

**The influence of exogenous cortisol manipulation on parental investment and reproductive
success in nest guarding male smallmouth bass**

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Abstract

Parental care, providing post-fertilization care to a developing brood, is a beneficial reproductive behaviour commonly found in teleost fishes. Parental care is a challenging period for fish, and stress may exacerbate the challenges during parental care, potentially leading to negative fitness consequences. In Chapter 2, I explored the interactive effects of elevated cortisol, body size, and brood size have on smallmouth bass parental care behaviours and reproductive success. I found that elevated cortisol had an influence on reproductive success. In Chapter 3, I examined the influence of elevated cortisol on swimming behaviours and locomotor activity level, monitored at a fine scale over 70 hours. I found that fish with elevated cortisol exhibited reduced locomotor activity and parental care swimming behaviours compared to controls. The results of Chapter 2 and Chapter 3 contribute to the growing body of literature illustrating the effects of elevated cortisol on teleost reproduction.

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Co-Authorship Statement

Chapter 2: Parental care in a stressful world: Do brood size and body size influence parental care behaviours and reproductive success in cortisol-manipulated nest-guarding male smallmouth bass?

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This manuscript will be submitted to a peer reviewed journal. The project was conceived by Algera and Cooke. Field work was completed by Algera, Zolderdo, and Cooke. Data analysis and interpretation was conducted by Algera, Gutowsky, and Cooke. Manuscript was written by Algera, and all co-authors contributed with feedback.

Chapter 3: Misbehaving fathers: the effects of elevated cortisol on locomotor activity and parental care behaviour of nesting male smallmouth bass

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Chapter 1 – General Introduction

Parental Care

Many vertebrate taxa provide extended post fertilization care during brood development brood, which is advantageous in terms of fitness; parents benefit by increasing chances of offspring survival, thus increasing the chances of passing on their genes to future generations (Clutton-Brock 1991). Parental care occurs in mammals (Gubernick and Klopfer 1981), birds (Tullberg et al. 2002), reptiles (Shine et al. 1997; Platt and Thorbjarnarson 2000), amphibians (Summers 1992; Tumulty et al. 2014), and fish (Blumer 1982; Gross and Sargent 1985).

Parental care takes four major forms: no care, sole male care (paternal), sole female care (maternal), and bi-parental (both parents). Dominant parental care forms differ among taxa. Fish exhibit the greatest diversity of parental care forms of all vertebrate taxa (Clutton-Brock 1991; Webb et al. 1999), particularly in the teleost fishes (Gross and Sargent 1985; Goodwin et al. 1998), providing many model species for parental care research (Amundsen 2003). Paternal care is the most common parental care form among teleost species exhibiting parental care (Blumer 1982). Parental care behaviours

In order to maximize lifetime fitness, present reproductive investment must be weighed against future reproductive potential. If there are not enough resources (e.g., energetic resources) available for reproduction prior to the breeding season, making reproduction not feasible or the costs of current reproductive investment place survival in jeopardy, choosing to forego current reproductive efforts in favor of self-preservation and investment into future reproduction would be advantageous (Williams 1966; Wingfield and Sapolsky 2003). Trade-offs occur in resource allocation among growth, survival, and reproduction; resources allocated to one life history trait

are not available for investment into others (Williams 1966; Trivers 1972; Stearns 1989). Fish body size, an important factor in fish reproduction, illustrates how resource trade-offs link physiology, behaviour, and life history. A positive relationship exists between fecundity and female body size in some taxa (Raffetto et al. 1990), and body size is a sexually selected trait, with females preferring large males (Raffetto et al. 1990; Hanson et al. 2009c). Energetic investment into present reproduction cannot be allocated to somatic growth, which may reduce future fecundity (in females) or the ability to court mates (in males), thus reducing the capacity for future reproduction.

Stress Response in Fish

Fish are continually exposed to challenges and disturbances, which can act as a stressor. Stressors can be physical, chemical, and perceived, and threatens the homeostasis of an organism (Barton 2002). When a stressor is perceived and perturbs homeostasis, a stress response - a cascade of physiological and behavioural responses - is initiated to mitigate the effects of the stressor and return the system back to homeostasis (Wendelaar Bonga 1997; Mommsen et al. 1999). The physiological stress response involves stimulation of the neuroendocrine system and activation of the hypothalamus-pituitary-interrenal (HPI) axis, the stress axis in fish (Wendelaar Bonga 1997). At the onset of the stress response, catecholamines involved in the “flight or fight” response (e.g., adrenaline) are released. The hypothalamus releases corticotropin releasing factor (CRF), which then stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary, triggering interrenal cells in the kidney to synthesize glucocorticoids (GCs), a group of steroids known for energy mobilisation and anti-inflammatory properties (Mommsen et al. 1999). Cortisol, a GC that plays a primary role in the stress response in fish, is beneficial at basal levels, essential to normal body function and maintenance (Wendelaar Bonga 1997; Busch and

Hayward 2009). Acute stressors produce a primary stress response characterized by elevated circulating GC (e.g., cortisol) levels above what is required for body maintenance functions (Wendelaar Bonga 1997; Barton 2002). Secondary physiological responses occur when circulating GCs uptake into the blood and tissue, which can produce alterations to metabolic activity, osmoregulation, and immune function (Barton 2002). If elevated GC levels continue to persist, tertiary (whole organism) effects with fitness implications can occur such as alterations to behaviour, somatic growth, reproduction, and even mortality (Wendelaar Bonga 1997; Barton 2002). Glucocorticoids and other stress axis hormones can interact with the hypothalamic-pituitary-gonadal (HPG) axis, responsible for reproductive related physiology and behaviours in vertebrates, thus affecting several aspects of reproduction across vertebrate taxa. Stress hormones have been found to decrease or inhibit GnRH and sex hormone release (testosterone, 11-ketotestosterone, and estradiol), fecundity, egg quality, courtship behaviour, and affect timing of reproductive functions (Rivier and Rivest 1991; Schreck et al. 2001; Hayward and Wingfield 2004; Charmandari et al. 2005; Schreck 2010).

GC Manipulation

To test the mediating and modulating roles of various stress steroids and hormones, many studies focus on elevating GC levels through confinement, handling, dietary restriction, and environmental factors (e.g., heat/cold shock). Elevating GCs through these methods provides a natural reaction from a perceived stressor and are typically sufficient in eliciting an acute stress response (O'Connor et al. 2011). However, if a chronic stress response is desired, this may involve repeated interactions, manipulations, and handling of the organism(s), and thus may not be an appropriate or feasible approach when using wild animals. Experimentally elevating GCs exogenously through dietary additions, implants, and injections are increasingly common

methods to chronically elevate GCs in wild animals (Sopinka et al. 2015), minimizing human influence and interaction with wild study species. Once validated, using exogenous GC manipulations in controlled experiments can be a powerful tool in elucidating effects of stressors in behaviour and life history trade-offs in wild fishes (Crespi et al. 2013; Sopinka et al. 2015; Crossin et al. 2016). Although experimentally elevating GCs mimics some aspects of the stress response, it should not be considered a complete substitution for a natural stress response; experimental manipulation is missing elements of the complete neuroendocrine cascade encompassed from an organism responding to a stimulus (perceived stressor). Researchers using experimentally elevated GCs in wild teleost fish have found changes in various physiological and behavioural related parameters such as increased protein metabolism (Liew et al. 2013) and decreased reproductive success (O'Connor et al. 2009; Dey et al. 2010), immune function (O'Connor et al. 2009), and activity level (O'Connor et al. 2010).

Study Species

Smallmouth bass (*Micropterus dolomieu*), a member of the *Centrarchidae* family, is distributed throughout North America (Scott and Crossman 1973). Smallmouth bass parental care is characterized by paternal care, where the male provides sole care for the developing brood (Blumer 1982). As the water temperature approaches ~15°C, males begin to stage in the littoral zone where they construct a nest, a saucer shaped depression, in rocky substrate (Ridgway 1988). After successfully courting a female, she deposits her eggs into the nest, which are fertilized and the male provides sole care for the developing brood for 4-6 weeks until offspring reach independence (Ridgway 1988; Cooke et al. 2006). While the brood is developing, nesting males actively monitor the nest area, vigorously defending their offspring day and night, engaging and chasing brood predators away from the nest area, removing debris

and dead eggs from the nest, and fans the eggs to ensure they are well oxygenated (Hinch and Collins 1991; Cooke et al. 2002). While under parental care, the progression of brood development can be categorized into 4 main developmental stages: the egg stage, egg-sac fry, swim-up fry, and free swimming fry (Cooke et al. 2002). Parental investment varies over the brood stages, with higher parental investment typically shown in the egg and egg-sac fry stages, and lowest in the free swimming fry stage (Ridgway 1988; Cooke et al. 2002). The parental care period is a challenging period for smallmouth bass, as reproduction is energetically costly (Gillooly and Baylis 1999; Mackereth et al. 1999; Cooke et al. 2002, 2006). Moreover, smallmouth bass forego active foraging (Hinch and Collins 1991), primarily relying on endogenous energy reserves accumulated over the previous year.

Elevated cortisol has been shown to interact with parental care in smallmouth bass. Previous research on smallmouth bass demonstrated that nesting smallmouth bass with exogenously elevated cortisol levels had significantly lower nest success compared to non-manipulated controls (O'Connor et al. 2009; Dey et al. 2010). These studies also assessed the influence of exogenously elevated cortisol on parental care behaviours (i.e., nest-tending and brood defence), but found no significant effects of cortisol treatment (O'Connor et al. 2009; Dey et al. 2010). However, these studies examined interactions between parental care behaviours and elevated cortisol independent of other known factors important in parental care and through a wide time window, warranting further investigation. Smallmouth bass is a model species for researching the nexus of elevated GCs and parental care. A wide range of body sizes of male smallmouth bass spawn each year (Steinhart and Lunn 2011) and the brood size a male receives can vary from hundreds to thousands of eggs (Kubacki 1992), which can affect parental investment and overall nest success (Ridgway 1988; Steinhart and Lunn 2011). Smallmouth bass

(or closely related Centrarchid species, largemouth bass *Micropterus salmoides*) parental care behaviours are well characterized in the literature (e.g., Ridgway 1988; Cooke et al. 2006; Hanson et al. 2009b; O'Connor et al. 2009; Dey et al. 2010; Zuckerman et al. 2013). Smallmouth bass parental care duration of several weeks allows for ample time for experimental cortisol elevation.

Research Objectives

The general objective of this research was to explore the effects of elevated cortisol on parental care behaviours and reproductive success using smallmouth bass as a model species. More specifically, in Chapter 2 the interactive influence among parental body size, brood size, and elevated cortisol on parental care behaviours and reproductive success were explored. By experimentally manipulating brood size and cortisol levels across a gradient of body sizes (large and small), I tested the hypothesis that nest guarding male smallmouth bass reproductive success and parental investment into the brood (e.g., brood defence, nest-tending) are influenced by parental body size, brood size and cortisol level. Larger (older) fish have more reproductive experience and have comparatively less future reproductive events than smaller (younger) fish (Williams 1966; Curio 1983; Steinhart et al. 2008; Steinhart and Lunn 2011), which may lead them to abandon a brood less frequently. Fish with a large body size benefit from energetic advantages (Glazier 2005), have higher energy reserves for reproductive investment (Mackereth et al. 1999), and being that large body size is a sexually selected trait, females prefer to mate with large males (Raffetti et al. 1990). Furthermore, a large brood has a higher reproductive value to male smallmouth bass compared to a small one (Ridgway 1989; Steinhart and Lunn 2011; Zuckerman et al. 2013). Consequently, I predicted the interaction of a large body size and brood size to produce the highest reproductive success and parental investment (in terms of

aggression and nest-tending behaviours). Based on observations by O'Connor et al. (2009) and Dey et al. (2010), smallmouth bass with elevated cortisol can exhibit a decreased body condition and immune function, so I predicted that fish with elevated cortisol would exhibit diminished nest success and parental investment (i.e., nest-tending and brood defence behaviours), favouring self-preservation over parental care duties. In Chapter 3 I examined whether experimental cortisol elevation alters the swimming behaviours and locomotor activity of parental smallmouth bass. To do so I combined exogenous cortisol manipulation with tri-axial accelerometers (Brownscombe et al. 2013, 2014) attached to nesting smallmouth bass for 3 days. I hypothesized that elevated cortisol would affect locomotor activity level and swimming behaviours of nesting smallmouth bass. I predicted that relative to controls, fish with elevated cortisol would exhibit reduced locomotor activity and swimming behaviours, with activity and behaviour changes magnified in fish with supra-physiological cortisol levels. Chapter 4 will discuss the findings from Chapters 2 and 3, highlighting potential future research opportunities.

Chapter 2 – Parental care in a stressful world: Do brood size and body size influence parental care behaviours and reproductive success in cortisol-manipulated nest-guarding male smallmouth bass?

