

**Cold Weather Event
Simulations on the Survival
of Larval Guadalupe Bass**

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**Management Data Series
No. 289
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INLAND FISHERIES DIVISION
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ABSTRACT

Guadalupe Bass are currently being produced at the A.E. Wood Hatchery to bolster populations in their native range. Larval fish are raised in outdoor ponds and reared to 38 mm (TL) before being stocked. During an extreme cold weather event occurring at the hatchery, ponds harboring Guadalupe Bass larvae had low returns. Hatchery biologists speculated that low returns could have been due to the cold weather event. In order to test the likelihood that larval fish could withstand a cold weather event we conducted two cold weather simulations during which larval Guadalupe Bass were exposed to 12-h treatments of 14°C or 16°C. No differences in survival occurred between larvae exposed to 16°C for 12-h and control ($P > 0.05$) or larvae exposed to 14°C for 12-h and controls ($P > 0.05$). Growth was significantly lower ($P < 0.05$) for fish exposed to 14°C, whereas fish exposed to 16°C no differences in growth were determined ($P > 0.05$).

INTRODUCTION

The Guadalupe Bass *Micropterus treculii* is a species of concern due to genetic introgression and competition with introduced stocks of Smallmouth Bass *Micropterus dolomieu* (Garrett 1991; Koppelman and Garrett 2002). A recent genetic survey discovered that introgression currently no longer occurs in the Lampasas and San Gabriel rivers and that recent stocking efforts have not led to depressed genetic variation (Bean et al. 2013). The Texas Parks and Wildlife Department conducts Guadalupe Bass culture as part of its Inland Fisheries stock enhancement program (Carmichael and Williamson 1986).

In the spring season, captive Guadalupe Bass adults are spawned in indoor raceways at the A.E. Wood Hatchery in San Marcos, Texas (AEW). Eggs are maintained indoors in ambient temperature incubation troughs throughout the hatching process. After the larvae have developed a functional digestive system, they are then stocked in outdoor rearing ponds and ready to feed. Previous survival data in 2014 suggests the possibility that cold weather events may have impacted survival. Current literature does not list a lower lethal temperature for larval Guadalupe bass.

In this study, we exposed Guadalupe bass fry (~7-mm in SL) to a simulated cold weather event, similar to a study conducted by McDonald et al. 2016. Throughout the temperature exposure, experimental and control fish were monitored for indicators of stress (i.e., erratic swimming), loss of equilibrium and death. These cold weather event simulations can give insight into the low temperature tolerance of larval Guadalupe Bass maintained at AEW.

METHODS

Fish spawning and transfer

Spawning and incubation of Guadalupe Bass occurred at AEW using standard hatchery protocols. First-feeding fry, commonly stocked into outdoor rearing ponds, were used for this study. Fry (n = 200) were transported within a 45-L ice chest to the Perry R. Bass Marine Fisheries Research Station (PRB), Palacios, Texas. Prior to live fish transport, water quality records (daily temperature, pH, and DO) were documented throughout the life of the hatched larvae; and the water used for transport was obtained from the same source. The PRB wetlab had a prepared tank filled with San Marcos River water from AEW (~100 gallons) for each trial.

Experimental design

Cold weather simulations were conducted in a similar manner to McDonald et al. 2016, using a temperature programmable environmental chamber (Luwa Environmental Specialties, model ES2000 C-LT-R, Raleigh, NC) in concert with a LabLine environmental chamber for controls. Recirculating systems were created using a polypropylene reservoir (55-L) equipped with a regulated submersible water pump to an aerated shallow water bath (15-L) a water volume of 40-L was cycled back through a 7.6-L biofilter. Duplicate systems were used within the environmental chamber and as controls (total 4 systems). Each water bath contained 14, 500-mL polypropylene jars with flow-through holes and a single plexiglass wall to separate two independent fish (to maximize space and prevent overeating), allowing for a total of 28 fish per

