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# An Investigation into the Effects of Elevated Water Hardness on Channel Catfish Egg Viability

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**Abstract:** Channel catfish (*Ictalurus punctatus*) are a popular sportfish across the United States and are often stocked to enhance fishing opportunities. There has been increased research into their life history, management, and population characteristics over recent decades. In a study conducted on channel catfish recruitment in Thunderbird Reservoir, Oklahoma, researchers found that recruitment was negatively associated with total annual water hardness, hypothesizing that larval fish survival decreased when water hardness was  $\geq 170$  mg/L. To test this hypothesis, we investigated the effects of water hardness on channel catfish egg hatch rates to determine if total water hardness impacts the survivability of larva. Fertilized eggs were obtained from the Holdenville State Fish Hatchery, Oklahoma and transferred to the Oklahoma Fishery Research Laboratory. Eggs were divided and placed in tanks of seven water hardness levels (78 [control], 100, 200, 300, 500, 1000, 3000 mg/L  $\text{CaCO}_3$ ). Overall survival, hatch rate, and larval abnormalities were recorded and analyzed for differences between hardness levels and fish. Water hardness did not influence survival or growth early in life in our study. However, we did observe that the spawning matrix deteriorated in higher hardness concentrations ( $\geq 500$  mg/L). Future studies should investigate the effects of water hardness on channel catfish survival post yolk-sac abortion to determine if mortality increases later in life and determine if water quality optima vary between catfish populations at smaller spatial extents. Future work examining the effects of varying water chemistry levels on egg/larval fish survival can replicate our methods, providing additional insight into the early life history of Channel Catfish or other catfish species.

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## Introduction

Channel catfish (*Ictalurus punctatus*) are popular among anglers, generally abundant, long-lived, commercially important species found throughout the United States (Bouska

et al. 2011). Original distribution of Channel catfish included the Mississippi River basin and Gulf States, north into southern Canada, and south into Mexico, but they can now be found across the Atlantic basin and west of the Rocky Mountains (Wellborn 1988). Federal and state agencies manage and stock channel catfish to enhance fishing opportunities for anglers

(Bodine et al. 2013).

There has been an increase in channel catfish aquaculture research and management literature in recent decades because of their popularity and commercial importance (Porath et al. 2021). Aquaculture studies have yielded information on pond preparation guidelines (Steeby and Brunson 1997), broodfish and hatchery care (Steeby and Avery 2005), and reproductive physiology (Tucker and Hargreaves 2004). Management focused studies have investigated ageing techniques (Hubert 1999, Buckmeier et al. 2002.), growth rates (Holland and Peters 1992, Shephard and Jackson 2006;), and overall population dynamics in Oklahoma and across the country (Shrader et al. 2003, Barada 2009, Bouska et al. 2011, Griffin et al. 2022.).

The Oklahoma study was completed on Thunderbird Reservoir, where channel catfish exhibited slow growth and recruitment was negatively associated with annual water total hardness, seemingly reducing year class strength when water hardness values exceeded 170 mg/L (Griffin et al. 2022). Other studies have found that comparatively higher concentrations of water hardness (150mg/L vs 70mg/L) lead to lower survival of larval fish (*Rhamdia quelen*; Silva et al. 2005) and hatching abnormalities occur at hardness levels  $\geq 300$ mg/L (*Clarias gariepinus*; Molokwu and Okpokwasili 2002). For channel catfish, Tucker and Steeby (1993) tested varying levels of water hardness, up to 100 mg/L, and recommended that hatchery water should contain a minimum of 10 mg/L for the best survival of embryos.

Because a gap exists between better survival of channel catfish embryos exposed to water hardness values  $\geq 10$ mg/L (Tucker and Steeby 1993) and reduced recruitment when water hardness values were  $\geq 170$ mg/L in Thunderbird Reservoir, Oklahoma (Griffin et al., 2022), further investigation was warranted. Therefore, the objectives of this study were to 1) investigate the effects of varying concentrations of water hardness on channel catfish egg hatch rates and 2) determine if higher total water hardness levels impact the survivability of larva.

