The metabolic and biochemical basis of vulnerability to recreational angling after three generations of angling-induced selection in a teleost fish

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Abstract: Although the selective pressures of commercial fishing are well known, few studies have examined this phenomenon in recreational fisheries. This study used a unique population of largemouth bass (*Micropterus salmoides*) with lines bred for low (LVF) and high (HVF) vulnerability to recreational angling. We evaluated whether differential vulnerability to angling was correlated with physiological traits, including metabolic rate, metabolic scope, anaerobic capacity, and biochemical response to exercise. Indeed, angling selection affected the metabolic rate of fish significantly. The standard metabolic rate was 10%, maximal metabolic rate was 14%, and metabolic scope was 16% lower for LVF compared with HVF. Following exhaustive exercise, LVF required 1 h for lactate levels to recover to control values, whereas HVF required 2 h. Anaerobic energy expenditure was significantly lower for LVF, a finding consistent with the observation that LVF swam at a steadier rate during exercise. Although the reasons behind vulnerability to angling are complex, the phenotypic trait "vulnerability to angling" appears to be linked to a suite of physiological traits, including metabolism and the capacity for anaerobic activity. Thus, angling-induced selection might alter the physiological characteristics of wild largemouth bass populations, with unknown outcomes for long-term population viability.

Résumé : Bien que l'on connaisse bien les pressions sélectives qui s'exercent dans les pêches commerciales, peu d'études se sont intéressés au phénomène dans la pêche sportive. Notre étude examine une population particulière d'achigans à grande bouche (*Micropterus salmoides*) qui possède des lignées sélectionnées pour leur vulnérabilité basse (LVF) ou élevée (HVF) à la pêche sportive. Nous avons déterminé si les différences de vulnérabilité à la pêche sportive sont en corrélation avec des traits physiologiques, tels que le taux métabolique, le profil métabolique, la capacité anaérobie et la réaction biochimique à l'exercice. En effet, la sélection par la pêche sportive affecte significativement le taux métabolique des poissons. Chez les poissons LVF, le taux métabolique standard, le taux métabolique maximal et le profil métabolique sont respectivement 10 %, 14 % et 16 % plus bas que chez les poissons HVF. Après un exercice épuisant, il faut 1 h pour que les concentrations de lactate retournent aux concentrations témoins chez les LVF, mais il faut 2 h chez les HVF. La dépense énergétique anaérobie est significativement inférieure chez les LVF, un résultat qui est compatible avec l'observation que les LVF nagent à un taux plus régulier durant l'exercice. Bien que les raisons qui expliquent la vulnérabilité à la pêche sportive soient complexes, le trait phénotypique « vulnérabilité à la pêche sportive » semble être relié à une série de traits

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physiologiques et en particulier au métabolisme et à la capacité d'activité anaérobie. Ainsi la sélection exercée par la pêche sportive peut modifier les caractéristiques physiologiques de populations sauvages d'achigans à grande bouche, avec des résultats inconnus sur la viabilité à long terme de la population.

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Introduction

Selectively harvesting fish populations for economically desirable traits generates directional selection pressures that can lead to evolutionary changes of life-history traits such as age and size at maturation, reproductive investment, or growth rate in heavily exploited stocks (Law 2000; Heino and Godø 2002; Kuparinen and Merilä 2007). Although much of the evidence for fishing-induced selection has been attributed to commercial marine fisheries, the potential also exists for recreational angling to generate similar selection pressures (Cooke and Cowx 2006; Lewin et al. 2006; Philipp et al. 2009). In inland freshwater systems with small, locally adapted stocks, angling mortality may be particularly intense (Post et al. 2002; Arlinghaus and Cooke 2005; Cooke and Cowx 2006). In a manner similar to commercial fishing, recreational angling can also be highly selective for size, species, and sex, but in addition, a suite of physiological and behavioural traits may be under equal or stronger selection pressures than those related to body size (reviewed in Lewin et al. 2006; Uusi-Heikkilä et al. 2008). For example, behaviours linked with size or sex might be under selection in recreationally important fish species, such as aggression in male largemouth bass (*Micropterus salmoides*) during the extended parental care period (Suski and Philipp 2004; Uusi-Heikkilä et al. 2008). Not only is there potential for selection against these traits upon harvest, but selection can also occur in catch-and-release fisheries when fish experience hooking or stress-related mortalities (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007) or physiological impairment of reproductive behaviour (Cooke et al. 2002*b*), which facilitates nest predation and loss of broods in species with nest guarding behaviour such as largemouth bass and smallmouth bass (*Micropterus dolomieu*; Kieffer et al. 1995; Cooke et al. 2002*b*). Limited information exists for the potential of fishing-induced selection to affect behavioural and underlying physiological traits, and this research area has been identified as one of the most pressing areas to advance the field of fishing-induced evolution (Uusi-Heikkilä et al. 2008).

