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[Article]

Physiology, Behavior, and Survival of Angled and Air-Exposed Largemouth Bass

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Abstract.—Catch-and-release practices are common in recreational fisheries, yet little is known about the behavior, physiology, and ultimate fate of released fish. We used a combination of radiotelemetry (external attachment) and nonlethal blood sampling (i.e., the blood concentrations of lactate and glucose and plasma concentrations of aspartate aminotransferase (AST), Na^+, K^+, and Cl^-) to assess the relationship between the prerelease physiological status and postrelease behavior and mortality of largemouth bass *Micropterus salmoides*. The experiments were conducted at two temperatures: approximately 15°C and 21°C. Immediately after capture by standard angling techniques, largemouth bass were exposed to air for 0 to 15 min to assess the consequences of air exposure at two moderate water temperatures. Fish exposed to air for long periods (approximately 10 min or more) had significantly higher concentrations of blood glucose 30 min after air exposure and took significantly longer to regain equilibrium than fish exposed for shorter periods (approximately 3 min or less). The responses of other physiological indicators were inconsistent. Interestingly, at lower water temperatures, males had greater initial concentrations of glucose and AST than females, revealing the importance of sexual differences in the response to angling stress. The fish exposed to air for longer durations tended to exhibit behavioral impairments and remained close to the release site longer than those exposed for short periods. Despite exposure to air for lengthy periods, no postrelease mortality was observed during the 5-d monitoring period. Although the two water temperatures that we used were moderate for this species, a number of sublethal differences (e.g., physiological disturbances and behavioral impairments) were evident in the longer-air-exposure treatment group, highlighting the need to minimize air exposure during catch-and-release angling to maintain the welfare of angled fish.

Catch-and-release angling is an increasingly common leisure activity as well as a form of recreational fisheries management throughout the world (Cooke and Cowx 2004; Arlinghaus et al. 2007a). Anglers may release fish either voluntarily or when mandated by harvest regulations with the assumption that the majority of the individuals will survive with negligible long-term consequences. However, much literature suggests that there can be considerable mortality after catch-and-release angling events even though the fish appear to be in good condition at the time of release (reviewed in Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007b). Recently, a number of studies have been conducted to assess the sublethal physiological consequences of angling-related stress on individual fish, and they have

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revealed that all angling practices elicit a physiological response (reviewed in Cooke and Suski 2005; Arlinghaus et al. 2007a). In particular, protracted air exposure (Ferguson and Tufts 1992; Suski et al. 2004; Schreer et al. 2005), high water temperatures (Wilkie et al. 1996, 1997; Anderson et al. 1998; Thorstad et al. 2003), and the interaction of the two (Gingerich et al. 2007; White et al. 2008) are regarded as the predominant stressors influencing fish survival (Cooke and Suski 2005). There is a need for further research to identify the air-exposure duration and water temperature thresholds for common sport fish so that anglers and fisheries managers can develop angling guidelines that minimize the mortality and sublethal effects from catch-and-release angling (Schreer et al. 2005; Arlinghaus and Hallermann 2007).

Telemetry is increasingly being used as a tool to assess postrelease behavior and survival (Cooke et al. 2002b; Donaldson et al. 2008), but only recently have there been appropriate tools for linking those metrics to individual physiological condition at release. Nonlethal biopsies (e.g., of blood and muscle tissue) can be performed on fish to assess their physiological condition before release, and telemetry devices can be used to determine the subsequent behavior and fate of the same individuals (Cooke et al. 2005; Moyes et al. 2006; Skomal 2007). Such an approach has recently been taken to study the consequences of commercial longline capture on fish physiology and survival (Moyes et al. 2006). When biotelemetry and nonlethal biopsy are coupled, they provide the scientist with an integrated perspective on the physiology, behavior, and fate of fish that are angled and released, linking individual condition to population-level issues (reviewed in Cooke and Schramm 2007). This mechanistic approach to evaluating the impacts of catch and release on the fish will enable managers to better assess the biological impacts (Arlinghaus et al. 2007a) and welfare impairments (Arlinghaus et al. 2007b; Cooke and Sneddon 2007). Indeed, such an approach is well suited to identifying realistic thresholds for air exposure and water temperature in recreational fishing.

In North America, and indeed much of the world, largemouth bass *Micropterus salmoides* is one of the most important game fish species (Duttweiler 1985; Schramm et al. 1991; Kerr and Kamke 2003) and one that is often released after capture (Quinn 1996). Anglers target largemouth bass for various reasons, often participating in competitive angling events in which fish experience a suite of physiological disturbances (Cooke et al. 2002c; Suski et al. 2003, 2004) and in which mortality rates can exceed 50% (Wilde 1998; Cooke et al. 2002c; Siepker et al. 2007). To date, however, no studies have attempted to link the individual physiology of angled largemouth bass with their postrelease behavior and fate. Therefore, we coupled telemetry and nonlethal biopsy for the first time to evaluate the combined effects of air-exposure duration and water temperature on largemouth bass physiology and postrelease behavior and survival and thereby developed a unique model that may be applicable to other sport fish species.

