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Failed Recruitment of Southern Toads (*Bufo terrestris*) in a Trace Element-Contaminated Breeding Habitat: Direct and Indirect Effects That May Lead to a Local Population Sink

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Abstract. We conducted a transplant study in which embryonic southern toads (*Bufo terrestris*) were held in a site polluted with coal ash (site AB; containing As, Cd, Cr, Cu, Se, and other elements) and a reference site (site R) through hatching and early larval development. To examine the remainder of the larval period, surviving larvae in AB were then transplanted to R and back-transplanted to AB, whereas surviving larvae from R were back-transplanted to R. Survival through early larval development was lower in AB than in R (34% versus 50%). However, site of hatching did not influence traits later in development (larval metabolic rate, larval morphology, duration of larval period, size at metamorphosis, or average hopping distance by metamorphs). Toads that spent the entire larval period in R had high rates of survival (70–94% of individuals transplanted after the embryonic period) regardless of where they spent the embryonic and early larval period. However, toads held in AB for the duration of the larval period suffered 100% mortality. Algal resources were scarce and their trace element concentrations high in AB compared to R, suggesting that mortality of larval toads resulted from a combination of direct toxicity (via sediment- and foodborne exposure) and indirect effects on resource abundance. The study suggests that the widespread practice of disposing of coal ash in open aquatic basins may result in sink habitats for some amphibian populations.

Amphibians have become increasingly important as indicators of environmental quality because of life history traits that may make them particularly susceptible to environmental contaminants and their important ecological roles in freshwater and terrestrial habitats (see Dunson *et al.* 1992). Most amphibians have complex life cycles, in which different life stages occupy distinct habitat types; thus, susceptibility to specific environmental stresses (*e.g.*, waterborne, soilborne, etc.) varies with life stage. Amphibians also assume very different ecological roles during different parts of their complex life cycle; thus, influences of contaminants on specific life stages would be expected to have broad ecological effects in more than one habitat type. Most frogs and toads, for example, have larval stages that are grazers of living and nonliving aquatic plant material, yet on metamorphosis, they become important predators in terrestrial habitats. Substantial effects of contaminants on the aquatic life stages might not only influence the ecology of the aquatic habitat but also carry over into ecological changes in surrounding terrestrial habitats via decreased recruitment of predatory juveniles.

Most amphibians have aquatic premetamorphic life stages consisting of a relatively short (hours to days) embryonic period followed by a substantially longer larval period, lasting from days to years, depending on species. The duration of the larval period thus puts amphibians at risk of chronic exposure to contaminants in water and sediment. Not only are larval amphibians faced with potential chronic abiotic stresses, but biotic stresses can also be quite severe. The larval period is a time of rapid growth and subsequently high nutritional requirements, which can lead to severe inter- and intraspecific competition, even in systems that support a healthy resource base (*e.g.*, Brockelman 1969; Alford and Wilbur 1985). Recruitment of juveniles must therefore reflect an interaction among abiotic and biotic stresses incurred during the embryonic and larval life stages (*sensu* Dunson and Travis 1991). Such an interaction may be important in contaminated breeding habitats; contaminants may affect larval amphibians directly, via influences on physiological, behavioral, and morphological traits (see Rowe *et al.* 1996, 1998a, 1996b; Raimondo *et al.* 1998; Hopkins *et al.* 2000), and indirectly via influences on resource abundance, thereby affecting competition.

Especially where breeding habitats are isolated, contamination of one or more sites could lead to breeding sinks, potentially having strong influence on local amphibian populations. A sink habitat, a net importer of individuals, acts to attract immigrants from other (source) habitats but produces relatively few emigrants (see Pulliam 1988). Because of the seasonal

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influxes of some amphibians into aquatic sites for breeding purposes, contamination of breeding sites could reduce breeding success, limiting recruitment of juveniles into the terrestrial habitat. If local source habitats do not produce the number of migrants necessary to overcome losses to these sink habitats, long-term population declines would be predicted. Such a scenario would be particularly of concern if contaminant conditions conducive to formation of sink habitats are widespread across the landscape, providing the potential for effects on multiple populations of amphibians.

