Behavioural and Metabolic Responses to the Cold in Winter-Dormant Fishes:

Reductions in Activity as the Key Strategy Underlying Energy Savings

by

Connor Reeve

Bachelor of Science, Honours, in Marine & Freshwater Biology,

University of Guelph, 2017

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

Masters of Science in Biology

in the Graduate Academic Unit of Biology

Supervisor:	Ben Speers-Roesch, PhD, Department of Biological Sciences, UNB Saint John
Examining Board:	Heather Hunt, PhD, Department of Biological Sciences, UNB Saint John, Chair
	Jim Kieffer, PhD, Department of Biological Sciences, UNB Saint John
	Tillmann Benfey, PhD, Department of Biology, UNB Fredericton

This thesis is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

April, 2020

ABSTRACT

Metabolic rate depression (MRD) is a reversible downregulation of standard (resting) metabolic rate (SMR) that facilitates survival in energy-limited environments. Previous studies suggesting interspecific variation in the capacity for MRD among winter-dormant fishes may be confounded by unaccounted variation in activity, which affects the accuracy of SMR estimates. When winter activity reductions were controlled for, a recent study on cunner (*Tautogolabrus adspersus*) found no MRD. I investigated whether inactivity is the central strategy underlying the metabolic phenotype of winter-dormant fishes. I characterized winter-dormant behaviour in four temperate fish species, finding that activity reductions are ubiquitous albeit varying in magnitude. I then investigated the relationship between activity and metabolic rate using video tracking and intermittent respirometry in two species (mummichog, *Fundulus heteroclitus*; pumpkinseed sunfish, *Lepomis gibbosus*) during acute cooling and after winter temperature acclimation. Low winter metabolic rates resulted from reduced activity combined with passive physicochemical effects of cooling, not MRD. Inactivity is the key energy saving strategy of winter-dormant fishes.

ACKNOWLEDGEMENTS

I am grateful for the various opportunities and experiences I was fortunate enough to obtain during my time at the University of New Brunswick, Saint John. I would like to acknowledge all of the individuals who have helped to facilitate this research. Notably, I would like to thank my supervisor, Dr. Ben Speers-Roesch, for his ongoing mentorship and support during all aspects of my degree. My appreciation also extends to all individuals of the Speers-Roesch lab for, not only their assistance in various projects and general fish husbandry, but also for creating a friendly and engaging work environment. I would also like to thank my supervisory committee members, Dr. Heather Hunt and Dr. Jim Kieffer, for their assistance and guidance. Additionally, I would like to acknowledge the Huntsman Marine Science Centre, Cooke Aquaculture, Atlantic Canada Eels Inc., and Rockwood Park for graciously supplying or permitting me to sample fish used in various experiments during this study. I also acknowledge Alvaro Rodriguez and the ToxTrac team for their assistance and recommendations that were imperative for video analysis. Finally, I am grateful for the funding provided through the NSERC Discovery programs and Vaughan Fellowship, which helped to support the research in this thesis.

Table of Contents

List of Figures

Figure 1.

Effects of acute cooling (1^{o}C/day) on activity-related behaviors and food consumption in four species of putatively winter-dormant fishes (Experiment 1) (Species data panels arranged vertically: cunner, n = 16, panels A, E, I; pumpkinseed sunfish, n = 12, panels B, F, J; mummichog, n = 16, panels C, G, K; American eel, n = 12, panels D, H). Data are means ± S.E.M. for spontaneous activity (panels A-C), vigilance (panel D), food consumption (panels E-H), and sheltering (panels I-K), open circles represent day-time measurements and closed circles represent night-time measurements. Food consumption was measured once daily as the percentage of the daily ration (~0.5%BW) consumed (gray closed circles, panels E – H). Spontaneous activity represents the average velocity in body lengths per minute (BL min^{-1}) of the fish over the full measurement period at each temperature. Day and night cold steady states (represented by dashed and solid lines, respectively) indicate the range of temperatures where behavior or feeding was not significantly different from the measurement at the coldest temperature within the same day or night-time period. Where panels only contain one cold steady state line, it is because there was only one clear and consistent steady state in either day or night. * indicates a significant difference between day and night-time values at a given temperature. American eel spontaneous activity could not be measured using the same methodology applied to the other species; instead, vigilance (see methods) was scored in response to cooling (panel D). Generalized linear mixed-effects models and Bonferroni post-hoc multiple comparisons tests were used to identify significant differences ($p<0.05$) for all behavioural measurements (see Table 5).

Figure 2. The diel cycle of spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) measured simultaneously in mummichog (n = 12) during acute cooling (~3^{o}C day^{-1}) and following 4-6 weeks acclimation to 2^{o}C and acute rewarming (warmed ~14^{o}C overnight) (Experiment 2). The closed black circles are the spontaneous activity (A) and $\dot{M}O_2$ (B) values for all fish during each measurement interval. The red closed circles are the mean ± S.E.M. values for each day and night-time period (represented by white and black bars, respectively) at each temperature. The red line represents the experimental temperature regime. Generalized linear mixed-effects models and Type II Wald chi-square tests were used to assess where significant affects occurred. Additionally, Bonferroni post-hoc multiple comparisons tests were used to identify significant differences ($p<0.05$).Spontaneous activity and $\dot{M}O_2$ were significantly affected by temperature ($\chi^2 = 103.806$, df = 9, $p<0.0001$; $\chi^2 = 989.295$, df = 9, $p<0.0001$, respectively), diel cycle ($\chi^2 = 82.071$, df = 1, $p<0.0001$; $\chi^2 = 19.370$ df = 1, $p<0.0001$, respectively), and their interaction ($\chi^2 = 54.916$, df = 8, $p<0.0001$; $\chi^2 = 24.747$, df = 8, $p<0.0017$, respectively).

Figure 3. The diel cycle of spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) measured simultaneously in pumpkinseed sunfish (n = 11) during acute cooling (~3^{o}C day^{-1}) and following 4-6 weeks acclimation to 2^{o}C and acute rewarming (warmed ~14^{o}C overnight) (Experiment 2). The closed black circles are the spontaneous activity (A) and $\dot{M}O_2$ (B) values for all fish during each measurement interval. The red closed circles are the mean ± S.E.M. values for each day and night-time period

(represented by white and black bars, respectively) at each temperature. The red line represents the experimental temperature regime. Generalized linear mixed-effects models and Type II Wald chi-square tests were used to assess where significant affects occurred. Additionally, Bonferroni post-hoc multiple comparisons tests were used to identify significant differences ($p<0.05$). Spontaneous activity and $\dot{M}O_2$ were significantly affected by temperature ($\chi^2 = 311.3361$, df = 9, $p<0.0001$; $\chi^2 = 1308.554$, df = 9, $p<0.0001$, respectively) and their interaction ($\chi^2 = 95.6660$, df = 8, $p<0.0001$; $\chi^2 = 17.619$, df = 8, $p<0.0243$, respectively); however, only $\dot{M}O_2$ was significantly affected by diel cycle ($\chi^2 = 77.686$, df = 1 $p<0.0001$).

Figure 4. The effect of acute cooling on metabolic rate (oxygen consumption rate, $\dot{M}O_2$) in all measurement intervals (i.e., routine metabolic rate) in mummichogs (A) (n = 12) and pumpkinseed sunfish (B) (n = 11) (Experiment 2). The data were fitted with an exponential regression and the slope of the line was used to calculate a single population-level Q_{10} value across all exposure temperatures without controlling for variation in spontaneous activity ($Q_{10} = e^{(0.1154 \cdot 10)} = 3.17$ and $Q_{10} = e^{(0.1653 \cdot 10)} = 5.22$, for mummichog and pumpkinseed sunfish, respectively).

Figure 5. The relationship between spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) for all mummichogs (n = 12) at all measurement intervals within each experimental temperature in response to acute cooling (Panel A) and 4-6 weeks acclimation to ~2.64ºC followed by acute rewarming to ~15.07ºC (Panel B) (Experiment 2). The statistical output from these relationships and average temperatures ± standard

deviation are reported in Table 4. Within each temperature, the relationship between spontaneous activity and $\dot{M}O_2$ was modeled exponentially using the following formula in order to calculate SMR: $y = ae^{(bU)}$, where a is SMR (extrapolated $\dot{M}O_2$ at zero activity), b is the slope, and U is spontaneous activity. The relationships shown here were modeled using all measurements for all fish within each temperature (enabling calculation of a single population-level SMR at each temperature); I also modeled the relationship within individuals to determine mean SMR (see Figure 7).

Figure 6. The relationship between spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) for all pumpkinseed sunfish (n = 11) at all measurement intervals within each experimental temperature in response to acute cooling (Panel A) and 4-6 weeks acclimation to ~2.66ºC followed by acute rewarming to ~14.24ºC (Panel B) (Experiment 2). The statistical output from these relationships and average temperatures ± standard deviation are reported in Table 4.Within each temperature, the relationship between spontaneous activity and $\dot{M}O_2$ was modeled exponentially using the following formula in order to calculate SMR: $y = ae^{(bU)}$, where a is SMR (extrapolated $\dot{M}O_2$ at zero activity), b is the slope, and U is spontaneous activity. The relationships shown here were modeled using all measurements for all fish within each temperature (enabling calculation of a single population-level SMR at each temperature); I also modeled the relationship within individuals to determine mean SMR (see Figure 8).

Figure 7. A) Standard metabolic rate (SMR) of mummichog during acute cooling from ~15^{o}C to ~2.5^{o}C and after 4-6 weeks acclimation to ~2.5^{o}C followed by acute re-warming to ~15^{o}C. SMR were calculated by fitting exponential regressions to the data for spontaneous activity vs. metabolic rate in individual fish and calculating extrapolated $\dot{M}O_2$ at zero activity (Experiment 2). The overlying black points represent the individual SMR values calculated for each fish. B) Thermal sensitivity of SMR in mummichogs during acute cooling (14.9^{o}C to 8.6^{o}C and 8.6 to 2.5^{o}C), acute re-warming of ~2.5^{o}C acclimated fish (2.6^{o}C to 15.0^{o}C), and for ~15^{o}C vs. ~2.5^{o}C acclimated fish. Q_{10} values were calculated for each individual fish using the individual's SMR values shown in Panel A. The dotted horizontal line represents my defined Q_{10} threshold indicating metabolic rate depression (i.e., $Q_{10} > 3.5$). Letters denote significant differences between groups identified using linear mixed effects models and Bonferroni post-hoc multiple comparisons tests ($p<0.05$). Data are means ± S.E.M., n=12.

Figure 8. A) Standard metabolic rate (SMR) of pumpkinseed sunfish during acute cooling from ~14^{o}C to ~2.5^{o}C and after 4-6 weeks acclimation to ~2.5^{o}C followed by acute re-warming to ~14^{o}C. SMR were calculated by fitting exponential regressions to the data for spontaneous activity vs. metabolic rate in individual fish and calculating extrapolated $\dot{M}O_2$ at zero activity (Experiment 2). The overlying black points represent the individual SMR values calculated for each fish. B) Thermal sensitivity of SMR in mummichogs during acute cooling (14.2^{o}C to 8.0^{o}C and 8.0 to 2.6^{o}C), acute re-warming of ~2.5^{o}C acclimated fish (2.7^{o}C to 14.2^{o}C), and for ~14^{o}C vs. ~2.5^{o}C acclimated fish. Q_{10} values were calculated for each individual fish using the individual's SMR values

shown in Panel A. The dotted horizontal line represents my defined Q_{10} threshold indicating metabolic rate depression (i.e., $Q_{10} > 3.5$). Letters denote significant differences between groups identified using linear mixed effects models and Bonferroni post-hoc multiple comparisons tests ($p<0.05$). Data are means ± S.E.M., n=11.

List of Tables

correspond to the data shown in Figure 1. Significant affects were calculated using type II Wald chi-square tests and ($p < 0.05$) are indicated in bold.

Table 6. Thermal sensitivity quotients (Q_{10}) of SMR of mummichog (means ± S.E.M., n = 12) using different methods of estimating SMR (Experiment 2). "$Q_{10\ (Ind\ Extrapolated)}$" and "$Q_{10\ (Grp\ Extrapolated)}$" refer to the Q_{10}'s calculated using SMR values estimated by extrapolating $\dot{M}O_2$ to zero activity in individuals (also reported in Figures 5 and 7) and across all experimental fish, respectively. "$Q_{10\ (Overlapping)}$" refers to Q_{10}'s calculated using comparisons of the average of each individual's $\dot{M}O_2$ values corresponding to a common, overlapping range of spontaneous activity (SA) across all temperatures. Due to differing overlapping ranges of SA, the Q_{10} between acclimated 14.9°C and acclimated 2.6°C could not be calculated (see methods). "$Q_{10\ (Lowest\ 20\ \dot{M}O_2)}$" and "$Q_{10\ (Lowest\ 20\ SA)}$" refer to the Q_{10}'s calculated by comparing the average of each individual's lowest 20 $\dot{M}O_2$ points at each temperature and by comparing the average $\dot{M}O_2$ associated with the lowest 20 SA measurements at each temperature, respectively. "$Q_{10\ (Average\ \dot{M}O_2)}$" refers to the Q_{10} calculated from the average of all $\dot{M}O_2$ values for each individual fish at each temperature. "$Q_{10\ (Extrapolated\ +\ \dot{M}O_2)}$" and "$Q_{10\ (Extrapolated\ +\ SA)}$" refer to the Q_{10}'s calculated using SMR values estimated by extrapolating $\dot{M}O_2$ to zero activity in individual fish and, where this relationship was insignificant, replacing the extrapolated SMR with the average of the lowest 20 $\dot{M}O_2$ points or with the value for average $\dot{M}O_2$ associated with the lowest 20 SA measurements, respectively, and in the same individual fish. Q_{10}'s above my defined threshold for metabolic rate depression (i.e. $Q_{10} > 3.5$) are bolded. The Q_{10} values for different temperature intervals were compared with linear mixed effects

models; italicized P-values represent a significant difference ($p < 0.05$). Where a significant difference was observed, values that share letters are not significantly different (Bonferroni post-hoc multiple comparisons tests, $p < 0.05$).

Table 7. Thermal sensitivity quotients (Q_{10}) of SMR of pumpkinseed sunfish (means ± S.E.M., n = 11) using different methods of estimating SMR (Experiment 2). See Table 6 caption for description of the different Q_{10} calculation methods and details of statistical analyses.

List of Symbols, Nomenclature or Abbreviations

mg: Milligram
g: Gram
kg: Kilogram
BW: Body weight
O_2: Oxygen
^oC: Degrees Celsius
hr: Hour
10L:14D: The photoperiod used for all experiments and holding conditions; 10 hours light, 14 hours dark.
fps: Frames per second
Hz: Hertz
min: Minute
nm: Nanometer
mm: Millimeter
cm: Centimeter
‰: Parts per thousand
BL: Body lengths
SA: Spontaneous activity
VS: Vigilance Score
n: Samples size
Chisq: Chi-squared test statistic
df: Degrees of freedom
vs: Versus
ANOVA: Analysis of variance
GLMM: Generalized linear mixed effects model
LMM: Linear mixed effects model
MRD: Metabolic rate depression
Q_{10}: The thermal sensitivity quotient
$\dot{M}O_2$: Oxygen consumption rate, a proxy of metabolic rate, measured in $mgO_2\ kg^{-1}\ hr^{-1}$

Chapter 1: General Introduction

The onset of winter at temperate to polar latitudes is associated with significant seasonal changes. For aquatic organisms, such as fish, these seasonal changes include annual temperature minimums throughout the water column, shorter photoperiod, the potential for reduced oxygen (mainly in freshwater environments), and often reduced density of food resources (Shuter *et al.,* 2012). These intra-annual variations are key, ecologically-important drivers of physiological and behavioural changes in fish that promote species coexistence and often alter many aspects of their biology and life-history (McMeans *et al*., 2019).

Temperature has profound physicochemical effects on biochemical and physiological processes, especially in ectotherms where body temperature is determined by the environmental temperature (Pörtner, 2002). Notably, enzymatic activity and protein synthesis are temperature-dependent processes, typically faster with warming and slower with cooling (Lewis and Driedzic, 2007; Angilletta, 2009). Additionally, the function of cellular membranes depends upon temperature (Hazel and Williams, 1990; Hazel, 1995; Angilletta, 2009). For example, cold temperatures slow the movements of phospholipids, increasing membrane rigidity and changing the membrane fluidity, which can impair cellular membrane function (Angilletta, 2009; Schulte, 2015). At thermal extremes, protein misfolding or membrane dysfunction can occur, in part explaining species thermal limits (Angilletta, 2009). Because enzyme-catalyzed biochemical reactions and membrane function underlie metabolism, warming typically speeds up and

cooling slows down metabolic rate, while also having effects on many other physiological processes (Angilletta, 2009; Schulte, 2015).

Decreased water temperature, for example, has profound physiological effects (Clarke, 2017). These can include, but are not limited to, reductions in muscle and swimming performance (Johnston and Temple, 2002; Rome, 1990), digestion (Legler *et al*., 2010; Shrable *et al*., 1969; Molnár and Tölg 1962), protein synthesis and thus growth (Clarke, 2017; Mathews and Haschemeyer, 1978), gametogenesis (Wood and McDonald, 1997; Kaya and Hasler, 1972), and cognition (Montgomery and MacDonald, 1990; Montgomery and McVean, 1986; Montgomery *et al*., 1982). The pervasive physiological effects of temperature therefore can have large implications for fish behaviours such as foraging, predator avoidance, and reproduction. Indeed, winter low temperatures can affect survival. Overwintering mortality is a common phenomenon which is particularly prevalent in juvenile fish and is more common at the poleward edge of a species range (Hurst, 2007). Thus, winter is a major bottleneck to the poleward migration and persistence of a species.

To cope with the challenges of winter including low temperature and reduced resource availability, fish living at temperate to polar latitudes have evolved three primary overwintering strategies. The first overwintering strategy involves the avoidance of winter altogether: migration. Many species choose to expend energy to move to warmer and more productive environments in winter, thus avoiding the physiologically unfavourable conditions imposed by cold temperatures and poor food availability. For example, swordfish (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*) in Canadian Atlantic waters engage in southern migrations along the Gulf Stream during the winter to

exploit the relatively high abundance of resources and warmer water temperatures (Block *et al*., 2001; Neilson *et* al., 2006).

The second overwintering strategy is compensation, which is a type of phenotypic plasticity that occurs in many species of fish that remain at poleward latitudes in winter. Compensation occurs when, following acclimation to low temperature, the fish adjust their physiology to counteract the slowing effects of cold on their function and performance. Typically, this is seen as an increase in physiological reaction rates which maintain performance at levels higher than would otherwise occur at the new cold temperature. Physiological compensation in response to low temperature has been and continues to be an area of substantial study. One common compensatory response detailed in teleosts is enhancement of metabolic rate and swimming performance in the cold, which helps to maintain active foraging and/or predator avoidance despite harsh winter conditions (Driedzic *et al*., 1996). Compensation of swimming performance involves modifications to the skeletal muscle, increased proportion of red muscle mass to support sustained swimming, increased enzymatic activities associated with aerobic energy production, increased mitochondrial density, and an increased importance of fatty acid use as opposed to carbohydrates as metabolic fuel (Eggington and Sidell, 1989; Guderley, 1990; Guderley and Gawlicka, 1992; Rodnick and Sidell, 1994; Sidell and Moerland, 1989). Enhancements of swimming performance may be accompanied by cardiac remodeling and an increase in relative heart mass, as sustained swimming and the recovery from burst swimming must be supported by cardiac performance (Farrell *at al*., 1988; Driedzic *et al*., 1996; Klaiman *et al*., 2011). These cardiorespiratory changes also support compensation of metabolic rate in the cold (Angilletta, 2009). Seasonal shifts in

metabolic rate in response to thermal changes is a common adaptive response through which animals can better match their metabolic phenotype to their new thermal environment (Angilletta, 2009). However, this compensation is rarely complete (i.e. rate in the cold remains lower than that in the warm), potentially because acclimation costs energy (Seebacher *et al*., 2015).

The third overwintering strategy involves the conservation of energy until the environmental conditions become favourable. This strategy is termed dormancy: a reversible seasonal phenotype characterized by inactivity, low body temperature, fasting, and low metabolic rates (Costa, 2017; Speers-Roesch *et al.*, 2018). Theoretically, dormancy involves a lack of compensation or potentially even inverse compensation, where fish may actively downregulate their metabolism to conserve energy in the cold. Whereas low temperature compensation is heavily studied in fish, dormancy is a relatively understudied overwintering strategy. Dormancy may facilitate the persistence of a species at the cold limit of their range; thus, it may permit the expansion of a species geographic range to the cold extreme of their thermal niche (Stuart-Smith et al., 2017). As such, dormancy has evolved multiple times among animals as a winter survival strategy to reduce energetic requirements in the face of a lack of resources and/or to minimize physiological impacts of the cold (McNab, 2002; Shuter, 2012).

Whereas winter dormancy in mammals (i.e., hibernation) is well-studied, little is known about winter dormancy in ectotherms despite its widespread use as an overwintering strategy (Costa *et al*., 2013; Crawshaw *et al*., 1982). A diverse range of high-latitude fish species demonstrate winter-dormant behaviour (Costa *et al*., 2013; Crawshaw, 1984; Crawshaw *et al*., 1982; Lemons and Crawshaw, 1985; Raposa, 2003;

Roberts, 1964; Sayer and Davenport, 1996; Speers-Roesch *et al.*, 2018; Targett, 1978; Tomie *et al*., 2013; Walsh *et al*., 1983), yet the underlying mechanisms are still unknown or debated.

Dormancy in fish and other ectotherms is often considered analogous to mammalian hibernation where profound metabolic rate depression (MRD) occurs (Costa *et al*., 2013; Speers-Roesch *et al*., 2018). MRD is an active, reversible depression of cellular energy turnover that lowers an organism's metabolic rate below that of the standard metabolic rate (SMR), decreasing overall energy requirements (Storey and Storey, 2004). These active changes involve a suppression of ATP-producing and ATP-consuming processes to reach a new lower net rate of ATP turnover that can be sustained over long periods of time (Storey and Storey, 2004). MRD thus requires a coordinated downregulation of ATP expensive processes such as ion pumping, gene transcription and translation, and growth and development, which is driven in large part by reversible protein phosphorylation within cells (Storey and Storey, 2004). MRD typically results in a metabolic rate lowered to between 5 – 40% of the SMR of the normal animal (Storey and Storey, 2004). SMR is defined as the minimum metabolic rate at a specified temperature necessary to sustain life in an ectothermic organism (Chabot *et al*., 2016). SMR, therefore, includes the cost of the internal processes related to organism homeostasis (e.g. maintenance of ion gradients, maintenance levels of protein turnover, etc.), but explicitly excludes many aspects of routine existence such as locomotor activity, digestion, and reproduction (Chabot *et al*., 2016; Clarke, 2017). In contrast, routine metabolic rate (RMR) includes the energetic cost of SMR and the cost of routine activity (Chabot *et al*., 2016). RMR can be at any level between SMR and maximum

metabolic rate (MMR) and therefore RMR can often be substantially larger than SMR (Chabot *et al*., 2016). For example, Nilsson (1993) quantified the cost of routine activity in goldfish (*Carassius auratus*) and found that activity constituted 67% of their RMR. Thus, reducing activity can decrease RMR, yielding energetic savings until SMR is reached at zero activity. However, MRD is required to decrease energy demands further, thus decreasing an organism's metabolic rate lower than their SMR (Guppy and Withers, 1999). MRD is an essential strategy that has evolved many times to allow animals to survive long exposures to energy-limited environments, such as winter or prolonged hypoxia (Guppy and Withers, 1999). The trade-off of MRD is reduced capacities for physiological performance, so MRD is associated with relatively inactive, non-feeding, non-growing states.

While MRD is well-known in hibernating endotherms, controversy exists over its involvement in dormant ectotherms including fish (Crawshaw, 1984; Ultsch, 1989; McNab, 2002; Tattersall and Ultsch, 2008). This is partially due to the difficulty of differentiating MRD from lethargy and slowed metabolism resulting from the passive physicochemical effects of cooling (McNab, 2002). Thus, the involvement of MRD in winter-dormant ectotherms can be determined using the thermal sensitivity quotient (Q_{10}) of metabolic rate over the transition from an active to dormant state (Speers-Roesch *et al*., 2018).

The Q_{10} is used to describe the effect of temperature on biological rate reactions, where Q_{10} is a unitless description of the fold-change in a biological rate over a standardized 10^{o}C change in temperature. The effect of temperature on whole animal metabolic rate in ectotherms, including fish, is relatively conserved among species and

taxonomic groups and is commonly associated with a Q_{10} = 2-3 (Peck, 2016; Clarke, 2017), which reflects the direct, passive physicochemical effects of temperature on underlying cellular biochemistry. Conversely, a greater change in metabolic rate in response to cooling, indicated by a Q_{10} > 3.5, typically indicates that the animal is utilizing MRD as their metabolic rate is suppressed to a greater extent than would be predicted from the passive thermal effects on metabolism alone (Crawshaw, 1984; Costa *et al*., 2013; Staples, 2016; Speers-Roesch *et al*., 2018).

