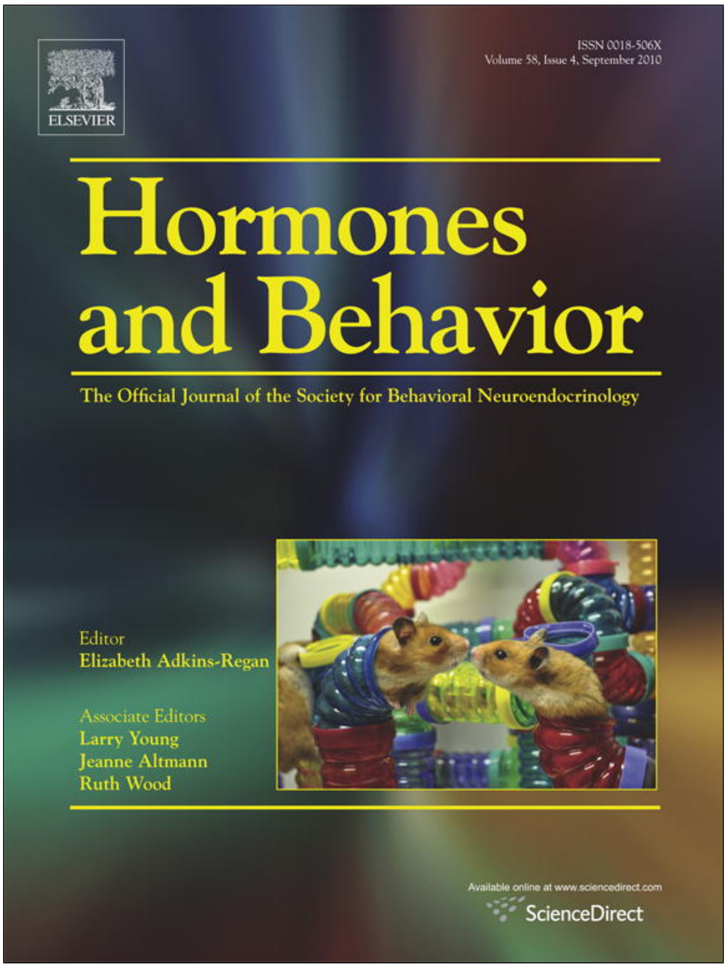

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Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care

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ABSTRACT

Proximate mediators of reproductive behaviors in vertebrates have a long history of study. In fishes, relatively few studies have focused on hormonal control of parental care, despite a comprehensive background on the general physiology of fishes, and the frequent occurrence of parental care behaviors. Studies on this taxon have repeatedly found that the relationships between androgens and paternal care do not follow the predictions made in the avian and mammalian literature. Glucocorticoids may also have a role in mediating parental behaviors, possibly through their role as regulators of metabolism. As such, we investigated the role of 11-ketotestosterone (11-KT) and cortisol in mediating parental effort of male smallmouth bass (*Micropterus dolomieu*) by manipulating hormone titers in wild fish. In smallmouth bass, males spawn annually with a single female and defend a single brood for up to 30 days. Treatment of parental fish with cyproterone acetate (CYA; an androgen receptor antagonist) resulted in a decrease in nest defense in response to a simulated brood predator; however, no changes in nest success, nest tending or biochemical indicators of nutritional status were detected. Treatment with exogenous cortisol did not change parental behavior, but did increase the rate of nest failure, possibly owing to the energetic cost of chronically elevated cortisol concentrations. We discuss these findings in the context of resource-driven trade-offs and highlight life history as an important factor controlling parental effort in species with costly parental care behaviors.

Introduction

Studies of parental investment in care-providing species have yielded an abundance of literature regarding the relationship between animal behavior and fitness. Many researchers have considered parental investment as a function of the specific costs to the parent and the survival and health of the offspring (Trivers, 1972, 1974). Other studies have focused on the physiological mechanisms through which parental care is mediated (see Storey et al., 2006). Endocrine systems play an important role in the initiation and control of reproductive activities, including parental care (e.g., Brown, 1993; Hunt et al., 1999), yet elucidation of specific roles for each hormone in relation to behavior has proven difficult (reviewed by Ziegler, 2000; see also Bender et al., 2008; Angelier and Chastel, 2009).

Androgens are often cited as critical agents in stimulating the reproductive activities of male vertebrates (Silverin, 1979; Stacey, 1983; Matty, 1985). In addition to their role in spermatogenesis (Singh et al., 1995), androgens are essential for the expression of certain reproductive behaviors, such as territory acquisition and nest building (e.g., Smith, 1969). In many fish, 11-ketotestosterone (11-KT) is considered to be the dominant male androgen (Kime, 1993; Borg, 1994). 11-KT is typically higher in male than female teleosts (e.g. Lokman et al., 2002) and is more effective than testosterone (T) in stimulating secondary sex characteristics, spermatogenesis and reproductive behaviors (reviewed by Borg, 1994). Although there is some agreement on the pre-reproductive importance of gonadal androgens, the roles of these hormones during parental care are more contentious. Classical views suggest that androgens are incompatible with parental care behaviors because they mediate aggressive male–male interactions in competitions for mates and territories (Wingfield et al., 1987, 1990). This 'challenge hypothesis' has been supported by studies in a variety of taxa, most notably birds (e.g. Silverin, 1980; Oring et al., 1989; Dittami et al., 1991; Ketterson and Nolan, 1992; Smith et al., 2005), but also mammals (Cavigelli and Pereira, 2000; Creel et al., 1997; Clark and Galef, 1999), amphibians (Townsend and Moger, 1987), reptiles (Moore, 1986; Klukowski and Nelson, 1998) and fishes (Pankhurst, 1990; Specker and Kishida, 2000; Hirschenhauser et al., 2004). Most evidence for the challenge hypothesis is based on observations of males with elevated