A globally widespread source of contaminants to amphibian breeding sites is the practice of discharging coal ash, a waste product of large-scale coal combustion enriched in trace elements (As, Cd, Cr, Cu, Ni, Se, and others), into open impoundments. A recent survey of 259 disposal facilities (associated with electric utilities having greater than 100 MW of coal-fired generating capacity) in 35 states in the United States found that 46% of the facilities disposed of ash in this way, accounting for about 1.8×10^7 tonne of ash entering aquatic habitats (EPRI 1997). In the disposal sites and aquatic systems receiving effluents from them, amphibians and other wildlife can accumulate trace elements and be affected lethally or sublethally (Brieger *et al.* 1992; Carlson and Adriano 1993; Lemly 1993; Gillespie and Baumann 1986; Skorupa 1998; Rowe *et al.* 1996, 1998a, 1998b; Hopkins *et al.* 1997, 1998, 1999a, 1999b, 2000). Thus, if the effects of coal ash on individuals are great enough to substantially reduce recruitment, this ubiquitous source of environmental contamination could have important ramifications for many populations of amphibians and other wildlife.

Recently, investigations of amphibians exposed to coal ash during the embryonic and larval periods have demonstrated effects on larval survival and performance. The bulk of recent studies examining effects of coal ash on amphibians has focused on aquatic life stages of the bullfrog (*Rana catesbeiana*; Rowe *et al.* 1996, 1998a, 1998b; Raimondo *et al.* 1998; Hopkins *et al.* 2000). Hatching success of embryonic bullfrogs was not affected by coal ash exposure, but survival of larvae over 80 days posthatching was reduced and average standard metabolic rates were elevated in larvae from the ash-polluted system compared to a reference site (Rowe *et al.* 1998a). Morphological and behavioral abnormalities have also been reported for larval bullfrogs chronically exposed to coal ash, influencing resource acquisition, growth, and predator avoidance (Raimondo *et al.* 1998; Rowe *et al.* 1996, 1998b; Hopkins *et al.* 2000). Thus, for bullfrogs, a variety of larval traits are modified by chronic exposure to coal ash, having potential cumulative influences on recruitment.

The recent findings of effects of coal ash exposure on larval bullfrogs prompted us to examine responses of the aquatic life stages of another amphibian, the southern toad (*Bufo terrestris*). Unlike bullfrogs, which remain near water bodies during adulthood, adult southern toads disperse into surrounding habitats, where they lead a terrestrial existence, returning to water only to breed. Influences of aquatic contaminants on recruitment of toads therefore would be expected ultimately to affect ecology of nearby terrestrial environments. During breeding events, adult southern toads are abundant in the coal ash–contaminated site used in this experiment, yet we have rarely observed late-stage larvae or recently metamorphosed individuals there (personal observation). Adult southern toads rapidly accumulate trace elements from terrestrial areas contaminated with coal ash (Hopkins *et al.* 1998) and experience endocrinological changes compared to reference animals (Hopkins *et al.* 1997, 1999a). However, the chronic effects of coal ash on recruitment of southern toads were unknown. Thus, we addressed four general questions with this study: (1) Are southern toads affected lethally by *in situ* exposure to coal ash during the embryonic and larval periods? (2) Do surviving individuals experience sublethal effects of coal ash during the larval or juvenile (postmetamorphic) periods? (3) Does the coal ash–contaminated site support a resource base adequate to sustain rapidly growing larval toads throughout the larval period? (4) Does a combination of lethal, sublethal, and indirect effects significantly reduce recruitment of juvenile toads in the contaminated site?

Materials and Methods

We conducted this study at a power and steam generating facility on the U.S. Department of Energy's Savannah River Site near Aiken, South Carolina. The facility discharges a slurried coal ash and water mixture through two settling basins and a drainage swamp; effluent then enters a tributary of the nearby Savannah River. Amphibians and other animals inhabiting the site experience elevated tissue concentrations of coal-related contaminants (Cherry and Guthrie 1978; Rowe *et al.* 1996; Hopkins *et al.* 1998, 1999b). Corresponding with the contaminant body burdens, numerous sublethal and lethal responses have been reported for amphibians (Rowe *et al.* 1996, 1998a, 1998b; Raimondo *et al.* 1998; Hopkins *et al.* 1997, 1999a, 2000) and other animals (Rowe 1998; Hopkins *et al.* 1999b) in this site.