Advances in the understanding of recreational fishing-induced evolution have recently occurred using largemouth bass as a model species. In an experimental population of largemouth bass that were specifically selected for differential vulnerability to angling over three generations, it was determined that vulnerability to angling is a genetically heritable trait (Philipp et al. 2009). Although it has been recognized for some time that certain individuals within a species are more vulnerable to angling than others, for example in largemouth bass (Bennett 1954) and brown trout (*Salmo trutta*) (McLaren 1970), this study was the first to demonstrate a heritable basis for individual differences in vulnerability to the angling gear (Philipp et al. 2009). Explanations for these variable responses have been related to different levels of wariness or boldness, and the differential ability of individual fish to learn from previous experiences (Anderson and Heman 1969; Beukema 1970; Garrett 2002). Alternative possibilities link the amount of nutrients required by fish with spatial and temporal foraging activity as means of encountering anglers' artificial lures more often (Philipp et al. 2009). Indeed, a study by Cooke et al. (2007) demonstrated that lower vulnerability to angling in largemouth bass was correlated with lower resting cardiac variables, which are measures known to approximate standard metabolic rate (SMR) (Satchell 1991). SMR represents the minimum energy demand for baseline organismal function (Fry 1957), and lower rates are less energetically costly (Brett and Groves 1979). It is conceivable that any decreases in demands for foraging activity for obtaining resources make these fish less susceptible to capture by anglers. If SMR is a driving force behind differential vulnerability to angling, behavioural differences and activity rates may also emerge as important traits correlated with metabolism or other physiological properties of the individual fish (Uusi-Heikkilä et al. 2008).

In physiologically stressful situations, fish can be exposed to anaerobic disturbances (Booth et al. 1995; Cooke et al. 2002*b*) that result in the depletion of muscle glycogen, adenosine triphosphate (ATP), and phosphocreatine (PCr) stores, as well as the production of lactate (Driedzic and Hochachka 1978; Milligan 1996). The severity of anaerobic disturbance can be determined during simulated exhaustive exercise (Milligan 1996; Kieffer 2000). In addition, an indication of overall anaerobic (i.e., lactate dehydrogenase (LDH)) and aerobic (i.e., citrate synthase (CS) and, cytochrome *c* oxidase (CCO)) capacity can be gained from muscle enzyme activity (Somero and Childress 1980; 1990). The duration of recovery has implications for the resumption of aerobic activity (reviewed in Goolish 1991). An evaluation of exercise performance, recovery, and muscle enzyme activities of fish differentially selected for vulnerability to angling may provide additional insight into the physiological traits under selection, as well as the potential consequences of fishing-induced selection for wild fish stocks.

The purpose of this study was to evaluate the physiological correlates of vulnerability to angling in two experimental lines of largemouth bass that were differentially selected for this trait, and that exhibit rather dramatic differences in catchability to rod-and-reel angling (Philipp et al. 2009). In fact, angling vulnerability was determined to be a heritable trait with a heritability of about 0.15 (Philipp et al. 2009). Specifically, we tested the hypothesis that low vulnerability fish (LVF) and high vulnerability fish (HVF) would have different metabolic rates and scopes. In terms of predictions, first based on previous findings where LVF had lower resting cardiac variables (Cooke et al. 2007), we predicted that LVF would display a lower SMR than would HVF. Second,

because fish with a lower SMR should correspondingly decrease their maximum metabolic rate (MMR; Priede 1985), we predicted that LVF and HVF would have comparable metabolic scopes. We also tested the hypothesis that LVF and HVF would have a different physiological response to exhaustive exercise. Based on the expectation that LVF would display a lower SMR with decreased energetic demands (Priede 1985), we predicted that LVF would have a reduced anaerobic capacity, as indicated by muscle lactate dehydrogenase (LDH), and accumulate less lactate than HVF. LVF would then be expected to require proportionally less time to recover from exhaustive exercise (Goolish 1991).

Materials and methods

Study animals

This study takes advantage of an artificial truncation selection experiment that began several decades ago at the Illinois Natural History Survey in the USA (Philipp et al. 2009). Beginning in 1977, largemouth bass in Ridge Lake (Illinois, USA, 39.40°N, 88.16°W; 7.1 ha surface area) were subjected to four consecutive seasons of angling, and catch histories of tagged individuals were recorded as part of a project evaluating the impact of catch-and-release angling (Burkett et al. 1986). Following these four seasons of angling, the reservoir was drained and the largemouth bass were collected. Based on an assessment of individual catch histories, two divergent experimental lines, each with two replicate lines, were selected for low and high vulnerability to angling (Philipp et al. 2009). Low vulnerability brood fish (LVF) were never captured across all four seasons, and high vulnerability brood fish (HVF) were captured more than four times in a single season (Philipp et al. 2009). Five pairs in each parental (P1) generation of each line were bred in separate experimental ponds to produce first (F1) generation offspring, which were then differentiated by pelvic fin clips (Philipp et al. 2009). The offspring from each replicated line (N = 200) were raised together in a common pond for three years until the individuals were large enough to be angled (Philipp et al. 2009). A selection procedure using experimental angling over one season was repeated on the F1 fish, and artificially selected LVF and HVF based on catch frequency were again separated into different experimental ponds for breeding (Philipp et al. 2009). The F2 generation offspring were raised in a manner similar to the F1 generation, and the same selection procedure was repeated until the F4 generation. The response to selection was found to increase with each generation, and LVF displayed a heightened response as compared with HVF (Philipp et al. 2009). The fish used in the current study were bred naturally in ponds in the spring of 2006 from the F3 generation, and they had not experienced any further artificial selection.

