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Environmental Toxicology and Chemistry, Vol. 19, No. 4, pp. 862–868, 2000

Printed in the USA
0730-7268/00 $9.00 + .00

INCIDENCE AND IMPACT OF AXIAL MALFORMATIONS IN LARVAL BULLFROGS (*RANA CATESBEIANA*) DEVELOPING IN SITES POLLUTED BY A COAL-BURNING POWER PLANT

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(*Received* 22 *February* 1999; *Accepted* 14 *July* 1999)

Abstract—Amphibian malformations have recently received much attention from the scientific community, but few studies have provided evidence linking environmental pollution to larval amphibian malformations in the field. We document an increased incidence of axial malformations in bullfrog larvae (*Rana catesbeiana*) inhabiting two sites contaminated with coal combustion wastes. In the polluted sites, 18 and 37% of larvae exhibited lateral curvatures of the spine, whereas zero and 4% of larvae from two reference sites had similar malformations. Larvae from the most heavily polluted site had significantly higher tissue concentrations of potentially toxic trace elements, including As, Cd, Se, Cu, Cr, and V, compared with conspecifics from the reference sites. In addition, malformed larvae from the most contaminated site had decreased swimming speeds compared with those of normal larvae from the same site. We hypothesize that the complex mixture of contaminants produced by coal combustion is responsible for the high incidence of malformations and associated effects on swimming performance.

Keywords—Coal combustion wastes Amphibians Selenium Malformations Behavior

INTRODUCTION

Recent field surveys have found high frequencies of malformations in metamorphosing or recently metamorphosed anurans [1–4]. Whereas examination of terrestrial life stages allows quantification of conspicuous appendicular malformations such as missing or supernumerary limbs and digits, many malformations can also be detected in larval anurans. Quantifiable malformations in developing amphibians include head, eye, and heart abnormalities as well as axial curvatures and abnormal gut coiling [5]. Amphibian larvae are commonly examined for malformations after experimental exposures in the laboratory [6–8]. Seldom, however, have amphibian larvae been included in field surveys of malformation frequencies. Because malformations in larvae can potentially decrease recruitment into the terrestrial environment, they may ultimately decrease the number of metamorphosing anurans available for sampling in impacted habitats. Therefore, when environmental conditions alter larval morphology, field surveys that examine only recently metamorphosed amphibians likely underestimate the presence and impact of malformations.

Field surveys and reciprocal transplant experiments have linked coal combustion wastes to developmental abnormalities in both fish and amphibian larvae. Aquatic organisms inhabiting coal ash–polluted sites are at risk because they accumulate extremely high concentrations of teratogenic trace elements, such as Cd, Cu, and Se, in their tissues [9,10]. Indeed, studies of fish have found bioaccumulation of Se accompanied by a high incidence of axial and fin malformations in species inhabiting coal ash–impacted sites [11]. High frequencies of oral abnormalities (96%) have been reported in bullfrog larvae (*Rana catesbeiana*) exposed to coal combustion wastes [9,12], and elevated tissue concentrations of multiple trace elements, including Se, have been associated with oral abnormalities in pollutant-exposed amphibian larvae [9].

Axial malformations are among the most common and easily detectable malformations in developing amphibian larvae [5,13]. Malformations such as kinked tails, scoliosis, lordosis, and kyphosis have repeatedly been documented in larval anurans exposed to xenobiotics in the laboratory [8,14] but only rarely in field studies [13]. In light of the evidence that coal combustion wastes cause axial malformations in fish and that amphibian larvae commonly exhibit similar malformations after laboratory exposures to other xenobiotics, we examined the axial morphology of amphibian larvae exposed to coal combustion wastes. This study documents the frequency of axial malformations within populations of bullfrogs developing at sites contaminated with coal combustion byproducts and compares such frequencies with those found at reference sites. In addition, we examine the impact of axial malformations on parameters of swimming performance.

