J Comp Physiol B (2010) 180:371–384
DOI 10.1007/s00360-009-0419-4

ORIGINAL PAPER

Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish

Andrew James Gingerich · David P. Philipp · Cory D. Suski

Received: 31 July 2009 / Revised: 23 October 2009 / Accepted: 3 November 2009 / Published online: 20 November 2009

Abstract The influence of feeding on swimming performance and exercise recovery in fish is poorly understood. Examining swimming behavior and physiological status following periods of feeding and fasting is important because wild fish often face periods of starvation. In the current study, researchers force fed and fasted groups of largemouth bass (*Micropterus salmoides*) of similar sizes for a period of 16 days. Following this feeding and fasting period, fish were exercised for 60 s and monitored for swimming performance and physiological recovery. Resting metabolic rates were also determined. Fasted fish lost an average of 16 g (nearly 12%) of body mass, while force fed fish maintained body mass. Force fed fish swam 28% further and required nearly 14 s longer to tire during exercise. However, only some physiological conditions differed between feeding groups. Resting muscle glycogen concentrations was twofold greater in force fed fish, at rest and throughout recovery, although it decreased in both feeding treatments following exercise. Liver mass was nearly three times greater in force fed fish, and fasted fish had an average of 65% more cortisol throughout recovery. Similar recovery rates of most physiological responses were observed despite force fed fish having a metabolic rate 75% greater than fasted fish. Results are discussed as they relate to largemouth bass starvation in wild systems and how these physiological differences might be important in an evolutionary context.

Keywords Exercise · Nutritional status · Recovery physiology

Communicated by I. D. Hume.

A. J. Gingerich (✉) · C. D. Suski
Department of Natural Resources and Environmental Science, University of Illinois, C-519 Turner Hall (MC-047), 1102 South Goodwin Ave., Urbana, IL 61801, USA
e-mail: andrewgingerich@hotmail.com

A. J. Gingerich · D. P. Philipp
Center for Aquatic Ecology, Institute of Natural Resource Sustainability, University of Illinois, Champaign, IL 61820, USA

Introduction

Biotic and abiotic conditions vary in aquatic ecosystems, potentially impacting the availability and accessibility of prey resources for fish. Many factors, including water temperature, salinity and turbidity, can act together or independently to impact the ability of a fish to capture prey (Goede and Narton 1990; Post and Parkinson 2001; Barton et al. 2002). Variation in prey consumption can have important impacts on nutritional status (defined here as the relative maintenance of liver and somatic energy stores, blood oxygen carrying capacity, and ionic balance) in fish, and nutritional status has been shown to vary with season (Adams et al. 1982), reproductive status (Brown and Murphy 2004), social dominance (McCarthy et al. 1992) and swimming performance (Kolok and Farrell 1994). As a result, nutritional status can vary across fish populations in different waterbodies, within individual fish from the same population, and within a given fish during seasonal and ontogenetic changes (Mackereth et al. 1999; Brown and Murphy 2004). Nutritional status can have pronounced impacts on the performance and physiological condition of fish across a number of different levels of organization. For example, the concentration of anaerobic fuels that are important for burst swimming, ionic status important for nerve conduction, and oxygen carrying tissues such as red

blood cells all have the potential to be impacted by the nutritional status of a fish. When fish are able to feed adequately, they can accumulate energy stores, maintain the activity rates of important enzymes (Deng et al. 2004), conserve swimming ability and exhibit increases in both length and weight (Mendez and Wieser 1993). Following periods of starvation, fish can lose energy reserves, demonstrate elevated concentrations of blood glucose (hyperglycemia) (Scarabello et al. 1991) and experience mass loss (Sheridan and Mommsen 1991; Vijayan and Moon 1992; Navarro and Gutierrez 1995). Further, fish have been shown to reduce activities of anaerobic and aerobic enzymes (Sullivan and Somero 1983; Yang and Somero 1993) and blood oxygen carrying capacity (Rios et al. 2002) after prolonged periods of fasting. The response of resting metabolic rate to fasting in fishes has not been clearly defined to date (see Scarabello et al. 1991; Alsop and Wood 1997), preventing a clear understanding of the relationship between these two variables.

Changes to performance and physiological condition that result from reduced nutritional status can have important ecological implications for wild fishes. For example, individual fish having higher energy stores exhibit greater over-winter survival than conspecifics with lower energy (Miranda and Hubbard 1994), and reduced nutritional status can motivate foraging activities putting a foraging fish at risk of predation (Garvey et al. 2004). In addition, when fish feed effectively they have an increased ability to find mates (Druker 1996; Plaut 2001), are more capable of avoiding predators (Videler 1993; Reidy et al. 1995; Watkins 1996) and therefore, potentially have a fitness advantage over food-deprived conspecifics (Plaut 2001). Of course, all of these observations can be highly species specific.

To date, mechanistic links between fish nutritional status, associated changes in physiological parameters and the resulting changes in individual performance have not been clearly identified. For example, although previous work has demonstrated a decrease in anaerobic energy stores when fish are starved (Scarabello et al. 1991; Keiffer and Tufts 1998), studies have not clearly shown that this reduced energy content is responsible for declines in ecologically relevant performance or function such as swimming ability, the maintenance of ionic balance or the ability to recover from stress-induce disturbances. Furthermore, while fish have been shown to reduce activities of anaerobic and aerobic enzymes (Sullivan and Somero 1983; Yang and Somero 1993) and blood oxygen carrying capacity (Rios et al. 2002) after prolonged periods of fasting, it has yet to be shown that alterations in physiological parameters have any impact on ecologically important metrics such as swimming performance or the ability to recover from stress-induced disturbances, both of which can be important for free-swimming fish in the wild.

The goals of the following study were to determine the impact of nutritional status on (1) the performance of largemouth bass (*Micropterus salmoides*), defined as metabolic rate and swimming ability, (2) the source and magnitude of physiological disturbances following exercise, and (3) the ability of largemouth bass to return to baseline physiological conditions following exercise-induced disturbances. To achieve these goals, we used a force feeding and fasting regime to manipulate nutritional status in two groups of fish that were exposed to a common exercise challenge. Results will provide a mechanism to link nutritional status and ecologically relevant performance, generating an improved understanding of how wild fish can be affected by periods of starvation.

Materials and methods

Fish husbandry

On 13 April 2008, 127 wild largemouth bass (mass = 134 ± 2 g standard error (SE); starting mean length = 221 ± 1 mm SE) were obtained from the Illinois Natural History Survey. Largemouth bass were of mixed origin collected from various locations across Illinois, and all fish had been held for 2–4 months in outdoor earthen ponds stocked with fathead minnows (*Pimephales promelas*) as forage prior to experiments. Fish were removed by draining the pond and dip netting fish at the collection box. Collected largemouth bass were taken to the aquatic research facility at the University of Illinois where they were weighed to the nearest *g*, given an individual fin clip and randomly placed in one of five outdoor 960 L tanks. Tanks were connected to a 0.04 ha earthen research pond using a submersible pump (McMaster-Carr 42945K29, Atlanta, GA, USA), and water supplied to the tanks was returned to the earthen pond for filtration and removal of waste. Tanks were measured daily for temperature (mean = 15.21 ± 0.31°C) and dissolved oxygen (mean = 9.80 ± 0.18 mg/L) with a portable meter (YSI, 550A Yellow Springs Instruments, Irvine, CA, USA), and a commercially available kit (Aquarium Pharmaceuticals Inc, LR8600, Chalfont, PA, USA) confirmed that total ammonia remained <0.25 ppm throughout the study. Each of the five 960 L tanks contained approximately 25 largemouth bass, and the tanks all contained fish from a single experimental treatment: two tanks contained largemouth bass from the 'force fed' treatment, two tanks contained largemouth bass from the 'fasted' treatment, and the final tank contained fish that were not disturbed during the experiment, hereafter referred to as non-handled controls (NHC).

Feeding and fasting

Starting 14 April 2008, largemouth bass ($N = 50$) assigned to the 'force fed' treatment were collected in dip nets from the 960 L tanks every other day and force fed a standard maintenance ration of food at a rate of 1.6–3.0% average body mass. This feeding treatment lasted for 16 days (until 30 April) and was intended to provide sufficient energy to prevent weight loss (Congleton and Wagner 2006). Food consisted of dense culture fish food (40% protein, Aquatic Ecosystems Inc., Chalfont, PA, USA) mixed with deionized water to form a paste. Food was administered using a 25 mL syringe outfitted with a soft, flexible tube that was inserted orally into the gullet of the fish. Feeding was halted 48 h prior to sampling to allow for the digestion of food prior to experiments.

