Articles

Effects of Temperature, Total Dissolved Solids, and Total Suspended Solids on Survival and Development Rate of Larval Arkansas River Shiner

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Abstract

Decreases in the abundance and diversity of stream fishes in the North American Great Plains have been attributed to habitat fragmentation, altered hydrological and temperature regimes, and elevated levels of total dissolved solids and total suspended solids. Pelagic-broadcast spawning cyprinids, such as the Arkansas River Shiner *Notropis girardi*, may be particularly vulnerable to these changing conditions due to their reproductive strategy. Our objectives were to assess the effects of temperature, total dissolved solids, and total suspended solids on the developmental and survival rates of Arkansas River Shiner larvae. Results suggest temperature had the greatest influence on the developmental rate of Arkansas River Shiner larvae. However, embryos exposed to the higher levels of total dissolved solids and total suspended solids reached developmental stages earlier than counterparts at equivalent temperatures. Although this rapid development may be beneficial in fragmented waters, our data suggest it may be associated with lower survival rates. Furthermore, those embryos incubating at high temperatures, or in high levels of total dissolved solids and total suspended solids resulted in less viable embryos and larvae than those incubating in all other temperature, total dissolved solid, and total suspended solid treatment groups. As the Great Plains ecoregion continues to change, these results may assist in understanding reasons for past extirpations and future extirpation threats as well as predict stream reaches capable of sustaining Arkansas River Shiners and other species with similar early life-history strategies.

Keywords: pelagic-broadcast spawning cyprinids, semi-buoyant eggs, threatened species, developmental series, Great Plains cyprinids

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Running Title: Factors Affecting Larval Arkansas River Shiner Survival and Development Rate

Introduction

The rivers and streams flowing through the Great Plains of central North America that once hosted high levels of biodiversity are now considered among the most imperiled biomes on the continent (Dodds et al. 2004). The native ichthyofauna of the Great Plains has

69 experienced high levels of local extirpations influenced by changes in the physicochemical
70 environment (Hoagstrom et al. 2011). For example, average temperature within the Great
71 Plains ecoregion has increased by approximately 0.8°C relative to the 1970s and is predicted
72 to continue to increase by another 1.0-7.5°C by the end of the century (Fields et al. 2007; Karl
73 et al. 2009). Increases in air and water temperatures interact with anthropogenic water
74 withdrawals to intensify stream drying and concentrate total dissolved solids (TDS; Hammer
75 1971; Williamson et al. 1999; Dodds et al. 2004). Total suspended solids (TSS) within Great
76 Plains will continue to increase due to soil erosion after intense rainfall events (Leemans and
77 Kleidon 2002). While the changes projected for Great Plains rivers do not seem to present an
78 improved outlook for already imperiled fishes, there is little information available to
79 understand how species might respond to these predicted changes.
80 The plight of pelagic broadcast-spawning, or pelagophilic, cyprinids inhabiting the
81 rivers of the Great Plains ecoregion represents a nexus where anthropogenic modifications,
82 including climate change, may affect their early life-history stages and ultimately determine
83 their persistence. Pelagophilic cyprinids are a reproductive guild of approximately 20 species
84 of small-bodied (<7 cm) fishes that produce semi-buoyant, non-adhesive embryos (Moore
85 1944; Bestgen et al. 1989; Platania and Altenbach 1998; Bonner and Wilde 2000; Perkin and
86 Gido 2011). Although multiple factors have been proposed as contributing to unsuccessful
87 reproduction in pelagic broadcast-spawning cyprinids, much still remains unknown about how
88 projected physicochemical changes in temperature, TDS, and TSS, might affect development
89 and survival of their early life-history stages.
90 Arkansas River Shiner *Notropis girardi* was listed as a federally threatened species in
91 1998 pursuant to the US Endangered Species Act (ESA 1973, as amended; USFWS 1998).

