

## Circulating androgens are influenced by parental nest defense in a wild teleost fish

Constance M. O'Connor · Kathleen M. Gilmour ·  
Glen Van Der Kraak · Steven J. Cooke

Received: 22 September 2010/Revised: 11 January 2011/Accepted: 29 January 2011/Published online: 16 February 2011  
© Springer-Verlag 2011

**Abstract** While social interactions influence vertebrate endocrine regulation, the dynamics of regulation in relation to specific behaviors have not been clearly elucidated. In the current study, we investigated whether androgens (testosterone) or glucocorticoids (cortisol) play a functional role in aggressive offspring defense behavior in wild smallmouth bass (*Micropterus dolomieu*), a teleost fish with sole paternal care. We measured circulating testosterone and cortisol concentrations in plasma samples taken from parental males following a simulated nest intrusion by a common nest predator, the bluegill sunfish (*Lepomis macrochirus*). To understand whether endocrine regulation changes across the parental care period, we looked both at males guarding fresh eggs and at males guarding hatched embryos. Plasma testosterone levels increased in males subjected to a simulated nest intrusion when compared to sham controls. Testosterone concentrations in males guarding embryos were lower than in males guarding fresh eggs, but circulating testosterone was positively correlated

with the level of aggression towards the nest predator at both offspring development stages. However, there was no increase in cortisol levels following a simulated nest intrusion, and no relationship between cortisol and any measured parameter. These results suggest that androgens play an important role in promoting aggressive nest defense behavior in teleost fish.

**Keywords** Cortisol · Androgens · Testosterone · Challenge hypothesis · Parental care

### Introduction

The challenge hypothesis, proposed in a classic paper by Wingfield et al. (1990), provides a framework for understanding patterns of androgen regulation across the vertebrates. As originally outlined, the challenge hypothesis was based on the premise that elevated androgens are important in male vertebrates for mate attraction, territory defense, and reproduction, but are generally incompatible with paternal care (Silverin 1986; Hegner and Wingfield 1987). Thus, the endocrine response of a male to a territorial challenge during paternal care will depend on several factors, notably the frequency of territorial challenges, the mating system, and the level of paternal care. For example, an individual with few territorial challenges, a monogamous mating system, and high paternal care is expected to maintain androgens at relatively low levels, and respond to a territorial challenge by rapidly and transiently increasing circulating androgens. Alternately, an individual with frequent territorial challenges, a polygamous mating system, and little to no paternal care is expected to maintain continually high androgen concentrations, and a further increase in response to a territorial challenge may not be

---

C. M. O'Connor (✉) · S. J. Cooke  
Fish Ecology and Conservation Physiology Laboratory,  
Ottawa-Carleton Institute of Biology, Carleton University,  
1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada  
e-mail: coconno4@connect.carleton.ca

K. M. Gilmour  
Department of Biology, University of Ottawa,  
30 Marie Curie, Ottawa, ON K1N 6N5, Canada

G. Van Der Kraak  
Department of Integrative Biology, College of Biological  
Sciences, University of Guelph, 50 Stone Road East,  
Guelph, ON N1G 2W1, Canada

S. J. Cooke  
Institute of Environmental Science, Carleton University,  
1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada

possible (Wingfield et al. 1990). This general pattern has been observed in a range of vertebrates (see reviews for birds, Wingfield et al. 1990; fish, Hirschenhauser et al. 2004; across taxa, Hirschenhauser and Oliveira 2006).

One postulate of the challenge hypothesis is that androgens are involved only in conspecific aggression (e.g., competition over mates or territories), while other types of aggression (e.g., anti-predator aggression) are regulated by different proximate mechanisms (Wingfield et al. 1990). An interesting exception may be aggressive offspring defense by parental care-providing teleost fish. In fish, parental care is thought to have arisen from ancestral territory defense (Gross and Sargent 1985), and unlike in other taxa, parental care often continues despite high androgen levels (e.g., Desjardins et al. 2005; Dey et al. 2010).

