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Chronic Exposure to Coal Fly Ash Causes Minimal Changes in Corticosterone and Testosterone Concentrations in Male Southern Toads *Bufo terrestris*

C. K. Ward,[1] M. T. Mendonça[2]

[1]Department of Biology, Auburn University Montgomery, P. O. Box 244023, Montgomery, Alabama 36124-4023
[2]Department of Biological Sciences, Auburn University, 331 Funchess Hall, Auburn, Alabama 36849

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Abstract. More than 50% of the electricity in the United States is produced by coal-burning power plants. The byproduct of coal-burning plants is coal fly ash, which contains increased concentrations of trace metals and is disposed of in collection basins. Southern toads (*Bufo terrestris*) frequently use these basins for reproduction. Male toads were collected in spring 2001 and 2002 from an ash basin and a reference site and divided into four groups: toads collected at the control site and maintained on (1) control substrate and food or (2) ash and contaminated food and toads collected at the ash site and maintained in (3) control or (4) ash conditions. Blood was collected periodically during 5 months to determine testosterone and corticosterone concentrations. Reference to ash toads exhibited a significant, transient increase in corticosterone at 4 weeks, but neither corticosterone nor testosterone continued to increase beyond this time. In contrast, toads caught and maintained on ash did not exhibit increased corticosterone. Testosterone in these toads appeared to be unrelated to ash exposure. This unexpected lack of a corticosterone response and no effect on testosterone suggests that toads chronically exposed to trace metals can acclimate to a polluted environment, but they may still experience subtle long-term consequences.

The generation of electricity by coal-fired plants produces large quantities of waste-containing pollutants that are becoming ubiquitous in the environment. Coal combustion waste (*i.e.*, coal fly ash) contains <60 trace metals (Birge 1978). Coal fly ash is frequently mixed with water and pumped into a series of large (*e.g.*, approximately 1 km^2) settling basins for ultimate disposal. The ash forms a layer of silt at the bottom of these basins (Gutherie and Cherry 1979). Settling basins often serve as habitats for aquatic organisms, including a number of invertebrates, turtles, fish, alligators, frogs, and toads (Gutherie and Cherry 1979). Vertebrates and invertebrates sequester numerous trace metals from coal fly ash, including arsenic, lead, aluminum, selenium, and cadmium (Hopkins *et al*. 1997, 1999; Rowe 1998). Coal fly ash has been documented to act as a stressor (Hopkins *et al*. 1997) causing a variety of deleterious effects (*e.g.*, increase in standard metabolic rate, endocrine disruption, and developmental abnormalities [Hopkins *et al*. 1997; Rowe 1998, 1998a, 1998b; Hopkins *et al*. 1999a]).

The normal stress response is a highly conserved adaptive suite of physiologic mechanisms that allow organisms to mitigate environmental perturbations for the short term. Exposure to a stressor typically causes increases in plasma glucocorticoid concentrations, which mobilize glucose stores, increase metabolism, and can temporarily decrease some functions such as the inflammatory response to prevent swelling. Stress can also inhibit reproductive behavior, which can decrease energy loss in the short term (Bonga 1997). This series of responses is generally considered adaptive. However, chronic exposure (*i.e.*, more than a few hours or days) to a stressor leads to a prolonged increase in glucocorticoids, resulting in secondary maladaptive effects, including a decrease in reproductive hormone concentrations and behavior, increased susceptibility to disease, and weight loss because of decreases in feeding and channeling of more energy for body maintenance (Bonga 1997). Furthermore, visceral pathologies, such as stomach ulcers and necrosis of the liver and kidneys, have also been observed in response to chronic exposure to a stressor and a prolonged stress response (Bonga 1997) This suite of symptoms and pathologies mimics those that have been documented for exposure to trace metals such as cadmium, selenium, mercury, aluminum, and arsenic in fish (Larsson *et al*. 1985).