Although they are among the better studied of the pelagophilic cyprinids, uncertainties regarding their ecological requirements and response to changing environmental conditions limit conservation and recovery planning (Wilde 2002). This species is endemic to the Arkansas River basin but has been extirpated from much of its historic range over the past 30 years (Worthington et al. 2014). Although numerous factors have been proposed to explain this rapid range wide decline, there is still a need to better understand how the changing physicochemical environment may also be contributing. For example, reductions in stream flow have negative effects on reproductive success of Arkansas River Shiners (Durham and Wilde 2009), but the mechanisms responsible for decreasing reproductive success remain unclear. In addition to influencing transport times and retention, reductions in stream flow could produce changes in physicochemical conditions unrelated to current velocity that may affect reproductive success of pelagophilic cyprinids. Numerous studies have demonstrated the effects that physicochemical conditions, such as temperature, TDS, and TSS have on the abundance and persistence of Arkansas River Shiner and other pelagophilic cyprinids (Matthews and Hill 1980; Polivka 1999; Ostrand and Wilde 2002; Ostrand and Wilde 2004), but the effects on their developmental rate and larval survival are not well understood. A thorough understanding of how Arkansas River Shiners respond to temperature, TDS, and TSS during their early life history is an important component necessary for developing an effective conservation program that is responsive to the dynamic environment of the Great Plains. Therefore, the objectives for our study were to 1) assess the effects of temperature, TDS, and TSS on the developmental and survival rates of Arkansas River Shiner embryos and larvae; 2) create an early life-history stages identification guide for Arkansas River Shiner.

Methods

Collection of Arkansas River Shiner

We captured adult Arkansas River shiner in June 2012 using seines from (1) the South Canadian River at the US-283 crossing near Roll, Oklahoma (*n*=54; personnel from U.S. Fish and Wildlife Service) and (2) the Pecos River approximately 10 km upstream from the US-70 crossing in Chaves County, New Mexico (*n*=56; personnel from Museum of Southwestern Biology). We transported all individuals (*n*=110) by truck using small aerated tanks to the U.S. Fish and Wildlife Service Tishomingo National Fish Hatchery in Tishomingo, Oklahoma. We housed the broodstock in a shaded flow-through raceway system maintained at ambient water temperatures (19.9°C - 27.9°C) and photoperiod cycle of 15-h light and 9-h dark. Hatchery personnel fed the Arkansas River Shiner freeze-dried *Tubifex* and frozen chironomid larva (bloodworms) daily.

Induction of spawning

We induced spawning following an established protocol for captive breeding Arkansas River Shiners (K. Graves, Hatchery Manager, U.S. Fish and Wildlife Service, pers. comm.). Briefly, we anesthetized the fish by immersing them into a 25-mg/L tricaine methanosulfate (Western Chemical, Inc., Ferndale, Washington) solution. To determine sex, we applied pressure to the abdomen of each fish sufficient to express gametes then separated individuals by sex into two 37.9-L aquaria (Aqueon, Franklin, Wisconsin) maintained at ambient temperature. We allowed the fish to recover undisturbed for 36-h before inducing them to spawn.

On July 26, 2012 at approximately 0830 hours, we induced spawning in 10 adult Arkansas River Shiners of each gender. To induce spawning, we anesthetized fish 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. Approximately 1.0 ml of a 100-mg/L solution of ground carp pituitary extract (Argent Laboratories, Redmond, Washington) dissolved in sterile saline was injected along the ventral midline near the anus of each fish. After injection, we held all fish in a 10.8-L plastic tub until equilibrium was regained. Then, we transferred the recovered adults to a 37.9-L aquarium and left undisturbed to allow reproduction to occur. As a result, all embryos from all adult pairings were mixed together.

Distribution of embryos

Once spawning was finished and the perivitelline space began to fill, the embryos became semi-buoyant and were ready for transfer to the eight temperature-TDS-TSS treatment beakers. For each treatment, we collected embryos from the spawning tanks using a clear PVC pipe with a 12.7-mm diameter attached to a clear flexible polyurethane tube of the same diameter, and placed into a full 100-ml graduated cylinder. Once the embryos displaced 1.0 ml of water from the graduated cylinder, we transferred the contents to a 240-ml glass treatment beaker using a 3-ml plastic pipette. Preliminary work determined that approximately 45 Arkansas River Shiner embryos occupied a volume of 1.0 ml (Mueller 2013). We therefore estimated that 45 embryos were exposed to each treatment.

