RESEARCH ARTICLE

Sex-Specific Consequences of Experimental Cortisol Elevation in Pre-Reproductive Wild Largemouth Bass

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ABSTRACT

Experimental implants were used to investigate the effect of elevated cortisol (the primary stress hormone in teleost fish) on energetic and physiological condition prior to reproduction in male and female largemouth bass (*Micropterus salmoides*). Fish were wild-caught from lakes in Illinois, and held in experimental ponds for the duration of the study. Between 9 and 13 days after cortisol treatment, and immediately prior to the start of the reproductive period, treated and control animals were sampled. Females exhibited lower muscle lipid content, lower liver glycogen content, and higher hepatosomatic indices than males, regardless of treatment. Also, cortisol-treated females had higher hepatosomatic indices and lower final mass than control females, whereas males showed no differences between treatment groups. Finally, cortisol-treated females had higher gonadal cortisol concentrations than control females. In general, we found evidence of reduced energetic stores in female fish relative to male fish, likely due to timing differences in the allocation of resources during reproduction between males and females. Perhaps driven by the difference in energetic reserves, our data further suggest that females are more sensitive than males to elevated cortisol during the period immediately prior to reproduction. *J. Exp. Zool. 319A:23–31, 2013.* © 2012 Wiley Periodicals, Inc.

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The stress response is initiated to increase the chance of survival and promote recovery following a challenging event (Wingfield et al., '98; Sapolsky et al., 2000). However, if challenges are persistent or repeated, the stress response becomes chronically activated, and the same physiological processes that initially promoted survival and recovery become deleterious (Sapolsky et al., 2000; Romero, 2004). In either case, the stress response

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inhibits processes not immediately involved in the survival of the individual, including the suppression of reproductive function (e.g., Silverin, '86; Wingfield and Silverin, '86; Greenberg and Wingfield, '87; Pankhurst and Van Der Kraak, '97; Schreck et al., 2001) and parental care behavior (e.g., Silverin, '86; Clutton-Brock, '91). The mechanisms underlying suppression of reproductive function during a stress response include actions of glucocorticoid stress hormones (cortisol in teleost fish) on the hypothalamic–pituitary–gonadal (HPG) axis that is responsible for the secretion of hormones that influence important reproductive processes such as sexual development, reproductive cycles, and reproductive behavior (see reviews by Greenberg and Wingfield, '87; Moore and Jessop, 2003; Fuzzen et al., 2011). Glucocorticoid actions that are independent of the HPG-axis also may inhibit reproduction (e.g., behavioral effects mediated through brain receptors; Sapolsky et al., 2000).

Although the negative relationship between stress and reproduction is well supported across a variety of taxa, it is not ubiquitous. In particular, individuals with valuable current reproductive opportunities (e.g., individuals with few lifetime reproductive opportunities, with few future reproductive opportunities, or with particularly high-quality offspring) would be predicted to maintain reproductive investment even when faced with a challenge. This "resistance to stress hypothesis" (Wingfield and Sapolsky, 2003) is based on life-history theory; that is, individuals should make optimal trade-offs between current and future reproductive opportunities to maximize fitness (Williams, '66). The mechanisms proposed to underlie resistance to stress and the maintenance of reproductive function during key reproductive periods are varied, and include attenuation of the glucocorticoid response to a challenge, decreased sensitivity to the effects of elevated glucocorticoid hormones, or compensation by other systems for the effects of elevated glucocorticoid hormones (Wingfield and Sapolsky, 2003). Collectively, these physiological changes enable some individuals to maintain reproductive capabilities during key reproductive periods, even when faced with a challenge (see reviews by Angelier and Chastel, 2009; Bókony et al., 2009).

