EFFECTS OF TEMPERATURE, SALINITY, AND SUSPENDED SOLIDS ON THE EARLY LIFE HISTORY STAGES OF ARKANSAS RIVER SHINER

by

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ABSTRACT

Arkansas River shiner (ARS) *Notropis girardi* is a federally-threatened cyprinid native to rivers and streams of the Arkansas River Basin. It is a member of a reproductive guild of pelagic broadcast-spawning cyprinids that release non-adhesive, semi-buoyant eggs during an extended spawning season. For successful reproduction, ARS eggs require a combination of sufficient current velocity to remain suspended in the water column and substantial lengths of free-flowing rivers for successful reproduction. Environmental factors such as temperature, total dissolved solids, and total suspended solids have the potential to influence egg buoyancy and developmental rate, thereby affecting the minimal current velocity and stream fragment length required to support ARS populations. However, how environmental factors and fragmentation interact to affect ARS reproductive success is still unclear. I assessed the effects temperature, total dissolved solids, and total suspended solids have on the developmental rate of ARS early life-history stages and the minimal current velocity needed for ARS eggs to remain suspended within a water column. Results show that developmental rate of ARS eggs and larvae were positively correlated with increases in temperature. Eggs incubating in the highest total suspended solid and total dissolved solid treatments developed at a faster rate than counterparts developing at the same temperature but with lower levels of total suspended solids and total dissolved solids. Temperature was negatively correlated and total suspended solids were positively correlated with the minimum current velocity needed to keep ARS eggs in suspension. Together they explained 42% of the variation in current velocity for this experiment. Also, total dissolved solids had no effect on the current velocity and treatments with the highest total suspended solids required the highest flow velocities. The first 24–48 hr free-float period for eggs of ARS and other pelagic broadcast-spawning cyprinid is crucial for growth and development. However, if at any point during this time they experience current velocities lower than what has been recorded in my results eggs could sink to the bottom leading to unsuccessful reproduction. More importantly, as the Great Plains ecoregion experiences changes due to both climate and land, use these

results could help predict stream reaches capable of sustaining their early life-history strategies.

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INTRODUCTION

Rivers and streams that once flourished in the Great Plains of North America are now considered among the most endangered biomes on the continent (Dodds et al. 2004). These prairie rivers and streams hosted high levels of biodiversity, including a large number of endemic species. Human activity has directly altered the integrity of these rivers and streams through channelization, pollution, dams, and other watershed modifications (Cross et al. 1985, Rabeni and Sowa 1996). Human activities also have indirect effects on these rivers and streams as evidenced by the changing climate of the region. Relative to the 1970s baseline, average temperature within the Great Plains has already increased about 0.8°C and is projected to increase anywhere from 1-7.5°C by the end of the century (Field et al. 2007; Karl et al. 2009). Future projections suggest that suspended solids within the river system will increase due to soil erosion after intense rainfall events associated with changes in climate (Leemans and Kleidon 2002). As air and water temperatures increase, stream drying will intensify and the already noted decline for spring refugia for fish species will be exacerbated (Dodds et al. 2004). As a result of these alterations and disturbances, the rivers and streams of the Great Plains will experience changes in flow regimes, as well as habitat availability and connectivity (Brown et al. 2007). The alterations already experienced in prairie streams and rivers have resulted in the decline of endemic species and projections of future conditions do not show improvements of this outlook. A thorough understanding of how the biology of these species interacts with the changing physicochemical environment and landscape is imperative to developing effective conservation programs for these species.

The plight of pelagic broadcast-spawning cyprinids in the Great Plains ecoregion represents a nexus point where these changes may affect their early life history stages and ultimately determine their persistence. There are at least 12 species of pelagic broadcast-spawning cyprinids representing a reproductive guild of small-bodied (<5−7 cm TL) fishes that produce semi-buoyant, non-adhesive eggs (Moore 1944, Bestgen et al. 1989, Platania and Altenbach 1998, Bonner and Wilde 2000,

Perkin and Gido 2011). This reproductive strategy is generally uncommon among North American cyprinids, but seems to have arisen repeatedly in several lineages of cyprinids inhabiting prairie streams and rivers. The majority of North American cyprinids release negatively-buoyant eggs over a structurally-complex substrate, such as rock or gravel, where they remain until the completion of development (Balon 1975, Johnston and Page 1992). Pelagic broadcast-spawning cyprinids release eggs into the water column that then float downstream as they develop (Moore 1944, Bestgen et al. 1989, Platania and Altenbach 1998, Bonner and Wilde 2000, Perkin and Gido 2011). Eggs of these species require substantial lengths of free-flowing river (> 100 km) before they complete development and are capable of lateral movement into low current velocity nursery habitats (Bonner and Wilde 2000, Perkin and Gido 2011). Although eggs of pelagic broadcast-spawning cyprinids can take 24–48 hrs to complete development and hatch (Moore 1944), numerous factors can influence length of river needed to sustain their early life history, such as temperature (Moore 1944, Perkin and Gido 2011), drift rate, channel morphology (Bonner and Wilde 2000, Kehmeier et al. 2007), and current velocity. In combination, these factors contribute to determining the length of free-flowing river needed for successful reproduction.

Unsuccessful reproduction among members of this guild is generally attributed to two broad categories, fragmentation and altered stream flow. Pelagic broadcast-spawning cyprinids require lengths capable of supporting their 24–48 hrs of drift before their eggs hatch into larvae and are capable of horizontal movement (Platania et al. 2005). Drifting is an important component of these species life cycle as it keeps the eggs and larvae from being abraded by shifting sand substrates and renders them less vulnerable to predation by demersal species (Moore 1944). However, Perkin and Gido (2011) found that the average fragment length in the Great Plains was consistently less than the length needed for members of this guild to complete development (Bonner and Wilde 2000, Perkin and Gido 2011). Durham and Wilde (2006) indicated continuous reproductive success during the extended spawning season for members of this guild except during periods of no flow, but there is controversy about whether

these species need high flow pulses to spawn or if they spawn independent of flow. A lack of flow could lead to delayed reproduction, resulting in shortened growing season and increased susceptibility to overwinter mortality for young-of-year (Henderson et al. 1988, Shuter and Post 1990, Durham and Wilde 2005).

Although studies have identified multiple potential contributors to unsuccessful reproduction in pelagic broadcast-spawning cyprinids, much still remains unknown about the full range of factors, such as temperature, conductivity, suspended solids, and flow, affect entrainment and development of early life-history stages. These factors have the potential to influence egg buoyancy, developmental rate and thus the minimal current velocity and stream fragment length required to support these species. For example, Cowley et al. (2005) demonstrated that the Rio Grande silvery minnow *Hybognathus amarus* eggs incubated at high levels of total dissolved solids hatched earlier than those incubated in lower levels at the same temperature. This may alter the length of river needed for these species to complete development. However, it is still unclear whether this variability in river length would be beneficial or detrimental to these species.

Arkansas River shiner (ARS) *Notropis girardi* is among the better studied pelagic broadcast-spawning cyprinids, yet there is still uncertainty regarding the reasons for its decline. This small (<5.0 cm TL) cyprinid is endemic to the Arkansas River Basin and was found throughout Oklahoma, western Arkansas, southern Kansas, northern Texas, and northwest New Mexico (Cross 1967b, Robinson and Buchanan 1988, Miller and Robison 2004, Thomas et al. 2007). However, ARS has been extirpated from much of its original range including Arkansas (Robinson and Buchanan 1988) and Kansas (Cross et al. 1985, Haslouer et al. 2005). Collections in Oklahoma have shown that this species is currently restricted to the South Canadian River (Pigg 1991, Pigg et al. 1999). Records from Texas and New Mexico have also indicated a decline in this species (Sublette et al. 1990, Bonner et al. 1997, Bonner and Wilde 2000). In 1998, the U.S. Fish and Wildlife Service listed ARS as a threatened species under the Endangered Species Act (Ryan 2004).

The decline of ARS has been attributed to a suite of environmental changes (i.e. temperature, variability in flow, etc.) and habitat fragmentation resulting in unsuccessful reproduction. In particular, stream segments of insufficient length to allow the completion of development has been implicated in the decline of ARS populations (Bonner and Wilde 2000, Perkin and Gido 2011). However, this critical stream length likely varies with several factors including: 1.) interaction between the physicochemical environment and characteristics of ARS early life-history stages and 2.) distribution/location of suitable habitat within a river segment. Temperature, total dissolved solids, and total suspended solids may alter the buoyancy and developmental rates of eggs and larvae and thus influence habitat requirements, such as stream fragment length and minimum current velocities (Cowley et al. 2005). These environmental factors may lead to reductions in the minimum current velocity necessary to keep the eggs in suspension which may reduce drift length.

