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

1999). 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

115 hormones (i.e., cortisol). Once validated in controlled experiments, exogenous glucocorticoid
116 (GC) manipulations can be a powerful tool in assessing the effects of stressors and life history
117 variations in wild fishes (Crespi et al. 2013; Sopinka et al. 2015). Using exogenous cortisol
118 manipulations, O'Connor et al. (2009) experimentally raised cortisol titers in fish to further
119 challenge nesting male largemouth bass. Relative to controls the cortisol-manipulated fish
120 exhibited greater declines in physiological status, showed evidence of immune function
121 impairment, and had higher rates of nest abandonment. However, the authors used a narrow
122 range of fish sizes.

123 Building on the approach used by O'Connor et al. (2009), we conducted a study to
124 determine the influence of body size and brood size on parental care behaviors and reproductive
125 success, and the extent to which outcomes varied between control and cortisol-manipulated fish.
126 We selected smallmouth bass (*Micropterus dolomieu*) as a model species because they maintain
127 a presence on their nest for several weeks, allowing ample time for experimentation,, and their
128 stress response, parental care behaviors, and nest success are well characterized in the literature
129 (Ridgway 1988; Hanson et al. 2009b; O'Connor et al. 2009; Dey et al. 2010). We hypothesized
130 that parental body size, brood size and cortisol level (i.e., experimental cortisol elevation relative
131 to controls), influence parental care behaviors and reproductive success in nesting males. We
132 predicted that the interaction of a large body size and brood size will produce the highest
133 reproductive success and parental investment (in terms of aggression and tending behaviors)
134 given the past reproductive experience of larger (older) fish, the energetic advantages afforded
135 from a large body size, and the potential reproductive value of a large brood. Furthermore, larger
136 fish have comparatively less future reproductive events than smaller (younger) fish, presumably
137 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, focussing 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

161 based on extensive aging work on a nearby system (i.e., O'Connor et al. 2012). A large brood
162 size was a nest having an egg score of 4 and 5, whereas an egg score of 1 or 2 was considered a
163 small brood (Kubacki 1992). Nests selected for the study were randomly distributed in similar
164 habitats (rocky substrate) in 0.5 to 1.5 m water depth.

165 Behavioral assessments were designed to test a guarding male's nest tending and
166 aggression behaviors. Tending was assessed first, followed by aggression. To assess the tending
167 and vigilance, a tending score was developed where each nest guarding male was visually
168 observed by a diver situated > 3 meters from the nest, limiting disturbance to the guarding male.
169 After a 60 s acclimation period, the diver recorded how many times the male was within 1 meter
170 of his nest on 20 s increments for a total of 3 minutes [giving a total possible score of 9; as per
171 Gravel and Cooke (2009)]. An aggression score was developed to test the nest guarding male's
172 defensive aggression towards a common brood predator. Aggression score was assessed using a
173 bluegill sunfish (*Lepomis machrochirus*) between 130-140 mm total length (TL) contained in a 4
174 L glass jar placed directly adjacent to the nest (Hanson et al. 2009b). After an acclimation period
175 of 30 s, a diver situated > 3 meters from the nest observed the engagement of the male with the
176 brood predator, recording the duration of direct jar contact (in seconds), number of strikes to the
177 jar, mouth flares, and charges at the jar for a total of 60 seconds (Hanson et al. 2009b). After the
178 behavioral assessments, experimental fish were angled from the nest using rod and reel, and
179 placed into a foam-lined trough containing fresh lake water for TL measurement (all fish were
180 angled and measured) and administered a cortisol treatment if applicable. The angling fight time
181 was minimized (< 20 s) in order to limit stress from capture and exhaustive exercise. No
182 anesthesia was needed to sedate the fish while in the trough given that they were calm when in
183 supine position.

184 Cortisol treated fish received 10 mg kg^{-1} of cortisol (Hydrocortisone 21-hemisuccinate,
185 Sigma Aldrich Corp.) suspended in a cocoa butter vehicle via intraperitoneal injection with a 16-
186 gauge needle. Fish were injected with 0.005 mL per gram of fish body weight. Hydrocortisone
187 21-hemisuccinate is commonly used to experimentally elevate cortisol levels and is known to
188 produce post receptor effects comparable to endogenously produced cortisol in teleost fishes
189 (Pickford et al. 1970; Chan and Woo 1978; Foster and Moon 1986, Kiilerich et al. 2007).
190 Exogenous cortisol manipulation used in this study is a validated (for the same species, in the
191 same watershed, at the same temperatures, using the same field methods and lab assays, by the
192 same research group) method for elevating cortisol levels in smallmouth bass for 5-6 days
193 (Gamperl et al. 1994; O'Connor et al. 2009; Dey et al. 2010). Mean post-treatment cortisol levels
194 are expected to range from 750-2250 ng mL^{-1} in black bass (O'Connor et al. 2009, Dey et al.
195 2010). For context, cortisol levels of control black bass engaged in parental care are typically
196 <50 ng mL^{-1} (Dey et al. 2010). No sham treatment (injection containing only cocoa butter) was
197 used in the experimental design due to inconsistent cortisol responses of sham treated fish (see
198 DiBattista et al. 2005). Though mass was not directly measured, a log_{10} transformed length-
199 weight relationship equation from smallmouth bass in Opinicon Lake, a lake within the same
200 system, was used to calculate fish weight from the TL measurements of experimental fish
201 receiving cortisol treatment (Dey et al. 2010); the equation was log_{10} mass = -7.1004 x
202 3.884(log_{10} TL) with mass reported in grams and TL in millimeters. Time away from the nest
203 was minimized; angling and cortisol administration took less than 120 s, and fish were released
204 within 5 m of their nest post-treatment. Throughout the treatment process a diver guarded the
205 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

