THESIS

THERMAL IMPACTS ON THE EARLY LIFE HISTORY OF SAUGER (*SANDER CANADENSIS*)

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2019

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ABSTRACT

THERMAL IMPACTS ON THE EARLY LIFE HISTORY OF SAUGER (*SANDER CANADENSIS*)

Sauger *(Sander canadensis),* a large North American member of the family Percidae, often exhibit sporadic recruitment governed by a variety of biotic and abiotic factors. This episodic reproductive success is emblematic in Percids, as it has been documented across a wide geographic area for multiple Percid species. Temperature, the most influential abiotic variable, directly affects Percid recruitment, physiology, and distribution, while simultaneously modifying many other factors that govern population dynamics such as food abundance. The Wind and Bighorn River drainages of Wyoming, among the highest elevation tributaries of the Missouri River basin, remain a stronghold for two native Sauger populations. These populations are among the slowest growing and longest lived in the entire native range and provide an important recreational angling resource. While recent population trends have been positive (2011-2016), conditions in the past decade have resulted in poor recruitment, with only older age classes present in the annual Wyoming Game and Fish Department (WGFD) surveys of the Wind River population (2002-2011). Agency concern over poor recruitment resulted in the initiation of artificial spawning operations in both drainages, with the aim of bolstering these populations. Sauger hatch percentages from these spawning efforts were highly variable, and generally low across all years (0-75%). One hypothesis advanced to explain the observed variable egg survival was that river temperatures leading up to and during the spawn were outside of optimal ranges.

Our research explored how temperature affects multiple stages in reproduction including adults, embryos, and prolarvae. Specifically, we tested the effect of temperatures in the range of

10-24°C on the survival and rates of development of Sauger embryos and prolarvae (phase I and II). In the incubation experiment, hatch rates were low for all treatments (<22%). Sauger embryos displayed appreciable thermal plasticity and no differences in hatch percentage were detected in the range of 12.2-18.9°C. Statistically lower hatch rates were detected for our 10°C and diel fluctuating (17-22°C) treatments compared to all others. Hatch timing and duration was inversely related with temperature. We developed a regression model to estimate temperature units (TU's) necessary to reach hatching and duration of hatch (days) based on average temperature. Our results indicate that optimal incubation temperature for Sauger is near 14.5°C.

Sauger prolarvae survived at high rates (>90%) to the onset of exogenous feeding in all treatments ≥ 18°C, although pronounced mortality associated with the time that yolk was completely absorbed, suggested that starvation occurred, despite offering brine shrimp (*Artemia nauplii*) daily. Prolarvae in the 12 and 15°C treatments survived at a statistically lower rate to the onset of exogenous feeding and fed poorly after. We suggest that optimal temperature for prolarvae survival is in the range of 18-24°C with the caveat that rapid mortality can result around the time of yolk absorption if larvae do not successfully feed exogenously. Growth rate (SL) was positively associated with temperature. Time to reach exogenous feeding was negatively associated with average temperature. We developed a regression model predicting TU's necessary for larvae to reach exogenous feeding based on average temperature conditions.

We also exposed wild adult Sauger to two pre-spawning temperature treatments, approximately six weeks before the expected spawn date. One treatment was an above average, stochastic thermal scenario (fluctuating), while the other was a gradually warming treatment expected to be near optimal for spawning (control). We evaluated impacts on egg viability, as determined by fertilization and hatch percentages. We also determined egg energy density as a measure of egg quality. We found no differences in fertilization and hatch percentages between

treatments, perhaps due to a small sample size (n = 7 spawns). Similarly, there was no difference in mean egg energy density between treatments. Adult mortality was greater in the fluctuating treatment compared to the control (44.4% vs. 26.3%) and females were disproportionately affected.

Our research explicitly defines thermal criteria, that will provide managers with guidelines to understand how temperature may influence recruitment in the wild, in addition to providing thermal recommendations for artificial spawning and/or hatchery operations in the future.

ACKNOWLEDGMENTS

It takes a village to successfully complete a graduate project—from those who dream up and implement the project, to those who provide expertise, and even those who simply offer love and support along the way. First and foremost, I want to thank my best friend, who also happens to be my wife, Laurel, for her enduring support throughout this process. She always inspires me to be the best version of myself and kept my feet on the ground when the going was tough.

I thank my research committee, Brett Johnson, Cameron Ghalambor and Mark Smith for their valuable input on my project. I feel sincerely gracious to have had Dr. Chris Myrick as my advisor. Chris has supported me without exception, was always available to offer his expertise, and was quick to share a fishing story or two (over a cold one or two). Chris is the gold standard advisor and professor, but most importantly, Chris is a phenomenal father, friend, and mentor. This department and university benefits disproportionately from Chris's dedication and passion for what he does.

This project would not have been possible without the funding and logistical support from Wyoming Game and Fish Department (WGFD). Mark Smith, Paul Gerrity, Joe Skorupski, Sam Hochhalter, and Kristopher Holmes were a joy to interact with and instrumental along every step of this process, from project inception, to squeezing Sauger on the Bighorn River. WGFD set aside countless hours of their packed schedules to execute field sampling procedures, provide input on my experiments and ensure that I had everything necessary to be successful. I appreciate the opportunity I have had to interact and learn from these professional and great characters and experience the unique resource they manage.

I was incredibly fortunate to have had some of the best undergraduate research technicians on this side of the Mississippi. Katie Rohwer, Kira Paik, Chase Garvey, and Kevin

Fitzgerald were all critically important to completing this project and remained steadfast, dedicated, and reliable in spite of the many "speed bumps" encountered along the way. They all shouldered a great amount of responsibility and I have little doubt that all of these great young professionals will be leaders in our field.

I also want to thank all of my fellow graduate students, all of whom I consider great friends and stellar human beings. Alex Townsend, Tyler Swarr, Chris Kotalik, Cat DeVlaming, Cole Brittain, Rachel Jones, Ben Vaage, and Collin Farrell were all available to help at the drop of a hat. Many hours were spent in the "dungeons" of Wagar scheming our projects, bouncing ideas off one-another, and grinding out coursework. The relationships forged in grad school are fire-hardened and I look forward to decades of productive working associations and genuine friendships.

I am also grateful to Colorado Parks and Wildlife for providing support in my research. David Harris, Carrie Tucker, and Mandi Brandt offered culture expertise and were gracious enough to provide some Walleye eggs to help refine my experimental methods. CPW fish pathologists/aquatic veterinarians John Drennan, Vicki Milano, and Colby Wells provided critical fish health information that kept my project on track. Others who contributed to this project were Bill Pate, who spent many hours providing help in the Fisheries Ecology Lab. Ann Hess provided critical help with statistics. Jake Cammack provided GIS help and brotherly encouragement and Sage Cammack consistently wagged her tail and reminded me that there was a pond conveniently located adjacent to the lab (optimal "lab" habitat). Cheers to all!

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CHAPTER 1: INFLUENCE OF TEMPERATURE ON SURVIVAL AND DEVELOPMENT OF EGGS AND PROLARVAE

INTRODUCTION

Temperature has long been recognized as the master variable affecting fish physiology, wherein metabolic rates and energy budgeting are directly tied to temperature in the vast majority of fishes (Brett 1971). Metabolic processes in fishes are evolutionarily linked to long-term, specific thermal regimes, and thus alterations in these thermal regimes can lead to deleterious physiological changes (Pörtner and Knust 2007). Because biochemical reactions are temperature dependent, virtually all aspects of fish physiology are influenced by changing temperature including growth, reproduction, and activity (Ficke et al. 2007). The temperatures of freshwater ecosystems are sensitive to wide range of modifications including those in hydrology (dams, irrigation, diversions), landscape alteration (urbanization, deforestation, agriculture) and climate change. In North America, mean annual stream temperatures have warmed at rates between 0.009 and 0.07°C per year, correlated with rising air temperatures (Kaushal et al. 2010). Global temperatures have warmed approximately 1°C from pre-industrial times and, given the current trajectory, are expected to rise another 0.5°C by the year 2040 (Allen et al. 2018). Changes in mean temperature may be accompanied by increased frequency of extreme climatic events, leading to more stochastic thermal regimes that will in turn influence freshwater ecosystems (Mantua et al. 2010; Steel et al. 2012; Whitney et al. 2016). The responses of fish to these changes will be largely dependent on the magnitude of the temperature deviations and will vary among species because of differences in thermal tolerances and adaptive potentials.

Sauger (Percidae: *Sander canadensis*), occupy the cool-water guild of freshwater fishes and have an intermediate range of temperature tolerance (Manguson et al. 1978; Hokanson 1977). While adult Sauger may have sufficient thermal plasticity to cope with a relatively wide seasonal range of temperatures and diel fluctuations, prolonged exposure to steady temperatures and/or fluctuations outside of the optimal range may impact reproduction, which is often the most thermally-sensitive phase of a fishes' life cycle. Under the most extreme conditions, temperature–related mortality of eggs or larval stages may be acute, though chronic thermal conditions may eventually cause mortality via interactions between developmental rate and resource availability. For example, elevated temperatures cause increases in metabolic rate in larval fish, leading to faster yolk absorption and the need to start successful exogenous feeding at an earlier stage. Hokanson and Kleiner (1974) found that the median period between hatch and death of unfed Yellow Perch (Percidae; *Perca flavescens*) was nine and 21 days at 19.8°C and 10°C respectively, suggesting that higher temperatures constrain larvae to feed sooner. This is in contrast to colder temperatures, which delay the exhaustion of endogenous energy reserves, thereby providing more time for behavioral adaptation to exogenous feeding (Hokanson 1977; Smith Jr. and Koenst 1975).

Sauger, a native species of special concern and popular gamefish in Wyoming, have experienced drastic declines across their native range in the recent past (Hesse 1994; McMahon and Gardner 2001; Nelson and Walburg 1977), including in the Wind River in north-central Wyoming (Figure 1-1). The estimated Sauger population sizes in the Little Wind River and Popo Agie River declined by 73% between 2002 and the 2009 – 2010 sampling period (Gerrity 2013). These declines were thought to be driven largely by poor recruitment, as suggested by the increased prevalence of larger and older size classes of fish. The Bighorn River population has remained relatively robust, though spawning operations were conducted to increase angling

opportunity in Bighorn Reservoir (Hochhalter 2015). Supplemental stocking of Sauger fry, produced from wild-caught broodstock, was initiated to attempt to bolster recruitment in both populations, though efforts were hampered by variable (and often low) survival of eggs collected during wild spawning operations. The poor egg survival coincided with elevated and/or highly variable temperatures leading up to, and during the spawn (Figure 1-2), especially in 2013, 2015, and 2016[1]. This led to the hypothesis that temperature excursions outside of the putative optimum range may adversely impact reproductive success at one or more life stages, including spawning adults, embryos, and larvae.

This study was designed to improve our understanding of how Sauger reproduction responds to various thermal conditions. Our objectives were to test the effects of a wide range of ecologically relevant temperatures on the survival of fertilized eggs (i.e., embryos) collected in the wild and the resulting prolarvae, in addition to determining how embryo and larval development are influenced by temperature. Through this research, we hope to clarify how thermal conditions may drive trends in the recruitment of wild populations of Sauger, in addition to how temperature influences hatch percentages and larval survival during artificial spawn takes and associated hatchery operations.

METHODS

Field Sampling and Spawning Procedures

In May 2017 and May 2018 Wyoming Game and Fish Department (WGFD) and Colorado State University (CSU) personnel collected and spawned wild Sauger on the Bighorn River, near

[1] The region also experienced a significant drought from 2000 to 2008; unfortunately, there are no continuous stream temperature data for this period.

Basin, WY. This section of the Bighorn is large (mean wetted width: 40 m) and characterized by low gradient (approximately 0.93 m:km), high turbidity, and a mix of substrate types (silt to cobble). The Bighorn R. Sauger population was chosen due to its robust size compared to other Wyoming sites and because known spawning areas were accessible. Sauger were collected by jet boat electrofishing using a 75A ETS control unit (ETS, Madison WI, USA) powered by a 6500W generator. Two anode booms were oriented near parallel to the boat and shocker output was set between 10 – 14 amps and 200 – 300 volts; these settings varied from day to day depending on river conditions. Deep, near shore habitats were thoroughly sampled to target spawning aggregations. Upon capture, fish were sexed, measured, and marked with individual Floy Tags. Fish were sorted in the following manner:

- Males that expressed milt with gentle pressure to the abdomen were considered ripe and held in 1135-L tanks on shore, while green and immature males were returned to the river.
- Females judged to be intermediate-soft or soft, based on the WGFD rating system (Table 1-1) were injected with hormone and kept in holding tanks. All other females (green and immature) were released.

The capture of a surplus of ripe males obviated the need to inject males and induce final maturation of gametes in both years. In contrast, ripe females were rarely captured so hormone injections were used to trigger final maturation (Hochhalter 2015). In 2017, 15µg/kg OvaRH® (Syndel, Ferndale, WA USA), a synthetic salmon GnRH, was used. Fish were injected with a single dose in the dorsal musculature. In 2018, OvaRH® was unavailable during the spawning period, so two intramuscular injections (48 h apart) of 1650 IU/kg Chorulon® (Merck, Kenilworth, NJ, USA), a human chorionic gonadotropin (hCG) were used. In both 2017 and 2018, injected females and ripe males were held onshore in a set of three 1135-liter insulated hatchery tanks that were kept at or near air-saturated dissolved oxygen levels using compressed

O_2 delivered through fine pore oxygen diffusers. Water for the holding tanks was taken from the river. Females were assessed early each morning and once adequate numbers of females were ripe, they were spawned on site.

Ripe fish were spawned utilizing the dry method following WGFD standard protocols. In 2017, sperm motility was confirmed in all spawning males to verify reproductive viability and two males were used to fertilize the eggs of each female. In 2018, sperm motility was not checked because excess males were captured and at least three males were used per female to ensure that fertilization occurred. A bentonite clay slurry was added to newly fertilized eggs to reduce clumping of the adhesive Sauger eggs. Eggs were water–hardened with gentle aeration for 1.5 hours, prior to being transported to the CSU Foothills Fisheries Laboratory (FFL) in 34-L insulated water coolers. Water coolers were equipped with three levels of nylon mesh to utilize vertical extent of container. This ensured that only one layer of eggs existed on each level to provide adequate oxygenation and reduce clumping. During travel, temperature was monitored approximately hourly and small amounts of chlorine-free ice were added to maintain temperatures within 1°C of the spawning temperature (13-15°C).

Effects of Incubation Temperature on Egg Survival and Hatch Timing

The first part of the study (Phase I - 2017) addressed how water temperature affected the hatching success and rate of development of Sauger eggs. Fixed temperature treatments of 10, 12.2, 14.4, 16.7 and 18.9°C were used, along with a fluctuating treatment (diel fluctuation from 17 to 22°C). These treatments were selected in consultation with WGFD to encompass the thermal ranges observed on the Wind and Bighorn Rivers during egg incubation.

Two batches of fertilized Sauger eggs were transported back to the FFL in Fort Collins for incubation. The first and second batch of eggs consisted of spawns of three and seven females respectively, for a total of 10 spawns. Upon arrival at the FFL, Sauger eggs were

tempered to their target incubation temperatures at a rate not exceeding 1°C/h. Eggs (2.5 ml) were loaded into eight, 500-mL miniature upwelling incubators (Figure 1-3) for each of the six temperature treatments (n = 8; total number of incubators = 48). Each incubator received 600 mL/min of air-saturated and temperature-controlled water. Water temperatures were kept within 0.6°C of the desired levels with Love® model 16B-33 digital controllers controlling Asco® two-way solenoid valves that regulated the mixture of well water with either heated or chilled water. Hobo© temperature loggers within each treatment tank recorded the thermal conditions throughout the incubation period. All incubators were covered with black plastic to reduce ambient light.

To reduce the possible loss of eggs to opportunistic fungi (e.g., *Saprolegnia* spp.) each incubator was treated with 500 ppm H_2O_2 for 15 minutes as per Colorado Parks and Wildlife standard practices (David Harris, CPW, personal communication) each day until eggs were eyed. Dead eggs were removed and counted daily. Hatched larvae swam through the incubator outlet into an individual collection container. Collection containers were emptied daily, and larvae were euthanized using 250 mg/L of pH-buffered tricaine methanesulfonate, counted, and preserved in 80% ethanol for subsequent analyses. These methods gave us a clear picture of when hatching started and ended for each jar and allowed us to quantify hatch percentages for each jar.

Data Analyses: Our predictor variable for this experiment was temperature treatment with a primary response variable of percent hatch of Sauger eggs. Data were analyzed using one-way ANOVAs with incubators as our experimental unit using RStudio, version 1.0.136. Spawning "batch" was employed as a blocking variable such that each batch appeared within each temperature treatment in a random complete block design; doing so allowed us to account for batch variability within the model. Secondarily, we were interested in how temperature

influenced the timing and duration of hatch. We adopted time to "peak hatch" as our response variable to assess hatch timing, defined as the day that the greatest number of larvae hatched within each incubation jar. Duration of hatch was defined as the time between the first and last observed hatch for each incubation jar. Additionally, we computed the temperature units (TU's) to reach peak hatch, as defined as the summed daily mean temperature above 0°C (e.g., 10 days at 10°C=100 TU's). We developed a logarithmic regression to predict TU's necessary to reach peak hatch based on average incubation temperature using Microsoft® Excel 16.21.1.

Effects of Post-Hatch Temperature on Survival and Development of Prolarvae

The purpose of the second part of the study (Phase II – 2018) was to quantify the effects of temperature on the development and survival of larval Sauger from hatching until they absorbed their yolk and started exogenous feeding. To maximize ecological relevance, temperature treatments encompassed the full range of temperatures observed within and across years, during the period that prolarvae are present in the river. To avoid possible confounding effects of different incubation temperatures, we incubated wild Bighorn River Sauger eggs at 14.5°C. Based on our egg incubation experiment, this was near optimal temperature.

Two batches of fertilized Sauger eggs were transported to the FFL for incubation. The first and second batch of eggs consisted of spawns of four and seven females respectively, for a total of 11 spawns. Eggs were incubated in a series of eight covered 2-liter upwelling incubators (four per batch). Each day, eggs received flow-through treatments of H_2O_2 (500 ppm for 15 minutes) to control fungus. Dead eggs and large clumps of eggs were removed to prevent fungal outbreaks and maintain optimal conditions for developing embryos. Once the majority of eggs hatched (three days after first hatch), larvae were randomly assigned to one of six rearing temperature treatments (12, 15, 18, 21 and 24°C, and diel fluctuation from 17-22°C). Larvae

were tempered at 1°C/hour to the rearing temperature to reduce thermal shock and unintended physiological stress and/or mortality. After tempering, larvae were stocked into six replicate 4-L tanks (250 fish per tank) per temperature treatment (three tanks per batch). Two additional tanks in each temperature treatment (one for each batch) were stocked with 350 larvae. These surplus tanks were used to obtain daily subsamples to track developmental changes throughout the course of the experiment and were not considered replicates in the analyses, but rather representative of developmental stage. Each tank received continuous flows of air-saturated water at the treatment temperature at a rate of approximately 240 ± 20 ml/min and was equipped with small spray bars to increase oxygenation, provide current, and disperse any oil on the surface that could potentially impede proper gas bladder inflation (Summerfelt and Johnson 2015). Tanks were covered with black plastic to ensure that ambient light levels were low and similar between treatments. Throughout the experiment, dead larvae were removed from the replicate containers each day and counted. In addition, approximately 10 larvae were removed from each subsample tank at 1-2 day intervals and euthanized in MS-222. These larvae were examined at 20X magnification to identify developmental stage. Following evaluation, larvae were preserved in 10% formalin for later analyses. Development of subsampled larvae was considered representative of the larvae in our experimental replicates because of the identical thermal conditions.

Due to the inherent variability in time to hatch, early hatching larvae were more developmentally advanced and started to feed exogenously before later hatching larvae. All treatments were offered live feed (*Artemia nauplii*) *ad libitum* when the mouths of larvae in subsamples appeared developed. This helped to prevent starvation of exogenously feeding larvae so that any observed mortality could be attributed to thermal conditions, and not food availability. All subsamples were checked for onset of exogenous feeding (i.e., yolk sac

absorbed, mouth open). This gave us a ratio of prolarvae (yolk sac present) to postlarvae (yolk sac absorbed) (Summerfelt and Johnson 2015) that helped track the rate of development. When 100% of the subsampled larvae had no visible yolk remaining or when the rate of cannibalism reached 10 fish/day, all corresponding experimental larvae were euthanized, counted and preserved to determine percent post-hatch survival to the onset of exogenous feeding and the size at first feeding.

Preserved specimen analyses: Preserved larvae from subsample jars were examined at 20X magnification. Measurements of standard length (mm) and yolk area (mm^2) were made using NIH ImageJ 1.8.0 (U.S. National Institute of Health, Bethesda, Maryland, USA). Additionally, each larva was examined for the presence/absence of a yolk sac. These metrics enabled us to track developmental changes through time for each treatment.

Data Analyses: Our primary objective was to study how temperature influences survival and development timing of prolarvae to the stage where the yolk sac was exhausted and exogenous feeding commenced. Because there was inherent variability in developmental stage among individuals within a given treatment, it was necessary to define a time that larvae were considered to be exogenously feeding, to avoid arbitrary comparisons between treatments. This gave us the ability to standardize between temperature treatments and make survival comparisons at a similar stage of development. Using subsampled larval measurements, we evaluated the depletion of yolk through time for each respective treatment and identified the day at which 50% or more of subsampled larvae no longer had any visible yolk remaining, defined as $D_{0\text{-}y}$ (day zero-yolk). We considered $D_{0\text{-}y}$ to represent the day that most larvae needed to feed exogenously to avoid starvation. Once $D_{0\text{-}y}$ was identified for each treatment, the percent survival of larvae was computed for all the respective replicates, herein referred to as "percent survival to exogenous feeding." Due to heteroscedasticity of the data, we chose to use a non-

parametric approach to compare survival between treatments using a Kruskal-Wallis one-way ANOVA, followed by a pairwise Wilcoxon's test to evaluate differences between treatments, using RStudio, version 1.0.136. To evaluate changes in the rate of development we assessed changes in yolk area and standard length using logarithmic regressions produced in Microsoft® Excel 16.21.1.

RESULTS

Phase I Spawning Study - Effects of Incubation Temperature on Egg Survival and Hatch Timing

Treatment temperatures remained within 0.3°C of target temperatures for the duration of the experiment, with only two exceptions. On July 6th, the temperature dropped approximately 1.2°C in one hour in the 14.4°C treatment due to a mechanical malfunction. This mild deviation did not appear to influence the results, as no unusual spike in egg mortality was observed. Secondly, on June 1st an unknown error occurred in the program of the digital temperature controller of the fluctuating treatment, causing the temperature to drop approximately 10°C in one hour. While, this was a drastic drop, it did not appear to largely affect our results, as we observed no subsequent spike in egg mortality and embryos continued to hatch normally following the error. In addition, the majority of larvae (73%) had hatched prior to the malfunction.