Spawning habitat requirements for ARS have not been adequately documented and defined to date, which is a factor impeding development of effective conservation and recovery plans (Polivka 1999). While the location of spawning habitats within a river segment likely has little impact on the total distance required to sustain ARS early life history, it may determine the effective habitat length available to the species and ultimately the suitability of a given stream fragment. Creating a complete developmental series for eggs and larvae of ARS would enable a more accurate hindcasting of when and where ARS were spawning. Most surveys for ARS usually include the collection of their eggs in the field, so this developmental series will enable collectors to back track to estimated spawning locations. Obtaining a better understanding of when and where ARS spawn would enable refinements of habitat suitability models to better direct limited conservation and recovery funds. Moore (1944) created a developmental series of ARS, but many stages of development and the interaction between developmental rate and physicochemical conditions were left unidentified. These results will allow for identification of estimated spawning habitats

for ARS and potentially other *Notropis* species if they occupy similar niches in other river systems and have similar reproductive strategies.

While these factors may have important consequences to understanding the recruitment and persistence of ARS populations, the effects of physicochemical factors on ARS developmental rate, egg buoyancy, and hatch rate are not well understood. The objective of my experiment was to assess the effects of temperature, total dissolved solids (TDS), and total suspended solids (TSS) on the developmental rate and buoyancy of ARS eggs and larvae. Understanding how these factors affect the early life-history stages could potentially explain their consistency in decreasing populations throughout the Great Plains ecoregion. With these results, it is possible to better predict stream reaches capable of supporting ARS populations, particularly as landscape use and climatic patterns continue to change within the region.

METHODS

Collection of ARS and induction of spawning.—On 28 June 2012 ARS were collected with personnel from the U.S. Fish and Wildlife Service Oklahoma Ecological Services Field Office. ARS (n=54) were seined from the South Canadian River at the US-283 crossing near Roll, Oklahoma (Roger Mills County). The Museum of Southwestern Biology Division of Fishes in Albuquerque, New Mexico also provided ARS (n=56) collected from the Pecos River approximately 10 km upstream from the US-70 crossing in Chaves County, New Mexico on 6 June 2012. All individuals were transported to Tishomingo National Fish Hatchery in Tishomingo, Oklahoma where they were housed in a shaded flow-through raceway system. Fish were maintained at ambient temperature (19.9°C−27.9°C) and photoperiod (approx. 15:9 light:dark) during the study.

Spawning was induced multiple times through prior knowledge of successful spawning of ARS in laboratory settings (Kerry Graves, U.S. Fish and Wildlife Service, pers. comm.). Briefly, fish were removed from the raceway and anesthetized by immersing them in a 25-mg/L tricaine methanosulfate (MS-222; Western Chemical, Inc., Ferndale, Washington) solution. Sex was determined by applying

gentle pressure to the abdomen of each fish to express gametes. Individuals were then segregated by sex into two 37.9-L aquaria and allowed to recover undisturbed for 48 hrs. To induce spawning, fish were anesthetized and after loss of equilibrium (1−2 min), each individual was then transferred to a wet gauze pad cradle on the stage plate of a dissection microscope. A 0.5-cc insulin syringe with a 31-gauge needle was used to deliver carp pituitary extract. Injected at the ventral midline near the anus of each fish was a dosage of approximately 1.0 mL/g wet-body mass. After injection, the fish were then held in a 10.8-L plastic tub until equilibrium was regained, then transferred into 37.9-L holding tanks and left undisturbed to allow for reproduction to occur.

Temperature, TDS, and TSS experimental set up and design.— Two treatment levels each of temperature (25°C, 30°C), TDS (1,000 mg/L, 6,000 mg/L), and TSS (0 mg/l, 3,000 mg/l) were selected to represent the high and low levels of each variable that ARS may experience during their spawning season in the Canadian River Basin. Temperature treatment levels were selected to encompass a range of values were successful spawning has been observed in laboratory settings (Platania and Altenbach 1998; K. Graves, U.S. Fish and Wildlife Service, pers. comm.) and the range of temperatures observed in the Canadian River during ARS spawning season (May-August). Treatment levels for TDS and TSS were selected to represent a typical value and an extreme value of each from the Canadian River based on reports by Pigg et al. (1999).

Temperature was maintained by a 50-W submersible heater and thermostat (Aqueon Products, Franklin, Wisconsin) placed on the bottom in each of the two aquaria. Within these aquaria, 250-ml glass beakers were used to house the TDS-TSS treatment groups. TDS levels were established through the addition of the appropriate amount of Instant Ocean seawater mix (Spectrum Brands, Cincinnati, Ohio). Red clay was added to obtain the desired TSS levels (Hazelton and Grossman 2009), and was kept in suspension by placing large air stones at the bottom of each beaker. Every hour, each beaker was gently mixed by hand to ensure the eggs and solids remained in

suspension during the experiments. This mixing was subtle to ensure it did not cause any disturbances to the development of ARS eggs.

Effects of Temperature, TDS, and TSS on the developmental rate of ARS eggs and larvae.—On 26 July 2012 at approximately 08:30, spawning was induced in ten males and ten female ARS as described above. Spawning activity began approximately 10 hours later. A subsample of eggs was fixed and preserved in 3% formalin immediately post fertilization, and the remainder of eggs were equally distributed among the temperature-TDS-TSS treatments (Figure 1). Previous work established that approximately 45 ARS eggs displaced a volume of 1.0 ml. I used this volumetric method to ensure that approximately the same number of eggs was placed into each treatment leve1 (approximately 90 eggs). A subsample of five ARS eggs was then collected from treatments at 1 hr, 2 hrs, 4 hrs, 8 hrs, 12 hrs, 16 hrs, 24 hrs, 36 hrs, and 48 hrs post fertilization and fixed in 3% formalin.

I used an Olympus SZX16 dissecting microscope (Olympus Corporation, Tokyo, Japan) equipped with an Infinity 1 digital camera (Lumenera Corporation, Ottawa, ON Canada) to capture digital images of all preserved early life-history stages at a magnification of 11.5X. Images were enhanced to aid in determining developmental stage using the unsharp mask filter and the brightness adjustment feature of Image J v.1.46 (Abràmoff et al. 2004). The chorion, embryo, or larvae were measured for each individual using Image J. Assignments of developmental stage were based on the criteria presented by Moore (1944) for ARS and supplemented with similar information from other cyprinids, such as rosyface shiner *Notropis rubellus* (Reed 1958), zebrafish *Danio rerio* (Kimmel et al. 1995), Asian carps (Yi et al. 1988, Chapman 2007), and spottail shiner *Notropis hudsonius* (Jones et al. 1978b).

Creation of a developmental series for ARS eggs and larvae.—Photos taken to assess the developmental rate of ARS were also used to create a developmental series. The treatments with the lowest TDS and TSS (TDS 1,000 mg/L, TSS 0 mg/L) were selected for this experiment along with both temperatures (25°C, 30°C).

Construction of flow apparatus and experimental design.—I used a flow apparatus as described in Mueller et al. (*in review*) to assess the minimum current velocities needed to keep ARS eggs in suspension under different temperature, TSS, and TDS treatment levels. Briefly, my flow apparatus consisted of a submersible pond pump, a riser stem, a ball valve, and a clear PVC observation and measurement chamber (Figure 2). Once constructed, this flow apparatus allowed for fine adjustments of flow at very low current velocities (≥ 0.0002 m/s). Hash marks were drawn on the PVC chamber to represent different lengths in meters so further calculations could be made to obtain current velocities. In order to manipulate temperature and TDS, eight tubs, approximately 38.9-L were used in order to fully submerge our flow apparatus during trials. The appropriate amount of Instant Ocean seawater mix was added to each tub to obtain our three different TDS levels (1,000 mg/L; 3,000 mg/L; 6,000 mg/L; Figure 3).

