Environmental Pollution 224 (2017) 810–819

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Accumulation of coal combustion residues and their immunological effects in the yellow-bellied slider (*Trachemys scripta scripta*)☆

David L. Haskins [a, b, *], Matthew T. Hamilton [a, b], Amanda L. Jones [a], John W. Finger Jr. [a, c], Robert B. Bringolf [b], Tracey D. Tuberville [a, b]

[a] Savannah River Ecology Lab, University of Georgia, Drawer E, Aiken, SC 29802, USA
[b] D.B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, USA
[c] Dept. of Biological Sciences, Auburn University, Auburn, AL 36849, USA

ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form
3 January 2017
Accepted 17 January 2017
Available online 8 March 2017

Keywords:
Reptile
Bioaccumulation
Coal combustion residues
Ecoimmunology
Nondestructive sampling

ABSTRACT

Anthropogenic activities such as industrial processes often produce copious amounts of contaminants that have the potential to negatively impact growth, survival, and reproduction of exposed wildlife. Coal combustion residues (CCRs) represent a major source of pollutants globally, resulting in the release of potentially harmful trace elements such as arsenic (As), cadmium (Cd), and selenium (Se) into the environment. In the United States, CCRs are typically stored in aquatic settling basins that may become attractive nuisances to wildlife. Trace element contaminants, such as CCRs, may pose a threat to biota yet little is known about their sublethal effects on reptiles. To assess the effects of CCR exposure in turtles, we sampled 81 yellow-bellied sliders (*Trachemys scripta scripta*) in 2014–2015 from CCR-contaminated and uncontaminated reference wetlands located on the Savannah River Site (Aiken, SC, USA). Specific aims were to (1) compare the accumulation of trace elements in *T. s. scripta* claw and blood samples between reference and CCR-contaminated site types, (2) evaluate potential immunological effects of CCRs via bacterial killing assays and phytohaemagglutinin (PHA) assays, and (3) quantify differences in hemogregarine parasite loads between site types. Claw As, Cd, copper (Cu), and Se (all $p \leq 0.001$) and blood As, Cu, Se, and strontium (Sr; $p \leq 0.015$) were significantly elevated in turtles from CCR-contaminated wetlands compared to turtles from reference wetlands. Turtles from reference wetlands exhibited lower bacterial killing ($p = 0.015$) abilities than individuals from contaminated sites but neither PHA responses ($p = 0.566$) nor parasite loads ($p = 0.980$) differed by site type. Despite relatively high CCR body burdens, sliders did not exhibit apparent impairment of immunological response or parasite load. In addition, the high correlation between claw and blood concentrations within individuals suggests that nonlethal tissue sampling may be useful for monitoring CCR exposure in turtles.

1. Introduction

Coal combustion residues (CCRs) are common by-products of energy production globally, are produced in large quantities, and contain high levels of potentially toxic trace elements such as arsenic (As), cadmium (Cd), and selenium (Se; Rowe et al., 2002). The United States produces over 130 million tons of CCRs per year and prior to 1980 approximately 66% of CCRs were placed into aquatic settling basins (Rowe et al., 2002). As of 2010, 40% of these wastes were still being disposed into aquatic settling basins (ACAA, 2010). Trace element contaminants from CCR wastes held in these settling basins can enter the environment in a variety of ways. Coal combustion residues can leach into groundwater, be released from runoff or discharges, or escape as a result of the collapse of retaining walls (Rowe et al., 2002; Ruhl et al., 2012; Van Dyke et al., 2013). Unintentional releases do not have to occur for CCRs to negatively affect local biota. Aquatic settling basins can become attractive nuisances by attracting many species of wildlife for activities such as foraging and reproduction, thereby placing them at risk of exposure to potentially toxic levels of trace elements (Bryan et al., 2012; Lemly and Skorupa, 2012).

Previous work has found that reptiles inhabiting CCR-

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author. Savannah River Ecology Lab, University of Georgia, Drawer E, Aiken, SC 29802, USA.
E-mail address: davidhaskins44@att.net (D.L. Haskins).

http://dx.doi.org/10.1016/j.envpol.2017.01.048
0269-7491/

contaminated environments accumulate significant amounts of CCR trace elements (Hopkins et al., 1999; Nagle et al., 2001; Roe et al., 2004). High CCR trace element concentrations are known to cause deleterious effects on reproduction, metabolic processes, and survival in aquatic species including fish, amphibians, and invertebrates (Hopkins et al., 1999, 2013; Rowe et al., 2002). Furthermore, CCR trace elements such as As and Se can cause immunotoxic effects in fish and birds in controlled exposure studies (Ghosh et al., 2006; Fairbrother et al., 1994). However, field studies that focus specifically on CCR exposure and its impact on the immune response are sparse. Because mounting an immune response is energetically costly, disruption of metabolic processes and energy allocation due to CCR exposure could lead to compromised immune responses or impair ability to regulate parasite loads (Martin et al., 2010). Immune function can also be negatively impacted by parasite loads in vertebrates (Graham, 2002; Martin et al., 2010). Furthermore, little is known overall regarding how trace element contaminants could negatively affect the reptilian immune system, and studies assessing the toxic risk of these contaminants in reptiles have only started in the last two decades (Keller et al., 2006).