Hatch percentages were low for all experimental treatments, ranging from 12.7% for the fluctuating treatment to a high of 21.7% for the 14.4°C treatment (Figure 1-4), with statistical differences in hatch percentage existing between temperature treatments (one-way ANOVA, $P<0.001$). Hatching rates in the 10°C and fluctuating treatments were similar to each other but

were lower than in the other treatments. The mortality that led to the low hatch percentages typically occurred within the first seven days of the experiment (Figure 1-5). A significant batch effect was observed with the first batch of eggs having higher survival than the second (one-way ANOVA, $P<0.001$). There were significant differences in time to reach peak hatch (one-way ANOVA, $P<0.001$), with the fastest hatching times observed in the warmer treatments (Figure 1-6). All treatments had statistically different days to reach peak hatch except for the 18.9°C and fluctuating treatments. Interestingly, there were also differences in the temperature units (TU's) needed to reach peak hatch (one-way ANOVA, $P<0.001$), ranging from a low of 146 TUs for the 17-22°C treatment (with the warmest average temperature of 19.5°C) to a high of 219 TUs for the 10°C treatment (Figure 1-7). Duration of hatch was also significantly different between temperature treatments (one-way ANOVA, $P<0.001$) with lower temperatures corresponding to longer hatch durations (Figure 1-7).

Phase II Prolarval Study – Effects of Post-Hatch Temperature on Survival and Development of Prolarvae

While not specifically quantified, hatch percentages of fertilized eggs were low (<30%) for each of the eight upwelling jars, but we still produced a surplus of hatched larvae for the study. The mean size (± SD) of larvae at hatch was 6.46 ± 0.23 mm SL. Despite daily treatments with H_2O_2, fungus attacked eggs in each jar, necessitating the removal of clumped (affected) eggs at least twice per day to prevent further spread. Temperatures remained consistent at 14.5°C (± 0.2°C) throughout the incubation period and within 0.5°C of the treatment temperatures once the larval Saugers were transferred to the experimental tanks.

Larval Sauger developed at drastically different rates, with fish in warmer treatments increasing in size (SL) and depleting their yolk sacs faster (Figures 1-8 and 1-9). Interestingly, standard length in the fluctuating treatment (17-22°C) was greater on all experimental days than

other treatments, suggesting better growth resulted from the mean temperature of 19.5°C. For all treatments, the rate of growth (increase in SL) decreased over time. This decrease in the growth rate was especially pronounced in the 12°C and 15°C treatments, where fish exhibited negative growth towards the end of the experiment.

Temperature units necessary to reach exogenous feeding were not uniform across treatments. This metric was inversely related with average temperature, as approximated by a logarithmic regression (Figure 1-10). As summarized in Table 1-2, time of exogenous feeding occurred at drastically different times. For example, fish in the 24°C treatment reached exogenous feeding after three experimental days, while those in the 12°C treatment reached that milestone after 13 days. Very few larvae in the coldest two treatments (12°C and 15°C) were observed exogenously feeding on brine shrimp and cannibalism was uncommon. In contrast, in all other treatments a majority of larvae were observed feeding and cannibalism became a frequent occurrence during the final days of the experiment despite the provision of satiation rations of brine shrimp.

Average survival of prolarvae remained relatively high (>90%) for all warmer treatments (18, 21, 24 and 17-22°C) until there was a sudden and sharp decline (Figure 1-9). This increase in mortality corresponded to the time that larval fish had utilized most or all of their yolk sac and likely starved because they did not encounter enough live feed or because they failed to transition preying on live feed (Figure 1-9). We detected statistical differences in survival to the onset of exogenous feeding (Kruskal-Wallis chi-squared = 29.117, $P<0.001$), with the 12°C and 15°C treatments surviving at a lower rate than all other treatments, although they did not differ from each other. All other treatments experienced similar survival to the onset of exogenous feeding with the exception of the 18°C and 24°C treatment, whereby the 24°C larvae survived at a slightly greater rate (Figure 1-11).

DISCUSSION

Over two seasons, we successfully measured the effects of temperature on Sauger egg incubation and larval development. As expected, temperature influenced survival of embryos and prolarvae, though not necessarily in the ways that we predicted. In general, we anticipated that the relationship between temperature and survival for both eggs and prolarvae would follow an inverse parabolic function for the fixed temperature treatments, whereby highest survival would be associated with intermediate temperature treatments and taper off towards the upper and lower ends. We anticipated that diel fluctuations would negatively impact survival as compared to intermediate fixed temperatures because of the thermal excursions into high suboptimal temperatures. Our data did not fully support these predictions. Generally, both embryos and prolarvae displayed a wider range of thermal tolerance than we expected or was reported by Koenst and Smith Jr. (1976) (Table 1-3). Our egg incubation experiment hatch percentages in warmer fixed temperatures (16.7°C and 18.9°C) were not statistically different than other fixed treatments we tested (12.2°C and 14.4°C), within the reported optimal range (12-15°C) according Koenst and Smith Jr. (1976). These warmer temperatures exhibited slightly lower average hatch percentage than our 14.4°C treatment, though the differences were not statistically significant.

We did detect a negative impact of colder treatments. Our results clearly indicate that there is a threshold in embryo survival between 10°C and 12.2°C, as these were statistically different from one another (11.9% vs. 19.7% hatch, respectively). Unusually cold spring temperatures may result from hypolimnetic releases from Boysen Reservoir in the upper reaches of the Bighorn River. This departure from the natural thermal regime may cause historical

spawning habitat to be thermally incompatible for Sauger reproduction, thus restricting spawning to lower reaches with roughly natural thermal regimes.

Interestingly, diel fluctuations of 17-22°C appear to impair hatching success, as we observed statistically lower hatch rates for the diel treatment compared to steady temperature treatments in the range of 12.2-18.9°C, even though the mean temperature was approximately 19.1°C. Our study was not designed to determine whether this impact resulted from the fluctuations themselves, from exceeding a thermal threshold at the upper end of the fluctuation (near 22°C) for a period each day, or from some other indirect effect. There were more eggs colonized by fungus in the diel treatment than in other treatments, suggesting that conditions may have been more conducive to fungal growth and it is known that some types of fungus (e.g., *Saprolegnia*) are more active and virulent at higher temperatures (Hatai et al. 1990; Oláh and Farkas 1978).

Temperature also had pronounced effects on the growth and development of the larval Sauger, with more rapid increases in size, depletion of yolk reserves and transitions to exogenous feeding at the higher temperatures. Interestingly, the larvae in the fluctuating temperature treatment grew faster/larger than did those in the steady temperature treatments. Average standard length was greater on all days in the diel treatment compared to other treatments. This increased growth may have resulted from greater metabolic efficiency resulting from the synergism between the timing of feeding and temperature. Brine shrimp were only offered during the day, which corresponded with warmer temperatures in the diel treatment (peak temperature reached at 18:00 hrs). Temperature then decreased throughout the night, lowering metabolic demand and the necessity to feed until morning when it began to rise again at 06:00 hrs. This temperature regime may have presented an energetic advantage compared to larvae in warmer static temperatures, who likely needed almost constant feed availability to exceed basic

metabolic demands and experience rapid and consistent growth. This observation is further supported by the greater mortality rate experienced in the 21°C and 24°C treatments, compared to the diel treatment, following the disappearance of yolk and start of exogenous feeding. This suggests that larvae in these treatments operated closer to their energetic limit where a delay in finding live feed could have lethal consequences. If we would have consistently provided live feed throughout a 24-hour period, as is sometimes done with "green water culture" (Stuart and Drawbridge 2011), growth and survival may have improved in these treatments, though we did provide *ad libitum* rations of larval brine shrimp during the day.

Larval Sauger in the 12 and 15°C treatments experienced considerable mortality before the onset of exogenous feeding was reached. We did not consistently inspect all mortalities for the presence of yolk but observed that some had yolk remaining while others did not. This suggests that mortality may have resulted from direct physiological effects of cold temperatures for some individuals and through starvation for others. Interestingly, 12 - 15°C was near optimal for the development of Sauger embryos, yet this thermal niche did not translate to the post-hatch larvae. This suggests that Sauger thermal optima show ontogenetic shifts and considerable warming is beneficial in the period between incubation and prolarval stages. This is corroborated by the findings of Busch et al. (1975), who found that year class strength in the closely-related Walleye is correlated with the rate of spring warming.

Koenst and Smith Jr. (1976) reported an optimal temperature range from 15 - 21°C for Sauger larvae. Our results are similar, but we suggest that the optimal range is between 18 - 24°C because larvae in the 15°C treatment experienced considerably lower survival before exogenous feeding commenced (80%) and fed poorly after its onset. Prolarvae in the 18°C treatment also exhibited statistically lower survival to the onset of exogenous feeding (95%), compared to those reared at 24°C (98.7%), though we would not consider this biologically significant. Further,

larvae within the 18°C treatment exhibited high growth, suggesting they fed well, and may have maintained high survival to the postlarval stage. Our proposed optimal range is nuanced by the fact that suitable forage must not be limiting following the onset of exogenous feeding. Our data clearly indicate that at warmer temperatures, rapid mortality can result if the availability of zooplankton of the right size and quantity is limited.

In addition to differences in survival and growth, temperature drastically modified the timing of hatch and rate of development. Rate of development through embryogenesis and prolarval stages is of critical importance to eventual recruitment to the population as timing of these events is inextricably linked with other ecological variables, as is shown in Figure 1-12. Prolarvae developed much faster both in terms of growth and rate of yolk absorption as rearing temperature increased. Based on our observations, faster development is a large advantage for prolarvae. Smaller, less developed larvae were vulnerable to cannibalism by their larger counterparts and would be gape limited in terms of the size of prey they could consume. In the wild, cannibalism and intra-specific competition may serve as a density-dependent mechanism, impacting recruitment, especially if appropriate forage is limiting (Jonas and Wahl 1998). Additionally, faster growth may promote survival in larval Sauger by helping them to exceed the gape limits of other predators.

As predicted, temperature accelerated hatching and development in both experiments. The inverse relationship between average temperature and TU's necessary to reach peak hatch and exogenous feeding suggests that monitoring TU's to predict hatch timing may vary depending on how cold or warm the average conditions are. We suggest using our proposed regressions (Figures 1-7 and 1-10) to predict the timing of critical stages in development (hatch and exogenous feeding).

In addition, Sauger hatch duration was inversely correlated with temperature, with highly synchronous and short duration hatch timings under warmer conditions. This may represent an evolutionary adaptation to exploit beneficial conditions, mediated through warmer temperatures (e.g., zooplankton blooms). Conversely, when faced with lower environmental temperatures, it may prove more adaptive to exhibit less synchronous hatching to increase the probability that a subset of a cohort will encounter favorable conditions — this is essentially a form of thermal bet hedging.

Interestingly, despite the use of careful husbandry techniques, hatch percentages were consistently low, which is similar to the results of past WGFD spawn operations as reported in Hochhalter (2015). The timing of egg mortality suggests that that some eggs were not successfully fertilized or that there is a critical stage during early embryogenesis where Sauger are susceptible to high mortality. Unfortunately, we did not evaluate fertilization percentages during this study, though our experience with laboratory-spawned Sauger suggests that there may have been wide variability in fertilization rates between individuals (See Chapter 2).

The pattern of early egg mortality matches what is frequently reported in the literature (Heidinger et al. 1997; Kamler 2005). For example, Latif et al. (1999) observed 80% of the total mortality in Walleye (*Sander vitreus*) eggs occurred at 50-100 hours after fertilization. The authors concluded that this timing corresponded with the transition of germinal layers to organs and could be a sensitive period in embryogenesis. While some early mortality may be common, the overall magnitude of mortality we observed seems to be unusual for Sauger culture, especially when compared to the congeneric Walleye, where it is common for Colorado Parks and Wildlife to have survival to hatch exceeding 80%. We believe that high embryo mortality may be associated with other poorly understood factors, potentially contributing to compromised gamete quality. Because reproductive physiology is a product of a diverse array of intrinsic and

extrinsic factors, it is inherently complex to troubleshoot. Despite low hatch percentages, we successfully executed both experiments, and can offer valuable insights about the early life history for these populations of Sauger.

Ecological Relevance

The primary objective of our experiment was to investigate whether temperatures outside of optimal conditions could directly increase mortality in embryos and prolarvae. While we did indeed detect a direct mortality effect, we found that both early life stages are somewhat resilient to a wide range of temperatures, and only extreme conditions may cause direct mortality in nature. It may be most relevant for managers to consider how temperature, within sub-lethal ranges, modifies survival through its synergistic effects with rates of development and numerous other biophysical variables (Engel et al. 2000; Koonce et al. 1977). We consider a variety of established recruitment hypotheses and consider how temperature may interact to drive interannual variability in Wyoming Sauger populations.

Critical Period and Match-Mismatch Hypotheses: First proposed by Hjort (1914), the Critical Period Hypothesis states that mortality is highest and most variable for the egg and larval stages of fish. This variability is decisive in dictating annual recruitment success. Within these life stages, the most sensitive interval, known as the "critical period", is when larvae utilize all their yolk and are constrained to feed exogenously. If adequate feeding does not take place, high mortality can occur (Figure 1-13) and consequently, poor year classes may development (Houde 2016). Our larval Sauger results support the hypothesis of a "critical period" as mortality rates increased drastically in all treatments following the absorption of yolk, likely due to starvation. Similarly, Li and Mathias (1982) observed high mortality of cultured larval Walleye in the period between yolk and oil globule consumption. While ecological and artificial culture conditions differ drastically, we expect that a "critical period" exists in wild Sauger populations

and may help to explain variable recruitment strength. Our research demonstrates that warmer temperatures may compress the critical period by forcing larvae to transition to external food resources quickly or face starvation. In contrast, cooler temperatures extend endogenous reserves and may prolong the critical period, lending more time for completing the transition to exogenous feeding before starvation occurs. However, at cold temperatures we observed dysfunctional feeding behavior and evidence that starvation was still a major source of mortality (albeit more delayed), suggesting a tradeoff may exist.

Building upon Hjort's Critical Period Hypothesis, Cushing (1990) proposed the Match-Mismatch Hypothesis, predicting that recruitment of fishes largely depends upon the degree of synchronicity between plankton production and the timing of exogenous feeding in fish larvae (Figure 1-14). Cushing observed that plankton blooms were more temporally variable than the spawn timing of many fishes, likely because spawn timing is largely regulated by photoperiod (consistent between years), with temperature exerting proximal controls (Houde 2016). This dynamic can create conditions whereby zooplankton are abundant throughout the critical stage of larval development leading to adequate growth and strong recruitment—a match. In contrast, if the timing of the zooplankton bloom does not strongly coincide with the onset exogenous feeding in larvae, high mortality can result leading to poor recruitment, or complete year class failure—a mismatch. Larval Sauger rely on zooplankton as their primary food source (Nelson 1968b) and are likely dependent upon plankton production timing and transport to areas with favorable forage densities. Temperature plays a critical role in this phenomenon as it controls the timing of plankton blooms, larval development rates, and timing of exogenous feeding. Under natural conditions, it is likely that Sauger evolved to maximize temporal and spatial overlap between larvae and zooplankton; in anthropogenically modified systems these events may be decoupled, leading to changes in recruitment success. Further investigation is necessary

to see if this relationship may be significant in the Wind and Bighorn River systems of Wyoming.

In addition to the Critical Period Hypothesis, Hjort (1914) proposed a second hypothesis suggesting that recruitment is influenced by the transport of eggs and larvae into favorable or unfavorable environments. Sauger prolarvae are relatively weak swimmers and are dispersed easily by currents (Bozek et al. 2011). Flow regime, characterized by the magnitude, frequency, duration, and timing of flow (Poff et al. 1997) likely has ramifications for transporting and entraining Sauger embryos and larvae in variable habitat types where survival may increase or decrease. In the Wind and Bighorn River populations, larval Sauger are likely transported into reservoirs (Boysen and Bighorn) below lotic spawning sites and may be entrained in shallow, littoral areas near the inlet. In years with large spring floods, larvae may be transported quickly to these favorable nursery areas, potentially reducing the risk of starvation, predation, and cannibalism leading to better recruitment. Roseman et al. (2005) found that currents in Lake Erie tend to concentrate zooplankton and larval Walleye in similar locations, providing good forage opportunities. Transport to areas of low zooplankton density may lead to increased incidence of predation and cannibalism (Jonas and Wahl 1998). This biophysical coupling likely influences Sauger recruitment in the Bighorn/Wind drainages through similar mechanisms.

Reservoir management may strongly influence nursery habitat. During favorable water years, reservoir levels are higher, providing quality littoral habitat where conditions may be more compatible for larval growth and survival. In contrast, low reservoir levels may be associated with minimal littoral habitat, leaving larvae vulnerable to multiple sources of mortality including starvation, predation, and cannibalism. Similarly, large fluctuations in reservoir levels and or high exchange rates may negatively influence recruitment (Walburg 1972). Transport dynamics may interact strongly with temperature as warmer temperatures will cause faster growth and

development leading to improved swimming ability and selection of favorable environments. In our study, we observed earlier formation of the swim bladder at warmer temperatures. This physiological event causes larval Sauger to become positively buoyant and more active. This may significantly modify transport dynamics under wild conditions.

Stage Duration: Development and growth are inextricably linked with temperature, with warmer temperatures associated with shorter stage durations and faster growth. Among 38 populations assessed, total length of age-one Walleye was positively correlated with growing degree days (Bozek et al. 2011). As supported by our findings, colder temperatures suppress egg and larval development rates which could subject embryos to sources of mortality for longer time periods such as predation, abrasion, or transport to unsuitable areas (Bozek et al. 2011). Similar to most fishes, early feeding in larval Sauger is regulated by gape limit. Galarowicz et al. (2006) found that larval Walleye shift their diet throughout development and select food items that are most profitable for growth. As observed in our larval Sauger study, larvae will attempt to consume their counterparts at the absolute limits of their gape extent (Figure 1-15). This highly predatory nature suggests that wild Sauger exhibit opportunistic piscivory even as larvae, though zooplankton and other invertebrates may also remain important prey items (Chipps et al. 2011). With changing size and forage dynamics, Sauger also exploit different habitats. This shift corresponds to physiological changes in retinal structure in *Sander spp.* (Ali et al. 1977). Specifically, the development of the tapetum lucidum increases scotopic vision and may help Sauger to utilize darker habitats, avoid predators, and exploit other forage resources. In Walleye, this reflective retinal layer is present at 37 mm in length but may not be fully developed until 140 mm (Bozek et al. 2011).

Because Wind and Bighorn River Sauger exist at the highest elevations within the species (Amadio 2003), they may be subject to highly variable temperatures between years and

therefore variable stage duration lengths. Temperature-mediated changes in stage duration may explain variable recruitment success, especially when interactions with other biophysical factors are considered.

Management Implications

Environmental Monitoring: Our research provides a variety of thermal criteria that managers may use to help predict recruitment in wild Sauger populations. Peak spawn date may be estimated using a ratio of spawned (spent) females to gravid females. By monitoring temperature following the peak spawn date, a reasonable estimate of average hatch timing may be derived. Specifically, peak hatch likely takes place in the range of 150-220 daily temperature units after fertilization, depending on specific conditions. If average daily temperatures are near 14.5°C, hatch will take place at approximately 180 temperature units (about twelve days). Prolonged periods of cold (<12°C) during incubation may inhibit overall hatch percentages. These conditions will also drastically slow the rates of development, prolong hatch duration, and may take closer to 220 daily temperature units to reach peak hatch (16-22 days). Conversely, it may only take 150 daily temperature units (7-8 days) to reach peak hatch if average conditions are near or above 19°C. We recommend monitoring river temperatures following spawning and determining average temperature over the incubation period. Using our developed logarithmic relationship (Figure 1-7), average temperature may be used to predict the approximate TU's necessary to reach peak hatch. Based on estimated TU's to reach peak hatch, a reasonable estimate of hatch date may be back calculated.

Large diel fluctuations may also inhibit hatching, especially if the upper limits meet or exceed 22°C. We believe that fluctuations of similar magnitude (5°C), but lower average are less likely to negatively impact embryos. While we did not directly test this assertion with

Sauger, Schneider et al. (2002) found no impact in hatching of Walleye eggs subject to temperature swings of 14°C. Because of their physiological similarities with Walleye, Sauger embryos are likely resistant to relatively large diel fluctuations, so long as they stay within thermal tolerance limits. It is possible that specific stages of embryogenesis (e.g., organogenesis) are more sensitive to fluctuating temperatures, while other stages are largely unaffected. Further research on the effects of diel fluctuations during embryogenesis may help to support or refute this prediction.

Similar to predicting average hatch timing, a reasonable estimate of the time to reach exogenous feeding may be produced by monitoring environmental temperatures. We used the time that 50% of subsampled larvae had no remaining yolk as a proxy for the start of exogenous feeding in each respective treatment. These values may serve as a guideline for predicting the time that most larvae may be constrained to feed exogenously, which may represent a "critical period" in Sauger recruitment. Time to reach exogenous feeding may be variable and is influenced by temperature dynamics and activity patterns of larval Sauger. Based on our results, exogenous feeding may commence anywhere between 95 and 164 TU's after hatch, depending on average thermal conditions. Similar to our Sauger hatching results, our larval Sauger data suggest that TU's to reach exogenous feeding are inversely related with average temperature. This may be due to the synergistic effects imposed by increasing temperature whereby both standard metabolic rate and activity increase at warm temperatures. After computing a reasonable estimate of average temperature during the prolarval stage, we recommend using the logarithmic equation we developed (Figure 1-10) to estimate TU's after hatch to reach exogenous feeding. With this information, a reasonable date may be estimated for the onset of exogenous feeding for each year class. While we have illustrated that TU's to reach exogenous feeding are dependent on average temperature, available historical data suggests that average

temperatures in the Wind and Bighorn drainages during the prolarval stage are rarely below 16°C. In the future, cold average conditions may become even more rare in the face of warming climate. Under this assumption, managers can expect exogenous feeding to take place in the range of 75-92 TU's. Timing of development could be combined with estimates of zooplankton abundance, analyses of transport dynamics and characterization of habitat availability to model recruitment strength. Drift net sampling and inspection of larvae may be used to corroborate our estimates of hatch timing and onset of exogenous feeding. Prolonged periods of cold (<15°C) during the prolarval stage may directly increase mortality and cause larvae to feed poorly, leading to starvation.

