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[Article]

Factors Contributing to the Physiological Disturbance in Walleyes during Simulated Live-Release Angling Tournaments

SHAUN S. KILLEN

Ocean Sciences Centre and Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador A1C 5S7, Canada

CORY D. SUSKI

Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

STEVEN J. COOKE

Institute of Environmental Science and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

DAVID P. PHILIPP

Center for Aquatic Ecology, Illinois Natural History Survey, and Department of Natural Resources and Environmental Sciences, University of Illinois, 607 East Peabody Drive, Champaign, Illinois 61820, USA

BRUCE L. TUFTS*

Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Abstract.—The goal of the current study was to examine the physiological disturbances in walleyes *Sander vitreus* that occurred during the different phases of a live-release angling tournament. To achieve this, we took blood and white muscle samples from walleyes during experiments that simulated different aspects of a live-release tournament (angling, live-well confinement, and weigh-in procedure). In accordance with recent findings for largemouth bass *Micropterus salmoides*, the physiological disturbance experienced by tournament walleyes results mainly from two distinct bouts of anaerobic metabolism occurring during angling and the weigh-in procedure. These periods are characterized by large increases in white muscle and plasma lactate concentrations, reductions in white muscle ATP, phosphocreatine, and glycogen and a significant cardiac disturbance. The physiological effects of angling are already well understood, but the physiological disturbance that occurs during the weigh-in procedure of tournaments has received little attention. In view of the results from our tournament simulations, a final set of experiments was conducted to examine more closely the bout of anaerobic metabolism experienced by walleyes during the tournament weigh-in. The results of these experiments showed that the magnitude of the physiological disturbance in tournament-caught walleyes is directly related to the duration of air exposure occurring during the weigh-in. Based on these findings, we suggest that to reduce the physiological disturbance experienced by tournament-caught walleyes, tournament organizers should strive to minimize air exposure during the weigh-in procedure.

In recent years, the popularity of competitive angling events has increased dramatically (Schramm et al. 1991; Kerr 1999; Kerr and Kamke 2003). In an effort to conserve the fisheries resource, the vast majority of these events have adopted a live-release policy. In live-release tournaments, competitors strive to keep captured fish alive until they can be weighed at the end of the tournament day. Following weigh-in, surviving fish are returned as quickly as possible to the water body from which they were angled.

During a live-release angling tournament, many factors contribute to physiological disturbances in fish. For example, angling may involve severe exercise, as well as air exposure during hook removal (Payer et al. 1989; Gustaveson et al. 1991; Ferguson and Tufts 1992; Kieffer et al. 1995). To keep fish alive during the angling day, anglers typically retain fish in on-board live wells that are designed to allow fish to recover from angling in fresh lake water. However, fish may be exposed to several stressors while being held in live wells. These include hypoxia (Hartley and Moring 1993), temperature change (Plumb et al. 1988), crowding (Cooke et al. 2002), and accumulation of metabolic wastes (Kwak and Henry 1995). Fish may also experience an additional physiological disturbance during the weigh-in process (Suski et al. 2004). At the

* Corresponding author: tuftsb@biology.queensu.ca

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weigh-in, fish are normally confined in water-filled plastic bags while being transferred from the live-well to the weigh-in staging area, and are then air-exposed while being weighed on a scale.

Several authors have suggested procedural guidelines for reducing stress among fish during tournaments (Schramm and Heidinger 1988; Goeman 1991; Wilde 1998; Gilliland 2002; Suski et al. 2004). Only recently, however, have efforts been made to quantify the physiological disturbance present in tournament fish. Suski et al. (2003) found that largemouth bass *Micropterus salmoides* sampled at the end of tournaments show a large metabolic disturbance characterized by a profound depletion of muscle energy stores and accumulation of lactate in plasma and white muscle. More recent studies have revealed that most largemouth bass probably recover from angling if provided with adequate live-well conditions, but that typical tournament weigh-in procedures are responsible for most of the metabolic disturbance present in tournament-caught largemouth bass (Suski et al. 2004). Specifically, those authors noted that the period of air exposure during the weigh-in is an important contributor to the physiological disturbance observed in tournament-caught largemouth bass.

Currently, little is known about the physiological condition of other species during live-release angling tournaments, although reports on tournament mortality (Goeman 1991; Fielder and Johnson 1994; Hoffman et al. 1996) suggest that walleyes *Sander vitreus* may be more sensitive to typical tournament procedures than largemouth bass. Killen et al. (2003) found that tournament walleyes show a profound metabolic disturbance similar to that of tournament largemouth bass, but also show some evidence of plasma ion loss. While this information is useful, the relative contribution of the different components of a live-release angling tournament towards the physiological disturbance in walleyes remains unknown.

Based on this background, the objective of this study was to quantify the physiological disturbance in walleyes that occurred during the different phases of a live-release angling tournament. First, we sampled walleyes during experiments that simulated different aspects of a live-release tournament (angling, live-well confinement, weigh-in procedure). Based on the results of Suski et al. (2004), we hypothesized that walleyes would recover from angling stress during live-well confinement, but would be sensitive to stressors encountered during the tournament weigh-in. Second, we performed a series of experiments that were specifically designed to vary the duration of air exposure received by the walleyes during a simulated weigh-in. For these experiments, we hypothesized that the length of time spent exposed to air would be directly related to the magnitude of the physiological disturbance in tournament-caught walleyes. This study also represents an effort to expand our understanding of the physiological responses of different fishes to a variety of stressors.

Methods

The tournament simulation study consisted of two complimentary series of experiments: blood and muscle sampling and cardiac measurements. Both series of simulation experiments involved treatment groups that were designed to isolate the physiological effects of the three key stages of a typical tournament day: angling, live-well confinement, and the weigh-in.