Effects of temperature, TDS, and TSS on developmental rate

We chose two treatment levels of temperature (25°C, 31°C), TDS (1,000 mg/L, 6,000 mg/L), and TSS (0 mg/L, 3,000 mg/L) to represent the expected range of typical and projected environmental conditions that Arkansas River Shiner in the Canadian River experience during their spawning season. We selected temperature treatment levels to encompass a range in which successful spawning was observed in laboratory settings (Platania and Altenbach 1998; K. Graves, U.S. Fish and Wildlife Service, pers. comm.) and were within the range of mean monthly water temperatures observed in the Canadian River during Arkansas River Shiner spawning season (April 2011-July 2012, Gage Number 07227500; U.S. Geological Survey). We selected the high treatment levels for TDS and TSS to represent high values observed in the Canadian River (TDS=6,450 mg/L, TSS=3,033 mg/L; Pigg et al. 1999; Gage Number 07227500; U.S. Geological Survey). We used the 25°C-1,000 mg/L TDS-0 mg/L TSS level as the control for the experiment as it represented the same conditions as the spawning tanks.

We created the temperature treatments by adding a 50-W submersible heater and thermostat (Aqueon Products, Franklin, Wisconsin) to the bottom of two 37.9-L aquaria that were filled with approximately 6.5 L of water. We placed the heater horizontally on the backside of the tank and we filled the tanks with water to just below the tops of the beakers so no additional water would get into our treatments. We placed a single 304.8-mm long air diffuser (Top Fin 12-Inch Aquarium Air Stone, PetSmart, Phoenix, Arizona) horizontally in each aquarium in front of the heater to help circulate water and ensure that temperature was uniform throughout the tank. Using a portable fish tank thermometer (Top Fin Digital Aquarium Thermometer, PetSmart, Phoenix, Arizona) we checked the temperature in the tank randomly throughout the study. In both aquaria, at the opposite end of the heater we placed

four 250-ml glass beakers. These beakers housed the TDS-TSS treatment groups. We checked the temperature within each beaker frequently during the study using our portable fish tank thermometer. The high TDS level was established through the addition of Instant Ocean seawater mix (Spectrum Brands, Cincinnati, Ohio). We used commercially packaged Moroccan red clay, i.e., rhassoul, to obtain the desired TSS levels as described by Zamor and Grossman (2007) and Hazelton and Grossman (2009). Both the seawater mix and red clay were weighed to the nearest 0.01 g using a CQT core compact portable electronic balance (Adam Equipment, Inc., Danbury, Connecticut) and added to a premeasured volume of water. To ensure the red clay and Arkansas River Shiner embryos remained in suspension for the duration of the study, we placed a 25.4-mm air diffuser (Grreat Choice Mini Air Stone, PetSmart, Phoenix, Arizona, USA) at the bottom of all eight beakers. In addition to the air diffuser, we used a glass stir rod to gently stir the water in each beaker once per hour to re-suspend anything that may have settled to the bottom.

We collected embryos from each beaker at 1, 2, 4, 8, 12, 16, 24 and 36-h post-fertilization using a plastic pipette and placed into 1.5-ml plastic clear snap cap containers containing a 3% formalin solution. We labeled all containers with the appropriate temperature-TDS-TSS treatment and held in ziploc bags separating them by hours post-fertilization. At the 36-h mark, we drained all beakers into a fine mesh net to ensure no living larvae were left in our treatment beakers. We added any remaining larvae to our 36-h post fertilization results. We used a fine mesh net with a mesh size of 0.09 cm to minimize abrasion while collecting the remaining larvae. During each collection hour, when more than five embryos were collected in a pipette, we retained them all to ensure that potentially damaged embryos were not returned to the treatment beakers. This resulted in inconsistent

sample sizes for each sampling period (Table S1). Furthermore, we were unable to acquire a sufficient number of embryos for a fully-replicated factorial design from induced spawning. Therefore, Arkansas River Shiner developmental data are represented by a single, unreplicated experiment.