Aquaculture: Our findings may help to increase hatchery efficiency and maximize the production of viable Sauger for supplemental stocking. Because eggs within the Wind/Bighorn populations exhibit poor and variable survival, all precautions must be taken to optimize artificial spawning procedures. Similar to Koenst and Smith Jr. (1976), we suggest that 14.5°C is near optimal temperature for incubating Sauger eggs, although temperatures in the range of 12-19°C may not greatly impair hatch percentages. At optimal temperature, peak hatch will likely commence around 180 TU's (12 days). Warmer incubation temperatures will shorten the duration of hatch (i.e. time from first to final hatch) and may help to streamline production. At optimal temperature, hatch duration should last for approximately four to five days. Based on our results, fluctuations should be minimized, especially in scenarios where temperature rises above 20°C. Further research is warranted to see if fluctuations with cooler averages could negatively impact hatching in Sauger eggs, though we anticipate little effect on hatching if fluctuations remain within the range of 12-19°C.

Despite daily treatments of eggs with H_2O_2, we observed a considerable increase in fungus (e.g., *Saprolegnia spp*.) at warmer temperatures treatments (>17°C), especially in the diel

treatment. Because we used low-volume incubation jars (500 mL), we opted to use static H_2O_2 treatments for the sake of simplicity. Flow-through treatments may have been more effectual, as active chemical concentrations may have remained higher using this method (David Harris, CPW, personal communication). Because fungal infections can significantly increase mortality, we recommend daily flow-through treatments using H_2O_2 or formalin until eggs reach the eyed stage. This is especially relevant at temperatures greater than approximately 17°C.

Upon hatch, larval Sauger should be slowly transitioned to warmer temperatures to avoid temperature shock. Optimum temperatures for prolarvae are likely within the range 18-21°C. They will tolerate and may even thrive at warmer temperatures (up to 24°C), given adequate available forage. It is likely that maximum consumption and growth will be realized at temperatures near the physiological tolerance of this species and thus warm temperatures should be used cautiously. Additionally, at warmer temperatures exogenous feeding is quickly reached and rapid starvation may ensue if larvae are unable to transition to feed. In our study, many larvae began eating zooplankton before they had completely absorbed their yolk sack, similar to the findings of Nelson (1968a). To ensure that larvae have time to behaviorally adapt to feeding and that early developing larvae do not starve, zooplankton should be presented during early larval stages and provided at all times of the day, perhaps by using green water culture techniques. We used *Artemia nauplii* (brine shrimp) in our experiment and witnessed many larvae effectively feeding in our warmer treatments (>15°C). Some larvae in our 15°C treatment fed during the end of the experiment, though it was highly variable among individuals. Exogenous feeding in the 12°C treatment was close to non-existent. Experimenting with smaller food items such as rotifers may have helped to stimulate feeding at colder temperatures if gape limit was inhibiting effective feeding, though we recommend avoiding colder temperature altogether for prolarvae when possible. Across all treatments, we observed that larvae

preferentially fed on actively swimming brine shrimp and did not feed well once brine shrimp had died and settled to the bottom. Offering freshly-hatched brine shrimp, frequently throughout a 24-h period would likely optimize feeding for larval Sauger. Aquaculture operations should take special care to optimize larval and associated zooplankton densities, as cannibalism becomes problematic in early ontogeny.

Conclusion

Spawning stock size often fails to adequately describe trends in recruitment in many fishes and Sauger are likely no exception. Sauger exhibit high fecundity (33,000-106,000 eggs/kg) (Carlander 1997), and average survival to maturity is likely extremely low, even during successful years of recruitment. An analysis of Walleye populations concluded that survival from egg to age-one is on the order of 0.01% (Baccante and Colby 1996). Because of the tremendous numbers of potential recruits, survival of early life stages plays a critical role in establishing year class strength. This recruitment is influenced by a diverse combination of biological, physical and chemical mechanisms, all of which can be affected by temperature through direct and indirect pathways. Under optimal conditions, relatively small numbers of spawners may establish strong year classes of Sauger. In our laboratory study, we isolated temperature and its direct effects on the survival and development of embryos and prolarvae. Our findings suggest that direct mortality may increase under extreme thermal scenarios—conditions that may become more common in the face of climate change and other anthropogenic modifications. Perhaps more importantly are the ways in which temperature controls physiological rates of development and modifies forage dynamics, activity levels, predator/prey interactions and effects of pathogens. While more challenging to investigate, these complex interactions may influence growth, stage duration, and transport dynamics and may help to explain variable mortality rates of early life stages. Understanding recruitment in natural

systems is a complicated, lengthy and often frustrating endeavor. Our research provides a small, yet important informational component, to aid managers in understanding how temperatures may impact natural reproduction and artificial propagation dynamics of this unique native species.

TABLES

Table 1-1.— WGFD criteria used to assess reproductive development in Sauger during artificial spawn-takes.

Category	Indicators
Firm	Abdomen firm; ovipositor not extended
Intermediate	Abdomen moderately soft; ovipositor not extended
Intermediate-Soft	Abdomen moderately soft to soft; ovipositor moderately extended
Soft	Abdomen soft; ovipositor fully extended; may express eggs, but eggs do not flow freely when pressure is applied to abdomen
Ripe	Abdomen soft; ovipositor fully extended; eggs flow freely when pressure is applied to abdomen

Table 1-2.— Time and temperature units (TU's) to reach exogenous feeding for each treatment, as defined by the day that at least 50% of subsampled larvae had no visible yolk remaining.

Treatment (°C)	Time to exogenous feeding (experimental days)	TU's post-hatch to exogenous feeding (°C)
12	13	164
15	8	134
18	4	86
21	3	75
24	3	75
17-22	4	92

Table 1-3.— Proposed optimum temperatures based on survival for various early life stages of Sauger.

Life stage	Optimum temperature (Koenst and Smith 1976)	Optimum temperature (present study)
Fertilization	9-15°C	Not tested
Incubation	12-15°C	12-19°C
Larvae	15-21°C	18-24°C

FIGURES

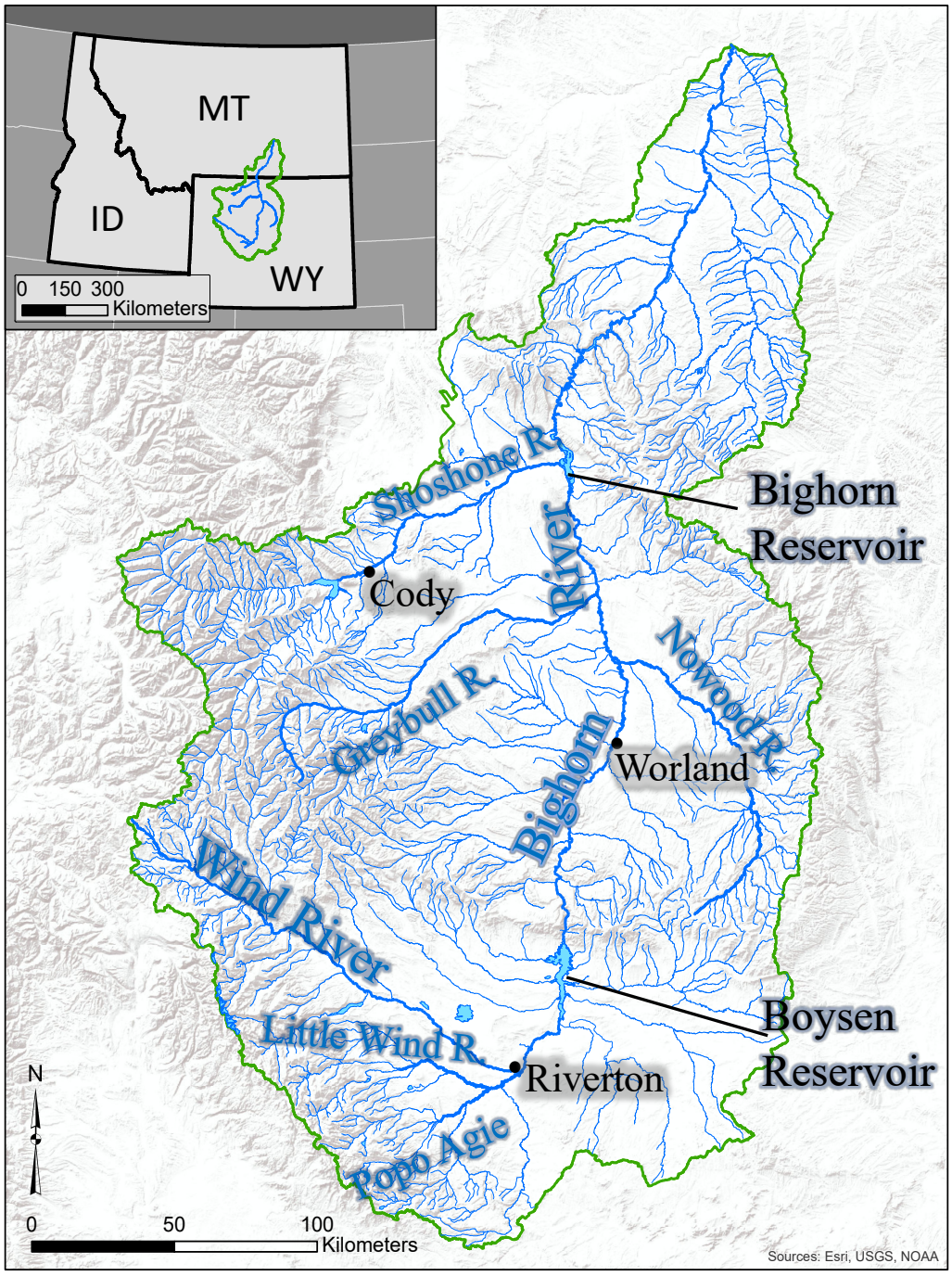

Figure 1-1.— Map of the Wind and Bighorn River system of Wyoming where Sauger populations of interest for the present study exist.

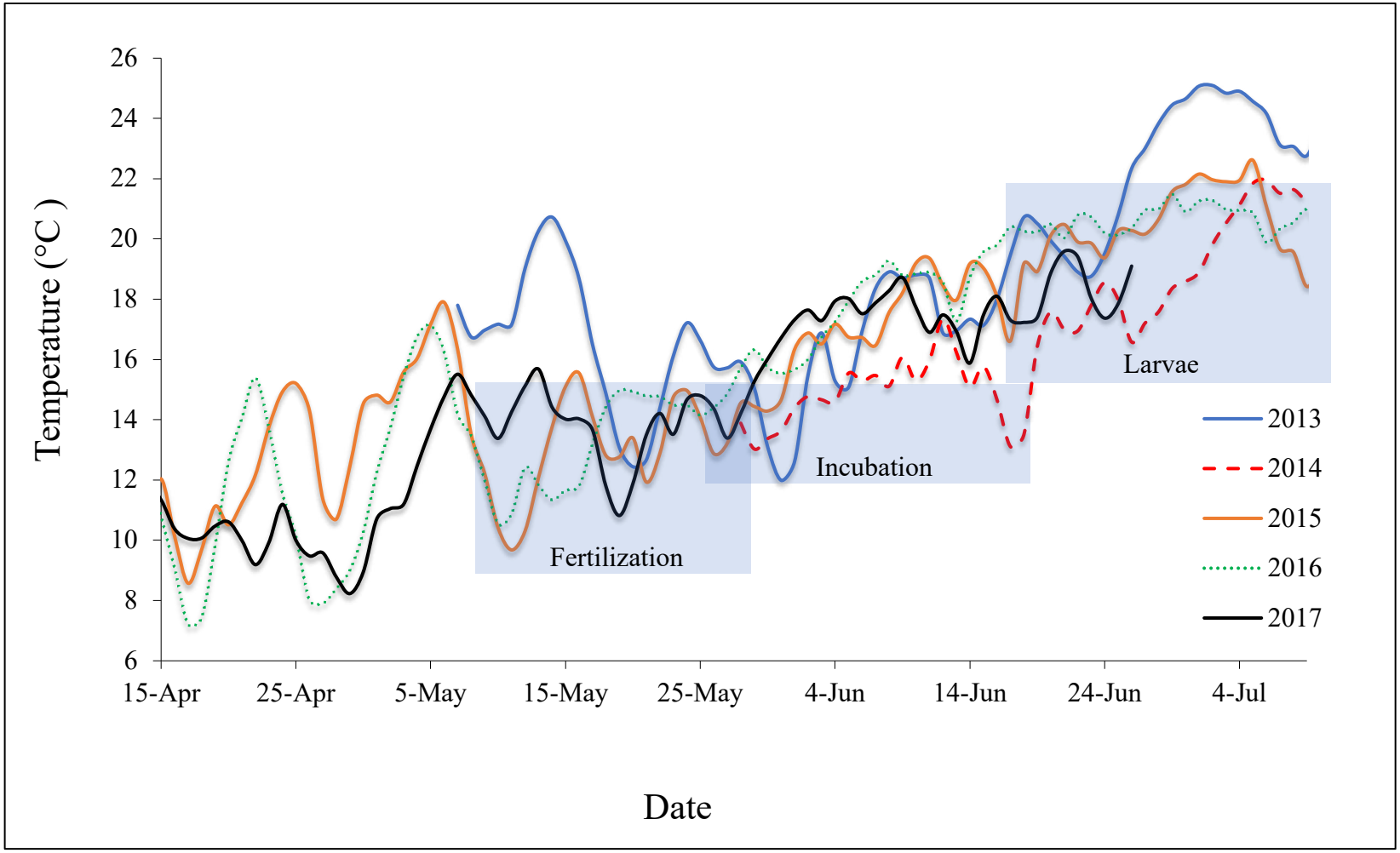

Figure 1-2.— Temperatures on the Bighorn River, near Manderson, WY during the April – July period when Sauger are in pre-spawn, spawn, and post-spawning phases. Boxes indicate the different thermal optimums and timing for various life stages as defined by Koenst (1976). The vertical height of the box defines the thermal optima and horizontal dimension defines approximate timing of each event.

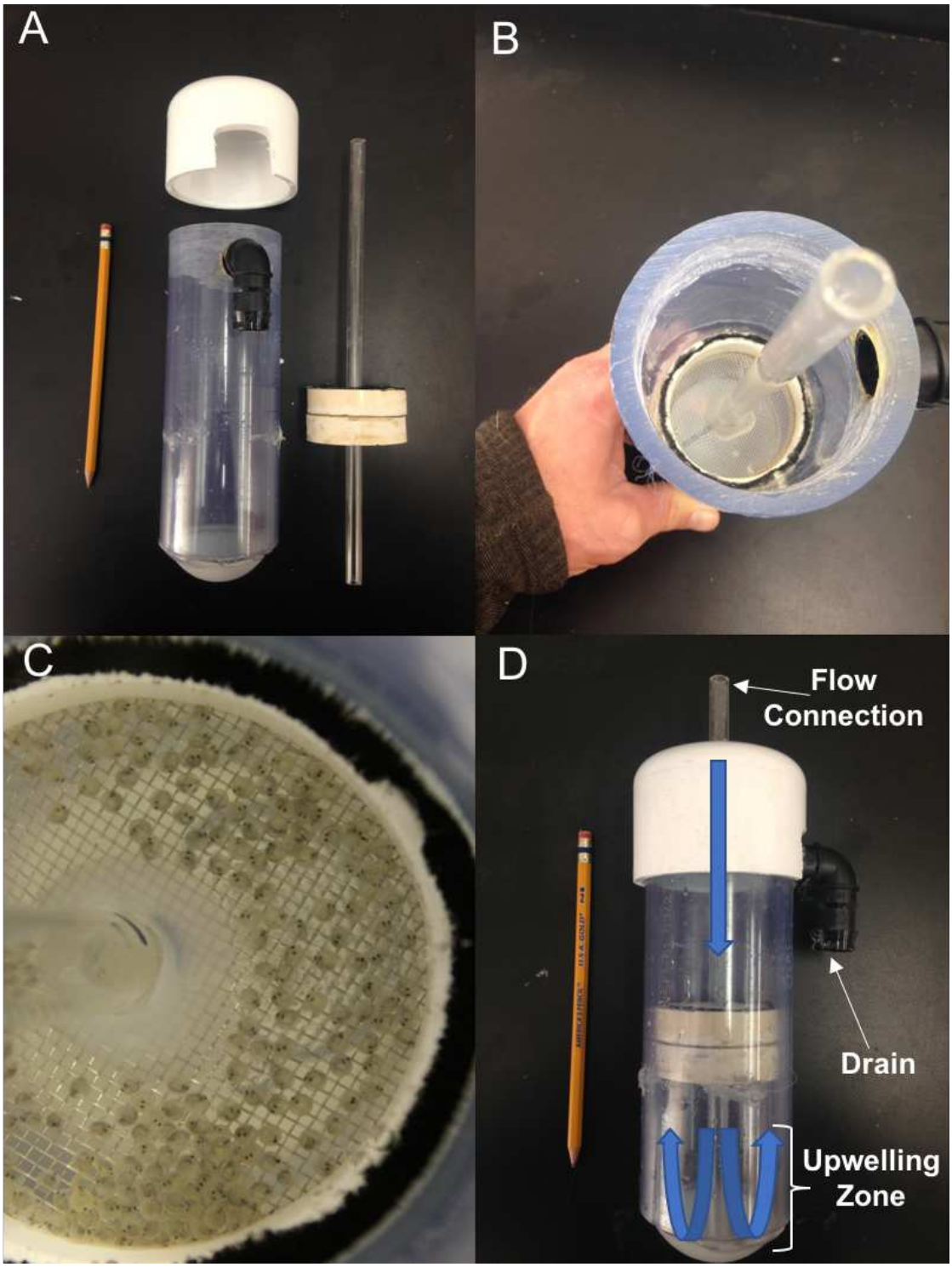

Figure 1-3.— Miniature upwelling incubation Jars. (A) shows individual incubator pieces including lid, jar, and inner disk apparatus (pencil shown for scale). (B) shows top view (without lid) of jar with inner disk apparatus inserted. (C) shows close-up of eyed Walleye eggs sitting on the stainless-steel mesh of the inner disk apparatus. (D) Illustrates fully assembled incubation jar. Blue arrows represent flow direction.

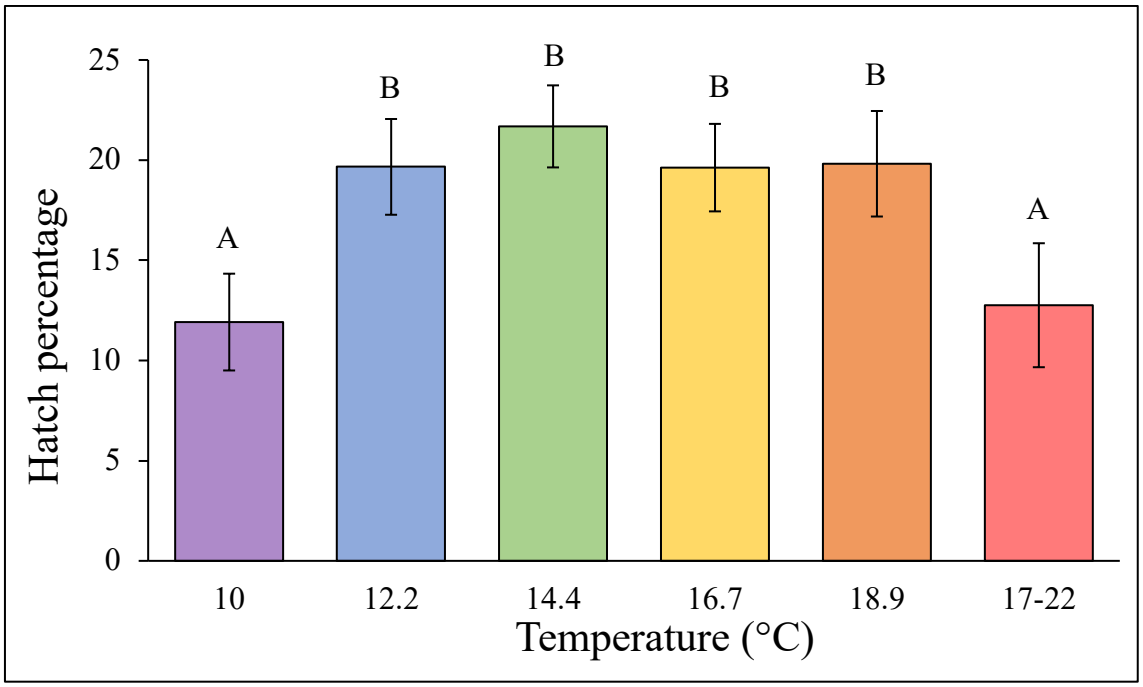

Figure 1-4.— Bar plot of average hatch percentage (± SEM) across replicates within each treatment for the egg incubation experiment. Different letters represent statistical significance.

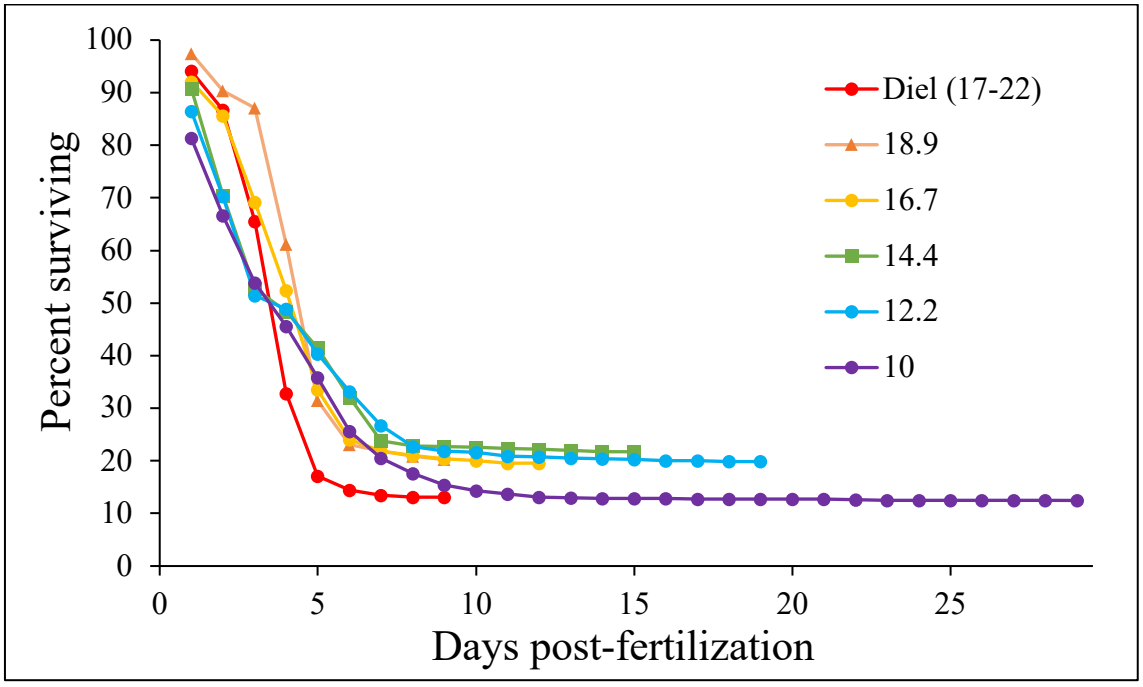

Figure 1-5.— Survivorship of Sauger embryos incubated at steady temperatures of 10 to 18.9°C or under a fluctuating thermal regime (17 – 22°C). Each point represents the average survival proportion across all replicates within a treatment for a given day.