The current study investigated the influence of experimental cortisol elevation prior to reproduction on wild largemouth bass (*Micropterus salmoides*). Cortisol implants were used to increase circulating cortisol concentrations to levels typical of an endogenous stress response in this species (O'Connor et al., 2009). Although glucocorticoid elevation represents only one component of the stress response (Mommsen et al., '99; Sapolsky et al., 2000; Romero, 2004), it is an accepted approach to explore the consequences of stress in a controlled, mechanistic manner (Gamperl et al., '94). Largemouth bass are long-lived fishes (~20 years) with few natural predators as adults. However, heavy predation upon the young requires diligent parental care during early development (Coble, '75; Brown, '84). This parental care falls entirely to the male, who guards the eggs and developing offspring for approximately 30 days after spawning in the spring (Brown, '84). During parental care, male bass exhibit both behavioral insensitivity to elevated glucocorticoids (O'Connor et al., 2009; Dey et al., 2010), and an attenuated cortisol response to a stressor (O'Connor et al., 2011a). However, little is known about the effects of stress on reproduction in females, or in males outside of the parental care period. Although exposure to angling-related stressors prior to reproduction decreased offspring quality in largemouth bass (Ostrand et al., 2004), the mechanisms mediating this effect remain unknown. Sex-specific effects may be important for largemouth bass given the differential time course of energy allocation and different behavioral roles for male and female bass during reproduction. Specifically, females invest heavily in the production of eggs prior to reproduction, while males invest in costly parental care behaviors following spawning (e.g., fanning, nest defense, Cooke et al., 2002, 2006). In the present study, cortisol was elevated for 5 days in the early spring, approximately 2 weeks prior to natural reproduction in our study population, and the physiology of cortisol-treated versus control male and female fish was examined 9–13 days later. Given that females at this reproductive stage have already invested heavily in egg production, while males have yet to invest, we predicted that females would be highly resistant to stress (i.e., show fewer effects of cortisol treatment) relative to males.

MATERIALS AND METHODS

Study Site and Animals

All largemouth bass were wild-caught during 2008 from Sam Dale Lake ($n = 25$; 38°32′N, 88°35′W), Lake Shelbyville ($n = 25$; 39°25′N, 88°46′W), or Ridge Lake ($n = 28$; 39°24′N, 88°09′W) in central Illinois, USA. An initial group of fish for use in a pilot study ($n = 8$) to find an appropriate cortisol treatment dose was caught from Sam Dale Lake on March 23, while the experimental fish ($n = 70$) were caught from all three lakes between March 24 and 29. All animals were captured by electrofishing, and were transported in aerated 500 L insulated tanks to the Sam Parr Biological Station (38°43′N, 88°45′W), where they were given 24–48 hr to recover in 1,000 L flow-through tanks (up to 12 fish per tank) supplied with fresh water from the adjacent Forbes Lake and housed under an open-sided shelter. Water temperature throughout the study was 9–11°C.

Pilot Study

Fish were distributed into size-matched treatment groups: cortisol-treated ($n = 4$) or control ($n = 4$). Cortisol-treated fish were placed in a water-filled trough with the ventral side exposed, and given a 5 mL kg^{-1} intracoelomic injection of 10 mg mL^{-1} hydrocortisone 21-hemisuccinate (Sigma H4881; Sigma–Aldrich, Inc., St. Louis, MO) emulsified in melted cocoa butter. Control fish were not injected but were otherwise handled identically. The objective of this experiment was to investigate the effect of

elevated cortisol concentrations on the physiology of pre-reproductive fish. To this end, non-invasive methods of elevating circulating cortisol concentrations (e.g., feeding fish pellets impregnated with cortisol) would have been optimal, since any handling stress is eliminated. However, such methods are not practical in a field setting, and so single slow-release injections were employed. With this injection method, handling and injection themselves constitute a stressor, and so unsurprisingly, sham treatment (i.e., the injection of the vehicle without the cortisol) has been shown to raise circulating cortisol to levels intermediate between minimally handled controls and cortisol-treated fish (e.g., O'Connor et al., 2009, 2010, 2011b; Dey et al., 2010). Thus, with this experimental protocol, sham treatment represents what is effectively an "intermediate" stress group, and therefore a sham-treated group was not included in the current experiment.

