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The effect of body size on post-exercise physiology in largemouth bass

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Abstract Variation in individual size has important consequences for a number of characteristics of fish, which can impact fish populations. The impact of fish size on recovery following exercise, however, is poorly understood, with little information existing on the recovery of ionic/osmotic variables. The goal of this study was to quantify not only how allometry impacts the magnitude of physiological disturbance following burst exercise in largemouth bass (*Micropterus salmoides*), but also how allometry impacts the time required for exercise-induced disturbances to return to baseline levels. To accomplish this goal, two size classes of largemouth bass (large = 772–1,441 g total weight, mean = 1,125 g; small = 93–238 g, mean = 148 g) were exercised for 60 s and allowed to recover for 0, 1, 2, or 4 h before being sampled for plasma and white muscle. Large largemouth bass exhibited elevated concentrations of plasma glucose and sodium relative to small fish following a common exercise challenge. Large fish required additional time to clear metabolic disturbances in plasma and failed to restore potassium to basal levels even following 4 h of recovery, indicating an improved ability of the smaller fish to recover from disturbances. Results are further discussed in the context of physiological ecology and fitness for largemouth bass.

Keywords Stress · Disturbance · Recovery · Allometry · Scaling

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Introduction

The body size of organisms spans 21 orders of magnitude, ranging from mycoplasmas to whales (Schmidt-Nielsen 1984). Consequently, biological studies involving allometry are widespread. Processes such as metabolism, heart rate, enzyme content of cells, and movement rates all have been shown to vary with size across a range of organisms (Von Bertalanffy 1957; Dial et al. 2008). These allometric characteristics remain consistent, (e.g., metabolic rates decreasing with increasing size) even when considering the animal kingdom's great diversity in anatomy, physiological processes, environmental adaptation, and life history strategies (Von Bertalanffy 1957).

For fishes, variation in individual size has been shown to have important consequences for population-level parameters, with larger individuals often

having a fitness advantage relative to smaller conspecifics. For example, larger fish in a population have been shown to have increased survival probabilities (Hutchings 1994), improved quality of nest sites (van den Berghe and Gross 1989), an improved ability to attract mates (Foote 1988), higher fecundity (Wootton 1998), higher fertilization success (Hutchings and Myers 1988), and elevated brood defense (Suski and Philipp 2004) relative to smaller individuals, which often translates into allometric fitness benefits for larger conspecifics (Hutchings 1991). Individual size also has important consequences for a number of physiological properties of fish. Research has demonstrated that larger individuals show a reduced relative cost of swimming (Schmidt-Nielsen 1972), lower mass-specific metabolic rates (Kieffer and Tufts 1998), lower activity rates of certain enzymes (Davies and Moyes 2007), and an increased concentration of anaerobic fuel in muscle (Kieffer et al. 1996). Considerably less is known, however, about how these allometric differences in physiological properties scale up to influence performance, potentially providing larger individuals with fitness advantages.

One aspect of fish performance that is ecologically relevant and may contribute to this allometric advantage relates to burst activity (exercise), coupled with the associated recovery from the exercise-induced disturbances. Burst swimming in fish is fueled by anaerobic metabolism, and the ability of fish to perform burst exercise represents the integration of an individual's physiology, morphology, and biochemistry (Plaut 2001). More importantly, allometric differences described above may influence the ability of differently sized fish to perform burst exercise required for activities such as avoiding unfavorable conditions, escaping predators, and capturing prey, therefore impacting fitness (Plaut 2001). Additionally, accumulated physiological disturbances (i.e., metabolic wastes) associated with burst activity can impair a fish's ability to perform consecutive burst swims, making them more susceptible to predation, highlighting a potential advantage for accelerated recovery from exercise (Milligan and Wood 1986; Goolish 1991; Jain et al. 1998; Danylchuk et al. 2007). Despite this ecological importance, studies on the impacts of allometry on physiological recovery from exercise in fishes are surprisingly rare. Previous work that has been performed on this topic has largely focused on the magnitude of disturbance in muscle metabolites immediately after exercise (Kieffer et al. 1996). Comparatively few studies have examined the time required for recovery, with few investigations examining ionic/osmotic parameters (Kieffer 2000). Improving our understanding of how allometry can impact recovery from exercise would help elucidate processes potentially limiting performance and influencing fitness.

The goal of the current study was to quantify not only the impact of size on the magnitude of physiological disturbances following burst exercise in fish, but also how allometry influences the rate of recovery from burst exercise, with a particular emphasis on the recovery dynamics of osmotic/ionic disturbances in plasma. Due to their reduced cost of swimming (Schmidt-Nielsen 1972), larger individuals were predicted to experience reduced physiological disturbances and accelerated recovery rates relative to smaller individuals, providing yet another size-based advantage for larger fish.