Effects of Temperature, TDS, and TSS on the current velocity required to keep ARS eggs in suspension.—On 06 December 2012 at approximately 10:00, I injected ten male and ten female ARS with carp pituitary extract to induce spawning. Spawning activity began approximately 30 hours later and once the eggs became semi-buoyant they were siphoned out and approximately 20 eggs were then placed into eight beakers and incubated at 21°C to ensure that eggs were all at approximately the same level of development during trials (Figure 4). It was assumed that once the eggs made contact with the controlled temperatures within each of the nine tubs, the eggs would instantaneously react and quickly reach thermal equilibrium. Once the eggs were placed into the TDS-TSS beakers they were left undisturbed for 4 hrs before trials began.

After 4 hrs, one egg at a time from each beaker was placed into the flow apparatus. Fine adjustments were made to the valves until the egg remained stationary within the PVC chamber for approximately 1 min. I then recorded the distance from the bottom of the chamber to where the egg remained stationary in meters. Once this was accomplished, the apparatus was turned off and the PVC chamber was removed

from the pump. Lastly, the chamber was reassembled onto the pond pump and the apparatus was turned back on. A timer was started as soon as the water began to fill up the chamber until it reached the location where the egg was stationary. The distance in meters was then divided by the time in seconds it took the water to fill up the column to obtain the current velocity for each egg.

Data analysis.—In order to assess the effects of temperature, TDS and TSS on the developmental rate cumulative percentages for each stage per temperature-TDS-TSS treatment group was calculated. To ensure there was no misidentification, I combined the gastrulation stages as well as the tail bud and caudal find stages together. Lengths were recorded for the embryo and chorion or larval length for each picture. A four parameter logistic curve was fit for each temperature-TDS-TSS treatment at each developmental stage using SigmaPlot 11.0 (Systat Software, San Jose, CA) and the following equation:

$$\mathrm{P_{dev}} = min + \frac{(max - \min)}{(1 + (\frac{x}{EC50})^{(-hillslope)}}$$

Where $\mathrm{P_{dev}}$ is the proportion of individuals at or exceeding a developmental stage at time = *x*, max and min are the maximum and minimum proportion of individuals that reached or exceeded a developmental stage at time = *x* respectively, *EC*50 corresponds to the time required for 50% of the population to reach or exceed a given developmental stage, and *hillslope* describe the steepness of the curve. In this model the minimum and maximum were held constant at 0 and 1 respectively. I evaluated the effects of temperature, TDS, and TSS on the developmental rate of ARS eggs and larvae using analysis of variance (ANOVA) with EC-50 value as the dependent variable and stage, temperature, TDS and TSS as independent effects.

The effects of temperature, TDS, and TSS on the minimum current velocity needed to keep ARS eggs in suspension was assessed using analysis of covariance (ANCOVA) with TDS as a covariate and temperature and TSS as independent variables. Post hoc tests were performed using Duncan's multiple range tests with a *p*-value of 0.05. I ensured that there were no violations of parametric assumptions before

running my analysis by running Shapiro-Wilk, Kolmogorov-Smirnov, and Cramer-von Mises test. All analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Developmental series for ARS eggs and larvae.—In total, 183 pictures were taken of ARS eggs and larvae and these were classified into 14 developmental stages. Chorion, embryo, or larval length was recorded for each individual (Table 1-3). However, small sample sizes at each of the temperature-TDS-TSS treatment groups at each stage resulted in no noticeable differences in lengths among groups. In general, early development of ARS is rapid and most individuals were entering the late gastrula or neurula stage within 4 hrs post-fertilization. The 14 developmental stages were distinctive from one another and were characterized as follows:

512−cell stage (512-S; Figure 5-A). This stage marks the beginning of the mid-blastula transition, during which the blastodisc forms a high mound and the blastodermal cells are arranged into relatively smooth layers. This stage was recorded only during 1 hr post-fertilization and by the 2−hr sample all individuals had surpassed the 512−cell stage.

Late blastula (LB; Figure 5-B). The blastodisc has flattened and the blastodermal cells align with the yolk cell to form an oval shape. This stage was recorded 1−2 hrs post-fertilization.

Early gastrula (EG; Figure 5-C). The blastoderm starts to surround the yolk cell covering about 30% of the entire distance between the animal and vegetal poles. This stage can also be referred to as 30%-epiboly and was recorded 1−2 hrs post-fertilization.

Mid-gastrula (MG; Figure 5-D). The blastoderm is now covering 50% of the total distance between the animal and vegetal poles. This stage can also be referred to as 50%-epiboly and was recorded 1−2 hrs post-fertilization.

Late gastrula (LG; Figure 5-E). Recorded 2−4 hrs post-fertilization, this is the final gastrula stage recorded, also referred to as 75%-epiboly. At this stage the blastoderm is now covering 75% of the entire distance between the animal and vegetal pole.

Neurula (N; Figure 5-F). The blastoderm covers almost the entire distance between the animal to vegetal poles. The small portion of the yolk cell near the vegetal pole not covered by the blastoderm margin is the yolk plug. This stage was recorded at 4 hr post-fertilization.

Blastopore closure (BC; Figure 5-G). The blastoderm has now completely covered the yolk cell. This stage is also known as 100%-epiboly. At this stage the head became larger and more visible. This stage was recorded at 2−4 hrs post-fertilization.

Somite appearance (SA; Figure 5-H). Somites first became visible midway between the animal and vegetal poles at this stage. This stage was recorded 4−8 hrs post-fertilization.

Optic-primordium (OP; Figure 5-I). The eye became visible and is a long oval shape during this stage. The area of the yolk that is not encircled formed a straight line and additional somites became visible This stage was the most variable in its timing from this sample occurring in samples taken 8 hrs post-fertilization.

Tail bud (TB; Figure 5-J). Recorded from 8−16 hrs, at this stage the end of the tail begins separation from the yolk sac.

Caudal fin (CF; Figure 5-K). The tail has completely separated from the yolk sac and the embryo and yolk sac become elongated. This stage was recorded 8-24 hrs post-fertilization.

Otolith appearance (OA; Figure 5-L). At this stage the otolith became visible posterior to the eye, the eye begins to obtain coloration, and hatching occurred. The yolk sac became smaller than that in the previous stage and is more elongated. This stage was recorded from 24−48 hrs post-fertilization.

Melanoid eye (ME; Figure 5-M). Only recorded at 36 hr post-fertilization, the eyes take on a brownish color and became very distinct. The yolk sac continues shrinking and is smaller than that at the otolith appearance stage.

Gas-bladder emergence (GBE: Figure 5-N). Collected at 24 hr post-fertilization, during this stage, the gas-bladder became visible and the yolk sac completely disappears. In my specimens for this stage the mouth was preserved open.

Developmental rate of ARS eggs and larvae.—Temperature-TDS-TSS had little effect on developmental rate of ARS eggs and larvae during the earliest developmental stages. However as development progressed, there was a noticeable difference among treatment groups in (Figure 6). As early as the second developmental stage (late blastula), a difference in developmental rates became apparent between the two temperatures. Eggs and larvae that were developing in the highest TDS-TSS treatment groups developed at a faster rate than those developing in other treatment groups. There were not enough preserved specimens to obtain EC-50 values for the gas bladder emergence stage.

As expected, my analysis showed that stage had the greatest effect on the EC-50 values ($F_{8,49}$=35.01, P<0.0001). However, temperature ($F_{1,49}$=13.14, P=0.0007) and the interaction between TDS-TSS also had shown to have an effect on the EC-50 values ($F_{1,49}$=6.40, P=0.0146). EC-50 values estimated for each stage at for each treatment group suggested that eggs and larvae in the highest TDS-TSS treatment groups (Figure 7) were developing at a faster rate than counterparts in the lower TDS-TSS treatment groups at a given temperature ($F_{10,49}$=30.06, P=<0.0001, R^2=0.860).

ARS minimum current velocity.—I conducted 35 trials using a single ARS egg at a time (Table 4), but these trials were not equally distributed among all treatment combinations due to time constraints. There were two instances where more than three trials were performed; this was due to one of the original trials producing an outlier resulting in variation within a particular group (Table 4).

I found that TSS and temperature explained 42% of the variation in the current velocity needed to keep ARS eggs in suspension ($F_{2,32}$=11.76, P=0.0001, R^2=0.424). My results show that at the higher temperature, the minimum current velocity was less variable and required lower current velocities to remain in suspension for all of my

TSS treatment groups (Figure 8). The ARS eggs from the highest TSS treatments required the higher current velocities than those from lower TSS treatments at any given temperature (TSS*temp: $F_{1,32}$=6.90, P=0.0131; TSS: $F_{1,32}$=16.62, P=0.0003; Table 5). The minimum current velocity needed to keep eggs in suspension did not vary depending on TDS treatment groups ($F_{1,31}$=0.15, P=0.70).

