The effect of temperature on sea lamprey (*Petromyzon marinus*): ecological and cellular implications

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Abstract

Understanding how species respond to thermal challenges is important for forecasting future species distributions, and to provide information for population management. Sea lamprey (*Petromyzon marinus*), a jawless representative of a basal branch of the vertebrates, successfully invaded the Laurentian Great Lakes in the 20th century to the detriment of the local fishery, resulting in an intensive population control effort since the late 1950s. Sea lamprey are therefore an excellent study species not only for the practical implications of population management, but also as an outgroup to the vertebrates and for testing predictions of invasion theory. Here, the critical thermal maximum (CTMax) of a native population of sea lamprey larvae from New Brunwick was determined in individuals acclimated to 5 °C, 13.5 °C, and 20 °C, and gene expression profiles were determined via reverse-transcription quantitative polymerase chain reaction (qPCR) for genes involved in the cellular stress response (CSR) in response to an acute thermal shock. Subsequently, similar experiments were performed on an invasive landlocked population of sea lamprey larvae acclimated to similar temperatures, and a population comparison was performed. The CTMax results demonstrated that sea lamprey possess a relatively low acclimation capacity, as their CTMax increased only 0.12 °C for each 1 °C increase in acclimation temperature in both populations; however, the landlocked population had a consistently higher CTMax temperature. Sublethal thresholds appear to exist at ~24 °C – 26 °C and ~30 °C in the New Brunswick population, where the CSR is initiated and then shifts to an extreme response, respectively. Invasion theory predicts increased plasticity in invasive populations, and evidence for this was found in the transcription profiles of heat shock proteins in the landlocked population relative to the New Brunswick population. Temperatures above 30 °C are already being recorded in historical sea lamprey spawning streams in the southern end of

their native distribution, suggesting that a northerly range shift may occur. Despite their greater upper thermal tolerance, this suggests the landlocked population may be more susceptible for future warming, as they already exploit their entire range in the Great Lakes Basin.

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Dedication

This thesis is dedicated to my Mother and Father.

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ARR	Acclimation response ratio
cDNA	Complimentary DNA
CNRQ	Calibrated normalized relative quantity
CSR	Cellular stress response
Ct	Threshold cycle
CTMax	Critical thermal maximum
CV	Coefficient of variation
GLFC	Great Lakes Fisheries Commission
mRNA	Messenger RNA
NF	Normalization factor
NRQ	Normalized relative quantity
RQ	Relative quantity

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Chapter 1: General Introduction

Temperature is a primary environmental parameter influencing the ecology and physiology of ectotherms (Fry 1971; Hochachka and Somero 1971; Brett 1971). Aquatic ectotherms are particularly affected as their body temperature closely follows that of the surrounding water (Stevens and Sutterlin 1976). As a result of climate change, average global temperatures are projected to continue to increase (IPCC 2014). Species will therefore be faced with modified thermal conditions in their current habitat and will have to respond accordingly. Depending on species' capacity to respond to their new thermal regime, they may be forced to shift their distribution towards new, more favourable habitats, or if their ability to disperse is limited, they will need to adapt their thermal tolerance (Perry et al. 2005; Parmesan 2006; Daufresne and Boët 2007; Cheung et al. 2009; Somero 2010; Hoegh-Guldberg and Bruno 2010; Fuller et al. 2010; Doney et al. 2012). If species are unable to adequately respond to these changing conditions, they could be faced with extirpation. Understanding how species respond to thermal challenges will be a vital part of predicting species-specific impacts of future climate change (Perry et al. 2005; Parmesan 2006; Cheung et al. 2009; Somero 2010; Doney et al. 2012; Seebacher et al. 2015).

1.1 Upper Thermal Limits

Upper thermal tolerance estimates have generally been produced by one of two experimental methods; the incipient lethal temperature, or the critical thermal maximum (CTMax). These approaches provide a common methodology, allowing for comparative investigations in acute upper thermal limits within habitats and between related species or

populations, permitting insight into adaptive differences (Somero 2010; Comte and Olden 2017). The incipient lethal temperature provides an estimate of the temperature at which 50 % of a population dies within a defined time frame (e.g., two weeks; Potter and Beamish 1975), similar to toxicological estimates of lethal concentrations. This is a static measurement where fish are acclimated at some ecologically-relevant temperature, then introduced as a group to one of several aquaria at a greater, fixed temperature, and observed over time. Each aquarium provides a single data point of mortality rate, and the temperature for 50 % mortality is estimated via a regression (Potter and Beamish 1975; Beitinger et al. 2000). The CTMax is a dynamic measurement where fish are placed in a container at their acclimation temperature and exposed to a constant temperature increase (often 0.3 °C min^{-1}) until a repeatable, near-lethal event is observed, at which point fish are rescued, returned to their acclimation temperature, and monitored for survival (Beitinger et al. 2000; Morgan et al. 2018). The endpoint chosen depends on the biology of the species being investigated; for teleosts and crustaceans, a common endpoint is loss of equilibrium (Beitinger et al. 2000; Vinagre et al. 2015), while for snails, detachment temperature can be used (Johansson and Laurila 2017). Due to the constant rate of temperature change, this method can overshoot temperatures which would be lethal under natural conditions, but it does provide a useful estimate of acutely lethal upper thermal limits (Beitinger et al. 2000).

Examining the capacity for species to adjust their CTMax through acclimation to different temperatures provides an opportunity to quantify species' acclimation capacity (Stillman 2003). A review of thermal tolerance data of freshwater fishes from North America found that the mean (±SD) acclimation response ratio (ARR) was 0.41 (±0.07) with a range

$$Equation\ 1.1: ARR = \frac{\Delta CTMax}{\Delta Acclimation\ temperature}$$

of 0.27-0.50 across 20 species representing nine families (Beitinger et al. 2000). A more recent

review of thermal tolerance data for 82 freshwater fish species, from 27 families, found a mean ARR of 0.37 (±0.16), with a total range of 0.07-0.91 (Comte and Olden 2017). Other investigations in a number of temperate fishes have produced ARRs similar to the means reported in these reviews, including mummichog (*Fundulus heteroclitus*: 0.36-0.41), longjaw mudsucker (*Gillichthys mirabilis*: 0.28), common killifish (*Fundulus heteroclitus*: 0.3-0.4), Chinese sucker (*Myxocyprinus asiaticus*: 0.50), rock carp (*Procypris rabaudi*: 0.50), and lake sturgeon (*Acipenser fulvescens*: 0.18-0.31) (Fangue et al. 2006; Logan and Somero 2010; Healy and Schulte 2012; Zhou et al. 2019; William Bugg, pers. comm.).

Traditionally, tropical species have been hypothesized to possess a reduced capacity to compensate for increased acclimation temperature because they inhabit relatively thermally stable environments, and the potential for a trade-off where acclimation capacity is reduced in favour of a high upper thermal tolerance (Beitinger et al. 2000; Stillman 2003; Carveth et al. 2006; Gunderson and Stillman 2015). This is true for some fish species; for example spotted barb (*Barbodes binotatus*) and greater scissortail (*Rasbora caudimaculata*), freshwater fish native to Thailand, have ARR values of 0.05 and 0.09, respectively (Tongnunui and Beamish 2017), while common triplefin (*Forsterygion lapillum*) and the guppy (*Poecilia reticulata*) have ARRs of ~0.17, and 0.13-0.15, respectively (Chung 2001; McArley et al. 2017). There have, however, been several recent studies performed on tropical fish which puts this assumption into question. In the same study on Thai fish, two additional species tested, a danio (*Devario acrostomus*) and *Mystacoleucus chilopterus*, demonstrated ARRs of 0.26 and 0.21, respectively (Tongnunui and Beamish 2017). Six tropical freshwater fish species examined across three continents demonstrated ARR values between 0.33 and 0.43 (Lapointe et al. 2018), while the Nile perch (*Lates niloticus*), Egyptian mouthbreeder (*Pseudocrenilabrus multicolor*), and the green neon

tetra (*Paracheirodon simulans*) have ARRs of 0.30, 0.43, and 0.50, respectively (McDonnell and Chapman 2015; Chrétien and Chapman 2016; Campos et al. 2017). Similarly, Antarctic fishes, which inhabit one of the most thermally stable environments on Earth, are hypothesized to have reduced acclimation capabilities for similar reasons as tropical species, but experimental data for eight Antarctic notothenoid fish species revealed ARRs between 0.21 and 0.55 (Bilyk and DeVries 2011). These values align neatly with those previously described for temperate species (Beitinger et al. 2000; Comte and Olden 2017), indicating that environmental variability may not dictate acclimation capacity.

1.2 The Cellular Stress Response

The cellular stress response (CSR) is a highly conserved defensive response to any stressor that has the potential to inflict macromolecular damage (e.g. heat, cold, ultraviolet radiation, acidic or alkaline conditions, salinity, pressure), present in nearly all extant cellular life (Kültz 2005). The magnitude of the response is proportional to the severity of the stressor, resulting in a tiered response involving stress inducible transcription factors (e.g. immediate early genes), molecular chaperones, proteolytic enzymes, interruptions in the cell cycle, or in severe cases, induction of apoptosis (Lindquist and Craig 1988; Morimoto 1998; Kültz 2005; Logan and Somero 2011; Jeffries et al. 2018). Generally, the tiers of the CSR proceed as follows: 1) in response to a mild stressor, some upregulation of immediate early genes and molecular chaperones (e.g. heat shock proteins [HSPs]) would be expected, in order to repair any minor damage and mitigate further damage to macromolecular structure; 2) in response to a more severe stressor, the immediate early genes and molecular chaperones would be upregulated, while proteases and cell cycle regulators will begin to be upregulated as well. If proteins are

denatured to a degree where they cannot be simply repaired, they will need to be sequestered and broken down, while damage to DNA molecules would require the arrest of the cell cycle to prevent the potential inheritance of harmful mutations in any daughter cells; 3) finally, in cases of extreme stress, cells may employ a 'survival' strategy, where all of the previously mentioned processes will be upregulated in the short term, but if the stressor persists those may be abandoned, and programmed cell death (i.e. apoptosis) may occur.

Transcriptomics and reverse-transcription quantitative polymerase chain reaction (qPCR) approaches allow for investigations into the changes in specific gene transcript (i.e., messenger RNA [mRNA]) abundance in tissues following stress events, allowing researchers to directly detect which genes have altered expression under experimental conditions in both model and non-model species (Råbergh et al. 2000; Vornanen et al. 2005; Logan and Somero 2011; Long et al. 2012; Jeffries et al. 2012, 2014, 2016, 2018; Logan and Buckley 2015; Komoroske et al. 2015; Li et al. 2017). These investigations have revealed several genes which appear to consistently respond to thermal stress in a wide range of species, including the immediate early genes (*JUN, FOS*), molecular chaperones (*HSP*s), genes involved in proteolysis (*UBQ*s), cell cycle regulators (*CDK*s), and genes involved in apoptotic pathways (*CASP*s). A broad thermal tolerance is the result of regulating the CSR in both long-term and acute exposure to a thermal stressor. Eurytherms can mount a strong response, as measured by the magnitude of change in expression of genes related to the CSR, while stenotherms have a reduced capability to differentially regulate their gene expression (Logan and Buckley 2015).

In this thesis, expression patterns of seven genes involved in the CSR were evaluated: three transcription factors, three heat shock proteins (HSPs), and a proteolytic enzyme. *HSF1* is an important transcription factor regulating expression of HSPs and other elements of the heat

shock response, and has recently been demonstrated to itself be inducible in response to heat shock in a sea cucumber (Morimoto 1998; Xu et al. 2016). *JUN* is a heat-inducible gene with a heat shock element in its promoter region, and therefore it has been demonstrated to be activated by HSF1 in human cells (Sawai et al. 2013). Among the many downstream pathways it influences, JUN has been shown to regulate progression of the cell cycle at the G1 to S transition through negative regulation of p53, and to prevent apoptosis (Wisdom 1999; Schreiber et al. 1999). *EPAS1B* (hypoxia inducible factor 2) is a transcription factor activated by reduced cellular oxygen levels, which stimulates expression of genes involved in hypoxia response (Baudel et al. 2017). *HSP90B1* is a member of the 90 kDa heat shock protein family primarily located on melanosomes and the endoplasmic reticulum (Chen et al. 2005). The majority of 90kDa heat shock protein targets are signal transduction proteins; for example, HSF1 is kept in its inactive monomer state by HSP90 (Young et al. 2001). *SERPINH1* encodes a 47 kDa collagen-specific molecular chaperone located in the endoplasmic reticulum (Hosokawa et al. 1998; Widmer et al. 2012). *HSPA9* is a constitutive member of the 70kDa heat shock protein family primarily located in the mitochondria, a molecular chaperone which aids in protein folding as well as cell proliferation (Kaul et al. 2002; Liu et al. 2013; Asea 2018). Suppression of *HSPA9* (mortalin) in human cells results in a senescent-like growth arrest, leading to cell death, while overexpression leads to an extended life span in cultured human cells (Kaul et al. 2000, 2002; Wadhwa et al. 2004). *MMP2* is a membrane-bound proteinase involved in remodeling the extracellular matrix (Sariahmetoglu et al. 2007).

There have been criticisms regarding the use of mRNA transcript abundance as a proxy for protein abundance, stemming from the often low correlation between gene transcript and protein concentrations ($r \approx 0.64$) (Vogel and Marcotte 2012). Much of this disparity in the

predictive power of mRNA abundance on protein abundance is attributed to post-transcriptional and -translational regulation, as well as differences in the half-life of mRNA and peptide molecules (Vogel and Marcotte 2012). On the other hand, studies have also demonstrated a relatively high correlation between mRNA and protein abundance in individual *Escherichia coli* cells (r = 0.77) (Taniguchi et al. 2010), and yeast cells subjected to an osmotic shock demonstrated an r of 0.88 between mRNA and protein abundance for transcripts which increased in abundance, while transcripts that decreased in abundance had a very low correlation with protein (r = 0.30) (Lee et al. 2011). In human tissues, a high gene-specific RNA-to-protein correlation has been demonstrated (r = 0.85-0.90), but gene-specific RNA-to-protein ratio varies widely, from as low as 200 up to 220,000 (Edfors et al. 2016). Regardless of the degree to which mRNA abundance influences protein abundance, an increase in mRNA transcripts will generally result in an increase in the corresponding protein. In this thesis research, the goal was not to identify specific levels of expression, rather it was to examine the relative response patterns of genes involved in the cellular stress response. This does not require a one-to-one relationship between a transcript and its product to be able to make informative comparisons between groups.

1.3 Invasion Theory and Phenotypic Plasticity

Invasive species are a global problem, causing great financial strain on the agriculture and fisheries sectors, degrading aquatic infrastructure, and contributing to local biodiversity loss (Lodge 1993; Karatayev et al. 2007; Catford et al. 2009; Engel et al. 2011; Blackburn et al. 2011). Aided by anthropogenic landscape modification and global trade, species are capable of being transported or distributing themselves across previously impassable barriers into novel territories (Kim and Mandrak 2016). There is much interest in identifying common traits of

successful invasive species, and through these traits, identifying species which have the potential to become invasive (Zambrano et al. 2006; Hayes and Barry 2008; DeVaney et al. 2009; Onikura et al. 2011). Phenotypic plasticity is a feature hypothesized to be advantageous to species colonizing novel environments and therefore is predicted to increase in a founding population relative to a source population (Yeh and Price 2004; Parsons and Robinson 2006; Ghalambor et al. 2007; Torres-Dowdall et al. 2012; Morris et al. 2014). In the literature regarding invasive species, greater plasticity is commonly assumed to be among the contributing factors to their success (Marchetti et al. 2004; Smith 2009; Engel et al. 2011) and has been tested experimentally on several occasions on a variety of taxa (Sexton et al. 2002; Braby and Somero 2006; Lockwood et al. 2010; Engel et al. 2011; Lockwood and Somero 2011; van Kleunen et al. 2011; Pusack et al. 2016; Wellband and Heath 2017; Pickholtz et al. 2018).

Fundamentally, phenotypic plasticity results from differential gene expression, which can be directly compared across populations using molecular tools (Schlichting and Smith 2002). Reaction norms of gene transcript abundance in relation to different environmental parameters can vary between invasive and native populations, with invasion theory predicting greater plasticity, and therefore greater variability, in the invasive population. Greater ARR values, corresponding to a steeper positive relationship between CTMax and acclimation temperature, would also indicate increased plasticity and would be predicted for an invasive relative to a native population.

1.4 Sea Lamprey

Lampreys (Petromyzontida) are one of two extant classes of jawless fishes, which diverged from the vertebrate lineage some 500 million years ago, and from hagfishes (Myxini),

the other extant class of jawless fish, ~450 million years ago (Smith et al. 2013; McCauley et al. 2015; Evans et al. 2018). Sea lamprey (*Petromyzon marinus*) are an anadromous native fish of the North Atlantic, historically spawning in tributaries extending from Newfoundland to Florida in the West Atlantic, and from Norway to Spain in the East Atlantic (Dempson and Porter 1993; Hansen et al. 2016). Following a larval phase, which may last approximately 3-8 years, sea lamprey undergo a metamorphosis into a free-swimming juvenile, which travels downstream to the marine or lacustrine environment and parasitizes larger aquatic species for 1-2 years, and then returns upstream to spawn prior to dying (Applegate 1950; Beamish 1980; Hansen et al. 2016; Evans 2017). Sea lamprey are very fecund, with egg production estimates from single females ranging from 44,000 from smaller-bodied landlocked populations (Manion and Hanson 1980), to over 200,000 in the native, anadromous populations (Beamish 1980). The parasitic phase of the lifecycle is very notable as the mouth morphology differs greatly from that present in other fish, and sea lamprey are known to parasitize a wide variety of species, including numerous fish, shark, and whale species (Farmer et al. 1975; Beamish et al. 1979; Beamish 1980; Johnson and Anderson 1980; Halliday 1991; Wilkie et al. 2004; Gallant et al. 2006; Nichols and Tscherter 2011; Silva et al. 2014). During the mid-20th century, sea lamprey successfully invaded Lakes Erie and the upper Laurentian Great Lakes, contributing to drastic declines in the fisheries of nearly all commercially-important species (Smith 1968; Morman et al. 1980; Bence et al. 2003; Irwin et al. 2012; Hansen et al. 2016). Studies suggest that each parasitic sea lamprey is capable of killing 6.6-20.3 kg of prey fish, with greater prey mortality occurring at higher water temperatures (Bence et al. 2003; Swink 2003). In response to the damage done to the Great Lakes fisheries, a successful bi-national control effort has been put in

place by the governments of Canada and the United States of America (Heinrich et al. 2003; McLaughlin et al. 2003; Schleen et al. 2003; Siefkes 2017).

Larval sea lamprey, or ammocoetes, inhabit the substrate of their natal streams, preferring slow-flowing depositional zones with a soft, sand-silt substrate (Young et al. 1990; Fodale et al. 2003; Slade et al. 2003; Dawson et al. 2015). Burrowed in the substrate, they filter feed on detritus, diatoms, and bacteria (Manion and McLain 1971; Sutton and Bowen 1994). The growth rate of ammocoetes is highly variable depending on a number of environmental factors, including temperature, ammocoete density, and stream productivity (Purvis 1980; Murdoch et al. 1992; Dawson et al. 2015; Evans 2017). Ammocoetes must remain in the substrate until they have accumulated enough energy reserves to undergo the energetically costly process of metamorphosis. Due to the rearrangements which occur to the digestive system they are unable to feed during the metamorphic process, which can take several months; therefore, the age at metamorphosis is highly dependent on the rate of growth and lipid accumulation (Youson 2003). The relatively static distribution of ammocoetes, both geographically and temporally, make this life stage an ideal target for management actions (Morman et al. 1980; Dawson et al. 2015; Siefkes 2017).

Parasitic-phase sea lamprey have been recorded in a wide variety of habitats and conditions, ranging from freshwater to saltwater, depths up to 4099m, distances up to 815km off the coast, and water temperatures from -0.6 °C to 25 °C (Farmer et al. 1977; Haedrich 1977; Beamish 1980; Morman et al. 1980; Silva et al. 2014). The distribution of the parasitic juvenile life stage is highly dependent on the movements of its host, resulting in the exposure to these diverse environmental conditions. Once attached to prey, sea lamprey appear to prefer to remain attached for as long as possible; for example, under laboratory conditions sea lamprey have been

observed to feed on lake trout (*Salvelinus namaycush*) until the host is killed, up to 14.4 d, while in the wild a sea lamprey was observed attached to a Minke whale (*Balaenoptera acutorostrata*) for 87 consecutive days (Farmer et al. 1975; Nichols and Tscherter 2011). Therefore, juvenile sea lamprey may have a reduced capability to actively choose their environment, making the ability to tolerate a wide range of abiotic stressors important at this life stage to exploit each prey for as long as possible before detaching and seeking a new host. Sea lamprey ammocoetes also have a limited ability to choose their environmental conditions, being restricted within their natal stream system, and thus they also have a need to be resilient against environmental perturbations which may impact their homeostasis.

Temperature plays a key role in multiple facets of sea lamprey life history. Under laboratory conditions, embryonic development requires stable temperatures between 15.6 °C and 23 °C for the fish to develop into a burrowing larva (Piavis 1961; McCauley 1963; Rodríguez-Muñoz et al. 2001). However, embryonic development has been recorded in streams with temperatures fluctuating from 10 °C to 18.3 °C (Manion and Hanson 1980), and 16.1-26.1 °C (Applegate 1950). There is also evidence that sex determination in sea lamprey is influenced by growth rate, which could in turn be influenced by temperature (Johnson et al. 2017). No study which has assessed lower thermal tolerance in sea lamprey ammocoetes was found, but they survive several years in stream sediment and so likely tolerate temperatures close to freezing, while their incipient upper lethal temperature has been estimated to be 31.4 °C in the laboratory (Potter and Beamish 1975), while their distribution in tributaries of the Great Lakes has historically been limited to those streams which have a maximum annual temperature of 31.1 °C (Morman et al. 1980). Ammocoete mean chronic (3 d) thermal preference has been estimated to be 13.6 °C (±0.17 °C) (Reynolds and Casterlin 1978), while acute preferences appear to shift

from 20.8 °C in the summer to 16.8 °C in the winter (Holmes and Lin 1994). Evidence suggests that the initiation of metamorphosis is linked to the springtime rise in temperatures, while metamorphosis has an optimum temperature around 21 °C, and can proceed within the range of 13-25 °C (Youson 2003). Post-metamorphic downstream migration has been strongly linked to stream temperature in the River Rhine, with the bulk of migration occurring in the spring at temperatures of 9 °C to 12 °C (Baer et al. 2018). Parasitic juveniles have been kept successfully in the laboratory at temperatures between 4 °C and 20 °C (Farmer et al. 1977), and have been observed at temperatures ranging from -0.6 °C to 22 °C (Beamish 1980; Morman et al. 1980), but upper thermal limits are unknown. Upstream spawning migrations are closely correlated with river temperature, beginning once stream temperatures exceed those of the lake or ocean, with peak migration occurring at ~15 °C (Applegate 1950; Beamish 1980; Morman et al. 1980; Binder et al. 2010). Finally, spawning has been observed at temperatures from 10.0 °C to 26.1 °C (Manion and Hanson 1980).

As temperatures in the Great Lakes basin have increased over the past century (Austin and Colman 2007, 2008), and global temperatures continue to rise (Hodgkins et al. 2003; Hayhoe et al. 2007; IPCC 2014), it will be important to understand how species react to their new reality. Understanding how these warming conditions will affect sea lamprey is important for projecting future management requirements, as stakeholders from the Great Lakes seek to continue to control them while European managers are attempting to restore populations (Mateus et al. 2012; Cline et al. 2014). In the Great Lakes basin, warmer temperatures have been occurring earlier in the year, resulting in a corresponding shift in the timing of sea lamprey spawning migrations over the past ~40 years (McCann et al. 2018). Lake Superior has warmed significantly since 1980, which parallels an increase in lamprey size and feeding rate over the

same period (Cline et al. 2014). Knowledge of how these increasing temperatures will impact sea lamprey ammocoetes is important so that we can better predict whether warmer temperatures will encourage population growth or contraction at this life stage.

In the only previous work examining the molecular response of sea lamprey to heat shock, Wood et al. (1998, 1999) found increases in HSP70 and HSP90 protein abundances in gill, liver, and intestine tissues following 1h of thermal shock in ammocoetes, metamorphic, and post-metamorphic sea lamprey. Unfortunately, the specific members of the HSP70 and HSP90 families which were observed in this study are unknown, as they were identified by one-dimensional gel electrophoresis. Wood et al. (1999) did demonstrate that sea lamprey require a relatively large increase in temperature to induce the CSR as compared to most teleosts, about 13-16 °C above their acclimation temperatures (9 °C and 13 °C), indicating that lamprey may have a high resistance to thermal protein denaturation.

A robust CSR may have also played a role in the apparent ease with which sea lamprey were able to colonize Lake Erie and the upper Laurentian Great Lakes following the expansion of the Welland canal in 1932 (Morman et al. 1980). Invasion theory predicts that successful invasive species will generally demonstrate greater plasticity than non-invasive species (Yeh and Price 2004; Parsons and Robinson 2006; Ghalambor et al. 2007). Wellband and Heath (2017) proposed that the differential success of two closely related species of invasive goby in the Great Lakes could be at least partly attributable to their abilities to alter gene expression in response to environmental stress. The relatively more successful round goby (*Neogobius melanostomus*) demonstrated a greater number of differentially expressed genes related to maintenance of homeostasis as compared to the tubenose goby (*Proterorhinus semilunaris*), a species with a restricted distribution in the Great Lakes, in response to a thermal challenge. Sea lamprey,

through a relatively high tolerance to abiotic factors, may then have been pre-adapted for successful invasion of the Great Lakes.

Chapter 2: Response in gene expression to an acute heat shock in an anadromous population of sea lamprey

2.1 Abstract

How species respond to short-term thermal challenges can be used as a proxy to evaluate their overall thermal tolerance. RT-qPCR was used to evaluate the expression of several genes involved in the cellular stress response of sea lamprey (*Petromyzon marinus*), an invasive species in the Great Lakes which currently experiences temperatures up to ~30 °C in some larval-hosting streams. Larval sea lamprey acclimated to 5 °C, 13 °C, and 20 °C were exposed to thermal stresses of 26 °C and 2 °C below the acclimation-specific CTMax (~30.5 °C, 31.5 °C, 32.5 °C, respectively), for one and four hours. Sea lamprey rapidly upregulated an inducible transcription factor (*JUN*) 10 to 40-fold and important molecular chaperones *(HSP90B1*, *SERPINH1A*) 10 to 100-fold. A recovery pattern was also observed in transcription factor expression following the 4 h 26 °C exposure, where expression returned to control levels. These results indicate that acclimation-independent physiological thresholds may exist in larval sea lamprey, where the cellular stress response transitions first from typical homeostatic-maintenance to a mild stress response at ~26 °C, followed by another transition between 26 °C and ~30 °C to a more extreme cellular survival response. Due to climate change, the frequency of temperatures meeting and exceeding 30 °C will increase, potentially leading to a shift in sea lamprey larval distribution along the North American Atlantic coast.

2.2 Introduction

Environmental temperature has a great influence on aquatic ectothermic species at all levels of biological organization, as their body temperatures closely parallel water temperature (Fry 1971; Hochachka and Somero 1971; Stevens and Sutterlin 1976; Somero 2010). Due to global climate change, marine and freshwater temperatures are expected to continue to rise in the future, which may pose a threat to aquatic ectotherms (IPCC 2014). Two factors which will dictate how species are ultimately impacted by increased temperatures are 1) species' upper thermal limits, and 2) species' capacity to adjust their thermal limits in response to thermal challenges (Stillman 2003; Somero 2010).

Upper thermal tolerance estimates are commonly produced by the critical thermal maximum (CTMax) method, a non-lethal and fairly rapid procedure with a high degree of repeatability (Beitinger et al. 2000; Morgan et al. 2018). Measuring the change in CTMax over a range of acclimation temperatures (acclimation response ratio – ARR) can also provide an estimate of species' acclimation ability (Beitinger et al. 2000; Stillman 2003; Comte and Olden 2017). Most temperate freshwater fish previously studied have a CTMax ARR ~0.40 (range 0.07-0.91) (Beitinger et al. 2000; Comte and Olden 2017).

$$Equation\ 2.1: ARR = \frac{\Delta CTMax}{\Delta Acclimation\ temperature}$$

Another method of estimating species' thermal tolerance limits and acclimation capacity is to investigate the response of organisms acclimated to different temperatures to various levels of sublethal thermal stress. The cellular stress response (CSR) is a defensive response to any stressor which has the potential to inflict macromolecular damage, including heat (Kültz 2005). The CSR can be directly measured by comparing relative gene transcript (mRNA) abundance at

different levels of heat shock through reverse-transcription quantitative polymerase chain reaction (qPCR) (Logan and Somero 2011; Logan and Buckley 2015; Komoroske et al. 2015; Jeffries et al. 2016, 2018). The CSR is tiered to the severity of the response, whereby a mild stress event will elicit a relatively mild response geared towards repairing minor macromolecular damage and preventing additional damage, primarily via upregulation of heat shock proteins (HSPs). A severe stress event producing irreversible cellular damage will elicit a severe response, ultimately resulting in programmed cell death (Lindquist and Craig 1988; Morimoto 1998; Kültz 2005; Logan and Somero 2011; Jeffries et al. 2018). While the CSR acts along a spectrum, it is possible to define threshold levels of a stressor. In the case of thermal stress, specific temperatures may trigger response patterns which correspond to a mild, middling, or extreme response, allowing investigators to identify temperatures which, although not necessarily lethal, may have long-term fitness implications (Logan and Somero 2011; Jeffries et al. 2018). Multiple sublethal thermal thresholds were identified in the delta smelt (*Hypomesus transpacificus*) based on the pattern of gene expression involved in the CSR (Komoroske et al. 2015; Jeffries et al. 2018). Unlike the CTMax, sublethal thresholds can identify temperatures at which species experience a mild stress from which they can recover relatively quickly, or temperatures which induce a severe response, and though not acutely lethal, may result in delayed recovery and ultimately reduced fitness (Jeffries et al. 2018).

Acclimation history is hypothesized to have an impact on the temperatures required to initiate certain tiers of the CSR. Individuals acclimated to higher temperatures should only experience cellular stress at relatively higher temperatures than individuals acclimated to lower temperatures (Schulte et al. 2011). This can be quantified as the temperature at which certain suites of genes are significantly upregulated, termed the initiation temperature. In studies of the

heat shock response, this has traditionally been the temperature at which HSP mRNA or protein abundance increases (Wood et al. 1998; Buckley and Hofmann 2002; Logan and Somero 2011), but could also apply to genes involved in other tiers of the stress response.

The aim of the present research was to evaluate the thermal tolerance of sea lamprey (*Petromyzon marinus*) ammocoetes, and to examine gene expression patterns from elements of the CSR. First, providing an estimate of the CTMax for this species at a range of acclimation temperatures was required, as this is a commonly used method to estimate upper lethal temperatures which is currently missing from the sea lamprey literature. It was expected that the CTMax would be slightly higher than the incipient lethal temperatures measured previously (Potter and Beamish 1975; Beitinger et al. 2000). Assessing CTMax at a range of acclimation temperatures also provided an estimate of their ARR, a measurement of acclimation capacity. Due to their broad thermal scope (~32 °C) and temperate habitat, it was expected that larval sea lamprey would demonstrate a similar acclimation capability as other temperate ectothermic fish species, measured as an ARR ~0.3-0.5. Second, expression patterns of selected genes from the CSR in response to different levels of acute heat shock via qPCR were assessed. Two acute heat shock temperatures, 26 °C and the acclimation-specific CTMax-2 °C, and two exposure durations, 1 h and 4 h, were assessed to evaluate the speed, magnitude, and tier of the stress response. Given their capability for withstanding environmental stressors, transcription patterns consistent with a robust stress response were expected, similar to those of other highly tolerant species. At the relatively mild stress temperature, it was predicted that HSPs would be upregulated, as has been previously found in this species at temperatures around 26 °C (Wood et al. 1999). At the more extreme temperature, it was expected that an upregulation of genes involved in more severe responses, including proteolytic enzymes, and transcription factors, in

addition to increased HSP expression would be observed. It was also expected that acclimation history would impact these responses – individuals acclimated to greater temperatures should show evidence of reduced cellular stress compared to those acclimated to lower temperatures.

2.3 Materials and Methods

2.3.1 Animal Collection and Holding

Anadromous sea lamprey ammocoetes were collected over a 5 d period (June 12-16, 2017) by backpack pulsed-DC electrofishing (LR-20B, Hoskin Scientific Ltd.) in tributaries of the Richibucto River, NB, Canada (Fig. 2.1). Animals were then transported to Wilfrid Laurier University, Waterloo, ON, sorted according to size, and transferred into 110 L acclimation tanks. Ammocoetes used in the present study were between 65 mm and 112 mm in length, with a mean (±SD) of 85.1 mm (±10.7 mm). The 390 anadromous ammocoetes were haphazardly distributed into three 110 L fiberglass aquaria, with approximately 10 cm of sand provided as a burrowing substrate and received a continuous flow of aerated well water (mean flow rates of 0.96 L min^{-1}, 2.80 L min^{-1}, and 0.78 L min^{-1} in the cold, moderate, and warm tanks, respectively. Dissolved oxygen >75 % saturation). Ammocoetes were held under a 12 h light-12 h dark cycle and were fed a slurry of Baker's yeast at a rate of 1 g $animal^{-1}$ once per week.

Each holding aquarium was designated as either cold, moderate, or warm, with target acclimation temperatures of 5 °C, 12 °C, or 19 °C, respectively. These temperatures are representative of a range of realistic temperatures experienced under natural conditions (Fig. 2.2). Temperatures were achieved and maintained through a combination of either a chiller (Coralife) or heating rods (Eheim Jager Aquarium Thermostat Heater 100W), and the controlled flow of incoming water. Temperature was raised or lowered at a rate of approximately 1 °C day^{-}

[1]. The mean (±SD) temperatures of the acclimation conditions were 5.0 °C (±0.3 °C), 13.5 °C (±0.9 °C), and 20.3 °C (±1.5 °C). Maintaining the temperature in the warm acclimation tank was challenging due to fluctuations in the flow rate of the system. The acclimation period lasted a minimum of 16 d prior to experimentation. While both length and mass were greatest in the cold acclimation group (ANOVA: p=0.005, and p=0.007 respectively), no difference was detected in the condition factors between acclimation groups (ANOVA, p=0.7).

$$Equation\ 2.2: Condition\ Factor\ K = 10^6(\frac{mass}{length^3})$$

2.3.2 CTMax Procedure

Three 2 L glass beakers were filled with ~10 cm of sand then topped to 1400 mL with water from the relevant acclimation tank. Long forceps were used to rake through the sediment in the acclimation tanks to flush out individual ammocoetes, which were collected and placed individually into a beaker, within a 60 L glass aquarium. The cold, moderate, and warm acclimation groups had sample sizes of 15, 16, and 15, respectively. An airstone was placed into each beaker, a temperature probe was inserted into the sediment to record sediment temperature while minimizing disturbance to the burrowed ammocoete. Thirty minutes after introduction to the beaker, or once all ammocoetes were burrowed into the sediment (which typically occurred within a few minutes), 12 L of moderate water was added to the aquarium. Between three and six heating rods (number varied due to the wattage of individual rods), set to their maximum temperature were plugged in, and an aquarium pump was turned on. Beaker water and sediment temperature were recorded every 10 min, rising at a mean rate (±SD) of 0.16 °C min^{-1} (±0.03 °C min^{-1}), with the rate slowing as the water temperature approached the heating bars' maximum

temperatures. All sediment temperature probes were later standardized against the probe used to measure water temperature, and adjusted sediment temperatures are reported.