individual recirculating system. At the start of each experiment, 28 fish fry were randomly selected and placed within the 14 jars per recirculating system. Target sample sizes were 56 experimental fish and 56 control fish. Experiments began once all fish were randomly placed in each recirculating system. All fish were fed a daily diet of live *Artemia* throughout the experiment. Experimental fish were kept at the start temperature (22°C) for 24-h so that any immediate mortalities caused by transportation could be replaced with acclimated reserve fish prior to the actual experiment. After this 24-h monitoring period the temperature drop began to decrease at a rate of $\Delta = 0.5^\circ\text{C}/\text{h}$ to a target temperature of 16°C (Experiment 1) or 14°C (Experiment 2) and kept at this temperature for a period of 6-h. Temperatures were then increased $\Delta = 0.25^\circ\text{C}/\text{h}$ to the original start temperature 22°C, and maintained for an additional 12-h. Control water temperatures were maintained at around 22°C using glass aquarium heaters within the recirculation tanks. Experiment 1 was conducted on April 7, 2015 and Experiment 2 was conducted on April 13, 2015. Water temperature was monitored using submerged thermistors (Tidbit, Hoboware, Onset, Bourne, Massachusetts). Fish were monitored for survival periodically throughout the entire study for mortalities (every 4 hours). If a mortality was observed, the fish was immediately removed and measured to nearest mm. Water quality was recorded daily with the use of a hand-held meter (YSI Pro-plus with Quatro probe YSI, Yellow Springs, Ohio); water parameters consisted of temperature (°C), dissolved oxygen (mg/L), saturated dissolved oxygen (%), and pH. Un-ionized ammonia was monitored daily using a colorimeter (Colorimeter II Hach, Loveland, Colorado). If ammonia levels became greater than 0.01-mg/L in any recirculating system, a dosage of 5-mL of ammonia remover (Amquel® Kordon LLC, Hayward, California) was added to each system by the lab personnel.

Statistical analyses

Survival analysis was used to examine the effects of cold temperature treatment on mortality. The response variable was time until death for these experiments. Our analysis included right-censored data (individuals that did not experience death) because we had survival in each experiment. Survival curves were created using the Survival Distribution Function (Proc LIFETEST) along with a comparison of mortality between life stages using a Log rank test with the Šidák multiple-comparison adjustment. Fish sizes of experimental vs. controls were compared using a Mann-Whitney U statistic. All statistical analyses were performed using the Statistical Analysis System (SAS) version 9.2 (SAS Institute, Inc., Cary NC), and statistical significance was considered to occur at $\alpha = 0.05$.

RESULTS AND DISCUSSION

16°C Test

Water quality (mean \pm SD) for the control was pH 8.6 ± 0.18 ; dissolved oxygen 8.4 ± 0.23 mg/L; ammonia (NH_3) <0.01 mg/L; and for treatment was pH 8.6 ± 0.03 ; dissolved oxygen 9.0 ± 0.7 mg/L; ammonia <0.01 mg/L. Water temperatures were maintained at 21.3°C for treatment tanks and temperatures then dropped at a rate of 0.37°C for 14-h and was maintained at $15.5 \pm 0.32^\circ\text{C}$ for 12-h and temperatures then increased 0.24 for 20-h before reaching 21.2°C and maintained for another 24-h. Control water temperatures were maintained at $21.7 \pm 0.18^\circ\text{C}$ for the entire trial (Figure 1).

Twelve fish died within the treatment tanks of the 16°C test whereas seven fish died within control tanks. The 16°C temperature treatment resulted in 20.7% mortality whereas the control tanks resulted in 12.3% mortality, <9% difference. Log-ranked tests of survival curves detected no differences between control and experimental survival exposed to 16°C for 12-h ($P = 0.2571$). Minimal differences in mortalities between control and treatments suggest that batch fitness may have resulted in mortalities rather than a treatment effect. Surviving fish sizes displayed no significant differences in sizes ($P = 0.133$) when comparing experimental fish vs. control fish (Figure 2).

14°C Test

Water quality (mean \pm SD) for the control was pH 8.7 ± 0.068 ; dissolved oxygen 8.0 ± 0.67 mg/L; ammonia (NH₃) <0.01 mg/L; and for treatment was pH 8.7 ± 0.05 ; dissolved oxygen 8.9 ± 1.07 mg/L; ammonia <0.01 mg/L. Water temperatures were maintained at 21.4°C for treatment tanks and temperatures then dropped at a rate of -0.45°C for 16-h and was maintained at 14.0 ± 0.24 °C for 12-h and temperatures then increased 0.25 for 22-h before reaching 20.2°C and maintained for another 24-h. Control water temperatures were maintained at 21.7 ± 0.11 °C for the entire trial (Figure 3).

