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Physiological Responses of Walleyes to Live-Release Angling Tournaments

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Abstract.—This study examines the physiological impacts of live-release angling tournament practices on walleyes *Sander vitreus* (formerly *Stizostedion vitreum*). Blood and white muscle samples were taken from walleyes following the weigh-in at several live-release angling tournaments in southern Ontario. The tournament walleyes had significantly elevated plasma cortisol levels in comparison with those of the control walleyes, indicating that tournament practices elicit a significant stress response in the tournament fish. Increased creatine phosphokinase and lactate dehydrogenase activities revealed that walleyes experience a moderate degree of cellular damage during live-release angling tournaments. In contrast to recent research with largemouth bass *Micropterus salmoides*, the tournament walleyes showed evidence of plasma ion loss. The tournament walleyes also experienced dramatic reductions in white muscle energy stores (phosphocreatine, ATP, and glycogen) and corresponding increases in plasma and white muscle lactate levels when compared with those of the control fish. These metabolic changes were similar in magnitude to the changes previously observed in largemouth bass following tournaments and suggest that current tournament procedures may commonly cause relatively large bouts of anaerobic metabolism in fish.

Competitive angling events are increasingly widespread throughout North America, with at least 30,000 events occurring annually (Shupp 1979; Duttweiler 1985; Schramm et al. 1991). Several benefits of these events have been cited, including economic benefits to the communities and the promotion of the fisheries resource (Schramm et al. 1991). Angling tournaments have also had a major impact on the popularity of catch-and-release angling. The vast majority of these events involve a live-release format in which an effort is made to release all of the fish caught at the end of each tournament day.

Despite their potential societal benefits, live-release angling tournaments may have negative biological impacts (Shupp 1979; Schramm et al. 1991; Hayes et al. 1995; Wilde 1998). In particular, significant fish mortality has been documented following some live-release angling tournaments. The estimates of total mortality range from 22% to 80% for walleyes *Sander vitreus* (formerly *Stizostedion vitreum*) (Goeman 1991; Fielder and Johnson 1994; Hoffman et al. 1996), and 0–61% for largemouth bass *Micropterus salmoides* (Wilde 1998). Several studies have attempted to correlate tournament mortality with environmental factors such as season (Lee et al. 1993; Kwak and Henry 1995), wind speed (Goeman 1991; Fielder and Johnson 1994), and air or water temperature (Schramm et al. 1985; Schramm et al. 1987; Bennett et al. 1989). In addition, the effects of organizational procedures on tournament mortality have been examined (Lee et al. 1993; Weathers and Newman 1997). To date, however, little information exists on the impact of tournaments on the physiology of important freshwater recreational species.

Suski et al. (2003) recently described the physiological changes that occur in largemouth bass as a result of live-release angling tournaments. This study showed that the disturbances associated with tournaments cause a large increase in the plasma levels of the hormone cortisol in largemouth bass, as well as profound changes in white muscle metabolites, but there was no evidence of significant ion losses or cell damage. At this time, however, it is not known whether the physiological profile of other species following tournaments would be similar to that of largemouth bass.

Walleyes are another popular target species for live-release angling tournaments throughout North America. Several lines of evidence—including earlier reports on mortality (Goeman 1991; Fielder and Johnson 1994; Hoffman et al. 1996) and the anecdotal observations of fisheries managers and tournament organizers—indicate that walleyes may be more sensitive to stressors than species

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such as largemouth bass in a tournament environment. At present, however, the underlying reasons for these apparent interspecies differences are largely unknown due to the absence of relevant physiological information for species such as walleyes.

Our objective was to examine the physiological condition of walleyes following live-release angling tournaments. We hypothesized that angling tournament practices would cause a significant physiological disturbance in walleyes. Because walleyes seem to be more sensitive to tournament procedures, we also predicted that this disturbance may be somewhat different in nature, or greater in magnitude, than Suski et al. (2003) recently documented for largemouth bass. The information gained from this study may be used to minimize the impact of tournaments on the physiology and survivorship of important recreational species such as walleye.