Materials and methods

The model organism for this study was the largemouth bass (*Micropterus salmoides*). In September 2007, two size classes of largemouth bass were collected by electroshocking the lakes in central Illinois and transported to the Kaskaskia Biological Station (near Sullivan, IL). The large size class of fish ranged from 385 to 463 mm total length (mean = 419 ± 4 mm SE, $N = 30$) and 772–1,441 g total weight (mean = 1,123 g ± 6 g SE, $N = 30$). The small size class of fish ranged from 203 to 273 mm total length (mean = 233 ± 3 mm SE, $N = 30$) and 93–238 g total weight (mean = 148 g ± 2 g SE, $N = 30$). Differences in both total length and total weight were significantly different (*t* tests, $P < 0.05$). More importantly, the use of two distinct size classes of fish that span the range of sizes used in the current study has previously been used for studies of allometry and burst exercise physiology with fish (Kieffer et al. 1996; McDonald et al. 1998). At the Kaskaskia Biological Station, all largemouth bass were first allowed to recover from transport in static, aerated outdoor holding tanks where they were regularly fed fathead minnows (*Pimephales promelas*). Following a 10-day recovery period, fish were

moved indoors and held under natural photoperiods in aerated tanks (540 l) filled with re-circulated, filtered water. Water in these indoor tanks was maintained at 20.3 ± 0.2°C. Dissolved oxygen was kept at 7.3 ± 0.2 mg l^{-1}, verified with a YSI 85 oxygen meter (Columbus, OH). A commercially available kit (Model # 33D, Aquarium Pharmaceuticals Inc., Chalfont, PA) was used to confirm that total ammonia levels (T_{amm}) remained below one part per million throughout the holding and sampling period. Prior to experimentation, all fish were fasted 48–60 h.

Control fish and sampling

To generate resting control values for physiological variables, six fish of each size class were transferred from indoor holding tanks to individual, aerated, darkened chambers supplied with circulating water. Two different sizes of containers were used for each size class of fish, with containers approximately 11 l used for the small size class, while containers of approximately 68 l were used for the large size class. Fish were allowed to acclimate to these darkened individual chambers for 24 h. After 24 h, the flow of water to each chamber was terminated, and each fish was lethally anesthetized with an overdose of anesthetic (250 mg l^{-1} 3-aminobenzoic acid ethyl ester methanesulphonate [MS-222] buffered with 500 mg l^{-1} $NaCO_3$ as per Summerfelt and Smith 1990; Suski et al. 2006). Upon cessation of ventilation, fish were weighed to the nearest gram, measured to the nearest mm (total length), and blood withdrawn from the gill arch using a 1.0 ml syringe rinsed with lithium heparin (Houston 1990). Blood was centrifuged for 120 s at 2,000*g* immediately after extraction. Plasma was separated from erythrocytes using a disposable transfer pipette, divided into three 100 µl aliquots in 1.5 ml microcentrifuge tubes, and immediately frozen in liquid nitrogen. In addition, a portion of white epaxial muscle (approximately 50–100 g) was excised from behind the left operculum and above the lateral line using a razor blade, freeze clamped with aluminum tongs (pre-cooled in liquid nitrogen), wrapped in aluminum foil, and immediately frozen in liquid nitrogen (Suski et al. 2006). For storage prior to analyses, all samples (plasma and muscle) were transferred from the field to an ultra cold freezer (<−75°C) using a liquid nitrogen-filled dewar.

Burst exercise challenge

To generate physiological disturbances, both size classes of largemouth bass were removed from indoor holding tanks and chased by tail pinching for 1 min in a circular tank >2 m in diameter similar to methods in earlier studies (Suski et al. 2006; Redpath et al. 2010). Previous studies have shown that a chase duration of 1 min is an adequate duration of time to induce significant physiological disturbances, including studies with largemouth bass (Wood et al. 1983; Milligan 1996; Suski et al. 2003, 2007a, b), and is also relevant to the life of largemouth bass, which are an important sport fish routinely angled for this duration (Milligan 1996; Suski et al. 2003, 2007a, b). Following this period of exercise, some fish were immediately transferred to a container of water with a lethal dose of anesthetic and sampled for blood and white muscle as described above. This made up group "0 h", i.e., the amount of time provided for recovery before sampling the fish for blood and muscle.

To quantify the length of time required to recover following exercise, other largemouth bass were first exercised for 1 min as described above and then transferred to individual, aerated chambers supplied with re-circulated water, where they were allowed to recover for either 1, 2, or 4 h (Suski et al. 2006). Following these various time points, the flow of water to chambers was stopped; a lethal dose of anesthetic was added to each chamber; and fish were sampled for blood and white muscle as described above. Sample sizes for this study were six fish per treatment group, which is common for studies of this nature (Wang et al. 1994; Suski et al. 2007a, b; Wood et al. 2010).

Laboratory analysis

Plasma potassium (K^+) and sodium (Na^+) concentrations were quantified with a digital flame photometer (Cole-Parmer Instrument Company, Model 2655-00, Chicago, IL), whereas plasma chloride (Cl^-) concentrations were determined using a digital chloridometer (Labconco, Model 4425000, Kansas City, MO). Plasma glucose and lactate concentrations were determined enzymatically based on the methods of Lowry and Passonneau (1972) using a microplate spectrophotometer (Molecular Devices, Spectra Max Plus 384, Model # 05362, Union City, CA). Commercially

available kits were used to determine concentrations of both plasma cortisol (Assay Designs, Kit # 900-071, Ann Arbor, Michigan) and plasma hemoglobin (Bio-Assay Systems, QuantiChrom Hemoglobin Assay Kit, DIHB-250, Hayward, CA). The activity of lactate dehydrogenase (LDH; enzyme number 1.1.1.27) in plasma was determined using standard kinetic spectrophotometric techniques based on the methods of Wroblewski and LaDue (1955).