We conducted a two-part transplant experiment in which we tested the effects of exposure to the contaminated habitat during (a) the embryonic and early posthatching periods and (b) the entire premetamorphic period of southern toads. Response variables were survival through the early larval period (stages 25–27, Gosner 1960; when larvae begin actively swimming and feeding), survival to metamorphosis, time to and size at metamorphosis, larval metabolic rates (per Rowe *et al.* 1998a), frequency of oral deformities (per Rowe *et al.* 1996, 1998b) and spinal curvatures (per Hopkins *et al.* 2000) in larvae, and hopping distance of metamorphs. We predicted that modifications from normal in the above responses would reflect duration of exposure to pollutants (least impaired to most impaired: individuals held in a reference pond, individuals held in contaminated site only through stages 25–27, individuals held in contaminated site for entire embryonic and larval period). We also measured abundance and trace element content of resources (periphyton) among sites to examine relationships between site-specific resource characteristics and survival of larval toads.

Transplant Experiment, Part I

During a spring breeding event, we collected four egg masses (< 6 h old) from a pond with no known history of pollution. Eggs were returned to the laboratory and counted into 24 groups of 150 eggs (6 groups per clutch × 4 clutches). Three groups from each clutch were placed in 19-L polyethylene and 2-mm mesh enclosures (see Rowe and Dunson 1995) in the D-Area settling basin (site AB) or in a historically uncontaminated reference pond (site R) located about 4 km from AB and having a similar thermal regime (Rowe *et al.* 1998a) and water pH (pH ~ 6.4–6.8 for both sites; personal observation). Thus, we used 12 enclosures at each site, comprised of 3 replicates from each egg mass. Enclosures were placed in the sites 15 days prior to addition of animals and contained enough sediment to cover the bottom. Eggs

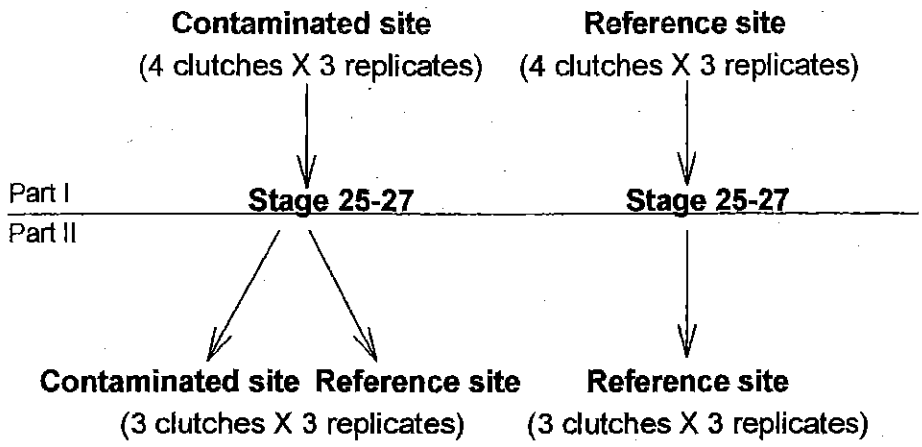

Fig. 1. An illustration summarizing the design of the transplant experiment

were checked at 1- to 2-day intervals until they had hatched and reached stages 25–27 (Gosner 1960), 9 days after transplantation of eggs. We then brought the larvae to the laboratory and counted them to determine survival. Animals beyond those needed for Part II of the study were used for measurement of physiological and morphological responses (see below).

Transplant Experiment, Part II

For the second portion of the study, we used larvae from three clutches from Part I to reduce the number of cages required. We excluded the clutch that differed the most from the mean survival to stages 25–27 in each site. We used 18 cages in site R to hold animals hatched in site AB and site R (3 clutches × 3 replicates = 9 cages each), and we used 9 cages in AB, which received animals hatched in that site only (see design summary in Figure 1). The fractional design was used in Part II because a transplant of animals hatched in site R into site AB would have provided little information about natural exposure regimes (*e.g.*, it is very unlikely that an embryo would hatch in uncontaminated conditions and find itself in a contaminated pond). However, we did include the treatment in which embryos hatched in site AB were then transplanted to site R, because this treatment allowed us to compare potential residual effects of exposure to contaminants only during the embryonic period with total effects experienced during both the embryonic and larval periods (Figure 1). The design thus allowed us to maximize the information gained within prevailing logistical constraints.