Abstract

Parental care is an advantageous reproductive behaviour as the fitness of the caregiver is increased through improving the chances of its offspring's survival. Parental care occurs in a variety of teleost fishes. The body size of parental fish and the size of their brood can affect nest abandonment decisions, where compared to smaller fish with smaller broods, larger fish with larger broods typically invest more energy into reproductive events because they have less future reproductive potential. Though essential for basal and body maintenance functions, when glucocorticoid hormones (e.g., cortisol) are chronically elevated, as can occur during stress, fish may experience impairments in behaviour and immune function leading to compromised health and condition. Anthropogenic stressors during parental care can lead to elevated stress, therefore making it necessary to understand how stress influences an already challenging period. Using smallmouth bass as a model, a gradient of body sizes and experimentally manipulated brood size (i.e., reducing large broods and supplementing small broods) and cortisol levels (i.e., elevated via slow-release intraperitoneal cocoa butter implant containing cortisol versus controls), I tested the hypothesis that nest guarding male smallmouth bass reproductive success and parental care behaviours (i.e., aggression, nest-tending) are influenced by parental body size, brood size and cortisol level. Overall, there was a relationship between cortisol treatment and nest success in which larger fish exhibited lower success when cortisol levels were elevated. Brood size had a significant effect on fish tending behaviour, independent of cortisol level and body size. Lending partial support to my hypothesis, the results of this study indicate that guarding male smallmouth

bass reproductive success is influenced by cortisol level, and tending behaviour is affected by brood size.

Introduction

Many taxa focus their reproductive efforts into a single reproductive period each year, exhibiting reproductive behaviours to maximize reproductive success. Parental care, defined here as the post-fertilization behaviours undertaken to care for offspring during vulnerable early developmental stages, increases the probability of offspring survival, thus increasing the fitness of the parents (Clutton-Brock 1991). Life history theory predicts trade-offs in resource allocation between growth, survival, and reproduction (Williams 1966; Trivers 1972). Current reproductive investment must also be weighed against the potential for future reproductive events; if survival is placed in jeopardy due to the cost of a current reproductive event, such as parental care during a breeding season, it may be advantageous to forgo current reproductive efforts to maximize future reproductive potential (Williams 1966; Wingfield and Sapolsky 2003). Such trade-offs effectively link behaviour, physiology and life-history (Zera and Harshman 2001).

Parental care behaviours occur in a variety of teleost fishes (Blumer 1982) making them ideal models to study parental care (Amundsen 2003). Though not common among marine teleosts, parental care occurs in about 60% of freshwater fish species (Gross and Sargent 1985) and they exhibit a diversity of parental care forms (Goodwin et al. 1998). Teleost parental care can be broadly categorized into tending behaviours and aggression behaviours. Tending behaviours are those involved in caring for the brood, such as fanning eggs to ensure they are well oxygenated and removing debris and dead eggs from the nest (Ridgway 1988). Aggression behaviours typically encompass brood defense, such as chasing away potential brood predators (Ridgway 1988).

The parental care period is a challenging time for parent(s) and their offspring, as fish are exposed to a variety of biotic and abiotic challenges. This is exemplified by the centrarchid fishes, a family for which sole paternal care is ubiquitous (Cooke et al. 2006, 2008). Within the *Centrarchidae*, parental care has been particularly well studied for black bass (*Micropterus spp.*; i.e., smallmouth bass, *Micropterus dolomieu*, and largemouth bass, *Micropterus salmoides*). Black bass begin their nesting season in the early spring when the weather is often highly variable, choosing nesting sites located within shallower waters in the littoral zone (Cooke et al. 2006). Adverse conditions produced from inclement weather can reduce nest success in fish (Goff 1985; Steinhart et al. 2005). Brood predator burden can be very high in some aquatic systems (Steinhart et al. 2004; Gravel and Cooke 2009), affecting parental care behaviours, energy expenditure (Steinhart et al. 2004; Gravel and Cooke 2009) and nest success (Zuckerman and Suski 2013). This is compounded by the fact that nest-guarding males must remain vigilant 24 hours per day, expending substantial energy patrolling the nest area (Hinch and Collins 1991; Cooke et al. 2002), all the while curtailing food intake due to a combination of reduced feeding opportunities and suppressed appetite (Hanson et al. 2009a). Black bass must also deal with anthropogenic stressors during the parental care period. The littoral zones where bass nest are often subject to habitat degradation from shoreline alterations (Wagner et al. 2006) and noise from boats (Graham and Cooke 2009). In addition, because black bass are the most popular sportfish in North America, they are also subject to intense angling pressure (Quinn and Paukert 2009). Because of their heightened aggression during the care period, bass are extremely vulnerable to angling (Kieffer et al. 1995; Philipp et al. 1997; Suski and Philipp 2004). Even short periods of absence for fish that are captured and released can lead to brood depredation and thus abandonment (Kieffer et al. 1995; Philipp et al. 1997). Angling also causes stress that

reduces their ability to defend their brood once released (Cooke et al. 2001; Suski et al. 2003). Clearly the parental care period for nesting bass is among the most challenging periods in the life of a black bass.

The costs of reproduction and basis for trade-offs in parental care decisions are influenced by the body size of the parent. Body size is a product of life history traits, ontogeny, and genetics (Stearns 1983; Winemiller and Rose 1992), and is an important factor in reproduction. Larger black bass typically show increased reproductive success and parental investment (Wiegmann and Baylis 1995; Lunn and Steinhart 2010; Gingerich and Suski 2011; Steinhart and Lunn 2011), likely attributable to greater body condition (Hinch and Collins 1991) and past breeding experience (Curio 1983), thus being better prepared to deal with the energetic demands of reproduction associated with parental care duties (Gillooly and Baylis 1999). Body size has a positive relationship with brood size, another important factor in black bass reproduction, in which larger males receive more eggs than smaller males (Ridgway 1989; Philipp et al. 1997; Suski and Philipp 2004; Gingerich and Suski 2011) through attracting and mating with larger females (Wiegmann et al. 1992). Brood size has implications for nest success and parental investment in care-giving fish. When brood size is large, parental investment remains high and nest abandonment frequency is much lower relative to when brood size is reduced (Ridgway 1989). Experimentally manipulating the brood size of wild fish, essentially simulating a nest depredation event, allows for greater exploration of the importance of brood size as a factor in parental investment and reproductive success. Studies decreasing the brood size of nesting male black bass through egg removal, essentially simulating a nest depredation event in the wild, found that nest success and parental investment decreased relative to control fish with larger unaltered brood sizes (Ridgway 1989; Suski et al. 2003; Zuckerman et al. 2014).

Augmented broods, those that are initially small yet become larger broods, occur for nesting black bass in cases where a second female spawns with the male, or when “creching” occurs where broods from different parents merge yet protection is provided by a single parent. The increased brood sizes appear to have a positive effect on nesting black bass, as Ridgway (1989) observed that experimentally increasing brood sizes through adding offspring to the nest led to increases in parental investment.

Although a number of studies have examined the influence of brood size, body size, or their combined influence on parental care in black bass (e.g., Ridgway 1989; Mackereth et al. 1999; Suski et al. 2003; Steinhart and Dunlop 2008; Hanson et al. 2009b; Lunn and Steinhart 2010; Steinhart and Lunn 2011; Gingerich and Suski 2012; Zuckerman et al. 2014), comparatively little is known about how stress modulate parental care behaviours and reproductive success. For the purposes of this study, stress is defined as a rise in circulating stress hormones (i.e., cortisol). Given that anthropogenic activity (e.g., disturbance, habitat degradation) in littoral zones where many parental-care providing fish nest is continuing to increase, it is conceivable that anthropogenic stress will likely exacerbate the energetic costs of an already challenging period. Once validated in controlled experiments, using exogenous GC manipulations can be a powerful tool in assessing effects of stressors (Sopinka et al. 2015) and life history variations (Crespi et al. 2013) in wild fishes. Using exogenous cortisol manipulations, O’Connor et al. (2009) experimentally raised cortisol titers in fish to further challenge nesting male largemouth bass. Relative to controls the cortisol-manipulated fish exhibited greater declines in physiological status, showed evidence of immune function impairment, and had higher rates of nest abandonment. However, the authors used a narrow range of fish sizes.

Building on the approach used by O'Connor et al. (2009), I conducted a study to determine the influence of body size and brood size on parental care behaviours and reproductive success, and the extent to which outcomes varied between control and cortisol-manipulated fish. I selected smallmouth bass (*Micropterus dolomieu*) as a model species because they maintain a presence on their nest for several weeks, allowing ample time for experimentation, their nests are readily accessible and easy to locate in the littoral zone, and most importantly their stress response, parental care behaviours, and nest success are well characterized in the literature (Hanson et al. 2009b; O'Connor et al. 2009; Dey et al. 2010). I hypothesized that parental body size, brood size and cortisol level (i.e., experimental cortisol elevation relative to controls), influence parental care behaviours and reproductive success in nesting males. I predicted the interaction of a large body size and brood size to produce the highest reproductive success and parental investment (in terms of aggression and tending behaviours) given the past reproductive experience of larger (older) fish, the energetic advantages afforded from a large body size, and the potential reproductive value of a large brood. Furthermore, larger fish have comparatively less future reproductive events than smaller (younger) fish, presumably leading them to abandon a brood less frequently. Due to the declines in physiological status and body condition associated with elevated circulating cortisol from exogenous cortisol manipulation, fish with elevated cortisol were predicted to exhibit decreased reproductive success and diminished parental investment, choosing to abandon their nests in favour of their own survival.

Methods

Sampling was conducted in late May through to mid June in 2014 in Charleston Lake, in the Gananoque watershed and Big Rideau Lake, and Sand Lake in the Rideau River system. The three lakes are similar in their aquatic community composition (Gravel and Cooke 2009).

Surface water temperatures ranged from 15 to 19° C throughout the experimental duration. At these temperatures male smallmouth bass are known to construct a nest in the littoral zone, attract a female, mate, fan eggs, and defend the brood for several weeks (Ridgway 1988). All research was conducted in accordance with Animal Care Protocol # B12-08 authorized by Carleton University and the Canadian Council on Animal Care.

Nest guarding males were located through snorkel surveys using a trained team of snorkelers. If a nest guarding male's length was estimated as falling within appropriate size ranges (i.e., large, > 420 mm or small, < 330 mm), their brood size (egg score) was also visually estimated. Egg score is a qualitative, highly repeatable assessment categorizing egg counts from 1 through 5, with 1 being few eggs and 5 being thousands of eggs (Kubacki 1992; Philipp et al. 1997; Suski et al. 2003; Zuckerman et al. 2014). The experimental design of this study purposefully avoided the inclusion of fish with an average body size and brood size, focussing on large and small body sizes and egg scores. Although I did not age fish, in this region the "large" fish would have been ~8 to 14 years old, and "small" fish would have been ~ 4 to 7 years based on extensive aging work on a nearby system (i.e., O'Connor et al. 2012). A large brood size was a nest having an egg score of 4 and 5, whereas an egg score of 1 or 2 was considered a small brood (Kubacki 1992).

Behavioural assessments were designed to test a guarding male's nest tending and aggression behaviours. Tending was assessed first, followed by aggression. In order to assess the tending and vigilance of each nest guarding male, a tending score was developed where each nest guarding male was visually observed by a diver situated > 3 meters from the nest, limiting disturbance to the guarding male. After a 60 s acclimation period, the diver recorded how many times the male was within 1 meter of his nest on 20 s increments for a total of 3 minutes [giving

a total possible score of 9; as per Gravel and Cooke (2009)]. An aggression score was developed to test the nest guarding male's defensive aggression towards a common brood predator.

Aggression score was assessed using a bluegill sunfish (*Lepomis machrochirus*) between 130-140 mm total length (TL) contained in a 4 L glass jar placed directly adjacent to the nest (Hanson et al. 2009b). After an acclimation period of 30 s, a diver situated > 3 meters from the nest observed the engagement of the male with the brood predator, recording the duration of direct jar contact (in seconds), number of strikes to the jar, mouth flares, and charges at the jar for a total of 60 seconds (Hanson et al. 2009b). After the behavioural assessments, experimental fish were angled from the nest using rod and reel, and placed into a foam-lined trough containing fresh lake water for TL measurement (all fish were angled and measured) and given a cortisol treatment if applicable. The angling fight time was minimized (using heavy fishing gear, angling time < 20 s) in order to limit stress from capture and exhaustive exercise.

Cortisol treated fish received 10 mg kg⁻¹ of cortisol (Hydrocortisone 21-hemisuccinate, Sigma Aldrich Corp.) suspended in a cocoa butter vehicle via intraperitoneal injection with a 16-gauge needle. Fish were injected with 0.005 mL per gram of fish body weight. This is a validated (for the same species, in the same watershed, at the same temperatures, using the same field methods and lab assays, by the same research group) method for elevating cortisol levels in smallmouth bass for 5-6 days (Gamperl et al. 1994; O'Connor et al. 2009; Dey et al. 2010), with post-treatment cortisol levels are expected to range from 750-2250 ng mL⁻¹ (O'Connor et al. 2009, Dey et al. 2010). I decided not to measure circulating cortisol because the expected cortisol level range (750-2250 ng mL⁻¹) represents a high physiological or supra-physiological level, allowing for confidence of cortisol uptake into tissues. No sham treatment (injection containing only cocoa butter) was used in the experimental design because a sham treatment may result in

elevated cortisol, presenting difficulties in distinguishing between a sham effect and a treatment effect. Additionally, sham treatments can be difficult to interpret due to inconsistent cortisol responses of sham treated fish (see DiBattista et al. 2005). Though mass was not directly measured, a \log_{10} transformed length-weight relationship equation from smallmouth bass in Opinicon Lake, a lake within the same system, was used to calculate fish weight from the TL measurements of experimental fish receiving cortisol treatment (Dey et al. 2010); the equation was $\log_{10} \text{ mass} = -7.1004 + 3.884(\log_{10} \text{ TL})$ with mass reported in grams and TL in millimeters. Time away from the nest was minimized; angling and cortisol administration took less than 120 s, and fish were released within 5 m of their nest post-treatment. Throughout the treatment process a diver guarded the nest from brood predators in the guarding male's absence.

