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Thermal Tolerance of Alligator Gar (*Atractosteus spatula*)

A THESIS

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Jory Bartnicki

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Thermal Tolerance of Alligator Gar (*Atractosteus spatula*)

A THESIS
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By Chris Butler 19 Nov 21
Chris Butler Committee Chairperson

19 Nov 2021
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19 Nov 2021
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Table of Contents

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List of Figures

Chapter I: An alternative, low-cost method to chill water for critical thermal minima trials

Chapter II: Critical thermal minima of alligator gar (*Atractosteus spatula*, [Lacépède, 1803]) during early life stages

Chapter III: Use of multiple climate change scenarios to predict future distributions of alligator gar (*Atractosteus spatula*)

- Figure 1: *The current modeled distribution for alligator gar. The legend shows probability of occurrence, the red shade represents >0.5 probability.*
- Figure 2: *The future model projections for alligator gar under four Shared Socio-economic Pathways (SSPs) climate scenarios. The legend shows probability of occurrence, the areas in red represents a >0.5 probability.*
- Figure 3: *Plotted centroids are the center of the projected range of alligator gar. The current range is shown along with the four projected climate change scenarios.*

List of Tables

Chapter I: An alternative, low-cost method to chill water for critical thermal minima trials

Chapter II: Critical thermal minima of alligator gar (*Atractosteus spatula*, [Lacépède, 1803]) during early life stages

Chapter III: Use of multiple climate change scenarios to predict future distributions of alligator gar (*Atractosteus spatula*)

- Table 3: *The best model runs with variables that best explain alligator gar distribution. Beta (β) is the regularization multiplier. The natural log of probability of the data present in the model is given by the log likelihood. AICc is a small-sampled corrected AIC score; the top model is shown along with the four closest performing models are shown. Delta AICc is the difference between the top model and other model scores. The wAICc is the model weight score which is calculated from the relative likelihood for each model, divided by the total relative likelihood for all models that were considered. Area under the curve (AUC) is a metric to evaluate model accuracy.*
- Table 4: *Distance that the centroid for alligator gar moves for each climate projection scenario and the rate of movement per decade*

- Table 5: *Range of values from response curves for the variables used in the current projection of alligator gar where there is >50% suitability. Elevation is meters above sea level, Bio 10 is mean temperature of warmest quarter, Bio 11 is mean temperature of coldest quarter, and inundation is percent of floodplain covered.*

Abstract

ABSTRACT OF THESIS

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ABSTRACT:

Alligator gar belong to an ancient lineage that dates back over 215 million years. Historically, alligator gar populations occurred in the Mississippi River basin from Illinois (USA) to Mexico. Alligator gar populations have been reduced or extirpated throughout much of their range, and introductory programs have been implemented by some state and federal agencies attempting to reestablish historical populations. Efforts have been aided by studies investigating many ecological and life-history aspects of this species. However, thermal minima has yet to be evaluated for alligator gar. The goal of this thesis is to provide quantified estimates of alligator gar minima temperatures and to forecast potential range distribution based on current and predicted future climate scenarios. Newly hatched alligator gar were collected from a hatchery. The gar were acclimated to three different aquarium temperatures (21°C, 24.5°C, and 28°C) and then the water temperature was slowly decreased until the gar lost equilibrium. Variables (temperature[°C], total length [mm], and weight [g]) were tested for correlation and logistic regression models were used to determine the relationship between loss of equilibrium temperature and length or weight. Akaike's information criterion scores were used to evaluate the best model. Additionally, alligator gar occurrence data was obtained from published papers, historical photos, field observations, and by downloading existence records from the Global Biodiversity Information Facility. These occurrences were combined with bioclimatic variables, terrestrial, aquatic and elevation data and then run in Maxent to estimate a current range and to forecast potential future ranges under different predicted climate scenarios. All variables were tested for correlation and Akaike’s information criterion scores in addition to receiver operating characteristics were used to evaluate models. Mean critical thermal minima temperatures obtained for each acclimation were 11.57°C, SD = 1.39 (21°C), 12.83°C, SD = 1.47 (24.5°C), and 14.16°C, SD = 1.60 (28°C). Alligator gar suitable habitat is expected to increase and shift to the north under all climate scenarios. The furthest shift north occurs under the most extreme warming scenario. This study provides the first quantified estimates of juvenile alligator gar thermal minima temperatures and fills a gap in alligator gar life history. The results from this study can be used in future studies aimed at conservation and management of alligator gar. Additionally, findings from this study can aid in determining the future northern extent of alligator gar distribution. Future research should investigate critical thermal minima of alligator gar larger than 519mm (the largest individual used in this study). Also, the survival of alligator gar that have been introduced at the northern edge of current projected suitable habitats should be followed to verify recruitment.

Introduction

Alligator gar are members of a prehistoric lineage that dates back over 215 million years (Scarnecchia, 1992). Alligator gar are also one of the largest and oldest living fish species in North America, living to ages > 50 years and growing to weights > 100 kg (Fernando et al., 2016; Snow and Long, 2015). Historically, alligator gar were in the Mississippi River drainage basin from Illinois to Mexico (Allen et al., 2020). Alligator gar utilize many different habitats, including rivers, reservoirs, and gulf estuaries (Ferrara, 2001). However, alligator gar populations have declined throughout most of their native range (Fernando et al., 2016; Porta et al., 2019).

Because of the recent decline of alligator gar populations, several conservation agencies have started stocking programs to reintroduce alligator gar into habitats where they have been locally extirpated (Mendoza et al., 2002; Hilsabeck et al., 2017; Porta et al., 2019). Also, the recent decline has led to the investigation of life history and ecological aspects of alligator gar. Some of these studies have looked at individual movements (Buckmeier, Smith & Daugherty, 2013; Militello, 2013; Solomon, Phelps & Herzog, 2013), early life history (Brinkman, 2008; Snow & Long, 2015; Snow et al., 2018), recruitment success (Buckmeier et al., 2017; Snow et al., 2018), development (Aguilera et al., 2002; Mendoza et al., 2002; Long, Snow & Porta, 2020), and critical thermal maxima (CT_{max}; Fernando et al., 2016). However, a critical thermal minimum (CT_{min}) has yet to be evaluated in alligator gar of any size. Also, it is unknown how anthropogenic induced climate change may impact future alligator gar distribution.

The goal of this study is to provide the first estimates of alligator gar minima temperatures and to determine how future climate change will potentially impact the suitable habitat of alligator gar. Chapter one of this study consists of the device that was developed to perform minima trials on alligator gar. Chapter two consists of collecting juvenile alligator gar from a hatchery setting and then acclimating them to three different testing temperatures and performing thermal minima trials. Chapter three consists of estimating the current and future distribution of alligator gar using occurrence records and future climate projections. This data was used in conjunction with alligator gar occurrence records to develop a model that would forecast how future climate scenarios would impact the suitable habitat of alligator gar. The results obtained during this study will help to contribute to the management and conservation of alligator gar.

Chapter I

An alternative, low-cost method to chill water for critical thermal minima trials

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RH: Simple, low-cost method to chill water

ABSTRACT

Water temperature is an important abiotic factor that impacts physiological processes in fish. Attempts to quantify thermal tolerance of fishes exposed to various temperatures have been performed since the 1800s. A common approach to test the thermal tolerance of fishes is the critical thermal method. Traditionally this method has required access to large and expensive lab equipment. However, many individuals may not have direct access to such equipment, making critical thermal studies difficult-to-impossible to conduct. We present a simple and cost-friendly alternative device to use for critical thermal minima trials. This device uses a cooler with a copper line and copper coil to chill water within an aquarium. A pump and valves control the water flow as it passes from the aquarium through the copper. This allows the user to lower the water temperature in the aquarium from 28^{o} C to 0^{o} C at a steady rate of 0.23^{o} C min^{-1}. The device allows those with limited funding or without access to traditional lab equipment the ability to perform critical thermal minima trials, expanding our collective understanding of life histories of fish and their potential vulnerabilities to increasingly altered thermal environments.

Keywords: Critical thermal minima; Fish; Methods; Temperature; Water cooling

INTRODUCTION

Water temperature is the most important abiotic factor to impact the physiological processes of fish and determine their distributions (Coutant, 1976; Beitinger, Bennett, & McCauley, 2000). Physiological processes of fish influenced by water temperature include reproductive timing, metabolism, growth rate, overall fitness, and mortality (Beitinger et al., 2000; Donaldson, Cooke, Patterson, & Macdonald, 2008; Long, Snow, & Porta, 2020). Water temperature also influences the threshold tolerance of fish to a variety of harmful stimuli (Coutant, 1976; Beitinger & McCauley, 1990). Rapid water temperature changes in excess of a fish's specific tolerance may result in mortality (Donaldson et al., 2008). Even sublethal exposure can alter behavior, resulting in a higher risk of predation and having decreased foraging success (Donaldson et al., 2008; Snow, Shoup, & Porta, 2018). Studies on how water temperature influences fish species dates back to the 1800s (Currie, Bennett, & Beitinger, 1998; Beitinger et al., 2000).

Common methods to test temperature tolerance of fish include incipient lethal temperature (ILT) technique (Fry, 1947), chronic lethal (CL) methodology (Fields, Lowe, Kaminski, Whitt, & Philipp, 1987; Zale & Gregory, 1989) and critical thermal (CTM) methods (Beitinger et al., 2000; Cowles & Bogert, 1944). CTM is deemed to be the most ecologically relevant test, as fish in their natural environment may be exposed to similar temperature variations, rather than the extreme temperature changes seen in both ILT and CL trials (Beitinger et al., 2000; Bennett & Judd, 1992; Currie et al., 1998). In critical thermal minima (CT_{min}) trials, a sample of fish is acclimated to a specific temperature and then subjugated to a linear decrease in temperature until a predefined sublethal endpoint, usually loss of equilibrium (LOE) is reached (Currie et al., 1998; Barker 2015; Fernando, Lochmann, & Haukenes, 2016). Water temperature is not required to maintain or reach a defined range of temperatures during CT_{min} trials. However, it is important that the decrease in temperature remains linear until the fish reaches a defined endpoint (Beitinger et al., 2000). The CTM methodology can also be used to establish temperature tolerance for threatened species (Beitinger et al., 2000) because the trials do not require large quantities of fish and individuals typically recover from LOE once returned to original acclimation temperature.

There has been more research on determining thermal maximums of fishes rather than thermal minimums. A review of temperature tolerances by Beitinger et al. (2000) found that CT_{max} had been reported for 108 fish species compared to only 37 species reported for CT_{min}. Additionally, Fernando et al. (2016) state that CT_{max} trials are easy to perform, and equipment is readily available. The discrepancy between the two trials may exist from the cost difference that is associated with each trial, or from an assumed need to focus on thermal maximums in a warming climate. However, warming climates can lead to increased variability in daily temperature extremes (Stott, 2016) which can potentially affect cold tolerance. For example, Fangue and Bennett (2003) found that Atlantic stingrays sacrificed heat tolerance to increase cold tolerance because low temperature extremes posed the greatest threat. Shultz, Zuckerman, and Suski (2016) found that thermal tolerance varied significantly between seasons in shallow water marine fishes. Consequently, the study of thermal minimums in fish needs to continue, especially when considering that temperature related fish kills in nature commonly result from exposure to low temperatures (Currie et al., 1998; Donaldson et al., 2008; Barker, 2015).