Temperatures were recorded for two different events; complete emergence from the sediment (i.e. the emergence temperature), and the CTMax. CTMax was determined by a lack of response to a series of three gentle tail pinches administered with a pair of forceps. At this point individuals were removed from the experimental beaker and mass and lengths were recorded. Larvae were then placed in a recovery beaker with moderate well water, an airstone, and polyester pillow stuffing to provide an artificial burrowing substrate and kept for observation for a minimum of 12 h. Recovery appeared to occur rapidly following re-introduction to the ~13 °C recovery beaker. Survivors were placed in another 110 L flow-through tank and not used for any subsequent experimentation.

2.3.3 Thermal Challenge and Sampling Procedure

Three thermal regimes were targeted; a handling control maintained as closely as possible to the acclimation conditions, a common stressor of 26 °C, and a common relative extreme stressor of 2 °C below the acclimation-specific CTMax. Actual measured exposure temperatures are shown in Table 2.1. Exposures lasted either 1 h or 4 h, with a typical sample size of 10 ammocoetes (range 8-11) for each sampling period.

Water from the relevant acclimation tank was used to fill 2 L glass beakers to 1400 mL, and an airstone and some polyester stuffing was provided as a burrowing substrate. In preliminary tests, sea lamprey ammocoetes did not react behaviourally to increasing temperatures when burrowed in polyester stuffing but did when allowed to burrow in a sand

substrate; therefore, polyester stuffing was used instead of sand in the gene expression trials. This reduced the likelihood of any behavioural responses which may have altered metabolic demands, or otherwise had an influence on gene expression patterns. Ammocoetes were collected from their acclimation tanks using a trawling method – dragging a wide-mouth net through the substrate in the acclimation tank and separating the ammocoetes from the substrate in the net. Ten ammocoetes were placed in each beaker, with two beakers placed inside each 60 L glass aquarium. One beaker was designated as the 1 h exposure group, while the other contained the 4 h exposure group. Ammocoetes were given a 1 h habituation period to the beakers prior to the start of any experimental manipulation to allow them time to burrow into the polyester substrate and to reduce potential handling effects on their gene expression.

Following the habituation period, the aquarium was filled with 12 L of moderate water. In the two thermal challenge regimes, an aquarium pump and three to five heating rods (number varied due to the wattage of individual rods) were plugged in. The heating rods were all set to their maximum temperature except one, set at the target temperature. This was done in order to mimic the heat ramp from the CTMax trials, but once the temperature was achieved, only one heating bar was required to maintain the temperature. Beaker water temperature was frequently monitored, and once the target was achieved adjustments were made as necessary to maintain the temperature as closely as possible to the target. The 1 h and 4 h exposure times began once the desired temperature was achieved, which generally took approximately 1 h. The handling control group was treated in the same manner as the others up to the end of the beaker habituation period. The aquarium temperature was then brought as close as possible to the acclimation temperature, using moderate water, ice, and room-temperature water. The handling control groups were given a 1 h mock warm-up period to ensure consistency across treatments.

Therefore, total time spent by ammocoetes in the experimental setup varied from 3 h to over 6 h. Three aquaria were run simultaneously, one at each thermal regime, with staggered start times to allow time for sampling. In this way, all six unique treatments were completed for each acclimation group in a single day. All thermal challenges were completed over consecutive days. Due to a high mortality rate in the initial warm-acclimation, 4 h CTMax-2 °C treatment (60 % mortality), this exposure was repeated the following day (30 % mortality), and results were pooled.

Following the completion of the experimental exposure, the relevant beaker was removed from the water bath, and the 10 ammocoetes were placed in a lethal dose of MS-222 (1.5 g L^{-1}, buffered with 3 g of $NaHCO_3$). Following mass and length measurements, ammocoetes were placed on ice in preparation for dissection. Intestine and liver tissues were removed from each individual and placed in 0.5 mL of RNAlater (Thermo Fisher). Dissections were completed as rapidly as possible to reduce any potential RNA degradation. Tissue samples were kept at 4 °C for 24 h, and then stored at -80 °C. Tissue samples were transported from Wilfrid Laurier University to the University of Manitoba, Winnipeg, MB, on dry ice and stored at -80 °C upon arrival. Ten individuals were sampled directly from the acclimation tanks before the thermal challenges began, and again after all were completed. These individuals were euthanized in MS-222 immediately and followed the same sampling technique as previously described.

2.3.4 RNA Extraction and cDNA Synthesis

Total RNA was extracted from tissue samples using the PureLink RNA Mini Kit (Thermo Fisher, Cat. #12183025), according to the manufacturer's instructions with some minor modifications described here. The tissue sample was thawed on the bench and placed into a

round-bottom 2 mL homogenization tube with 0.6 mL of provided homogenization buffer and a sterile metal bead. Tissues were placed in a TissueLyser LT (Qiagen, Cat. #85600) set to maximum speed for between 2 min to 8 min, depending on the tissue type. The resulting homogenate was spun down for 2 min at maximum speed on a centrifuge (MicroCL 21, Thermo Fisher, Cat. # 75002466) to concentrate any solid pieces of tissue at the base of the tube. Following centrifugation, 350 µL of liquid homogenate was removed and mixed with 350 µL of 70 % ethanol in RNase-free water. All 700 µL of this mixture was then placed in a spin column and spun down for 1 min at maximum speed. The flow-through was discarded, 700 µL of wash buffer I was added to the spin column, and the spin column was gently shaken to rinse the walls and lid of the column with wash buffer I. The column was then centrifuged at maximum speed for 1 min, and the flow-through was discarded. 500 µL of wash buffer II was then added to the spin column, which was again gently shaken to rinse the interior with wash buffer II and centrifuged for 1 min at maximum speed, and the resulting flow-through was discarded. This wash step was performed twice. The empty column was then spun down in the centrifuge for 2 min at maximum speed to dry any remaining buffers from the membrane. 30 µL of ultrapure water heated to 60 °C was then placed onto the membrane and allowed to incubate at room temperature for 1 min before being spun down for 2 min at maximum speed.

RNA quantity and quality were assessed on a NanoDrop One (Thermo Fisher, Cat. # ND-ONE-W), then on a 1 % agarose gel using SYBR safe (Thermo Fisher, Cat. # S33102) and an ultraviolet transilluminator. All RNA samples demonstrated a 260/230 absorbance ratio between 1.24 and 2.47 (mean = 2.18), and a 260/280 absorbance ratio of 2.05 – 2.26 (mean = 2.14). Concerns regarding some samples' low 260/230 absorbance ratios were allayed by outlier analysis on the final dataset, visually via boxplots and statistically via Dixon Tests. These

demonstrated that none of the samples with low 260/230 ratios were outliers in the qPCR analyses; therefore, they were retained in the analysis. RNA samples were stored at -80 °C.

Complementary DNA (cDNA) was produced using a Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase (Thermo Fisher, Cat. #K1672), according to manufacturer's instructions. An appropriate volume of sample was added to bring the total RNA content of each reaction to 1000 ng. A corresponding volume of ultrapure water was added to each reaction to bring the total volume to 8 µL, then 1 µL each of the 10x dsDNase buffer and the dsDNase enzyme solution was added to each well to bring the total reaction volume to 10 µL. The 96-well plate was then covered and spun down briefly before being placed in a SimpliAmp Thermal Cycler (Thermo Fisher, Cat. # A24811) for a 2 min incubation at 37 °C. The plate was then spun down again briefly and placed on ice, then 10 µL of a master mix of two parts ultrapure water, two parts 5x Reaction Mix, and one-part Maxima Enzyme Mix was added to each reaction. This was followed by another brief centrifuge of the plate and incubation in the thermocycler for 10 min at 25 °C, 30 min at 50 °C, 5 min at 85 °C, and a hold at 4 °C. 280 µL of ultrapure water was then added to each well to dilute the cDNA to an appropriate level for use in subsequent qPCR. cDNA plates were stored at -20 °C.

2.3.5 Primer Design

Gene sequences for both reference genes and genes of interest were downloaded from the Ensembl database for sea lamprey (Zerbino et al. 2018). Annotations of some sequences used here were altered in more recent releases after primers were designed. Gene names for the sequences used have been updated to reflect the most current Ensembl release (v.95). Primers were designed following the protocol by(Thornton and Basu 2015; Table 2.2). Briefly, primer

sequences were produced for each target sequence using both Primer3Plus version 2.4.2 (Untergasser et al. 2012) and NCBI Primer-BLAST (Ye et al. 2012) online tools, and evaluated for both predicted amplicon secondary structures and predicted primer self and cross structures using the IDT UNAFold (Owczarzy et al. 2008) and Premier Biosoft Beacon Designer (Free Edition) online tools. Primers were ordered from IDT (Coralville, Iowa, USA), suspended to 100 mM in ultrapure water and stored at -20 °C.

2.3.6 qPCR Protocol

qPCR reactions were assembled using Maxima SYBR Green qPCR Master Mix (Thermo Fisher, Cat. # K0252). Forward and reverse primers for each target sequence were diluted into a single 6 µM solution, and diluted cDNA samples were thawed on ice. The background reporter ROX was added to the SYBR Green Master Mix at a ratio of 2.5 µL ROX per 1250 µL of SYBR Green Master Mix. A master mix for each gene was created with six parts SYBR Green Master Mix with added ROX, and one-part 6 µM forward and reverse primer solution. The master mix was kept on ice in the dark until ready for use. 5 µL of cDNA sample was added to each reaction well on a 384-well plate. Three no template control samples (ultrapure water) and three pooled cDNA samples were also used for each gene on each plate, to verify a lack of reagent contamination and for use as inter-run calibrators, respectively. A reverse transcriptase negative control was not used, as a double-stranded DNAse treatment was performed during cDNA synthesis; therefore, it was assumed that no double stranded DNA was present. The plate was then briefly centrifuged before each reaction received 7 µL of the appropriate master mix, bringing the total reaction volume to 12 µL. Following another brief centrifugation, plates were loaded into a QuantStudio 5 Real-Time PCR System (Thermo Fisher, Cat. # A28140). qPCR

plate layouts and cycling conditions were designed using the QuantStudio Design and Analysis Software v1.4.2 (Thermo Fisher). The following cycling parameters were used: 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C, and finally a melt curve stage consisting of 15 s at 95 °C, one min at 60 °C, and a slow increase back to 95 °C at a rate of 0.075 °C s^{-1} with continuous data collection. Three genes were run over two 384-well plates for each tissue, while *JUN* had all samples of a single tissue run simultaneously on the same plate. A haphazard subset of PCR products were examined on a 1 % agarose gel with SYBR safe to confirm size of amplicons matched predicted size.

2.3.7 qPCR Data Analysis

qPCR data was first inspected using the QuantStudio software to check for any run errors such as multiple melt curves, evaporation from any wells, contamination of the negative template control samples, etc. Expression of genes of interest was calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Raw cycle threshold (Ct) values were then analyzed following the framework of normalizing to three reference genes, with some modifications to include the use of an inter-run calibrator (Hellemans et al. 2007). All statistical tests were performed in R v3.2.3 (R Core Team 2018). A normalization factor (NF) for each sample was subsequently calculated by dividing the geometric mean of the RQs of all three reference genes for a sample by the geometric mean of all RQs of all reference genes for all samples on plate A. The normalized relative quantity (NRQ) of gene A for each sample was then calculated by dividing the RQ by the NF. To measure variation in the reference genes, the coefficient of variation (CV) was calculated for each reference gene on plate A by dividing the standard error of the mean of the gene A NRQs by the mean of the gene A NRQs on plate A. CV is generally below 25 % for

stably expressed reference genes (Hellemans et al, 2007). The CV for reference genes used in the present study were all <22 % (mean CV = 15.9 %).

Genes of interest followed a similar normalization procedure. The minimum Ct value of each gene from that plate was subtracted from each samples' raw Ct value to give a minimum corrected ΔCt value. The resulting value was used to calculate the RQ of transcript by the equation $2^{-\Delta Ct}$. Using the sample-specific NF calculated from the reference genes, the gene of interest NRQ was calculated by dividing the RQ by the NF. Inter-run calibrators were then used to normalize results for the same gene between plates, using methods similar to the calculation of the NF. The geometric mean of gene A, plate A inter-run calibrator NRQs was divided by the geometric mean of gene A inter-run calibrator NRQs on both plates A and B to give the calibration factor (CF) for gene A on plate A. Calibrated normalized relative quantities (CNRQ) of gene A was obtained by dividing the NRQ of gene A by the CF for gene A. These were then log2-transformed and grouped into experimental treatments. Finally, the log2 CNRQ value for each sample was normalized against the mean log2 from samples taken directly from the moderate acclimation tank by subtracting the latter from the former, giving a ΔΔCt value. Statistics were run on these values after multiplying by -1, while figures were produced with the relative fold-change, calculated as $2^{-\Delta\Delta Ct}$.

Two-way type-III ANOVAs were performed for each gene target, using the three acclimation levels and three experimental levels as the two factors. A separate analysis was performed on each treatment duration, using an alpha of 0.05 for statistical significance. *A priori* contrasts were identified prior to testing the model to examine differences between acclimation groups within experimental treatments, and between experimental treatments within acclimation groups to the handling control. Due to the large number of tests (18), a Bonferroni correction was

applied to the p-values for the contrasts, thus an adjusted alpha of <0.0028 was used for statistical significance. Relative gene expression was also analyzed between samples taken directly from the acclimation tanks and the 1 and 4 h handling control treatments. These were analyzed via two-way ANOVAs for each gene as well, with 12 *a priori* defined contrasts between treatment durations within acclimation groups and between acclimation groups within the acclimation sampling point. Again, a Bonferroni correction was applied to p-values for the contrasts, thus an alpha of <0.0042 was used. Principal component analyses (PCA) were conducted on expression data from all genes of interest from all experimental treatments to examine overall trends in each tissue.

2.3.8 Potomac River Temperature

Daily thermal data from a historically ammocoete-bearing tributary (Beamish 1980), the Potomac River, were compiled to examine annual thermal characteristics (Fig. 2.2). Daily mean water temperature data recorded by a probe 0.3m from the river substrate between 2006 and 2017 was acquired from the United States Geological Survey's (USGS) National Water Information System database. Annual thermal data was superficially examined for completeness, and years which appeared to be missing substantial data as well as all provisional data were discarded.

2.4 Results

2.4.1 CTMax

Mean CTMax (±SEM) of the cold, moderate, and warm acclimation groups were 32.5 °C (±0.2 °C), 33.4 °C (±0.1 °C), and 34.4 °C (±0.1 °C), respectively, corresponding to an ARR of 0.12, while mean sediment temperatures at emergence were 31.6 °C (±0.3 °C), 33.0 °C (±0.2 °C), and 33.5 °C (±0.2 °C), respectively (Fig. 2.3). CTMax data demonstrated a significant difference between acclimation groups (ANOVA; $F=60.17$, $p<0.0001$), and *a priori* defined contrasts revealed differences between the cold and moderate acclimation groups ($t=-5.05$, $p<0.0001$), and the moderate and warm acclimation groups ($t=-6.17$, $p<0.0001$). ANOVA of the emergence results also uncovered a significant difference between acclimation groups ($F=17.12$, $p<0.0001$), while *a priori* contrasts revealed significant differences between the cold and moderate acclimation groups ($t=-4.10$, $p=0.0002$), but no significant difference between the moderate and warm acclimation groups ($t=-1.53$, $p=0.13$).

2.4.2 Gene Expression

<u>Handling controls vs acclimation samples</u>

Gene expression of the handling control treatments after 1 and 4 h were compared with samples taken directly from each acclimation tank (Appendix A: Fig. A.1, A.2). Relative to acclimation samples, *JUN* transcript expression rose significantly in the cold acclimation group after 4 h in both liver ($t=-3.03$, $p=0.04$) and intestine ($t=-5.29$, $p<0.0001$), and after 1 h in the intestine ($t=-4.16$, $p=0.0009$). An increase was also observed in *JUN* transcript expression in the intestine samples from the moderate acclimation group following a 1 h handling control ($t=-4.63$, $p=0.0002$). *MMP2* expression in intestine decreased significantly from acclimation samples in

the 4 h moderate (t=3.413, p=0.01) and warm (t=3.07, p=0.04) acclimation groups, and was also expressed lower in the 1 h moderate acclimation group relative to the acclimation samples (t=3.348, p=0.01).

Transcription factors

Of the three transcription factors selected for this study, only *JUN* demonstrated a significant increase in expression following the acute heat shock treatments (Fig. 2.4 E, F; Fig. 2.5 E, F; Tables 2.3, 2.4). Expression of *JUN* increased rapidly in both tissues following 1 h of heat shock at the common 26 °C stressor, tiered to the acclimation history (the cold group demonstrated the greatest increase, followed by the moderate group, while the warm group displayed a negligible increase), but displayed a recovery to basal expression levels following 4 h exposure to the 26 °C treatment. Meanwhile, no such recovery in expression is observed following 4 h at the extreme CTMax-2 °C stressor in liver tissue, suggesting that the *JUN* pathway is very much still a necessary component of the extreme heat stress response. A decrease in expression is noted in the intestine following 4 h at the extreme CTMax-2 °C treatment.

Expression of *HSF1* remained relatively stable after 1 h in both tissues, while a general trend of decreasing expression with treatment severity was observed following 4 h of heat shock (Fig. 2.4 C, D; Fig. 2.5 C, D; Tables 2.3, 2.4). There was also no evidence of hypoxic stress during any treatment, as *EPAS1B* expression remained unchanged throughout the exposures, although it should be noted that there was a clear segregation based on acclimation history, with the cold group generally displaying the lowest expression level, and the warm group generally with the greatest expression (Fig. 2.4 A, B; Fig. 2.5 A, B; Tables 2.3, 2.4).

Molecular chaperones

A classic heat shock response was observed in the expression of *HSP90B1*, with an upregulation in response to increasing thermal stress (Fig. 2.6 A, B; Fig. 2.7 A, B; Tables 2.3, 2.4). The cold-acclimated group demonstrated no change in *HSP90B1* expression following the 1 h exposures in either tissue as compared to the handling control, while the warm and moderate acclimation groups demonstrated significant upregulation after 1 h in the face of a relatively lower temperature increase (26 °C vs. 12 °C temperature increase). It should be noted, however, that *HSP90B1* expression was greatest in the cold-acclimation group in both the handling control and 26 °C treatments after 1 h. After 4 h, liver tissue expression increased more in the 26 °C treatment than the CTMax-2 °C treatment relative to the handling control. Meanwhile in the intestine, following 4 h of heat shock, *HSP90B1* expression increased with treatment severity. *HSP90B1* expression in the warm acclimation group was only upregulated in the CTMax-2 °C treatment following 1 h exposure, and no change in expression was observed in the cold acclimation group (Fig. 2.6, 2.7; Tables 2.3, 2.4). Meanwhile the moderate acclimation group demonstrated a two and three-fold increase in response to the 26 °C treatment in intestine and liver tissue, respectively, and a four and 10-fold upregulation in response to the CTMax-2 °C treatment. Following 4 h the liver expression levels are similar across acclimation groups, with upregulation of 23 to 30-fold observed in the 26 °C treatment and 11 to 16-fold upregulation in the CTMax-2 °C treatment.

The response of *HSPA9* was muted, especially in the intestine which revealed no treatment effect (Fig. 2.6 C, D; Fig. 2.7 C, D; Tables 2.3, 2.4). In liver tissue following the 4h exposures, a peak in expression is observed in the 26 °C treatment from both the cold and

moderate acclimation groups, while the CTMax-2 °C treatment shows no difference with the handling control.

SERPINH1A displays an acclimation-dependent response in expression to the different heat shock treatments (Fig. 2.6 E, F; Fig. 2.7 E, F; Tables 2.3, 2.4). Tissue samples taken directly from the acclimation tanks demonstrate significant differences in expression, with obvious upregulation in the warm acclimation group, and low expression levels in the cold group (Appendix A: Fig. 2). Following the 1 h treatments of heat shock the cold and moderate groups displayed dramatic upregulation with the treatment temperature, while *SERPINH1A* expression in the warm group rose only moderately with stress severity. Following 4 h of treatment, all acclimation groups have dramatically upregulated *SERPINH1A* expression with treatment severity, but the warm group still lags significantly behind the others.

Proteolytic enzymes

MMP2 expression remained steady across nearly all treatments (Figs. 2.8, 2.9; Tables 2.3, 2.4). Significant upregulation compared to the handling control in the cold acclimation group occurs following 1 h at the CTMax-2 °C treatment in liver tissue.

Principal components analysis

The PCA evaluating overall liver gene expression patterns revealed responses to treatment severity along both PC1 (34.3 % variance explained) and PC2 (19.2 % variance explained), and acclimation-specific responses along PC2 (Fig. 2.10). Handling control treatments were negatively correlated to PC1, while CTMax-2 °C treatments were positively

correlated. The cold acclimation group follows similar patterns overall as the other two but is shifted positively along PC2. The loading plot indicates that *EPAS1B, HSPA9,* and *MMP2* expression have the greatest impact along PC2, and these tended to be differentially expressed in the cold acclimation group relative to the others (Fig 2.10 inset).

The PCA performed on intestine gene expression data similarly shows a response pattern to treatment severity along both PC1 (32.9 % variance explained) and PC2 (25.1 % variance explained), but no consistent separation based on acclimation is apparent (Fig. 2.11). Again, handling controls are negatively correlated to PC1, and CTMax-2 °C treatments are positively correlated. The CTMax-2 °C 4 h treatment also shows a negative correlation with PC2 in the moderate and cold acclimation groups.

2.5 Discussion

From the present investigation, three main conclusions may be inferred. First, sea lamprey ammocoetes can rapidly and dramatically differentially regulate important elements of the CSR in response to an acute thermal stressor, and in accordance with the severity of the stressor. Second, sea lamprey ammocoetes appear to have a limited ability to thermally acclimate relative to other fish species, as the mild difference in CTMax across acclimation temperatures and correspondingly low ARR attest. Also, differences in mRNA expression patterns in the suite of genes examined here across acclimation groups were predominantly observed following the short 1 h exposure, with few differences observed following 4 h. Finally, based on the expression patterns of the genes in response to the temperatures used here, physiological thresholds appear to exist at ~24 °C – 26 °C and between 26 °C and ~30 °C, at which the cellular processes

involved in the stress response initiate, and then shift to a severe stress response, respectively. Again, these physiological thresholds appear to be acclimation-independent.

2.5.1 CTMax and Acclimation Response Ratio

In the present study, acclimation temperature played a smaller role in thermal tolerance than expected, as sea lamprey demonstrated an ARR of 0.12, increasing their CTMax from 32.5 °C to 34.4 °C over a 15.3 °C increase in acclimation temperature. This result is in contrast to most temperate fish species previously studied, which possess ARRs between ~0.3 and ~0.5 (Fry 1971; Beitinger et al. 2000; Fangue et al. 2006; Logan and Somero 2010; Healy and Schulte 2012; Zhang and Kieffer 2014; Komoroske et al. 2014; Comte and Olden 2017; Rodgers et al. 2019; W. Bugg, pers. comm.), although exceptions do exist such as the Amargosa River pupfish (*Cyprinodon nevadensis amargosae*) with an ARR of 0.13 (Feldmeth et al. 1974). This pupfish is endemic to Death Valley, California, and is adapted to significant diel and annual fluctuations in temperature, with daily temperatures ranging from 9.5 °C – 12.8 °C in winter months to 27.0 °C – 42.0 °C in the summer (Shrode 1975). This species also displays one of the greatest CTMax values recorded among fish species at 43.3 °C (Feldmeth et al. 1974), and as Comte and Olden (2017) demonstrate, species which tolerate the greatest temperatures tend to have a reduced ability to shift their CTMax via acclimation, which may help to explain this exception.

Previously, a low ARR has been noted on the acute preferred temperature of sea lamprey ammocoetes (0.19), relative to other fish species (0.41-0.84; Holmes and Lin 1994). Meanwhile, investigations on incipient lethal temperatures across various acclimation temperatures in sea, brook (*Lampetra planeri*), and pouched lamprey (*Geotria australis*) ammocoetes have all found limited changes in response to acclimation temperature, with ARR values of 0.08, 0.05-0.1, and

0.05, respectively (Potter and Beamish 1975; Macey and Potter 1978). These authors also noted the low influence of acclimation temperature on the incipient lethal temperature, considering that most other fish species studied until that time tended to have incipient lethal temperature ARRs around 0.33 (Fry 1971), mirroring those measured in the CTMax experiments involving temperate fishes. Making meaningful comparisons of this response across a variety of species from a wide range of thermal backgrounds is difficult; however, the fact that all lampreys appear unable to adjust their upper thermal limit according to their acclimation history implies that the ability to thermally compensate through acclimation has been reduced in the Petromyzontida. Alternatively, it could also be a derived trait of the jawed fishes which was subsequently lost in a few lineages. A review of the plasticity of upper thermal tolerance across 82 freshwater fish species within 27 families of jawed fishes found upper thermal tolerance to be correlated to thermal habitat, while ARR was uncorrelated with habitat thermal conditions but related to phylogeny (Comte and Olden 2017). The present study represents an outgroup for the Comte and Olden (2017) review, which used shortnose sturgeon (*A. brevirostrum*) as their outgroup. It would be informative to examine the ARR of representatives from other early branches of the chordates (lancelets, ascidians, salps, hagfish) to evaluate and compare their acclimation capacity.

A previous investigation into thermal tolerance of sea lamprey larvae indicated that a gradual increase in temperature (daily increase of 0.3 °C – 0.5 °C) begins to elicit larval emergence from the sediment at 30 °C in individuals acclimated to 25 °C, while the same experiment demonstrated 100 % mortality by 32.3 °C (Potter and Beamish 1975). In the present study, sediment temperature was recorded at the moment of complete ammocoete emergence to quantify a behavioural response similar to an agitation temperature, as defined in McDonnell and

Chapman (2015). Sea lamprey ammocoetes tended to emerge from the substrate during a rapid increase in temperature within ~1 °C of their CTMax temperature, and franticly swim around the beaker presumably looking for a thermal refuge. On occasion, they would reburrow moments later at sediment temperatures near or over 30 °C, similar to behavioural reports from Potter and Beamish (1975). The limited number of acclimation temperatures evaluated here makes it difficult to elucidate a pattern; however, based on the data available, emergence temperature appears to begin to plateau with an increase in acclimation temperature. It is possible if another acclimation group was added at a greater temperature, 25 °C for example, that emergence temperature may continue the same trajectory and be comparable to that observed in the warm group here, continuing to diverge from the CTMax temperature.

Interpretation of the emergence responses recorded here must be done with caution, however, as this behaviour was only noted during the CTMax trials when ammocoetes were provided with sand as a substrate – no agitation behaviour was observed in the gene expression exposures or preliminary CTMax trials when polyester stuffing was provided as a burrowing substrate, including those which reached temperatures of 32.5 °C, resulting in ammocoete mortality. The polyester tended to float near the surface, had constant aeration from an airstone underneath, and would have warmed at the same rate as the surrounding beaker water, while the sand substrate warmed faster than the beaker water and may have been more oxygen-limiting. Therefore, emergence from the sediment may have been a result of a small thermal or oxygen gradient between the sediment and the relatively cooler, likely oxygen-richer beaker water, rather than an escape response due to temperature alone. It should also be considered that in a streambed under natural conditions a strategy employed by ammocoetes to escape thermal stress could be to burrow deeper into the substrate, rather than emerging, exposing themselves to

potential predation and expending energy by swimming to find a thermal refuge. In that case, thermal and oxygen gradients would also be present, with both variables decreasing as substrate depth increases. Therefore, burrowing deeper may be an effective short-term strategy for escaping thermal stress, but would be ineffective if stressful conditions persist. Also, just as the CTMax method can overestimate upper thermal limits, it may have overestimated the initiation temperature of the agitation response. According to the present data, the response is not initiated until temperatures close to the CTMax, at which point escaping without fitness consequences may prove difficult. Therefore, this behavioural response may be a desperate "last-ditch" attempt to escape stressful situations, and not a routine response to stressful conditions.

2.5.2 Gene Expression Response to Acute Thermal Shock

Of the transcription factors examined here, *JUN* transcripts demonstrated the most drastic differential regulation, increasing ~20 to 45-fold following 1 h of the extreme CTMax-2 °C treatment, and ~4 to 9-fold in the 26 °C treatment. This was followed by a recovery pattern after 4 h exposed to the 26 °C treatment, while the CTMax-2 °C treatment held steady at a ~34-36-fold upregulation. It should be noted that this gene was sensitive to handling, displaying significant upregulation in the handling control compared with samples taken directly from the acclimation tanks; therefore, the treatment effect may be overestimated here. The recovery pattern observed at the lesser 26 °C stressor indicates that the JUN product synthesized by the rapid upregulation observed at 1 h was adequate to respond to the acute thermal stress, and consequently by 4 h excess *JUN* expression was no longer necessary. This is especially remarkable in the cold-acclimated group, which following a 4 h exposure to a 21 °C increase in temperature have no further need to activate downstream pathways influenced by *JUN*.

Meanwhile, in response to the extreme stressor, *JUN* pathways remain crucial for the response in liver tissue but less so in the intestine, as expression levels decreased from 1 to 4 h. These results concur with previous studies on the response of *JUN* expression to heat shock in delta smelt and Chinook salmon (*Oncorhynchus tshawytscha*) (Tomalty et al. 2015; Komoroske et al. 2015; Jeffries et al. 2016).

Among the many downstream pathways *JUN* influences, it plays a role in cell cycle progression at the G1 to S transition and can prevent cells from undergoing apoptosis (Wisdom 1999; Schreiber et al. 1999). Therefore, the differential *JUN* expression revealed in the present study may suggest that upon initial acute heat shock, even to a relatively minor thermal stress (26 °C), sea lamprey alter the regulation of the cell cycle in both liver and intestine cells and prevent apoptosis. *JUN* expression returns to basal levels after 4 h at 26 °C, but the increased expression observed in the first hour could be a response to the heat ramp rather than the final temperature achieved. In the face of an extreme stressor (e.g. CTMax-2 °C), an increase in molecular chaperones alone may not be sufficient to cope with the thermal stress, and therefore *JUN*-mediated pathways continue to be exploited following the end of the heat ramp.

Both *HSP90B1* and *SERPINH1A* demonstrated significant upregulation in liver and intestine, with the magnitude of upregulation proportional to the change in temperature. Acclimation history made a temporal impact on their expression, as the warm-acclimation group did not upregulate expression of these chaperones after 1 h at 26 °C, but upregulation was apparent after 4 h. These results for *HSP90B1* agree with similar investigations in a wide variety of other fish species, including pink (*O. gorbuscha*), sockeye (*O. nerka*), Chinook, and Atlantic salmon (*Salmo salar*), zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), hybrid channel (*Ictalurus punctatus*) and blue catfish (*I. furcatus*), and

longjaw mudsucker (*Gillichthys mirabilis*) (Wang et al. 2007; Logan and Somero 2011; Long et al. 2012; Liu et al. 2013; Jeffries et al. 2014; Tomalty et al. 2015). However, the magnitude of the response (i.e. the fold-change in expression) is greater in the present study relative to these other investigations. For example, the greatest increase in longjaw mudsucker *HSP90B1* expression occurred in the group acclimated to 19 °C following exposure to a constant heat ramp sampled at 39 °C and was upregulated approximately 4-fold (Logan and Somero 2011), compared with the approximately 30-fold upregulation presented here. The dramatic, 100-fold upregulation of *SERPINH1A* observed here has also been observed in rainbow trout (*O. mykiss*) in a similar study (Wang et al. 2016), while the lower magnitude of response has been recorded in similar investigations on sockeye, pink, and Chinook salmon, delta smelt (*Hypomesus transpacificus*), rainbow trout, and catfish, providing further evidence that *SERPINH1A* is a strong indicator of heat stress in fish (Jeffries et al. 2012, 2014; Liu et al. 2013; Tomalty et al. 2015; Komoroske et al. 2015; Verleih et al. 2015; Tan et al. 2016). Upregulation of *SERPINH1A*, along with seven other specific genes, has recently been proposed as a sensitive molecular biomarker indicating chronic thermal stress in fish (Akbarzadeh et al. 2018), and the differentiation observed here in the handling control treatment suggests these same genes may be applicable for sea lamprey ammocoetes as well.

An interesting point to note is that the cold acclimation group demonstrated no significant change in *HSP90B1* expression following 1 h in either tissue in response to either stress treatment. It was, however, expressed in both the liver and intestine at a greater level than both the moderate and warm acclimation groups in the handling control and it was expressed at a greater level than the warm acclimation group in the 1 h 26 °C treatment. This suggests that *HSP90B1* may be upregulated in response to chronic exposure to 5 °C in sea lamprey. In

Drosophila, exposure to a cold shock has been found to increase heat tolerance (Sejerkilde et al. 2003), and a similar stress-hardening effect could be at play here. Threespine stickleback (*Gasterosteus aculeatus*) exposed for nine weeks to 8 °C demonstrate higher HSP90 protein abundance relative to a 20 °C treatment group (Teigen et al. 2015). A relative abundance of HSP90 product already present in the cold acclimation group may have buffered the response to an acute heat shock during the first hour, making additional *HSP90B1* transcription unnecessary. HSF1 is kept in an inactive monomer by chaperone proteins, including HSP70 and HSP90, preventing HSF1 from forming its active trimer and binding the heat shock element, promoting transcription of other heat shock proteins (Morimoto 1998; Young et al. 2001; Li et al. 2017). Those HSPs preventing the formation of the active HSF1 complex disassociate with the HSF1 monomers in the presence of denatured proteins; therefore, a relatively greater basal expression of *HSP90B1* could suppress additional *HSP90B1* expression by maintaining a greater ratio of HSF1 in its inactive monomer state at relatively higher temperatures. After 4 h, the buffering effect of the extra *HSP90B1* may no longer be sufficient to respond to the stressor, and HSF1 monomers have been released by HSP90B1 allowing the formation of the active HSF1 trimer, resulting in *HSP90B1* transcription to be upregulated in earnest. This pattern seems to corroborate the results of Wood et al. (1999) that found an increase in acclimation temperature from 9 °C to 13 °C resulted in a lowering of the initiation temperature of HSP90 protein synthesis. The thermal preference of sea lamprey ammocoetes according to a choice experiment lies between 13.6 °C and 14.5 °C (Reynolds and Casterlin 1978), and therefore it is possible that at 9 °C, sea lamprey experience some mild cellular stress, causing constitutively expressed HSP90 to be present at a greater basal level compared to the 13 °C group, buffering the need to produce more HSP90 until exposure to a relatively greater heat shock.

