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Reviewed work(s):
Source: *Physiological and Biochemical Zoology*, Vol. 85, No. 3 (May/June 2012), pp. 209-218
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/665272
Accessed: 04/05/2012 16:25

Reproductive History and Nest Environment Are Correlated with Circulating Androgen and Glucocorticoid Concentrations in a Parental Care–Providing Teleost Fish

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Accepted 2/4/2012; Electronically Published 4/3/2012

ABSTRACT

Using a long-term study population of wild smallmouth bass *Micropterus dolomieu* in a connected river-lake system, we investigated whether circulating glucocorticoid (cortisol) and androgen (testosterone) concentrations are influenced by reproductive investment and nesting environment in fish providing nest-guarding paternal care. For all individuals, we collected measures of reproductive history and the value of current parental care. We assessed nest environment and monitored individuals to quantify seasonal reproductive success. Finally, we measured circulating cortisol concentrations following a standardized stressor and circulating testosterone concentrations. Using general linear models, we found that poststress circulating cortisol concentrations were positively related to water temperature and were higher in fish nesting in the river than in the lake. Circulating testosterone concentrations were negatively related to water temperature and were higher in reproductively experienced fish. When considering the factors that influence reproductive success, we found that only parental size was positively related to current nest success. In summary, the results demonstrate that nesting environment is correlated with parental stress responses during parental care, while reproductive history and nesting environment are correlated with circulating androgen concentrations. Collectively, these results offer insight into the roles of both glucocorticoid and androgen steroid hormones during parental care in teleost fish.

Introduction

Life-history theory is based on the premise that individuals must allocate resources among competing functions (e.g., growth, immune function, reproduction). Individuals that make optimal allocations maximize lifetime fitness (Stearns 1989, 1992; Roff 2002). Investment in current versus future reproduction is one important life-history trade-off because energy allocated toward current reproduction cannot be directed toward growth and self-maintenance, thereby decreasing future reproductive potential (Williams 1966). Owing to its role in governing behavior, physiology, and the responses of individuals to their environment, the endocrine system is increasingly put forward as a proximate mediator of such life-history trade-offs (Zera and Harshman 2001; Ricklefs and Wikelski 2002).

Potential endocrine mediators of the life-history trade-off between current and future reproduction are glucocorticoid and reproductive hormones. Glucocorticoid hormones serve many functions in vertebrates (Mommsen et al. 1999; Sapolsky et al. 2000) but are best known for initiating a suite of physiological and whole-animal changes collectively termed a "stress response," which serves to promote individual survival during and after exposure to a challenging event (Wingfield et al. 1998; Romero 2004). The stress response is therefore adaptive and critical for self-preservation, but it can also suppress other important functions, including reproduction (Sapolsky et al. 2000; Wingfield and Sapolsky 2003; Angelier and Chastel 2009). The underlying mechanisms involve the actions of glucocorticoids at various locations within the hypothalamic-pituitary-gonadal axis, which is responsible for the synthesis and secretion of reproductive hormones (Greenberg and Wingfield 1987; Moore

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Physiological and Biochemical Zoology 85(3):209–218. 2012. © 2012 by The University of Chicago. All rights reserved. 1522-2152/2012/8503-1100$15.00.
DOI: 10.1086/665272

and Jessop 2003; Fuzzen et al. 2011). As a result of the negative relationship between the glucocorticoid stress response and reproductive hormones, an attenuation of the glucocorticoid stress response during key reproductive periods is thought to be an adaptive mechanism maintaining investment in current reproduction at the potential expense of survival and future reproductive opportunities (Wingfield and Sapolsky 2003). Baseline levels of glucocorticoids also serve important biological functions and may play a role in preparing an organism to appropriately respond to a future challenge (Sapolsky et al. 2000; Romero 2004). Whereas elevation of glucocorticoids in response to stress inhibits reproduction, increases in baseline glucocorticoid levels may benefit reproductive activity by mobilizing energy reserves for the challenges associated with mating or parental care (Moore and Jessop 2003; Romero 2004). Therefore, both baseline glucocorticoid levels and the extent of glucocorticoid elevation during a challenge are thought to mediate the trade-off between current and future reproduction and may function in a complementary fashion (Romero 2004).

On the other hand, elevated levels of hormones important for reproduction (e.g., testosterone, oestradiol, luteinizing hormone, prolactin) are considered indicative of increased reproductive investment. Elevated levels of these hormones are found to improve reproductive performance while reducing resource allocation to self-maintenance functions (Hegner and Wingfield 1987; Angelier and Chastel 2009). Elevated levels of reproductive hormones have also been found to suppress the glucocorticoid stress response itself (Pottinger et al. 1995, 1996; Fuzzen et al. 2011). The opposing relationships between glucocorticoids and the hypothalamic-pituitary-gonadal axis at the endocrine level (Greenberg and Wingfield 1987; Fuzzen et al. 2011) thus result in trade-offs between competing system-level functions (Ricklefs and Wikelski 2002), and interactions between endocrine states may be a proximate mechanism mediating optimal resource investment between current and future reproduction (Wingfield and Sapolsky 2003; Angelier and Chastel 2009).

