

Original Articles

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] \pm 1 SD: 29.95 \pm 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

sample. Each beaker was filled with either 80 ml of stream water where the salamander was collected (2012, 2013) or 100 ml of well water (2014). We attempted to maintain a stable temperature by placing the beakers into approximately 3 cm of stream water within a cooler. At the end of the 1-h hormone leaching period (the time spent in the beaker), salamanders were removed and water was poured into labeled 120-ml high-density polyethylene containers. Water samples were then placed in a cooler with ice packs until they were frozen in the laboratory at -20°C (within 3 h). Before being released, salamanders were photographed against a standardized grid to obtain SVL measurements (Gabor et al., 2016). Using photographic identification (Bendik et al., 2013) we determined that we did not recapture the same salamanders at any time throughout the experiment. We also collected either 80 ml (2012, 2013) or 100 ml (2014) of stream water at each site to measure background CORT (the exogenous, background concentration of CORT in the stream) using a beaker. All supplies (e.g., beakers, Nalgene sleeves, containers) were cleaned with 95% EtOH and rinsed with DI water before use and gloves were worn throughout.

2.2. Baseline CORT in rural and urban sites

We considered “baseline” CORT release rates to be the values we measured after individual salamanders were placed in beakers for the 1-h leaching period (following methods described above). CORT release rates obtained soon after capture can be considered baseline values and are significantly lower than CORT release rates after exposure to a stressor (Davis and Gabor, 2015; Gabor et al., 2013; Gabor et al., 2016; Reedy et al., 2014). We visited sites between 5 and 7 June 2012 (17–19 individuals per site), 8–13 May 2013 (16–20 individuals per site), and 23 May–18 June 2014 (7–20 individuals per site). All CORT release rates were ln-transformed to meet the statistical assumptions of our models (a summary of untransformed values are provided in the figures). We used a linear mixed model to examine differences in ln CORT across development (urban, rural) with years as fixed effects and site as a random factor.

2.3. Stress responsiveness in rural and urban sites

In 2013 and 2014 we measured the stress responsiveness CORT release rates to agitation from a different set of individuals from these sites. After placing each salamander into a beaker within a beaker box (with cardboard inserts to keep beakers separated), we lifted the box and gently agitated the beakers for one min every three min during the 1-h hormone leaching period (following Gabor et al., 2016). In amphibians, an agitation test has commonly been used to measure HPI responsiveness (Belden et al., 2007; Gabor et al., 2016; Glennemeier and Denver, 2002a). We obtained stress responsiveness CORT release rates from 5 to 12 individuals per site in 2013 and 7–15 individuals per site in 2014. In 2014, we did not obtain stress responsiveness from three sites because we were unable to collect enough salamanders (Barrow, Trib 4, WTP4). We used a linear mixed model to examine the effect of agitation on ln CORT release rates to determine whether urban sites showed muted stress responsiveness. Treatment (baseline, agitated), development (urban, rural), and year were modeled as fixed effects (including all interactions) and site was included as a random effect.

2.4. Hormone extraction

We maintained hormone samples at -20°C for less than three months until the hormone assays were performed (Ellis et al., 2004; Gabor et al., 2013; Gabor et al., 2016). We extracted water-borne hormones (following Gabor et al., 2016). We re-suspended the dried hormone residue for all salamander and stream water samples in a 400 μl solution of 95% enzyme-immunoassay (EIA) buffer (Cayman Chemicals Inc., Ann Arbor, MI, USA) and 5% EtOH. We re-suspended the well water samples in 260 μl solution (95% EIA buffer and 5%

EtOH). We measured CORT samples in duplicate using a CORT EIA plate (Cayman Chemicals Inc.) on a spectrophotometer plate reader at 405 nm (BioTek ELX800). We multiplied the CORT concentrations (pg/ml) by the re-suspension volume to obtain total CORT (pg) within each sample. We then subtracted the value of the background CORT sample (either from the stream or well water) from the salamander CORT value to determine the amount of CORT released by each salamander. However, our measurement of stream background CORT was greater than the measurement of baseline CORT for most samples from a single site in 2013 (Barrow), which resulted in negative numbers after subtracting background CORT value from each sample. These values could not be ln-transformed. Our results did not change when we included these data by adding a constant to all values. Therefore, we chose to present our results excluding these data from this population in 2013. To control for differences in CORT release rates based on size, we divided salamander CORT values by SVL, yielding a CORT release rate in units of pg/SVL/h. To standardize the background CORT value, we divided the amount (pg) by the initial sample volume (80 or 100 ml) to give the concentration of CORT in the stream or well water (pg/ml of water). The use of water-borne CORT samples for *E. tonkawae* has been previously validated by (Gabor et al., 2016). The sensitivity of the CORT EIA plates ranged from 17.90–29.96 pg/ml and all samples were well above the sensitivity of the relative plates. We ran a total of 12 EIA plates and based on our control samples intra-plate variation ranged from 0.47–16.6% and inter-plate variation was 12.95%.