A second group of largemouth bass ($N = 50$) was assigned to the 'fasted' treatment and were handled in a manner identical to fish in the force fed treatment, but were not given any food (i.e., fish were collected in dip nets every second day for 16 days, an empty syringe was inserted into their gullet, and they were then subsequently released into the appropriate tank). Groups of fasted and force fed fish were rotated among tanks to negate potential tank effects. The 'NHC' treatment consisted of 25 largemouth bass placed in a tank that was identical to the force fed and fasted fish, but the NHC were not netted during the 16-day period. All tanks (including non-handled control tank) were siphoned four times over the 16-day treatment period to remove accumulated solid wastes.

Control fish and sampling

To generate resting control values for physiological variables, six largemouth bass from each of the three experimental treatments (feeding, fasted, and NHC) were transferred from holding tanks to individually aerated, darkened chambers provided with recirculating pond water. Fish were allowed to acclimate to these darkened individual chambers for 24 h. Following acclimation to the chambers, the flow of water to each chamber was terminated and each fish was lethally anesthetized with an overdose of anesthetic (250 mg/L 3-aminobenzoic acid ethyl ester methanesulphonate (MS-222) buffered with 500 mg/L $NaCO_3$) (Summerfelt and Smith 1990; Suski et al. 2006). Following cessation of ventilation, fish were weighed to the nearest g, measured to the nearest mm [total length (TL)], identified by fin clip, and blood was withdrawn from the gill arch using a 1 mL syringe rinsed with lithium heparin (Houston 1990). A small volume of whole blood was placed in a heparinized capillary tube and spun for 120 s using a hematocrit centrifuge to generate hematocrit values [% packed cell volume (PCV)], and an additional aliquot of approximately 100 μL of whole blood was placed in a microcentrifuge tube for later quantification of hemoglobin. The remaining blood (approximately 0.7 mL) was immediately transferred to a 1.5 mL microcentrifuge tube and spun at $2{,}000 \times g$ for 120 s. Plasma was subsequently separated from erythrocytes using a disposable transfer pipette, and divided into three 100 μL aliquots in labeled 1.5 mL microcentrifuge tubes that were immediately flash 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 razorblade, freeze clamped with aluminum tongs pre-cooled in liquid nitrogen, wrapped in labeled aluminum foil, and immediately frozen in liquid nitrogen (Suski et al. 2006). Finally, whole livers were removed from each fish and weighed to the nearest hundredth g. All collected samples (plasma, muscle and liver) were transferred from liquid nitrogen to an ultra cold freezer (<-75°C) at the University of Illinois daily after sampling.

Treatment fish

To induce physiological disturbances, both force fed and fasted groups of largemouth bass were removed from holding tanks and chased by tail pinching for 1 min in a small circular tank, a technique previously shown to be effective at inducing significant physiological disturbances in fish (Wood et al. 1983; Gustaveson et al. 1991; Wood 1991; Suski et al. 2003; Portz 2007). The circular chase tank was divided into quarter sections using lines on the bottom of the tank, and, during the 1 min exercise period, the number of quarter lines crossed by the chased fish was summed, and the time taken to achieve exhaustion was also recorded. Previous work has shown these techniques to be an effective surrogate for critical swimming speed (U_{crit}), and allows for a relative comparison of swimming ability across treatment groups (Plaut 2001). Exhaustion was determined to have occurred when the tail of the chased fish was pinched twice without eliciting a burst swimming response. Following 1 min of exercise, 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.

To assess differences in physiological recovery profiles between force fed and fasted largemouth bass, fish from both treatments were first exercised for 1 min and then quickly transferred to individual, aerated chambers supplied with recirculated water as described above. Exercised fish were allowed to recover for either 1, 2, or 4 h in aerated boxes supplied with reciruclated water, and were then sampled for blood and muscle as described above.

Resting metabolic rate

Over four nights (2–5 May 2008), the resting metabolic rates (RMR) of eight force fed and eight fasted largemouth bass were determined using computerized, intermittent-flow respirometry (LoligoSystems, Hobro, Denmark) (Steffensen 1989). The system consisted of four glass chambers (200 mm long × 62 mm inner diameter; 0.57 L) outfitted with fiber optic oxygen probes immersed in a 140 L tank of aerated pond water maintained at 20°C (±0.5°C). Notably this temperature was slightly higher than our feeding and fasting treatment temperature (15.21°C ± 0.31). Any potential bias in metabolic rates due to increased water temperatures during this part of the experiment should have been applied equally to both force fed and fasted fish. Change in oxygen concentration (α) for each chamber was calculated as slope ($\Delta O_{2saturation}/\Delta t$), and oxygen consumption rate ($\dot{M}O_2$, mg O_2 kg^{-1} h^{-1}) for each fish was calculated by

$$\dot{M}O_2 = \alpha V_{resp} \beta M_b^{-1}$$

where V_{resp} is the volume of each glass chamber minus the volume of the fish (L), β is oxygen solubility (adjusted nightly for both temperature and barometric pressure), and M_b is the fish mass (kg) prior to placing in respirometer chamber. During each trial, the coefficient of determination (r^2) for all slope measurements was >0.95, and oxygen concentration in each chamber was recorded every 2 s. Experiments were designed such that oxygen consumption in each individual chamber was quantified with 15 min cycles that consisted of an 8 min measurement phase, a 4 min flush period to replace water in each chamber, and a 3 min wait period following each flushing prior to commencing measurements. During each measurement period, water from the chambers was continually recirculated across the fiber-optic oxygen probes to ensure adequate mixing, and all calculated dissolved oxygen values were corrected for background oxygen consumptions generated for each specific fish and chamber prior to commencing experiments. Fiber optic oxygen probes were calibrated with oxygen-free water and fully aerated water regularly throughout experiments, and data were recorded with AutoResp software (Version 1.4, Steffensen 1989; Schurmann and Steffensen 1997).

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, USA), while plasma chloride (Cl^-) concentrations were determined using a digital chloridometer (Labconco, Model 4425000, Kansas City, MO, USA). Plasma glucose and lactate concentrations were determined enzymatically following the methods of Lowry and Passonneau (1972) using a microplate spectrophotometer (Molecular Devices, Spectra Max Plus 384, Model # 05362, Union City, CA, USA). Commercially available kits were used to determine concentrations of both plasma cortisol (Assay Designs, Kit # 900-071, Ann Arbor, Michigan) and whole blood hemoglobin (BioAssay Systems, QuantiChrom Hemoglobin Assay Kit, DIHB-250, Hayward, CA, USA). The activity of lactate dehydrogenase (LDH; EC 1.1.1.27) in plasma was determined using standard kinetic spectrophotometric techniques and based on the methods of Wroblewski and LaDue (1955). Mean cell hemoglobin concentration (MCHC) was calculated by:

$$(\%\mathrm{Hct}/\mathrm{Hb}) \times 100$$

where %Hct is the percent hematocrit measurement at the time of blood drawing, and Hb is the whole blood hemoglobin concentration in g/dL generated in the laboratory using a commercially available kit (BioAssay Systems DIHB-250, Hayward, CA, USA).

White muscle lactate phosphocreatine (PCr), and adenosine triphosphate (ATP) concentrations were measured following the enzymatic methods of Lowry and Passonneau (1972) after first grinding frozen muscle with a mortar and pestle under liquid nitrogen and extracting metabolites according to the procedure described in Booth et al. (1995). Muscle water content was quantified by drying tissue samples at 80°C for 48 h and comparing wet mass to dried mass.

Activity of citrate synthase (CS; EC 2.3.3.1) in white muscle was determined by diluting frozen ground muscle in 10 volumes homogenization buffer (20 mmol/L Hepes, 1 mmol/L EDTA, 0.1% Triton X-100, pH·7.2) using a T 25 digital *ULTRA-TURRAX* homogenizer (IKA, Wilmington, NC, USA). Homogenates were assayed within 15 min of homogenization without centrifugation. Enzyme activities were assayed in quadruplicate using a 96-well plate spectrometer at 25°C and assayed in 50 mmol/L Tris (pH·8.1–8.2), 0.10 mmol/L 5,5-dithiobis(2-nitro-benzoic acid), 0.15 mmol/L acetyl CoA, and 0.50 mmol/L oxaloacetate (Bergmeyer 1965; Davies and Moyes 2007).

