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The efficacy of assisted ventilation techniques for facilitating the recovery of fish that are exhausted from simulated angling stress

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ABSTRACT

Employing science-based best angling practices is important for sustainable catch-and-release fisheries. In situations where fish lose equilibrium (unable to maintain upright posture to swim in a coordinated manner), anglers often provide assisted ventilation by hand, which typically involves maneuvering fish to move water over the gills until equilibrium is regained. However, it is unclear whether these tactics are effective at facilitating physiological and behavioural recovery and improving survival. Here we tested the efficacy of assisted ventilation techniques in two freshwater species popular for angling, largemouth bass and brook trout. Fish were captured by angling with rod and reel, and subsequently air exposed until equilibrium was lost. Treatments included maneuvering fish in a back-and-forth manner or in a constant forward motion, which were compared to controls that did not experience assisted ventilation. In largemouth bass, physiological stress values (i.e., blood glucose, lactate, pH, hematocrit) and rates of equilibrium regain were not significantly different between treatments, while all fish survived a 24-h holding period. In brook trout, fish maneuvered in a back-and-forth manner regained equilibrium fastest, but differences between treatments were not statistically significant. Further, once equilibrium was regained, brook trout often spent extended periods resting on the bottom, and likely had limited capabilities to avoid predators. We found little evidence of any physiological or behavioural benefits of two common assisted ventilation techniques that would result in improved fish survival or fitness with largemouth bass or brook trout in recreational angling scenarios. However, releasing fish in poor condition may lead to greater predation risk, so retaining fish with minimal handling until swimming capabilities return is likely the most advisable course of action.

1. Introduction

Catch-and-release angling is a popular conservation strategy predicated on the assumption that released fish will survive and experience limited fitness consequences (Wydoski, 1977; Arlinghaus et al., 2007). Fish are released to comply with mandated regulations (i.e., harvest regulations) or as a voluntary action reflecting conservation ethic of anglers (Cowx, 2002; Brownscombe et al., 2014a). However, due to angling-related physiological stress and injury, fish fitness and survival can be impacted (Cooke and Schramm, 2007). The outcome of angling events varies greatly due to angler behaviour and environmental conditions. Therefore developing and applying best angling practices (i.e. those that maintain the welfare status of fish) is essential for sustainable recreational fisheries (Cooke and Suski, 2005; Brownscombe et al., 2016).

Despite the best intentions of anglers, there are still cases where fish experience significant stress as a result of the additive effects of the fight and handling, mediated by environmental conditions (Cooke and Suski, 2005; Arlinghaus et al., 2007). When stressors are extreme (which is a relative species-specific construct) it can lead

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to fish exhaustion and behavioural impairment. While behavioural impairment is challenging to measure post-release, reflex tests are a good predictor of behavioural impairment and mortality in angled fish (Brownscombe et al., 2013, 2014b). The most obvious reflex indicator of exhaustion is when fish are unable to maintain upright posture (equilibrium) or swim away from the angler in a coordinated manner. In these instances fish have a much lower probability of survival due to the physiological stress or post-release predation by opportunistic predators (Danylchuk et al., 2007; Raby et al., 2012). When fish are unable to swim away, anglers often hold fish by hand and maneuver them in various patterns through the water in an attempt to assist the fish in regaining equilibrium (Pelletier et al., 2007). In lentic systems (i.e. lakes, reservoirs), these assisted recovery techniques typically involve moving the fish back-and-forth in the water, or in a constant forward motion in a figure-8 or circular pattern. In riverine systems, it is common to hold fish facing into the flow to provide assisted ventilation. However, Robinson et al. (2013, 2015) found no evidence that this technique improves survival or migration success in sockeye salmon (*Oncorhynchus nerka*). To our knowledge, no studies have ever tested the efficacy of assisted ventilation techniques at reducing stress or improving fish survival in lentic systems. Fish gill lamellae are designed to uptake oxygen from anteriorly-sourced water flow using a counter-current gas exchange system (Gilmour, 1997), so it is generally believed that constant forward movement is more efficient for oxygen uptake, while back-and-forth movement may be detrimental (Pelletier et al., 2007).