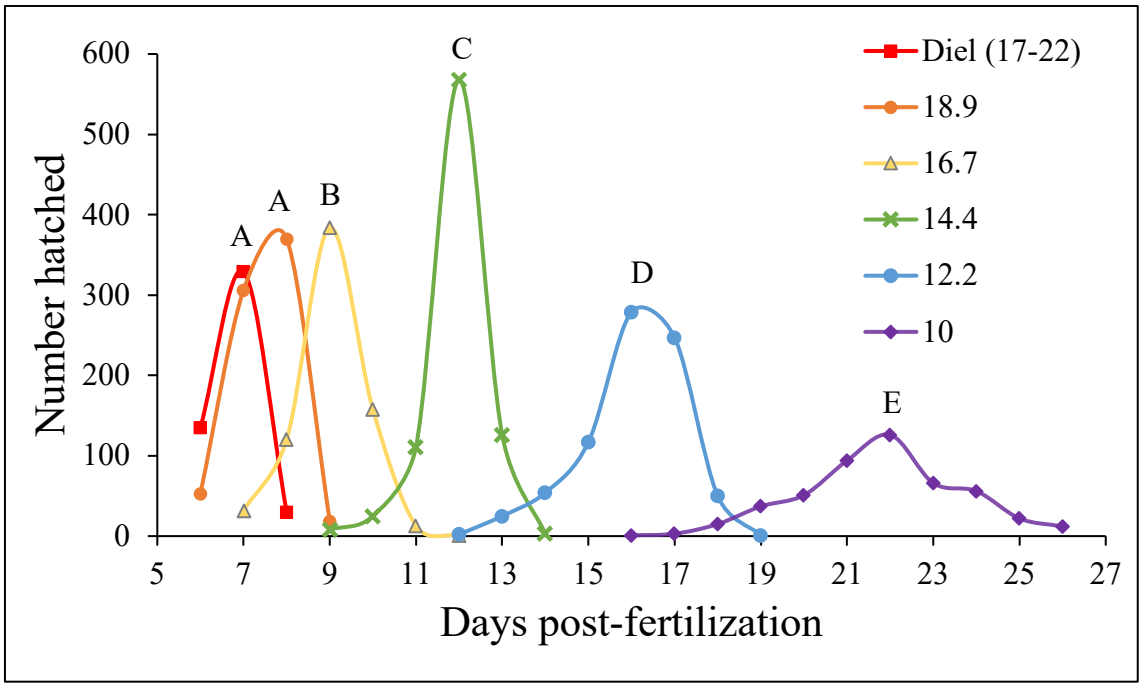

Figure 1-6.— Effects of incubation temperature (°C) on the hatch timing of larval Sauger. Each point represents the cumulative number of larvae hatched across all replicates in a treatment for a given day. Statistically significant differences between times to peak hatch are indicated by different letters. Note that the duration of hatch also varied significantly between treatments.

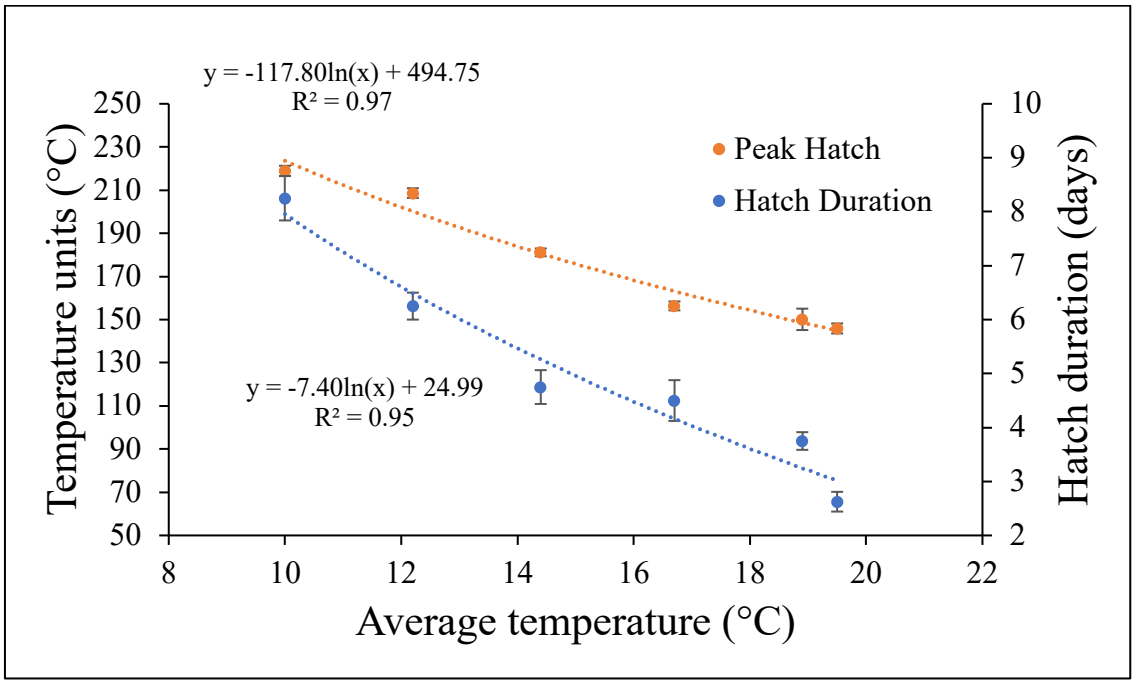

Figure 1-7.— Average temperature units to reach peak hatch(± SEM) and hatch duration (± SEM) among different average temperatures in the egg incubation experiment.

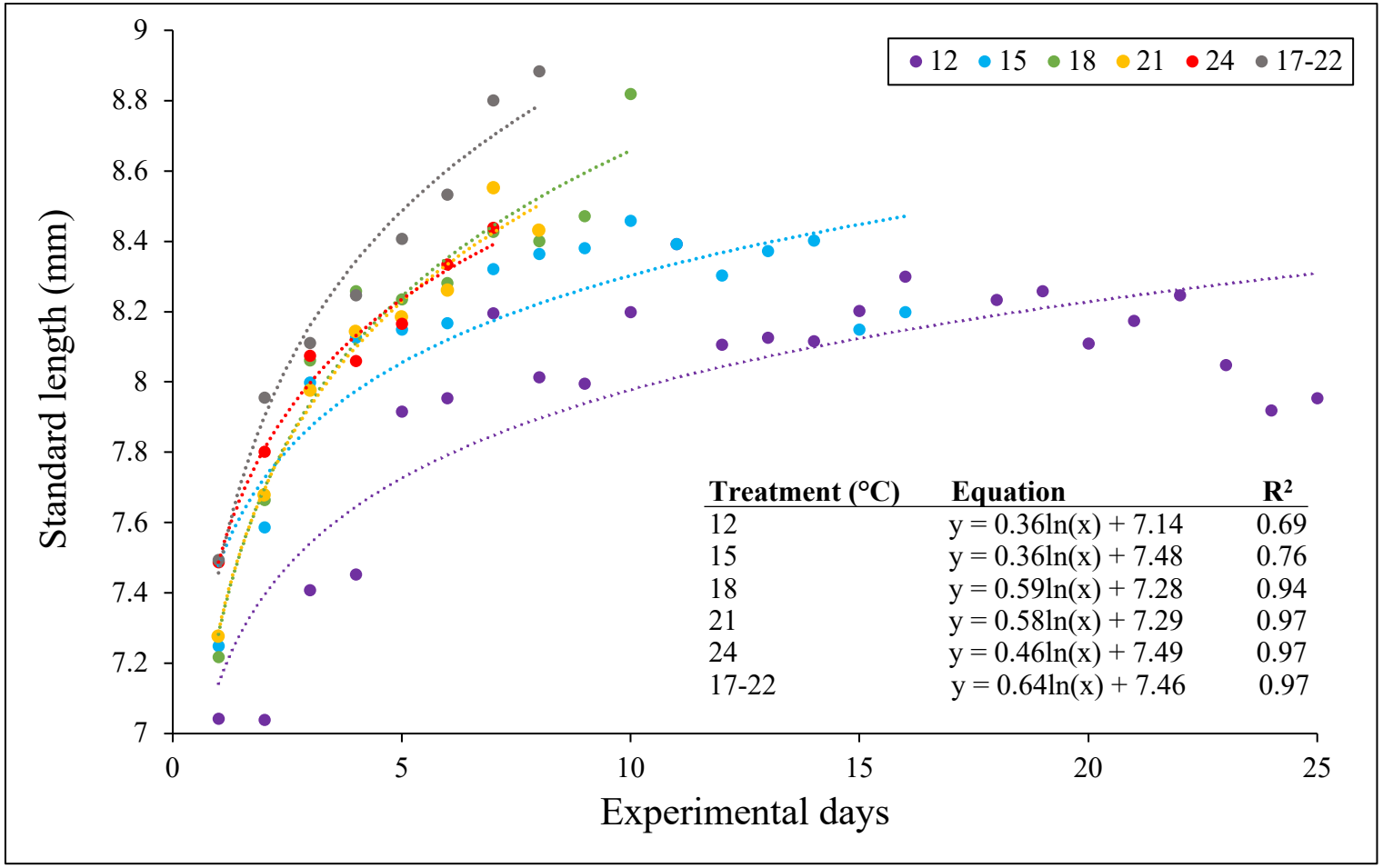

Figure 1-8.— Effects of temperature treatment (°C) on the size (SL) of larval Sauger. Each point represents the mean value of all subsampled larvae in a treatment for a given day. Lines represent logarithmic regressions. Equations and associated R^2 values can be found in provided table.

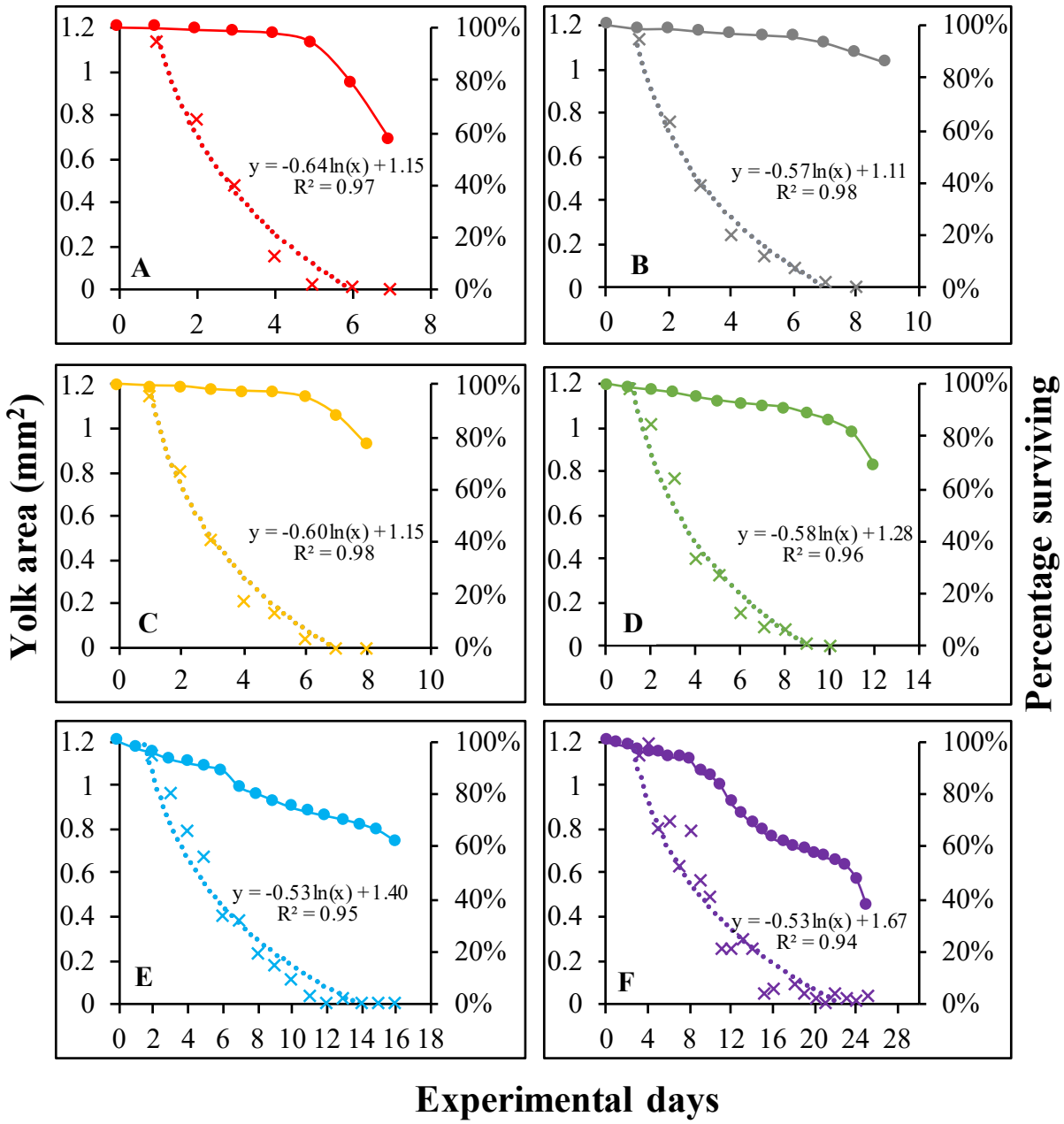

Figure 1-9.— Survivorship and change in yolk area of larvae in all experimental treatments (A: 24°C, B: 17-22°C, C: 21°C, D: 18°C, E: 15°C, F: 12°C). Dots represent the average survival percentage across replicates for each day of the experiment. X's represent the average yolk area of all subsampled larvae for each day of the experiment. Dotted lines and associated equations are logarithmic regressions for yolk area.

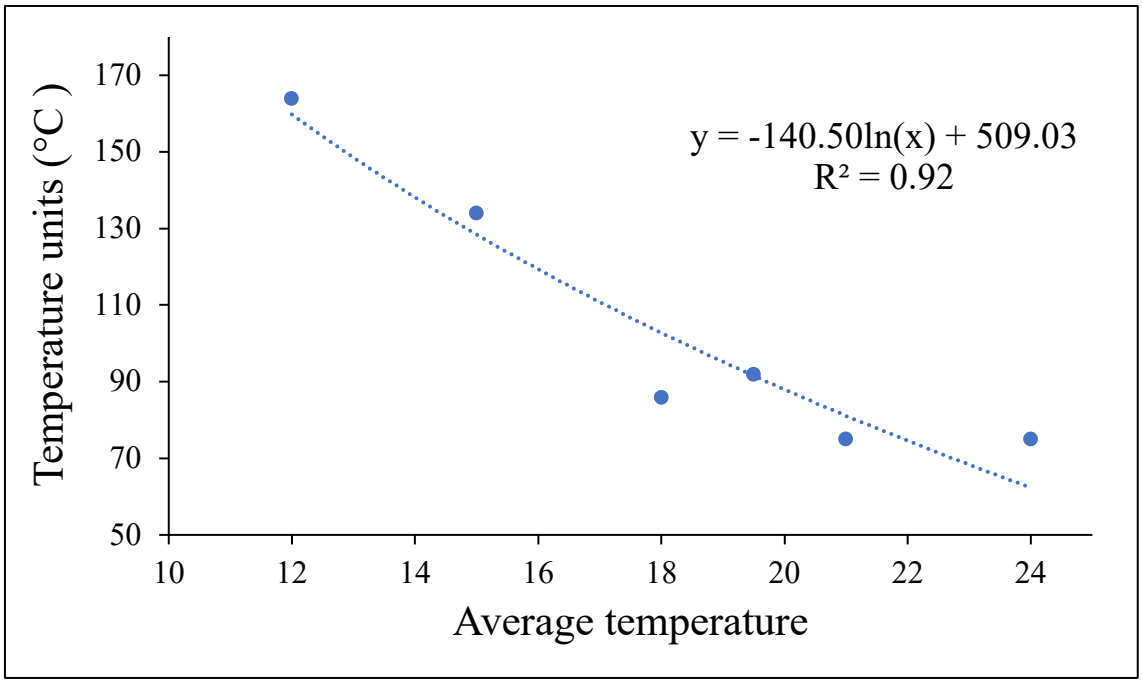

Figure 1-10.— Approximate post-hatch temperature units required to reach exogenous feeding in experimental Sauger larvae as a function of average temperature. Each point was derived by identifying the day that 50% of subsampled larvae had no visible yolk remaining for each respective treatment and calculating the associated temperature units of this day. The line and associated equation is a logarithmic regression of the points.

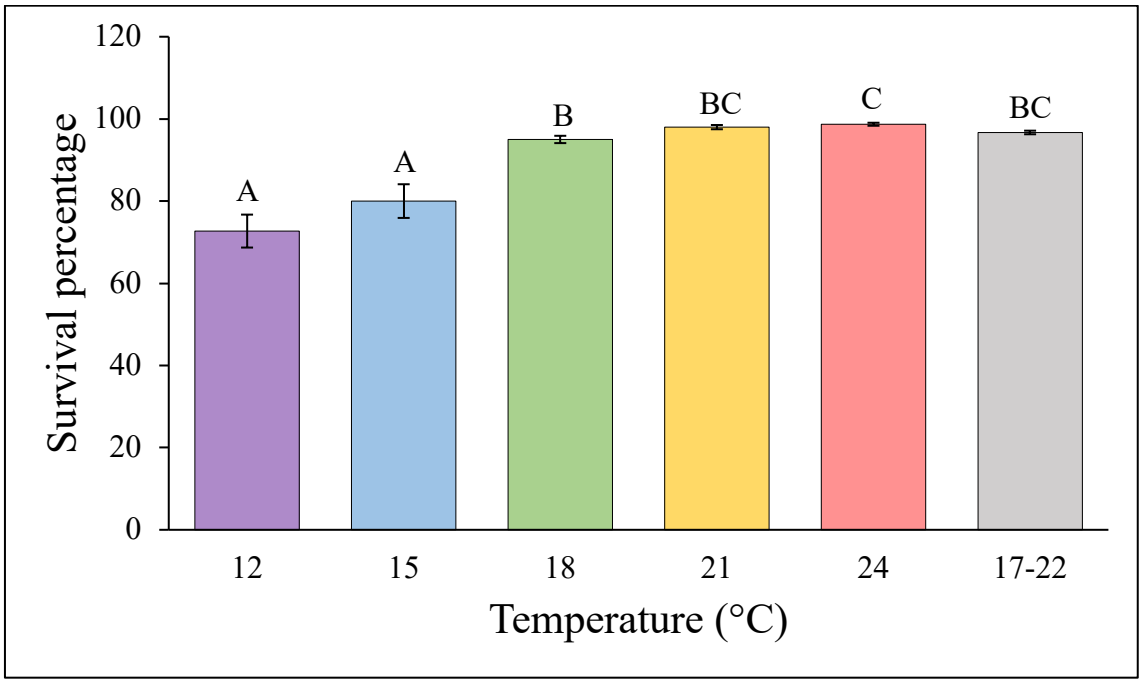

Figure 1-11.— Comparison of the survival of larval Sauger reared at different temperatures to the onset of exogenous feeding (± SEM). Different letters represent statistical significance.

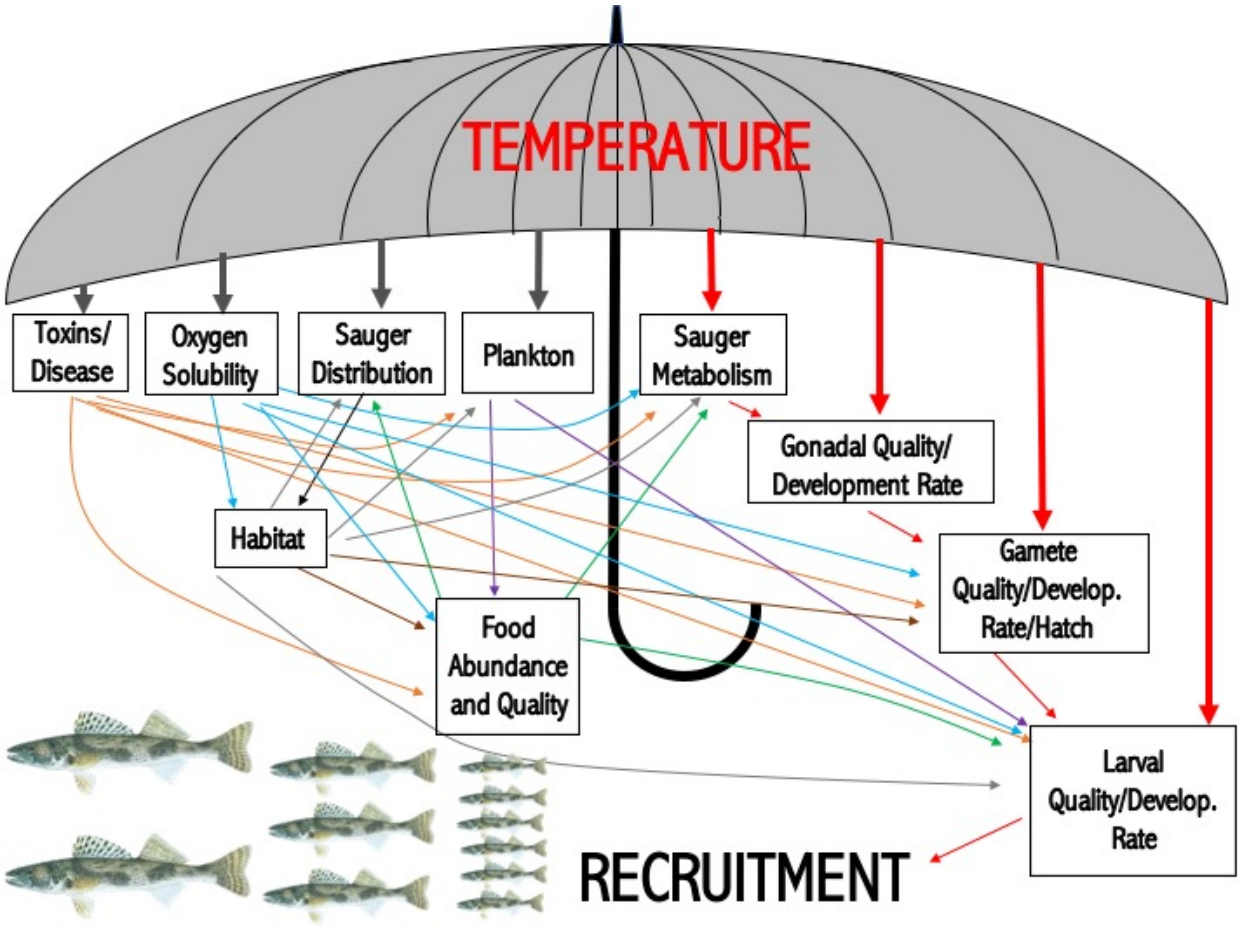

Figure 1-12.— Conceptual figure representing temperature influences on key Sauger recruitment variables. Arrows show the direction of influence. Bold arrows point to variables directly affected by temperature, while thin arrows represent indirect influences. Arrow color is unique for each variable identified. The right side of the umbrella and all red arrows represent variables of particular interest for this study.