291 decisions. Though not explicitly measured here, cortisol-treated black bass have been found to
292 exhibit plasma hyperglycemia and hypercholesterolaemia (O'Connor et al. 2009; Dey et al.
293 2010; Zolderdo et al. 2016), indicating that smallmouth bass in this study may be experiencing
294 elevated metabolic activity from cortisol treatment. Black bass are capital breeders and an
295 increase in metabolic activity may lead to decreased body condition during this already
296 energetically demanding period. Other indicators of degraded physiological condition from
297 elevated cortisol include observations of altered immunocompetence through decreased
298 lymphocyte levels (Zolderdo et al. 2016) and significantly higher *Saprolegnian* infections
299 (O'Connor et al. 2009) in black bass. Zolderdo et al. (2016) also found increased reactive oxygen
300 species (8-OHdG) present in cortisol treated fish relative to controls, indicating signs of
301 oxidative stress in response to elevated cortisol. Nest success probability decreased as fish body
302 size increased in cortisol treated fish guarding a large brood (Figure 1). Though not statistically
303 significant, this may indicate that cortisol can disproportionately influence nest success in large
304 fish relative to small fish. Large black bass have been shown to take longer to recover from an
305 exercise induced disturbance (Gingerich and Suski 2012) and have higher endogenous energy
306 reserves at the start of the spawning season (Wiegmann et al. 1997), and thus may experience
307 relatively greater declines in body condition. Furthermore, small body-sized black bass use a
308 different spawning strategy than large black bass to produce a successful brood, which may be
309 advantageous in mediating potential declines in physiological condition. Small black bass tend to
310 begin nesting later in the reproductive season compared to large fish (Wiegmann et al. 1997) and
311 may feed more frequently during parental care to supplement their comparative lack of energy
312 reserves (Hinch and Collins 1991). Additionally, eggs develop more quickly in warmer water
313 (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

337 was experimentally reduced to a much smaller brood size of an egg score of ≤ 2. Interestingly,
338 despite increased parental investment (i.e., nest-tending) relative to fish guarding large broods,
339 fish with a small brood did not exhibit a higher probability of nest success (Table 1). The
340 reproductive value of the remaining brood is also an important factor in black bass nest
341 abandonment decisions (Zuckerman et al. 2014). Individuals may value broods differently
342 according to environmental thresholds. Predator burden can be high in the lakes used in this
343 study (Gravel and Cooke 2009), and fish residing in higher latitude lakes, such as those in
344 southeastern Ontario, can have comparatively limited future reproductive capacities (Ridgway
345 1989; Shaw and Allen 2014). Moreover, cumulative investment and physiological thresholds,
346 such as energy availability and body condition, are central in parental investment decisions
347 (Trivers 1972; Wingfield and Sapolsky 2003). Fish in this study may be placing a high value on
348 what brood remains, and consequently are increasing parental investment (i.e., nest-tending in
349 this study) to ensure some reproductive success, attempting to maximize past investment,
350 regardless of the energetic demands of parental care and the lower reproductive value of a
351 smaller brood.

352 Teleost fish exhibiting parental care show much variation in their response to natural and
353 experimental brood reductions. Reductions in black bass brood size are shown to lead to
354 decreased parental investment (Ridgway 1989). Repudiating our prediction and what is prevalent
355 in the literature, the results of this study show that small brood sizes could receive higher
356 parental investment than larger ones. This study further highlights the complexity in parental
357 investment decisions, how they are individual-specific, and are likely due to relative energetic
358 cost and storage (Stearns 1989). The growing body of research exploring the interaction between
359 reproduction and stress have demonstrated varied results (Sopinka et al. 2015), indicating that

360 parental care trade-offs and stress is highly complex and remains poorly understood. Increasing
361 shoreline development and many human activities are a source of environmental disturbances
362 such as chemical, light, and noise pollution (Graham and Cooke 2009; Brüning et al. 2015),
363 translating to increased human-induced stressors in the littoral zone (Wagner et al. 2006). In
364 addition, given that black bass are the most popular sportfish in North America, angling-induced
365 stress before or during the parental care period is common (Philipp et al. 1997; Suski et al. 2003).
366 It stands to reason that the increasing presence of human-induced stressors in aquatic ecosystems
367 will further magnify the stress associated with parental care in this already challenging period.
368 Thus, understanding how reproductive success and parental care behaviors interact with
369 increased GCs (i.e., stress) and life history traits is imperative. Studies such as this one that
370 experimentally manipulate GC levels have the potential to reveal how anthropogenic stressors
371 will mediate parental care behavior (including decisions to abandon a developing brood or
372 forego reproduction), reproductive success and fitness in wild animals.
373

374 **REFERENCES**

375 Amundsen T. 2003. Fishes as models in studies of sexual selection and parental care. J Fish Biol
376 63:17–52.

377 Bates D., M. Maechler, B. Bolker, and S. Walker. 2014. _lme4: Linear mixed-effects models
378 using Eigen and S4_. R package version 1.1-7.

379 Blumer L.S. 1982. A bibliography and categorization of bony fishes exhibiting parental care.
380 Zool J Linn Soc 76:1–22.

381 Bolker B.M., M.E., Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and
382 J.S.S.White. 2009. Generalized linear mixed models: a practical guide for ecology and
383 evolution. Trends Ecol Evol 24, 127–135.

384 Brüning A., F. Hölker, S. Franke, T. Preuer, and W. Kloas. 2015. Light pollution affects
385 circadian rhythms of European perch but does not cause stress. Sci Total Environ 516–522.

386 Chan D.K.O. and N.Y.S. Woo. 1986. Effect of cortisol on the metabolism of the eel, Anguilla
387 japonica. Gen Comp Endocrin 35:205-215.

388 Clutton-Brock T.H., 1991. The evolution of parental care. Princeton University Press.

389 Cooke, S.J., D.P. Philipp, J.F. Schreer, and R.S. McKinley. 2000. Locomotory impairment of
390 nesting male largemouth bass following catch-and-release angling. N Am J Fish Manage
391 20:968-977.

392 Cooke S.J., D.P. Philipp, D.H. Wahl, and P.J. Weatherhead. 2006. Energetics of parental care in
393 six syntopic centrarchid fishes. Oecologia 148:235–249.

394 Cooke S.J., P.J. Weatherhead, D.H. Wahl, and D.P. Philipp. 2008. Parental care in response to
395 natural variation in nest predation pressure in six sunfish (Centrarchidae: Teleostei) species.
396 Ecol Freshw Fish 17: 628–638.

397 Crespi E.J., T.D. Williams, T.S. Jessop, and B. Delehanty. 2013. Life history and the ecology of
398 stress: how do glucocorticoid hormones influence life-history variation in animals? Funct
399 Ecol 27:93–106.

400 Crossin G.T., O.P. Love, S.J. Cooke, and T.D. Williams. 2016. Glucocorticoid manipulations in
401 free-living animals: considerations of dose delivery, life-history context, and reproductive
402 state. Funct Ecol 30:116-125.