2.5. Environmental variables

To gain a further understanding of the resulting patterns of CORT release rates in *E. tonkawae* during our study, we performed several post-hoc data analyses. We explored whether our grouping factors (year and development) were associated with water quantity and water quality variables at each site. We measured conductivity as specific conductance corrected to 25°C , pH, and water temperature using a Hydrolab MiniSonde 5. We also obtained discharge measurements within two weeks of CORT sampling for each stream. We did not include elevation because it varies minimally in Austin, Texas with a range of 130–305 m above sea level and most of our sites were around 180 m. We conducted two-factor ANOVAs with year, development and their interaction for each dependent environmental variable and followed up using Tukey’s HSD ($\alpha = 0.05$) to test specific group comparisons where applicable. We also examined the relationships among environmental variables and mean baseline CORT release rates from each site-year using linear regressions. Given the relatively small sample size per year and development group ($n = 2$ or 3), single measurements have a large effect. We therefore interpret these results with caution.

2.6. Statistical packages

All analyses were performed using R (R Core Team, 2013). Linear mixed models were estimated with the lme4 package (Bates et al., 2015) and models were assessed using Akaike’s information criterion (Burnham and Anderson, 2002) corrected for small sample size (AICc) as calculated by package AICcmodavg (Mazerolle, 2016). We visually examined residuals from the mixed models for obvious patterns or outliers to assess model fit. We did not observe any problems with heterogeneity of variance upon examination of the residuals from the models.

3. Results

Based on the most optimal model as determined by AICc, ln-transformed baseline CORT release rates for *E. tonkawae* differed between urban and rural sites, but the effect was inconsistent among years (Tables 2 and 3).

Table 2
Model selection results for baseline CORT release rates of *Eurycea tonkawae* sampled across urban and rural sites (Development) and Years (2012–2014).

	K^a	AICc ^b	$\Delta AICc^c$	ω^d	logLik
Development × Year	8	380.8	0	0.99	−182.07
Year	5	391.97	11.17	0	−190.85
Development + Year	6	392.8	12	0	−190.21
Development	4	394.72	13.92	0	−193.27

^a Number of parameters estimated in each model.

^b Akaike's information criterion adjusted for small sample size.

^c Difference between the AIC value for candidate model and the AIC value of the best approximating model.

^d Akaike weights. Probability that candidate model is the best-approximating model.

Table 3

Predicted values from fixed effects and 95% confidence intervals from the most optimal model, baseline CORT release rates ~Year × Development, with Site as a random effect for *Eurycea tonkawae*.

Group	Estimate	95% CI ^a
2012 Rural	0.51	0.23–0.78
2012 Urban	1.11	0.82–1.39
2013 Rural	0.28	0.00–0.55
2013 Urban	0.95	0.60–1.30
2014 Rural	0.71	0.47–0.94
2014 Urban	0.66	0.41–0.90

^a Confidence intervals include uncertainty from fixed effects only.

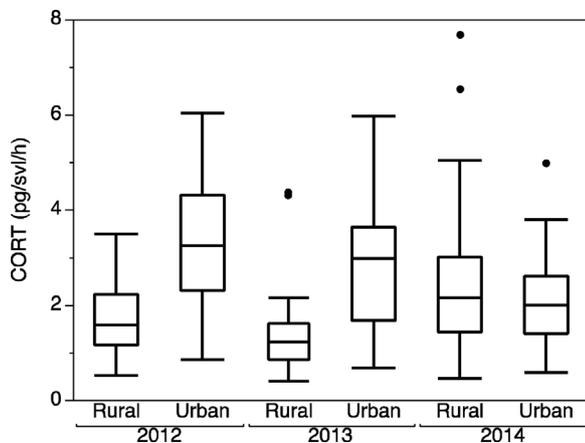


Fig. 1. Box-and-whisker plot of baseline CORT release rates for *Eurycea tonkawae* (standardized by SVL) in rural and urban sites across years.

