Possible Alteration of Circadian Rhythms in Bats at a Heavy Metal Contaminated Site

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Abstract: The ability of organisms to exhibit daily patterns of behavior and hormone regulation is deeply connected to the changing light levels over any 24 hour solar cycle. Both morphological and physiological mechanisms allow for proper timing of key behaviors such as emergence and sleep so as to maximize benefits and minimize risks to survival. We were interested in how heavy metal contamination may affect circadian activity levels in wild bat populations. Specifically we hoped to determine if the emergence time of bats within the Tar Creek Superfund Site (TC) in northeastern Oklahoma differed from that at two uncontaminated locations within the Oologah Wildlife Management Area (OWMA, Oklahoma). We recorded emergence times visually and by using an acoustical bat detector and compared them to sunset times at each location. We found emergence times of bats at TC occurred significantly later ($p = 0.022$, $df = 3$) than at the combined locations within OWMA.

Introduction

The existence of circadian rhythmicity in regulation of hormonal and behavioral activity of organisms ranges from single-celled forms to higher vertebrates, which points to the importance of this daily phenomenon to survivability and persistence (Young and Kay 2001). This circadian rhythmicity is controlled in mammals by a region of the brain, inside the hypothalamus called the suprachiasmatic nucleus (SCN), that regulates the rhythmic expression of genes by a series of complex feedback mechanisms (Zelinski et al. 2014). Information about time of day projects from the retina through the retinohypothalamic tract (RHT), via glutamate, directly into the SCN (Gompf et al. 2015; Schmoll et al. 2011). In this way the circadian regulation of hormonal activity important to metabolism, sleep-wake cycles, and health follows or entrains to changing light and dark cycles of any given 24 hour period (Schmoll et al. 2011). Research implicates alterations to these circadian regulations or abilities in many human diseases and disorders such as diabetes and cancer (Zelinski et al. 2014). Likewise, circadian regulation of hormones is important to reproduction and health of developing offspring (Boden et al. 2013; Zelinski et al. 2014).

Wild animals are exposed to many different environmental toxicants. Heavy metals such as lead (Pb), cadmium (Cd), and zinc (Zn), when found in the environment, are particularly dangerous because of the risk they pose to development and proper function of the central nervous system of animals (Tokar et al. 2013). Pb is a neurotoxin that, among other effects, inhibits growth of synapses in the brain (Neal et al. 2011). Cd is both a neurotoxin and endocrine disrupting chemical and affects many aspects of behavior and central nervous system function (Huang et al. 2015; Leret et al. 2003).

These metals may also pose a risk to important mechanisms involved in entrainment of circadian processes in organisms experiencing chronic or acute environmental exposure.

Experiments have shown effects by these metals on various components of the circadian system in animals. For example, Rojas-Castañeda et al. (2007, cited in Rojas-Castañeda et al. 2011) found chronic Pb exposure changes circadian activity levels of Wistar rats (*Rattus norvegicus*). This effect may be due to alterations to photoentrainment mechanisms that might occur as Pb accumulates in the retina (Fox and Boyes 2013) and, at chronically low exposure levels, induces rod photoreceptor death (He et al. 2000, 2003). Increases in the time it takes to adjust to changes in light levels may result from this environmental Pb exposure (Fox and Boyes 2013). Furthermore, both Pb and Cd impair glutamate transport (Borisova et al. 2011) which is the main neurotransmitter released from the RHT into the SCN (Gompf et al. 2015). Pb also increases DNA methylation of genes relevant to behavior in animals (Nye et al. 2015). Finally, early Pb exposure affects development and physical properties of the SCN itself (Rojas-Castaneda et al. 2011). Cd may also affect circadian regulatory mechanisms because in Wistar rats it decreased the expression of two major clock genes, Per 1 and Per 2, involved in regulation of circadian cycles (Cano et al. 2007).