Sixteen fish died within the treatment tanks of the 14°C test whereas twelve fish died within control tanks. The 14°C temperature treatment resulted in 27% mortality whereas the control tanks resulted in 21.4% mortality, <6% difference. Log-ranked tests of survival curves detected no differences between control and experimental survival exposed to 16°C for 12-h ($P = 0.4673$). Surviving fish sizes were significantly different between experimental and control fish ($P = 0.029$), control fish being larger than experimental fish (Figure 2).

CONCLUSIONS

Laboratory exposed larval Guadalupe Bass fed a diet of live artemia had similar survival rates when exposed to short-term durations (12-h exposure) of cold exposure to 14°C or 16°C. Growth was significantly lower for fish exposed to 14°C when compared to controls whereas, 16°C exposure resulted in no difference in growth. This study had some limitations due to the lack of replication, but we feel that good control survival warranted these data to be reported. Further testing is necessary to determine if cooler temperatures and/or longer duration cold fronts may result in mortality and/or slowed growth.

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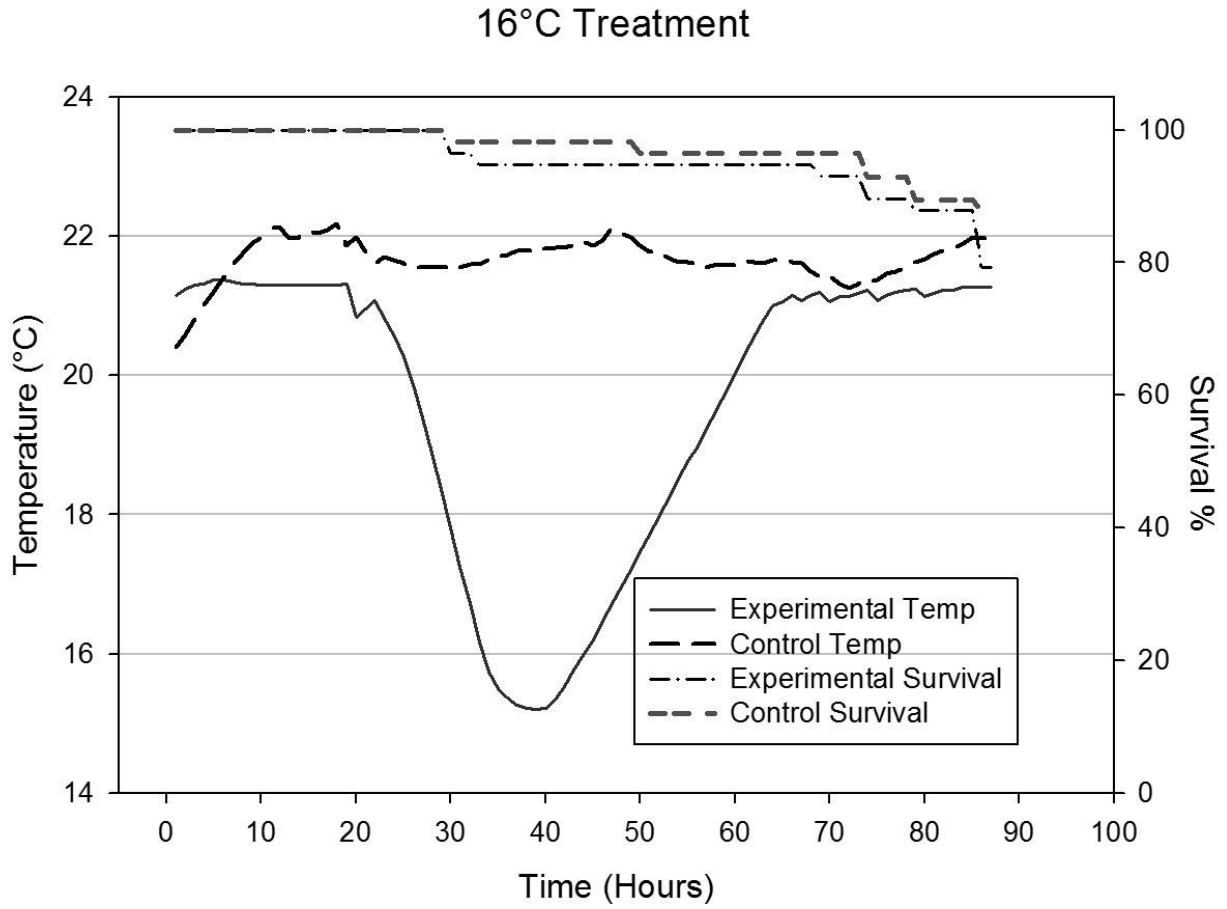