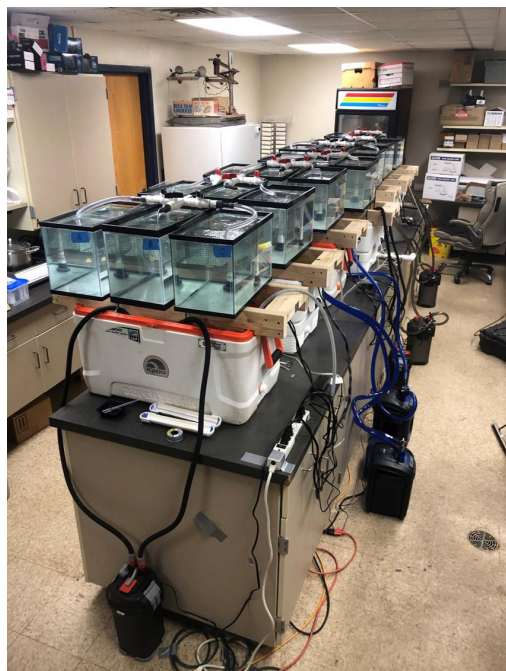
Proc. Okla. Acad. Sci. 102: pp 29 - 38 (2022)

## Methods

Fertilized channel catfish eggs from three individual fish were obtained from the Holdenville State Fish Hatchery in Holdenville, Oklahoma. Eggs were produced in accordance with the methods listed in Steeby and Avery (2005). After incubating for ~24 hours at the hatchery, eggs were transferred to the Oklahoma Fishery Research Laboratory, divided, placed in experimental tanks of seven water hardness levels (78 [control], 100, 200, 300, 500, 1000, 3000 mg/L CaCO<sub>3</sub>), and brought up to and held at 28 °C for the remainder of the experiment. Overall survival, hatch rate, and larval abnormalities were recorded and analyzed for differences between hardness levels.

## Study Design

Twenty-one 19-liter aquariums (Aqueon Standard Glass Aquarium Tank, Aqueon Products, Franklin, WI) were placed on top of seven 68 L coolers (Igloo Marine Ultra 72, Igloo Products Corp., Katy, TX) in three rows of seven (rows A, B, and C; Figure 1). Each



**Figure 1. Image of tank setup showing seven treatment levels (cooler/tank complex) and three replicates (A, B, and C).**

individual aquarium and cooler were filled with 19 and 57 L of water, respectively, for a total of 114 L of water per system. Pre-soaked baskets built with galvanized hardware cloth (Galvanized Steel Hardware Cloth, 6.35 mm square mesh, Blue Hawk, Lowe's) were used to support egg masses to help ensure adequate circulation (Figure 2). Treatment water for each hardness level was aerated, heated, and filtered in a cooler then pumped through a manifold into three individual aquariums to circulate (Figure 1). We used one heater (ViaAqua 300-Watt Quartz Glass Submersible Heater), filter (Fluval 207 Performance Canister Filter, Rolf C. Hagen Corp., Mansfield, MA; Top Fin CF60 Canister Filter, United Pet Group, Earth City, Missouri; Marineland Magnum Polishing Internal Canister Filter and Marineland Magniflow 220 Canister Filter, Spectrum Brands Pet, LLC, Blacksburg, VA), and water pump (Ecoplus Eco-396 Submersible Pump, Hawthorne Gardening Company, Vancouver, WA) per cooler. We used one aerator (Sweetwater Model SL56, Aquatic Eco-Systems Inc., Apopka, FL) with a manifold that split to each of the seven coolers for aeration. Total hardness (mg/L), pH, dissolved

