Determining the thermal preferences of Carmine Shiner (*Notropis percobromus*) and Lake Sturgeon (*Acipenser fulvescens*) using an automated shuttlebox

J.A. Stol, J.C. Svendsen, and E.C. Enders

Oceans and Science
Central and Arctic Region
Fisheries and Oceans Canada
Freshwater Institute
Winnipeg, MB
R3T 2N6

2013

Canadian Technical Report of Fisheries and Aquatic Sciences 3038

Fisheries and Oceans
Pêches et Océans

Canada

Canadian Technical Report of
Fisheries and Aquatic Sciences 3038

2013

DETERMINING THE THERMAL PREFERENCES OF CARMINE SHINER (*Notropis percobromus*) AND LAKE STURGEON (*Acipenser fulvescens*) USING AN AUTOMATED SHUTTLEBOX

by

J.A. Stol[1], J.C. Svendsen[2], and E.C. Enders[2]

Oceans and Science, Central and Arctic Region
Fisheries and Oceans Canada
Freshwater Institute
Winnipeg, MB
R3T 2N6

---

[1] School of Environment and Sustainability, Royal Roads University, Victoria, BC, V9B 5Y2
[2] Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB, R3T 2N6

Cat. No. Fs 97-6/3038E   ISSN 1488-5379

Correct citation for this publication:

Stol, J.A., Svendsen, J.C. and Enders, E.C. 2013. Determining the thermal preferences of Carmine Shiner (*Notropis percobromus*) and Lake Sturgeon (*Acipenser fulvescens*) using an automated shuttlebox. Can. Tech. Rep. Fish. Aquat. Sci. 3038: vi + 23 p.

ABSTRACT

Stol, J.A., Svendsen, J.C. and Enders, E.C. 2013. Determining the thermal preferences of Carmine Shiner (*Notropis percobromus*) and Lake Sturgeon (*Acipenser fulvescens*) using an automated shuttlebox. Can. Tech. Rep. Fish. Aquat. Sci. 3038: vi + 23 p.

This report summarizes the results of a series of laboratory-based behavioural temperature preference experiments using an automated electronic shuttlebox system. We tested two species of fish: Carmine Shiner (*Notropis percobromus*) and Lake Sturgeon (*Acipenser fulvescens*). These species were selected because they are either listed or under consideration for listing under the Species at Risk Act, and because they represent two very different taxonomic groups that have distinct evolutionary histories, behaviours, and body forms.

Carmine Shiner appeared to behaviourally thermoregulate in the shuttlebox. The mean preferred temperature was 23.6 ± 1.4 ºC. This temperature is within the range experienced by Carmine Shiner in their natural environment. Due to their small size and highly mobile nature, Carmine Shiner were a suitable species to test in the shuttlebox system, and further studies could be completed to test the thermal preference under a variety of conditions.

The preferred temperature of Lake Sturgeon could not be determined using the shuttlebox. This species did not behaviourally select a preferred temperature within the range that was achievable in this study (approximately 9 - 22 ºC), possibly due to a wide temperature tolerance range. It is also possible that although this species exhibits behavioural thermoregulation in the natural environment, the individuals did not learn to behaviourally thermoregulate in the shuttlebox environment in the time frame of the experiment.

RÉSUMÉ

Stol, J.A., Svendsen, J.C. and Enders, E.C. 2013. Déterminer les préférences thermiques de la tête carmin (*Notropis percobromus*) et de l'esturgeon jaune (*Acipenser fulvescens*) en utilisant un «shuttlebox» automatisé. Can. Tech. Rep. Fish. Aquat. Sci. 3038: vi + 23 p.

Ce rapport résume les résultats d'une série d’expériences en laboratoire basés sur la préférence thermique à l'aide d'un système électronique automatisé «shuttlebox». Nous avons testé deux espèces de poissons: La tête carmin (*Notropis percobromus*) et l'esturgeon jaune (*Acipenser fulvescens*). Ces espèces ont été choisies parce que la tête carmin est déclarée menacée et le statu de l'esturgeon jaune est présentement examiné afin de déterminer si ce dernier sera ajouté à la liste des espèces en péril. Egalement, les deux espèces représentent des groupes taxonomiques très différents qui ont des histoires évolutives, des comportements et des formes corporelles très distincts.

Le comportement et les mouvements de la tête carmin dans le «shuttlebox» démontrent que cette dernière recherche une température d’eau préférée. La température moyenne préférée est de 23,6 ± 1,4 ºC. Cette température est située à l’intérieur de l’écart des températures de son environnement naturel. En raison de sa petite taille et sa grande mobilité, la tête carmin est une espèce très appropriée pour les expériences de «shuttlebox». D'autres études pourraient être complétées afin d’analyser la préférence thermique sous une variété de conditions physiques.

Par contre, la température préférée de l'esturgeon jaune n'a pas pu être déterminée en utilisant le «shuttlebox». Cet espèce n'a pas sélectionné une température préférée dans l’écart de température (environ 9 à 22 ºC) qu’il nous a été possible d’atteindre avec le setup expérimental que nous avions en place. Nous pensons que la vaste tolérance thermique de l’esturgeon jaune face à son environnement pourrait expliquer ceci. Il est également possible que, bien que cette espèce présente une thermorégulation comportementale dans son milieu naturel, les individus n'ont pas développé ce comportement dans le «shuttlebox» au cours de l'expérience.

TABLE OF CONTENTS

LIST OF FIGURES

1 INTRODUCTION

Temperature plays a vital role in a fish's environment, influencing everything from metabolic rates and growth to species distributions (Ferguson, 1958; Jobling, 1981; Tsuchida, 1995; White, Phillips & Seymour, 2006). Temperature, like cover, substrate or food availability, is a component of a fish's habitat; fish exist within a "thermal niche" that can be defined by optimal conditions for growth, lethal limits or behavioural preferences (Magnuson, Crowder & Medvick, 1979). Temperature can be viewed as a consumable ecological resource that is subject to interspecific and intraspecific competition (Magnuson et al., 1979). Being highly mobile organisms in a thermally heterogeneous environment, fish can exert control over their body temperature through behavioural mechanisms, relocating themselves to more favourable conditions. Behavioural homeostasis is valuable to fish, as even small temperature changes can have significant impacts on metabolism, fluid-electrolyte balance, and acid-base relationships (Crawshaw, 1977).

Considerable effort has been devoted to studying behavioural thermoregulation in fish species (Beitinger & Magnuson, 1979; Khan & Herbert, 2012; Petersen & Steffensen, 2003; Ward, Hensor, Webster & Hart, 2010), as this may be important for determining habitat selection in the field. When given options among waters of differing temperatures in a laboratory setting, fish gravitate towards a narrow temperature range. In the short term, during approximately the first two hours, preferred temperature is strongly influenced by acclimation temperature and is sometimes referred to as the acute preferred temperature (Reynolds & Casterlin, 1979). Over longer periods of time (generally 24 hours), fish will move towards a final species-specific preferred temperature (T_{pref}), also called the final preferendum (Fry, 1947; Golovanov, 2006; Jobling, 1981; Reynolds & Casterlin, 1979; Schurmann, Steffensen & Lomholt, 1991). Fry (1947) defined T_{pref} as either the temperature at which all individuals of a species will congregate, regardless of their thermal experience, or the temperature at which the preferred temperature is equal to the acclimation temperature. Fish that have selected a given water temperature within a gradient do not necessarily remain at this temperature, but may continue to explore waters of higher or lower temperature. Therefore, it has been proposed that T_{pref} is better expressed as a zone, rather than as a fixed temperature (Jobling, 1981).

Coutant (1977) provided a summary of studies on temperature selection in fish. He found that species-specific temperature preference and avoidance had been clearly shown in field and laboratory results for many species. To date, approximately 250 species of fish have been found to demonstrate an ability to actively select a specific thermal zone (Golovanov, 2006). For example, Rainbow Trout (*Oncorhynchus mykiss*) prefer a temperature of 16.1 °C (Schurmann et al., 1991) and Arctic Char (*Salvelinus alpinus*) prefer 11.5 to 11.8 °C in spring and summer, with its preferred temperature dropping to 8.7 °C in winter (Mortensen, Ugedal & Lund, 2007). Satinfin Shiner (*Notropis analostanus)* prefer a temperature of 27.17 °C (Cincotta & Stauffer, 1984).

