

Effects of competition and coal-combustion wastes on recruitment and life history characteristics of salamanders in temporary wetlands

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Abstract

Amphibians in natural systems must cope with a number of biotic and abiotic stressors that can potentially interact with pollutants to influence toxicity. Although interactive effects of short-lived pesticides with various environmental stressors have been studied, how persistent and bioaccumulative compounds such as metals interact with natural stressors to influence amphibians remains unexplored. We exposed the salamander *Ambystoma talpoideum* to coal-combustion wastes (a complex mixture of metals and metalloids, hereafter referred to as CCW) at low and high larval density throughout aquatic development in mesocosms simulating temporary wetlands. CCW and high density reduced survival to metamorphosis by 57–77% and 85–92%, respectively, and the effects of these two factors together were additive. Reduced metamorphosis was due in part to larval mortality prior to initiation of pond drying, but CCW and high density also extended the larval period, causing mortality of larvae that were not ready to metamorphose before the pond dried. To our knowledge, this is the first demonstration of a pollutant-induced extension of larval period leading to reduction in amphibian recruitment. Metamorphs were smaller in high density compared to low in reference ponds, but those from CCW emerged at similar sizes irrespective of density, suggesting less-than-additive effects of density and CCW on metamorph size. The adverse responses of salamanders to CCW were likely due to direct toxicity, as *A. talpoideum* metamorphs accumulated high concentrations of a suite of trace elements (As, Se, Sr, and V), and also to indirect effects on the community food web. We conclude that in no case did the addition of a natural stressor (high density) exacerbate CCW-related effects, but that the effects of CCW alone can be detrimental to larvae of salamanders that breed in temporary ponds.

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1. Introduction

Environmental pollutants are believed to be one of the greatest threats to the health of amphibian populations (Stuart et al., 2004), but the degree to which species or populations are affected may depend on a number of non-toxicological factors. For example, both abiotic (e.g., temperature, UVB-radiation) and biotic (predators, pathogens, competition) factors are known to interact with pollutants to influence contaminant effects in amphibian larvae (Zaga et al., 1998; Taylor et al., 1999; Boone and Bridges, 1999; Boone and Semlitsch, 2001; Relyea and Mills, 2001; Metts et al., 2005). While important for establishing cause and effect relationships and identifying mechanisms

of toxicity, studies examining the impact of environmental contaminants under highly controlled conditions (e.g., stable water levels and constant uncontaminated food rations in a laboratory environment) are often not representative of complex field exposure conditions and thus risk drawing oversimplified conclusions not always applicable to populations in natural systems (Sibly, 1999; Hopkins et al., 2004).

Many amphibians breed in temporary wetlands and must cope with dramatic fluctuations in both abiotic and biotic conditions characteristic of these habitats. Water level changes, and perhaps most importantly complete drying, put limitations on the length of time allowed for growth and development of aquatic larval stages. In theory, pollutant-induced reductions in development and/or growth rates could prevent individuals from metamorphosing in temporary wetlands, but whether pollutant-induced extensions of the larval period actually reduce the number of individuals recruited into terrestrial life stages

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has not been demonstrated. Another common natural stressor in temporary wetlands is larval density, which can vary widely within and among years according to water level fluctuations, variance in adult breeding population size, predator density, and other factors (Semlitsch et al., 1996). Increased density is known to reduce larval growth and survival to metamorphosis, and increase time to metamorphosis in amphibians, all of which influence recruitment into the adult population (Wilbur, 1976; Semlitsch, 1982; Scott, 1990). In populations regulated by density-dependent factors, the addition of an environmental contaminant could influence contaminant effects in one of three ways outlined by Forbes et al. (2001). Stress caused by the toxicant could either exacerbate density-dependent effects (more-than-additive), ameliorate density-dependent effects (less-than-additive), or the effects of contaminants and density could operate independently of one another (additive), but the nature of the interaction between toxicant and density ultimately depends on the life history type of the species in question and which life history traits are affected by the contaminant (Forbes et al., 2001).

The combustion of coal by the power industry generates one of the largest solid waste streams in the world. Roughly 123 million tonnes of coal-combustion wastes (CCW), which is a highly concentrated mixture of trace elements (As, Cd, Cu, Ni, Pb, Se, Sr, Zn, and others), is produced annually in the United States (USDOE, 2003). The quantity of waste produced, along with its toxic constituents, poses significant challenges for its disposal. Currently, one-third of CCW is disposed in aquatic basins where amphibians may breed (Rowe et al., 2002). The disposal of CCW in this manner has been identified as a factor compromising the health of aquatic-breeding amphibians, and potentially resulting in localized declines. Numerous species of amphibians inhabiting such systems accumulate high concentrations of trace elements and exhibit a variety of adverse responses (Rowe et al., 1996, 1998; Hopkins et al., 1997, 1999, 2000a,b, 2006; Snodgrass et al., 2004), including complete recruitment failure (Rowe et al., 2001). We may expect that amphibian responses to trace element contamination may differ in the presence of natural stressors because competition and food limitation have been shown to modify biological responses to trace element exposure in other organisms (fish: Hopkins et al., 2002; polychaetes: Johnston and Keough, 2003; Hopkins et al., 2004), and numerous studies demonstrate how short-lived organic contaminants (e.g., pesticides) interact with density to impact amphibians (Boone and Semlitsch, 2001, 2002; Boone and James, 2003; Mills and Semlitsch, 2004; Metts et al., 2005). However, to our knowledge, none have investigated how density interacts with complex contaminant mixtures that do not degrade (e.g., CCW) to affect amphibians.