Frozen muscle was ground with a mortar and pestle under liquid nitrogen. Metabolites from ground muscle were extracted according to the procedure described in Booth et al. (1995). Extracts were subsequently used for determining the concentrations of lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP) following the enzymatic methods of Lowry and Passonneau (1972). Muscle water content was quantified by drying tissue samples at 80°C for 48 h and comparing wet mass to dried mass.

The total anaerobic energy expenditure (AEE) in the white muscle of fish from each size class was expressed in terms of ATP equivalents according to

$$\mathrm{AEE} = (\Delta \text{ lactate } \times 1.5) + \Delta \text{ ATP} + \Delta \text{ PCr}$$

where Δ represents the difference between control and exercise values, 1.5 units of ATP are generated per unit of lactate, and 1 unit of PCr is equal to 1 unit of ATP (McDonald et al. 1998).

Statistical analyses

Comparison of physiological response variables was conducted using a two-way analysis of variance (ANOVA) (main effects: size class and recovery duration, as well as their interaction). Main effects of ANOVAs were not interpreted when the interaction term in the two-way ANOVA was significant, but were interpreted when the interaction was not significant. An LSMeans Tukey HSD *post hoc* test was used to separate means where appropriate (Zar 1999). All statistical analyses were performed using JMP version 7.0 (SAS Institute, Cary, NC). The level of significance (α) for all tests was 0.05.

Results

One minute of exercise did not induce any time-specific differences in concentrations of plasma glucose for either size class of fish (Fig. 1a; Table 1, $P > 0.05$). Across all sampling periods combined, however, the large size class of fish did exhibit plasma glucose concentrations that were significantly higher than the small size class of fish (Fig. 1a; Table 1, $P < 0.05$). For several time points following exercise, differences in plasma glucose concentrations for large fish were almost twice that of small fish (Fig. 1a). Although plasma cortisol concentrations were similar between fish sizes across all recovery durations, large fish showed a 40% decrease in plasma cortisol relative to control values immediately following exercise that returned to resting by 1 h of recovery (Fig. 1b; Table 1, $P < 0.05$). For both size classes of largemouth bass, the activity of

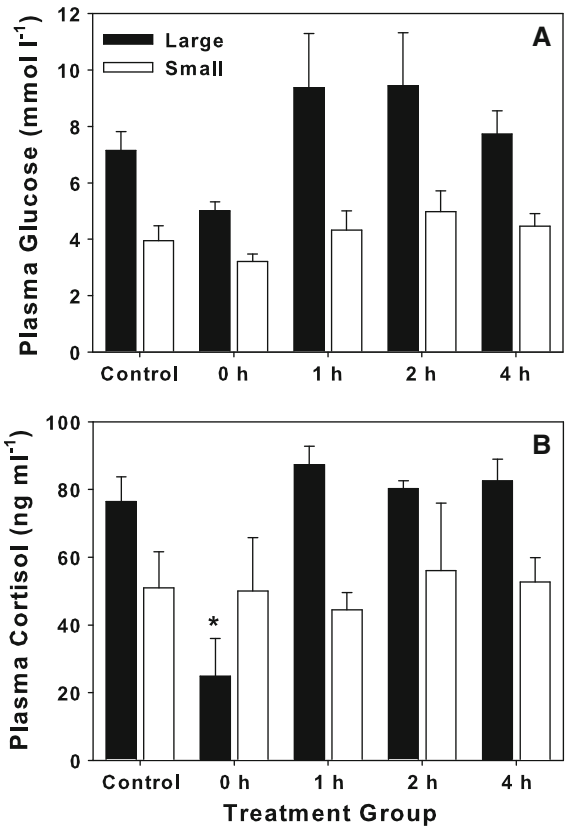

Fig. 1 Plasma glucose concentration (**a**) and cortisol concentration (**b**) in small and large largemouth bass exercised for 1 min and recovered for up to 4 h in ambient oxygenated water. Differences in concentrations of glucose and cortisol were statistically significant across size classes, with concentrations higher for large fish (ANOVA $P < 0.05$). Six fish were sampled to generate each bar

Table 1 Results of two-way ANOVAs examining the influence of size class (large vs. small) and recovery time (1, 2 or 4 h) following 1 min of exercise for physiology in largemouth bass