Following a protocol adapted from Suski et al. (2003), all fish received a brood size manipulation using gentle suction with a rubber bulb pipette within 24 to 48 hours after initial behavioural assessment. The delay in performing brood size manipulations was to minimize nest abandonment due to having eggs removed from the nest combined with angling stress and cortisol treatment. Large broods (egg score 4 and 5) were reduced to a small brood size (egg score 1 or 2), while small broods were supplemented from other nests to become large broods. All brood manipulations occurred during fresh egg and early egg sac fry offspring development stage, typically 0-5 days after the male receives eggs. Due to an already complex study design and the known details regarding natural brood size and the responses of black bass in terms of nest success and parental investment prevalent in the literature, I chose to forego inclusion of size-neutral manipulations as controls.

Tending and aggression scores were re-assessed 5 days later on all fish using the same protocol outlined above. Following behavioural re-assessments, nests were monitored every

second day for the duration of the parental care period. A nest was considered abandoned when no guarding male and no brood were present in or around the nest, whereas a nest was deemed successful if the offspring reached the free swimming fry development stage which cannot occur without the presence of the parental male (Ridgway et al. 1991; Philipp et al. 1997).

Statistical Analyses

Nest success, aggression score, and tending score were each tested in a generalized linear mixed model (GLMM) where for my study design, multiple observations from each individual prescribed that fish ID be included as a random effect. Prior to analysis, data were first plotted and explored for outliers, multicollinearity, and relationships. Nest success (yes/no, binomial distribution) was modeled as a function of body size (total length, TL, standardized by subtracting the mean and dividing by the standard deviation), final brood size (large vs. small) cortisol treatment (cortisol versus no cortisol), and lake (Big Rideau, Charleston, Sand). Two-way interactions included Cortisol Treatment x Brood Size, Cortisol Treatment x TL, and Brood Size x TL. All fish received a brood size manipulation treatment in the experimental design for nest success.

The aggression and tending score models included the same fixed and random effects structure used in the nest success GLMM, with the exception of an observation-level random effect term to account for overdispersion in the residuals. Aggression and tending scores were modeled assuming a Poisson and binomial distribution, respectively. Models were verified by plotting the residuals against the fitted values, against all the factors, and assessed for overdispersion (i.e., the occurrence of more variance in the data than predicted by a statistical model; Bolker et al. 2009). All statistical analyses were conducted using the package “lme4” (Bates et al. 2014) in R statistical software (R Core Development Team 2013, version 3.0.1).

Although statistical significance was considered based on the approximated test statistics, trends in the fitted values and their potential biological significance were of primary interest.

Results

A total of 93 fish were included in nest success and behaviour analyses. Overall, 55 fish ranging from 251-330 mm TL were designated as small fish, while 43 fish ranging from 420-505 mm TL were designated as large fish. All fish had their brood size manipulated and a total of 50 fish also received cortisol treatment. Trends in the response variables (nest success, tending score) were generated for each lake (Big Rideau, Charleston, Sand), and to best represent the data, incorporated all fixed factors co-efficients that were tested in the GLMMs: brood size and cortisol treatment as categorical variables, and TL as a continuous variable (used as co-variate).

Nest Success

Overall, there was a relationship between nest success probability and cortisol treatment, with control fish (i.e., NC, no cortisol) exhibiting significantly higher nest success probability than fish treated with cortisol (Table 2-1). No relationship was evident between nest success and any of the other tested explanatory variables (Table 2-1). In each lake the control fish showed a positive trend between body size and nest success probability regardless of brood size, whereas a negative trend was evident in cortisol treated fish with a large brood size (Figure 2-1).

Aggression and Tending Scores

No relationships were found between aggression score and the tested explanatory variables (Table 2-1). For tending score, the coefficient for small brood sizes was significant and the fitted values for this model indicated that fish with a small (experimentally reduced) brood size had higher tending scores (i.e., spent a higher proportion of time within 1 m of their nest) compared to fish with large (experimentally supplemented) broods. Compared to fish with small broods, the tending score of fish with large broods decreased more rapidly with increasing fish

size in each of the lakes (Figure 2-2). Coefficients for cortisol treatment, fish size, and their interactions were not significant in the model for tending score (Table 2-1).

Discussion

This study explored parental body size, brood size, and experimentally elevated cortisol levels, with particular focus on testing the combined interactions of these, as factors influencing reproductive success and parental care behaviours. As predicted, cortisol treatment had an overall negative effect on nest success (Table 2-1), with control fish exhibiting higher nest success than the cortisol treatment group (Figure 2-1). In each lake, males guarding a large brood size exhibited decreased nest success with increasing body size (Figure 2-1), though the tested interaction term between cortisol treatment and body size was not significant (Table 2-1). Tending score was significantly affected by brood size (Table 2-1), with fish guarding small broods exhibiting a higher tending score than those guarding a large brood. The tending score of fish with small broods remained relatively unchanged, while fish with large broods decreased with increasing fish size (Table 2-1; Figure 2-2). Despite my predictions, no relationships were found between the tested explanatory variables and aggression score (Table 2-1).

Cortisol-treated fish, particularly larger fish guarding a large brood, tended to exhibit decreased nest success compared to large control fish (Table 2-1; Figure 2-1), consistent with other studies (O'Connor et al. 2009; Dey et al. 2010). My results suggest that glucocorticoids such as cortisol interact with reproduction, contributing to nest abandonment decisions to forego current reproductive efforts in favour of survival (Wingfield et al. 1998). Although I did not explicitly measure circulating cortisol in this experiment, others have validated the use of cortisol treatments on black bass (O'Connor et al. 2009; Dey et al. 2010). In the current study, the single exogenous cortisol implant experimentally elevated circulating cortisol to high physiological levels for 5 to 6 days (Gamperl et al. 1994; O'Connor et al. 2009), mimicking the cortisol-dependent physiological effects akin to chronic stress observed in teleosts (Wendelaar Bonga

1997). Although cortisol is a key component of the GC response, elevations in cortisol alone do not perfectly emulate stress. In normal situations the stress response would be initiated after sensory perception of a perceived stressor and associated activation of the HPI axis (Wendelaar Bonga 1997). Nonetheless, experimental cortisol elevation is a useful strategy for understanding how stress influences wild animals (Sopinka et al. 2015; Crossin et al. 2016).

Parental care behaviours investigated in this study, namely aggression and tending behaviours, are ubiquitous in centrarchid parental care (Cooke et al. 2006). None of the tested factors had an effect on nest guarding male aggression (Table 2-1). Parental male aggression remaining unaffected by cortisol treatment demonstrates consistency with other black bass parental care studies (O'Connor et al. 2009, 2011; Dey et al. 2010). Tending score, measured as time spent within 1 m of the nest, was driven by brood size (Table 2-1), insofar that fish with a small (experimentally reduced) brood size exhibited a higher tending score compared to fish with a large (experimentally increased) brood (Figure 2-2). This was an unexpected result, as nest guarding black bass are known to decrease parental investment (Ridgway 1989) and increase nest abandonment (Hanson et al. 2007; Zuckerman et al. 2014) in response to experimentally reduced broods. However, initial brood size, not tested as an explanatory variable in this study, plays a role in assessing the value of the brood; Zuckerman et al. (2014) found that fish with a larger initial brood size (prior to devaluation) were less likely to abandon their nest than those with a small initial brood size. In this study, fish with a small brood size originally had a large brood size (egg score ≥ 4), which was experimentally reduced to a much smaller brood size of an egg score of ≤ 2 . Interestingly, despite increased parental investment (i.e., nest-tending) relative to those fish guarding large broods, fish with a small brood did not exhibit a higher probability of nest success (Table 2-1). The reproductive value of the remaining brood is also an important

factor in black bass nest abandonment decisions (Zuckerman et al. 2014). Individuals may value broods differently according to environmental thresholds such as predator burden and breeding season length. Predator burden can be high in the lakes used in this study (Gravel and Cooke 2009), and fish residing in higher latitude lakes, such as those in southeastern Ontario, can have comparatively limited future reproductive capacities (Ridgway 1989; Shaw and Allen 2014). Moreover, cumulative investment and physiological thresholds, such as energy availability and body condition, are central in parental investment decisions (Trivers 1972; Wingfield and Sapolsky 2003). Fish in this study may be placing a high value on what brood remains, and consequently are increasing parental investment (i.e., nest-tending in this study) to ensure some reproductive success, attempting to maximize past investment, regardless of the energetic demands of parental care and the lower reproductive value of a smaller brood.

Teleost fish exhibiting parental care show much variation in their response to natural and experimental brood reductions. Reductions in black bass brood size are shown to lead to decreased parental investment (Ridgway 1989). Repudiating my prediction and what is prevalent in the literature, the results of this study show that small brood sizes could receive higher parental investment than larger ones. This study further highlights the complexity in parental investment decisions, how they are individual-specific, and are likely due to relative energetic cost and storage (Stearns 1989). The growing body of research exploring the interaction between reproduction and stress have demonstrated varied results (Sopinka et al. 2015), indicating that parental care trade-offs and stress is highly complex and remains poorly understood. Increasing shoreline development and many human activities are a source of environmental disturbances such as chemical, light, and noise pollution (Graham and Cooke 2009; Brüning et al. 2015), translating to increased human-induced stressors in the littoral zone (Wagner et al. 2006). In

addition, given that black bass are the most popular sportfish in North America, angling-induced stress before or during the parental care period is common (Philipp et al. 1997; Suski et al. 2003). It stands to reason that the increasing presence of human-induced stressors in aquatic ecosystems will further magnify the stress associated with parental care in this already challenging period. Thus, understanding how reproductive success and parental care behaviours interact with increased GCs (i.e., stress) and life history traits is imperative. Studies such as this one that experimentally manipulate GC levels have the potential to reveal how anthropogenic stressors will mediate parental care behaviour (including decisions to abandon a developing brood or forego reproduction), reproductive success and fitness in wild animals.

Tables

Table 2-1 – Model, estimate (standard error), and p-values for each term. Coefficients with significant p-values are denoted in boldface. N=93 fish for each analysis. NC is no cortisol.

| Model | Fixed Effects | Estimate (\pm SE) | Z-value | P-value |
|------------------|------------------------------------|----------------------|--------------|------------------|
| Nest Success | Intercept | 0.17 (0.49) | 0.354 | 0.724 |
| | Cortisol (NC) | 1.57 (0.74) | 2.128 | 0.033 |
| | Brood Size (Small) | 0.24 (0.65) | 0.364 | 0.716 |
| | TL.std | -0.44 (0.41) | -1.077 | 0.282 |
| | Lake (Charleston) | -0.89 (0.53) | -1.696 | 0.090 |
| | Lake (Sand) | 0.92 (1.22) | 0.753 | 0.451 |
| | Cortisol (NC) x Brood Size (Small) | -0.81 (0.98) | -0.827 | 0.408 |
| | Cortisol (NC) x TL.std | 0.81 (0.50) | 1.610 | 0.107 |
| | Brood Size (Small) x TL.std | 0.36 (0.49) | 0.732 | 0.464 |
| Aggression Score | Intercept | 3.13 (0.11) | 27.63 | <0.001 |
| | Cortisol (NC) | -0.23 (0.15) | -1.485 | 0.137 |
| | Brood Size (Small) | 0.09 (0.15) | 0.615 | 0.539 |
| | TL.std | -0.09 (0.09) | -0.993 | 0.321 |
| | Lake (Charleston) | -0.11 (0.11) | -0.919 | 0.358 |
| | Lake (Sand) | -0.02 (0.22) | -0.079 | 0.937 |
| | Cortisol (NC) x Brood Size (Small) | 0.07 (0.21) | 0.325 | 0.745 |
| | Cortisol (NC) x TL.std | -0.03 (0.10) | -0.254 | 0.799 |
| | Brood Size (Small) x TL.std | 0.10 (0.10) | 0.936 | 0.349 |
| Tending Score | Intercept | 2.43 (0.34) | 7.190 | <0.001 |
| | Cortisol (NC) | 0.62 (0.51) | 1.209 | 0.227 |
| | Brood Size (Small) | 1.01 (0.51) | 1.994 | 0.046 |
| | TL.std | -0.35 (0.27) | -1.275 | 0.202 |
| | Lake (Charleston) | 0.35 (0.38) | 0.926 | 0.355 |
| | Lake (Sand) | 0.11 (0.75) | 0.154 | 0.878 |
| | Cortisol (NC) x Brood Size (Small) | -1.04 (0.71) | -1.461 | 0.144 |
| | Cortisol (NC) x TL.std | -0.22 (0.35) | -0.629 | 0.529 |
| | Brood Size (Small) x TL.std | 0.16 (0.35) | 0.454 | 0.650 |

