The Effects of Fishing-Induced Selection on Physiological and Life-History Traits in Largemouth Bass (*Micropterus salmoides*): A Recreational Angling Perspective

By

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Dedication

To Alex, for opening my eyes to the beauty and wonder of the underwater world, and for never failing to support and encourage me. To Mom and Dad, for teaching me to get my hands dirty, to respect nature in all seasons, and to follow my dreams. Thanks to each and every one of my colleagues in the Cooke Lab for sharing advice, humour, and friendship. Special thanks to Steve for paving the way and being an inspirational role model.

Abstract

Humans are increasingly affecting the sustainability of fisheries by way of fishing-induced selection, leading to shifting gene frequencies and evolutionary changes. This thesis examines the potential effects of fishing-induced selection on the trait of vulnerability to angling in a recreational fishery. Field and laboratory experiments were carried out to determine the relationships between vulnerability to angling and metabolism, anaerobic capacity, life history, and energetics. Largemouth bass (*Micropterus salmoides*) selected for high vulnerability to angling (HVF) had higher metabolic rates and a broader metabolic scope, and they experienced more severe physiological disturbances following exhaustive exercise. Fish selected for low vulnerability to angling (LVF) demonstrated higher growth rates accompanied by a lower investment in gonadal development. While energy stores did not differ, HVF displayed higher levels of plasma magnesium, which is indicative of increased feeding. Overall, this research identifies novel associations between physiological and life-history traits and differential vulnerability to angling from the perspective of a recreational fishery.

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Co-Authorship

Chapter 2: Metabolic Correlates of Vulnerability to Angling in a Teleost Fish: Insights from Physiology and Biochemistry. T.D. Redpath, S.J. Cooke, C.D. Suski, R. Arlinghaus, P. Couture, D.H. Wahl, and D.P. Philipp

While this study is my own, the research was undertaken as part of a collaborative effort, and each co-author played a valuable role in its completion. Specifically, the experimental lines of largemouth bass were created by Wahl and Philipp. The project was conceived by Redpath, Cooke, and Philipp. Laboratory experiments were designed by Redpath, Cooke, Suski, and Couture, and all laboratory work was conducted by Redpath with the assistance of Suski and Couture. All data analysis was conducted by Redpath. Data were interpreted by Redpath, Cooke, Suski, and Couture. All writing was conducted by Redpath. All co-authors provided comments and feedback on the manuscript. The manuscript is in preparation for submission to the *Journal of Experimental Biology*.

Chapter 3: Life-History Traits and Energetic Status in Relation to Vulnerability to Angling in a Teleost Fish. T.D. Redpath, S.J. Cooke, R. Arlinghaus, D.H. Wahl, and D.P. Philipp

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Glossary

AEE	anaerobic energy expenditure
ANOVA	analysis of variance
ATP	adenosine triphosphate
CCO	cytochrome *c* oxidase
CPK	creatine phosphokinase
CS	citrate synthase
EC	Enzyme Commission
EDTA	ethylenediaminetetraacetic acid
EMG	electromyogram
EPOC	excess post-exercise oxygen consumption
GSI	gonadosomatic index
HEPES	4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid
HSD	honestly significant difference
HSI	hepatosomatic index
HVF	high vulnerability fish
IU	international units
KCl	potassium chloride
KOH	potassium hydroxide
LDH	lactate dehydrogenase
LVF	low vulnerability fish
MMR	maximum metabolic rate
MR	metabolic rate

Mo_2	oxygen consumption
NADH	nicotinamide-adenine dinucleotide reduced
PCr	phosphocreatine
PFK	phosphofructokinase
Po_2	oxygen partial pressure
RMR	routine metabolic rate
SE	standard error
SMR	standard metabolic rate
TL	total length
WT	weight

Chapter 1: General Introduction

It is largely accepted by scientists that natural selection has played a dominant role in shaping the evolutionary paths of fish species (reviewed in Robinson and Schluter 2000). However, over-exploitation by humans is increasingly being recognized for altering the dynamics of many marine fish populations. Commercial fisheries tend to target certain life-history traits, and corresponding changes in size, maturation, and reproduction have resulted in the phenomenon of fisheries-induced selection (reviewed in Kuparinen and Merilä 2007). Although the mechanisms of selection are similar in recreational angling situations, few studies have examined this issue from a recreational perspective. It is important for fisheries managers to be aware of the potential implications of fisheries-induced evolution on recreational populations, as the resulting changes could affect genetic diversity, harvestable biomass, and ultimately fish population sustainability (Philipp et al. 2008). The overall objective of this thesis is to examine the effects of fisheries-induced selection from a recreational angling perspective. A brief overview of the critical concepts related to evolution and selection (natural and artificial) will be presented by way of a general introduction. The current state of scientific knowledge on fisheries-induced selection will also be summarized. Most of the existing studies have focused on the effects on marine fish species from a life-history perspective without examining the corresponding links to traits at other biological levels. It is important to consider the effects that selection could have from physiological and behavioural perspectives (Garland 2003), as these traits can also have long-term implications for the overall condition and survival of the fish. A second objective of this thesis is to determine the physiological and life history-based traits that are correlated

with differential vulnerability to angling. The general introduction will continue by providing an overview of several traits that serve as appropriate metrics to study the underlying mechanisms of vulnerability to angling. The traits that will be presented include: metabolism, aerobic and anaerobic activity, life-history, and energetics. This introductory chapter will conclude by presenting basic information on the model system and some general objectives and hypotheses.

Natural Selection and Evolution

Biologists and naturalists alike have long been intrigued by the diversity of life on the planet and by the capacity of organisms to respond to environmental changes. When Charles Darwin put forth his elegantly simple yet fundamental ideas of natural selection to explain the processes of adaptation and speciation in 1859, the foundation was laid for our understanding of the principles of evolutionary change. A central tenet of Darwin's work proposed that the distribution of traits in a natural population changes over time, as individuals more suited to their environment achieve greater reproductive success and pass their traits onto the next generation (Darwin 1859). A trait may be considered as any characteristic of an individual that can be measured or categorized (Nager et al. 2000), such as growth rate, running speed, and colouration. In any given population, there are many different phenotypes (observed values of a trait), which are composed of genetic factors (the genotype) (Nager et al. 2000). Phenotypes are expressed in response to complex interactions that occur between genetic and environmental influences, enabling individuals to maximize their chances for reproductive success (Nager et al. 2000). When the frequency of phenotypes with a hereditable basis in a population

changes, (which may or may not involve changes in gene frequencies), a step is taken on the evolutionary pathway (Pigliucci and Kaplan 2006).

Trait Heritability

Across a gradient of environmental conditions, a variety of phenotypes underlain by a single genotype will be acted on by natural selection (Nager et al. 2000). However, unless a trait has a heritable basis, changes in the phenotype will not lead to evolution (Nager et al. 2000; Pigliucci and Kaplan 2006). Heritability is defined as the proportion of phenotypic variance that is attributed to additive genetic variance, and it demonstrates the amount of genetic variability in a trait that may be passed on to the next generation (Hoffmann 2000). The breeder's equation is a simple formula that is often used to describe the phenotypic response to selection, such that $R = h^2 S$, where R = the response to one generation of selection, h^2 = heritability, and S = the selection differential, or the difference between the phenotypic mean of the breeder individuals and the whole population (reviewed in Garland 2003). The response to selection tends to be greater when heritability is higher and there is a wider selection differential (Garland 2003; Hill and Caballero 1992). The breeder's equation is useful in making short-term predictions for an individual population, but it is not very reliable over longer evolutionary time-scales or for extrapolating to other populations (Hill and Caballero 1992; Pigliucci and Kaplan 2006). The equation only considers a single trait at once, and selection becomes quite complicated by the interactions between traits and changing heritabilities as genes and gene frequencies become altered (Pigliucci and Kaplan 2006). Often, the most reliable and informative method for studying the relationships between traits at the

phenotypic and genotypic levels is to conduct a selection experiment in a controlled environment.

Selection Experiments

Purpose of Selection Experiments

The discovery that selectively breeding individuals with certain desirable features could produce more offspring with these same features has long been acknowledged and exploited by agriculturalists. Indeed, this type of artificial selection is largely responsible for the domestication of plants and animals that led to an enhanced rate of food production (Hill and Caballero 1992). From the perspectives of modern comparative biology and evolutionary physiology, selection experiments can provide insight into the past evolution of complex traits and on the factors that will continue to shape and constrain their future evolutionary trajectories (Swallow and Garland 2005). This understanding is based primarily on current selection intensities and the extent of heritable variation and co-variation (Garland 2003). Selection experiments can also be used to monitor changes across generations, test hypotheses regarding physiological evolutionary principles, and to unravel links between the form and function of organisms (Swallow and Garland 2005). These types of studies are valuable as they can be conducted within a manageable time frame [(generally five generations or less) (Hill and Caballero 1992)], and they take place under controlled and repeatable conditions, thus eliminating much of the uncertainty present in wild environments (Garland 2003). Selection experiments also tend to alter many gene frequencies and act on many gene loci, and they are considered representative of changes that might occur naturally

(Garland 2003). Selection can be undertaken solely on individuals, within a family, or on entire families, depending on the genetic expression of the targeted trait (Garland 2003).

Conducting a Selection Experiment

The main principle of selection experiments involves breeding individuals of a captive population that display a particular trait at the whole-organism level (typically behavioural, size-based, or reproductive) (Garland 2003; Swallow and Garland 2005). Artificial truncation selection is a technique by which individuals in each generation are assessed according to the mean value of a phenotypic trait, and the high and/or low fractions are then chosen as breeders for the next generation (Garland 2003). In any selection experiment, it is crucial to include a line of randomly bred individuals to control for random genetic changes and mutations, and to determine the extent of the selection differential (Garland 2003). Ideally, each line (control and selected) should be duplicated (Garland 2003; Hill and Caballero 1992), as these replicate lines can lead to the discovery of various adaptive responses that may occur under intense selection (Garland 2003). Selection experiments are generally carried out over several generations to enhance the trait divergence, so it is more efficient to use organisms with a relatively short lifespan and a smaller body size (Garland 2003). Hundreds of individuals per generation will be required to ensure an adequate mix of genetic material and to avoid issues related to inbreeding. The selected trait and the body size of the organisms may also dictate the type of enclosures that the individuals should be housed in. For example, if the experiments are targeting reproductive or social behaviours, an artificial laboratory environment may compromise natural interactions. In this instance, it may be beneficial

to use a common garden design with naturalized ponds or islands (Garland 2003). The selection of a trait expressed at the whole-organism level is inevitably linked to various other secondary traits. Upon adequate divergence of the selected and control lines, a better understanding of the genetic basis and the mechanisms that drive complex physiological and morphological traits may be realized (Garland 2003; Swallow and Garland 2005).

Fishing-Induced Selection and Evolution

A large-scale, unrestrained selection experiment is currently underway in many of the most productive commercial fishing grounds in marine environments. It is well established that many commercially harvested fish stocks are declining at an unsustainable rate (Jackson et al. 2001; Pauly et al. 1998; Vincent and Hall 1996), and there is now increasing recognition that remaining fish display phenotypic traits that are shifting the population towards earlier maturity with smaller body sizes at maturation (reviewed in Kuparinen and Merilä 2007). Depending on the mesh size of the nets and on the type of gear used, fish of a targeted size, age, maturity, or sex are removed at a higher rate than others (Law 2000). Many of these phenotypic traits have a heritability of 20-30% (Kuparinen and Merilä 2007), and any changes with a genetic origin are likely to result in an evolutionary response.

Although it is often difficult to find direct evidence of evolutionary changes in natural fish populations due to complex genetic and environmental interactions (Law 2007), controlled laboratory experiments have been able to demonstrate the evolutionary effects of size-selective harvesting. A line of Atlantic silversides (*Menidia menidia*)

showed significant declines in weight and biomass when the largest 90% of individuals were removed (Conover and Munch 2002), and genetic correlations were found between adult length, egg diameter, length at hatch, and juvenile length (Munch et al. 2005). High mortality rates, due to predation, in experimental populations of guppies (*Poecilia reticulata*) favoured selection towards earlier maturity at smaller sizes (Reznick and Ghalambor 2005). It is clear that evolutionary changes are possible in an experimental situation, as these examples of shifting genotypic frequencies illustrate the core definition of evolution (Pigliucci and Kaplan 2006).

Many studies on natural populations of fish have documented phenotypic changes in traits that are known to be heritable. Some early studies focused on maturation and reproduction in the northeast Arctic cod (*Gadus morhua*) (Jørgensen 1990) and the North Sea plaice (*Pleuronectes platessa*) (Rijnsdorp 1993). More recently, high rates of phenotypic divergences in age and size at maturation in five populations of grayling (*Thymallus thymallus*) were attributed to evolutionary adaptation (Haugen and Vøllestad 2001). Connections between strong selection differentials, heritability, and a declining trend in body size have also been made in populations of Atlantic cod (*Gadus morhua*) (Swain et al. 2007). A variety of modeling techniques (e.g. probabilistic maturation reaction norms, Dieckmann and Heino 2007) are now being used to tease apart the effects phenotypic, genotypic, and environmental changes on the evolution of fish populations (reviewed in Kuparinen and Merilä 2007). Any evolutionary changes to life-history traits will affect recruitment and the future harvestable biomass, and the implications for conservation biology are quite serious.

Fishing-induced evolution is not always easy to prove, but there is plenty of evidence to suggest that significant changes are occurring in marine fish populations as a direct result of commercial exploitation. Selective harvesting is not limited to commercial fishing, however, as recreational fishing has been described as having similar principles (Cooke and Cowx 2006). Indeed, anglers often target fish of a specific size (Aas et al. 2000; Petering et al. 1995) or sex (Casselman 1975; Pérez et al. 2005), as well as the behaviours and traits that are indirectly linked with size and sex (reviewed by Lewin et al. 2006). Although there have been few studies on the impacts of fishing-induced selection from a recreational perspective, information is emerging on life-history trait selection in some species of recreational fish. A recent study by Dunlop et al. (2005) revealed that changes in maturation patterns of smallmouth bass (*Micropterus dolomieu*), whereby increasingly rapid growth at young ages led to earlier maturation, were driven by phenotypic plasticity in growth rates. If these phenotypic traits are heritable and a strong selection pressure is introduced, the potential exists for evolutionary changes to occur.

