The Effects of High Temperature, Low Dissolved Oxygen, and Asian Tapeworm Infection on Growth and Survival of the Topeka shiner, *Notropis topeka*

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTERS SCIENCE

March 2006

Acknowledgments

I am grateful to many people who have contributed to this research and to my graduate school experience. First, I would like to thank my advisor, Dr. Ira Adelman, who is an exceptional mentor and role model. Also, thanks to my committee members, Dr. Bruce Vondracek and Dr. Jay Hatch for their invaluable guidance.

For funding this project, I thank the Minnesota Department of Natural Resources, Division of Ecological Services, especially Rich Baker, and the United States Fish and Wildlife Service, Endangered Species Program, for a grant provided under Section 6 of the Endangered Species Act. I thank Rich Cook and others at the Lost Valley Fish Hatchery (Missouri Department of Conservation), and Scott Campbell with the University of Kansas for providing Topeka shiners. Thanks also to Andrew Goodwin of the University of Arkansas for identifying tapeworms, and to Drew Mitchell of Stuttgart National Aquaculture Research Center, for recommending worming treatment.

Thanks to several professors at the University of Minnesota, including Dr. Peter Sorensen, Dr. Ray Newman, Dr. George Spangler, Dr. Andrew Simons, Dr. Anne Kapuscinski, Dr. Yossi Cohen, and Dr. Sandy Weisberg, who have offered lab space, statistical advice, and other guidance. Also, thanks to the departmental office staff, including Nancy Rothman, Abby Lloyd, Rachel Jones, Mary Barfield, Rosalyn Zippa, and Jo Schroeder. Many thanks to Jay Maher for his great attitude, ingenuity, and selfless commitment to fisheries research at the University of Minnesota, and to Mark Hove, Rachel Jones, Matt Hennen, and Thor Eide for their assistance in the laboratory. Thanks also to my fellow graduate students! I am grateful to many of you, but particularly to Dr. Dalma Martinovic, Dr. Marilyn Sherman, Jared Fine, Lance Vrieze, Sean Sisler, Ben Thwaits, Branda Long, Leah Sharpe, Mike Simone, and Nick Schlesser. Also, thanks to Carl Cabak, Nick Gidmark, and Kristan Maccaroni for lab assistance.

Finally, I would like to thank my friends and family. My parents, Alan and Patricia Davis, showed me the beauty of the natural world which is the inspiration for my career, and helped to give me the opportunity for higher education. Also thanks to my three sisters, Jennie Kelly, Alita Miller, and Laura Strader, who have supported and encouraged me constantly. Last but not least, thanks to my husband, Karl Koehle, for being my sounding board and best friend, and for always making life interesting.

Abstract

The Topeka shiner (*Notropis topeka*) is an endangered fish species, historically described as inhabiting cool, headwater prairie streams. However, Topeka shiners recently have been found in off-channel habitats with high temperatures and low dissolved oxygen levels. To determine if Topeka shiners can tolerate conditions in these off-channel habitats for extended periods of time, I determined their critical thermal maximum (CTM), optimum temperature for growth, and lower lethal dissolved oxygen level (96hr LC_{50}). Also, I studied the effects of reduced oxygen as well as Asian tapeworm infection (*Bothriocephalus acheilognathi*) on growth. Topeka shiners have a CTM of 39 C at a 31 C acclimation temperature and their optimum temperature for growth is about 27 C. Topeka shiners are capable of growth at dissolved oxygen concentrations as low as 2 mg L^{-1}, but in some circumstances at a considerably lower rate than at dissolved oxygen concentrations at or above 4 mg L^{-1}. Their 96-hour LC_{50} for dissolved oxygen at 26 C is 1.2 mg L^{-1}. Finally, growth is reduced by the presence of Asian tapeworms. Asian tapeworm is not only a threat to fish health but use of infected fish in restoration programs is prohibited. Overall, temperature and oxygen are probably not responsible for Topeka shiner population declines and are not limiting factors in most off-channel habitats. Judging by the abundance of Topeka shiners in off-channel habitats, these habitats may be population sources, rather than sinks, and thus may be important to Topeka shiner populations.

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Introduction

The Topeka shiner (*Notropis topeka*) is an endangered minnow species native to North American prairie streams in Minnesota, South Dakota, Iowa, Nebraska, Missouri, and Kansas (Hatch 2001). Over the past century the Topeka shiner's range has decreased by 80%, with more than 50% of this decline within the past 25 years (Tabor 1998). Habitat loss may be one of the main reasons for their decline, but habitat suitability information for Topeka shiners is limited. Topeka shiners have recently been found in abundance in off-channel habitats in Minnesota. These off-channel habitats may be sources, rather than sinks, for shiner populations (Pulliam, 1988; Dahle, 2001) and it is vital to understand how Topeka shiners use these habitats. The continued existence of this species is important to the larger goal of protecting native biodiversity in Midwest prairie stream ecosystems.

Information is now available about some aspects of Topeka shiner life history, but questions still remain about their habitat requirements. Topeka shiners are small (<75 mm) omnivorous cyprinids (Hatch and Besaw 2001). Spawning occurs between early May and late August, and is often associated with sunfish (*Lepomis spp.*) nests (Dahle 2001; Kerns and Bonneau 2002; Stark et al. 2002). Historically, Topeka shiners were reported to live in cool, clear headwater stream pools with substrates of gravel, rubble, clay hardpan, or bedrock, sometimes with a thin layer of silt. Many of these streams were intermittent during dry summers, but were maintained by groundwater seepage (Minckley and Cross 1959). Current descriptions of Topeka shiner habitat in Kansas and Missouri agree with historical descriptions. Topeka shiners are found in pools often connected by water seeping through the gravel (Hrabik 1996; Kerns and Bonneau 2002;

Stark et al. 2002) with generally good water quality (Bayless et al. 2003). However, in addition to those habitats, populations of Topeka shiners in Minnesota have been reported to inhabit and spawn in off-channel oxbow lakes and excavated pools, as well as in streams carrying high sediment loads (Dahle 2001; Hatch 2001). Kuitunen (2001) reported that Topeka shiners in Minnesota streams were more often collected in clearer, vegetated backwater areas than in more turbid stream channels. The presence of Topeka shiners in these off-channel habitats has been found to depend on a variety of factors, including flooding events (Berg et al. 2004). Off-channel habitat is often maintained by groundwater or seepage from the main stream channel (Berg et al. 2004), but it is unclear whether temperature and dissolved oxygen levels in degraded streams and off-channel habitat are suitable for Topeka shiners. Because these factors may be limiting in off-channel habitats, this study was undertaken to determine tolerance of Topeka shiners to high temperature and low oxygen.

A few anecdotal observations have been made on Topeka shiner temperature tolerance, but no information has been published for Topeka shiner low dissolved oxygen tolerance. In Kansas, Kerns and Bonneau (2002) reported that Topeka shiners were usually the last fish to succumb to deteriorating conditions in drying pools; temperatures reached 28.5 C during their study, and more extreme temperatures likely occurred. Also in Kansas, Topeka shiners were observed in temperatures up to 29 C (Schrank et al. 2001). In Minnesota, Hatch (2001) reported observing Topeka shiner spawning behavior at 31 C. Specifically in off-channel habitat in Minnesota, Dahle (2001) reported finding shiners in water that was 24 C in August 1998.

During the course of the experiments to determine temperature and oxygen tolerance, I discovered that some fish were infected with the Asian tapeworm (*Bothriocephalus acheilognathi*) as a result of exposure at the hatchery where they were spawned. Asian tapeworms are intestinal parasites of teleosts (Hoffman 1998). These tapeworms were probably introduced to the United States in the 1960s with grass carp (*Cyprinus idella*), imported for aquatic vegetation control (Mitchell 2004). The life cycle of Asian tapeworms involves an intermediate stage with a copepod host, so a population of infected copepods was probably the mechanism of exposure in the hatchery ponds. Although Asian tapeworm infection has not yet been observed in wild populations of Topeka shiners, hatchery infections could threaten rehabilitation efforts. Widespread introduction of Asian tapeworms into a threatened population could be devastating. Further study is needed because few studies have quantified the effects of Asian tapeworm infection on fish growth.

Information about Topeka shiner tolerance of high temperature, low dissolved oxygen, and tapeworm infection need to be better understood in order to protect critical habitats and ensure the survival of this species. Therefore, the objectives of this study were: 1) to determine the optimum temperature for growth, 2) to estimate the upper lethal temperature [as critical thermal maximum (CTM)] after acclimation to high temperature, 3) to determine the effect of reduced dissolved oxygen on growth, 4) to estimate the lower lethal concentration of dissolved oxygen, and 5) to determine the effect of Asian tapeworm infection on growth and CTM.