Various techniques and rates of change have been used by previous authors to perform CT_{min} trials. Many thermal trials require specialized equipment that can be costly, and each piece of equipment has caveats for use when dealing with rates of temperature change. Some studies have used ultra-cold freezers that cost between \$1,000 - \$20,000+ USD (Heath, Bennett, Kennedy, & Beitinger, 1994), others have used thermoregulators that start around \$2,000 USD (McClanahan, Feldmuth, Jones, & Stoltz, 1986), and some have used portable water chillers that cost approximately \$3,000 USD (Currie et al., 1998). Temperature change during trials needs to be rapid enough so that fish do not gain temperature tolerance, yet slow enough that core body temperature of fish does not diverge from water temperatures (Becker & Genoway, 1979; Beitinger et al., 2000). A temperature change of 0.3^{o} C per minute was proposed as a standard rate in evaluation of thermal maximum trials (Becker & Genoway, 1979). However, chilling water linearly is difficult, especially as water temperature nears 0^{o} C. Consequently, varying rates of change have been accepted for use in CT_{min} experiments, ranging from 0.01 to 0.5^{o} C min^{-1} (Bennett & Beitinger, 1997; Hockett & Munduhl, 1988; Jennings, 1991; McClanahan et al., 1986).

Herein, we present a simple, low-cost approach to perform CT_{min} trials. The cooling device is constructed with affordable, common components including an aquarium, copper tubing, and a cooler, yet the device can attain a steady linear decrease in temperature. This device provides an accessible and inexpensive method for performing CT_{min} trials, which could advance this field of study by overcoming the budgetary and access hurdles typical of traditional CT_{min} lab equipment.

MATERIALS AND METHODS

Materials are easy to obtain, relatively inexpensive, and construction should only take about a day. Tools needed for construction include a knife, PVC pipe cutter or saw, a drill, tape and pliers, materials needed are found in Table 1. The device is composed of an aquarium and cooler component, where water is chilled linearly by flowing from the aquarium through a closed system of tubes and copper that are housed in the cooler (Fig. 1). Assembly of the aquarium portion, wherein the fish will be placed and CT_{min} trials conducted, should be completed before the cooler portion so that hoses can be easily fitted together. The aquarium contains a water pump, that passes water through tubing connected to the copper line and copper coil, and a PVC inflow pipe where water returns to the aquarium after passing through the copper. The aquarium also has an acrylic and fiberglass mesh divider that keeps fish separate from the pump intake. Holes were drilled into the inflow pipe and acrylic sheet allowing water to mix uniformly inside the aquarium. The PVC inflow pipe was capped using a 12.7 mm PVC cap to force water through the drilled holes. Silicone (aquarium safe) was used to attach components in place. Styrofoam insulation was taped to all sides of the aquarium to resist heat from ambient room temperature and to reach a 0^{o} C water temperature. The front piece of insulation was cut so that it covered only the bottom half of the glass, creating a viewing window that allows fish to be monitored during trials.

A cooling unit, housed in a nearby cooler, is used to manipulate the water temperature of the aquarium. The cooler houses the copper line that is used during the early chilling phase of aquarium water, and the copper coil that is used during the latter stage of cooling (Fig. 1). A water pump inside the cooler promotes uniform cooling by circulating water and mixing the ice cubes and salt (NaCl) that are added during defined times

(Table 2). The addition of salt is needed to lower the freezing point inside the cooler and achieve 0° C inside the aquarium near the end of the trial. PVC vinyl tubing is used to attach the aquarium pump to the copper lines (Fig. 1, 2). Zip ties can be used to cinch tubing to the copper line and copper coil in order to prevent leaking.

Through trial and error, we found the following protocols to achieve a linear cooling rate, with step-by-step instructions reported in Table 2. This device can be operated by a single user; however, a second individual can be useful for monitoring potential endpoint criteria in fish being tested while the first person focuses on operation of the device. To operate the device, begin by filling the aquarium with water until it is 38 mm from the top of aquarium. Then fill the cooler so water completely covers the copper coil. The water pump inside the aquarium should not begin operating until the trial is ready to commence. Initially, water should flow through the copper line with the valve to the copper coil shut. After 75 minutes the temperature inside the cooler needs to be raised so that the copper coil valve can be opened and the valve to the copper line closed (Table 2). The purpose for raising the temperature inside the cooler is to prevent a rapid decline in temperature and allow for a continued linear temperature decrease inside the aquarium. Starting temperature inside the cooler should be approximately 11° C colder than that of the aquarium starting temperature. The initial trial of the cooling device was performed with an aquarium temperature of 28° C, and the starting temperature of the water in the cooler was 17° C. Water in the cooler will rise from ice melt and will need to be drained periodically so the water inside the cooler does not overflow.

After specifics were determined on how to operate the cooling device (Table 2), we performed five trial runs at room temperature. The water temperatures in the aquarium, and in the cooler, were monitored and recorded every five minutes during each trial using a YSI pro 2030 meter (YSI/Xylem,Inc., Yellow Springs, OH). A timing and instruction sheet was placed next to the aquarium in order to monitor time and keep a log of actions completed.

RESULTS

Unit construction took roughly four hours once all required supplies were obtained. Total cost of necessary equipment to construct one device was under $250 USD. During the trials, the rate of decrease in water temperature during aquarium trials was Temperature (^{o} C) = 27.46 + (-0.23*Time), $r^2 = 0.9933$ (Fig. 3). Temperature in the cooler declined from 17^o C to ~2.5^o C during the first 75 minutes and was then raised to 8^o C before opening the copper coil valve so that water continued to cool linearly within the aquarium (Fig.4). Temperature in the cooler then declined to -1.3^o C through the end of the trial (Fig. 4).

DISCUSSION

A starting temperature of 28^o C and ending temperature of 0^o C was used to encompass a wide range of acclimation and endpoint temperatures found in past trials. For example, largemouth bass (*Micropterus salmoides*, [Lacépède, 1802]) acclimated to 25^o C had an endpoint temperature of 7.3^o C (Currie et al., 1998), and sheepshead minnow (*Cyprinodon variegatus*, [Lacépède, 1802]) acclimated to 21^o C lost equilibrium at 6.9^o C (Bennett & Beitinger, 1997). Temperature during trials declined at a rate of 0.23^o C min^{-1} which falls between the proposed 0.3^o C min^{-1} (Becker & Genoway, 1979) and 0.1^o Cmin^{-1} used in past CT_{min} trails (Bennett & Beitinger, 1997). Critical thermal trials frequently overshoot the actual temperature where physiological disorder ensues (Beitinger et al., 2000). The slower rate of water cooling during this study, would allow for the body temperature of fish to better match the surrounding water temperature, potentially minimizing the endpoint overshoot seen with faster CT_{min} rates. Considering the size of the aquarium used, and that the appropriate rate of change for critical thermal studies is a function of fish size (Stevens & Fry, 1974), this device should be suitable for use with small-bodied freshwater fishes.

There are a few caveats to consider for the operation of this cooling device. Trials were conducted at room temperature (21^o C); if the device is operated at different ambient temperatures, then observed changes in temperatures will likely differ from this study. The cooling device is more prone to user error than costlier, automated technologies; thus, several practice runs should be conducted before performing trials with fish. We also found that the timing of ice addition, the quantity of ice added and altering when the copper coil valve opened had the greatest influences on the cooling rate. To prevent from having to weigh ice before every addition, a feed scoop was used where one scoop of ice equaled 1 kg. The copper coil must be completely covered by water or ice will develop inside the copper during operation. Ultimately, users could modify our protocols to attain other desired cooling rates through trial-and-error.

The amount of ice needed to conduct each trial run required 32 kg of ice to decrease the water temperature from 28^{o} C to 0^{o} C. In total we used a total of 160 kg of ice for five trials, which could be problematic if access to ice is limited. However, this quantity of ice should not be needed for most trials considering nearly half (47%) of the ice used during trials was used to achieve temperatures lower than 6^{o} C. For example, Currie et al. (1998) found the endpoint temperature for largemouth bass acclimated to 25^{o} C to be 7.3^{o} C, which would have only required 13 kg of ice. Furthermore, sheepshead minnows acclimated to 21^{o} C would require 12 kg of ice to reach an endpoint temperature of 6.9^{o} C (Bennett & Beitinger, 1997). Considering these examples are from two warm water fish species, larger quantities of ice should be expected when determining CT_{min} of cold-water fish species. For example, 26 kg of ice would be needed for rainbow trout (*Oncorhynchus mykiss*, [Walbaum, 1792]) acclimated to 20^{o} C to reach LOE at 2^{o} C (Currie et al., 1998). A possible alternative to ice cubes is the use of plastic bottles filled with water and then frozen. This could potentially produce desired results, allowing for a reusable ice source.

An alteration to consider for potential use in experiments is an evaluation of different coolers to either lower cost or to limit amount ice needed. The Igloo coolers (Nostalgia Products LLC., Green Bay, WI) used in this study are costly and it seems reasonable that similar, less expensive coolers would produce comparable results, thus lowering the overall cost per unit of the device. Further, if ice needed is a concern, multiple aquarium systems could be run inside a single larger cooler, eliminating the need for multiple coolers, and potentially reducing the amount of ice needed. Although, outside the scope of this study, one would need to conduct trail runs to ensure an appropriate linear rate of decrease in water temperature is obtainable.

Field trials could be performed if this device was altered for portability and rates at different ambient temperatures were observed. To simplify the use of this device, PVC hoses could be shortened and fitted with quick connect features that would allow for quick assembly and disassembly. The discharge pipe could be mounted so that it does not protrude out the top of the aquarium, consequently allowing the aquarium to fit inside the cooler for transport. Field trials could potentially be useful in determining thermal minima of endangered or threatened species where extended handling times are unwanted. The cooling device and methods outlined here provide an affordable pathway for conducting CT_{min} trials. This methodology should appeal to individuals without access or the storage space for the large and costly equipment that is traditionally needed for CT_{min} trials and for those wanting to perform field trails. If performed correctly this device will aid in determining critical thermal minima of many fish species, an area where continued expansion of knowledge is currently needed. Ultimately this device will allow for a better and more complete understanding of fish species’ life histories.

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CONFLICT OF INTEREST

There is no conflict of interest declared in this article.

DATA ACCESSIBILITY

All data used are present in this manuscript.