Methods

Sampling sites.—Seven live-release walleye tournaments were visited on lakes in southern Ontario, Canada, in the summers of 1999, 2000, and 2001. Three sites (Belleville, Picton, and Deseronto) were visited on the Bay of Quinte, Lake Ontario; the other sampling sites were Sturgeon Lake, Lake Scugog, and Rice Lake (which was visited on two separate occasions). At three of the seven tournaments, walleyes were terminally sampled for blood and white muscle (Belleville, Picton, and Lake Scugog). At the remaining tournaments, fish were sampled for blood only and then released (Rice Lake [both visits], Deseronto, and Sturgeon Lake).

All tournaments were held during the spring or early summer (April–early June), and surface water temperatures ranged from 17°C to 25°C. The tournaments were competitions for the greatest combined weight of fish caught (five fish maximum), and the organizational procedures and rules of the tournaments resembled those of previous descriptions in that only artificial baits were used, anglers were penalized for dead fish, fish had to comply with a minimum length limit, and anglers reported to a common weigh-in point at the conclusion of the angling day (Kwak and Henry 1995).

Sampling protocol.—At each tournament, walleyes were randomly selected for sampling after having gone through the weigh-in procedure, but before being placed in the live-release boat (N = 7–13). Fish were sampled on-site (within 5 min after being weighed in), and their total length varied between 390 and 570 mm.

During sampling, a heparinized (50 IU/mL sodium heparin) syringe was used to collect approximately 3 mL of blood by caudal puncture. The fish sampled for blood only were not anesthetized so they could be subsequently released following sampling. Most fish were quite lethargic after having gone through the tournament process, and this method of sampling does not significantly affect the physiological variables of fish under these conditions (Suski et al. 2003). Blood plasma was immediately separated by centrifugation at 10,000 rpm for 2 min (Eppendorf 5415 C minicentrifuge, Brinkmann). Both the plasma supernatant and remaining pellet were immediately frozen on dry ice until they could be returned to the laboratory and stored at −80°C. Prior to sampling, the fish that were terminally sampled for muscle and blood were individually anesthetized in a buffered solution of 3-aminobenzoic acid ethyl ester (250 mg/L MS-222 and 500 mg/L $NaHCO_3$). Immediately following total anesthesia (approximately 1 min), blood was sampled as previously described. A white muscle sample (2–10 g) was then collected from the epaxial musculature behind the operculum above the lateral line, immediately freeze-clamped with precooled aluminum tongs and placed in liquid nitrogen.

For comparison with the samples collected at tournaments, the control samples were obtained by two different methods. First, walleyes were collected by angling from Lake Ontario and were placed in laboratory holding tanks to recover for at least 48 h. Individual fish were then placed in aerated, darkened Perspex boxes, where they rested quietly for an additional 48 h before being sampled (flow-through water temperature = 20°C). Immediately before sampling, the water flow into the boxes was stopped and anesthetic was added. Following full anesthesia, the fish were sampled for blood and white muscle as was described for the tournament walleyes (N = 8). A second set of control plasma values was also obtained by sampling walleyes for blood immediately following angling (N = 6). These angled controls were sampled rapidly to precede the cortisol response normally encountered with this type of disturbance (Barton 2002). The main purpose of this angled control group was to provide additional information about the plasma cortisol levels of walleye in more natural environments. The fish in this control group consisted of wild walleyes angled from Lake Ontario and farmed walleyes angled from outdoor,

earthen ponds (Leonard's Walleye Culture, Hartington, Ontario). Fish were landed as quickly as possible, and approximately 3 mL of blood were sampled immediately without anesthesia. The time between hooking the fish and the completion of sampling was between 30 s and 1 min.

Plasma and white muscle analysis.—Plasma samples were thawed and analyzed for lactate using Sigma reagents and the appropriate standards (kit 826-A, Sigma Chemical Co., St. Louis, Missouri). Plasma glucose was measured using a hexokinase enzymatic assay (Sigma Chemical Co., St. Louis, Missouri). Cortisol concentrations were determined using a commercially available radioimmunoassay kit (kit TKC01,Coat-a-Count, Diagnostic Products Corp., Los Angeles, California) with the appropriate intra- and interassay standards. This assay involves the competitive binding of plasma cortisol and ^{125}I-labeled cortisol to cortisol-specific antibody coated tubes. Plasma chloride concentrations were determined with a CMT10 chloride titrator (Radiometer, Copenhagen, Denmark), and plasma osmolality was quantified using a freezing-point depression osmometer (model 3M0, Advanced Instruments, Inc., Norwood, Massachusetts). Plasma creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activities were determined according to the methods of Hørder et al. (1990) and Wróblewski and LaDue (1955), respectively, using kinetic assays with commercially available reagents (Sigma Chemical Co., St. Louis, Missouri). Due to a limited amount of plasma for some individuals (which resulted mainly from the occasional clotting of blood samples during centrifugation), not all of the walleyes sampled at the tournaments were analyzed for plasma CPK and LDH activity.

