Hot and already bothered:

exploring effects of warming waters on an imperiled freshwater fish,

Pugnose Shiner (Notropis anogenus)

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ABSTRACT

Climate change has emerged as an increasingly critical threat to freshwater systems that may affect freshwater fishes through direct effects on their thermal physiology, but also indirectly by altering the magnitude of biotic interactions and/or the relative abundance of cohabiting species. Understanding the direct and indirect effects of temperature represents a significant knowledge gap that may be vital to effective conservation strategies for freshwater fishes. This is especially true for already imperiled species because these taxa tend to be more sensitive to changes in their environment and are already contending with other, and potentially interactive, stressors. In this thesis, I used a combination of ecophysiology and field-based techniques to examine effects of elevated water temperature on Pugnose Shiner, *Notropis anogenus*, an endangered fish species in Canada.

In Chapter 1, I investigated effects of temperature on critical thermal maximum (CT_{max}) and standard metabolic rate (SMR) in juvenile Pugnose Shiner reared for 4 months across five different temperatures. CT_{max} was measured under normoxia and acute exposure to hypoxia to test for oxygen sensitivity of upper thermal limits in this species. CT_{max} increased with elevated water temperature. Agitation temperature (temperature at which fish show behavioural signs of thermal stress) also increased with rearing temperature and occurred on average 4.32°C above acclimation temperatures. CT_{max} was lower when fish were acutely exposed to hypoxia during CT_{max} trials and showed no increase between 19 and 25°C. Surprisingly, CT_{max} under acute hypoxia increased sharply at the highest temperature. SMR increased between 16 and 25°C, and then declined significantly at 28°C, indicating that the ability of Pugnose Shiner to reverse thermodynamic effects on SMR is limited and there was no evidence of long-term thermal compensation. Interestingly, gill size (e.g. total gill filament length, hemibranch area) increased

with long-term exposure to high temperature, which may increase oxygen uptake capacity and help to fuel increased metabolic demands.

In Chapter 2, I explored the role of temperature, other abiotic factors, and co-occurring species in shaping the fine-scale distribution of Pugnose Shiner within Thompson's Bay in the upper St. Lawrence River, Ontario. Multivariate analyses and occupancy modelling were used to assess how environmental variables and species co-occurrence accounted for variation in the distribution of Pugnose Shiner. Environmental variables, including temperature, were significant predictors of community composition. Pugnose Shiner was associated with clear, cool sites far from the inner bay with high vegetation density, including *Chara vulgaris*. Pugnose Shiner was negatively associated with Brook Silverside (*Labidesthes sicculus*), Largemouth Bass (*Micropterus salmoides*), Pumpkinseed (*Lepomis gibbosus*), and juvenile *Lepomis* spp. This has important conservation implications because some native centrarchids are increasing in abundance with climate warming, which may threaten persistence of rare and imperiled cyprinids, including Pugnose Shiner.

Although Pugnose Shiner showed little evidence of thermal compensation (SMR increased with acclimation temperature), it did show acclimation capacity in terms of thermal tolerance and increased gill size. Captive rearing may provide opportunities for thermal hardening in reintroduction efforts, and targeted hunting of expanding species may buffer Pugnose Shiner from the negative effects of elevated water temperature. Overall, my research highlights the importance of examining multiple traits at multiple scales of biological organization to quantify effects of elevated water temperature on freshwater fishes and guide conservation recommendations for imperiled species.

RÉSUMÉ

Les changements climatiques représentent une menace de plus en plus importante pour les systèmes d'eau douce et peut affecter les poissons à la fois par des effets directs sur leur physiologie thermique, mais aussi indirectement, en modifiant l'ampleur ou les interactions biotiques et / ou l'abondance relative des espèces voisines. Comprendre les effets directs et indirects de la température constitue une lacune importante qui peut être vitale pour établir des stratégies de conservation efficaces pour les poissons d'eau douce. Cela est particulièrement vrai pour les espèces déjà en péril car ces taxons ont tendance à être plus sensibles aux changements de leur environnement, et qui interagissent déjà avec d'autres facteurs potentiels de stress. Dans cette thèse, j'ai utilisé une combinaison d'écophysiologie et de techniques de terrain pour examiner les effets de l'elevation de la température de l'eau sur le méné camus, *Notropis anogenus*, une espèce menacée au Canada.

Dans un premier chapitre, j'ai étudié les effets de la température sur le maximum thermique critique (CT_{max}) et le taux métabolique standard (SMR) chez le méné camus juvénile, élevé pendant 4 mois à cinq températures différentes. La CTmax a été mesurée sous normoxie et exposition aiguë à l'hypoxie afin de tester la sensibilité à l'oxygène de la limite thermique supérieure (CT_{max}) chez cette espèce. La CT_{max} a augmenté avec la température de l'eau. La température d'agitation (température à laquelle les poissons montrent des signes comportementaux de stress thermique) a également augmenté avec la température d'élevage et s'est établie en moyenne 4.32°C au-dessus des températures d'acclimatation. La CT_{max} était plus basse lorsque les poissons étaient exposés à une hypoxie aiguë et ne présentait aucune augmentation entre 19 et 25°C. Étonnamment, la CT_{max} sous hypoxie aiguë a fortement augmenté à la température la plus élevée. Le SMR a augmenté entre 16 et 25°C, puis a diminué de manière significative à 28°C, indiquant que la capacité des individus à inverser les effets thermodynamiques sur le RSM est limitée entre 16 et 25°C. Il est intéressant de noter que la taille des branchies (longueur totale du filament des branchies, région de l'hémibranche) augmente avec l'exposition à long terme à une température élevée, ce qui peut augmenter la capacité d'absorption de l'oxygène et faciliter la réponse des individus à des demandes métaboliques accrues.

Au chapitre 2, j'ai exploré le rôle de différents facteurs, tels que la température, d'autres facteurs abiotiques et de l'abondance d'espèces concurrentes, sur la répartition à petite échelle du méné camus dans la baie Thompson (fleuve du Saint-Laurent, Ontario). Des analyses multivariées et une modélisation de l'occupation ont été utilisées pour évaluer la manière dont les variables environnementales et la cooccurrence d'espèces expliquaient la répartition du méné camus. Les variables environnementales, y compris la température, étaient des prédicteurs significatifs de la composition de la communauté. Le méné camus était associé avec des sites clairs et frais éloignés de la baie intérieure, où la densité de la végétation était élevée, y compris *Chara vulgaris*. Le méné camus était négativement associé avec le crayon d'argent (*Labidesthes sicculus*), l'achigan à grande bouche (*Micropterus salmoides*), le crapet soliel (*Lepomis gibbosus*) et des jeunes *Lepomis* spp. Ces observations ont des implications importantes pour la conservation car le centrage de certaines centrarchidés indigènes augmente avec le réchauffement climatique, ce qui peut menacer la persistance de cyprinidés rares et en péril, y compris le méné camus.

Bien qu'il y ai peu d'indications que le méné camus fasse preuve de compensation thermique (le SMR augmentait avec la température d'acclimatation), les individus ont démontré une capacité d'acclimatation en termes de tolérance thermique et une augmentation de la taille

des branchies. En termes d'action de conservation, l'élevage en captivité peut permettre le durcissement thermique (« thermal hardening ») des individus dans des efforts de rapatriement / réintroduction. De plus, la chasse ciblée d'espèces en expansion peut protéger le méné camus des effets négatifs de la température élevée de l'eau. Dans l'ensemble, nos recherches ont mis en évidence l'importance d'examiner plusieurs traits à plusieurs échelles d'organisation biologique, afin de quantifier les effets de l'élévation de la température de l'eau sur les poissons d'eau douce, et de guider les recommandations de conservation des espèces en péril.

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PREFACE

Thesis Format

This thesis has been written in manuscript-format, as permitted by McGill University. The titles of the two manuscripts in this thesis are as follows:

- Coping with climate warming: phenotypic plasticity in an endangered minnow in response to elevated water temperature
- 2. Examining the emerging threat of climate change to an endangered minnow

The first chapter has been submitted to Aquatic Conservation and is currently under review. The second chapter will be submitted to Endangered Species Research. General introductory material, a linking statement, and general conclusions provide cohesion throughout the thesis. All references were formatted following Ecology guidelines and citation style for consistency and are listed together at the end of the thesis.

Author Contributions

This research and thesis program were carried out under the supervision of Dr. Lauren J. Chapman in the Department of Biology at McGill University and Dr. Nicholas E. Mandrak in the Department of Biological Sciences at University of Toronto Scarborough. It represents the results of my own independent research. I planned, designed and carried out all field and laboratory experiments personally, and then compiled, interpreted and reported results. Both chapters have been co-authored by Dr. Chapman and Dr. Mandrak who contributed to the design, execution, statistical analyses, and writing by providing valuable feedback and assistance.

GENERAL INTRODUCTION

Freshwater fishes are among the most imperiled taxa on the planet

The Earth is currently experiencing a sixth mass extinction, with the global rate of species loss tens to hundreds of times greater than ever before in human history (Barnosky et al. 2011, Dirzo et al. 2014, Ceballos et al. 2015, McCallum 2015). A recent report published by the International Panel of Biodiversity and Ecosystem Services (IPBES) estimated that up to 1 million species of plants and animals are currently threatened with extinction (IPBES 2019). However, limited conservation research on freshwater fishes represents a significant knowledge gap in our understanding of global biodiversity trends (Clark and May 2002, Darwall and Freyhof 2016). Freshwater and diadromous fishes constitute 23% of vertebrate diversity and 48% of fish diversity (Fricke et al. 2019), but less than half of all known species have been assigned a conservation status by the IUCN Red List (IUCN 2019). This data deficit meant that freshwater fishes were not factored into the extinction risk calculations in the IPBES report, which instead relied on rates of imperilment in more well-studied groups (IPBES 2019). Of the freshwater and diadromous fishes that have been assessed by the IUCN, 28% are currently categorized as critically endangered, endangered, or vulnerable, a conservative estimate that places freshwater fishes among the most imperiled vertebrate groups on the planet (Darwall and Freyhof 2016, IUCN 2019). Rates of imperilment in freshwater fishes are high, in part, due to the fact that freshwater ecosystems are disproportionally affected by multiple and pervasive stressors (Dudgeon et al. 2006, Ormerod et al. 2010, Carpenter et al. 2011, WWF 2014).

Freshwater comprises 0.01% of the Earth's water supply, with lakes, reservoirs and rivers occupying less than 2.5% of Earth's surface area (Lehner and Döll 2004), although exact estimates may vary (McManamay et al. 2018). Despite this small total surface area, freshwater systems are affected by upstream landscape processes, such as deforestation and erosion, and act as sinks for terrestrial run-off, including pollution (Thieme et al. 2010). Furthermore, freshwater systems are under intense competition with humans for water resources such as hydropower, irrigation, and transport (Thieme et al. 2010, Vorosmarty et al. 2010, WWF 2016). Habitat destruction and degradation, overexploitation, pollution, and invasive species are the main causes of freshwater fish declines globally (Arthington et al. 2016, Closs et al. 2016); however, climate change is a prominent emerging threat for freshwater species (Reid et al. 2019) that may act independently but, more likely, compound the effects of other anthropogenic stressors on already vulnerable taxa (Ficke et al. 2007, Arthington et al. 2016, Heino et al. 2016).

Climate change is an emerging threat to freshwater fishes

Over the course of the century, global climate change is projected to increase the average atmospheric temperature between 1 and 5°C with most pronounced temperature increases at high latitudes (IPCC 2013). Rising atmospheric temperatures and associated impacts of climate change are projected to affect freshwater fishes in numerous ways. Direct stressors include elevated water temperatures, decreased dissolved oxygen, altered hydrological regimes, and increased toxicity of pollutants (Ficke et al. 2007, Myers et al. 2017). Warming waters and increased frequency of extreme thermal events mean that fishes may be exposed to temperatures beyond their thermal limits, resulting in potentially harmful effects (Pörtner and Farrell 2008). Increased temperatures and extreme thermal variability as a result of climate change are

especially significant for ectotherms such as fishes that rely on the external environment to regulate their body temperature (McNab 2002) and, consequently, their metabolic rate (Fry 1967).

Elevated water temperatures can affect fish at multiple levels of biological organisation including molecular, physiological, whole organism, population, and community responses. These include changes in gene expression (Goldspink 1995, Brian et al. 2008, Windisch et al. 2014), organ remodelling (Sollid and Nilsson 2006, Keen et al. 2017, Wu et al. 2017, Nyboer and Chapman 2018), growth and condition (McCormick and Molony 1995, Munday et al. 2008, Healy and Schulte 2012), fitness and performance traits (Pankhurst 1997, Pörtner et al. 2001, Herbing 2002), and species distributions (Comte et al. 2013, Alofs et al. 2014, Comte et al. 2014, Heino et al. 2016, Ruiz-Navarro et al. 2016). Freshwater fishes will likely be more exposed to temperature increases than marine fishes because freshwater systems are shallower and have a smaller thermal buffering capacity (O'Reilly et al. 2015, FAO 2018). Furthermore, given the physical restrictions of their environment, freshwater fishes often have limited dispersal capacity (Woodward et al. 2010). Thus, to cope with rapidly changing thermal regimes, freshwater fishes that need to respond *in situ* must adjust through genetic adaptation and/or phenotypic plasticity. Highly sensitive, highly exposed, and simultaneously contending with other (and potentially interactive) stressors, freshwater fishes may be particularly vulnerable to extinction due to rising temperatures.

Climate change may affect freshwater fishes through direct effects on their thermal physiology, but also indirectly by altering the magnitude or biotic interactions and/or the relative abundance of co-habiting species. For example, Wenger et al. (2011) projected that rising temperature, among other factors, will decrease the availability of suitable habitat for native

Cutthroat Trout (*Oncorhynchus clarkii*) and non-native Brook Trout (*Salvelinus fontinalis*), Rainbow Trout (*Oncorhynchus mykiss*), and Brown Trout (*Salmo trutta*) in the interior western United States by 2080; in addition, associations of Cutthroat Trout with the other three species will result in an additional suitable habitat reduction of 26%. Additionally, Jackson and Mandrak (2002) predicted that a range expansion of Smallmouth Bass (*Micropterus dolomieu*) across Ontario under climate-change scenarios would extirpate 25 000 populations of four cyprinid prey species by 2100. Understanding the direct and indirect effects of temperature represents a significant knowledge gap that may be vital to effective conservation strategies for freshwater fishes.

Trait-based research may improve conservation of freshwater fishes dealing with climate change

In response to a significant knowledge gap on the effects of climate change on freshwater fishes, there has been growing research on trait-based responses to elevated water temperatures, both in the field and in the lab. Trait-based approaches usually refer to multi-species vulnerability assessments using species-specific characteristics (Pacifici et al. 2015, Foden et al. 2019) and can improve predictions of climate-change effects on freshwater fishes (Sunday et al. 2012, Chessman 2013, Comte et al. 2014). However, species-specific thermal tolerance data are still lacking for most freshwater fishes making it challenging to improve predictive modelling. Even among the relatively well-studied freshwater fishes of North America, much of the thermal tolerance research has been directed towards species of commercial and recreational value, such as Largemouth Bass (*Micropterus salmoides*) and Rainbow Trout (e.g., Beitinger et al. 2000, Myers et al. 2017). Few studies have investigated the thermal tolerance of already imperiled species, even though these species tend to be more sensitive to changes in their environment. This taxonomic bias not only limits our understanding of how fishes respond to temperature, but may also limit the effectiveness of conservation efforts of imperiled species.

In Canada, in light of this critical knowledge gap, there has been increased effort to understand causal threat mechanisms and integrate trait-based research into recovery strategies for freshwater fishes at risk. This was formalized in 2017 under the Canadian Freshwater Species at Risk Network (FWSARNet), a collaboration between Fisheries and Oceans Canada (DFO) and academic researchers at 10 universities in Canada and the United States. The goal of research generated within the FWSARNet was to direct conservation action and improve the recovery of imperiled freshwater fishes and mussels in Canada by focusing on two core themes: 1) "Field, lab, and analytical experiments to better understand threat mechanisms (causative factors) and impacts (consequences of causative factors), including relationships between multiple interacting stressors and species responses, such as changes to vital rates"; and, 2) "Developing captive rearing techniques for listed freshwater fishes and mussels, including research to understand mating systems and the development of experimental populations" (FWSARNet 2018). This research project was developed within the inaugural year of FWSARNet and focuses on the effects of elevated water temperature on the imperiled Pugnose Shiner, Notropis anogenus, an endangered species in Canada.

Pugnose Shiner is an endangered species in Canada

Canada's abundant fresh waters are home to over 200 species, sub-species, and distinct populations of freshwater and diadromous fishes (Burkhead 2012, Rainer 2017, IUCN 2019). However, over one third are currently threatened by extinction or national extirpation (Darwall and Freyhof 2016, Government of Canada 2019). This includes Pugnose Shiner, *Notropis*

anogenus (Forbes 1885), a small North American fish in the family Cyprinidae (carps and minnows). Pugnose Shiner has a slender, pale-yellow and silver body with a dark lateral stripe that extends onto the snout (Holm et al. 2010). Its characteristic feature is a tiny, upturned mouth that distinguishes it from other blackline shiners: Blackchin Shiner (*Notropis heterodon*), Blacknose Shiner (*Notripis heterolepis*), and Bridle Shiner (*Notropis bifrenatus*) (Holm et al. 2010). Indeed, the Greek scientific name *anogenus* means "without a chin." Unlike most shiners, Pugnose Shiner also has a black peritoneum (Page and Burr 2011). The maximum total length is 60 mm for females and 50 mm for males (Holm and Mandrak 2002). Little is known about the life history of Pugnose Shiner; however, the maximum life span is up to 3 years (Becker 1983). It is an open substrate, non-guarding spawner and likely spawns in mid-June in Ontario, Canada (Leslie and Timmins 2002). Pugnose Shiner feeds on plants, algae, small leeches, cladocerans, and trichopterans (COSEWIC 2013).

Pugnose Shiner is found in the Great Lakes and Upper Mississippi River basins, in clear, low-flow, and highly vegetated waters (Holm and Mandrak 2002). The species is found in shallow sites of lakes and slow-moving streams and is typically associated with dense submerged vegetation, including plants such as *Chara*, *Vallisneria*, and *Elodea* spp. (COSEWIC 2013). Pugnose Shiner is highly sensitive to water quality and seems to be especially intolerant of turbidity; Pugnose Shiner acclimated to low levels of turbidity (<10 NTU) exhibited a decline in maximum swim speed, hypoxia-tolerance, and schooling behaviour (Gray et al. 2014, Gray et al. 2016).

Pugnose Shiner is rare across its range (Bailey 1959, COSEWIC 2013, NatureServe 2018) and is frequently reported as being absent from sites with apparently suitable habitat (e.g. Schmidt 2018). The IUCN Red List classifies Pugnose Shiner as Least Concern (IUCN 2019),

although many experts consider the species to be Threatened (Jelks et al. 2008). In Canada, Pugnose Shiner are only found within the province of Ontario, and the species is assessed as Threatened by COSEWIC (COSEWIC 2013) and listed as Endangered under Schedule 1 of the Species at Risk Act. Within the province, it has been extirpated from three sites: Gananoque River, Point Pelee, and Rondeau Bay (COSEWIC 2013). Pugnose Shiner is not federally listed in the United States, but its status varies by state; the species is listed as Extirpated in Ohio, Critically Imperiled in Illinois, Indiana, Iowa, and North Dakota, and Imperiled in Michigan, Minnesota, New York, and Wisconsin (NatureServe 2018).

Population sizes and trends of Pugnose Shiner are not known. However, Pugnose Shiner distribution is highly fragmented (COSEWIC 2013), and populations are genetically distinct (McCusker et al. 2014b). This suggests limited connectivity among sites and greater vulnerability to extirpation as a result of low dispersal capacity and genetic factors. Furthermore, habitat reductions are occurring across the species' range that pose a significant risk for this highly sensitive species. Habitat destruction and degradation, including aquatic vegetation removal, sediment/nutrient loading, and lakeshore development, have been identified as the greatest threats to Pugnose Shiner (COSEWIC 2013). Climate change may represent an emerging threat for this already vulnerable species, but this has remained largely unexplored.

Thesis objectives

The goal of my thesis was to explore the effects of elevated water temperature on the physiology and distribution of Pugnose Shiner, and to provide recommendations for conservation action. This thesis is presented in two chapters. In Chapter 1, I examined phenotypic plasticity in critical thermal maximum (CT_{max}) and standard metabolic rate (SMR) in juvenile Pugnose

Shiner captured from a brood stock pond at the State University of New York (SUNY) Cobleskill. To explore acclimation capacity at ecologically relevant temperatures and timescales, juvenile Pugnose Shiner were reared for 4 months across five different temperatures representative of their current thermal range in Ontario. To test for oxygen sensitivity of upper thermal limits in this species, CT_{max} was measured under normoxia and acute exposure to hypoxia. Moving from whole-organism to population and community responses to elevated water temperatures, Chapter 2 builds upon Chapter 1 by examining the role of temperature, other abiotic factors, and co-occurring species in shaping the distribution of Pugnose Shiner within Thompson's Bay in the upper St. Lawrence River, Ontario. Multivariate analyses and a habitat occupancy model were used to assess the extent to which environmental variables and species co-occurrence accounted for variation in the fine-scale distribution of Pugnose Shiner within Thompson's Bay. I end with a concluding discussion that highlights the major findings in light of broader related literature and I consider next steps for research and recovery action for Pugnose Shiner in Canada.

CHAPTER 1

Title: Coping with climate warming: phenotypic plasticity in an imperiled minnow in response to elevated water temperature

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ABSTRACT

Climate change has emerged as an increasingly important threat to freshwater systems. To cope with rapidly changing thermal regimes, freshwater fishes must either relocate or adjust through genetic adaptation and/or phenotypic plasticity. Short-term responses to elevated water temperature have been well studied in freshwater fishes; however, far less is understood about change induced by long-term exposure. This study investigated the effects of rearing temperature on critical thermal maximum (CT_{max}) and standard metabolic rate (SMR) in Pugnose Shiner, Notropis anogenus. Juvenile Pugnose Shiner were reared for 4 months across five different ecologically relevant temperatures. CT_{max} was measured under normoxia and acute exposure to hypoxia to test for oxygen sensitivity of upper thermal limits in this species. CT_{max} increased with elevated water temperature. Agitation temperature (temperature at which fish show behavioural signs of thermal stress) also increased with rearing temperature and occurred, on average, 7.63°C below the CT_{max} (or 4.32°C above acclimation temperatures). CT_{max} was lower when fish were acutely exposed to hypoxia and showed no acclimation capacity between 19 and 25°C. Surprisingly, CT_{max} under acute hypoxia increased sharply at the highest temperature. SMR increased between 16 and 25°C, and then declined significantly at 28°C, indicating that the ability of Pugnose Shiner to reverse thermodynamic effects on SMR is limited, and there was no evidence of long-term thermal compensation. Gill size (e.g. total gill filament length, hemibranch area) increased with long-term exposure to high temperature, which may increase oxygen uptake capacity and fuel the increased metabolic demands of warmer waters.

INTRODUCTION

The Earth is currently experiencing a sixth mass extinction, in which the global rate of species loss is tens to hundreds of times greater than natural extinction rates (Barnosky et al. 2011, Dirzo et al. 2014, Ceballos et al. 2015, McCallum 2015). While the global status of freshwater fishes is largely unknown due to limited conservation research, there is a tremendous richness of freshwater fishes (over 16 000 species) (Fricke et al. 2019), and they rank among the most imperiled vertebrate groups on the planet (Darwall and Freyhof 2016, IUCN 2019). This is a result of freshwater ecosystems being disproportionally affected by multiple and pervasive stressors (Dudgeon et al. 2006, Ormerod et al. 2010, Carpenter et al. 2011, WWF 2014). Climate change is an emerging threat that may compound the effects of other anthropogenic stressors on already vulnerable freshwater fish species (Ficke et al. 2007, Arthington et al. 2016, Heino et al. 2016).