At the conclusion of our study, we transferred the plastic bags holding all Arkansas River Shiner embryos to Texas Tech University for further analysis of developmental stage. We used a dissecting microscope (SZX16, Olympus Corporation, Tokyo, Japan) equipped with a digital camera (Infinity 1, Lumenera Corporation, Ottawa, ON Canada) to capture images of all preserved specimens under 11.5X magnification. To aid in the determination of developmental stages, we enhaced images by using the unsharp mask filter and the brightness adjustment feature of the ImageJ v.1.46 software (Abràmoff et al. 2004).

We based assignments of developmental stages primarily on the criteria presented by Moore (1944) for Arkansas River Shiner and supplemented with descriptions of other species in the Cyprinidae family (i.e., Spottail Shiner *Notropis hudsonius*, Jones et al. 1978; Speckled Chub *Macrhybopsis aestivalis*, Bottrell et al. 1964; Rosyface Shiner *Notropis rubellus*, Reed 1958; Zebrafish *Danio rerio,* Kimmel et al. 1995; Grass Carp *Ctenopharyngodon idella*, Black Carp *Mylopharyngodon piceus*, Silver Carp *Hypophthalmichthys molitrix*, and Bighead Carp *Hypophthalmichthys nobilis*, Yi et al. 1988; Chapman 2007). Although some of these species have different reproductive strategies compared to Arkansas River Shiner, we expected that the stages of early development would be consistent among cyprinids.

Results

Images (*n*=227) of Arkansas River Shiner embryos (pre-hatching developmental stages) and larvae (post-hatching developmental stages) were classified into 14 distinct developmental stages (Table 1, Figure 1). Hatching occurred between the caudal fin appearance stage and the otolith appearance stage (Figure 1). When analyzing our developmental rate data, stages that lacked an adequate number of replicates were combined with others, dropping our total number of stages to nine (Figure 2, Table S1). To obtain larger sample sizes, we combined early, mid, and late gastrula together as gastrula stage, tail bud and caudal fin together as tail bud stage, and otolith appearance, melanoid eye, and gas bladder emergence together as otolith appearance stage. Early development of Arkansas River Shiner was rapid, with most individuals entering the late gastrula or neurula stages within 4-h post-fertilization and hatching within 24-36 hours. Embryos from the 31°C temperature treatment tended to be more advanced in their development at a given time than their counterparts incubating at 25°C (Figure 2). Individuals in the highest temperature-TDS-TSS treatment group began hatching as early as 8-h post-fertilization (Figure 2). Embryos developing at higher TDS and TSS treatment levels tended to reach developmental stages earlier than those at lower TDS-TSS treatment levels in the same temperature (Figure 2). Furthermore, the total number of embryos and larvae classified into developmental stages from the lower temperature treatment levels (*n*=144) was almost double those from the higher temperature treatment levels (*n*=83) due to a lack of individuals at the later sampling periods. These results suggest that Arkansas River Shiners raised in the 25°C treatments experienced a mortality rate of approximately 20% compared to about 54% for the individuals exposed to the 31°C treatments.

Discussion

Our study represents an initial step in the process of determining how the changing physicochemical environment of the rivers of the Great Plains ecoregion may be affecting the development and survivability of Arkansas River Shiners. With the climate and landscape use changes being made to the Great Plains we can expect to experience increases in temperature, TDS, and TSS within our river systems. The presence and abundance of pelagophilic cyprinids, such as Arkansas River Shiner, exhibit a strong correlations with these physicochemical factors. For example, Ostrand and Wilde (2002) found that Plains Minnow *Hybognathus placitus*, Smalleye Shiner *Notropis buccula*, and Sharpnose Shiner *Notropis oxyrhynchus* relative abundances were greatest at sites in the upper Brazos River in Texas with low TDS and high TSS. Furthermore, the distribution of Arkansas River Shiner in the South Canadian River in Oklahoma was negatively correlated with both temperature and TDS (Polivka 1999). Our results suggest that changes in the temperature, TDS, and TSS in the rivers and streams of the Great Plains ecoregion may affect the persistence of these species by influencing the developmental rate and survival of early life-history stages.

