

Testing Temperature Preference and Critical Thermal Maximum Across Walleye Populations

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Abstract

Intraspecific variation in fish, particularly temperature preference and critical thermal maximum (CT_{max}), can reflect the vulnerability of species to extreme warming events in the face of climate change. Using fingerling fish, we examined the temperature preferences of three Michigan walleye (*Sander vitreus*) populations across a latitudinal gradient using a shuttle-box arena. We then tested the CT_{max} of the same fish. We found no significant difference in temperature preference across the test populations; however, the CT_{max} of the northern population was about 0.6°C lower than one of the southern populations. We found several limitations in the use of shuttlebox experiments to reliably capture differences in thermal preferences for walleye. Additionally, we developed a new method for calculating preferred temperature which excludes periods where fish do not appear to regulate temperature. With this new method, we found an adjusted preferred temperature for Michigan walleye juveniles of $22.75 \pm 2.59^{\circ}\text{C}$ (mean \pm SD); this closely mirrors the reported temperature of optimal growth. The average estimated CT_{max} was $33.71 \pm 0.43^{\circ}\text{C}$. Results suggest that walleye from northern populations may be more vulnerable to extreme warming events than southern populations.

Keywords: Intraspecific variation, temperature preference, critical thermal maximum, thermal tolerance, walleye; *Sander vitreus*, Great Lakes Basin

Introduction

Anthropogenic climate change stands to drastically alter aquatic ecosystems (Meyer et al., 2007). Freshwater ecosystems are already experiencing a disproportionate decline in biodiversity yet are under-studied when compared to terrestrial and marine ecosystems (Collen et al., 2009; Di Marco et al., 2017). Modeling studies predict increasing water temperatures in freshwater lakes (Mortsch and Quinn, 1996; Sharma et al., 2007; Winslow et al., 2017). For example, average surface water temperatures in the Laurentian Great Lakes are expected to increase between 3 and 5°C by the end of the century (Hayhoe et al., 2010; Collingsworth et al. 2016). Other studies predict changes in thermal stratification, ice cover, water level, and thermal habitat (Kraemer et al., 2015, Woolway et al., 2021, Woolway et al., 2022). Warming water temperatures have far-reaching consequences for freshwater fishes, which comprise one-fifth of all vertebrate species on the planet (Helfman et al., 2009). As aquatic ectotherms, temperature fluctuations and stressors pose severe threats to fish since their body temperatures sync to the surrounding water temperatures and they are limited in their ability to disperse toward more favorable thermal habitats (Cossins & Bowler, 1987). Fishes are adapted to exist in certain thermal ranges, deviations from which can lead to declines in growth or fecundity, limited metabolic performance, and in some extreme cases, death (Neubauer and Anderson, 2019). Predicted future climate change will likely further test species' ability to adapt to altered conditions and may cause declines in abundance (Lynch et al., 2010; Prakash, 2021).

Intraspecific variation, among individuals or populations, in thermal traits may mitigate the impacts of climate change. Intraspecific variation can be driven by a variety of mechanisms including mutations or natural selection leading to genotypic differences (Des Roches et al., 2017, Doyle et al., 2011; Meffe et al., 1995; Perry et al., 2005). Variation within a population or species may also result from phenotypic plasticity, alternative expressions of genes caused by environmental pressures (Stearns, 1989). Developmental plasticity, where conditions during early life stages have persistent effects on phenotype, has been poorly studied relative to reversible plasticity, flexible changes in physiological phenotypes as a result of environmental exposures in the time range of days to months (Schulte et al., 2011; Le Roy, Loughland & Seebacher, 2017; McKenzie et al., 2020). Regardless of origin, intraspecific variation can cause significant differences in the ability of organisms to survive in their ecological niche or adapt to changes within their ecosystems. Past research supports the idea that these sources of variation influence thermal preference and tolerance among fishes and can impact population demography across generational time scales (Bennett, Duarte, Marbà & Wernberg, 2019; McKenzie et al., 2020). Further, intraspecific variation may allow some species to adapt and survive in warming waters (Dobosenski, Heald & Hrabik, 2022; Leggett & Carscadden, 1978). If populations differ in temperature tolerances, some may be more resilient to extreme warming events while the others may fall victim to mass mortality events. Over time, differences in temperature tolerance and preference may lead to the extirpation of less resilient populations. A lack of differentiation in thermal performance among populations might mean the entire species is equally susceptible to warming impacts. Moreover, understanding the mechanisms by which species adapt, via genetics or developmental plasticity, can guide implications for conservation and management of

species. Changes in management strategies could take the form of selectively stocking warming water bodies with populations resistant to extreme warming events or adjusting the rearing conditions of brood stock in order to influence their temperature preference.

Temperature preference and critical thermal maximum (CT_{max}) are two measures of thermal physiological traits that can help us understand a fish's sensitivity to warming temperatures. Temperature preference is the temperature range in which an organism congregates, or in which most time is spent (Reynolds and Casterlin, 1979). A fish's temperature preference often correlates with the temperature at which an organism's aerobic scope is largest or near largest (Cossins & Bowler, 1987). A large aerobic scope provides a fish with a greater ability to direct energy resources towards growth and reproduction compared to temperatures at which its aerobic scope is limited (Fry, 1971). Temperature preference also aligns with components of a fish's realized niche, revealing thermal guilds where it might successfully survive and reproduce (Magnuson, Crowder, and Medvick, 1979). It is unknown if temperature preference is a heritable trait or whether it has the capacity to adjust to long-term shifts in climate (Paranjpe et al., 2013). One study found that three-spine stickleback (*Gasterosteus aculeatus*) prefer 13°C water despite multigenerational exposure to geothermally warmed waters (Pilakouta et al., 2023). A fish's CT_{max} is the maximum temperature a fish can withstand before it experiences a loss of equilibrium and is a proxy for resilience to thermal stress (Cossins & Bowler, 1987; Sunday et al., 2014). CT_{max} is highly correlated with an organism's optimum growth temperature (Jobling 1981; Rodnick et al., 2004). This metric may reflect a fish's ability to withstand ever more common extreme warming events. Also, CT_{max} is a heritable trait, able to be passed down through several generations (Doyle et al., 2011; Meffe et al., 1995; Perry et al., 2005).

Our study seeks to determine whether walleye populations (*Sander vitreus*) differing in genetic background and rearing conditions possess differences in temperature preference and CT_{max}. Previous research on walleye has identified optimal temperatures for growth, thermal preferences, and thermal tolerances, however, none of these studies have examined variation in thermal metrics among populations (Lester et al., 2004; Hasnain et al., 2010). Other studies have examined the potential impacts of climate change on walleye including predicted shifts in walleye distribution and possible declines in abundance (Lynch et al., 2010; Hansen et al., 2017). Studying temperature preference and CT_{max} of Michigan walleye will provide us with better insight into whether climate change, including increasing water temperatures and more frequent extreme warming events, will differentially impact walleye populations. We hypothesize that walleye that originate from higher latitude populations and cooler rearing temperatures will have lower preferred temperatures and lower CT_{max}. Understanding intraspecific variation in walleye thermal performance could inform stocking and rearing practices by MDNR, providing mechanisms for improving the resilience of walleye populations to ongoing warming and extreme weather.

Methods and Materials

Study species and system

We chose walleye as our study species because of their history and management strategy in Michigan. Walleye are a game species with deep historical and cultural significance in the state of Michigan. They are native to the Laurentian Great Lakes and naturally reproducing walleye populations are found in many inland lakes in Michigan (Herbst et al., 2021). In addition, around 10.5 million walleye are currently stocked throughout the state annually (Herbst et al., 2021). Michigan Department of Natural Resources (MDNR) began stocking walleye throughout the state in 1882, and since 2000 state managers have maintained genetic diversity with annual egg-take operations from 10 brood sources (Herbst et al., 2021). These locations are selected for egg-take to sustain production and genetic integrity because they represent genetically distinct populations that are viewed as being representative of populations inhabiting the state's two different peninsulas (Herbst et al., 2021). The egg-take creates genetically distinct rearing groups while the growth of young of year in rearing ponds creates a gradient of environmental conditions ideal for testing for phenotypic plasticity caused by environmental factors.

Fish and Holding Facilities

Our experimental fish were hatched from two gamete sources, one in the Muskegon River (LP) (43.22°N) in the Lower Peninsula and the other in Little Bay de Noc (UP) in the Upper Peninsula (45.78°N). Fertilized eggs are reared at hatcheries, and after hatching, walleye are transported to over 30 rearing ponds across the state (Herbst et al., 2021). Our experimental fish were collected from three ponds spread across a latitudinal gradient. The coldest and northernmost pond was in the Upper Peninsula near the city of Menominee (hereafter referred to as MN) (45.22°N) and was stocked with walleye from the UP source hatched at the Thompson State Fish Hatchery. The remaining two ponds were in the Lower Peninsula and stocked with walleye from the LP population hatched at the Wolf Lake State Fish Hatchery. The intermediate latitude pond was near Bay City (BC) (43.66°N), and the southernmost pond was located near the city of Dearborn (DB)(42.58°N). Walleye grew in these ponds from April-June 2021 after which they were collected and transported to laboratory facilities at the University of Michigan in Ann Arbor, Michigan.