During spring and summer 2013 we conducted both visual and acoustical observations of bat activity along Tar Creek (TC; N 36°57.495, W 094°50.731) within the Tar Creek Superfund Site, which is contaminated with toxic levels of Pb, Cd, and Zn (USEPA 2010), and at two uncontaminated locations, Plum Creek (PL; N 36°35.5063, W 095°32.4197) and Panther Creek (PA; N 36°37.747, W 095°31.372), located within the Oologah Wildlife Management Area (OWMA). We compared emergence time, relative to sunset, of bats at TC, PL, and PA. We hypothesized that emergence times would differ at TC when compared to the two uncontaminated sites. Emergence of bats, like all mammals, is in part determined by the phase of endogenous feedback mechanisms controlled by the SCN (Zelinski et al. 2014). Because Pb and Cd affect these mechanisms, as outlined above, and impair the reception of light information by the SCN (Borisova et al. 2011), thus reducing the capacity of animals to properly entrain circadian cycles to changing photoperiods, it is plausible that sleep-wake cycles in exposed animals could be temporally shifted compared to unexposed animals. Because of this potentially reduced ability to photoentrain their sleep-wake cycles to daily changes in photoperiod, we predicted that emergence time, an indicator of the sleep-wake cycle, would occur later in the evening for bats within TC compared to emergence times at the two uncontaminated sites.

Methods

This study was conducted simultaneously with a companion study (Eguren 2014) in which bats were captured with mist nets set across streams at the same locations and dates. Bats captured in mist nets were removed and identified to species in hand. Total number of each captured bat species for each location was recorded. Straight-line distances between sites were determined using the ruler tool in Google Earth Pro. The contaminated site (TC) is separated from the uncontaminated Plum Creek (PL) by 72.8 km and from Panther Creek (PA) by 70.1 km. The two uncontaminated sites are approximately 3.2 km apart. Data collection occurred on separate nights May-August 2013. Twelve nights were spent afield (TC = 8, PL = 2, PA = 2). Mist nets were opened and observation began 30 minutes before sunset and continued for 5 hours or until 1:00 am. On each night of observation we sat about 2 m from each creek adjacent to mist nets set across the creek while holding a bat detector (Convergence Tech Inc., Belfry Bat Detector) pointed directly upward toward the sky. This detector is rated as capable of detecting echolocation calls at distances of 75 to 200 ft (Convergence Tech, Inc. 2015). The same bat detector was used for each sampling night. An observation of a bat was defined as visual observation of a bat around the nets or a clear repeating auditory signal from the bat detector. The time of the first observation (visual or acoustic) of a bat was recorded for each night.

We defined emergence time as the number of minutes after sunset time for each sampling location (USNO 2014) of each recorded first bat observation. The mean emergence time (min) of all sampling nights was determined for each location. To compare mean emergence time of bats at the two uncontaminated sites a two-tailed Student's t-test was used. A one-tailed Student's t-test was conducted in statistical calculations of mean emergence time between contaminated and combined uncontaminated locations.

To estimate local habitat composition at each site, satellite images, dated 23 April 2013, of each sampling location was obtained using Google Earth Pro (GE). Using the ruler tool in GE a circle of radius 250 m was drawn centered on each observation location for each site. Three habitat types were defined: forest, water, and open. Forest was defined as unbroken tracts of tree canopy and isolated clumps of trees. Isolated individual trees were not included. Water was defined as streams or ponds containing water. Open was considered as everything (including roads) that did not fit into forest or water. There were no residential areas within or near any of the sampling sites. In GE, individual polygons were fitted around forest and water habitat within each circle at each location. The surface area (m^2) within each polygon denoting habitat types within each circle was determined. The proportion of each habitat type contained within each circle was then determined and compared across sites.