Cortisol, the primary glucocorticoid in teleosts, also may play an important role in mediating aggressive nest defense behavior in teleost fish. Glucocorticoids have been implicated in mobilizing the energy reserves required for energetically costly behaviors such as nest defense (Nelson 2000). However, glucocorticoids also play an important role in mediating stress (Wendelaar-Bonga 1997; Mommensen et al. 1999; Sapolsky et al. 2000), and high levels of glucocorticoids are often associated with reproductive suppression (Schreck et al. 2001; Fuzzen et al. 2011), reduced annual reproductive activity (e.g., Magee et al. 2006), and nest abandonment (e.g., O'Connor et al. 2009).

The current study investigated the potential roles of circulating androgens and cortisol in aggressive nest defense behavior in a teleost fish, the smallmouth bass (*Micropterus dolomieu*). Specifically, we determined whether a simulated intrusion by a potential nest predator would elicit an androgen or a cortisol response in parental care-providing fish. To understand whether the androgen or cortisol response is maintained across parental care, we studied smallmouth bass guarding fresh eggs versus embryos. Smallmouth bass spawn annually, with males establishing territories and clearing an area of substrate for the nest, and then providing sole paternal care to a single brood of offspring for approximately 6 weeks (Coble 1975). With this social system, the challenge hypothesis predicts high androgen levels during territory establishment, and decreasing levels across parental care, a pattern that has been documented in the smallmouth bass (O'Connor et al. 2011). The challenge hypothesis further predicts that a conspecific territorial challenge during parental care will elicit a transient and rapid androgen response. If androgens perform a functional role in aggressive nest defense during parental care, then we predict that a simulated nest intrusion by a nest predator (i.e., a small fish that is not a threat to the territory or male) should elicit an increase in circulating androgen

concentrations. Further, if cortisol liberates stored energy reserves to fuel aggression, then an increase in circulating cortisol levels following a simulated nest intrusion should also occur.

## Materials and methods

### Study animals and field data collection

On May 25 and 26, 2008 (water temperature 15–16°C), male smallmouth bass guarding nests with fresh eggs (0–1 day after laying and fertilization) were identified on Charleston Lake, a public lake that is part of the Gananoque River system in eastern Ontario (44°32'N, 75°59'W). All nests were individually marked with a numbered tile, and the brood size of each nest was classified according to egg score. The egg score is a standard and highly repeatable measure of the relative number of eggs within a nest, and ranges from 1 (low, <500) to 5 (high, >4,000; Philipp et al. 1997; Suski et al. 2003). For standardization, only males guarding nests with intermediate egg scores of 3–4 were included in this study.

A subset of parental fish was sampled immediately. Fish were first subjected to a simulated nest predator intrusion. Bluegill sunfish (*Lepomis macrochirus*) are common nest predators of smallmouth bass, and simulated nest intrusions of bluegill sunfish elicit aggressive nest defense behaviors (e.g., Hanson et al. 2009). A bluegill sunfish (150–200 mm) in a 3.8-L glass jar was placed in the nest by a snorkeler who then retreated to a distance of approximately 2 m for 3 min of behavioral observations. Aggressive behaviors were defined as by O'Connor et al. (2009). Briefly, a “yawn” is when the parent opens and closes its mouth in the direction of the glass jar, a “charge” is when the parent moves rapidly towards the jar but without making physical contact, and a “hit” refers to the smallmouth bass making physical contact with the jar. Snorkelers recorded the sum of all three aggressive behaviors for each individual during the 3 min period. After 3 min, the snorkeler removed the jar. After 25 min, a standard time to allow circulating cortisol concentrations to rise following a stressor (e.g., O'Connor et al. 2011) and also sufficient for androgens to increase following a simulated territorial intrusion in cichlid fish (e.g., Desjardins et al. 2005), fish were quickly captured by rod-and-reel angling and sampled for 1 mL of blood by caudal puncture using lithium-heparinized vacutainers (B.D. Vacutainer, Franklin Lakes, NJ) and 21 gauge, 38 mm needles. Only fish that could be captured and blood sampled within 5 min of the first angling attempt were included in the study. Fish were measured (total length, TL) and released. In total, blood samples were obtained from 24 males

[TL = 399.1 ± 8.7 mm (mean ± SE)] guarding fresh eggs. Blood samples were also obtained from 6 males (TL = 413.8 ± 17.4 mm) selected for a “sham” treatment, in which an empty jar was placed in the nest. On 5 June 2008 (water temperature 17.5°C), smallmouth bass guarding newly hatched embryos (TL = 399.2 ± 9.8 mm,  $N = 19$ ) were subjected to a simulated nest intrusion and sampled for blood as described above.