We conducted an experiment in outdoor mesocosms simulating temporary wetlands to determine whether CCW and different levels of larval density interact to influence aspects of metamorphosis in the salamander *Ambystoma talpoideum*. We hypothesized that CCW would reduce zooplankton prey, reduce salamander survival to and size at metamorphosis, and lengthen the larval period. We predicted that extending the larval period would decrease the number of individuals that would successfully metamorphose prior to complete drying. We also

hypothesized that the impact of CCW would be more severe at high densities, where the intensity of competition for limited resources is higher.

2. Methods

2.1. Study species

The mole salamander, *A. talpoideum*, is common throughout much of the southeastern coastal plain of the United States, with disjunct populations occurring in eight southern states (Petranka, 1998). *A. talpoideum* adults migrate to aquatic habitats from surrounding terrestrial sites to breed during winter and early spring. Following courtship in the water, females oviposit between 10 and 1000 eggs, and soon after mating and oviposition, adults return to terrestrial habitats where they remain until the next breeding season (Semlitsch and Gibbons, 1985). Within 30–40 days, eggs hatch into aquatic, carnivorous larvae that prey on a variety of small vertebrates and microinvertebrates (Semlitsch, 1985; Taylor et al., 1988). After 4–5 months in the pond, aquatic larvae are capable of metamorphosing into terrestrial subadults that migrate to terrestrial habitats where they will remain until mature. However, larvae may remain aquatic and reproduce as paedomorphs if the pond does not dry (Semlitsch, 1985).

2.2. Experimental design

We created aquatic mesocosms in 24 polyethylene cattle tanks (1.85 m diameter, 1480 L volume) in a field at the Savannah River Ecology Laboratory in Aiken County, South Carolina, USA. Mesocosms were fitted with adjustable standpipes and covered with screen mesh lids to exclude potential predators and competitors. Mesocosms were arranged in a randomized block design with four treatments reflecting combined exposure to contaminated (CCW) or reference (REF) sediment at either low (L) or high (H) larval density. Each of the four treatments (CCW L, REF L, CCW H, REF H) had six replicates, and two replicates from each treatment were randomly allocated to each of six blocks.

In November 2003, mesocosms were filled with 95 L of dry sediment to provide a benthos approximately 5 cm deep. Sediments with low organic content were collected either from a basin receiving CCW or from a historically unpolluted section of upper three runs creek (REF). For a description of the CCW-polluted site, refer to Hopkins et al. (1997) and Roe et al. (2005). We then added 1000 L of well water, 1.5 kg of dry leaf litter, and 17 g of finely ground Purina Rabbit Chow[®] to each mesocosm. We also added equal volumes of pond water with concentrated zooplankton and periphyton collected from several nearby unpolluted wetlands on three occasions between 24 November and 9 December. To supplement zooplankton collected from natural ponds, we added equal amounts of *Daphnia magna* and *Daphnia pulex* obtained from Carolina Biological Supply Co. (Burlington, NC).

In January 2004, we captured adult *A. talpoideum* in pitfall traps along a drift fence during their breeding migration to an

unpolluted wetland, Flamingo Bay, in Aiken County, SC, USA. To induce courtship and oviposition, we paired six females with two males each in 10 L aquaria containing leaf litter and well water. Following oviposition, eggs (none older than four-cell stage) were collected and kept overnight in an environmental chamber at 10 °C for approximately 24 h. On 19 January 2004, eggs from the six clutches were mixed to homogenize genetic variation before being separated into groups of 12 (low density) or 36 (high density). The groups were then randomly assigned to the sediment treatments. Eggs were added to mesocosms in floating, flow-through containers that kept the eggs submerged approximately 10 cm deep suspended over, but not in direct contact with the sediment. As eggs are typically deposited on leaf litter or attached to twigs (Semlitsch and Gibbons, 1985), direct contact with sediments would not likely occur in basins that have revegetated. Eggs were monitored daily for hatching and mortality, and dead embryos were removed to prevent fouling of neighboring eggs.

Because egg mortality was uneven among mesocosms (see Section 3), we redistributed larvae to achieve the desired starting densities for larval exposure. Larvae from their respective sediment treatments (to which they were exposed as eggs) were pooled in a large aquarium and allowed to mix for 1.5 h. We then haphazardly split larvae from each sediment treatment into groups of 11 and 33 individuals to represent low and high densities, respectively. Densities of 11–33 larvae per 1000 L are within the range of larval densities found in natural breeding ponds (Semlitsch, 1987a). Larvae were then released into mesocosms on 2 March 2004.