Oxygen consumption and metabolic rates

In October 2007, a sub-sample of juvenile LVF and HVF were removed randomly from a common experimental pond at the University of Illinois in Champaign–Urbana and transported to the Kaskaskia Biological Station, near Sullivan, Illinois (39.60°N, 88.61°W). There were no significant differences between the weights for the two groups [LVF, 95.0 ± 6.7 g (mean ± standard error of the mean, SEM) (N = 8); HVF, 78.8 ± 8.3 g (N = 8); P = 0.15], but there was a significant difference between the total lengths (LVF, 200 ± 4.0 mm; HVF, 172 ± 11.4 mm; P = 0.04). Prior to experimentation, all fish were held for a minimum of 60 h in a recirculating tank (~300 L) at water temperatures of 20 ± 0.8 °C, dissolved oxygen levels of 8.2–8.8 mg·L^{-1}, ammonia levels of less than 3.0 ppm, a pH of 7.6, and a natural photoperiod (12 h light : 12 h dark). The fish were not fed for the duration of the experimental trials, which lasted for one week.

Oxygen consumption (standard metabolic rate, SMR) of the two lines was measured using computerized, intermittent flow-through respirometry (Loligo Systems, Tjele, Denmark) with a configuration similar to that described by Herskin (1999). Briefly, four glass chambers (volume = 0.7 L) were immersed side by side in a 175 L tank maintained at 20.5 ± 1 °C. The water in the tank was aerated and filtered continuously through charcoal and a UV-sterilizer. Water was recirculated continuously through each chamber using small fountain pumps, which were driven by AutoResp™ 4 software (Loligo Systems, Tjele, Denmark) installed on a personal computer. The system alternated between a 5 min closed, re-circulating measurement phase, a 4 min open flushing phase, with a 1 min delay period (Herskin 1999).

Variations in oxygen partial pressures ($P\text{o}_2$) were measured by fiber optic oxygen probes connected to an OXY-4 fiber optic oxygen instrument (Loligo Systems, Tjele, Denmark). $P\text{o}_2$ was recorded every second during the 5 min measuring phase, and a linear regression was calculated between $P\text{o}_2$ and time. The slope of the regression line (k) was used to calculate oxygen consumption according to the equation:

$$M\text{o}_2 = kV_{\text{resp}} \cdot M^{-1}\alpha,$$

where $M\text{o}_2$ is the oxygen consumption in mg O_2·kg^{-1}·h^{-1}, V_{resp} is the volume of the respirometry chamber in L, M is the fish mass in kg, and α is the solubility of O_2 in water at the experimental temperature (Cruz-Neto and Steffensen 1997). All slopes used in the calculations were derived from equations where $r^2 > 0.95$. This method generated one $M\text{o}_2$ data point every 10 min.

Experimental trials consisted of placing two LVF and two HVF in individual chambers during the afternoon. The fish were left undisturbed for an afternoon and an overnight period (18–20 h), during which time the AutoResp™ 4 software controlled the measuring and flushing phases and recorded $M\text{o}_2$. SMR was determined for each individual fish by averaging the lowest six $M\text{o}_2$ values recorded between approximately 2100–0800, and is representative of a 1 h period (McKenzie et al. 2003). Routine metabolic rate (RMR) was determined by averaging the six $M\text{o}_2$ values recorded between approximately 1845–1945, after the fish had acclimated to their chambers and had resumed normal daylight activities.

Upon completion of the SMR trials, the elevation of $M\text{o}_2$ following exercise was assessed, generating a factor similar to "excess post-exercise oxygen consumption" (EPOC; Gaesser and Brooks 1984), which enabled us to evaluate recovery patterns. Previous work has used swimming respir-

ometry and the continuous monitoring of recovering fish over time to generate data on MMR and EPOC (Jain et al. 1998; Farrell 2007). However, largemouth bass are regarded as poor swimmers, and their lifestyle is more consistent with a "sit-and-burst" approach to movement (Kieffer and Cooke 2009). In the current study, we calculated EPOC in a manner that allows a relative comparison of the metabolic scope devoted to recovery following exhaustive exercise. As such, our approach yielded an approximation of EPOC and it was not possible to calculate the area under the curve (as per Farrell 2007) given our protocol. For this measurement, the largemouth bass were individually removed from their chambers and placed in an aerated tank (~60 L), where they were forced to exercise for 2 min by having their tails pinched to induce burst swimming. Tail pinching is a standard method of exhaustively exercising fish to generate physiological disturbances (Wood 1991; Wang et al. 1994). Largemouth bass exhaust quickly relative to salmonids (Kieffer and Cooke 2009), so the 2 min exercise period was considered a substantial metabolic challenge for the fish. Following the 2 min exercise period, the largemouth bass were immediately returned to their chambers and their oxygen consumption rates were recorded over a 2 h period. The highest $M\text{o}_2$ value recorded during the post-exercise period was used as a measure of the MMR for each individual. Metabolic scope was calculated as the difference between the MMR and the lowest $M\text{o}_2$ measured overnight. For the 2 h recovery period, the EPOC values were expressed as a percentage of the metabolic scope calculated at 30 min intervals with an initial 15 min value.

Exercise and muscle metabolites

In July 2007, a random sub-sample of LVF and HVF were removed from an experimental pond at the University of Illinois in Champaign–Urbana and transported to the Sam Parr Biological Station near Kinmundy, Illinois (38.77°N, 88.85°W). There were no significant differences between the weights for the two groups [LVF, 13.3 ± 0.4 g (mean ± SEM) (N = 49); HVF, 12.7 ± 0.2 g (N = 47); P = 0.14], but there was a significant difference between the total lengths (LVF, 110 ± 0.9 mm; HVF, 106 ± 0.6 mm; P = 0.002). Prior to experimentation, fish were held for a minimum of 48 h in recirculating tanks (~600 L) at a density of 7–8 fish per tank. Water temperatures in the tanks were 23–24.5 °C, dissolved oxygen levels were 8.2–8.5 mg·L^{-1}, ammonia levels were less than 0.25 ppm, and a natural photoperiod (14 h light : 10 h dark) was maintained. These fish were not fed for the duration of the experimental trials, which lasted 10 d.