We observed higher urban baseline CORT release rates than rural baseline CORT in both 2012 and 2013, but baseline CORT release rates between urban and rural sites did not differ in 2014 (Fig. 1; Table 3).

On average, all sites showed stress responsiveness to agitation indicated by the importance of a treatment (baseline, agitated) effect among our candidate models (Fig. 2; Table 4), suggesting that salamanders at urban sites were not compromised in their ability to mount a stress response to agitation. We also observed a year by development interaction consistent with our baseline CORT release rates results.

Stream background CORT exhibited a similar pattern to salamander baseline CORT release rates, where urban sites exhibited higher values compared to rural sites the first two years (2012: adjusted $P = 0.019$; 2013: adjusted $P = 0.001$), but not for the third year (2014: adjusted $P = 0.40$; Fig. 3a; Supplemental Material Table 1). Stream background CORT was positively correlated with mean baseline CORT release rates ($t = 2.344$, $P = 0.036$; Fig. 3b; Supplemental Material Table 2).

Conductivity was higher for urban sites compared to rural ones, and this effect was consistent across years ($F_{1,13} = 32.57$, $P = 0.0002$; Supplemental Material Table 1), but there was no relationship between

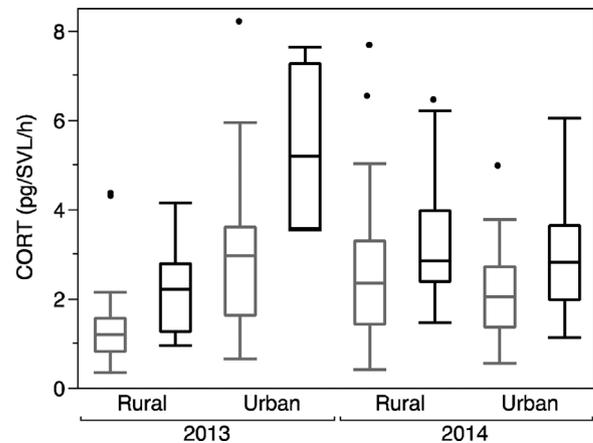


Fig. 2. Box-and-whisker plot of baseline (grey) and agitation (black) CORT release rates for *Eurycea tonkawae* (standardized by SVL) in rural and urban sites across years.

Table 4

Model selection results for difference in baseline and stress response (agitation) CORT release rates (treatment: Trt) across rural and urban sites (Development) and Years (2013, 2014) for *Eurycea tonkawae*.

	K^a	AICc ^b	$\Delta AICc^c$	ω^d	logLik
Development × Year + Trt	7	391.97	0	0.95	−188.75
Development × Year × Trt	10	397.88	5.91	0.05	−188.48
Development + Year + Trt	6	405.28	13.31	0	−196.47
Trt × Year + Development	6	407.38	15.41	0	−196.46
Trt	4	411.04	19.07	0	−201.44
Development × Year	6	421.66	29.69	0	−204.66

^a Number of parameters estimated in each model.

^b Akaike's information criterion adjusted for small sample size.

^c Difference between the AIC value for candidate model and the AIC value of the best approximating model.

^d Akaike weights. Probability that candidate model is the best-approximating model.

conductivity and mean baseline CORT release rates ($t = 1.26$, $P = 0.230$; Supplemental Material Table 2). Temperature, pH, and discharge did not exhibit any statistically significant patterns among year and development factors (Supplemental Material Table 1). Temperature, pH, conductivity, and discharge did not show a relationship with mean baseline CORT release rates (Supplemental Material Table 2).

4. Discussion

Eurycea tonkawae from urban sites are exposed to a suite of potential stressors associated with the “urban stream syndrome” (Walsh et al., 2005), including degraded water quality (e.g., elevated conductivity, run-off, wastewater effluent) and altered hydrologic regimes. For example, pollutants such as polycyclic aromatic hydrocarbons (PAH) are prevalent in some Austin, Texas streams (Diaz and Diago, 2014; Scoggins et al., 2007). Additionally, organochlorines and polybrominated diphenyl ether have been shown to bioaccumulate in *E. tonkawae* from urban sites (Diaz and Diago, 2014). In this study, we found evidence that salamanders in urban streams have elevated baseline CORT release rates compared to those in rural streams. Elevated baseline CORT may be indicative of persistent stressors within urban streams where populations of *E. tonkawae* have declined (Bendik et al., 2014; Bowles et al., 2006). Elevated baseline CORT may also have direct effects on *E. tonkawae* such as affecting mating behavior (Davis and Leary, 2015) antipredator behavior (Davis et al., 2017) or acting as an immunosuppressant (Falso et al., 2015; Fonner et al., 2017).