Lab Acclimation and Husbandry

Upon arrival at the lab, fish were isolated to check for disease and acclimated to study temperatures. The fish were quarantined for a period, during which holding temperatures were raised to 21 °C by adjusting temperatures by 1°C per day from the fish's pond temperature. We maintained a photoperiod of 16 hours of light and 8 hours of dark lasting from 11 PM to 7 AM. We fed walleye ad-libitum a combination of blood worm (*Glycera sp.*) and a commercially prepared pellet food (Mazuri® LS Aquatic Carni-Blend Diet 1mm) once daily (about 1-8% of body weight). Prior to starting experiments, walleye were acclimated for at least four weeks to 21°C, their optimal temperature for growth reported in the literature (Lester et al., 2004). We used recirculating 'zebra-fish racks' with 9.5 l tanks for holding fish in isolation for short-terms during fasting and recovery, before and after experiments. Individuals resided in the 9.5 l tanks

between 3-7 days between shuttlebox trials and critical thermal maximum trials and between critical thermal maximum trials and tissue sampling.

Design and Set Up of Shuttlebox and Critical Thermal Maximum Systems

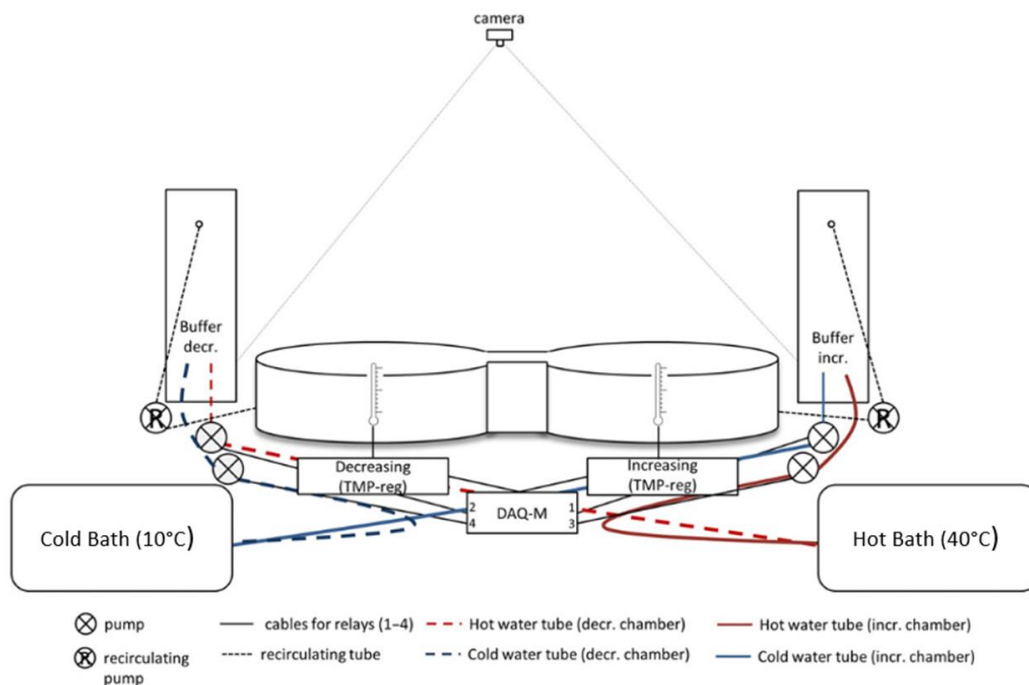


Figure 1. Design of shuttlebox system (chambers and buffers not shown to scale) (Macnaughton et al., 2018).

We performed temperature-preference experiments using a Loligo shuttlebox system (Figure 1), similar to previous studies (Killen, 2014). The system consisted of two cylindrical chambers (40 cm diameter) connected by a narrow channel (10 cm), allowing for the shuttling of fish between the two compartments in response to thermal stimulus (Loligo, Christensen, Norin, Tabak, van Deurs & Behrens, 2020). Continuous circular current was maintained in each chamber by pumping water from two raised buffer tanks. Each chamber was filled with water to a depth of approximately 8 cm. Air stones were placed in each buffer tank to increase dissolved oxygen concentration. Temperature probes connected to temperature regulation instruments recorded the temperature within each probe vessel, which were placed in-line between hot- and cold-water buffer tanks and shuttlebox chambers. We controlled water temperature in the shuttlebox vessels with a series of pumps connected to a DAQ-M instrument that was turned on and off by ShuttleSoft software (Loligo, Shuttle Box System Temperature, 2017). This system maintained temperature limits between 12 and 31°C, the latter of which is the upper lethal limit for walleye (Hasnain et al., 2010). We set the lower limit to 12°C because it was the lowest temperature we could consistently reach with our cold baths and was well below previously reported preferred

temperature for Walleye (Wismer and Christie, 1987; Danzmann et al., 1991; Hasnain et al., 2010). To prevent fish from jumping out of the experimental arena, a 1×1 cm-fine plastic mesh cover was added to the shuttlebox. For day and night recording by a uEye USB 2.0 camera, the arena was lit from above and with fluorescent lighting and below with fluorescent lighting scattered through translucent covers. A black-out curtain isolated the shuttlebox system from the rest of the laboratory to minimize photoperiod shifts and other disturbances (Shuttle Box System Temperature, 2017).

A camera was placed over the shuttle box to record fish position and allow ShuttleSoft to adjust temperature based on the location of the fish. The camera tracked fish position approximately every second for the duration of trials, relaying the location of the fish to ShuttleSoft. Using the software, we limited the rate of temperature changes to 4°C per hour. The system adjusted the temperature by adding water from the warm or cool reservoir depending on whether the fish occupied the chamber designated as “increasing” or “decreasing”. Fish regulated their ambient temperature by shuttling back and forth between chambers. When the fish occupied the cooler chamber, cold water was pumped into both chambers. Conversely, occupying the warmer chamber caused heated water to pump into the shuttlebox.

After measuring and weighing a fish, we placed the fish in the shuttlebox system to acclimate to the system for one hour. We selected the acclimation length of one hour as it is the median reported acclimation time in Christensen et al. (2021). Harman et al. 2020 also found different acclimation periods had no effect on temperature preference, though it affected variation within experimental groups. During the acclimation period, the temperature of the shuttlebox system was kept static, averaging 21°C with a 2°C buffer. After the acclimation period, the software was switched to dynamic mode, which allowed for the fish’s movement to change the system temperature as the overhead camera tracked the fish’s position. Each trial ran 23 hours. We selected 23 hours for trial length to allow enough time for fish to gravitate towards their preferred temperature multiple times if fish stopped regulating their temperature. Several studies demonstrated stable preference measurements within 24 hours of experimentation (Killen 2014; Macnaughton et al. 2018; Harman et al. 2020). At the onset of each trial, water temperature in each cooler chamber sat at 20°C while the warmer chamber’s temperature was 22°C . We maintained a temperature difference of 2°C between the chambers. Every week, we measured dissolved oxygen levels, water conductivity, salinity, pH, nitrogen levels, chlorine, and water hardness. We changed over 50% of the water by volume between trials, and drained the whole system every two weeks and washed it to remove microorganisms. The overhead camera captured fish position in the shuttlebox arena approximately every second for the entire trial duration. After trials concluded, fish were placed in the 9.5-L tanks and held for 3-7 days, during which time they were fed and reacclimated to 21°C . We successfully tested 30 fish in shuttlebox trials: 7 from the Dearborn rearing pond, 11 from the Bay City pond, and 12 from the Menominee pond. We excluded 18 shuttlebox trials due to trial failure. Trial failures include instances where the shuttlebox system failed to accurately track temperature, the camera failed to monitor fish position in the shuttlebox, or the death of a fish. Test subjects which were part of a failed trial were not subjected to a CTmax test. An additional 5 test subjects were removed

because they did not exhibit enough shuttling behavior required to compute adjusted temperature preference values (see more about this measure below).

After completing the thermal preference experiments, we tested the CTmax of each fish. One at a time, individuals were transferred to a 20-gal (dimensions 13 x 13 x 30 in) aerated aquarium filled with 21°C water. After fasting and acclimating to the experimental tank overnight, temperature was increased from 21°C at a rate of 0.3 °C per minute until each individual reached their CTmax (loss of equilibrium continuously for 5 s). Temperature was adjusted with a 1000W titanium immersion heater controlled by an Aqua Logic TR115SN Single Stage Digital Controller.