Increased temperatures and extreme thermal variability as a result of climate change are especially significant for ectotherms, such as fishes, that rely on the external environment to regulate their body temperature (McNab 2002) and, consequently, their metabolic rate (Fry 1967). Freshwater fishes will likely be more exposed to temperature increases than marine fishes because freshwater systems are shallower and have a smaller thermal buffer capacity (O'Reilly et al. 2015, FAO 2018). Furthermore, given the physical restrictions of their environment, freshwater fishes often have limited dispersal capacity (Woodward et al. 2010). Highly sensitive, highly exposed, and simultaneously contending with other (and potentially interactive) stressors, freshwater fishes may be particularly vulnerable to extinction due to rising temperatures.

The critical thermal maximum (CT_{max}), a non-lethal metric of thermal tolerance, is often used to assesses the vulnerability of ectotherms to climate change. Theoretical underpinnings of

 CT_{max} suggest that rare events that increase temperatures beyond the CT_{max} of a species may shape population persistence and/or biogeography with climate change (Sunday et al. 2012, Sunday et al. 2014). Species with a CT_{max} well above environmental temperatures are assumed to be relatively well-buffered from the negative effects of warming waters. However, CT_{max} is a highly plastic trait, especially in aquatic systems (Gunderson and Stillman 2015), and risk of extinction may be over-estimated among studies that do not account for this plasticity. Similarly, measurements of standard or resting metabolic rates (SMR or RMR, respectively) may be used as another metric to assess the threat of global warming, as individual fitness may decline as SMR/RMR increases with rising temperatures (Pörtner et al. 2001). However, metabolic rates can also exhibit thermal compensation (also known as reverse plasticity) where SMR and RMR decline after an acclimation period to warmer waters, thus reducing the metabolic costs of increased temperature (Seebacher et al. 2014). Therefore, the ability of an ectotherm to persist in elevated temperatures may depend, at least in part, on their ability to physiologically acclimate by increasing thermal tolerance and/or reducing metabolic rates. Furthermore, it may also depend on their ability to cope with other environmental stressors that interact with climate warming.

One stressor that is likely to interact with elevated water temperature is aquatic hypoxia. Hypoxia occurs naturally in many systems characterized by low light and low levels of mixing (e.g. dense swamps, profundal waters of deep lakes), but hypoxia is increasing in extent and frequency of occurrence driven by influxes of waste and fertilizers that accelerate eutrophication and subsequent bacterial decomposition of organic matter (Diaz and Rosenberg 2008). Furthermore, eutrophication and associated hypoxia will likely be exacerbated by warming waters (Feuchtmayr et al. 2009, Moss 2011). The effects of climate warming in aquatic ectotherms may be compounded by exposure to hypoxia because both stressors affect aerobic

metabolism (McBryan et al. 2013). Specifically, the oxygen demands of an ectotherm increase with ambient temperature, while aquatic hypoxia limits oxygen supply making it more difficult to meet temperature-driven increases in oxygen requirements. There is a growing body of theoretical and empirical research that suggest a functional association between hypoxia and thermal tolerance, often explored under the framework of the oxygen- and capacity-limited thermal tolerance concept (OCLTT, Pörtner and Farrell 2008, Pörtner 2010). Briefly, OCLTT suggests that under warming water temperature, the maximum rate of oxygen consumption increases, exceeding the capacity of the cardio-respiratory system to supply oxygen to tissues, manifested as a reduction in aerobic scope (AS - difference between the standard and maximum metabolic rate) and, in some cases, a reduction in CT_{max} (Pörtner et al. 2017). Consequently, the upper thermal limits of fishes and other aquatic animals are thought to be highly sensitive to hypoxia. While there is a mounting body of literature on acclimation potential in CT_{max} and SMR/RMR, most studies have focused on short-term or acute exposures. However, plasticity in thermal responses can act over much longer time frames – developmental, transgenerational, or multi-generational - and short-term acclimation may interact with developmental plasticity, partially or fully irreversible phenotypic plasticity during early ontogeny (Kinne 1962), to affect thermal tolerance (Schulte et al. 2011). In addition, studies of thermal acclimation in juvenile fishes are few and, similarly, have focused primarily on short-term exposure periods (e.g. 7 - 40 days, Walsh et al. 1998, Ziegeweid et al. 2007, Wang et al. 2008, Brahmane et al. 2014, Komoroske et al. 2014). This represents a significant knowledge gap because early developmental life stages may be most sensitive to thermal processes (Komoroske et al. 2014) and subject to non-genetic irreversible changes (Kinne 1962). The latter was demonstrated in a study by Schaeffer and Ryan (2006) of juvenile Zebrafish (Danio rerio) reared under two

different temperatures for over 100 days. They found that developmental exposure to higher temperatures had an irreversible impact on CT_{max} ; they detected significant differences between rearing temperature regimes even after subsequent short-term acclimations (Schaefer and Ryan 2006). In a second study of developmental plasticity, Donelson et al. (2011) reared Spiny Chromis Damselfish (*Acanthochromis polyacanthus*) under three temperature regimes (mean temperature in wild populations, +1.5°C, +3°C) and found that fish exhibited reduced RMR after being reared at the highest temperature compared to fish reared under lower temperatures and acutely exposure to the high temperature. Both of these studies emphasize the importance of developmental plasticity in coping with thermal stress. However, the threat mechanisms of many imperiled freshwater fishes are poorly understood, which may hinder recovery efforts. This information may be particularly pertinent in captive-breeding programs for imperiled fishes that may need to consider the rearing environment in relocation or repatriation programs.

In this study, the effect of elevated rearing temperature on the thermal tolerance and metabolic rate in juvenile Pugnose Shiner, *Notropis anogenus*, was examined. Pugnose Shiner is a rare North American fish in the family Cyprinidae (carps and minnows) and is federally protected as an endangered species in Canada. Within Canada, Pugnose Shiner is found in isolated populations (McCusker et al. 2014b) in clear, shallow, and highly vegetated habitats in Ontario (Holm and Mandrak 2002). Turbidity has been identified as a primary cause of decline in several imperiled Canadian fish species, including in this environmentally sensitive species (Gray et al. 2014, Gray et al. 2016), although other potential stressors remain poorly studied. Pugnose Shiner may be vulnerable to climate change because its small body size and presumed weak swimming ability would limit natural range expansion (Chu et al. 2005), although specific

thermal responses have never been investigated, and unknown thermal limits have been identified as a "major knowledge gap" for this species (McCusker 2016).

The capacity to which juvenile Pugnose Shiner are able to acclimate to elevated water temperatures was examined by comparing CT_{max} and SMR after long-term exposure to a range of five ecologically relevant temperatures. Mass-relationships were quantified at the end of the rearing period to detect significant changes in fish condition across temperature regimes. The effect of acute hypoxia (low dissolved oxygen) exposure on CT_{max} was also quantified, given that nutrient loading and eutrophication represents a medium to high threat across all populations of Pugnose Shiner (COSEWIC 2013). Notably, Lake Erie, which hosts a robust population of Pugnose Shiner near Long Point, has experienced massive algal blooms in recent years and associated large-scale fish die-offs (Rao et al. 2014, Watson et al. 2016). To examine potential mechanisms underlying phenotypic plasticity in tolerance and SMR, gill size was quantified based on the prediction that greater oxygen demands under elevated water temperature might lead to the development of larger gills.

METHODS

Fish collection and experimental set-up

Pugnose Shiner was collected from a 0.57-acre broodstock pond at the SUNY Cobleskill Experimental Pond Complex (42° 40' 18.07"N, 74° 29' 53.494"W) and transported to McGill University on October 24, 2017. This pond stock was derived from 65 adult Pugnose Shiner collected from Sodus Bay, NY in October 2015 (Robbins et al. 2018). In the lab, young of the year (YOY) were distinguished from adults based on small size (total length < 20 mm) and were distributed among twenty 52 L aquaria. Each tank was equipped with a sponge filter, an oxygen diffuser, a plastic plant, and sand. Tanks were fully aerated, pH was held at 7.5 ± 0.5 , and conductivity was kept between 200-300 uS, parameters informed by the Cobleskill broodstock pond conditions. The laboratory was held under a 15:9 hr light:dark cycle to mimic the July photoperiod of Cobleskill, NY. Fish were fed frozen bloodworm *ad libitum* once daily, and water-quality parameters (including ammonia and nitrite) were monitored daily. Water changes (10-20 L) were conducted every three days or when necessary. Temperature was maintained at $16^{\circ}C \pm 0.5^{\circ}C$ for three months prior to the experimental rearing period. Seven to ten weeks prior to starting the acclimation experiment, 182 fish were measured (mean SL(cm) = 2.33 ± 0.02 SE, mean mass (g) = 0.207 ± 0.007 SE), with 160 of these used in the temperature treatments. Fish were not measured again prior to starting the treatments to minimize stress to this sensitive species.

During the experimental period, aquaria were randomized to five rearing temperatures: 16°C, 19°C, 22°C, 25°C, and 28°C. These values were selected to encompass the summer thermal range of Pugnose Shiner in Ontario (DFO *unpublished data*). Four aquaria were assigned to each temperature, and densities were standardized to eight fish per aquarium. Of these eight fish, four were used for CT_{max} trials, two were used for SMR trials, and the remainder were euthanized for immunohistochemistry studies (not reported here). Aquaria assigned to temperatures 19°C and above were warmed using individual aquarium heaters at a rate that did not exceed more than 1°C per day. Aquaria were held at these rearing temperatures (± 0.2°C) between 101 and 133 days (mean: 111.8 days). No mortality was observed during the experimental period.

Condition

To examine the effect of rearing temperature on size and condition, standard length (SL) and mass were measured for a subset of fish (N = 110) after the 4-month rearing period. The relative condition factor (K_n) was determined using the equation

$$K_n = \frac{mass_{obs}}{mass_{pred}}$$

where predicted mass was calculated using the equation $mass_{pred} = a \times SL^b$ (Le Cren 1951). The coefficient *a* and scaling exponent *b* were determined from the least squares linear regression of mass and standard length, both log-transformed. A K_n value of 1 represented the average condition, therefore individuals with $K_n > 1$ were considered to be in better condition than average, whereas those with $K_n < 1$ were in worse condition. It should also be noted that, by the end of the 4-month acclimation period, there was a range in body size within all tanks (average difference within tanks between the smallest and largest fish was 1.65 cm and 1.23 g). Furthermore, observations on fish behaviour within tanks indicated dominance of some larger fish that may have influenced the growth of others. Therefore, the smaller fish were used for CT_{max} trials and the larger fish for the SMR trials to better match respirometer volume and CT_{max} chamber volume to fish size. Larger fish were also used for the gill dissections as the fine-scale measurements were deemed more accurate on the larger fish.

Critical thermal maximum (CT_{max})

The critical thermal maximum (CT_{max}) was quantified as loss of equilibrium (LOE), following a method described in earlier studies that gradually increased water temperature at a rate low enough to not shock the fish, but high enough to prevent acclimation (Becker and Genoway 1979, Lutterschmidt and Hutchinson 1997, McDonnell and Chapman 2015). After

being starved for 48 h, two of the smaller fish from the same rearing tank (< 0.8 g) were transferred to separate baskets in an experimental tank at their treatment temperature. The larger fish were maintained for estimates of SMR. Bubble wrap was placed on the surface of the water in each basket to prevent jumping and to reduce gas exchange. Fish were acclimated for 3 h before the trials began. During trials, fish were subjected to a constant increase in water temperature at a rate of 0.33°C/min. Water temperature and dissolved-oxygen concentration were continuously monitored by probes and regulated by a control unit and software (TEMP-REG, OXY-REG, Witrox 4, AutoResp; Loligo Systems). When fish exhibited LOE, the individual was removed from its basket and placed in an aerated tank at its rearing temperature to recover. After a recovery period, mass and standard length (SL) were measured, and fish were returned to their rearing tank. Trials were conducted under either high oxygen (normoxic) or low oxygen (hypoxic) conditions. During normoxic trials, the experimental tank was supplied with constant aeration to maintain high dissolved-oxygen (DO) levels (>95%) throughout. During hypoxic trials, DO was gradually reduced during the 3-hour acclimation period at an approximate rate of 6.5% every 15 minutes until concentrations reached 22% by bubbling nitrogen gas through the system. The hypoxia condition was selected as 22% saturation because it represented a potentially stressful condition but was higher than the average critical tension (Pcrit) of Pugnose Shiner, as determined in a previous study (Gray et al. 2016). The OXY-REG probe was calibrated at 0% and 100% at each rearing temperature. Each trial was recorded by a webcam to minimize disturbance. Time-stamped webcam footage was reviewed and cross-referenced with the software output file to validate the temperature at multiple behavioural thresholds, including CT_{max} and agitation temperature (T_{ag}). T_{ag} was first described by McDonnell and Chapman (2015) as the temperature at which a fish starts exhibiting avoidance behaviour that occurs before

fish lose equilibrium during a CT_{max} trial. In this study, fish were initially at rest under cover or moving slowly throughout the basket. However, as temperatures increased, individuals would begin to quickly dart around the basket in an agitated manner. Often, individuals would display intermittent periods of agitation and rest before reaching CT_{max} ; T_{ag} was recorded as the first agitation period lasting longer than 40 seconds. T_{ag} was proposed as an ecologically relevant metric that may be used to quantify the onset of thermal stress. Given that there is no standard agitation methodology used to quantify T_{ag} , a 40 second period was selected to discriminate between short, passive movements and longer, active avoidance behaviours. The agitation window was calculated as the difference between CT_{max} and T_{ag} , and McDonnell and Chapman (2015) suggested that larger agitation windows represent a wider range of stressful thermal conditions.

To quantify the degree of plasticity in CT_{max} , the acclimation response ratio (ARR) was calculated under both normoxic and hypoxic trials. ARR_{CTmax} is defined as the change in CT_{max} across the range of acclimation temperatures and has been used as an estimate of thermal plasticity (Claussen 1977). When the change in CT_{max} across temperatures is consistent, ARR_{CTmax} is equal to the slope of the linear regression of the relationship between CT_{max} and temperature across all individuals and all treatments. While ARR has not specifically been applied to T_{ag} before, it was calculated in the same way as ARR_{CTmax} and was used similarly to estimate the plasticity in agitation temperature.

Standard metabolic rate (SMR)

Metabolic rates (MO₂) were obtained using Loligo AutoResp intermittent flow-through respirometry equipment and software (Loligo Systems). The system included a 14 mL glass chamber, plastic tubing, and flush pumps for a total system volume of 183 mL. When the system was open, the chamber was continuously flushed with aerated water. When the system was closed, oxygen concentrations declined as the fish respired. The oxygen and temperature in the experimental tank surrounding the chamber were monitored and regulated using TEMP-REG and OXY-REG probes, while the oxygen within the chamber was measured using a Fibox3 fibre-optic oxygen probe and optical oxygen sensor (Loligo Systems). Oxygen probes were calibrated at each treatment temperature. To account for background respiration, the rate of oxygen depletion in the empty chamber was calculated over a 15-minute closed interval before each metabolic trial; the average control rate was -3.49 %/h. Fish were starved for 48 h to ensure a post-absorptive state. Before being put in the chamber, fish were measured (SL) and weighed (g). Only individuals > 0.80 g were used because the chamber was too large to accurately detect metabolic rates in smaller fish. Metabolic rates were measured for individual fish at their rearing temperature. Trials were run overnight and lasted a minimum of 14 h (average duration of 16 h 41 min). The rate of oxygen depletion was calculated over 20-minute closed measure periods, alternating with a 10-minute flush period and a 2-minute wait period. The control rate was subtracted from the measured rate to calculate final MO₂ values, and trials were only included if the control rate was < 35% of the measured rate. Standard metabolic rate (SMR) was estimated from the minimum oxygen uptake rate (MO_{2Min}), calculated as the mean of the lowest 10% MO_2 rates. Given that metabolic rate scales with body size, SMR was mass-adjusted using the equation:

$$SMR = MO_{2Min} \times \left(\frac{mass_{mean}}{mass_{obs}}\right)^{b}$$

where *b* was calculated from the slope of the least squares linear regression of mass and MO_{2Min} , both log-transformed. To quantify the response of SMR to rearing temperatures, the Q_{10}
coefficient was calculated. Q₁₀ is the change in metabolic rate over a change in temperature of 10°C (McNab 2002) and was calculated from the equation:

$$Q_{10} = \left[\frac{SMR_2}{SMR_1}\right]^{10/(T_2 - T_1)}$$

Q₁₀ was calculated using the mean SMR at each temperature, between each increment (e.g., 16-19°C, 19-22°C, 22-25°C, and 25-28°C) and also derived from the slope of the linear regression across the full temperature range (16-28°C).

Gill morphometrics

To examine the effects of rearing temperature on gill size, the two largest fish in each tank from the lowest (16°C) and highest (28°C) temperature treatments were culled and euthanized using a solution of clove oil and ethanol (mean mass = 1.50 g, mean SL = 4.30 cm). Gills were dissected out from the branchial basket on one side of the fish, and the four gill arches were separated. Both sides of each arch (hemibranch) were photographed using a Lumenera Scientific Infinity camera attached to a dissecting microscope. Measurements of six gill metrics were made using ImageJ v. 1.50i and measured to the nearest 0.001 mm or 0.01 mm². These included total gill filament length (TGFL), average gill filament length (AFL), total number of filaments (TNF), total area of the hemibranchs (THA), average length of the base of the gill hemibranch (ABL), and average density of gill filaments (AFD). TGFL was quantified by measuring the length of every fifth filament, using the average of two measures to estimate the length of the four filaments between them, summing the lengths of the filaments for side of the branchial basket. AFL was also quantified using this procedure, but the total filament length was divided by the number of filaments. TNF was quantified by counting the number of filaments on all four arches. THA was quantified by estimating the area around the filaments for each side of

each hemibranch for all four arches and summing these values. The ABL was estimated as the length of the base of each hemibranch expressed as an average of all hemibranchs. The AFD was then calculated by dividing the total number of filaments on the gill hemibranch by the length of the base of the hemibranch and averaging over all hemibranchs. All gill metrics were expressed for one side of the branchial basket.

Gill metrics were mass-adjusted using the equation:

$$Trait_{adj} = Trait_{raw} \times \left(\frac{mass_{mean}}{mass_{obs}}\right)^{b}$$

where *b* was calculated from the slope of the least squares linear regression of mass and the raw gill measurement, both log-transformed. One gill trait (number of gill filaments) exhibited an effect of temperature on *b*; specifically, there was an effect of mass of number of filaments in fish reared at 28°C but not at 16°C. However, the size distribution of fish was unequal between these two temperatures (e.g. smaller fish were sampled from 28°C: mean mass of 1.84±0.19 SE g at 16°C and 1.25± 0.11SE g at 28°C) and once mass was standardized (e.g. fish less than 1.3 g were removed), mass effects were no longer significant (b \approx 0). Therefore, the effect of rearing temperature on number of gill filaments was examined using this smaller subset and values were not mass adjusted.

Statistical analyses

All analyses were performed using open-source packages (R Core Team 2018). ANOVA was used to test for differences in CT_{max} , agitation temperature, and thermal window with rearing temperature and test condition (oxygen) as fixed factors, tank as a random factor nested within temperature, and including the interaction between rearing temperature by test condition. The effects of body mass on CT_{max} were tested by including it as a covariate, but there was no

significant effect, and it was removed from the models. ANOVA was used to test for differences in mass-adjusted SMR, again with rearing temperature as a fixed factor and tank as a random factor nested within temperature. Post-hoc Tukey tests were performed to assess differences between pairs of rearing temperatures and, for CT_{max} trials, oxygen treatment within each rearing temperature. Finally, ANOVA was used to test for differences in five mass-adjusted gill metrics and one non-mass-adjusted gill metric (number of gill filaments), with rearing temperature (16°C and 28°C only) as a fixed factor and tank as a random factor nested within temperature.

RESULTS

Body size and condition

While there was a range in body size within all tanks at the end of the rearing period (average difference within tanks between the smallest and largest fish was 1.65 cm and 1.23 g), there was no effect of rearing temperature on mass or standard length (P=0.5042 and P=0.4744, respectively) (Table 1). Relative condition factor was calculated from the equation $K_n = mass_{obs}/0.0095 \times SL^{3.37}$ where a = 0.0095 and b = 3.37. There was no significant effect of rearing temperature on condition (P = 0.1169), although fish tended to exhibit better condition at 16°C (Table 1).

CT_{max} and T_{ag}

Rearing temperature, oxygen treatment, and the interaction term had a highly significant effect on CT_{max} (Table 2). Under normoxia, CT_{max} increased from 30.21°C in fish reared at 16°C to 38.21°C in at 28°C (Table 3, Figure 1). The acclimation response ratio (ARR_{CTmax}), estimated from the slope of the linear regression between CT_{max} and rearing temperature, was 0.66. Under

hypoxic conditions, CT_{max} ranged from 26.49°C in fish reared at 16°C to 34.77°C in fish reared at 28°C, but CT_{max} did not increase across all temperature treatments (Table 3, Figure 1). Specifically, there was no change in CT_{max} across the three intermediate temperatures (ARR_{CTmax} of 0), 19°C, 22°C, and 25°C and then an increase in CT_{max} at 28°C.

There was a strong effect of both rearing temperature and test DO on T_{ag}, but no significant interaction was detected (Table 2). Under normoxia, T_{ag} ranged from 19.71°C in fish reared at 16°C to 31.25°C at 28°C and averaged 7.63 degrees lower than the CT_{max} (Table 3, Figure 2). Under hypoxia, T_{ag} ranged from 17.41°C in fish reared at 16°C to 28.64°C at 28°C and averaged 6.56 degrees lower than the CT_{max} (Table 3, Figure 2). Under both normoxia and hypoxia, T_{ag} generally increased with temperature and had a similar ARR of 0.94 and 0.86, respectively. However, under hypoxic conditions, some fish were agitated before temperatures increased, likely the result of progressive reductions in oxygen saturation during the 3-h acclimation period. As a result, it is likely that agitation temperatures are not representative of the onset of thermal stress in some hypoxic trials. A surface-dwelling behaviour was observed in most hypoxic trials, but not in normoxic, trials (Table 4). This behaviour was characterized by consistent ventilation near the surface of the water. While this may be representative of aquatic surface respiration (ASR), a widely documented response to hypoxia in fishes (Chapman and McKenzie 2009), it was not possible to validate the behaviour as ASR because the bubble wrap over each basket may have prevented fish from accessing the surface. Interestingly, in contrast to all other temperatures under hypoxia, this behaviour was rarely observed at 19°C (Table 4).

There was an effect of temperature and DO on agitation window (Table 2), with fish generally exhibiting a larger window at lower rearing temperatures and under normoxic conditions (Table 3, Figure 3). However, agitation window increased between 25°C and 28°C

under both normoxic and hypoxic conditions. There was no significant interaction between temperature and DO; however, as mentioned above, it is likely that agitation temperatures were not representative of the onset of thermal stress in some hypoxic trials.

Standard metabolic rate (SMR)

There was a strong effect of rearing temperature on mass-adjusted standard metabolic rate (SMR) (P << 0.001, Table 5). SMR generally increased between 16°C and 25°C, but then showed a significant decline after 25°C (Table 5, Figure 4). Q₁₀ values between each rearing temperature increment ranged between 0.5834 and 3.2796 (Table 6). The Q₁₀ across the full range of temperatures (16°C and 28°C) calculated by the difference in means was 1.5710, while the Q₁₀ calculated by values estimated from a linear regression was 1.6146. The slope of the linear regression across all five temperatures was 0.013869 and across only positive intervals (i.e. not including 28°C) was 0.021824.

