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ARTICLE

Do live-well additives influence the physiological and behavioral recovery of Largemouth Bass?

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Abstract

Objective: There is an ongoing effort to enhance the welfare and survival of black bass *Micropterus* spp. released after angling tournaments. Live-well additives are substances added to live-well water that are intended to help fish recover during retention. Aside from claims made by manufacturers, little information is available on the additives' effectiveness for recovery or their potential welfare consequences. Thus, our objective was to test whether live-well additives influence fish physiology and postrelease behavior.

Methods: Two techniques were used to test the influence of live-well additives on the welfare of angled Largemouth Bass *M. nigricans* (LMB) held in a live well with lake water (control) or one of three additive products. Prior to fish release, pop-off biologging packages were fastened to a subset of LMB to monitor behavior (locomotor activity, depth, and water temperature use) for 5 min ($n=61$) and blood samples were taken from another subset of LMB to assess their physiology ($n=47$). We obtained baseline ($n=9$) blood samples from LMB immediately upon capture.

Result: Regardless of treatment, postrelease locomotor activity increased with increasing time spent in the live wells. Live-well additive type alone did not have an influence on the postrelease locomotor activity. Further, LMB retained in the live wells had greater blood glucose and lactate concentrations relative to baseline LMB, suggesting that fish did not recover from angling during retention in live wells. Other than elevated plasma chloride levels of LMB held in one of the live-well additives, plasma chloride and sodium concentrations for LMB in live wells with additives and for those in the control live well did not differ, suggesting that the LMB had not recovered while retained.

Conclusion: Our results suggest that the live-well additives tested did not enhance recovery or reduce confinement stress of LMB retained in live wells under the tested circumstances. Additional research on live-well additives is needed given that our findings did not align with the claims made by the manufacturers of these products. We suggest that anglers intending to retain fish in live wells should use fresh, well-oxygenated lake water.

KEYWORDS

behavior, black bass, catch and release, physiology, tournament

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INTRODUCTION

Competitive angling events have been around since about 1950, and in the early 1990s there were over 10,000 black bass *Micropterus* spp. tournament events occurring annually in North America (Duttweiler 1985; Schramm et al. 1991b). Although there are no recent tallies of the number of events, black bass competitive angling events clearly remain popular. These events generate economic benefits by supporting local retailers, restaurants, hotels, and rental accommodations (Schramm et al. 1991a). Given the sheer volume of such events and because competitive events usually target the largest and most valuable (i.e., sexually mature) individuals within a fish population, concerns have been raised regarding the influence that tournaments may have on the sustainability of black bass populations (Holbrook 1975; Barnhart 1989; Suski and Philipp 2004; Schramm and Gilliland 2015). Although evidence supports low mortalities of black bass, especially Largemouth Bass *M. nigricans* (LMB), released from tournaments (e.g., Wilde 1998; Allen et al. 2008; Sass et al. 2018), the extent to which sublethal stressors encountered during tournaments may indirectly influence the sustainability of black bass populations (e.g., via reductions in reproductive output and recruitment) is still unclear (Driscoll et al. 2007; Hysmith et al. 2014; Sylvia et al. 2021).

Anglers, industry partners, and manufacturers have recently taken an interest in identifying methods and strategies to mitigate stress and further reduce mortality of black bass during tournament events (Schramm and Gilliland 2015). Typically, live-release black bass tournaments allow anglers to fish for 6–8 h to catch their heaviest five-fish limit. There is a strong incentive for anglers to keep captured fish alive in their live wells during tournament hours so that fish can be brought to the central weigh-in station in a good health standing (i.e., alive), as there are typically strict penalties associated with moribund fish. The primary purpose of live wells during tournaments is to keep fish alive while confined so that they can be culled as needed (e.g., smaller fish are released as bigger fish are captured and eventually brought to the weigh-in station and released alive; Holbrook 1975). A secondary purpose of live wells is to facilitate the recovery of angled fish and help alleviate physiological disturbances induced by angling (Suski et al. 2004). Live wells are simply water-filled tanks onboard fishing boats and are outfitted with pumps and aerators that can draw fresh water from the surface of the water body, and can also recirculate water within the tank. This way, oxygen levels within the live well can remain elevated, either through the addition of fresh lake water or through aeration of existing water. Maintenance of good water quality during live-well confinement plays a vital role in facilitating recovery and reducing the physiological disturbances (Cooke et al. 2002; Suski et al. 2003, 2004), behavioral alterations, and mortality of black bass (Plumb et al. 1988; Gilliland 2002; Vanlandeghem et al. 2010). Conversely, the potential accumulation of sublethal stressors associated with poor water quality and fish density/activity during live-well confinement can have a negative influence on the survival rate of fish (Schramm et al. 1987; Kwak and Henry 1995). Conditions in the live well during confinement may become increasingly problematic to the point of mortality, as the severity of stressors increases with elevated water temperatures (Suski et al. 2006); low dissolved oxygen, creating hypoxia (Hartley and Moring 1993); overcrowding and the subsequent transfer of disease (Schramm et al. 2006); elevated activity rates from boat wakes and wave activity (Suski et al. 2005; Brooke et al. 2019); and the increase of metabolic wastes and ammonia buildup (Kwak and Henry 1995). Therefore, angler knowledge related to water quality and live-well management is an important aspect influencing the survival of black bass during live-release tournaments when fish are confined to a live well (Edwards et al. 2004). With a strong emphasis placed on keeping fish alive for the sustainability of a population and to avoid strict penalties associated with dead fish during tournaments, anglers, tournament organizations, and industry partners alike have made efforts to minimize the potential stressors related to live-well confinement.