Even though we were unable to produce a sufficient number of embryos for a fully replicated factorial experiment, the protocols used to spawn Arkansas River Shiner for this study did produce viable embryos. We experienced difficulties collecting a species listed as threatened, which resulted in delayed collections and a smaller than expected sample size. Additionally, previous spawning attempts by these fish may have reduced the number of gametes each individual could produce. Future work should induce as many individuals of Arkansas River Shiner as possible at the beginning of their spawning season. This would help increase the number of viable embryos and also allow for multiple spawning events to occur

during their spawning season if needed. As our study came to a close, meeting our goal of collecting five embryos in each exposure group became more difficult to achieve for those embryos incubated at 31°C. Although we used the same volumetric method as described in our methods for placing embryos into each beaker, this technique had its limitations and did not ensure that all beakers would start with the exact same number of embryos. These results may suggest that although higher temperatures result in faster development, which may be beneficial in shorter river segments, this faster development seemed to also be associated with higher levels of mortality. Though our study did not directly assess mortality rates at regular intervals in each treatment, the number of embryos and larvae recovered for classification at the end of treatment allowed an estimate of the mortality rates associated with different temperatures.

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, a similar relationship between TDS and developmental rate has been described in the Rio Grande Silvery Minnow *Hybognathus amarus* (Cowley et al. 2005). When incubating in high levels of TDS, Rio Grande Silvery Minnows hatched hours earlier than individuals at the same temperature and lower TDS levels. However, the mechanism(s) driving this relationship has not yet been determined. Embryos of another cyprinid, Zebrafish, hatched earlier when exposed to elevated TDS levels produced by inorganic limestone and limestone in suspension than control groups (Reis 1969).

Sorensen et al. (1977) found that increases in suspended solid loads tend to coincide with decreases in dissolved oxygen, which could lead to hypoxic stress during development in many fish species. However, the timing of exposure during early development to increases in

296 TSS may contribute to how the species are affected. For example, Muncy (1979) found that
297 embryos of warm-water fishes show no effects of exposure to high levels of TSS during water
298 hardening of the eggs, but these same levels could result in mortalities when oxygen demands
299 of the embryos were greater during later stages of development. Siefert et al. (1974) found
300 that Smallmouth Bass *Micropterus dolomieu* development was accelerated by low dissolved
301 oxygen concentrations associated with increased TSS. In salmonids, fry survival can decrease
302 as much as 3.4% for each 1% increase in fine sediment (Cederholm et al. 1981). Although
303 rapid development for Arkansas River Shiner and other pelagophilic cyprinids could be a
304 strategy of survival (Platania and Altenbach 1998), the oxygen demands associated with the
305 higher metabolic rates that are likely necessary to support rapid development may render
306 these species more susceptible to mortality or other negative effects from elevated levels of
307 TDS and TSS.
308 Although Arkansas River Shiner embryos reached developmental milestones faster in
309 higher temperatures, future work addressing the survival of their early life-history stages
310 under a range of conditions is warranted. Under the projected changes due to global climate
311 change, a better understanding of how they are affected by the ever-changing environment of
312 the Great Plains, including changes in temperature, TDS, and TSS is needed. The distribution
313 of adult Arkansas River Shiners seem to be negatively correlated with TDS and TSS
314 (Matthews and Hill 1980; Reash and Pigg 1990; Polivka, 1999), and our results suggest that
315 this may be in part due to high mortality rates in their embryo and larvae stages. A better
316 understanding of how shifting physicochemical conditions, such as temperature, TDS, and
317 TSS affect the early life-history stages of different species is important. Such an
318 understanding of different species will allow us to better predict stream reaches capable of

supporting these species even as water quality in the rivers and streams of the Great Plains continue to change.

Supplemental Material

Table S1. Developmental stage of Arkansas River Shiner *Notropis girardi*, eggs and larvae incubating in different temperature (25°C, 31°C), total dissolved solid (TDS; 1,000 mg/L, 6,000 mg/L), and total suspended solid (TSS; 0 mg/L, 3,000 mg/L) treatment groups sampled during the first 36 hours post-fertilization at Tishomingo National Fish Hatchery in Tishomingo, Oklahoma on July 26-27, 2012. Developmental stages include 512-cell stage (512-S), late blastula (LB), gastrula (G), neurula (N), blastopore closure (BC), somite appearance (SA), optic primordium (OP), tail bud (TB), and otolith appearance (OA).