Tournament Simulation for Blood and White Muscle Measurements

Adult walleyes used for blood and muscle analyses were obtained by angling from various lakes in southern Ontario. After capture, walleyes (mean total length = 482.3 ± 11.4 mm) were transported to the aquatic facility at Queen's University in aerated holding tanks. Once at the laboratory walleyes were held in large plastic holding tanks containing water from the City of Kingston's municipal supply. Water flowing continuously through the tanks was aerated. dechlorinated, and maintained at 11°C. Walleyes were allowed to acclimate for at least 5 d before experimentation and showed no visible signs of stress during the holding period. Previous work has shown that the physiological parameters quantified in this study typically take from 12 to 24 h to recover following disturbances such as angling or transport (Wood 1991; Milligan 1996; Suski et al., 2006). Treatment descriptions for the tournament simulation in which walleyes were sampled for blood and white muscle are described below.

Angling simulation.—To simulate the exercise associated with hook-and-line angling, individual walleyes ($n = 6$) were carefully netted from a common holding tank and manually chased in a circular tank for 1 min. Previous work has shown this to be an accepted method for exhausting fish and replicating the physiological disturbances associated with angling (Wood 1991; Suski et al. 2004). Following exercise, the fish were immediately anesthetized using 250 mg/L of tricaine methanesulfonate (MS-222) buffered with 500 mg/L $NaCO_3$. We then obtained blood samples by caudal vessel puncture using an 18-gauge needle and a heparinized syringe. Blood was drawn from the vessel, transferred to a 1.5-mL microcentrifuge tube, and immediately centrifuged for 1 min. We then drew the plasma (supernatant) from the corpuscular fraction

of the blood using a pipette. Both plasma and erythrocytes were stored in the field in liquid nitrogen and transferred to a −80°C freezer in the laboratory. For white muscle sampling, about 5–10 g of white muscle were removed from the epaxial musculature behind the operculum and above the lateral line, immediately freeze-clamped, and placed in liquid nitrogen. This method of tissue preparation results in preservation of energy metabolites and prevents the accumulation of waste products from sampling-induced activity (Wang et al. 1994). Once in the laboratory, white muscle samples were stored at −80°C until processing. The time required for the collection of blood and tissue after the fish had ceased to ventilate following anesthesia was normally <30 s.

Live-well simulation.—Walleyes were first subjected to the exercise regime described for the angling simulation above, then transferred (three fish at a time) to a live well (81.5 × 26.5 × 22.5 cm) within a recreational fishing boat ($n = 6$ for each treatment group). To simulate the experience of walleyes caught early in a tournament day, these fish remained in the live well for 6 h as the boat was driven on Lake Ontario at various speeds. Approximately 5 min out of every half-hour was spent driving at speeds fast enough to cause large degrees of bow movement and live-well disturbance. The live well used a spray method of aeration and was turned on and off for alternating 10-min periods. Walleyes were anesthetized and sampled for blood and white muscle after the 6 h live-well confinement period.

Weigh-in simulation.—Walleyes ($n = 6$) were subjected to the angling and live-well treatments as previously described but were then removed from the live well and held for 5 min in a clear plastic bag containing 15 L of lake water (one fish per bag). These fish were not intentionally exercised beyond that of the angling treatment described above. The plastic bag was then emptied into a laundry basket that allowed the water to drain. The fish remained in the basket, where they were air-exposed for 1 min, and were then anesthetized and sampled.

All of the above treatment groups were compared with control walleye ($n = 7$). For the control treatment, individual walleye held in the laboratory were placed in aerated and darkened Perspex boxes receiving a continuous flow of water for 48 h before sampling. Immediately before sampling, water flow into the boxes was stopped, and anesthetic was added. Following full anesthesia, fish were sampled for blood and white muscle as described above.

At this point, it is important to note that there was a significant temperature difference between the water in which the walleyes were exercised to simulate angling (11°C) and the temperature of the water in the live-well simulation (22 ± 1°C). In a real tournament situation, however, it is common for the fish to experience a significant temperature change when they are angled from deep water and then placed in a live well filled with surface water. At the time of these experiments, for example, the difference in water temperature between the depths that walleyes are commonly angled in our area of Lake Ontario (7–10 m) and the surface was about 10–12°C (YSI Submersible Temperature Probe, YSI, Inc., Yellow Springs, Ohio). Although the temperature difference used in the tournament simulation was substantial, it was, therefore, a realistic representation of the temperature differences that walleyes may experience in live-release angling tournaments.

Tournament Simulation for Measurement of Cardiac Variables

The second series of tournament simulation experiments examined cardiac variables and was conducted at the Queen's University Biological Station (QUBS) on Lake Opinicon, Ontario. Walleyes used in the experiments to examine cardiac variables were supplied by Leonard's Walleye Culture, Hardington, Ontario. Fish were collected from outdoor earthen ponds by both angling and seining, and immediately transported to the aquatic holding facility at QUBS. Walleyes were allowed to acclimate for 48 h in 75-L aquaria supplied with a continuous flow of lake water before experimentation. The water temperature of the holding tanks at QUBS and the temperatures of Lake Opinicon during the experimental treatments were about 22°C.

The surgical procedures and the cardiac output apparatus used in these experiments are described by Cooke et al. (2001). Each walleye was anesthetized with 60 mg/L clove oil emulsified with ethanol (9:1 [ethanol : clove oil]) until the fish had lost equilibrium and was nonresponsive. The fish was then transferred to a surgery table, and water containing a maintenance concentration of anesthetic (30 mg/L clove oil) was pumped over the gills. A flexible silicone cuff-type Doppler flow probe (subminiature 20 MHz piezoelectric transducer; Iowa Doppler Products, Iowa City, Iowa), sized to match the diameter of the blood vessel, was then placed around the ventral aorta, which was accessed by making a small incision in a thin membrane within the opercular cavity. The lead wire from the probe was then sutured to the side of the fish in six locations to prevent the cuff from shifting. The Doppler flow probe was attached to a flowmeter (545C-4 Directional Pulsed Doppler Flowmeter, Bioengineering, University of Iowa, Iowa City, Iowa) and

a digital strip-chart recorder (LabVIEW 4.0.1, National Instruments Corporation, Austin, Texas) to monitor cardiac output, stroke volume, and heart rate. Following surgery, fish were allowed to recover in holding tanks for 18–24 h before experimentation.