Variable	Source	*df*	SS	*F*	*P* value	Variable	Source	*df*	SS	*F*	*P* value
Plasma	Entire model	9	21,924.9	3.7	0.0012	Plasma	Entire model	9	40,640.2	2.2	0.036
[Cortisol]	Recovery time	4	8,138.8	3.1	0.023	[LDH]	Recovery time	4	17,903.3	2.2	0.082
(ng mg l^{-1})	Size class	1	5,676.3	8.7	0.0048	(U l^{-1})	Size class	1	1,082.5	0.5	0.47
	Size × recovery time	4	8,109.8	3.1	0.023		Size × recovery time	4	19,394.6	2.4	0.06
	Error	50	32,528.1				Error	49	99,328.3		
Plasma	Entire model	9	279.7	5.2	<0.0001	Muscle	Entire model	9	1,315.9	9.9	<0.0001
[Glucose]	Recovery time	4	71.4	3.0	0.028	[PCr]	Recovery time	4	1,135.6	19.3	<0.0001
(mmol l^{-1})	Size class	1	189.2	31.6	<0.0001	(μmol g^{-1})	Size class	1	76.4	5.2	0.027
	Size × recovery time	4	19.1	0.8	0.53		Size × recovery time	4	103.8	1.8	0.15
	Error	50	299.1				Error	50	735.4		
Plasma	Entire model	9	28.9	17.7	<0.0001	Muscle	Entire model	9	170.1	2.2	0.035
[K^+]	Recovery time	4	7.3	10.0	<0.0001	[ATP]	Recovery time	4	123.8	3.7	0.011
(mEq l^{-1})	Size class	1	12.7	69.8	<0.0001	(μmol g^{-1})	Size class	1	1.0	0.1	0.73
	Size × recovery time	4	8.9	12.3	<0.0001		Size × recovery time	4	45.3	1.3	0.27
	Error	50	9.1				Error	50	423.5		
Plasma	Entire model	9	37,086.0	23.6	<0.0001	Plasma	Entire model	9	620.7	12.7	<0.0,001
[Na^+]	Recovery time	4	16597.7	23.8	<0.0001	[Lactate]	Recovery time	4	557.0	25.6	<0.0001
(mEq l^{-1})	Size class	1	13,535.6	77.6	<0.0001	(mmol l^{-1})	Size class	1	28.7	5.3	0.026
	Size × recovery time	4	6,952.6	10.0	<0.0001		Size × recovery time	4	35.0	1.6	0.19
	Error	50	8,726.4				Error	50	272.5		
Plasma	Entire model	9	2,843.4	1.4	0.23	Muscle	Entire model	9	954.1	15.8	<0.0001
[Cl^-]	Recovery time	4	1,877.0	2.0	0.10	[Lactate]	Recovery time	4	766.6	28.5	<0.0001
(mEq l^{-1})	Size class	1	19.3	0.1	0.77	(μmol g^{-1})	Size class	1	133.5	19.9	<0.0001
	Size × recovery time	4	947.1	1.0	0.40		Size × recovery time	4	54.0	2.0	0.11
	Error	50	11,564.2				Error	50	336.1		
Plasma	Entire model	9	833.1	0.7	0.71	Fish	Entire model	9	14,468,074.0	89.4	<0.0001
[Hemoglobin]	Recovery time	4	279.1	0.5	0.72	Mass	Recovery time	4	101,917.0	1.4	0.24
(mg dl^{-1})	Size class	1	292.1	2.2	0.15	(g)	Size class	1	14,256,450.0	792.7	<0.0001
	Size × recovery time	4	275.0	0.5	0.72		Size × recovery time	4	109,707.0	1.5	0.21
	Error	48	6,388.6				Error	50	899,220.0		

Table 1 continued

Variable	Source	*df*	SS	*F*	*P* value	Variable	Source	*df*	SS	*F*	*P* value
Muscle	Entire model	7	3,479.5	12.5	<0.0001						
[AEE]	Recovery time	3	2,696.0	22.7	<0.0001						
(μmol g^{-1})	Size class	1	495.0	12.5	0.001						
	Size × recovery time	3	288.5	2.4	0.07						
	Error	40	1,586.6								

df degrees of freedom, *SS* sum of squares

LDH in plasma, as well as the concentration of hemoglobin in plasma, remained unchanged throughout all treatments in this study (Fig. 2; Table 1, $P > 0.05$).