Using this approach, previous studies have argued both for (Roberts, 1964; Targett, 1978; Walsh *et al*., 1983; Sayer and Davenport, 1996; Costa *et al*., 2013) and against (Crawshaw *et al*., 1982; Lemons and Crawshaw, 1985; Costa *et al*., 2013; Speers-Roesch *et al*., 2018) the presence of MRD in winter-dormant fish species. In many cases, however, the interpretation of MRD in winter dormancy may be confounded by unaccounted variation in activity levels. Activity is rarely measured in conjunction with measurements of routine or standard metabolic rate (Chabot *et al*., 2016). Considering that routine activity is a major contributor to RMR in fish (Nilsson *et al*., 1993), it is possible that the previously described metabolic reduction attributed to MRD may have resulted simply from inactivity in combination with the physicochemical effects of cooling on their underlying metabolism. In fact, a recent study demonstrated that when variation in spontaneous activity is controlled for in cunner (*Tautogolabrus adspersus*), a winter-dormant model species previously reported to engage in MRD, the decrease in metabolic rate during winter dormancy was explained by the physicochemical effects of cold alone (Speers-Roesch *et al*., 2018). In other words, MRD does not occur in winter-

dormant cunner and instead their low winter metabolic rates arise from reduced activity combined with passive physicochemical effects of cooling (Speers-Roesch *et al*., 2018).

An outstanding question is whether this inactivity-based energy savings strategy, in which MRD is lacking, is specific to cunner or if it is common among winter-dormant fish. I hypothesize that diminished activity is the central convergent mechanism underlying the metabolic phenotype of winter-dormant fish. If there is convergence for this behavioural modulation of metabolic rate to reduce energy expenditure in winter-dormant fish species, then I expect to see that a phylogenetically diverse range of winter-dormant species will show metabolic rates in winter that are explained solely by reductions in activity combined with passive physicochemical effects of cold. In other words, when variation in activity across temperatures is controlled for in order to obtain an accurate estimate of SMR, I expect the thermal sensitivity of SMR to be explained by the physicochemical effects of the cold alone (i.e. $Q_{10} < 3.5$). Conversely, if metabolic rate depression is involved during winter dormancy in some species, I would expect to see SMR decrease to a greater extent than could be explained by simply the physicochemical effects of cooling (i.e. I will observe a $Q_{10} > 3.5$)

In my thesis I sought to address this outstanding question and provide a template for assessing the behavioural and metabolic mechanisms underlying winter dormancy in fish for future work. A mechanistic understanding of how temperature impacts activity and metabolism in winter-dormant ectotherms is important. Winter dormancy is an understudied phenology in fish living at temperate to polar latitudes that may engage in this overwintering strategy for up to half their lifespan. Thermal constraints of cold on activity and metabolism of animals can have major life history and ecological

consequences (Gunderson and Leal, 2016; Williams *et al*., 2015). Additionally, a better understanding of the factors that induce variability in metabolism, such as the often overlooked influence of activity, is valuable for informing metabolic theories of ecology that depend upon the assumption of predictable thermal effects on metabolism (Clarke, 2017; ,van der Meer, 2006; Huey *et al*., 2011).

Chapter 2: Inactivity as the Convergent Mechanism Underlying the Metabolic Phenotype of Winter-Dormant Fishes

ABSTRACT

Winter dormancy is a key seasonal response in many ectotherms, characterized by inactivity, fasting, and low metabolic rates at winter low temperatures. The involvement of metabolic rate depression (MRD) in winter-dormant ectotherms, including many temperate fishes, is controversial. MRD is a reversible downregulation of standard (resting) metabolic rate (SMR) that allows survival in energy-limited environments. Previous studies have suggested considerable interspecific variation in the capacity for MRD in winter-dormant fish. However, some of these studies may suffer from confounding influences of temperature-dependent variation in activity on metabolic rate, which affects the accuracy of SMR estimates. Recent work on cunner (*Tautogolabrus adspersus*), a winter-dormant model fish species, showed that the low winter metabolic rate was explained not by MRD, but rather by inactivity combined with the passive, physicochemical effect of cold on SMR. I hypothesize that inactivity is the central,

convergent mechanism underlying the metabolic phenotype of winter-dormant fish. Using automated video tracking, I first investigated species-specific thresholds for winter dormancy, defined as major reductions in activity, increased sheltering, and feeding cessation with cooling below specific temperatures, in four phylogenetically diverse teleost species known or reported to engage in winter dormancy: cunner, pumpkinseed sunfish (*Lepomis gibbosus*), American eel (*Anguilla rostrata*), and mummichog (*Fundulus heteroclitus*). All species showed reductions in activity and cessation of feeding, but the magnitude of change and dormancy threshold temperature varied among species. An increase in sheltering was observed in cunner, pumpkinseed sunfish and American eel, but not mummichog. The relationship between activity and metabolic rate was then measured simultaneously using video tracking and automated respirometry in mummichog and pumpkinseed sunfish during an acute cooling period and long-term acclimation to winter temperatures. There was a strong relationship between activity and metabolic rate at all temperatures, and cooling was associated with reduced activity and consequently reduced metabolic rate. When variation in activity was controlled for across temperatures, the effect of temperature on metabolic rate including SMR indicated passive physicochemical influences only (mean Q_{10}<3.5). Thus, in pumpkinseed sunfish and mummichog, as for cunner, winter metabolic rates came from reduced activity combined with passive physicochemical effects of cooling, not MRD. A growing body of evidence suggests inactivity is the key energy saving strategy of winter-dormant fishes.

INTRODUCTION

Winter dormancy is a common strategy among animals to cope with the challenging cold and energy-limited winter periods in temperate to polar latitudes (McNab, 2002). Winter dormancy is a reversible seasonal phenotype characterized by inactivity, low body temperature, fasting, and a low metabolic rate (McNab, 2002; Shuter *et al*., 2012; Boyles *et al*., 2013; Speers-Roesch *et al*., 2018). Winter dormancy may be a useful strategy to facilitate the persistence of a species at the cold limit of its range and could be viewed as a tactic to expand a species geographic ranges to the lower limit of thermal niche (Stuart-Smith et al., 2017).

Many temperate fish species engage in dormancy during overwintering (Roberts, 1964; Nyman, 1972; Targett, 1978; Crawshaw *et al*., 1982; Walsh *et al*., 1983; Crawshaw, 1984; Lemons and Crawshaw, 1985; Sayer and Davenport, 1996; Raposa, 2003; Costa *et al*., 2013; Tomie *et al*., 2013; Speers-Roesch *et al*., 2018). For example, American eel and brown bullhead (*Ameiurus nebulosus*) have been reported to refrain from eating and remain buried within the sediment during the winter months (Nyman, 1972; Walsh *et al*., 1983; Crawshaw *et al*., 1982). Additionally, many centrarchid species, such as largemouth (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*), have been characterized as winter-dormant; exhibiting marked decreases in activity and minimal feeding in the cold (Lemons and Crawshaw, 1985; Cooke and Philipp, 2009). However, only a relatively small number of species has been investigated and few studies have directly measured dormant behaviour. Thus, as a whole, winter-dormant behaviour and physiology in fish is still poorly characterized and understood, especially in a comparative context.

The involvement of metabolic rate depression (MRD) in winter-dormant fish is also controversial. MRD is an active, reversible depression of resting cellular energy turnover that lowers an animal's metabolic rate to well below their standard or basal (i.e. resting) metabolic rate. MRD is a common strategy to facilitate the persistence of animals during energy limited periods (Storey and Storey, 2004). Winter dormancy in ectotherms is often considered analogous to mammalian hibernation where profound MRD is common (Crawshaw, 1984; Costa *et al.*, 2013). However, excluding certain species that overwinter in anoxic waters (e.g., some freshwater turtles; Staples, 2016), controversy exists over the involvement of MRD in winter-dormant ectotherms that overwinter under normoxic conditions (Ultsch, 1989; McNab, 2002). This is partially due to the difficulty of differentiating MRD from lethargy and slowed metabolism at cold temperatures (McNab, 2002).

The involvement of MRD in winter-dormant ectotherms can be assessed using the thermal sensitivity quotient (Q_{10}) of metabolic rate over the transition from an active to dormant state (Speers-Roesch *et al*., 2018). The effect of temperature on whole-animal metabolic rate in ectotherms, including fish, is relatively conserved among species and taxonomic groups and is commonly associated with a Q_{10} = 2-3 (Peck, 2016; Clarke, 2017), which reflects the direct, passive physicochemical effects of temperature on underlying cellular biochemistry. At low temperatures, typical Q_{10} values for metabolic rate tend to be slightly higher compared with warmer temperatures (Clarke, 2017). Therefore, conservatively, a cold-induced decrease in metabolic rate with a Q_{10} >3.5 has commonly been taken to indicate MRD, where the animal is actively suppressing its metabolic rate to a greater extent than would be predicted to arise from passive thermal

effects on metabolism alone (Crawshaw, 1984; Costa *et al*., 2013; Staples, 2016; Speers-Roesch *et al*., 2018).

Using this approach, previous studies have argued both for (Roberts, 1964; Targett, 1978; Walsh *et al*., 1983; Sayer and Davenport, 1996; Costa *et al*., 2013) and against (Crawshaw *et al*., 1982; Lemons and Crawshaw, 1985; Costa *et al*., 2013; Speers-Roesch *et al*., 2018) the presence of MRD in various winter-dormant fish species, suggesting considerable interspecific variation in the capacity for MRD. For example, studies on winter-dormant brown bullhead and largemouth bass reported typical physicochemical effects of temperature on metabolic rate (Q_{10} = 2-3) associated with inactivity and lethargy, suggesting that dormancy may simply be a phase of inactivity where SMR is maintained (Crawshaw *et al*., 1982; Crawshaw, 1984; Lemons and Crawshaw, 1985). Alternatively, studies on winter-dormant American eel and temperate wrasses (Labridae) have reported disproportionately large decreases in metabolic rate with cooling to winter temperature (Q_{10} values of 4.1 and 7.9-10.4, or more), which these authors took as evidence for involvement of MRD (Walsh *et al*., 1983; Sayer and Davenport, 1996; Costa *et al*., 2013;).

Interpretation of these previous studies is complicated because many of them may suffer from confounding, unaccounted influences of variation in activity on their estimates of standard metabolic rate (SMR; i.e., resting metabolic rate). As MRD is a depression of metabolic rate below SMR, identification of the involvement of MRD in winter dormancy is dependent upon accurate measurements of SMR at each test temperature. However, SMR can be difficult to measure because of the difficulty of obtaining metabolic rate measurements on absolutely still, resting fish (Chabot *et al*.,

2016). Ideally, simultaneous measurement of activity alongside metabolic rate can be done to ensure that SMR is estimated at times when the fish are resting, yet this is rarely done (Chabot *et al*., 2016). Alternatively, SMR can be estimated by making metabolic rate measurements at times when the fish is presumed to be inactive, such as at night in diurnal fish (i.e., when the fish is normally quiescent) or using a small subset of the lowest metabolic rate measurements (Chabot *et al*., 2016). However, none of the previous studies arguing for the presence of MRD in certain winter-dormant fishes have used such approaches. Yet, spontaneous activity can cause substantial elevations of metabolic rate above SMR in fishes (Nilsson *et al*., 1993). Thus, given that winter dormancy is characterized by inactivity, compared with warm temperatures, reductions in spontaneous activity could explain the large decreases in metabolic rates observed during dormancy.

In fact, a recent study demonstrated that when variation in spontaneous activity is controlled for in cunner, a winter-dormant species previously described to engage in MRD (Q_{10} = 7.9-10.4) (Costa *et al*., 2013), the decrease in metabolic rate during winter dormancy was explained by the physicochemical effects of cold alone (Q_{10}=2-3) (Speers-Roesch *et al*., 2018). An outstanding question is whether these results are specific to cunner or whether, as I hypothesize, diminished activity is the central convergent mechanism underlying the metabolic phenotype of winter-dormant fish.

To test this hypothesis, I first assessed the species-specific dormant behaviours in four species of temperate fishes previously reported to be winter-dormant and representing a broad phylogenetic range (cunner, pumpkinseed sunfish, mummichog, and American eel). I used day and night videography followed by automated computer tracking to measure how spontaneous activity, feeding, and sheltering behaviour change

in response to acute cooling from active, warm temperatures to dormant, cold temperatures. I then investigated the relationship between spontaneous activity and metabolic rate during acute cooling and long-term acclimation to winter low temperatures in two of the species (pumpkinseed sunfish and mummichog) to obtain accurate estimates of SMR and determine whether MRD or inactivity explains low metabolic rates in winter dormancy in fishes.

METHODS

Experimental Species

The study species were mummichog (*Fundulus heteroclitus,* family Fundulidae), cunner (*Tautogolabrus adspersus*, family Labridae), pumpkinseed sunfish (*Lepmomis gibbosus,* family Centrarchidae), and American eel (*Anguilla rostrata,* family Anguillidae). Adult mummichog were collected from Sam Orr Pond, Bocabec, New Brunswick in fall 2017 using a combination of minnow traps and seining. Adult pumpkinseed sunfish were collected in fall 2018 from Lily Lake, Saint John, New Brunswick, with permission from Rockwood Park, using a combination of trap netting and seining. Fishes were collected under DFO permit 349289. Juvenile cunner were obtained from a captive breeding program at the Huntsman Marine Science Centre (HMSC) in January 2018 (F1 offspring of wild caught parents reared in 2017, stock origin: Saint Mary's Bay, Nova Scotia). Juvenile American Eel were supplied by Atlantic Canada Eels Inc. in May 2018 and were wild-caught elvers returning to freshwater from the ocean.

Fish were maintained for a minimum of 4 weeks prior to experimentation in holding tanks supplied with flow-through freshwater (pumpkinseed sunfish and American eel) or recirculating filtered seawater (mummichog and cunner) at the University of New Brunswick, Saint John (UNBSJ). The holding tanks contained numerous PVC pipe shelters. Mummichog, cunner, and pumpkinseed sunfish were fed dry pellets (1.5mm, Gemma, Skretting, St. Andrews, New Brunswick, Canada) and American eel were fed bloodworms (San Francisco Bay Freeze Dried Bloodworms Fish Food, San Francisco Bay Brand, Newark, California, USA). All fish were fed to satiation every second day (3-4 times per week). Water temperature of the holding tanks was maintained at 14^{o}C ± 0.6^{o}C for cunner, mummichog, and pumpkinseed sunfish, and 17^{o}C ± 0.6^{o}C for American eel. A higher holding temperature was used for American eel because several studies have suggested that American eel enter dormancy at a relatively warmer temperature; a warmer holding temperature provided a larger range of warm-to-cold experimental temperatures (Nyman, 1972; Riley *et al*., 2011; Westerberg and Sjöberg, 2015). The fish were maintained under a winter photoperiod (10L:14D). The light period included a simulated sunrise and sunset (30 min each) to minimize potential effects of sudden light changes on behaviour (Ryu *et al*. 2020). The 10L:14D photoperiod was used because it occurs in New Brunswick and Nova Scotia when water temperatures are cooling in the fall (October) and fish are presumably preparing to enter winter dormancy, and in the middle of the winter when water temperatures are lowest and fish would be in winter dormancy (February).

All experimental work described was approved by the Institutional Animal Care Committee of University of New Brunswick, Saint John, following the standards and guidelines outlined by the Canadian Council on Animal Care.

Experiment 1: Behavioural Responses to Cooling to Winter Temperature

In order to identify the threshold temperature for the onset of dormant behaviours (i.e. inactivity, feeding cessation, increased sheltering), I measured spontaneous activity, food consumption, and sheltering behaviour in each species during acute cooling ($1^{o}C$ day^{-1}) from their respective acclimation temperature to a winter low temperature. Due to the elongate shape, undulating swimming, and small size of American eels in Experiment 1, I could not use the activity tracking software to measure changes in spontaneous activity or sheltering. Instead, I scored their "vigilance", which assessed their activity out of the shelter as well as their alertness within the shelter (see below).

The system to measure behaviour consisted of a clear acrylic aquarium (101cm x 68 cm x 15 cm) standing on upright clear acrylic pipes and illuminated below by four infrared lamps (940 nm). There was no indication that the fish could sense this light wavelength, based on personal observations of fish behaviour (i.e., no changes in behaviour with the sudden onset of the infrared lights), which is consistent with observations by Speers-Roesch *et al.* (2018). A white translucent sheet of acrylic was placed directly underneath the acrylic aquarium to diffuse the infrared light. Mounted above the aquarium was an infrared-sensitive digital video recording system (640 x 480p, 10-15 fps; IDS Camera, IDS Imaging, Obersulm, Germany) that enabled day and night video recordings of fish behaviour.

Within the acrylic aquarium were plastic arenas (6 for pumpkinseed sunfish, 12 for American eel, or 16 for cunner and mummichog), each for an individual fish and each fitted with a section of PVC pipe for shelter (see Table 1). The arenas were plastic boxes matched to fish size to minimize confinement stress and allow for sufficient room for exploratory behaviour; the shelters were sized to match fish length and height (Table 1). The behavioural arenas were individually plumbed with tubing carrying water from a seawater or freshwater recirculating system (depending on the species) initially maintained at the species-specific holding acclimation temperature using a commercial water chiller (1/3 horsepower Arctica, JBJ Chillers, St. Charles, MO). The water height in each arena was between 5 cm and 11 cm depending on the species (5 cm for American Eel, 8 cm for cunner and mummichog, and 11 cm for pumpkinseed sunfish). Each arena had overflow holes that drained to the outer acrylic tank, and this in turn drained to a sump from which water was recirculated to the arenas following filtration and chilling.

For cunner, mummichog, and American eel, a single experimental trial was run; however, due to their larger size, pumpkinseed sunfish were measured in two separate, sequential trials to reach an adequate sample size (cunner, n = 16; pumpkinseed sunfish, n = 12; mummichog, n = 16; American eel, n = 12). Fish were placed individually into the plastic arenas and a sheet of clear acrylic was placed on top of all arenas to prevent fish escape. The fish were exposed to a winter photoperiod (10L:14D), including simulated 30 min sunrise/sunset. The experimental setup was surrounded by black plastic bags and several of the overhead lights in the lab were blacked out to minimize the effect of bright light on the fish's behaviour. The fish were given 2-4 days to become accustomed to their experimental arenas. The trial began with a 24-hour measurement at the fish's respective

acclimation temperature (i.e., a complete light-dark cycle). The fish were then cooled every morning at a rate of ~1°C/day from their acclimation temperature (14°C for mummichog, cunner, and pumpkinseed sunfish; 17°C for American eel) to ~2°C for cunner, pumpkinseed sunfish, and American eel and ~1°C for mummichog. The daily 1°C cooling took about 30 minutes, during which time feeding counts from previous day were carried out (see below). Therefore, the fish were exposed to each temperature for approximately 24 hours. At each temperature, the behavioural parameters (see below) were measured over the day and night and were averaged to obtain a single day and night measurement for each fish.

The temperature within the acrylic aquarium was recorded using a Traceable digital thermometer (Cole-Parmer Canada Company, Montreal, Quebec, Canada) which measured temperature in real-time and recorded the highest and lowest temperatures experienced during the 24-hour period (i.e., representing the hysteresis of the chiller). An average daily temperature was calculated by averaging temperatures recorded in the morning (~9:00), evening (~17:00) and the highest and lowest temperatures recorded by the thermometer. The average daily temperatures each species experienced during their respective acute cooling trials can be found in Table 2.

Throughout each trial, fish were fed a ration of ~0.5% body weight (BW) of feed pellets or blood worms (for American eels) every morning. Food consumption was determined at each temperature by counting the remaining pellets or worms collected 24 hours later, before the next cooling step. Spontaneous activity and sheltering behaviour were calculated from day and night video recordings using automated tracking software (ToxTrac, v2.84; Rodriguez *et al.*, 2017, Rodriguez *et al.*, 2018). The first and last hour

and a half of the daytime and nighttime periods were removed from the measurement period to reduce the effect of disturbances (i.e., feeding and/or cleaning the lids) or light changes on fish behaviour (daytime measurement period = 9:00 – 16:00, nighttime measurement period = 19:00 – 6:00). The pixel-to-distance calibration necessary for appropriate calculations of distance moved was conducted using known distances and pixel measurements in ImageJ (Version 1.52a, National Institutes of Health, USA; Schneider *et al.*, 2012). My measurements of spontaneous activity represent the average speed of the fish over the day or night at each temperature, calculated as the total distance moved over that period (as measured by ToxTrac) divided by the total corresponding time and standardized to the fish's total length (average body lengths moved min^{-1}, BL min^{-1}). Sheltering behaviour was quantified by using ToxTrac's calculated invisible time over the day or night at each temperature, because periods of invisibility (i.e. periods where the software could not find the fish based on the defined detected threshold limits) occurred when the fish were inside their opaque PVC shelter. Activity within the shelter was assumed to be zero because the shelter size was matched closely to the fish size, thus providing minimal space for movement.

Vigilance was qualitatively scored for American eels during day and night at each temperature using the video recordings. At every 30 min point, a single minute of video was visually assessed where each eel was scored using the following rubric: 1 = fish out of shelter, 0.5 = head out of the shelter, 0 = fish fully enclosed within the shelter. These 30 min values were averaged for each eel across the day or night at each temperature. American eel, under normal temperatures and conditions, are generally active at night, when they forage, but will spend their daytime sheltering within burrows or in spaces

among rocks or bottom debris (Tomie, 2013). Additionally, Nyman (1972) noted that eels often protruded their heads from their shelters and that, with decreasing temperature, fewer heads became visible from their shelters. Thus, this method is a suitable alternative for assessing American eel activity.

Experiment 2: The Effect of Acute Cooling and Cold Acclimation on the Relationship Between Spontaneous Activity and Metabolic Rate in Mummichog and Pumpkinseed Sunfish

I simultaneously measured metabolic rate and spontaneous activity in mummichog and pumpkinseed sunfish (Table 3) during acute cooling (~3^{o}C/day) and after 4-6 weeks of cold acclimation (~2.5^{o}C). A faster cooling rate was used in Experiment 2, relative to Experiment 1 (1^{o}C/day), to minimize the fasting duration of the fish (they were not fed within the respirometers). Using these data, I estimated the thermal sensitivity of metabolic rate at known and comparable levels of activity, including extrapolated zero activity (i.e., SMR). This approach enabled me to control for the influence of activity on metabolic rate and estimate the thermal sensitivity of SMR in order to ascertain if a MRD was involved in winter dormancy (i.e., $Q_{10} > 3.5$ for SMR over the active to dormant transition). Prior to experimentation, fish were individually marked with visible implant elastomer tags (Northwest Marine Technology Inc., Anacortes, Washington, USA) to enable repeated measurements on the same fish. The fish were transferred from their holding tanks to one of two species-specific acclimation systems each consisting of three separate glass aquaria (4 fish per aquarium, n= 12) containing PVC pipe shelters. The three aquaria in each system were supplied with

recirculating, temperature-controlled (Arctica chiller) filtered and aerated water with salinity and temperature appropriate for the species (full-strength sea water, ~15^{o}C for mummichog; freshwater, ~14^{o}C for pumpkinseed sunfish). Fish were maintained under a winter photoperiod (10L:14D including a 30 min sunrise/sunset) and fed to satiation every other day. Fish were held in the acclimation system for at least 4 weeks before experimentation and fasted for a period of 48-72 hours prior to transfer to the experimental setup for measurement of metabolic rate and spontaneous activity during acute cooling.

For acute cooling, four experimental trials were run per species with three fish per trial (n=12 fish per species). For each trial, the three fish were transferred into individual custom-made circular acrylic respirometers that were placed in a clear acrylic water bath maintained under the same photoperiod and water conditions as the acclimation system. As in Experiment 1, the experimental setup was surrounded by black plastic bags and several of the overhead lights in the lab were blacked out to minimize the effect of bright light on the fish's behaviour . This water bath was supplied with recirculated water that was temperature controlled using both an Arctica chiller (1/3 horsepower Arctica, JBJ Chillers, St. Charles, MO) and an Arctic A25 refrigerated circulator (Thermo Scientific, Newington, NH, USA). Once placed in the respirometer, the fish were allowed to recover overnight for ~8-10 hours, after which the fish had returned to a stable metabolic rate, and the measurement period began. The fish were then exposed to a stepwise acute cooling exposure while metabolic rate and spontaneous activity were simultaneously recorded continuously both day and night (see below). The fish were first kept at their acclimation temperature for 2 days (i.e., ~15^{o}C for mummichog and ~14^{o}C for

pumpkinseed sunfish), following which they were cooled at a rate of 3^{o}C per day until the fish reached ~2.5^{o}C. The cooling was initiated at ~17:30 every day and took between 15 and 60 min to cool (depending on the temperature; i.e., it took longer for the system to reach colder set points). Thus, nearly 24 hours of metabolic rate and spontaneous activity was measured at each temperature.

Following the acute cooling exposure, the fish were transferred back to their acclimation systems which were pre-cooled to a winter low temperature of ~2.5^{o}C. The fish were acclimated to ~2.5^{o}C for a period of 4-6 weeks under winter photoperiod, during which they were provided food every second day (3-4 times per week). Following acclimation, the same subset of 3 fish were transferred back to their respective respirometers for repeated measurements of metabolic rate and spontaneous activity at ~2.5^{o}C and after an acute re-warming to ~14 or ~15^{o}C. As for the acute cooling exposure, fish were fasted 48-72 hrs prior to transfer to the experimental setup and had a period of ~8-10 hours to recover from handling prior to recording. Metabolic rate and spontaneous activity were measured for two days at ~2.5^{o}C. The fish were then acutely warmed from ~2.5^{o}C to their initial warm acclimation temperatures (i.e., ~15^{o}C for mummichog and ~14^{o}C for pumpkinseed sunfish) over ~6-8 hrs and, once the temperature had stabilized, recordings of metabolic rate and spontaneous activity continued for one full day/night cycle. The average temperatures ± S.D. each species was exposed to during Experiment 2 can be found in Table 4.