Studies on fishing-induced selection have mainly focused on life-history traits, possibly due to the ease of documenting them at the population level. In laboratory-based selection experiments, a variety of traits in categories including behaviour, performance, morphology, and physiology have been studied (Swallow and Garland 2005). In a series of experiments using *Drosophila*, Roff and Mousseau (1987) attributed high heritabilities to morphological and physiological traits and low heritabilities to life-history and behavioural traits. However, life-history traits likely have higher heritabilities than originally estimated by Roff and Mousseau (1987), as only a few traits

(longevity, fecundity, and development time) were considered, and the heritability ranges for each trait were quite wide (Hoffmann 2000). Also, behaviour often fluctuates, resulting in less stable measurements, and behavioural traits are actually believed to evolve much faster than suggested by a low heritability (Hoffmann 2000). Life-history and behavioural traits play a vital role in many selection experiments. Ongoing selection experiments by Garland and colleagues on the evolution of behaviour and morpho-physiological traits are taking place in house mice (*Mus domesticus*) selected for high levels of voluntary wheel running (Swallow et al. 1998). A variety of traits have been examined in connection to the activity levels of these mice, including metabolic rate and energy expenditure (Vaanholt et al. 2007), aerobic capacity (Rezende et al. 2006), muscle enzyme activities (Houle-Leroy et al. 2000), body mass at maturity (Swallow et al. 1999), and food intake and body composition (Swallow et al. 2001). These traits represent different levels of biological organization, and they can also offer insights into the potential evolutionary trajectories of fish populations subject to strong selection pressures.

Traits Studied in Selection Experiments

Metabolism

A fundamental aspect of physiological performance in any animal, including fish, is the relative efficiency by which energy is metabolized. Metabolism reflects the chemical processes within the body that convert ingested food into a source of energy to perform at all types of organismal functions (Brett and Groves 1979; Schmidt-Nielsen 1998). Energy expenditure in fish is often estimated indirectly in a respirometer chamber

that measures the decline in dissolved oxygen concentration over time (Brett and Groves 1979). Use of these respirometry tunnels creates a controlled environment in which metabolic rates can be determined under a variety of conditions. Metabolic rate in fish is known to vary with body size and temperature (reviewed in Brett and Groves 1979). The amount of activity that a fish engages in also leads to fluctuations in energy expenditure. Standard metabolic rate represents the minimal energy demand for maintenance requirements to keep the fish alive, and routine metabolic rate encompasses short bouts of spontaneous activity that take place while the fish is feeding, digesting, and swimming normally (Brett and Groves 1979; Fry 1957). Active metabolic rate is recognized as the maximal level of sustained activity that takes place during episodes of chasing and escaping, and it is limited by the aerobic capacity of the fish (Brett and Groves 1979). Rates of energy metabolism vary substantially between individual fish and have direct implications for the swimming performance of a fish in situations where sustained or maximum swimming speeds are required.

Aerobic and Anaerobic Activity

In their natural environments, fish are motivated to swim quickly or slowly for a variety of reasons. Rapid swimming movements are used to escape from predators, capture elusive prey, and pursue or attract mates; slower, sustainable swimming is used to forage for non-elusive food and for daily or seasonal migrations within a body of water (reviewed in Gibb and Dickson 2002). In teleost (bony) fish, swimming locomotion is partitioned between the red and white muscle. Red muscle powers sustained, aerobic swimming, it is positioned mainly along the body's lateral line, and it is composed of

slow, oxidative fibers (reviewed in Gibb and Dickson 2002). White muscle provides the power required for short-duration sprint swimming typically associated with anaerobic activity, it represents most of the axial musculature, and it is composed of fast, glycolytic fibers (reviewed in Gibb and Dickson 2002). High intensity sprint swimming requires high energy phosphates delivered by adenosine triphosphate (ATP) and phosphocreatine (PCr), and it results in a depletion of muscle glycogen and tissue energy stores (ATP and PCr) and the anaerobic production of lactate (Driedzic and Hochachka 1978; Milligan 1996; Wang et al. 1994). The response to exhaustive swimming within the tissue metabolites (lactate, ATP, and PCr) is well documented for salmonids (Milligan 1996) and largemouth bass (*Micropterus salmoides*) (Suski et al. 2004). Tissue lactate values peak immediately after the high intensity event and then slowly decline during recovery until reaching control values 4-8 h later (Milligan 1996). Tissue energy stores (ATP and PCr) drop to within 40-90% of control values immediately following high intensity activity and then quickly return to control values 2 h later (Milligan 1996). The capacity for anaerobic activity in fish is indicated by the glycolytic enzyme lactate dehydrogenase, and the capacity for aerobic activity is indicated by the mitochondrial enzymes citrate synthase and cytochrome *c* oxidase (Somero and Childress 1980, 1990). Simulation of exhaustive exercise in a controlled setting is a common technique for measuring the physiological response to anaerobic disturbance and for determining the recovery rate (Kieffer 2000; Milligan 1996). The duration of recovery has implications for the resumption of aerobic swimming activity and the ability to undertake subsequent high-intensity anaerobic activity (reviewed in Goolish 1991).

Life-History Characteristics

In the overall management of a fishery, life-history traits related to growth, survival, and reproduction for a given species are fundamental aspects to quantify. These factors have direct implications for yield (harvestable biomass) and catch-per-unit-effort, and they are relatively straightforward to observe. Life-history traits are closely intertwined, and the partitioning of growth and overall growth rate strongly influences reproductive criteria such as the time to maturation, age at maturation, and fecundity (Calow 1985). Growth rate and body size are generally correlated with reproductive effort and fecundity in many fish species, but if reproduction diverts too many resources away from somatic growth, future fecundity will decrease (Wootton 1985). When attempting to predict the life-history patterns that will be the most advantageous in a particular environment, a major assumption is that a trade-off exists between the present and future reproductive efforts (Wootton 1985). Based on variations in life-history traits such as age-specific mortality and fecundity, it is theoretically possible to predict reproductive effort (Wootton 1985). Another important aspect of life-history theory relates to optimizing selection, whereby individuals that display an intermediate value of a trait should have greater reproductive success than individuals at either the smaller or larger ends of the trait spectrum (Sinervo 2000). As well, trade-offs exist in terms of the quality and quantity of offspring that are produced, whereby selection favours fecundity in instances of large clutches and survival in instances of large offspring (Sinervo 2000). From a natural selection perspective, the aim is to manage the costs of reproduction and achieve an optimum balance between current fecundity and future survival (Sinervo 2000).

Energy Utilization and Nutrition

Adequate nutrition is essential for fish to perform the various functions described above. Without access to quality forage resources and prey items, less energy would be available to metabolize and direct towards growth, reproduction, and locomotion. The food resources consumed by fish are partitioned either towards the formation and repair of body tissues (anabolism) or the production of energy (catabolism) (Brett and Groves 1979; Phillips 1969). Carbohydrates, lipids, and proteins are substances used by the body for growth and the production of energy; minerals, vitamins, water, and oxygen are substances that support those critical processes without directly being responsible (Phillips 1969). Although not all species of fish utilize these food-derived substances in exactly the same manner, some general trends do exist. Lipids and carbohydrates are used most often by fish as sources of energy, although carbohydrates are generally not used very efficiently nor found in high levels in natural diets (Brett and Groves 1979; Phillips 1969). Proteins are mainly allocated towards tissue growth, and the protein requirements for fish vary with the stages of the life cycle (Phillips 1969). More protein is required by juveniles and during periods when water temperatures are optimal, as growth takes place more quickly (Phillips 1969). Adults approaching the spawning season also require more protein and lipids to assist in the formation of gonads (Phillips 1969). While fish can lose considerable amounts of protein and lipids without serious side-effects, maintaining a water balance of approximately 75-80% is absolutely critical to their survival (Phillips 1969). Comparisons in food consumption and nutrition between individual fish can be made by separating these various body constituents and by calculating their energy densities (Breck 2008).

Purpose and General Hypotheses

The purpose of this thesis is to examine the effects of fisheries-induced selection from a recreational angling perspective. In this study, the overall trait targeted by selection is vulnerability to angling. Two experimental lines of largemouth bass (*Micropterus salmoides*) bred for high and low vulnerability to angling via artificial truncation selection will be used throughout the experiments. After four generations of selection over a thirty-year period, it was established that the trait of angling vulnerability in these largemouth bass displayed a realized heritability of 0.15 (Philipp et al. 2008). This heritability is comparable to values for life-history traits in other fish species considered to be moderately heritable (e.g. Atlantic salmon, *Salmo salar* Garcia de Leaniz et al. 2007). The traits that are correlated with vulnerability to angling will be evaluated in terms of physiology, energetics, and life history (summarized in the above paragraphs). Chapter 2 will present a study investigating the correlations between vulnerability to angling, metabolism, and the anaerobic response to exhaustive exercise. It is hypothesized that high vulnerability fish will have a higher standard metabolic rate and a greater anaerobic capacity than low vulnerability fish. Chapter 3 will present a study investigating the correlations between vulnerability to angling, growth rate, energetics, and nutritional status. It is hypothesized that the growth rates will be similar between the two groups, but that high vulnerability fish will have higher energy densities and nutritional indices. The results of these experiments will offer insights into the mechanisms behind differential vulnerability to angling including potential differences in behaviours. These experiments are unique, as they will evaluate the physiological and energetic mechanisms that have the potential to be affected by fishing-induced selection

from a recreational perspective. Chapter 4 will highlight the management implications of the findings and will present future research opportunities. Overall, this thesis will attempt to uncover the potential evolutionary consequences associated with recreational angling.

Chapter 2: Metabolic Correlates of Vulnerability to Angling in a Teleost Fish: Insights from Physiology and Biochemistry

Abstract

Although the selective pressures of commercial fishing are well known, few studies have examined this phenomenon in recreational fisheries. This study used a population of largemouth bass (*Micropterus salmoides*) with lines bred for high (HVF) and low (LVF) vulnerability to angling that were previously established from artificial truncation selection experiments. The main objective was to evaluate if differential vulnerability to angling was correlated with physiological traits including metabolic rate, metabolic scope, anaerobic capacity, and biochemical response to exercise. Standard metabolic rate was 11% higher, and maximal metabolic rate was 16% higher for HVF compared to LVF, with a metabolic scope that was 20% higher for HVF. A similar proportion of this scope was utilized by both HVF and LVF during recovery. Although immediately following exhaustive exercise, muscle lactate concentrations were 44% higher for HVF compared to LVF, both groups had returned to control levels after 2 h of recovery. Anaerobic energy expenditure (in ATP equivalents) was also significantly higher for HVF, a finding that is consistent with the observation that during exercise, HVF exhibited bursting activity more frequently than LVF. Significant differences were not detected between HVF and LVF for tissue energy stores (e.g. ATP, PCr) or aerobic (e.g. CS, CCO) and anaerobic (e.g. LDH) enzyme activities. Although the inherent reasons behind differential vulnerability to angling are complex, selection for these opposing phenotypes also appears to selecting for a suite of physiological traits, including

metabolism and the capacity for anaerobic activity. These phenotypic traits (and any others that may be linked with vulnerability to angling) are at risk of being altered in recreational fish populations when angled fish are harvested or experience post-release mortality, emphasizing the need for incorporating evolutionary principles into fisheries management activities.

Introduction

Selectively harvesting fish populations for economically desirable traits generates directional selection pressures that can lead to evolutionary changes in heavily exploited stocks (reviewed in Heino and Godø 2002; Hutchings and Fraser 2008; Jørgensen et al. 2007; Kuparinen and Merilä 2007; Law 2000). Changes to life-history traits resulting from selective commercial fishing pressures have been documented for growth rate (e.g. grayling, *Thymallus thymallus* Haugen and Vøllestad 2001), age and size at maturation (e.g. Atlantic cod, *Gadhus morhua* Olsen et al. 2004), and reproductive investment (e.g. Atlantic cod Yoneda and Wright 2004). Recently, it has become evident that in addition to phenotypic changes, these trait changes are resulting in shifting genotypic frequencies (Jørgensen et al. 2007) and may be evolutionary in nature (Kuparinen and Merilä 2007). It is also acknowledged that these evolutionary changes have occurred quite rapidly, over the span of a few decades (Stockwell et al. 2003). Not only does fishing-induced selection have the potential to influence the sustainability of commercial fisheries, but whole marine ecosystems are at risk of being drastically altered relatively quickly (Jørgensen et al. 2007). As a result, conservation programs need to incorporate

evolutionary principles into the development of sustainable management strategies (Ashley et al. 2003; Francis et al. 2007; Jørgensen et al. 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. 2008). In inland freshwater systems with small, locally adapted stocks and high angling activity that leads to fish mortality, selective pressures may be particularly intense (Arlinghaus and Cooke 2005; Cooke and Cowx 2004, 2006; Post et al. 2002). In a manner similar to commercial fishing, recreational angling can also be highly selective for size, sex, and a suite of other physiological and behavioural traits (reviewed in Lewin et al. 2006). For example, certain behaviours linked with size and sex may be selected against, such as aggression in male largemouth bass (*Micropterus salmoides*) during parental care (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 (Arlinghaus et al. 2007; Bartholomew and Bohnsack 2005; Muoneke and Childress 1994) or physiological impairment of reproductive behaviour (Cooke et al. 2002b).