MATERIALS AND METHODS

Study sites

Larval bullfrogs were collected at four sites (two polluted by and two unpolluted by coal combustion wastes) during the study. The two polluted sites are associated with a coal-burning power plant located on the Savannah River Site, which is a National Environmental Research Park located near Aiken, South Carolina, USA. The plant discharges sluiced fly and bottom ash into a series of open settling basins that drain into a 2-ha swamp. From the swamp, water flows into Beaver Dam Creek, which is a tributary of the Savannah River (Fig. 1). As water moves through the system, suspended ash precipitates, thereby resulting in extremely high sediment concentrations of trace elements, including As, Cd, Cr, Cu, and Se [15,16]. Moreover, biota inhabiting the settling system incorporate high

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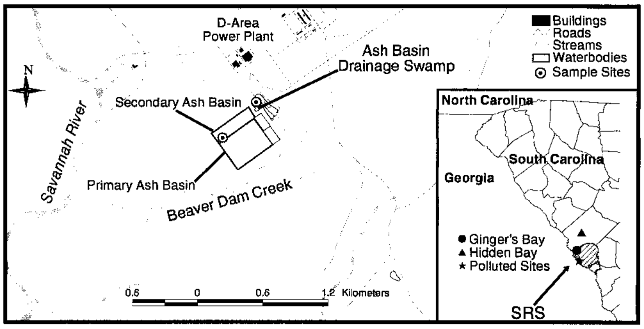

Fig. 1. Location of sample sites in Aiken County, South Carolina, USA. Within the coal ash–polluted system, bullfrog larvae (*Rana catesbeiana*) were sampled in the secondary settling basin and in the drainage swamp. Inset: Location of reference sites, Ginger's Bay and Hidden Bay (Aiken Bay), in relation to the polluted system and the Savannah River Site (SRS).

concentrations of potentially toxic trace elements into their tissues [9,10,16].

Reference sites located in Aiken County, South Carolina, USA, have historically been uncontaminated by coal combustion byproducts (Fig. 1). One of the reference sites, Ginger's Bay, is a 1.5-ha Carolina bay located approximately 13.5 km from the polluted sites on the Savannah River Site. This bay has been extensively sampled for amphibians in the past and has no known anthropogenic input of contaminants. The second reference site, Hidden Bay, is a 1.0-ha temporary wetland within the Carolina Bay Nature Reserve, which is an area designated as a public park within the Aiken city limits. The wetland is not contaminated with coal combustion byproducts, but it does receive moderate runoff from nearby paved parking lots.

Animal collection

Larval bullfrogs were collected using minnow traps during 1998 between June 8 and July 4. In the polluted system, larvae were collected from the secondary basin and the drainage swamp (Fig. 1). After capture, larvae were returned to the laboratory, where mass, developmental stage [17], and presence or absence of axial malformations were determined. Documented axial malformations were lateral curvatures of the spine originating at the midpoint or near the base of the tail (Fig. 2). To prevent individuals from being recaptured, larvae were held in field enclosures (constructed at each sampling site) before their release at the end of the study.

Trace element determination

Four larval bullfrogs exhibiting normal axial morphology from each site (individual wet mass, $>$8.0 g) were returned to the laboratory, where they were allowed to void gut contents for 48 h. Larvae were then sacrificed for determination of whole-body trace element concentrations. In addition, three surface sediment samples were taken from each study site. Tissue and sediment samples were lyopholized and homogenized before being sent to the University of Georgia Crop and Soil Science Center (Athens, GA, USA). The samples were digested with HNO_3-H_2O_2 and then analyzed for trace elements by using inductively coupled plasma mass spectroscopy. In addition to trace element concentrations, pH, water temperature, and dissolved oxygen were also determined for each site.