Blood samples were held in water–ice slurries for no more than 1 h, and then centrifuged at 10,000g for 5 min (Compact II Centrifuge, Clay Adams, NJ). Plasma samples were flash frozen in liquid nitrogen and stored at –80°C until analysis.

### Hormone analysis

Cortisol concentrations were determined using a commercial radioimmunoassay kit (ImmunoChem Cortisol <sup>125</sup>I RIA kit; MP Biomedicals, Orangeburg, NY) previously validated for teleost fish (Gamperl et al. 1994). All samples were run in a single assay. Intra-assay variability (% CV) was 7.3%. For the measurement of testosterone (T), plasma samples were extracted three times using 5 ml ethyl acetate. The dried extract was resuspended in phosphate-buffered saline (pH 7.6) containing 0.3% gelatine following the methods outlined in McMaster et al. (1992). T content was measured in duplicate by <sup>3</sup>H-radioimmunoassay using antibodies provided by Medicorp (AS0116, Medicorp Inc., Montréal, QC). Cross-reactivity of the T antibody with 11-KT was 7.5%. All samples were run in a single assay, and intra-assay variability was 5.2%. There was insufficient plasma to run a T assay for two males guarding fresh eggs (resulting in a sample size of 22) and for one male guarding embryos (resulting in a sample size of 18).

### Statistical analysis

No difference in male TL was found among the groups ( $F_{2,46} = 0.314$ ,  $p = 0.73$ ). Therefore, this parameter was not included as a covariate, and Student's  $t$  tests were used to compare aggression scores, circulating cortisol levels, and circulating T levels between fish guarding fresh eggs subjected to the simulated nest intrusion, and the sham group. To investigate whether the intensity of aggression was related to hormone levels, analysis of covariance (ANCOVA) models were run with T or cortisol as the independent variable, offspring development stage (eggs or embryos) as the dependent variable, and aggression (aggressive behaviors in 3 min) as the covariate. The interaction between group and aggression was included in the model.

For all tests, residuals were tested for normal distribution using goodness-of-fit tests, and for homogeneity of

variance using Levene's test or by visual inspection (Zar 1999). Assumptions were met in all but one case (see “Results and discussion”). All analyses were performed in the statistical packages JMP, version 7.0.1 (SAS Institute Inc., Cary, NC). The level of significance for all tests ( $\alpha$ ) was 0.05. All results are stated as mean ± standard error.

## Results and discussion

Did parental smallmouth bass respond to the bluegill as a nest intrusion?

Parental smallmouth bass exhibited aggressive behavior towards a bluegill in a jar, and showed no behavioral response to an empty glass jar placed in the nest (Table 1). This difference was statistically significant ( $t_{28} = -6.019$ ,  $p < 0.01$ ), although the uniform zero scores in the sham treatment group violated the test's assumptions.

Is there a role for androgens in aggressive nest defense?

Fish subjected to a simulated brood predator displayed higher circulating T levels than those subjected to the empty glass jar ( $t_{26} = -2.481$ ,  $p = 0.02$ ; Table 1). Furthermore, a significant positive correlation was detected between circulating T level and the intensity of the aggressive response (full ANCOVA model  $R^2 = 0.555$ ,  $F_{3,36} = 14.963$ ,  $p < 0.01$ ; aggression term in ANCOVA  $F_{1,1} = 4.821$ ,  $p = 0.03$ ; Fig 1a). As found in previous studies in this species (O'Connor et al. 2011) and in the plainfin midshipman fish (*Porichthys notatus*; Knapp et al. 1999), circulating T concentrations were higher in fish guarding eggs than in fish guarding embryos (offspring development stage term in ANCOVA  $F_{1,1} = 9.873$ ,  $p < 0.01$ ). There was no significant influence of offspring development stage on the positive correlation between circulating T concentration and aggression (offspring development stage × aggression interaction term in ANCOVA  $F_{1,1} = 0.749$ ,  $p = 0.39$ ). Collectively, these results demonstrate that circulating T is elevated following aggressive nest defense, and this elevation correlates with the intensity of aggressive behavior.