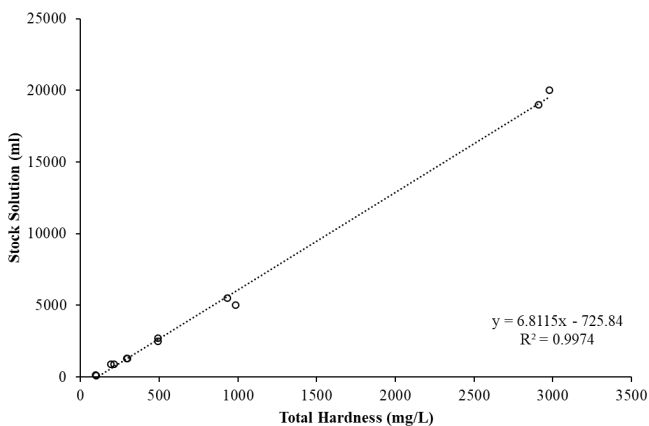
oxygen (mg/L), ammonia (ppm), salinity (ppt), specific conductivity ( $\mu\text{S}/\text{cm}$ ), and temperature (C) were monitored throughout the experiment to ensure water quality was adequate and that treatment levels remained consistent (Table 1). Water was added or changed as needed to maintain a constant volume and to sustain negligible ammonia levels ( $\leq 3.8$  ppm; see Colt and Tchobanoglous 1976).

We used City of Norman, Oklahoma tap water as source water and treated at a rate of 10 ml per 114 L to remove chloramines, chlorine, and detoxify heavy metals (API Tap Water Conditioner, Mars Fishcare North America, Inc. Chalfont, PA). Treated water was stored in six clean 208 L plastic drums (with lids) and mixed accordingly with 70:30 Ca to Mg stock solution (the naturally occurring ratio in our source water) to achieve the desired total hardness level (Table 1). Our stock solution was created by adding 700 g of calcium chloride and 300 g of magnesium carbonate (Reagent Grade, Innovating Science, Aldon Corp., Avon, NY) to 20 L of deionized water, mixing, then boiling for 30 minutes. This stock solution was allowed to cool then added at an incremental rate to achieve the desired hardness level for each treatment (Figure 3). Unmixed treated tap water was used for the control (Table 1). Sand shiners (*Notropis stramineus*,  $n = 60$ ) were held 48 hours in six aquariums with baskets in place to ensure the effectiveness of the tap water conditioner and determine if the galvanized basket material had a negative effect. No deaths were observed after 48 hours and fish were released.



**Figure 2.** Image showing galvanized mesh egg basket.

Eggs from three different individuals (replicates, tank rows A, B, C) were split and placed into tanks according to treatment level (Figure 1; Tucker and Steeby 1993, Molokwu and Okpokwasili 2002.). An initial test was aborted early due to substantial die off, likely caused by an overabundance of eggs. For the second trial, a mass of approximately 50 eggs was weighed, number estimated, then placed in baskets in each tank. Dead eggs were counted and removed as needed. The duration of incubation (hours), number of hatched eggs, number of larval abnormalities, mean length



**Figure 3. Ratio of stock solution to total hardness (mg/L) level when the stock solution is mixed with 208 L of source water (total hardness = 87 mg/L).**

second day post-hatch, and final mean length was recorded for each tank. Throughout the entire process, waste was removed via siphoning to prevent ammonia build up and reduce stress.

### Analysis

Three growth intervals were determined similar to Molokwu and Okpokwasili (2002): the egg interval (initial placement in tanks to beginning of hatch), hatching interval (duration in which eggs were hatching), and yolk-sac interval (post-hatch until yolk-sacs were fully absorbed). For each growth interval, percent survival was determined. Lengths of larva (subset of 10 from each tank) were measured two days post-hatch and at final yolk sac absorption. Percent mortality ( $\sin^{-1}\sqrt{\%}$  transformed) during the egg interval, hatch interval, and duration of the experiment (overall) along with growth (length at yolk sac absorption – larval length two days post-hatch;  $\log_e$ -transformed) were analyzed using two-way analysis of variance (ANOVA) without replication (Zar 1999). The assumptions of each ANOVA were assessed using a Shapiro-Wilk's normality test (Shapiro and Wilk 1965) and a Bartlett's test for homogeneity of variances (Bartlett 1937). If ANOVA detected a significant difference within either of our treatment groups (i.e., fish, hardness), we used a Duncan's multiple range test (DMRT; Tucker and Steeby 1993) *post hoc* to determine which means were significantly different. All statistical tests were conducted using program R 4.2.1 (R Core Team

2022) and *post hoc* tests were performed via the "bartlett.test()" function in the agricolae package (Mendiburu 2021). The threshold for statistical significance was  $\alpha = 0.05$  for all tests.