To simulate the drying of temporary wetlands and induce metamorphosis (defined as resorption of gills), we lowered water levels by adjusting the standpipes. Beginning on 21 May 2004, water levels were lowered 5 cm per week. The drying regime mimicked that of nearby natural wetlands, many of which dry in summer (June–August; Semlitsch, 1985). We suspended three minnow traps in each mesocosm such that the trap opening was even with the water level. Traps were checked daily for metamorphs from 11 May until 1 July 2004, when water levels dropped below 10 cm. At this point, we considered mesocosms uninhabitable for salamander larvae because water temperature exceeded 40 °C, and dead larvae were floating to the surface in large numbers. At this point, the remaining water was drained and mesocosms were thoroughly searched for remaining salamanders.

2.3. Data collection

We measured important developmental and life history endpoints including hatching success, survival to initiation of drying, proportion of larvae metamorphosing, time to metamorphosis, as well as snout–vent length (SVL) and mass at metamorphosis. All individuals unaccounted for at the end of the experiment were assumed to have died early in the experiment before the initiation of drying (before the first water level lowering), and we refer to these as “died before initiation of drying”. Larvae that were known to have died throughout pond drying (from initiation of water level lowering to complete drying) are referred to

as “died during drying”. Time to metamorphosis was measured as the number of days elapsed from initiation of larval exposure to metamorphosis.

We measured water temperature, dissolved oxygen, conductivity, pH, and chlorophyll concentration in each mesocosm on four occasions (22 January, 2 March, 15 April, and 20 May). We continued to sample water temperature weekly throughout water drawdown. To assess food availability, zooplankton (comprised of mostly cladoceran and copepods; see Section 3) were sampled three times during the study (2 March, 15 April, and 20 May) by submerging a 3.3-L Van Dorn bottle to a depth of 15–25 cm at two consistent locations in each mesocosm. The two samples were pooled and filtered through an 80 µm mesh sieve, preserved in 95% ethanol, stained, then identified and counted under 25× magnification to estimate abundance.

Sediment and water samples from one location in each mesocosm were collected immediately prior to water drawdown. Approximately 50 g of sediment was collected by hand and sealed in sterile whirlpaks, and approximately 50 mL of water was collected by submerging a plastic bottle 15–25 cm deep. Once all metamorphs were collected and frozen, we selected a random subset of up to three metamorphs from each mesocosm for trace element analysis; in mesocosms where fewer than three larvae metamorphosed, all metamorphs were kept for analysis. All samples were immediately frozen (–50 °C) for storage.

Whole bodies of metamorphic salamanders were lyophilized and sediments were oven-dried prior to homogenization for trace element analysis. Water was filtered (1.2 µm) and acidified before analysis. Concentrations of eight trace elements (As, Cd, Cu, Ni, Pb, Se, Sr, and V) were determined using an inductively coupled plasma-mass spectrometer (Perkin-Elmer, Norwalk, CT, USA). We chose this suite of trace elements because they typically occur in high concentrations in the sediments and biota at CCW-contaminated sites (Hopkins et al., 1998; Roe et al., 2005). For a detailed description of digestion and trace element analysis procedures, refer to Hopkins et al. (2004). Mean percent recovery in certified reference materials (TORT-2 lobster hepatopancreas, NRCC, Canada) for sediments and salamander tissue ranged from 80 to 112%, and mean method detection limits ranged from 0.009 to 3.592 ppm. Tissue and sediment trace element concentrations (ppm) are presented on a dry mass basis, while water concentrations are presented in ppb.

2.4. Statistical analyses

Statistical analyses were performed using SPSS Version 11.5 (SPSS, 1999). We examined the assumptions of homogeneity of variances and normality; where deviations from these assumptions were detected, the appropriate transformations were made. Tank-specific mean values for all variables were used in analyses. For all tests, we initially included block as a fixed factor in the model, but where not significant, statistical tests were then run with block dropped from the model. When multivariate tests indicated significant results, we used univariate ANOVA to further explore each variable separately. Statistical significance was accepted at the $\alpha = 0.05$ level, and means are presented ± 1 standard error (S.E.).

To examine survival to initiation of drying and proportion metamorphosing, we used a MANOVA with sediment, density, and block as the main effects. In the above analysis, survival to initiation of drying was calculated as the number of larvae that died during drying plus those that metamorphosed. To examine effects on metamorph size (mass) and time to metamorphosis, we used a MANCOVA with sediment, density, and block as main effects, and survival to initiation of drying as the covariate. Hatching success was examined with univariate ANOVA with sediment, density, and block as the main effects. We chose not to include hatching success in the above MANOVAs because recently hatched larvae were redistributed into new tanks between embryonic and larval exposures, and thus variables collected during embryonic exposures would be independent of variables collected during larval exposure. We then examined metamorph body condition among treatments using ANCOVA, with sediment, density, and block as the main effects, mass as the dependent variable, and SVL as the covariate. This analysis assumes an animal in good condition would have a greater mass than an animal of the same SVL in poor condition. Hatching success, survival to initiation of drying, and proportion metamorphosing were all arcsine square-root transformed, while mass and SVL were log₁₀-transformed. Because mass and SVL were correlated (see Section 3), we chose to use only mass at metamorphosis in statistical tests examining size.