Statistical analyses

Comparisons of blood and muscle parameters across nutritional status and recovery durations were conducted using a two-way analysis of variance (ANOVA, main effects: nutritional status, recovery time) followed by LSMeans Tukey HSD post hoc test where appropriate (Zar 1999). Differences in starting mass, mass change, liver mass, swimming and performance (number of lines crosses and time to exhaustion) across treatment groups were

assessed using t tests (paired for mass comparisons and unpaired for swimming performance) (Zar 1999). The mean $\dot{M}O_2$ for each fish was determined by selecting the six lowest $\dot{M}O_2$ values for each fish held in the respirometry chamber. Mean $\dot{M}O_2$ values for fed and fasted fish were compared using an unpaired t test (Schurmann and Steffensen 1997). Finally, resting physiological variables from the three different control (non-exercised) groups (force fed, fasted, and non-handled control) were compared as independent variables in a one-way ANOVA to quantify the impacts of repeated handling on treatment fish. All statistical analyses were performed using JMP version 7.0 (SAS Institute, Cary, NC, USA), and the level of significance (α) for all tests was 0.05.

Results

Nutritional condition and performance

Prior to commencing the 16-day feeding and fasting treatments, largemouth bass in the fasted and force fed groups were similar in mass ($P > 0.05$; Table 1). Following the 16-day experimental period, force fed fish weighed more than fasted fish ($P < 0.05$; Table 1). Specifically, force fed fish maintained body weight over 16 days of feeding (mass loss of 0.20 g or 0.30%), whereas fasted largemouth bass lost an average of 16 g (12% mass reduction). Force fed largemouth bass had a smaller mass difference between their initial and final weight and also had a lower percent body mass change ($P < 0.05$; Table 1). In addition, following 16 days of experimental treatment, the liver masses of force fed fish were more than three times greater than livers from fasted fish ($P < 0.05$; Table 1).

Fasting resulted in a significant decrease of individual performance, with force fed largemouth bass crossing nearly 30% more lines during 60 s of exercise than fasted largemouth bass, and force fed largemouth bass swam for over 40% longer than fasted fish before reaching exhaustion ($P < 0.05$; Fig. 1). Force fed fish also had resting metabolic rates that were 1.75× greater than fasted fish ($P < 0.05$; Table 1).

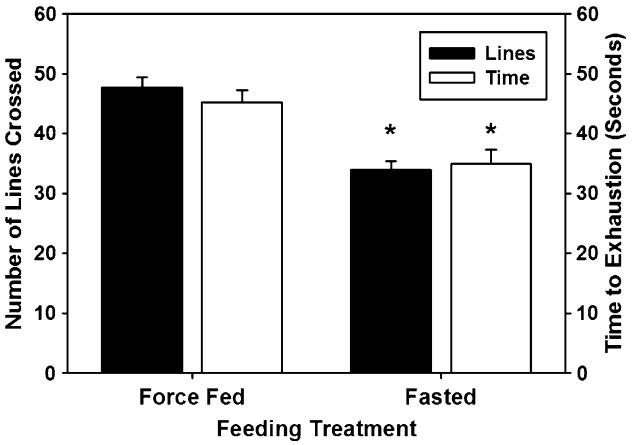

Fig. 1 Performance differences between force fed and fasted largemouth bass (number of quarter lines crossed and time to exhaustion during 60 s of forced swimming). Bass were force fed and fasted for 16 days. An *asterisk* represents a significant difference between fed and fasted fish (t test, $P < 0.05$). Twenty-four force fed and 24 fasted fish were used to generate bar means

Exercise and recovery

Two-way ANOVAs revealed differences in the primary and secondary stress response following exercise for the two groups of fish. Fasted largemouth bass had a greater overall primary stress response evidenced by 65% more plasma cortisol throughout recovery compared to force fed fish ($P < 0.05$; Fig. 2a; Table 2). Fasted fish had twofold more plasma glucose immediately after exercise than fed fish, however, this value was not significantly different from resting conditions ($P < 0.05$; Fig. 2b).

Force fed and fasted largemouth bass showed no difference in plasma sodium concentrations across all recovery treatments ($P > 0.05$; Table 2). Force fed and fasted fish had similar resting plasma chloride values. Pooling

Table 1 Mass changes, performance metrics and metabolic responses in largemouth bass following 16 days of either force feeding or fasting

Variable	Force fed	Fasted	T test results
Starting mass (g)	133 (2)	137 (3)	$t_{58} = 1.24$, $P = 0.89$
Final mass (g)	133 (3)	121 (2)	$t_{58} = -3.1$, $P <$ **0.05***
Mass difference between start and final (g)	−0.2 (0.8)	−16.2 (0.6)	$t_{58} = -16.2$, $P <$ **0.05***
Change in body mass (%)	−0.3 (0.6)	−11.8 (0.3)	$t_{58} = -17.3$, $P <$ **0.05***
Final liver mass (g)	2.91 (2.86)	0.93 (0.03)	$t_{58} = -18.3$, $P <$ **0.05***
RMR $\dot{M}O_2$ (mg/g per h) $N = 8$/group	113 (6)	64.5 (2.2)	$t_{14} = 8.1$, $P <$ **0.05***

Force fed fish received a maintenance ration of food (approximately 1.6–3.0% body weight) for 16 days, while fasted fish were handled but not fed. $N = 30$ for each group unless otherwise stated. Values are mean values ± SE

Bold values indicate $P < 0.05$

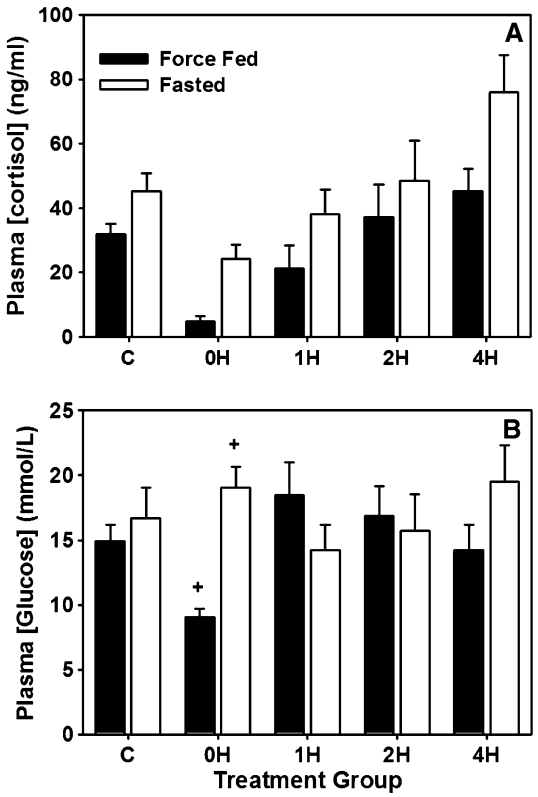

Fig. 2 Plasma cortisol concentration (**a**) and glucose concentration (**b**) in force fed and fasted largemouth bass exercised for one min and recovered up to 4 h. Force feeding and fasting treatments lasted for 16 days. A *plus sign* represents a value that is significantly different across feeding treatments at a specific recovery time (significant interaction, two-way ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were sampled to generate each bar. Cortisol had a significant treatment effect with fasted fish having an average of 65% more cortisol before and after exercise than force fed fish (grouping recovery durations)

recovery treatments, concentrations of chloride in plasma were 14.6% higher in force fed fish than in fasted fish ($P < 0.05$; Table 2). Following 1 min of exercise, force fed largemouth bass experienced a 30% increase in chloride, relative to controls but this disturbance returned to resting conditions with 1 h of recovery ($P < 0.05$; Fig. 3b). In contrast, after exercise, fasted fish showed no chloride disturbance throughout recovery. Immediately following exercise, both force fed and fasted fish displayed twofold increases in plasma potassium relative to control values ($P < 0.05$; Fig. 3c). By 1 h after chasing, exercise-induced changes in plasma potassium had returned to control values. No significant changes in muscle water content were found for either group across any time period ($P > 0.05$; Table 2).