T_{pref} correlates with optimal temperature for growth and since T_{pref} is faster and easier to determine, this measure has also been proposed as a proxy for determining optimal growth temperatures (Jobling, 1981; Kellogg & Gift, 1983). T_{pref} is also the temperature that can influence habitat selection in the natural environment. A species can benefit from being able to discern between microhabitats with differing temperatures because this allows individuals to select habitats where they will be exposed to optimal temperature conditions.

Water temperature needs to be considered along with other abiotic variables such as water velocity, depth, cover or substrate when determining the suitability of aquatic habitat and assessing potential impacts of future changes to habitat. This requires an understanding not only of a species' tolerance to temperature extremes, but also its ability to sense and select habitat based on the presence of a preferred range of water temperatures.

The objective of this study was to determine the feasibility of using an automated shuttlebox system to find the behavioural thermal preferences of two freshwater fish species that are at-risk in Manitoba. The first species, the Carmine Shiner (*Notropis percobromus*), was legally listed as a threatened species under the Species at Risk Act (SARA) in 2003. Carmine Shiner are slender, elongate minnows that were recently differentiated from a Manitoba population of Rosyface Shiner (*Notropis rubellus*) (Wood, Mayden, Matson, Kuhajda & Layman, 2002). Carmine Shiner are found in Manitoba but have not been recorded elsewhere in Canada. Carmine Shiner have been captured in fast flowing creeks and small rivers, including the Winnipeg, Bird, Whitemouth, and Birch Rivers (Carmine Shiner Recovery Team, 2007). Their biology, life history, distribution, abundance, habitat requirements, and physiology are not well understood. Much of what is known about this species is based on studies of Rosyface Shiners captured outside of the range of Carmine Shiner. One laboratory-based temperature preference study of Rosyface Shiner found that T_{pref} varied from 14.8 °C to 26.8 °C depending on the acclimation temperature (Cherry, Dickson & Cairns Jr, 1975). However, these tests were of such short duration (40 min) that they likely reflect the acute preferred temperature and not the final temperature preferendum.

Lake Sturgeon (*Acipenser fulvescens*) is one of Canada's largest freshwater fishes and has a long history of commercial exploitation and scientific study. It was once distributed throughout the Great Lakes, Mississippi River, and Hudson Bay drainages (Harkness & Dymond, 1961). Over the past century, the size and distribution of Lake Sturgeon populations have been severely reduced as a result of fishing, habitat loss, and degraded water quality (Cleator et al., 2010; Harkness & Dymond, 1961). Today, known Canadian populations of Lake Sturgeon range from the North Saskatchewan River in Alberta, to Hudson Bay in the north, and east to the St Lawrence River estuary (DFO, 2010). Long-lived, slow to mature, and infrequent spawners, Lake sturgeon are especially vulnerable to overfishing and habitat degradation (Boreman, 2002).

Lake Sturgeon have been identified by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as endangered. Fisheries and Oceans Canada (DFO) is considering listing this species under SARA. Researchers and managers generally agree that a high priority should be placed on studies directed at early life stages of Lake Sturgeon (DFO, 2007; Secor, Anders, Van Winkle & Dixon, 2002; Velez-Espino & Koops, 2008). This project was designed in part to address an identified data gap in our knowledge of Lake Sturgeon by studying a component of the habitat requirements of juveniles.

An automated shuttlebox was used to conduct the experiments. Variations of the shuttlebox system have been used to determine the preferred temperatures of a number of species (Mortensen et al., 2007; Reynolds & Casterlin, 1977; Schurmann & Steffensen, 1992; Schurmann et al., 1991; Staaks, Kirschbaum & Williot, 1999) as well as to assess turbidity and salinity preferences (Meager & Utne-Palm, 2008; Serrano, Grosell & Serafy, 2010). Shuttleboxes are essentially two-chambered tanks, with a temperature gradient between the chambers. These automated systems use overhead cameras and motion tracking software to monitor a fish's movements between the warmer and cooler chambers, and automatically raise or lower the water temperature of the entire system based on the fish's position. In this way, a fish can regulate its own body temperature by shuttling back and forth between the chambers. In theory, a fish would continue to choose one temperature over another by swimming back and forth between chambers until it arrived at a final preferred temperature. It would then continue shuttling back and forth between the chambers to maintain its environment at this temperature. The median occupied temperature is generally considered T_{pref}.

Other methods for determining preferred temperature are based on allowing fish to distribute themselves within a temperature gradient, or selecting between two or more chambers containing water of different temperatures. A review of laboratory methods for determining temperature preferences was completed by McCauley (1978), who found that variability due to fish age, size, season, physiological condition, and social factors likely influence the resulting T_{pref} values more than experimental artifacts such as different techniques, apparatus, or statistical measures.

The temperature preference of either study species, Carmine Shiner and Lake Sturgeon, has not previously been described through laboratory-based studies. An automated shuttlebox system has also not previously been used to study either species. Therefore, the primary goal of this study was to assess its effectiveness and ease of use, including recommendations for future research.

2 METHODS

2. 1 ANIMAL HUSBANDRY

2.1.1 Carmine Shiner

Carmine Shiner were captured from the Birch River, Manitoba in October 2011 (Figure 1). To reduce handling stress and mortality that can occur at higher temperatures, fish collection was timed to coincide with water temperatures of less than 15 ºC. Carmine

Shiner were transported to the DFO Freshwater Institute in Winnipeg, Manitoba and held in 100 L glass aquaria in temperature controlled rooms to acclimate for a period of one to two months. Each tank was equipped with three separate filtering water pumps, two air stones, bottom gravel, and aquatic vegetation. Water was replaced (30%) once a week using de-chlorinated water. Water temperature was maintained at 20 °C for the duration of the acclimation period. Carmine Shiner were fed *ad libitum* every one to two days with a combination of frozen brine shrimp and Tetrafin goldfish flakes. DFO staff were responsible for fish capture, care, and feeding. Individual Carmine Shiner were not marked, but were transferred into a different holding tank once they had been used in the experiment. Therefore, no shiner was tested more than once. Fish body mass (mean ± S.D.) was 2.04 ± 0.25 g and total body length was 6.8 ± 0.36 cm.

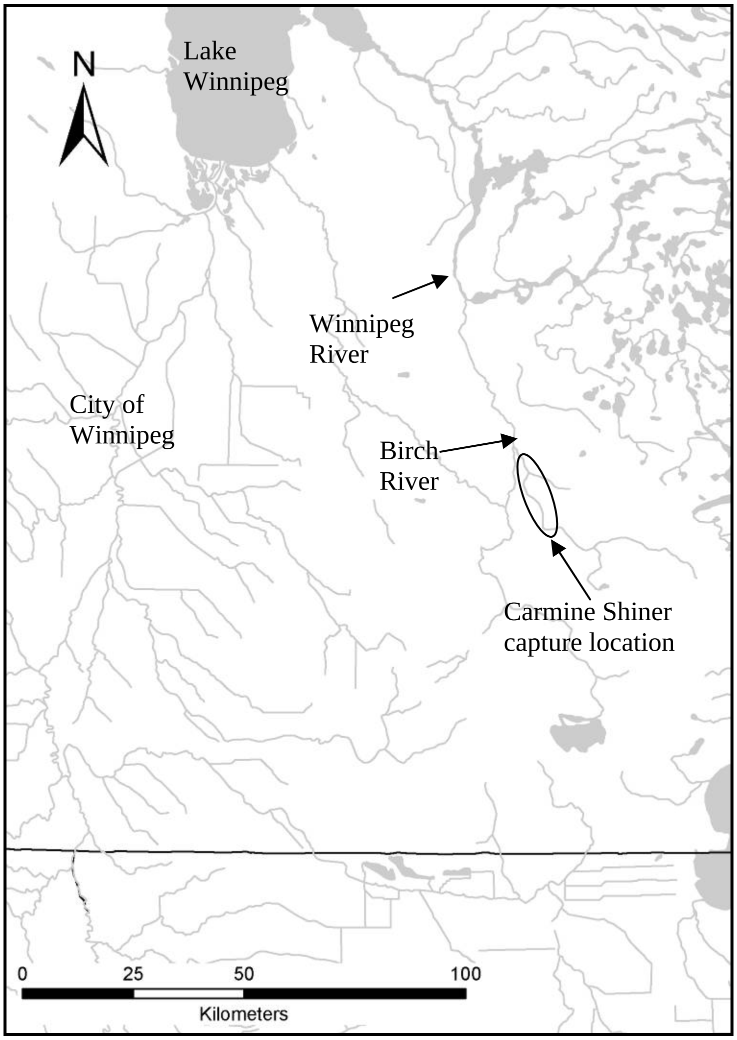

Figure 1. Carmine Shiner (*Notropis percobromus*) capture location on the Birch River, Manitoba. Map courtesy of D. Watkinson, Fisheries and Oceans Canada.

