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TEMPERATURE IMPACTS ON EMBRYONIC AND LARVAL DEVELOPMENT OF  
YELLOW PERCH (*PERCA FLAVESCENS*)

by

Anne Linkenheld

A Thesis Submitted in  
Partial Fulfillment of the  
Requirements for the Degree of

Master of Science  
in Freshwater Sciences and Technology

at

The University of Wisconsin-Milwaukee

May 2019

## ABSTRACT

### TEMPERATURE IMPACTS ON EMBRYONIC AND LARVAL DEVELOPMENT OF YELLOW PERCH (*PERCA FLAVESCENS*)

by

Anne Linkenheld

The University of Wisconsin-Milwaukee, 2019  
Under the Supervision of Professor Osvaldo Jhonatan Sepulveda-Villet

Early life stages of fishes are critical stages due to their importance in enhancing recruitment. Given the high mortality through the embryonic and larval stages, managers have started investigating factors that impact these stages. Environmental factors, such as water temperature, have been found to play a larger role in early life survival. Climate change predications will be more apparent in northern temperate systems like the Great Lakes. Yellow perch (*Perca flavescens*) are an important sport fish in the region whose populations have been declining since the 1980s. The development of yellow perch as an aquaculture species has occurred in order to meet consumer demands. Yellow perch recruitment is highly erratic due to the species dependence on spring water temperatures. With warming waters occurring earlier in the seasons, it is unsure how wild yellow perch will adapt. The literature suggests that warming water temperatures could either improve or hinder yellow perch recruitment through early life stages. Even in aquaculture, larval survival is still low in tank cultured yellow perch. An importance has been placed on finding one rearing methodology that yields the highest production of larvae. The objective of this study was to determine how variations in temperature regimes during the egg incubation period would impact embryonic and larval development in yellow perch. Four different temperature treatments were used in this study. The results of this study confirm that water temperatures severely impact embryonic development and incubation

periods of yellow perch. This study reveals that yellow perch are better adapted to withstand acute cold shifts in water temperature than acute warming events. The incorporation of cold shocks could yield higher percentages of viable larvae in tank cultured yellow perch if used in union with a gradual warming of water temperature during incubation. Climate change could potentially hinder an already struggling Lake Michigan yellow perch population causing a higher demand on producing more cultured yellow perch.

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## CHAPTER 1: INTRODUCTION

### 1.1 Early Life History Dynamics of Fishes

Life history of fishes have been studied for years in ecological systems. However, gaps in early life adaptations due to natural and anthropogenic stressors have become apparent as management needs have changed (Young et al. 2006). All fish populations undergo drastic shifts in habitats and trophic position throughout ontogeny, it is widely accepted that some life history stages are more critical due to overall impacts on the population (Wiedenmann and Essington 2006). The stage of life history that defines year class strength for fish does vary depending on the environment and species, however the bottleneck for recruitment often occurs during the first-year post-spawn for many species such as: yellow perch, African sharp-tooth catfish (*Clarias gariepinus*), and herring (*Clupea harengus* m.). (Kaemingk et al. 2014; Prokešová et al. 2015; Arula et al. 2016). The first year of development includes the of egg, larval, and early-juvenile life history stages, which are collectively referred to as the early life history stages (Jůza et al. 2010; Garrido et al. 2015; Zhang et al. 2017). It is commonly accepted that a high mortality rate (~99%) occurs through these early life developmental stages, specifically during the embryonic and larval stages compared to juvenile stages for yellow perch (Kaemingk et al. 2014; Bogner et al. 2016), European sardine (*Sardina pilchardus*) (Garrido et al. 2015), lake sturgeon (*Acipenser fulvescens*) (Forsythe et al. 2013) and northern pike (*Esox lucius*) (Vindenes et al. 2016). Massive mortality events can drastically affect a single year class which has a larger impact on short-lived species compared to long-lived species (Ohlberger and Langangen 2015).

As a consequence of high mortality rates in early life stages, many fish populations are dominated by few year classes rather than an even distribution of all year classes (Jůza et al.

2010; Ohlberger and Langangen 2015). Being able to accurately estimate larval and juvenile stages is an important factor in estimating adult populations for many species (Wiedenmann and Essington 2006; Jůza et al. 2010). The high mortality observed in the larval stages of fish is regarded to be size-dependent (Garrido et al. 2015; Feiner et al. 2016; Dembkowski et al. 2017). Offspring size variation is largely attributable to environmental conditions experienced in early life (Feiner et al. 2016) Larger size at hatch has been associated with increased survivability through the larval period of development (Garrido et al. 2015; Pagel et al. 2015; Dembkowski et al. 2017). Garrido et al. (2015) found that the probability of survival is closely related to the size of larvae at hatch. Larger larval size is often associated with being stronger swimmers that are less susceptible to predation (Bondarenko et al. 2015). Even the smallest improvement in early life survival could increase recruitment in both marine and freshwater fishes (Landsman et al. 2011; Weber et al. 2011; Garrido et al. 2015).

Due to the low survival in the early life stages, investigating mechanisms that impact growth variation in these stages will improve the knowledge of factors that impact recruitment dynamics (Pagel et al. 2015). Understanding recruitment dynamics is a challenge faced by fisheries professionals due to the complex factors that impact recruitment in fish populations (Redman et al. 2011; Bogner et al. 2016; Garrido et al. 2015; Zhang et al. 2017). There are several recruitment hypotheses that fisheries managers use, such as, “stock-recruitment” hypothesis that emphasizes the importance of density-dependent impacts on spawning stocks. Other hypotheses place higher focus on how growth during the larval and juvenile stages impacts survival (Zhang et al. 2017). However, no single hypothesis universally explains the variation in annual recruitment (Mangel et al. 2009; Zhang et al. 2017). Annual recruitment of fish can be highly variable (Bogner et al. 2016; Dembkowski et al. 2017). Fish recruitment is influenced by

many variables that determine the production and survival through critical early life stages (Collingsworth et al. 2017). While biological factors such as maternal effects (i.e. female size, egg and yolk sac size, etc.) are present in fishes, their impacts on recruitment variations are rather small compared to environmental conditions (i.e. water temperatures, wind disturbance, eutrophication, etc.) experienced during early life stages (Wiedenmann and Essington 2006; Landsman et al. 2011; Zhang et al. 2017). Feiner et al. (2016) documented that walleye exhibit maternal effects, however, the study also concluded that winter water temperature influenced the egg sizes produced by females.

## 1.2 Temperature Impacts on Early Life Stages

There is interest in increasing the understanding of the influence that climate change may have on population and reproductive dynamics of fish (Young et al. 2006; Lyons et al. 2015). Climate change could alter the physical and thermal environments during spawning season as well as through early life phases (Lyons et al. 2015; Lee et al. 2016; Collingsworth et al. 2017). Environmental conditions experienced during these early life stages could shape future fitness and abundance of a species at later life stages (Bogner et al. 2016; Rutherford et al. 2016). During the critical processes of embryonic and larval development, young individuals are sensitive to environmental conditions (Morgan and Rasin 1982; Vindenes et al. 2016; Rutherford et al. 2016). However, it has been found that fish eggs can tolerate temperature changes of  $\pm 6^{\circ}\text{C}$  without incurring serious negative impacts (Landsman et al. 2011). Rutherford et al. (2016) found that walleye (*Sander vitreus*) eggs were sensitive to temperature changes within the first 10 days post-fertilization. Embryos exposed to a heat shock could become immunocompromised and larval size could be negatively impacted (Lee et al. 2016).