Force fed and fasted largemouth bass had similar resting concentrations of white muscle PCr and ATP (Fig. 4a, b). Immediately following exercise, both force fed and fasted fish consumed similar amounts of available PCr (approximately 66.7%) and ATP (63.6%) (Table 2; Fig. 4a). These changes to both ATP and PCr were restored by 1 h of recovery (Fig. 4a, b). In contrast, force fed fish had more than double the amount of muscle glycogen than fasted fish prior to exercise, consuming 55% of their glycogen stores after exercise, compared to 73% of glycogen consumed for fasted fish. Both force fed and fasted fish partially restored glycogen after 4 h of recovery ($P < 0.05$; Fig. 4c; Table 2). Immediately following exercise, the concentration of lactate in muscle of both force fed and fasted largemouth bass increased approximately 14-fold relative to resting values ($P < 0.05$; Fig. 5; Table 2). There were no differences in the rate of muscle lactate clearance between the two feeding treatments ($P > 0.05$; Fig. 5; Table 2).

Indicators of oxygen carrying capacity (whole blood hemoglobin, hematocrit and MCHC) showed no significant differences between force fed and fasted fish following exercise and throughout recovery ($P > 0.05$; Table 2). Indicators of tissue damage (activity of LDH in plasma) and indicators of erythrocyte rupturing (plasma hemoglobin) did not vary with treatment group ($P > 0.05$; Table 2). In addition, no differences existed between force fed and fasted largemouth bass with respect to the activity of citrate synthase in muscle ($P > 0.05$; Table 2).

Control groups

One-way ANOVAs comparing control (resting) largemouth bass from the force fed, fasted, and non-handled fasted fish (NHC) treatments showed significant influences of our regular handling regime on several physiological parameters. The NHC group had greater resting plasma chloride concentrations than force fed and fasted fish, and also had lower muscle water content than fasted largemouth bass ($P < 0.05$; Table 3). NHC's exhibited less than half of the resting plasma glucose than force fed and fasted fish, and nearly twice as much cortisol in plasma ($P < 0.05$; Table 3). NHC's and fasted largemouth bass had similar glycogen, ATP, and PCR concentrations in white muscle, however, the NHC group had significantly higher resting muscle lactate than force fed largemouth bass. Force fed fish, fasted fish and NHC's had no differences in resting hematocrit, hemoglobin or MCHC. All body mass changes were similar between NHC's and fasted fish, while force fed fish had increased body mass, and liver mass following 16 days of feeding ($P < 0.05$; Table 3). The activity of citrate synthase in white muscle was similar between force fed, fasted and NHC largemouth bass ($P > 0.05$; Table 3).

Discussion

The 16-day force feeding and fasting treatment used in the current study generated two nutritionally distinct groups of

Table 2 Statistical results examining the impact of force feeding and fasting on physiological responses in largemouth bass

Variable	Source	*df*	SS	*F*	*P*
Plasma [Cortisol] (ng/ml)	Entire model	9	19,557.7	5.9	**<0.0001***
	Recovery time	4	13,803.8	9.3	**<0.0001***
	Feed treatment	1	5,050.0	13.7	**<0.0001***
	Recovery × feed	4	704.0	0.5	0.75
	Error	50	18,493.2		
Plasma [Glucose] (mmol/L)	Entire model	9	511.1	2.1	**0.040***
	Recovery time	4	56.6	0.5	0.72
	Feed treatment	1	83.0	3.1	0.085
	Recovery × feed	4	371.5	3.5	**0.014***
	Error	50	1,344.5		
Plasma [Cl^-] (meq/L)	Entire model	9	4,506.2	4.6	**<0.0001***
	Recovery time	4	1,105.3	2.5	0.051
	Feed treatment	1	1,402.1	12.9	**<0.0001***
	Recovery × feed	4	1,944.1	4.5	**<0.0001***
	Error	47	5,094.8		
Plasma [Na^+] (meq/L)	Entire model	9	1,1231.2	2.5	**0.018***
	Recovery time	4	5,182.1	2.6	**0.046***
	Feed treatment	1	26.8	0.1	0.82
	Recovery × feed	4	6,083.3	3.1	**0.025***
	Error	48	23,688.3		
Plasma [K^+] (meq/L)	Entire model	9	53.7	21.8	<0.0001*
	Recovery time	4	50.6	46.1	<0.0001*
	Feed treatment	1	0.4	1.5	0.23
	Recovery × feed	4	2.6	2.3	0.068
	Error	48	13.2		
Muscle [PCr] (mmol/g)	Entire model	9	1,289.4	4.0	**<0.0001***
	Recovery time	4	1,014.2	7.1	**<0.0001***
	Feed treatment	1	31.8	0.9	0.35
	Recovery × feed	4	243.4	1.7	0.16
	Error	50	1,784.1		
Muscle [ATP] (mmol/g)	Entire model	9	114.1	7.1	**<0.0001***
	Recovery time	4	99.2	13.8	**<0.0001***
	Feed treatment	1	5.3	3.0	0.091
	Recovery × feed	4	9.6	1.3	0.27
	Error	50	89.6		
Muscle [Lactate] (mmol/g)	Entire model	9	1,549.1	39.0	**<0.0001***
	Recovery time	4	1,524.6	86.3	**<0.0001***
	Feed treatment	1	9.7	2.2	0.14
	Recovery × feed	4	14.8	0.8	0.51
	Error	50	220.7		
Muscle [Glycogen] (g)	Entire model	9	3,328.1	19.6	**<0.0001***
	Recovery time	4	1,392.2	18.5	**<0.0001***
	Feed treatment	1	1,864.8	99.1	**<0.0001***
	Recovery × feed	4	61.7	0.8	0.52
	Error	49	922.4		

Table 2 continued

Variable	Source	*df*	SS	*F*	*P*
Blood [Hemoglobin] (mg/dL)	Entire model	9	42.5	1.1	0.40
	Recovery time	4	18.4	1.0	0.39
	Feed treatment	1	3.0	0.7	0.41
	Recovery × feed	4	19.2	1.1	0.37
	Error	48	210.8		
Plasma [Hemoglobin] (mg/dL)	Entire model	9	0.3	2.3	**0.028***
	Recovery time	4	0.2	3.0	**0.026***
	Feed treatment	1	0.0	0.0	0.96
	Recovery × feed	4	0.1	2.2	0.081
	Error	50	0.8		
Blood	Entire model	9	803.9	3.0	**0.0075***
Hematorcrit (%)	Recovery time	4	521.8	4.5	**<0.0001***
	Feed treatment	1	88.0	3.0	0.089
	Recovery × feed	4	231.7	2.0	0.11
	Error	47	1,368.2		
MCHC	Entire model	9	386.2	0.9	0.55
	Recovery time	4	244.3	0.1	0.71
	Feed treatment	1	6.8	0.1	0.30
	Recovery × feed	4	135.1	0.7	0.60
	Error	47	2,281.9		
Muscle [CS] activity (IU/min/g of wet mass)	Entire model	9	10.9	0.8	0.61
	Recovery time	4	5.1	0.8	0.51
	Feed treatment	1	4.4	2.9	0.093
	Recovery × feed	4	1.3	0.2	0.93
	Error	48	72.2		
Plasma [LDH] (U/L)	Entire model	9	132,526.2	4.5	**<0.0001***
	Recovery time	4	108,126.5	8.2	**<0.0001***
	Feed treatment	1	5,781.7	1.8	0.19
	Recovery × feed	4	18,051.1	1.4	0.26
	Error	48	158,337.0		
Water content (%)	Entire model	9	32.4	0.8	0.58
	Recovery time	4	12.9	0.8	0.56
	Feed treatment	1	10.2	2.4	0.13
	Recovery × feed	4	9.3	0.5	0.70
	Error	50	212.6		

Force fed fish received a maintenance ration of food (approximately 1.6–3% body weight) for 16 days, while fasted fish were handled similarly but not fed. Fish were subsequently exercised for 1 min and allowed to recover for 1, 2 or 4 h. Each variable represents a two-way ANOVA effects tests and interactions

