The relationships between morphology, physiology, performance and individual fitness in two species of teleost fish

By

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A thesis submitted to the Faculty of Science in fulfillment of the thesis requirement for the degree of

Doctor of Philosophy in Biology

Ottawa Carleton Institute of Biology

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Ottawa, Ontario, Canada

Dedication

Many thanks to everyone who made this thesis possible. To my family, for supporting me throughout what at times seemed like a never ending task. To my advisor, for giving me the opportunity to excel at being a research biologist. To my friends, old and new, for the much needed laughter and shoulders to lean on over the years. To my various fishing partners, for reminding me why I first started in the field of fish biology. To all the fish who participated in these studies, for being willing to take a lure. To the future, full of days in the lab and the field. And, finally, to a lifetime of great days on the water when your cast lies out gently, your fly floats correctly, the fish are on the rise, your leader does not break, and dusk turns into night leaving you with the familiar longing for one more cast.

Abstract

The zoological literature is replete with theoretical links between individual physiological condition and fitness, though these links have rarely been demonstrated empirically. The goal of this thesis was to identify relationships between variation in morphology, performance, and physiology and differential individual fitness among smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*M. salmoides*), a pair of species noted for extended, uniparental male care for developing broods. My first studies noted that differences in male bass swimming performance across parental care (PC) were related to specific body shapes. Additionally, I tracked changes in nutritional condition (primarily related to indicators of recent foraging) across PC. Elements of these studies were combined to determine if female bass select certain males based on body shapes indicative of nutritional condition at the time of spawning. While body shape (specifically overall length and body stoutness) were preferred by females, no nutritional indicators were significantly related to female mate choice. Finally, to determine the causes and consequences of voluntary anorexia by males during PC, I performed a set of manipulation experiments aimed at determining the role of the appetite hormone ghrelin in PC. The results of this study showed that voluntary anorexia is maintained through a combination of low levels of appetite hormones across PC likely combined with receptor insensitivity to appetite hormones (as demonstrated through hormonal manipulations). Ultimately, I noted that swimming performance of males that had fed to satiation decreased, likely as a result of the demands of specific dynamic action. As no empirical links between organismal physiology and fitness were noted in these studies, I present a series of recommendations for future studies in the field of evolutionary physiology.

Acknowledgements

As with all modern studies in the field of biology, the projects within this thesis required the assistance of a small army of fellow researchers. First, I wish to thank my co-authors, Alfonso Abizaid, Steven Cooke, Caleb Hasler, and Cory Suski, for providing support and feedback throughout the process of generating manuscripts based on the findings of this thesis. Second, I wish to thank the members of the Cooke Lab who provided assistance in the field (typically in the cold spring waters of multiple Canadian lakes), especially Michelle Caputo, Alison Colotelo, Jake Davis, Cody Dey, Michael Donaldson, Andrew Gingerich, Ashley Graham, Marie Ange Gravel, Caleb Hasler, Constance O'Connor, Amanda O'Toole, Tara Redpath, Rana Sunder, and Lisa Thompson. Finally, I wish to thank my committee membersf Lenore Fahrig and Katie Gilmour, for guidance throughout the course of my thesis research. The research contained within this thesis was funded through NSERC, the Canadian Foundation for Innovation, Carleton University, and the University of Illinois at Urbana Champaign. Additional logistical support was provided by the staff of Queen's University Biological Station, especially Frank Phelan.

Co-Authorship

Chapter 2: Morphological correlates of swimming activity in wild largemouth bass (*Micropterus salmoides*) in their natural environment. K.C. Hanson, C.T. Hasler, C.D. Suski, and S.J. Cooke

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-authors. Specifically, the project was conceived by Hanson, Hasler, and Cooke. Telemetry and field work was conducted by Hanson and Hasler. Telemetry data processing was performed by Hanson and Hasler. All data analysis was conducted by Hanson. Data were interpreted by Hanson, Hasler, Suski and Cooke. All writing was conducted by Hanson. All coauthors provided comments and feedback on the manuscript. The manuscript has been published with the following citation:

Hanson, K.C., C.T. Hasler, C.D. Suski, and S.J. Cooke. 2007. Morphological correlates of swimming performance of nest-guarding male largemouth bass (*Micropterus salmoides*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 148:913-920.

Chapter 3: Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus* spp.) relative to stage of offspring development. K.C. Hanson and S.J. Cooke.

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-author. Specifically, the project was conceived by Hanson and Cooke. Field work was conducted by Hanson and Cooke. Data were interpreted by Hanson and Cooke. All writing was conducted by Hanson. All coauthors provided comments and feedback on the manuscript. The manuscript has been published with the following citation:

Hanson, K.C., and S.J. Cooke. Accepted. Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus* spp.) relative to stage of offspring development. Journal of Comparative Physiology B. 00:00-00.

Chapter 4: Why does size matter? A test of the benefits of female mate choice in a teleost fish based on morphological and physiological indicators of male quality. K.C. Hanson and S.J. Cooke

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-author. Specifically, the project was conceived by Hanson and Cooke. Field work was conducted by Hanson and Cooke. Data were interpreted by Hanson and Cooke. All writing was conducted by Hanson. All coauthors provided comments and feedback on the manuscript. The manuscript has been accepted for publication at *Physiological and Biochemical Zoology*.

Chapter 5: Causes and consequences of voluntary anorexia during the parental care period of male smallmouth bass (*Micropterus dolomieu*) in relation to brood development. K.C. Hanson, A. Abizaid, and S.J. Cooke

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-authors. Specifically, the project was conceived by Hanson, Abizaid and Cooke. Field work was conducted by Hanson and Cooke. Laboratory analyses were conducted by Abizaid. Data were interpreted by Hanson, Abizaid, and Cooke. All writing was conducted by Hanson. All coauthors provided comments and feedback on the manuscript. The manuscript is in preparation for submission to the Journal of Experimental Biology.

Chapter 6: The relationship between individual physiological traits and fitness in wild fish: Myth or reality? K.C. Hanson and S.J. Cooke

While this study is my own, the work is a collaborative effort and required the valuable assistance of my co-author. Specifically, the project was conceived by Hanson and Cooke. All writing was conducted by Hanson. All coauthors provided comments and feedback on the manuscript. The manuscript is in been accepted for publication at the Journal of Fish Biology.

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Chapter 1: General Introduction

Between 1831 and 1836, Charles Darwin served as the naturalist on H.M.S. Beagle and, during the ship's circumnavigation of the globe, chronicled the diversity of the flora and fauna that was encountered thereby setting the framework for one of the most important and unifying theories in biological science. Darwin (1895) observed that variation in traits will be acted upon by natural selection in which beneficial adaptations lead to increased reproductive success (fitness) for individuals which express those traits, ultimately, providing the opportunity for evolution to occur. Darwin (1895) proposed that natural selection was the driving force behind speciation around the globe, an assertion that is widely accepted within the scientific community, though at times this remains quite controversial amongst the general public. Since the genesis of the theory of evolution in the work of Darwin (1895), the field of evolutionary biology has primarily focused on the relationships between individual fitness and morphological, behavioural, and life history traits (Endler, 1986; Lessells, 1991).

Relationship between Morphology and Fitness

Indeed, Darwin's first proposed examples of adaptive radiation of a species related to specific interactions between environmental factors and morphological variation which lead to fitness advantages for some individuals and eventually adaptive radiation into separate species (Darwin, 1895). Of the many examples from this work, perhaps the most well known is that of "Darwin's finches", a group of related species of Passerine birds that, while similar in overall appearance, displayed striking differences in beak morphology indicating that there were up to 14 separate species present in the Galapagos islands (Darwin, 1859). As such, it was proposed that natural selection in the form of annual changes in forage type and abundance acted upon variation in beak morphology in an ancestral form of the finches, leading to adaptive radiation of the multiple extant species (Darwin, 1859). Since this seminal work, the concept that natural selection on various traits leading to fitness

differences among individuals has become widely accepted in the biological sciences and has formed the foundation for the modern fields of evolutionary biology and ecology.

Relationship between Performance and Fitness

In the early 1980's, researchers focused on discerning a paradigm by which to organismal ecological performance is the functional link between individual fitness and morphological traits (Arnold, 1983). Adoption of the paradigm advocated by Arnold (1983) (Figure 1.1) has resulted in multiple research studies focused on determining the fitness value of morphological traits through the relationship to variation in organismal performance (Kingsolver et al., 2001; Irschick, 2003). In particular, much of this research has focused on the relationships between locomotory performance and individual fitness primarily in herpetofauna (Irschick, 2003). In a stellar example of this type of research, sprint speed of collared lizards (*Crotaphytus collaris*) was found to be under heavy sexual selection only in males due to the fact that increased sprint speeds allowed individuals to defend larger home ranges (Peterson and Husak, 2006). In turn, defending a larger home range would increase the number of females encountered by a male, thereby increasing individual fitness (Peterson and Husak, 2006). Both of these studies represent classic examples of how the proximate questions dealing with how traits influence performance have been well researched, though the ultimate question of how performance influences fitness has rarely been evaluated, particularly outside of the laboratory (Mayr, 1961; Irshick, 2003; Peterson and Husak, 2006; Husak and Fox, 2008).

Relationship between Behaviour and Fitness

As a natural extension of the abovementioned research, evolutionary biologists have also focused on measuring the influence of natural selection on individual behavioural metrics. In general, this work functions on the belief that natural selection acts upon individual differences in adapted behaviours, which in turn is comprised by variation in individual phenotypic (morphological or

physiological) traits (Arnold, 1983). As such, it should be possible to measure the relative fitness of individual behaviour through laboratory and field techniques (Arnold, 1983). Similarly, the extant variation in phenotypic traits provides the raw material upon which natural selection can act (Arnold, 1983; Kingsolver et al., 2001). Individual variation in complex behaviours such as territory acquisition/defense (Maguire, 2006; Husak and Fox, 2008), mate choice (Wong and Condolin, 2005; Barbosa and Magurran, 2006), parental care (Weber and Olsson, 2008), brood parasitism (Gross, 1979; Lyon and Eadie. 2008) and dispersal (Doligez and Part, 2008) have all been linked to individual fitness of individuals within a population of various species.

Relationship between Physiology and Fitness

Similar to the abovementioned examples, the concept of evolutionary physiology (see Garland and Carter, 1994; Feder et al., 2000) is originally rooted in the work of Charles Darwin. Evolutionary physiologists have noted that there are existing patterns in variation of physiological traits and evidence for the heritability of these traits (Feder, 1987, Spicer and Gaston, 1999). As such, the raw material for natural selection is present within populations, which has led many researchers to infer that natural selection should and does act upon physiological traits (Feder, 1987; Spicer and Gaston, 1999). Within this framework, it has been theorized that, since natural selection acts upon the whole organism traits such as morphology, behaviour or performance, variation in related microscale physiological traits should be also be reflected in differential fitness between individuals. While this argument is logically sound and has been put forth many times in the literature, there is a paucity of studies that have empirically tested the relationship between individual physiological variation and individual fitness (Endler, 1986; Feder, 1987; Feder, 2000). Rather, most often, the relationships have been inferred from data (Spicer and Gaston, 1999; Feder, 2000; Irschick, 2003). As such, there have been repeated calls for research projects aimed at providing stringent empirical evidence for a relationship between physiological diversity and individual fitness (Feder, 1987; Spicer and Gaston, 1999; Feder, 2000).

Given this framework, the goal of this thesis work was to use innovative field techniques to investigate both correlative and causal relationships between physiological parameters and individual fitness in wild teleost fishes. In particular, this work will focus on the uniparental care period of male black bass (*Micropterus* spp.) for two reasons. First, failure to successfully maintain parental behaviours can lead to brood abandonment by the male and no individual fitness for the season. Second, the reproductive period in black bass is noted for being extremely energetically and nutritionally demanding for individual males and variation in individual energetic and nutritional status varies between individuals throughout parental care.

Model Species

For the purpose of this thesis, two species of temperate, teleost fish (largemouth bass [*Micropterus salmoides*] and smallmouth bass [*M. dolomieu*]) were chosen as models to investigate the relationships between behaviour, morphology, physiology and variation in individual fitness. These species make interesting models for a number of reasons. First, both species have wide native ranges covering extensive portions of North America (Scott and Crossman, 1973) and have been introduced throughout the world (Robbins and MacCrimmon, 1974; ISSG, 1999). Within their ranges, both species are extremely economically valuable as sport fish, generating millions of dollars in revenue (U.S. Fish and Wildlife Service, 2002).

Of most importance to this research, both species exhibit a life history trait wherein male fish provide extended parental care for the developing brood. Though there are differences in spawning habitat and thermal preferences, in general both species behave quite similarly with respect to parental care. In spring following the melting of winter ice cover, when water temperatures reach ~15°C, male bass move to the littoral zone of lakes, select territories, and construct nests (saucer like depressions in the substrate) as the site of later courtship, egg deposition, and parental care (Kramer and Smith, 1962; Ridgway, 1988). Females move into the littoral zone, choose a spawning partner, deposit eggs in the

nest, and depart the area with no further parental involvement (Kramer and Smith, 1962; Coble, 1975). Males then provide parental care in the form of brood maintenance (fanning the eggs or fry to prevent smothering through silt deposition) and defense from potential predators (typically small bodied fish) that can last up to month as the brood develops to independence (Ridgway, 1988). This time period is quite energetically costly to the male as foraging typically ceases (Hinch and Collins, 1991; Mackereth et al., 1999) concomitant with hyper activity associated with brood defense and maintenance (Cooke et al., 2002). As a result, males are required to power all parental activities through endogenous energy stores accumulated during the previous fall (with possible supplementation during the winter and the brief period following ice melt but preceding the spawn (Mackereth et al., 1999). Collectively, the parental care period offers researchers unique insight into the relationships between morphology, behaviour, performance and fitness during a time period where a male bass participates in a series of behaviours (courtship, spawning, brood defense) powered through a finite reserve of endogenous energy to secure a successful reproductive event (and subsequent fitness).

Goals

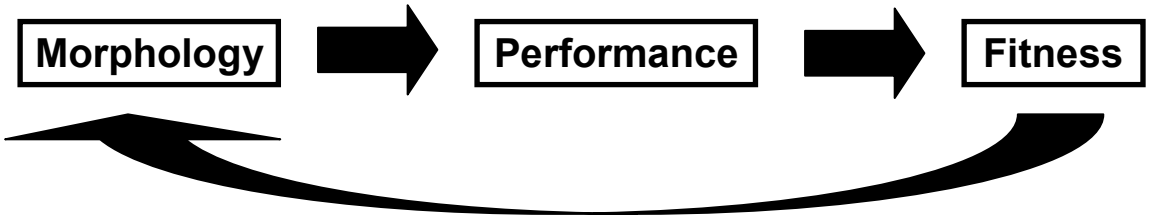
This thesis will follow the history of the field of evolutionary physiology. Chapter 2 will deal with the relationship between organismal performance (swimming performance) of wild largemouth bass during the parental care period. Chapter 3 will provide insight into the variation in nutritional factors across the parental care period in smallmouth bass as well as provide a validation of field physiological tools to be used in later studies. Chapter 4 will link physiological variation in nutritional factors to variation in a behaviour (female mate choice) that relates to fitness differentials between individual males. Chapter 5 will investigate the causal relationship between a single physiological factor (an appetite hormone) and a parental behaviour (voluntary anorexia) known to affect the nutritional status of an individual male, and, subsequently, individual fitness. Finally, Chapter 6 will

125 revisit the history of evolutionary physiology and highlight some of the limitations of current research,

126 methods to overcome these limitations, and future directions for research in this field.

Figure

Figure 1.1: Theoretical framework outlining how variation in individual morphological traits leads to individual fitness differences among individuals within a population (adapted from Arnold 1983).



Chapter 2: Morphological correlates of swimming activity in wild largemouth bass (*Micropterus salmoides)* in their natural environment

Abstract

Individual variation in morphology has been linked to organismal performance in numerous taxa. Recently, the relationship between functional morphology and swimming performance in teleost fishes has been studied in laboratory experiments. In this study, we evaluate the relationship between morphology and swimming activity of wild largemouth bass (*Micropterus salmoides*) during the reproductive period, providing the first data derived on free-swimming fish not exposed to forced swim trials in the laboratory. Sixteen male largemouth bass were angled from their nests, telemetered, and subsequently monitored by a whole-lake acoustic hydrophone array with sub-meter accuracy. Additionally, eleven morphological measurements were taken from digital images of each fish. A principal components analysis of the morphological measurements described 79.8% of the variance. PC1 was characterized by measures of overall body stoutness, PC2 was characterized by measures of the length and depth of the caudal region, and PC3 characterized individuals with relatively large anterior portions of the body and relatively small caudal areas. Of these variables, only PC3 showed significant relationships to swimming activity throughout the parental care period. PC3 was negatively correlated with multiple measures of swimming activity across the parental care period. Furthermore, swimming performance of individual male bass was noted to be repeatable across the parental care period indicating that this phenomenon extends beyond the laboratory.

Introduction

As a behaviour, locomotion is required for survival by most animal species (Ricklefs and Miles, 1994; Domenici and Blake, 1997; Plaut, 2001; Vincent et al., 2005; Husak, 2006), and individual variation in locomotory performance is often correlated with individual variation in morphological characteristics in a variety of taxa (Garland, 1984; Brana, 2003; Fitzpatrick et al., 2005; Husak, 2006). In fish, functional morphology has been shown to relate to variation in the swimming ability of individuals (Kolok, 1992a; Pettersson and Hedenstrom, 2000; Boily and Magnan, 2002; Standen et al., 2002; Lauder and Drucker, 2004; Fisher et al., 2005; Blake et al., 2005; Ohlberger et al., 2006). It has been postulated that increased swimming ability associated with morphological differences may be advantageous in many situations such as predator prey interactions, arduous migrations, defending territories or offspring, and habitat use (Fuiman, 1994; Wintzer and Motta, 2005; Gibb et al., 2006; Ohlberger et al., 2006). Unfortunately, most assessments of the relationship between swimming performance and morphology have been confined to the laboratory partially due to the difficulty of accurately quantifying swimming ability in the wild (Hawkins and Quinn, 1996; Farrell et al., 1998; Martínez et al., 2001; Ojanguren and Brana, 2003; Lee et al., 2003; Macnutt et al., 2006).

Recent advances in biotelemetry have allowed researchers to record movements of animals in the field with fine resolution, especially in the aquatic environment (Lucas and Baras, 2000; Cooke et al., 2004b; Cooke et al., 2004c). The advent of three dimensional acoustic positioning systems have allowed researchers unprecedented capabilities to monitor the behaviour of wild individuals over extended time periods (Lucas and Baras, 2000; Cooke et al., 2004b; Cooke et al., 2004c; Cooke et al., 2005b). Transmitters can be positioned with sub-meter accuracy every few seconds (Niezgoda et al., 2002; Cooke et al., 2005b), and currently the use of these systems is limited to a handful of locations around the world (Niezgoda et al., 2002; Cooke et al., 2005b). One such telemetry array has been used to monitor the behaviour of largemouth bass (*Micropterus salmoides*) year round in a Canadian lake

(Cooke et al., 2005b), and provides a unique opportunity to assess fish morphology and performance relationships in the wild.

This study aimed to relate fish morphology to swimming activity of largemouth bass. For several reasons that will become apparent, we focused on the reproductive period. When water temperatures reach 14°C in spring, male largemouth bass move to the littoral zone and construct nests (saucer shaped depressions in the substrate) in which egg deposition and fertilization occur (Kramer and Smith, 1962). After spawning, the male largemouth bass becomes sole parental care giver by actively guarding the nest from possible brood predators as well as fanning the brood to provide proper oxygenation and prevent sedimentation (Kramer and Smith, 1962). To successfully raise the brood, these male largemouth bass will continue to provide parental care until the brood becomes independent, which can often require one month (Kramer and Smith, 1962; Ridgway, 1988). The parental care period is recognized as one of the most stressful and energetically costly times of a male bass's life due to the fact that the male is extremely active making movements in a localized area above and adjacent to the nest (Cooke et al., 2002) and can not forage normally to replenish energy lost in said movements (Hinch and Collins, 1991; Mackereth et al., 1999; Cooke et al. 2002). As such, we believed that individual variation in morphology as it related to locomotory performance as well as overall body condition would affect the swimming ability of a male largemouth bass during the reproductive period. Individuals characterized by morphometric measures that correlated with improved hydrodynamics and increased propulsion were expected to exhibit higher swimming speeds than other fish. Also, due to the energetic constraints during the parental care period, it was expected that individuals that were characterized by morphology that indicated increased body condition and pre spawn energy stores would be more active than others. Also, we predicted that largemouth bass swimming behaviour in the wild would be repeatable throughout the parental care period as has been noted in laboratory studies on this species (Kolok, 1992b).

Methods

Study Site

This study was carried out from May 1st to June 5th, 2005 on Warner Lake, eastern Ontario (44°31'N, 76°20'W). Warner Lake is an 8.3 hectare research lake wholly enclosed on Queen's University Biological Station (QUBS) property and is the site of a telemetric ecological observatory. The lake shoreline is characterized by extensive littoral zone featuring fallen timber and some submergent and emergent macrophyte growth. Further details on the lake structure and community can be found in Suski (2000) and Hanson et al., (2007). The backbone of the ecological observatory is a fixed underwater acoustic telemetry array, and system details can be found in Niezgoda et al., (2002) and Hanson et al. (2007). Briefly, 13 permanently moored hydrophones configured in optimal geometry monitor telemetered fish movements throughout the lake. Hydrophones are connected to two on shore, multi-port MAP_600 (Lotek Wireless Inc.) receivers through fixed cabling. The system relies upon code division multiple access (CDMA) technology that encodes data transmitted from tags and allows for sub-meter positioning due to the elimination of signal collision events and subsequent data loss. Sub-meter positioning of transmission events results from previous differential GPS surveys (±0.2 m) of hydrophone locations (Niezgoda et al., 2002). Positions calculated with as few as four hydrophones show sub-meter accuracy within the footprint of the array and accuracy of greater than one meter outside of the footprint. As more hydrophones are added to each position solution, error significantly decreases (Niezgoda et al., 2002). Received data are stored on flash cards on site and later transferred to a personal computer for processing.

Study Animals

Starting on May 9th, 2004, snorkel surveys of the littoral zone were conducted daily to locate largemouth bass that were actively guarding nests. Upon locating an active bass nest (one that contained a guarding male and eggs), the snorkeler placed a numbered PVC tile near the nest and

recorded nest location, nest depth, and number of eggs within the nest (visual, categorical assessment ranging from low of 1 to high of 5; Suski and Philipp 2004). A total of 16 males, each located guarding 1-day-old eggs, were used in this study. These fish were collected by angling the day after original location of the nest. Each fish was briefly angled (< 10 sec) from the nest and placed in a cooler of fresh lake water. Individuals were then removed from the cooler and held flat on a spatially referenced tray and digitally photographed (Sony DSC-P1, 3.3 megapixel) from 1m directly above. Fish were also measured for total length (mean ± SD, 415.7 ± 33.0mm, range, 320-447mm) and gape (to the nearest mm measured by opening the mouth with calipers) before being returned to the cooler. Subsequently, fish were placed in a foam lined surgery trough that was filled with fresh lake water for transmitter attachment. Acoustic transmitters (Model CTP-M11-25, 11mm x 25mm, mass 23.9g, signal transmission rate 2.5 seconds, Lotek Wireless Inc.) were externally attached to the nesting largemouth bass by a wire passed through the dorsal musculature (approximately 2mm below the dorsal fin) using two hypodermic needles (21 gauge, 1.5") (Cooke, 2003). Applied transmitters weighed less than 2-3% of the body weight so as to avoid an effect of the tag on individual behaviour (Winter 1983; Brown et al., 1999). A rubber backing plate was positioned on the opposite side of the fish to prevent injury from the wire. Fish were then released within 5m of the nest. The total amount of time required for both surgery and the capture of a digital image was less than 2 minutes. After release, fish movements were remotely monitored by the abovementioned acoustic telemetry array, and daily snorkeling surveys determined if the fish was present on the nest. Data recording was terminated when an individual left the nest area as a result of successfully raising a brood or abandoning the nest.

Data Processing and Analysis

Data processing details may be found in Niezgoda et al. (2002) and Hanson et al. (2007). Briefly, raw positional data were loaded into the program BioMAP (v. 2.1.12.1; Lotek Inc.) and then subjected to an internal two dimensional positioning engine. Fish position estimates computed by the

telemetry equipment will have a precision level dictated by hydrophone geometry, fish tag location and the underlying temporal resolution of the receiver (Niezgoda et al., 2002). Invariably, estimate records will also contain spurious outliers that are artifacts of signal measurement, propagation anomalies and the mechanics of position estimation. To prepare data for further analysis two treatments are applied to position estimate records. The first treatment identifies and removes outliers based on a statistical outlier removal technique that separates samples on the basis of significance with respect to the underlying trend (Coifman and Wickerhauser, 1995). The second treatment smoothes the trajectory of position estimates by means of an adaptive trend filter (Wakeling et al., 2002). Information on the movements of each individual across the parental care period was determined by querying the fully filtered data set for each day the individual was present guarding the nest on a daily basis. Measures of daily maximum swim speed and daily distance traveled were calculated for each day the individual was on the nest (day defined as starting with the point to 00:00 hrs and ending with the closest signal to 23:59:59 hrs). Two dimensional distances between successive XY positions were calculated (with the assumption that the fish maintained constant depth) and summed across the day to determine the daily distance traveled. Subsequently, the mean daily distance traveled (a metric describing the amount of voluntary activity per day) was determined for each individual across the parental care period and used in analysis. Also, the maximum daily swim speed (defined as the fastest rate of travel between two successive XY positions) was calculated for each individual as a metric describing burst swimming behaviour associated with chasing off potential brood predators. Again, the mean swim speed was calculated by individual for further analysis as a measurement of voluntary swimming speed. Unfortunately, no field derived metric analogous to critical swimming speed could be constructed from the available data.

Additionally, to analyze data on a finer scale, analyses were carried out on positional data gathered on the fourth day of parental care for each fish. At this time period, broods have developed from eggs to egg sac fry and this transition has been noted as a time where largemouth bass are highly

active (Cooke et al., 2002). Also, we standardized the behaviour of males with respect to parental investment by testing a specific day during the nest guarding period that is related to brood development. These analyses were also performed due to the fact that individual fish guarded their broods for various time periods ranging from 4 to 25 days. For this day, maximum swimming speed and distance traveled were calculated by the methods stated above for each individual. Also, mean swimming speed was determined as the average of all instantaneous swimming speeds calculated across the day for each fish.

Digital images of individuals were measured for a suite of morphological characteristics (Fig. 2.1) using the program ImageJ (Abramoff et al., 2004). The following dimensions, modified from Hawkins and Quinn (1996), were measured: head depth 1 (HD1); head depth 2 (HD2); body depth at posterior aspect of the dorsal fin (PELVDF); origin of the pelvic fin to posterior aspect of the soft dorsal fin (PELVSD); origin of the anal fin to posterior aspect of the soft dorsal fin (ANSD); origin of the anal fin to the top of caudal flexure (ANC1); insertion of the anal fin to bottom of the caudal flexure (ANC2); posterior aspect of the soft dorsal fin to top of the caudal flexure (SDC1); posterior aspect of the soft dorsal fin to bottom of the caudal flexure (SDC2); and the caudal flexure depth (CFD). Morphological measures were resolved to the nearest millimeter. Additionally, gape and total length (measured at the time of capture) were used in subsequent analysis.

Statistical Analysis

All analyses were performed in the statistical package JMP IN v 4.0 and the level of significance for all tests (α) was assessed at 0.05 (Zar, 1999). All values presented represent means ± S.D. unless otherwise noted. To determine if size played a role in swimming performance during the spawning period, least squares linear regressions of total length by mean maximum swimming speed and mean daily distance traveled were performed (Ojanguran and Brana, 2003). To remove the possible effects of allometric growth on morphological measurements, the residuals of the least squares

linear regression of log transformed traits on log transformed fish lengths were used in subsequent principal components analysis (Tabachnick and Fidell, 1989; Hawkins and Quinn, 1996; Ojanguren and Brana, 2003). The Kaiser-Guttman criteria (or latent root criteria) was used to determine which principal factors would be retained for later analysis (Kaiser, 1960). Principal factors with eigenvalue scores of greater than 1 were subsequently used to determine the relationship between morphology and swimming behaviour (Kaiser, 1960). Least squares linear regression between principal factors and both mean maximum swimming speed and mean daily distance traveled was then performed (Hawkins and Quinn, 1996; Ojanguren and Brana, 2003). *Post-hoc* power analyses were conducted using the observed effect size and variance to determine the power of each regression as well as the least number of samples required to determine significant differences given these effect sizes, and are presented with P-values to aid in data interpretation (Thomas, 1997).

The repeatability of swimming performance (both maximum swimming speed and daily distance traveled) of individuals across the parental care period was tested by conducting Spearman's coefficient of rank correlation tests on measures of swimming behaviour from the first full day of monitoring and the last full day of monitoring (Kolok, 1992b; Zar, 1999). If there was significant correlation between the rank order of individual swimming behaviours across the parental care period, this performance was repeatable. To aid in data interpretation, *post hoc* power analyses were conducted using observed effect size and variance and using predetermined effect size (5%) and the observed variance (Thomas, 1997).