DISCUSSION

Developmental series for ARS eggs and larvae.—My results suggest that classifying ARS eggs and larvae into developmental stages is fairly straightforward. When looking at these preservations under a microscope, developmental characteristics that aid in the separation of stages are fairly distinct. Furthermore, my results are largely consistent with the basic description of ARS development provided by Moore (1944) with the addition of classifying ARS eggs and larvae into different developmental stages. Further, my data provides a higher level of temporal resolution than that of previous studies and additionally, contrasted development at two temperature treatments. Not surprisingly, eggs incubating at higher temperatures developed faster than those incubating at lower temperatures. However, this fluctuation in developmental rate did not seem to affect the size of the eggs and larvae as the stages were of similar sizes regardless of temperature. Mean lengths recorded in this study were similar to those of six other species of pelagic broadcast-spawners (Platania and Altenbach 1998), which is not surprising given the similarities in life-history and body size among the members of this guild.

To my knowledge, there are currently no published descriptions of the development of other pelagic broadcast-spawning minnows. However, comparing my results with *Notropis* species from other reproductive guilds, it seems that ARS eggs and larvae develop at a faster rate. Development of rosyface shiner *Notropis rubellus* (Reed 1958), satinfin shiner *Cyprinella analostana*, bridle shiner *Notropis bifrenatus*, ironcolor shiner *Notropis chalybaeus* and spot tail shiner *Notropis hudsonius* (Jones et al. 1978a) all appear to be considerably slower than that of ARS, even when

accounting for the lower incubation temperatures (19-24°C). For example, ARS ranged from 512–stage to gastrula stage at one hour post fertilization, while these other *Notropis* species were proceeding through their first or second cleavage stage. Furthermore, these *Notropis* species did not hatch until several days post fertilization while ARS hatched one day post fertilization. Slower development may be due to the different reproductive strategies employed by these species. While they differ somewhat in their particulars, all of the above listed species are lithophilic, or in the case of brindle shiner, phytophilic (Balon 1975), meaning that their eggs complete development on or near the bottom. This reproductive strategy may require slower developmental rates, and thus lower oxygen demands, given that the eggs are stationary and dissolved oxygen may be a limiting factor in development (Sorensen 1977). The developing eggs and larvae of pelagic broadcast-spawning cyprinids, such as ARS, are unlikely to be limited by dissolved oxygen while suspended in the water column and thus are capable of supporting the rapid development necessary to limit downstream transport. However, overtime this downstream transport without some form of behavioral mediation, such as upstream spawning migrations, will result in populations gradually shifting downstream and potentially out of preferred habitats (Bonner 2011). Platania and Altenbach (1998) suggested that this rapid development is a strategy of survival for fishes in the Great Plains ecoregion to avoid suffocation by the sand and silt substrates characteristic of their habitat. Several authors have suggested reproduction for pelagic broadcast-spawning cyprinids occur during periods of elevated flows (Moore 1944, Lehtinen and Layzer 1988, Taylor and Miller 1990). If this is accurate, these increases in flow would coincide with increases in current velocities within the water column and thus a need for rapid development to avoid fragments in the river and allow for long distance dispersal (Platania and Altenbach 1998, Bestgen et al. 2010).

With the detailed developmental series created in this study, it may be possible to collect eggs and larvae in the field, estimate their age, and hindcast to estimated spawning habitats. Previous studies have used daily growth increments from otoliths

to estimate the time of spawning of pelagic broadcast-spawning cyprinids, such as central stoneroller *Campostoma anomalum*, southern redbelly dace *Phoxinus erythrogaster*, white sucker *Catostomus commersoni* (Beckman and Schulz 1996), sharpnose shiner *Notropis oxyrhynchus*, smalleye shiner *Notropis buccula*, plains minnow *Hybognathus placitus*, shoal chub *Macrhybopsis hyostoma*, and Red River pupfish *Cyprinodon rubrofluviatilis* (Durham 2007). However because daily increment formation starts only after hatching, using these estimates to hindcast potential spawning locations or correlate spawning activity with hydrological events may be biased due to need to extrapolate the length of time an individual was drifting prior to hatching. Previous studies used estimates ranging from 24-28 hrs (Moore 1944; Platania and Altenbach 1998; Perkin and Gido 2011). However, my results suggest these fish require 24-48 hrs depending on temperature to complete development. This information should allow for more accurate estimates of spawning times and locations to be made from otolith studies of ARS and potentially other broadcast-spawning cyprinids.

Determining spawning habitats will allow for the identification of stream reaches capable of supporting ARS and may also lead to explanations for reductions and extirpations in stream reaches thought to be of sufficient length. Bonner and Wilde (2000) and Perkin and Gido (2011) found that the estimated fragment length needed to sustain ARS early life-history stages was around 220 river km. However, that fragment length assumes that the fish are spawning at the upstream end which is unknown and unlikely. If optimal spawning habitats in reaches thought to be of sufficient length do not occur until further downstream, the length of river available for free float would be reduced, which could lead to unsuccessful reproduction.

Developmental rate of ARS eggs and larvae.—My results show that the median developmental rate of ARS eggs and larvae was negatively correlated with temperature and the data suggests that TDS and TSS may also be negatively correlated with the median time it took to reach or exceed a developmental stage. However, these data are represented by only a single, unreplicated experiment due to difficulty

acquiring a sufficient number of eggs during an induced spawning event. Therefore, my results should be interpreted and applied cautiously until further work is done to replicate this experiment. These replicate samples will aid in fully resolving the interactive effects of temperature-TDS-TSS on ARS eggs and larvae.

While a strong relationship between temperature and developmental rate was expected, the increased developmental rate associated with elevated TDS and TSS treatments was not anticipated. However, finding a positive correlation between TDS and developmental rate is consistent with the relationship seen in Rio Grande silvery minnow (Cowley et al. 2005). When incubating in high levels of TDS, Rio Grande silvery minnow hatched hours earlier than individuals at the same temperature and lower TDS levels. Another cyprinid, zebrafish *Danio rerio*, experienced faster hatching when exposed to inorganic limestone and limestone in suspension (Reis 1969). Increases in suspended solid loads tend to coincide with decreases in dissolved oxygen thus leading to hypoxic stress during development in many fish species (Sorensen 1977). For example, Siefert et al. (1973) found that smallmouth bass *Micropterus dolomieu* development was accelerated by low dissolved oxygen concentrations associated with increased TSS. Combined with elevated temperatures, increases in TDS and TSS could result in accelerated development in ARS, potentially allowing successful reproduction in stream fragments thought to be of insufficient length.

Although increases in developmental rate associated with elevated temperature, TDS, and TSS may allow pelagic broadcast-spawning cyprinids to persist in areas once thought unable to sustain their reproductive strategy, it is still unclear if these increases correlate with survival. For example, difficulty in obtaining enough individuals to fulfill sampling requirements indicated higher mortalities in those treatments of the highest levels of temperature-TDS-TSS. However, this study was not specifically designed to assess mortality and future work should address the survival of ARS early life-history stages under a range of conditions because suspended solid loads naturally occurring in river systems have shown to cause major mortalities to

embryos of warm water fishes (Sorensen 1977). A better understanding of how shifting physicochemical conditions, such as temperature, TDS, and TSS effect the early life-history stages of different species will allow us to better predict stream reaches capable of supporting them, especially as we begin to experience changes in climate and land use patterns.

ARS minimum current velocity.—Estimates of minimum current velocities necessary to keep eggs in suspension suggest ARS egg buoyancy was negatively correlated with temperature and positively correlated with TSS. Basic physics suggest the addition of TDS would increase the density of the water, resulting in lower current velocities needed to keep ARS eggs in suspension. However, my data showed TDS had minimal effect on buoyancy. It is possible that the effect of TDS is minor relative to those of temperature and TSS, but more likely the lack of an observed relationship was due to the fact that all eggs were fertilized and hardened at a common TDS level (TDS=730–750 mg/L) before transferring to the different treatment levels. Platania and Altenbach (1998) found that once spawned, these eggs take between 10–30 minutes for the previtelline space to fill with water. Once this occurs the eggs are likely impermeable to water and ions and experience little change when transferred to beakers of different TDS levels (Lewis and Leitritz 1976). To fully assess whether TDS contributes to the minimum current velocity needed to keep ARS eggs in suspension, eggs should be spawned and allowed to harden in tanks at the experimental treatment levels for TDS.