After reaching their CTmax, fish were euthanized by an overdose of MS-222. We then weighed each fish and measured the total length. Fish from the Bay City pond averaged 152.5 mm in length (SD = 11.43 mm) and 33.64 g (SD = 5.46 g). Fish from the Dearborn pond averaged 177.86 mm (SD = 12.35 mm) and 39.30 g (SD = 7.97 g). Fish from the Menominee pond averaged 187.58 mm (SD = 14.39 mm) and 44.10 g (SD = 11.62 g).

Data Analysis

Several metrics were calculated using data on fish position in the shuttle box across the experimental period. The movement of fish was recorded by counting the number of instances where a fish switched chambers. ShuttleSoft calculated the (minimum) total distance a fish swam over the trial duration (in cm) and the temperature preference (T_{pref}) of each fish as the median temperature occupied throughout the trial. Calculating temperature preference from the median selected temperature instead of the mean is useful when the trial data is skewed by unusual events, such as periods of little or no movement by test subjects (Schurmann et al., 1991; Christensen et al. 2021). The calculated total distance a fish swam was a minimum estimate, because the fish could take a circuitous route between successive camera shots, rather than the assumed straight-line movement.

Previous shuttlebox experiments in the literature often assume that test subjects actively regulate their temperature throughout the entire trial with perhaps a small gravitation time to reach their preferred temperature (Christensen et al., 2021). In order to separate active periods from irregular periods of sitting which were not associated with time of day or experimental duration (Figure 5 and see results below), we calculated an *adjusted preferred temperature* (*aT_{pref}*; in °C), which captured the preferred temperature of the fish when it actively regulated its temperature. We first designated periods of activity, or shuttling periods, as periods with half-hour moving windows in which the fish switched chambers at least 25 times; these periods contained evidence of the fish exhibiting temperature preference and selection. We then identified all shuttling periods in each dataset. Using the shuttling periods for each trial, we calculated the mean temperature each fish occupied across all shuttling periods, as recorded by ShuttleSoft, and took the mean of those values across all fish.

In order to determine the strength of relationships between the variables, we computed the correlation coefficients between length, weight, distance and all of our response metrics and ran Pearson correlation tests of the significance of correlation coefficients. We also ran a paired *t*-test between *T*_{pref} and *aT*_{pref} to look for differences between the metrics. We then performed linear regression between our response variables and potential covariates as our predictor variables. To determine if a fish's rearing pond influenced its length, weight, or distance traveled, we performed analyses of variance (ANOVAs). Finally, in order to examine differences in temperature responses across ponds, while considering potential covariates, we performed separate analyses of covariance (ANCOVAs) for each response variable: *T*_{pref}, *aT*_{pref}, and *CT*_{max}. We started each ANCOVA model including weight and distance traveled as well as interactions between rearing pond and weight, rearing pond and distance, and distance and weight. We then iteratively simplified our models to remove insignificant interactions. We performed TukeyHSD post hoc analyses of any significant models.

Finally, we performed four power analyses on unadjusted and adjusted temperature preference datasets. The first series determined if our sample sizes were large enough to reliably detect a difference in preference between ponds. The second set determined the needed sample size to achieve 80% power given our experimental means and variance. The third set of power analyses looked at the power level of a one- and two-degree difference between experimental groups given our experimental sample size (~8). The final set determined the sample size needed to achieve 80% power in three experimental groups with a one-degree difference in mean. We tested for the power to detect a one-degree difference across ponds because this would approximately reflect temperature differences across inland lakes in the Upper and Lower Peninsula (modeled lake surface water temperatures from 1961-1990 averaged 25.1°C in the LP and 23.9°C in UP; J. Breck, personal communication, modeled after Shuter et al. (1983)). All analyses were performed in Rstudio using the *car*, *multcomp*, *stats*, and *Superpower* packages (Rstudio Team, 2022).

Results

Rearing Pond	Length (mm)	Weight (g)	Preferred Temperature (°C)	Adjusted Preference (°C)	CT_{max} (°C)	Mean Distance Traveled (m)	Shuttling Count
Dearborn	177.86 (12.35)	39,30 (5.46)	20.00 (6.29)	23.64 (2.68)	33.73 (0.39)	892.06 (833.25)	1902.45 (1891.4)
Bay City	152.5 (11.43)	33.64 (5.46)	20.63 (4.62)	21.82 (2.80)	33.76 (0.44)	1544.63 (1162.99)	803 (767.82)
Menominee	187.58 (14.39)	44.10 (11.62)	21.39 (6.17)	23.27 (2.24)	33.64 (0.48)	1693.27 (1496.72)	872.83 (775.37)

Table 1. Summary statistics of measured variables. Standard deviations are in parentheses.

Fish weight and length were strongly correlated ($r = 0.93$), as expected. Consequently, we used only weight, as a size covariate, in further analyses (Figure 2). Unadjusted preferred temperature (T_{pref}) was moderately correlated with its adjusted preferred temperature (aT_{pref}) ($r = 0.68$), and total distance traveled ($r = 0.60$) (Appendix Figure A1). aT_{pref} was moderately correlated with total distance traveled ($r = 0.45$). CT_{max} did not have a correlation higher than 0.3 with any variable.

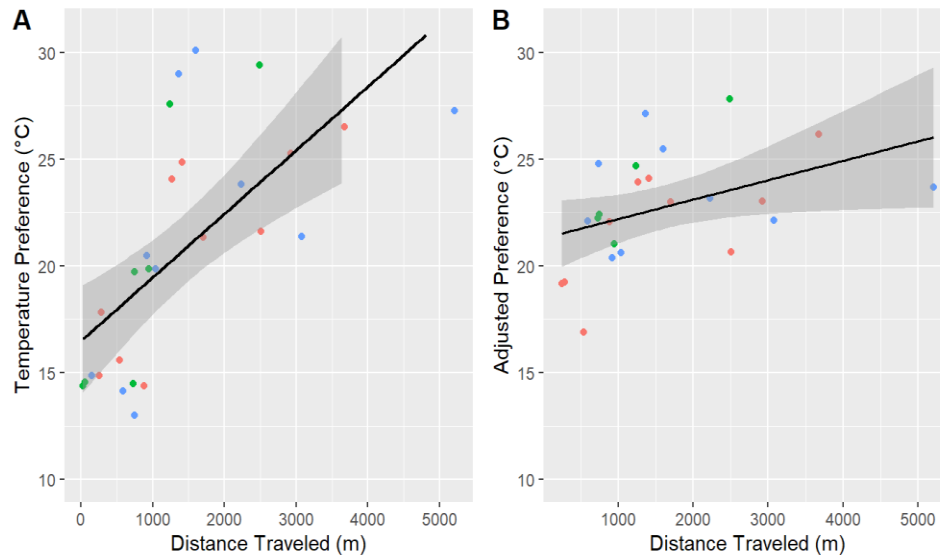


Figure 2. Scatter plots of distance traveled by fish during trial with (A) T_{pref} preference and (B) aT_{pref} . The color of the symbols indicates the rearing pond location: red is Bay City; blue is Menominee; green is Dearborn.

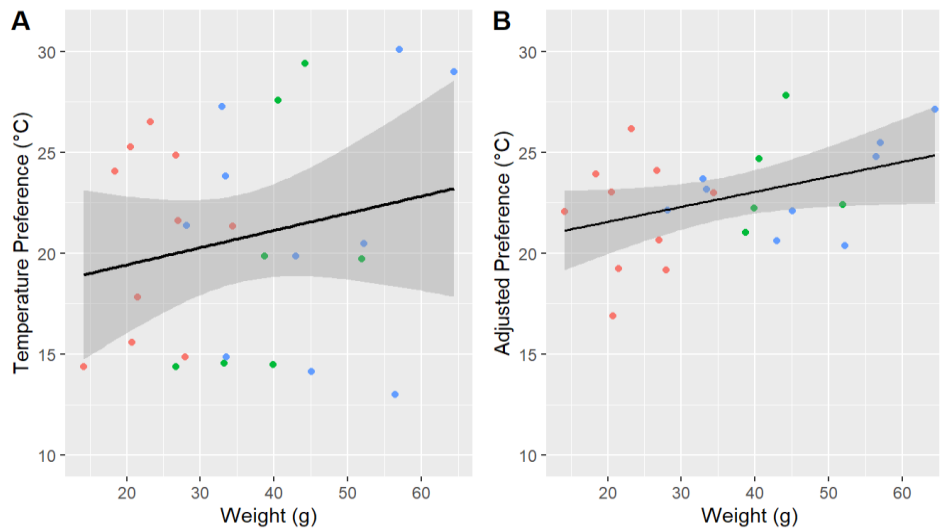


Figure 3. Scatter plots of fish weight and (A) T_{pref} and (B) aT_{pref} . The color of the symbols indicates the rearing pond location: red is Bay City; blue is Menominee; green is Dearborn.