Muscle lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP) were determined as outlined by Kieffer et al. (1995). Approximately 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 ethylenediaminetetraacetic acid [EDTA]) and mixed with a vortex mixer. The resulting slurry was centrifuged and immediately neutralized (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 Co., St. Louis, Missouri) were used with each assay. White muscle glycogen was determined according to the method of Hassid and Abraham (1957). Approximately 250 mg of frozen muscle was placed directly into 1.0 mL of 30% KOH and digested in a boiling water bath. The resulting pellet was washed with ethanol, resuspended in water, and digested with amyloglucosidase to convert muscle glycogen to glucose. The quantity of muscle glycogen was then determined by following the enzymatic digestion of glucose in the presence of hexokinase using a spectrophotometer. All analyses for muscle PCr, ATP and glycogen were performed in duplicate in order to increase accuracy.

Statistical analysis.—All values are reported as means ± SE. Due to nonnormal distributions, plasma CPK and LDH activities for tournament walleyes were compared with those of the laboratory controls using Mann–Whitney *U*-tests. The data for all other tournament variables were analyzed using a one-factor analysis of variance (ANOVA) to detect significant differences between the groups. A Dunnet's post hoc test was then used to compare individual tournaments and the tournament mean (the combined mean for all fish sampled at all tournaments) with that of the control groups. The level of significance (α) for all tests was 0.05.

Results

Plasma Analyses

Following the tournaments, walleyes experienced significant changes in several plasma constituents. The cortisol levels of walleyes sampled immediately after angling were low (2.2 ng/mL ± 0.7) and assumed to be representative of unstressed, free-swimming fish (Figure 1A). Tournament fish plasma cortisol levels were between 30- and 60-fold greater than these ''angled'' controls, and approximately twofold greater than the laboratory control group. Of the seven tournaments sampled, the mean plasma cortisol levels in fish from six were significantly different from the angled controls, and three were significantly higher than values for the laboratory controls. Plasma glucose levels were elevated in walleyes from all tournaments, but only one tournament showed a statistically significant increase in plasma glucose (Figure 1B).

Plasma osmolality varied among fish from the different tournaments, and the combined mean from all tournaments was not significantly different from that for either of the control groups (Figure 2A). Similarly, plasma chloride concentrations