Fish were placed singly in divided raceways supplied with fresh water from Forbes Lake. At 24 hr, approximately 2.5 mL of blood was quickly withdrawn by caudal puncture into lithium-heparinized 3 mL vacutainer-style syringes (B.D., Franklin Lakes, NJ). The time between netting fish and blood sample collection was <3 min. Blood samples were placed in ice-water slurries for no more than 2 hr, then centrifuged at 10,000*g* for 5 min (Compact II Centrifuge, Clay Adams, NJ). Plasma samples were flash-frozen in liquid nitrogen and stored at −80°C until analysis. Twenty-four hours after injection of the implant, cortisol treatment was effective in raising circulating cortisol concentrations to 157 ± 47 ng mL^{-1}, as compared to the control group where cortisol concentrations were 6 ± 3 ng mL^{-1}. This difference was statistically significant (pilot study fish, t-ratio = 3.18, df = 6, $P = 0.01$). Furthermore, circulating levels achieved by the implants were consistent with endogenous levels found in largemouth (O'Connor et al., 2009) and the congeneric smallmouth bass (*Micropterus dolomieu*; Morrissey et al., 2005; O'Connor et al., 2011a) following stressors such as catch-and-release angling, confinement, or exhaustive exercise. Thus, the cortisol dose tested in the pilot trial was deemed to be effective and was used for the experimental treatment group.

Experimental Protocol

Between March 26 and 30, study fish were netted out of holding tanks, weighed, and placed in a water-filled trough with the ventral side exposed. Fish were outfitted with a passive-integrated transponder (PIT) tag (12.5 mm × 2.0 mm) injected into the coelomic cavity for individual identification. Fish were then size- and sex-matched into treatment groups (sex was confirmed later by dissection): cortisol-treated ($n = 35$) or control ($n = 35$). Cortisol-treated fish were given a 5 mL kg^{-1} intracoelomic injection of 10 mg mL^{-1} cortisol emulsified in melted cocoa butter as above, while control fish were not injected. Fish were then released into 0.4 ha drainable research ponds (35 fish per pond, balanced by experimental treatment and treatment date) at Sam Parr Biological Station.

Sampling Protocol

On April 7 and 8, the research ponds were drained (one pond each day). Fish were held in raceways for up to 6 hr between pond draining and sampling. Bass were netted out of raceways, placed in a water-filled trough with the ventral side exposed, and approximately 2.5 mL of blood was withdrawn by caudal puncture as described above. Blood samples were processed as described above. Fish were then euthanized by cephalic blow, weighed, and scanned for a PIT tag using a PIT tag reader (Biomark, Boise, ID). The fish were placed on ice for no more than 1 hr and stored at −20°C for no more than 36 hr before dissection.

During dissection, solidified cocoa butter was removed from the coelomic cavity of cortisol-treated fish and weighed to confirm dose. Gonads were removed and weighed, and sex was recorded. Liver and the eviscerated carcass were also weighed. Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as tissue mass divided by eviscerated body mass (see Barton, 2002). Samples of gonad and liver were placed in duplicate 2 mL tubes (Eppendorf, Mississauga, ON), while a white muscle sample (approximately 2 cm^2) was obtained from the dorsal musculature and wrapped in household aluminum foil. All tissue samples were flash-frozen in liquid nitrogen and stored at −80°C until further analysis.

Analytical Procedures

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., '94). All plasma samples were measured in a single assay. Intra-assay variability (% CV) was 2.9%. Cortisol levels were also measured in ovarian tissue. Steroids were extracted in duplicate from 1 g samples of homogenized wet gonadal tissue using 3 × 5 mL diethyl ether, and resuspended in phosphate buffer (pH 7.6), following the protocols outlined in McMaster et al. ('92). Extracted cortisol concentrations were measured in duplicate for all samples in a single assay using the commercial kit described above. Variability was 5.8%.