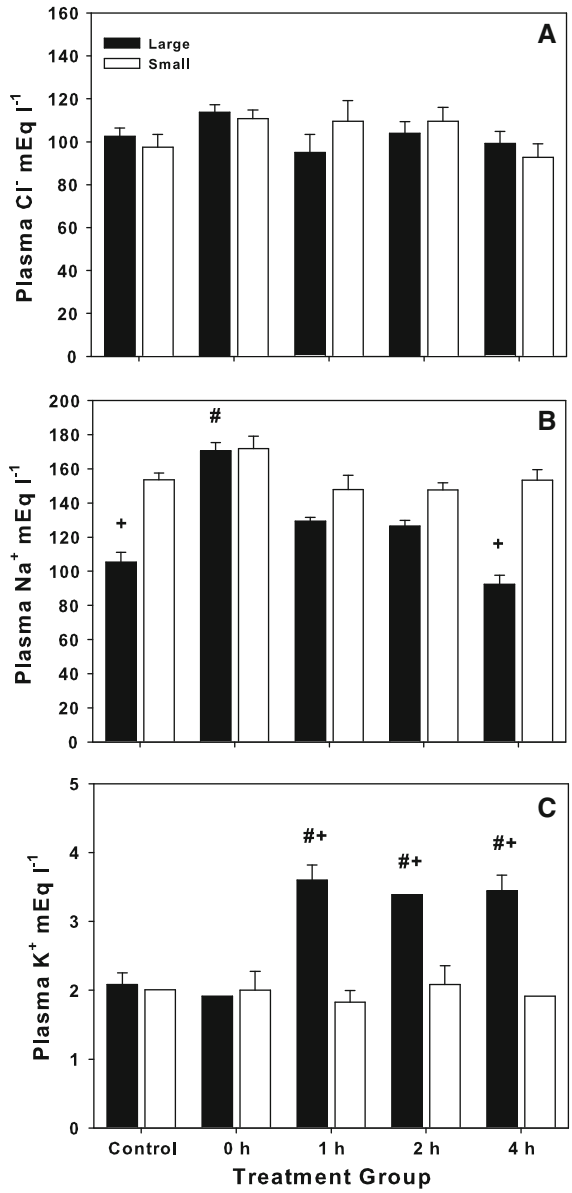

Fig. 2 Plasma chloride (**a**), sodium (**b**) and potassium (**c**) concentration in small and large largemouth bass exercised for 1 min and recovered for up to 4 h in ambient oxygenated water. A *pound sign* (#) represents a significant difference from the control value within a size class. A *plus sign* (+) represents a value that is significantly different between size classes at a specific time point (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

Neither 1 min of exhaustive exercise nor 4 h of recovery, induced any significant changes to plasma chloride concentrations for either size class of largemouth bass (Fig. 3a; Table 1, $P > 0.05$). In addition, there were no observed changes in either plasma sodium concentration (Fig. 3b; Table 1, $P > 0.05$) or plasma potassium concentration at any point in the study within the small size class of largemouth bass (Fig. 3c; Table 1, $P > 0.05$). While there were no differences across size classes in sodium concentrations at 0 h post-stress, plasma sodium concentrations in large fish increased by approximately 70% relative to the control treatment immediately following exercise and then returned to resting levels following 1 h of recovery (Fig. 3b; Table 1, $P < 0.05$). Large fish also exhibited a near doubling of plasma potassium concentrations at 1 h post-exercise, and this disturbance did not return to control levels even after 4 h of recovery (Fig. 3c; Table 1, $P < 0.05$).

One minute of exercise did not result in significant differences in muscle ATP concentrations across the two size classes of largemouth bass (Fig. 4a; Table 1, $P > 0.05$). Immediately following 1 min of exercise, both size classes of largemouth bass consumed over 70% of white muscle PCr stores relative to control concentrations (Fig. 4a; Table 1, $P < 0.05$). White muscle PCr was restored to control levels for both size classes within 1 h of recovery, but differences were detected across size classes, with concentrations of muscle PCr for the large size class significantly lower than that of small largemouth bass (Fig. 4b; Table 1). Exercise resulted in an increase in plasma

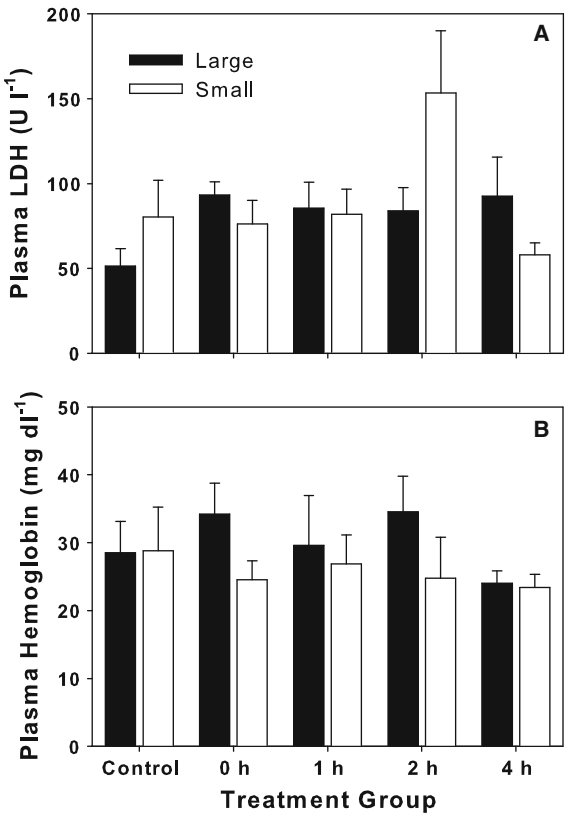

Fig. 3 Plasma lactate dehydrogenase activity (**a**) and hemoglobin concentration (**b**) in small and large largemouth bass exercised for 1 min and allowed to recover up to 4 h in ambient oxygenated water. There were no significant differences across size classes or sampling points. Six fish were used to generate each bar