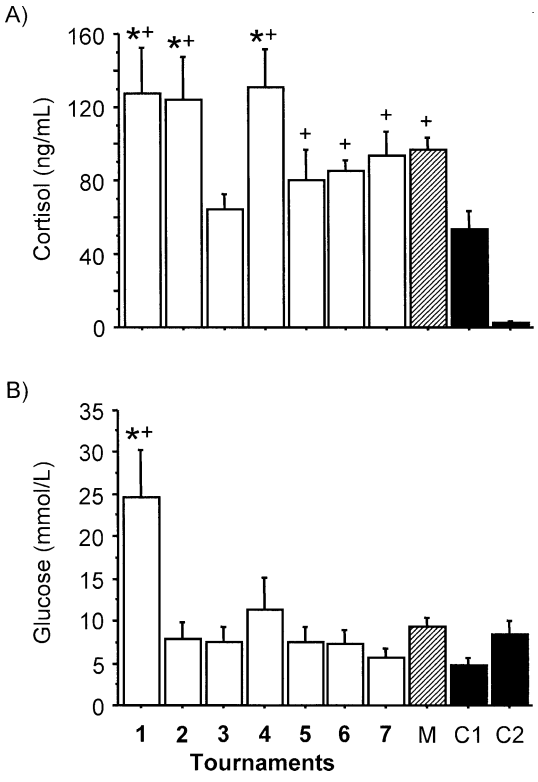

FIGURE 1.—Plasma **(A)** cortisol and **(B)** glucose of walleyes sampled after live-release angling tournaments. The white bars represent mean values for walleyes sampled following tournaments at (1) Belleville, (2) Picton, (3) Lack Scugog, (4) Deseronto, (5) Rice Lake–1st visit, (6) Rice Lake–2nd visit, and (7) Sturgeon Lake (N = 7–13). The striped bar indicates the combined mean for all tournaments (M). The shaded bars represent the mean values for the resting laboratory controls (C1, N = 8) and the angled controls (C2, N = 6). An asterisk denotes a significant difference from the angled control (ANOVA, Dunnet's test; $P < 0.005$). Values are presented as means ± SEs.

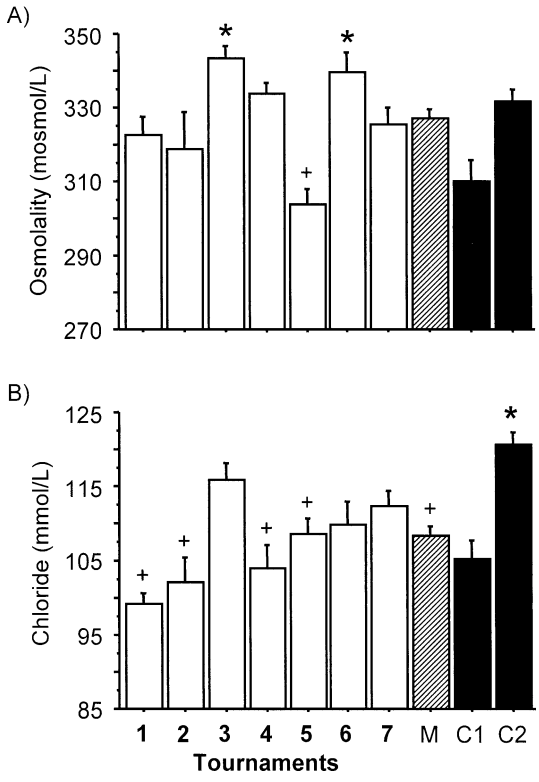

FIGURE 2.—Plasma **(A)** osmolality and (**B)** chloride of walleyes sampled after live-release angling tournaments. An asterisk denotes a significant difference from the laboratory control and a plus sign indicates a significant difference from the angled control (ANOVA, Dunnet's test; $P < 0.05$). Values are presented as means ± SEs. See the caption to Figure 1 for additional details.

in tournament walleyes were not significantly different from that for the resting laboratory control walleyes (Figure 2B). In walleyes from four of the individual tournaments, as well as the combined mean for all tournaments, however, the mean plasma chloride concentration was significantly lower than that for the angled control group.

The plasma CPK activity of the tournament walleyes was not significantly different from that of the resting laboratory controls (Table 1). In contrast, the plasma LDH activity of the tournament walleyes was fourfold higher than that of the resting laboratory controls (Table 1).

White muscle energy stores were significantly depleted in fish from all tournaments when compared with that of the resting laboratory controls. The mean concentrations of PCr, ATP, and glycogen for the tournament walleyes were reduced to 24, 44, and 17% of their respective values in control walleyes (Figure 3). Plasma lactate concentrations were also elevated 4–16 times among the tournament walleyes when compared with those of the laboratory controls (Figure 4B). Sim-

TABLE 1.—Plasma creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activity for walleyes sampled under control conditions (N = 8) or after live-release angling tournaments (CPK: N = 43; LDH: N = 54). Values were compared using a Mann–Whitney U-test and are presented as means ± SEs.

Enzyme	Tournament (IU/L plasma)	Control (IU/L plasma)	P-value
CPK	1360 ± 440	290 ± 77	0.351
LDH	672 ± 72	166 ± 60	0.001