A similar response is observed in *SERPINH1A* expression in the warm acclimation group. Basal expression was greatest in the warm group and expression increases only slightly in response to the CTMax-2 °C treatment following 1 h, while dramatic upregulation is observed in response to both 26 °C and CTMax-2 °C treatments after 4 h. Again, this is likely due to greater expression in response to the chronic 20 °C exposure, leading to a shielding of any immediate protein denaturation in the first hour of acute heat shock, but requires an additional increase in expression to cope with a prolonged exposure to the thermal stressor.

Generally, an increase in acclimation temperature is associated with an increase in initiation temperature of various elements of the heat shock response (Fangue et al. 2011). Longjaw mudsucker and tidepool sculpins (*Oligocottus maculosus*) both demonstrate an increase in heat shock inducible gene initiation temperature with an increase in acclimation temperature, mimicking the increase observed in CTMax (Buckley and Hofmann 2002, 2004; Logan and Somero 2011; Fangue et al. 2011). Similar patterns have also been identified in relatively stress-intolerant species such as the delta smelt (Komoroske et al. 2015). Previous investigations in sea lamprey revealed a negative interaction between acclimation temperature and initiation temperature of HSP70 and HSP90 in liver tissue, with initiation temperatures of 29 °C and 25 °C observed from 9 °C and 13 °C acclimation groups respectively (Wood et al. 1999). In the present study, after 1 h of exposure to 26 °C, no *HSP90B1* upregulation is observed in either the cold or warm acclimation groups, while an increase is observed in the moderate group, in both tissues. If no further timepoints were examined, this would appear to align with the results from Wood et al. (1999), where an increase in acclimation temperature (cold vs moderate) results in a decrease in HSP90 induction temperature. However, following 4 h of exposure, all acclimation groups demonstrate a similar *HSP90B1* expression pattern. *SERPINH1A* expression shows the opposite

trend, with an increase in acclimation temperature resulting in an increase in induction temperature after 1 h, but no difference in induction temperature between acclimation groups after 4h exposure. It is possible that due to the small number of treatment temperatures used, changes in initiation temperatures between acclimation groups were simply missed. It is also possible that, because the duration of the thermal stress in these other studies was shorter and typically included a recovery period, a similar pattern to that observed here may have resulted had similar methods been used here.

Here, the response of *HSPA9* was muted in the intestine and liver following 1 h exposures but demonstrated a peak after 4 h at 26 °C in liver tissue. This is consistent with results on mammalian models, which have shown *HSPA9* to not be heat inducible (Kaul et al. 2002); however, this is in direct contrast to the response of *HSPA9* observed in the longjaw mudsucker and Chinook salmon, which both demonstrate a significant upregulation in gill tissue in response to a constant heat ramp (Logan and Somero 2011; Tomalty et al. 2015). While *HSPA9* was significantly upregulated here, differences in the magnitude of upregulation observed here and in these other studies could be due to the tissue type examined: it is possible that *HSPA9* is more inducible in gill tissues than in liver or intestine.

HSF1 is also not responsive to the acute heat shock treatments. This could be because HSF1 product is already present at sufficient levels in the cell in an inactive form under non-stress conditions and is converted into the active trimer by accumulation of misfolded proteins; therefore, no additional *HSF1* transcription is required to respond to the acute heat shock.

2.5.3 Physiological Thresholds

The transcriptional responses uncovered here imply the presence of two sublethal thermal thresholds in sea lamprey ammocoetes which are slightly altered by acclimation, one around 24-26 °C, and another around 30 °C. The tier of CSR appears to alter around these two temperatures; first, around 24 °C – 26 °C, inducible heat shock proteins are upregulated after 4 h of exposure similarly across acclimation temperatures; and secondly, around 30 °C, large heat shock protein expression is decreased (relative to 26 °C), while processes influencing cell cycle regulation and apoptosis are dramatically upregulated. Following a 4 h exposure to the acute heat shock treatments, expression of nearly all genes was remarkably similar across acclimation groups. No differences were detected between *JUN, HSP90B1,* or *HSPA9* expression in liver tissue of any acclimation groups at either 26 °C or CTMax-2 °C, and no difference existed in *SERPINH1A* expression at 26 °C. In the intestine more differences were observed, but the overall expression pattern was similar. Regardless of whether a sea lamprey was acclimated to 5 °C and experienced a 21 °C increase in temperature or whether it was acclimated to 20 °C and experienced a 6 °C temperature increase, 4 h of exposure prompted very similar transcriptional responses of the heat-inducible genes examined here. This hints at the presence of a physiological threshold existing around 26 °C at which point protein denaturation becomes problematic for sea lamprey ammocoetes despite acclimation history.

Wood et al. (1999) found HSP70 and HSP90 production in various tissues of sea lamprey ammocoetes acclimated to 9 °C or 13 °C was initiated in response to a 1 h heat shock of ~24 °C – 25 °C followed by a 2 h recovery period. The authors interpreted this result as indicating that a relatively large temperature increase (11°C – 15 °C) was required to initiate a CSR. In light of the results presented here, I would suggest instead that this reinforces the idea of an acclimation-

independent physiological threshold around 24 °C – 26 °C. A similar acclimation-independent threshold for HSP induction has been described in channel catfish, which increase HSP70 synthesis in response to temperatures of 32.5 °C in liver tissue cultured from animals acclimated to 7 °C, 15 °C, and 25 °C (Koban et al. 1987). This would correspond to the onset temperature of the heat shock response, as outlined by Jeffries et al. (2018), indicating a mildly stressful condition, but one which can be recovered from with minimal long-term fitness implications. Meanwhile, one of the only studies examining the metabolic rate and metabolic scope of sea lamprey ammocoetes provides further evidence of a physiological threshold ~24 °C – 26 °C (Holmes and Lin 1994). Individuals acclimated to 25 °C had the lowest standard metabolic rate, active metabolic rate, and the lowest metabolic scope of any acclimation group tested (7 °C, 10 °C, 15 °C, and 20 °C), in addition to the lowest swimming speed and duration (Holmes and Lin 1994).

Potter and Beamish (1975) calculated an ultimate incipient lethal temperature of 31.4 °C for sea lamprey ammocoetes, while experimental incipient lethal temperatures for ammocoetes acclimated to 5 °C and 25 °C was 29.5 °C and 31 °C respectively. Potter and Beamish (1975) also performed a preliminary experiment on sea lamprey acclimated to 25 °C to evaluate approximate lethal temperatures, raising tank temperature at a rate of 0.3 °C – 0.5 °C per day. Sea lamprey ammocoetes began to die at a temperature of 31.0 °C, and final deaths occurred at 32.3 °C. Meanwhile McCauley (1963) was able to acclimate sea lamprey ammocoetes to 30 °C, but they displayed an incipient lethal temperature of 31.5 °C. During the 4 h heat shock trials performed here, temperatures of 32.5 °C and 31.9 °C were lethal to 60 % and 30 % of ammocoetes acclimated to 20 °C respectively, while exposure to 31.4 °C did not induce any mortality in the 13 °C acclimation group within 4 h. Meanwhile, inverse U-shaped expression

patterns consistent across acclimation groups were observed in liver *HSP90B1* and *HSPA9* expression after 4 h, with greater expression induced in response to the 26 °C treatment relative to the greater thermal challenge presented by the CTMax-2 °C treatment. Combined with the sustained elevated *JUN* expression in the 4 h CTMax-2 °C treatment in all acclimation groups, these data point toward a shift in the emphasis of the CSR from maintenance of homeostasis to potentially more serious cell cycle disruptions and inhibition of apoptosis around a temperature of 30 °C.

On the scale of sublethal thresholds described by Jeffries et al. (2018), this response pattern insinuates the presence of another acclimation-independent physiological threshold at ~30 °C where cells transition from a damage control and prevention response predominantly mediated by HSPs to ensuring short-term survival and minimizing permanent damage. I would further suggest that the thermal safety margin for sea lamprey ammocoetes between initiation of this extreme tier of CSR and the lethal threshold is very narrow. As mentioned previously, ammocoetes acclimated to 13.5 °C had a 100 % survival rate after 4 h exposure to 31.4 °C, while ammocoetes acclimated to 20 °C had a 70 % survival rate when exposed to 31.9 °C for 4 h. This implies the presence of a hard upper acutely lethal thermal ceiling around 32 °C for sea lamprey ammocoetes which again, appears to be independent of acclimation history.

2.5.4 Summary

This study demonstrated that sea lamprey are capable of mounting a rapid, dramatic change in gene expression in order to respond to an acute thermal stressor. Acclimation history has an influence on the induction temperature and severity of certain responses; however, overall gene expression patterns were very similar among acclimation groups. A more detailed

transcriptomic study would be useful in evaluating a broader range of gene families and functions differentially expressed during acute thermal stress. The present study provides a first attempt at understanding the transcript-level response to high temperatures in sea lamprey ammocoetes.

2.6 Figures and Tables

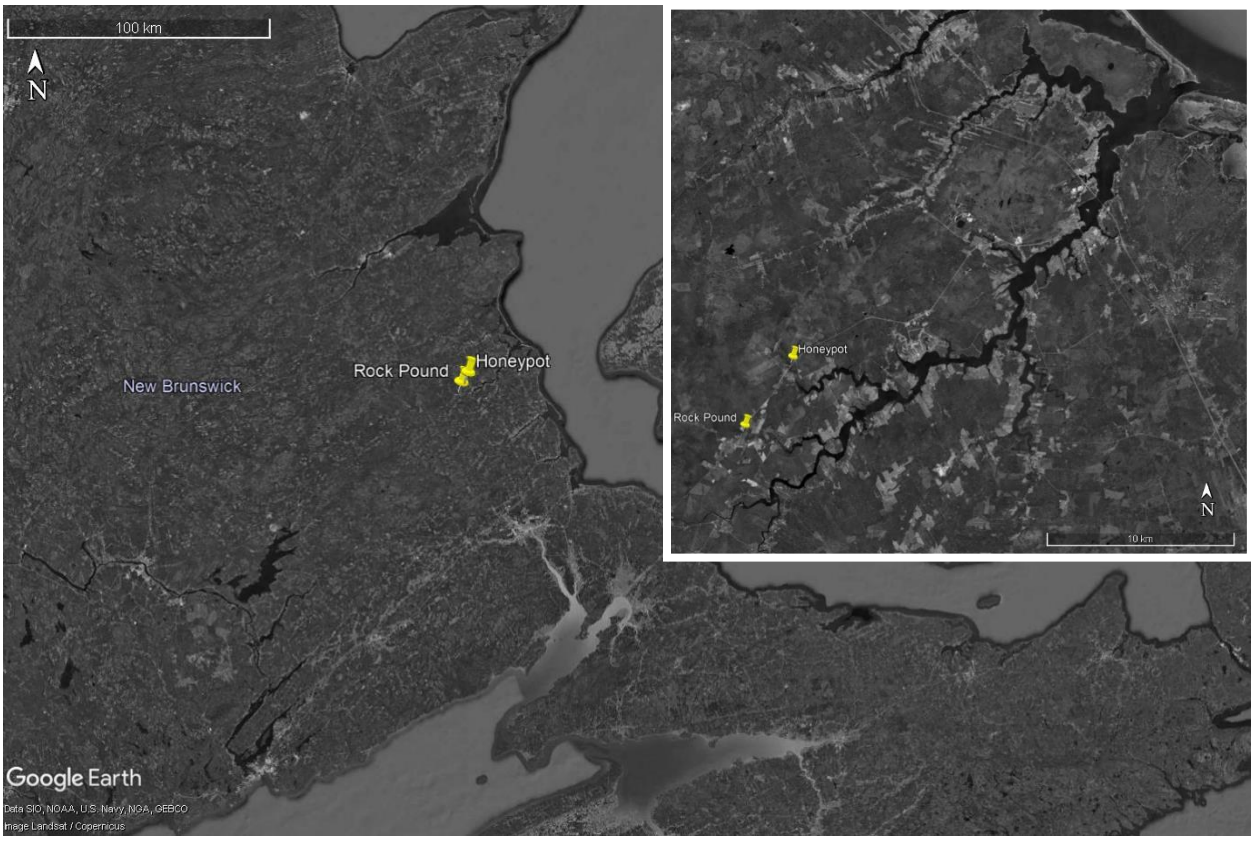


Figure 2.1. Sea lamprey ammocoete collection sites on the Richibucto River in New Brunswick, Canada.

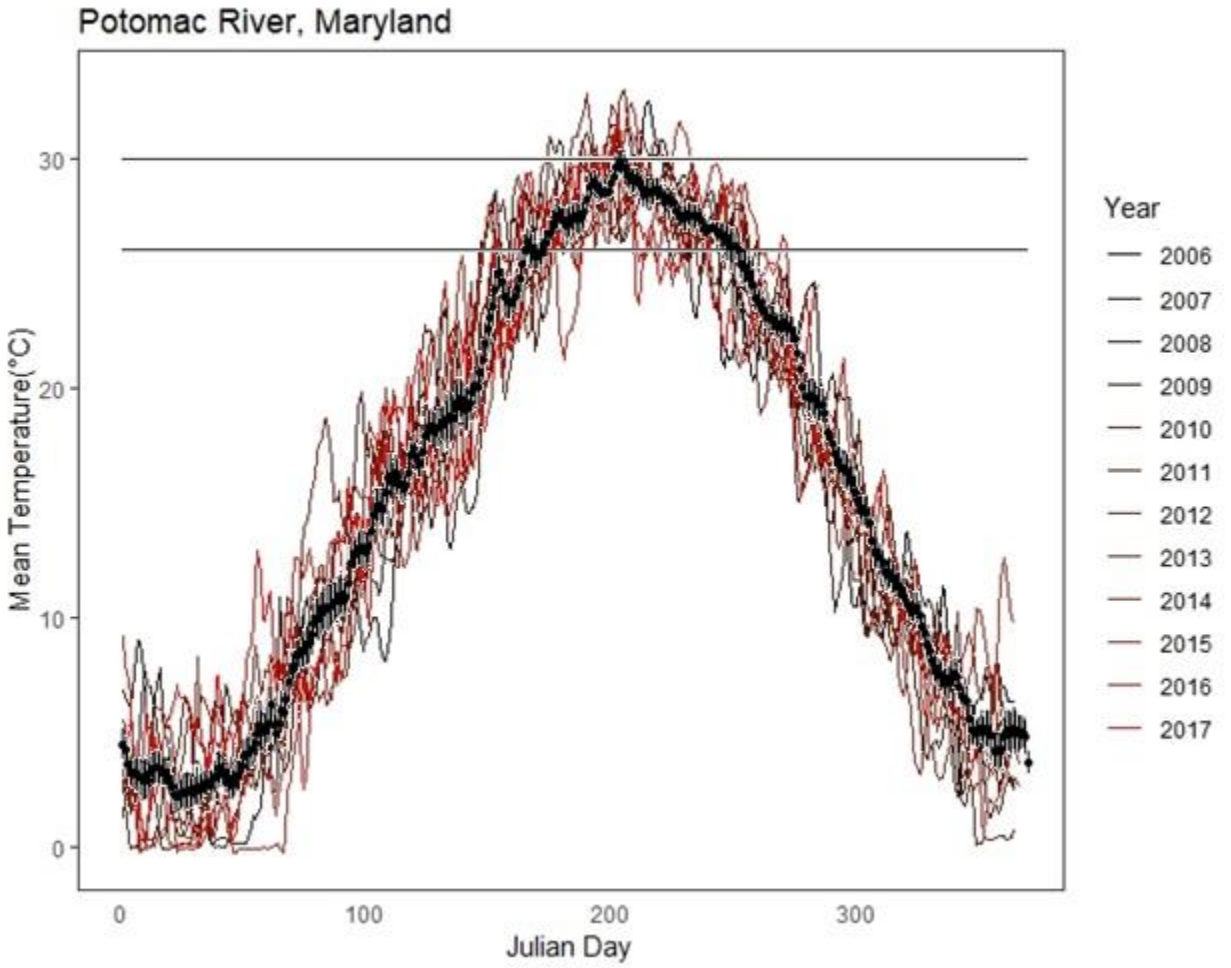


Figure 2.2. Daily mean temperature of the Potomac River, a tributary of ammocoete-producing streams in Maryland, USA, measured 1 ft from the river bottom. Horizontal lines indicate the putative thermal thresholds corresponding to the initiation of the heat shock response (26°C) and the initiation of the severe stress response (30°C) in sea lamprey ammocoetes. Data retrieved from the publicly available USGS database. Years are coloured along a gradient in chronological order, with 2006 in black and 2017 in red. Points indicate daily mean temperature values across all years, with error bars representing SEM.

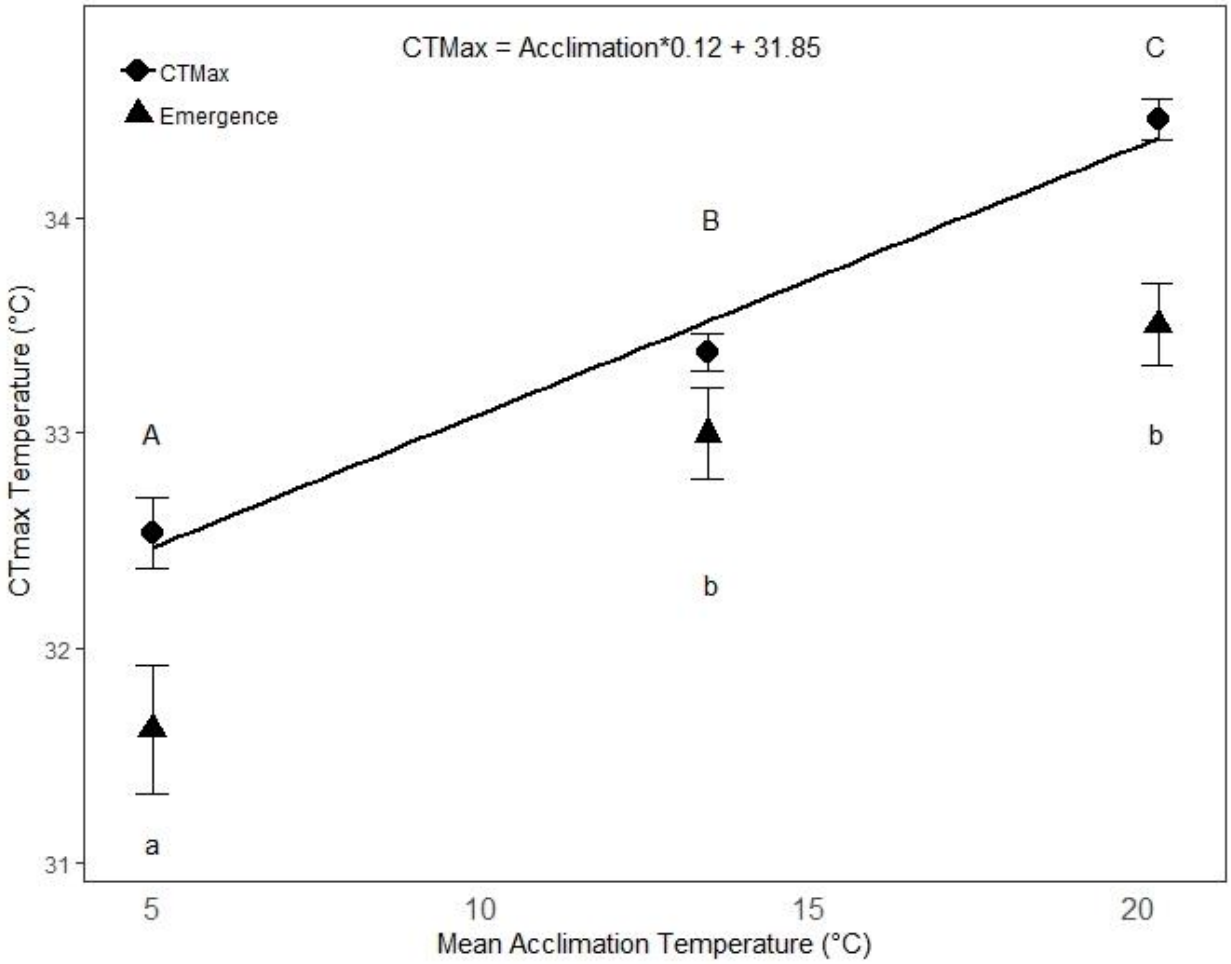


Figure 2.3. Mean (±SEM) CTMax (circles) increases ~0.12°C per 1°C increase in acclimation temperature. Emergence temperature (triangles) do not rise linearly but appear to be approaching a plateau. Capital letters denote statistical significance as revealed by planned contrasts between adjacent CTmax mean values ($p<0.05$). Lower-case letters denote the same between emergence temperatures.

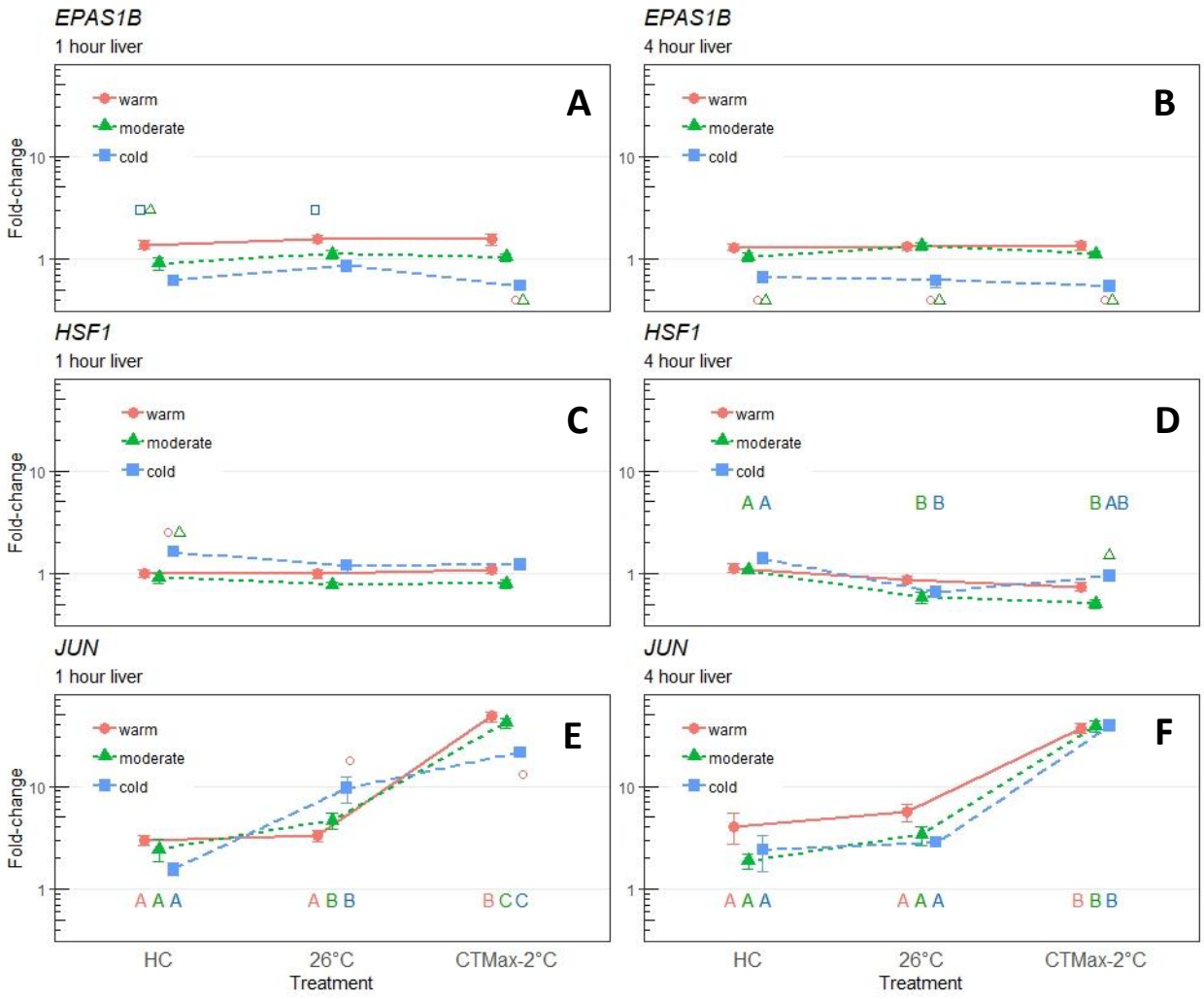


Figure 2.4. Mean (±SEM) relative fold-change in transcription factor mRNA expression in liver tissue from the acute heat shock experiment after both 1 (A, C, E) and 4 h (B, D, F), normalized to samples taken directly from the moderate acclimation tank (not shown). *EPAS1B* (A, B) expression is stratified according to acclimation history but is otherwise unaffected across both timepoints. *HSF1* (C, D) demonstrates a slight decreasing trend with increasing treatment temperature after 4 h. *JUN* (E, F) is upregulated following 1 h but demonstrates a recovery pattern in the 26°C treatment following 4 h. *JUN* is also the most sensitive to handling, as demonstrated by the increased expression in the moderate handling control. Coloured letters indicate statistically significant differences in expression between treatments within acclimation groups. The data point closest to an open symbol is different from the corresponding acclimation group at that treatment level.

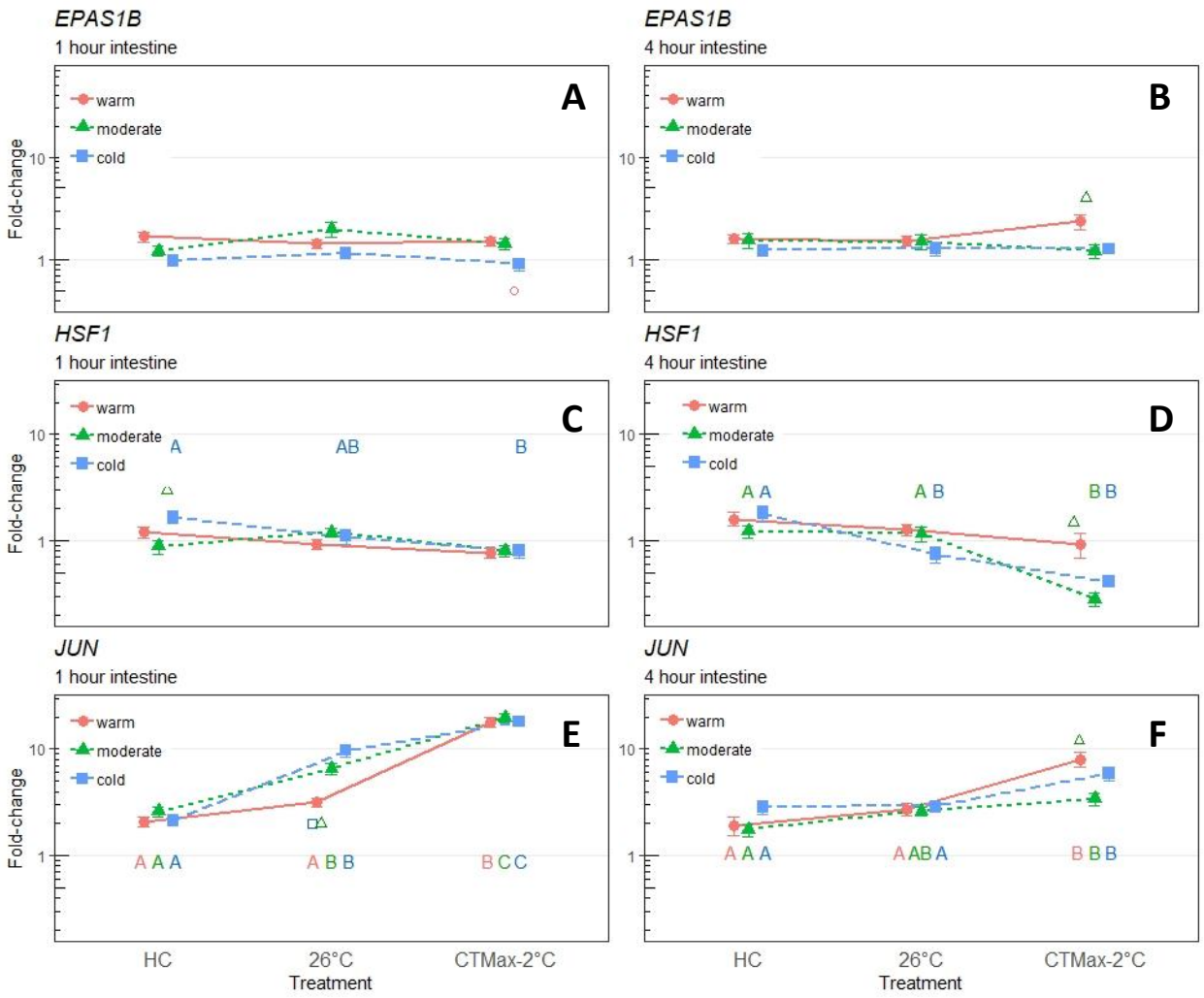


Figure 2.5. Mean (±SEM) relative fold-change in transcription factor mRNA expression in intestine from the acute heat shock experiment after both 1 (A, C, E) and 4 h (B, D, F), normalized to samples taken directly from the moderate acclimation tank (not shown). *EPAS1B* (A, B) expression is unaffected by either acclimation history or treatment temperature across both timepoints. *HSF1* (C, D) demonstrates a decreasing trend with increasing treatment temperature after 4 h. *JUN* (E, F) is upregulated following 1 h but demonstrates a recovery pattern in the 26°C treatment following 4 h. Coloured letters indicate statistically significant differences in expression between treatments within acclimation groups. The data point closest to an open symbol is different from the corresponding acclimation group at that treatment level.

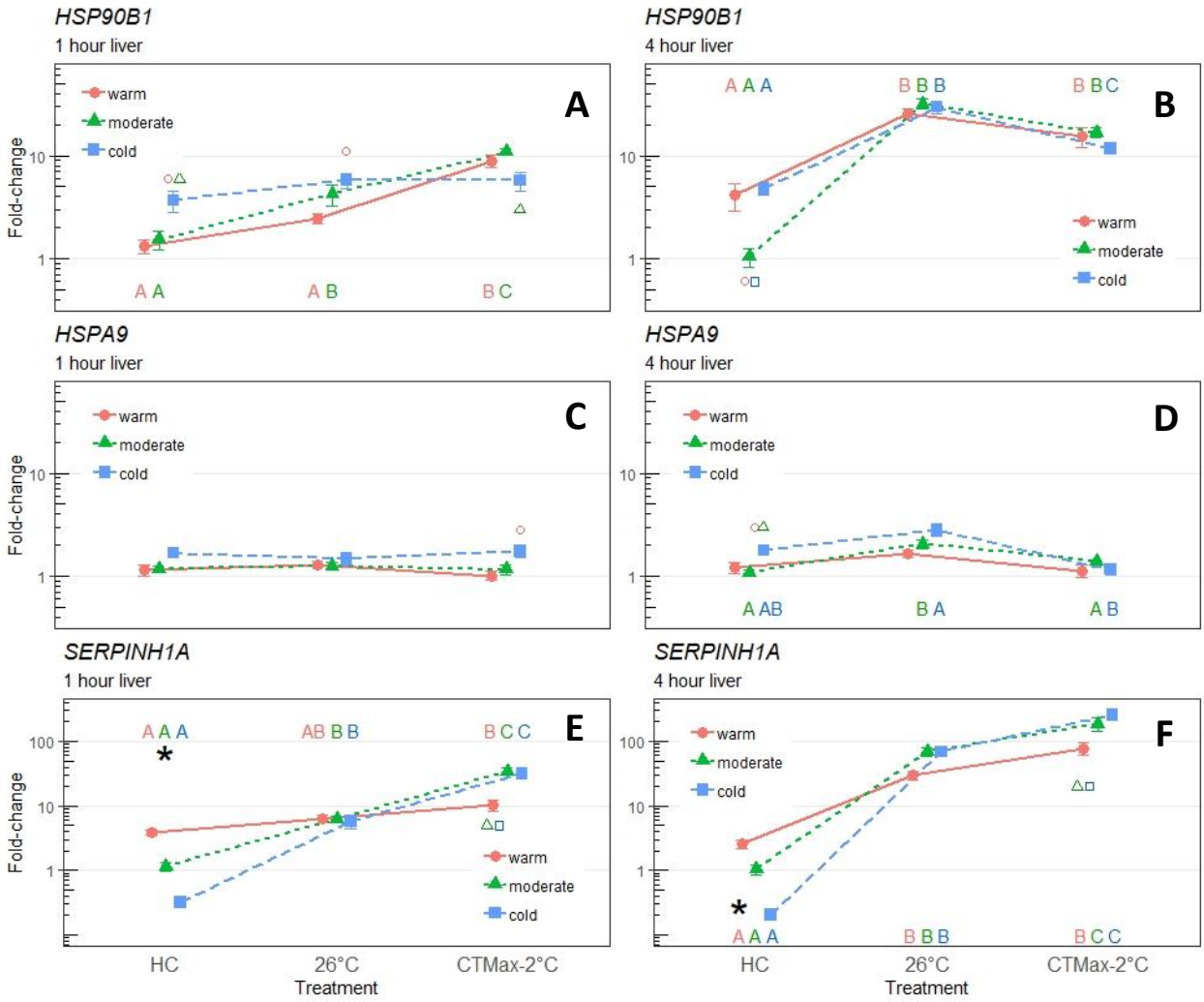


Figure 2.6. Mean (±SEM) relative fold-change in molecular chaperone mRNA expression in liver tissue from the acute heat shock experiment after both 1 (A, C, E) and 4 h (B, D, F), normalized to samples taken directly from the moderate acclimation tank (not shown). *HSP90B1* (A, B) expression generally rises with treatment temperature after 1 h, while it demonstrated an inverse U-shaped pattern after 4 h with a peak at the 26°C treatment. *HSPA9* (C, D) is unaffected after 1 h but again demonstrated an inverse U-shaped pattern after 4 h. *SERPINH1A* (E, F) expression rises with treatment temperature rapidly. It should be noted that all genes are presented on the same scale except for *SERPINH1A*, which required a much larger scale. Coloured letters indicate statistically significant differences in expression between treatments within acclimation groups. The data point closest to an open symbol is different from the corresponding acclimation group at that treatment level.