Bold values indicate $P < 0.05$

largemouth bass. Fish that received a food injection of 1.6–3.0% body weight every second day were able to maintain body mass over 16 days, whereas fish fasted over this same period lost an average of 16 g of body mass and experienced a 66% reduction in liver mass. In addition, fasted largemouth bass had half as much resting white muscle glycogen stores and exhibited nearly 65% more plasma cortisol throughout recovery relative to force fed fish. Mass differences between the feeding groups likely resulted from liver and muscle lipid and glycogen energy consumption, and catabolism of somatic protein (Sullivan and Somero 1983; Sargent et al. 1989). Previous studies have documented similar amounts of resting muscle glycogen declines following five to seven d of fasting in rainbow trout (*Oncorhynchus mykiss*) (Scarabello et al. 1991; Keiffer and Tufts 1998) and following 21 days of fasting in coho salmon (*Oncorhynchus kisutch*) (Sheridan and Mommsen 1991). In addition, carp (*Cyprinus carpio*)

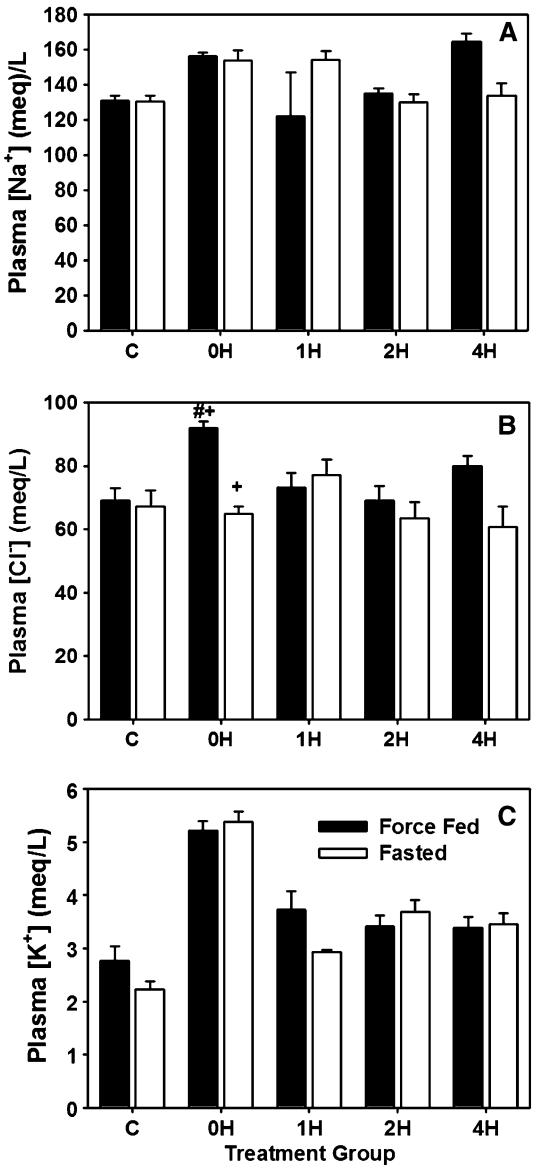

Fig. 3 Plasma sodium (**a**) chloride (**b**) and potassium (**c**) concentration in force fed and fasted largemouth bass exercised for 1 min and recovered up to 4 h. A *pound sign* represents a significant difference from the control value within a size class at a specific recovery time. A *plus sign* represents a value that is significantly different across feeding treatments at a specific recovery time (significant interaction, two-way ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

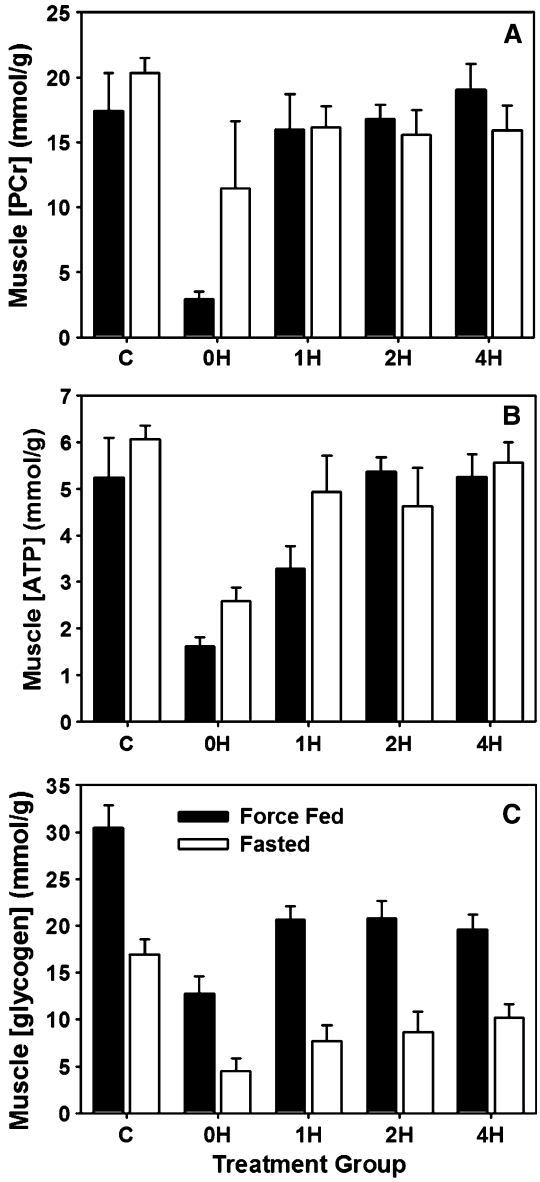

Fig. 4 Muscle phosphocreatine (PCr) (**a**) adenosine triphosphate (ATP) (**b**) and Glycogen (**c**) concentration in force fed and fasted largemouth bass exercised for 1 min and recovered up to 4 h. There was no interaction for feeding treatment and recovery group, although there was similar and significant consumptions of these energies by both force fed and fasted fish, which were restored at similar rates. Force fed fish had twice as much glycogen as fasted fish, regardless of recovery duration (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

starved for 19 days show liver mass reductions of 31%, with researchers attributing these declines to the mobilization of liver glycogen stores to fuel aerobic tissues (Sullivan and Somero 1983; Sargent et al. 1989; Blasco et al. 1992). Together, results showed that a 16-day cycle of force feeding and fasting generated two nutritionally distinct groups of largemouth bass.

The primary stress response and portions of the secondary stress response exhibited by exercised largemouth bass in the current study was strongly influenced by feeding treatments. Prior to and after exercise, the concentration of plasma cortisol in fasted fish was 65% higher than force fed fish. Conversely, force fed and fasted largemouth bass showed no significant difference in concentrations of both anions and cations in plasma despite differences in handling, feeding regimes and cortisol concentrations. Cortisol plays an important role in fish during periods of fasting, but

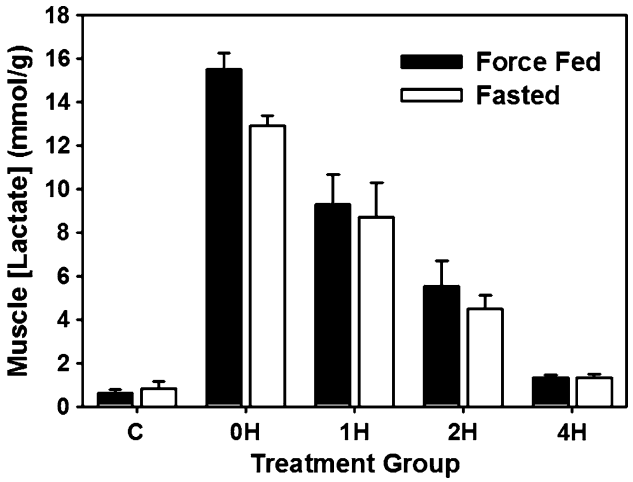

Fig. 5 Muscle lactate concentration in force fed and fasted largemouth bass exercised for one min and recovered up to 4 h. Force fed and fasted fish had similar changes in the production and clearance of lactate throughout recovery (ANOVA and LSMeans Tukey HSD, $P < 0.05$). Six fish were used to generate each bar

its exact role and mechanism has yet to be clearly defined (Van der Boon et al. 1991). Rainbow trout denied food for 30 days exhibit increases in plasma cortisol relative to fed conspecifics (Vijayan and Moon 1992), and elevated concentrations of cortisol in plasma due to starvation have been linked to loss of plasma potassium ions in eels (*Anguilla japonica*) (Chan and Woo 1978). Cortisol has a high affinity for receptor binding in the hepatic system (Henderson and Garland 1980) often resulting in amino acid and carbohydrate catabolism in the liver and peripheral muscle of teleosts (Van der Boon et al. 1991). Thus, increased cortisol concentrations may be one of the mechanisms responsible for catabolism of liver and muscle energy stores and therefore, likely played an important role in the mass loss of starved largemouth bass in the current study. The magnitude of the cortisol response in starved fish has also been shown to vary across species, by study design and the type of stressors provided. For example, some studies report increased plasma cortisol in starved fish following a stressor (Vijayan and Moon 1992), whereas others show cortisol concentrations that are of similar magnitude for fed and starved fish subjected to identical stressors (Barton et al. 1988; Bandeen and Leatherland 1997). These discrepancies were likely caused by species-specific response to stressors (Sheridan and Mommsen 1991) and/or differences in experimental methods across studies (Ruane et al. 2002). Taken together, the increased primary stress response in starved largemouth bass likely played an important role in the mobilization of energy stores, but has no effect on ionic balance following 16 days of starvation.