Average T_{pref} was 20.75°C ($sd = 5.48^{\circ}\text{C}$) between the three stocks, aT_{pref} was 22.75°C ($sd = 2.59^{\circ}\text{C}$), and average CT_{max} was 33.71°C ($sd = 0.43^{\circ}\text{C}$). T_{pref} was 20.00°C ($sd = 6.29^{\circ}\text{C}$), 20.63°C ($sd = 4.62^{\circ}\text{C}$), and 21.39°C ($sd = 6.17^{\circ}\text{C}$) for the DB, BC, and MN stocks, respectively. The aT_{pref} was 23.64°C ($sd = 2.68^{\circ}\text{C}$), 21.82°C ($sd = 2.80^{\circ}\text{C}$), and 23.27°C ($sd = 2.24^{\circ}\text{C}$) for the DB, BC, and MN stocks, respectively. CT_{max} values were 33.73°C ($sd = 0.39^{\circ}\text{C}$), 33.76°C ($sd = 0.44^{\circ}\text{C}$), and 33.64°C ($sd = 0.48^{\circ}\text{C}$) for the DB, BC, and MN stocks, respectively (Figures 2 and 3).

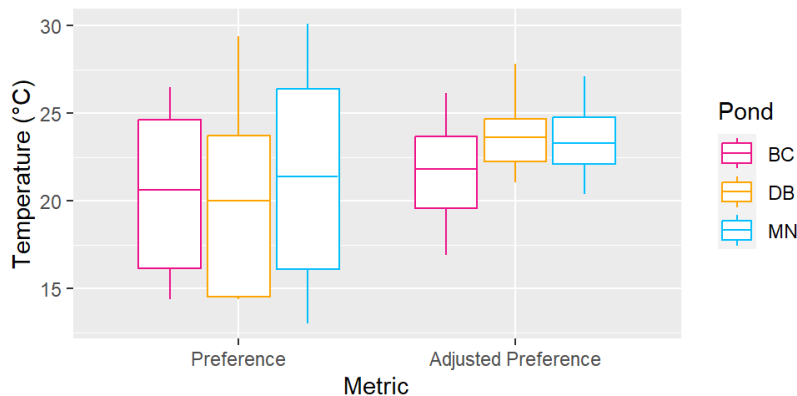


Figure 3. Boxplots of response variables sorted by rearing pond. Center lines represent the mean of each pond.

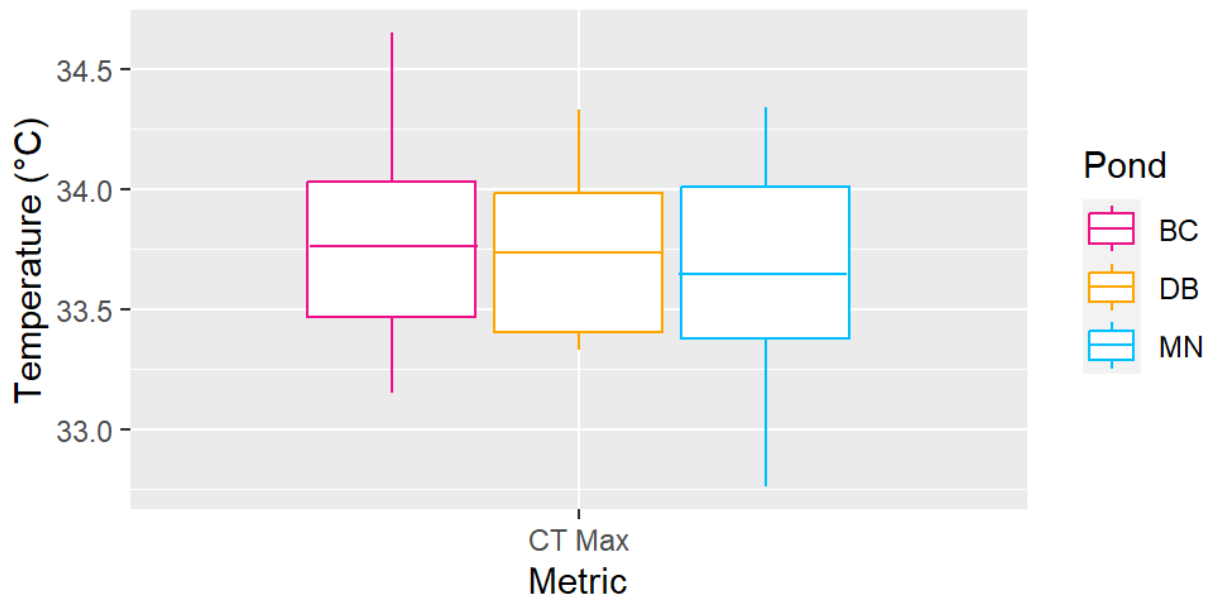


Figure 4. Boxplots of CT_{max} results sorted by rearing pond. Center lines represent the mean.

Fish exhibited a variety of activity levels during preference trials. Distance swam during preference trials ranged from 31.11 to 5203.02 m with a mean of 1430.50 m and a standard deviation of 1230.23 m. No statistically significant differences in distance swam existed among

rearing ponds (p -value = 0.406). We found a positive relationship between distance swam and both T_{pref} and aT_{pref} (p -values 0.0001 and 0.0407, respectively). For each additional 100 m swam during a preference trial, T_{pref} increased by 0.298°C and aT_{pref} increased by 0.0908°C .

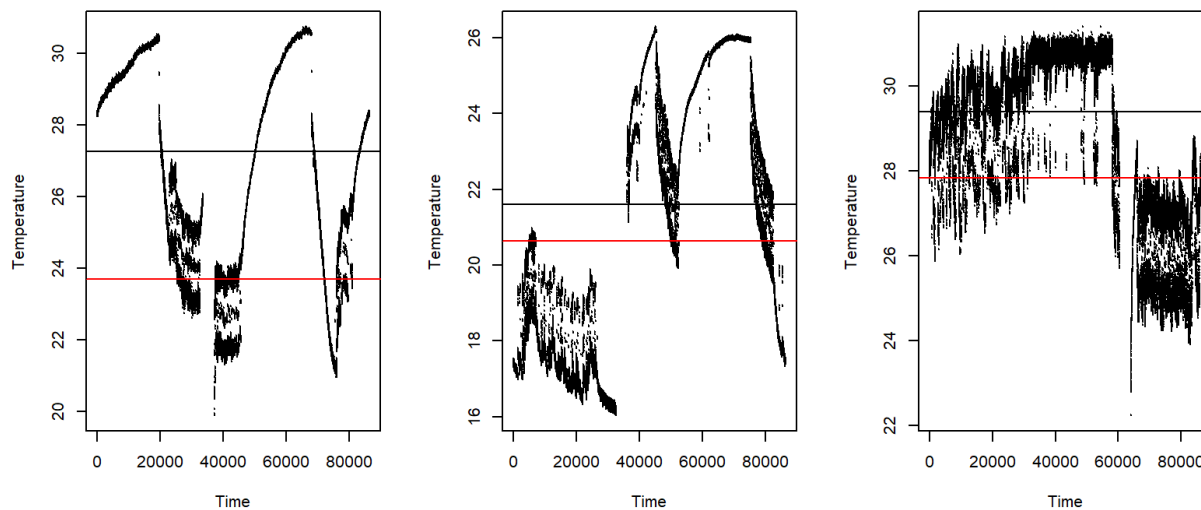


Figure 5. Examples of the behavior spectrum. From left to right: shy, semi-bold, and bold fish. Black horizontal lines represent T_{pref} . Red horizontal lines represent aT_{pref} . Shuttling periods appear as thick bands indicating movement between chambers which had a two-degree temperature differential.

We found no significant interactions among predictors. After running a simplified ANCOVA with T_{pref} as our response variable, we found no statistically significant difference in preference between rearing ponds. The p -value of the difference between the BC and DB ponds was 0.3612 while the p -value of the relationship between the BC and MN was 0.0699. However, there were significant positive relationships between a fish's weight and T_{pref} (p -value = 0.005) and distance traveled and T_{pref} (p -value < 0.0001). For each gram and meter increase in weight and distance traveled, respectively, T_{pref} increased by 0.234°C and $3.396 \times 10^{-5}^{\circ}\text{C}$, respectively.

The results from our analysis of adjusted preferred temperature followed a similar pattern to T_{pref} . There were no statistically significant interaction effects between rearing pond and weight (p -value = 0.391) and distance (p -value = 0.304) in the aT_{pref} ANCOVA. The simplified ANCOVA showed no significant difference in aT_{pref} among the rearing ponds (p -value of relationship between BC and DB pond = 0.6486, p -value of relationship between BC and MN ponds = 0.1514) though weight and distance traveled both possessed statistically significant relationships with aT_{pref} (p -values of 0.005 and < 0.001, respectively); each unit increase in weight (g) and distance traveled (cm) corresponded to increases in aT_{pref} by 0.15°C and $1.36 \times 10^{-5}^{\circ}\text{C}$.