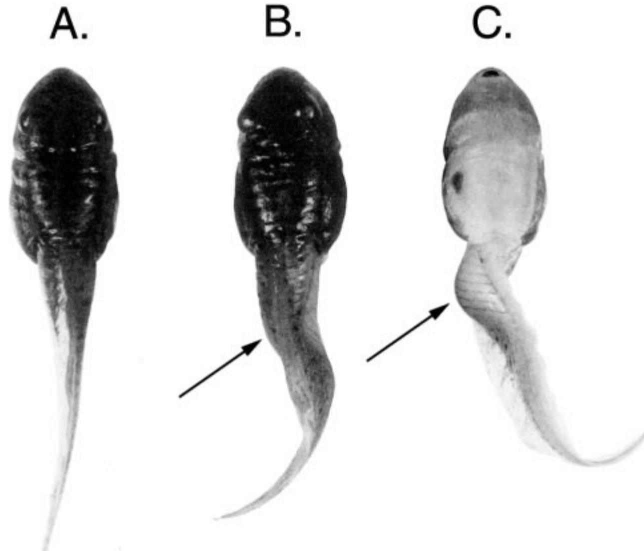

Fig. 2. Typical axial malformation found in bullfrog larvae (*Rana catesbeiana*) developing in sites polluted with coal combustion wastes. Malformations with varying degrees of severity were found, but all consisted of a lateral flexure of the tail originating at the midpoint or near the tail base. (**A**) Dorsal view of a normal bullfrog larva. (**B**) Dorsal view of the lateral curvature of the spine in a bullfrog larva exposed to coal ash. (**C**) Ventral view of the lateral curvature of the spine in a bullfrog larva exposed to coal ash.

Swimming performance parameters

Swimming performance trials used malformed larvae from the polluted secondary basin and larvae without axial malformations (hereafter referred to as normal larvae) from the secondary basin, drainage swamp, and Hidden Bay. Larvae from Ginger's Bay were not used for swimming performance trials because the wetland dried during the latter portion of the study (late July). In addition, malformed larvae from the drainage swamp and Hidden Bay were not included in the trials because of the low frequencies of malformation at these sites. Larvae were collected in minnow traps and returned to the laboratory, where they were held individually in 500 ml of artificial soft water (25°C) for 24 h before the swimming trials [18]. Larvae selected for swimming trials (mean mass, 3.61 $\pm$ 0.18 g) were at developmental stage 25 [17].

The same 3-m swimming track used by Raimondo et al. [12] was used in this study. The track was marked every centimeter and filled with artificial soft water (25°C). Larvae were placed in the track beneath a mesh holding cage until they remained motionless. The cage was then lifted, and an initial prod was administered with a blunt probe. All prods were delivered at the base of the tail on the right side of the body. Swimming was defined as any conspicuous tail movement, and prods were administered whenever swimming stopped. Larvae were allowed to swim for 1 m and were then returned to individual containers refreshed with artificial soft water. Each larva was used in three swimming trials, and each trial was separated by 1 h. Trials were videotaped to obtain precise measurements.

Both swimming responsiveness and swimming speed were used as indicators of overall swimming performance. The number of prods required to induce larvae to swim 1 m was used as a measure of swimming responsiveness. Swimming speed was the time required for a tadpole to swim one complete meter. When individuals stopped before swimming one com-

Table 1. Summary of axial malformation frequencies in larval bullfrogs (*Rana catesbeiana*) sampled from four sites in Aiken County, South Carolina, USA

Site	n[a] Sampled	n[a] Malformed	% Malformed
Secondary ash basin[b]	443	163	36.79
Drainage swamp[b]	615	109	17.72
Hidden Bay	722	32	4.43
Ginger's Bay	350	0	0.00

[a] n = number.
[b] Denotes sites polluted by coal combustion wastes.

plete meter, the time that an individual spent motionless was removed from the total time required to complete the full meter. By using the frame-by-frame advance on the VCR, measurements of swimming speeds were accurately obtained to within 0.03 s.

Data analysis

The percentages of malformed larvae from the four sites were compared using a chi-square analysis. Trace element levels in the sediments and in bullfrog tissue were tested for normality and homoscedasticity using normal probability plots and Hartley's test, respectively. Data were log transformed before analysis. Mean sediment and tissue concentrations were compared using one-way analysis of variance. Because seven trace elements were compared from the same samples, a sequential Bonferroni adjustment was used to adjust the critical levels downward. Tukey's pairwise comparisons were used to identify significantly different groups. Mean water temperature, pH, and dissolved oxygen at the four sites were compared using one-way analysis of variance followed by Tukey's pairwise comparisons [19].