Figure 1. Survival curves of Guadalupe Bass exposed to a temperature treatment of 16°C for 12-h and a control. Water temperatures (°C) are given for the treatment and control.

Fish Sizes x Treatment

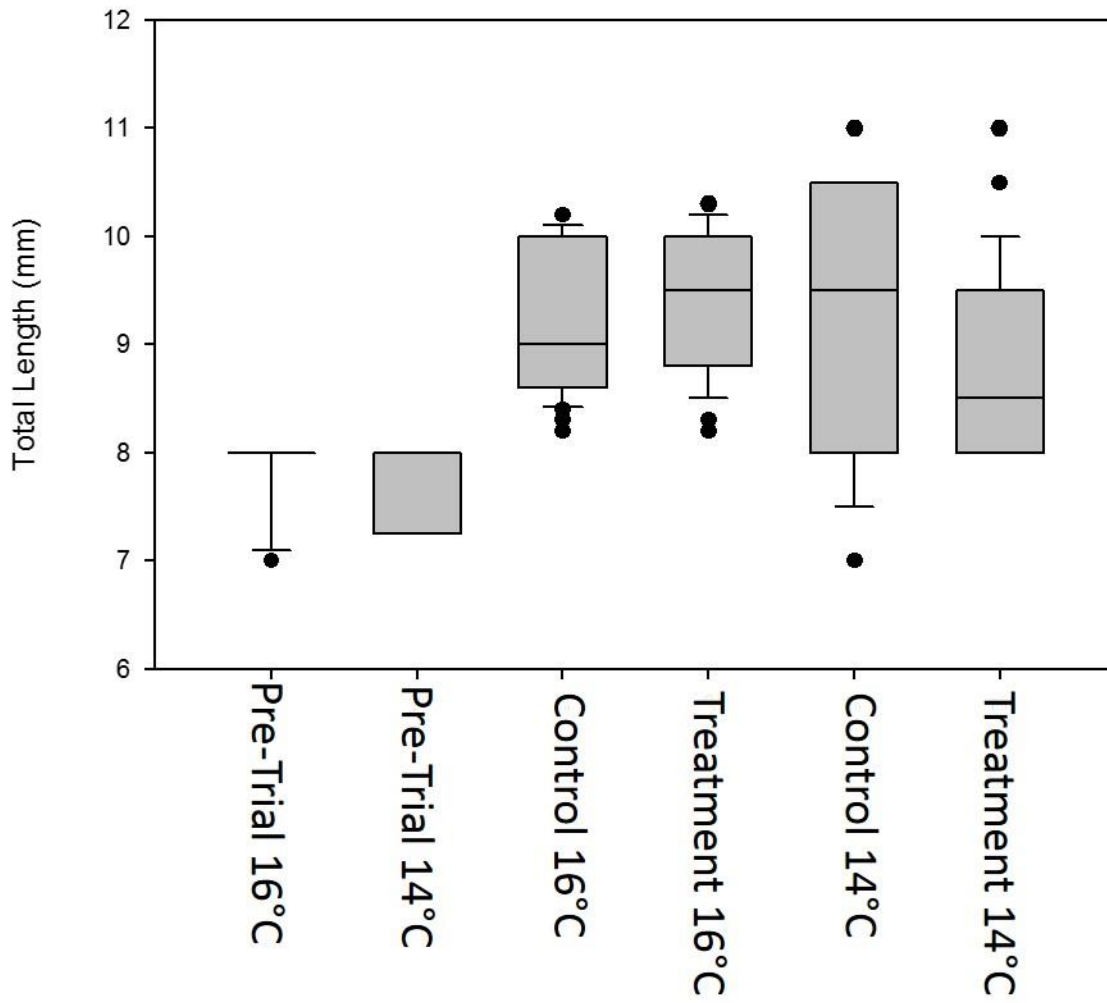


Figure 2. Box plots demonstrating total length (mm) of Guadalupe Bass at start of trials (pre-trial) for both trials (16°C and 14°) and the result of sizes of survivors post-study for controls and treatments.