To generate control (resting) physiological values, individual LVF and HVF were removed from the holding tanks and placed in darkened holding chambers (0.8 L) continuously supplied with aerated water. Fish were left undisturbed for a 24 h period, at which time a lethal dose of anesthetic (250 mg·L^{-1} of tricaine methanesulfonate buffered by 500 mg·L^{-1} of sodium bicarbonate) (Summerfelt and Smith 1990) was introduced into the chamber. Once fish had completely lost all reactivity (~2 min), all white muscle above the lateral line and behind the operculum (~1 g) was removed using a razor blade. Muscle samples were immediately freeze-clamped with aluminum tongs pre-cooled in liquid nitrogen and then stored in liquid nitrogen until processing (Booth et al. 1995).

To induce a physiological disturbance, an exhaustive exercise regime was applied to another group of LVF and HVF. Fish were transferred from a common holding tank to an aerated circular container (20 L) where they were chased by tail pinching for 2 min. Following the 2 min exercise regime, one group of fish (both LVF and HVF) was immediately transferred into a solution of buffered anesthetic (2 L) for euthanization and white muscle samples were removed as previously described. Three additional groups of largemouth bass (both LVF and HVF) were also exercised for 2 min and then placed into individual holding chambers and allowed to recover for 1, 2, or 4 h. Following the recovery period, buffered anesthetic was introduced into each chamber for euthanization and white muscle samples were removed as described above.

Tissue metabolite assays

White muscle tissue was prepared for metabolic assays by following the methods of Booth et al. (1995). Briefly, 0.5 g of frozen muscle was ground under liquid nitrogen using a mortar and pestle. The powdered tissue was first combined with a solution of 8% perchloric acid and 1 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA), and then with a volume of neutralizing solution (containing 2 mol·L^{-1} potassium hydroxide, 0.4 mol·L^{-1} potassium chloride, and 0.3 mol·L^{-1} imidazole). The resulting supernatant was stored at –80 °C to be assayed later. Analyses of tissue lactate, PCr, and ATP concentrations were performed in duplicate on the prepared muscle extracts using the enzymatic assay techniques of Lowry and Passonneau (1972). Water content of the muscle samples was determined as outlined in Suski et al. (2003).

The total anaerobic energy expenditure (AEE) in the white muscle of fish from each exercise treatment group was expressed in terms of ATP equivalents according to the following equation:

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

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 (Pearson et al. 1990; McDonald et al. 1998*a*).

Tissue enzyme and protein assays

White muscle samples from a separate group of control (no exercise) fish were prepared for enzymatic assays using the homogenization technique described in Rajotte and Couture (2002). The homogenization buffer contained 20 mmol·L^{-1} 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 1 mmol·L^{-1} EDTA, and 0.1% Triton X-100. All enzyme activity determinations were performed in duplicate at 24 °C (the optimum temperature for growth of largemouth bass) using a UV/Vis spectrophotometer (Varian Cary 100, Varian Inc., Palo Alto, California, USA). Tissue dilutions and cofactor concentrations were selected from assay optimizations. Enzyme assays were conducted (in the following order) for citrate synthase (EC 4.1.3.7; CS), lactate dehydrogenase (EC 1.1.1.27; LDH), and cytochrome *c* oxidase (EC 1.9.3.1; CCO). The wavelengths and millimolar

Table 1. Metabolic rates ($mg\,O_2 \cdot kg^{-1} \cdot h^{-1}$) and body weight (g) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass, presented as mean ± SEM.

	Vulnerability line			
Parameter	HVF	LVF	Test statistic	*P* value
Body weight	79±8.3	95±6.7	T = –1.53	0.15
Standard metabolic rate (SMR)	115±3.6	104±3.3	T = 2.42	0.029
Maximal metabolic rate (MMR)	280±11.8	241±7.5	T = 2.85	0.014
Metabolic scope	167±9.4	140±6.0	T = 2.51	0.026
Routine metabolic rate (RMR)	189±26.5	130±3.0	U = –0.95	0.35

Note: $N = 8$ for all values, except metabolic scope for HVF, where $N = 7$. T denotes the use of a *t* test, and U denotes the use of a Mann–Whitney *U* test.

extinction coefficients identified for CS, LDH, and CCO were identical to those used in Couture et al. (1998), with the exception of the millimolar extinction coefficient for cytochrome *c*, which was 18.7. The reactions were linear over the 5 min period used for the calculation of enzyme activity, and the results are expressed in international units (micromole of substrate converted to product per minute) per gram of tissue mass. Assay conditions for CS, LDH, and CCO were as described in Pelletier et al. (1994), with the following modifications. For LDH, 0.16 $mmol \cdot L^{-1}$ nicotinamide adenine dinucleotide reduced (NADH) and 1.0 $mmol \cdot L^{-1}$ pyruvate were used; and for CS, 0.1 $mmol \cdot L^{-1}$ acetyl CoA and 0.15 $mmol \cdot L^{-1}$ oxaloacetate were used. For CCO, the pH was adjusted to 7.5. The protein content of the muscle extracts was determined using the bicinchoninic acid method of Smith et al. (1985) with bovine serum albumin as a standard.