Metabolic rate was estimated by measuring oxygen consumption rate ($\dot{M}O_2$, $mgO_2\ kg^{-1}\ hr^{-1}$) using automated intermittent-closed optical respirometry (4 channel FireSting with 4 temperature extension module, PyroScience, Aachen, Germany). Each

respirometer was fitted with an individual optode and temperature probe to measure the within-chamber temperature-compensated oxygen level. The respirometer water was mixed by recirculation through an Eheim water pump (Eheim 300, 5L/min, clamped to a low flow with plastic screw clamps turned a specific number of rotations to achieve a consistent flow). The stop and flush periods were modified depending on water temperature (5 min flush/15 min stop period at ~14-15^{o}C and ~11^{o}C; 5 min flush/25 min stop period at ~8^{o}C; 5 min flush/40 min stop period at ~5^{o}C; 5 min flush/55 min stop period at ~2.5^{o}C). $\dot{M}O_2$ was measured from the slope of the decline in water oxygen content during the closed period; the slopes were extracted using LabChart (Version 8.1.13, ADInstruments, Colorado Springs, Colorado, USA). The first 5 min of each stop period (i.e., the equilibration period following flush) was excluded from slope calculation. A blank respirometer containing no fish was run simultaneously alongside the fish to estimate and correct for background respiration.

To measure spontaneous activity simultaneously with metabolic rate, the respirometers were illuminated from below with infrared lights (940 nm) and video recorded with infrared-sensitive cameras, as described previously. For each interval of time where metabolic rate was calculated for a given fish, the associated spontaneous activity was also calculated from the video of the fish within the respirometer over the same interval. Spontaneous activity was calculated using ToxTrac as described in the Methods for Experiment 1. ToxTrac is sensitive enough to track minor postural adjustments or the fish being slightly buffeted by the recirculating water flow, which we did not consider to be active spontaneous movement of the fish itself. Thus, we removed these effects for each fish by calculating individual “inactive control values”. These

inactive control values were calculated by measuring each individual fish's movement in ToxTrac over 3 periods (~10 mins each) of known inactivity (i.e., visually assessed from video) and were subtracted from all of the measurements of spontaneous activity for each fish, thus removing the influence of minor uncontrolled movement on their measurements.

Data Analysis and Statistics

For all analyses, statistical significance was accepted at $p < 0.05$ and all values presented in the text are means ± standard errors of the mean (S.E.M.), unless otherwise noted.

Experiment 1: Behavioural Responses to Cooling to Winter Temperature

Measurements of day and nighttime behaviour (i.e., spontaneous activity, sheltering, feeding, and vigilance) were obtained for each fish at each temperature during the acute cooling trial. The behavioural changes in response to acute cooling during night and day were assessed using generalized linear mixed effects models (GLMM; family = Gamma, link = inverse) in RStudio (Version 1.2.1335, RStudio Inc., Boston, MA, USA; http://www.rstudio.com) using the function glmer. To assess changes in spontaneous activity and sheltering behaviour in response to cooling as well as diel cycle (night vs. day), GLMMs (family = Gamma, link = inverse) were run using individual spontaneous activity or sheltering data related to experimental temperature in combination with day and night periods, with fish as a random factor nested within trial where necessary (i.e., multiple trials were not run for all species). Changes in feeding behaviour in response to acute cooling were determined using GLMMs of individual feeding data related to

temperature with fish as a random factor nested within trial where necessary. In some instances, in order to fit the GLMMs to the data, rescaling of the data (dependent variable/1000) was performed. Significant effects were determined by using the Anova function in RStudio which calculated p-values using Type II Wald chi-square tests. After fitting GLMMs to these various data, Bonferroni post-hoc multiple comparisons tests were completed using the estimated marginal means, or emmeans, package (Singman *et al*., 2019).

Each species-specific winter dormancy threshold temperature was defined by the fish reaching a steady state that was not significantly different from the measurement at the coldest temperature (e.g., ~2^{o}C). Thus, below the dormancy threshold temperature the behavioural measurements, in response to further cooling, would not differ significantly. Additionally, when possible, dormancy threshold temperatures were supplemented with calculations of mean inactive and mean fasting temperatures. These are defined as the average temperature at which individual fish entered an inactive or fasting state, respectively.

Experiment 2: Effects of Temperature on Diel Cycles of $\dot{M}O_2$ and Spontaneous Activity

Measurements of $\dot{M}O_2$ and spontaneous activity in each fish were averaged across all measurement intervals for that fish and I calculated an average day and night-time value for $\dot{M}O_2$ and corresponding spontaneous activity for each fish at each experimental temperature. To determine whether a diel cycle existed for $\dot{M}O_2$ and spontaneous activity and whether temperature had a significant effect on this diel cycle, GLMMs (family = Gamma, link = inverse) were applied in RStudio using the function glmer. Individual

averaged $\dot{M}O_2$ or spontaneous activity measurements were related to temperature in combination with day and night-time periods with fish as a random factor nested within trial. Significant effects were determined by using the Anova function in RStudio which calculated p-values using Type II Wald chi-square tests. After fitting GLMMs to these various data, Bonferroni post-hoc multiple comparisons tests were completed using the estimated marginal means, or emmeans, package (Singman *et al*., 2019).

Experiment 2: Estimating Thermal Sensitivities (Q_{10}) of Standard Metabolic Rate (SMR) and Metabolic Rate at a Similar Activity Level

Acute Thermal Sensitivity of Group Routine $\dot{M}O_2$

The thermal sensitivity of $\dot{M}O_2$ in response to acute cooling in mummichog and pumpkinseed sunfish was first assessed without controlling for variation in activity by exponentially relating all measured $\dot{M}O_2$ values to their corresponding temperature measurements. By doing so, the thermal sensitivity (Q_{10}) of acute cooling of routine $\dot{M}O_2$ in both species could be calculated from the slope of the exponential curve over the full range of temperatures measured using the following equation; $Q_{10} = e^{(Slope \text{ of } exponential\ equation \cdot 10)}$. Calculation of this Q_{10} provides a useful comparison for highlighting the importance of controlling for temperature-dependent variation in activity when estimating the effect of temperature on different levels of metabolic rate.

Controlling for the Effects of Variation in Activity on Metabolic Rates

I used several approaches to control for the effect of variation in activity on metabolic rate during cooling and acclimation to winter cold, in order to obtain estimates

of SMR (i.e., metabolic rate at zero activity) at each temperature or of metabolic rate at a similar level of activity at each temperature. The thermal sensitivity of SMR or activity-controlled metabolic rate was then determined using the Q_{10} equation (see below), in order to determine if, when contributions of activity were removed or controlled for, there was evidence of an active depression of metabolic rate (i.e., $Q_{10} > 3.5$) or simply passive physicochemical effects of cooling ($Q_{10} = 2\text{-}3$).

Estimating SMR of individual fish

The primary approach used to calculate SMR at each temperature in each species involved controlling for variation in spontaneous activity by correlating measurements of spontaneous activity with their corresponding $\dot{M}O_2$ values for all measurement intervals for each individual fish at each experimental temperature (Prism 6, GraphPad Software, Inc. San Diego, California, USA). The resulting relationship was described using the exponential equation, $ae^{(bU)}$, where a is SMR (extrapolated $\dot{M}O_2$ at zero activity), b is the slope of the exponential regression, and U is spontaneous activity. Thus, for each individual fish, several exponential regressions were generated to determine its SMR at each experimental temperature (i.e., ~14^{o}C, ~11^{o}C, ~8^{o}C, ~5^{o}C, ~2.5^{o}C, 4-6 week acclimated ~2.5^{o}C, and acutely warmed ~14^{o}C). This relationship provides a robust method of making predictions beyond the measured range, which is particularly beneficial for estimating SMR by extrapolating $\dot{M}O_2$ values as a function of zero spontaneous activity (Korsmeyer *et al.*, 2002).

Estimates of SMR in individual fish were secondarily calculated by averaging its lowest 20 $\dot{M}O_2$ values at each temperature or by averaging the $\dot{M}O_2$ values corresponding

to the lowest 20 spontaneous activity points at each temperature. I calculated SMR using these secondary methods to assess whether less rigorous methods of SMR estimation, relative to the extrapolation method described above, would nevertheless provide a consistent result.

In instances where the exponential regression extrapolation approach did not show a significant relationship for a specific fish at a given temperature (e.g., because of a low spread in activity values), these extrapolated SMR values were replaced with values calculated by the secondary methods of individual SMR calculation to determine if there was any effect on my estimate of Q_{10}. In all of these instances, changing these values had no effect on the overall average Q_{10} value (See Tables 6 and 7).

Estimating group SMR

To support the analysis using individual SMR estimates, a single group SMR value at each temperature was also calculated. This was done using the extrapolated $\dot{M}O_2$ at zero activity calculated from exponential regressions using all measurements of spontaneous activity and corresponding $\dot{M}O_2$ across all individual fish for a species at each temperature.

Metabolic rate at a similar level of activity (activity-controlled metabolic rate)

In addition to estimating SMR, I calculated the $\dot{M}O_2$ of individual fish at each temperature within a narrow, overlapping range of spontaneous activity that occurred at all temperatures. However, due to differing levels of spontaneous activity measured in the pre- and post-acclimation trials, different narrow overlapping ranges had to be used to calculate the thermal sensitivity of acute cooling (between 0.8 – 1.8 BL min^{-1} for both

mummichog and pumpkinseed sunfish) and acute re-warming (between 2.0 – 2.5 BL min^{-1} and 1.0 – 1.5 BL min^{-1} for mummichog and pumpkinseed sunfish, respectively), and the thermal sensitivity between ~14-15ºC acclimated and ~2.5ºC acclimated mummichog and pumpkinseed sunfish could not be calculated. These values allowed for thermal sensitivity analysis of $\dot{M}O_2$ values where the contribution of variation in activity across temperature has been controlled for.

Thermal sensitivity analysis

The thermal sensitivities of SMR and activity-controlled metabolic rate were assessed by calculating the thermal sensitivity quotient (Q_{10}) for both individual fish and group values. Q_{10} values were calculated for multiple temperature intervals. During acute cooling, Q_{10} was calculated for the full temperature interval as well as the warm and cold halves of the thermal change, which generally bracketed the temperature of onset of dormancy behaviours (i.e., ~14ºC to ~2.5ºC, ~14ºC to ~8ºC, ~8ºC to ~2.5ºC). Q_{10} was also calculated for the acute re-warming (i.e., 4-6 week acclimated 2.5ºC to acutely warmed 14ºC), for comparison to the acute cooling. Finally, Q_{10} was calculated for the acclimation to winter low temperature (~14ºC acclimated to 4-6 week acclimated ~2.5ºC). These temperature comparisons were chosen to elucidate whether MRD, if present, occurs acutely in fish or if the onset of MRD may be delayed (i.e., after acclimation). Alternatively, the comparison of ~14ºC and ~2.5ºC acclimated animals could indicate a reduced thermal sensitivity, suggesting compensation following cold acclimation.

Statistical analysis of metabolic rates and their thermal sensitivity

The effect of temperature on SMR in pumpkinseed sunfish and mummichog was tested with a linear mixed-effects model that included, repeated measures (lmer function in R Studio) followed by post hoc multiple comparisons test (emmeans function, R studio). The same test was used to determine if Q_{10} was similar or different among the various temperature intervals identified above.

RESULTS

Experiment 1: Quantifying Species-Specific Winter-Dormant Behaviour

Acute cooling yielded significant, species-specific changes in spontaneous activity, sheltering behaviour, and feeding behaviour. *Cunner*

Cunner spontaneous activity was significantly affected by the decreasing temperature, diel period, and their interaction (Figure 1A, Table 5). Their spontaneous activity was significantly higher during the daytime relative to the nighttime between 14.2^{o}C and 8.2^{o}C (Bonferroni post-hoc, $p < 0.05$), but below this threshold there were no significant differences in day or nighttime spontaneous activity. Daytime spontaneous activity significantly decreased by over 95% from 14.2^{o}C to 8.2^{o}C (Bonferroni post-hoc, $p < 0.0001$). At and below 7.1^{o}C, excluding some negligible nighttime movement at 4.9^{o}C, activity was zero in all fish day and night. The average inactive temperature (i.e., the average temperature where each individual fish became and remained inactive) was 7.3 ± 0.6^{o}C. Individual variation existed, however, with some cunner becoming inactive

at considerably warmer temperatures (e.g., two cunner entered and remained in an inactive state at 13.0°C and 10.3°C, respectively).

Cunner feeding behaviour significantly decreased with cooling from 14.2°C to 7.1°C (feed consumed decreased from ~65% to ~20% of ration consumed) (Bonferroni post-hoc, $p < 0.0001$) and all fish fasted below 5.9°C (Figure 1E, Table 5). The mean fasting temperature was similar to that of their mean inactive temperature (i.e., temperature where the fish began and remained in a state of fasting). Fish on average ceased feeding and remained in a fasted state at 7.5 ± 0.4°C; however, some fish began to fast at considerably warmer temperatures (two fish fasted at 10.9°C and 10.3°C, respectively).

The proportion of time cunner spent within their shelters was significantly affected by temperature and by an interaction of temperature and diel period (Figure 1I, Table 5). While sheltering was significantly higher in the nighttime at 14.2°C and 13.0°C, below 13.0°C cunner spent most of, if not all of, their time sheltering regardless of day or nighttime period (ranging from ~90% between 11.9°C to 10.3°C and ~100% between 8.1°C to 1.8°C) (Bonferroni post-hoc, $p < 0.0001$).

Pumpkinseed Sunfish

Pumpkinseed sunfish spontaneous activity was significantly affected by temperature, diel period, and their interaction (Figure 1B, Table 5). Pumpkinseed sunfish spontaneous activity was significantly higher during the daytime relative to nighttime from 14.1°C to 5.9°C (Bonferroni post-hoc, $p < 0.05$). With further cooling to 3.0°C and below, this diel pattern shifted, such that nighttime spontaneous activity increased and

was significantly higher than daytime spontaneous activity, although activity was still very low (Bonferroni post-hoc, $p < 0.05$). Both day and nighttime spontaneous activity significantly decreased in response to cooling. Daytime pumpkinseed sunfish spontaneous activity decreased from 14.1°C to 9.1°C (~84% decrease in spontaneous activity) (Bonferroni post-hoc, $p < 0.001$), below which their level of activity did not change relative to the measurement at 2.2°C. Similarly, nighttime spontaneous activity decreased by ~82% in response to cooling to 2.2°C. However, unlike daytime spontaneous activity, nighttime spontaneous activity was significantly higher at 14.1°C and 12.9°C and significantly lower between 9.6°C and 5.9°C relative to the measurement at 2.2°C (Bonferroni post-hoc, $p < 0.05$).

Acute cooling caused a significant reduction in pumpkinseed sunfish feed consumption (Figure 1F, Table 5). Food consumption decreased by 60% from 14.1°C to 4.8°C (Bonferroni post-hoc, $p < 0.001$). Below 4.8°C, pumpkinseed sunfish feed consumption did not significantly change but continued to decrease on average; however, while the majority of the pumpkinseed sunfish had ceased feeding by the coldest temperature, a few fish continued to feed.

Pumpkinseed sunfish sheltering behaviour was found to be significantly affected by temperature, diel period, and their interaction(Figure 1J, Table 5). Pumpkinseed sunfish spent significantly more time in their shelters at night relative to the day at temperatures from 14.1°C to 9.1°C (Bonferroni post-hoc, $p < 0.05$). Below 9.1°C, pumpkinseed sunfish spent a similar amount of time in their shelters during the day and night, excluding 3.0°C and 2.2°C where fish spent significantly more time in their shelters during the day (~90% vs. 60% of time their spent in shelter) (Bonferroni post-hoc, $p <$

0.001). Both day and nighttime sheltering behaviour increased in response to decreasing temperature. Daytime sheltering behaviour significantly increased from 14.1°C to 9.6°C (from ~25% to ~60% of time spent in shelter, respectively), relative to the measurement at 2.2°C, then remained relatively stable from 9.1°C to 2.2°C (ranging from ~70% to ~80% of time spent in shelter) (Bonferroni post-hoc, $p < 0.05$). Nighttime sheltering behaviour significantly increased from 14.1°C to 12.9°C and then plateaued from 11.9°C to 4.0°C (i.e., no significant changes in spontaneous activity between these temperatures) (Bonferroni post-hoc, $p < 0.05$). However, at 3.0°C and 2.2°C nighttime sheltering behaviour decreased significantly from the measurement at 4.0°C (sheltering decreased ~25-30%) (Bonferroni post-hoc, $p < 0.05$).

Mummichog

Mummichog spontaneous activity was significantly affected by temperature and diel period but was not significantly affected by their interaction (Figure 1C, Table 5). Higher spontaneous activity was observed during the night at each temperature with significantly higher nighttime activity observed from 14.3°C to 12.0°C and from 5.9°C to 3.1°C (Bonferroni post-hoc, $p < 0.05$). Nighttime spontaneous activity decreased 69% from 14.3°C to 4.1°C (Bonferroni post-hoc, $p < 0.05$) and, at 3.1°C and below, their level of nighttime activity remained unchanged . Mummichog daytime spontaneous activity decreased by 16% from 14.3°C to 7.1°C (Bonferroni post-hoc, $p < 0.05$). At 5.9°C and below, the low level of daytime activity did not significantly differ from the measurement at 1.1°C.

Acute cooling caused mummichog feed consumption to significantly decrease from 14.3°C to 4.1°C (100% to ~20% of ration consumed, respectively) (Bonferroni post-hoc, $p < 0.05$) and from 3.1°C to 1.1°C all mummichog had ceased feeding (Figure 1G, Table 5). Mummichog's mean fasting temperature was 4.4 ± 0.3°C.

The proportion of time mummichog spent in their shelters was significantly affected by temperature and diel period but was not affected by their interaction (Figure 1K, Table 5). Mummichog spent significantly more time within their shelters during the daytime (~50-60% of the time) than the nighttime (~25-40% of the time) between 13.1°C and 1.9°C (Bonferroni post-hoc, $p < 0.05$). Unlike cunner and pumpkinseed sunfish, there was little change in daytime or nighttime sheltering behaviour in response to acute cooling, except for the final daytime measurement at 1.1°C where mummichog spent significantly less time within their shelter, relative to all other temperatures (Bonferroni post-hoc, $p < 0.05$).

American Eel

American Eel vigilance was significantly affected by temperature, diel period, and their interaction (Figure 1D, Table 5). Eels were largely nocturnal, with significantly higher vigilance observed during the nighttime at most temperatures, excluding 3.4°C and 2.5°C (Bonferroni post-hoc, $p < 0.05$). Daytime vigilance was generally higher at warmer temperatures; however, this varied to some extent. Significantly higher daytime vigilance was observed at 17.3°C, 15.9°C, 10.2°C, and 8.0°C, relative to the 2.5°C measurement (Bonferroni post-hoc, $p < 0.05$). Nighttime vigilance stayed relatively stable from 17.3°C to 10.2°C, but from 9.2°C to 4.0°C nighttime vigilance significantly decreased by ~40%

(Bonferroni post-hoc, $p < 0.05$). At 3.0°C and below, American eel nighttime vigilance decreased markedly (vigilance decreased by ~72% from 4.0°C to 3.4°C) and remained at this nadir at 2.5°C (Bonferroni post-hoc, $p < 0.05$).

Acute cooling significantly affected American eel food consumption (Figure 1H, Table 5), decreasing from 95% to 20% of ration consumed at 17.3°C to 6.0°C, respectively (Bonferroni post-hoc, $p < 0.05$). Below 6.0°C, food consumption did not significantly differ from that measured at 2.5°C (Bonferroni post-hoc, $p > 0.05$), however, eels continued to consume slightly less, and feeding ceased completely at 3.4°C and 2.5°C. The mean fasting temperature was 5.5 ± 0.4°C, and there was individual variation in fasting temperature (ranging from 8.0°C to 3.4°C).

Experiment 2: The Effect of Acute Cooling and Cold Acclimation on the Relationship Between Spontaneous Activity and Metabolic Rate in Mummichog and Pumpkinseed Sunfish

Mummichog Activity and $\dot{M}O_2$

Mummichog spontaneous activity in Experiment 2 was significantly affected by temperature, diel period, and their interaction (Figure 2). As in Experiment 1, mummichog demonstrated a nocturnal activity pattern, with significantly higher nighttime spontaneous activity at all temperatures (Figure 2A) (Bonferroni post-hoc, $p < 0.05$). Mummichog daytime spontaneous activity decreased with cooling from 14.9°C to 11.6°C (daytime spontaneous activity decreased ~45%) then remained relatively stable from 8.6°C to 2.5°C (Bonferroni post-hoc, $p < 0.05$). After acclimation to ~2.5°C for 4-6 weeks, both the first and second measurement days at ~2.5°C yielded significantly higher

daytime spontaneous activity relative to the initial acutely cooled 2.5°C measurement (spontaneous activity increased by ~129% and ~49%, respectively), with the first measurement day being significantly higher than the second (Bonferroni post-hoc, $p < 0.05$). Following acute re-warming to 15.1°C the mummichog spontaneous activity did not significantly change, relative to the ~2.5°C post-acclimation measurements and was significantly lower than the first measurement at 14.9°C (spontaneous activity was ~33% lower) (Bonferroni post-hoc, $p < 0.05$).

Mummichog nighttime spontaneous activity was less affected by acute cooling relative to mummichog daytime activity. While the nighttime spontaneous activity measured at 2.5°C was significantly lower than the nighttime spontaneous activity measured at 14.9°C (spontaneous activity was ~56% lower at 2.5°C) (Bonferroni post-hoc, $p < 0.05$), nighttime spontaneous activity did not significantly differ between 11.6°C and 2.5°C; though nighttime spontaneous activity did continue to decrease slightly in response to the cooling (spontaneous activity decreased by ~32% from 11.6°C to 2.5°C). There was no significant change in nighttime spontaneous activity following acclimation to ~2.5°C relative to the initial acutely cooled 2.5°C measurement (Bonferroni post-hoc, $p > 0.05$). Additionally, following acute re-warming, there was no significant change in spontaneous activity relative to the acclimated ~2.5°C measurements (Bonferroni post-hoc, $p > 0.05$).

Mummichog $\dot{M}O_2$ significantly changed in response to temperature, diel period, and an interaction of these factors (Figure 2B). Like mummichog spontaneous activity, mummichog $\dot{M}O_2$ was higher on average during the nighttime period compared with the daytime period; however, nighttime $\dot{M}O_2$ was only significantly higher at the acute 2.5°C

measurement and for both nights of ~2.5ºC measurements following acclimation (Bonferroni post-hoc, $p < 0.05$). Both day and nighttime $\dot{M}O_2$ followed a similar trend in response to acute cooling, acclimation, and acute re-warming. Both day and nighttime $\dot{M}O_2$ decreased significantly from 14.9ºC to 2.5ºC (decreased by 76% and 73% from 14.9ºC to 2.5ºC, respectively) (Bonferroni post-hoc, $p < 0.001$). Following acclimation, day and nighttime $\dot{M}O_2$ was initially significantly higher on the first day/night measurement ($\dot{M}O_2$ was ~25% and 52% higher following acclimation in the day and night, respectively) (Bonferroni post-hoc, $p < 0.01$); however, on the second day/night measurement the $\dot{M}O_2$ values had returned to a similar level, relative to the initial acutely cooled 2.5ºC measurement. Mummichog day and nighttime $\dot{M}O_2$ then significantly increased in response to acute re-warming, relative to the post-acclimation ~2.5ºC measurements ($\dot{M}O_2$ increased by ~200-350% in both day and nighttime measurements) (Bonferroni post-hoc, $p < 0.0001$).

Pumpkinseed Sunfish Activity and $\dot{M}O_2$

Pumpkinseed sunfish spontaneous activity was significantly affected by temperature and its interaction with diel period during Experiment 2 (Figure 3). While the diel period was found to not significantly affect pumpkinseed sunfish spontaneous activity, significant differences between day and nighttime spontaneous activity were observed at several of the temperatures measured. Interestingly, as evidenced by a significant interaction, the diel cycle changed with decreasing temperature, such that at warmer temperatures pumpkinseed sunfish were more active during the day and at colder temperatures they were more active during the night. Spontaneous activity was higher

during the day for 14.2°C, 11.1°C, and at 14.2°C following acute warming; however, spontaneous activity was only significantly higher during the daytime at ~14°C (both prior to cooling and after acute re-warming) (Bonferroni post-hoc, $p < 0.05$). Alternatively, spontaneous activity was higher during the night at 8.0°C, 5.3°C and at all measurements at ~2.5°C (both prior to and after acclimation to ~2.5°C); however, there was little difference between 8.0°C day and nighttime periods. Significantly higher nighttime spontaneous activity was observed at 5.3°C, 2.6°C, and for the first ~2.5°C measurement, following acclimation to ~2.5°C (Bonferroni post-hoc, $p < 0.05$).

Similar to the results in Experiment 1, pumpkinseed sunfish spontaneous activity decreased in response to the acute cooling. Their daytime spontaneous activity significantly decreased by 77% from 14.2°C to 2.6°C (Bonferroni post-hoc, $p < 0.001$). Following acclimation to ~2.5°C for 4-6 weeks, pumpkinseed sunfish daytime spontaneous activity significantly increased by 85-106% when compared with the acute 2.5°C (Bonferroni post-hoc, $p < 0.0001$). Daytime spontaneous activity following acute warming to 14.2°C, while significantly higher than the initial acutely cooled 2.6°C (spontaneous activity was ~127% higher at 14.2°C) (Bonferroni post-hoc, $p < 0.0001$) daytime measurement, did not significantly differ from the daytime spontaneous activity recorded during both days following acclimation to ~2.5°C.

Nighttime spontaneous activity was significantly higher at 14.2°C and 11.1°C, relative to the measurement at 2.6°C (Bonferroni post-hoc, $p < 0.05$). From 8.0°C to 2.6°C nighttime spontaneous activity remained unchanged. Nighttime spontaneous activity was significantly higher for both nights following acclimation to ~2.5°C, relative to the initial acutely cooled 2.6°C night (nighttime spontaneous activity increased by

~96% and 53%, respectively) (Bonferroni post-hoc, $p < 0.05$). Interestingly, after acute re-warming to ~14°C, nighttime spontaneous activity was significantly lower by 30-50% compared with those recorded for both ~2.5°C days following acclimation (Bonferroni post-hoc, $p < 0.05$).