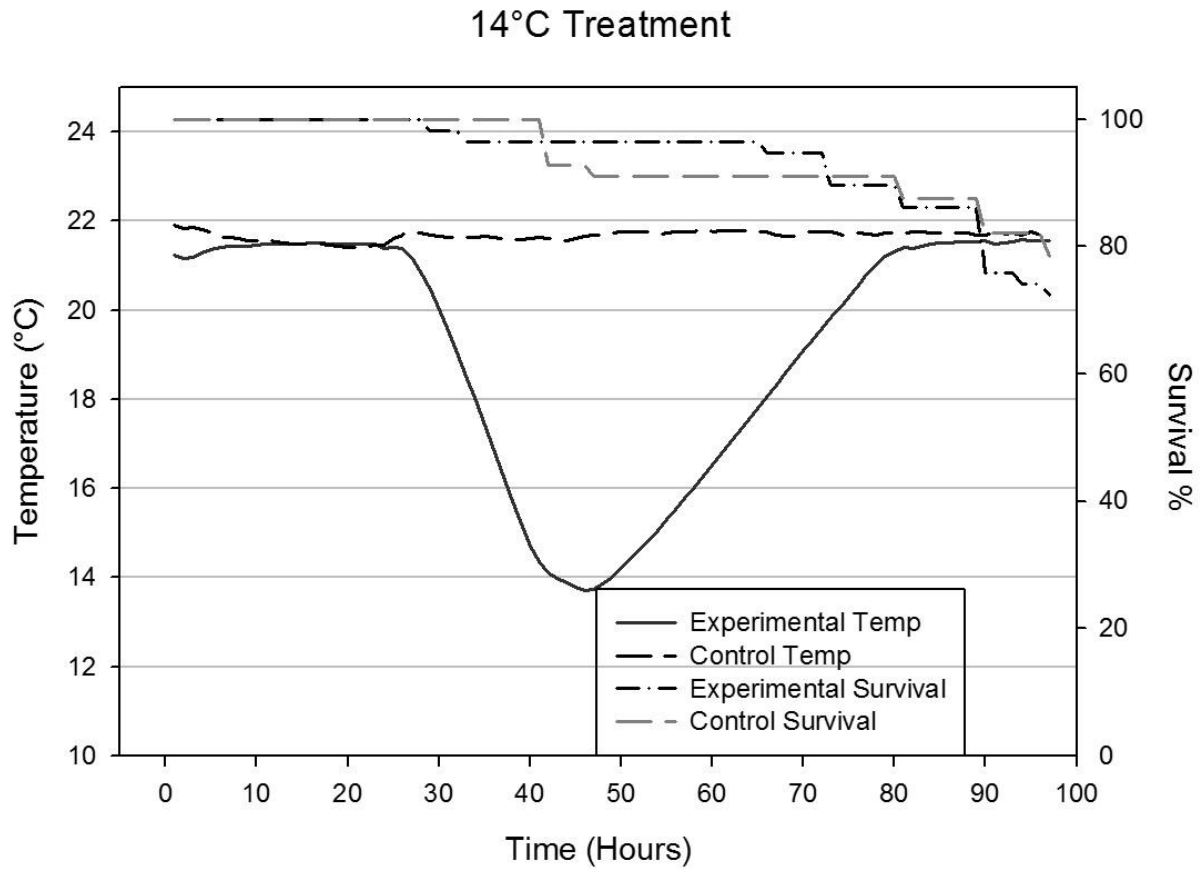


Figure 3. Survival curves of Guadalupe Bass exposed to a temperature treatment of 14°C for 12-h and a control. Water temperatures (°C) are given for the treatment and control.

Texas Parks and Wildlife Department
4200 Smith School Road, Austin, Texas 78744

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