403 Curio E. 1983. Why do young birds reproduce less well? Ibis (Lond 1859) 125:400–404.

404 Dey C.J., C.M. O'Connor, K.M. Gilmour, G. Van Der Kraak, S.J. Cooke, and G. Van Der Kraak.
405 2010. Behavioral and physiological responses of a wild teleost fish to cortisol and androgen
406 manipulation during parental care. Horm Behav 58:599–605.

407 DiBattista J.D., H. Anisman, M. Whitehead, and K.M. Gilmour. 2005. The effects of cortisol
408 administration on social status and brain monoaminergic activity in rainbow trout
409 Oncorhynchus mykiss. J Exp Biol 208:2707–2718.

410 Donaldson E.M., 1981. The pituitary-interrenal axis as an indicator of stress in fish, in:
411 Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London/New York, pp. 11-47.

412 Foster G.D. and T.W. Moon. 1986. Cortisol and liver metabolism of immature American eels,
413 Anguilla rostrate (LeSueur). Fish Physiol Biochem 1:113-124.

414 Gamperl A.K., M.M. Vijayan, and R.G. Boutilier. 1994. Experimental control of stress hormone
415 levels in fishes: techniques and applications. Rev Fish Biol Fish 4:215–255.

416 Gillooly J.F. and J.R. Baylis. 1999. Reproductive success and the energetic cost of parental care
417 in male smallmouth bass. J Fish Biol 54:573–584.
418 Gingerich A.J. and C.D. Suski. 2011. The role of progeny quality and male size in the nesting
419 success of smallmouth bass: Integrating field and laboratory studies. Aquat Ecol 45:505–515.
420 Gingerich A.J. and C.D. Suski. 2012. The effect of body size on post-exercise physiology in
421 largemouth bass. Fish Physiol Biochem. 38:329-340.
422 Goff G.P. 1985. Environmental influences on annual variation in nest success of smallmouth
423 bass, Micropterus dolomieui, in Long Point Bay, Lake Erie. Environ Biol Fishes 14:303–307.
424 Goodwin N., S. Balshine-Earn, and J.D. Reynolds. 1998. Evolutionary transitions in parental
425 care in cichlid fish. Proc Royal Soc Lond B 265:2265–2272.
426 Graham A.L. and S.J. Cooke. 2009. The effects of noise disturbance from various recreational
427 boating activities common to inland waters on the cardiac physiology of a freshwater fish, the
428 largemouth bass (Micropterus salmoides). Aquat Conserv 18:1315–1324.
429 Gravel M.A. and S.J. Cooke. 2009. Influence of inter-lake variation in natural nest predation
430 pressure on the parental care behaviour of smallmouth bass (Micropterus dolomieu). Ethology
431 115:608–616.
432 Hanson K.C., A. Abizaid, and S.J. Cooke. 2009a. Causes and consequences of voluntary
433 anorexia during the parental care period of wild male smallmouth bass (Micropterus
434 dolomieu). Horm Behav 56:503–509.
435 Hanson K.C., S.J. Cooke, C.D. Suski, and D.P. Philipp. 2007. Effects of different angling
436 practices on post-release behaviour of nest-guarding male black bass, Micropterus spp . Fish
437 Manag Ecol 141–148.

438 Hanson K.C., C.M. O'Connor, G. Van Der Kraak, and S.J. Cooke. 2009b. Paternal aggression
439 towards a brood predator during parental care in wild smallmouth bass is not correlated with
440 circulating testosterone and cortisol concentrations. Horm Behav 55:495–499.
441 Hinch S. and N. Collins. 1991. Importance of diurnal and nocturnal nest defense in the energy
442 budget of male smallmouth bass: insights from direct video observations. Trans Am Fish Soc
443 120:37–41.
444 Kieffer J.D., M.F. Kubacki, F.J.S. Phelan, D.P. Philipp, and B.L. Tufts. 1995. Effects of catch-
445 and-release angling on nesting male smallmouth bass. Trans Am Fish Soc 124:70–76.
446 Kiilerich P., K. Kristiansen, and S.S. Madsen. 2007. Cortisol regulation of ion transporter mRNA
447 in Atlantic salmon gill and the effect of salinity on the signaling pathway. J Endocrin
448 194:417-427.
449 Lunn B.D. and G.B. Steinhart. 2010. Effect of brood reduction on nest abandonment of
450 smallmouth bass. Trans Am Fish Soc 139:586–592.
451 Mackereth R.W., D.L.G. Noakes, and M.S. Ridgway. 1999. Size-based variation in somatic
452 energy reserves and parental expenditure by male smallmouth bass, *Micropterus dolomieu*.
453 Environ Biol Fishes 56, 263–275.
454 O'Connor C.M., B.L. Barthel, K.M. Gilmour, D.P. Philipp, G. Van Der Kraak, and S.J. Cooke.
455 2012. Reproductive history and nest environment are correlated with circulating androgen and
456 glucocorticoid concentrations in a parental care-providing teleost fish. Physiol Biochem Zool
457 85:209–18.
458 O'Connor C.M., K.M. Gilmour, R. Arlinghaus, G. Van Der Kraak, and S.J. Cooke. 2009. Stress
459 and parental care in a wild teleost fish: insights from exogenous supraphysiological cortisol
460 implants. Physiol Biochem Zool 82:709–719.

461 O'Connor C.M., K.M. Gilmour, G. Van Der Kraak, and S.J. Cooke. 2011. Circulating androgens
462 are influenced by parental nest defense in a wild teleost fish. J Comp Physiol A 197:711–715.
463 Philipp D.P., C.A. Toline, M.F. Kubacki, D.B.F. Philipp, and F.J.S. Phelan. 1997. The impact of
464 catch-and-release angling on the reproductive success of smallmouth bass and largemouth
465 bass. North Am J Fish Manag 17:557–567.

466 Pickford G.E., P.K.T. Pang, E. Weinstein, J. Torretti, E. Hendler, and F.H. Epstein. 1970. The
467 response of hypophysectomised cyprinodont, Fundulus heteroclitus, to replacement therapy
468 with cortisol: effects on blood serum and sodium-potassium activated adenosine
469 triphosphatase in the gills, kidney, and intestinal mucosa. Gen Comp Phys 14:524-534.