Methods

All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee (protocol B-05-06). All research occurred on Lake Opinicon, Ontario (44°31′N, 76°20′W). Adult largemouth bass were angled during two distinct thermal periods: May 3–10, 2006 ($N = 27$ fish; water temperature, 15.1 ± 0.5°C [mean ± SE]), and June 4–12, 2006 ($N = 31$; 21.3 ± 1.0°C). The water temperature was obtained from temperature probes installed in Lake Opinicon at a depth of 3.3 m. The total length (TL) of the fish used in the first thermal period ranged from 272 to 431 mm (mean ± SE = 339 ± 9 mm) and in the second thermal period ranged from 239 to 462 mm (351 ± 8 mm).

Sampling.—The fish were angled using standard rod-and-reel outfits suitable for the capture of largemouth bass. Because our study involved monitoring postrelease behavior at a common release site (44°33′56″N, 76°19′24″W), all angling was conducted at least 1 km from this release site. All fish were hooked on artificial lures and experimentation was limited to fish in which the lure could be easily removed (as per Cooke et al. 2001). Once hooked, the fish was quickly reeled into the boat (standardized angling time of 20 s) and submerged ventral side-up into an onboard, V-shaped sampling trough filled with fresh lake water (Cooke et al. 2005). No anesthetic was used because this would have altered their blood chemistry and postrelease behavior and would not represent angler handling practice (Cooke et al. 2005). Once in the trough, a set of wet hands held the fish motionless while a blood sample was collected using the nonlethal caudal venipuncture technique (Houston 1990). Approximately 1 mL of blood was withdrawn from the vessels in the caudal hemal arch into a 3-mL Vacutainer (38 mm, 21.5 gauge) within 1 min of being placed in the sampling trough (Cooke et al. 2005). Light pressure was applied to the puncture site after phlebotomy to facilitate clotting.

Initially, blood samples were placed in an ice water slurry until they could be processed (within a maximum of 10 min). Lactate and glucose levels were

measured in the field on whole blood by adding 10 μL of blood to handheld glucose (Accu-check glucose meter, Roche Diagnostics Corporation, Indianapolis, Indiana) and lactate (Lactate Pro LT-1710 portable lactate analyzer, Arkray Inc., Kyoto, Japan) meters. Appropriate standards and calibrations were used with meters before analysis as per the manufacturer guidelines. These field meters have been shown to produce results for fish and other animals that are comparable to laboratory values (e.g., Morgan and Iwama 1997; Wells and Pankhurst 1999; Pyne et al. 2000; Venn Beecham et al. 2006), and even if there are minor deviations in values from laboratory assays, the relative differences among treatments are useful (Morgan and Iwama 1997; Mizock 2002; Venn Beecham et al. 2006). After the concentrations of lactate and glucose were measured, the blood sample was transferred to a centrifuge (Clay Adams Compact II Centrifuge) and immediately spun at 10,000 × gravity for 5 min. The plasma was then separated from the cellular portion of the blood using a pipette, and the plasma was stored in a liquid nitrogen dewar at a minimum of −80°C. The vials remained in liquid nitrogen until laboratory processing, which occurred within 6 months of sample collection. Laboratory analyses were conducted to determine plasma aspartate aminotransferase (AST; enzyme number 2.6.1.1; IUBMB 1992), Na^+, K^+, and Cl^- concentrations via a Roche Hitachi 917 analyzer (Basal, Switzerland) and relevant Roche reagent. Sample sizes varied among tested variables because the strict sampling protocols did not always allow for the required volume of blood to be acquired from every individual fish. As such, analysis was prioritized among acquired samples. Analysis was based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standard reference model.

Air-exposure treatment protocol.—After undergoing phlebotomy, fish were randomly assigned to an air-exposure treatment (ranging from 1 to 900 s) that aimed to incorporate all levels of angler handling skills or placed in the control group (0 s of air exposure). After treatment, each fish was placed in an onboard holding container for a standardized duration of 30 min, allowing for maximum physiological response to occur (Suski et al. 2006). During the 30-min period, qualitative and quantitative observations were made of the fish, including loss of equilibrium, time to regain equilibrium, and opercular rate (obtained 5 min after air exposure). After the 30-min period, a second blood sample was obtained using the method described above. Fish were then measured (TL, mm) and externally sexed, the latter of which was possible because the study took place at the beginning of the breeding season, when vent characteristics were sexually dimorphic.

Behavioral analysis.—While the fish was still in the V-shaped processing trough, a micro-radiotelemetry tag (2-g mass, 20-cm antenna, 9-d life expectancy) was externally attached (Cooke 2003). The radiotelemetry attachment device consisted of a backing plate placed on two 22-gauge hypodermic needles that were mounted on 3-mL syringes (Cooke 2003). The needles were pushed through the dorsal musculature, ventral to the junction of the soft and spiny dorsal fins, and 20-gauge surgical stainless steel attachment wires were threaded through the transmitter and backing plate and inserted into the lumen of the needles (Cooke 2003). From the opposite side of the fish, the wires were pulled out, the needles were removed, a second backing plate was placed against the fish and the excess wires were cut off and carefully secured (Cooke 2003). In addition to a radiotelemetry device, a uniquely colored anchor tag (Floy Manufacturing) was placed into the dorsal musculature, ventral to the spiny dorsal fin, allowing for visual identification of free-swimming fish. After the attachment, the fish were released at a standardized location. Radio receivers (SRX_400, Lotek Wireless, Newmarket, Ontario; and IC-R20, Icom America Inc., Irvine, California) were used to track the fish until they departed the bay in which they were released (approximate distance, 400 m).