For each larval bullfrog, swimming speed and number of prods were represented by the mean value from three swimming trials. Parameters of swimming performance were tested for normality and homoscedasticity using normal probability plots and Hartley's test, respectively. Data were log transformed before analysis. The effects of mass and tail length on swimming speed and swimming responsiveness were tested within each site as well as with all sites combined using regression analysis. Because no significant correlation was found between swimming parameters and mass or tail length (in all cases, $p > 0.22$), comparisons of swimming speed and responsiveness were performed using one-way analysis of variance rather than analysis of covariance. The effect of malformations on swimming parameters was determined within the secondary ash basin by comparing malformed and normal individuals. In addition, the effect of the site of origin on swimming performance was determined by comparing normal larvae from all sites. Tukey's pairwise comparisons were used to identify significantly different groups [19].

RESULTS

Malformations

The incidence of axial malformations (Fig. 2) was significantly different among the four sites sampled in this study ($\chi^2 = 305.05$; $p < 0.0001$; Table 1). Malformation frequencies at the two reference sites were less than 5% (Table 1). Of the 1,058 larvae sampled from the polluted system (i.e., secondary ash basin and drainage swamp combined), more than 25% exhibited axial malformations. When the secondary basin and the swamp were examined separately, however, the secondary ash basin had twice the number of malformed larvae compared with the downstream drainage swamp (36.8% vs 17.7%, respectively). Based on these findings, the two polluted sites were treated independently of one another. The two sites were connected by a culvert, but water flowed from the secondary basin into the swamp through a vertical standpipe before traveling over a significant drop in elevation. This unidirectional flow served as a barrier against the upstream movement of sediments and larvae from the drainage swamp to the basins. Therefore, the lower frequency of malformations in the swamp could potentially be an overestimate if any downstream movement of larvae occurred between the sites.

Trace elements and water chemistry

Sediment concentrations of all measured trace elements were significantly higher in the two coal ash–polluted sites compared with Ginger's Bay (Table 2). Sediment levels of trace elements were generally low in Hidden Bay, but the site did have higher Cr levels than the two polluted sites. In addition, levels of Cr, Sr, and V were significantly higher in sediments from Hidden Bay compared with sediments from Ginger's Bay. With the exception of Sr, trace element concentrations were not different between the polluted secondary ash basin and the polluted drainage swamp. Mean As and Cu concentrations in the secondary basin were nearly double those in the drainage swamp, but these differences were not statistically significant (Table 2).

Tissue concentrations of trace elements were generally

Table 2. Trace element concentrations of sediment (ppm dry mass) from four sites in Aiken County, SC, USA (n = 3 samples from each site)

Trace element	Secondary ash basin[a,b]	Drainage swamp[a]	Hidden Bay	Ginger's Bay	p[c]
Arsenic	49.39 ± 1.06A[d]	28.94 ± 9.38A	2.10 ± 0.32B	1.99 ± 0.19B	<0.001
Cadmium	0.72 ± 0.02AB	1.38 ± 0.66A	0.18 ± 0.09BC	0.08 ± 0.03C	0.003
Chromium	23.85 ± 1.33A	22.04 ± 2.94A	42.84 ± 3.73B	7.88 ± 1.36C	<0.001
Copper	84.72 ± 8.61A	43.50 ± 19.80AB	19.33 ± 2.40BC	9.20 ± 0.31C	<0.001
Selenium	6.11 ± 0.30A	7.11 ± 1.11A	0.42 ± 0.19B	0.78 ± 0.06B	<0.001
Strontium	106.39 ± 7.40A	40.97 ± 4.79B	18.89 ± 1.82C	8.63 ± 0.95D	<0.001
Vanadium	45.83 ± 2.63A	41.35 ± 2.58A	45.13 ± 4.72A	16.50 ± 1.71B	<0.001

[a] Denotes sites polluted with coal combustion wastes.
[b] Data are provided as mean ± SE.
[c] Minimum critical value as determined by Bonferroni sequential adjustment is $p < 0.007$.
[d] Trace element levels followed by the same letters do not differ significantly from one another.