For many vertebrates, parental care represents a major reproductive investment (Clutton-Brock 1991). Life-history theory predicts that if the endocrine system is a proximate mechanism mediating investment between current and future reproduction (Zera and Harshman 2001; Ricklefs and Wikelski 2002), then endocrine activity during parental care should be correlated with the value of an individual's current reproductive opportunity relative to future opportunities. Thus, individuals providing care to valuable current offspring or with few opportunities for future reproduction should display elevated baseline glucocorticoid levels (Romero 2004) and attenuated glucocorticoid stress responses when faced with a challenge during parental care (Wingfield and Sapolsky 2003; Angelier and Chastel 2009) relative to individuals with less valuable current offspring or more opportunities for future reproduction. Correspondingly, individuals providing care to valuable current offspring or with few opportunities for future reproduction are expected to exhibit higher circulating levels of reproductive hormones relative to individuals with less valuable current offspring or more opportunities for future reproduction (Wingfield and Sapolsky 2003; Goymann et al. 2007; Angelier and Chastel 2009). Previous research has provided empirical support for such correlations between reproductive investment and endocrine state (Angelier et al. 2006, 2007*a*, 2007*b*; Heidinger et al. 2006; Lendvai et al. 2007). However, examples remain restricted to studies in birds.

In this study, we explored the potential for glucocorticoids and androgens to mediate life-history trade-offs. To address this overarching objective, we determined whether circulating glucocorticoid and androgen concentrations were correlated to reproductive investment in the direction predicted by life-history theory. We conducted a population-level field-based study in the smallmouth bass *Micropterus dolomieu*, a long-lived iteroparous freshwater fish common in North America. Males provide annual sole parental care to a single brood for up to 6 wk during the spring; care consists of guarding eggs and then free-swimming larvae until the offspring have developed antipredator behaviors (Cooke et al. 2006). This research was conducted on a population of smallmouth bass that has been the subject of long-term monitoring (>15 yr), and the reproductive history of all nesting males is known (Barthel et al. 2008; B. L. Barthel, unpublished data). For all individuals, we assessed a suite of variables related to life history and reproductive investment: parental size, the number of previous nests, brood size, water temperature, and nest location. Larger smallmouth bass attract larger females (Hanson and Cooke 2009), obtain more eggs in their nests (Suski and Philipp 2004), and defend offspring more successfully than smaller fish (Wiegmann and Baylis 1995). Larger individuals in the congeneric largemouth bass *Micropterus salmoides* also have proportionally higher investment in gonads (gonadosomatic index) than smaller bass (Brown and Murphy 2004), and we therefore considered parental size a measure of parental quality and parental investment. The number of previous nests provided reproductive history. For nest-guarding fish such as the smallmouth bass, larger broods result in higher reproductive success per brood (Smith and Wootton 1995). Thus, we used brood size as the measure of the potential value of current parental care. In smallmouth bass, larger males tend to nest earlier in the year (Ridgway et al. 1991). The factors that limit smaller males to nesting later in the year remain unclear, but evidence suggests that smaller males need to feed for a longer period in the spring to build up the energy stores necessary to maintain parental care behavior (Ridgway et al. 1991). Early nesting is associated with lower water temperatures, which are in turn correlated with lower cortisol stress responses (e.g., Barton and Schreck 1987; Pottinger and Carrick 2000; O'Connor et al. 2011*b*). Thus, the timing of reproduction has the potential to influence endocrine parameters, and we recorded water temperature at the nest. Finally, smallmouth bass in this population exhibit high nest-site fidelity throughout their reproductive lives to either a lake or a river section of the study site, and males nesting in the two systems exhibit divergent life-history strategies (Barthel et al. 2008). The river is a more variable environment, and males nesting in the river have a lower mean age and size at maturity and lower nest success than males nesting in the lake (Barthel et al. 2008). We therefore included nesting location (river or lake) as an additional variable that could influence life-history trade-offs and therefore reproductive investment.