Figures

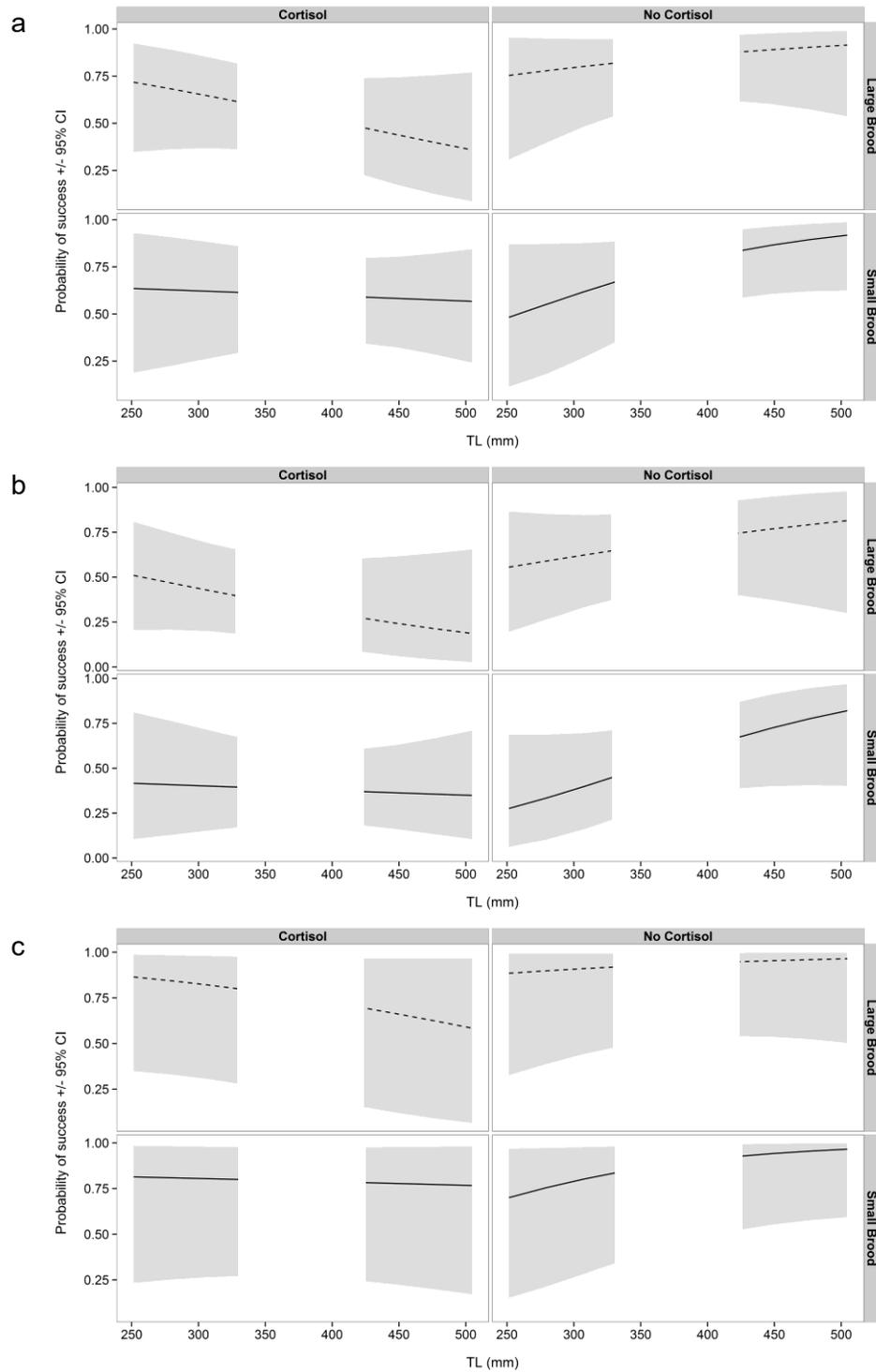


Figure 2-1: Probability (\pm 95% CI) of male smallmouth bass nest success according to body size (TL), brood size, and cortisol treatment in a) Big Rideau Lake b) Charleston Lake, and c) Sand Lake. The vertical facets show large and small brood sizes.

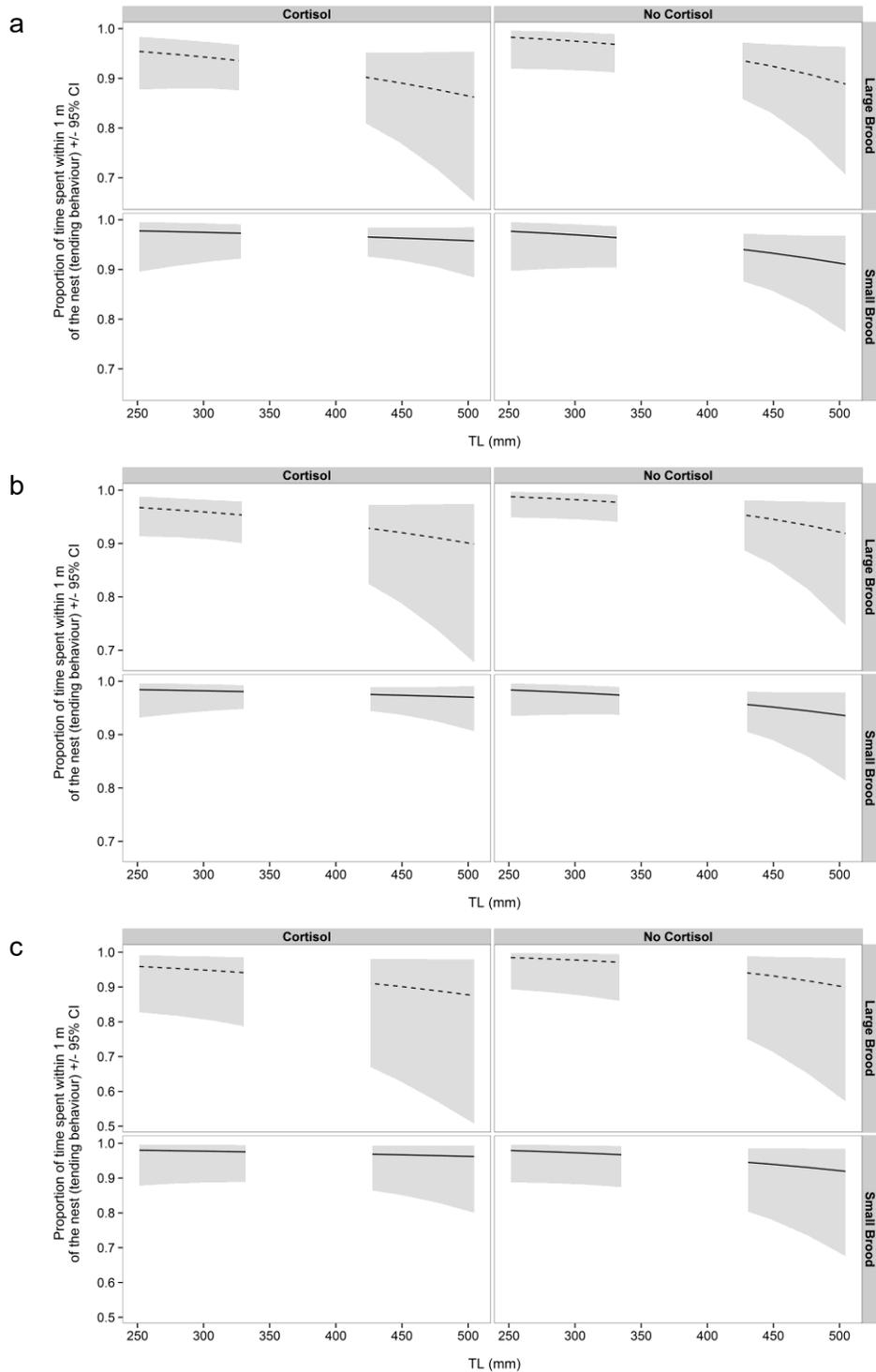


Figure 2-2: Proportion (\pm 95% CI) of time nest guarding male smallmouth bass spent within 1 m of the nest (i.e., tending behaviour) as measured over the course of 3 minutes in a) Big Rideau Lake b) Charleston Lake, and c) Sand Lake. The vertical facets show large and small brood sizes.

Chapter 3 – Misbehaving fathers: the effects of elevated cortisol on locomotor activity and parental care behaviour of nesting male smallmouth bass.

Abstract

Paternal care, where the male provides sole care for the developing brood, is a common form of reproductive investment among teleost fish and ubiquitous in the *Centrarchidae* family.

Throughout the parental care period, nesting males expend energy undertaking a variety of swimming behaviours, such as burst and routine swimming, vigilantly monitoring the nest area and protecting the brood from predators. This already challenging period is presumably made even more difficult if fish are exposed to additional challenges such as those arising from human disturbance. Here I examined whether experimental elevation of the stress hormone cortisol would alter the swimming behaviours and locomotor activity of nesting male smallmouth bass (*Micropterus dolomieu*). To do so I combined exogenous cortisol manipulation (intracoelomic injection) with tri-axial accelerometers attached to nesting male smallmouth bass for 3 days, to test the hypothesis that elevated cortisol would affect locomotor activity level and swimming behaviours of nesting smallmouth bass. I predicted that elevated cortisol would reduce locomotor activity and swimming behaviours relative to control fish, with the extent of the alteration magnified for fish with supra-physiological cortisol levels. Cortisol-treated fish exhibited significantly reduced levels of locomotor activity, burst swimming behaviours and routine swimming, and significantly increased resting behaviour compared to control fish that did not receive cortisol treatment. Temporal effects (study hour, diel period) were found to have a significant effect on activity level and swimming behaviours as well. My results suggest that cortisol treated fish were altering their behaviour in response to elevated cortisol, thus exhibiting

decreased parental investment in their brood, which could have potential fitness implications. This research contributes to the growing body of literature using experimental cortisol manipulation to explore the potential effects of elevated cortisol on fitness of wild animals.

Introduction

As part of their reproductive strategy, many taxa provide extended parental care for their developing offspring. By providing parental care, defined here as the post-fertilization behaviours exhibited while caring for offspring, the parent(s) increase the chances of offspring survival, thus maximizing the potential for reproductive success and fitness of the parents (Clutton-Brock 1991). Parental care occurs in a variety of taxa including mammals (Gubernick and Klopfer 1981), birds (Tullberg et al. 2002), reptiles (Shine et al. 1997; Platt and Thorbjarnarson 2000), amphibian (Summers 1992; Tumulty et al. 2014), and fish (Blumer 1982; Gross and Sargent 1985). Of all vertebrate taxa, fish exhibit the greatest diversity of parental care modes (Clutton-Brock 1991; Webb et al. 1999) including paternal care (sole male care), maternal care (sole female care), biparental care (both parents), and combinations thereof (Gross and Sargent 1985; Goodwin et al. 1998). Parental care is particularly prevalent among teleost fishes, with paternal care being the most common mode (Blumer 1982).

Paternal care is ubiquitous in fish in the freshwater centrarchidae family; this phenomenon has been particularly well-studied for the black bass (*Micropterus spp.*). Reproduction is energetically costly for male black bass (Gillooly and Baylis 1999; Mackereth et al. 1999; Cooke et al. 2002, 2006). Black bass rely on endogenous energy reserves through the parental care period, which lasts 4-6 weeks (Ridgway 1988; Cooke et al. 2006). Elevated locomotor activity level must be maintained by nesting males throughout parental care (Cooke et al. 2002) in order to vigorously defend their developing offspring from persistent brood

predators, which are often omnipresent and able to quickly consume an entire brood if the male is not vigilant (Steinhart et al. 2004; Gravel and Cooke 2009; Zuckerman and Suski 2013). Nesting black bass that exhibit relatively higher parental investment into their brood defense and nest-tending behaviours are known to have a higher probability of reproductive success compared to those exhibiting diminished defensive and nest-tending behaviours (Ridgway 1998). Considerable amounts of time are devoted to nest-tending activities (i.e., fanning eggs, removing debris and dead eggs from the nest) to ensure eggs remain well oxygenated and healthy (Hinch and Collins 1991). Time consuming parental care duties and alterations in appetite hormones both lead to reduced food intake which means that care is largely driven by endogenous energy stores. Due to the challenges associated with paternal care and the trade-offs between current and future reproductive success (Williams 1966; Trivers 1972), premature nest abandonment is not uncommon, particularly in fish with limited energy reserves (e.g., small fish or those in poor condition; Gingerich and Suski 2011) or those with small broods (Zuckerman et al. 2014).