Water temperatures can impact early survival of fishes (Landsman et al. 2011; Vindenes et al. 2016; Collingsworth et al. 2017; Zhang et al. 2017). Water temperatures often dictate hatching and survival of most larval fish (Rutherford et al. 2016). Major die-offs could be an effect of extreme climatic events (heat waves, hurricanes, severe cold snaps, floods, etc.) which are projected to increase in frequency due to climate change (Ohlberger and Langangen 2015). Infrequent thermal shocks (heat or cold) during the embryonic and larval phases could impact behavior after hatching and cause large larval mortality events (Landsman et al. 2011; Patrick et al. 2013; Feiner et al. 2016; Lee et al. 2016). Larval big-scale sand smelt (*Atherina mochon*) exposed to thermal shocks during embryonic development showed a modified schooling behavior (Lee et al. 2016). Extreme climate may impact species differently (Young et al. 2006; Ohlberger and Langangen 2015). If perturbations persist long-term, life history stages could change resulting in shifts in spawning phenology impacting offspring size and reproductive success (Feiner et al. 2016; Collingsworth et al. 2017). Pacific salmon species (i.e. chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus nerka*) salmon) stocks with warmer natal stream temperatures will better adapt to predicted increases in stream temperatures (Young et al. 2006).

Few studies have investigated how temperature impacts hatch phenology (duration of hatching including peak hatch) (Lyons et al. 2015; Bogner et al. 2016). Generally, the reproductive season remains stable, occurring in the same season each year. However, fish populations may shift timings of spawning within normal reproductive seasons (Bogner et al. 2016). Variation in spawning timing could have an impact on subsequent recruitment (Bogner et al. 2016). Hatching earlier in the season is often assumed to increase the fitness of offspring for species such as pumpkinseed (*Lepomis gibbosus*) (Murphy et al. 2012), northern pike

(Bondarenko et al. 2015; Pagel et al. 2015), and yellow perch (Farmer et al. 2015). Increasing fluctuations in water temperatures during incubation and hatching periods could disfavor early hatching offspring if food availability is scarce (Patrick et al. 2013; Pagel et al. 2015). Projected climate-driven changes to thermal conditions may produce faster spring warming and longer stratification in lakes. A change in reproductive phenology could vary based on species specific requirements for ovarian development and dependence on climate-driven environmental cues (Collingsworth et al. 2017). Such shifts have been documented in fish populations in lakes, rivers, estuarine, and oceanic environments (Taylor 2008; Collingsworth et al. 2017). In Lake Erie, both walleye and yellow perch spawn earlier following a warm winter with an early spring onset compared to cold winters with a delayed spring onset (Farmer et al. 2015). Prolonged incubation periods due to colder water temperatures in northern pike had low larval survivorship as well as an increase in malformed larvae (Bondarenko et al. 2015).

Studies have continually emphasized the relevance of temperature in variation of size and growth in early life stages (Pagel et al. 2015). Temperature is known to impact potential food that is available to larval fish, therefore the time of spawning and hatching plays a critical role in the trajectory of growth for offspring (Lyons et al. 2015; Collingsworth et al. 2017). In centrarchid species, early hatching results in faster growth which increases predation avoidance (Pagel et al. 2015). Warm-water condition may promote higher growth in some species (Collingsworth et al. 2017). Faster growth rates could reduce the time that larval fish spend in a vulnerable ontogenetic stage (Dembkowski et al. 2017). Many recruitment hypotheses postulate that faster growth in early life stages creates higher survival and increased recruitment in later life stages (Dembkowski et al. 2017).

### 1.3 Yellow Perch in the Great Lakes Region

In the Great Lakes region, yellow perch are a favored recreational sport fish and important commercial species in areas where they are abundant (Marsden and Robillard 2004; VanDeHey et al. 2013; Kaemingk et al. 2014; Dembkowski et al. 2016; Collingsworth et al. 2017). Yellow perch are typically found in the shallower, more productive basins around the lakes, such as Green Bay (Lake Michigan) and the western basin of Lake Erie (Collingsworth et al. 2017). During the 1980s recreational and commercial fisheries expanded, increasing harvest of the species by 10-fold. Very little coordination occurred between state agencies on management of recreational harvests to prevent over-harvesting of the species (Marsden and Robillard 2004). Agencies first documented dwindling numbers of age-0 fish after 1988 and restrictions to commercial fishing on Lake Michigan occurred in 1994 when it was evident that recruitment was not improving (Marsden and Robillard 2004). By 1997, commercial fisheries for yellow perch were closed on Lake Michigan proper and managers were faced with an aging and declining population without understanding the cause of the decline (Marsden and Robillard 2004). Over the last decades, yellow perch recruitment has become exceedingly erratic (Redman et al. 2011). As abundance of yellow perch declined, the sex ratio became skewed towards males due to overexploitation of faster growing and larger females before all commercial fishing was either closed or restricted on the species (Redman et al. 2011). Presently, Lake Michigan only has one commercial fishery open in the Green Bay waters, with restrictions during spawning season (Mecozzi 2008) while a majority of commercial caught yellow perch comes from the Canadian waters of Lake Erie (Purchase et al. 2005).

In the Great Lakes, yellow perch recruitment variability is dependent on spring temperatures on a broad scale but biotic factors at a local scale (e.g. food availability and

predator abundance) also are a source of differentiation in recruitment (VanDeHey et al. 2013; Kaemingk et al. 2014; Bogner et al. 2016; Dembkowski et al. 2017). Yellow perch spend 30-60 days as pelagic larvae during early life stages, which is unusual for a freshwater species (Weber et al. 2011). Due to their prolonged pelagic phase, yellow perch larvae are susceptible to offshore conditions, such as water temperatures or currents (Redman et al. 2011; Weber et al. 2011). In general, successful hatching increases when water temperatures gradually warm for yellow perch (Jansen et al. 2009). Yellow perch and Eurasian perch (*Perca fluviatilis*) year-class strength is the highest during springs with stable, warm water temperatures while low year-class strength is connected to springs with water temperature fluctuations (Jansen et al. 2009; Weber et al. 2011). Yellow perch spawning events often occur in very narrow time periods, some have been documented as lasting only five days. (Kaemingk et al. 2014; Bogner et al. 2016). For this reason, yellow perch are more susceptible to weak year classes or failures (Weber et al. 2011; Bogner et al. 2016). In the Great Lakes, a few year classes typically dominate the population structure of yellow perch (Marsden and Robillard 2004). Ohlberger and Langangen (2015) found that populations that are only dominated by a few year classes are at a higher risk of a population crash with little chance of recovery. This type of collapse has already been documented in the Lake Michigan yellow perch population. The collapse could be due to both bottom-up and top-down effects on yellow perch in the Great Lakes (Janssen et al. 2014; Roloson et al. 2016; Zhang et al. 2017). The recovery of threatened populations is determined by the resilience of reproduction and subsequent recruitment of the populations (Mangel et al. 2009).

Climate change may have a negative effect on yellow perch recruitment during embryonic development by altering the incubation and hatching period of larval yellow perch (Collingsworth et al. 2017; Dembkowski et al. 2017). Water temperature is believed to play a



primary role in yellow perch reproduction and early life survival (Jansen et al. 2009; Redman et al. 2011). There have been previous studies that claim potential warming waters may benefit yellow perch in the Great Lakes (Collingsworth et al. 2017). However, a study by Farmer et al. (2015) found that warm winters may reduce egg quality and embryonic development leading to a decrease in hatching success of yellow perch. Yellow perch would be expected to shift their spawning period to earlier in spring as a result of the prediction of earlier spring onsets (Collingsworth et al. 2017). Lyons et al. (2015) documented earlier spawning in Lake Michigan yellow perch in response to earlier spring onset with advancements by 1.8 to 6.8 days per decade since the 1980s. The shift that yellow perch demonstrate following a warm winter is somewhat constrained (only advancing by a week) relative to a thermal regime shift (advancement by three weeks) causing a mis-match with prey (Farmer et al. 2015; Collingsworth et al. 2017).

