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MANAGEMENT BRIEF

Physiological and Behavioral Responses of Age-0 Muskellunge during Simulated Stocking in Elevated pH Water

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Abstract
Successful stocking of Muskellunge *Esox masquinongy* into reservoirs is hampered by a variety of stressors that can affect poststocking survival, such as elevated pH conditions common to eutrophic water bodies. We quantified Muskellunge survival, behavior, and physiological responses during a simulated stocking event where age-0 Muskellunge were netted, held on a hauling truck for 2 h, and then released into water with either the same pH (8.4) or one of two elevated pH levels (8.6 or 9.8). Following the hauling simulation, Muskellunge exhibited an upregulation of plasma-associated stress responses. Stocking into high-pH water did not cause mortality, but Muskellunge stocked into pH 9.8 showed signs of stress through ion imbalances and increased surfacing behavior. Four hours after stocking there were indications of recovery with reduced indicators of physiological stress, but Muskellunge did not fully recover in that time. Our study suggests that elevated pH alone may not result in short-term (≤4 h postrelease) poststocking mortality of age-0 Muskellunge but does add to the cumulative stress incurred during the stocking process.

Supplemental stocking of sport fish is an important management tool to maintain recreational fisheries in waters that do not support natural recruitment (Cowx 1994), with the success of any stocking program dependent upon the health and survival of the individuals released (Pitman and Gutreuter 1993). A variety of stressors, such as handling, confinement, and transport, can affect postrelease survival of hatchery-reared fish (Mather et al. 1986; Harmon 2009; Sampaio and Freire 2016). Stocking success also depends on how well hatchery-reared fish can acclimate to new physiochemical water conditions (Hartman and Preston 2001). Thus, understanding how differences in physiochemical conditions between hatchery and recipient ecosystems affect postrelease stress and survival of hatchery-reared fish is critical for successfully maintaining recreational fisheries supported by stocking.

Elevated pH is a common water quality concern within eutrophic reservoirs and has been shown to negatively impact fish stocking success (Murray and Ziebell 1984; Yesaki and Iwama 1992; Beklioglu and Moss 1995; Wagner et al. 1997). In eutrophic reservoirs, elevated pH occurs as a result of dense populations of phytoplankton that, through photosynthesis, deplete carbon dioxide faster than it can be replenished by respiration or diffusion, causing a shift in equilibrium from hydrogen ions to hydroxyl ions, raising pH (Boyd 1979; Pearce et al. 2017).

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Hatchery fish reared in pH-neutral water and not conditioned to water with elevated pH can thus be subjected to pH shock following the abrupt transfer to reservoirs with elevated pH, resulting in increased physiological stress and potential mortality (Lloyd and Jordan 1964; Murray and Ziebell 1984; Wagner et al. 1997; Scott et al. 2005). In elevated pH, fish can upregulate cortisol and glucose as part of the stress response, excrete bicarbonate through a chloride bicarbonate exchanger to stabilize internal pH, and increase lactate production (Wilkie and Wood 1991; Yesaki and Iwama 1992; Sampaio and Freire 2016). Additionally, elevated pH can result in the inability to excrete ammonia, leading to an accumulation of ammonia in the blood that, coupled with stressed behavior in an attempt to avoid unhealthy water conditions, can result in death (Wilkie and Wood 1991, 1995; Yesaki and Iwama 1992; Suski et al. 2007).

Fisheries for Muskellunge *Esox masquinongy* are often supplemented or entirely supported by stocking (Wingate 1986; Margenau 1992; Szendrey and Wahl 1996). Therefore, mitigating stocking stressors is vital for successful Muskellunge management. Several factors have been shown to influence poststocking survival of Muskellunge, including hatchery rearing techniques (Larscheid et al. 1999; McKeown et al. 1999), size of fish released (Hanson et al. 1986; Margenau 1992; McKeown et al. 1999), number and size of predators (Carline et al. 1986), and prey type and abundances in recipient waters (Gillen et al. 1981; Carline et al. 1986; Hanson et al. 1986; Margenau 1992). In particular, stocking methods (i.e., dip-net handling and confinement) and water temperature have been proven to be important determinants of stocked Muskellunge survival (Mather et al. 1986). Despite substantial research, Muskellunge stocking success is still highly variable and typically low, requiring further research into other potential stressors, such as pH shock (Stein et al. 1981; Owensby et al. 2017). Several eutrophic reservoirs stocked with Muskellunge have pH levels of 8.5–10.0, which can pose a risk of increased short-term poststocking physiological stress and mortality (Illinois Natural History Survey, unpublished data). While pH limits for many fish have been identified (Peterson et al. 1989; West et al. 1997; Tierney 2016), limits for Muskellunge have not been identified, precluding us from defining safe pH targets for stocking.

The objectives of this study were to quantify the short-term physiological and behavioral responses of age-0 Muskellunge released into waters with elevated pH representative of stocking conditions experienced in eutrophic reservoirs. Our approach was to simulate a stocking event wherein hatchery-reared Muskellunge were transferred to a hauling truck for an extended period and then released into water with either the same pH as the hauling water or one of two elevated pH levels. Indicators of stress were assessed through behavioral observations, survival, and blood-based physiological parameters. This study will help define the role that elevated pH might have on the short-term survival and stress of hatchery-reared Muskellunge.