Methods

General procedures

Three experiments were conducted to determine the effect of temperature on growth of Topeka shiners (T-1, T-2, and T-3) and two experiments were conducted to determine the effect of oxygen on growth (O-1 and O-2). Also, experiments to determine the critical thermal maximum (CTM) (Becker and Genoway 1979) were conducted on fish used in T-1, T-2, and T-3 (CTM-1, -2, and -3). The effects of Asian tapeworm infection on growth and CTM were determined in experiments T-3 and CTM-3. Finally, experiments were conducted on fish used in experiments O-1 and O-2 to determine lethal oxygen concentrations for fish previously acclimated to low dissolved oxygen (LC_{50}-1 and LC_{50}-2).

All experiments except the CTM determinations were conducted using a flow-through system with water supplied by a deep dedicated well. Topeka shiners used in experiments T-1, CTM-1, O-1, and LC_{50}-1 were obtained from the Lost Valley Fish Hatchery (LVFH) Missouri, and Topeka shiners used in experiments T-2, CTM-2, T-3, CTM-3, O-2, and LC_{50}-2 were from the University of Kansas (KU). All fish came from an original breeding population collected from Deep Creek at Pillsbury Crossing (T11S, R9E, Sec 5), Riley County, Kansas.

No tapeworms were ever detected in the fish from LVFH. The fish from KU were infected with the Asian tapeworm because grass carp had previously been held in the rearing ponds and thus infected the intermediate host copepods. For experiments T-3, CTM-3, and O-2, and LC_{50}-2, Praziquantel (manufactured by Bayer as Droncit) was used to rid the fish of the tapeworms. Praziquantel dosage was 1.5 mg L^{-1} for 24 hours as

recommended by Mitchell (2004) with fish density of about 0.15 g L^{-1}. Microscopic examination of the gastrointestinal track of over 60 fish treated with Praziquantel indicated 100% removal of tapeworms with no fish mortality and no observed stress. Post-experiment dissection to determine the presence or absence of tapeworms was performed on all fish from experiments T-1, T-2, T-3 and CTM-3, and on randomly sampled fish from experiment O-1. Fish from experiment O-2 were not dissected for tapeworms because by that time the Praziquantel treatment was assumed to be 100% effective.

At the beginning of the acclimation period of all growth experiments, Topeka shiners of both sexes were distributed to test aquaria in a stratified-random manner so that every aquarium received one fish before any aquarium received a second fish, and so on. Growth was measured as specific growth rate (SGR) over 28 to 30 days as percent growth per day (SGR = [ln (final measure – initial measure)] / [number of days elapsed between measurements]). At the beginning and end of each experiment, weight was recorded to the nearest 0.1 g and total length was recorded to the nearest 0.5 mm. Before these measurements were taken, food was withheld for 24 hours. During the measuring procedure, fish were anaesthetized with 100 mg L^{-1} tricaine methanesulfate (MS-222). At the beginning of some experiments, individual fish were identified using either one or two fin clips. Preliminary experiments not reported here showed that fin clips did not significantly lower Topeka shiner growth rate over 30 days. Shiners were fed frozen brine shrimp (*Artemia salina*) once per day during holding periods, two to three times per day during acclimation periods, and three times per day to satiation during test periods. Deaths during growth experiments were recorded as missing data points. Photoperiod for

experiment T-1 was 16h light: 8h dark, but for all other experiments photoperiod was 9h light: 15h dark. A shorter daylength was used in an attempt to suppress reproductive behavior. Fluorescent lights provided illumination during daylight hours.

During all experiments except CTM determinations, flow rate to each aquarium was regulated with pipette tips. Desired test temperatures were achieved by mixing warm and cold water, and by using 200- and 300-watt Marineland Visi-Therm Deluxe submersible heaters in mixing chambers. Foam insulation was placed around aquaria and tubing to maintain high water temperatures. Onset Stowaway Tidbit temperature loggers were placed in each aquarium to monitor temperature, and records were later downloaded to Microsoft Excel using BoxCar Pro software. Temperature and dissolved oxygen were also monitored daily during experiments using digital thermometers and a YSI dissolved oxygen meter. The oxygen meter was calibrated using the manufacturer's air calibration procedure and periodically checked against dissolved oxygen concentrations using the Winkler method (APHA 1998).

Optimum Temperature for Growth

The goals of experiments T-1, T-2, and T-3 were to determine the Topeka shiner's optimum temperature for growth. Optimum temperature for growth is important information because a high level of somatic fish growth indicates that the fish is able to put large amounts of energy towards growth. Optimum temperature for growth may not indicate optimum temperature for all aspects of the fish's life, but it does indicate that metabolism is efficient; the fish is at a temperature high enough for growth, yet low enough to avoid thermal stress.

To determine the optimum temperature for growth, growth rate was measured over a range of five treatment temperatures with two replicates of each treatment (Table 1). Each replicate consisted of one 20-liter aquarium for a total of 10 aquaria. Flow rate to each aquarium was 600 ml min^{-1}.

Experiment T-1.— In this 30-day experiment, 14 Topeka shiners (13 months old) were placed in each replicate treatment, for totals of 10 aquaria and 140 fish. Before the experiment began, water in all aquaria was at the fish's acclimation temperature (22 C), and temperatures were changed daily over 10 days to reach desired treatment levels. Fish were not individually marked for identification; therefore, specific growth rate was calculated from the mean initial and final weights and lengths of fish in each replicate treatment. At the conclusion of the experiment, all fish from the two highest temperature treatments were used in experiment CTM-1.

TABLE 1.—Mean and standard deviation (in parenthesis) of at least 50 randomly sampled temperature (C) logger data points for experiments T-1, T-2, and T-3, for all treatments and replications (Rep).

	Treatment									
	1		2		3		4		5	
Experiment	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
T-1	12.7 (0.24)	12.8 (0.26)	18.0 (0.24)	18.2 (0.24)	23.3 (0.24)	23.4 (0.24)	27.8 (0.18)	27.7 (0.21)	33.0 (0.35)	32.9 (0.41)
T-2	12.3 (0.26)	13.0 (0.53)	17.1 (0.81)	17.9 (0.88)	22.9 (0.63)	22.4 (0.67)	26.8 (0.41)	27.0 (0.41)	31.1 (0.40)	31.3 (0.34)
T-3	14.5 (0.43)	14.7 (0.52)	19.4 (0.53)	19.9 (0.50)	24.4 (0.48)	24.5 (0.50)	29.6 (0.17)	29.6 (0.26)	34.4 (0.39)	34.8 (0.25)

Behavior was observed once a day on nine different days, and aquaria were observed in random order for 10 seconds each. Swimming activity in each aquarium was subjectively classified as high, medium, or low, and was given a score of 3, 2, or 1,

respectively. Reproductive behavior (subjectively defined here as a male actively chasing other fish out of his territory) was also classified in each aquarium as high, medium, or low, corresponding to scores of 3, 2, or 1, and the number of males exhibiting chasing behavior was recorded. The reproductive activity score was calculated by adding the reproductive behavior score to the number of mature males (those with reddened fins, which were chasing others out of their territory).

Experiment T-2.— In this 28-day experiment, 8-month old Topeka shiners infected with Asian tapeworms were used, although the tapeworms were not discovered until after the test was completed. The fish were randomly distributed among five treatments with two replicates (Table 1). Each replicate contained 10 fish, for a total of 10 aquaria and 100 fish. All aquaria began at the acclimation temperature of the fish (23 C), and temperatures were changed daily over 8 days to reach desired treatment levels. Fish were given either one or two fin clips at the time of initial measuring to identify individuals. Initial and final weights and lengths from each fish were used to calculate specific growth rate for individual fish. At the conclusion of the experiment, all fish from the two highest temperature treatments were used in experiment CTM-2.

Experiment T-3.— In this 28-day experiment, 12-month old Topeka shiners from the same cohort as fish used in experiment T-2 were randomly distributed among five treatments with two replicates (Table 1). Each replicate contained 14 fish, for a total of 10 aquaria and 140 fish. In each replicate, seven fish had been treated with Praziquantel 2 weeks prior to the start of the experiment to rid them of tapeworms. The other seven fish were not treated and were assumed to be 100% infected. All aquaria began at the fish's holding temperature (20 C), and water temperatures were changed daily over 13

days to reach desired treatment temperatures. Fish were fin-clipped to identify individuals, so that growth of infected fish could be compared with that of non-infected fish. Specific growth rates for individual fish were determined as in T-1 and T-2. At the conclusion of the experiment, all fish from the 30 C temperature treatment were used in experiment CTM-3.