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Figure 1: *Schematic showing components used during construction of cooling device with arrows illustrating water flow. Quantity needed for the items found in the legend are in Table 1. PVC tubing lengths used in our device are A=254mm, B=533mm, C=89mm, D=457mm, E=1080mm, F=699mm, G=89mm, H=216mm.*

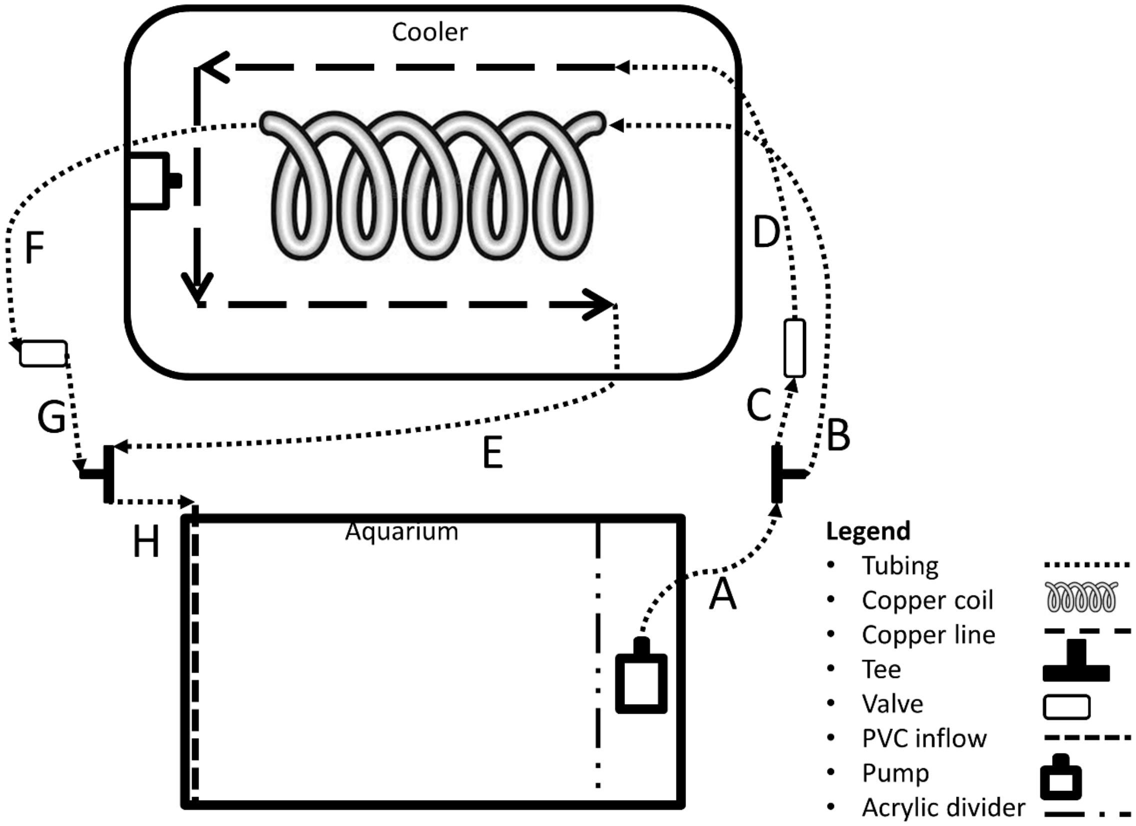

Figure 2: *Overhead view of cooler (top) next to aquarium view (bottom) showing PVC tubing attachment to valves, tees, copper coil and copper line. PVC labels are the same as those found in Figure 1. The placement of both water pumps, PVC inflow pipe and acrylic/mesh divider inside aquarium can also be seen.*

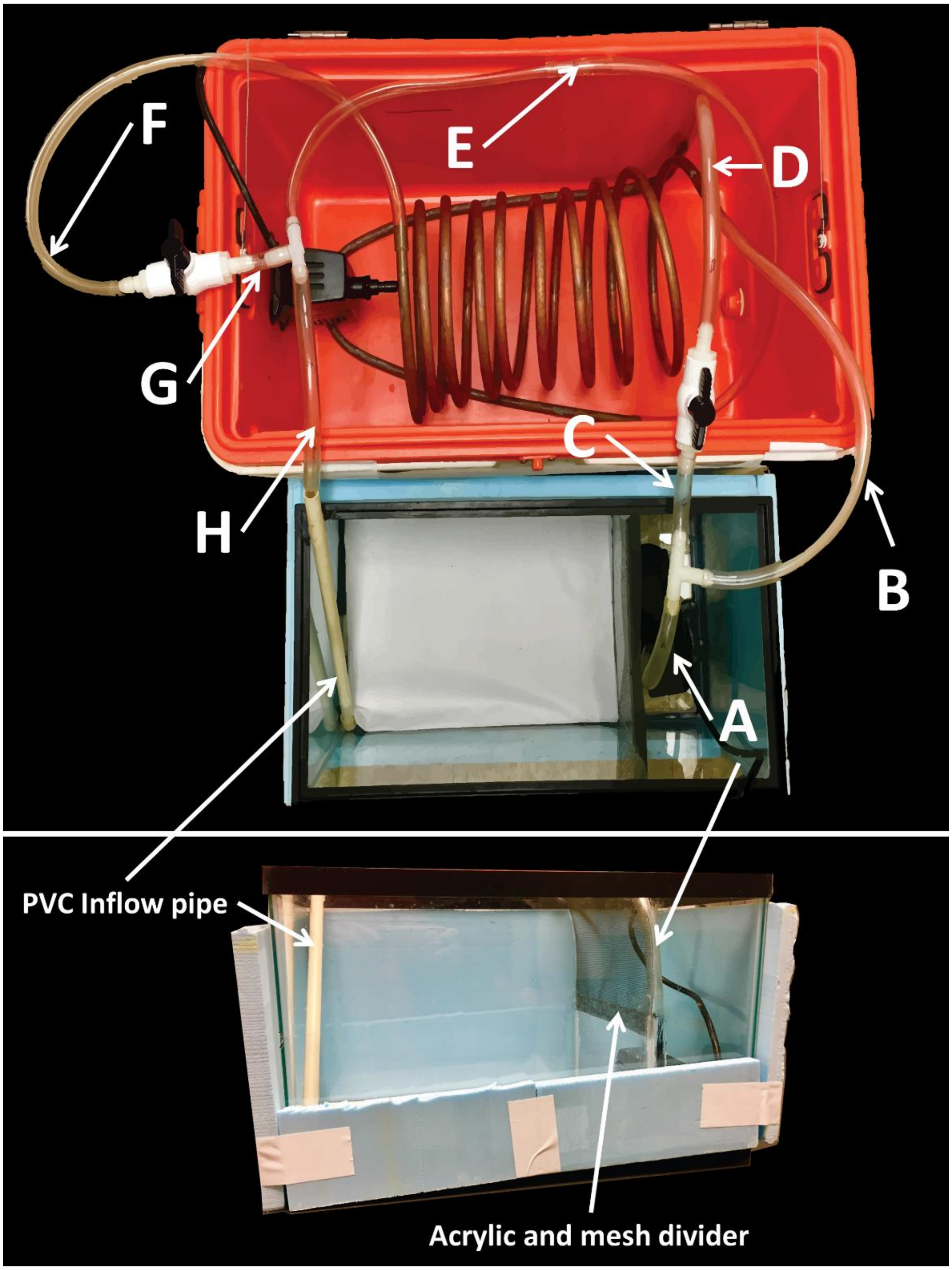

Figure 3: *Rate of change in aquarium water temperature during 5 trial runs using simple linear regression. Trend line represents best fit between water temperature and time. Temperature (^{o} C) = 27.46 + (-0.23*Time), r^2 = 0.9933.*

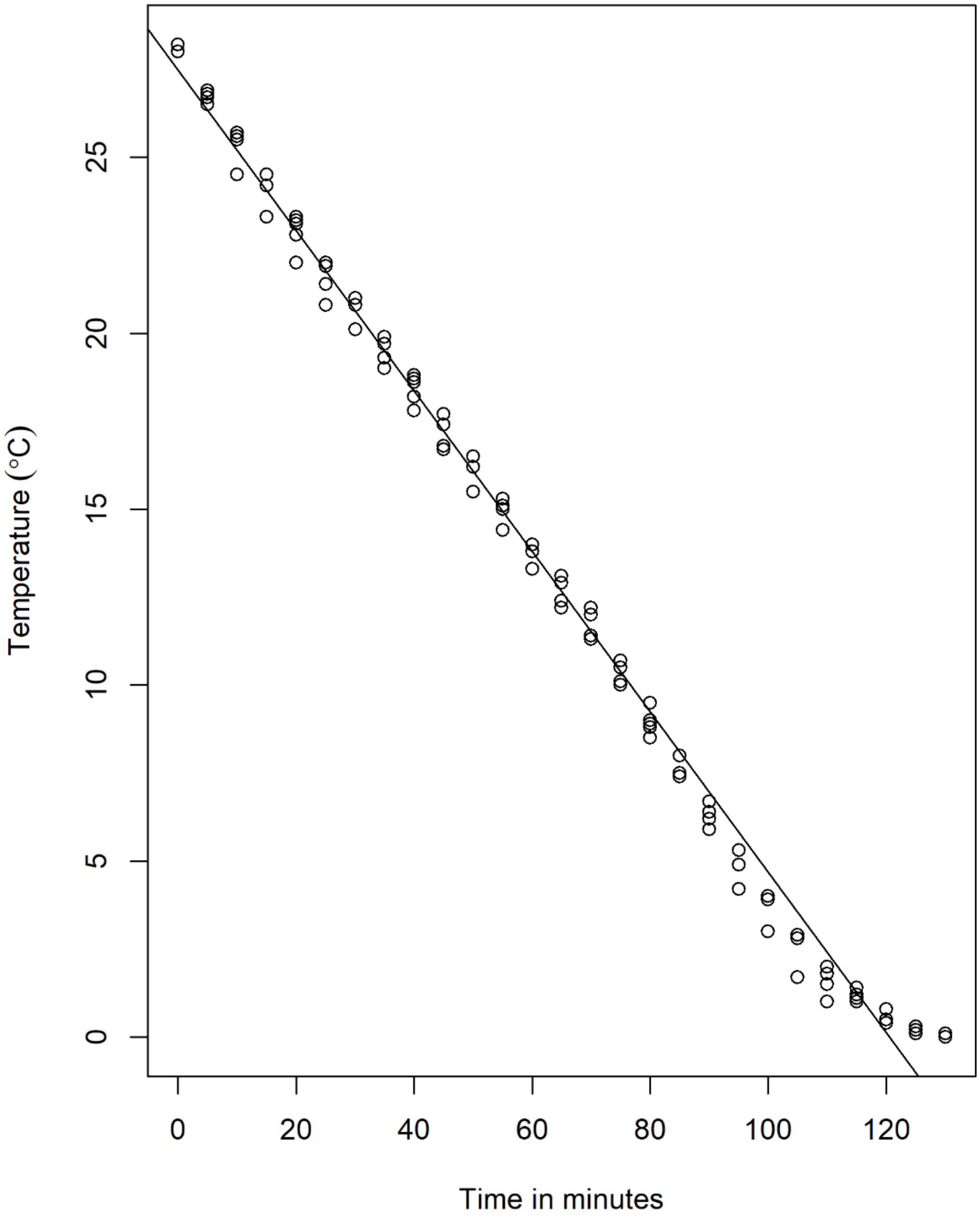

Figure 4: *Temperatures recorded inside cooler during 5 trial runs. Raise in temperature at 75 minutes is due to addition of warm water (Table 2) to raise cooler temperature to ~ 8 °C.*