Table 3. Whole body trace element concentrations (ppm dry mass) of larval bullfrogs (*Rana catesbeiana*) from four locations in Aiken County, SC, USA ($n = 4$ samples from each site)

Trace element	Secondary ash basin[a,b]	Drainage swamp[a]	Hidden Bay	Ginger's Bay	p[c]
Arsenic	33.10 ± 3.23A[d]	15.09 ± 2.18B	1.54 ± 0.20C	1.59 ± 0.27C	<0.001
Cadmium	5.47 ± 0.72A	1.59 ± 0.40B	1.14 ± 0.12B	0.39 ± 0.22C	<0.001
Chromium	18.25 ± 1.75A	3.49 ± 1.31B	4.30 ± 1.04B	4.11 ± 0.81B	<0.001
Copper	116.72 ± 9.37A	29.07 ± 4.33B	12.31 ± 4.20C	11.79 ± 0.58C	<0.001
Selenium	20.25 ± 2.11A	27.93 ± 1.80A	2.82 ± 0.70B	1.69 ± 0.29B	<0.001
Strontium	39.89 ± 4.42A	88.50 ± 7.03B	22.29 ± 2.87C	27.50 ± 2.02CA	<0.001
Vanadium	17.32 ± 2.12A	6.65 ± 1.26AB	3.74 ± 1.50B	5.43 ± 1.46B	0.006

[a] Denotes sites polluted with coal combustion wastes.
[b] Data are provided as mean ± SE.
[c] Minimum critical value as determined by Bonferroni sequential adjustment is $p < 0.007$.
[d] Trace element levels followed by the same letters do not differ significantly from one another.

highest in larvae from the polluted basin, intermediate in larvae from the polluted drainage swamp, and lowest in larvae from the two reference sites (Table 3). Concentrations of Se and V, however, were not significantly different in larvae from the two polluted sites. In addition, the Sr concentration was significantly higher in larvae from the swamp than in larvae from the basin (Table 3). When the two reference sites were compared, only the Cd concentration was found to be significantly higher in larvae from Hidden Bay compared with larvae from Ginger's Bay (Table 3).

Because water chemistry could potentially affect larval morphology, we measured dissolved oxygen, pH, and water temperature at each of the four sites. There was no difference in dissolved oxygen between sites. Depending on location, water depth, and time of day, however, dissolved oxygen was highly variable within each site, ranging between 3.5 and 7.0 mg/L. Based on three water samples at each site, no significant difference in mean water temperature was found between the sites ($p = 0.71$). A significant difference in pH between sites, however, was found ($p < 0.01$). Ginger's Bay had a significantly lower pH (5.57) than the secondary basin, drainage swamp, and Hidden Bay (6.72, 6.97, and 6.86, respectively).

Swimming performance

Site of origin did not appear to affect swimming speed, but the presence of axial malformations significantly slowed pollutant-exposed larval bullfrogs. Larvae with axial malformations from the secondary basin swam significantly slower than normal larvae from the same site ($p = 0.015$, $F_{1,24} = 6.81$; Fig. 3). No significant difference in the swimming speeds of normal larvae from the three sites was found ($p > 0.05$; Fig. 3).

Within the polluted secondary basin, larvae with axial malformations did not respond differently to prodding compared with normal larvae ($p > 0.05$; Fig. 4). Normal larval responsiveness, however, was significantly affected by site of origin ($p < 0.001$, $F_{2,35} = 12.86$; Fig. 4). Larvae from Hidden Bay were as much as 150% more responsive to prodding than larvae from the two polluted sites. No significant difference in the responsiveness of normal larvae from the two polluted sites was found (Fig. 4).