Gill morphometrics

There was a significant effect of rearing temperature on almost all metrics of gill size (Table 7). Fish reared at 28°C were characterized by a larger total hemibranch area, a larger total and average gill filament length, a longer hemibranch base, and more gill filaments (P < 0.05, Figure 5). The density of the gill filaments was marginally smaller in the high temperature treatment (P=0.076, Figure 5).

DISCUSSION

In Pugnose Shiner, there was a significant increase in thermal tolerance after long-term exposure to elevated water temperature, which may partly reflect gill plasticity that increases oxygen-uptake capacity. However, exposure to acute hypoxia reduced thermal tolerance indicating sensitivity of upper thermal limits to dissolved oxygen availability in this imperiled fish. Additionally, Pugnose Shiner did not show evidence of thermal compensation in SMR at higher temperatures. These findings suggest that Pugnose Shiner may increasingly become at risk of extinction with climate change and that recovery efforts should incorporate strategies to mitigate the threat of elevated water temperatures.

Plasticity in upper thermal tolerance under normoxia

Under normoxic conditions, CT_{max} increased by 0.66°C per 1°C increase in rearing temperature and ranged from 30.2°C in the 16°C treatment to 38.2°C in the 28°C treatment. To our knowledge, this study represents the first thermal tolerance data for blackline shiners or endangered minnows; however, other North American *Notropis* species have been examined. For example, Smale and Rabeni (1995) measured the CT_{max} of Rosyface Shiner (*Notropis rubellus*,) and Bigmouth Shiner (*Notropis dorsalis*) collected from small streams in Missouri acclimated for 63 – 160 days to 26°C and recorded CT_{max} values of 35.5°C and 36.6°C, respectively. While these values are similar to the CT_{max} of 36.0°C recorded for Pugnose Shiner acclimated to 25°C in our study, Smale and Rabeni did not detect any significant differences across all 34 species included within their study, suggesting that exposure to a similar thermal regime may be more predictive of thermal tolerance than similar phylogeny.

Fewer studies have tested CT_{max} at multiple temperatures in *Notropis* species, therefore limited data exist for acclimation capacity (ARR_{CTmax}) in this genus. In a recent meta-analysis Comte and Olden (2017) documented an average ARR_{CTmax} of 0.37 with a range between 0.07 and 0.91 across 82 freshwater fish species. Supporting previous work by Smale and Rabeni (1995) and others, Comte and Olden found that CT_{max} correlated with temperatures experienced by species across their range, but they did not observe the same relationship in ARR_{CTmax}. Among the 35 cyprinid species included in Comte and Olden's meta-analysis, only one *Notropis* species was included, Spottail Shiner (Notropis hudsonius), whose ARR_{CTmax} was calculated from ten separate studies and best modelled by a quadratic response curve. One of the Spottail Shiner studies included in Comte and Olden's meta-analysis acclimated the species to temperatures of 5, 10, 15, 20, 25, and 30°C and observed CT_{max} of 27.7, 30.2, 31.2, 33.3, 35.5, and 37.7°C, respectively, which represents an overall linear ARR_{CTmax} of 0.40 (Ecological Analysts 1978). The ARR_{CTmax} of juvenile Pugnose Shiner (0.66) is higher than the ARR_{CTmax} of Spottail Shiner and substantially higher than the average reported for a large group of fishes in Comte and Olden's meta-analysis. This suggests that juvenile Pugnose Shiner have a relatively high acclimation capacity in upper thermal tolerance. Given that Pugnose Shiner is an imperiled species that appears to be more sensitive to environmental conditions than other, more common congeners (Gray et al. 2014, Gray et al. 2016), it was expected that they might exhibit a low level of plasticity in thermal tolerance. The apparently high ARR_{CTmax} result in this sensitive species was surprising; however, a similarly high ARR_{CTmax} was also observed in Hardhead Minnow (Mylopharodon conocephalus), a species of special concern in California. Specifically, juvenile and adult Hardhead Minnow, exhibited an ARR_{CTmax} of 0.53°C and 0.49°C, respectively, after more than 30 days of acclimation across four different temperatures (Thompson et al. 2012). Due

to limited research, factors associated with interspecific variation in thermal acclimation capacities are unclear, and the relative sensitivities of already imperiled species to elevated temperatures are also unresolved. However, unlike CT_{max}, phylogeny likely plays a role in ARR_{CTmax} (Comte and Olden 2017), and Pugnose Shiner may experience more similar acclimation capacity to closely related taxa (e.g. other blackline shiners in the genus *Notropis* and cyprinid species) than to more distantly related groups. Examining the CT_{max} and ARR_{CTmax} in other species of blackline shiner represents an exciting avenue of future research that could help elucidate trends in ARR_{CTmax} within the *Notropis* genus and also shed light on the relative acclimation capacities of imperiled species and their more common congeners.

Comparisons of CT_{max} and ARR_{CTmax} among species and studies must also be considered in light of the exposure time to the thermal regime. In this study, juvenile Pugnose Shiner were reared for 4 months, whereas most studies of thermal acclimation capacity have used short-term acclimation periods (2-4 weeks). The longer-term developmental exposure in this study may have resulted in higher CT_{max} or a greater ARR_{CTmax} than would be the case if exposure time was shorter and/or focused on adult fish. In a recent study, the CT_{max} for adult Pugnose Shiner from the same population acclimated to short-term (2-week) exposures to a similar temperature range showed lower CT_{max} values ranging from 29.8°C at 16°C to 36.0°C at 28°C (McDonnell and Chapman *unpublished data*). Although this could reflect life-stage differences in thermal tolerance, it may also, and more likely, reflect the long-term developmental exposure in the current study.

Finally, in this study, we did not detect any body size effect on CT_{max} . Body size may affect CT_{max} based on differences in surface area to volume ratio, where heat diffusion time may be longer in larger fish, resulting in a higher CT_{max} (Becker and Genoway 1979). However,

studies that have examined this body size to CT_{max} relationship have yielded mixed results (i.e. positive, negative, and no correlation) and the effects may be species-specific (e.g. Cox 1974, Barrionuevo and Femandes 1995, Ospina and Mora 2004). Therefore it is not possible to discern whether the observed lack of correlation between body size and CT_{max} was due to true independence or due to our overall small mass range based on a priori selection of smaller individuals for CT_{max} trials.

Plasticity in agitation temperature and window under normoxia

While CT_{max} is a widely used metric to assess thermal tolerance, negative effects of elevated water temperature on fitness-related traits can occur at temperatures below CT_{max} (Anttila et al. 2013, Munoz et al. 2014). T_{ag} was first proposed as an ecologically relevant metric of thermal stress by McDonnell and Chapman (2015), who suggested that Tag represented a thermal-avoidance behaviour that might come at the expense of other fitness-related behaviours such as feeding or predator avoidance. In their study, McDonnell and Chapman found evidence of acclimation in CT_{max} but not in T_{ag} for a widespread African cichlid (Pseudocrenilabrus *multicolor*), resulting in an overall increase in agitation window at higher temperatures. Unlike McDonnell and Chapman, Pugnose Shiner showed very high acclimation capacity in Tag under normoxia; the acclimation response ratio (ARR) in T_{ag} was 0.94, well above the ARR_{CTmax} of 0.66 and close to a "perfect" acclimation of 1. As a consequence of higher acclimation in T_{ag} than CT_{max}, there was an overall decline in agitation window at higher temperatures. Interestingly, T_{ag} occurred fairly close to acclimation temperatures (average difference of 4.32°C), indicating that even modest increases in temperature elicit an avoidance response in Pugnose Shiner.

Standard metabolic rate (SMR)

The SMR of juvenile Pugnose Shiner increased with temperature with an overall Q10 across rearing temperatures (16°C to 28°C) of 1.57 (difference in means) or 1.61 (linear regression); however, the SMR fell significantly at 28°C. Recalculated for the range between 16°C and 25°C, the Q10 was 2.18, higher than the average Q10 of 1.95 reported for a diversity of freshwater fishes (Seebacher et al. 2014). Some fishes exhibit metabolic thermal compensation whereby they compensate for the direct thermodynamic effects of increasing water temperature on standard metabolic rate, maintaining a relatively stable SMR over a broad range of ambient temperatures. For example, when Sandblom et al. (2014) transferred Shorthorn Sculpin (Myoxocephalus scorpius) from 10°C to 16°C, the 82% increase in SMR during the first week of exposure was reduced to pre-exposure levels after eight weeks of acclimation. Similarly, in their study of the Atlantic Halibut (*Hippoglossus hippoglossus*) transferred from 10°C to 16°C, Gräns et al. (2014) found that the initial rise in SMR at higher temperature was reversed after 14 weeks. Such plastic responses to thermal shifts may buffer the effects of climate warming by allowing time for genetic adaption to occur, and thermal acclimation capacity is increasingly considered a key determinant of resilience of fishes to climate change (Somero 2010, Sandblom et al. 2016).

Although Pugnose Shiner shows high acclimation capacity in CT_{max} , its ability to reverse thermodynamic effects on SMR is limited, and there was no evidence of long-term thermal compensation. This may limit its ability to persist in warming waters, particularly if increases in nutrient availability or acquisition do not match the increase in SMR. Healy and Schulte (2012) found that Mummichog (*Fundulus heteroclitus*) was unable to maintain body mass during longterm acclimation to high temperatures, which they suggested reflected a mismatch between rates of nutrient availability and increased SMR. In contrast, *Enteromius neumayeri* exposed to elevated water temperatures in the lab exhibited higher routine metabolic rates (RMR) but, in the wild, individual growth rates between two streams with different thermal profiles were similar, suggesting that increased prey consumption likely supported increased RMR in warm stream residents (Fugère et al. 2018). In our study, no evidence was found for loss of condition with increased SMR in Pugnose Shiner and no mortality over the course of the experiment, which suggests rates of nutrient acquisition were adequate to compensate for the increased SMR. SMR did drop significantly at the highest temperature treatment of 28°C. This may represent thermal compensation at this highest temperature treatment or, alternatively, the drop in SMR may reflect metabolic-rate depression, a temporary suppression of energy turnover in response to environmental stress (Guppy and Withers 1999). This pattern of SMR decline at a very high temperature was also observed in the African cichlid Pseudocrenilabrus multicolor acclimated for one week to a range of temperatures (McDonnell and Chapman 2016). This decline in SMR at 28°C is especially important as rearing temperatures were based on the current summer thermal range of Pugnose Shiner in Ontario (based on DFO capture data between 2002 - 2016); therefore, signs of metabolic depression at 28°C may suggest that Pugnose Shiner is vulnerable to negative effects of elevated water temperature within its current range. Future studies may want to examine acclimation temperatures representative of climate-warming scenarios, when Pugnose Shiner are exposed to temperatures beyond 28°C.

This study did not quantify the aerobic scope of Pugnose Shiner, which may have provided a greater understanding of thermal optimum and the threat of climate change as maximum metabolic rates (MMR, metabolic rate during maximum sustainable exercise) may exhibit less plasticity than basal metabolic rates (SMR, RMR) (Sandblom et al. 2016). However, Pugnose Shiner is presumed to be a weak swimmer (COSEWIC 2013) and, therefore, MMR may

not provide a good estimate of aerobic capacity (Clark et al. 2013). Additionally, while SMR or RMR (resting metabolic rate) is related to body mass by scaling component "b" (Clark and Johnston 1999), this "b" value may be related to intraspecific lifestyle factors such as activity level (Killen et al. 2010). Furthermore, some studies have observed that juvenile salmonids with higher SMR or RMR experienced higher growth rates and were found to be more dominant than those with lower metabolic rates, even after controlling for initial size (Metcalfe 1995, Yamamoto et al. 1998). Therefore, it is possible that a priori selection of larger individuals for SMR trials may have biased results by including individuals with higher metabolic rates.

Plasticity in the size of the gill

The increase in CT_{max} and high ARR_{CTmax} in Pugnose Shiner, as well as the increase in SMR, may relate to underlying changes in mechanisms and efficiency of oxygen uptake and delivery from the environment to the tissues. In Pugnose Shiner, several gill-size metrics were greater in fish reared at 28°C than in fish reared at 16°C, including gill filament length, area of the hemibranch, length of the hemibranch base, and number of filaments. Although these measures do not directly estimate gill surface area, they have been shown in other studies to correlate with gill surface area both within (Crispo and Chapman 2010) and among (Palzenberger and Pohla 1992) species. Phenotypic plasticity in gill size in response to elevated water temperature is not surprising given high levels of plasticity observed in fish gills in response to hypoxic rearing environments, where large gills in low-DO reared fish are presumably an adaptive mechanism to increase oxygen-uptake capacity. For example, *Pseudocrenilabrus multicolor* showed an increase in total gill-filament length, total gill surface area, and other gill metrics in response to rearing under hypoxia versus normoxia (Chapman et al. 2008, Crispo and Chapman 2010). High levels of developmental plasticity

in gill size and shape have also been reported for other haplochromine cichlids (Rutjes 2006, Chapman et al. 2007). Another mechanism by which fishes have been shown to modify the size of the gill surface area is via gill remodelling, which involves either the expansion of the cell mass between the lamellae on the gill filaments, referred to as the interlamellar cell mass (ILCM), that decreases the effective gill surface area, or a reduction of the ILCM through apoptosis that exposes the gill epithelium and increases oxygen-uptake capacity (Sollid et al. 2005, Nilsson 2007, Tzaneva et al. 2011, Nilsson et al. 2012). While limited, there is emerging research on the effects of temperature on gill plasticity that suggests that gill remodelling can happen rapidly in response to elevated water temperatures (Sollid et al. 2005, McBryan et al. 2016, Wu et al. 2017).

Increased gill size is only one of many potential mechanisms that may facilitate thermal acclimation capacity in Pugnose Shiner. For example, cardiac remodelling has been observed in several species in response to fish reared under average and elevated water temperatures, whereby warmer water induced a smaller relative ventricular mass and a higher percent of compact myocardium which may increase the efficiency of oxygen delivery (Klaiman et al. 2011, Anttila et al. 2013, Gräns et al. 2014, Keen et al. 2017, Nyboer and Chapman 2018). The mechanisms underlying thermal acclimation capacity represent an important area for future study of Pugnose Shiner and imperiled fishes in the face of climate warming.

Acute exposure to hypoxia

Physiological responses to elevated water temperature observed in the lab do not account for the full extent of ecological complexity and may not coincide with observed responses in the wild. One way to account for ecological complexity in acclimation studies is to examine the effects of multiple stressors. Exposure to acute hypoxia limited CT_{max} , reduced ARR_{CTmax} to zero across three intermediate temperatures, induced the onset of agitation behaviour (Tag) at lower temperatures (potentially even at rearing temperatures), and was further manifested in a novel surface-dwelling behaviour. These observations suggest that thermal tolerance is oxygen limited at 22% DO saturation, a finding that supports the oxygen- and capacity-limited thermal tolerance (OCLTT) concept (Pörtner 2001). However, only one level of hypoxia was used in this study, a level slightly above the recorded critical oxygen tension for this species. In a recent study, Ern et al. (2016) found that the CT_{max} of Lumpfish (*Cyclopterus lumpus*) and Red Drum (*Sciaenops* ocellatus) was independent of oxygen availability over a wide range of oxygen levels despite a large reduction in aerobic scope. The CT_{max} of both species fell sharply under extreme hypoxia, and the first sign of reduced CT_{max} was observed slightly above the P_{crit} for Lumpfish and slightly below for Red Drum. Since our measures of CT_{max} in Pugnose Shiner were estimated at an oxygen level close to the P_{crit}, we cannot evaluate oxygen dependency at higher levels. Ern et al. (2016) proposed a new metric, PCT_{max} , the oxygen level below which CT_{max} declines, which would be useful to estimate in future studies of Pugnose Shiner thermal tolerance across multiple levels of DO.

Interestingly, CT_{max} under hypoxia increased significantly at 28°C, after no increase at rearing temperatures between 19°C and 25°C. SMR also showed a change in direction of response at 28°C. This suggests that the mechanism(s) of thermal acclimation above 25°C are different than the one(s) at lower rearing temperatures and may buffer Pugnose Shiner from acute hypoxia stress. McBryan et al. (2016) found that warm acclimation almost completely reversed reductions in hypoxia tolerance (critical oxygen tension – P_{crit}) in Mummichog, supporting the hypothesis that mechanisms of thermal acclimation may buffer species from hypoxia stress. In Pugnose Shiner, a reduced metabolic rate at 28°C coincides with increased

 CT_{max} , but may reflect metabolic depression that could limit the ability of the fish to perform other aerobic functions.

Conservation implications

While Pugnose Shiner showed high acclimation capacity in CT_{max} and T_{ag}, T_{ag} occurred close to rearing temperatures, suggesting that Pugnose Shiner are behaviourally sensitive to increases in water temperature. This may have strong ecological relevance if avoidance behaviour above T_{ag} exposes fish to sub-optimal habitat and/or predators. Furthermore, individuals did not exhibit metabolic compensation in SMR and likely suffer from metabolic depression beyond rearing temperatures of 25°C, an especially important finding given that such temperatures are within the current range of Pugnose Shiner in Ontario. This suggests that climate warming may already pose a risk to population persistence in this imperiled species, which may be exacerbated as temperatures continue to rise. Furthermore, the apparent divergent responses between CT_{max} and SMR (e.g. high acclimation vs. no compensation) highlights the importance of examining multiple traits associated with thermal tolerance to assesses species-specific responses to climate change.

These findings suggest that Pugnose Shiner may increasingly become at risk of extinction with climate change and that recovery efforts should incorporate strategies to mitigate this threat. However, unlike other stressors, such as habitat destruction, that can be mitigated through local actions such as wetland restoration and improved connectivity, significant reductions in greenhouse-gas emissions requires global action and is, therefore, beyond the scope of individual species recovery efforts. Furthermore, even under the most stringent greenhouse-gas mitigation scenario, the mean annual global temperature is expected to increase by at least 1.5°C relative to

pre-industrial temperature by the end of the century (IPCC 2013). As a consequence, imperiled freshwater fishes will increasingly confront higher annual temperatures as well as more frequent extreme hot temperature events. Identifying interactions between elevated temperature and other stressors provides an opportunity for local interventions. For example, these results show that acute hypoxia reduces thermal tolerance in Pugnose Shiner. Therefore, reducing nutrient loading across Pugnose Shiner habitat may simultaneously mitigate the threat of eutrophication, while promoting population persistence under climate-change scenarios. This research also suggests potential for more adaptive strategies, including the introduction of more thermally tolerant individuals. Encouragingly, Pugnose Shiner has previously been the focus of a successful introduction effort; in September 2016, Chaumont Bay was stocked by the New York State Department of Environmental Conservation using individuals from the SUNY Cobleskill and size distribution in 2018 sampling suggested successful reproduction (Haynes et al. 2019). While these efforts might be effective under current climate scenarios, as warming progresses, captive breeding under higher temperature regimes during development or across multiple generations, or identifying source populations with greater thermal tolerance may bolster the success of introduction efforts. However, the fitness trade-offs of higher thermal tolerance need to be explored, and these knowledge gaps may hinder the efficacy of such introduction efforts. Furthermore, caution should be taken when extrapolating responses from individuals in this study to all Pugnose Shiner populations, given that physiological traits are widely affected by local adaptation (Eliason et al. 2011, Kelly et al. 2011, Sorte et al. 2011). Additional studies may be necessary to assess thermal sensitivity and acclimation capacity across different populations of Pugnose Shiner.

This study was among the first to examine developmental plasticity in thermal tolerance of a fish. Higher CT_{max} at higher temperatures suggest that phenotypic plasticity may help facilitate persistence of Pugnose Shiner under global warming. However, no evidence for compensation in SMR suggests limited acclimation capacity across multiple traits. It is proposed that one mechanism of developmental acclimation to elevated water temperature is gill plasticity, whereby larger gills facilitate greater oxygen uptake to keep pace with a rise in metabolic rates and/or a decline in oxygen saturation. It is further hypothesized that there exists a threshold effect above 25°C, where the mechanisms of thermal acclimation may shift. It is likely that this reflects metabolic depression, but this will require further study. Identifying source populations with greater thermal tolerance or implementing captive breeding under higher temperature regimes may improve the success of introduction efforts and promote population persistence with climate change, but fitness trade-offs in thermal tolerance should be examined.

Tables

Table 1. Mean mass (g), standard length (SL, cm), and relative condition factor (K_n) of juvenile Pugnose Shiner (n=110) across five different rearing temperatures. Predicted masses were calculated from the equation $a \times SL^b$ (a = 0.0095 and b = 0.6021). No significant differences between rearing temperature were found in mass (P=0.5042), SL (P=0.4744), or K_n (P=0.1169).

Rearing Temperature (°C)	n	No. of Acclimation Days	Mean Mass _{obs} ± SE (g)	Mean SL ± SE (cm)	Mean Mass _{pred} ± SE (cm)	Mean K _n ± SE (cm)
16	21	>150	0.81 ± 0.13	3.49 ± 0.16	0.75 ± 0.12	1.085 ± 0.028
19	21	103-111	0.93 ± 0.15	3.72 ± 0.18	0.96 ± 0.15	0.972 ± 0.032
22	22	104-111	0.70 ± 0.11	3.46 ± 0.13	0.71 ± 0.10	0.972 ± 0.027
25	22	102-113	0.68 ± 0.12	3.35 ± 0.16	0.68 ± 0.11	1.000 ± 0.026
28	24	115-133	0.68 ± 0.10	3.38 ± 0.14	0.67 ± 0.10	1.012 ± 0.020

Table 2. Results of mixed-model ANOVAs testing for differences between five rearing temperatures and acute hypoxia in thermal tolerance metrics (CT_{max} , T_{ag} , and thermal window) of juvenile Pugnose Shiner. Bold type indicates significance at $\alpha = 0.05$ and <<0.001 indicates that the P-value was much less than 0.001. Degrees of freedom and F-statistic were calculated using Satterthwaite's method.

Oxygen	Parameter	Effect	F	df	Р
Normoxic	CT _{max}	Temp	128.12	4, 14.342	<<0.001
	T_{ag}	Temp	13.224	4, 12.733	0.0002
	Window	Temp	2.1447	4, 12.895	0.1335
Hypoxic	CT_{max}	Temp	29.412	4, 15.599	<<0.001
	T_{ag}	Temp	76.447	4, 30	<<0.001
	Window	Temp	12.271	4, 14.441	<<0.001
Full	CT_{max}	Temp x DO	11.584	4, 45.601	<<0.001
		Temp	88.018	4, 14.969	<<0.001
		DO	362.168	1, 45.637	<<0.001
	T_{ag}	Temp x DO	0.6151	4, 46.670	0.6539
		Temp	42.5201	4, 15.001	<<0.001
		DO	31.7919	1, 46.981	<<0.001
	Window	Temp x DO	0.8173	4, 47.123	0.5206
		Temp	7.1574	4, 15.969	0.0017
		DO	5.5390	1, 51.383	0.0225
		DO	5.5390	1, 51.383	0.0225

Table 3. Number of days reared under temperature treatments, mean mass (g), mean standard
length (SL, cm), mean CT_{max} (°C), mean T_{ag} (°C), and mean agitation window (°C) of juvenile
Pugnose Shiner under normoxic (>95% saturation) or hypoxic (22% saturation) conditions after
a 4-month rearing period (no. of days). Significant differences (P<0.05) between CT_{max} within
each oxygen condition are denoted by lettered subscripts. Significance between oxygen
conditions at each temperature are represented by an asterisk (*). Note that one fish was
excluded from the T_{ag} and agitation window dataset (hypoxic, 19°C) relative to the CT_{max}
dataset.