The evolutionary implications of angling pressure have recently been emphasized in an experimental population of largemouth bass that were specifically selected for differential vulnerability to angling (Philipp et al. 2008). Upon examining the response to selection over three generations, it was determined that, for this population of largemouth bass, vulnerability to angling is a genetically heritable trait (Philipp et al. 2008). Although it has been recognized for some time that certain individuals within a

species are more vulnerable to angling than others (e.g. largemouth bass, *Micropterus salmoides* Bennett 1954; brown trout, *Salmo trutta* McLaren 1970), this study was the first to demonstrate a heritable basis for these differences (Philipp et al. 2008). 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. 2008). Indeed, a recent study by Cooke et al. (2007) demonstrated that higher vulnerability to angling in largemouth bass was correlated with higher 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 higher rates are more energetically costly (Brett and Groves 1979). It is conceivable that increased foraging activity to obtain more resources makes these fish more susceptible to capture by anglers. If SMR is a driving force behind differential angling vulnerability, behavioural differences and activity rates may also emerge as important factors (Uusi-Heikkilä et al. 2008).

SMR indicates the baseline energetic requirements of an individual fish, and maximum metabolic rate (MMR) is the energy expenditure that takes place during maximum sustained activity (Brett and Groves 1979). The range bound by SMR and MMR has been described as the metabolic scope (Fry 1957), and this zone is where normal, spontaneous aerobic activity related to feeding metabolism and foraging activity takes place (Brett and Groves 1979; Tandler and Beamish 1981). When SMR increases,

mainly due to heightened energy requirements, MMR tends to shift upwards as well, thus preserving the scope available for routine activities (Priede 1985). In cases where the scope is wider than expected, an individual's capacity to withstand a physiologically stressful situation, such as a predator-prey interaction or a catch-and-release angling event, would be extended (Killen et al. 2007).

In physiologically stressful situations, fish are typically exposed to anaerobic disturbances (Booth et al. 1995; Cooke et al. 2002b) that result in the depletion of muscle glycogen, ATP, and 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 (Kieffer 2000; Milligan 1996). In addition, an indication of overall anaerobic capacity can be gained from the enzyme activity of muscle lactate dehydrogenase (LDH) (Somero and Childress 1980, 1990). The duration of recovery has implications for the resumption of aerobic activity (reviewed in Goolish 1991). Because citrate synthase (CS) and cytochrome *c* oxidase (CCO) influence the rate of recovery from exhaustive exercise, the activities of these enzymes can provide an indication of aerobic capacity (Somero and Childress 1980, 1990). An evaluation of exercise performance, recovery, and muscle enzyme activities of fish differentially selected for vulnerability to angling may provide some insight into the physiological traits under selection as well as the consequences of fishing-induced selection from a recreational perspective.

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 (Philipp et al. 2008). Specifically, we tested the

hypothesis that high vulnerability fish (HVF) and low vulnerability fish (LVF) would have different metabolic rates and metabolic scopes that shifted accordingly. First, based on previous findings where HVF had higher resting cardiac variables (Cooke et al. 2007), we predicted that HVF would display a higher SMR than would LVF. Second, because fish with a higher SMR should correspondingly increase their MMR (Priede 1985), we predicted that HVF and LVF would have comparable metabolic scopes. We also tested the hypothesis that HVF and LVF would have a different physiological response to exhaustive exercise. Based on the expectation that HVF would display a higher SMR with increased energetic demands (Priede 1985), we predicted that HVF would have a heightened anaerobic capacity, as indicated by LDH, and accumulate more lactate than LVF. HVF would then be expected to require proportionally more time to recover from exhaustive exercise (Goolish 1991). In gaining an understanding of the physiological correlates of vulnerability to angling, it will be possible to determine additional traits that may be selected against in a recreational fishery and evaluate the potential management implications from an eco-evolutionary perspective.

Materials and Methods

Study Animals

The model species chosen for these experiments was the largemouth bass, because it is subjected to high levels of angling pressure in North America (Pullis and Laughland 1999). This study takes advantage of a unique artificial truncation selection experiment that began several decades ago at the Illinois Natural History Survey (Philipp et al. 2008). Beginning in 1977, largemouth bass in Ridge Lake (39.40 °N, 88.16 °W;

7.10 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. 1984). Following these four seasons of angling, the lake 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 high and low vulnerability to angling (Philipp et al. 2008). 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. 2008). Five pairs in each parental (P1) generation of each line were bred in separate experimental ponds to produce F1 generation offspring, which were then differentiated by pelvic fin clips (Philipp et al. 2008). 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. 2008). A selection procedure using experimental angling over one season was repeated on the F1 fish, and HVF and LVF were again separated into different experimental ponds for breeding (Philipp et al. 2008). The F2 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 to HVF (Philipp et al. 2008). The fish used in this research were bred naturally in ponds in the spring of 2006 as part of an F4 generation, and they had not experienced any further artificial selection.

Oxygen Consumption and Metabolic Rates

In October 2007, a sub-sample of HVF and LVF 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, IL (39.60 °N, 88.61 °W). There were no significant differences between the weights for the two groups [HVF, 78.8 ± 8.3 g (mean ± SE) (N = 8); LVF, 95.0 ± 6.7 g (N = 8); P = 0.15], but there was a significant difference between the total lengths (HVF, 172 ± 11.4 mm; LVF, 200 ± 4.0 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 ºC, 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 was measured using computerized, intermittent flow-through respirometry (Loligo Systems) with a similar configuration as described by Herskin (1999). Briefly, four plexiglass chambers (volume = 0.7 L) were immersed side by side in a 175 L tank that was maintained at 20.5 ± 1ºC. The water in the tank was aerated and filtered continuously through charcoal and a UV-sterilizer. Water was recirculated continuously through each chamber using Eheim pumps, which were driven by the AutoResp^{TM} 4 software 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 (Po_2) were measured by oxygen probes connected to an OXY-4 fiber optic oxygen instrument. Po_2 was recorded every second

during the 5 min measuring phase, and a linear regression was calculated between Po_2 and time. The slope of the regression line (k) was used to calculate oxygen consumption according to the equation:

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

where $M\mathrm{o}_2$ = oxygen consumption in mg O_2 kg^{-1} h^{-1}, V_{resp} = volume of the respirometry chamber in L, M = fish mass in kg, and α = 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 $M\mathrm{o}_2$ data point every 10 min.

Experimental trials consisted of placing two HVF and two LVF in individual chambers during the afternoon. The fish were left undisturbed for an overnight period (18-20 h), during which time the AutoResp™ 4 software controlled the measuring and flushing phases and recorded $M\mathrm{o}_2$. SMR was determined for each individual fish by averaging the lowest six $M\mathrm{o}_2$ values recorded between approximately 21:00 – 08:00, and is representative of a 1 h period (McKenzie et al. 2003). Routine metabolic rate (RMR) was determined by averaging the six $M\mathrm{o}_2$ values recorded between approximately 18:45 – 19:45, when fish had acclimated to their chambers and had resumed normal daylight activities.

Upon completion of the SMR trials, the elevation of $M\mathrm{o}_2$ following exercise was assessed, generating a factor termed ‘excess post-exercise oxygen consumption’ (EPOC; Gaesser and Brooks 1984). 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 (Wang et al. 1994; Wood 1991). 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_{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_{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. This method was used to demonstrate the relative amount of the metabolic scope that is devoted to recovery following exhaustive exercise.

Exercise and Muscle Metabolites

In July 2007, a sub-sample of HVF and LVF were removed randomly from an experimental pond at the University of Illinois in Champaign-Urbana and transported to the Sam Parr Biological Station near Kinmundy, IL (38.77 °N, 88.85 °W). There were no significant differences between the weights for the two groups [HVF, 12.7 ± 0.2 g (mean ± SE) (N = 47); LVF, 13.3 ± 0.4 g (N = 49); P = 0.14], but there was a significant difference between the total lengths (HVF, 106 ± 0.6 mm; LVF, 110 ± 0.9 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 ºC, 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 days.

To generate control (resting) physiological values, individual HVF and LVF 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), the entire portion of white muscle (~ 1 g) above the lateral line and behind the operculum was removed using a razor blade (~ 1 min). The muscle samples were immediately freeze-clamped with aluminum tongs pre-cooled in liquid nitrogen and then stored in liquid nitrogen (Booth et al. 1995).

To induce a physiological disturbance, an exhaustive exercise regime was applied to another group of HVF and LVF. Fish were transferred from a common holding tank to an aerated treatment container (20 L) where they were chased by tail pinching for 2 min to induce exhaustive exercise. Following the 2 min exercise regime, one group of fish (both HVF and LVF) 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 (both HVF and LVF) 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 1mM EDTA, and then with a volume of neutralizing solution (containing 2 M KOH, 0.4 M KCl, and 0.3 M Imidazole). The resulting supernatant was stored at – 80 ºC 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:

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

where Δ represents the difference between control and exercise values, 1.5 units of ATP are generated per unit of lactate, and 1 unit of PCr is equal to 1 unit of ATP (McDonald et al. 1998a; Pearson et al. 1990).

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 mM HEPES, 1 mM EDTA, and 0.1% Triton X-100. All enzyme activity determinations were performed in

duplicate at 24 ºC (the optimum temperature for growth of largemouth bass) using a UV/Vis spectrophotometer (Varian Cary 100). 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 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 (μmol of substrate converted to product per min) per g 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 mM NADH and 1.0 mM pyruvate were used; and for CS, 0.1 mM acetyl CoA and 0.15 mM 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

For all data, 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 HVF and LVF for SMR, MMR, RMR, metabolic scope, and tissue enzymes using *t*-tests. Where the assumption of equality of variance was not met, the non-parametric Mann-Whitney U test was used. An arcsine square-root transformation

was carried out on the EPOC data, because it was expressed as a percentage of the metabolic scope. These EPOC values were compared between HVF and LVF across recovery periods using a two-way repeated measures analysis of variance (ANOVA). The mean concentrations of tissue metabolites and anaerobic energy expenditures were compared between HVF and LVF across recovery periods using a two-way ANOVA. For the two-way ANOVA analyses, the statistical differences between pairs were evaluated using the conservative Tukey-Kramer HSD test. All *t*-tests and the two-way repeated measures ANOVA were carried out using SPSS 11.0 (SPSS Inc.). The two-way ANOVAs were carried out using JMP 4.0 (SAS Institute). Values are reported as means (± SE) and the level of significance (α) used for all tests was 0.05.

Results

HVF and LVF differed significantly in terms of mass-specific SMR and MMR. SMR was 11% greater and MMR was 16% greater for HVF than for LVF (Table 2-1). The metabolic scope was approximately 20% greater for HVF than for LVF (Table 2-1). An initial comparison of RMR between HVF and LVF revealed a 45% greater value for HVF than for LVF. Due to substantially unequal variances between the RMR for HVF and LVF (Levene's test, $P < 0.05$), a non-parametric test was conducted, which was non-significant (Table 2-1).

Within 15 min after achieving maximal $M\dot{O}_2$, both HVF and LVF were devoting 80-84% of their metabolic scope to recovery (Figure 2-1). 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 (Figure

2-1). No significant differences were detected between HVF and LVF at any of the recovery intervals (Figure 2-1).

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 with the same vigour as HVF.

Exhaustive exercise for 2 min resulted in a six-fold increase in tissue lactate concentrations for HVF relative to resting controls (Figure 2-2A; Table 2-2). For LVF, the increase in tissue lactate concentrations over control values was three and a half times, resulting in a significant difference between HVF and LVF immediately following exercise (Figure 2-2A; Table 2-2). By 1 h following exercise, the tissue lactate concentrations for HVF and LVF had decreased to within three and a half times and two times the control values, respectively, but these differences were no longer statistically significant (Figure 2-2A; Table 2-2). By 2 h following exercise, tissue lactate concentrations for both HVF and LVF had returned to resting control values, where they remained for the remainder of the recovery profile (Figure 2-2A; Table 2-2). Immediately following exhaustive exercise, white muscle ATP and PCr concentrations for both HVF and LVF decreased by 80-100% relative to resting control values (Figure 2-2B, 2-2C; Table 2-2). Due to the high amount of variability between individuals, however, differences across treatment groups were not statistically significant. Following 1 h of recovery, tissue ATP concentrations were approaching control values for HVF and

LVF, and they returned to control levels following 2 h of recovery (Figure 2-2B; Table 2-2). Following 1 h of recovery, tissue PCr concentrations had returned to control values and remained there for the entire recovery profile (Figure 2-2C; Table 2-2). Mean water content of the white muscle was 78.5% (range 71.0 – 81.5%) and did not differ significantly between HVF and LVF ($P = 0.48$).

Immediately following exhaustive exercise, the AEE for HVF was over two thirds greater than the AEE for LVF (Figure 2-3). Following 1 h of recovery, however, there was no longer a significant difference between the AEE of both groups (Figure 2-3).

The activity of LDH, an indicator of anaerobic capacity, did not differ between HVF and LVF, nor did the activities of CS and CCO, which are indicators of aerobic capacity (Table 2-3). Power analyses revealed that the values for β were very low, with the highest reported value at about 0.2 for CCO (Table 2-3).

