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MANAGEMENT BRIEF

Influence of Ice Angler Culling Practices on Bluegill Physiological Stress Responses and Mortality

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Abstract

Decreasing bag limits is a management mechanism for enhancing size structure of Bluegill *Lepomis macrochirus*. However, restrictive bag limits can promote culling, where an angler returns a live fish to the water in exchange for another. Little is known about the effect of culling on ice-angled fishes. Our objective was to compare the effects of Bluegill confinement methods (reference, ice well, and bucket) and holding durations (0, 1, 2, or 5 h) on changes in water quality parameters (dissolved oxygen, pH, carbon dioxide, and water temperature) and Bluegill stress physiology (blood glucose and plasma cortisol), reflex responses (reflex action mortality predictor [RAMP] scores), and mortality while ice angling. In February 2018, 182 Bluegills were angled through the ice and randomly assigned a confinement method and holding duration. Bluegill blood glucose levels were higher in both confinement methods than reference fish at 2-h and 5-h holding durations. Bluegills had higher blood glucose levels in buckets than ice wells at 1 and 2 h, but they had higher blood glucose levels in ice wells at 5 h. Water temperature was warmer in buckets than ice wells at all holding durations, while ice wells were cooler than ambient lake temperature. Bucket pH was higher than the lake at 2 h, and ice well pH was higher than the lake at 1 and 2 h. Bluegill RAMP scores were similar across all holding durations and confinement methods, but they were elevated in individuals held for the 24-h mortality assessment. Two Bluegill mortalities occurred for fish held in ice wells. Our results suggest that confinement method and holding duration while ice angling can result in altered Bluegill blood glucose concentrations, water temperatures, and pH concentrations but that culling while ice angling might not result in mortality. Consequently, culling practices may be compatible with and not negate the intended benefits of reduced Bluegill bag limits.

Catch-and-release angling is a popular recreational activity driven by changes in angler conservation ethics and more restrictive harvest regulations (Cowx 2002; Cooke and Cowx 2004; Bartholomew and Bohnsack 2005). Benefits of catch-and-release angling include enhanced recreational quality, increased catch rates, and improved size structure (Hubert and Quist 2010). However, success of catch-and-release practices relies on the assumption that released fish will survive after experiencing stressors associated with capture, handing, and confinement (Arlinghaus et al. 2007). The catch-and-release process can directly influence changes in fish stress physiology (e.g., changes in blood glucose and plasma cortisol concentrations) via handling practices (e.g., Pottinger 1998; Meka and McCormick 2005) and indirectly via changes associated with confinement water quality (e.g., water temperature, ammonia, carbon dioxide, and dissolved oxygen; Caldwell and Hinshaw 1994; Meka and McCormick 2005). Prolonged exposure to stressors associated with catch-and-release angling can result in altered reflexes, behavior, and mortality (Cooke et al. 2003; Davis 2007; Raby et al. 2012, 2014). Thus, understanding the influence of angler practices on fish stress physiology, water quality, and survival can enhance fish management and conservation.

Catch-and-release angling is popular during both open-water (Bettoli and Osborne 1998; Suski et al. 2003; Bartholomew and Bohnsack 2005) and frozen-water

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(i.e., ice angling Margenau et al. 2003; Cooke and Cowx 2004; Louison et al. 2017a, 2017b) seasons. However, most of the research assessing the effects of angling on alterations to fish stress physiology, behavior, and survival has taken place during open-water periods (e.g., Cooke et al. 2002, 2003; Bettinger et al. 2005; Brill et al. 2008; Wedemeyer and Wydoski 2008) when water temperatures and fish metabolic rates are at their highest (Winter et al. 2018). Comparatively, there is less information concerning the effects of catch-and-release ice angling on fish stress physiology, behavior, and survival when water temperatures and metabolic rates are their lowest (but see Louison et al. 2017a, 2017b; Twardek et al. 2018; Winter et al. 2018; Bieber et al. 2019; Logan et al. 2019). Yet, an appreciable number of ice-angled fishes are released (Margenau et al. 2003; Schroeder and Fulton 2014), including following competitive live-release ice-angling events (e.g., the Brainerd Jaycees Ice Fishing Extravaganza in Gull Lake, Minnesota [http://icefishing.org]; Reel Fun Ice Fishing Tournament in Grand Lake, Michigan [http://www.icefishingalpena.com]; and Northern Challenge Fishing Derby in Tupper Lake, New York [https://www.tupperlake.com/events/northern-challenge-fishing-derby]). Furthermore, recreational ice anglers are increasingly incorporating culling practices due to changes in fishing regulations, such as decreases in bag limits (i.e., the number of fish an angler is permitted to harvest). Culling occurs when an angler returns a captured and confined fish to the water in exchange for a fish captured at a later time (Kerr and Kamke 2003; Isermann and Paukert 2010). While there is increasing insight into physiological responses of fish captured through the ice, little is known about the post-release survival of these fish (but see Twardek et al. 2018) and how it may vary across different angler culling practices, such as duration of holding time and type of confinement. Therefore, it is important to evaluate whether various ice-angling practices such as confinement method and holding duration influence stress and mortality of fishes to understand whether fish culled by recreational anglers or fish released following ice-angling tournaments survive. Information regarding the effects of culling practices can develop best angler practices to improve the survival of ice-angled fish (e.g., Cooke and Suski 2005; Cooke and Schramm 2007).