Although TSS showed a relationship with the minimum current velocity needed to keep ARS eggs in suspension, there were minimal differences in the current velocities for all temperatures when incubating in 1,500 mg/L of TSS. The reason behind this still remains largely unknown, but may be due to the eggs being largely unaffected by suspended solid loads at this concentration. Rio Grande silvery minnow eggs showed increases in specific gravity when incubated in salinity and suspended solids treatment groups compared to those incubated in well water (Cowley et al. 2005). Although this study did not assess the current velocity needed to keep the eggs in suspension, these increases in specific gravity may coincide with increases in

current velocity. A more recent study suggested that Rio Grande silvery minnow is not a true pelagic broadcast spawning minnow (Medley and Shirey 2013). Instead, it may primarily be a demersal spawning species whose eggs have achieved a state of semi-buoyancy only in rivers with high TSS loads. Therefore, eggs developing in rivers with higher TSS loads should require less current velocities to keep them in suspension. My results align closely with the findings by Cowley et al. (2005), which showed that higher loads of TDS and TSS resulted in increases in egg specific gravity. Although this study did not look at current velocity needed to keep the eggs in suspension, increases in specific gravity should coincide with increases in velocity. However, there is a need for future studies to look at the mechanisms by which TSS influence egg buoyancy and the minimum current velocity necessary to keep them in suspension.

Understanding how changes in physiochemical conditions affect ARS egg and larval developmental rates and buoyancy provides insights into how these stages interact with their environment to determine fragment length necessary to support successful reproduction. My results suggest that ARS eggs require 24–48 hrs of free float before they hatch and are capable of horizontal movement, depending upon temperature, TDS, and TSS. If at any time during this crucial free float period current velocities drop below the values presented in my results, eggs may sink and become susceptible to being covered or abraded by the shifting sand substrates characteristic of prairie streams (Cross 1967a, b, Lee et al. 1980, Miller and Robison 2004). Ultimately, this may lead to reoccurring unsuccessful reproduction amongst members of this guild and thus explain reasons for declines. However, the current velocities need to keep the eggs in suspension are extremely low. Hypothetically, my data suggest ARS requires a fragment length of approximately 2.5 km if that fragment consisted of a simple, straight channel exhibiting laminar flow at the maximum current velocity (0.014 m/s) needed to keep eggs in suspension observed during this study. This is two orders of magnitude less than the minimum fragment length estimated by Perkin and Gido (2011). It is important to note that even under low flow conditions,

the minimum current velocities reported in this study are unlikely to occur within a stream channel and may even be exceeded in eddies and off-channel habitats. Therefore, current velocity is unlikely to be a factor limiting the reproductive success of ARS.

However, the ability of ARS eggs to remain in suspension with low current velocities may represent a property important for retaining offspring within suitable habitat. It has been hypothesized that the eggs of Pecos bluntnose shiner *Notropis simus pecosensis* and Rio Grande silvery minnow are entrained in off-channel habitat on the floodplain during their downstream transport, thus keeping offspring in suitable habitat and reducing the fragment length needed for successful reproduction (Medley et al. 2007; Widmer et al. 2010). While these findings are not without controversy (Medley et al. 2009; Zymonas and Propst 2009), it does offer a potential explanation for why pelagic broadcast-spawning cyprinids may exhibit peaks in reproductive activity associated with high flow pulses (Moore 1944). However, changes in flow regimes associated with changing land and water use patterns, impoundments, and altered precipitation patterns can increase channelization, decrease the complexity of river geomorphology (Poff and Ward 1990; Bunn and Arthington 2002) and in return most likely increase downstream transport of semi-buoyant eggs (Dudley and Platania 2007). In addition, large reservoirs and smaller dams created in the Great Plains has changed the magnitude and variability of flow, as well as narrowed the stream channels (Williams and Wolman 1984; Friedman et al. 1998; Wilde 2010) and thus reducing habitat that would be very beneficial for these species, i.e. accessibility to backwater habitat, eddies, etc. If these eggs had accessibility to backwater habitats and eddies this may help reduce the fragment length. Therefore, with faster flowing water and less complex river fragments it is unlikely the eggs would be entrained in these habitats and still require great distances of fragment length to successfully grow into larvae.

Reinert et al. (2004) and Kehmeier (2007) indicate that gellan beads have similar specific gravities as fish eggs and they do not change physically with additions

of salinity. Therefore, gellan beads are commonly used to evaluate drift dynamics of pelagic broadcast-spawning cyprinid eggs (Dudley and Platania 1999). However, Mueller et al. (*in review*) demonstrated that gellan beads required less current velocities and became less variable within higher TDS levels. My results suggest that gellan beads and ARS egg may respond differently to the same physicochemical conditions. The behavior of gellan beads may not be directly comparable to that of fish eggs and thus the scale and goals of the study are likely important considerations in determining whether gellan beads will serve as an adequate proxy.

Conclusions.—With climate and landscape use changes made to the Great Plains rivers and streams we can expect to experience increases in temperature, TDS, and TSS within our river systems. Alterations already made to the area have resulted in major declines of endemic species, such as pelagic broadcast-spawning cyprinids. My results suggest their reproduction may not be successful in fragments predicted to be of sufficient length due to reproductive behavior and the interaction between the egg and the physicochemical environment. For example, the spawning habitat of ARS is not known. However, the inherent assumption when predicting whether a stream fragment is of sufficient length is that the fish spawn at the upstream end of the fragment. If the fish spawn anywhere else, then the effective length of that fragment can be dramatically reduced. Further, a better understanding of the complex interplay between the egg and the external environment is needed. In particular, how the observed changes in buoyancy associated with temperature, TDS, and TSS play out in a riverine environment. Obtaining this knowledge on how these reproductive factors interact with the changing physicochemical environment and landscape will enable us to better predict stream reaches capable of supporting these species as well as build predictive models that aid in conservation and management efforts needed to protect and restore their populations.

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Table 1. Chorion length measures from vegetal pole to animal pole of preserved Arkansas River shiner eggs. All temperature, total dissolved solid, and total suspended solid treatment groups were combined together for each stage.

Stage	***N***	**Mean**	**Std. Dev.**	**Minimum**	**Maximum**
512-S	19	2.1478	0.2754	1.5800	2.5170
BC	6	2.0350	0.3620	1.4780	2.3760
CF	12	2.2238	0.1862	2.0570	2.5560
EG	21	2.1072	0.3114	1.3220	2.5710
LB	12	2.0532	0.4307	0.9350	2.5370
LG	15	2.0203	0.3389	0.9450	2.4440
MG	10	1.9574	0.3012	1.4150	2.3320
N	12	2.1964	0.1664	1.8930	2.3950
OP	4	2.1148	0.1759	1.8830	2.3020
OV	2	1.9025	0.1039	1.8290	1.9760
SA	9	2.0851	0.1711	1.8980	2.4630
TB	7	2.1130	0.1717	1.9270	2.3900

Table 2. Embryo length measures from vegetal pole to animal pole of preserved Arkansas River shiner eggs. All temperature, total dissolved solid, and total suspended solid treatment groups were combined together for each stage.

Stage	***N***	**Mean**	**Std. Dev.**	**Minimum**	**Maximum**
512-S	22	1.0403	0.1482	0.7510	1.3270
BC	7	0.8911	0.0840	0.7810	1.0500
CF	15	1.6043	0.4213	1.1280	2.3700
EG	29	0.9654	0.2582	0.7850	2.2390
LB	14	1.0163	0.1238	0.8920	1.3600
LG	14	0.9135	0.0728	0.8150	1.0870
MG	12	0.9018	0.1125	0.7240	1.1440
N	14	0.9528	0.0963	0.8150	1.1280
OP	4	1.0838	0.1623	0.8850	1.2820
OV	3	1.2130	0.0950	1.1130	1.3020
SA	10	1.0343	0.1451	0.8680	1.3620
TB	10	1.2122	0.1244	0.9940	1.3690

Table 3. Larval length measures of preserved Arkansas River shiner larvae. All temperature, total dissolved solid, and total suspended solid treatment groups were combined together for each stage.