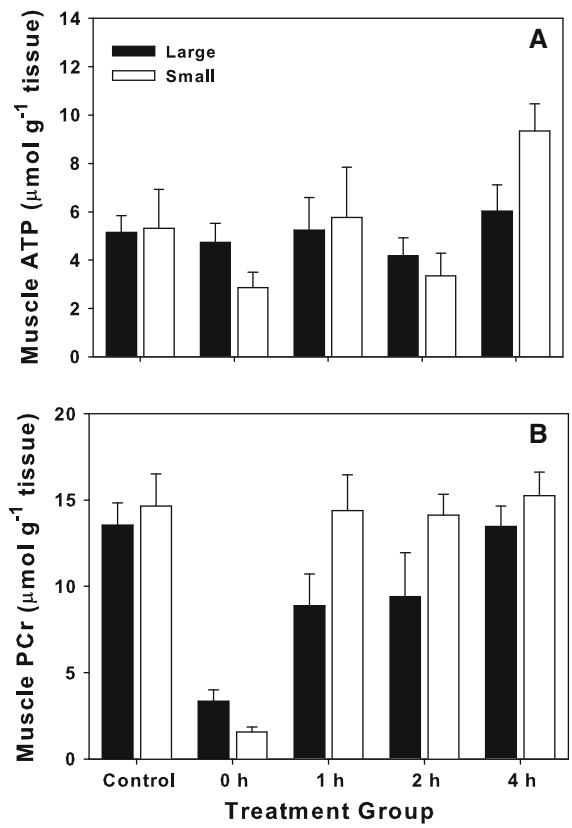

Fig. 4 Muscle adenosine triphosphate (ATP) (**a**) and phosphocreatine (PCr) (**b**) concentration in small and large largemouth bass exercised for 1 min and recovered for up to 4 h in ambient oxygenated water. Differences in ATP concentration did not vary statistically. Concentrations of PCr varied significantly across size classes, with large fish having significantly lower concentrations of (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

and white muscle lactate concentration of approximately threefold and tenfold, respectively, for both the large and small size classes of largemouth bass, with lactate concentrations in both plasma and muscle significantly greater for the large size class of fish (Fig. 5c; Table 1). Even after 4 h of recovery, concentrations of muscle lactate in large fish were over fourfold greater than that of small fish, while concentrations of plasma lactate were approximately 25% greater than that of small fish (Fig. 5c). The anaerobic energy expenditure of large fish was significantly greater than the energy expenditure of small fish, although there was no significant interaction between size classes and sampling at a specific time point (Fig. 6; Table 1). There was no difference

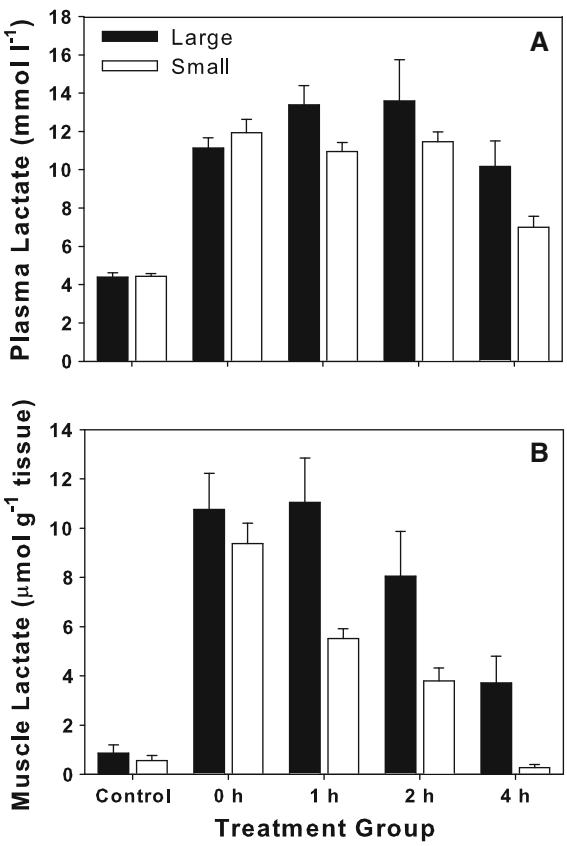

Fig. 5 Plasma (**a**) and muscle (**b**) lactate concentration in two size classes of largemouth bass exercised for 1 min and then recovered for up to 4 h in ambient oxygenated water. A *pound sign* (#) represents a significant difference from the control value within a size class (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Concentrations of lactate in muscle were significantly lower for the large size class (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

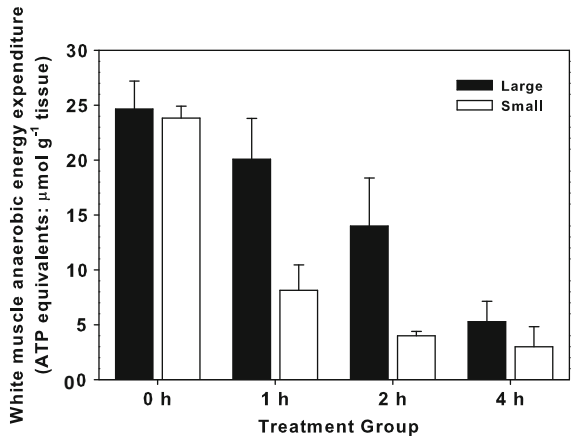

Fig. 6 White muscle anaerobic energy expenditure (AEE) for small and large largemouth bass exercised for 1 min and recovered for up to 4 h in ambient oxygenated water. White muscle AEE concentrations significantly across size classes, with large fish having significantly higher expenditure of (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

in muscle water content across any treatment group ($P > 0.05$, data not shown).