Thirty individuals were placed into each cage (70 L cage volume, 0.32 m^2 bottom area, constructed of polyethylene and 2 mm mesh). Note that the density of larvae used (94/m^2 bottom surface area) was well below the density at which conspecifics were reported to show any indication of competitive effects on growth or survival when caged for the entire larval period (> 278/m^2 bottom surface area; Brockelman 1969). The cages were in place in the study sites (with ≈ 1 cm of sediment in the bottom) for 30 days prior to use to allow a resource base to develop. After a period of 2 weeks during which cages were left undisturbed, we placed floating substrates in each cage and began monitoring cages at 2-day intervals for presence of metamorphs. Metamorphs (defined as having left the water and having a tail of < 2 mm length) were brought to the laboratory where snout to vent length (SVL) was measured to the nearest 0.1 mm and wet mass determined to the nearest 1 mg, and hopping performance trials were conducted (see below).

Measures of Physiology, Morphology, and Performance

Physiology and Morphology. After Part I of the study, two larvae from each replicate that contained survivors were used for determination of standard metabolic rate (SMR). SMR was determined by measuring oxygen consumption in resting larvae that had been held unfed for 3 days to void stomach contents. Oxygen consumption (at 25°C) was measured on a computer-controlled, closed-circuit respirometer (Columbus Instruments Micro-Oxymax) following a protocol similar to that used by Rowe *et al.* (1998a). Each channel of the respirometer independently sampled hourly rates of oxygen consumption by the pair of larvae removed from each cage. Larvae that were not used for determination of SMR or for Part II of the study were examined under a dissecting microscope for presence of oral deformities (per Rowe *et al.* 1996, 1998b).

Performance: Metamorphs collected from the second portion of the experiment were held unfed for at least 1 day until tail resorption was complete, following which we measured SVL and wet mass. We then measured hopping distance of individuals following a prod in the posterior by a blunt probe. Hopping trials were conducted in a 24°C laboratory where animals were placed on a laboratory bench top covered in white paper. The paper allowed us to mark the position of the posterior of the individual prior to prodding and after hopping. Linear hopping distance was measured to the nearest 0.1 mm. Each animal was prodded to hop three times within a 2-min interval.

Measurement of Resource Abundance and Trace Element Content

In each site, we placed replicate 11 × 9 cm Plexiglas plates 1 month prior to breeding by toads, and collected them 3 months later during the period of peak metamorphosis to quantify algal resource abundance and trace element concentration. In each site (AB and R), groups of three plates were placed in each of two areas (subsites), to provide some estimate of spatial heterogeneity within sites. Plates were suspended vertically in the same orientation and in water of similar depth among sites and areas. At the time of removal, plates were rinsed with deionized water and brought to the laboratory, where all material was scraped from the surface of both sides and weighed for wet and dry mass. Scraped material was oven-dried (55°C); a subsample of dry material from each plate was analyzed for coal ash–related trace elements using ICP-MS, and an additional subsample was combusted

Table 1. Survival of toads through the embryonic/early larval period (experiment Part I) and through metamorphosis (experiment Part II)

Site of Embryonic Development		Survival (%)
A. Experiment Part I—Survival through embryonic and early posthatching period		
AB		34.0 ± 5.1
R		50.6 ± 6.6
	Site of Larval Development	
	AB	R
B. Experiment Part II—Survival through metamorphosis		
AB	0.0 ± 0	94.4 ± 2.8
R	DNT	70.4 ± 9.5

Values are mean % survived ± 1 SE. Percentages are based on numbers survived per 150 embryos transplanted (Part I) or per 30 larvae transplanted (Part II). DNT = did not test.

at 500°C in a muffle furnace for 24 h for determination of organic content.

Data Analyses

All analyses were conducted on mean responses per replicate. Wet mass, SVL, time to and size at metamorphosis, and proportion survived were compared among treatment groups using mixed-model ANOVAs, in which clutch was a random variable and site was a fixed variable. Data for proportion survived were normalized by transformation by arcsines prior to analysis. All larval toads placed in site AB for Part II of the study died. Thus, only for animals held in R during Part II were we able to test the effects of hatching site, larval site, and clutch on responses of metamorphs. The effects of embryonic site and clutch on survival to metamorphosis for animals held in R for the second part of the experiment were determined using mixed-model ANOVA (clutch = random, embryonic site = fixed). Average hopping distances of metamorphs from each treatment group were compared using mixed-model ANOVAs (clutch = random variable) after preliminary analyses indicated that hopping distance did not vary significantly with the size variables measured (mass, SVL, or mass-to-SVL ratio). Average SMRs were compared using a mixed-model ANCOVA with wet mass as a covariate and clutch as a random variable, since linear regression indicated that oxygen consumption was correlated with total wet mass ($F_{1,16} = 6.42$, $p = 0.022$, $r^2 = 24.2\%$). The slopes describing the relationship between SMR and wet mass did not differ between treatments. Prior to analysis, measurements of oxygen consumption for each animal were ranked and the highest 50% of measurements were excluded (Rowe *et al.* 1998a). This was done to estimate the metabolic rate of starved animals at rest; since we could not monitor animals for activity during oxygen measurements, using the truncated data set for analysis should exclude values that were elevated due to activity (Rowe *et al.* 1998a). Oral and spinal deformities were absent in all animals and did not require statistical analyses.