METHODS

Age-0 Muskellunge used in this experiment ranged from 164 to 240 mm total length (mean $\pm$ SE = 209 $\pm$ 1.3 mm) and were produced at the Illinois Department of Natural Resources (IDNR) Jake Wolf Memorial Fish Hatchery (Mason County, Illinois) from broodstock collected from Spring Lake, Manito, Illinois. Two weeks before the start of the experiment, 130 Muskellunge were equally distributed across 13 identical, indoor, concrete flow-through raceways (i.e., 10 fish per raceway) and fed Fathead Minnows *Pimephales promelas*. Raceways were $5.5 \times 1 \times 0.85$ m (85,000 L) and continuously supplied with hatchery water from the onsite Solar Pond, a 0.86-ha shallow pond. Throughout the experiment, water was maintained at approximately 22–23°C and pH 8.4 (hardness 140 mg/L), conditions similar to those experienced by age-0 Muskellunge during fall stocking events in Illinois. Three days prior to the experiment, all Fathead Minnows were removed from the raceways to eliminate any possible dietary influences on the experiment.

To quantify the effects of increased pH on physiological indicators of stress, it was necessary to establish a baseline for blood parameters. Baseline values for physiological parameters were obtained on the day of the simulated stocking by stopping the flow of water to one raceway and then anesthetizing the 10 Muskellunge in the raceway with 60 ppm eugenol (clove oil; Sigma-Adlrich, St. Louis, Missouri) until fish lost equilibrium (approximately 4 min; Anderson et al. 1997). Once equilibrium was lost, individuals were removed from the raceway one by one, measured for total length to the nearest millimeter, and oriented ventral side up on a foam V-shaped surgery board. Approximately 0.5 mL of blood was collected by caudal puncture using a 22-G needle and heparinized syringe (sodium heparin, Sigma-Addlrich #SRE0027). Blood was transferred to a 1.5-mL microcentrifuge tube and immediately centrifuged at 10,000 $\times$ gravity for 2 min. Plasma was separated from the cellular portion of the blood using a pipette. The plasma was flash-frozen and held in liquid nitrogen until delivered to the laboratory and stored at −80°C until processing.

Simulation of the stocking process followed typical procedures used by IDNR hatchery staff. Age-0 Muskellunge (120 individuals) were netted from the remaining raceways, placed into buckets, and carried to an oxygenated 416-L hauling truck with pH 8.4. Fish density in the hauling tank (0.312 fish/L) was a typical density used by IDNR hatchery staff when transporting Muskellunge to stocking locations. While the Muskellunge were held on the hauling truck, 12 raceways ($5.5 \times 1 \times 0.85$ m; 85,000 L) were filled with fresh hatchery water and pH was adjusted to treatment levels using sodium hydroxide (Duda Diesel, Decatur, Alabama; Wilkie and Wood 1991; Wagner et al. 1997). Four raceways each were adjusted to a nominal pH of 8.6 and 9.8. Initially, there were four raceways assigned as controls and left at a pH of 8.4 (i.e., no change from initial conditions); however, because water conditions fell outside treatment parameters for one of these raceways, only three raceways at this pH level were used. Treatments were selected to encompass the range of pH levels found in reservoirs stocked with Muskellunge in Illinois (Raman and Twait 1994; Illinois Natural History Survey, unpublished data). Water in the raceways was static during the experiment, and an aerator was added to each raceway to facilitate mixing to maintain pH and dissolved oxygen conditions throughout the experiment. Temperature and pH were monitored every hour with a YSI Pro-Solo (YSI, Yellow Springs, Ohio) and PH60 digital pH meter (Apera Instruments, Columbus, Ohio) (Table S1 in the Supplemental Materials provided in the online version of this article).

After 2 h, a typical hauling duration, 110 Muskellunge were netted from the hauling truck, placed in buckets, and carried inside the hatchery, where they were released into the 11 raceways (10 fish/raceway). Fish size did not differ across treatments (one-way analysis of variance: $F_{7,65} = 1.61$, $P = 0.15$). To separate transportation and handling stress from the effects of pH treatment, 10 fish were not stocked into raceways but instead netted from the hauling truck and immediately anesthetized in a 60-ppm eugenol bath, and blood was drawn nonlethally as described for the baseline measurements. One and four hours after stocking, two raceways from each pH treatment were anaesthetized and five Muskellunge from each raceway were sampled for blood following the same methods as the baseline treatment (10 fish per pH level × time step combination). The fish not sampled for blood in the raceways were used to quantify survival. Order of fish sampling did not influence plasma response variables (one-way analyses of variance: $F_{9,61} > 0.46$, $P > 0.25$). Treatment pH 8.4 (i.e., no change from rearing or hauling pH) 1 h postrelease only had one raceway ($N = 5$ fish). To monitor behavioral responses, cameras (GoPro model Hero 4) were mounted above six raceways, two per pH level, to quantify surfacing behavior during the first hour following stocking. Surfacing was defined as a Muskellunge disturbing the water's surface but not jumping out of the water. Throughout the experiment pH levels generally decreased due to the evaporation of water and the absorption of carbon dioxide from the air (Table S1).