Despite prolonged periods of starvation and force feeding, several physiological parameters remained similar across our two groups of largemouth bass, and were

Table 3 Physiological differences in control, force fed, fasted, and non-handled largemouth bass following 16 days of treatment

Variable	Force fed controls	Non-handled controls (NHC)	Fasted controls	ANOVA result
Muscle water content (%)	79.4 (0.2) AB	79.4 (0.3) B	80.4 (0.3) A	**$F_2 = 4.5$, $P = 0.029$***
Plasma [Cl^-] MEQ	69.1 (3.8) B	98.9 (2.9) A	67.3 (5.0) B	**$F_2 = 21.4$, $P < 0.0001$***
Plasma [Na^+] MEQ	130.8 (2.9)	138.5 (7.1)	130.6 (3.1)	$F_2 = 0.9$, $P = 0.42$
Plasma [K^+] MEQ	2.8 (0.3) AB	3.1 (0.2) A	2.2 (0.2) B	**$F_2 = 5.3$, $P = 0.018$***
Plasma cortisol (ng/mL)	31.9 (3.1) A	87.9 (20.8) B	45.2 (5.5) AB	**$F_2 = 5.4$, $P = 0.017$***
Plasma glucose (mmol/L)	14.9 (1.3) A	7.97 (1.2) B	16.7 (2.3) A	**$F_2 = 7.6$, $P = 0.0053$***
Muscle glycogen (mmol/g tissue)	30.4 (2.5) A	19.3 (2.3) B	16.9 (1.6) B	**$F_2 = 11.3$, $P = 0.0010$***
Lactate (mmol/g tissue)	0.6 (0.2) B	1.9 (0.4) A	0.8 (0.3) AB	**$F_2 = 4.4$, $P = 0.032$***
ATP (mmol/g tissue)	5.2 (0.9)	7.9 (0.9)	6.1 (0.3)	$F_2 = 3.3$, $P = 0.063$
PCr (mmol/g tissue)	17.4 (2.9)	27.9 (4.1)	20.3 (1.2)	$F_2 = 3.3$, $P = 0.066$
Hematocrit	41.0 (1.3)	41.8 (0.9)	45.3 (1.9)	$F_2 = 2.5$, $P = 0.11$
Whole blood Hemoglobin (g/dL)	8.7 (0.3)	9.6 (0.5)	8.9 (0.6)	$F_2 = 0.6$, $P = 0.55$
MCHC (g/dL)	20.9 (1.3)	22.7 (1.2)	20.0 (2.0)	$F_2 = 0.8$, $P = 0.49$
Citrate synthase activity (IU/min/g wet mass)	2.9 (0.6) A	3.7 (0.4) A	2.6 (0.6) A	$F_2 = 1.0$, $P = 0.39$
Difference between starting and final mass (g)	1.50 (1.38) A	−13.60 (1.50) B	−17.70 (1.09) B	**$F_2 = 61.3$, $P < 0.0001$***
Change in mass (%)	1.06 (1.08) A	−10.30 (1.15) B	−12.20 (0.47) B	**$F_2 = 61.9$, $P < 0.0001$***
Liver mass (g)	2.71 (0.14) A	0.69 (0.06) B	0.90 (0.04) B	**$F_2 = 139$, $P < 0.0001$***

Force fed fish received an oral injection of food for 16 days, fasted fish were handled similarly but did not receive food and NHC were left undisturbed in tanks for 16 days. $N = 6$ for each treatment group. Values are expressed as mean ± SE. Letters represent similarities and differences among treatments

Bold values indicate $P < 0.05$

DF degrees of freedom

conserved possibly because of their ecological and biological importance. More specifically, white muscle citrate synthase activity, muscle ATP and PCr concentrations, plasma hemotocrit, plasma hemoglobin, and plasma MCHC were all similar for force fed and fasted largemouth bass. In addition, with the exception of a plasma chloride increase observed in force fed fish immediately following exercise, no treatment-specific differences in ionic disturbance following exercise were observed between the two feeding regimes. The maintenance of ionic balance for freshwater fish is an energetically expensive activity due to the concentration gradient that exists between plasma and fresh water (Randall et al. 1972; Gonzalez and McDonald 1992). The fact that both treatment groups of largemouth bass in the current study maintained a similar concentration of plasma ions despite a reduction in energy stores is perhaps not surprising as a loss of ionic balance would almost certainly result in mortality (Wood et al. 1983). Concentrations of ATP and PCr in muscle are the first fuels consumed during anaerobic swimming such as predator avoidance and prey capture, and together represent anaerobic potential (Keiffer et al. 1996; Kieffer 2000). The preservation of anaerobic potential despite prolonged periods of fasting likely represents an evolutionarily important adaptation that would allow fish to avoid predation and capture prey even after bouts of starvation (Kieffer 2000).

In our study, activities of citrate synthase in white muscle did not change following force feeding and fasting treatments, and this variable may have remained constant because 16 days may be an insufficient length of time to alter enzyme activities. For example, after 24 weeks of fasting, sablefish (*Anoplopoma fimbria*) demonstrate a 75% reduction in enzyme activities in white muscle (lactate dehydrogenase and pyruvate kinase) (Sullivan and Somero 1983). Similarly, Yang and Somero (1993) showed that activities of anaerobic enzymes such as lactate dehydrogenase (LDH) change more drastically than aerobic enzymes such as CS after 90–105 days of starvation in Scorpaenid fishes. In the wild, muscle LDH activities differ between populations of yellow perch (*Perca flavescens*) and lake trout (*Salvelinus namaycush*), and these differences have been attributed to local adaptations driven by the availability of prey resources (Sherwood et al. 2002). Oxygen carrying capacity was also preserved in starved largemouth bass compared to fed individuals, but, as with enzyme activity rates, this similarity across treatment groups could be a function of a relatively short experimental duration. For example, wolf fish (*Hoplias malabaricus*) show reductions in haematocrit after 150 days of starvation (Rios et al. 2002), but 42 days of starvation does not affect hemoglobin and hematocrit concentrations in European eel (*Anguilla anguilla*) (Caruso et al. 2008). Clearly, even after prolonged starvation several physiological parameters remain similar across two largemouth bass feeding groups and these conservations are likely necessary to sustain predator avoidance, prey capture, and general biological needs.

Whole animal performance including metabolic rates and swimming performance, were influenced greatly by 16 days of force feeding and fasting in largemouth bass. Force fed fish swam 29% further and 40% longer, and had resting metabolic rates that were 75% greater than starved largemouth bass. Clearly, resting metabolic rate is strongly influenced by the nutritional status of largemouth bass, and likely declined in starved fish due to an attempt to conserve remaining energy stores and minimize energy investment in growth (Wieser et al. 1992). Similar to the results of our study, $\dot{M}O_2$ rates in satiated rainbow trout increased by 68% relative to fasted conspecifics, and by 30% relative to rainbow trout fed a maintenance ration (Alsop and Wood 1997). Likewise, a depression in metabolic rates of 60–66% was found in both spotted scorpion fish (*Scorpaena guttata*) and short-spine thornyhead (*Sebastolobus alascanus*) that were fasted for more than 90 d (Yang and Somero 1993). Some studies, however, have demonstrated a lack of metabolic declines after periods of starvation in salmonid fishes (Scarabello et al. 1991). Inconsistency in the response of metabolic rates to starvation across studies might be attributed to species-specific differences and/or differences in experimental method. Swimming ability has previously been shown to be negatively affected by decreased feeding. For example, U_{Crit} (critical swimming speed) was reduced by 15% in starved rainbow trout compared to fed individuals (Alsop and Wood 1997). Decreased swimming performance in fasted largemouth bass were likely due to reduced muscle glycogen stores, which prevented continued burst (anaerobic) swimming after PCr and ATP stores in muscle had been consumed (Kieffer 2000). It is not likely that reduced swimming performance was influenced by the ability to transport oxygen, or differences in the activity of CS, as enzyme activity rates, blood hemoglobin concentration and hematocrit values were similar across both feeding treatments. Together, swimming and metabolic performance in largemouth bass is strongly affected by feeding and fasting and appears to be driven by differences in anaerobic potential.