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androgen levels decreasing their provisioning of young (e.g., Hegner and Wingfield, 1987) while increasing sexual (i.e., song rates; Ketterson et al., 1992) and aggressive (i.e., territory size; Chandler et al., 1994) behaviors. Few studies have focused on androgen mediation of other more immediate measures of parental care, such as nest defense (but see Cawthorn et al., 1998; Desjardins et al., 2008; Hanson et al., 2009).

Defense of the nest from heterospecific brood predators is an inherently aggressive behavior that is thought to have evolved in fish from territory defense (Gross and Sargent, 1985). The few studies that have examined the relationship between nest defense and androgens have noted that a negative relationship does not exist (Cawthorn et al., 1998; Hanson et al., 2009) and in fact, androgens may facilitate nest defense in some species (e.g. *Neolamprologus pulcher*, Desjardins et al., 2008).

Stress may play an important role in regulating parental investment in vertebrates (Pereyra and Wingfield, 2003; Love et al., 2004). Glucocorticoids (GCs) have been implicated as key mediators in mobilizing the energy reserves required for energetically costly behaviors such as nest defense (Nelson, 2000), but high levels of GCs also have been linked to increases in nest failure (i.e., abandonment: Love et al., 2004; Magee et al., 2006; O'Connor et al., 2009). The role of stress hormones in fish reproductive behavior has received surprisingly little attention with only a few studies having documented the fluctuations of endogenous GCs in relation to parental care (Knapp et al., 1999; Pankhurst et al., 1999; Buchner et al., 2004; Bender et al., 2006; Magee et al., 2006; Bender et al., 2008) although parental care is present in over 20% of fish families (Gross, 2005).

The smallmouth bass (*Micropterus dolomieu*) is a long-lived centrarchid fish endemic to lakes and rivers in eastern North America. Smallmouth bass males typically spawn annually with a single female and exhibit solitary, protracted parental care (Coble, 1975). In early spring, mature males actively seek and defend a territory in which they construct a nest and court a female. The female departs after spawning while the male remains to care for the brood for up to 6 weeks (Ridgway, 1988). Although adult smallmouth bass have few natural predators, their developing broods are heavily preyed upon; vigilant parental care is required for offspring to survive to independence (Philipp et al., 1997; Gravel and Cooke, 2009). Parental care in smallmouth bass consists of two main activities: brood maintenance (egg fanning to improve aeration and remove silt) and nest defense (agonistic interactions with heterospecific brood predators).

The present study aimed to investigate the role of androgens and GCs across the parental care period of smallmouth bass by using exogenous manipulation of hormone titers. Supra-physiological doses of cortisol during parental care were found to increase nest abandonment in largemouth bass (*Micropterus salmoides*) without attenuating parental care behaviors (O'Connor et al., 2009). The present study employed a more physiologically relevant cortisol dose. Administration of cyproterone acetate (CYA), a commonly used androgen receptor antagonist, was used to investigate the role of androgens in mediating parental care. Because androgens are hypothesized to inhibit parental care, we predicted that treatment with CYA would increase nest defense aggression and brood maintenance.

Materials and methods

Experimental animals and treatments

All fish were sampled under an Ontario Ministry of Natural Resources Scientific Collection Permit issued to S.J.C. and handled in accordance with the guidelines of the Canadian Council on Animal Care as administered by Carleton University (B09-12). From May 7 to 9, 2009, 50 male smallmouth bass (*M. dolomieu*) guarding nests with fresh eggs (0–1 day old) were identified by a snorkeling survey in the littoral zone of Sand Lake (N 44 33.418, W 76 14.558), in eastern Ontario, Canada. Angling for bass is prohibited by Ontario provincial law at this time, and we saw no evidence that experimental fish were targeted (i.e., no illegal angling observed) or caught (no evidence of hook wounds) by recreational anglers. The snorkeler recorded nest location and egg score, a visual assessment of brood size ranging from 1 (low) to 5 (high) (Philipp et al., 1997). Nests were marked with numbered polyvinyl chloride (PVC) tiles for identification and fish were assigned to one of three treatment groups: control, CYA or cortisol.