Data for resources (wet and dry mass and organic content) were analyzed using nested, mixed-model ANOVAs, in which subsite was a random factor nested within site, a fixed factor. Prior to analysis, data for wet mass were transformed by $\log_{10}$ to satisfy assumptions of normality and homoscedasticity, and organic content was transformed by arcsine square roots. Trace element concentrations were not analyzed statistically, due to the extreme bimodal characteristics of the data.

Results

Survival to stages 25–27 (Part I of study) was significantly lower in AB than in FP ($F_{1,16} = 85.38$, $p = 0.003$; Table 1). Survival through Part I of the study was also affected by clutch ($F_{3,16} = 15.80$, $p = 0.024$), but the interaction between site and clutch was not significant ($F_{3,16} = 0.04$, $p = 0.989$). Oxygen consumption by larvae was not affected by the treatments (site $F_{1,19} = 0.97$, $p = 0.332$; clutch $F_{3,9} = 0.32$, $p = 0.813$; site * clutch $F_{3,9} = 0.20$, $p = 0.895$; covariate [mass] $F_{1,9} = 1.05$, $p = 0.332$). Oral and spinal deformities were absent in all animals, regardless of the site in which they hatched.

There was 100% mortality for larvae transplanted to AB for Part II of the study (Table 1). Survival to metamorphosis of toads transplanted to site R for Part II of the study was significantly affected by the site in which the animals had spent Part I ($F_{1,12} = 25.69$, $p = 0.037$); those hatched in AB survived better as larvae in R than did those hatched in R (Table 1). There was also an effect of clutch ($F_{2,12} = 27.56$, $p = 0.035$) on survival to metamorphosis of larvae held in site R, but the interaction of clutch and embryonic site was not significant ($F_{2,12} = 0.45$, $p = 0.648$). Time to metamorphosis was independent of clutch ($F_{2,12} = 5.90$, $p = 0.145$), site of embryonic exposure ($F_{1,12} = 1.16$, $p = 0.395$), and the interaction term ($F_{2,12} = 2.23$, $p = 0.150$). Mass and SVL at metamorphosis were independent of site of embryonic development (mass: $F_{1,12} = 1.29$, $p = 0.374$; SVL: $F_{1,12} = 0.050$, $p = 0.846$), clutch (mass: $F_{1,12} = 3.02$, $p = 0.249$; SVL: $F_{1,12} = 0.720$, $p = 0.580$), and the interaction terms (mass: $F_{2,12} = 2.24$, $p = 0.149$; SVL: $F_{2,12} = 3.63$, $p = 0.059$). Average hopping distance was also independent of manipulated variables ($p = 0.303$–0.957).

Mean wet mass of material attached to sampling plates (potential resources) was significantly lower at AB than at R ($F_{1,8} = 49.34$, $p = 0.020$; Table 2). There was significant spatial heterogeneity in wet mass within sites ($F_{2,8} = 18.31$, $p = 0.001$), but this was not the case for dry mass ($F_{1,8} = 1.06$, $p = 0.389$) or organic content ($F_{1,7} = 0.28$, $p = 0.763$; Table 2). Dry mass of potential resources did not differ among sites ($F_{1,8} = 4.34$, $p = 0.173$), but percent organic content was significantly higher in R than in AB ($F_{1,7} = 4465.6$, $p < 0.001$; Table 2). Concentrations of all measured trace elements were exceptionally high in attached material collected in AB compared to R (up to 15× higher; Table 3) and were substantially higher than previously reported concentrations in sediments (Table 3).