Parental care is a challenging period for fish including black bass. In addition to the previously noted increased energetic demand associated with heightened locomotor activity while having reduced food intake, parental fish must endure other challenges such as increased vulnerability to piscivorous predators (e.g., heron, osprey) due to their nesting locations in the littoral zone, and inclement weather which can expose the parent and developing offspring to variation in temperature, sediment and currents/wave action. This already challenging period for black bass may be magnified through various anthropogenic environmental disturbances and stressors which are all too common in freshwater systems and include shoreline development and associated habitat alterations (Wagner et al. 2006), ecological light pollution in the littoral zone (Foster et al. 2016 in submission), noise and wave disturbances from vessels powered by

combustion engines (Graham and Cooke 2009) and fisheries interactions (e.g., catch-and-release angling; Philipp et al. 1997; Suski et al. 2003). If a disturbance disrupts homeostasis (i.e., initiates a stress response), circulating stress steroids, glucocorticoids (GCs), rise above basal levels for a period of time (Wendelaar Bonga 1997; Mommsen et al 1999). Given that parental care is already regarded as a challenging period for nesting black bass (Cooke et al. 2002), these additional challenges have the potential to cause stress and alter behaviour and energy use.

An increasingly common approach to study how anthropogenic challenges may alter the energetics and behaviour of wild animals including fish is through experimental GC manipulation (Sopinka et al. 2015; Crossin et al. 2016). Researchers have experimentally elevated GCs and revealed that largemouth bass and smallmouth bass treated with cortisol tend to exhibit elevated nest abandonment relative to controls (e.g., O'Connor et al. 2009; Dey et al. 2010). However, alterations in behaviour of nesting males prior to abandonment have not been detected (e.g., O'Connor et al. 2009; Dey et al. 2010) which may be a consequence of the fact that behaviour is typically assayed by use of snorkelers who monitor simple behaviours for several minutes (e.g., response to a brood predator model, monitoring time spent on nest, willingness to engage predators), only during daylight conditions. Some researchers that have studied black bass parental care behaviour and energetics independent of GC manipulation have used video cameras (Hinch and Collins 1991; Cooke et al. 2006), but cameras have a restrictive field of view and are heavily influenced by water clarity and light (Struthers et al. 2015). New developments in biologging technology enable researchers to affix tri-axial accelerometers to fish to monitor fine-scale behaviours and energy use of free-swimming fish such that animals can be studied day and night with greater temporal resolution (e.g., Brown et al. 2013).

Given the above, I was interested in understanding how disturbances associated with human activity influence the behaviour and energy use of wild fish engaged in already-challenging and costly paternal care duties. To that end, the objective of this study was to explore the influence of elevated cortisol on swimming behaviours and relative locomotor activity level of nesting black bass. I hypothesized that elevated cortisol would affect locomotor activity level and swimming behaviours of nesting smallmouth bass. Since nesting fish with experimentally elevated cortisol are known to exhibit decreased nest success (e.g., O'Connor et al. 2009; Dey et al. 2010), and nest-tending and brood predator defence are fundamental locomotor based behaviours linked to reproductive success, I predicted that nesting males with elevated cortisol levels will exhibit reduced locomotor activity and swimming behaviours compared to control fish that did not receive any cortisol treatment. Furthermore, I predict the extent of the alteration to be magnified for fish with supra-physiological cortisol levels. Here I used accelerometer loggers affixed to nesting fish to measure locomotor activity level and characterize swimming behaviours in response to experimentally elevated cortisol levels.

Methods

Nesting male smallmouth bass were located through snorkel surveys and angled from the nest, with fight time minimized (< 20 s) to reduce angling associated stress. Following capture, fish were placed into a foam-lined trough containing fresh lake water and measured for total length (TL). Following the general methods of Brownscombe et al. (2014), accelerometers were firmly attached externally through the dorsal musculature using 13.6 kg Dacron line and a backing pad on the right side of the fish under the soft area of the dorsal fin. To limit fish handling time mass was estimated from known length-mass relationships for fish in Lake Opinicon (Dey et al. 2010); the equation is $\log_{10} \text{ mass} = -7.1004 + 3.884(\log_{10} \text{ TL})$, mass is

reported in grams and TL in millimeters. After accelerometer attachment, fish included in the cortisol treatment group received a 5 mL kg⁻¹ intraperitoneal injection of cocoa butter impregnated with cortisol (Hydrocortisone 21-hemisuccinate, Santa Cruz Biotechnology) using a 16-gauge needle. Three cortisol treatment levels were used in the experimental design, fish that didn't receive any cortisol treatment (control fish), fish that received a low (5 mg mL⁻¹) concentration, and fish that received a high (10 mg mL⁻¹) concentration. A sham treatment was not included in the experimental design due to inconsistent cortisol responses of sham treated fish (see DiBattista et al. 2005). Furthermore, a sham treatment is not as useful in studies examining elevated cortisol, as a sham treatment may result in elevated cortisol, presenting difficulties in distinguishing between a sham effect and a treatment effect. Prior to release fish (with tag affixed) were rotated along known axes (forward pitching motion and rolled to the right) in a cooler containing fresh lake water, noting the corresponding times to aid in calibrating accelerometers. The capture, tagging procedure, cortisol administration (if applicable), and accelerometer calibration encompassed less than 6 minutes. Fish were released within 5 m of the nest. A diver guarded the nest in the male's absence until the male resumed parental behaviours, typically less than 5 minutes. All nesting males were ≥ 390 mm total length (TL), and had an egg score of ≥ 3 , attempting to limit premature nest abandonment. Egg score is a repeatable qualitative measure of the amount of offspring categorized as 1 through 5, with 1 indicating a nest containing hundreds of eggs, 3 indicating an average amount of eggs, and 5 indicative of a nest containing several thousands of eggs (Kubacki 1992; Philipp et al. 1997).

After 3 days fish were recaptured using rod and reel (angling fight time < 20 s), placed into a foam-lined trough containing fresh lake water, a blood sample was taken, and the accelerometer removed. Time from capture to blood sampling was < 120 seconds for each fish.

Blood samples (approximately 1 mL) were taken from the caudal vasculature using a 22 mm 21-gauge needle on a 4 mL heparinized vacutainer (B.D. Vacutainer, Franklin Lakes, NJ) and immediately placed into a water-ice slurry for no longer than 90 minutes. Blood was centrifuged at 15,000 rpm for 120 seconds and blood plasma was extracted from samples using 1 mL disposable transfer pipettes, frozen in liquid nitrogen, and stored in -80°C until analysis. Cortisol analysis was conducted using a radioimmunoassay kit (ImmuChem, MP Biomedicals) with isotope I-125, which was counted for total gamma radiation on a Perkin Elmer 2470 Wizard2 automatic gamma counting unit. Inter-plate variation was 10.0% and intra-plate variation was 1.5% and 2.8%. Cortisol treatment groups were categorized based on their measured cortisol levels with control being considered as 0 to 50 ng ml^{-1} , reflective of basal levels of cortisol (Morrissey et al. 2005; Hanson et al. 2009b; Dey et al. 2010; O'Connor et al. 2011); low cortisol as > 50 up to 1000 ng ml^{-1} , documented in fish during a stress response (Morrissey et al. 2005; O'Connor et al. 2011); and high cortisol as $> 1000\text{ ng ml}^{-1}$, a supra-physiological level (O'Connor et al. 2009; Dey et al. 2010). A one-way ANOVA was conducted to highlight significant differences in circulating cortisol concentrations between treatment groups. Measured cortisol values were \log_{10} transformed to meet assumptions for ANOVA testing.

Two accelerometer models were used (model X16mini, 17 g in air, 25 Hz recording frequency, $\pm 16\text{ g}$ range, 2048 count/g sensitivity; model X8M-3, 15 g in air, 25 Hz recording frequency, $\pm 8\text{ g}$ range, 1024 count/g sensitivity; Gulf Coast Data Concepts, Waveland, MS) programmed to continuously record acceleration measurements at a frequency of 25 Hz on 3 axes (x = heave, y = surge, z = sway). Static (gravity) and dynamic (fish movement) acceleration were separated by a weighted smoother over each axis at a 2 s interval, found to be appropriate for small teleost fishes (Brownscombe et al. 2013, 2014). Average weight of accelerometers

including backing plates and tape was ~ 28 g in air, corresponding to less than 2% of fish weight for all fish in this study, minimizing tagging burden (Brown et al. 1999). Furthermore, numerous visual observations confirmed that swimming behaviours were not impeded by accelerometer attachment, with tagged fish continuing to exhibit the same general swimming behaviours as untagged fish (Algera, Personal Observations).

Swimming behaviours, namely routine swimming, burst swimming, and resting were included in analysis. Routine swimming behaviours included general swimming to monitor the nest area and turns. Burst swimming differs from routine swimming by being characterized as aggressive, short duration, and energetically demanding swimming events, exhibited by nesting fish chasing away a brood predator. Resting behaviours included stationary and limited locomotor activities such as sitting atop the nest, and performing nest checks to remove dead eggs and debris. Swimming behaviours of several fish were visually observed by divers with digital watches synchronized to the accelerometer, noting the time and duration that a fish exhibited behaviours of interest. Visually observed behaviours served to develop criteria for a trainer data set via a classification tree. Predictor variables in the classification tree model included the mean and standard deviation for each of the following: dynamic acceleration for each axis (x, y, z), pitch, and roll. The final classification tree model was trimmed to include three nodes (one node for each swimming behaviour), and was cross-validated, resulting in a 5% misclassification error. To classify routine swimming, burst swimming, and resting behaviours on unobserved fish, accelerometer outputs were run through the classification tree model (Brownscombe et al. 2014). Each predicted behaviour was classified at a probability of ≥ 0.70 from the classification tree criteria output. Overall dynamic body acceleration (ODBA), commonly used to quantify relative activity level (Brownscombe et al. 2013, 2014), was

calculated as the absolute sum of the dynamic acceleration of the three axes (X, Y, Z). Metabolic rates highly correlate with ODBA in a variety of taxa, including fish (Halsey et al. 2009; Gleiss et al. 2011; Wright et al. 2014). The frequency of each behaviour, as well as mean ODBA was calculated for each study hour. The first hour of the study period was removed in order to standardize analyses and control for any short term impacts of the capture and handling procedures. The first hour of the study period was removed in order to standardize analyses and control for any short term impacts of the capture and handling procedures. Moreover, it would require ~1 hr for cortisol injections to raise circulating cortisol levels while the capture-induced cortisol elevation would be starting to decline by that time (Suski et al. 2006).

Statistical Analyses

Effects of cortisol level on swimming behaviours (burst swimming, resting, routine swimming) and locomotor activity level (ODBA) were tested using linear mixed effects models with cortisol level (no cortisol, low cortisol, high cortisol), diel period (day, night), hour of study period, and their interaction terms as fixed effects. Multiple observations on the same fish prescribed that fish ID be included as a random factor in each model. From the model including all explanatory variables and interactions, a stepwise backward model selection with single term deletions using likelihood ratio tests was conducted; first the significance and optimal structure of the random component was established with restricted maximum likelihood estimation (REML), and then the optimal structure of the fixed component was established with maximum likelihood estimation (ML), as recommended by Zuur et al. (2009). Model validation was conducted as per Zuur et al. (2009). Due to patterns in the residuals, rank transformation was applied to the swimming behaviour response variables (Conover and Iman 1976) resulting in greater model fit. Significance was tested at $\alpha=0.05$. Accelerometer data analysis was conducted

using IGOR Pro 6.0 (WaveMetrics Inc., Lake Oswego, OR) with the Ethographer package (Sakamoto et al. 2009). Statistical analyses were conducted using R software (v. 3.2.3, R Foundation for Statistical Computing, Vienna, Austria).

Results

Sample size for each of the analyses included 6 control (no cortisol), 5 low cortisol, and 5 high cortisol treatment fish for statistical testing. After 3 days, circulating cortisol was lowest in the control group (Table 3-1) ranging from 10 to 19 ng mL⁻¹. The low cortisol treatment group included fish with measured circulating cortisol values ranging from 128 to 897 ng mL⁻¹ and the high cortisol treatment group with measured values ranging from 1527 to 4277 ng mL⁻¹. Cortisol values were significantly different among all treatment groups (ANOVA; $F = 127.7$, $df = 2$, p -value < 0.0001 ; Tukey's HSD; p -value < 0.001 , all cases; Table 3-1).

Locomotor Activity Level (ODBA)

The treatment groups all followed similar activity level trends throughout the study period, steadily increasing and peaking in activity level around hour 40 (Figure 3-1). Throughout the study duration, cortisol treated fish exhibited decreased locomotor activity levels compared to controls until hour 60, where activity levels of control fish decreased, converging with the cortisol treated fish (Figure 3-1). Linear mixed model testing revealed a significant effect of cortisol treatment, diel period, study hour, and their interaction terms (with exception of diel x hour) on nesting smallmouth bass locomotor activity level (ODBA) (Table 3-2).

Swimming Behaviours

Control fish maintained a consistent frequency of burst swimming per hour throughout the study period (Figure 3-2). Compared to control fish, cortisol treated fish performed fewer

burst swims for the first 20 hours, after which they began to exhibit similar frequencies to controls (Figure 3-2). Linear mixed model testing revealed a significant effect of diel period, study hour, cortisol treatment x diel interaction term, and a cortisol treatment x study hour interaction term on nesting smallmouth bass burst swimming frequency (Table 3-2).

Similar trends in resting behaviour were observed between control and cortisol treatment fish throughout the study period, however, compared to control fish, cortisol treatment fish spent more time resting per hour averaged across the entire study period (Figure 3-3). Linear mixed model testing revealed a significant effect of diel period, study hour, a cortisol treatment x diel interaction term, and a cortisol treatment x study hour interaction term on nesting smallmouth bass burst swimming frequency (Table 3-2).