Cortisol-treated fish ($n = 15$) received an intraperitoneal injection of cocoa butter (5 mL kg^{-1} body weight) impregnated with 10 mg mL^{-1} hydrocortisone 21-hemisuccinate (Sigma H4881; Sigma-Aldrich Inc., St. Louis, MO). This widely used method elevates circulating cortisol concentrations for 5–6 days (Gamperl et al., 1994). Similarly, CYA-treated fish ($n = 15$) received an intraperitoneal injection of cocoa butter (2 mL kg^{-1} body weight) impregnated with 10 mg mL^{-1} cyproterone acetate (Sigma C3412; Sigma-Aldrich Inc., St. Louis, MO). Previous studies have administered CYA via daily intraperitoneal injections (e.g., Chowdhury and Joy, 2001), aquarium water (e.g., Sharpe et al., 2004) or food (e.g.,Navarro-Martin et al., 2009), methods that were not possible in the current study. Consequently, the CYA dose used in the current study was estimated from mammalian studies (e.g., Neumann and Kalmus, 1991). Control fish were handled in the same manner as treated fish (see below), but did not receive an intraperitoneal injection. Based on the arguments put forward by DiBattista et al. (2005) concerning variable cortisol responses in sham-treated animals, a sham treatment group (i.e., fish injected with cocoa butter alone) was not included in the experimental design.

Experimental protocol

Once nesting bass with fresh eggs (0–1 day old) had been identified and marked, parental behavior was assessed. First, fish were scored by a snorkeler for brood tending every 30 s for 5 min. A fish received a “tending score” of 0 when it was more than 1 m away from the nest or a score of 1 when it was within 1 m of the nest. This score is modified from O'Connor et al. (2009) and Gravel and Cooke (2009), and was used as a proxy of brood tending behavior (i.e. egg fanning). Second, the snorkeler placed a glass jar (volume = 3.78 L) containing a small (<180 mm in length) bluegill sunfish, a common brood predator, directly on the nest. The snorkeler then recorded the number of ‘hits’ performed by the parental male on the jar in a 60 s period, where a ‘hit’ was defined as the male making physical contact with the jar. This score is a standard measure of parental care behavior (Fitzgerald and Caza, 1993), with more aggressive parents receiving a higher score.

Immediately following behavioral assessments, all fish were captured by rod and reel angling either from a boat or by the snorkeler. Heavy action angling equipment was utilized to minimize fight time (typically <20 s), thereby minimizing stress and anaerobic exercise associated with capture (Kieffer, 2000; Cooke and Suski, 2005). Upon landing, fish were placed in a foam-lined trough filled with fresh lake water. Total length was recorded and any external injuries were noted. A small (approximately 1.25 mL) blood sample was quickly withdrawn from the caudal vessels with a 21-gauge needle and 3 mL vacutainer-style syringe containing lithium heparin as an anti-coagulant (B.D. Vacutainer, Franklin Lakes, NJ). To avoid the use of anesthesia (required to accurately weigh fish), mass was estimated from total length using the relationship $\log_{10}$ mass $= -4.91466 + 3.02635$ ($\log_{10}$ total length) where mass was reported in grams and length in millimeters (Bayley and Austen, 1987). This relationship was verified by weighing a subset of the experimental fish ($n = 21$, $R^2 = 0.92$). Fish were assigned to one of the three treatment groups in a successive manner and treated accordingly (see above). Assigning animals to treatment in this manner did not yield differences in total length among groups (one-way ANOVA, $F_{2,\ 46} = 1.262$, $p = 0.532$, mean $= 410 \pm 5$ mm). During handling and treatment a snorkeler remained at the nest site to protect the brood from predation. Fish were typically released within 3 min of capture and returned to their nests within 5 min of release.

Four days post-treatment (May 11–13, 2009), parental behavior was again assessed using the metrics described above. This sampling point coincided with egg hatching; all developing broods were within 1 day (±) of hatching. A subset ($n = 6$) of each treatment group was recaptured by rod and reel angling and blood was sampled as described above to assess the efficacy of hormone treatments and physiological status. Again, nests were guarded by a snorkeler until the parental fish returned. Seventeen to 19 days post-treatment (May 26, 2009), a snorkeling survey was conducted to assess nest abandonment rates (presence or absence of a brood in the nest). On this date, the developing fry in all nests were heavily pigmented and yolk sacs were barely visible. This developmental stage occurs immediately prior to the swim-up fry stage (Cooke et al., 2002) and will be termed the 'black fry' stage. Parental care behaviors were not quantified; at this stage parental males increase foraging activity and are more widely distributed making the behavioral metrics unreliable (Cooke et al., 2002). A second subset ($n = 6–8$) of fish, different individuals from those sampled during egg hatching, was captured, and blood was sampled.

Analytical techniques

Blood samples were held on ice until processed. Blood glucose was measured in the field using a handheld blood glucose meter that had previously been validated for use in fish (Accucheck Compact Plus, Roche; Beecham et al., 2006; Cooke et al., 2008). Blood was centrifuged at 10,000× *g* for 5 min, plasma was separated into three aliquots and flash frozen in liquid nitrogen prior to storage at −80 °C.