Critical Thermal Maximum

The goal of this group of experiments (CTM-1, -2, and -3) was to determine upper lethal temperatures of Topeka shiners acclimated to high temperatures. The fish used in these experiments had been acclimated to high temperatures as a result of undergoing high temperature treatments in growth tests T-1, T-2, or T-3. The apparatus used to determine CTM was an aerated 10-liter static-water aquarium. An MGW Lauda MS heater/ chiller/ mixer, with precise temperature control, was placed in one end. A small mesh enclosure was placed in the other end that allowed the fish access to the surface but kept the fish away from the heater's circulating motor. The initial aquarium temperature was set at the fish's acclimation temperature. One, two, three, or four fish along with a digital thermometer probe were placed in the mesh container and fish were given one minute to acclimate before temperature was increased. Water temperature was raised 0.3 C per minute, and the CTM endpoint was reached when the fish could no longer regain equilibrium (Becker and Genoway 1979). CTM values were recorded for individual fish.

Experiment CTM-1.— Twenty-eight Topeka shiners from the 28 C treatment and 23 fish from the 33 C treatment from experiment T-1 were used in this experiment, 12

days after the conclusion of the growth experiment. During the holding period between T-1 and CTM-1 fish were held at 28 and 31 C, respectively.

Experiment CTM-2.— Sixteen Topeka shiners from the 27 C treatment and 14 fish from the 32 C treatment from experiment T-2 were used in this CTM determination, conducted within 1 week after conclusion of the growth experiment.

Experiment CTM-3.— Nineteen fish without Asian tapeworms and six fish with Asian tapeworms from the 30 C treatment in T-3 were used within 1 week after conclusion of the growth experiment (presence or absence of Asian tapeworm was verified through dissection after completing the experiment). Fish from the highest treatment temperature (35 C) in T-3 were not used because of high mortality at that temperature.

Effect of Dissolved Oxygen on Growth

The goals of experiments O-1 and O-2 were to determine the effect of reduced dissolved oxygen concentration on growth for Topeka shiners at relatively high temperatures. Dissolved oxygen concentration was controlled using an oxygen ladder modified from Brungs (1971). Briefly, cold well water was heated in a head tank and vigorously aerated to bring dissolved gases to atmospheric equilibrium. Water at the desired test temperature then flowed by gravity to a stripping column in which nitrogen was bubbled through the water in a counter current direction to reduce the dissolved oxygen concentration to near zero. From the stripping column, water flowed to the top of the oxygen ladder, then flowed over baffles separating individual compartments, and finally to a drain. As water moved over the baffles it gradually absorbed oxygen from the

atmosphere. Airstones were placed in some of the compartments of the ladder to increase the rate of oxygen absorption. Water was withdrawn from the compartments of the ladder where the desired treatment concentrations had been attained.

For all three experiments, the desired treatment concentrations were saturation, 5, 4, 3, and 2 mg L^{-1} of dissolved oxygen. However, temperature fluctuations as well as calcium deposits and algal growth in the tubing caused some variability in the actual dissolved oxygen concentrations (Table 2). Experimental chambers were 40-liter aquaria with water inflow positioned diagonally opposite water outflow. Fish were individually identified with unique fin clips. Specific growth rate was measured as described in the temperature experiments above. After conclusion of the experiments, fish from the two lowest dissolved oxygen levels were used in the LC_{50} determinations.

TABLE 2.—Mean dissolved oxygen concentrations (mg L^{-1}) for the five treatments in experiments O-1, and O-2, with standard error in parenthesis.

		Treatment				
Experiment	Fish age (months)	1	2	3	4	5
O-1	19	2.02 (0.0396)	2.91 (0.0593)	3.87 (0.0419)	4.78 (0.0560)	6.14 (0.0865)
O-1	7	2.42 (0.0474)	3.12 (0.0615)	4.31 (0.0530)	5.23 (0.0581)	6.27 (0.0861)
O-2	18	2.42 (0.0527)	3.46 (0.0445)	4.31 (0.0552)	5.23 (0.0833)	6.27 (0.0426)

Experiment O-1.— This 28-day experiment was conducted at a temperature of 24.8 C (± 0.8 C) based on the findings of experiment T-1. This temperature was considered at the time of O-1 to be optimum for growth. Both 19–month old and 7-

month old Topeka shiners were used in each of the five treatments. Plastic divider screens placed parallel to the shorter sides of the aquarium separated older and younger fish. The position of older and younger fish in relation to inflow and outflow was randomly selected in one replicate and was the opposite arrangement in the other replicate. Dissolved oxygen and temperature were measured in each compartment. The compartments varied slightly in both temperature and oxygen, so the actual dissolved oxygen levels varied slightly between groups of fish. Each replicate contained 12 fish in each age group, for a total of 24 fish per aquarium. Water flow rate was 270 ml min^{-1}. During the 11-day acclimation period, the initial oxygen concentrations of between 3.3 and 7.6 mg L^{-1} were gradually lowered to the desired treatment concentrations while temperature was increased from 21 C to 25 C. Dissolved oxygen started in this range of concentrations because the oxygen ladder's design did not allow for multiple water withdrawals from the same compartment in the oxygen ladder. The 7-month-old fish had Asian tapeworm infection but this was not discovered until midway through the test. The 19-month-old fish did not have tapeworm infection. Fish from both age groups were randomly chosen for dissection at the end of the experiment to verify the level of tapeworm infection.

Experiment O-2.— This 28-day experiment was designed to examine the relationship between dissolved oxygen (Table 2) and Topeka shiner growth at a temperature higher than the optimum temperature for growth. The temperature during this experiment was 28.2 C (± 0.6 C). Eighteen-month old fish were used in each of the five treatment levels, and the aquaria were not divided because only the one age group was used. These fish were previously infected with Asian tapeworm, but treatment of all

fish with Praziquantel had eradicated the infection. Each replicate contained 20 fish, and flow rate to each aquarium was 300 ml min^{-1}. During the 7-day acclimation period, temperatures were slowly increased from 26 C, and oxygen was adjusted to the final treatment concentrations starting from a range between 3.1 and 7.1 mg L^{-1} as in experiment O-1.

Four separate behavior trials were conducted in which activity was measured by counting line-crossings. A narrow side of each aquarium was divided into quarters using colored tape, and each time the fish in question crossed a tape line, it was counted as a line-crossing. Three different "chaser" fish and three "non-chaser" fish were observed for 30 seconds in each aquarium during each trial. "Chaser" fish were selected as being aggressive male fish that were most actively chasing other fish. "Non-chaser" fish were fish that were judged subjectively to be among the least active fish in the aquarium.

Lower Lethal Dissolved Oxygen Concentrations

Two 96-hour LC_{50} determinations were conducted to determine lower lethal oxygen concentrations. Dissolved oxygen was measured twice per day using the Winkler method (APHA 1998) and averaged for calculation of LC_{50} values (Table 3).

Experiment LC_{50}-1.— This test began 11 days after the conclusion of O-1, and used fish from the two lowest dissolved oxygen treatments. Fish remained at lowered dissolved oxygen levels between the growth test and LC_{50}-1. The arrangement of older and younger fish in relation to inflow and outflow was randomized as in experiment O-1, and age groups were separated with plastic screens. Fish were exposed to three dissolved oxygen concentrations with two replicates each (Table 3). Each aquarium contained

eight fish from each age group. Flow rate to each aquarium was 390 ml min^{-1}, and temperature was between 24.5 C (± 0.5 C). Plastic covers were placed on the surface of the water but not sufficiently tight to prevent fish from having slight access to the water surface. Fish were not fed during the 96 hours, and deaths were recorded every 24 hours.

Experiment LC_{50}-2.— This test began 2 days after the conclusion of O-2 and used fish from the lowest two dissolved oxygen treatments. Four treatments with two replicates were used (Table 3), for a total of eight 40-liter aquaria. Each aquarium contained either 8 or 9 fish. Flow rate to each aquarium was 300 ml min^{-1}, and temperature was 27.8 C (± 0.4 C). Plastic covers with foam insulation around the edges prevented fish from gaining access to the surface more effectively than in LC_{50}-1. Fish were not fed during the 96 hours, and deaths were recorded every 24 hours.

TABLE 3.—Mean and standard error (in parenthesis) of dissolved oxygen concentrations (mg L^{-1}) for treatments in experiments LC_{50}-1, and LC_{50}-2.