#### 1.4 Importance of Yellow Perch in Aquaculture

Wild populations of yellow perch in Lake Michigan have diminished to the point where commercial and recreational harvest of the species is much reduced (Brown et al. 2002). Restrictions and closures of commercial fisheries coupled with an increasing demand for yellow perch has fueled the interest in generating yellow perch as an aquaculture species (United States Department of the Interior (USDI) 1995; Food and Agriculture Organization of the United Nations 2010; Rosauer et al. 2011). Yellow perch aquaculture has become a focal point in areas where the species once was prominent: The Great Lakes region (Kolkovski and Dabrowski 1998; Brown et al. 2002; Suchocki and Sepulveda-Villet 2019). However, even with a high demand for yellow perch, the yellow perch aquaculture industry in the United States does not have high enough production to meet current demands on its own (Rosauer et al. 2011). It typically takes 15-18 months in ponds or 9-12 months in recirculating systems for yellow perch to reach harvest

size (150g) (Rosauer et al. 2011). The grow out period for yellow perch is comparable to channel catfish and hybrid striped bass but the harvest size is much larger for the other species compared to yellow perch (Rosauer et al. 2011). This has pushed research into growth rates to the forefront of yellow perch aquaculture (Malison 2003; Rosauer et al. 2011). Up to now, most studies have looked into improving culture conditions during the grow out periods (e.g. optimal temperature and increased photoperiods) in addition to selecting genetic strains that have higher growth rates (Rosauer et al. 2011). Yellow perch aquaculture largely has focused on how to manipulate adult yellow perch by changing spawning periods to out of season (Malison 2003; Rosauer et al. 2011). Very little research has been conducted on early life stages of yellow perch for aquaculture.

Yellow perch have a unique egg strand form which breaks down during incubation creating a challenge for rearing compared to other cultured fish (Hart et al. 2006). The common practice in yellow perch aquaculture is incubating egg ribbons by steadily increasing the water temperature by 1°C every two days (Hart et al. 2006). However, some researchers believe that this method causes larvae to hatch too early leading to developmental problems and higher mortality (Hart et al. 2006). Incubating at a steady temperature could vary the rate of embryonic development by increasing or decreasing the incubation period (Hart et al. 2006). Egg incubation practices for yellow perch aquaculture need more research to determine a single method that will yield the best survival (Hart et al. 2006). Survival through the larval stage in cultured yellow perch is still extremely low (Suchocki and Sepulveda-Villet 2019). When using tank culture, many researchers and commercial producers have yet to consistently maintain high larval survival (Hart et al. 2006). Some research has suggested that survival rates of 70% or higher can be achieved in tank culture, but methods for achieving this high of survival has not been

published (Hart et al. 2006). It is commonly accepted, even in cultured yellow perch, that larval growth is highly variable (Brown et al. 1996; Hart et al. 2006). Variable hatch rates occur in yellow perch which could be the source of larval growth variation seen in aquaculture (Hart et al. 2006). In aquaculture, predatory species have been known to become cannibalistic during the early larval stages (Marsden and Robillard 2004; Pagel et al. 2015; Naumowicz et al. 2017). Cannibalism could be an effect of variation in growth within cohorts (Naumowicz et al. 2017; Schaefer et al. 2017). Researchers need to look into improving physical metrics in tank culture practices that could increase larval survival and decrease growth variations (Hart et al. 2006).

### 1.5 Objectives and Hypotheses

Water temperature plays a crucial role in yellow perch early life stages. Previous research either suggests warming waters may benefit or inhibit yellow perch populations. Given that wild populations in Lake Michigan have already severely declined, interest in developing yellow perch aquaculture has grown over the decades. However, cultured yellow perch embryonic and larval survival are still below optimal levels. Further developing techniques to ensure the highest survival rate through these early life stages is needed in yellow perch aquaculture before it can achieve commercial success.

The objective of this study was to determine how variations in temperature regimes during the egg incubation period would impact embryonic and larval development in yellow perch. To achieve this, yellow perch were hatched and observed throughout the full larval stage. Several hypotheses were addressed within this objective: 1) Temperature treatments will not impact egg or larval mortality throughout incubation 2) Temperature treatments will not impact production of larvae 3) Temperature treatments will not impact the incubation period (i.e. time of

hatch out of larvae) 4) Temperature treatments will not impact initial size, growth, or final size of larvae.

## CHAPTER 2: METHODS

### 2.1 Yellow Perch Gamete Collection

Gametes were collected from the laboratory of Fred Binkowski at the University of Wisconsin-Milwaukee School of Freshwater Sciences (Milwaukee, Wisconsin). The strain of yellow perch used in this study was a Northeast River strain broodstock originating from wild yellow perch gametes from the Northeast River in Chesapeake Bay, Maryland. The broodstock used in this study is the F-1 generation and has been managed for six years at the School of Freshwater Sciences.

Milt was collected from three different males and pooled together eliminating any potential male variation over the course of the study. An immobilization solution (.33M sucrose solution) was added to preserve the milt ensuring all eggs were fertilized with the same milt (Glogowski et al. 1999; Miller et al. 2018). Once egg ribbons were acquired from five different females, each female was kept for weight and length measurements. Each egg skein was fertilized with 50 mL of the preserved milt solution. Prior to splitting each egg ribbon up into experimental replicates, total weight and volume of each ribbon was recorded.

### 2.2 Experimental Design

The experiment was a randomized block design with the blocking factor as females. This was done to account for variation in females while primarily testing how temperature impacts embryonic and larval development. Egg ribbons were divided into four segments. One segment from each female was put into each of the four temperature treatments (Fig. 1). The temperature treatments were as follows:

- Steady Treatment: eggs were held at 16°C for entire incubation period.
- Standard Treatment: Common gradient increase in temperature throughout incubation starting at 12°C increasing by 1-2°C every 3-4 days.
- Heat Shock Treatment: on day six of incubation eggs were exposed to 20°C temperatures for 16 hours to mimic a heat wave event.
- Cold Shock Treatment: on day six of incubation eggs were exposed to 10°C temperatures for 16 hours to mimic a cold snap event.

Temperature treatments were set up on individual sump systems to run all the treatments at the same time. Each temperature treatment system contained five 3.5L plastic buckets housed in 25-gallon tank inserts (Fig. 1). Each female egg segment was kept separate per treatment and was randomly assigned to a bucket. Once larvae started hatching out, a select number from each replicate were moved to 6L buckets housed in 25-gallon tank inserts on the same sump system (Fig. 2).

### 2.3 Data Collection

Initial egg parameters were collected the day of spawning (Oct. 9<sup>th</sup>, 2018). Five one-milliliter samples of each egg ribbon were collected to estimate total egg count, egg volume ( $\text{mm}^3$ ), oil drop volume ( $\text{mm}^3$ ), and fertilization rates (%) prior to separation into replicates. At the end of incubation, three egg ribbon samples per segment were collected to quantify egg mortalities: dead eggs: completely opaque egg (%), failed larvae: eyed up larvae but opaque (%), and viable larvae: eyed up larvae and translucent or a ruptured empty egg (%) (Fig. 3). Larval parameters were collected once hatch out occurred. Initial lengths of larvae were taken day of hatch out. Larvae were moved into grow out buckets held at the same temperature for the

duration of the larval phase (28 days; Mansueti 1964), and mortalities were recorded. Larval lengths were measured from recovered mortalities every four days for growth rates. All mortalities for each replicate were preserved in formalin in case any samples needed to be re-evaluated. At the end of the larval period all surviving larvae from each replicate were measured for final length. Estimated mortality (%) was calculated to account for any discrepancies between initial number, Observed Mortalities (recovered dead larvae) (%), and Survivals (%).

$$\text{Observed Mortalities} = \frac{\text{Recovered Mortalities}}{\text{Total Mortalities}} \times 100$$

$$\text{Est. Mortalities} = \frac{\text{Initial Count of Larvae} - \text{Final Count of Larvae} - \text{Observed Mortalities}}{\text{Total Mortalities}} \times 100$$

$$\text{Survival} = \frac{\text{Final Count of Larvae}}{\text{Initial Count of Larvae}} \times 100$$

All egg and larval measurements were taken using ImageJ (version 1.52k, January 2019) image analysis software (National Institute of Health, Bethesda, MD, <http://imagej.nih.gov/ij>). Digital images were taken using Canon EOS RebelT5i camera.