To examine differences in environmental variables among treatments, we used repeated-measures MANOVA with log₁₀-transformed water temperature, dissolved oxygen, conductivity, pH, and chlorophyll concentration as dependent variables and sediment type, density, and block as main effects. Effects on zooplankton abundance were examined using repeated-measures MANOVA, with log₁₀-transformed cladoceran and copepod abundance as the dependent variables and sediment type, density, block as the main effects. Even after logarithmic transformations, neither environmental variables nor zooplankton abundances satisfied the assumption of sphericity (the variances of the differences between treatments were not equal). Consequently, we used the *P*-values reported for the Greenhouse–Geisser estimate of epsilon.

Trace element concentrations in metamorphs, sediment, and water were examined using MANOVAs, with element concentrations as the dependent variables and sediment type, density, and block as main effects. For statistical comparisons, trace element concentrations below the instrument’s detection limit (BDL) were given a value of half the minimum detection limit for the element. For sediment, As, Cd, Se, and V concentrations were BDL in most REF samples, while no elements were BDL for water or tissue.

3. Results

3.1. Effects on salamanders

All viable eggs hatched within 44 days of being introduced to mesocosms. Hatching success ranged from 67 to 80%, but did not differ between sediment types or densities after accounting for a significant block × density interaction (sedi-

Table 1

Summary of MANOVA results for various life history traits for *Ambystoma talpoideum* exposed to coal-combustion wastes (CCW) at high and low density in simulated temporary ponds

	Hypothesis, error d.f.	Wilk’s λ	<i>F</i>	<i>P</i>
Survivorship to drying, proportion metamorphosing				
Sediment	2, 19	0.53	8.47	0.002
Density	2, 19	0.26	27.57	<0.001
Sediment × density	2, 19	0.69	4.33	0.028
Block effects		>0.41	<2.07	>0.100
Size at metamorphosis, time to metamorphosis				
Sediment	2, 12	0.99	0.06	0.941
Density	2, 12	0.57	4.58	0.033
Sediment × density	2, 12	0.57	4.60	0.034
Covariate (survival)	2, 12	0.61	3.79	0.053
Block effects		>0.54	<1.81	>0.203

Block effects include main effect and all interactions of block with other factors.

ment: $F_{1,12} = 0.83$, $P = 0.380$; density: $F_{1,12} = 0.05$, $P = 0.832$; sediment × density: $F_{1,12} = 0.63$, $P = 0.442$; density × block: $P = 0.037$).

Larvae began to metamorphose on 26 May after 83 days of larval development, which was 5 days following the initial water drawdown. We detected an interaction between sediment and density on proportion of individuals surviving to initiation of drying and proportion metamorphosing, but block effects were not significant (Table 1). The interaction was due only to effects on survival to initiation of drying (ANOVA; sediment: $F_{1,20} = 1.63$, $P = 0.217$; density: $F_{1,20} = 14.09$, $P = 0.001$; sediment × density: $F_{1,20} = 8.83$, $P = 0.008$), where high density reduced the proportion of individuals surviving to initiation of drying in REF but had no effect in CCW (Fig. 1). Proportion of larvae metamorphosing depended upon both sediment type and larval density, but not their interaction (ANOVA; sediment: $F_{1,20} = 16.97$, $P = 0.001$; density: $F_{1,20} = 58.03$, $P < 0.001$; sediment × density: $F_{1,20} = 3.87$, $P = 0.063$; Fig. 1). More larvae survived to metamorphosis in REF than CCW, and more metamorphosed at low density than high (Fig. 1). Of larvae that

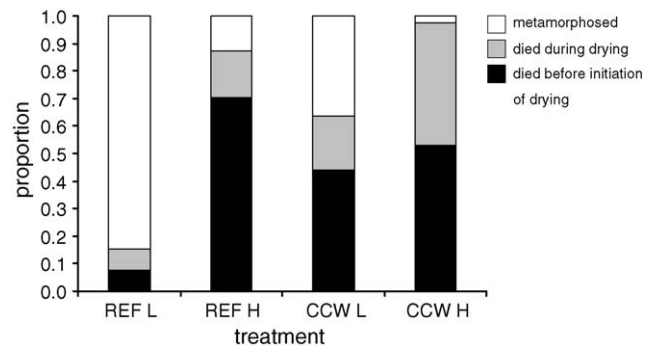


Fig. 1. Proportion of *Ambystoma talpoideum* larvae that died before initiation of pond drying, that died during pond drying, or that survived to metamorphosis after exposure to sediments from either a coal ash-polluted settling basin (CCW) or a reference site (REF) at low (L) and high (H) larval density in mesocosms simulating temporary wetlands. Note that the overall proportion of individuals that survived up to initiation of pond drying was examined by combining those that died during pond drying with those that successfully metamorphosed.