Unforeseen mortality caused by catch-and-release ice angling is important to understand, as harvest rates for sport fishes can remain constant (Deroba et al. 2007) or increase (Teisl et al. 1993; Radomski 2003; Isermann et al. 2005) during the ice-angling season. Bluegill *Lepomis macrochirus* is an important sport and food fish (Edison et al. 2006) that can experience significant harvest during open-water and ice seasons (Reed and Parsons 1999). For instance, the exploitation rate of quality-sized Bluegills (>150 mm total length) was 35% of the population within the first month of the opening of the Mid Lake fishery in Wisconsin, USA (Goedde and Coble 1981). Additionally, Bluegills in Lake Onalaska, Wisconsin, USA, accounted for 91% (31,696 kg) of the winter creel in 1976–1977 (Rach and Meyer 1982). Historically, fisheries managers considered harvest regulations for Bluegills unnecessary (Coble 1988) because angler harvest was thought to be important for mitigating density-dependent responses (decreased growth and poor size structure associated with high abundance; Krumholz 1946; Tomcko and Pierce 2011). However, recent literature suggests that excessive harvest of Bluegills can be a driver of undesirable size structure (Drake et al. 1997; Beard and Essington 2000; Crawford and Allen 2006) characterized by an abundance of small and similarly sized individuals (Hubert and Quist 2010). Consequently, some state agencies have started decreasing angler bag limits to improve Bluegill size structure (e.g., Beard et al. 1997; Jacobson 2005; Edison et al. 2006; Rypel 2015). For instance, the Wisconsin Department of Natural Resources developed regulations aimed at improving panfish size structure in 94 systems by decreasing bag limits to as low as 15 panfish per day with no more than 5 of one species (Wisconsin Department of Natural Resources 2015). Even more conservatively, the Minnesota Department of Natural Resources proposed increasing the number of special-regulation lakes for sunfish (bag limits of 5–10 fish per day) from 57 to 200–250 lakes by 2023 (Minnesota Department of Natural Resources 2020). Research in Illinois (Edison et al. 2006) and Nebraska (Paukert et al. 2002) suggests that anglers are more likely to support more restrictive regulations with the possibility of improved Bluegill size structure.

While reduced bag limits may curtail the number of fish harvested, it may also promote angler culling, as individuals larger than those confined may be captured after the bag limit is reached. Ice anglers use various methods to confine their catch, including buckets or ice wells (holes cut into the ice surface that hold fish and exchange water with the lake), that may have differential effects on culled fish stress physiology and survival. Research on Largemouth Bass *Micropterus salmoides* (Kwak and Henry 1995; Siepker et al. 2007), Smallmouth Bass *M. dolomieu* (Gravel and Cook 2008), and Walleye *Sander vitreus* (Goeman 1991; Reeves and Bruesewitz 2007) catch-and-release angling during open-water periods indicates that mortality can occur as a result of culling. If survival of culled fish is low, bag limits intended to preserve stocks may be less effective (Post et al. 2002). Therefore, understanding the effects of different holding treatments on fish stress physiology and survival is critical for successful angler culling and implementing more conservative harvest regulations.

Ice angling (Louison et al. 2017b) and exercise at low water temperatures (Winter et al. 2018) induce physiological changes in Bluegills indicative of stress. However, information concerning how ice-angling confinement methods influence water quality, fish stress physiology, and survival is scarce. Identifying confinement methods that reduce fish stress and increase survival will improve fish welfare and benefit restrictive harvest regulations. Therefore, our objectives were to compare the effects of confinement methods (ice well and bucket) and holding duration (0, 1, 2, and 5 h) on changes in water quality parameters (dissolved oxygen, pH, carbon dioxide, and water temperature), Bluegill physiological stress responses (blood glucose and plasma cortisol), reflex responses (reflex action mortality predictor [RAMP] scores), and mortality rates. Confined Bluegills were hypothesized to have higher blood glucose and plasma cortisol concentrations, reflex impairments, and mortality rates than released fish. Additionally, we hypothesized that Bluegills held in buckets would have higher blood glucose and plasma cortisol concentrations than those held in ice wells, and buckets would have lower dissolved oxygen concentrations and higher water temperatures relative to ice wells. Insights from this research will provide valuable information on whether confinement methods and holding duration influence the stress and survival rates of culled Bluegills during ice angling

METHODS

Study overview.— On February 22 and 25, 2018, 182 Bluegills (average TL = 167 mm; SD = 16) from Twin Anchors Pond in Colo, Iowa, USA were ice-angled between 0700 and 1800 via rod and reel. The 2 d of sampling had high temperatures of 1.1°C and 6.7°C, low temperatures of −3.9°C and −4.4°C, and average temperatures of −1.1°C and 0.6°C. All fishing rods had 1.36-kg test line and were baited with a #14 tungsten jig tipped with a wax worm. Five anglers participated on the February 22 and 18 anglers on February 25, with fishing depths ranging from 1.5 to 4.3 m.