Experiment	Fish Age (months)	Treatment			
		1	2	3	4
LC_{50}-1	20	0.62 (0.0478)	0.87 (0.0436)	2.16 (0.0450)	-
LC_{50}-1	8	0.64 (0.0592)	0.88 (0.0417)	2.17 (0.0460)	-
LC_{50}-2	19	0.46 (0.0499)	0.80 (0.0395)	1.15 (0.0174)	1.98 (0.0547)

Data Analysis

Optimum temperature.— Optimum temperatures for growth were calculated by plotting mean SGR values for each replicate vs. mean aquarium temperature for experiment T-1, and by plotting the median SGR value for each replicate vs. mean aquarium temperature for experiments T-2 and T-3. Median values were used because they eliminated the decision of whether or not to remove outliers, while adequately representing the central tendency of fish in the sample; however, median values could not be used in analysis of experiment T-1 because individual fish were not identified. Mean values weighted for number of fish per replicate were used for experiment T-1. Third order polynomial regression was performed in Arc, a statistical regression program (Cook and Weisberg, 1999) using the Delta method (Weisberg, 2005) to calculate estimates and standard errors for each experiment's optimum temperature for growth. Third order regression was used because it gave the best fit based on R-squared values. A separate linear regression was performed in Arc on data from experiment T-3 to examine significant effects of tapeworm infection on fish growth. Chi-square tests were performed for T-1, T-2, and T-3 to compare survival rate between treatments for each experiment. Also, two t-tests were used to compare survival rates between fish with and without tapeworm infection for experiment T-3; one t-test included all five treatments, and the other excluded the highest temperature treatment (35 C) because of low survival.

Activity scores recorded during T-1 were first examined visually for time trends (e.g. a time trend exists if one aquarium shows consistent increase in activity over time) and then replicates were averaged and examined visually for any patterns occurring between treatments. Each aquarium's score was then averaged over all observations,

resulting in 10 data points total (n=2 for each of five treatments). One-way ANOVAs were then performed in Statistica (StatSoft, Inc., 1997) to determine if temperature had a significant effect on either swimming activity or reproductive behavior.

Critical Thermal Maximum.— Estimates of mean, standard deviation, and standard error of the mean were calculated for each experiment. Different acclimation temperature groups were separated for experiments CTM-1 and CTM-2, and fish treated and not treated for tapeworm were separated for CTM-3. To test for significant differences in CTM between groups, t-tests were performed in Microsoft Excel.

Dissolved Oxygen Effect on Growth and Behavior.— Specific growth rates for each fish were calculated in experiments O-1 and O-2. For experiment O-1, a split-plot design ANOVA was used for analysis in the following procedure. First, a model including oxygen level, fish age group, and interactions between aquarium and oxygen level as well as age and oxygen level was fitted in Arc to examine within-treatment differences and effect of age on growth. Next, separate one-way ANOVAs and post-hoc Tukey tests were performed in Statistica for each age group to examine only effect of dissolved oxygen level on growth. For experiment O-2, a one-way ANOVA and post-hoc Tukey test was performed in Statistica to test for effect of dissolved oxygen on growth.

The behavior observations (line crossings) that were recorded during O-2 were analyzed with a 2-way ANOVA and post-hoc Tukey test in Statistica to determine significant differences in activity between oxygen treatment levels and between 'chaser' and 'non-chaser' fish. Finally, Chi-square tests were performed for both O-1 and O-2, comparing survival rates between treatments for each experiment.

Lethal Oxygen Concentrations.— For both LC_{50}-1 and LC_{50}-2, the number of deaths at 96 hours was used to calculate LC_{50} values using the binary method (Newman 1995).

Results

Optimum Temperature and Effect of Asian Tapeworm on Growth

Experiment T-1.— The optimum temperature for growth as SGR in weight (i.e. mass) was 18.0 C (SE 0.348), and for length 18.4 C (SE 0.604) (Table 4, Figure 1). All treatments had 100% survival except for the highest treatment temperature (33 C) in which 28 of 29 fish survived. Temperature had no significant effect on fish survival (P=0.417). Dissections revealed no tapeworms in any of the fish. Reproductive behavior was significantly higher at the 23 C treatment and above (P<0.05), and activity was significantly higher at two highest temperature treatments compared to the lowest temperature treatment (P<0.05) (Figure 2). No time trends were found for any of the behavior observations.

Experiment T-2.— The optimum temperature for growth in weight was 32.1 C (SE 2.32), and 29.5 C (SE 1.36) for length. However, the highest mean treatment temperature was 31.2 C and therefore the estimate for weight is an extrapolation. The highest growth rate for weight occurred at the highest temperature tested (Figure 3, Table 4). Survival was 90%, 75%, 90%, 85%, and 70% for the five treatments in order of increasing temperature, and no significant effect of temperature was found on fish survival rate (P=0.346). Post-experiment dissections revealed that Asian tapeworms

were present in fish in all treatments, with 94%, 89%, 100%, 94%, and 43% infection in the five treatments, in order of increasing temperature.

TABLE 4.— Optimum temperature for growth (SE in parenthesis), treatment temperature where highest specific growth rate (SGR) occurred, and that treatment's mean SGR (%/day) in experiments T-1, T-2, and T-3.

Experiment	Measure	Fish age (months)	Optimum Temperature (C)	Temperature with highest mean SGR (C)	Highest Mean SGR
T-1	Weight	13	18.0 (0.348)	18.1	0.804
T-1	Length	13	18.4 (0.604)	18.1	0.137
T-2	Weight	8.5	32.0 (2.32)	31.2	2.04
T-2	Length	8.5	29.5 (1.35)	31.2	0.507
T-3	Weight, no worms	12	27.4 (0.811)	24.4	2.21
T-3	Length, no worms	12	27.1 (0.757)	24.4	0.606
T-3	Weight, with worms	12	28.1 (0.429)	29.6	1.42
T-3	Length, with worms	12	26.3 (1.63)	24.4	0.342

Experiment T-3.— Fish that had been treated with Praziquantel, and therefore were free of tapeworms during the experiment, had an optimum temperature for growth in weight of 27.4 C (SE 0.811) and 27.1 C (SE 0.757) for growth in length (Figure 4, Table 4). For fish that were infected with tapeworms (those that had not received the Praziquantel treatment), the optimum temperature for growth in weight was 28.1 C (SE 0.429), and for length it was 26.3 C (SE 1.63) (Figure 4, Table 4). In weight, growth rate

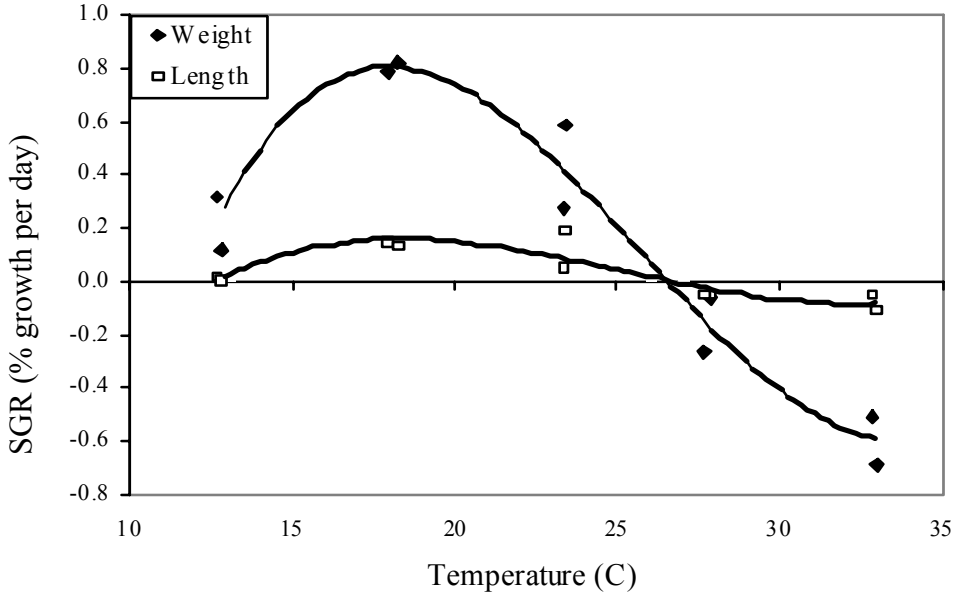


FIGURE 1.—Relationship of specific growth rate (SGR) to temperature for weight and length in experiment T-1. Regression equation for weight is $y = 0.0007x^3 - 0.0567x^2 + 1.1331x - 9.0468$, $R^2=0.952$. Regression equation for length is $y = 0.0002x^3 - 0.0137x^2 + 0.321x - 2.2413$, $R^2=0.815$.