Table 2. Final simplified ANCOVA of the effect of rearing pond and weight (g) on walleye $T_{pref}(^{\circ}C)$

	Estimate	Std Error	t-value	Pr(> t)
Intercept	9.87	2.64	3.744	0.0011
DB Rearing Pond	-2.13	2.28	-0.931	0.3612
MN Rearing Pond	-4.69	2.46	-1.905	0.0699
Weight	0.23	0.08	2.789	0.0107
Distance	0.003	<0.001	5.356	<0.0001

We also found no significant interaction between weight and rearing pond in predicting CT_{max} (p -value = 0.999). After running a simplified model, we found significant differences in the CT_{max} between the MN and BC rearing ponds (p -value = 0.0141). CT_{max} was $0.65^{\circ}C$ lower for MN fish than BC fish ($SE = 0.244^{\circ}C$) after accounting for size. Weight significantly increased with CT_{max} (p -value = 0.0113). For every 1-g increase in weight, CT_{max} increased by $0.02^{\circ}C$.

Table 3. Final simplified ANCOVA of the effect of rearing pond and weight (g) on walleye $aT_{pref}(^{\circ}C)$

	Estimate	Std Error	t-value	Pr(> t)
Intercept	16.18	1.73	9.356	<0.0001
DB Rearing Pond	-0.70	1.51	-0.463	0.6486
MN Rearing Pond	-2.35	1.57	-1.495	0.1514
Weight	0.15	0.05	2.780	0.0119
Distance	<0.001	< 0.001	3.476	0.0025

Our power analyses showed that we would need more replicates to detect a significant difference in T_{pref} and aT_{pref} among ponds given our experimental data. Using the estimated marginal means from our models, which account for differences in size and distance traveled, we observed the largest differences in each preference metric when comparing fish from BC and MN ponds

(which differed by 4.7°C in T_{pref} and 2.4°C in aT_{pref}). Our trials involved 12, 11 and 7 replicates from the MN, BC and DB ponds, respectively. Our power to detect a difference in T_{pref} between BC and MN ponds was only 35.89%; to reach 80% power would require 23 replicates from each of the MN and BC ponds. Our power to detect a difference between BC and MN ponds in aT_{pref} was 40.77%; to reach 80% power, would require 20 replicates from each of the MN and BC ponds.

Table 4. Final simplified ANCOVA of the effect of rearing pond and weight (g) on walleye CT_{max} (°C)

	Estimate	Std Error	t-value	Pr(> t)
Intercept	33.26	0.24	140.41	<0.0001
DB Rearing Pond	-0.44	0.24	-1.863	0.0743
MN Rearing Pond	-0.65	0.25	-2.639	0.0141
Weight	0.02	0.01	2.733	0.0113

Discussion

Examining juvenile walleye which are grown for stocking, we found significant differences in thermal tolerance (CT_{max}) between fishes from different genetic stocks which also experienced different rearing conditions (Table 4, Figure 4). We found no difference in CT_{max} between DB and BC fish, both of which originate from the same genetic stock (Muskegon River), suggesting plasticity (or at least these different rearing conditions) may play a limited role in determining thermal tolerance. Conversely, we found a significant difference ($p = 0.014$) of 0.61°C between BC and MN fish, which originate from separate genetic stocks (Table 4). There is previous research linking thermal tolerance to genetic variation. Evidence of heritable differences in thermal tolerance have been found among separate populations of sockeye salmon (Chen et al., 2013). Differences in thermal tolerance are usually linked to genetic adaptations. Our experimental fish came from two populations separated by 150 miles in Lake Michigan, but walleye occurs from Alabama to the Northwest Territories of Canada (Page and Burr, 1991). Our results suggest that a larger latitudinal gradient may lead to greater differences in CT_{max} and should be tested in the future.

The positive relationship we found between weight and CT_{max} has not, to our knowledge, previously been reported (0.024°C increase in CT_{max} / g increase in weight; p -value = 0.006). In contrast, prior research investigating the relationship between size and thermal tolerance found a

negative relationship (Atkinson, 1994). As fish increase in size, their tolerance to higher temperatures decreases (Leiva et al., 2019) and is thought to be the result of a difference between oxygen requirements and the capacity of the cardiovascular system to transport oxygen (Pörtner and Knust, 2007). Additionally, organisms with faster growth rates are more sensitive to warmer temperatures and possess lower thermal tolerances (Roze et al., 2013). Since differences in growth rates can reflect trade-offs between somatic and reproductive growth and the fish used in our experiments were juveniles, the relationship between CT_{max} and growth rate observed by Roze et al. (2013) could be related to the fish's age.

While we did not identify significant differences in walleye thermal preference among rearing ponds, we did observe more than two degrees difference in the estimated marginal means of northern and southern populations (Table 5). The lack of statistically significant differences in preference among ponds could be the result of our small sample size as our power analysis found that increasing replication to around 20 individuals per sample group would be necessary to reliably detect differences in thermal preference. If a two-degree difference in thermal preference truly exists within Michigan walleye, these differences could have significant implications for thermal habitat occupation, metabolic rate, and growth. Additionally, other work has demonstrated differences in metabolic rates and aerobic scope across Michigan walleye (Jackson et al., in review).

Table 5. Table of estimated marginal means after accounting for differences in weight (g) and distance traveled (m).

Pond	Estimate (°C)	SE	CI lower bound	CI upper bound	Weight (g)	Distance Traveled (m)
BC	23.8	0.94	21.8	25.7	35.9	1599
DB	23.1	0.98	21.0	25.1	35.9	1599
MN	21.4	0.90	19.5	23.3	35.9	1599

Differences in thermal preference may be driven by genetic variation and selection through time, phenotypic plasticity mediated by environmental conditions, or maternal effects. Overall, previous research in ectotherms has failed to find differences in thermal preference driven by genetic adaptation. A study using stickleback in geothermally warmed water found that thermal preference did not change from the ancestral state even after several centuries (Pilkouta et al., 2023). Researchers testing the thermal preference of brown anole lizards (*Anolis sagrei*) found no differences in and no heritability of thermoregulatory behavior in two populations from contrasting thermal environments (Logan et al., 2018). While studying side-blotched lizards (*Uta stansburiana*), Paranjpe et al. (2013) found that maternal effects, rather than genetic variation, determine offspring's thermal preference. Similarly, researchers studying thermal tolerance and preference in *Drosophila subobscura* found low heritability of thermal preference, suggesting a low evolutionary response for the trait (Castañeda et al., 2013).

Interestingly, individual walleye appear to have highly variable activity levels which seem to impact their thermal preference and our ability to measure that preference. Based on in-situ

observations, as well as the shuttling behavior and distance traveled metrics, walleye exhibited a variety of activity levels across a continuum. On one end of the spectrum are “bold” fish, who actively shuttled between chambers, regulating their temperatures for the majority of trials. On the other end of the spectrum lie “shy” fish, who regulated their temperatures less than half of the time; some of which moved hardly at all. Previous studies have described similar archetypes in which bold fish exhibit higher amounts of exploratory and foraging behavior compared to shy fish (Sneddon, 2003; Jolles et al., 2019). Other research suggests that variable behavior between individuals may be the result of differences in metabolism (Rupia et al. 2016). In our experiments, reductions in activity level occurred at both high and low temperature extremes ($< 14^{\circ}\text{C}$ and $< 28^{\circ}\text{C}$). Additionally, we found significant variation in activity level (mean distance traveled in 23 h = 1430.5 m, SD = 1230.2 m, a mean swimming speed of 1.03 m/min, but we found no differences in boldness among ponds, as indicated by these measures. T_{pref} and aT_{pref} , however, increased with distance traveled (p -value < 0.001 in analyses of unadjusted and adjusted preference) suggesting that bold fish may have higher preferred temperatures. Telemetry research in the Laurentian Great Lakes has shown some Walleye will migrate long distances, while others do not, suggesting that Walleye vary in migratory and perhaps exploratory behaviors (McKee et al., 2022). Studying differences in behavioral profiles and their impacts on thermal preference as well as determining if differences in behavior exist between populations is another potentially fruitful area of research.

It is possible that walleye activity in our experiments is not representative of natural behaviors. Our experiments maintained constant light levels of around 1000 lux over 24 h to facilitate movement tracking, which relied upon contrast between the fish and their background. Walleye are usually most active during twilight periods and reduce movement in clear water when exposed to light levels above 800 lux (Ryder, 1977). Further, in our shuttlebox some lights were below the tank, so some light came from below, an unnatural direction that may have affected Walleye behavior.

In order to address variable activity levels in our experimental fishes, we developed a new metric of thermal preference. A recent literature review found that the mean, median, and mode object temperatures are among the many metrics previously used to describe thermal preference (Christensen et al., 2021). None of the previous metrics, however, distinguish between periods of active shuttling and periods of inactivity (energy conservation). Not accounting for inactivity can bias measures of thermal preference and increase variability within trial groups, reducing the power of experiments and increasing the number of replicates needed (Macnaughton et al., 2018). Our aT_{pref} metric removed periods where fish are not actively regulating their temperature. While the differences between T_{pref} and aT_{pref} were statistically insignificant (paired t -test p -value = 0.149), aT_{pref} better matched previously reported temperature preference of 22.5°C (Wisner and Christie, 1987; Danzmann et al., 1991; Hasnain et al., 2010; mean aT_{pref} was 22.75°C compared to mean T_{pref} of 20.75°C). Moreover, there was noticeably less variation within aT_{pref} (SD = 2.59°C) in comparison to T_{pref} (SD = 5.48°C).