The objective of this study was to test the efficacy of commonly employed assisted ventilation techniques at facilitating physiological and behavioural recovery and improving survival of recreationally angled fish. We tested two different techniques with two freshwater fish species that support popular recreational fisheries, largemouth bass (*Micropterus salmoides*; Quinn and Paukert, 2009) and brook trout (*Salvelinus fontinalis*; Power, 1980), in an effort to determine the best course of action when fish show signs of exhaustion and behavioural impairment from recreational angling. For largemouth bass we examined secondary physiological stress responses, reflex impairment, and mortality. Due to logistical constraints at the field site, for brook trout we examined only reflex impairment.

2. Methods

2.1. Largemouth bass recovery

This experiment was conducted at Queen's University Biological Station (QUBS) Lake Opinicon (44° 35′ 6.4″, −76° 17′ 47.7″) in Ontario, Canada between 01-05-2015 and 04-05-2015 at water temperatures from 15 to 22 °C. Largemouth bass were angled from a single shallow embayment using 2-m-long, medium-strength fishing rods and reels equipped with 6.8 kg break-strength braided fishing line. Terminal tackle included a 1/0 octopus hook, baited with a 15 cm wacky-rigged plastic worm. Upon capture, largemouth bass were air exposed in a rubberized net until equilibrium was lost. Based on initial tests and previous literature (Thompson et al., 2008), a minimum of 10 min of air exposure was required to cause largemouth bass to lose equilibrium at these water and air temperatures. In order to avoid placing fish in water to test equilibrium periodically, an initial reflex test was applied prior to testing equilibrium. The initial reflex test involved grabbing the fish by the lower jaw; the fish flexing its body indicated a positive response. Starting at 10 min of air exposure, initial reflex tests were conducted every 2 min until fish were unresponsive. Equilibrium was then tested by flipping the fish upside down in water; a positive response was indicated by the fish righting itself within 3 s. If the response was positive, air exposure continued under the same initial reflex test procedure until equilibrium was lost.

Upon equilibrium loss, additional reflex tests were conducted using RAMP methods (Davis, 2010). Five predictors were measured: tail grab, body flex, equilibrium, head complex impairment, and vestibular-ocular response (VOR). These predictors were selected because they are strong indicators of fish behavioural impairment and mortality, and also feasible for anglers to adopt (Davis 2010; Raby et al., 2012; Brownscombe et al., 2013; Brownscombe et al., 2016). Tail grab was tested by grabbing the fish's tail in water; an attempt to escape indicated a positive response. Body flex was tested by holding the fish in air by the center of the body; flexing in attempt to escape indicated a positive response. A positive head complex response was indicated by regular opercular movement in water. Vestibular-ocular response (VOR) was tested by rolling the fish side-to-side; a positive response was indicated by the eyes moving to track level. Each indicator was scored as 0 = unimpaired and 1 = impaired and overall RAMP scores were calculated as the proportion of indicators impaired.

After RAMP assessment, fish were treated with one of three assisted ventilation techniques: control (n = 25), forward motion (n = 24), or back-and-forth (n = 24) for up to 3 min in 90 l holding containers. Fish in the control treatment were left untouched aside from equilibrium checks. Fish in the forward motion treatment were held by the center of the body while maneuvering the fish in a circular pattern in constant forward motion to generate anteriorly sourced water flow. While anglers typically manoeuvre fish in an 'S shape' or 'figure-8', the circular motion was more feasible in the holding containers and generated similar anteriorly sourced water flow. Fish in the back-and-forth treatment were held in the same manner as the forward motion treatment but manoeuvred forward and backward through the water. Equilibrium was checked every 20 s, and the recovery period concluded once equilibrium was regained, up to a maximum of 3 min.