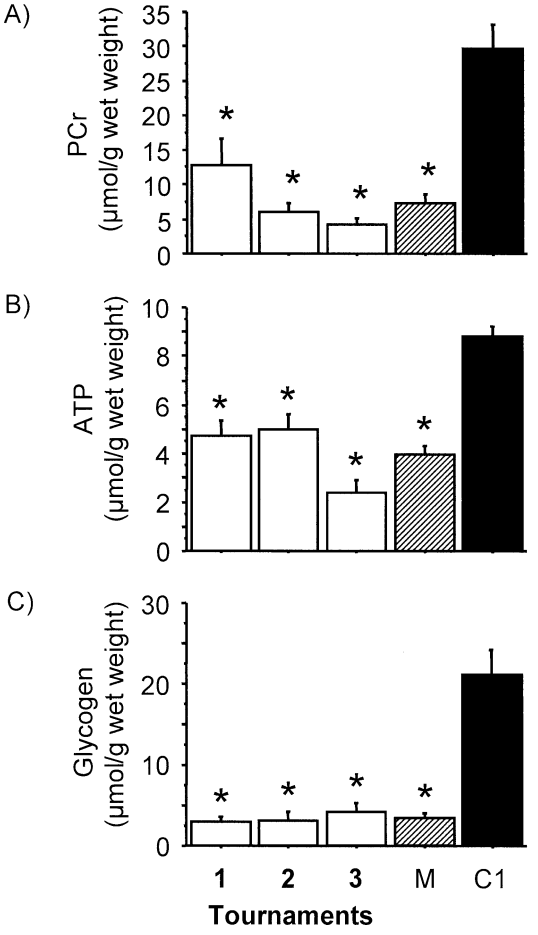

FIGURE 3.—**(A)** White muscle phosphocreatine (Pcr), **(B)** adenosine triophosphate (ATP), and **(C)** glycogen of walleyes sampled after live-release angling tournaments at (1) Belleville, (2) Picton, and (3) Lake Scugog (N = 9–12). The striped bar indicates the combined mean for all tournaments (M). All tournament values are compared with resting laboratory control measurements (C1, N = 8). An asterisk denotes significant difference from the laboratory control (ANOVA, Dunnet's test; $P < 0.05$). Values are presented as means ± SEs.

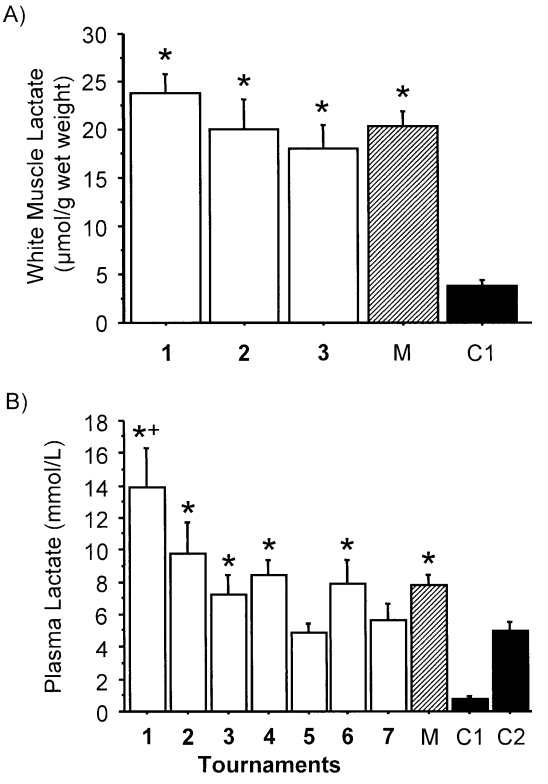

FIGURE 4.—**(A)** White muscle lactate and **(B)** plasma lactate of walleyes sampled after live-release angling tournaments. White muscle samples were collected at three of these tournaments: (1) Belleville, (2) Picton, and (3) Lake Scugog (N = 9–12). Plasma samples were obtained from walleyes captured in tournaments at (1) Belleville, (2) Picton, (3) Lake Scugog, (4) Deseronto, (5) Rice Lake–1st visit, (6) Rice Lake–2nd visit, and (7) Sturgeon Lake (N = 7–13). The striped bar indicates the combined mean for all tournaments (M). The shaded bars represent mean values for the resting laboratory controls (C1, N = 8), and the angled controls (C2, N = 6). An asterisk denotes significant difference from the laboratory control (ANOVA, Dunnet's test; $P < 0.05$). Values are presented as means ± SEs.

ilarly, white muscle lactate was significantly increased at all tournaments, with the tournament mean being over five times higher than that measured for resting laboratory controls (Figure 4A).