470 Quinn, S. and C. Paukert. 2009. Centrarchid Fisheries. In: Cooke S.J., Philipp D.P. (Eds.),
471 Centrarchid fishes - Diversity, biology, and conservation. John Wiley & Sons, West Sussex,
472 UK, pp. 312-339.

473 R Core Development Team. 2013. R: a language and environment for statistical computing. R
474 Foundation for Statistical Computing, Vienna. http://www.R-project.org/.

475 Ridgway M.S. 1988. Developmental stage of offspring and brood defense in smallmouth bass
476 (Micropterus dolomieu). Can J Zool 66:1722–1728.
477 Ridgway M.S. 1989. The parental response to brood size manipulation in smallmouth bass.
478 Ethology 80:47–54.
479 Ridgway M.S., B.J. Shuter, and E.E. Post. 1991. The relative influence of body size and
480 territorial behaviour on nesting asynchrony in male smallmouth bass, Micropterus dolomieu
481 (Pisces: Centrarchidae). J Anim Ecol 60:665–681.
482 Shaw S.L. and M.S. Allen. 2014. Localized spatial and temporal variation in reproductive effort
483 of Florida Bass. Trans Am Fish Soc 143:85–96.

484 Sopinka N.M., L.D. Patterson, J.C. Redfern, N.K. Pleizier, C.B. Belanger, J.D. Midwood, G.T.
485 Crossin, and S.J. Cooke. 2015. Manipulating glucocorticoids in wild animals: basic and
486 applied perspectives. Conserv Physiol 3(1): cov031 doi:10.1093/conphys/cov031
487 Stearns S. 1989. Trade-offs in life-history evolution. Funct Ecol 3:259–268.
488 Steinhart, G.B. and E.S. Dunlop. 2008. Should I stay or should I go? Optimal parental care
489 decisions of a nest-guarding fish. Evol Ecol Res 10, 351–371.
490 Steinhart G.B., N.J. Leonard, R.A. Stein, and E.A. Marschall. 2005. Effects of storms, angling,
491 and nest predation during angling on smallmouth bass (Micropterus dolomieu) nest success.
492 Can J Fish Aquat Sci 62:2649–2660.
493 Steinhart G.B. and B.D. Lunn. 2011. When and why do smallmouth bass abandon their broods?
494 The effects of brood and parental characteristics. Fish Manag Ecol 18:1–11.
495 Steinhart G.B., M.E. Sandrene, S. Weaver, R.A. Stein, and E.A. Marschall. 2004. Increased
496 parental care cost for nest-guarding fish in a lake with hyperabundant nest predators. Behav
497 Ecol 16:427–434.
498 Suski C.D. and D.P. Philipp. 2004. Factors affecting the vulnerability to angling of nesting male
499 largemouth and smallmouth bass. Trans Am Fish Soc 133:1100–1106.
500 Suski C.D., J.H. Svec, J.B. Ludden, F.J.S. Phelan, and D.P. Philipp. 2003. The effect of catch-
501 and-release angling on the parental care behavior of male smallmouth bass. Trans Am Fish
502 Soc 132:210–218.
503 Trivers, R.L. 1972. Parental investment and sexual selection, in: B. Campbell, (Ed.), Sexual
504 Selection and the Descent of Man. Heinemann, London, pp. 139-179.
505 Wagner T., A.K. Jubar, and M.T. Bremigan. 2006. Can habitat alteration and spring angling
506 explain largemouth bass nest success? Trans Am Fish Soc 135:843–852.

507 Wendelaar Bonga S.E. 1997. The stress response in fish. Physiol Rev 77:591–625.

508 Wiegmann D., J.R. Baylis, and M. Hoff. 1992. Sexual selection and fitness variation in a
509 population of smallmouth bass, Micropterus dolomieui (Pisces: Centrarchidae). Evolution
510 46:1740–1753.

511 Wiegmann D.D. and J.R. Baylis. 1995. Male body size and paternal behaviour in smallmouth
512 bass, Micropterus dolomieui (Pisces: Centrarchidae). Anim Behav 50:1543–1555.

513 Wiegmann, D.D., J.R. Baylis, and M.H. Hoff. 1997. Male fitness, body size and timing of
514 reproduction in smallmouth bass, Micropterus dolomieui. Ecology 78:111-128.

515 Williams G.C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's
516 principle. Am Nat 100:687–690.

517 Wingfield J.C., D.L. Maney, C.W. Breuner, J.D. Jacobs, S. Lynn, M. Ramenofsky, and R.D.
518 Richardson. 1998. Ecological bases of hormone-behavior interactions: the "emergency life
519 history stage." Am Zool 38:191–206.

520 Wingfield J.C. and R.M. Sapolsky. 2003. Reproduction and resistance to stress: when and how. J
521 Neuroendocrinol 15:711–724.

522 Zera A.J. and L.G. Harshman. 2001. The physiology of life history trade-offs in animals. Annu
523 Rev Ecol Syst 32:95–126.

524 Zolderdo A.J., D.A. Algera, M.J. Lawrence, K.M. Gilmour, M.D. Fast, J. Thuswaldner, W.
525 Willmore, and S.J. Cooke. 2016. Stress, nutrition and parental care in a teleost fish: exploring
526 mechanisms with supplemental feeding and cortisol manipulation. J Exp Biol 219:1237-1248.

527 Zuckerman Z.C., D.P. Philipp, and C.D. Suski. 2014. The influence of brood loss on nest
528 abandonment decisions in largemouth bass Micropterus salmoides. J Fish Biol 84:1863–1875.

Zuckerman Z.C. and C.D. Suski. 2013. Predator burden and past investment affect brood abandonment decisions in a parental care-providing teleost. Funct Ecol 27:693–701.

532 **Figure Captions**

533

534 Figure 1 – Probability (± 95% CI) of male smallmouth bass nest success according to body size

535 (TL), brood size, and cortisol treatment in a) Big Rideau Lake b) Charleston Lake, and c) Sand

536 Lake. The vertical facets show large and small brood sizes.

537

538 Figure 2 – Proportion (± 95% CI) of time nest guarding male smallmouth bass spent within 1 m

539 of the nest (i.e., tending behavior) as measured over the course of 3 minutes in a) Big Rideau

540 Lake b) Charleston Lake, and c) Sand Lake. The vertical facets show large and small brood

541 sizes.