We found across-year variation in baseline CORT release rates, indicating that elevated levels are not temporally consistent. Our use of

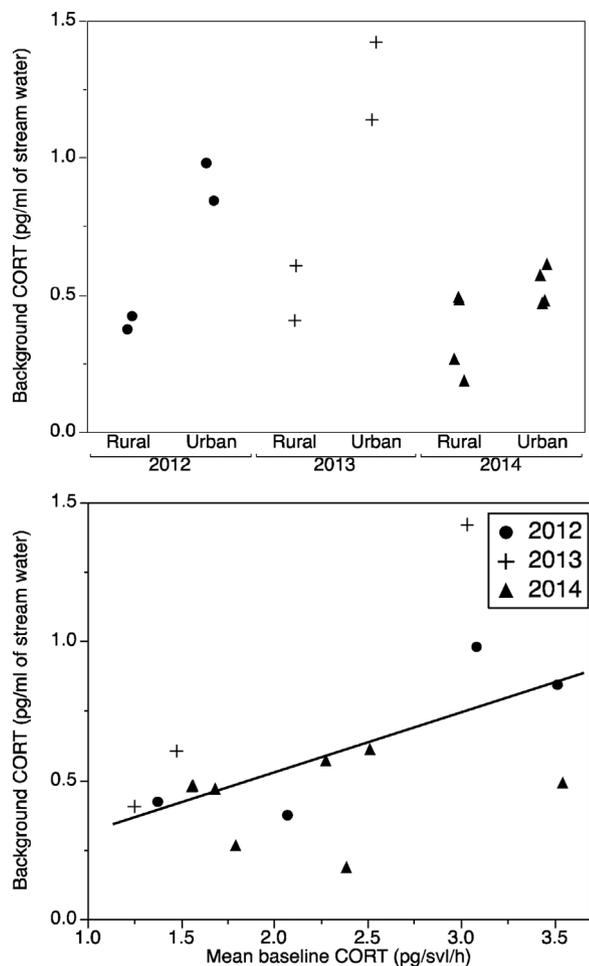


Fig. 3. Relationship between stream background CORT and (A) development across years for each site (2012 = circles, 2013 = plus, 2014 = triangles) and (B) mean baseline CORT release rates of *Eurycea tonkawae*.

different water sources for the CORT leaching period may have resulted in different baseline CORT release rates in 2014. Initially, we used stream water to leach hormones because we reasoned that placing salamanders in novel water chemistry may act as an additional stressor. However, high levels of background CORT in the stream water interfered with our ability to resolve low concentrations of salamander CORT. We therefore switched to leaching in water with low background CORT (well water) for the final year of sampling. This change may have inadvertently affected the salamander baseline CORT release rates. The background CORT in the well water (January: 0.19 pg/ml; June: 0.20 pg/ml) was the same or lower than the background CORT found at all stream sites (range: 0.19–1.43 pg/ml). Salamanders from urban populations may have released less CORT when leached in well water, possibly due to improved water quality (e.g., lower exogenous CORT, decreased conductivity). Conversely, rural populations may have negatively responded to small changes in water chemistry when added to the well water. Although specific conductivity of the well water was higher or the same as the rural sites and lower than most of the urban sites, differences of several hundred $\mu\text{S}/\text{cm}$ are unlikely to be biologically meaningful for *Eurycea* (e.g., Poteet and Woods, 2006). Lower overall stream background CORT values in 2014, or other unmeasured variables (e.g., PAH and organochlorines), may have mediated these differences. Although we did not observe differences in stream discharge among years, spring rains and flooding earlier in the year may have influenced both salamander baseline CORT and stream background CORT, resulting in the year-effect we observed.