Results and Discussion

There was only one observation period in which bats were neither seen nor heard. In order to account for any seasonal variation in emergence and activity times (Hayes 1997) only data from months in which the contaminated and uncontaminated sites were both sampled were included in analysis (July and August). This resulted in one observation being discarded for May from TC. Due to flooding at two of the three sites, there were no observations made during June. We did not attempt to identify bats to species based on calls picked up on the acoustical detector; however, the most commonly collected species at each location in the companion study (Eguren 2014) was the Eastern Red Bat (*Lasiurus borealis*). On the nights and locations in which we recorded observations, 12 total bats were captured across all three sites. Eleven of them (91.7%) were *L. borealis*. The average number of minutes after sunset until the first bat was observed for TC, PL, and PA was 77.50 ± 40.17 minutes (n = 6), 26.00 ± 7.07 minutes (n = 2), and 16.50 ± 0.71 minutes (n = 2), respectively. Emergence times at PL and PA were not statistically different ($p = 0.31$, $df = 1$) so observations from these locations were combined. The mean time of emergence for combined uncontaminated locations was 21.25 ± 6.85 min (n = 4). Bat emergence time at TC was significantly later ($p = 0.022$, $df = 3$) than emergence time of bats at the combined uncontaminated sites (Fig. 1).

Forest habitat comprised 29.2% (TC), 53.7% (PL), and 48.7% (PA) of total surface area contained within the local landscape of each location. Water habitat comprised 4.5% (TC), 3.5% (PL), and 2.8% (PA). Open habitat comprised 66.3% (TC), 42.8% (PL), and 48.5% (PA) (Fig. 2).

We found that bats at a site contaminated with the heavy metals Pb, Cd, and Zn did exhibit a different pattern of emergence compared to bats at uncontaminated locations as hypothesized. Bats from the contaminated site had a mean emergence time significantly later than bats from the combined uncontaminated reference sites ($p = 0.022$, $df = 3$). However, variability in emergence times was nearly 6 times higher (5.86x) at TC compared to the uncontaminated sites. This may be because individual bats at TC experience differential exposures to the contaminants thus adding to variability in behavioral outcomes. Eguren (2014) found that *L. borealis* collected from TC had liver Pb levels that ranged from 0.002-0.026 µg/g but liver Pb in bats from PA and PL ranged from below detection limits to 0.014 µg/g. Levels of Pb in water at TC (0.935 ± 0.658 mg/L) were elevated compared to PL (0.219 ± 0.153 mg/L) but not PA (1.579 ± 1.356 mg/L—Eguren 2014). Also, Cd levels in water were much higher at TC (6.546