We investigated the cortisol stress response and circulating androgen (testosterone) concentrations as potential endocrine correlates of variation in reproductive investment. Circulating androgens are key steroids for reproduction in male fish. Levels generally decline during parental care relative to levels observed during courtship and mating, which suggests that these hormones are important in territory establishment and mating (see review in Oliveira et al. 2002). However, the existing literature also supports a role for androgens during parental care. Treatment with an androgen receptor antagonist decreased nest defense behavior in smallmouth bass (Dey et al. 2010). Furthermore, circulating testosterone concentrations were found to be higher in individuals providing greater levels of parental care in a cichlid fish (*Neolamprologus pulcher*; Desjardins et al. 2008), and testosterone concentrations were positively correlated with nest defense behavior in smallmouth bass (O'Connor et al. 2011*a*). Conversely, cortisol is the primary glucocorticoid in teleost fish (Mommsen et al. 1999), and some evidence suggests that regulation of the cortisol stress response may negatively influence parental investment in smallmouth bass. The cortisol stress response is attenuated during parental care in smallmouth bass (O'Connor et al. 2011*b*), indicating that a robust cortisol stress response is incompatible with the maintenance of parental care behaviors in this species or that activation of the hypothalamic-pituitary-gonadal axis during parental care suppresses the cortisol stress response or both. In any case, we predicted for this study that within the parental care–providing fish, the extent of attenuation of the cortisol stress response would correlate with reproductive investment. We predicted that a robust cortisol stress response and low circulating testosterone concentrations would be correlated with decreased reproductive investment, while an attenuated cortisol stress response and high circulating testosterone would be correlated with increased reproductive investment in our study population.

To summarize, if regulation of the cortisol stress response and circulating testosterone concentration underlies variation in reproductive investment, then the extent of the cortisol stress response should be negatively correlated with reproductive investment (Wingfield and Sapolsky 2003; Bokony et al. 2009)

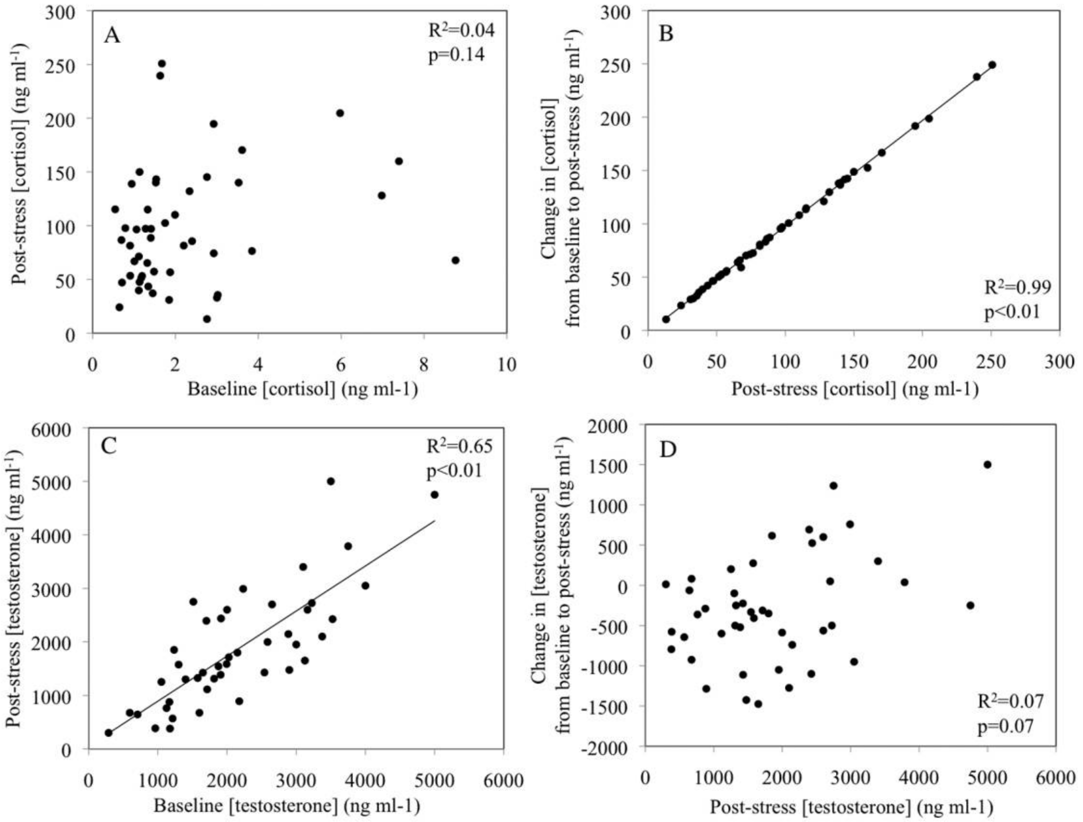

Figure 1. Relationships between baseline and poststress circulating steroid concentration (*A*, *B*) and between poststress circulating steroid concentration and the steroid hormone response to a standardized stressor (*C*, *D*; i.e., the difference between poststress and baseline circulating hormone concentrations) in smallmouth bass *Micropterus dolomieu*. Relationships are depicted for cortisol (*A*, *C*) and testosterone (*B*, *D*). The explained variance (r^2) and significance (*P*) are noted on each graph. Data are from a subset of fish collected for a previous study (O'Connor et al. 2011*b*).