### Results

The initial number of eggs per tank ranged from 43-64 (Table 2). Hatching began five days post placement and took ~48 hours to complete across all tanks. Mean hatch and final survival ranged from 96.7-99.3 and 85-94.7 percent, respectively across treatment levels (Table 2). Mean larval total length measured at two days post hatch and after complete absorption of the yolk sac ranged 10.9-11.7 mm and 13.3-14.3 mm, respectively (Table 3). Two larval abnormalities were observed. One fish in treatment group 100 A and one in 200 B hatched roughly 48 hours prior to the rest of the fish (regardless of treatment level) and both died. Interestingly, we observed that the spawning matrix in higher treatment levels ( $\geq 500$  mg/L, but particularly at 3000 mg/L) began breaking down early (two days prior to the beginning of the hatch period) to the point where eggs were spread out and some were lost through the basket and laying in the bottom of the tank (A-spawning matrix intact, B-spawning matrix deteriorated; Figure 4).

ANOVA results revealed no significant differences for egg interval mortality or growth



**Table 1.** Mean (SD) values of water quality parameters for each total water hardness (mg/L) treatment level.

Treatment Level	Parameters						
	Total Hardness (mg/L)	pH	Dissolved Oxygen (mg/L)	Ammonia (ppm)	Salinity (ppt)	Specific Conductivity (µS/cm)	Temperature (C)
<b>Control</b>	87 (3)	7.48 (0.41)	7.01 (0.46)	0.25 (0.21)	0.3 (0.05)	617 (106)	27.6 (0.3)
<b>100</b>	103 (5)	7.91 (0.19)	7.11 (0.18)	0.73 (0.23)	0.23 (0.02)	483 (33)	27.8 (0.5)
<b>200</b>	238 (12)	7.91 (0.12)	6.99 (0.25)	0.34 (0.29)	0.4 (0.02)	829 (47)	27.8 (0.4)
<b>300</b>	293 (13)	7.98 (0.07)	7.01 (0.23)	1.36 (0.65)	0.51 (0.03)	1035 (57)	27.6 (0.6)
<b>500</b>	506 (19)	7.89 (0.16)	6.9 (0.22)	0.68 (0.36)	0.8 (0.04)	1607 (80)	27.8 (0.5)
<b>1000</b>	1008 (68)	7.93 (0.15)	6.98 (0.28)	0.27 (0.17)	1.4 (0.07)	2712 (133)	27.4 (0.7)
<b>3000</b>	3044 (158)	7.41 (0.29)	6.85 (0.22)	0.89 (0.2)	4.24 (0.19)	7715 (329)	27.9 (0.2)

within fish ( $df = 2$ ,  $F$  range = 2.18 – 2.42, all  $P > 0.05$ ) or hardness level ( $df = 6$ ,  $F$  range = 1.56 – 1.71, all  $P > 0.05$ ) groups. Hatch interval and overall mortality were determined to be significantly different between fish ( $df = 2$ ,  $F$  range = 5.62 – 8.11, all  $P < 0.05$ ) but similar across hardness levels ( $df = 6$ ,  $F$  range = 0.63 – 1.81, all  $P > 0.05$ ). DMRT results suggested that Fish B (mean = 0.19) had significantly higher hatching mortality than fish C (mean = 0.02); however, both mortality rates were similar to fish A (mean = 0.11; Figure 5). Interestingly, DMRT results suggested that Fish B (mean = 0.36) had

significantly higher overall mortality than fish A (mean = 0.20); however, both mortality rates were similar to Fish C (mean = 0.28; Figure 5). Shapiro-Wilk's normality test confirmed transformed-residuals were normally distributed in all ANOVA models ( $W$  range = 0.43 – 0.98, all  $P > 0.05$ ). Bartlett's test confirmed homogeneity of variance for transformed mortality and growth rates between fish ( $df = 2$ ,  $K^2$  range = 0.45 – 3.71, all  $P > 0.05$ ) and hardness ( $df = 6$ ,  $K^2$  range = 0.73 – 9.62, all  $P > 0.05$ ) groups.