Understanding the differences in fish thermal tolerance and preference can have important implications for fisheries management and climate resilience. Differences in CT_{max} suggest that

northern walleye may be a little more vulnerable to extreme warming events than southern walleye in Michigan. As climate continues to warm and extreme warming events become more common, managers might consider stocking southern genetic stock further north as they will be more well adapted to these conditions. Differences in the thermal preferences between ponds are less clear; however, if differences do exist, these differences could be used to inform the types of lakes where stocking might be most successful. Lakes with smaller surface areas less than 163 acres and average depths shallower than 5 m are more sensitive to warming than larger, deeper lakes (Wehrly et al., 2012). Increasing water temperatures in these smaller lakes may cause walleye to migrate away from the nearshore in search of preferred thermal habitats (Coutant, 1987; Hofman and Fischer, 2002). Conversely, walleye may instead choose to continue to reside in water outside of their thermal preference and optimum, potentially leading to increases in the standard metabolic rate, decreases in growth rates, lowering of their aerobic scopes, and therefore reducing their fitness. Lakes with larger surface areas and greater average depths will be more resistant to warming, as they have greater thermal inertia (Woolway et al., 2016). A significant proportion of stocked lakes support little or no walleye recruitment, and the amount of thermally suitable lakes for walleye are expected to decrease by 2070 (Van Zuiden and Sharma, 2016; Herbst et al., 2021). Walleye recruitment and abundances in inland Wisconsin lakes have decreased and are expected to further decrease (Hansen et al., 2017). Declines in recruitment and abundance related to warming temperatures may lead to an increased reliance on stocked walleye if more lakes become unable to support natural recruitment. Better understanding and documentation of when lakes become unsuitable for walleye will help fish biologists inform the expectations of anglers and reduce the stocking of unsuitable lakes. Therefore, it is important to continue investigating the relationship between the variation of thermal traits in walleye and their underlying mechanisms.

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Appendix.

Figure A1. Correlation matrix of study variables.