As an indication of energetic status (Vijayan et al., '91), hepatic glycogen content was measured in duplicate using the anthrone method of Wedemeyer and Yasutake ('77). Intra-assay variability was 7.6%. Lipid content of muscle tissue was measured in duplicate using a methanol–chloroform extraction following the modified technique of Bligh and Dyer (Bligh and Dyer, '59; Smedes and Askland, '99) as detailed in Gravel et al. (2010). Variability of the duplicates was 10.8%.

Finally, as additional indicators of feeding and condition (Congleton and Wagner, 2006; Hanson and Cooke, 2009), plasma concentrations of cholesterol, triglycerides, total protein, and magnesium were quantified using a Roche Hitachi 917 analyser

(Roche, Basal, Switzerland). These measurements followed the 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, and were carried out by Idexx Laboratories, Inc. (Markham, ON).

Statistical Analysis

A Student's *t*-test was used to determine differences between cortisol-treated and control fish from the pilot study. For the experimental data, a series of general linear models (GLMs) was first run to ensure that holding time prior to sampling did not influence the physiological parameters of interests (final mass, GSI, HSI, hepatic glycogen content, muscle lipid content, and plasma cholesterol, triglycerides, total protein, magnesium, plasma cortisol concentration, and ovarian tissue cortisol concentration). Any physiological parameter influenced by holding time prior to sampling was deemed an inappropriate endpoint for our experiment, and excluded from further analysis. A series of GLMs was then run to assess the influence of our treatment (cortisol-treated or control), sex (male or female), initial mass, and interaction terms on the appropriate physiological parameters of interest (final mass, GSI, HSI, hepatic glycogen content, muscle lipid content, and plasma cholesterol, triglycerides, total protein, and plasma cortisol concentrations). A similar GLM was run for ovarian tissue cortisol content, but excluding sex and the associated interaction terms as predictor variables. Tukey's honestly significant difference (HSD) post hoc tests were run following GLMs with significant interaction terms to determine where among the groups the differences lay. All variables were scaled by their standard deviation and centered by their means to make estimates comparable for all model terms and to improve interpretability of interaction effects (Schielzeth, 2010). For all models and all model terms, $\alpha = 0.05$. Unless otherwise noted, values are presented as mean $\pm$ 1 standard error of the mean (SEM). All analyses were performed using R version 2.14.0.

RESULTS

Holding time between pond draining and individual sampling had a significant effect on circulating magnesium concentrations ($F_{1,68} = 6.08$, $P = 0.02$) and cholesterol concentrations ($F_{1,68} = 5.11$, $P = 0.03$). Therefore, circulating magnesium and cholesterol concentrations were removed from further analyses. Holding time did not influence the other measured parameters (all $P > 0.19$; Supplementary Table 1).

In the experimental fish, sampled 9–13 days post-injection, cortisol-treated fish displayed lower plasma cortisol concentrations than control fish ($F_{1,62} = 4.99$; $P = 0.03$; Table 1; Fig. 1A), which is likely an effect of chronic stress on subsequent stress responses. Circulating cortisol concentrations were elevated in all animals due to acute stress caused by the capture method (i.e.,

Table 1. Means $\pm$ standard error of the means (SEMs) for physiological parameters measured in cortisol-treated and control male and female largemouth bass (*Micropterus salmoides*; $n = 19$ female and 16 male control fish, 22 female and 13 male cortisol-treated fish).

