Parental care in a stressful world: experimentally elevated cortisol and brood size manipulation influence nest success probability and nest-tending behavior in a wild teleost fish.

Keywords: glucocorticoid; cortisol; nest success; aggression; nest-tending; teleost; smallmouth bass

Running Head: Parental care in a stressful world

What is already known:
- Glucocorticoids interact with reproduction such that when cortisol, the primary stress hormone in fish, is chronically elevated, reproductive success is impaired.
- Large fish typically have higher nest success than smaller fish due to several metabolic and sexually-selected advantages.
- Parental fish with a large brood typically provide higher levels of parental investment.

What this study adds:
- First time using experimentally elevated cortisol to examine the interactive effects of body size, brood size, and glucocorticoids on reproductive success and parental care behaviors in wild fish.
- Larger fish with a large brood exhibited lower nest success than smaller fish when cortisol levels were elevated.
ABSTRACT

Parental care is an advantageous reproductive behavior 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 metabolism and body maintenance functions, when glucocorticoid hormones (e.g., cortisol) are chronically elevated, as can occur during stress, fish may experience impairments in behavior 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), we 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 behavior, independent of cortisol level and body size. Lending partial support to our hypothesis, the results of this study indicate that guarding male smallmouth bass reproductive success is influenced by cortisol level, and tending behavior is affected by brood size.
INTRODUCTION

Many taxa focus their reproductive efforts into a single reproductive period each year, exhibiting specialized behaviors intended to maximize reproductive success. Parental care, defined here as the post-fertilization behaviors undertaken to care for offspring, 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 (Trivers 1972; Williams 1966). 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 behavior, physiology and life-history (Zera and Harshman 2001).

Parental care behaviors occur in a variety of teleost fishes (Blumer 1982), encompassing a diversity of parental care forms (Goodwin et al. 1998), thus making fish ideal models to study parental care (Amundsen 2003). Teleost parental care can be broadly categorized into nest-tending (e.g., fanning eggs, removing debris from the nest) and aggression (e.g., brood defence) behaviors.

The parental care period is a challenging time for parent(s) and 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, 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 affect parental care behaviors, energy expenditure (Gravel and Cooke 2009; Steinhart et al. 2004) 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. The littoral zone is often subject to habitat degradation from shoreline alterations (Wagner et al. 2006) and noise from boats (Graham and Cooke 2009). Additionally, black bass are the most popular sportfish in North America, and are subjected to intense angling pressure (Quinn and Paukert 2009). Because of their heightened aggression during parental care, nesting bass are vulnerable to angling (Kieffer et al. 1995; Philipp et al. 1997; Suski and Philipp 2004). Even short absences from the nest can lead to brood depredation and thus nest 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. 2000; Suski et al. 2003). Clearly the parental care period 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 parental body size. 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 associated with parental care (Gillooly and Baylis
Body size has a positive relationship with brood size, another important factor in black bass reproduction. 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 is lower relative to a reduced brood size (Ridgway 1989). Studies decreasing the brood size of nesting male black bass, 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, 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. 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 modulates parental care behaviors and reproductive success. 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. For the purposes of this study, stress is defined as a rise in circulating stress
hormones (i.e., cortisol). Once validated in controlled experiments, exogenous glucocorticoid (GC) manipulations can be a powerful tool in assessing the effects of stressors and life history variations in wild fishes (Crespi et al. 2013; Sopinka et al. 2015). 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), we conducted a study to determine the influence of body size and brood size on parental care behaviors and reproductive success, and the extent to which outcomes varied between control and cortisol-manipulated fish. We 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., and their stress response, parental care behaviors, and nest success are well characterized in the literature (Ridgway 1988; Hanson et al. 2009b; O’Connor et al. 2009; Dey et al. 2010). We hypothesized that parental body size, brood size and cortisol level (i.e., experimental cortisol elevation relative to controls), influence parental care behaviors and reproductive success in nesting males. We predicted that the interaction of a large body size and brood size will produce the highest reproductive success and parental investment (in terms of aggression and tending behaviors) 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, court and mate with a female, 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.

Parental Care Behaviors and Reproductive Success

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, focusing on large and small body sizes and egg scores. Although we 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). Nests selected for the study were randomly distributed in similar habitats (rocky substrate) in 0.5 to 1.5 m water depth.