	Rearing Temp. (°C)	n	No. of Days	Mean Mass ± SE (g)	Mean SL ± SE (cm)	Mean CT _{max} ± SE (°C)	Mean T _{ag} ± SE (°C)	Mean Window ± SE (°C)
	16	6	>150	0.41 ± 0.47	2.97 ± 0.47	$30.21 \pm 0.47^{A*}$	$19.71 \pm 1.31^{\text{A}}$	$10.50 \pm 1.35^{\text{A}}$
ia	19	4	101-104	0.32 ± 0.21	2.80 ± 0.21	$32.04 \pm 0.21^{B*}$	$25.11 \pm 2.21^{B*}$	$6.93 \pm 2.01^{\mathrm{B}}$
ormox	22	7	103-106	0.39 ± 0.17	3.01 ± 0.17	$34.50 \pm 0.17^{C*}$	$26.38 \pm 1.34^{\text{B}*}$	$8.12 \pm 1.33^{\mathrm{AB}}$
N	25	8	102-105	0.35 ± 0.17	2.86 ± 0.17	$36.00 \pm 0.17^{\text{D}*}$	$29.92 \pm 0.76^{\circ}$	$6.07 \pm 0.79^{\mathrm{B}}$
	28	8	115-129	0.37 ± 0.13	2.93 ± 0.14	$38.21 \pm 0.13^{E*}$	$31.25 \pm 0.75^{C*}$	$6.96 \pm 0.80^{\mathrm{B}}$
	16	7	>150	0.42 ± 0.54	2.96 ± 0.53	$26.49\pm0.53^{\rm a}$	17.41 ± 0.23^{a}	9.08 ± 0.54^{a}
a	19	7	108-111	0.42 ± 0.48	3.11 ± 0.48	$29.70 \pm 0.48^{\mathrm{b}}$	22.12 ± 0.91^{b}	7.71 ± 0.66^{ab}
Hypoxi	22	8	109-112	0.46 ± 0.27	3.20 ± 0.27	29.76 ± 0.27^{b}	23.48 ± 0.55^{bc}	$6.28 \pm 0.36^{\rm bc}$
	25	7	108-112	0.35 ± 0.63	2.91 ± 0.63	29.17 ± 0.63^{b}	$25.36 \pm 0.11^{\circ}$	$3.82 \pm 0.61^{\circ}$
	28	7	119-133	0.34 ± 0.22	2.86 ± 0.23	$34.77 \pm 0.22^{\circ}$	28.64 ± 0.25^{d}	$6.13 \pm 0.25^{\rm bc}$

Table 4. Number of individual Pugnose Shiner per rearing temperature exhibiting surfacedwelling behaviour (SDB) during thermal tolerance trials under hypoxic conditions (n=36). No surface-dwelling behaviour was observed under normoxic conditions (n=33).

Rearing Temperature (°C)	No. of Trials with SDB	No. of Trials without SDB
16	7	0
19	1	6
22	8	0
25	5	2
28	5	2

Table 5. Number of days reared under temperature treatments, mean mass (g), mean standard length (SL, cm), and mean mass-adjusted standard metabolic rate (SMR, mg 0_2 per h of juvenile Pugnose Shiner (n=31) determined by intermittent respirometry. Significant differences in mean SMR between rearing temperatures (p<0.05) are denoted by lettered subscripts. Raw SMR values were mass-adjusted to the mean mass of all fish (1.40 g) based on a log-mass to log-SMR ratio (b = 0.6021).

Rearing Temperature (°C)	n	No. of Days Under Treatment Conditions	Mean Mass ± SE (g)	Mean SL ± SE (cm)	Mean SMR ± SE (mg 02 per hr)
16	5	>150	1.66 ± 0.01	4.50 ± 0.01	$0.1997 \pm 0.0105^{\text{A}}$
19	5	103-111	1.54 ± 0.02	4.52 ± 0.01	$0.2566 \pm 0.0149^{\mathrm{B}}$
22	6	104-111	1.24 ± 0.01	4.17 ± 0.01	$0.2828 \pm 0.0095^{\rm B}$
25	7	102-113	1.35 ± 0.02	4.34 ± 0.02	$0.4038 \pm 0.0173^{\circ}$
28	8	115-133	1.28 ± 0.01	4.24 ± 0.01	$0.3435 \pm 0.0064^{\mathrm{B}}$

Table 6. Q10 values calculated from the difference in mean mass-adjusted SMR of juvenile Pugnose Shiner across the range of rearing temperatures. Overall Q10 was calculated across the full range (16°C and 28°C) and across all positive increments (16°C and 25°C).

Rearing Temperature (°C)	Q10		
16-19	2.3033		
19-22	1.3824		
22-25	3.2796		
25-28	-0.5834		
16-28	1.5710		
16-25	2.1858		

Table 7. Results of ANOVAs testing for differences between rearing temperatures of 16°C or 28°C on 5 mass-adjusted gill metrics and 1 non-mass-adjusted metric (number of gill filaments) of juvenile Pugnose Shiner. Bold type indicates significance at $\alpha = 0.05$. Degrees of freedom and F-statistic calculated using Satterthwaite's method.

Gill Metric	F	df	р
Hemibranch Area	25.913	1,5	0.0038
Base Length	23.244	1,5	0.0048
Total Filament Length	22.003	1,5	0.0054
Average Filament Length	16.163	1,5	0.0101
Filament Density	4.9624	1,5	0.0764
Number of Filaments	30.943	1,3	0.0115

Figures



Figure 1. Mean CT_{max} (±SE) of juvenile Pugnose Shiner under normoxic (circles, n=33) and hypoxic (diamonds, n=36) conditions after a 4-month rearing period under five different temperatures (P_{temp X oxygen} << 0.001). Significant differences (P<0.05) between CT_{max} within each oxygen condition are denoted by lettered subscripts. Significance between oxygen conditions at each temperature are represented by an asterisk (*).



Figure 2. Mean T_{ag} (±SE) of juvenile Pugnose Shiner under normoxic (circles, n=33) and hypoxic (diamonds, n=36) conditions after a four-month rearing period under five different temperatures (P_{temp} << 0.001, P_{oxygen} << 0.001). Significant differences (P<0.05) between T_{ag} within each oxygen condition are denoted by lettered subscripts. Significance between oxygen conditions at each temperature are represented by an asterisk (*).



Figure 3. Mean agitation window (\pm SE) of juvenile Pugnose Shiner under normoxic (circles, n=33) and hypoxic (diamonds, n=35) conditions after a four-month rearing period under five different temperatures (P_{temp}=0.0017, P_{oxygen}=0.022). Significant differences (P<0.05) between agitation window within each oxygen condition are denoted by lettered subscripts. Significance *between* oxygen conditions at each temperature are represented by an asterisk (*).



Figure 4. Mean mass-adjusted SMR (\pm SE) of juvenile Pugnose Shiner (n=31) after a four-month rearing period under five different temperatures (P_{temp} << 0.001). Significant differences (P<0.05) between mass-adjusted SMR between temperatures are denoted by lettered subscripts. Values were mass-adjusted to a common body mass of 1.4 g.



Figure 5. Mean values (\pm SE) of 5 mass-adjusted gill metrics and 1 non-mass-adjusted metric (number of gill filaments) of juvenile Pugnose Shiner (n=14) after a four-month rearing period under 16°C or 28°C. Raw values were mass-adjusted to the mean mass of all fish (1.50 g) based on a log-mass to log-gill metric ratio. Significant differences (P<0.05) between mass-adjusted SMR between temperatures are denoted by an asterisk (*).

Supplementary Information



Figure S1. Tank set-up for Pugnose Shiner lab-rearing experiments

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LINKING STATEMENT

In the first chapter of this thesis, I demonstrated that Pugnose Shiner exhibit significant plasticity in CT_{max} and gill morphology in response to 4-month developmental exposure to elevated water temperatures. However, these responses were dampened by exposure to acute hypoxia. Furthermore, thermal compensation in SMR was low (Q10 = 2.19) between 16 and 28°C). Together, these results show variation in acclimation capacity among traits and an oxygen dependency in thermal tolerance, stressing the importance of measuring multiple traits when evaluating response to thermal stress. While physiological acclimation likely represents an important survival strategy for freshwater fishes contending with warming environments, behavioural thermoregulation, whereby an organism exploits habitat heterogeneity to minimize thermal stress, may also play a role. Interestingly, during CT_{max} experiments, Pugnose Shiner exhibited agitation behaviour close to acclimation temperatures, suggesting that they are sensitive to even small increases in temperature and may try to avoid them in the wild. While Gunderson and Stillman (2015) suggested that aquatic ectotherms exhibit greater phenotypic plasticity in thermal tolerance due to apparent reduced opportunities for behavioural thermoregulation than terrestrial ectotherms, there have been multiple documented examples of freshwater fishes exploiting habitat heterogeneity to stay cool, for example utilizing groundwater upwelling in streams (e.g. Nielsen et al. 1994, Sutton and Soto 2012) or diurnal vertical migration to deeper, cooler layers in lakes (e.g. Brandt 1980, Jensen et al. 2006). Identifying thermal refugia and opportunities for behavioural thermoregulation in the wild may represent an important conservation tool for this sensitive species. Furthermore, due to ecological complexity, lab studies may not be representative of responses in a field setting. Notably, the functional niche, the range of environmental conditions that allow a species to persist, can differ from the

realized niche as the result of species interactions (Peterson et al. 2011). Species interactions may be especially determinate of community structure across fine-scale spatial resolutions (Godsoe et al. 2017) and will likely be affected by climate change (Kordas et al. 2011). Therefore, in the second chapter, I explore the distribution of Pugnose Shiner and species associations within a single Pugnose Shiner habitat patch, Thompson's Bay in the upper St. Lawrence River in Ontario, Canada. I consider my findings in terms of thermal refugia and potential opportunities for behavioural thermoregulation as well as significant species associations that may be affected by climate warming. This field study compliments my lab study in Chapter 1 by incorporating ecological complexity to explore the effect of temperature on the imperiled Pugnose Shiner.

CHAPTER 2

Title: Examining the emerging threat of climate change to an endangered minnow

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ABSTRACT

Species distributions are shaped by abiotic habitat factors and species interactions at multiple scales. Climate change may directly alter species interactions if species differ in their thermal niche and thermal tolerance or indirectly affect distributions by altering the magnitude of biotic interactions and/or the relative abundances of species. Understanding the direct and indirect effects of elevated water temperature represents a significant knowledge gap for many imperiled species, which may be vital for effective conservation strategies. The goal of this study was to quantify the role of temperature, other abiotic factors, and co-occurring species in shaping the fine-scale distribution of the endangered Pugnose Shiner (Notropis anogenus) within a single population in Thompson's Bay in the upper St. Lawrence River, Ontario, Canada. A field survey was conducted in late summer 2018 to explore the distribution of Pugnose Shiner during the warmest period of the year. Multivariate analyses and occupancy modelling were used to assess how environmental variables and species co-occurrence accounted for variation in the distribution of Pugnose Shiner. Environmental variables, including temperature, were identified as predictors of community composition. Pugnose Shiner was associated with clear, cool sites with high vegetation density, including Chara vulgaris, far from the inner bay. Pugnose Shiner occurred in habitats characterized by a wide range of summer temperatures (23.9 - 28.5°C) and temperature loggers placed in a subset of sites where Pugnose Shiner were captured showed strong diel shifts (maximum range of 1.03°C to 6.66°C). Pugnose Shiner was negatively associated with Brook Silverside, Largemouth Bass, Pumpkinseed, and juvenile *Lepomis* spp. This has important conservation implications because some native centrarchid predators are increasing in abundance with climate change, which may threaten persistence of rare and imperiled cyprinids, such as Pugnose Shiner.

INTRODUCTION

Temperature has long been identified as a primary driver of species distributions and abundances (Hutchins 1947). Thus, it is not surprising that global warming is inducing range shifts across all biomes, including terrestrial (Parmesan et al. 2000, Hickling et al. 2005, Chen et al. 2011), marine (Sorte et al. 2010, Poloczanska et al. 2013, Poloczanska et al. 2016), and freshwater ecosystems (Heino et al. 2009, Comte et al. 2013, Alofs et al. 2014, Comte et al. 2014, Heino et al. 2016). Elevated water temperatures represent a particularly pronounced threat to shallow freshwater habitats that are highly exposed to atmospheric warming (O'Reilly et al. 2015, FAO 2018) and to freshwater ecotherms, such as fishes, that are highly sensitive to their thermal environment (Fry 1967, McNab 2002). Furthermore, the ability of freshwater species to track preferred water temperatures in a changing climate may be restricted by the relatively fragmented nature of freshwater habitats (Woodward et al. 2010). Limited dispersal capacity in the face of climate change represents an additional barrier to the persistence of highly exposed and highly sensitive freshwater fishes that already rank among the most imperiled taxa on the planet (Dudgeon et al. 2006, Carpenter et al. 2011, IUCN 2019).

While freshwater fishes may be limited in opportunities for range shifts in response to global warming, they may be able to persist within existing habitat patches by exploiting microhabitat variation and thermal refugia. For example, temperature has been identified as a significant factor characterizing the distribution of fish species within short stream reaches (Baltz et al. 1987, Grossman and Freeman 1987, Wichert and Lin 1996). Furthermore, the use of thermal refugia in lotic systems, including coldwater patches from tributaries and groundwater upwelling, has been widely documented in salmonids both in the field (e.g. Nielsen et al. 1994, Ebersol 2001, Sutton et al. 2007, Sutton and Soto 2012) and in experimental settings (e.g. Hitt et

al. 2016, White et al. 2019). In deep lentic systems, fish may move into deeper, cooler waters during the day as a response to high surface water temperatures in the summer (e.g. Brandt 1980, Rowe and Chisnall 1995, Jensen et al. 2006). By providing opportunities for behavioural thermoregulation within a warming environment, microhabitat heterogeneity and thermal refugia may buffer freshwater fishes from the negative effects of climate change and promote population persistence.

Sensitive species, including many fishes already imperiled, may be vulnerable to extinction from climate change as a result of direct thermal stress (Ficke et al. 2007, Heino et al. 2016), but may also be indirectly affected by increased predation and/or competition due to increased abundance and/or distribution of more tolerant species (Comte et al. 2013). For example, Jackson and Mandrak (2002) predicted that human-assisted range expansion of Smallmouth Bass (Micropterus dolomieu) across Ontario under climate-change scenarios would extirpate 25 000 populations of four cyprinid prey populations by 2100. Additionally, Wenger et al. (2011) projected that negative associations between native Cutthroat Trout (Oncorhynchus *clarkii*) and three other non-native trout species would reduce suitable habitat in the interior western United States by an additional 26% by 2080. While poorly studied, the indirect effects of temperature on biotic interactions and species associations have been identified as potentially more important than direct effects in structuring aquatic communities under climate change (Kordas et al. 2011), and recent meta-analyses and research papers have urged scientists and managers to better integrate community-level effects of climate change into conservation planning in aquatic systems (Daufresne and Boët 2007, Gilman et al. 2010, Kordas et al. 2011, Morecroft et al. 2012).

Both the direct and indirect effects of climate change have been poorly studied in freshwater species at risk in Canada, including Pugnose Shiner (*Notropis anogenus*). Pugnose Shiner is a small minnow (family Cyprinidae) native to the Great Lakes and Upper Mississippi River basins (COSEWIC 2013). Pugnose Shiner was assessed as threatened in Canada (COSEWIC 2013) and receives protection as an endangered species under Schedule 1 of the federal Species at Risk Act. Within Canada, Pugnose Shiner is found only in Ontario in isolated populations (McCusker et al. 2014b) in clear, shallow, and highly vegetated habitats (Holm and Mandrak 2002). Turbidity has been identified as a primary cause of decline in this environmentally sensitive species (COSEWIC 2013, Gray et al. 2014, Gray et al. 2016), but climate change may represent an emerging threat. Recent studies have examined the response of Pugnose Shiner to short-term (2 weeks) and long-term (4 months) exposures to elevated water temperatures (Chapter 1; McDonnell et al. unpublished data) and found that Pugnose Shiner can adjust its critical thermal maximum under higher temperatures, but show little evidence of thermal compensation (i.e. their standard metabolic rate increased at a high rate with increased water temperature) (Chapter 1). Given its small body size and presumed weak swimming ability (COSEWIC 2013), Pugnose Shiner is not likely to expand its range into other watersheds with climate change (Chu et al. 2005). This means that Pugnose Shiner may have to rely on behavioural thermoregulation, physiological acclimation, genetic adaptation, and/or thermal refugia to respond to warming water.

The goal of this study was to quantify the role of temperature, other abiotic factors, and co-occurring species in shaping the fine-scale distribution of the threatened Pugnose Shiner (*Notropis anogenus*) within a single population in Thompson's Bay in the upper St. Lawrence River, Ontario, Canada. Nearshore habitats and sheltered embayments within the upper St.
Lawrence River, including within the Thousand Islands region, have high biotic integrity (Hoyle and Yuille 2016) and support many sensitive/intolerant species (Carlson 1997, Henning et al. 2014). Furthermore, it may be the largest continuous expanse of high-quality Pugnose Shiner habitat in Canada (Fisheries and Oceans Canada 2010). This stretch of the river supports a robust meta-population of Pugnose Shiner within the Thousand Island region (McCusker et al. 2014a), and Thompson's Bay has specifically been identified as the largest and most suitable habitat patch in (McCusker et al. 2014a, McCusker et al. 2017). While extinction risk for Pugnose Shiner within the upper St. Lawrence River is low (McCusker et al. 2017), the mean annual water temperature in the St. Lawrence River has risen by over 1.3°C since 1960 (Hudon et al. 2009). Increasing temperatures may degrade the quality of Pugnose Shiner habitat and could represent a threat to population persistence within the St. Lawrence River. Thompson's Bay likely represents an important stronghold for the species; thus, understanding how the emerging threat of climate change might affect this population will be especially important for conservation purposes.

METHODS

Field survey

A field survey of Thompson's Bay in the St. Lawrence River, ON (44°24'40" N / -75°54'00" W) was conducted between July 26 and August 9, 2018. The study area was selected along the northern shore of the bay between 44°24'21" N / -75°54'34" W and 44°25'04"/-75°53'39" W and up to 5 seconds (~200 m) from shore to include a range of microhabitats and to avoid populated areas along the southern shore. We did not evaluate the presence of onshore development on broad-scale patterns of distribution within the bay, but rather focused on microhabitat use. Within the study area, sites were numbered along a coordinate grid system (1" by 1") and were randomly selected for sampling using a random-number generator. Sampling was conducted during daylight hours between 0900 and 1900. At each site, the environment was characterized based on water-chemistry parameters, water depth, and the extent and type of vegetation. Water temperature, dissolved-oxygen concentration, and water turbidity were measured using a YSI probe (YSI ProDSS Multiparameter Water Quality Meter). These metrics were measured at a single depth (~1/2m below surface) in the water column. Thermal stratification was not observed during an initial sampling period between July 16 to 25, 2018; therefore, our measurements were assumed to be representative of the entire water column. Water depth and depth to the top of submerged vegetation were measured using a weighted line. The density of aquatic vegetation visible from the sampling point was quantified as low (0-33% coverage), medium (33-66%), or high (66-100%). All vegetation was identified to species or genus.

Fishes were sampled using a 10-m bag seine (3-mm mesh) that was either deployed behind a 5 m jon boat or by walking parallel to the shoreline where the water was too shallow for boat access. In total, 71 sites were sampled by boat seine, and 17 sites were sampled by walking the seine. The area of deployment was approximately 100 m², estimated by walking 10 m with a 10-m seine or by GPS coordinates during boat seines. Each site was sampled with three seine hauls. To reduce capture and handling stress, fishes were counted and released after each seine haul. While we cannot account for replacement, it is unlikely that fishes were recaptured because fishes were released outside of the sampling site between seine hauls. Careful attention was paid to distinguish Pugnose Shiner from similar species, especially Blacknose Shiner (*Notropis heterolepis*) and Blackchin Shiner (*Notropis heterodon*), which look very similar to the untrained

eye (Holm et al. 2010). We did not sample within dense emergent *Typha* spp. stands along the perimeter of the bay nor within a dense patch of water lilies at the end of the bay because they did not represent the preferred habitat of Pugnose Shiner (Fisheries and Oceans Canada 2010), and we wanted to characterize micro-habitat distribution within a high-quality habitat patch. In addition, within the main bay area, some sites with thick, muddy sediments and dense water lilies, which prevented proper deployment of the bag seine, could not be sampled. However, the large sample size and coverage allowed us to characterize Pugnose Shiner occurrence and abundance across both the inner and outer bay, which we defined as the area located within and outside of the southern peninsula (inner and outer, respectively, Figure 3). While we did not measure water velocity, the inner bay was likely characterized by little to no water flow while the outer bay, which was exposed to the St. Lawrence River, likely experienced increased water mixing.

Thermal profile of Pugnose Shiner habitat

To further investigate the thermal niche of Pugnose Shiner in Thompson's Bay, four temperature loggers (HOBO Water Temperature Pro v2 Data Logger - U22-001) were deployed between August 3 and August 12 to collect diel water temperature data. Loggers were deployed at or near sites where Pugnose Shiner had been captured by our sampling within the bay (Figure 3); thus, we assume that the diel loggers reflect temperatures likely to be experienced by Pugnose Shiner during the warmest part of the year, although it is possible that Pugnose Shiner seek different microhabitats at night. Two HOBO loggers (temperature recorded once a minute) were positioned at Pugnose Shiner capture sites close to shore (nearshore, less than 30 m from shore), and two were positioned at sites farther away from shore (offshore, greater than 30 m from shore) to investigate spatial and temporal effects of temperature at sites occupied by Pugnose Shiner. All sites were in the outer bay area, which was the habitat where Pugnose Shiner was most abundant.

Statistical Analysis

To examine species associations, the environmental correlates of Pugnose Shiner presence/abundance, and the significance of both environmental and species covariates on Pugnose Shiner distribution, I used a principal components analysis (PCA) fitted with environmental correlates, and I developed an occupancy model. Analyses were run using opensource packages within the software R (R Core Team 2018).

Multivariate analysis

PCA was conducted to examine community composition and species correlations in Thompson's Bay using the package '*vegan*' (Oksanen et al. 2019). A PCA is a form of unconstrained ordination that uses a set of variables to generate new orthogonal axes (principal components) that explain as much variation as possible within the dataset. Prior to conducting the PCA, fish abundance data were Hellinger-transformed to control for low counts and zero inflation (Borcard et al. 2011). Significant axes were selected based on the Kaiser-Guttman criterion, which identifies axes with eigenvalues greater than 1. To explore the relationship between habitat characteristics and community composition, candidate environmental variables were selected based on previous Pugnose Shiner studies and included both continuous and categorical variables. Environmental variables were fit to the matrix of the first two axes of the PCA by conducting 9999 permutations. Significant components (p < 0.05) were retained, and

squared correlation coefficients (r^2) were calculated for each variable. Prior to fitting, continuous variables were scaled and centered (z-scored) and Pearson correlations were calculated. When correlations were identified (|r| > 0.7), one of the variables was removed from the model. The results of the PCAs were represented on a correlation biplot (scaling 2) to visually examine the relationship of Pugnose Shiner to other species and environmental variables within Thompson's Bay. Components appearing in similar ordination space were interpreted as being positively correlated, while those opposite to each other were negatively correlated, and perpendicular components were not correlated.