Discussion

Following 1 min of forced exercise, largemouth bass exhibited changes to several tissue and ionic parameters, with significant differences exhibited across the two size classes examined. More specifically, large largemouth bass exhibited plasma glucose concentrations that were more than twice that of smaller conspecifics throughout the experiment and showed a 70% increase in plasma sodium concentration following exercise. All of these disturbances were either significantly reduced, or else absent, in the smaller size class, despite the same duration of exercise. Large largemouth bass also showed significantly greater total anaerobic capacity than smaller individuals, evidenced by significantly greater concentrations of lactate in both muscle and plasma and greater expenditure of anaerobic energy in white muscle (anaerobic equivalents) (Goolish 1991), as well as a short-term decrease in plasma cortisol concentration immediately following exercise. Previous studies have demonstrated allometric variation in a number of physical properties in fishes, with larger individuals having an increased gill surface area (Oikawa

and Itazawa 1985), elevated activities of anaerobic enzymes (Davies and Moyes 2007), and increased buffering capacity of blood and muscle (Nelson and Magnuson 1987) compared with smaller conspecifics, highlighting the potential for size-specific physiological responses to stressors. Earlier work has shown that concentrations of both Na^+ and K^+ in plasma increase following exercise in fish, with the source of these cations being either intracellular metabolic waste (Wang et al. 1994), erythrocytic Na^+/K^+ exchangers (Borgese et al. 1987), or gill tissue (Wood and LeMoigne 1991). In the current study, increases in plasma Na^+ and K^+ following exercise were similar in magnitude to earlier work, but only for the large size class of largemouth bass. Because we did not observe any differences in muscle water content (Milligan and Wood 1986; Parkhouse et al. 1987; Wang et al. 1994) either within or among treatments, nor was there evidence of hemolysis from red cell rupturing, it is likely that the observed ionic disturbances in large individuals resulted from true changes in plasma ion concentrations and were not artifacts of fluids shifting between intra- and intercellular compartments. Work by Kieffer et al. (1996) did not report allometric differences in total anaerobic capacity (i.e., lactate production) for two size classes of exercised largemouth bass, contrary to findings in the current study (Goolish 1991; Kieffer et al. 1996). This discrepancy in results likely occurred because the study by Kieffer et al. (1996) used smaller fish in their large size category [range for Kieffer et al. (1996) 290–360 mm fork length compared with a range of 385–463 mm total length in the current study], and these researchers did not sample fish during recovery, providing a coarse examination of total anaerobic capacity. Cortisol is the primary stress hormone produced by fishes, whereas glucose is an aerobic fuel produced as part of the secondary stress response used by tissues such as gill and heart (Mazeaud et al. 1977; Wendelaar Bonga 1997). The decrease in plasma cortisol concentration of large fish can likely be attributed to uptake of cortisol by tissues such as liver or muscle (Mommsen et al. 1999). Both the control and post-exercise values of cortisol and glucose are within the range of values previously reported for largemouth bass (and other fish species) (Milligan 1996; Suski et al. 2007a, b; VanLandeghem et al. 2010) and are well below values for individuals displaying a chronic stress response (Suski et al. 2003). Together, results from the current study indicate that different size classes of largemouth bass exhibit differences in the immediate physiological response to forced exercise, with large bass exhibiting a greater degree of osmotic/ionic disturbances in plasma and total anaerobic capacity than smaller individuals.

In addition to the differences observed in their immediate response to exercise, the large and small size classes of largemouth bass exhibited differences in delayed physiological responses following exercise, with smaller fish recovering faster than large fish. The current study showed that concentrations of lactate in the plasma and muscle of small largemouth bass were lower than large fish throughout recovery despite similar post-exercise values, as were concentrations of plasma glucose. Similarly, concentrations of plasma K^+ in large largemouth bass were double that of control values at four hours post-exercise, while K^+ disturbances in small largemouth bass did not differ from control values. As reviewed in Bœuf and Payan (2001), osmotic regulation accounts for 20 to >50% of the resting energy expenditure of several freshwater fishes, indicating a relatively large energetic cost to correct altered ionic status. Accelerated recovery rates of metabolites in smaller fish have previously been attributed to smaller individuals having a greater per gram metabolic rate than larger individuals, or else an increased reliance on aerobic processes during burst swimming, thereby resulting in accelerated returns to homeostasis (Wakefield et al. 2004; Ohlberger et al. 2005). An alternative explanation, however, is that smaller fishes have a smaller diameter of muscle fibers relative to larger individuals resulting in a greater surface area per unit length for the muscle fibers, permitting facilitated metabolic exchange of nutrients and accelerated clearance of waste products (Weatherly 1990). In addition, even though the two size classes of largemouth bass in this study produced similar quantities of white muscle lactate on a per gram of tissue basis, when this difference is scaled up across the entire mass of white muscle within the fish, substantial differences are realized. More specifically, assuming that white muscle constitutes 90% of the mass of our fish (Goolish 1991; Sänger and Stoiber 2001), large largemouth bass would have produced approximately five times more total lactate than small largemouth bass, and current results did demonstrate elevated