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519 **Table 1.** Assigned developmental stages for the first 36 hours post-fertilization of Arkansas River Shiner *Notropis girardi* embryos
520 and larvae along with its associated time frame and a brief description that aided in identifying each stage. Hatching occurred between
521 the caudal fin appearance stage and the otolith appearance stage. All work was conducted at Tishomingo National Fish Hatchery in
522 Tishomingo, Oklahoma on July 26-27, 2012.
523

Developmental stage	Time post-fertilization (hours)	Description
512-cell stage (512-S)	<1	Beginning of the mid-blastula transition; blastodisc forms a high mound; blastodermal cells arranged into relatively smooth layers (Figure 1A).
late blastula (LB)	1-2	Blastodisc flattened; blastodermal cells align with the yolk cell and form an oval shape (Figure 1B).
early gastrula (EG)	1-2	Blastoderm covers about 30% of distance between animal and vegetal poles, i.e., 30%-epiboly (Figure 1C).
mid-gastrula (MG)	1-2	Blastoderm covers about 50% of distance between animal and vegetal poles, i.e., 50%-epiboly (Figure 1D).
late gastrula (LG)	2-4	Blastoderm covers about 75% of distance between animal and vegetal poles, i.e., 75%-epiboly (Figure 1E).
neurula (N)	4	Blastoderm covers almost the entire distance between the animal to vegetal poles; small uncovered portion of the yolk cell near the vegetal pole is the yolk plug (Figure 1F)
blastopore closure (BC)	2-4	Blastoderm completely covers the yolk cell, i.e., 100%-epiboly; head becomes larger and more distinct (Figure 1G).
somite appearance (SA)	4-8	Somites become visible midway between the animal and vegetal poles (Figure 1H).
optic primordium (OP)	8	Eyes become visible and are elliptical in shape; yolk area not encircled by embryo forms a straight line; additional somites visible (Figure 1I).
tail bud (TB)	8-16	End of tail separates from yolk sac (Figure 1J).
caudal fin (CF)	8-24	Tail completely separated from yolk sac; embryo and yolk sac become elongated (Figure 1K).