Most studies examining the effects of trace metals in animals use acute, high-dose exposures to determine the LD_{50}s of different specific metals. However, they are not representative of actual environmental exposure, which usually involves exposure to chronic, sublethal doses of a combination of metals. A more realistic model for assessing the impact of environmental metals is to determine the consequences of chronic, sublethal exposure (Sparling *et al*. 2000).

Most studies examining the "chronic" effects of trace metals have defined such an exposure to be a period between 10 to 30 days (Mahmoud *et al*. 1989; Moore *et al*.

Correspondence to: C. K. Ward; *email:* cward3@mail.aum.edu

1991; Groten *et al*. 1994; Oner *et al*. 1995; Hall and Van Ham 1998; Hu *et al*. 2000; Kobayashi *et al*. 2000; Butler and Roesijadi 2001). This exposure length has been found to cause significant changes in corticosterone and testosterone levels (Mahmoud *et al*. 1989; Moore *et al*. 1991; Hu *et al*. 2000), weight loss or gain (Hu *et al*. 2000), histopathology (Groten *et al*. 1994; Kobayashi *et al*. 2000), hematology (Hall and Van Ham 1998), and protein levels (Oner *et al*. 1995). Likewise, many short studies (10 to 30 days) examined mortality where the final end point was death or LT_{50} values. However, when chronic exposure to trace metals exceeded 30 days, studies usually observed relatively small (Pickering and Stewart 1984; Lemly 1993; Hopkins *et al*. 2002a) or no (Schreck and Lorz 1978; Hopkins *et al*. 2002a) effects. These latter results would indicate some acclimation to the stress caused by toxicant exposure. Exceptions to this generalization occur if exposure coincides with development. Studies in which organisms are exposed to trace metals during critical stages of development have found long-term morphologic and physiologic effects (Brodeur *et al*. 1997; Rowe *et al*. 1998a).

In this study, we examined long-term, sublethal exposure of amphibians to coal combustion waste. Previous studies of adult male southern toads *Bufo terrestris* found that free-ranging individuals in coal fly ash basins have higher corticosterone concentrations than animals in noncontaminated environments after a possible 2 months of exposure to ash environment (Hopkins *et al*. 1997). These data suggested that exposure to ash can be a stressor. We examined the effects of a longer exposure period (*i.e.*, 4 months), in finer detail (by means of a more frequent sampling regime), of coal fly ash using a microcosm paradigm on the toads. This experimental design allowed us to more fully characterize the impact of ash exposure on reproductive and stress hormone patterns.

Materials and Methods

Animal Capture

Adult male Southern toads were captured by hand at the Savannah River Site (Aiken, SC) in early spring: March and April 2001 ($n = 96$) and 2002 ($n = 168$). Adult toads (identified by call patches and nuptial pads) were collected from either the ash basin area on the Savannah River Site (as described in the Introduction; see Gutherie and Cherry 1979) or a reference site approximately 15 km from the ash basin. Mass (mean mass 15.0 ± 0.2 g) was measured on a digital balance (0.01 g accuracy), and an initial blood sample was taken (see Blood Sampling). All toads were then assigned an individual identification number on a toe clip.

Housing and Experimental Design

Within two days of capture, toads were transported to Auburn University (Auburn, AL) and transferred into microcosms (208-L Rubbermaid [Rubbermaid, Inc., Fair Lawn, OH] containers with screen lids). Each microcosm contained 70% sediment (coal fly ash collected from the capture site or play sand purchased from a local hardware store) and 30% water by area. Toads were placed in microcosm with sediment equivalent to the type on which they were captured. There were 60 total microcosms (30 ash and 30 sand controls), each housing three toads. Sediment was covered by pine straw, 5 cm deep, and a 10 × 25-cm piece of pine bark was placed in the microcosm for shelter. All microcosms were located outdoors under a shade-cloth tent and subject to ambient conditions.