Similar to the magnitude of disturbances following exercise, force fed and fasted largemouth bass recovered from exercise-induced disturbances at similar rates. Despite declines in muscle glycogen following starvation and exercise, force fed and fasted fish had only returned 2/3 of their muscle glycogen to resting by 1, 2 and 4 h. Similarly, by 1 h of recovery both force fed and fasted fish had completely restored depleted concentrations of muscle ATP and PCr in muscle despite large differences in resting

metabolic rates between feeding treatments. Previous research has shown that recovery from exercise is a complex activity fueled by the aerobic consumption of lipids (Richards et al. 2002). Earlier work with largemouth bass has shown that recovery from exercise is impaired by elevated concentrations of ambient ammonia, cold water temperatures, hypoxia and hyperoxia (Suski et al. 2006). As well, forcing largemouth bass to swim against a gentle current after exercise for prolonged periods does not result in improved recovery times dissimilar to previous work with salmonid fishes (Suski et al. 2007a, b). In the current study, recovery from exercise was not influenced by nutritional status or the energy content of the largemouth bass; this observation could be a product of evolutionary adaptation, as fish likely need to recover from exercise induced disturbances quickly to engage in bust swimming activities with minimal delay (chase prey or avoid predation) (Kieffer 2000).

Repeated handling did not appear to cause severe negative physiological consequences on largemouth bass as resting physiological variables were similar between fasted handled and fasted non-handled (NHCs) treatments. Most importantly, handled fasted fish and NHC's lost similar amounts of weight and liver mass (approximately 1 g per day of fasting in overall mass). Oxygen carrying potential (hematocrit, whole blood hemoglobin, MCHC), concentrations of ions in plasma (plasma sodium, potassium) and energy stores (glycogen, ATP, PCr) were also similar between the two fasted groups. NHCs had 30% more plasma chloride than resting handled controls (force fed and fasted bass) and was likely a result of chloride loss in the handled groups as prolonged stressors, such as the handling regime used in the current study, have previously been shown to cause ion loss in freshwater fish (Carmichael et al. 1984; Gonzalez and McDonald 1992). In addition, NHCs had 50% less resting plasma glucose and twice as much resting plasma cortisol compared to fasted and force fed treatments, suggesting an increased primary stress response for NHC's, or perhaps, a dampening of physiological responses in handled fish due to repeated handling (Jentoft et al. 2005). The impacts of starvation on plasma glucose concentrations in fish have been shown to vary across studies. For example, resting plasma glucose has been shown to increase following starvation of rainbow trout and coho salmon due to a catabolism of glycogen stores (Scarabello et al. 1991; Sheridan and Mommsen 1991). Barton et al. (1988) showed a reduced plasma glucose concentration following handling stress in Chinook salmon (*Oncorhynchus tshawytscha*) starved for 20 days compared to fed conspecifics, suggesting this was related to an inability to mobilize glucose due to reductions in glycogen stores induced by starvation. Clearly, the cortisol and glucose response in starved largemouth bass is strongly influenced by the kind of additional stressor(s) (i.e., handling, air exposure, exercise, hauling) applied, and likely represents one explanation for the many discrepancies that in the literature on this topic. Together, repeated handling caused little additional physiological decline beyond disturbances induced by 16 days of starvation.

The influence of varied nutritional status on individual performance can have important ecological applications for wild largemouth bass. Factors such as water temperature, prey abundance, and turbidity can vary both across aquatic ecosystems, as well as spatially within different systems, providing varied levels of prey resources to largemouth bass (Goede and Narton 1990; Barton et al. 2002). In addition, factors such as reproductive status (Brown and Murphy 2004), social dominance (McCarthy et al. 1992), and swimming performance (Kolok and Farrell 1994) can all combine to create differences in rates of food acquisitions for fish. These factors can vary nutritional status across individuals within the same population. Currently, it is unclear what advantage wild fish with superior nutritional status would have over food-deprived fish, or how wild fish would respond to different kinds of stressors during periods of fasting. The current study, however, suggests that fasting has important impacts on the primary stress response, energy stores and performance of wild largemouth bass. Fed largemouth bass show improved swimming performance relative to starved conspecifics, which can result in wild fish having competitive advantages over those who struggle to forage successfully (Plaut 2001). Enhanced food intake, therefore, might act as a positive feedback mechanism providing greater future food consumption for individuals that have fed (Druker 1996). Research has also shown that, when fish feed they are more successful at reproduction due to increased energy stores, which allow them to search for mates (Druker 1996), and are more capable of avoiding predators (Videler 1993; Reidy et al. 1995; Watkins 1996). Energetically costly or vulnerable periods, for largemouth bass, might include the end of winter and during the reproductive period (Adams et al. 1982; Hinch and Collins 1991; Mackereth et al. 1999; Brown and Murphy 2004) because at these time fish drastically reduce feeding and therefore might be sensitive periods for fish facing stressors. Considering the natural variation in food consumption and energy stores in wild fish, fed fish might have competitive advantage over food deprived individuals that might be weaker swimmers.

Many ecological-specific questions remain despite our description of energy loss and swimming performance declines in largemouth bass. Future research on starvation might consider how many days of starvation can occur prior to an individual fish experiencing disruptions to ionic and oxygen transport systems, and if repeated swimming challenges, similar to those that might occur during

predator avoidance, result in a decline in individual performance. Fish size is also an important variable to consider in future feeding and fasting studies, as larger fish typically have greater energy stores, lower metabolic rates, and reduced swimming costs than smaller fish (Kieffer 2000) and therefore might be more prepared for periods of starvation. Together our results indicate that largemouth bass show energy losses after fasting, and this reduction in nutritional status has a dramatic effect on swimming performance, metabolic rates and some energy stores. In the future, these results need to be considered in ecological contexts as fish often undergo periods of fasting or low prey availability.

Acknowledgments All of the experiments described in this study were conducted in accordance with the regulations and policies of the University of Illinois Office of Laboratory Animal Research. This project was supported by the United States Department of Agriculture Cooperative State Research Education and Extension Service by McIntire-Stennis funds through project ILLU-875-328, the Illinois Natural History Survey and the Illinois Department of Natural Resources. Illinois Natural History Survey staff, specifically Jimmy "Mac" McNamara and James "Jamie" Hopper, are thanked for their assistance with fish husbandry facilities.

References

Adams SM, McLean RB, Parrotta JA (1982) Energy partitioning in largemouth bass under conditions of seasonally fluctuating prey availability. Trans Am Fish Soc 111:549–558

Alsop DH, Wood CM (1997) The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 200:2337–2346

Bandeen J, Leatherland JF (1997) Transportation and handling stress of white suckers raised in cages. Aquac Int 5:358–396

Barton BA, Schreck CB, Fowler LG (1988) Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile Chinook salmon. Prog Fish Cult 50:16–22

Barton BA, Morgan JD, Vijayan MM (2002) Physiological and condition-related indicators of environmental stress in fish. In: Adams SA (ed) Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, pp 111–148

Bergmeyer HU (1965) Methods of enzymatic analysis. Academic Press, New York

Blasco J, Fernandez J, Gutierrez J (1992) Fasting and refeeding in carp. *Cyprinus carpio* L.: the mobilization of reserves and plasma metabolite and hormone variations. J Comp Physiol B 162:539–546

Booth C, Patel S, Bennion GR, Potten CS (1995) The isolation and culture of adult mouse colonic epithelium. Epithelial Cell Biol 4:76–86

Brown ML, Murphy BR (2004) Seasonal dynamics of direct and indirect condition indicies in relation to energy allocation in largemouth bass *Micropterus salmoides*. Ecol Fresh Fish 13:23–36

Carmichael GJ, Tomasso JR, Simco BA, Davis KB (1984) Confinement and water-quality induced stress in largemouth bass. Trans Am Fish Soc 113:767–777