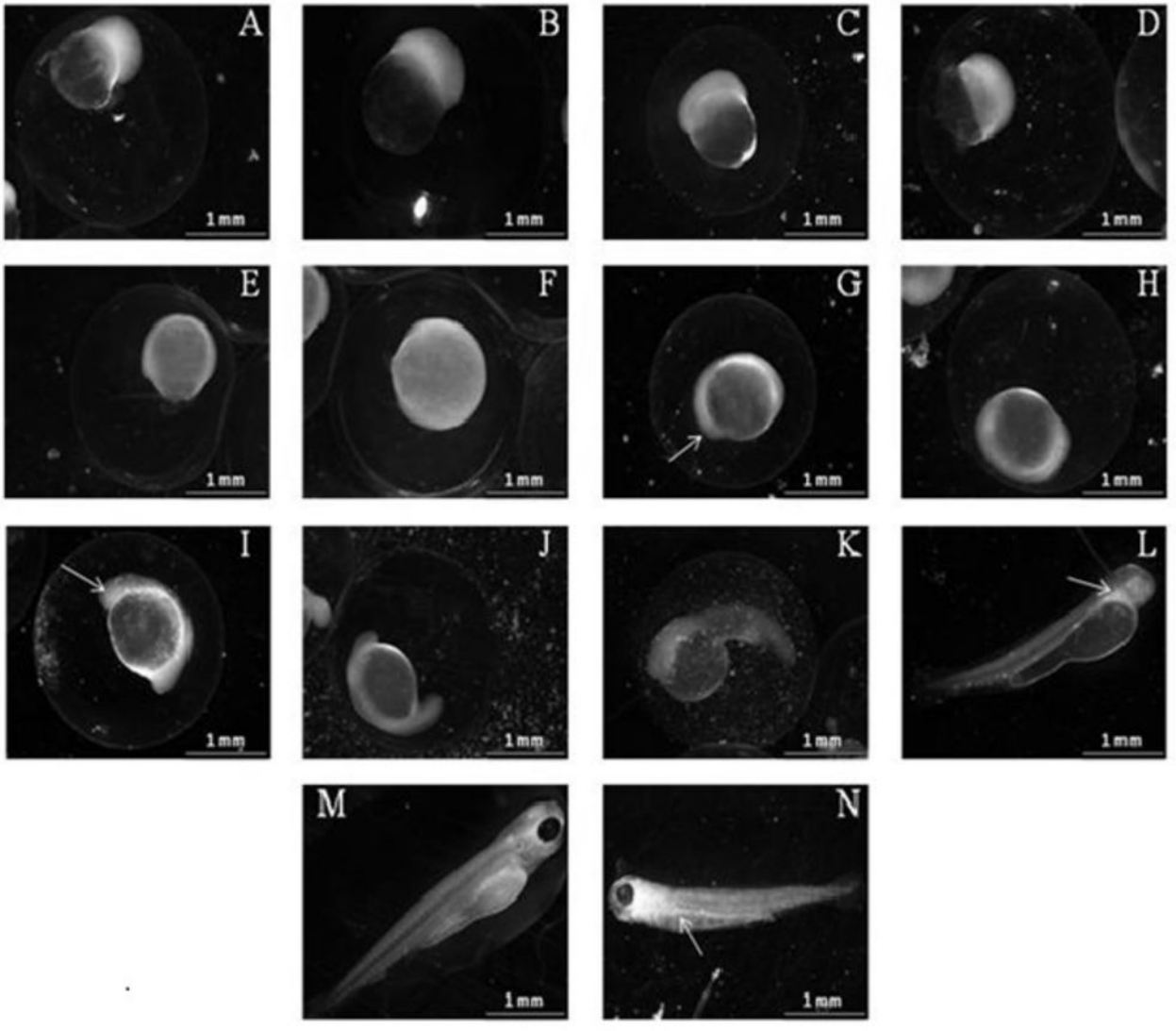
otolith appearance (OA)	24-36	Post-hatching; otolith visible posterior to the eye; eye pigments begin to appear; yolk sac becomes smaller and more elongated (Figure 1L).
melanoid eye (ME)	36	Eyes become very distinct and brown in color; yolk sac continues shrinking and elongating (Figure 1M).
gas bladder emergence (GBE)	24-36	Gas-bladder becomes visible; yolk sac completely absorbed (Figure 1N).

524

Figure captions

Figure 1. Stages of embryonic and larval development of Arkansas River Shiner *Notropis girardi*: (A) 512-cell, (B) late blastula, (C) early gastrula, (D) mid-gastrula, (E) late gastrula, (F) neurula, (G) blastopore closure, (H) somite appearance, (I) optic primordium, (J) tail bud, (K) caudal fin, (L) otolith appearance, (M) melanoid eye, (N) gas bladder emergence. Hatching occurred between the caudal fin appearance stage (K) and the otolith appearance stage (L). All work was conducted at Tishomingo National Fish Hatchery in Tishomingo, Oklahoma on July 26-27, 2012.