Collection and holding protocols.— Confinement methods consisted of an 18.9-L bucket and a 1.1-L ice well that was cut in the ice surface and connected to the lake. After angling, Bluegills ($N = 137$) were randomly assigned to a treatment. Treatments consisted of a combination of confinement method and holding duration (1, 2, or 5 h), or fish were immediately processed and returned to the lake (hereafter referred to as "reference Bluegills"). Treatments were based on common practices of ice anglers in the region. An additional 44 Bluegills were randomly held in an 18.9-L bucket or ice well for 2 h or immediately released into a mortality monitoring cage for 24 h. Each bucket was filled with 3.8 L of lake water at the time of Bluegill capture. All buckets used were identical in size (18.9 L) and color (blue) to avoid any potential bias (Barcellos et al. 2009). Ice wells were drilled with a 20.3-cm-diameter auger and included a solid bottom except for a 1.5-cm hole in the base of the well to allow for water exchange with the lake.

Blood collection and analysis.— Blood samples were drawn either immediately postcapture (≤1 min) or 1, 2, or 5 h later. The entire blood draw process took less than 30 s for each Bluegill and should have had a limited effect on reflex responses. A 23-gauge needle with a vacutainer containing 143 units of sodium heparin was inserted ventrally adjacent to the anterior of the anal fin and 400 μL of whole blood was removed (Ball and Weber 2017). Each vacutainer was inverted 10 times to mix the sodium heparin and whole blood before being placed into an ice slurry. After collection of all blood samples, a sterile disposable pipette was used to remove 0.3 μL of whole blood that was placed directly onto a FreeStyle Lite test strip (FreeStyle Lite Meter, Abbott Diabetes Care, Alameda, California) to measure blood glucose concentrations (Cousineau et al. 2014; Ball and Weber 2017). The remaining sample was placed in a 14-mL centrifuge tube and spun at 3,500 revolutions per minute for 10 min to separate plasma from red blood cells. Plasma from the top of the sample was removed with a clean, disposable pipette and placed in a microfuge tube and frozen at −80°C for future plasma cortisol processing in the laboratory at Iowa State University.

A Cortisol ELISA kit (Enzo Life Sciences, Farmingdale, New York), a competitive immunoassay, was used to measure quantitative plasma cortisol concentrations (Grausgruber and Weber 2021). The kit uses a monoclonal antibody that competitively binds to plasma cortisol. A standard curve was developed in conjunction with the enzymatic sample reactions. After an incubation time of 2 h, the binding reaction was stopped, and the yellow color generated was read on a microplate reader at 405 nm. Plasma cortisol concentrations were based on the developed standard curve (Chard 1995). Difficulty obtaining sufficient quantities of blood for blood glucose and plasma cortisol analysis in the cold weather environment limited the sample size ($N = 41$ Bluegills) for plasma cortisol.

Reflex impairment assessment.— Immediately before release, all Bluegills were evaluated for the presence of five reflexes that are consistently present in fish in favorable conditions (RAMP scores; Davis 2005, 2007; Raby et al. 2012). Each reflex was assessed categorically (0 = unimpaired, 1 = impaired) in a conservative matter. If the evaluator doubted whether the reflex was present, the response recorded was impaired. Reflexes tested included tail grab, body flex, head complex, vestibular–ocular response, and orientation. Evaluation of the tail grab response consisted of the handler attempting to grab the Bluegill's tail with the fish submerged in an 18.9-L bucket containing lake water. Bluegills attempting to immediately burst swim away upon contact indicated an unimpaired response. The evaluator assessed fish body reflex by holding the Bluegill out of water using two hands wrapped around the middle of the body. An unimpaired response occurred if the Bluegill actively attempted to struggle free. An unimpaired head complex response occurred if a Bluegill exhibited an irregular ventilation pattern (5 s) when held out of the water. Irregular ventilation patters are observable by watching the opening and closing of the lower jaw. To evaluate the vestibular–ocular response, the evaluator turned the fish on its side (i.e., on a lengthwise axis) out of water. The eye rolling to maintain level pitch and appearance to track the evaluator indicated an unimpaired vestibular–ocular response. Finally, the orientation reflex was evaluated by inverting each Bluegill in an 18.9-L bucket containing lake water just below the surface: an unimpaired response consisted of the fish righting itself within 3 s. The entire reflex assessment took approximately 30 s to complete. Reflex assessments took place before release and after blood sample collection. For each Bluegill, an overall RAMP score was calculated as the proportion of impaired reflexes (Davis 2007).