Toads were acclimated in microcosms containing their capture sediment for 1 month before being transferred to the experimental sediment. Acclimation was to insure that all toads were healthy, eating, and exposed to the same environmental conditions before sampling. Blood sampling began 1 week after transfer to experimental sediment in 2001 and 3 days after transfer in 2002. Toads were assigned into one of four groups ($n = 24$/group for 2001 and $n = 42$/group for 2002). Toads acclimated to control (C) or ash (A) sediments were then transferred to microcosms containing control (C → C, A → C) or ash (C → A, A → A) sediments. No toad remained in its original microcosm. Toads were fed weekly (approximately 10 crickets/toad) with crickets fed either a control diet of cat food or dry cat food chow contaminated with coal fly ash (50:50 mixture by volume determined empirically to increase metal concentrations in crickets to within field levels; Table 1) depending on treatment sediment. Crickets raised on coal fly ash accumulated significant amounts of metals (Table 1). Metals concentrations in ash crickets were on average increased 570% higher than control concentrations (Table 1).

This microcosm experimental paradigm resulted in toads transferred from a reference site to ash microcosms accumulating significant amounts of trace metals (*e.g.*, up to a 1000% increase from capture) within 4 weeks of exposure. Toads moved from the ash site to ash microcosms also accumulated significant amounts of trace metals within 4 months of exposure. The concentrations of the metals continued to increase in ash-exposed toads throughout the experiment (Ward *et al*. submitted).

Blood Sampling

In 2001, animals were monitored from May 1 to September 9. Blood samples (0.1 ml) were taken (details later) at capture and on a 3-week cycle thereafter. Each experimental group of toads was randomly assigned to one of two subgroups ($n = 12$/group), and blood samples were taken on consecutive weeks. Samples were not taken during the third week (Fig. 1). This sampling regime resulted in each toad being bled only once every 3 weeks, thus minimizing handling and the potential stress of repeated sampling. In 2002 (April 26 through June 19), each experimental group of toads was divided into four subgroups ($n = 10$/group). Blood samples were taken from each group consecutively, so that alternate subgroups of toads were bled every day for the first 8 days and every week for the next 4 weeks. Two subgroups were combined, and the new groups ($n = 20$/group) were bled on consecutive weeks; as in 2001, there was no bleed during the third week. This sampling regime allowed us to examine long-term exposure in 2001 and focus on short-term (first month) exposure in 2002 (Fig. 1).

All blood samples were taken by way of cardiac puncture using a 26.5-gauge heparinized needle. Blood samples were stored on ice (< 2 hours) until they were centrifuged at 7000 rpm for 5 minutes. The plasma was then removed by pipette and frozen at −4°C.

Hormone Quantification

Plasma corticosterone and testosterone levels were quantified using a standard competitive binding radioimmunoassay as described in

Table 1. Metal concentrations for crickets raised on either a control or ash diet[a]

Metal	Control	Ash	Difference (%)	*p* Value	*z* Value
Al	19.0	451	2280	<0.001	19.9
Ba	0.872	12.9	1379	0.001	3.24
Zn	255	347	54	<0.001	5.11
As	BDL	2.62	N/A	0.11	1.62
Sr	1.96	6.27	219	0.13	1.50
Cu	20.4	29.6	44	0.20	1.30
V	0.248	2.28	820	0.20	1.28
Ni	1.50	0.615	143	0.55	0.61
U	BDL	0.340	N/A	0.56	0.58
Co	0.435	0.049	783	0.58	0.55
Pb	0.155	0.607	291	0.60	0.52
Cr	1.27	0.605	109	0.63	0.49
Se	0.653	.959	46	0.81	0.24
Rb	4.11	4.74	15	0.83	0.21
Cs	0.013	0.058	344	0.86	0.17
Cd	0.105	0.074	−29	0.94	−0.073

BDL = below detectable limit.
[a] Crickets were analyzed as a homogenized group. Means are presented in ppm.
p and *z* values are from unpaired Student *t*-test.

