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Seasonal variation in baseline and maximum whole-body glucocorticoid concentrations in a small-bodied stream fish independent of habitat quality

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ABSTRACT

Alterations to natural habitats are becoming more common due to changes in anthropogenic land use. As such, there is increasing interest in determining how wild animals adapt and respond to environmental stressors. The glucocorticoid (GC) stress response enables animals to react appropriately to environmental challenges but can be affected by many factors, two of which are habitat quality and time of year (i.e., season). This study tested whether baseline and maximum (stress-induced) whole-body cortisol concentrations varied in relation to habitat quality and season using wild central mudminnows (*Umbra limi*) collected from two connected streams differing in habitat quality in each of four seasons. Overall, baseline and maximum cortisol levels did not differ significantly between the two systems but there was evidence of a seasonal effect. Baseline cortisol levels in the fall and summer were significantly ($P < 0.01$) lower than those in winter and spring and maximum cortisol levels in the summer were significantly lower ($P < 0.01$) than those in the spring. Inconsistent with the prevailing paradigm, our results indicate that habitat quality does not always influence baseline GCs or the stress response. In contrast, baseline and maximum GCs in this species do vary seasonally. As such, seasonality should be considered in the interpretation of stress response data especially when using small-bodied stream fish as biological indicators.

1. Introduction

Human activities, past and present, have led to dramatic changes in the biosphere (Vitousek et al., 1997). As the world's population continues to grow and human development accelerates, natural habitats are becoming severely altered. Given the direct relationship between habitat quality and organismal health and condition (Huey, 1991), degraded habitats can result in alterations to organismal physiology (Wingfield, 2005), which has the potential to influence population-level processes (Calow and Forbes, 1998; Ricklefs and Wikelski, 2002; Fefferman and Romero, 2013). Recent studies have begun to unravel some of the specific mechanisms by which changes in habitat quality influence resident biota (Wikelski and Cooke, 2006; Cooke et al., 2013). Evaluating individual stress physiology is one way to quantify the effects of human activities, such as habitat degradation on vertebrates (Homyack, 2010; Baker et al., 2013), thus serving as a useful indicator of ecosystem health (Dale and Beyeler, 2001).

Glucocorticoids (GCs) are found in all vertebrates and changes in GC levels play an important role in responding to and overcoming environmental challenges (Sapolsky et al., 2000). GCs are secreted as a result of activation of the hypothalamic–pituitary–adrenal (HPA) axis in reptiles, birds and mammals, and the hypothalamic–pituitary–interrenal (HPI) axis in fishes and amphibians (Bonga, 1997; Reeder and Kramger, 2005). Corticosterone (or cortisol for fish) is the primary GC stress hormone in vertebrates. This axis is activated when an organism experiences an actual or perceived stressor in its environment (Sapolsky et al., 2000). The acute stress response is widely considered to be beneficial as it is seen across all vertebrates and assists in reacting appropriately to potentially lethal encounters (Wingfield et al., 1998; Breuner et al., 2008). However, it is also accepted that long-term elevation of GCs by chronic stress can be severely detrimental to the health, reproduction and survival of an organism (Breuner et al., 2008). Baseline GC levels regulate basic survival needs (e.g., feeding behaviour, locomotor activity and metabolism; Landys et al., 2006) and maximum GC levels are those that can be measured following the physiological response to a challenge or acute stressor (Sapolsky et al., 2000). Baseline samples are collected immediately following capture (typically within 3 min; Romero and Reed, 2005) and then a stress-induced value is measured some period

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thereafter following exposure to a stressor (e.g., timed handling stressor or air stressor in fish) corresponding to when the GC response is maximal. It is generally considered that lower baseline GC levels indicate an individual or population that is in better condition or exposed to less stress than those with higher baseline GC levels (Bonier et al., 2009a; Busch and Hayward, 2009). As mentioned previously, an acute stress response is beneficial to an organism, though a response that elicits a quick increase of GCs followed by a rapid decrease back to baseline GC levels would intuitively serve the organism better. Such a response would allow animals to respond appropriately to a threat but return to normal functioning quickly, thus avoiding the consequences of chronic stress (Breuner et al., 2008; Angelier and Wingfield, 2013).

Researchers over the past several decades have explored the effects of habitat quality on baseline and stress-induced GCs, particularly among mammals, birds, and herpetofauna. However, the results have been inconclusive and indicate context specificity. In spotted salamanders (*Ambystoma maculatum*), for example, the relationship between stress physiology and habitat quality is sex-specific (Newcomb Homan et al., 2003). Hopkin and DuRant (2011) evaluated baseline and maximum GCs of eastern hellbenders (*Cryptobranchus alleganiensis*) from two stream reaches with differing habitat quality and found no habitat-related differences in GC levels. In the small rodent, the degu (*Octodon degus*), habitats with good cover quality, low ectoparasite loads, and increased food availability were associated with lower baseline and maximum GCs (Bauer et al., 2013). Marra and Holberton (1998) studied baseline and maximal GCs in American redstarts (*Setophaga ruticilla*) from two different habitat types and identified seasonal differences in GCs. Some studies have found no relationship between habitat and GCs, some have found seasonal differences, and in some cases sex differences have been observed. Clearly, interpreting GC concentrations in wild animals is a complex process (Johnstone et al., 2012; Dantzer et al., 2014; Crossin et al., 2015). Interestingly, there are relatively few studies that have examined the influence of habitat quality on wild fish, nor done so across seasons.