Control fish consistently performed more routine swimming behaviours per hour than cortisol treated fish (Figure 3-4). Routine swimming increased in each treatment group for the first 30 hours of the study period, and then steadily decreased, eventually converging at around hour 60 (Figure 3-4). Linear mixed model testing revealed a significant effect of the cortisol x diel period interaction term, cortisol x study hour interaction term, and a cortisol treatment x diel period x study hour 3-way interaction term, on nesting smallmouth bass routine swimming (Table 3-2).

Discussion

In this study I tested whether experimental cortisol elevation induced changes in nesting male smallmouth bass locomotor activity level and swimming behaviours to gain further insight into the effects of cortisol, a key stress hormone in fish, on parental care investment.

Experimentally elevated cortisol treatment induced changes to locomotor activity levels in nesting smallmouth bass (Table 3-2); as predicted, throughout the study period cortisol treated

nesting smallmouth bass exhibited lower locomotor activity level compared to control fish that did not receive cortisol treatment (Figure 3-1). Cortisol treatment also affected smallmouth bass swimming behaviours (bursting, resting, and routine swimming) depending on temporal factors (Table 3-2), mirroring locomotor activity levels, as cortisol treated fish performed fewer burst and routine swimming behaviours and spent more time resting compared to control fish (Figures 3-2 to 3-4). The effect of cortisol on swimming behaviours and activity level was also dependent on time (study hour) (Table 3-2), with greater effects in the initial 20 to 30 hours following release (Figures 3-1 to 3-4). Diel period had an effect on smallmouth bass activity levels and all swimming behaviours (Table 3-2) such that fish were less active and performed fewer behaviours at night compared to the day.

As predicted, nest tending smallmouth bass receiving a cortisol treatment exhibited lower locomotor activity level throughout the study period compared to control fish (Figure 3-1). Lower locomotor activity level in cortisol treated fish was likely driven by changes in the swimming behaviours, as they performed fewer routine and burst swims (Figures 3-2; 3-4). Studies experimentally manipulating cortisol in rainbow trout (Gregory and Wood 1999) and common carp (Liew et al. 2013) found no effect of chronically elevated cortisol on swimming performance in these species. However, fish in those studies were forced to swim when provided with swimming performance challenges rather than observing behaviours as I did in this study. It is possible that swimming function per se was not impaired by cortisol in nesting smallmouth bass, but rather elevated cortisol may be affecting their behavioural choices (i.e., motivation and investment).

Parental care is costly to nesting black bass (Cooke et al. 2002), contributing to lost body mass (Gillooly and Baylis 1999) and depleted endogenous energy reserves (Mackereth et al.

1999; Cooke et al. 2006). Parental care is also inherently stressful as fish partake in activities that involve engaging with nest predators (Hanson et al. 2009c); in such instances cortisol mobilizes energy reserves to help fish deal with such stress (Wendelaar Bonga 1997; Mommsen et al. 1999). Cortisol treated fish in this study had elevated cortisol levels measured at 3 days, surpassing a duration typically seen during an acute stress response in fish (Gamperl et al. 1994), which can have deleterious effects on body condition and functions (Wendelaar Bonga 1997). O'Connor et al. (2009) found that nesting black bass with elevated cortisol levels had increased Saprolegnial infections compared to controls. Using experimentally elevated cortisol levels in common carp (*Cyprinus carpio*), Liew et al. (2013) found liver and muscle protein mobilization in resting fish. Thus, nesting smallmouth bass with elevated cortisol may still be expending energy reserves even while resting, which could promote decreases in health and condition and thus lead to premature nest abandonment. Collectively, the results of this study suggest that the cortisol treated fish may be saving energy by reducing activity level, favoring self maintenance and survival (i.e., future reproductive potential) over the continued parental investment associated with continuing to care for the current developing brood (Wingfield et al. 1998).

Overall dynamic body acceleration, used to estimate activity level in this study, is highly correlated with energy use in teleost fishes (Gleiss et al. 2011; Wright et al. 2014). Control fish, serving as an indicator of the typical activity level and behaviours characteristic of parental care for nesting black bass, exhibited greater locomotor activity level, burst swimming, and routine swimming than cortisol treated fish. Cooke et al. (2002) estimated that nesting black bass swim upwards of 40 km day⁻¹ monitoring their nest area with electromyogram telemetry transmitters. Gravel and Cooke (2013) found that when predation pressure increased, guarding males spent more time performing burst swims, an essential behaviour in protecting the nest from brood

predators. Reduced monitoring and nest defense behaviours exhibited by cortisol treated fish (i.e., reduced routine and burst swimming), and increased time spent resting may indicate deficient or impaired parental investment, which may ultimately have fitness related impacts through reduced nest success with the current brood.

There were temporal effects found in study hour and diel period (Table 3-2). Study hour had a significant effect on locomotor activity level and swimming behaviours. Observed differences in control and cortisol treated nesting smallmouth bass activity level and swimming behaviours between are evident right from the study onset (hour 2), suggesting that cortisol uptake was rapid. The effect of study hour is driven by differences in activity level and swimming behaviours between treatment groups in the first 20-30 hours of the study. The control fish activity level and swimming behaviours converging with cortisol treated fish could reflect a known general decrease in parental care behaviour in black bass (Cooke et al. 2002) coupled with a reduction in circulating levels of cortisol. There were differences in night time and day time activity level and swimming behaviours (Table 1). At night all treatment groups of nesting smallmouth bass exhibited reduced locomotor activity, bursting and routine swimming, while increasing the time spent resting. The reductions in locomotor activity and swimming behaviours at night are not surprising, as nesting smallmouth bass have fewer brood predators to engage. Although there are nocturnal brood predators (e.g., bullhead catfish) present in the study lake system (Keast 1978), the predator burden by these nocturnal species would be negligible in comparison to centrarchids (*Lepomis spp.*), the dominant brood predators in lakes within this system (Keast 1978; Cooke and Gravel 2013), which are primarily active during daylight hours (Emery 1973).

Taken together, the corresponding locomotor activity levels and swimming behaviour responses suggest that physiologically relevant circulating cortisol levels reflective of a stress response (i.e., low cortisol group in this study) and supra-physiological levels (i.e., high cortisol group in this study) produce a similar behavioural impairment in nesting smallmouth bass.

Assessing parental care behaviours in response to elevated cortisol has been explored in other research, but results were inconsistent and assessed from a narrow time window (e.g., snorkel observations of behaviour for a matter of minutes; O'Connor et al. 2009; Dey et al. 2010). This study is the first to examine locomotor activity level and parental care behaviours in response to elevated cortisol at a fine scale and over a long period of time, and adds to the growing body of literature illustrating the effects of elevated cortisol levels on teleost reproduction.

Many species that provide parental care construct their nest in the littoral zone near the shoreline (e.g., Keast et al. 1978; Cooke et al. 2006). Due to the proximity of anthropogenic shoreline development, fish in nearshore areas would have highest exposure to added challenges and stressors such as shoreline development, increased light pollution, and boat operation and noise. Clearly, exhibiting appropriate behavioural responses will become increasingly important in the face of increased anthropogenic environmental alterations in aquatic ecosystems. My study focused only on a single reproductive event and it is well known that fish will trade-off the value of their current brood with the potential for future reproductive success. Longer-term studies examining the consequences of cortisol elevation during parental care on lifetime fitness is needed. Similarly, more work on understanding how elevated cortisol at other periods in fish life-history may influence parental care success and decisions and ultimately influence their lifetime fitness.

Tables

Table 3-1: Circulating cortisol measured 3 days post-treatment. Cortisol values are reported as mean (\pm SE). Control N=6, Low cortisol N=5, High cortisol N=5. Different letters denote significant differences among treatments.

| Treatment | Cortisol (ng mL ⁻¹) |
|---------------|---------------------------------|
| Control | 14.8 (1.5)a |
| Low cortisol | 537.0 (153.2)b |
| High cortisol | 2690.6 (468.6)c |

Table 3-2: Significant factors from linear mixed models testing the effects of cortisol treatment (Cortisol), diel period (Diel), and study hour (Hour) on nesting smallmouth bass locomotor activity level (ODBA) and swimming behaviours (bursting, resting, routine). Significant terms (at $\alpha = 0.05$) are denoted in boldface font. For each analysis Control N=6, Low Cortisol N=5, High Cortisol N=5.

| Response | Factor | numDF, denDF | F-value | p-value |
|--------------------------|-------------------------------|--------------|-----------|-------------------|
| Activity Level (ODBA) | Intercept | 1, 1042 | 550.5132 | <0.0001 |
| | Cortisol | 2, 13 | 6.7971 | 0.0095 |
| | Diel | 1, 1042 | 65.9412 | <0.0001 |
| | Hour | 1, 1042 | 14.4757 | 0.0002 |
| | Cortisol : Diel | 2, 1042 | 9.5431 | 0.0001 |
| | Cortisol : Hour | 2, 1042 | 6.1543 | 0.0022 |
| | Diel : Hour | 1, 1042 | 0.8705 | 0.3510 |
| | Cortisol : Diel : Hour | 2, 1042 | 3.7604 | 0.0236 |
| Burst Swimming | Intercept | 1, 1042 | 236.952 | <0.0001 |
| | Cortisol | 2, 13 | 2.076 | 0.1650 |
| | Diel | 1, 1042 | 70.078 | <0.0001 |
| | Hour | 1, 1042 | 70.474 | <0.0001 |
| | Cortisol : Diel | 2, 1042 | 16.248 | <0.0001 |
| | Cortisol : Hour | 2, 1042 | 14.560 | <0.0001 |
| | Diel : Hour | 1, 1042 | 0.360 | 0.5486 |
| | Cortisol : Diel : Hour | 2, 1042 | 0.240 | 0.7868 |
| Resting | Intercept | 1, 1042 | 96.93293 | <0.0001 |
| | Cortisol | 2, 13 | 2.42299 | 0.1275 |
| | Diel | 1, 1042 | 13.15975 | 0.0003 |
| | Hour | 1, 1042 | 33.16124 | <0.0001 |
| | Cortisol : Diel | 2, 1042 | 16.41496 | <0.0001 |
| | Cortisol : Hour | 2, 1042 | 25.58306 | <0.0001 |
| | Diel : Hour | 1, 1042 | 2.41865 | 0.1202 |
| | Cortisol : Diel : Hour | 2, 1042 | 3.69113 | 0.0253 |
| Routine Swimming | Intercept | 1, 1042 | 111.94144 | <0.0001 |
| | Cortisol | 2, 13 | 2.35887 | 0.1337 |
| | Diel | 1, 1042 | 1.55661 | 0.2124 |
| | Hour | 1, 1042 | 1.74903 | 0.1863 |
| | Cortisol : Diel | 2, 1042 | 17.36858 | <0.0001 |
| | Cortisol : Hour | 2, 1042 | 18.93310 | <0.0001 |
| | Diel : Hour | 1, 1042 | 1.24739 | 0.2643 |
| | Cortisol : Diel : Hour | 2, 1042 | 3.80603 | 0.0225 |