Figure 1-13.— Critical period hypothesis, conceptual model. (A) No critical period; constant mortality rate from age 0 to 100 days; (B) Critical period; >90% mortality occurs from starvation during the days at which first-feeding must be established (orange shading). Modified from Houde (2002).

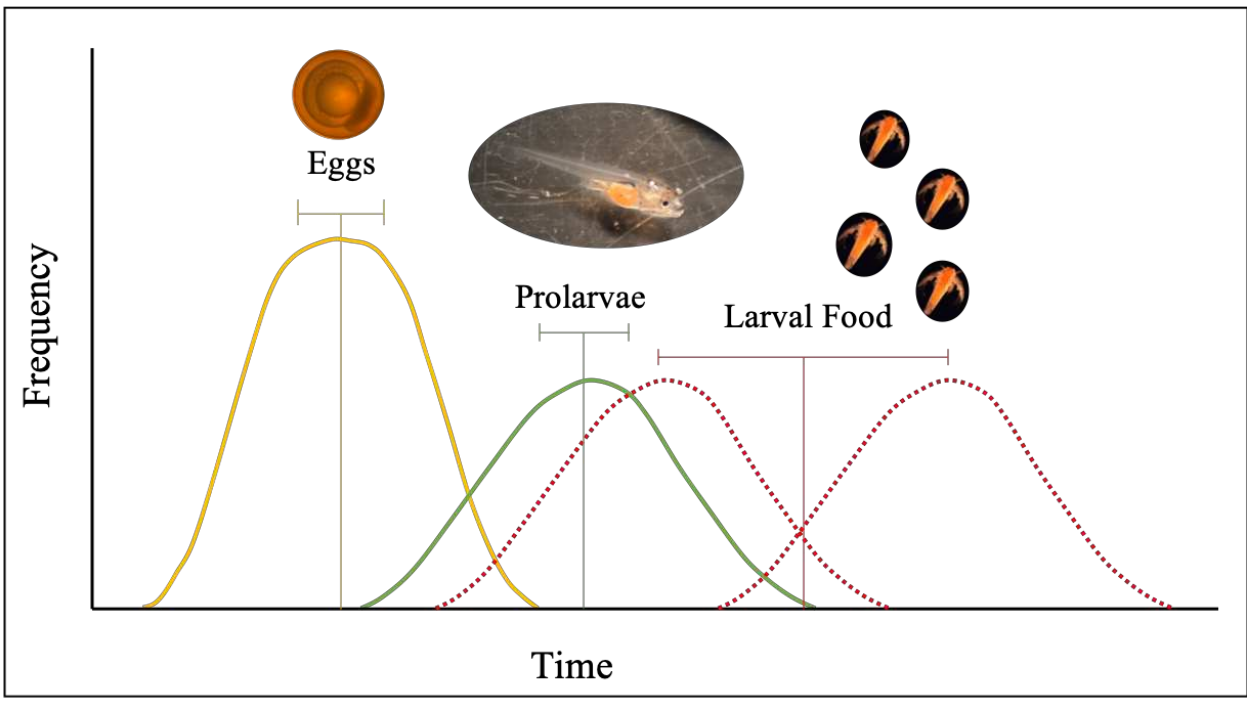

Figure 1-14.— The match/mismatch hypothesis. The production of eggs, larvae and larval food are depicted as distributions in time. The match or mismatch is represented by the overlap in time between the production of fish larvae and that of their food. Error bars express the annual variation of each biological component. (Modified from Cushing 1990).

Figure 1-15.— Larval Sauger (left) attempting to cannibalize its less fortunate counterpart (right). Object above larvae is included for scale and has a width of approximately 1.2 mm.

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CHAPTER 2: EFFECTS OF PRE-SPAWNING TEMPERATURE ON SAUGER REPRODUCTIVE OUTPUT AND SUBSEQUENT HATCH SUCCESS

INTRODUCTION

A desired pinnacle of fisheries management is the ability to understand and predict recruitment, given its complexity and difficulty to study (Cushing 1975; Houde 2016; Ludsin et al. 2014). While vexing, the capability to forecast recruitment is of great interest to managers in their quest to provide quality commercial, sport, and conservation fisheries alike. In many cases, spawner abundance is, at best, only loosely correlated with recruitment—10-fold interannual variability is not uncommon among fish populations (Jakobsen et al. 2016). Fishes in the family Percidae are no exception to this rule, commonly displaying boom or bust recruitment dynamics. For example, Walleye *Sander vitreus* can experience 12 to 74-fold variations in year class strength (Koonce et al. 1977). In the closely-related Sauger, *Sander canadensis* it was shown that no correlation exists between numbers of spring larvae and fall age-0 larvae, suggesting that conditions during early life history are decisive in regulating recruitment (Pitlo Jr. 2002). As in all populations of fish, this reproductive variability is largely attributed to a suite of abiotic and biotic factors that strongly influence the spawning and early ontogeny of fishes.

While there are numerous other factors regulating reproduction in Percids, evidence suggests that thermal conditions are strongly correlated with year class strength (Hokanson 1977; Koenst and Smith Jr. 1976; Koonce et al. 1977). Temperature may influence reproductive success via direct pathways (i.e., physiological and physicochemical effects) and indirect pathways such as food abundance and habitat availability (Figure 2-1). Regardless of the pathways these effects are modulated through, temperature can influence every stage in the

reproductive process from adult spawners, to eggs, to juveniles. It is generally reported that gradual warming during incubation and larval development correlates with strong year classes in Percids. Busch et al. (1975) found a significant correlation between year class strength of Walleye in Lake Erie and the rate of warming during incubation and larval development.

Percids, classified as a cool-water species, are geographically widespread due in part to their high degree of thermal plasticity (Hokanson 1977; Kitchell et al. 1977). Despite their broad thermal tolerances, extreme fluctuations in temperature and/or temperatures that depart the optimal ranges could potentially affect reproduction and population stability. These effects may become more common in the face of extreme conditions predicted to occur with global climate change (Allen et al. 2018), and in anthropogenically modified thermal regimes that result from dams, habitat degradation, and water extraction (Whitney et al. 2016). This is further complicated by widespread losses in connectivity due to artificial barriers, whereby fish may be unable to migrate to areas with ideal thermal conditions.

Sauger are native fish of special concern and an important sportfish in the Wind and Bighorn Rivers of Wyoming and, as such, are monitored closely. The Wind River population has experienced protracted declines in abundance, especially of young age classes, suggesting that in certain years, a recruitment bottleneck has been in operation. The Bighorn River Sauger population has remained robust, though supplemental stocking of Sauger was initiated in attempt to enhance the sport fishery within Bighorn Reservoir. For both Wind and Bighorn systems, fisheries managers undertook artificial spawning operations to produce larval Sauger in hatcheries for stock enhancement purposes. These operations were hampered by highly variable, but generally low survival of eggs and larvae (Table 2-1). Years of low wild recruitment and poor hatchery production of fry were accompanied by river thermal regimes that were highly variable and often outside of what is considered optimal temperature (Figure 2-2). One

hypothesis advanced to explain the poor wild recruitment and hatchery production during this period was that temperatures leading up to the spawn were adversely influencing gamete quality, leading to depressed numbers of viable fry.

While a large body of research has focused on effects of temperature on egg and larval stages of fish, less emphasis has been placed on understanding how parental temperature exposure during the period leading up to final gamete maturation may influence reproductive physiology (but see Pankhurst and Munday 2011). Temperature may influence adults through multiple pathways since it is the primary determinant of metabolic rates in ectotherms. Temperature-mediated reproductive effects are known to act on hormonal cascades—specifically, the hypothalamic-pituitary-gonadal (HPG) axis. Endocrine processes are subject to temperature-dependent reaction rates that influence not only the timing of hormonal synthesis, but also the structure of hormones (Pankhurst and Munday 2011). Along with other ecological factors, temperature controls the timing of Sauger spawning and may modify gamete quality, having implications for both natural recruitment and artificial spawning operations.

Our study took a novel approach for large Percids by exposing wild adult Sauger to two different pre-spawning thermal regimes in a laboratory setting. One regime was a simple, gradual warming trend, representative of average thermal conditions in the wild, while the second treatment was a stochastic simulation of warmer than average, but highly fluctuating temperatures. Our primary objective was to spawn fish in both treatments and quantify reproductive success by comparing egg hatching rates and quality. Based on anecdotal evidence in the field, we hypothesized that the stochastic temperature treatment would temper spawning success by interfering with temperature-mediated pathways during final gamete maturation for male and female Sauger (ovulation and spermiation). We believed these effects would propagate into poor fertilization and hatch percentages, in addition to reduced egg quality—our primary

metrics of interest. Holding these fish overwinter in the laboratory increased the overall degree of parameter control; however, because these fish were wild, the degree of control over some of the parameters that influenced egg quality, hatching success, and larval quality was still variable (Figure 2-3).

METHODS

Broodstock Collection and Transport

On September 13th and 14th 2017, CSU and WGFD biologists captured adult Sauger on the Bighorn River between the town of Basin, WY and Bighorn Reservoir using electrofishing boats. Captured Sauger were weighed, measured and marked with a Floy Tag near the spiny dorsal fin. We intended to collect approximately 40 sexually mature fish (30 females and 10 males) for our experiment, a number that represented the holding capacity of the Sauger system at the CSU Foothills Fisheries Laboratory. Fish were provisionally sexed by the collection team based on sexual dimorphism, wherein females generally obtain greater maximum size than males, while males become sexually mature at smaller sizes (Bozek et al. 2011).

Fish were transported to the laboratory in a large (1,140 L) hatchery truck supplied by WGFD. Ice was added to depress transportation temperatures slightly below river temperatures (~21°C) to mitigate the stress response experienced by transplanted fish. We added sodium chloride (NaCl; 7 g/L) to reduce the energetic costs of osmoregulation and decrease the likelihood of stress-induced mortality (Barton and Zitzow 1995). Compressed O_2 delivered through fine-pore diffusers kept dissolved oxygen levels close to 100% saturation.

Culture System Design and Broodstock Management

Upon arrival to the CSU Foothills Fisheries Laboratory (FFL), Sauger were divided among three tanks, with roughly equal numbers of males and females per tank. Males and females were combined in case there were hormonal signals that assist in reproductive development (i.e., served as spawning cues). The adult Sauger holding system consisted of round tanks (1,100 L) receiving 18 $L \cdot min^{-1}$ of air-saturated, ambient temperature, filtered, and UV-irradiated surface water from College Lake. This helped to maintain a natural temperature regime, roughly parallel to that of the capture location. One-half inch PVC spray bars created a slight current, provided supplemental aeration, and provided opportunities for fish to exercise, while aiding with the self-cleaning aspect of the tanks. Each tank was equipped with an external standpipe. Initially, PVC pipe segments and plastic vegetation were added to each tank to provide refugia and minimize the perception of overcrowding, but the pipes were eventually removed after concerns that fish were receiving abrasions. Tanks were covered with black Coroplast™ lids that obscured 90% of the surface and reduced ambient light levels. Fish were kept under a photoperiod matching that of the collection area (44.38°N). Medium-pore air diffusers were used to help maintain tank dissolved oxygen levels ≥ 80% of saturation as confirmed by weekly checks. Similarly, pH, hardness, alkalinity, nitrate, and nitrite were frequently monitored to ensure that fish were exposed to near optimal conditions.

Feeding: Sauger are predators and at the sizes collected, highly piscivorous. Fish were fed live prey (juvenile Rainbow Trout *Oncorhynchus mykiss*) immediately after arrival at the FFL; we attempted to transition them from live prey to a frozen fish diet (Atherinidae: silversides) over a period of approximately three weeks but were ultimately unsuccessful. Due to the risk of starvation, we elected to forgo attempts to transition broodstock to frozen feed and fed them live fish for the remainder of the study. Rainbow trout were fed Oncor® fry diet (Skretting USA) to

ensure that experimental Sauger received adequate nutrition for high quality gamete production. Sauger were fed daily, *ad libitum*, to eliminate competition for food and to ensure that all fish had an opportunity to eat.

Fish Health: Tanks were siphoned daily and scrubbed weekly to maintain cleanliness. Because experimental Sauger came from the wild, and likely carried various external parasites, 12 $g \cdot L^{-1}$ salt baths were frequently used (about once per week) as a prophylactic measure. This treatment helps to detach parasites and maintains slime coat integrity—a fishes first line of defense (Horner 1996). Similar treatments have been used successfully on captive Walleye and Sauger broodstocks housed at the Northern Aquaculture Demonstration Facility at the University of Wisconsin Stevens Point (Greg Fischer, personal communication). Despite our preventative measures, low levels of mortality were experienced throughout the holding period; these appeared to be linked to the presence of external parasites (e.g., *Gyrodactylus* spp.) and associated secondary bacterial infections. Kordon® Rid Ich Plus (Active ingredients: formalin and malachite green) and formalin baths were utilized with all experimental fish following consultations with fish pathologists. We followed the labeled dosage when using Rid Ich Plus and administered formalin baths at a concentration of 125 ppm; both treatments were administered as needed. Additionally, tanks were scrubbed and disinfected using a bleach solution monthly to eliminate pathogen buildup through time.

Experimental Design and Temperature Treatments

From September to February all three tanks were held at a common temperature (ambient lake water). In February, a fourth tank was added to the system and fish were randomly re-assigned to experimental tanks (n= 2 tanks per treatment), ensuring that each tank had equal distribution of sexes.

Each treatment system (described below) consisted of a pair of experimental tanks

receiving water from a common thermal mixing head tank. Each head tank was equipped with Love® model 16B-33 digital controllers that controlled Asco® two-way solenoid valves regulating the mixture of well water and heated or chilled water. Both head tanks received a similar mixture of well and lake water to maintain consistent water chemistry between treatments.

Temperature treatment selection: We collaborated with WGFD Biologists to select two pre-spawn temperature treatments to begin on April 1st (approximately 6-7 weeks before expected spawn date, also known as Day 1). Our first treatment, hereafter called the "control" treatment, was a gradually warming scenario, representative of average thermal conditions observed in the Bighorn River, based upon temperature data from 2001, 2002, and 2013-2017 (all available data). Simply, the control treatment was a linear regression through all available temperature data points for the Bighorn River near Basin, WY. Our second treatment, referred to as the "fluctuating" treatment, was a simulation of a warming average temperature incorporating extremely fluctuating spring temperatures whereby large weekly fluctuations of about 7°C were overlaid by diel fluctuations of 5°C (Figure 2-4). To maximize our chances of detecting an effect, while maintaining ecological relevance, the fluctuating treatment was modeled after the most extreme temperature scenarios observed on Bighorn River between 2013 and 2017 and repeated for a longer duration.

Hormonal injection: To maximize the likelihood of spawning our experimental fish, Chorulon® (hCG) was utilized to induce final maturation of gametes in both males and females. Two doses (1650 IU·kg $^{-1}$) were injected 48 hours apart into the dorsal musculature. This regimen is used successfully on wild Sauger at the Milford State Fish Hatchery, KS (Daric Schneidewind, Kansas Department of Wildlife and Parks, personal communication). Initial injections for each treatment group were given at approximately 500 temperature units after Day

1 to ensure equivalent thermal history between groups. Temperature units (TU's) are defined as the summed daily mean temperature above 0°C (e.g., 10 days at 10°C=100 TU's). First injection dates were on Day 35 and Day 42 for the fluctuating and control groups respectively. Injected fish were checked every other day for reproductive development using the criteria proposed by WGFD (Table 2-2). When females became soft and the ovipositor fully extended with pressure, fish were checked daily in anticipation of reaching ovulation.

Spawning procedure: As soon as males expressed milt with pressure to the abdomen, a sample of sperm was taken and assessed under a microscope at 400 power to ensure that sperm were motile and ostensibly viable. A single, reproductively viable male was selected at random and used to spawn an individual female. Males were not used on more than one female, therefore each group of eggs corresponded to a unique family pair. Females were spawned with selected males as soon as they became ripe using the "dry method." Because only a small number of eggs were needed for experimentation, only eggs from the middle third of the ovary were fertilized. Anecdotal evidence suggests that these eggs are of the highest quality. In short, eggs and milt were stripped into a dry stainless-steel bowl. Water was added to initiate fertilization and eggs and sperm were gently mixed with a goose feather for 90 seconds. Following fertilization, a bentonite clay slurry was added to remove natural adhesive properties and reduce egg clumping. After rinsing the eggs thoroughly, they were placed in water hardening buckets with gentle aeration for two hours. During this period, eggs were gradually tempered from their respective spawning temperature to the target incubation temperature of 14.5°C.

At the conclusion of the experiment, adult Sauger were weighed, measured and euthanized in 250 mg/L of pH-buffered tricaine methanesulfonate. Otoliths were removed, sectioned and aged under a dissecting scope, using standard aging protocols.

Incubation procedure: Following water hardening, eggs (1.5 ml/jar) were randomly stocked into

four to six miniature upwelling incubation jars (see Chapter 1 for a detailed description) receiving 750 ± 10 $mL \cdot min^{-1}$ of air-saturated temperature-controlled water. Multiple jars per female were used to avoid "putting all of our eggs in one basket." Eggs received a static treatment of 500 ppm H_2O_2 for 15 minutes to control fungal infections. Four to six hours after fertilization a random subsample of at least 100 surplus eggs were evaluated under a microscope at 40 power to estimate fertilization percentage. During this time period eggs were easily identified as fertilized by the presence of peaked blastomeres (typically 2-6 cells; Figure 2-5) so that estimates of percent fertilization could be calculated for each individual spawn.

As in our egg incubation experiment (Chapter 1; Phase I), dead eggs were removed from the incubators and counted daily throughout the experiment. Upon the initiation of hatching, larvae swam through the incubator outlet and into an individual collection container. Collected larvae were euthanized daily using 250 mg/L of pH-buffered tricaine methanesulfonate, before counting and preservation in 10% formalin for subsequent analyses. These methods gave us a clear picture of time to the start and end of egg hatching for each jar. By quantifying all the dead eggs, and hatched larvae, we could census the starting number of eggs and therefore accurately calculate percent hatch for each jar.

Egg quality analyses: Because only a small proportion of eggs were needed for incubation procedures, surplus eggs were available for additional analyses. We were primarily interested in two indicators of egg quality - egg size and energy content, as these are correlated with egg quality (Czesny and Dabrowski 1998; Shaw et al. 2018). A subsample of 150 fertilized and water-hardened eggs were measured in a Von Bayer trough (Piper et al. 1982) to estimate total diameter. By dividing the total length of all eggs (cumulative diameter) by the number of eggs sampled, we could approximate average diameter (mm) per egg.

To measure energy content, a subsample of ca. 1200 unfertilized eggs from each female.

were frozen in scintillation vials before being dried at 37°C for approximately 48 hours, until all water content was evaporated. Dried eggs were pressed into a tablet using a pellet press and weighed (mg) using a precision balance. The energy content of four egg tablets per female were determined using a Model 6725 semimicro oxygen bomb calorimeter (Parr Instrument Company, Moline, IL, USA); a known standard was run frequently to ensure that the machine maintained calibration throughout the process.

Data Analyses: Individual spawning pairs were the experimental units for this study. Our primary variable of interest was the mean hatch percentage for each female, calculated by averaging the hatch percentage of all miniature upwelling jars (n = 4 to 6) that were stocked with that female's eggs. The effect of temperature treatment on hatch percentage, fertilization rates, time to ripen post-injection, and time to peak hatch were evaluated using two sample t-tests. Peak hatch was defined as the day that the greatest number of larvae hatched within each incubation jar. Similarly, we compared egg quality as defined by egg diameter and egg energy density using two-sample t-tests. We performed a linear regression to evaluate the relationship between fertilization percentage and hatch percentage, and used a power regression to evaluate the relationship between female weight and egg size. Similarly, we evaluated whether hatch percentage was related to female size using a linear regression. All statistical tests were performed in RStudio 1.0.136 and regressions were developed in Microsoft® Excel 16.21.1.

RESULTS

Broodstock Condition and Survival

Forty-four adult Sauger collected during two days of electrofishing on the Bighorn River were transported back to the Foothills Fisheries Laboratory. Of these 44 fish, 11 were provisionally identified as males, 22 were provisionally identified as female and the sexes of the remaining 11 were questionable. We hoped that a significant proportion of the questionable fish were mature females, so we could approach or exceed our goal of 10 males and 30 females. Fish husbandry techniques were largely successful and 37 (84%) of broodstock survived through the holding period to the first day of the experiment on April 1st (Day 1). Of the survivors, 24 increased in weight (>10 g), ten lost weight (>10 g) and four were unchanged (± 10 g), over the approximate six-month interval between capture and the first day of the experiment. Following the initiation of the two thermal regimes, mortality increased with 13 mortalities (11 females and two males) occurring between Day 1 and Day 16. Most of the female mortalities contained large ovaries with developed oocytes. Of the 13 mortalities, eight occurred in the fluctuating treatment and five in the control (Figure 2-6).

Temperature Control: All experimental fish experienced a common temperature (Figure 2-7) until Day 1, when we began to apply the selected thermal regimes. As shown in Figure 2-4, actual temperatures closely matched target temperatures throughout the duration of the experiment, with a few exceptions. Peak temperatures were intentionally changed to stay < 20°C on Day 40 and Day 46 due to a high mortality event experienced on Day 34 when the planned temperature exceeded 21°C for the first time. We reasoned that a tolerance threshold was crossed and if temperatures continued to exceed 21°C, as initially planned, feared there was a greater probability of losing most or all the broodstock. Control treatment temperatures

remained within 0.5°C of target temperatures for the entire experiment except on Day 27, when a power outage triggered a 2.9°C rise over three hours before it was resolved. During the egg incubation period temperatures remained at 14.5 ± 0.2°C throughout the duration of the experiment.