After the recovery period fish were held for 1 h prior to blood sampling to assess physiological stress, as secondary stress levels typically peak around 1 h post-stressor in the blood of largemouth bass (Suski et al., 2007). For blood sampling, fish were transported to a sloped trough filled with lake water where ~1 mL of blood was extracted via the caudal venipuncture of each fish using an 18-gauge syringe and 3 mL Vacutainer® (75 USP lithium heparin). Blood was immediately stored on ice and later analyzed using point-of-care devices effective with fish (Cooke et al., 2008; Stoot et al., 2014) for glucose (in millimoles per litre; Accu-Chek Compact Plus), lactate (in millimoles per litre; Nova Biomedical, MA, USA), and pH (HI-99161; Hanna Instruments, RI, USA). Hematocrit (erythrocyte volume fraction) was measured by spinning whole blood at 5000 rpm for 5 min in capillary tubes (CritSpin, MA, USA). Lastly, fish were given a fin clip unique to each treatment and transported back to QUBS where they were placed in a 1.22 m × 1.22 m × 1.22 m floating net pen to monitor mortality for 24 h. All fish were released after experimentation.

2.2. Brook trout recovery

This experiment was conducted on Collins Lake in Kenauk Nature Reserve, Quebec, Canada (45° 44′ 38.0″, −74° 48′ 24.7″) on 06-10-2015 and 07-10-2015 at water temperatures ranging from 13 to 14 °C. Brook trout were stocked from hatcheries near Mont-Tremblant in 06-2015 and 09-2015. Fish were captured using in-line spinner lures (size 2 or 3) with barbed treble hooks and held overnight in a net pen (1.2 × 1.2 × 1.2 m, maximum density 20 fish) as a part of another study examining angling-related mortality. The following day, brook trout that were not deeply hooked and had no visible signs of injury or behavioural impairment were included in this study.

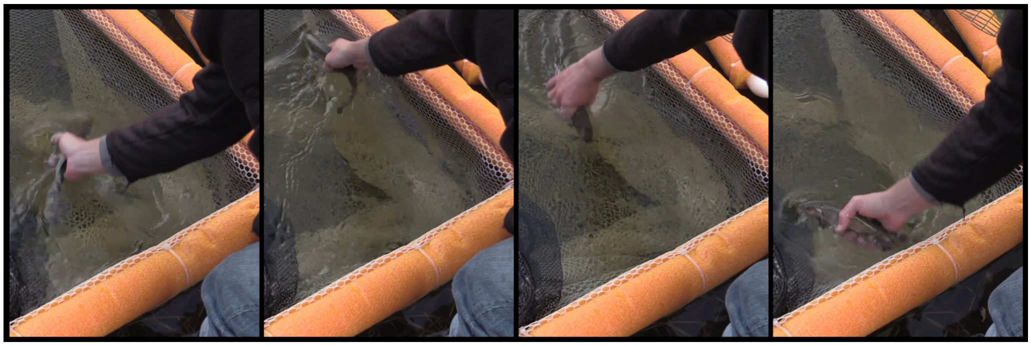

Fig. 1. Brook trout maneuvered in a figure-8 pattern through the water in an attempt to assist ventilation.

In order to elicit equilibrium loss, brook trout were air exposed in a rubberized net for a standardized 8-min period, as initial tests suggested this was a sufficient amount of time to cause equilibrium loss. After air exposure, fish were tested for the same five RAMP indicators used on largemouth bass, and separated into the same treatment groups, control (n = 21), back-and-forth motion (n = 21), and forward motion (n = 21). However, the forward motion treatment was manoeuvred in a figure-8 pattern rather than the circular pattern used with largemouth bass, and all recoveries took place in a net pen in the lake (See Fig. 1 for visualization of recovery procedure). Equilibrium was checked every 20 s, and the recovery period concluded once equilibrium was regained. Fish were released immediately after experimentation, and visually observed for up to 10 min near the release site.