Physiological parameter	Sex	Cortisol	Control
Final mass (g)	Male	611.9 $\pm$ 94.6	804.0 $\pm$ 91.5
	Female	812.5 $\pm$ 87.7	660.2 $\pm$ 87.4
Gonadosomatic index (%)	Male	0.46 $\pm$ 0.06	0.55 $\pm$ 0.04
	Female	6.04 $\pm$ 0.73	5.41 $\pm$ 0.49
Hepatosomatic index	Male	1.43 $\pm$ 0.05	1.41 $\pm$ 0.08
	Female	2.36 $\pm$ 0.10	2.14 $\pm$ 0.07
Hepatic [glycogen] (mg g^{-1})	Male	47.1 $\pm$ 9.2	49.9 $\pm$ 7.4
	Female	20.3 $\pm$ 2.0	17.6 $\pm$ 2.6
Muscle lipid (%)	Male	3.78 $\pm$ 0.16	3.64 $\pm$ 0.27
	Female	3.13 $\pm$ 0.11	3.28 $\pm$ 0.13
Plasma [cortisol] (ng mL^{-1})	Male	97.7 $\pm$ 35.8	207.0 $\pm$ 47.8
	Female	106.2 $\pm$ 19.9	154.2 $\pm$ 31.7
Plasma [triglycerides] (mmol L^{-1})	Male	2.62 $\pm$ 0.57	2.45 $\pm$ 0.50
	Female	2.54 $\pm$ 0.39	2.65 $\pm$ 0.34
Plasma [total protein] (g L^{-1})	Male	43.2 $\pm$ 1.4	41.6 $\pm$ 1.3
	Female	41.0 $\pm$ 1.1	40.9 $\pm$ 1.6
Gonad [cortisol] (ng mg^{-1})	Female	111.8 $\pm$ 53.4	11.1 $\pm$ 0.8

For full statistical details regarding differences among groups, see Results Section and Supplementary Table 2.

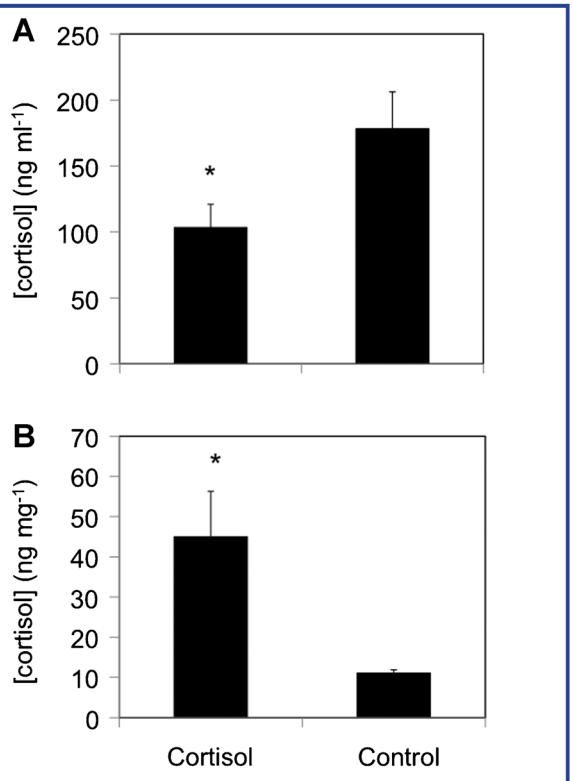

Figure 1. (A) Plasma and (B) ovarian cortisol concentrations in cortisol-treated and control largemouth bass (*Micropterus salmoides*). Blood and tissues samples were collected 9–13 days following treatment with a cortisol implant. Values are means ± standard error of the means ($n = 35$ in each treatment group). Asterisks indicate statistical differences between treatment groups. For full statistical details, see Results Section and Supplementary Table 2.