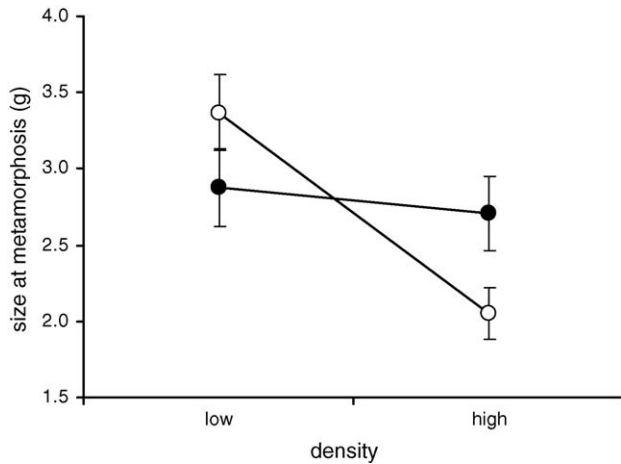


Fig. 2. Size at metamorphosis for *A. talpoideum* raised at high and low density in mesocosms containing sediments from either a coal ash-polluted settling basin (CCW; black) or a reference site (REF; white).

survived to initiation of drying but that failed to metamorphose, the majority (88%) died during the final week of the experiment when water levels dropped below 10 cm.

After adjusting for survival, metamorph size and time to metamorphosis were also affected by an interaction between density and sediment type, but no block effects were detected (Table 1, Fig. 2). The interaction was due only to effects on size (ANOVA; sediment: $F_{1,13}=0.09$, $P=0.764$; density: $F_{1,13}=6.22$, $P=0.027$; sediment \times density: $F_{1,13}=5.76$, $P=0.032$), as metamorphs emerged larger from low density compared to high in REF, but at similar sizes in both densities in CCW (Fig. 2). Mean time to metamorphosis ranged from 101.4 ± 4.2 days in REF low density to 110.7 ± 1.6 days in the CCW high density treatments, but timing of metamorphosis did not depend on either sediment or density (ANOVA; sediment: $F_{1,13}=0.004$, $P=0.950$; density: $F_{1,13}=0.634$, $P=0.440$; sediment \times density: $F_{1,13}=848$, $P=0.374$).

Snout-vent length was a significant predictor of body mass, and after the relationship between SVL and mass was accounted for, neither sediment, density, block, nor any interactions between dependent variables and the covariate influenced body mass, indicating similar body condition among treatments (SVL: $F_{1,14}=46.80$, $P<0.001$, sediment: $F_{1,14}=1.43$, $P=0.257$; density: $F_{1,14}=1.29$, $P=0.280$; all interactions: $F_{1,14}<1.44$, $P>0.255$, block effects: $P>0.202$).

3.2. Environmental conditions and zooplankton community

Environmental conditions changed over the course of the study, and changes depended on an interaction of sediment type and density after accounting for block effects (time: Wilks' $\lambda=0.00$, $F_{15,89}=811.29$, $P<0.001$; time \times sediment: Wilks' $\lambda=0.03$, $F_{15,89}=15.90$, $P<0.001$; time \times density: Wilks' $\lambda=0.71$, $F_{15,89}=0.77$, $P=0.710$; time \times sediment \times density: Wilks' $\lambda=0.46$, $F_{15,89}=1.95$, $P=0.028$; time \times density \times block: $P=0.003$). Between-subjects tests indicated differences between sediment treatments in chlorophyll, temperature, and conductivity, but not dissolved oxygen or pH (chlorophyll:

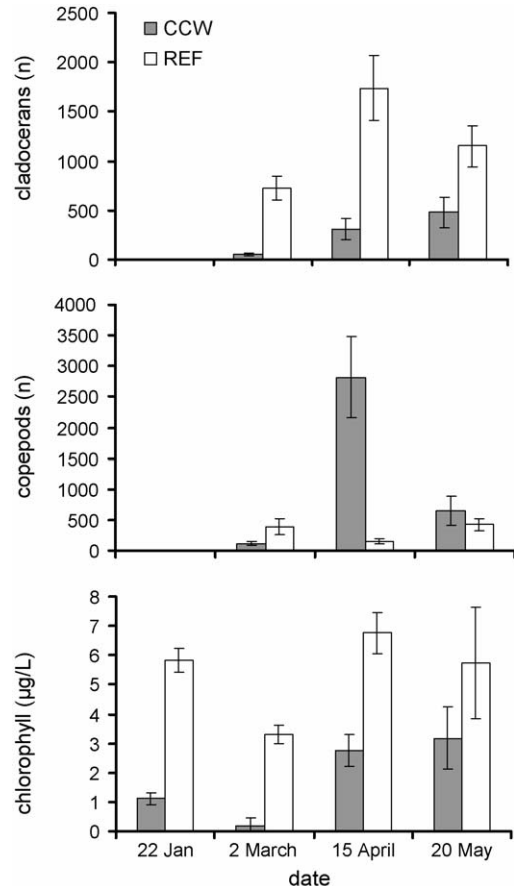


Fig. 3. Chlorophyll concentrations and relative abundance of zooplankton prey in experimental mesocosms containing sediments from either a coal ash-polluted settling basin (CCW) or a reference site (REF).