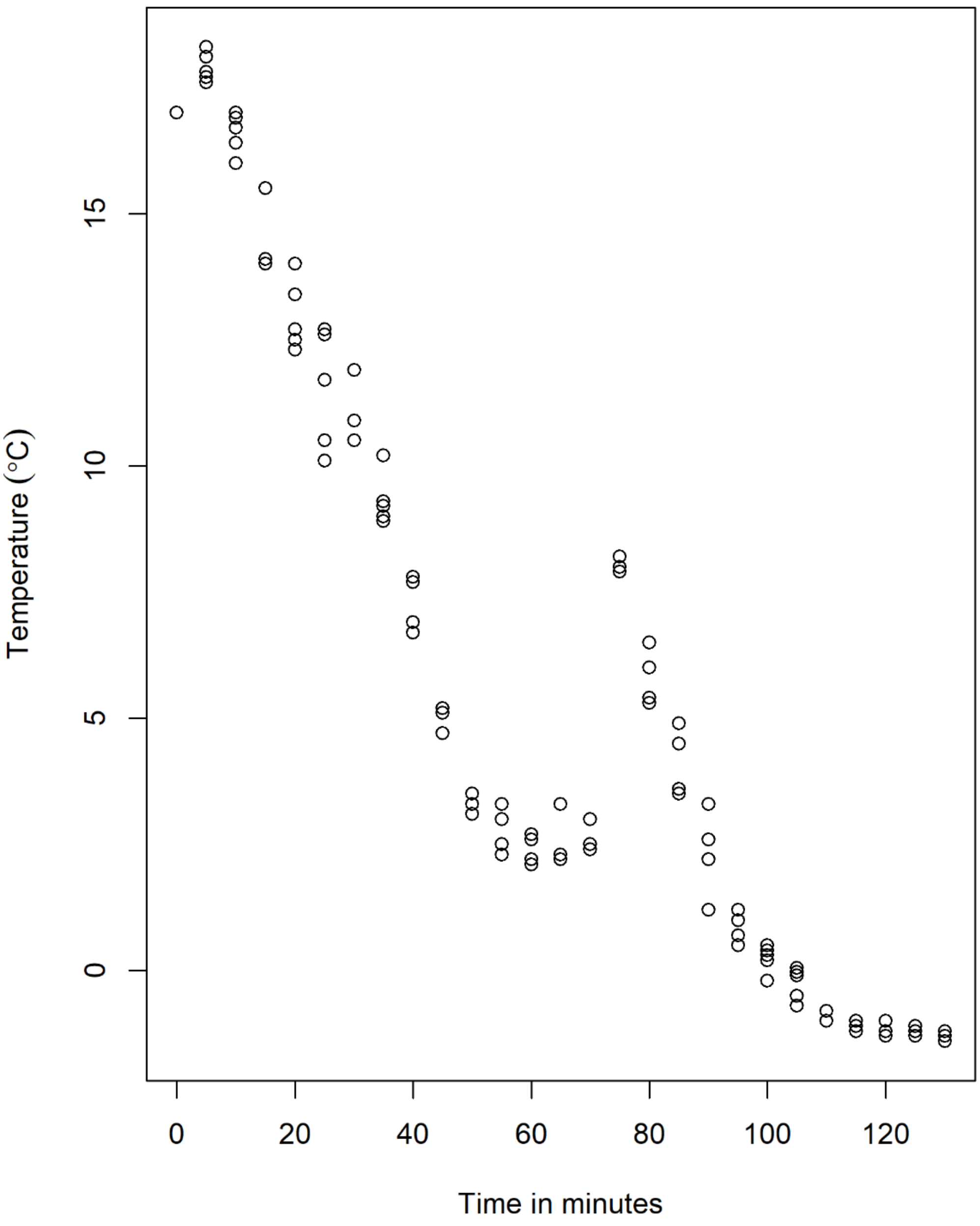

Table 1: *Materials needed, and total quantities used to construct cooling apparatus for CT_{min} trials. Items are separated into component location.*

Supplies and dimensions	**Quantity (Dimensions)**	**Approx. Cost (USD)**
	Cooler components	
68 Liter cooler	1	$90
12.7 mm Copper coil	1 (6096 mm)	$40
12.7 mm Copper line	1 (1067 mm)	$10
1499 LPH water pump	1	$10
	Aquarium components	
12.7 mm PVC pipe	1 (439 mm)	$2
12.7 mm PVC cap	1	$1
Fiberglass screen mesh	1 (191 mm X 241 mm)	$7
1499 LPH water pump	1	$10
37.85 Liter aquarium	1 (514 mm X 267 mm X 319 mm)	$30
Aquarium safe silicone(80mL)	1	$4
19mm Styrofoam insulation	1 (19 mm X 2438 mm X 1219 mm)	$13
Acrylic sheet	1 (165 mm X 241 mm)	$8
	Tubing components	
12.7 mm Male insert adapter	4	$2
12.7 mm Plastic tee fitting	2	$1
12.7 mm Female ball valve	2	$5
15.9 mm OD PVC vinyl tubing	1 (6096 mm)	$8
9.5 mm OD PVC vinyl tubing	1 (102 mm)	$3
203 mm Zip Ties	4	$6

Table 2: *The order of operations completed with resulting temperatures after each step. The starting aquarium water temperature = 28° C and starting cooler water temperature = 17° C.*

Time in minutes	**Action required to cooler**	**Temperature inside aquarium° C (SD)**
5	Add 1 kg of ice cubes	26.74 (0.09)
10	Add 1 kg of ice cubes	25.4 (0.15)
15	Add 1 kg of ice cubes	24.14 (0.49)
20	Add 2 kg of ice cubes, drain to initial	22.88 (0.53)
25	No action	21.6 (0.50)
30	Add 1 kg of ice cubes	20.6 (0.46)
35	Add 2 kg of ice cubes	19.56 (0.40)
40	Add 2 kg of ice cubes, Drain to initial	18.42 (0.41)
45	Add 2 kg of ice cubes	17.2 (0.43)
50	Add 1 kg of ice cubes	15.98 (0.45)
55	No action	14.84 (0.42)
60	Drain to initial	13.68 (0.36)
65	No action	12.7 (0.38)
70	No action	11.78 (0.40)
75	Raise temperature in cooler to 8^{o} C by adding warm tap water (~49° C). Then open copper coil and shut off copper line.	10.36 (0.30)
80	Add 2 kg of ice cubes, drain to initial	8.94 (0.36)
85	Add 2 kg of ice cubes	7.58 (0.24)
90	Add 4 kg of ice cubes	6.28 (0.29)
95	Add 5 kg of ice cubes	4.92 (0.45)
100	Add 6 kg of ice cubes and 0.5 kg of salt (NaCl)	3.74 (0.42)
105	Add 0.5 kg of salt (NaCl)	2.64 (0.52)
110	Break up ice sheet on surface	1.66 (0.42)
115	No action	1.16 (0.15)
120	No action	0.5 (0.1)
125	No action	0.02 (0.04)

Chapter II

Critical thermal minima of alligator gar (*Atractosteus spatula*, [Lacépède, 1803]) during early life stages

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RH: CT_{min} of juvenile alligator gar

SUMMARY

Water temperature controls a plethora of morphological and metabolic functions in fishes and studies examining thermal tolerance of fishes and the responses of fishes to changing water temperatures have been conducted for over a century. Although there has been a renewed interest in the management and conservation of alligator gar, currently there are no data regarding the thermal minima of the species. In this study, we obtained alligator gar juveniles from a hatchery setting, acclimated these fish to three separate water temperatures (21, 24.5, and 28ºC) and then subjected them to critical thermal minima trials to quantify lower thermal tolerance. Critical thermal minima results were found to be related with acclimation temperature and total length. Less developed gar experienced equilibrium loss at higher temperatures than larger, more developed gar. Also, alligator gar in the coldest acclimation trials (21ºC) were able to tolerate the lowest temperatures. The mean CT_{min} temperature values obtained for each acclimation trial were 11.57 ºC, SD = 1.39 (21ºC), 12.83 ºC, SD = 1.47 (24.5ºC), and 14.16 ºC, SD = 1.60 (28ºC). This study helps to fill an important data gap in alligator gar life history, and our results could be beneficial for management of alligator gar and determining potential future range distributions.

Keywords: **Alligator gar, Critical thermal minima**

INTRODUCTION

Alligator gar (*Atractosteus spatula* [Lacépède, 1803]) are members of a prehistoric lineage dating back 215 million years (Scarnecchia, 1992), and are a long-lived (>50 years), long to mature (5-10+ years), and highly fecund (>4000 eggs per kg of bodyweight) species (Ferrara, 2001; Brinkman, 2008). Historically, populations occurred in the Mississippi River drainage basin from Illinois (USA) to Mexico (Ferrara, 2001; Fernando, Lachmann & Haukenes, 2016). However, throughout much of their range, alligator gar populations have been reduced or extirpated (Ferrara, 2001; Fernando et al., 2016; Porta, Snow & Graves, 2019). Leading contributors to alligator gar population decline include overexploitation and habitat degradation (Scarnecchia, 1992; Buckmeier, 2008; Brinkman, 2008; van der Most & Hudson, 2017). Alligator gar rely on a narrow range of water temperatures, environmental conditions, and hydrologic factors for successful recruitment (Brinkman, 2008; Buckmeier, Smith, Daugherty & Bennett, 2017; Snow, Porta, Simmons Jr. & Bartnicki, 2018). Anthropogenic alterations in riverine systems, such as channelization, appear to be harmful to alligator gar because of their reliance on floodplains for spawning (Brinkman, 2008; Snow & Long, 2015; Buckmeier et al., 2017; van der Most & Hudson, 2017). Higher recruitment of alligator gar occurs when water temperatures are between 20-30^{o}C and inundation of floodplain vegetation lasts for periods longer than five days (Buckmeier et al., 2017), indicating the importance of suitable water temperature and accessible habitat for reproduction.

In response to the decline of alligator gar populations, several conservation agencies have implemented stocking programs to reintroduce alligator gar into areas where they have been locally extirpated (Mendoza et al., 2002; Hilsabeck et al., 2017; Porta et al., 2019). These efforts have been aided by the recent elucidation of many ecological and life-history aspects of this species, including individual movements (Buckmeier, Smith & Daugherty, 2013; Militello, 2013; Solomon, Phelps & Herzog, 2013), early life history (Brinkman, 2008; Snow & Long, 2015; Snow et al., 2018), recruitment success (Buckmeier et al., 2017; Snow et al., 2018), development (Aguilera et al., 2002; Mendoza et al., 2002; Long, Snow & Porta, 2020), and critical thermal maxima (CT_{max}; Fernando et al., 2016). However, a critical thermal minimum (CT_{min}) has yet to be evaluated in alligator gar of any size. Fernando et al. (2016) suggests that CT_{max} tolerance is uniform across all alligator gar populations. Despite this assertion, hatching rate of alligator gar eggs and development of juveniles varies significantly depending on water temperature, and some eggs fail to hatch when exposed to a water temperature of $15.5^{o}C$ (Long et al., 2020).

Critical thermal methodology (CTM) is an ideal method for testing temperature tolerances of threatened or endangered fish, because large quantities of fish are not required and fish are not sacrificed during trials. During CTM trials fish are exposed to linear rates of temperature change until a non-lethal endpoint, either onset muscle spasms (OS) or loss of equilibrium (LOE) is achieved (Beitinger, Bennett & McCauley, 2000). Understanding water temperature impact on fish is critical because effects of temperature on fishes are pervasive across biological scales, influencing spatial distributions of species and regulating numerous physiological processes within fish (Coutant, 1979; Beitinger et al., 2000). Temperature effects on fish include metabolic functions, growth rates, reproductive timing, and overall fitness and mortality (Coutant, 1976; Donaldson et al., 2008; Snow, Shoup & Porta, 2018). In nature, rapid decreases in temperature result in temperature-related fish kills (Donaldson, Cooke, Patterson & Macdonald, 2008; Barker, 2015). This is because fish gain heat tolerance more rapidly than cold tolerance and increases in temperature allow fish to escape warming waters whereas declining temperatures cause sluggishness in fish and inhibit the ability to escape (Currie, Bennett & Beitinger, 1998; Beitinger et al., 2000). Knowledge of the lower thermal limits of alligator gar would provide insight into when and where to stock individuals, potentially increasing success of reintroduction efforts. The objective of this study was to determine CT_{min} of juvenile alligator gar acclimated to three different water temperatures (21, 24.5, and $28^{o}C$).