Plasma cortisol concentrations were determined in a single assay using a commercial radioimmunoassay kit (MP Biomedicals, Orangeburg, NY) previously validated for fish (see Gamperl et al., 1994). Intra-assay variability was 4.6%. 11-KT was extracted from 200 μl of plasma using 3 × 5 mL diethyl ether and re-suspended in phosphate-buffer (pH 7.6) containing 0.1% gelatin and 0.01% thimersol. The amount of 11-KT in the plasma extracts was measured by radioimmunoassay as per McMaster et al. (1992) using an 11-KT antibody from Helix Biotech (Vancouver, BC). Inter- and intra-assay variabilities were 8.7% and 4.8%, respectively.

Plasma concentrations of several parameters, previously implicated as indicative of fish nutritional status in nesting smallmouth bass, were also determined (Lall, 2002; O'Connor et al., 2009; Hanson and Cooke, 2009). Plasma total protein, cholesterol, triglyceride and magnesium concentrations were quantified using a Roche Hitachi 917 analyzer (Basal, Switzerland) according to the International Federation of Clinical Chemistry and Laboratory Medicine standard reference model. All techniques followed procedural guidelines for standardization and quality assurance established by the Veterinary Laboratory Association Quality Assurance Program and the Canadian Food Inspection Agency External Proficiency Panel. These analyses were carried out by Idexx Laboratories Inc. (Markham, Canada).

Statistical analyses

Data are presented as mean values ± standard error (SE). All analyses were performed using JMP 8.0.1 software with a level of significance (α) of 0.05 unless otherwise stated. Residuals were examined for normal distribution and heterogeneity of variance using the Shapiro–Wilk goodness-of-fit and Levene's test, respectively. When data were non-normally distributed, the entire data set was rank transformed prior to analysis (Conover and Iman, 1981; Zar, 1999). Analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test, where appropriate, was used to assess the statistical significance of treatment effects on physiological and biochemical parameters. Nest abandonment rate and egg score were analyzed by Pearson's chi-square test (Zar, 1999). Pre-treatment values were analyzed separately and comparisons were made among treatment groups (using ANOVA or chi-square tests as appropriate). This approach was necessary due to the nature of the experimental design (different subsets of fish were recaptured at hatching and at the black fry stage) as well as the large discrepancy in sample sizes between pre- and post-treatment data which made repeated measures ANOVAs unsuitable.

Results

Efficacy of hormone manipulation

Prior to treatment, no differences in cortisol (one-way ANOVA, $F_{2,46} = 2.745$, $p = 0.253$, mean $= 2.70 \pm 0.22$ ng mL^{-1}) or 11-KT (one-way ANOVA, $F_{2,46} = 0.396$, $p = 0.675$, mean $= 10.28 \pm 0.82$ ng mL^{-1}) levels were detected among treatment groups. To assess the effects of hormone manipulation on endocrine titers, plasma hormone concentrations were analyzed by two-way ANOVA using sampling period and treatment as factors. Residuals of post-treatment plasma cortisol concentrations were non-normally distributed and hence data were rank transformed prior to analysis. As expected, a significant effect of treatment (two-way ANOVA, $F_{2,30} = 9.16$, $p = 0.0008$; Fig. 1A) but not sampling period ($F_{1,30} = 0.7167$, $p = 0.404$) was detected for plasma cortisol concentrations, and no interaction effect was present

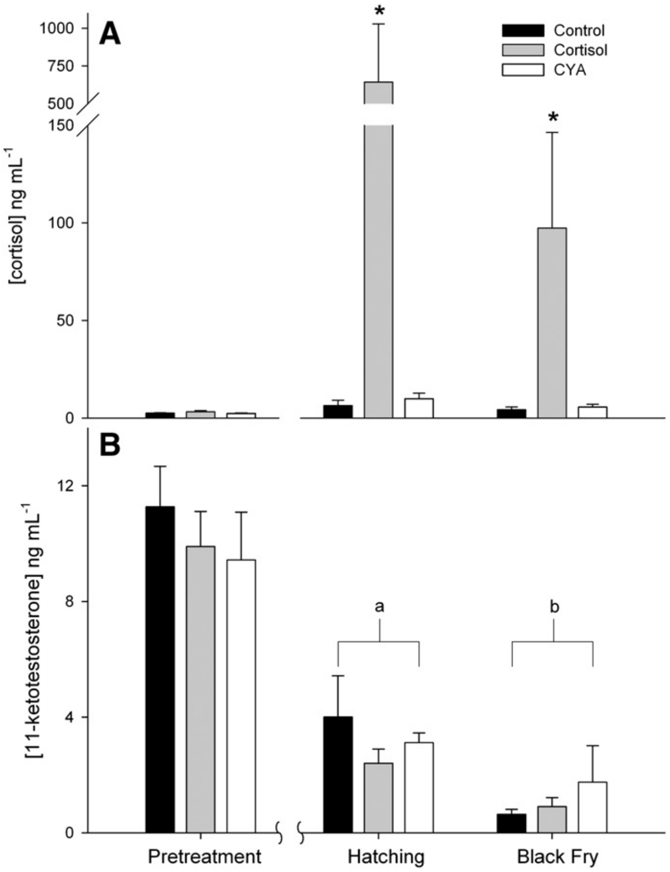

Fig. 1. The effects of hormone manipulation and parental care on circulating concentrations of (A) cortisol and (B) 11-ketotestosterone (11-KT) in smallmouth bass (*M. dolomieu*). Data are means (±SE) for untreated control fish ($n = 6–7$) as well as fish treated with slow-release intraperitoneal implants of cortisol ($n = 6$) or cyproterone acetate (CYA; $n = 5–6$). Pre-treatment values did not differ among treatment groups (one-way ANOVAs). Post-treatment, plasma cortisol concentrations were significantly elevated when compared to controls or CYA-treated fish (indicated by asterisks). Plasma 11-KT concentrations were significantly affected by sampling period (indicated by letters); see text for details of the statistical analysis.