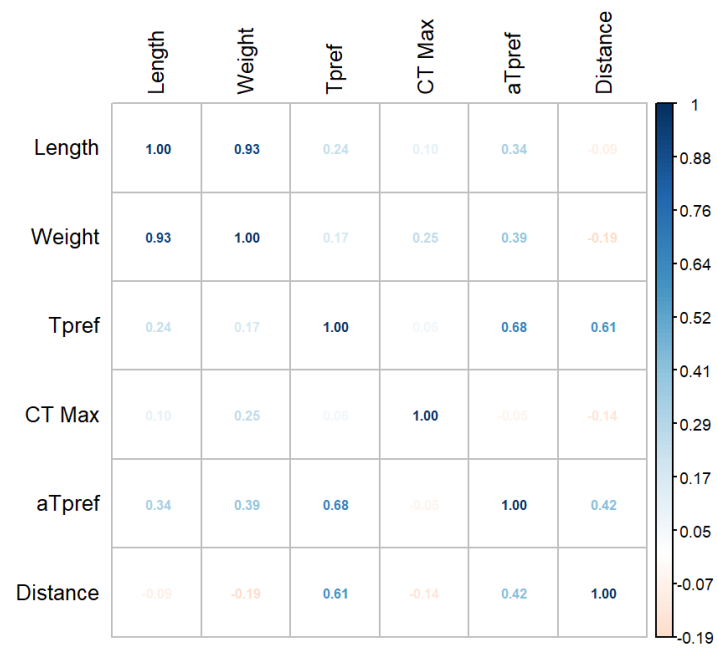
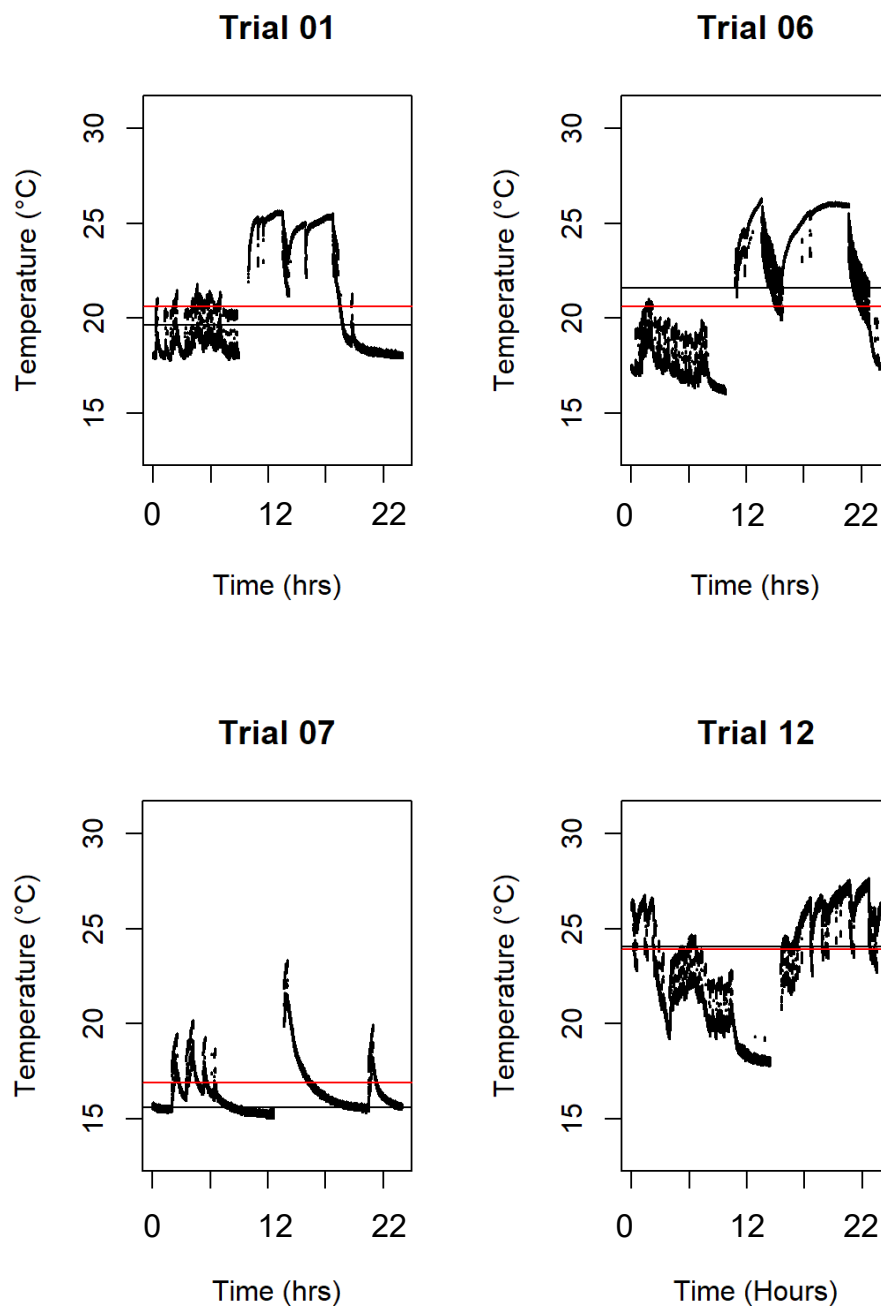
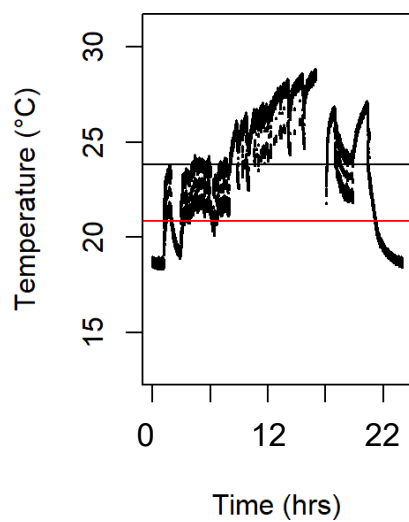
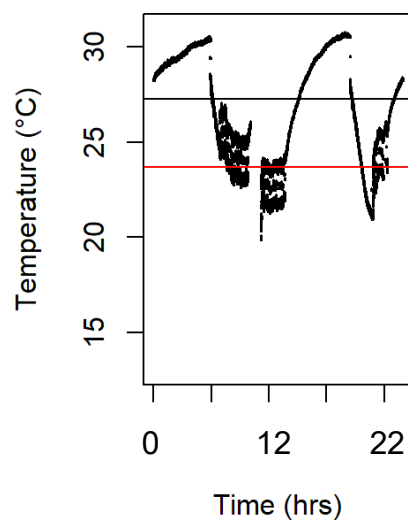
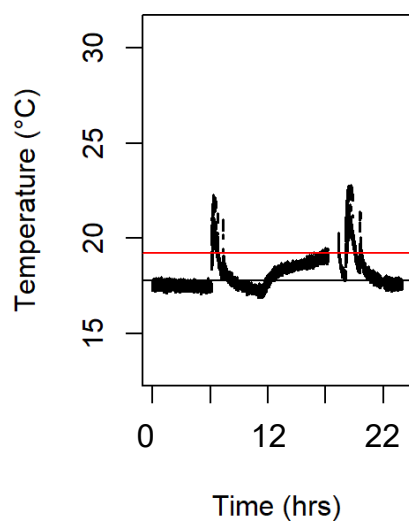
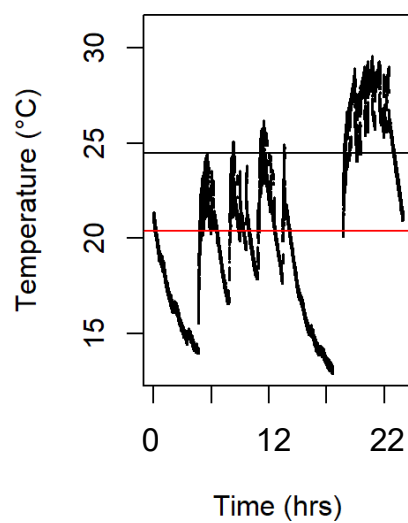
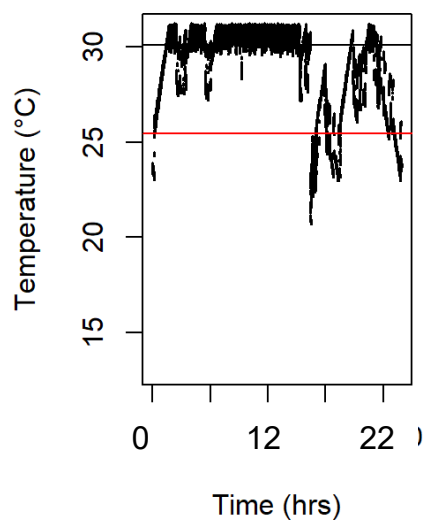
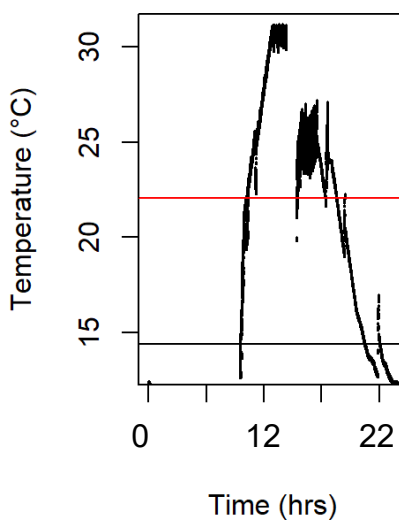
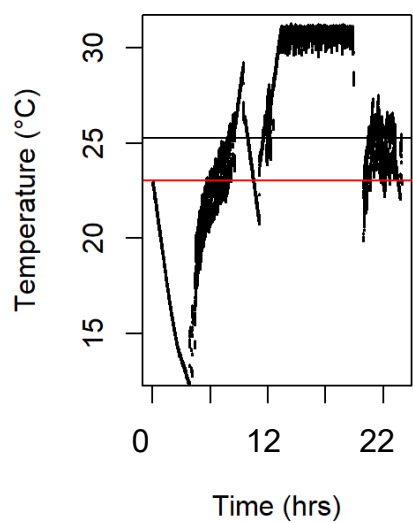
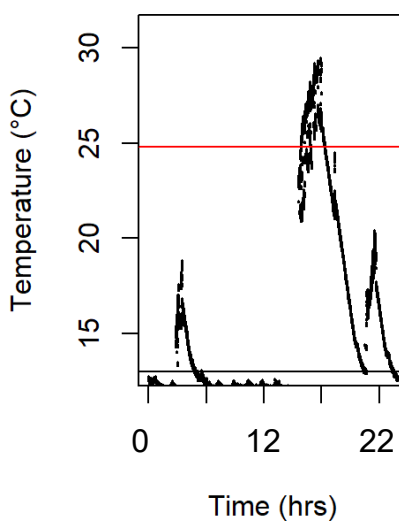
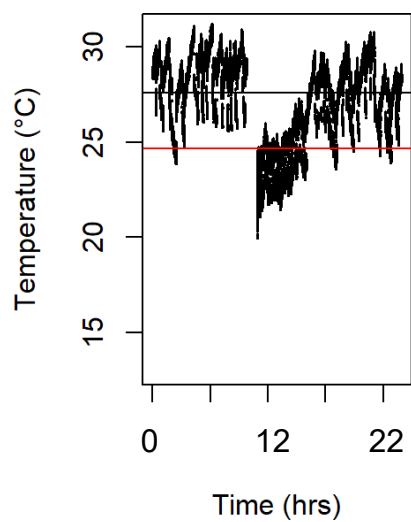
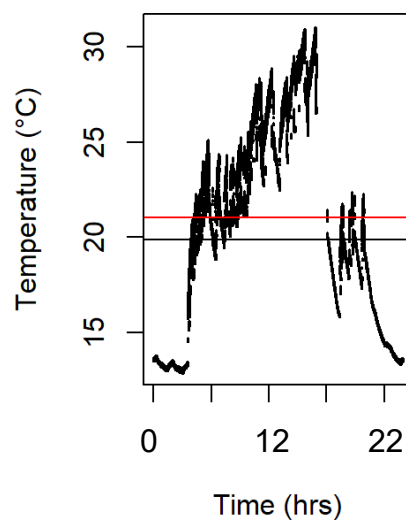
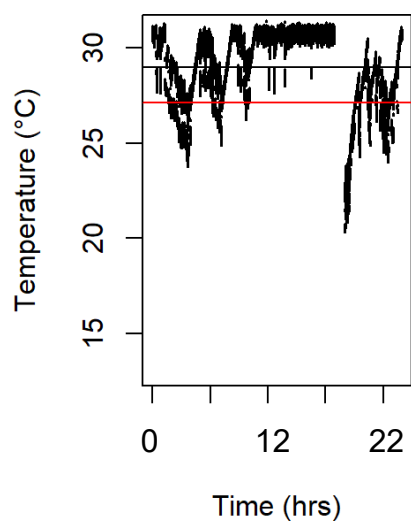
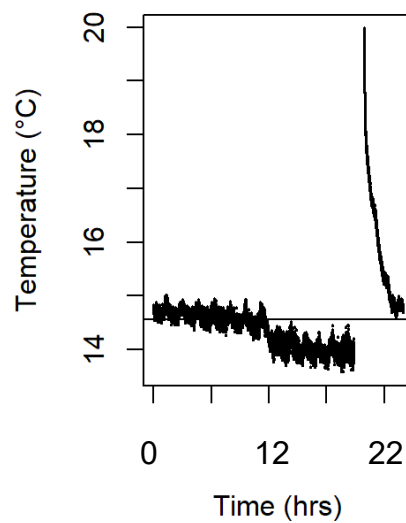


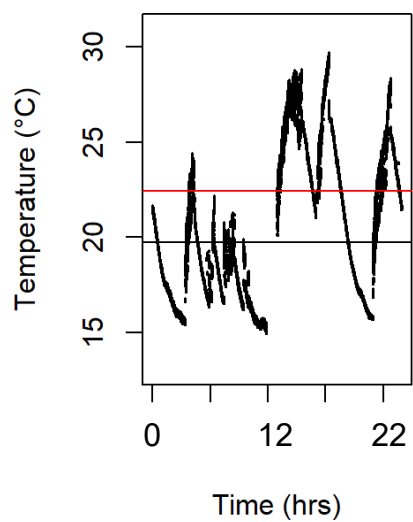
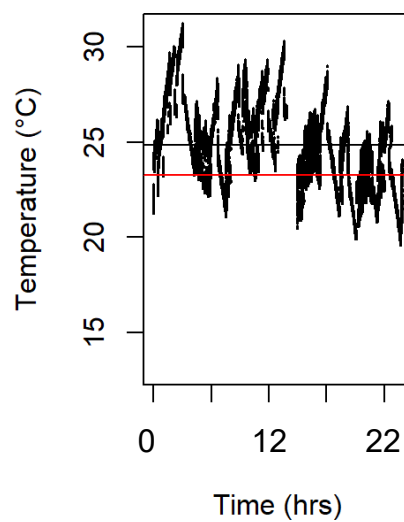
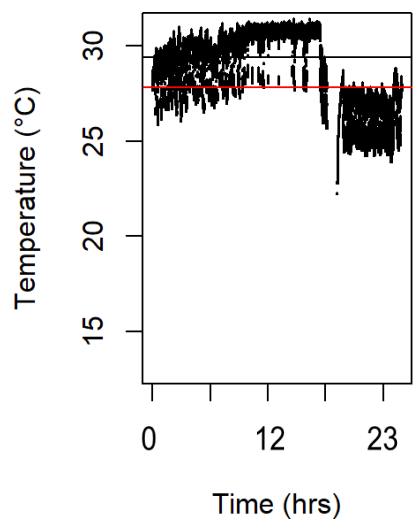
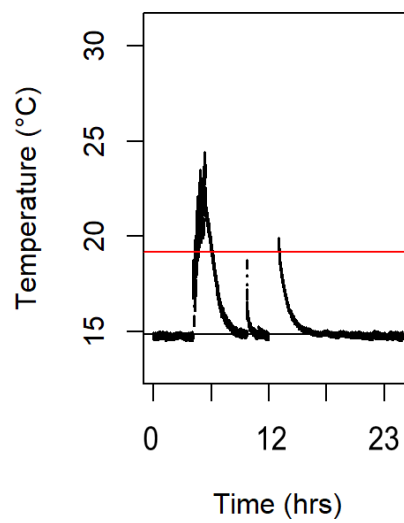
Figure A2. Plots of water temperature versus time for the shuttlebox trials of juvenile Walleye. Fertilized eggs came from two different source populations in Michigan (Little Bay de Noc and Muskegon River), were hatched in two different hatcheries, and reared in three different rearing ponds.