Water quality parameters.— Water quality parameters (dissolved oxygen, pH, water temperature, and carbon dioxide) were measured concurrently with blood draws and RAMP assessments. Reference Bluegill water samples consisted of sampling the water directly in the lake 0.5 m below the ice. Dissolved oxygen (mg/L), pH, and water temperature (°C) were quantified with a HACH Multi HQ 40d meter (HACH, Loveland, Colorado). A LaMotte carbon dioxide DRT kit (LaMotte, Chestertown, Maryland) was used to quantify carbon dioxide concentrations (mg/L). The carbon dioxide DRT kit uses a titration method where two drops of 1% phenolphthalein indicator were mixed with 20 mL of sample water. A series of 1-mL drops of carbon dioxide reagent B (4253DR) were added to the solution and gently swirled until a faint pink color was produced and persisted for 30 s. The amount of carbon dioxide reagent B used in the titration represented the carbon dioxide concentration in ppm (mg/L).

Assessing mortality.— In addition to the 132 Bluegills used for the blood analysis portion of the experiment, 44 additional Bluegills (reference = 15; bucket = 14; ice well = 15) were held in either an 18.9-L bucket or ice well for 2 h or immediately released (reference Bluegill) into a cylindrical mesh cage (height: 1.9 m; diameter 0.9 m) underneath the ice and held for 24 h. Fin clips differentiated Bluegills from each treatment (Coble 1988). After a 24-h holding duration, the number of deceased Bluegills was recorded, and viable individuals underwent the reflex impairment assessment protocol as previously described then released.

Statistical analyses.— Due to an incomplete factorial design (i.e., reference fish that were immediately released instead of confined could not be held for any period of time), linear models blocked by sample day with treatment means representing a combination of holding duration and confinement method (e.g., bucket, 2 h postcapture; ice well, 0 h postcapture, etc.) were used to assess whether there were differences in water quality parameters (water temperature, dissolved oxygen, pH, and carbon dioxide) and Bluegill blood glucose concentrations. Thus, for each linear model, seven treatments were compared for each response variable (Y_{ij}), and all models had a similar structure:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij},$$

where μ is the overall treatment mean, τ_i is the effect of the ith treatment, β_j is the random effect of day, and ε_{ij} is experimental random error. All models were evaluated in R Studio (R Core Team 2017). Post hoc evaluations of linear model assumptions assessed the independence, normal distribution, and equal variance of residuals (McDonald 2014). All model assumptions were met; thus, transformations were not necessary. The "emmeans" package (Lenth 2018) used Tukey's correction for multiple comparisons to compare treatment differences of linear models. Due to the small sample size of plasma cortisol samples across treatments ($N = 41$) we did not have sufficient data to conduct the aforementioned analysis. Instead, we assessed mean values (±1 SE) for the limited number of observations per treatment ($n = 2$–14).

Individual reflex impairment (RAMP) data are proportional and violate the assumptions of a linear model. Therefore, we analyzed these data using a nonparametric Kruskal–Wallis test that is an alternative to a parametric one-way ANOVA. Post hoc multiple comparisons were made using a pairwise Wilcox test with a Benjamini–Hochberg correction that helps avoid type I errors. The package "dplyr" (Wickham et al. 2021) was used to run nonparametric reflex impairment tests and post hoc analysis. Binary logistic regression models with number of Bluegill mortalities as the dependent variable and confinement methods as the independent variable were used to evaluate the number of Bluegill mortalities across confinement methods (2-h bucket, 2-h ice well, and reference Bluegill).

RESULTS

Water Quality

Water quality parameters were significantly different among confinement treatments (water temperature: $F_{6,\ 124} = 60.82$, $P \leq 0.0001$; pH: $F_{6,\ 131} = 5.63$, $P \leq 0.0001$; dissolved oxygen: $F_{6,\ 125} = 3.99$, $P \leq 0.0011$; carbon dioxide: $F_{6,\ 86} = 6.60$, $P \leq 0.0001$). Mean water temperatures in buckets were significantly higher than the lake at all holding durations except at 5 h (Figure 1A). Conversely, mean water temperatures in ice wells were significantly lower