METHODS

The Tishomingo National Fish Hatchery (TNFH) provided the alligator gar for this experiment. The Oklahoma Department of Wildlife used experimental gill nets to collect broodstock used to produce young from Lake Texoma, Oklahoma, during the winter of 2018-2019 (Binion et al., 2015; Schlechte et al., 2016). TNFH held adults in 0.6 hectare ponds until they were ready to be induced for spawning. In order to facilitate spawning, TNFH pulled adult alligator gar from holding ponds once water temperature reached 24°C. TNFH injected these gar with a LHRH (luteinizing hormone releasing hormone) analog and then placed them into recreational-grade swimming pools (4.6 m in diameter x 1.2 m deep) that contained black willow (*Salix nigra*, [Marshall, 1785]) branches as spawning substrate (Porta et al., 2019). Three days after hatching, we collected the juvenile alligator gar from TNFH and transported them to the Oklahoma Fisheries Research Lab. To reduce stress, we transported alligator gar inside tanks that contained the same water that the fish were being held in at TNFH. We divided and placed gar into 37.85 L holding aquaria that were at 21°C (room temperature), 24.5°C, or 28°C. We chose temperatures based on Long et al. (2020) who examined developmental rates of alligator gar eggs with these temperatures. We acclimated gar to these testing temperatures for 72 hours. Acclimation periods were short compared to other CT_{min} studies because of the fast growth rates of alligator gar. We conducted trials weekly from the end of May to early July.

We provided holding tanks with constant aeration and achieved higher acclimation temperatures (24.5 and 28°C) by using Aquatop 300-watt submersible heaters (Aquatop, Brea, CA). We fed alligator gar <80 mm in length a mixture of freeze dried plankton (*Euphausia pacifica*, [Hansen, 1911]), and Purina pellet feed (LAND O' LAKES, INC., Arden Hills, MN) daily and for gar >80 mm in length we used a combination of pellet feed and fathead minnows (*Pimepjales promelas*, [Kendall, 1903]). We fed all gar daily until satiated and removed excess food, waste, and water via siphoning. We then refilled aquaria with fresh well water that we raised to each acclimation temperature. Once CT_{min} trials were ready to be conducted, we selected multiple alligator gar from each acclimation holding aquaria and placed the gar into similar temperature experimental aquaria. An alternative, low-cost way to cool water was used for our CT_{min} trials, detailed in Bartnicki et al. (2021). In brief, we used an EcoPlus 396 water pump (Hawthorne Gardening Co., Vancouver, WA) to pass aquarium water through a series of copper tubes housed inside a cooler. We decreased water temperature at a rate of 0.3°C $^{min-1}$, which we monitored using a YSI pro 2030 meter (YSI/Xylem,Inc., Yellow Springs, OH). We evaluated gar using the loss of equilibrium (LOE) methodology (Becker, Genoway &

Schneider, 1979; Beitinger et al., 2000; Fernando et al., 2016), and water temperature was decreased until all gar in a trial reached LOE. We used a three second count to determine the LOE endpoint (Fernando et al., 2016). Once an individual gar lost equilibrium, we recorded the temperature where LOE occurred and then removed the individual gar and weighed and measured them. We then placed the gar into a room temperature holding tank (21ºC) where we monitored them during recovery.

We tested measured variables (including temperature [ºC], total length [mm], weight [g]; Table 1) for correlation using a Pearson's correlation test. Total length and weight were correlated at r = 0.949, and only one variable was used. We coded LOE temperatures with a dummy binary variable (0 = no effect, 1 = loss of equilibrium). We used logistic regression models to determine the relationship between the temperature where LOE occurred and either total length or weight for each acclimation trial temperature (21, 24.5, and 28 ºC). We analyzed the models using Akaike's information criterion (AICc) scores in addition to receiver operating characteristic (ROC) curves to evaluate the accuracy of the model used (Butler et al., 2016). The logistic regression containing total length performed better (AICc = 3037.9) than the logistic regression with weight (AICc = 4469.3). All analyses were performed using XLSTST 2020 (Addinsoft Inc., New York City, NY). All significance tests were evaluated at $P \leq 0.05$.

RESULTS

A total of 500 juvenile alligator gar ranging from 14 – 519 mm (median = 38 mm) and weighing from 0.01 – 690 g (median = 0.25 g) were used in acclimation temperature trials (21ºC = 167, 24.5 ºC = 167, and 28 ºC = 166). The mean CT_{min} temperature values obtained for each acclimation trial were 11.57 ºC, SD = 1.39 (21ºC), 12.83 ºC, SD = 1.47 (24.5ºC), and 14.16 ºC, SD = 1.60 (28ºC). The coldest temperature (8.2 ºC) where LOE occurred was from an alligator gar that was 501 mm in length, weighed 580 g and was acclimated to 21ºC. The warmest temperature (20.5 ºC) where LOE occurred was from an alligator gar that was 14 mm in length, weighed 0.1 g, and was acclimated to 24.5 ºC. A full range of LOE temperatures by length and acclimation temperatures were plotted and fitted with a two-phase decay line with model equation represented (Figure 1). All alligator gar fully recovered after trials and exhibited no signs of distress.

The best logistic regression model indicated that temperature (°C), total length (mm) and acclimation temperatures (21, 24.5, and 28ºC) significantly affected the temperature where LOE of alligator gar occurred (Table 2). The AUC (area under the curve) for the model was 0.948. No significant difference was detected for total length between acclimation trials (Table 3). Furthermore, as total length increased the effect of temperature where LOE ensued decreased (Figure 1), illustrating that smaller less developed gar (<100 mm) are more likely to experience LOE during changes in water temperature than larger individuals (Figure 2). The probability that LOE occurred for any individual gar decreased by approximately one percent for every 1 mm increase in total length (odds ratio 0.967).

We found significant differences among the three acclimation trials (Table 4), and as acclimation temperatures increased, the temperature where LOE ensued in alligator gar increased as well (Figure 3). For example, 94% of juvenile alligator gar had already experienced LOE at 13ºC for an acclimation temperature of 28 ºC, but only 30% of gar had experienced LOE at 13ºC for an acclimation temperature of 21ºC. Additionally, 28ºC acclimation temperatures had the strongest effect on LOE temperatures, with alligator gar being 3.63 times more likely to lose equilibrium for every 1ºC decrease in temperature (odds ratio 3.627). The effect of declining temperature decreased as acclimation temperatures decreased (24.5ºC odds ratio = 3.429 and 21ºC odds ratio = 2.984).

DISCUSSION

To our knowledge this is the first study to quantify CT_{min} in alligator gar. Acclimation temperatures were significantly different from one another and directly affected where LOE temperatures in alligator gar occurred. However, the effect of declining water temperatures was minimized as alligator gar grew in length. This finding suggests that alligator gar are more vulnerable during early development.

Similarly, different water temperatures affect early development of other Lepisosteidae species. For example, tropical gar (*Atractosteus tropicus*, [T. N. Gill, 1863]) larvae took longer to hatch at 25°C (80 hr) after fertilization compared to 35°C (21 hr; Marquez 1998). At 26°C Cuban gar (*Atractosteus tristoechus*, [Bloch & Schneider 1801]) hatched after 111 hr and began free swimming after 4 days in comparison to 30°C water temperature where Cuban gar hatched after 87 h and larvae were free-swimming in 3 days (Comabella, Hurtado, Canabal & Garcia-Galano, 2014). Likewise, alligator gar and spotted gar (*Lepisosteus oculatus*, [Winchell 1864]) hatching and free-swimming times were slowest at 15.5°C and fastest at 32.2°C (Long et al., 2020). Additionally, alligator gar exhibited 0% hatching success at the coldest temperature of 15.5°C (Long et al., 2020). The effect of lower water temperatures during early development could affect development and survival, thus impacting recruitment.

The comparatively higher CT_{min} values (~19°C) that were seen in smaller, less-developed individuals within high acclimation trials suggests that alligator gar are most vulnerable during early development. Early life history of alligator gar is complex, and larvae develop as follows: lecithotrophic (yolk sac nutrition only) at 1-4 days after hatching (DAH), lecithoextrophic (yolk sac and exogenous nutrition; = swim-up stage) at 5-8 DAH, and exotrophic (exogenous feeding only) at 9 DAH (Mendoza et al., 2002). Alligator gar in the beginning trials were still in their lecithotrophic and lecithoexotrophic stages and retained their adhesive suction discs. We observed that the gar could no longer remain attached to aquarium walls after the water temperature dropped below 18.5°C. This observation may indicate heightened vulnerability to temperature changes during early development. The loss of ability to attach to vegetation or woody debris early in life could increase vulnerability to predation by fish or other predators.

Spawning areas of alligator gar are frequently very shallow and are typically only available during seasonal floods that inundate terrestrial vegetation on the surrounding floodplain (Snow & Long, 2015; Buckmeier et al., 2017; van der Most & Hudson, 2017). Adult alligator gar select for the warmest water accessible on floodplains and are often cut off from the main river or reservoir as water levels recede after flooding events (Allen, Kimmel & Constant, 2020; van der Most & Hudson, 2017). These shallow areas experience large temperature fluctuations during diurnal heating and cooling events during the spring because the ground temperature has not warmed enough to provide thermal buffering (Jacobs, Heusinkveld, Kraai & Paaijmans, 2008). Our findings suggest that juvenile alligator gar are vulnerable during this time, especially in a changing climate with increased thermal variability, where a late season cold front could impact recruitment success by subjugating fish to lower threshold temperatures. For example, alligator gar that hatch in colder temperatures have a reduced free-swimming percentage, and an extended time to free-swimming stage, potentially increasing vulnerability to predation (Long et al., 2020). Exposure to sublethal temperatures can also impact survival given that fish weakened from stress have decreased foraging success and a higher risk of predation (Coutant, 1976; Donaldson et al., 2008; Snow et al., 2018). In areas where juvenile alligator gar have access to thermal refugia during these temperature fluctuations, the idiosyncrasies of early gar development may hinder their ability to escape (Long et al. 2020).

This study provides the first quantified estimates of juvenile Alligator Gar thermal minima temperatures. The CT_{min} values obtained during this study may support why northern alligator gar populations rely on late season flooding events for higher recruitment (Allen et al., 2020) and further explicate the findings of Buckmeier et al. (2017) where lower year class recruitment was found in the Trinity River, Tx (USA) during April and May even when suitable flooding events occurred during those months. Reproduction and survival of larval alligator gar at the northern edge of its range may be aided by a warming climate, however yearly recruitment may be impacted by extreme temperature fluctuations and other ancillary effects beyond temperature (Ficke, Myrick & Hansen, 2007). Results from this study can be applied to future studies aimed at conservation and management of alligator gar. For example, our findings may aid in delineating the potential northern extent of alligator gar distribution given current and projected climate data. Considering that water temperature accounts for up to 50% of the variation in survival and growth (Chambers & Trippel 1997), future studies should focus on how extreme weather events (i.e., late season cold fronts, cold rain events) impact water temperatures across floodplain habitats that are used by juvenile alligator gar during early development. Additionally, because our largest individual was 519 mm TL, further investigation of CT_{min} should be completed on alligator gar > 519 mm TL to better understand the impact of cold or declining water temperatures on larger alligator gar. The information obtained during this study will further contribute to the management and conservation practices of alligator gar.

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CONFLICT OF INTEREST

There is no conflict of interest declared in this article.

DATA AVAILABILITY STATEMENT

All data used are present in this manuscript.