Statistical analyses

Normality was assessed using a one-sample Kolmogorov–Smirnov test, and homogeneity of variance was assessed using the Levene's test. Comparisons were made between LVF and HVF for SMR, MMR, RMR, metabolic scope, and tissue enzymes using *t* tests. Where the assumption of equality of variance was not met, the nonparametric Mann–Whitney *U* test was used. An arcsine square-root transformation was carried out on EPOC data, because they were expressed as a percentage of metabolic scope. These EPOC values were compared between LVF and HVF across recovery periods using a two-way repeated measures analysis of variance with individual fish entered as a random effect, and both treatment and recovery time entered as fixed effects. The mean concentrations of tissue metabolites and anaerobic energy expenditures were compared between LVF and HVF across recovery periods using a two-way analysis of variance (ANOVA; main effects: vulnerability group and recovery time). For the two-way ANOVAs, when there was a significant difference between HVF and LVF coupled with a significant treatment effect but no significant interaction, a post hoc *t* test was used to identify differences between HVF and LVF. For situations following the two-way ANOVA with no significant HVF/LVF effect, no significant interaction, but a significant treatment effect, a Tukey–Kramer honestly significant difference (HSD) post-hoc test was used to compare individual recovery times with each other (independent of HVF/LVF effects). For situations following the two-way ANOVA with a significant interaction combined with a significant group and (or) treatment effect, data were split and examined within lines with a Tukey–Kramer HSD post-hoc test to identify differences within lines at different recovery points (Sokal and Rohlf 1995). For the two-way ANOVAs involving muscle metabolites, EPOC, and AEE, all models were initially run independently with individual fish weight and length as covariates; because neither of these covariates had a significant influence on the dependent variable in any case, length and weight were omitted from subsequent models reported below. All *t* tests were carried out using SPSS 11.0 (SPSS Inc., Chicago, Ill.). All ANOVAs were carried out using JMPIN 7.0.1 (SAS Institute Inc., Cary, North Carolina). Values are reported as means (± SEM), and the level of significance (α) used for all tests was 0.05.

Results

LVF and HVF differed significantly in terms of mass-specific SMR and MMR. SMR was 10% lower and MMR was 14% lower for LVF than for HVF (Table 1). The metabolic scope was approximately 16% lower for LVF than for HVF (Table 1). An initial comparison of RMR between LVF and HVF revealed a 31% lower value for LVF than for HVF. Owing to substantially unequal variances between the RMR for LVF and HVF (Levene's test, $P < 0.05$), a nonparametric test was conducted, which was nonsignificant (Table 1).

Within 15 min after achieving maximal $\dot{M}O_2$, both LVF and HVF were devoting 80%–84% of their metabolic scope to recovery (Fig. 1). The amount of the metabolic scope utilized for EPOC declined to 57% at the 35 min recovery interval, where it then remained relatively stable (within 34%–52%) for the duration of the recovery period (Fig. 1).

During the 2 min exercise trials, differential swimming patterns were observed between HVF and LVF. Upon being chased by tail grabbing, HVF exhibited rapid and erratic burst swimming, whereas LVF did not burst swim as readily. HVF also appeared to reach exhaustion (in terms of being unable to respond to chasing) quicker than LVF. LVF were more often able to sustain some degree of swimming for the duration of the exercise period, although they failed to burst swim with the same vigour as HVF.

Immediately following exhaustive exercise, white muscle ATP and PCr concentrations for both LVF and HVF decreased by 80%–100% relative to resting control values (Figs. 2*a* and 2*b*; Table 2). Owing to variability between individuals, however, differences across treatment groups were not statistically significant. Following 1 h of recovery, tissue

Fig. 1. Percent of metabolic scope utilized during recovery from exhaustive exercise by high vulnerability (HVF) and low vulnerability (LVF) largemouth bass. Filled bars represent HVF and open bars represent LVF. Values are mean ± SEM, where $N = 7$ for HVF and $N = 8$ for LVF. The effects tests for the two-way repeated measures ANOVA are as follows: group, df = 1, $F = 0.002$, $P = 0.97$; treatment, df = 4, $F = 134.6$, $P < 0.001$; interaction, df = 4, $F = 4.5$, $P = 0.003$. Dissimilar letters a, b, and c denote significant differences between pairs of HVF across recovery times, and dissimilar letters w, x, y, and z denote significant differences between pairs of LVF across recovery times (Tukey–Kramer honestly significant difference test, $P < 0.05$).