Discussion

This study demonstrated some metabolic and behavioural correlates of differential vulnerability to angling in largemouth bass following four generations of artificial selection. Specifically, large differences in metabolic capacity were observed between the two experimental lines. HVF were found to exhibit a higher SMR than LVF, a trend that is consistent with the higher cardiac output and heart rate found for HVF by Cooke et al. (2007). The combination of a higher SMR and a higher MMR following exhaustive exercise enabled HVF to attain a broader metabolic scope than LVF. This finding is in contrast to the smaller cardiac scope found for HVF 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 HVF require a greater metabolic scope to account for increases in foraging activities to satisfy their energetic demands (Priede 1985), which have been estimated to be 40% greater than those of LVF (Cooke et al. 2007). A study with a population of three-spined sticklebacks (*Gasterosteus aculeatus*) offers support for this argument, as the active migratory morph displayed higher SMR, higher active MR, and a larger metabolic scope than the resident non-migratory morph (Tudorache et al. 2007). Metabolic scope can also be an indicator of the potential range for aerobic activity (Bishop 1999), 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 (Bishop 1999) related to aggression (Metcalfe et al. 1995) and parental care (Cooke et al. 2002a) better than LVF. Although aggression was not directly evaluated in this study, research on Atlantic salmon (*Salmo salar*) has linked higher SMR with more aggressive, dominant behaviour (Cutts et al. 1998). The potential for inherent aggression, coupled with higher

SMR and a need for increased food intake, may help explain why HVF are more likely to strike at artificial lures compared to 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 HVF and LVF in terms of the tissue metabolite (lactate) and energy stores (ATP and PCr) following exhaustive exercise were comparable to results in the literature (reviewed in Milligan 1996), there were some notable variations. The differences observed between HVF and LVF were most pronounced immediately following exhaustive exercise. HVF experienced tissue lactate levels that were 44% higher than LVF, which contributed to their 63% greater AEE. AEE is regarded as a measure of realized anaerobic capacity (McDonald et al. 1998b), because it encompasses the maximal amount of lactate production and depletion of the tissue energy stores. In experiments featuring sprint-trained rainbow trout (*Salmo gairdneri*), trained fish achieved 30% higher lactate concentrations (Pearson et al. 1990) and nearly double the AEE (McFarlane and McDonald 2002) compared with untrained fish following exhaustive exercise. While this was a plastic response to training, the values found for HVF were at least partly genetic because selection based on vulnerability to angling occurred over four generations without any training. In addition to exhibiting physiological characteristics similar to those of sprint-trained fish, the swimming behaviour of HVF during the exercise simulation differed from that of LVF. HVF engaged in higher incidences of burst swimming accompanied by faster startle responses, whereas LVF maintained a steadier swimming pattern during manual chasing. These observations, along with measured variables of lactate and AEE, suggest that HVF

have a greater anaerobic capacity than LVF. 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). Enhanced bursting ability may also assist juveniles with predator avoidance (Fuiman and Magurran 1994), and adult male largemouth bass may be better equipped to defend their broods from predation (Cooke et al. 2002a).

Despite enhanced anaerobic capacity and burst swimming ability of HVF, not all of the physiological variables were strongly associated with differential vulnerability to angling. 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 HVF and LVF in terms of LDH activity. Additional enzymes assist in generating anaerobic metabolic power (Somero and Childress 1980) and could be related to the greater anaerobic capacity observed in HVF. Creatine phosphokinase (CPK) generates ATP in the initial seconds of burst swimming (Driedzic and Hochachka 1978). Phosphofructokinase (PFK) is found at the beginning of the glycolytic pathway (Driedzic and Hochachka 1978), and PFK was strongly correlated with burst swimming in adult sticklebacks (Garenc et al. 1999). Glycogen stores and the overall glycolytic capacity to produce pyruvate can also influence anaerobic power (Somero and Childress 1990). Indeed, the differences in lactate accumulation between sprint-trained and

untrained fish in studies by both Pearson et al. (1990) and McFarlane and McDonald (2002) were attributed to an increased use of exogenous glucose. Although the enzymatic activity of LDH did not provide evidence of a higher anaerobic capacity for HVF, perhaps the increased lactate accumulation in HVF can be related to the availability of more 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 HVF and LVF 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). Interestingly, HVF appear to have an enhanced ability to clear lactate, as this value recovered to control levels within the same timeframe as LVF, despite peaking at a higher concentration following exhaustive exercise. The sprint-trained trout studied by Pearson et al. (1990) also recovered rapidly following exercise, and this was attributed to enhanced lactate clearing. In contrast, the potential for a higher aerobic capacity in LVF is suggested by the observations of their sustained swimming ability. The two opposing aerobic strategies provide a possible explanation for the identical recovery profiles generated for ATP and PCr following exercise. A clear distinction has been made between the initial anaerobic response to exhaustive exercise for HVF and LVF, 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 distinct physiological traits are correlated with experimental lines of largemouth bass intentionally selected for high and low vulnerability to angling.

To conclude, some individuals within a population of fish are more vulnerable to angling than others, and this vulnerability appears to 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. 2008). 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, these physiological traits were strongly correlated with differential vulnerability to angling, which has important 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, potentially affecting their performance of certain behaviours, based on these physiological parameters (Uusi-Heikkilä et al. 2008). This study demonstrates the potential for evolutionary changes to occur within a population of fish exposed to recreational angling, not only on the metabolic traits that have been identified, but also on the life-history characteristics (Calow 1985) and behaviours (Careau et al. 2008) that they support. These findings complement previous studies on fishing-induced evolution from a commercial context (Jørgensen et al. 2007; Kuparinen and Merilä 2007). Recreational fisheries managers are urged re-evaluate management strategies to address the impact of selection through angling, because these potential evolutionary changes may hinder the ability of stocks to cope with other natural selection pressures (Edeline et al. 2007).

Tables

Table 2-1. Metabolic rates ($mgO_2\ kg^{-1}\ h^{-1}$) and body weight (g) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass, presented as mean (± SE). $N = 8$ 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.

	Vulnerability			
Parameter	**High**	**Low**	**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
Maximum 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

Table 2-2. Effects tests for two-way ANOVAs used to compare tissue metabolites for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass. Group refers to HVF and LVF, and treatment refers to the recovery period following exhaustive exercise.

Response	**Source**	**Degrees of freedom**	**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

Table 2-3. Enzyme activities (IU g wet mass^{-1}) and protein concentrations (mg g^{-1}) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass, presented as mean (± SE), where $N = 8$.

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

Note: Least significant *N* represents a power of β = 0.5.

Figures

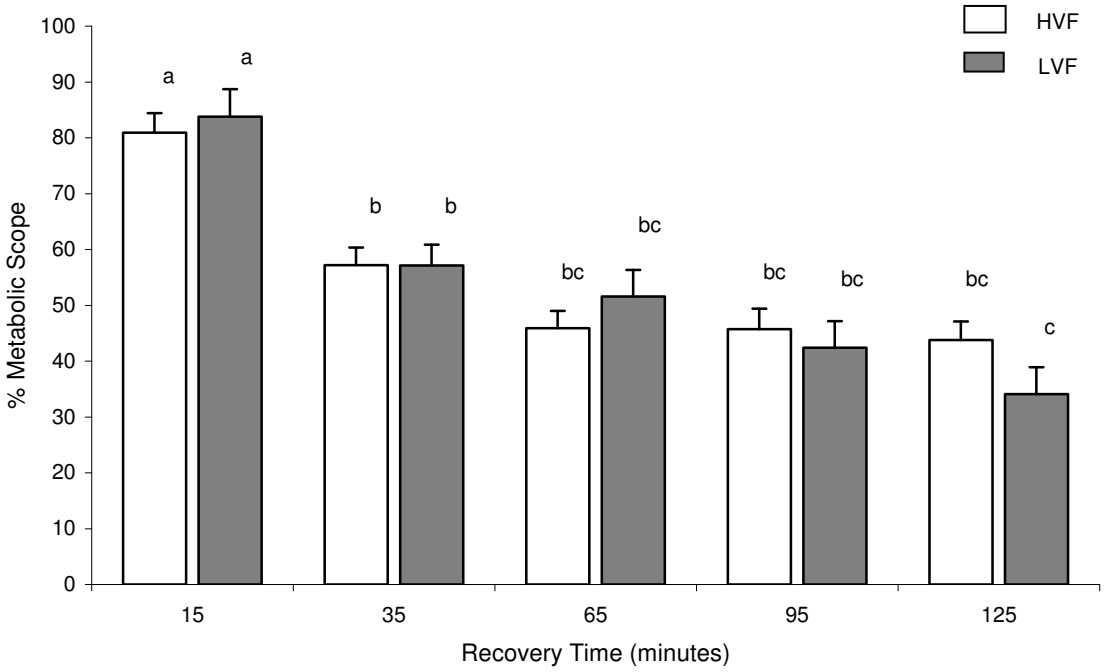
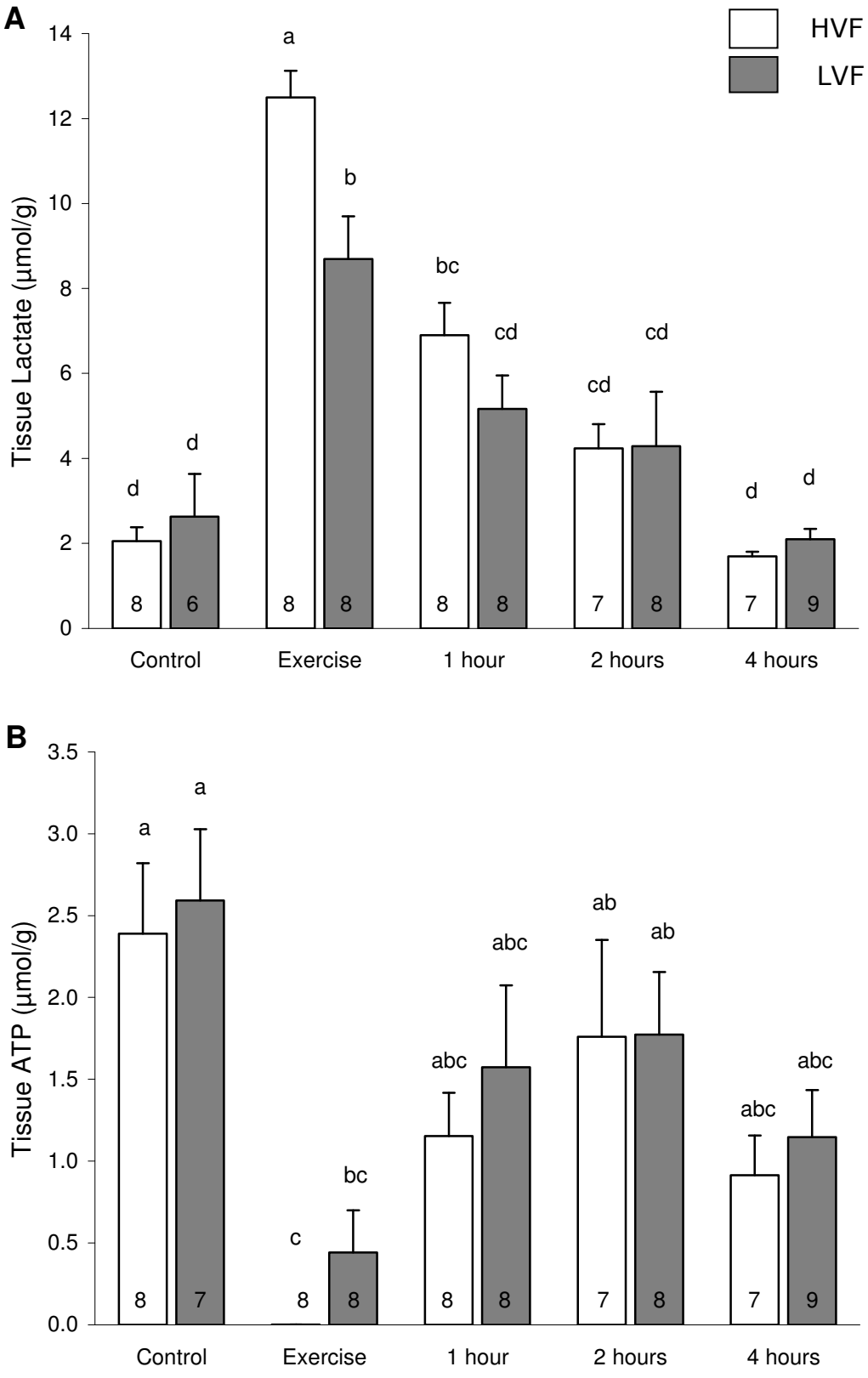


Figure 2-1. Mean (+SE) percent of metabolic scope utilized during recovery from exhaustive exercise by high vulnerability (HVF) and low vulnerability (LVF) largemouth bass. $N = 7$ for both HVF and LVF. The effects tests for the two-way repeated measures ANOVA are as follows: group, $df = 1$, $F = 0.05$, $P = 0.84$; treatment, $df = 2.04$, $F = 109.3$, $P < 0.01$; interaction, $df = 2.14$, $F = 112.5$, $P = 0.03$. Dissimilar letters denote significant differences between pairs at and across recovery times (Tukey-Kramer HSD test, $P < 0.05$).


A
HVF
LVF
Tissue Lactate (µmol/g)
0
2
4
6
8
10
12
14
a
b
bc
cd
cd
cd
d
d
d
d
8
6
8
8
8
8
7
8
7
9
Control
Exercise
1 hour
2 hours
4 hours
B
Tissue ATP (µmol/g)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
a
a
c
bc
abc
abc
ab
ab
abc
abc
8
7
8
8
8
8
7
8
7
9
Control
Exercise
1 hour
2 hours
4 hours

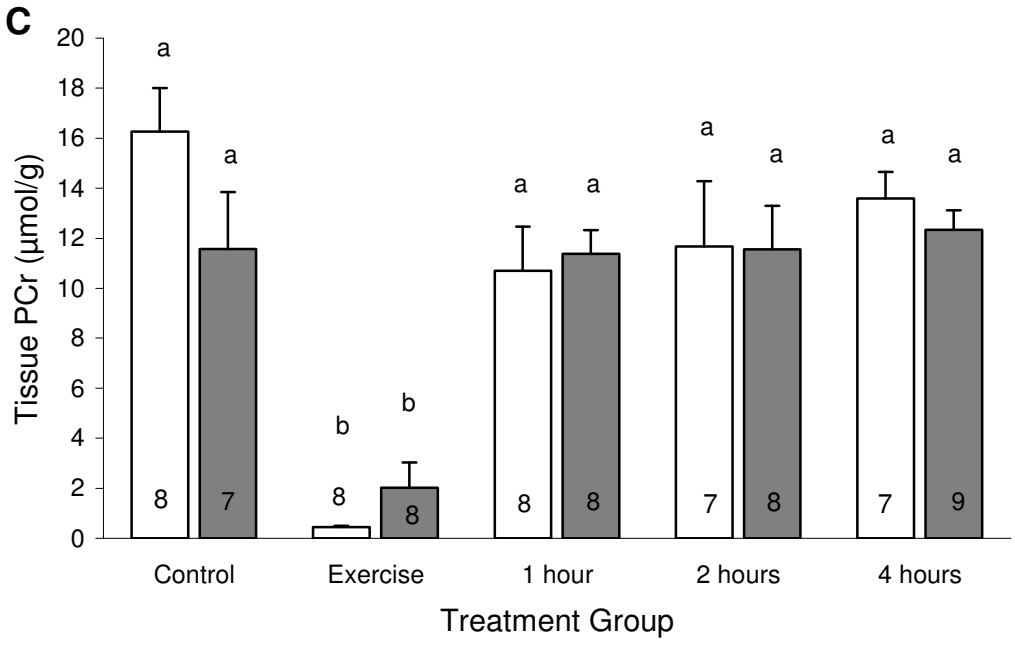


Figure 2-2. Mean (+ SE) tissue concentrations of (**A**) lactate, (**B**) ATP, and (**C**) phosphocreatine (PCr) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass following exhaustive exercise and recovery. The sample size for each treatment is shown on the bars. Dissimilar letters denote significant differences between pairs at and across recovery times (Two-way ANOVA, Tukey-Kramer HSD test, $P < 0.05$). ANOVA results are shown in Table 2-2.