Table1

Table 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 (±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

Figure1

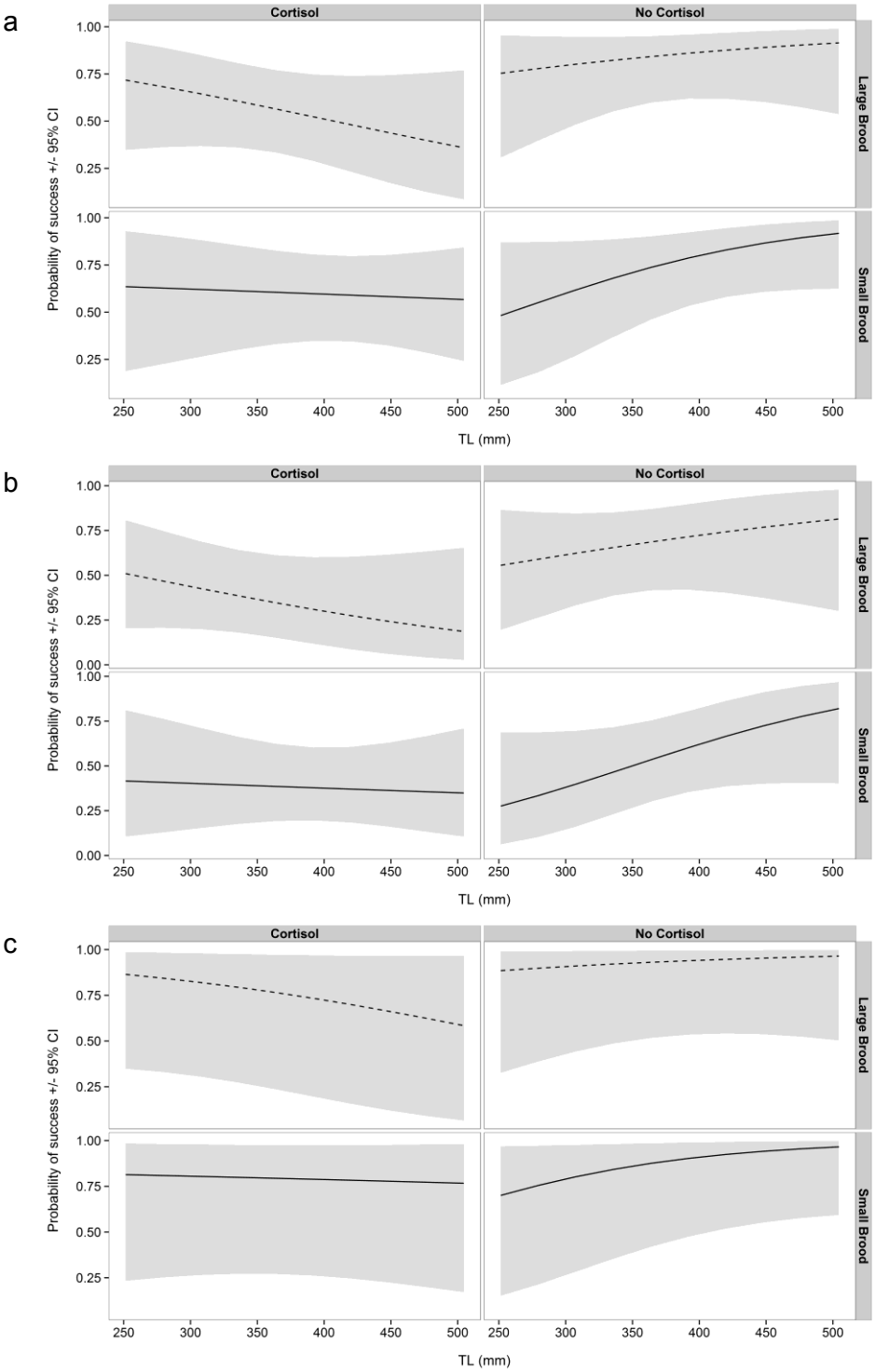

a
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
1.00
0.75
0.50
0.25
250
300
350
400
450
500
TL (mm)
b
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
1.00
0.75
0.50
0.25
0.00
250
300
350
400
450
500
TL (mm)
c
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
1.00
0.75
0.50
0.25
250
300
350
400
450
500
TL (mm)