**Figure 4.** Image (A) shows a clumped egg mass indicative of control tanks with a water hardness of 87 mg/L and image (B) illustrates the observed breakdown of the egg mass spawning matrix in the treatment tanks with water hardness  $\geq 500$  mg/L.

**Table 2. Survival of channel catfish eggs and larva incubated in different treatments of total water hardness (mg/L).**

Treatment Level	Egg interval			Hatch interval			Yolk sac interval					
	Initial # of eggs			Survival # to hatch (percent)			Survival # to end of hatch (percent)			Final survival (percent)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	48	56	50	48 (100)	52 (93)	49 (98)	47 (98)	48 (92)	49 (100)	47 (98)	46 (82)	47 (94)
100	54	64	48	53 (98)	50 (78)	41 (85)	53 (100)	48 (96)	41 (100)	51 (94)	48 (75)	41 (85)
200	49	47	51	46 (94)	44 (94)	46 (90)	44 (96)	42 (96)	46 (100)	44 (90)	41 (87)	46 (90)
300	47	51	55	44 (94)	50 (98)	55 (100)	44 (100)	47 (94)	54 (98)	44 (94)	46 (90)	54 (98)
500	51	52	48	51 (100)	48 (92)	48 (100)	51 (100)	47 (98)	48 (100)	51 (100)	47 (90)	45 (94)
1000	51	56	46	51 (100)	53 (95)	42 (91)	48 (94)	52 (98)	42 (100)	49 (96)	53 (95)	42 (91)
3000	43	59	52	40 (93)	56 (95)	50 (96)	39 (98)	55 (98)	50 (100)	40 (93)	54 (92)	47 (90)

**Table 3. Mean larval total length in mm (range, 2nd day post hatch and final) for fish A, B, and C treated at various levels of total water hardness.**