Occupancy modelling

A habitat occupancy model was created using the package '*unmarked*' (Version 0.12-0, Fiske and Chandler 2011) and "*RPresence*" (Version 2.12.34, MacKenzie and Hines 2017) following the modelling framework developed by MacKenzie et al. (2002). This approach accounts for imperfect detection (i.e. when detection probabilities are less than 1) by fitting a single-season occupancy model based on zero-inflated binomial models where

observed occupancy ~ true occupancy(ψ) × detection probability (p)

In this occupancy model, the probability of detection (p) is estimated from repeat surveys at a given site that, in turn, is incorporated into the probability of occupancy (ψ) . Both ψ and p can incorporate site covariates by fitting generalized linear models $(\hat{\psi} \text{ and } \hat{p})$. This model assumes that sites are closed throughout the duration of the survey and independent from one another. Given the scale of our survey, there was likely fish movement among sampling sites, but bias was mitigated by treating successive hauls as repeat sampling events and short sampling windows (average time spent sampling each site was 31 minutes). Using capture data from

repeat seine hauls, multiple occupancy models were created for Pugnose Shiner in Thompson's Bay. Candidate models incorporated environmental covariates identified as important to Pugnose Shiner from the literature. Environmental covariates included turbidity (Gray et al. 2014, Gray et al. 2016), depth (McCusker et al. 2014a, McCusker 2016), vegetation density (Holm and Mandrak 2002, Fisheries and Oceans Canada 2010, McCusker et al. 2014a), the presence of submerged macrophytes Chara vulgaris, Vallisneria americana (Holm and Mandrak 2002), and/or Myriophyllum spp. (Lyons 1989), and temperature and dissolved oxygen, which had not yet been explicitly investigated. Conductivity was included because of observed occurrences of Pugnose Shiner in areas of the St. Lawrence with high conductivity and high pH (McKenna and David 2017). Water velocity has previously been identified as an important predictor of Pugnose Shiner distribution (McCusker et al. 2014a, Haynes et al. 2019) but was not directly measured in our survey. However, water velocity was approximated by calculating the distance between sample sites and the innermost site, based on the assumption that sites within the inner bay would have little to no water velocity, whereas sites in the outer bay were exposed to flow in the main river. To account for diel variation, time of day was also included as a covariate in occupancy analyses. Detection probabilities were further explored with gear type and removal, a vector that incorporated occupancy information from previous hauls as covariates.

Given the abundance of environmental variables, occupancy models were developed using forward-selection approaches where covariates were initially incorporated into models individually, and only covariates in the best performing models were included in subsequent multi-covariate models. Interaction effects between covariates were also included in some models. Given a relatively small sample size, models did not incorporate more than three covariates for either detection or occupancy probabilities. While typical occupancy models are

not able to incorporate temporally fluctuating parameters, such as temperature and oxygen, hauls were successive and usually completed within 30 minutes of each other. Therefore, these variables were assumed to be constant across repeated sampling events within a randomly selected site, and they were included in models as covariates of occupancy $(\hat{\psi})$. Models were compared using Akaike's Information Criterion (AIC), which ranks models according to their ability to explain the most amount of variation (high goodness of fit) with the fewest variables (low degrees of freedom). Goodness of fit was measured on the model with the lowest AIC score using the Pearson chi-square statistic in a parametric bootstrap with 1000 simulation. The amount of variation explained by the model was estimated using Nagelkerke's R-squared index by comparison against the null model (i.e. with no covariates). Post-hoc, the model was back transformed to predict probability of detections using different sampling techniques and the probability of occupancy given specific environmental conditions. Another assumption of occupancy modelling is the independence of sites. However, as in most species distribution modelling, spatial autocorrelation (SAC) across sites is likely to be present. The 'RPresence' and 'unmarked' programs do not assess or control for SAC, and it was not accounted for in the occupancy models. While SAC may result in the overestimation of the importance of covariates (Legendre 1993) or misrepresentation of uncertainty estimates (Guélat et al. 2018), the effects of SAC may also be quite negligible (Hawkins 2012, Thibaud et al. 2014). Furthermore, spatial effects were partially accounted for by including distance from inner bay as a covariate.

RESULTS

Fish community composition

Environmental and fish abundance data were collected at 88 sites in Thompson's Bay. Summary statistics of all environmental variables are summarized in Table 1. Fish capture data included 25 species and 5443 individuals (Figure 3). Largemouth Bass, Yellow Perch (Perca *flavescens*), and Pumpkinseed were the most ubiquitous species, captured at 73, 67, and 64 sites of the total of 88 sites, respectively. Three species were captured at only one site: Black Crappie (Pomoxis nigromaculatus), Bowfin, (Amia calva), and Common Carp (Cyprinus carpio). A PCA identified 10 significant axes that explained a cumulative 66.3% of the variation in species abundances at each site. A biplot of the two most significant axes (R^2 values of 0.134 and 0.093) indicated that Pugnose Shiner was positively associated with other species of blackline shiner (Blackchin Shiner (Notropis heterodon), Blacknose Shiner (Notropis heterolepis), and juvenile shiners that could not be identified to species) and Round Goby (Neogobius melanostomus) and Yellow Perch (Figure 5). There were not any strong negative correlations with other species, although Largemouth Bass, Pumpkinseed, juvenile Lepomis spp. and Brook Silverside appeared weakly negatively correlated with Pugnose Shiner. Significant correlation was detected between pH and oxygen. Therefore, pH was removed. Significant variables in a matrix of the two most significant PCA axes of fish community distribution, in order of weight were: distance, depth, Chara vulgaris, vegetation density, turbidity, temperature, Elodea spp., and Nymphaea spp. (Table 2, Figure 6). Pugnose Shiner was associated with clear, cool sites far from the inner bay with high vegetation density, including Chara vulgaris (Figure 6).

Occupancy modelling

We captured 120 Pugnose Shiner across at 25 sites (Figure 2). However, we were not able to distinguish among some juvenile shiners, so this focuses on individuals greater than

approximately 20 mm TL. With no covariates, true occupancy was estimated at 0.324, slightly higher than the observed/naïve occupancy of 0.284 (25 out of 88 sites). Probability of detection in this null model was 0.503. Detection probability was best modelled by incorporating vegetation density, while models that incorporated sampling method (boat seine vs. walking seine) and a removal factor performed worse than the null model (Table 3). Specifically, detection probabilities were higher in sites with medium and high vegetation densities. When time of day was included in occupancy models, they had among the highest AIC scores, suggesting that time of day did not strongly affect our results and that sampling was sufficiently randomized across the bay to homogenize time of day effects. The best occupancy model included distance, conductivity, and the presence of Chara vulgaris (Table 4). Specifically, the probability of Pugnose Shiner occupancy increased with distance from the inner bay and with the presence of Chara vulgaris, and decreased with increasing conductivity (Figure 7). Simulated datasets from the model were not significantly different from the refitted model (bootstrap X^2 pvalue of 0.398) and, therefore, the model was considered a good fit and the amount of variance explained in Pugnose Shiner presence was 30% (Nagelkerke's R² of 0.30).

Thermal profile in Pugnose Shiner habitat

Data collected from HOBO temperature loggers deployed in Pugnose Shiner habitat, August 3 to August 12 revealed daily temperature fluctuations between 1.03° C and 6.66° C (Mean = 2.97 + 0.22 SE) (Figure 4), with daily lows around 0700 h and highs peaking around 1600-1700 h across all four sites. Interestingly, offshore sites greater than approximately 30 m from shore (HOBOs 2 and 4) were cooler and exhibited smaller diel fluctuations in water temperature (mean daily amplitude of 2.18°C) than nearshore sites within 30 m from shore (HOBOs 1 and 3, mean daily amplitude of 3.75°C) (Figure 4).

DISCUSSION

Effective conservation of freshwater species at risk in Canada should address local threats, especially for species with discrete and fragmented populations. Thompson's Bay has previously been identified as a critical habitat patch for Pugnose Shiner in the upper St. Lawrence (McCusker et al. 2014a) and may support one of the largest populations of Pugnose Shiner in the world (Fisheries and Oceans Canada 2010). This study identified significant environmental variables and species associations that correlate with the fine-scale distribution of Pugnose Shiner within Thompson's Bay. Furthermore, given that climate change has been identified as a particular threat to freshwater species, such as Pugnose Shiner, that occupy shallow waters sensitive to rising atmospheric temperature, the thermal profile of Pugnose Shiner habitat was examined. These findings could inform population-level conservation approaches that address local threats, notably the direct and indirect effects of rising temperature on Pugnose Shiner in Thompson's Bay

Community composition and environmental correlates

Within Thompson's Bay, species distributions were relatively continuous but Pugnose Shiner exhibited positive associations to sites with excellent water quality and weak negative associations with some native species. Consistent with the small spatial scale of our survey, there was limited clustering of species along PCA axes, suggesting that species distributions across the sampled area were relatively continuous. Our sampled area was restricted to nearshore sites along a stretch of shoreline less than 2 km long, within which we observed limited environmental variation. For example, all sampled sites were less than 2 m deep, only three sites exhibited turbidity levels greater than 2 NTU, and only one site had dissolved oxygen concentrations less than 6 mg/L. These observations highlight that Thompson's Bay represents a relatively small geographic area through which fishes can potentially move. Furthermore, low turbidity, high dissolved-oxygen concentrations, and an abundance of submerged aquatic macrophytes including *Chara vulgaris* confirm Thompson's Bay as a high-quality habitat patch for Pugnose Shiner (McCusker et al. 2014a).

While environmental variability across sites may have been low, a few habitat characteristics emerged as being significant predictors of fish community distribution in Thompson's Bay. Consistent with known habitat requirements (Holm and Mandrak 2002, Fisheries and Oceans Canada 2010), Pugnose Shiner was specifically associated with clear, cool sites far from the inner bay, with high vegetation density, including the presence of *Chara* vulgaris. The strong association between Chara vulgaris and Pugnose Shiner is likely due to a variety of factors, as in the following examples including feeding, refuge habitat, and improved water quality. Pugnose Shiner forage on aquatic vegetation such as *Chara* spp. (Becker 1983, Holm and Mandrak 2002) and also feed on cladocerans and other small invertebrates that tend to be more abundant in or near vegetated sites (Schriver et al. 1995, Jeppesen et al. 2010). Furthermore, Chara vulgaris can form dense underwater mats and was frequently observed as the dominant submerged aquatic vegetation (SAV) in high vegetation density sites in Thompson's Bay. High-density SAV has been linked to reduced predation of juvenile or smallbodied fishes (Anderson 1984, Rozas and Odum 1988, Savino and Stein 1989a, b). In addition to providing refuge, Chara spp. has been specifically linked to improved water transparency (Van

den Berg et al. 1998, De Backer et al. 2010), which may, partly, be why Pugnose Shiner, a species sensitive to turbidity is often associated with *Chara* spp. (Holm and Mandrak 2002).

Despite limited overall multivariate grouping of species across Thompson's Bay, Pugnose Shiner was associated with other species of blackline shiner, Yellow Perch, and Round Goby. The positive association of Pugnose Shiner with other blackline shiners supports previous observations of co-occurrence (Holm and Mandrak 2002), especially with Blackchin Shiner (Carlson 1997, Haynes et al. 2019). For example, in a 1993-1994 survey in the St. Lawrence River, Carlson noted that "when blackchin shiner were collected, there was a high probability of catching pugnose shiner" (1997). Positive associations between Pugnose Shiner and Yellow Perch have previously been identified in other embayments in the upper St. Lawrence River (McKenna and David 2017) and are likely reflections of similar preferred vegetated habitat. Interestingly, adult Yellow Perch are piscivorous (Knight et al. 1984, Paszkowski and Tonn 1994, Fullhart et al. 2002) and are likely predators of Pugnose Shiner and other small-bodied fishes in the bay. To our knowledge, associations between Pugnose Shiner and Round Goby have not previously been identified; this observation was surprising given that, in contrast to Pugnose Shiner, Round Goby is benthic and prefers rock and gravel substrate (Brownscombe and Fox 2012).

In addition to these positive associations, Pugnose Shiner was found to be weakly negatively associated with Largemouth Bass, Pumpkinseed, juvenile *Lepomis* spp., and Brook Silverside. The negative association of Pugnose Shiner presence with Largemouth Bass was not surprising given that Largemouth Bass is a highly effective predator of small-bodied fishes (Brown et al. 2009), although we only captured young-of-the-year (YOY) and juveniles that did not exceed approximately 12 cm TL. The absence of larger fish likely reflects similar habitat

preferences of juvenile fishes in littoral areas such as in Thompson's Bay; and while predation is unlikely at this small size, it is possible that competition may be present between juvenile Largemouth Bass and Pugnose Shiner. Negative associations of Pugnose Shiner with Brook Silverside is likely a reflection of different habitat associations, with Brook Silverside a surface feeder that prefers open-water sites with little vegetation (Killgore et al. 1991). Interestingly, both Pumpkinseed and juvenile *Lepomis spp.* appear to share similar habitat preferences as Pugnose Shiner with an affinity for highly vegetated sites (Laughlin and Werner 1980, Brown and Colgan 1982), although Pumpkinseed is a habitat generalist that appears to be widely tolerant to a range of environmental conditions (Keast et al. 1978, Henning et al. 2014, King 2014). The similar habitat preferences, but negative associations, between these species suggest that interspecific interactions may play a role in structuring the distribution of Pugnose Shiner in Thompson's Bay, although this was not explicitly explored within this survey.

The negative associations of Pugnose Shiner with three centrarchid species are especially revealing giving that Largemouth Bass, Pumpkinseed, and juvenile *Lepomis* spp. were among the most abundant and widely distributed species across the survey area. Notably, Largemouth Bass was the most ubiquitous fish across Thompson's Bay, captured at 83% of sites. The dominance of centrarchids is consistent with recent surveys conducted across other protected nearshore areas in the upper St. Lawrence River (Henning et al. 2014, Haynes et al. 2019). While these surveys, as well as our own, reflect sampling efforts within a single season, centrarchid species have expanded in range (Cazelles et al. 2019) and abundance (Finigan et al. 2018) across Ontario over the last few decades. Notably, a recent study examining patterns of fish community homogenization in Ontario documented increased presence of Bluegill by 140%, Pumpkinseed by 49.5%, and Largemouth Bass by 20% across 524 lakes between two time periods (1965-1982)

and 2008-2012) (Cazelles et al. 2019). Additionally, a smaller study across 22 lakes in southeastern Ontario showed that even at sites where centrarchid species were historically present, abundances have dramatically increased over a 45-year period to the point where communities have shifted from being cyprinid-dominated to centrarchid-dominated (Finigan et al. 2018). In this paper, the authors related this shift in relative abundances to changing land use as well as a slight increase in mean annual air temperature. Emerging research suggests that Pugnose Shiner declines in Ontario are correlated with increased abundance of some centrarchids (Fisheries and Oceans Canada, unpublished). While we do not have a sufficient formal sampling history within Thompson's Bay to explore changing fish abundances quantitatively, local residents in Thompson's Bay reported a decline in minnows in the area over the last decade(s) (L. Potts. August 2018. Personal communications). These qualitative observations suggest that trends in Thompson's Bay may be consistent with broader patterns across Ontario. It also identifies local knowledge as a potentially valuable monitoring tool, even for non-gamefish species. Finally, within this study, Largemouth Bass was associated with warmer sites, suggesting that temperature may also structure community spatially as well as temporally. Given the weak negative associations identified between Pugnose Shiner and other centrarchid species in this study, and their inverse relationships with temperature, increasing abundances and/or range expansions of centrarchids may threaten persistence of Pugnose Shiner in Thompson's Bay, despite an abundance of high-quality habitat. These changes may be facilitated or exacerbated by climate change, although predicting the specific effects of climate change on local fish assemblages is challenging given the wide-range of effects on both abiotic and biotic conditions acting at multiple spatial and temporal scales (Tonn 1990, Ficke et al. 2007, Rahel et al. 2008, Rahel and Olden 2008, Comte et al. 2013).

Pugnose Shiner occupancy modelling

We found that the probability of Pugnose Shiner occupancy in Thompson's Bay increased with distance from the inner bay and in the presence of *Chara vulgaris*, and decreased with increasing conductivity. These findings are largely consistent with previous findings (Holm and Mandrak 2002, Fisheries and Oceans Canada 2010) and other surveys in the upper St. Lawrence, including a survey across four bays in New York state (Haynes et al. 2019). The scale of my survey was most closely aligned with a study conducted by researchers at SUNY Brockport between 2015 and 2016, which examined the associations between Pugnose Shiner, physiochemical factors, and other fish species in two bays in the upper St. Lawrence River (Chippewa Bay and Goose Bay) and one bay in Lake Ontario (Sodus Bay) to examine the suitability of a currently unoccupied bay in Lake Ontario (Chaumont Bay) for reintroduction of Pugnose Shiner (Haynes et al. 2019). They identified an abundance of *Chara vulgaris* as well as a slowly moving current (not measured, but qualitatively described) as important habitat characteristics for Pugnose Shiner in the two bays in the St. Lawrence River. While we also did not measure water velocity directly, distance from the inner bay was incorporated as a proxy variable. In Thompson's Bay, the importance of current is perhaps best illustrated by the clear spatial division between Pugnose Shiner presence in the outer bay and near absence in the inner bay where only three individuals were captured. While Pugnose Shiner is negatively associated with high water velocities (McCusker et al. 2014a), some water exchange likely facilitates population persistence by moderating environmental extremes, including hypoxia and elevated water temperatures, although these effects should be explored further. It should be also be noted that the survey by Haynes et al. (2019) was conducted with different sampling techniques, many at higher precision that our own. For example, they sampled fishes both with beach seining and

electrofishing (we only relied on seining) and quantified vegetation density through stem counts of plant species within a 1 m² quadrat, while our survey relied on a cruder estimate of vegetation density (high, medium, low), which did not distinguish between relative plant abundances. Therefore, the congruence of our results not only highlights the importance of these environmental characteristics to Pugnose Shiner, but also suggests that sampling effort can be reduced without compromising scientific accuracy in the absence of resources.

While our findings were consistent with surveys at similar spatial scales, they diverged slightly from models at larger scales. The site of our survey (Thompson's Bay) was previously incorporated into a distribution model at a larger spatial scale, by modelling suitable habitat in the upper St. Lawrence River based on 2D models of depth and velocity, and on models of submerged aquatic vegetation from depth-by-density relationships (McCusker et al. 2014a). The authors of that study showed that habitat suitability in the upper St. Lawrence River increased with increased vegetation, decreased water velocity, and decreased depth. Within this framework, Thompson's Bay was identified as the most suitable habitat patch; however, no distinction was made between the inner and outer bay. Our findings, along with those presented by Haynes et al. (2019), suggest that some water velocity is important to Pugnose Shiner and identify inner bay sites as unsuitable habitat. These observations could help refine the McCusker et al. model (2014a), and the description of critical habitat (Fisheries and Oceans Canada 2012), by incorporating water velocity as a non-linear correlate with some intermediate optimum.

The effect of conductivity on Pugnose Shiner has been less well explored. We had initially included this parameter based on a survey by McKenna and David (2017) that described Pugnose Shiner habitat among sites where conductivity was higher than average on the American side of the St. Lawrence River. However, we observed that within Thompson's Bay,

Pugnose Shiner occupancy declined as conductivity increased. Previous studies have shown that conductivity gradients can affect fish species distributions (Kimmel and Argent 2010, Janetski and Ruetz III 2015), although the range of values recorded within our survey was relatively small $(211.4 - 290.7 \,\mu\text{S})$. It is possible that conductivity was related to another unmeasured variable that affects Pugnose Shiner distribution, but this requires additional investigation.

Thermal niche of Pugnose Shiner in Thompson's Bay

At the small spatial resolution of Thompson's Bay, temperature did not emerge as an important covariate of Pugnose Shiner presence specifically, although it did emerge as a predictor of overall fish community composition. Pugnose Shiner was captured in sites with water temperatures ranging from 23.9 - 28.5°C, which largely covered the range of temperatures observed across all sites (23.1 - 29.8°C). In areas where Pugnose Shiner was captured, water temperature exhibited substantial diel variation (mean daily amplitude of 2.97°C across four HOBO loggers); however, there were also spatial effects. Specifically, offshore sites (HOBO loggers 2 and 4) were cooler and exhibited smaller diel fluctuations in water temperature (mean daily amplitude of 2.18°C) than onshore sites (HOBOs 1 and 3, mean daily amplitude of 3.75°C). This spatial pattern has also been documented in bays on the American side of the St. Lawrence River and Lake Ontario where shallow, nearshore sites experienced greater daily variation than deeper offshore sites (Haynes et al. 2019). This is notable because it suggests that offshore sites may be slightly buffered from warming effects (for example, as a result of increased water flow); however, we did not detect a difference in Pugnose Shiner abundance between offshore and nearshore sites in the outer bay, which may be related to low overall capture data. High diel temperature variation across sites in Thompson's Bay where Pugnose Shiner was caught is

notable for this apparently thermally-sensitive species. In Chapter 1, we observed that juvenile Pugnose Shiner had limited ability to reverse thermodynamic effects on standard metabolic rate (SMR), represented by a high Q_{10} (the ratio between two rates over a 10°C interval) after a 4month rearing under a range of water temperatures. This was also observed in a 2-week acclimation study on adult Pugnose Shiner (McDonnell et al. unpublished). McDonnell et al. also observed reduced survivorship of Pugnose Shiner at the highest acclimation temperatures of 28 and 31°C, suggesting that Pugnose Shiner in Thompson's Bay are already experiencing temperatures that may impose significant stress. Elevated SMR at higher temperatures may limit the ability of fishes to persist in warming waters by affecting various fitness-related traits, including condition (e.g. Healy and Schulte 2012). Furthermore, during thermal tolerance trials, Pugnose Shiner exhibited a stress response to moderate increases in temperature; specifically, the onset of agitation behaviour occurred close to acclimation temperatures (average difference of 4.32°C) (Chapter 2). While the rate of warming during thermal tolerance (CT_{max}) trials was higher than diel rates of warming, some fish in our lab study exhibited agitation behaviour well within the range of temperatures experienced by Pugnose Shiner within a single day in Thompson's Bay. The reliance of Pugnose Shiner on shallow habitats with an abundance of submerged vegetation likely restrict it to these highly thermally variable sites. As climate change increases both mean annual temperature and extreme thermal events (IPCC 2013), Pugnose Shiner is likely to become even more exposed to the threat of elevated water temperatures.

Projected effects of climate change on Pugnose Shiner in Thompson's Bay

The direct effects of temperature on Pugnose Shiner distribution and community composition in Thompson's Bay were marginal. However, Pugnose Shiner was largely captured

in the outer bay, a spatial pattern replicated across multiple bays within the St. Lawrence River (Haynes et al. 2019). These sites are likely characterized by higher water velocities, which may buffer Pugnose Shiner from atmospheric warming by facilitating water mixing between Thompson's Bay and the St. Lawrence River. However, this study only characterized diel variation between nearshore and offshore sites within the outer bay where the abundance of Pugnose Shiner was highest, and we did not examine differences between the inner and outer bay in the context of sites that varied in Pugnose Shiner density. In the outer bay, Pugnose Shiner was exposed to large diel fluctuations in temperature, which are projected to increase with climate warming (IPCC 2013). One of the most significant environmental features characterising the distribution of Pugnose Shiner was dense patches of *Chara vulgaris*, suggesting that opportunities for behavioural thermoregulation may be limited by the extent of submerged macrophytes. This means that Pugnose Shiner may be confined to shallow waters that will be increasingly exposed to higher thermal fluctuations that, in turn, may affect population persistence. Understanding diel and seasonal movement patterns in response to temperature and how Pugnose Shiner may exploit thermal heterogeneity in their habitat will be an important topic of future research. Furthermore, while the indirect effects of temperature on species interactions were not obvious within the scope of this study, Pugnose Shiner exhibited negative associations with Largemouth Bass, Pumpkinseed, and juvenile Lepomis spp. These observations compliment emerging research that suggests that Pugnose Shiner declines are correlated with increased abundance of some centrarchids (Fisheries and Oceans Canada unpublished). In the context of climate change, this is significant because centrarchids appear to be increasing in abundance with rising temperatures (Finigan et al. 2018).