Stage	***N***	**Mean**	**Std. Dev.**	**Minimum**	**Maximum**
CF	2	2.3995	0.1662	2.2820	2.5170
GBE	4	3.0185	0.0097	3.0090	3.0310
ME	12	2.7745	0.0205	2.7600	2.7890
OA	15	3.1775	0.2967	2.7660	3.7130
OP	14	2.9253	0.2436	2.6020	3.3030

Table 4. Minimum current velocity needed to keep Arkansas River shiner eggs in suspension at different combinations of temperature, total dissolved solids (TDS), and total suspended solids (TSS).

N	**Temperature (°C)**	**TDS (mg/L)**	**TSS (mg/L)**	**Mean current velocity (m/S)**	**Std. dev.**
2	21	3000	0	0.0004	0.0000
2	25	1000	0	0.0028	0.0011
2	25	6000	0	0.0051	0.0026
2	31	1000	0	0.0012	0.0008
2	31	6000	0	0.0002	0.0001
1	21	1000	1500	0.0012	.
2	21	3000	1500	0.0014	0.0011
4	31	3000	1500	0.0012	0.0014
5	21	1000	3000	0.0093	0.0044
3	21	3000	3000	0.0070	0.0058
1	21	6000	3000	0.0059	.
2	25	1000	3000	0.0057	0.0067
2	25	6000	3000	0.0082	0.0019
2	31	1000	3000	0.0012	0.0013
2	31	6000	3000	0.0032	0.0001

Table 5. Minimum current velocity needed to keep Arkansas River shiner eggs in suspension at different combinations of temperature and total suspended solid treatment groups.

N	**Temperature (°C)**	**TSS (mg/L)**	**Mean current velocity (m/S)**	**Std. dev.**
3	21	0	0.0020	0.0028
3	21	1500	0.0014	0.0008
9	21	3000	0.0082	0.0045
4	25	0	0.0040	0.0021
4	25	3000	0.0069	0.0043
4	31	0	0.0007	0.0078
4	31	1500	0.0012	0.0002
4	31	3000	0.0022	0.0014

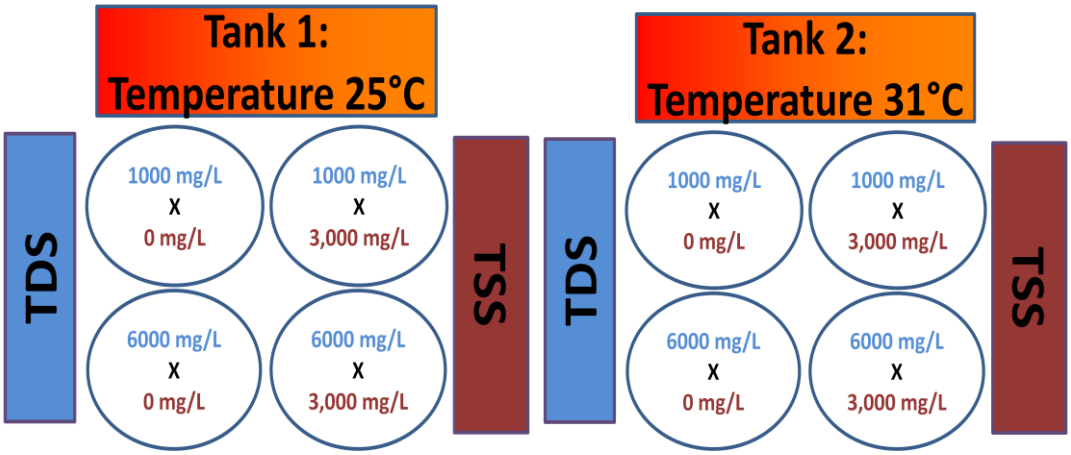

Figure 1: Developmental rate experimental design set up representing two aquaria that controlled two temperature levels, and within each aquaria, four beakers that represented different total dissolved solid (TDS) and total suspended solid (TSS) treatment levels where Arkansas River shiner eggs remained for the duration of the study before 5 eggs from each beaker were placed in vials containing 3% formalin initially after fertilization then again at 0.5 hr, 1 hr, 2 hrs, 4 hrs, 8 hrs, 12 hrs, 16 hrs, 24 hrs, 36 hrs, and 48 hrs.

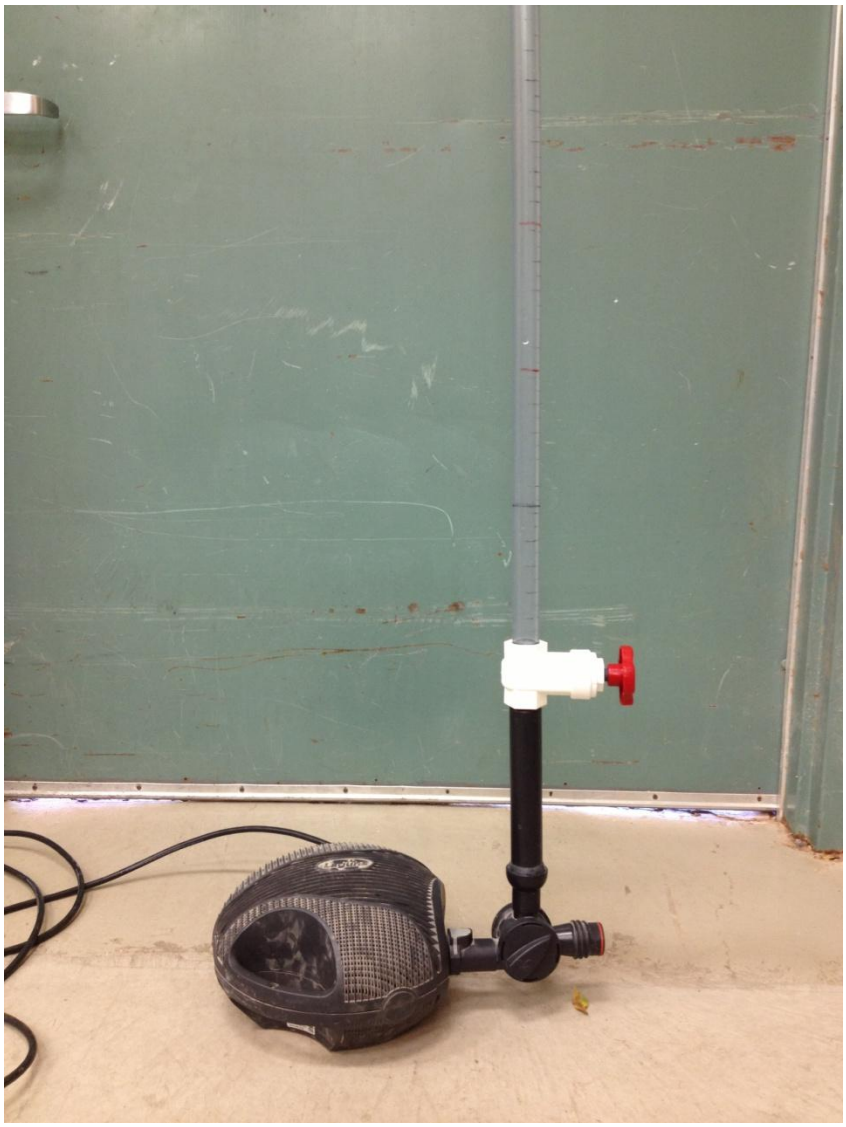

Figure 2. The flow apparatus, created by Mueller et al. (2012; *in review*) using a submersible pond pump, riser stem, ball valve, and clear PVC chamber. This apparatus was used to measure the minimum current velocity needed to keep Arkansas River shiner eggs in suspension four hours after fertilization.