($F_{2,30}=0.1500$, $p=0.861$). Cortisol treatment, but not CYA treatment, significantly elevated circulating cortisol levels over those measured in control fish. The levels achieved in cortisol-treated fish were comparable to endogenous cortisol levels generated by exhaustive swimming in smallmouth bass (330 ± 40 ng mL^{-1}; O'Connor et al., 2009). Values for all fish not treated with cortisol were under 20 ng mL^{-1}, with 90% of fish having values under 10 ng mL^{-1}, suggesting negligible effects of handling or injection on cortisol levels. Plasma 11-KT concentrations were also rank transformed and did not differ among treatment groups (two-way ANOVA, $F_{2,28}=1.573$, $p=0.225$; Fig. 1B). 11-KT concentrations declined as parental care progressed ($F_{1,28}=30.69$, $p<0.0001$; Fig. 1B); no significant interaction between treatment and sampling period was detected ($F_{2,28}=0.1885$, $p=0.829$).

Quantification of parental care behavior

Parental care behaviors were assessed during the fresh egg (i.e., pre-treatment) and egg hatching sampling periods. No differences among groups in egg score were detected (chi-square test, $\chi^2(8)=9.649$, $p=0.291$, mean = 2.98 ± 0.13, $n=50$) an important consideration since brood size is known to influence parental behaviors in smallmouth bass (Ridgway, 1988; Hanson et al., 2009). Data for nest defense aggression were rank transformed prior to analysis. In addition, fish that hit the jar fewer than three times were removed from analysis ($n=2$ control fish, $n=1$ CYA fish) owing to concern that these fish did not recognize the confined bluegill as a nest predator. No differences in nest defense were detected pre-treatment (one-way ANOVA, $F_{2,46}=0.050$, $p=0.951$, mean = 17.53 ± 1.08 hits), but post-treatment, CYA-treated fish performed significantly fewer hits than control fish (one-way ANOVA, $F_{2,13}=4.62$, $p=0.030$; Fig. 2) with cortisol-treated fish being intermediate between the other groups. Tending scores were also rank transformed prior to analysis. No differences were detected among groups pre-treatment (one-way ANOVA, $F_{2,46}=2.046$, $p=0.359$, mean = 10.54 ± 0.12) or post-treatment (one-way ANOVA, $F_{2,17}=4.16$, $p=0.125$; mean = 10.79 ± 0.10 tending score). The rate at which parental male fish abandoned their developing broods differed among groups (chi-square test, $\chi^2(2)=18.05$, $p<0.001$, Fig. 3). Cortisol-treated, but not CYA-treated fish abandoned at a higher frequency than control fish. Because Magee et al. (2006) noted important hormonal differences between nest-tending male bluegill that were known to re-nest later in the breeding season and those that were not, we repeated all the analyses above excluding males that were known to abandon. Performing the analyses in such a way did not change the conclusions of this study.

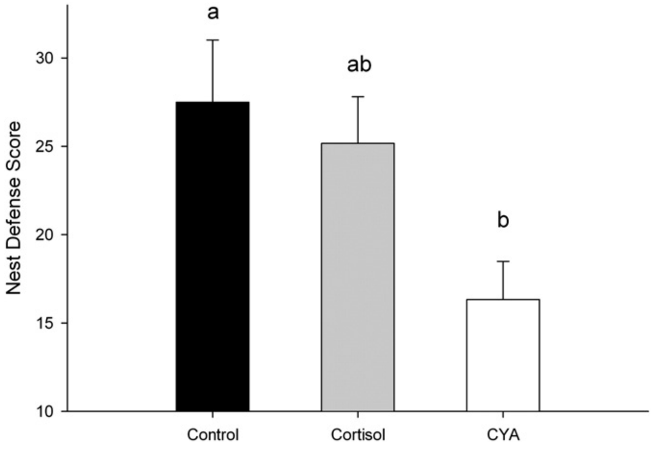

Fig. 2. Nest defense aggression scores for parental male smallmouth bass (*M. dolomieu*) at egg hatching. Data are means (±SE) for untreated control fish ($n=4$) as well as fish treated with slow-release intraperitoneal implants of cortisol ($n=6$) or cyproterone acetate (CYA; $n=6$). Groups that share a letter are not significantly different from one another (one-way ANOVA); see text for details of statistical analysis.

