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Regulation of hypothalamic–pituitary–interrenal axis function in male smallmouth bass (*Micropterus dolomieu*) during parental care

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ABSTRACT

Male smallmouth bass (*Micropterus dolomieu*) provide sole parental care until offspring reach independence, a period of several weeks. During the early parental care period when males are guarding fresh eggs (MG-FE), cortisol responsiveness is attenuated; the response is re-established when males reach the end of the parental care period and are guarding free-swimming fry (MG-FSF). It was hypothesized that attenuation of the cortisol response in male smallmouth bass during early parental care reflected modulation of hypothalamic–pituitary–interrenal (HPI) axis function. Male smallmouth bass were sampled at the beginning (MG-FE) and end of the parental care period (MG-FSF), before and/or 25 min after exposure to a standardized stressor consisting of 3 min of air exposure. Repeated sampling of stressed fish for analysis of plasma cortisol and adrenocorticotropic hormone (ACTH) levels was carried out. Males significantly elevated both plasma cortisol and ACTH levels when guarding free-swimming fry but not during early parental care. Control and stressed fish were terminally sampled for tissue mRNA abundance of preoptic area (POA) and hypothalamic corticotropin-releasing factor (CRF) as well as head kidney melanocortin 2 receptor (MC2R), steroidogenic acute regulatory protein (StAR) and cytochrome P450 side chain cleavage enzyme (P450scc). No significant differences in either hypothalamus CRF or head kidney P450scc mRNA abundance were found across parental care stages or in response to stress. However, POA CRF mRNA abundance and interrenal cell MC2R and StAR mRNA abundances failed to increase in response to stress in MG-FE. Thus, the attenuated cortisol response in males guarding fresh eggs may be explained by hypoactive HPI axis function in response to stress. The present is one of few studies, and the first teleost study, to address the mechanisms underlying resistance to stress during the reproductive/parental care period.

1. Introduction

All centrarchid fish provide sole male parental (i.e., paternal) care although the level of parental investment varies markedly among species (Warren, 2009). Male smallmouth bass (*Micropterus dolomieu*) invest extensively in parental care because males provide care to offspring over a period of 4–6 weeks in the late spring and early summer. During this period, males exert considerable energy providing nest defence, either directly by aerating the eggs and defending the nest from predators, or indirectly by limiting their foraging opportunities (Cooke et al., 2006, 2002; Ridgway, 1988; Ridgway et al., 1991). Indeed, although they rarely move more than 3 m away from the nest, telemetry revealed that bass swim the equivalent of over 40 km/day (Cooke et al., 2002). The energy exerted during this period can negatively impact adult growth as well as survival probability over the following winter (Ridgway et al., 1991). Owing to these energy limitations, a life-history trade-off exists between investing in defence of a current brood (current reproductive outcome) and investing in growth and survival (future reproductive outcome) (Williams, 1966).

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; cAMP, 3′-5′-cyclic adenosine monophosphate; cDNA, complementary deoxyribonucleic acid; CRF, corticotropin-releasing factor; P450scc, cytochrome P450 side chain cleavage enzyme; DEPC, diethylpyrocarbonate; EDTA, ethylenediaminetetraacetic acid; HPA, hypothalamic–pituitary–adrenal; HPI, hypothalamic–pituitary–interrenal; MG-FE, males guarding fresh eggs; MG-FSF, males guarding free-swimming fry; MC2R, melanocortin 2 receptor; mRNA, messenger ribonucleic acid; POA, preoptic area; RM ANOVA, repeated measures ANOVA; RNA, ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of the mean; StAR, steroidogenic acute regulatory protein.

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Indeed, reproductive holidays (i.e., when fish skip reproduction in one or more years despite being sexually mature) are common for smallmouth bass, which is presumably a reflection of this trade-off (Barthel et al., 2008; Gravel et al., 2010).

A rise in glucocorticoid levels in response to a stressor initiates a sequence of physiological effects that are important to the survival of an organism (Wendelaar Bonga, 1997). Cortisol is the main glucocorticoid in teleost fish, and acts to mobilize energy resources to cope with increased energy demand during stress and to restore homeostasis (Barton, 2002; Mommsen et al., 1999; Wendelaar Bonga, 1997). Stress-induced levels of cortisol are adaptive, but when stressors are severe or prolonged, cortisol can negatively impact reproductive function (reviewed by Fuzzen et al., 2011; Schreck, 2010; Schreck et al., 2001). For example, nest abandonment in male smallmouth bass increased when cortisol levels were raised exogenously (Dey et al., 2010; O'Connor et al., 2009). Owing to the negative impacts of high cortisol levels on current reproductive outcome, 'resistance to stress' (reviewed by Wingfield and Sapolsky, 2003) may allow for successful reproduction in fish invested in defending a current brood. O'Connor et al. (2011) found that male smallmouth bass attenuated their cortisol response to a standardized stressor at the early stages of parental care, with the stress response being re-established toward the end of the parental care period. The aim of the present study was to investigate the mechanisms underlying attenuation of the cortisol response during early parental care in smallmouth bass.