Discussion

Several previous studies have investigated the potential impacts of live-release angling tournaments on wild fish populations. However, most of these studies have focused on quantifying tournament mortality. While providing useful information, these earlier studies provided little insight into the underlying physiological changes that occur in fish during live-release tournaments. Understanding the physiological changes that take place in fish during tournaments provides important additional information about the nature of the disturbances that various species experience during these events and may also indicate ways to minimize these disturbances. To our knowledge, this study represents the first thorough investigation into the physiological impacts of live-release angling tournaments on walleyes.

Plasma cortisol and glucose are among the most commonly measured indicators of stress in fish.

Cortisol is secreted during the primary stress response in fish and affects a number of physiological processes, including the mobilization of energy reserves (Gamperl et al. 1994; Wendelaar Bonga 1997). In general, walleyes sampled at tournaments showed greatly elevated plasma cortisol levels, especially when compared with the angled controls. It is noteworthy, however, that the cortisol levels in tournament walleye are within the same range as, or less than, those observed in walleye following other types of stresses (Barton and Zitzow 1995; Forsberg et al. 2001; Barton 2002; Barton et al. 2003). The plasma glucose levels of the tournament walleyes were also about double those of the resting laboratory control walleyes. During stress, circulating glucose is thought to be mainly used by red muscle and cardiac tissue (West et al. 1993; Mommsen et al. 1999). Taken together, the observed changes in plasma cortisol and glucose among tournament walleyes indicate that tournaments elicit a substantial stress response in these fish. In the laboratory control walleyes, the plasma cortisol levels were also much greater than those in walleyes sampled immediately after angling in more natural environments. Even though these fish were quietly resting in well-oxygenated water, confinement in the black holding boxes probably contributed to the increased cortisol levels in the laboratory control walleye. These results indicate that plasma cortisol levels are very responsive to almost any type of disturbance, even when considerable effort is made to provide optimal environmental conditions for the fish.

Changes in osmotic balance have also been frequently used to assess the magnitude of physiological disturbances, or stresses, in fish. A number of factors contribute to plasma osmolality, however, and changes in this plasma constituent may be difficult to interpret. In the present study, the tournament mean for plasma osmolality was not significantly different than that for the laboratory control walleyes, although there was a trend for increased plasma osmolality in fish from several of the tournaments that were sampled. The concentration of plasma chloride, which is an important contributor towards plasma osmolality, was also not significantly different between tournament and control walleyes (Figure 2B). These results are interesting because prolonged severe stressors typically result in a loss of ions (such as plasma chloride) across the gills of freshwater fishes (Carmichael et al. 1984). Although fish may be subject to numerous disturbances during a typical tournament day, these results suggest that, in general, tournaments may not represent a severe chronic stress for walleyes.

For several reasons, the potential impact of tournaments on ion losses in walleyes probably deserves further study. Although the mean chloride values for the tournament walleyes did not show significant declines, there were some individual tournament fish with plasma chloride levels that were markedly reduced when compared with those of the control fish. Numerous previous studies have also shown that acute bouts of anaerobic activity cause a significant hemoconcentration in fish due to water shifts from the plasma and into the white muscle (Holeton et al. 1983; Milligan and Wood 1986; Wood 1991). These changes will cause an apparent rise in plasma ion levels and would explain, for example, why the values for plasma chloride in the group of angled control walleyes are greater than those in the laboratory control walleyes. Based on the observed changes in muscle metabolites, one would expect a significant hemoconcentration in walleyes after tournaments, but the anticipated increase in plasma chloride levels was not observed in these situations. These results could be explained by the possibility that there is some degree of chloride loss occurring concurrently in walleyes during tournaments. At this time, however, further experiments to directly determine unidirectional ion fluxes in walleyes under these conditions are probably required before any final conclusions can be made. Also, the present results indicate that substantial net losses of ions are probably not a serious problem for most walleyes during tournaments.