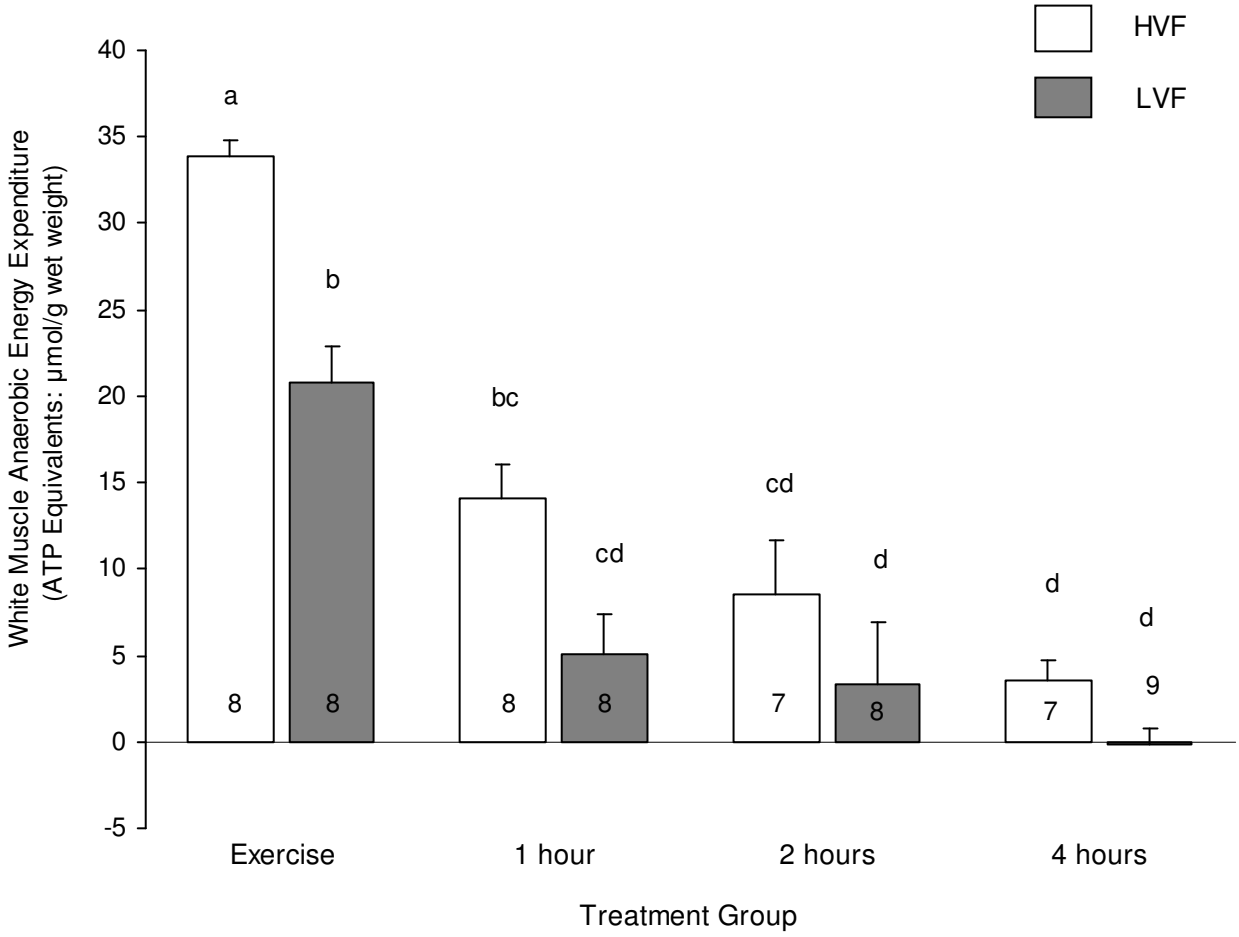


Figure 2-3. Mean (+ SE) white muscle anaerobic energy expenditure for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass following exhaustive exercise and recovery. The sample size for each treatment is shown on the bars. The effects tests for the two-way ANOVA are as follows: group, $df = 1$, $F\ ratio = 24.9$, $P < 0.01$; treatment, $df = 4$, $F\ ratio = 53.1$, $P < 0.01$; interaction, $df = 4$, $F\ ratio = 1.84$, $P = 0.15$. Dissimilar letters denote significant differences between pairs at and across recovery times (Tukey-Kramer HSD test, $P < 0.05$).

Chapter 3: Life-History Traits and Energetic Status in Relation to Vulnerability to Angling in a Teleost Fish

Abstract

In recreational fisheries, a correlation has been established between fishing-induced selection pressures and the metabolic traits of individual fish. This study used a population of largemouth bass (*Micropterus salmoides*) with high (HVF) and low (LVF) vulnerability lines that were previously established from artificial truncation selection experiments. The main objective was to evaluate if differential vulnerability to angling was correlated with growth, energetics, and nutritional condition. Significant differences were detected between HVF and LVF for length and weight in two experimental ponds. Absolute growth rate was found to be between 9-17% higher for LVF compared to HVF over a six-month period in three experimental ponds. The gonadosomatic index in females was higher for HVF compared to LVF. Although energy stores (measured using body constituent analysis) varied across ponds, no significant differences were observed between HVF and LVF. In addition, both groups were consuming the same prey items as evidenced by stomach content analysis. Plasma magnesium levels were found to be significantly higher for HVF, indicating that they may be feeding more than LVF. The inherent reasons behind differential vulnerability to angling are complex, and selection for these opposing phenotypes appears to also select for a trade-off between somatic growth and gonadal investment. These traits are important from a life-history perspective, and alterations to their frequency as a result of fishing-induced selection

could alter fish population structure. These findings further emphasize the need to incorporate evolutionary principles into fisheries management activities.

Introduction

An emerging issue in fisheries science concerns the awareness that fishing pressure is a selective force that is influencing the direction of evolutionary change (reviewed in Heino and Godø 2002; Jørgensen et al. 2007; Kuparinen and Merilä 2007; Law 2000). The specific traits that are under selection in commercial fishing are often dictated by type and size of gear, due to correlations with body size (Law 2000). Fishing-induced selection has not been as extensively studied in a recreational context, and the complexity of factors that determine the vulnerability of an individual fish to capture make it more difficult to discern the specific traits that are under selection. Uusi-Heikkilä et al. (2008) emphasized that behavioural traits rather than life-history traits are more likely to determine an individual's vulnerability to angling. Behaviours that are likely to influence vulnerability include: wariness or boldness (Anderson and Heman 1969; Garrett 2002), aggression (Cooke et al. 2007), ability to learn (Askey et al. 2006; Beukema 1970), and spatial and temporal foraging activity (Philipp et al. 2008). If the behaviours and other phenotypic traits that make a fish vulnerable to angling have a genetic basis, selection pressure can be exerted upon harvest (Lewin et al. 2006) and when hooking or stress-related mortality occurs in catch-and-release events (Arlinghaus et al. 2007; Bartholomew and Bohnsack 2005; Muoneke and Childress 1994). Angling-induced selection is also conceivable without mortality, for example in cases of impairment during reproduction through catch-and-release angling (Cooke et al. 2002b).

Consequences that may become apparent as a result of these lethal and non-lethal events acting on highly vulnerable individuals could include decreased catch rates and reduced reproductive output of the population (Philipp et al. 2008). Knowledge of the specific traits under selection is crucial to understand how evolutionary changes may be manifested in terms of growth, survival, and reproductive capabilities of recreational fish populations, and to develop management strategies that account for potential changes on population dynamics and viability (Ashley et al. 2003; Francis et al. 2007; Jørgensen et al. 2007).

The vast majority of research into the evolutionary consequences of fishing-induced selection has focused on the effects of commercial marine fishing from a life-history trait perspective (Jørgensen et al. 2007; Kuparinen and Merilä 2007). Changes to life-history traits have been documented in commercially exploited fish species for growth rate (e.g. grayling, *Thymallus thymallus* Haugen and Vøllestad 2001), age and size at maturation (e.g. Atlantic cod, *Gadhus morhua* Olsen et al. 2004), and reproductive investment (e.g. Atlantic cod Yoneda and Wright 2004). Similar finding have been reported in model species used in laboratory selection experiments (e.g. Atlantic silversides, *Menidia menidia* Munch et al. 2005; guppies, *Poecilia reticulata* Reznick and Ghalambor 2005). Relatively few studies on fishing-induced selection have been conducted in recreational fisheries (Lewin et al. 2006). Available research has shown that vulnerability to angling is a genetically heritable trait in an experimentally-selected population of largemouth bass (*Micropterus salmoides*), with a realized heritability of 0.15 (Philipp et al. 2008). Recent studies on fish with differential vulnerability to angling have focused on the physiological correlates of this vulnerability. This research has

revealed that largemouth bass with higher levels of vulnerability have higher resting cardiac variables (Cooke et al. 2007), higher standard metabolic rates, and a broader metabolic scope for activity (T.D. Redpath, S.J. Cooke, C.D. Suski, R. Arlinghaus, P. Couture, D.H. Wahl, and D.P. Philipp, unpublished manuscript). While these finding have provided some insight into the traits linked to vulnerability to angling in a recreational fish species, the effects of these metabolic differences on characteristics such as growth and resource consumption have not been evaluated.

Growth is of central importance in fisheries management, and its allocation rate towards somatic and reproductive tissues influences the time to maturation, reproductive investment, and harvestable biomass of a fish population (Calow 1985). Growth rate and body size are traits known to have moderate levels of heritability (reviewed for Atlantic salmon, *Salmo salar*, in Garcia de Leaniz et al. 2007), and any selection that occurs may affect the stability and productivity of a fish population. The amount of food resources consumed will naturally influence growth rate, and any increases in standard metabolic rate will be accompanied by higher energetic costs (Priede 1985). It is therefore necessary to increase food consumption to maintain the same growth rates as individuals with lower intrinsic metabolic rates (Brett and Groves 1979; Jobling 1985). Differences in food consumption can be detected by examining the body constituents (lipid, protein, trace minerals, and water content) and associated energy densities (Breck, 2008). As well, an examination of blood-based nutritional condition indicators that reflect energy reserves can provide further insight into the overall health of the fish (Congleton and Wagner 2006). An evaluation of growth rates, energetics, and nutritional indicators in fish differentially selected for vulnerability to angling may provide further insight into the

diversity of traits under selection as well as the consequences of selection from a fisheries management perspective.

The purpose of this study was to evaluate growth, energetics, and nutritional status in two experimental lines of largemouth bass with differential vulnerability to angling. Specifically, we tested the hypothesis that high vulnerability fish (HVF) and low vulnerability fish (LVF) would have different growth rates. First, based on previous findings that HVF have higher standard metabolic rates (Redpath et al., unpublished manuscript), which may lead to a greater energy intake (Brett and Groves, 1979), we predicted that HVF and LVF would have similar growth rates in a common garden experiment. Second, we tested the hypothesis that the energetic and nutritional indicators for HVF and LVF would differ. Based on the assumption that HVF are consuming additional food resources to sustain their metabolic requirements, we predicted that HVF would have higher energy densities and nutritional indices than LVF.

Materials and Methods

Study Animals

The model species chosen for these experiments was the largemouth bass, because it is subjected to high levels of angling pressure in North America (Pullis and Laughland 1999). This study takes advantage of a unique artificial truncation selection experiment that began several decades ago at the Illinois Natural History Survey (Philipp et al. 2008). Beginning in 1977, largemouth bass in Ridge Lake (39.40 °N, 88.16 °W; 7.10 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. 1984). Following these four seasons of angling, the lake 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 high and low vulnerability to angling (Philipp et al. 2008). 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. 2008). Five pairs in each parental (P1) generation of each line were bred in separate experimental ponds to produce F1 generation offspring, which were then differentiated by pelvic fin clips (Philipp et al. 2008). 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. 2008). A selection procedure using experimental angling over one season was repeated on the F1 fish, and HVF and LVF were again separated into different experimental ponds for breeding (Philipp et al. 2008). The F2 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 to HVF (Philipp et al. 2008). The fish used in this research were bred naturally in ponds in the spring of 2006 as part of an F4 generation, and they had not experienced any further artificial selection.