Caruso G, Maricchiolo G, Micale V, Genovese L, Caruso R, Denaro MG (2008) Physiological responses to starvation in the European eel (*Anguilla anguilla*): effects on haematological, biochemical, non-specific immune parameters and skin structures. Fish Physiol Biochem. doi:10.1007/s10695-008-9290-6

Chan DKO, Woo NYS (1978) Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. Gen Comp Endocrinol 35:205–215

Congleton JL, Wagner T (2006) Blood-chemistry indicators of nutritional status in juvenile salmonids. J Fish Biol 69:473–490

Davies R, Moyes CD (2007) Allometric scaling in centrarchid fish: origins of intra- and inter-specific variation in oxidative and glycolytic enzyme levels in muscle. J Exp Biol 210:3798–3804

Deng L, Zhang WM, Lin WM, Cheng CHK (2004) Effects of food deprivation on expression of growth hormone receptor and proximate composition in liver of black seabram *Acanthopagrus schlegeli*. Comp Biochem Physiol B 137:421–432

Druker EG (1996) The use of gait transition speed in comparative studies of fish locomotion. Am Zool 36:555–566

Garvey JE, Ostrand KG, Wahl DH (2004) Energetics, predation, and ration affect size-dependent growth and mortality of fish during winter. Ecology 85:2860–2871

Goede RW, Narton BA (1990) Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. In: Adams SA (ed) Biological indicators of stress. American Fisheries Society, Bethesda, pp 93–108

Gonzalez RJ, McDonald DG (1992) The relationship between oxygen consumption and ion loss in a freshwater fish. J Exp Biol 163:317–332

Gustaveson AW, Wydowski RS, Wedemeyer GA (1991) Physiological response of largemouth bass to angling stress. Tran Am Fish Soc 120:629–636

Henderson IW, Garland HO (1980) General, comparative and clinical endocrinology of the adrenal cortex. Academic Press, London

Hinch SG, Collins NC (1991) Importance of diurnal and nocturnal nest defense in the energy budget of male smallmouth bass: insights from direct video observations. Trans Am Fish Soc 120:657–663

Houston AH (1990) Blood and circulation. In: Shreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 237–334

Jentoft S, Aastveit AH, Torjesen PA, Andersen O (2005) Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol A 141:353–358

Kieffer JD (2000) Limits to exhaustive exercise in fish. Comp Biochem Physiol A 126:161–179

Keiffer JD, Tufts BL (1998) Effects of food deprivation on white muscle energy reserves in rainbow trout (*Oncorhynchus mykiss*): the relationships with body size and temperature. Fish Phys Biochem 19:239–245

Keiffer JD, Ferguson RA, Tompa JE, Tufts BL (1996) Relationship between body size and anaerobic metabolism in brook trout and largemouth bass. Trans Am Fish Soc 125:760–767

Kolok AS, Farrell AP (1994) Individual variation in the swimming peroformance and cardiac performance of Northern squawfish, *Ptychocheilus oregonensis*. Phys Zool 67:706–722

Lowry OH, Passonneau JV (1972) A flexible system of enzymatic analysis. Academic Press, New York

Mackereth RW, Noakes DLG, Ridgeway MS (1999) Size-based variation in somatic energy reserves and parental expenditure by male smallmouth bass, *Micropterus dolomieu*. Environ Biol Fish 56:263–275

McCarthy ID, Carter CG, Houlihan DF (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Biol 41:257–263

Mendez G, Wieser W (1993) Metabolic responses to food deprivation and refeeding in juveniles of *Rutilus rutilus* (*Teleostei:Cyprinidae*). Environ Biol Fish 36:73–81

Miranda LE, Hubbard WD (1994) Length-dependent winter survival and lipid composition of age -0 largemouth bass in Bay Springs Reservoir, Mississippi. Trans Am Fish Soc 123:80–87

Navarro I, Gutierrez J (1995) Fasting and starvation. In: Honchachka OW, Mommsen TP (eds) Biochemistry and molecular biology of fishes. Elsevier, Amsterdam, pp 393–434

Plaut I (2001) Crtical swimming speed: its ecological relevance. Comp Biochem Physiol A 131:41–50

Portz DE (2007) Fish-holding-associated stress in Sacramento River Chinook salmon (*Oncorhynchus tshawytscha*) at south delta fish salvage operations: effects on plasma constituents, swimming performance, and predator avoidance. Ph.D. thesis. University of California Davis, Davis, California, USA

Post JR, Parkinson EA (2001) Energy allocation strategy in young fish: allometry and survival. Ecology 82:1040–1051

Randall DJ, Baumgarten D, Malyusz M (1972) The relationship between gas and ion transfer across the gills of fishes. Comp Biochem Physiol A 41:629–637

Reidy SP, Nelson JA, Tang Y, Kerr SR (1995) Post-exercise metabolic rate in Atlantic Cod (*Gadus morhua*) and its dependence upon the method of exhaustion. J Fish Biol 47:377–386

Richards JG, Heigenhauser GJF, Wood CM (2002) Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. Am J Physiol Regul Integr Comp Physiol 282:R89–R99

Rios FS, Kalinin AL, Rantin FT (2002) The effects of long-term food deprivation on respiration and haematology of the neotropical fish *Hoplias malabaricus*. J Fish Biol 61:85–95

Ruane NM, Huisman EA, Komen J (2002) The influence of feeding history on the acute stress response of common carp (*Cyprinus carpio*). Aquaculture 210:245–257

Sargent J, Henderson RG, Tocher DR (1989) The lipids. In: Halver JE (ed) Fish nutrition. Academic Press, New York, pp 154–218

Scarabello M, Wood CM, Heigenhauser GJF (1991) Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis. Can J Zool 69:2562–2568

Schurmann H, Steffensen JF (1997) Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. J Fish Biol 50:1166–1180

Sheridan MA, Mommsen TP (1991) Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon (*Oncorhynchus kisutch*). Gen Comp Endochrinol 81:473–483

Sherwood GD, Pazzia I, Moeser A, Hontela A, Rasmussen JB (2002) Shifting gears: enzymatic evidence for the energetic advantage of switching diet in wild-living fish. Can J Fish Aquat Sci 59:229–241

Steffensen JF (1989) Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiol Biochem 6:49–59

Sullivan KM, Somero GN (1983) Size and diet related variations in enzuymatic activity and tissue composition in the sablefish, *Anoplopoma Fimbria*. Biol Bull 164:315–326

Summerfelt RC, Smith LS (1990) Anesthesia, surgery and related techniques. In: Shreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 213–272

Suski CD, Killen SS, Morrissey MB, Lund SG, Tufts BL (2003) Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. North Am J Fish Manage 23:760–769

Suski CD, Killen SS, Kieffer JD, Tufts BL (2006) The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. J Fish Biol 68:120–136

Suski CD, Kieffer JD, Killen SS, Tufts BL (2007a) Sub-lethal ammonia toxicity in largemouth bass. Comp Biochem Physiol A 146:381–389

Suski CD, Cooke SJ, Tufts BL (2007b) Failure of low-velocity swimming to enhance recovery from exhaustive exercise in largemouth bass (*Micropterus salmoides*). Physiol Biochem Zool 80:78–87

Van der Boon J, Van den Thillart GEEJM, Addink ADF (1991) The effects of cortisol administration on intermediary metabolism in teleost fish. Comp Biochem Physiol A 100:47–53

Videler JJ (1993) Fish swimming. Chapman & Hall, London

Vijayan MM, Moon TW (1992) Acute handling stress alters hepatic glycogen metabolism in food deprived rainbow trout (*Oncorhynchus mykiss*). Can J Fish Aquat Sci 49:2260–2266

Watkins TB (1996) Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. Physiol Zool 69:154–167

Wieser W, Krumschnabel G, Ojwang-Okwor JP (1992) The energetics of starvation and growth after refeeding in juveniles of three cyprinid species. Environ Biol Fish 33:63–71

Wood CM (1991) Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. J Exper Bio 160:285–308

Wood CM, Turner JD, Graham MS (1983) Why do fish dies after severe exercise? J Fish Biol 22:189–201

Wroblewski F, LaDue JS (1955) Lactic dehydrogenase activity in blood. Proc Soc Exp Biol Med 90:210–213

Yang TH, Somero GN (1993) Effects of feeding and food deprivation on oxygen consumption, muscle protein concentration. J Exp Biol 181:213–232

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, New Jersey