Discussion

The results of this experiment indicate that the lack of recruitment of southern toads from the contaminated habitat results primarily from direct or indirect effects of conditions in the contaminated site on larval survival, rather than from effects on embryonic survival. In the reference site the probability of mortality declined sharply after the free-swimming stage was

Table 2. Abundance of materials collected from resource sampling plates suspended in two areas (subsites) within each study site

Site	Subsite	Wet Mass (g/cm^2)	Dry Mass (g/cm^2)	Organic Content (%)
AB	1	0.0281 ± 0.0012	0.0031 ± 0.0002	39.2 ± 2.0
	2	0.0346 ± 0.0027	0.0027 ± 0.0002	38.2 ± 3.3
R	1	0.3031 ± 0.0165	0.0021 ± 0.0002	80.5 ± 1.2
	2	0.1781 ± 0.0134	0.0025 ± 0.0004	79.8 ± 1.4

n = 3 plates per subsite, except for organic content in AB subsite 2, in which n = 2 due to loss of one sample during combustion. Note that the similarity in dry mass among sites reflects the large amounts of ash that adhered to the plates in site AB (see text).
Values are means ± 1 SE.

Table 3. Concentrations of coal ash–related trace elements in material collected on sampling plates (organic and inorganic material available to grazing larvae). Sediment trace element concentrations in the study sites (from Rowe 1998) are included for comparison

Site	Ag	As	Ba	Cd	Co	Cr	Cu	Ni	Pb	Se	Sr	Zn
A. Material collected from sampling plates (potential resources for grazers)												
AB	0.618	140.84	597.9	20.08	273.4	46.94	550.1	356.71	21.576	11.854	163.7	1161.0
	(0.146)	(4.88)	(14.9)	(1.19)	(18.1)	(1.77)	(25.6)	(23.90)	(0.608)	(0.140)	(10.5)	(65.6)
R	0.241	8.189	295.18	0.4675	75.02	6.820	6.809	10.558	7.042	1.037	29.678	74.01
	(0.098)	(0.184)	(7.92)	(0.0492)	(3.72)	(0.162)	(0.175)	(0.322)	(0.49)	(0.098)	(0.702)	(6.32)
B. Sediments (from Rowe 1998)												
AB	NR	39.638	NR	0.252	NR	10.869	18.386	13.735	6.457	4.383	NR	NR
		(2.704)		(0.011)		(0.815)	(1.310)	(0.957)	(0.500)	(0.188)		
R	NR	0.341	NR	0.032	NR	7.022	4.036	1.107	4.224	0.104	NR	NR
		(0.008)		(0.001)		(0.186)	(0.097)	(0.125)	(0.079)	(0.005)		

Concentrations are in ppm dry mass.
n = 6 plates per site, 3 sediment samples per site.
Values are means (1 SE).
NR = not reported.
Number of decimal places represents resolution of measurements at the measured concentration.

reached, whereas in the contaminated site the probability of mortality increased as larval development proceeded. Larval mortality may have reflected cumulative impacts of contaminants; if chronic exposure was required to induce mortality, we would expect to observe most mortality during the larval period, rather than during the preceding, relatively brief embryonic period.

Effects of clutch on survival to hatching indicate that there may be parental factors (genetic or nongenetic) that vary in the study populations that may significantly affect hatching success. However, clutch-specific factors did not influence the response to contaminants, illustrated by the lack of a significant interaction between clutch and site. Embryonic survival was lower in the contaminated site than in the reference site, and there was a significant difference in larval survival based on embryonic exposure; contaminant-exposed embryos transplanted to the uncontaminated site had greater survival than unexposed embryos back-transplanted to the same uncontaminated site. Taken together, these results might indicate that embryonic exposure to contaminants reduced the hatchling pool to only the most stress-resistant individuals, and these individuals were better able to tolerate any other stresses that may have been present in the uncontaminated reference site. Such an unexpected result cannot be rigorously examined based on the protocol of this study, but it is especially interesting in the light of recent discussions regarding hormesis (*e.g.*, Forbes 2000).

Our measurements of resource abundance in the study sites suggest that larval mortality may have resulted from more complex phenomena than simply duration of exposure to contaminants. The increased risk of mortality in the contaminated habitat corresponded with the stages of development during which individuals were actively foraging. Thus, food quality (trace element concentration) and abundance may have had some influence on larval survival.