The objective of this study was to determine how habitat quality and season affected baseline and maximal (i.e., stress-induced) GC levels in a small-bodied freshwater fish. The model species in this study was the central mudminnow (*Umbra limi*) collected from Watts Creek and Kizell Municipal Drain in Kanata, Ontario, Canada. Central mudminnows (family: Umbridae) are commonly found in freshwater streams, lakes, and ponds of central and eastern North America (Scott and Crossman, 1973). Central mudminnows are typically found in areas with low current and high vegetative cover (Peckham and Dineen, 1957; Martin-Bergmann and Gee, 1985). The two sampling locations in this study join at a confluence and sampling was done 150 m upstream of this confluence in both streams. Previous studies in this area demonstrated that this species travels very little between the two adjoining streams (Bliss et al., 2015). This particular combination of movement and location permitted testing of how habitat quality plays a role in subsequent responses to a standardized stressor while controlling for factors such as weather events, temperature, etc. In this context Kizell Drain was considered to be the more degraded system due to lower levels of sinuosity, habitat complexity, cover, and types of sediment (Goldstein and Meador, 2005; Walsh et al., 2005; Bliss et al., 2015). Although recent advances in techniques allow collection of small sample volumes to estimate plasma GC levels (Sheriff et al., 2011), it is not possible to obtain a sufficiently large blood sample volume for a cortisol assay from small organisms such as the central mudminnow, so a whole-body cortisol measure was conducted (Feist et al., 1990; de Jesus et al., 1991). Given that multiple samples (i.e., baseline and maximum) could not be collected from the same individual, it is not possible to measure individual responsiveness (i.e., the difference between maximum and baseline values) but much information can be obtained from the available whole-body samples.

This work is based on the expectation that organisms in degraded habitats, presumably in poorer condition, will display increased baseline GC levels (Bonier et al., 2009a) and a depressed response when exposed to a standardized stressor (i.e., Hontela et al., 1992; Norris et al., 1999). This experiment tested the hypothesis that the central mudminnow population collected from Kizell Drain, a more disturbed stream (Bliss et al., 2015), would display higher baseline GC levels and lower maximum GC levels than the population collected from Watts Creek, a relatively less disturbed stream. Moreover, we tested the hypothesis that these GC responses would vary across seasons given the manifold effects of water temperature on fish (Fry, 1947) and the strong influence of the reproductive period on GCs and GC response (Wingfield and Sapolsky, 2003). Specifically, we predicted that winter would be among the most challenging periods (i.e, higher baseline levels). We also predicted that during the reproductive period baseline GCs would be elevated while the stress response (maximum) would be depressed consistent with theory (Wingfield and Sapolsky, 2003).

2. Methods

2.1. Study area

Watts Creek and Kizell Municipal Drain (45°20′42″N, 75°52′19″W) are located in Kanata, a suburb of Ottawa, Ontario, Canada. Watts Creek is a tributary of the Ottawa River and collects stormwater from surrounding residential areas, including the aforementioned Kizell Drain. All collections took place at minimum 150 m upstream from the confluence of Watts Creek and Kizell Drain to avoid any possible fish movement between sites. Kizell Drain is narrower, shallower, more channelized, and shows lower habitat complexity and cover than Watts Creek (Bliss et al., 2015). For these reasons, as in previous studies (e.g., Bliss et al., 2015), Kizell is considered a more degraded stream when compared to Watts Creek.

Fish for this study were collected on October 24 (fall), December 2 (winter) of 2013, and May 20 (spring) and July 14 (summer) of 2014. Twenty fish were sampled as encountered during electrofishing (backpack shocking, Model 12, Smith-Root, Vancouver, WA, USA) from both Kizell Drain and Watts Creek except for the winter sampling for which only 19 fish were sampled from both Kizell and Watts. The electrofishing crew worked upstream and ceased shocking upon netting of a central mudminnow greater than 35 mm. Shocking commenced again at least 2 m upstream of the last capture location to reduce the likelihood of sampling fish that had already experienced a shock. For both Kizell Drain and Watts Creek, 10 fish were stunned using a cerebral percussion immediately after capture and stored in liquid nitrogen within 3 min (representing baseline cortisol levels; Romero and Reed, 2005). Meanwhile, the other 10 sampled fish were exposed to a 3 min standardized air stressor in a dampened bucket (as per O'Connor et al., 2011) followed by 27 min in ~2 L of water. At 30 min, these fish were stunned as above and stored in liquid nitrogen (representing maximum cortisol). The 30 min time period was selected as it appears to be the most typical period at which maximum values are attained in freshwater temperate fish (reviewed in Barton, 2002). As fish were encountered by electrofishing, they were alternatively assigned to either baseline or maximum groups. All samples were stored in a −80 °C freezer until analysis could be done.

2.2. Cortisol analysis

Whole frozen fish were crushed using a mortar and pestle and liquid nitrogen to keep the samples frozen resulting in a powdery extract. Total lipid extraction was performed using the Folch method (Folch et al., 1957) optimized for central mudminnow. First, 30 mL of Folch solution (2 chloroform:1 methanol v/v) was added and the mixture homogenized (Polytron homogenizer; Kinematica, Luzern, Switzerland) for 2 min. After sitting for 20 min, 10 mL KCl with 5 mM EDTA was added and the extract allowed to settle for another 20 min. The lipid fraction was removed using a pipette and transferred to a test tube. The