$F_{1,20}=39.33$, $P<0.001$; temperature: $F_{1,20}=7.90$, $P=0.011$; conductivity: $F_{1,20}=397.90$, $P<0.001$; dissolved oxygen: $F_{1,20}=0.02$, $P=0.898$; pH: $F_{1,20}=0.01$, $P=0.994$; block effects: $P>0.126$). Mean chlorophyll concentrations were higher in REF than CCW throughout the experiment, with differences tending to be greatest earlier in the experiment (REF 5.2–16.5 times higher than CCW in January and March) compared to the end (REF 1.8–2.4 times higher than CCW in April and May; Fig. 3). Water temperature and conductivity differences between sediment treatments tended to be more consistent throughout, with mean temperatures between 0.21 and 0.59°C higher in REF than CCW, and mean conductivity values 3.0–3.1 times higher in CCW than REF. Mean temperatures ($^\circ\text{C}$) ranged from 8.46 ± 0.14 (CCW) and 8.82 ± 0.18 (REF) at the beginning of the experiment to 30.90 ± 0.21 (CCW) and 31.11 ± 0.15 (REF) at the final sampling period prior to water drawdown, while mean conductivity ($\mu\text{S}/\text{cm}$) ranged from 111 ± 4 (CCW) and 37 ± 1 (REF) at the beginning of the experiment to 187 ± 6 (CCW) and 62 ± 2 (REF) at the final sampling period. Mean dissolved oxygen (% saturation) ranged from 99.9 to 77.2 at the initial and final sampling, while mean pH ranged from 7.1 to 8.1.

The most numerically abundant groups of zooplankton in the mesocosms were copepods and cladocerans, which together

Table 2

Trace element concentrations in water, sediment, and whole bodies of recently metamorphosed *A. talpoideum* from experimental mesocosms containing sediments from either a coal ash-polluted settling basin (CCW) or a reference site (REF)

	Water (ppb)		Sediment (ppm)		Metamorphs (ppm)	
	REF	CCW	REF	CCW	REF	CCW
As	0.80 ± 0.06	24.43 ± 1.94	BDL	124.09 ± 6.55	0.20 ± 0.03	3.15 ± 0.72
Cd	0.01 ± 0.01	0.08 ± 0.04	BDL	4.22 ± 0.70	0.08 ± 0.03	1.37 ± 0.85
Cu	2.58 ± 0.21	2.42 ± 0.30	1.01 ± 0.23	106.66 ± 3.19	5.69 ± 0.53	7.06 ± 2.65
Ni	0.75 ± 0.08	1.23 ± 0.22	0.32 ± 0.08	106.31 ± 4.16	0.64 ± 0.11	0.93 ± 0.18
Pb	0.09 ± 0.01	0.07 ± 0.01	0.89 ± 0.12	26.86 ± 1.51	0.69 ± 0.27	0.38 ± 0.17
Se	0.21 ± 0.02	1.83 ± 0.12	BDL	10.53 ± 0.65	1.66 ± 0.13	34.69 ± 2.71
Sr	7.54 ± 0.62	346.05 ± 17.38	1.01 ± 0.09	392.89 ± 31.05	49.37 ± 3.77	250.29 ± 16.38
V	0.35 ± 0.09	1.80 ± 0.28	BDL	103.33 ± 5.00	0.24 ± 0.02	0.61 ± 0.08

Concentrations were compared between treatments using MANOVA, and further explored with univariate ANOVA. Water concentration (ppb) differed between treatments for all elements except Cu and Pb, sediment concentration (ppm) differed for all elements, and tissue concentration (ppm) differed for all elements except Cd, Cu, Ni, and Pb. BDL indicates values below instrument detection limits.

comprised 98% of all invertebrates in our samples. Copepod and cladoceran densities ranged from 17 to 427 and 8 to 264 individuals per liter, respectively. Abundances of both copepods and cladocerans changed throughout the study, and changes depended on sediment treatment but not density or block (time: Wilks' $\lambda = 0.48$, $F_{4,78} = 8.63$, $P < 0.001$; time \times sediment: Wilks' $\lambda = 0.56$, $F_{4,78} = 6.52$, $P < 0.001$; time \times density: Wilks' $\lambda = 0.87$, $F_{4,78} = 1.40$, $P = 0.241$; time \times sediment \times density: Wilks' $\lambda = 0.96$, $F_{4,78} = 0.42$, $P = 0.791$; block effects: $P > 0.063$). Between-subjects tests indicated zooplankton abundance differed between sediment types, but not density or block (copepod: sediment, $F_{1,20} = 14.57$, $P = 0.001$; density, $F_{1,20} = 0.12$, $P = 0.734$; sediment \times density, $F_{1,20} = 0.37$, $P = 0.548$; cladocera: sediment, $F_{1,20} = 36.67$, $P < 0.001$; density, $F_{1,20} = 0.13$, $P = 0.727$; sediment \times density, $F_{1,20} = 0.87$, $P = 0.362$; block effects $P > 0.059$). Cladoceran abundance was reduced by 54–93% in CCW compared to REF throughout, whereas copepod abundance was similar between sediment types for the first and last sampling periods. However, copepods were more abundant in CCW than in REF during the middle sampling period (Fig. 3).