anaerobic energy expenditure for large fish. Therefore, it may take more time for lactate accumulations to clear in larger largemouth bass. Although not investigated in this study, the rate of muscle lactate clearance in large fish may be hindered further by allometric differences in enzyme activities. In several fish species, larger individuals have lower activities of citrate synthase than smaller individuals, a difference that can result in dissimilar recovery rates (Childress and Somero 1990a; Davies and Moyes 2007). Together, results from the current study demonstrate that large largemouth bass experience prolonged recovery times following disturbance relative to small largemouth bass, especially relating to the clearance of metabolic wastes.

Despite several size-specific responses, there were instances where small and large largemouth bass exhibited similar physiological responses to forced exercise. Both large and small largemouth bass consumed similar relative amounts of muscle PCr and ATP following exercise and had similar concentrations of LDH in plasma following exercise. Previous studies investigating forced exercise in salmonid fishes [Atlantic salmon (*Salmo Salar*) (Wakefield et al. 2004), brook trout (*Salvelinus fontinalis*) (Kieffer et al. 1996), and rainbow trout (Goolish 1989)] noted that PCr consumption was greater in larger individuals compared with smaller individuals, that larger individuals experienced a greater acid-base disturbance relative to smaller individuals, and that larger fish also had elevated lactate production relative to smaller conspecifics. These differences in metabolic disturbances across size classes of salmonid fishes were attributed to either an elevated cost of swimming (i.e., increased power requirement) (Schmidt-Nielsen 1972; Goolish 1991), increased anaerobic enzyme activity (increases in lactate dehydrogenase and creatine phosphokinase), and/or decreased aerobic potential (citrate synthase activity) (Ferguson and Tufts 1992) for larger individuals. These differences might have led to a greater consumption of energy stores (Kieffer et al. 1996) and potentially increased production of anaerobic waste products for larger fish. The fact that these allometric differences were not observed in this study using largemouth bass suggests that species-specific differences in lifestyle between non-migratory largemouth bass and highly migratory species such as salmonid fishes may be responsible for those differences.

Largemouth bass are adapted to a sedentary lifestyle with reliance on ambush tactics to obtain prey compared with more active, migratory salmonid fishes. As such, largemouth bass typically experience brief, intermittent movement patterns with few daily large-scale movements (Demers et al. 1996), and the relative amount of white (anaerobic) muscle exceeds 90% of the trunk in centrarchid fishes (Davies and Moyes 2007) compared with 70% for salmonids (Sänger and Stoiber 2001). Furthermore, the activity of LDH in white muscle, which is indicative of potential for anaerobic glycolysis, can vary across fish species that have different locomotory habits. For example, white muscle LDH activity is typically greater for active and migratory rainbow trout relative to sedentary, benthic fish species such as the Dover sole (*Microstomus pacificus*) (Childress and Somero 1990b). Unlike what has been reported in rainbow trout, prolonged low-velocity swimming in largemouth bass failed to facilitate recovery from exercise, indicating that differences in recovery patterns across species are likely a result of different lifestyle preferences (Suski et al. 2007a, b). Finally, greater acceleration for centrarchid fishes relative to salmonid fishes [i.e., bluegill (*Lepomis macrochirus*) compared with rainbow trout] further emphasizes reliance on ambush tactics relative to cruising (Webb 1978). Together, these findings suggest that the varied evolutionary pressures found in differing environments inhabited by salmonids as compared to centrarchids have resulted in the different physiological responses following exercise, including a lack of allometric responses to exercise-induced disturbances to muscle energy stores.

Our study emphasizes the need to ensure a narrow size distribution of subjects for studies examining stress responses in fish and cautions against applying findings relating to stress and disturbance to conspecifics that differ in size. For example, previous studies of exercise, stress, and disturbance in rainbow trout by Pagnotta and Milligan (1991) used fish that ranged from 100 to 250 g. Similar work by Primmett et al. (1986) used rainbow trout that ranged from 200 to 300 g, while Wood et al. (1983) used rainbow trout that ranged from 200 to 400 g. By comparison, largemouth bass in this study ranged from 93 to 1,441 g across both size classes. In many of these earlier studies, a narrow size range of fish was likely used intentionally to control for allometric

differences in physiological responses (e.g., Wakefield et al. 2004). Results, however, are often then applied across a broad range of fish sizes and even across different fish species with different ecological roles and lifestyles (i.e., sedentary vs. active, migratory vs. non-migratory, etc.).

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