Data analysis.—Although the air-exposure data were initially collected in a continuous manner, upon preliminary analysis we determined that categorizing the data was more appropriate to the analysis of the physiological and behavioral impairments pertaining to catch-and-release angling. Thus, the air-exposure data were grouped into three categories: (1) control (0 s exposure), (2) low (179 ± 95 s [mean ± SE); range = 18–332 s), and (3) high (598 ± 194 s: range = 369–900 s).

All physiological data were assessed for normality by means of probability plots. Homogeneity of variance was assessed with Levene's test (no outliers were present), and the normality of the residuals was tested using predicted plots. Nonnormal data (AST, K^+, baseline lactate, and baseline glucose) were $\log_{10}$ transformed. Arcsine-square-root transformations were used for all percentage data derived from the calculation of the percent change in the physiological parameter concentrations after air exposure. A two-way analysis of variance (ANOVA) was used to assess whether there were differences in fish total length between treatment groups (air exposure and temperature as factors). Two-way ANOVAs and Tukey post hoc tests were also used to examine the interactive

TABLE 1.—Comparison of biological and physiological variables between male (M) and female (F) largemouth bass angled at cool (15.1 ± 0.4°C) and warm (21.3 ± 1.1°C) water temperatures, just before air-exposure treatment. Analyses were conducted via two-way analysis of variance (ANOVA) with sex and temperature as the effects. Bolded values indicate significant models; N = number of fish sampled.

Variables	Cool water [mean (SE)]	N	Warm water [mean (SE)]	N	ANOVA output		
					Temperature	Sex	Interaction
Total length (mm)							
F	329.3 (13.4)	14	365.2 (12.2)	13	$F = 1.09$, $P = 0.30$	$F = 0.06$, $P = 0.81$	$F = 2.50$, $P = 0.12$
M	350.2 (12.3)	13	341.2 (11.1)	18			
Lactate (mmol/L)							
F	3.8 (0.6)	14	1.4 (0.2)	13	**$F = 113.81$, $P < 0.001$**	$F = 2.60$, $P = 0.12$	**$F = 7.10$, $P = 0.01$**
M	4.2 (0.5)	13	1.3 (0.2)	18			
Glucose (mmol/L)							
F	2.2 (0.2)	14	2.2 (0.2)	13	**$F = 5.44$, $P = 0.030$**	$F = 1.20$, $P = 0.28$	$F = 2.50$, $P = 0.12$
M	3.0 (0.3)	13	2.2 (0.1)	18			
Aspartate aminotransferase (units/L)							
F	118.0 (32.2)	10	55.2 (11.6)	9	**$F = 27.24$, $P < 0.001$**	$F = 1.34$, $P = 0.25$	$F = 3.83$, $P = 0.058$
M	231.9 (40.1)	9	51.25 (11.5)	12			
Sodium (mmol/L)							
F	164.0 (1.7)	9	158.3 (1.4)	8	$F = 3.30$, $P = 0.08$	$F = 0.48$, $P = 0.50$	$F = 1.27$, $P = 0.27$
M	160.4 (3.2)	9	159.1 (0.8)	10			
Potassium (mmol/L)							
F	4.1 (0.4)	9	3.9 (0.3)	8	$F = 2.43$, $P = 0.13$	$F = 0.57$, $P = 0.46$	$F = 1.05$, $P = 0.31$
M	4.5 (0.2)	9	3.8 (0.2)	10			
Chloride (mmol/L)							
F	111.4 (3.3)	9	103.8 (7.8)	8	$F = 0.28$, $P = 0.60$	$F = 0.03$, $P = 0.86$	$F = 1.25$, $P = 0.25$
M	105.3 (3.1)	9	108.2 (3.5)	10			

effects of sex and water temperature on the baseline physiological status, the percent change in physiological status after air exposure at the two water temperatures, and the differences in behavioral responses (e.g., time to regain equilibrium, opercular rate, and time to leave the release site) to air exposure at two water temperatures. Equilibrium loss after air exposure at the two water temperature groups was assessed using a nominal logistic fit with the effect evaluated using a Wald test. All statistical assessments were conducted using JMP IN 4.0.4 (SAS Institute, Cary, North Carolina) and the significance was set at $\alpha = 0.05$ (Zar 1984).

Results

The total length of fish did not vary among air-exposure groups (two-way ANOVA: $F = 0.11$, df = 2, $P = 0.895$) or water temperature groups ($F = 1.2$, df = 1, $P = 0.284$) (Table 1). The water temperature × sex interaction was significant for baseline lactate concentration, lactate concentrations being significantly higher for the 15.1°C water temperature group, particularly in males (Table 1). Fish angled at 15.1°C had significantly higher glucose concentrations than fish angled at 21.3°C, regardless of their sex. The baseline concentration of AST for male largemouth bass was elevated significantly during the cool water treatment period. Ion concentrations did not significantly differ among sexes or treatment groups.

All control fish maintained equilibrium throughout the entire experiment, whereas all fish exposed to long durations of air exposure lost equilibrium for a minimum of 2 s and a maximum of 1,800 s (Table 2). Fish angled at 15.1°C and subjected to the low-duration air exposure took significantly less time (maximum = 240 s) to regain equilibrium than fish angled at 21.3°C and subjected to the same air exposure (maximum = 1,200 s). Three fish in the lengthy-air-exposure group had not regained equilibrium after the 30 min monitoring period.