Table 1: Summary of data collected for 131 male smallmouth bass *Micropterus dolomieu* captured while guarding fresh eggs in Miller's Lake and its upstream river section of the Mississippi River, Ontario, Canada, in May and June 2009

	Mississippi River ($n = 71$)				Miller's Lake ($n = 60$)			
Variable	Mean	Median	SEM	Range	Mean	Median	SEM	Range
Total length (mm)	252.2	239	5.29	195.0–405.0	284.5	267.5	7.61	196.0–482.0
Number of previous nests	.7	0	.15	.0–5.0	.8	0	.22	.0–7.0
Brood size (egg score)	2.3	2	.1	1.0–4.0	2.5	2	.1	1.0–5.0
Water temperature (°C)	15.5	15.5	.08	14.5–17.0	15.4	15.5	.09	14.0–16.5
Date (d)	May 30	June 2	.77	May 17–June 14	May 30	June 1	.85	May 17–June 7
Poststress [cortisol] (ng mL^{-1})	280.3	283.5	17.77	14.4–811.7	183.3	183.9	12.7	6.1–508.7
[Testosterone] (ng mL^{-1})	1	.8	.09	.4–3.2	.9	.8	.09	.4–3.9

and circulating testosterone concentrations should be positively correlated with reproductive investment (Angelier and Chastel 2009). We therefore predicted that larger males with more previous reproductive seasons nesting in the lake at cooler water temperatures and guarding larger broods should display lower poststress cortisol concentrations and higher circulating testosterone concentrations than smaller younger males with less experience nesting at warmer water temperatures and guarding smaller broods.

Material and Methods

Study Site and Animals

The study was conducted in a short reach of the Mississippi River of Ontario (Frontenac County, Ontario, Canada; 44°57′N, 76°43′W). For a full description of the study site, see Barthel et al. (2008). In brief, the study site is composed of distinct upstream riverine (Mississippi River) and downstream lacustrine (Miller's Lake) habitats separated by a <1-m waterfall that is not a barrier to smallmouth bass movement. The study site is bordered upstream by a >2-m waterfall that is a barrier to smallmouth bass and downstream by a series of rapids and waterfalls. Radio telemetry studies have not detected fish movement out of the study site in either direction (Barthel et al. 2008). Although smallmouth bass show high nest-site fidelity to either the river or the lake during the reproductive period, most fish overwinter in the lake (Barthel et al. 2008). The study site is relatively isolated, and the system receives negligible fishing pressure.

Selection of Sampling Protocol

The fish used in this study were important as individuals within a long-term (>15 yr) study population on the reproductive ecology of smallmouth bass. To minimize the effect of our sampling protocol, we were limited to collecting only a single blood sample per fish. To understand the information provided by a single blood sample, we first obtained both baseline and poststress cortisol and testosterone values from a separate population of fish. These fish were originally captured as part of a separate study; for full details, please refer to O'Connor et al. (2011*b*). Briefly, fish were captured while guarding fresh eggs and immediately sampled for blood by caudal puncture. They then were subjected to a 3-min standardized capture-and-restraint protocol followed by a 25-min recovery before being sampled for blood again by caudal puncture. The 3-min standardized capture-and-restraint protocol, blood sampling protocols, and the handling and assaying of blood samples for cortisol concentration were all identical to the procedures used for this study and are fully described below. For the purposes of this study, the relationships between baseline and poststress cortisol and testosterone concentrations were explored using regression models to assess the relationship between baseline and poststress steroid concentration and the relationship between poststress steroid concentration and the steroid response to the standardized stressor (i.e., the difference between poststress and baseline concentrations). Only values obtained from unmanipulated control smallmouth bass providing care to eggs <24 h old were used ($n = 49$).

These data demonstrated that in smallmouth bass providing parental care to fresh eggs, baseline circulating cortisol concentrations were consistently low with little variation (fig. 1*A*). The very low variation in baseline circulating cortisol concentrations may be the result of a "floor" effect of the 3-ng mL^{-1} detection limit of the cortisol assay (O'Connor et al. 2011*b*). Poststress cortisol concentration was unrelated to baseline circulating cortisol concentration (fig. 1*A*) but was an accurate measure of the cortisol stress response (i.e., the change in cortisol concentration from baseline to poststress; fig. 1*B*). On the other hand, poststress testosterone concentrations more closely reflected baseline testosterone concentrations (fig. 1*C*) than the ability of parents to maintain testosterone concentrations following a stressor (fig. 1*D*). Thus, being limited to a single sample, we opted to collect only a poststress sample because it provided an accurate measure of the cortisol stress response (fig. 1*B*) and a reasonable measure of baseline circulating androgen concentration (fig. 1*C*).