Management recommendations

A federal recovery strategy for Pugnose Shiner in Canada was developed in 2012 (Fisheries and Oceans Canada) and was recently updated to reflect progress made on action items since 2012 (Fisheries and Oceans Canada *unpublished*). While the recovery strategy outlines the importance of identifying and mitigating key threats such as sediment loading, SAV removal, and invasive species, it does not address the emerging threat of climate change. Climate change is likely to be a prominent stressor for freshwater fishes, such as Pugnose Shiner, that are simultaneously contending with the direct and indirect effects of rising water temperatures. Failing to account for the effects of climate change may jeopardize ongoing conservation efforts of rare and imperiled freshwater fishes in Canada. For example, the expansion of centrarchids, including predators such as Largemouth Bass, with climate change may significantly impact Pugnose Shiner, even under suitable habitat conditions. To mitigate negative interactions, increased fishing pressure on expanding species may be a potential conservation measure (McGinn 2002). Furthermore, accounting for species co-occurrence may be an important step in facilitating reintroduction, repatriation, and assisted range expansions of imperiled species (Lamothe et al. 2019).

The long-term recovery goal for Pugnose Shiner in Canada, as identified by the 2012 Recovery Strategy (Fisheries and Oceans Canada), is to "maintain self-sustaining populations at existing locations and restore self-sustaining populations to historic locations, where feasible." In Canada, populations of Pugnose Shiner are discrete, isolated, and genetically distinct (McCusker et al. 2014b), emphasizing the importance of population-level conservation approaches that address local threats. This study examined the distribution of Pugnose Shiner within Thompson's Bay and identified local environmental and species associations that had not previously been

explored in one of the most important habitat patches in the upper St. Lawrence River (McCusker et al. 2014a) . Encouragingly, my finding that Pugnose Shiner distribution was correlated with water velocity (inferred using distance from inner bay) and the presence of *Chara vulgaris* was consistent with other studies on Pugnose Shiner in the St. Lawrence River, including a survey conducted at a similar scale (embayment-level) on the American side of the St. Lawrence River (Haynes et al. 2019). The striking congruence between the results from my survey and the Haynes et al. survey emphasizes the importance of these two habitat features for Pugnose Shiner populations in the upper St. Lawrence River. Furthermore, these results can be used to improve the accuracy of a habitat-suitability model developed for the upper St. Lawrence river (McCusker et al. 2014a) by adjusting the water-velocity parameter to incorporate a water-velocity optima, which should be more precisely quantified in future studies.

The sampled area in Thompson's Bay was largely dominated by clear, highly vegetated, and highly oxygenated patches, which is ideal Pugnose Shiner habitat. However, with the study area, Pugnose Shiner was restricted to outer bay sites with slow water velocities and likely lower diel oxygen and temperature variability, suggesting that this species may be sensitive to environmental fluctuations that are expected to increase with climate change. Furthermore, Pugnose Shiner was negatively associated with some fish species, including predators, projected to increase in range and abundance under future climate-warming scenarios. These findings suggest that, while Thompsons' Bay represents an important stronghold for Pugnose Shiner, emerging threats such as climate change likely threaten this population and will require active monitoring and implementation of recovery strategies to conserve this endangered species.

Tables

Table 1. Summary statistics of continuous environmental variables across 88 sites in a survey forPugnose Shiner conducted in Thompson's Bay, Ontario, Canada between July 26 and August 8,2018.

Parameter	Mean	Median	Minimum	Maximum
Water Temperature (°C)	26.32	26.3	23.6	29.7
Oxygen (mg/L)	9.48	9.29	5.97	17.06
Turbidity (NTU)	0.46	0	0	12.5
Conductivity (µS)	253.7	253.2	211.4	290.7
pH	8.23	8.26	7.52	8.77
Depth (m)	1.1	1.07	0.38	1.80

Table 2. Significance of environmental variables that were fit to the matrix of the first two axes of the PCA by conducting 9999 permutations. Significant components (p < 0.05) are indicated in bold.

Parameter	Туре	r ²	p-value
Temperature	Vector	0.0778	0.0323
Oxygen	Vector	0.0195	0.4368
Turbidity	Vector	0.0831	0.0364
Conductivity	Vector	0.0405	0.1744
Depth	Vector	0.2791	0.0001
Distance	Vector	0.3140	0.0001
Vegetation Density	Factor	0.1028	0.0019
Chara	Factor	0.1122	0.0001
Vallisneria	Factor	0.0260	0.0977
Myriophyllum	Factor	0.0096	0.4428
Elodea	Factor	0.0417	0.0268
Nymphaea	Factor	0.0465	0.0165
Typha	Factor	0.0112	0.3714

Table 3. Summary of detection models in Thompson's Bay, St. Lawrence River, Ontario,

 selected using AIC including covariates of vegetation density (veg), sampling technique (gear),

 and a removal factor (rem). Descriptions of environmental and sampling variables are provided

 in Table S1.

Model	AIC	ΔΑΙϹ	Akaike weight	npar	-2*LL
ψ(.), <i>p</i> (veg)	205.39	0.00	0.2990	4	197.39
$\psi(.), p(.)$	206.33	0.94	0.1807	2	202.33
$\psi(.), p(\text{veg} + \text{gear})$	206.40	1.01	0.1800	5	196.4
ψ(.),p(gear)	207.38	1.99	0.1110	3	201.38
$\psi(.), p(veg + rem)$	190.35	2.00	0.1100	5	197.39
ψ(.),p(rem)	208.31	2.92	0.0700	3	202.31
$\psi(.), p(gear + rem)$	209.26	3.87	0.0430	4	201.26

Table 4. Summary of the top 17 occupancy models for Pugnose Shiner in Thompson's Bay, St. Lawrence River, Ontario, selected using AIC. The top models included distance from inner bay (dist), conductivity (cond), and the presence of *Chara vulgaris* (char). Descriptions of environmental and sampling variables are provided in Table S1.

Model	AIC	ΔΑΙϹ	Akaike weight	npar	-2*LL
$\psi(\text{dist} + \text{cond} + \text{char}), p(\text{veg})$	188.75	0.00	0.2366	7	174.75
$\psi(\text{dist} + \text{cond} * \text{char}), p(\text{veg})$	188.87	0.12	0.2229	8	172.87
$\psi(\text{dist} + \text{cond}), p(\text{veg})$	188.96	0.21	0.2133	6	176.96
$\psi(\text{dis} * \text{cond}), p(\text{veg})$	190.24	1.48	0.1127	7	176.24
$\psi(dis * cond + char), p(veg)$	190.35	1.59	0.1066	8	174.35
$\psi(ext{dist} * ext{cond} * ext{char}), p(ext{ve}g)$	192.48	3.72	0.0367	11	170.48
$\psi(\text{dist} + \text{char}), p(\text{veg})$	193.85	5.10	0.0185	6	181.85
$\psi(\text{dist} + \text{turb}), p(\text{veg})$	194.56	5.80	0.0130	6	182.56
$\psi(ext{dist} * ext{char}), p(ext{veg})$	194.95	6.20	0.0107	7	180.95
$\psi({ m dist}), p({ m veg})$	195.20	6.44	0.0094	5	185.20
$\psi(\text{dist} + \text{veg}_{\text{dens}}), p(\text{veg})$	197.43	8.68	0.0031	7	183.43
$\psi(\text{cond} + \text{char}), p(\text{veg})$	197.62	8.87	0.0028	6	185.62
$\psi(ext{cond} * ext{char}), p(ext{veg})$	198.02	9.26	0.0023	7	184.02
$\psi(ext{cond}), p(ext{veg})$	200.48	11.72	0.0007	5	190.48
$\psi(\text{char}), p(\text{veg})$	200.52	11.76	0.0007	5	190.52
$\psi(turb), p(veg)$	201.94	13.18	0.0003	5	191.94
$\psi(.), p(\text{veg})$	205.39	16.64	0.0001	4	197.39

Figures



Figure 1. Sites sampled for Pugnose Shiner in Thompson's Bay, St. Lawrence River, Ontario, Canada between July 26 and August 8, 2018. Locations of 4 HOBO temperature loggers deployed between August 3 and 12 are indicated in red: H1 and H3 were considered nearshore sites (less than 30 m from shore), while H2 and H4 were offshore (greater than 30 m from shore). The inner bay and outer bay are roughly delineated by the dashed blue line.



Figure 2. Distribution of Pugnose Shiner in Thompson's Bay, ON. Sites where Pugnose Shiner were present are indicated by a closed circle and sites where Pugnose Shiner were absent are represented by an open circle. The relative size of the circle corresponds to the number of individuals captured (minimum abundance = 1, maximum = 14). A total of 120 Pugnose Shiner were caught, across 25 sites.



Figure 3. Fish abundance by species (A) and total number of sites occupied by each species (B) across 88 sites in Thompson's Bay, in Thompson's Bay, St. Lawrence River, Ontario, sampled between July 26 and August 8, 2018.



Figure 4. Diel temperature data, recorded once every minute, from four HOBO loggers deployed in in Thompson's Bay, St. Lawrence River, Ontario, between August 3 and August 12, 2018. Nearshore sites appear in orange (HOBO 1 = light orange, HOBO 3 = dark orange) and offshore sites appear in blue (HOBO 2 = light blue, HOBO 4 = dark blue). Locations of HOBO loggers are indicated in Figure 3.



Figure 5. Biplot of the two most significant axes of Hellinger-transformed fish abundance data in Thompson's Bay, St. Lawrence River, Ontario sampled between July 26 and August 8, 2018. Species appear in black and are represented by species codes (Table S3). The angles between arrows represent species correlations. Pugnose Shiner (PNS) is underlined in red and is positively associated with juvenile shiners (N_juv), Blackchin Shiner (BCS), Blacknose Shiner (BNS), Round Goby (RG), and Yellow Perch (YP) and negatively associated with Brook Silverside (BS), juvenile *Lepomis* spp. (L_juv), Largemouth Bass (LMB), and Pumpkinseed (PS).



Figure 6. Biplot of the two most significant axes of Hellinger-transformed fish abundance data in Thompson's Bay, St. Lawrence River, Ontario sampled between July 26 and August 8, 2018. Species appear in black and are represented by species codes (Table S3). Significant environmental variables (p < 0.05) appear in red and are represented by variable codes (Table S1). Continuous environmental variables (depth, distance, temperature, and turbidity) are associated with a vector where the length of the arrow represents the relative strength of the predictor. Factors (vegetation density, *Chara vulgaris, Elodea* spp., and *Nymphaea* spp.) are represented by factor averages. Pugnose Shiner (PNS) is associated with sites characterized by a large distance from the inner bay (Dist), low temperature (Temp), low turbidity (Turb), high vegetation density (vegH), and the presence of *Chara vulgaris* (c1).



Figure 7. Probability of detection (*p*) of Pugnose Shiner in Thompson's Bay, St. Lawrence River, Ontario, as a function of vegetation density (A). Probability of occupancy (ψ) as a function of: B) distance from inner bay, C) conductivity, and D) presence of *Chara vulgaris*. Figures that represent factors (A and D) are shown with standard error bars and figures with continuous variables (B and C) include 95% confidence intervals (shaded grey area).

Supplementary Information

Table S1. Environmental variables collected during the survey period in in Thompson's Bay, St. Lawrence River, Ontario sampled between July 26 and August 8, 2018. Only a subset was used in multivariate analyses and occupancy models.

Variable	Code	Туре	Unit	Description	
Water Temperature	Те	Continuous	°C	Water temperature at mid-depth at sample point	
Oxygen	Ox	Continuous	mg l ⁻¹	Dissolved oxygen concentration at mid-depth at sample point	
Turbidity	Tu	Continuous	NTU	Turbidity at mid-depth at sample point	
pН	рН	Continuous		pH at mid-depth at sample point	
Conductivity	Co	Continuous	μS	Conductivity at mid-depth at sample point	
Depth	De	Continuous	m	Depth to substrate at sample point	
Depth to vegetation		Continuous	m	Depth to top of dominant submerged vegetation at sample point	
Distance	Di	Continuous	m	Distance between sample point and site furthest in the inner bay	
Air Temperature	AT	Continuous	°C	Air temperature	
Wind Speed	WS	Continuous	m hr-1	Wind speed	
Vegetation Density	Veg	Ordinal	High, medium, or low	Vegetation density estimated at sample point	
Chara	Ch	Nominal/Binary	0 (absent), 1 (present)	Presence of Chara vulgaris	
Vallisneria	Va	Nominal/Binary	0 (absent), 1 (present)	Presence of Vallisneria americana	
Myriophyllum	Му	Nominal/Binary	0 (absent), 1 (present)	Presence of watermilfoils, Myriophyllum spp.	
Elodea	El	Nominal/Binary	0 (absent), 1 (present)	Presence of <i>Elodea</i> spp.	
Nymphaea	Ny	Nominal/Binary	0 (absent), 1 (present)	Presence of water lilies, <i>Nymphaea spp</i> .	
Typha	Ту	Nominal/Binary	0 (absent), 1 (present)	Presence of cattails, Typha spp.	
Sampling technique	gear	Nominal	Walking, Boat	Sampling technique using the same 10-m bag seine (3-mm mesh)	
Removal	rem	Nominal/Binary	0 (no removal), 1(removal)	Reflecting removal of fish captured during previous seine hauls	

Table S2. Pearson's correlations for continuous environmental variables used in multivariate analyses and occupancy models for Pugnose Shiner in Thompson's Bay, St. Lawrence River, Ontario, sampled between July 26 and August 8, 2018. Significant correlations (|r| > 0.7) are indicated in bold.

	Temperature	Oxygen	Turbidity	pН	Conductivity	Depth	Distance
Temperature		0.38	0.06	0.09	-0.07	-0.37	-0.23
Oxygen			-0.07	0.74	-0.56	-0.33	0.06
Turbidity	_			-0.27	-0.04	-0.15	-0.08
pH					-0.50	0.00	0.12
Conductivity			_			0.16	-0.10
Depth							-0.07
Distance					-		

Table S3. Species captured in in Thompson's Bay, St. Lawrence River, Ontario sampledbetween July 26 and August 8, 2018.

Common Name	Scientific Name	Species Code
Banded Killifish	Fundulus diaphanus	BK
Black Crappie	Pomoxis nigromaculatus	BC
Blacknose Shiner	Notropis heterolepis	BNS
Blackchin Shiner	Notropis heterodon	BCS
Bluegill	Lepomis macrochirus	BG
Bluntnose Minnow	Pimephales notatus	BM
Bowfin	Amia calva	BF
Brook Silverside	Labidesthes sicculus	BS
Brown Bullhead	Ameiurus nebulosus	BBH
Central Mudminnow	Umbra limi	СММ
Common Carp	Cyprinus carpio	CC
Emerald Shiner	Notropis atherinoides	ES
Golden Shiner	Notemigonus crysoleucas	GS
Grass Pickerel	Esox americanus vermiculatus	GP
Juvenile Lepomis spp.	Lepomis spp.	L_juv
Juvenile shiner	Notropis spp.	N_juv
Largemouth Bass	Micropterus salmoides	LMB
Mimic Shiner	Notropis volucellus	MS
Pugnose Shiner	Notropis anogenus	PNS
Pumpkinseed	Lepomis gibbosus	PS
Rock Bass	Ambloplites rupestris	RB
Round Goby	Neogobius melanostomus	RG
Tadpole Madtom	Noturus gyrinus	ТМ
Yellow Bullhead	Ameiurus natalis	YBH
Yellow Perch	Perca flavescens	YP



Figure S1. North shore of Thompson's Bay, St. Lawrence River, Ontario, facing west.



Figure S2. A typical dense submerged macrophyte bed that characterized much of the habitat across Thompson's Bay, St. Lawrence River, Ontario. *Chara vulgaris* was frequently the dominant SAV species in Pugnose Shiner habitat.
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GENERAL CONCLUSION

Currently, there are 101 species, populations, and sub-populations of freshwater fishes classified as endangered, threatened, or of special concern in Canada (Government of Canada 2019), reflecting the global phenomenon of high rates of imperilment in freshwater taxa. While pollution, invasive species, and natural-system modifications are currently listed as main threats to freshwater fishes across the country (McCune et al. 2013), climate change represents an emerging threat. The effects of climate change are multidimensional, multi-scalar, and interactive; therefore, the impacts on freshwater fishes are hard to predict. However, elevated water temperature likely represents a significant physiological stressor for ectotherms, such as fishes, that rely on the external environment to regulate their body temperature and, consequently, their metabolic rate. Understanding effects of elevated water temperature on imperiled species may be critical to their persistence, because these taxa tend to be more sensitive to changes in their environment and are already contending with other, and potentially interactive, stressors. While climate change has been identified as a threat to many species at risk in Canada (McCune et al. 2013), to our knowledge, no studies have explicitly investigated the physiological effects of elevated water temperature on imperiled freshwater fishes in Canada.

This thesis explored the effects of elevated water temperature on Pugnose Shiner (*Notropis anogenus*), a federally endangered species. Across its range, Pugnose Shiner occupies shallow nearshore habitats with low thermal buffer capacity and is, therefore, likely to be highly exposed to rising atmospheric temperatures. Due to its small body size and perceived weak swimming ability, Pugnose Shiner is unlikely to expand its range in response to climate change (Chu et al. 2005). Therefore, the ability of Pugnose Shiner to persist within a warming environment will likely depend on its ability to behaviourally or physiologically acclimate

through thermal refuge seeking, phenotypic plasticity, and/or adapt through genetic change. However, studies examining both plastic and genetic responses in the lab may miss aspects of ecological complexity that may facilitate or hinder population persistence in the wild. For example, habitat heterogeneity may provide opportunities for behavioural thermoregulation. At the same time, changing distribution and abundance of other species and/or the strength of species interactions may affect the abundance and distribution of Pugnose Shiner. In this thesis, I explored the effects of elevated water temperature on Pugnose Shiner by exploring phenotypic plasticity across multiple traits as well as on fine-scale distribution and species associations in a single population. By combining both lab- and field-based approaches, I attempted to provide a more comprehensive assessment of the risk that elevated water temperatures and climate change may have on Pugnose Shiner. Furthermore, I provided specific conservation recommendations with the intention that this research be used to support effective recovery strategies.

In Chapter 1, I demonstrated that Pugnose Shiner show high acclimation capacity to elevated water temperatures over developmental time scales in some traits but not others. Specifically, while Pugnose Shiner exhibit high acclimation capacity in critical thermal maxima (CT_{max}) , its ability to reverse thermodynamic effects on standard metabolic rate (SMR) is limited. Furthermore, exposure to acute hypoxia reduces CT_{max} of Pugnose Shiner, suggesting that thermal tolerance in this species is oxygen limited; at least at DO concentrations of 22%. Gill size increased with long-term exposure to high temperature, which may increase oxygen uptake capacity and fuel increased metabolic demands. In terms of conservation approaches, we proposed mitigating the effects of local stressors, such as eutrophication, that will likely interact with elevated water temperatures as well as exploring opportunities for improving thermal resistance during captive rearing and re-introduction efforts and/or identifying source

populations with greater thermal tolerance that may bolster the success of introduction efforts. Future studies should examine the trade-offs associated with acclimation to elevated water temperatures and continue to explore the interaction of temperature with other stressors, including the oxygen-dependency of thermal tolerance at different levels of hypoxia and/or exploring interactions with turbidity. Furthermore, to our knowledge, this study represents the first time that juvenile Pugnose Shiner have been successfully maintained long-term in a lab environment and, therefore, provides promise for captive-breeding programs.

In Chapter 2, I showed that environmental and species associations are important in shaping Pugnose Shiner distribution at the local population scale. Pugnose Shiner was associated with clear, cool sites far from the inner bay with high vegetation density, including *Chara vulgaris*. Pugnose Shiner showed a weak negative association with Largemouth Bass (*Micropterus salmoides*), Pumpkinseed (*Lepomis gibbosus*), and juvenile *Lepomis* spp. This has important conservation implications because native centrarchids are becoming more abundant with climate change, which may threaten the persistence of rare and imperiled cyprinids including Pugnose Shiner. I highlighted the importance of considering species interactions during species recovery planning. Future studies should examine species associations across the range of Pugnose Shiner, especially outside of the St. Lawrence River, to explore population-specific associations.

Given its sensitivity to environmental stressors, such as turbidity, and its strong dependence on submerged macrophytes, the occurrence of Pugnose Shiner is considered to be an indicator of good habitat quality (Schneider 2002). However, given limited information on the responses of other co-occurring *Notropis* and cyprinid species, its relative sensitivity to elevated water temperature is unknown. As a result, the degree to which the responses of Pugnose Shiner

to elevated water temperature are similar or predictive of responses in other closely related species is unknown. Future studies would benefit from examining species-specific thermal responses in other blackline shiner, both in the lab and in the field, to assess the relative sensitivities of imperiled vs. non-imperiled congeners to elevated water temperatures. Such studies would shed light on the extent to which recovery actions that mitigate the threat of elevated water temperature for Pugnose Shiner are also likely to benefit other species, especially other small-bodied cyprinids.

Of course, the effects of climate change on freshwater fishes are not limited to changes in thermal regimes. Climate change will also likely result in decreased dissolved oxygen availability, altered hydrological regimes, increased stratification, and increased toxicity of pollutants, among other changes (Ficke et al. 2007, Crozier and Hutchings 2014). Furthermore, human responses to climate change are likely to exacerbate these stressors (Vörösmarty et al. 2000, Xenopoulos et al. 2005). Responses to climate change will occur across multiple traits and at multiple scales of biological organization. In many ways, management of imperiled species in the face of climate change is challenged by having to contend with many unknowns. This research attempted to integrate complex responses by examining mechanistic responses to elevated water change over a developmental time scale, by including interactions of elevated water temperature with another stressor (hypoxia), as well as by exploring responses to temperature at the individual- and population-level; however, it did not capture the full extent of Pugnose Shiner responses to climate change.

To account for uncertainty in the face of a changing climate, adaptive conservation strategies are increasingly being emphasized in the management of imperiled aquatic species (Rahel et al. 2008, Lawler 2009, Hollowed et al. 2011, Green et al. 2014, Lamothe and Drake

2019). Approaches identified in the previous studies include, but are not limited to, habitat modifications to buffer effects of climate change, early detection and eradication of invasive species, as well as more controversial efforts such as assisted range shifts. In addition to implementing adaptive management, building human capacity will play an important role in the conservation of imperiled species, including the development of multi-national conservation policies and collaboration between multiple stakeholders (Collares-Pereira and Cowx 2004, McClanahan et al. 2008, Hermoso and Clavero 2011). While specific conservation actions are likely to be context-dependent, on both the intensifying climate and biodiversity crises, experts agree that urgent action is needed to avoid the most destructive effects of climate change and biodiversity loss (IPCC 2014, IPBES 2019). Given the immediate and intensifying threat of multiple stressors in freshwater ecosystems (Dudgeon et al. 2006, Ormerod et al. 2010, Carpenter et al. 2011, WWF 2014), conservation of Pugnose Shiner and other imperiled fishes in Canada should be based on the best available information but also be adaptive to change.