## 2.4 Statistical Analyses

Statistical analyses were carried out with JMP Pro 14 (SAS Institute, Cary, NC). Skein weight (g), egg count, egg volume (mm<sup>3</sup>), oil drop volume (mm<sup>3</sup>), and the oil drop volume to total egg volume ratio were assessed to determine relationships between one another using a linear regression. Egg and oil drop volumes were calculated using diameters assuming a spherical shape:

$$\text{volume} = \frac{1}{6} \pi d^3$$

The ratio of oil drop volume to total egg volume was calculated using:

$$\frac{\text{oil drop volume (mm}^3\text{)}}{\text{total egg volume (mm}^3\text{)}}$$

To determine if there was a difference between ribbon parameters described above between temperature treatments unpaired *t*-tests were conducted. Randomized block *F*-test with a Tukey-Kramer HSD post-hoc test was used to analyze embryonic development dependent variables: percent viable larvae, failed larvae, and dead eggs. Randomized block *F*-test with Tukey-Kramer HSD post-hoc tests were used with larval dependent variables: time to hatch, percent survival (%), observed and estimated mortality (%), initial size (mm), average growth rate (mm/day), and final size (mm) (See Appendix B). The formula used to calculate average growth rates:

$$\frac{\sum \left( \frac{\Delta \text{Length (mm)}_{\text{Day } 1-5}}{\# \text{ of days}} \right), \left( \frac{\Delta \text{Length (mm)}_{\text{Day } 6-9}}{\# \text{ of days}} \right), \dots, \left( \frac{\Delta \text{Length (mm)}_{\text{Day } 26-28}}{\# \text{ of days}} \right)}{\text{total \# of days (28)}}$$

For all statistical analyses, means were considered significantly different when  $p < 0.05$ .



## CHAPTER 3: RESULTS

### 3.1 Temperature and Embryonic Development

Egg ribbon sections weighed between 72.5 to 90.9 g among the replicates, which could vary total egg amounts between replicates. Mean weights ( $\pm$ SD) for the standard, steady, heat shock, and cold shock temperature treatments were as follows: 80.8 g ( $\pm$ 4.7); 82.7 g ( $\pm$ 7.6); 79.5 g ( $\pm$ 5.2); 85.1 g ( $\pm$ 5.2) (Table 1a). There was no significant difference between temperature treatments for both weights of egg ribbon sections and egg amounts. A positive relationship emerged between weight of skein section (x) and the number of eggs (y) ( $y = 280.2x + 607.6$ ,  $r^2 = 0.23$ ,  $df = 19$ ,  $p = 0.033$ ). Total number of eggs ranged from 18,500 to 30,960 per replicate. All other egg parameters (e.g. egg volume, oil drop volume, and oil drop to total egg volume ratio) showed no significant difference between temperature treatments (Table 1b). There was a negative relationship between average egg volumes (x) and the ratio of oil drop to total egg volume (y) ( $y = 0.04 - 0.01x$ ,  $r^2 = 0.79$ ,  $df = 19$ ,  $p < 0.0001$ )

Temperature treatment did not have a significant impact on the percent of dead eggs at the end of the incubation period ( $p = 0.0958$ ,  $F_{3,12} = 2.66$ ). Temperature treatment had a significant impact on the percent of failed larvae at the end of the incubation period ( $p = 0.0005$ ,  $F_{3,12} = 12.5$ , Fig. 4a, See Appendix A). Tukey tests showed the cold shock treatment average percent of failed larvae (0.8%) was significantly lower than the steady treatment average (22.9%) ( $p = 0.0011$ ) and the heat shock average (17.6%) ( $p = 0.0092$ ). The standard treatment average (3.8%) was also significantly lower than the steady treatment average ( $p = 0.0036$ ) and the heat shock treatment average ( $p = 0.0312$ ). The cold shock treatment had the lowest percent of failed larvae (0.8%) while the steady treatment had the highest percent (22.9%) (Table 2). Temperature

treatment had a significant impact on the percent of viable larvae ( $p = 0.0425$ ,  $F_{3,12} = 3.71$ , Fig. 4b, See Appendix A). The cold shock treatment average (49.6%) was significantly higher than the heat shock treatment average (19.9%) ( $p = 0.0325$ ) (Table 2).

### 3.2 Temperature and Larval Development

The heat shock temperature treatment and final size variable were not included in the larval analyses due to poor survival. Temperature treatment did have a significant impact on the time it took for larvae to successfully hatch out ( $p < 0.001$ ,  $F_{2,8} = 76$ , Table 3, Fig. 5, See Appendix A). The steady treatment larvae started hatching after 10 days post-fertilization (dpf) and finished hatching after 11 days which was significantly faster than both the standard treatment ( $p < 0.001$ ) and the cold shock treatment ( $p < 0.001$ ). The standard treatment larvae started hatching out 12 dpf and finished hatching out after 13 days. The cold shock treatment larvae took the longest, hatching out after 13 dpf which was significantly longer than the standard treatment ( $p < 0.0148$ ). Temperature treatment did not have an impact on the initial size of larvae ( $p = 0.116$ ,  $F_{2,8} = 2.85$ ). Temperature treatment did not have an impact on observed mortality and estimated mortality ( $p = 0.96$ ,  $F_{2,8} = 0.03$ ). Temperature treatment did not impact the percent of surviving larvae ( $p = 0.35$ ,  $F_{2,8} = 1.19$ ). Temperature treatment did not impact the average growth rate of larvae ( $p = 0.16$ ,  $F_{2,8} = 2.31$ ).

## CHAPTER 4: DISCUSSION

### 4.1 Role of Temperature on Embryonic Development

Our study found that temperature does have an impact on embryonic development of yellow perch. Temperature did not cause an increase or decrease of egg mortality between treatments. We believe this is due to the importance that fertilization plays in initial development. However, it has been documented that variable thermal shocks have increased the mortality of incubating eggs of warm-water fish species (Murphy et al. 2012). Yellow perch egg ribbons can result in erratic mortality patterns both in the wild and culture setting. However, variation in egg mortality could be minimized in yellow perch given how dependent the species is on water temperature (Marsden and Robillard 2004; Bogner et al. 2016). Further investigation into the factors impacting egg mortality may provide higher survival for cultured yellow perch as well as providing insight for wild populations.

Water temperature had the most impact on the percent of failed larvae. The cold shock treatment had the lowest percent of failed larvae while both the heat shock and the steady treatment had the highest percent failed. The cold shock treatment appeared to negate the failed larvae phenomenon that occurs in yellow perch embryonic development. Our findings contradict previous studies that claim acute cold fronts decreases the survival of yellow perch embryos and subsequently lower numbers of larvae are produced (Longhenry, M.S. 2006; Jolley, Ph.D. 2009). The standard treatment also demonstrated low percent of failed larvae. Hokanson and Kleiner (1974) reported that yellow perch hatching percentage is the highest when water temperature rises by 0.5-1°C per day from an initial optimal temperature (5-10°C). This supports the wide acceptance that a slow steady increase in temperature during incubations periods is optimal both in wild and cultured yellow perch.

We found temperature had a significant overall impact on the number of viable larvae produced. Jansen et al. (2009) also found that a decrease in water temperature simulating a spring cold-front had very little effect on the hatching success of yellow perch eggs. Landsman et al. (2014) found that both smallmouth bass (*Micropterus dolomieu*) and largemouth bass eggs were more resilient to acute cold changes in temperature than acute warm water changes. However, in colder than optimal temperatures the abundance of viable larvae decreases thus impacting overall survival of walleye larvae (Rutherford et al. 2016). In 2005 and 2009 average water temperatures during yellow perch spawning were low (2005: 11°C; 2009: 12.4°C) at Little Tail Point, Green Bay, a known spawning location for yellow perch. The following survey at that location for age-0 perch showed high abundances (2005: 7,310; 2009: 9,815). The heat shock treatment had the lowest percent of viable larvae in addition to causing three skein sections to completely fail by producing no viable larvae. Three days post-temperature exposure, the heat shock treatment embryonic development either sped up or completely failed. Data from Little Tail Point, Green Bay showed that 2007 and 2012 were unusually hot years (2007: 16°C; 2012: 16.9°C) during yellow perch spawning and larval periods. Those years, age-0 yellow perch were at very low abundance (2007: 702; 2012: 985). Given the results of our study and others, it is evident that yellow perch may have adapted to withstand substantial cold shocks during embryonic development (Jansen et al. 2009; VanDeHey et al. 2013). It has been documented that walleye are also very resilient to cold temperature fluctuations (Rutherford et al. 2016). Our findings do support Farmer et al. (2015) that yellow perch embryonic survival could be susceptible to short, warm winters that are predicted to increase in occurrence.