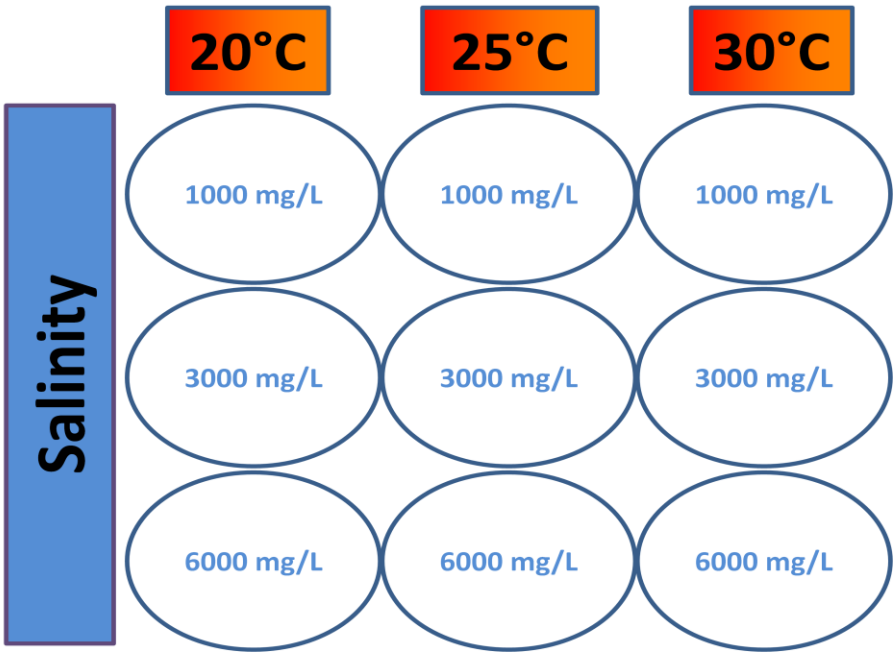

Figure 3. The flow apparatus, as described by Mueller et al. (*in review*) was fully submerged into nine tubs filled with three temperature groups (20°C, 25°C, and 30°C) and three total dissolved solid groups (1,000 mg/L, 3,000 mg/L, 6,000 mg/L). Four hours post fertilization trials began to determine the effects temperature and TDS had on the minimum current velocity required to keep Arkansas River shiner eggs in suspension.

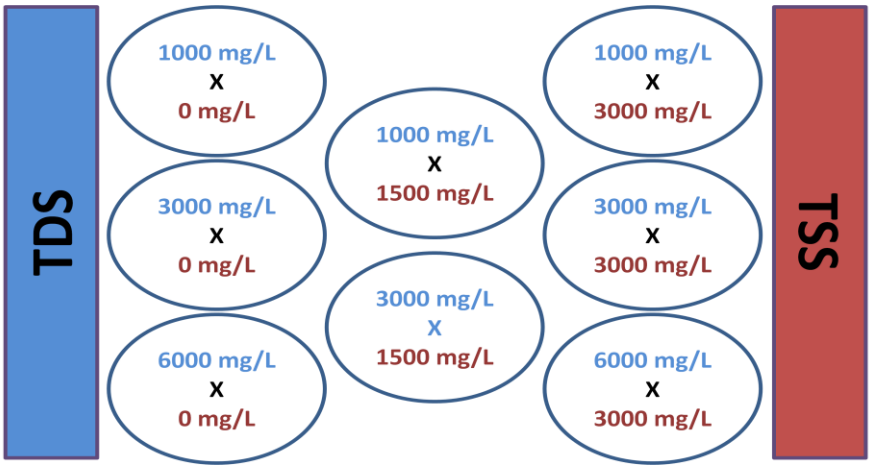

Figure 4. Experimental design used to determine the effects temperature, total dissolved solids (TDS), and total suspended solids (TSS) have on the minimum current velocity Arkansas River shiner (ARS) eggs. For four hours, ARS eggs incubated within one aquaria at ambient temperature (20°C) and randomly distributed into nine beakers used to represent the TDS (1,000 mg/L, 3,000 mg/L, 6,000 mg/L) and TSS (0 mg/l, 1,500 mg/L, 3,000 mg/L) treatment levels.

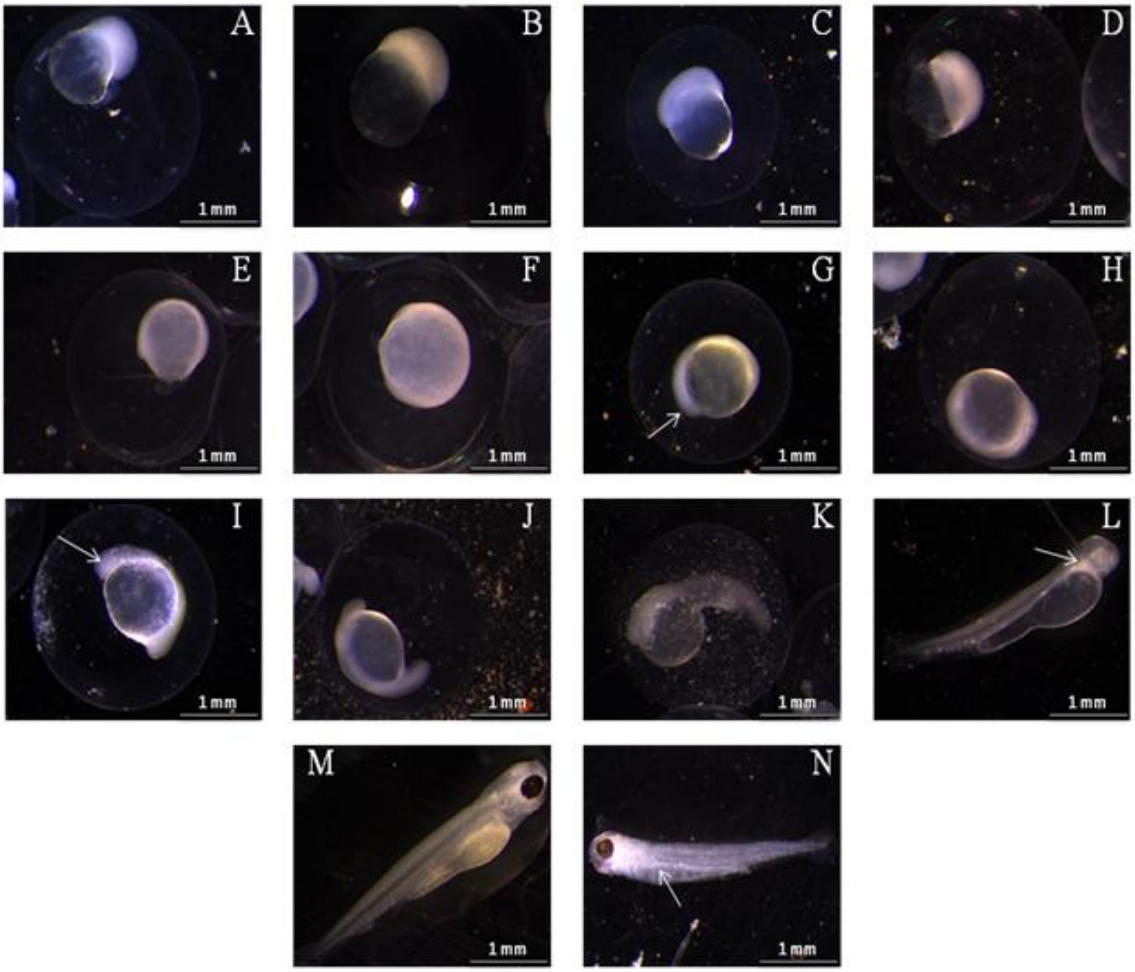

Figure 5. Stages of embryonic and larval development of Arkansas River shiner: (A) 512-cell, (B) late blastula, (C) early gastrula, (D) mid-gastrula, (E) late gastrula, (F) neurula, (G) blastopore closure, (H) somite appearance, (I) optical primordium, (J) tail bud, (K) caudal fin, (L) otolith appearance, (M) melanoid eye, (N) gas bladder emergence.