3.3. Trace elements

Trace element concentrations in water differed between sediment treatments but not density after accounting for block effects (sediment: Wilks' $\lambda = 0.001$, $F_{5,8} = 434.11$, $P < 0.001$; density: Wilks' $\lambda = 0.303$, $F_{5,8} = 1.44$, $P = 0.358$; sediment \times density: Wilks' $\lambda = 0.430$, $F_{5,8} = 0.82$, $P = 0.614$; sediment \times density \times block: $P = 0.004$), while concentrations in sediment differed between sediment treatments, but not density or block (sediment: Wilks' $\lambda = 0.001$, $F_{8,13} = 1617.13$, $P < 0.001$; density: Wilks' $\lambda = 0.765$, $F_{8,13} = 0.50$, $P = 0.836$; sediment \times density: Wilks' $\lambda = 0.719$, $F_{8,13} = 0.53$, $P = 0.737$; block effects: $P > 0.141$). Trace element concentrations in sediment were higher in CCW than REF for all elements (in all cases $F_{8,15} > 139.40$, $P < 0.001$; Table 2). Concentrations of seven elements (As, Cd, Ni, Pb, Se, Sr, and V) were significantly higher in water from CCW compared to REF (in all cases $F_{1,12} > 6.43$,

$P < 0.026$), whereas Cu concentrations were similar between sediment types ($F_{1,12} = 1.13$, $P = 0.308$).

Whole body concentrations of trace elements in metamorphic salamanders differed between sediment treatments, but larval density had no effect after accounting for significant block effects (sediment: Wilks' $\lambda = 0.000$, $F_{1,8} = 9439.24$, $P = 0.008$; density: Wilks' $\lambda = 0.001$, $F_{1,8} = 132.30$, $P = 0.067$; sediment \times density: Wilks' $\lambda = 0.002$, $F_{1,8} = 57.52$, $P = 0.102$; sediment \times block: $P = 0.048$; sediment \times density \times block: $P = 0.038$). Individual ANOVAs indicated that concentrations of four elements (As, Se, Sr, and V) in metamorphs from the CCW treatment were significantly elevated above those from REF (in all cases $F_{1,8} > 22.34$, $P < 0.001$; Table 2), but concentrations of Ni, Cu, Cd, and Pb were similar in metamorphs between sediment types (in all cases $F_{1,15} < 2.03$, $P > 0.192$; Table 2).

4. Discussion

A. talpoideum accumulated a suite of trace elements and exhibited adverse responses to contaminant exposure, high larval density, and the interaction of these two factors, but not always as predicted. Moreover, both high density and CCW caused large numbers of *A. talpoideum* to extend larval period and fail to metamorphose before complete pond drying, but such reductions in recruitment may not have been detected under stable water levels. Taken together, our results suggest that the type of interaction observed between environmental contaminants and natural stressors in *A. talpoideum* depends on the life history endpoint in question.

Survival to metamorphosis is an endpoint of critical importance to populations of temporary pond-breeding amphibians, as the number of metamorphs directly determines the number of individuals recruited into the adult population that can potentially contribute to population-level processes by reproducing (Berven, 1990; Beebee et al., 1996). Exposure to CCW during larval development, but not embryonic development, reduced the number of *A. talpoideum* that metamorphosed by 57–77% at low and high densities, respectively. Although other studies

have shown CCW to reduce survival to metamorphosis in anurans (frogs and toads; Rowe et al., 2001; Snodgrass et al., 2004), this is the first to demonstrate such effects in salamanders.

The ability of most larval amphibians to metamorphose is contingent upon both survival through larval development and attainment of a minimum size to initiate metamorphosis (35 mm SVL for *A. talpoideum*; Wilbur and Collins, 1973; Semlitsch and Wilbur, 1988). Reduced metamorphosis in this study may be a direct consequence of trace element toxicity, as salamanders accumulated high levels of at least four trace elements that were significantly elevated in the water and sediment of CCW mesocosms (Table 2). Although comparisons to other studies are potentially complicated by trophic level differences, concentrations of most elements in *A. talpoideum* were similar to those of field-exposed metamorphic amphibians from the same contaminated site (Hopkins et al., 1998; Snodgrass et al., 2003; Roe et al., 2005), but notably higher than those in laboratory exposures where supplemental uncontaminated food was provided (Snodgrass et al., 2003, 2004). Tissue concentrations of elements of toxicological concern in *A. talpoideum* were at least comparable to those accumulated by aquatic animals that directly linked CCW exposure with adverse effects including mortality, reduced growth rate, and developmental arrest (Hopkins et al., 2000a,b; Snodgrass et al., 2004), all of which could contribute to the lower metamorphic rates of CCW-exposed salamanders in this study.