With the following exceptions, the experimental treatments for the tournament simulation to monitor cardiac variables were identical to those used in the previous tournament simulation experiments ($n = 8$ for rest and exercise groups, $n = 7$ for live-well and weigh-in groups, and $n = 6$ for recovery group). The live wells consisted of eight plastic containers (about 70 L in volume, 60 × 40 cm, each with a tight fitting lid) placed on a small pontoon boat. In this manner, several fish could be subjected to experimentation (in separate live wells), without the lead wires from the flow probes becoming tangled. Each live well was equipped with a section of plastic tubing passing through the lid of the container, and the tubing from four live wells was connected in series to a fountain pump submerged into Lake Opinicon at a depth of about 0.75 m. The plastic tubing was positioned in such a way that, when the fountain pumps were turned on, water could be sprayed into the live well providing aeration along with fresh water, and the lids of the tanks could remain closed. Overflow holes were drilled into the side of the plastic containers so that the volume of water contained in the live well was about 50 L. Throughout the experiment, fish were supplied with fresh aerated water three times per hour for about 10 min. Each live well contained one subject fish monitored for cardiac output (mean body mass = 638 ± 46.2 g), and one additional fish (292 ± 2.54 g) which increased the density of fish in the live well. After each stage of the simulated tournament walleyes were monitored for 5 min to obtain measurements of cardiac activity. Resting values before experimentation were obtained by monitoring the walleyes contained in the 75-L holding aquaria at QUBS. After experimentation fish were returned to the holding tanks at QUBS and their cardiac activity was measured after 24 h of recovery.

Following all experimental treatments for cardiac monitoring, fish were euthanatized with an overdose of anesthetic (180 mg/L clove oil), and a postmortem calibration was conducted to convert Doppler shift (in volts) to actual blood flow (mL/min). Pig blood perfused through the aorta was used to calibrate the probes over a range of flow rates encompassing those recorded during the trials. Reference flow rates were analyzed with linear least squares regression.

Air Exposure Experiments

The adult walleyes (mean total length = 455 ± 19.28 mm) used for the air exposure experiments were obtained by angling from the Trilakes region of southern Ontario and transported to the aquatic facility at Queen's University in aerated holding tanks. Once at the laboratory fish were held in large plastic holding tanks containing water from the City of Kingston's municipal supply. The water flowing continuously through the tanks was aerated, dechlorinated and maintained at a temperature of 11–12°C. Walleyes were allowed to acclimate for 5 d before experimentation and showed no visible signs of stress during the holding period.

Experimental protocol.—Individual walleyes ($n = 6$ for each treatment group, $n = 7$ for resting walleyes) were carefully netted from the common holding tank and transferred (three fish per trial) into a portable live well (35 × 35 × 87 cm). The live well was supplied with continuously flowing water (12°C), which was aerated by passing it through a section of perforated plastic tubing located about 5 cm above the water surface in the live well. Fish remained undisturbed for 4 h, at which time individual fish were removed from the live well and held for 5 min in a clear, plastic, transfer bag containing 15 L of water. The transfer bag was then emptied into a basket that allowed the water to drain. Fish remained in the basket and were exposed to air for either 30 s or 90 s. Another group of fish received the same live well and transfer bag treatment, but was not air exposed during the weigh-in simulation. Instead, these fish were placed into a plastic container full of water for 90 s. Of the three fish in the live well during each trial, one fish was subjected to each of the three weigh-in treatments (water, 30 s of air, or 90 s of air). This procedure was repeated six times for a total of six individuals per experimental group. Immediately following the weigh-in treatments individual fish were anesthetized and sampled for blood and muscle as described previously. Samples collected for the three air exposure treatments were also compared with control fish sampled using darkened Perspex boxes as described above (water temperature = 12°C).

To investigate the additional possibility that acute hypoxia may have contributed to the physiological disturbance observed during confinement of the walleyes in the transport bags, further experiments were performed to measure oxygen depletion in the plastic transport bags commonly used at tournament weigh-ins. The walleyes (mean body mass = 731 ± 20.2 g) were carefully netted from a common holding tank (12°C) and placed directly into clear, plastic, transfer bags containing 15 L of water at 12°C. Experiments were performed with the bags containing one fish, three fish, or five fish (for a total of three trials at 12°C). During each trial, oxygen levels in the bags were measured at 1 min intervals for 6 min by means of

a YSI 55 dissolved oxygen probe. Timing began when all fish for a given trial were placed into the plastic transfer bag. A second set of experiments was performed in an identical manner, except that the water in the plastic bags was 22°C, and before experimentation the walleyes had been held in a holding tank at 22°C.

Plasma and Muscle Analyses

Plasma samples were thawed and analyzed for lactate using Sigma reagents and the appropriate standards (Sigma Chemical Co., St. Louis, Missouri). Plasma chloride concentrations were determined with a CMT10 chloride titrator (Radiometer, Copenhagen, Denmark), and plasma osmolality was quantified using a freezing-point depression osmometer (Advanced Instruments Inc., model 3M0).

Muscle lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP) were determined as outlined by Kieffer et al. (1995). About 1–2 g of muscle was ground under liquid nitrogen with a precooled mortar and pestle. The ground muscle powder was added to four volumes of ice-cold 8% perchloric acid (PCA and 1 mM EDTA) and mixed by means of a vortex mixer. The resulting slurry was centrifuged and immediately neutralized with a buffer containing 2 mM KOH, 0.4 mM KCl, and 0.4 mM imidazole. Following the methods of Lowry and Passonneau (1972), lactate, PCr, and ATP concentrations were assayed enzymatically using these neutralized PCA extracts. Appropriate metabolite standards (Sigma Chemical) were used in each assay. For measurement of white muscle glycogen, about 250 mg of frozen muscle was placed directly into 1.0 mL of 30% KOH and digested in a boiling water bath. Glycogen was isolated as described by Hassid and Abraham (1957), and measured as free glucose following digestion with amyloglucosidase.

White muscle samples collected during the weigh-in experiments were also analyzed for intracellular pH (pH_i) according to the methods of Pörtner et al. (1990) using a capillary pH electrode adjusted to 12°C and connected to a a pH meter (Radiometer PHM 84 Research pH Meter).