#### 4.2 Role of Temperature on Larval Development

We found that temperature did determine the incubation period of yellow perch egg ribbons. The steady treatment hatched out only after 10 days of incubation. While the heat shock was not included due to lack of replication power, the two ribbons segments that produced larvae hatched out after between 10 to 11 days of incubation. The cold shock treatment was the only treatment that all ribbon segments hatched out on the same day (after 13 days of incubation). The cold shock treatment larvae development was normal although slower than the other treatments while the steady and heat shock larvae developed faster than normal for yellow perch.

Bondarenko et al. (2015) found that when incubation temperatures fell outside the normal range for northern pike it resulted in a shift of the hatching period by either prolonging or accelerating embryonic development. Previous studies also have documented that low temperatures during incubation slows development and growth of eggs as well as larvae (Morgan and Rasin 1982; Murphy et al. 2012). Bogner et al. (2016) found higher larval yellow perch abundances when hatching periods were initiated earlier in the year when temperatures are cooler. Following a warm winter, yellow perch have been documented spawning up to a week earlier which constrains optimal hatching by spawning in warmer waters (Farmer et al. 2015; Collingsworth et al. 2017). Lyons et al (2015) found that yellow perch in Lake Michigan were shifting spawning time in order to align with preferred spawning temperature. However, yellow perch may not be adjusting spawning time fast enough to keep pace with projected warming water temperatures (Farmer et al. 2015). Walleye have been documented shifting its reproductive phenology by two months earlier to match with preferred water temperatures in Lake Erie (Collingsworth et al. 2017). Prokešová et al. (2015) also found that colder than average water temperature increased the duration of the incubation period in African sharp-tooth catfish, further supporting our evidence that colder temperatures increased incubation periods by prolonging embryonic

development. Warm water species like centrarchids have also been documented having earlier spawning and hatch-out when water temperatures were warmer than average (Murphy et al. 2012).

We believe the low survival documented through the larval period impacted our ability to determine if temperature had an impact on larval development. The heat shock treatment produced low numbers of larvae and was not included in any statistical analyses for larval development. In addition to having to remove final size variable since some buckets had no survival by the end of the larval period. We believe that the low survival of larvae in our study was due to a high amount of cannibalism rather than the temperature treatments. Cannibalism is well documented in many fish species especially in early life stages (Pagel et al. 2015; Naumowicz et al. 2017). Cannibalism is highly prominent in predatory species used in aquaculture (Naumowicz et al. 2017). Pagel et al. (2015) found that the earlier onset of cannibalism in northern pike resulted in a growth advantage since fast, early growth is positively related to survival. Schaefer et al. (2017) found that a wide variation in growth and size of larvae promotes cannibalism within cohorts. Cannibalism is a major problem in commercial production of predatory fish and ranges from 15% to 90% of individuals (Naumowicz et al. 2017). Previous studies suggest that short and warm winters could weaken annual recruitment of yellow perch by decreasing initial larval survival (Weber et al. 2011; Farmer et al. 2015) However, warm spring and summer temperatures have been linked to positive yellow perch and walleye recruitment across the Great Lakes (Collingsworth et al. 2017). The predictions of more dynamic weather patterns during spring may only increase the occurrence of erratic recruitment patterns in yellow perch populations (Bogner et al. 2016). In order to determine if climate change will impact initial

larval survival more studies will need occur with higher replication in order to account for potential cannibalism related mortalities.

While survival through the larval phase was low due to cannibalism, we did start to see some trends in initial size and growth of larvae. The steady treatment hatched out the earliest and was initially the largest (5.36 mm). However, it had a slower growth rate (0.07 mm/day) compared to the cold shock treatment (0.105 mm/day) and the standard treatment (0.108 mm/day). Previous studies support the claim of yellow perch larvae that hatch out later exhibit faster growth rates than larvae with earlier hatch dates (Weber et al. 2011; Bogner et al. 2016). Kaemingk et al. (2014) also found water temperature and hatch date had a positive impact on larval yellow perch growth rates. Studies have also found that in centrarchids, early hatching has increased growth advantages (Pagel et al. 2015). Growth rates commonly determine survival of larval yellow perch in northern regions due to the shorter growing seasons (Weber et al. 2011). Dembkowski et al. (2016) found that environmental conditions experienced during the post-emergence period could play a larger role in larval yellow perch abundance than those during spawning and incubation periods. A reduction of reproductive success has been found in wild yellow perch following short warm winters by a decline in hatching success and smaller larval sizes at hatch-out (Weber et al. 2011; Farmer et al. 2015). Pagel et al. (2015) found that northern pike that hatched out early in the season under cooler water temperatures did not grow as well as late-season larvae that hatched when water temperatures were warming to more favorable growth conditions. Similar results have been documented in walleye larvae where total length of larvae increased as temperatures warmed (Rutherford et al. 2016). Further replication is needed in order to confirm the trend of water temperature impacting initial length and growth rates in larval yellow perch.

### 4.3 Conclusions

These results provide evidence that yellow perch embryonic development is highly dependent on water temperature during the incubation period. The cold shock treatment has a low percent of failed larvae and the high percent of viable larvae. The standard treatment which is commonly used to rear yellow perch in aquaculture had a low percent of failed larvae in addition to having a high percent of viable larvae. Given how well both treatments performed, aquaculture practices could still use a gradual increase of water temperatures but have cooler initial start temperatures. The heat shocks results show that yellow perch are not well adapted to acute warm temperature changes. This treatment clearly damaged developing embryos in addition to causing massive mortality events. This suggests that wild yellow perch will not be able to adapt spawning phenology enough to keep up with increasing water temperatures predicted to occur with climate change.

This study also confirms that temperature impacts the incubation period. We found that warmer temperature treatments (steady and heat shock) shortened the incubation period while the cold shock and standard treatments were significantly longer. The cold shock treatment all hatched on the same day potentially decreasing growth variation and increasing food competition. Further research would need to occur in order to determine if synchronized hatching can help eliminate growth variation causing higher rates of cannibalism. The steady treatment larvae hatched out the earliest and were larger with slower growth rates compared to the cold shock and standard treatments. However, more research with higher replication will be needed in order to conclude if water temperature has an impact on larval development.



Table 1a. Egg ribbon metrics by treatment. Mean egg ribbon weights ( $\pm$ SD) per temperature treatment. Egg counts and fertilization rates per temperature treatment were calculated using ImageJ.

Treatment	Ribbon Weight (g)	Egg Count	Fertilization Rate (%)
Steady	82.7 ( $\pm$ 7.6)	23,500	99.95
Standard	80.8 ( $\pm$ 4.7)	23,300	99.95
Cold Shock	85.1 ( $\pm$ 5.2)	23,700	99.95
Heat Shock	79.5 ( $\pm$ 5.2)	23,800	99.95

Table 1b. Egg ribbon metrics by female yellow perch used in study. Averages per temperature treatment were all the same since each female had a ribbon section in each treatment. Egg and oil drop volumes were calculated based on diameter measurements using ImageJ. Oil drop to total egg volume ratio was calculated based per female.