Growth Assessment

In April 2007, age 1 largemouth bass [HVF, N = 161, TL = 71 ± 0.5 mm (mean ± SE), WT = 4.7 ± 0.1 g; LVF, N = 161, TL = 66 ± 0.5 mm, WT = 3.6 ± 0.01 g; where $P <$

0.01 for TL and WT] were removed from a common garden experimental pond (0.1 ha) at the University of Illinois in Champaign-Urbana. The fish were then re-stocked into four smaller experimental ponds (0.04 ha), with each pond containing 40 HVF and 40 LVF to provide ample space for growth across the summer season. Each pond was stocked with fathead minnows (*Pimephales promelas*), providing forage for the largemouth bass. Naturalized populations of benthic invertebrates, zooplankton, and occasionally terrestrial invertebrates provided additional food sources. Our initial experimental design had four ponds as replicates (to control for a pond effect) to assess absolute growth over a six-month period. We intended to remove a sub-sample of fish from each pond in July and again in October. However, due to unforeseen mortalities over the course of the spring and early summer, one pond (Pond C) was entirely lost and the remaining three ponds saw declines in overall numbers. To ensure that we would have sufficient sample sizes at both sampling periods, we abandoned the original strategy to use each pond as a replicate. Rather, fish sampled in July (by seine net) were taken randomly from Pond B, and fish sampled in October (by draining down the ponds) were taken randomly from Pond A and Pond D. Data were compared across ponds, because it was not possible to separate potential seasonal changes from potential pond effects. Change in length (mm) since April was calculated by subtracting the initial mean length (based on $N = 40$) for either HVF or LVF (from each pond) from the total length of each individual sampled in July and October, as it was not possible to uniquely mark individual fish. Absolute growth rate was determined by dividing the change in length by the number of days, expressed as mm day $^{-1}$ (Jobling 1985; Leitner et al. 2002).

Dissections and Blood Sampling

All largemouth bass sampled from each pond had their lengths recorded to the nearest mm, and their weights recorded to the nearest 0.1 g. Fulton's condition factor was calculated according to the following equation:

$$K_{TL} = [(100{,}000)(W)]/L^3$$

where W = weight in grams, and L = total length in millimeters (Lagler 1956). A sub-sample of fish collected from the common pond (in April) was euthanized by cerebral percussion (HVF, $N = 17$; LVF, $N = 15$). The liver was removed and weighed, and the carcass was homogenized in a food chopper and frozen in airtight bags at – 20 °C for future energetic analyses. Sub-samples of fish were collected from Pond B (in July) (HVF, $N = 13$; LVF, $N = 13$) and Pond A (in October) (HVF, $N = 4$; LVF, $N = 10$), and they were euthanized using concentrated anesthetic (250 mg L^{-1} of tricaine methanesulfonate buffered by 500 mg L^{-1} of sodium bicarbonate) (Summerfelt and Smith 1990). Sampling proceeded once the fish had lost equilibrium and ceased ventilation (~ 2 min). Blood samples (1 mL) were taken from the caudal vein with a 1.9 cm, 26-gauge needle and a syringe previously rinsed with a solution of heparinized saline to prevent coagulation (Houston 1990; Russell 1990). The blood was immediately transferred to a 1.5 mL microcentrifuge tube and centrifuged at 6000 rpm for 2 min. The plasma (supernatant) was separated from the blood cells, stored in liquid nitrogen, and transferred to a – 80 °C freezer in the laboratory.

Dissections were undertaken on the fish collected from ponds B and A to separate the liver, gonads, stomach, and intestines. The weight of all organs was recorded to the nearest 0.01 gram. Liver weight and gonad weight (females only) were expressed as a

percentage of the total body weight to yield the hepatosomatic index (HSI) and the gonadosomatic index (GSI) (Coelho and Erzini 2006). The contents of the stomach were examined, and the basic types of forage were identified (i.e. invertebrate, minnow). The presence or absence of prey types within each stomach enabled a comparison between HVF and LVF in terms of preferred forage over approximately the previous 24 h (Franssen and Gido 2006). The remainder of the carcass was coarsely homogenized in a food processor and frozen at – 20 °C for future energetic analyses.

Energetic Analyses

To ensure an accurate assessment of the whole body lipid stores, a portion (6-8 g) of each homogenized carcass was ground into a powder under liquid nitrogen using a mortar and pestle (Booth et al. 1995). Samples (2 g) of the powdered carcasses were dried overnight (18 h) to a constant mass at 80 °C. The dried samples were then crushed using a glass pestle, and a portion (0.2 g) was used in the lipid extraction procedure. The lipid content of the whole body (fish from all ponds) and the livers and gonads (Pond A) was determined using the Smedes and Askland (1999) modification of the chloroform-methanol extraction technique developed by Bligh and Dyer (1959). Briefly, the samples were combined with chloroform, methanol, and water in a 1:2:0.8 mL ratio and placed in an ultrasonic bath (Fisher Scientific FS20) for 15 min. Additional amounts of chloroform and water were added, and the samples were centrifuged at 1500 rpm for 10 min (VWR Clinical 50 Centrifuge). The solvent layer of chloroform (containing the lipids) was removed using a pipette and filtered through sodium sulfate and quartz wool, and the extraction procedure was repeated on the supernatant. The extracted lipids were left

overnight (to allow the chloroform to evaporate), dried for 1 h at 60 °C, and weighed to calculate the percent of lipids by dry mass. These values for the dried samples were then expressed in terms of percent of lipid by wet mass. Each individual fish was analyzed in duplicate, and the variation between the percentages for the sub-samples was never greater than 2%.

The remaining whole body constituents (water, protein, and trace mineral) were analyzed following the methods outlined in Crossin and Hinch (2005). A representative sample (2 g) of each homogenized carcass was selected (avoiding large pieces of bone and skin), and they were weighed and dried overnight (18 h) to a constant mass at 80 °C. Once dry, the samples were re-weighed to determine the percent of water by wet mass. The dried samples were then combusted for 2 h in a muffle furnace at 500-600 °C, and the remaining ash was weighed to determine the percentage of trace minerals by wet mass. Each individual fish was analyzed in duplicate, and the variation between the percentages of the sub-samples was never greater than 2%. The percent of body protein was determined by the relationship $C_P = 100(C_W + C_A + C_L)$, where C_W, C_A, and C_L = percent water, ash, and lipid, respectively (Berg et al. 1998; Hendry et al. 2000).

Fish energy density was calculated according to the following equation:

$$d = fD_f + pD_p,$$

where d = energy density in MJ kg^{-1}, f and p = fraction of lipids and proteins from the samples, expressed in g kg^{-1}, D_f and D_p = energy density of lipids and protein in fish, expressed in MJ g^{-1} (Breck 2008). The energy density values for lipids and proteins were derived from the values presented for fish in Brett and Groves (1979), where lipids in fish contain 0.0362 MJ g^{-1} and proteins in fish contain 0.0201 MJ g^{-1}.

Nutritional Indicator Analyses

Plasma from HVF and LVF sampled from Pond B were analyzed for concentrations of biochemical components that form an index of nutritional condition in fish (total protein, cholesterol, triglycerides, calcium, and magnesium) (Congleton and Wagner 2006; Wagner and Congleton 2004). These analyses were conducted on a Roche Hitachi 917 analyzer (Basal, Switzerland) and based upon the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standard reference model. To ensure proper quality control, all assays followed procedural guidelines for standardization and quality assurance established by the Veterinary Laboratory Association Quality Assurance Program, College of American Pathologists, and the Canadian Food Inspection Agency External Proficiency Panel.

Statistical Analysis

For all data, normality was assessed using a one-sample Kolmogorov-Smirnov test and homogeneity of variance was assessed using the Levene's test. The comparisons of growth rates, whole body constituents, and energetic indices between HVF and LVF across ponds were conducted using two-way analysis of variance (ANOVA). The statistical differences between groups and ponds were evaluated using the student's *t*-test. The mortalities that occurred in the ponds were expressed as percent survival and compared using a Mann-Whitney U non-parametric test (due to small sample sizes). The comparisons between HVF and LVF for nutritional indicators (sampled from Pond B) and organ lipids (liver and gonad - sampled from Pond A) were conducted using *t*-tests. The comparison of the stomach contents between HVF and LVF was conducted using a

chi-square test. Arcsine square-root transformations were carried out on all proportional data (for whole body constituents and survival). All analyses were carried out using JMP 7.0 (SAS Institute). Values are reported as means (± SE) and tests are interpreted at a significance level (α) of 0.05. Due to multiple comparisons between similar parameters, sequential Bonferroni corrections were performed (see Table 3-2; Rice 1989). In Tables 3-1 and 3-2, the uncorrected *P*-values are presented, and the results are interpreted based on the more conservative Bonferroni level of significance. Results deemed non-significant based on these conservative criterion may in fact still be important (Cabin and Mitchell 2000; Moran 2003).

Results

Many of the results highlighted below demonstrate changes between the ponds, which were each sampled at different periods throughout the growing season. As the possibility that these changes are due to individual pond effects rather than seasonal differences cannot be eliminated, the results will be presented according to the ponds. LVF exhibited consistently higher absolute growth rates than HVF across ponds, with growth rates that were between 8.7-17.3% greater (Figure 3-1). When the experiments were initiated from the common pond in April, the total length and weight for HVF was 9% and 30% greater than for LVF (Tables 3-1 and 3-2). When Pond B was seined (in July), HVF and LVF demonstrated similar measurements, but when Pond A was drained (in October), the total length and weight for LVF was 6% and 16% greater than for HVF (Tables 3-1 and 3-2). While total length was significantly different between HVF and LVF without the Bonferroni correction ($P = 0.009$), it merely approached significance

when assessed under the more conservative Bonferroni criterion ($\alpha = 0.006$) (Table 3-2). Fulton's condition factor varied across the ponds, and it differed significantly between HVF and LVF overall without the Bonferroni correction ($P = 0.044$) (Tables 3-1 and 3-2). However, when interpreted using the Bonferroni correction, this apparent trend was no longer significant. In terms of the percent of fish surviving in each pond across the six-month period, no significant differences were found between HVF and LVF [HVF, 21 ± 12.0 % (mean ± SE) ($N = 4$); LVF, 36 ± 13.6 % ($N = 4$); $P = 0.47$] (Table 3-3).

The HSI was higher for fish sampled from the common pond as compared to Pond B and Pond A, but there were no differences between HVF and LVF (Tables 3-1 and 3-2). Female HVF demonstrated a higher GSI compared to LVF in both Pond B and Pond A (Tables 3-1 and 3-2). An assessment of the stomach contents revealed that the majority of individuals (85%) were consuming aquatic invertebrates in both Pond B and Pond A. The chi-square test demonstrated no differences between the stomach contents of the HVF and the LVF for either pond (Pond B: calculated $\chi^2 = 0.62$, critical $\chi^2 = 3.84$; Pond A: calculated $\chi^2 = 2.0$, critical $\chi^2 = 3.84$).

Energy stores, as assessed by whole body constituent analysis (in terms of percent lipid, protein, trace minerals, and water), and the calculated energy density values varied significantly across the ponds (Tables 3-1 and 3-2). Although percent lipid values and energy densities were higher and water content was lower for fish in Pond B compared to the common pond, these values were stable between Pond B and Pond A (Table 3-1). Although percent protein and trace mineral values were similar between fish in the common pond and Pond B, these values varied between Pond B and Pond A (Table 3-1). No differences were observed between HVF and LVF for any of the whole body

constituents or energy densities (Tables 3-1 and 3-2). There were no differences between the percentage of lipids within the livers and the gonads of the HVF and LVF collected from Pond A (Table 3-4).

From the suite of plasma biochemical indicators that were assessed as an indication of nutritional condition (total protein, cholesterol, triglycerides, calcium, and magnesium), only magnesium displayed a significant difference between HVF and LVF (Table 3-4). The levels of magnesium were greater for HVF as compared to LVF (Table 3-4).

Discussion

This study revealed that life-history traits are correlated with vulnerability to angling in largemouth bass in a manner that conflicts with the findings in commercial marine fisheries, where selection has mainly been size-based (Kuparinen and Merilä 2007; Law 2007). Specifically, differences in growth rates and GSI were observed between the two experimental lines of largemouth bass. When initially sampled in the common pond, HVF displayed a greater length and weight than LVF. However, for Pond B (sampled in July) these differences were no longer evident, and for Pond A (sampled in October) the initial trend had reversed, with LVF displaying a greater length and weight than HVF. When assessed against the baseline values, LVF achieved higher absolute growth rates (mm day $^{-1}$) than HVF across all three experimental ponds. The total amount of energy ingested by a fish is partitioned towards growth (somatic and reproductive), metabolism (standard, feeding, and active), and excretion (which remains constant) (Brett and Groves 1979; Calow 1985). HVF have a higher standard metabolic

rate (SMR) (Redpath et al., unpublished manuscript), which must be balanced by increasing energy intake if the growth rate is to remain stable (Brett 1979; Brett and Groves 1979). Any increased foraging to obtain additional food will encompass a greater portion of the active metabolism (Priede 1985), because locomotion in fish requires considerable amounts of energy (Brett and Groves 1979). The slower growth rate found in HVF may indicate that they are unable to consume sufficient food resources [estimated to be 40% more than control largemouth bass (Cooke et al. 2007)] to compensate for their increased metabolism. Alternatively, the potential increase in foraging activity by HVF could resulting in less available scope for feeding metabolism and digestion processes, result in decreased growth (Priede 1985). Although HVF have shown a greater metabolic scope for activity (Redpath et al., unpublished manuscript), it may not be broad enough to account for the increased energy requirements of a higher standard metabolic rate and related foraging activity. In juvenile sand sharks (*Carcharhinus plumbeus*), it has been shown that 34-100% of the metabolic scope is required to sustain routine metabolic rates, a factor that is thought to result in slower growth rates (Dowd et al. 2006). We assumed that food resources were not a limiting factor, given the low densities of fish introduced into each pond. Without a measurement of actual food consumption, it is difficult to determine ingestion rates and relative allocation towards metabolic requirements. Regardless of the metabolic constraints, HVF and LVF have demonstrated different growth rates, and any change in allocation of energy may affect a variety of life-history traits (Calow 1985).