Results

Entire reproductive period

There was no relationship between size and swimming activity metrics including mean (mean maximum swimming speed $R^2 = 0.0002$, $F_{1,14} = 0.002$, $P = 0.9639$) across the parental care period in nest guarding male bass, as revealed by linear regression (Table 2.1). However, principal components

analysis produced three factors describing 79.8% of the variance in the morphological variables surveyed in this study (Table 2.2). Principal component 1 (PC 1) was characterized by high positive factor loadings for HSD2, PELVDF, PELVSD, ANSD, ANC1, ANC2 and CFD (Table 2.2), representing overall body stoutness and accounting for 51.1% of the variance. SDC1 and SDC2 had high positive factor loadings for principal component 2 (PC 2) (Table 2.2). This factor accounted for 18.6% of the variance and mainly described the length and depth of the caudal region and potential for propulsion ability. Lastly, principal component three accounted for 10.1% of the variance and described stoutness of the anterior portion of the fish (high positive factor loadings for HD1, HD2, and gape) and skinniness in the posterior portion of the fish (high negative factor loadings for ANC2 and CFD) (Table 2.2). Of the principal components formulated, only PC 3 explained significant proportions of the variation in swimming performance of largemouth bass across the parental care period (Table 3). PC 3 was negatively correlated with mean daily distance traveled (R^2= 0.330, $F_{1,14}$ ratio = 6.883, P = 0.020; Table 2.3, Fig. 2.2). The other two principal components did not statistically correlate with swimming performance during the parental care period, though statistical power was generally low ($1 - \beta < 0.70$), suggesting that larger sample sizes would be needed to find significant differences (Tables 2.3). Across the parental care period, maximum daily swimming speed was found to be repeatable by individual bass (Spearman's rho = 0.570, P = 0.0213; Fig. 2.3), but daily distance traveled was not (Spearman's rho = 0.131, P = 0.6287).

Egg sac fry stage

To determine the proportion of variation associated with differences in morphology, the three principal components were regressed against maximum swimming speed, mean swimming speed, and distance traveled of each individual on the fourth day of nesting (when broods had developed from eggs to the egg sac fry) (Table 2.4). PC 3 was negatively correlated with mean swimming speed at this stage of brood development (R^2= 0.410, $F_{1,14}$ ratio = 9.738, P = 0.0075) (Table 2.4, Fig. 2.4A). Also,

PC 3 was negatively correlated with distance traveled on this day (R^2= 0.329, $F_{1,14}$ ratio = 6.826, P = 0.0201) (Table 2.4, Fig. 2.4B). Again, no statistically significant correlations between the other two principal components and swimming performance on the fourth day of nest guarding, though it should be noted that statistical power was generally low ($1 - \beta < 0.70$), suggesting that larger sample sizes would be needed to find significant differences (Tables 4).

Discussion

Though there is an abundance of literature relating to the relationships between morphology and swimming performance (generally defined as sprint performance or endurance), to date, there is a lack of information regarding the similar relationships between voluntary activity (similar to the measures of this study) and functional morphology. Unfortunately, it is currently impossible to construct a metric of field based activity that is analogous to laboratory based sprint or critical swimming speeds. However, recent research has noted that individual variation in sprint performance is positively correlated with variation in voluntary activity in fish (McDonald et al., 2007). Due to the possible existence of correlation between sprint and voluntary swimming activities, the remainder of this discussion will draw upon literature concerning the relationships between morphology and sprint performance to guide interpretation of the data on voluntary activity generated through this study.

Of all the morphological measurements evaluated in this study, those summarized by PC3 were negatively correlated with swimming activity of largemouth bass during the reproductive period. PC3 was strongly correlated with measures of the larger size of the head and smaller size of the caudal region (Table 2.2). There are several biomechanical interpretations of why this particular suite of morphological characteristics would influence swimming behaviour. First, the negative relationship may be due to simple hydrodynamic inefficiency. A large head would increase the drag experienced by an individual while swimming, thereby slowing the fish (Weihs and Webb, 1983). Similarly, Boily and Magnan (2002) showed that swimming costs were higher for individual yellow perch characterized

by stout body shapes most likely as a result of hydrodynamic drag. Additionally, largemouth bass swim by undulating the body and caudal fin (the subcarangiform swimming mode), ultimately achieving propulsion via lateral movements of the caudal region of the body (Webb, 1993; Johnson et al., 1994). The negative relationship between an undersized caudal region and swimming performance has been shown in multiple teleost fish species (Hawkins and Quinn, 1996; Ojanguren and Brana, 2003). Second, the morphological relationships evidenced by PC3 may relate to the nutritional status of the bass during the spawning period. Studies of the morphology of fish subjected to starvation have repeatedly noted that as an individual fish starves and consumes internal energy stores, overall body shape changes (Gwak et al., 1999; Smith et al., 2006). The resultant body shape is characterized by a large head relative to the posterior end of the individual (Gwak et al., 1999; Smith et al., 2006). As such, it is possible that PC3 is related to the nutritional status of male largemouth bass at the beginning of the spawn. In northern latitudes (where this study was carried out), immediately prior to spawning, bass have spent the winter under ice presumably not feeding and relying on energy stores (Crawshaw, 1984; Mackereth et al., 1999). Immediately following winter, bass enter another time of energy depletion, the reproductive period (Mackereth et al., 1999).

During the parental care period, male largemouth bass are highly active while defending and maintaining their brood, and only forage opportunistically, which can lead to an energy deficit at this time period of an individual's life (Kramer and Smith, 1962; Ridgway, 1988; Hinch and Collins, 1991; Mackereth et al., 1999; Cooke et al., 2002). As such, males are thought to primarily live off internal energy stores while partaking in parental care activities (Hinch and Collins, 1991; Ridgway and Shuter, 1994; Mackereth et al., 1999). Individuals that start the parental care period that have already experienced starvation (as indicated by the body morphology summarized by PC3) would have less energy to expend on care activities and may curtail swimming movements, both in distance traveled as well as in rate of movement, to conserve energy stores for later use. In multiple fish species, starvation disturbs the physiological status of an individual due to the breakdown of muscle tissue and disruption

of proteins associated with locomotor performance (Loughna and Goldspink, 1984; Beardall and Johnston, 1985; van Dijk et al., 2002; Simpkins et al., 2003; Lapointe et al., 2006). In a number of fish species, starvation has been positively correlated with reductions in swimming activity and reduction in activity levels similar to what was seen in this study (Wieser et al., 1992; van Dijk et al., 2002). Additionally, Kolok (1992b) noted that there was a positive correlation between condition index and swimming performance in winter acclimated largemouth bass.

Lastly, studies have provided evidence that there is a genetic basis to swimming performance in fishes that would be unrelated to morphology. In multiple studies, it has been noted that the hierarchies of swimming performance between individuals is generally conserved even in the face of various biotic and abiotic stressors. Swimming performance has been noted to be a heritable trait in fish (Nicoletto, 1995; Garenc et al., 1998) and that performance is imbedded within individual phenotypic variation that can be acted upon by selective pressure (Ghalambhor et al., 2003). Kolok (1992a) found that largemouth bass swimming performance was repeatable over a range of temperatures, indicating that the best swimmers maintained their performance regardless of ambient temperature. Martinez et al. (2001) found that cod (*Gadus morhua*) maintained individual hierarchies of sprint speed even through periods of starvation. Similarly, across this study, the hierarchy of individual swimming behaviour, measured as maximum daily swim speed at the start and end of parental care, was conserved. All of these facts lend credence to the genetic basis of swimming performance in fish.

In summary, we provide some of the first evidence from the wild that morphology is correlated with swimming activity. Also, although well documented in the laboratory, until now little information of the repeatability of swimming behaviour has shown for wild fish. As transmitter technology becomes more advanced, extremely rapid transmission rates (on the order of sub-second) with increased longevity will allow researchers to focus on seasonal variation of swimming behaviour. Coupling this technology with non-lethal physiological sampling will also allow researchers to couple field based estimates of swimming behaviour with individual differences in physiological and energetic

427 status.

428 **Tables**

429 Table 2.1: Results of simple linear regressions of total length by mean maximum swimming speed,
430 mean daily distance traveled, median swimming speed and median daily distance traveled.

Response Variable	R^2	Parameter Estimate	d.f.	F-ratio	*P*	Observed Power	Least Significant Number
Mean maximum swimming speed (m/s)	0.0002	0.001	1, 14	0.002	0.964	0.050	28946
Mean daily distance traveled (m)	0.024	4.07	1, 14	0.339	0.570	0.085	184

431

Table 2.2: Loading of the morphological measurements into three principal factors by principal components analysis (PC 1, PC 2, and PC 3). Variables that contribute maximally to each factor are in bold.

	PC 1	PC 2	PC 3
Eigenvalue	5.622	2.045	1.108
HD1	0.289	0.022	**0.569**
HD2	**0.371**	-0.012	**0.302**
PELDVF	**0.350**	-0.210	0.022
PELVSD	**0.383**	-0.237	-0.163
ANSD	**0.367**	-0.259	-0.192
ANC1	**0.312**	-0.014	-0.078
ANC2	**0.302**	0.262	**-0.358**
SDC1	0.053	**0.592**	0.289
SDC2	0.180	**0.558**	-0.002
CFD	**0.338**	0.257	**-0.346**
Gape	0.194	-0.189	**0.428**
% Variance Explained	51.1	18.6	10.1

436 Table 2.3: Results of simple linear regressions of principal components vs. swimming performance

437 factors.

Factor	Response Variable	R^2	Parameter estimate	d.f.	F-ratio	*P*	Observed Power	Least Significant Number
Mean maximum swimming speed (m/s)	PC 1	0.010	-0.116	1, 14	0.143	0.711	0.064	434
	PC 2	0.157	-0.759	1, 14	2.602	0.129	0.324	26
	PC 3	0.172	-1.079	1, 14	2.899	0.111	0.355	24
Mean daily distance traveled (m)	PC 1	0.089	104.921	1, 14	1.373	0.261	0.194	47
	PC 2	0.010	-56.964	1, 14	0.135	0.718	0.064	457
	PC 3	0.330	-454.163	1, 14	6.883	***0.020***	0.685	12

438

439 Table 2.4: Results of simple linear regressions of principal components vs. maximum swimming speed

440 on the fourth day of nest guarding.

Factor	Response Variable	R^2	Parameter Estimate	d.f.	F ratio	*P*	Observed Power	Least Significant Number
Maximum swimming speed (m/s)	PC 1	0.119	0.223	1, 14	1.883	0.192	0.249	35
	PC 2	0.138	-0.398	1, 14	2.233	0.157	0.286	30
	PC 3	0.026	-0.237	1, 14	0.380	0.547	0.088	164
Mean swimming speed (m/s)	PC 1	0.012	0.002	1, 14	0.177	0.681	0.068	350
	PC 2	0.015	0.003	1, 14	0.212	0.653	0.071	293
	PC 3	0.410	-0.023	1, 14	9.738	***0.008***	0.827	9
Distance traveled (m)	PC 1	0.095	198.602	1, 14	1.476	0.245	0.205	44
	PC 2	0.010	105.621	1, 14	0.139	0.715	0.064	446
	PC 3	0.329	-831.580	1, 14	6.876	***0.020***	0.684	12

Figures

Figure 2.1: Morphological traits measured for use in principal components analysis of the swimming performance of wild largemouth bass in Warner Lake, Ontario. 1. Head depth 1 (HD1), 2. head depth 2 (HD2), 3. body depth at the insertion of the spiny dorsal fin (PELVDF), 4. insertion of the pelvic fin to the posterior aspect of the soft dorsal fin (PELVSD), 5. insertion of the anal fin to the posterior aspect of the soft dorsal fin (ANSD), 6. insertion of the anal fin to the top caudal flexure (ANC1), 7. insertion of the anal fin to the bottom caudal flexure (ANC2), 8. posterior aspect of the soft dorsal fin to the top caudal flexure (SDC1), 9. posterior aspect of the soft dorsal fin to the bottom caudal flexure (SDC2), and 10. caudal flexure depth.

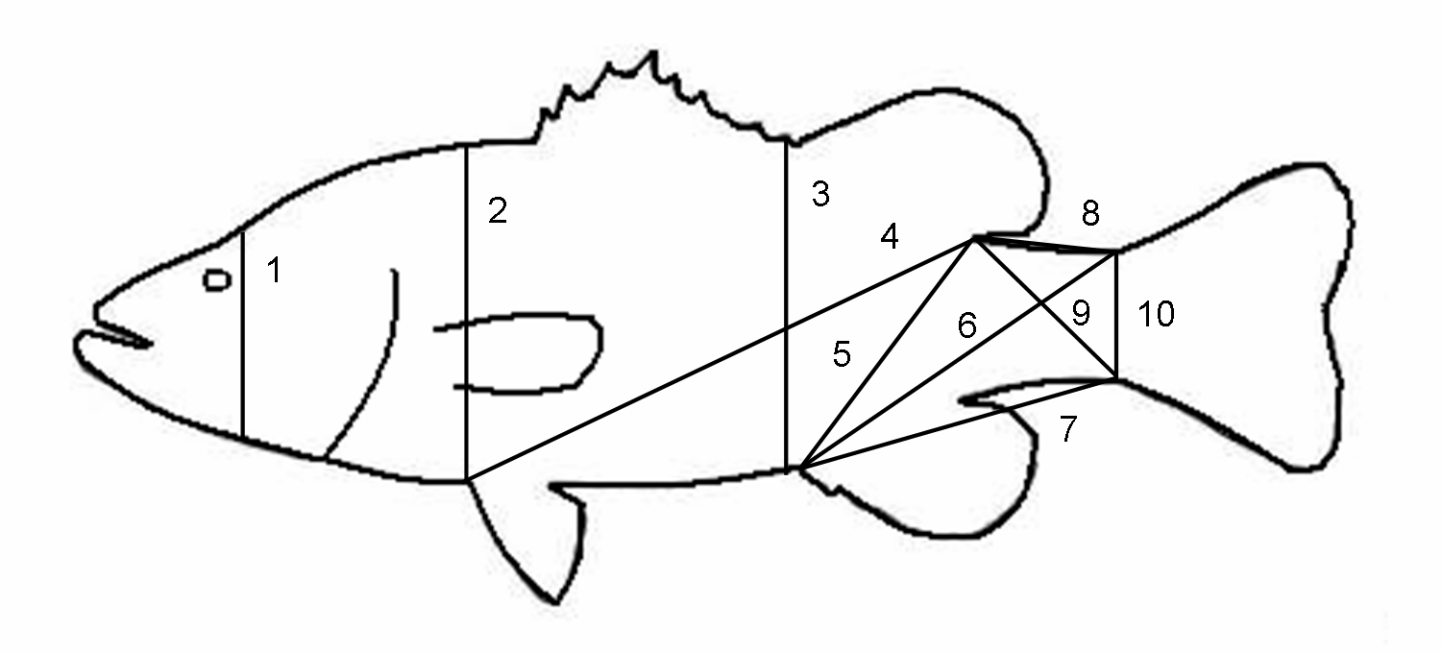
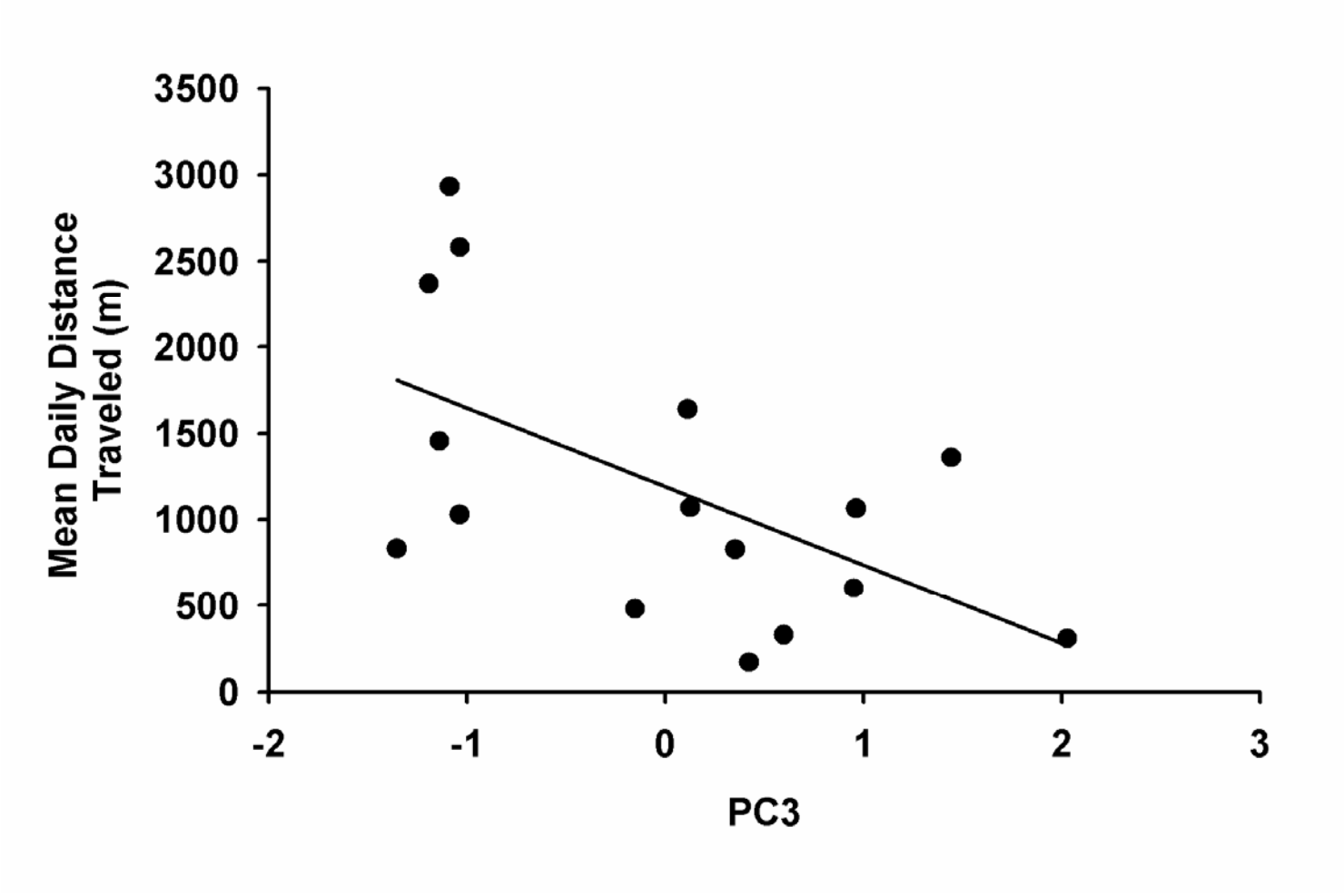


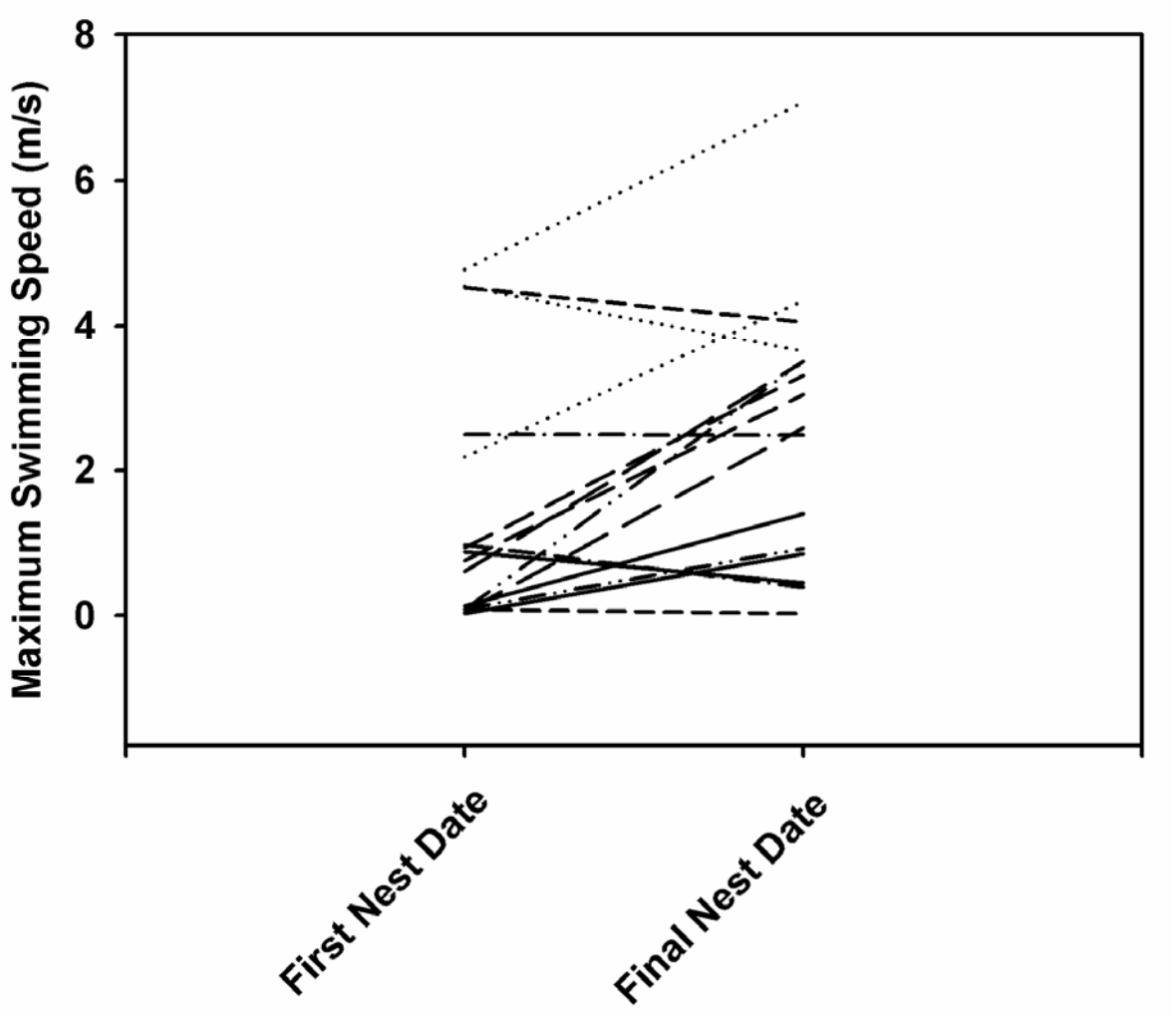
Figure 2.2: Linear regressions of PC 3 by mean daily distance traveled of nest guarding male largemouth bass across the parental care period (R^2= 0.330, $F_{1,14}$ ratio = 6.883, P = 0.020).



454 Figure 2.3: Repeatability of maximum daily swim speed across the parental care period by 16

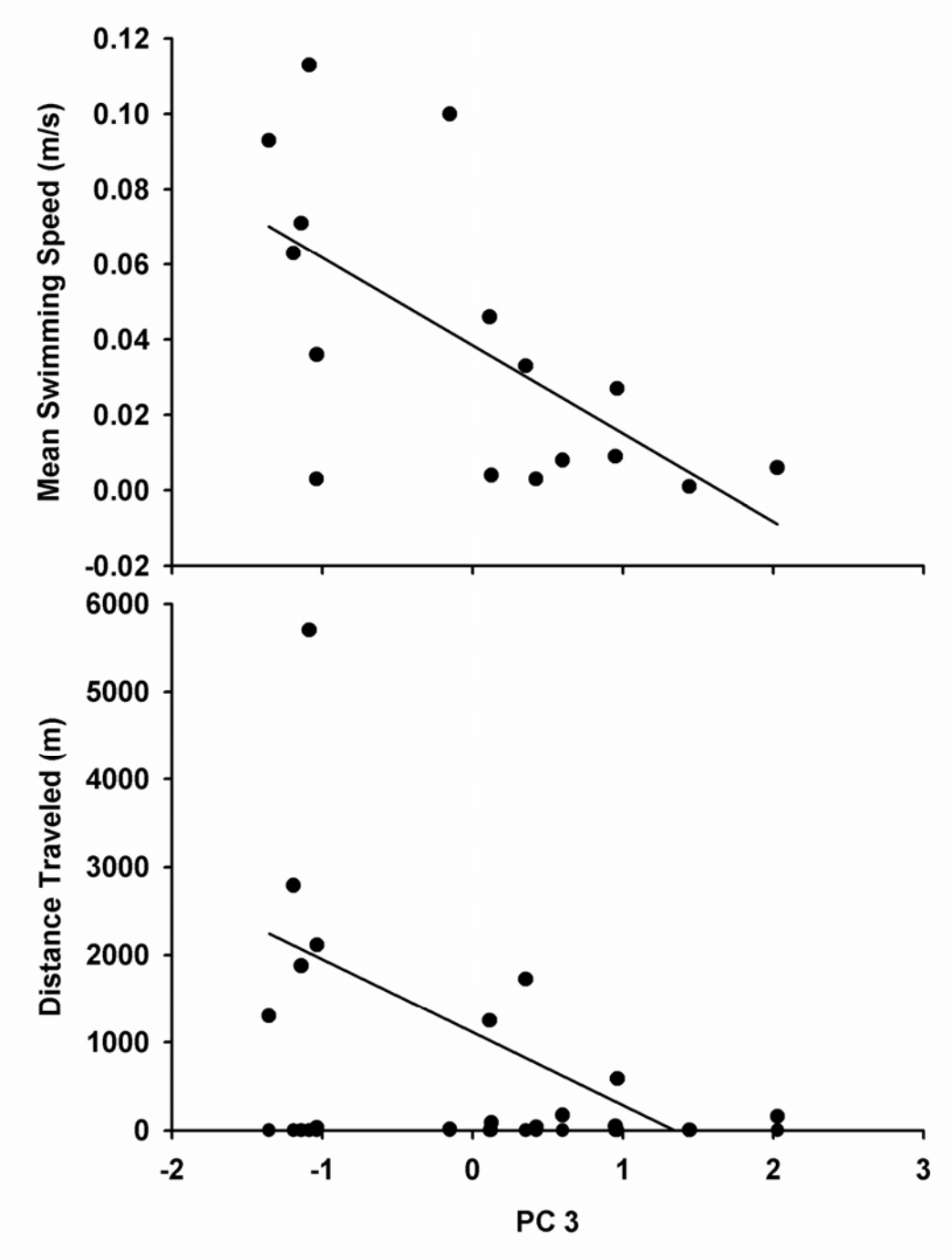
455 largemouth bass in Warner Lake (Spearman's rho = 0.570, $P = 0.0213$).

456



457

458 Figure 2.4: Linear regression of principal component three by measurements of swimming
459 performance on the fourth day of parental care by nest guarding male largemouth bass (A. mean
460 swimming speed [R^2= 0.410, $F_{1,14}$ ratio = 9.738, P = 0.0075], and B. distance traveled [R^2= 0.329, $F_{1,14}$
461 ratio = 6.826, P = 0.0201]).
462



463

Chapter 3: Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus spp*.) relative to stage of offspring development

Abstract

Parental care requires a complex integration of physiology and behaviour, yet little is known about the physiological and energetic consequences or correlates of these behaviours. Using two species of male black bass (smallmouth bass, *Micropterus dolomieu*; largemouth bass, *M. salmoides*) as a model, the focus of this study was to determine the biochemical and hematological indicators of change in nutritional status and potential for chronic stress. This was accomplished by randomly sampling individuals at 4 stages across parental care. Additionally, a subset of individuals was repeatedly sampled at three brood development stages to track changes in biochemical factors within the individual. Though there were changes in physiological factors across parental care in randomly sampled fish of both species (declines in plasma glucose in largemouth bass; decreases in hematocrit and plasma chloride in smallmouth bass), repeated sampling of individuals was determined to be a more appropriate sampling technique due to natural variability in biochemical factors among individual fish. Repeated sampling of smallmouth bass did not adversely influence physiological metrics or brood abandonment. However, there were higher incidences of nest abandonment in repeatedly sampled largemouth bass. Amongst the repeatedly sampled smallmouth bass, nutritional indicators such as plasma triglyceride levels decreased indicating individual fasting across the majority of parental care. Increases in plasma calcium and magnesium towards the end of care indicated that feeding most likely resumed when the brood was close to independence after ~ three weeks of care. Lastly, several indicators of chronic stress, such as plasma glucose and chloride levels, increased throughout the parental care period. These sublethal stressors are indicative of decreasing body condition associated with prolonged activity and fasting which may have marked impacts on the ability of an individual to continue parental care for the current brood and impact subsequent individual fitness. Further research

489 into the mechanistic relationships between behaviour, physiology, and energetics during the parental
490 care period will provide a better understanding of the decisions by individuals facing multiple trade
491 offs that ultimately lead to differences in individual fitness.

Introduction

Several syntheses have explored the links between fitness and morphology, behaviour, and life history (Endler, 1986; Lessells, 1991). However, there is a paucity of research investigating the relationship between individual physiological variation, behaviour, and individual fitness, even though these links have been theorized (Endler, 1986; Feder, 1987; Ricklefs and Wikelski, 2002). Generally, links between physiological variation and fitness have been inferred from data rather than implicitly tested (Spicer and Gaston, 1999). Amongst behaviours, parental care requires a complex integration of physiology and behaviour (mediated by the endocrine system) to secure individual fitness, yet little is known about the physiological consequences of these behaviours. Parental care represents a trade off between multiple interests of the adult providing the care. Adult individuals sacrifice their own health and body condition (Horak et al., 1999; Steinhart et al., 2005), at the risk of mortality (Sabat, 1994), as well as other opportunities to mate (both current and future) to ensure increased survival of offspring and subsequent fitness (Williams, 1966; Gross and Sargent, 1985; Sargent et al., 1987). As the brood develops towards independence and the probability of individual survivorship increases, the care-giving adult should adjust the amount of care given in favor of minimizing current costs to conserve future reproductive opportunities (Williams, 1966; Gross and Sargent, 1985; Gross, 2005).