Cortisol elevation reflects activation of the hypothalamic–pituitary–interrenal (HPI) axis in fish (HP-adrenal in other vertebrates). When the HPI axis is activated, corticotropin-releasing factor (CRF) is released from the preoptic area (POA) of the brain to the pituitary corticotropes (reviewed by Bernier et al., 2009; Flik et al., 2006; Lederis et al., 1994). Stimulation of corticotropes by CRF causes the release of adrenocorticotropic hormone (ACTH), the main secretagogue of cortisol (reviewed by Lederis et al., 1994; Wendelaar Bonga, 1997). Circulating ACTH binds to melanocortin 2 receptors (MC2R), G protein-coupled receptors on interrenal cells in the head kidney (Aluru and Vijayan, 2008), a structure homologous to the mammalian adrenal gland. Binding of ACTH to MC2R activates a cAMP-signaling cascade that facilitates the movement of cholesterol to the inner mitochondrial membrane, via steroidogenic acute regulatory protein (StAR), where it is cleaved to pregnenolone by cytochrome P450 side chain cleavage enzyme (P450scc) in the first and rate-limiting step of cortisol synthesis (Aluru and Vijayan, 2008; Hagen et al., 2006; Mommsen et al., 1999). Because key mediators of cortisol synthesis are expected to increase in response to a stressor (e.g., Aluru and Vijayan, 2006, 2008; Jeffrey et al., 2014) it was hypothesized that attenuation of the cortisol response in male smallmouth bass during early parental care was a result of modulation of HPI axis activity.

To test the above hypothesis, male smallmouth bass guarding nests were sampled during early and late parental care, when the cortisol response is, respectively, attenuated and re-established (O'Connor et al., 2011). Circulating cortisol and ACTH levels were assessed together with mRNA abundance of POA and hypothalamus CRF and head kidney MC2R, StAR and P450scc in males pre- and post- exposure to a stressor.

2. Materials and methods

2.1. Experimental animals

In the spring of 2012, male smallmouth bass [$N = 27$, total length = 36.8 ± 0.9 cm (mean ± SEM); size range = 29.5–46.0 cm] guarding nests were identified by snorkelling on Charleston Lake in eastern Ontario. Males were sampled on two occasions representing the beginning (May 17, 2012; water temperature 16 °C) and end (May 29, 2012; water temperature 20 °C) of the parental care period when males were guarding fresh eggs (MG-FE; $N = 13$) and free-swimming fry (MG-FSF; $N = 14$), respectively. Only males with an egg or fry score of 3–4 (where the maximum score of 5 represents a nest of >4000 eggs/fry and the minimum score of 1 represents a nest of <500 egg/fry) were sampled (O'Connor et al., 2011).

Male smallmouth bass were either sampled as unstressed controls ($N = 14$) or were exposed to a standardized stressor ($N = 13$). Males were angled using a standard rod-and-reel with barbless hooks and a rubber mesh landing net and immediately were placed in a foam-lined trough filled with fresh lake water (O'Connor et al., 2011). Once a fish was hooked, it was landed and placed in the trough within 20 s and total length was measured. A baseline blood sample (1.5 ml) was collected immediately from all fish by caudal puncture using EDTA-coated 3 ml vacutainers (21G needle; BD Vacutainer). Subsequently, fish were either euthanized as controls or subjected to 3 min of air exposure in a damp, foam-lined plastic tub. Stressed fish were allowed to recover in individual tubs filled with fresh lake water until a second blood sample (O'Connor et al., 2011) was collected 25 min post-stress, and again until 2 h post-stress, when fish were euthanized. O'Connor et al. (2011) validated this approach and reported that the peak GC responsiveness occurred 25 min post-stressor. Fish were euthanized via cerebral percussion in accordance with approved standard operating procedures approved by institutional animal care committees (protocol # B10-09, Carleton University) and in accordance with the guidelines of the Canadian Council on Animal Care for the use of animals in research and teaching. Hypothalamus, POA and head kidney tissue were collected immediately after phlebotomy (after blood withdrawal for control fish and 2 h post-stressor for stressed fish). Blood samples were centrifuged at 10,000*g* for 5 min in the field. Plasma and tissue samples were flash frozen in liquid nitrogen and stored at −80 °C for later analysis of plasma cortisol and ACTH levels or mRNA abundance of HPI axis-related genes.

2.2. Hormone analysis

Circulating levels of cortisol and ACTH were determined by radioimmunoassay using commercially available kits (MP Biomedicals). All samples were analyzed together in a single assay where intra-assay variability (% CV) was 6.79% for ACTH. In our hands, the intra-assay variability for cortisol is typically 7.3% (Jeffrey et al., 2014). These kits have been validated for use on teleost samples (Doyon et al., 2006; Gamperl et al., 1994; Lim et al., 2013; O'Connor et al., 2011).

2.3. RNA and first strand cDNA synthesis

RNA extraction and cDNA synthesis were performed as in Jeffrey et al. (2012). Briefly, total RNA was extracted from tissues (10–100 mg) using TRIzol reagent (Invitrogen) according to the manufacturer's protocol, and quantified using a NanoDrop® ND-1000 UV–Vis Spectrophotometer. Tissues were homogenized by repeatedly passing the solution of TRIzol and tissue though syringes with 18G and 23G needles. Prior to cDNA synthesis, 1 μg of POA and hypothalamus, and 2 μg head kidney RNA were treated with deoxyribonuclease I (amplification grade, DNase; Invitrogen) according to the manufacturer's protocol. The cDNA was synthesized by reverse transcription using 100 U of RevertAid H Minus M-MuLV Reverse Transcriptase (Fermentas) and 0.2 μg of random hexamer primer (IDT ReadyMade Primer) according to the manufacturer's protocol.