2.1.2 Lake Sturgeon

Juvenile Lake Sturgeon (age 2+) were hatched and raised by staff and students at the University of Manitoba Animal Holding Facility. The juveniles were progeny of wild-caught Lake Sturgeon from the Winnipeg River population, captured immediately downstream of the Point du Bois generating station (Figure 2). The Lake Sturgeon used for the temperature preference study were smaller than average for their age, as they had been fed a reduced ration during the previous winter as part of an unrelated study. These fish were selected despite being used in a past experiment because they were the only individuals available that fit the shuttlebox dimensions.

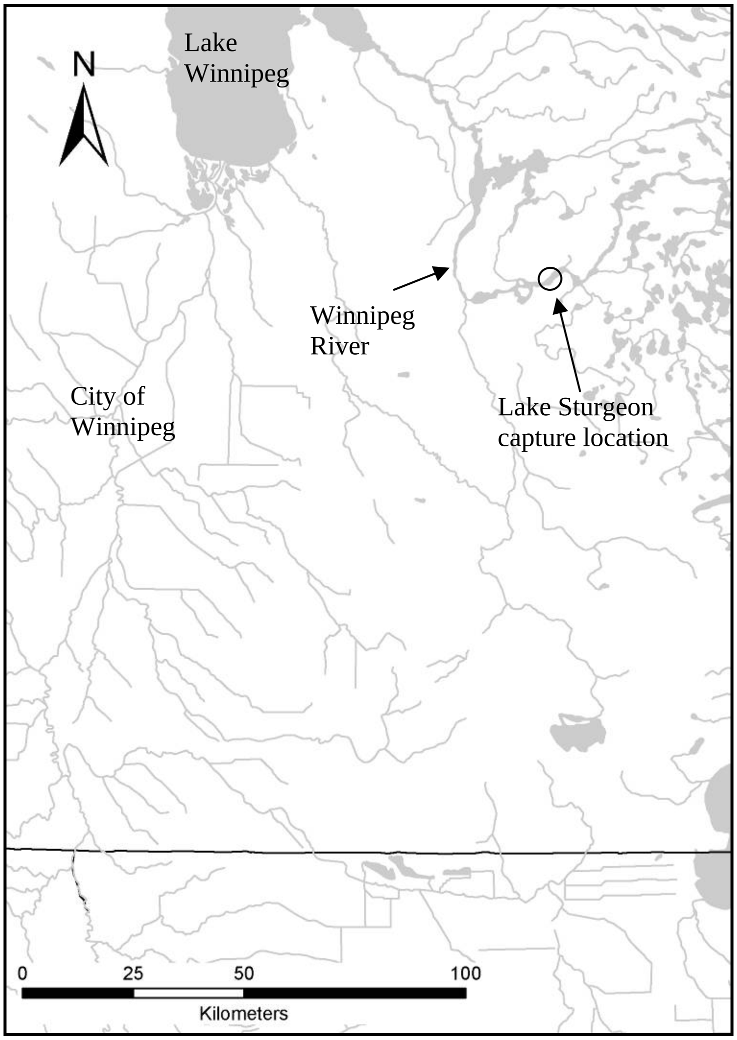

Figure 2. Adult Lake Sturgeon (*Acipenser fulvescens*) capture location on the Winnipeg River, Manitoba. Map courtesy of D. Watkinson, Fisheries and Oceans Canada.

Lake Sturgeon were marked with individual colour codes using Visual Implant Elastomer tags from Northwest Marine Technology, Inc. (Figure 3). This tagging method was chosen because tags were easy to implant and do not impede future growth or swimming ability. Throughout this study, Lake Sturgeon were fed a ration of bloodworm, squid, and commercial trout pellets (2.5 pt Martin Mills Classic Sinking) at a rate of 1% body weight two times per day. Holding tanks were supplied with continuous, temperature-controlled fresh water maintained at 17 °C. All fish were weighed and measured prior to each experiment. Fish body mass (mean ± S.D.) was 51.4 ± 17.6 g, and total body length was 23.5 ± 2.5 cm.

Figure 3. Visual Implant Elastomer tags (vertical orange bars) on the ventral side of the rostrum of a juvenile Lake Sturgeon *(Acipenser fulvescens)*.

2.2 SHUTTLEBOX SETUP

We conducted temperature preference experiments using automated electronic shuttleboxes built and supplied by Loligo Systems (Tjele, Denmark). Adapting the method introduced by Neill et al. (1972), the shuttleboxes consisted of two-chambered tanks that had a narrow passage connecting the chambers (Figure 4). A continuous circular current was maintained in each chamber by pumping water into two buffer tanks that were placed above the shuttlebox (Figure 5). Water then flowed back to the chambers by gravity. To minimize waste products buildup, water filters were placed in each buffer tank and water was changed between experiments. Buffer tanks were insulated using polystyrene foam panels to minimize the effect of ambient room temperature on water temperature.

Figure 4. Shuttlebox tank used to test Carmine Shiner (*Notropis percobromus*) temperature preference.

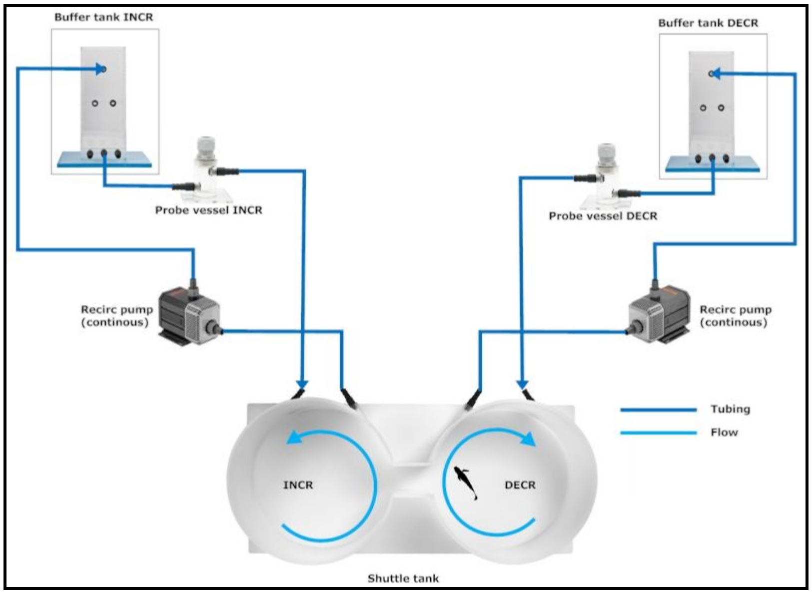

Figure 5. A representation of the shuttlebox components used to maintain a continuous circular current. Figure source: www.loligosystems.com.

For Carmine Shiner experiments, a 67.0 cm long by 29.5 cm wide shuttlebox with a 5.0 cm wide by 8.0 cm long passage between the two chambers was used. Chamber diameter was 29.5 cm. Water depth was approximately 10 cm.

For Lake Sturgeon experiments, a 110.0 cm long by 50.0 cm wide shuttlebox with a 7.5 cm wide by 10.0 cm long passage between the two chambers was used. Chamber diameter was 50.0 cm. Water depth was 10 to 15 cm. The shuttlebox size was selected based on the connecting passage width. Lake Sturgeon do not normally fold their pectoral fins flat against their bodies, but rather hold them straight out from their sides. As a result, the connecting passage width needed to accommodate not only the

Lake Sturgeon’s body but also the extended pectoral fins. Otherwise, Lake Sturgeon may avoid the passage due to its restrictive size.

Temperature probes (Pt-100, accuracy ± 0.15 °C) connected to TMP-REG temperature instruments, both from Loligo Systems, were used to record the temperature in each chamber. For Carmine Shiner experiments, temperature probes were placed within probe vessels connected in series between the buffer tanks and the shuttlebox (Figure 5). For Lake Sturgeon experiments, temperature probes were secured to the sides of each chamber and recorded water temperature approximately 2 cm from the sides, which corresponded to the typical swimming track of the sturgeon. The probe locations shown in Figure 5 were initially attempted. However, in this configuration, water temperature recorded by the probes differed by as much as 1 ºC from temperature within the chambers. The rate of temperature change in the chambers lagged behind that recorded by the probes, likely due to the larger flow rate to total water volume ratio, when compared to the Carmine Shiner shuttlebox.