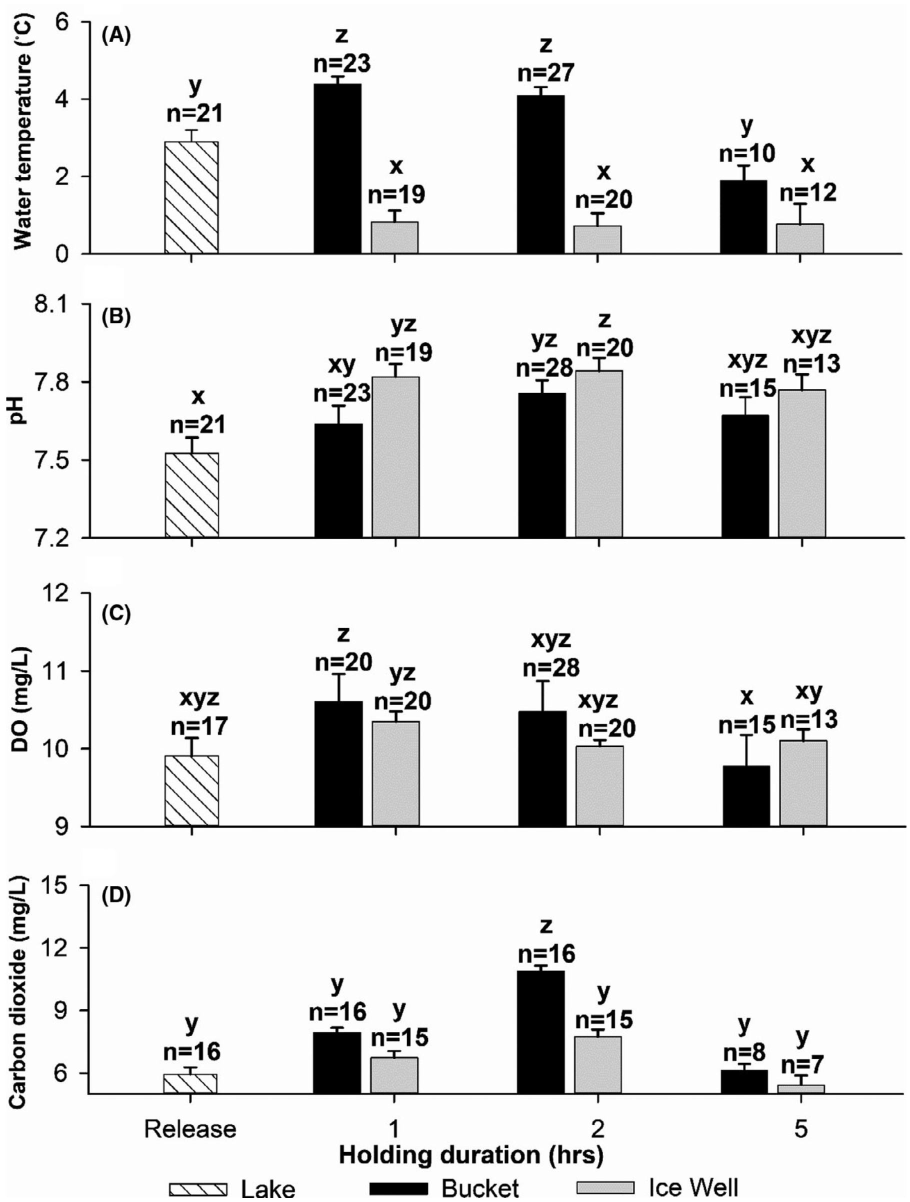

FIGURE 1. **(A)** Mean (±SE) water temperature (°C), **(B)** pH, **(C)** dissolved oxygen (mg/L) and **(D)** carbon dioxide (mg/L) values for different holding durations (1, 2, and 5 h) in different confinement methods (lake = white with diagonal lines; bucket = black; ice well = light gray). Sample size (*n*) is displayed above each bar, and bars sharing similar letters are not significantly different from one another.

than temperatures in the lake and buckets at all holding durations (Figure 1A). Mean pH was significantly higher in ice wells than the lake at 1 and 2 h but similar to buckets and the lake at 5 h (Figure 1B). Confinement method had little effect on mean dissolved oxygen or carbon dioxide across all holding durations (Figure 1C, D).

Blood Glucose and Plasma Cortisol Response

Blood glucose ($F_{6, 124} = 20.76$, $P \leq 0.0001$) concentrations were significantly different among confinement treatments. Mean blood glucose concentrations of Bluegills held for 2 and 5 h in both confinement methods and Bluegills held for 1 h in buckets were all significantly higher than reference Bluegills (Figure 2A). Bluegills held in buckets had higher blood glucose concentrations than ice wells at 1 and 2 h, while ice wells contained Bluegills with higher blood glucose concentrations than buckets at 5 h (Figure 2A). Mean (±SE) Bluegill plasma cortisol concentrations held for 1 h in buckets (3.30 ± 0.46 ng/mL) were more than two times higher than fish held in ice wells (1.35 ± 0.35 ng/mL) and reference Bluegills (1.54 ± 0.54 ng/mL; Figure 2B). Additionally, mean (±SE) plasma cortisol