Field Data Collection

During May and June 2009, the study site was surveyed by snorkeling every 24–52 h. For the long-term data set, all nests were identified as in previous years. Because androgens decline

Table 2: Results of scaled general linear models explaining variation in circulating steroid hormone concentrations and reproductive success for 131 male smallmouth bass *Micropterus dolomieu* captured while guarding fresh eggs in Miller's Lake and its upstream river section of the Mississippi River, Ontario, Canada, in May and June 2009

Parameter and model term	t	P
Poststress [cortisol] (ng mL^{-1}):[a]		
TL (mm)	−.95	.345
Reproductive experience	1.79	.077
Brood size (egg score)	.77	.441
Location (river or lake)	**−3.63**	**<.001**
Water temperature (°C)	**4.96**	**<.001**
[Testosterone] (ng mL^{-1}):[b]		
TL (mm)	−1.36	.178
Reproductive experience	**2.82**	**.005**
Brood size (egg score)	−1.65	.101
Location (river or lake)	.61	.546
Water temperature (°C)	**−3.68**	**<.001**
Reproductive success:[c]		
Poststress [cortisol] (ng mL^{-1})	1.72	.087
[Testosterone] (ng mL^{-1})	.12	.907
TL (mm)	**2.11**	**.037**
Reproductive experience	.12	.902
Brood size (egg score)	−.71	.477
Location (river or lake)	1.40	.165
Water temperature (°C)	.71	.480

Note. TL = total length. Bold indicates significant model terms within significant models ($\alpha = 0.05$).

[a] $r^2 = 0.35$, $F_{5,128} = 13.62$, $P < 0.001$.

[b] $r^2 = 0.22$, $F_{5,128} = 7.07$, $P < 0.001$.

[c] $r^2 = 0.11$, $F_{7,126} = 2.16$, $P = 0.042$.

across parental care in smallmouth bass (i.e., androgens are highest while guarding eggs and decline as offspring develop; O'Connor et al. 2011*b*), only parental fish guarding nests with fresh eggs (<24 h old) were used in this study. The date that eggs were laid was recorded. All nests were marked with a numbered tile and assigned an egg score, which is a standard measure of the number of eggs within a nest and ranges from 1 (<500 eggs) to 5 (>4,000 eggs; Philipp et al. 1997). Focal parental males were then captured by targeted rod-and-reel angling. For standardization, the duration between hooking and landing the fish was always <15 s. Once on the boat, fish were then subjected to a standardized 3-min air emersion stressor (O'Connor et al. 2011*b*). Following the standardized stressor, males were held singly for 25 min in 48-L coolers filled with freshwater to allow circulating cortisol concentrations to rise (Mommsen et al. 1999; O'Connor et al. 2011*b*). A single poststress 1-mL blood sample was then withdrawn by caudal puncture into lithium-heparinized 3-mL vacutainer-style syringes (Becton, Dickinson, Franklin Lakes, NJ). As part of the long-term data set, all fish in this system are fitted with an intracoelomic passive integrated transponder (PIT) tag (12.5 mm × 2.0 mm) when they are first captured. For this study, as in previous years, all fish were scanned for a PIT tag using a reader (Biomark, Boise, ID), and a new tag was inserted in first-time nesting males. Fish were measured for total length (TL), scales were collected for aging (Jearld 1983; Barthel et al. 2008), and fish were fin clipped so that snorkelers could visually confirm that the parental fish was correctly captured from each nest. Handling time for PIT tagging, TL measurement, and collection of scales was always <2 min. Fish were released at their nest, and water temperature at the nest was recorded. Snorkeling surveys were continued until the end of the reproductive season to assess reproductive success. When a male raised a brood until the offspring were able to feed exogenously and evade predators, the nesting attempt was considered successful, while abandonment of the offspring before this development stage was considered unsuccessful (Philipp et al. 1997). In total, 255 parental males were found guarding nests at various developmental stages in the system and were included as part of the long-term data set. Of these, 131 fish were identified and captured on fresh eggs and were included in this study.

Hormone Analysis

Plasma cortisol concentration was determined using a commercial kit (ImmunoChem Cortisol ^{125}I RIA kit; MP Biomedicals, Orangeburg, NY) previously validated for teleost fish (Gamperl et al. 1994). All plasma samples were measured in a single assay. To calculate intra-assay variability, six known-concentration standards and 20 plasma samples were measured in duplicate. Intra-assay variability was 3.6% (coefficient of variation). Measurements of testosterone followed the methods outlined by McMaster et al. (1992). Testosterone content was measured in duplicate for all samples by ^{3}H-radioimmunoassay using antibodies provided by Medicorp (Montreal). Cross-reactivity of the testosterone antibody with 11-ketotestosterone was 7.5%. For testosterone, interassay variability was calculated from 12 known-concentration standards measured in triplicate and duplicate plasma samples and was 5.2%. Intra-assay variability was measured using the 12 known-concentration standards and a laboratory standard and was 4.4%.