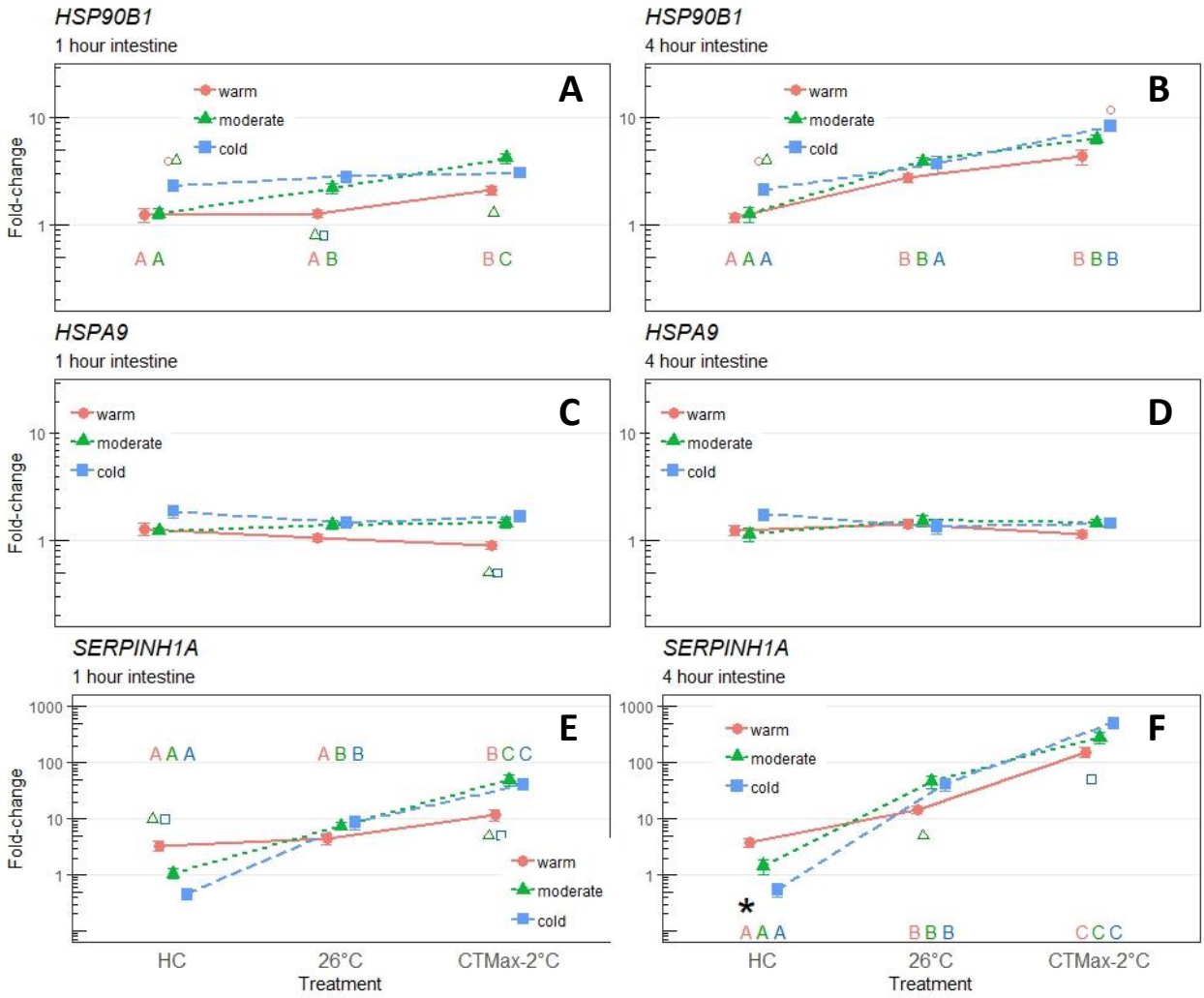


Figure 2.7. Mean (±SEM) relative fold-change in molecular chaperone mRNA expression in intestine from the acute heat shock experiment after both 1 (A, C, E) and 4 h (B, D, F), normalized to samples taken directly from the moderate acclimation tank (not shown). *HSP90B1* (A, B) expression generally rises with treatment temperature after 1 h, while a strong increase with temperature is observed after 4 h. *HSPA9* (C, D) is unaffected by either acclimation or treatment temperature after 1 and 4 h. *SERPINH1A* (E, F) expression rises with treatment temperature rapidly, and to a remarkable extent after 4 h. It should be noted that all genes are presented on the same scale except for *SERPINH1A*, which required a much larger scale. Coloured letters indicate statistically significant differences in expression between treatments within acclimation groups. The data point closest to an open symbol is different from the corresponding acclimation group at that treatment level.

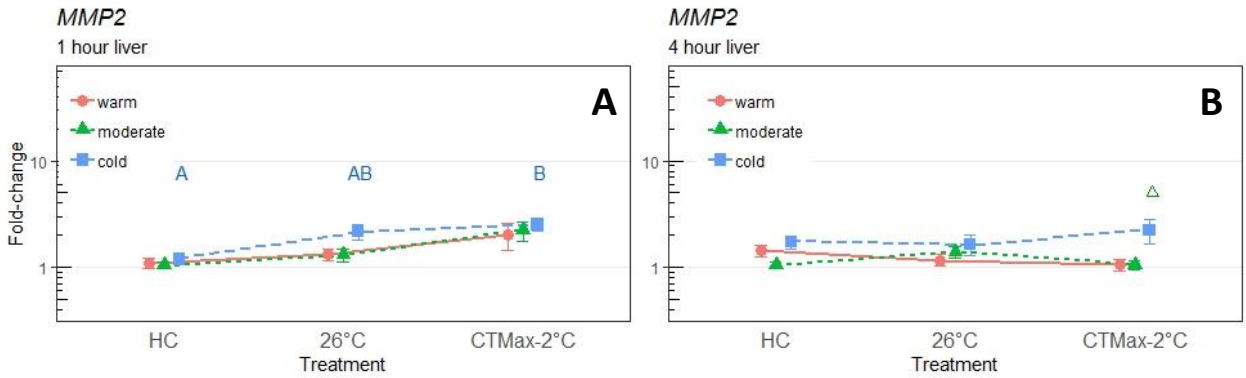


Figure 2.8. Mean (±SEM) relative fold-change in *MMP2* mRNA expression in liver tissue from the acute heat shock experiment after both 1 (A) and 4 h (B), normalized to samples taken directly from the moderate acclimation tank (not shown). Expression tends to increase slightly with treatment temperature after 1 h but no treatment effect was detected after 4 h. Coloured letters indicate statistically significant differences in expression between treatments within acclimation groups. The data point closest to an open symbol is different from the corresponding acclimation group at that treatment level.

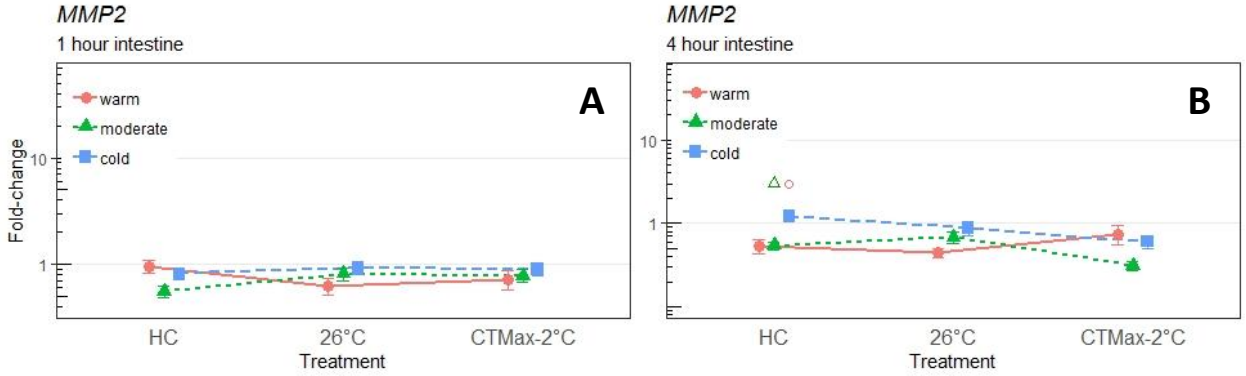


Figure 2.9. Mean (±SEM) relative fold-change in *MMP2* mRNA expression in intestine from the acute heat shock experiment after both 1 (A) and 4 h (B), normalized to samples taken directly from the moderate acclimation tank (not shown). Expression appears unaffected by either acclimation history or treatment temperature after both 1 and 4 h. Coloured letters indicate statistically significant differences in expression between treatments within acclimation groups. The data point closest to an open symbol is different from the corresponding acclimation group at that treatment level.

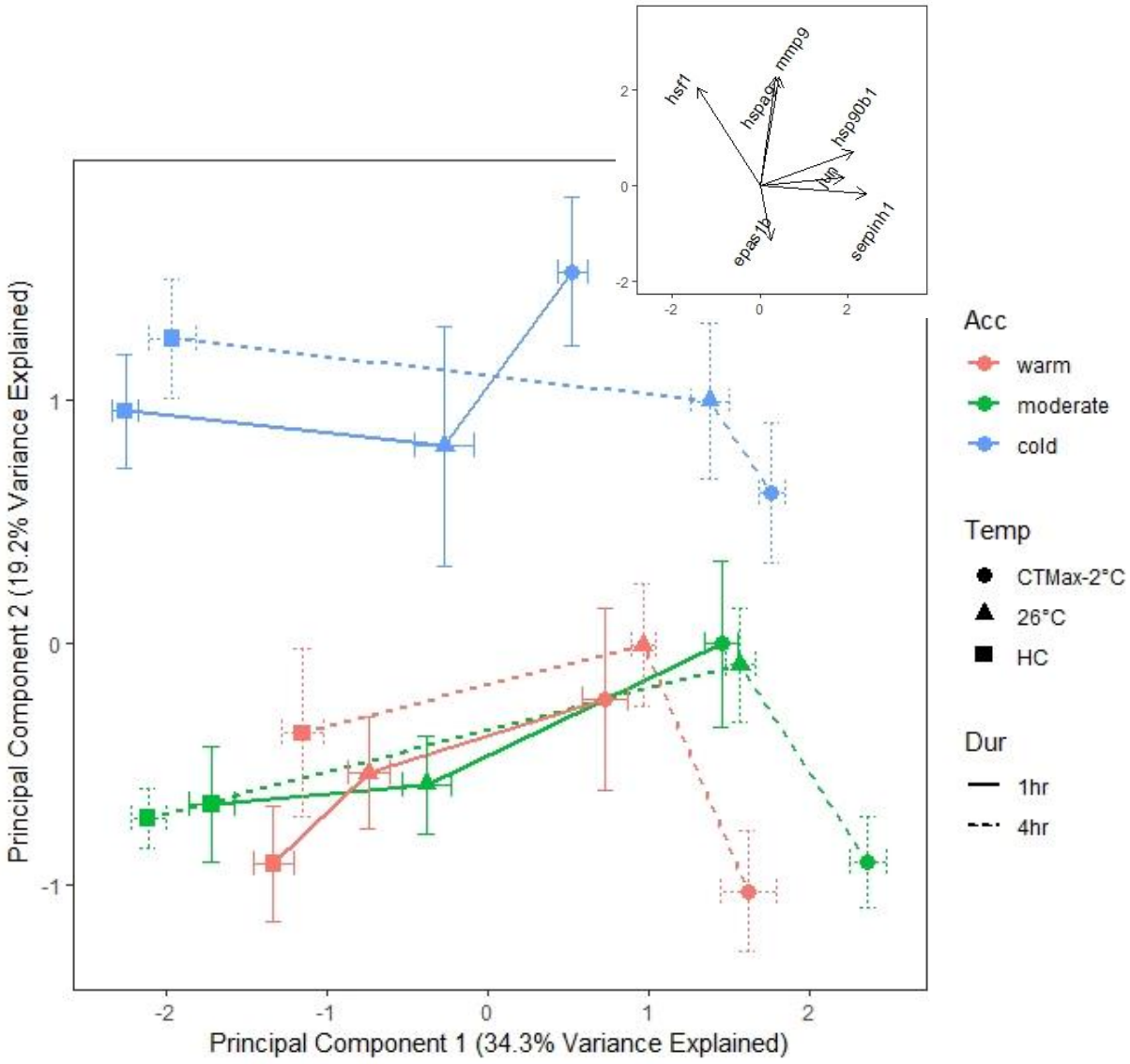


Figure 2.10. Principal Component analysis on liver gene expression results, with the loading plot inset. Mean values (±SEM) for each experimental group presented, with squares for the handling controls, triangles for the 26°C treatment, and circles for the CTMax-2°C treatment. Cold, moderate, and warm acclimation groups are demarcated by blue, green, and red symbols, respectively. Solid lines connect observations made after 1 h, dashed lines connect the 4 h timepoints within each acclimation group. In the loading plot, *HSPA9* and *MMP2* are overlapped.

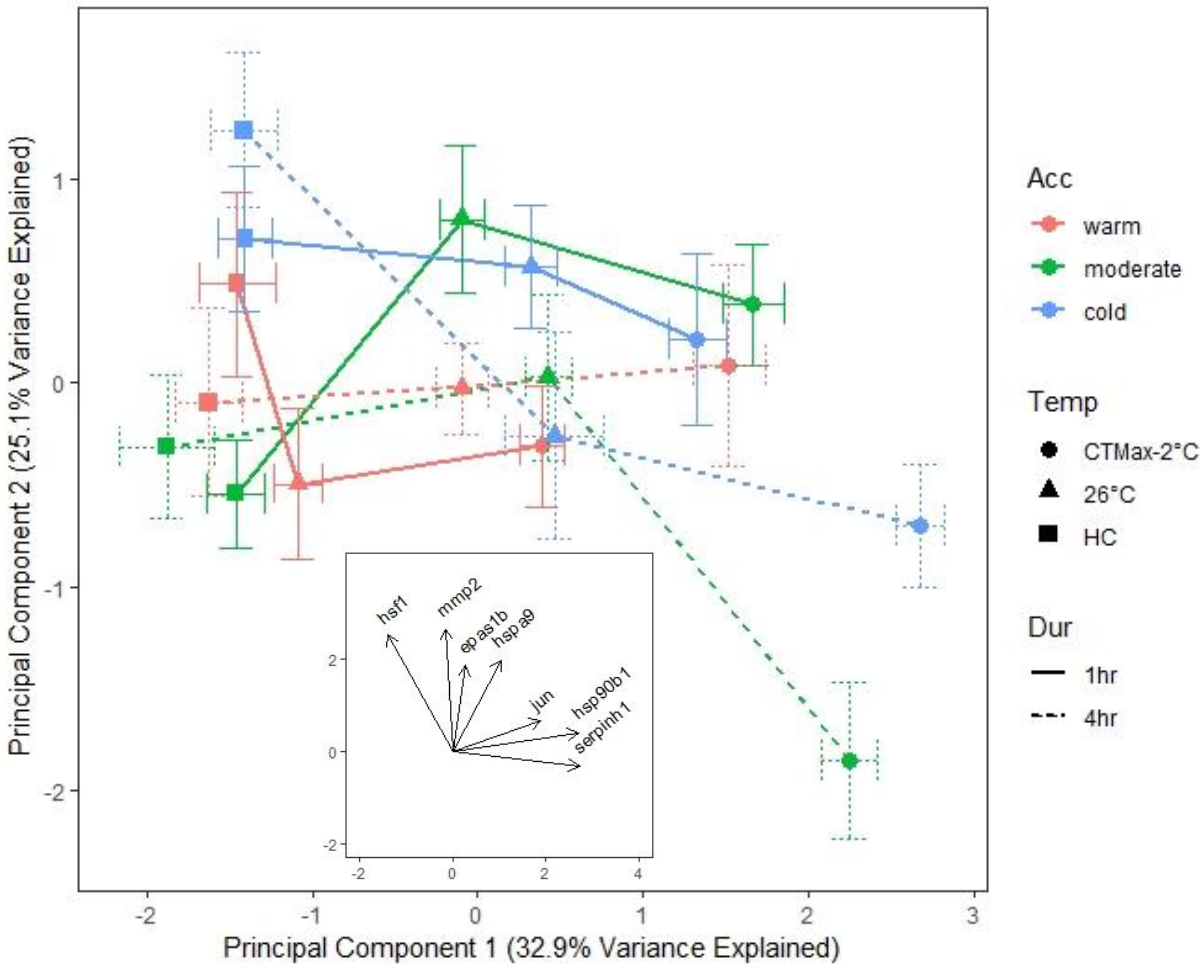


Figure 2.11. Principal component analysis on the intestine data with the loading plot inset. Mean values (±SEM) for each experimental group presented, with squares for the handling controls, triangles for the 26°C treatment, and circles for the CTMax-2°C treatment. Cold, moderate, and warm acclimation groups are demarcated by blue, green, and red symbols, respectively. Solid lines connect observations made after 1 h, dashed lines connect the 4 h timepoints within each acclimation group.

Table 2.1. Target and actual recorded temperatures in the acute heat shock treatments. Handling control (HC) treatments were intended to be as close as possible to the acclimation temperature. Each acclimation group's specific Critical Thermal Maximum (CTMax) was used to calculate the severe stressor temperature.

Acclimation group	Intended Treatment	Actual mean treatment temperature (°C)(±SD)	Increase from acclimation temperature (°C)
Cold	1 hr HC	5.4 (±1.2)	0.4
	4 hr HC	5.8 (±1.5)	0.8
	1 hr 26°C	25.8 (±0.7)	20.8
	4 hr 26°C	25.9 (±0.4)	20.9
	1 hr CTMax-2°C	31.1 (±0.4)	26.1
	4 hr CTMax-2°C	30.7 (±0.5)	25.7
Moderate	1 hr HC	15.3 (±0.3)	2.3
	4 hr HC	15.4 (±0.4)	2.4
	1 hr 26°C	25.4 (±1.6)	12.4
	4 hr 26°C	26.1 (±0.8)	13.1
	1 hr CTMax-2°C	31.7 (±0.6)	18.7
	4 hr CTMax-2°C	31.4 (±0.4)	18.4
Warm	1 hr HC	20.2 (±0.1)	0.2
	4 hr HC	20.0 (±0.1)	0.0
	1 hr 26°C	26.5 (±0.6)	6.5
	4 hr 26°C	26.3 (±0.5)	6.3
	1 hr CTMax-2°C	33.3 (±0.2)	13.3
	4 hr CTMax-2°C	32.5 (±0.5) (40% survival)	12.5
		31.9 (±0.9) (70% survival)	11.9

Table 2.2. Information on genes and primers used in the present study. Accession numbers are available on the current Ensembl release, v.95. *JUN* and *HSF1* are unannotated in the current Ensembl database but were annotated as such when the database was first accessed to design primers (v.90). These sequences were BLASTed in a different sea lamprey genome (SIMRbase) which has them annotated as *JUN* and *HSF1*. The *EPAS1B* sequence is annotated as such in Ensembl v.95 but is annotated as *HIF1A* in the SIMRbase genome.

Functional group	Gene Code	Full gene name	Accession No.	Efficiency	Primer sequence
Transcription factor	*JUN*	Jun proto-oncogene, AP-1 transcription factor subunit	ENSPMAT00000004097.1	101%	F-ATG AAC TCC AGG ATG GAA GC R-GGA GAG GCT CTT TTT CAT TGC
	HSF1	(Predicted) Heat shock transcription factor 1	ENSPMAT00000005090.1	101%	F-CAT GGG CAG GAT CAT AAG TG R-TCA TGG TGT TGA GTC GGT TG
	EPAS1B	Endothelial PAS domain protein 1b	ENSPMAT00000010244.1	99.5%	F-TAC GAA TGC CTT GCG ACA AC R-TGT TGA AGT GGT GGA CAT GC
Molecular Chaperone	*HSP90B1*	Heat shock protein 90 Beta family member 1	ENSPMAT00000001245.1	99.5%	F-TCA CGT TGG TGC TCA AGG AA R-GGT TCT TCA ACG GTC TCG GT
	HSPA9	Heat shock protein family A (hsp70) member 9	ENSPMAT00000007246.1	98.5%	F-CCA ACC CAA ACA ACA CCT TC R-TTC TGC ACA TCG GAG TCA TC
	SERPINH1A	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1a	ENSPMAT00000008277.1	97%	F-ATG CAT CGC ACA GGC TTC TA R-TTG AGC TGC ATC TCC AGC AG
Proteolytic Protein	*MMP2*	Matrix metallopeptidase 2	ENSPMAT00000003005.1	98.5%	F-GCC CAA GTA CGA GGT GTG TA R-GCG CAT GTG CAT CTT GTT CT
Ribosomal protein (Reference)	*RPL8*	Ribosomal protein L8	ENSPMAT00000009691.1	100%	F-GCG CTC ACA ACA AAC ACA AG R-CGT GGA TTA TGT CCT TGA CG
	RPL13A	Ribosomal protein L13A	ENSPMAT00000007151.1	100%	F-ATC GCC TGA AGG TGT TTG AC R-TAC TGC AAA CTT GCG TGT GG
	RPS6	Ribosomal protein s6	ENSPMAT00000008266.1	98%	F-AGA TCC GCA AGC TCT TCA AC R-TTT GCC TTC CTT GGT GAG TG

Table 2.3. Summary table of planned contrasts in two-way ANOVAs for liver gene expression data. Bonferroni-corrected p-values presented here. **Bold** numbers indicate p<0.05, *italics* indicate 0.05<p<0.1.

	Defined Contrasts		P-values						
			HSF1	*EPAS1B*	*JUN*	*HSP90B1*	*HSPA9*	*SERPINH1A*	*MMP2*
1 hr exposure	Warm	25°C vs HC	1.0	1.0	1.0	0.130214	1.0	8.63e-1	1.0
		25°C vs Ctmax-2°C	1.0	1.0	**<2e-16**	**4.57e-5**	9.68e-1	9.25e-1	1.0
		CTMax-2°C vs HC	1.0	1.0	**<2e-16**	**3.36e-10**	1.0	**2.67e-3**	1.0
	Moderate	25°C vs HC	1.0	1.0	**3.16e-2**	**6.46e-3**	1.0	**9.53e-11**	1.0
		25°C vs Ctmax-2°C	1.0	1.0	**9.59e-14**	**1.51e-4**	1.0	**1.17e-9**	1.0
		CTMax-2°C vs HC	1.0	1.0	**<2e-16**	**1.69e-11**	1.0	**<2e-16**	1.72e-1
	Cold	25°C vs HC	3.95e-1	1.0	**1.99e-7**	4.94e-1	1.0	**<2e-16**	4.87e-1
		25°C vs Ctmax-2°C	1.0	1.79e-1	**6.41e-4**	1.0	1.0	**3.84e-10**	1.0
		CTMax-2°C vs HC	8.68e-1	1.0	**<2e-16**	8.02e-1	1.0	**<2e-16**	**4.73e-2**
	Handling Control	Warm vs Moderate	1.0	**4.78e-2**	1.0	1.0	1.0	**1.84e-6**	1.0
		Moderate vs Cold	**1.17e-3**	8.47e-1	1.0	**2.82e-2**	2.58e-1	**4.12e-6**	1.0
		Warm vs Cold	**2.45e-2**	**3.78e-5**	1.01e-1	**7.66e-3**	*6.07e-2*	**<2e-16**	1.0
	26°C	Warm vs Moderate	1.0	5.13e-1	1.0	1.0	1.0	1.0	1.0
		Moderate vs Cold	3.80e-1	1.0	7.20e-1	1.0	1.0	1.0	1.0
		Warm vs Cold	1.0	**5.38e-3**	**2.79e-2**	**4.60e-2**	1.0	1.0	1.0
	CTMax-2°C	Warm vs Moderate	3.80e-1	3.14e-1	1.0	1.0	1.0	**2.21e-6**	1.0
		Moderate vs Cold	*5.52e-2*	**1.17e-3**	1.22e-1	**3.80e-2**	1.04e-1	1.0	1.0
		Warm vs Cold	1.0	**6.35e-8**	**1.91e-2**	6.85e-1	**5.21e-3**	**2.11e-5**	1.0
4 hr exposure	Warm	25°C vs HC	1.0	1.0	2.48e-1	**1.89e-12**	2.38e-1	**5.52e-13**	1.0
		25°C vs Ctmax-2°C	1.0	1.0	**8.11e-9**	2.75e-1	*7.45e-2*	1.06e-1	1.0
		CTMax-2°C vs HC	1.02e-1	1.0	**3.20e-14**	**5.75e-8**	1.0	**<2e-16**	1.0
	Moderate	25°C vs HC	**3.29e-4**	5.37e-1	6.50e-1	**<2e-16**	**3.30e-3**	**<2e-16**	1.0
		25°C vs Ctmax-2°C	1.0	1.0	**4.08e-13**	1.15e-1	5.43e-1	**6.30e-3**	1.0
		CTMax-2°C vs HC	**1.20e-05**	1.0	**<2e-16**	**<2e-16**	1.0	**<2e-16**	1.0
	Cold	25°C vs HC	**5.57e-06**	1.0	8.64e-1	**5.80e-11**	1.57e-1	**<2e-16**	1.0
		25°C vs Ctmax-2°C	1.21e-1	1.0	**3.36e-13**	**4.97e-3**	**1.87e-5**	**1.74e-4**	1.0
		CTMax-2°C vs HC	2.08e-1	1.0	**2e-16**	**1.87e-3**	1.17e-1	**<2e-16**	1.0
	Handling Control	Warm vs Moderate	1.0	8.34e-1	1.0	**4.80e-5**	1.0	**2.74e-2**	1.0
		Moderate vs Cold	1.0	**6.80e-3**	1.0	**8.93e-9**	**3.74e-2**	**8.16e-8**	4.73e-1
		Warm vs Cold	1.0	**2.44e-6**	1.0	1.0	*5.78e-2*	**2.40e-14**	1.0
	26°C	Warm vs Moderate	*6.90e-2*	1.0	1.0	1.0	1.0	1.06e-1	1.0
		Moderate vs Cold	1.0	**3.35e-8**	1.0	1.0	1.0	1.0	1.0
		Warm vs Cold	6.40e-1	**1.19e-7**	9.57e-1	1.0	*8.36e-2*	*6.57e-2*	1.0
	CTMax-2°C	Warm vs Moderate	1.60e-1	1.0	1.0	1.0	8.70e-1	**6.34e-3**	1.0
		Moderate vs Cold	**5.10e-4**	**2.01e-9**	1.0	1.0	1.0	1.0	*5.61e-2*
		Warm vs Cold	1.0	**3.28e-9**	1.0	1.0	1.0	**9.19e-5**	**4.17e-2**

Table 2.4. Summary table of planned contrasts in two-way ANOVAs on intestine gene expression data. Bonferroni-corrected p-values presented here. **Bold** numbers indicate p<0.05, *italics* indicate 0.05<p<0.1.

	Defined Contrasts		P-values						
			HSF1	*EPAS1B*	*JUN*	*HSP90B1*	*HSPA9*	*SERPINH1A*	*MMP2*
1 hr exposure	Warm	25°C vs HC	1.0	1.0	1.81e-1	1.0	1.0	1.0	3.11e-1
		25°C vs Ctmax-2°C	1.0	1.0	**<2e-16**	**4.11e-2**	1.0	**2.25e-2**	1.0
		CTMax-2°C vs HC	3.56e-1	1.0	**<2e-16**	**9.77e-3**	4.60e-1	**1.01e-3**	1.0
	Moderate	25°C vs HC	8.51e-1	2.58e-1	**4.82e-6**	**2.14e-2**	1.0	**3.72e-8**	1.0
		25°C vs Ctmax-2°C	3.48e-1	1.0	**6.91e-9**	**3.10e-3**	1.0	**3.98e-7**	1.0
		CTMax-2°C vs HC	1.0	1.0	**<2e-16**	**3.46e-9**	1.0	**<2e-16**	1.0
	Cold	25°C vs HC	7.09e-1	1.0	**7.57e-12**	1.0	1.0	**3.04e-13**	1.0
		25°C vs Ctmax-2°C	1.0	1.0	**2.50e-3**	1.0	1.0	**6.31e-5**	1.0
		CTMax-2°C vs HC	**2.88e-3**	1.0	**<2e-16**	1.0	1.0	**<2e-16**	1.0
	Handling Control	Warm vs Moderate	1.0	1.0	1.0	1.0	1.0	**3.93e-3**	3.79e-1
		Moderate vs Cold	**1.37e-2**	1.0	1.0	**8.70e-3**	1.46e-1	*8.24e-2*	1.0
		Warm vs Cold	1.0	*5.60e-2*	1.0	**1.52e-3**	1.72e-1	**3.31e-8**	1.0
	26°C	Warm vs Moderate	1.0	1.0	**7.25e-4**	**1.82e-2**	1.0	9.19e-1	1.0
		Moderate vs Cold	1.0	2.18e-1	5.64e-1	1.0	1.0	1.0	1.0
		Warm vs Cold	1.0	1.0	**2.91e-7**	**2.01e-4**	6.19e-1	6.60e-1	3.63e-1
	CTMax-2°C	Warm vs Moderate	1.0	1.0	1.0	**1.23e-3**	**2.49e-2**	**1.08e-4**	1.0
		Moderate vs Cold	1.0	1.26e-1	1.0	1.0	1.0	1.0	1.0
		Warm vs Cold	1.0	**3.66e-2**	1.0	4.35e-1	**6.79e-4**	**1.25e-3**	1.0
4 hr exposure	Warm	25°C vs HC	1.0	1.0	5.10e-1	**2.97e-4**	1.0	**3.07e-4**	1.0
		25°C vs Ctmax-2°C	5.28e-1	7.01e-1	**3.43e-5**	7.90e-1	1.0	**1.60e-9**	1.0
		CTMax-2°C vs HC	*7.18e-2*	1.0	**2.35e-9**	**6.24e-8**	1.0	**<2e-16**	1.0
	Moderate	25°C vs HC	1.0	1.0	6.26e-1	**7.80e-8**	9.56e-1	**<2e-16**	1.0
		25°C vs Ctmax-2°C	**8.91e-7**	1.0	1.0	3.35e-1	1.0	**2.53e-6**	*6.54e-2*
		CTMax-2°C vs HC	**2.24e-7**	1.0	**2.75e-2**	**1.57e-12**	1.0	**<2e-16**	3.30e-1
	Cold	25°C vs HC	**8.88e-3**	1.0	1.0	3.36e-1	1.0	**<2e-16**	1.0
		25°C vs Ctmax-2°C	6.08e-1	1.0	**2.02e-2**	**8.51e-5**	1.0	**6.58e-11**	1.0
		CTMax-2°C vs HC	**2.95e-6**	1.0	**3.25e-3**	**2.56e-9**	1.0	**<2e-16**	*5.99e-2*
	Handling Control	Warm vs Moderate	1.0	1.0	1.0	1.0	1.0	**2.87e-2**	1.0
		Moderate vs Cold	1.0	1.0	7.02e-1	**3.56e-2**	1.23e-1	**3.04e-2**	**2.39e-2**
		Warm vs Cold	1.0	1.0	6.32e-1	**4.21e-2**	6.77e-1	**7.33e-8**	**2.46e-3**
	26°C	Warm vs Moderate	1.0	1.0	1.0	1.0	1.0	**2.94e-2**	1.0
		Moderate vs Cold	8.87-1	1.0	1.0	1.0	1.0	1.0	1.0
		Warm vs Cold	2.57e-1	1.0	1.0	1.0	1.0	1.25e-1	2.52e-1
	CTMax-2°C	Warm vs Moderate	**5.47e-4**	**1.65e-2**	**2.15e-3**	4.36e-1	1.0	1.0	1.22e-1
		Moderate vs Cold	1.0	1.0	1.78e-1	1.0	1.0	7.53e-1	2.31e-1
		Warm vs Cold	3.06e-1	*7.61e-2*	1.0	**6.50e-3**	1.0	**9.81e-3**	1.0

Chapter 3: Thermal tolerance in a New Brunswick anadromous population and a landlocked population of sea lamprey

3.1 Abstract

Invasion theory predicts that invasive populations often display increased tolerance to a range of environmental stressors. Sea lamprey (*Petromyzon marinus*), an anadromous fish species native to the North Atlantic Ocean, successfully invaded the Laurentian Great Lakes of North America in the early-mid-20th century, to the detriment of local fish populations. A successful, binational control program between the US and Canada has been in place since the 1950s to control sea lamprey populations, primarily utilizing the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). In the present study, New Brunswick and landlocked populations of sea lamprey ammocoetes were evaluated for their upper thermal tolerance using a critical thermal maximum (CTMax) methodology, at a range of ecologically-relevant acclimation temperatures. Responses to acclimation conditions were also examined for both populations through examination of mRNA expression of eight target genes, seven of which are involved in the cellular stress response (*EPAS1B*, *HSF1*, *JUN*, *HSPA9*, *HSP90B1*, *SERPINH1A*, *MMP2*), and *UGT*, a gene encoding an enzyme involved in detoxifying the lampricide TFM. The landlocked population demonstrated a consistently greater CTMax than the New Brunswick population, ~0.8 °C greater at any given acclimation temperature. The landlocked population also demonstrated increased plasticity in *JUN*, *HSP90B1*, and *HSPA9* in response to the different temperature treatments. Expression of *UGT* in the liver of the landlocked population was also differentially expressed under moderate conditions relative to the New Brunswick population, which may influence how the landlocked population processes the lampricide TFM.

3.2 Introduction

Invasive populations are hypothesized to demonstrate greater phenotypic plasticity in relation to both native heterospecifics and source population conspecifics (Yeh and Price 2004; Parsons and Robinson 2006; Ghalambor et al. 2007; Torres-Dowdall et al. 2012; Lande 2015). Plasticity in temperature tolerance and expression of cellular stress proteins have been specifically suggested to be beneficial to invasive species success (Henkel et al. 2009; Zerebecki and Sorte 2011; Hill et al. 2013; Bates et al. 2013).

Estimates of upper thermal tolerance are commonly produced by the critical thermal maximum (CTMax) method, a dynamic measurement where individuals are placed in a container at their acclimation temperature and exposed to a constant temperature increase (typically ~0.3 °C min^{-1}) until a repeatable, sublethal event is observed, at which point they are rescued, returned to their acclimation temperature, and monitored for survival (Beitinger et al. 2000). Measuring the change in CTMax over a range of acclimation temperatures (acclimation response ratio – ARR) can provide an estimate of species' acclimation ability, a reaction norm which can quantify phenotypic plasticity (Beitinger et al. 2000; Stillman 2003; Comte and Olden 2017).

$$Equation\ 3.1: ARR = \frac{\Delta CTMax}{\Delta Acclimation\ temperature}$$

Investigating traits between invasive vs native populations of a single species can provide clues as to how species may adapt to changing environments (Moran and Alexander 2014). Only one study was found which compared thermal tolerance between invasive and native populations of a single species. Rooke et al. (2017) examined thermal physiology, including CTMax, between a native pumpkinseed (*Lepomis gibbosus*) population in Canada with an invasive population in Spain. In contrast to expected results, negligible differences in CTMax were found

between populations at a range of acclimation temperatures, despite the invasive, Spanish population, living in a generally warmer climate than the native Canadian population.

Other studies have compared thermal tolerance between invasive and native species in the same habitat (Carveth et al. 2006; Henkel et al. 2009; Zerebecki and Sorte 2011; Bates et al. 2013). These studies have provided evidence that invasive species generally demonstrate greater thermal tolerance, or a greater upper thermal limit than non-invasive species. For example, CTMax was assessed in 18 fish species from Arizona, and four of the six species with the greatest CTMax were invasive (Carveth et al. 2006). The delta smelt (*Hypomesus transpacificus*), a native fish to the San Francisco Estuary, was also recently shown to have a significantly lower CTMax than both Mississippi silversides (*Menidia beryllina*) and largemouth bass (*Micropterus salmoides*), which both have successful invasive populations in the system (Davis et al. 2019). Extensive work has been performed on the blue mussel, *Mytilus galloprovincialis*, a native of the Mediterranean Sea which has invaded the Pacific coast of North America, with multiple studies demonstrating its increased capacity to withstand higher temperatures (Braby and Somero 2006; Schneider 2008; Lockwood and Somero 2011), salinities (Braby and Somero 2006), and air exposure (Schneider 2008) relative to native heterospecifics. A transcriptomic study revealed minor differences in the transcriptional response to an acute thermal shock between this invasive species and a closely related native, *M. trossulus*, which it is displacing on the California coast (Lockwood et al. 2010). These authors credit four genes that demonstrated differential regulation between species – three oxidative stress genes and *HSP24* – for the higher heat tolerance observed in the invader compared to its native relative.