Statistical Analysis

For the tournament simulation experiments, a one-way analysis of variance (ANOVA) was used to detect significant differences among the treatment groups. A Dunnet's post hoc test was then used to compare treatment groups with the control. Results from the cardiac output portion of the tournament simulation experiment were compared using a one-way repeated measures ANOVA followed by a Dunnet's test (Sokal and Rohlf 1995). To test the assumptions of ANOVA, the residuals of our data were tested for homogeneity, independence, and normality. Homogeneity and independence of residuals were confirmed by observing the absence of a pattern in residual-fit plots. Normality of the residuals was assessed by means of histograms and Kolmogorov–Smirnov normality tests (Sokal and Rohlf 1995).

For the air exposure experiments, a one-way ANOVA was used to detect significant differences among the treatment groups. To better understand the effect of air exposure duration, a post hoc Tukey–Kramer test was used for pairwise comparisons between treatments. For the rates of oxygen decrease in transport bags, a two-way analysis of covariance (ANCOVA) was performed for each temperature using the General Linear model (using fish number as an explanatory variable and time [min] as a covariate; Minitab 13.1). The interaction between fish number and time was of interest in this study. All values are reported as means ± 1 SE and the level of significance (α) for all statistical tests was 0.05.

Results

Tournament Simulation

Plasma analysis.—Plasma osmolality and plasma chloride concentrations peaked immediately following exercise, and both were significantly different from resting control values (Figure 1). Plasma osmolality and chloride then returned to control levels following the live-well treatment and remained at this level for the remainder of the experiment (Figure 1A, B).

After exercise, the white muscle lactate concentrations of the experimental walleyes were about 12 times higher than those in the control walleyes, and plasma lactate concentrations were 4.5 times higher than in the control group (Figure 2). White muscle and plasma lactate returned to control levels during live-well confinement, but were increased significantly following the weigh-in treatment.

White muscle analysis.—All of the measured white muscle energy stores showed significant declines following exercise, with PCr, ATP, and glycogen being 37, 49, and 42% of resting values, respectively (Figure 3). Levels of all three white muscle energy stores recovered during live-well confinement to near-resting levels. Following the weigh-in, white muscle PCr and ATP concentrations were only 60% and 69% of resting values, respectively. White muscle glycogen also decreased during the weigh-in treatment, but this change was not significant from control levels.

Cardiac variables.—Cardiac output increased by almost 80% following exercise but returned to control levels during live-well confinement (Figure 4A). The

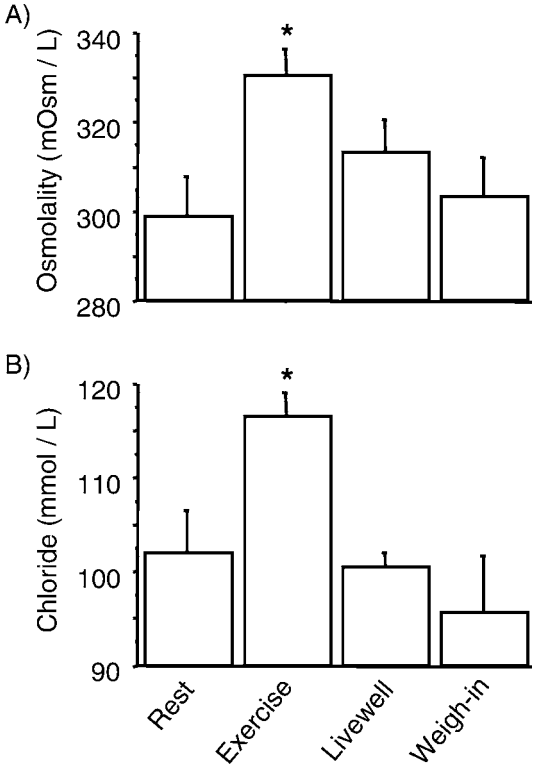

FIGURE 1.—**(A)** Plasma osmolality and **(B)** chloride for walleyes at different stages during a live-release angling tournament simulation. Values are means + SEs; $n = 7$ for all groups except the weigh-in treatment, for which $n = 6$. Asterisks indicate significant differences from the resting control (ANOVA: Dunnet's test, $P < 0.05$).

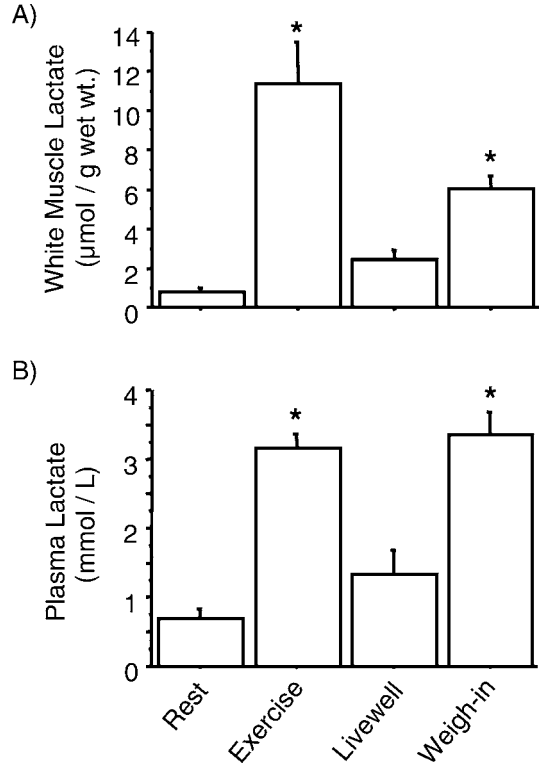

FIGURE 2.—**(A)** White muscle lactate and **(B)** plasma lactate for walleyes at different stages during a live-release angling tournament simulation. See the caption to Figure 1 for additional details.

weigh-in treatment caused another increase in cardiac output similar in magnitude to that observed following exercise, and after 24 h of recovery time, cardiac output returned to control levels. The changes in cardiac output with each treatment were largely due to changes in heart rate, as stroke volume showed no significant changes during the experiment (Figures 4B, C).