2.3. Data analysis

For the largemouth bass experiment, fish sizes (total length), air exposure durations, and water temperatures were compared between treatments with one-way ANOVAs. Blood physiology metrics (blood glucose, lactate, pH, and hematocrit) were compared between the three treatments as well as baseline values using one-way ANOVAs. Time until equilibrium regain was compared between treatments using Cox proportional hazard regression analysis because the data were censored at a 180 s maximum.

For the brook trout experiment, fish sizes (total length) and time until equilibrium regain were compared between treatments using one-way ANOVAs. For all ANOVAs where significant differences were found, Tukey's HSD post hoc tests were applied. Assumptions of normality, homogeneity, and for the Cox hazard regression, proportionality and nonlinearity were checked prior to analysis. The level of significance for all analyses was set at $\alpha = 0.05$, and all analyses were conducted using R Studio version 0.99.447 (RStudio Team, 2015). Survival analysis was conducted using the 'survival' package (Therneau, 2015). All data are reported as mean ± SE unless otherwise specified.

3. Results

3.1. Largemouth bass recovery

Largemouth bass (n = 74, 36.6 ± 5.1 cm total length) angling durations were variable (31 ± 3.6 s), but were not significantly different between treatments (one-way ANOVA; $F_{2,71} = 1.3$, $p = 0.28$). Air exposure durations required for largemouth bass to lose equilibrium were relatively long (19.8 ± 2.3 min), and not significantly different between treatments ($F_{2,71} = 0.23$, $p = 0.80$). Fish sizes were also not significantly different between treatments ($F_{3,93} = 0.89$, $p = 0.45$).

There were significant differences in all physiological values when comparing treatments and baseline physiological values, including blood glucose ($F_{3,93} = 19.9$, $p < 0.001$), lactate ($F_{3,93} = 271$, $p < 0.001$), pH ($F_{3,93} = 11.2$, $p < 0.001$), and hematocrit ($F_{3,93} = 3.6$, $p = 0.02$). Blood glucose, lactate, and pH were significantly higher in all three treatments than baseline values, but the treatments were not different from each other (Fig. 2). Hematocrit was significantly higher in control fish than baseline.

A greater number of fish regained equilibrium in the forward motion treatment during the first 2 min. However, all treatments were similar by the end of the 3-min monitoring period (Fig. 3A). There were no significant differences in time to equilibrium regain between treatments (Cox proportional hazard regression; $\chi^2 = 1.17$, $p = 0.56$). All fish had regained equilibrium prior to the blood sampling 1 h later, and all fish survived during the 24-h holding period.

3.2. Brook trout recovery

Brook trout (n = 65, 31.2 ± 3.7 cm total length) sizes were not significantly different between treatments ($F_{2,62} = 0.59$, $p = 0.56$). Compared to largemouth bass, brook trout recovered equilibrium relatively quickly (Fig. 3B). Fish in the back-and-forth treatment generally regained equilibrium fastest and controls the slowest, however, there was no significant difference in time to equilibrium regain between treatments (one-way ANOVA; $F_{2,62} = 1.5$, $p = 0.24$). Once equilibrium was regained, fish often rested on the bottom for an extended period (2–10 min) prior to resuming swimming behaviour.

4. Discussion

Despite the best intentions of anglers, there are scenarios where angled fish become exhausted and thus unable to swim away from the angler, a condition that can lead to mortality (Danylchuk et al., 2007; Raby et al., 2014). Here we aimed to determine if maneuvering fish through the water, a common practice amongst anglers, is an effective method of facilitating physiological and behavioural recovery, and improving fish survival in largemouth bass and brook trout. Overall, we found limited evidence that maneuvering fish in a constant forward motion or back-and-forth confers any fitness benefit, with similar physiological stress values and reflex impairment to control fish that were not maneuvered.