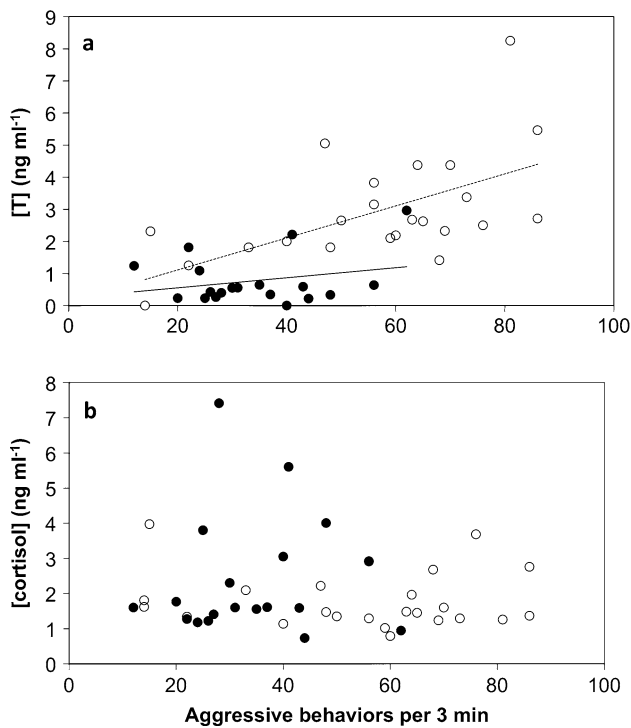
Hanson et al. (2009) measured circulating T levels in smallmouth bass immediately (less than 5 min) following a simulated nest intrusion by a bluegill sunfish and failed to detect a relationship between parental aggression and circulating T concentration. The apparent discrepancy in results between Hanson et al. (2009) and the current study is explained by the different time courses of sampling: T concentrations in Hanson et al. (2009) likely reflected baseline levels, while T concentrations in the current study

**Table 1** Behavioral responses and circulating steroid [cortisol and testosterone (T)] concentrations in male parental smallmouth bass (*Micropterus dolomieu*) presented with either a simulated (bluegill

sunfish in a glass jar placed in the nest) or sham (empty jar placed in the nest) nest intrusion

	Bluegill present	Empty jar	<i>t</i> statistic	<i>p</i> value
Aggressive behaviors per 3 min	<b>54.8 ± 4.5 (24)</b>	<b>0.0 ± 0.0 (6)</b>	<b>6.019</b>	<b>0.01</b>
[T] (ng ml <sup>-1</sup> )	<b>3.10 ± 0.34 (22)</b>	<b>1.39 ± 0.33 (6)</b>	<b>2.481</b>	<b>0.02</b>
[cortisol] (ng ml <sup>-1</sup> )	1.8 ± 0.2 (24)	1.8 ± 0.5 (6)	0.028	0.97

Mean and standard error of the mean are presented, with sample size in brackets. Bold text indicates statistically significant differences between fish presented with the simulated nest intrusion and fish presented with the sham control (Student's *t* tests,  $\alpha = 0.05$ )



**Fig. 1** Circulating **a** testosterone (T) and **b** cortisol concentrations following a simulated nest intrusion for male parental smallmouth bass (*Micropterus dolomieu*) guarding fresh eggs (*empty symbols*) or embryos (*filled symbols*). T concentrations were positively correlated to the level of aggression, while cortisol concentrations were unrelated to aggression (ANCOVA tests,  $\alpha = 0.050$ )