To improve the water quality in the live wells during confinement of black bass, it has become common practice for anglers to use live-well additives or water conditioners (Cooke et al. 2002; Suski et al. 2005, 2006; Ostrand et al. 2011). For instance, given that mortality is often related to warm water temperatures (Schramm et al. 1987; Plumb et al. 1988; Wilde 1998) and given evidence suggesting that live-well temperatures do not deviate from the epilimnion, which is generally the warmest part of the water during summer tournaments (Sullivan et al. 2015), anglers will often add ice to live wells to reduce water temperature. However, the addition of ice can result in delayed physiological recovery, behavioral alterations, and mortality due to

Impact statement

Live-well additives do not improve recovery or reduce stress for Largemouth Bass in live wells. Anglers that hold fish in their live wells should consider using fresh lake water that is well oxygenated (i.e., keep live wells recirculating) rather than using live-well additives.

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cold shock (Suski et al. 2006; Donaldson et al. 2008). Ostrand et al. (2011) also showed that even when water temperatures exceeded 30°C, the addition of ice, salt, or a combination of both did not reduce mortality in LMB. Another mitigation method is diffusing pure oxygen into the live-well water (at rates that do not create supersaturation), which has been shown to reduce mortality of confined fish compared to live wells with just recirculating lake water (Gilliland 2002), but recovery can be hindered if the water is hyper-oxygenated (Suski et al. 2006; Shultz et al. 2011). Chemical water conditioners are also used to treat live-well water, with the intent of reducing negative impacts on fish. For example, Plumb et al. (1988) showed that survival rates of fish increased in live wells that were treated with chemical water conditioners. However, Cooke et al. (2002) found that chemical water conditioners used in live wells with black bass resulted in delayed physiological recovery, potentially leading to reduced survival rates. Furthermore, it has been shown that simply maintaining good water quality in the live well during confinement (e.g., providing fish with ample fresh, ambient water to maintain adequate dissolved oxygen and ambient water temperatures) without supplemental additions can be sufficient to ensure recovery from stressors related to tournament events (Furimsky et al. 2003; Suski et al. 2004). Because the evidence regarding the use of live-well additives for black bass is equivocal, further work is needed to define the physiological and behavioral consequences (whether positive or negative) associated with exposure to live-well additives (i.e., many such products are purported to reduce stress in and expedite the recovery of fish) during and after confinement to inform best practices that should be used by tournament anglers to minimize negative influences on black bass while retained in live wells.

Stressors that are present during live-release tournament events and subsequent live-well confinement can lead to physiological and biochemical changes that cause behavioral alterations and reduce the ability of fish to perform certain functions (Schreck 1990; Schreck et al. 1997; Suski et al. 2004). When the homeostasis of fish is threatened, there is a cascade of responses to cope with the perceived disturbance (Barton and Iwama 1991; Barton 2002). The severity of the acute stress event, or the presence of a stress event, can be measured by the increase in circulating levels of plasma cortisol in the bloodstream (Barton and Iwama 1991; Wendelaar-Bonga 1997), with spikes in circulating cortisol beginning and peaking at about 1–2 h after the original stress event (i.e., hooking) and only decreasing with negative feedback on the hypothalamic–pituitary axis (Fryer and Peter 1977; Wendelaar-Bonga 1997). Additionally, stress created from tournament angling involves a degree of exhaustive exercise, resulting in a depletion in the muscle energy stores and an increase in blood-bound glucose (Suski et al. 2004), which is stimulated by an increase in cortisol (Kieffer 2000; Suski et al. 2003). Concentrations of circulating lactate increase due to the accumulation of metabolic waste from anaerobic activity, including exercise or the exposure of fish to air or hypoxia (Kieffer 2000; Suski et al. 2003). Behavioral traits in fish are closely related to the physiological changes that occur, which also makes the assessment of behavior a valuable method for measuring the degree of stress in fish (Schreck 1990; Schreck et al. 1997; Cooke et al. 2002). Altered swimming behavior due to a stress event is evident with deviation in routine movements (frequency and duration of movements), swimming speed, distance traveled, position in the water column, displacement patterns, the ability to maintain position in water current, and the ability to return to the site of capture (Schreck 1990; Calfee et al. 2016). After being captured and confined to a live well, fish may suffer from cognitive impairment and become disorientated upon release (Cooke et al. 2014). As a result of these impairments, fish may have a reduced ability to seek refuge and may become subject to an increased risk of predation (Danylchuk et al. 2007; Arlinghaus et al. 2009; Brownscombe et al. 2013; Cooke et al. 2014). New technologies—specifically the use of triaxial accelerometer sensors—have been useful for investigating the short-term postrelease behavior of fish after various handling practices. Biologgers have the capacity to store data internally and can be equipped with acceleration, temperature, and pressure (depth) sensors (Halsey et al. 2009; Gleiss et al. 2011; Brownscombe et al. 2013; Wright et al. 2014). This technology has been an effective tool for determining the overall dynamic body acceleration (ODBA) from the fine-scale swimming activity of fish after release—a proxy for understanding the locomotor activity and field metabolic rate of fish (Wilson et al. 2006; Gleiss et al. 2011; Brownscombe et al. 2018). Furthermore, fish are ectotherms living in a three-dimensional world, and they possess the ability to select preferred temperatures and depths. Therefore, temperature selection and depth selection are important and relevant factors that can indicate the welfare status and health condition of a fish upon release.

Our objective was to test whether commercially available live-well additives influenced the physiology and postrelease behavior of LMB—a popular tournament species in North America. We captured LMB from a lake in eastern Ontario and placed them in a live well with one of three live-well additives or in a live well with fresh lake water (control). We then quantified blood physiology (i.e., lactate, glucose, chloride [Cl^-], and sodium [Na^+]) during recovery, along with evaluating postrelease behavior (i.e.,

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swimming activity, depth, and temperature selection) by using externally attached biologgers. We assessed three different commercial live-well additives, and we also had a control live well that was filled with ambient lake water. Findings from this study will provide anglers, tournament organizers, and managers with best live-well management practices to maximize the welfare of black bass during live-release tournaments and, in doing so, reduce the likelihood of mortality.