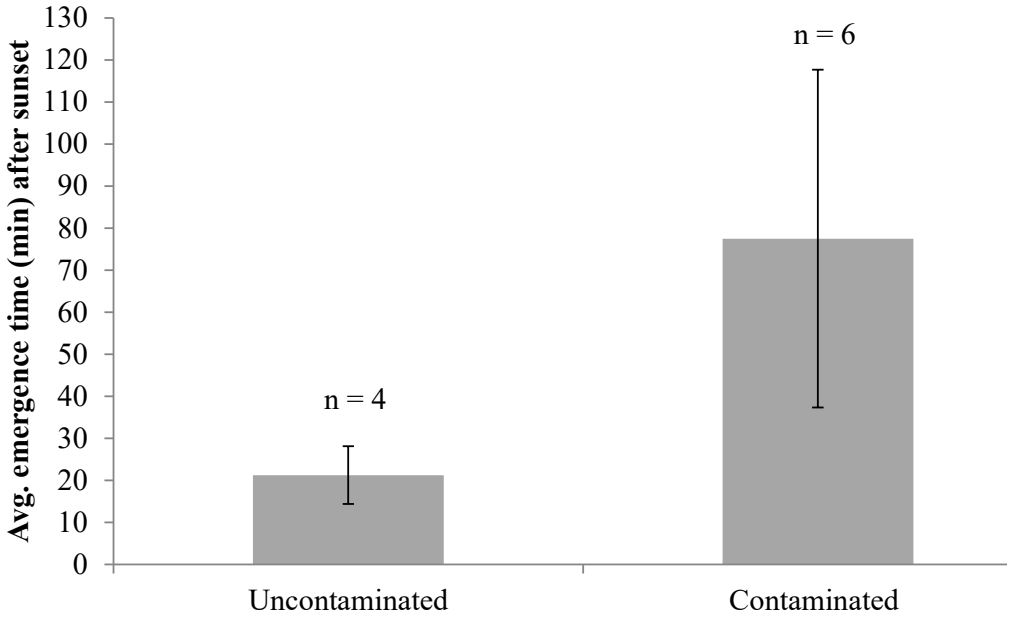

Figure 1. The average number of minutes after sunset that bats emerged at the contaminated site was significantly different (p = 0.022, df = 3) from that at the combined uncontaminated sites. Standard deviations are indicated by the bars.

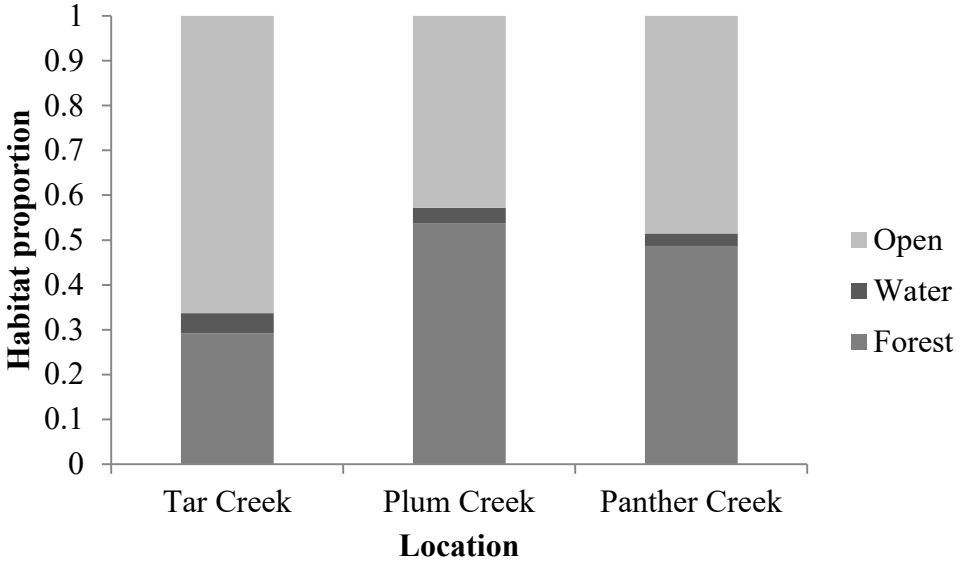

Figure 2. The proportion of habitat types (forest, water, open) found within a 250 m radius at each location. Tar Creek had less forest habitat and more water and open habitats when compared to each of the reference sites.

± 0.952 µg/L) compared to PA (0.353 ± 0.148 µg/L) and PL (0.038 ± 0.004 µg/L—Eguren 2014). Furthermore, Eguren (2014) found Cd levels in insects were 34% higher at TC and another contaminated location compared to the two uncontaminated sites in this study.

One of the outcomes of low level chronic Pb exposure is apoptosis of the rod photoreceptors (He et al. 2000, 2003). Of the three types of photoreceptive cells in the retina of mammals, rods contribute the most to sensitivity of photoentrainment of circadian cycles (Altimus et al. 2010; Lucas et al. 2012). It is possible bats experiencing retinal damage due to rod photoreceptor cell death, induced by chronic Pb exposure, would exhibit reduced sensitivity to changes in light levels and therefore require more light in order to properly photoentrain their circadian cycles. Behavioral effects could include later emergence or more variable emergence times in the evening than unexposed bats. Future studies could determine if bats at Tar Creek are in fact experiencing retinal deficits due to photoreceptor apoptosis. Also Gompf et al. (2015) found that mice without glutamate transmission from intrinsically photosensitive retinal ganglionic cells (ipRGC) had greatly reduced abilities of photoentrainment. Therefore, it is possible bats experiencing Pb and Cd exposures may exhibit poor photoentrainment abilities due to reduced or impaired glutamate transmission induced by these toxicants (Borisova et al. 2011).