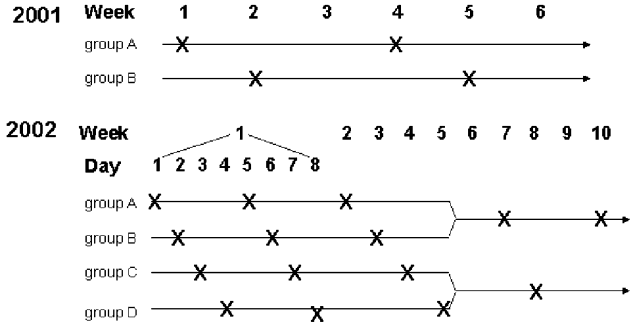

Fig. 1. Time line for blood sampling for 2001 and 2002. X = indicates sampling

Mendonça *et al*. (1996). Ether extraction was used to separate steroid hormones from the plasma. Antibodies were purchased from Esoterix (Calabasas Hills, CA) and used with standard charcoal–dextran separation technique. Intra-assay variation was 4.34% for testosterone and 3.71% for corticosterone. Interassay variation was 10.9% for testosterone and 13.0% for corticosterone (Chard 1995).

Statistics

All hormone data were tested for heterogeneity of variance and determined to be homogeneous by *F* max test ($p < 0.01$) and therefore repeated analyses of variance were used for statistical analysis on untransformed data (Sokal and Rohlf 1995). Hormone concentrations among treatments for each time period were analyzed with an unpaired Student *t* test. Total comparisons among treatments for all time periods were analyzed with repeat ANOVA. In 2002, samples taken on days 1 through 8 did not differ significantly ($p > 0.5$) within the groups and were combined to increase sample size. Data were given as means ± SE. Statistics were calculated Statview version 5.0.1 and SAS version 9.1 (SAS, Cary, NC).

Results

Hormones

Corticosterone. In 2001, plasma corticosterone concentrations did not differ between sites at capture ($\bar{x}_{ash} = 8.29 \pm 1.54$ ng/ml and $\bar{x}_{control} = 8.44 \pm 2.3$, $p = 0.32$, $df = 34$, $F = 0.98$). There was no significant difference among groups with time ($p = 0.25$, $df = 18$, $F = 1.2$). Only one group, toads transferred from a control to an ash environment (C → A), exhibited a significant transient increase in corticosterone greater than controls and only during the fourth week of exposure (32.2 ± 6.3 vs. 10.54 ± 4.5 ng/ml, $p = 0.03$, $df = 18$, $t = 2.36$); they returned to low levels (9.80 ± 2.78 ng/ml) by the seventh week (Fig. 2A).

In 2002, toads captured at the reference site had significantly higher corticosterone concentrations than toads at the ash site ($\bar{x}_{ash} = 16.2 \pm 2.0$ ng/ml and $\bar{x}_{control} = 38.1 \pm 2.5$, $p < 0.0001$, $df = 119$, $t = -5.80$). There was no significant difference among the groups with time ($p = 0.06$, $df = 263$, $F = 1.72$). Toads at the reference site were calling, exhibiting reproductive behavior, and were known to have produced egg masses. No such behaviors were observed at the ash basin site. In this second year, blood sampling frequency was increased during the first 4 weeks of exposure to determine if there was an earlier or more extended peak in corticosterone that was not previously detected < in 2001. The increased sampling regime did not show any changes in corticosterone other than the significant peak observed at 4 weeks as in 2001 ($p = 0.01$, $df = 26$, $t = 2.63$ for C → A to C → C) (Fig. 2B). In 2002, corticosterone was monitored until October; these data did not differ from those collected in 2001, and no additional corticosterone peak was observed.

Testosterone. In 2001, plasma testosterone concentrations did not differ between sites at capture ($\bar{x}_{ash} = 45.9 \pm 14.0$ and $\bar{x}_{control} = 36.8 \pm 11.1$ ng/ml, $p = 0.61$, $df = 33$, $t = -0.51$).