fig 1

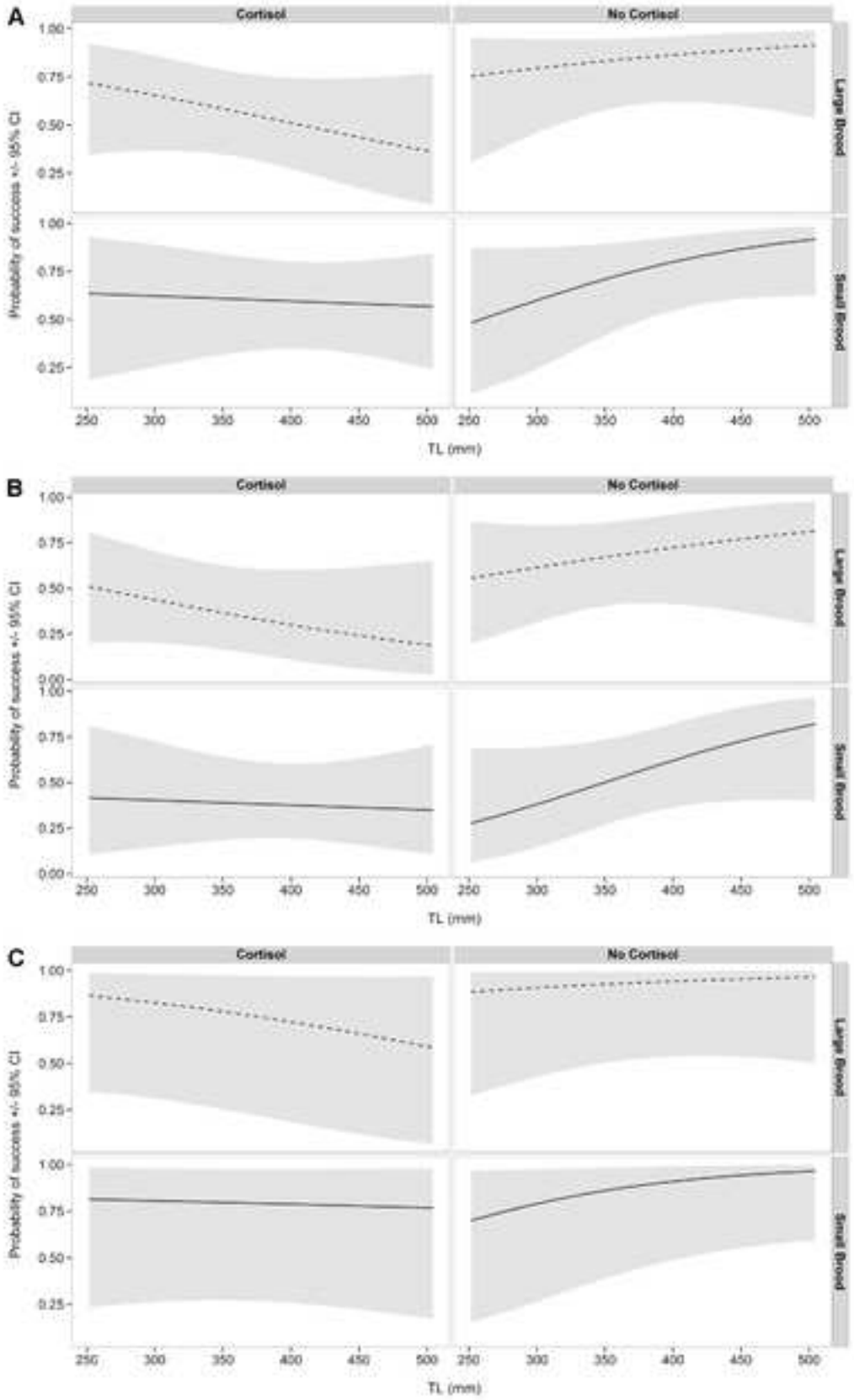

A
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
1.00
0.75
0.50
0.25
250
300
350
400
450
500
TL (mm)
B
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
1.00
0.75
0.50
0.25
0.00
250
300
350
400
450
500
TL (mm)
C
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
1.00
0.75
0.50
0.25
250
300
350
400
450
500
TL (mm)

fig 1

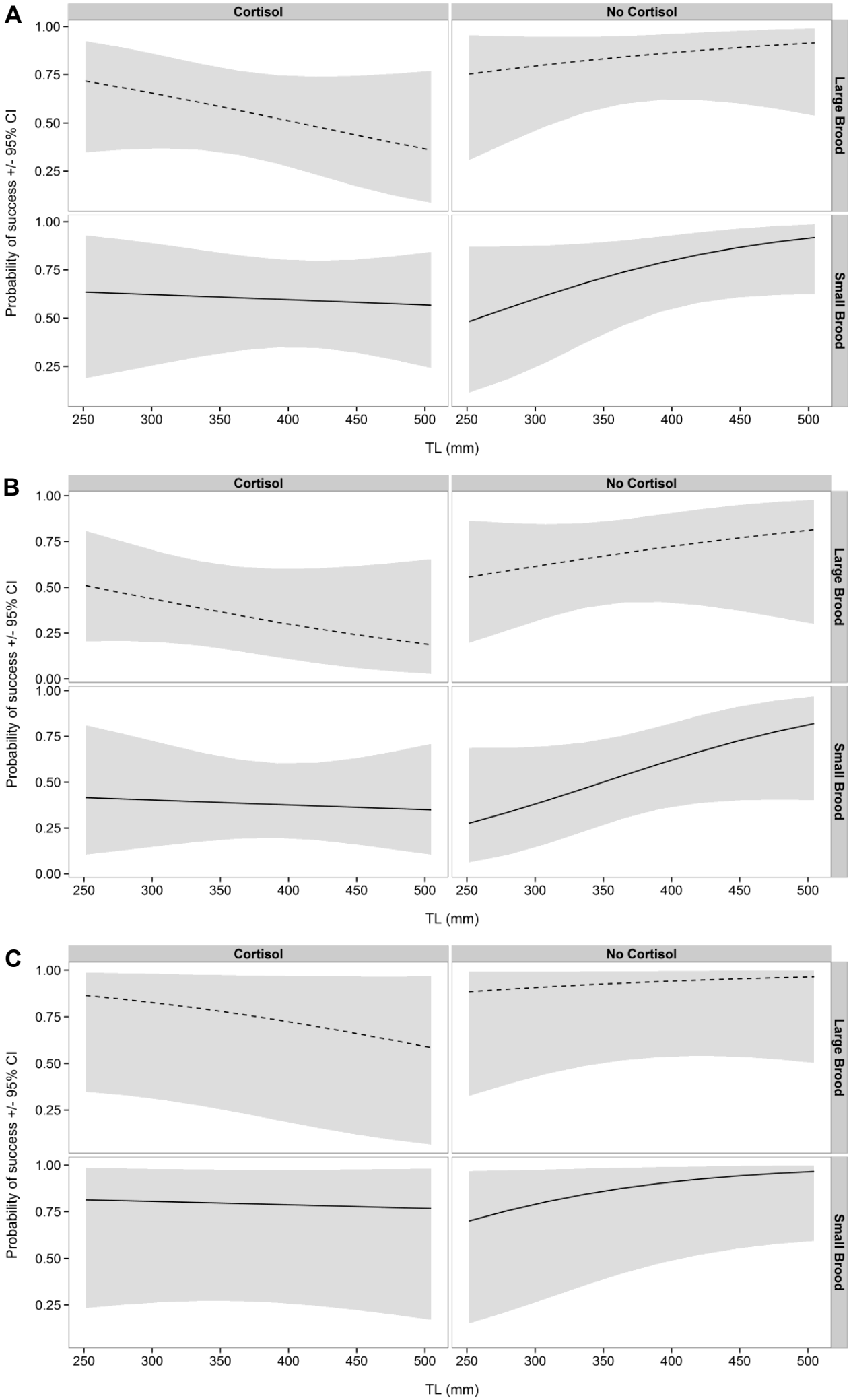

A
B
C
Cortisol
No Cortisol
Large Brood
Small Brood
Probability of success +/- 95% CI
TL (mm)
1.00
0.75
0.50
0.25
0.00
250
300
350
400
450
500