The pattern of elevated baseline CORT release rates in urban

streams was consistent with that of stream background CORT. Stream background CORT could originate from salamanders or other aquatic vertebrates (e.g., fish and turtles), or from the landscape via sewage effluent/leaks or terrestrial animals (e.g., wild mammals, pets, and birds). While cortisol is the dominant stress hormone in fish and most mammals, mammals still excrete CORT albeit at lower rates than cortisol (Koren et al., 2012) and CORT can be measured from their feces (Touma et al., 2004). These possible sources, combined with greater impervious cover at urban sites, may lead to run-off containing more exogenous CORT in these streams. Furthermore, urban catchments contain both municipal sewer lines that occasionally leak (e.g., a sewer line runs through Barrow tributary) as well as septic systems (e.g., Trib 6) that leach effluent. Positive feedback between stream background CORT and baseline CORT may explain higher baseline CORT release rates for *E. tonkawae* in urban streams, because amphibians can uptake exogenous CORT from water (Belden and Kiesecker, 2005; Belden et al., 2010; Glennemeier and Denver, 2002b).

We did not find a pattern of chronic stress or muted stress response across urban populations of *E. tonkawae* compared to rural populations. *Eurycea tonkawae* from urban and rural populations had responsive HPI axes as indicated by their ability to mount a CORT stress response (Romero, 2004; Romero et al., 2009). In contrast to our results, other studies have found that urban populations of various species show muted or no stress responsiveness compared to undeveloped sites (French et al., 2008; Gendron et al., 1997; King et al., 2016; Owen et al., 2014; Romero and Wikelski, 2002). Our results could indicate that either the salamanders are not chronically stressed and/or that they have habituated to repetitive urban stressors (Cyr and Romero, 2009). To test the latter hypothesis we would need to inject salamanders with adrenocorticotropin hormone (ACTH) to measure adrenal responsiveness to test for desensitization or exhaustion (Cyr and Romero, 2009) and or examine recovery time from acute stressors (Wingfield, 2013).

Evidence for amphibian declines due to landscape disturbance are well documented (Barrett and Price, 2014; Scheffers and Paszkowski, 2012; Willson and Dorcas, 2003), yet the mechanisms that drive these declines remain poorly understood (Barrett and Price, 2014). Several authors have argued for the need to establish the connection between land use, habitat quality, and physiological performance to aid managers in forming proactive conservation strategies (Cooke and Suski, 2008; Ellis et al., 2012; Jeffrey et al., 2015). Our results contribute to this goal by demonstrating a link between urbanization and the physiological status of *E. tonkawae*, and expand our knowledge of the potential mechanisms affecting population decline in this species. We have demonstrated how a rapid, non-invasive measure of stress may be a useful diagnostic tool for assessing the physiological health of populations. However, it is important to determine whether elevated levels of baseline CORT at the levels we observed are ultimately detrimental to individual fitness. Future studies should continue to explore the possibility of measuring baseline CORT as a proxy for individual fitness and background CORT as a non-invasive ecological indicator. Further, proactive conservation strategies should involve prioritization of high-risk populations (e.g., those with highest levels of background CORT and/or with impaired HPI responsiveness) and identification of the drivers of physiological stress.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2017.10.047>.