DISCUSSION

Malformations and pollution

Numerous factors, including heritable traits, unsuitable water chemistry, radiation, and toxic substances, may cause vertebral malformations in aquatic biota [20]. The nature of this survey did not enable us to address heritable factors, and the results of this study indicate that water temperature, pH, and dissolved oxygen are not likely contributors to spinal malformations. In fact, of the water chemistry variables we measured, the only significant difference found was decreased pH in one of the reference sites (Ginger's Bay). Therefore, we suspect that high concentrations of toxic trace elements in the polluted sediments were the causative agents of the observed larval malformations. In general, the polluted sites had much higher sediment concentrations of trace elements, such as Se, As, Cd, Cu, and Sr, compared with the reference sites (Table 2). Moreover, larval bullfrogs from the polluted sites incorporated high concentrations of trace elements in their tissues, whereas larvae from the reference sites tended to have lower body burdens of toxic elements (Table 3). Because pollutant-exposed larvae

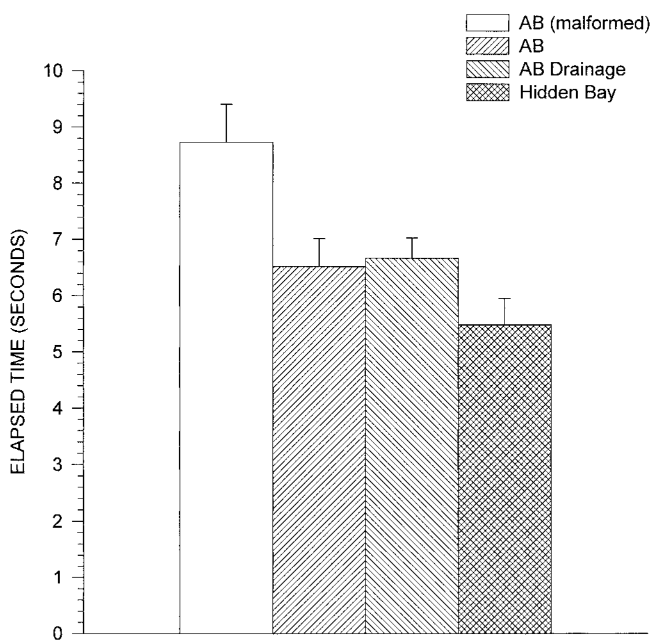

Fig. 3. Elapsed time (s) necessary for bullfrog larvae (*Rana catesbeiana*) to swim one complete meter. Swimming speeds of malformed larvae from the polluted secondary ash basin (AB malformed, $n = 13$) were compared with swimming speeds of normal larvae from the secondary basin (AB, $n = 13$). In addition, comparisons of swimming speed were made between normal larvae from the secondary basin (AB), the ash basin drainage swamp (AB Drainage, $n = 11$), and Hidden Bay ($n = 14$). All values are expressed as mean ± SE ($p < 0.05$).

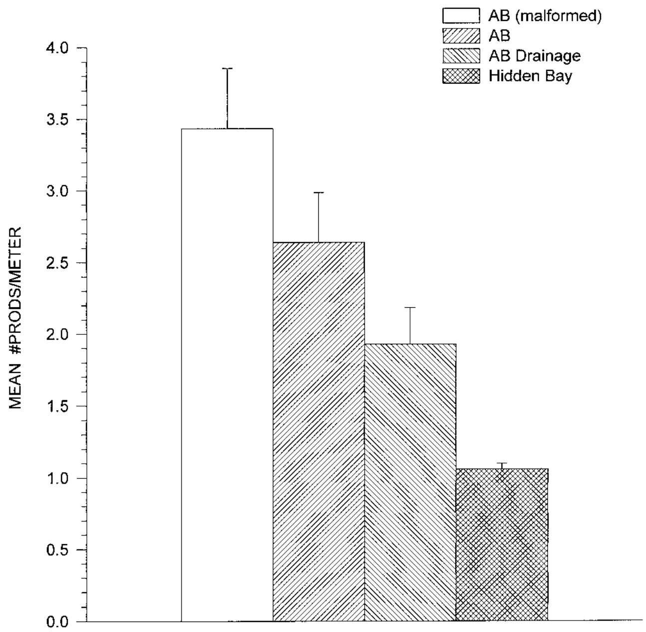

Fig. 4. Number of prods necessary to stimulate bullfrog larvae (*Rana catesbeiana*) to swim one complete meter. Responsiveness of malformed larvae from the polluted secondary ash basin (AB malformed, $n = 13$) was compared with responsiveness of normal larvae from the secondary basin (AB, $n = 13$). In addition, comparisons of swimming responsiveness were made between normal larvae from the secondary basin (AB), the ash basin drainage swamp (AB Drainage, $n = 11$), and Hidden Bay ($n = 14$). All values are expressed as mean ± SE ($p < 0.05$).