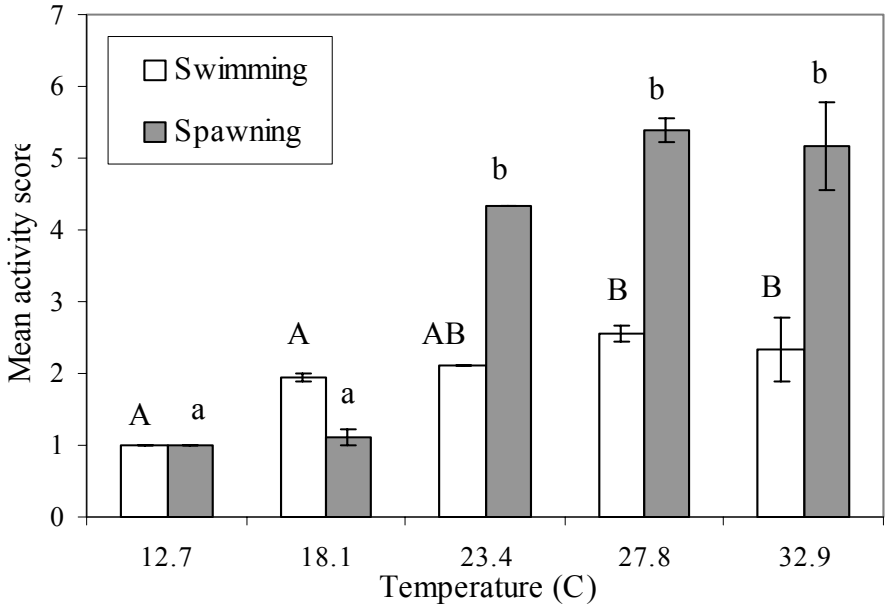


Figure 2.— Observations of behavior during experiment T-1. Reproductive-related activity and general swimming activity were scored as high (3), medium (2) or low (1). Number of reproductive males was added to reproductive activity score. Results shown are mean scores for each of the five treatment temperatures. Letters indicate significantly different growth rates, as determined by post-hoc Tukey tests.

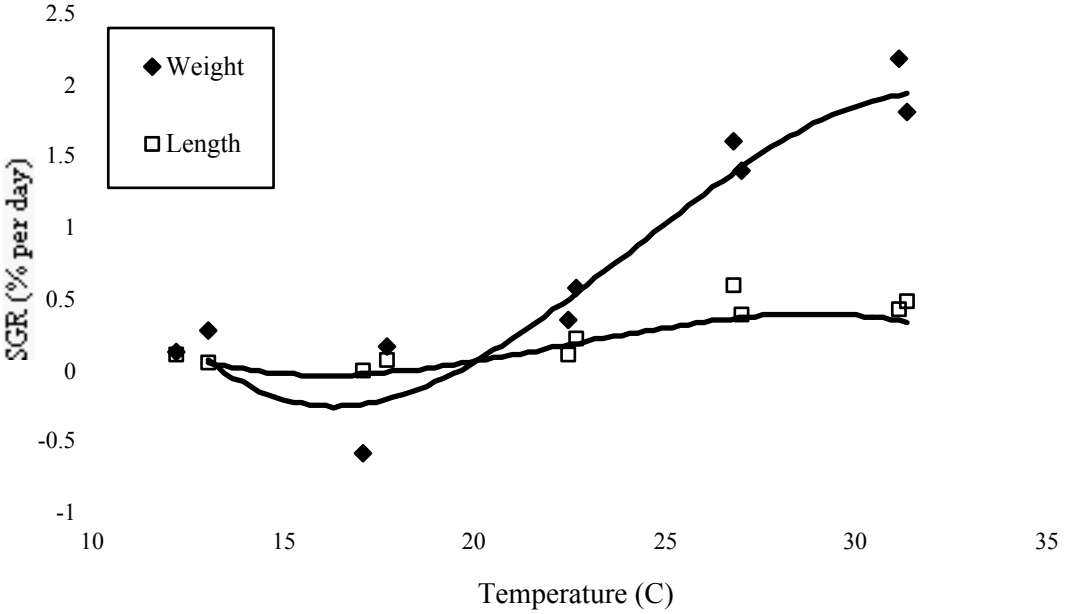


FIGURE 3.— Relationship of specific growth rate (SGR) to temperature for weight and length in experiment T-2. Regression equation for weight is $y = -0.0012x^3 + 0.0832x^2 – 1.7942x + 11.862$, $R^2=0.937$. Regression equation for length is $y = -0.0004x^3 + 0.0276x^2 – 0.5698x + 3.6862$, $R^2=0.864$.

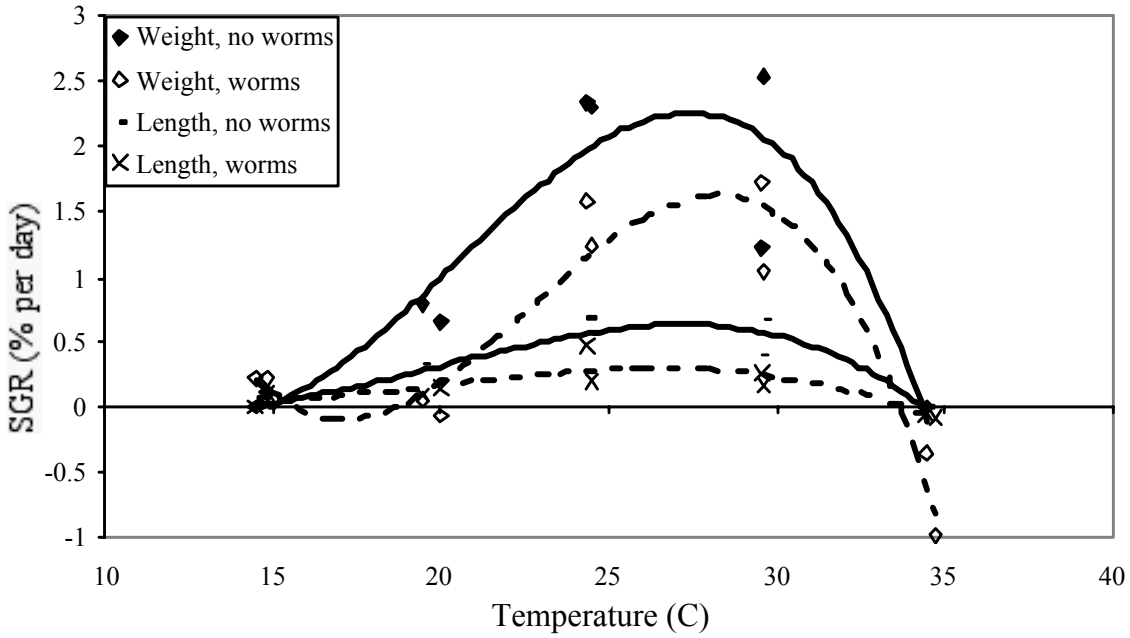


FIGURE 4.— Relationship of specific growth rate (SGR) to temperature for weight and length in experiment T-3. Regression equation for weight, without tapeworms, is $y = -0.0017x^3 + 0.1039x^2 - 1.8766x + 10.521$, $R^2=0.838$; for weight, with tapeworms, $y = -0.0025x^3 + 0.1677x^2 - 3.564x + 24.17$, $R^2=0.883$; for length, without tapeworms, $y = -0.0004x^3 + 0.0251x^2 - 0.4356x + 2.3458$, $R^2=0.869$; and for length, with tapeworms, $y = -0.000202x^3 + 0.0112x^2 – 0.188x + 0.9954$, $R^2=0.657$.

was reduced by 42.2% at 20 C, 38.9% at 25 C, and 26.5% at 30 C; in length, growth rate was reduced by 56.0% at 20 C, 45.1% at 25 C, and 61.2% at 30 C. These values were calculated by comparing median values at each treatment temperature. Growth rate was not reduced at 15 C, and too few fish survived the 35 C treatment for a valid growth rate estimate to be calculated for percent growth rate reduction. Post-experiment dissections revealed no tapeworms in fish that had undergone Praziquantel treatment. Asian tapeworms were present in fish that had not received Praziquantel, with 77%, 89%, 83%, and 54% infection in the lowest four temperature treatments, in order of increasing temperature. For the 35 C treatment, no Asian tapeworms were found upon dissection at the conclusion of the experiment. Survival rate for the four lowest treatment temperatures of fish without tapeworms was 100%, and was 21% for the 35 C treatment. Survival rate for fish with tapeworms was 93%, 64%, 86%, 79%, and 21% in the five treatments in order of increasing temperature. Temperature had a significant effect on fish survival of fish both with (P<0.0001) and without (P=0.0005) tapeworm infection. There was no significant difference in survival between fish with and without tapeworms when all treatments were considered (P=0.239). However, when the highest temperature (35 C) was excluded from the analysis, fish without tapeworms had a significantly higher survival rate (P=0.005). Growth rate was reduced in fish with tapeworm infection.