Figure 2. Effect of temperature, total dissolved solids (TDS), and total suspended solids (TSS) on the time of appearance post-fertilization of nine developmental stages in Arkansas River Shiner *Notropis girardi.* The work was completed at Tishomingo National Fish Hatchery in Tishomingo, Oklahoma on July 26-27, 2012. The stages are as follows: 512-cell (512-S), late blastula (LB), gastrula (G), neurula (N), blastopore closure (BC), somite appearance (SA), optic primordium (OP), tail bud (TB) and otolith appearance (OA). The height of each bar indicates the sample size for each temperature-TDS-TSS treatment group. Sample size varied in part due to difficulty collecting embryos and larvae and having to eliminate damaged samples. Hatching occurred between the tail bud stage and the otolith appearance stage.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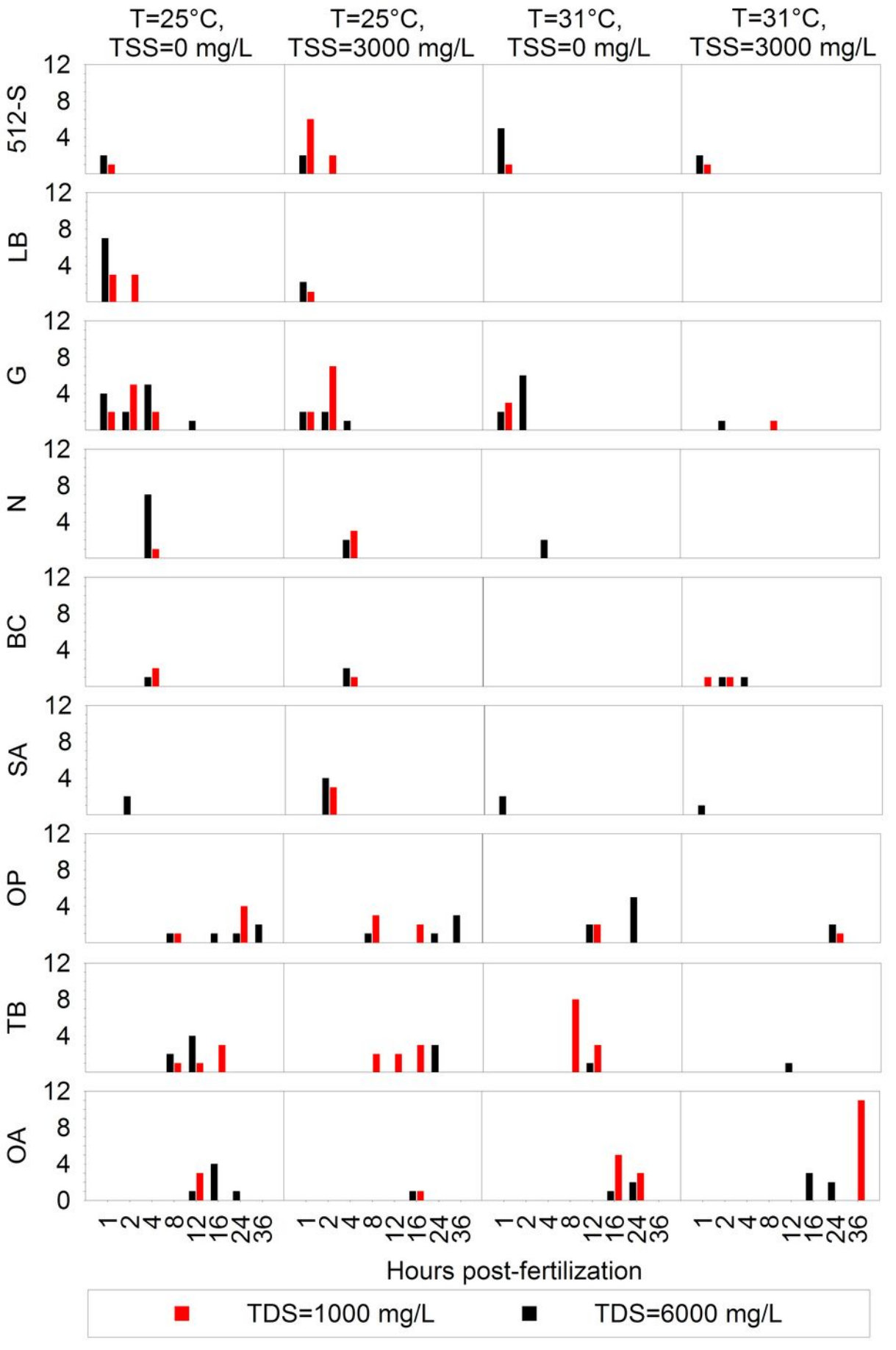
T=25°C, TSS=0 mg/L
T=25°C, TSS=3000 mg/L
T=31°C, TSS=0 mg/L
T=31°C, TSS=3000 mg/L
512-S
LB
G
N
BC
SA
OP
TB
OA
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4
0
1
2
4
8
12
16
24
36
Hours post-fertilization
TDS=1000 mg/L
TDS=6000 mg/L