Due to the physical exercise during the angling event and subsequent air exposure, fish experience anoxia and deplete readily available energy stores (Cooke and Suski, 2005; Arlinghaus et al., 2007; Cook et al., 2015), which in extreme cases can lead to a loss of motor control, resulting in behavioural impairment. The primary stress response involves the release of catecholamines and corticosteroids, which have cascading effects in the body, including mobilization of energy stores and immune system suppression

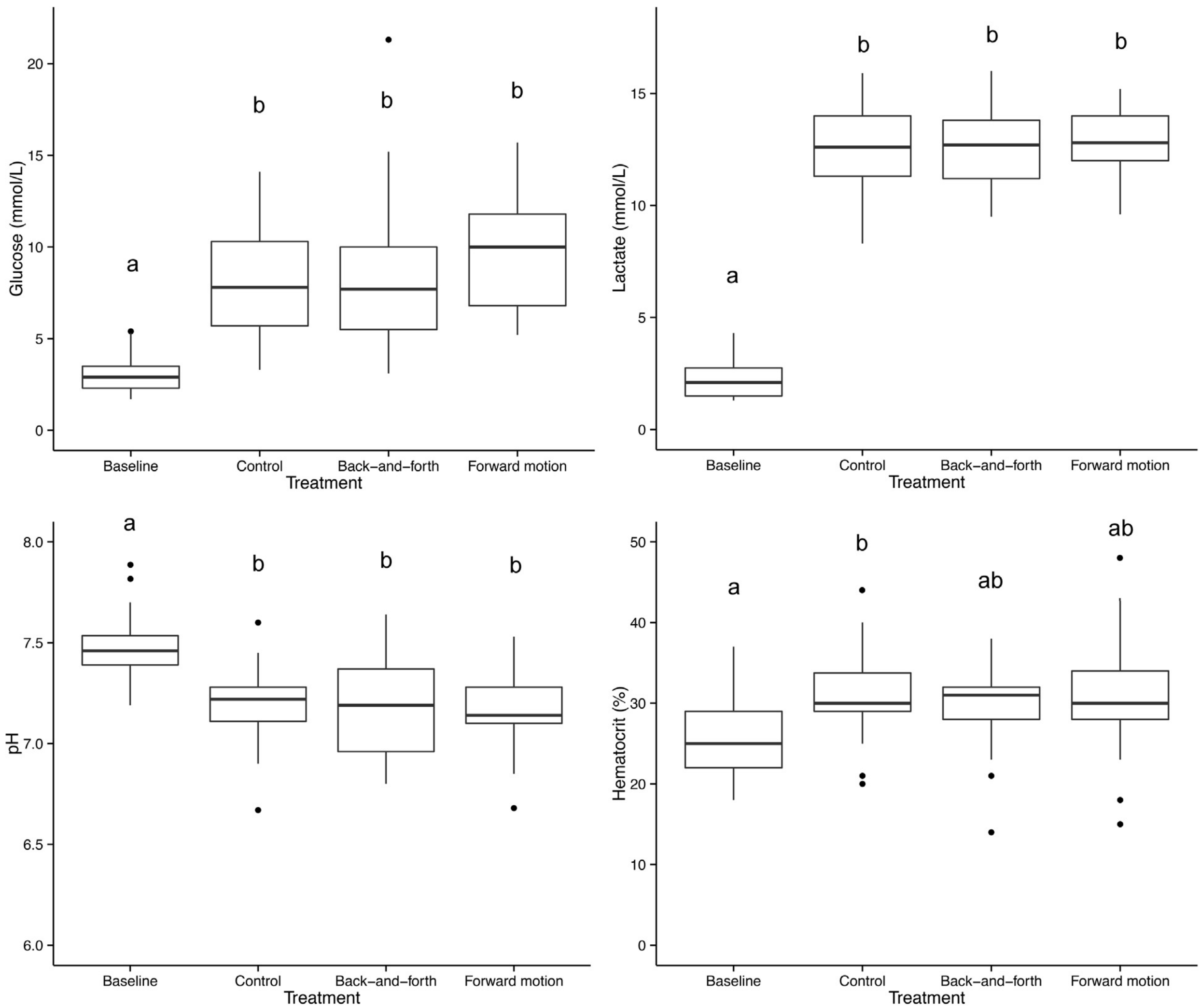

Fig. 2. Largemouth bass secondary stress physiological measures across baseline, control, back-and-forth recovery, and forward motion assisted ventilation treatments. Dissimilar letters indicate statistically significant ($p < 0.05$) differences based on analysis of variance (ANOVA) models and Tukey's post-hoc tests; see methods and results for full details).