Behavioral assessments were designed to test a guarding male’s nest tending and aggression behaviors. Tending was assessed first, followed by aggression. To assess the tending and vigilance, 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 behavioral 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 administered a cortisol treatment if applicable. The angling fight time was minimized (< 20 s) in order to limit stress from capture and exhaustive exercise. No anesthesia was needed to sedate the fish while in the trough given that they were calm when in supine position.
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. Hydrocortisone 21-hemisuccinate is commonly used to experimentally elevate cortisol levels and is known to produce post receptor effects comparable to endogenously produced cortisol in teleost fishes (Pickford et al. 1970; Chan and Woo 1978; Foster and Moon 1986, Kiilerich et al. 2007).

Exogenous cortisol manipulation used in this study 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). Mean post-treatment cortisol levels are expected to range from 750-2250 ng mL\(^{-1}\) in black bass (O’Connor et al. 2009, Dey et al. 2010). For context, cortisol levels of control black bass engaged in parental care are typically <50 ng mL\(^{-1}\) (Dey et al. 2010). No sham treatment (injection containing only cocoa butter) was used in the experimental design 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 \(\log_{10} \text{mass} = -7.1004 \times 3.884(\log_{10} \text{TL})\) 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 behavioral 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, we 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 behavioral 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 our 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 behavior 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.

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 1). No relationship was evident between nest success and any of the other tested explanatory variables (Table 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 1).

Aggression and Tending Scores

No relationships were found between aggression score and the tested explanatory variables (Table 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). Coefficients for cortisol treatment, fish size, and their interactions were not significant in the model for tending score (Table 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 behaviors. As predicted, cortisol treatment had an overall negative effect on nest success (Table 1; Figure 1). In each lake, males guarding a large brood size exhibited decreased nest success with increasing body size (Figure 1), though the tested interaction term between cortisol treatment and body size was not significant (Table 1). Tending score was significantly affected by brood size (Table 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 1; Figure 2). Despite our prediction, no relationships were found between the tested explanatory variables and aggression score (Table 1).

Cortisol-treated fish, particularly larger fish guarding a large brood, tended to exhibit decreased nest success compared to large control fish (Table 1; Figure 1), consistent with other studies (O’Connor et al. 2009; Dey et al. 2010). Our results suggest that GCs such as cortisol interact with reproduction, contributing to nest abandonment decisions to forego current reproductive efforts in favour of self-preservation (Wingfield et al. 1998). Similar to other research exploring the influence of elevated cortisol on black bass parental care (O’Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016), cortisol-treated fish in this study continued to guard their nests beyond the expected elevated cortisol duration (i.e., cortisol elevated for 5-6 days, Gamperl et al. 1994; O’Connor et al. 2009), suggesting that tertiary effects from elevated cortisol or other factors not measured in the present study may be responsible for nest abandonment. We surmise that declining physiological condition is likely contributing to nest abandonment.
decisions. Though not explicitly measured here, cortisol-treated black bass have been found to exhibit plasma hyperglycemia and hypercholesterolaemia (O’Connor et al. 2009; Dey et al. 2010; Zolderdo et al. 2016), indicating that smallmouth bass in this study may be experiencing elevated metabolic activity from cortisol treatment. Black bass are capital breeders and an increase in metabolic activity may lead to decreased body condition during this already energetically demanding period. Other indicators of degraded physiological condition from elevated cortisol include observations of altered immunocompetence through decreased lymphocyte levels (Zolderdo et al. 2016) and significantly higher Saprolegnia infections (O’Connor et al. 2009) in black bass. Zolderdo et al. (2016) also found increased reactive oxygen species (8-OHdG) present in cortisol treated fish relative to controls, indicating signs of oxidative stress in response to elevated cortisol. Nest success probability decreased as fish body size increased in cortisol treated fish guarding a large brood (Figure 1). Though not statistically significant, this may indicate that cortisol can disproportionately influence nest success in large fish relative to small fish. Large black bass have been shown to take longer to recover from an exercise induced disturbance (Gingerich and Suski 2012) and have higher endogenous energy reserves at the start of the spawning season (Wiegmann et al. 1997), and thus may experience relatively greater declines in body condition. Furthermore, small body-sized black bass use a different spawning strategy than large black bass to produce a successful brood, which may be advantageous in mediating potential declines in physiological condition. Small black bass tend to begin nesting later in the reproductive season compared to large fish (Wiegmann et al. 1997) and may feed more frequently during parental care to supplement their comparative lack of energy reserves (Hinch and Collins 1991). Additionally, eggs develop more quickly in warmer water (i.e., towards the latter half of spawning season), providing them the opportunity to successfully
raise a brood in a comparatively shorter time frame and presumably using less energy than large fish. This may explain the tendency for decreased nest success in cortisol treated large fish relative to cortisol treated small fish in this study. The expected exogenously elevated cortisol levels of fish in this study mimics 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 is initiated after sensory perception of a stressor and 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 behaviors investigated in this study, namely aggression and tending behaviors, are ubiquitous in centrarchid parental care (Cooke et al. 2006). None of the tested factors had an effect on nest guarding male aggression (Table 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 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). This was an unexpected result, as 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 than those with a small initial brood size. In this study, fish with a small brood originally had a large brood (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 fish guarding large broods, fish with a small brood did not exhibit a higher probability of nest success (Table 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. 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 our 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 behaviors 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 behavior (including decisions to abandon a developing brood or forego reproduction), reproductive success and fitness in wild animals.
REFERENCES