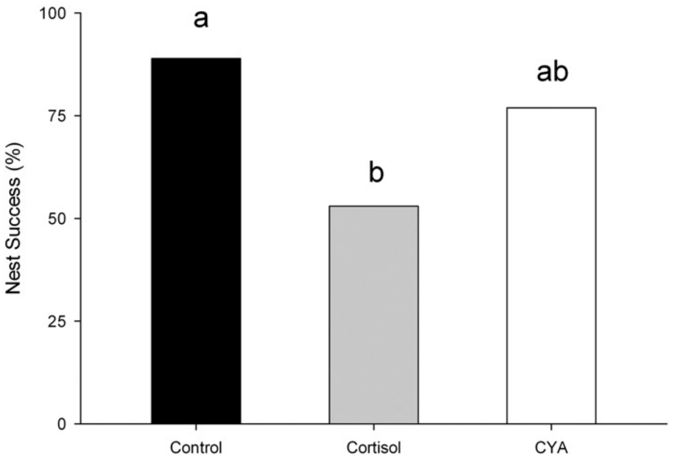

Fig. 3. Nest success of smallmouth bass (*M. dolomieu*) treated with slow-release implants of cortisol ($n=13$) or CYA ($n=13$), or untreated controls ($n=18$). Nest success was defined as the percentage of males within a group that raised a brood to the black fry stage. Groups that share a letter are not significantly different from one another (chi-square test); see text for details of statistical analysis.

Plasma indicators of nutritional status

Plasma biochemical correlates of parental care in black bass have received specific attention in other studies (i.e., Hanson and Cooke, 2009). As such, this study focused on the effect of hormone manipulation on the variables considered. Prior to treatment, no differences were detected among treatment groups for any variable (Table 1). Analysis of rank-transformed plasma glucose concentrations revealed a strong effect of treatment (two-way ANOVA; $F_{2,32}=15.307$, $p<0.0001$; Table 1), with cortisol-treated fish exhibiting significantly higher values than control or CYA-treated fish. A trend for plasma glucose concentrations to decrease across sampling periods was observed, but was not statistically significant ($F_{1,32}=2.723$, $p=0.109$; Table 1) and no interaction between treatment group and sampling period was present ($F_{2,32}=0.2422$, $p=0.786$). Plasma total protein did not differ among treatment groups (two-way ANOVA, $F_{2,27}=1.8187$, $p=0.182$; Table 1) but was significantly affected by sampling period ($F_{1,27}=4.569$, $p=0.042$), with fish

Table 1

Plasma indicators of nutritional status prior to and following manipulation of hormone titers in parental male smallmouth bass (*M. dolomieu*). Mean values with standard error are presented for fish treated with cortisol ($n=11$ pre-treatment and 4–5 post-treatment) or cyproterone acetate (CYA; $n=12$ pre-treatment and 6 post-treatment) along with untreated control fish ($n=13$ pre-treatment and 6–7 post-treatment). Pre-treatment values did not differ among treatment groups (one-way ANOVAs; $P>0.05$ in all cases). Post-treatment values were analyzed by two-way ANOVA with treatment group and sampling period as factors. In no case was the interaction of these two factors significant. Thus, values indicated with bold type are significantly different from the control group (an effect of treatment group), whereas italics indicates a significant difference between the two post-treatment sampling periods (see text for details of statistical analysis).

Variable (mmol/L)	Sampling period	Control	Cortisol	CYA
Cholesterol	Pre-treatment	11.42 ± 0.62	13.11 ± 0.54	12.46 ± 0.74
	Hatching	10.32 ± 0.89	**13.20 ± 1.09**	10.63 ± 0.89
	Black Fry	*12.07 ± 1.25*	***15.45 ± 3.04***	*13.52 ± 2.75*
Glucose	Pre-treatment	3.83 ± 1.87	3.91 ± 3.07	2.83 ± 0.98
	Hatching	2.40 ± 0.67	**4.86 ± 0.74**	2.20 ± 0.67
	Black Fry	2.90 ± 0.69	**6.53 ± 0.79**	2.37 ± 0.79
Magnesium	Pre-treatment	1.06 ± 0.03	1.13 ± 0.05	1.08 ± 0.04
	Hatching	0.96 ± 0.04	1.06 ± 0.05	1.06 ± 0.04
	Black Fry	1.00 ± 0.03	1.01 ± 0.04	1.07 ± 0.03
Total Protein	Pre-treatment	43.85 ± 0.97	45.18 ± 1.33	44.17 ± 1.01
	Hatching	40.67 ± 1.08	41.50 ± 1.33	41.67 ± 1.08
	Black Fry	*42.14 ± 1.40*	*46.74 ± 1.85*	*42.33 ± 1.51*
Triglycerides	Pre-treatment	3.49 ± 0.35	4.17 ± 0.41	3.18 ± 0.38
	Hatching	2.93 ± 0.39	1.96 ± 0.47	2.46 ± 0.39
	Black Fry	2.08 ± 0.29	2.19 ± 0.39	2.24 ± 0.31

guarding black fry having higher values than those with less-developed broods; again, no significant interaction between factors was detected ($F_{2,27} = 1.30$, $p = 0.284$). Plasma cholesterol levels were significantly influenced by both treatment group (two-way ANOVA, $F_{2,27} = 4.886$, $p = 0.015$; Table 2) and sampling period ($F_{1,27} = 8.246$, $p = 0.008$), but no interaction effect was present ($F_{2,27} = 0.1971$, $p = 0.822$). Post-hoc tests revealed cholesterol concentrations to be significantly higher in cortisol-treated fish compared to control fish, with CYA-treated fish being intermediate. Cholesterol concentrations were also significantly higher for fish guarding black fry than for those guarding eggs at the point of hatching. Plasma triglyceride and magnesium concentrations were unaffected by treatment or sampling period (two-way ANOVA, $F_{5,27} = 0.9258$, $p = 0.480$ for triglycerides and $F_{5,27} = 1.347$, $p = 0.275$ for magnesium; Table 1).

