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Urbanization is associated with elevated corticosterone in Jollyville Plateau salamanders

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ABSTRACT

Urbanization has the potential to induce major changes in freshwater systems. Expected increases in human populations will likely amplify these changes and lead to the overall degradation of habitat quality within these systems. Such habitat alterations may function as stressors that can affect glucocorticoid stress hormones in freshwater vertebrates. Examining changes in physiological stress may provide early warning indicators of environmental threats and provide insights into the sub-lethal effects of habitat degradation. The threatened, obligate aquatic, Jollyville Plateau salamander (*Eurycea tonkawae*) is found in urbanized and rural catchments within central Texas and has experienced population declines in heavily urbanized areas. We tested the prediction that salamanders from urbanized sites would have different levels of baseline corticosterone (CORT) and muted or no stress responsiveness (to an external stressor, agitation) compared to salamanders from rural sites. We collected water-borne hormones to measure baseline CORT release rates ($n = 3$ years) and stress responsiveness ($n = 2$ years) in salamanders inhabiting urbanized and rural sites. We also measured "background" CORT from stream water alone at each visit. For the first two years we found that baseline CORT was higher in urbanized sites but not in the third year. Across years and populations, salamanders showed stress responsiveness, suggesting that, even if physiological stress is higher in urbanized areas, it has not resulted in the impairment of the hypothalamic–pituitary–interrenal axis. Background CORT was higher in urban than in rural streams and was positively correlated with mean baseline CORT of salamanders across populations and years. Our results contribute to the goal of finding early warning indicators of environmental threats by demonstrating a relationship between urbanization and the physiological status of *E. tonkawae*, using a rapid, non-invasive measure of stress.

1. Introduction

Increasing human populations have resulted in land-use conversion, with agricultural and urban development being the two most pervasive threats to ecosystems and biodiversity (Czech et al., 2000; Liu et al., 2003; McKinney, 2002; Vitousek et al., 1997). Habitat destruction can have large impacts on freshwater ecosystems (Allan, 2004; Olden et al., 2010; Paul and Meyer, 2001; Wang et al., 1997), wherein the extinction rates for aquatic species are five times higher than for terrestrial species (Ricciardi and Rasmussen, 1999). As human populations continue to grow and use more resources, the quality of natural landscapes is expected to decline further (Foley et al., 2005; Rees, 1999), and therefore, it is important for land managers to understand the impact of anthropogenic habitat alteration on the health of freshwater species. For example, run-off from urbanization alters temperature, water chemistry, and hydrology in freshwater ecosystems, resulting in negative impacts to biodiversity (Allan, 2004; Czech et al., 2000). These alterations may function as stressors that can elevate or dysregulate glucocorticoid (GC) stress hormones in vertebrates (Ellis et al., 2012; French et al., 2008; Hayes et al., 2006; King et al., 2016; Larson et al., 1998; McMahon et al., 2011) and yield persistent effects on individual health (Boekelheide et al., 2012; Martin et al., 2010; Rohr et al., 2013).

The GC stress response has been successfully used to assess an organism's physiological response to disturbance (reviewed in Dickens and Romero, 2013; Ellis et al., 2012; Jeffrey et al., 2015). An acute stress response consists of mobilizing energy stores and suppressing both growth and reproduction in vertebrates (Greenberg and Wingfield, 1987) and is an adaptive mechanism to maintain homeostasis (Romero, 2004; Sapolsky et al., 2000). However, severe and prolonged stressors (e.g. food limitation, anthropogenic disturbance) may result in

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pathology and ultimately death. Chronic stress reduces reproduction, depresses immune function, suppresses growth (McEwen and Wingfield, 2003; Romero, 2004), and can lead to either permanently elevated or depressed baseline levels of circulating GCs (Sapolsky et al., 2000). Further, chronic stress can result in muted or no responsiveness to stressors owing to impaired hypothalamic–pituitary–interrenal (HPI) axis functionality, which can ultimately result in homeostatic failure (Romero, 2004; Romero et al., 2009). In sum, an individual's GCs affect their physiological performance, behavioral performance, and fitness which may be associated with population dynamics (reviewed by Dantzer et al., 2014).

Prior studies of ectotherms have not found consistent patterns for GC levels of populations exposed to anthropogenic stressors, but these populations often show higher baseline GCs in response to anthropogenic disturbances (reviewed by Dantzer et al., 2014) and show muted stress responsiveness (a change in GC levels in response to an acute stressor). Muted stress response may be due to acclimation to stressors, chronic stress inhibiting stress response, or selection for low stress responsiveness in stressful environments. Lower baseline corticosterone (CORT; primary GC in amphibians and reptiles) in disturbed areas is seen in the ornate tree lizard (*Urosaurus ornatus*; French et al., 2008) and mudpuppies (*Necturus maculosus*; Gendron et al., 1997) but no difference in baseline CORT is seen in copperheads (*Agkistrodon contortrix*; Owen et al., 2014) and marine iguanas (*Amblyrhynchus cristatus*; Romero and Wikelski, 2002) of disturbed and undisturbed areas. However, all these studies saw muted or lowered stress responsiveness when these species were found in disturbed areas compared to undisturbed areas. Because the physiological response to stress can vary widely, understanding both baseline and stress-induced GCs is necessary when evaluating the potential impacts of an environmental stressor on animal populations (Cyr and Romero, 2009; Dickens and Romero, 2013; King et al., 2016; Wingfield, 2013).