(Wendelaar Bonga, 1997; Barton, 2002). Here, we did not measure these responses directly, but instead measured secondary stress responses including blood glucose and lactate concentrations, hematocrit (% red blood cell volume), and blood pH, which are generally considered to be good proxies of physiological stress levels (Cooke and Suski, 2005). Presumably, had maneuvering the fish through the water facilitated greater oxygen uptake, then less anaerobic metabolism would have occurred, resulting in reduced secondary stress responses. However, we observed similar secondary stress levels in maneuvered fish and controls. These findings are consistent with those from Robinson et al. (2013), who found that assisted ventilation (via holding fish facing water flow in riverine systems) has little effect on secondary stress responses in sockeye salmon. This may be because extended handling times in maneuvered fish caused greater stress responses (Meka, 2004), which counteracted any physiological benefit to the maneuvering. We also noted that in all treatments, fish actively respired by beating their opercula rapidly. Oxygen uptake may be nearly maximized via natural respiration in fish that actively ventilate, such as largemouth bass and brook trout. Recovery tactics could be more beneficial for obligate ram ventilators such as tuna and mackerel.

Here we used air exposure as the primary stressor to induce equilibrium loss. Although largemouth bass were angled, fight times were minimal and fish were vigorous upon landing. There may be differences in physiological stress responses caused by exercise and air exposure (e.g., increased lactic acid in the muscles with exercise; Wood, 1991). However, air exposure is the major cause of equilibrium loss in angled fish (Cooke and Suski, 2005; Cook et al., 2015). Brownscombe et al. (2014c) found fight duration was not the primary driver of equilibrium loss or physiological stress in largemouth bass when using appropriate fishing gear for the species. In this study fish did require longer air exposure times to lose equilibrium than in typical angling scenarios due to low water temperatures (relative to the species upper tolerance). Future research could explore whether recovery tactics are more beneficial at higher water temperatures when equilibrium loss is more common in angling scenarios.

While there was no observed physiological benefit to assisted ventilation techniques, it is also important to consider behavioural impairment, which can lead to increased predation risk by opportunistic predators (Danylchuk et al., 2007; Cooke et al., 2014; Brownscombe et al., 2014b). Interestingly, the back-and-forth treatment resulted in the fastest equilibrium regain in brook trout, as well as a subset of the fastest recovering largemouth bass; however, overall these differences were not statistically significant. Further, upon release we observed that fish often rested on the bottom without moving for up to 10 min. In these cases, fish may be able to swim away from the handler, but are still not very capable