METHODS

Fish capture

All LMB were captured from Big Rideau Lake (44°43.887′N, 76°13.975′W) in eastern Ontario between July 5 and August 10, 2021. Surface water temperatures ranged between 20.7°C and 30.6°C. Fish were captured by means of angling with artificial lures that were actively fished in a manner consistent with angling tournaments. Rods ranged between medium power to extra heavy power depending on the artificial lure and location. Spinning and baitcasting reels were equipped with braided fishing line that ranged between 4.5- and 22.7-kg test. The strength of the fishing line was paired with a rod having the appropriate level of power (i.e., heavier line was used for heavier rods). Artificial lures that were used to capture LMB had a single barbed hook or multiple barbed hooks and are commonly used by tournament anglers. Hooked fish were brought to the boat immediately in an attempt to minimize fight duration, typically in less than 20 s. Captured LMB were landed by hand (i.e., no net was used), and the hook was immediately removed by hand or with the aid of hemostats (if needed) in less than 10 s. Deeply hooked fish were omitted from the study. Once the hook(s) were removed, each LMB was placed in a water-filled trough onboard the boat for total length measurement (mm), and the fish received an external anchor tag for identification. From there, the LMB was either placed into one of the live wells (i.e., a live well filled with lake water or containing a live-well additive) for up to 8 h (the maximum duration for which a fish would be held during a tournament) for behavioral assessment or blood sampling. The LMB that were held for blood sampling were only held for a maximum of 2 h (as per Suski et al. 2006). The holding period for LMB used in postrelease behavior assessment was not standardized, and these fish were held for various durations (a maximum of 8 h in the live well). The Lund Renegade 1775 boat used for the study was equipped with a 95-L live well at the rear of the boat and a 45-L live well at the front. Both live wells were kept on recirculation for the entirety of the day, thus allowing water to remain oxygenated. However, to avoid dilution of the live-well additive, no new water was added to the live wells during the angling day. The rear live well was used as the additive live well and never exceeded five LMB, while the front live well acted as the control (i.e., was filled with lake water) and never exceeded two LMB.

Treatments

A specific live-well additive that was selected for use at the beginning of each sampling day was the only live-well additive used for that day (i.e., only one trial with one live-well additive was conducted per day). The first seven LMB captured that day with a total length of at least 305 mm were randomly assigned to a live well, and their use in blood physiology assessment or postrelease behavior assessment was also assigned. Five treatments were used, which consisted of three different live-well additives designated X, Y, and Z (X was a liquid product, whereas Y and Z were powder products; all three were purchased in May 2021); a live well full of fresh lake water (control); and a baseline (only used for blood sampling). The live-well products selected for study were among the products that we perceived to be the most popular (based on our experiences with availability, discussions with tournament participants and organizers, and social media posts). For LMB in the baseline treatment, blood was immediately drawn after length measurement. Live wells were full of fresh lake water, and additives were added to the live well as per the manufacturers' suggestions (instructions on the bottles; 12.4 mL of product X, 12.3 mL of product Y, or 12.3 mL of product Z).

Biologging and tag attachment

Behavioral data for locomotory activity, depth, and temperature selection were collected using Axy-Depth biologgers (12 × 31 × 11 mm; 7.5 g in air; TechnoSmArt). Acceleration data were recorded in three axes (surge [A_x], sway [A_y], and heave [A_z] with respect to attachment orientation) at a frequency of 25 Hz and an 8-bit resolution. Temperature sensing in this biologger has a resolution of ±0.1°C, while the pressure sensor (depth) has a resolution of ±5 cm. Absolute dynamic acceleration was obtained by using a 2-s box smoother to remove the static acceleration (gravity) from the dynamic acceleration (animal movement) as described by Shepard et al. (2008) and Brownscombe et al. (2018). The ODBA (i.e., locomotor activity) of fish movement was then obtained by summing the absolute dynamic acceleration in all three axes (A_x, A_y,

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and A_z; Wilson et al. 2006; Halsey et al. 2011) during the entire monitoring period.

The Axy-Depth biologgers were epoxied into a cylindrical piece of balsa *Ochroma pyramidale* wood with just enough buoyancy that the biologging package was positively buoyant and would float. Additionally, the biologging package was equipped with a small radio transmitter (NanoTag; 15.0×8.2mm, 1.5g in air; Lotek) that was used to locate the biologging package after it was released from the fish. The float package was attached to the LMB using two plastic zip-ties that were fastened to a dissolvable ring (Figure 1). A Pep-O-Mint Life Savers candy (Mars, Inc.) was used as the dissolvable ring to which the zip-ties were fastened. To reduce the rate at which the Life Savers dissolved, we covered them in Plasti Dip (Plasti Dip International) and then removed a small area of the Plasti Dip to allow for the candy to dissolve. Once the Life Savers candy had dissolved, the biologging float package floated to the surface and was retrieved by the aid of the radio transmitter. The postrelease locomotor activity of LMB was monitored for a 5-min period due to the limitation of the dissolvable link and as reported by LaRochelle (2022). All LMB that were monitored for their postrelease behavior were released at the same location, which was near a weedy bay with a drop-off (up to 12m deep), where fish could seek refuge in the vegetation or could seek deep water.

Blood sampling

To test whether specific live-well additives influenced the physiological recovery of LMB, blood samples were taken after 2h of live-well confinement (Suski et al. 2006). Blood parameters generally peak before 2h and are on a recovery trajectory by the 2-h mark (Suski et al. 2006), making this a logical single time point on the recovery continuum to test whether the products enhanced recovery and reduced stress (shifting toward the baseline; as per Suski et al. 2006). The baseline blood sampling occurred immediately after capture (<2min) to ensure accurate baseline blood parameters. Lawrence et al. (2018) revealed that glucose, lactate, and cortisol values measured in blood sampled from angled fish within 3min of hooking reflected the baseline values of fish prior to capture. These fish were not confined in a live well for the 2-h period. Blood samples were taken at random and were taken every day. Individuals that were sampled for blood physiology were different than the individuals used for postrelease behavior assessment. Blood was collected from LMB in a water-filled trough, and the blood was drawn from the caudal vessel between the anal and caudal fins. Each sample used a new, 21-gauge Eclipse blood collection needle attached to a one-use holder. Once the needle entered the fish, a 4-mL BD Vacutainer blood collection tube was placed into the one-use holder, and contact with the needle was made to collect blood. The needle was kept in the fish until approximately 2mL of blood were collected in the BD Vacutainer tube. The tube was then carefully inverted three times, allowing blood to encounter the heparin within the tube to prevent coagulation. Immediately upon collection, a small, disposable plastic pipette was used to draw about 0.1mL of blood from the collection tube. The blood collection tube was immediately capped, labeled accordingly, and placed into a cooler containing an ice-water slurry. A small amount of the blood that was drawn into the pipette was placed on a Contour Next EZ Glucose meter strip for glucose quantification and on a Nova Biomedical Lactate Plus meter strip for lactate quantification. These devices have previously been validated for use in fish (Stoot et al. 2014).