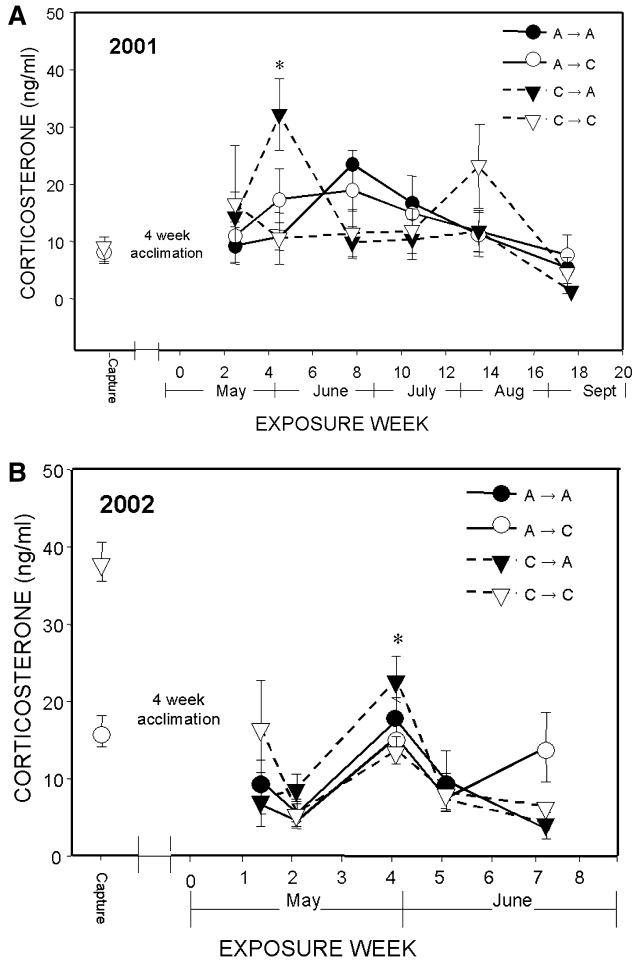

Fig. 2. (**A**) Circulating corticosterone concentrations of male Southern toads in 2001 moved from control (C) or ash (A) environments to C or A microcosms. (**B**) In 2002, four groups of toads were bled on alternating days for the first week, and data were combined. (Closed circle) A → A. (Open circle) A → C. (Open triangle) C → C. (Closed triangle) C → A. Values expressed as means ± 1 SE. *Significant ($p = 0.03$ for 2001 and $p = 0.01$ for 2002) difference

Testosterone differed significantly among groups and by time ($p = 0.05$, $df = 249$, $F = 2.52$) during the entire experiment. This significant difference was driven by the first 6 weeks of exposure ($p < 0.001$, $df = 104$, $F = 8.11$). Toads captured at the reference site, regardless of which substrate they were transferred to, experienced significantly decreased testosterone compared with those collected at the ash basin ($\bar{x}_{ash} = 26.5 \pm 4.1$ and $\bar{x}_{control} = 12.7 \pm 4.0$ ng/ml, $p < 0.0001$, $df = 104$, $F = 22.2$). After the sixth week, there was no significant difference between the groups ($p = 0.34$, $df = 151$, $F = 1.12$). Toads that were captured at the reference site, regardless of which substrate they were transferred to, experienced significantly decreased testosterone compared with those collected at the ash basin ($\bar{x}_{ash} = 26.5 \pm 4.1$ and $\bar{x}_{control} = 12.7 \pm 4$ ng/ml, $p = 0.001$, $df = 263$, $F = 11.1$) (Fig. 3A).

In 2002, testosterone levels for ash toads at capture and toads collected from reference sites did not differ significantly (66.06 ± 9.27 ng/ml vs. 98.64 ± 12.46 ng/ml, $p = 0.13$, $df = 74$, $F = -1.55$; Fig. 3B). Testosterone differed significantly among groups and by time ($p = 0.01$, $df = 296$, $F = .56$). No pattern was apparent in 2002 as a result of capture sediment.