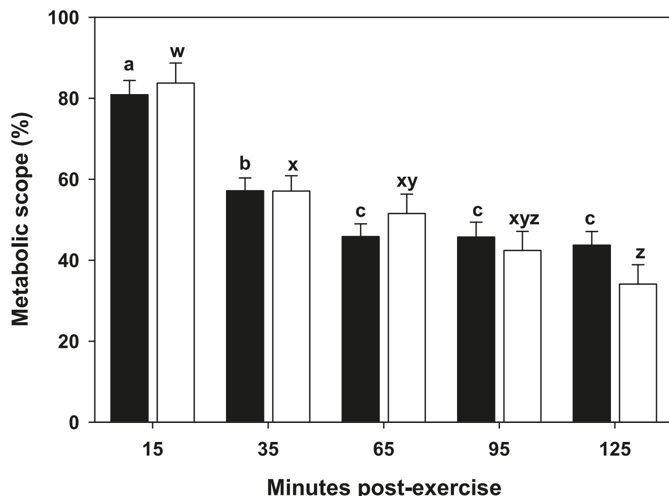
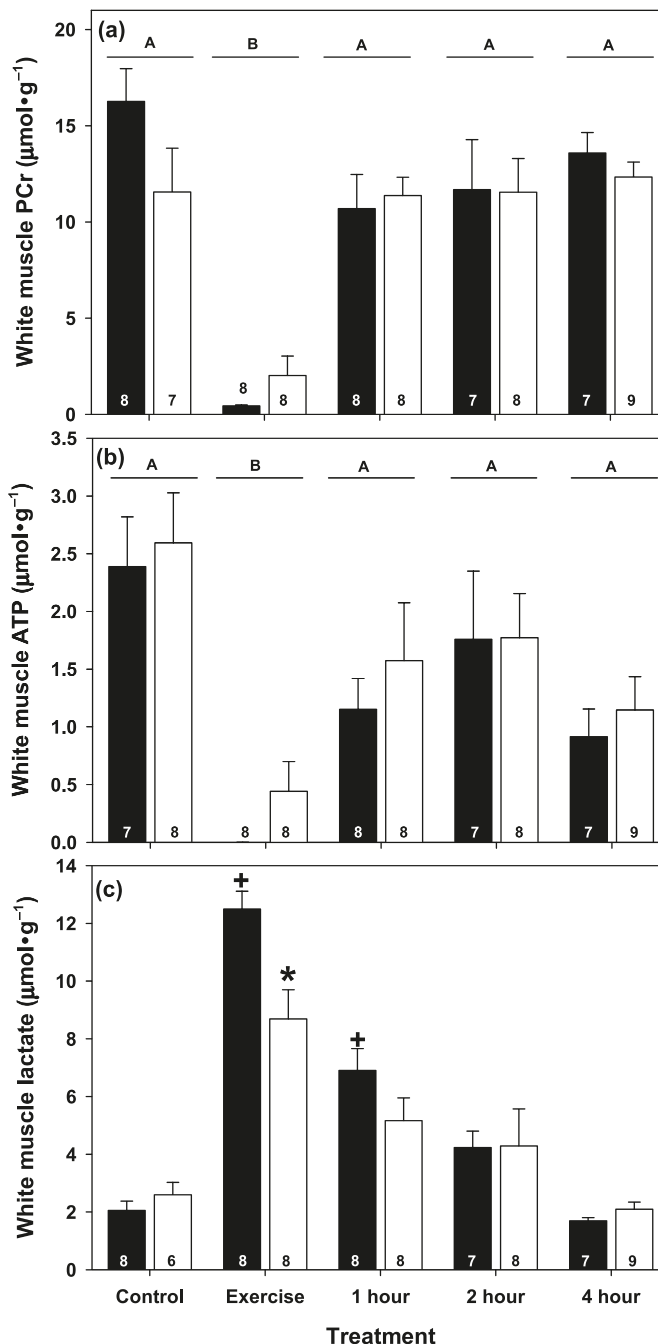


Fig. 2. Concentrations of (*a*) PCr, (*b*) ATP, and (*c*) lactate in the white muscle of high vulnerability (HVF) and low vulnerability (LVF) largemouth bass following exhaustive exercise and recovery. Filled bars represent HVF and open bars represent LVF. Values are mean ± SEM, with the sample size for each treatment shown on the bars. For PCr and ATP, differences at individual recovery periods (independent of vulnerability) are denoted by upper case letters joined by a horizontal line (two-way ANOVA), Tukey–Kramer honestly significant difference (HSD) test, $P < 0.05$). For lactate, differences between a recovery period and the within-line control group are indicated by a plus sign (+) for HVF, and an asterisk (*) for LVF line (two-way ANOVA, Tukey–Kramer HSD test, $P < 0.05$). ANOVA results are shown in Table 2.



ATP concentrations were approaching control values for LVF and HVF, and they returned to control levels following 2 h of recovery (Fig. 2*b*; Table 2). Following 1 h of recovery, tissue PCr concentrations had returned to control values and remained there for the entire recovery profile (Fig. 2*a*; Table 2). Mean water content of the white muscle was 78.5% (range 71.0%–81.5%) and did not differ significantly across the different treatments, time periods, or their interaction ($P > 0.05$). Exhaustive exercise for 2 min resulted in an increase in tissue lactate that was 3.5× greater than resting control values for LVF (Fig. 2*c*; Table 2). For HVF, there was a six-fold increase in tissue lactate concentrations over control values (Fig. 2*c*; Table 2). By 1 h following exercise, the tissue lactate concentrations for LVF were no longer significant from resting control values (Fig. 2*c*; Table 2). By 2 h following exercise, tissue lactate concentrations for both LVF and HVF had returned to resting control values, where they remained for the remainder of the recovery profile (Fig. 2*a*; Table 2).

At all measurement points following the exhaustive exercise protocol, the AEE for LVF was significantly lower than the AEE for HVF (Fig. 3). White muscle AEE declined with recovery times, and there was no significant interaction between recovery time and angling vulnerability (Fig. 3; Table 2).