FIGURE 1 Largemouth Bass with a pop-off biologging package (A), which was equipped with a biologger that included a tri-axial accelerometer, temperature sensor, and pressure sensor. The biologging package was also equipped with a radio transmitter. The package was attached to the ventral side of the fish and was fastened to a dissolvable ring (B), which allowed the pop-off package to detach from the fish and float to the water surface for retrieval.

At the end of the field sampling day, all blood collection tubes were removed from the ice-water slurry and placed into a centrifuge. Samples were spun for 5min to separate plasma from red blood cells. Using a disposable plastic pipette, plasma was collected and placed into small, 1-mL vials for plasma chloride and plasma sodium analyses. A new plastic pipette was used for each sample to avoid cross contamination. The 1-mL vials were immediately placed into liquid nitrogen until analyses could be conducted in the laboratory. Plasma chloride and plasma sodium were quantified via commercially available assay kits (chloride: BioAssay Systems, Catalog Number DICL-250, Lot Number CC05A27; sodium: MyBioSource, Catalog Number MBS2540574, Lot Number UX050X681157) following the manufacturers' protocols. Plasma chloride was diluted 1:40 and plasma sodium was diluted 1:2 with double-distilled water (Appendix Table A.1).

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Data analysis

Postrelease behavior

All analyses were conducted in R version 4.1.3 via R Studio version 2022.02.1, and all figures were produced using the ggplot2 package (Wickham 2016). A one-way analysis of variance (ANOVA) was used to test whether there was a difference in fish total length or time held in live wells across treatments. To test for variables with the strongest influence on postrelease activity, backwards elimination was conducted using the "step" function from the lmerTest package (Kuznetsova et al. 2017). This technique initially started with a fully parameterized model that included all possible predictor variables of interest, and variables were then removed to achieve the model that best explained trends in locomotor activity by selecting the model with the lowest Akaike's information criterion (AIC) score (Zuur et al. 2009; Richards 2015). The initial, fully parameterized model contained the total locomotor activity (i.e., total ODBA) summed across the 5-min postrelease monitoring period as the response variable, along with the following predictors: live-well additive type, postrelease water temperature selected by LMB, postrelease depth selection, fish total length, angling date, the interaction of live-well additive type with postrelease depth selection, the interaction of live-well additive type with postrelease water temperature, the interaction of live-well additive type with time held in the live well, and the interaction of live-well additive type with fish total length.

After we identified the top model by using AIC, a single generalized linear model (Gaussian) was generated using the "glm" function to visualize relationships among variables. This generalized linear model was fitted with locomotor activity (i.e., ODBA) as the response variable and had the following predictor variables: live-well additive type, postrelease depth, postrelease water temperature, time retained in the live well, the interaction of live-well additive type with postrelease depth, and the interaction of live-well additive type with postrelease water temperature. Factors and interactions with factors were followed up with a Tukey post hoc test using the "emmeans" function in the emmeans package (Lenth 2023) to further examine for the differences within the factors or interaction terms with factors within the model. The level of significance (α) for all models was $p < 0.05$.

Blood physiology

A one-way ANOVA using the "aov" function was fitted with fish total length (mm) as the response variable and live-well additive as the predictor variable to test for differences in total length across treatments involving blood sampling. If results from the one-way ANOVA were significant, a Tukey post hoc test using the "glht" function from the multcomp package (Hothorn et al. 2008) was used to separate means and identify differences in fish total length among live-well additives. Four blood physiology models were fitted using the glm function with blood glucose, lactate, chloride, or sodium as the response variable and live-well additive type (including control and baseline) and LMB total length as predictor variables. A Tukey post hoc test was used to examine for differences in means and further explore differences in blood parameters across live-well additives.

RESULTS

Postrelease behavior

In total, 61 LMB were captured (mean total length ± SD = 385 ± 37 mm), subjected to one of the three live-well additive treatments or the control, and monitored for 5 min postrelease. The total length of LMB ($F_{57,3} = 1.180$, $p = 0.325$) and the time spent in the live well (Table 1; $F_{57,3} = 0.262$, $p = 0.853$) did not differ across live-well additive types. Overall, LMB were held in live wells for 231 ± 69 min (mean ± SD). After performing backwards elimination on the initial, fully parameterized model, the top model that best explained postrelease locomotor activity included

TABLE 1 Duration of time for which Largemouth Bass were held in a live well across the treatment types (a live well with fresh lake water [control] or containing one of three live-well additives [X, Y, or Z]) for the fish that were monitored for postrelease behavior. Sample size (*n*) per treatment, number of replicates, mean time (min) that fish were held in the live well (with standard deviation [SD]), the minimum and maximum time held, and the mean total length (TL; with SD) of Largemouth Bass per treatment are shown.