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Figure 1: *Plot of loss of equilibrium (LOE) temperatures experienced by alligator gar at different lengths. A best fit line with a corresponding equation is included for each acclimation temperature (21°C [blue], 24.5°C [black], and 28°C [red]).*

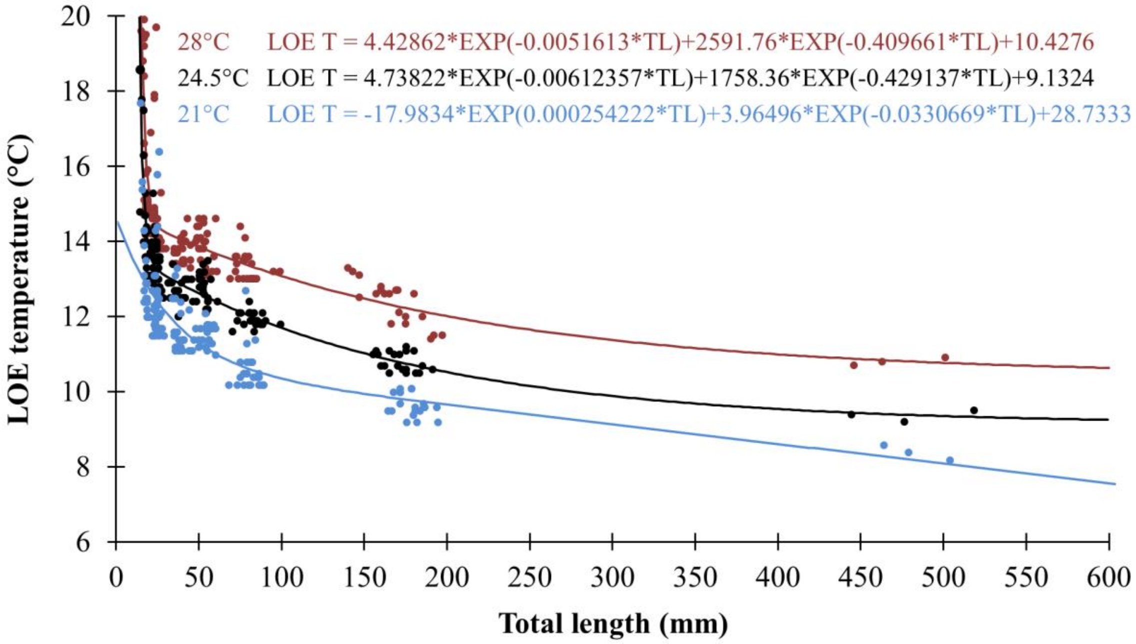

Figure 2: *Logistic regression model displaying the relationship between the total length (mm) of an alligator gar and the likelihood that loss of equilibrium is experienced during all temperature changes in any acclimation temperature trial (21, 24.5, and 28°C). Dashed lines represent 95% confidence intervals.*

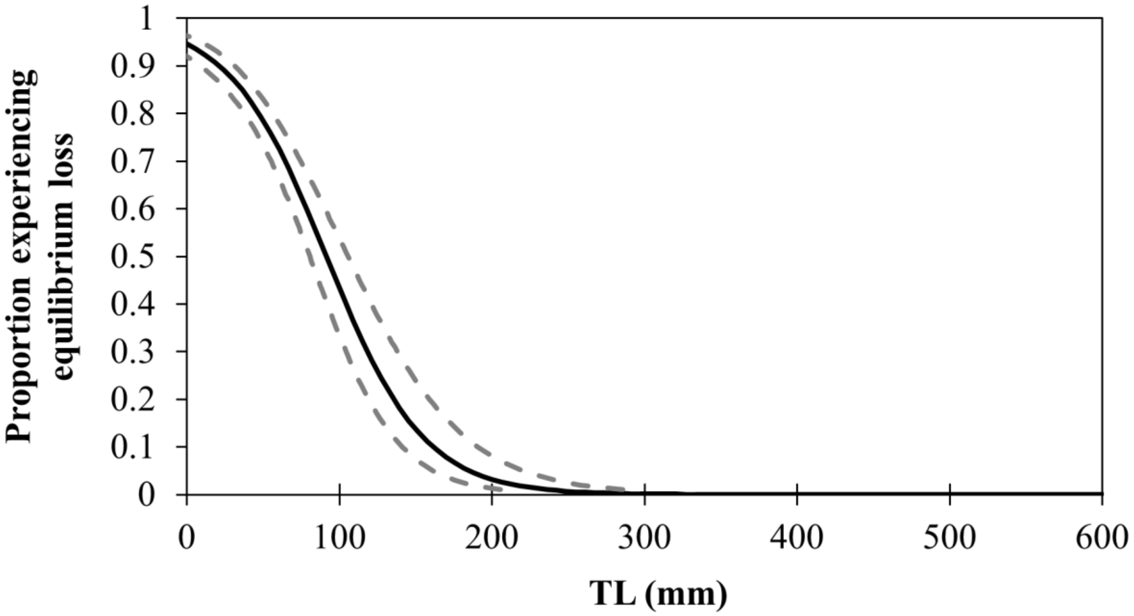

Figure 3: *Logistic regression model displaying relationship between acclimation temperatures (21°C [blue], 24.5°C [black], and 28°C [red]) and the probability that an alligator gar will experience loss of equilibrium (LOE) at a specific temperature (°C). Note the reversed x-axis signifying cooling water temperatures. Dashed lines represent 95% confidence intervals.*

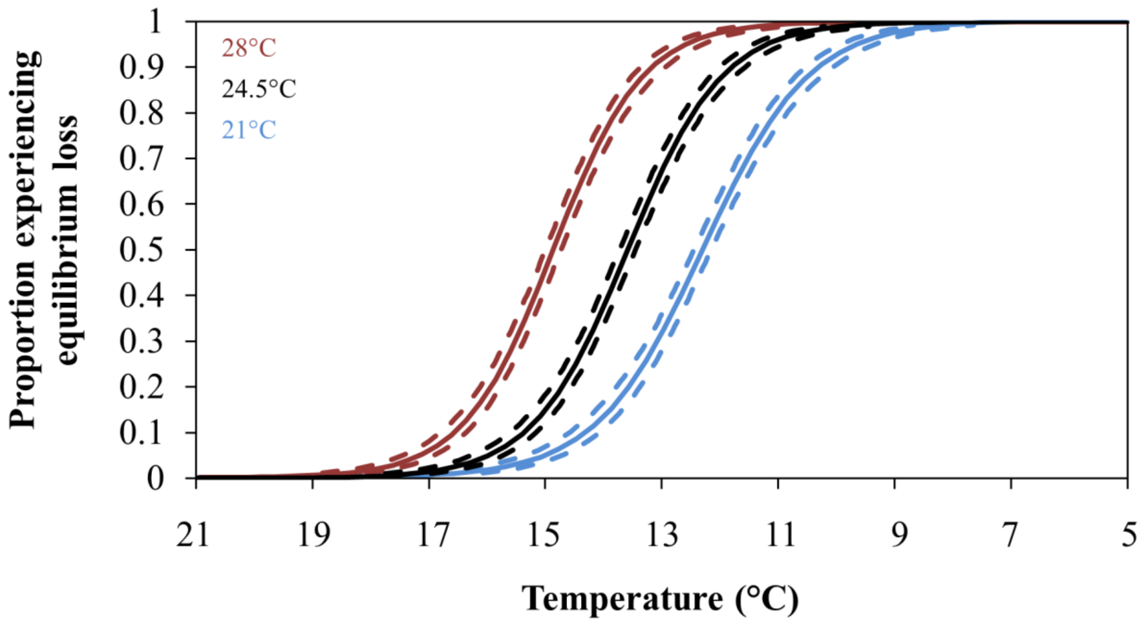

Table 1: *A Pearson correlation matrix of variables used in the multiple logistic regression model. The variables with values in bold show a strong correlation (≥ |0.80|).*

Variables	Temperature (°C)	TL (mm)	Weight (g)
Temperature (°C)		-0.721	-0.572
TL (mm)	-0.721		**0.949**
Weight (g)	-0.572	**0.949**	

Table 2: *Results of the multiple logistic regression model examining loss of equilibrium of alligator gar in relation to temperature (°C), total length (mm), and acclimation temperature (21, 24.5, and 28 °C). Variables with P-values in bold were significant at an alpha level = 0.05.*

Variables	X^2	df	*P*-value
Temperature (°C)	301.66	1	< **0.01**
TL (mm)	23.46	1	< **0.01**
Acclimation temp.	184.31	2	< **0.01**

Table 3: *Results of the multiple logistic regression model comparing total length (mm) of alligator gar between each acclimation temperature trial (21, 24.5, and 28 °C). Acclimation trial comparisons with P-values in bold had a significant difference between total length at alpha level = 0.05.*

TL for each acclimation temperature	X^2	df	*P*-value
21°C vs 24.5°C	0.099	1	0.75
21°C vs 28°C	0.134	1	0.71
24.5°C vs 28°C	0.001	1	0.97

Table 4: *Results of the multiple logistic regression model comparing loss of equilibrium of alligator gar between acclimation temperature (21, 24.5, and 28 °C). Acclimation trial comparisons with P-values in bold were significantly different between trials at an alpha level = 0.05.*

Acclimation trial comparison	X^2	df	*P*-value
21°C vs 24.5°C	50.01	1	**< 0.01**
21°C vs 28°C	112.06	1	**< 0.01**
24.5°C vs 28°C	55.32	1	**< 0.01**

Chapter III

Use of multiple climate change scenarios to predict future distributions of alligator gar (*Atractosteus spatula*)

Use of multiple climate change scenarios to predict future distributions of alligator gar (*Atractosteus spatula*)

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ABSTRACT

Climate change is expected to cause extinction in vulnerable species and reduce available habitat for others. Freshwater communities are particularly vulnerable considering they are confined to fragmented habitats. Alligator gar is a species that may be impacted by changes in climate. We created a species distribution model to provide insight about the projected range for alligator gar in the United States under four different climate change outcomes (SSP126, SSP245, SSP370, SSP585). Our models suggest that the most suitable habitat will shift from where alligator gar are currently found and move to the northeast by 2080. Under the most severe climate scenario, the centroid for alligator gar range will shift north-northeast ward nearly 900 km from the current location in central Louisiana to south-central Illinois. Freshwater fish have limited dispersal capabilities, and alligator gar will not be able to populate newly suitable habitats without fishery manager influence. Additionally, it is likely that local extinctions will occur in areas where alligator gar currently reside considering that suitable habitat is projected to decline across their current range. However, the impacts of decreasing suitability may not be initially noticed because alligator gar are a long-lived species and larger individuals will likely persist across their current range.

Keywords: **Alligator gar, climate change, distribution, ecological niche model, Maxent**

INTRODUCTION

Biological shifts in community compositions, species distributions and phenology have been linked to anthropogenic climate change (Butler et al., 2016; Lynch et al., 2016). Anthropogenic induced climate change is expected to increase the likelihood of extinction in vulnerable species (Burkhead, 2012; Butler & Larson 2020), and reduce suitable habitat availability for many other species (Butler et al., 2016; Murawski, 2011; Taylor et al. 2017). There have been many studies investigating how climate change will influence marine populations, and many communities are expected to shift towards the poles (Mohseni et al., 2003).

Inland fish communities are particularly susceptible to anthropogenic alterations, especially those that are confined to fragmented habitats (Krabbenhoft et al., 2020; Taylor et al., 2017). Over the last century freshwater fish have experienced the fastest extinction rate among all vertebrates (Burkhead, 2012). Climate change is expected to alter hydrologic regimes by impacting water temperatures, dissolved oxygen, salinity concentrations and the timing, frequency, and severity of flow events (Bond et al., 2011; Roghair & Adams, 2019; Xenopoulos et al., 2005). Understanding how fish communities are impacted by a changing climate combined with anthropogenic alterations, such as stream modification and increased water usage, is imperative for conservationist and fisheries managers alike.