fig 2

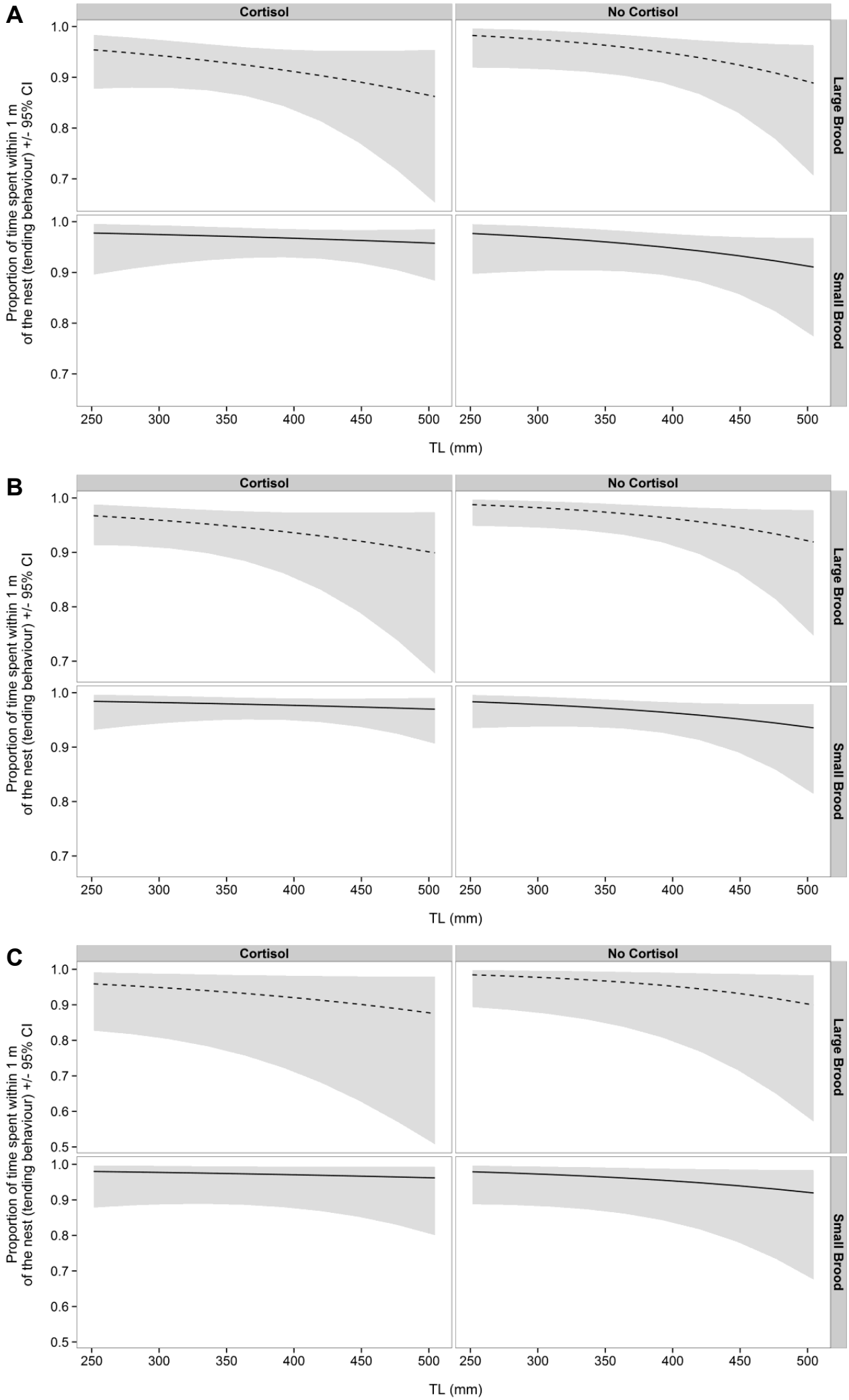

A
B
C
Cortisol
No Cortisol
Large Brood
Small Brood
Proportion of time spent within 1 m of the nest (tending behaviour) +/- 95% CI
TL (mm)
250
300
350
400
450
500
1.0
0.9
0.8
0.7
0.6
0.5