had tissue concentrations of teratogenic trace elements comparable to those levels known to induce malformations in other aquatic organisms, our discussion focuses on the potential effect of coal ash–derived elements on amphibian morphology [11]. We cannot eliminate, however, the possibility that coal-derived organic pollutants contributed to the morphological anomalies. Most of these organic compounds are volatilized during combustion, but future investigations examining the availability of such compounds would be valuable.

Bullfrog larvae from the two reference sites were exposed to low concentrations of trace elements, had low tissue concentrations of trace elements, and exhibited few axial malformations (Tables 1 to 3). Based on the sediment concentrations of trace elements, Ginger's Bay was a more pristine reference site than Hidden Bay (Table 2). Chromium levels were particularly elevated in the sediments of Hidden Bay, probably because of runoff from the nearby parking lots. Despite the elevated sediment concentrations of Cr, Sr, and V in Hidden Bay, larval bullfrogs from this site had low tissue concentrations of these trace elements (Table 3). Concomitant with low tissue concentrations of trace elements, the incidence of malformations found at Hidden Bay and at Ginger's Bay were a fraction of those frequencies found at the two polluted sites (Table 1). In fact, both bays had malformation frequencies within the range of reference sites and laboratory controls reported in other studies [8,13,21].

Differences in the frequency of malformations between the two polluted sites (secondary basin, 36.8%; downstream drainage swamp, 17.7%) may result from the differential accumulation of trace elements by larvae. With the exception of Sr, sediment concentrations of trace elements at the two polluted sites were not significantly different (Table 2). Whole-body concentrations of As, Cd, Cr, and Cu, however, were higher in larvae from the secondary basin than in larvae from the drainage swamp, thus suggesting possible differences in the availability or uptake of elements at the two sites.

We suspect differences in contaminant concentrations caused the disparity between malformation frequencies at the two polluted sites, but other ecological factors may have contributed to the observed dissimilarities. For instance, the community structure of the drainage swamp is more complex than that of the basin, and it includes an array of predators, most notably largemouth bass, that are absent from the settling basin (personal observations). Other predators, such as water snakes, are present in both polluted sites but have much higher densities in the drainage swamp (personal observations). Because malformations significantly impacted swimming performance (discussed later), malformed larvae may be more susceptible to predation. Therefore, fewer malformed individuals may have been available for sampling in the drainage swamp because of selective predation on the less agile, malformed larvae.

Of the trace elements having elevated levels in larval bullfrogs from the two polluted sites, Se is the most frequently documented teratogen. Laboratory studies indicate that as many as 50% of developing amphibians exposed to Se exhibit malformed heads and spinal curvatures [22]. Previous studies on fish exposed to coal combustion wastewater suggest that Se might also be responsible for various spinal malformations, including scoliosis, lordosis, and kyphosis [11,23]. As many as 70% of fish exposed to coal combustion byproducts exhibited malformations in one study, whereas the prevalence of malformations in reference populations never exceeded 4% [11]. Moreover, axial and fin malformations were among the most common malformations observed. Other, less prevalent malformations in fish included cataracts, exopthalmos, and head deformities [11,23].

Selenium is a potential cause of the malformations observed in the amphibian larvae, but other pollutants are also capable of contributing to these malformations. The effluent produced by coal combustion is an extremely complex mixture of trace elements, including elevated concentrations of Cd and Cu [16], both of which can also be teratogenic [24–26]. For instance, studies on fish have found that Cd exposure induces vertebral malformations in as many as 70% of exposed individuals [26]. Likewise, the majority (92%) of fathead minnows exposed to Cu exhibit developmental malformations [24]. Other elements, such as Sr, a calcium analogue, could alter calcium metabolism in amphibian larvae and contribute to observed spinal malformations. Therefore, vertebrates inhabiting coal ash–polluted sites may exhibit developmental abnormalities via complex interactions created through simultaneous exposure to a suite of potential teratogens.