Discussion

The role of androgens in mediating paternal care in fish is not well defined. Parental care in fish is predominantly male-only (Gross, 2005), thus fish provide a system in which to study endocrine correlates of parental behavior that contrasts with the primarily female-only or bi-parental avian and mammalian models. As in other teleost species that have been examined (e.g., *Lepomis macrochirus*, Kindler et al., 1989; *Porichthys notatus*, Knapp et al., 1999; *Gasterosteus aculeatus*, Pall et al., 2002a see also, Oliveira et al., 2002), circulating androgen levels in smallmouth bass decrease as parental care progresses. Whether the 11-KT profile is more complex that these data suggest will require sampling with a higher frequency. For example, 11-KT levels in bluegill sunfish drop sharply after spawning but transiently increase during egg hatching (Magee et al., 2006), a pattern than would have been missed in the current study. Regardless, the fact that 11-KT levels are maintained above non-breeding baseline concentrations (Yambe et al., 2004) even 5–6 days after spawning suggests that androgens may play a role in mediating paternal care.

In agreement with this possibility, treatment with CYA, a potent androgen receptor antagonist (Neumann and Kalmus, 1991), resulted in a decrease in nest defense aggression towards a simulated brood predator. The impact of elevated androgens has been examined in several studies, and it has been repeatedly demonstrated that elevated androgens do not necessarily decrease parental investment in male fish (Pall et al., 2002b; Hirschenhauser et al., 2004; Ros et al., 2004; Rodgers et al., 2006; Desjardins et al., 2008). On the other hand, only one other study to date has examined the effect of androgen ‘knock-down’ on male parental behaviors (Pall et al., 2002b). That study found that castration of male three-spined sticklebacks (*Gasterosteus aculeatus*) did not affect parental care intensity measured in terms of egg fanning rates; nest defense was not, however, considered. Few studies have used CYA as a tool to investigate androgen mediation of teleost behavior (but see Kindler et al., 1991; Oliveira et al., 2009) and we are aware of only one study that has used this anti-androgen in free-living fish (Kindler et al., 1991). The approach used in the current study (slow-release implants dissolved in cocoa butter) has not been used previously for CYA administration but is based on methods used for cortisol (from Gamperl et al., 1994). Although the primary action of CYA is to block androgen receptors, CYA has also been shown to decrease circulating androgen concentrations by acting in an anti-gonadotropic manner (Namer, 1988). Several studies (e.g. Kindler et al., 1991) have measured the efficacy of CYA treatment through this effect (i.e., a decrease in circulating androgens), however, the current study demonstrates that relevant behavioral changes can occur as a result of CYA treatment without detectable changes in circulating androgens (see also Oliveira et al., 2009).

The reduction in nest defense aggression with androgen receptor blockade in the current study was somewhat surprising, given that circulating T levels during parental care were not correlated with parental aggression in smallmouth bass (Hanson et al., 2009). However, many researchers have noted the greater importance of 11-KT over T in terms of regulating reproductive physiology and behavior in fishes (e.g., Fosteir et al., 1983; Lily and Stacey, 1983; Borg, 1994, Hirschenhauser et al., 2004). Although CYA treatment caused changes in paternal behavior, no differences were detected among the plasma parameters considered. Therefore, the effects of CYA treatment on paternal behavior are likely the result of direct action of the androgen receptor antagonist rather than indirect effects associated with a decrease in physiological performance. A growing body of evidence suggests that aggressive nest defense behaviors towards heterospecific brood predators are not regulated in the same manner as other paternal behaviors. For example, Neff and Knapp (2009) note that circulating T and 11-KT are not related to egg fanning, egg pecking, rim circling or chases of brood predators but there is an inverse relationship between these two androgens and aggression towards a simulated brood predator. In contrast, Kindler and colleagues (1991) found that bluegill treated with 11-KT tended to be more aggressive towards a simulated brood predator, although this difference was not statistically significant due to high variance. Furthermore, Desjardins et al. (2008) demonstrated a positive relationship between androgen levels and a composite measure of parental care which included defense of young. The current study provides further evidence for a positive relationship between androgens and aggressive nest defense in fish.