Critical Thermal Maximum

In experiment CTM-1, fish acclimated to 28 C had a mean CTM of 38.9 C (n=27) (Table 5). Fish acclimated to 31 C had a mean CTM of 39.6 C (n=23). The CTM values for these two acclimation groups were significantly different (P<0.0001). Fish in

experiment CTM-2 acclimated to 27 C had a mean CTM of 38.7 C (n=16) and fish acclimated to 31.5 C had a mean CTM of 39.8 C (n=14). These two groups were also significantly different (P<0.0001). In experiment CTM-3, fish with tapeworms (n=19) as well as fish without tapeworms (n=6) both had mean CTM values of 39.5 C. No significant difference in CTM was found for fish with and without tapeworm infection (P=0.467).

Effect of Dissolved Oxygen on Growth

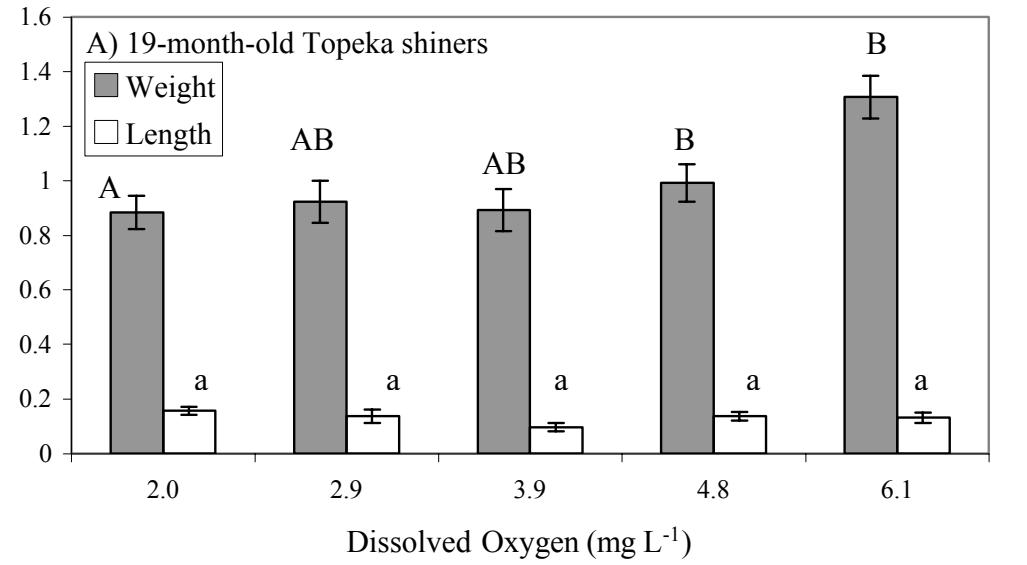
Experiment O-1.— Age was a significant factor for growth in length (P<0.01) but there was no significant difference in growth in weight. For the 19-month-old shiners, dissolved oxygen had a significant effect on growth in weight (P=0.0003) and weight was significantly higher at 6 mg L^{-1} and 5 mg L^{-1} than at 2 mg L^{-1} (P<0.05) (Figure 5a). Growth in length for the 19-month-old shiners was not significantly affected by oxygen level. For the 7-month-old shiners, variability was high and no significant differences were observed in growth rates (Figure 5b).

Survival rates for the 19-month-old fish were 100%, 96%, 100%, 96%, and 96% for the five treatments in order of increasing dissolved oxygen concentration with no significant effect of dissolved oxygen on survival (P=0.73). Survival rates for the 7-month-old fish were 96%, 88%, 92%, 92%, and 88% in order of increasing dissolved oxygen treatments with no significant effect of dissolved oxygen on survival (P=0.84). Post-experiment dissections revealed no tapeworms present in 19-month-old fish and 89% infection in 7-month-old fish (i.e. 16 of 18 randomly sampled 7-month-old fish over the three highest dissolved oxygen treatments of were infected with tapeworms).

TABLE 5.—Critical thermal maxima from experiments CTM-1, CTM-2, and CTM-3. *P*-values from one-tailed t-tests indicate comparisons between acclimation groups in CTM-1 and CTM-2, and between groups with and without tapeworms in CTM-3.

Experiment	*n*	Acclimation Temp. (C)	CTM Mean	CTM SD	CTM Minimum	CTM Maximum	*P*
CTM-1	23	31	39.6	0.273	38.5	39.9	} <0.0001
	27	28	38.9	0.203	38.5	39.3	
CTM-2	14	31.5	39.8	0.181	39.4	40.1	} <0.0001
	16	27	38.7	0.203	38.4	39.1	
CTM-3 (worms)	19	30	39.5	0.340	38.3	39.9	} 0.467
(no worms)	6	30	39.5	0.082	39.4	39.6	

Experiment O-2.— Dissolved oxygen concentration had a significant effect on growth in weight (P=0.0001) for 18-month-old fish (Fig. 6). Growth in weight was significantly lower for the 2.4 and 3.5 mg L^{-1} dissolved oxygen treatments than the 4.3 mg L^{-1} dissolved oxygen treatment and higher (P<0.05) (Figure 6). The effect of dissolved oxygen on growth in length was not significant. Survival rates were significantly different among treatments (P=0.01) with 93%, 98%, 95%, 78%, and 95% surviving in order of increasing dissolved oxygen concentration. Tapeworm infection was not determined by dissection, but was assumed to be 0% for the duration of the experiment because of the efficacy of the Praziquantel treatment observed in other experiments.



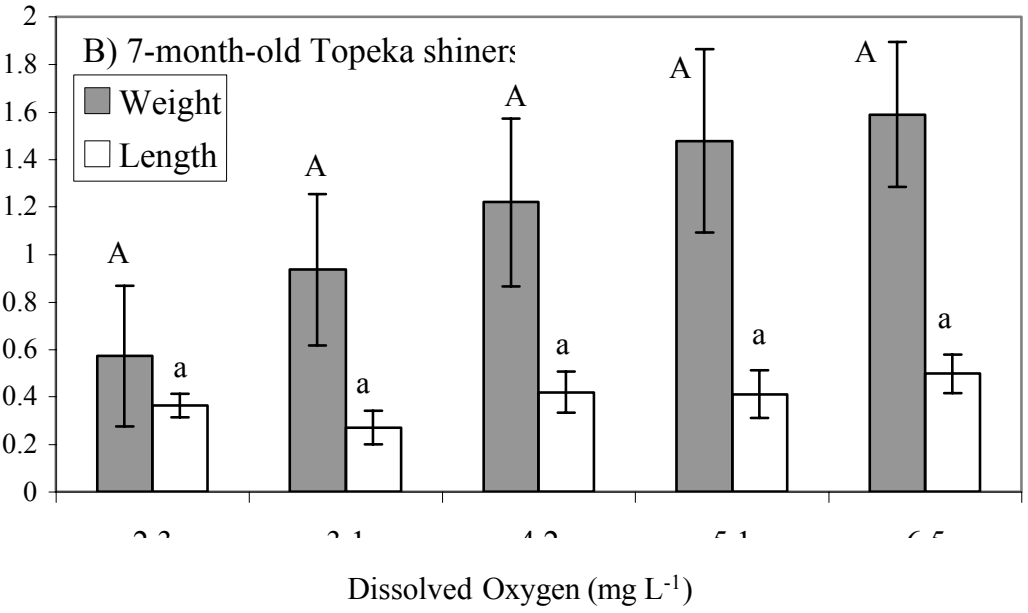


FIGURE 5.—Mean specific growth rates (SGR) (+/- SE) of 19-month old fish (A) and 7-month old fish (B) in relation to dissolved oxygen concentration in experiment O-1. Letters indicate significantly different SGR between treatments as determined by post-hoc Tukey tests.