Parental care, especially uniparental male care, is a widespread reproductive behaviour amongst teleost fishes ranging from simple forms such as concealment of eggs to complex forms such as rearing the brood within the body cavity of an adult or live bearing of young (Gross and Sargent, 1985). While the energetic costs of parental care have been studied for a number of species (e.g., Sargent and Gross, 1986; Coleman and Fischer, 1991; Mackereth et al., 1999), little information is currently available about changes to the physiological status of the adult across the parental care period. The majority of previous work on the physiology of parental care in fishes has focused on the endocrine correlates of paternal care (e.g., Knapp et al., 1999; Páll et al., 2002, 2005; Magee et al., 2006; Rodgers et al., 2006). One study has documented the differences in muscle enzyme activity between parental versus bachelor

517 male fish (Guderley and Guevara, 1998), but to our knowledge no studies have documented variation
518 in nutritional physiology and biochemistry of individual fish across the parental care period.
519 Furthermore, no studies have repeatedly sampled the same fish throughout the parental care period to
520 document changes at the level of the individual, an approach that has the potential to elucidate inter-
521 individual variation.

522 Both largemouth (*Micropterus salmoides*) and smallmouth bass (*M. dolomieu*), collectively
523 termed 'black bass', exhibit extended parental male care. Black bass are an ideal model for the study
524 of the physiology of parental care in the wild because individual fish can be repeatedly captured via
525 angling (to enable tissue sampling), are large enough to enable the collection of tissue samples (relative
526 to many of the smaller-bodied fishes that have been the focus of behaviour-oriented parental care
527 studies; e.g., cichlids, sticklebacks), have been well-studied with respect to parental care behaviour and
528 energetics providing sufficient information to interpret physiological findings, and because their
529 reproductive success can be easily visually quantified. For both species, when water temperatures
530 reach approximately 14°C in spring, male bass move into the littoral zone where nest construction (the
531 digging out of saucer shaped depressions in the substrate), courtship, spawning, and egg deposition and
532 fertilization occur (Kramer and Smith, 1962; Ridgway, 1988). After spawning, the female bass leaves
533 the area of the nest while the male bass initiates parental care in the form of active nest defense from
534 potential brood predators as well as fanning the brood to provide proper oxygenation and prevent
535 sedimentation (Hinch and Collins, 1991). The male bass will continue to participate in parental care
536 activities until the brood becomes independent, which can often require one month (Cooke et al.,
537 2006b).

538 The parental care period of black bass has been noted to be one of the most energetically
539 demanding time periods of an individual's life (Hinch and Collins, 1991; Cooke et al., 2002; Cooke et
540 al., 2006b). While guarding the nest, individuals greatly curtail foraging activities due to the fact that
541 they are unable to leave the brood unattended (Hinch and Collins, 1991). At the same time, nest

guarding male fish are also some of the most active fish in the population as localized movements on and around the nest equate to movements over tens of kilometers per day (Hinch and Collins, 1991; Cooke et al., 2002; Hanson et al., 2007a). Male fish engaged in parental care must rely on endogenous energy reserves to fuel activity during this time (Mackereth et al., 1999). Nest guarding male bass continually move about the nest, executing tight turns to remain above the nest as well as sculling all fins at the same time to remain stationary above the nest while providing oxygenation and preventing silt deposition on the brood (Hinch and Collins, 1991; Cooke et al., 2002). As such, it has been theorized that the combination of reliance on endogenous energy supply with increased energy consumption from nest guarding activities results in a continual decline in the energetic and nutritional status of nest guarding males across the parental care period (Mackereth et al., 1999). Drastic declines in endogenous energy reserves can lead to brood abandonment as the current brood is abandoned to secure future reproductive success (Trivers, 1972; Sargent and Gross, 1986). Additionally, it has been theorized that individual survival through the following winter may be compromised if internal energy reserves are over-utilized (Mackereth et al., 1999).

Using nest-guarding male black bass as a model, the objective of the present study was to determine the nature and magnitude of the energetic and nutritional decline and associated stress physiology across the parental care period through the use of non-lethal sampling. We predicted that blood based indicators of nutritional and energetic status would change as the brood developed and the adult male remained on the nest unable to forage normally and fueling activity through endogenous energy reserves. Additionally, we predicted that patterns in hematology and plasma biochemistry indicative of chronic stress would be evident as parental care progressed. We also tested the utility of repeated blood sampling of individuals across the parental care period. Specifically, we predicted that repeated sampling of individuals would more accurately show the decline of nutritional indicators across parental care than would comparing the means of separate, randomly sampled groups, while not causing detrimental effects to individuals.

Methods

Field Techniques

This study was carried out from May 1st to June 1st, 2006 on Lake Opinicon, eastern Ontario, Canada (44°30'N, 76°20'W). Daily snorkel surveys of the littoral zone were conducted to locate largemouth and smallmouth bass that were actively guarding nests with newly deposited eggs. Upon locating an active bass nest (defined as male guarding newly deposited [< 1 day old] eggs), the snorkeler placed a numbered PVC tile near the nest and recorded nest location, nest depth, and number of eggs within the nest (visual, categorical assessment ranging from low of 1 to high of 5; Suski and Philipp, 2004). At the time of nest discovery, individuals were randomly assigned to sampling groups. Control fish were not handled beyond that as described above to provide a baseline estimate of nest abandonment within the lake for each species. Subsets of individuals were sampled at each of the four brood developmental stages (eggs [sampled within 1 day of spawning], egg sac fry [newly hatched embryos, approximately 1.5 weeks after spawning], swim up fry [larvae begin to swim >0.5m above the nest, approximately two weeks after spawning], and free swimming fry [larvae swim < 1m above and around the nest, prior to independence, approximately three weeks after spawning]). Fish were captured using heavy-action recreational fishing equipment that could be used to angle fish from the boat or underwater (by the diver). In total, 41 largemouth bass (total length mean ± s.d.; 381 ± 40mm) and 50 smallmouth bass (total length mean ± s.d.; 366 ± 38mm) were blood sampled for this study. All fish were landed within 20 sec of hooking to minimize non-parental care related anaerobic exercise. During the entire period that angled fish were held on the boat, they were always in water. Upon capture, fish were quickly blood sampled by the caudal puncture method using a 1.5", 21 gauge vacutainer syringe (Houston 1990) while being held within a foam lined trough containing fresh lake water. Up to 1.5mL of blood (representing approximately 3.7% of total blood volume) was collected in a 3mL, flat-bottomed vacutainer containing lithium heparin to prevent blood coagulation. Total length

592 was recorded as well as the presence or absence of any injury. Individuals were then released within
593 5m of the nest in less than 2 minutes. During the sampling procedure, a snorkeler remained at the nest
594 site and defended the brood until the male returned (typically in under 5 minutes). Blood samples were
595 centrifuged immediately at 10000x gravity for 5 minutes (Clay Adams Compact II Centrifuge).
596 Hematocrit was assessed in the field by measuring the volume of red blood cells by volume of total
597 liquid on centrifuged blood collection tubes using micrometer calipers. Plasma samples were stored in
598 liquid nitrogen for subsequent analysis. Individuals in the last treatment group, repeatedly sampled
599 fish, were sampled at each stage of brood development (with the exception of the swim up fry stage).
600 At the final stage of brood development, due to the fact that fish at this stage roam across large areas
601 and capture by angling becomes ineffective, fish were captured by a snorkeler using a spear gun.
602 Following sampling, fish were euthanized by cerebral percussion. After non-lethal sampling, a
603 snorkeler revisited each nest every 2 days to record presence or absence of the male as well as the
604 progression of the brood through developmental stages.
605
606 *Lab Analyses*
607 Samples were analyzed for concentrations of various biochemical constituents indicative of
608 individual nutritional status (alkaline phosphatase [ALP; enzyme number 3.1.3.1], aspartate
609 transaminase [AST; enzyme number 2.6.1.1], creatine kinase [CK; enzyme number 2.7.3.2], lactate
610 dehydrogenase [LDH; enzyme number 1.1.1.27], total protein, phosphorous, triglycerides, cholesterol,
611 and glucose) as well as ions (Mg^{+}, Ca^{++}, Cl^{-}, Na^{+}, K^{+}) (Wagner and Congleton, 2004; Congleton and
612 Wagner, 2006). In previous work conducted on Pacific salmonids (*Oncorhynchus* spp.) these
613 biochemical constituents have been shown to reflect the short and long term nutritional status of
614 individual fish subjected to fasting or feeding (Wagner and Congleton, 2004; Congleton and Wagner,
615 2006). In particular, we measured variables that have been shown to respond to fasting and feeding
616 activity (ALP, CK, total protein, phosphorous, triglycerides, cholesterol, Mg^{+}, and Ca^{++}; Lall, 2002;

Wagner and Congleton, 2004; Congleton and Wagner, 2006) as well as indicators of tissue damage (AST, LDH; Morrissey et al., 2005), and chronic stress (glucose, Cl^-, Na^+, and K^+; Wendelaar Bonga, 1997; Barton, 2002). All biochemical 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 (performed by laboratory personnel at Vita-Tech, Markham, ON, Canada) followed procedural guidelines for standardization and quality assurance established by the Veterinary Laboratory Association Quality Assurance Program, New York State Department of Health, College of American Pathologists, and the Canadian Food Inspection Agency External Proficiency Panel.

Statistical Analysis

All analyses were performed in the statistical package JMP IN v 4.0 and the level of significance for all tests (α) was assessed at 0.01 to minimize Type I error associated with multiple statistical tests (Zar, 1999). All values presented represent means ± S.E. unless otherwise noted. Normality and heterogeneity of variance of initial physiological data was assessed to determine whether variables needed to be transformed before analysis. Non-normal variables were log-10 transformed prior to subsequent analysis. To determine differences in physiological variables between brood stages, one way ANOVA's followed by Tukey's HSD *post hoc* tests were employed (Zar, 1999). In instances where homogeneity of variance was violated, Welch's ANOVA was utilized (Zar, 1999). To determine the utility of repeated sampling, the means of each physiological variable from the repeated sampling events were compared to the means of randomly sampled fish from the same brood developmental period through paired t-tests (Zar, 1999). Additionally, nest abandonment rates between repeated sampling groups and natural whole lake abandonment were analyzed by Chi Square contingency table analysis (Zar, 1999). Multiple comparisons across proportions were performed to determine significant differences in abandonment rates according to methods in Zar (1999).

Repeatedly sampled fish were analyzed separately, but in a similar manner. Repeated measures ANOVA's (or Welch's ANOVA as described above) and Tukey's HSD *post hoc* tests were used to determine significant differences between sampling periods.

Results

Randomly sampled fish

Overall, very few parameters differed significantly over the course of parental care in both largemouth and smallmouth bass (Tables 3.1, 3.2). For largemouth bass across the parental care period, there were only significant alterations in one blood biochemistry variable. Specifically, blood glucose levels in individuals significantly increased after the egg stage of brood development ($P < 0.01$; Fig. 3.1, Table 3.3). All other physiological variables did not show any differences across brood development (Table 3.3).

In smallmouth bass, changes in the levels of hematocrit and chloride levels were noted across parental care. Hematocrit was highest at the commencement of parental care ($41.69 \pm 2.45\%$) and declined throughout brood development, reaching its lowest level at the free-swimming fry stage ($29.78 \pm 2.30\%$; $P < 0.01$; Fig. 3.2, Table 3.3). Chloride levels followed a pattern in which levels declined to the lowest levels during the free swimming fry stage when compared to the egg and egg sac fry stages ($P < 0.01$; Fig. 3.2, Table 3.3).

Validation of repeated sampling

Due to high levels of brood abandonment, only comparisons between physiological variables in the second repeated sampling event and control fish at the egg sac fry stage could be performed for largemouth bass. Repeatedly sampled largemouth bass showed decreased levels of phosphorous when compared to controls ($P < 0.01$; Fig. 3.3, Table 3.4). No significant differences were detected for other blood biochemistry variables ($P > 0.01$, Table 3.4).

Similarly, at the same stage of brood development, several differences were noted between values from smallmouth bass that were sampled for the second time and control smallmouth bass at the egg sac fry stage. Specifically, smallmouth bass sampled twice showed had decreased levels of magnesium when compared to control fish at the same brood development stage ($P < 0.01$; Fig. 3.3, Table 3.4). No significant differences were detected for other blood biochemistry variables ($P > 0.01$, Table 3.4). When comparing smallmouth bass sampled for a third time to control fish at the free swimming fry stage, no significant differences in values of physiological variables were noted ($P > 0.01$, Table 3.4).

Finally, when compared to natural nest abandonment, repeated sampling was found to increase brood abandonment in largemouth bass ($\chi^2 = 9.31$, d.f. =2, $P < 0.01$; Fig. 3.4). Specifically, brood abandonment amongst repeatedly sampled fish at the third sampling period increased to > 80%, more than double the natural abandonment rate (Fig. 3.4). Though there were statistically significant differences between repeated sampling abandonment rates and natural abandonment rates for smallmouth bass, the final abandonment rate did not increase significantly above control rates for repeatedly sampled fish ($\chi^2 = 25.93$, d.f. = 2, $P < 0.01$; Fig. 3.4).

Repeated Sampling

Between the egg and egg sac fry brood development stages, repeatedly sampled largemouth bass did not vary in physiological parameters (Table 3.5). Due to the increased levels of brood abandonment amongst repeatedly sampled largemouth bass, no statistical analyses could be performed that included fish at the free swimming fry stage. Conversely, smallmouth bass showed differences in multiple physiological parameters. In particular, magnesium levels decreased in the egg sac fry stage as compared to the egg and free swimming fry stages ($P < 0.01$; Fig. 3.5, Table 3.5). Chloride and hematocrit decreased across the parental care period ($P < 0.01$; Fig. 3.5, Table 3.5). Lastly, plasma

691 calcium levels increased between the egg sac fry and free swimming fry stages ($P < 0.01$; Fig. 3.5,
692 Table 3.5).
693
694 **Discussion**
695 Evidence of changing nutritional status across the parental care period at the population level
696 (i.e., randomly sampled fish that were not repeatedly sampled) was difficult to obtain in the current
697 study despite the fact that we predicted such alterations given the high levels of parental care activity
698 (i.e., brood defense and nest aeration; Hinch and Collins, 1991; Cooke et al., 2002, 2006) and reduced
699 foraging activities (Hinch and Collins, 1991; Mackereth et al., 1999). Previous studies have
700 documented large individual variation in biochemical nutritional indicators (Congleton and Wagner,
701 2006). Similarly, in this study, extensive individual variation was noted in the majority of parameters
702 measured (Tables 3.1, 3.2). For example, individual values for the enzyme creatine kinase (in U/L)
703 ranged from a low of 608 for to a high of 15870 within one sampling period (i.e., at the swim up fry
704 stage; Tables 3.1, 3.2), though measurements of the enzymatic variables in the current study (especially
705 LDH and CK) may be influenced by sampling strategy (i.e., blood collection via the caudal vasculature
706 can cause elevations in these parameters; Morrisey et al., 2005). AST is a more reliable metric given
707 this blood sampling approach (Morrisey et al., 2005) and it showed similar patterns. Regardless, with
708 natural variation of this magnitude within the measured biochemical parameters, only large effects
709 could be resolved via statistical testing. Many nutritional changes across the parental care period may
710 not be sufficiently large to be noticed with this degree of background variation. Such variation is
711 common in physiological studies and may be indicative of individual differences in behaviour and
712 fitness and reflective of differences in genotype, environment, or individual health and condition
713 (Bennett, 1987). For example, in the current study, local environmental conditions (water temperature,
714 wave activity, oxygen levels) and nest predator burdens undoubtedly varied from nest to nest, which
715 may have contributed to variation in organismal behaviour and physiological status.

716

717 *Comparison of sampling techniques*

718 In the current study, two separate sampling methods (randomly sampling individuals once at a
719 given brood stage or repeatedly sampling individuals at each brood stage) were employed. Repeated
720 sampling had a negative effect on parental care behaviour in largemouth bass. Largemouth bass
721 subjected to repeated sampling had nest abandonment rates that were approximately 2.5 times higher
722 than the natural abandonment rate for largemouth bass in the lake (Fig. 3.4). Smallmouth bass,
723 however, did not abandon nests at any higher rates than natural nest abandonment (Fig. 3.4). This
724 increased abandonment by largemouth bass relative to smallmouth bass can be attributed to a
725 difference in parental care investment due to egg size and value (Sargent et al., 1987) and is consistent
726 with parental investment and life-history theory (Cooke et al., 2006b). These findings are also
727 consistent with data from catch-and-release studies that reveal that largemouth bass tend to have higher
728 post-angling abandonment rates than smallmouth bass (Hanson et al 2007b). Also, this could reflect
729 interspecific variation in response to stress, though largemouth bass are generally regarded as being
730 less sensitive to hypoxia and stress than smallmouth bass (Furimsky et al., 2003). Due to the higher
731 incidence of nest abandonment of largemouth bass relative to smallmouth bass, repeated sampling of
732 largemouth bass at the free swimming fry stage was impossible.

733 To test the utility of repeatedly sampling fish without having the sampling alter physiological
734 and nutritional condition, we compared the values found for each repeated sampling period to control
735 values determined by singly sampling fish at the analogous brood development stage. The only
736 detectable biochemical differences between repeatedly sampled fish and singly sampled fish occurred
737 at the second sampling period which coincides with the egg sac fry brood stage. Specifically,
738 repeatedly sampled largemouth bass had lower levels of plasma phosphorous than singly sampled fish
739 (Table 3.4; Fig. 3.3), and repeatedly sampled smallmouth bass had lower levels of plasma magnesium
740 than singly sampled fish (Table 3.4; Fig. 3.3), though the reasons for these differences are unclear.

Additionally, there were no differences in any hematology or biochemical parameters between repeatedly sampled smallmouth bass at the third blood sampling period and singly sampled fish at the free swimming fry brood stage (Table 3.4). The lack of differences between repeated and singly sampled fish indicates that repeated sampling does not have a marked effect on the biochemical parameters measured in this study. In our study, between 3 to 7 days elapsed between repeat sampling periods. Another commonly cited explanation for changes in physiological metrics across stages of offspring development is that environmental conditions were variable. However, the only environmental factor that changed modestly across the parental care period was water temperature (increasing ~3°C between the first and last sampling periods). It was not possible to control for this thermal variation, but these temperatures (both the range and absolute values) are all well within the tolerances of both species and would be considered moderate. As such, we will discuss results from both randomly and repeatedly sampled fish together in the context of changes in physiology across parental care.

Indications of fasting and resumption of feeding

We noted several changes in biochemical parameters that indicate that individuals fasted for the beginning portion of parental care and resumed feeding by the time the brood developed into free swimming fry. Plasma triglyceride levels decreased throughout the parental care period, though this result was not statistically significant at $\alpha = 0.01$ (Table 3.5). Currently, research indicates that parental care is powered through endogenous energy reserves, primarily muscle energy stores in the form of lipids (Mackereth et al., 1999). Recent research has indicated that circulating levels of lipids in the blood stream are indicative of nutritional status and internal energy stores of the individual as well as recent feeding activity (Wagner and Congleton, 2004; Congleton and Wagner, 2006; Polakof et al., 2007). Therefore, the decline of plasma triglyceride across the parental care period is an indicator of

765 extended fasting and is consistent with videographic observations for smallmouth bass during nesting
766 (Hinch and Collins, 1991).

767 Additionally, hematocrit levels decreased from the commencement of parental care to the egg
768 sac fry stage and then remained stable through to the end of sampling in both randomly and repeatedly
769 sampled fish (Figs. 3.2, 3.5). Due to the fact that whole blood is being removed from the animal,
770 decreases in hematocrit may be caused by sampling technique. However, the pattern of hematocrit
771 decline within repeatedly sampled fish is consistent with a similar decline amongst singly sampled fish.
772 Since this pattern is conserved through both sampling strategies, we believe that the fluctuations in
773 hematocrit are due to a physiological response to parental care rather than our sampling practices.
774 Consistent with the idea forage intake is markedly decreased during parental care, decreases in
775 hematocrit may be indicative of the use of internal energy stores (rather than exogenous forage) to
776 power parental care activities at the cost of maintaining tissues such as replacing senescent erythrocytes
777 (Rios et al., 2005).

778 Plasma magnesium levels also fluctuated in a manner indicative of fasting in the current study.
779 Interestingly, the pattern of change in plasma magnesium may also be indicative that fasting only
780 occurs during the first two weeks of parental care and normal foraging resumes during the free
781 swimming fry stage (approximately the third week of parental care). Plasma magnesium decreased by
782 0.16 mmol/L (~16% change from the baseline value at the egg stage) at the egg sac fry stage of brood
783 development and then, by the free swimming fry stage, increased back to the levels at the
784 commencement or parental care (Fig. 3.5). Plasma magnesium is also a required mineral for enzymatic
785 processes in teleost fishes and is primarily recruited from dietary sources (Lall, 2002). In fasted
786 salmonids, circulating magnesium levels decreased in response to fasting (Congleton and Wagner,
787 2006) similar to what was seen in the present study. However, the magnitude of change was greater for
788 salmonids. Incidentally, much research has focused on the role of water temperature in influencing
789 circulating magnesium levels, specifically in the fact that low temperatures tend to decrease levels of

790 plasma magnesium within fish (Burton, 1986; Congleton and Wagner, 2006). As bass spawning
791 coincides with increasing water temperatures in the spring (Kramer and Smith, 1962; Ridgway, 1988),
792 decreases in plasma magnesium are more likely to be attributable to the effects of fasting rather than
793 ambient temperature. Additionally, the increase of plasma magnesium at the free swimming fry stage
794 to levels similar to those found at the commencement of parental care are indicative of increased
795 feeding during this time.

796 Besides fluctuations in plasma magnesium levels, other biochemical metrics indicate that bass
797 may resume feeding towards the end of parental care. By the free swimming fry stage in brood
798 development, the fry have moved into a loosely associated group that fans out over a larger area
799 (Friesen and Ridgway, 2000), forcing the male to swim over larger distances to guard the brood and
800 thereby increasing the area over which a male may encounter and consume prey items (Cooke et al.,
801 2002). Circulating calcium levels increased by approximately 0.2 mmol/L (~7%) in parental males by
802 the free swimming fry stage of brood development (Fig. 3.5). In general, most teleost fish satisfy
803 calcium requirements through the absorption of mineralized calcium from dietary sources (Lall 2002),
804 so increases in circulating levels may be due to digestion of forage. Additionally, increases in
805 circulating calcium levels may be bolstered by the response of the organism to long term fasting in
806 which internal reserves of calcium are mobilized to maintain homeostasis (Yamada, 1956; Ikeda et al.,
807 1974; Persson, 1997). Calcium is required for various metabolic functions within the body such as
808 nerve transmission, cell membrane function and integrity, and enzyme activity (Lall 2002) as well as
809 the formation of hard structures such as scales and the skeletal system (which may account for up to
810 95% of body calcium; Berg, 1968; Fleming, 1974; Persson, 1997). Further supporting the idea that
811 feeding resumes by the end of parental care, total protein levels remained relatively consistent across
812 parental care (Table 3.5). In a study of fasting salmonids, Congleton and Wagner (2006) noted that
813 total circulating protein levels decreased dramatically after the first three weeks of fasting. These
814 decreases in circulating plasma protein are thought to be due to the digestion of endogenous proteins

for metabolism when outside sources of protein usually derived from forage are unavailable (Sauer and Haider, 1979; Navarro and Gutiérrez, 1995; Rehulka, 1993; Wagner and Congleton, 2004). In the present study, no decreases in the levels of plasma protein were noted at the free swimming fry stage (roughly three weeks from the onset of parental care), possibly indicative of the resumption of feeding (and intake of exogenous protein) by this time period to preserve homeostasis in the individual.

Indications of chronic stress

Indicators of chronic stress in parental individuals varied considerably as parental care progressed. In randomly sampled largemouth bass, only plasma glucose varied significantly across stages of brood development, representing a 30% increase (0.6 mmol/L) above the baseline values at the egg stage (Fig. 3.1). Increased plasma glucose is among a suite of commonly measured indicators of chronic stress and acute stress in fishes (Wedemeyer et al., 1990; Mommsen et al., 1999; Barton, 2002) as glucose levels increase as energy reserves are mobilized in response to an acute stressor (Wendelaar Bonga, 1997; Barton, 2002). In previous studies of fish nutrition, increases in plasma glucose have been frequently attributed to stress due to handling (Wagner and Congleton, 2004; Congleton and Wagner, 2006) and plasma glucose levels typically peak about one hour after exposure to an acute stressor (Milligan, 1996; Mommsen et al., 1999; Barton, 2002). In the current study, handling effects would not be detected given that fish were sampled within seconds of capture, which is reflected by the fact that glucose levels recorded in this study are within the typical range of lab controls for black bass in previous studies (Suski et al., 2003).

In addition, randomly sampled smallmouth bass showed declines in levels of plasma chloride of ~12mmol/L (representing a 10% decrease from the egg stage by the end of parental care) (Fig 3.2). Decreases in plasma ion concentrations, such as chloride, often reflect hydromineral imbalances that can result in osmoregulatory dysfunction (Mazeaud and Mazeaud, 1981; Barton and Iwama, 1991; McDonald and Milligan, 1997; Wendelaar Bonga, 1997; Wagner and Congleton, 2004). Similar to the

randomly sampled fish mentioned above, plasma chloride decreased by ~17mmol/L (representing a 14% decrease from the egg stage) across the entire parental care period within the group of repeatedly sampled smallmouth bass (Fig. 3.5). There is a possibility that the repeated handling of these individual fish across the approximately three week-long sampling period could account for the stress response noted in the data. We believe this is not the case due to the fact that a similar pattern in decline in plasma chloride was noted in the singly sampled fish, providing support that these changes are a response to the chronic stress associated with parental care. Furthermore, stress associated with recreational fishing practices (i.e., our capture technique), including ionic imbalances, are rectified within hours and certainly within days for black bass (Gustaveson et al., 1991; Suski et al., 2004; Suski et al., 2006). Together, the fluctuations in plasma glucose and ion concentrations suggest that parental care behaviours represent a chronic stress to the individual for the duration of parental care.

Conclusions

In summary, our results have shown that hematology and biochemical factors associated with endogenous energy stores and parental condition vary across parental care. Interestingly, a rise in indicators of feeding at the free swimming fry stage denotes the resumption of feeding as the brood gains independence. Additionally, factors associated with the response to chronic stress increase across parental care. Overall, changes in nutritional status across the parental care period can have marked impacts on individual fitness (Pottinger, 1999). Currently, it is believed that parental care giving male bass largely power brood defense and maintenance behaviours through the use of endogenous energy stores (Mackereth et al., 1999). Many of the biochemical parameters measured in this study reflect either metabolism of these endogenous energy reserves in response to fasting or mobilization of nutrients from ingested food (Congleton and Wagner, 2006). Individuals characterized by nutritional indices that indicate poor relative condition prior to spawning, or increased use of energy reserves during parental care relative to conspecifics, may run the risk of expending energy reserves prior to the

865 independence of the brood. Also, the combined sublethal effects of energy depletion coupled with
866 chronic stress could prove to be lethal to the individual. In such a case, the male should abandon the
867 current brood at a cost of any current fitness to ensure his own survival and future reproductive
868 opportunities in keeping with the William's Principle (Williams, 1996, Sargent and Gross, 1986).
869 Continuing research on parental care behaviour and its underlying physiological and energetic costs
870 and consequences will help to elucidate the links between physiology, behaviour and fitness. This
871 work will afford researchers a better understanding of the tradeoffs encountered by the individual that
872 dictate parental decisions and, ultimately, differences in individual fitness.

873 **Tables**

874 Table 3.1. Physiological variables (mean ± SD with range) measured in largemouth bass treatment groups across this study.

Physiological Variable	Egg	Egg sac fry single sample	Swim up fry single sample	Free swimming fry single sample	Repeated sampling 2 (egg sac fry stage)
ALP (U/L)	20.88 ± 1.53 (17 - 30)	18.18 ± 1.24 (9 - 25)	18.67 ± 0.49 (17 - 20)	24.14 ± 3.32 (5 - 30)	16.38 ± 1.87 (5 - 22)
AST (U/L)	63.13 ± 16.32 (23 - 165)	71.55 ± 9.96 (19 - 120)	81.67 ± 42.96 (21 - 295)	99.71 ± 37.70 (22 - 250)	39.38 ± 6.75 (13 - 78)
Calcium (mmol/L)	2.85 ± 0.07 (2.65 – 3.22)	2.84 ± 0.03 (2.73 – 3.00)	2.93 ± 0.05 (2.75 – 3.06)	2.94 ± 0.10 (2.59 – 3.23)	2.87 ± 0.07 (2.57 – 3.10)
Chloride (mmol/L)	109 ± 3.31 (96 - 116)	112 ± 2.08 (98 - 118)	104 ± 3.43 (93 - 112)	105 ± 3.25 (98 - 116)	106 ± 1.86 (97 - 112)
Cholesterol (mmol/L)	13.08 ± 0.90 (10 – 16.2)	15.49 ± 1.08 (11.2 – 21.5)	13.03 ± 0.55 (11.2 – 14.8)	14.16 ± 0.62 (12.6 – 16.8)	12.34 ± 0.97 (8.40 – 17.1)
CK (U/L)	5939 ± 1749 (355 - 14807)	7526 ± 1017 (126 - 12365)	4242 ± 2400 (608 - 15870)	5505 ± 2644 (1054 - 20940)	2569 ± 882 (409 - 7337)
Glucose (mmol/L)	2.03 ± 0.09 (1.80 – 2.6)	2.21 ± 0.12 (1.80 – 3.0)	2.37 ± 0.20 (1.90 – 3.2)	2.63 ± 0.10 (2.30 – 3.0)	2.46 ± 0.16 (1.60 – 3.0)
Hematocrit (proportion)	0.35 ± 0.03 (0.27 – 0.5)	0.25 ± 0.01 (0.19 – 0.31)	0.27 ± 0.03 (0.24 – 0.46)	0.32 ± 0.03 (0.18 – 0.37)	0.28 ± 0.02 (0.21 – 0.34)
LDH (U/L)	461 ± 96 (113 - 847)	576 ± 105 (74 - 1429)	940 ± 666 (94 - 4240)	1106 ± 564 (122 - 3990)	230 ± 47 (57 - 398)
Magnesium (mmol/L)	1.17 ± 0.02 (1.08 – 1.27)	1.21 ± 0.02 (1.13 – 1.33)	1.22 ± 0.03 (1.09 – 1.35)	1.18 ± 0.05 (1.00 – 1.37)	1.16 ± 0.02 (1.08 – 1.27)
Phosphorous (mmol/L)	2.15 ± 0.08 (1.9 – 2.5)	2.28 ± 0.09 (2.1 – 2.9)	2.12 ± 0.16 (1.7 – 2.8)	1.97 ± 0.23 (1.5 – 3.1)	1.95 ± 0.06 (1.7 – 2.2)
Potassium (mmol/L)	3.43 ± 0.017 (2.9 – 4.0)	3.8 ± 0.13 (3.3 – 4.6)	4.02 ± 0.38 (3.3 – 5.8)	3.96 ± 0.19 (3.5 – 4.5)	3.48 ± 0.12 (3.2 – 4.2)
Sodium (mmol/L)	158.83 ± 1.25 (154 - 163)	159.60 ± 1.23 (153 - 166)	160.67 ± 1.31 (156 - 164)	158.60 ± 2.73 (154 - 169)	158.50 ± 1.46 (152 - 164)
Total Protein (g/L)	37.88 ± 0.93 (33 - 42)	39.00 ± 0.75 (37 - 45)	37.83 ± 0.87 (35 - 40)	37.71 ± 1.38 (32 - 44)	38.38 ± 1.95 (31 - 50)
Triglycerides	1.19 ± 0.49	0.83 ± 0.08	0.63 ± 0.09	0.58 ± 0.10	0.59 ± 0.07

(mmol/L)	(0.43 – 4.55)	(0.49 – 1.27)	(0.45 – 1.04)	(0.27 – 0.90)	(0.38 – 0.90)

875 Table 3.2. Physiological variables (mean ± SD with range) measured in smallmouth bass treatment groups across this study.

Physiological Variable	Egg	Egg sac fry single sample	Swim up fry single sample	Free swimming fry single sample	Repeated sampling 2 (egg sac fry stage)	Repeated sampling 3 (free swimming fry stage)
ALP (U/L)	38.30 ± 10.29 (13 - 120)	20.00 ± 3.09 (8 - 30)	11.25 ± 4.01 (5 - 23)	33.38 ± 9.27 (8 - 90)	21.50 ± 6.33 (9 - 76)	27.10 ± 5.70 (8 - 61)
AST (U/L)	232 ± 67 (44 - 620)	219 ± 53 (48 - 470)	69 ± 21 (24 - 112)	149 ± 37 (45 - 357)	97 ± 22 (38 - 274)	157 ± 45 (40 - 469)
Calcium (mmol/L)	2.65 ± 0.07 (2.44 – 2.91)	2.54 ± 0.01 (2.50 – 2.60)	2.55 ± 0.04 (2.45 – 2.64)	2.76 ± 0.08 (2.54 – 2.95)	2.60 ± 0.03 (2.45 – 2.74)	2.85 ± 0.05 (2.67 – 3.09)
Chloride (mmol/L)	120 ± 2.79 (113 - 132)	120 ± 1.49 (115 - 126)	115 ± 1.68 (113 - 120)	108 ± 2.55 (102 - 116)	113 ± 2.15 (104 - 123)	104 ± 3.58 (90 - 121)
Cholesterol (mmol/L)	11.2 ± 0.70 (8.4 – 14.9)	13.51 ± 0.84 (10.5 – 16.9)	12.73 ± 1.27 (9.5 – 15.1)	13.10 ± 0.84 (10.1 – 16.2)	11.14 ± 0.63 (8.60 – 14.2)	10.91 ± 0.63 (8.50 – 15.5)
CK (U/L)	7809 ± 2745 (1298 - 24920)	8986 ± 3052 (1178 - 24091)	2056 ± 704 (370 - 3465)	3895 ± 1500 (650 - 12845)	5398 ± 2497 (1043 - 27560)	4361 ± 1920 (4 - 17210)
Glucose (mmol/L)	2.35 ± 0.11 (1.9 – 2.8)	2.53 ± 0.26 (1.70 – 3.9)	2.83 ± 0.19 (2.3 – 3.2)	3.11 ± 0.19 (2.30 – 3.9)	2.57 ± 0.17 (2.2 – 3.6)	2.97 ± 0.09 (2.4 – 3.3)
Hematocrit (proportion)	0.42 ± 0.02 (0.32 – 0.57)	0.35 ± 0.02 (0.29 – 0.42)	0.35 ± 0.03 (0.30 – 0.41)	0.30 ± 0.03 (0.19– 0.38)	0.31 ± 0.02 (0.22 – 0.39)	0.33 ± 0.02 (0.22 – 0.50)
LDH (U/L)	1776 ± 649 (169 - 5780)	1432 ± 429 (210 - 2870)	457 ± 154 (142 - 809)	833 ± 269 (218 - 2280)	669 ± 302 (169 - 3350)	762 ± 292 (1 - 2653)
Magnesium (mmol/L)	1.10 ± 0.02 (1.04 – 1.19)	1.12 ± 0.02 (1.04 – 1.16)	1.03 ± 0.03 (0.98 – 1.11)	1.08 ± 0.04 (0.99 – 1.23)	0.95 ± 0.03 (0.70 – 1.04)	1.06 ± 0.03 (0.96 – 1.23)
Phosphorous (mmol/L)	2.57 ± 0.10 (2.3 – 2.9)	2.31 ± 0.09 (2.0 – 2.7)	2.18 ± 0.06 (2.0 – 2.3)	2.18 ± 0.07 (2.0 – 2.4)	2.24 ± 0.09 (1.8 – 2.6)	2.29 ± 0.09 (2.0 – 2.8)
Potassium (mmol/L)	3.2 ± 0.15 (2.8 – 3.8)	3.53 ± 0.18 (3.2– 4.4)	3.38 ± 0.17 (2.9 – 3.6)	3.6 ± 0.19 (3.0 – 4.0)	3.33 ± 0.14 (2.6 – 4.1)	4.43 ± 0.56 (3.2 – 7.8)
Sodium (mmol/L)	157 ± 1.95 (153 - 166)	156 ± 1.01 (153 - 159)	158 ± 1.32 (154 - 160)	160 ± 1.02 (156 - 162)	152 ± 0.82 (149 - 158)	156 ± 1.05 (150 - 160)
Total Protein (g/L)	41.9 ± 1.46 (35 - 49)	43.14 ± 0.67 (41 - 46)	40.8 ± 2.66 (33 - 45)	42.7 ± 1.41 (37 - 47)	40.9 ± 1.00 (37 - 47)	40.1 ± 1.30 (35 - 49)
Triglycerides (mmol/L)	2.44 ± 0.23 (1.40 – 3.41)	2.69 ± 0.44 (1.12 – 4.69)	2.76 ± 0.24 (2.24 – 3.40)	2.11 ± 0.45 (1.06 – 4.21)	2.22 ± 0.26 (1.38 – 4.17)	1.61 ± 0.18 (1.12 – 3.05)

876

Table 3.3. Comparison of nutritional indicators of nest guarding male largemouth and smallmouth bass (*Micropterus* spp.) randomly sampled across four stages of brood development during the parental care period (eggs, egg sac fry, swim up fry, and free swimming fry) in Lake Opinicon, Ontario.

	Largemouth bass			Smallmouth bass		
Physiological Variable	d.f.	F-ratio	P-value	d.f.	F-ratio	P-value
ALP (U/L)	3, 28	0.81†	0.51	3, 26	3.25	0.04
AST (U/L)	3, 28	0.22	0.88	3, 26	1.53	0.23
Calcium (mmol/L)	3, 25	0.73	0.55	3, 17	2.51†	0.14
Chloride (mmol/L)	3, 23	1.88†	0.19	3, 17	6.30	***<0.01***
Cholesterol (mmol/L)	3, 27	1.78†	0.18	3, 24	1.87	0.16
CK (U/L)	3, 28	0.69†	0.58	3, 25	1.66	0.20
Glucose (mmol/L)	3, 27	5.80†	***<0.01***	3, 22	3.31	0.04
Hematocrit (proportion)	3, 28	3.88	0.02	3, 26	5.20	***<0.01***
LDH (U/L)	3, 28	0.20	0.90	3, 24	1.82†	0.20
Magnesium (mmol/L)	3, 25	0.56	0.65	3, 18	1.67	0.21
Phosphorous (mmol/L)	3, 26	1.48	0.24	3, 18	3.88†	0.05
Potassium (mmol/L)	3, 23	1.44	0.26	3, 17	1.21	0.34
Sodium (mmol/L)	3, 23	0.30	0.83	3, 17	1.47	0.26
Total Protein (g/L)	3, 27	0.47	0.71	3, 22	0.41	0.76
Triglycerides (mmol/L)	3, 27	1.64†	0.23	3, 24	0.95	0.43

Note: Italicized and boldfaced statistical output indicates significant differences at $\alpha = 0.01$. If variances were homogeneous for these data, analyses were conducted with one-way ANOVA; otherwise, Welch ANOVA was used.

†Denotes use of Welch ANOVA.

887 Table 3.4. Contrast between the second sampling of repeatedly sampled nest guarding
888 male largemouth and smallmouth bass (Micropterus spp.) at the egg sac fry and free
889 swimming fry brood development stages with control values for fish randomly sampled
890 fish in Lake Opinicon, Ontario.

891

	Largemouth bass egg sac fry stage			Smallmouth bass egg sac fry stage			Smallmouth bass free swimming fry stage		
Physiological Variable	d.f.	t-value	P-value	d.f.	t-value	P-value	d.f.	t-value	P-value
ALP (U/L)	17	0.86†	0.43	16	-0.19	0.85	16	-0.49	0.63
AST (U/L)	17	-2.20	0.04	16	-2.21	0.04	16	-0.22	0.83
Calcium (mmol/L)	16	0.39	0.70	14	1.71†	0.11	12	0.86†	0.42
Chloride (mmol/L)	16	2.15†	0.05	14	-2.26	0.04	11	-0.83	0.43
Cholesterol (mmol/L)	16	2.17†	0.05	15	-2.29	0.04	15	-2.21	0.04
CK (U/L)	17	2.23†	0.04	15	-1.06	0.31	16	-1.12	0.28
Glucose (mmol/L)	16	1.27†	0.23	15	0.14	0.89	15	-0.77	0.45
Hematocrit (proportion)	17	1.28	0.22	16	-1.82	0.09	16	0.86	0.40
LDH (U/L)	17	-2.57	0.02	15	1.58†	0.14	16	0.69†	0.50
Magnesium (mmol/L)	16	-1.71	0.11	15	-4.19	***<0.01***	12	-0.40	0.70
Phosphorous (mmol/L)	16	-3.22	***<0.01***	15	0.59†	0.57	12	0.99†	0.35
Potassium (mmol/L)	16	-1.89	0.08	14	0.98	0.35	11	-1.14	0.28
Sodium (mmol/L)	16	-0.58	0.57	14	-2.77	0.02	11	-2.11	0.06
Total Protein (g/L)	16	-0.48	0.64	15	-1.68	0.11	15	-1.34	0.20
Triglycerides (mmol/L)	16	2.15†	0.05	15	-0.83	0.42	15	-0.92	0.37

892

893 **Note:** Italicized and boldfaced statistical output indicates significant differences at $\alpha =$
894 0.01. If variances were homogeneous for these data, analyses were conducted with one-
895 way ANOVA; otherwise, Welch ANOVA was used.

896 †Denotes use of Welch ANOVA.

Table 3.5. Comparison of nutritional indicators of repeatedly sampled nest guarding male largemouth bass (Micropterus salmoides) across two stages of brood development (eggs and egg sac fry) and smallmouth bass (Micropterus dolomieu) across three stages of brood development during the parental care period (eggs, egg sac fry, and free swimming fry) in Lake Opinicon, Ontario.

	Largemouth bass			Smallmouth bass		
Physiological Variable	d.f.	F-ratio	P-value	d.f.	F-ratio	P-value
ALP (U/L)	1, 14	1.83	0.20	2, 27	1.75	0.20
AST (U/L)	1, 14	1.62	0.23	2, 27	1.28	0.30
Calcium (mmol/L)	1, 13	0.08	0.92	2, 22	8.17	***<0.01***
Chloride (mmol/L)	1, 12	0.41	0.67	2, 21	7.21	***<0.01***
Cholesterol (mmol/L)	1, 14	0.25	0.78	2, 27	0.04	0.95
CK (U/L)	1, 14	1.06	0.37	2, 27	3.07	0.06
Glucose (mmol/L)	1, 14	2.90	0.09	2, 25	5.54	0.01
Hematocrit (proportion)	1, 14	2.45	0.12	2, 27	6.79	***<0.01***
LDH (U/L)	1, 14	3.36	0.06	2, 26	1.93	0.17
Magnesium (mmol/L)	1, 13	1.35	0.29	2, 22	6.61	***<0.01***
Phosphorous (mmol/L)	1, 14	2.18	0.15	2, 22	3.15	0.06
Potassium (mmol/L)	1, 12	0.04	0.96	2, 21	4.66	0.02
Sodium (mmol/L)	1, 12	0.10	0.90	2, 21	4.82	0.02
Total Protein (g/L)	1, 14	0.04	0.96	2, 25	0.48	0.62
Triglycerides (mmol/L)	1, 14	1.79	0.20	2, 27	4.93	0.02

Note: Italicized and boldfaced statistical output indicates significant differences at $\alpha = 0.01$.

Figures

Figure 3.1. Changes in plasma glucose levels in randomly sampled nest guarding male largemouth bass across four stages of brood development (egg, egg sac fry, swim up fry, and free swimming fry) during the parental care period. Letter assignments of 'a' and 'b' denote significant ($P < 0.01$) differences among brood development stages for largemouth bass. Error bars show mean ± 1 S.E.

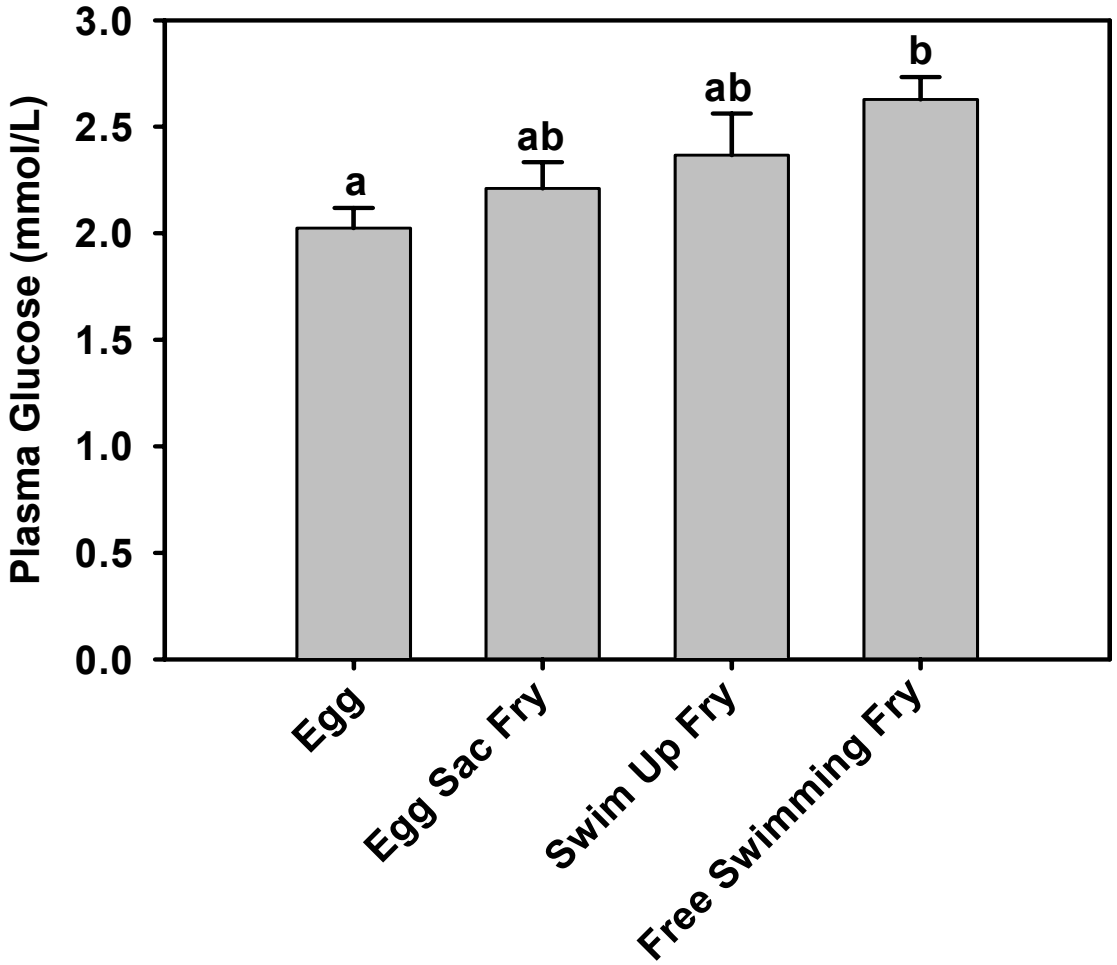


Figure 3.2. Changes in A.) hematocrit and B.) plasma chloride levels in randomly sampled nest guarding male smallmouth bass across four stages of brood development (egg, egg sac fry, swim up fry, and free swimming fry) during the parental care period. Letter assignments of 'a' and 'b' denote significant ($P < 0.01$) differences among brood development stages for smallmouth bass. Error bars show mean ± 1 S.E.

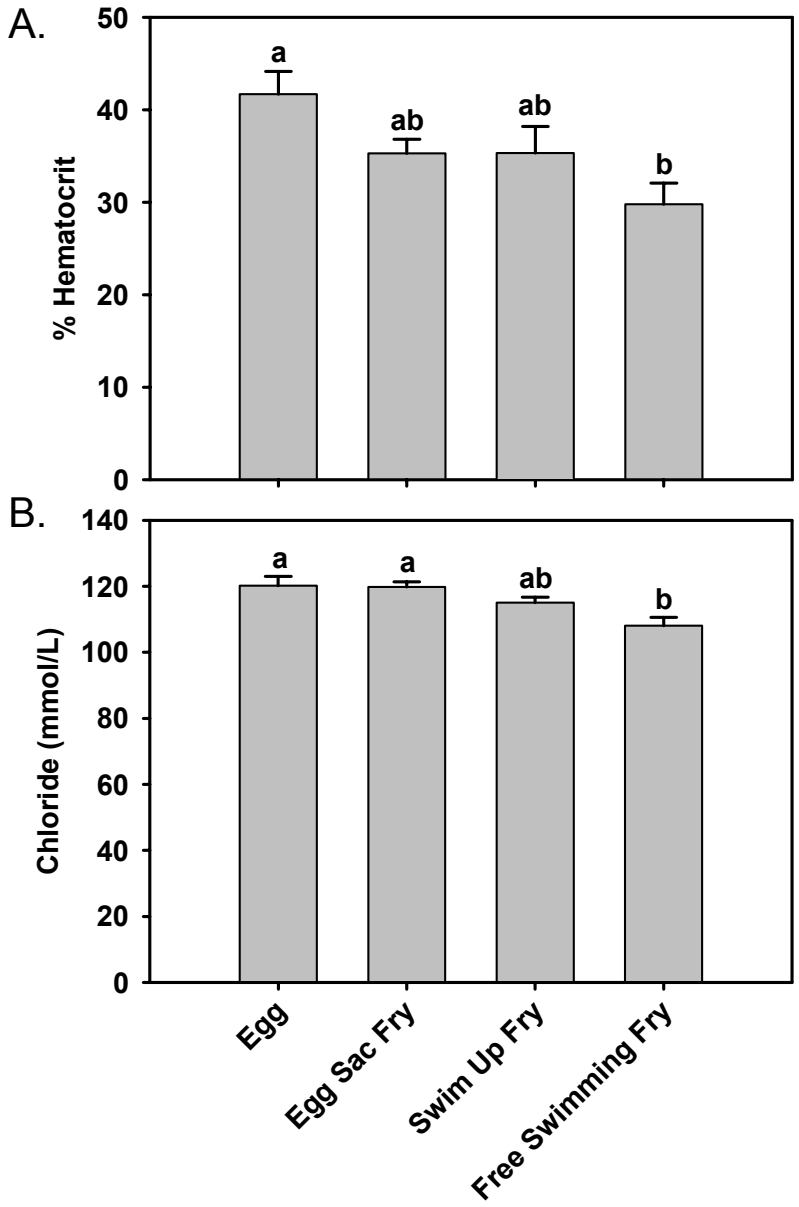
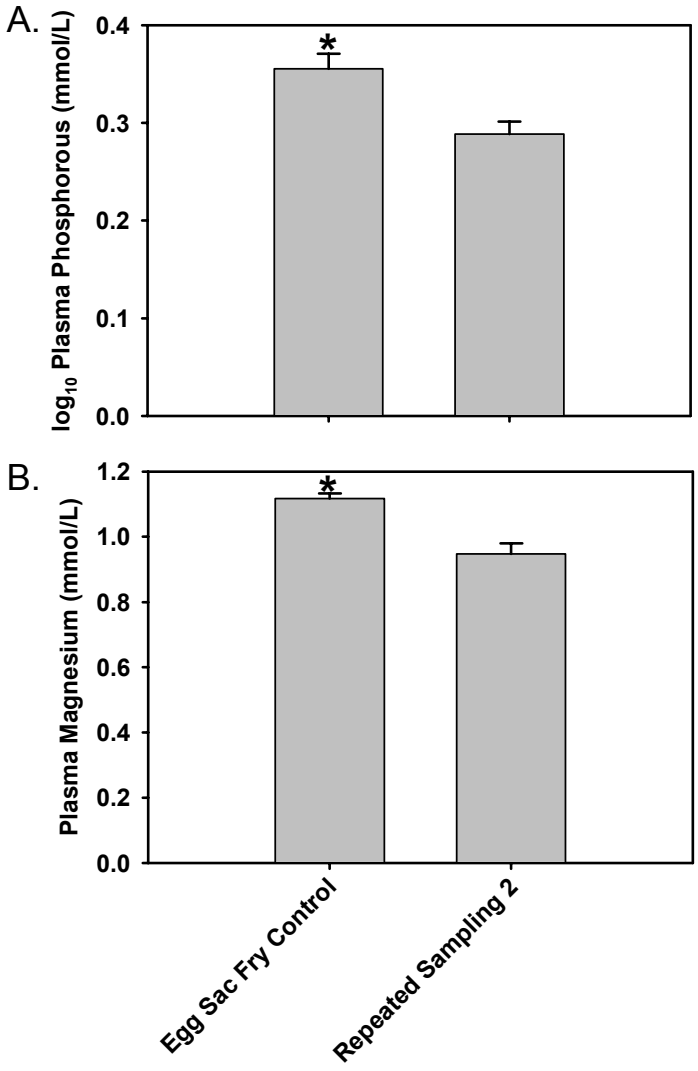
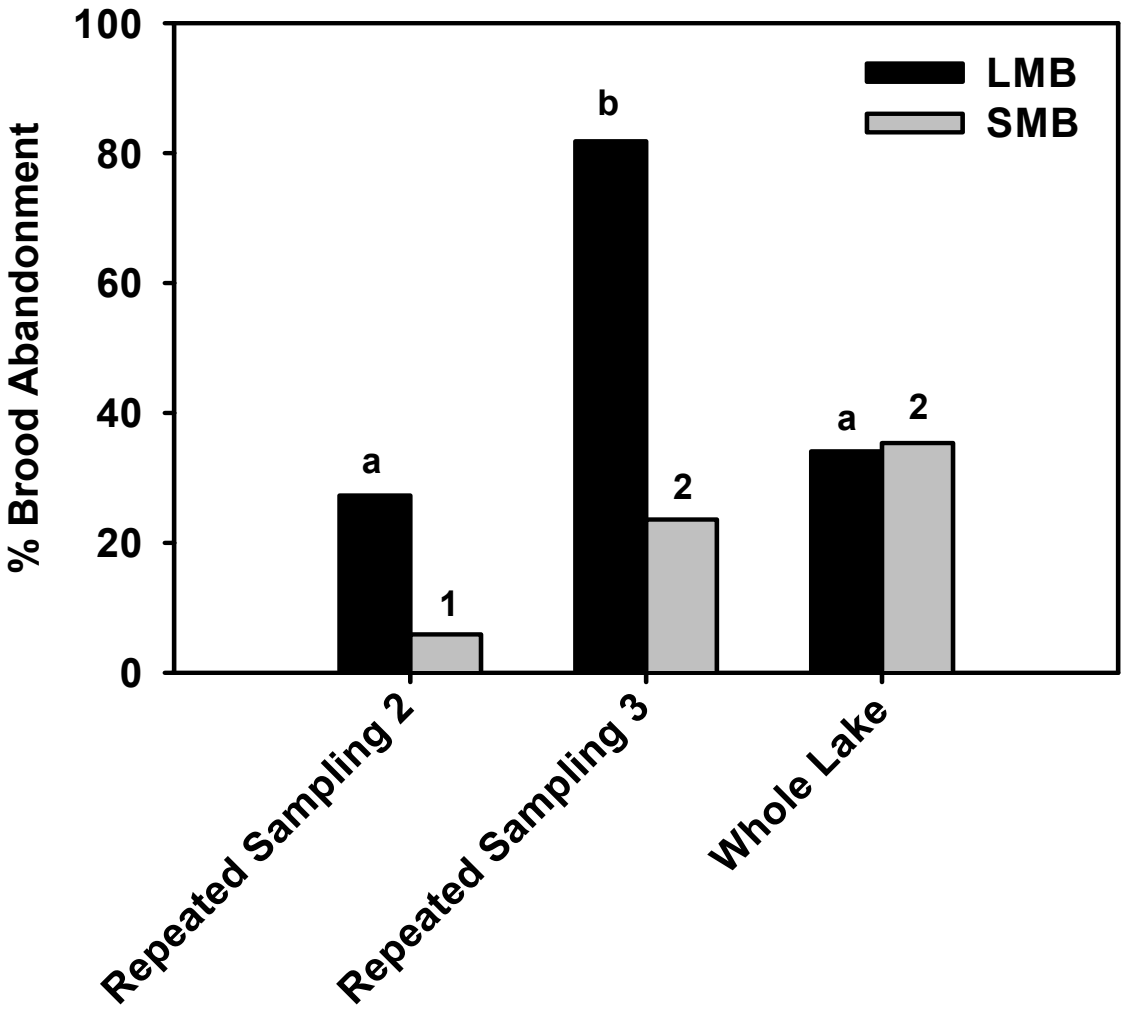


Figure 3.3. Comparison of A.) plasma phosphorous levels of nest guarding male largemouth bass and B.) plasma magnesium levels nest guarding male smallmouth bass between the second sampling of repeatedly sampled individuals with control values for fish randomly selected fish both at the egg sac fry brood development stage. Assignment of an asterisk (*) denotes significant ($P < 0.01$) differences between repeatedly and randomly sampled fish. Error bars show mean ± 1 S.E.

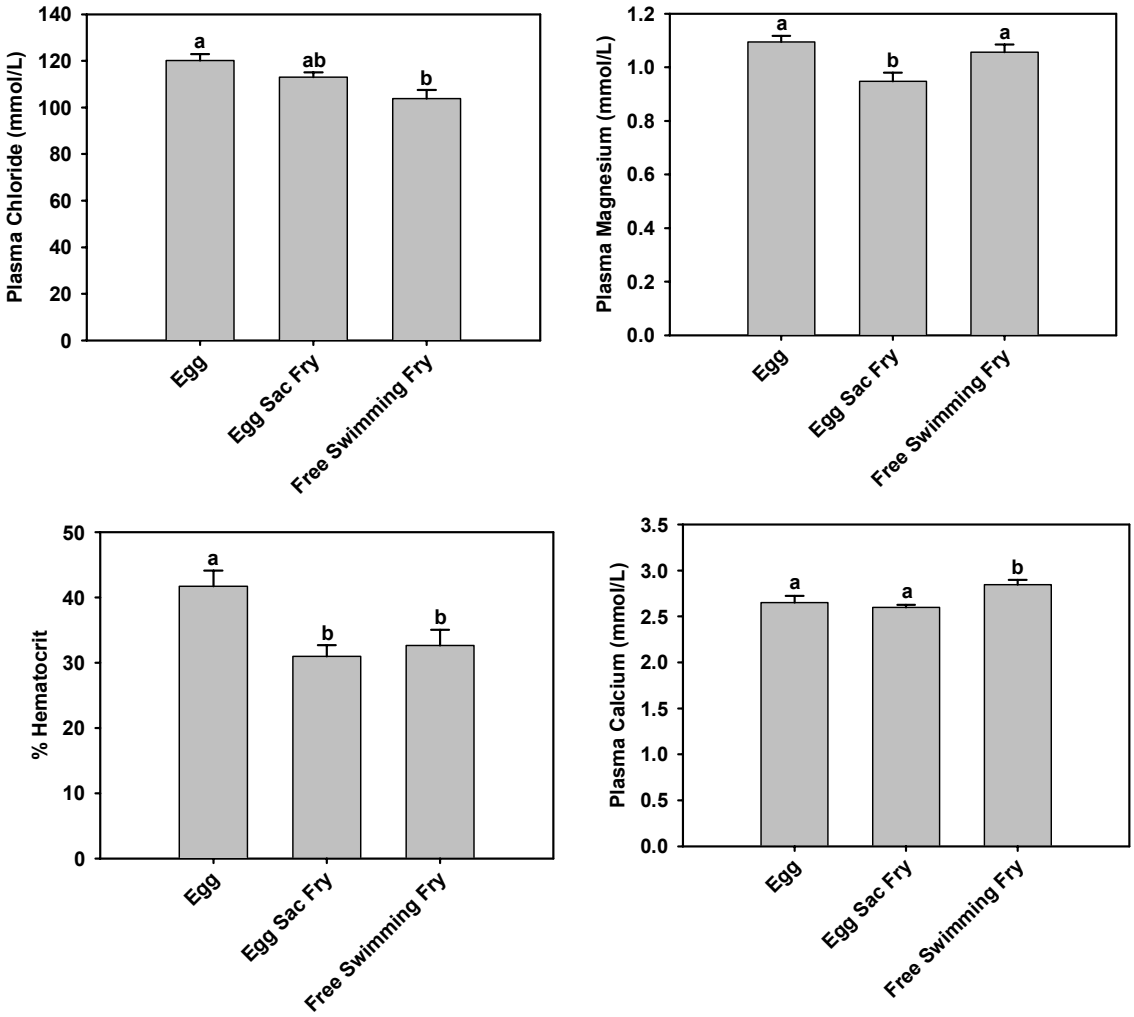


928 Figure 3.4. Abandonment rates of repeatedly sampled largemouth and smallmouth bass compared to
929 non-sampled bass (representing natural, whole-lake abandonment levels). Letter assignments of 'a',
930 and 'b' denote significant ($P < 0.01$) differences among groups for largemouth bass, and number
931 assignments of '1' and '2' denote significant differences among groups for smallmouth bass.
932



933

Figure 3.5. Comparison of plasma A.) chloride, B.) magnesium, C.) hematocrit, and D). calcium levels between three repeated sampling periods of nest guarding male smallmouth bass at the egg, egg sac fry, and free swimming fry brood development stages. Letter assignments of 'a' and 'b' denote significant ($P < 0.01$) differences among brood development stages for smallmouth bass. Error bars show mean ± 1 S.E.



Chapter 4: Why does size matter? A test of the benefits of female mate choice in a teleost fish based on morphological and physiological indicators of male quality

Abstract

In female mate choice, a female chooses a reproductive partner based on direct or indirect benefits to the female. While sexual selection theory regarding female mate choice is well developed, there are few mechanistic studies of the process by which females evaluate reproductive partners. Using paternal care providing smallmouth bass (*Micropterus dolomieu*) as a model, the purpose of this study was to determine the relationship between female mate choice and the morphological and physiological status of chosen males. This was accomplished by locating nests within one day of spawning and categorizing brood size (indicator of female mate choice) followed by capture of parental males which were blood sampled (for nutritional analyses), digitally photographed (for morphometric analyses), and released. Principal components analysis (PCA) of morphometric measurements described 72.7% of the variance associated with body morphology and generated three principal components (PC's) indicative of fusiform body shape, increased posterior size, and body stoutness. PCA of nutritional indicators described 75.4% of the variance associated with physiological metrics and generated two PC's indicative of plasma mineral content (Ca++ and Mg+) and energetic condition (total protein, triglyceride, and cholesterol). Male total length and body stoutness were the only significant predictors of female mate choice. Interestingly, no nutritional indicators were predictive of female mate choice, and there were no direct relationships between morphological variables and nutritional physiology indicators. Further research is needed to elucidate the mechanistic relationships between morphology and nutritional physiology (especially in relation to the parental care period) of individual fish to determine the basis of female mate preference.

Introduction

The role and consequences of sexual selection have been extensively discussed in the field of evolutionary biology (Darwin, 1871; Andersson, 1994; Johnstone, 1995; Andersson and Iwasa, 1996; Lailvaux and Irschick, 2006). Biological diversity ranging from the gross scale of speciation (Coyne and Orr, 2004) to the fine scale of differences in body ornaments or plumage coloration (Berglund et al., 1996) is thought to be a direct result of sexual selection. Female mate choice, whereby a female selects a mate based on perceived benefits to the female, is a key process within the realm of sexual selection (Andersson, 1994). Females may choose a mate based upon direct material benefits such as nuptial gifts or parental care from the male (Kirkpatrick, 1982; Reynolds, 1996; Vahed, 1998; Pizzari, 2003) or based on secondary sexual characteristics that are indicative of indirect benefits (such as good genes or superior health [Andersson, 1994; Andersson and Iwasa, 1996; Kirkpatrick, 1996; Møller and Alatalo, 1999]) that should benefit offspring survivability. However, recent syntheses have noted that most studies take an ethological or life history approach which leaves many mechanistic questions unanswered (Lailvaux and Irschick, 2006; Irschick et al., 2007). In particular, work on female mate choice in a number of species across multiple taxa has repeatedly elucidated traits in males that females choose which are correlated with reproductive success, though rarely is the mechanistic basis of these correlations clear (Irschick et al., 2007).

Smallmouth bass (*Micropterus dolomieu*), a teleost fish species, serve as an interesting model to study female mate choice due to their protracted paternal care period and lack of exaggerated male secondary sexual characteristics. In spring when the water temperature reaches ~15°C, male bass construct nests in the littoral zone which are the site of courtship and egg deposition (Coble, 1975; Ridgway, 1988). After spawning, females leave the vicinity of the nest and the male assumes the role of sole parental care giver (Cooke et al., 2006b). Parental care, consisting of brood maintenance and defense, typically lasts a month and is highly energetically demanding as males are extremely active and unable to forage normally (Hinch and Collins, 1991; Mackereth et al., 1999; Cooke et al., 2002).

989 During this period, parental care activities are powered primarily by endogenous energy reserves
990 accrued prior to the preceding winter (Mackereth et al., 1999). Parental care theory suggests that if
991 parental male energy levels decrease to a point that could threaten the potential for future reproduction,
992 the individual should abandon the current brood (Trivers, 1972; Sargent and Gross, 1986). Previous
993 work has indicated that male body size and body energy reserves are positively related at the onset of
994 parental care and that large males with high energy reserves (assessed using proximate body
995 composition analysis) provide parental care for longer durations when compared to smaller
996 counterparts (Mackereth et al., 1999). Based on this finding, it has been speculated that female
997 preference for large males is due to the ability of large males to use more energy reserves in parental
998 care than smaller conspecifics (Wiegmann and Baylis, 1995). Additionally, multiple studies have
999 noted that brood size is positively related to male size (Philipp et al., 1997; Suski and Philipp, 2004;
1000 Barbosa and Magurran, 2006). Since offspring survival is enhanced by parental care performance
1001 (Sargent and Gross, 1986), female choice for male characteristics demonstrative of the ability to
1002 perform parental care for extended time periods (i.e., larger body size) would increase female
1003 reproductive success.

1004 The goal of this study was to determine the relationships between morphological measures,
1005 nutritional physiology indicators, and female mate choice (measured as number of eggs in the nest of
1006 an individual male) at the onset of parental care in wild smallmouth bass. We predicted that females
1007 would choose males in better condition (indicated by increased plasma borne indicators of energetic
1008 and nutritional status) as these males would be most likely to successfully raise a brood and represent
1009 the best choice for female investment. We predicted that female choice would be based on male size
1010 (with larger males with stouter body shapes preferred) as overall body size is an honest signal of energy
1011 reserves in parental bass (Mackereth et al., 1999). Consequently, larger, more preferred males should
1012 also show increased biochemical indicators of nutritional and energetic status compared to less
1013 preferred males.

Methods

Field Techniques

This study was carried out from May 24th to June 5th, 2007 on Charleston Lake, eastern Ontario, Canada (44°32'14"N, 75°59"48"W). To eliminate confounding factors associated with a trend in which larger males spawn earlier during the spawning period (typically lasting 3 weeks [Wiegmann et al., 1992; Kubacki et al., 2002]), all sampling of males was conducted during the first three days of spawning in a lake where we had previously observed a wide range of size among parental males even early in the spawning period. At the beginning of every sampling day (lasting from May 24th to May 26th), snorkel surveys of the littoral zone (typically less than 1m water depth) were conducted to locate smallmouth bass that were actively guarding nests with newly deposited eggs (1 or 2 day old). Upon locating an active bass nest, the snorkeler placed a numbered polyvinyl chloride (PVC) tile near the nest and recorded nest location, nest depth, and number of eggs within the nest (visual, categorical assessment ranging from low of 1 to high of 5; Suski and Philipp, 2004). Fish were then captured using heavy-action recreational fishing equipment that could be used to angle fish from the boat or underwater (by the diver). All fish were landed within 20 sec of hooking to minimize non-parental care related anaerobic exercise. Upon capture, fish were placed supine in a foam lined sampling trough filled with fresh lake water and quickly blood sampled by the caudal puncture method using a 1.5", 21 gauge vacutainer syringe (Houston, 1990). Approximately 1.5mL of blood was collected in a 3mL vacutainer containing lithium heparin to prevent blood coagulation and was then placed into a water-ice slurry. Additionally, total length was measured and presence or absence of injury was noted. Individuals were transferred to a flat, foam lined, spatially referenced tray and digitally photographed (Pentax Optio WPI, 6 megapixel, Pentax Imaging Company, Golden, CO, U.S.A.) from 0.60m directly above. Individuals were then released within 5m of the nest. During the sampling procedure (191 ± 5s), a snorkeler remained at the nest site and defended the brood until the male returned (typically in

under 5 minutes). In total, 86 male bass were sampled. Blood samples were centrifuged (after sampling six fish) at 10,000x gravity for 5 minutes (Clay Adams Compact II Centrifuge) and plasma samples were stored in liquid nitrogen for subsequent analysis. Snorkel surveys to determine presence or absence of the male were conducted 7 and 10 days after sampling which roughly corresponded to the end of larval stage of brood development. Presence of the male on the nest at this time was used as a measure of parental care success as after the hatching of eggs, parental males provide less vigilant parental care and are more prone to abandoning the nest as the brood becomes increasingly independent (Sargent and Gross, 1986; Ridgway, 1988; Cooke et al., 2002).

Lab Analyses

Samples were analyzed for concentrations of various blood-borne biochemical constituents that have been previously identified as indicative of individual energetic and nutritional status (total protein, triglycerides, and cholesterol) as well as dietary minerals (phosphorus, magnesium, and calcium) (Wagner and Congleton, 2004; Congleton and Wagner, 2006; Hanson and Cooke, 2009). All biochemical 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. 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.

Digital Image Analysis

Digital images of individuals were measured for a suite of morphological characteristics (Fig. 4.1) using the program ImageJ (Abramoff et al., 2004). The following metrics, as modified from Hawkins and Quinn (1996) and detailed in Hanson et al. (2007c), were quantified to the nearest mm: head depth 1 (HD1); head depth 2 (HD2); body depth at posterior aspect of the dorsal fin (PELVDF);

origin of the pelvic fin to posterior aspect of the soft dorsal fin (PELVSD); origin of the anal fin to posterior aspect of the soft dorsal fin (ANSD); origin of the anal fin to the top of caudal flexure (ANC1); insertion of the anal fin to bottom of the caudal flexure (ANC2); posterior aspect of the soft dorsal fin to top of the caudal flexure (SDC1); posterior aspect of the soft dorsal fin to bottom of the caudal flexure (SDC2); and the caudal flexure depth (CFD).

Satistical Analyses

To remove the possible effects of allometric growth on morphological measurements (Table 4.1), the residuals of the least squares linear regression of log transformed traits on log transformed fish lengths were used in subsequent principal components analysis with varimax rotation (Kaiser, 1960; Tabachnick and Fidell, 1989; Hawkins and Quinn, 1996; Ojanguren and Brana, 2003). The Kaiser-Guttman criteria (or latent root criteria) was used to determine which principal factors would be retained for later analysis (Kaiser, 1960). Only principal factors with eigenvalue scores of greater than 1 were used to determine the relationship between morphology and egg scores (Kaiser, 1960). Physiological variables (Table 4.2) were subjected to principal components analysis in the same manner as described above (Kaiser, 1960; Tabachnick and Fidell, 1989).

To determine which traits female smallmouth bass preferred, a nominal logistic regression of egg score by principal components from both morphological and physiological measures, as well as total length, was performed (Zar, 1999). Least squares linear regression was employed to determine if there was a relationship between morphological and physiological variables (as represented by the above derived principal component scores as well as total length) (Zar, 1999). To determine if there were differences in parental care success between individuals of different sizes, the mean size of successful parental males was compared to the mean size of parental males who abandoned their brood through a t-test (Zar, 1999). To aid in data interpretation, *post hoc* power analyses were conducted using observed effect size and variance (Thomas, 1997). All analyses were performed in the statistical

package JMP v 7.0 and the level of significance for all tests (α) was assessed at 0.05. All values presented represent means ± S.E. unless otherwise noted.

Results

Principal Components Analyses

Principal components analysis on morphological measurements produced three factors describing 72.7% of the variance in the morphological variables surveyed in this study (Table 4.3). Morphological principal component 1 (MPC 1) was characterized by high positive factor loadings for PELVSD, ANSD, ANC1, ANC2 and CFD (Table 4.3), representing a fusiform body shape and accounting for 26.6% of the variance. SDC1 and SDC2 had high positive factor loadings for morphological principal component 2 (MPC 2) while PELVSD had a high negative factor loading (Table 4.3). This factor accounted for 22.7% of the variance and mainly described the length and depth of the caudal region (potential for propulsion ability). Lastly, morphological principal component three (MPC 3) accounted for 23.4% of the variance and described overall body stoutness with high positive factor loadings for HD1, HD2, PELDVF and ANSD (Table 4.3). Principal components analysis of physiological variables produced two factors describing 65.5% of the variation in physiological measurements from this study (Table 4.4). Physiological principal component 1 (PPC 1) was characterized by high factor loadings for Ca^{++}, Ma^{+}, P and total protein and represented plasma mineral content (Table 4.4). Physiological principal component 2 (PPC 2) was characterized by high factor loadings for total protein, triglycerides, and cholesterol and represented plasma lipid content (Table 4.4).

Correlates of Female Mate Choice

Overall, only 24 % of the variance associated with female mate choice was described by the variables included in this study (Nominal logistic regression; d.f. = 24, $\chi^2 = 57.65$, $P < 0.001$, observed

power = 0.98). MPC 3 (body stoutness) (Nominal logistic regression; d.f. = 4, $\chi^2 = 9.60$, P = 0.048) was positively correlated with female mate choice (Table 5; Fig. 1). Total length was also positively correlated with female mate choice (Nominal logistic regression; d.f. = 4, $\chi^2 = 32.79$, $P < 0.001$; Table 4.5; Fig. 4.2). Interestingly, no physiological variables were significantly predictive of egg score (Table 4.5) and statistical power for the nominal logistic regression was high (observed power = 0.98). Due to the fact that there was no direct relationship between female mate choice and biochemical indicators of nutritional status, we investigated the possibility that physiological variables were directly influencing morphological principal component scores. However, there were no significant relationships between either PPC 1 or PPC 2 and any of the morphological variables included in this study, though the observed power of these analyses was generally low (Table 4.6). There were no differences in size between fish which abandoned the brood prematurely (410 ± 20 mm) and fish that successfully raised the brood (418 ± 6 mm) (t-test; d.f. = 85, t-value = 0.49, P-value = 0.63; Table 4.7). Additionally, there were no relationships between any of the morphological or physiological metrics and brood abandonment, though the observed power of these analyses was generally low (Table 7).

Discussion

Mate choice is a complex behavior that requires that a female be able to reliably evaluate the direct or indirect benefits of mating with a particular male (Andersson, 1994; Lailvaux and Irschick, 2006; Irschick et al., 2007). For this to occur there needs to be some cue that the female favors that relates to the status of the male (Wiegmann and Baylis, 1995; Maynard-Smith and Harper, 2003). In the current study, females preferred larger males as evidenced by the positive relationship between brood size and multiple metrics of body shape (total length, body stoutness, size of the posterior end of the body). These findings are consistent with previous studies that have linked brood size to male size in smallmouth bass (Ridgway, 1988; Wiegmann and Baylis, 1995; Mackereth et al., 1999; Suski and Philipp, 2004). Though the relationship between male body size and brood size was noted in these

1139 studies, the mechanistic rationale behind the preference for larger males was not tested. In the current
1140 study, we predicted that larger males would be preferred because they would be in better energetic and
1141 nutritional condition at spawning and, therefore, would be able to withstand the nutritional declines
1142 associated with parental care and would not abandon the brood.

1143 Preference for larger males could be related to the energetic dilemma encountered by a parental
1144 male bass. The parental care period is characterized by intense activity such as brood defense and
1145 maintenance (Hinch and Collins, 1991; Cooke et al., 2002) that is powered through endogenous energy
1146 reserves (Mackereth et al., 1999) since foraging is limited to a small area around the nest and prey
1147 intake is greatly curtailed (Hinch and Collins, 1991; Cooke et al., 2002). As a result, premature
1148 exhaustion of endogenous energy reserves renders the male unable to continue parental care and the
1149 current brood will be abandoned (and consumed by brood predators) as an act of self preservation to
1150 maintain the possibility for future reproductive activity (Trivers, 1972; Sargent and Gross, 1986;
1151 Philipp et al., 1997). Previous work has noted that larger males (as measured by total length) typically
1152 have increased energy stores when compared to smaller males at the onset of spawning, though the
1153 relationship to female preference was not investigated (Ridgway and Friesen, 1992; Mackereth et al.,
1154 1999). Additionally, it has been theorized that large males would be preferred because the loss of
1155 energy reserves associated with parental care would be a lower proportion of overall endogenous
1156 energy reserves than that of small conspecifics partaking in the same behaviour (Shuter et al., 1980;
1157 Wiegmann and Baylis, 1995). In previous studies, circulating levels of triglycerides and cholesterol
1158 have been shown to decline in response to starvation in Pacific salmonids (Wagner and Congleton,
1159 2004; Congleton and Wagner, 2006) and during parental care in black bass (Hanson and Cooke, 2009).
1160 Additionally, fluctuations in dissolved minerals due to starvation have been noted in parental black
1161 bass as minerals acquired from forage are no longer available and the body depleted internal resources
1162 (Hanson and Cooke, 2009). In the current study, no biochemical measures of nutritional or energetic
1163 status as measured at the beginning of parental care were directly reflective of female preference (Table

4.5). Additionally, there were no correlations between morphometric measures and biochemical measures of nutrition or energetic status (Table 4.6). The lack of a relationship between female preference and circulating indicators of energetic and nutritional status may be the result of two situations. First, morphology may actually not be an honest signal of male energetic status as predicted, and female preference for larger males in this system would not be indicative of energetic or nutritional differences between males at the commencement of parental care. Second, all spawning males may initiate spawning with similar levels of mobilized lipids and minerals (as measured in the current study), but only larger males with increased endogenous energy reserves (Mackereth et al., 1999) may be able to maintain these levels across the entirety of parental care. Currently, the exact relationship between plasma borne nutritional indicators and total endogenous energy reserves as well as differences in rates of change of circulating indicators of nutrition between different sizes of fish is not clearly understood, largely due to the challenges of obtaining estimates of gross somatic energy without lethally sampling fish. However, there are also other potential direct or indirect benefits to the female of choosing a large male.

The quality of parental care that offspring receive may be a possible indirect benefit gained by the female for choosing a larger male mate. Parental care activities increase offspring survival at the cost of adult condition (Gross and Sargent, 1985; Clutton-Brock, 1991; Sargent and Gross, 1986). Larger males have been shown to provide more rigorous parental care for longer durations of time than small males because larger fish are in better condition at the commencement of spawning (Wiegmann and Baylis, 1995; Mackereth et al., 1999). Additional work has shown that large male bass are more aggressive nest defenders, though this finding is confounded by the fact that larger males typically have a larger parental investment due to increased brood sizes (Suski and Philipp, 2004). Though we had no direct measure of quality of parental care, we did monitor premature nest abandonment by all males in this study and there were no relationship between the size of the parental male and premature nest abandonment rates. It is possible that large males are at an advantage when defending the brood

against possible predation as larger male bass could potentially consume small brood predators themselves. Additionally, as large males typically spawn first, these individuals may monopolize optimal spawning and rearing territories (Ridgway et al., 1991; Wiegmann et al., 1992), though, currently, no studies have documented differences in female preference based upon male spawning location and habitat.

Though not tested in the current study, two final mechanisms may account for the correlation between body size and female preference. First, larger body size may be indicative of superior genetic quality of the male and females that successfully mate with large males then indirectly benefit from having offspring that inherit the favored genotype of the father (Andersson, 1994; Møller and Alatalo, 1999; Hunt et al., 2004; Neff and Pitcher, 2005). Second, since fish exhibit indeterminate growth, size is typically an indication of age of the individual. A female preference for increased male body size may be a result of a preference for males which would have previous parental care experience and could possibly be dominant in their mating system (Wiegmann et al., 1992; Jacob et al., 2007), though the advantages of mating with an older male are not clearly understood.

Mate choice represents a complex interplay of signaling on the part of the chosen sex and evaluation on the part of the choosy sex. The ultimate result that female smallmouth bass preferred larger males with distinctive body shapes is consistent with a wide body of literature on both fish and other taxa (Wiegmann et al., 1992; Husak and Fox, 2006; Lailvaux and Irschick, 2006; Jacob et al., 2007; Salvador et al., 2007). Likely, the preference for larger males is a result of body size being an honest signal of male quality (Maynard-Smith and Harper, 2003). However, the proximate mechanisms behind this choice remain unknown and are likely a result of a complex interplay between direct (e.g., male parental care performance) and indirect benefits (e.g., good genes) to the female (Barbosa and Magurran, 2006). Future studies that include measures of physiological and nutritional status across a range of animal models will help to reveal the extent to which the pattern that we observed in this study (i.e., the apparent lack of relationship between parental male physiology and

1214 eggs received as a proxy for female selection) may be a general rule. Furthermore, it would be
1215 interesting to replicate such a study in a year or study system where resources are extremely limited
1216 (e.g., drought, long winter) and where there is a wide range in organismal condition.

Tables

Table 4.1: Morphological measurements (mean ± S.D.) measured from nest guarding male smallmouth bass at the commencement of parental care in Charleston Lake, Ontario, separated by brood size [egg score ranging from a low of 1 to a high of 5]). Morphological measures were modified from Hawkins and Quinn (1996) and are detailed in Hanson et al., (2007), were quantified to the nearest mm: head depth 1 (HD1); head depth 2 (HD2); body depth at posterior aspect of the dorsal fin (PELVDF); origin of the pelvic fin to posterior aspect of the soft dorsal fin (PELVSD); origin of the anal fin to posterior aspect of the soft dorsal fin (ANSD); origin of the anal fin to the top of caudal flexure (ANC1); insertion of the anal fin to bottom of the caudal flexure (ANC2); posterior aspect of the soft dorsal fin to top of the caudal flexure (SDC1); posterior aspect of the soft dorsal fin to bottom of the caudal flexure (SDC2); and the caudal flexure depth (CFD).

	ES 1 (N = 5)	ES 2 (N = 10)	ES 3 (N = 22)	ES 4 (N = 33)	ES 5 (N = 17)
HD1	5.1 ± 0.8	6.2 ± 0.9	6.8 ± 1.0	6.5 ± 0.8	7.2 ± 0.6
HD2	8.5 ± 1.2	10.6 ± 1.4	11.7 ± 1.7	11.5 ± 1.4	12.5 ± 1.0
PELVDF	9.0 ± 1.0	11.2 ± 1.5	12.4 ± 1.9	12.3 ± 1.6	13.3 ± 1.2
PELVSD	14.8 ± 1.1	18.8 ± 2.7	19.9 ± 3.0	19.9 ± 2.5	21.4 ± 2.0
ANSD	7.4 ± 0.6	9.4 ± 1.2	10.0 ± 1.4	10.2 ± 1.2	10.9 ± 0.9
ANC1	11.3 ± 0.6	13.8 ± 1.2	14.3 ± 1.7	14.4 ± 1.5	15.4 ± 1.2
ANC2	9.5 ± 0.2	11.5 ± 1.2	11.7 ± 1.4	11.9 ± 1.3	12.5 ± 1.4
SDC1	5.4 ± 0.8	6.2 ± 0.8	6.6 ± 1.2	6.7 ± 0.8	6.9 ± 1.0
SDC2	6.9 ± 0.8	8.2 ± 0.9	8.6 ± 1.2	8.8 ± 1.0	9.3 ± 1.1
CFD	4.2 ± 0.5	5.2 ± 0.5	5.4 ± 0.7	5.5 ± 0.7	5.8 ± 0.6
Total Length	328.2 ± 23.7	398.5 ± 43.8	423.9 ± 51.1	418.5 ± 47.4	446.5 ± 37.1

Table 4.2: Physiological measurements (mean ± S.D.) measured from nest guarding male smallmouth bass at the commencement of parental care in Charleston Lake, Ontario, separated by brood size [egg score ranging from a low of 1 to a high of 5]).

	ES 1 (N = 5)	ES 2 (N = 10)	ES 3 (N = 22)	ES 4 (N = 33)	ES 5 (N = 17)
Calcium (mmol/L)	2.60 ± 0.35	2.70 ± 0.22	2.62 ± 0.30	2.66 ± 0.30	2.70 ± 0.31
Magnesium (mmol/L)	1.07 ± 0.07	1.24 ± 0.07	1.07 ± 0.15	1.17 ± 0.13	1.20 ± 0.19
Phosphorus (mmol/L)	1.40 ± 0.10	1.55 ± 0.34	1.41 ± 0.25	1.37 ± 0.30	1.35 ± 0.22
Total Protein (g/L)	39.00 ± 6.92	43.40 ± 4.14	41.18 ± 6.10	41.90 ± 5.62	43.88 ± 5.86
Triglyceride (mmol/L)	2.79 ± 1.03	2.90 ± 0.84	2.97 ± 0.88	3.18 ± 0.83	3.07 ± 0.95
Cholesterol (mmol/L)	12.00 ± 4.09	12.8 ± 1.51	12.38 ± 2.75	13.51 ± 2.85	14.83 ± 3.38

Table 4.3: Loading of the morphological measurements into three principal factors by principal components analysis (MPC 1, MPC 2, and MPC 3). Variables that contribute maximally to each factor are in bold. Morphological measures were modified from Hawkins and Quinn (1996) and are detailed in Hanson et al., (2007), were quantified to the nearest mm: head depth 1 (HD1); head depth 2 (HD2); body depth at posterior aspect of the dorsal fin (PELVDF); origin of the pelvic fin to posterior aspect of the soft dorsal fin (PELVSD); origin of the anal fin to posterior aspect of the soft dorsal fin (ANSD); origin of the anal fin to the top of caudal flexure (ANC1); insertion of the anal fin to bottom of the caudal flexure (ANC2); posterior aspect of the soft dorsal fin to top of the caudal flexure (SDC1); posterior aspect of the soft dorsal fin to bottom of the caudal flexure (SDC2); and the caudal flexure depth (CFD).

	MPC 1	MPC 2	MPC 3
Eigenvalue	2.660	2.269	2.342
HD1	-0.308	0.113	**0.665**
HD2	0.051	0.098	**0.895**
PELDVF	0.133	0.028	**0.850**
PELVSD	**0.592**	**-0.527**	0.200
ANSD	**0.633**	-0.292	**0.537**
ANC1	**0.849**	0.177	0.009
ANC2	**0.751**	-0.062	-0.099
SDC1	0.026	**0.932**	0.085
SDC2	0.157	**0.911**	0.166
CFD	**0.695**	0.382	-0.054
% Variance Explained	26.6	22.7	23.4

Table 4.4: Loading of the physiological measurements into three principal factors by principal components analysis (PPC 1, and PPC 2). Variables that contribute maximally to each factor are in bold.

	PPC 1	PPC 2
Eigenvalue	2.726	1.478
Calcium (mmol/L)	**0.896**	0.183
Magnesium (mmol/L)	**0.777**	0.188
Phosphorus (mmol/L)	**0.582**	-0.253
Total Protein (g/L)	**0.700**	**0.593**
Triglyceride (mmol/L)	-0.065	**0.732**
Cholesterol (mmol/L)	0.202	**0.796**
% Variance Explained	45.4	20.1

Table 4.5: Results of simple nominal regression of both morphological and physiological principal components vs. female mate choice (as measured by brood size of individual parental male smallmouth bass).

Source	d.f.	χ^2	P-value
MPC 1 (Large Posterior)	4	9.40	0.051
MPC 2 (Fusiform)	4	2.22	0.696
MPC 3 (Stoutness)	4	9.60	***0.048***
PPC 1 (Minerals)	4	3.45	0.486
PPC 2 (Lipids)	4	5.12	0.277
Total Length	4	28.08	***< 0.001***

Table 4.6: Relationships between morphological principal components and physiological principal components in parental smallmouth bass.

		d.f.	F	P-value	Observed Power
PPC 1 (Minerals)	MPC 1 (Large Posterior)	1, 84	0.95	0.33	0.16
	MPC 2 (Fusiform)	1, 84	0.16	0.69	0.07
	MPC 3 (Stoutness)	1, 84	<0.001	0.99	0.05
	Total Length	1, 84	0.22	0.64	0.07
PPC 2 (Lipids)	MPC 1 (Large Posterior)	1, 84	0.13	0.72	0.07
	MPC 2 (Fusiform)	1, 84	0.75	0.39	0.14
	MPC 3 (Stoutness)	1, 84	1.59	0.23	0.24
	Total Length	1, 84	1.29	0.26	0.22

Table 4.7: Relationships between morphological and physiological principal components and the presence of the parental male smallmouth bass on the nest 10 days after sampling.

Source	d.f.	t-value	P-value	Observed Power
MPC 1 (Large Posterior)	85	1.66	0.10	0.38
MPC 2 (Fusiform)	85	1.19	0.24	0.22
MPC 3 (Stoutness)	85	-0.67	0.50	0.10
PPC 1 (Minerals)	84	-0.27	0.79	0.06
PPC 2 (Lipids)	84	0.10	0.92	0.05
Total Length	85	0.49	0.63	0.08

Figures

Figure 4.1. The relationship between MPC3 (indicative of overall male body stoutness) and female mate preference (as measured by brood size [egg score ranging from a low of 1 to a high of 5]).

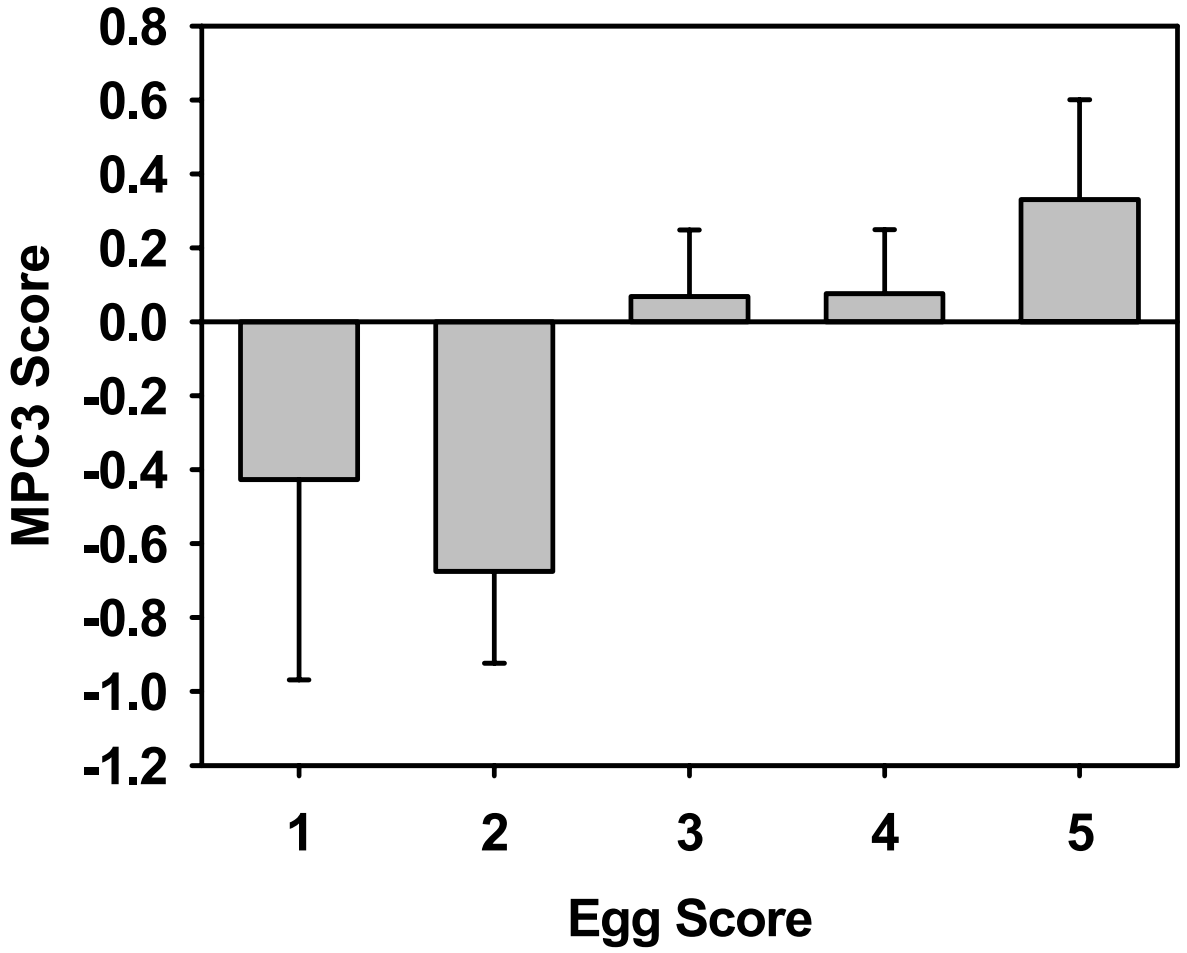
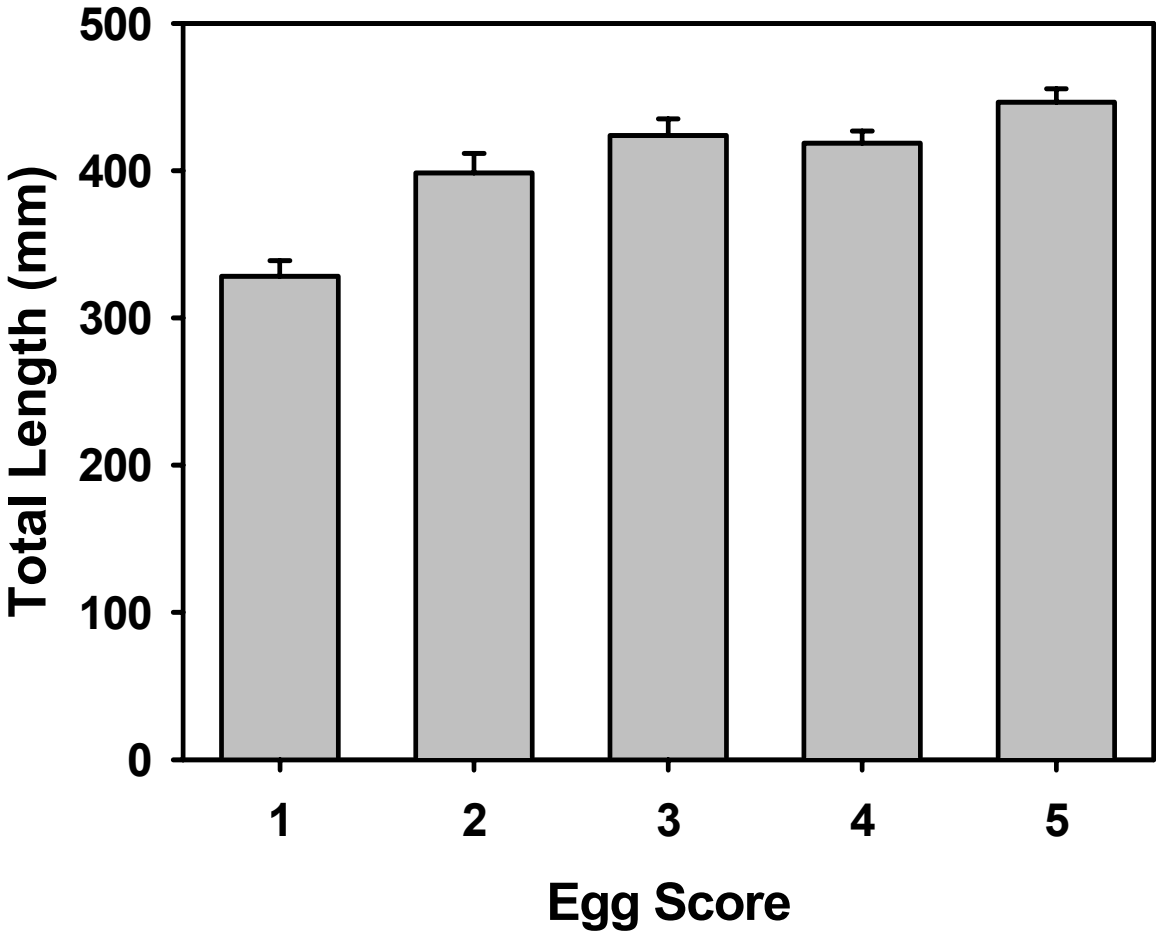


Figure 4.2. The relationship between parental male smallmouth bass total length and female mate preference (as measured by brood size [egg score ranging from a low of 1 to a high of 5]).



Chapter 5: Causes and consequences of voluntary anorexia during the parental care period of wild male smallmouth bass (*Micropterus dolomieu*)

Abstract

By definition, parental care behaviours increase offspring survival, and individual fitness, at some cost to the parent. In many species, the cost of parental care is often a decline in condition and energy reserves of the care-giving parent. In smallmouth bass (*Micropterus dolomieu*), parental males provide sole care for a developing brood often exceeding a month. This care involves a dramatic increase in activity as the male defends the brood from predation coupled with decreased foraging and a subsequent decline in endogenous energy reserves and nutritional condition. To date, no mechanisms have been proposed for the lack of voluntary foraging, though regulation of appetite hormones such as ghrelin have been documented to affect feeding behaviour in other fish species. To determine the mechanism by which smallmouth bass cease feeding during parental care, we documented baseline fluctuations in plasma ghrelin concentrations. Plasma ghrelin concentrations were lowest during the early stages of parental care before increasing when the brood developed to independence (a time when feeding has been noted to resume). Additionally, we performed an intervention experiment whereby plasma ghrelin levels of a subset of fish were artificially increased through an injection of rodent ghrelin at the onset of parental care. Despite measuring a significant increase in plasma ghrelin when the brood developed from eggs to larvae (approximately one week after injection), we noted no differences in plasma borne indicators of recent foraging activity or nutritional status indicating that voluntary anorexia is possibly reinforced by receptor insensitivity to appetite hormones during this time period. Finally, we assessed the ultimate consequences of foraging behaviour by feeding a subset of fish to satiation and measuring post-prandial changes in swimming performance and aggression. Fish fed to satiation showed significant decreases in burst swimming ability as well as aggressiveness towards potential brood predators. Voluntary anorexia during smallmouth bass parental care is an

adaptive behaviour that avoids potentially deleterious declines in swimming performance and parental aggression apparently through a modulation of production and reception of appetite hormones including ghrelin.

Introduction

Broadly defined, parental care behaviours are defined as any investment into offspring after initial fertilization and serve the function of increasing offspring survival, typically at some cost to the parent (Williams, 1966; Trivers, 1972; Reynolds, 1996). As a behaviour, parental care has evolved in numerous species in multiple taxa (Gross and Sargent, 1985; Reynolds, 1996; Møller and Cuervo, 2000; Mas and Kolliker, 2008) and various forms have been documented from simple behaviours such as concealment of fertilized eggs (Gross and Sargent, 1985) to highly complex behaviours such as extended internal gestation followed by feeding of offspring through lactation (Martin, 2007), and teaching of offspring for years (Thornton and Raihani, 2008). Often, concomitant with other parental costs such as decreased opportunity for mating (Magrath and Komdeur, 2003), care givers often expend energy during care which can result in a decrease in condition of the parent (Coleman and Fische,r 1991; Smith and Wooton, 1995; Reynolds, 1996; Webb, 2002). Though trends in declining condition of parents have been documented, the mechanisms whereby organisms regulate energy utilization during parental care to maximize offspring survival and individual fitness are still largely unknown.

Teleost fish species exhibit a wide range of parental care behaviours (Blumer, 1982; Gross and Sargent, 1985). Of the various forms of parental care, smallmouth bass (*Micropterus dolomieu*) exhibit the most common teleost behaviour, namely uniparental male care (Gross and Sargent, 1985). In spring, when water temperatures reach approximately 15°C, male bass construct nests (small, saucer shaped depressions) in the littoral zone which serves as the site of courtship and fertilization (Coble, 1975; Ridgway, 1988). Shortly after fertilization, the female departs and the male provides care for the developing brood in the form of protection from potential brood predators and maintenance of the nest

site to prevent silt deposition and to aerate the nest site (Coble, 1975; Ridgway, 1988). During this parental care period, which lasts until the brood is independent (typically ~ 1 month), male bass are highly active while defending the brood (Hinch and Collins, 1991; Cooke et al., 2002). Although the fish restrict their activity to a localized area (e.g., 10 m^2), they can swim as much as 41 km per day while defending the nest (Cooke et al., 2002). In addition, 20% of the time is spent with the male engaged in high intensity activity (i.e., >80% of critical swimming speed; Cooke et al 2002). Concomitant with this increase in activity, males dramatically decrease foraging (Hinch and Collins, 1991) and suffer drastic declines in energy reserves and nutritional condition as endogenous resources are catabolized to power this activity (Mackereth et al., 1999; Cooke et al., 2006b; Hanson and Cooke, 2009). Opportunistic feeding has been shown at very low levels that would not compensate for energy loss associated with parental care activities (Hinch and Collins, 1991; Steinhart et al., 2005), though manipulative experiments have revealed that smallmouth bass can be fed supplemental food while on the nest (Ridgway and Shuter, 1994). While cessation of foraging is a common, and presumably adaptive, feature of bass parental care, to date no research has clarified the proximate causes and ultimate consequences of voluntary anorexia during this time period.

Cessation of foraging behaviour can be induced through modulation of various gut hormones (Badman and Flier, 2005; Abizaid and Horvath, 2008). Amongst these appetite hormones, ghrelin has been previously noted to relate to feeding behaviour and lipid deposition in a number of vertebrate species (Unniappan et al., 2004; Unniappan and Peter, 2004; Matsuda et al., 2006; Shephard et al., 2007; Kaiya et al., 2008). Particularly relevant to this study, experimentally induced increases in plasma ghrelin levels have been noted to stimulate foraging behaviour in a number of teleost fishes (Unniappan and Peter, 2004; Kaiya et al., 2008). Ghrelin also stimulates anabolic metabolism, principally the storage of lipids for later use as endogenous energy reserves (Riley et al., 2005; Unniappan and Peter, 2004; Kaiya et al., 2008), and increases in production of growth hormones (Unniappan et al., 2002; Unniappan and Peter, 2004; Kaiya et al., 2008). In the only study of the

relationship between gut hormones and energy utilization during the reproductive period in fish, fluctuations in plasma ghrelin immuno-reactive peptide levels have been noted across the spawning period in burbot (*Lota lota*) corresponding to decreased foraging prior to and during spawning and resumption of feeding afterwards (Mustonen et al., 2002). Since parental care in smallmouth bass is marked by a lack of foraging and catabolism of endogenous energy reserves, decreases in ghrelin production and receptor sensitivity could be the possible mechanism to induce this state.

In accordance with previous work on feeding and nutrition during parental care, we predicted that endogenous plasma ghrelin levels would be lowest during the egg and egg sac fry brood development stages (during times of restricted foraging and increased catabolic demands) and increase during the free swimming fry stage (indicating resumption of foraging by the parental male and increased lipid deposition). If we noted an increase in indicators of recent foraging and increased nutritional status of fish treated with exogenous ghrelin, we predicted that parental male bass suppress ghrelin production to maintain a state of voluntary anorexia during parental care. However, if treatment with exogenous ghrelin produced no changes in nutritional status, parental bass would apparently be reducing responsiveness to appetite hormones to cease foraging. Functionally, we predicted that cessation of foraging by nesting male bass is necessary to avoid decreases in swimming ability that would occur during digestion of prey items, thereby making a parental male unable to aggressively defend the brood from potential predators. In laboratory studies, researchers have documented that post-prandial blood flow to the gut increases which reduces swimming performance (Thorarensen and Farrell, 2006) and increases heart rate and oxygen consumption (Eliason et al., 2008). As such, we predict that fish which ingest high numbers of prey items should experience decreases in their ability to engage and chase brood predators after feeding.

Materials and Methods

Baseline Sampling of Endogenous Ghrelin across Parental Care

To determine natural appetite hormone fluctuation, sampling occurred from May 27th to June 12th, 2008 in a lake in eastern Ontario, Canada (44° 32' N, 76° 00' W). Snorkel surveys of the littoral zone were conducted to locate smallmouth bass that were actively guarding nests with newly deposited eggs (> 1 day old) at the commencement of the study. Upon location of an active bass nest, the snorkeler placed a numbered polyvinyl chloride (PVC) tile near the nest and recorded nest location and number of eggs within the nest (visual, categorical assessment ranging from low of 1 to high of 5; Suski and Philipp, 2004). At this point, individual fish were captured via heavy-action recreational fishing equipment from either the boat or underwater (by the diver) and landed at the boat in under 20 sec to minimize physiological disturbance related to anaerobic exercise. Fish were then placed in a foam lined sampling trough filled with fresh lake water and non-lethally blood sampled by the caudal puncture method using a 1.5", 21 gauge vacutainer syringe (Houston, 1990). Approximately 1.5mL (representing approximately 3.7% of total blood volume) of blood was collected in a 3mL, flat-bottomed vacutainer treated with lithium heparin to prevent blood coagulation and was then placed into a water-ice slurry. Blood samples were centrifuged immediately at 10,000x gravity for 5 min (Clay Adams Compact II Centrifuge). Two separate blood samples were stored in liquid nitrogen for later analysis: an unmodified sample to be used to for nutritional analysis as described in Hanson and Cooke (2008) and a sample for ghrelin analysis preserved with 10μL p-hydroxymercuribenzoic acid (PHMB) and 10 μL HCl per 1mL of plasma to reduce protease activity. Finally, total length was measured prior to release of the individual within 5m of the nest (sampling time mean ± S.D.; 128 ± 49sec). During the time that the fish were removed from the nest, the diver protected the nest from potential brood predators using a blunt pole. The preceding sampling procedure was repeated at three stages of brood development representing the entirety of the parental care period in smallmouth bass. Briefly, the brood development stages at which the male was sampled were fresh eggs (sampled within 1 day of spawning; n = 49), egg sac fry (newly hatched embryos, approximately 1.5 weeks after spawning; n =

14), and free swimming fry (larvae swim < 1m above and around the nest, prior to independence, approximately three weeks after spawning; n = 12).

Samples were analyzed for concentrations of plasma-borne ghrelin which has previously been identified as a primary appetite hormone in a number of fish species (Unniappan and Peter, 2004; Kaiya et al., 2008). Plasma samples were assayed in duplicate to determine the content of active (acylated) ghrelin using a commercially available radioimmunoassay (RIA) kit (Millipore, Billerica, Massachusetts). All samples were assayed together and had an intra-assay variability of 9.5%.

To determine the differences in plasma appetite hormone concentrations between stages of brood development during parental care, the mean plasma ghrelin concentrations of each brood development group were compared by one way analysis of variance (ANOVA) (Zar, 1999). All analyses were performed in the statistical package JMP v 7.0 and the level of significance for all tests (α) was assessed at 0.05. All values presented represent means ± S.D. unless otherwise noted.

Experimental Manipulation with Exogenous Ghrelin

To determine the role of ghrelin in regulating voluntary anorexia during parental care, a manipulation experiment was conducted concurrently with endogenous ghrelin sampling. Location of individual bass nests, capture of animals, blood sampling for nutritional and ghrelin analyses, and sample storage followed the methods described above. After capture at the egg stage, but prior to release, individual bass were randomly placed in one of two treatment groups. Fish in the control group (n = 11) were released without further intervention. Fish in the exogenous ghrelin group (n = 12) were intraperitoneally injected with rodent ghrelin (via 1", 21 gauge hypodermic needle at a dosage of 100μg of ghrelin [dissolved in physiological saline] per kg of fish). Previous studies have noted that injections of rat ghrelin at similar dosages induce feeding behaviour in teleost fishes (Shepherd et al., 2007). After experimental intervention, fish were released at the site of the nest. All experimental fish were recaptured when the brood developed to the egg sac fry stage and blood sampled for nutritional

and ghrelin analyses. There is a closed season for bass fishing during the reproductive period so it was illegal for members of the public to target or harvest fish from the study site.

In the laboratory, samples were analyzed for concentrations of plasma-borne biochemical indicators of individual nutritional status (total protein, triglycerides, and cholesterol) as well as dietary minerals (phosphorus, magnesium, and calcium) and enzymatic indicators of recent feeding ([alkaline phosphatase [ALP; enzyme number 3.1.3.1]) (Wagner and Congleton, 2004; Congleton and Wagner, 2006; Hanson and Cooke, 2009). All biochemical analyses were conducted on a Roche Hitachi 917 analyzer (Basal, Switzerland) based upon the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standard reference model. All nutritional 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. Plasma ghrelin levels were analyzed concurrently with samples from the previous portion of the study.

To determine the effect of exogenous ghrelin on feeding activity, the mean plasma concentrations of ghrelin and each nutritional factor mentioned above were compared between brood development stages by repeated measures analysis of variance (Repeated measures MANOVA) (Zar, 1999). All analyses were performed in the statistical package JMP v 7.0 and the level of significance for all tests (α) was assessed at 0.05. All values presented represent means ± S.D. unless otherwise noted.

Swimming Performance Experiment

To determine the effect of feeding and digestion on swimming performance, a separate study was conducted between May 15th and 17th, 2008. In total, 27 male smallmouth bass were included in the study. Following nest location, fish were randomly assigned to the following two treatment groups: 1) a group that was not fed, 2) a group that was fed local crayfish (*Orconectes virilis*) until satiation and

then sampled 3 hours after feeding, and 3) a group that was fed crayfish until satiation and then sampled 24 hours after feeding. When appropriate, fish were then captured via standard recreational angling gear in less than 10 seconds, placed in an annular swim flume filled with fresh lake water (Portz, 2007), and chased to exhaustion by application of tactile stimulus to the caudal region of the fish to induce burst swimming (Kieffer, 2000). Swim trials were digitally recorded (by a camera mounted directly above the flume) and analyzed to determine the number of burst swimming events (a measure of maximum swimming performance combining both burst and sustained swimming) and time elapsed prior to exhaustion (a measure of aerobic swimming performance) (Beamish, 1978; Drucker, 1996; Portz, 2007). Fish were then released within 5m of their nest. This protocol for swimming trials allowed fish to be returned to the nest without long term removal which would be required if we were using other swimming protocols such as critical swimming speed tests. Moreover, the swim flume was of sufficient size that it could be safely mounted on our 24 foot research vessel. Differences between burst swimming performance between the treatment groups was assessed by one-way ANOVA and Tukey's *post-hoc* test (Zar, 1999). The same statistical method was applied to determine differences in time to exhaustion between treatment groups (Zar, 1999).

Aggression Experiment

To determine the effect of foraging and exogenous ghrelin on parental aggression towards a potential brood predator, 21 male smallmouth bass guarding eggs were located on May 28th, 2008. Upon location of individual nests, a snorkeler subjected each male to an aggression test wherein a glass jar (volume = 3.78L) containing a small nest predator (bluegill, *Lepomis macrochirus*, TL = 172 ± 29mm) was placed on the rim of the nest and the number of aggressive acts ('hits' when a male made physical contact with the jar) performed by the parental male in a one minute time period was enumerated. Fish were then randomly assigned to the following two treatment groups: a control group (n = 7) that was not treated and, the ghrelin injection group (n = 7) described above. Fish from the

ghrelin treatment group were captured via standard recreational angling gear in under 10 seconds and placed in a foam lined surgery trough filled with fresh lake water and were intraperitoneally injected following study protocols. Twenty four hours later, a snorkeler relocated each nest and fed parental males to satiation by placing dropping crayfish into the area of the nest. Three hours after feeding, each individual was subjected to the aggression test.

Differences between the weight of crayfish (g) consumed by individuals in each treatment group were assessed by Students t-test (Zar, 1999). Additionally, simple linear regression was used to determine the relationship between the weight of crayfish consumed and percent change in aggression between the two days (Zar, 1999).

Results

Baseline Sampling of Endogenous Ghrelin across Parental Care

Baseline plasma ghrelin levels fluctuated across the parental care period in relation the stage of brood development (One-way ANOVA, d.f. = 2, 72, $F = 16.56$, $P < 0.001$; Table 5.1, Fig. 5.1). Specifically, plasma ghrelin levels were lowest during the egg (55.71 ± 25.32 pg/ml) and egg sac fry stages (82.83 ± 29.59 pg/ml) and the highest during the free swimming fry stage (207.61 ± 200.30 pg/ml) of brood development.

Experimental Manipulation with Exogenous Ghrelin

Twenty four hours after injection, plasma ghrelin levels (as measured in a subset of wild fish) were increased to 382.92 ± 182.49 pg/ml, almost seven times greater than the mean value of 55.71 ± 25.32 pg/ml found for fish at the egg stage that were not subjected to intervention. Prior to exogenous ghrelin manipulation, there were no significant differences between groups in plasma ghrelin levels at the egg stage of brood development (Repeated measures MANOVA; Tables 5.1, 5.2). Additionally, indicators of nutritional status and recent foraging activity did not differ significantly between groups at

the egg stage (Tables 5.2, 5.3). Following injection, plasma ghrelin levels in ghrelin injected fish increased ~170% from the egg stage to the egg sac fry stage (Repeated measures MANOVA; Tables 5.1, 5.2; Fig. 5.2). However, no blood borne indicators of nutritional status or recent foraging activity differed between groups (Tables 5.2, 5.3).

Swimming Performance Experiment

Fish that were not fed showed a higher number of burst swimming events than either group of fed fish indicating a loss of swimming performance as a result of digestion (One-way ANOVA, d.f. = 2, 24, F = 3.45, P = 0.048, Fig. 5.3A). Additionally, swimming performance is impaired by digestion starting as early as 3 hours after feeding and lasting for up to 24 hours after feeding (One-way ANOVA, d.f. = 2, 24, F = 3.45, P = 0.048, Fig. 5.3A). However, there was no difference in time elapsed until exhaustion between the treatment groups (One-way ANOVA, d.f. = 2, 23, F = 1.96, P = 0.16, Fig. 5.3B).

Feeding Experiment

There were no differences in crayfish consumption (g) between the control (20.30 ± 15.67g) and ghrelin injected (8.79 ± 13.86g) treatment groups (Student's t-test, d.f. = 8, t = 1.31, P = 0.22). Amongst fish that ingested food items, there was a negative relationship between the weight of crayfish consumed and percent change in aggression between the two days, with fish that consumed greater a greater amount of crayfish showing reductions in aggression towards a potential brood predator (Simple Linear Regression, d.f. = 12, F = 8.09, P = 0.016, Fig. 5.4).

Discussion

In the current study, plasma ghrelin levels were lowest during the egg (55.71 ± 25.32 pg/ml) and egg sac fry (82.83 ± 29.59 pg/ml) stages of brood development before increasing to 207.61 ±

200.30 pg/ml during the free swimming fry stage (Fig. 5.1). During the early stages of parental care when the male defends the brood in a localized area around the nest, foraging behaviour dramatically decreases (Ridgway, 1988; Hinch and Collins, 1991; Cooke et al., 2002). Previous research has speculated that decreased foraging behaviour is a result of decreased opportunity of finding suitable forage in the area of the nest (Ridgway, 1988; Hinch and Collins, 1991), though individual bass have been noted to remove potential food items (small bodied fishes, invertebrates) from the vicinity of the nest without consuming them (David Philipp and Steven Cooke, personal communication). As ghrelin has been noted to be a hormonal cue initiating voluntary foraging in teleost fishes (Unniappan and Peter, 2004; Volkoff et al., 2005), decreases in plasma ghrelin may be necessary to induce voluntary anorexia during parental care.

Concomitant with this decline in foraging, parental male bass are extremely active (Cooke et al., 2002) with localized movements powered through mobilization of endogenous energy reserves in the form of muscle and liver lipid stores (Mackereth et al., 1999). The sum total of this energetic dilemma featuring a massive increase in activity with a massive decrease in energy uptake through foraging is loss of endogenous energy reserves (Mackereth et al., 1999; Cooke et al., 2006b), decreases in indicators of nutritional physiology (Hanson and Cooke, 2009), and potential loss of body mass (Cooke et al., 2002). Reduction of ghrelin levels would allow parental male bass to enter the catabolic state described above as plasma ghrelin levels have been shown to be positively related to lipid deposition in teleost fishes (Unniappan and Peter, 2004). High plasma ghrelin levels are simply incompatible with the requirements of parental care (specifically the need for energy utilization and decreased foraging) of male bass.

As the brood develops to the free swimming fry stage, the fry develop the ability to swim and spread out across a large area which the male patrols to defend the brood, thereby increasing the probability that a male will encounter a suitable forage item (Friesen and Ridgway, 2000). At this time, increases in blood borne nutritional factors such as dietary minerals indicate that males begin to forage

at this time (Mackereth et al., 1999; Steinhart et al., 2005; Hanson and Cooke, 2009). The timing of the increase in minerals derived from foraging corresponds to the timing of the increase in plasma ghrelin during the free swimming fry stage (Fig. 5.1). Mustonen et al. (2002) attributed similar increases in appetite hormone levels following spawning in burbot as a mechanism to increase appetite and foraging behaviour in spawned individuals to replenish exhausted energy stores. In the current study, we also believe increases in ghrelin production would be necessary to initiate increased foraging following parental care and enter into an anabolic state to replenish endogenous energy reserves. Additionally, it has been theorized that complete over depletion of energy reserves during a single parental care period can be linked to individual mortality during the following winter (Mackereth et al., 1999). As such, it may be necessary for bass to resume foraging and lipid deposition as soon as the brood becomes independent to ensure survival through the year and the possibility of future reproductive opportunities.

This study also provides evidence that parental male bass show receptor insensitivity to ghrelin during the early stages of parental care. Previous studies have noted that the structure and function of ghrelin is highly conserved amongst vertebrates (Kaiya et al., 2008) and multiple researchers have used exogenous injections of rodent ghrelin to induce physiological changes and voluntary feeding in teleost fishes (Unniappan and Peter, 2004; Volkoff et al., 2005). In the current study, treatment fish were subjected exogenous injections of rodent ghrelin at the egg stage of brood development that resulted in artificially increased plasma ghrelin concentrations (382.92 ± 182.49 pg/ml hours after injection) which persisted for at least one week until the brood developed to the egg sac fry stage (Fig. 5.2). However, ghrelin injected fish did not feed at elevated levels twenty four hours after injection when compared to controls (Fig. 5.4), though studies that have documented the orexigenic effects of ghrelin in fish have either measured voluntary foraging in the first hour after exposure (Matsuda et al., 2006; Miura et al., 2007) or did not show an effect at a longer time scale (Jonsson et al., 2007). Additionally, ghrelin treated fish showed no significant differences in plasma values of multiple nutritional and energetic indicators of fasting (Congleton and Wagner, 2006; Hanson and Cooke, 2009). In effect, even though

the hormonal cue to increase foraging and switch to an anabolic state was present in the blood stream at levels similar to those at the end of parental care, no physiological indicators of feeding were noted indicating that the action of the hormone was likely blocked. This resistance to the action of ghrelin could be reinforced through receptor insensitivity (growth hormone secretagogue receptor type 1a [GHS-R1a]) in the hypothalamus as receptor expression has been shown to be positively related to ghrelin levels (Camiña, 2006). Additionally, though not measured in the current study, other endocrine factors, such as leptin and growth hormone, may be involved in potentially inducing receptor insensitivity (Camiña, 2006). Functionally, it appears that individual parental bass regulate foraging and energy utilization through a combination of cessation of production of ghrelin coupled with redundant receptor insensitivity.

Ultimately, anorexia during the parental care period may be an adaptive behaviour that prevents loss of offspring through brood predation. Multiple studies have noted that swimming performance and digestion are temporally incompatible due to constraints on imparted through competing requirements for blood flow (Thorarensen et al., 1993; Alsop and Wood, 1997; Farrell et al., 2001). Digestion of food items requires a shift in blood flow to the viscera from the swimming musculature that can often last for over 24 hours after ingestion (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Thorarensen et al., 1993; Thorarensen and Farrell, 2006). This increase in blood flow is required for a myriad of processes required for catabolism of food items and results in an increase in metabolic rate referred to as specific dynamic action (SDA) (reviewed in McCue, 2006). In rainbow trout (*Oncorhynchus mykiss*), Alsop and Wood (1997) showed that, as fish exhibit an absolute maximum oxygen consumption, increases in SDA following feeding reduce the portion of the scope for activity that can be devoted to swimming metabolism due to digestive requirements reducing the amount of oxygen available to swimming muscles, thereby reducing critical swimming speed. Similarly, in smallmouth bass, Beamish (1974) noted that increases in oxygen consumption due to digestion of a 4% ration mirrored increases in oxygen consumption required to swim at up to 2.5 body lengths per

second. In the current study, we noted that repeated burst swimming activity decreased in fish that were fed to satiation twenty four hours earlier when compared to unfed controls (Fig. 5.3A). Repeated burst swimming, as calculated in the current study, is a measure of the maximum swimming performance of the animal consisting of anaerobic muscular activity (the repeated burst swim events; Beamish 1978). As such, both decreased blood flow to swimming musculature coupled with the metabolic demands of SDA would impact the maximum swimming performance of the individual by impairing the ability to maintain swimming metabolism and preventing further anaerobic swimming activity (Alsop and Wood, 1997). During parental care, parental bass regularly engage in burst swimming to chase potential brood predators from the vicinity of the nest (Hinch and Collins, 1991; Cooke et al., 2002). Failure to vigorously defend the brood in this manner results in reproductive failure as the male will often abandon a brood that has been severely depredated (Philipp et al., 1997; Steinhart et al., 2004). We also noted that the amount of prey consumed was negatively related to changes in aggression towards a simulated brood predator (Fig. 5.4). Even though supplemental foraging at high levels could mitigate the energetic decline experienced by adult males, this would occur at a potential cost to offspring survival and individual fitness. Given that parental care aims to maximize offspring survival at a cost to the condition of the parent (Trivers, 1972; Gross and Sargent, 1985; Gross, 2005), the ultimate cost of supplemental foraging to offspring survival negates any benefits to individual fitness incurred through engaging in parental care. As such, voluntary anorexia would be a required component of parental care by a male bass to successfully raise a brood.

In conclusion, voluntary anorexia during the parental care period in smallmouth bass appears to be an adaptive behaviour aimed at avoiding decreases in swimming ability and parental aggressiveness that are needed to defend the brood and that could lead to potential decreases in reproductive success and fitness if impaired. This behaviour seems to be modulated through a combination of declines in production of ghrelin coupled with a decrease in receptor sensitivity to this appetite hormone. Further research into the mechanism of induced voluntary anorexia during parental care should focus on the

role of complimentary appetite hormones such as leptin or endocrine factors such as cortisol, as well as studies at the level of the receptor and genes.

Tables

Table 5.1: Parental male smallmouth bass plasma ghrelin concentrations at three stages of brood development during parental care. Values are presented as mean ± S.D. with minimum and maximum in parentheses.

	Egg	Egg sac fry	Free swimming Fry
Non-injected control fish (pg/mL)	55.71 ± 25.33 (15.10 – 164.90) N = 49	82.83 ± 29.59 (31.19 – 133.90) N = 14	207.61 ± 200.31 (64.67 – 798.40) N = 12
Saline injected fish (pg/mL)	38.71 ± 16.04 (15.1 – 70.39) N = 8	74.49 ± 33.26 (37.62 – 124.80) N = 8	N/A
Exogenous ghrelin injected fish (pg/mL)	59.59 ± 37.12 (23.57 – 164.9) N = 12	101.15 ± 51.27 (38.14 – 226.70) N = 12	N/A

Table 5.2: Results of repeated measure multiple analysis of variance (MANOVA) comparing indicators of nutrition and recent foraging activity among two groups of parental smallmouth bass (one uninjected control group and one group injected with rodent ghrelin) across the first two stages of brood development (egg, egg sac fry). Significant differences at $\alpha = 0.05$ are indicated by bold and italicized font.

	Source	d.f.	F-ratio	P-value
Ghrelin	Brood Stage	1, 20	4.29	0.05
(pg/mL)	Treatment	1, 20	3.09	0.09
	Brood Stage*Treatment	***1, 20***	***6.19***	***0.02***
ALP (U/L)	Brood Stage	1, 21	13.16	0.002
	Treatment	1, 21	0.02	0.90
	Brood Stage*Treatment	1, 21	0.04	0.84
Calcium	***Brood Stage***	***1, 21***	***10.83***	***< 0.001***
(mmol/L)	Treatment	1, 21	0.07	0.80
	Brood Stage*Treatment	1, 21	0.88	0.36
Cholesterol	Brood Stage	1, 21	0.81	0.38
(mmol/L)	Treatment	1, 21	0.29	0.60
	Brood Stage*Treatment	1, 21	0.05	0.83
Magnesium	***Brood Stage***	***1, 21***	***41.20***	***< 0.001***
(mmol/L)	Treatment	1, 21	0.13	0.73
	Brood Stage*Treatment	1, 21	2.80	0.11
Phosphorous	***Brood Stage***	***1, 21***	***8.65***	***< 0.001***
(mmol/L)	Treatment	1, 21	0.51	0.48
	Brood Stage*Treatment	1, 21	0.13	0.73
Triglycerides	Brood Stage	1, 21	0.73	0.40
(mmol/L)	Treatment	1, 21	0.05	0.83
	Brood Stage*Treatment	1, 21	2.27	0.15
Total Protein	***Brood Stage***	***1, 21***	***6.07***	***0.03***
(g/L)	Treatment	1, 21	0.41	0.53
	Brood Stage*Treatment	1, 21	0.06	0.81

1627 Table 5.3: Concentrations of plasma borne indicators associated with nutritional status and recent foraging activity for three groups of
1628 experimentally manipulated nest guarding male smallmouth bass (non manipulated controls, fish injected with physiological saline,
1629 and fish injected with rodent ghrelin) at two stages of brood development during parental care. Values are presented as mean ± S.D.
1630 with minimum and maximum in parentheses.

	Egg - Control	Egg – Saline injected	Egg – Ghrelin injected	Egg sac fry - Control	Egg sac fry – Saline injected	Egg sac fry – Ghrelin injected
ALP (U/L)	21.64 ± 9.61 (13 – 40) N = 11	19.38 ± 8.42 (10 – 33) N =8	21.75 ± 13.83 (9 – 48) N =12	12.92 ± 5.42 (7 – 27) N =11	12.63 ± 2.56 (9 – 16) N =8	12.42 ± 4.34 (6 – 22) N =12
Calcium (mmol/L)	2.48 ± 0.11 (2.29 – 2.70) N =11	2.89 ± 1.23 (2.34 – 5.91) N =8	2.52 ± 0.15 (2.22 – 2.81) N =12	2.59 ± 0.11 (2.43 – 2.75) N =11	3.20 ± 1.66 (2.44 – 7.28) N =8	2.59 ± 0.18 (2.41 – 3.04) N =12
Cholesterol (mmol/L)	11.95 ± 2.84 (7.3 – 17.3) N =11	10.4 ± 1.85 (8.2 – 13.2) N =8	11.18 ± 3.79 (5.3 – 16.5) N =12	11.93 ± 2.71 (8.3 – 17.0) N =11	10.33 ± 1.79 (6.9 – 12.4) N =8	11.38 ± 3.63 (5.7 – 17.5) N =12
Magnesium (mmol/L)	1.19 ± 0.12 (1.04 – 1.41) N =11	1.18 ± 0.21 (1.01 – 1.65) N =8	1.25 ± 0.16 (1.01 – 1.51) N =12	1.08 ± 0.06 (1.00 – 1.19) N =11	1.17 ± 0.33 (0.9 – 1.96) N =8	1.06 ± 0.07 (0.95 – 1.12) N =12
Phosphorous (mmol/L)	1.76 ± 0.35 (1.3 – 2.5) N =11	1.85 ± 0.50 (1.4 – 3.0) N =8	1.81 ± 0.30 (1.4 – 2.4) N =12	1.58 ± 0.13 (1.4 – 1.8) N =11	1.91 ± 0.75 (1.4 – 3.7) N =8	1.66 ± 0.20 (1.4 – 2.0) N =12
Triglycerides (mmol/L)	2.72 ± 0.50 (1.91 – 3.76) N =11	3.64 ± 3.33 (1.95 – 11.76) N =8	2.45 ± 0.46 (1.51 – 3.28) N =12	2.39 ± 0.63 (1.78 – 3.68) N =11	3.71 ± 4.03 (1.19 – 13.48) N =8	2.54 ± 1.05 (1.18 – 4.80) N =12
Total Protein (g/L)	41.00 ± 2.57 (37 – 44) N =11	38.63 ± 2.45 (36 – 44) N =8	40.25 ± 3.86 (34 – 46) N =12	40.83 ± 2.31 (37 – 43) N =11	39.88 ± 3.98 (37 – 48) N =8	39.25 ± 3.89 (33 – 47) N =12

Figures

Figure 5.1: Changes in baseline plasma ghrelin levels in nest guarding male smallmouth bass across three stages of brood development (egg, egg sac fry, and free swimming fry) during the parental care period. Letter assignments of "a" and "b" denote significant ($P < 0.05$) differences among brood development stages. Error bars show mean ± S.E.

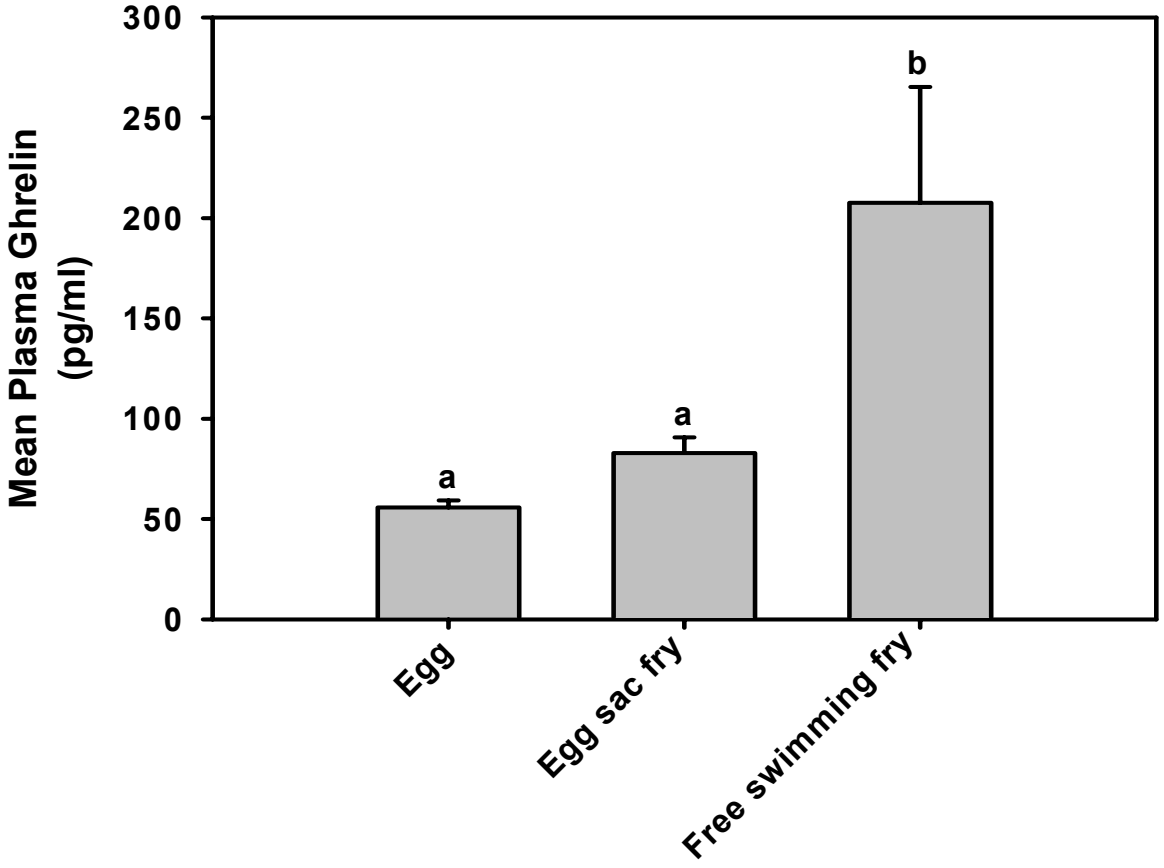


Figure 5.2: Changes in plasma ghrelin levels in nest guarding male smallmouth bass across two stages of brood development (egg [dark bars] and egg sac fry [light bars]) subjected to exogenous ghrelin injection during the parental care period. Letter assignments of "a" and "b" denote significant ($P < 0.05$) differences among brood development stages. Error bars show mean ± S.E.

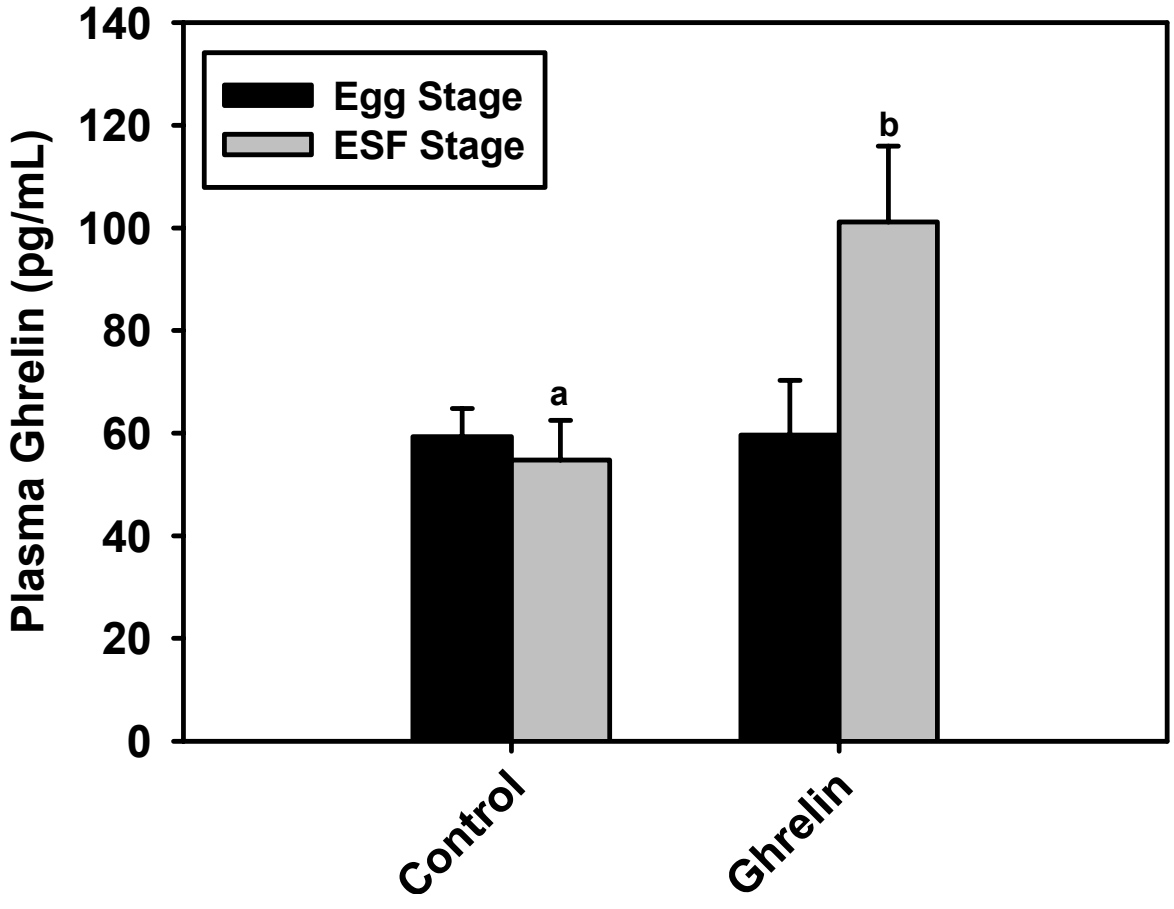
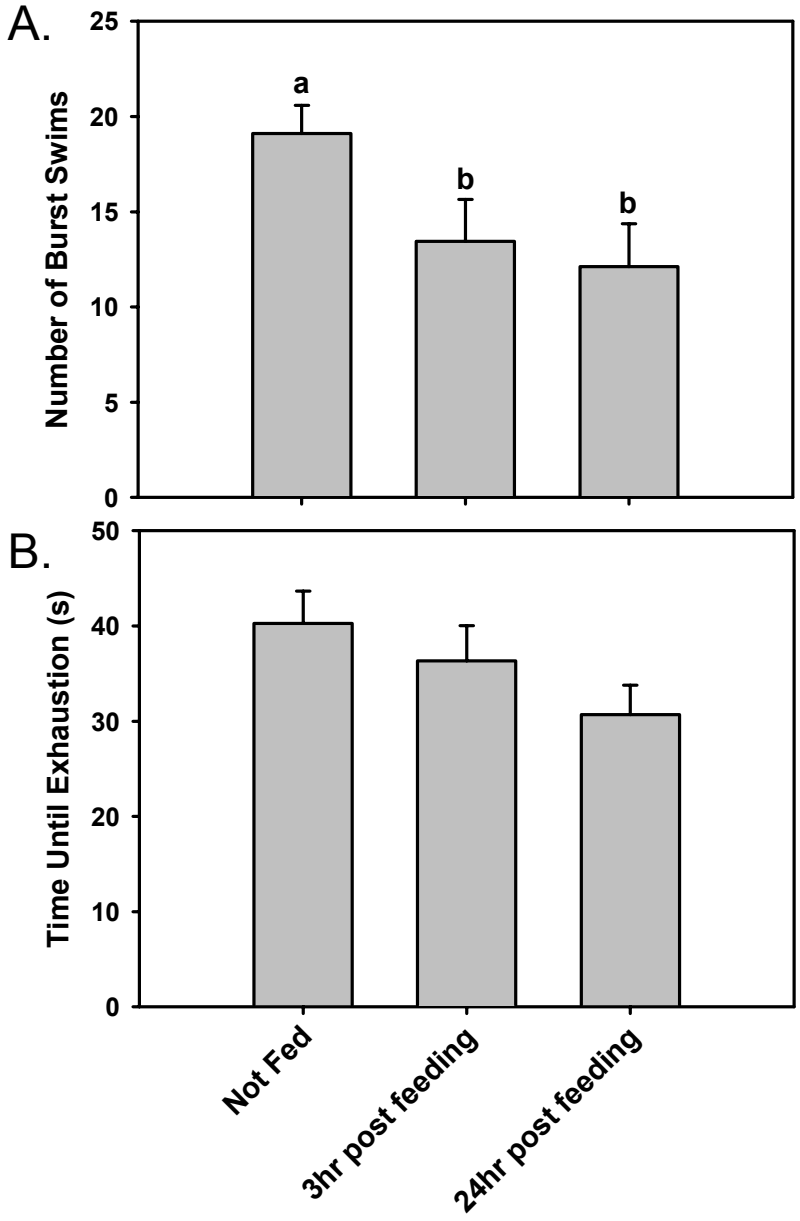
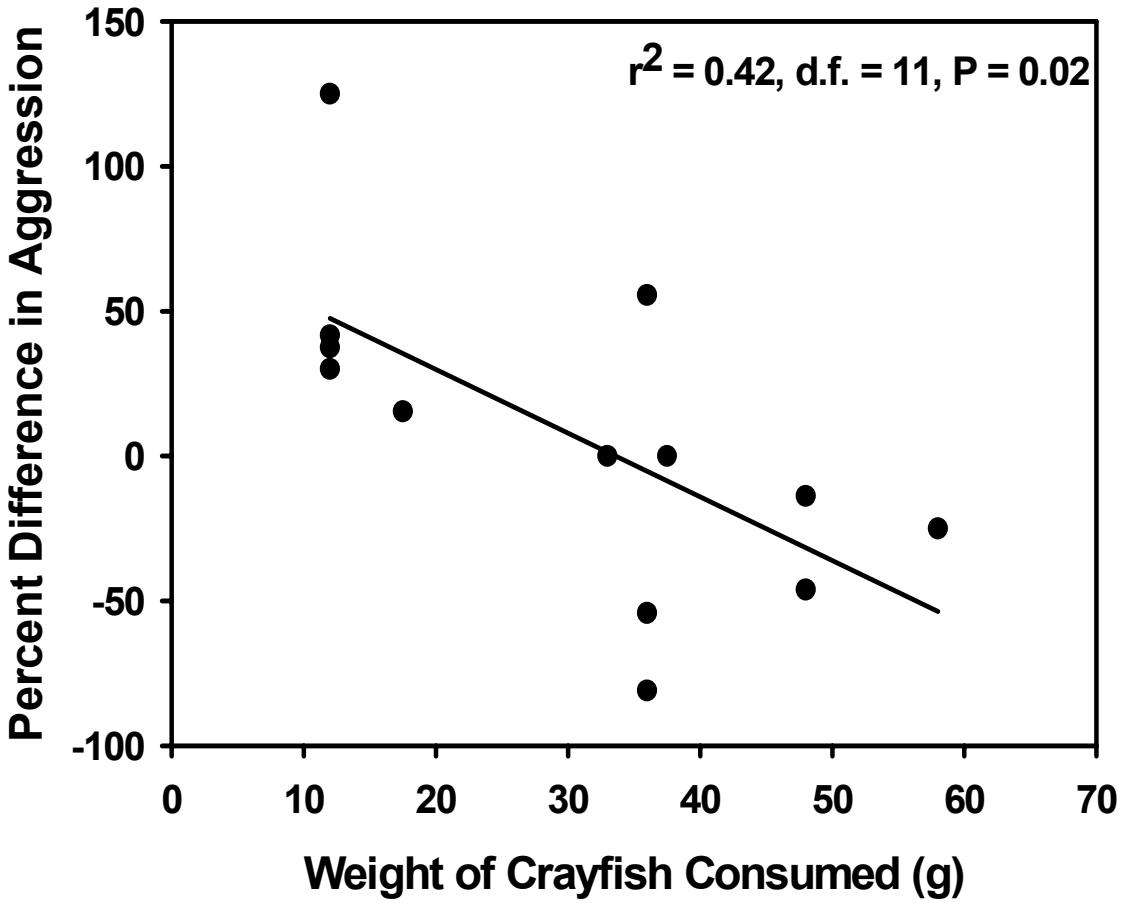


Figure 5.3: Changes in swimming performance (A. number of burst swims, B. time until exhaustion [s]) among nest guarding male smallmouth bass subjected to swimming trials at three time periods (non fed controls, three hours after feeding to satiation, 24 hors after satiation). Letter assignments of "a" and "b" denote significant ($P < 0.05$) differences among brood development stages. Error bars show mean ± S.E.



1651 Figure 5.4: Percent change in nest guarding male smallmouth bass aggression towards a
1652 simulated brood predator the parental care period 24 hours after feeding to satiation.



1653

Chapter 6: The relationship between individual physiological traits and fitness in wild fish: Myth or reality?

Abstract

It has been widely acknowledged that heritable variation in physiological traits exist among individuals in a population providing the raw material upon which natural selection can act. To date, there are few examples in the literature linking variation in physiology to fitness differences among individuals. Historically, researchers contended that the apparent lack of a clear relationship was the result of poor experimental design and inference of fitness relationships from observational data. As a result, significant effort has been devoted to the design of manipulative experiments, though demonstrable links between fitness and physiological variation continue to be elusive. Indeed, in our own work spanning the reproductive migration of Pacific salmon (*Oncorhynchus* spp.) and parental care in black bass (*Micropterus* spp.), we often fail to detect significant influences of organismal physiology on fitness, or find that such relationships are subtle, complex (e.g., often inter-related with behaviour and morphology), and context dependent (e.g., life-history, seasonal). We provide a set of limitations that may continue to affect the ability to determine the extent to which organismal physiology is related to the fitness of fish and other animals in the wild including a current lack of understanding of the full range of physiological diversity, potential concerns with the scale at which physiological measurements are performed, and the complications of collating and interpreting the results from field and laboratory studies. We also provide possible solutions to circumvent these limitations by merging the established fields of behavioural

ecology and field physiology and adopting new sampling regimes and advanced technologies. For wild fish, innovations in non-lethal biopsy, biotelemetry and field intervention experiments hold the most promise for identifying and understanding the elusive physiology-fitness interface. As human activities continue to stress aquatic systems around the globe, there is an increasing urgency for studying and understanding how physiological diversity at the level of the individual can affect fitness, demography, and evolutionary processes.

Introduction and Focus

The concept of evolutionary physiology (see Garland and Carter, 1994; Feder et al.,, 2000) is originally rooted in the work of Charles Darwin. Darwin (1895) observed that variation in traits will be acted upon by natural selection thereby favoring certain traits over others and leading to increased reproductive success (fitness) for individuals who express those traits and, ultimately, providing the opportunity for evolution to occur. Similar to the work of Darwin (1895), evolutionary biologists have primarily focused on the relationships between fitness and morphological, behavioural, and life history traits (Endler, 1986; Lessells, 1991). Within this framework, it is widely accepted that natural selection operates on the whole-organism level that requires the interplay of multiple organismal features ranging from microscale physiological processes to macroscale traits such as whole-body morphology or individual behaviour. However, there is a paucity of research investigating the relationship between individual physiological variation and individual fitness, even though these links have long been theorized (Endler, 1986; Feder, 1987; Feder, 2000) or inferred from data (Spicer and Gaston, 1999; Feder, 2000; Irschick, 2003). Existing patterns in variation of physiological traits and evidence for the heritability of these traits suggest that the raw material for natural selection is present within populations leading to the inference that natural selection acts upon physiological traits (Feder, 1987; Spicer and Gaston, 1999). Given that this individual variation in a number of physiological parameters exists, a logical conclusion is that the variation in these traits should relate to differences in individual fitness.

The goals of this paper will be to determine the state of current research with respect to the relationship (or lack thereof) between organismal physiology and individual

fitness, illustrate potential limitations in current research, and provide suggestions to overcome these issues. For the purposes of this paper, we focus on wild fish using semelparous Pacific salmon (*Onchorhynchus* spp.) and iteroparous black bass (*Micropterus* spp.) as models, being mindful of research on other taxa and general theoretical constructs and paradigms. We contend that our perspectives have broader utility beyond wild fish and can be adopted by researchers studying a wide range of animal taxa.

Teleost fishes as a model

Our rationale for focusing on fish is partly as a pragmatic convenience in that we have been studying the relationship between physiology and fitness in these animals for a number of years and in a number of different contexts. However, wild fish, and our two models, are also useful comparative models in that Pacific salmon are capital breeders while black bass are iteroparous. Beyond the sheer numbers of species and wide geographical distributions, teleost fish make ideal research models for a number of reasons. For a vertebrate taxa, fish species have an enormous diversity of life history traits (Adams, 1980; Thorpe, 1990; Winemiller and Rose, 1992; Rochet, 2000; King and McFarlane, 2003; Young et al.,, 2006). This variation in life history traits includes species body size (Alm, 1959), generation time (Beverton and Holt, 1959), and sexual dimorphism in multiple morphological and physiological traits (Hanson et al.,, 2008b). Fish also show a great diversity of reproductive behaviours including age at maturation, breeding systems (Alm, 1959; Beverton, 1992; McCann and Shuter, 1997) and intensity and diversity of parental care tactics (Gross and Sargent, 1985) which lend to the

potential for a vast array of differences in reproductive physiology. Many fish species also exhibit plastic phenotypic response to a number of biotic and abiotic factors (Klementsen et al., 2003). Finally, for ease of research, some species may be acclimated to the lab environment enabling highly controlled experimental designs (Stickney and Kohler, 1990; Young et al., 2006). Additionally, many behaviours, perhaps best illustrated by long distance migrations of Pacific salmon (Hinch et al., 2005; Wilcove and Wikelski, 2008), require a complex integration of behaviour, performance, and physiology by the organism to survive and secure fitness.

In addition to the biological rationale outlined above, as a result of the economic (i.e., sale of protein [Burger, 2002] or recreational fisheries [Arlinghaus and Cooke, 2008]) and ecosystem value (Holmlund and Hammer, 1999) of fish, they are subject to intensive harvest and fisheries management strategies (Pauly et al., 2002; Pauly et al., 2003), perhaps more so than any other vertebrate taxa. An important component of fisheries management activities is being able to predict how different management regimes will influence demographics, something that makes physiology a potentially useful tool if there is a relationship between physiology and fitness (see Young et al., 2006). Current conservation strategies have begun to acknowledge the usefulness of physiological measures to guide studies of fish and ecosystem health with the goal of sustainably managing stocks or species in the face of environmental and anthropogenic disturbances (Pauly et al., 2002; Wikelski and Cooke, 2006; Young et al., 2006). However, the integration of physiological data into population dynamic models is currently problematic due to the lack of established relationship between physiological traits and fitness. Rapid assessment of differential fitness via non-lethal physiological

measures would be an incalculably valuable tool for the management of economically valuable fish species. Fish, particularly in inland waters (e.g., Allan et al., 2005), are one of the most imperiled group of wild organisms, facing threats from human-induced disturbance, environmental change, and stress, increasing the urgency for studying and understanding if and how physiological variation and stress at the level of the individual can affect fitness and population processes.

Limitations of current research

Empirical testing

An often cited reason for the lack of clearly documented relationships between individual fitness and physiological traits is the lack of adequate empirical testing to identify such relationships (Arnold, 1983). Even though the theoretical framework for many modern tests of these relationships was described in the early 1980's (Arnold, 1983), through the late 1990's there were calls (e.g., Bennett, 1987; Feder, 1987) for more strenuous empirical testing rather than the more prevalent pattern descriptions present in the literature. At that time, through adaptive reasoning, most studies would argue that variation in physiology was rooted in variation in physiological traits between individuals of an ancestral form that was acted upon by natural selection, thereby leading to differential fitness amongst individuals of a species and subsequent evolution (Feder, 1987). Thus, a common facet of comparative physiology at the time was to contrast two unlike species that varied drastically in physiology and a corresponding behaviour or life history trait and then infer that this variation was the result of natural selection having acted upon ancestral individuals (Feder, 1987). Unfortunately, in nearly all studies, the

1776 actual relationship between individual variation in physiology and subsequent fitness of
1777 extant species was not empirically tested (Feder, 1987; Spicer and Gaston, 1999).
1778 Recently, as evidenced through numerous literature reviews (Feder et al., 2000; Irschick,
1779 2003; Kingsolver and Huey, 2003) there has been an increased usage of properly
1780 designed field and laboratory tests of the relationship between fitness and physiology,
1781 primarily through the intermediate step of organismal performance.
1782 Another potential limitation with current research is the analytical approach
1783 applied to physiological datasets. Due to commonly utilized statistical techniques (e.g.,
1784 based on means), researchers often disregard variation in measurements due to the aptly
1785 termed, 'tyranny of the golden mean' (Kolok, 1999; Williams, 2008). Previously,
1786 variation between individuals was often disregarded in many ecological and
1787 physiological studies as statistical noise or measurement error, and thereby not
1788 considered as real (Bennett, 1987) despite the fact that this variation had been noted for
1789 some time (Prosser, 1955). Recently, researchers have begun to note that individual
1790 variation exists and it is rooted in differences of genetics, development, and the interplay
1791 of these factors with current environmental conditions and can have significant
1792 ramifications for overall organismal performance (Bennett, 1987; Spicer and Gaston,
1793 1999; Ghalambhor et al., 2003). Moreover, many current studies have noted that these
1794 patterns of individual variation in physiological traits may result in differential fitness
1795 between individuals, allowing for empirical tests of the relationship between individual
1796 physiology and fitness.
1797
1798 *Range of variation and sampling concerns*

Variation in single physiological mechanisms may occur within a broad range of functionally optimal values before a precipitous drop into dysfunction at a certain threshold. For example, the functional window of concentrations of inorganic minerals within an organism may fluctuate greatly, with little impacts on organismal homeostasis until a certain upper or lower threshold is crossed and the organism suffers from dysfunction in enzymatic processes possibly leading to mortality (Lall, 2002). Similar patterns have been noted in metabolic scope for activity (Fry, 1971; Priede, 1985), oxygen consumption (Farrell and Steffensen, 1987; Lee et al., 2003), and cardiovascular performance (Priede, 1977; Farrell, 1996). If organismal homeostasis has a broad functional window bounded by an upper and lower threshold, only extreme variation in a physiological trait would result in a fitness differential through individual mortality, therefore sampling of living, non-moribund animals does not show an accurate range of variation in said physiological trait. This notion interconnects with sampling stratagems commonly employed by researchers. Typically, sampling of wild animals focuses on active (and presumably healthy) individuals through the capture of active animals using a passive restraining device (e.g., netting or angling for fish [Hayes et al., 1996]) or observation of animals partaking in 'normal' behaviours (e.g., reproduction, feeding, migration, etc.). In general, animals that would be suffering from moderate to severe physiological dysfunction may not be active enough to be captured using the above methods or partaking in normal behaviours that would make them obvious to observers and would thereby be excluded from analyses resulting in little to no information gathered on individuals of this type.

For example, studies of Pacific salmon spawning migrations have focused specifically on determining variation in many physiological traits (primarily regarding bioenergetics, osmoregulatory status, and reproductive hormones) that lead to abnormal migration timing and failure to spawn in sockeye salmon (*O. nerka* [Walbaum]) (Cooke et al., 2008a). While these studies have noted some relationships between physiological variables and fitness (as measured by the successful arrival of an individual at or near the spawning grounds) (e.g., Cooke et al., 2004a; Cooke et al., 2006a), these studies are constrained by the fact that capture methods (such as commercial netting in the ocean and river) focus on healthy individuals actively participating in migration, and tend to disregard the most physiologically compromised and moribund individuals as these individuals are unlikely to participate in migratory behaviours. Additionally, individuals that fail to migrate at all are never captured. Research at the spawning grounds focus on both healthy and moribund individuals (i.e., fish that die before expelling their gametes), but individuals that survive to reach the spawning grounds represent a minor subset of the overall population. Though a large proportion of individuals are sampled at any given time, the full extent of variation in any parameter can not be sufficiently measured given the current technology and capture techniques. In this instance, researchers are limited by these techniques and can not overcome this obstacle without advances in technology allowing for detection and capture of individuals who fail to participate in migration and succumb to mortality in the high seas for currently unknown reasons.

Research into the links between physiological variation and individual fitness in black bass are similarly hampered by sampling concerns. Again, studies have noted variation in s a host of physiological traits during the parental care period in bass

(cardiovascular performance [Cooke, 2004]; energetic and nutritional factors [Hanson and Cooke, 2009]), though the physiological variation documented by these studies is again constrained by sampling stratagems. Research during the parental care period of bass focuses on male bass that were successful in territory establishment, spawning, and initiating parental care (Hanson and Cooke, 2009) and males that were unable to successfully complete any of these activities are not sampled. Due to the extreme energetic requirements of parental care (Cooke et al., 2002; Cooke et al., 2006a), it has been theorized that the subset of individuals that successfully spawn is dictated by individual energetic status prior to the reproductive period (Mackereth et al., 1999). If this is the case, by sampling only animals involved in parental care, researchers have likely inadvertently selected a subset of the population with a relatively homogenous physiological status when compared to the population at large.

Scale of variation

Strong evidence linking differences in individual fitness to physiological processes is still scant and may be attributed to a variety of other reasons. As mentioned above, many studies of fitness differential between individuals focus on macroscale, whole organism traits such as locomotory performance (Kingsolver and Huey, 2003; Irschick, 2003; Husak and Fox, 2006; Peterson and Husak, 2006), personality (Dall et al., 2004), and social dominance (Booth, 1995). It has long been recognized that organismal performance, particularly locomotory performance, is influenced by underlying physiological mechanisms (Prosser, 1955; Kolok, 1999; Feder, 2000; Ghalambor et al., 2003). Additionally, evolutionary biologists have long accepted the fact that adaptive

1867 evolution of physiological and morphological traits occurs through the natural selection
1868 of whole organism performance (Bartholomew, 1958; Huey and Stevenson, 1979;
1869 Arnold, 1983; Huey, 1983; Feder, 2000; Ghalambor et al., 2003). Research into the
1870 selection of traits on the suborganismal level generally includes empirical tests of whole
1871 organism performance as the mechanistic link between phenotypic traits and adaptive
1872 evolution (Irshick, 2003; Cooke et al., 2006a; Peterson and Husak, 2006).
1873 Within this framework, the role of a single physiological trait is not well
1874 understood nor has it been properly tested. While differences in single physiological
1875 measures (i.e., a single biochemical constituent) amongst individuals may exist in a
1876 readily measureable and statistically testable form, resulting statistical significance may
1877 not be rooted in biological significance due to subtle interactions with many other single
1878 traits/parameters required to elicit a whole organism response that would be subject to
1879 natural selection. In short, due to subtle interaction among a host of processes to elicit a
1880 whole organism phenotype, it may be possible that variation in no single physiological
1881 trait in and of itself may be capable of eliciting differences in individual fitness. Hence,
1882 measuring a single variable would not provide a proverbial "smoking gun". If this is the
1883 case, macroscale variables such as those described above may be the finest scale
1884 resolution to which physiological variation can be acted upon by natural selection and, as
1885 such, should be the natural focus of research efforts.
1886 In the case of Pacific salmon, Cooke et al. (2006) examined the physiological
1887 correlates of migration failure (which in turn leads to a complete loss in fitness for the
1888 individual) in sockeye salmon. Sockeye salmon characterized by high plasma values of
1889 lactate, glucose, cortisol, Na^+ and osmolality generally failed successfully enter the river

on their way to natal spawning grounds (Cooke et al., 2006a). Additionally, all fish characterized by low energetic status as well as females with high values of circulating reproductive hormones typically suffered higher mortality in the river (Cooke et al., 2006a). Similarly, swimming speed during migration was affected by a host of physiological variables (again including energetic status, plasma ion levels, and reproductive status) but rarely in a consistent manner that would allow a clear cut relationship between a single physiological variable and migration performance (Hanson et al., 2008a). While no single physiological variable was significantly predictive of mortality or performance, macroscale groupings of traits (e.g., energetic status, osmoregulatory function, reproductive development) were linked to variation in individual fitness in a stark and absolute manner that lead to mortality and no fitness for some individuals in the population (Cooke et al., 2006a; Cooke et al., 2008a; Hanson et al., 2008a).

Similarly, in black bass, we have repeatedly noted relationships between whole organism traits and the potential for individual fitness differentials. Mate preference (Hanson and Cooke, In Review) and locomotory performance during the parental care period (Hanson et al., 2007) have both been linked to morphological differences amongst individuals. Additionally, studies have theorized that individual physiological status (mainly energetic status) should be predictive of fitness through the correlate of successfully raising a brood to independence (Mackereth et al., 1999; Cooke et al., 2002; Hanson and Cooke, 2009). However, a clear correlative link between parental care performance (defined as successfully raising a brood) and any physiological variable has yet to be established, reinforcing the idea that variation in single physiological traits may

1913 be masked by subtle and complex interactions with other traits and macroscale
1914 physiological systems may be the lowest level of variation appropriate for testing in
1915 research programs.
1916
1917 *The relevance of 'certain' laboratory findings in an 'uncertain' world and vice versa*
1918 As the field of evolutionary physiology has grown, disagreements have arisen
1919 between researchers trying to integrate results from studies conducted under varying
1920 conditions. In particular, primarily as a result of moving physiological sampling
1921 techniques from laboratories to the field, the validity and relevance of common methods
1922 when applied to natural systems such as individual fitness have been challenged (Pough,
1923 1989). It is now accepted that laboratory studies may not be accurate representations of
1924 organismal performance in nature (Irschick and Garland, 2001; Irschick, 2003; Peake and
1925 Farrell, 2004). Field ecologists often take umbrage with the lack of realism imparted by a
1926 closed laboratory environment with control of all variables save for the variable of
1927 interest and the frequent use of somewhat domesticated animals. Moreover, field
1928 ecologists often question of the results of these studies when compared to the wild in
1929 which multiple uncontrolled variables affect the organism at every given moment
1930 (Irschick and Garland, 2001; Irschick, 2003). A particularly relevant example relates to
1931 the very common measurement of prolonged swimming performance in fish, namely
1932 critical swimming speed (Brett, 1964), defined as aerobic swimming lasting 20s to
1933 200min and resulting in fatigue (Beamish, 1978). The prevailing wisdom indicates that
1934 individual survival and fitness differences will only occur and be quantifiable with
1935 measures of swimming performance at or near maximum performance (Drucker, 1996;

Plaut, 2001; Reidy et al., 2000), and to achieve this state researchers have subjected fish to forced swimming trials to determine maximum performance capabilities during prolonged exercise (Brett, 1964; Beamish, 1978; Kolok, 1999). This research tool has provided invaluable calculations of inter- and intra-specific swimming capacities, but recently the applicability of these findings to natural systems has been questioned (Plaut, 2001). In particular, ecologists have noted that animals would ideally avoid performing at maximum for any extended period of time and that activity resulting in total fatigue and an inability to move would most likely result in mortality (Plaut, 2001). Additionally, critical swimming speed estimates fail to account for other interactions with variables such as behavioural modification that occur in the wild and would affect the fitness of an individual (Brauner et al., 1994). This, therefore, brings into question the ecological relevance of critical swimming speed and it's use as a viable indicator of performance differences in the wild (Plaut, 2001; Nelson et al., 2002). That said, a recent study has revealed that volitional activity is correlated with maximum swimming capacity in rainbow trout (McDonald et al., 2007), and recent research has noted that field based measures of swimming performance in largemouth bass are repeatable within stable seasonal conditions (Hanson et al., In Review).

As is the case in most contentious situations, there is another distinct side to the story whereby laboratory physiologists have raised issues with field based assessments of physiological variables. By its very nature, field research occurs in an uncontrollable environment with many factors beyond the control of the researcher that may be acting upon a physiological trait. Due to this fact, causality may often be incredibly difficult, if not impossible, to ascertain leading to many studies providing only correlative evidence

(Wright, 1921). To revisit the example of critical swimming speed, recall that laboratory studies established that variation in critical swimming speed exists (Kolok 1999) and relevant differences in swimming performance leading to variation in individual survival will only occur and be quantifiable at or near maximum performance (Drucker, 1996; Plaut, 2001; Reidy et al., 2000). Other studies have noted that the variation in swimming performance as measured in the laboratory is heritable (Ghalambhor et al., 2003; Claireaux et al., 2007). Combined, these data suggest that laboratory measures of critical swimming speed in a number of species are reflective of relevant variation among individuals that can be acted upon and maintained through whole organism selection (Ghalambhor et al., 2003). Conversely, to date, measures of volitional swimming in the wild have only been correlated to Darwinian fitness in highly active planktivorous fishes (Plaut, 2001). Additionally, swimming performance can be affected by a host of environmental conditions that are uncontrollable in the field (Beamish, 1978), thereby making determination of a causal link between voluntary swimming performance and individual fitness extremely difficult. As such, laboratory measures of swimming performance continue to be advocated as ecologically relevant measurements (summarized in Plaut, 2001). Without further research to establish correlations between variation in volitional swimming and variation in critical swimming performance or fitness, many assumed links between volitional swimming activity and fitness measured in the field are tenuous.

Possible Solutions

Sampling the full extent of physiological diversity

While it must be conceded that current research efforts have described a large portion of the range of physiological variation of various species as well as animals participating in many behaviours, researchers tend to ignore and avoid sampling organisms in extremely poor condition. Though counterintuitive at first glance, sampling of moribund animals could provide a much clearer picture of the true scope of physiological diversity with regards to the realm of dysfunction in physiological traits that often leads to a complete lack of individual reproductive opportunities (and fitness) due to mortality. Superficially, the simple solution to this situation is to design studies with appropriate controls that include sampling a portion of the population not involved in the focal behaviour as well as clearly moribund individuals. Results derived in a binary manner by comparing moribund individuals with healthy individuals may provide a more simplified and easy to analyze comparison to base future research into the full range of physiological diversity. Unfortunately, in many research systems, detecting and capturing animals not involved in a focal behaviour or moribund individuals would be quite difficult (as mentioned above), and proper sampling strategies would need to be planned out by experts for each study.

Pairing field and laboratory studies

Previously, we discussed the separation that can occur between laboratory and field physiologists. While minor disagreements may abound between practitioners in these two realms, it has become increasingly apparent that the only way to properly assess the relationship between physiological traits and fitness is through an integration of field and laboratory techniques. Recent reviews (Irschick, 2003) and issues of

prominent journals (summarized in Kingsolver and Huey, 2003) have echoed earlier calls for adapting theoretical models, such as that proposed for relationships between morphology, performance, and fitness in Arnold (1983), that advocate designing studies with complimentary laboratory and field components. To summarize, the general idea is that both field and laboratory techniques have complementary strengths that should be combined to adequately describe how a physiological trait can relate to whole organism fitness. In particular, laboratory studies can provide background information on a given trait including the extent and importance of individual variation upon which natural selection can act. Complementary field components can assess the mechanisms by which natural selection acts upon a given trait *in situ*. The combination of these studies, therefore, provides information describing the proximate relationships between a physiological trait and natural selection allowing researchers to understand the ultimate relationship between physiological variation and fitness differentials between individuals.

To this end, recent research studies have begun to adopt the methods first described in Arnold (1983) in two prominent designs. In the first, researchers monitor some behaviour in one set of unrestrained animals in the field to determine behavioural or performance differences amongst individuals relative to some metric (environmental, social, behavioural indices) that could impart a fitness differential on those individuals. A complementary laboratory study on a separate set of animals of the same species is employed to determine the role of physiological traits of the behaviours in the wild. The results of the two phases are then compiled to yield a continuum of data starting with lab measurements of a physiological trait through ecological performance of individuals to a final estimation of how that physiological trait could influence fitness. Multiple

researchers have employed this framework in relating physiological traits to fitness through the intermediary of performance (Arnold, 1983). For example, in a measure of swimming performance related to abiotic environmental conditions, Nelson et al. (2003) measured flow conditions in the wild and then performed laboratory based estimates of swimming performance on fish captured in a variety of stream habitats. Similarly, in a study of the behavioural responses to hypoxia in largemouth bass, Hasler et al. (2009) first measured movement and activity of wild individuals in relation to natural fluctuations in dissolved oxygen. A complimentary laboratory study was employed to experimentally manipulate dissolved oxygen levels to determine behavioural responses (Hasler et al., 2009).

The second general type of study utilizes physiological sampling of an animal involved in a particular behaviour to create a snapshot of an organism's physiological status which can be compared to various metrics of performance, behaviour, and fitness. Many of the Pacific salmon examples from this review utilize this method whereby adult salmon are intercepted along the migratory route, physiologically sampled to produce a characterization of each individual, and then released with implanted telemetry devices that allow researchers to assess various metrics of behaviour and survival/fitness (summarized in Cooke et al., 2008a). Due to the magnitude of scale of the salmon migration in both physical size as well as numbers of salmon, recapture of individual fish is usually impossible (but see Figure 1 in Cooke et al., 2008a) requiring researchers to sample new sets of individuals at various locations or stages of migration. Though less than ideal, this initial physiological characterization serves as the sole source of physiological data on the migrating individuals and is subsequently used to determine the

influence of organismal physiology on behaviour and fate for the duration of the migration after sampling and tagging (Cooke et al., 2005a). Similar study designs have been applied to research of the physiological changes associated with parental care in black bass (Hanson and Cooke, 2009; Hanson and Cooke, In Review). However, because bass participating in parental care occupy a discrete area around a nest and the brood with little straying from this location, individuals can be repeatedly sampled to determine fluctuation in physiological parameters in relation to changing parental care behaviours (Hanson and Cooke, 2009).

Field physiology

Due to the difficulties associated with working with unrestrained animals in the wild, researchers have developed a sub discipline named 'field physiology' that promotes non-invasive techniques for sampling physiological parameters in the wild while causing minimal stress that would alter the behaviour of that animal (Costa and Sinervo, 2004). Owing to advances in laboratory procedures requiring miniscule amounts of tissue to perform most modern physiological assays, small biopsies of multiple tissues such as blood, gill, and muscle can be taken from an individual with minimal impact on behaviour and survivability (Cooke et al., 2005a). This sampling regime can provide a comprehensive snapshot of an organism's physiological status at the time of capture. Mobile devices capable of analyzing physiological samples have also been developed and afford field researchers the opportunity to near instantaneously analyze samples with accurate and consistent results similar to laboratory assays. In particular, meters capable of measuring from whole blood glucose levels indicative of the secondary stress response

as well as lactate levels indicative of both the secondary stress response and anaerobic activity (Morgan and Iwama, 1997; Wells and Pankhurst, 1999; Pyne et al., 2000; Venn Beecham et al., 2006; Thompson et al., 2008), and measurements of minerals and ions (Mandelman and Farrington, 2007; Cooke et al., 2008b). Additionally, handheld devices such as bioelectrical impedance assessment meters (Cox and Hartman, 2005; Willis and Hobday, 2008) and microwave energy meters (Crossin and Hinch, 2005) now allow researchers the ability to make non-lethal assessments of energy density analogous to proximate body composition analyses which required lethal sampling of organisms. These new tools will allow researchers to properly pair field studies with laboratory work allowing for comprehensive studies of the links between physiological variation and subsequent fitness. Additionally, through incorporating non-lethal and non-invasive sampling strategies, researchers can repeatedly sample individuals (Hanson and Cooke, 2009) allowing for insight into the changing physiological status of an organism in response to a changing environment, ontogeny, or life-history status.

Genomic techniques

Increasingly, physiologists are utilizing genomic techniques as a method of rapidly analyzing multiple physiological systems within a single organism to determine individual differences. Genomic techniques allow the researcher to determine patterns of gene expression in many physiological systems (on the order of thousands of individual genes) from a single sample from an individual (Klaper and Thomas, 2004; Thomas and Klaper, 2004). Genomic approaches enable the researcher to resolve differences in thousands of biochemical pathways between groups rather than the standard few

pathways analyzed in traditional bioassays. This approach uniquely lends itself to initial data mining to determine gross scale differences in systems that can later be examined using more in depth bioassays. Additionally, through testing an entire genome at one time, researchers have an unprecedented view of interactions between multiple genes that would not be resolved by traditional bioassays.

Currently, genomic techniques have been applied with great success in research programs investigating the biochemical causes of migration failure (hence, reproductive failure) in sockeye salmon (Miller et al., 2007; Cooke et al., 2008a). To date, this work has noted physiological changes along the migration route associated with entry into freshwater and reproductive maturation (Miller et al., 2007). Research has also noted gross scale variation in gene expression between fish that survive to arrive on spawning grounds and fish that die en route suggesting that fish are predisposed to their fate prior to river entry (Cooke et al., 2008a). To date, however, the precise function of genes that are differentially expressed between the groups has yet to be resolved (Cooke et al., 2008a). Regardless, this research demonstrates the utility of genomic techniques to determine differences in a host of physiological systems between groups of individuals with different levels of fitness.

Technological advances aiding in sampling

Supplemental to field physiology, technological advances enable researchers to implant an animal with a device capable of continuously monitoring performance and/or physiological metrics in the wild. Advances in technology now allow researchers unprecedented access to affordable, reliable, and advanced biotelemetry and biologging

devices that are capable of measuring both metrics of organismal performance (movement, activity) as well as physiological traits (body temperature, muscle activity) in unrestrained animals in the natural environment (Cooke et al., 2004b). These technologies have been discussed at length in recent reviews (Cooke et al., 2004b; Block, 2005), so we will briefly summarize advances in this paper. A common complaint of metrics of field locomotory performance was that non-visual measures of activity of unrestrained animals in the wild were underestimates of actual activity and movements (Ovidio et al., 2000; Løkkeberg et al., 2002; Hanson et al., 2007). Currently, there have been several deployments of telemetry systems capable of monitoring fish implanted with transmitters capable of transmitting location data every few seconds (Niezgoda et al., 2002; Hanson et al., 2007) capable of generating activity estimates with unprecedented accuracy. Additionally, advances in sensor technology now allow researchers to collect physiological and performance data from telemetered individuals. Fine scale estimates of physiological process such as muscle contraction and blood flow as well as estimates of localized activity can be measured by a host of innovative biotelemetry techniques summarized in Cooke et al. (2004b). Additionally, a number of telemetry tags can have optional sensors included allowing researchers to measure multiple environmental variables (e.g., temperature, pressure, light, salinity) as the animal faces these conditions in the wild (Cooke et al., 2004b). These devices enable researchers to measure physiological processes in wild organisms as well as measure many of the possible environmental factors that can influence these processes. Finally, advances in the ability to measure elemental signatures in tissues (including stable isotope analysis and otolith microchemistry) have given researchers the ability to back calculate habitat preferences

(e.g., Bradbury et al., 2008), movements (e.g., Miller and Shanks 2004), foraging behaviour (e.g., Vander Zanden et al., 1997), and growth rates (e.g., Neilson and Campana, 2008) without the need for surgical intervention.

Implications for other taxa

While the current manuscript focused on teleost fish, in particular salmonids and centrarchids, the core message translates well to research conducted on other taxa. In particular, sampling constraints will vary drastically between taxa both in the capture methods as well as what tissues can be sampled non-lethally. However, given that these difficulties can be magnified when working with fish (organisms living in a completely foreign environment that are difficult to observe first hand, let alone capture), we are confident that researchers can adapt the ideas espoused in this manuscript to almost any model species provided that they are of sufficient body size. Additionally, research utilizing higher vertebrate models may be better able to determine links between parental physiology and fitness due to the relatively higher chances of a single offspring surviving to maturity in these taxa. In fish, any individual fry spawned during a reproductive event has an exceedingly low probability of surviving to adulthood (due to high mortality in early life history stages linked to exogenous factors such as climate, predation, etc.) which could potentially swamp out effects of inherited physiological characteristics from parents. For fish, there is a need to understand the relative role of stochastic environmental variation and physiology on organismal fitness.

Conclusions

The field of evolutionary physiology is a growing, multidisciplinary effort that fuses concepts from the diverse areas of evolutionary biology, genetics, and systematics with ongoing research into extant physiological variation (Feder et al., 2000). Though the field has progressed from the original flaws of its infancy discussed by Feder (1987), some of these flaws persist. There still exists a tendency to infer the fact that individual variation in traits should lead to differential fitness without including explicit tests of these inferences, though researchers working in this field have begun to conduct studies on the direct links between variation in physiology and variation in fitness (Feder et al., 2000; Irschick, 2003; Cooke et al., 2006a; Peterson and Husak, 2006). As highlighted throughout this manuscript, research focusing on the reproductive physiology of individuals has perhaps the most promise for providing insight into the relationships between organismal physiological processes and individual fitness. Future research would do well to focus on empirical testing of links between physiology and fitness through the use of innovative techniques applied to both healthy and moribund individuals. Comprehensive programs such as these will afford researchers in the field of evolutionary physiology both the mindset and tools to finally empirically test the exact relationship between the individual variation in physiological traits and variation fitness that is acted upon by natural selection leading to evolution.

Chapter 7: Future Research Directions

The field of evolutionary physiology is a multidisciplinary effort, borrowing from the fields of evolutionary biology, genetics, and systematics with ongoing research into extant physiological variation (Feder et al., 2000). In its infancy, multiple fundamental flaws (discussed in Feder, 1987) plagued the field and hampered the efforts of researchers. To a large degree, these issues have been noted and avoided in current research efforts, though a few continue to be stumbling blocks. Chief amongst them, researchers continue to infer the fact that individual variation in traits should lead to differential fitness without explicitly testing this inference (Feder et al., 2000; Irschick, 2003). In a promising trend, recent studies have begun to focus on the direct links between variation in physiology and differential fitness (Feder et al., 2000; Irschick, 2003; Cooke et al., 2006a; Peterson and Husak, 2006).

As highlighted throughout this thesis, research focusing on the reproductive physiology of individuals has perhaps the most promise for providing insight into the relationships between organismal physiological processes and individual fitness. In the research contained within this thesis, I continually focused solely on healthy individuals already engaged in reproduction. As such, it is possible that these individuals represent a very minor subset of the physiological diversity inherent in the population, thereby decreasing the ability to resolve differences between individuals as related to fitness. Future research should widen the focus beyond individuals already reproductively active. Preliminary examinations of differences in physiological parameters between reproductive and non-reproductive individuals would allow researchers a coarse view of

which physiological conditions are necessary to even attempt to reproduce in a given year.

Additionally, continued pairing of controlled laboratory experiments with complementary field estimates of fitness is required. Laboratories afford researchers a controlled environment wherein precise measures of organismal physiology and performance may be performed. Pairing laboratory results with novel field based techniques will allow for non speculative measurements of fitness and ecologically relevant conclusions to be drawn. Researchers must also continue to embrace the rapid technological advances that have improved the precision, cost effectiveness, and field suitability of physiological measurements which enable comparison between laboratory and field settings. Comprehensive programs that utilize careful and creative experimental designs wedded with powerful new methodologies hold the most promise to empirically test the exact relationship between individual variation in physiological traits and fitness.

Specifically related to studies of Centrarchid fishes, several areas of research hold promise for determining a link between variation in physiological parameters and fitness differentials between individuals. First, I believe that research should focus on the condition of males that spawn relative to males that do not as a first step to understanding which physiological parameters most accurately represent the reproductive capabilities of an animal. Second, as currently there are no studies in this area due to the focus on uniparental male care, research should focus on physiological differences between females at the time of egg deposition. Finally, in relation to Chapter 5, I believe future research should focus on interactions between multiple endocrine cues (e.g., ghrelin, leptin, cortisol, etc.) in relation to male nutritional condition across parental care as well

as parental aggression and swimming performance. In all of these studies, particular care should be given to remain within the paradigm established in Chapter 1 whereby the ultimate link between an individual physiological parameter and organismal fitness is some field based measure of performance. Studies such as these would allow for a clearer understanding of the physiological correlates of individual fitness in Centrarchid fishes.

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