Pumpkinseed sunfish $\dot{M}O_2$ was significantly affected by temperature and diel period (Figure 3). Similar to the results for pumpkinseed sunfish spontaneous activity, their $\dot{M}O_2$ diel cycle was altered with decreasing temperature. $\dot{M}O_2$ was significantly higher during the day from 14.2°C to 11.1°C and after acute rewarming to 14.2°C (Bonferroni post-hoc, $p < 0.05$).

Day and nighttime $\dot{M}O_2$ of pumpkinseed sunfish yielded similar decreases in response to decreasing temperature. Day and nighttime $\dot{M}O_2$ decreased significantly by 14.2°C to 2.6°C ($\dot{M}O_2$ decreased by ~88% and 78%, respectively) (Bonferroni post-hoc, $p < 0.01$). Following acclimation to ~2.5°C for 4-6 weeks, pumpkinseed sunfish day and nighttime $\dot{M}O_2$ significantly increased by ~50-100%, relative to the initial acute measurement at 2.6°C. Additionally, in response to acute re-warming to 14.2°C, both day and nighttime $\dot{M}O_2$ significantly increased (day and nighttime $\dot{M}O_2$ increased by 238-300% and ~132-194%, respectively), relative to the first and second post-acclimation to ~2.5°C measurements (Bonferroni post-hoc, $p < 0.0001$).

Experiment 2: Acute Thermal Sensitivity of Group Routine $\dot{M}O_2$

For comparison to my analyses of metabolic rate where variation in activity was controlled for (see below), the thermal sensitivity in response to acute cooling was first calculated without taking into consideration variation in spontaneous activity. Q_{10}s was

calculated by exponentially relating temperature with all measurements of $\dot{M}O_2$ from all the tested fish for a given species. A strong significant effect of temperature on routine $\dot{M}O_2$ was observed in mummichog and pumpkinseed sunfish ($R^2 = 0.46$, df = 4644, $p < 0.0001$ and $R^2 = 0.57$, df = 4023, $p < 0.0001$, respectively). Using this approach, the thermal sensitivity of routine $\dot{M}O_2$ was associated with a Q_{10} of 3.17 for mummichog and a Q_{10} of 5.22 in pumpkinseed sunfish (Figure 4).

Experiment 2: Relationship between Spontaneous Activity and Metabolic Rate

Measurements of spontaneous activity and $\dot{M}O_2$ were related exponentially to obtain estimates of group and individual SMR (i.e., extrapolated zero-point activity measurements of $\dot{M}O_2$) at each experimental temperature. When assessing group SMR (i.e., plotting all measurements of spontaneous activity and $\dot{M}O_2$), strong significant relationships between spontaneous activity and $\dot{M}O_2$ at each experimental temperature were observed in both mummichog (Figure 5, Table 4) and pumpkinseed sunfish (Figure 6, Table 4). When assessing each fish's individual SMR, the majority of exponential relationships between spontaneous activity and $\dot{M}O_2$ yielded significant relationships ($p < 0.05$) while a few did not reach significance (individual relationships not shown). However, as mentioned in the methods, in instances where the exponential regression did not reach significance for a specific fish at a given temperature, these extrapolated SMR values were replaced with values calculated by the secondary methods of individual SMR calculation and in all instances changing these values had no significant effect on the overall average Q_{10} value.

Experiment 2. Thermal Sensitivity of Standard Metabolic Rate and Activity-Controlled Metabolic Rate

Mummichog SMR, calculated from the individual extrapolated zero-activity $\dot{M}O_2$ values, significantly decreased in response to acute cooling (SMR decreased ~74% on average from 14.9ºC to 2.5ºC) and remained unchanged following 4-6 weeks acclimation to ~2.5ºC (Figure 7, Panel A) (Bonferroni post-hoc, $p < 0.05$). Re-warming significantly increased SMR (SMR increased ~386% following re-warming) to a level similar to the initial SMR measurement at 14.9ºC. The thermal sensitivity of extrapolated individual SMR did not differ significantly among all acute cooled, acclimated, or acute re-warming temperature intervals and in all instances the average Q_{10} was less than 3.5 (Figure 7B). The same result was found when using SMR calculated from the 20 $\dot{M}O_2$ values associated with each individual's lowest 20 spontaneous activity measurements at each temperature (Table 6). This result was also consistent with the average Q_{10} values calculated using the individual $\dot{M}O_2$ values within an overlapping activity range (i.e., activity-controlled metabolic rate) at each temperature; however, due to differing levels of spontaneous activity between pre and post ~2.5ºC acclimated mummichog, this method could not be used to calculate the thermal sensitivity between ~15ºC acclimated and ~2.5ºC acclimated fish. It should also be noted that these values were similar to the group Q_{10} values (Q_{10} = 2.65 – 3.28) calculated from extrapolated SMR using all measurements of $\dot{M}O_2$ and spontaneous activity (Figure 5, Table 6), however, due to the singular nature of this calculation, statistical testing could not be applied to these values.

Conversely, the thermal sensitivity of SMR calculated from the individual average 20 lowest $\dot{M}O_2$ measurements significantly differed depending on the specific

temperature interval over which Q_{10} was calculated (Table 6). The thermal sensitivity of the individual average $\dot{M}O_2$ measurements also differed depending on the specific temperature interval over which Q_{10} was calculated. However, while Q_{10} may have been more variable using these methods of SMR determination, in both instances the majority of the Q_{10} values were less than 3.5. A marginal exception to this was the average Q_{10} in response to acute warming (acutely warmed from acclimated 2.6^{o}C to 15.1^{o}C) calculated from each individual fish's average lowest 20 $\dot{M}O_2$ measurements, where Q_{10} = 3.52 ± 0.18.

Pumpkinseed sunfish SMR, calculated from individual extrapolated zero-activity $\dot{M}O_2$, significantly decreased by ~46% in response to acute cooling from 14.2^{o}C to 2.6^{o}C (Figure 8A) (Bonferroni post-hoc, $p < 0.05$). Following 4-6 weeks acclimation to ~2.5^{o}C, SMR remained unchanged. Re-warming significantly increased SMR by ~3-fold . to a level similar to the initial SMR measurement at 14.2^{o}C (Bonferroni post-hoc, $p < 0.05$). The thermal sensitivity of pumpkinseed sunfish individual extrapolated SMR did not differ significantly among all acute cooled, acclimated, or acute re-warming temperature intervals and in all instances the average Q_{10} was less than 3.5 (Figure 8B, Table 7). The same result was found for Q_{10} calculated using the individual $\dot{M}O_2$ values within an overlapping activity range (Table 7); however, like mummichog, this method could not be used to calculate the thermal sensitivity between ~14^{o}C acclimated and ~2.5^{o}C acclimated fish due to differing levels of spontaneous activity between pre- and post ~2.5^{o}C acclimation fish. Also, like mummichog these values were similar to the group Q_{10} calculation (Q_{10} = 2.45 – 3.19) (Figure 6, Table 7); however statistical analysis could not be performed with these values.

The calculation of Q_{10} using approaches that did not directly control for the effect of variation in spontaneous activity on pumpkinseed sunfish $\dot{M}O_2$ (e.g. Q_{10} values calculated from each individual's average lowest 20 $\dot{M}O_2$ values at each temperature, or the $\dot{M}O_2$ values associated with each individual's lowest 20 spontaneous activity measurements at each temperature, or the using the mean $\dot{M}O_2$ of all fish at each temperature), were found to significantly differ depending on the specific temperature interval over which Q_{10} was calculated (Table 7). Additionally, when using these methods of SMR estimation that did not control for the effect of variation in spontaneous activity on $\dot{M}O_2$, the Q_{10} values in response to acute cooling were all greater than 3.5.

DISCUSSION

My findings support the hypothesis that inactivity, and not metabolic rate depression, is the key strategy underlying energetic savings in winter-dormant fishes. In all four of the putatively winter-dormant study species investigated (cunner, pumpkinseed sunfish, mummichog, and American eel), cooling caused reductions in spontaneous activity, accompanied by reductions in feeding. The threshold temperatures and magnitude of the reductions in activity differed among species, suggesting that winter dormant behaviourial responses are species-specific and that winter dormancy must be carefully defined. Regardless, at all temperatures and in both acute and acclimated exposures, spontaneous activity was strongly correlated with metabolic rate in the two study species (mummichog and pumpkinseed sunfish) in which it was investigated, as found previously in cunner (Speers-Roesch et al., 2018). Reductions in activity with temperature therefore were associated with reduced metabolic rate. The influence of

temperature-dependent variation in activity on metabolic rate was controlled for by estimating SMR from extrapolated zero-point activity of the activity-metabolic rate relationship or by calculating metabolic rates within a narrow overlapping activity range common to all temperatures ("activity-controlled metabolic rate"). The thermal sensitivity of extrapolated SMR or activity-controlled metabolic rate indicated passive physicochemical effects of cold alone ($Q_{10} < 3.5$), even after 4-6 weeks of cold acclimation. In other words, there was no evidence of a disproportionately large decrease in SMR that would be expected if MRD was involved. This discovery is consistent with Speers-Roesch *et al*.'s (2018) similar single-species analysis of cunner dormancy, and also with the few previous studies on winter-dormant fishes where consideration was given to the potential influence of activity levels on metabolic rate (Crawshaw, 1984; Crawshaw *et al*., 1982; Lemons and Crawshaw, 1985). Overall, there is now stronger evidence that energetic savings of winter dormancy in fishes result from inactivity and the passive physicochemical effects on metabolism, not MRD.

Species-Specificity of Winter Dormant Behaviour

To my knowledge, my study is the first to comprehensively quantify winter-dormant behaviour in a broad range of putatively winter-dormant fish species. My four study species have been previously reported to be winter-dormant (Roberts, 1964; Targett, 1978; Walsh *et al*., 1983; Sayer and Davenport, 1996; Speers-Roesch *et al.*, 2018), so I predicted that large reductions in activity and feeding, accompanied by increases in sheltering, would be observed in all species at temperatures below species-specific "winter dormancy thresholds". Winter dormancy threshold temperatures have

been inferred for several dormant species based on anecdotal observations (Walsh *et* al., 1983; Costa *et al*., 2013; Westerberg and Sjöberg, 2015; Speers-Roesch *et al*., 2018); however, winter dormancy threshold temperatures are rarely, if ever, directly measured. My findings from Experiment 1 show that the patterns of winter dormant behaviour vary among species, with the most pronounced dormant behaviour occurring in cunner and the least pronounced in mummichog.

Cunner Behavioural Changes in Response to Acute Cooling

When cooled to winter temperatures, cunner demonstrated a classic winter-dormant behavioural phenotype, including inactivity, sheltering, and fasting. Cunner became inactive at 7.3 ± 0.6^{o}C on average, and below 7.1^{o}C all individuals were virtually inactive, consistent with previous reports of winter inactivity in wild cunner (Green and Farwell, 1971, Bradbury and Green 1997) and laboratory cunner held at 1.0^{o}C (Speers-Roesch *et al.*, 2018).

The large decrease in activity with cooling corresponded with an increase in sheltering until at 2.9^{o}C and 1.8^{o}C all cunner spent 100% of their time hidden within their shelters. Cunner and other temperate wrasse species commonly engage in sheltering behaviour, often hiding in small rock crevices, especially at night (Green and Farwell, 1971; Sayer *et al*., 1994; Sayer and Davenport, 1996; Bradbury and Green, 1997; Arendt *et* al., 2001). While nighttime sheltering was observed in this study, cunner also spent over half of the daytime within their shelter even at the warmest temperatures. It is possible that the winter photoperiod used in my study (10L:14D) elicited more sheltering

than may normally be seen at 14.2^{o}C in summer, as wild cunner spend increasingly more time in shelter with decreasing photoperiod (Arendt *et* al., 2001).

Food consumption in cunner decreased with acute cooling and, on average, cunner ceased feeding at 7.5 ± 0.4^{o}C, with none feeding at 5.9^{o}C and below. Fasting in winter-dormant cunner has been previously reported but is rarely quantified (Green and Farwell, 1971; Bradbury and Green, 1997; Speers-Roesch *et al*., 2018). One recent study that quantified the feed consumption in cunner at cold temperatures found that cunner did not eat or fed at an extremely low level, which caused negative growth below approximately 9^{o}C (Watson and Rowsey, Unpublished). Surprisingly, some of the fish exhibited inactivity at relatively high temperatures yet continued to consume feed below their inactive temperature. Therefore, some spontaneous activity during the morning feeding period (~1 hr) was missed by my method of analysis; however, it is unlikely that there would have been substantial activity during this period in the absence of food, especially at colder temperatures.

The distinct winter dormant inactive and fasting threshold temperatures suggest that the cunner's dormancy threshold temperature is approximately 7^{o}C. Previous studies on seasonal responses of wild Newfoundland cunner suggested that the winter dormancy threshold temperature of cunner is 5^{o}C (Bradbury and Green, 1997; Green and Farwell, 1971). The cunner used in these experiments were from a cultured population originating from a Nova Scotian strain, so it is possible that the difference in the winter dormancy thresholds is a result of adaptive responses to differing thermal regimes experienced by northern and southern populations. Alternatively, the higher dormancy threshold I measured could stem from my use of an acute cooling exposure; perhaps, if the

temperature was cooled over a longer time period, similar to the previous seasonal studies of wild cunner, a lower threshold temperature would be observed.

Pumpkinseed Sunfish Behavioural Changes in Response to Acute Cooling

The behavioural response to acute cooling in pumpkinseed sunfish was broadly similar to that of cunner, although the magnitude of change was not as extreme. Pumpkinseed sunfish spontaneous activity decreased with declining temperature until, at 7.9^{o}C, a minimally active steady state was reached. Unlike cunner, however, pumpkinseed sunfish never reached inactivity. The response of pumpkinseed sunfish activity to cooling to winter temperatures is similar to that of other, closely related centrarchid species. Green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), and largemouth bass (*Micropterus salmoides*) showed marked reductions in spontaneous activity to very low levels after acclimation to temperatures below 7^{o}C (Lemons and Crawshaw, 1984; Tschantz *et al*., 2002). Many centrarchids appear to minimize activity at winter temperatures.

Like cunner, the reduction in activity with cooling coincided with an increase in sheltering. However, unlike cunner, pumpkinseed sunfish never spent the entirety of their time within their shelters at cold temperatures, which is consistent with the low levels of spontaneous activity measured at these same temperatures. Strangely, there was a slight decrease in sheltering observed at 3.0^{o}C and 2.2^{o}C in pumpkinseed sunfish. Little work has been done to quantify the overwintering sheltering or area selection in centrarchid fish species (Cooke and Philipp, 2009). Centrarchid overwintering habitat selection likely includes the movement to deeper, slow-moving waters (Cooke and Philipp, 2009), but

habitat selection can vary greatly among species and sometimes within species (Carlson, 1992; Cunjack, 1996; Lyons and Kanehl, 2002; Karchesky and Bennett, 2004; Cooke and Philipp, 2009). One available study investigating overwintering habitat selection on freshwater fishes in the Credit River system found pumpkinseed sunfish near woody debris, cobble-boulders, and aquatic vegetation (Cunjak, 1996), inferring they at least select habitat near shelter, which is consistent with our findings. Regardless, whether within shelter or adjacent to it, activity of pumpkinseed sunfish remains very low at winter temperatures.

Pumpkinseed sunfish feeding decreased in response to cooling. They consumed 100% of the ration until 7.9^{o}C, below which there was a marked decrease in food consumption to a very low level. Unlike cunner, some pumpkinseed sunfish never ceased feeding even at the coldest temperature, a result consistent with previous studies on centrarchid overwinter feeding. Among three closely related species of sunfishes living in the Mississippi River, all continued to feed during winter, albeit at a lower level (Snyder and Peterson, 1999; VanderKooy *et al*., 2000). Lemons and Crawshaw (1985) also noted similar feeding behaviour in largemouth bass in response to cold, noting that their food intake decreased significantly to a low level when exposed to temperatures below 10^{o}C. Additionally, decreased feeding and foraging behaviour has been reported for pumpkinseed sunfish at cooler temperatures, with the caveat that these studies failed to reach the cold temperatures of my study and as such could only report that decreases in feeding/foraging were observed when water temperatures dropped below 10^{o}C to 15^{o}C (Hathaway, 1927; Collins and Hinch, 1993).

These results are consistent with the existing literature which has shown that pumpkinseed sunfish and similar species of sunfish generally reduce activity in response to the cold but still maintain some activity and a low level of feeding (Hathaway, 1927; Collins and Hinch, 1993; Snyder and Peterson, 1999; VanderKooy *et al*., 2000; Tschantz *et al*., 2002). While cunner show distinct inactive and fasting threshold temperatures, in pumpkinseed sunfish these temperatures are less clear. Although pumpkinseed sunfish appear to reach a minimally active steady state that may be indicative of the onset of dormancy at 7.9^{o}C, the dormancy threshold is less clear for this species due to the fact that many of the pumpkinseed sunfish continued to feed at much lower temperatures. Therefore, the assignment of a dormancy threshold temperature for this species is challenging when considering the ambiguity surrounding their overwintering behaviour and may be unwarranted due to the lack of certain dormant characteristics (i.e., inactivity and fasting).

Mummichog Behavioural Changes in Response to Acute Cooling

Mummichog displayed significant reductions in spontaneous activity in response to acute cooling; however, unlike the other species tested, there was no clear cold steady state and the decrease in activity with cooling was not as marked as in cunner and pumpkinseed sunfish. Additionally, mummichog sheltering behaviour was relatively unaffected by cooling, also in contrast to the sheltering responses of cunner and pumpkinseed sunfish. Despite several studies investigating mummichog's overwintering behaviour, their behaviour remains ambiguous due to conflicting results. To date, studies have reported that mummichog overwintering behaviours include migrating offshore into

salt water bays, migrating to more saline portions of creeks, migrating to less saline waters, migrating to tidal salt marsh pools, not migrating at all, and overwinter burying (Chidester, 1920; Fritz *et al*., 1975; Smith and Able, 1994; Haplin, 1997; Raposa, 2003). Due to these conflicting results, Fritz *et al.* (1975) suggested that mummichog may be polytypic in their overwintering behaviour.

Similar to the other species, mummichog feeding decreased in response to cooling to 3.1^{o}C, at which temperature all fish had ceased feeding (mean fasting temperature = 4.4 ± 0.3^{o}C). Virtually nothing is known about how mummichog feed during the winter months. Chidester (1920) examined the gut contents of mummichog during the winter and found that, in fish that were active, the gut contents were largely algal matter. It is possible that the fasting observed at the coldest temperatures in this study results from the acute temperature stress and with acclimation the mummichog would begin to feed again. In fact, Reeve, Vrooman, and Speers-Roesch (unpublished results) showed that while mummichog feeding is significantly reduced when acclimated to low temperatures, they will still engage in a low level of feeding at 2-3^{o}C. While this may also be the case for cunner and American eel (see below), there have been several studies that suggest that cunner and American eel remain in a fasting state following acclimation (Nyman, 1972; Walsh, 1983; Rowsey, Watson and Speers-Roesch, unpublished results).

American Eel Behavioural Changes in Response to Acute Cooling

American eel demonstrated decreasing vigilance (see methods) with decreasing temperature, indicating a greater affinity for sheltering and reduced alertness at colder temperatures. In particular, a marked reduction in vigilance was observed at 3.4^{o}C and

2.5°C, where the majority of the eels were completely hidden within their shelters for the entirety of the day and nighttime periods. This decrease in vigilance is consistent with several other studies detailing American eel or similar anguillid species overwintering behaviour (Sinha and Jones, 1967; Nyman, 1972; Walsh *et al*., 1983; Westerberg and Sjöberg, 2015). Additionally, several studies have described the presence of burying behaviour in American eel, and similar anguillid species, during their dormant periods, in which they remain within their burrows for the duration of the winter (Walsh *et al*., 1983; Smith and Saunders, 1955; Tomie, 2013).

Interesting, Nyman (1972) used a similar method to assess behavioural changes in response to cooling in the European eel (*Anguilla anguilla*). Nyman recorded the number eels swimming, partly buried (head out of substrate), or buried at several temperatures in an acute cooling trial. Nyman found that with decreasing temperature there was increasing burying behaviour and that below 8°C all eels had buried. Nyman also noted fewer heads visible from their burrows with decreasing temperature and that no heads were visible below 6.4°C. Similarly, Walsh *et al*. (1983) reported that American eel buried into the mud at temperatures of <5°C. Riley *et al*. (2011) and Westerberg and Sjöberg (2015) indirectly measured dormant behaviour in the European eel using PIT tags and temperature and depth biologgers, respectively. Westerberg and Sjöberg (2015) reported that the onset of dormancy occurred at about 9.1°C but varied among individuals from 4.5°C to 12.4°C and Riley *et al*. (2011) reported that the European eel became inactive below 10°C. However, these studies quantified the onset of dormancy either by the absence of PIT tag detections or by the absence of depth changes; therefore, it is

likely that minimal movement at cold temperatures was missed which may explain why their reported dormant temperatures are relatively higher than mine.

The marked decrease in vigilance below 3.4^{o}C coincided with the onset of fasting, consistent with an onset of dormancy below this temperature. There is limited research on winter feeding in eels. Sinha and Jones (1967) examined the stomach contents of European eel in the winter and found that they generally did not eat, or if they did it was very little. Walsh *et al*. (1983) reported that American eels and European eels cease feeding below 5^{o}C and 8^{o}C, respectively (Nyman, 1972; Walsh et al 1983). Thus, the fasting temperature recorded in this study is lower; however, neither study directly measured feeding and, based on the result from Sinha and Jones (1967), it's possible that American eel may engage in some opportunistic feeding above ~3^{o}C.

Similar to cunner, American eel were found to reach a virtually inactive state (demonstrated by significantly low vigilance) at 3.4^{o}C. Also, like cunner upon reaching this temperature, all fish had ceased feeding, although some ceased feeding at warmer temperatures (mean fasting temperature = 5.5 ± 0.4^{o}C). Therefore, it appears that the winter dormancy threshold temperature in American eel is ~3-4^{o}C, though this sheltering/fasting behaviour has generally been noted at slightly warmer temperatures in previous studies (Nyman, 1972; Walsh *et al*., 1983; Riley *et al*., 2011; Westerberg and Sjöberg, 2015).

The Classification of Overwintering Strategies

My comprehensive analysis of dormant behaviour across a range of phylogenetically diverse species indicates that winter dormancy behaviour is species-

specific and occurs along a spectrum of varying magnitude. Additionally, while clear winter-dormant threshold temperatures occurred for some species, this was not clear in others. A reappraisal of how we define winter dormancy and other overwintering responses in fishes is warranted.

I propose that overwintering strategies be classified as either winter-active, winter-lethargic, or winter-dormant. Whereas winter activity and winter dormancy represent the active, foraging and inactive, fasting ends of the overwintering spectrum, respectively, winter lethargy represents an intermediate overwintering strategy. Where winter-activity is defined by relatively high level of activity, continued foraging, and the absence of sheltering at winter low temperatures, winter-lethargy is defined by a marked reduction in activity, a low level of opportunistic feeding, and the selection of an overwintering area rather than a specific shelter (e.g., a low flow area of a river), and winter-dormancy is defined by inactivity, fasting, and sheltering.

For example, Arctic charr (*Salvelinus alpinus*) and yellow perch (*Perca flavescens*) are good examples of winter-active species as they remain relatively active and continue to forage throughout the winter (Sullivan, 1986; Brännäs and Wiklund, 1992). Alternatively, the results from this study suggest that pumpkinseed sunfish and mummichog are examples of winter-lethargic species. Due to the maintenance of some feeding and a low level of activity at low temperatures, pumpkinseed sunfish appear to be winter-lethargic rather than winter-dormant fish. Additionally, mummichog activity remained at a higher level relative to the other species tested in the cold and, although fasting was observed at the coldest temperatures, a separate study showed that a low level of feeding during the cold is not uncommon (Reeve, Vrooman, and Speers-Roesch,

unpublished results). Thus, it appears that mummichog, like pumpkinseed sunfish, are a winter-lethargic species rather than a winter-dormant species. However, it should be noted that there is a larger scope of activity that fits within winter-lethargy, relative to winter-dormancy, and based on the results it appears that pumpkinseed sunfish showed a relatively greater "dormant" response than mummichog. Furthermore, the results from this study, and consistent with several other studies, suggest that cunner and American eel are characteristic winter-dormant species; both cunner and American eel entered an inactive state, sheltered, and fasted at winter low temperatures (Sinha and Jones, 1967; Green and Farwell, 1971; Nyman, 1972; Walsh, 1983; Sayer and Davenport, 1996; Bradbury and Green, 1997; Riley *et al*., 2011; Westerberg and Sjöberg, 2015; Speers-Roesch *et al*., 2018).

Therefore, it appears that while cunner and American eel show "classical" winter dormancy, pumpkinseed sunfish appear to use overwintering behaviour consistent with the definition of winter lethargy. Winter lethargy may also be a more apt description of the behaviours exhibited by many other temperate fish species, especially centrarchid species, which are often broadly described as winter-dormant despite an abundance of evidence that shows such species engage in low level feeding and activity during the winter months (Hathaway, 1927; Lemons and Crawshaw, 1985; Collins and Hinch, 1993; Snyder and Peterson, 1999; VanderKooy *et al*., 2000; Tschantz *et al*., 2002; Karchesky and Bennett, 2004; Cooke and Philipp, 2009). Thus, the description of dormancy must be reassessed in some winter dormant species.