At 15.1°C the opercular rate decreased with increasing air exposure, whereas at 21.3°C the opercular rate was intermediate for controls, highest in the low-air-exposure group and lowest in the high-air-exposure group (Table 3). In all instances, air exposure had a significant affect on the opercular rate of the largemouth bass (Table 3).

The physiological response to air exposure was inconsistent across water temperature and air-exposure groups. Regardless of the air-exposure duration, the mean change in lactate and AST concentrations were significantly lower at 15.1°C than at 21.3°C (Table 3). Conversely, fish in different water temperature groups did not have significantly different mean glucose concentrations; however, fish in different air-exposure groups did (Table 3). Generally, the mean change in glucose concentrations was highest in the low-air-exposure group, the greatest change in glucose

TABLE 2.—Relationship between time to regain equilibrium (s) and various physiological and behavioral indicators of largemouth bass angled at cool and warm water temperatures (see Table 1). Analyses were conducted using linear regression. Bolded values indicate significant models.

Indicator	Temperature (°C)	R^2	N	P-value
Change in lactate concentration (%)	Cool	0.04	27	0.294
	Warm	0.00	30	0.841
Change in glucose concentration (%)	Cool	0.00	27	0.958
	Warm	0.133	30	0.048
Change in aspartate aminotransferase concentration (%)	Cool	0.00	18	0.781
	Warm	0.09	20	0.207
Change in sodium concentration (%)	Cool	0.01	16	0.656
	Warm	0.00	12	0.926
Change in potassium concentration (%)	Cool	0.02	15	0.601
	Warm	0.00	12	0.854
Change in chloride concentration (%)	Cool	**0.29**	**15**	**0.041**
	Warm	0.03	12	0.567
Opercular rate (beats/min)	Cool	0.00	14	0.984
	Warm	**0.324**	**15**	**0.027**
Time to leave (h)	Cool	0.00	19	0.903
	Warm	0.08	22	0.217

concentration occurring in the low-air-exposure group at 21.3°C (Table 3). Ion status varied inconsistently with air exposure and water temperature. However, in general the change in sodium ion concentrations increased with increasing air exposure and water temperature, whereas the K^+ concentrations decreased in both water temperature groups at high air exposures (Table 3). The change in Cl^- concentration after air exposure was significantly lower at 15.1°C than at 21.3°C (Table 3).

The time that it took for the fish to leave the release site ranged from 1 to 73 h. Although the result was not statistically significant, fish exposed to long periods of air exposure at 21.3°C took longer to leave the release site than fish exposed to short periods of air exposure at 15.1°C (Table 3). Despite prolonged air exposures, no immediate or delayed postrelease (within 5 d) mortality was observed.

Discussion

The mortality of largemouth bass associated with catch-and-release angling is generally thought to be low, except in unusual situations where injury is substantial, environmental conditions are extreme (e.g., high water temperature), or handling is excessive (Muoneke and Childress 1994; Cooke and Suski 2005; Arlinghaus et al. 2007a). The interactive effects of independent stressors such as elevated water temperatures (Wilkie et al. 1997; Thorstad et al. 2003) and prolonged air exposure (Ferguson and Tufts 1992; Cooke et al. 2001; Arlinghaus and Hallermann 2007) can have physiological consequences on largemouth bass in catch-and-release settings and may lead to higher mortality. Indeed, consistent with this idea, the current study revealed that the interactive effects of angling at elevated water temperatures and high air-exposure duration resulted in pronounced alterations in postcapture physiology and some alterations in post-release behavior.

Blood samples taken as soon as a fish is landed provide insight into its baseline physiological condition because the physiological variables measured in this study have a delayed response time of several minutes (Suski et al. 2006). At about 15°C, fish had significantly greater baseline concentrations of blood lactate, glucose, and plasma AST concentrations than fish at about 21°C. Largemouth bass begin to spawn in Lake Opinicon as soon at the water temperatures reach about 14°C (Cooke, personal observations). As such, the first sampling period coincided with the largemouth bass spawning season. Although nesting fish were not directly targeted in this study, nesting males are extremely vulnerable to capture because they provide parental care for their brood by defending their nests against brood predators and fan their eggs to provide oxygen and keep them free of silt (Cooke et al. 2002a). Egg-fanning and brood-guarding cause nesting male bass to have high activity levels and frequently use burst swimming to chase potential predators (Cooke et al. 2002a). Although never studied explicitly for nesting largemouth bass, this anaerobic activity could lead to an increase in lactate accumulation, as has been observed in numerous fish species after burst exercise (Cooke and Suski 2005). Interestingly, female fish also had elevated baseline lactate concentrations at about 15°C. It is possible that the observed elevated lactate concentrations in both male and female bass is attributed to the spawning season that corresponded with the first sampling period for this study. In addition to nest guarding activities, males do not actively feed

TABLE 3.—Comparison of biological and physiological variables among largemouth bass angled at cool and warm water temperatures (see Table 1) and exposed to air for 0 (control), 179 ± 95 (low), or 598 ± 195 s (high). In this analysis, the change in physiological variables refers to the change between the two blood sampling periods (immediately after capture and 20 min after air-exposure treatment). Analyses were conducted via two-way analysis of variance (ANOVA), with air exposure and temperature as the effects. Bolded values indicate significant models; N = number of fish sampled.