When critical knowledge gaps are filled for imperiled species, the transformation of scientific knowledge into effective conservation actions is no longer limited by what we should do, but rather what we are willing to do. Practically, this often translates into an issue of cost, what we are willing to spend in order to recover species and habitats. In Canada, conservation of imperiled species is realized through the individual and collective efforts of government agencies and conservation authorities, NGOs such as Wildlife Conservation Society Canada (WCS Canada) and World Wildlife Fund Canada (WWF-Canada), Indigenous groups, and engaged citizens. However, federal protection granted through the Species at Risk Act (SARA) is often first line of defence for species at risk of extinction in Canada. Funding for the implementation of recovery actions mandated by the Act is delivered, as are all federal budgets, through tax-

payer dollars. As a result, SARA itself is explicit about cost-efficiency and the long-term recovery goal for Pugnose Shiner is to "maintain self-sustaining populations at existing locations and restore self-sustaining populations to historic locations, *where feasible*" (Fisheries and Oceans Canada 2012). Therefore, effective conservation of Pugnose Shiner not only relies on quality science to inform recovery actions but also on what are Canadians are willing to spend to reach these recovery targets.

Interestingly, while economic valuation of freshwater species is sparse (Grantham and Rudd 2015), Pugnose Shiner was recently included among other freshwater fish species at risk within a public survey to assess the non-use value of little-known species. This 2011 study surveyed over 400 respondents and determined that Ontario residents were willing to pay an average \$25.11 per household per year for recovery efforts of Pugnose Shiner (Rudd et al. 2016). Even though willingness to pay (WTP) surveys may inflate species valuation, the authors noted that when extrapolated across all households in Ontario "even with a 50-70% downward recalibration of current WTP values for little-known species, their aggregate value would still be in the tens of millions of dollars per year." In other words, Ontarians place significant value on poorly-known imperiled freshwater fishes that have little to no economic utility, including Pugnose Shiner, which justifies investing federal resources under SARA into conserving and restoring these species. Interestingly, the same survey highlighted that respondents were willing to pay more for the recovery of a guild of little-known freshwater fishes at risk than for the restoration of wetland habitats; in other words, pay more when conservation activities were directed towards species than towards habitats. These results likely point to an effect known as the 'iconisation' of species, whereby simply naming a species breaks down intangible notions of biodiversity and removes anonymity, making people more willing to assume responsibility for

the conservation of species (Jacobsen et al. 2008). While the reasons for conserving Pugnose Shiner are numerous and varied (e.g. as an indicator of good habitat conditions, as a contributor to overall biodiversity), this study suggests that citizens are willing to pay a substantial amount to restore freshwater fish species at risk and that just knowing that there is a fish out there called Pugnose Shiner provides incentive for conservation among stakeholders.

Pugnose Shiner is a small freshwater fish poised at the intersection of the global biodiversity and climate crises. The research generated from this thesis filled critical knowledge gaps on the thermal biology of this endangered species and also identified specific conservation recommendations for Pugnose Shiner contending with both the direct and indirect effects of warming waters. As a general contribution to the knowledge of the effects of climate change on freshwater fishes, it highlighted the importance of accounting for multiple traits in assessing acclimation potentials, of executing lab studies over ecologically relevant time scales and at different life stages, of accounting for interactions of additional stressors, and of examining responses across multiple scales of biological organization. Conservation of Pugnose Shiner and other imperiled freshwater fishes in Canada will increasingly depend on adaptive conservation strategies and collaboration with multiple stakeholders, dynamic initiatives that will inevitably require substantial input of expertise, time, and funds. However, and perhaps most encouragingly, Canadians seem to agree: it is worth it.

LITERATURE CITED

- Alofs, K. M., D. A. Jackson, and N. P. Lester. 2014. Ontario freshwater fishes demonstrate differing range-boundary shifts in a warming climate. Diversity and Distributions 20:123-136.
- Anderson, O. 1984. Optimal foraging by largemouth bass in structured environments. Ecology **65**:851-861.
- Anttila, K., R. S. Dhillon, E. G. Boulding, A. P. Farrell, B. D. Glebe, J. A. Elliott, W. R. Wolters, and P. M. Schulte. 2013. Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. Journal of Experimental Biology 216:1183-1190.
- Arthington, A. H., N. K. Dulvy, W. Gladstone, and I. J. Winfield. 2016. Fish conservation in freshwater and marine realms: status, threats and management. Aquatic Conservation: Marine and Freshwater Ecosystems 26:838-857.
- Bailey, R. M. 1959. Distribution of the American cyprinid fish *Notropis anogenus*. Copeia **1959**:119-123.
- Baltz, D., B. Vonderacek, L. Brown, and P. Moyle. 1987. Influence of temperature on microhabitat choice by fishes in a California stream. Transactions of the American Fisheries Society 116:12-20.
- Barnosky, A. D., N. Matzke, S. Tomiya, G. O. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth's sixth mass extinction already arrived? Nature 471:51-57.
- Barrionuevo, W. R., and M. N. Femandes. 1995. Critical thermal maxima and minima for curimbatá, Prochilodus scrofa Steindachner, of two different sizes. Aquaculture Research 26:447-450.
- Becker, C. D., and R. G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes 4:245-256.
- Becker, G. C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison, Wisconsin.
- Beitinger, T. L., W. A. Bennett, and R. W. McCauley. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes **58**:237–275.
- Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical ecology with R. Springer, New York, New York.
- Brahmane, M. P., K. K. Krishnani, B. Sarkar, B. Sajjanar, S. Kumar, A. D. Nakhawa, and P. S. Minhas. 2014. Growth, thermal tolerance and oxygen consumption in rohu, *Labeo rohita* early fry acclimated to four temperatures. African Journal of Agricultural Research 9:854-858.
- Brandt, S. B. 1980. Spatial segregation of adult and young-of-the-year alewives across a thermocline in Lake Michigan. Transactions of the American Fisheries Society **109**:469-478.
- Brian, J. V., C. A. Harris, T. J. Runnalls, A. Fantinati, G. Pojana, A. Marcomini, P. Booy, M. Lamoree, A. Kortenkamp, and J. P. Sumpter. 2008. Evidence of temperature-dependent effects on the estrogenic response of fish: implications with regard to climate change. Science of the Total Environment **397**:72-81.

- Brown, J. A., and P. W. Colgan. 1982. The inshore vertical distribution of young-of-year *Lepomis* in Lake Opinicon, Ontario. Copeia **1982**:958-960.
- Brown, T. G., B. Runciman, S. Pollard, and A. D. A. Grant. 2009. Biological synopsis of largemouth bass (*Micropterus salmoides*). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2884. Fisheries and Oceans Canada, Nanaimo, British Columbia.
- Brownscombe, J. W., and M. G. Fox. 2012. Range expansion dynamics of the invasive round goby (*Neogobius melanostomus*) in a river system. Aquatic Ecology **46**:175-189.
- Burkhead, N. M. 2012. Extinction rates in North American freshwater fishes, 1900–2010. BioScience **62**:798-808.
- Carlson, D. M. 1997. Status of the pugnose and blackchin shiners in the St. Lawrence River in New York, 1993–95. Journal of Freshwater Ecology **12**:131-139.
- Carpenter, S. R., E. H. Stanley, and M. J. Vander Zanden. 2011. State of the world's freshwater ecosystems: physical, chemical, and biological changes. Annual Review of Environment and Resources **36**:75-99.
- Cazelles, K., T. Bartley, M. M. Guzzo, M.-H. Brice, A. S. MacDougall, J. R. Bennett, E. H. Esch, T. Kadoya, J. Kelly, S.-i. Matsuzaki, K. A. Nilsson, and K. S. McCann. 2019. Homogenization of freshwater lakes: recent compositional shifts in fish communities are explained by gamefish movement and not climate change. Global Change Biology 25:4222-4233.
- Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human–induced species losses: entering the sixth mass extinction. Science Advances 1:e1400253.
- Chapman, L., J. Albert, and F. Galis. 2008. Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. The Open Evolution Journal 2:75-88.
- Chapman, L., T. DeWitt, V. Tzaneva, and J. Paterson. 2007. Interdemic variation in the gill morphology of a eurytopic African cichlid. Pages 209-225 *in* Proceedings of the 9th International Symposium on Fish Physiology, Toxicology, and Water Quality. United States Environmental Protection Agency.
- Chapman, L. J., and D. J. McKenzie. 2009. Behavioral responses and ecological consequences. Pages 26-77 in J. G. Richards, A. P. Farrell, and C. J. Brauner, editors. Hypoxia in Fishes. Elsevier, San Diego, California.
- Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. Science **333**:1024-1026.
- Chessman, B. C. 2013. Identifying species at risk from climate change: traits predict the drought vulnerability of freshwater fishes. Biological Conservation **160**:40-49.
- Chu, C., N. E. Mandrak, and C. K. Minns. 2005. Potential impacts of climate change on the distributions of several common and rare freshwater fishes in Canada. Diversity and Distributions 11:299-310.
- Clark, J. A., and R. M. May. 2002. Taxonomic bias in conservation research. Science **297**:191-192.
- Clark, T. D., E. Sandblom, and F. Jutfelt. 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. Journal of Experimental Biology 216:2771-2782.
- Clarke, A., and N. M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology **68**:893-905.

- Claussen, D. L. 1977. Thermal acclimation in ambystomatid salomanders. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology **58**:333-340.
- Closs, G. P., P. L. Angermeier, W. R. T. Darwall, S. R. Balcombe, G. P. Closs, M. Krkosek, and J. D. Olden. 2016. Why are freshwater fish so threatened? Pages 37-75 *in* G. P. Closs, M. Krkosek, and J. D. Olden, editors. Conservation of freshwater fishes. Cambridge University Press, Cambridge.
- Collares-Pereira, M., and I. Cowx. 2004. The role of catchment scale environmental management in freshwater fish conservation. Fisheries Management and Ecology **11**:303-312.
- Comte, L., L. Buisson, M. Daufresne, and G. Grenouillet. 2013. Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. Freshwater Biology 58:625-639.
- Comte, L., J. Murienne, and G. Grenouillet. 2014. Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes. Nature Communications **5**:5023.
- Comte, L., and J. D. Olden. 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Global Change Biology **23**:728-736.
- COSEWIC. 2013. COSEWIC assessment and status report on the Pugnose Shiner, *Notropis anogenus* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario.
- Cox, D.K. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. Pages 158–163 in J.W. Gibbons & R.R. Sharitz, editors. Thermal Ecology. U.S. Atomic Energy Commission, Savannah, Georgia.
- Crispo, E., and L. J. Chapman. 2010. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. Journal of Evolutionary Biology **23**:2091-2103.
- Crozier, L. G., and J. A. Hutchings. 2014. Plastic and evolutionary responses to climate change in fish. Evolutionary Applications 7:68-87.
- Darwall, W. R. T., and J. Freyhof. 2016. Lost fishes, who is counting? The extent of the threat to freshwater fish biodiversity. Pages 1-36 *in* G. P. Closs, M. Krkosek, and J. D. Olden, editors. Conservation of freshwater fishes. Cambridge University Press, Cambridge.
- Daufresne, M., and P. Boët. 2007. Climate change impacts on structure and diversity of fish communities in rivers. Global Change Biology **13**:2467-2478.
- De Backer, S., S. Van Onsem, and L. Triest. 2010. Influence of submerged vegetation and fish abundance on water clarity in peri-urban eutrophic ponds. Hydrobiologia **656**:255-267.
- Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**:926-929.
- Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, and B. Collen. 2014. Defaunation in the Anthropocene. Science **345**:401-406.
- Donelson, J. M., P. L. Munday, M. I. McCormick, and G. E. Nilsson. 2011. Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. Global Change Biology 17:1712-1719.
- Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163-182.
- Ebersol, J. L. 2001. Heterogeneous thermal habitat for northeast Oregon stream fishes. Thesis, Oregon State University, Corvallis, Oregon.

- Ecological Analysts. 1978. Hudson River thermal effects studies for representative species. Final Report to Central Hudson Gas & Electric Corporation, Consolidated Edison Company of New York, Inc., Orange and Rockland Utilities, Inc., Middleton, New York.
- Eliason, E. J., T. D. Clark, M. J. Hague, L. M. Hanson, Z. S. Gallagher, K. M. Jeffries, M. K. Gale, D. A. Patterson, S. G. Hinch, and A. P. Farrell. 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332:109-112.
- Ern, R., T. Norin, A. K. Gamperl, and A. J. Esbaugh. 2016. Oxygen dependence of upper thermal limits in fishes. Journal of Experimental Biology **219**:3376-3383.
- FAO. 2018. Impacts of climate change on fisheries and aquaculture: synthesis of current knowledge, adaptation and mitigation options. FAO Fisheries and Aquaculture Technical Paper 627. Food and Agriculture Organization of the United Nations, Rome.
- Feuchtmayr, H., R. Moran, K. Hatton, L. Connor, T. Heyes, B. Moss, I. Harvey, and D. Atkinson. 2009. Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental hypertrophic shallow lake mesocosms. Journal of Applied Ecology 46:713-723.
- Ficke, A. D., C. A. Myrick, and L. J. Hansen. 2007. Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries **17**:581-613.
- Finigan, P. A., N. E. Mandrak, and B. L. Tufts. 2018. Large-scale changes in the littoral fish communities of lakes in southeastern Ontario, Canada. Canadian Journal of Zoology 96:753-759.
- Fisheries and Oceans Canada. 2010. Recovery potential assessment of Pugnose Shiner (*Notropis anogenus*) in Canada. Department of Fisheries and Oceans Canadian Science Advisory Secretariat Science Advisory Report 2010/025. Fisheries and Oceans Canada, Winnipeg, Manitoba.
- Fisheries and Oceans Canada. 2012. Recovery strategy for the Pugnose Shiner (*Notropis anogenus*) in Canada (proposed). Species at Risk Act Recovery Strategy Series. Fisheries and Oceans Canada, Ottawa, Ontario.
- Fiske, I. J., and R. B. Chandler. 2011. unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. Journal of Statistical Software **43**:1-23.
- Foden, W. B., B. E. Young, H. R. Akçakaya, R. A. Garcia, A. A. Hoffmann, B. A. Stein, C. D. Thomas, C. J. Wheatley, D. Bickford, and J. A. Carr. 2019. Climate change vulnerability assessment of species. Wiley Interdisciplinary Reviews: Climate Change 10:e551.
- Forbes, S. A. 1885. Description of new Illinois fishes. Bulletin of the Illinois State Laboratory of Natural History **2**:135-139.
- Fricke, R., W. N. Eschmeyer, and R. Van der Laan. 2019. Eschmeyer's Catalog of Fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatma in.asp (25/05/2019).
- Fry, F. E. J. 1967. Responses of vertebrate poikilotherms to temperature. Pages 375-409 *in* A. H. Rose, editor. Thermobiology. Academic Press, New York, New York.
- Fugère, V., T. Mehner, and L. J. Chapman. 2018. Impacts of deforestation-induced warming on the metabolism, growth and trophic interactions of an afrotropical stream fish. Functional Ecology 32:1343-1357.
- Fullhart, H. G., B. G. Parsons, D. W. Willis, and J. R. Reed. 2002. Yellow perch piscivory and its possible role in structuring littoral zone fish communities in small Minnesota lakes. Journal of Freshwater Ecology 17:37-43.

- FWSARNet. 2018. Canadian Freshwater Species at Risk Research Network. https://fwsarnet.ca/ (08/01/2019).
- Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt. 2010. A framework for community interactions under climate change. Trends in Ecology and Evolution 25:325-331.
- Godsoe, W., J. Jankowski, R. D. Holt, and D. Gravel. 2017. Integrating biogeography with contemporary niche theory. Trends in Ecology and Evolution **32**:488-499.
- Goldspink, G. 1995. Adaptation of fish to different environmental temperature by qualitative and quantitative changes in gene expression. Journal of Thermal Biology **20**:167-174.
- Government of Canada. 2019. Species at Risk Public Registry: A to Z species index. https://wildlife-species.canada.ca/species-risk-registry/sar/index/default_e.cfm (08/08/2019).
- Grantham, R., and M. Rudd. 2015. Current status and future needs of economics research of inland fisheries. Fisheries Management and Ecology **22**:458-471.
- Gray, S. M., F. M. E. Bieber, L. H. McDonnell, L. J. Chapman, and N. E. Mandrak. 2014. Experimental evidence for species-specific response to turbidity in imperilled fishes. Aquatic Conservation: Marine and Freshwater Ecosystems 24:546-560.
- Gray, S. M., L. H. McDonnell, N. E. Mandrak, and L. J. Chapman. 2016. Species-specific effects of turbidity on the physiology of imperiled blackline shiners *Notropis* spp. in the Laurentian Great Lakes. Endangered Species Research **31**:271-277.
- Green, A. L., L. Fernandes, G. Almany, R. Abesamis, E. McLeod, P. M. Aliño, A. T. White, R. Salm, J. Tanzer, and R. L. Pressey. 2014. Designing marine reserves for fisheries management, biodiversity conservation, and climate change adaptation. Coastal Management 42:143-159.
- Grossman, G. D., and M. C. Freeman. 1987. Microhabitat use in a stream fish assemblage. Journal of Zoology **212**:151-176.
- Gräns, A., F. Jutfelt, E. Sandblom, E. Jönsson, K. Wiklander, H. Seth, C. Olsson, S. Dupont, O. Ortega-Martinez, I. Einarsdottir, B. T. Björnsson, K. Sundell, and M. Axelsson. 2014.
 Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO2 in Atlantic halibut. The Journal of Experimental Biology 217:711-717.
- Gunderson, A. R., and J. H. Stillman. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proceedings of the Royal Society B: Biological Sciences 282:20150401.
- Guppy, M., and P. Withers. 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biological Reviews 74:1-40.
- Guélat, J., M. Kéry, and N. Isaac. 2018. Effects of spatial autocorrelation and imperfect detection on species distribution models. Methods in Ecology and Evolution 9:1614-1625.
- Hawkins, B. A. 2012. Eight (and a half) deadly sins of spatial analysis. Journal of Biogeography **39**:1-9.
- Haynes, J. M., J. J. Maharan, and K. L. Barrett. 2019. Population and habitat characteristics of the Pugnose Shiner, *Notropis anogenus*, in four bays of Lake Ontario and the St. Lawrence River, New York. New York.
- Healy, T. M., and P. M. Schulte. 2012. Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). Physiological and Biochemical Zoology 85:107-119.

- Heino, J., J. Erkinaro, A. Huusko, M. Luoto, G. P. Closs, M. Krkosek, and J. D. Olden. 2016. Climate change effects on freshwater fishes, conservation and management. Pages 76-106 in G. P. Closs, M. Krkosek, and J. D. Olden, editors. Conservation of freshwater fishes. Cambridge University Press, Cambridge.
- Heino, J., R. Virkkala, and H. Toivonen. 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biological Reviews of the Cambridge Philosophical Society **84**:39-54.
- Henning, B. F., K. L. Kapuscinski, and J. M. Farrell. 2014. Nearshore fish assemblage structure and habitat relationships in protected and open habitats in the upper St. Lawrence River. Journal of Great Lakes Research **40**:154-163.
- Herbing, I. H. v. 2002. Effects of temperature on larval fish swimming performance: the importance of physics to physiology. Journal of Fish Biology **61**:865-876.
- Hermoso, V., and M. Clavero. 2011. Threatening processes and conservation management of endemic freshwater fish in the Mediterranean basin: a review. Marine and Freshwater Research **62**:244-254.
- Hickling, R., D. B. Roy, J. K. Hill, and C. D. Thomas. 2005. A northward shift of range margins in British Odonata. Global Change Biology **11**:502-506.
- Hitt, N., E. Snook, and D. Massie. 2016. Brook trout use of thermal refugia and foraging habitat influenced by brown trout. Canadian Journal of Fisheries and Aquatic Sciences 74.
- Hollowed, A. B., M. Barange, S. Ito, S. Kim, H. Loeng, and M. A. Peck. 2011. Effects of climate change on fish and fisheries: forecasting impacts, assessing ecosystem responses, and evaluating management strategies. ICES Journal of Marine Science 68:984-985.
- Holm, E., and N. E. Mandrak. 2002. Update COSEWIC status report on the Pugnose Shiner *Notropis anogenus* in Canada, *in* COSEWIC assessment and update status report on the Pugnose Shiner *Notropis anogenus* in Canada. Ottawa.
- Holm, E., N. E. Mandrak, and M. E. Burridge. 2010. The ROM field guide to freshwater fishes of Ontario. Royal Ontario Museum, Toronto, Ontario.
- Hoyle, J. A., and M. J. Yuille. 2016. Nearshore fish community assessment on Lake Ontario and the St. Lawrence River: a trap net-based index of biotic integrity. Journal of Great Lakes Research **42**:687-694.
- Hudon, C., A. Armellin, P. Gagnon, and A. Patoine. 2009. Variations in water temperatures and levels in the St. Lawrence River (Québec, Canada) and potential implications for three common fish species. Hydrobiologia 647:145-161.
- Hutchins, L. W. 1947. The bases for temperature zonation in geographical distribution. Ecological Monographs 17:325-335.
- IPBES. 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. IPBES secretariat, Bonn, Germany.
- IPCC. 2013. Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA.
- IPCC. 2014. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland.
- IUCN. 2019. The IUCN Red List of Threatened Species. http://www.iucnredlist.org (24/05/2019).

- Jackson, D. A., and N. E. Mandrak. 2002. Changing fish biodiversity: predicting the loss of cyprind biodiversity due to global climate change. Pages 89-98 in American Fisheries Society Symposium 32. American Fisheries Society, Bethesda, Maryland.
- Jacobsen, J. B., J. H. Boiesen, B. J. Thorsen, and N. Strange. 2008. What's in a name? The use of quantitative measures versus 'Iconised'species when valuing biodiversity. Environmental and Resource Economics **39**:247-263.
- Janetski, D. J., and C. R. Ruetz III. 2015. Spatiotemporal patterns of fish community composition in Great Lakes drowned river mouths. Ecology of Freshwater Fish **24**:493-504.
- Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. Mandrak, F. McCormick, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. J. Warren. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372– 407.
- Jensen, O. P., T. R. Hrabik, S. J. Martell, C. J. Walters, and J. F. Kitchell. 2006. Diel vertical migration in the Lake Superior pelagic community. II. Modeling trade-offs at an intermediate trophic level. Canadian Journal of Fisheries and Aquatic Sciences **63**:2296-2307.
- Jeppesen, E., M. Meerhoff, K. Holmgren, I. González-Bergonzoni, F. Teixeira-de Mello, S. A. Declerck, L. De Meester, M. Søndergaard, T. L. Lauridsen, and R. Bjerring. 2010. Impacts of climate warming on lake fish community structure and potential effects on ecosystem function. Hydrobiologia 646:73-90.
- Keast, A., J. Harker, and D. Turnbull. 1978. Nearshore fish habitat utilization and species associations in Lake Opinicon (Ontario, Canada). Environmental Biology of Fishes 3:173-184.
- Keen, A. N., J. M. Klaiman, H. A. Shiels, and T. E. Gillis. 2017. Temperature-induced cardiac remodelling in fish. Journal of Experimental Biology **220**:147-160.
- Kelly, M. W., E. Sanford, and R. K. Grosberg. 2011. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proceedings of the Royal Society B: Biological Sciences 279:349-356.
- Killen, S.S., C. Atkinson, D. S. and Glazier. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology Letters 13:184-193
- Killgore, K., J. Hoover, and R. Morgan II. 1991. Habitat value of aquatic plants for fishes. Washington, D.C.
- Kimmel, W. G., and D. G. Argent. 2010. Stream fish community responses to a gradient of specific conductance. Water, Air, and Soil Pollution **206**:49-56.
- King, G. D. 2014. Nutritional condition and stress response of fishes along a gradient of habitat quality in the St. Lawrence River: physiological consequences of anthropogenic habitat degradation. Thesis, University of Illinois at Urbana-Champaign, Urbana, Illinois.
- Kinne, O. 1962. Irreversible nongenetic adaptation. Comparative Biochemistry and Physiology 5:265-282.
- Klaiman, J. M., A. J. Fenna, H. A. Shiels, J. Macri, and T. E. Gillis. 2011. Cardiac remodeling in fish: strategies to maintain heart function during temperature change. PLOS One 6:e24464.