Thermal Sensitivity of SMR and Activity-Controlled Metabolic Rate: No Evidence of MRD in Mummichog or Pumpkinseed Sunfish

My primary finding was that the low metabolic rates of pumpkinseed sunfish and mummichog at winter temperatures did not result from MRD but rather from reduced activity in combination with the physicochemical effects of cooling on their underlying metabolism. When SMR was estimated by extrapolating metabolic rate to zero activity (Korsmeyer *et al.*, 2002), or when the influence of variation in activity on $\dot{M}O_2$ was controlled for by using the average $\dot{M}O_2$ over a narrow overlapping range of spontaneous activity, the thermal sensitivity (Q_{10}) of metabolic rate in response to cooling for pumpkinseed sunfish (Q_{10} = 2.67 – 3.27) and mummichog (Q_{10} = 2.55 – 3.17) indicated typical passive thermal metabolic response to cold. (Tables 6 and 7, respectively). Furthermore, the metabolic rates of pumpkinseed sunfish and mummichog remained unchanged after several weeks of acclimation at winter low temperature, arguing against the possibility of a delayed onset of MRD as seen in certain estivating amphibians (Hillman *et al*., 2009). The absence of cold compensation of SMR in mummichog is also consistent with Healy *et al*. (2017) and has also been reported for winter-dormant cunner (Speers-Roesch *et al*., 2018). Fish that minimize activity in winter may not benefit from thermal compensation of SMR, which is reported in more active species (Peterson and Anderson, 1969; Evans, 1990). It is likely that previous reports of high thermal sensitivity of metabolic rate in pumpkinseed sunfish (Q_{10} = 6.0; Roberts, 1964) and mummichog (Q_{10} = 4.42; Targett, 1978) in response to cooling to winter temperatures were confounded by variation in activity.

Crawshaw and his colleagues were the first to suggest that quiescence and temperature-related decreases in metabolic rate may explain the low metabolic rates in winter dormant fish species (Crawshaw *et al*., 1982; Crawshaw, 1984; Lemons and Crawshaw, 1985); however, they were cautious in concluding that inactivity was the primary contributor to the low metabolic rates, as they did not investigate the relationship between these variables (Crawshaw, 1984). Their research on the thermal sensitivity of brown bullhead and largemouth bass metabolic rate in response to cooling to winter temperatures demonstrated typical passive effects of cooling on both species' metabolism (i.e. Q_{10} = 2-3), and, thus they concluded that MRD was not involved during these species' dormant periods. These results have been largely misinterpreted as evidence of species-specific responses due to an abundance of contrasting studies implying the involvement of MRD during other species dormancy (i.e. $Q_{10} > 3.5$).

Speers-Roesch *et al.* (2018) corroborated Crawshaw's early work in a recent study on winter-dormant cunner and were the first to quantify the primary role of inactivity in driving winter low metabolic rates. Speers-Roesch *et al.* (2018) found that, when variation in activity was controlled for, the cold-induced metabolic decrement, previously thought to arise from a MRD (Costa *et al*., 2013), instead resulted from inactivity in the cold and the physicochemical effects of cooling. My findings extend this conclusion to two additional putatively winter-dormant species, the pumpkinseed sunfish and mummichog. Taken together, and considering the diverse phylogenetic range of fishes examined, the results of Crawshaw and colleagues (Crawshaw *et al*., 1982; Lemons and Crawshaw, 1985), Speers-Roesch *et al.* (2018), and my present study reinforce the conclusion that MRD is not involved in winter dormant fishes. Rather, these

studies provide strong support for my hypothesis that inactivity or major reductions in activity, combined with passive physicochemical cooling effects, is the key strategy underlying the low metabolic rates of winter-dormant fishes.

Compared with MRD, inactivity may be an optimal energy savings strategy for ectotherms in cold environments. Essentially, behavioural suppression of activity is a simple response that exploits the passive cooling effects on metabolism, obviating the evolution of more complex active downregulation of metabolism via MRD. Activity is a highly flexible trait; alterations in activity can happen more quickly and with fewer physiological modifications than MRD. Thus, activity modulation may allow species to more easily exit their dormant state under stressful conditions (i.e., predation, hypoxia, etc.) or to exploit temporarily favourable environmental conditions (i.e., opportunistic foraging). Indeed, the reductions in activity exhibited during dormancy have been proposed to be facultative rather than obligate in a number of dormant fish species (Kolok, 1991; Cooke and Philipp, 2009; Hasler, 2009). Thus, lethargic or dormant fish species may simply prioritize reducing activity in the cold in order to minimize energy expenditure. This prioritization may form the basis of a consistent adaptive response within species, for example in cunner where a striking dormancy is observed. On the other hand, lethargic species in particular may show more flexibility in their overwintering behaviour depending on prey assemblages, temperature, energy reserves, and the presence of predators (Micucci *et al*., 2003; Garvey *et al*., 2004). For example, Karchesky and Bennett (2004) showed individual variation in overwintering activity among largemouth bass: some remained inactive within an overwintering area, while others migrated freely between overwintering areas. Additionally, they also noted that

some largemouth bass continued to move within overwintering areas during their dormant period. The inherent flexibility of activity level makes it a particularly useful strategy to conserve energy in dynamic overwintering environments.

Activity-related Caveats for SMR Estimation

Q_{10} values were only elevated when comparing metabolic rates where variation in activity was not controlled for (e.g. Figure 4), in particular in pumpkinseed sunfish. Essentially, higher activity at warmer temperatures and lower activity at cold temperatures caused higher and lower metabolic rates to be recorded, respectively, inflating the difference between metabolic rates at the two temperatures. The influence of activity on metabolic rate and estimates of SMR should not be underestimated and should always be considered in studies on metabolic rate of fishes. Many researchers use an average lowest subset of $\dot{M}O_2$ measurements to estimate SMR when activity is not directly measured (Chabot *et al*., 2016). A general assumption is that these low $\dot{M}O_2$ values represent periods of inactivity and that variation among them represents variation in SMR, with the possibility of potentially underestimating SMR if too small a subset is used (Chabot *et al*., 2016). My results, however, suggest a caveat to this approach. At warmer temperatures, fishes were rarely inactive for the duration of the metabolic rate measurements; for example, the average of the lowest 20 spontaneous activity measurements for pumpkinseed sunfish and mummichog at 14-15C was 1.62 ± 0.42 BL min^{-1} and 0.89 ± 0.86 BL min^{-1}, respectively. Thus, at warmer temperatures the lowest $\dot{M}O_2$ measurements may not actually reflect periods of inactivity and, contrary to previous assumptions, may be overestimating SMR.

Due to this type of SMR overestimation, high Q_{10} values ($Q_{10} > 3.5$) were observed in pumpkinseed sunfish when comparing estimates of SMR that did not truly equalize variation in activity (i.e., not $\dot{M}O_2$ extrapolated to zero activity or active-controlled metabolic rate). Mummichog only showed a $Q_{10} > 3.5$ when comparing SMR estimates from the average lowest 20 $\dot{M}O_2$ measurements during acute rewarming. This difference between species may exist due to differing effects of temperature on each species' spontaneous activity. Pumpkinseed sunfish spontaneous activity changed more than mummichog in response to cooling. This is especially evident when looking at the average individual lowest 20 spontaneous activity measurements in each species, where pumpkinseed sunfish saw a 58% decrease and mummichog only saw a 9% decrease in their average individual lowest 20 spontaneous activity measurements in response to cooling. Thus, it appears that while using the lowest subset of spontaneous activity or $\dot{M}O_2$ measurements may have been appropriate for the accurate calculation of Q_{10} for mummichog, this method was inappropriate for pumpkinseed sunfish due to the larger change in spontaneous activity with cooling. Therefore, recording simultaneous measurements of $\dot{M}O_2$ and activity is a robust method of ensuring an accurate estimate of SMR has been reached, and although this method is onerous, the benefits likely outweigh the costs.

Correlating metabolic rate with simultaneous measurements of spontaneous activity is challenging, and consequently my study is one of few that have directly investigated this relationship in fishes (Lucas and Priede, 1992; Zimmerman and Kunzman, 2001; Tudorache *et al*., 2009; Steinhausen *et al*., 2010; Speers-Roesch *et al*., 2018), despite the general understanding that voluntary movements influence

measurements of metabolic rate (Nilsson *et al*., 1993; Chabot *et al*., 2016). My study, and Speers-Roesch *et al*. (2018), have highlighted the importance of measuring variation in activity, especially for the accurate determination of SMR. Accurate estimates of the relationship between activity and metabolic rate, and how they are influenced by temperature, are needed to inform physiological and ecological theories that rely on the assumption of predictable universal thermal effects on metabolism among animals, which are often broadly applied to model and predict the impacts of climate change (van der Meer, 2006; Dillon *et al*., 2010; Pörtner, 2010; Clarke, 2017).

Conclusions

My salient discovery was that the thermal sensitivity of SMR or activity-controlled metabolic rates during acute cooling and long-term acclimation to winter low temperature in both pumpkinseed sunfish and mummichog indicated the influence of physicochemical effects of cooling alone. There was no evidence of an active, disproportionately large decrease in SMR that would be expected if metabolic rate depression was involved. Rather, cold-induced reductions in activity lower metabolic rate close to SMR, which combined with passive cooling effects, allow for maintenance of a low energy expenditure in winter. This result is consistent with the previous work by Crawshaw and colleagues (Crawshaw *et al*., 1982; Crawshaw, 1984; Lemons and Crawshaw, 1985) on largemouth bass and brown bullhead and Speers-Roesch *et al*. (2018) on cunner. These previous studies are the only others to consider the influence of activity on winter metabolic rates in winter-dormant fishes, and in all cases, MRD is absent and only passive effects of cold are apparent.

Thus, it appears that inactivity is the convergent energy savings strategy underlying the metabolic phenotype in winter-dormant fish species. The energetic savings of dormancy result from inactivity and the passive physicochemical effects on metabolism. It is likely that previous studies reporting high thermal sensitivity ($Q_{10} > 3.5$) of metabolic rate in winter-dormant fishes are confounded by the unaccounted influence of variation of activity levels on their estimates of SMR. My findings underscore the importance of considering the influence of activity on metabolic rate and estimates of SMR. Finally, my comprehensive analysis of the behavioural responses to cooling in a diverse range of putatively winter-dormant fish species reveals that the involvement and magnitude of characteristic winter-dormant behaviours is species-specific, and that winter dormancy must be carefully defined.

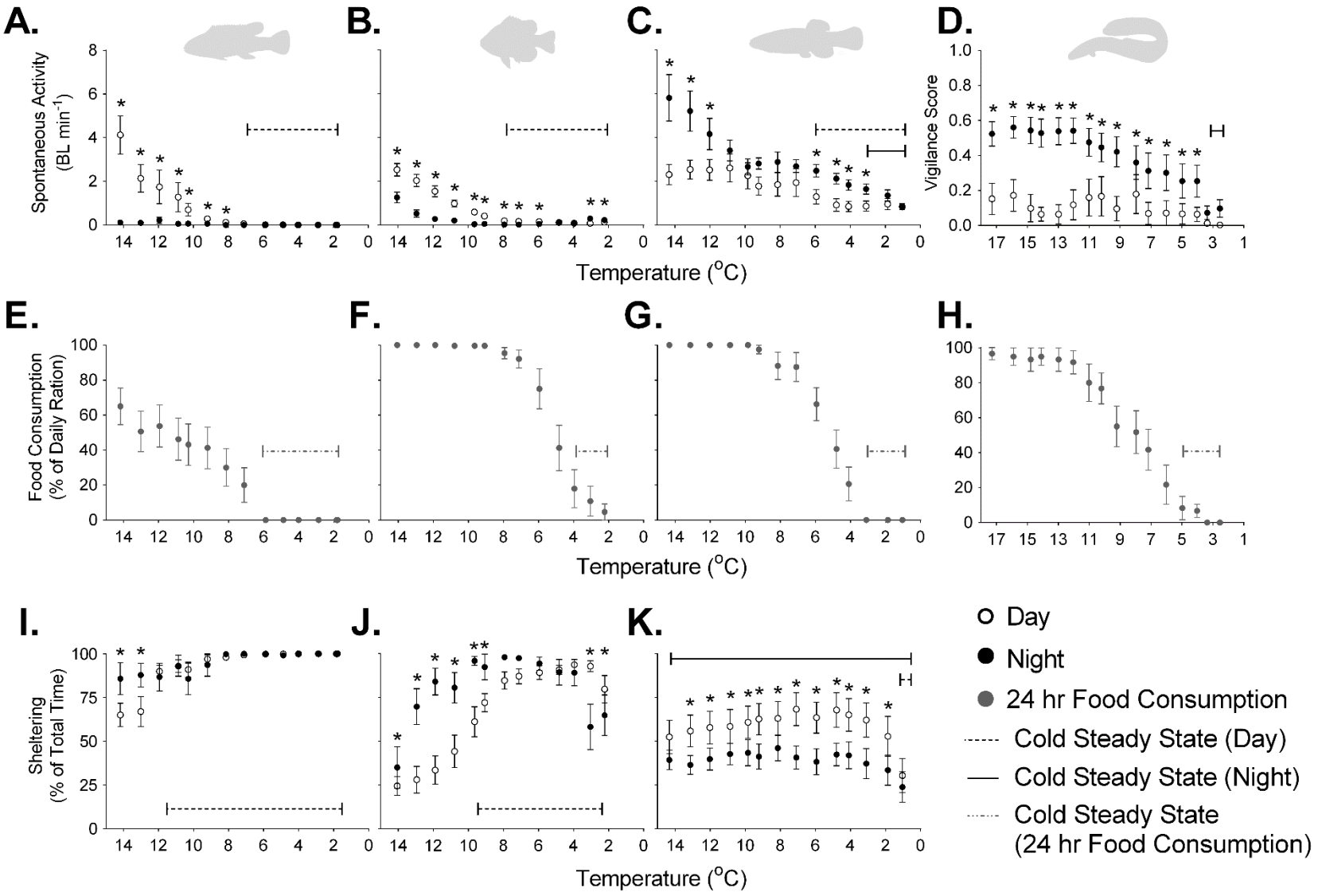
A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
Spontaneous Activity (BL min-1)
Vigilance Score
Food Consumption (% of Daily Ration)
Sheltering (% of Total Time)
Temperature (°C)
Day
Night
24 hr Food Consumption
Cold Steady State (Day)
Cold Steady State (Night)
Cold Steady State (24 hr Food Consumption)

Figure 1 (previous page). Effects of acute cooling (1^{o}C/day) on activity-related behaviors and food consumption in four species of putatively winter-dormant fishes (Experiment 1) (Species data panels arranged vertically: cunner, n = 16, panels A, E, I; pumpkinseed sunfish, n = 12, panels B, F, J; mummichog, n = 16, panels C, G, K; American eel, n = 12, panels D, H). Data are means ± S.E.M. for spontaneous activity (panels A-C), vigilance (panel D), food consumption (panels E-H), and sheltering (panels I-K), open circles represent day-time measurements and closed circles represent night-time measurements. Food consumption was measured once daily as the percentage of the daily ration (~0.5%BW) consumed (gray closed circles, panels E – H). Spontaneous activity represents the average velocity in body lengths per minute (BL min^{-1}) of the fish over the full measurement period at each temperature. Day and night cold steady states (represented by dashed and solid lines, respectively) indicate the range of temperatures where behavior or feeding was not significantly different from the measurement at the coldest temperature within the same day or night-time period. Where panels only contain one cold steady state line, it is because there was only one clear and consistent steady state in either day or night. * indicates a significant difference between day and night-time values at a given temperature. American eel spontaneous activity could not be measured using the same methodology applied to the other species; instead, vigilance (see methods) was scored in response to cooling (panel D). Generalized linear mixed-effects models and Bonferroni post-hoc multiple comparisons tests were used to identify significant differences ($p<0.05$) for all behavioural measurements (see Table 5).

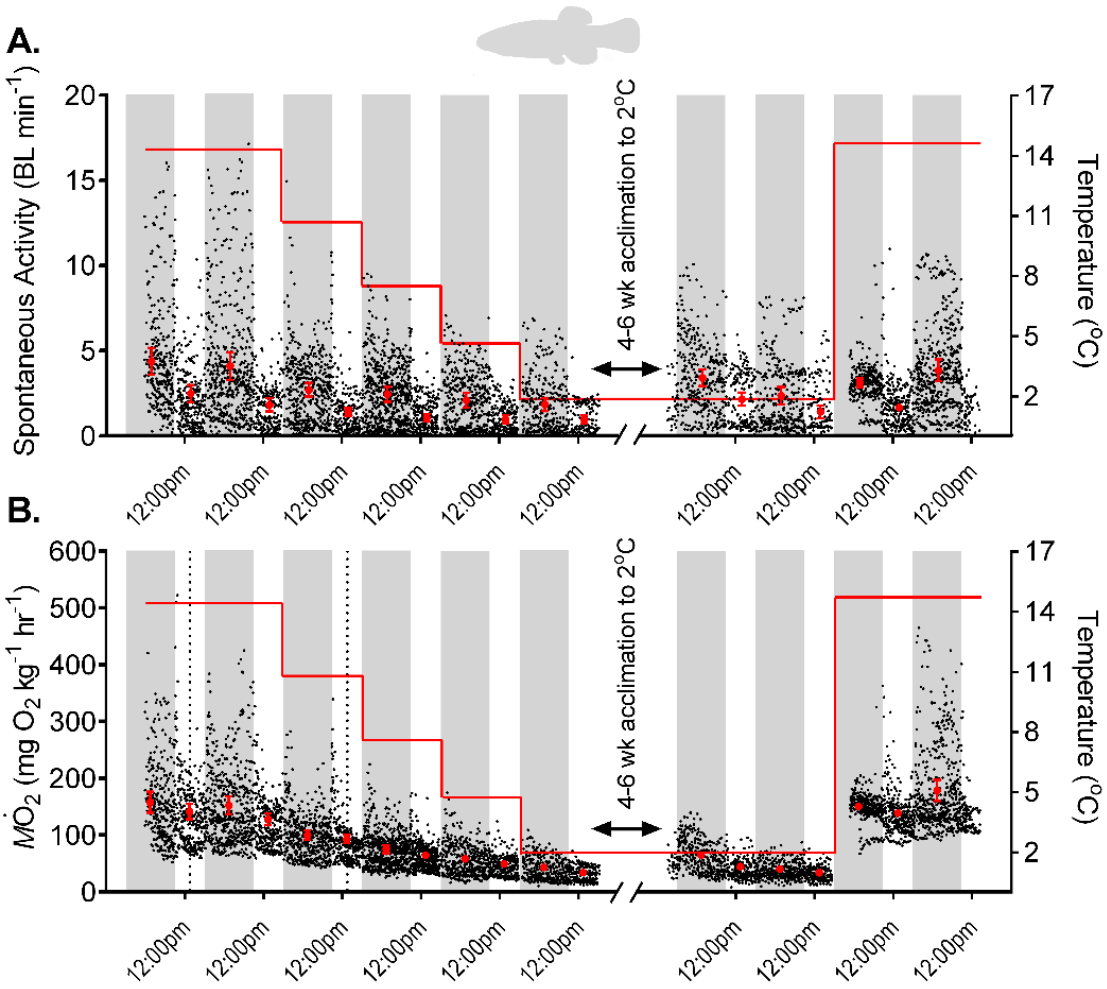

Figure 2. The diel cycle of spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) measured simultaneously in mummichog (n = 12) during acute cooling (~3ºC day^{-1}) and following 4-6 weeks acclimation to 2ºC and acute rewarming (warmed ~14ºC overnight) (Experiment 2). The closed black circles are the spontaneous activity (A) and $\dot{M}O_2$ (B) values for all fish during each measurement interval. The red closed circles are the mean ± S.E.M. values for each day and night-time period (represented by white and black bars, respectively) at each temperature. The red line represents the experimental temperature regime. Generalized linear mixed-effects models and Type II Wald chi-

square tests were used to assess where significant affects occurred. Additionally, Bonferroni post-hoc multiple comparisons tests were used to identify significant differences ($p<0.05$).Spontaneous activity and $\dot{M}O_2$ were significantly affected by temperature ($\chi^2 = 103.806$, df = 9, $p<0.0001$; $\chi^2 = 989.295$, df = 9, $p<0.0001$, respectively), diel cycle ($\chi^2 = 82.071$, df = 1, $p<0.0001$; $\chi^2 = 19.370$ df = 1, $p<0.0001$, respectively), and their interaction ($\chi^2 = 54.916$, df = 8, $p<0.0001$; $\chi^2 = 24.747$, df = 8, $p<0.0017$, respectively).

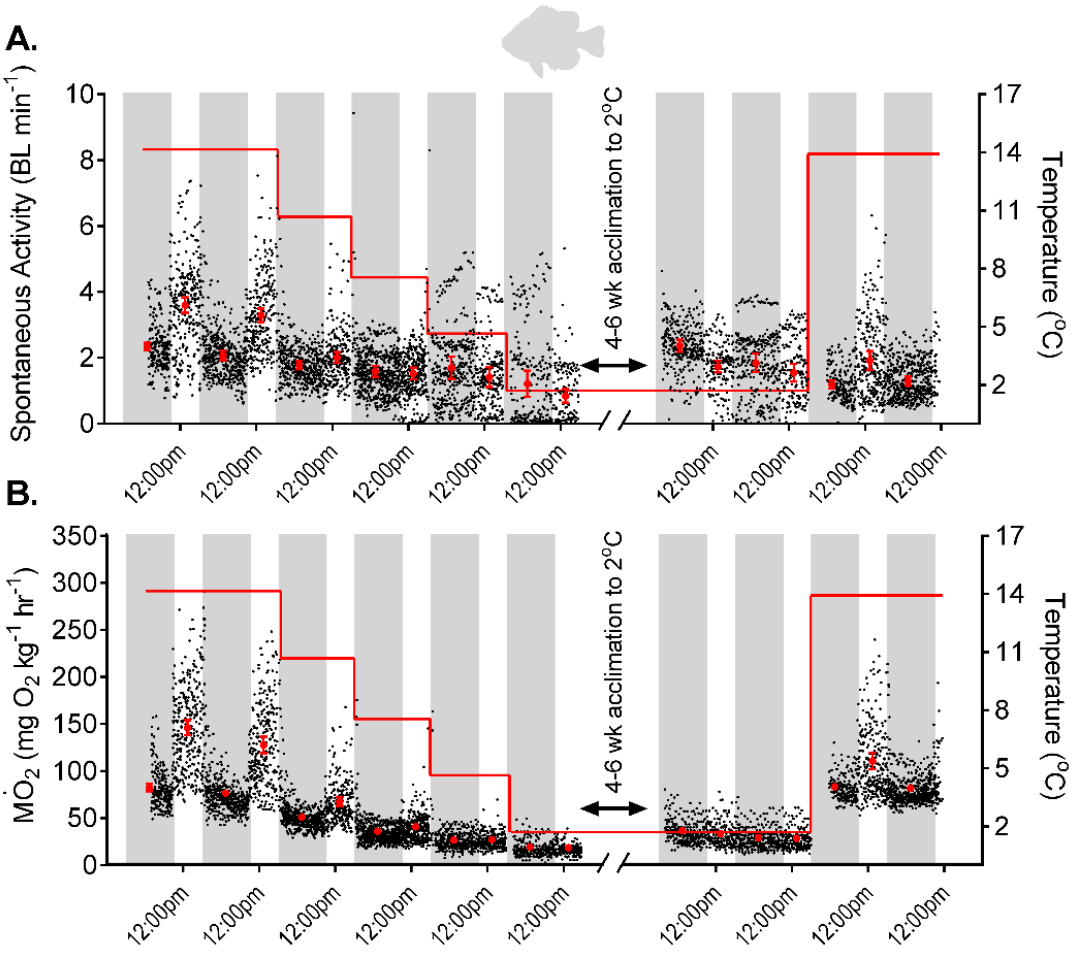

Figure 3. The diel cycle of spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) measured simultaneously in pumpkinseed sunfish (n = 11) during acute cooling (~3°C day^{-1}) and following 4-6 weeks acclimation to 2°C and acute rewarming (warmed ~14°C overnight) (Experiment 2). The closed black circles are the spontaneous activity (A) and $\dot{M}O_2$ (B) values for all fish during each measurement interval. The red closed circles are the mean ± S.E.M. values for each day and night-time period (represented by white and black bars, respectively) at each temperature. The red line represents the experimental temperature regime. Generalized linear mixed-effects models

and Type II Wald chi-square tests were used to assess where significant affects occurred. Additionally, Bonferroni post-hoc multiple comparisons tests were used to identify significant differences ($p<0.05$). Spontaneous activity and $\dot{M}O_2$ were significantly affected by temperature ($\chi^2 = 311.3361$, df = 9, $p<0.0001$; $\chi^2 = 1308.554$, df = 9, $p<0.0001$, respectively) and their interaction ($\chi^2 = 95.6660$, df = 8, $p<0.0001$; $\chi^2 = 17.619$, df = 8, $p<0.0243$, respectively); however, only $\dot{M}O_2$ was significantly affected by diel cycle ($\chi^2 = 77.686$, df = 1 $p<0.0001$).