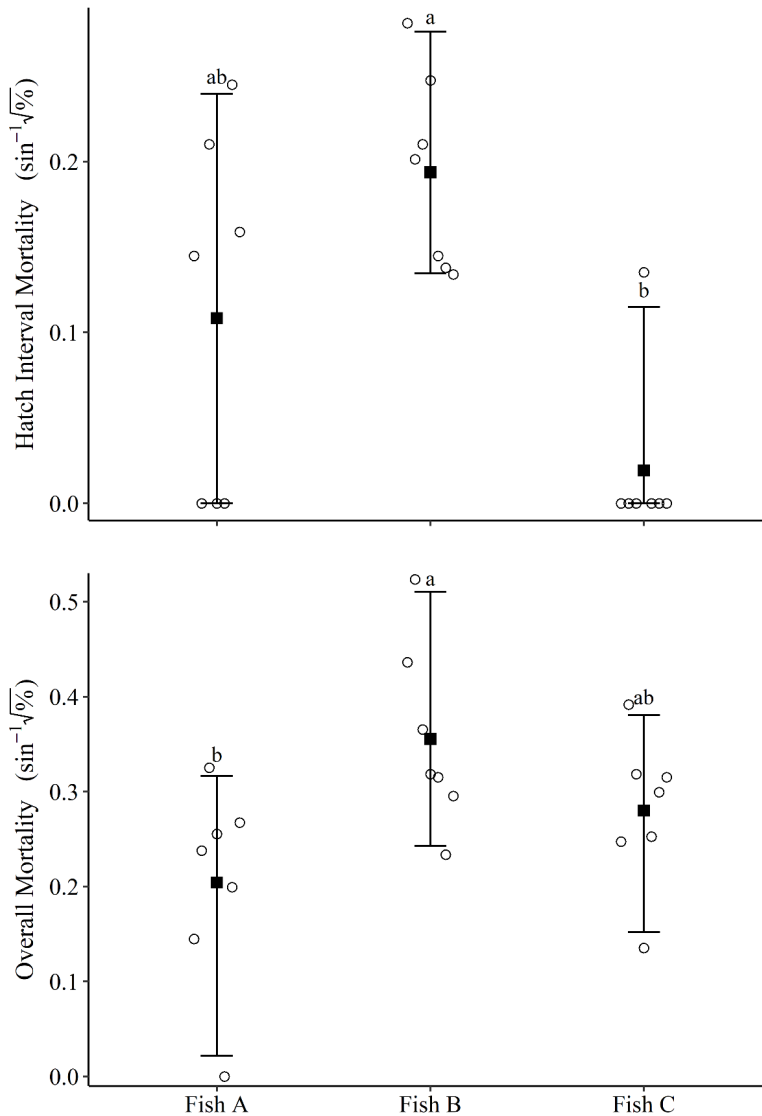
Treatment Level	Mean total length (range)					
	2nd day post hatch			Final		
	A	B	C	A	B	C
Control	11 (10-12)	11.4 (10-12)	11.6 (11-12)	13.5 (13-14)	13.6 (13-14)	14.3 (14-15)
100	11.6 (11-13)	11.3 (11-13)	11.4 (11-12)	13.9 (13-15)	13.4 (13-14)	13.6 (13-14)
200	11.5 (11-12)	10.9 (10-12)	11 (10-12)	14.1 (13-15)	13.6 (13-14)	13.6 (13-14)
300	11.1 (10-12)	10.9 (10-12)	10.9 (10-12)	13.6 (13-14)	13.4 (13-14)	13.6 (13-14)
500	11.1 (10-13)	11.4 (11-12)	11 (10-12)	13.8 (13-15)	13.7 (13-15)	13.4 (13-14)
1000	11.7 (11-13)	11.6 (11-12)	11.2 (10-12)	13.6 (13-14)	13.8 (13-15)	13.8 (13-15)
3000	11.4 (10-12)	11.4 (11-12)	10.7 (10-12)	13.3 (13-14)	13.9 (13-15)	13.4 (13-15)

## Discussion

Our results suggest water hardness did not influence channel catfish survival or growth early in life (i.e., egg to yolk-sac absorption). These findings appear to contradict those of Griffin et al. (2022) *prima facie*, as their results suggested water hardness influenced channel catfish recruitment in Thunderbird Reservoir, OK. However, these contrasting results may be the result of the life interval observed. Our study measured the effects of water hardness on survival up to yolk-sac absorption, whereas Griffin et al. (2022) investigated recruitment variation based on hardness exposure over the first year of life. Increased water hardness may

increase mortality post yolk-sac absorption as external feeding requires greater energy expenditure and increased water hardness has been known to have adverse physiological effects on channel catfish (Buentello and Gatlin 2001). Future studies should investigate the effects of water hardness on channel catfish survival post yolk-sac absorption to determine if mortality increases after the ontogenetic shift to exogenous consumption.

Channel catfish eggs used in this study come from hatchery brood stock, not wild populations. Prior research has noted that there are distinct genetic differences between wild and domestic stocks (Simmons et al. 2006). Furthermore,



**Figure 5. Observed hatch interval and overall percent mortality ( $\sin^{-1}\sqrt{\%}$  transformed) for Fish A, B, and C across all hardness levels (circles). Mean mortality (squares) and 95% confidence intervals are included for each fish. Lowercase letters indicate statistical differences in hatch interval or overall mortality between fish.**

domestic channel catfish exhibit different growth and reproductive characteristics than their wild counterparts (Broussard and Stickney 1981, Bondari 1984). Future work should determine if the results of water chemistry (e.g., hardness, salinity, temperature) studies conducted using domesticated stocks can be applied to wild populations. This is especially important given our results disagree with prior work on a wild channel catfish population (see Griffin et al.

2022). The methodology outlined within this study can be used to determine if wild and domestic stocks exhibit different responses (e.g., reduced survival, lower growth) to water chemistry variation.