Statistical Analysis

Data were examined using general linear models. First, to explore the influence of life history and environmental parameters on endocrine parameters, models were constructed with poststress cortisol concentration and testosterone concentration as dependent variables and parental size (TL in mm), reproductive history (number of previous nests), brood size (egg score), location (river or lake), and water temperature (°C) as independent variables. To understand the influence of endocrine parameters, life history, and environmental parameters on reproductive success, a general linear model was constructed with reproductive success as the dependent variable and poststress cortisol concentration, testosterone concentration, TL, number of previous nests, egg score, location, and water temperature as independent variables. TL, poststress cortisol concentration, and testosterone concentration were log transformed to meet the assumptions of normal distribution and homogeneity of variances. Owing to the number of first-time nesting males (i.e., males with no previous nests), reproductive history was considered a dichotomous variable with males categorized as either "experienced" or "inexperienced." All variables were scaled by their standard deviation and centered by their means to make estimates comparable for all model terms (Schielzeth 2010). For all models and model terms, $\alpha = 0.05$. All analyses were performed using R, version 2.14.0.

Results

The data collected from the 131 parental male fish are summarized in table 1. Using general linear models, we found that water temperature and nest location (river or lake) both explained a significant proportion of the variance in poststress circulating cortisol concentrations (table 2). As evidenced by effect sizes, changes in both water temperature and nest location greatly influenced poststress circulating cortisol concentrations (fig. 2). Water temperature was positively related to poststress circulating cortisol concentration, and poststress circulating cortisol concentration was higher in fish nesting in the river than in the lake (figs. 2, 3). We found that the number of previous nests and water temperature both explained a significant proportion of the variance in circulating testosterone concentrations (table 2; fig. 2). Water temperature was negatively related to circulating testosterone concentration, while experienced parents had higher circulating testosterone concentrations than inexperienced parents (figs. 2, 4). Finally, only TL significantly influenced annual reproductive success in these fish (table 2). TL was positively related to reproductive success (fig. 2).

Discussion

The results of this study demonstrate that poststress circulating cortisol concentrations are correlated with measures of nest environment and circulating testosterone concentrations are correlated with measures of nest environment and reproductive investment in smallmouth bass providing parental care to fresh eggs. We discuss insights gained from this data set on the roles of cortisol and androgen regulation during parental care in smallmouth bass.

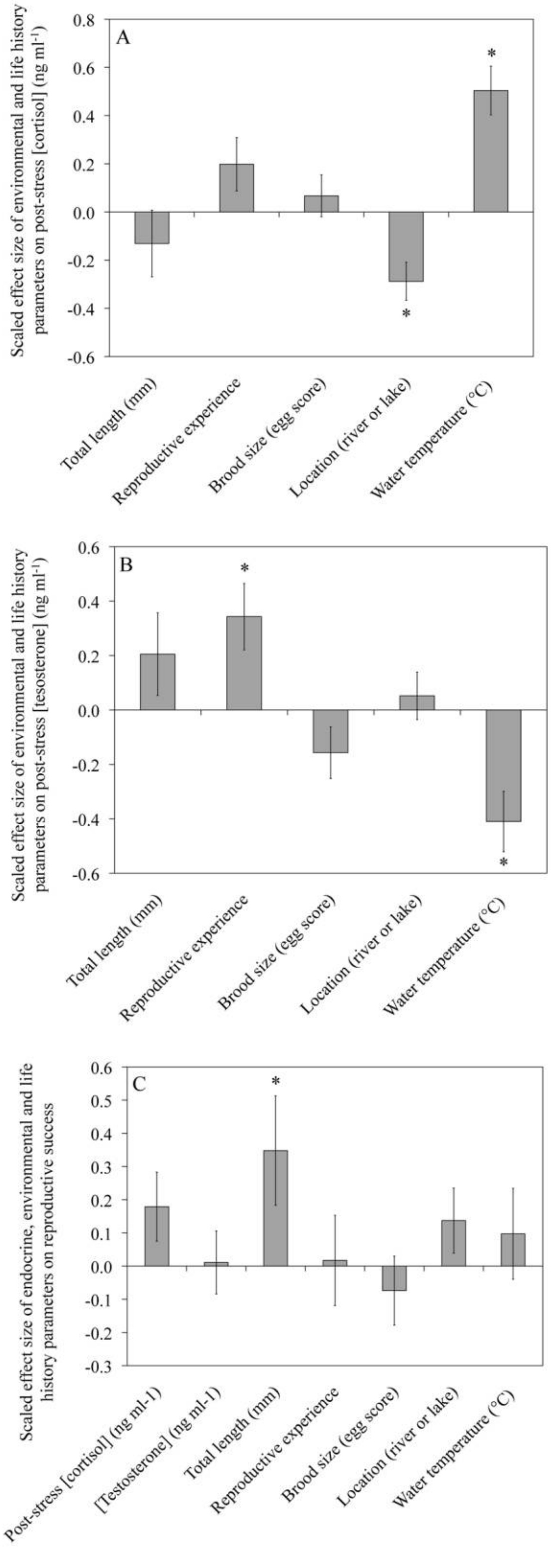

Figure 2. Effect size and standard error of the effect size for each of the relevant parameters on poststress circulating cortisol concentration (*A*), circulating testosterone concentration (*B*), and reproductive success (*C*) in scaled general linear models for 131 male smallmouth bass *Micropterus dolomieu* captured while guarding fresh eggs in Miller's Lake and its upstream river section of the Mississippi River, Ontario, Canada, in May and June 2009. Asterisks represent effects that were significant in the scaled general linear models. See table 2 for statistical details.