Figures

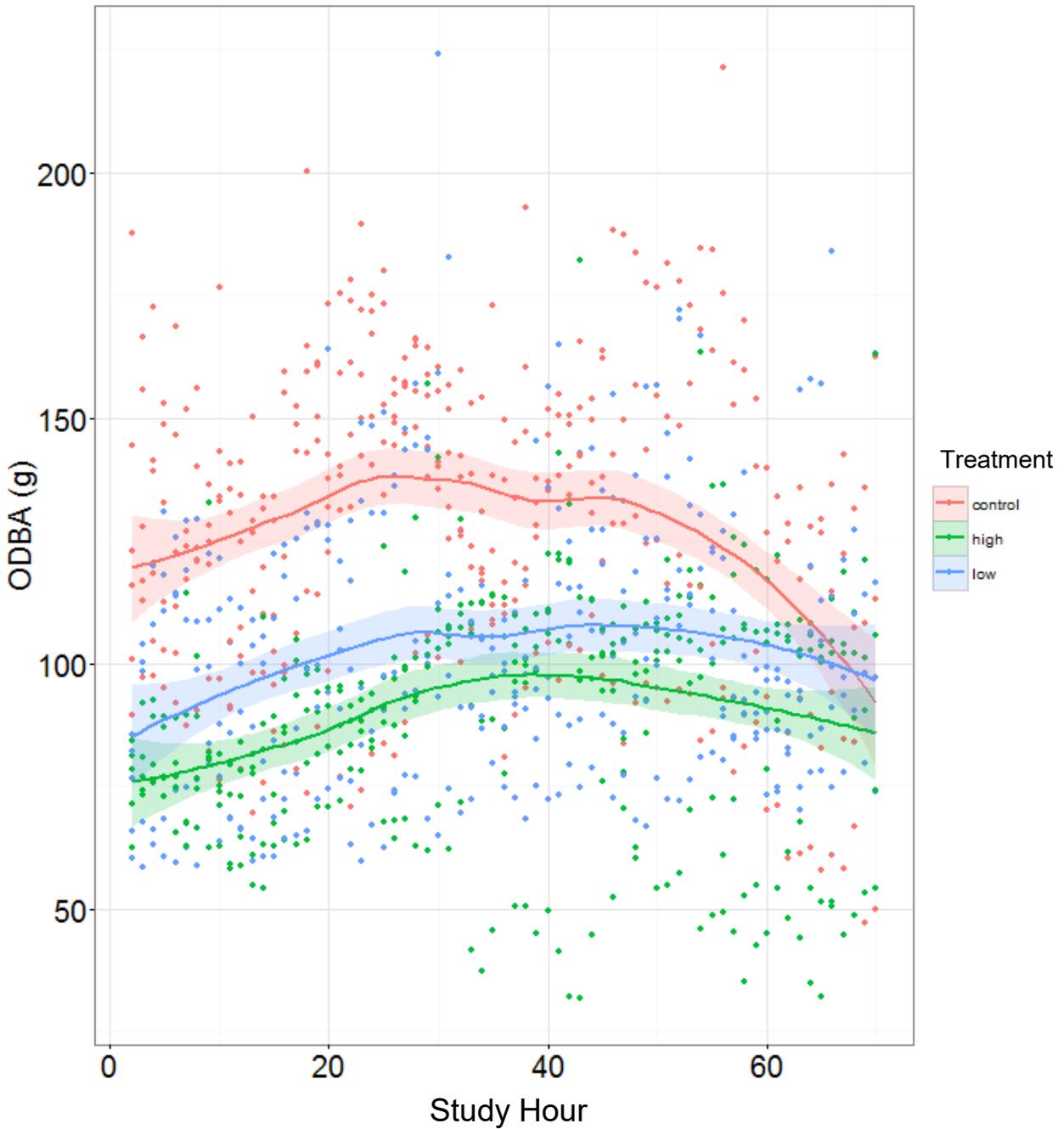


Figure 3-1: Smallmouth bass locomotor activity (ODBA), over the course of the study period (70 hours). Control (red), Low Cortisol (blue) and High Cortisol (green) treatment groups are fit with a loess smoother, shown with 95% confidence intervals surrounding lines. Control N=6, Low Cortisol N=5, High Cortisol N=5.

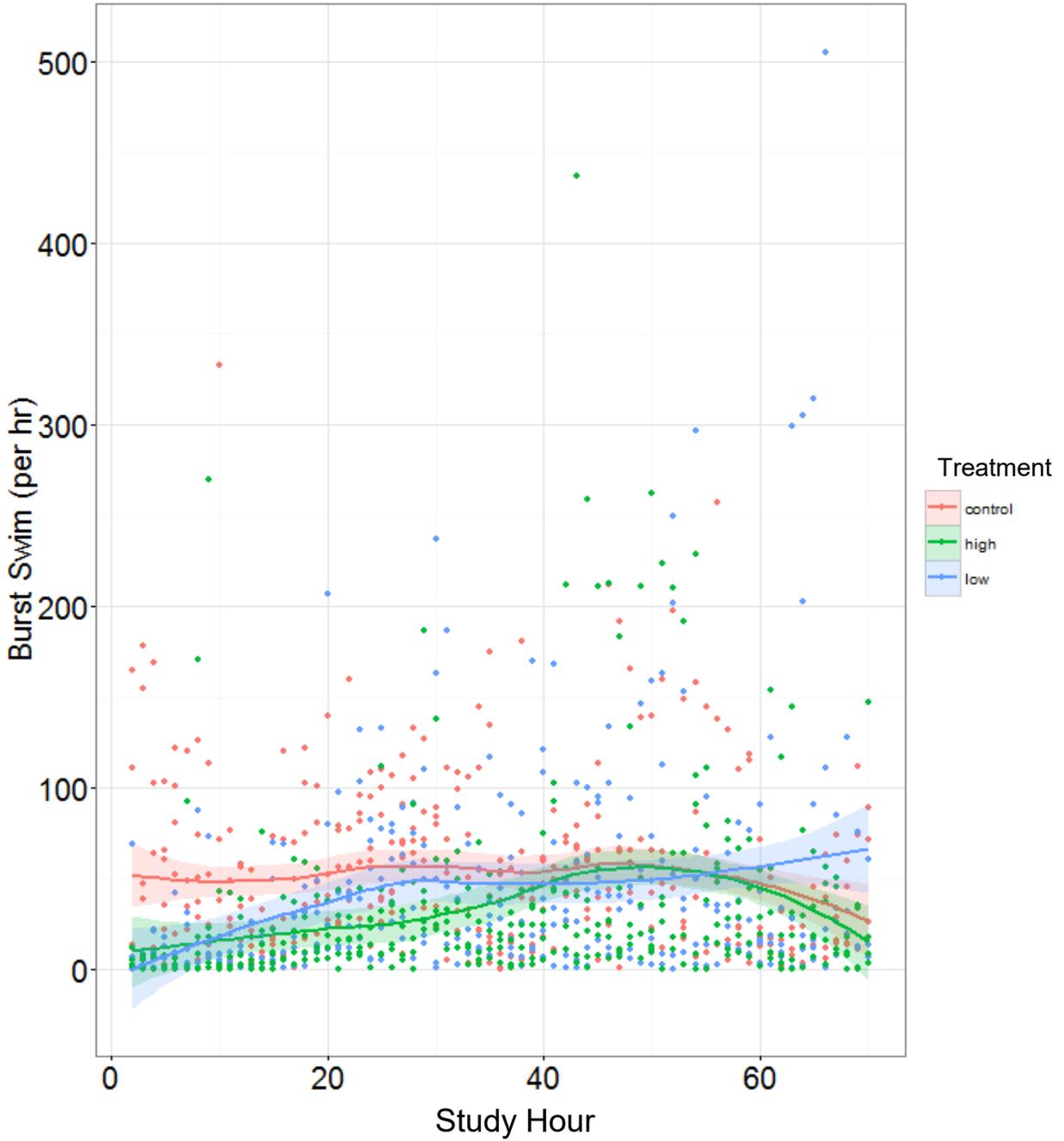


Figure 3-2: Smallmouth bass burst swimming behaviour over the course of the study period (70 hours). Control (red), Low Cortisol (blue) and High Cortisol (green) treatment groups are fit with a loess smoother, shown with 95% confidence intervals surrounding lines. Control N=6, Low Cortisol N=5, High Cortisol N=5.

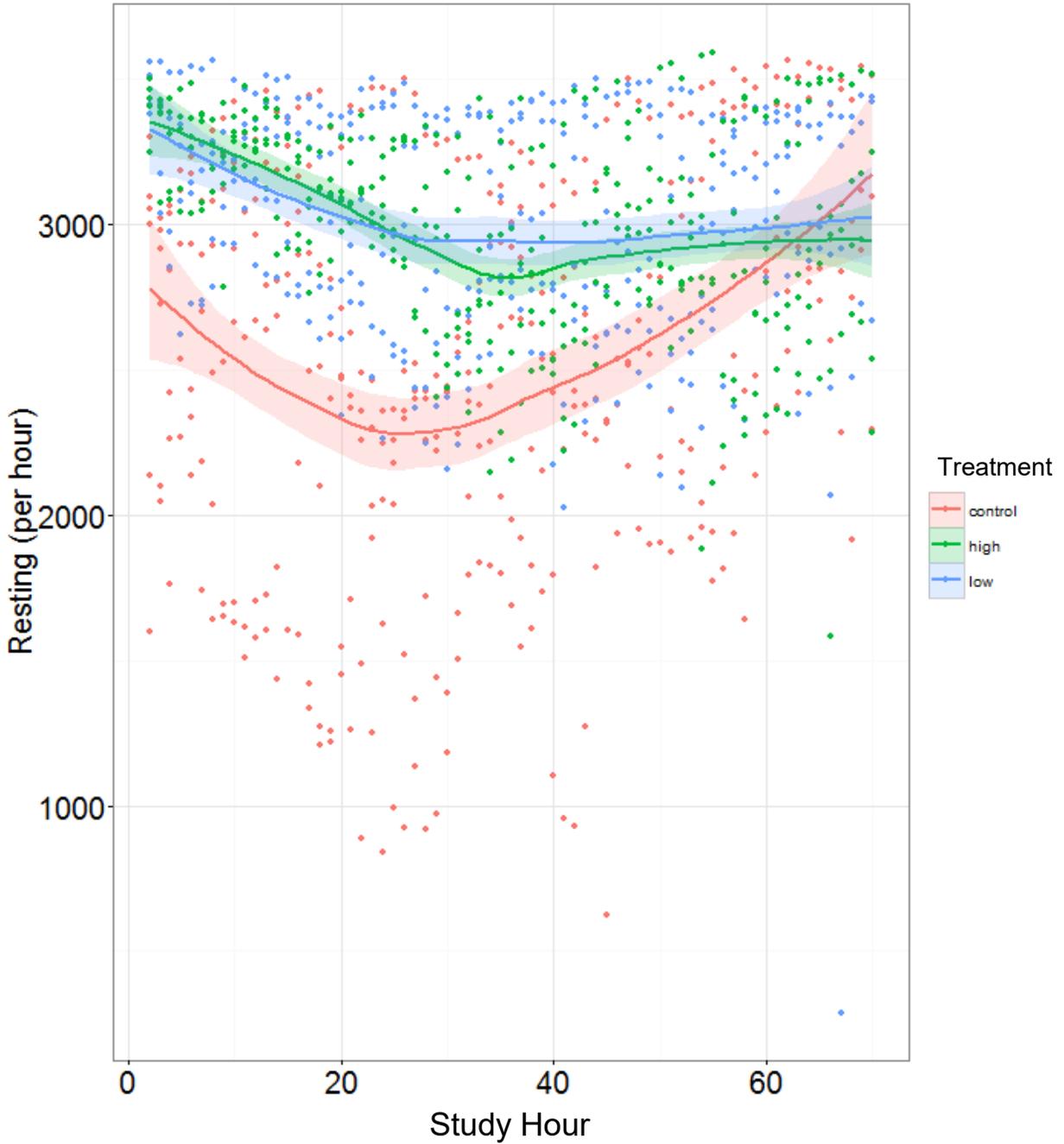


Figure 3-3: Smallmouth bass resting behaviour over the course of the study period (70 hours). Control (red), Low Cortisol (blue) and High Cortisol (green) treatment groups are fit with a loess smoother, shown with 95% confidence intervals surrounding lines. Control N=6, Low Cortisol N=5, High Cortisol N=5.

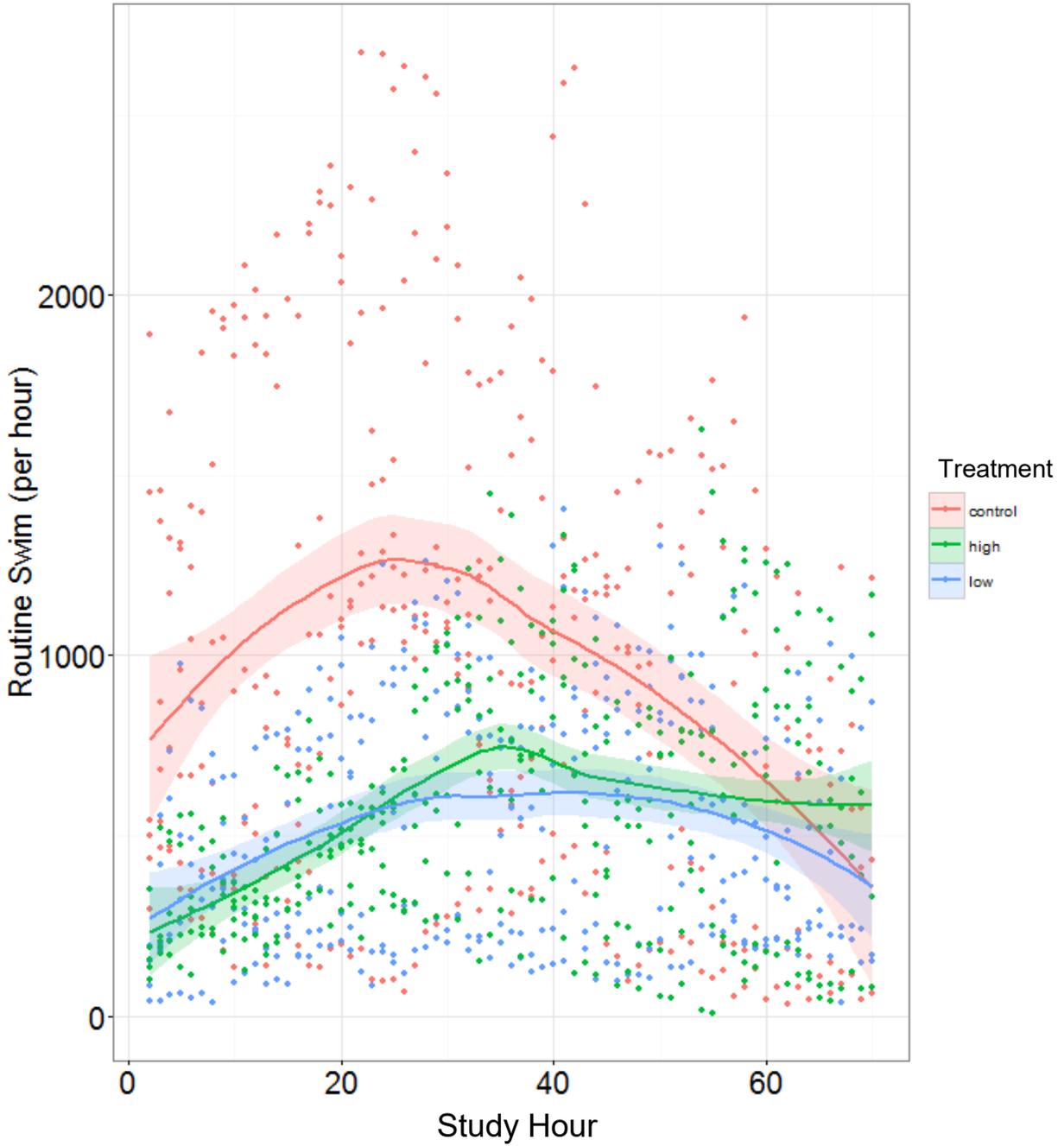


Figure 3-4: Smallmouth bass routine swimming behaviour over the course of the study period (70 hours). Control (red), Low Cortisol (blue) and High Cortisol (green) treatment groups are fit with a loess smoother, shown with 95% confidence intervals surrounding lines. Control N=6, Low Cortisol N=5, High Cortisol N=5.