Injections: On Days 35 and 37 all fish in the fluctuating treatment were injected with Chorulon® (7 Males, 5 Females), and three females and four males reached final maturation. On Days 42 and 44 all fish in the control treatment were injected (10 males, 6 females) resulting in four ripened females and 10 males. Females in the control treatment reached final maturation at a mean of 6.75 days after the first injection vs. 6.00 days for the fluctuating treatment. Time to ripen was not statistically different between treatments (two sample t-test, p=0.49)

Fertilization, survivorship and hatch: Due to high mortality during the immediate pre-spawning phase, fewer females than anticipated were available for spawning. A summary of parameters of spawned fish are provided in Table 2-3. There was no significant difference between the mean (± SD) fertilization rate for the control (51.56% ± 23.74%) and that of the fluctuating treatment (63.61% ± 19.77%) (two sample t-test, p=0.51). Average hatch percentages were 13.15% ± 9.19% and 27.78 ± 18.6% for the control and fluctuating groups respectively (Figure 2-8), but differences were not statistically significant (two sample t-test, p= 0.299). Virtually all egg mortality during the incubation period for both treatments occurred within the first three days (Figure 2-9). As expected, there was a positive correlation between fertilization percentage and hatch percentage ($R^2 = 0.62$; Figure 2-10), and hatch percentages were appreciably lower than fertilization percentage. As shown in Figure 2-11, no difference in time to hatch was detected between experimental groups (two sample t-test, p = 0.44). Peak hatch occurred at an average of 12.5 ± 0.6 days post-fertilization for the control group and at 12 ± 1.0 days for the fluctuating group.

Egg quality: Egg diameter did not differ between treatments (two sample t-test, p = 0.41); control eggs averaged 1.72 ± 0.1 mm in diameter and eggs from the fluctuating treatment averaged 1.65 ± 0.1 mm. There was a weak positive correlation between female weight and egg total diameter ($R^2 = 0.25$; Figure 2-12). No differences in energy density were detected between groups (two sample t-test, p=0.88). The fluctuating treatment had an average energy density of 28.42 ± 0.26 J·Mg dry weight $^{-1}$ and that of the control group was 28.36± 0.61 J·Mg dry weight^{-1}.

DISCUSSION

Analysis of Results

We were able to successfully hold wild-caught Sauger under laboratory conditions, expose them to artificial thermal regimes over a matter of months, and induce final maturation using hormonal (hCG) injections. Unfortunately, heavy mortality during the immediate pre-spawning phase of the experiment limited our ultimate sample size (n=7 spawns), limiting our ability to draw robust inferences from the data. Nevertheless, there are still several valuable insights that can be gained from our experiment.

There was no indication that the fluctuating thermal regime adversely impacted reproduction, other than possibly by triggering pre-spawning mortality when $T_W > 21°C$, when compared to the idealistic warming scenario experienced by the control group. Contrary to our predictions, eggs derived from spawning pairs in the fluctuating treatment had higher average hatch percentages than those in the control group (27.8% vs. 13.2%), though these results lacked statistical significance, likely due to high variability within each treatment, probably stemming from the small sample size. We were somewhat surprised by the lower hatch rates in the control given that pre-spawn and incubation temperatures should have been near the published and

experimentally-derived optima for Sauger. It is possible that Sauger may be adapted to natural diel fluctuations and that such fluctuations may serve as a cue for spawning and help regulate endocrine cascades. Similarly, there was some indication that larval Sauger may benefit from fluctuating temperatures, as they grew and fed better when exposed to these conditions (17-22°C; see Ch. 1). Interestingly, our earlier results (reported in Ch. 1) showed that *eggs* incubated at this temperature were adversely impacted and hatched at a lower rate than all other treatments, except the 10°C treatment. An alternative explanation is that the low hatch rates across both treatments were due to a variable that all experimental Sauger experienced. In prior field spawn-takes and during this captive spawning experiment, fish were held in captivity prior to spawning and injected with hormones, potentially compromising gamete quality.

High variability in hatch percentage, irrespective of treatment, is similar to results observed during artificial spawn takes on the Wind and Bighorn River projects, whereby different batches (group of fish spawned in the same day) of eggs can have significantly different eye-up percentages, even within the same year, suggesting high variability in female egg quality. As with the wild spawn operations, we observed low average hatch percentage among all spawns.

The female that had the greatest hatch percentage (45.6%) came from the fluctuating treatment and had a large influence on the higher average hatch rate of this treatment. Interestingly, this female was also the smallest, youngest and had the lowest condition (relative weight =79.1) of all the females spawned; she also had the smallest average diameter eggs (1.53 mm). These observations run contrary to the literature norms, which suggest that offspring survival can be positively correlated with maternal size, age, and condition, often referred to as maternal influences—the phenotypic and genetic contribution passed from mother to offspring. For example, Venturelli et al. (2010) showed that in closely related Walleye, offspring survival

increased with egg size. Similarly, Moodie et al. (1989) found that larger Walleye eggs contained high concentrations of polyunsaturated fatty acids and resulting larvae exhibited better growth, lower deformity, and lower mortality. Some studies suggest that Walleye egg size is positively related to maternal size and age (Johnston and Leggett 2002; Wiegand et al. 2007), though this relationship may be highly variable among populations (Moles et al. 2008). While there was a weak positive relationship between female weight and egg diameter, there was no indication that females with larger eggs had improved hatch percentages in our experiment. Also, there was no indication that pre-spawning temperature influenced the size of eggs. Given our small sample size, this is not surprising, as the primary determinant of egg size was likely maternal size and age (Johnston and Leggett 2002; Kamler 2005). It would likely take a large sample of all representative size classes to determine if pre-spawning temperature influences egg size.

We used calorimetry to determine the energy density for the eggs from each individual that spawned, with the assumption that energy density is a metric of egg quality that relates to maternal energy endowment to offspring, yet we found no differences between the two treatments. Similar to Johnston (1997), we assumed that higher energy density values may be indicative of greater lipid content. We hypothesized that because hatch percentages and larval size would correlate positively with egg energy density, exposure to the fluctuating temperature scenario might reduce the amount of energy allocated to the eggs due to increased maternal metabolic demands and reduced aerobic scope of fluctuating vs. control adult females. This hypothesis was not supported by our data. One potential explanation for the lack of differences in egg energy density between treatments is the timing of the thermal manipulations. Maternal energy is largely allocated to the ovaries during the fall and winter, when a spike in gonadosomatic index (GSI) is accompanied by the important process of vitellogenesis, or when the egg yolk and associated oils are integrated into the oocyte (Malison et al. 1994). Had thermal

manipulations occurred during this time, there is a chance that we would have seen differences in energy density and egg size between treatments. However, while we recognize that more thermally sensitive time periods exist, we wanted to specifically address whether spring temperature regimes prior to spawning could influence spawning success because these were the conditions in question from Wyoming.

Similar to our earlier study (Chapter 1), the majority of egg mortality occurred within the first three days. Based on our fertilization results, some of these eggs were not successfully fertilized, while others died during early embryogenesis. Early mortality of eggs is a common finding in the experimental literature (Heidinger et al. 1997; Kamler 2005). A spike in mortality is commonly reported between eye-up and hatch, though this did not occur in our experiment.

Parental Temperature Impacts on Reproduction

Reproductive maturation is regulated through the hypothalamic pituitary gonadal axis. Annual variation in photoperiod and temperature are the primary environmental cues regulating the timing of maturational events in Percids (Wang et al. 2010). Temperature largely influences reproduction via rate-determining effects, with warmer temperatures corresponding to faster reaction rates and earlier spawning in Percids. This is supported by the latitudinal progression of the spawning season from South to North in Percids. Indeed, in our study, fish in the fluctuating temperature ripened an average of almost one day earlier following hormonal injections compared to those in the control treatment, though this difference was not statistically significant. In our experiment, hormonal injections likely obscured the effect of temperature on final maturation timing. The timing of spawning is significant, as it can interact with other important ecological variables such as hydraulic conditions, habitat availability, predator/prey dynamics, and food resources—all impactful variables in the recruitment process.

Not only does temperature modify the timing of reproductive events, but it can also influence the structure and function of hormones involved in regulating reproduction. Hormonal processes can be inhibited at low temperatures (Pankhurst and Munday 2011). For example, Distefano et al. (1997) implicated periodic releases of cold water from Harry S. Truman Dam in Missouri as one of the main causes of chronic stress in Walleye, leading to reproductive dysfunction and subsequent recruitment failure in this population. The Wind and Bighorn Rivers are snowmelt driven systems that can experience periods of depressed temperatures during runoff that coincides with the spawning period of Sauger (Figure 2-13). Because this is a natural phenomenon, Sauger are likely well-adapted and we do not expect these cold temperatures to negatively impact reproduction. Based on anecdotal evidence by WGFD during past spawn takes, we reasoned that higher and/or more variable temperatures are more likely to affect reproduction (Paul Gerrity, WGFD, personal communication).

At higher than normal temperatures, conformational changes in a variety of hormones and associated receptors have been reported for temperate fishes throughout the process of gametogenesis and final maturation (Pankhurst and Munday 2011). The absolute timing of the temperature event will dictate the specific effect seen in reproduction. In closely related Walleye, vitellogenesis is completed in early winter and final maturation (ovulation and spermiation) is induced by rising temperatures in the spring. Due to similarities in spawning biology, we expect that Sauger undergo these events at similar timelines. Given the timing of our experiment, we expect that any interruptions in normal reproductive function, induced by thermal conditions, would have occurred during germinal vesicle breakdown (final maturation), regulated by maturation-inducing steroids, likely 17,20β-dihydroxy-4-pregnen-3-one (17,20βP), as reported for Walleye (Barry et al. 1995; Pankhurst et al. 1986). As reviewed in Pankhurst and King (2010), in salmonids high temperature exposure in the final weeks before spawning

inhibited the synthesis of the same maturation-inducing steroid (17,20βP) and impeded ovulation. It is unknown how, or if these results may translate to Sauger, and we did not undertake any measurements of hormonal concentrations.

Admittedly, in our experiment temperature effects may have been obscured by injecting fish with hCG, as hormonal therapies have been suggested as a means to mitigate suboptimal thermal conditions. For example, Rainbow Trout (*Oncorhynchus mykiss*) held at 12°C prior to spawning required a single dose of synthetic luteinizing hormone (LHRHA) to ovulate while those held at 18°C required two doses (Pankhurst et al. 1996). We initially intended to let fish mature naturally, without the use of hormones, but reasoned that captivity-induced stress, combined with repeated handling stress would likely preclude final maturation in experimental broodstock. This decision was supported by numerous agencies with experience spawning wild Sauger (Daric Schneidewind, Kansas Department of Wildlife and Parks, Personal Communication). Because hormonal injections were utilized, we limited our ability to make inference to wild spawning populations, though our procedures have direct relevance to artificial spawn-takes, where hormone injections are utilized.

While our primary focus was on egg quality, it is also possible that sperm quality may be impacted by pre-spawning temperature. Considerably less research has focused on the issue of sperm quality as a potential variant in reproductive success, though sperm number, swimming speed, and swimming longevity have all been identified as potential factors regulating fertilization success, especially in the face of sperm competition (Burness et al. 2004; Casselman et al. 2006). Temperature has been identified as an important environmental regulator in spermiation, but little information exists about how pre-spawning temperature may impact the overall quality of sperm of Percids. In other species, there is some evidence that sperm quality metrics are influenced by holding and fertilization temperatures (Cosson et al. 1985; Van Look

2001; Williot et al. 2000). It is likely that this is an important variable in the viability of Sauger sperm as well.

We assessed motility to ensure that viable sperm existed in each of the males used in the experiment. This analysis was a simple binary analysis (i.e. sperm movement detected vs. sperm movement not detected) and likely inadequately described variability in sperm quality between males. While oversimplified, this approach is used during wild-spawn takes to confirm male viability. When possible, multiple males are utilized to fertilize each group of eggs, increasing the probability of successful fertilization, in addition to diversifying genetics in the resulting offspring. In contrast, we only used one randomly selected, viable male to fertilize the eggs of each female due to logistical constraints. Given that each male was completely stripped of milt, we expected that surplus sperm was available to fertilize the eggs in each bowl, and therefore any reduction in fertilization was not likely to be a result of low sperm number. All spawning pairs had some fertilization and successful hatching, suggesting that each male selected was indeed viable at some level; however, there were some spawning pairs that had surprisingly low fertilization percentages (<50%).

The specific reproductive mechanisms that might be influenced by temperature were not addressed in this study, though there is a large body of evidence that temperature can exert a strong influence on parental reproductive physiology through a variety of pathways. Although we did not show a temperature effect in the present study, our results do not imply that temperature is not an important variable in reproduction of Wind and Bighorn River Sauger populations — rather, it does not appear to be the "silver bullet" in explaining past recruitment failures and culture challenges. It is probable that other variables are impacting reproductive success—some of which may act synergistically with temperature. Further examination of these variables is warranted.

Stress and Hormonal Manipulation

Similar to past WGFD wild-spawn takes, low hatch percentages were observed across all experiments conducted as part of this study. Holding fish in a captive environment and inducing final maturation with hormone injections could impact reproduction; they were common-threads in all the aforementioned spawning events. It stands to reason that these factors may be implicated in the lack of success in wild spawn takes, though we lack comparisons of spawning success of naturally ripened fish, not held in these conditions. Stress has been implicated to impact reproduction in a variety of fishes, including Percids. Stress responses can have short term, adaptive benefits for fish by increasing oxygen delivery to tissues and increasing energetic potential to help fish escape the stressor. If the stressor is prolonged these same physiological effects can become maladaptive, largely explained by the continued stimulus of the hypothalamo-pituitary-interrenal axis, leading to increases in the steroid cortisol than can adversely impact growth, immunocompetence, and reproduction (Pankhurst and Munday 2011). Impacts in fishes vary from altered spawn timing to complete inhibition of spawning (Schreck et al. 2001).

In early WGFD attempts to spawn Sauger on the Wind and Bighorn Rivers (2011-2012), fish were not injected with hormones; instead they were held in net pens within the river and checked daily for ripeness. This approach resulted in low proportions of fish that ripened and somewhat high levels of mortality, indicative of chronic stress. Adding the OvaRH and/or hCG injections and holding fish onshore in a more stable environment markedly increased the proportion of fish that ripened, though adult mortality remained an issue, as did the variable and generally low hatch percentages (Hochhalter 2015). This observation suggests that utilizing hormones may help to bypass any reproductive inhibitions created by stress, though gamete quality remains in question. Ironically, injections themselves may increase stress in fish.

Falahatkar and Poursaeid (2014) showed that plasma cortisol concentrations increased in female Pikeperch (*Sander lucioperca*) following injection with hCG, relative to those injected with saline, carp pituitary, or LHRHA. They also showed that stress responses varied between sexes, with females being more sensitive to stress overall. Barry et al. (1995) showed that Walleye injected with saline (control) experienced increases in cortisol, and never ovulated, suggesting that handling stress and/or captive conditions may have precluded normal maturation. They attributed reproductive inhibition to chronic stress that likely interfered with the HPG axis at the level of the brain or pituitary. Findings from these studies seem to be corroborated by WGFD wild spawn-takes, where mortality was higher in fish that were injected vs. those that were not, suggesting that hormonal injection in combination with captive conditions may induce stress-related mortality. What is unclear, however, is whether Sauger gamete quality may be compromised around the time of final maturation, as a result of stress or hormonal injection, in spite of successful induction of ovulation. Percid research suggests that hormonal injections in post-vitellogenic females can induce ovulation and consistently produce hatch percentages comparable to naturally ripened fish. Hormonal manipulations are quite common in the Percid aquaculture industry and are often used in combination with photoperiod and thermal adjustments to advance the timing of spawning, as reviewed by Fontaine et al. (2015). It is possible that wild broodstock may be more vulnerable to stress caused by these methods, compared to their domesticated counterparts. However, hormonal injection in wild Sauger have been used successfully in other locations. For example, the Milford State Fish Hatchery (KS), captures wild Sauger broodstock during the early spring and holds them in large hatchery tanks. Human chorionic gonadotropin is administered to both males and females causing them to ripen weeks before normal spawn time. This approach yields satisfactory results, as hatch percentages consistently range from 60-80% (Daric Schneidewind, KDWPT, personal communication).

While the specific mechanism is unclear, it appears that Wind and Bighorn River Sauger populations may either exhibit a higher sensitivity to artificial spawning methodologies or may be reproductively compromised by other factors.

Effect of Parasites

In addition to the stress of captivity, we know that our fish were impacted by two common parasites: *Gyrodactylus salaris* and *Ichthyoboda necator.* Infection rates were classified as severe based on consultation with Colorado Parks and Wildlife Aquatic Pathologist John Drennan, who assayed gill tissue and epidermis from a mortality. It was determined that this infection likely contributed to the mortalities experienced during the holding period, by damaging gill tissue and leading to respiratory and osmoregulatory dysfunction and/or by creating vulnerabilities to secondary bacterial or viral infections. In response, we increased the use of prophylactic salt baths and in limited instances subjected all experimental fish to formalin baths to control the outbreak. Frustratingly, *Gyrodactylus* were detected on mortalities found following formalin treatments, suggesting that either treatment was not 100% effective and/or pathogens were being reintroduced to the holding tanks despite our biosecurity measures, and multiplying through time. We believe that frequent salt treatments were helpful in controlling the outbreak, though they did not eliminate it entirely, possibly because our thermal regimes provided ideal conditions for the parasites.

Jansen and Bakke (1991), found that the potential for population growth in *Gyrodactylus salaris* was positively associated with increases in temperature over the range of 6.5-19°C. This might explain why broodstock mortality remained relatively low during the winter holding period and increased in the spring when temperatures began to rise, including during experimental manipulations. During the experimental period, females were disproportionally affected by mortality (11 of 13). Many of these females were larger and older fish containing

well developed, post-vitellogenic ova, suggesting that reproductive females may be especially vulnerable to added stressors, as suggested in (Falahatkar and Poursaeid 2014). These mortalities reached a pinnacle on Day 33 (May 3rd; informally known as the Macabre May Massacre), when temperatures crested 20°C in the fluctuating treatment and four large females died. Impacted fish were observed struggling to ventilate their gills prior to death, suggesting respiratory or osmoregulatory stress, perhaps as a result of damaged gill tissue. Dissolved oxygen concentrations remained above 80% saturation during this event, so we do not believe that death resulted from hypoxic conditions. While it is unknown what degree parasite load played in mortality, it was probably a contributing factor. Even for fish that survived to spawn, sub-lethal effects may have existed, namely in the form of chronic stress. Unfortunately, conclusions are largely circumstantial in the absence of data about infection rates and thorough examination of the causes of death. Because fish densities are much lower in the natural setting, we would expect that infection rates are somewhat low in Wind and Bighorn River Sauger populations. We do not anticipate external parasites to be a major factor influencing the natural spawning success or artificial spawn-takes, unless environmental conditions are conducive to an outbreak of a non-specific parasite similar to *Gyrodactylus*.

Recommendations for Management and Future Research

This research project has helped identify several practical considerations. With regards to temperature, captured adult fish should be maintained at 14 – 18°C, conducive to prompt final maturation, but not so high (approaching 20°C) where stress and mortality may be drastically increased. It is unknown how this observation may translate to wild Sauger, though persistent river temperatures above 20°C may result in spawning inhibition if thermal refugia cannot be found. Reasonable diel fluctuations in holding temperature are unlikely to negatively impact broodstock fish. In our experiment, fish were exposed to diel fluctuations of 5°C throughout the

course of the experiment, and no impact in spawning quality was observed relative to the control treatment; however, broodstock mortality was greater in the fluctuating treatment as compared to the control treatment (44.4% vs. 26.3%). This suggests that stochastic thermal conditions may impose considerable stress on spawning Sauger, especially for females who were disproportionately affected in our experiment (87.5% of mortalities in fluctuating treatment).

The effects of parental stress on Sauger broodstock remain unknown, but it is reasonable to suggest that stress should be reduced to the degree possible. This may be especially relevant for reproductive females that are ostensibly more vulnerable to stress because of their elevated energetic demands. During field collection, water quality should be maintained in holding tanks by frequently exchanging water, using supplemental oxygen, and adding 7 $g \cdot l^{-1}$ NaCl to the water to reduce osmotic stress. In our experiment, Sauger tolerated extended salt baths of up to 20 $g \cdot L^{-1}$ so there should be little risk in maintaining isotonic conditions over multiple days. It may also be helpful to reduce light levels during transport to the boat ramp by utilizing dark covers over the tanks. The use of anaesthetics prior to handling and injections may help to limit the magnitude and duration of the stress responses. Using river water within the holding tanks, as opposed to hatchery water, may benefit broodstock by maintaining natural water chemistry. It may be helpful to let some natural light into holding tanks to maintain natural photoperiod cues. Crowding should be minimized in holding tanks to the degree possible, especially for females that are likely to spawn.

After spawning, checking fertilization rates could be a quick and cost-effective way to estimate hatch percentages. Fertilized and unfertilized eggs can be easily identified four to six hours post-fertilization by noticeably peaked cell divisions. As shown in Figure 2-10, a relationship could be developed to use fertilization percentage as an indicator of eventual hatch percentage similar to Heidinger et al. (1997). This could give managers a method whereby they

could eliminate unproductive spawns and/or determine whether additional field collections were necessary.

It would be valuable to compare hatch percentages from females that have ripened naturally to those that have received injections and been held in captivity, though it is acknowledged that capturing adequate numbers of naturally ripe females has proved difficult. Evidence suggest that current electrofishing methodologies may be targeting mostly staging fish that are near, but not fully ripe. Capturing ripe females in active spawning areas may be limited by some sort of physical limitation (e.g. depth, cover, etc.). The use of other sampling protocols (e.g., gillnets, trapnets, trammel nets) to see if more ripe females can be encountered could be worthwhile. This would enable statistically viable comparisons to be made and potentially shed light on whether current spawning techniques are leading to compromised gamete viability. Additionally, the effect of hormonal injections might be further examined by comparing reproductive success across multiple hormone types and doses.

High variability in hatch percentage across individual females seems to be a common occurrence. While paternal effects may play a role in this variability, we believe it is more likely that egg quality is the primary determinant of reproductive success. In the future, it would be useful to obtain additional metrics on each female spawner (e.g., egg quality, plasma cortisol levels, disease, etc.) and track reproductive success through time (e.g., fertilization, hatch, deformity, larval size and growth). Paternal influences could be controlled by spawning each female with a standard volume of milt from a single viable male, or group of males. These procedures may help to elucidate important trends in brood stock quality and help to identify high quality females. It could help to prioritize other areas of further inquiry, based on observed trends. In our study, we obtained various quality attributes of our broodstock including size, age, relative weight, egg size and egg energy density. With only seven data points, correlations are

tenuous at best and prone to outliers. By increasing the sample size, some interesting patterns may emerge.