The alligator gar (*Atractosteus spatula*) is a fish that might be impacted by climate change and anthropogenic alterations. Alligator gar are one of the largest and oldest living fish species in North America, and inhabit many different habitats, including rivers, reservoirs, and gulf estuaries (Scarnecchia, 1992; Snow and Long, 2015). Alligator gar require submerged vegetation, usually on an inundated river floodplain, for successful spawning and recruitment (Allen et al., 2020; Buckmeier et al., 2017; Snow et al., 2018), and flooding events in April and May resulted in lower recruitment of alligator gar compared to floods that occurred later in the season (Buckmeier et al., 2017). Considering that climate change is expected to reduce water availability, though river discharge (Xenopoulos et al., 2005) successful spawning events may become increasingly limited. Water temperature pre- and post-hatch is also important, cooler temperatures lead to longer incubation times and alligator gar eggs exposed to water temperature of 15.5°C failed to hatch. In critical thermal minima trials, > 90% of juvenile alligator gar experienced equilibrium loss by 10°C when acclimation temperature was 21°C (Bartnicki et al., 2021). Also, water temperatures that are too hot can be detrimental for development as alligator gar displayed development abnormalities when exposed to 32.2°C incubation temperatures (Long et al., 2020).

Other studies have investigated alligator gar life histories and anthropogenic alteration to rivers. However, investigation into the impact of anthropogenic induced climate change on the species has yet to be investigated. Our goal was to use Maxent to accurately determine where alligator gar occur presently. We then projected habitat suitability under four different climate scenarios to potentially determine how alligator gar range may be impacted by the effects of climate change.

METHODS

We downloaded records for alligator gar from the Global Biodiversity Information Facility (GBIF.org [5 June 2020] GBIF Occurrence Download https://doi.org/10.15468/dl.hjp8hu) and combined them with occurrences from published papers, historical photos, and field observations. We used Maxent to create a species distribution model delineating the current existing range and projecting potential future ranges under a variety of climate change scenarios. We removed duplicate records and trimmed the remaining occurrences so that there was only one occurrence point per 25 km^2. We downloaded 19 bioclimatic variables (Table 1) from WorldClim (Fick & Hijmans, 2017) at a resolution of 2.5 arc-minutes (25 km^2). We obtained terrestrial and aquatic raster data (Table 2) from the HydroSHEDS database (Lehner et al., 2008). We set the spatial extent north to south from southern Canada to central Mexico and east to west from the Atlantic coastline to the Rocky Mountains in the United States.

We employed the methods that are outlined by Butler and Larson (2020) and Butler et al. (2016), and only used the variables that had the highest gain when used in isolation (contained the most predictive information) and the variables that contained unique predictive information. We followed the regularization approach used in ENMtools (Warren et al. 2021) and used small sample corrected variant of Akaike's information criterion (AICc) scores to evaluate models (Warren & Seifert, 2011). We used all possible combinations of variables that did not contain high multicollinearity (e.g., $|r| < 0.8$). We used 70% of occurrence records for training, 30% for model validation, and 10,000 background points. To create receiver operation characteristic (ROC) curves we plotted sensitivity against 1 – specificity and then used 10-fold cross-validation training and testing AUC (area under the curve) scores to evaluate the accuracy of the model. To determine which models best described the current distribution of alligator gar we used AICc scores and model weights in combination with AUC scores.

We projected the potential future distribution of alligator gar at 2.5 arc minutes (25 km^2). We utilized the model that best predicted the current distribution of the species and combined it with future climate projections, to year 2080, that used CMIP6 data from WorldClim (Fick & Hijmans, 2017). We evaluated four scenarios that used eight Global Climate Models (BCC-CSM2-MR, CNRM-CM6-1, CNRM-ESM2-1, CanESM5, IPSL-CM6A-LR, MIROC-ES2L, MIROC6, MRI-ESM2-0) under four Shared Socio-economic Pathways (SSPs) scenarios (SSP126, 245, 370 and 585), where each scenario differs by the amount of carbon dioxide that is emitted into the atmosphere during the 21st century, with SSP126 having the least emissions and SSP585 containing the most emissions. We averaged models to project suitability for alligator gar under each SSP scenario to the year 2080. Following Butler et al. (2016), we separated results into five suitability categories: 0-10% suitable, 10-20% suitable, 20-35% suitable, 35-50% suitable, and >50% suitable. We generated response curves for variables in the top model to reveal values where suitability was higher than 50%.

RESULTS

The model that best explained alligator gar distribution contained the variables mean temperature of warmest quarter (BIO 10), mean temperature of coldest quarter (BIO 11), elevation and inundation (Table 3). Areas containing at least 50% suitability or greater included the Mississippi River valley in western Tennessee and northern Arkansas southward to the Gulf of Mexico (Figure 1). The Louisiana and Texas coastlines as well as the Red, Sabine, Neches, Trinity, Brazos, Colorado, Guadalupe, San Antonio, Nueces, and Rio Grande rivers also showed at least 50% suitability. The mean AUC scores for the best model were training AUC = 0.960 and testing AUC = 0.959 (Table 3).

Under all future projections, highly suitable habitat increases and shifts northward (Figure 2). Alligator gar centroids shift to the north and east in all climate projection scenarios (Table 4), moving from central Louisiana (Current), into Tennessee and Kentucky, and reaching as far north as Illinois (Figure 3). Suitability within the current range of alligator gar decreases under all future climate scenarios. Range of values obtained from response curves for suitability > 50% were 0 – 22.5 m for elevation, 27.5 – 29.5°C for mean temperature of warmest quarter (BIO 10), 10.25 – 20.75°C for mean temperature of coldest quarter (BIO 11), and 1.5 – 92.5% for inundation (Table 5).

DISCUSSION

The estimated current range of alligator gar that was produced by Maxent was comparable to their actual range. However, some highly suitable habitat is projected an area that is outside the current range of alligator gar and is located along the eastern coast of Florida, Georgia, and South Carolina. Maxent has predicted suitable habitat outside native ranges for plants and animals in past studies (Ficetola et al., 2009; Wilson et al., 2009), including turtles (Butler et al., 2016) and palm trees (Butler & Larson, 2020). The Appalachian Mountains and Florida peninsula separate the uninhabited suitable area for alligator gar from the inhabited area and may have acted as a geographic barrier that prohibited eastern expansion. Overall, the highest predicted occurrence was located within alligator gar's geographic range.

The northward shift of alligator gar suitability is not surprising considering that both marine and freshwater fish communities are expected to be pushed towards the poles as the earth's climate changes (Brander, 2007; Murawski, 2011). Although habitat suitability is expected to increase in all climate change models, actual movement of alligator gar populations may not be able to disperse into these new habitats. Alligator gar, and all fish in general, have limited dispersion capabilities (Potts et al., 2019) and may not be able to expand into new territories with the shift in suitable habitat. Movement will be hindered by dams, reservoirs, and other anthropogenic modifications (Buckmeier, 2008; Militello, 2013). State agencies and fishery managers should consider stocking fish to introduce populations into expanding habitats that alligator gar cannot disperse into otherwise.

These results reveal a potential outcome of climate change on alligator gar range. However, land use is occurring at a fast rate (Comte et al., 2021; Taylor et al., 2017) and may not be conducive for alligator gar to occupy some of the projected suitable habitat, as alligator gar require some specific hydrologic conditions for spawning purposes (Allen et al., 2020; Buckmeier et al., 2017; Snow et al., 2018). Ultimately, these results provide insight into potential shifts of alligator gar habitat and can be used to direct ongoing conservation efforts.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

None declared.

Table 5: *Bioclimatic variables used during this study*

Variable	**Definition**
BIO 1	Annual mean temperature
BIO 2	Mean diurnal range (mean of monthly [max temp. - min temp.])
BIO 3	Isothermality (BIO 2 / BIO 7) x 100
BIO 4	Temperature seasonality (standard deviation x 100)
BIO 5	Max temperature of warmest month
BIO 6	Min temperature of coldest month
BIO 7	Temperature annual range (BIO 5 - BIO 6)
BIO 8	Mean temperature of wettest quarter
BIO 9	Mean temperature of driest quarter
BIO 10	Mean temperature of warmest quarter
BIO 11	Mean temperature of coldest quarter
BIO 12	Annual precipitation
BIO 13	Precipitation of wettest month
BIO 14	Precipitation of driest month
BIO 15	Precipitation seasonality (coefficient of variation)
BIO 16	Precipitation of wettest quarter
BIO 17	Precipitation of driest quarter
BIO 18	Precipitation of warmest quarter
BIO 19	Precipitation of coldest quarter

Table 6: *Soil, aquatic, and elevation variables used during this study.*

Variable	Definition
Elevation	Elevation above sea level in meters
Inundation	Represents three states of land surface inundation extents: mean annual minimum (permanently inundated), mean annual maximum (seasonally inundated), and long-term maximum (areas affected by extreme flood events)
Stream Gradient	Ratio between the elevation drop within the river reach (i.e. the difference between min. and max. elevation along the reach) and the length of the reach
Discharge	Annual minimum and maximum discharges were derived from the 12 long-term average monthly flow values (1971-2000), i.e. they represent the flow of the lowest or highest month within the average year
Limnicity	Percent lake area in a given spatial unit
Degree of Regulation (DoR)	Index of how strongly a dam or set of dams can affect the natural flow regime of downstream river reaches
Sand Fraction in Soil	Contains spatial predictions for a selection of soil properties (at six standard depths) including sand
Clay Fraction in Soil	Contains spatial predictions for a selection of soil properties (at six standard depths) including clay
River Area	Surface area of entire river reach (width x length)
River Volume	Water volume for each river reach (width x depth x length)
Terrain Slope	Gradient of terrain surface
Freshwater Habitat Types	Biogeographic regionalization of Earth's freshwater biodiversity based on regional expert knowledge

Table 7: *The best model runs with variables that best explain alligator gar distribution. Beta (β) is the regularization multiplier. The natural log of probability of the data present in the model is given by the log likelihood. AICc is a small-sampled corrected AIC score; the top model is shown along with the four closest performing models are shown. Delta AICc is the difference between the top model and other model scores. The wAICc is the model weight score which is calculated from the relative likelihood for each model, divided by the total relative likelihood for all models that were considered. Area under the curve (AUC) is a metric to evaluate model accuracy.*

Variables	Beta (β)	Log-likelihood	AICc score	ΔAICc	wAICc	Mean Training AUC	Mean Testing AUC
Elevation, BIO 10, BIO 11, Inundation	1.00	-4086.12	8350.71	0	1.0	0.960	0.959
Elevation, BIO 4, BIO 10, Inundation	1.00	-4082.54	8371.96	21.25	0.00	0.962	0.960
Elevation, BIO 6, BIO 10, Inundation	1.00	-4101.34	8399.91	49.19	0.00	0.962	0.959
Elevation, BIO 7, BIO 10, Inundation	1.00	-4114.76	8477.09	123.37	0.00	0.960	0.958
Elevation, BIO 1, Inundation	1.50	-4225.86	8541.444	190.73	0.00	0.949	0.947

Table 8: *Distance that the centroid for alligator gar moves for each climate projection scenario and the rate of movement per decade*

Scenario projection	Distance (km) and direction from current	Rate per decade (km/decade)
Current	0	-
SSP126	442.99 (NE)	73.83
SSP245	616.42 (NNE)	102.74
SSP370	749.38 (NNE)	124.90
SSP585	899.08 (NNE)	149.85

Table 5: *Range of values from response curves for the variables used in the current projection of alligator gar where there is >50% suitability. Elevation is meters above sea level, Bio 10 is mean temperature of warmest quarter, Bio 11 is mean temperature of coldest quarter, and inundation is percent of floodplain covered.*

Variable	Range of values
Elevation	0 – 22.5 m
Bio 10	27.5 – 29.5°C
Bio 11	10.25 – 20.75°C
Inundation	1.5 – 92.5%