Variables	Air exposure	Cool water [mean (SE)]	N	Warm water [mean (SE)]	N	ANOVA output		
						Temperature	Air exposure	Interaction
Change in lactate concentration (%)	Control	315.4 (83.2)	9	635.0 (96.8)	13	**$F = 58.78$, $P = <0.01$**	$F = 1.31$, $P = 0.29$	$F = 2.02$, $P = 0.16$
	Low	197.5 (78.9)	9	843.7 (99.2)	9			
	High	150.6 (35.2)	9	911.5 (162.37)	8			
Change in glucose concentration (%)	Control	185.6 (28.4)	9	229.8 (31.9)	13	$F = 1.24$, $P = 0.28$	**$F = 4.62$, $P = 0.02$**	$F = 0.20$, $P = 0.82$
	Low	277.9 (55.1)	9	301.8 (39.9)	9			
	High	208.2 (26.8)	9	274.8 (38.6)	8			
Change in aspartate aminotransferase concentration (%)	Control	−2.27 (55.9)	3	307.0 (126.9)	8	**$F = 4.86$, $P = 0.04$**	$F = 0.40$, $P = 0.68$	$F = 0.43$, $P = 0.66$
	Low	64.2 (45.0)	7	288.2 (172.6)	6			
	High	20.1 (57.8)	7	321.2 (290.0)	6			
Change in sodium concentration (%)	Control	3.2 (2.1)	3	5.7 (2.0)	4	$F = 4.05$, $P = 0.06$	$F = 0.27$, $P = 0.77$	$F = 0.09$, $P = 0.91$
	Low	3.5 (1.6)	6	8.1 (0.3)	3			
	High	4.8 (2.1)	6	7.4 (1.2)	5			
Change in potassium concentration (%)	Control	−7.2 (28.6)	3	17.1 (23.6)	4	$F = 0.21$, $P = 0.66$	$F = 0.19$, $P = 0.83$	$F = 0.25$, $P = 0.78$
	Low	14.7 (15.3)	6	4.2 (5.4)	3			
	High	−5.8 (11.1)	6	−9.4 (9.4)	5			
Change in chloride concentration (%)	Control	−1.7 (1.4)	3	3.0 (0.9)	4	**$F = 4.8$, $P = 0.04$**	$F = 0.11$, $P = 0.90$	$F = 1.26$, $P = 0.31$
	Low	0.39 (1.5)	6	4.3 (3.4)	2			
	High	0.66 (1.9)	6	1.8 (2.3)	5			
Time to regain equilibrium (s)	Control	0.1 (0)	9	0.1 (0.2)	13	$F = 0.01$, $P = 0.92$	**$F = 7.66$, $P < 0.01$**	$F = 0.049$, $P = 0.95$
	Low	60.2 (29.1)	9	160.9 (130.5)	9			
	High	780.3 (224.4)	9	554.7 (199.0)	9			
Opercular rate (beats/min)	Control	50 (2.9)	3	54 (2.6)	4	**$F = 5.58$, $P = 0.027$**	$F = 2.86$, $P = 0.08$	$F = 0.59$, $P = 0.56$
	Low	49 (4.9)	5	63 (2.3)	4			
	High	42 (2.3)	6	50 (5.3)	7			
Time to leave (h)	Control	39 (11.5)	3	19 (6.9)	5	$F = 1.42$, $P = 0.26$	$F = 0.52$, $P = 0.48$	$F = 0.40$, $P = 0.67$
	Low	39.9 (8.7)	7	22.1 (7.5)	9			
	High	36.3 (9.3)	9	46.5 (9.5)	8			

during the parental care period and, thus, the elevated glucose concentrations are probably attributed to a mobilization of endogenous energy reserves. This study found that male fish had significantly greater baseline AST concentrations than females did. In a study on the physiological consequences of decompression, Morrissey et al. (2005) found AST to be the most useful indicator of the extent of permanent tissue damage because it is highly correlated with other tissue damage indicators, including plasma lactate dehydrogenase (enzyme number 1.1.1.27 IUBMB 1992) and creatine phosphokinase (2.7.3.2). Because AST is an intracellular enzyme that is predominately located in the heart and liver, its appearance in plasma is a common and reliable indicator of permanent tissue damage in vertebrates (Morrissey et al. 2005). Although it is difficult to attribute the permanent tissue damage to one casual factor, the findings of our study suggest that the elevated AST concentrations could be linked with male spawning and nest-guarding activities or the emergence from the difficult winter period. Although much of this discussion is speculative, the key finding is that there were seasonal and intersexual differences in baseline physiology that must be considered when conducting research on catch-and-release angling because this can confound field research.

Although no mortality was observed in this study, sublethal physiological impairments were found after air exposure at both water temperatures. Suski et al. (2006) identified that elevation in water temperature caused biochemical and respiratory responses to catch-

and-release stressors that function suboptimally in largemouth bass. Consequently, anaerobic by-products may accumulate within the individual because tissues and metabolic processes are required to rely on anaerobic metabolism for energy production (Hochachka 1991). In our study, the increase in the percent change of blood lactate concentrations between temperature groups suggests that air exposure and elevated water temperature combined with the exercise associated with the angling event resulted in increased anaerobic metabolism. Air exposure has been found to cause a rapid and significant increase in lactate and glucose concentrations (Arends et al. 1999). Furthermore, gas exchange is severely inhibited in air-exposed fish because of the collapse of the gill lamellae and a reduction of functional gill surface area, leading to the increased percent change in lactate concentrations we observed (Ferguson and Tufts 1992; Arends et al. 1999; Suski et al. 2004; Killen et al. 2006). Some studies have found that physiological stress responses (e.g., lactate, glucose) are more pronounced at warmer water temperatures (Gustaveson et al. 1991; Wilkie et al. 1997). This, however, was not clearly evident in our study, but this may reflect the fact that the warmest temperature was close to (but below) the optimal temperature for the species.