- Knight, R. L., F. J. Margraf, and R. F. Carline. 1984. Piscivory by walleyes and yellow perch in western Lake Erie. Transactions of the American Fisheries Society **113**:677-693.
- Komoroske, L. M., R. E. Connon, J. Lindberg, B. S. Cheng, G. Castillo, M. Hasenbein, and N. A. Fangue. 2014. Ontogeny influences sensitivity to climate change stressors in an endangered fish. Conservation Physiology 2:cou008.
- Kordas, R. L., C. D. G. Harley, and M. I. O'Connor. 2011. Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. Journal of Experimental Marine Biology and Ecology 400:218-226.
- Lamothe, K. A., A. J. Dextrase, and D. A. R. Drake. 2019. Characterizing species co-occurrence patterns of imperfectly detected stream fishes to inform species reintroduction efforts. Conservation Biology **33**:1 392-1403.
- Lamothe, K. A., and D. A. R. Drake. 2019. Moving repatriation efforts forward for imperilled Canadian freshwater fishes. Canadian Journal of Fisheries and Aquatic Sciences **76**:1914-1921.
- Laughlin, D. R., and E. E. Werner. 1980. Resource partitioning in two coexisting sunfish: Pumpkinseed (*Lepomis gibbosus*) and Northern Longear Sunfish (*Lepomis megalotis peltastes*). Canadian Journal of Fisheries and Aquatic Sciences **37**:1411-1420.
- Lawler, J. J. 2009. Climate change adaptation strategies for resource management and conservation planning. Annals of the New York Academy of Sciences **1162**:79-98.
- Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology **20**:201-219.
- Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology 74:1659-1673.
- Lehner, B., and P. Döll. 2004. Development and validation of a global database of lakes, reservoirs and wetlands. Journal of Hydrology **296**:1-22.
- Leslie, J. K., and C. A. Timmins. 2002. Description of age 0 juvenile pugnose minnow *Opsopoeodus emiliae* (Hay) and pugnose shiner *Notropis anogenus* (Forbes) in Ontario. Burlington, Ontario.
- Lutterschmidt, W. I., and V. H. Hutchinson. 1997. The crictical thermal maximum: history and critique. Canadian Journal of Zoology **75**:1567-1574.
- Lyons, J. 1989. Changes in the abundance of small littoral-zone fishes in Lake Mendota, Wisconsin. Canadian Journal of Zoology **67**:2910-2916.
- MacKenzie, D., and J. Hines. 2017. RPresence. R package version 2.12.34.
- MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248-2255.
- McBryan, T. L., K. Anttila, T. M. Healy, and P. M. Schulte. 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. Integrative and Comparative Biology **53**:648-659.
- McBryan, T. L., T. M. Healy, K. L. Haakons, and P. M. Schulte. 2016. Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. Journal of Experimental Biology 219:474-484.
- McCallum, M. L. 2015. Vertebrate biodiversity losses point to a sixth mass extinction. Biodiversity and Conservation **24**:2497-2519.
- McClanahan, T., J. Cinner, J. Maina, N. Graham, T. Daw, S. Stead, A. Wamukota, K. Brown, M. Ateweberhan, and V. Venus. 2008. Conservation action in a changing climate. Conservation Letters 1:53-59.

- McCormick, M. I., and B. W. Molony. 1995. Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. Marine Ecology Progress Series **118**:59-68.
- McCune, J. L., W. L. Harrower, S. Avery-Gomm, J. M. Brogan, A.-M. Csergő, L. N. Davidson, A. Garani, L. R. Halpin, L. P. Lipsen, and C. Lee. 2013. Threats to Canadian species at risk: an analysis of finalized recovery strategies. Biological Conservation 166:254-265.
- McCusker, M. R. 2016. Species distribution model of the Pugnose Shiner (*Notropis anogenus*) in Long Point Bay, with evaluation of climate change and *Phragmites australis* impact scenarios. Department of Fisheries and Oceans, Burlington, Ontario.
- McCusker, M. R., J. M. R. Curtis, N. R. Lovejoy, and N. E. Mandrak. 2017. Exploring uncertainty in population viability analysis and its implications for the conservation of a freshwater fish. Aquatic Conservation: Marine and Freshwater Ecosystems **27**:780-788.
- McCusker, M. R., N. E. Mandrak, S. Doka, E. L. Gertzen, J. F. v. Wieren, J. E. McKenna, D. M. Carlson, and N. R. Lovejoy. 2014a. Estimating the distribution of the imperiled pugnose shiner (*Notropis anogenus*) in the St. Lawrence River using a habitat model. Journal of Great Lakes Research 40:980-988.
- McCusker, M. R., N. E. Mandrak, B. Egeh, and N. R. Lovejoy. 2014b. Population structure and conservation genetic assessment of the endangered Pugnose Shiner, *Notropis anogenus*. Conservation Genetics 15:343-353.
- McDonnell, L. H., and L. J. Chapman. 2015. At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. Conservation Physiology **3**:cov050.
- McDonnell, L. H., and L. J. Chapman. 2016. Effects of thermal increase on aerobic capacity and swim performance in a tropical inland fish. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology **199**:62-70.
- McGinn, N. A. 2002. Fisheries in a changing climate. American Fisheries Society Symposium 32. American Fisheries Society, Bethesda, Maryland.
- McKenna, J. E., and A. David. 2017. Evaluation of threatened, endangered, and rare fish and communities of the Saint Lawrence River and its tributaries.
- McManamay, R. A., N. A. Griffiths, C. R. DeRolph, and B. M. Pracheil. 2018. A synopsis of global mapping of freshwater habitats and biodiversity: implications for conservation. Pages 59-87 *in* L. Hufnagel, editor. Pure and applied biogeography. InTech Open.
- McNab, B. K. 2002. The physiological ecology of vertebrates: a view from energetics. Cornell University Press, Ithaca, New York.
- Metcalfe, N. B., A. C. Taylor, and J. E. Thorpe. 1995. Metabolic rate, social status and lifehistory strategies in Atlantic salmon. Animal Behaviour **49**:431-436.
- Morecroft, M. D., H. Q. P. Crick, S. J. Duffield, and N. A. Macgregor. 2012. Resilience to climate change: translating principles into practice. Journal of Applied Ecology **49**:547-551.
- Moss, B. 2011. Allied attack: climate change and eutrophication. Inland Waters 1:101-105.
- Munday, P., M. Kingsford, M. O'callaghan, and J. Donelson. 2008. Elevated temperature restricts growth potential of the coral reef fish *Acanthochromis polyacanthus*. Coral Reefs 27:927-931.
- Munoz, N. J., K. Anttila, Z. Chen, J. W. Heath, A. P. Farrell, and B. D. Neff. 2014. Indirect genetic effects underlie oxygen-limited thermal tolerance within a coastal population of chinook salmon. Proceedings of the Royal Society B: Biological Sciences 281:20141082.

- Myers, B. J. E., A. J. Lynch, D. B. Bunnell, C. Chu, J. A. Falke, R. P. Kovach, T. J. Krabbenhoft, T. J. Kwak, and C. P. Paukert. 2017. Global synthesis of the documented and projected effects of climate change on inland fishes. Reviews in Fish Biology and Fisheries 27:339-361.
- NatureServe. 2019. Notropis anogenus. NatureServe Explorer: An online encyclopedia of life. NatureServe, Arlington, Virginia. http://explorer.natureserve.org/ (08/04/2019)
- Nielsen, J. L., T. E. Lisle, and V. Ozaki. 1994. Thermally stratified pools and their use by steelhead in northern California streams. Transactions of the American Fisheries Society **123**:613-626.
- Nilsson, G. E. 2007. Gill remodeling in fish a new fashion or an ancient secret? Journal of Experimental Biology **210**:2403-2409.
- Nilsson, G. E., A. Dymowska, and J. A. W. Stecyk. 2012. New insights into the plasticity of gill structure. Respiratory Physiology and Neurobiology **184**:214-222.
- Nyboer, E. A., and L. J. Chapman. 2018. Cardiac plasticity influences aerobic performance and thermal tolerance in a tropical, freshwater fish at elevated temperatures. Journal of Experimental Biology **221**:jeb178087.
- O'Reilly, C. M., S. Sharma, D. K. Gray, S. E. Hampton, J. S. Read, R. J. Rowley, P. Schneider, J. D. Lenters, P. B. McIntyre, B. M. Kraemer, G. A. Weyhenmeyer, D. Straile, B. Dong, R. Adrian, M. G. Allan, O. Anneville, L. Arvola, J. Austin, J. L. Bailey, J. S. Baron, J. D. Brookes, E. de Eyto, M. T. Dokulil, D. P. Hamilton, K. Havens, A. L. Hetherington, S. N. Higgins, S. Hook, L. R. Izmest'eva, K. D. Joehnk, K. Kangur, P. Kasprzak, M. Kumagai, E. Kuusisto, G. Leshkevich, D. M. Livingstone, S. MacIntyre, L. May, J. M. Melack, D. C. Mueller-Navarra, M. Naumenko, P. Noges, T. Noges, R. P. North, P.-D. Plisnier, A. Rigosi, A. Rimmer, M. Rogora, L. G. Rudstam, J. A. Rusak, N. Salmaso, N. R. Samal, D. E. Schindler, S. G. Schladow, M. Schmid, S. R. Schmidt, E. Silow, M. E. Soylu, K. Teubner, P. Verburg, A. Voutilainen, A. Watkinson, C. E. Williamson, and G. Zhang. 2015. Rapid and highly variable warming of lake surface waters around the globe. Geophysical Research Letters 42:10,773-710,781.
- Oksanen, J. F., G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R.
 B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, and H. Wagner.
 2019. vegan: community ecology package. R package version 2.5-4.
- Ormerod, S. J., M. Dobson, A. G. Hildrew, and C. R. Townsend. 2010. Multiple stressors in freshwater ecosystems. Freshwater Biology **55**:1-4.
- Ospina, A. F. and C. Mora. 2004. Effect of body size on reef fish tolerance to extreme low and high temperatures. Environmental Biology of Fishes **70**:339-343.
- Pacifici, M., W. B. Foden, P. Visconti, J. E. Watson, S. H. Butchart, K. M. Kovacs, B. R. Scheffers, D. G. Hole, T. G. Martin, and H. R. Akcakaya. 2015. Assessing species vulnerability to climate change. Nature Climate Change 5:215.
- Page, L. M., and B. M. Burr. 2011. Peterson field guide to freshwater fishes of North America north of Mexico. Houghton Mifflin Harcourt Publishing Company, New York, New York.
- Palzenberger, M., and H. Pohla. 1992. Gill surface area of water-breathing freshwater fish. Reviews in Fish Biology and Fisheries **2**:187-216.
- Pankhurst, N. 1997. Temperature effects on the reproductive performance of fish. Page 159 *in* C.M. Wood and D. G. MacDonald, editors. Global warming: implications for freshwater and marine fish. Cambridge University Press, Cambridge.

- Parmesan, C., T. L. Root, and M. R. Willig. 2000. Impacts of extreme weather and climate on terrestrial biota. Bulletin of the American Meteorological Society **81**:443-450.
- Paszkowski, C. A., and W. M. Tonn. 1994. Effects of prey size, abundance, and population structure on piscivory by yellow perch. Transactions of the American Fisheries Society 123:855-865.
- Peterson, A. T., J. Soberón, R. G. Pearson, R. P. Anderson, E. Martínez-Meyer, M. Nakamura, and M. B. Araújo. 2011. Ecological niches and geographic distributions (MPB-49). Princeton University Press, Princeton, New Jersey.
- Poloczanska, E. S., C. J. Brown, W. J. Sydeman, W. Kiessling, D. S. Schoeman, P. J. Moore, K. Brander, J. F. Bruno, L. B. Buckley, M. T. Burrows, C. M. Duarte, B. S. Halpern, J. Holding, C. V. Kappel, M. I. O'Connor, J. M. Pandolfi, C. Parmesan, F. Schwing, S. A. Thompson, and A. J. Richardson. 2013. Global imprint of climate change on marine life. Nature Climate Change 3:919-925.
- Poloczanska, E. S., M. T. Burrows, C. J. Brown, J. García Molinos, B. S. Halpern, O. Hoegh-Guldberg, C. V. Kappel, P. J. Moore, A. J. Richardson, D. S. Schoeman, and W. J. Sydeman. 2016. Responses of marine organisms to climate change across oceans. Frontiers in Marine Science 3:62.
- Pörtner, H.-O., and A. P. Farrell. 2008. Physiology and climate change. Science 322:690-692.
- Pörtner, H.-O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften **88**:137-146.
- Pörtner, H.-O. 2010. Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213:881-893.
- Pörtner, H.-O., B. Berdal, R. Blust, O. Brix, A. Colosimo, B. De Wachter, A. Giuliani, T. Johansen, T. Fischer, and R. Knust. 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). Continental Shelf Research 21:1975-1997.
- Pörtner, H.-O., C. Bock, and F. C. Mark. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. Journal of Experimental Biology **220**:2685-2696.
- R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rahel, F. J., B. Bierwagen, and Y. Taniguchi. 2008. Managing aquatic species of conservation concern in the face of climate change and invasive species. Conservation Biology 22:551-561.
- Rahel, F. J., and J. D. Olden. 2008. Assessing the effects of climate change on aquatic invasive species. Conservation Biology **22**:521-533.
- Rainer, R. B., B.; Blaney, S.; Enns, A.; Henry, P.; Lofroth, E.; Mackenzie, J. 2017. On guard for them: species of global conservation concern in Canada. Ottawa, ON.
- Rao, Y. R., T. Howell, S. B. Watson, and S. Abernethy. 2014. On hypoxia and fish kills along the north shore of Lake Erie. Journal of Great Lakes Research **40**:187-191.
- Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. J. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden, S. J. Ormerod, J. P. Smol, W. W. Taylor, K. Tockner, J. C. Vermaire, D. Dudgeon, and S. J. Cooke. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94:849-873.

- Robbins, B., J. R. Foster, B. C. Lehman, J. M. Ratchford, and M. W. Soukup. 2018. Pond culture of the endangered Pugnose Shiner (*Notropis anogenus*). Student Research Showcase 2018. SUNY Cobleskill, Cobleskill, New York.
- Rowe, D., and B. Chisnall. 1995. Effects of oxygen, temperature and light gradients on the vertical distribution of rainbow trout, *Oncorhynchus mykiss*, in two North Island, New Zealand, lakes differing in trophic status. New Zealand Journal of Marine and Freshwater Research 29:421-434.
- Rozas, L. P., and W. E. Odum. 1988. Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. Oecologia 77:101-106.
- Rudd, M. A., S. Andres, and M. Kilfoil. 2016. Non-use economic values for little-known aquatic species at risk: comparing choice experiment results from surveys focused on species, guilds, and ecosystems. Environmental Management 58:476-490.
- Ruiz-Navarro, A., P. K. Gillingham, and J. R. Britton. 2016. Predicting shifts in the climate space of freshwater fishes in Great Britain due to climate change. Biological Conservation 203:33-42.
- Rutjes, H. A. 2006. Phenotypic responses to lifelong hypoxia in cichlids. Leiden University, Leiden, Netherlands.
- Sandblom, E., A. Gräns, M. Axelsson, and H. Seth. 2014. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. Proceedings of the Royal Society B: Biological Sciences 281:20141490.
- Sandblom, E., T. D. Clark, A. Gräns, A. Ekstrom, J. Brijs, L. F. Sundstrom, A. Odelstrom, A. Adill, T. Aho, and F. Jutfelt. 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nature Communications 7:11447.
- Savino, J. F., and R. A. Stein. 1989a. Behavior of fish predators and their prey: habitat choice between open water and dense vegetation. Environmental Biology of Fishes **24**:287-293.
- Savino, J. F., and R. A. Stein. 1989b. Behavioural interactions between fish predators and their prey: effects of plant density. Animal Behaviour **37**:311-321.
- Schaefer, J., and A. Ryan. 2006. Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. Journal of Fish Biology **69**:722-734.
- Schmidt, K. 2018. In search of Minnesota's missing fishes. American Currents 43:15-31.
- Schneider, J. C. 2002. Fish as indicators of lake habitat quality and proposed applications.
- Michigan Department of Natural Resources, Fisheries Division, Ann Arbor, Michigan. Schriver, P., J. Bøgestrand, E. Jeppesen, and M. Søndergaard. 1995. Impact of submerged
- macrophytes on fish-zooplanl phytoplankton interactions: large-scale enclosure experiments in a shallow eutrophic lake. Freshwater Biology **33**:255-270.
- Schulte, P. M., T. M. Healy, and N. A. Fangue. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integrative and Comparative Biology **51**:691-702.
- Seebacher, F., C. R. White, and C. E. Franklin. 2014. Physiological plasticity increases resilience of ectothermic animals to climate change. Nature Climate Change 5:61-66.
- Smale, M. A., and C. F. Rabeni. 1995. Hypoxia and hyperthermia tolerances of headwater stream fishes. Transactions of the American Fisheries Society **124**:698-710.
- Sollid, J., and G. E. Nilsson. 2006. Plasticity of respiratory structures adaptive remodeling of fish gills induced by ambient oxygen and temperature. Respiratory Physiology & Neurobiology **154**:241-251.

- Sollid, J., R. E. Weber, and G. E. Nilsson. 2005. Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. Journal of Experimental Biology **208**:1109-1116.
- Somero, G. N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. The Journal of Experimental Biology **213**:912-920.
- Sorte, C. J., S. J. Jones, and L. P. Miller. 2011. Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. Journal of Experimental Marine Biology and Ecology **400**:209-217.
- Sorte, C. J. B., S. L. Williams, and J. T. Carlton. 2010. Marine range shifts and species introductions: comparative spread rates and community impacts. Global Ecology and Biogeography **19**:303-316.
- Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2012. Thermal tolerance and the global redistribution of animals. Nature Climate Change **2**:686-690.
- Sunday, J. M., A. E. Bates, M. R. Kearney, R. K. Colwell, N. K. Dulvy, J. T. Longino, and R. B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences of the United States of America 111:5610-5615.
- Sutton, R., and T. Soto. 2012. Juvenile coho salmon behavioural characteristics in Klamath river summer thermal refugia. River Research and Applications **28**:338-346.
- Sutton, R. J., M. L. Deas, S. K. Tanaka, T. Soto, and R. A. Corum. 2007. Salmonid observations at a Klamath River thermal refuge under various hydrological and meteorological conditions. River Research and Applications **23**:775-785.
- Thibaud, E., B. Petitpierre, O. Broennimann, A. C. Davison, and A. Guisan. 2014. Measuring the relative effect of factors affecting species distribution model predictions. Methods in Ecology and Evolution **5**:947-955.
- Thieme, M. L., E. Turak, P. McIntyre, W. Darwall, K. Tockner, J. Cordeiro, and S. H. M. Burchart. 2010. Freshwater ecosystems under threat: the ultimate hotspot. Pages 123-143 *in* R. A. Mittermeier, T. Farrell, I. J. Harrison, A. J. Upgren, and T. Brooks, editors. Fresh water: the essence of life. CEMEX and iLCP, Boulder, Colorado.
- Thompson, L. C., N. A. Fangue, J. J. C. Jr., D. E. Cocherell, and R. C. Kaufman. 2012. Juvenile and adult hardhead thermal tolerances and preferences: Temperature preference, critical thermal limits, active and resting metabolism, and blood-oxygen equilibria. University of California, Davis.
- Tonn, W. M. 1990. Climate change and fish communities: a conceptual framework. Transactions of the American Fisheries Society **119**:337-352.
- Tzaneva, V., S. Bailey, and S. F. Perry. 2011. The interactive effects of hypoxemia, hyperoxia, and temperature on the gill morphology of goldfish (*Carassius auratus*). American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 300:R1344-R1351.
- Van den Berg, M. S., H. Coops, M.-L. Meijer, M. Scheffer, and J. Simons. 1998. Clear water associated with a dense Chara vegetation in the shallow and turbid Lake Veluwemeer, The Netherlands. Pages 339-352 in E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen, editors. The structuring role of submerged macrophytes in lakes. Springer New York, New York, NY.

- Vorosmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467:555-561.
- Vörösmarty, C. J., P. Green, J. Salisbury, and R. B. Lammers. 2000. Global water resources: vulnerability from climate change and population growth. Science **289**:284-288.
- Walsh, S. J., D. C. Haney, C. M. Timmerman, and R. M. Dorazio. 1998. Physiological tolerances of juvenile robust redhorse, *Moxostoma robustum*: conservation implications for an imperiled species. Environmental Biology of Fishes 51:429-444.
- Wang, Y. S., Z.-D. Cao, and S.-J. Fu. 2008. Thermal tolerance of juvenile *Silurus meridionalis*. Chinese Journal of Ecology **27**:2136-2140.
- Watson, S. B., C. Miller, G. Arhonditsis, G. L. Boyer, W. Carmichael, M. N. Charlton, R. Confesor, D. C. Depew, T. O. Höök, S. A. Ludsin, G. Matisoff, S. P. McElmurry, M. W. Murray, R. Peter Richards, Y. R. Rao, M. M. Steffen, and S. W. Wilhelm. 2016. The re-eutrophication of Lake Erie: harmful algal blooms and hypoxia. Harmful Algae 56:44-66.
- Wenger, S. J., D. J. Isaak, C. H. Luce, H. M. Neville, K. D. Fausch, J. B. Dunham, D. C. Dauwalter, M. K. Young, M. M. Elsner, B. E. Rieman, A. F. Hamlet, and J. E. Williams. 2011. Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. Proceedings of the National Academy of Science (PNAS) 108:14175-14180.
- White, S. L., B. C. Kline, N. P. Hitt, and T. Wagner. 2019. Individual behaviour and resource use of thermally stressed brook trout *Salvelinus fontinalis* portend the conservation potential of thermal refugia. Journal of Fish Biology **95**:1061-1071.
- Wichert, G. A., and P. Lin. 1996. A species tolerance index for maximum water temperature. Water Quality Research Journal **31**:875-893.
- Windisch, H. S., S. Frickenhaus, U. John, R. Knust, H.-O. Pörtner, and M. Lucassen. 2014. Stress response or beneficial temperature acclimation: transcriptomic signatures in Antarctic fish (*Pachycara brachycephalum*). Molecular Ecology 23:3469-3482.
- Woodward, G., D. M. Perkins, and L. E. Brown. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B: Biological Sciences 365:2093-2106.
- Wu, C.-B., Z.-Y. Liu, F.-G. Li, J. Chen, X.-Y. Jiang, and S.-M. Zou. 2017. Gill remodeling in response to hypoxia and temperature occurs in the hypoxia sensitive blunt snout bream (*Megalobrama amblycephala*). Aquaculture 479:479-486.
- WWF. 2014. Living Planet Report: species and spaces, people and places. Gland, Switzerland.
- WWF. 2016. Living Planet Report 2016: risk and resilience in a new era. Gland, Switzerland.
- Xenopoulos, M. A., D. M. Lodge, J. Alcamo, M. Märker, K. Schulze, and D. P. Van Vuuren. 2005. Scenarios of freshwater fish extinctions from climate change and water withdrawal. Global change biology 11:1557-1564.
- Yamamoto, T., H. Ueda, and S. Higashi. 1998. Correlation among dominance status, metabolic rate and otolith size in masu salmon. Journal of Fish Biology **52**:281-290.
- Ziegeweid, J. R., C. A. Jennings, and D. L. Peterson. 2007. Thermal maxima for juvenile shortnose sturgeon acclimated to different temperatures. Environmental Biology of Fishes **82**:299-307.