Figure Captions

Figure 1 – Probability (± 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 – Proportion (± 95% CI) of time nest guarding male smallmouth bass spent within 1 m of the nest (i.e., tending behavior) 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.
<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed Effects</th>
<th>Estimate (±SE)</th>
<th>Z-value</th>
<th>P-value</th>
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<td></td>
<td><strong>Cortisol (NC)</strong></td>
<td><strong>1.57 (0.74)</strong></td>
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<td></td>
<td>Cortisol (NC)</td>
<td>0.62 (0.51)</td>
<td>1.209</td>
<td>0.227</td>
</tr>
<tr>
<td></td>
<td><strong>Brood Size (Small)</strong></td>
<td><strong>1.01 (0.51)</strong></td>
<td><strong>1.994</strong></td>
<td><strong>0.046</strong></td>
</tr>
<tr>
<td></td>
<td>TL.std</td>
<td>-0.35 (0.27)</td>
<td>-1.275</td>
<td>0.202</td>
</tr>
<tr>
<td></td>
<td>Lake (Charleston)</td>
<td>0.35 (0.38)</td>
<td>0.926</td>
<td>0.355</td>
</tr>
<tr>
<td></td>
<td>Lake (Sand)</td>
<td>0.11 (0.75)</td>
<td>0.154</td>
<td>0.878</td>
</tr>
<tr>
<td></td>
<td>Cortisol (NC) x Brood Size (Small)</td>
<td>-1.04 (0.71)</td>
<td>-1.461</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>Cortisol (NC) x TL.std</td>
<td>-0.22 (0.35)</td>
<td>-0.629</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>Brood Size (Small) x TL.std</td>
<td>0.16 (0.35)</td>
<td>0.454</td>
<td>0.650</td>
</tr>
</tbody>
</table>
Figure 1

(a) Cortisol vs. No Cortisol
(b) Cortisol vs. No Cortisol
(c) Cortisol vs. No Cortisol

Probability of success ± 95% CI over TL (mm)

Legend:
- Upper Bound
- Lower Bound
- Small Bound
- Big Bound
fig 1

A

Cortisol

No Cortisol

Large Brood

Small Brood

Probability of success +/- 95% CI

TL (mm)

250 300 350 400 450 500

B

Cortisol

No Cortisol

Large Brood

Small Brood

Probability of success +/- 95% CI

TL (mm)

250 300 350 400 450 500

C

Cortisol

No Cortisol

Large Brood

Small Brood

Probability of success +/- 95% CI

TL (mm)

250 300 350 400 450 500
Figure 2: The graphs illustrate the proportion of time spent within 1 m of the nest (bending behavior) for different TL (mm) values comparing cortisol (Cortisol) and no cortisol (No Cortisol) conditions. The graphs are divided into subplots labeled A, B, and C, each representing different nest sizes (Large Brood and Small Brood). The shaded areas indicate 95% CI.