Studies have also examined differences in thermal tolerance within a species across its geographic range (Hart 1952; McCauley 1958; Matthews 1986; Gaston and Spicer 1998; Fangue

et al. 2006; Pereira et al. 2017; Dammark et al. 2018; Chen et al. 2018). These have returned mixed results, with some species demonstrating physiological differentiation between geographically separated populations, while others do not. For instance, no variation in CTMax was found in red shiner (*Notropis lutrensis*) across ~10° of latitude in the United States (Matthews 1986), while two populations of arctic charr (*Salvelinus alpinus*), separated by ~8° latitude showed a roughly 0.5 °C difference in upper lethal temperature at two different acclimation temperatures (McCauley 1958). Results from these types of experimentation are dependent on a variety of external factors, including microenvironment conditions, the length of time the population has been established in each area, the level of genetic drift between populations, and possible transgenerational and developmental plasticity effects. Regardless, these projects can illustrate intraspecific variation, as well as species-specific capacity to withstand environmental change through local adaptation (Moran and Alexander 2014). Fangue et al. (2016) examined both CTMax and gene expression profiles of five heat shock proteins in response to an acute heat stress in geographically disparate populations of the common killifish (*Fundulus heteroclitus*). They found that the southern population, which inhabits a generally warmer environment than the northern population against which they were compared, had a consistently greater CTMax (Fangue et al. 2006).

An increase in transcriptional plasticity was demonstrated in derived freshwater threespine stickleback (*Gasterosteus aculeatus*) when compared with nearby marine populations in response to a chronic thermal challenge (Morris et al. 2014). This lends support to the hypothesis that populations evolve an increase in plasticity in response to colonization of novel environments. A similar result was demonstrated in a pair of invasive gobies in the Great Lakes, with the more widespread round goby (*Neogobius melanostomus*) demonstrating greater

transcriptional plasticity than the relatively more restricted, less successful tubenose goby (*Proterorhinus semilunaris*; Wellband and Heath 2017).

Sea lamprey (*Petromyzon marinus*) are a classic example of an invasive species (*sensu* Valéry et al. 2008). Native to tributaries on both coasts of the North Atlantic Ocean, they were first reported in Lake Erie in 1921 following renovations of the Welland Canal, which allowed them to bypass the significant physical barrier of Niagara Falls (Aron and Smith 1971). Sea lamprey were then free to colonize Lakes Huron, Michigan, and Superior, with initial observations recorded in 1937, 1936, and 1938, respectively, and were abundant in all of the Great Lakes by the 1950s to the detriment of local fish species and fisheries (Applegate 1950; Smith 1968; Lawrie 1970; Smith and Tibbles 1980). There is some debate surrounding the origin of sea lamprey in Lakes Ontario, Champlain, and the Finger Lakes of New York, with some authors suggesting sea lamprey invaded these lakes via the Erie and Champlain Canals (Aron and Smith 1971; Eshenroder 2009, 2014), while evidence from mitochondrial haplotype and nuclear microsatellite analyses suggest colonization occurred following glacial retreat ~12,500 years ago (Wigley 1959; Daniels 2001; Waldman et al. 2004, 2006, 2009; Bryan et al. 2005; D'Aloia et al. 2015).

Bryan et al. (2005) demonstrated that colonization of the upper Great Lakes by sea lamprey came about by a series of population bottlenecks. Genetic bottlenecks are common in invasive populations, but somewhat counterintuitively may be beneficial, as it can purge deleterious alleles responsible for inbreeding depression, and may increase additive genetic variation (Lindholm et al. 2005; Dlugosch and Parker 2008; Facon et al. 2011; Hodgins et al. 2018). The authors speculate that a small number of pioneering sea lamprey were able to continuously invade novel territories, resulting in a founder effect which produced the

bottlenecked population structure observed today. These founder effects may have arisen because some individuals were better able to tolerate the newly invaded environment, either through novel mutations, or through existing genetic variation. However, it could also be due to distribution patterns of juveniles and migrating adults, population size and density, or other considerations not having to do with environmental tolerance. Due to the relatively short time scale required for colonization of the entire Great Lakes basin, it is possible that a small number of the most pre-adapted individuals became the source population for the next lake in the invasion sequence (Bryan et al., 2005). With their high fecundity rates, the successful reproduction of a small number of individuals could have led to a relatively large population in only a few generations.

Since the 1950s, an international coalition between the United States and Canada has been implementing control measures to attempt to reduce sea lamprey populations in the Great Lakes. The most successful control method used to date has been the application of 3-trifluoromethyl-4-nitrophenol (TFM) to ammocoete-hosting streams (Smith and Tibbles 1980; Birceanu et al. 2009, 2011), a pesticide lethal to all lamprey species at concentrations which normally have little impact on other fish species (although, see Boogaard et al. 2003; McLaughlin et al. 2003; Birceanu et al. 2014; Sakamoto et al. 2016; Birceanu and Wilkie 2018). The lethal effect of TFM stems from its ability to uncouple mitochondrial oxidative phosphorylation after accumulating to appropriate concentrations in body tissues, interfering with ATP production (Birceanu et al. 2011). Once existing ATP has been depleted, anaerobic pathways are exploited to produce more ATP, leading to sharp declines in glycogen stores and increases in lactate, with death following once neural activity can no longer be energetically supported (Birceanu et al. 2009). Metabolic rate has been demonstrated to have a positive

relationship with TFM uptake (Tessier et al. 2018), and so temperature may also affect TFM uptake indirectly, as greater temperatures lead to higher metabolic rates (Holmes and Lin 1994). There exists a concern regarding the potential for sea lamprey to develop a resistance to TFM (Dunlop et al. 2018). TFM is processed in the liver by uridine 5'-diphospho-glucuronosyltransferase (UGT), an enzyme which is quite active in bony fish, but expressed at low levels with a low affinity for TFM in lampreys (Kane et al. 1994).

The sequence of invasion, from Lake Ontario to Lake Erie, then on to the upper Great Lakes, implies that a high degree of plasticity of thermal tolerance may have been required in the founding population. Lake Erie is the shallowest and warmest of the five Great Lakes and was subject to severe environmental degradation due to anthropogenic modification of the surrounding landscape (Sullivan et al. 2003; Sgro and Reavie 2018). Colonization of Lake Erie likely represented the greatest filter for the invasion front, which could help to explain the 15-year lag between the first sea lamprey observations from Lake Erie to the other upper Great Lakes. Fifteen years represents approximately two to three generations, during which time sea lamprey may have responded to the challenging environment by a heritable increase in tolerance to a number of environmental parameters, including temperature (Levis and Pfennig 2016).

In the present study, upper thermal limits and expression profiles of genes involved in the cellular stress response (CSR) and TFM detoxification between a native, Atlantic population of sea lamprey from New Brunswick, Canada, and a landlocked population from a Lake Huron stream were compared. If the landlocked sea lamprey population has greater plasticity in thermal tolerance, as invasion theory would predict, then their acclimation history would have a greater impact on their CTMax compared with a New Brunswick anadromous population, resulting in a higher ARR. It was also expected that a greater upper thermal limit overall would be observed in

the landlocked population, as estimated by CTMax. Further, it was expected that increased transcriptional plasticity would be observed, resulting in differential regulation of elements of the cellular stress response in the landlocked population, such as higher initiation temperatures, or greater variation in expression levels of molecular chaperones across chronic temperature treatments. Initiation temperature refers to the temperature at which a significant change in protein or mRNA transcript level is detected. *UGT* expression between populations was also examined to test whether the landlocked population may be responding to lampricide treatments by upregulating basal *UGT* expression, especially at higher temperatures when TFM uptake is expected to be higher.

3.3 Materials and Methods

Many of the methods are similar to those in Chapter Two. To minimize repetition, only methods unique to this chapter are presented and the reader can refer to Chapter Two for commonalities.

3.3.1 Animal Collection and Holding

Anadromous sea lamprey ammocoetes used were the same as described in Chapter Two. Landlocked sea lamprey ammocoetes were collected by United States Fish and Wildlife Service (USFWS) personnel using backpack pulsed-DC electrofishing from the Chippewa River, MI in April 2018 (Fig. 3.1). The Chippewa is a tributary of the Saginaw River, which empties into Lake Huron. Ammocoetes were held in 200 L tanks at the Hammond Bay Biological Station (Millersburg, MI) supplied with Lake Huron water, with 5 cm of sand provided as a burrowing

substrate. Ammocoetes were fed a slurry of brewer's yeast once per week. Animals were shipped to Wilfrid Laurier University in early June 2019, and 50 ammocoetes were housed in each of three 110 L aquaria, with approximately 10 cm of sand provided as a burrowing substrate and received a continuous flow of aerated well water (flow rate of 0.5 L min^{-1} – 2.0 L min^{-1}). Ammocoetes were held under a 12 h:12 h light-dark cycle and were fed a slurry of baker's yeast at a rate of 1 g $animal^{-1}$ once per week. Landlocked ammocoetes measured 57 mm – 106 mm in length, with a mean (±SD) of 78.0 mm (±13.0 mm).

Each holding aquarium was designated as either cold, moderate, or warm, with target acclimation temperatures of 5 °C, 12 °C, or 19 °C, respectively. Temperatures were achieved and maintained through a combination of either a chiller (Coralife) or heating rods (Eheim Jager Aquarium Thermostat Heater 100 W), and the controlled flow of incoming water. Temperature was raised or lowered at a rate of approximately 1 °C day^{-1}. Actual acclimation temperatures (±SD) for the New Brunswick individuals in 2017 were 5.0 °C (±0.3 °C), 13.5 °C (±0.9 °C), and 20.3 °C (±1.5 °C), while the landlocked individuals were acclimated to 5.0 °C (±0.4 °C), 14.8 °C (±0.5 °C), and 21.0 °C (±1.9 °C). A two-way ANOVA revealed differences in acclimation temperatures between both acclimation groups ($F=2581.88$, $p<0.0001$), and populations ($F=14.42$, $p=0.0002$). *A priori* defined contrasts within acclimation groups across populations revealed the only difference between populations was in the moderate acclimation group ($t=4.71$, $p<0.0001$). Maintaining the temperature in the warm acclimation tank was challenging due to fluctuations in the flow rate of the system. The warm acclimation tank for the landlocked population had to be euthanized and restarted with fresh animals after flow to the tank ceased overnight, and the temperature rose to 29.1 °C one week into the acclimation period. The acclimation period lasted a minimum of 16 d prior to experimentation in all cases.

3.3.2 Stream Temperature

Daily thermal data from known ammocoete-bearing tributaries along the Northeast Coast of the United States (from Maine to Delaware) and downstream of the collection site for the landlocked population, from the Saginaw River, were collected to evaluate whether they are comparable (Fig. 3.2). Daily mean water temperature data for coastal tributaries confirmed to house sea lamprey ammocoetes (Beamish 1980) were mined from the United States Geological Survey's (USGS) National Water Information System database. All sites found were included for visual representation. Annual thermal data was superficially examined for completeness, and years which appeared to be missing substantial data and all provisional data were discarded. Each site contributed between four and 44 years' worth of daily data (Table 3.1). Statistical analysis was not deemed to be appropriate or necessary on these data as a variety of uncontrolled factors exist between sites (depth of thermal probe, elevation, shade cover, proximity to infrastructure, instrument calibration, etc.) and because the New Brunswick population was not collected from any of the locations with temperature data. Temperature data for the Richibucto River is available from the New Brunswick Surface Water Quality Data Portal; however, it is extremely limited, with a total of 54 observations recorded over 16 years. These data were plotted with the Saginaw River data to provide a direct comparison in thermal characteristics experienced by the New Brunswick and landlocked populations used here (Fig. 3.2). Annual stream thermal characteristics appear to be very similar between both areas.

3.3.3 CTMax Procedure

Three 2 L glass beakers were filled with ~10 cm of sand and then topped to 1400 mL with water from the relevant acclimation tank. Long forceps were used to rake through the sediment in the acclimation tanks to flush out individual ammocoetes, which were collected and placed in their own beaker, within a 60 L glass aquarium. The New Brunswick cold, moderate, and warm acclimation groups had a sample size of n=15, 16, and 15, respectively, while the landlocked groups had n=14, 15, and 19, respectively. New Brunswick ammocoetes acclimated to moderate conditions (n=16) were also tested in the summer of 2018 to verify whether any observed differences in CTMax were due to the new heating rods used in 2018, or any potential temporal variation due to the approximately year-long gap between testing populations. An airstone was placed into each beaker, a temperature probe was inserted into the sediment and kept in place to get accurate sediment temperature readings while minimizing disturbance to the burrowed ammocoete. Half-an-hour after introduction to the beaker, or once all ammocoetes were burrowed into the sediment, 12 L of moderate water was added to the aquarium. Between one and six heating rods (number varied due to the wattage of individual rods: Eheim Jager Aquarium Thermostat Heater, 100 W; Finnex Deluxe Titanium Tube Heater, 300 W), set to their maximum temperature were plugged in, and an aquarium pump was turned on. Beaker water and sediment temperature were recorded every 10 minutes, rising at a mean (±SD) rate of 0.17 °C min^{-1} (±0.02 °C min^{-1}).

Different heating rods were used on the different populations, leading to a concern that any apparent differences in CTMax could be an artifact of different rates of heating (Appendix A: Table A.1). There is evidence in other species that different rates of temperature increase does not have an impact on the CTMax estimate (Zhang et al. 2017); however, differences in heating

rates were tested between populations within acclimation groups to check for statistical evidence of divergence in the heating rates. Since the rate of temperature increase fell as the water temperature approached the upper maximum of the heating bars, both time and temperature variables were normalized via log transformations to increase their linearity. The mean slope of regressions performed separately for each CTMax trial were not found to be significantly different across populations within any acclimation group (cold: t=1.60, p=0.13; moderate: t=0.23, p=0.82; warm: t=-1.01, p=0.32; Appendix A: Fig. A.3, Table A.2). An additional CTMax experiment was run on a group of New Brunswick ammocoetes acclimated to moderate conditions in 2018, collected from the same streams as the 2017 New Brunswick ammocoetes. This test was run to verify whether inter-annual, and therefore inter-populational, comparisons would be valid. CTMax procedure followed that outlined in Chapter Two.

Attempts were made to use animals of roughly the same size in each case, but unfortunately differences in the sizes were discovered (Appendix A: Table A.3, Fig. A.4). A two-way ANOVA on Condition Factors (Henson et al. 2003) uncovered a significant difference between acclimation groups, but subsequent *a priori* defined contrasts were unable to uncover the source of variation (Appendix A: Tables A.4, A.5).

$$Equation\ 3.2: Condition\ Factor\ K = 10^6(\frac{mass}{length^3})$$

Two-way ANOVAs on both length and weight uncovered further differences, and subsequent contrasts confirmed that the landlocked warm acclimation group had the smallest mean lengths and weights (Appendix A: Tables A.4, A.6, A.7). This was likely because the warm landlocked acclimation group had to be euthanized after flow ceased to this tank and had to be restarted with ammocoetes which were left over from the initial shipment from the

USFWS. These leftover individuals would have not been selected initially due to their being outside the ideal size range. Linear regressions were performed to verify whether any relationship existed between CTMax and condition factor, weight, or length. A weak positive relationship was found between CTMax and condition factor when all data was pooled (adj R^2=0.07, F=8.40, p=0.005; Appendix A: Fig. A.5), meanwhile the only group which demonstrated a significant relationship was the New Brunswick warm acclimation group (adj R^2=0.33, F=7.40, p=0.02; Appendix A: Fig. A.6); all others were not statistically significant. A weak negative relationship was indicated between CTMax and length when all data was pooled (adj R^2<0.01, F=4.75, p=0.03; Appendix A: Fig. A.7). When analyzed individually, only the New Brunswick warm acclimation group had a significant negative relationship with CTMax (adj R^2=0.34, F=7.75, p=0.017), while a significant positive relationship was also detected in the landlocked moderate group (adj R^2=0.22, F=5.03, p=0.04; Appendix A: Fig. A.8). No relationship was detected between mass and CTMax when all data was pooled or when each acclimation group was analyzed separately (Appendix A: Figs. A.9, A.10). Taken together, the slight differences observed in animal size between groups likely had minimal impact on any subsequent measurements.

Due to differences in the acclimation temperatures of the two populations, CTMax data comparisons between populations were made using an ANCOVA, with mean acclimation temperature as the covariate.

3.3.4 Tissue Sampling Procedure

Ten individuals were sampled directly from the acclimation tanks following the CTMax trials. Sampling procedure followed that outlined in Chapter Two.

3.3.5 RNA Extraction and cDNA Synthesis

RNA extractions and cDNA synthesis followed the procedures outlined in Chapter Two. All landlocked RNA samples demonstrated a 260/230 absorbance ratio between 1.44 and 2.44 (mean = 2.15), and a 260/280 absorbance ratio between 2.0 and 2.26 (mean = 2.12). Concerns regarding some samples' low 260/230 absorbance ratios were allayed by outlier analysis on the final dataset, visually via boxplots and statistically via Dixon Tests. These demonstrated that none of the samples with low 260/230 ratios were outliers; therefore, they were retained in the analysis.

3.3.6 Primer Design

Primers used here were the same as in Chapter Two except for *UGT*, which was designed using the framework described in Chapter Two (Table 3.2).

3.3.7 qPCR Protocol and Data Analysis

The qPCR protocol and data analysis were performed as per Chapter Two. To measure variation in the reference genes, the coefficient of variation (CV) was calculated for each reference gene on plate A by dividing the standard error of the mean of the gene A normalized relative quantities (NRQs) by the mean of the gene A NRQs on plate A. CV is generally below 25 % for stably expressed reference genes (Hellemans et al. 2007). The CV for reference genes used in the present study were all <29.5 % (mean CV = 19.7 %). Statistics were run on the log-base-two NRQ values, while figures were produced with the NRQ values.

Two-way type-III ANOVAs were performed for each gene target, using the three treatment levels and two populations as the two factors. A separate analysis was performed on each tissue. *A priori* contrasts were identified prior to testing the model to examine differences between treatments within populations, and between populations within treatments. Due to the large number of tests (9), a Bonferroni correction was applied when testing for significance in the contrasts.

3.4 Results

3.4.1 CTMax

Mean CTMax (±SEM) of the New Brunswick sea lamprey population acclimated to cold, moderate, and warm conditions were 32.5 °C (±0.2 °C), 33.4 °C (±0.1 °C), and 34.5 °C (±0.1 °C) respectively, while the landlocked populations had mean CTMax values of 33.3 °C (±0.1 °C), 34.4 °C (±0.2 °C), and 35.2 °C (±0.1 °C), respectively (n = 14-19; Fig. 3.3; Table 3.3). There were statistically significant differences in CTMax temperatures between acclimation temperatures within each population ($p<0.001$ in all contrasts), while differences were also observed between populations within each acclimation group ($p<0.003$ in all contrasts). Because acclimation temperatures differed within the moderate acclimation groups between populations, an ANCOVA was employed to examine potential differences between populations. Linear models were fit to both the New Brunswick ($y = 0.1243x + 31.85$) and landlocked populations ($y = 0.1196x + 32.67$), and the model slopes and intercepts were sequentially compared through ANOVA, revealing no difference in slopes between populations ($F=0.07$, $p=0.79$), but a significant difference in intercept ($F=48.55$, $p<0.001$).

An additional CTMax trial was performed on New Brunswick individuals (n=16) acclimated to moderate temperatures using identical methods to those used for the landlocked population to evaluate whether this method led to an increased CTMax estimate, regardless of the lack of evidence that the heating rate differed (Fig. 3.3). This group had a mean CTMax (±SEM) of 33.9 °C (±0.1 °C), 0.21 °C greater than the CTMax estimate for their mean acclimation temperature generated by the original New Brunswick linear model, but 0.55 °C lower than the predicted value from the landlocked model. A two-tailed t-test revealed a significant difference between the landlocked moderate and 2018 New Brunswick moderate acclimation groups (t=2.69, p=0.015).

3.4.2 Gene Expression

Transcription factors

HSF1 expression was not found to be differentially expressed between the two populations across temperature treatments in either tissue (Table 3.4; Fig. 3.4 B, C). A greater expression level was observed in the cold compared to the moderate treatment in the intestines from the New Brunswick population (t=-3.15, p=0.024).

EPAS1B expression demonstrated similar trends between the two populations overall, but expression was higher in the New Brunswick population in response to the warm treatment in liver tissue (t=-3.62, p=0.006; Fig. 3.4 A, B). Expression generally increased with treatment temperature. In all cases the warm treatment had a greater *EPAS1B* expression than the cold treatment (New Brunswick t=-6.28, p<0.0001; Landlocked t=-2.89, p=0.05). The moderate treatment demonstrated opposite reactions in the two tissues; in the intestine, expression levels

matched those of the cold treatment; in the liver, expression levels matched those of the warm treatment.

JUN expression demonstrated different trends between populations. In the New Brunswick population there was no change in *JUN* expression between any treatment temperature in either tissue. *JUN* expression in the landlocked population increased with treatment temperature significantly between the cold and moderate treatments in both tissues (intestine: $t=3.94$, $p=0.002$; liver: $t=3.30$, $p=0.015$; Fig. 3.4 E, F). In intestine, a difference in expression level between populations was noted in the cold treatment, with lower expression in the landlocked population ($t=-5.39$, $p<0.0001$), while expression levels were nearly identical in the warm treatment. No differences between populations were observed in the liver samples.

Heat shock proteins

HSP90B1 expression followed the same general patterns in response to treatment between both tissues and populations, exhibiting a U- or L-shaped pattern with the lowest expression in the moderate treatment (Fig. 3.5A, B). Intestine samples demonstrated greater expression in the cold treatment compared with the moderate temperature in both populations (New Brunswick: $t=-4.51$, $p=0.0003$; landlocked: $t=4.89$, $p<0.0001$), while no difference was detected between the moderate and warm treatments. Liver samples did not show significant differences in *HSP90B1* expression among different treatments in the New Brunswick population, while the U-shape is very distinct in the landlocked population, with significant differences between each treatment (all $t<-3.73$, $p<0.0042$). Population differences in expression were also detected in the liver samples in the warm treatment, as the landlocked population had a greater expression level ($t=4.91$, $p<0.0001$).

HSPA9 expression patterns differed between tissues and populations (Fig. 3.5 C, D). Intestine samples were nearly indistinguishable between populations, with greatest expression in the cold treatment as compared to the moderate (New Brunswick: t=-4.46, p=0.0003; landlocked: t=-6.63, p<0.0001) and warm treatments (New Brunswick: t=3.64, p=0.006; landlocked: t=5.48, p<0.0001). No changes in *HSPA9* expression were noted in the New Brunswick liver tissue with respect to treatment temperature, while a U-shape was evident in the landlocked samples, with the moderate treatment demonstrating the lowest expression with respect to both the cold (t=-5.05, p<0.0001) and warm (t=-4.67, p=0.0002) treatments. Landlocked moderate liver *HSPA9* expression was significantly lower than the New Brunswick moderate group (t=-4.00, p=0.0017).

SERPINH1A expression patterns were again consistent between treatments and population in the intestine, rising with temperature (Fig. 3.5 E, F). The warm treatment displayed significantly greater expression than the moderate treatment in the intestine (New Brunswick: t=-5.95, p<0.0001; landlocked: t=-3.21, p=0.02), while no statistical difference was detected between cold and moderate treatments. *SERPINH1A* expression patterns in the liver were also consistent between populations, rising with temperature. A difference was apparent in the activation temperature, however, as the New Brunswick population significantly upregulated *SERPINH1A* with each increase in treatment temperature (all t>6.36 or t<-3.46, all p<0.0094), while the landlocked population significantly upregulated *SERPINH1A* only in response to the warm treatment (all t<-7.13, p<0.0001).

Proteolytic enzyme – *MMP2*

MMP2 varied relatively little across treatment groups, with no changes detected in New Brunswick intestine tissue or landlocked liver tissue (Fig. 3.6 A, B). A decrease in expression was observed in the intestine of warm landlocked individuals, relative to moderate (t= 4.18,

p=0.001) and cold (t=3.77, p=0.004) treatments. In New Brunswick liver tissue, the cold treatment demonstrated borderline significantly greater expression than moderate (t=-2.89, p=0.05).

Detoxifying enzyme – UGT

UGT expression remained unchanged across treatments in the landlocked population in both tissues (Fig. 3.6 C, D). The New Brunswick intestine showed an increase in expression with temperature as the warm treatment expressed *UGT* at a greater rate than the cold treatment (t =-4.16, p=0.001). Similarly, the warm treatment had greater expression in the liver than the moderate treatment in the New Brunswick population (t=-4.73, p=0.0001). The landlocked population demonstrated elevated expression relative to the New Brunswick population in the moderate liver samples (t=3.76, p=0.004).

Principal components analysis

The PCAs performed separately on liver and intestine tissues for all genes of interest both demonstrate that PC1 contains variation predominately from the response to treatment temperature, and to a lesser extent population differences (liver PC1 explains 27.04 % variance; intestine PC1 explains 34.05 % variance; Figs. 3.7, 3.8). PC2 reveals further variation in response to treatment temperature as well as population differences within treatments (liver PC2 19.6 % variance explained; intestine PC2 explains 23.72 % variance).

In the liver PCA, the cold treatment group of both populations are indistinguishable, negatively related to PC1, and positively related to PC2. The pattern then clearly changes between populations as the treatment temperature increases. The landlocked population demonstrates a U-shaped pattern, with the moderate treatment negatively related to both PCs, and the warm treatment positively related to both. Contrarily, the moderate treatment in the New

Brunswick anadromous population is centered on both PCs, and the warm treatment is positively related to PC1 and central on PC2. The intestine PCA is largely similar but appears to be tilted about 45° compared to the liver PCA. The temperature treatments follow a J-shaped pattern, with the cold treatment group negatively related to PC1 and in the middle of PC2, the moderate treatment group positively related to PC1 and negatively related to PC2, and the warm group positively related to both PCs. Population differences are evident in the cold group, with the landlocked population more negatively related to PC1, and the warm group, with the New Brunswick anadromous population being more positively related to PC2.

3.5 Discussion

Overall results were compatible with most of the predictions. The landlocked population demonstrated a greater upper thermal tolerance than the New Brunswick population, although the prediction that the slope of the acclimation-CTMax temperature relationship (ARR) would differ was not supported. Greater phenotypic plasticity was confirmed in the gene expression patterns of the landlocked population, with greater flexibility in *JUN*, *HSP90B1*, and *HSPA9* expression. In the liver, expression of *UGT* in the landlocked population was greater under moderate conditions than the New Brunswick population.

3.5.1 CTMax

The CTMax trials demonstrated a linear pattern, with each population's ARR = 0.12, while the landlocked population's CTMax values were consistently shifted ~0.8 °C higher. The repeated CTMax result from an moderate-acclimated New Brunswick group in 2018, performed

on animals collected from the same stream as the New Brunswick population tested in 2017, verified the presence of population-level differences and dispelled concerns over comparing the interannual CTMax results. This greater thermal tolerance observed in the landlocked population corresponds to predictions made with current invasion theory, i.e. that invasive populations will generally demonstrate increased tolerance to environmental stressors (Zerebecki and Sorte 2011; Hill et al. 2013; Bates et al. 2013). Interestingly, no difference existed between populations' ARR, implying that the landlocked population has not developed greater plasticity in their upper thermal tolerance. This finding was surprising as populations colonizing novel habitats are hypothesized to demonstrate greater plasticity relative to con- and hetero-specifics (Yeh and Price 2004; Scoville and Pfrender 2010; Davidson et al. 2011; Lande 2015). Petromyzontiformes in general appear to have a reduced capacity to adjust their upper thermal tolerance through acclimation (Chapter Two), and therefore this trait may not be ideal for detecting potential changes in plasticity in this species. It is possible that the genetic underpinnings which allow for flexibility of the ARR have been lost from this lineage, and therefore there was no opportunity to modify the ARR in the landlocked population.

In one of the only other studies comparing thermal tolerance between invasive and native populations within a species, no difference was found in CTMax at a range of acclimation temperatures between a native pumpkinseed population from Canada and an invasive population sourced from Spain, despite the invasive population being subjected to a generally warmer climate (Rooke et al. 2017). This discrepancy could stem from several sources. First, the Spanish climate from which the invasive pumpkinseed population was obtained in the Rooke et al. (2017) study experiences a narrower range of annual temperatures than the native population used. Decreased annual temperature fluctuations have been demonstrated to be linked to decreased

upper thermal tolerance in fish (Feminella and Matthews 1984; Strange et al. 2002). Second, the experiments were performed on F1 and/or F2 generations reared under common conditions over several years which were started from ~20 individuals per population. This would have allowed for founder effects or even some selection to have occurred and would have weakened any transgenerational factors or developmental plasticity which could be operating within the wild population in the invasive range.

Previously, rapid evolution of thermal tolerance has been demonstrated in founding populations of threespine stickleback. Within three generations of being introduced to a small, artificial freshwater pond, marine-sourced sticklebacks demonstrated a 2.5 °C decrease in their critical thermal minimum, to a temperature comparable to that observed in natural freshwater populations (Barrett et al. 2011). Due to the remarkable fecundity of sea lamprey (44,000-200,000 eggs per female: Beamish 1980; Manion and Hanson 1980), a similarly rapid adaptational response, albeit likely smaller in magnitude, may have occurred early in their invasion of the Great Lakes. In a similar study, Fangue et al. (2006) reported that common killifish from a southern population have a CTMax ~1.5 °C higher at a range of acclimation temperatures, compared to a population from the northern edge of their distribution. This trend held true for multiple northern and southern populations tested. This is a greater difference than the ~0.8 °C difference observed in sea lamprey, potentially due to the greater divergence in thermal conditions between these two killifish populations, the greater length of time these killifish populations have been reproductively isolated from each other, as well as this species' extreme plasticity.

3.5.2 Gene Expression

Overall, few differences in transcription factor expression patterns were noticed between populations. *EPAS1B* (hypoxia-inducible factor 2 [in a new version of the sea lamprey genome this sequence is annotated as hypoxia-inducible factor 1; Smith et al. 2018]) expression followed similar patterns between populations, with expression generally increasing with temperature. This could be due to the positive effect temperature has on sea lamprey metabolic rates (Holmes and Lin 1994). The warmest group would have the greatest metabolic rate and therefore the greatest oxygen demand, while at the same time, water tends to hold less dissolved oxygen as temperatures increase, creating a scenario where the warm individuals could be under slightly greater oxygen stress than the cold and moderate treatment individuals. By no means were they under hypoxic conditions, but a slight oxygen limitation in the warm relative to the cold treatment is not unexpected. While only statistically significant in the liver in warm treatment samples, the landlocked population tended to have lower expression in all treatments. This could indicate a greater tolerance to oxygen limitation, as *EPAS1B* is not being induced as much relative to the New Brunswick population.

An interesting population difference is clear in the expression patterns of *JUN* in response to the different temperature treatments. The New Brunswick population had a stable, constant level of expression regardless of the different treatment temperatures, while the landlocked population showed an increase in *JUN* expression with temperature. This suggests increased transcriptional plasticity in the landlocked population, as they reduce the expression of *JUN* at low temperatures, and increase it as required at greater temperatures. The differences observed in *JUN* expression between populations must be interpreted with caution however, as *JUN* expression in sea lamprey was previously demonstrated to be sensitive to handling

(Appendix A: Fig.1). This makes *JUN* a good candidate for assessing acute stress in sea lamprey ammocoetes on the order of minutes to hours but may be less reliable for determining chronic responses.

Few differences were noted in heat shock protein expression between populations in the intestine, as expression levels and patterns were closely matched. Alternatively, liver tissue demonstrated interesting differences between populations. *HSP90B1* and *HSPA9* both remained unchanged across treatments in the New Brunswick population, while both demonstrated downregulation in the landlocked population in response to a moderate temperature. Sea lamprey ammocoetes have demonstrated a temperature preference of 13.6 °C (±0.17 °C), and therefore may experience the least homeostatic disturbance at this temperature (Reynolds and Casterlin 1978), while some protein denaturation may be occurring under the cold and warm treatments which requires relatively greater expression of these heat shock proteins. While the expression of *SERPINH1A* did not differ significantly between populations, differences in the trends are apparent. A lower initiation temperature appears to be present in the New Brunswick population, which elevated liver *SERPINH1A* expression in the moderate treatment, while the landlocked population only elevated expression in the warm treatment. A forking of the trend is also present in the intestine, as expression in the cold and moderate treatments nearly overlap in both populations, while a spike occurs in the New Brunswick population relative to the landlocked in the warm treatment, with nearly double the *SERPINH1A* expression.

Zerebecki and Sorte (2011) found that an invasive tunicate species from the California coast demonstrated greater thermal tolerance and greater HSP70 protein expression in response to a heat shock compared with a native tunicate. These authors speculate that the increased responsiveness of HSP70 could be directly linked to the increased thermal tolerance observed.

Increased basal levels of HSC70 and HSP70 proteins have also been implicated in increased thermal tolerance in livebearer (*Poeciliopsis*) fish (Dilorio et al. 1996). Fangue et al. (2006) were able to demonstrate differences in gene expression between two populations of common killifish, a northern and more thermally-tolerant southern population. The normally constitutively expressed *HSC70* gene expression increased in response to heat shock in the southern, but not northern population. Unfortunately, here, the full heat shock experiment which had previously been completed on the New Brunswick population (Chapter Two) was not repeated on the landlocked population. Comparisons in gene expression patterns were only made in response to the different acclimation conditions and are more reflective of basal expression levels. Fangue et al. (2006) showed no differences in gene expression between samples taken directly from their acclimation tanks in any gene except *HSP90β*, which had greater expression in the more thermally tolerant southern population. They used a single acclimation temperature for their gene expression experiment, 20 °C, a comfortable temperature for both populations. Around this acclimation temperature, the present study also found greater *HSP90B1* expression in the more thermally tolerant landlocked sea lamprey ammocoetes. In both species, 20 °C is expected to be a non-stressful temperature; therefore, increased expression of *HSP90B1* in the more thermally tolerant population could indicate their greater capacity to withstand additional temperature increases relative to the less thermally tolerant population.

Increased activation temperature of HSPs has been associated with greater thermal tolerance in other species subjected to an acute thermal stressor (Buckley and Hofmann 2002, 2004; Logan and Somero 2011; Fangue et al. 2011; Komoroske et al. 2015). Here, opposite initiation patterns emerged for *SERPINH1A* and *HSP90B1* in response to chronic temperature treatments, both of which were previously demonstrated to be inducible in response to an acute

heat shock (Chapter Two). The landlocked population had a higher initiation temperature relative to the New Brunswick anadromous population for *SEPRINH1A*, and a lower initiation temperature for *HSP90B1*. Therefore, drawing conclusions linking initiation temperature in these two genes to overall thermal tolerance is difficult and may not be appropriate based on the limited data here.