Conclusions

The thermal regime experienced by adult Sauger matters for natural recruitment, and artificial spawn-operations on the Wind and Bighorn Rivers. Temperature dictates the timing of reproductive maturation, can alter endocrine structure, and strongly interacts with a variety of important ecological factors. We were unable to detect significant differences in hatch percentages between Sauger held in two different pre-spawning temperature scenarios, possibly because of our small sample sizes. We did observe low, but highly variable hatch percentages among individuals, similar to WGFD spawning operations. While impactful, temperature alone may not explain the problems experienced during artificial spawn operations, nor the years of low natural recruitment. Consistent with the current understanding of recruitment dynamics of fisheries, Sauger reproduction is inherently complex and is undoubtedly regulated through a variety of biotic and abiotic factors. The Wind and Bighorn River Sauger have consistently proved challenging to culture, even when carefully executed, well-established spawning approaches are employed. Added investigation into the roles of stress and hormonal injection are warranted. Furthermore, additional variables governing the quality of gametes must be considered to narrow our understanding of factors affecting the hatch rate of Wind and Bighorn River Sauger eggs, including parental nutritional state, disease status, contamination, and stress. As postulated by Rachel Carson, "nothing in nature exists alone." Because reproduction is the integrator of numerous exogenous and endogenous processes, a multipronged approach should be considered to advance our understanding of the reproductive processes of Wyoming Sauger.

TABLES

Table 2-1.— Summary of Bighorn River, Wyoming spawning operation results from 2011 to 2013. Data are from Hochhalter (2015).

Year	Number of females captured	Number spawned	Number of eggs collected (millions)	Range of eye-up percentage across all takes	Number of fingerlings stocked	Percent survival (egg-fingerling)
2011	416	19	1.5	40-71	48,000	3.2
2012	561	48	6	0-10	0	0
2013	144	15	1.2	0-77	105,000	8.8
2014	165	61	7.1	0-46	176,000	2.5

Table 2-2.— WGFD criteria used to assess reproductive development in Sauger during artificial spawn-takes.

Category	Indicators
Firm	Abdomen firm; ovipositor not extended
Intermediate	Abdomen moderately soft; ovipositor not extended
Intermediate-Soft	Abdomen moderately soft to soft; ovipositor moderately extended
Soft	Abdomen soft; ovipositor fully extended; may express eggs, but eggs do not flow freely when pressure is applied to abdomen
Ripe	Abdomen soft; ovipositor fully extended; eggs flow freely when pressure is applied to abdomen

Table 2-3.— Summary of morphometrics and reproductive success of Sauger spawning pairs used in this study. All fish were collected from the Bighorn River in September 2017, held in captivity in the Foothills Fisheries Facility, and spawned in May 2018.

Pair	Tag number	Treatment	Sex (M/F)	Total length (mm)	Weight (g)	Relative weight	Age	Fertilization percentage	Hatch percentage
C1	8359	Control	F	522	1175	79.6	6	73.1	24.1
	8292		M	477	870	88.2	7		
C2	8288	Control	F	592	1963	89.0	12	48.8	16.5
	8342		M	489	1025	89.5	11		
C3	8341	Control	F	535	1313	82.2	5	19.3	2.8
	8350		M	426	455	76.8	13		
C4	8294	Control	F	493	1067	86.7	7	65.0	9.2
	5484		M	457	455	76.7	13		
T1	8298	Treatment	F	500	1221	94.8	7	74.0	29.2
	4338		M	502	920	72.0	12		
T2	8340	Treatment	F	582	1844	88.3	12	40.8	8.5
	8345		M	447	704	81.3	11		
T3	8296	Treatment	F	486	930	79.1	4	76.0	45.6
	2933		M	447	664	75.5	11		

FIGURES

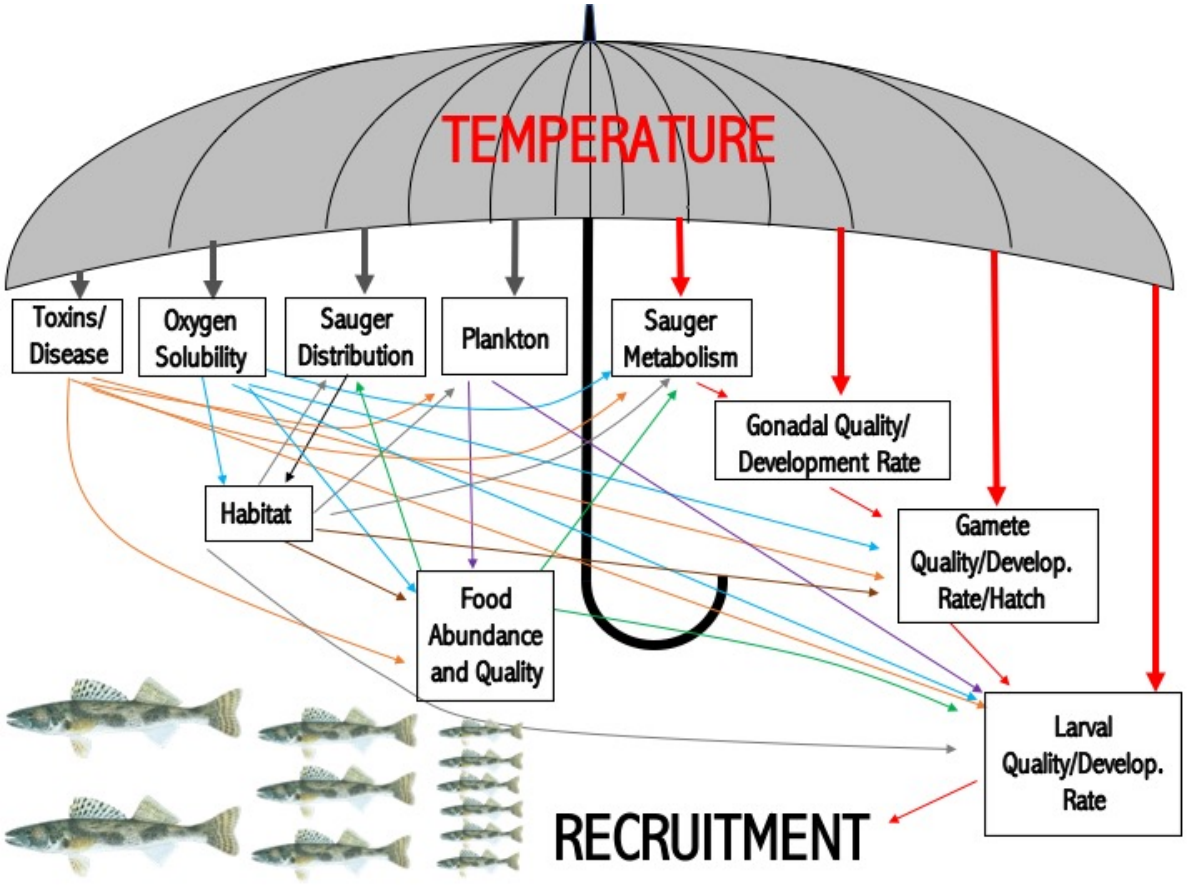

Figure 2-1.— Conceptual figure representing the umbrella of temperature influences on key Sauger recruitment variables. Arrows show the direction of influence. Bold arrows point to variables directly affected by temperature, while thin arrows represent indirect influences. Arrow color is unique for each variable identified. The right side of the umbrella and all red arrows represent variables of particular interest for this study.

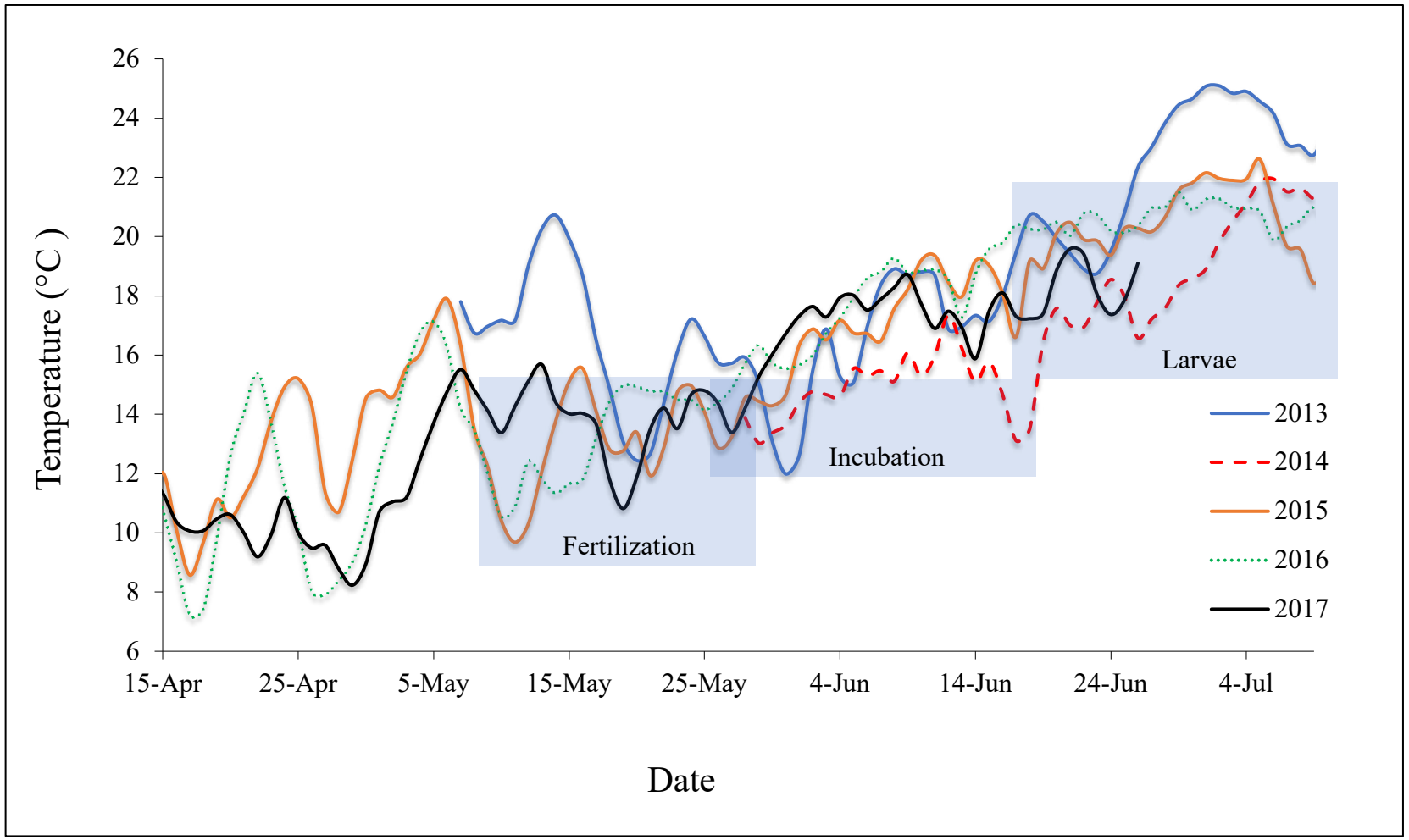

Figure 2-2.— Temperatures on the Bighorn River, near Manderson, WY during the April – July period when Sauger are in pre-spawn, spawn, and post-spawning phases. Boxes indicate the different thermal optimums and timing for various life stages as defined by Koenst (1976). The vertical height of the box encompasses the accepted thermal optima for that stage, while the horizontal dimensions define the approximate timing of each stage.

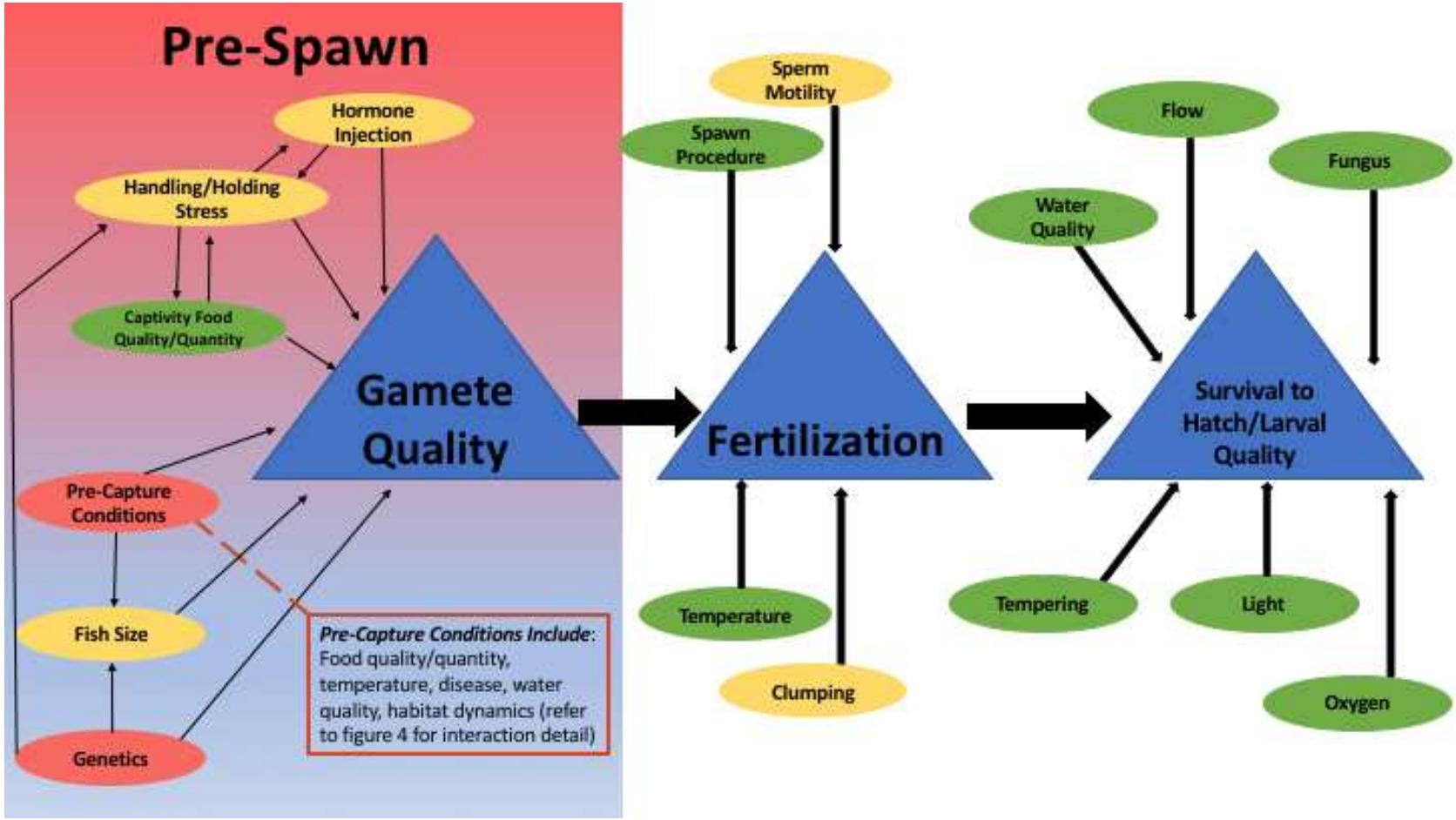

Figure 2-3.— Phase III: Effects of pre-spawning temperature on female Sauger reproductive output, subsequent hatch success, and larval quality. This conceptual model illustrates the variables and associated interactions for this study. Arrows point towards the direction of influence. Moving from left to right roughly represents the chronological order of variable introduction. Blue triangles represent variables of primary research interest, while ovals represent influential external variables. Colors signify different levels of control as follows: variables that cannot be controlled (pink), partially controlled (yellow), fully controlled (green). Red-blue color gradient represents the interval of thermal experimentation.

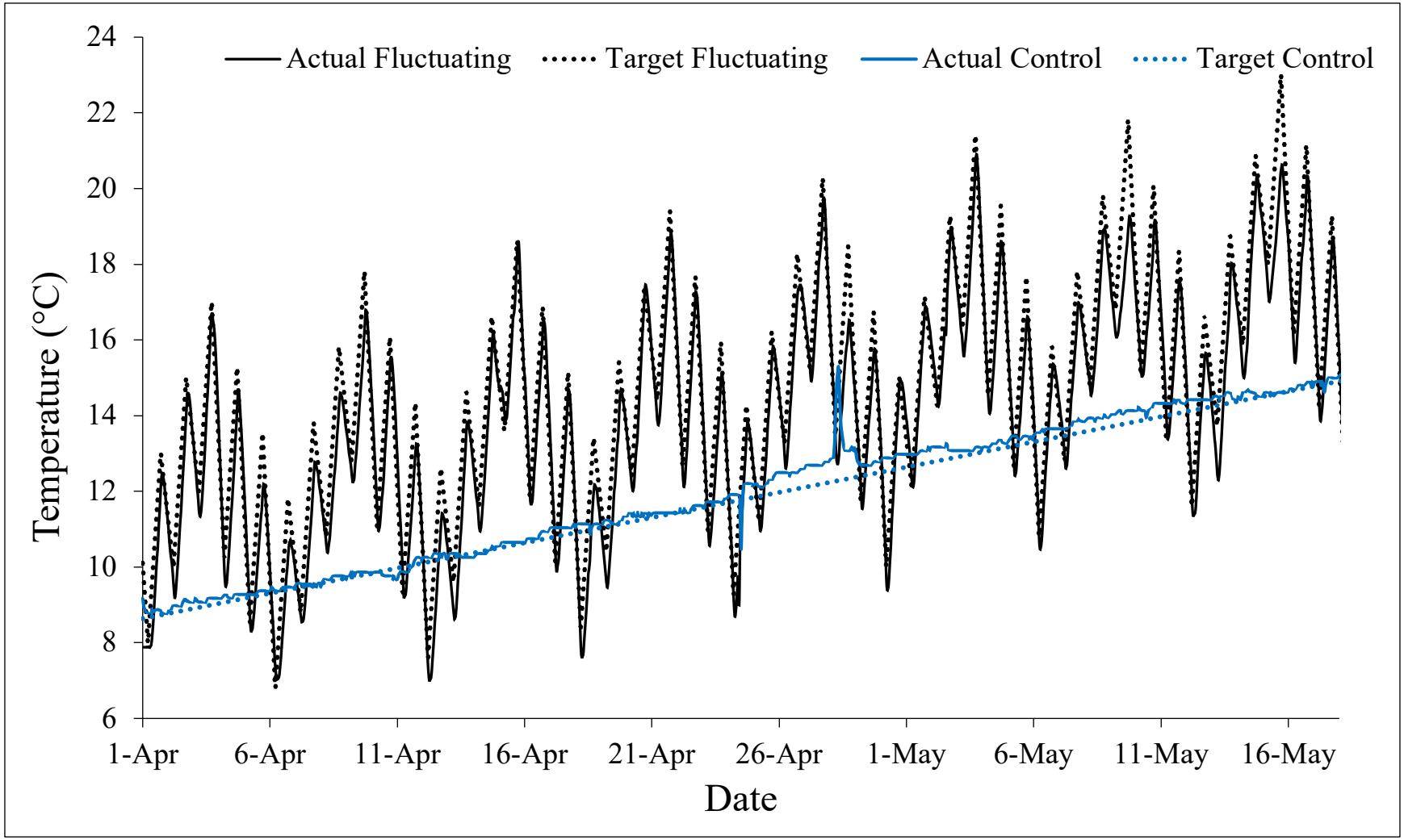

Figure 2-4.— Comparison of target and actual temperatures for each treatment throughout the duration of the phase III experiment.

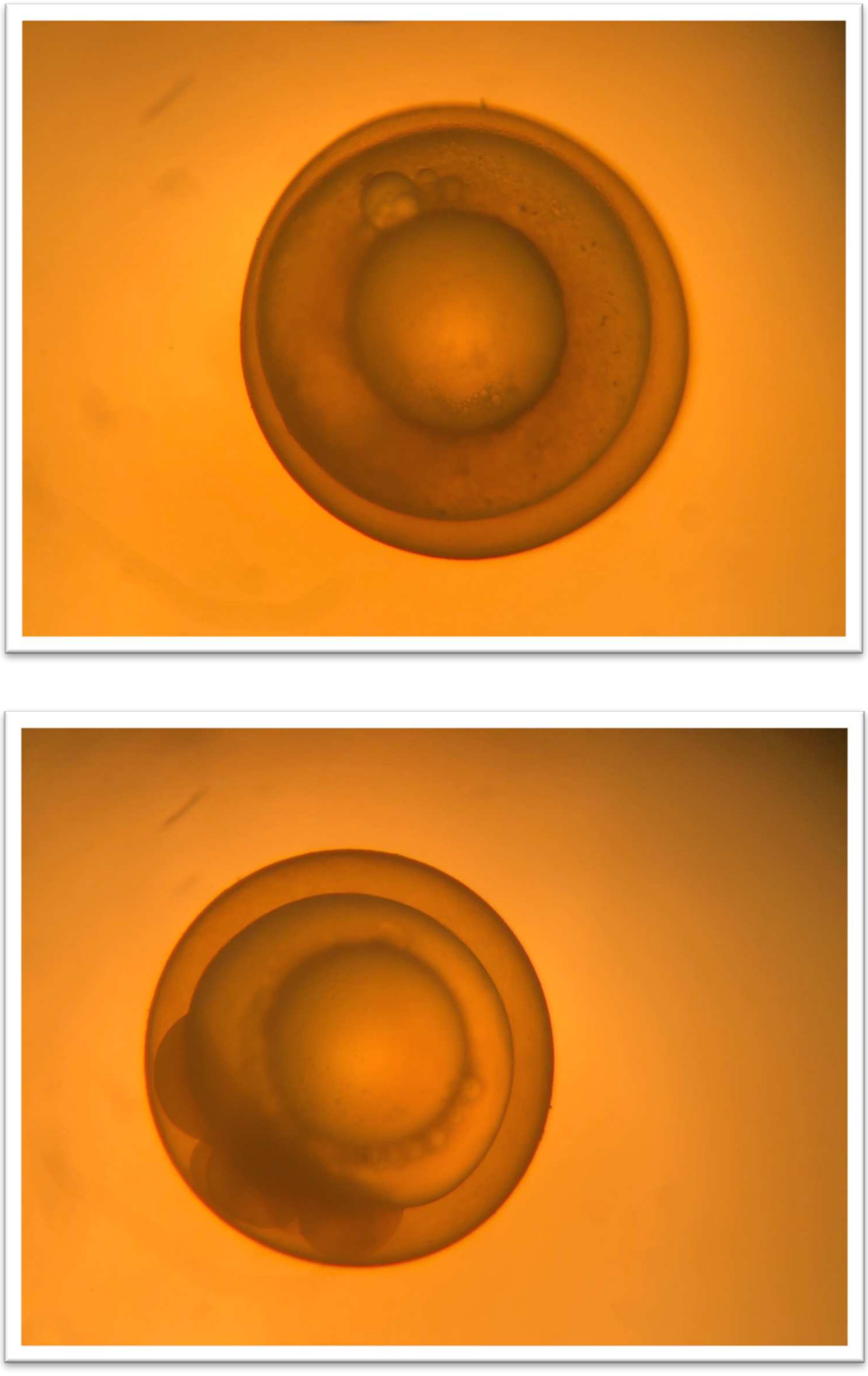

Figure 2-5.— Comparsion of unfertilized (top) and fertilized (bottom) Sauger eggs approximately four hours after fertilization.