Female Length (mm)	Avg. Egg Volume (mm <sup>3</sup> )	Avg. Oil Drop Volume (mm <sup>3</sup> )	Oil Drop to Total Egg Volume Ratio
293	2.40	0.05	0.02
302	3.10	0.04	0.01
326	2.10	0.05	0.02
320	2.70	0.06	0.02
309	3.20	0.04	0.01

Table 2. Embryonic development of each treatment at the end of the incubation period. Viable larvae include alive formed larvae and ruptured eggs indicating successful hatch. Dead eggs are completely opaque eggs. Failed larvae are completely opaque formed larvae that died before hatch-out. Counts of each category were performed with ImageJ. Tukey-Kramer HSD post hoc results are in parentheses next to treatments means. Treatment means that share the same letter are not significantly different from one another.

Treatment	Percent Viable Larvae (%)	Percent Dead Eggs (%)	Percent Failed Larvae (%)
Steady	27.9 (ab)	49.2	22.9 (a)
Standard	30.8 (ab)	65.4	3.8 (b)
Cold Shock	49.6 (a)	49.6	0.8 (b)
Heat Shock	19.9 (b)	62.5	17.6 (a)

Table 3. Average larval development metrics of each temperature treatment. Average incubation period was calculated using the start of hatch-out through the end. Initial lengths ( $\pm$ SD) and growth rate samples were measured using ImageJ. Percent (%) survival and mortalities were calculated from initial and final counts. Growth rate was calculated from samples taken every 4 days for 28 days. Tukey-Kramer HSD post hoc results are in parentheses next to treatments means. Treatment means that share the same letter are not significantly different from one another.

Treatment	Incubation Period	Initial Count	Initial Length (mm)	Final Count	% Survival	% Mortality (Observed)	% Mortality (Estimated)	Growth Rate (mm/day)
Steady	10.5 (c)	<b>332 Total</b> (66 average)	5.30 ( $\pm$ 0.30)	<b>6 Total</b> (1.2 average)	2.30	52.7	47.9	0.10
Standard	12 (b)	<b>330 Total</b> (66 average)	4.98 ( $\pm$ 0.36)	<b>1 Total</b> (0.2 average)	0.27	55.0	45.0	0.11
Cold Shock	13 (a)	<b>375 Total</b> (75 average)	4.96 ( $\pm$ 0.27)	<b>4 Total</b> (0.8 average)	1.07	53.6	46.4	0.12

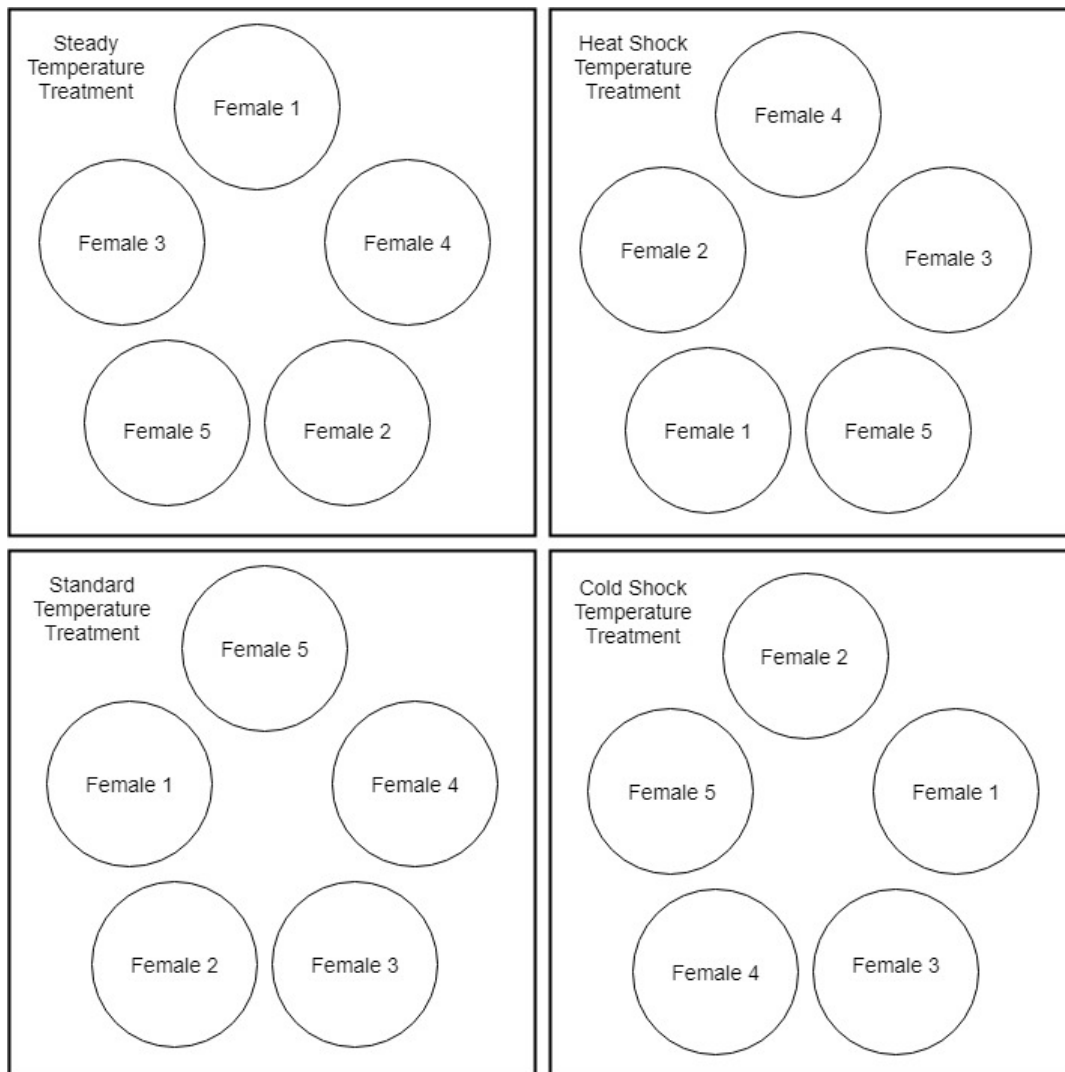


Figure 1. Embryonic Development Experimental Design. Egg ribbons from each female were randomly assigned to a replicate within each treatment block. Each temperature treatment was separate from the others. Embryonic development was observed during the incubation period until larvae started to hatch-out.