Water temperature was controlled through a series of pumps that were turned on and off by the ShuttleSoft software. To increase the temperature, water was pumped from the buffer tanks, through tubing connected to a stainless steel coil placed in a hot water bath, and back to the buffer tanks (Figure 6). A thermostatic bath (LAUDA Alpha A 24, Lauda-Königshofen, Germany) was used for Carmine Shiner experiments, while a Canlab model W3241-2 hot water bath was used for Lake Sturgeon experiments. To cool the water, it was pumped from the buffer tanks to coils placed into a cold water bath, then back to the buffer tanks. A Forma Scientific model CH/P Temperature Control System was used to cool the water for Carmine Shiner experiments. A LAUDA Alpha RA 24 thermostatic bath was used for Lake Sturgeon experiments. Water temperature in each chamber was controlled independently, thereby maintaining the desired temperature gradient between the chambers.

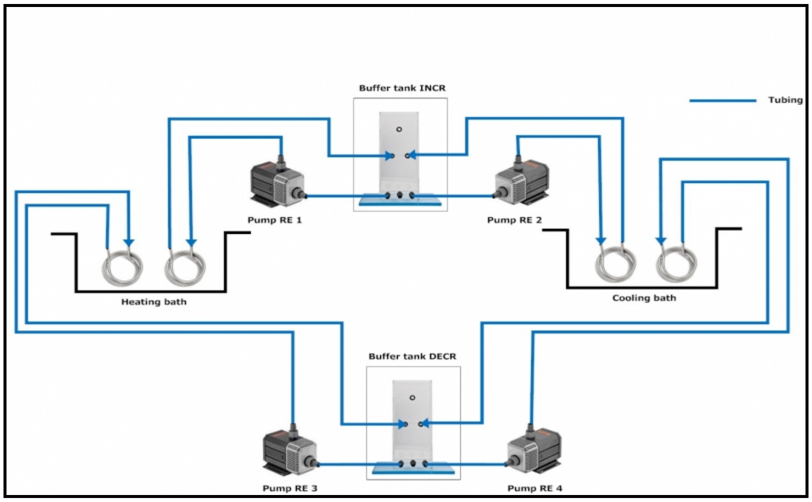

Figure 6. A representation of the shuttlebox components used to control water temperature. Figure source: www.loligosystems.com.

A video camera (uEye 1640-C, Imaging Development Systems, Dimbacher, Germany) was placed above the shuttlebox. The camera was connected to a PC computer, allowing the ShuttleSoft software to continuously track individual fish movements (Figure 7). The camera was able to sense infrared light for night recording. Overhead fluorescent lighting was used during daylight hours, and infrared lights placed below the shuttlebox were used at night for Lake Sturgeon experiments. For Carmine Shiner experiments, the camera was placed on a tripod above the shuttlebox. For Lake Sturgeon experiments, we suspended the camera from the ceiling, approximately 2 m above the shuttlebox, to capture the full width of the tank.

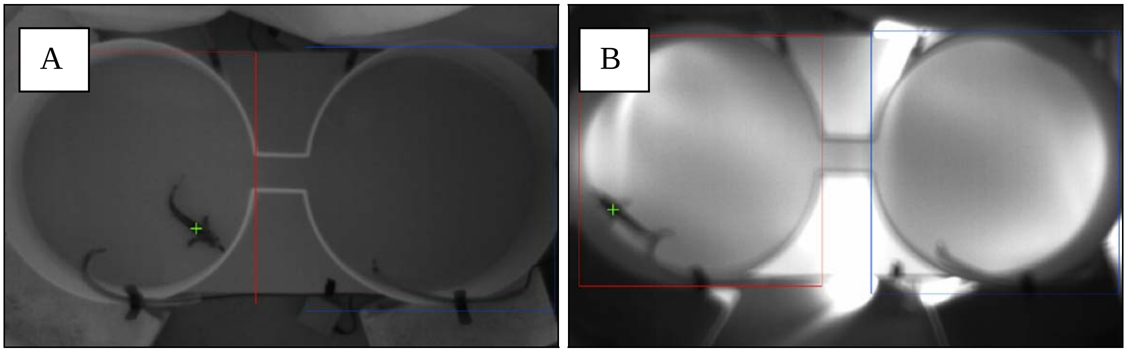

Figure 7. Images of a juvenile Lake Sturgeon in the shuttlebox, as captured by the overhead video camera. The tank was illuminated by fluorescent light during the day (A) and infrared light during the night (B).

Water temperature in each shuttlebox chamber could either be maintained at a fixed value (static setting) or controlled by the fish’s movements (dynamic setting). Under the dynamic setting, if the fish moved to the warmer or “INCR” chamber, the software would track the position and cause the system temperature to be raised by turning on pumps that forced water through hot water coils. If the fish moved to the cooler or “DECR” chamber, the system temperature was lowered by pumping water through cold water coils. The maximum rate of temperature change was 4 °C per hour. This was based on the assumption that if the water temperature changed too quickly, fish may not have time to sense and react to the change to avoid being exposed to temperature extremes. If the temperature changed too slowly, fish may slowly acclimate and not sense the need to escape to a more desirable temperature. The temperature range that could be achieved by the system, while maintaining a 2 °C gradient between the two tanks, was 6 °C to 32 °C for the Carmine Shiner setup.

The success of the ShuttleSoft software at controlling the water temperature appeared to be dependent on the shuttlebox size. The larger shuttlebox used for Lake Sturgeon experiments had a significantly greater water volume than the system used to test Carmine Shiners. The cold water bath did not have the capacity to cool the water at the desired rate outside of a narrow range. Once warm water was pumped through the coils in the cold water bath, the coolant temperature would rise and it could no longer effectively cool the shuttlebox water. Also, in the Lake Sturgeon shuttlebox there was greater heat exchange between the two chambers, which compromised the system’s ability to maintain the desired temperature gradient. The desired rate of temperature change (4 °C per hour) could only be maintained between 14 °C and 20 °C. At higher or lower temperatures, the rate of change gradually decreased until the system could no longer raise or lower the temperature while maintaining a 2 °C gradient between the chambers. The cold water bath used for Carmine Shiner experiments was larger and had a greater cooling capacity.

To increase the range of temperatures that could be achieved for Lake Sturgeon experiments, the hot and cold baths were removed and the system was hooked up directly to the on-demand hot and cold water supply available in the University of Manitoba Animal Holding Facility. The system was no longer closed, as fresh water was pumped in as needed to adjust the temperature. This setup increased the effective range slightly; the system could now achieve temperatures from 9 to 22 °C, with the desired rate of temperature change maintained between 13 and 22 °C (below 13 °C, the rate of change became significantly slower than 4 °C per hour). This also removed the concern about waste products building up in the system over the course of each trial, since water was continually being flushed. Dissolved oxygen concentration was not measured.

A curtain was hung around the shuttleboxes to prevent visual disturbances and ambient noise was kept to a minimum at all times.

ShuttleSoft recorded three temperatures once a second during the experiments: temperature in the INCR tank, temperature in the DECR tank, and “Object Temperature”, which was the temperature in the chamber where the fish was located at any given time.

2.3 EXPERIMENTAL PROCEDURE

2.3.1 Carmine Shiner

Eight Carmine Shiner were tested. The sample size was comparable to previous temperature preference experiments, for example Staaks et al. (1999) selected n=4, Reynolds & Casterlin (1977) n=6, Petersen & Steffensen (2003) n=8, and Beitinger (1974) n=10.

Following a 24 h starvation period, individual Carmine Shiner were placed into the shuttlebox in the evening and lights were turned off for 8 h. The full acclimation period was 12 h. Half of the fish were first introduced to the INCR chamber, the other half were placed first in the DECR chamber, alternating between experiments. Each Carmine Shiner was observed to explore both chambers during the first few minutes. During the acclimation period, the temperature was maintained at 19 °C in the DECR chamber and 21 °C in the INCR chamber (static setting). The experimental phase was run on dynamic setting for 10 to 12 h under fluorescent lights during the day. At the end of the 22 to 24 h period, fish were transferred to a different holding tank, so that each fish was not used more than once. For consistency, 10 h of data was used to calculate T_{pref} for each fish.

2.3.2 Lake Sturgeon

Lake Sturgeon were starved for 24 h prior to being placed in the shuttlebox. A total of six Lake Sturgeon were tested for 48 h each. Lake Sturgeon were allowed to acclimate for 24 h as per Schurmann et al. (1991). Periodic monitoring during the acclimation period confirmed that they were able to move freely between the two chambers. The chamber that was the INCR side was alternated between each experiment. The starting temperature was set to 16 °C in the DECR chamber and 18 °C in the INCR chamber. The system was set to dynamic during the acclimation period. Following the acclimation period, the experimental phase was continued for 24 h.