Air Exposure Experiments

White muscle energy stores generally decreased with increasing amounts of handling and air exposure. White muscle PCr levels for all treatments were significantly lower than control values (Figure 5A). Levels of PCr declined with longer periods of air exposure, but the differences within the air exposure treatments themselves were not significant. White muscle ATP concentrations also decreased with increasing duration of air exposure (Figure 5B). Declines observed during the water treatment and after 30 s of air exposure were not significantly different from those at rest. However, 90 s of air exposure resulted in a significant ATP depletion when compared with resting levels, with concentrations dropping by about 23%. White muscle glycogen levels for all treatments were significantly lower than those at rest (Figure 5C).

White muscle lactate concentrations showed a significant increase relative to control values after 30 s of air exposure and were 15 times greater than control levels after 90 s of air exposure. Plasma lactate concentrations of all air-exposure treatments were significantly greater than control values and showed a gradual increase with longer periods of air exposure. The differences observed between air-exposure treatments were not significant (Figure 6B).

White muscle pH_i was highest at rest, and significant acidosis occurred as air exposure increased beyond 30 s. (Figure 7). In addition, pH_i after the water treatment was significantly greater than after the 90 s air-exposure treatment (Figure 7).

The rates of oxygen decrease in transport bags were significantly greater with increasing numbers of fish at both 12°C and 22°C (Figure 8; Table 1). Overall, the rates of oxygen decrease were more rapid at 22°C than

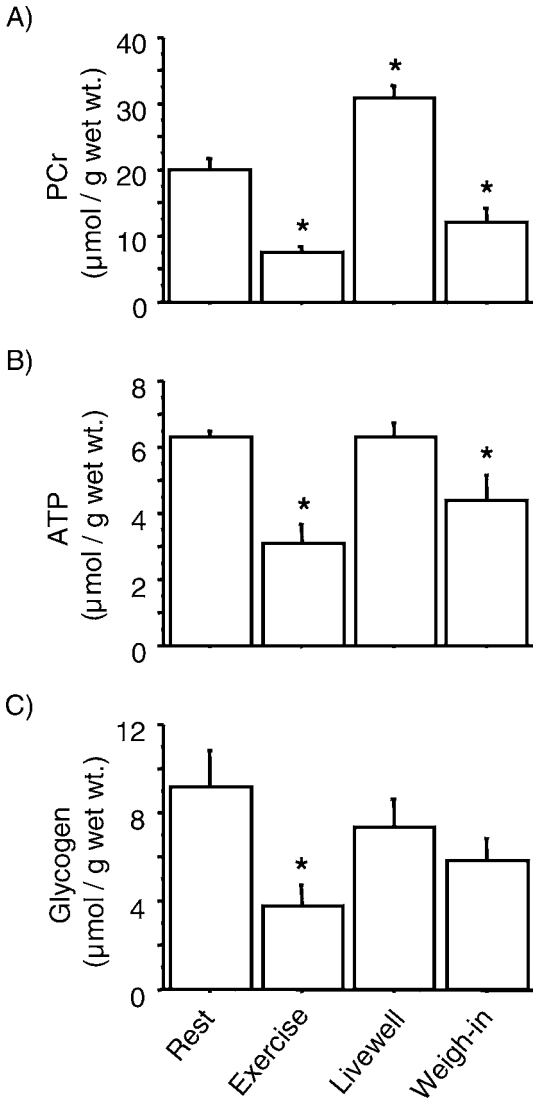

FIGURE 3.—**(A)** White muscle phosphocreatine (PCr), **(B)** adenosine triphosphate (ATP), and **(C)** glycogen for walleyes at different stages during a live-release angling tournament simulation. See the caption to Figure 1 for additional details.

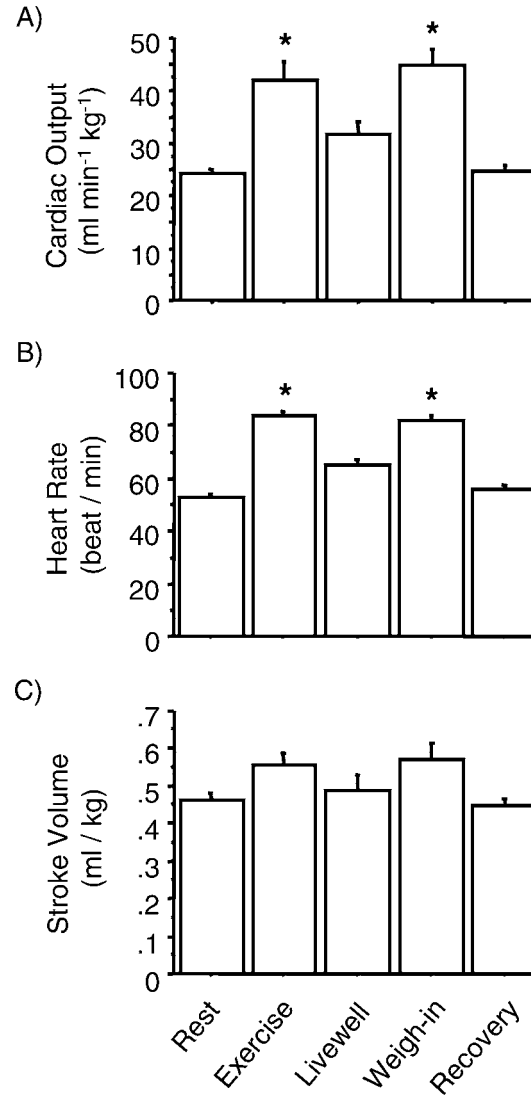

FIGURE 4.—**(A)** Cardiac output, **(B)** heart rate, and **(C)** stroke volume for walleyes at different stages during a live-release angling tournament simulation. Values are means + SEs; $n = 8$ for the rest and exercise groups, $n = 7$ for the live-well and weigh-in groups, and $n = 6$ for the recovery group. Asterisks indicate significant differences from the resting control (ANOVA: Dunnet's test, $P < 0.05$).

at 12°C. Also, due to temperature-related differences in oxygen solubility in water, initial water oxygen concentrations (mg/L) were lower at 22°C than at 12°C.