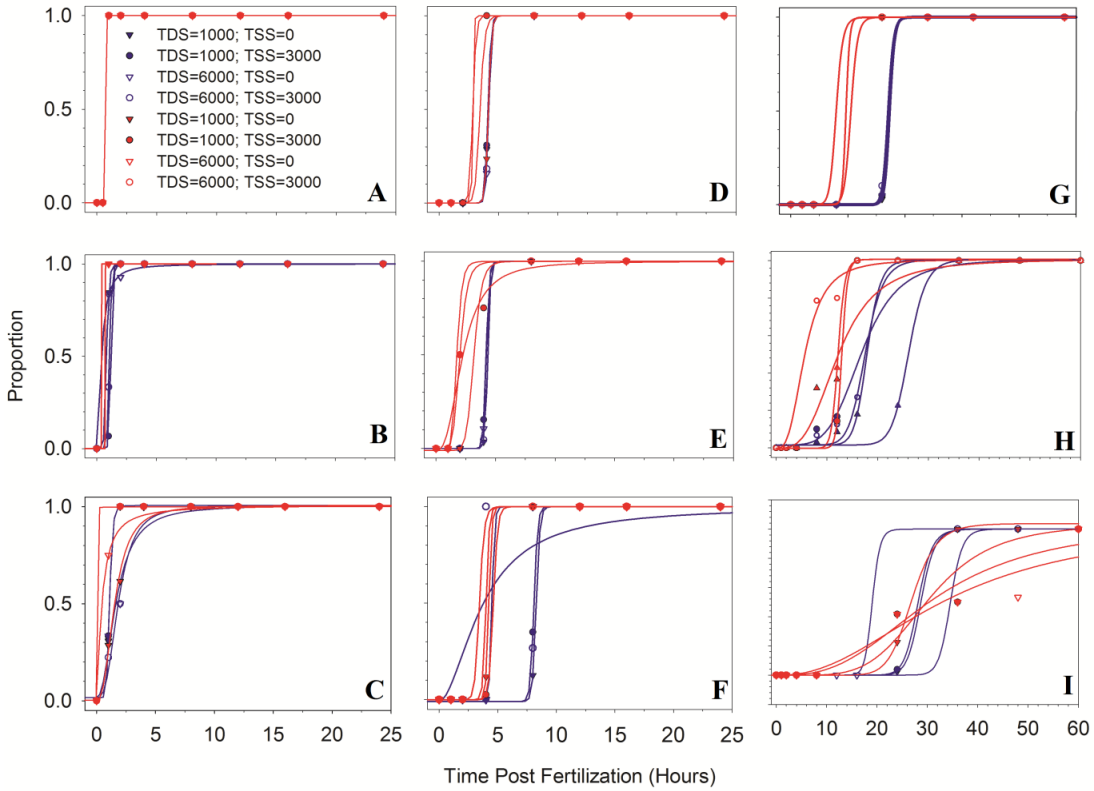

Figure 6.Non-linear regression with a global curve fitting line for each temperature (Red=25°C, Blue=31°C), total suspended solid, and total dissolved solid treatment group at each developmental stage. (A) 512-cell, (B) late blastula, (C) gastrula, (D) neurula (E) blastopore closure, (F) somite appearance, (G) optical primordium, (H) tail bud/caudal fin, (I) otolith appearance.

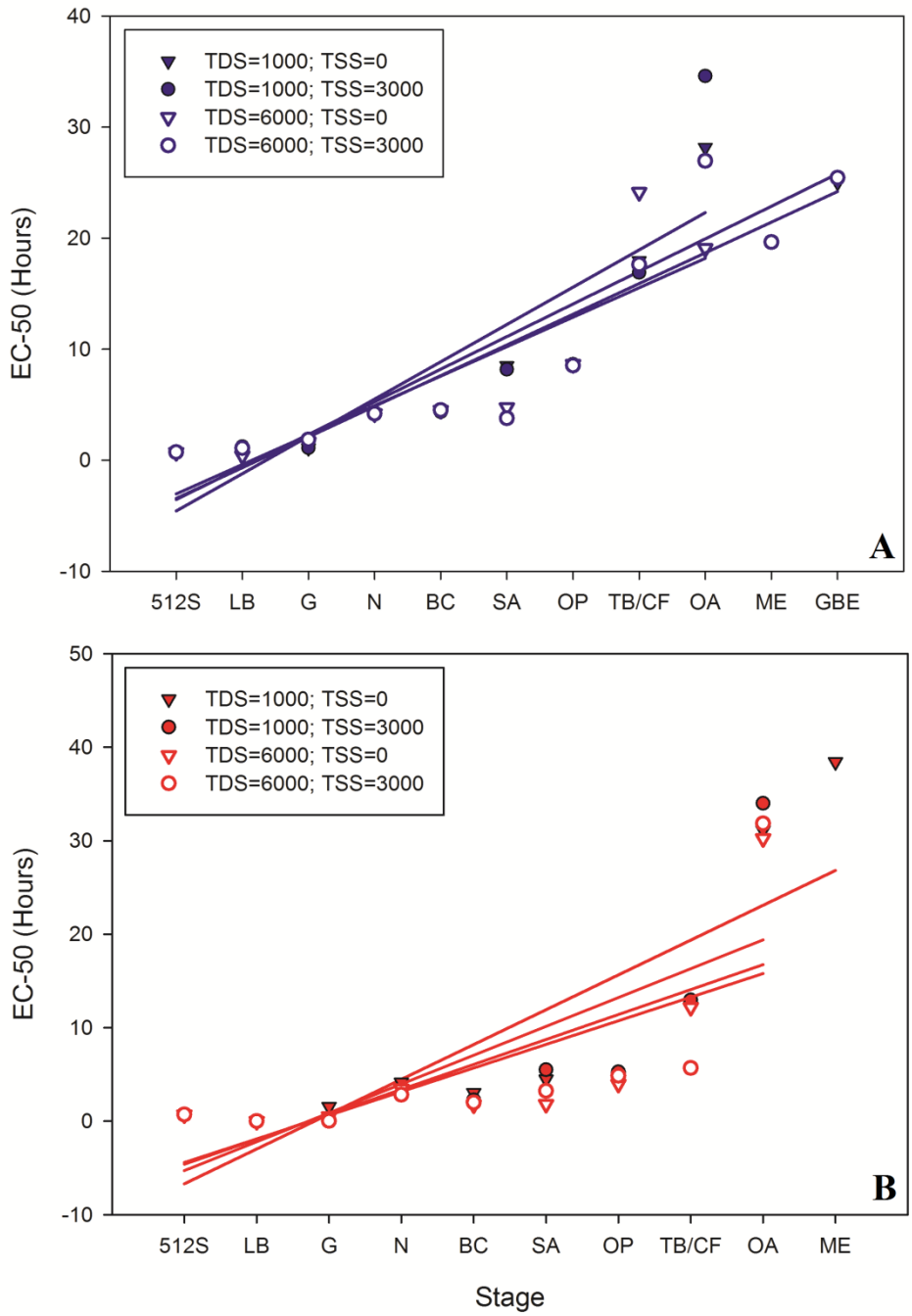

Figure 7. Predicted EC-50 values (hours) for each total dissolved solids, total suspended solids treatment group at 25°C (A) and 31°C (B) per stage of development of Arkansas River shiner eggs and larvae. EC-50 values represents when 50% of the eggs and larvae have reached or exceeded a given stage.

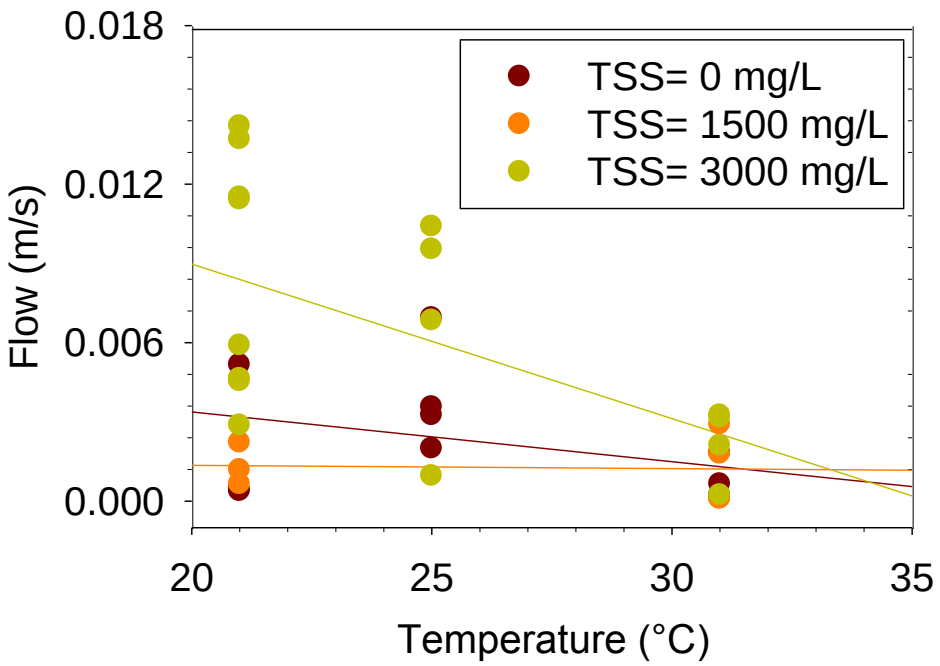

Figure 8. Effects temperature and total suspended solids (TSS) on minimum current velocity needed to keep Arkansas River shiner eggs in suspension.