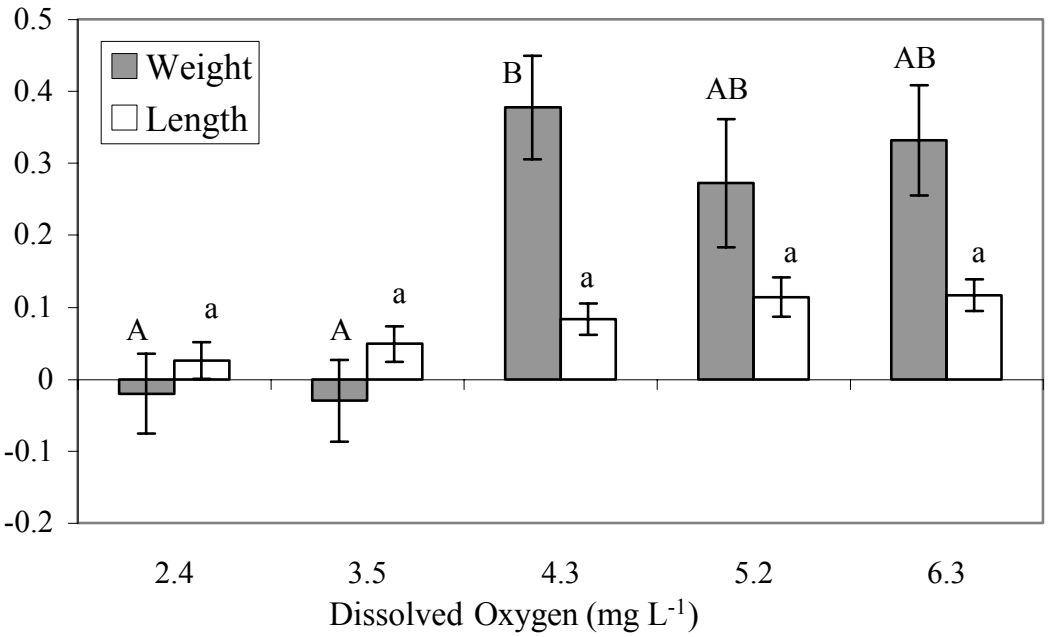


FIGURE 6.—Mean specific growth rates (SGR) (+/- SE) for weight and length in experiment O-2 for 18-month old fish. Letters indicate significantly different SGR between treatments as determined by post-hoc Tukey tests

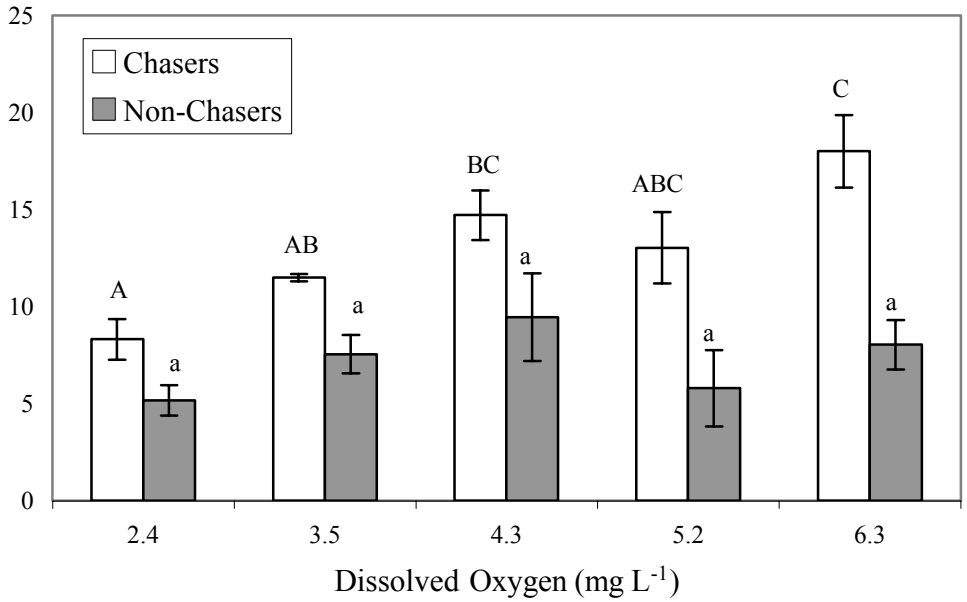


FIGURE 7.— Summary of behavior observations in experiment O-2. Mean number of line crossings are shown (+/- SE) for four different observational periods for "chaser" and "non-chaser" fish. Letters indicate significant differences.

In the analysis of behavior, there were significant effects of oxygen levels on line crossings for 'chaser' fish (P=0.001) (Fig. 6). Significant differences occurred in line crossings between 2 and 4 mg L^{-1}, between 2 and 6 mg L^{-1}, and between 3 and 6 mg L^{-1} treatments (Figure 7). Dissolved oxygen level did not affect line crossings by 'non-chaser' fish (P=0.31).

Lower Lethal Dissolved Oxygen Concentrations

In experiment LC_{50}-1, the 96-hour LC_{50} value for the 19-month old fish acclimated to oxygen concentrations between 2.0 and 2.9 mg L^{-1} was 1.16 mg L^{-1}, with a 95% confidence interval of 0.62 - 2.16 mg L^{-1}. No 96-hour LC_{50} value was calculated for the 7-month old fish because the binary method of calculation requires at least one treatment level with 100% survival and one with 100% mortality, and at no treatment was there 100% mortality. In experiment LC_{50}-2, the 96-hour LC_{50} value for fish acclimated to oxygen concentrations between 2.4 and 3.5 mg L^{-1} was 1.26 mg L^{-1}, with a 95% confidence interval of 0.80 - 1.98 mg L^{-1}.

Discussion

High temperature and low dissolved oxygen alone are probably not factors in Topeka shiner population declines. Topeka shiners were able to survive and grow in conditions of both high temperature and low dissolved oxygen. Published historical information describing Topeka shiner habitat as being cool prairie streams does not seem to adequately describe their possible range of habitats. A combination of factors other

than high temperature and low oxygen are probably responsible for local Topeka shiner extirpations.

Although little information was previously available about temperature and oxygen tolerance of the Topeka shiner, information on other *Notropis* species suggests considerable tolerance of high temperatures. In Ohio, Mundahl (1990) reported observing *Notropis chrysocephalus* (striped shiners) and *N. stramineus* (sand shiners) surviving in habitats with water temperatures up to 39.5 C. However, shade may have provided lower temperatures in some areas of those habitats. CTM for *Notropis* species indicates values are as high as 39.65 C for *N. lutrensis* (red shiner) after fish were acclimated to 30 C (Rutledge & Beitinger 1989) (Table 6, adapted from Beitinger et al. 2000).

Additionally, as summarized in Coutant (1977) a few studies have described preferred temperatures and optimum temperature for growth for species in the genus *Notropis*. Cherry et al. (1975) acclimated *N. rubellus* (rosyface shiner) and *N. spilopterus* (spotfin shiner) to a range of temperatures and reported a preferred temperature for each level of acclimation. When acclimated to 27 C, on average *N. rubellus* preferred 26.8 C and *N. spilopterus* preferred 28.1 C. Similarly, Barans and Tubb (1973) tested both young and adult *N. atherinoides* (emerald shiners) for preferred temperature after being acclimated to water between 20 and 25 C. Both age groups preferred temperatures between 21 and 23 C. Kellogg and Gift (1983) demonstrated that preferred temperature was related to optimum temperature for growth in the four species they tested. One of these species was *N. atherinoides*. They report *N. atherinoides*' preferred temperature as being 29 C after acclimation to 25 C, and they report an optimum temperature for growth

for *N. atherinoides* of 27.3 C. Previously, McCormick and Kleiner (1976) studied optimum temperature for growth for *N. atherinoides*, and reported a value of 28.9 C. These past studies show that the genus *Notropis* has other members with high temperature tolerance, which supports our findings for *N. topeka.*

Little information is available about low dissolved oxygen tolerance in *Notropis* spp. Smale and Rabeni (1995) reported critical dissolved oxygen tolerances (dissolved oxygen concentration at which opercular movement and ventilation ceases, conducted at 26 C over a 4 to 6 hour period) for *N. rubellus, N. nubilus, N. dorsalis, and N. stramineus* (rosyface shiner, Ozark minnow, bigmouth shiner , and sand shiner) as 1.49, 1.45, 1.02, and 0.93 mg L^{-1}, respectively. Ostrand and Wilde (2001) reported dissolved oxygen concentrations at which loss of equilibrium occurred over about 15 minutes for *N. buccula* and *N. oxyrhynchus* as 2.11 and 2.66 mg L^{-1}, respectively. Results indicate that Topeka shiners have similar high-temperature, low-oxygen tolerances as other species of *Notropis*.

Growth rates differ quite a bit between the three experiments in which I determined optimum temperature for growth. Although statistically it is not possible to draw a comparison between experiments, it is important to note the difference. One possible explanation for this difference is that the three groups of fish had different thermal and photoperiod histories, which could affect their circadian or circannual rhythms. Seasons, because of differing photoperiod, affect fish growth, and that factor could have affected the results. Another factor to consider is fish age, which is discussed in more detail below in regards to experiment T-2.

TABLE 6.—Critical thermal maxima for *Notropis* spp. adapted from Beitinger et al. (2000). Entries include pretest acclimation temperature (C), rate of temperature increase during CTM trial (ΔT ºCmin^{-1}), reported test endpoint (OS=onset of muscular spasms, LOE=loss of equilibrium), mean CTM and standard deviation of all CTMs tested, *n*=number of fish tested, and reference as given by Beitinger et al. (2000).