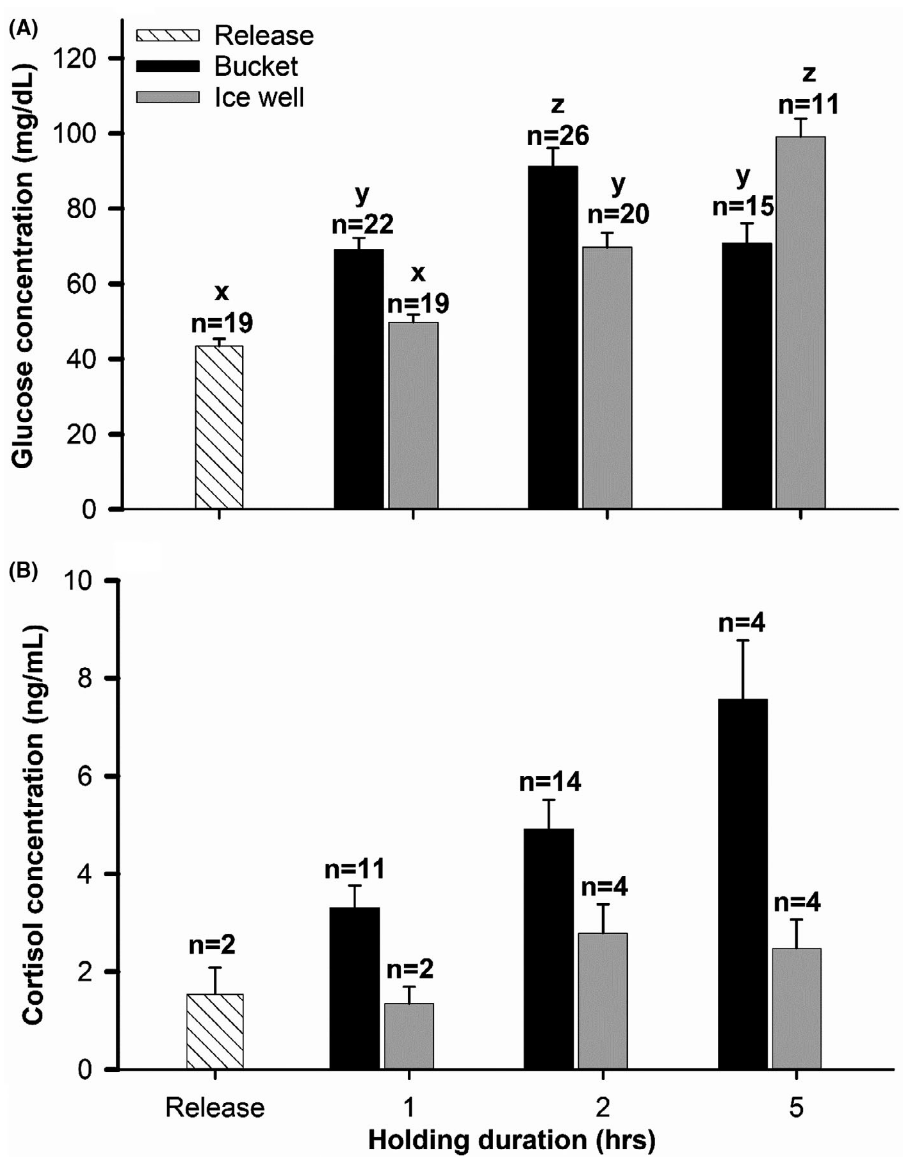

FIGURE 2. Mean (±SE) Bluegill **(A)** blood glucose (mg/dL) and **(B)** plasma cortisol (ng/mL) concentrations in different holding durations (1, 2, and 5 h) and in different confinement methods (release = white with diagonal lines; bucket = black; ice well = light gray). Sample size (*n*) is displayed above each bar, and bars of blood glucose sharing similar letters are not significantly different from one another.

concentrations for Bluegill held for 2 h in buckets (4.92 ± 0.60 ng/mL) and ice wells (2.79 ± 0.59 ng/mL) were numerically higher than reference Bluegills. At 5 h, mean (±SE) Bluegill plasma cortisol concentrations were more than four times higher for fish held in buckets (7.57 ± 1.20 ng/mL) than reference Bluegills (Figure 2B).

RAMP Scores

Following confinement, Bluegill average RAMP scores varied from 0.21 to 0.67 and significantly differed across treatments ($\chi^2 = 51.06$, $P < 0.0001$). Bluegills held for 24 h had the highest mean RAMP scores relative to Bluegills held at all other holding durations and were significantly different than reference Bluegills (Figure 3). Conversely, all confinement methods within holding durations contained Bluegills with similar mean RAMP scores in addition to being similar to reference Bluegills (Figure 3). There were no observed temporal differences within any of the confinement methods.

Mortality

No immediate mortalities occurred during holding. Two mortalities occurred within 24 h postangling for Bluegills held in ice wells, whereas zero mortalities occurred