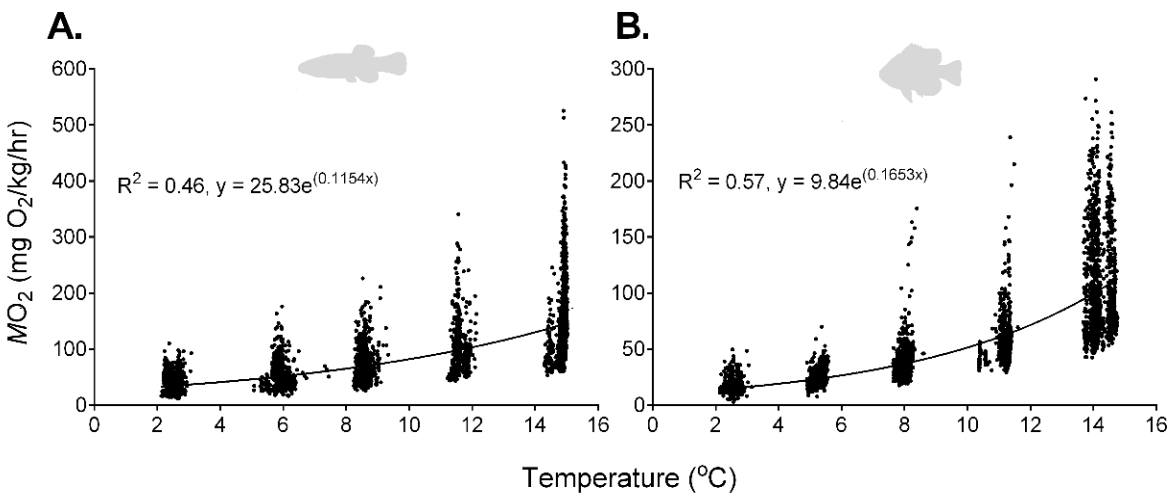

Figure 4. The effect of acute cooling on metabolic rate (oxygen consumption rate, $\dot{M}O_2$) in all measurement intervals (i.e., routine metabolic rate) in mummichogs (A) (n = 12) and pumpkinseed sunfish (B) (n = 11) (Experiment 2). The data were fitted with an exponential regression and the slope of the line was used to calculate a single population-level Q_{10} value across all exposure temperatures without controlling for variation in spontaneous activity ($Q_{10} = e^{(0.1154 \cdot 10)} = 3.17$ and $Q_{10} = e^{(0.1653 \cdot 10)} = 5.22$, for mummichog and pumpkinseed sunfish, respectively).

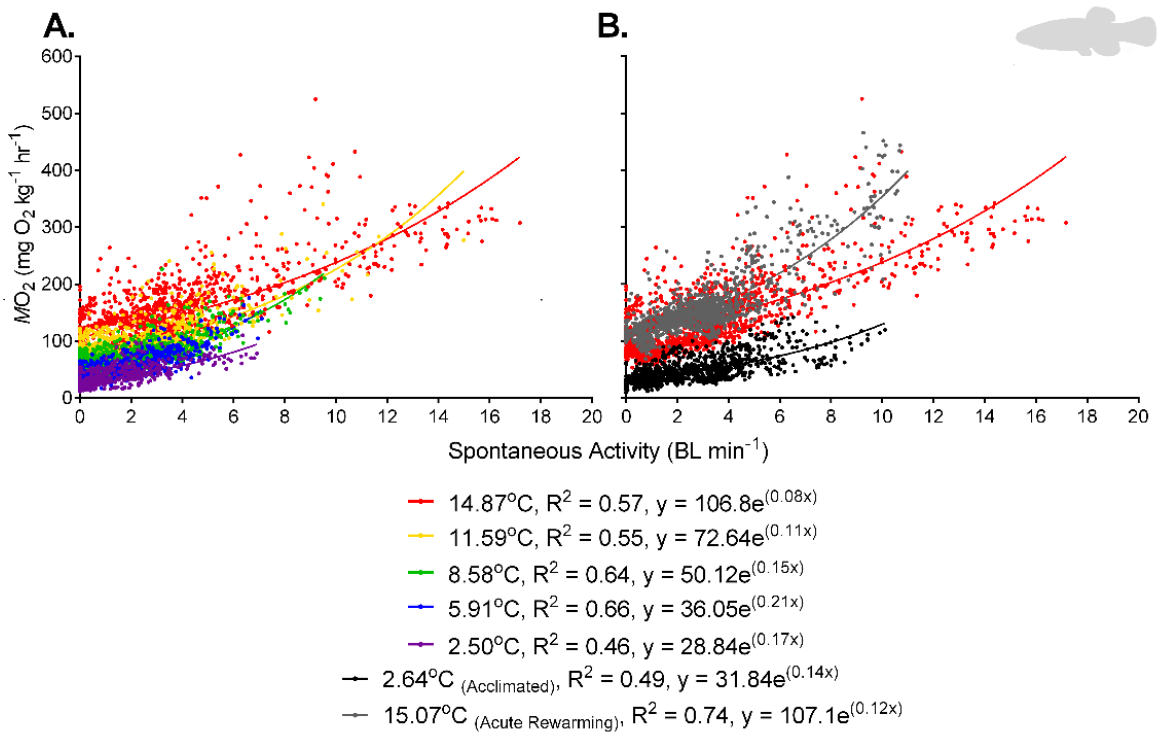

Figure 5. The relationship between spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) for all mummichogs (n = 12) at all measurement intervals within each experimental temperature in response to acute cooling (Panel A) and 4-6 weeks acclimation to ~2.64ºC followed by acute rewarming to ~15.07ºC (Panel B) (Experiment 2). The statistical output from these relationships and average temperatures ± standard deviation are reported in Table 4. Within each temperature, the relationship between spontaneous activity and $\dot{M}O_2$ was modeled exponentially using the following formula in order to calculate SMR: $y = ae^{(bU)}$, where a is SMR (extrapolated $\dot{M}O_2$ at zero activity), b is the slope, and U is spontaneous activity. The relationships shown here were modeled using all measurements for all fish within each temperature (enabling calculation of a single population-level SMR at each temperature); I also modeled the relationship within individuals to determine mean SMR (see Figure 7).

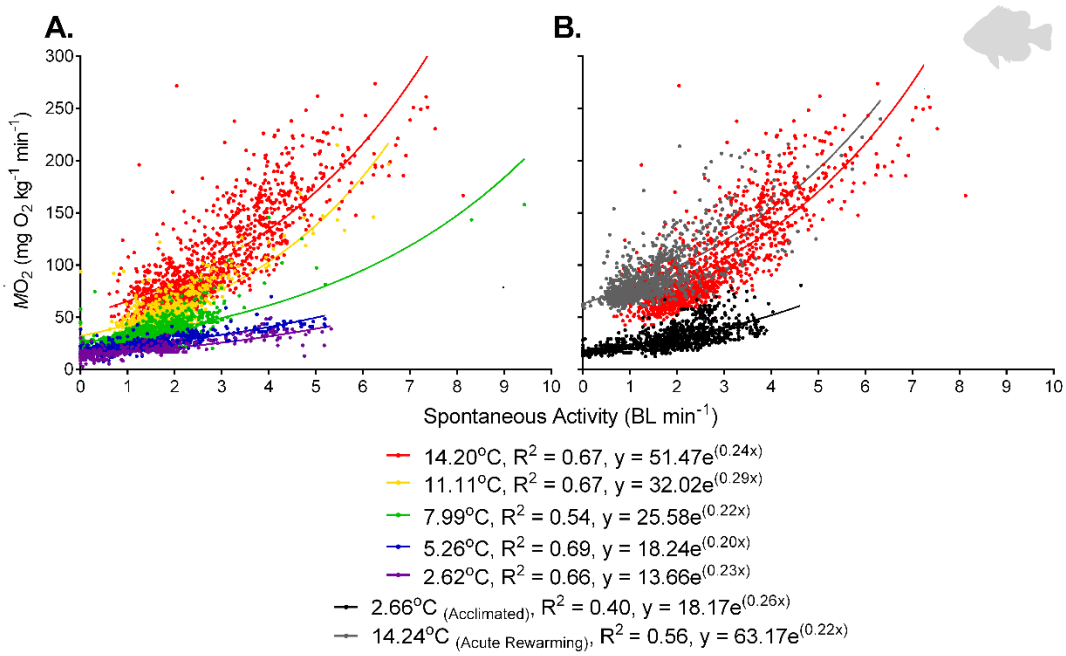

Figure 6. The relationship between spontaneous activity and metabolic rate (oxygen consumption rate, $\dot{M}O_2$) for all pumpkinseed sunfish (n = 11) at all measurement intervals within each experimental temperature in response to acute cooling (Panel A) and 4-6 weeks acclimation to ~2.66°C followed by acute rewarming to ~14.24°C (Panel B) (Experiment 2). The statistical output from these relationships and average temperatures ± standard deviation are reported in Table 4.Within each temperature, the relationship between spontaneous activity and $\dot{M}O_2$ was modeled exponentially using the following formula in order to calculate SMR: $y = ae^{(bU)}$, where *a* is SMR (extrapolated $\dot{M}O_2$ at zero activity), *b* is the slope, and *U* is spontaneous activity. The relationships shown here were modeled using all measurements for all fish within each temperature (enabling calculation of a single population-level SMR at each temperature); I also modeled the relationship within individuals to determine mean SMR (see Figure 8).

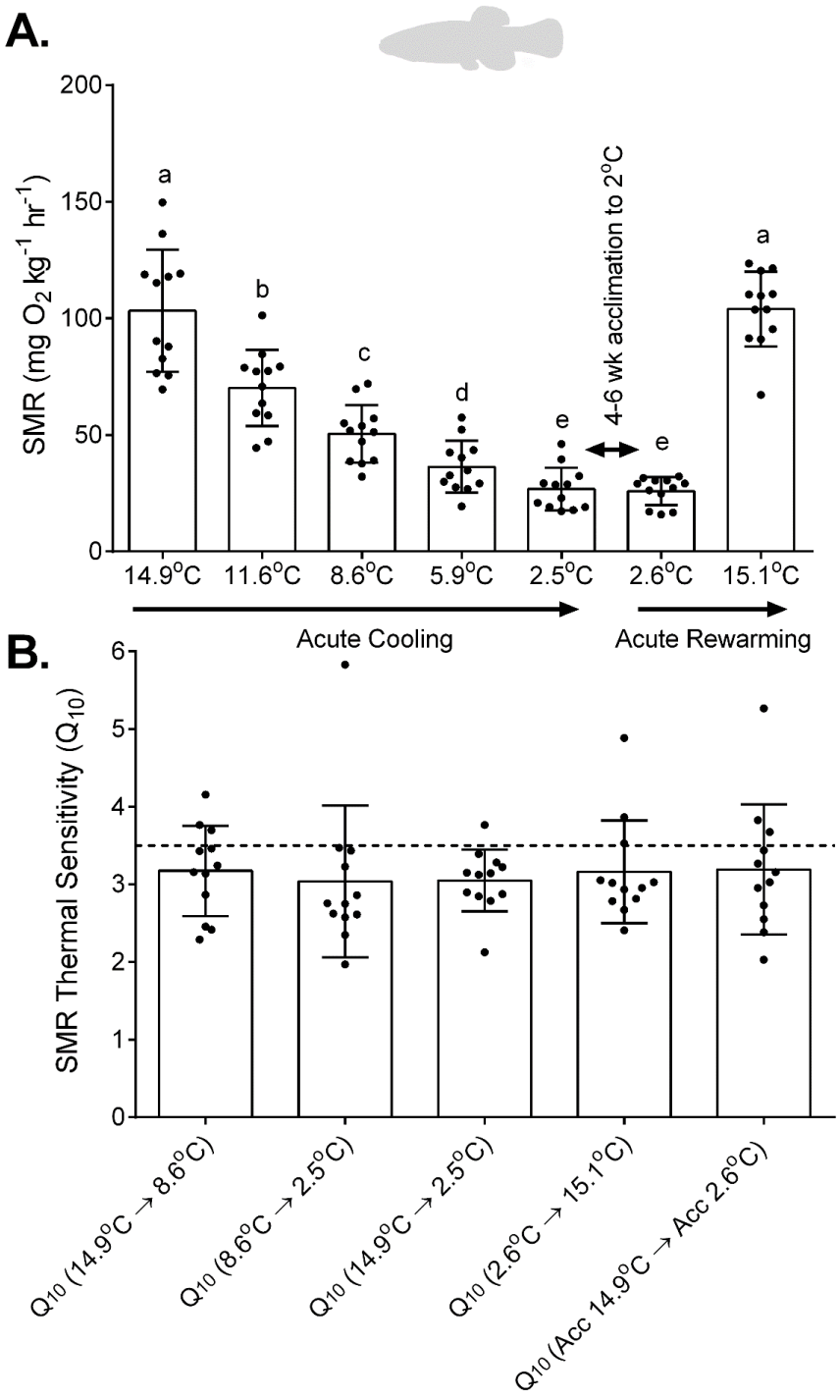
A.
SMR (mg O_2 kg^{-1} hr^{-1})
200
150
100
50
0
a
b
c
d
e
4-6 wk acclimation to 2°C
e
a
14.9°C
11.6°C
8.6°C
5.9°C
2.5°C
2.6°C
15.1°C
Acute Cooling
Acute Rewarming
B.
SMR Thermal Sensitivity (Q_{10})
6
5
4
3
2
1
0
Q_{10} (14.9°C → 8.6°C)
Q_{10} (8.6°C → 2.5°C)
Q_{10} (14.9°C → 2.5°C)
Q_{10} (2.6°C → 15.1°C)
Q_{10} (Acc 14.9°C → Acc 2.6°C)

Figure 7 (previous page). A) Standard metabolic rate (SMR) of mummichog during acute cooling from ~15ºC to ~2.5ºC and after 4-6 weeks acclimation to ~2.5ºC followed by acute re-warming to ~15ºC. SMR were calculated by fitting exponential regressions to the data for spontaneous activity vs. metabolic rate in individual fish and calculating extrapolated $\dot{M}O_2$ at zero activity (Experiment 2). The overlying black points represent the individual SMR values calculated for each fish. B) Thermal sensitivity of SMR in mummichogs during acute cooling (14.9ºC to 8.6ºC and 8.6 to 2.5ºC), acute re-warming of ~2.5ºC acclimated fish (2.6ºC to 15.0ºC), and for ~15ºC vs. ~2.5ºC acclimated fish. Q_{10} values were calculated for each individual fish using the individual's SMR values shown in Panel A. The dotted horizontal line represents my defined Q_{10} threshold indicating metabolic rate depression (i.e., $Q_{10} > 3.5$). Letters denote significant differences between groups identified using linear mixed effects models and Bonferroni post-hoc multiple comparisons tests ($p<0.05$). Data are means ± S.E.M., n=12.

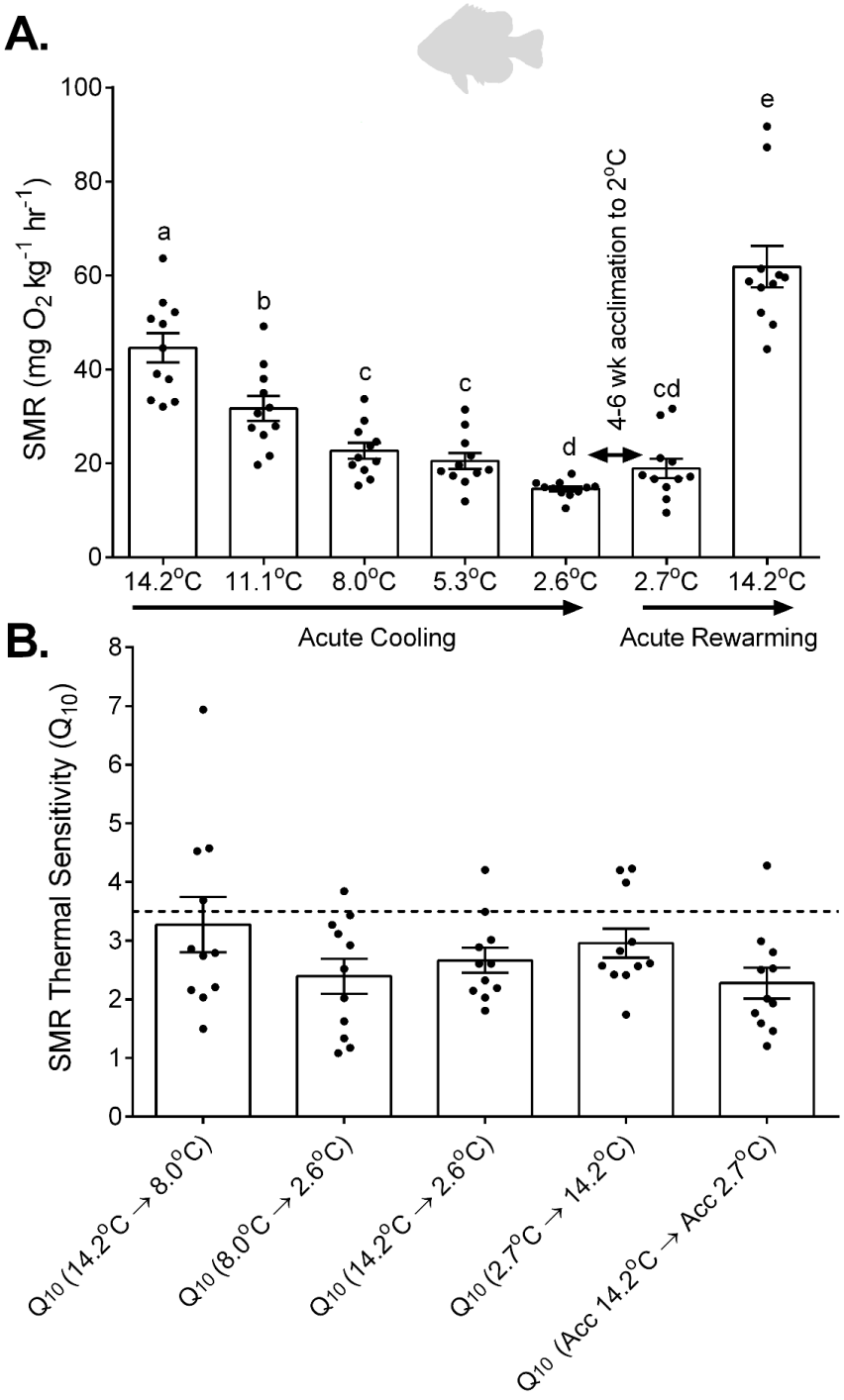
A.
SMR (mg O_2 kg^{-1} hr^{-1})
100
80
60
40
20
0
a
b
c
c
d
cd
e
4-6 wk acclimation to 2°C
14.2°C
11.1°C
8.0°C
5.3°C
2.6°C
2.7°C
14.2°C
Acute Cooling
Acute Rewarming
B.
SMR Thermal Sensitivity (Q_{10})
8
7
6
5
4
3
2
1
0
Q_{10} (14.2°C → 8.0°C)
Q_{10} (8.0°C → 2.6°C)
Q_{10} (14.2°C → 2.6°C)
Q_{10} (2.7°C → 14.2°C)
Q_{10} (Acc 14.2°C → Acc 2.7°C)

Figure 8 (previous page). A) Standard metabolic rate (SMR) of pumpkinseed sunfish during acute cooling from ~14ºC to ~2.5ºC and after 4-6 weeks acclimation to ~2.5ºC followed by acute re-warming to ~14ºC. SMR were calculated by fitting exponential regressions to the data for spontaneous activity vs. metabolic rate in individual fish and calculating extrapolated $\dot{M}O_2$ at zero activity (Experiment 2). The overlying black points represent the individual SMR values calculated for each fish. B) Thermal sensitivity of SMR in mummichogs during acute cooling (14.2ºC to 8.0ºC and 8.0 to 2.6ºC), acute re-warming of ~2.5ºC acclimated fish (2.7ºC to 14.2ºC), and for ~14ºC vs. ~2.5ºC acclimated fish. Q_{10} values were calculated for each individual fish using the individual's SMR values shown in Panel A. The dotted horizontal line represents my defined Q_{10} threshold indicating metabolic rate depression (i.e., $Q_{10} > 3.5$). Letters denote significant differences between groups identified using linear mixed effects models and Bonferroni post-hoc multiple comparisons tests ($p<0.05$). Data are means ± S.E.M., n=11.

Table 1. Length and weight of study species and dimensions of the behavioural arenas and shelters used for behavioural assessments (Experiment 1).

Species	Total Length (cm)	Weight (g)	Arena Dimensions (cm)	Shelter Size (Length x internal diameter)
Cunner	6.4 ± 0.1	5.1 ± 0.3	20.2 x 15.6 x 9.7	8 cm x 1.9cm PVC
Pumpkinseed Sunfish	11.8 ± 0.2	30.0 ± 0.7	38.3 x 24.1 x 14.1	15 cm x 1.9cm PVC
Mummichog	6.6 ± 0.2	5.7 ± 0.4	20.2 x 15.6 x 9.7	8 cm x 1.9cm PVC
American Eel	9.8 ± 0.2	1.1 ± 0.1	20.2 x 15.6 x 9.7	10 cm x 1.3cm PVC

Cunner, n = 16; pumpkinseed sunfish, n = 12; mummichog, n = 16; American eel, n = 12. Data are presented in means ± S.E.M.

Table 2. The average daily temperatures recorded during each species' acute cooling trial in Experiment 1. Data are presented as means ± S.D.

Duration (Days)	Cunner	Pumpkinseed Sunfish	Mummichog	American Eel
1	14.18 ± 0.19ºC	14.06 ± 0.20ºC	14.32 ± 0.21ºC	17.29 ± 0.17ºC
2	13.01 ± 0.23ºC	12.94 ± 0.19ºC	13.13 ± 0.16ºC	15.90 ± 0.18ºC
3	11.94 ± 0.14ºC	11.89 ± 0.23ºC	12.02 ± 0.16ºC	14.78 ± 0.27ºC
4	10.87 ± 0.12ºC	10.78 ± 0.12ºC	10.86 ± 0.12ºC	14.10 ± 0.20ºC
5	10.30 ± 0.12ºC	9.64 ± 0.20ºC	9.84 ± 0.20ºC	12.98 ± 0.16ºC
6	9.21 ± 0.16ºC	9.06 ± 0.38ºC	9.22 ± 0.17ºC	12.03 ± 0.21ºC
7	8.15 ± 0.19ºC	7.94 ± 0.35ºC	8.13 ± 0.16ºC	11.01 ± 0.23ºC
8	7.12 ± 0.23ºC	7.12 ± 0.38ºC	7.09 ± 0.26ºC	10.24 ± 0.31ºC
9	5.89 ± 0.28ºC	5.94 ± 0.38ºC	5.94 ± 0.26ºC	9.22 ± 0.23ºC
10	4.87 ± 0.29ºC	4.82 ± 0.24ºC	4.79 ± 0.26ºC	7.96 ± 0.28ºC
11	4.01 ± 0.21ºC	3.96 ± 0.28ºC	4.09 ± 0.09ºC	7.18 ± 0.34ºC
12	2.89 ± 0.17ºC	3.04 ± 0.26ºC	3.10 ± 0.16ºC	6.02 ± 0.25ºC
13	1.79 ± 0.25ºC	2.23 ± 0.33ºC	1.87 ± 0.11ºC	4.98 ± 0.31ºC
14	NA	NA	1.08 ± 0.42ºC	4.01 ± 0.31ºC
15	NA	NA	NA	3.37 ± 0.28ºC
16	NA	NA	NA	2.54 ± 0.35ºC

Table 3. Sample size, length, and the pre- and post-acclimation weight of study species, as well as the average volume of the 3 respirometers used for metabolic measurements in mummichog and pumpkinseed sunfish (Experiment 2). Note that the average pre-acclimation weight represents the weights of the fish during the acute cooling trial and the average post-acclimation weight represents the weights of the fish that were remeasured after 4-6 weeks of acclimation to ~2.5^{o}C and subsequently acutely re-warmed.

Species	Sample Size	Total Length (cm)	Pre-Acclimation Weight (g)	Post-Acclimation Weight (g)	Respirometer Volume (ml)
Mummichog	12	9.1 ± 0.2	8.7 ± 0.5	8.3 ± 0.5	252.4 ± 5.1
Pumpkinseed Sunfish	11	12.3 ± 0.1	28.5 ± 1.2	26.8 ± 1.0	1496.7 ± 12.1

Data are presented in means ± S.E.M.

Table 4. The average temperatures that mummichog and pumpkinseed sunfish were exposed to in Experiment 2 as well as the statistical output obtained from the exponential relationships made within GraphPad Prism. Data are presented as means ± S.D.

Species	Temperature	R^2	df	p-Value
Mummichog	14.87 ± 0.09°C	0.57	1429	< 0.0001
	11.59 ± 0.12°C	0.55	808	< 0.0001
	8.58 ± 0.10°C	0.64	1051	< 0.0001
	5.91 ± 0.16°C	0.66	721	< 0.0001
	2.50 ± 0.16°C	0.46	523	< 0.0001
	2.64 ± 0.20°C (Acclimated)	0.49	1108	< 0.0001
	15.07 ± 0.10°C (Acute Rewarming)	0.74	1258	< 0.0001
Pumpkinseed Sunfish	14.20 ± 0.26°C	0.67	1242	< 0.0001
	11.11 ± 0.22°C	0.67	688	< 0.0001
	7.99 ± 0.14°C	0.54	977	< 0.0001
	5.26 ± 0.15°C	0.69	596	< 0.0001
	2.62 ± 0.18°C	0.66	416	< 0.0001
	2.66 ± 0.13°C (Acclimated)	0.40	957	< 0.0001
	14.24 ± 0.20°C (Acute Rewarming)	0.56	981	< 0.0001

Table 5. (See Over) Summary of statistical outputs obtained from GLMMs examining the effects of diel cycle (Day vs. Night) and acute cooling on spontaneous activity, sheltering, and food consumption of four species of putatively winter-dormant fishes. These analyses correspond to the data shown in Figure 1. Significant affects were calculated using type II Wald chi-square tests and ($p < 0.05$) are indicated in bold.