The difference between the response of *UGT* expression between populations is especially interesting, although difficult to interpret. This is a key enzyme in the detoxification of the lampricide, TFM, employed as a piscicide in the Great Lakes to control sea lamprey ammocoete populations (Kane et al. 1994). In the present study, *UGT* expression was unchanged by treatment temperatures in the landlocked individuals but was expressed at greater levels relative to the New Brunswick population in the liver under moderate (~13 °C) conditions. A recent investigation showed that the uptake rate of TFM in sea lamprey ammocoetes increases with increased metabolic rate, as measured by oxygen consumption (Tessier et al. 2018). Since this ectotherm's metabolic rate is directly controlled by environmental temperature, warmer temperatures should increase the efficacy of TFM. Increased respiration and heart rates at higher temperatures would increase the rate of TFM uptake and accumulation in body tissues, and the greater metabolic demand would result in faster depletion of ATP reserves. The Great Lakes Fishery Commission (GLFC) takes full advantage of ice-free conditions, applying TFM to Great Lakes tributaries from April through October (GLFC SOP Appendix H, 2018). The fact that *UGT* is expressed at significantly greater levels in the landlocked population under moderate conditions (~13 °C – 14 °C) suggests a selective pressure may be at work; however, given the evidence, it is unlikely that TFM is the culprit behind selection. If adaptation to TFM were responsible, one would expect to see elevated *UGT* expression across all temperature treatments

relative to the New Brunswick population. UGT is involved in the breakdown of various phenolic compounds, which can come from a variety of natural or artificial sources, including pharmacological drugs, other pesticides, and by-products of petroleum industries (Kane et al. 1994). Therefore, this increased *UGT* expression could be a result of the population bottlenecks and subsequent variation fixes in the landlocked population as it expanded through the relatively polluted Great Lakes system. This expression pattern may have originated in the original Lake Erie founder population, which would have encountered a polluted lake and tributaries in the early days of the invasion (Sullivan et al. 2003).

A drawback of the present study design, however, lies in the fact that no age or sex verification could be performed on the larval sea lamprey. The anadromous juveniles and adults can grow larger than their landlocked kin, but recently metamorphosed juveniles appear to be of a similar size between both populations (Hansen et al. 2016). Animals used were also too small to rapidly sex individuals as they were sampled; therefore, it is unknown whether the ratios of males to females may have differed between populations or treatment groups. In some species age has been shown to be negatively correlated with temperature tolerance (Bowler and Terblanche 2008), while sex has been demonstrated to have an impact on expression of heat shock protein genes (Dammark et al. 2018) and thermal tolerance (Jeffries et al. 2012), while both age and sex have an impact in others (Winne and Keck 2005). Controlling for age and development stage was attempted by using the same approximate size range for both populations.

3.5.3 Summary

In conclusion, an invasive, landlocked population of sea lamprey ammocoetes had a consistently higher CTMax than a New Brunswick anadromous population at a range of acclimation temperatures. They also demonstrate increased plasticity in *JUN*, *HSP90B1*, and *HSPA9* in response to different chronic treatments at a range of ecologically-relevant temperatures. Expression of *UGT* in the liver of the landlocked population was also differentially expressed under moderate conditions relative to the New Brunswick population, which may influence how the landlocked population processes the lampricide TFM.

3.6 Figures and Tables

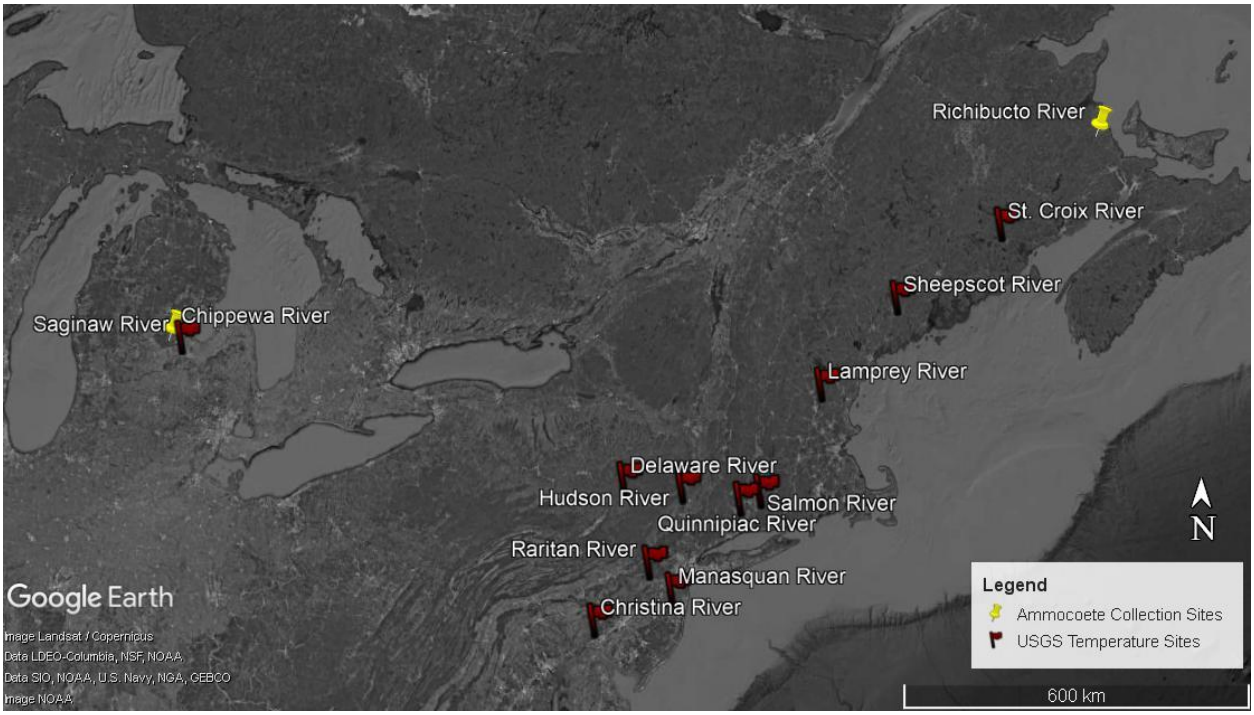
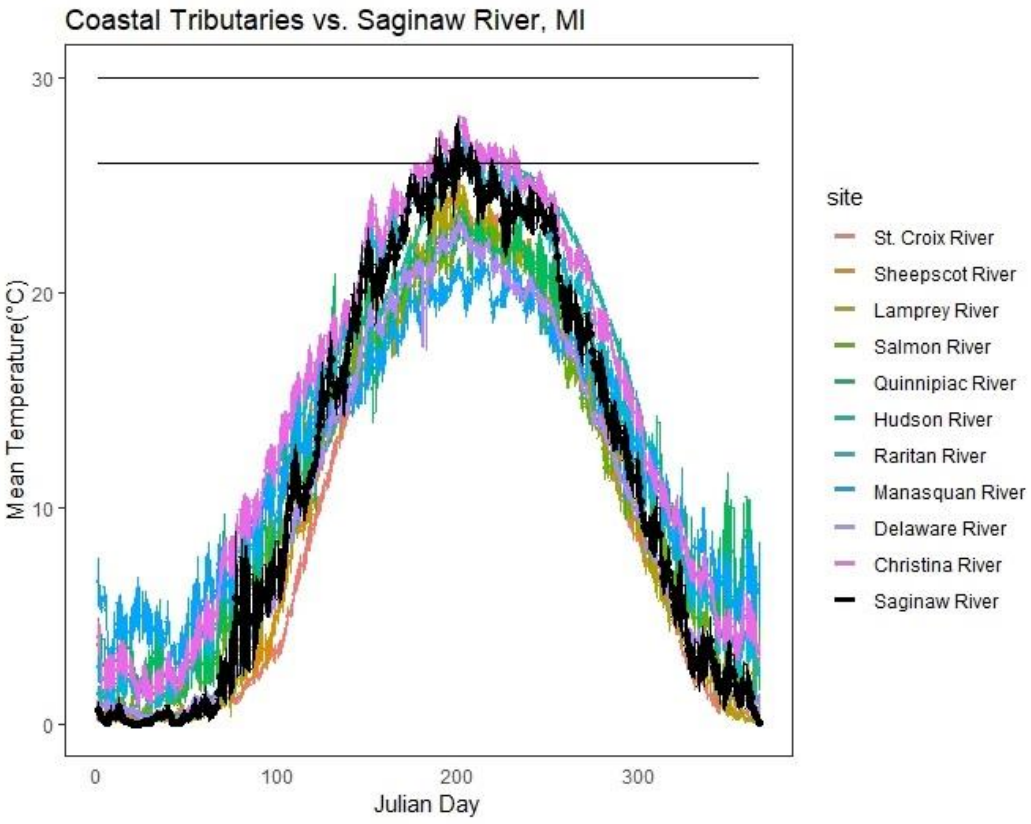


Figure 3.1. Sea lamprey ammocoete collection sites (Yellow) and USGS water temperature monitoring sites (red). Ammocoetes from the native population were collected from tributaries of the Richibucto River, New Brunswick in 2017, while the landlocked population were collected from the Chippewa River, Michigan, in 2018.


Coastal Tributaries vs. Saginaw River, MI
Mean Temperature(°C)
Julian Day
0
10
20
30
0
100
200
300
site
St. Croix River
Sheepscot River
Lamprey River
Salmon River
Quinnipiac River
Hudson River
Raritan River
Manasquan River
Delaware River
Christina River
Saginaw River

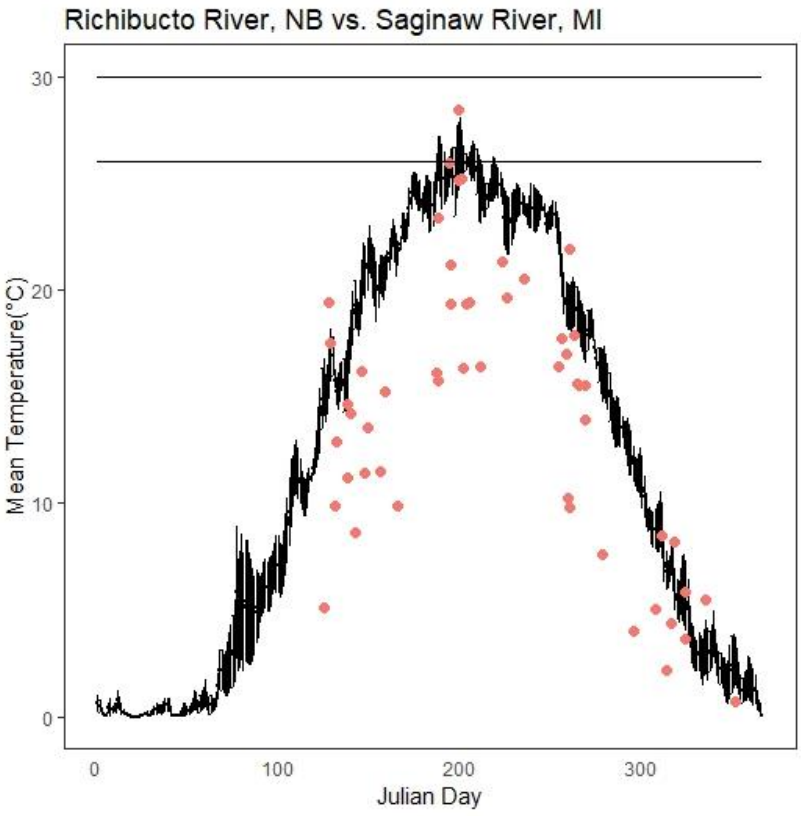

Richibucto River, NB vs. Saginaw River, MI
Mean Temperature(°C)
Julian Day
0
10
20
30
0
100
200
300

Figure 3.2. Mean (±SEM) daily temperature for tributaries of ammocoete-bearing streams of the Atlantic Coast from Maine to Delaware, and the tributary of the source stream of the landlocked population used in the present study, the Saginaw River (black). Horizontal lines were drawn at 26°C and 30°C to denote putative temperature thresholds proposed in Chapter Two for initiation of a mild heat shock response and initiation of a severe heat shock response. Annual thermal regimes in these areas are highly comparable overall. Data was retrieved from publicly available USGS records and from the Province of New Brunswick's water quality database.

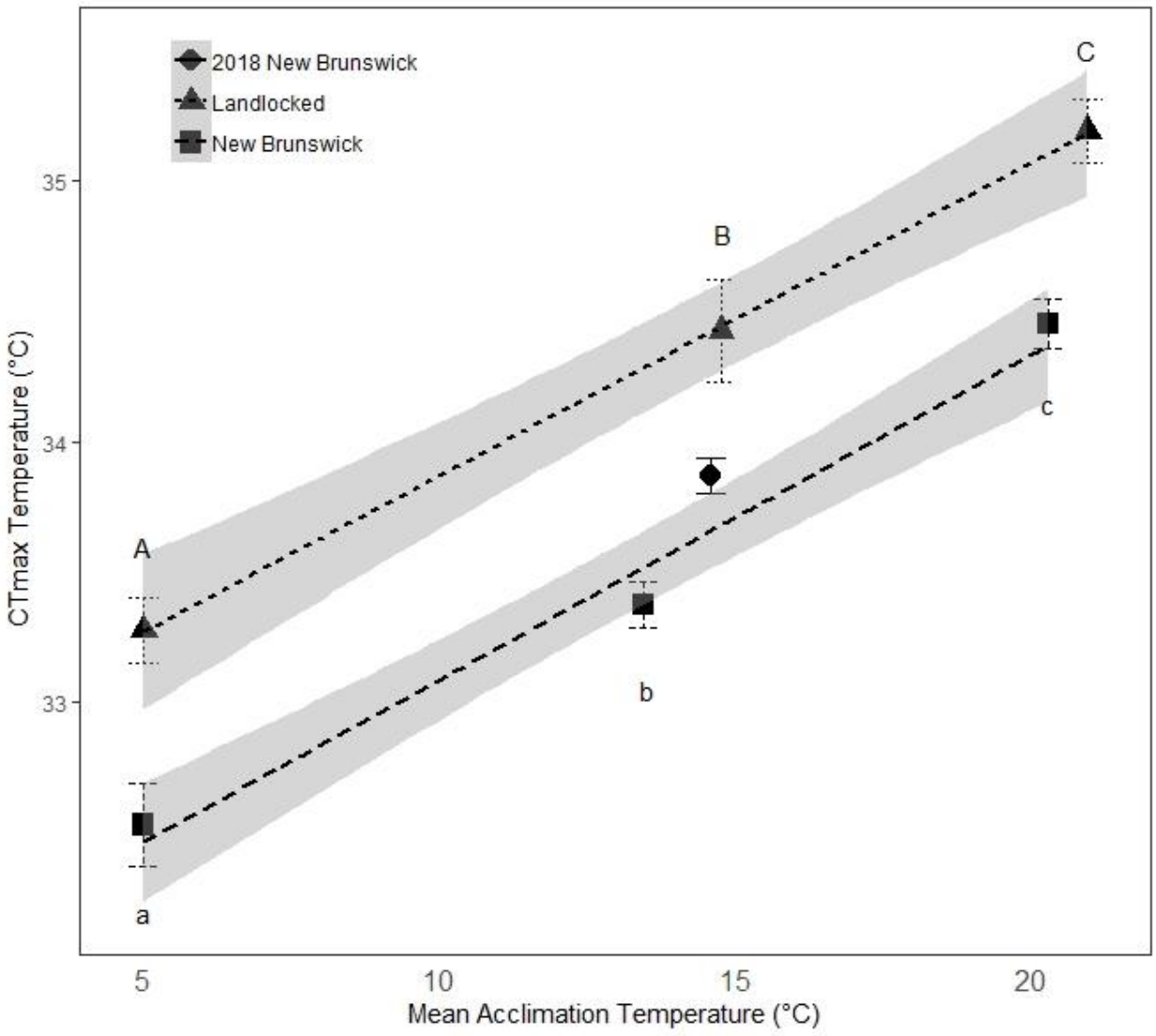


Figure 3.3. Mean CTMax (± SEM) values of sea lamprey acclimated to cold, moderate, and warm temperatures from landlocked (squares, large dashed line) populations are greater than those from New Brunswick (triangles, small dashed line) populations. Regression lines demonstrate a parallel trend for the effect of acclimation on CTMax, while the intercept for the landlocked population is significantly greater. Capital letters denote significant differences between acclimation temperatures in the landlocked population, while small letters show the same for the New Brunswick population. Due to a small difference in CTMax protocols used between years, an additional moderate New Brunswick group was tested following the methods used for the landlocked population in the summer of 2018 (circle). This CTMax estimate falls very close to the predicted value for the New Brunswick population.

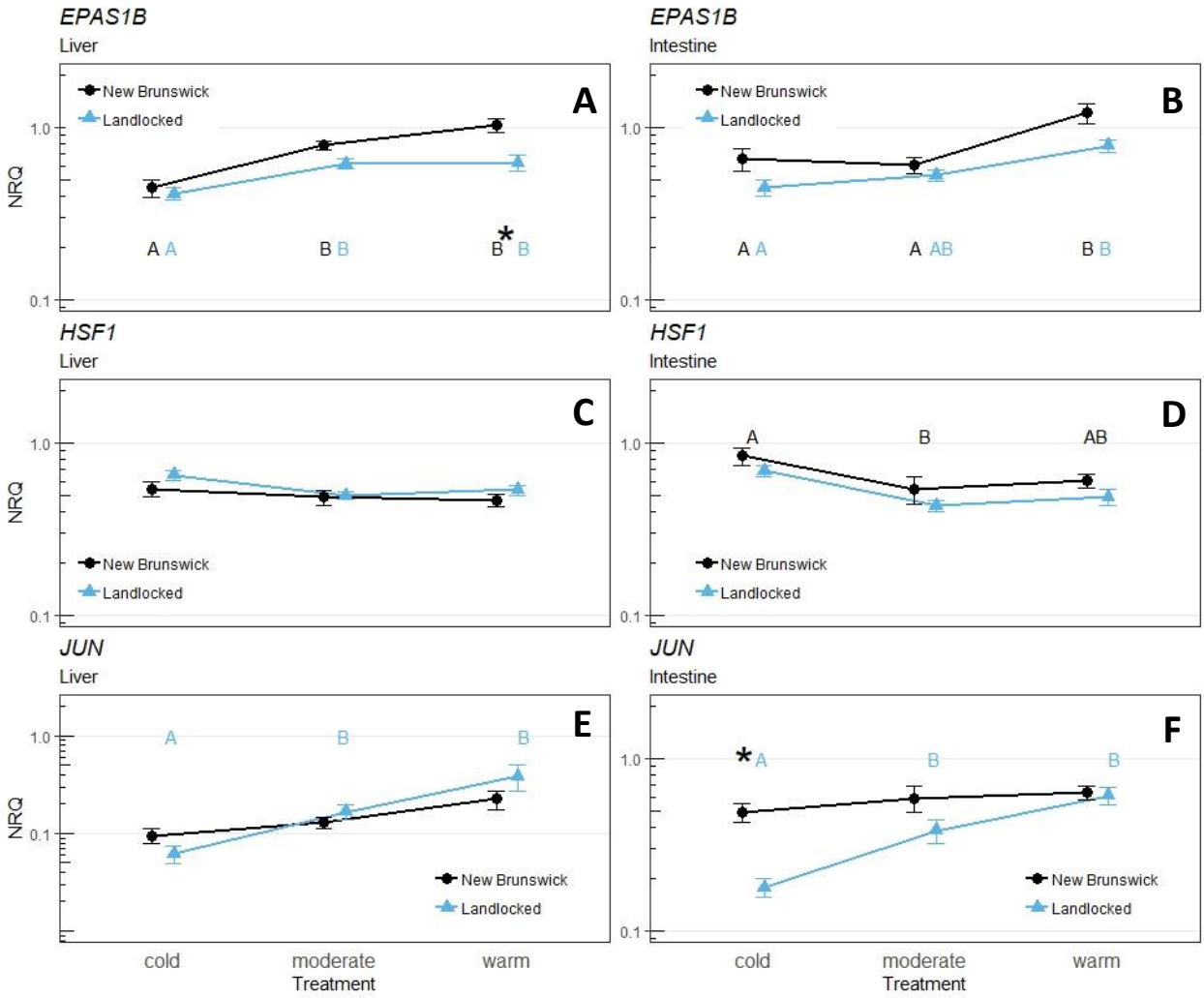


Figure 3.4. Mean (±SEM) transcription factor expression relative to the three control genes (normalized relative quantity – NRQ) from the liver (A, C, E) and intestine (B, D, F) tissues from both the New Brunswick anadromous (black circles) and landlocked (blue triangles) populations. Capital letters denote statistically significant differences within populations across treatments, corresponding to the colour of the population. Asterisk denotes statistically significant differences between populations within a treatment. Note the different scale used for the liver *JUN* data (E).

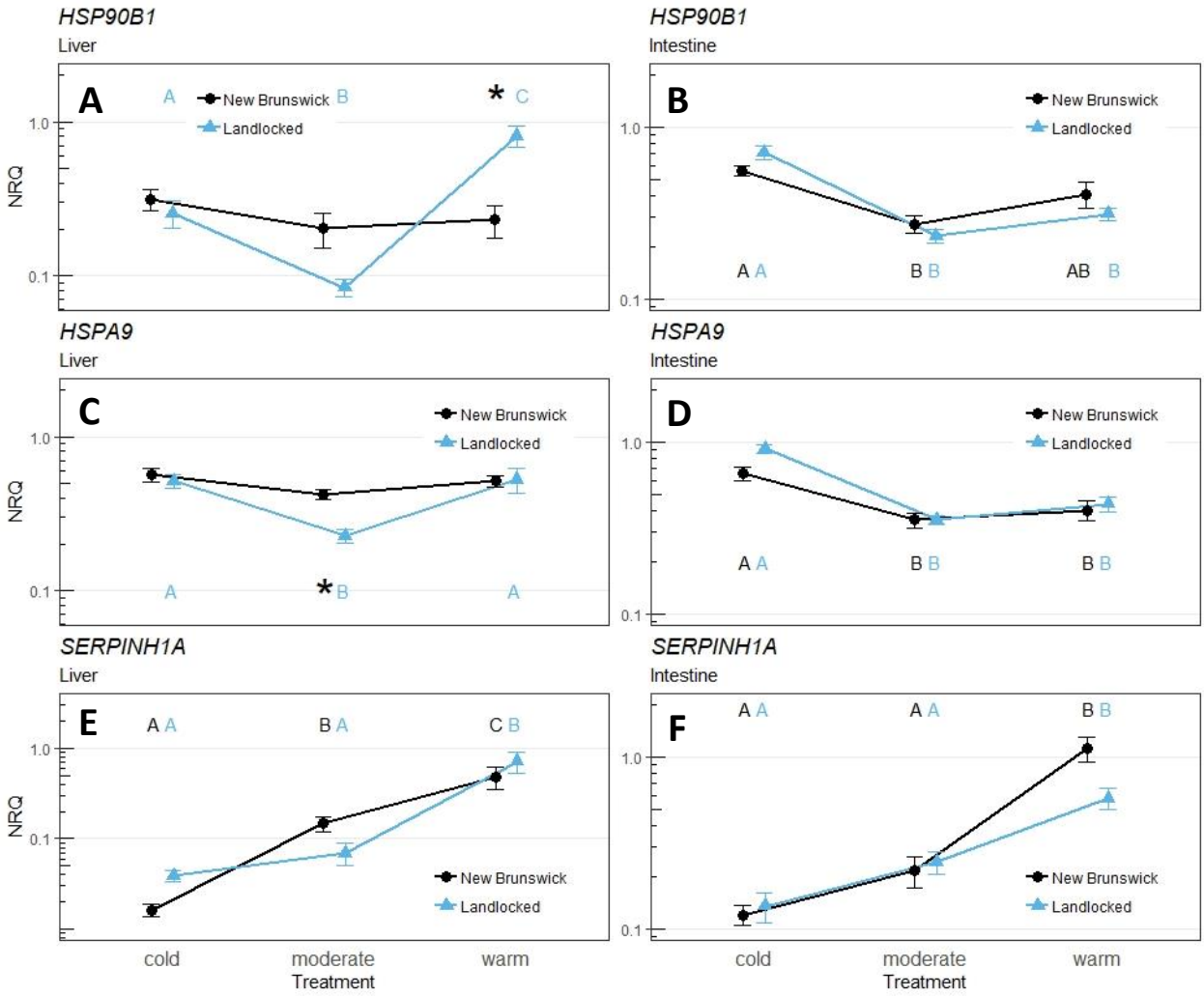


Figure 3.5. Mean (±SEM) molecular chaperone expression relative to the three control genes from the liver (A, C, E) and intestine (B, D, F) tissues from both the New Brunswick anadromous (black circles) and landlocked (blue triangles) populations. Capital letters denote statistically significant differences within populations across treatments, corresponding to the colour of the population. Asterisk denotes statistically significant differences between populations within a treatment. Note the different scale used for the *SERPINH1A* liver data (E).

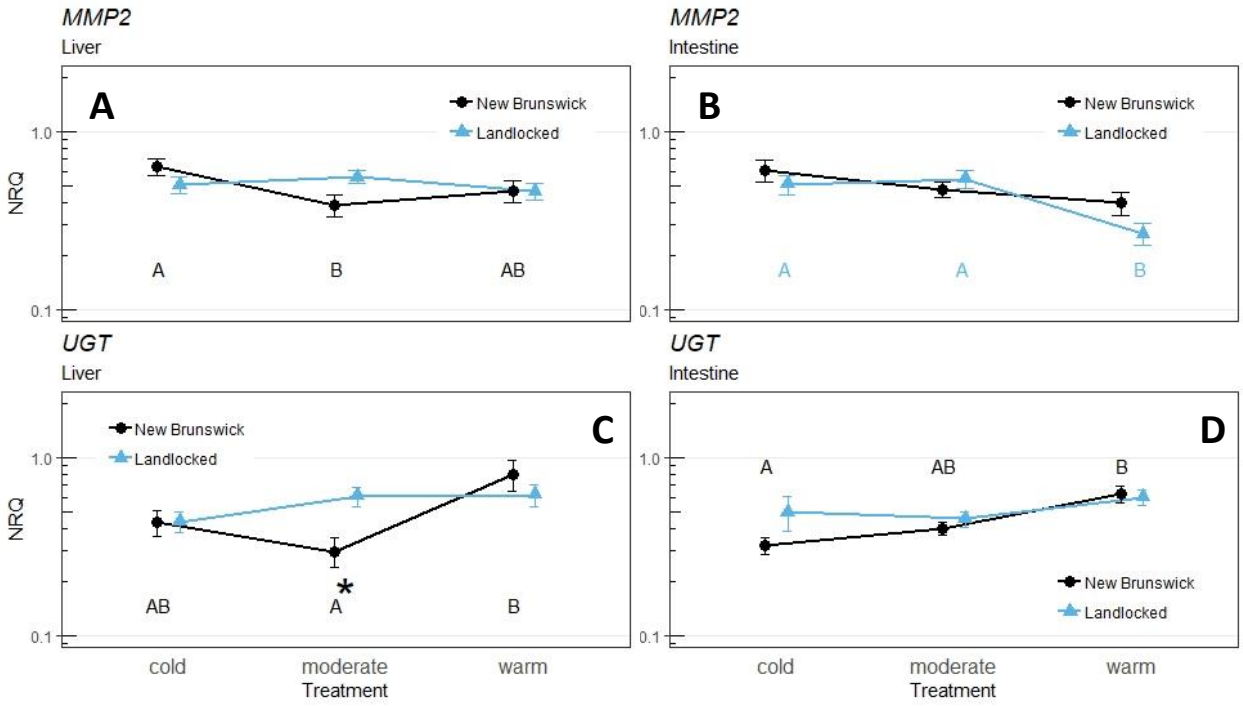


Figure 3.6. Mean (±SEM) *MMP2* and *UGT* expression relative to the three control genes from the liver (A, C) and intestine (B, D) tissues from both the New Brunswick anadromous (black circles) and landlocked (blue triangles) populations. Capital letters denote statistically significant differences within populations across treatments, corresponding to the colour of the population. Asterisk denotes statistically significant differences between populations within a treatment.

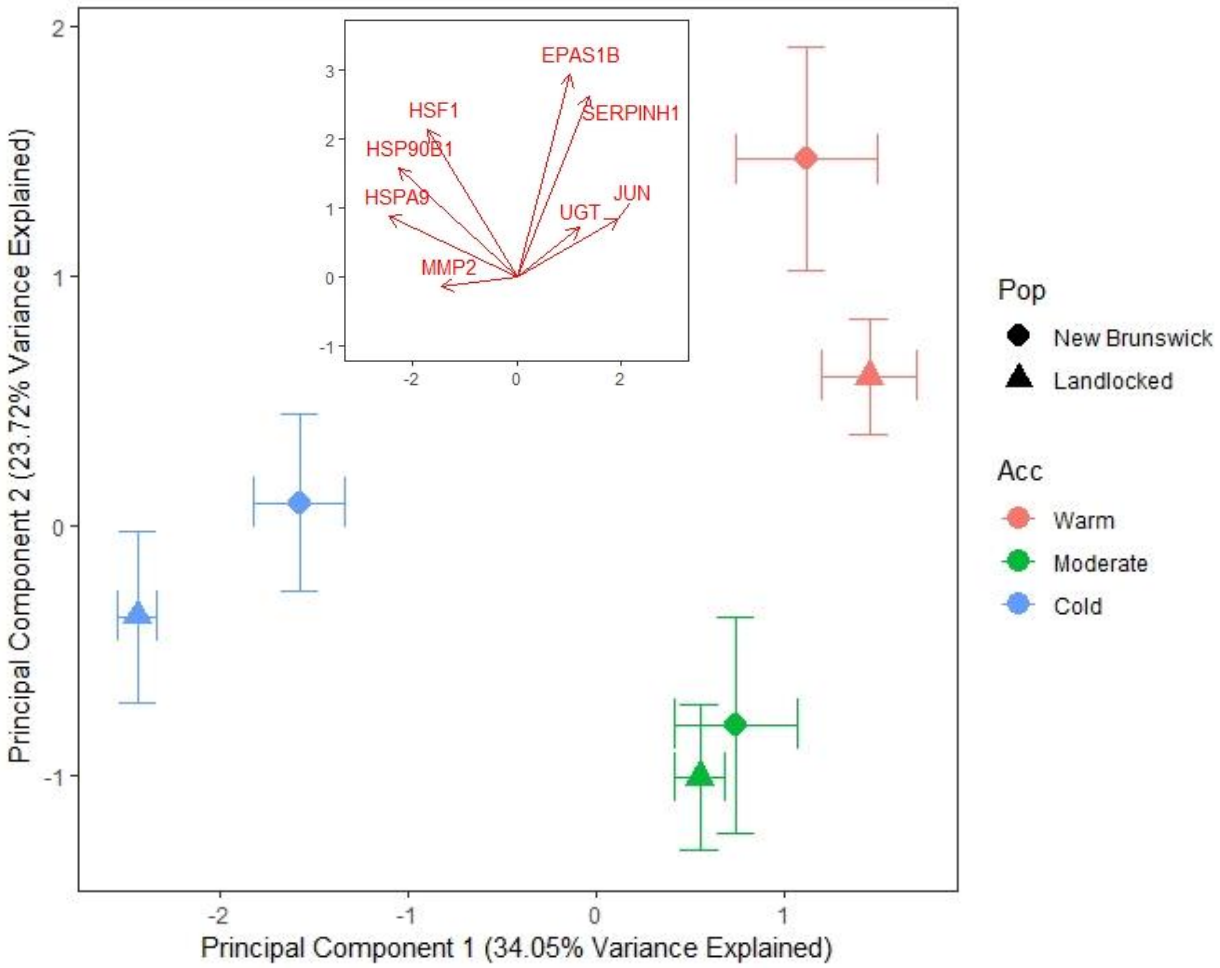


Figure 3.7. Principal components analysis on intestine gene expression results, with the corresponding biplot inset. Mean values (±SEM) for each experimental group presented, with triangles for the landlocked population and circles for the New Brunswick anadromous population. Cold, moderate and warm treatments are presented in blue, green, and red, respectively.

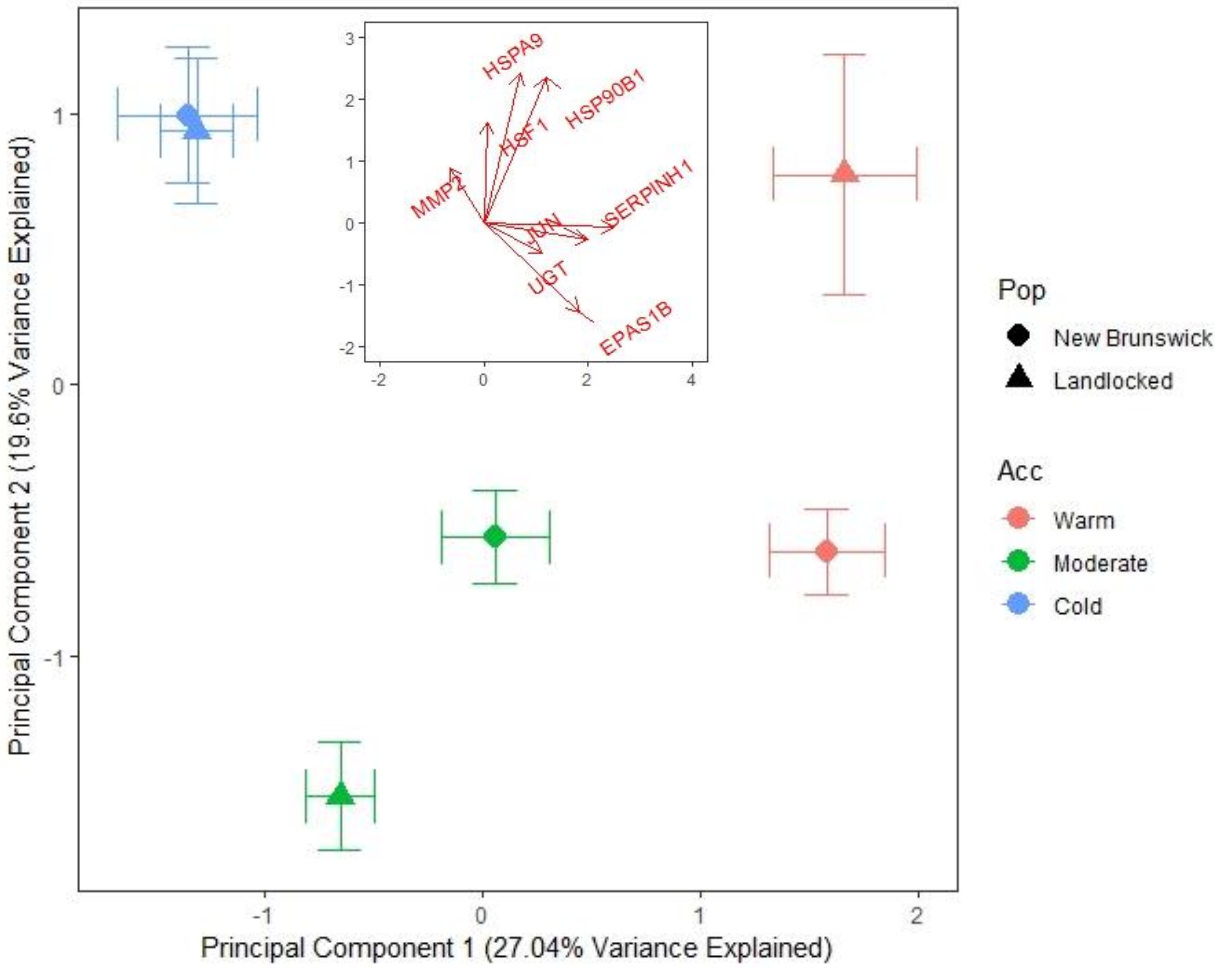


Figure 3.8. Principal components analysis on liver gene expression results, with the associated biplot inset. Mean values (±SEM) for each experimental group presented, with triangles for the landlocked population and circles for the New Brunswick anadromous population. Cold, moderate and warm treatments are presented in blue, green, and red, respectively.