(collected 25 min after disturbance) reflect post-intrusion levels. Collectively, the results strongly suggest that circulating androgen concentrations transiently increase during parental care in response to a threat to offspring, and play a role in aggressive nest defense. This is supported by a previous study in which parental smallmouth bass treated with an androgen receptor antagonist (cyproterone acetate) displayed reduced aggression towards a simulated nest predator when compared with control paternal fish (Dey et al. 2010). To the best of our knowledge, this is the first example of an androgen increase in a teleost fish in response to what is strictly a threat to the offspring rather than to a conspecific territorial challenge. Overall,

however, this finding is consistent with the challenge hypothesis (Wingfield et al. 1990).

Is there a role for cortisol in aggressive nest defense?

Cortisol levels of smallmouth bass subjected to the simulated nest intrusion did not differ from those subjected to the empty glass jar ( $t_{28} = 0.028$ ,  $p = 0.97$ ; Table 1); all parental smallmouth bass exhibited low plasma cortisol concentrations (Fig. 1b). Moreover, neither aggressive behavior nor offspring development stage affected these uniformly low plasma cortisol values (full ANCOVA model  $F_{3,39} = 0.701$ ,  $R^2 = 0.051$ ,  $p = 0.56$ ; Fig 1b). Thus, in the smallmouth bass, cortisol does not appear to play a role in aggressive nest defense behavior. This finding is interesting because aggressive nest defense is energetically costly (Cooke et al. 2006) and cortisol mobilizes energy reserves during a stress response (Mommensen et al. 1999). Our results imply that a nest predator does not invoke a stress response, and that aggression and stress are uncoupled in this system. This result is consistent with previous results in parental plainfin midshipman fish, showing no relationship between offspring development stage and circulating cortisol (Knapp et al. 1999), but contrasts with aggressive social interactions, where cortisol is elevated and may play a significant role in mobilizing energy reserves (e.g., Gilmour et al. 2005). Parental aggression may also be a measure of parental quality (e.g., Tolonen and Korpimäki 1994).

The positive correlation between aggression towards a nest predator and circulating T concentration provides support for the hypothesis that androgens play a role in aggressive nest defense behavior in teleost fish. The androgen response to nest predators resembled that proposed by the challenge hypothesis for male–male conspecific territorial challenges. However, we found no evidence that cortisol functioned in aggressive nest defense behavior in this system.

**Acknowledgments** All fish were sampled under an Ontario Ministry of Natural Resources Scientific Collection Permit and handled in accordance with the guidelines of the Canadian Council on Animal

Care as administered by Carleton University and the Queen's University Biological Station. This research was supported by NSERC Discovery Grants to S.J.C., K.M.G., and G.V.D.K., and a Research Achievement Award from Carleton University to S.J.C. C.M.O. was supported by an Ontario Graduate Scholarship, and by Carleton University. The authors also wish to thank members and friends of "Cooke Country" for assistance with fieldwork, particularly Jake Davis, Cody Dey, Eric Fontaine, Marie-Ange Gravel, Kyle Hanson, Sean Landsman, Sarah McConnachie, Rana Sunder, Alex Wilson, Samantha Wilson, and Claire Yick. Finally, many thanks to Cody Dey and Jacquie Matsumoto for their invaluable assistance with androgen assays.