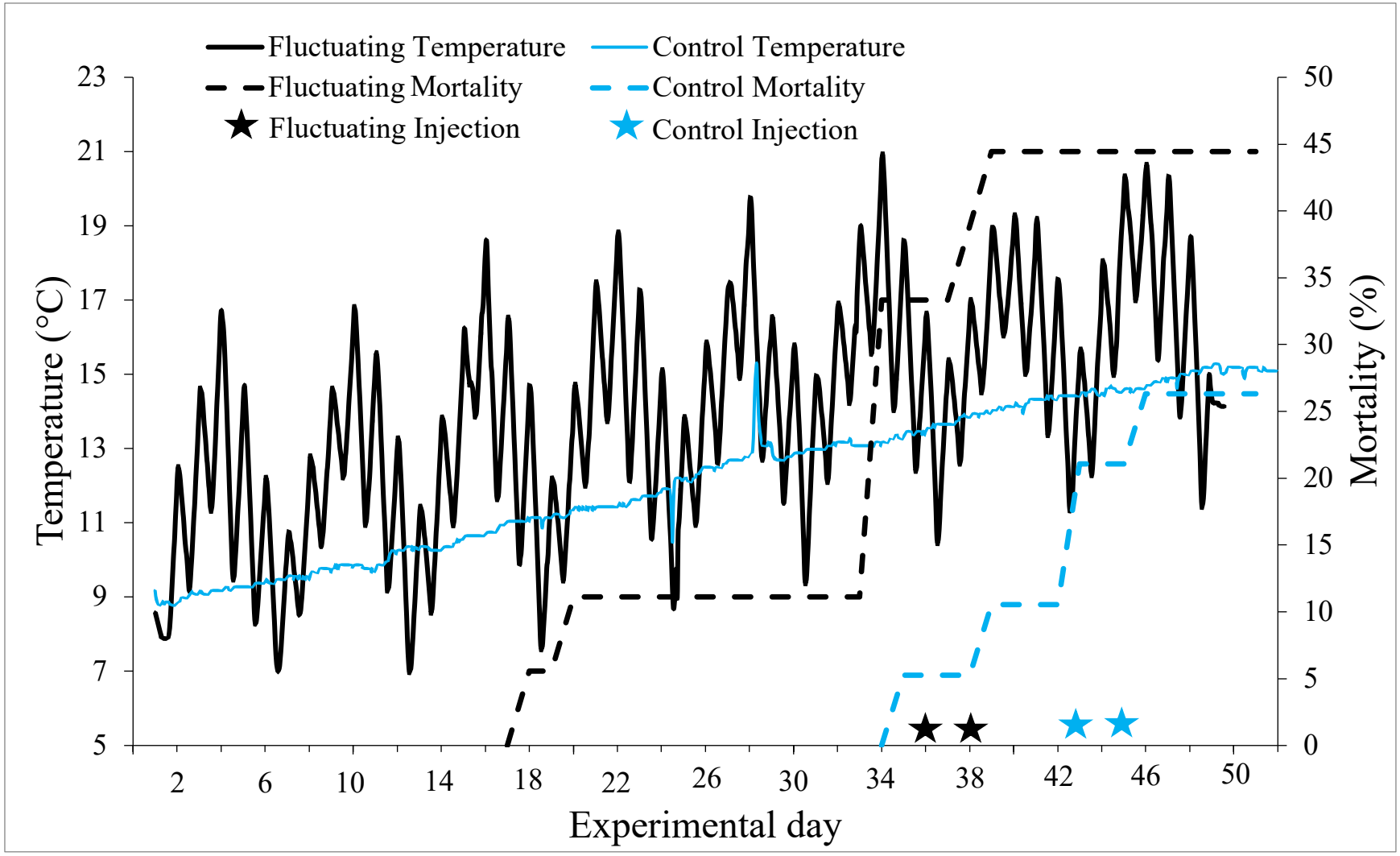

Figure 2-6.— Temperature (left axis) and mortality of experimental Sauger (right axis). Stars represent days that Sauger were injected with hCG.

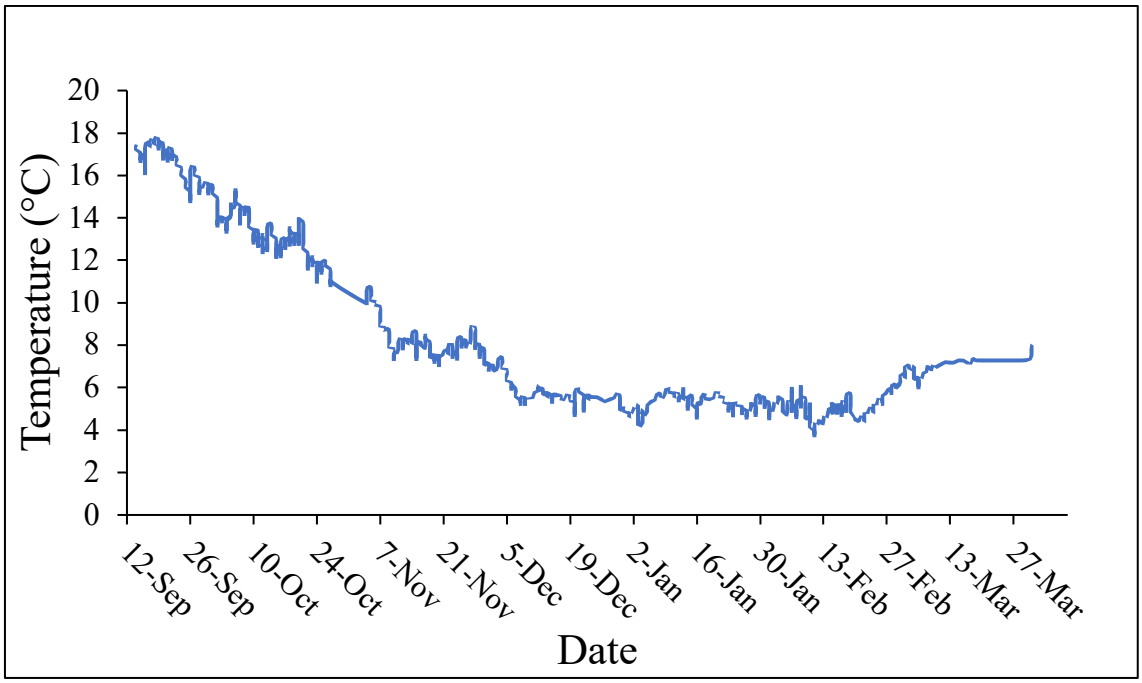

Figure 2-7.— Captive holding temperature of all experimental Sauger until Day 1 of the experiment (April 1st).

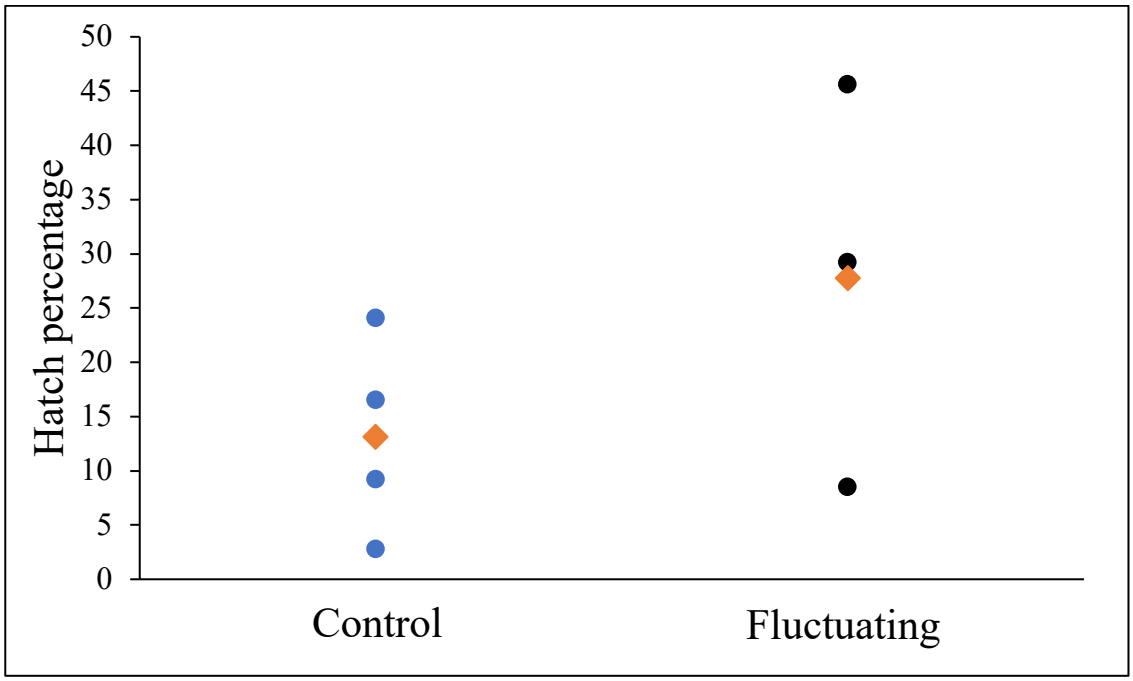

Figure 2-8.— Hatch percentage of each spawning pair for a given temperature treatment (represented by dots). Red diamonds represent the mean of each treatment.

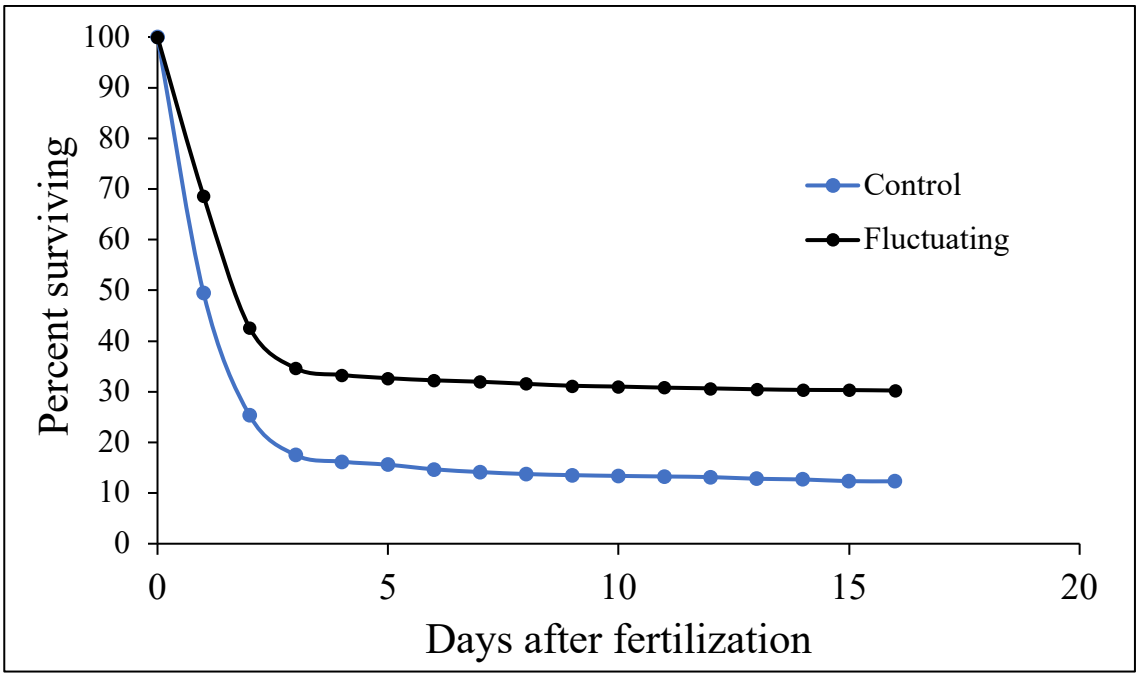

Figure 2-9.— Survival of Sauger eggs as a function of time post-fertilization. Lines represent all of the the eggs incubated across multiple spawning pairs, for each respective treatment.

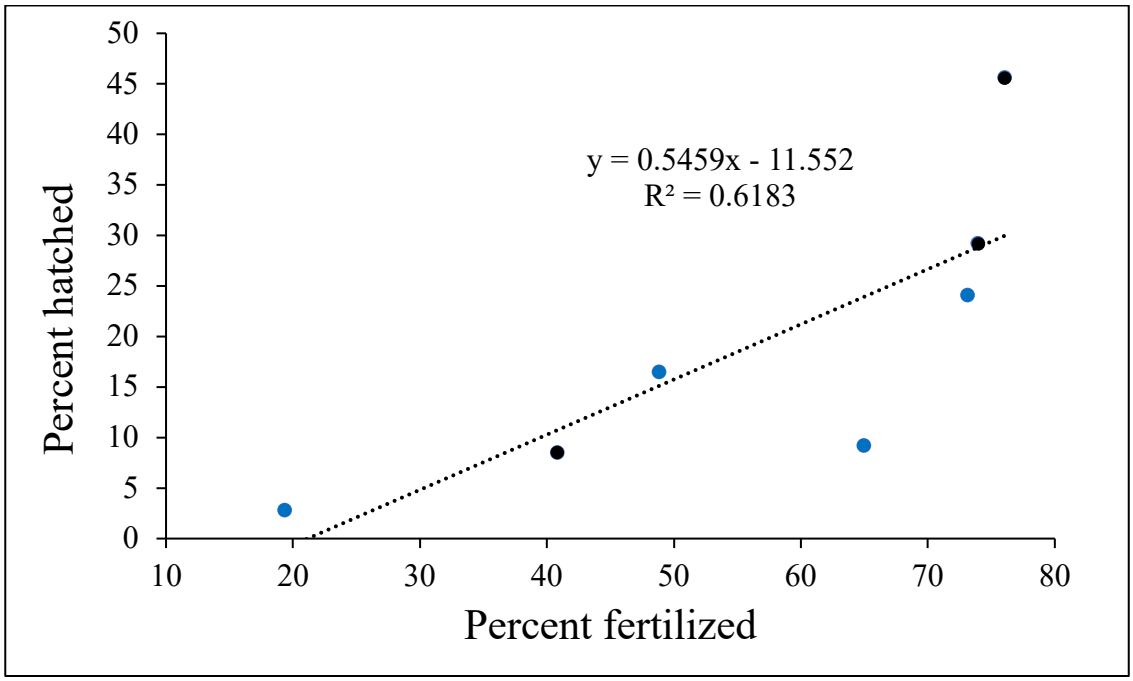

Figure 2-10.— Comparison of hatch percentage and fertilization percentage for Sauger eggs. Each point represents the values for an individual spawning pair for the control (blue) and fluctuating treatment (black). Linear regression is derived from all points (control and fluctuating).

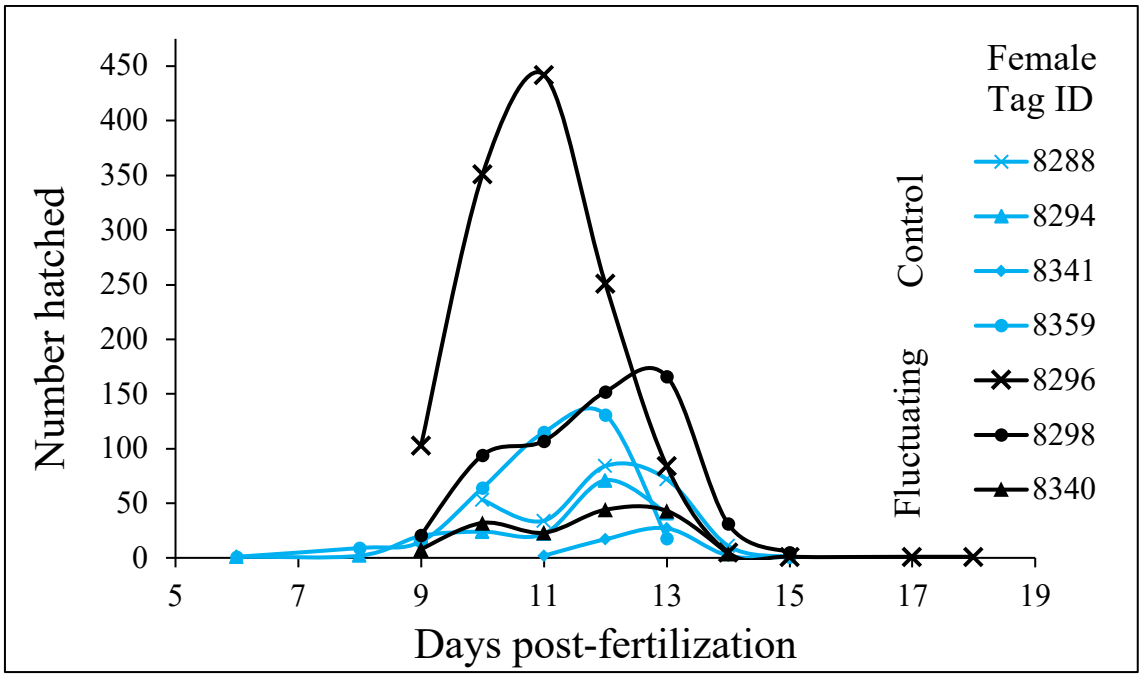

Figure 2-11.— Effects of adult holding temperature on the hatch timing of Sauger eggs from four females in the control treatment and three females from the fluctuating treatment. Each line represents the offspring of an individual female.

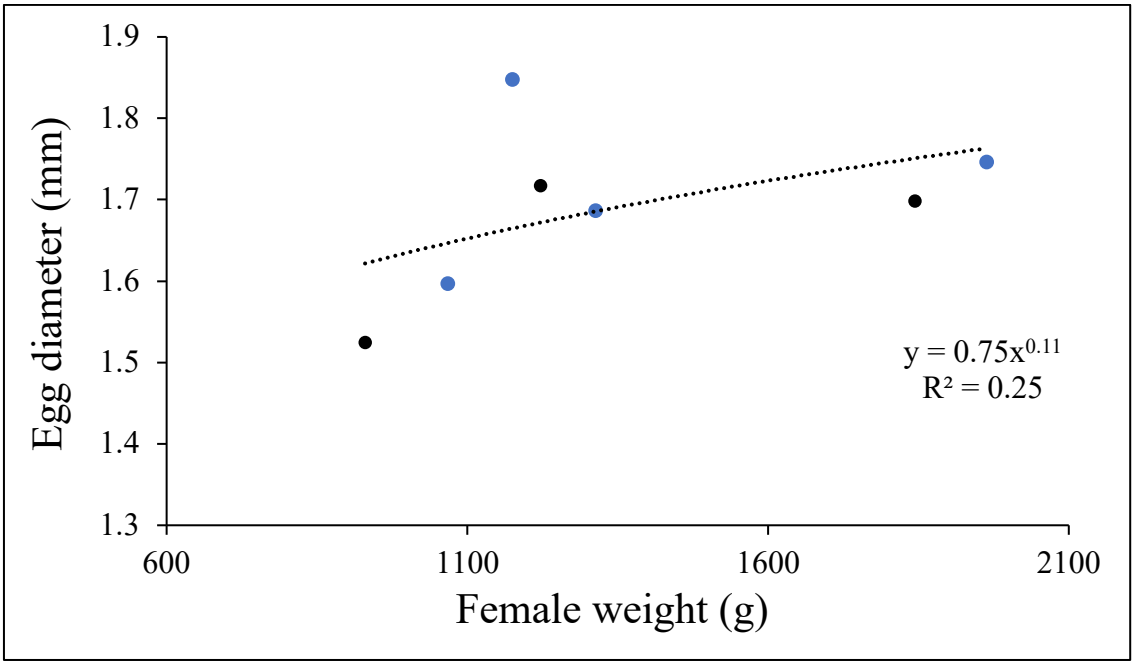

Figure 2-12.— Egg diameter as a function of female weight. Each point corresponds to the average total egg diameter (using the Von Baer Method) for each individual female that was spawned. Females in the control treatment represented by blue dots and females in fluctuating treatment, black dots. Power regression derived from all points (control and fluctuating).

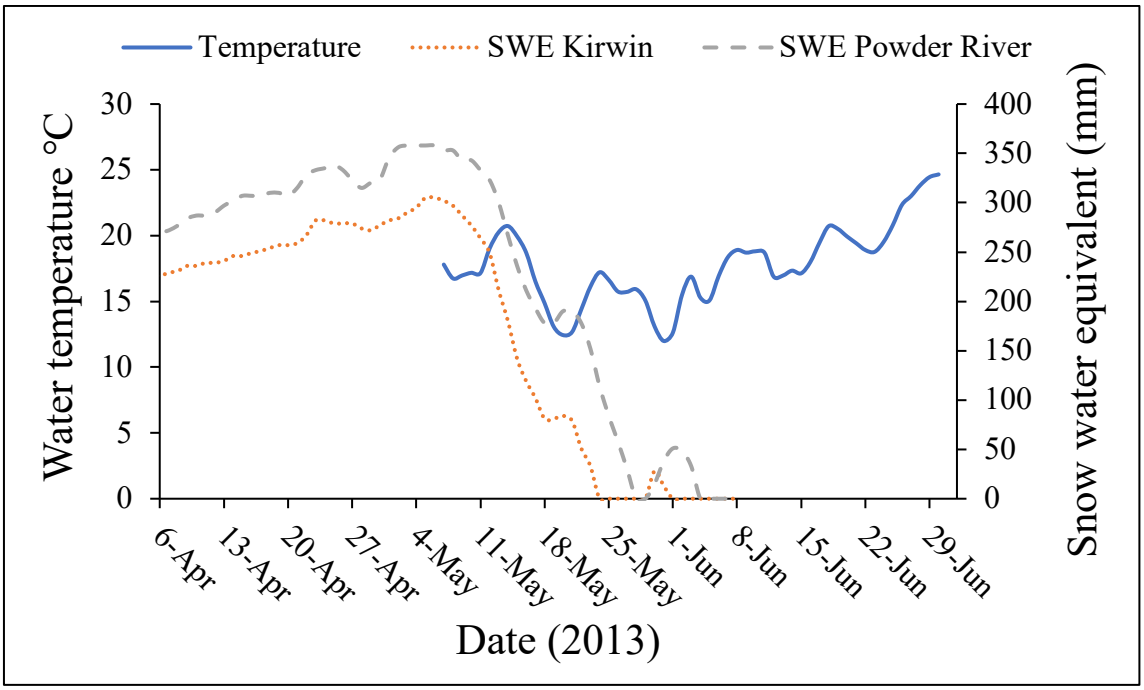

Figure 2-13.— Bighorn River water temperature in Manderson, WY (left axis) and snow water equivalent (right axis) for two snotel sites in the headwaters of the Bighorn River during spring of 2013.

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