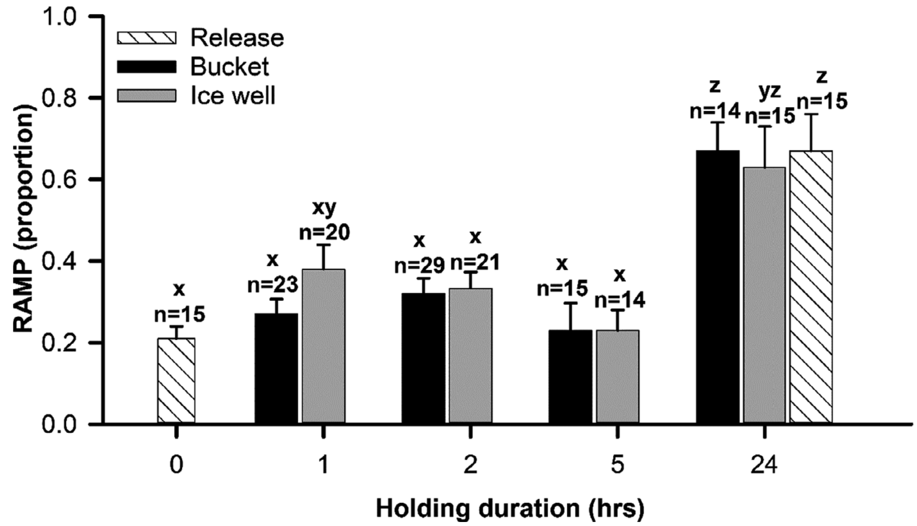

FIGURE 3. Mean (±SE) Bluegill proportional reflex action mortality predictor (RAMP) scores for treatments with different holding durations (1, 2, 5, and 24 h) in different confinement methods (release = white with diagonal lines; bucket = black; ice well = light gray). A score of 0 indicates no impairment, while 1 is impaired. Because individuals could not be held and released, released fish are only included in 0-h and 24-h durations. Sample size (*n*) is displayed above each bar, and bars sharing similar letters are not significantly different from one another.

for Bluegill held in buckets or reference fish. However, there was no effect of treatment on the post-angling mortality rates of Bluegills (ice well versus buckets: $Z = -0.004$; $P = 0.90$; ice well versus reference fish: $Z = -0.004$; $P = 0.90$).

DISCUSSION

Confinement method and holding duration influenced water temperatures and pH concentrations, but they had a lesser effect on carbon dioxide and dissolved oxygen concentrations. Water temperatures were significantly lower in ice wells than the lake, perhaps due to the insulating properties of ice (Patterson and Hamblin 1988). However, temperatures in buckets were significantly higher than the lake, possibly due to their blue color that may have absorbed energy from the sunlight, thus warming the water. Water temperature disparities among treatments compared to the lake were a maximum of +1.5°C for buckets and a minimum of −2.1°C in ice wells. Similarly, during summer months, water temperatures in insulated live wells were up to 3°C warmer than the study system (Plumb et al. 1988). Ice wells at 1 and 2 h and buckets at 2 h had elevated pH levels; however, pH levels never reached concentrations that are detrimental to Bluegill welfare (Carlson 1984). Additionally, dissolved oxygen concentrations decreased through time in ice wells and buckets but never approached lethal concentrations (0.25 mg/L) for Bluegills at low water temperatures (2.5–4.0°C; Petrosky and Magnuson 1973). Furthermore, increases in water temperatures of buckets may have induced increases in metabolic rates such as respiration (Beamish 1964; Brett 1964) that could have resulted in decreases of dissolved oxygen concentrations and pH as well as increases in carbon dioxide concentrations (Wedemeyer 2001).

Changes in water quality parameters can affect physiological stress responses via the direct relationship between water temperature and metabolic rates (Bonga 1997). Throughout holding durations, we observed considerable differences in blood glucose concentrations among treatments. For instance, Bluegills held in buckets had blood glucose concentrations that differed from ice wells and the lake at all holding durations. Specifically, Bluegill blood glucose concentrations were higher for those held in buckets at 1 and 2 h than ice wells. At 5 h, individuals held in ice wells had higher blood glucose concentrations than those held in buckets. Only Bluegills held in ice wells for 1 h had similar blood glucose concentrations to reference fish. A challenge inherent to research evaluating physiological changes of fishes is a delayed secondary stress response. Since fish are ectotherms, water temperature plays a critical role in physiological response times (Schreck et al. 2016). Previous ice-angling experiments have reported delays in physiological responses because cooler water temperatures reduce fish metabolic rates (Louison et al. 2017a, 2017b). Similarly, we found that Bluegill blood glucose concentrations in this study were lower than those recorded in previous studies during warmer periods (e.g., Cousineau et al. 2014). Discrepancies in blood glucose concentrations of Bluegills held in buckets compared to those held in ice wells may stem from the significant differences in water temperatures. At cooler water temperatures, fish experience reductions in the magnitude of physiological changes as well as delays in stress responses, such as blood glucose and plasma cortisol (Barton and Schreck 1987; Lankford et al. 2003). Therefore, the increased water temperatures in buckets could have decreased the delay in the blood glucose response observed in Bluegills held in colder ice wells. Although we observed variability in blood glucose responses of Bluegills held in different confinement methods, differences in plasma cortisol concentrations were not as prevalent. Challenges associated with extracting a large enough blood sample for whole-blood glucose and plasma cortisol analysis limited our sample size for plasma cortisol, and that limited our ability to conduct meaningful statistical analysis. Additionally, biochemical and physiological responses to stressors do not simultaneously increase and decrease with exposure to stressors. Thus, physiological responses (e.g., blood glucose and plasma cortisol) portray the collective response associated with angling, confinement, and holding. Consequently, peaks in whole-blood glucose concentrations could partially be due to a delayed response from angling stress or stressors associated with confinement method and holding duration. In regard to physiological stress responses, our findings are consistent with previous research on ice-angled Bluegills (Louison et al. 2017b) and other species such as Northern Pike *Esox lucius* (Louison et al. 2017a). The reduced stress responses observed in our study suggest that Bluegills caught and released via ice angling are less susceptible to detrimental physiological responses than those caught during warmer months.