Discussion

In our experiment, toads kept on ash sediment significantly accumulated a variety of trace metals within 4 weeks of exposure. The top four metals that increased in control-to-ash–exposed toads at 4 weeks were Tl, Sr, V, and Se (1514%, 1270%, 628%, and 594% increase over capture, respectively; Table 2) (Ward *et al*. submitted). The only metal in this list that has been extensively studied for toxic physiologic effects is Se. Se is known to cause liver and reproductive dysfunction as well as metabolic stress (Chang 1996). Tl, Sr, and V have been tested for LD_{50} and are known to be toxic at increased concentrations, such as those found in ash basin environments, but the physiologic effects of these metals have not been well studied. The concentrations of metals found in toads in this study are within an order of magnitude of levels found in previous studies from this site (Hopkins *et al*. 1998). Any differences seen in the levels of metals found in toads between the studies are likely attributable to numerous factors, including differences in metal concentrations in the parent coal, the age of the coal fly ash, the pH of the water, the weather, and industrial processes that could incrementally increase the concentrations of metals in effluent. Because toads in this study accumulated a substantial trace metal load and maintained the load for an extended period of time (5 months) with no difference in survival among the groups, this experimental microcosm paradigm resulted in a chronic sublethal exposure of the toads to a combination of metals (Ward *et al*.). Thus, these metals were bioavailable in concentrations known, at least in tadpoles and adults, to show physiologic effects (*i.e.*, deformities and changes in standard metabolic rate in tadpoles and abnormal hormone profiles in adults) (Hopkins *et al*. 1997; Rowe *et al*. 1998a, 1998b). However, male toads exposed to ash in our microcosm paradigm experienced minimal hormonal disruption. Corticosterone, despite frequent sampling, only exhibited a transitory yet significant increase in week 4. Testosterone was increased in 2001 in ash-collected animals and corresponded with capture sediment, whereas in 2002 testosterone was more affected by sediment exposure in the microcosms.

A previous study on free-ranging toads in an ash basin environment found that corticosterone and testosterone levels were significantly increased when sampled twice (*i.e.*, June/July and August) during the summer (Hopkins *et al*. 1997). Corticosterone in reference toads were 27.68 ± 4.8 and 7.67 ± 1.3 ng/ml in June/July and August, respectively, whereas ash toads had corticosterone levels of approximately 37 and 65 ng/ml during the same time period. Testosterone levels were 29.7 ± 6.1, 4.46 ± 1.1 ng/ml for reference toads, whereas ash toads exhibited testosterone levels of 34.64 ± 3.7, 64.86 ± 11.4 ng/ml (Hopkins *et al*. 1997) for June/July and August, respectively. Unlike the previous field study, corticosterone only exhibited a transient significant increase in C → A male toads, and corticosterone was not significantly