To determine whether the disturbance resulting from tournaments causes cell damage in walleyes, we also examined the activities of CPK and LDH in plasma. These enzymes are normally confined within the intracellular space of several tissue types (with high concentrations being found in the cardiac and skeletal muscle of fish), and elevated plasma activity is therefore commonly used as an indicator of cell damage (Clarkson 1992; Escher et al. 1999). For example, the plasma CPK activity can be increased several thousand times above resting levels in humans following severe exercise (Clarkson et al. 1992). In the present study, the plasma CPK activity appeared to be somewhat elevated among tournament walleyes, but this increase was not significant when compared to the laboratory control walleyes. In contrast, the plasma LDH activity among tournament walleyes was significantly elevated in comparison with that of the laboratory controls. However, the magnitude

of this increase in LDH activity was still much less than that found in other investigations involving fish where significantly mortality has been observed (Balint et al. 1997; Escher et al. 1999). Taken together, the changes in the plasma activity of these enzymes indicate that walleyes may experience some degree of cell damage during a tournament, but the magnitudes of these changes are not comparable to those in studies where substantial cell damage has occurred or where fish mortality has been documented.

In contrast to many of the other physiological variables monitored in this study, the metabolic status of walleyes changed greatly following tournaments. The post-tournament profile for white muscle energy reserves in walleyes indicates that the tournament process typically results in a large metabolic disturbance. Walleyes sampled after tournaments had greatly reduced levels of the three main fuel sources present in white muscle: PCr, ATP, and glycogen. The posttournament levels of these white muscle energy reserves were also very similar to those in studies where fish have been exercised to complete exhaustion (Dobson and Hochachka 1987; Kieffer et al. 1996; Milligan 1996). In addition, tournament walleyes exhibited large increases in muscle and blood lactate concentrations (Figure 4). Lactate is an end product of anaerobic metabolism that increases in concentration during severe exercise or hypoxia (Milligan and Wood 1986; Boutilier et al. 1988; Milligan 1996). In tournaments, walleyes undergo a bout of exercise during angling but typically have several hours to recover in the live well before being sampled at the end of the tournament. Moreover, fish that are captured in tournaments are usually brought to the boat very quickly, and there is normally no attempt to play the fish to complete exhaustion. Thus, other factors in the tournament process—in addition to angling—probably contributed to this large metabolic disturbance. For example, adverse live well conditions (e.g., hypoxic water, nitrogenous waste accumulation, or temperature change) may prevent normal recovery that would otherwise occur following angling. Walleyes may also experience a large metabolic disturbance during the bag confinement, handling, and air exposure associated with the weigh-in procedure. Although the exact causes of the large metabolic disturbance present in tournament walleyes are currently unknown, this should probably be an important focus of future research. This profound change in metabolic status may represent one of the most important physiological consequences of tournaments for walleyes.

Taken together, the results from the present study suggest that walleyes and largemouth bass differ in their physiological responses to tournament practices (Suski et al. 2003). In contrast to largemouth bass, walleyes do not exhibit large increases in plasma osmolality following tournaments but do show some evidence of net ion losses from the plasma. These differences could be explained by the fact that ion losses across the gills during tournaments are greater in walleyes. The gill permeability of black basses has been shown to be relatively low compared with that of other teleost fish (McDonald et al. 1991), which provides additional support for this possibility, but further experiments are definitely required in this area.

The physiological responses of walleyes and largemouth bass to tournaments are also similar in some important ways. Both species have markedly elevated levels of plasma cortisol and display a profound metabolic disturbance after tournaments. The profound metabolic disturbance in both species is particularly noteworthy and suggests that the current format of tournaments may commonly cause fish to become severely anaerobic. If this is indeed the case, one can speculate that the differences in sensitivity towards tournament stressors that appear to exist between these two species may simply reflect their different capacities to tolerate hypoxia. Further studies are therefore underway in order to determine whether popular tournament species such as largemouth bass and walleyes have markedly different sensitivities towards hypoxia.

In conclusion, our results indicate that walleyes experience a significant physiological disturbance during live-release angling tournaments. In contrast to previous studies on largemouth bass (Suski et al., in press), walleyes suffer moderate cell damage and ion loss during these events. The most striking features of the physiological disturbance following tournaments are very similar between largemouth bass and walleyes, however, and involve a large increase in plasma cortisol and a profound metabolic disturbance. The latter indicates that severe bouts of anaerobic activity may be one of the most important physiological consequences of the current tournament format for these and similar species of fish. Additional experiments are currently underway to further explore this issue and to determine what aspects of tournament procedures are most important in this regard.

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