Changes in plasma electrolytes have been identified as one of the most serious physiological consequences for fish from stress associated with catch-and-release angling (Suski et al. 2004). When fish are stressed they generally experience a reduction in chloride cell activity, an increase in respiration, and changes in the resorption of electrolytes by the kidney tubules caused by cortical production (Wydoski et al. 1976). The only significant change that occurred was that the plasma chloride ion concentration was significantly lower at 15°C than at 21°C. The decrease in plasma chloride concentrations could be an artifact of the chronic stress of overwintering and spawning, as previously discussed.

Equilibrium impairment assessments can be valuable in the evaluation of sublethal effects because the maintenance of equilibrium relies on a suite of coordinated activities by multiple organ systems (Cooke and Philipp 2004; Danylchuk et al. 2007; Gingerich et al. 2007). We found that the duration of air exposure was significantly related to the time it took for a fish to regain equilibrium. This pattern has also recently been documented for smallmouth bass *M. dolomieu* and largemouth bass in a laboratory setting where fish were exposed to catch-and-release simulations (White et al. 2008). Our study highlights the importance for anglers and managers to reduce the amount of air exposure a fish receives during a catch-and-release event.

Opercular beat rate is commonly used in physiological and behavioral studies to assess the extent of sublethal disturbance (e.g., Gingerich et al. 2007; White et al. 2008). In our study, opercular rates were significantly lower at 15°C than at 21°C, as would be expected given the temperature dependence of metabolism and respiration (Fry 1971). Opercular rates did not differ significantly between air-exposure-duration groups. The amplitude of the opercular beats was not measured, so it is possible that fish were able to modulate the volume of water movement by altering both frequency and amplitude. Indeed, Barreto and Volpato (2004) found that opercular rate is a good behavioral metric because of its sensitivity to stress. However, their research showed that the opercular rate was not proportional to the time of imposition of the stressor, and thus, the results obtained in our study should be interpreted cautiously.

We found that the time it took fish to vacate the release site varied with air exposure and water temperature. Specifically, the group that took the longest time to leave the release site was the group exposed to long air-exposure periods at the highest water temperature. However, none of the differences were significantly different because of substantial individual variation. Delayed departure from the release site could be associated physiological disturbance, such as an accumulation of anaerobic metabolites (i.e., lactate) and general problems with the maintenance of homeostasis (e.g., ionic status). Nonetheless, this expected pattern (i.e., link between magnitude of physiological disturbance and postrelease behavior) was not clearly evident in our study. Although sublethal physiological impairment after air exposure should theoretically lead to increasing the probability of individual fish death postrelease, there are still few empirical examples. In fact, there appears to be a general incongruence between the physiological disturbance and both behavioral impairments and mortality, as previously documented in the context of commercial bycatch (Davis et al. 2001; Moyes et al. 2006). This finding may extend to recreational fisheries.

Conclusions

This study demonstrate that the combined effects of air exposure and water temperature can result in changes in the physiology and behavior of largemouth bass in a catch-and-release context that are indicative of welfare impairments. Although we observed no mortality, it is possible that replicating the study at higher water temperatures would induce mortality,

particularly for fish exposed to air for longer durations. The two water temperatures that we used were reasonably moderate and were well below the optimal temperature (e.g., the temperature at which largemouth bass experience optimal performance) and far below temperatures that would exceed the thermal tolerances of largemouth bass. The fact that some of the physiological indicators in our study yielded results that were inconsistent with predictions may reflect the considerable interindividual and intersexual variation in baseline physiology. This, however, does not mean that exposing largemouth bass to air for periods of up to 15 min is consistent with good fisheries practice in a catch-and-release context. Even short durations of air exposure may lead to sublethal physiological disturbances (summarized in Cooke and Suski 2005), compromising the well being and welfare of the fish (Arlinghaus et al. 2007b; Cooke and Sneddon 2007). We urge all anglers to adopt a risk-averse strategy and eliminate or minimize air exposure by using barbless hooks, unhooking fish in water, having pliers or unhooking devices accessible, etc. (Cooke et al. 2001; Arlinghaus and Hallermann 2007). At higher water temperatures, even short durations of air exposure could lead to mortality in black bass and other species. However, there is still a paucity of catch-and-release research on the interactive effects of different stressors across a broad range of water temperatures.

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References

Anderson, W. G., R. Booth, T. A. Beddow, R. S. McKinley, B. Finstad, F. Okland, and D. Scruton. 1998. Remote monitoring of heart rate as a measure of recovery in angled Atlantic salmon, *Salmo salar* (L.). Hydrobiologia 371/372:233–240.

Arends, R. J., J. M. Mancera, J. L. Munoz, S. E. Wendelaar Bonga, and G. Flik. 1999. The stress response of gilthead seabream (*Sparus aurata L.*) to air exposure and confinement. Journal of Endocrinology 163:149–157.