The indirect effects of contaminants can be equal to or more severe than direct toxicity (Fleeger et al., 2003). Like previous studies, both primary productivity (chlorophyll) and invertebrate prey resources were reduced in CCW (Cherry et al., 1979; Rowe et al., 2001; Hopkins et al., 2004; Fig. 3). Reductions in zooplankton, which are algal consumers, likely resulted from the indirect effects of CCW on primary production. Had CCW impacted zooplankton primarily through direct toxicity, we would expect chlorophyll concentrations to have increased in CCW compared to REF due to a release from zooplankton foraging pressure in contaminated conditions as has been documented with pesticides (Boone and Semlitsch, 2002), but we observed the opposite. The only period when zooplankton abundance in CCW was higher than REF was 15 April, but at this time CCW mesocosms were comprised mostly of small copepod nauplii, whereas large cladocerans were abundant in REF throughout the entire study, an observation that suggests species of zooplankton responded differently to CCW. In hindsight, we believe that measures of biomass, which reflect both prey abundance and size, would have more accurately tracked food availability. Contaminant-induced changes to zooplankton community composition could also impact salamander foraging success, as larval *A. talpoideum* selectively prey on large cladocerans and consume few cyclopoid copepods even when copepods are in high abundance (Taylor et al., 1988). However, despite evidence that CCW altered the community food web, we detected no effect of salamander density on zooplankton abundance, which suggests food resources may not have been a limiting factor in our mesocosms and that direct toxicity may be primarily responsible for reduced metamorphosis.

Like contaminant exposure, a three-fold increase in density significantly reduced the number of larvae that metamorphosed.

Reduced metamorphosis at high density resulted in part from higher rates of early larval mortality (i.e., prior to initiation of drying), but many individuals still alive at the end of exposure were incapable of metamorphosing and died as a result of complete pond drying (Fig. 1). High larval density likely increased exploitative competition for food and interference competition, where large individuals inhibit smaller ones through aggressive interactions (Wilbur, 1976; Petranka and Sih, 1986; Scott, 1990). However, although the combination of high density and CCW proved least conducive for metamorphosis, we did not detect an interaction between them, suggesting these two factors operated independently of one another and the sum of their effects were approximately additive. Metts et al. (2005) found similar additive effects of density and the insecticide carbaryl on metamorphosis in carnivorous salamanders, but Boone and Semlitsch (2001, 2002) demonstrated that carbaryl ameliorated the effects of increased density for anuran amphibians, which are omnivorous filter or scraper feeders that compete with zooplankton for algal food resources. Differences in the interactive effects of competition and environmental contaminants among amphibians appear to depend upon the trophic level of the species in question, and perhaps whether the contaminant impacts individuals primarily through direct or indirect avenues.

Given the high trace element concentrations of salamanders and reductions in zooplankton prey, we expected salamanders in CCW to metamorphose at smaller sizes than from REF, but this was not the case. Instead, we observed a less-than-additive interaction between CCW and density, where increased density reduced metamorph size by 39% in REF but by only 6% in CCW. However, it should be noted that while increased density ameliorated the effects of CCW on metamorph size, this combination of factors resulted in only 3% of larvae metamorphosing, the lowest of any treatment. A contaminant-induced reduction in survival that removes a portion of the larval population but that releases the few surviving individuals from density-dependent effects has been hypothesized as an explanation for less-than-additive effects of contaminants and density on growth (Forbes et al., 2001; Hooper et al., 2003; Metts et al., 2005). However, that larval mortality prior to the initiation of pond drying was actually slightly higher in REF than CCW at high density suggests the less-than-additive effects on metamorph size were not necessarily related to a contaminant-induced release from density effects (Fig. 1). An explanation for the less-than-additive effect of density and CCW on metamorph size observed in this study remains elusive.

Timing of metamorphosis was similar among treatments and coincided closely with the initial water drawdown. That metamorphic timing was similar among treatments is likely a consequence of the stimulatory effect of pond drying on metamorphosis. In natural ponds, timing of metamorphosis in *A. talpoideum* is strongly correlated with the date the pond dries (Semlitsch, 1987b). In our study, metamorphosis was intentionally triggered by water drawdown mimicking the drying of a temporary wetland, which resulted in a relatively synchronous emergence of those larvae that were large enough and physiologically capable to initiate metamorphosis. Our mesocosms held water for 122 days, which is within the range of hydroperiods

for natural ponds in the region (Pechmann et al., 1989), and the high rates of metamorphosis in the REF low density treatment (85%) indicate that this hydroperiod is of sufficient length to allow larvae to attain adequate size to metamorphose in favorable conditions. The large proportion of individuals in CCW and high density treatments that survived to initiation of pond drying but that failed to metamorphose indicates that both high density and CCW lengthened larval period, but the hydroperiod did not allow for such extensions. Animals that extended larval development succumbed to the extreme conditions associated with drying wetlands. Thus, our experimental design allowed us to explore the impact of natural and anthropogenic stressors under conditions similar to natural temporary wetlands, where animals do not always have the luxury of additional time to grow and complete larval development.

5. Conclusions

The results of this investigation and others demonstrate the benefits of assessing a population's response to pollution in the context of natural environmental variation and potential interactive effects with natural stressors. The effects of a natural stressor (density) and a pollutant (CCW) on metamorphosis were additive, while the effects on size at metamorphosis were less-than-additive. The effects of CCW and density on *A. talpoideum* were most evident in reduced metamorphosis, as numerous larvae in these conditions failed to metamorphose before complete pond drying and died from the hostile conditions associated with pond drying. That size and body condition at metamorphosis were not influenced by CCW is likely because size itself is such a critical factor in amphibian metamorphosis, and because our drying regime did not allow for extension of larval growth and development. We conclude that high density did not exacerbate contaminant-related effects, but the effects of CCW alone on recruitment in amphibian populations may be severe, especially in temporary wetlands.

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