Figure 4: *The current modeled distribution for alligator gar. The legend shows probability of occurrence, the red shade represents >0.5 probability.*

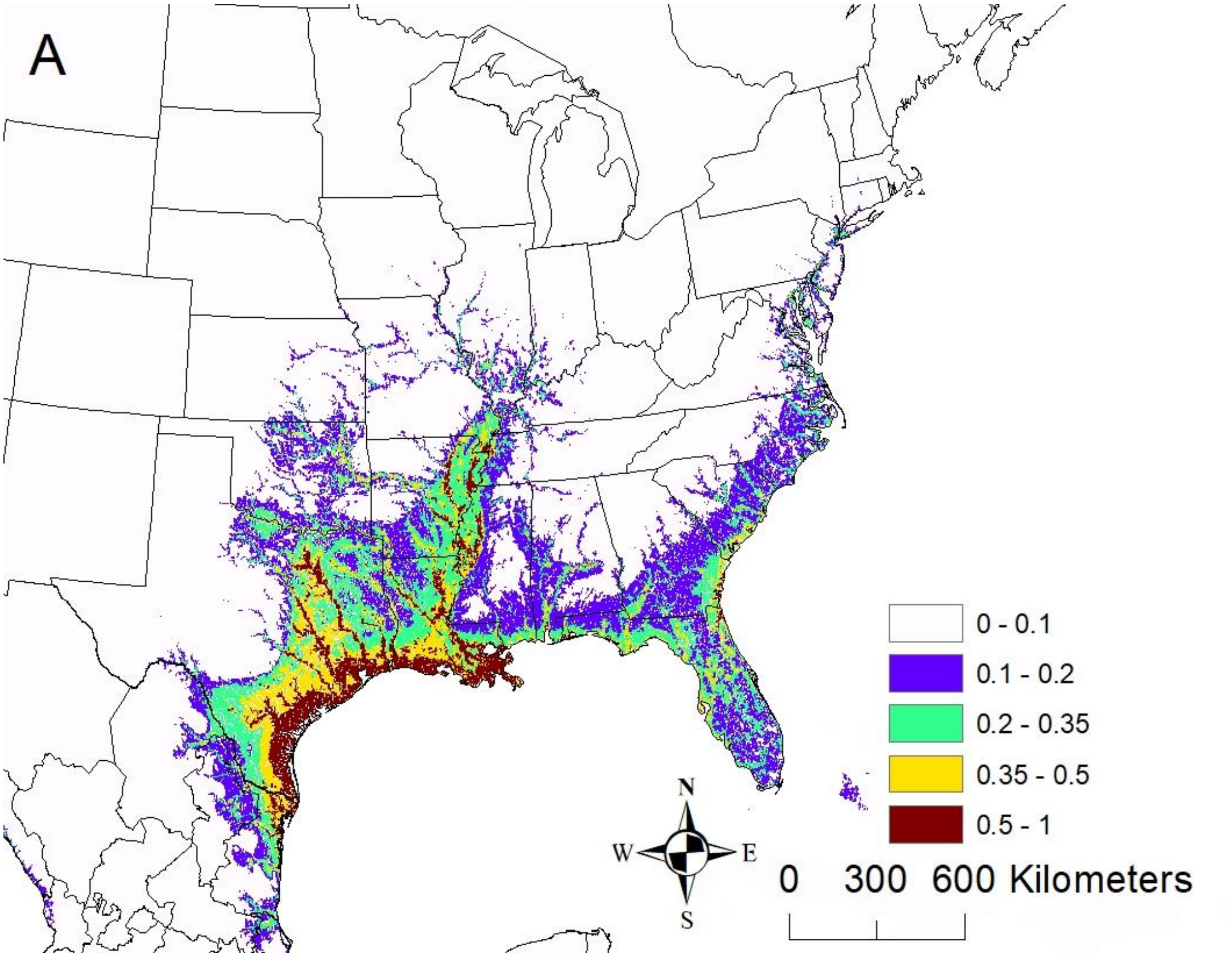


Figure 5: *The future model projections for alligator gar under four Shared Socio-economic Pathways (SSPs) climate scenarios. The legend shows probability of occurrence, the areas in red represents a >0.5 probability.*

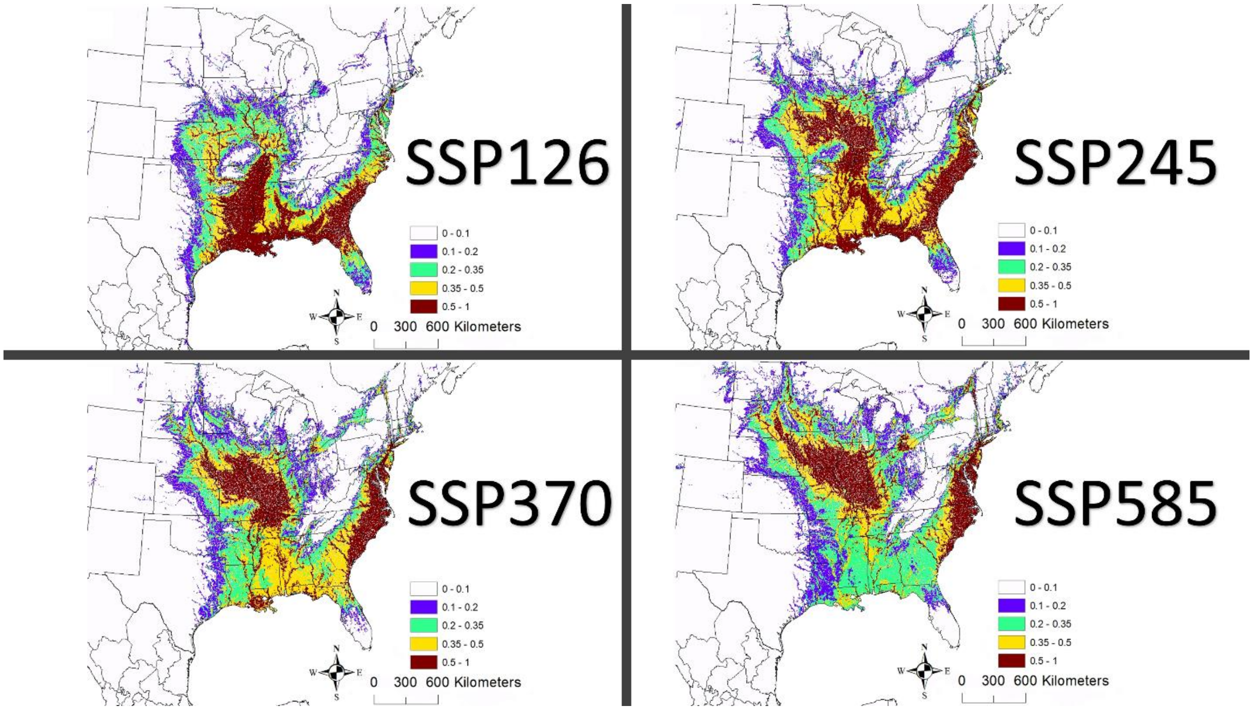
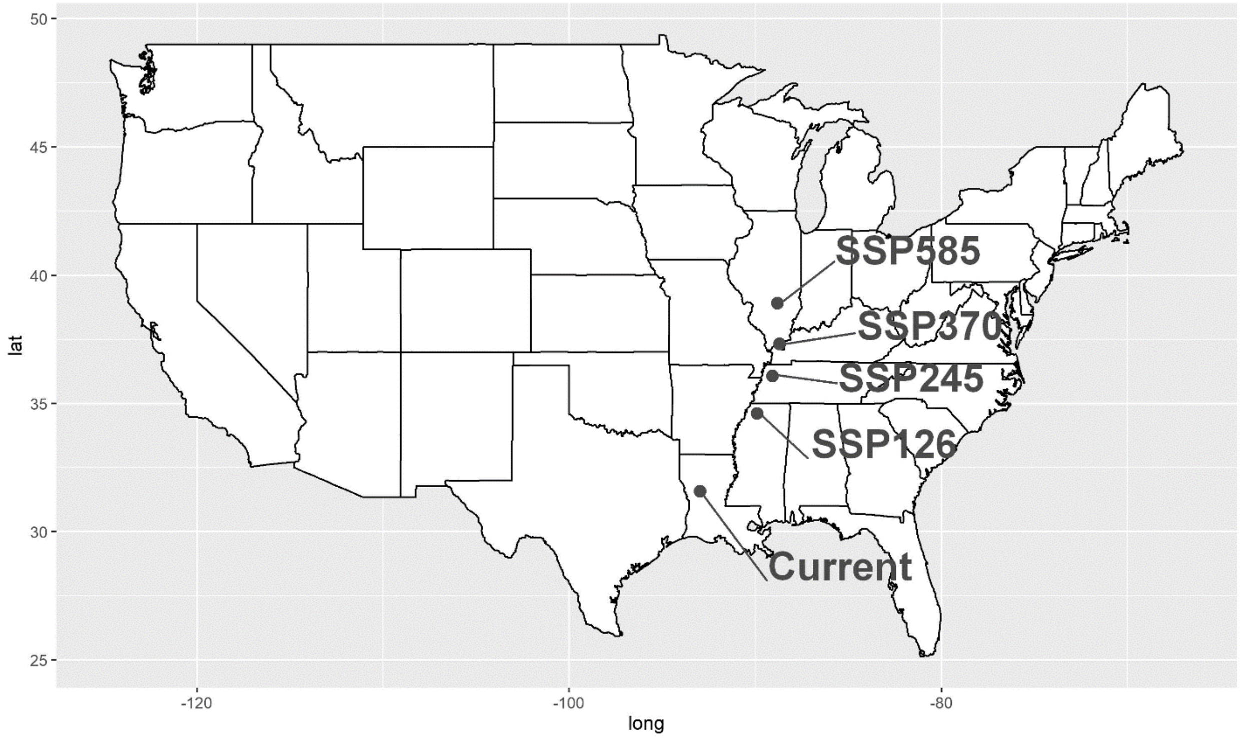


Figure 6: *Plotted centroids are the center of the projected range of alligator gar. The current range is shown along with the four projected climate change scenarios.*



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Summary

This study provides the first estimates of alligator gar thermal minima temperatures. The thermal minima temperatures obtained may help to support the findings of other studies. Including why northern alligator gar populations rely on late season floods for spawning success (Allen et al., 2020), or why lower year class recruitment was found in the Trinity River, TX during April, and May even when suitable flooding events occurred for spawning activity (Buckmeier et al., 2017). This study also reveals the potential future range of alligator gar because of climate change. The forecasted range can help fishery managers direct ongoing conservation efforts of the species. The reproduction and survival of alligator gar will likely be supported at the current northern edge of its range because of climate change.

Considering that water temperature accounts for up to 50% of the variation in survival and growth (Chambers & Trippel 1997), future studies should focus on how extreme weather events (i.e., late season cold fronts, cold rain events) impact water temperatures across floodplain habitats that are used by juvenile alligator gar during early development. Additionally, because the largest individual in this study was 519 mm TL, further investigation of CT_{min} should be completed on alligator gar > 519 mm TL to better understand the impact of cold or declining water temperatures on larger alligator gar. Since climate change is projected to shift the suitable habitat for alligator gar northward, it is imperative fishery managers consider stocking fish to introduce populations of alligator gar into expanding habitats that they cannot disperse into otherwise. Future studies should evaluate these introduced populations to insure survival and reproduction. Ultimately, the findings of this study will assist in ongoing and future management of alligator gar.