Although our hypothesis is supported by this study, the low sample size at each location and the fact that this study was conducted during only one brief sampling period means that we don't know if this pattern of behavior is consistent from year to year or if it is a consistent representation of bat behavior at each site. Future studies should greatly increase sample sizes at each location and compare results for longer sampling periods from multiple years. Bat activity levels vary greatly through time (Hayes 1997). While we only included results of observations conducted during the same months, and we tried to visit each site in sequence so that there was little change in weather or moon phase during each observation period, a future study could improve upon this design by simultaneous data gathering at each location in order to compare results of observations at each site on the same evening (Hayes 1997). This would allow greater control of other factors that may contribute to timing of bat emergence such as moon phase (Thomas and Jacobs 2013), vegetation structure (Russo et al. 2007), presence of predators (Lima and O'Keefe 2013), and insect prey abundance (Thomas and Jacobs 2013).

Bat activity patterns also typically exhibit some species dependence (Kunz 1973). Possibly our results are simply due to differing community structure of bats at each site. However, Eguren (2014) did not find differences in diversity or number of bats captured at the same locations during her study and *L. borealis* was the most commonly encountered species (92.5%, n = 37) at all three sites over a period of two years. This indicates the population structure of bats at each site is similar. Future studies could focus on passive acoustic sampling surveys and using bat call identification software to better assess community structure at each site (Coleman et al. 2014).

TC contained different proportions of forest, water, and open habitat within a 250 m radius of our sampling location compared to the two uncontaminated sampling sites. TC generally contained less forest and more water and open habitat than either of the uncontaminated locations. It is unclear if this difference influenced emergence times. Russo et al. (2007) showed that emergence of bats occurred later in more loose canopy structure compared to dense canopy structure due to increased light levels remaining later in the evening in central Italy. Therefore, the later emergence time detected in bats at TC may be due to the occurrence of more open habitat compared to the two uncontaminated sites. However, all sampling locations in this study were in similar immediate local habitat and adjacent to areas of dense forest.

Because the community structure of bats at each of our locations appears to be dominated by *L. borealis*, it is important to understand

how habitat structure and roosting ecology of this tree-roosting species affects its detection and emergence time (O'Keefe et al. 2009). A consistent finding in studies containing analysis of *L. borealis* activity patterns is that they are frequently among the first bats active after sunset (Caire et al. 1988; Kunz 1973). Also, open areas and streams adjacent to hardwood forest edges appear to be important foraging habitats for Eastern Red Bats (Limpert et al. 2007; O'Keefe et al. 2009). These habitat conditions are present at each of our sampling locations and include forests dominated by hardwood trees such as oaks (Phelps and McBee 2009). Therefore, each of our sampling locations represent quality foraging habitat for the most frequently detected bat species that consistently exhibits among the earliest emergence times of bat species in the area.

In conclusion, this study serves as an intriguing observation suggesting possible behavioral consequences of exposure to environmental heavy metals. While it is possible emergence times differed as a result of detrimental effects of heavy metal exposure there are also other possible causal agents involved that were not controlled for in this study. Our results should encourage future studies that take into account these agents as well as possible physiological, functional, and morphological changes in bats exposed to environmental heavy metal contamination.

Acknowledgments

This paper represents work done in partial fulfillment of requirements for ZOOL 4700 Independent Studies by JJL. This research was supported in part by the Oklahoma State University Collection of Vertebrates, and by grants to R. E. Eguren from Bat Conservation International, Sigma Xi, Oklahoma State University Women's Faculty Council, and the Bryan P. Glass Student Fellowship in Zoology. We thank R. E. Eguren, C. Hodges, M. Reidy, K. Negrón, A. Scofield, and H. McCartney for field assistance. We are especially grateful to B. Rush, and Oologah Wildlife Management Area, S. Cox, and J. Gillham for land use permission.

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Received August 14, 2015 Accepted October 29, 2015