References

- Allan, J.D., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annu. Rev. Ecol. Syst.* 35, 257–284.
- Barrett, K., Price, S.J., 2014. Urbanization and stream salamanders: a review conservation options, and research needs. *Freshw. Sci.* 33, 927–940.
- Barrett, K., Helms, B.S., Guyer, C., Schoonover, J.E., 2010. Linking process to pattern: causes of stream-breeding amphibian decline in urbanized watersheds. *Biol. Conserv.* 143, 1998–2005.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48.
- Belden, L.K., Kiesecker, J.M., 2005. Glucocorticosteroid hormone treatment of larval treefrogs increases infection by *Alaria* sp trematode cercariae. *J. Parasitol.* 91, 686–688.
- Belden, L.K., Moore, I.T., Wingfield, J.C., Blaustein, A.R., Beaupre, S.J., 2005. Corticosterone and growth in Pacific treefrog (*Hyla regilla*) tadpoles. *Copeia* 2005, 424–430.
- Belden, L.K., Rubbo, M.J., Wingfield, J.C., Kiesecker, J.M., 2007. Searching for the physiological mechanism of density dependence: does corticosterone regulate tadpole responses to density? *Physiol. Biochem. Zool.* 80, 444–451.
- Belden, L.K., Wingfield, J.C., Kiesecker, J.M., 2010. Variation in the hormonal stress response among larvae of three amphibian species. *J. Exp. Zool. Part A Ecol. Genet. Physiol.* 313A, 524–531.
- Bendik, N.F., Meik, J.M., Gluesenkamp, A.G., Roelke, C.E., Chippindale, P.T., 2013. Biogeography, phylogeny, and morphological evolution of central Texas cave and spring salamanders. *BMC Evol. Biol.* 13, 1–18.
- Bendik, N.F., Sissel, B.N., Fields, J.R., O'Donnell, L.J., Sanders, M.S., 2014. Effect of urbanization on abundance of Jollyville Plateau salamanders (*Eurycea tonkawae*). *Herpetol. Conserv. Biol.* 9, 206–222.
- Bendik, N.F., 2017. Demographics, reproduction, growth, and abundance of Jollyville Plateau salamanders (*Eurycea tonkawae*). *Ecol. Evol.* 7, 5002–5015.
- Boekelheide, K., Blumberg, B., Chapin, R.E., Cote, I., Graziano, J.H., Janesick, A., Lane, R., Lillycrop, K., Myatt, L., States, J.C., Thayer, K.A., Waalkes, M.P., Rogers, J.M., 2012. Predicting later-life outcomes of early-life exposures. *Environ. Health Perspect.* 120, 1353–1361.
- Bowles, B.D., Sanders, M.S., Hansen, R.S., 2006. Ecology of the Jollyville Plateau salamander (*Eurycea tonkawae*: Plethodontidae) with an assessment of the potential effects of urbanization. *Hydrobiologia* 553, 111–120.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multi-model Inference: A Practical Information-theoretical Approach*. Springer, New York.
- Chambers, D.L., 2011. Increased conductivity affects corticosterone levels and prey consumption in larval amphibians. *J. Herpetol.* 45, 219–223.
- Chen, C.Y., Hathaway, K.M., Folt, C.L., 2004. Multiple stress effects of vision[®] herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands. *Environ. Toxicol. Chem.* 23, 823–831.
- Cooke, S.J., Suski, C.D., 2008. Ecological restoration and physiology: an overdue integration. *Bioscience* 58, 957–968.
- Cyr, N.E., Romero, L.M., 2009. Identifying hormonal habituation in field studies of stress. *Gen. Comp. Endocrinol.* 161, 295–303.
- Czech, B., Krausman, P.R., Devers, P.K., 2000. Economic associations among causes of species endangerment in the United States: associations among causes of species endangerment in the United States reflect the integration of economic sectors, supporting the theory and evidence that economic growth proceeds at the competitive exclusion of nonhuman species in the aggregate. *Bioscience* 50, 593–601.
- Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conserv. Physiol.* 2.
- Davis, D.R., Gabor, C.R., 2015. Behavioral and physiological antipredator responses of the San Marcos salamander, *Eurycea nana*. *Physiol. Behav.* 139, 145–149.
- Davis, D.R., DeSantis, D.L., Gabor, C.R., 2017. Antipredator behavior of the Barton Springs salamander (*Eurycea sosorum*) in response to aquatic invertebrates: potential consequences of habitat restoration. *Hydrobiologia* 795, 129–137.
- Davis, A.G., Leary, C.J., 2015. Elevated stress hormone diminishes the strength of female preferences for acoustic signals in the green treefrog. *Horm. Behav.* 69, 119–122.
- Diaz, P., Diago, A., 2014. Salamander Toxicity Project. Texas Fish and Wildlife Conservation Office Monthly Report. May.
- Dickens, M.J., Romero, L.M., 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. *Gen. Comp. Endocrinol.* 191, 177–189.
- Ellis, T., James, J.D., Stewart, C., Scott, A.P., 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. *J. Fish Biol.* 65, 1233–1252.
- Ellis, R.D., McWhorter, T.J., Maron, M., 2012. Integrating landscape ecology and conservation physiology. *Landsc. Ecol.* 27, 1–12.
- Falso, P.G., Noble, C.A., Diaz, J.M., Hayes, T.B., 2015. The effect of long-term corticosterone treatment on blood cell differentials and function in laboratory and wild-caught amphibian models. *Gen. Comp. Endocrinol.* 212, 73–83.
- Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. *Science* 309, 570–574.
- Fonner, C.W., Patel, S.A., Boord, S.M., Venesky, M.D., Woodley, S.K., 2017. Effects of corticosterone on infection and disease in salamanders exposed to the amphibian fungal pathogen *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* 123, 159–171.
- French, S.S., Fokidis, H.B., Moore, M.C., 2008. Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban–rural gradient. *J. Comp. Physiol. B* 178, 997–1005.