Resource abundance was exceptionally low in the contaminated site compared to the reference site (Table 2). Wet mass and organic content of material attached to sampling plates was significantly greater in the reference area than in the contaminated area, but dry mass did not differ between sites. The great difference in organic content of attached material between sites suggests that much of the material collected on the plates in the contaminated site was nonliving material, such as ash particles adhered to the plates, providing little or no nutritional value to the larvae. Our results are in agreement with a previous study examining aufwuchs in the ash-contaminated system, which also showed that majority of material attached to submerged slides was abiotic in nature (Newman *et al.* 1985).

In addition to low resource abundance in the contaminated habitat, the organic material that was available as periphyton contained very high concentrations of trace elements (*e.g.*, resource quality was low; Table 3). Moreover, trace element concentrations were much higher in attached material than in surface sediments in the contaminated site (Table 3). Because the sediments in the contaminated site were almost entirely composed of ash, the elevated concentrations in attached material compared to sediments suggests that there was substantial

accumulation of trace elements by living resources. The resource measurements suggest that recruitment of juvenile toads was reduced by direct effects of sediment- and foodborne contaminants on larval toads, combined with challenges imposed by food limitations. Thus, mortality of larval toads was likely the result of several direct and indirect pathways by which contaminants exerted influences on the system.

Conditions in the coal ash–contaminated area appear to have affected premetamorphic toads differently than had been reported for another amphibian, the bullfrog. Primarily, whereas larval bullfrogs can inhabit the contaminated area for long periods of time and experience multiple, sublethal effects (Rowe *et al.* 1996, 1998a, 1998b, Raimondo *et al.* 1998; Hopkins *et al.* 2000), toads were far more likely to be affected lethally. Hatching success of toads was reduced in the contaminated site, but this was not the case for bullfrogs (Rowe *et al.* 1998a). Moreover, whereas 15% of bullfrogs survived 80 days beyond hatching in the contaminated site (Rowe *et al.* 1998a), all toads died prior to metamorphosis, about 58 days after hatching (mean time to metamorphosis in the reference site). There are no quantitative data on recruitment success of bullfrogs with which we can compare results of the current study. However, we have occasionally observed recently metamorphosed bullfrogs near the contaminated site, suggesting that some bullfrogs may be successfully recruited from that site (personal observation). Although such comparisons among studies are necessarily qualitative, perhaps southern toads are more sensitive to conditions as a whole (contaminants, resources, other unmeasured factors) in the contaminated site than are bullfrogs.

The complete failure in recruitment of toads in the contaminated site indicates that the coal ash–contaminated area may serve as a breeding sink (*sensu* Pulliam 1988) for toads from the local population. Over several years we have observed repeated breeding events in which large numbers of adult southern toads enter the contaminated site to breed, yet we have rarely observed late-stage larvae or metamorphs in the site. This experiment suggests that the observed lack of recruits in the contaminated area is due to lethal impacts of conditions in the site on larval toads. It thus appears that the breeding populations of toads using the contaminated sites are themselves recruits from populations breeding in other, uncontaminated sites. These adults migrate during breeding events to the contaminated site where they experience reproductive failure.

The overall importance of the contaminated site in modifying the ecology of nearby terrestrial areas remains unknown. Local uncontaminated breeding sites (such as our reference site) might export a sufficient number of recruits to nearby habitats such that overall impacts of the contaminated site are minimal. On the other hand, if recruitment failure following breeding by large numbers of toads in the contaminated site is a recurring event and migration from other sites cannot compensate for the lost cohorts, we would expect toad densities in surrounding habitats to be reduced. Given the trophic importance of some amphibians in the ecology of terrestrial systems (for example, see Burton and Likens 1975a, 1975b), substantial reductions in one or more species might be expected to have important, ecological ramifications. The potential long term influence of contaminant-induced changes in amphibian breeding and recruitment dynamics on larger ecological phenomena needs to be examined rigorously.

Conclusion

Contaminated aquatic systems may act as sinks for amphibian populations by attracting adults that subsequently experience poor reproductive success. For animals such as amphibians that have complex life cycles, the effects of contaminants on aquatic life stages could carry over into effects on terrestrial systems, via loss of recruits to the terrestrial habitat. This study implicates conditions in an open coal ash disposal system as having severe effects on recruitment of southern toads, apparently via a complex interaction of direct and indirect effects. Given recent concerns about amphibian population declines, we suggest that investigators examine the role of contaminated habitats as potential recruitment sinks influencing local amphibian populations.

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