Species	Acclimation temperature	ΔT	End point	Mean CTM	SD	*N*	Reference
N. atherinoides	25	1.0	OS	37.6	0.40	10	Matthews & Maness (1979)
	10	1.0	OS	34.1	0.65	8	Lutterschmidt & Hutchinson (1997)
N. buccula	25	0.5	LOE	39.65	0.23	15	Ostrand & Wilde (2001)
	30	0.5	LOE	36.5-37.9	-	15	Ostrand & Wilde (2001)
N. chrysocephalus	24(field)	0.5-0.8	LOE	36.2	1.0	6	Mundahl (1990)
	11(field)	1.0	LOE	30.8	1.8	8	Hockett & Mundahl (1988)
N. cornutus	15	1.0	OS	31.9	0.48	8	Schubauer et al. (1980)
	15(Dec)	1.0	OS	30.6	0.97	10	Kowalski et al. (1978)
	15(Mar)	1.0	OS	32.0	0.54	16	Kowalski et al. (1978)
N. cummingsae	8	1.0	LOE	29.0	1.3	-	McFarlane et al. (1976)
	8	0.1	LOE	28.0	1.1	-	McFarlane et al. (1976)
N. dorsalis	26	0.017	LOE	36.6	0.49	9	Smale & Rabeni (1995)
N. girardi	25	1.0	OS	38.6	0.32	10	Matthews & Maness (1979)
N. lutipinnis	13	1.0	LOE	30.0	1.2	-	McFarlane et al. (1976)
	13	0.1	LOE	29.0	0.7	-	McFarlane et al. (1976)
N. lutrensis	30	0.3	LOE	39.65	0.23	10	Rutledge & Beitinger (1989)
N. nubilus	26	0.017	LOE	36.2	0.62	9	Smale & Rabeni (1995)
N. oxyrhynchus	25	0.5	LOE	36.5-37.9	-	15	Ostrand & Wilde (2001)
	30	0.5	LOE	39.2	0.2	15	Ostrand & Wilde (2001)
N. rubellus	15	1.0	OS	31.8	0.51	5	Kowalski et al. (1978)
	26	0.017	LOW	35.3	0.23	7	Smale & Rabeni (1995)
N. spilopterus	11(field)	1.0	LOE	31.8	1.8	26	Hockett & Mundahl (1988)
N. stramineus	15(4 seasons)	1.0	OS	32.3-33.0	0.45-0.68	10-18	Kowalski et al. (1978)
	26	0.017	LOE	37.0	0.27	6	Smale & Rabeni (1995)

The estimate of 18 C in T-1 was probably not an accurate estimate for the Topeka shiner's optimum temperature (Figure 2). At higher temperatures and long daylengths, mature fish became sexually active, with considerable chasing of subordinate fish by dominant males. High energy expenditures resulting from this intense reproductive

activity at or above 23 C probably reduced energy available for growth, resulting in slower growth of sexually active fish and a lower estimate for temperature of optimum growth. Subsequent experiments using a shorter daylength reduced the reproductive behavior considerably, although all chasing behavior was not eliminated. In both of the experiments with shorter daylength, the optimum temperature for growth was at least 9 C higher than with longer daylength (and more reproductive activity), which supports the conclusion that the optimum of 18 C was an underestimate.

It appears that an accurate estimate of optimum temperature for growth lies between 27 and 31 C, regardless of whether growth is measured as length or weight. However, the 31 C estimate may be too high because of the presence of tapeworms. It is clear from the experiment comparing infected and non-infected fish that tapeworms reduce growth rate. However, lower parasite loads at higher temperatures suggest that tapeworms could not tolerate the highest temperature, as demonstrated in experiment T-2 where only 43% of the fish at 31 C had tapeworms, compared to between 89 and 100% infection at all lower temperatures. Similarly, tapeworm infection in experiment T-3 for fish that had not been treated with Praziquantel was 54% at 30 C and 0% at 35 C, compared with 77%, 89 %, and 83% infection at 15, 20, and 25 C, respectively. While I could not document exactly when fish shed tapeworms during the test, it is likely that the reduction in parasite load increased their subsequent growth rate. Thus, the high growth rate of infected fish at 31 C in experiment T-2 was relatively higher than fish at 27 C, which had a 94% tapeworm infection at the end of the experiment. This relatively higher growth rate at 31 C than at 27 C would cause the estimate of optimum temperature for growth to be high. Also, younger fish were used in experiment T-2 (in which all fish

were initially infected with tapeworm). Age of fish is an important factor to consider for at least two reasons. First, younger fish do not exhibit reproductive behavior, although individuals still exhibit some chasing behavior; second, younger fish also could have different growth potential than adults, resulting in a higher optimum temperature for growth. However, there is no way to separate effect of age from effect of tapeworm infection in the experiment, as no experiment was performed on young fish without tapeworm infection. The best estimate, therefore, is that optimum temperature for growth is probably closer to 27 than 31 C for Topeka shiners. The estimate of 27 C is similar to other species in the genus *Notropis*.

Overall, the presence of tapeworms reduced fish growth rate and survival. A reduction in growth has been reported for several other fish species parasitized by tapeworm (Brouder 1999; Pulkkinen and Valtonen 1999; Sirois and Dodson 2000; Saksvik et al. 2001). Hoffman (1998) suggests that mortality due to tapeworms is not often a major concern, but this study indicated reduced growth and higher mortality and suggests that infected fish should not be used for population rehabilitation. Furthermore, because the Asian tapeworm is an exotic parasite, regulations prohibit the release of infected fish in waters where the parasite is not already present.

CTM values were similar to the highest values reported for other *Notropis* species (Table 6). Although I did not test for the Topeka shiner's ultimate upper incipient lethal temperature (UULT; the maximum tolerable temperature regardless of acclimation temperature) it is probably between 33 and 35 C. This is suggested by the results, which showed that 97% of fish survived temperatures of 33 C, compared with 80% mortality at 35 C over 30 days. Additionally, a high CTM value supports the estimate of a high

optimum temperature for growth, because for many fish, the optimum temperature for growth is relatively close to the CTM value. Overall, survival at temperatures as high as 33 C with a CTM of 39 C is further evidence that Topeka shiners can tolerate high temperatures.

Topeka shiners are also tolerant of low dissolved oxygen levels. Although a high level of variability was present not only in the oxygen levels themselves (Table 2) but in growth of individual fish, results still showed that growth occurred at dissolved oxygen levels as low as 2 mg L^{-1}. Growth in length and weight was observed for both younger and older fish at 2 mg L^{-1}. Although the results for effect of oxygen on growth were not exactly the same between the two oxygen-growth experiments, the general downward trend of growth rate with reduced oxygen suggests that Topeka shiner growth is dependent on oxygen concentration. At oxygen levels below about 4 mg L^{-1}, reduction in growth may be substantial. Results also show some evidence that activity level, including reproductive behavior, decreased as dissolved oxygen level decreased (Figure 7). Inhibition of reproductive behavior could impact isolated populations.

Results from the lower lethal dissolved oxygen tests support oxygen-growth test results, showing that Topeka shiners are quite tolerant of low oxygen conditions. However, three aspects of experimental conditions should be noted for the lower lethal dissolved oxygen tests. One aspect is that the first experiment was disrupted twice over the 96-hour period because the compressed nitrogen gas tank used for stripping oxygen from the water ran out, allowing oxygen levels in the experimental tanks to rise for a maximum of about 4 hours. Another aspect from the first test is that although tanks were equipped with plastic covers floating on the surface of the water, fish still had access to

the surface via small gaps between the covers and the sides of the tank, and most fish in the treatments below 2 mg L^{-1} congregated there during the experiment, taking advantage of the slightly more oxygenated surface layer of water. Finally, the second experiment was at a slightly higher temperature than the first (about 28 C vs. about 25 C). In spite of these three differences, the estimates of 1.26 and 1.16 mg L^{-1} for 96-hour LC_{50} values obtained from the two experiments are similar, and the estimate of 1.2 mg L^{-1} for a 96-hour LC_{50} value is probably valid.

Tolerance of high temperatures and low oxygen may result from adaptation to the demanding conditions of naturally occurring seasonal drought associated with prairie streams; however, other *Notropis* species show similar tolerances and some are not prairie headwater species, so the hypothesis about adaptation to prairie streams may be more complicated.

Topeka shiner tolerance of high-temperature and low-oxygen conditions supports the idea that off-channel habitats may be population sources, rather than population sinks. Certain microhabitats may allow populations to persist by supplying organisms to the areas in the habitat from which organisms are frequently lost (Pulliam 1988). The off-channel habitats may provide refuges for Topeka shiners in times of drought. When water again becomes abundant the off-channel habitats reconnect, at least temporarily, to the main stream, allowing Topeka shiners to repopulate the stream. This study gives new information about physiological limits of the Topeka shiner that should be applied in the protection of known Topeka shiner habitat, including off-channel habitats, and could help to ensure success of future population restoration efforts.

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