Table 3.1. USGS and Province of New Brunswick water quality monitoring site information for sites used to gather tributary temperature data. A total of 54 observations were available for the Richibucto River, an average of 3.4 observations per year.

	Site ID	River	Latitude	Longitude	Duration of Record (# years used)
Atlantic (USGS)	1021050	St. Croix River	45°10'12"	67°17'48"	1972-2017 (44)
	1038000	Sheepscot River	44°13'22"	69°35'38"	1974-76, 2004-15 (11)
	1073319	Lamprey River	43°02'29"	71°12'06"	2008-2017 (8)
	1193500	Salmon River	41°33'08"	72°26'59"	1975-1992 (16)
	1196500	Quinnipiac River	41°27'00.95"	72°50'28.59"	2014-2017 (4)
	1372058	Hudson River	41°39'03"	73°56'42"	1992-2017 (26)
	1400500	Raritan River	40°33'20"	74°34'58"	2008-2017 (10)
	1408000	Manasquan River	40°09'41"	74°09'17"	1969-74, 2015-2017 (9)
	1427510	Delaware River	41°45'24.3"	75°03'26.7"	1975-2017 (37)
	1480065	Christina River	39°42'38.3"	75°36'31.4"	2006-2017 (12)
Huron	04157005	Saginaw River	43°25'19"	83°57'07"	2012-2016 (5)
Atlantic (NB)	Richibucto River above Smiths Corner Bridge (New Brunswick)		46.51	-65.163	2003-2018 (16)

Table 3.2. Information on genes and primers used in the present study. Accession numbers are available on the current Ensembl release, v.95. *JUN* and *HSF1* are unannotated in the current Ensembl database but were annotated as such when the database was first accessed to design primers (v.90). These sequences were BLASTed in a different sea lamprey genome (SIMRbase) which has them annotated as *JUN* and *HSF1*. The *EPAS1B* sequence is annotated as such in Ensembl v.95, but is annotated as *HIF1A* in the SIMRbase genome.

Functional group	Gene Code	Full gene name	Accession No.	Efficiency	Primer sequence
Transcription factor	*JUN*	Jun proto-oncogene, AP-1 transcription factor subunit	ENSPMAT00000004097.1	2.02 101%	F-ATG AAC TCC AGG ATG GAA GC R-GGA GAG GCT CTT TTT CAT TGC
	HSF1	(Predicted) Heat shock transcription factor 1	ENSPMAT00000005090.1	2.02 101%	F-CAT GGG CAG GAT CAT AAG TG R-TCA TGG TGT TGA GTC GGT TG
	EPAS1B	Endothelial PAS domain protein 1b	ENSPMAT00000000148.1	1.99 99.5%	F-TAC GAA TGC CTT GCG ACA AC R-TGT TGA AGT GGT GGA CAT GC
Molecular Chaperone	*HSP90B1*	Heat shock protein 90 Beta family member 1	ENSPMAT00000001245.1	1.99 99.5%	F-TCA CGT TGG TGC TCA AGG AA R-GGT TCT TCA ACG GTC TCG GT
	HSPA9	Heat shock protein family A (hsp70) member 9	ENSPMAT00000007246.1	1.97 98.5%	F-CCA ACC CAA ACA ACA CCT TC R-TTC TGC ACA TCG GAG TCA TC
	SERPINH1A	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1a	ENSPMAT00000008277.1	1.94 97%	F-ATG CAT CGC ACA GGC TTC TA R-TTG AGC TGC ATC TCC AGC AG
Proteolytic Protein	*MMP2*	Matrix metallopeptidase 2	ENSPMAT00000003005.1	1.97 98.5%	F-GCC CAA GTA CGA GGT GTG TA R-GCG CAT GTG CAT CTT GTT CT
Ribosomal protein (Reference)	*RPL8*	Ribosomal protein L8	ENSPMAT00000009691.1	2.0 100%	F-GCG CTC ACA ACA AAC ACA AG R-CGT GGA TTA TGT CCT TGA CG
	RPL13A	Ribosomal protein L13A	ENSPMAT00000007151.1	2.0 100%	F-ATC GCC TGA AGG TGT TTG AC R-TAC TGC AAA CTT GCG TGT GG
	RPS6	Ribosomal protein s6	ENSPMAT00000008266.1	1.96 98%	F-AGA TCC GCA AGC TCT TCA AC R-TTT GCC TTC CTT GGT GAG TG

Table 3.3. Acclimation and CTMax temperatures for the New Brunswick anadromous and landlocked sea lamprey populations, and regression coefficients for the linear models of the relationship between the two for each population (CTMax = α + β*Acclimation).

Treatment	Acclimation Temperatures °C (±sd)		CTMax °C (±SE)		Regression coefficients (±SE)		
	New Brunswick	*Landlocked*	*New Brunswick*	*Landlocked*		*New Brunswick*	*Landlocked*
Cold	5.0 (± 0.3)	5.0 (± 0.4)	32.5 (± 0.2)	33.3 (± 0.1)	**β**	0.1243 (±0.01)	0.1196 (±0.01)
Moderate	13.5 (± 0.9)	14.8 (± 0.5)	33.4 (± 0.1)	34.4 (± 0.2)	**α**	31.845 (±0.16)	32.674 (±0.20)
Warm	20.3 (± 1.5)	21.0 (± 1.9)	34.5 (± 0.1)	35.2 (± 0.1)	**Adj. R^2**	0.7309	0.6442

Table 3.4. Summary table of planned contrasts in two-way ANOVAs on liver and intestine gene expression data. Bonferroni-corrected p-values presented here. **Bold** numbers indicate $p<0.05$, *italics* indicate $0.05<p<0.1$.

	Defined Contrasts		P-values							
			HSF1	*EPAS1B*	*JUN*	*HSP90B1*	*HSPA9*	*SERPINH1A*	*MMP2*	*UGT*
Liver	LL vs NB	Warm	1.0	**5.87e-3**	1.0	**8.07e-5**	1.0	1.0	1.0	1.0
		Moderate	1.0	6.56e-1	1.0	1.01e-1	**1.72e-3**	1.36e-1	2.21e-1	**3.73e-3**
		Cold	5.98e-1	1.0	1.0	2.68e-1	1.0	*8.94e-2*	1.0	1.0
	NB	Cold v Moderate	1.0	**2.55e-4**	1.0	2.68e-1	1.0	**4.01e-7**	**4.96e-2**	4.25e-1
		Moderate v Warm	1.0	8.41e-1	1.0	1.0	1.0	**9.34e-3**	1.0	**1.48e-4**
		Cold v Warm	1.0	**5.41e-7**	1.01e-1	1.0	1.0	**1.13e-12**	4.73e-1	*8.29e-2*
	LL	Cold v Moderate	3.12e-1	**3.98e-2**	**1.54e-2**	**4.11e-3**	**4.76e-5**	1.0	1.0	9.68e-1
		Moderate v Warm	1.0	1.0	1.0	**3.49e-10**	**1.82e-4**	**2.20e-8**	1.0	1.0
		Cold v Warm	9.86e-1	*5.00e-2*	**8.04e-5**	**3.09e-4**	1.0	**2.72e-10**	1.0	9.32e-1
Int.	LL vs NB	Warm	1.0	1.09e-1	1.0	1.0	1.0	4.93e-1	2.31e-1	1.0
		Moderate	1.0	1.0	2.87e-1	1.0	1.0	1.0	1.0	1.0
		Cold	1.0	4.79e-1	**1.44e-5**	1.0	1.55e-1	1.0	1.0	2.43e-1
	NB	Cold v Moderate	**2.40e-2**	1.0	1.0	**3.19e-4**	**3.77e-4**	8.78e-1	1.0	1.0
		Moderate v Warm	1.0	**5.80e-4**	1.0	7.05e-1	1.0	**1.83e-6**	1.0	1.19e-1
		Cold v Warm	1.0	**7.07e-4**	9.83e-1	*8.00e-2*	**5.54e-3**	**3.41e-9**	1.91e-1	**1.02e-3**
	LL	Cold v Moderate	1.46e-1	1.0	**2.14e-3**	**1.2e-7**	**1.49e-7**	1.66e-1	1.0	1.0
		Moderate v Warm	1.0	1.49e-1	*6.38e-2*	7.02e-1	1.0	**1.99e-2**	**9.78e-4**	7.62e-1
		Cold v Warm	5.23e-1	5.26e-3	**9.95e-8**	**8.56e-5**	**1.04e-5**	**5.69e-6**	**3.67e-3**	9.39e-1

Chapter 4: General Discussion

To the best of my knowledge, this study was the first to evaluate CTMax for sea lamprey at any life stage. The CTMax of some species align well with thermal limits observed under natural conditions (Jeffries et al. 2018). In the case of sea lamprey ammocoetes, however, this does not hold true. Previous investigations on their upper thermal limit using incipient lethal temperature predicted that a temperature of 31.4 °C would kill 50 % of a sea lamprey population within two weeks (Potter and Beamish 1975). Based on the present results, exposure to 32.5 °C for four hours is lethal to 60 % of a population acclimated to 20 °C, while four hours at 31.9 °C is lethal to 30 % of the same population, insinuating the presence of a concrete thermal ceiling around 32 °C which is lethal to sea lamprey ammocoetes on the order of hours. This further suggests that the CTMax method, while a useful tool to estimate acutely lethal temperatures in a population, may have limited applicability in sea lamprey ammocoete management because it overshoots lethal temperatures by a few degrees at this life stage, as has been previously reported for other species (Beitinger et al. 2000). The CTMax is a useful method to rapidly estimate relative acutely lethal temperatures, and to compare thermal tolerance between species or populations, but from a population management perspective however, it is best viewed as one of many tools that can be used to estimate thermal limits, and it is most useful when used with other parameters including thermal distribution in the wild, behavioural temperature preference and agitation experiments, and physiological indicators of sublethal thresholds (Jeffries et al. 2018).

It is possible that the endpoint selected, lack of response to a physical stimulus, was not appropriate for estimating the CTMax, but there were few other repeatable alternatives. During the preliminary CTMax trial runs it was observed that after emergence from the sediment, while the ammocoetes were rapidly swimming around the beaker, they would occasionally perform a

so-called ‘loop-de-loop’, swimming in a tight vertical circle in an uncoordinated manner. Consideration was given to recording the onset temperature of this behaviour, but unfortunately it was not consistently exhibited, and with multiple individuals being run simultaneously the likelihood of missing the onset of this behaviour would have been too high without recording video of the trials.

In Chapter Two, the existence of two physiological thresholds which exist around 24 °C – 26 °C and ~30 °C were proposed, and evidence of an apparently hard upper acutely lethal temperature around 32 °C was found. The threshold ~24 °C – 26 °C corresponds to the initiation temperature of heat shock protein expression, as previously described by Wood et al. (1999) and reinforced with the present data, as well as a decline in metabolic rates (Holmes and Lin 1994). The first proposed threshold represents the temperature where inducible elements of the cellular stress response are initiated to maintain cellular homeostasis. The second threshold corresponds to the point at which the cellular stress response shifts away from a homeostasis maintenance, or ‘routine’ stress response, and towards a severe stress response, or a survival response. This is supported in the present data by the relative decrease in expression of some molecular chaperones in the CTMax-2 °C compared with the 26 °C treatments, combined with the sustained high expression of the immediate early gene *JUN*. While the low resolution of the present study somewhat restricts the ability to accurately estimate the threshold temperature, the threshold is more likely closer to 30 °C than to 26 °C given that mean escape response temperatures for all acclimation groups were above 30 °C, with the lowest response initiated at 28.2 °C. This upper, sublethal threshold also agrees with the highest temperature to which sea lamprey ammocoetes have been acclimated (30 °C: McCauley 1963) and is very close to both chronic (31.4 °C) and acute upper thermal limits (~32 °C).

The landlocked sea lamprey ammocoete population demonstrated a higher upper thermal tolerance than the New Brunswick anadromous population, as measured by CTMax, but no change in thermal tolerance plasticity, as the relationship between acclimation temperature and CTMax remained constant at 0.12. Along with low acclimation response ratios to incipient lethal temperatures in sea lamprey, brook lamprey, and pouched lamprey (Potter and Beamish 1975; Macey and Potter 1978), this suggests that a low thermal acclimation capacity may be common in Petromyzontiformes. While invasion theory predicts an increase in phenotypic plasticity, the relationship between acclimation temperature and CTMax appears inflexible in sea lamprey, and therefore the genetic raw material may have been inadequate to increase plasticity of this trait. Meanwhile, transcriptional plasticity, itself a measurement of phenotypic plasticity, does appear to have increased in the landlocked population, as demonstrated by the differential expression of *HSP90B1*, *HSPA9*, *JUN*, *UGT*, and the different expression pattern of *SERPINH1A*.

Previously, sessile organisms have been found to possess a reduced ability to adjust their heat stress response induction temperature compared with mobile species, as it is thought that a broad thermal tolerance is achieved at the cost of reduced acclimation capability (Barua and Heckathorn 2004). Sea lamprey ammocoetes are not strictly sessile, as they can move through stream substrate and can swim surprisingly well, yet in this respect they act as a sessile species displaying an ability to tolerate a wide range of temperatures, but do not appear to shift induction temperatures through thermal acclimation. Once they have metamorphosed into free-swimming parasitic juveniles, sea lamprey are likely to experience much more rapid temperature fluctuations, and need to be able to respond appropriately. Sea lamprey are known to feed on a huge variety of sea life, including fish, baleen whales (Beamish, 1980; Nichols and Hamilton, 2004; Nichols and Tscherter, 2011), and sharks (Wilkie et al., 2004; Gallant et al., 2006). Some

of these prey species, especially in the case of baleen whales, basking sharks, and Greenland sharks, dive to significant depths before returning to the surface, resulting in dramatic, rapid temperature fluctuations (Herdendorf and Berra, 1995; Gore et al., 2008). Indeed, a juvenile sea lamprey was caught by trawlers at a depth of 4099 m, approximately 400 km off the coast of New England (Haedrich, 1977). If this sea lamprey was caught at this depth, instead of at an intermediate depth while raising the net, this individual would have to be coping with multiple stressors, including barometric and cold stress. Investigations in *Saccharomyces cerevisiae* and *Escherichia coli* have demonstrated overlap between the heat shock response and the response to hydrostatic pressure and have also demonstrated that exposure to heat shock increases survival to high pressure (Iwahashi et al., 1991; Bartlett et al., 1995; Aertsen et al., 2004). The ability to mount a robust CSR to a variety of potential stressors is therefore important during the parasitic juvenile phase as well as the more sedentary larval phase, and the ability to mount a strong CSR is likely conserved throughout the sea lamprey lifecycle.

4.1 Potential Ecological Consequences

Nursery streams for sea lamprey ammocoetes regularly reach temperatures of 26 °C in their native habitat, and temperatures approaching the incipient lethal temperature of 31.4 °C (Potter and Beamish 1975) have been recorded in downstream tributaries of natal streams. For example, the Potomac River is fed by several historically ammocoete-producing streams (Beamish 1980). Between 2007 and 2016, the highest average daily water temperature recorded 30.5 cm from the riverbed in the Potomac River just West of Washington D.C. was 32.8 °C, while it has spent an average of 79.5 d (range 61 d – 94 d), and 10.6 d (range 0 d – 24 d) annually at or above an average temperature of 26 °C and 30 °C, respectively. As presented in

Chapter Two, sea lamprey ammocoetes initiate the CSR near these temperatures. With global temperatures expected to continue to rise, the occurrence of these temperatures in historical sea lamprey spawning streams is likely to continue to increase (IPCC 2014).

If the substrate in sea lamprey ammocoete-hosting streams reach temperatures at or above the proposed physiological threshold ~24 °C – 26 °C, cellular resources will need to be redirected from normal metabolism and growth processes into synthesizing molecular chaperones to protect against macromolecular damage. As more time is spent at these temperatures, impacts on development could occur, resulting in altered growth rates, delayed metamorphosis, extended generation times, and ultimately loss of certain streams as larval habitat. On the other hand, these negative effects could potentially be offset by a longer growing season. However, if river and substrate temperatures begin to regularly cross the ~30 °C threshold during summer months, eliciting an increase in the tier of CSR from a routine stress response to a survival response, sea lamprey ammocoete fitness could be compromised.

Meanwhile, the temperature at which escape responses were initiated during the CTMax trials (lowest temperature: 28.2 °C – cold acclimation group; 31.1 °C – warm acclimation group) or those reported previously (Potter and Beamish 1975), were very close to the proposed severe threshold temperature ~30 °C. While the escape behaviour described here may not be observed under natural conditions, where heating rates would likely be lower, and the option to burrow into deeper, cooler substrate exists (although deeper substrate becomes more hypoxic: Williams and Hynes 1974; Strommer and Smock 1989; Atkinson et al. 2008), it represents a proxy for the initiation temperature of an agitation response. Behavioural avoidance responses to increased temperature may therefore be limited to conditions very close to sea lamprey ammocoetes'

ultimate thermal limits, making a robust cellular stress response important to avoid permanent or lingering damage resulting from thermal stress events.

Together, this could result in changes to future sea lamprey distribution as they begin to avoid streams which become too warm to successfully produce ammocoetes and shift into cooler streams, which will likely manifest in an abandonment of streams along their southern range, and an expansion northward (Sunday et al. 2012, 2015). I would therefore predict that the New Brunswick population will not be negatively impacted by rising temperatures, as they are not restricted from expanding northwards. On the other hand, the landlocked population may be at greater risk of population declines. While they have a modestly higher upper thermal tolerance compared to New Brunswick sea lamprey ammocoetes (~0.8 °C), the landlocked population has a greatly reduced capacity to expand their range. Currently sea lamprey ammocoetes have only been detected in ~8 % of tributaries of the Great Lakes Basin, but there is no evidence that temperature is the limiting factor restricting their use of the other 92 %. Therefore, if future warming makes streams in the southern Great Lakes unsuitable for rearing ammocoetes, then the density of currently infested streams in the northern Great Lakes should increase. As the sea lamprey population becomes more concentrated, resource managers will be able to target fewer streams for control efforts, with greater overall impact on the population.

4.2 Future Directions

The main drawback of the present study is the limited number of genes examined. Ideally, a full transcriptomic study could be performed, or at least a wider swath of genes involved in more processes could have been examined, which may help elucidate what processes are being differentially regulated in response to the varying levels of thermal stress. Here, three

transcription factors, three molecular chaperones, and one proteolytic enzyme were selected, of which about half demonstrated a transcriptional response to experimental treatments. It would be beneficial to test additional genes involved in the cell cycle (e.g. CDKNs), apoptosis (e.g. CASPS), more proteolytic enzymes (e.g. UBQs), and transcription factors involved in different response pathways. Another aspect that was not examined here was the pattern of gene expression following recovery from thermal stress. Recovery patterns would be useful to more clearly define sublethal thresholds where recovery is either delayed or impossible, ultimately leading to fitness consequences (Jeffries et al., 2018). The physiological thresholds proposed here indicate transitions in the tier of cellular stress response, but it is unknown if once the thermal stressor is lifted how soon, or if, cellular processes will return to normal.

In order to make a more robust comparison between the New Brunswick and landlocked populations, the acute heat shock experiment should be performed on the landlocked population. This would allow for more inferences into differences in thermal tolerance between populations, including whether the initiation temperature of genes is shifted, whether expression patterns indicative of stressor threshold levels are shifted, and whether one population is capable of mounting a stronger response than the other. These could help determine the source of the increased thermotolerance observed in the landlocked population. In the best case, a full transcriptomic study could be performed on both populations. In addition to the interesting results this would generate in terms of differential gene expression, it would also allow inference into population structuring between the populations and could potentially allow a glimpse into whether the landlocked sea lamprey population has undergone any rapid adaptation within the Great Lakes.

Future research on the thermal tolerance of sea lamprey ammocoetes should include more thermal stress temperatures. The present study used only two heat stress temperatures, limiting the ability to pinpoint threshold temperatures. Combining this data with that previously published by Wood et al. (1999), provides a more accurate estimate of the initiation temperature for the heat shock response; however, the transition from routine stress response to a severe cellular survival response cannot be accurately stated. A future study performing heat shocks at smaller temperature intervals from 24 °C to 31 °C on ammocoetes acclimated to different temperatures could verify the impact, or lack thereof, that acclimation history has on initiation temperature of the heat shock response, or transition temperatures from different tiers of a CSR.

Another interesting angle which could be explored is a more fine-scale evaluation of thermal tolerance in different sea lamprey populations. Based on the differences observed between the landlocked and New Brunswick population in the present study, I suspect there would be a difference in thermal tolerance across the range of sea lamprey. Comparing populations from Georgia or the Carolinas to those from Delaware or New Jersey, to Newfoundland could paint a picture of adaptive capacity in native sea lamprey. Further, quantifying thermal tolerance of populations from Lakes Ontario and Erie could provide hints as to whether Lake Erie, the warmest of the Great Lakes, acted as a strong filter to invasion, which had to be overcome prior to colonization of the rest of the Great Lakes.

4.3 Summary

In conclusion, this study demonstrated that sea lamprey are capable of mounting a rapid, dramatic change in gene expression in order to respond to an acute thermal stressor. Acclimation history has an influence on the induction temperature and severity of certain responses; however,

overall gene expression patterns are similar. Evidence for sublethal thresholds around 24 °C – 26°C and 30 °C were uncovered in the gene expression patterns of New Brunswick sea lamprey ammocoetes, which appeared to be independent of acclimation history. The invasive, landlocked population of sea lamprey ammocoetes demonstrated a consistently higher CTMax than a New Brunswick anadromous population at a range of acclimation temperatures. The landlocked population also demonstrated increased plasticity in *JUN*, *HSP90B1*, and *HSPA9* in response to different chronic treatments at a range of ecologically-relevant temperatures. Expression of *UGT* in the liver of the landlocked population was also differentially expressed under moderate conditions relative to the New Brunswick population, which may influence how the landlocked population processes the lampricide TFM.

Literature Cited

Akbarzadeh A, Günther OP, Houde AL, et al (2018) Developing specific molecular biomarkers for thermal stress in salmonids. BMC Genomics 19:. doi: 10.1186/s12864-018-5108-9

Applegate V (1950) Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. Ph.D Thesis, University of Michigan

Aron WI, Smith SH (1971) Ship canals and aquatic ecosystems. Sci New Ser 174:13–20

Asea AAA (2018) Regulation of heat shock protein responses. Springer Berlin Heidelberg, New York, NY

Atkinson BL, Grace MR, Hart BT, Vanderkruk KEN (2008) Sediment instability affects the rate and location of primary production and respiration in a sand-bed stream. J North Am Benthol Soc 27:581–592. doi: 10.1899/07-143.1

Austin J, Colman S (2008) A century of temperature variability in Lake Superior. Limnol Oceanogr 53:2724–2730. doi: 10.4319/lo.2008.53.6.2724

Austin JA, Colman SM (2007) Lake Superior summer water temperatures are increasing more rapidly than regional air temperatures: A positive ice-albedo feedback. Geophys Res Lett 34:. doi: 10.1029/2006GL029021

Baer J, Hartmann F, Brinker A (2018) Abiotic triggers for sea and river lamprey spawning migration and juvenile outmigration in the River Rhine, Germany. Ecol Freshw Fish 27:988–998. doi: 10.1111/eff.12409

Barrett RDH, Paccard A, Healy TM, et al (2011) Rapid evolution of cold tolerance in stickleback. Proc R Soc B Biol Sci 278:233–238. doi: 10.1098/rspb.2010.0923

Barua D, Heckathorn SA (2004) Acclimation of the temperature set-points of the heat-shock response. J Therm Biol 29:185–193. doi: 10.1016/j.jtherbio.2004.01.004

Bates AE, McKelvie CM, Sorte CJB, et al (2013) Geographical range, heat tolerance and invasion success in aquatic species. Proc R Soc B Biol Sci 280:20131958–20131958. doi: 10.1098/rspb.2013.1958

Baudel MASM-A, Rae MT, Darlison MG, et al (2017) Preferential activation of HIF-2α adaptive signalling in neuronal-like cells in response to acute hypoxia. PLOS ONE 12:e0185664. doi: 10.1371/journal.pone.0185664

Beamish FWH (1980) Biology of the North American anadromous sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 37:1924–1943. doi: 10.1139/f80-233

Beamish FWH, Potter IC, Thomas E (1979) Proximate composition of the adult anadromous sea lamprey, *Petromyzon marinus*, in relation to feeding, migration and reproduction. J Anim Ecol 48:1. doi: 10.2307/4096

Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ Biol Fishes 58:237–275. doi: 10.1023/A:1007676325825

Bence JR, Bergstedt RA, Christie GC, et al (2003) Sea lamprey (*Petromyzon marinus*) parasite-host interactions in the Great Lakes. J Gt Lakes Res 29:253–282. doi: 10.1016/S0380-1330(03)70493-6

Bilyk KT, DeVries AL (2011) Heat tolerance and its plasticity in Antarctic fishes. Comp Biochem Physiol A Mol Integr Physiol 158:382–390. doi: 10.1016/j.cbpa.2010.12.010

Binder TR, McLaughlin RL, McDonald DG (2010) Relative importance of water temperature, water level, and lunar cycle to migratory activity in spawning-phase sea lampreys in Lake Ontario. Trans Am Fish Soc 139:700–712. doi: 10.1577/T09-042.1

Birceanu O, McClelland GB, Wang YS, et al (2011) The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) uncouples mitochondrial oxidative phosphorylation in both sea lamprey (*Petromyzon marinus*) and TFM-tolerant rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol Part C Toxicol Pharmacol 153:342–349. doi: 10.1016/j.cbpc.2010.12.005

Birceanu O, McClelland GB, Wang YS, Wilkie MP (2009) Failure of ATP supply to match ATP demand: The mechanism of toxicity of the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), used to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes. Aquat Toxicol 94:265–274. doi: 10.1016/j.aquatox.2009.07.012

Birceanu O, Sorensen LA, Henry M, et al (2014) The effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on fuel stores and ion balance in a non-target fish, the rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol Part C Toxicol Pharmacol 160:30–41. doi: 10.1016/j.cbpc.2013.10.002

Birceanu O, Wilkie MP (2018) Post-exposure effects of the piscicide 3-trifluoromethyl-4-nitrophenol (TFM) on the stress response and liver metabolic capacity in rainbow trout (*Oncorhynchus mykiss*). PLOS ONE 13:e0200782. doi: 10.1371/journal.pone.0200782

Blackburn TM, Pyšek P, Bacher S, et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339. doi: 10.1016/j.tree.2011.03.023

Boogaard MA, Bills TD, Johnson DA (2003) Acute toxicity of TFM and a TFM/niclosamide mixture to selected species of fish, including lake sturgeon (*Acipenser fulvescens*) and mudpuppies (*Necturus maculosus*), in laboratory and field exposures. J Gt Lakes Res 29:529–541. doi: 10.1016/S0380-1330(03)70514-0

Bowler K, Terblanche JS (2008) Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol Rev 83:339–355. doi: 10.1111/j.1469-185X.2008.00046.x

Braby CE, Somero GN (2006) Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). J Exp Biol 209:2554–2566. doi: 10.1242/jeb.02259

Brett JR (1971) Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am Zool 11:99–113. doi: 10.1093/icb/11.1.99

Bryan MB, Zalinski D, Filcek KB, et al (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. Mol Ecol 14:3757–3773. doi: 10.1111/j.1365-294X.2005.02716.x

Buckley BA, Hofmann GE (2002) Thermal acclimation changes DNA-binding activity of heat shock factor 1 (HSF1) in the goby *Gillichthys mirabilis*: implications for plasticity in the heat-shock response in natural populations. J Exp Biol 205:3231–3240

Buckley BA, Hofmann GE (2004) Magnitude and duration of thermal stress determine kinetics of *hsp* gene regulation in the goby *Gillichthys mirabilis*. Physiol Biochem Zool 77:570–581. doi: 10.1086/420944

Campos DF, Jesus TF, Kochhann D, et al (2017) Metabolic rate and thermal tolerance in two congeneric Amazon fishes: *Paracheirodon axelrodi* Schultz, 1956 and *Paracheirodon simulans* Géry, 1963 (Characidae). Hydrobiologia 789:133–142. doi: 10.1007/s10750-016-2649-2

Carveth CJ, Widmer AM, Bonar SA (2006) Comparison of upper thermal tolerances of native and nonnative fish species in Arizona. Trans Am Fish Soc 135:1433–1440. doi: 10.1016/j.jbusres.2007.02.011

Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib 15:22–40. doi: 10.1111/j.1472-4642.2008.00521.x

Chen B, Piel WH, Gui L, et al (2005) The HSP90 family of genes in the human genome: Insights into their divergence and evolution. Genomics 86:627–637. doi: 10.1016/j.ygeno.2005.08.012

Chen Z, Farrell AP, Matala A, Narum SR (2018) Mechanisms of thermal adaptation and evolutionary potential of conspecific populations to changing environments. Mol Ecol 27:659–674. doi: 10.1111/mec.14475

Cheung WWL, Lam VWY, Sarmiento JL, et al (2009) Projecting global marine biodiversity impacts under climate change scenarios. Fish Fish 10:235–251. doi: 10.1111/j.1467-2979.2008.00315.x

Chrétien E, Chapman LJ (2016) Tropical fish in a warming world: thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. Conserv Physiol 4:cow062. doi: 10.1093/conphys/cow062

Chung KS (2001) Critical thermal maxima and acclimation rate of the tropical guppy *Poecilla reticulata*. Hydrobiologia 462:253–257

Cline TJ, Kitchell JF, Bennington V, et al (2014) Climate impacts on landlocked sea lamprey: Implications for host-parasite interactions and invasive species management. Ecosphere 5:art68. doi: 10.1890/ES14-00059.1

Comte L, Olden JD (2017) Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Glob Change Biol 23:728–736. doi: 10.1111/gcb.13427

D'Aloia CC, Azodi CB, Sheldon SP, et al (2015) Genetic models reveal historical patterns of sea lamprey population fluctuations within Lake Champlain. PeerJ 3:e1369. doi: 10.7717/peerj.1369

Dammark KB, Ferchaud AL, Hansen MM, Sørensen JG (2018) Heat tolerance and gene expression responses to heat stress in threespine sticklebacks from ecologically divergent environments. J Therm Biol 75:88–96. doi: 10.1016/j.jtherbio.2018.06.003

Daniels RA (2001) Untested assumptions: The role of canals in the dispersal of sea lamprey, alewife, and other fishes in the Eastern United States. Environ Biol Fishes 60:309–329

Daufresne M, Boët P (2007) Climate change impacts on structure and diversity of fish communities in rivers. Glob Change Biol 13:2467–2478. doi: 10.1111/j.1365-2486.2007.01449.x

Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol Lett 14:419–431. doi: 10.1111/j.1461-0248.2011.01596.x

Davis BE, Cocherell DE, Sommer T, et al (2019) Sensitivities of an endemic, endangered California smelt and two non-native fishes to serial increases in temperature and salinity: implications for shifting community structure with climate change. Conserv Physiol 7:. doi: 10.1093/conphys/coy076

Dawson HA, Quintella BR, Almeida PR, et al (2015) The ecology of larval and metamorphosing lampreys. In: Docker MF (ed) Lampreys: Biology, Conservation and Control. Springer Netherlands, Dordrecht, pp 75–137

Dempson JB, Porter TR (1993) Occurrence of sea lamprey, *Petromyzon marinus*, in a Newfoundland River, with additional records from the Northwest Atlantic. Can J Fish Aquat Sci 50:1265–1269. doi: 10.1139/f93-143

DeVaney SC, McNyset KM, Williams JB, et al (2009) A tale of four "carp": Invasion potential and ecological niche modeling. PLoS ONE 4:e5451. doi: 10.1371/journal.pone.0005451

Dilorio P, Holsinger K, Schultz RJ, Hightower LE (1996) Quantitative evidence that both Hsc70 and Hsp70 contribute to thermal adaptation in hybrids of the livebearing fishes *Poeciliopsis*. Cell Stress Chaperones 1:139–147

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. doi: 10.1111/j.1365-294X.2007.03538.x

Doney SC, Ruckelshaus M, Emmett Duffy J, et al (2012) Climate change impacts on marine ecosystems. Annu Rev Mar Sci 4:11–37. doi: 10.1146/annurev-marine-041911-111611

Dunlop ES, McLaughlin R, Adams JV, et al (2018) Rapid evolution meets invasive species control: the potential for pesticide resistance in sea lamprey. Can J Fish Aquat Sci 75:152–168. doi: 10.1139/cjfas-2017-0015

Edfors F, Danielsson F, Hallström BM, et al (2016) Gene-specific correlation of RNA and protein levels in human cells and tissues. Mol Syst Biol 12:883. doi: 10.15252/msb.20167144

Engel K, Tollrian R, Jeschke JM (2011) Integrating biological invasions, climate change and phenotypic plasticity. Commun Integr Biol 4:247–250. doi: 10.4161/cib.4.3.14885

Eshenroder RL (2009) Comment: Mitochondrial DNA analysis indicates sea lampreys are indigenous to Lake Ontario. Trans Am Fish Soc 138:1178–1189. doi: 10.1577/T08-035.1

Eshenroder RL (2014) The role of the Champlain Canal and Erie Canal as putative corridors for colonization of Lake Champlain and Lake Ontario by sea lampreys. Trans Am Fish Soc 143:634–649. doi: 10.1080/00028487.2013.879818

Evans TM (2017) Measuring the growth rate in three populations of larval lampreys with mark–recapture techniques. Trans Am Fish Soc 146:147–159. doi: 10.1080/00028487.2016.1249292

Evans TM, Janvier P, Docker MF (2018) The evolution of lamprey (Petromyzontida) life history and the origin of metamorphosis. Rev Fish Biol Fish 28:825–838. doi: 10.1007/s11160-018-9536-z

Facon B, Hufbauer RA, Tayeh A, et al (2011) Inbreeding Depression Is Purged in the Invasive Insect Harmonia axyridis. Curr Biol 21:424–427. doi: 10.1016/j.cub.2011.01.068

Fangue NA, Hofmeister M, Schulte PM (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. J Exp Biol 209:2859–2872. doi: 10.1242/jeb.02260

Fangue NA, Osborne EJ, Todgham AE, Schulte PM (2011) The onset temperature of the heat-shock response and whole-organism thermal tolerance are tightly correlated in both laboratory-acclimated and field-acclimatized tidepool sculpins (*Oligocottus maculosus*). Physiol Biochem Zool 84:341–352. doi: 10.1086/660113

Farmer G., Beamish FW., Robinson G. (1975) Food consumption of the adult landlocked sea lamprey, *Petromyzon marinus*, L. Comp Biochem Physiol A Physiol 50:753–757. doi: 10.1016/0300-9629(75)90141-3