Figure2

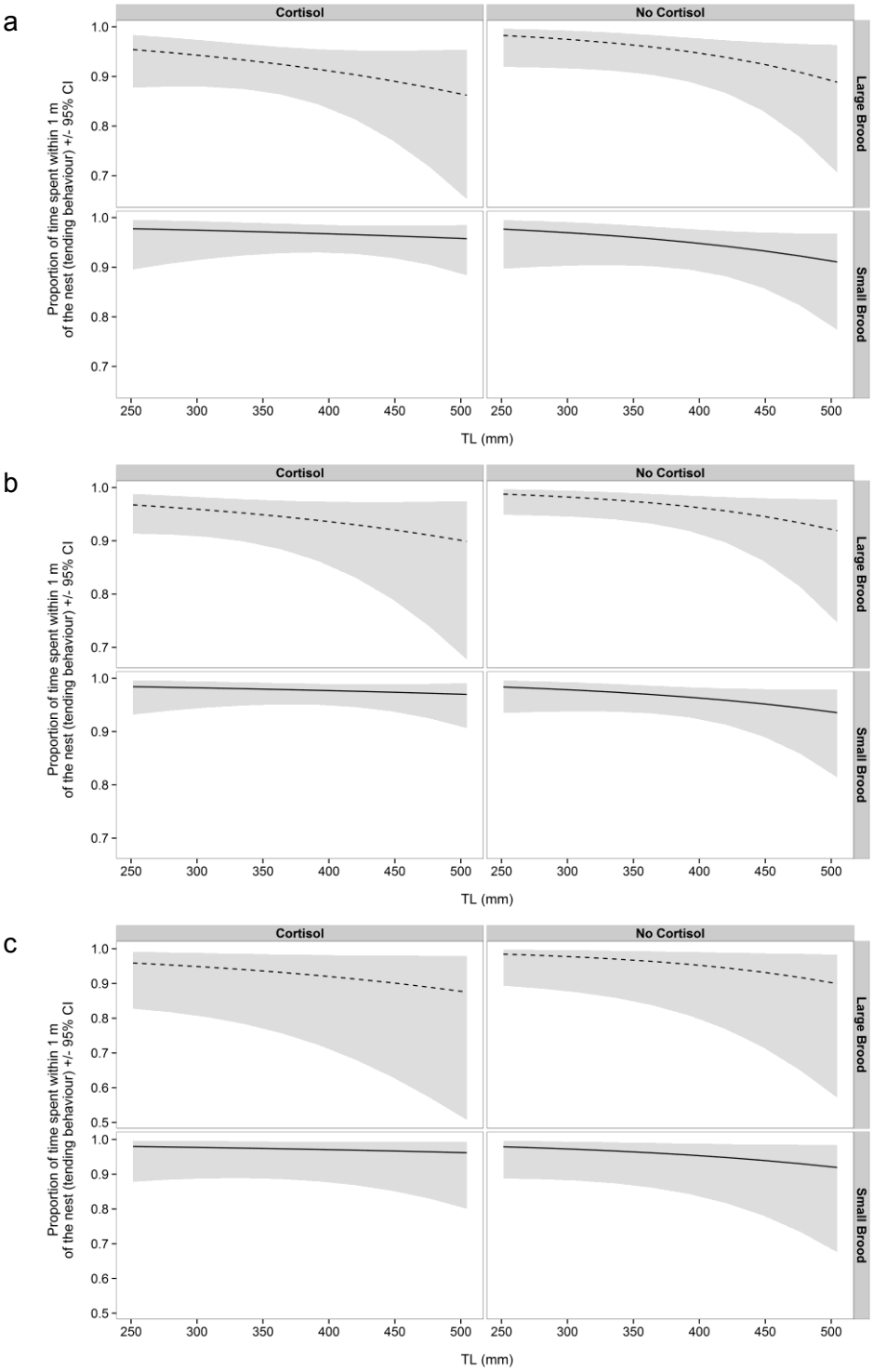
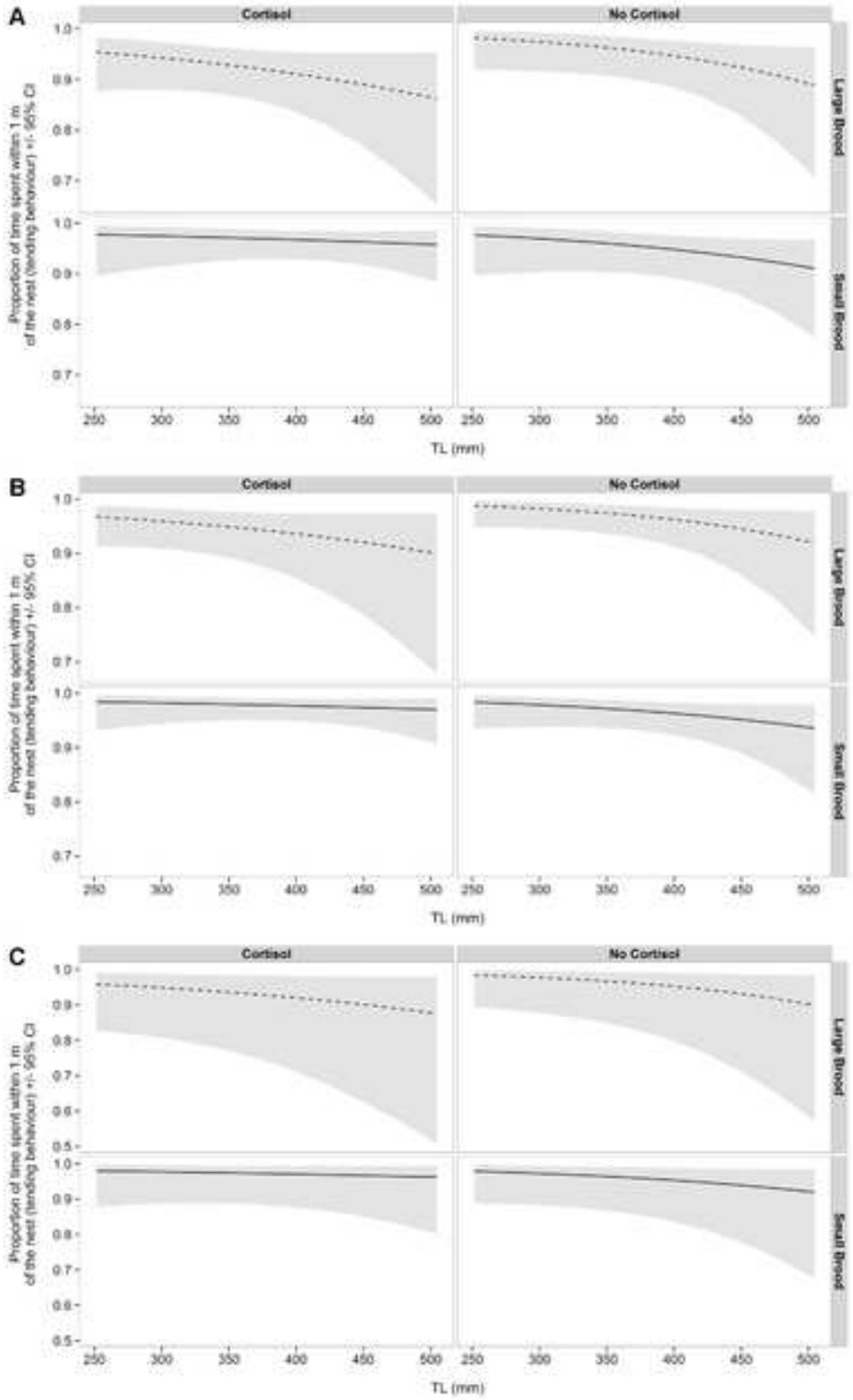

a
b
c
Cortisol
No Cortisol
Large Brood
Small Brood
Proportion of time spent within 1 m of the nest (tending behaviour) +/- 95% CI
TL (mm)
250
300
350
400
450
500
1.0
0.9
0.8
0.7
0.6
0.5

fig 2


A
Cortisol
No Cortisol
Large Brood
Small Brood
Proportion of time spent within 1 m of the nest (tending behaviour) +/- 95% CI
TL (mm)
B
Cortisol
No Cortisol
Large Brood
Small Brood
Proportion of time spent within 1 m of the nest (tending behaviour) +/- 95% CI
TL (mm)
C
Cortisol
No Cortisol
Large Brood
Small Brood
Proportion of time spent within 1 m of the nest (tending behaviour) +/- 95% CI
TL (mm)