Discussion

Tournament Simulation

The results of the white muscle and cardiac analysis complement those of Suski et al. (2004) and provide further evidence that the metabolic disturbance present in tournament fish occurs in two acute phases. First, walleyes that were exercised to simulate angling exhibited large depletions of PCr, ATP, and glycogen with a corresponding increase in blood and muscle lactate. These types of changes are often observed in fish that are exercised to exhaustion and have undergone bouts of anaerobic activity (Wood 1991; Milligan 1996; Kieffer 2000). For example, in this study, 1 min of exercise resulted in an increase in white muscle lactate of about 12-fold above control values,

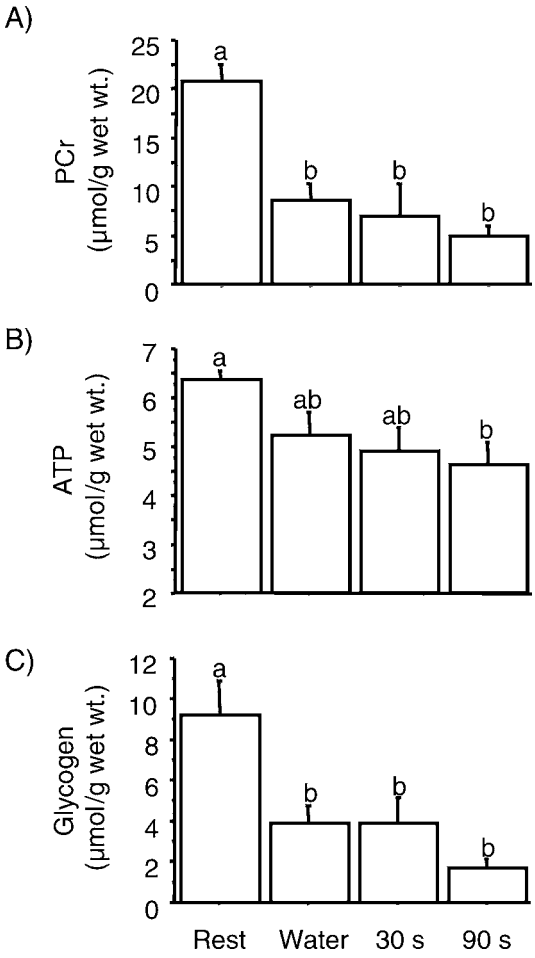

FIGURE 5.—**(A)** White muscle phosphocreatine (PCr), **(B)** adenosine triphosphate (ATP), and **(C)** glycogen for walleyes subjected to a weigh-in simulation that involved either no air exposure (fish were placed in water), 30 s of air exposure, or 90 s of air exposure. Also shown are values collected from resting walleyes. Walleyes in all groups were acclimated to 12°C. Values are means + SEs; $n = 6$ for all treatment groups except resting walleyes, for which $n = 7$. Significant differences between groups are indicated by different letters (ANOVA: Tukey–Kramer test, $P < 0.05$).

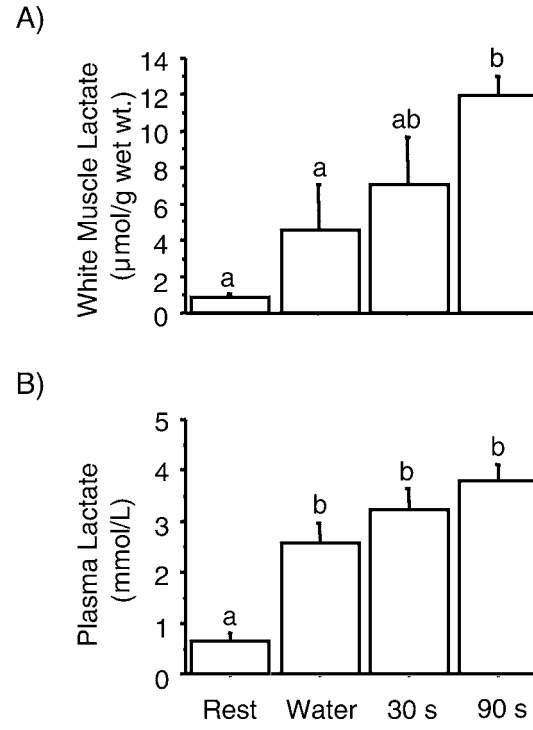

FIGURE 6.—**(A)** White muscle lactate and **(B)** plasma lactate for walleyes subjected to a weigh-in simulation that involved either no air exposure, 30 s of air exposure, or 90 s of air exposure. Also shown are values collected from resting walleyes. See the caption to Figure 5 for additional details.

while Suski et al. (2004) noted a seven-fold increase in muscle lactate above control values for largemouth bass exercised for 1 min. Angling also caused large increases in heart rate and cardiac output, which are indicative of increased oxygen demands (Schreer et al. 2001). The second phase of anaerobic activity occurred during the weigh-in simulation. At this time, the walleyes showed another large drop in white muscle energy reserves (PCr and ATP), increases in white muscle and plasma lactate, and changes in cardiac variables that were similar to those observed following the angling simulation. This likely occurred due to the anoxia resulting from air exposure during weighing, as well as during handling and confinement in the plastic transport bags. For these reasons, the angling and the weigh-in phases of a tournament are probably the most

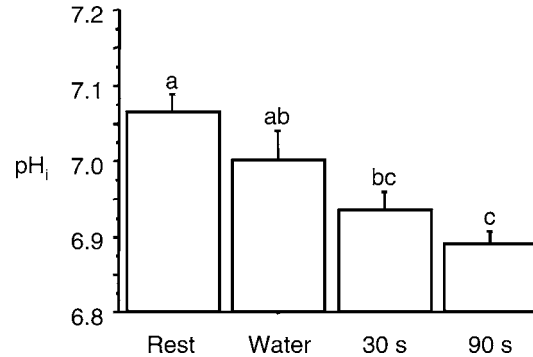

FIGURE 7.—White muscle intracellular pH (pH_i) for walleyes subjected to a weigh-in simulation that involved either no air exposure, 30 s of air exposure, or 90 s of air exposure. Also shown are values collected from resting walleyes. See the caption to Figure 5 for additional details.