Independent Factor	Dependent Factor	Cunner			Pumpkinseed Sunfish			Mummichog			American Eel		
		Chisq	**df**	**p**	**Chisq**	**df**	**p**	**Chisq**	**df**	**p**	**Chisq**	**df**	**p**
Spontaneous Activity	Temperature	3600.68	14	**<0.0001**	2490.64	14	**<0.0001**	312.520	15	**<0.0001**	NA		
	Day/Night	338.33	1	**<0.0001**	272.03	1	**<0.0001**	54.517	1	**<0.0001**			
	Interaction	2311.39	14	**<0.0001**	1132.40	14	**<0.0001**	16.412	14	0.2889			
Food Consumption	Temperature	377.14	14	**<0.0001**	682.52	14	**<0.0001**	860.89	15	<0.0001	668.46	17	**<0.0001**
Sheltering	Temperature	159.0956	14	**<0.0001**	272.743	14	**<0.0001**	61.9898	15	**<0.0001**	NA		
	Day/Night	3.1676	1	0.0751	21.794	1	**<0.0001**	1108.84	1	**<0.0001**			
	Interaction	47.8360	14	**<0.0001**	222.444	14	**<0.0001**	6.1437	14	0.9627			
Vigilance	Temperature	NA			NA			NA			224.203	17	**<0.0001**
	Day/Night										418.734	1	**<0.0001**
	Interaction										43.641	14	**0.0004**

Chisq is the chi-squared test-statstic and df are the degrees of freedom

Table 6. (See Over) Thermal sensitivity quotients (Q_{10}) of SMR of mummichog (means ± S.E.M., n = 12) using different methods of estimating SMR (Experiment 2). "$Q_{10\ (Ind\ Extrapolated)}$" and "$Q_{10\ (Grp\ Extrapolated)}$" refer to the Q_{10}'s calculated using SMR values estimated by extrapolating $\dot{M}O_2$ to zero activity in individuals (also reported in Figures 5 and 7) and across all experimental fish, respectively. "$Q_{10\ (Overlapping)}$" refers to Q_{10}'s calculated using comparisons of the average of each individual's $\dot{M}O_2$ values corresponding to a common, overlapping range of spontaneous activity (SA) across all temperatures. Due to differing overlapping ranges of SA, the Q_{10} between acclimated 14.9°C and acclimated 2.6°C could not be calculated (see methods). "$Q_{10\ (Lowest\ 20\ \dot{M}O_2)}$" and "$Q_{10\ (Lowest\ 20\ SA)}$" refer to the Q_{10}'s calculated by comparing the average of each individual's lowest 20 $\dot{M}O_2$ points at each temperature and by comparing the average $\dot{M}O_2$ associated with the lowest 20 SA measurements at each temperature, respectively. "$Q_{10\ (Average\ \dot{M}O_2)}$" refers to the Q_{10} calculated from the average of all $\dot{M}O_2$ values for each individual fish at each temperature. "$Q_{10\ (\ Extrapolated\ +\ \dot{M}O_2)}$" and "$Q_{10\ (Extrapolated\ +\ SA)}$" refer to the Q_{10}'s calculated using SMR values estimated by extrapolating $\dot{M}O_2$ to zero activity in individual fish and, where this relationship was insignificant, replacing the extrapolated SMR with the average of the lowest 20 $\dot{M}O_2$ points or with the value for average $\dot{M}O_2$ associated with the lowest 20 SA measurements, respectively, and in the same individual fish. Q_{10}'s above my defined threshold for metabolic rate depression (i.e. Q_{10} >3.5) are bolded. The Q_{10} values for different temperature intervals were compared with linear mixed effects models; italicized P-values represent a significant difference ($p < 0.05$). Where a significant difference was observed, values that share letters are not significantly different (Bonferroni post-hoc multiple comparisons tests, $p < 0.05$).

	14.9°C → 8.6°C	**8.6°C → 2.5°C**	**14.9°C → 2.5°C**	**Acc 2.6°C → 15.0°C**	**Acc 14.9°C → Acc 2.6°C**	**P-Value**
Q_{10} (Ind Extrapolated)	3.17 ± 0.17	3.04 ± 0.12	3.05 ± 0.12	3.16 ± 0.19	3.19 ± 0.24	0.9711
Q_{10} (Grp Extrapolated)	2.65	3.28	2.93	2.90	2.42	NA
Q_{10} (Overlapping SA)	2.83 ± 0.15	2.55 ± 0.25	2.64 ± 0.20	3.26 ± 0.13	NA	0.612
Q_{10} (Lowest 20 $\dot{M}O_2$)	3.24 ± 0.27	2.59 ± 0.17	2.87 ± 0.15	**3.52 ± 0.18**	3.24 ± 0.23	0.1189
Q_{10} (Lowest 20 SA)	3.17 ± 0.26^{acd}	2.57 ± 0.19^{b}	2.82 ± 0.15^{abd}	3.15 ± 0.14^{cd}	2.96 ± 0.20^{d}	*0.0053*
Q_{10} (Average $\dot{M}O_2$)	3.28 ± 0.27^{a}	2.79 ± 0.17^{ab}	2.97 ± 0.13^{ab}	2.68 ± 0.10^{ab}	2.48 ± 0.13^{b}	*0.0181*
Q_{10} (Extrapolated + $\dot{M}O_2$)	3.12 ± 0.17	3.06 ± 0.29	3.04 ± 0.13	3.14 ± 0.19	3.21 ± 0.24	0.9897
Q_{10} (Extrapolated + SA)	3.22 ± 0.18	2.81 ± 0.32	2.94 ± 0.13	3.18 ± 0.20	3.17 ± 0.24	0.4967

Table 7. (See Over) Thermal sensitivity quotients (Q_{10}) of SMR of pumpkinseed sunfish (means ± S.E.M., n = 11) using different methods of estimating SMR (Experiment 2). See Table 6 caption for description of the different Q_{10} calculation methods and details of statistical analyses.

	14.2°C → 8.0°C	8.0°C → 2.6°C	14.2°C → 2.6°C	Acc 2.7°C → 14.2°C	Acc 14.2°C → Acc 2.7°C	p-Value
Q_{10} (Ind Extrapolated)	3.27 ± 0.47	2.40 ± 0.30	2.67 ± 0.21	2.96 ± 0.25	2.30 ± 0.26	0.0712
Q_{10} (Grp Extrapolated)	3.08	3.19	3.13	2.93	2.45	NA
Q_{10} (Overlapping SA)	3.25 ± 0.18	3.13 ± 0.37	3.10 ± 0.17	2.68 ± 0.14	NA	0.2595
Q_{10} (Lowest 20 $\dot{M}O_2$)	**3.97 ± 0.36[a]**	**3.95 ± 0.51[a]**	**3.78 ± 0.23[a]**	2.75 ± 0.20[b]	2.61 ± 0.16[b]	*0.0041*
Q_{10} (Lowest 20 SA)	**3.69 ± 0.34[a]**	**3.99 ± 0.34[a]**	**3.74 ± 0.23[a]**	2.59 ± 0.15[b]	2.49 ± 0.18[b]	*<0.0001*
Q_{10} (Average $\dot{M}O_2$)	**5.61 ± 0.41[a]**	**4.08 ± 0.38[b]**	**4.79 ± 0.33[c]**	2.92 ± 0.25[d]	3.01 ± 0.17[d]	*<0.0001*
Q_{10} (Extrapolated + $\dot{M}O_2$)	3.36 ± 0.46	2.92 ± 0.51	2.93 ± 0.29	2.90 ± 0.25	2.29 ± 0.26	0.2399
Q_{10} (Extrapolated + SA)	3.18 ± 0.50	2.70 ± 0.21	2.70 ± 0.21	2.90 ± 0.25	2.28 ± 0.27	0.341

Chapter 3: General Discussion

My thesis sought to outline the metabolic and behavioural phenotypes associated with fish overwintering dormancy in order to determine if inactivity combined with the passive effects of cooling, rather than MRD, is the primary mechanism underlying the energetic savings during dormancy. My principal finding was that MRD was not involved in pumpkinseed sunfish or mummichog winter dormancy; inactivity combined with passive cooling effects underlie low metabolic rates in winter in these two species. Additionally, I discovered that significant reductions in spontaneous activity in response to cold temperatures was a common response among putatively winter-dormant species, suggesting that there is convergence among overwintering fish to reduce activity in order to accrue large energy savings in the cold. Therefore, my hypothesis that diminished activity is the central convergent mechanism underlying the metabolic phenotype of winter dormant fish was supported.

Another main goal of this study was to provide a better understanding of dormant behaviour in overwintering fish by examining the behavioural responses to cooling in several putatively winter dormant species (cunner, pumpkinseed sunfish, mummichog, and American eel). Specifically, I examined whether several characteristic dormant behaviours (i.e., inactivity, fasting, and sheltering) are common to these species. In response to acute cooling, a few common responses across species became apparent. Notably, reductions in activity were observed in all species and this also typically coincided with reductions in feeding. Dampening of diel cycles was another common response to cooling, due to cold-induced reductions in activity during the normal diel period of heightened activity. Yet, a key result of my study was that my study species

showed considerable interspecific variation in the magnitude of their dormant behaviours. In response to acute cooling, I observed a spectrum of dormant behaviours, suggesting that the behavioural phenotype of winter-dormant fish is species-specific, and that winter dormancy must be carefully defined.

Effect of Temperature on Diel Cycles

All study species showed some degree of diel cycling of behaviours and metabolic rate in Experiments 1 and 2. The diel light cycle between night and day has great ecological importance as it can govern cycles of many important organismal traits including activity (Boujard and Leatherland, 1992; Reebs, 2002), foraging and feeding (Landless, 1976; Boujard and Leatherland, 1992; Collins and Hinch, 1993), metabolism (Nixon and Gruber, 1988; Thetmeyer, 1997; Speers-Roesch *et al*., 2018), hormone levels (Peter *et al*., 1978; Speiler and Noeske, 1984), migration (Neilson and Perry, 1990; Mehner, 2012), and habitat preference (Hanych *et al*., 1983; Crook *et al*., 2001). At warm temperatures, cunner and pumpkinseed sunfish were primarily diurnal while mummichog and American eel were primarily nocturnal. Excluding mummichog, these results are consistent with previous literature describing these species' diel activity (Collins and Hinch, 1993; Tomie, 2013; Speers-Roesch *et al*., 2018).

Mummichog are usually considered diurnal species, although few studies have directly measured diel patterns of activity this species. Using an ultrasonic movement-detection system, Kavaliers (1980) found mummichogs were light active. Less directly, Weisberg *et al*. (1981) and Baker-Dittus (1978) found that mummichog guts were typically fuller during the day, inferring diurnal activity for feeding. Alternatively, Butner

and Brattstorm (1960) suggested that mummichog feeding cycle is tide dependent. In this study mummichog were tested at full strength sea water (~35 ‰), whereas other studies often test mummichog in brackish water, so it is possible that salinity may have an influenced their diel cycles of activity. Alternatively, the different populations of mummichog investigated in my study and by others may exhibit polytypic diel cycles of activity as a result of varying environmental conditions between their locations. Our initial investigations on this suggest that photoperiod and salinity have no effect on diel cycle, so population differences may explain the conflicting results to date (E. Senathirajah, C. Reeve, and B. Speers-Roesch, unpublished).

Whereas diel cycles of activity are strongly entrained in mammals and birds, fish diel activity is considered more plastic and within-species or even within-individual variation in diel behaviour is often observed (Reebs, 2002). An interesting result from Experiment 1, and found again in Experiment 2, was the dampening of diel rhythms in response to acute cooling in all study species. This dampening is reflected by my observation of significant interactions between the effect of diel cycle and temperature on behaviours and $\dot{M}O_2$ measured in Experiments 1 and 2, except for mummichog spontaneous activity and sheltering in Experiment 1. The dampening of diel rhythms results from the reductions in activity during each species' more active diel period, such that night and day values become similar and low. Dampening or alterations of diel behaviour in response to winter cooling appears to be common among both winter-active and winter-dormant fishes. In cunner, their well-developed dormancy results in inactivity at all times of day, such that their diurnal activity pattern disappears at cold, dormant temperatures (Speers-Roesch et al. 2018). Even winter-active salmonids become less

diurnal and/or more nocturnal at the end of the summer and through the fall (Heggenes *et al*., 1993; Riehle and Griffith, 1993; Amundsen *et al*., 2000; Bremset, 2000; Jakober *et al*., 2000). Godin (1984) noted that under summer temperatures and photoperiod pink salmon (*Oncorhynchus gorbuscha*) exhibited diurnal behaviour, but under winter conditions pink salmon daytime activity decreased to levels similar to those of the nighttime, which matches the responses of my study species. Additionally, Fraser *et al*. (1993,1995) demonstrated that cold-exposed Atlantic salmon (*Salmo salar*) suppressed daytime activity and became increasingly nocturnal even when under a summer photoperiod. Thus, it appears that a reduction in activity during the active diel phase may be a common response to cooling among fish and in some species a shift in the diel phase of activity may be observed.

Notably, in both experiment 1 and 2 at the coldest temperatures (~2^{o}C – 3^{o}C), pumpkinseed sunfish had higher nighttime spontaneous activity relative to the daytime, even after several weeks of cold acclimation, and in contrast to their diurnal activity at warm temperatures. To my knowledge this is the first evidence of diel changes in the activity of centrarchids in response to cold temperatures. Fraser *et al*. (1994,1995) proposed that the dampening or alteration of diel cycling may relate to predator-prey interactions. Under warm conditions, higher metabolic rates facilitate predator avoidance behaviours and thus fish can risk foraging during the daytime, when foraging success is typically greater. However, under cold conditions, reduced metabolic rate and swimming speeds makes fish more vulnerable to endothermic diurnal predators, therefore making nocturnal foraging favourable. While temperature-induced diel changes appear to be relatively common, we know little about the underlying mechanisms.

The Spectrum of Overwintering Strategies in Fishes

Dormancy is normally defined by a specific suite of behavioural characteristics (i.e., inactivity, fasting, sheltering, low metabolic rates at winter low temperatures). My findings on four species of previously reported winter-dormant fish, however, showed a species-specific spectrum of behaviours, rather than a single distinct phenotype characteristic of dormancy. At low temperatures, activity was zero or near zero in cunner and eel, which is similar to observations on brown bullhead (Crawshaw *et al*., 1982). Strong sheltering behaviour was also seen in all of these species. Activity remained at a low level in pumpkinseed sunfish, similar to largemouth bass, with strong sheltering in general (Lemons and Crawshaw, 1985). Mummichog were the most active and rarely sheltered, but still showed large reductions in activity compared to warm temperatures. Regarding food consumption, cunner, mummichog, and eels fasted at low temperatures, whereas some pumpkinseed sunfish, as well as largemouth bass (Lemons and Crawshaw, 1985), continued feeding at a low level (Figure 1). Therefore, while appropriate for some species (e.g., cunner, American eel, brown bullhead), winter dormancy may not be an appropriate classification for a number of previously described winter-dormant species (e.g., largemouth bass, pumpkinseed sunfish, mummichog). Thus, the description of dormancy must be reassessed in some winter dormant species.

The term dormancy was originally developed to describe the hibernation responses of endotherms because associated decreases in body temperature and metabolic rate are so profound as to abolish any resemblance to their normal state. As such, dormancy may be less applicable among overwintering ectotherms, where tolerance and/or compensation for broad ranges of body temperature are more typical (Crawshaw,

1982). Furthermore, involvement of behavioural reductions in activity for energy savings, rather than MRD, may make the behavioural phenotype of overwintering fish more flexible. Certain fish species do exhibit dormancy, such as cunner and perhaps American eel, but many described winter-dormant species do not display all the characteristics of dormant behaviour. Based on my study, winter dormancy seems to be particularly ill-suited to describe the overwintering phenotype of mummichogs and perhaps pumpkinseed sunfish. Several other researchers have noted that many winter-dormant species are not always completely sedentary (Crawshaw, 1982; Hanson *et al*., 2007; Cooke and Philipp, 2009) yet these species remain termed "winter-dormant species".

Correct definitions of winter dormancy and the characterization of overwintering behaviour and physiology are important. For example, the misinterpretation of dormant behaviour among centrarchid species has led to inaccuracies in some existing bioenergetics models, which are important in influencing fishery and conservation management (Brandt and Hartman, 1993; Hansen *et al*., 1993; Cooke and Philipp, 2009). Currently, many bioenergetics models assume that centrarchid species cease feeding and remain largely inactive during the winter (Wright *et al*., 1999; Cooke and Philipp, 2009). Wright *et al*. (1999) showed that the prevailing bioenergetics models fare poorly in predicting the responses of largemouth bass to winter conditions, likely due to the underestimated role of predation and feeding in influencing overwinter survival.

One possible cause of these kinds of problems is that overwintering strategies have been characterized in dichotomous terms of winter-dormant (i.e., inactivity, fasting, near-constant sheltering) and winter-active (i.e., compensation: maintained or slightly reduced activity and feeding, little to no sheltering). Thus, species that simply

demonstrate one of the characteristics of either of these groupings, for example reduced activity during the winter, may be pigeonholed into the dormancy group. In reality, the overwintering strategy of many fish species may fall in between the extremes of winter-dormant and winter-active phenotypes. I propose winter lethargy as the intermediate overwintering strategy between winter dormancy and winter activity. Winter lethargy is characterized by a marked reduction in spontaneous activity to a new minimally active steady state, a low level of opportunistic feeding, and often the selection of a defined overwintering area (e.g., low flow area of a river) rather than a specific shelter or burrow like you may see in a winter dormant species at winter low temperatures (Green and Farwell, 1971; Crawshaw, 1982; Crawshaw *et al*., 1984; Sayer *et al*., 1994; Sayer and Davenport, 1996; Bradbury and Green, 1997; Arendt *et* al., 2001; Tomie *et al*., 2013).

Various considerations must be made when assigning a fish species to a winter-dormant, winter-lethargic, or winter-active strategy. For example, considering that reductions in activity and feeding are relatively common even among "winter active" fish species, one must be cognizant of the gradient of overwintering activity and feeding that currently exists. Salmonids are often regarded as the characteristic "winter-active" species; however, salmonid activity and feeding are only partially compensated during the winter and reductions in routine activity and feeding are common (Peterson and Anderson, 1969; Cunjak *et al*., 1998; Bremset, 2000). Some species of salmonids maintain relatively higher levels of activity and feeding in the winter that can sometimes equate to winter growth (e.g. brook trout, *Salvelinus* fontinalis, rainbow trout, *Oncorhynchus mykiss*, and Arctic char, *Salvelinus alpinus*) (Cunjak *et al*., 1987; Brännäs and Wiklund, 1992); however, other studies on salmonid winter behaviour indicate a

decrease in feeding and activity associated with a decrease in body condition (Gardiner and Geddes, 1979; Cunjak *et al*., 1987; Cunjak *et al*., 1988).Conversely, true winter active species do exist, though they appear to be rare. For example, burbot (*Lota lota*) are a uniquely winter active fish, displaying their highest activity in the cold, on account of being cold adapted; they spend the summer in a dormant state (Carl, 1995; Binner *et al*., 2008). Difficulty also arises when attempting to determine the winter behaviour of relatively sedentary fish species. For example, Northern pike (*Esox lucius*) show little difference between winter and summer activity; however, they are often considered a relatively cold "active" species due to continued winter foraging (Diana, 1980; Cook and Bergersen, 1988; Jepsen *et al*., 2001; Koed *et al*., 2006).

An important distinction between these overwintering strategies may be the degree of change observed between the summer and winter months. For example, since Northern pike show a smaller degree of change between summer and winter in both activity and foraging, they may be classified as winter active. Alternatively, cunner show a profound shift in behaviour as the seasons change, altering their behaviour from a relatively active and foraging state to profoundly inactive and fasting state. Many centrarchid species show a lesser degree of change, relative to cunner, but still show marked reductions in activity and feeding during the winter and, therefore, represent a largely winter-lethargic group of fish species (Cooke and Philipp, 2009).

Perhaps the clearest distinction between winter-dormant, winter-lethargic, and winter-active species may be differences in foraging behaviour. Notably, as I defined earlier, winter dormant species engage in profound fasting, winter-lethargic species engage in a low level of opportunistic feeding, and winter-active species continue to

forage throughout the winter. Therefore, using this definition, cunner and American eel would be considered winter-dormant species due to an absence of feeding; mummichog pumpkinseed sunfish, and likely most other centrarchid species would be winter-lethargic species due to a low level of opportunistic feeding (Lemons and Crawshaw, 1985; Cooke and Philipp, 2009); and Arctic char, brook trout, and rainbow trout would be winter-active species due to continued winter foraging, even if growth is not optimal (Cunjak *et al*., 1987; Brännäs and Wiklund, 1992).

Importantly, the degree of overwinter feeding, and thus our allocation of overwintering strategies to species, may be influenced by a species' capacity to lay down energy stores and efficiently use them prior to and during winter. A study comparing lipid storage and use in largemouth bass and yellow perch, showed that yellow perch, which do not rely on the storage of lipids prior to the onset of winter, fare poorly when deprived of food during the winter relative to largemouth bass (Sullivan, 1986). Therefore, yellow perch must continue to forage throughout the winter and must rely on winter food availability, whereas largemouth bass can rely primarily on their internal energy reserves. Metcalfe and Thorpe (1992) showed that winter appetite in young-of-the-year Atlantic salmon was regulated by nutritional state (i.e., increased consumption when energy stores became depleted) and a similar mechanism has been proposed for governing the appetite in centrarchid species (Cooke and Philipp, 2009). Thus, it seems that the storage and utilization of energy may play a role in the determination of species-specific overwintering behaviour; however, more research is warranted.

Winter is an ecologically important time of the year for temperate fish species and the physiological challenges that come about as a result of winter impart major

constraints on the range expansions and northern persistence of a population (Hurst, 2007). Recent climate models suggest that global warming will increase North American air temperatures, subsequently warming aquatic temperatures (Meisner *et al*., 1987; Eaton and Scheller, 1987; de Stasio *et al*., 1996; Fang and Stefan, 1998). It is believed that this predicted temperature rise will result in decreased winter severity at higher latitudes, possibly leading to the poleward expansion of southern fish species (Meisner 1987; Regier 1989, Shuter and Post, 1990; Lyons *et al*., 2010; Alofs *et al*., 2014). It is also probable that the relative abundance of various species will be impacted due to alterations in thermal niches, prey availability, and dissolved oxygen concentrations as a result of these climatic changes (Meisner *et al*., 1987; Lyons *et al*., 2010). Winter has a pronounced impact on the life-history, survival, and distribution of many fish species. Without a good understanding of fish winter behaviour and ecology, the effect of global warming on these species cannot be accurately predicted (Hurst, 2007; Gunderson and Leal, 2016; Williams *et al*., 2015). While more research is necessary to address these large questions, the results from this study provide a basic understanding of how temperate fish species behave and the metabolic changes that likely occur in the wild during the winter.

BIBLIOGRAPHY

Alofs KM, Jackson DA, Lester NP. Ontario freshwater fishes demonstrate differing range-boundary shifts in a warming climate. *Diversity Distrib*. 20: 123-136.

Amundsen PA, Gabler HM, Herfindal T, Riise LS. 1999 Feeding chronology of Atlantic salmon parr in subarctic rivers: consistency of nocturnal feeding. *J Fish Biol*. 56: 676-686.

Angilletta, MJ. 2009 Thermal adaptation: a theoretical and empirical synthesis. Cambridge, UK: Oxford University Press.

Arendt MD, Lucy JA, Evans DA. 2001 Diel and seasonal activity patterns of adult tautog, *Tautoga onitis* in lower Chesapeake Bay, inferred from ultrasonic telemetry. *Environ Biol Fishes*. 62: 379-391.

Bajer PG, Chizinski CJ, Sorensen PW. 2011 Using the Judas technique to locate and remove wintertime aggregations of invasive common carp. *Fisheries Manage Ecol*. 18: 497-505.

Baker-Dittus AM. 1978 Foraging patterns of three sympatric killifish. *Copeia*. 1978(3): 383-389.

Binner M, Kloas W, Hardewig I. 2008 Energy allocation in juvenile roach and burbot under different temperature and feeding regimes. *Fish Physiol Biochem*. 34: 103-116.

Block BA, Dewar H, Blackwell SB, Williams TD, Prince ED, Farwell CJ, Boustany A, Teo SL, Seitz A, Walli A, Fudge D. 2001 Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science.* 293: 1310–1314.

Boyles JG, Thompson AB, McKechnie AE, Malan E, Humphries MM, Careau V. 2013 A global heterothermic continuum in mammals. *Glob Ecol Biogeogr*. 22: 1029-1039.

Boujard T, Leatherland JF. 1992 Circadian rhythms and feeding time in fishes. *Environ Biol Fish*. 35: 109-131.

Bradbury C, Green JM. 1997 Daily and seasonal activity patterns of female cunner, *Tautogolabrus adspersus* (Labridae), in Newfoundland. *Fish Bull*. 95:646-652.

Brännäs E, Wiklund B. 1992 Low temperature growth potential of arctic charr and rainbow trout. *Nordic J Freshw Res*. 67: 77-81.

Brandt SB, Hartman KJ. 1993 Innovative approaches with bioenergetics models: future application to fish ecology and management. *Trans Amer Fish Soc*. 122: 731-735.

Bremset G. 2000 Seasonal and diel changes in behaviour, microhabitat use and preferences by young pool-dwelling Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. *Environ Biol Fishes*. 59: 163-179.

Butner A, Brattstrom BH. 1960 Local movement in Menidia and Fundulus. *Copeia*. 1960(2): 139-141.

Carl LM. 1995 Sonic tracking of burbot in Lake Opeongo, Ontario. *Trans Amer Fish Soc*. 124: 77-83.

Carlson DM. 2011 Importance of wintering refugia to the largemouth bass fishery in the Hudson River estuary. *Journal of Freshwater Biology*. 7(2): 173-180.