The extended duration of Lake Sturgeon experiments compared to Carmine Shiner experiments was due to the observed behavioural differences between the two species. Carmine Shiner were very active in the shuttlebox. Data recorded during initial trials showed that they appeared to be actively controlling the temperature within 24 h. By comparison, Lake Sturgeon swam much more slowly and made significantly fewer transits between the INCR and DECR chambers. Trials were initially conducted using the same time duration as in the Carmine Shiner experiments: 12 h of acclimation followed by 12 h of testing. However, Lake Sturgeon did not appear to actively “shuttle” back and forth between the chambers, and as a result the temperature fluctuated widely. The duration of both the acclimation phase and the experimental phase were therefore increased allowing Lake Sturgeon more time to learn to thermoregulate by moving between chambers. Based on advice from the shuttlebox’s designers, the acclimation period was set to dynamic rather than static, to allow more time for Lake Sturgeon to sense the changing temperature, with the intent of motivating them to seek out preferred conditions.

Lake Sturgeon have been observed to be more active at night (G. Anderson*, pers. comm.*). Therefore, it was important to maintain a diurnal light cycle to capture night time activity as well as to minimize stress. Overhead fluorescent lights were on from 8 am to 8 pm. Infrared lights were the only source of illumination at night (8 pm to 8 am).

2.4 DATA HANDLING

ShuttleSoft recorded the water temperature of the occupied chamber throughout the experiment. The median occupied temperature during the experimental phase was used as a measure of T_{pref} for each fish. The median occupied temperature is generally considered the best indicator of preferred temperature because the mean is affected by extreme or unusual values if the data distribution is skewed and the mode is affected by the size of the class intervals should the data distribution show a broad plateau (Schurmann et al. 1991).

The final T_{pref} for each species was the mean of the median occupied temperatures for each individual. Graphs of occupied temperature over time were also examined to look for individual patterns of temperature selection.

ShuttleSoft recorded data points once a second, resulting in very large files over the course of the experiments. Data analysis was conducted using the R software package, as the amount of data was too large to be analyzed in MS Excel.

3 RESULTS

3. 1 CARMINE SHINER

During the experimental phase the occupied chamber temperature ranged from 15.5 to 31.4 ºC (Figures 8 and 9). The mean minimum temperature occupied by a fish during the experimental phase (± S.D.) was 17.8 ± 1.8 ºC. The mean maximum temperature was 28.8 ± 1.5 ºC. Individual T_{pref} ranged from 21.7 to 25.8 ºC. The final mean T_{pref} was 23.6 ± 1.4 ºC.

Some Carmine Shiner were very active, making hundreds of passes between chambers. By spending little time in either chamber before passing back to the other side, these fish kept the system temperature relatively constant for many hours. For example, Carmine Shiner 02 made frequent passes for just over four hours, and then began spending relatively more time in the warmer chamber (Figure 8). The system temperature rose until hour eight, when the frequency of passes increased and the temperature of the system dropped to a similar range as during hours one through four. T_{pref} for this fish was 21.6 ºC. Carmine Shiner 07 was initially spending relatively more time in the warm chamber, but by hour eight was also making quick passes between chambers (Figure 8). In contrast, some of the Carmine Shiner spent over an hour in one chamber or the other (for example Carmine Shiner 03, Figure 8). The distribution of occupied temperatures is shown in Figure 9.

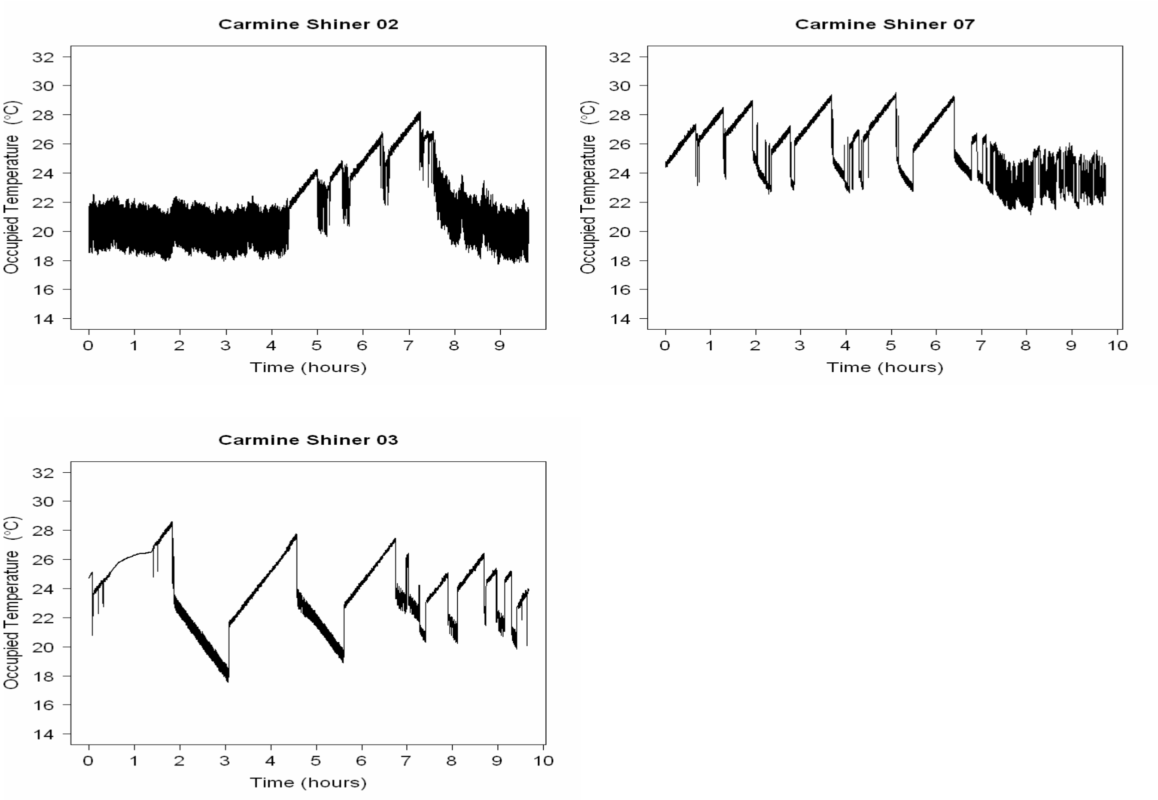

Figure 8. Occupied temperature over time for three Carmine Shiner in the automated shuttlebox.