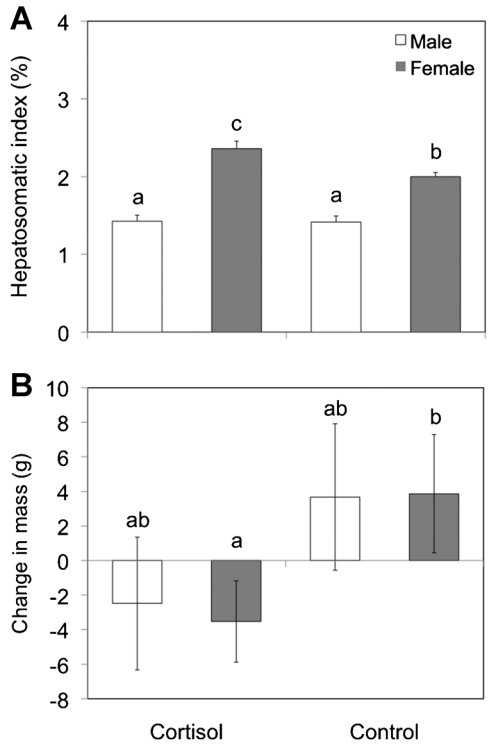

Figure 2. (A) Hepatosomatic index and (B) difference between final and initial mass in cortisol-treated and control male and female largemouth bass (*Micropterus salmoides*). Values are presented as means ± standard error of the means ($n = 19$ female and 16 male control fish, 22 female and 13 male cortisol-treated fish). Different letters indicate statistical differences between groups. For full statistical details, see Results Section and Supplementary Table 2.

draining experimental ponds and collecting fish), and were elevated to a greater extent in control than cortisol-treated animals. However, higher cortisol levels were detected in the ovaries of cortisol-treated females than control females ($F_{1,62} = 2.25$, $P = 0.03$; Table 1; Fig. 1B). There was no influence of sex (in the case of circulating plasma concentrations), initial mass, or the interaction effects on either parameter (all $P > 0.25$; Supplementary Table 2).

GSI was higher in female than male fish ($F_{1,62} = 83.43$, $P < 0.001$; Table 1), and influenced by initial mass of the individuals ($F_{1,62} = 8.02$, $P < 0.01$), but there was no influence of cortisol treatment or any interaction effects (all $P > 0.14$; Supplementary Table 2). There was a significant effect of the interaction between sex and treatment on HSI, with post hoc tests showing that HSI was higher in cortisol-treated females than control females, and with no difference between treatments in male fish ($F_{1,62} = 3.20$, $P = 0.04$; Table 1; Fig. 2A). Regardless of treatment, HSI was higher in females than males ($F_{1,62} = 27.09$, $P < 0.001$; Table 1; Fig. 2A). None of the other effects on HSI were significant (all $P > 0.24$; Supplementary Table 2). Finally, there was a significant effect of initial mass on final mass ($F_{1,62} = 12176$, $P < 0.001$) and there was a significant effect of the interaction among initial mass, sex, and treatment on final mass ($F_{1,62} = 6.02$, $P = 0.02$; Table 1). Post hoc tests revealed that females in the cortisol-treated group lost more mass than females in the control group, whereas there was no difference in mass lost between cortisol-treated and control males over the experiment (Fig. 2B). None of the other effects on final mass were significant (all $P > 0.20$; Supplementary Table 2).

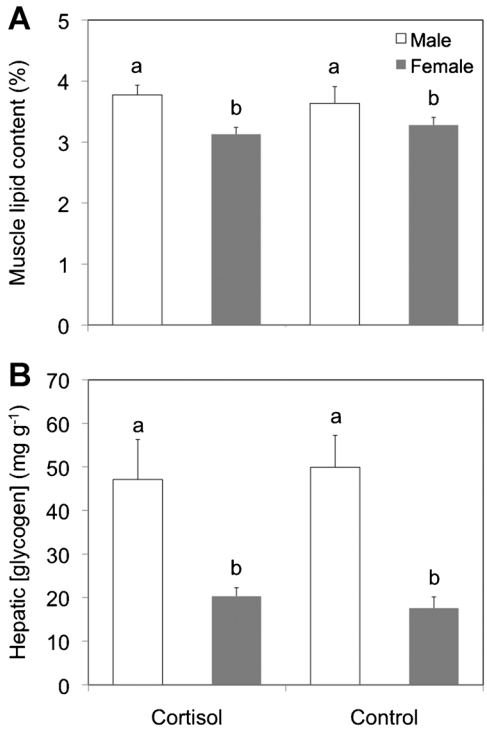

Figure 3. (A) Muscle lipid content and (B) hepatic glycogen concentration cortisol-treated and control male and female largemouth bass (*Micropterus salmoides*). Values are presented as means ± standard error of the means (n = 19 female and 16 male control fish, 22 female and 13 male cortisol-treated fish). Different letters indicate statistical differences between groups. For full statistical details, see Results Section and Supplementary Table 2.