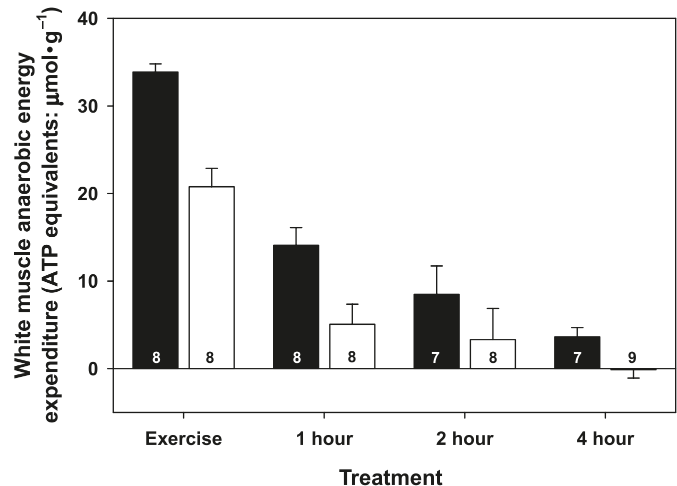
The activity of LDH, an indicator of anaerobic capacity, did not differ between LVF and HVF, nor did the activities of CS and CCO, which are indicators of aerobic capacity (Table 3). Power analyses revealed that the values for β were very low, with the highest reported value at about 0.2 for CCO (Table 3).

Table 2. Effects tests for two-way ANOVAs used to compare tissue metabolites for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass.

Source	df	*F* ratio	*P* value
Lactate			
Group	1	3.56	0.064
Treatment	4	44.4	<0.01
Interaction	4	3.12	0.021
ATP			
Group	1	1.27	0.26
Treatment	4	10.5	<0.01
Interaction	4	0.11	0.98
PCr			
Group	1	0.63	0.43
Treatment	4	23.1	<0.01
Interaction	4	1.27	0.29
AEE			
Group	1	24.9	<0.0001
Treatment	3	53.1	<0.0001
Interaction	3	1.8	0.15

Note: Body weight was excluded from the final model because it was a nonsignificant co-variate ($P > 0.05$). Group refers to HVF and LVF, and treatment refers to the recovery period following exhaustive exercise.

Fig. 3. White muscle anaerobic energy expenditure (AEE) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass following exhaustive exercise and recovery. AEE was significantly higher for HVF by comparison with LVF. Filled bars represent HVF and open bars represent LVF. Values are mean ± SEM, with the sample size for each treatment shown on the bars. ANOVA results are shown in Table 2. The effects tests for the two-way ANOVA are as follows: group, df = 1, $F = 24.9$, $P < 0.0001$; treatment, df = 3, $F = 53.1$, $P < 0.001$; interaction, df = 3, $F = 1.8$, $P = 0.15$.



Discussion

Following three generations of artificial selection for differential angling vulnerability, differences in metabolic capacity were observed between two experimental lines of largemouth bass. LVF were found to exhibit a lower SMR than HVF, a trend that is consistent with the lower cardiac output and heart rate found for LVF by Cooke et al. (2007). The combination of a lower SMR and a lower MMR following exhaustive exercise limited LVF to a narrower metabolic scope than HVF. This finding is in contrast to the broader cardiac scope found for LVF by Cooke et al. (2007), where cardiac output and heart rate were used to approximate metabolic scope. The relationship between heart rate and oxygen consumption (used here to determine metabolic rates) is not always linear or constant within a single species, and the relationship can be further confounded by stress (potentially induced by the cardiac surgery) and environmental factors (Thorarensen et al. 1996). It is generally accepted that fish compensate for a higher SMR by increasing their MMR sufficiently to maintain metabolic scope (Priede 1985); however, a higher SMR is known to be more energetically costly and thereby requires a greater food intake (Brett and Groves 1979). It is possible that LVF have acquired a lower metabolic scope in response to the decreased foraging activities necessary to meet their lower energetic demands (Priede 1985), which have been estimated to be 30% lower than those of HVF (Cooke et al. 2007). A study with a population of three-spined sticklebacks (*Gasterosteus aculeatus*) offers support for this argument: the resident, nonmigratory morph displayed a lower SMR, a lower active MR, and a narrower metabolic scope than the active, migratory morph (Tudorache et al. 2007). Under food limited situations the lower SMR might actually be beneficial for LVF and result in elevated growth rate over growth by HVF. This prediction agrees with a study by Redpath et al. (2009) on juvenile LVF and HVF. In that study LVF in ponds were found to grow faster than HVF, which on first sight might seem counterintuitive but can be explained by the lower metabolic costs that LVF have compared with HVF fish. Metabolic scope can also be an indicator of the potential range for aerobic activity (Priede 1985), and an enhanced scope can assist in recovery after burst-type anaerobic activity (Killen et al. 2007). Despite the larger metabolic scope displayed by HVF, both groups devoted nearly identical proportions of their scopes to facilitate recovery from exhaustive exercise and to repay oxygen debts. When not forced to function at the limit of their aerobic capacity, a greater scope may still enable HVF to sustain energetically costly behaviours, such as parental care, better than LVF.