Behavior

Behavioral responses to pollutants have recently become recognized as sensitive and meaningful indicators of organismal health [27,28]. Behavioral parameters often provide insight into how pollutant-induced biochemical changes are expressed in an organism. Furthermore, behavioral responses illustrate the effects of sublethal exposure to pollutants on ecological interactions. Parameters of swimming performance, such as endurance, spontaneous activity, responsiveness, and sprint speed, can directly impact ecological parameters in amphibians, such as growth, timing of and size at metamorphosis, recruitment, and predator avoidance [28–31].

In addition to a high incidence of axial malformations in larvae from the polluted sites, we found that larvae with lateral spinal flexures exhibited decreased swimming performance. Malformations appeared to disrupt the normal tail movements that provide forward propulsion for the organism. As a result, malformed larvae swam 34% slower than normal individuals from the same site (Fig. 3). Moreover, malformed larvae swam nearly 60% slower than normal larvae from the reference site. Malformations clearly impact the mechanical movements necessary for swimming speed, but no significant effect of malformations on responsiveness to stimuli was found (Fig. 4).

The site at which larvae developed also had a significant effect on swimming performance. Normal larvae from all three sites exhibited similar swimming speeds (Fig. 3). Larvae from the two coal ash–polluted sites, however, were as much as three times less responsive to a physical stimulus than individuals from Hidden Bay (Fig. 4). In some cases, multiple prods were necessary to initiate swimming in larvae from the polluted sites. Our findings corroborate those of another recent study [12] that documented similar reduced responsiveness in bullfrog larvae exposed to coal ash. It is possible that conditions in the coal ash–polluted habitat are deleterious to the neural and/or neuromuscular responses involved in the flight response.

For a developmental malformation to have ecological implications, it must adversely impact some aspect of an organism's biology or alter the manner in which the organism interacts with its environment. To our knowledge, no studies to date have quantified the impact of amphibian malformations on parameters of swimming performance. The current study illustrates that a common pollutant-induced malformation in larvae can significantly affect larval performance. In polluted habitats where the frequency of these malformations is increased, a higher percentage of the local population may be less efficient at evading and/or detecting predators because of decreased swimming capabilities. Thus, pollutant-induced alterations in morphology may not be directly lethal, but they may ultimately decrease the recruitment of individuals to the terrestrial environment by negatively impacting larval swimming performance.

SUMMARY

Larval bullfrogs from coal ash–polluted sites had increased tissue concentrations of trace elements and higher frequencies of malformations compared with conspecifics from reference sites. The fact that malformations apparently impact swimming performance suggests that pollutant-induced malformations could ultimately impact larval survival in the polluted habitats. Future studies that quantify the effect of malformations on predation rates would be valuable in further establishing a link between morphological anomalies and larval survivorship. We have identified very few malformations in recently metamorphosing amphibians from the ash-polluted habitat (personal observations), but such high rates of spinal curvatures and abnormal oral structures [9], which negatively impact larval performance and growth rates, respectively, indicate that larval malformations may be as detrimental to local amphibian populations as grossly malformed terrestrial life stages.

In conjunction with many other studies [9,10,12,16,32–34], our results further implicate coal combustion byproducts as being disruptive agents to the biology of amphibians. Morphological, behavioral, and physiological abnormalities in amphibians exposed to coal combustion wastes have been identified, but the mechanisms by which these disruptions occur remain unknown. The status of amphibian populations as well as the ubiquity of coal combustion wastes make this a critical area for future research.

Acknowledgement—We thank Caralyn Zehnder, Chris Rowe, Deno Karapatakis, David Scott, and Laura Janecek. The City of Aiken and the City of Aiken Open Land Trust allowed us to sample Hidden Bay. We also thank Michelle Davis, Chris Beck, John Roe, Joel Snodgrass, and Tom Phillipi. W.A. Hopkins and J. Congdon were supported by the U.S. Department of Energy Financial Assistance Award DE-FC09-96SR18546 to the University of Georgia Foundation.

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