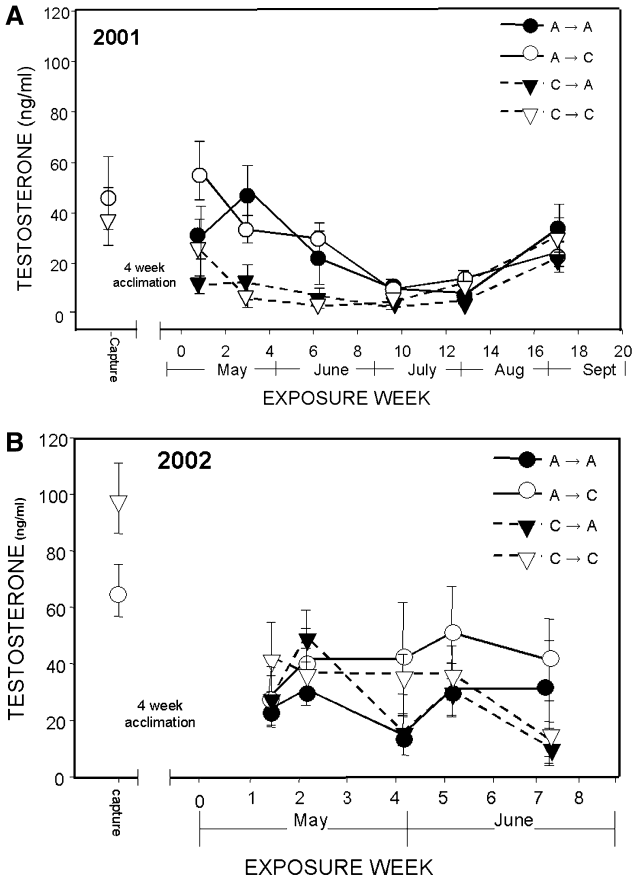

Fig. 3. (**A**) Circulating testosterone levels of male Southern toads in 2001 moved from control (C) or ash (A) environments to C or A microcosms. (**B**) In 2002, four groups of toads were bled on alternating weeks, and data were combined. (Closed circle) A → A. (Open circle) A → C. (Open triangle) C → C. (Closed triangle) C → A. Values expressed asmeans ± 1 SE

increased in the A → A toads in both years of the study. Thus, we did not see the prolonged increase in corticosterone observed in the field-collected animals despite more frequent sampling during a longer exposure interval.

However, the results of our microcosm study do mirror those of an enclosure study by Hopkins *et al*. (1997) in which animals were transferred from a reference site to an ash environment. Hopkins *et al*. (1997) were also unable to replicate the chronic increase in corticosterone seen in free-ranging individuals when they transferred unexposed toads to enclosures in the ash basin environment. Toads in ash enclosures experienced a transitory, significant peak in corticosterone at 10 days (similar to what we observed after 4 weeks of exposure). Just as in our microcosm study, these increased corticosterone concentrations did not approach concentrations observed in the field (*i.e.*, 20 and 50 ng/ml in the Hopkins enclosure and in the microcosm in our study, respectively, vs. 80 ng/ml in field-collected males) and did not remain increased during the 12-week experiment. Thus, the results seen in the microcosm and enclosure studies and those seen in the field survey differ. It is likely that the microcosm and enclosure conditions shielded the toads from other environmental stressors, such as decreased food availability and predation, and thus allowed toads to adapt to the pollutant (see Discussion later).

In the first year of our study, the testosterone results paralleled what Hopkins *et al*. (1997) observed in the field. Throughout the experiment, testosterone levels in ash-collected toads (*e.g.*, A → A and A → C) remained increased above those of toads collected at the reference site. However, in the second year, our testosterone results better matched what Hopkins *et al*. (1997) observed in their enclosure study: Testosterone decreased in animals exposed to ash (A → A and C → A). The timing of these experiments in relation to the initiation of breeding (*i.e.*, movement into the ash basin and increase in testosterone) may explain why there is a difference in how toads react hormonally to trace metal exposure.

In all of the studies encompassing Southern toads exposed to coal fly ash, toads displayed seemingly contradictory physiologic responses. Many studies on a variety of vertebrates have shown that chronically increased corticosterone levels suppress testosterone and reproductive behavior (Bonga 1997). However, free-ranging, ash-exposed toads had high body levels of trace metals and experienced significantly increased corticosterone as well as testosterone concentrations relative to controls and also displayed reproductive behavior (*e.g.*, calling and amplexus [Hopkins *et al*. 1997]). This combination of anomalous responses suggests that ash-exposed toads are not experiencing chronic stress as a result of ash exposure but rather are experiencing a shorter, transient corticosterone response and then acclimating to the polluted environment. This is also supported by the lack of response in ash-exposed toads; both C → A and A → A toads had significantly increased levels of metals—up to 5000% the concentration found in control toads at capture—and neither group showed any other measured physiologic effect of exposure such as weight loss or differences in metabolic measures (Ward *et al*. 2006).

Other coal fly ash exposure studies have shown a similar lack of physiologic effects in laboratory experiments. For example, water snakes collected in ash basins had significantly increased standard metabolic rates compared with those collected in reference areas (Hopkins *et al*. 1999a). However, when water snakes were brought into the laboratory, housed in aquaria, and fed a diet of ash-contaminated or control mice, there was no significant difference in standard metabolic rate that could be accounted for by exposure (Hopkins *et al*. 2002a). It is likely that a laboratory diet may mask some of the effects of exposure by providing excess energy for acclimation. This may explain why toads exposed to ash in microcosms and in field enclosures (Hopkins *et al*. 1999b), both provided with supplemental food, did not exhibit the increase in corticosterone levels seen in field-collected toads.