Chapter 4 – General Discussion

Findings and Implications

There is a growing body of literature exploring the role of glucocorticoids (GCs), hormones and steroids responsible for the stress response across vertebrate taxa, during parental care. When interacting, handling, and manipulating an organism multiple times, producing a natural chronic stress response is not always feasible or appropriate, particularly when using wild animals. Experimental elevation of GCs is an increasingly commonly used tool, providing insight into the interactions between stress and reproduction in wild animals (Sopinka et al. 2015; Crossin et al. 2016). Using experimentally elevated cortisol, my thesis research suggests that elevated cortisol interacts with reproduction in teleosts, influencing parental care behaviours and reproductive success in wild fish. In Chapter 2, using a gradient of body sizes, manipulating brood sizes, and experimentally elevating cortisol (through cortisol injected implants), I explored the interaction between elevated cortisol, body size, and brood size as they relate to parental care behaviours and reproductive success in nesting smallmouth bass. This is the first study to explore interactive effects of cortisol level, body size, and brood size on parental care behaviours and reproductive success in wild fish using a wide range of body sizes combined with experimentally manipulated cortisol levels. In Chapter 3, using cortisol implants combined with tri-axial accelerometers attached to smallmouth bass for 3 days, I tested if elevated cortisol affected locomotor activity level, burst swimming, routine swimming, and resting behaviour. This is the first study to examine the effects of elevated cortisol on parental care behaviours at a fine scale and longer time period in wild fish. It is also the first study to use accelerometer biologgers to characterize the behaviour of an animal during parental care.

There are many factors linked to smallmouth bass reproductive success. Through my thesis research, I found that elevated cortisol had a negative effect on smallmouth bass reproductive success (Chapter 2), consistent with other similar studies (O'Connor et al. 2009; Dey et al. 2010). I did not explicitly measure circulating cortisol for the study in Chapter 2, but other studies using the same species, dose, and experimental protocols found that cortisol was elevated (O'Connor et al. 2009; Dey et al. 2010). Moreover, in Chapter 3 circulating cortisol was measured and found to be elevated, so I am confident in concluding that cortisol played a role in the decreased nest success probability of cortisol-treated fish in Chapter 2. Body size and brood size are important factors in smallmouth bass parental care (Ridgway 1989; Ridgway 1991; Mackereth et al. 1999; Suski and Ridgway 2007). The interactions of body size and brood size with cortisol level were explored in Chapter 2. Although none of the relevant interaction terms had a significant effect on smallmouth bass nest success, there was a tendency for larger cortisol-treated fish guarding large broods to exhibit lower nest success probabilities compared to small fish (Chapter 2). Many physiological factors have been found to scale with body size in teleost fish (Kieffer et al. 1996; Kieffer and Tufts 1998; Wakefield et al. 2004; Glazier 2005; Davies and Moyes 2007; Salze et al. 2014). Despite numerous studies incorporating body size in experimental design, there is no evidence of a size-based stress response (at least among adults), so it is unlikely that elevated cortisol is interacting directly with body size; multiple factors acting together are likely responsible. Drawing from the literature, elevated cortisol, implicated in energy mobilisation (Wendelaar Bonga 1997), combined with larger fish incurring greater swimming penalties (Ohlberger et al. 2005) could contribute to a more rapid decline in body condition compared to their smaller counterparts, leading larger fish to more readily opt to discontinue parental care (for that breeding season) in favour of survival (Wingfield et al. 1998).

Swimming behaviours are fundamental in parental care because they define the ability of the nesting male to tend, monitor, and defend the brood and nest area. In Chapter 2, cortisol did not have an effect on aggression or nest-tending behaviours of nesting smallmouth bass. However, these behaviours were examined through a narrow time window (i.e., upwards of 3-5 minutes spent at each nest, assessed visually by divers). In Chapter 3, I examined parental care behaviours in response to elevated cortisol at a fine scale and for a much longer duration using tri-axial accelerometer biologgers. Contrary to the results of Chapter 2, the results of Chapter 3 suggest that cortisol has an effect on parental care behaviours, particularly those involved in actively defending the brood, as the cortisol treated fish performed fewer burst and routine swims and spent more time resting than control fish. Elevated cortisol may change behaviour choices rather than affect swimming capacity, as other research suggests that cortisol does not affect swimming performance (Gregory and Wood 1999; Liew et al. 2013). The results from Chapter 2 and Chapter 3 differ in their conclusions in terms of the effects of cortisol on parental care behaviour, demonstrating the importance of temporal scale when assessing behaviour. Due to the finer scale analysis, I have more confidence in the results from Chapter 3, suggesting that elevated cortisol can have an influence on parental care behaviour. The Chapter 2 methodologies in assessing aggression and nest-tending behaviours are based on previous research by O'Connor et al. (2009) and Dey et al. (2010), assessing the behaviours on the 5th day after receiving cortisol treatment. These studies found that experimentally elevated cortisol did not affect parental care behaviours. Chapter 3 found differences in parental care swimming behaviours in cortisol-treated fish relative to controls, but had converged with control fish by the end of the study, suggesting that the five-day time window used to assess parental care behaviours in O'Connor et al. (2009), Dey et al. (2010), and Chapter 2 was too long to detect differences.

Corresponding to the swimming behaviour results, locomotor activity declined in cortisol-treated fish compared to control fish (Chapter 3). I did not calibrate accelerometer outputs to metabolic rate in a swim tunnel, enabling energy expenditure estimation. Removal of fish from the nests to calibrate the loggers would have led to nest failure through depredation. However, overall dynamic body acceleration (ODBA), used to quantify locomotor activity level, is strongly correlated with metabolic rates in fish (Gleiss et al. 2011; Wright et al. 2014), so the lower locomotor activity level is likely indicative of lower levels of energy expenditure in the cortisol-treated fish. Elevated cortisol is linked to energy mobilisation in some teleost species, even when resting (Liew et al. 2013). Parental care is energetically costly for fish (Cooke et al. 2002), contributing to decreased body condition (Gillooly and Baylis 1999; Mackereth et al. 1999). Taken together, the decreased parental care swimming behaviours and locomotor activity (Chapter 3) and decreased reproductive success (Chapter 2) may be attributed to depleted energetic resources in cortisol-treated fish. Nesting smallmouth bass may be reducing parental investment (through reduced swimming behaviours, increased nest abandonment) (Wingfield et al. 1998) in response to elevated cortisol, as it may induce additional energetic demands during an already challenging period.

Other factors independent of cortisol treatment were found to have an effect on smallmouth bass nest success and parental care behaviours. In Chapter 2, males guarding small broods spent more time within 1 m of the nest (i.e., exhibited a higher tending score) compared to those guarding large broods. Brood size is known to have implications for parental investment, typically investment increases with an increasing brood size (Ridgway 1989; Zuckerman et al. 2014), but responses to brood size manipulations are variable. Steinhart and Lunn (2011) found no effect of brood size reduction on smallmouth bass nest success in some aquatic systems.

Temporal factors, diel period and study hour, affected locomotor activity and swimming behaviours in Chapter 3.

Limitations and Future Perspectives

The research in this thesis has some notable shortcomings. In Chapter 2, there is no control group where eggs are moved but there is no change in brood size. This would have strengthened the experimental design, providing a baseline of parental care behaviour (nest-tending and aggression) and nest success to compare to, rather than comparing large/small brood size response variables among relative to each other. Having brood size manipulation-neutral controls in Chapter 2 would allow me to determine if nest success or behavioural responses were attributable to brood size manipulations or due to other factors. Another limitation in Chapter 2 and 3 is the lack of a sham injection treatment, which may have shown any effects of just the injection and cocoa butter implant. For example, perhaps the cocoa butter implant impedes swimming to a degree. Small sample sizes are a limitation in both Chapter 2 and 3. A large number of smallmouth bass were sampled in Chapter 2 for testing main effects ($N = 93$). However, due to the complex experimental design in Chapter 2, large sample sizes of each combination of interaction terms (among body size, brood size, and cortisol level) were difficult to obtain, reducing statistical power. Similarly, in Chapter 3 the cortisol treatment groups suffered from small sample sizes. Nonetheless, I think the results provide valuable insights, and suggest further investigation into the role elevated cortisol has in reproduction is warranted.

Research experimentally elevating GCs in fishes has largely focussed on cortisol (Sopinka et al. 2015). Exogenously elevated cortisol mimics some aspects of the stress response, but does not account for the complete neuroendocrine cascade. Exploring the role of hormones and steroids upstream of cortisol would serve to improve understanding of other stress hormones

implicated in the stress response. Comparatively less research has focussed on other hormones and steroids upstream of cortisol in the stress response pathway. Corticotropin releasing hormone (CRH) has been demonstrated to affect locomotor activity (Clements et al. 2002) and movement (Clements and Schreck 2004) in fish. Using tri-axial accelerometers biologgers and experimental manipulations (as in Chapter 3), CRH may be a good candidate for researching interactions of stress and parental investment.

Chapter 2 results indicated decreased nest success probability in cortisol treated fish, but no effects of cortisol treatment on parental care behaviours were found, which I thought was peculiar. The results from Chapter 3 indicated changes in swimming behaviours and locomotor activity level, likely attributable to elevated cortisol, but reproductive success was not tracked. Future research could explore the links between elevated cortisol, parental care behaviours (via swimming behaviours) and reproductive success; such a study would potentially indicate which behaviours are important for nest success, how frequently they are being performed, and the energetic demands of those behaviours. This could be accomplished using cortisol treatment and tri-axial accelerometers (as in Chapter 3), combined with tracking reproductive success. Incorporating other factors such as body size and brood size (as in Chapter 2) would be possible, though achieving statistically relevant sample sizes may be difficult given the complex design; another model species may be better suited.

Tri-axial accelerometers were an effective tool for quantifying locomotor activity and swimming behaviours, but some limitations were evident in my approach. When developing the trainer data set for the classification tree, the time differences between the diver's visual observations of swimming behaviours and the accelerometer outputs caused me to lose confidence and exclude some of the visually observed behaviours. To permit multiple viewings

and reduce ambiguity, capturing behaviours on digital video via action cameras (Struthers et al. 2015) would prove beneficial.

My original intention was to include fanning, foraging, and nest checks in the behaviour analyses. Due to the placement of the accelerometer on the fish (below soft dorsal fin) and because smallmouth bass primarily use their pectoral fins for fanning, I was unable to reliably differentiate fanning from resting, so it was excluded from analyses. Foraging events and nest checks are primarily reflected on the pitch (Y) axis. With slight variation in accelerometer placement on each fish, I was not confident in the pitch axis criteria generated by the classification tree algorithms; specifically, I was concerned that the classification tree criteria would over- or underestimate foraging and nest checks on unobserved fish. Due to the low locomotor level involved in foraging and nest checks, so I considered them as resting behaviour. Different accelerometer placement on the fish or higher sample frequency (more samples per second) may have enabled me to characterize fanning, foraging, and nest check behaviours.

Locomotor activity was quantified in Chapter 3 using ODBA, but energetics were not estimated. Parental care energetic have been studied using electromyogram activity monitors (Cooke et al. 2002), and estimating energetics using tri-axial accelerometers has successfully been conducted on other teleost species (e.g., bonefish, *Albula spp*; Brownscombe et al. 2014). Future research on energetic implications of elevated GCs on parental care swimming behaviours could be conducted using tri-axial accelerometers and experimental GC manipulation (as in Chapter 3) by calibrating accelerometers on fish in a swim tunnel. Rather than parental males, non-reproductive conspecifics could be used to calibrate accelerometers in swim tunnel trials, eliminating the time away from nest for parental males.

Conclusion

My thesis contributes to the growing body of research focused on understanding how elevated cortisol can impact reproduction in wild animals. The results illustrate the complex interaction between elevated cortisol and parental investment, demonstrated through altered swimming behaviours, locomotor activity level, and decreased reproductive success, which may have fitness implications. In species exhibiting parental care, demonstrating the appropriate responses to stress will be imperative, especially in the face of increasing anthropogenic environmental disturbance and alteration.

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