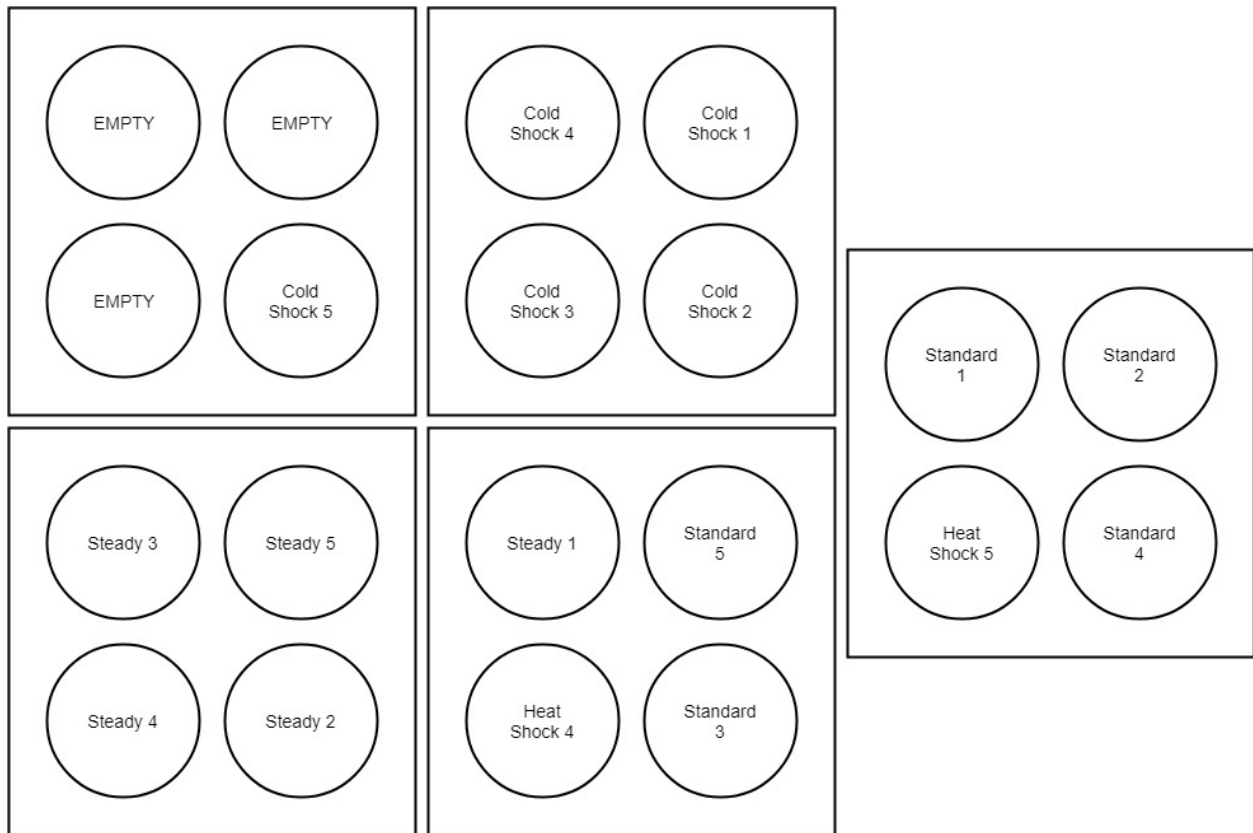


Figure 2. Larval Development Experimental Design. Once larvae hatched out they were moved into larger buckets for grow out observations. Larval buckets were set up as hatch-out occurred. Maximum number of larvae were moved from each replicate. “Empty” buckets indicate three buckets that were unused due to the heat shock treatment causing three replicates to completely fail to produce larvae. All the replicates were on the same temperature system.

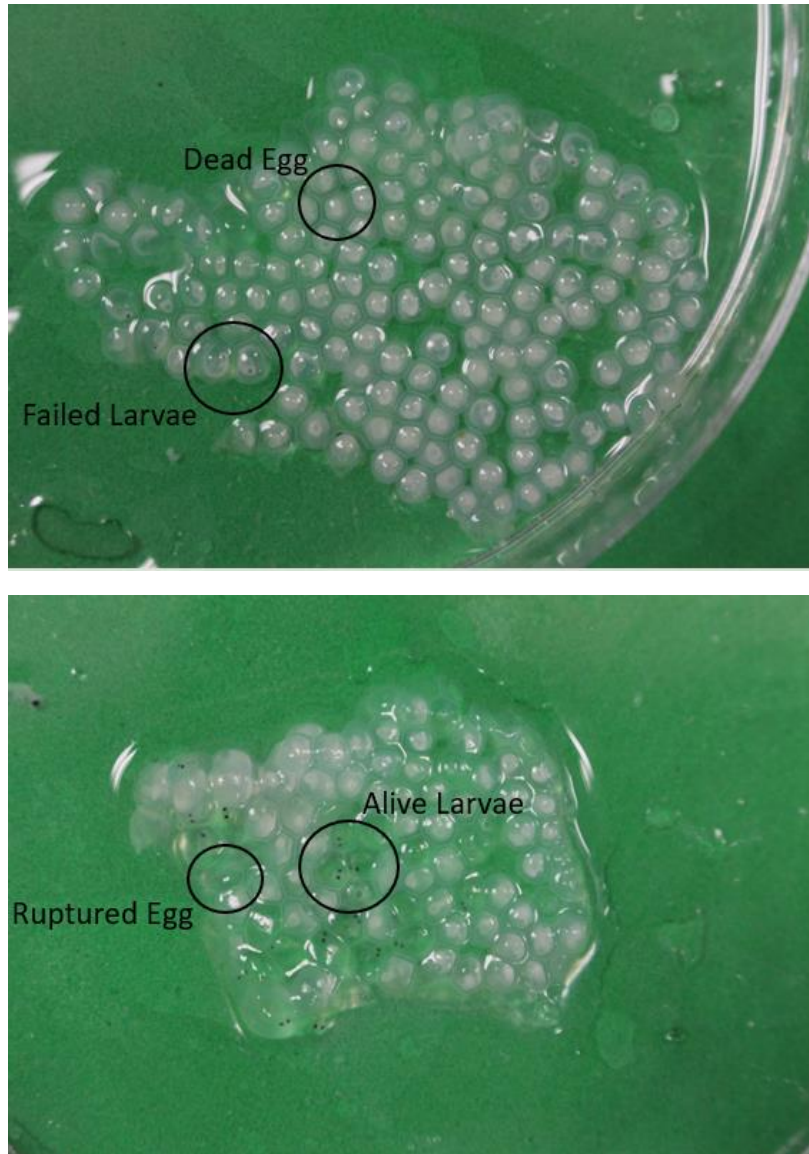


Figure 3. Photographic Examples of Embryonic Development at the end of incubation. Images were taken using Canon EOS RebelT5i camera. Top image shows what counted as a dead egg and as failed larvae. The bottom image shows what counted as viable larvae.

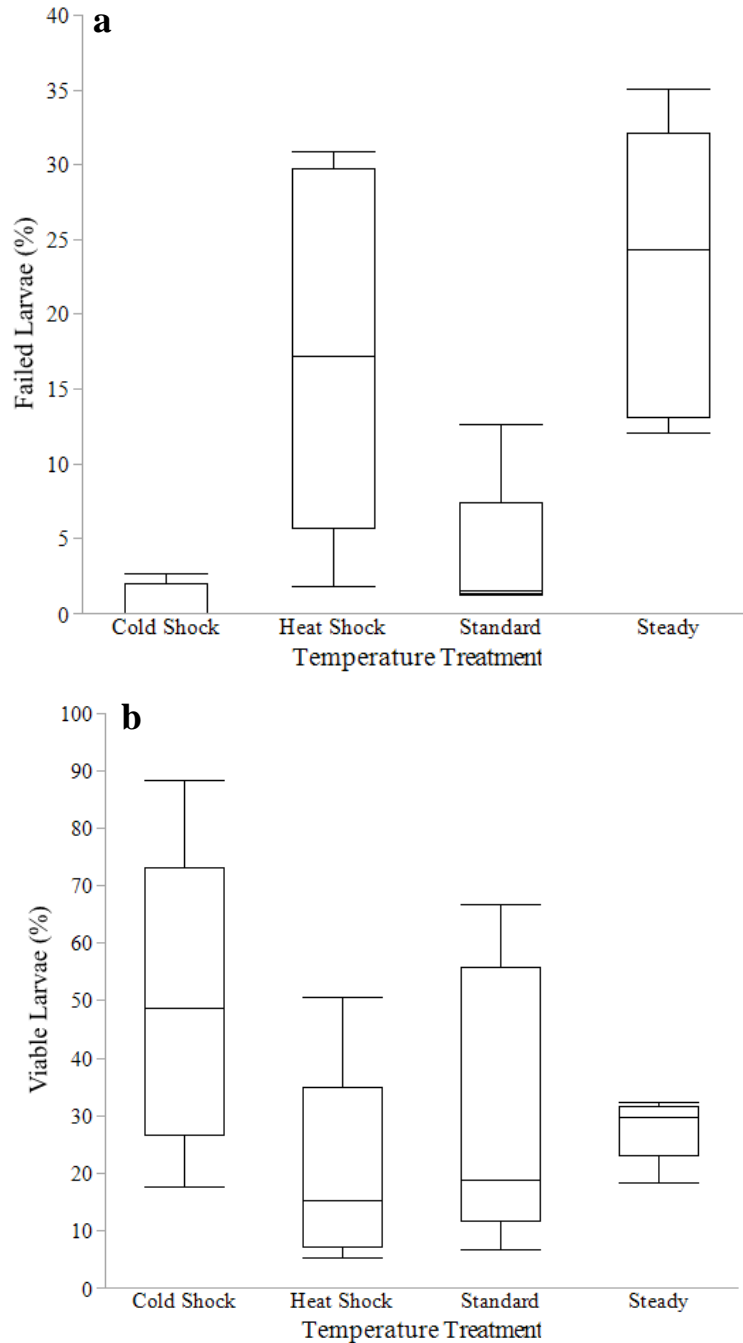


Figure 4. a) Percent (%) failed larvae at the end of incubation per temperature treatment. Interquartile range, maximum percent and minimum percent are shown for each treatment. Randomized Block  $F$ -test revealed temperature treatments did have an effect on the percent of failed larvae ( $p = 0.0005$ ,  $F_{3,12} = 12.5$ ) b) Percent (%) of viable larvae at the end of incubation per temperature treatment. Interquartile range, maximum percent and minimum percent are shown for each treatment. Randomized Block  $F$ -test revealed temperature treatments did have an effect on the percent of viable larvae ( $p = 0.0425$ ,  $F_{3,12} = 3.71$ ) (See Appendix C)