Arlinghaus, R., S. J. Cooke, J. Lyman, D. Policansky, A. Schwab, C. D. Suski, S. G. Sutton, and E. B. Thorstad. 2007a. Understanding the complexity of catch and release in recreational fishing: an integrative synthesis of global knowledge from historical, philosophical, social, and biological perspectives. Reviews in Fisheries Science 15:75–167.

Arlinghaus, R., S. J. Cooke, A. Schwab, and I. G. Cowx. 2007b. Fish welfare: a challenge to the feelings-based approach, with implications for recreational fishing. Fish and Fisheries 8:57–71.

Arlinghaus, R., and J. Hallermann. 2007. Effects of air exposure on mortality and growth of undersized pikeperch, *Sander lucioperca*, at low water temperatures, with implications for catch-and-release fishing. Fisheries Management and Ecology 14:155–160.

Barreto, R. E., and G. L. Volpato. 2004. Caution for using ventilatory frequency as an indicator of stress in fish. Behavioural Processes 66:43–51.

Bartholomew, A., and J. A. Bohnsack. 2005. A review of catch-and-release angling mortality with implications for no-take reserves. Reviews in Fish Biology and Fisheries 15:129–154.

Cooke, S. J. 2003. External attachment radio transmitters do not affect the parental care behaviour of rock bass. Journal of Fish Biology 62:965–970.

Cooke, S. J., and I. G. Cowx. 2004. The role of recreational fishing in global fish crises. BioScience 54:857–859.

Cooke, S. J., G. T. Crossin, D. A. Patterson, K. K. English, S. G. Hinch, J. L. Young, R. F. Alexander, M. C. Healey, G. Van Der Kraak, and A. P. Farrell. 2005. Coupling noninvasive physiological assessment with telemetry to understand interindividual variation in behaviour and survivorship of sockeye salmon: development and validation of a technique. Journal of Fish Biology 67:1342–1358.

Cooke, S. J., K. M. Dunmall, J. F. Schreer, and D. P. Philipp. 2001. The influence of terminal tackle on physical injury, handling time, and cardiac disturbance of rock bass. North American Journal of Fisheries Management 21:265–274.

Cooke, S. J., and D. P. Philipp. 2004. Behavior and mortality of caught-and-released bonefish (*Albula* spp.) in Bahamian waters, with implications for a sustainable recreational fishery. Biological Conservation 118:599–607.

Cooke, S. J., D. P. Philipp, and P. J. Weatherhead. 2002a. Parental care patterns and energetics of smallmouth bass, *Micropterus dolomieu*, and largemouth bass, *M. salmoides*, monitored with activity transmitters. Canadian Journal of Zoology 80:756–770.

Cooke, S. J., and H. L. Schramm. 2007. Catch-and-release science and its application to conservation and management of recreational fisheries. Fisheries Management and Ecology 14:73–79.

Cooke, S. J., J. F. Schreer, K. M. Dunmall, and D. P. Philipp. 2002b. Strategies for quantifying sublethal effects of

marine catch-and-release angling: insights from novel freshwater applications. Pages 121–134 *in* J. A. Lucy and A. L. Studholme, editors. Catch and release in marine recreational fisheries. American Fisheries Society, Symposium 30, Bethesda, Maryland.

Cooke, S. J., J. F. Schreer, D. H. Wahl, and D. P. Philipp. 2002c. Physiological impacts of catch-and-release angling practices on largemouth bass and smallmouth bass. Pages 489–512 *in* D. P. Philipp and M. S. Ridgway, editors. Black bass: ecology, conservation, and management. American Fisheries Society, Symposium 31, Bethesda, Maryland.

Cooke, S. J., and L. U. Sneddon. 2007. Animal welfare perspectives on catch-and-release recreational angling. Applied Animal Behaviour Science 104:176–198.

Cooke, S. J., and C. D. Suski. 2005. Do we need species-specific guidelines for catch-and-release recreational angling to conserve diverse fishery resources? Biodiversity and Conservation 14:1195–1209.

Danylchuk, A. J., S. E. Danylchuk, S. J. Cooke, T. L. Goldberg, J. B. Koppelman, and D. P. Philipp. 2007. Postrelease mortality of bonefish, *Albula vulpes*, exposed to different handling practices during catch-and-release angling in Eleuthera, The Bahamas. Fisheries Management and Ecology 14:149–154.

Davis, M. W., B. L. Olla, and C. B. Schreck. 2001. Stress induced by hooking, net towing, elevated sea water temperature, and air in sablefish: lack of concordance between mortality and physiological measures of stress. Journal of Fish Biology 58:1–15.

Donaldson, M. R., R. Arlinghaus, K. C. Hanson, and S. J. Cooke. 2008. Enhancing catch-and-release science with biotelemetry. Fish and Fisheries 9:79–105.

Duttweiler, M. W. 1985. Status of competitive fishing in the United States: trends and state fisheries policies. Fisheries 10(5):5–7.

Ferguson, R. A., and B. L. Tufts. 1992. Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*): implications for "catch and release" fisheries. Canadian Journal of Fisheries and Aquatic Sciences 49:1157–1162.

Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. Pages 1–98 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology, volume VI. Academic Press, New York.

Gingerich, A. J., S. J. Cooke, K. C. Hanson, M. R. Donaldson, C. T. Hasler, C. D. Suski, and R. Arlinghaus. 2007. Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. Fisheries Research 86:169–178.

Gustaveson, A. W., R. S. Whydoski, and G. A. Wedemeyer. 1991. Physiological response of largemouth bass to angling stress. Transactions of the American Fisheries Society 120:629–636.