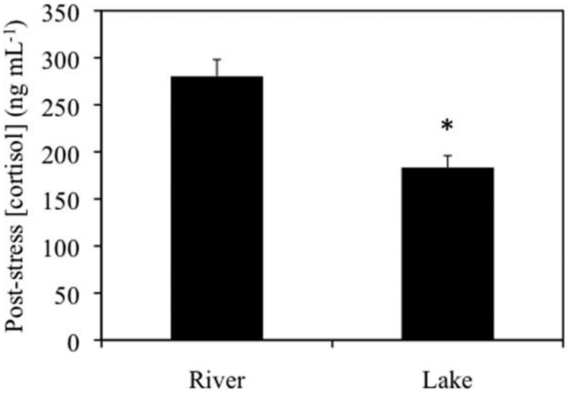

Figure 3. Significant relationship found using scaled general linear models between poststress circulating cortisol concentration and nest location for 131 male smallmouth bass *Micropterus dolomieu* captured while guarding fresh eggs in Miller's Lake and its upstream river section of the Mississippi River, Ontario, Canada, in May and June 2009. See table 2 for statistical details.

What Can We Learn about the Roles of Cortisol and Androgens during Parental Care?

We found that poststress circulating cortisol concentration is correlated with water temperature, while circulating testosterone concentration is correlated with water temperature and reproductive history. Nesting earlier in the season provides clear advantages because it improves offspring survival through their first summer (Pine et al. 2000), gives offspring a longer growing season before their first winter (Wiegmann et al. 1992), and in turn increases first-winter survival (Biro et al. 2004). The results of this study suggest that the cooler water temperatures faced by early nesters may also facilitate the physiological capacity of fish to provide parental care. There is evidence from previous literature that increased androgen concentrations and a decreased cortisol stress response may benefit parental care–providing smallmouth bass. In fish, circulating testosterone concentration is higher in individuals providing greater levels of parental care in both laboratory (Desjardins et al. 2008) and field (O'Connor et al. 2011*a*) settings. Based on the available information, it is likely that fish gain multiple benefits by nesting at cooler temperatures both in terms of increased offspring survival and in terms of increased physiological capacity for parental care. Mechanistic studies detailing the downstream effects of the relationship between physiological parameters and water temperature are necessary to draw definitive conclusions regarding the potential benefits of lower water temperature for parental care behavior in smallmouth bass.

Factors beyond water temperature are also likely to affect endocrine regulation during parental care. In particular, circulating testosterone concentration was positively correlated with reproductive history. These results are consistent with previous literature showing higher reproductive hormone levels in older parents (Angelier et al. 2007*a*; Angelier and Chastel 2009). These endocrine factors may contribute to the pattern of increased reproductive success at cooler water temperatures for older, larger smallmouth bass. Three hypotheses have been put forward to explain the consistently demonstrated positive relationships among reproductive investment, parental age, and reproductive hormone levels (Curio 1983; Forslund and Pärt 1995). "Parental constraint" posits that young animals are physiologically incapable of investing heavily in offspring; "parental restraint" suggests that older animals increasingly invest in offspring as future reproductive opportunities become limited; and "selection" proposes that lower-quality animals succumb earlier in life than higher-quality animals, and the result is a higher-quality group of older parents (Curio 1983; Forslund and Pärt 1995). All three hypotheses make many of the same predictions, and drawing conclusions regarding ultimate drivers therefore becomes challenging. However, the lack of relationship between endocrine parameters and brood size in this study can be viewed as indirect support for the parental constraint or selection hypotheses rather than the restraint hypothesis (Curio 1983). These results are consistent with previous research showing that manipulating brood size does not affect androgen or cortisol concentrations in smallmouth bass (O'Connor et al. 2011*b*). Collectively, these results suggest that androgen parameters may reflect an increased physiological capability (either as a result of increased capacity with age or as a result of selection) in larger, older parents rather than sensitivity to current reproductive potential.