Laboratory analyses.— Plasma chloride, sodium, and cortisol concentrations were determined using commercially available assay kits (BioAssay Systems, Hayward, California; MyBioSource, San Diego, California; Enzo Live Sciences, Farmingdale, New York). Plasma ammonia and glucose concentrations were determined enzymatically following published procedures (Lowry and Passonneau 1972; Kun and Kearney 1974). Data on the accuracy and precision of laboratory assays was based on Sheriff et al. (2011) and Andreasson et al. (2015) and are provided in Table S2.

Data analysis.— Plasma variables were visually assessed for a raceway effect (i.e., differences among replicate raceways within treatments) by examining the data spread for each response variable and identifying whether or not any raceway observations were outliers. A raceway effect may have been present only for plasma glucose; however, upon closer inspection, removing each of the raceways individually did not influence the overall results (Figure S1 in the Supplemental Materials provided in the online version of this article). As a result, raceways for each treatment were pooled for analysis and individual fish were treated as replicates.

Data for time postrelease were analyzed using a two-way analysis of variance (ANOVA) to evaluate the significance of pH treatment (control, 8.6, or 9.8) and time (1 or 4 h postrelease) as main effects, as well as their interaction. When statistical differences in pH treatments were detected, pairwise comparisons between pH treatments were conducted within time points using a Tukey–Kramer honestly significant difference test. Model assumptions were verified by plotting residuals versus fitted values and versus each covariate in the model (Zuur et al. 2010; Zuur and Ieno 2016). Cortisol data were natural log transformed prior to being included in models to normalize residual distribution. Given that fish from the hauling truck were not sampled across multiple time points, baseline and hauling simulation treatments were not included in the two-way ANOVA models. Instead, baseline, hauling simulation, and treatment mean values and 95% confidence intervals were compared for potential overlap (Ott 2015; Trushenski et al. 2019).

Surfacing behavior was quantified by counting the number of surfacing events that occurred within the first hour following stocking. A generalized linear model using a Poisson error distribution, a distribution appropriate for count data, was used to compare the occurrences of surfacing events across the three pH treatments, with a Tukey–Kramer honestly significant difference test if a treatment main effect was significant. All statistical analyses were performed using R Studio version 1.3.1093 (Boston, Massachusetts), and the level of significance (α) for all tests was 0.05.

RESULTS

After being loaded and held on the hauling truck for 2 h, Muskellunge plasma glucose was elevated by 14% and cortisol was elevated by 56% relative to baseline values (Figure 1). One hour poststocking, plasma cortisol concentrations remained significantly elevated relative to baseline and were similar to levels measured in fish used in the hauling simulation. Four hours postrelease, plasma cortisol concentrations were lower than values observed 1 h postrelease (Figure 1; $F_{1,46} = 7.14$, $P = 0.01$). Plasma glucose concentrations remained elevated both 1 and 4 h postrelease into raceways with no significant effect of time (Figure 1; $F_{1,48} = 0.88$, $P = 0.35$). There were no significant differences across pH treatments in either plasma cortisol (Figure 1A; $F_{1,46} = 0.26$, $P = 0.8$) or glucose concentrations (Figure 1B; $F_{1,48} = 7.14$, $P = 0.24$), with no interaction between time and pH treatment for either cortisol ($F_{2,46} = 0.83$, $P = 0.4$) or glucose ($F_{2,48} = 2.1$, $P = 0.13$).

Irrespective of pH, plasma sodium concentrations were elevated 4 h postrelease relative to plasma concentrations 1 h after stocking (Figure 2; $F_{1,48} = 14.16$, $P = 0.0005$). Independent of time following release, plasma sodium concentrations of Muskellunge stocked into raceways with pH 9.8 were significantly lower than those stocked into the pH 8.6 treatment (Figure 2; $P = 0.03$). Sodium levels 1 and 4 h postrelease were not different from either baseline or hauling simulation values, and there was no significant time and pH treatment interaction ($F_{2,48} = 2.82$, $P = 0.07$). Plasma chloride concentrations were significantly lower 4 h after stocking relative to Muskellunge held in raceways for only 1 h, independent of pH treatment (Figure 2B; $F_{1,47} = 14.56$, $P = 0.0004$). However, Muskellunge held in water with pH 9.8 displayed chloride concentrations that were significantly lower than Muskellunge held at pH 8.6 ($P = 0.001$), independent of time. There was no significant interaction between time and pH treatment on plasma chloride ($F_{2,47} = 1.34$, $P = 0.27$). Most treatments (time or pH) were not different from baseline values; only Muskellunge in pH 9.8 water after 4 h were outside of the baseline's 95% confidence interval, with approximately 22% lower plasma chloride concentrations (Figure 2B). Plasma ammonia concentrations did not differ across time ($F_{1,48} = 2.5$, $P = 0.12$) or pH treatment ($F_{1,48} = 0.01$, $P = 0.9$), and there was no significant interaction between time and pH treatment (Figure 3; $F_{1,48} = 2.5$, $P = 0.12$). Four hours after stocking, there was a decrease in plasma ammonia concentrations of approximately 10–15% relative to baseline, but plasma ammonia did not drop below the baseline treatment 95% confidence intervals. Age-0 Muskellunge surfaced most frequently in pH 9.8 raceways and the least in pH 8.6, with intermediate frequency in pH 8.4 (Figure 4; Table S3). No Muskellunge died during the experiment.