In spite of recent advances in the field of reptilian ecotoxicology, sublethal effects associated with chronic exposure to trace element contaminants are relatively unknown. Thus, this study explored the sublethal effects of CCR trace elements on freshwater turtles. Because recent research has suggested that red-eared sliders (*Trachemys scripta elegans*) rely more heavily on innate immune responses rather than adaptive responses (Zimmerman, 2013), we investigated the potential effects of exposure to CCR trace elements on the innate immune responses in wild caught *T. s. scripta*, focusing on two commonly used immunological assays – bacteria killing assays and phytohaemagglutinin skin assays (described further below). Our specific objectives were to: (1) quantify the accumulation of CCR-associated trace elements (As, Cd, Cr, Cu, Se, Sr) in tissues corresponding to different exposure time scales (blood and claw); (2) compare innate immunological responses of sliders inhabiting CCR-contaminated wetlands and reference wetlands; and (3) determine if parasite burdens differ based on exposure to CCR trace elements.

2. Methods

2.1. Study species

The yellow-bellied slider (*T. s. scripta*) is a freshwater turtle common throughout southeastern United States and is a habitat generalist (Gibbons, 1990a). Yellow-bellied sliders, like all sliders, tend to have small annual home ranges (although they may move long distances over their lifetime; Morreale et al., 1984), exhibit high site fidelity, are long-lived (20–30 years in the wild), and feed in middle to high trophic levels (Gibbons, 1990a; Tucker, 2001; Parmenter and Avery, 1990). These life history characteristics, along with their wide geographic distribution (North America to South America; Gibbons, 1990a), make slider turtles, including *T. s. scripta*, an excellent species for contaminant studies. Slider turtles also experience an ontogenetic diet shift – juveniles tend to be carnivorous but become more omnivorous as adults (Parmenter and Avery, 1990), potentially altering patterns of dietary exposure and accumulation of contaminants over their lifetime. On the Savannah River Site (SRS) where our study was conducted (see below), *T. s. scripta* is the most abundant freshwater turtle species (an estimated 61 individuals/ha of aquatic habitat) and accounts for almost 50% of all freshwater turtle biomass (Congdon et al., 1986).

2.2. Study site

Our study was conducted on the Savannah River Site (SRS), an 800-km^2 Department of Energy facility located in the southeastern Coastal Plain in west-central, South Carolina, USA. The SRS supports a variety of wetland types, including isolated wetlands (Carolina bays), farm ponds, streams, and bottomland hardwood swamps. A small proportion of these wetlands have been impacted by site operations (*e.g.*, the production of nuclear materials, power production), resulting in wetlands with varying contaminant histories. One of the major sources of industrial contaminants on the SRS was former coal-burning power plants, which produced CCRs that were disposed in surface impoundments (aquatic settling basins). Our study was conducted in the D-Area ash basin system, which has been well-characterized in previous studies (Hopkins et al., 1999; Nagle et al., 2001). Coal fly ash was discharged from the power plant into receiving basins, then into large primary and secondary basins (Fig. 1), with particulates separating and settling out as water moved through the system. Several natural wetlands in close proximity (<0.1 km) to the D-Area settling basins receive run-off from the basins. In addition, turtles are known to move between the settling basins and associated wetlands. Thus, for our study D-area turtles were collected from the primary ash settling basin and from wetlands 'A' and 'B' adjacent to D-area (see Fig. 1). Reference animals were collected from 10 natural wetlands on the SRS that have not historically received CCR-effluents and that were 2.4–24 km away from D-area and other CCR basins.

2.3. Field sampling and sample collection

During May–September 2014 and May–July 2015, *T. s. scripta* were captured with hoop nets baited with sardines and/or creamed corn. Traps were checked once daily, and all turtles were transported to the Savannah River Ecology Laboratory (SREL) for processing. Morphological data including plastron length (PL; to nearest 1 mm), weight (to nearest 2 g), and sex were recorded for each individual. Each turtle was permanently marked by notching or drilling a unique combination of marginal scutes (Cagle, 1939; Gibbons, 1990b). Whole blood and claw samples were collected to provide a short and long-term measurement of contaminant exposure, respectively (Aresco, 2005; Van Dyke et al., 2013). Tips of claws (2–3 mm) from the left forelimb were collected from each turtle and stored at −60 °C until analysis. Blood samples totaling no more than 1% of the animal's body weight, generally <1–1.5 mL, were collected via the subcarapacial sinus with a 27 or 25-gauge needle (Hernandez-Divers et al., 2002). After setting aside half of each whole blood sample for storage at −60 °C for subsequent trace element analysis, a drop of whole blood was also used to make blood smears, which were fixed with 100% methanol (VWR International, Radnor, PA) for *Haemogregarina* parasite quantification. Blood smears were collected opportunistically in 2014 and for all turtles in 2015.

The remaining blood sample was then aliquoted into lithium heparin tubes (Becton Dickson, San Antonio, TX, USA) or SealRite© 1.5 mL microcentrifuge tubes (USA Scientific Inc., Ocala, FL, USA) and centrifuged (Heathrow Scientific LLC, Vernon Hills, IL, USA) at 6000 RPM to collect plasma for the bactericidal assay. Plasma samples were stored at −60°C until further analysis. Finally, phytohaemagglutinin (PHA) skin assays were performed on individuals having at least 100 mm PL. Biological samples were only collected and PHA assays performed for turtles at their first capture during the study period. Assays are described further below.