- Gabor, C.R., Fisher, M.C., Bosch, J., 2013. A non-invasive stress assay shows that tadpole populations infected with *Batrachochytrium dendrobatidis* have elevated corticosterone levels. *PLoS One* 8, e56054.
- Gabor, C.R., Zabierek, K.C., Kim, D.S., Alberici da Barbiano, L., Mondelli, M.J., Bendik, N.F., Davis, D.R., 2016. A non-invasive water-borne assay of stress hormones in aquatic salamanders. *Copeia* 2016, 172–181.
- Gendron, A.D., Bishop, C.A., Fortin, R., Hontela, A., 1997. In vivo testing of the functional integrity of the corticosterone-producing axis in mudpuppy (Amphibia) exposed to chlorinated hydrocarbons in the wild. *Environ. Toxicol. Chem.* 16, 1694–1706.
- Glennemeier, K.A., Denver, R.J., 2002a. Developmental changes in interrenal responsiveness in anuran amphibians. *Integr. Comp. Biol.* 42, 565–573.
- Glennemeier, K.A., Denver, R.J., 2002b. Small changes in whole-body corticosterone content affect larval *Rana pipiens* stress components. *Gen. Comp. Endocrinol.* 127, 16–25.
- Greenberg, N., Wingfield, J.C., 1987. Stress and reproduction: reciprocal relationships. In: Norris, D.O., Jones, R.E. (Eds.), *Hormones and Reproduction in Fishes, Amphibians and Reptiles*. Plenum, New York, USA, pp. 461–489.
- Hayes, T.B., Case, P., Chui, S., Chung, D., Haeffele, G., Haston, K., Lee, M., Mai, V.P., Marjuoa, Y., Parker, J., Tsui, M., 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? *Environ. Health Perspect.* 114, 40–50.
- Jeffrey, J.D., Hasler, C.T., Chapman, J.M., Cooke, S.J., Suski, C.D., 2015. Linking landscape-scale disturbances to stress and condition of fish: implications for restoration and conservation. *Integr. Comp. Biol.* 55, 618–630.
- King, G.D., Chapman, J.M., Cooke, S.J., Suski, C.D., 2016. Stress in the neighborhood: tissue glucocorticoids relative to stream quality for five species of fish. *Sci. Total Environ.* 547, 87–94.
- Kloas, W., Urbatzka, R., Opitz, R., Wurtz, S., Behrends, T., Hermelink, B., Hofmann, F., Jagnytsch, O., Kroupova, H., Lorenz, C., Neumann, N., Pietsch, C., Trubiroha, A., Van Ballegooy, C., Wiedemann, C., Lutz, I., 2009. Endocrine disruption in aquatic vertebrates. *Ann. N.Y. Acad. Sci.* 1163, 187–200.
- Koren, L., Whiteside, D., Fahlman, S., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., Wynne-Edwards, K., 2012. Cortisol and corticosterone independence in cortisol-dominant wildlife. *Gen. Comp. Endocrinol.* 177, 113–119.
- Larson, D.L., McDonald, S., Fivizzani, A.J., Newton, W.E., Hamilton, S.J., 1998. Effects of the herbicide atrazine on *Ambystoma tigrinum* metamorphosis: duration, larval growth, and hormonal response. *Physiol. Zool.* 71, 671–679.
- Liu, J.G., Daily, G.C., Ehrlich, P.R., Luck, G.W., 2003. Effects of household dynamics on resource consumption and biodiversity. *Nature* 421, 530–533.
- Martin, L.B., Hopkins, W.A., Mydlarz, L.D., Rohr, J.R., 2010. The effects of anthropogenic global changes on immune functions and disease resistance. In: Ostfeld, R.S., Schlesinger, W.H. (Eds.), *Year in Ecology and Conservation Biology*, pp. 129–148.
- Mazerolle, M.J., 2016. Package 'AICcmodavg'.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43, 2–15.
- McKinney, M.L., 2002. Urbanization, biodiversity, and conservation. *Bioscience* 52, 883–890.
- McMahon, T.A., Halstead, N.T., Johnson, S., Raffel, T.R., Romansic, J.M., Crumrine, P.W., Boughton, R.K., Martin, L.B., Rohr, J.R., 2011. The fungicide chlorothalonil is nonlinearly associated with corticosterone levels, immunity, and mortality in amphibians. *Environ. Health Perspect.* 119, 1098–1103.
- Meyer, J.L., Paul, M.J., Taulbee, W.K., 2005. Stream ecosystem function in urbanizing landscapes. *J. North Am. Benthol. Soc.* 24, 602–612.
- Neuman-Lee, L.A., Stokes, A.N., Greenfield, S., Hopkins, G.R., Brodie Jr., E.D., French, S.S., 2015. The role of corticosterone and toxicity in the antipredator behavior of the rough-skinned newt (*Taricha granulosa*). *Gen. Comp. Endocrinol.* 213, 59–64.
- Olden, J.D., Kennard, M.J., Leprieux, F., Tedesco, P.A., Winemiller, K.O., García-Berthou, E., 2010. Conservation biogeography of freshwater fishes: recent progress and future challenges. *Divers. Distrib.* 16, 496–513.
- Owen, D.A.S., Carter, E.T., Holding, M.L., Islam, K., Moore, I.T., 2014. Roads are associated with a blunted stress response in a North American pit viper. *Gen. Comp. Endocrinol.* 202, 87–92.
- Paul, M.J., Meyer, J.L., 2001. Streams in the urban landscape. *Annu. Rev. Ecol. Syst.* 32, 333–365.
- Poteet, M.F., Woods, H.A., 2006. Physiological, Survival, and Growth Responses of *Eurycea nana* to Variation in Levels of Conductivity and Dissolved Oxygen; Including Physiological Responses of *Eurycea sosorum* to Conductivity and Dissolved Oxygen. Technical Progress Report to the Barton Springs/Edwards Aquifer Conservation District, Austin, Texas, USA.
- Pottinger, T.G., Williams, R.J., Matthiessen, P., 2016. A comparison of two methods for the assessment of stress axis activity in wild fish in relation to wastewater effluent exposure. *Gen. Comp. Endocrinol.* 230, 29–37.
- R Core Team, 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reedy, A.M., Edwards, A., Pendlebury, C., Murdaugh, L., Avery, R., Seidenberg, J., Aspbury, A.S., Gabor, C.R., 2014. An acute increase in the stress hormone corticosterone is associated with mating behavior in both male and female red-spotted newts, *Notophthalmus viridescens*. *Gen. Comp. Endocrinol.* 208, 57–63.
- Rees, W.E., 1999. FORUM: Consuming the earth: the biophysics of sustainability. *Ecol.*