Aquatic organisms face a suite of potential stressors associated with the "urban stream syndrome" (Meyer et al., 2005; Walsh et al., 2005). One major impact on urban streams is stormwater run-off, but other stressors include sanitary sewer overflow and wastewater treatment plant effluent. Pottinger et al. (2016) found that three-spined sticklebacks (*Gasterosteus aculeatus*) had a positive relationship between the release rates of water-borne cortisol and the concentration of wastewater treatment effluent across sites. Stormwater runoff and sewage effluent may facilitate the movement of exogenous GCs found in the environment. Some steroids such as GCs may be present in elevated amounts (in the water) due to effluent from sewage treatment plants (Kloas et al., 2009), surface run-off (e.g., from urine and feces of terrestrial vertebrates), and/or from natural aquatic sources such as those released by aquatic vertebrates. Exposure to low levels of exogenous CORT (i.e., in the water) increases endogenous CORT and slows development in tadpoles (Belden et al., 2005; Belden et al., 2010; Glennemeier and Denver, 2002b) and affects endogenous CORT in salamanders (Neuman-Lee et al., 2015). Further, other environmental variables associated with the urban stream syndrome may stress organisms such as pH (Chen et al., 2004), conductivity (Chambers, 2011), and water velocity (Barrett et al., 2010).

Individual-level metrics of population health, such as physiological stress, may help in understanding how the effects of anthropogenic disturbance contribute to population decline. While community and population level demographic indicators are commonly used to understand landscape effects on populations, these methods usually result in detection of problems only after declines have occurred (Ellis et al., 2012; Jeffrey et al., 2015). Examination of physiological stress may provide early warning indicators of problems and potentially help determine their cause.

In this study, we examined physiological stress (via integrative measures of CORT) in the federally threatened, obligate aquatic Jollyville Plateau salamander (*Eurycea tonkawae*). We measure CORT because it is the main amphibian GC and increased CORT is immunosuppressive in amphibians (Falso et al., 2015; Fonner et al., 2017). Similar to other stream dwelling salamanders (Barrett and Price, 2014), *E. tonkawae* has experienced population declines in urbanized stream catchments in Austin, Texas (Bendik et al., 2014). We used a non-invasive technique (Gabor et al., 2013; Gabor et al., 2016) to assess baseline CORT release rates and stress responsiveness of *E. tonkawae* from urbanized and non-urbanized streams during late spring 2012–2014 to examine the relationship between physiological status and urbanization. We predicted different baseline CORT release rates between urbanized and non-urbanized populations and a muted stress response in urbanized populations due to prolonged exposure to potential stressors associated with urbanization. In addition, we quantified the amount of exogenous CORT (herein, background CORT) found in stream water, and measured pH, temperature, conductivity, and discharge at each site.

2. Materials and methods

2.1. Sample sites and hormone collection methods

We worked with populations of *Eurycea tonkawae* in the Bull Creek Watershed in Travis County, Texas, USA from 2012 to 2014 (Table 1). We obtained water-borne hormone samples from *E. tonkawae* at two urbanized (Barrow Hollow, Trib 4) and two non-urbanized (Franklin, Lanier) sites from 2012 to 2014. In 2014 we sampled two additional urbanized (Trib 6, Troll) and non-urbanized (Ribelin, WTP4) sites. Stream sites were categorized based on the amount of impervious cover (< 20% for non-urbanized, > 20% urbanized) calculated as the percent of total area of road and building surfaces within each surface catchment. We chose a threshold that evenly splits sample sites above and below the average impervious cover value among sites (mean = 18%); this threshold also corresponds to biologically significant effects in other stream-dwelling salamanders (Willson and Dorcas, 2003). Herein we refer to non-urbanized as rural and urbanized as urban.

We obtained water-borne hormones from *E. tonkawae* at each site from 1000 to 1430 h during sampling events in the late spring (exact dates provided in following subsections). We collected adult salamanders (mean snout–vent length [SVL] ± 1 SD: 29.95 ± 4.5 mm; Bendik, 2017) under cover objects in the stream (i.e., rocks, woody debris) and captured them using hand-held nets. Determination of sex proved challenging in *E. tonkawae*, but prior studies with two closely related species, *E. nana* and *E. sosorum*, found no significant difference in baseline CORT values for males and females (Gabor et al., 2016). No salamanders collected were gravid, as the breeding season for *E. tonkawae* primarily occurs from September–March (Bendik, 2017). We attempted to collect 20 salamanders per site because Gabor et al., (2016) found that for *E. tonkawae*, sample sizes of 15 or 16 individuals have enough power to detect a significant difference, but final sample sizes were site-dependent (samples sizes provided in following subsections). When we caught a salamander, we immediately (< 1 min) placed it into a 250-ml glass beaker lined with a perforated inner Nalgene sleeve to allow easy removal of the salamander from the water

Table 1
Names, locations and description of the collection sites for *Eurycea tonkawae* in central Texas.

Site	Percent Impervious Cover	Development	Year(s) Sampled
Barrow Hollow	27	Urban	2012–2014
Trib 4	28	Urban	2012–2014
Trib 6	25	Urban	2014
Troll	37	Urban	2014
Franklin	0.05	Rural	2012–2014
Lanier	6.5	Rural	2012–2014
Ribelin	4.1	Rural	2014
WTP4	17	Rural	2014