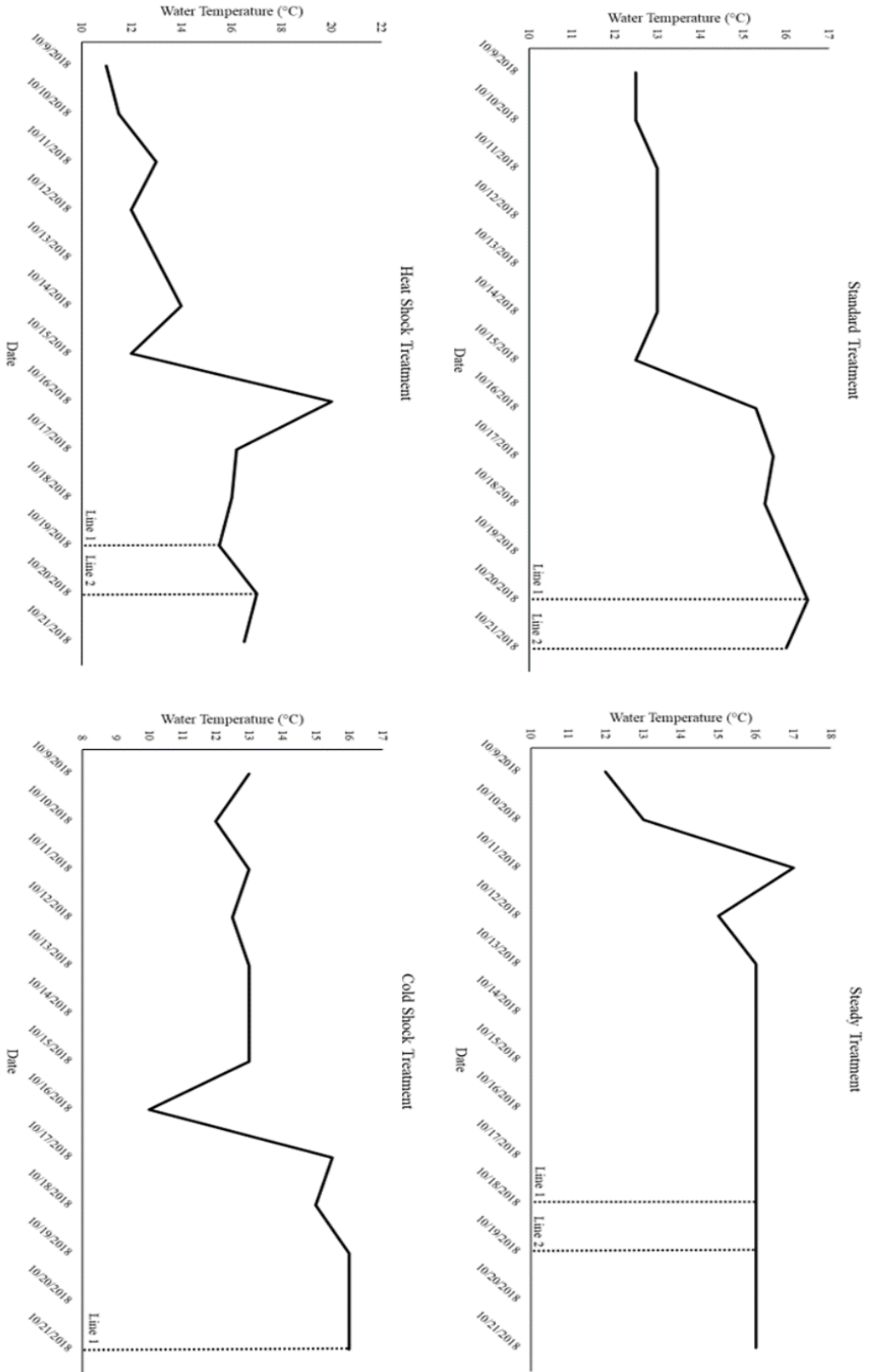


Figure 5. Total duration of incubation periods for each temperature treatment. Line 1 indicates the start of the hatching period for each treatment. Line 2 indicates the end of the hatching period if it extended multiple days. Randomized Block *F*-test revealed that temperature did have a significant effect on incubation period ( $p < 0.001$ ,  $F_{2,8} = 76$ ). Heat shock treatment was included for comparison purposes but was not included in the statistical analysis.



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APPENDIX A: Randomized Block *F*-test ANOVA table for 1) percent of failed larvae at the end of incubation period 2) percent viable larvae at the end of incubation period 3) duration of incubation period

1. Percent of Failed Larvae Randomized Block ANOVA table

Source	df	SS	MS	F
Treatment	3	1709.9	569.9	12.5
Female (Block)	4	550.4	137.6	3.02
Error	12	546.0	45.5	
Total	19	2806.3		

2. Percent of Viable Larvae Randomized Block ANOVA table

Source	df	SS	MS	F
Treatment	3	2373.5	791.2	3.71
Female (Block)	4	4067.0	1016.7	4.76
Error	12	2558.5	213.2	
Total	19	8999.0		

3. Duration of Incubation Period Randomized Block ANOVA table

Source	df	SS	MS	F
Treatment	2	17.7	8.8	76
Female (Block)	4	1.06	0.27	2.2
Error	8	0.93	0.12	
Total	14	19.7		

APPENDIX B: Randomized Block  $F$ -test  $F$ -values and  $p$ -values for 1) embryonic development variables 2) larval development variables \* indicates significantly different means when  $p < 0.05$

1. Embryonic Development Variables

Dependent Variables	F-critical	F-value	$p$ -value
Percent Dead Eggs	3.49	2.65	0.0958
Percent Failed Larvae	3.49	12.5	0.0005*
Percent Viable Larvae	3.49	3.71	0.0425*

2. Larval Development Variables

Dependent Variables	F-critical	F-value	$p$ -value
Duration of Incubation Period	4.45	76	0.00001*
Percent Survival	4.45	1.18	0.35
Observed and Estimated Mortality	4.45	0.036	0.96
Initial Size	4.45	2.85	0.116
Growth Rates	4.45	2.31	0.161

APPENDIX C: Tukey-Kramer HSD post-hoc test results for 1) percent of failed larvae at the end of incubation period 2) percent viable larvae at the end of incubation period 3) duration of incubation period. Treatment means that share a letter are not significantly different.

1. Percent of Failed Larvae at the end of Incubation

Treatment	Tukey-Kramer HSD result	Mean
Steady	A	22.9
Heat Shock	A	17.6
Standard	B	3.8
Cold Shock	B	0.8

2. Percent of Viable Larvae at the end of Incubation

Treatment	Tukey-Kramer HSD result	Mean
Cold Shock	A	49.6
Standard	AB	30.8
Steady	AB	27.8
Heat Shock	B	19.9

3. Duration of Incubation Period

Treatment	Tukey-Kramer HSD result	Mean
Cold Shock	A	13
Standard	B	12.2
Steady	C	10.4