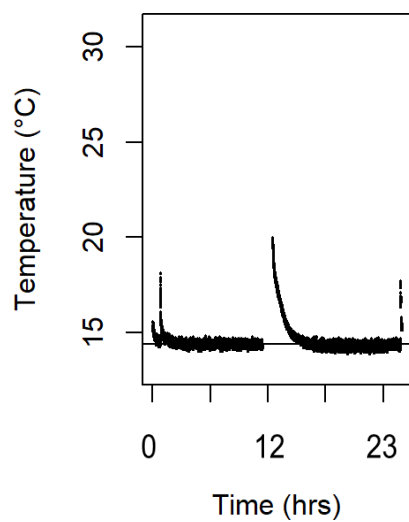
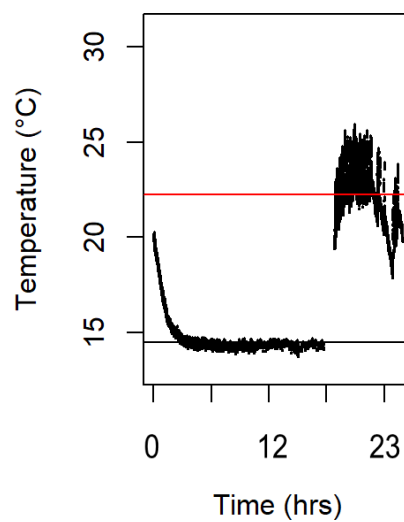
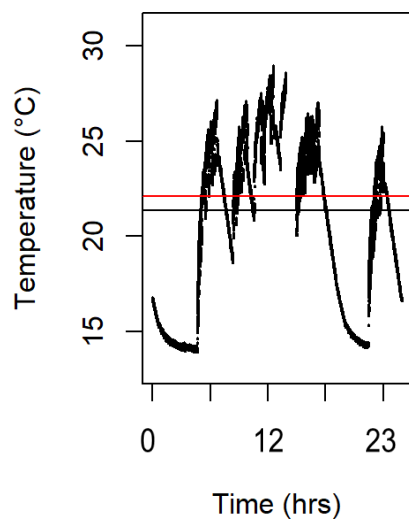
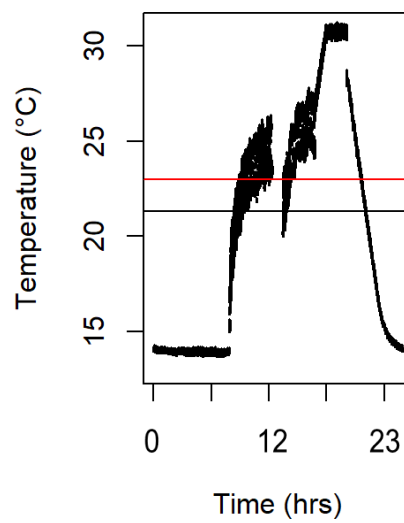


Trial 13**Trial 15****Trial 16****Trial 22**

Trial 24**Trial 26****Trial 28****Trial 29**

Trial 31**Trial 33****Trial 34****Trial 35**

Trial 36**Trial 37****Trial 38****Trial 39**

Trial 41**Trial 42****Trial 44****Trial 45**

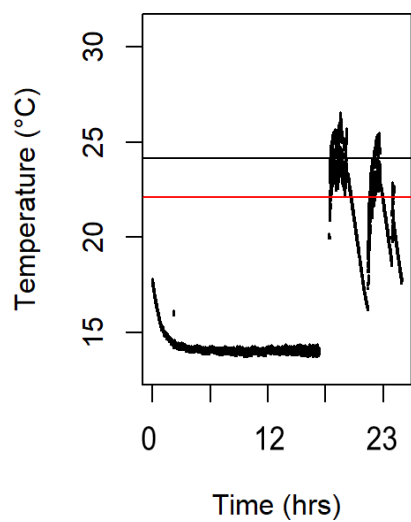
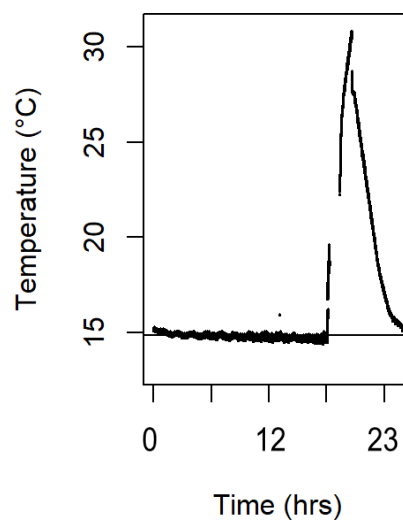
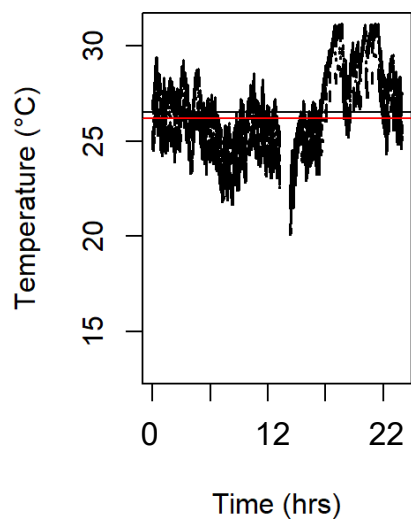
Trial 46**Trial 47****Trial 48**

Table A1. Data table of study variables for each trial.

Trial Number	Rearing Pond	Length (mm)	Weight (g)	Tpref (°C)	CT max (°C)	aT_{pref} (°C)	Distance (m)
1	MN	181	42.9	19.87	33.98	20.62	1043.28
6	BC	160.5	27.01	21.61	34.65	20.64	2508.04
7	BC	149	20.75	15.59	34.17	16.90	537.82
8	BC	148	21.35	21.09	33.3	20.86	621.02
12	BC	141	18.45	24.06	34.02	23.92	1270.16
13	MN	173	33.45	23.82	33.71	23.17	2229.94
15	MN	175	32.91	27.26	33.61	23.69	5203.82
16	BC	146	21.52	17.81	33.62	19.25	286.71
19	MN	199	47.67	12.55	33.19	14.45	530.08
22	MN	196	52.18	20.49	34.1	20.38	920.30
24	MN	204	56.97	30.1	33.44	25.46	1599.89
26	BC	133	14.19	14.38	33.32	22.07	884.33
27	MN	182	35.59	22.77	33.88	22.84	592.10
28	BC	147	20.57	25.26	33.61	23.02	2922.04
29	MN	174	56.4	12.99	34.12	24.79	743.62
31	DB	182	40.55	27.58	33.85	24.67	1237.17
33	DB	171	38.69	19.86	34.12	21.03	949.78
34	MN	214	64.37	29	34.34	27.12	1367.32
35	DB	169	33.2	14.56	33.46	NA	57.23
36	DB	198	51.87	19.72	33.7	22.42	750.24
37	BC	163	26.73	24.84	33.15	24.11	1408.73
38	DB	181	44.19	29.39	34.33	27.83	2491.44
39	BC	164	27.94	14.87	34.04	19.17	253.77
41	DB	160	26.68	14.39	33.35	NA	31.11
42	DB	184	39.9	14.47	33.33	22.25	727.45
44	MN	172	28.17	21.36	33.04	22.12	3080.69
45	BC	172	34.42	21.33	33.89	22.99	1701.58
46	MN	201	45.09	14.15	33.55	22.10	590.85
47	MN	180	33.48	14.87	32.76	NA	153.00
48	BC	154	23.18	26.5	33.61	26.18	3673.17