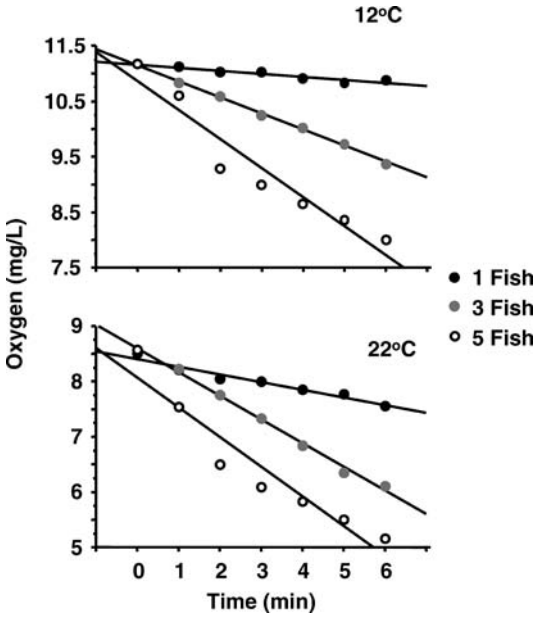

FIGURE 8.—Changes in water oxygen concentrations in plastic transport bags over time with varying numbers of walleyes at 12°C and 22°C.

important contributors towards the physiological disturbance in tournament walleyes.

A loss of ionically charged constituents from plasma is a commonly used indicator of physiological disturbance in fish, particularly under conditions of prolonged or "chronic" stress (Carmichael et al. 1984; McDonald and Milligan 1992; Killen et al. 2003). In this study, plasma osmolality and chloride increased during the angling simulation, which was likely due to hemoconcentration caused by water movement out of the plasma and an osmotic gradient generated by lactate accumulation in white muscle (Holeton et al. 1983; Milligan and Wood 1986; Wood 1991). However, osmotic balance then returned to control levels by the end of the live-well and weigh-in simulations. These results agree with Killen et al. (2003) who found little difference in plasma osmolality between walleyes sampled following an angling tournament and those from resting laboratory controls. However, Killen et al. (2003) suggested that one should expect hemoconcentration from fish sampled following an angling tournament (based on increased levels of white muscle lactate). The absence of hemoconcentration in their study may be explained by net losses of ions, such as Cl^- or H^+ from the plasma. However, the sampling regime of our study suggests otherwise. Plasma osmolality and chloride values obtained following the live-well treatment were collected prior to the bout of anaerobic activity experienced during the weigh-in simulation and represent ion levels in the absence of hemoconcentration. White muscle lactate was at control levels following live-well confinement and previous studies have shown walleyes to exhibit low levels of activity during live-well confinement, thereby facilitating recovery from angling-induced exercise (Suski et al. 2005). Plasma osmolality and chloride did not differ from controls values at this time, suggesting that there was no net loss of ions during the experiment up to this point. Stressors encountered during the weigh-in simulation may cause ion losses in walleyes, but this seems unlikely because disturbances from the angling and weigh-in simulations were similar in magnitude, and walleyes were able to make a full recovery following the angling simulation. Future experiments should explore this possibility further by examining changes in osmotic balance in the hours following the weigh-in procedure. Suski et al. (2004) found no evidence for significant plasma ion loss in largemouth bass at any stage of a tournament simulation, and observed that total plasma osmolality had returned to normal after 24 h of recovery. Taken together, it would appear that plasma ion loss is not a serious problem for most tournament-caught fish.

Interestingly, the walleyes in our study were able to make an almost complete recovery from the metabolic disturbance caused by angling while being held in the live well despite the angling activities and boat driving

TABLE 1.—General linear model results for the decrease in oxygen content in plastic transport bags containing varying amounts of walleyes at 12°C and 22°C. Linear regression equations were calculated using the time spent in plastic transport bags (t; min) as a covariate. Asterisks indicate significant differences ($P < 0.05$); daggers indicate that the lower-order terms cannot be evaluated independently because the interaction term is significant.

Temperature (°C)	Number of fish	Equation	Term	P	F
12	1	$O_2 = -0.056t + 11.16$	Time	<0.001*†	124.1
	3	$O_2 = -0.29t + 11.15$	Number of fish	<0.001*†	22.29
	5	$O_2 = -0.523t + 10.8$	Time × number of fish	<0.001*	38.76
22	1	$O_2 = -0.138t + 8.41$	Time	<0.001*†	207.81
	3	$O_2 = -0.429t + 8.59$	Number of fish	0.085†	2.92
	5	$O_2 = -0.534t + 8.06$	Time x number of fish	<0.001*	21.73

that occurred during confinement. Similar results were obtained by Suski et al. (2004) using largemouth bass. Despite these observations, and although the current experiments show that the largest physiological disturbances occur during angling and the weigh-in procedure, the importance of proper live-well maintenance cannot be overlooked. Appropriate live-well water quality is crucial to facilitate recovery from angling. The replenishment of energy stores following exhaustive exercise, such as that caused by angling, appears to be fueled by lipid oxidation (Richards et al. 2002) and requires an environment with adequate oxygen supply. At present, however, there is great variability in the types of live wells that may be used by tournament competitors. For example, tournament professionals often have very large live wells (or multiple live wells) that aerate automatically on a schedule. The water quality in these live wells would likely be similar to that of the present study and would facilitate the recovery of captured fish. On the other hand, many amateur or novice competitors have live wells that must be aerated manually by switching on pumps that supply fresh water. In such cases, optimal water quality may not always be achieved if individual anglers do not adhere to rigid aeration schedules. Low oxygen levels and high waste accumulation are additional stressors that could impair recovery from angling and even cause an additional physiological disturbance (Hartley and Moring 1993; Kwak and Henry 1995). Thus, the importance of proper live-well conditions should still be emphasized.