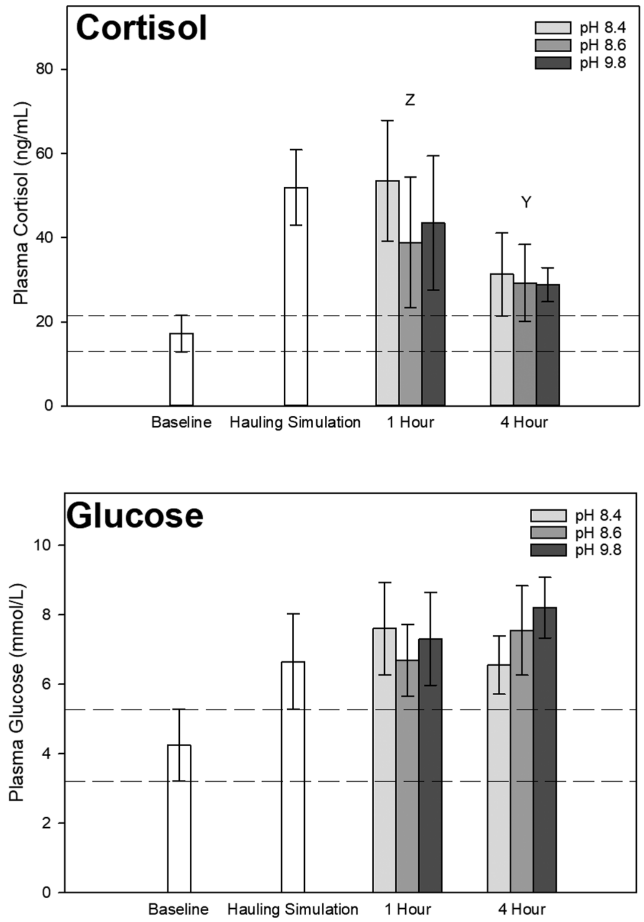
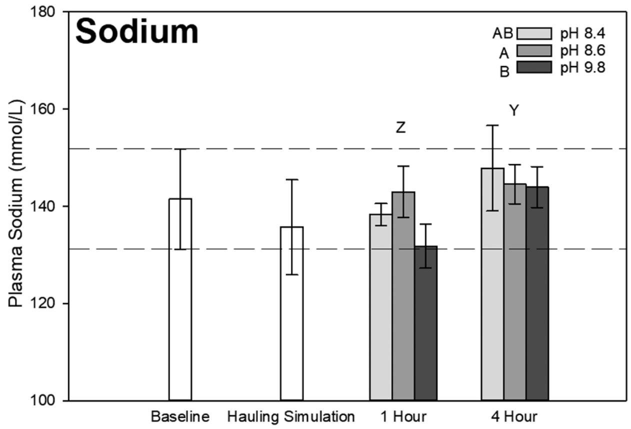


FIGURE 1. Plasma cortisol and glucose concentrations for juvenile Muskellunge prior to stocking simulation (baseline), confined on a hauling truck for 2 h (hauling simulation), and 1 and 4 h after release into one of three pH conditions. Error bars represent 95% confidence intervals, with horizontal dashed lines extended from baseline confidence intervals for visual comparison. Differing letters (Z, Y) represent a significant difference between the 1- and 4-h postrelease periods (two-way ANOVA: $P < 0.05$). Sample sizes ranged from 4 to 10.

DISCUSSION

The hauling scenario simulated in the current study caused significant physiological disturbances for Muskellunge, with increases in cortisol and glucose concentrations and decreases in chloride and sodium concentrations relative to control fish. When fish undergo vigorous activity from netting, handling, and air exposure, anaerobic respiration can increase, resulting in the release of stress hormones, such as cortisol, and an increased reliance on anaerobic glycolysis in muscle; the generation of protons can lead to a concomitant reduction in blood pH (Wood 1991; McDonald and Milligan 1997). Internal physiological mechanisms are used to cope with these disturbances. Sodium-proton exchange mechanisms, for example, are used to excrete protons, coupled with a bicarbonate-



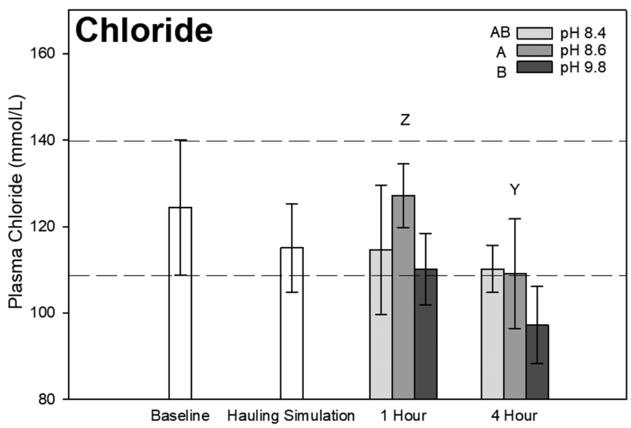


FIGURE 2. Plasma sodium and chloride concentrations for juvenile Muskellunge prior to stocking simulation (baseline), confined on a hauling truck for 2 h (hauling simulation), and 1 or 4 h after release into one of three pH conditions. Error bars represent 95% confidence intervals, with horizontal dashed lines extended from baseline confidence intervals for visual comparison. Differing letters represent a significant difference of pH treatments (A, B) and sampling times (Z, Y). Sample sizes ranged from 5 to 10.

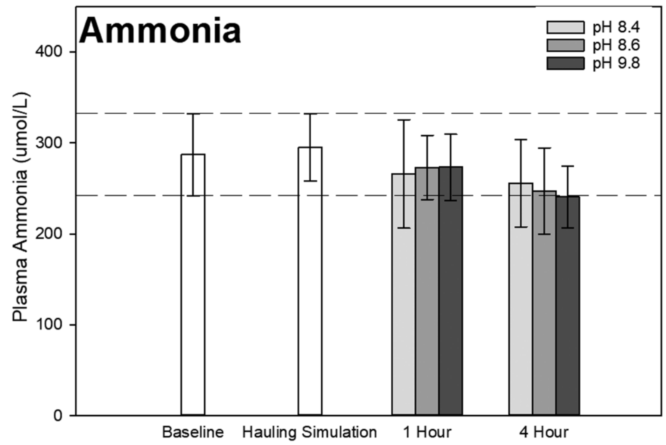


FIGURE 3. Plasma ammonia concentrations for juvenile Muskellunge prior to stocking simulation (baseline), confined on a hauling truck for 2 h (hauling simulation), and 1 and 4 h after release into one of three pH conditions. Error bars represent 95% confidence intervals, with horizontal dashed lines extended from baseline confidence intervals for visual comparison. Sample sizes ranged from 4 to 10.

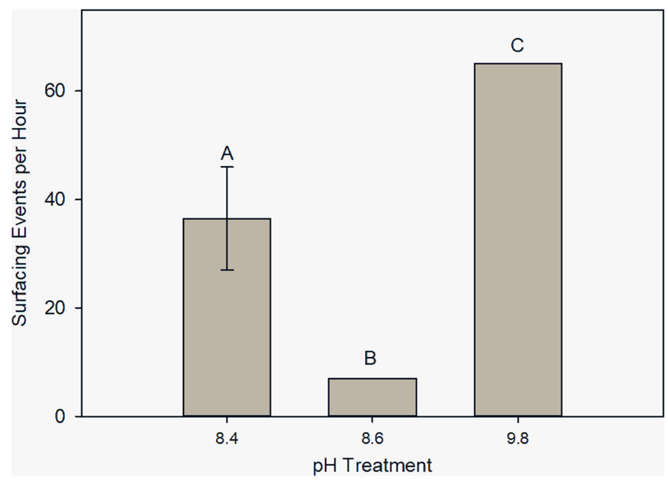


FIGURE 4. Number of surfacing events by juvenile Muskellunge during the first hour following release into one of three pH conditions. Differing letters represent significant differences between pH treatments (generalized linear model: $P < 0.05$).

chloride ion exchange to buffer blood from decreases in pH (Marshall 2002; Dymowska et al. 2012). However, there is a metabolic cost of diverting energy from normal metabolic functions to the functions used to cope with stress, and when the costs are forced beyond the fish's normal limits, mortality can occur (Pedersen et al. 2006; Harmon 2009).

In this study no morality occurred; however, increased stress hormones and ion imbalances were observed, similar to previous hauling and stocking studies (Miles et al. 1974; Davis and Parker 1990; Sink and Neal 2009) and during live-release tournaments (Suski et al. 2004). Furthermore, 4 h following stocking, cortisol concentrations returned to baseline levels, suggesting that Muskellunge were starting to recover from the handling stress. However, glucose and sodium concentrations that had been elevated following the hauling simulation had not returned to baseline levels, suggesting that Muskellunge likely had not fully recovered from the hauling simulation and that physiological disturbances beyond 4 h could further compromise survival. Barton et al. (2003) found similar results in Walleye *Sander vitreus* stocked in 12–15°C waters in that fish started to recover within the first 24 h but took longer than 24 h to fully recover from ion imbalances. Barton (2000) found signs of recovery in several salmonid species 24–48 h poststocking, with glucose returning to baseline levels, but cortisol remained elevated 48 h poststocking. Furthermore, Rainbow Trout *Oncorhynchus mykiss* in 11–12°C have been shown to take 8 d to fully recover from stocking (Barton et al. 1980). Though the Muskellunge in our study experienced significant physiological stress during stocking and were unable to fully recover 4 h after stocking, there were signs of recovery and a reduction in stress during the monitoring period.

Against the background signals of stress and disturbance from the hauling simulation, pH stress from exposure to high pH was still detectable. At pH 9.8, there were significantly higher plasma sodium concentrations and lower chloride concentrations relative to pH 8.6. Furthermore, there was a loss of chloride by 4 h post release relative to 1 h postrelease, a common response to elevated pH (Wilkie and Wood 1995; Wagner et al. 1997). Similarly, surfacing behavior was highest in pH 9.8 but lowest in pH 8.6 during the first hour following stocking. This difference in surfacing behavior suggests that there may be a threshold response, where at lower pH, changes there are minimal impacts, but as pH increases to pH 9.8, there are significant behavioral effects. Under field conditions, more frequent surfacing behavior may lead to increased predation by birds and mammals as observed in other stocking events (Weber and Weber 2020). Though high pH can require several hours or days to become physiologically significant, a strong short-term pH response would have been expected to result in increased cortisol and glucose (Wagner et al. 1997), a loss of sodium ions (Wilkie and Wood 1991, 1995), an increase in plasma ammonia (Wilkie and Wood 1991, 1995; Yesaki and Iwama 1992), and mortality (Yesaki and Iwama 1992; Wilkie et al. 1993; Belkioglu and Moss 1995). Furthermore, water alkalinization would have affected the transport of ammonia, sodium, and chloride ions in the branchial epithelium and a number of compensatory physiological adjustments would have been required to cope with the elevated pH. Though small increases in pH induced limited stress in our study, pH 9.8 produced significant ion imbalances and behavioral disturbance.

Results from this study suggest that elevated pH alone may not cause short-term (i.e., ≤4 h) poststocking mortality of Muskellunge but likely contributes to stress in concert with handling and hauling stressors. Physiological disturbances often occur over a period of several hours to days; therefore, measuring the physiological and behavioral responses of Muskellunge stocked into elevated pH over a longer time course would be a valuable future study. Some sources of stress, such as netting, handling, and crowding in the hauling tank, are unavoidable during stocking. However, good hauling and transport procedures should mitigate the effects of these stressors. A greater concern when stocking Muskellunge may be the cumulative effect of multiple sublethal stressors incurred during the entire stocking process and additional water quality parameters (Schreck 1981; Mather et al. 1986; Harmon 2009; Diana et al. 2017). Eutrophic reservoirs may experience additional water quality issues along with elevated pH, including warm temperatures, low dissolved oxygen concentrations, and elevated ammonia levels (Blann et al. 2009; Pearce et al. 2017). For example, ammonia toxicity increases with increasing pH and temperature and can be more problematic for stocked fish when coupled with elevated pH (Thurston et al. 1981; Miron et al. 2008). Thus, acclimation to conditions unique to recipient water bodies can be critical for survival of stocked fish (Murray and Ziebell 1984; Trushenski et al. 2019). Additionally, future studies are needed to quantify the interactive effects of pH and other water quality parameters on Muskellunge poststocking physiological stress and survival, as well as measuring pH thresholds and long-term effects of Muskellunge stress responses.

In this study there was no mortality following stocking Muskellunge into waters with elevated pH; however, there were significant physiological and behavioral disturbances. When combining stocking and pH-induced stress with other water quality issues in reservoirs, such as high temperature and ammonia, there will likely be increased stress responses and possible mortality. Thus, when stocking Muskellunge, there is a need to monitor the pH of recipient waters to avoid additional stress and increase stocking success.

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REFERENCES

Anderson, W. G., R. S. McKinley, and M. Colavecchia. 1997. The use of clove oil as an anesthetic for Rainbow Trout and its effects on swimming performance. North American Journal of Fisheries Management 17:301–307.

Andreasson, U., A. Perret-Liaudet, L. J. C. van Waalwijk, K. van Doorn, D. Blennow, S. Chiasserini, T. Engelborghs, S. Fladby, N. Genc, H. B. Kruse, L. Kuiperij, P. Kulic, B. Lewczuk, B. Mollenhauer, L. Mroczko, E. Parnetti, M. M. Vanmechelen, B. Verbeek, H. Winblad, M.-S. Zetterberg, and C. E. Teunissen. 2015. A practical guide to immunoassay method validation. Frontiers in Neurology 6:1–8.

Barton, B. A. 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. North American Journal of Aquaculture 62:12–18.

Barton, B. A., A. H. Haukenes, B. G. Parsons, and J. R. Reed. 2003. Plasma cortisol and chloride stress responses in juvenile Walleyes during capture, transport, and stocking procedures. North American Journal of Aquaculture 65:210–219.

Barton, B. A., R. E. Peter, and C. R. Paulencu. 1980. Plasma cortisol levels of fingerling Rainbow Trout *Salmo gairdneri* at rest, and subjected to handling, confinement, transport, and stocking. Canadian Journal of Fisheries and Aquatic Sciences 37:805–811.

Beklioglu, M., and B. Moss. 1995. The impact of pH on interactions among phytoplankton algae, zooplankton and perch *Perca fluviatilis* in a shallow, fertile lake. Freshwater Biology 33:497–509.

Blann, K. L., J. L. Anderson, G. R. Sands, and B. Vondracek. 2009. Effects of agricultural drainage on aquatic ecosystems: a review. Critical Reviews in Environmental Science and Technology 39:909–1001.

Boyd, C. 1979. Water quality in warmwater fish ponds. Auburn University Agriculture Experimental Station, Auburn, Alabama.

Carline, R. F., R. A. Stein, and L. M. Riley. 1986. Effects of size at stocking, season, Largemouth Bass predation and forage abundance on survival of tiger muskellunge. Pages 151–167 *in* G. E. Hall, editor. Managing muskies. American Fisheries Society Special Publication, Bethesda, Maryland.

Cowx, I. G. 1994. Stocking strategies. Fisheries Management and Ecology 1:15–30.

Davis, K. B., and N. C. Parker. 1990. Physiological stress in Striped Bass: effect of acclimation temperature. Aquaculture 91:349–358.

Diana, M. J., C. P. Wagner, and D. H. Wahl. 2017. Differences in stocking success among geographically distinct stocks of juvenile Muskellunge in Illinois lakes. North American Journal of Fisheries Management 37:633–643.

Dymowska, A. K., P.-P. Hwang, and G. G. Goss. 2012. Structure and function of ionocytes in the freshwater fish gill. Respiratory Physiology and Neurobiology 84:282–292.

Gillen, A. L., R. A. Stein, and R. F. Carline. 1981. Predation by pellet-reared tiger muskellunge on minnows and Bluegills in experimental systems. Transactions of the American Fisheries Society 110:197–209.

Hanson, A. D., M. D. Staggs, S. L. Sterns, D. J. Johnson, and L. M. Andrews. 1986. Survival of stocked Muskellunge eggs, fry, and fingerlings in Wisconsin Lakes. Pages 216–228 *in* G. E. Hall, editor. Managing muskies: a treatise on the biology and propagation on Muskellunge in North America. American Fisheries Society, Special Publication 15, Bethesda, Maryland.

Harmon, T. S. 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Reviews in Aquaculture 1:58–66.

Hartman, K. J., and B. Preston. 2001. Stocking. Pages 661–686 *in* G. Wedemeyer, editor. Fish hatchery management. American Fisheries Society, Bethesda, Maryland.

Kun, E., and E. B. Kearney. 1974. Ammonia. Pages 1802–1806 *in* H. U. Bergmeyer, editor. Methods of enzymatic analysis, 2nd edition. Academic Press, New York.

Larscheid, J., J. Christianson, T. Gengerke, and W. Jorgensen. 1999. Survival, growth, and abundance of pellet-reared and minnow-reared Muskellunge stocked in northwestern Iowa. North American Journal of Fisheries Management 19:230–237.

Lloyd, R., and D. H. M. Jordan. 1964. Some factors affecting the resistance of Rainbow Trout to acid waters. International Journal of Air and Water Pollution 8:393–403.

Lowry, O. H., and J. V. Passonneau. 1972. A flexible system of enzymatic analysis. Academic Press, New York.

Margenau, T. L. 1992. Survival and cost-effectiveness of stocked fall fingerling and spring yearling Muskellunge in Wisconsin. North American Journal of Fisheries Management 12:484–493.

Marshall, W. S. 2002. Na^+, Cl^-, Ca^{2+}, Zn^{2+} transport by fish gills: retrospective review and prospective synthesis. Journal of Experimental Zoology 293:264–283.

Mather, M. E., R. A. Stein, and R. F. Carline. 1986. Experimental assessment of mortality and hyperglycemia in tiger muskellunge due to stocking stressors. Transactions of the American Fisheries Society 115:762–770.

McDonald, G., and L. Milligan. 1997. Ionic, osmotic and acid-base regulation in stress. Pages 119–145 *in* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, editors. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, UK.

McKeown, P. E., J. L. Forney, and S. R. Mooradian. 1999. Effects of stocking size and rearing method on Muskellunge survival in Chautauqua Lake, New York. North American Journal of Fisheries Management 19:249–257.

Miles, H. M., S. M. Loehner, D. T. Michaud, and S. L. Salivar. 1974. Physiological responses of hatchery reared Muskellunge *Esox masquinongy* to handling. Transactions of the American Fisheries Society 103:336–342.

Miron, D. D. S., B. Moraes, A. G. Becker, M. Crestani, R. Spanevello, V. L. Loro, and B. Baldisserotto. 2008. Ammonia and pH effects on some metabolic parameters and gill histology of Silver Catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture 277:192–196.

Murray, C. A., and C. D. Ziebell. 1984. Acclimation of Rainbow Trout to high pH to prevent stocking mortality in summer. Progressive Fish-Culturist 46:176–179.

Ott, L., and M. Longnecker. 2015. An introduction to statistical methods and data analysis, 7th edition. Cengage Learning, Boston, Massachusetts.

Owensby, D. P., J. A. Rice, and D. D. Aday. 2017. Mortality, dispersal, and habitat use of stocked juvenile Muskellunge in two western North Carolina rivers. North American Journal of Fisheries Management 37:108–121.

Pearce, A. R., L. G. Chambers, and E. A. Hasenmueller. 2017. Characterizing nutrient distributions and fluxes in a eutrophic reservoir, Midwestern United States. Science of the Total Environment 581:589–600.

Pedersen, S. F., M. E. O'Donnell, S. E. Anderson, and P. M. Cala. 2006. Physiology and pathophysiology of Na^+/H^+ exchange and Na^+-K^+-$2Cl^-$ cotransport in the heart, brain, and blood. American Journal of Physiology Regulatory, Integrative and Comparative Physiology 291:R1–R25.