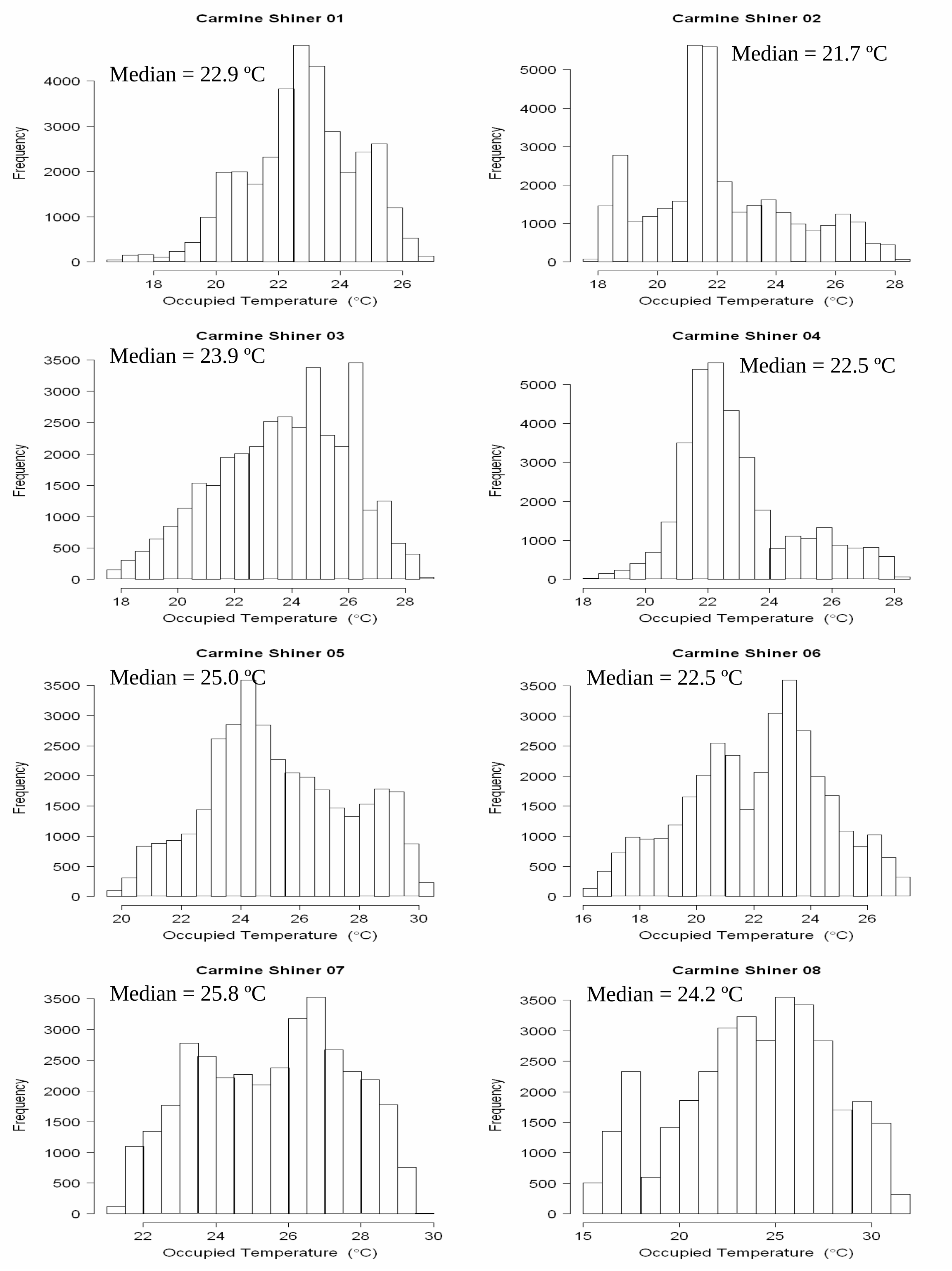

Figure 9. Distribution of occupied temperature for all Carmine Shiner.

3. 2 LAKE STURGEON

During the experimental phase, occupied water temperature ranged from 9.6 to 21.6 ºC. The mean minimum temperature was 11.9 ± 2.9 ºC. The mean maximum temperature was 18.9 ± 3.7 ºC. Individual T_{pref} ranged from 10.0 to 21.3 ºC. The final mean T_{pref} was 15.0 ± 5.1 ºC.

The temperature occupied by Lake Sturgeon varied widely between the system's minimum and maximum capacities. Often, the sturgeon stayed within one chamber or the other for extended periods. This was evident when viewing the graphs of occupied temperature over time. For example, Lake Sturgeon 03 remained in the cold chamber for several hours, resulting in the system cooling to its minimum temperature (Figure 10). This Lake Sturgeon then moved to the warm chamber and the system temperature increased to its maximum. Finally, it moved back to the cold chamber and the system temperature again decreased to its minimum. T_{pref} for this fish was 11.2 ºC. However, by examining the graph of temperature over time, it was evident that the Lake Sturgeon was in fact not shuttling between chambers and did not appear to be selecting for a temperature within the range achievable by the system. Lake Sturgeons 05 and 07 remained primarily in the warm chamber, while Lake Sturgeons 08 and 12 remained primarily in the cold chamber.

The data distribution was highly variable among individuals, as can be seen in the histograms presented in Figure 11.

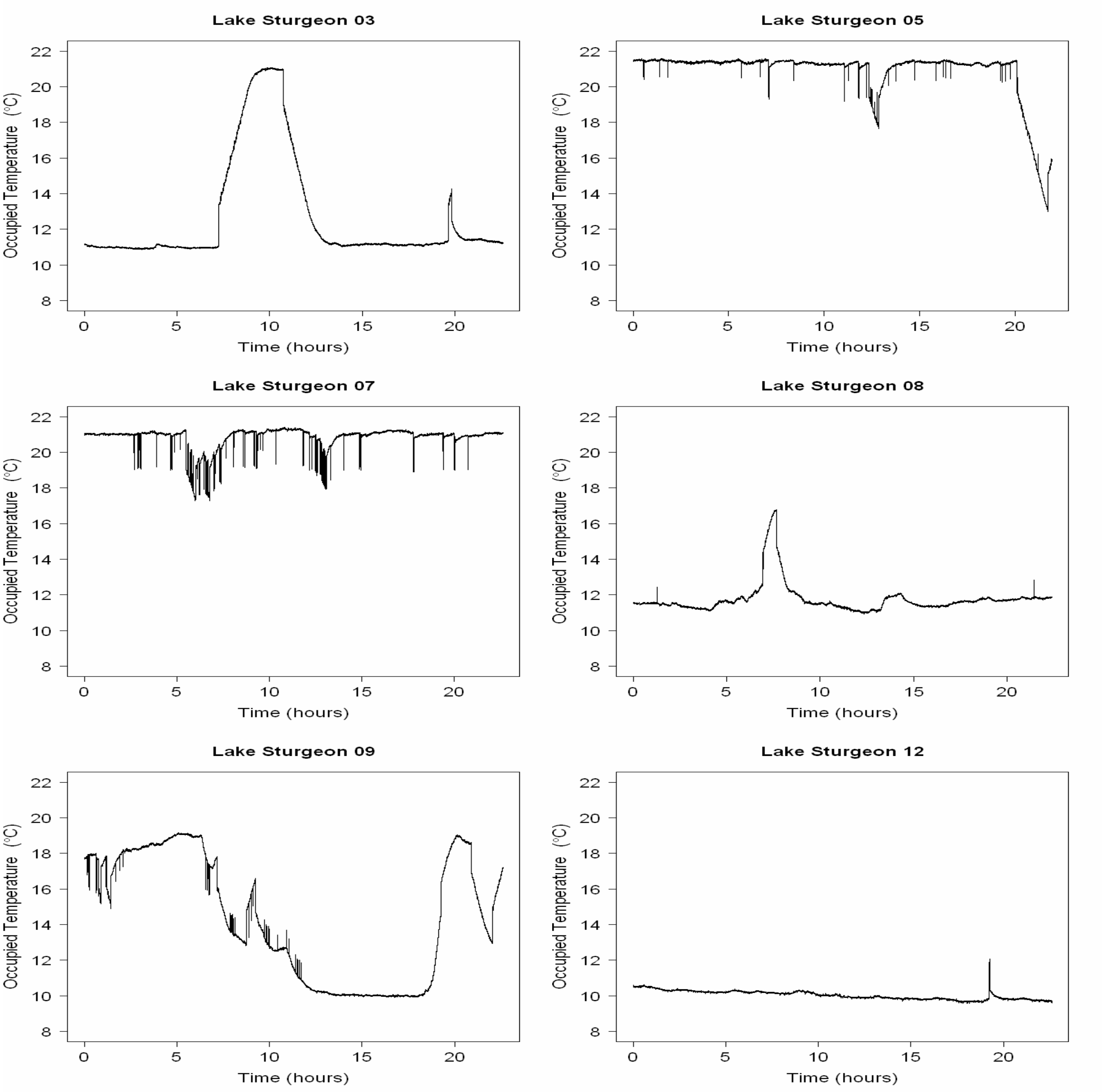

Figure 10. Occupied temperature over time for Lake Sturgeon in the automated shuttlebox.

.