It is possible that androgens mediate trade-offs between different parental behaviors. In centrarchid fishes, parental care is comprised of brood maintenance (i.e., fanning eggs, removing silt) and aggressive interactions with brood predators. Since time and energy expended on one behavior cannot be spent on the other; an increase in aggressive chasing of nest predators could negatively affect brood development via a reduction in egg fanning. Ros et al. (2004) demonstrated such a time budget trade-off between territorial behavior and paternal behavior and suggested that androgens could indirectly influence parental investment of male fish by influencing the relative time spent on competing activities. Since CYA treatment caused fish to respond less aggressively to a putative nest predator these individuals should have had more energy available to allocate towards non-aggressive parental behaviors (i.e. brood tending). Although we did not observe any changes in the time spent tending (via tending score) as a result of pharmacological treatment, it is possible that a more sensitive measure of brood care (e.g. video analysis of fanning rates) would reveal such a trade-off between aggressive and non-aggressive parental behaviors.

Much less is known about the role of GCs in teleost parental care. Only a handful of studies have assessed stress hormones during these behaviors (Knapp et al., 1999; Pankhurst et al., 1999; Buchner et al., 2004; Bender et al., 2006; Magee et al., 2006; Bender et al., 2008; Neff and Knapp, 2009) and we are aware of only one study that manipulated cortisol in the wild and evaluated parental care behavior of fish (O'Connor et al., 2009). Our results indicate that male smallmouth bass maintain the intensity of parental care behavior even when faced with a chronic increase in circulating cortisol concentrations; findings similar to those for largemouth bass (O'Connor et al., 2009). The plasma cortisol levels measured in the current study 4 days post-treatment were comparable to endogenous post-stress levels found in smallmouth bass (O'Connor et al., 2009). However, cortisol levels had not decreased to baseline values by 15–17 days post-treatment. This chronic increase in circulating GCs is not typical of a primary stress response but does serve as a useful manipulation for investigating the role of GCs in mediating parental behavior.

Physiological and behavioral resistance to stress during parental care has been observed in avian and mammalian systems. Resistance to stress is suggested to be an adaptive response to increase current reproductive success at a potential cost of reduced survival of the individual (Boonstra and Boag, 1992). Consequently, resistance to stress during reproduction is predicted to be most intense in animals with short breeding seasons, a relatively large number of offspring and few additional chances to breed

(Wingfield and Sapolsky, 2003). Smallmouth bass certainly fit some of these criteria; this species spawns at a narrow range of water temperatures and estimates of age suggested that fish used in this study averaged 9–10 years old with some fish being >15 years old (MacMillan et al., 2002; Barthel et al., 2008) suggesting that many individuals in this study had relatively few remaining opportunities to breed. Wingfield and Sapolsky (2003) postulated several mechanisms by which individuals might resist stress during reproduction. Broadly, these mechanisms fit into two categories: attenuation of GC release to a stressor or a reduction in physiological and behavioral responses to circulating GCs. The current study aimed to investigate whether circulating GCs modulated parental behavior and the fact that no changes in parental behavior were detected, even in the face of a chronic increase in circulating cortisol, is interesting. In a variety of bird species, treatment with exogenous corticosterone caused a reduction in provisioning of the young (Silverin, 1986; Kitaysky et al., 2001; Almasi et al., 2008; Horton and Holberton, 2009) even when corticosterone levels remained below those observed during a stress response (as little as 15–20 ng mL^{-1}; Horton and Holberton, 2009). The cortisol treatment used in the current study was clearly costly, as nearly 50% of fish treated with cortisol abandoned their brood by the black fry stage of development, compared to 11% for controls. O'Connor et al. (2009) suggested that cortisol leads to nest abandonment because cortisol drives both resource trade-offs and results in compromised immune function. Although immune function was not quantified in this study, cortisol treatment did yield plasma hyperglycaemia and hypercholesterolaemia, secondary stress responses consistent with an increase in catabolism of already limited endogenous energy stores (Mommsen et al., 1999). The limited evidence available suggests that male smallmouth bass are able to behaviorally resist high levels of cortisol during parental care but the energetic demands imposed by chronically high levels of GCs (through the cortisol manipulation in this study) may contribute to increased rates of nest failure.

The findings of studies investigating the role of hormones in mediating parental care in fish frequently deviate from the predictions made in the avian and mammalian literature. These differences may reflect, at least in part, inherent environmental and life history differences among taxa. Most notable is that parental care in fish is predominantly male-only (Gross, 2005), whereas most studies of paternal care in birds and mammals focus on species with bi-parental care. In smallmouth bass, this male-only parental care precludes further reproductive opportunities (Coble, 1975). In such cases, the circumstances that promote androgen-mediated trade-offs between sexual or aggressive behavior and parental care are diminished because competition for mates is weak or non-existent during brood development. Consistent with this hypothesis, behavioral-insensitivity to T during parental care in males of some avian species has been noted (reviewed by Lynn, 2008). Some evidence suggests that this insensitivity is adaptive in species having a short breeding season (see Lynn, 2008 for explanation) and in species where reductions in male parental care would significantly reduce reproductive success (Lynn et al., 2005). Behavioral insensitivity (resistance) to stress hormones is also predicted to be most intense in individuals with few, short breeding opportunities and in those for which maintaining parental care is essential to offspring survival (Wingfield and Sapolsky, 2003). A variety of fish species fit these criteria, especially as male-only parental care can be considered to be the most extreme form of offspring dependence on paternal behavior. Taken together, these considerations suggest that life history, especially the form of parental care, can have a significant influence on the interaction between circulating hormone levels and paternal care.

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