Peterson, R. H., K. Coombs, J. Power, and U. Paim. 1989. Responses of several fish species to pH gradients. Canadian Journal of Zoology 67:1566–1572.

Pitman, V. M., and S. Gutreuter. 1993. Initial poststocking survival of hatchery-reared fishes. North American Journal of Fisheries Management 13:151–159.

Rama, R. K., and R. M. Twait. 1994. Water quality characteristics of Lake Bloomington and Lake Evergreen. Illinois State Water Survey, Contract Report 569, Champaign, Illinois.

Sampaio, F. D. F., and C. A. Freire. 2016. An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. Fish and Fisheries 17:1055–1072.

Schreck, C. B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. Pages 295–321 *in* A. D. Pickering, editor. Stress and fish. Academic Press, London.

Scott, D. M., M. C. Lucas, and R. W. Wilson. 2005. The effect of high pH on ion balance, nitrogen excretion and behaviour in freshwater fish from an eutrophic lake: a laboratory and field study. Aquatic Toxicology 73:31–43.

Sheriff, M. J., B. Dantzer, B. Delehanty, R. Palme, and R. Boonstra. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166:869–887.

Sink, T. D., and J. W. Neal. 2009. Stress response and posttransport survival of hybrid Striped Bass transported with or without clove oil. North American Journal of Aquaculture 71:267–275.

Stein, R. A., R. F. Carline, and R. S. Hayward. 1981. Largemouth Bass predation on stocked tiger muskellunge. Transactions of the American Fisheries Society 110:604–612.

Suski, C. D., J. D. Kieffer, S. S. Killen, and B. L. Tufts. 2007. Sub-lethal ammonia toxicity in Largemouth Bass. Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology 146:381–389.

Suski, C. D., S. S. Killen, S. J. Cooke, J. D. Kieffer, D. P. Philipp, and B. L. Tufts. 2004. Physiological significance of the weigh-in during live-release angling tournaments for Largemouth Bass. Transactions of the American Fisheries Society 133:1291–1303.

Szendrey, T. A., and D. H. Wahl. 1996. Size-specific survival and growth of stocked Muskellunge: effects of predation and prey availability. North American Journal of Fisheries Management 16:395–402.

Thurston, R. V., R. C. Russo, and G. A. Vinogradov. 1981. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Environmental Science and Technology 15:837–840.

Tierney, K. B. 2016. Chemical avoidance responses of fishes. Aquatic Toxicology 174:228–241.

Trushenski, J. T., D. A. Larsen, M. A. Middleton, M. Jakaitis, E. L. Johnson, C. C. Kozfkay, and P. A. Kline. 2019. Search for the smoking gun: identifying and addressing the causes of postrelease morbidity and mortality of hatchery-reared Snake River Sockeye Salmon smolts. Transactions of the American Fisheries Society 148:875–895.

Wagner, E. J., T. Bosakowski, and S. Intelmann. 1997. Combined effects of temperature and high pH on mortality and the stress response of Rainbow Trout after stocking. Transactions of the American Fisheries Society 126:985–998.

Weber, R. E., and M. J. Weber. 2020. Yearling Muskellunge poststocking behavior and survival in two central Iowa reservoirs with contrasting habitat availability. North American Journal of Fisheries Management 40:1263–1275.

West, D. W., J. A. T. Boubée, and R. F. G. Barrier. 1997. Responses to pH of nine fishes and one shrimp native to New Zealand freshwaters. New Zealand Journal of Marine and Freshwater Research 31:461–468.

Wilkie, M. P., G. K. Iwama, and C. Wood. 1993. The physiological responses of the Lahontan Cutthroat Trout *Oncorhynchus clarkii henshawi*, a resident of highly alkaline Pyramid Lake (pH 9.4), to challenge at pH 10. Journal of Experimental Biology 175:173–194.

Wilkie, M. P., and C. M. Wood. 1991. Nitrogenous waste excretion, acid-base regulation, and ionoregulation in Rainbow Trout *Oncorhynchus mykiss* exposed to extremely alkaline water. Physiological Zoology 64:1069–1086.

Wilkie, M. P., and C. M. Wood. 1995. Recovery from high pH exposure in the Rainbow Trout: white muscle ammonia storage, ammonia washout, and the restoration of blood chemistry. Physiological Zoology 68:379–401.

Wingate, P. 1986. Philosophy of Muskellunge management. Pages 199–202 *in* G. E. Hall, editor. Managing muskies: a treatise on the biology and propagation on Muskellunge in North America. American Fisheries Society, Special Publication 15, Bethesda, Maryland.

Wood, C. M. 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. Journal of Experimental Biology 160:285–308.

Yesaki, T. Y., and G. K. Iwama. 1992. Survival, acid-base regulation, ion regulation, and ammonia excretion in Rainbow Trout in highly alkaline hard water. Physiological Zoology 65:763–787.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3–14.

Zuur, A. F., and E. N. Ieno. 2016. A protocol for conducting and presenting results of regression-type analyses. Methods in Ecology and Evolution 7:636–645.

SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.