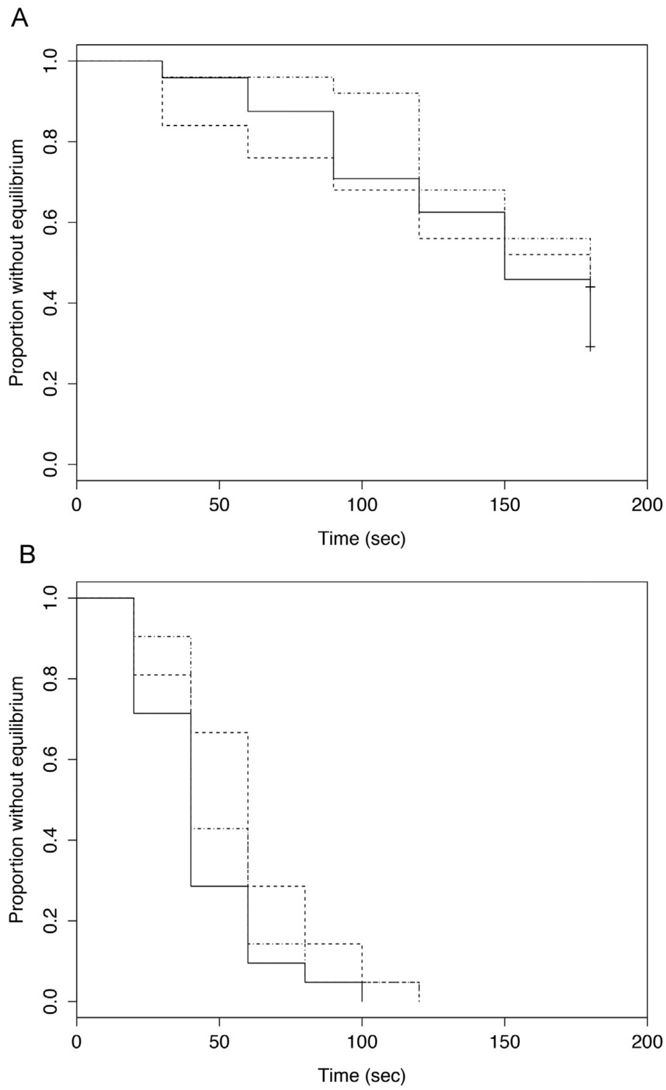

Fig. 3. Time to equilibrium regain in (A) largemouth bass and (B) brook trout exposed to control (no recovery; dash line), back-and-forth (solid line), and forward motion (dot-dash line) assisted ventilation treatments.

of avoiding opportunistic predators (see Cooke and Philipp, 2004; Danylchuk et al., 2007). Based on our findings, when fish show signs of behavioural impairment (indicated through loss of equilibrium or other RAMP tests), the best course of action is likely retaining the fish for a short period in a live well or recovery bag with ambient oxygen levels (e.g. 15 min for bonefish; Brownscombe et al., 2013) to allow fish to recover before releasing them back into the wild.

When designing this experiment, we elected to hold the fish by the body during recovery maneuvers instead of the mouth in order to test a generalizable method that could be apply to fish with any mouth dentition. However, during forward motion treatments, we noticed the fish often had their mouths partially closed, impeding the flow of water to some degree. For species such as largemouth bass, it is possible to hold fish by the lower jaw between the thumb and forefinger during maneuvering, which may better facilitate oxygen uptake. Future research may explore this option as a more effective recovery method; however, it is difficult to apply to many species with sharper dentition.

In summary, the assisted ventilation techniques tested here, involving maneuvering fish through the water by hand in back and forth or constant forward motion, were not effective at facilitating physiological or behavioural recovery in largemouth bass. Similarly, these techniques did not significantly improve rates of equilibrium regain in brook trout. Further, while in some instances maneuvering brook trout helped them regain equilibrium faster, they were likely still unprepared to avoid opportunistic predators. Therefore, we suggest that in instances where fish are unable to swim away from the angler but must be released, holding the fish in a livewell or fish recovery bag in ambient oxygen levels for a short period prior to release, without direct extended handling by the angler, is likely the best course of action to maximize survival. This study represents, to our knowledge, the first test of the efficacy of assisted ventilation techniques in lentic systems involving maneuvering fish by hand. These experiments were conducted at moderate water temperatures, and assisted ventilation may be more beneficial at higher water temperatures when oxygen is more limited, or with other species (e.g., obligate ram ventilators). We did not measure blood gases (i.e. oxygen and carbon dioxide), which would provide a more direct measure of the physiological impacts of these techniques, and should be the focus of further studies. Similarly, more detailed physiological studies that evaluate the time course of recovery (see Cooke et al., 2013) may provide additional insight into the relative merits of different recovery tactics.

Contributions

Brownscombe conceived and designed this project, provided funding, conducted sampling, data analysis, and wrote the manuscript. Parmar, Almeida, Giesbrecht, Batson, Chen, and Wesch contributed to study design, data collection, data analysis and manuscript preparation. O'Connor and Ward contributed to study design and manuscript preparation. Cooke contributed to funding, study design and manuscript preparation.

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