Muscle lipids ($F_{1,62} = 10.71$, $P = 0.001$; Table 1; Fig. 3A) and liver glycogen ($F_{1,62} = 35.52$, $P < 0.001$; Table 1; Fig. 3B) were both higher in males than females, with no influence of treatment, initial mass, or the interaction effects on either parameters (all $P > 0.10$; Supplementary Table 2). There was no effect of treatment, sex, initial mass, or the interaction effects on plasma triglyceride or total protein levels (all $P > 0.07$; Table 1; Supplementary Table 2).

DISCUSSION

Female largemouth bass were more affected by cortisol treatment than male largemouth bass, suggesting that female bass are more vulnerable to stress before reproduction than males. Furthermore, regardless of treatment, male bass had greater energetic reserves than females, suggesting that males may have greater energetic stores prior to reproduction than females. Collectively, these results suggest that physiological limitations may constrain the capacity of female largemouth bass to cope with an additional challenge during the pre-reproductive period.

Sex Differences in Energetic Reserves

Hepatic glycogen and muscle lipid content are both measures of stored energy (Sheridan, '88, '94; Sheridan and Mommsen, '91; Hemre et al., 2002; Gilmour et al., 2012). Lipid metabolism in particular provides the majority of energy for growth, reproduction, and movement in fish (Tocher, 2003). We found both hepatic glycogen and muscle lipid content were higher in males than females. This result probably reflects the different time courses of reproductive investment for male versus female largemouth bass (Brown and Murphy, 2004). Before spawning, females must invest in the production of eggs, which are costly to produce and high in lipids (Tocher, 2003). Males, however, invest in reproduction after spawning, when they perform costly parental care behaviors (e.g., fanning, nest defense; Cooke et al., 2002) that limit feeding opportunities and require the catabolism of stored energy reserves (Cooke et al., 2006). The results of the present study are consist with the premise that before reproduction, females have already depleted stored glycogen and lipids for use during egg production, while males are in the process of storing glycogen and lipids for use during upcoming parental care.

Sex Differences in Response to Cortisol Treatment

Although hepatosomatic index (HSI) is often taken as a proxy of stored energy reserves in fish, with larger values indicating higher stored energy reserves (Campbell and Love, '78; Wootton et al., '78), HSI correlates poorly with more direct measures of stored energy such as liver glycogen (Chellappa et al., '95). Furthermore, HSI is often higher in fish living in contaminated areas (e.g., Khan and Billiard, 2007), in fish with higher parasite burdens (e.g., Tierney et al., '96), or in diseased fish (e.g., Řehulka and Minařík, 2007). We add to this emerging and complex picture of HSI, with HSI being higher in females than males, and higher in cortisol-treated females than control females. Since females had lower stored energy reserves, and cortisol-treated females lost more total mass over the course of the experiment than control females, high HSI in this case appears to reflect relatively consistent liver mass, and a concurrent loss in body mass.