Treatment	*n*	Replicates	Mean time (min)	SD (min)	Minimum time (min)	Maximum time (min)	Mean TL (mm)	SD (mm)
Control	16	16	233	57	145	339	378	37
X	14	9	225	75	134	352	399	41
Y	14	8	244	73	131	367	383	36
Z	16	9	224	74	127	412	378	33

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live-well additive type, postrelease depth, postrelease water temperature, time held in the live well, the interaction of live-well additive type with postrelease depth, and the interaction of live-well additive type with postrelease water temperature as predictor variables (Table 2). Live-well additive type alone did not influence the postrelease locomotor activity of LMB (Table 3). The duration of time spent in the live well prior to release had a significant influence on postrelease locomotor activity (Table 3; Figure 2; $r=0.28$), as LMB that were held in the live well longer during the angling day showed increased activity after release, independent of treatment. Furthermore, the water temperature selected postrelease did not have a significant influence on postrelease locomotor activity ($F_{55,1}=3.227$, $p=0.079$). There was a significant interaction effect of treatment and postrelease depth selection on the postrelease locomotor activity of LMB (Table 3; Figure 3; $F_{48,1}=3.787$, $p=0.016$). Locomotor activity of LMB that were retained in live-well additives increased as the depth selected increased relative to LMB that were retained in the control live well, for which postrelease locomotor activity exhibited minimal changes with increasing depth (Figure 3). However, the Tukey post hoc test did not separate out one treatment as being different from the others (Table 4). Finally, the interaction of treatment with postrelease water temperature selection did not have a significant effect on the postrelease locomotor activity of LMB ($F_{51,1}=2.761$, $p=0.052$).

Blood physiology

Overall, 56 LMB (mean total length ± SD = 363 ± 48 mm) were analyzed for their blood physiology: 47 fish were blood sampled 2 h into their live-well confinement, whereas nine fish were sampled immediately after angling. The ANOVA model indicated a difference in total length of LMB across treatments ($F_{51,4}=2.613$, $p=0.046$; Table 5), but the Tukey post hoc test did not detect differences across treatments ($p>0.05$). The total length of LMB did not influence the concentration of blood glucose or blood lactate after 2 h of holding in a live well, regardless of the live-well treatment (Table 6). There was no influence of LMB total length on plasma chloride ($F_{48,1}=3.591$, $p=0.064$) or plasma sodium ($F_{54,1}=3.273$, $p=0.077$). Treatment had a significant influence on blood glucose (Figure 4A; $F_{51,4}=6.851$, $p<0.001$) and blood lactate (Figure 4B; $F_{51,4}=10.482$, $p<0.001$)

TABLE 2 Output of backwards elimination for the model with postrelease locomotor activity (i.e., overall dynamic body acceleration [ODBA]) of Largemouth Bass as the response variable. "Depth" represents the depth selected during the postrelease monitoring period, and "Water temp" represents the postrelease water temperature selected. "Held" represents the total time that the fish remained in the live well prior to release. "Additive" represents the live-well additive type used in the live well. The number of model parameters is represented by the *K* symbol, while log(*L*) represents the log likelihood used to identify the model fit. Akaike's information criterion (AIC) is shown, with ΔAIC representing the difference in AIC score between the top model and the respective model. The model selected for statistical analysis is presented in bold.

Model	*K*	Log(*L*)	AIC	ΔAIC
Additive + Depth + Water temp + Held + (Additive × Depth) + (Additive × Water temp)	**13**	**−184.3**	**396.6**	**0.0**
Additive + Depth + Water temp + Held + Total length + (Additive × Depth) + (Additive × Water temp)	14	−183.7	397.5	0.9
Additive + Depth + Water temp + Held + Total length + (Additive × Depth) + (Additive × Water temp) + (Additive × Held)	17	−183.0	402.0	5.4
Additive + Depth + Water temp + Held + Total length + (Additive × Depth) + (Additive × Water temp) + (Additive × Total length)	17	−183.1	404.7	8.1
Full model	49	−164.8	423.6	27.0
Additive + Depth + Water temp + Held + Total length	8	−194.7	407.4	10.8
Additive + Depth + Water temp + Held	7	−194.8	405.6	9.0
Additive	4	−201.3	412.5	15.9
Depth	2	−199.6	405.1	8.5
Water temp	2	−198.8	403.6	7.0
Held	2	−198.8	405.5	8.9
Total length	2	−202.3	410.6	14.0
Additive × Depth	8	−197.5	412.9	16.3
Additive × Water temp	8	−194.7	407.4	10.8
Null model	1	−202.3	408.6	12.0

TABLE 3 Output of a generalized linear model for the postrelease behavior of Largemouth Bass. Treatment consisted of a control and three different live-well additives. "Depth" represents the postrelease water depth selected by the fish. Similarly, "Water temp" represents the postrelease water temperature selected. "Held" represents the total time that the fish spent in the live well prior to being released for a postrelease monitoring period. Significant variables are shown in bold.

Response variable	df	Deviance	Residual df	Residual deviance	*F*-value	*p*-value
Treatment	3	91.50	57	2621.6	0.973	0.413
Depth	**1**	**260.07**	**56**	**2361.5**	**8.297**	**0.006**
Water temp	1	101.17	55	2260.4	3.227	0.079
Held	**1**	**139.94**	**54**	**2120.4**	**4.463**	**0.040**
Treatment × Depth	**3**	**356.13**	**48**	**1504.6**	**3.787**	**0.016**
Treatment × Water temp	3	259.67	51	1860.8	2.761	0.052

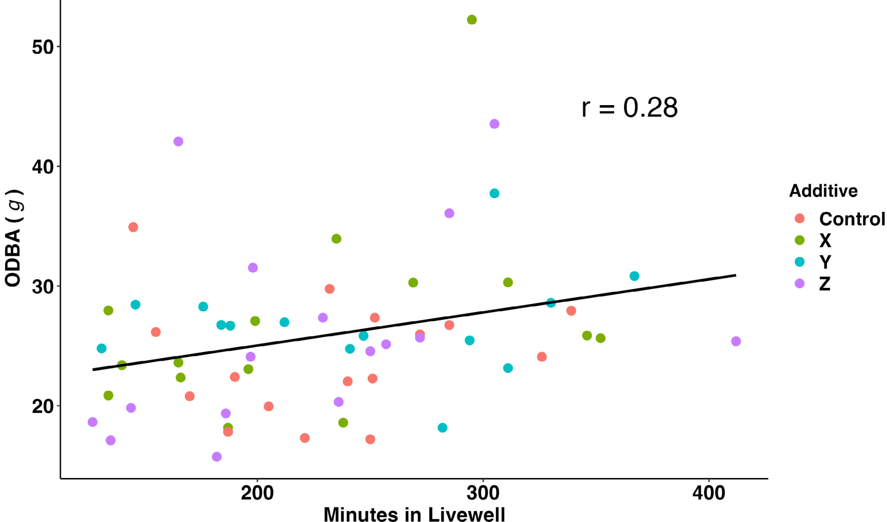

FIGURE 2 Postrelease locomotor activity (i.e., overall dynamic body acceleration [ODBA]) of Largemouth Bass after being held (min) in a live well containing one of three live-well additives (X, Y, or Z) or in a control live well with fresh lake water. The solid line represents the line of best fit ($y = 19.49x + 0.03$).