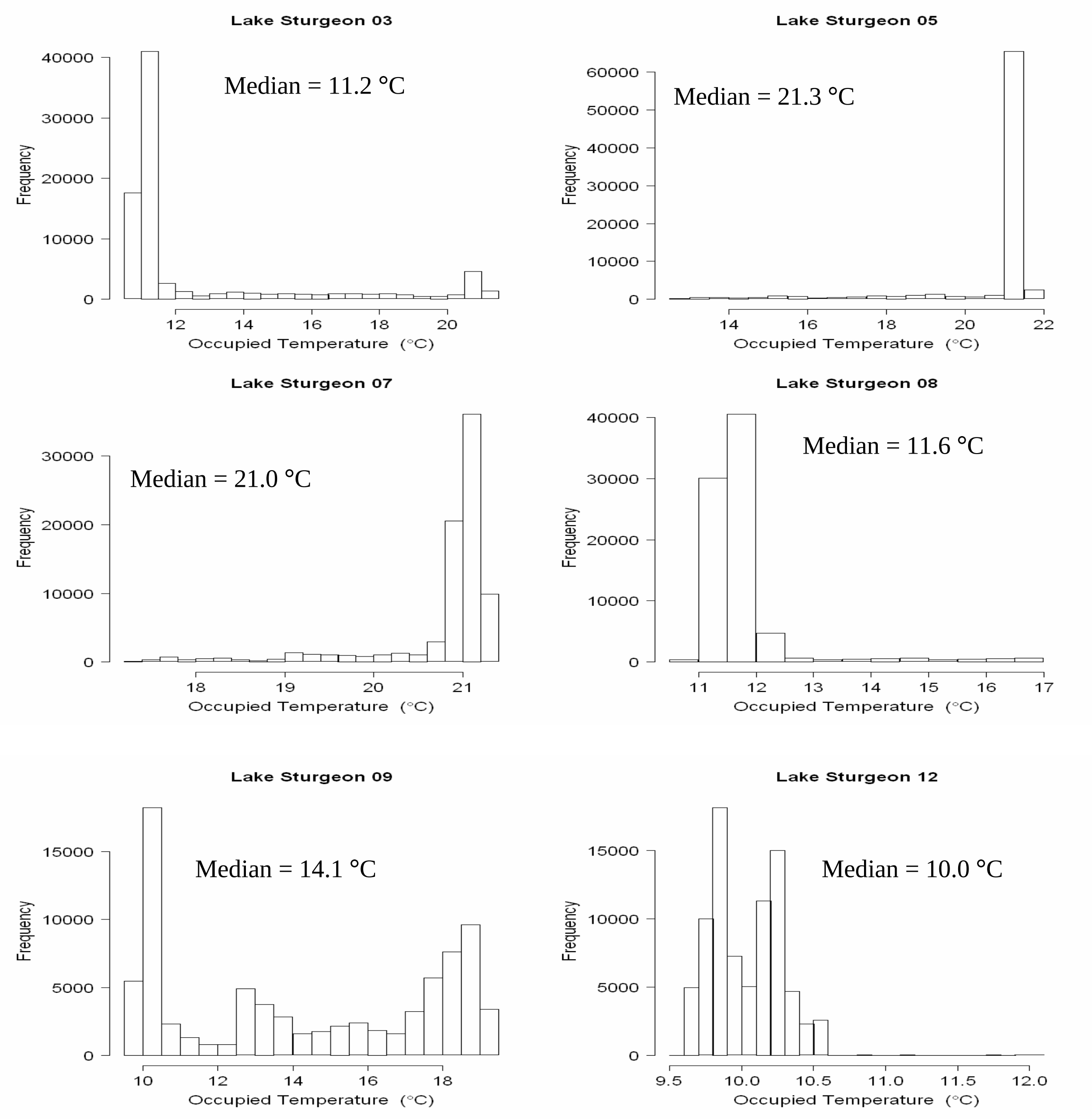

Figure 11. Distribution of occupied temperature for Lake Sturgeon.

4 DISCUSSION

Carmine Shiner demonstrated a preference for a water temperature of 23.6 ºC. This was higher than the temperature at which they were captured (maximum 15 ºC) and higher than the acclimation temperature (20 °C). Although data reported here are based on a small sample size, the shuttlebox apparatus was successful as a means of determining preferred temperature.

DFO collected temperature data from the Birch River, where Carmine Shiner used for this study were captured, over the course of the ice-free period in 2011. The temperature ranged from 0.6 ºC in April to 26.2 ºC in July (D. Watkinson, *pers. comm*.). The preferred temperature recorded in these experiments was therefore within the range that this species would experience in its natural environment.

In the field, temperature, light, and oxygen levels can fluctuate from day to night as well as seasonally. T_{pref} may follow these cycles; for example T_{pref} can decrease in response to low oxygen levels (Schurmann, 1991). In order to determine the true T_{pref} for Carmine Shiner it will be necessary to conduct experiments over longer periods of time (minimum 24 h) to capture day and night behaviour. It may be useful to test T_{pref} under different lighting conditions and different acclimation temperatures to approximate seasonal effects. A larger sample size would also be favourable.

Further study of Carmine Shiner in their natural environment would complement this laboratory-based study. As has been demonstrated by others (e.g., Jobling, 1981), preferred temperature may correlate with the optimum temperature for growth. Therefore, the ability of Carmine Shiner to seek out and inhabit waters of this temperature may directly impact individual fitness. Carmine Shiner from watercourses of varying temperatures could be compared for size and condition to look for correlations between temperature and fitness. Water temperature can also impact the distribution of Carmine Shiner. A comparison between seasonal temperatures and instream distributions would be useful to better describe the critical habitat of this species.

Computer modeling of future temperature trends would also give insight into how Carmine Shiner may be affected, should instream temperatures increase significantly beyond their preferred temperature. Rosyface Shiner, closely related and until recently not differentiated from Carmine Shiner, are considered a warm water adapted species (Coker, Portt & Minns, 2001; Hasnain, Minns & Shuter, 2010; Houston, 1996). The Manitoba population of Carmine Shiner is currently at the northern limit of the species' range. A warming trend could increase habitat suitability north of its current limits should more northern watercourses fall within the species preferred temperature niche (Carmine Shiner Recovery Team, 2007).

Despite the extended duration of the experiments conducted with Lake Sturgeon, there was no indication that this species exhibited behavioural thermoregulation within the shuttlebox. Either this species has a wide range of temperature tolerance with no behavioural mechanism for selecting a preferred temperature within the range that was achievable by the shuttlebox, or the individuals did not learn to behaviourally thermoregulate by shuttling between the chambers within the timeframe of the experiment. In a previous study of European Sturgeon (*Acipenser sturio*), also conducted using an automated shuttlebox, the authors were also not able to demonstrate a temperature preference range or active thermoregulatory behaviour (Staaks et al., 1999).

Sturgeon are, in general, bottom dwellers in large rivers and lakes, where their environment may be fairly homothermal compared to species that inhabit shallower environments. Barth (2011) found that juvenile Lake Sturgeon in the Winnipeg River exhibited high site fidelity and occupied small home ranges. Well-developed behavioural thermoregulation in the juvenile life stage may not provide an adaptive advantage. In addition, Lake Sturgeon used for this experiment had been held in a laboratory environment for over two years. Their holding tanks did not experience more than minor temperature fluctuations during this time. Therefore, they would not have needed to seek out new habitats that were more thermally suitable. The individuals used for this experiment were also abnormally small for their age due to a previous feeding experiment. Their experimental history may have had an influence on their behaviour. These and other factors may have influenced the ability of Lake Sturgeon to exhibit behavioural thermoregulation within the shuttlebox environment.

5 RECOMMENDATIONS FOR FUTURE STUDIES

Over the course of these experiments, the experimental setup was altered many times to better meet the study objectives for the target species. The following recommendations should be considered by future researchers who wish to use similar shuttlebox systems to study preferred temperatures of these or other aquatic species.

- Measure the minimum passage width that the study animals can pass through, particularly with species that do not regularly fold their pectoral fins flat against their sides. It is desirable to minimize water exchange between the two chambers by using the smallest passage width possible, while providing enough space for the fish to pass between the chambers.

- Use infrared lights at night so that a normal light/dark cycle can be maintained, thereby reducing stress to the fish. The change in contrast when switching from florescent lights to infrared may require that the settings on ShuttleSoft be manually adjusted (i.e. the experimenter may have to be present when the lighting type is changed).

- Ensure the ceiling height in the lab is sufficient to allow the camera to be affixed at an appropriate height above the shuttlebox. Larger tanks may require that the camera be mounted at a height of up to 2 m. Mounting height will depend in part on the type of camera lens.

- Use the smallest shuttlebox possible for the study species. Larger shuttleboxes require a larger volume of water and it becomes more difficult to maintain the desired temperatures. High capacity heaters and chillers may be required should large volume shuttleboxes be used.

- If using the temperature probe vessels supplied with the Loligo shuttlebox, verify that the readings accurately reflect the temperature within the chambers.
- If available, temperature controlled rooms are ideal for ensuring that the ambient temperature does not hamper the ability of the system to maintain control over the water temperature.
- Install water filters in the buffer tanks to remove nitrogenous wastes. Tubing should also be cleaned periodically as it provides an ideal growth medium for algae.

ACKNOWLEDGEMENTS

This project was made possible through the generous support of the following people. G. Anderson, M. Saboraki, C. Klassen, and D. Watkinson, as well as reviewers P. Blanchfield and K. Hedges. The work was supported by grants from the Fisheries Enhancement Fund of the Manitoba Water Stewardship Division and the SARA Program of Fisheries and Oceans Canada. The authors would also like to thank T. Smith and the staff of the University of Manitoba Animal Care Facility, and F. McCann from Fisheries and Oceans Canada, for their technical support.