Interestingly, neither of the endocrine parameters was correlated with current reproductive success. Only TL was significantly correlated with reproductive success, and this relationship was relatively weak. Circulating poststress cortisol concentration was a marginally nonsignificant factor but in the direction opposite our predictions (i.e., increased poststress cortisol concentration was associated with increased reproductive success). This is a finding that warrants further research and consideration. Our results show that circulating cortisol

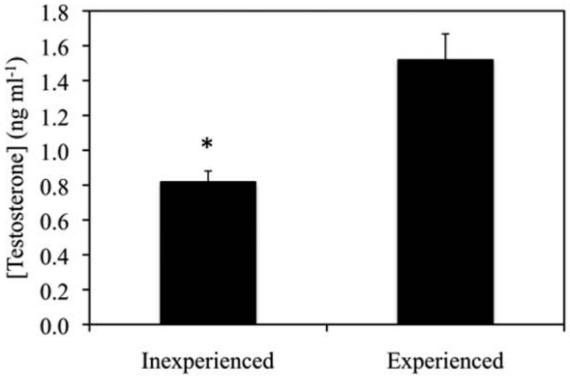

Figure 4. Significant relationship found using scaled general linear models between circulating testosterone concentration and reproductive experience for 131 male smallmouth bass *Micropterus dolomieu* captured while guarding fresh eggs in Miller's Lake and its upstream river section of the Mississippi River, Ontario, Canada, in May and June 2009. See table 2 for statistical details.

and testosterone concentrations are correlated with measures of reproductive investment in the directions predicted by life-history theory. However, these relationships operate within a network of other relationships, including nesting environment, and are not directly correlated with reproductive success.

Why Is the Cortisol Stress Response Higher in River-Nesting Fish?

In this connected river-lake study system, males show high nest-site fidelity, and there is divergence in life-history traits between the two locations. Males nesting in the river are younger and smaller at maturity than fish nesting in the lake and have lower annual reproductive success; however, lifetime reproductive success does not differ between the locations (Barthel et al. 2008). In this study, poststress cortisol concentrations were higher in fish nesting in the river than in the lake. This difference in cortisol responsiveness may reflect differences in environmental variables between the two locations. Additional research that examines more specifically the many variables that differ between the two locations (e.g., water flow, predation rates, turbidity) is necessary to fully understand differences in endocrine regulation between the two locations.

An alternate explanation is that there may be differences in the characteristics of the fish themselves between the two locations. Differences in stress-coping styles have been documented in fish (Pottinger and Carrick 1999; reviewed in Schjolden and Winberg 2007). Proactive individuals are more active, are more aggressive, are routine forming, and exhibit higher sympathetic activity in response to a stressor, whereas reactive individuals freeze and exhibit higher hypothalamic-pituitary-interrenal responses following a stressor but are more responsive to environmental changes and exhibit more behavioral flexibility (Coppens et al. 2010). Thus, it is possible that reactive fish (i.e., fish that display high cortisol response to stress) nest in the more unpredictable riverine environment at higher rates than proactive fish (i.e., fish that display low cortisol response to stress). Further research investigating whether the differences in cortisol responsiveness are maintained year-round—including during winter, when all fish aggregate in the lake (Barthel et al. 2008)—would be helpful as a first step in investigating this possibility.

Limitations of a Correlative Data Set

In this study, many variables were highly correlated. Larger males are also more experienced, tend to guard more eggs, and nest earlier in the year at colder water temperatures (Barthel et al. 2008). Unfortunately, there is no statistical method that can entirely disentangle these variables to reliably identify which among the variables are the driving factors influencing poststress cortisol and circulating testosterone concentrations. While our general linear models can identify large and potentially important effects, as with all correlative data sets, experimental manipulation of various parameters is necessary to determine causal relationships among these variables.

Summary

The results of this study demonstrated that nesting environment was correlated with poststress cortisol concentrations while nesting environment and reproductive history were correlated with testosterone concentrations during parental care in a population of wild teleost fish. The resultant relationships contributed to a pattern of higher reproductive success in more experienced males that reproduced earlier in the year at cooler water temperatures. Thus, this study provides support for higher androgen concentration as a signal of greater reproductive investment and for increased androgen concentration as a potential mechanism driving increasing reproductive success with age. This study suggests that while the cortisol stress response varied among the parental care–providing fish, it was correlated more strongly with environmental variables than with life-history traits. This study identified previous nesting experience and water temperature as important factors correlated with endocrine parameters during parental care. The results provide the framework and ecological relevance for further experimental work in which life history and environmental variables should be manipulated to better understand the driving factors influencing patterns of hormone regulation in relation to reproductive investment.

Acknowledgments

Work was conducted in accordance with the guidelines of the Canadian Council for Animal Care administered through Carleton University and under the authority of permit B09-12. This research was supported by Natural Sciences and Engineering Research Council Discovery Grants to S.J.C., K.M.G., and G.V.D.K.; a Research Achievement Award from Carleton University to S.J.C.; and Illinois Natural History Survey support to D.P.P. C.M.O. was supported by a Grant-in-Aid-of-Research award from the Society for Integrative and Comparative Biology, an Ontario Graduate Scholarship, and Carleton University. Logistical support was provided by the Queen's University Biological Station. We wish to thank Zac Blevins, Niels Carlson, Alison Colotelo, Cody Dey, Sean Landsman, and Lianne Nowell for their help in the field and Cody Dey and Jacquie Matsumoto for their invaluable assistance with the hormone assays.

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