Reflex action mortality predictors have become a popular tool among researchers for the rapid assessment of postcapture fish vitality (i.e., the capacity for survival) by quantitatively linking the reflex stress response to fitness outcomes (Davis 2007, 2010). From a practical perspective, RAMP measures the impairment of normal reflexes that provide a measure of vitality (Davis 2005). The premise behind the reflex impairment method is that stress state and likelihood for postrelease mortality can be predicted by noting the absence or presence of specific organismal reflexes that are normally expressed in unstressed individuals (Davis 2005). Reflex action mortality predictors scores of Bluegills showed no variation among the designated holding durations. However, we did observe higher RAMP scores for Bluegills held for the 24-h mortality assessment, suggesting that holding fish for extended durations under the ice can result in altered behavior possibly due to increased stress. Bluegills may be held for extended periods by recreational or tournament ice anglers who are culling fish with the goal of acquiring the largest fish for their bag. Live-release ice-fishing tournaments are widespread in many northern states, such as Michigan (e.g., the Reel Fun Ice Fishing Tournament in Grand Lake), New York (e.g., the Northern Challenge Fishing Derby in Tupper Lake), and Minnesota (e.g., the Brainerd Jaycees Ice Fishing Extravaganza in Gull Lake). Prolonged holding during these live-release ice-angling events may unintentionally increase fish stress and mortality, circumventing their fundamental goal of successful release.

We observed no immediate mortality (0, 1, 2, or 5 h) and minimal delayed mortality (2 individuals; 24 h) associated with ice-angled Bluegills. The two mortalities that did occur were both confined in an ice well. Previous research on catch-and-release Bluegills during the summer months suggests that the highest water temperatures produce the greatest number of mortalities (Cooke et al. 2003; Gingerich et al. 2007). Similarly, increases in Largemouth Bass (Ostrand et al. 2011; Sylvia and Weber 2019), Walleye (Loomis et al. 2013), and Sockeye Salmon *Oncorhynchus nerka* (Gale et al. 2011) angling mortalities have been attributed to increases in water temperatures. In the current study, ice wells had significantly lower water temperatures than buckets and the lake at all holding durations. The reduction in water temperature from the lake to the ice wells may have reduced the ability of Bluegills to adequately acclimate its metabolic functions, as Bluegill acclimation at lower temperatures (<5°C) is repressed (Roots and Prosser 1962). Similarly, Suski and Ridgway (2009) found metabolic responses of centrarchids are reduced during winter. The lower water temperatures found in the ice wells may also promote cold shock stress, where a fish experiences a rapid decrease in water temperature that results in cascading physiological stress responses and potentially death (Donaldson et al. 2008). Therefore, confinement methods such as ice wells that result in colder water temperatures during ice angling may increase Bluegill stress associated with catch-and-release angling.

In the current study, we found differences in water quality parameters and physiological stress responses across confinement methods. However, our results may have been influenced by several factors. For instance, differences in body size can affect the physiological stress response of fish (Meka and McCormick 2004; Brownscombe et al. 2014). The influence of fish size on physiological stress responses was not evaluated in this study due to the lack of variability of fish length (average TL = 167 mm; SD = 16); a Bluegill population with more variable size structure may display differences in stress responses. Secondary stress responses such as blood glucose and lactate typically peak following 1 h after exposure to a stressor in fishes (Bracewell et al. 2003; Jentoft et al. 2005; Suski et al. 2007). Therefore, our measurements of blood glucose levels in reference fish may have been biased low. However, indicators of the primary stress response, such as plasma cortisol, are generally not delayed (Schreck et al. 2016), giving us a reliable measurement of reference fish stress associated with angling. Air temperatures during this study were mild, and temperatures during ice-angling events are often much colder. Decreased temperatures during ice angling could affect the water quality parameters in the confinement methods, ultimately influencing Bluegill stress physiology and mortality. For example, cold shock stress may be more prevalent in colder temperatures, as water in a confinement method may be close to or at freezing temperatures. Future research evaluating colder temperatures during ice angling may be beneficial.

Reductions in Bluegill bag limits to improve size structure (Beard et al. 1997; Reed and Parsons 1999; Edison et al. 2006; Rypel 2015) may promote angler culling. However, variations in Bluegill confinement method and holding duration before culling may influence the survival of released Bluegills after ice angling We observed differences in water quality parameters (e.g., water temperature and pH) and Bluegill physiological parameters (e.g., whole-blood glucose) using different confinement methods and durations. Changes in water quality parameters, especially water temperature, can have a cascading effect on metabolic rates and physiological responses that may influence mortality. Understanding the influence of confinement method on catch-and-release angling has the potential to improve Bluegill fisheries management practices by increasing the survival rates of culled individuals. Our results suggest that buckets are a favorable confinement method compared to ice wells. Holding Bluegills in ice wells may expose fish to low water temperatures that negatively influence physiological stress responses. However, it appears that culled ice-angled Bluegill survival is high regardless of confinement method or duration.

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