This study highlights the importance of monitoring mortality over various life stages (e.g., egg, hatch, larva). No mortality differences were observed between fish at the egg stage

while fish C progeny exhibited the lowest mortality during the hatch interval, and fish B progeny exhibited the lowest mortality by the end of the study. To the best of our knowledge, this is the first study to document a significant difference between interval specific mortality between the same species of catfish. However, variation in interval specific mortality due to water chemistry (specifically salinity) has been noted between species of catfish (Abass et al. 2017). The variation in interval specific mortality between conspecifics, the consistently higher mortality of fish B from the hatching interval on, and the relatively stable density of individuals within treatments suggest differences may be due to genetic or epigenetic variation between individuals. However, further study would be required to determine if genetic or epigenetic variations are the source of differential mortality between channel catfish.

Genetic variation in channel catfish stocks is poorly understood within Oklahoma. Genetic information from Mexico (Lara-Rivera et al. 2019) and Alabama (Simmons et al. 2006) show that distinct genetic stocks can exist. Furthermore, genetic differentiation between channel catfish generally increases with spatial distance and is influenced by site-specific effects (Sotola et al. 2017). This suggests that there is potential for genetic variation within channel catfish populations in Oklahoma. If genetic differences do exist, there is potential for variation in water quality optima (e.g., salinity, temperature, hardness) between populations. At broader spatial extents (i.e., northern United States vs southern United States), differences in critical thermal maxima have been documented between channel catfish strains (Stewart and Allen 2014). Future work should determine if water quality optima vary between catfish populations at smaller spatial extents (e.g., Oklahoma) and if there is a genetic or epigenetic basis for such regulation.

Water chemistry can exhibit varying relationships on fish vital rates (e.g., mortality, growth) due to interactions between variable causing confounding effects. Prior studies have documented that lower water hardness increases

copper-induced mortality (Perschbacher and Wurts 1999) and mitigates sub-lethal effects (e.g., reduced growth) of chronic ammonia exposure (Sinha et al. 2022) in channel catfish. Additionally, our observation that the spawning matrix deteriorated in higher hardness concentrations might be a clue. Male channel catfish remain with the egg mass after fertilization to guard and fan water over the eggs (Tucker and Hargreaves 2004). In higher concentrations of water hardness, the breakdown of the egg matrix would likely allow the eggs to be either covered in silt, or fanned out of the spawning cavity, where they would be susceptible to predation. Future work should determine the impact of accelerated breakdown of the spawning matrix on eggs (i.e., eggs falling into silt/sediment, vulnerability to predation).

The results of this study help to contextualize the findings of Tucker and Steeby (1993), who recommended that water supply for hatchery rearing have a minimum hardness of 10 mg/L. These findings may be exclusive to channel catfish considering that other species, such as the silver catfish (*Rhamdia quelen*), had lower post hatch survival in higher water hardness trials (Silva et al. 2003). Our study did not find any difference in hatching or larval success based on hardness level contrasting with the findings of Griffin et al. (2022), where recruitment success was negatively correlated with higher water hardness values. Following this study, we recommend that fishery managers continue to follow past guidelines, such as those produced by Tucker and Steeby (1993), for channel catfish rearing. However, if supply water has high levels of hardness ( $\geq 500$  mg/L) managers should use caution when handling, transporting, or incubating egg masses. Future studies on how water hardness impacts the uptake of potential toxins should be considered for channel catfish. Additionally, future work examining the effects of varying water chemistry levels on egg/larval fish survival can replicate our methods for experimental design.

## Acknowledgments

The authors thank Shelby Jeter, Alexis



Whiles, and Michael Richardson for their assistance with laboratory work. We thank Graham Montague for reviewing an earlier version of this manuscript. We also thank Dr. Mostafa Elshahed and anonymous reviewers for providing edits that improved this manuscript. Financial support for this publication was provided by Sport Fish Restoration Program grants F-5-C-51 and F-43-D-30 to the Oklahoma Department of Wildlife Conservation.

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Submitted August 16, 2022 Accepted November 12, 2022