6 REFERENCES

Barth, C. C. (2011). *Ecology, behavior, and biological characteristics of juvenile lake sturgeon, Acipenser fulvescens, within an impounded reach of the Winnipeg River, Manitoba, Canada.* PhD, University of Manitoba, Winnipeg.

Beitinger, T. L. & Magnuson, J. J. (1979). Growth rates and temperature selection of Bluegill, *Lepomis macrochirus*. *Transactions of the American Fisheries Society, 108*(4), 378-382. doi: 10.1577/1548-8659(1979)108<378:gratso>2.0.co;2

Boreman, J. (2002). Sensitivity of North American sturgeons and paddlefish to fishing mortality. In V. Birstein, J. Waldman & W. Bemis (Eds.), *Sturgeon Biodiversity and Conservation* (Vol. 17, pp. 399-405): Springer Netherlands.

Carmine Shiner Recovery Team (2007). *Recovery strategy for the Carmine Shiner (Notropis percobromus) in Canada*. Ottawa: Fisheries and Oceans Canada.

Cherry, D. S., Dickson, K. L. & Cairns Jr, J. (1975). Temperatures selected and avoided by fish at various acclimation temperatures. *Journal of the Fisheries Research Board of Canada, 32*(4), 485-491. doi: 10.1139/f75-059

Cleator, H., Martin, K. A., Pratt, T. C., Barth, C. C., Corbett, B., Duda, M. & Leroux, D. (2010). Information relevant to a recovery potential assessment of lake sturgeon: Winnipeg River-English River populations (DU5). *Canadian Science Advisory Secretariat Research Document, 2010/84*.

Coker, G., Portt, C. & Minns, C. (2001). Morphological and ecological characteristics of Canadian freshwater fishes. *Canadian Manuscript Report of Fisheries and Aquatic Sciences, 2554*(iv), 89.

Coutant, C. C. (1977). Compilation of temperature preference data. *Journal of the Fisheries Research Board of Canada, 34*, 739-745.

Crawshaw, L. I. (1977). Physiological and behavioral reactions of fishes to temperature change. *Journal of the Fisheries Research Board of Canada, 34*(5), 730-734. doi: 10.1139/f77-113

DFO (2007). Proceedings of the lake sturgeon recovery planning workshop; 28 February to 1 March 2006. *DFO Canadian Science Advisory Secretariat Proceedings Series, 2007*(030).

DFO (2010). Recovery potential assessment of Lake Sturgeon: Red-Assiniboine Rivers - Lake Winnipeg populations (Designatable Unit 4). *Canadian Science Advisory Secretariat Science Advisory Report, 2010/051*.

Ferguson, R. G. (1958). The preferred temperature of fish and their midsummer distribution in temperate lakes and streams. *Journal of the Fisheries Research Board of Canada, 15*(4), 607-624. doi: 10.1139/f58-032

Fry, F. E. J. (1947). Effects of the environment on animal activity. *University of Toronto Studies, Biological Series 55* (Vol. 68, pp. 1-68).

Golovanov, V. (2006). The ecological and evolutionary aspects of thermoregulation behavior on fish. *Journal of Ichthyology, 46*(0), S180-S187. doi: 10.1134/s0032945206110075

Harkness, W. J. K. & Dymond, J. R. (1961). *The lake sturgeon: the history of its fishery and problems of conservation*. Ottawa: Ontario Department of Lands and Forests.

Hasnain, S. S., Minns, C. K.& Shuter, B. J. (2010). Key ecological temperature metrics for Canadian freshwater fishes. *Climate Change Research Report CCRR-17*: Ontario Ministry of Natural Resources.

Houston, J. (1996). The status of the rosyface shiner, *Notropis rubellus*, in Canada. *Canadian Field-Naturalist, 110*(03), 489-494.

Jobling, M. (1981). Temperature tolerance and the final preferendum—rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology, 19*(4), 439-455.

Kellogg, R. L.,& Gift, J. J. (1983). Relationship between optimum temperatures for growth and preferred temperatures for the young of four fish species. *Transactions of the American Fisheries Society, 112*(3), 424-430. doi: 10.1577/1548-8659(1983)112<424:rbotfg>2.0.co;2

Khan, J. R. & Herbert, N. A. (2012). The behavioural thermal preference of the common triplefin (*Forsterygion lapillum*) tracks aerobic scope optima at the upper thermal limit of its distribution. *Journal of Thermal Biology, 37*(2), 118-124. doi: 10.1016/j.jtherbio.2011.11.009

Kwain, W. & McCauley, R. W. (1978). Effects fo age and overhead illumination on temperature preffered by underyearling rainbow trout, *Salmo gairdneri*, in a vertical temperature gradient. *Journal of the Fisheries Research Board Canada, 35*, 1430-1433.

Magnuson, J. J., Crowder, L. B. & Medvick, P. A. (1979). Temperature as an ecological resource. *American Zoologist, 19*(1), 331-343. doi: 10.1093/icb/19.1.331

Meager, J. & Utne-Palm, A. (2008). Effect of turbidity on habitat preference of juvenile Atlantic cod, *Gadus morhua*. *Environmental Biology of Fishes, 81*(2), 149-155. doi: 10.1007/s10641-007-9183-z

Mortensen, A., Ugedal, O. & Lund, F. (2007). Seasonal variation in the temperature preference of Arctic charr (*Salvelinus alpinus*). *Journal of Thermal Biology, 32*(6), 314-320.

Neill, W. H., Magnuson, J. J. & Chipman, G. G. (1972). Behavioral thermoregulation by fishes: A new experimental approach. *Science, 176*(4042), 1443-1445.

Petersen, M. F. & Steffensen, J. F. (2003). Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. *The Journal of Experimental Biology, 206*, 359-364. doi: 10.1242/jeb.00111

Reynolds, W. W. & Casterlin, M. E. (1977). Temperature preferences of four fish species in an electronic thermoregulatory shuttlebox. *The Progressive Fish-Culturist, 39*(3), 123-125. doi: 10.1577/1548-8659(1977)39[123:tpoffs]2.0.co;2

Reynolds, W. W. & Casterlin, M. E. (1979). Behavioral thermoregulation and the “final preferendum” paradigm. *American Zoologist, 19*(1), 211-224. doi: 10.1093/icb/19.1.211

Schurmann, H. & Steffensen, J. F. (1992). Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod, *Gadus morhua* L. *Journal of Fish Biology, 41*, 927-934.

Schurmann, H., Steffensen, J. F. & Lomholt, J. P. (1991). The influence of hypoxia on the preferred temperature of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology, 157*, 75-86.

Secor, D. H., Anders, P. J., Van Winkle, W. & Dixon, D. A. (2002). Can we study sturgeons to extinction? What we do and don't know about the conservation of North American sturgeons. *American Fisheries Society Symposium, 00*, 183-189.

Serrano, X., Grosell, M. & Serafy, J. E. (2010). Salinity selection and preference of the grey snapper *Lutjanus griseus*: field and laboratory observations. *Journal of Fish Biology, 76*(7), 1592-1608.

Staaks, G., Kirschbaum, F. & Williot, P. (1999). Experimental studies on thermal behaviour and diurnal activity rhythms of juvenile European sturgeon (*Acipenser sturio*). *Journal of Applied Ichthyology, 15*(4-5), 243-247.

Tsuchida, S. (1995). The relationship between upper temperature tolerance and final preferendum of Japanese marine fish. *Journal of Thermal Biology, 20*(1–2), 35-41. doi: 10.1016/0306-4565(94)00024-d

Velez-Espino, L. A. & Koops, M. A. (2008). *Recovery potential assessment for lake sturgeon (Acipenser fulvescens) in Canadian designatable units*. Canadian Science Advisory Secretariat Research Document 2008/007.

Ward, A. J. W., Hensor, E. M. A., Webster, M. M. & Hart, P. J. B. (2010). Behavioural thermoregulation in two freshwater fish species. *Journal of Fish Biology, 76*(10), 2287-2298. doi: 10.1111/j.1095-8649.2010.02576.x

White, C. R., Phillips, N. F. & Seymour, R. S. (2006). The scaling and temperature dependence of vertebrate metabolism. *Biology Letters, 2*, 125-127.

Wood, R. M., Mayden, R. L., Matson, R. H., Kuhajda, B. R. & Layman, S. R. (2002). Systematics and biogeography of the *Notropis rubellus* species group (Teleostei: Cyprinidae). *Bulletin Alabama Museum of Natural History, 22*, 37-80.