Chabot D, Steffensen JF, Farrell AP. 2016 The determination of standard metabolic rate in fishes. *J Exp Biol.* 88: 81-121.

Chidester FE. 1920 The behaviour of Fundulus heteroclitus on the salt marshes of New Jersey. *Am Nat*. 54(635): 551-557.

Clark KL, Ruiz GM, Hines AH. 2003 Diel variation in predator abundance, predation risk and prey distribution in shallow-water estuarine habitats. *J Exp Mar Biol Ecol.* 287: 37-55.

Clarke A. 2017 Principles of Thermal Ecology: Temperature, Energy, and Life. Cambridge, UK: Oxford University Press.

Collins NC, Hinch SG. 1993 Diel and seasonal variation in foraging activities of pumpkinseeds in an Ontario pond. *Trans Amer Fish Soc*. 122(3): 357-365.

Cook MF, Bergersen EP. 1988 Movements, habitat selection, and activity periods of northern pike in Eleven Mile Reservoir, Colorado. *Trans Amer Fish Soc.* 177(5): 495-502.

Cooke SJ, Philipp DP. 2009 Centrarchid Fishes. Chichester, UK: Blackwell Publishing Ltd.

Costa IA, Driedzic WR, Gamperl K. 2013 Metabolic and cardiac responses of cunner *Tautogolabrus adspersus* to seasonal and acute changes in temperature. *Physiol Biochem Zool* 86:233-44.

Crawshaw LI. 1984 Low-temperature dormancy in fish. *Am J Physiol* 246: R479-86.

Crawshaw LI, Lemons DE, Parmer M, Messing JM. 1982 Behavioural and metabolic aspects of low temperature dormancy in the brown bullhead, *Ictalurus nebulosus*. *J Comp Physiol B* 148:41-47.

Crook DA, Robertson AI, King AJ, Humphries P. 2001 The influence of spatial scales and habitat arrangement on diel patterns of habitat use by two lowland river fishes. *Oecologia*. 129(4): 525-533.

Cunjak RA. 1988 Behaviour and microhabitat of young Atlantic salmon (*Salmo salar*) during winter. *Fish Aquat Sci*. 45: 2156-2160.

Cunjak RA. 1996 Winter habitat of selected stream fishes and potential impacts from land-use activity. *Can J Fish Aquat Sci*. 53(Sppl. 1): 267-282.

Cunjak RA, Curry A, Power G. 1987 Seasonal energy budget of brook trout in streams: implications of a possible deficit in early winter. *Trans Amer Fish Soc*. 116(6): 817-828.

Cunjak RA, Prowse TD, Parrish DL. 1998 Atlantic salmon (*Salmo salar*) in winter: "the season of parr discontent"? *Can J Fish Aquat Sci*. 55: 161-181.

de Stasio BT, Hill DK, Kleinhans JM, Nibbelink NP, Magnuson JJ. 1996 Potential effects of global climate change on small north-temperate lakes: physics, fish, and plankton. *Limnol Oceanogr*. 41(5): 1136-1149.

Diana JS. 1980 Diel activity pattern and swimming speeds of Northern pike (*Esox lucius*) in Lac Ste. Anne, Alberta. *Can J Fish Aquat Sci*. 37: 1454-1458.

Dillon ME, Wang G, Huey RB. 2010 Global metabolic impacts of recent climate change. *Nature*. 467: 704-706.

Driedzic WR, Bailey JR, Sephton DW. 1996 Cardiac adaptations to low temperature in non-polar teleost fish. *J Exp Biol*. 275: 186-195.

Eaton JG, Scheller RM. 1996 Effects of climate warming on fish thermal habitat in streams of the United States. *Limnol Oceanogr*. 41(5): 1109-1115.

Egginton S, Sidell BD. 1989 Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am J Physiol*. 256: Rl-R9.

Evans DO. 1990 Metabolic thermal compensation by rainbow trout: effects on standard metabolic rate and potential usable power. *Trans Amer Fish Soc*. 119: 585-600.

Fang X, Stefan HG. 1998 Potential climate warming effects on ice covers of small lakes

in the contiguous U.S. *Cold Regions Science and Technology*. 27: 119-140.

Farrell AP, AM Hammons, MS Graham, GF Tibbits. 1988. Cardiac growth in rainbow trout, *Salmo gairdneri. Can. J. Zool.*, 66:2368-2373.

Flaherty CM, Sass GC, Stiles KE. 2003 Human mercury toxicity and ice angler fish consumption: are people eating enough to cause health problems? *Risk Anal*. 23(3): 497-504.

Fraser NHC, Heggenes J, Metcalfe NB, Thorpe JE. 1995 Low summer temperature cause juvenile Atlantic salmon to become nocturnal. *Can J Zool*. 73: 446-451.

Fraser NHC, Metcalfe NB, Thorpe JE. 1993 Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proc Biol Sci*. 252(1334): 135-139.

Fritz ES, Meredith WH, Lotrich VA. 1975 Fall and winter movements and activity level of the mummichog, Fundulus heteroclitus, in a tidal creek. *Chesapeake Sci*. 16(3): 211-215.

Gardiner, WR, Geddes P. 1980 The influence of body composition on the survival of juvenile salmon. *Hydrobiologia*. 69: 67-72.

Godin JG. 1983 Temporal variations in daily patterns of swimming activity and vertical distribution in juvenile pink salmon (*Oncorhynchus gorbuscha*). *Can J Zool*. 62: 72-79.

Green JM, Farwell M. 1971 Winter habits of the cunner, *Tautogolabrus adspersus* (Walbaum 1792), in Newfoundland. *Can J Zool*. 49: 1497-1499.

Guderley, H., and A. Gawlicka. 1992 Qualitative modification of muscle metabolic organization with thermal acclimation of rainbow trout *(Oncorhynchus mykiss). Fish Physio. Biochem*, 10:123-132.

Guppy M, Withers P. 1999 Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol Rev*. 74(1): 1-40.

Gunderson AR, Leal M. 2016 A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Biol Rev* 79:409-27.

Hanson KC, Cooke SJ, Suski CD, Niezgoda G, Phelan FJS, R Tinline, Philipp DP. 2007 Assessment of largemouth bass (*Micropterus salmoides*) behaviour and activity at multiple spatial and temporal scales utilizing a whole-lake telemetry array. *Hydrobiologia*. 58: 243-256.

Hansen MJ, Boisclair D, Brandt SB, Hewett SW, Kicthell JF, Lucas MC, Ney JJ. 1993 Application of bioenergetics models to fish ecology and management: where do we go from here? *Trans Amer Fish Soc*. 122: 1019-1030.

Hanych DA, Ross MR, Magnien RE, Suggars AL. 1983 Nocturnal inshore movement of the mimic shiner (*Notropis vollucellus*): a possible predator avoidance behaviour. *Can J Fish Aquat Sci*. 40: 888-894.

Haplin PM. 1997 Habitat use patterns of the mummichog, *Fundulus heteroclitus*, in New England. I. Intramarsh variation. *Estuaries*. 20(3): 618-625.

Hathaway ES. 1927 The relation of temperature to the quantity of food consumed by fishes. *Ecology*. 8(4): 428-434.

Hazel JR. 1995 Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu Rev of Physiol*. 57: 19-42.

Hazel JR, Williams EE. 1990 The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog Lipid Res.* 29: 167-227.

Healy TM, Chung DJ, Growther KG, Schulte PM. 2017 Metabolic and regulatory responses involved in cold acclimation in Atlantic killifish, *Fundulus heteroclitus*. *J Comp Physiol*. 187: 463-475.

Heggenes J, Krog OMW, Lindas OR, Dokk JG, Bremnes T. 1993 Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. *J Anim Ecol.* 62(2): 295-308.

Helfman GS. 1986 Diel distribution and activity of American eels (*Anguilla rostrata*) in a cave-spring. *J Fish Aquat Sci*. 43: 1595-1605.

Hillman SS, Withers PC, Drewes RC, Hillyard SD. 2009 Ecological and environmental physiology of amphibians. Oxford, UK: Oxford University Press.

Huey RB, Kingsolver JG. 2011 Variation in universal temperature dependence of biological rates. *Proc Natl Acad Sci USA.* 108: 10378 – 10378

Hurst TP. 2007 Causes and consequences of winter mortality in fishes. *J Fish Biol*. 71: 315-345.

Jakober MJ, McMahon TE, Thurrow RF. 2000 Diel habitat partitioning by bull charr and cutthroat trout during fall and winter in Rocky Mountain streams. *Environ Biol Fishes*. 59: 79-89.

Jepsen N, Beck S, Skov C, Koed A. 2001 Behaviour of pike (*Esox lucius* L.) >50cm in a turbid reservoir and in clearwater lake. *Ecol Freshw Fish*. 10: 26-34.

Johnson PB, Hasler AD. 1977 Winter aggregations of carp (*Cyprinus carpio*) as revealed by ultrasonic tracking. *Trans Amer Fish Soc*. 106(6): 556-559.

Johnston IA, Temple GK. 2002 Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. *J Exp Bio*. 205(15): 2305-2322.

Kaya CM, Hasler AD. 1972 Photoperiod and temperature effects on the gonads of green sunfish, *Lepomis cyanellus*, during the quiescent, winter phase of its annual sexual cycle. *Trans Amer Fish Soc.* 101(2): 270-275.

Kavaliers M. 1980 Social groupings and circadian activity of the killifish, *Fundulus heteroclitus*. *Biol Bull*. 158: 69-76.

Kelly, NI Alzaid A, Nash GW, Gamperl AK. 2014 Metabolic depression in cunner (*Tautogolabrus adspersus*) is influenced by ontogeny, and enhances thermal tolerance. *PLoS ONE*. 9: e114765

Klaiman JM, Fenna AJ, Shiels HA, Macri J, Gillis TE. 2011 Cardiac Remodeling in fish: strategies to maintain heart function during temperature change. *PLoS ONE*. 6(9): 1-11

Kobler A, Klefoth T, Wolter C, Fredrich F, Arlinghaus R. 2008 Contrasting pike (*Esox lucius* L.) movement and habitat choice between summer and winter in a small lake. *Hydrobiologia*. 601: 17-27.

Koed A, Balleby K, Mejhede P, Aarestrup K. 2006 Annual movement of adult pike (*Esox lucius* L.) in a lowland river. *Ecol Freshw Fish*. 15: 191-199.

Kolok AS. 1991 Temperature compensation in two centrarchid fishes: Do winter-quiescent fish undergo cellular temperature compensation? *Trans Am Fish Soc.* 120:52-57.

Korsmeyer KE, Steffensen JF, Herskin J. 2002 Energetics of median and paired fin swimming, body and caudal fin swimming, and gait transition in parrotfish (*Scarus schlegeli*) and triggerfish (*Rhinecathus aculeatus*). *J Exp Biol*. 205: 1253-1263.

Landless PJ. 1975 Demand-feeding behaviour of rainbow trout. *Aquaculture*. 7: 11-25.

Lemons DE, Crawshaw, LI. 1985 Behavioural and metabolic adjustments to low

temperatures in the largemouth bass (*Micropterus* salmoides). *Physiol Zool* 58:175-180.

Lucas MC, Priede IG. 1992 Utilization of metabolic scope in relation to feeding and activity by individual and grouped zebrafish, *Brachydanio rerio* (Hamilton-Buchanan). *J Fish Biol.* 41: 175-190.

Lyons J, Stewart JS, Mitro M. 2010 Predicted effects of climate warming on the distribution of 50 stream fishes in Wisconsin, USA. *J Fish Biol*. 77: 1867-1898.

Marshall WS, Tait JC, Mercer EW. 2016 Salinity preference in the estuarine teleost fish mummichog (*Fundulus heteroclitus*): halocline behaviour. *Physiol Biochem Zoo*. 89(3): 225-232.

Mathews RW, Haschemeyer AEV. Temperature dependency of protein synthesis in toadfish liver *in vivo*. *Comp Biochem Physiol B Biochem Mol Biol*. 61(4): 479-484.

Mehner T. 2012 Diel vertical migration of freshwater fishes – proximate triggers, ultimate causes and research perspectives. *Freshw Biol*. 57: 1342-1359.

Meisner DJ, Goodier JL, Regier HA. 1987 An assessment of the effects of climate warming on great lakes basin fishes. *J Great Lakes Res*. 13(3): 340-352.

Metcalfe NB, Thorpe JE. 1992 Anorexia and defended energy levels in over-wintering juvenile salmon. *J Anim Ecol*. 61(1): 175-181.

McMeans BC, McCann KS, Guzzo, MM, Bartley TJ, Bieg C, Blanchfield P, Fernandes T, Giacomini H, Middel T, Rennie M, Ridgway M, Shuter B. 2019 Winter in water: differential responses and the maintenance of biodiversity. bioRxiv. Preprint

McNab BK. 2002 The Physiological Ecology of Vertebrates: A View From Energetics. New York, USA: Cornell University Press.

Miranda LE, WD Hubbard. 1994. Winter survival of age-0 largemouth bass relative to size, predators, and shelter. *N Am J Fish Manage.* 14: 790–796.

Molnár G, Tölg I. 1962 Relation between water temperature and gastric digestion of largemouth bass (*Micropterus salmoides*). *J Fish Res Bd Can*. 19(6): 1005-1012.

Montgomery JC, MacDonald JA. 1990 Effects of temperature on nervous system: implications for behavioural performance. *Amer J Physiol*. 259 R191-R196.

Montgomery JC, McVean AR. 1987 Brain function in Antarctic fish: activity of central

vestibular neurons in relation to head rotation and eye movement. *J Comp Physiol A*. 160: 289-293.

Montgomery JC, McVean AR, McCarthy D. 1982 The effects of lowered temperature on spontaneous eye movements in a teleost fish. *Comp Biochem Phys A*. 75(3): 363-368.

Munther GL. Movement and distribution of smallmouth bass in the middle snake river. *Trans Amer Fish Soc*. 99(1): 44-53.

Neilson JD, Perry RI. 1990 Diel vertical migrations of marine fishes: an obligate or facultative process? *Adv Mar Biol*. 26: 115-168.

Neilson JD, Smith S, Royer F, Paul SD, Porter JM, Lutcavage M. 2009 Investigations of horizontal movements of Atlantic swordfish using pop-up satellite archival tags. In: Neilson JD, Arrizabalaga H, Fragoso N, Hobday A, Lutcavage M, Sibert J, eds. Tagging and tracking of Marine Animals with Electronic Devices. Dordrecht, Netherlands: Kluwer Academic Press.

Nilsson GE, Rosen PR, Johansson D. 1993 Anoxic depression of spontaneous locomotor activity in crucian carp quantified by a computerized imaging technique. *J Exp Biol* 180:153-162.

Nixon AJ, Gruber SH. 1988 Diel metabolic and activity patterns of the lemon shark (*Negaprion brevirostris*). *J Exp Zool*. 248: 1-6.

Nyman L. 1972 Some effects of temperature on eel (Anguilla) behavior. Institute of Freshwater Research (Drottningholm). 52: 90-102.

Peck LS. 2016 A cold adaptation to the sea. *TREE* 31:13-26.

Peter RE, Hantela A, Cook AF, Raulencu CR. 1978 Daily cycles in serum cortisol levels in the goldfish: effects of photoperiod, temperature, and sexual condition. *Can J Zool*. 56: 2443-2448.

Peterson RH, Anderson JM. 1969 Influence of temperature change on spontaneous locomotor activity and oxygen consumption of Atlantic salmon, *Salmo salar*, acclimated to two temperatures. *J Fish Res Bd*. 26: 93-109.

Pörtner, HO. 2002 Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp Biochem Physiol A*. 132: 739-761.

Pörtner, HO. 2009 Oxygen- and capacity-limitation of thermal tolerance: a matrix for

integrating climate-related stressor effects in marine ecosystems. *J Exp Biol*. 213: 881-893.

Rach JF, Meyer FP. 1982 Winter harvest of bluegills in 1976-1977 from lake Onalaska, Wisconsin. *N Am J Fish Manage*. 2: 28-32.

Raposa K. 2003 Overwintering habitat selection by the mummichog, *Fundulus heteroclitus*, in a Cape Cod (USA) salt marsh. *Wetlands Ecol Manag* 11:175-182.

Reebs SG. 2003 Plasticity of diel and circadian activity rhythms in fishes. *Rev Fish Biol Fisher*. 12: 349-371.

Reichle MD, Griffith JS. 1993 Changes in habitat use and feeding chronology of juvenile rainbow trout (*Oncorhynchus mykiss*) in fall and the onset of winter in Silver Creek, Idaho. *Can J Fish Aquat Sci*. 50: 2119-2128.

Richardson NE, McCleave JD. 1974 Locomotor activity rhythms of juvenile Atlantic salmon (*Salmo salar*) in various light conditions. *Biol Bull*. 147: 422-432.

Riley WD, Walker AM, Bendall B, Ives MJ. 2011 Movements of the European eel (*Anguilla anguilla*) in a chalk stream. *Ecol Freshw*. 20: 628-635.

Roberts JL. 1964 Metabolic responses of fresh-water sunfish to seasonal photoperiods and temperatures. *Helgo Wiss Meer* 9:459-473.

Rodnick, KJ, BD Sidell. 1994 Cold acclimation increases carnitine palmitoyltransferase I activity in oxidative muscle of striped bass. *Am J Physiol*, 266: R405-412.

Rodriguez A, Zhang H, Klaminder J, Brodin T, Andersson M. 2017 ToxId: an algorithm to track the identity of multiple animals. *Sci Rep.* 7(1): 14774.

Rodrigues A, Zhang H, Klaminder J, Brodin T, Andersson PL, Andersson M. 2019 ToxTrac: a fast and robust software for tracking organisms. *Methods Ecol Evol.* 9(3): 460-464.

Rome LC. 1990 Influence of temperature on muscle recruitment and muscle function *in vivo*. *Am. J. Physiol.* 259, R210–R222.

RStudio Team. 2018 RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Ryu HS, Song JA, Park H, Choi YJ, Choi CY. 2020 Physiological and oxidative stress response of goldfish *Carassius auratus* induced by a light dimming system. *Fish Physiol Biochem*. 46: 585-595.

Sayer MDJ, Cameron KS, Wilkinson G. 1994 Fish species found in the rocky sublittoral during winter months as revealed by the underwater application of the anesthetic quinaldine. *J Fish Biol*. 44: 351-353.

Sayer MDJ, Davenport J. 1996 Hypometabolism in tropid goldsinny wrasse subjected to rapid reductions in seawater temperature. *J Fish Biol*. 49:64-75.

Schneider CA, Rasband WS, Eliceiri KW. 2012 NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 9(7): 671-675.

Seebacher F, White CR, Franklin CE. 2015 Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim Change*. 5:61-66.

Shoup DE, Wahl DH. 2008 The effect of largemouth bass predation on overwinter survival of two size-classes of age-0 bluegills. *Amer Fish Soc*. 137: 1063-1071.

Shrable JB, Tiemeier OW, Deyoe CW. 1969 Effects of temperature on rate of digestion by channel catfish, *Prog Fish-Cult*. 31(3): 131-138.

Shulte PM. 2015 The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol*. 218: 1856-1866.

Shuter BJ, Finstad AG, Helland IP, Zweimüller I, Hölker F. 2012 The role of winter phenology in shaping the ecology of freshwater fish and their sensitivities to climate change. *Aquatic Sci* 74:637-657.

Shuter BJ, Post JR. 1990 Climate, population viability, and the zoogeography of temperate fishes. *Trans Amer Fish Soc*. 119(2): 314-336.

Sidell, B.D., and T.S. Moerland (1989) Effects of temperature upon muscle function and locomotory performance in teleost fishes. In: Advances in Comparative and Environmental Physiology. C.P. Mangum, ed. Springer-Verlag, New York, Vol. 5, pp. 115-156.

Sinha VRP, Jones JW. 1967 On the food of the freshwater eels and their feeding relationship with the salmonids. *J Zoo Lond*. 153: 119-137.

Smith KJ, Able KW. 1994 Salt-marsh tide pools as winter refuges for the mummichog, *Fundulus heteroclitus*¸in New Jersey. *Estuaries*. 17(18): 226-234.

Smith MW, Saunders JW. 1955 The American eel in certain freshwaters of the maritime provinces of Canada. *J Fish Res Bo Canada*. 12(2): 238-269.

Snyder DJ, Peterson MS. 1999 Foraging and prey selection by bluespotted sunfish

Enneacanthus gloriosus (Holbrook) in backwater, vegetated ponds in coastal Mississippi. *J Freshw Ecol*. 14: 187-196.

Speers-Roesch B, Norin T, Driedzic WR. 2019 The benefit of being still: energy savings during winter dormancy in fish arise from inactivity and the cold, not metabolic rate depression. *Proc Biol Sci*. 285(1886): 20181593.

Speiler RE, Noeske TA. 1984 Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. *Trans Amer Fish Soc*. 115: 528-539.

Staples, JF. 2016 Metabolic flexibility: hibernation, torpor, estivation. *Compr Physiol*. 6: 737-771.

Steinhausen MF, Steffensen JF, Andersen NG. 2010 The effects of swimming pattern on the energy use of gilthead seabream (*Sparus aurata* L.) *Mar Freshw Behav Phys*. 43(4): 227-241.

Storey KB, Storey JM. 2004 Metabolic rate depression in animals: transcriptional and translational controls. *Biol Rev* 79:207-233.

Stuart-Smith RD, Edgar GJ, Bates AE. 2017 Thermal limits to the geographic distributions of shallow-water marine species. *Nature Ecology & Evolution* 1: 1846-1856.

Sullivan KM. 1986 Physiology of feeding and starvation in overwintering freshwater fish. *Dev Environ Biol Fish*. 7: 259-268.

Targett TE. 1978 Respiratory metabolism of temperature acclimated *Fundulus heteroclitus*: zones of compensation and dependence. *J Exp Mar Biol Ecol* 32:197-206.

Tattersall GJ, Ultsch GR. 2008 Physiological ecology of aquatic overwintering in ranid frogs. *Biol Rev* 83:119-140.

Thetmeyer H. 1997 Diel rhythms of swimming activity and oxygen consumption in *Globiusculus flavencens* (Fabricius) and *Pomatoschistus minutus* (Pallas) (Teleostei: Gobiidae). *J Exp Mar Biol Eco*. 218: 187-198.

Tomie JPN, Cairns DK, Courtenay SC. 2013 How American eels *Anguilla rostrata* construct and respire in burrows. *Aquat Biol*. 19: 287-296.

Tudorache C, Jordan AD, Svendsen JC, Domenici P, DeBoeck G, Steffensen JF. 2009

Pectoral fin beat frequency predicts oxygen consumption during spontaneous activity in a labriform swimming fish (*Embiotoca lateralis*). *Environ Biol Fish*. 84: 121-127.

Ultsch GR. 1989 Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. *Biol Rev* 64:435-515.

van der Meer J, 2006 Metabolic theories in ecology. *Trends Ecol Evol*. 21:136-140.

VanderKooy KE, Rakocinski CF, Heard RW. 2000 Trophic relationships of three sunfishes (*Lepomis* spp.) in an estuarine bayou. *Estuaries*. 23: 621-632.

Walsh PJ, Foster GD, Moon TW. 1983 The effects of temperature on metabolism of the American eel *Anguilla rostrata* (LeSueur): compensation in the summer and torpor in the winter. *Physiol Zool* 56:532-540.

Weisberg SB, Whalen R, Lotrich VA. 1981 Tidal and diurnal influence on food consumption of a salt marsh killifish *Fundulus heteroclitus*. *Mar Biol*. 61: 243-246.

Westerberg H, Sjöberg N. 2015 Overwintering dormancy behaviour of the European eel (*Anguilla anguilla* L.) in a large lake. *Ecol Freshw*. 24: 532-543.

Williams CM, Henry HA, Sinclair BJ. 2015 Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biol Rev* 90:214-235.

Wood CM, McDonald DG. 1997 Global warming: Implications for freshwater and marine fish. Cambridge, UK: Cambridge University Press.

Zimmerman C, Kunzmann A. 2001 Baseline respiration and spontaneous activity of sluggish marine tropical fish of the family Scorpaenidae. *Mar Ecol Prog Ser*. 219: 229-239.

Curriculum Vitae

Candidate's full name: Connor Reeve

Universities attended: University of Guelph, 2013-2017, Bachelors of Science, Honors, in

Marine & Freshwater Biology

University of Guelph, 2017

Publications: NA

Conference Presentations:

Reeve, C., Speers-Roesch, B. 2019. Is Inactivity the convergent mechanism underlying the metabolic phenotype in winter dormant fish? (Oral). 2018. Atlantic Regional Comparative Physiology Workshop, St. Andrews, Canada.

Reeve, C., Rowsey, L., Speers-Roesch, B. 2019. Is Inactivity the convergent mechanism underlying the metabolic phenotype in winter dormant fish? (Oral). Society of Experimental Biology, Seville, Spain.

Reeve, C., Rowsey, L., Speers-Roesch, B. 2019. Is Inactivity the convergent mechanism underlying the metabolic phenotype in winter dormant fish? (Oral). Canadian Society of Zoologists, Windsor, Canada.

Reeve, C., Speers-Roesch, B. Behavioural responses to the cold in winter-dormant fishes (Oral). 2018. Atlantic Regional Comparative Physiology Workshop, St. Andrews, Canada.

Reeve, C., Rowsey, L., Speers-Roesch, B. 2018. Inactivity as the convergent mechanism underlying the metabolic phenotype in winter dormant fish. (Oral). Canadian Society of Zoologists, St. John's, Canada.

Reeve, C., Rowsey, L., Speers-Roesch, B. 2017. Is inactivity the central mechanism underlying the energetic savings of winter dormancy in fishes? (Oral). Atlantic Regional Comparative Physiology Workshop, St. Andrews, Canada.