Hochachka, P. W. 1991. Design of energy metabolism. Pages 325–251 *in* C. L. Prosser, editor. Environmental and metabolic animal physiology. Wiley, New York.

Houston, A. H. 1990. Blood and circulation. Pages 273–334 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

IUBMB (International Union of Biochemistry and Molecular Biology). 1992. Enzyme nomenclature 1992. Academic Press, San Diego, California.

Kerr, S. J., and K. K. Kamke. 2003. Competitive fishing on freshwaters of North America: a survey of Canadian and U.S. jurisdictions. Fisheries 28(3):26–31.

Killen, S. S., C. D. Suski, S. J. Cooke, D. P. Philipp, and B. L. Tufts. 2006. Factors contributing to the physiological disturbance in walleyes during simulated live-release angling tournaments. Transactions of the American Fisheries Society 135:557–569.

Mizock, B. A. 2002. Point-of-care testing of blood lactate. Laboratoriums Medizin 26:77–81.

Morgan, J. D., and G. K. Iwama. 1997. Measurements of stressed states in the field. Pages 247–268 *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.

Morrissey, M. B., C. D. Suski, K. R. Esseltine, and B. L. Tufts. 2005. Incidence and physiological consequences of decompression in smallmouth bass after live-release angling tournaments. Transactions of the American Fisheries Society 134:1038–1047.

Moyes, C. D., N. Fragoso, M. K. Musyl, and R. W. Brill. 2006. Predicting postrelease survival in large pelagic fish. Transactions of the American Fisheries Society 135:1389–1397.

Muoneke, M. I., and W. M. Childress. 1994. Hooking mortality: a review for recreational fisheries. Reviews in Fisheries Science 2:123–156.

Pyne, D. B., T. Boston, D. T. Martin, and A. Logan. 2000. Evaluation of the Lactate Pro blood lactate analyser. European Journal of Applied Physiology 82:112–116.

Quinn, S. 1996. Trends in regulatory and voluntary catch-and-release fishing. Pages 152–162 *in* L. E. Miranda and D. R. DeVries, editors. Multidimensional approaches to reservoir fisheries management. American Fisheries Society, Bethesda, Maryland.

Schramm, H. L., Jr., M. L. Armstrong, N. A. Funicelli, D. M. Green, D. P. Lee, R. E. Manns, Jr., B. D. Taubert, and S. J. Walters. 1991. The status of competitive sport fishing in North America. Fisheries 16(3):4–12.

Schreer, J. F., D. M. Resch, M. L. Gately, and S. J. Cooke. 2005. Swimming performance of brook trout after simulated catch-and-release angling: looking for air exposure thresholds. North American Journal of Fisheries Management 25:1513–1517.

Siepker, M. J., K. G. Ostrand, S. J. Cooke, D. H. Wahl, and D. P. Philipp. 2007. A review of the effects of catch-and-release angling on black bass, *Micropterus* spp.: implications for conservation and management of populations. Fisheries Management and Ecology 14:91–101.

Skomal, G. B. 2007. Evaluating the physiological and physical consequences of capture on postrelease survivorship in large pelagic fishes. Fisheries Management and Ecology 14:81–89.

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.

Suski, C. D., S. S. Killen, J. D. Kieffer, and B. L. Tufts. 2006. The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. Journal of Fish Biology 68:120–136.

Suski, C. D., S. Killen, M. B. Morrissey, S. G. Lund, and B. L. Tufts. 2003. Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. North American Journal of Fisheries Management 23:760–769.

Thorstad, E. B., T. F. Næsje, P. Fiske, and B. Finstad. 2003. Effects of hook and release on Atlantic salmon in the River Alta, northern Norway. Fisheries Research 60: 293–307.

Venn Beecham, R., B. C. Small, and C. D. Minchew. 2006. Using portable lactate and glucose meters for catfish research: acceptable alternatives to established laboratory methods? North American Journal of Aquaculture 68:291–295.

Wells, R. M. G., and N. W. Pankhurst. 1999. Evaluation of simple instruments for the measurement of blood glucose, lactate, and plasma protein as stress indicators in fish. Journal of the World Aquaculture Society 30:276–284.

White, A. J., J. F. Schreer, and S. J. Cooke. 2008. Behavioral and physiological responses of the congeneric largemouth (*Micropterus salmoides*) and smallmouth bass (*M. dolomieu*) to various exercise and air exposure durations. Fisheries Research 89:9–16.

Wilde, G. R. 1998. Tournament-associated mortality in black bass. Fisheries 23(10):12–22.

Wilkie, M. P., M. A. Brobbel, K. Davidson, L. Forsyth, and B. L. Tufts. 1997. Influences of temperature upon the postexercise physiology of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 54:503–511.

Wilkie, M. P., K. Davidson, M. A. Brobbel, J. D. Kieffer, R. J. Booth, A. T. Bielak, and B. L. Tufts. 1996. The physiology and survival of wild Atlantic salmon (*Salmo salar*) following angling in warm summer waters. Transactions of the American Fisheries Society 125:572–580.

Wydoski, R. S., G. A. Wedemeyer, and N. C. Nelson. 1976. Physiological responses to hooking stress in hatchery and wild rainbow trout (*Salmo gairdneri*). Transactions of the American Fisheries Society 105:601–606.

Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey.