Teleost fish providing parental care: individual and intergenerational costs and consequences of nest predation pressure

By

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Dedication

I would like to dedicate this thesis to my family, especially to my parents, siblings and husband for always supporting me and giving me the best work-life balance anyone could hope for.

Abstract

Predation pressure is an important ecological variable which can influence the morphology, behaviour, physiology and survival of prey species. A particular focus has been given to individuals engaged in reproduction because traits or behaviours associated with reproduction often make them more prone to predation. For many taxa, reproduction also involves specialized parental care behaviours. Under these circumstances, individuals engaged in parental care may not be directly threatened by predators, but guard vulnerable offspring. However, parental care often imposes physiological and energetic costs on parents which can influence their survival. In this thesis, I tested whether variation in nest predation pressure had consequences for parents, as well as offspring. I used a teleost fish that provides male-only parental care as a model (smallmouth bass, *Micropterus dolomieu*). I used six populations which differed in predation pressure to test a number of hypotheses. First, I tested whether variation in nest predation pressure influenced parental care behaviour. I found that males from populations with increased nest predation pressure were more often engaged in antipredator behaviours relative to males from populations with lower predation pressure. Second, I tested whether variation in nest predation pressure influenced the cost of providing care. Traditional energetic approaches (i.e., lipid analysis) showed that energy status declined during parental care for all populations but individuals from populations with increased predation pressure did not lose relatively more energy stores. An *in-situ* approach (i.e., electromyogram telemetry) showed that males from the populations at the opposite extremes of predation pressure differed in overall swimming activity. Third, I tested whether variation in predation pressure influenced indicators of performance in parental males. I found that males from all six populations had similar indicators of swimming performance, a proxy for parental care. Finally, I tested whether nest predation pressure influenced the antipredator behaviour of offspring. Here, I found that offspring from all populations were similarly able to avoid an introduced nest predator. From a physiological perspective, offspring from the site of highest predation pressure had lower active metabolic rates and recovered more quickly from a simulated predator attack compared to offspring from the population with the lowest predation pressure.

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PREFACE

I have opted to write this thesis in the Integrated Article Thesis form, as permitted by Carleton University regulations. The four manuscripts that have been included in this thesis are titled:

1. Influence of inter-lake variation in natural nest predation pressure on the parental care behaviour of smallmouth bass (*Micropterus dolomieu*)
2. Does nest predation pressure affect the energetic cost of nest guarding in a teleost fish?
3. Brood predation pressure does not influence parental enzyme activities related to swimming performance
4. Behavioural and physiological consequences of nest predation pressure for larval fish

The first chapter is published in the journal *Ethology*. The second chapter has been submitted to the *Comparative Biochemistry and Physiology, Part A.* The third chapter is published in the journal *Comparative Biochemistry and Physiology*, *Part A.* The final chapter has been accepted for publication in the journal *Behavioral Ecology.* Copyright permissions have been granted for the specific journals and are included as a separate document from this thesis.

Contributions of co-authors

This thesis represents the results of my own independent research. All data collection, data analyses and writing was conducted by myself. All chapters are co-authored by my thesis supervisor, Dr. Steven J. Cooke. Dr. Cooke contributed to the design and execution of these chapters. The manuscripts were written by myself and Dr. Cooke provided feedback and edits on all them. Chapter 3 was co-authored by Dr. Patrice Couture from the Institut National de la Recherche Scientifique. Dr. Couture provided support and training for the lab work and also provided guidance with data interpretation and gave feedback on the manuscript. Chapter 4 was co-authored by Dr. Cory Suski from the University of Illinois at Urbana-Champaign. Dr. Suski provided training and logistical support for the respirometry equipment and provided feedback on the manuscript. All co-authors have granted me permission to use these manuscripts in this thesis.

GENERAL INTRODUCTION

Predation pressure is an important ecological variable which can influence prey individuals in two specific ways. First, predation can have a strong selective force on prey physiology, behaviour and morphology by increasing mortality rates in individuals that do not possess these traits (e.g., Giles and Huntingford 1984; Petrin et al. 2010). Alternatively, the response of prey to predators can be plastic and changes in prey behaviour, morphology or physiology can simply be a response to current predation conditions (e.g., Bronmark and Miner 1992; McPeek et al. 2001; Verdolin 2006). For decades, scientific research has examined the interactions between predators and preys and has identified numerous ways in which predators can directly or indirectly influence prey fitness (reviewed by Lima and Dill 1990; Lima 1998; Apfelbach et al. 2005). In many instances, research efforts have focused on prey animals engaged in reproduction, and the specialized role that predators can have during this time (reviewed by Zuk and Kolluru 1998). Reproducing individuals are often more susceptible to predation due to physical characteristics associated with reproduction such as pregnancy, ornamentation or nuptial colouration (Magnhagen 1991). In addition, visual, olfactory and auditory cues used by reproductively active individuals to attract mates or display mate quality are also used by predators to detect potential prey (Zuk and Kolluru 1998). As a result, many studies have sought to describe the trade-offs that exist between reproductive success and the risk of individual predation (e.g., Magnhagen 1991; Forsgen 1992; Bernal et al. 2007; Kim et al. 2009).

For a wide variety of taxa (e.g., insects, fishes, amphibians, reptiles, birds and mammals) reproduction also involves engaging in specialized parental care behaviours such as nest preparation, brood guarding and food provisioning (reviewed in Clutton-Brock 1991). These behaviours have evolved to increase the probability of offspring survival when offspring face difficult environmental conditions such as extreme temperatures, low food availability or increased predation pressure (Clutton-Brock 1991). Although these specialized behaviours improve offspring survival, they also involve costs to parents (e.g., loss of mass, Townsend 1986; Moreno 1989; Marconto et al. 1993, depletion of energy stores, Fitzgerald et al. 1989; Gilloly and Baylis 1999; Mackereth et al. 1999, reduced immunocompetence, Hanssen et al. 2003, reduced future breeding opportunities, Coleman et al. 1985). Modeling studies have established that environmental variation should influence the costs of providing parental care (Carlisle 1982; Webb et al. 2002) but only a few studies have tested how variation in ecologically relevant variables can influence the costs of reproduction for parental-care providing individuals (e.g., parasite load, Delope et al. 1993, parental food limitation, Boggs and Ross 1993, or in the aquatic environment, dissolved oxygen concentrations, Jones and Reynolds 1999; Hale et al. 2003, and salinity, Hale and St Mary 2007).

For iteroparous individuals (i.e., individuals that reproduce more than once), lifetime fitness depends on their own survival, as well as the survival of offspring. Parents must balance the energetic and physiological costs associated with providing care without compromising their own survival, as well as increase the probability of offspring survival. As an ecological variable, predation pressure has the ability to dramatically influence both of these components of fitness. Avian ecologists have long been interested in how predation pressure influences clutch sizes (Slagsvold 1984; Doligez and Clobert 2003; Eggers et al. 2006) and parental behaviour (Ghalambor and Martin 2002;

Fontaine and Martin 2006, Eggers et al. 2008). Predation pressure has also been widely accepted as an important component of the life-history evolution of birds (Martin 1995; Ghalambor and Martin 2001) but the role of predation pressure has been largely ignored in other taxa that provide parental care. Birds, like mammals, provide offspring with food, warmth and protection, a form of depreciable parental care where the benefits for an individual offspring decrease as the brood size increases (Clutton-Brock 1991). Contrary to birds and mammals, the ultimate goal of parental care for fish is guarding eggs or developing offspring from predators, a form of undepreciable care. Other ecological differences such as offspring growth rate, survival, egg characteristics (size and number) and the abundance of aquatic predators, seem to make fish more susceptible to nest predation than birds (Magnhagen 1992), thus making fish particularly interesting to examine for differential effects of predation pressure. In this thesis, I propose to examine the costs and consequences associated with a natural gradient in nest predation pressure using a teleost fish species that provides sole-paternal care (the smallmouth bass, *Micropterus dolomieu*) as a model. Research efforts will focus on both parents and offspring.

Adult smallmouth bass (> 250 mm, Scott and Crossman 1973) are often the top predator in aquatic systems and face little risk of individual predation besides threats imposed by anglers or the occasional avian predator. In this species, males build nests, court and spawn with females and guard eggs and developing offspring from predation (Scott and Crossman 1973; Ridgway 1988). The parental care period typically lasts four weeks or longer (Ridgway 1988). During this time, males engage in energetically costly activities and significantly reduce their food intake (Hinch and Collins 1991; Hanson et al. 2009c). Nesting males have been shown to be more active than non-nesting individuals (Cooke et al. 2002) and individuals engaged in parental care lose energy stores across the parental care period (Gillooly and Baylis 1999; Mackereth et al. 1999). In many populations, nests left briefly unattended are quickly depredated by aquatic predators (Kieffer et al. 1995; Philipp et al. 1997). Because parental care is known to be energetically costly for this species and because nest predation pressure has the ability to drastically influence fitness, smallmouth bass make an interesting model with which to test for costs and consequences associated with nest predation pressure.

In my first chapter I tested if differences in nest predation pressure influenced parental care behaviour. In nesting birds, parents decrease nest visitation rates when nest predation pressure increases (Ghalambor and Martin 2002) because the conspicuous feeding of the nesting parent or nestlings can attract nest predators (Martin et al. 2000). Unlike birds, to date there is no evidence that parental care activities of nesting fish can influence the behaviour of nest predators. Conversely, large fish species will actively and aggressively defend their nest from natural and model nest predators (Ridgway 1988; Urban 1991; Steinhart et al. 2005) while smaller bodied fishes (e.g., common goby, *Pomatoschistus microps)* increase the individual risk of predation when nest predation pressure increases (Magnhagen and Vestergaard 1991). Nest predation pressure was assessed in six lakes within a narrow geographical region (< 50 km). I predicted that nesting males from populations with higher nest predation pressure would be more often engaged in antipredator activities relative to nesting males from populations with lower predation pressure.

In Chapter 2, I used traditional energy metrics as well as an *in-situ* approach to test for the differential costs of providing parental care across a gradient in nest predation pressure. The costs of reproduction are well documented (e.g., Bell 1980; Stearns 1989; Magnahagen 1991), as are the costs associated with parental care (e.g., Smith and Wooton 1995; Webb et al. 2002; Hanssen et al. 2003). As stated above, modeling studies have established that environmental variation should influence the cost of providing parental care but relevant ecological variables are rarely considered in this type of research (but see Hale et al. 2003; Steinhart et al. 2005). Here, I predicted that the energetic cost of providing care would be greater for nesting males from populations with higher nest predation pressure than males from populations with lower nest predation pressure.

In Chapter 3, I tested whether nest predation pressure influenced parental physiology. Specifically, I wanted to test if nest predation pressure would influence indicators of swimming performance. Most research on the evolutionary or plastic responses of prey to predators has focused on the morphological (e.g., Bronmark and Miner 1992; Mikolajewski et al. 2006) or behavioural (e.g., Giles and Huntingford 1984; Relyea 2002) consequences of predation pressure. In contrast, variation in prey physiological traits was been much less documented (reviewed by Kingsolver et al. 2001; Siepielski et al. 2009). However, physiological traits have been linked to individual performance in other ecologically relevant environments (e.g., habitat characteristics, Sullivan and Somero 1983; Amaral et al. 2008, prey communities, Kaufman et al. 2006; Selch and Chipps 2007). In addition, physiological traits such as enzyme activities have been linked to relevant indicators of individual performance (e.g., escape speed and swimming performance) for a number of taxa (Guderley 2004). Because nesting smallmouth bass must actively defend their nest from predators, I predicted that physiological traits (i.e., enzyme activities) associated with swimming performance would be higher in males from lakes with higher predation pressure relative to males from lakes with low predation pressure.

In my final data chapter, I focused on the intergenerational behavioural and physiological consequences of nest predation pressure. Local adaptation in antipredator behaviour is well-documented (Giles and Huntingford 1984; Magurran et al. 1993; Relyea 2002), while evidence for local physiological adaptations related to predation pressure are generally lacking (but see Strobbe et al. 2010). Research has focused on how parental behaviour can influence the predation of offspring in nesting species (Martin et al. 2000) but we know little about how offspring behaviour or physiology could be influenced by variation in nest predation pressure. Although the offspring of nesting species are often guarded by vigilant parents, parents are not always present (e.g., Kieffer et al. 1995) and there may still exist important selective forces that promote enhanced antipredator behaviour or physiological performance of larvae in populations where nest predation pressure is high. Here, I predicted that larvae from populations with increased nest predation pressure would display enhanced antipredator behaviour and physiological performance relative to larvae from populations with low nest predation pressure.

Chapter 1: Influence of inter-lake variation in natural nest predation pressure on the parental care behaviour of smallmouth bass (*Micropterus dolomieu*)

Gravel MA, Cooke SJ. 2009. Influence of inter-lake variation in natural nest predation pressure on the parental care behaviour of smallmouth bass (*Micropterus dolomieu*). Ethology. 115: 608-616

Abstract

Predation risk has the ability to greatly influence the behaviour of reproducing individuals. In large long-lived species with low risk of predation for parents, reproductive behaviours often involve caring for offspring (i.e. defending broods from predators) and these behaviours are essential for offspring survival. Our objectives were to test for the presence of natural variation in nest predation pressure in an aquatic environment for a species that provides sole-paternal care, smallmouth bass (*Micropterus dolomieu*), and to determine if natural variation in predation pressure influences parental care behaviour. We used snorkeler observations and a series of metrics to assess predation pressure and parental care behaviour in six lakes within a narrow geographical range. Lakes differed in all predation pressure metrics: number of predators in proximity to nest when males were present, time to predator arrival and number of predators that consumed eggs when males were absent and total number of nests that was preyed upon. Similarly, parental behaviour varied between lakes. Parental smallmouth bass spent more time engaged in anti-predator defences in lakes with high predation pressure, while males from low predator pressure lakes remained close to their nest. Conversely, males from lakes with low and high predation pressure showed a similar willingness to defend their nests during simulated nest predation events. Our results show that natural variation in aquatic nest predation pressure across multiple lakes can be significant and has the ability to influence baseline parental care behaviour. Such variation provides opportunities to study the costs and consequences of parental care and to evaluate how this could influence demography and community interactions in aquatic systems.

Introduction

Predation is often considered one of the greatest potential costs of reproduction (Magnhagen 1991). Reproducing animals can be physically impaired during reproduction (e.g., pregnancy, ornamentation), but it is generally their reproductive behaviour such as mate searching, mate signalling or mate calling which makes them more vulnerable to predation (reviewed by Lima and Dill 1990). Indeed, much literature has focused on how predators use the olfactory, auditory and visual cues of reproducing individuals to increase prey detection (reviewed by Zuk and Kolluru 1998). As a result, a wide range of studies have sought to describe the behavioural adjustments made by individuals to cope with the trade-offs that exist between reproductive success and predator avoidance (e.g., intersexual response to auditory predator cues in frogs, Bernal et al. 2007, predation pressure affects mate choice in colourful fish, Forsgren 1992; Gong and Gibson 1996).

Alternatively, predation can have little effect on the survival of reproducing individuals, but an important effect on the survival of the developing offspring. In large, long-lived species that provide parental care and have low risk of individual predation, efforts are devoted to caring for offspring (e.g., defending broods from predators). Based on of the level of parental investment, these behaviours can be rather costly and include a suite of potential consequences such as loss of mass (Townsend 1986; Moreno 1989), depletion of energy stores (Steinhart et al. 2005) and reduced future breeding opportunities (Coleman et al. 1985). In this case, the interactions with potential brood predators do not directly influence parent survival, but has the potential to influence the costs and consequences of providing care. Avian ecologists have long been interested in how predation pressure affects clutch size (e.g., Slagsvold 1984; Doligez and Clobert 2003), and have more recently examined how parental behaviour may influence offspring survival. There is strong evidence that an increase in parental care activities such as the feeding of nestlings or incubating female can increase nest predation rates (Martin et al. 2000). In addition, birds will reduce their nest visitation rates (Ghalambor and Martin 2002), become more cryptic or reduce clutch size (Eggers et al. 2006) when nest predation pressure is elevated.

Contrary to birds, there is little evidence that the parental care activities of fish are used as cues by nest predators. As such, fish seem to have evolved different optimal strategies as a response to changes in predation pressure and will often become more aggressive (Ridgway 1988; Ongarato and Snucins 1993) or increase their individual risk taking (Magnhagen and Vestergaard 1991) when nest predation pressures increase. This, in addition to fundamental ecological differences between birds and fish, make fish an interesting model to examine the influence of offspring predation pressure on the costs and consequences of parental care (Amundsen 2003). For example, by providing offspring with food and warmth, birds (and other animals) provide a form of depreciable parental care, where the individual offspring benefits decrease as the brood size increases.

Conversely, the most common type of care in fish is guarding eggs or developing offspring from potential predators (Gross and Sargent 1985), a form of undepreciable care (Clutton-Brock 1991). Other ecological differences such as growth rate, survival, egg characteristics (size and number) and the abundance of aquatic predators, seem to make fish more susceptible to nest predation than birds (Magnhagen 1992), thus making fish particularly interesting to evaluate the differential effects of nest predators.

Here we use the smallmouth bass (*Micropterus dolomieu*; teleostei: centrachidae) as a model to evaluate the effects of variation in natural nest predation pressure across six lakes within a narrow geographical range. In this species, adults are often the top predator in the system and have low risk of adult predation besides threats imposed by anglers or the occasional bird of prey (Scott and Crossman 1973). Males provide sole parental care for up to six weeks (Ridgway 1988), where they perform energetically costly activities (Cooke et al. 2002; Cooke et al. 2006) such as egg fanning to provide oxygen and prevent silt deposition, as well as brood defence. Similar to other animals, parental smallmouth bass perform a limited suite of nesting behaviours. They may be away from their nest (performing other behaviours such as foraging), on or near their nest (tending their eggs by fanning or vigilance) or actively chasing away nest predators (Ridgway 1988). If a smallmouth bass leaves his nest unattended for a short period of time, brood predation may occur (Kieffer et al. 1995; Philipp et al. 1997; Steinhart et al. 2004). In addition, smallmouth bass will actively and aggressively defend their nest from natural and model nest predators (Ridgway 1989; Urban 1991). The native range of smallmouth bass encompasses much of eastern and central North America (Scott and Crossman 1973) and thus includes a wide range of natural variation in environmental conditions, including predation pressure (Hinch and Collins 1991; Steinhart et al. 2005). A previous study has evaluated the interspecific variation of nest predation pressure within a single lake among six syntopic centrachid fishes (including smallmouth bass; Cooke et al. 2008), and between two lakes with and without an invasive nest predator (Steinhart et al. 2005). However, little is known about the natural variation in nest predation pressure among populations and if such variation influences the costs and consequences of parental care. Such information could provide insight into the extent of intraspecific variation in organismal behaviour and its ecological and evolutionary basis.

As such, our goal was to examine how natural variation in nest predation pressure influences parental care behaviour in smallmouth bass. We tested for the presence of variation in nest predation pressure across six lakes within a narrow geographic range where other environmental variables (e.g., climate drivers) would presumably be similar. Once the predation pressure gradient was established, we tested two hypotheses. First, we hypothesized that “baseline” parental care behaviour would be influenced by nest predation pressure. We predicted that smallmouth bass in lakes with high predation pressure would spend more time on their nest and engaged in antipredator responses than fish in low predation pressure lakes. Second, we hypothesized that “elicited” antipredator responses would not be influenced by predation pressure. Because the fitness consequences of allowing a nest predator to consume ones offspring are so great, we expected nesting males to recognize a nest predator and actively defend his nest from the intrusion irrespective of the population level of predation pressure. Collectively, this study will provide the first data on the variation in natural nest predation pressures across multiple lakes and the consequences of such variation on parental care behaviour. Such studies are urgently needed to understand the interface between community ecology and individual behaviour, yielding a more mechanistic ecology and predictive ethology (Altmann and Altmann 2003).

Methods

Study area

Smallmouth bass were studied in six lakes in the spring of 2007 (<50 km between most distant lakes) within the same ecoregion in southeastern Ontario (Upper Rideau Lake, Charleston Lake, Indian Lake, Newboro Lake, Opinicon Lake and Sand Lake). The last smallmouth bass stocked in Ontario was in 2000 and hatchery production (at the provincial level) has been negligible since the 1930's, hence there should be minimal influence from supplementation (Kerr 2006).

In the spring when temperatures reach approximately 15°C, male bass move into the littoral zone where they sweep out a nest in the substrate with their caudal fin, court females, spawn, and then provide parental care to the brood until the offspring become independent. Due to the ecological differences between lakes such as depth and turbidity, lakes warm differentially and allow for temporal variation during the reproductive season. Peak spawning dates, even within a small geographic region such as southeastern Ontario, can vary by approximately 10 days (Kubacki et al. 2002) enabling research to take place in multiple lakes within a small geographic area in a single season. Lakes were chosen due to their close proximity to each other (less than 50 km) and the indication that they showed inherent variation in nest predation pressure (based on interviews of biologists with the Ontario Ministry of Natural Resources and local sunfish researchers, Frank Phelan and David Philipp). As the reproductive season began, snorkelers swam a subset of the littoral zone of each lake to identify the location of approximately 30 nesting males on eggs ($\leq$ 4 days). Distances swam in each lake ranged between 0.5 and 3 km. Snorkelers estimated the egg score in each nest (a categorical metric from a low of 1 to a high of 5, Kubacki et al. 2002) and age of eggs (fresh eggs are golden with a visible oil droplet and gradually whiten within a few days). Factors such as male size, number and age of eggs are known to affect the behaviour of nest guarding males (Ridgway 1988; 1989; Suski et al. 2003) and were considered in analyses. Individual nests were identified with a numbered marker. Study sites within a lake were selected based upon previous research by our team or colleagues and focused on areas with appropriate spawning substrate (i.e., coble and gravel). All sampling occurred from May – June 2007. All observations were collected at the egg stage when predation pressure can be quite high because fresh eggs are energetically valuable to predators and can be easily captured because they are immobile.

Lake predation pressure

We used several metrics to establish the level of nest predation pressure in the six lakes. All observations were made by a snorkeler and were recorded on dive-slates. The first metric directly quantified the predation pressure of each smallmouth bass nest. This study was performed in conjunction with others (Gravel, unpublished data) which required relocating unmanipulated individuals at a later date. For this reason, only a proportion of individuals were used in this study. To select individuals, one of every three nests were chosen along transects swam by snorkelers. Overall, ten smallmouth bass nests were chosen from the nests previously marked by the snorkeler and were observed for 15 min with the snorkeler positioned 3 m from the nest. There was a short 1–2 min acclimation period but typically this distance was sufficient in preventing the disturbance of normal parental care behaviour. Only 5 of 59 fish reacted to the snorkeler and were removed from the baseline behaviour analysis. At 30 s intervals, the snorkeler recorded the number and species of nest predators that were within 2 m of the nest. The maximum number of predators within the 15-min period was determined for each nest since individual predators could not be identified and reporting means would be ambiguous. We considered fish to be potential nest predators if they had been previously reported as being such for bass nests in the literature or if we had observed them doing so. For the purpose of our study that list included bluegill (*Lepomis macrochirus*), pumpkinseed (*L.gibbosus*), rock bass (*Ambloplites rupestris*), yellow perch (*Perca flavescens*), black crappie (*Pomoxis nigromaculatus*), largemouth bass (<15 cm) (*Micropterus salmoides*) and conspecifics (<15 cm). The 2 m distance was chosen in order to ensure the same amount of visibility across all lakes. Moreover, previous studies of smallmouth bass have revealed that bass actively defend against predators within 2 m of the nest (e.g., Cooke et al. 2008). After the conclusion of the 15-min observation period the fish was removed from the nest by rod and reel. The snorkeler observed the nest area for an additional 15-min period and noted the time elapsed between the removal of the parent and the arrival of the first nest predator. At each one minute interval the snorkeler would also note the number and species of nest predators present and engaged in consumption of eggs at the abandoned nest.

Baseline parental care

Baseline parental care was measured during the initial 15 min sampling time and on the same 10 randomly sampled fish described above. At 30 s intervals, the snorkeler noted the activity being performed by the guarding male. Activity was a categorical measurement and fish could be performing only one activity at each time interval: 1) away from nest (> 2 m from nest) and/or not visible to the snorkeler 2) on nest or within 2 m of nest or 3) engaged in an antipredator behaviour.

Elicited antipredator response

Prior to the removal of nesting males, antipredator behaviours were elicited using a predatory sunfish (*Lepomis spp*) (mean total length ± SE; 149.25 ± 7.54 mm) placed in a glass jar. Smallmouth bass display three types of aggressive behaviours when encountering nest predators: yawn (males open their mouths and flare their branchiostegal membranes), rush (males quickly swim towards predator but do not strike) and hit (males make physical contact with the predator by striking or biting) (Suski et al. 2003). To elicit an antipredator response, the nest predator was placed 1 m from the nest for 30 s and then placed within the nest for 30 s. During this time, the snorkeler counted the number and type of aggressive behaviours made by the male towards the nest predator. The effect of distance was identical for all males across all lakes (data not shown), thus antipredator behaviours for both distances were summed for statistical analysis.

Statistical Analysis

All analyses were performed in JMP 7.0.1 (SAS Institute Inc. Cary, North Carolina) and the level of significance (α) for all tests was 0.05. All figures display means ± SE unless otherwise indicated. One-way analysis of variances (ANOVAs) were used to test for differences between the six lakes for each response variable (predation pressure metrics and staged intrusion) and for other traits that may have influenced predation pressure and/or parental behaviour such as male total length (TL), egg score and egg age. Data were tested for normality and heterogeneity of variance prior to analyses. Most response variables could not be transformed to fit the normal distribution and thus a non-parametric Kruskal-Wallis test was performed. These analyses were then followed by parametric or non-parametric multiple comparisons, respectively (Zar 1999). We used a univariate time-to-event (survival) analysis to test for differences in time to depredation as some nests were never preyed upon and data needed to be censored (i.e., censorship in a time-to-event analysis takes into consideration that the event did not occur within the given observation time). We used a 6 x 3 contingency table to compare the time spent performing each baseline behaviour across the six lakes and examined standardized residuals from this analysis [$R=(F_0-F_e)/\sqrt{F_e}$] to determine which cells, if any, had a major influence ($-1.96 \geq R \geq 1.96$) on significance (Haberman 1973).

Results

Male size, egg score and egg age

Nesting smallmouth bass did not differ in TL between lakes ($F_{5,53} = 0.9$, $p = 0.48$) (range: 394 mm – 497 mm; mean ± SE: 408.3 ± 5.7 cm). Egg score categories ranged from 2–4 and differed between lakes (Kruskal-Wallis, $\chi^2 = 12.2$, df = 5, $p = 0.03$, Fig. 1-1). Egg age did not differ between lakes (Kruskal-Wallis, $\chi^2 = 5.4$, df = 5, p = 0.37). Variation in the number of eggs did not affect the number of predators in proximity to the nest in the presence of males (Kruskal-Wallis, $\chi^2 = 2.4$, df = 5, p = 0.30) or the level of aggression of males during a staged nest predator invasion ($F_{5,51} = 0.65$, p = 0.53).

Nest predation pressure

Potential and actual nest predators documented in this study were bluegill, pumpkinseed and rock bass. Over 95% of predators identified in all observations were *Lepomis spp*. and thus individual predator species effects were not included in analyses and all predator species were grouped. The number of nest predators in proximity to a nest in the presence of a guarding male (perceived predator abundance) varied across lakes (Kruskal-Wallis, $\chi^2 = 23.1$, df = 5, p = 0.0003; Fig. 1-2a). Here, Sand Lake and Opinicon Lake had the highest perceived predation pressure, while Upper Rideau Lake had the lowest. Similarly, time to depredation (Time-to-event analysis, $\chi^2 = 25.6$, df = 5, p = 0.0001) and the proportion of nests which were preyed upon were also influenced by lake (Fig. 1-3). By the first minute, 60% of nests from Opinicon Lake were attacked by a nest predator, while it took over five minutes for most of the lakes to reach 30% predation. At the end of the 15 min period, Upper Rideau Lake had the lowest number of nests preyed upon (3/10) and Opinicon Lake had the greatest number of nests that were attacked by predators (9/10). The number of predators present after male removal (actual predation pressure) also differed between lakes (Kruskal-Wallis, $\chi^2 = 13.7$, df = 5, p = 0.02; Fig. 1-2b). Opinicon Lake had the greatest number of predators arrive after removal, while Upper Rideau Lake had the lowest.

Parental care behaviour

The proportion of time spent performing baseline parental behaviours differed between lakes (χ^2 = 83.9, df = 10, $p < 0.0001$; Fig. 1-4). Males from Opinicon Lake were most often engaged (11%) in antipredator behaviours, followed by Indian Lake (5%), and Sand Lake (4%). Residuals from the contingency analysis (Table 1-1) revealed that time spent away from nest in Upper Rideau Lake and Opinicon Lake and time engaged in antipredator activities from Opinicon Lake were significantly over-represented in the sample, while time spent engaged in antipredator activities were significantly under-represented in Upper Rideau Lake and Charleston Lake (Table 1). The number of yawns and hits performed by parental males towards a staged nest predator also varied between lakes (yawns; Kruskal-Wallis, χ^2 = 18.65, df = 5, p = 0.002) (hits; $F_{5,53}$ = 3.6, p = 0.007), while the number of rushes did not (Kruskal-Wallis, χ^2 = 5.1, df = 5, p = 0.4) (Fig. 1-5).

Discussion

Predation is considered an intense selective force able to influence the morphology and behaviour of organisms. In addition to its evolutionary importance, it is also relevant on ecological time scales, where organisms make behavioural adjustments in response to predation threats in their own lifetime (reviewed by Lima and Dill 1990). Although manipulative experiments have elucidated the behavioural consequences of predation pressure (e.g., Fontaine and Martin 2006; Eggers et al. 2008), little work has examined the ecological significance of natural variation in predation pressure. Our study showed clear evidence for natural variation in nest predation pressure within a narrow geographic range. Lakes were relatively constant in their “rankings” such that lakes that showed evidence for high predation pressure in one metric, ranked similarly for other metrics. Lakes that were statistically highest in predation pressure metrics were Opinicon Lake and Sand Lake, while Upper Rideau Lake was consistently the lowest. Of particular note is the proportion of nests that were preyed upon when parental males were removed. In Opinicon Lake, 90% of nests had eggs eaten by predators while Upper Rideau Lake only had 30% (Fig. 1-3). Hence, the perceived predator abundance in the presence of males (Fig. 1-2a) was representative of the actual number of nests which would be preyed upon in their absence (Fig. 1-2a and 1-3).

In addition to this apparent natural variation in nest predator pressure, our study demonstrates behavioural consequences of this natural phenomenon. Time spent performing parental behaviours differed between lakes and these differences were consistent with nest predation pressure gradient. Our analysis tested whether males from all lakes spent the same amount of time engaged in each form of parental care behaviour. Opinicon Lake, which showed one of the highest means in predation pressure metrics (Fig. 1-2) had males spend more time engaged in antipredator behaviours than expected (Table 1-1, Fig. 1-4). Males from lakes with low predation pressure such as Upper Rideau Lake and Charleston Lake spent significantly less time than expected engaged in antipredator behaviours (Table 1-1, Fig. 1-4). Males from both predation pressure extremes (high, Opinicon Lake and low, Upper Rideau Lake) spent more time than expected away from their nests (Table 1-1). Although these results appear similar, our snorkeling observations enabled us to distinguish between two distinct behaviours. Males from Opinicon Lake were out of snorkeler view due to the performance of antipredator behaviours (largely chasing), while males from Upper Rideau Lake were occasionally further than 2 m from nest but were not engaged in antipredator behaviours. It is possible that males from Upper Rideau Lake may simply be less bold towards snorkelers or other intrusions than males from other lakes due to low predation pressure and high visibility.

Conversely, our predator simulation data does not show evidence of “shy” individuals in lakes with low predation pressure. Here, parental smallmouth bass from Upper Rideau Lake showed similar or even increased willingness to defend against an introduced nest predator than males from other lakes (Fig. 1-5). As predicted, parental males from all lakes were generally similarly willing to defend their eggs from staged predator intrusion. The significant difference between the number of hits performed by males from Upper Rideau Lake and Charleston Lake, both with relatively low predation pressure, was unexpected and is difficult to explain. The higher number of hits by males from Upper Rideau Lake could be due to their higher energetic condition relative to conspecifics in systems with higher predation pressure and that are regularly engaged in chasing predators. However, it is currently not possible for us to determine if this is this case with our current data. What is important to note is that lakes from predation pressure extremes typically grouped together. Moreover, we demonstrated that the vast majority of males (57/59) were able to detect and perform antipredator behaviours during a staged intrusion, independent of nest predation pressure. This work supports other studies that have highlighted the importance of plasticity in the predator response (Ghalambor and Martin 2002).

Our study has demonstrated the importance of natural variation in nest predation pressure and its potential consequences on parental care behaviour and also provides opportunities for future research into natural variation of nest predation pressure. Recent work has shown that community structure can have significant consequences on physiological indicators of performance in natural systems (Kaufman et al. 2006). This study showed intraspecific variation in top predator (walleye, *Sander vitreus*) enzyme activities in relation to energetically demanding and ecological relevant activities such as prey capture. Another study has highlighted the loss of energy stores or increased energetic demand in a system with a novel predator (Steinhart et al. 2005). Thus, it is highly probable that fish providing parental care across a natural predator gradient not only demonstrate differential behavioural responses as we report here, but also exhibit associated physiological and energetic responses.

Although the nest predation pressure is believed to be relatively stable in this system (i.e., we used historical data and local knowledge to find lakes with variation in nest predation pressure), we recognize that systematic annual sampling has not yet been performed. As such, there also is opportunity for inter-annual variability in predation pressure which may have behavioural implications. Knowledge of the stability of predation pressure in a system across multiple years would help with understanding the evolutionary basis of antipredatory behaviour and parental care. Our results suggest that there is potential for important natural variation in nest predation pressure within a narrow geographical range and that natural variation in nest predator can indeed affect the behaviour of parental care providing species. Although it can be powerful to manipulate predation pressure during the reproductive period in order to test for behavioural adjustments, it is also of interest to examine how individuals respond in a more natural environment. Our study is comprehensive as it tests not only for the presence of variation in natural predation pressure, but also examines the effects of this variation on a species whose main goal during reproduction is the protecting of offspring.

Tables

Table 1-1 Residuals from 6 x 3 contingency analysis between lakes and baseline parental care behaviour (1: away from nest, 2: within 2 m of nest and 3: engaged in an antipredator behaviour). Bold-faced residuals are considered significant ($p < 0.05$) if above the standardized residual of +1.96 or below -1.96 (reprinted with permission of *Ethology*)

Lake	Activity		
	1	2	3
Big Rideau	**3.08**	0.31	**-3.16**
Charleston	-1.78	0.66	**-2.15**
Indian	-1.35	0.05	0.70
Newboro	-1.57	0.50	0.70
Opinicon	**2.99**	-1.50	**5.69**
Sand	-1.35	0.17	-0.12

Figures

Figure 1-1 Proportion of nests with different egg scores (ES) within each lake (reprinted with permission of *Ethology*)

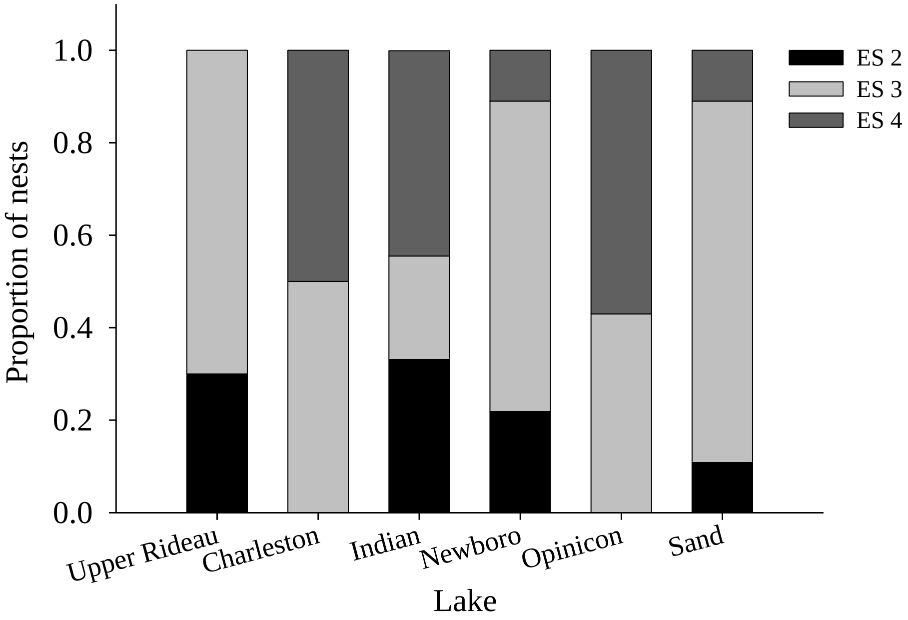


Figure 1-2 Perceived predation pressure (male present) (A) and actual predation pressure (male absent) (B) in six lakes $\leq$ 50 km radius in SE Ontario (Upper Rideau Lake, n = 10; Charleston Lake, n = 10, Indian Lake, n = 10; Newboro Lake, n = 9, Opinicon Lake, n = 10; Sand Lake, n = 10). Dissimilar letters denote significant differences between means (Tukey *post-hoc* test, p < 0.05). (reprinted with permission of *Ethology*)

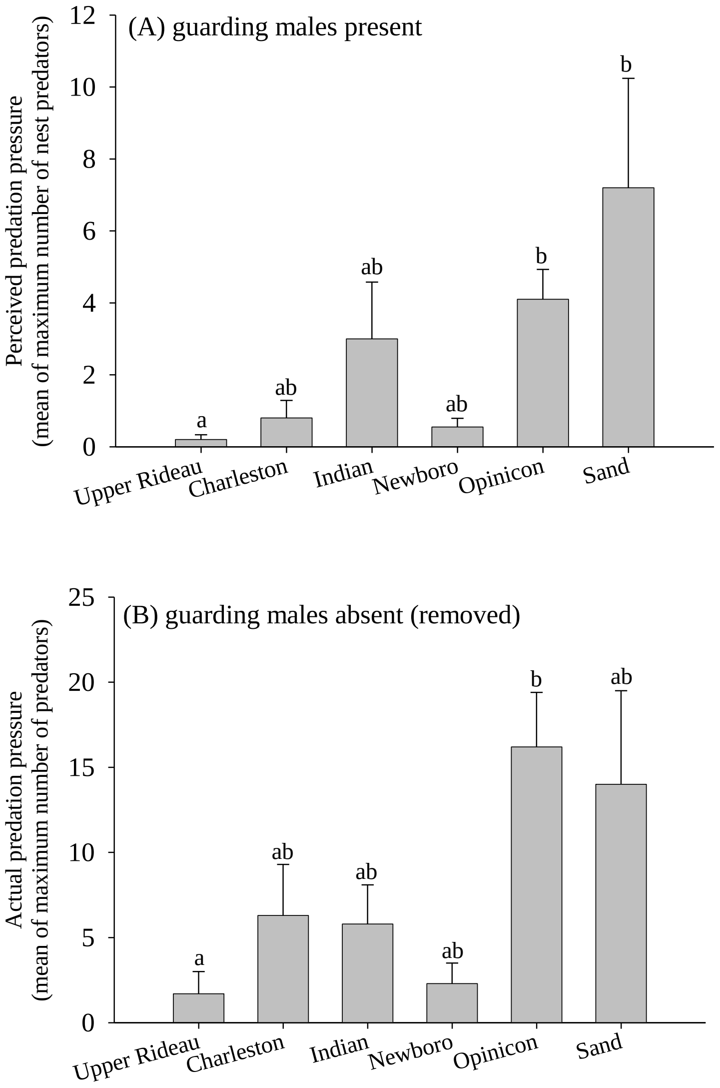


Figure 1-3 Time to depredation expressed as proportion of nests preyed upon within a 15-min period following the removal of nest guarding males in six lakes (≤ 50 km radius) in SE Ontario (Upper Rideau Lake, n = 10; Charleston Lake, n = 10, Indian Lake, n = 10; Newboro Lake, n = 9, Opinicon Lake, n = 10; Sand Lake, n = 10). (reprinted with permission of *Ethology*)

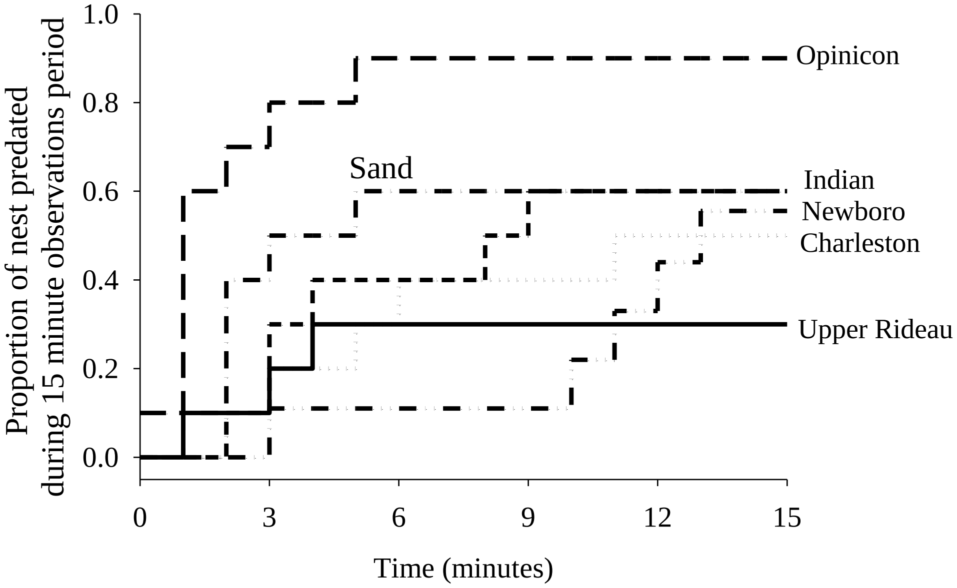


Figure 1-4 Proportion of time nest guarding smallmouth bass spent performing distinct parental care behaviours in six lakes (≤ 50 km radius) in SE Ontario. Sample sizes are shown on individual bars. (reprinted with permission of *Ethology*)

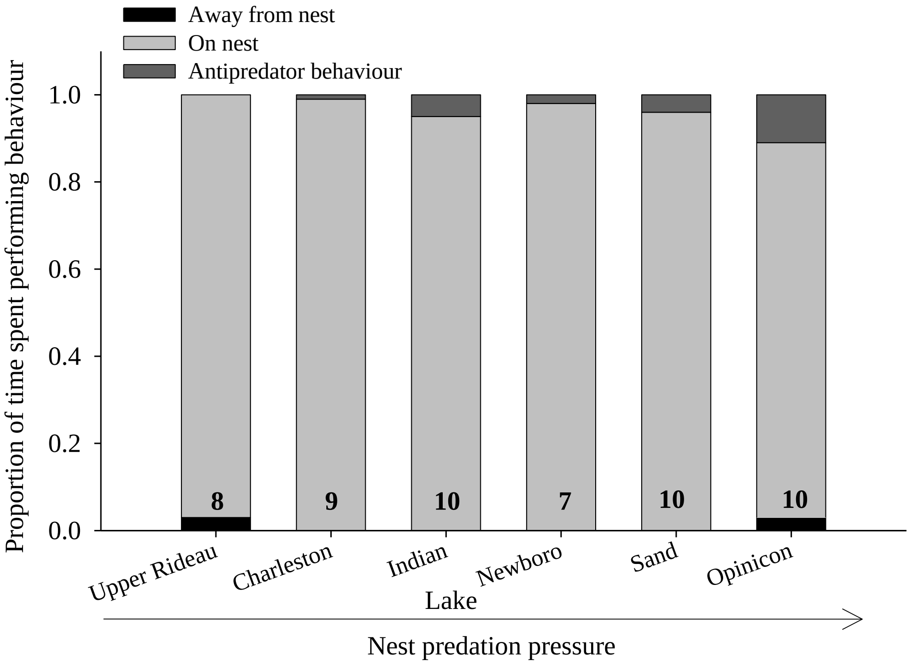
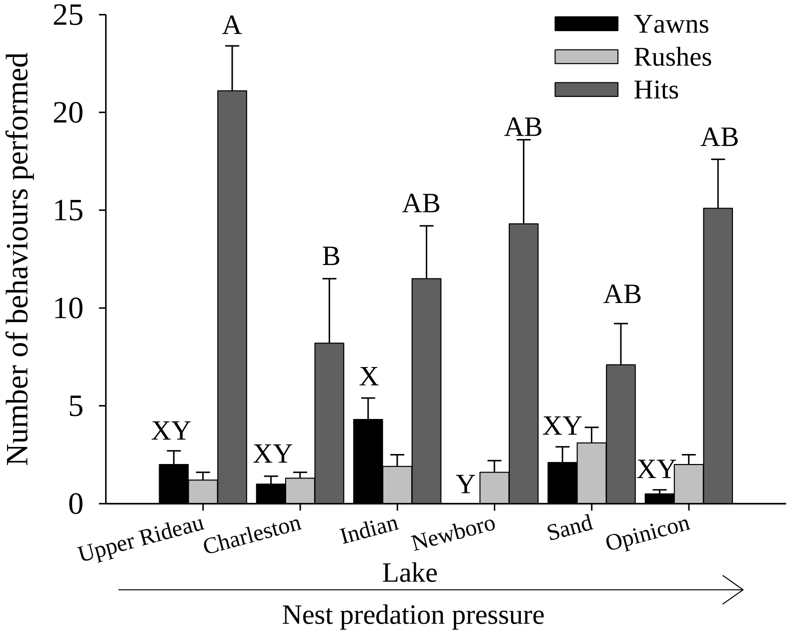


Figure 1-5 Mean number of antipredator behaviours performed by parental smallmouth bass towards a staged nest predator (*Lepomis spp*) (Upper Rideau Lake, n = 10; Charleston Lake, n = 10, Indian Lake, n = 10; Newboro Lake, n = 9, Opinicon Lake, n = 10; Sand Lake, n = 10). Dissimilar letters denote significant differences for a given antipredator behaviour (Tukey *post-hoc* test, $p < 0.05$). (reprinted with permission of *Ethology*)



Chapter 2: Does nest predation pressure affect the energetic cost of nest guarding in a teleost fish?

Gravel MA, Cooke SJ. In review. Does nest predation pressure affect the energetic cost of nest guarding in a teleost fish? Comp. Biochem. Physiol. A.

Abstract

The energetic costs of providing parental care are widely documented, but rarely do studies consider the role of ecological variation. Here, we tested if variation in nest predation pressure influenced the energetic costs of parental care in smallmouth bass, a teleost fish species that provides lengthy paternal care. First, we documented that nest predation pressure varied among the six lakes studied and was consistent across a three year period. We used a combination of traditional proximate body composition (PBC) analyses and electromyogram (EMG) telemetry to quantify activity costs of nesting fish across these populations. The traditional approach revealed declines in energy stores across the parental care period but showed no evidence of an increased energetic cost to parents from populations with higher nest predation pressure. EMG telemetry revealed that parents at the site of highest predation exhibited a 10% increase in overall locomotor activity relative to the parents from lowest predation pressure and spent twice as much time engaged in burst swimming activities when guarding young offspring. These differences in overall activity, a large contributor to the energy use of fish, may translate into longer recovery times and decreased future reproductive opportunities, particularly for temperate species where the growing season is limited.

Introduction

Reproduction is considered a costly activity (Bell 1980), because individuals must allocate a limited amount of resources to growth, maturation and reproduction. Allocating resources to reproduction instead of other functions is often referred to as the "physiological cost" of reproduction and has played an important role in defining life-history theory (Reznick 1992). Measuring the cost of reproduction within a single environment has been shown in a wide range of taxa (e.g., plants, insects, birds, reptiles and fish; Obeso 1993; Hutchings 1994; Lee et al. 1996; Madsen and Shine 2000; Almbro and Kullberg 2009). These costs have been measured in a variety of ways and often examine the trade-offs between reproductive success and growth (e.g., Obeso 1993; Cox and Calsbeek 2010), locomotor performance (e.g., Lee et al. 1996; Almbro and Kullberg 2009) and longevity (e.g., Rose and Charlesworth 1981). For taxa which provide parental care, reproduction can be particularly costly since it also involves energetically demanding behaviours such as offspring feeding, providing heat to offspring and guarding offspring for predators (Clutton-Brock 1991). Tending broods can cause loss of mass (Moreno 1989; Townsend 1986; Marconato et al. 1993), depletion of energy stores (Fitzgerald et al. 1989; Gillooly and Baylis 1999; Mackereth et al. 1999) and reduced future breeding opportunities (Coleman et al. 1985).

Theoretical models have revealed that environmental influences should affect the costs of providing parental care (Carlisle 1982; Webb et al. 2002) but natural variation in the environment is typically not considered a primary factor. Changes in the costs of parental care associated with different environments have been confirmed by only a handful of taxon-specific empirical studies (Boggs and Ross 1993; Delope et al. 1993; Jones and Reynolds 1999; Green and McCormick 2005; Steinhart et al. 2005; Hale and St Mary 2007). Most often these studies consider the effect of adult food limitation, but some studies have examined the increased costs associated with parasite load (Delope et al. 1993) or in the aquatic environment, low oxygen conditions (Jones and Reynolds 1999) and salinity (Hale and St Mary 2007). Predator abundance and predation pressure can greatly vary across the range of a species (Fontaine et al. 2007; Gravel and Cooke 2009), and the inability of a parent to protect its brood has severe fitness consequences (Philipp et al. 1997; Conway and Martin 2000). Thus, natural variation in nest predation pressure should influences the costs of providing parental care. We tested this prediction in a teleost fish, which provides extended nest guarding behaviour, the smallmouth bass (*Micropterus dolomieu).*

Smallmouth bass provide male-only parental care which typically lasts four weeks (Ridgway 1988). During this time they fan eggs to help prevent silt deposition, as well as guard eggs and developing offspring from potential nest predators. Due to their parental responsibilities, they have limited opportunities for feeding and must primarily rely on endogenous stores (Hinch and Collins 1991; Mackereth et al. 1999). There are evidences that parental care is energetically costly in this species. Nest guarding smallmouth bass are extremely active (Cooke et al. 2002), lose mass (Gillooly and Baylis 1999) and energy stores (Mackereth et al. 1999). Moreover, providing parental appears to impose a recovery period in which the male must actively feed and replace lost energy stores (Mackereth et al. 1999). In this study, we set out to test whether the energetic consequences for parental smallmouth bass vary across populations which naturally differ in nest predation pressure. We predicted that males from lakes with high nest predation pressure would show higher energetic costs relative to males from lakes with lower predation pressure. First, however, we had to test whether predation pressure was consistent over multiple years in a given waterbody and if it varied among lakes. We then measured the energetic costs of providing care using two techniques. First, we used a more traditional approach (e.g., proximate body composition – PBC) which compared energy stores between the onset of parental care and the end of parental care. This approach allowed us to compare the energy status of different individuals across many populations and examine the effects of predation pressure on the energy status of parents. Our second technique was an individual-based approach which permitted us to examine the overall activity of the same individual across the parental care period in relation to a particular predation habitat. When combined, these techniques have the potential to reveal whether natural variation in the environment influences the cost of providing parental care and more specifically if there are increased energetic costs associated with sites of high nest predation pressure.

Methods

Study site and species

The study took place on six lakes in south-eastern Ontario within the Rideau and Gananoque River watersheds. We showed elsewhere (Gravel and Cooke 2009) that these lakes varied greatly in nest predation pressure. Variation in predation pressure was measured for three years (2007-2009) in order to establish whether variation in predation pressure across lakes remained consistent across years. Each spring, when water temperatures reach approximately 15 °C, snorkelers swam the littoral zones of the lakes to identify nesting smallmouth bass that were guarding fresh eggs ($n \geq 40$ per lake). Predation pressure was then measured on a random ($n = 10$) subset of these fish. Due to differences in depth and other lake characteristics lakes warm differentially. In consequence, the sampling dates for the smaller shallower lakes were between May 8 and 16th, while the larger, deeper lakes were sampled between May 20th and 30th. Metrics of predation pressure are extensively outlined in Gravel and Cooke (2009) and consisted of measuring the maximum number of predators in close proximity to the nest (2 m radius) when male was present (perceived predation pressure) and measuring the maximum number of predators which actively consumed eggs when the male was absent (actual predation pressure). The proportion of nests depredated as well as the time to nest predator arrival in the absence of the male where also noted. The maximum values of nest predators were used in statistical analysis because individual predators could not be identified and reporting means would be misleading. Predator composition was similar in all lakes and was composed of pumpkinseed sunfish (*Lepomis gibossus*), bluegill sunfish (*L. macrochirus)* and rock bass (*Ambloplites rupestri)*.

Energy stores

In the spring of 2007 we lethally sampled parental fish from the lakes on which the predation pressure metrics were measured. Fish were removed from their nest with conventional hook-and-line equipment and brought quickly to the boat and euthanized by cerebral percussion. Fish were placed in individual plastic bags and transported in a cooler back to the lab (up to 12 hours) and placed in a chest freezer (up to four days, -20

°C). Fish were thawed for dissections. We measured total weight, total length (TL), liver and gonad weight. Gonadosomatic (GSI) and hepatosomatic (HSI) indices were calculated with following equations: GSI = gonad weight body weight $^{-1}$ * 100 and HSI = liver weight body weight $^{-1}$ * 100. Eviscerated fish were ground in a manual meat grinder. All tissues were placed back in the chest freezer until lipid analysis.

Energy stores were measured in the eviscerated tissue as well as livers. Techniques were identical to those used by Gravel et al. (2010a). We measured lipid content using a methanol-chloroform extraction modified from Bligh and Dyer (1959) (Smedes and Askland 1999). Homogenized tissues (2 g) were dried at 80 °C overnight (16-20h) until a constant mass was attained. The dried samples were then reduced to a fine powder with a mortar and pestle and dissolved in a 1:2:0.8 ratio of chloroform, methanol and water and then extracted with a 2:2:1.8 ratio of the same solvents. A second extraction was then performed with 10% methanol in chloroform solution. Lipid products were evaporated and then weighed. Replicates were made for all samples and extractions were repeated when differences between samples were larger than 3%. The total lipid content for wet mass was then calculated by converting dry mass lipid values to wet mass lipid values $M_W = 100M_D(100\text{-}W)$ and $L_W = 100L_DM_W^{-1}$, where M_W is the initial wet mass of sample, M_D is mass of the dried sample, W is the water content, L_W is the lipid content (in percent) of wet sample and L_D is lipid content of dried sample (in percent). All percent lipid values were then converted into g lipid Kg body^{-1}.

Other body constituents (water, trace mineral and protein) were determined for the eviscerated whole body tissue but were not measured on livers due to their small size. The homogenized tissue (2 g) was dried at 80 °C overnight (16-20h) until a constant mass was attained and re-weighed to assess water content. The dried samples were then combusted for 2 h in a muffle furnace at 500 - 600°C. The resulting trace minerals (ash) were weighed to determine the percentage of ash by wet mass. Protein content was determined using the following equation: $C_P = 100(C_W + C_A + C_L)$, where C_P, C_W, C_A and C_L represent percent protein, water, ash and lipid respectively (Crossin and Hinch 2005).

Electromyogram (EMG) telemetry

Based on our previous work (Gravel and Cooke 2009; Gravel et al. 2010b), we chose lakes at both extremes of predation pressure to examine swimming activity of nesting smallmouth bass (highest predation pressure: Opinicon Lake, n = 7 males and lowest predation pressure: Upper Rideau Lake, n = 8 males). Lakes differed in size and mean depth (Opinicon Lake: 790 ha, 2.4 m; Upper Rideau Lake: 6500 ha, 17.4 m, Marleau 2007) and differentially warm which resulted in variation in fish spawning dates. Fish were implanted with transmitters as soon as they began guarding fresh eggs which was from May 6th to May 8th at the high predation site and May 26th at the low predation site. Fish with fresh eggs were identified by a snorkeler, caught by conventional hook-and-line techniques and brought to the boat for surgery. In the meantime, the snorkeler remained at the nest and defended the eggs from nest predators when necessary. Fish were anesthetized in a large container (50L) of fresh lake water and clove oil (60 ppm) emulsified in ethanol. Once fish lost equilibrium and showed no response to manual stimulus, they were weighed (g), measured for TL (mm) and transferred to the surgery trough on a moistened sponge, ventral side up. Gills were irrigated with a mixture of fresh lake water and clove oil (30 ppm) emulsified in ethanol. We made a small lateroventral incision (approx. 20 mm) in order to insert the EMG transmitter into the body cavity and the gold electrodes were placed internally into the red axial musculature, immediately below the lateral line. The incision was closed with PDS-II absorbable sutures (3/0, sterile; Ethicon, Somerville, NJ) and fish were placed another container (50 L) of fresh lake water to recover. Fish were released once they had regained equilibrium and reacted to mechanical stimulus (i.e., tail-grabbing). The snorkeler departed from the nest once the male had returned.

Fish were implanted with coded electromyogram (EMG) transmitters (Lotek Wireless Inc. New Market, Ontario, Canada). Two sizes of transmitter were implanted (Lotek, CEMG2-R11-18, 54 x 11 mm, 4.9 g – in water and Lotek, CEMG-R16-25, 62 mm x 16 mm, 13 g - in water) based on the total weight of the fish. Tags always represented < 2% of total fish weight. The transmitters were equipped with an antenna and a pair of electrodes affixed with gold tips (9 carat, 7 x 1 mm). The electrodes detect electromyogram signals within the red axial muscle and integrate this signal over a 2.5 s period, providing a mean EMG value along with a time stamp. This signal is transmitted to a receiver (SRX 600, Lotek Wireless Inc.) on shore which records the EMG value. The EMG value (min: 0, max: 50) is proportional to muscle activity. Similar types of transmitters have previously been effectively used on centrarchids (Cooke et al. 2001; 2002). Subtle differences in electrode placement as well as component performance of tags vary slightly between individuals and each tag was individually calibrated *in situ* (Cooke et al. 2004) to standardize the EMG values provided by the tags. Details are provided below.

Tags were calibrated while males were still guarding eggs. Calibrations were performed 2–4 days post-surgery for fish in Opinicon Lake and two days post-surgery for fish in Upper Rideau Lake. Calibrations were performed underwater by the same snorkeler for all individuals. The snorkeler set their digital watch to match the recording time on the receiver and observed the fish for five minutes, monitoring all of the swimming movements and writing them on a dive slate. The behaviours were recorded every five seconds. Behaviours observed were 1) swimming in place on/near nest or 2) chasing a predator (burst swimming). The fish was then chased by the snorkeler for 30-40 s to elicit burst swimming. A nest predator was then introduced into the nest to elicit an antipredator response. The nest predator consisted of a live bluegill sunfish which was placed in a large glass jar filled with fresh lake water. The behaviours were not randomized since observing the fish on his nest for baseline EMG values may have been affected by first introducing a predator or being chased by a snorkeler. The behaviours observed by the snorkeler were then matched with the EMG values recorded by the receiver and each behaviour was assigned a specific range of EMG values.

Previous work has shown that the behaviour of nest guarding fishes can change across the parental care period (Ridgway 1988; Cooke et al. 2002, 2008) and that stage specific effects are important. To clarify, the behaviour of parents guarding eggs may differ from the behaviour of parents guarding hatched eggs or free-swimming fry, typically parents reduce the intensity of care as offspring approach independence (Gross 2005). We categorized offspring development into three periods: eggs, eleutheroembryo (embryos) and pterygiolarvae (larvae) (Wallace 1972; Balon 1975). Nests were snorkeled every 1–3 days in order to determine the development time of the offspring.

Hatching date, pigment acquirement and the onset of swimming and exogenous feeding were noted. All the fish from the low predation site remained with their offspring until we ceased visiting the nests (24 days). One EMG tag from the high predation site could not be calibrated and sample sizes were reduced to n = 7. Conversely, all males from the high predation site did not guard their nests for the entire monitoring period (24 days). One male abandoned his nest before eggs hatched (guarded nest for 8 days) and two males abandoned their nest after offspring had hatched and began swimming freely (21 days). Unfortunately we also had a tag fail in a male that was present during all of parental care but was no longer detected by the receiver on the 6th day of nest guarding. Our sample sizes for the high predation site was thus n = 6 for males guarding eggs, n = 5 for males guarding embryos and n = 3 for males guarding larvae.

Statistical analysis

All statistical analyses were performed in J.M.P 8.0.2 (SAS Institute, Vary, NC, USA), SPSS 17.0 (IBM, Chicago, Illinois, USA) and R 2.11.1 (www.r-project.org). Data were tested for normality and homogeneity of variance. When appropriate, non-normal data were log10 transformed to attain normality. We compared predation pressure metrics between lakes and among years using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1995; Dytham 2003) because data were not normally distributed and could not be transformed. We tested if the time to nest predation pressure differed among lakes using a parametric survival analysis. We used two-way analysis of variance (ANOVAs) to test if parental males differed in length or weight, using lake and stage of parental care as main effects and then used an ANCOVA model to test if the relationship between TL and weight (both log transformed due to the curvilinear relationship between length and mass, Mackereth et al., 1999) differed between fish guarding eggs and larvae. Because there is a known positive relationship between male size (length) and energy stores (Mackereth et al. 1999; Steinhart et al. 2005), we used general linear models, using TL as a covariate, to test if the residuals from the relationship between TL and energy indices (all lakes grouped) differed between stages of parental care, across lakes and of greater interest, across the stages of parental care within lakes (interaction term). The level of significance (α) for all tests was assessed at 0.01 to minimize Type I error associated with multiple statistical tests (Zar 1999). If significant differences were found, we used the Tukey-Kramer post-hoc test to identify significantly different groups.

EMG data for each fish were calibrated with individual behaviours and standardized using an activity index to ensure that data could easily be compared given that there can be variation in tag performance (Cooke et al. 2004). The minimum EMG value observed during the calibration period (when snorkeler was monitoring fish and it was stationary) was assigned the value 0 and the maximum EMG value observed during calibrations when bursting was elicited was assigned a value of 1. EMG data from individual fish were then converted to standardized EMG values ($EMG_{standard}$) using the following equation: $EMG_{standard} = (EMG_{obs}-EMG_{min})/(EMG_{max}- EMG_{min})$, where EMG_{max} is the original highest observed EMG value, EMG_{min} is the lowest observed EMG value, EMG_{obs} is an EMG value between EMG_{min} and EMG_{max}. Thus all EMG values between EMG_{min} and EMG_{max} are replaced with values between 0 and 1. Less than 4% of the data were below EMG_{min} or above EMG_{max} and were not included in the analysis. We then used non-parametric kernel density smoothing to compare the distribution of $EMG_{standard}$ between sites of predation pressure for each stage of parental care (egg, embryos and larvae) (Bowman and Azzalini 1997). We first used the conservative normal optimal smoothing method to compute our smoothing parameter, $h = \sigma(4/3n)^{1/5} = 0.017$, where n is our sample size and σ is the standard deviation of the distribution but this produced a highly variable estimate due to clustering in the data (i.e., many data points for certain EMG values) (Bowman and Azzalini 1997). We thus increased the smoothing parameter to 0.05 and this was deemed appropriate because it properly described the structure of the data. We used a 2 x 2 contingency table to compare the proportion of data points which corresponded to burst swimming events and swimming on nest behaviours between the low and high predation sites and this was repeated for each stage of development (egg, embryos and larvae). Using a t-test, we also compared the mean proportion of time spent burst swimming between the sites of low and high predation, again relative to the period of parental care. For these two analyses, we used the mean EMG value associated with swimming on nest and values below this mean as a measure of “on nest” behaviour and EMG values above this mean were considered burst swimming behaviours. Values presented are means ± standard error (SE) unless otherwise indicated and the significance of EMG related statistical tests were evaluated at $\alpha = 0.05$.

Results

Predation pressure

Perceived and actual predation pressure differed among lakes but did not differ among years, or among lakes within years (Table 2-1, Fig. 2-1). Lakes with lower predation pressure (Upper Rideau, Charleston Lake and Indian lake) had very little predation pressure, where on average less than two individual predators could be seen near the nest when males were present or depredating the nest when males were absent. Lakes with the highest predation pressure (Sand Lake and Opinicon Lake) had on average four or five predators in close proximity to nests when males were present but had 10–15 predators consume eggs when males were absent. Thus the lake with the highest predation pressure (Opinicon Lake) has over a 100 fold increase in actual nest predation pressure relative to the lake with the lowest predation pressure (Upper Rideau Lake). Because year had no apparent effects on predation pressure metrics, time to nest predation was pooled for all years. Time to predation event differed significantly among lakes ($\chi^2 = 48.57$, $p < 0.0001$, Fig.2-2a), where the mean time to first predator arrival ranged from 4–10 min (uncensored data, i.e., mean of 10 min signifies no predation occurred). Generally, little to no predation occurred for Upper Rideau Lake and Charleston Lake. The proportion of nests depredated among the six lakes across the three years of data collection ranged from 7% predation (2 of 29 were depredated) to 72% predation (22 of 29 nests were depredated, Fig. 2-2b).

Energy stores

Male guarding eggs were on average 7% longer and 22% heavier than males guarding larvae (Mean ± SE; TL_{egg}: 408.0 ± 6.0 mm, TL_{larvae}: 381.0 ± 6.0 mm, $Weight_{egg}$: 989.0 ± 45.0 g, $Weight_{larvae}$: 806.0 ± 44.0 g) but male size did not differ between lakes (lake: $F_{5,1}$

= 1.81, p = 0.12, stage: $F_{1,1}$ = 9.60, p = 0.002, lake * stage: $F_{5,1}$ = 1.38, p = 0.24). Male weight was also related to the stage of parental care (lake: $F_{5,1}$ = 1.13, p = 0.35, stage: $F_{1,1}$ = 9.02, p = 0.003, lake * stage: $F_{5,1}$ = 1.36, p = 0.24). Because the relationship between TL and weight did not change between fish guarding eggs and fish guarding larvae (stage: $F_{1,1}$ = 1.01, p = 0.32; TL: $F_{1,1}$ = 2046.99, p < 0.0001; stage * TL: $F_{1,1}$ = 0.19, p = 0.66), the weight loss can be attributed to differences in size between groups and not to loss of mass. Some energy indices decreased across the parental care period (Fig. 2-3 and 2-4). Residual GSI was influenced by male TL (slope = -0.001) differed between lakes and decreased across the parental care period but did not differ between stages of parental care among lakes (Table 2-2, Fig.2-3A). All other energy indices were not influenced by male total length (Table 2-2). Residual HSI decreased across parental care and the relationship between HSI and stage of parental care changed between lakes (Table 2-2, Fig. 2-3B), with Opinicon and Newboro Lake showing the greatest decrease. Residuals of eviscerated whole body lipid did not change between the stages of parental care period or between lakes (Table 2-2, Fig. 2-3C). Residuals of liver lipid increased between the stages of parental care and differed between lakes but the influence of stage of parental care did not change between lakes (Table 2-2, Fig. 2-3D). Males from Opinicon and Upper Rideau Lake had the most negative residual liver lipids, while males from Charleston Lake had the most positive residuals. Residuals of eviscerated whole body water content differed between lakes (Table 2-2, Fig. 2-3E), where individuals from Sand Lake had the most positive residuals and individuals from Indian Lake had the most negative residuals. Residuals of ash content differed among lakes and between the parental care stages (Table 2-2, Fig. 2-3F), where ash content generally decreased across parental care and where Upper Rideau Lake had the most positive residuals and Newboro Lake had the most negative residuals. Residuals of eviscerated whole body protein were influenced by lake and by the stage of parental care (Table 2-2, Fig. 2-3G), with a generally increase in protein content across parental care with Indian Lake showing the most positive residuals and Sand Lake showing the most negative residuals. Generally, there was no strong relationship between lake-level predation pressure and energy status using conventional energy status metrics.

EMG telemetry

The kernel density estimates of $\mathrm{EMG}_{\mathrm{standard}}$ differed between males from the site of low predation and high predation for all stages of parental care ($p < 0.0001$, Fig.2-4). For the egg stage, we observed that the distrubtion of $\mathrm{EMG}_{\mathrm{standard}}$ from Upper Rideau Lake (low predation) males showed two peaks in their density estimates; the largest peak was near the lowest $\mathrm{EMG}_{\mathrm{standard}}$ value, while the second smaller peak was near the mid-point of the $\mathrm{EMG}_{\mathrm{standard}}$ values. Conversely, the distribution of $\mathrm{EMG}_{\mathrm{standard}}$ values from Opinicon Lake (high predation) males showed only one peak near the mid-point. The two distributions showed more overlap when males guard older hatched offspring, with the biggest change being that the Opinicon Lake distribution had more data points were being registered at lower $\mathrm{EMG}_{\mathrm{standard}}$ ($p < 0.0001$, Fig.4b). Finally, the estimates of density continued to significantly differ between the lakes when males guarded larvae ($p < 0.0001$, Fig.4c), where Upper Rideau Lake show two peaks compared to only one in Opinicon Lake. Parental males from the lake of high predation pressure had 9.5–10.5% more EMG values associated with burst swimming events while guarding eggs ($\chi^2 = 11$

185, $p < 0.00001$) and guarding embryos ($\chi^2 = 12\ 199$, $p < 0.00001$), while parental males from the low predation site had 6.3% more EMG values associated with burst swimming events when guarding larvae ($\chi^2 = 902$, $p < 0.0001$). Although not statistically significant, parental males from the high predation site spent over twice the amount of time burst swimming when guarding eggs ($t = 1.83$, $p = 0.10$, Fig. 2-5) and 40% more time engaged in burst swimming when guarding embryos ($t = 1.78$, $p = 0.12$, Fig. 2-5). There were no differences in the proportion of time engaged in burst swimming activity when males were guarding larvae ($t = 0.68$, $p < 0.51$; Fig. 2-5). Due to small sample sizes, the power of these tests was relatively low ($1-\beta = 0.53$, 0.42 and 0.13 for the period spent guarding eggs, embryos and larvae respectively).

Discussion

We set out to test whether an ecologically relevant environmental factor, nest predation pressure, could influence the energetic costs of providing parental care in a paternal-care providing species, the smallmouth bass. Our study revealed that nest predation pressure varied among lakes and was consistent across years emphasizing the potential for predation pressure to serve as a selective force. We found no support for our hypothesis that predation pressure influenced the cost of parental care when we used a traditional energy status approach. In contrast, when we used an approach that enabled us to examine the activity of free-swimming individuals through time, we detected differences in overall locomotor activity between low and high predation sites.

Consistent with other studies (Gillooly and Baylis 1999; Mackereth et al. 1999), the traditional energetic approach detected a decrease in energy status of parental males across the period of parental care. However, we failed to detect differences in the energetic costs of parental care despite the fact that nest predation pressure varied significantly among lakes. Previous work by Steinhart et al. (2005) compared the energy density and weight loss of parental male smallmouth bass between a lake with a hyperabundant invasive nest predator (Lake Erie), the round goby (*Neogobius melanostomus*) and a lake with lower natural nest predation pressure. Contrary to our results, their work showed a reduction in parental weight and body energy density in parents from the lake with increased predation pressure (i.e., hyperabundant invasive nest predators) relative to the lake with lower and native nest predation pressure. In Steinhart et al. (2004) they report that nest predation pressure was 4.3 round goby/min (males absent) for the Lake Erie population. Presented this way, our data shows that predation pressure for the lake with the highest predation pressure (Opinicon Lake) would be on average 9.7 predators/min (males absent). In addition, Steinhart (2005) reports that nesting males were involved 9 times more frequently in predator chases in the lake with round gobies relative to the lake without invasives. Our previous work has shown that males from the site of lowest predation pressure do not engage in any predator chases, while males from our high predation site can engage in 10 or more predator chases within a 10 min period (Gravel and Cooke 2009). If weight loss and energy loss is solely attributed to an increase in predator abundance and consequent predator chases, we would also expect males from Opinicon Lake to show similar changes in energy status. One interesting distinction between the populations in our study and the Lake Erie population may be the historical levels of predation pressure. Round goby were first discovered in Lake Eric in 1993 and their abundances have greatly increased since this introduction (Corkum et al. 2004). Because smallmouth bass demonstrate some nest site fidelity (Ridgway et al. 1991a; Barthel et al. 2008) and predator abundance could be consistent across many years, selection pressure may play a role in adaptation to local predation pressures. Perhaps males from the populations we sampled are better equipped to deal with increased predation pressure relative to populations with a relatively novel and abundant predator such as the round goby.

Although depletions in energy indicators did not reflect variation in predation pressure, individuals from different lakes showed variation in some indicators of energy status. Evidently there are other important lake-level effects, other than predation pressure, which influence some of the energy indices measured in this study. One important difference between these lakes is their size (Gravel et al. 2010b). Upper Rideau and Charleston Lake are large and deep lakes (surface area: 6482 hectares, mean depth: 12.3 m and surface area: 2518, mean depth: 17.4 m respectively) which provide different habitat, prey availability and composition than smaller shallower lakes (mean surface area ± SE and mean depth ± SE for Indian, Newboro, Sand and Opinicon Lakes: 737 ± 408 hectares and 5.1 ± 1.7 m). However, no clear pattern emerges between energy indicators and lake size. Parental males from the larger lakes do not consistently distinguish themselves from males from the smaller lakes. Dunlop et al. (2005) have demonstrated that difference in adult smallmouth bass densities can influence life-history traits (e.g., size at reproduction, reproductive investment) in recently divergent populations. Additional research would need to focus on these life-history parameters (e.g., growth rate, age at maturity), as well as ecological parameters such as prey communities and densities, in order to identify the lake-specific effects that may be driving the differences in energy status.

Previous research shows that the traditional sampling technique has been used with success to identify variation in the energy status of fish providing parental care (e.g., Fitzgerald et al. 1989; Mackereth et al. 1999). However, the nature of the traditional sampling technique has some important implications. Researchers have been considering the importance of individual variation in physiological studies for some time now (Bennett 1987; Spicer and Gaston 1999). Using this technique, individuals are lethally sampled and the variation in energy indices across the parental care period cannot be compared to the individuals' initial state. In addition, the males sampled near the termination of parental care are only composed of individuals that have successfully raised their broods. Males who have prematurely abandoned their nests due to loss of energy stores, nest predation or other causes are not included in this type of sampling. The variation that may exist in energy stores may only be obvious in males which need to prematurely abandon their broods. Successful males may simply have energy stores above this "abandonment threshold". At the end of parental care, the values of the energetic indicators may be inflated because the fish that have fallen below the critical level have already abandoned.

The individual-based approach provided us with some evidence that the energetic cost of providing parental care differed between sites of low and high predation pressure. First and as mentioned in the methods, we detected different abandonment rates between the sites of low and high predation. We observed 100% success at the low predation site and 71% success at site of high predation. Here, it appears that the sub-lethal effects caused by tagging are more pronounced at the high predation site. Monitoring simple abandonment rates may also a useful metric for examining the costs of providing care. Since nesting smallmouth bass are iteroparous and providing parental care is considered a substantial investment into current reproduction (Gross 2005), abandoning a current brood prematurely may be a good strategy to increase future reproductive opportunities (Coleman et al. 1985). Modeling studies have often been interested in predicting when parents should abandon their current broods (Carlisle 1982; Webb et al. 2002) and a recent such study by Steinhart et al. (2008) revealed that abandonment rates of parental smallmouth bass may increase when the probability of adult survival decreases and as the cost of parental care increases.

Further evidence to an increase cost of parental care in areas of high predation pressure comes from the activity data that was provided by EMG telemetry. First, the kernel density estimates emphasize the physiological differences between the groups. Aquatic offspring are believed to be particularly vulnerable at the egg stage (Ridgway 1988; Hinch and Collins 1991) and this is also where the density estimates most obviously differ. The density estimate for the low predation site is relatively narrow and left-skewed, and thus approaches the lowest EMG values recorded during the parental care period. The distribution of the EMG values from the high predation site is wider and more centralized. Therefore, during the period of extreme vulnerability for offspring, males from the site of high predation pressure are spending more time at higher values of $EMG_{standard}$ but also show greater variance in their $EMG_{standard}$ values. Second, the EMG data showed that parental males from the site of high predation pressure had 10% more data points associated with burst swimming and also spend almost double the amount of time engaged in these burst swimming events when guarding eggs or hatched eggs. Because locomotor activity is one of the largest contributors to energy use in fish (Boisclair and Leggett 1989), this may translate into higher reproductive costs in lakes with increased predation pressure. For a temperate species such as the smallmouth bass, this could translate into longer post-reproduction recovery times (e.g., need to consume more food for longer) or potential “reproductive holidays” (Dunlop et al. 2005; Barthel et al. 2008). Individuals may not acquire enough energy reserves to endure the winter months and spawn in the early spring. In consequence, they may need to delay reproduction to the following year. To precisely relate these locomotor differences to energetic differences it would be necessary to calibrate each EMG tag individually with a respirometer (Cooke et al. 2004). This would provide us with the relationship between EMG values and swim-speed and oxygen consumption. Unfortunately, it is not feasible to submit a parental male to these sorts of trials since its nest is difficult to artificially protect during long periods of time and the added stress of confinement and exercise are probably above and beyond what a male is willing to endure while providing parental care. Bioenergetics modeling can provide some insights. Previous modeling work has shown that nesting smallmouth bass show a 17.6% increase in specific respiration rate relative to non-nesting individuals and that this increase in activity should translate to a loss of mass if males do not consume any food during parental care (approximately 11.5% of body weight; Cooke et al. 2002). Steinhart et al. (2005) came to similar conclusions and showed that modifying the activity parameter in a bioenergetics model to match the weight loss found in a lake with hyperabundant invasive nest predators, would suggest that males at the site of high predation would expend six times more KJ per day than males from a lake with low predation pressure.

Alternatively, parents at sites of high predation pressure may simply need to increase their energy intake during parental care in order to maintain these higher activities. Although smallmouth bass drastically decrease their food intake during parental care and demonstrate voluntary anorexia (Hinch and Collins 1991; Hanson et al. 2009c) the level to which parents reduce food intake differs between populations (Gravel et al. 2010a). There is also clear evidence that consuming food during parental care impedes burst swimming ability and reduces aggressiveness towards brood predators (Hanson et al. 2009c). Our work shows that males from sites of high predation pressure are more often engaged in aggressive behaviours towards brood predators (Gravel and Cooke 2009), spend more time burst swimming than males from low predation sites but somehow show no difference in depletion of energy stores. Although gut content only provides a snapshot of overall food consumption, we were able to compare gut contents from the fish which were sampled for the traditional energetic approach and parents from the different lakes did not differ in gut content wet-weight. Recent work has shown that reproduction costs can be masked by individual differences in resource availability (Hamel et al. 2010). Fundamentally, differences in the abundance of small centrarchids (i.e., abundance of nest predators) are caused by differences in lake geomorphology and geochemistry which in turn influences habitat characteristics and food availability. These general habitat differences and their associated local adaptations may also play a role in individual condition and energy status of parental smallmouth bass.

Overall, our study showed that traditional indicators of energy status did not detect an increased cost of parental care in populations with higher predation pressure. An individual-based approached detected physiological differences in the swimming behaviours of parental males between sites of high and low predation pressure. Parents from the site of high predation pressure spend more time burst swimming and were more often engaged in high levels of activity. Because locomotor activity is an important contributor to energy use in fish, it is possible that these differences in activity translate into longer recovery times and decreased future reproductive opportunities, particularly for temperate species where the growing season is limited. Given that patterns of nest predation among lakes appear to be consistent across years, and that activity levels of nest guarding males vary among high and low predator lakes, our study reveals that predation pressure does indeed represent a physiological cost and thus has the potential to influence life-history. As such, there may be life-history consequences associated with different levels of nest predation pressure. Our study emphasizes the need to incorporate field-based estimates (i.e., repeated measures) of energy use rather than only relying on metrics determined from lethal sampling.

Tables

Table 2-1 Results from non-parametric two-way ANOVA (Scheirer-Ray-Hare extension of Kruskal-Wallis test) that compares predation pressure metrics from six lakes from 2007 to 2009. Significant differences are shown in bold.

Response	Source	d.f.	H	P-value
Perceived predation pressure	Lake	5	16.23	**0.01**
	Year	2	2.94	0.23
	Lake × Year	10	8.27	0.60
Actual predation pressure	Lake	5	28.13	< **0.0001**
	Year	2	2.25	0.32
	Lake × Year	10	7.61	0.67

Table 2-2 Results from General Linear Models, using total length (TL) as a covariate and testing the influence of predation pressure (lake), stage of offspring development and their interaction on the residuals from the regression between male TL and different energy indices. Significant differences are shown in bold.

Response	Source	d.f.	F	P-value
Residuals of GSI	TL	1	16.88	< **0.0001**
	Lake	5	8.53	< **0.0001**
	Stage	1	93.22	< **0.0001**
	Lake × Stage	5	1.27	0.28
Residuals of HSI	TL	1	2.76	0.10
	Lake	5	0.31	0.91
	Stage	1	24.53	< **0.0001**
	Lake × Stage	5	5.87	< **0.0001**
Residuals of eviscerated whole body lipid (g Kg^{-1})	TL	1	0.22	0.88
	Lake	5	5.63	0.11
	Stage	1	4.85	0.03
	Lake × Stage	5	0.71	0.62
Residuals of liver lipid (g Kg^{-1})	TL	1	0.51	0.48
	Lake	5	5.63	< **0.0001**
	Stage	1	10.06	**0.002**
	Lake × Stage	5	2.00	0.08
Residuals of eviscerated whole body water content	TL	1	0.12	0.73
	Lake	5	3.51	**0.006**
	Stage	1	0.01	0.91
	Lake × Stage	5	2.55	0.03
Residuals of eviscerated whole body ash content	TL	1	2.81	0.10
	Lake	5	7.35	< **0.0001**
	Stage	1	6.38	**0.01**
	Lake × Stage	5	1.15	0.34
Residuals of eviscerated whole body protein content	TL	1	2.62	0.11
	Lake	5	3.78	**0.003**
	Stage	1	10.78	**0.001**
	Lake × Stage	5	2.72	0.02

Figures

Figure 2-1 Perceived (male present) and actual (male absent) predation pressure in six lakes sampled from 2007 to 2009. Sample sizes are shown on bars. Dissimilar letters (A,B) indicates significant differences ($p < 0.05$) across lakes for perceived predation pressure while dissimilar letter (X,Y) indicates significant differences ($p < 0.05$) across lakes for actual predation pressure. (2007 predation pressure metrics are adapted from Gravel and Cooke 2009).

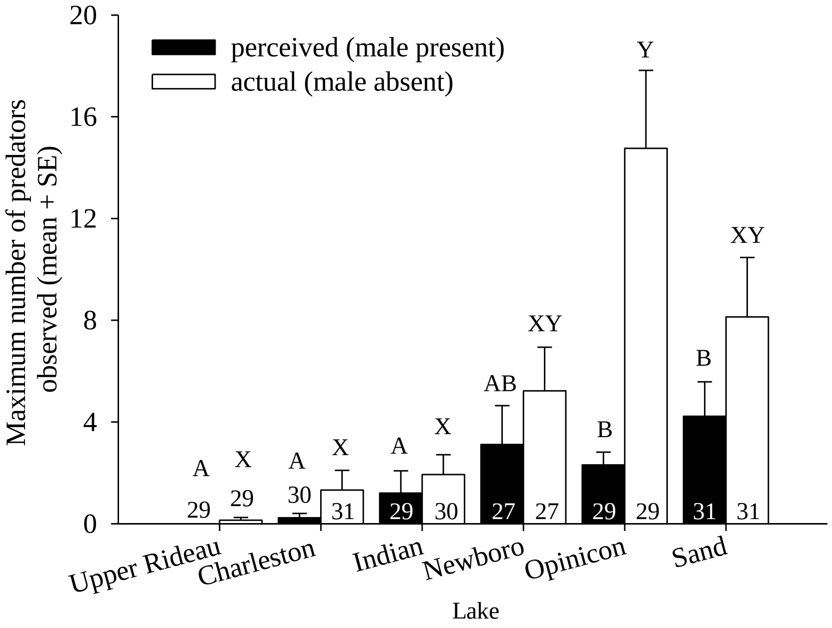


Figure 2-2 Time to nest predation (A) and proportion of nests predated (B) across six lakes from 2007 to 2009. Sample sizes are shown on bars.

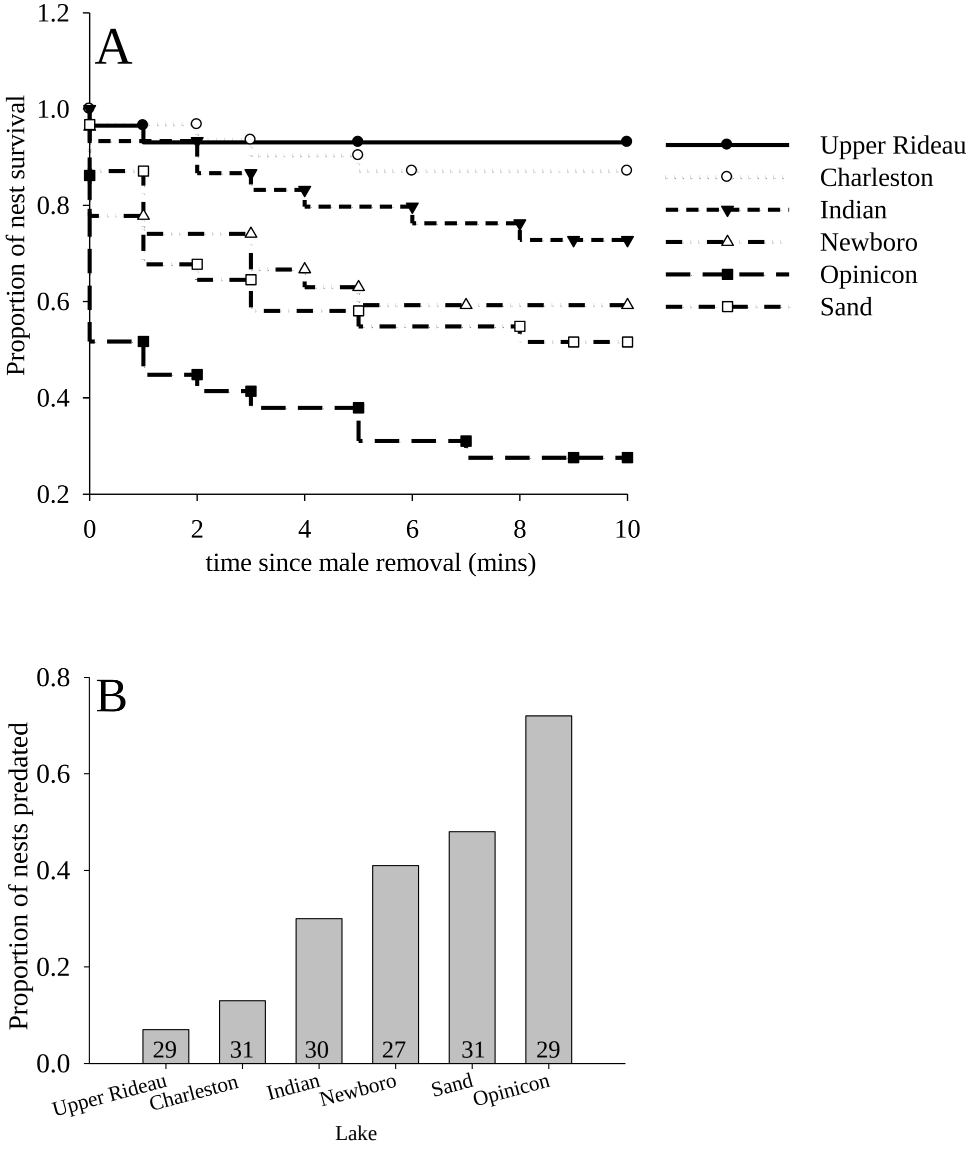
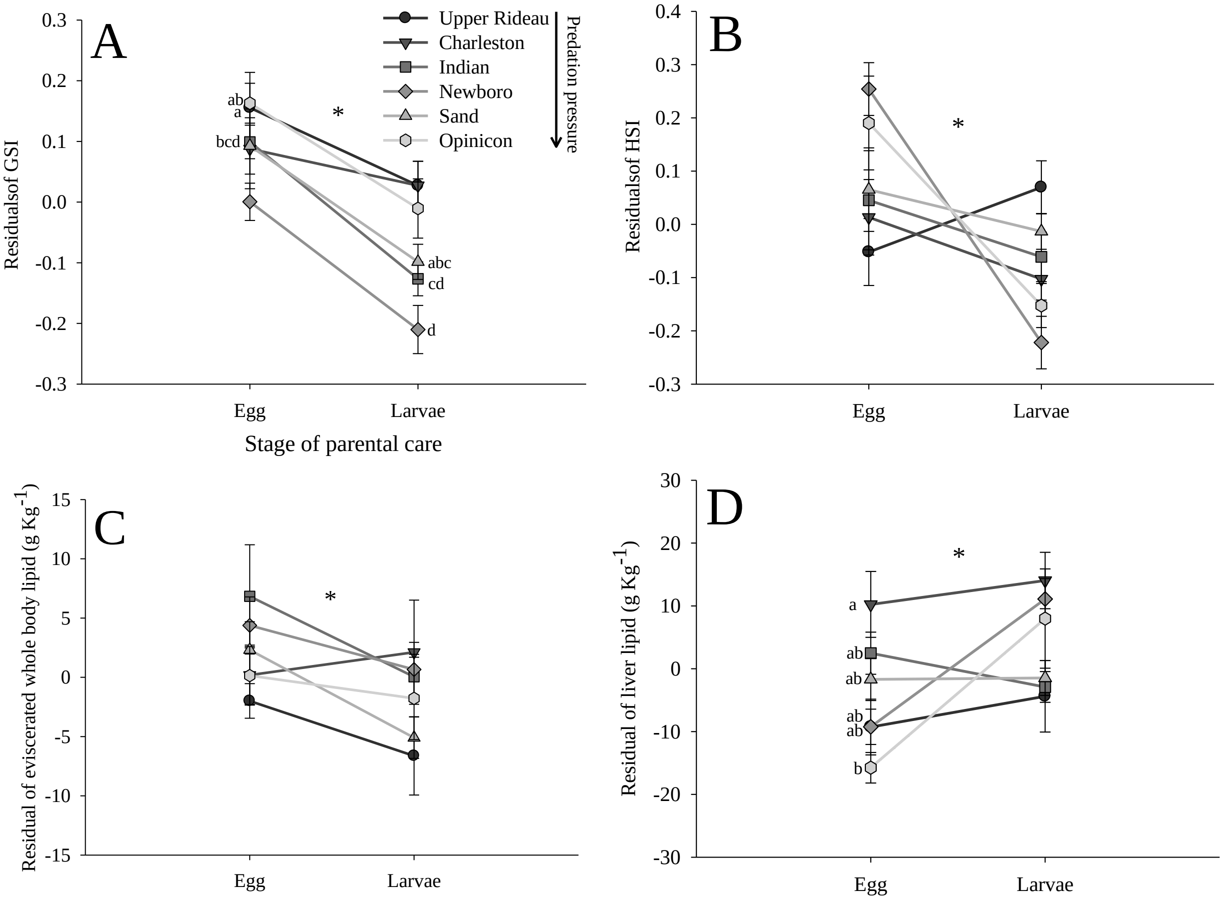


Figure 2-3 Residuals from regression between male smallmouth bass total length and GSI (A), HSI (B), eviscerated whole body lipid (C) and liver lipid (D), water content (E), ash content (F) and protein content (G) as a function of stage of parental care for six lakes that differ in predation pressure. Different letters (a,b,c,d) indicate significant differences between lakes ($p < 0.05$) and * indicates significant differences between the stages of



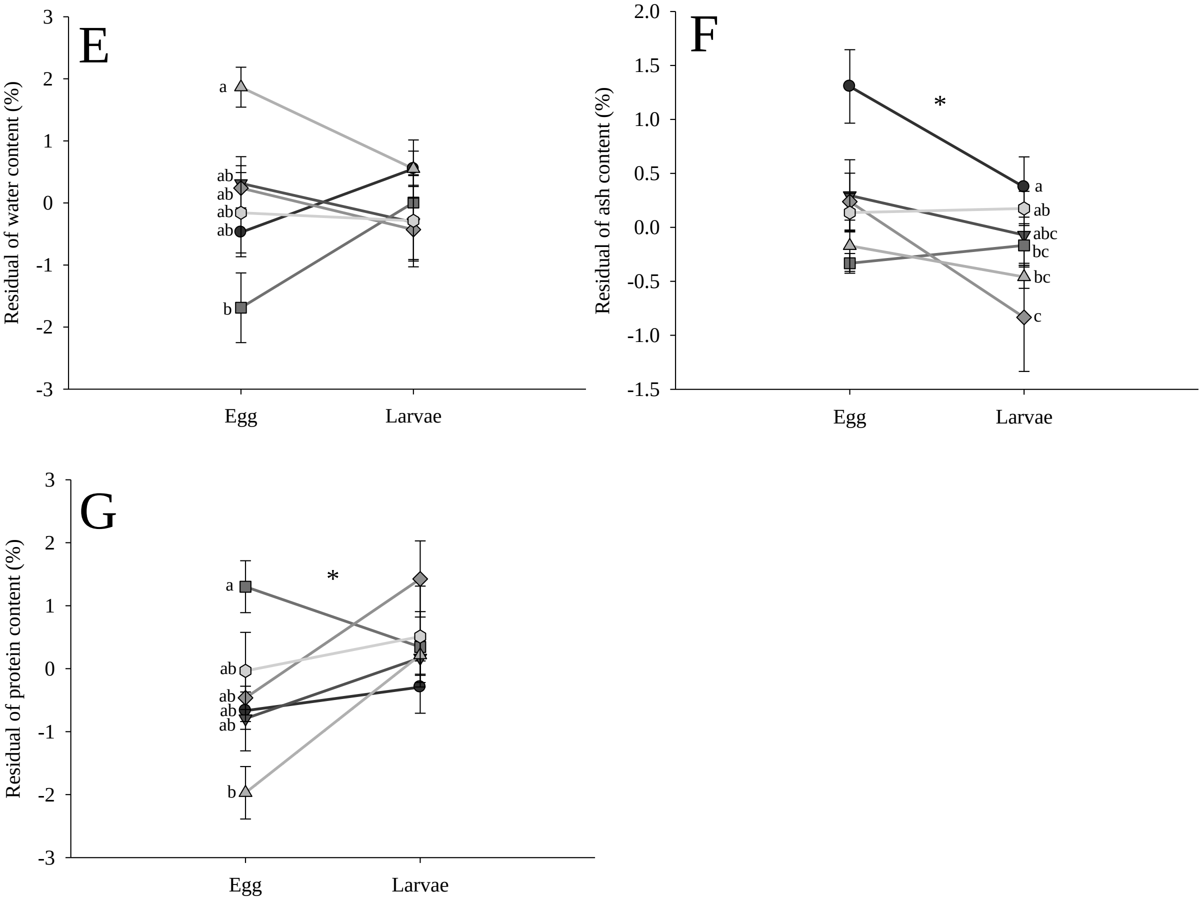

E
3
2
1
0
-1
-2
-3
Residual of water content (%)
a
ab
ab
ab
ab
b
Egg
Larvae
F
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Residual of ash content (%)
*
a
ab
abc
bc
bc
c
Egg
Larvae
G
3
2
1
0
-1
-2
-3
Residual of protein content (%)
*
a
ab
ab
ab
ab
b
Egg
Larvae

Figure 2-4 Kernel density estimates of standardized EMG values for Upper Rideau Lake (low predation pressure) and Opinicon Lake (high predation pressure) when parental males guarding eggs (A), embryos (B) and larvae (C)

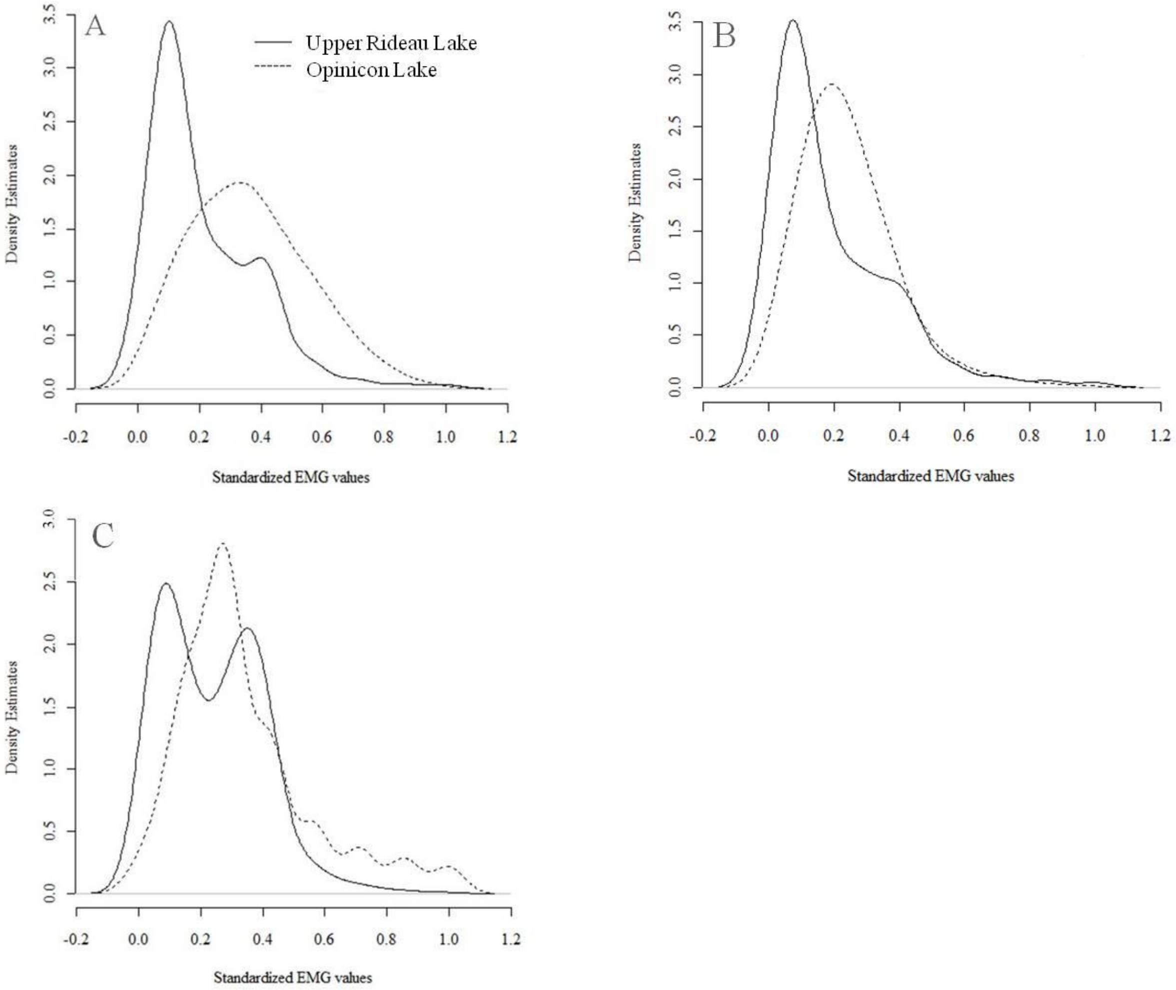
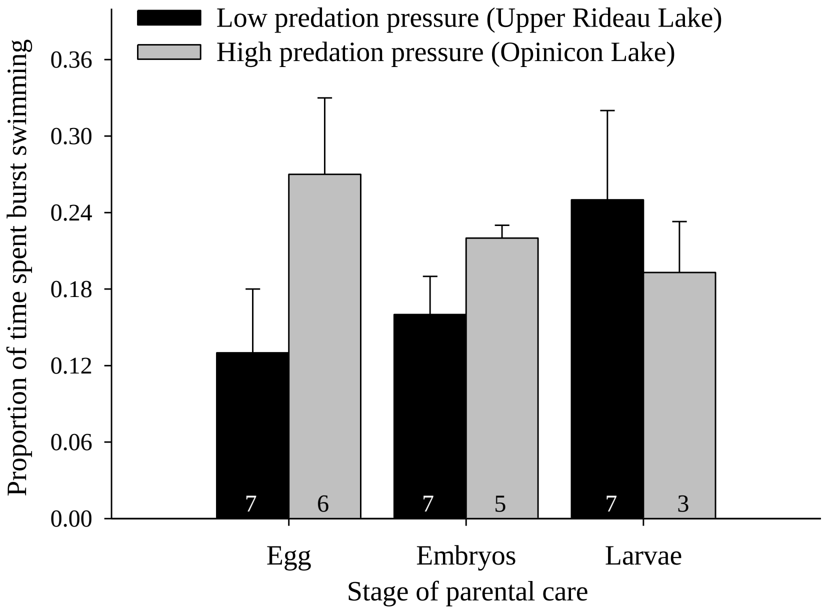


Figure 2-5 Proportion of time spent burst swimming for parental males from populations that differ in predation pressure when guarding eggs, embryos and larvae. Sample sizes are shown on bars.



Chapter 3: Brood predation pressure does not influence parental enzyme activities related to swimming performance

Gravel MA, Couture P, Cooke SJ. 2010. Brood predation pressure during parental care does not influence parental enzyme activities related to swimming activity in a teleost fish. Comp Biochem Physiol A. 155: 100-106

Abstract

Predation is considered one of the main costs to reproduction but is rarely examined from a physiological perspective. In particular, little is known about the influence of brood predation pressure on the physiology of parents engaged in care. Brood defence, even when there is no direct threat to the parent, can be costly as it requires constant vigilance and chasing predators to protect the developing brood and maintain parental investment (i.e., fitness). Our goal was to examine the influence of natural variation in nest predation pressure on the physiology of the teleost smallmouth bass *Micropterus dolomieu*, an animal that provides sole-paternal care for developing offspring. More specifically, we used indicators of anaerobic (lactate dehydrogenase [LDH]) and aerobic capacity (cytochrome C oxidase [CCO] and citrate synthase [CS]) in axial white muscle and pectoral red muscle to test for differences in antipredator performance of nest guarding males across six lakes with natural variation in nest predation pressure. Pectoral red muscle enzyme activities and protein concentrations were highly conserved among populations, while axial white muscle showed differences in LDH activities, CCO activities and protein concentrations. However, there was no evidence for higher

metabolic capacities in fish from lakes with increased brood predation pressure. Clearly, factors other than predation pressure have a greater influence on white muscle metabolic capacities. Additional research is needed to clarify the extent to which biotic and abiotic factors influence the enzyme activity and organismal performance in wild animals across their natural range.

Introduction

Predation is considered one of the greatest costs associated with reproduction (Magnhagen 1991). Most individuals engaged in reproductive activities become more prone to predation due to physical (e.g., ornamentation, pregnancy, nuptial coloration) or behavioural changes (e.g., mate searching, signaling and calling). As a result most research has explored how predators use the cues of reproducing individuals in order to exploit them (reviewed by Zuk and Kolluru 1998) or how animals engaged in reproduction reduce the risk of individual predation (reviewed by Lima and Dill 1990). Alternatively, there are many animals that face low risk of predation during reproduction but whose main goal during this time is to ensure the survival of vulnerable offspring. This specialized behaviour has evolved in most animal taxa (e.g., arachnids, insects, reptiles, fish, mammals, birds) and in environments where offspring face difficult environmental conditions such as limited food availability, extreme temperatures, low oxygen, or high levels of predation (Clutton-Brock 1991). Nest predator abundance and predation pressure can greatly vary across the reproductive range of a species (Steinhart et al. 2005; Fontaine et al. 2007; Gravel and Cooke 2009) and the inability of a parent to defend its brood has severe fitness consequences. As such, selective pressures should act on parental performance under these conditions.

The physiology associated with predation pressure has typically been examined from a stress response perspective. Natural variation in predation pressure influences the level of physiological stress response of prey species (Monclus et al. 2009) and much work is being accomplished to identify the physiological mechanisms involved in the growth/predation risk trade-off (Slos and Stoks 2008). Alternatively, the physiological response to variation in predation pressure may also relate to an individual's antipredator performance, such as escape speed or other antipredator defences. Several physiological tools have been identified as indicators of individual performance and have been used to link organisms to their ecological environment (e.g., Sullivan and Somero 1983; Kaufman et al. 2006; Selch and Chipps 2007). One of particular interest is the link between metabolic capacities, the locomotor performance of fish, and the factors that affect this relationship (reviewed by Guderley 2004). Muscle glycolytic (i.e., lactate dehydrogenase [LDH]) and aerobic (i.e., cytochrome C oxidase [CCO], citrate synthase [CS]) enzyme activities have been shown to be correlated with burst swimming speed and endurance swimming capacity in a variety of fish (e.g., Garenc et al. 1999; Martinez et al. 2003). These enzymes are also ecologically sensitive as they vary with habitat type (e.g., depth of occurrence Sullivan and Somero 1980), prey community (Kaufman et al. 2006; Selch and Chipps 2007) and predation pressure (Odell et al. 2003). These physiological indicators can thus be used as tools to examine questions that relate to performance and environmental variation such as predation pressure.

Here, we examine how nest predation pressure influences the physiology of parental care in a species that provides sole-paternal care, the teleost freshwater fish, smallmouth bass (*Micropterus dolomieu*). Parental care activities include fanning eggs to prevent silt deposition, maintaining vigilance while patrolling the nest area (using sustained swimming actions), and protecting offspring from nest predators by chasing away predators (with burst swimming events). In a system with relatively high nest predation pressure, Cooke et al. (2002) determined that parental smallmouth bass swam the equivalent of more than 40 km per day without leaving the immediate vicinity of the nest. Moreover, nearly 20% of the time bass were swimming at speeds in excess of 80% of critical swimming speeds indicative of anaerobic bursting to chase predators. Conversely, in a lake with very few nest predators, Hinch and Collins (1991) observed very few incidences of bursting activity though the fish were similarly vigilant in patrolling the nest area. The parental care period of smallmouth bass typically lasts four weeks (Ridgway 1988; Cooke et al. 2006) and is known to be physiologically and energetically demanding (Cooke et al. 2002; Cooke 2004; Hanson and Cooke 2009a). We hypothesize that natural variation in nest predation pressure has the ability to influence parental physiology. More specifically, we predicted that males from lakes with high predation pressure will exhibit greater anaerobic and aerobic muscle enzyme activities in their axial musculature than males from lakes with low predation pressure, due to the need for increased anaerobic burst swimming events associated with engaging predators and increased aerobic patrolling associated with nest vigilance and guarding. Conversely, we expect little difference in enzyme activities in the oxidative pectoral muscles of parental smallmouth bass as pectoral muscles are actively involved in egg fanning but most likely play an inconsequential role in burst swimming events or patrolling, which are typically used to deter nest predators. Studies that integrate animal behaviour and physiology are urgently needed to better understand the role of environmental variation on the performance (Altmann and Altmann 2003; Gilmour et al. 2005) and ultimately the fitness of individuals (Ricklefs and Wikelski 2002).

Material and methods

Study sites and sampling design

Fish were sampled from six lakes within a single ecoregion in southeastern Ontario, Canada: Upper Rideau Lake, Charleston Lake, Indian Lake, Newboro Lake, Opinicon Lake and Sand Lake. Study lakes were chosen due to inherent variation in nest predation pressure as documented and described in Gravel and Cooke (2009) with a series of metrics such as number of predators in proximity to nests, time to egg consumption in the absence of males and proportion of nests predated. By using non-parametric ranking tests, lakes were ordered from lowest to highest in nest predation pressure: Upper Rideau Lake < Newboro Lake ≤ Charleston Lake < Indian Lake < Sand Lake < Opinicon Lake.

Within this ecoregion, differences in lake depth and turbidity cause lakes to warm differentially, allow for temporal variation in peak spawning dates (Kubacki et al. 2002) and enable data collection within one spawning year. At the onset of spring, the six lakes were visited daily by snorkelers. Portions of the littoral zone were swum (approx. 1 to 3 km) and when present, parental males on fresh eggs were identified ($n \leq 30$) and nests were labeled with a numbered tile. All data collection occurred during May and June of 2007. Fish were sampled on fresh eggs and were collected by rod and reel (using heavy angling gear – all angling durations < 20 s) within 3 days of egg deposition for physiological analysis of adult males (n = 10 nesting adult males per lake).

Parental male fish were removed from their nest and placed in a foam-lined trough filled with fresh lake water for hook removal. Fish were then euthanized by cerebral percussion within 2 min of being on board the boat. Pectoral red muscle and axial white muscle samples were taken with a disposable scalpel, wrapped in foil, and immediately placed in liquid nitrogen until later transfer to a -80 ºC freezer. Pectoral red muscle was taken anterior and ventral to the pectoral fin, when it laid flat against the fish, while the axial white muscle sample was taken mid-way down the body, 1 cm above the lateral line.

Enzyme activities

White and red muscle samples were randomly chosen, thawed on ice, weighed and diluted 10 – fold with homogenizing buffer containing 20 mM HEPES, 1 mM EDTA, and 0.1% Triton X-100. Tissues were homogenized in plastic test tubes constantly immersed in ice with a Janke and Kunkel Ultra Turrax T25 homogenizer (Janke and Kunkel, Staufen, Germany) with 14 cm × 0.75 cm probe at maximal speed (24 000 RPM). All assays were performed in duplicate at 20 ºC on a UV/Vis spectrophotometer (Varian Cary 100; Varian Inc., Palo Alto, Calif.) with an assay volume of 1 mL. Substrate and cofactor concentrations were determined from assay optimization. Lactate dehydrogenase (LDH – EC 1.1.1.27), citrate synthase (CS – EC 4.1.3.7) and cytochrome C oxidase (CCO – EC 1.9.3.1) were measured as in Couture et al. (1998) with the following modifications. For LDH activity in white axial muscle and red pectoral muscle, dilutions of 1:1000 were made from the homogenized tissues. For CS activity in white axial muscles, assay conditions were changed to 0.1 acetylCoA. For CS activity in red pectoral muscles assay conditions were changed to 0.1 acetylCoA and 0.15 oxalacetate. For CCO activity in red pectoral muscle, dilutions of 1:1000 were made from the homogenized tissues. The reactions were linear over the 5 min period used for the calculation of enzyme activity, and the results are expressed in international units (IU; μmol of substrate converted to product per min) per g tissue mass. Protein concentrations were analyzed as in Lowry et al. (1951) and were determined against a bovine serum albumin (BSA) standard curve. Enzyme activities were also calculated as IU per mg of tissue protein, allowing us, by comparison to enzyme activities expressed on a wet weight basis, to examine whether differences in enzyme activities among groups were due to differences in tissue protein concentration or to up- or down-regulation of the enzymes examined.

Statistical analysis

All statistical analyses were performed using JMP 7.0 (SAS institute, Cary, North Carolina). Data were tested for normality and homogeneity of variance and non-normal data were log (axial muscle LDH and CS) or square-root (pectoral muscle CS and LDH) transformed to achieve normality. Where appropriate, transformed data were used for statistics but non-transformed data are always presented in figures. The relationship between enzyme activities and muscle protein concentration, as well as the relationship between enzyme activities, were tested using model I regressions. We used one-way ANOVAs to test for differences in mean enzyme activities and protein concentration of axial and pectoral muscle among lake populations. Tests were followed with planned multiple comparisons (Tukey-Kramer, *post-hoc*) when significant differences were present. The non-parametric Kruskal-Wallis test was used in one instance when homogeneous variance was not established (fish weight) and was followed by non-parametric multiple comparisons (Zar 1999). Values presented are means ± standard error (SE) and the significance of all tests was evaluated at $\alpha = 0.05$.

Results

Axial muscle

Parental smallmouth bass sampled for measurements of enzyme activities and protein concentrations did not differ in total length ($F_{5,52} = 0.87$, $p = 0.51$; mean ± SE: 408 ± 5.8 mm) or in total weight ($F_{5,52} = 0.98$, $p = 0.44$; mean ± SE: 996.8 ± 45.8 g). Axial enzyme activities were not significantly correlated to protein concentration (CCO: $R = 0.03$, $p = 0.81$; CS: $R = 0.02$, $p = 0.90$; LDH: $R = 0.22$, $p = 0.09$, Fig. 3-1A). Indicators of anaerobic capacity as indicated by LDH enzyme activities expressed per g wet mass, differed among populations ($F_{5,52} = 4.65$, $p = 0.001$), but contrary to our predictions, higher values of LDH activity were not associated with the highest predation pressure (Fig. 3-2A). Fish from lakes with low to intermediate predation pressure (Upper Rideau Lake and Charleston Lake) showed the highest LDH activities, while individuals from lakes with higher predation pressure (Opinicon Lake, Sand Lake and Indian Lake) showed intermediate LDH values (Fig. 3-2A). Of the indicators of aerobic capacity, CCO activity differed among populations ($F_{5,39} = 3.45$, $p = 0.01$, Fig. 3-2B), while CS did not ($F_{5,53} = 1.52$, $p = 0.2$, Fig. 3-2C). CCO failed to exhibit the pattern we had predicted with no clear trend between predation pressure and CCO activity. Axial muscle protein concentration differed among populations (Kruskal-Wallis: $\chi^2 = 13.21$, p = 0.02, Fig. 3-2D). Furthermore, differences among populations for LDH activities were reduced when activities were expressed per mg protein ($F_{5,52} = 2.12$, p = 0.08), while patterns remained the same for CS ($F_{5,53} = 1.6$, p = 0.19) and CCO ($F_{5,39} = 4.5$, p = 0.003). Axial LDH activities were not correlated to CCO or CS activities (LDH vs CCO: R = 17, p = 0.29; LDH vs CS: R = 0.08, p = 0.57) but CCO and CS activities were positively correlated (R = 0.54, p = 0.0001, Fig. 3-3A).

Pectoral muscle

Pectoral LDH activities were positively correlated with pectoral muscle protein concentration (R = 0.40, p = 0.004), while CCO and CS were not (CCO: R = 0.06, p = 0.67; CS: R = 0.001, p = 0.99, Fig. 3-1B). Consistent with our prediction, indicators of anaerobic and aerobic capacities of pectoral muscle did not differ among populations when expressed per g wet mass (LDH: $F_{5,45} = 1.56$, p = 0.19; CS: $F_{5,45} = 1.06$, p = 0.39; CCO: $F_{5,45} = 0.85$, p = 0.52, Fig. 2-4A-C) or mg protein (LDH: $F_{5,45} = 0.09$, p = 0.09; CS: $F_{5,45} = 1.12$, p = 0.36; CCO: $F_{5,45} = 0.63$, p = 0.07). Parental males from all lakes showed similar protein concentration in their pectoral muscles ($F_{5,45} = 0.88$, p = 0.50, Fig. 3-4D). Pectoral LDH activities were not correlated to CCO or CS activities (LDH vs CCO: R = 0.17, p = 0.29; LDH vs CS: R = 0.08, p = 0.57) but CCO and CS activities were positively correlated (R = 0.39, p = 0.005, Fig. 3-3B).

Discussion

Antipredator behaviours are well studied and relatively well understood (reviewed by Lima and Dill 1990). However, much less is known about the physiological consequences of predation pressure. Some attention has been placed on the physiological stress response of predation pressure (Scheuerlein et al. 2001) and recent work has explored the trade-offs between growth and predation risk (McPeek et al. 2001; Stoks et al. 2005). In this study, we set out to test if nest predation pressure could influence the physiological performance of a parental care providing species. In agreement with our hypotheses, we found variation in enzyme activities and protein concentration in the axial musculature of the parental smallmouth bass, but no variation of these parameters in the pectoral muscle. However, contrary to our expectations, variations in enzyme activities did not follow the gradient in nest predation pressure. Lakes with the lowest predation pressure often did not group together physiologically (e.g., Upper Rideau Lake and Newboro Lake, Fig. 3-2A) and there was evidence for fish from lakes with low predation pressure (e.g., Upper Rideau Lake and Charleston Lake, Fig. 3-2A) of having the highest indicators of anaerobic performance capacity. Such findings are contrary to our prediction which leads to several alternative explanations.

Although muscle enzyme activities provide information on a recent timescale (days-weeks, Nathanailides 1996) and our results represent the physiological condition of smallmouth bass providing parental care, it is possible that the enzyme activities of fish from these lakes differ for other ecological reasons. Over 95% of nest predators identified in Gravel and Cooke (2009) were *Lepomis spp*, which outside of the parental care period becomes one of the most common prey species of adult smallmouth bass in lentic centrarchid-dominated habitats (Keast 1978; Warren 2009). One possibility is that lower nest predator densities in individual lakes translate to overall lower sunfish densities throughout the active season. We presume such a relationship exists; however, there are no fisheries assessment data on which to evaluate this assumption. Work on other predatory fish show that enzyme activities are influenced by the size, abundance and type of prey (Sherwood et al. 2002; Kaufman et al. 2006). Ontogenetic diet shifts in yellow perch (*Perca flavescens*) result in lower LDH activities and decreased energetic costs for fish which switch from planktivory to piscivory (Sherwood et al. 2002). Similarly, the increase in size and energetic quality of prey reduces the LDH activities of predatory walleye (*Sander vitreus*, Kaufman et al. 2006). Lakes with low predation pressure such as Upper Rideau Lake and Charleston Lake may require foraging smallmouth bass to spend more time chasing fewer prey. Indeed, both of these lakes contain lake trout (*Salvelinus namaycush*) and have large areas that would be considered poor habitat for lepomids (i.e., deep points and rock shoals with minimal vegetation) but would be used by smallmouth bass to forage on crayfish or partially pelagic species such as yellow perch. Physiologically, the enzymatic indicators used in this study may be indicative of annual trends in food abundance rather than predation pressure during parental care. A simple way to explore this question would be to seasonally sample enzyme activities to test if lakes rank similarly across seasons. Work which has explored the influence of metal contaminants on enzyme activities of yellow perch has confirmed that regional differences are conserved throughout seasons (Couture et al. 2008).

The hypothesis of a link between low nest predation pressure and low prey availability does not clarify the discrepancies between some of the lakes with low predation pressure (Fig. 3-1A). The most obvious difference between Charleston Lake, Upper Rideau Lake and Newboro Lake is size. Charleston Lake and Big Rideau are large, deep lakes (2 500 ha; mean depth of 17.4 m and 6 500 ha; mean depth of 10.2 m respectively), while Newboro Lake is much smaller and shallower (1850 ha and mean depth of 3 m). Although the link between water body size and fish physiological indicators has received little attention, characteristics such as growth rate, condition factor, swimming intensity and consumption rate are known to influence physiological indicators, particularly LDH activities (e.g., Sullivan and Somero, 1983; Goolish, 1991; Pelletier et al. 1993). It has long been clear that habitat type and general feeding ecology influence enzyme activities interspecifically (Sullivan and Somero 1980; 1983), but intraspecific differences in enzyme activities which relate to habitat characteristic have only lately received any attention (Odell et al. 2003; Couture et al. 2008). Our data provides further evidence for intraspecific differences in enzyme activities across a range of habitats.

Variation in the activity of CCO and not CS among the populations studied may be due to the role of CCO in controlling the oxidation rate of lactate into glycogen in the white muscle after exercise (Goolish 1991). Smallmouth bass engaged in parental care are twice as active as non-nesting con-specifics and significantly increase levels of burst-swimming (Cooke et al. 2002; 2006). Since burst swimming events are related to nest predator chases and behavioural observations indicate that chases were more abundant in the lakes with higher predation pressure (Gravel and Cooke 2009) we would expect CCO activities to be elevated in lakes with high predation pressure. As with the pattern of LDH activity, this prediction did not hold.

The positive relationship between LDH activities and muscle protein concentration and the lack of differences between LDH enzyme activities among fish from different lakes when enzyme activities are expressed in mg protein support other work (Houlihan et al. 1988; Mendez and Wieser, 1993) which suggests that the cytosolic character of LDH enables it to become a source of protein during periods of fasting. The lack of relationship between the mitochondrial enzymes and muscle protein concentration further support this hypothesis. In our study, the higher levels of axial LDH activities in certain lakes, and the consequent higher anaerobic capacities, were probably achieved as a result of higher protein concentration and not specifically through the upregulation of anaerobic pathways. In contrast, the changes in axial CCO activity are unrelated to muscle protein concentration and are more likely a response to differences in metabolic demands among populations. The strong relationship between both mitochondrial enzymes supports that differences in these enzymes among populations most likely reflect changes in overall aerobic capacities, even if we are unable to identify the cause of this variation. Future studies focused on other lake-specific characteristics such as density, trophic structure or habitat availability may help to elucidate the relationship between organismal performance and enzyme capacities.

Although there is apparent variation in axial enzyme activities among lakes, pectoral enzyme activities were strongly conserved across the studied populations. The lack of variation in pectoral enzyme activities supports that the variation observed in axial enzyme activities reflect differences in physiological requirements among the populations studied. If differences in protein concentrations and enzyme activities simply reflected differences in protein synthesis between the six populations we would expect similar differences in the axial and pectoral muscles across lakes. However, we cannot exclude the possibility that axial muscle may inherently be more plastic since it is a location for protein storage when growth occurs, while oxidative pectoral muscle may simply be more conservative and less influenced by recent feeding.

Interestingly, this is one of the first studies to evaluate intraspecific variation in enzymatic activities in animals acclimatized to field conditions (but see Couture and Guderley 1990; Kaufman et al. 2006; Couture et al. 2008). Additional research is needed to clarify the extent to which biotic and abiotic factors influence the enzyme activity and organismal performance in wild animals across a range of ecosystems, contributing to the metabolic theory of ecology (Brown et al. 2004) and clarifying the potential role of these biochemical indicators as predictors of animal performance (Gibb and Dickson 2002). Although we do not fully understand the causes of differences in metabolic capacities, the evidence is strong that variations in metabolic capacities do reflect differences in biotic and abiotic components of the environment. Research that examines physiological variation among population across large spatial scales (e.g., “macrophysiology”; Osovitz and Hofmann 2007) will help us better understand the influences of external factors on fish metabolic capacities.

Figures

Figure 3-1 Relationships between axial muscle protein concentration and CCO, CS and LDH axial muscle activities (A), and between pectoral muscle protein concentration and CCO, CS and LDH pectoral muscle activities (B) (reprinted with permission of *Comparative Biochemistry and Physiology Part A*)

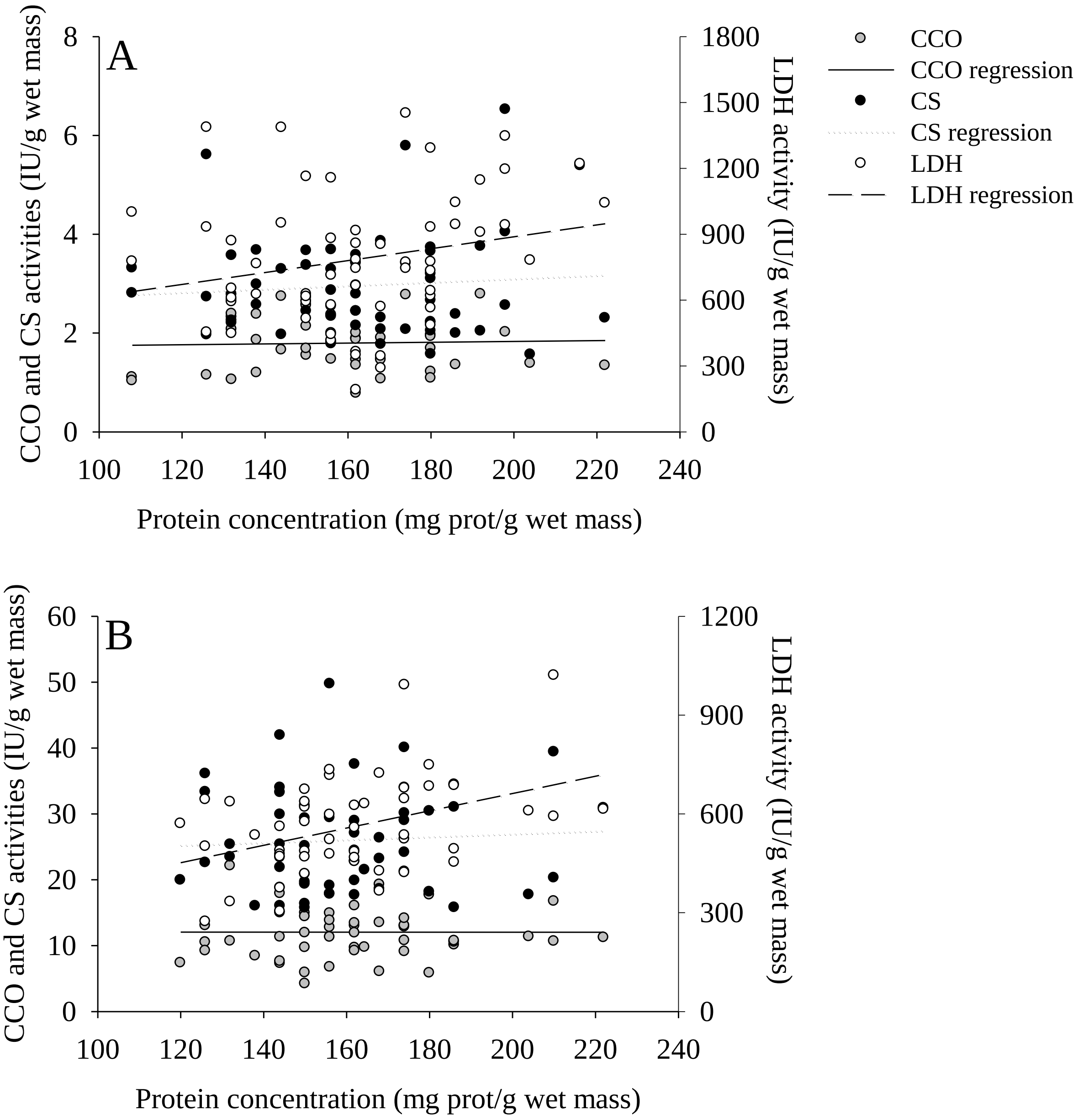


Figure 3-2 LDH (A), CS (B), CCO (C) enzyme activities and protein concentration (D) of axial white muscle of parental smallmouth bass across lakes with natural variation in nest predation pressure. Lakes are presented from lowest to highest predation pressure. Dissimilar letters denote significant differences between lakes within a given physiological parameter (Tukey *post hoc* test, $p < 0.05$). (reprinted with permission of *Comparative Biochemistry and Physiology Part A*)

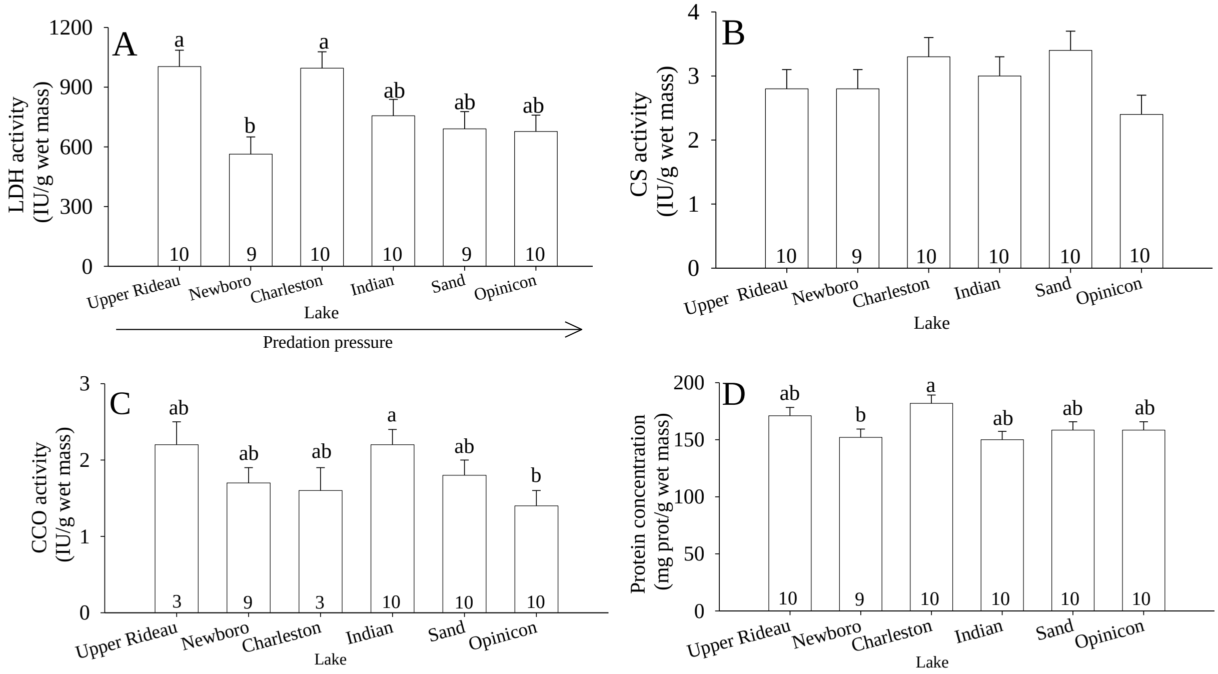


Figure 3-3 Relationship between axial muscle (A) and pectoral muscle (B) CCO and CS activities (reprinted with permission of *Comparative Biochemistry and Physiology Part A*)

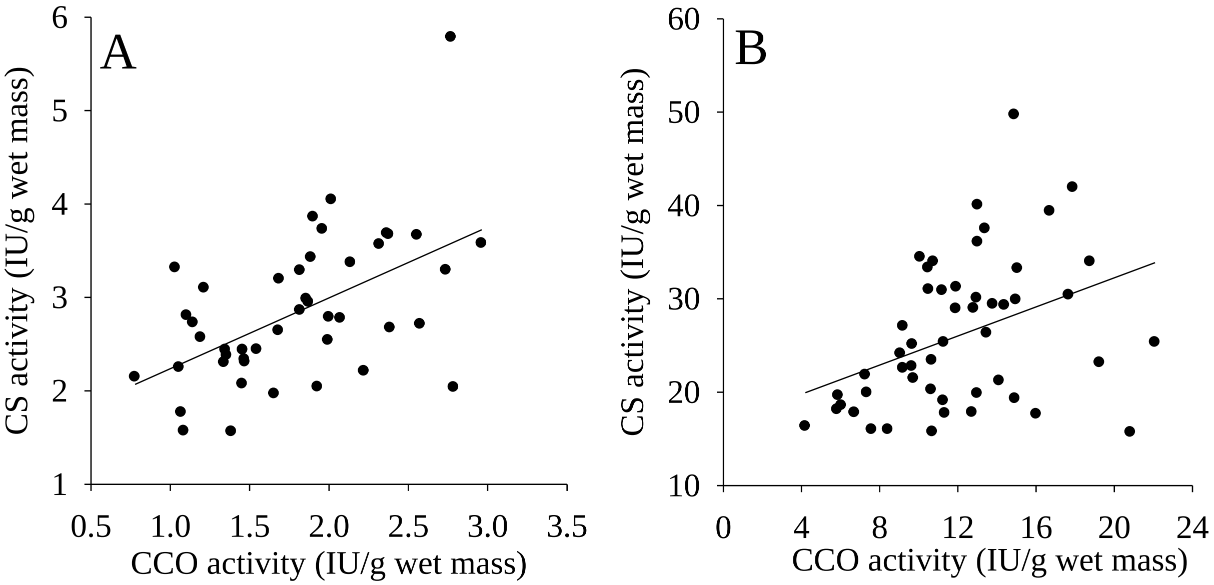
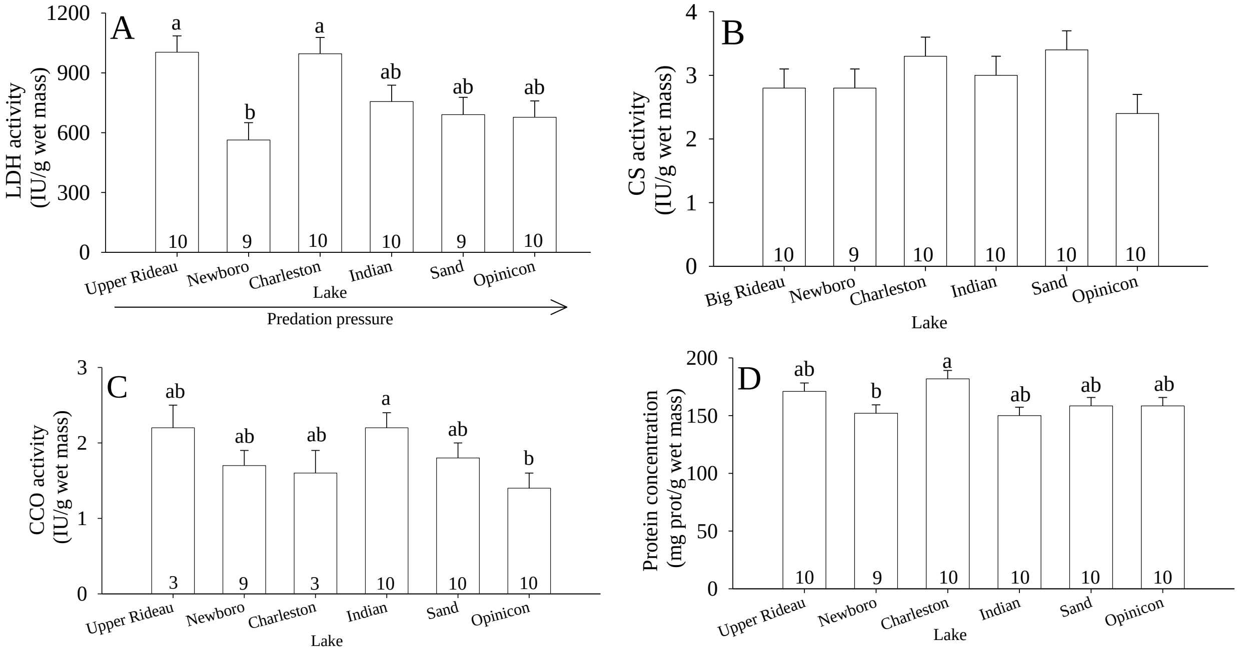


Figure 3-4 LDH (A), CS (B), CCO (C) enzyme activities and protein concentration of pectoral red muscle of parental smallmouth bass across lakes with natural variation in nest predation pressure. Lakes are presented from lowest to highest predation pressure. Dissimilar letters denote significant differences between lakes within a given physiological parameter (Tukey *post hoc* test, $p < 0.05$). (reprinted with permission of *Comparative Biochemistry and Physiology Part A*)



Chapter 4: Behavioural and physiological consequences of nest predation pressure for larval fish

Gravel MA, Cooke SJ. In press. Behavioral and physiological consequences of nest predation pressure for larval fish. Behav Ecol. XX: XX-XX

Abstract

Localized antipredator behaviours have been observed in a wide variety of taxa. Recent work has also shown that animals that provide parental care adjust their behaviour when faced with variation in offspring predation pressure. This variation in nest predation pressure may also influence the antipredator behaviour of offspring if improved antipredator behaviours can increase their probability of survival. We tested if a natural gradient in predation pressure influenced antipredator behaviours of larval teleost fish (smallmouth bass, *Micropterus dolomieu*). We examined the predator avoidance of wild larvae from six populations that differ in nest predation pressure and we also compared the recovery from a simulated predator attack of two populations at the opposite extremes of predation pressure. We found that larvae differed in their ability to avoid the nest predator but larvae from lakes of low predation pressure responded similarly to larvae from lakes of high predation pressure. Generally, older offspring were not significantly better at avoiding predators relative to younger offspring but we found a weak and significant positive correlation between the size of young offspring and their predator avoidance behaviour. The recovery from a simulated predation varied relative to predation pressure. Larvae from the site of high nest predation pressure exhibited

reduced rates of maximal oxygen consumption and recovered faster than larvae from the low predation pressure site. Thus, variation in nest predation pressure had little influence on the antipredator behaviour of offspring which are provided with parental care but may have important metabolic consequences.

Introduction

Predator-prey research has often focused on how prey assess and respond to the risk of predation within a particular ecological setting. Spatial and temporal variability in predation pressure can be important for prey decision-making, particularly when making choices that relate to habitat, foraging or reproduction (reviewed by Lima and Dill 1990). Alternatively, predation pressure can vary across broad-scale landscapes (e.g., populations), which provides opportunities for local adaptations in antipredator behaviours (Magurran et al. 1993). In many cases these localized antipredator behaviours are believed to be hereditary, but are also influenced by the current environment of individuals (Huntingford and Wright 1993). A wide-range of animals (e.g., spiders, amphibians, fish and birds, Giles and Huntingford 1984; Riechert and Hedrick 1990; Relyea 2002; Griesser and Nystrand 2009) have shown local antipredator behavioural adaptations to their predator environments. Recent work on species that provide parental care has shown that parents can also be sensitive to variation in nest predation pressure and will adjust their parental behaviours accordingly (Fontaine and Martin 2006; Gravel and Cooke 2009). Indeed, parents adjust their guarding behaviour because differences in nest predation pressure can greatly influence the survival of valuable offspring. Although the interactions between parental care behaviour and nest predation pressure have recently been examined in parental birds (Martin et al. 2000; Fontaine et al. 2007) and fish (Gravel and Cooke 2009), variation in predation pressure may also have important consequences for offspring. There are several ecological differences between birds and fish which make fish an interesting model for which to further examine the effects of nest predation pressure on offspring. First, the most common form of parental care for fish is guarding offspring, which can range from behaviours such as mouth-brooding to nest building and the defence from predators (Blumer 1982). These antipredator behaviours may be quite important in the learned behaviour of fish offspring (Tulley and Huntingford 1987). Second, the abundance of aquatic nest predators may be greater than the terrestrial environment (Magnhagen 1992), partially due to the fact that fish typically produce a great number of small eggs and these in turn are consumed by a great variety of predators. Third, larval fish antipredator defences develop early, are often innate, and are essential to juvenile survival (Fuiman and Magurran 1994). All of these characteristics make the larvae of parental care-providing fish species a good model for which to test for the effects of developmental environment on antipredator behaviour.

In this study we used the smallmouth bass (*Micropterus dolomieu*) as a model to evaluate the influence of predation pressure on the antipredator behaviour and physiological characteristics of larval fish. Smallmouth bass provide sole-paternal care for approximately four weeks, where males court and spawn with females, fan and guard eggs as well as developing offspring from potential nest predators (Ridgway 1988). Smallmouth bass are found across much of eastern and central North America (Scott and Crossman 1973) and thus inhabit a wide range of habitats, including those that differ in predation pressure (Steinhart et al. 2004; Steinhart et al. 2005; Gravel and Cooke 2009;

Gravel et al. 2010a; 2010b). In some populations, nest predation pressure can be so high that if a nest is left unattended, such is this case when a male is caught by an angler, it may be entirely consumed by predators within minutes (Kieffer et al. 1995; Suski et al. 2003; Steinhart et al. 2004; Gravel and Cooke 2009). For this reason, there is still a need for the early development of antipredator behaviours in offspring even though they may be well cared-for by vigilant males. The importance of antipredator behaviours is indisputable and the behavioural consequences of predation risk have been well examined in the literature (reviewed by Lima and Dill 1990). Unfortunately, little work has focused on the physiological implications which may also be associated with variation in predation pressure. Important relationships have been established between an individual’s physiology and other ecologically relevant variables such as prey availability (Kaufman et al. 2006) or migratory style (Wikelski et al. 2003; Pon et al. 2007) but rarely for predation pressure (but see Bell et al. 2010). Of the numerous tools that have been used to quantify the effects of environmental variation on animal behaviour, measures of metabolic rate (MR) may be among the most powerful. The flexibility of this tool enables researchers to measure resting and active MR, and also measure the MR of individuals that are performing specific activities or recovering from energetically costly challenges such as a simulated predator attack. Furthermore, slight environmental differences (e.g., temperature, pollution) are known to exert strong impacts on organisms’ MR (Schmidt-Nielsen 1984; McKenzie et al. 2007) and are generally conserved across species (Gillooly et al. 2001). As such, we propose to examine the influence of predation pressure on the antipredator behaviour of larval fish using two methods. First, we propose to use a strict behavioural approach to test if populations that differ in nest predation pressure respond similarly to an introduced nest predator. Second, we propose to use a physiological approach to test if predation pressure influences the rates of oxygen consumption following a simulated predator attack (i.e., exercise). Recovery from exercise is often used as an ecologically relevant indicator of survival (Czesny et al. 2003; Suski et al. 2007), can be a good measure of active metabolic rate (AMR) (Soofiani and Priede 1985) and is particularly relevant when individuals must continue to be vigilant to successive predation attempts.

Our first hypothesis tested the influence of variation in predation pressure on the predator avoidance behaviour of larval smallmouth bass. We tested the antipredator behaviour of larvae at two periods of larval development. We predicted that larval smallmouth bass from populations with increased nest predation pressure would have increased predator avoidance behaviours relative to larvae from populations with low predation pressure and that older larvae would better avoid predators (Brown 1984). Our second hypothesis tested if predation pressure could influence the recovery of larvae from a simulated predator attack. These populations could show physiological differences for two main reasons. First, larvae native to environments with high predation pressures may simply be in better physiological condition due to genetic and/or environmental training effects, and therefore may demonstrate accelerated recovery relative to larvae from low predation environments. Smallmouth bass demonstrate a certain level of inter-annual nest-site fidelity (Ridgway et al. 1991a; Barthel et al. 2008) and, as a result, parents may be producing offspring that are in better physiological condition in areas of high predation pressure for several generations. Larvae native to areas of high predation pressure may also be involved in a greater number of burst swimming events, which may lead to beneficial training effects (Pearson et al. 1990; Davison 1997). For both these reasons, we would expect larvae from sites with high predation pressure to have lower MRs and recovery more quickly from exercise than larvae from areas of low predation pressure. A second possibility is that there are negative sub-lethal impacts of predation pressure because most prey animals exhibit a physiological stress response to predators (e.g., Cockrem and Silverin 2002; Monclus et al. 2009). If increased predation pressure is viewed as a chronic stressor (as in Scheuerlein et al. 2001), we would expect larvae from high predation pressure environments to have sub-lethal metabolic costs associated with this chronic stressor such as higher MRs (Lankford et al. 2005) and an impaired ability to recovery from exercise relative to larvae from low predation pressure environments. When taken together, the results of this study will improve our understanding of both how predation pressure can shape behavioural properties of prey fish, but also how prey fishes can express differences in physiological characteristics that can lead to improved survival across varying predation levels.

Methods

Study site and predation pressure

In the springs of 2008 and 2009 snorkelers surveyed the shoreline of six lakes on the Rideau River and Gananoque River systems (Upper Rideau Lake, Charleston Lake, Indian Lake, Newboro Lake, Opinicon Lake and Sand Lake) in eastern Ontario, Canada, to locate smallmouth bass nests. Nests were individually marked using a numbered piece of polyvinyl chloride pipe. Experienced snorkelers estimated male total length (Suski et al. 2003) and these length estimates were later validated by catching some males by rod and reel (Suski and Philipp 2004; Gravel MA unpublished data). Larger males attract larger females, which results in larger males receiving a greater number of eggs per mating (Ridgway et al. 1991b; Philipp et al. 1997; Hanson and Cooke 2009b). Male and female size has the potential to influence offspring survival and behaviour if larger parents produce larger offspring, which is closely linked to size selective mortality and performance in juvenile fish (Sogard 1997) and thus must be considered in this study. These lakes were chosen based on their close proximity to each other (within a 50 km radius; Fig. 4-1) and due to previously documented variation in nest predation pressure (Gravel and Cooke 2009). Bluegill sunfish (*Lepomis macrochirus*), pumpkinseed sunfish (*L. gibbosus*), yellow perch (*Perca flavescens*), black crappie (*Pomoxis nigromaculatus*), largemouth bass (*M. salmoides*), rock bass (*Ambloplites rupestris*), and conspecifics have all been documented as nest predators for smallmouth bass (Scott and Crossman 1973). Our work has shown that the nest predators in this system are almost exclusively *Lepomis spp.*, that nest predation pressure varies greatly among these lakes and that nest predation pressure is consistent across years (Gravel MA unpublished data).

Offspring collection – predator avoidance

In 2008 and 2009, offspring were collected (only four lakes in 2008: Indian Lake, Newboro Lake, Opinicon Lake and Sand Lake) at approximately 7 to 8 days posthatch from a subset of nesting males (n = 9 to 13 nests per lake) in each of the four lakes where the exact date of egg deposition was known and the male had not been removed by researchers. This stage is termed “larvae” and it distinguished by a pigmented eye spot, free-swimming, and exogenously feeding larvae which have a much reduced yolk sac

(Wallace 1972; Balon 1975). Typically, the males had been guarding the developing offspring for 21 days at that point. In 2008 only, juveniles were again collected approximately 15 days posthatch (from the same nests when possible). Juveniles are distinguished by the visible presence of adult smallmouth bass structural elements (Wallace 1972; Balon 1975). That stage corresponded to approximately 28 days of parental care. It is around this time that parental care will soon be terminated and juveniles will remain in littoral habitats to fend for themselves. Ideally, offspring would be collected at identical ages across all lakes (e.g., degree days (DD) posthatch), but some variation was unavoidable due to the nature of running experiments with wild-caught animals and the fact that lakes warm differentially due to differences in depth and turbidity. Offspring were collected (n = 50) with an aquarium net, placed in a glass mason jar (750 mL) with a mesh screw top, filled with fresh lake water and kept in a cooler for transportation (up to 2 hours). Offspring were brought back to the Queen's University Biology Station (Chaffey's Lock, Ontario) where the mason jars were placed in a bath of temperature-regulated lake water (Opinicon Lake, 17 ± 1 ºC), allowing some water exchange between the water in the jars and lake-water bath. Air stones were also added to each Mason jar. Offspring were kept overnight and antipredator behaviour trials began the following morning.

Offspring collection – simulated predator attack

For the simulated predator attack experiment larvae were collected from two lakes at the extremes of the predation pressure gradient: Upper Rideau Lake (lowest predation pressure) and Opinicon Lake (highest predation pressure). A random subset of smallmouth bass nests was identified when males were guarding new eggs < 3 days old. At that time, the snorkeler also estimated male size. These nests (n = 8 for Upper Rideau Lake and n = 6 for Opinicon Lake) were monitored by a snorkeler every few days and larvae were collected (n = 50) in a manner identical to the methods described above. The larvae were held in the laboratory overnight in the aerated Mason jars at conditions described above, and experiments began the following day.

Because oxygen consumption and MR of fish embryos are strongly influenced by age and development stage (Wieser 1995; Hanel et al. 1996), and because development rate of smallmouth bass embryos is related to water temperature (Shuter et al. 1980), it was crucial that we standardized our sites by thermal age and development stage of larvae to ensure that any differences in MRs were not a result of differing development times. For this, the thermal age of larvae from each site was calculated by measuring degree-days (DD) following egg deposition, which was calculated by summing the mean hourly water temperatures from the day of spawning until the day of larvae collection (modified from Pawiroredjo et al. 2008). In Opinicon Lake, a temperature probe (model 105T thermocouple, Campbell Scientific, Edmonton, Alberta, Canada) measured hourly water temperature at 0.2 m and 3.3 m in areas adjacent to nesting smallmouth bass. We generated the mean values from these two probes because the depth of smallmouth bass nests is typically somewhere between 0.2 and 3.3 m (Rejwan et al. 1997). Thermal loggers (iButton ®, Maxim Integrated Products, Sunnyvale, CA, USA) were placed at the nest sites of two representative nests (one relatively shallow and one relatively deep) in Upper Rideau Lake when eggs were discovered, and water temperature data from these two thermo-loggers were downloaded soon after larvae collection and used to generate

DD for this site. The first 24 hours after egg deposition was excluded from degree day calculations because this data was only available from Opinicon Lake. A subset (n = 5) of larvae from each nest was blotted dry and weighed on an analytical balance (± 0.0001g) and a mean larval mass was generated for every nest.

Predator avoidance behaviour

All antipredator protocols were run between 830 and 1730 hours. Bluegill sunfish (n = 10) were caught by rod-and-reel and landed within 10 s on the morning of each trial and placed in a cooler (50 l) with fresh lake water. Individual bluegill were only used for one trial and total body length ranged between 92 and 141 mm (mean ± SE; 105.9 ± 1.0 mm). For this experiment, 35.5 l glass aquaria (25 × 29 × 49 cm) were modified into trial tanks. The aquaria were divided into uneven sections (⅓; predator and ⅔; larvae) by a removable opaque barrier and a permanent perforated transparent barrier. The offspring zone of the tank was subdivided into 3 sections by lines drawn on the inside of the tank and clearly visible to the observer. These lines created a grid and had sections nearest (N), middle (M) and farthest (F) from the predator enclosure. These tanks were covered in brown paper to avoid other visual disturbances and filled with fresh lake water before each trial. Tanks (n = 4) were placed alternatively with the predator-side and the prey-side facing the observer to evenly distribute overhead light or other tank effects. Offspring (n = 10 from each nest) were placed in the prey-side of a tank with the opaque barrier in place and were left to acclimatize for 10 to 15 min. The observer could run a trial with four nests simultaneously. Once offspring were acclimatized, the control trial began. The observer used a scan sampling technique to count the number of offspring in each grid (N, M and F) at 30 s intervals for a period of 5 min. At the end of the 5 min control, a bluegill was placed in the predator enclosure and the opaque barrier was carefully removed so as to not disturb the water and the offspring. Scan sampling resumed for another 5 min trial and the observer noted the location of the offspring within their enclosure at 30 s intervals. After the termination of the trial, individual bluegill were dip-netted from the aquaria, measured for total length (TL) and released back into the lake. Offspring were also measured for TL and weighed. Due to provincial regulations, larvae and juveniles could not be released into lake of origin once exposed to other lake water and were euthanized by anesthetic overdose (200 ppm of buffered MS-222).

Simulated predator attack

Larvae were exercised (n = 4 per nest) in Petri dishes (5 × 15 mm) filled with fresh lake water at 17 °C. Larvae were chased manually for 3 min with a micropipette by gently touching the posterior of the tail to mimic a predator attack and induce physiological disturbances resulting from a combination of exercise and the threat of predation (Wieser et al. 1985). Two of the four individuals were randomly selected and immediately placed into micro-respirometry chambers. Oxygen consumption was measured using computerized, intermittent flow-through respirometry (Loligo Systems, Hobro, Denmark, Steffensen 1989). The system consisted of four glass micro-chambers (700 µl) outfitted with fiber-optic oxygen probes immersed in a 45 l cooler filled with approximately 10 l of aerated lake water thermostatically maintained at 17.0 ± 1 °C. Change in oxygen concentration (α) for each chamber was calculated as slope ($\Delta O_{2\text{saturation}}/\Delta t$), and oxygen consumption rate ($\dot{M}O_2$, mg·O_2·kg^{-1}·h^{-1} individual^{-1}) for each chamber was calculated by

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

where V_{resp} is the volume of each glass chamber minus the volume of larvae (liter), β is oxygen solubility (adjusted regularly for both temperature and barometric pressure), and M_b is the mass of larvae (kilogram) prior to being placed in the respirometer chamber. The system alternated between a 240 s flush period that added fresh water to each chamber, followed by a 30 s wait period and a 360 s measure period where oxygen concentration in each chamber was measured every 2 s. Several preliminary trials were run prior to the experiment to determine the time necessary for oxygen consumption to decrease within the chambers without negatively impacting larvae (Spoor 1984), and also to define the time required to ensure oxygen concentrations returned to approximately 100% saturation following the flush period. One chamber was randomly assigned to remain empty to correct for background oxygen consumption within the system. To ensure confidence in generated data, only slopes that generated coefficients of determination (R^2 values) that were ≥ 0.99 were used for analyses, and, because some R^2 values were slightly below this criteria, sample sizes were reduced to $n = 8$ for Upper Rideau Lake and $n = 6$ for Opinicon Lake. For our experiment, larvae were allowed to recover from exercise for approximately 80 min (5000 s), during which time oxygen consumption was measured continuously (every 0.6–0.8 s). This measurement duration should be sufficient for recovery based on work performed with juvenile fishes (Gingerich et al. 2010). Although it would have been desirable to obtain data on standard MR (i.e. pre-disturbance values) for the same fish used for respirometry experiments, this was not done for several reasons. First, larvae remained active (swimming) within the chambers such that it was unlikely that we would have been able to obtain true standard MR estimates. Second, larvae were sensitive to repeated handling so it was not possible to first attempt to obtain standard MR data on fish and then remove them from the chambers, chase them, and then return them to chambers without resulting in physical injury.

Statistical analysis

All statistical analyses were performed with J.M.P. 7.0.1 (SAS Institute, Cary, NC, USA) and SPSS 15.0 (IBM, Chicago, IL, USA). Data were tested for normality and equality of variances. We used a one-way analysis of variance (ANOVA) to test for differences in male TL among the lakes that were used for the predator avoidance trials. We used t-tests to test for differences in male TL and larvae mass between the two lakes which were used for respirometry. We used a three-way repeated measures ANOVA with lake, time and larval stage as main effects for the 2008 predator avoidance trials. A two-way repeated measures ANOVA with lake and time as main effects was used to test the predator avoidance behaviour of larvae in 2009 and to compare the oxygen consumption of larvae following a simulated predator attack. The Mauchly's test of sphericity (i.e. test for equality of variances for repeated measures analyses) was not significant for all tests. Significance for all tests (α) was evaluated at 0.05 and data are shown as means ± SE unless otherwise indicated.

Results

Predator avoidance behaviour

There was no effect of sampling year on parental male TL ($F_{1,3}$ = 1.1, p = 0.30) and thus years were pooled for further analysis. Parental males used for the predator avoidance trials differed in TL ($F_{5,86}$ = 7.4, p < 0.0001, Fig. 4-2). There was an effect of year on larval length and weight and because all lakes were not sampled in both years, this data were analyzed separately. In 2008, larvae from different lakes differed in TL ($F_{3,40}$ = 6.1, p = 0.002; Fig. 4-3A) and weight ($F_{3,37}$ = 10.5, p < 0.0001; Figure 4-3B). Juveniles did not differ in TL ($F_{3,39}$ = 1.6, p = 0.20; Fig. 4-3A) or weight ($F_{3,37}$ = 2.2, p = 0.11; Fig. 4-3B). Both stages of offspring development spend significantly less time near the predator enclosure once the predator was introduced (Table 4-1, Fig. 4-3C) but there was no influence of lake or development stage on the predator avoidance behaviour of offspring (Table 4-1). In 2009, larvae used in the antipredator trials also differed in length ($F_{5,54}$ = 48.3, p < 0.0001, Fig. 4-4A) and weight ($F_{5,54}$ = 14.0, p < 0.0001, Fig. 4-4B) across lakes. Larvae from all lakes significantly reduced their time in the grid nearest the predator once the predator was introduced (Table 4-1, Fig.4-4C). Overall, there was also significant effect of lake on the predator avoidance behaviour of larval fish (Table 4-1, Fig. 4-4), but the data did not follow the predicted pattern because larvae from lakes with the highest predation pressure were not consistently better at avoiding predators than larvae from lakes of low predation pressure. Larvae from Newboro Lake had the greatest decrease in proportion of time spend in grid nearest to the predator once the predator was introduced, followed by Indian Lake and Upper Rideau Lake, which had very similar responses. Larvae from Opinicon Lake, Sand Lake and Charleston Lake had weaker responses to the predator. Interestingly, there was no significant relationship between male length and larval length (n = 92 R^2 = 0.0002, p = 0.91) or weight (n = 89, R^2 = 0.04, p = 0.06) and between male length and juvenile length (n = 28 R^2 = 0.004, p = 0.74) and weight (n = 27, R^2 = 0.005, p = 0.73). A post-hoc test revealed a weak but significant relationship between the TL of larvae and the difference between the proportion of time that larvae spent in the grid nearest the predator in the absence and presence of a nest predator (n = 104, R^2 = 0.08, p = 0.004, Fig. 4-5) but not for juveniles (n = 43, R^2 = 0.08, p = 0.07).

Recovery from simulated predation event

We collected larvae from our low predation site (Opinicon Lake) 24 and 25 days after egg deposition, which corresponded to embryos being 386 DD old. Our high predation site larvae (Upper Rideau Lake) were collected 24 days following egg deposition, which corresponded to a thermal age of 385 DD. Parental males from the two different sites did not differ in TL

(t = -0.71, p = 0.49, mean ± SE, Upper Rideau Lake: 385 ± 10 mm, Opinicon Lake: 377 ± 5 mm), but larvae from the low predation site (Upper Rideau Lake) were approximately 17% heavier than larvae from the high predation site (Opinicon Lake) (t = -2.54, p = 0.021, mean ± SE, Upper Rideau Lake: 0.012 ± 0.0014 g, Opinicon Lake: 0.0097 ± 0.0006 g) despite being almost identical in thermal age. When MR data for both sites were examined collectively, larvae from the low predation site had higher oxygen consumptions post-exercise compared with larvae from the high predation site (Table 4-1, Fig. 4-6). When examined on a finer scale, oxygen consumption of larval smallmouth bass following our standardized simulated predation event (i.e., 3 min of exercise) was influenced by time post-disturbance (Table 4-1). More specifically, oxygen consumption decreased following the disturbance period for both sites (Fig. 4-6), and the rate of recovery was steeper for the low predation site than the high predation site (Table 4-1, Fig. 4-6). There was no influence of male TL on the oxygen consumption of larvae ($R^2 = 0.135$, $p = 0.22$). Due to small differences in larval size between lakes we also ran the repeated measures model with larval weight as a covariate and it did not significantly contribute to the model ($F = 4.16$, d.f. = 1, $p = 0.07$).

Discussion

Local adaptation to variation in predation pressure has been observed in many species. Although parents have also shown localized antipredator behaviours, little work has focused on how offspring respond to variation in nest predation pressure. In circumstances where parents face little risk of predation but offspring predation pressure is high, selective pressures should not only act on the defensive behaviour of parents, but also on the antipredator behaviour of offspring. Our work has shown that a natural gradient of predation pressure has little influence on predator avoidance behaviour of larvae but may have important physiological implications.

Predation pressure did not influence the predator avoidance behaviour of larval and juvenile smallmouth bass. Offspring from the lake with the lowest predation pressure performed similarly to offspring from the lake with the highest predation pressure. Although we demonstrated behavioural differences between lakes that differed in predation pressure, we found no evidence for localized predator avoidance behaviour linked to differences in predation pressure. Many taxa have demonstrated population level antipredator tactics (e.g., Giles and Huntingford 1984; Riechert and Hedrick 1990;

Relyea 2002; Griesser and Nystrand 2009), particularly when adults of the population were tested. We predicted that in a system where it is the offspring that face immediate predation, we would also detect similar population level differences in offspring behaviour. Parents from lakes with high predation pressure could produce offspring that are able to better perform in these environments (i.e., adaptation) or simply because the environment itself enables offspring to become better performers (i.e., acclimation). In contrast to what we predicted, we found that offspring from a gradient of nest predation pressure were equally equipped to avoid predators. We propose two alternative hypotheses for this trend. First, in this particular system, offspring survival may not depend on performance. We tested the behaviour of offspring when they were still being guarded by a male parent and as they were developing avoidance as well as feeding skills. At this time, the antipredator behaviour of the parent may outweigh the ability of offspring to individually avoid predators. We find some support for this notion because parental behaviours are influenced by predation pressure. Nesting male smallmouth bass residing in lakes with high predation pressure spend more time engaged in antipredator activities than males from lakes with low predation pressure and are generally more active (Steinhart et al. 2005; Gravel and Cooke 2009; Gravel MA unpublished data). A second possibility relates to the cost of developing antipredator skills. The cost of developing and engaging in basic antipredator behaviour could be relatively low because offspring from all lakes were able to avoid the predator enclosure to similar degrees. Some of these similarities across populations may also be attributed to the shoaling nature of this behaviour because predator avoidance is often facilitated in groups when compared with individual avoidance (Godin 2002). Therefore, although offspring smallmouth bass demonstrated a clear ability to recognize and avoid potential predators, this ability was not influenced by the quantity of predators found in the natal rearing environment.

Although predation pressure did not clearly influence the antipredator behaviour of larval smallmouth bass, we did find a significant positive relationship between the performance of larvae and the TL of larvae.   Because we tested the behaviour of small fish, body size, which is typically accompanied by further neural development (Wallace 1972), can play an important role in the acquirement of antipredator behaviours. Within this reasoning, we would also expect older offspring (i.e., juveniles) to show increased predator avoidance behaviour. Although juveniles usually showed an increased predator avoidance (Figure 4-3C), there was no statistical distinction between their behaviour and larval behaviour. Ontogenetic shifts in antipredator behaviours are quite common (e.g., Pongracz and Altbacker 2000; Brown et al. 2002; Dangles et al. 2007) and are particularly relevant for species which provide parental care. Brown (1984) showed that young larvae from species which provide a relatively short-period of parental care (e.g., rock bass) showed significantly better antipredator avoidance than young larvae from species that provided relatively lengthy parental care (e.g., largemouth bass). Largemouth bass offspring only began avoiding predators when they were considered juveniles and which corresponded to the period when they would typically be abandoned by their parent. At the early larval stages largemouth bass spend more time foraging than older larvae and are believed to invest strongly into growth instead of predator avoidance, aided by the presence of a vigilant parent (Brown 1985). Our work does not show great support for the same shift for smallmouth bass. Although we found that larval smallmouth bass were able of actively avoiding an introduced predator, there was not a vast improvement in this avoidance from the larval to juvenile stage. There may not be as much room for improvement in the antipredator behaviour of smallmouth relative to largemouth bass because Brown (1984) found that larval largemouth bass showed reduced antipredator behaviours.

Our work also showed that predation pressure influences the ability of larval smallmouth bass to recover from exercise. Ideally, our study would have compared the physiological consequences of predation pressure across numerous populations of nesting smallmouth bass (see Gravel and Cooke 2009; Gravel et al. 2010b), but the direction of our results clearly supports the notion that individuals from high predation pressure environments recover from exercise more quickly, and are potentially in better physiological condition than individuals from the low predation pressure site. Hence, we found no evidence for our alternative prediction, which proposed that there may exist sublethal consequences of predation pressure. Sprint training is known to reduce energy loss during exercise as well as improve the swimming performance of fish and facilitate recovery following exercise (Pearson et al. 1990). In support of this, our work showed that larval smallmouth bass from a lake with high predation pressure had lower MRs during recovery as well as a steeper recovery slope than individuals from a lake with minimal predation pressure. Although 3 min of exercise is probably longer than the length of natural chase event, larvae were not chased to exhaustion and the physiological disturbance they experienced is still physiologically relevant. A possible consequence of this longer chase period may be that the physiological differences between the two groups are slightly more exaggerated than following a typical “natural” disturbance. Low

MRs (active and standard) are often considered an advantage as they allow for a greater metabolic scope (Cutts et al. 2002), which translates into a greater capacity for activity and recovery. Our experiment did not include the measurement of standard metabolic rate (SMR) for these two populations, but SMR and active metabolic rate (AMR) have been shown to be highly correlated in several species of juvenile fish (Cutts et al. 2002; Hansen and von Herbing 2009). If that is the case with larval bass, we would expect the larvae native to high predation sites to have lower SMRs, which may permit a greater scope for activity, as it does for other species (Cutts et al. 2002; Hansen and von Herbing 2009). This suggests that larvae from the site of high predation pressure are better physiologically equipped to respond to variation in their environment. Lower maintenance costs may enable fish to use this "surplus" energy in times of need, such as a predation attempt (Priede 1977). A larger scope for activity could translate into behavioural flexibility and allow individuals to perform a wide range of behaviours which rely on availability of metabolic scope. In contrast, larvae from the site of low predation pressure may be able to invest in higher maintenance costs due to fewer predation events. This may allow them to invest more heavily into growth and reduce the probability of size-selective mortality (Sogard 1997). In general, there is a need for research that examines the relationship between individual variation in behaviour and variation in individual physiology to help us better understand the behavioural and ecological consequences of physiological diversity.

Although there was no difference in male size between our sites of low and high predation pressure, there could be other indicators of male quality (i.e., genetic effects) that are contributing to the difference in larval performance (Patterson et al. 2004;

Pakkasmaa et al. 2006). Our work has shown that predation pressure is relatively stable across years (Gravel and Cooke in review) and because smallmouth bass demonstrate some level of nest-site fidelity (Barthel et al. 2008) , it is possible that parents from lakes with high predation pressure simply produce offspring better equipped to deal with their environment. Unfortunately, our work does not allow us to tease apart the potential benefits supplied from the predator rich environment (i.e., training effects) and the possible beneficial genetic effects. This type of system also provides little information about maternal effects as female smallmouth bass simply choose males, deposit eggs and then depart, making their collection in wild populations challenging. Consequently, although we know little about female quality, as well as the relationship between female quality and offspring size, survival and/or performance, we have some evidence that maternal effects may differ between these two populations due to our differences in offspring size. Larvae from Upper Rideau Lake were 17% heavier than larvae from Opinicon Lake. This could be an indication of investment into larger offspring because these larvae had only recently begun to exogenously feed and still had partial yolk sacs and it is unlikely that these weight differences could be attributed to differences in feeding habits. Unfortunately, this would be contrary to an *a priori* prediction that relates maternal investment and offspring size. We would expect females from sites of high predation pressure to invest in larger offspring than females from low predation pressure because larger offspring generally are better physical performers and have increased survival (Sogard 1997). Our work shows no support for this hypothesis. It is also possible that females from the site of low predation pressure are larger than females from the site of low predation pressure and simply produce larger eggs and offspring (Kamler 2005).

Our study examined whether a natural gradient in predation pressure would influence the antipredator behaviour of young fish that are provided with parental care. We found that predation pressure had little influence on the predator avoidance behaviour of larvae but that parent size may influence the performance of offspring. Furthermore, we found that recovery from a simulated predator attack differed between populations at the extremes of the predation pressure gradient. Taken as a whole, our work has shown that ecologically relevant environmental variation such as predation pressure has the ability to influence indicators of performance between populations. Further work that distinguishes between the roles of parental quality and environmental acclimation across these ecological gradients will help us better understand if or how these animals are adapted to these environments.

Tables

Table 4-1. Results from repeated measures ANOVA for the antipredator behaviour of larvae across a gradient of predation pressure and for the recovery from exercise of larvae from sites of low predation and high predation pressure. Significant differences are shown in bold. (reprinted with permission of *Behavioral Ecology*)

Response	Main effects	d.f.	F	p-value
Antipredator behaviour (2008)	Stage	1	1.3	0.27
	Lake	3	0.05	0.83
	Time	1	37.1	**<0.0001**
Antipredator behaviour (2009)	Lake	5	2.5	**0.04**
	Time	1	95.4	**<0.0001**
	Lake*Time	5	2.0	0.09
Recovery from exercise	Lake	1	12.7	**0.04**
	Time	6	5.4	**<0.0001**
	Lake*Time	6	4.3	**0.001**

Figures

Figure 4-1 Map of study area, showing the six lakes and the Queen's University Biological Station (reprinted with permission of *Behavioral Ecology*)

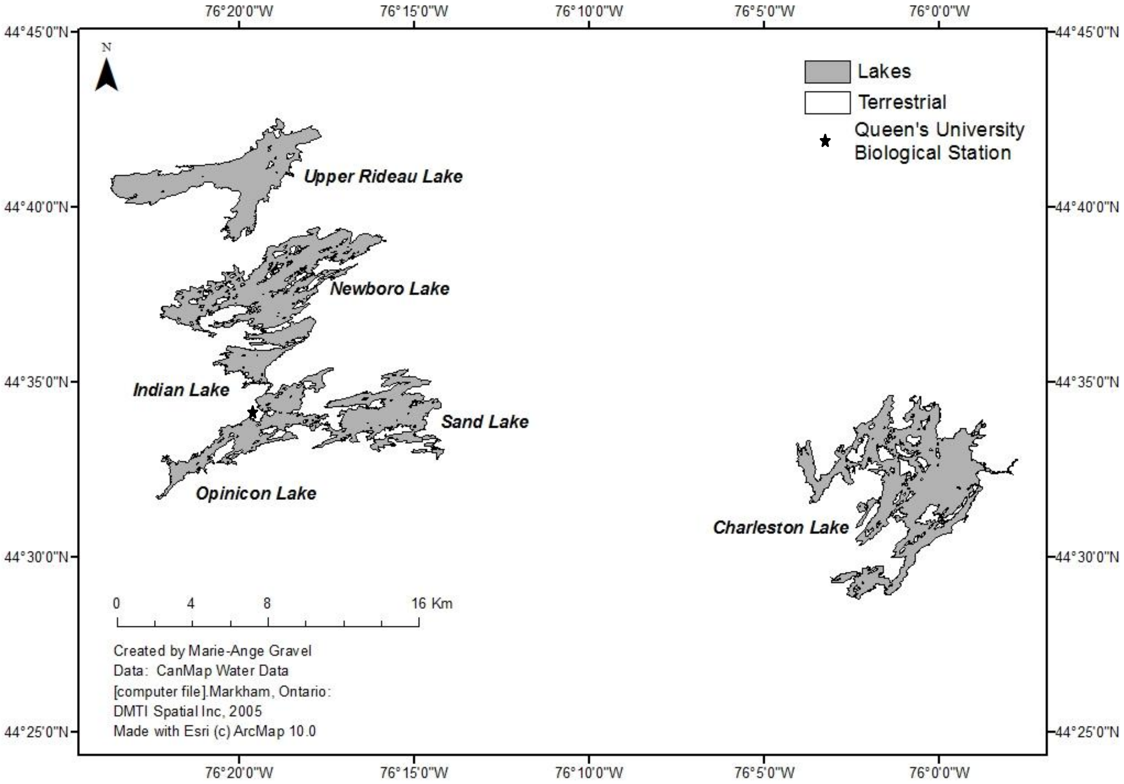


Figure 4-2 Smallmouth bass parental male total length (mean ± SE) collected from six different lakes in eastern Ontario that varied within level of predation pressure. Sample sizes are shown in brackets and dissimilar letters denote significant differences ($p < 0.05$). (reprinted with permission of *Behavioral Ecology*)

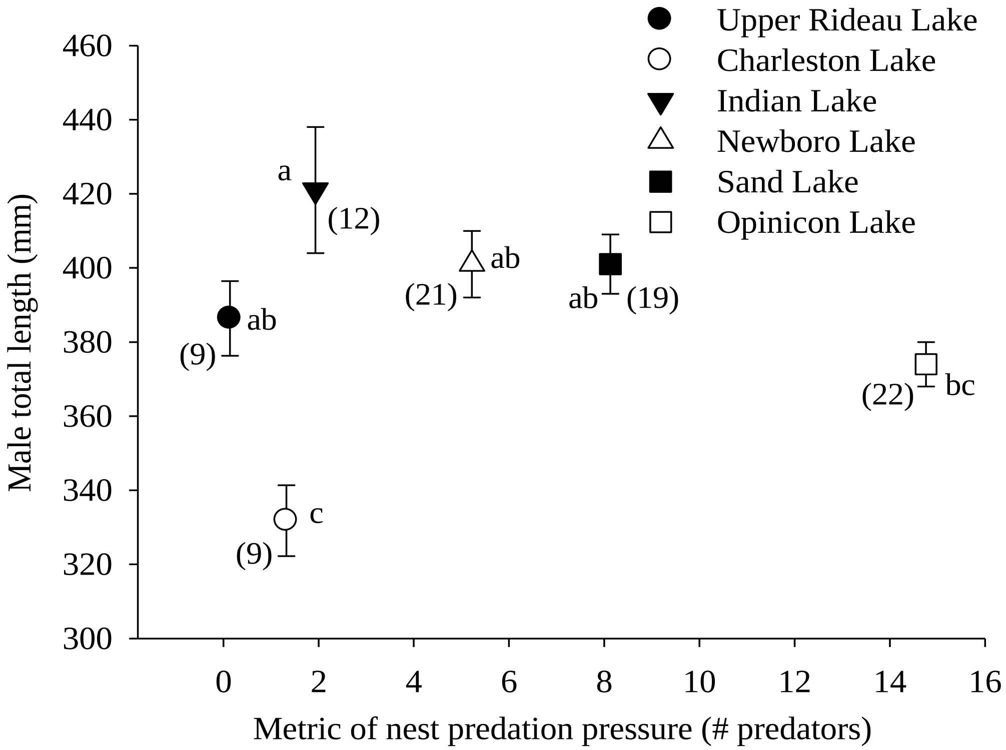


Figure 4-3 Larvae and juvenile total length (A), total weight (B) and difference (C) between the proportion of time spent in grid nearest predator enclosure in the presence of a predator and in the absence of a predator for larvae (closed symbols) and juveniles (open symbols) from four lakes sampled in 2008 which differ in predation pressure sampled. Showing mean ± SE for all parameters. Sample sizes are shown in brackets. (reprinted with permission of *Behavioral Ecology*)

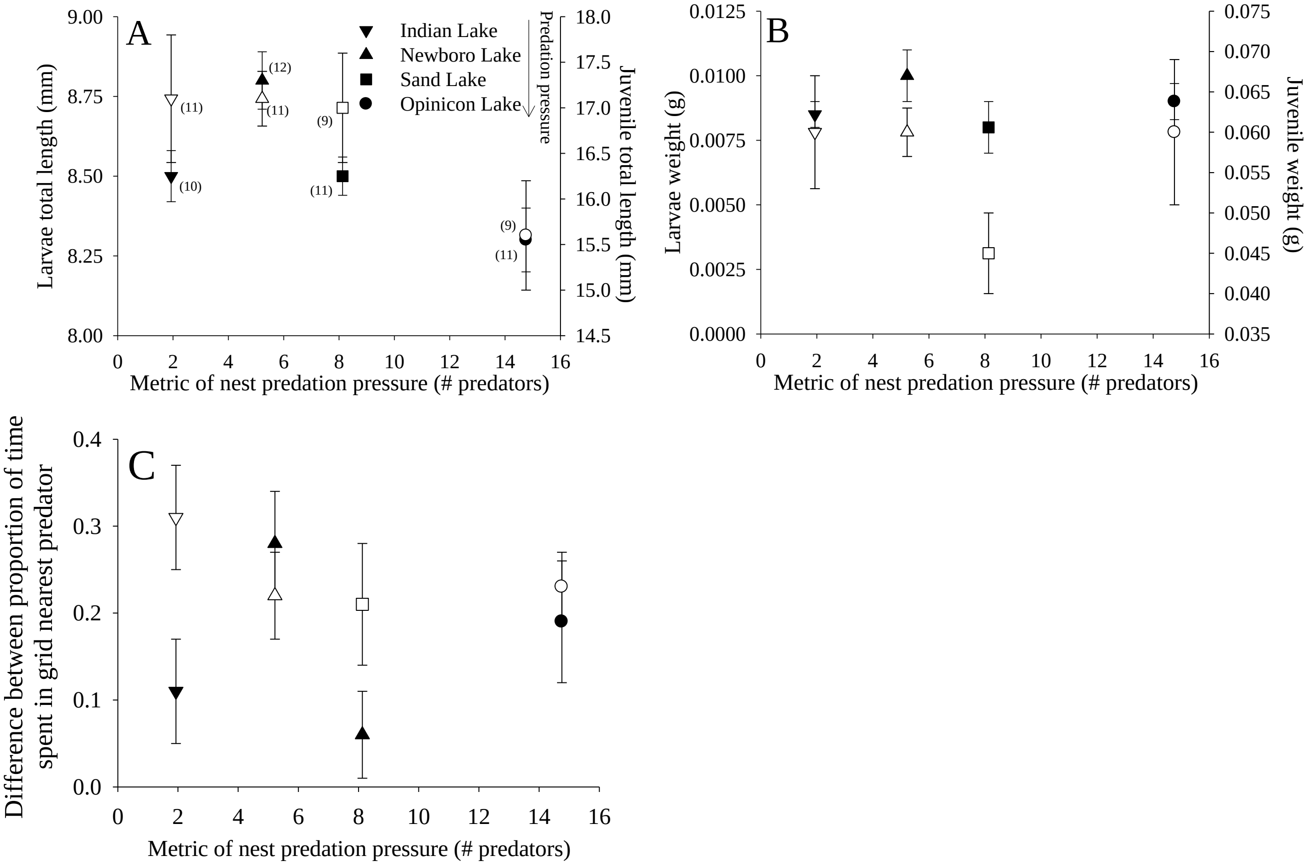


Figure 4-4 Larvae total length (A), total weight (B) and difference (C) between the proportion of time spent in grid nearest predator enclosure in the presence of a predator and in the absence of a predator for larvae from six lakes sampled in 2009 which differ in predation pressure. Showing mean ± SE for all parameters. Sample sizes are shown in brackets. (reprinted with permission of *Behavioral Ecology*)

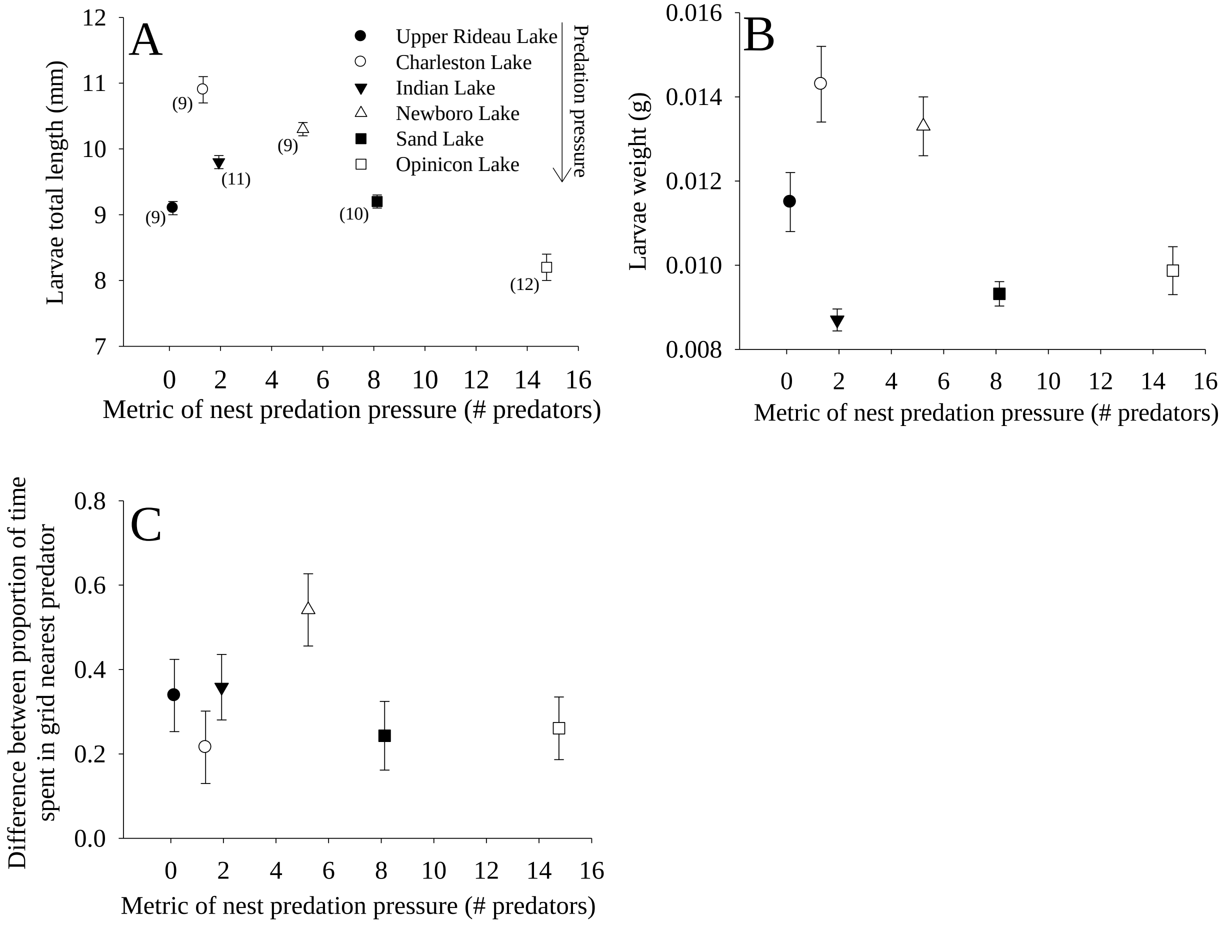


Figure 4-5 Relationship between larvae total length and the difference between the proportion of time larvae spend in the grid nearest the predator in the absence of predator and in the presence of a predator. Values above the dashed line show larvae from nests that moved away from the predator while values below the dashed line moved towards the predator. (reprinted with permission of *Behavioral Ecology*)

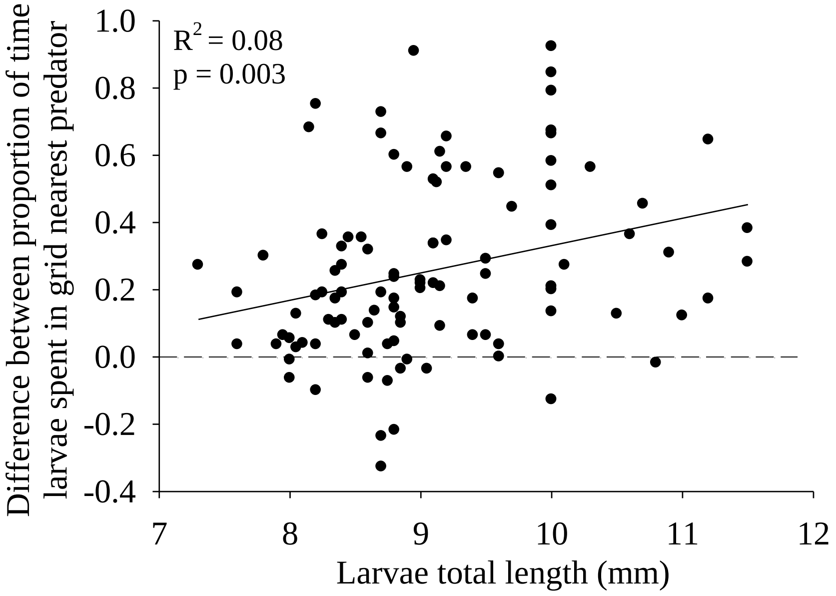
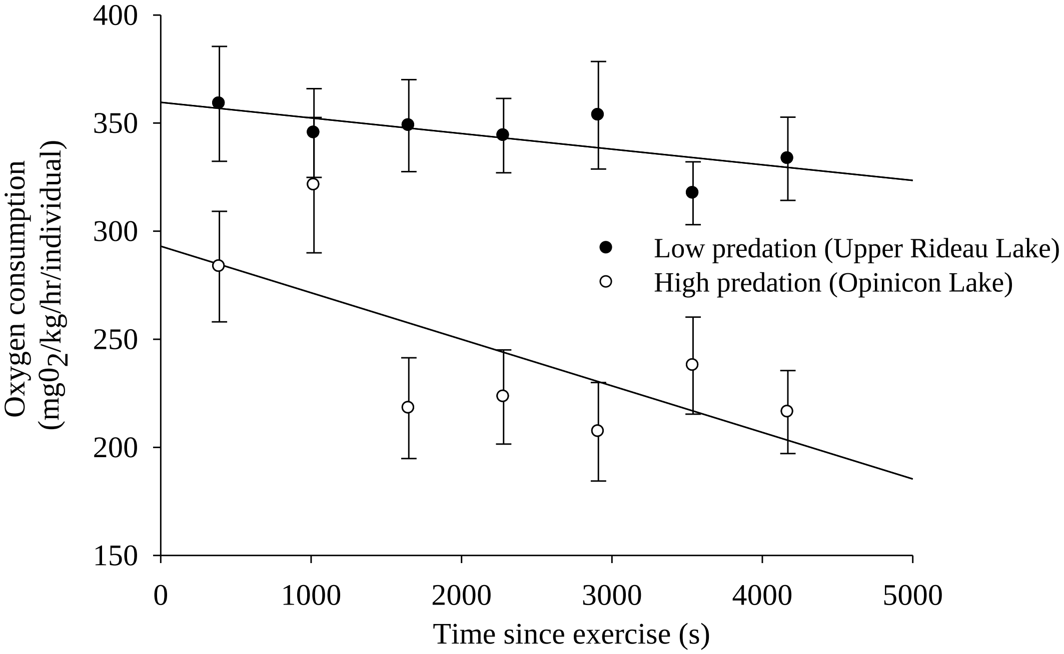


Figure 4-6 Oxygen consumption (mean ± SE) during post-exercise recovery of larval smallmouth bass from lakes with low and high predation pressure following a 3-min chase period. Sample sizes were n = 8 for Upper Rideau Lake and n = 6 for Opinicon Lake. (reprinted with permission of *Behavioral Ecology*)



GENERAL CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Predator-prey interactions have fascinated ecologists for decades (e.g., Elton and Nicholson 1942). However, there exist many ways in which these interactions have yet to be explored. One interesting line of questioning relates to individuals guarding vulnerable offspring but do not themselves face immediate risk of predation. Guarding offspring is energetically challenging, and thus offspring predation pressure has the ability to not only influence offspring survival, but also influence the behaviour and energetic of the parent providing care. In this thesis, I investigated whether offspring predation pressure influenced parental behaviour, energy status and indicators of swimming performance as well as offspring behaviour and recovery.

I found that parental behaviour was influenced by a natural gradient in offspring predation pressure. Parents from populations facing higher predation pressure spent more time engaged in antipredator behaviours then parents from lakes with lower predation pressure. However, males from different populations were equally willing to defend against a simulated predator attack (i.e., predator in a jar, Gravel and Cooke 2009). This provides evidence that the differences in antipredator behaviours are likely due to current environmental conditions. We have evidence that males from populations with lower predation pressure will actively defend their offspring from an introduced predator but we were unable to test if males from different populations differ in their capacity to defend against nest predators. We cannot disregard that there may also exist differences in performance (e.g., endurance, ability to recover from exercise) that distinguish males from populations of high nest predation pressure compared with males from populations with low predation pressure. Differences in behaviour, particularly active behaviours such as chasing nest predators, could also lead to differences in the energetic cost of providing parental care. As such, the second part of this thesis investigated how the energy status of parents was influenced by predation pressure.

Indicators of energy status (GSI, HSI, whole-body lipid and liver lipid) showed that parental care was energetically costly but that the cost associated with parental care did not change between populations that differed in nest predation pressure. Interestingly, these tools did detect lake-specific effects that appear unrelated to nest predation pressure. These lakes differ in size, geomorphology and geochemistry, which in turn influences habitat composition, including prey communities and nest predator assemblages. Thus, there may be multiple lake-specific effects that influence the energy status of parental males. Using a second physiological approach, I found that indicators of swimming performance (i.e., enzyme activities) were also not influenced by variation in nest predation pressure. Similar to the energy indices, enzyme activities showed lake-specific effects. Although these metrics relate information from a recent timescale (days to weeks), these lake-specific differences may simply be conserved across all four seasons (as in Couture et al. 2008) and reflect other relevant ecological differences between these populations such as prey availability. Sampling males of reproductive age from these different populations across four seasons would help determine if these lake-specific effects are present throughout the year or specifically arise during parental care.

While the traditional indicators of energy stores did not support an increased cost to parental care in lakes with higher predation pressure, the evidence provided by the *in-situ* approach showed support for this hypothesis. EMG telemetry demonstrated that parental males at the opposite extremes of predation pressure differed in their overall swimming activity. Because activity contributes significantly to the daily energetic budget of fishes (Boisclair and Leggett 1989), these differences in swimming activity could translate into differential energetic costs for these two populations. Collectively, these results indicate that using a traditional approach to assessing energy status may provide less information compared to repeated sampling of individuals. Further work is still needed to establish if these differences in activity can significantly contribute to the changes in the cost of parental care across populations.

In this thesis, I was also interested in identifying the intergenerational consequences of nest predation pressure. Research on other taxa that provide parental care (mainly birds) had previously established that nest predation pressure could influence parental care behaviour (e.g., Ghalambor and Martin 2002; Fontaine and Martin 2006) but there was little evidence that variation in nest predation pressure could also influence the antipredator behaviours of offspring. In this thesis I found that variation in predation pressure had little consequences on the antipredator behaviour of offspring. However, larger and older offspring (i.e., closer to being abandoned by their parent) showed improved antipredator behaviours when compared with younger offspring. While nest-guarding smallmouth bass are willing to increase the investment into antipredator behaviours when predation pressure increases (naturally or by artificial intrusion), the lack of variation in the antipredator behaviour of offspring is less clear. Because of their small size and limited neural development (Wallace 1972), it may simply be that all offspring are limited in their behavioural responses and are equally capable to move away from a disturbance. We have some evidence to support this since only ~ 40% of larvae (n = 740 from 37 nests, Fig. A-1, Appendix 1) displayed a startle response following a vibration stimulus (i.e. disturbing the water to simulate a predator attack) even though the startle response of larvae can be innate or develop very early in many groups of fishes (Fuiman and Magurran 1994). I was also able to raise a small subset of offspring in the lab from hatched eggs (n = 160 from 16 nests) and test their antipredator behaviours. Interestingly, larvae raised as orphans did not move away from the grid nearest to the predator once a predator was introduced (Fig. A-2, Appendix 1). The combination of results from wild-caught and lab-reared larvae provides evidence that the antipredator behaviour of larval smallmouth bass may not be innate and that environmental stimulus (from parents or from predators) may be necessary in order to develop "normal" antipredator responses.

From a physiological perspective, larvae from the site of highest predation pressure appear better equipped to recover from a simulated predator attack compared with larvae from the site of lowest predation pressure. The slope of recovery was steeper for larvae from the site of highest predation pressure and these larvae also had lower active MRs, most likely enabling them to have a larger scope for activity (Cutts et al. 2002; Hansen and von Herbing 2009). Larvae from other taxa and adult fish have displayed physiological responses to variation in predation pressure in addition to displaying a behavioural response (e.g., Relyea and Werner 1999; Relyea 2002; Woodley and Peterson 2003) but smallmouth bass larvae only displayed a physiological response. One possibility is that this physiological response (i.e., recovery from exercise) is more innate than a relatively more complex antipredator avoidance behaviour. There exists other physiologically mediated antipredator response such as the growth/predation risk trade-off that are not always accompanied by antipredator behaviours (McPeek et al.

2001). Earlier work by Brown (1984;1985) demonstrates that the antipredator response of larval congenerics (e.g., largemouth bass) does improve with age and our work (Gravel and Cooke In press) also supports this notion. Although my research did not show variation in the antipredator behaviour of juvenile smallmouth bass from different populations, my experiment did not include the two populations with the lowest predation pressure (Gravel and Cooke In press). Perhaps including a broader range of predation pressures may provide further information on the antipredator behaviour of juveniles. Together, the results from the larvae experiments indicate that larvae and juvenile fish from populations that differ in nest predation pressure display similar predator avoidance behaviours but larvae from populations at the extremes of predation pressure show physiological variation in their response to a simulated predator attack.

As with most research studies, this thesis generates a number of additional research questions. Of particular interest would be to tease apart the role of selection *vs* plasticity. It was clear that variation in nest predation pressure was able to influence the antipredator behaviours of parental males and that males from populations with lower predation pressure were willing to attack an introduced nest predator. Because smallmouth bass show substantial inter-annual nest-site fidelity (Ridgway et al. 1991a; Barthel et al. 2008) and predation pressure is locally consistent across years (Gravel MA unpublished data), male smallmouth bass from lakes with increased predation pressure may be better performers (e.g., reduced cost of antipredator behaviours, physiological processes underlying defense behaviours) than males from lakes with low predation pressure. One could test this by reciprocal translocation. If there were local adaptations, native males from populations with high predation pressure would have greater reproductive success than translocated males from populations with lower predation pressure. Conversely, in an environment of lower nest predation pressure native males and translocated males from populations with high nest predation pressure may have similar reproductive success since there is likely no additional costs imposed by reduced predation risk and if the local adaptations to increased predation pressure are not accompanied by other trade-offs (e.g., increased aggressivity that is unnecessary when predation is low). It would also be interesting to test if offspring show evidence of local adaptation. This thesis demonstrated that larvae from a site of high nest predation pressure recovered more quickly from a simulated predator attack and showed physiological characteristics that are consistent with a greater scope for activity compared with larvae from site of low predation pressure. These results could have arisen due to beneficial environmental training effects or local adaptation. I had very limited success in raising wild-caught larvae in the laboratory. Perhaps a different larval fish model would be best to tackle this research question or a natural large-scale pond experiment.

A second area of further research could explore the “tangible” costs of predation pressure. My research has shown that males from populations with increased predation pressure are more active and spend more time engaged in antipredator activities. Swimming activity contributes significantly to the bioenergetic costs of fish (Boisclair and Leggett 1989), but how important is an increase in activity that lasts four weeks or longer? Bioenergetics modeling has shown that the activity levels of parental smallmouth bass, coupled with limited feeding opportunities, should lead to significant weight loss (Cooke et al. 2002) and that increasing the cost of parental care leads to abandonment of the current brood (Steinhart et al. 2008). Although these modeling studies are informative, we know little about how individuals deal with increased costs in the wild. My research has provided some support for the notion that abandonment rates increase when the costs of parental increase (higher abandonment rates at the site of highest predation pressure, Gravel MA unpublished data), but we did not explicitly test abandonment rates across a gradient of predation pressure, which would provide some information about the trade-offs that occur within a given reproduction season. To gain knowledge on trade-offs that occur throughout an individual's lifetime, one would need to follow individuals over several years. In addition, "reproductive holidays" were mentioned many times during this thesis and one interesting way to test if the cost of providing parental care changes with predation pressure would be to compare the success of individuals in consecutive years. This would not be practical in large lake settings like those used in the thesis but could be performed on smaller populations, where all males can be accounted for (see Barthel et al. 2008).

In conclusion, this thesis has demonstrated that nest predation has consequences for both parents and offspring and that the energetic cost associated with parental care may be increased in populations with elevated nest predation pressure due to increases in parental activity. This thesis also used a number of tools to address ecological research questions on wild fish, primarily in the field. The integration of behavioural and physiological research tools enabled observations and mechanistic explanations that would have otherwise been overlooked. As ecological and evolutionary research questions become more complex, a comprehensive approach will be essential, particularly when studying animals in the wild.

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Appendix 1: Non-published data resulting directly from this thesis

Figure A-1 Proportion of larvae from smallmouth bass nests which showed startle response (□) and did not show (■) startle response across lakes with different predation pressure. Sample sizes (number of nests per lake) are shown on bars.

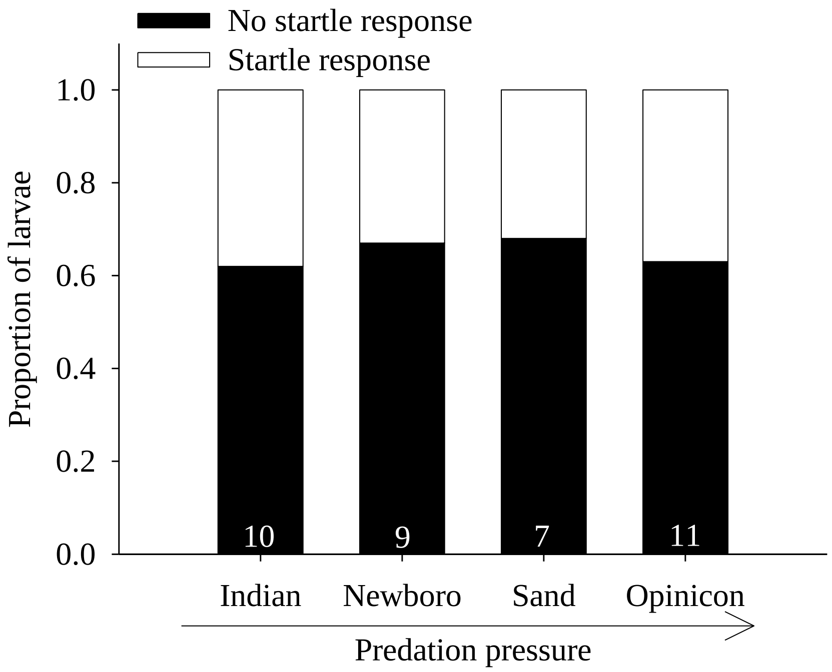
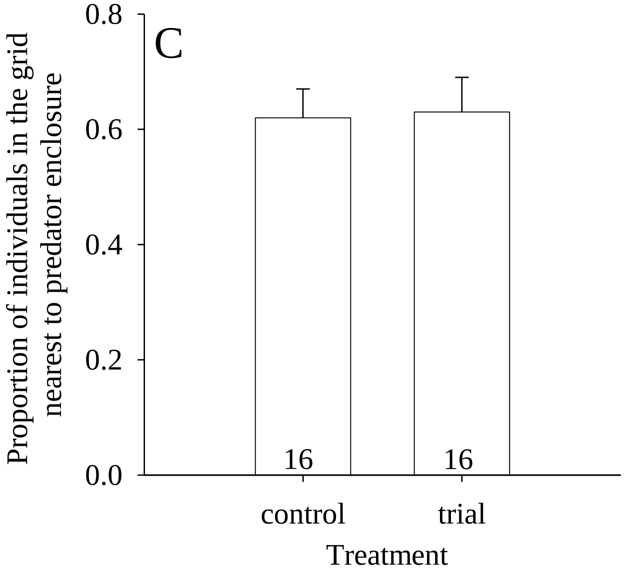


Figure A-2 Proportion of time spent in grid nearest predator enclosure in the absence (control) and presence (trial) of a predator for lab-reared larvae. Sample sizes (number of nests) are shown on bars



Appendix 2: Abstracts of other publications resulting directly from this thesis

1. **Gravel MA,** S.J. Cooke. 2008. Severity of barotrauma influences the physiological status, post-release behavior, and fate of tournament-caught smallmouth bass. N Am J Fish Manage. 28:607-617

Abstract

Much research on the fish physiological consequences of tournaments has been conducted to date and has provided anglers and tournament organizers with strategies for reducing stress and mortality. However, one aspect of tournaments that has received little attention is barotrauma. At a fall competitive angling event on Rainy Lake in northwestern Ontario, we evaluated the incidence of barotrauma among tournament-caught smallmouth bass (*Micropterus dolomieu*); we then tagged and released a subset of fish that had severe barotrauma indicators and compared physiology, postrelease behavior, and fate between these fish and those with negligible signs of barotrauma. Overall, 76% of fish had at least one sign of barotraurna (either hemorrhaging or swim bladder distention), but only 32% of fish had two or more indicators and were thus deemed to have severe barotrauma. When telemetered fish were released at a common site, we determined that fish with negligible signs of barotrauma evacuated the release site more rapidly than fish with severe barotrauma did. Some fish with barotraurna floundered at the surface when released, and one of these fish was subsequently hit and killed by a boat. At the end of the monitoring period, 20% of fish with severe barotrauma had died; two additional individuals (20%) that were still at the release site were moribund (failed to respond to diver stimuli). Conversely, we failed to observe any mortality in fish with negligible signs of barotrauma. All tournament fish had elevated levels of blood glucose and lactate. However, stress indices were higher in fish with barotrauma and tended to be highest among fish with barotrauma that died after release. This study revealed that the incidence of barotrauma in tournaments can be high; moreover, outside of a laboratory environment, a significant proportion of fish with severe barotrauma may die after release. Additional research is needed to determine the seasonal variation in incidence and consequences of barotrauma as well as the effectiveness of different depressurization techniques in the field that could be used during fishing tournaments.

2. Hanson KC, **Gravel MA,** Redpath T, Siepker MJ, Cooke SJ. 2008. Latitudinal variation in behavioral and physiological responses of nest guarding smallmouth bass (*Micropterus dolomieu*) to common recreational angling practices. Trans Am Fish Soc. 137:1558–1566

Abstract

Management policies related to catch-and-release (CR) angling of smallmouth bass (*Micropterus dolomieu*) vary widely across the geographic distribution of the species. Some jurisdictions, principally in the northern latitudes, prohibit or limit angling efforts that target nesting male smallmouth bass, whereas angling during the nesting period is generally unregulated in southern jurisdictions. Existing studies of individual-level angling impacts on nesting smallmouth bass have primarily been conducted in the north;

thus, the extent to which these findings are relevant to other regions is unknown. In the current study, we sought to systematically evaluate the rates of nest abandonment by nesting smallmouth bass subjected to common angling practices (CR treatment: brief angling and no exposure to air; air exposure [AE] treatment: exhaustive angling and 3 min of AE) and tournament practices (simulated tournament [ST] treatment: exhaustive angling, live-well retention, and 3 min of AE prior to release) across a latitudinal gradient encompassing virtually the entire south-north range of smallmouth bass (i.e., southern Missouri [MO], southern Ontario [SON], and northern Ontario [NON]) and compared these treatment groups with nonangled controls. We also quantified the extent to which physiological disturbance associated with angling varied across latitudes (peripheral populations [MO and NON] versus the intermediate-latitude population [SON]). Whole-blood lactate and glucose levels were highest in fish subjected to ST conditions, indicating increased stress; this pattern was conserved across all latitudes (although there was some evidence of intraspecific variation in stress response). Additionally, the pattern of brood abandonment was similar among fish at all three latitudes; ST fish exhibited the highest rates of nest abandonment (MO: control = 9.1%, CR = 0%, AE = 9.1 ST 100%; SON: control = 10%, CR = 10%, AE = 10%, ST = 50%; NON: control = 7.7%, CR = 0%, AE = 9.1%, ST = 50%). Interestingly, fish from the most southerly latitude, where regulations are the most liberal, abandoned nests at higher rates than did fish from the other latitudes. Collectively, these data reveal that the reproductive success of individual smallmouth bass can suffer from interaction with anglers, particularly in a tournament context, regardless of the region. Further study is needed to determine the extent to which individual nest success is relevant to recruitment and how this relationship varies across latitudes.

3. Nguyen V, **Gravel MA,** Hanson KC, Cooke SJ. 2009. The post release behaviour and fate of tournament-caught smallmouth bass after “fizzing” to alleviate distended swim bladders. Fish Res. 96: 313-318

Abstract

In recreational fishing, barotrauma occurs when fish that are angled from deep water are rapidly brought to the surface, causing a number of injuries and physiological alterations associated with gas expansion (such as distended swim bladders) that may impede swimming and prevent the fish from returning to depth. By deflating the swim bladder using a hypodermic needle (a process called "fizzing") fish typically can return to depth. However, little is known about its consequences and efficacy on wild fish. At a competitive smallmouth bass (*Micropterus dolomieu*) angling event on Rainy Lake in northwestern Ontario, we evaluated the effects of post-weigh in fizzing on the behaviour and short-term survival of three treatment groups after release: (i) barotrauma fish without fizzing (N = 8); (ii) barotrauma fish that were fizzed (N = 9); (iii) fish without signs of barotrauma that were fizzed (N = 10) (sham control). Small external radio transmitters were affixed to the fish and tracked for four days. Fish were released at a common site and we assessed their dispersion at specific distances from the release site (50, 250, and 2000 m). All fish survived the 4-day monitoring period. No differences were observed in the time it took each group to disperse from the release site.

Furthermore, there was no statistical evidence that fizzing influenced mean daily movements relative to controls, though a consistent trend was noted where fish that were fizzed displayed greater movement than non-fizzed fish with distended swim bladders. This study revealed that fizzing by trained experts is not detrimental to barotrauma fish. However, if done improperly there is risk to vital organs suggesting that there is merit in exploring other less invasive approaches to recompressing fish. Because our statistical power was generally low, further research is needed to determine whether fizzing should be encouraged or dissuaded to maintain the welfare status of the fish, decrease sublethal impairments, and reduce mortality.

4. **Gravel MA,** Couture P, Cooke SJ. 2010. Comparative energetics and physiology of parental care in smallmouth bass *Micropterus dolomieu* across a latitudinal gradient. J Fish Biol. 76: 280-300

Abstract

The energetic and physiological status of parental smallmouth bass *Micropterus dolomieu* was investigated across the majority of their latitudinal range at the onset and near the end of care. Variables such as tissue lipid stores, plasma indicators of nutritional status and chronic stress and white muscle were used to define energetic and physiological status. Results showed that northern males (48° N) were larger and heavier than mid-northern (44° N) and southern (36° N) latitude males. For a given body size, northern males had greater whole-body lipid across the parental care period and tended to feed more (based on gut contents) than mid-northern and

southern latitude conspecifics. Indicators of nutritional status were also highest in northern males. Conversely, the southern males exhibited the greatest capacity for biosynthesis across the entire parental care period as indicated by the highest level of nucleoside diphosphate kinase activities. Collectively, these finding suggest that the energetic costs and physiological consequences of care vary across latitudes, providing some of the first mechanistic evidence of how environmental conditions can influence both the ecological and physiological costs of reproduction for wild animals during parental care. The data also suggest that lake-specific processes that can vary independently of latitude may be important, necessitating additional research on fish reproductive physiology across landscapes.

Appendix 3: Abstracts of publications resulting from graduate courses

1. Suski CD, Cooke SJ, Danylchuk AJ, O'Connor CM, **Gravel MA**, Redpath T, Hanson KC, Gingerich AJ, Murchie KJ, Danylchuk SE, Goldberg TL. 2007. Physiological disturbance and recovery dynamics of bonefish (*Albula vulpe*s), a tropical marine fish, in response to variable exercise and exposure to air. Comp Biochem Physiol A. 148:664-673

Abstract

Current understanding of the stress response in fishes has largely come from studies of freshwater-adapted salmonids, with proportionately few comparative studies having examined marine fishes. The current study sought to quantify the magnitude of physiological disturbances, recovery dynamics, and post-exercise behaviour in bonefish (*Albula vulpes*; a tropical marine fish) exposed to several different exercise and air exposure regimens. Results showed that metabolic disturbances (lactate production, hyperglycemia) increased following exercise and exposure to air, and that the magnitude of metabolic disturbance was proportional to the duration of the stressful event. Fish required between 2–4 h to return to resting values. Exercise and exposure to air also resulted in significant increases in plasma Ca^{2+}, Cl^- and Na^+, but the magnitude of these ionic changes did not vary with exercise or exposure to air duration and required over 4 h to return to baseline levels. Mortality following exercise was observed only for fish that had been exposed to air for 3 min and not in fish that had been exposed to air for 1 min. Together, results from this study provide a physiological basis for management strategies that can improve the post-release survival of bonefish that have been caught during a catch-and-release angling event.

2. Hanson KC, **Gravel MA**, Graham A, Shoji A, Cooke SJ. 2008. Sexual Variation in Fisheries Research and Management: When Does Sex Matter? Rev Fish Sci. 16:421-436

Abstract

In fish, sex determination is a plastic process regulated by a relatively small number of genes that, in turn, leads to a cascade of organism level effects. In other animal taxa, intersexual variation is widespread and has implications in the realms of morphology, behavior, physiology, and bioenergetics. Although relatively well documented in the

literature focusing on mammals, birds, and reptiles, the degree to which sex-specific variation is considered is unknown in fish and fisheries research. We examined the scientific literature to evaluate the important sex-related differences in fish and highlighted why some of these differences are of great biological consequence. Sex-specific differences in morphology included sexual size dimorphism, external traits such as coloration, and internal anatomy such as neuron structure. Behavioral differences between the sexes are often linked to reproduction, but there are some documented differences (i.e., variation in aggression and predator avoidance) that are independent of the reproductive period. The potential for sex-related physiological differences are relatively unexplored for fish, although there is strong evidence for disparity in hormone regulation, stress, and immune responses between the sexes. Sex-related variation is also poorly examined in the field of bioenergetics despite the fact that differences in energy requirements and expenditure should and do vary between the sexes. A quantitative literature review of several prominent fisheries journals revealed that sex is often overlooked in fish and fisheries research (between 15 and 44% of articles), which may impair the ability of researchers to detect biologically relevant differences, which in turn can greatly affect management decisions. Although there has been a growing recognition that intra-specific variation (at the population level) is important in fisheries management and research, there is also a need to consider that intersexual diversity exists and is important to understand, conserve, and manage fish and fisheries resources.

Appendix 4: Statement of permission from co-authors

All co-authors have granted me permission to use co-authored manuscripts in this thesis.