- Econ. 29, 23–27.
- Ricciardi, A., Rasmussen, J.B., 1999. Extinction rates of North American freshwater fauna. *Conserv. Biol.* 13, 1220–1222.
- Rohr, J.R., Raffel, T.R., Halstead, N.T., McMahon, T.A., Johnson, S.A., Boughton, R.K., Martin, L.B., 2013. Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. *Proc. R. Soc. Biol. Sci. Ser. B* 280 20131502–20131502.
- Romero, L.M., Wikelski, M., 2002. Exposure to tourism reduces stress-induced corticosterone levels in Galápagos marine iguanas. *Biol. Conserv.* 108, 371–374.
- Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model—A new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* 55.
- Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol. Evol.* 19, 249–255.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89.
- Scheffers, B.R., Paszkowski, C.A., 2012. The effects of urbanization on North American amphibian species: identifying new directions for urban conservation. *Urban Ecosyst.* 15, 133–147.
- Scoggins, M., McClintock, N.L., Gosselink, L., Bryer, P., 2007. Occurrence of polycyclic aromatic hydrocarbons below coal-tar-sealed parking lots and effects on stream benthic macroinvertebrate communities. *J. North Am. Benthol. Soc.* 26, 694–707.
- Touma, C., Palme, R., Sachser, N., 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. *Horm. Behav.* 45, 10–22.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of earth's ecosystems. *Science* 277, 494–499.
- Walsh, C.J., Roy, A.H., Feminella, J.W., Cottingham, P.D., Groffman, P.M., Morgan, R.P., 2005. The urban stream syndrome: current knowledge and the search for a cure. *J. North Am. Benthol. Soc.* 24, 706–723.
- Wang, L., Lyons, J., Kanehl, P., Gatti, R., 1997. Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. *Fisheries* 22, 6–12.
- Willson, J.D., Dorcas, M.E., 2003. Effects of habitat disturbance on stream salamanders: implications for buffer zones and watershed management. *Conserv. Biol.* 17, 763–771.
- Wingfield, J.C., 2013. The comparative biology of environmental stress: behavioural endocrinology and variation in ability to cope with novel changing environments. *Anim. Behav.* 85, 1127–1133.