concentrations in LMB across the different treatments. Blood glucose and blood lactate concentrations were significantly elevated for all fish that were confined in live wells relative to fish in the baseline treatment (i.e., sampled immediately after angling), whereas blood glucose and lactate levels did not differ across the live-well treatments (Figure 4A,B; Table 6). Chloride concentrations were significantly greater for fish in the treatment with additive product Y relative to the control treatment ($z = 2.866$, $p = 0.034$), but there were no other differences in chloride concentrations across treatments (Figure 4C; Table 6). Treatment did not have an influence on plasma sodium concentrations in LMB (Figure 4D; Table 6).

DISCUSSION

The live-well additives tested in this study did not have an influence on the postrelease locomotor activity of LMB. Similar to previous findings from LaRochelle et al. (2022), our study found that postrelease locomotor activity increased with time spent in the live well. Assuming that conditions in the live well during retention are adequate (e.g., water chemistry, temperature, and dissolved oxygen) and if the fish do not have barotrauma, the live-well retention period can act as a period of recovery for black bass (Suski et al. 2004). Furthermore, the live-well additives tested in this study did not have an influence on the blood physiology of LMB. More specifically, the live-well additives did not enhance the recovery or reduce physiological disturbances in fish that were retained in live wells after capture.

Once hooked, fish begin to fight by engaging in burst swimming, attempting to escape the angling gear (Kieffer 2000). Burst swimming exceeds the aerobic capacity of muscle and is fueled by energy stores within the white muscle (Girard and Milligan 1992). Depending on the extent of the burst swimming activity (i.e.,

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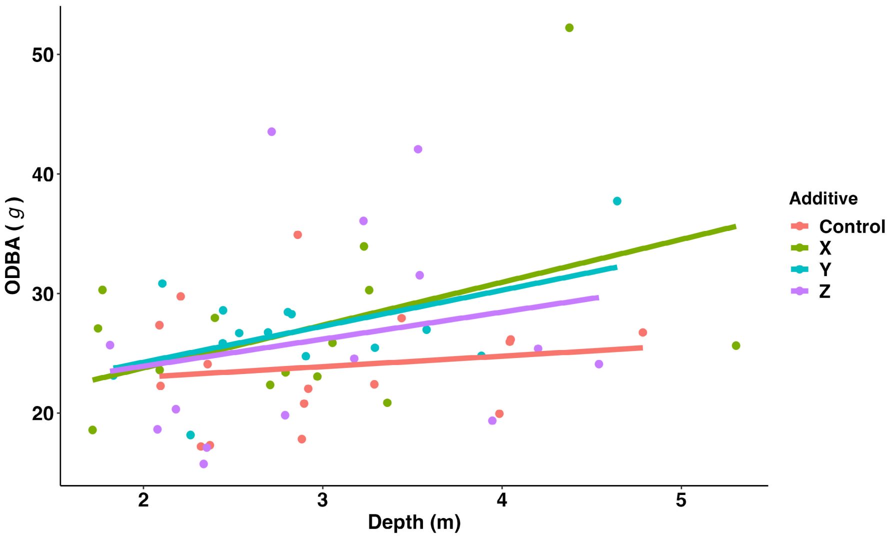

FIGURE 3 Interactive relationship between the overall dynamic body acceleration (ODBA) of Largemouth Bass and the postrelease depth selection across different live-well additives (X, Y, or Z). The lines represent the line of best fit respective of the live-well additive type.

TABLE 4 Tukey post hoc test output for the interaction effect of live-well additive type with postrelease depth selection on the postrelease locomotor activity of Largemouth Bass. Control represents the live well that was filled with fresh lake water, X was a liquid live-well additive product, and Y and Z were powder live-well additives.

Contrast	Estimate	SE	df	*t*-ratio	*p*-value
Control vs. X	−3.580	2.03	48	−1.760	0.305
Control vs. Y	−2.705	2.07	48	−1.309	0.526
Control vs. Z	−3.279	2.02	48	−1.625	0.374
X vs. Y	0.875	2.11	48	0.416	0.976
X vs. Z	0.300	2.05	48	0.147	0.999
Y vs. Z	−0.575	2.09	48	−0.275	0.993

TABLE 5 Total length (with standard deviation [SD]) of Largemouth Bass analyzed for blood physiology immediately after capture (i.e., angling baseline), after being placed in a live well with fresh lake water (i.e., control), or after being placed in a live well with one of three live-well additives (X, Y, or Z). The sample size and number of replicates per treatment are also presented.