Farmer GJ, Beamish FWH, Lett PF (1977) Influence of water temperature on the growth rate of the landlocked sea lamprey (*Petromyzon marinus*) and the associated rate of host mortality. J Fish Res Board Can 34:1373–1378. doi: 10.1139/f77-197

Feldmeth RC, Stone EA, Brown JH (1974) An increased scope for thermal tolerance upon acclimating pupfish (Cyprinodon) to cycling temperatures. J Comp Physiol 89:39–44. doi: 10.1007/BF00696161

Feminella JW, Matthews WJ (1984) Intraspecific differences in thermal tolerance of *Etheostoma spectabile* (Agassiz) in constant versus fluctuating environments. J Fish Biol 25:455–461. doi: 10.1111/j.1095-8649.1984.tb04892.x

Fodale MF, Bronte CR, Bergstedt RA, et al (2003) Classification of lentic habitat for sea lamprey (*Petromyzon marinus*) larvae using a remote seabed classification device. J Gt Lakes Res 29:190–203. doi: 10.1016/S0380-1330(03)70488-2

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS, Randall DJBT (eds) Fish Physiology - Environmental Relations and Behavior. Academic Press, pp 1–98

Fuller A, Dawson T, Helmuth B, et al (2010) Physiological mechanisms in coping with climate change. Physiol Biochem Zool 83:713–720. doi: 10.1086/652242

Gallant J, Harvey-Clark C, Myers RA, Stokesbury MJW (2006) Sea lamprey attached to a Greenland shark in the St. Lawrence Estuary, Canada. Northeast Nat 13:35–38. doi: 10.1656/1092-6194(2006)13[35:SLATAG]2.0.CO;2

Gaston KJ, Spicer JI (1998) Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarellus* (Crustacea: Amphipoda)? J Exp Mar Biol Ecol 229:265–276. doi: 10.1016/S0022-0981(98)00057-4

Ghalambor ACK, Mckay JK, Carroll SP, et al (2007) Adaptive Versus Non-Adaptive Phenotypic Plasticity and the Potential for Contemporary Adaptation in New Environments Published by : British Ecological Society Linked references are available on JSTOR for this article : Adaptive versus non-adaptive phenoty. Funct Ecol 21:394–407

Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B Biol Sci 282:20150401–20150401. doi: 10.1098/rspb.2015.0401

Haedrich RL (1977) A sea lamprey from the deep ocean. Copeia 1977:767. doi: 10.2307/1443185

Halliday RC (1991) Marine distribution of the sea lamprey (*Petromyzon marinus*) in the Northwest Atlantic. Can J Fish Aquat Sci 48:832–842. doi: 10.1139/f91-099

Hansen MJ, Madenjian CP, Slade JW, et al (2016) Population ecology of the sea lamprey (*Petromyzon marinus*) as an invasive species in the Laurentian Great Lakes and an imperiled species in Europe. Rev Fish Biol Fish 26:509–535. doi: 10.1007/s11160-016-9440-3

Hart JS (1952) Geographic variations of some physiological and morphological characters in certain freshwater fish. Univ Tor Biol Ser 60 79pp. doi: 10.1175/2010MWR3316.1

Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? Biol Invasions 10:483–506. doi: 10.1007/s10530-007-9146-5

Hayhoe K, Wake CP, Huntington TG, et al (2007) Past and future changes in climate and hydrological indicators in the US Northeast. Clim Dyn 28:381–407. doi: 10.1007/s00382-006-0187-8

Healy TM, Schulte PM (2012) Factors affecting plasticity in whole-organism thermal tolerance in common killifish (*Fundulus heteroclitus*). J Comp Physiol B 182:49–62. doi: 10.1007/s00360-011-0595-x

Heinrich JW, Mullett KM, Hansen MJ, et al (2003) Sea lamprey abundance and management in Lake Superior, 1957 to 1999. J Gt Lakes Res 29:566–583. doi: 10.1016/S0380-1330(03)70517-6

Hellemans J, Mortier G, Paepe AD, et al (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 14

Henkel SK, Kawai H, Hofmann GE (2009) Interspecific and interhabitat variation in hsp70 gene expression in native and invasive kelp populations. Mar Ecol Prog Ser 386:1–13. doi: 10.3354/meps08047

Henson MP, Bergstedt RA, Adams JV (2003) Comparison of spring measures of length, weight, and condition factor for predicting metamorphosis in two populations of sea lamprey (*Petromyzon marinus*) larvae. J Gt Lakes Res 29:204–213. doi: 10.1016/S0380-1330(03)70489-4

Hill MP, Chown SL, Hoffmann AA (2013) A predicted niche shift corresponds with increased thermal resistance in an invasive mite, Halotydeus destructor. Glob Ecol Biogeogr 22:942–951. doi: 10.1111/geb.12059

Hochachka PW, Somero GN (1971) Biochemical adaptation to the environment. In: Fish Physiology - Environmental Relations and Behavior. Academic Press, New York, NY

Hodgins K, Bock DG, Rieseberg LH (2018) Trait evolution in invasive species. Annu Plant Rev Online 1:1–37

Hodgkins G., Dudley R., Huntington T. (2003) Changes in the timing of high river flows in New England over the 20th Century. J Hydrol 278:244–252. doi: 10.1016/S0022-1694(03)00155-0

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328:1523–1528. doi: 10.1126/science.1189930

Holmes JA, Lin P (1994) Thermal niche of larval sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 51:253–262. doi: 10.1139/f94-026

Hosokawa N, Hohenadl C, Satoh M, et al (1998) HSP47, a collagen-specific molecular chaperone, delays the secretion of type III procollagen transfected in human embryonic kidney cell line 293: A possible role for HSP47 in collagen modification. J Biochem (Tokyo) 124:654–662. doi: 10.1093/oxfordjournals.jbchem.a022162

IPCC (2014) AR5 Synthesis Report: Climate Change 2014 — IPCC

Irwin BJ, Liu W, Bence JR, Jones ML (2012) Defining economic injury levels for sea lamprey control in the Great Lakes Basin. North Am J Fish Manag 32:760–771. doi: 10.1080/02755947.2012.685140

Jeffries KM, Connon RE, Davis BE, et al (2016) Effects of high temperatures on threatened estuarine fishes during periods of extreme drought. J Exp Biol 219:1705–1716. doi: 10.1242/jeb.134528

Jeffries KM, Fangue NA, Connon RE (2018) Multiple sub-lethal thresholds for cellular responses to thermal stressors in an estuarine fish. Comp Biochem Physiol -Part Mol Integr Physiol 225:33–45. doi: 10.1016/j.cbpa.2018.06.020

Jeffries KM, Hinch SG, Sierocinski T, et al (2012) Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*): Temperature Stress and Mortality in Sockeye Salmon. Ecol Evol 2:1747–1764. doi: 10.1002/ece3.274

Jeffries KM, Hinch SG, Sierocinski T, et al (2014) Transcriptomic responses to high water temperature in two species of Pacific salmon. Evol Appl 7:286–300. doi: 10.1111/eva.12119

Johansson MP, Laurila A (2017) Maximum thermal tolerance trades off with chronic tolerance of high temperature in contrasting thermal populations of *Radix balthica*. Ecol Evol 7:3149–3156. doi: 10.1002/ece3.2923

Johnson BGH, Anderson WC (1980) Predatory-phase sea lampreys (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:2007–2020. doi: 10.1139/f80-241

Johnson NS, Swink WD, Brenden TO (2017) Field study suggests that sex determination in sea lamprey is directly influenced by larval growth rate. Proc R Soc B Biol Sci 284:20170262. doi: 10.1098/rspb.2017.0262

Kane AS, Kahng MW, Reimschuessel R, et al (1994) UDP-glucuronyltransferase kinetics for 3-trifluoromethyl-4-nitrophenol (TFM) in fish. Trans Am Fish Soc 123:217–222. doi: 10.1577/1548-8659(1994)123<0217:UGKFTN>2.3.CO;2

Karatayev AY, Padilla DK, Minchin D, et al (2007) Changes in global economies and trade: The potential spread of exotic freshwater bivalves. Biol Invasions 9:161–180. doi: 10.1007/s10530-006-9013-9

Kaul SC, Reddel RR, Sugihara T, et al (2000) Inactivation of p53 and life span extension of human diploid fibroblasts by mot-2. FEBS Lett 474:159–164. doi: 10.1016/S0014-5793(00)01594-5

Kaul SC, Taira K, Pereira-Smith OM, Wadhwa R (2002) Mortalin: present and prospective. Exp Gerontol 37:1157–1164. doi: 10.1016/S0531-5565(02)00135-3

Kim J, Mandrak NE (2016) Assessing the potential movement of invasive fishes through the Welland Canal. J Gt Lakes Res 42:1102–1108. doi: 10.1016/j.jglr.2016.07.009

Koban M, Graham G, Prosser CL (1987) Induction of heat-shock protein synthesis in teleost hepatocytes: Effects of acclimation temperature. Physiol Zool 60:290–296. doi: 10.1086/physzool.60.2.30158653

Komoroske LM, Connon RE, Jeffries KM, Fangue NA (2015) Linking transcriptional responses to organismal tolerance reveals mechanisms of thermal sensitivity in a mesothermal endangered fish. Mol Ecol 24:4960–4981. doi: 10.1111/mec.13373

Komoroske LM, Connon RE, Lindberg J, et al (2014) Ontogeny influences sensitivity to climate change stressors in an endangered fish. Conserv Physiol 2:cou008–cou008. doi: 10.1093/conphys/cou008

Kültz D (2005) Molecular and Evolutionary Basis of the Cellular Stress Response. Annu Rev Physiol 67:225–257. doi: 10.1146/annurev.physiol.67.040403.103635

Lande R (2015) Evolution of phenotypic plasticity in colonizing species. Mol Ecol 24:2038–2045. doi: 10.1111/mec.13037

Lapointe D, Cooperman MS, Chapman LJ, et al (2018) Predicted impacts of climate warming on aerobic performance and upper thermal tolerance of six tropical freshwater fishes spanning three continents. Conserv Physiol 6:. doi: 10.1093/conphys/coy056

Lawrie AH (1970) The sea lamprey in the Great Lakes. Trans Am Fish Soc 99:766–774

Lee MV, Topper SE, Hubler SL, et al (2011) A dynamic model of proteome changes reveals new roles for transcript alteration in yeast. Mol Syst Biol 7:514. doi: 10.1038/msb.2011.48

Levis NA, Pfennig DW (2016) Evaluating 'plasticity-first' evolution in nature: Key criteria and empirical approaches. Trends Ecol Evol 31:563–574. doi: 10.1016/j.tree.2016.03.012

Li Y, Huang J, Liu Z, et al (2017) Transcriptome analysis provides insights into hepatic responses to moderate heat stress in the rainbow trout (*Oncorhynchus mykiss*). Gene 619:1–9. doi: 10.1016/j.gene.2017.03.041

Lindholm AK, Breden F, Alexander HJ, et al (2005) Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia: low diversity in invasive guppy populations. Mol Ecol 14:3671–3682. doi: 10.1111/j.1365-294X.2005.02697.x

Lindquist S, Craig EA (1988) the Heat-Shock Proteins. Campbell A 631–678. doi: 10.1146/annurev.ge.22.120188.003215

Liu S, Wang X, Sun F, et al (2013) RNA-Seq reveals expression signatures of genes involved in oxygen transport, protein synthesis, folding, and degradation in response to heat stress in catfish. Physiol Genomics 45:462–476. doi: 10.1152/physiolgenomics.00026.2013

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402–408. doi: 10.1006/meth.2001.1262

Lockwood BL, Sanders JG, Somero GN (2010) Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. J Exp Biol 213:3548–3558. doi: 10.1242/jeb.046094

Lockwood BL, Somero GN (2011) Invasive and native blue mussels (genus *Mytilus*) on the California coast: The role of physiology in a biological invasion. J Exp Mar Biol Ecol 400:167–174. doi: 10.1016/j.jembe.2011.02.022

Lodge DM (1993) Biological invasions: Lessons for ecology. Trends Ecol Evol 8:133–137. doi: 10.1016/0169-5347(93)90025-K

Logan C a, Somero GN (2011) Effects of thermal acclimation on transcriptional responses to acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). Am J Physiol Regul Integr Comp Physiol 300:R1373–R1383. doi: 10.1152/ajpregu.00689.2010

Logan CA, Buckley BA (2015) Transcriptomic responses to environmental temperature in eurythermal and stenothermal fishes. J Exp Biol 218:1915–1924. doi: 10.1242/jeb.114397

Logan CA, Somero GN (2010) Transcriptional responses to thermal acclimation in the eurythermal fish Gillichthys mirabilis (Cooper 1864). AJP Regul Integr Comp Physiol 299:R843–R852. doi: 10.1152/ajpregu.00306.2010

Long Y, Li L, Li Q, et al (2012) Transcriptomic characterization of temperature stress responses in larval zebrafish. PLoS ONE 7:e37209. doi: 10.1371/journal.pone.0037209

Macey DJ, Potter IC (1978) Lethal temperatures of ammocoetes of the Southern Hemisphere lamprey, *Geotria australis* Gray. Environ Biol Fishes 3:241–243. doi: 10.1007/BF00691950

Manion PJ, Hanson LH (1980) Spawning behavior and fecundity of lampreys from the upper three Great Lakes. Can J Fish Aquat Sci 37:1635–1640. doi: 10.1139/f80-211

Manion PJ, McLain AL (1971) Biology of larval sea lampreys of the 1960 year class, isolated in the Big Garlic River, Michigan, 1960-65. Gt Lakes Fish Commision Tech Rep 16:

Marchetti MP, Moyle PB, Levin R (2004) Alien fishes in California watersheds: characteristics of successful and failed invaders. 14:587–596

Mateus C, Rodríguez-Muñoz R, Quintella B, et al (2012) Lampreys of the Iberian Peninsula: distribution, population status and conservation. Endanger Species Res 16:183–198. doi: 10.3354/esr00405

Matthews (1986) Geographic Variation in Thermal Tolerance of a Widespread Minnow.Pdf. J Fish 28:407–417

McArley TJ, Hickey AJR, Herbert NA (2017) Chronic warm exposure impairs growth performance and reduces thermal safety margins in the common triplefin fish (*Forsterygion lapillum*). J Exp Biol 220:3527–3535. doi: 10.1242/jeb.162099

McCann EL, Johnson NS, Pangle KL (2018) Corresponding long-term shifts in stream temperature and invasive fish migration. Can J Fish Aquat Sci 75:772–778. doi: 10.1139/cjfas-2017-0195

McCauley DW, Docker MF, Whyard S, Li W (2015) Lampreys as diverse model organisms in the genomics era. BioScience 65:1046–1056. doi: 10.1093/biosci/biv139

McCauley RW (1963) Lethal temperatures of the developmental stages of the sea lamprey, *Petromyzon marinus* L. J Fish Res Board Can 20:483–490. doi: 10.1139/f63-036

McCauley RW (1958) Thermal Relations of Geographic Races of Salvelinus. Can J Zool 36:655–662. doi: 10.1139/z58-059

McDonnell LH, Chapman LJ (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. Conserv Physiol 3:cov050. doi: 10.1093/conphys/cov050

McLaughlin RL, Ellen Marsden J, Hayes DB (2003) Achieving the benefits of sea lamprey control while minimizing effects on nontarget species: Conceptual synthesis and proposed policy. J Gt Lakes Res 29:755–765. doi: 10.1016/S0380-1330(03)70529-2

Moran E V., Alexander JM (2014) Evolutionary responses to global change: Lessons from invasive species. Ecol Lett 17:637–649. doi: 10.1111/ele.12262

Morgan R, Finnøen MH, Jutfelt F (2018) CTmax is repeatable and doesn't reduce growth in zebrafish. Sci Rep 8:7099. doi: 10.1038/s41598-018-25593-4

Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 12:3788–3796. doi: 10.1101/gad.12.24.3788

Morman RH, Cuddy DW, Rugen PC (1980) Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1811–1826. doi: 10.1139/f80-224

Morris MRJ, Richard R, Leder EH, et al (2014) Gene expression plasticity evolves in response to colonization of freshwater lakes in threespine stickleback. Mol Ecol 23:3226–3240. doi: 10.1111/mec.12820

Murdoch SP, Docker MF, Beamish FWH (1992) Effect of density and individual variation on growth of sea lampreys (*Petromyzon marinus*) larvae in the laboratory. Can J Zool 70:184–188

Nichols OC, Tscherter UT (2011) Feeding of sea lampreys *Petromyzon marinus* on minke whales *Balaenoptera acutorostrata* in the St Lawrence Estuary, Canada. J Fish Biol 78:338–343. doi: 10.1111/j.1095-8649.2010.02842.x

Onikura N, Nakajima J, Inui R, et al (2011) Evaluating the potential for invasion by alien freshwater fishes in northern Kyushu Island, Japan, using the Fish Invasiveness Scoring Kit. Ichthyol Res 58:382–387. doi: 10.1007/s10228-011-0235-1

Owczarzy R, Tataurov AV, Wu Y, et al (2008) IDT SciTools: a suite for analysis and design of nucleic acid oligomers. Nucleic Acids Res 36:W163–W169. doi: 10.1093/nar/gkn198

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Parsons KJ, Robinson BW (2006) Replicated evolution of integrated plastic responses during early adaptive divergence. Evolution 60:801–813. doi: 10.1111/j.0014-3820.2006.tb01158.x

Pereira RJ, Sasaki MC, Burton RS (2017) Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. Proc R Soc B Biol Sci 284:20170236. doi: 10.1098/rspb.2017.0236

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308:. doi: 10.1126/science.1111322

Piavis GW (1961) Embryological stages in the sea lamprey and effects of temperature on development. Fish Bull Fish Wildl Serv 61:

Pickholtz RSM, Kiflawi M, Friedlander AM, Belmaker J (2018) Habitat utilization by an invasive herbivorous fish (*Siganus rivulatus*) in its native and invaded range. Biol Invasions 20:3499–3512. doi: 10.1007/s10530-018-1790-4

Potter IC, Beamish FWH (1975) Lethal temperatures in ammocoetes of four species of lampreys. Acta Zool 56:85–91. doi: 10.1111/j.1463-6395.1975.tb00084.x

Purvis HA (1980) Effects of temperature on metamorphosis and the age and length at metamorphosis in sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1827–1834. doi: 10.1139/f80-225

Pusack TJ, Benkwitt CE, Cure K, Kindinger TL (2016) Invasive Red Lionfish (*Pterois volitans*) grow faster in the Atlantic Ocean than in their native Pacific range. Environ Biol Fishes 99:571–579. doi: 10.1007/s10641-016-0499-4

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Råbergh C, Airaksinen S, Soitamo A, et al (2000) Tissue-specific expression of zebrafish HSF1. J Exp Biol 203:1817–1824

Reynolds WW, Casterlin ME (1978) Behavioral thermoregulation by Ammocoete larvae of the sea lamprey (*Petromyzon marinus*) in an electronic shuttlebox. Hydrobiologia 61:145–147. doi: 10.1007/BF00018745

Rodgers EM, Todgham AE, Connon RE, Fangue NA (2019) Stressor interactions in freshwater habitats: Effects of cold water exposure and food limitation on early-life growth and upper thermal tolerance in white sturgeon, *Acipenser transmontanus*. Freshw Biol 64:348–358. doi: 10.1111/fwb.13224

Rodriguez-Muncoz R, Nicieza AG, Brana F (2001) Effects of temperature on developmental performance, survival and growth of sea lamprey embryos. J Fish Biol 58:475–486. doi: 10.1111/j.1095-8649.2001.tb02266.x

Rooke AC, Burness G, Fox MG (2017) Thermal physiology of native cool-climate, and non-native warm-climate Pumpkinseed sunfish raised in a common environment. J Therm Biol 64:48–57. doi: 10.1016/j.jtherbio.2016.12.010

Sakamoto K, Dew WA, Hecnar SJ, Pyle GG (2016) Effects of lampricide on olfaction and behavior in young-of-the-year lake sturgeon (*Acipenser fulvescens*). Environ Sci Technol 50:3462–3468. doi: 10.1021/acs.est.6b01051

Sariahmetoglu M, Crawford BD, Leon H, et al (2007) Regulation of matrix metalloproteinase-2 (MMP-2) activity by phosphorylation. FASEB J 21:2486–2495. doi: 10.1096/fj.06-7938com

Sawai M, Ishikawa Y, Ota A, Sakurai H (2013) The proto-oncogene *JUN* is a target of the heat shock transcription factor HSF1. FEBS J 280:6672–6680. doi: 10.1111/febs.12570

Schleen LP, Christie GC, Heinrich JW, et al (2003) Development and implementation of an integrated program for control of sea lampreys in the St. Marys River. J Gt Lakes Res 29:677–693. doi: 10.1016/S0380-1330(03)70523-1

Schlichting CD, Smith H (2002) Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. Evol Ecol 16:189–211. doi: 10.1023/A:1019624425971

Schneider KR (2008) Heat Stress in the Intertidal: Comparing Survival and Growth of an Invasive and Native Mussel Under a Variety of Thermal Conditions. Biol Bull 215:253–264. doi: 10.2307/25470709

Schreiber M, Kolbus A, Piu F, et al (1999) Control of cell cycle progression by c-Jun is p53 dependent. Genes Dev 13:607–619. doi: 10.1101/gad.13.5.607

Schulte PM, Healy TM, Fangue NA (2011) Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr Comp Biol 51:691–702. doi: 10.1093/icb/icr097

Scoville AG, Pfrender ME (2010) Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. Proc Natl Acad Sci 107:4260–4263. doi: 10.1073/pnas.0912748107

Seebacher F, White CR, Franklin CE (2015) Physiological plasticity increases resilience of ectothermic animals to climate change. Nat Clim Change 5:61–66. doi: 10.1038/nclimate2457

Sejerkilde M, Sørensen JG, Loeschcke V (2003) Effects of cold- and heat hardening on thermal resistance in *Drosophila melanogaster*. J Insect Physiol 49:719–726. doi: 10.1016/S0022-1910(03)00095-7

Sexton JP, McKay JK, Sala A (2002) Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. Ecol Appl 12:1652–1660

Sgro GV, Reavie ED (2018) Lake Erie's ecological history reconstructed from the sedimentary record. J Gt Lakes Res 44:54–69. doi: 10.1016/j.jglr.2017.11.002

Shrode JB (1975) Developmental temperature tolerance of a Death Valley pupfish (*Cyprinodon nevadensis*). Physiol Zool 48:378–389. doi: 10.1086/physzool.48.4.30155663

Siefkes MJ (2017) Use of physiological knowledge to control the invasive sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes. Conserv Physiol 5:. doi: 10.1093/conphys/cox031

Silva S, Araújo MJ, Bao M, et al (2014) The haematophagous feeding stage of anadromous populations of sea lamprey *Petromyzon marinus*: low host selectivity and wide range of habitats. Hydrobiologia 734:187–199. doi: 10.1007/s10750-014-1879-4

Slade JW, Adams JV, Christie GC, et al (2003) Techniques and methods for estimating abundance of larval and metamorphosed sea lampreys in Great Lakes tributaries, 1995 to 2001. J Gt Lakes Res 29:137–151. doi: 10.1016/S0380-1330(03)70483-3

Smith BR, Tibbles JJ (1980) Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: History of invasion and control, 1936–78. Can J Fish Aquat Sci 37:1780–1801. doi: 10.1139/f80-222

Smith JJ, Kuraku S, Holt C, et al (2013) Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. Nat Genet 45:415–421. doi: 10.1038/ng.2568

Smith JJ, Timoshevskaya N, Ye C, et al (2018) The sea lamprey germline genome provides insights into programmed genome rearrangement and vertebrate evolution. Nat Genet 50:270–277. doi: 10.1038/s41588-017-0036-1

Smith LD (2009) The role of phenotypic plasticity in marine biological invasions. In: Rilov G, Crooks JA (eds) Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 177–202

Smith SH (1968) Species succession and fishery exploitation in the Great Lakes. J Fish Res Board Can 25:667–693. doi: 10.1139/f68-063

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers." J Exp Biol 213:912–920. doi: 10.1242/jeb.037473

Stevens ED, Sutterlin AM (1976) Heat transfer between fish and ambient water. J Exp Biol 65:131–145

Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. Science 301:65–65. doi: 10.1126/science.1083073

Strange KT, Vokoun JC, Noltie DB (2002) Thermal tolerance and growth differences in orangethroat darter (*Etheostoma spectabile*) from thermally contrasting adjoining streams. Am Midl Nat 148:120–128. doi: 10.1674/0003-0031(2002)148[0120:TTAGDI]2.0.CO;2

Strommer JL, Smock LA (1989) Vertical distribution and abundance of invertebrates within the sandy substrate of a low-gradient headwater stream. Freshw Biol 22:263–274. doi: 10.1111/j.1365-2427.1989.tb01099.x

Sullivan WP, Christie GC, Cornelius FC, et al (2003) The sea lamprey in Lake Erie: a case history. J Gt Lakes Res 29:615–636. doi: 10.1016/S0380-1330(03)70520-6

Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. Nat Clim Change 2:686–690. doi: 10.1038/nclimate1539

Sunday JM, Pecl GT, Frusher S, et al (2015) Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. Ecol Lett 18:944–953. doi: 10.1111/ele.12474

Sutton TM, Bowen SH (1994) Significance of organic detritus in the diet of larval lampreys in the Great Lakes Basin. Can J Fish Aquat Sci 51:2380–2387

Swink WD (2003) Host selection and lethality of attacks by sea lampreys (*Petromyzon marinus*) in laboratory studies. J Gt Lakes Res 29:307–319. doi: 10.1016/S0380-1330(03)70496-1

Tan E, Kinoshita S, Suzuki Y, et al (2016) Different gene expression profiles between normal and thermally selected strains of rainbow trout, *Oncorhynchus mykiss*, as revealed by comprehensive transcriptome analysis. Gene 576:637–643. doi: 10.1016/j.gene.2015.10.028

Taniguchi Y, Choi PJ, Li G-W, et al (2010) Quantifying E. coli proteome and transcriptome with single-molecule sensitivity in single cells. Science 329:533–538. doi: 10.1126/science.1188308

Teigen LE, Orczewska JI, McLaughlin J, O'Brien KM (2015) Cold acclimation increases levels of some heat shock protein and sirtuin isoforms in threespine stickleback. Comp Biochem Physiol A Mol Integr Physiol 188:139–147. doi: 10.1016/j.cbpa.2015.06.028

Tessier LR, Long TAF, Wilkie MP (2018) Influence of body size, metabolic rate and life history stage on the uptake and excretion of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) by invasive sea lampreys (*Petromyzon marinus*). Aquat Toxicol 194:27–36. doi: 10.1016/j.aquatox.2017.10.020

Thornton B, Basu C (2015) Rapid and simple method of qPCR primer design. In: Basu C (ed) PCR Primer Design. Springer New York, New York, NY, pp 173–179

Tomalty KMH, Meek MH, Stephens MR, et al (2015) Transcriptional response to acute Thermal exposure in juvenile chinook salmon determined by RNAseq. Genes Genomes Genet 5:1335–1349. doi: 10.1534/g3.115.017699

Tongnunui S, Beamish FW. (2017) Critical thermal maximum, temperature acclimation and climate effects on Thai freshwater fishes. EnvironmentAsia 10:109–117

Torres-Dowdall J, Handelsman CA, Reznick DN, Ghalambor CK (2012) Local Adaptation And The Evolution Of Phenotypic Plasticity In Trinidadian Guppies (Poecilia Reticulata). Evolution 66:3432–3443. doi: 10.1111/j.1558-5646.2012.01694.x

Untergasser A, Cutcutache I, Koressaar T, et al (2012) Primer3—new capabilities and interfaces. Nucleic Acids Res 40:1–12. doi: 10.1093/nar/gks596

Valéry L, Fritz H, Lefeuvre JC, Simberloff D (2008) In search of a real definition of the biological invasion phenomenon itself. Biol Invasions 10:1345–1351. doi: 10.1007/s10530-007-9209-7

van Kleunen M, Schlaepfer DR, Glaettli M, Fischer M (2011) Preadapted for invasiveness: do species traits or their plastic response to shading differ between invasive and non-invasive plant species in their native range?: Shade plasticity and invasiveness. J Biogeogr 38:1294–1304. doi: 10.1111/j.1365-2699.2011.02495.x

Verleih M, Borchel A, Krasnov A, et al (2015) Impact of thermal stress on kidney-specific gene expression in farmed regional and imported rainbow trout. Mar Biotechnol 17:576–592. doi: 10.1007/s10126-015-9640-1

Vinagre C, Leal I, Mendonça V, Flores AAV (2015) Effect of warming rate on the critical thermal maxima of crabs, shrimp and fish. J Therm Biol 47:19–25. doi: 10.1016/j.jtherbio.2014.10.012

Vogel C, Marcotte EM (2012) Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 13:227–232. doi: 10.1038/nrg3185

Vornanen M, Hassinen M, Koskinen H, Krasnov A (2005) Steady-state effects of temperature acclimation on the transcriptome of the rainbow trout heart. Am J Physiol-Regul Integr Comp Physiol 289:R1177–R1184. doi: 10.1152/ajpregu.00157.2005

Wadhwa R, Takano S, Taira K, Kaul SC (2004) Reduction in mortalin level by its antisense expression causes senescence-like growth arrest in human immortalized cells. J Gene Med 6:439–444. doi: 10.1002/jgm.530

Waldman J, Daniels R, Hickerson M, Wirgin I (2009) Mitochondrial DNA analysis indicates sea lampreys are indigenous to Lake Ontario: Response to comment. Trans Am Fish Soc 138:1190–1197. doi: 10.1577/T08-035R.1

Waldman JR, Grunwald C, Roy NK, Wirgin II (2004) Mitochondrial DNA analysis indicates sea lampreys are indigenous to Lake Ontario. Trans Am Fish Soc 133:950–960. doi: 10.1577/T03-104.1

Waldman JR, Grunwald C, Wirgin I (2006) Evaluation of the native status of sea lampreys in Lake Champlain based on mitochondrial DNA sequencing analysis. Trans Am Fish Soc 135:1076–1085. doi: 10.1577/T05-055.1

Wang J, Wei Y, Li X, et al (2007) The identification of heat shock protein genes in goldfish (*Carassius auratus*) and their expression in a complex environment in Gaobeidian Lake, Beijing, China. Comp Biochem Physiol Part C Toxicol Pharmacol 145:350–362. doi: 10.1016/j.cbpc.2007.01.018

Wang Y, Liu Z, Li Z, et al (2016) Effects of heat stress on respiratory burst, oxidative damage and SERPINH1 (HSP47) mRNA expression in rainbow trout *Oncorhynchus mykiss*. Fish Physiol Biochem 42:701–710. doi: 10.1007/s10695-015-0170-6

Wellband KW, Heath DD (2017) Plasticity in gene transcription explains the differential performance of two invasive fish species. Evol Appl 10:563–576. doi: 10.1111/eva.12463

Widmer C, Gebauer JM, Brunstein E, et al (2012) Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition. Proc Natl Acad Sci 109:13243–13247. doi: 10.1073/pnas.1208072109

Wigley RL (1959) Life history of the sea lamprey of Cayuga Lake, New York. Fish Bull Fish Wildl Serv 59:61

Wilkie MP, Turnbull S, Bird J, et al (2004) Lamprey parasitism of sharks and teleosts: high capacity urea excretion in an extant vertebrate relic. Comp Biochem Physiol A Mol Integr Physiol 138:485–492. doi: 10.1016/j.cbpb.2004.06.001

Williams DD, Hynes HBN (1974) The occurrence of benthos deep in the substratum of a stream. Freshw Biol 4:233–256

Winne CT, Keck MB (2005) Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex. Comp Biochem Physiol A Mol Integr Physiol 140:141–149. doi: 10.1016/j.cbpb.2004.11.009

Wisdom R (1999) c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. EMBO J 18:188–197. doi: 10.1093/emboj/18.1.188

Wood LA, Brown IR, Youson JH (1998) Characterization of the heat shock response in the gills of sea lampreys and a brook lamprey at different intervals of their life cycles. Comp Biochem Physiol A Mol Integr Physiol 120:509–518. doi: 10.1016/S1095-6433(98)10061-2

Wood LA, Brown IR, Youson JH (1999) Tissue and developmental variations in the heat shock response of sea lampreys (*Petromyzon marinus*): effects of an increase in acclimation temperature. Comp Biochem Physiol A Mol Integr Physiol 123:35–42. doi: 10.1016/S1095-6433(99)00035-5

Xu D, Sun L, Liu S, et al (2016) Molecular cloning of hsf1 and hsbp1 cDNAs, and the expression of hsf1, hsbp1 and hsp70 under heat stress in the sea cucumber *Apostichopus japonicus*. Comp Biochem Physiol B Biochem Mol Biol 198:1–9. doi: 10.1016/j.cbpb.2016.03.001

Ye J, Coulouris G, Zaretskaya I, et al (2012) Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13:134. doi: 10.1186/1471-2105-13-134

Yeh PJ, Price TD (2004) Adaptive Phenotypic Plasticity and the Successful Colonization of a Novel Environment. Am Nat 164:531–542. doi: 10.1086/423825

Young JC, Moarefi I, Hartl FU (2001) Hsp90: a specialized but essential protein-folding tool. J Cell Biol 154:267–274. doi: 10.1083/jcb.200104079

Young RJ, Kelso JRM, Weise JG (1990) Occurrence, relative abundance, and size of landlocked sea lamprey (*Petromyzon marinus*) ammocoetes in relation to stream characteristics in the Great Lakes. Can J Fish Aquat Sci 47:1773–1778. doi: 10.1139/f90-201

Youson JH (2003) The biology of metamorphosis in sea lampreys: Endocrine, environmental, and physiological cues and events, and their potential application to lamprey control. J Gt Lakes Res 29:26–49. doi: 10.1016/S0380-1330(03)70476-6

Zambrano L, Martínez-Meyer E, Menezes N, Peterson AT (2006) Invasive potential of common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) in American freshwater systems. Can J Fish Aquat Sci 63:1903–1910. doi: 10.1139/f06-088

Zerbino DR, Achuthan P, Akanni W, et al (2018) Ensembl 2018. Nucleic Acids Res 46:D754–D761. doi: 10.1093/nar/gkx1098

Zerebecki RA, Sorte CJB (2011) Temperature tolerance and stress proteins as mechanisms of invasive species success. PLoS ONE 6:. doi: 10.1371/journal.pone.0014806

Zhang Y, Kieffer JD (2014) Critical thermal maximum (CTmax) and hematology of shortnose sturgeons (*Acipenser brevirostrum*) acclimated to three temperatures. Can J Zool 92:215–221. doi: 10.1139/cjz-2013-0223

Zhang Y, Loughery JR, Martyniuk CJ, Kieffer JD (2017) Physiological and molecular responses of juvenile shortnose sturgeon (*Acipenser brevirostrum*) to thermal stress. Comp Biochem Physiol A Mol Integr Physiol 203:314–321. doi: 10.1016/j.cbpa.2016.10.009

Zhou L-Y, Fu S-J, Fu C, et al (2019) Effects of acclimation temperature on the thermal tolerance, hypoxia tolerance and swimming performance of two endangered fish species in China. J Comp Physiol B. doi: 10.1007/s00360-018-01201-9