We predicted that females, having already invested heavily in reproduction, would be highly resistant to stress, but instead females were more affected by cortisol treatment than males. There are three potential and non-mutually exclusive explanations for this result. The first potential explanation is that our data provides evidence for physiological limitations to stress coping, rather than results consistent with the resistance to stress hypothesis. The females, in poor physical condition relative to the males, may have been less physiologically capable of appropriately dealing with the additional challenge of prolonged

cortisol elevation. The second potential explanation is that males, in preparation for establishing territories and providing parental care, are highly resistant to stress before reproduction. Finally, a third potential explanation is that females might display resistance to stress by attenuating the cortisol stress response rather than displaying insensitivity to the effects of elevated circulating cortisol. Our results obtained using exogenous manipulation of cortisol levels demonstrate that female largemouth bass are sensitive to the effects of elevated cortisol before reproduction, but do not preclude the possibility that females would display resistance to stress in other aspects of the stress response. Female rainbow trout (*Oncorhynchus mykiss*) display an attenuated cortisol response to a stressor immediately before spawning (Schreck et al., 2001). Therefore, it is possible that female largemouth bass would display a similar attenuation of the cortisol stress response when faced with a stressor before reproduction. The sensitivity to cortisol that we documented could be mitigated by down-regulation of the cortisol response itself, and suggest an interesting direction for future investigation.

Effects of Chronic Cortisol Elevation Before Reproduction

Chronic stress before reproduction negatively affects offspring quality in rainbow trout (Campbell et al., '92) and largemouth bass (Ostrand et al., 2004), and our experiment provides a few potential mechanisms to explain these effects. First, we found lower circulating cortisol levels in the experimental cortisol-treated fish than control fish approximately 2 weeks following treatment. We expected to see elevated plasma cortisol concentrations in the control fish due to the acute stress of capture and handling. However, circulating cortisol levels are subject to negative feedback control (Wendelaar-Bonga, '97; Mommsen et al., '99; Barton, 2002). The most probable scenario is that the chronic elevation of circulating cortisol in the cortisol-treated fish suppressed a further acute stress response to capture and handling. This effect has been widely documented in a variety of fish species (e.g., Barton, 2002; Barton et al., 2005). The implication is that exposure to a chronic stressor inhibits the capacity of individuals to response in an adaptive way to a subsequent acute stressor (Wingfield et al., '98; Barton, 2002), and may be one mechanism by which performance is impaired during a chronic stressor.

More specific to reproduction, the ovaries of cortisol-treated females contained higher cortisol levels than the ovaries of control females, which clearly could provide a mechanism-linking stress before reproduction to impaired offspring development (Ostrand et al., 2004; Sloman, 2010). Evidence suggests that ovaries are protected from the effects of elevated circulating cortisol level (Schreck et al., 2001), and we therefore predicted that due to these protective mechanisms, cortisol concentrations would remain low in the ovaries of control fish despite the acute stress of capture and handling. Our result, that ovarian cortisol concentrations were low in control fish and elevated in cortisol-treated fish, suggests that while largemouth bass can and do protect ovaries against acute increases in circulating cortisol, these protective mechanisms may break down during chronic increases in cortisol. In turn, chronic cortisol elevation results in increases in ovarian cortisol concentrations, and provides a direct mechanism potentially linking chronic stress to impaired offspring development.

However, a caveat concerning the experimental protocol should be noted. We assumed that cortisol implanted into the body cavity was absorbed into the blood vessels, and that the higher cortisol levels measured in the ovaries were due to cortisol deposition via blood circulation. However, in female pre-reproductive fish, the ovaries occupy a large proportion of the body cavity. In many cases, the cortisol implant was in direct contact with the ovaries as well as the caeca, and direct transmission from the implant to the ovaries may have occurred. Therefore, further research is necessary to determine whether the elevated cortisol noted in largemouth bass ovaries in the current study would be replicated during a chronic endogenous stress response, and to determine how this would affect offspring development.

CONCLUSION

In general, we found evidence of reduced energetic stores in female fish relative to male fish, likely due to differences between males and females in the allocation of resources during reproduction. Perhaps driven by this difference, females appeared to be more sensitive than males to elevated cortisol during the period immediately prior to reproduction. These results suggest that physiological capability would limit the ability of females to cope with a secondary stressor during the pre-reproductive period, and provide a potential mechanism linking stress before reproduction to detrimental effects on offspring in largemouth bass.

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