The metabolic demands placed on the energy budget are maintained in balance with the requirements for growth, but trade-offs occur in the synthesis of tissues for

somatic and reproductive purposes (Calow 1985). Growth rate and body size are generally correlated with reproductive effort and fecundity in many species of fish, including three-spined sticklebacks (*Gasterosteus aculeatus*) and medaka (*Oryzias latipes*) (Wootton 1985). In situations where the reproductive effort diverts too many resources away from somatic growth, the future fecundity of an individual is likely to decrease (Wootton 1985). HVF displayed a smaller body size and a reduced growth rate, along with a higher GSI. It appears that HVF may be diverting more of their available energy towards earlier maturation rather than somatic growth. However, if their growth rate and size remain significantly smaller, this could result in a reduction in fecundity over the long term (Wootton 1985), and the opposite effect may be observed in LVF. In a heavily exploited stream containing fish less vulnerable to angling, mature female brook trout (*Salvelinus fontinalis*) exhibited slower growth rates and allocated more energy to gonadal tissue than more vulnerable fish from a less exploited stream (Nuhfer and Alexander 1994). Although these results are contrary to those in the current study, the brook trout were exploited based on size and faster-growing individuals were removed at a higher rate (Nuhfer and Alexander 1994). It has recently been hypothesized that for recreational fisheries, selection operates on behavioural traits rather than directly on body size-related traits (Uusi-Heikkilä et al. 2008). This corresponds the selection procedure in this study, whereby the largemouth bass were selected based on vulnerability to angling. Therefore, the predicted evolutionary changes in life-history traits for size-selective commercial fisheries may not correspond to changes in a recreational fishery.

Although HVF display a higher SMR and appear to be maturing more quickly than LVF, it is unknown whether these two traits are linked. In a study of convict cichlids (*Cichlasoma nigrofasciatum*), a small increase in metabolic rate was detected during the transition period between the juvenile and sexually mature stages (Fidhiany and Winckler 1998). The higher metabolic rate was attributed to internal changes occurring due to the development of the reproductive organs (Fidhiany and Winckler 1998). The largemouth bass in were still sexually immature, and it is unknown whether the observed differences into gonadal investment will be maintained as they mature. Rather than attempting to causally link SMR with maturation, it may be more appropriate to examine the effects that an increased metabolic rate could have on reproduction and fecundity. In instances where the resources for metabolism are limited, the formation of gametes is often given priority over somatic growth (Calow 1985). During periods of low energy intake, female largemouth bass have been shown to transfer energy from the soma to the ovaries (Wootton 1985). Due to their higher SMR and increased energy requirements, HVF may have more difficulty maintaining their ovarian growth during periods of low nutrient availability. Obviously, it is difficult to predict reproductive outcomes and fecundity for largemouth bass differentially selected for vulnerability to angling based on data from juvenile individuals. A thorough examination of mature HVF and LVF in terms of age at maturation, fecundity, and growth rate in relation to SMR and metabolic scope is necessary to fully understand the effects of fishing-induced selection from a life-history standpoint.

The amount of energy available to a fish has implications for growth, survival, and reproduction (Calow 1985), and the stored energy is reflected by the whole body

constituents (lipid, protein, trace minerals, and water) and the energy density (an index of energy contained in the lipid and protein) (Breck 2008). Recent feeding history and nutritional condition have typically been determined by analysis of body constituents and energy density (Elliott 1976), and they are now known to be correlated with certain biochemical factors circulating in the blood (Congleton and Wagner 2006; Wagner and Congleton 2004). The changes in body composition and energy density determined from the whole body constituent analysis for HVF and LVF across ponds over the six-month period were similar to expected trends during periods of growth. The percent of lipid and protein in brown trout (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*) has been shown to increase along with the size of the fish and the amount of food resources consumed, while the water content is proportionally reduced (Elliott 1976; Weatherley and Gill 1983). Although all body constituents and energy densities fluctuated across the ponds, no significant differences were observed between HVF and LVF in any of the ponds. Based on these similarities, HVF and LVF appear to be consuming a similar amount of food resources and deriving equivalent energy units. While most of the nutritional indicators assessed in the plasma did not differ, plasma magnesium levels were found to be significantly higher for HVF compared to LVF. Magnesium is essential in many enzymatic reactions for intermediary metabolism, and it is acquired mainly through dietary sources in freshwater fish (Lall 2002). In a recent study on largemouth bass across the parental care period, plasma magnesium levels remained relatively stable (Hanson and Cooke In Review), even though the opportunities for foraging were severely limited during this period (Hinch and Collins 1991). The apparent stability of plasma magnesium levels, even in periods of reduced food intake, is contrasted by the differences

between HVF and LVF. Since both HVF and LVF are consuming the same prey items, the higher plasma magnesium levels in HVF seem to indicate that they are consuming more food resources than initially estimated by the body constituent analysis. This finding supports the prediction that the higher SMR in HVF is more energetically costly and thus requires increased feeding. Fulton's condition factor (with or without the Bonferroni correction) provides additional evidence that the body condition of HVF is equivalent or perhaps even greater that that of LVF. The lower growth rate demonstrated by HVF in comparison to LVF does not appear to be attributed to differences in energy intake, but rather it seems to result from trade-offs in the synthesis of somatic and reproductive tissues.

In many ways, the life-history traits correlated with differential vulnerability to angling directly oppose the trends that occur under purely size-based selection. When larger fish are preferentially removed from a population, the classic evolution of life-history patterns is towards earlier maturation at smaller sizes with a higher reproductive effort at age (Edeline et al. 2007; Hutchings and Fraser 2008). However, direct selection on behaviour can also drive evolutionary changes in life-history traits (Biro and Post 2008). The artificial selection experiments for the largemouth bass were based on the trait of vulnerability to angling and were not size-dependant (Philipp et al. 2008). Although growth rates differ between the two experimental lines of largemouth bass, the body constituent and energy density analyses indicate that HVF and LVF are both consuming sufficient food resources. It appears that in HVF, a greater portion of the available energy is directed towards metabolic processes and gonad development rather than somatic growth. The implications of these findings for fish populations with

differential vulnerability to angling are quite striking. If individuals with HVF traits (higher standard metabolic rate, slower somatic growth, faster gonad development) are removed from a population at a faster rate, the traits of the remaining fish could begin to shift towards those characteristic of LVF (lower standard metabolic rate, faster somatic growth, slower gonad development). Given the potential of LVF for delayed maturation, and in combination with previous findings of decreased vigilance and activity during parental care (Cooke et al. 2007), removal of HVF could result in decreased recruitment to the overall population. The remaining fish will also become more difficult to catch, thus diminishing the quality of the fishery (Cooke et al. 2007). The implications of these changes could prove adverse from both a conservation biology and fisheries management perspective, and management strategies need to account for this issue prior to the onset of evolutionary changes that may be difficult to reverse (Law 2000; Stockwell et al. 2003).

Although this study focused on largemouth bass, other species that are angled recreationally have demonstrated variability in their vulnerability to angling (e.g. rainbow trout, *Oncorhynchus mykiss* Askey et al. 2006; brook trout, *Salvelinus fontinalis* Nuhfer and Alexander 1994). While heritability and selection pressures have not yet been assessed in these other species, it seems prudent to implement management strategies that strive to preserve the genetic traits that may be associated with an increased vulnerability to angling. Several recurring strategies are proposed when striving to conserve genetic diversity. One method is to restrict angling in certain portions of a lake or river by creating an aquatic protected area (Berkeley et al. 2004). This allows a refuge area where angling pressure is removed and fish with differing behavioural patterns and associated traits can flourish. A second option is to close the fishery entirely during the reproductive

period (Schramm et al. 1995) and to establish minimum-harvest limits that give all individuals an opportunity to reproduce, thus minimizing potential losses from the gene pool. Since much of recreational fishing involves catch-and-release angling, a third option is to reduce unintentional mortalities that result from hooking injuries and the associated physiological stress of an angling event (Cooke and Suski 2005). Long-term monitoring programs are still a necessary aspect of management strategies (Philipp et al. 2008). Without monitoring, it is difficult to determine if declining catch rates are due to a tangible reduction in numbers or to an increased presence of fish with low vulnerability to angling. It is important to implement management strategies as soon as possible to preserve the genetic diversity of recreational fish stocks and to avoid uncontrolled evolutionary changes (Francis et al. 2007; Heino and Godø 2002; Jørgensen et al. 2007; Law 2000).

Tables

Table 3-1. Comparison of energetics and body condition for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass across ponds for a six-month period presented as mean (± SE).

		Common Pond (April)			**Pond B (July)**			**Pond A (October)**		
Parameter	**Stock**	**Mean**	**SE**	***N***	**Mean**	**SE**	***N***	**Mean**	**SE**	***N***
Total length (mm)	HVF	71 a	0.5	161	163 b	2.3	13	247 c	4.9	4
	LVF	66 a*	0.5	161	168 b	3.3	13	262 c*	2.9	10
Weight (g)	HVF	4.7 a	0.1	161	56 b	2.3	13	229 c	17.4	4
	LVF	3.6 a*	0.01	161	60 b	5.7	13	265 c*	13.7	10
Fulton's condition factor	HVF	1.24 a	0.02	17	1.29 b	0.01	13	1.51 c	0.05	4
	LVF	1.19 a	0.02	15	1.24 b	0.04	13	1.46 c	0.03	10
Hepatosomatic index (%)	HVF	2.26 a	0.13	17	0.96 b	0.05	13	1.03 b	0.12	4
	LVF	2.30 a	0.25	15	1.05 b	0.09	13	0.93 b	0.04	10
Gonadosomatic index (%)	HVF	-	-	-	0.65 a	0.03	7	1.58 b	0.01	3
	LVF	-	-	-	0.49 a	0.02	8	1.32 b	0.11	6

Lipids (%)	HVF	2.22 a	0.11	17	3.48 b	0.11	13	3.79 b	0.32	4
	LVF	2.24 a	0.08	15	3.80 b	0.18	13	3.52 b	0.25	10
Protein (%)	HVF	17.1 a	0.17	17	17.6 a	0.11	13	18.4 b	0.97	4
	LVF	17.2 a	0.13	15	17.6 a	0.18	13	18.4 b	0.28	10
Trace minerals (%)	HVF	4.47 a	0.18	17	4.55 a	0.14	13	3.19 b	0.35	4
	LVF	4.29 a	0.12	15	4.56 a	0.21	13	3.62 b	0.28	10
Water (%)	HVF	76.2 a	0.27	17	74.4 b	0.22	13	74.7 b	1.26	4
	LVF	76.3 a	0.24	15	74.0 b	0.39	13	74.5 b	0.49	10
Energy density (MJ kg^{-1})	HVF	4.24 a	0.04	17	4.80 b	0.04	13	5.06 b	0.26	4
	LVF	4.27 a	0.04	15	4.92 b	0.07	13	4.97 b	0.11	10

Note: GSI was calculated for females only. Dissimilar letters indicate a significant difference across ponds for each stock, and * indicates a significant difference between HVF and LVF for a given pond ($\alpha = 0.05$; Student's *t*-test). ANOVA results are shown in Table 3-2.

Table 3-2. Results of a two-way ANOVA, with pond, stock, and the pond × stock interaction as effects, comparing high vulnerability (HVF) and low vulnerability (LVF) largemouth bass across a six-month period.

	Pond			**Stock**			**Pond × stock**		
Parameter	***F***	**df**	***P***	***F***	**df**	***P***	***F***	**df**	***P***
Total length (mm)	5667	2	**< 0.01**	6.95	1	0.009*	16.9	2	**< 0.01**
Weight (g)	4616	2	**< 0.01**	38.7	1	**< 0.001**	25.7	2	**< 0.01**
Fulton's condition factor	46.5	2	**< 0.01**	4.24	1	0.044*	0.01	2	0.99
Hepatosomatic index (%)	47.3	2	**< 0.01**	0.01	1	0.94	0.12	2	0.89
Gonadosomatic index (%)	188	1	**< 0.01**	10.1	1	**0.005**	0.62	2	0.44
Lipids (%)	67.5	2	**< 0.01**	0.02	1	0.89	1.20	2	0.31
Protein (%)	11.2	2	**< 0.01**	0.11	1	0.74	0.04	2	0.96
Trace minerals (%)	13.1	2	**< 0.01**	0.28	1	0.60	0.88	2	0.42
Water (%)	20.9	2	**< 0.01**	0.28	1	0.60	0.19	2	0.83
Energy density (MJ kg^{-1})	70.8	2	**< 0.01**	0.14	1	0.71	0.92	2	0.40

Note: Data tested by ANOVA are presented in Table 3-1. As multiple comparisons were conducted, Bonferroni corrections were applied. Significant values based on the criterion ($\alpha = 0.006$) are in boldface type. Significant values prior to Bonferroni corrections ($\alpha = 0.05$) are indicated by an asterisk.

Table 3-3. Survival rates for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass stocked in four experimental ponds.

Pond and stock	**Number stocked**	**Date sampled**	**Number of fish recovered**	**Percent survival (%)**
Pond A		11-Oct-07		
HVF	41		4	10
LVF	41		20	49
Pond B		30-Jul-07		
HVF	40		22	55
LVF	40		25	63
Pond D		16-Oct-07		
HVF	40		8	20
LVF	40		12	30

Table 3-4. Comparison of nutritional indicators and organ lipid levels between high vulnerability (HVF) and low vulnerability (LVF) largemouth bass presented as mean (± SE).

	Vulnerability					
Parameter	**High**	***N***	**Low**	***N***	**Test statistic**	***P*-value**
Total protein (g L^{-1})	31 ± 0.8	12	30 ± 0.8	11	T = 0.72	0.48
Cholesterol (mmol L^{-1})	8.7 ± 0.3	12	8.4 ± 0.3	11	T = 0.77	0.45
Triglycerides (mmol L^{-1})	3.55 ± 0.33	12	3.15 ± 0.38	11	T = 0.80	0.44
Calcium (mmol L^{-1})	3.51 ± 0.18	12	3.33 ± 0.04	11	U = -0.37	0.71
Magnesium (mmol L^{-1})	1.83 ± 0.11	12	1.52 ± 0.05	11	U = -2.34	**0.02**
Lipids - liver (%)	5.07 ± 0.32	4	5.49 ± 0.19	10	T = -1.16	0.27
Lipids - gonads (%)	4.25 ± 0.74	3	4.60 ± 0.23	6	T = -0.60	0.57

Note: Data were analyzed using a *t*-test (T) or a Mann-Whitney U test (U). Lipid results are for fish sampled from Pond A (October); all remaining results are for fish sampled from Pond B (July). Significant values based on the criterion ($\alpha = 0.05$) are in boldface type.