## References

- Coble DW (1975) Smallmouth bass. In: Clepper H (ed) Black bass ecology and management. Sport Fishing Institute, Washington, DC, pp 21–33
- Cooke SJ, Philipp DP, Wahl DH, Weatherhead PJ (2006) Energetics of parental care in six syntopic centrarchid fishes. *Oecologia* 148:235–249
- Desjardins JK, Hazeldon MR, Van Der Kraak G, Balshine S (2005) Male and female cooperatively breeding fish provide support for the "challenge hypothesis". *Behav Ecol* 17:149–154
- Dey CJ, O'Connor CM, Gilmour KM, Van Der Kraak G, Cooke SJ (2010) Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care. *Horm Behav* 58:599–605
- Fuzzen M, Bernier NJ, Van Der Kraak G (2011) Stress and reproduction. In: Norris DO, Lopez KH (eds) Hormones and reproduction in vertebrates, vol 1: Fishes. Elsevier, Toronto, pp103–117
- Gamperl AK, Vijayan MM, Boutilier RG (1994) Experimental control of stress hormone levels in fishes: techniques and applications. *Rev Fish Biol Fish* 4:215–255
- Gilmour KM, DiBattista JD, Thomas JB (2005) Physiological causes and consequences of social status in salmonid fish. *Integr Comp Biol* 45:263–273
- Gross MR, Sargent RC (1985) The evolution of male and female parental care in fishes. *Am Zool* 25:807–822
- Hanson KC, O'Connor CM, Van Der Kraak G, Cooke SJ (2009) Paternal aggression towards a brood predator during parental care in wild smallmouth bass is not correlated with circulating testosterone and cortisol concentrations. *Horm Behav* 55:495–499
- Hegner RE, Wingfield JC (1987) Effects of brood-size manipulation on parental investment, breeding success, and reproductive endocrinology of house sparrows. *Auk* 104:470–480
- Hirschenhauser K, Oliveira RF (2006) Social modulation of androgens in male vertebrates: meta-analysis of the challenge hypothesis. *Anim Behav* 71:265–277
- Hirschenhauser K, Taborsky M, Oliveira T, Canario AVM, Oliveira RF (2004) A test of the 'challenge hypothesis' in cichlid fish: simulated partner and territory intruder experiments. *Anim Behav* 68:741–750
- Knapp R, Wingfield JC, Bass AH (1999) Steroid hormones and paternal care in the plainfin midshipman fish (*Porichthys notatus*). *Horm Behav* 35:81–89
- Magee SE, Neff BD, Knapp R (2006) Plasma levels of androgens and cortisol in relation to breeding behavior in parental male bluegill sunfish, *Lepomis macrochirus*. *Horm Behav* 49:598–609
- McMaster M, Munkittrick K, Van Der Kraak G (1992) Protocol for measuring circulating levels of gonadal sex steroids in fish. *Can Tech Rep Fish Aquat Sci* 1836
- Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev Fish Biol Fish* 9:211–268
- Nelson RJ (2000) Homeostasis and behaviour. In: Nelson RJ (ed) An introduction to behavioural endocrinology. Sinauer Associates, Massachusetts, pp 447–496
- O'Connor CM, Gilmour KM, Arlinghaus R, Van Der Kraak G, Cooke SJ (2009) Stress and parental care in a wild teleost fish: insights from exogenous supraphysiological cortisol implants. *Physiol Biochem Zool* 82:709–719
- O'Connor CM, Yick CY, Gilmour KM, Van Der Kraak G, Cooke SJ (2011) The glucocorticoid stress response is attenuated but unrelated to reproductive investment during parental care in a teleost fish. *Gen Comp Endocrinol* 170:215–221
- Philipp DP, Toline CA, Kubacki MF, Philipp DBF, Phelan FJS (1997) The impact of catch-and-release angling on the reproductive success of smallmouth bass and largemouth bass. *N Am J Fish Manag* 17:557–567
- Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Rev* 21:55–89
- Schreck CB, Contreras-Sanchez W, Fitzpatrick MS (2001) Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* 197:3–24
- Silverin B (1986) Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period in the pied flycatcher. *Gen Comp Endocrinol* 64:67–74
- Suski CD, Svec JH, Ludden JB, Phelan FJS, Philipp DP (2003) The effect of catch-and-release angling on the parental behavior of male smallmouth bass. *Trans Am Fish Soc* 132:210–218
- Tolonen P, Korpimäki E (1994) Determinants of parental effort: a behavioural study in the Eurasian kestrel, *Falco tinnunculus*. *Behav Ecol Sociobiol* 35:355–362
- Wendelaar-Bonga SE (1997) The stress response in fish. *Physiol Rev* 77:591–625
- Wingfield JC, Hegner E, Dufty AM, Ball GF (1990) The "Challenge Hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 136:829–846
- Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Upper Saddle River