The overall lack of physiologic symptoms of exposure and long-term significant changes in both testosterone and corticosterone responses in microcosm metal–exposed toads suggest that toads that migrate into ash basins can adjust to the potentially stressful environment given that other long-term stressors are absent. In addition, some studies have shown that organisms have the ability to physiologically recover from initial exposure although the metals are still present (Haux and Larsson 1984; Mahmoud *et al*. 1989). Others have reported no effect of trace metal exposure at all (Schreck and Lorz 1978; Groten *et al*. 1994; Hopkins *et al*. 2002a). These results sug-

Table 2. Metal concentrations (ppm) and % change for the nine metals that significantly ($p < 0.1$) increased in toads collected at the control site and exposed to ash sediment in the microcosms for 4 weeks in 2001[a]

Metal	At Capture	4 week Ash Exposure	Change (%)	*p* Value	Student *t*-test
Tl	0.014 ± 0.001	0.226 ± 0.036	1514	<0.001	6.71
Sr	468 ± 72.2	6413 ± 3502	1270	0.005	3.30
V	0.394 ± 0.119	2.82 ± 1.01	628	0.025	2.51
Se	0.975 ± 0.334	6.77 ± 1.05	594	<0.001	6.25
U	0.022 ± 0.005	0.135 ± 0.057	573	0.04	2.26
As	0.640 ± 0.348	4.28 ± 1.01	569	0.003	3.52
Al	200.5 ± 53.2	962.6 ± 426.3	380	0.06	2.02
Cs	0.060 ± 0.011	0.142 ± 0.031	137	0.015	2.77
Rb	15.2 ± 4.27	30.6 ± 1.62	101	0.009	3.05

[a] Means are presented ± SE.
df for all samples is 14 (Ward *et al.* submitted).

gest that toads may be acclimating to the new environment. Acclimation may be possible by the use of protective mechanisms to lessen the effects of the trace metals. Stressor-specific protective mechanisms—such as metallothionein, which chelates divalent cationic metals (Bremner 1987) prominent in coal fly ash (Cherry and Gutherie 1977)—may be important in this acclimation, although this hypothesis has not been tested.

Although acclimation may be a likely explanation for the observed lack of endocrine dysfunction and other adult costs (*i.e.*, metabolic measures [Ward *et al*. submitted]) in toads of ash basin populations, there is still a cost from exposure. Tadpoles and eggs from toads experience decreased survivability (Rowe *et al*. 2001), and ranid tadpoles experience oral and spinoaxial deformities when exposed to coal fly ash (Hopkins *et al*. 1997; Rowe *et al*. 1998a). In addition, water snakes *Nerodia fasciata* (Hopkins *et al*. 1999a), prawns *Palaemonetes paludosus* (Rowe 1998), and lake chub suckers *Erymizon sucetta* (Hopkins *et al*. 2002b) experience increased standard metabolic rates. Although the ash basin environments are polluted with a combination of trace metals and have deleterious effects on juvenile amphibians, they appear to be otherwise suitable aquatic environments for adults (Gutherie and Cherry 1979).

Many anurans found in polluted environments may appear healthy and able to cope with the current environmental stressors (current study; Hopkins 1997) but any additional perturbations to the environment may place additional stress on the organism and compromise its ability to acclimate (Hopkins 2002b), causing decreases in local populations. It is important to understand how different organisms are physiologically responding to the myriad anthropogenic stressors that are now ubiquitous in some environments to properly manage the populations and determine if and/or how polluted environments need to be remediated. More research needs to be done on how long an organism can balance its own energetic needs with the energy required to ameliorate the effects of a stressor and its long-term effects.

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