Figure

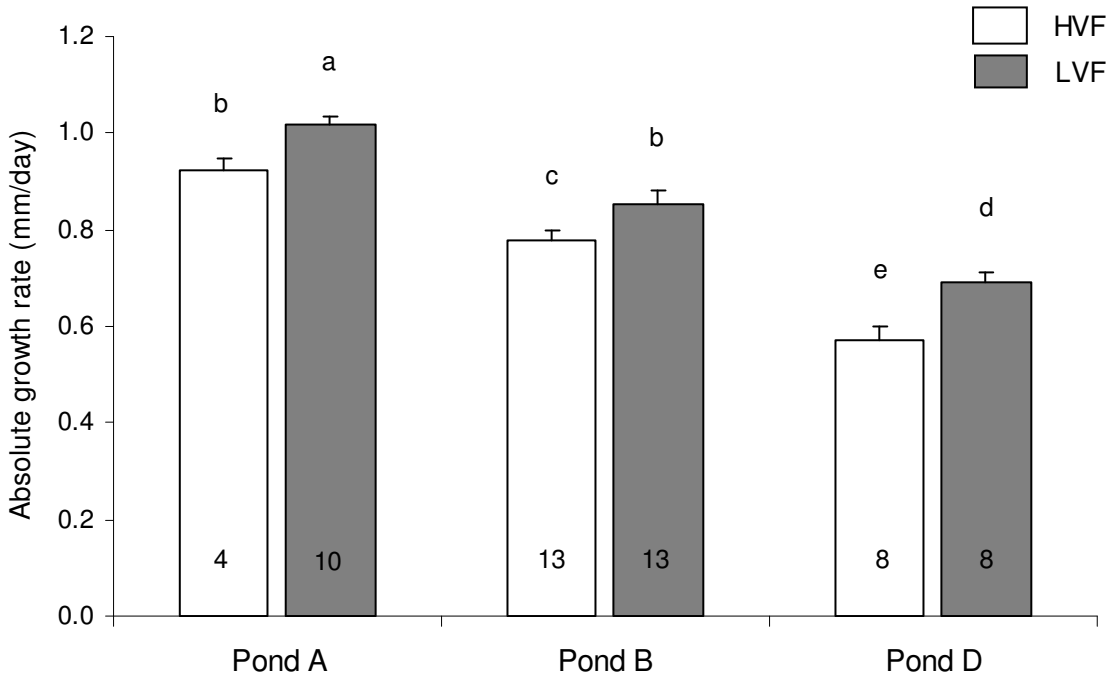


Figure 3-1. Mean (+SE) absolute growth rate (mm day^{-1}) for high vulnerability (HVF) and low vulnerability (LVF) largemouth bass. Pond B represents the period from April to July; Pond A and Pond D represent the period from April to October. Sample sizes are indicated on the bars. The effects tests from the two-way ANOVA are as follows: group, $df = 1$, $F = 21.1$, $P < 0.01$; pond, $df = 2$, $F = 73.1$, $P < 0.01$; interaction, $df = 2$, $F = 0.49$ $P = 0.61$). Dissimilar letters denote significant differences between pairs in and across ponds ($\alpha = 0.05$; Student's *t*-test).

Chapter 4: General Discussion

It has recently become evident that recreational angling has the capacity to induce trait-based selection pressures in a manner similar to trends documented in commercial fisheries (Kuparinen and Merilä 2007; Law 2007). The likelihood of capturing an individual fish by rod and reel depends in part upon its inherent level of vulnerability to angling. In the largemouth bass (*Micropterus salmoides*) used in this study, it had been previously determined that vulnerability to angling is a genetically heritable trait (Philipp et al. 2008). The purpose of this thesis was to identify the underlying biological traits that are correlated with differential vulnerability to angling by combining several physiologically-based laboratory and field studies. Specifically, the relationships between vulnerability to angling and metabolism, anaerobic capacity, growth rate, energetics, and nutritional status were examined.

Findings and Implications

In chapter 2, differences between fish with high (HVF) and low (LVF) vulnerability to angling were assessed in terms of metabolism and anaerobic capacity while at rest and following exhaustive exercise. In a previous study, differences in cardiovascular physiology following exercise were examined for HVF and LVF (Cooke et al. 2007). This study expands on the previous research by measuring metabolic rates directly and by determining the full extent of physiological disturbances following exercise within tissue metabolite and tissue energy stores. Standard metabolic rate was 11% higher, maximal metabolic rate was 16% higher, and metabolic scope was 20% higher for HVF compared to LVF. Immediately following exhaustive exercise, muscle

lactate concentrations were 44% higher for HVF compared to LVF, and both groups had returned to control levels after 2 h of recovery. Anaerobic energy expenditure was also significantly higher for HVF, and during exercise HVF exhibited burst swimming more frequently than LVF. These findings may have direct implications for the daily activities of fish, including energy acquisition and swimming performance when feeding or evading predators.

In chapter 3, differences between HVF and LVF were assessed in terms of growth rate, energetics, and nutritional status. Previous research on fishing-induced selection from a commercial perspective has often focused on growth rates and other life-history traits, as they provide valuable insights into the reproductive characteristics and influence the harvestable biomass (Haugen and Vøllestad 2001; Olsen et al. 2004). In this study, selection was carried out on the trait of vulnerability to angling rather than size, which tends to be the focus of studies in the commercial marine fishery sector (Kuparinen and Merilä 2007; Law 2007). Thus, the findings with respect to life-history traits tend to contrast those of previous studies. Growth rate was between 9-17% higher for LVF compared to HVF. The gonadosomatic index in females was higher for HVF compared to LVF. In terms of energy stores, no significant differences were observed between HVF and LVF, and both groups were consuming the same prey items. Plasma magnesium levels were significantly higher for HVF, indicating that they may be feeding more than LVF. These findings appear to be related to a trade-off between somatic growth and gonadal investment, and they may have important implications for the future reproductive success of these fish.

In general, these findings demonstrate that differential vulnerability to angling is correlated with a suite of physiological and life-history traits that often seem to be intimately linked with behaviour. While the largemouth bass used in these studies were part of a long-term selection experiment for differential vulnerability to angling, it is unknown how these physiological and life-history traits were expressed in the original parental (P1) generations. The original study that developed the HVF and LVF strains established vulnerability to angling as a heritable trait, and it was also noted that the response to selection was diverging with each generation (Philipp et al. 2008). It cannot, however, be assumed that the differences observed between the physiological and life-history traits for HVF and LVF in this study occurred in response to the original selection. As well, a lack of control lines in the original study makes it difficult to ascertain whether the observed changes are in fact related to selection, or if they are due to another factor such as genetic drift or inbreeding depression. The original study did include the creation of replicate lines, and this adds some strength to the correlations between the physiological and life-history traits that were examined and the general trait of differential vulnerability to angling.

It is important to recognize that vulnerability to angling is a genetically heritable trait in these experimentally-bred largemouth bass (Philipp et al. 2008), therefore shifting genotypic frequencies due to exploitation have the potential to bring about evolutionary changes (Nager et al. 2000; Pigliucci and Kaplan 2006). As evolutionary changes related to fishing pressure can occur rapidly over a few decades and are difficult to reverse (Law 2000; Stockwell et al. 2003), fisheries management strategies in the recreational sector must strive to minimize fisheries-induced selection. Harvesting recreationally-angled

largemouth bass is likely to result in the disproportionate exploitation of those more vulnerable to angling, along with their associated traits. A logical approach for minimizing exploitation would be to lower the numbers of fish that anglers are permitted to harvest (Kuparinen and Merilä 2007). Since fish with differential vulnerability to angling may reach maturation at different sizes, another proactive approach would be to raise the minimum size for harvest to allow most individuals the opportunity to reproduce several times prior to being exposed to harvest. The implementation of a maximum allowable size for harvest would also help preserve larger, older individuals, which are an important repository for genetic variability (Kuparinen and Merilä 2007). Other methods to address the issue of fishing-induced selection include the creation of aquatic protected areas in portions of a water body (Berkeley et al. 2004), the closure of a fishery during the reproductive period (Schramm et al. 1995), and the reduction of unintentional mortalities due to catch-and-release angling events (Cooke and Suski 2005). Throughout the implementation of these various strategies, managers and scientists should continue to monitor key life-history traits and evaluate the evolutionary status of the fishery (Kuparinen and Merilä 2007). New statistical techniques, such as probabilistic maturation reactions norms, may be utilized to determine the extent of the evolutionary changes that have taken place within a given stock (Dieckmann and Heino 2007). The use of rigorous genetic monitoring, including genetic markers linked to the affected genomic regions, can assist detecting evolutionary effects at an early stage (Kuparinen and Merilä 2007). By implementing these methods along with long-term monitoring programs, the genetic diversity of recreational fish stocks will be better preserved and the sustainability of fish populations will be improved (Philipp et al. 2008).

Future Research Directions

While the results of this thesis provide insight into some physiological and life-history traits that are correlated with vulnerability to angling, they raise additional questions regarding other closely linked traits. Some of the areas that require additional research include: swimming performance, activity levels, food consumption rates, and reproductive investment. HVF were observed to have a greater ability for burst swimming, and an assessment of their maximal swim speeds over short distances would provide further insight into their ability to evade predators and chase prey (Reidy et al. 2000). The use of a swim tunnel to measure critical and sustained swimming speeds would also clearly establish whether the capacity for aerobic activity differed between the two groups (Reidy et al. 2000). Daily activity levels are assumed to be higher for HVF based on their higher metabolic rate and increased energetic requirements. It is necessary to quantify these activity rates while the fish are behaving routinely in a natural environment, because any differences in activity patterns during daylight hours could also explain an increase in vulnerability to angling. The use of electromyogram (EMG) telemetry would be an appropriate method to quantify activity patterns in fish swimming freely in an experimental pond (Cooke et al. 2004a). Once calibrated, EMG transmitters provide data on locomotory activity, swimming speed, and the metabolic costs of activity by measuring bioelectrical voltage changes in fish muscle (Cooke et al. 2004b). The required energy consumption for sustaining the activity rates measured by the EMG transmitters could also be coupled with laboratory studies on feeding rates to gain more insight into differential energy partitioning and growth rates between the two groups of fish. Finally, the results of this study indicated that juvenile HVF were investing more

energy into gonadal development than LVF. Relevant studies related to reproductive energetics should continue as these fish mature to determine if the initial differences are preserved over the long term, and assessments of fecundity and reproductive success should also be carried out (Wootton 1985). These additional experiments would provide a more complete picture of the physiological and life-history traits that are associated with vulnerability to angling, and they would also provide more information regarding the consequences of any genetic changes to the population.

Vulnerability to angling clearly encompasses a suite of physiological and life-history traits, but the extent to which these traits are behavioural remains largely speculative. Recent studies have demonstrated that much of the inter-individual variation in physiological traits, including metabolic rate, can be attributed to specific animal personality types that are maintained over time and across situations (Careau et al. 2008; Réale et al. 2007). Examples of personality traits where individuals consistently behave towards one extreme or the other include: bold and shy, aggressive and non-aggressive, docile and untamed, and active and inactive (Réale et al. 2007). Similar types of behaviour also tend to be linked to an overall personality type, such that bold, aggressive individuals also possess a more exploratory nature than shy, docile individuals (Careau et al. 2008). While aggression and boldness are often attributed to fish with a high vulnerability to angling, future studies would benefit from a quantitative assessment of the personality types of high and low vulnerability fish. For example, aggressive behaviour may be studied using a mirror image stimulus and by observing social interactions between individual fish (Réale et al. 2007). In addition, physiological assays may be conducted in the laboratory on the male hormone 11- ketotestosterone which has

been linked to aggressive, dominant behaviour in fish (Oliveira et al. 1996). By linking the fields of physiology, behaviour, genetics, and ecology, researchers may gain a better understanding of the evolutionary changes likely to result from fishing-induced selection. As well, by continuing to study the traits linked to vulnerability to angling from a variety of biological levels, more insight into the mechanisms of selective exploitation may be gained.

Summary and Conclusions

1. **Fishing-induced selection has the potential to occur in a recreational fishery when individual fish are exploited based on the heritable trait of vulnerability to angling.** Many studies have been conducted on commercially-exploited marine fish to determine the consequences of fishing-induced selection and the potential for evolutionary changes. However, few studies have examined this phenomenon from the perspective of a recreational fish species even though the basis for selection is often similar. This thesis conducted a series of experiments on largemouth bass bred for differential vulnerability to angling over four generations, where vulnerability to angling was previously found to be a heritable trait.

2. **Results from respirometry and exhaustive exercise experiments show that largemouth bass with high vulnerability to angling display higher metabolic rates and a higher capacity for anaerobic activity.** These experiments demonstrated that HVF have a higher standard metabolic rate that LVF, and HVF

also attained a higher maximal metabolic rate and a greater metabolic scope following exhaustive exercise. An assessment of physiological variables following an exhaustive exercise simulation showed that HVF accumulated a greater concentration of tissue lactate and had higher anaerobic energy expenditures than LVF, and HVF recovered to the same control levels as LVF within the same timeframe. These results confirm the existence of metabolic differences between the two groups of fish while providing evidence of differing anaerobic capacities.

3. **Results from growth and energetics assessments and nutritional indicators show that largemouth bass with high vulnerability to angling display lower growth rates, invest more energy into gonadal development, and consume more food resources.** These experiments demonstrated that HVF have an absolute growth rate that is slower than LVF, yet female HVF invested more energy into gonadal development. Body constituent analyses demonstrated similar values in terms of lipid, protein, trace minerals, and water content between HVF and LVF, indicating that both groups are in the same relative condition. Plasma magnesium levels were found to be higher for HVF, indicating that they likely have an increased rate of feeding compared to LVF. These results provide evidence of differing life-history strategies and energy acquisition between the two groups of fish.

4. **Integrating the fields of physiology, behaviour, genetics, and ecology are important for understanding the mechanisms underlying differential vulnerability to angling.** By combining expertise from these various research areas, it will be possible to predict how genotypic frequencies may change in response to fishing-induced selection and to determine how the sustainability of fish populations may be affected.

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