The differences in metabolic capacities measured for largemouth bass selected for differential vulnerability to angling were accompanied by key changes in exercise performance. While the overall trends observed for LVF and HVF in terms of the tissue metabolite (lactate) and energy stores (ATP and PCr) following exhaustive exercise were comparable with results in the literature (reviewed in Milligan 1996), there were some notable variations. Following 1 h of recovery from exhaustive exercise, the lactate levels in LVF had returned to control values, while the lactate levels in HVF were still significantly higher than control values. LVF also demonstrated an AEE that was 39% lower than HVF. AEE is regarded as a measure of realized anaerobic capacity (McDonald et al. 1998*b*), because it encompasses the maximal amount of lactate production and depletion of the tissue energy stores. The swimming behaviour of LVF during the exercise simulation also differed from that of HVF. We observed qualitatively that HVF engaged in higher incidences of burst swimming accompanied

Table 3. Enzyme activities (IU, expressed as g·wet mass^{-1}) and protein concentrations (mg·g^{-1}) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass, presented as mean ± SEM, where N = 8.

Parameter	Vulnerability line: HVF	Vulnerability line: LVF	Test statistic (T)	*P* value	Power (β)	Least significant *N*
Lactate dehydrogenase	105.8±7.1	95.3±11.7	0.77	0.46	0.11	54
Citrate synthase	1.96±0.15	2.12±0.22	–0.59	0.57	0.08	91
Cytochrome *c* oxidase	1.51±0.08	1.71±0.15	–1.20	0.25	0.20	23
Proteins	162.8±4.2	155.3±9.5	0.73	0.48	—	—

Note: Least significant *N* represents a power of β = 0.5; (T) indicates the use of a *t* test.

by faster startle responses, whereas LVF maintained a steadier swimming pattern during manual chasing. These observations, along with the recovery rates for lactate and AEE, suggest that LVF have a lower anaerobic capacity than HVF. Given that the predation strategy of largemouth bass involves a series of short chases with fluctuating swimming velocities (Winemiller and Taylor 1987), this apparent difference in burst swimming ability should allow HVF to capture prey, and hence anglers' lures, more efficiently (Somero and Childress 1980). By contrast, decreased burst swimming ability in LVF may hinder juveniles in their efforts to avoid predators (Fuiman and Magurran 1994), and adult male LVF may not be equipped to defend their broods from predation as well as HVF (Cooke et al. 2002*a*).

Despite the decreased anaerobic capacity and burst swimming ability of LVF, not all of the physiological variables were strongly associated with differential vulnerability to angling, which is consistent with a growing body of literature that challenges the relationship between enzyme capacity and swimming performance (e.g., Gibb and Dickson 2002). Specifically, anaerobic and aerobic enzyme activities and tissue energy stores (ATP and PCr) across the recovery profile were statistically similar for both experimental lines. LDH is an enzymatic indicator of anaerobic capacity (Somero and Childress 1980; 1990), because it is responsible for the conversion of pyruvate to lactate and thus the anaerobic production of ATP (Driedzic and Hochachka 1978). There was no statistical difference between LVF and HVF in terms of LDH activity. Additional enzymes assist in generating anaerobic metabolic power (Somero and Childress 1980) and could be related to the decreased anaerobic capacity observed in LVF. Glycogen stores and the overall glycolytic capacity to produce pyruvate can also influence anaerobic power (Somero and Childress 1990). Although the enzymatic activity of LDH did not provide evidence of a lower anaerobic capacity for LVF, perhaps the decreased AEE in LVF can be related to the availability of less glucose or the activities of enzymes near the beginning of the glycolytic pathway.

An indication of aerobic capacity is provided by CCO and CS, and these enzymes also influence recovery rate (Somero and Childress 1980; 1990). No significant differences were observed between LVF and HVF for the enzyme activities of either CCO or CS. The oxidation rate of lactate into glycogen in the white muscle following exercise is controlled by the mitochondrial enzyme CCO (Goolish 1991), and it is a good indication of recovery rate in terms of lactate clearance (Somero and Childress 1980; 1990). LVF appear to have cleared lactate more quickly, as this metabolite returned to control values with 1 h, by comparison with the 2 h required by HVF. The potential for a higher aerobic capacity in LVF is further supported by the observations of their sustained swimming ability. However, the similarities between the aerobic enzymatic indicators in the two groups of fish make any conclusions difficult. A clear distinction has been made between the initial anaerobic response to exhaustive exercise for LVF and HVF, although further studies are required to determine whether the aerobic capacities are also different between the two strains of fish. Despite the questions that remain unanswered, this study provides evidence that experimental lines of largemouth bass intentionally selected for low and high vulnerability to angling exhibit differences in physiological capacity.

This study provides evidence that vulnerability to angling, a partially genetic trait (Philipp et al. 2009), may be related to a suite of physiological, and possibly behavioural, traits. Vulnerability to angling has been demonstrated to be a genetically heritable trait (h^2 = 0.15) for the largemouth bass used in this study (Philipp et al. 2009). The physiological traits that were evaluated were not measured for the original parental generations, so it is difficult to establish whether the observed differences in metabolic rates and anaerobic capacity occurred in response to selection. Nevertheless, the relationship that was identified between these physiological traits and differential vulnerability to angling has implications for the daily activities of fish in terms of energy acquisition and swimming performance. As a result, selection against high vulnerability to angling risks altering natural populations of recreationally exploited fish in physiological and behavioral terms, potentially affecting their performance of certain behaviours, based on these physiological parameters (Uusi-Heikkilä et al. 2008). Further research is needed on the question of whether the differences in metabolic rates have ecological consequences and affect fitness of fish selected for high and low vulnerability to angling. If this link is made, recreational fishing-induced evolution might have repercussions for wild fish stocks.

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