Air Exposure Experiments

While previous studies have shown weigh-ins to be physiologically challenging for tournament-caught fish (Killen et al. 2003; Suski et al. 2003, 2004), little attention has been paid to the relative physiological impact of the different weigh-in sections. This formed the basis for our air exposure experiments. Even without air exposure, walleyes displayed significant physiological disturbance after being transferred to the transport bags and confined for 5 min. These fish (the water treatment) had reduced white muscle energy stores when compared with resting walleyes (specifically PCr and glycogen), and significant increases in white muscle and plasma lactate after 30 and 90 s of air exposure, respectively. Most walleyes showed bursts of physical activity while in the transport bags, which probably were responsible in large part for the differences observed between the water treatment values and resting values. After transfer to the container of water, walleyes in the water treatment were very calm: it is unlikely any additional metabolic disturbance occurred during this time. It is also doubtful that differences between the resting walleyes and those from the water treatment were due to the initial handling and 4 h of live-well confinement that preceded the weigh-in simulation. It is also noteworthy that during the tournament simulation portion of this study, white muscle metabolites in exercised walleyes fully recovered during 6 h of confinement in a live well that was only periodically aerated. Walleyes used in these weigh-in experiments were not exercised prior to live-well confinement and received a constant supply of fresh water for aeration while in the live well. Thus, it is most likely that the white muscle metabolite status of the walleyes following the 4 h of live-well confinement was similar to that of walleyes at rest.

The metabolic disturbance that occurred during the period of bag confinement was further increased by subsequent air exposure. This is in agreement with previous studies examining the effects of air exposure on largemouth bass (Suski et al. 2003). It should be noted, however, that the magnitude of the resulting physiological disturbance increased with the amount of time spent air-exposed, even during very brief exposures (<90 s). In particular, levels of white muscle energy stores were significantly lower than control values following 90 s of air exposure. White muscle lactate levels after 90 s of air exposure were approximately 15-fold higher than at rest values, and threefold higher than the water treatment values. Air exposure in fish will cause an inhibition of gas exchange at the gills resulting in increased anaerobic metabolism (Boutilier 1990; Ferguson and Tufts 1992). Arends et al. (1999), for example, noted that the plasma lactate concentration of gilthead seabream *Sparus aurata* increased more than 2.5 times following 3 min of air exposure, while walleyes in our study showed almost a fourfold increase in plasma lactate after only 90 s of air exposure. The air exposure that occurs during a tournament weigh-in is usually accompanied by some physical activity while the fish is in the weigh-in basket (e.g. flips and tail flaps). These movements are a form of burst exercise and likely contribute to the physiological disturbance in air-exposed fish.

The air exposure experiments also caused changes in the white muscle pH_i of the walleyes. During anaerobic metabolism protons are produced by the dissociation of lactic acid and the hydrolysis of ATP (Hochachka 1991). Brief periods of air exposure (30 s) not only produced changes in white muscle levels of ATP and lactate, but also increased intracellular acidosis. A reduction in pH_i can cause an inhibition of cellular enzymes, muscle fatigue, and a reduced ability to recover from stress (Westerblad et al. 1991; Wood 1991; Mitton and McDonald 1994). According to Wood et al. (1983), the magnitude of the intracellular

acidosis may even be the ultimate cause of death among fish that are exercised to exhaustion, although studies involving Atlantic salmon *Salmo salar* documented a drop of up to 0.5 pH units without any fatalities (Wilkie et al. 1997). In the current study, walleyes experienced a drop of approximately 0.2 pH units. Thus, an increased intracellular acidosis in white muscle is another important consequence of air exposure during the weigh-in at live-release tournaments.

Confinement of walleyes in plastic transport bags resulted in a continuous decline of dissolved oxygen. Previous studies with walleyes have suggested that incipient oxygen thresholds (i.e., levels at which the effects of hypoxia are apparent at the behavioural level) range from 5.5 to 2.0 mg/L at 22°C (Scherer 1971), while smallmouth bass begin to show significant physiological disturbances when exposed to dissolved oxygen concentrations of about 5 mg/L (Furimsky et al. 2003). At both 12°C and 22°C, oxygen levels within transport bags declined faster as the number of fish increased, and the rate of oxygen decline at 22°C was higher than at 12°C. The weigh-in experiments used only one fish per bag at 12°C and so, acute hypoxia was probably not a contributing factor towards the physiological disturbance observed in these fish. In tournaments, however, it is very common for three or more fish to be held at a time in one transfer bag and our results suggest that fish may be exposed to acute hypoxia after only 5–6 min of confinement. This seems especially likely when water temperatures are warmer, not only due to increased rates of oxygen consumption by the fish (Cai and Summerfelt 1992), but also because warmer water contains less oxygen when saturated (Davis 1975). In such cases, the metabolic disturbance associated with bag confinement would likely be greater than that observed in this study.

Summary and Conclusions

The physiological disturbance observed in walleyes in this study is similar in magnitude to the changes that have been observed in previous experiments using largemouth bass (Suski et al. 2004). This study represents an attempt to comprehend the physiological responses of additional fish species. Taking into account other reports of a relatively high mortality rate for walleyes during live-release angling tournaments (Goeman 1991; Fielder and Johnson 1994; Hoffman et al. 1996), our findings suggest that walleyes may be more sensitive than largemouth bass to the physiological disturbances resulting from acute bouts of anaerobic metabolism. Further research is needed to understand the physiological mechanisms responsible for this potential difference in sensitivity between the two species. However, regardless of the species, it is clear that the most important contributors towards physiological disturbance in fish during live-release angling tournaments are angling and the air exposure associated with the weigh-in.

The fact that walleye tournaments contain two acute phases that involve significant anaerobic activity has important implications for fisheries managers and tournament organizers interested in minimizing fish stress during these events. In terms of the angling phase, our simulation (1 min of burst exercise) may have been longer than needed to produce the physiological disturbance that occurs in many situations. Most experienced tournament anglers are adept at minimizing the time between hooking the fish and releasing it into the live well. Clearly, this strategy has an important impact on the initial physiological condition of captured fish, and should be emphasized at all levels of tournament angling because the magnitude of physiological disturbance increases with angling duration (Gustaveson et al. 1991). However, other strategies could be used to minimize the magnitude of the disturbance caused by the weigh-in. For example, our evidence indicates that the bags used to contain the fish before being weighed should be aerated or supplied with fresh water, or the time that fish are allowed to be held in transport bags should be limited. This is most critical when water temperatures are warm. Perhaps the most significant area of concern during the weigh-in is the amount of time the fish are exposed to air while on the scale. The results of this study clearly indicate that this period of air exposure during the weigh-in must be minimized, or ideally eliminated, perhaps by weighing fish in water (Suski et al. 2004).

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