Treatment	*n*	Replicates	Mean TL (mm)	SD (mm)
Baseline	9	9	393	44
Control	13	13	369	46
X	11	9	377	71
Y	13	9	344	31
Z	10	6	338	18

anaerobic exercise) and depletion of energy stores in the white muscle tissue, exhaustion can occur (Girard and Milligan 1992). During burst swimming events, the white muscle of fish engages in fast-twitching action that is fueled by phosphocreatine, ATP, and glycogen (Sänger and Stoiber 2001). When these anaerobic fuels are used by white muscle during burst swimming events, they are converted to lactate by anaerobic glycolysis (Barton 2002). Once the burst swimming event has ended, muscle glycogen levels are diminished or depleted and lactate levels increase. However, physiological recovery occurs by glycogenic removal of lactate, which can be observed in fish when lactate returns to baseline levels, which did not occur for any of the retained fish in this study. For recovery to occur, it is important for lactate to be reduced and muscle glycogen to be replenished (Girard and Milligan 1992). One means of recovery from exercise is via muscle glycogenesis, whereby blood lactate is converted back into glycogen via the glycolysis cycle (Dinken et al. 2022). When an angling event occurs, glucose is mobilized into the bloodstream, supplying energy for aerobic tissues (e.g., brain and gills; Barton and Iwama 1991), which clearly occurred for the LMB retained in live wells for our study. Furthermore, previous research has shown that chloride and sodium ions increase immediately after exhaustive exercise, with ion concentrations returning to baseline levels during recovery (McDonald and Milligan 1992), which did not occur for LMB that were retained in the control live well or in the live wells containing additives.

Our results showed that use of the tested live-well additives during live-well confinement did not facilitate the physiological recovery of LMB compared to holding the fish in fresh lake water. There also was no difference in the postrelease locomotor activity of LMB held in live wells containing additives, further suggesting that the tested

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TABLE 6 Output of four generalized linear models for blood parameters in sampled Largemouth Bass. Treatments consisted of a baseline, control, and three different live-well additives, for a total of five treatments. Significant predictor variables are shown in bold.

Response variable or predictor variable	df	Deviance	Residual df	Residual deviance	*F*-value	*p*-value
Glucose						
Treatment	**4**	**31.673**	**51**	**57.797**	**6.851**	**<0.001**
Total length	1	0.009	50	57.788	0.008	0.929
Lactate						
Treatment	**4**	**312.373**	**51**	**373.820**	**10.482**	**<0.001**
Total length	1	1.313	50	372.500	0.176	0.676
Chloride						
Treatment	**4**	**4916.000**	**49**	**18,153.000**	**3.493**	**0.014**
Total length	1	1263.700	48	16,890.000	3.591	0.064
Sodium						
Treatment	4	946.010	50	20,478.000	0.604	0.662
Total length	1	1282.280	49	19,196.000	3.273	0.077

additive types alone did not facilitate the recovery of LMB retained in live wells. Although not significant, LMB that were held in live-wells treated with products X, Y, and Z had slightly greater postrelease locomotor activity while inhabiting deeper water relative to the LMB that were held in the control live well, suggesting that LMB retained in live-well additives were engaging in a fleeing (e.g., escaping a place of discomfort) behavior once they were released. Furthermore, LMB that were retained in a live well for a longer period tended to have greater postrelease locomotor activity relative to LMB that were released earlier. Based on these results and those previously reported by LaRochelle et al. (2022), it appears that the time held in the live well is more important for postcapture recovery (i.e., exhaustive exercise) of LMB than the product added to the live-well water.

Based on the elevated blood glucose and lactate levels for LMB retained in the live wells relative to levels for LMB from the baseline, our results suggest that live-well-retained LMB had not fully recovered from physiological disturbances by the time of sampling. This is not entirely surprising given that cortisol would presumably have been elevated and thus mobilized energy reserves. Similarly, lactate levels for LMB in the control live well and the live wells with additives were elevated compared to the baseline treatment and comparable with previous values from recovered fish in the literature (e.g., Suski et al. 2006). Interestingly, LMB that were retained in the control live well had significantly reduced plasma chloride concentrations relative to LMB that were held in the live well containing product Y. However, there were no other differences in plasma chloride or plasma sodium across the live-well additive types, indicating that changes in plasma chloride were equivocal. Plasma chloride ions in freshwater fish such as LMB increase immediately after exercise (McDonald and Milligan 1992; Suski et al. 2004), which likely explains the elevated chloride concentrations for LMB in the baseline treatment of our study. We suggest that (1) recovery did not occur for LMB that were retained in live wells with the tested additives and (2) fish held in live wells with additives experienced additional stressors relative to the control LMB based on elevated chloride concentrations. We speculate that fish in the live-well additive treatments and fish in the control had elevated chloride values from the capture event (i.e., exercise); however, the control fish progressed toward recovery, whereas fish held in the live-well additives did not. Similarly, plasma sodium ions appeared to increase immediately after capture, which is typical of an exhaustive exercise event such as angling (McDonald and Milligan 1992). Sodium values remained elevated for LMB in all treatments, suggesting that additives did not enhance fish recovery or reduce confinement stress during live-well retention relative to controls. Collectively, our results did not provide support that the tested live-well additives enhanced LMB recovery or reduced confinement stress while the fish were retained in live wells.

This study provided a foundation upon which to test the functionality of a novel pop-off tag for quantifying the postrelease behavior of fish in freshwater systems. Unlike saltwater applications, corrosive materials, such as galvanic metals, do not work in freshwater systems for the remote detachment and retrieval of a biologging package. The use of a hard candy as the dissolvable link had highly variable success, which ranged between the dissolvable link breaking immediately upon release and the link remaining on the fish for up to 1 h postrelease. We are confident that the data obtained during the initial 5 min

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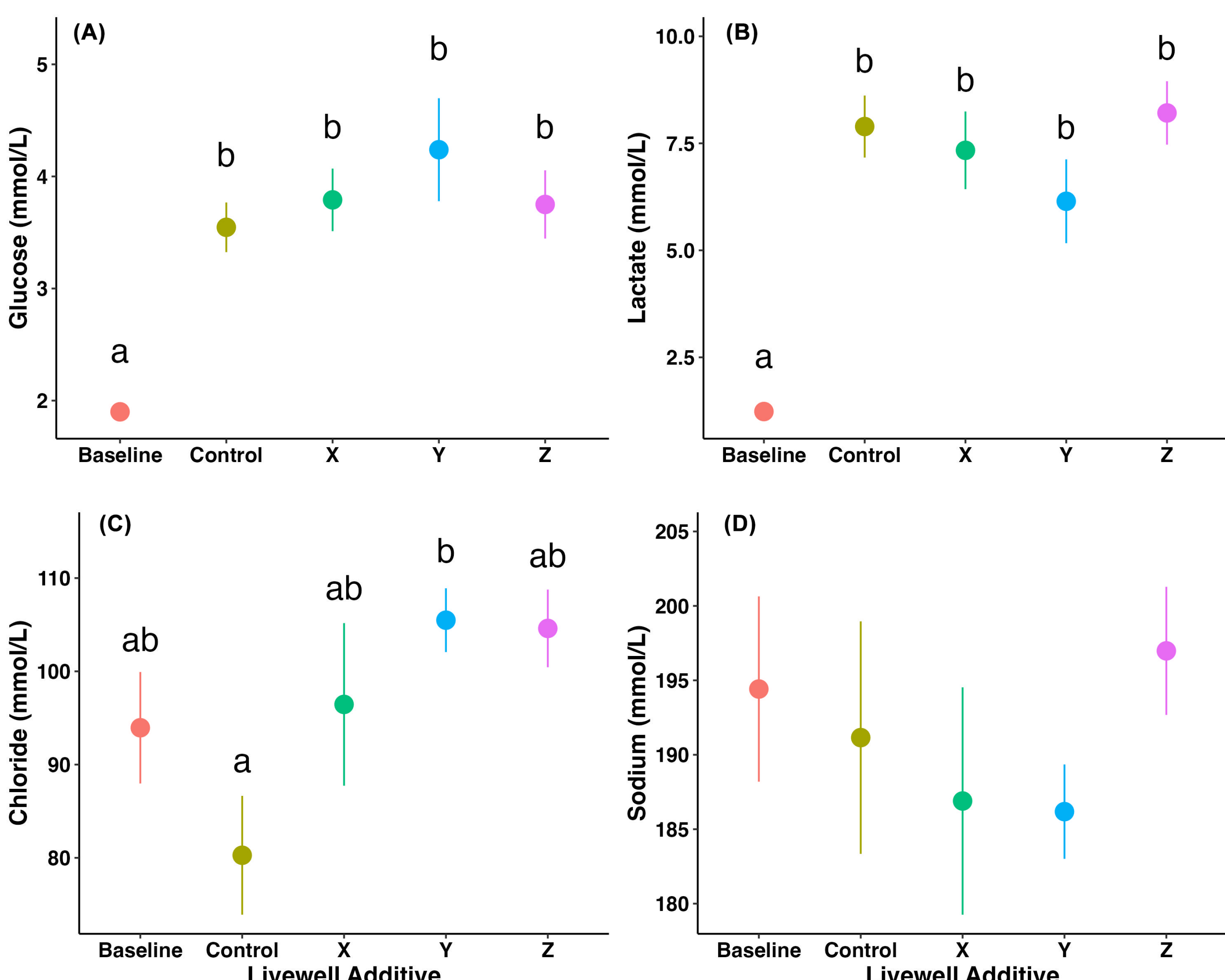

FIGURE 4 Concentrations (mmol/L) of (A) blood glucose, (B) blood lactate, (C) plasma chloride, and (D) plasma sodium of Largemouth Bass after being exposed to different live-well treatments (one of three additives [X, Y, or Z] or the control [fresh lake water]). Fish in the live-well treatments were sampled prior to release. Baseline values are for fish that were sampled immediately after capture by angling. Letters represent significant dissimilarities among treatment groups.

after release (previously suggested by LaRochelle 2022), in conjunction with the blood physiology data, are sufficient to draw conclusions on how the tested live-well additives influenced the recovery and confinement stress of LMB that were retained in live wells. Furthermore, previous studies have made connections between immediate reflex impairment levels and the long-term fate of fish, suggesting that impairments within the first 5 min are indicative of long-term fate (Raby et al. 2012). This issue clearly outlines the need for more research on (1) the relationship between ODBA and the stress level of fish and (2) the attachment duration needed for pop-off biologging packages to effectively monitor the postrelease behavior of fish after angling events. We also understand the potential issues (i.e., fish trying to get rid of the package; influences on fish buoyancy regulation) that may arise from fastening biologging packages to the fish, but we are confident that the data collected were precise given that all fish experienced the same degree of intrusiveness and positive buoyancy as a result of being tagged. For future studies considering the use of pop-off tags, we recommend employing a new material for a dissolvable link that would be more durable and would stay on the fish longer (e.g., catgut sutures).

Overall, our study indicates that the three evaluated live-well additives neither reduced physiological disturbances nor facilitated the recovery from exercise for LMB that were retained in a live well under the test conditions. There is still uncertainty related to the quality or benefits of the tested live-well additive products, compounded by the lack of government oversight, which could potentially lead to interbatch variations. Additionally, the health implications associated with keeping fish in the tested live-well additive products and then releasing the fish into areas

where humans may subsequently harvest them for consumption have not been defined. More research is needed to explore the potential risks associated with live-well additives and whether consumption of additive-exposed fish is harmful. Given the lack of evidence supporting the benefits of the tested live-well additive products, we recommend that anglers use fresh, well-oxygenated lake water when retaining fish in live wells unless future evidence emerges to the contrary. We encourage the manufacturers of live-well additives to be transparent regarding the contents of their products and to provide evidence supporting their claims. Until that happens, anglers should exercise great caution in using such products.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data is available upon reasonable request.

ETHICS STATEMENT

All research was conducted under a scientific permit issued by the Ontario Ministry of Natural Resources and Forestry, with approval from the Institutional Animal Care and Use Committee (Protocol 110558).

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APPENDIX A

Quality assurance metrics

TABLE A.1 Quality assurance metrics for sodium and potassium assays. Intraplate variance measures the coefficient of variation between duplicate wells of each sample. Interplate variance is the variation between identical samples across plates. Linearity informs where sample matrices inhibited reliable quantification after dilution, with a low r^2 indicating poor performance. Sensitivity indicates the lower limit of detection for the assay kit.

Metric	Chloride	Sodium
Intraplate variance (%)	2.5	2.1
Interplate variance (%)	3.4	0.1
Linearity (r^2)	0.96	0.98
Sensitivity	0.06	0.09

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