

Toxicity of coal-tar pavement sealants and ultraviolet radiation to *Ambystoma Maculatum*

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Abstract Polycyclic aromatic hydrocarbons (PAHs) can affect amphibians in lethal and many sublethal ways. There are many natural and anthropogenic sources of PAHs in aquatic environments. One potentially significant source is run off from surfaces of parking lots and roads that are protected with coal tar sealants. Coal tar is 50% or more PAH by wet weight and is used in emulsions to treat these surfaces. Break down of sealants can result in contamination of nearby waters. The toxicity of PAHs can be greatly altered by simultaneous exposure to ultraviolet radiation. This study exposes larvae of the spotted salamander (*Ambystoma maculatum*) to determine if coal tar sealant can have negative effects on aquatic amphibians and if coal tar toxicity is influenced by ultraviolet radiation. Spotted salamanders were exposed to 0, 60, 280 and 1500 mg coal tar sealant/kg sediment for 28 days. Half of the animals were exposed to conventional fluorescent lighting only and half were exposed to fluorescent lighting plus ultraviolet radiation. No significant mortality occurred during the experiment. Exposure to sealants resulted in slower rates of growth, and diminished ability to swim in a dose-dependent fashion. Exposure to ultraviolet radiation affected the frequencies of leukocytes and increased the incidence of micronucleated erythrocytes. There was an interactive effect of sealant and radiation on swimming behavior. We conclude that coal-tar sealant and ultraviolet radiation

increased sublethal effects in salamanders, and may be a risk to salamanders under field conditions.

Keywords Polycyclic aromatic hydrocarbons · Ultraviolet radiation · Sublethal toxicity · Salamanders · Behavior · Genotoxicity

Introduction

Polycyclic aromatic hydrocarbons (PAHs) can be toxic to amphibians. Harmful effects in amphibian larvae and other aquatic organisms include cancerous growths and cellular abnormalities (Eisler 2000), genotoxicity and micronucleated erythrocytes (Jaylet 1971; Fernandez and l'Haridan 1992; Gauthier et al. 1993), inhibition of growth and metamorphosis (Fernandez and l'Haridan 1994), edema and impaired gas exchange (McGrath and Alexander 1979), activation of mixed function oxidases or other cellular metabolic defense mechanisms (Schwen and Mannering 1982; Noshiro and Omura 1984), changes in activity, inability to swim properly, the appearance of skin lesions, and death (Hedtke and Puglisi 1982; Arfsten et al. 1996; Lefcort et al. 1997; Eisler 2000; Sparling 2000; and Blaustein et al. 2003). Amphibians can bioaccumulate PAHs (Grinfield et al. 1986; Vojinovic-Miloradov et al. 1996) but there is evidence that amphibians can develop an equilibrium in body concentrations after several days of exposure (Garrigues et al. 2004). Oxidation and UV radiation can degrade parent PAHs and either make them less toxic or increase their toxicity by many times (Monson et al. 1999, Albers 2003). For example, exposure of PAHs to ultraviolet (280–400 nm) radiation has increased toxicity by more than 100 times in bullfrogs (*Rana catesbeiana*) and the Spanish ribbed newt (*Pleurodeles waltl*) (Fernandez and

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l'Haridan 1992). However, not all PAHs respond in the same way to ultraviolet radiation (Diamond et al. 2000).

While there are many sources of PAHs in the environment, including natural occurrences, one potential anthropogenic source is runoff from pavements such as parking lots and roads (Mahler et al. 2005; Scoggins et al. 2007). The surfaces of these paved areas are often preserved with coal tar sealants. Coal-tar contains 50% or more PAHs by wet weight (U.S. Department of Health and Human Services 2002), and coal-tar-emulsion sealers can be 20 - 35% coal-tar (Mahler et al. 2003). The coal-tar that is applied to paved surfaces can chip or degrade and be transported into water ways via run-off where they can create localized areas of high concentrations (Mahler et al. 2003) that may be toxic to aquatic organisms.

One example of such an area is the city of Austin, Texas, USA. In 1995, elevated concentrations of PAHs were detected within sediments entering pools of Barton Springs, which provide the sole habitat for the federally endangered Barton Springs salamander (*Eurycea sosorum*, Chippindale et al. 1993; USFWS 1997). Evidence that sealants from paved surfaces might affect *E. sosorum* comes from several sources: 1) Barton Creek and Barton Springs Pool sediments can have up to 500 mg/kg of total PAH (Hayward et al. 2002), exceeding the U.S. Environmental Protection Agency's (EPA) Probable Effects Concentrations for aquatic species (MacDonald et al. 2000, Scoggins et al. 2007); 2) Hayward et al. (2002) showed that *Hyalella azteca*, a prey species for *E. sosorum*, experienced significant mortality when exposed to high concentrations of PAHs and ultraviolet radiation in the laboratory; 3) Scoggins et al. (2007) determined that areas downstream of some parking lots had significantly higher sediment concentrations than areas of the same streams above the parking lots and they demonstrated that aquatic invertebrate communities were diminished downstream; 4) Van Metre et al. (2000) found that wetland sediments near sealed lots had higher concentrations of PAHs than those near unsealed lots.

The primary objective of this study was to determine if coal-tar sealants with or without ultraviolet radiation can pose toxic risks to aquatic stages of salamanders. For this experiment we used larval spotted salamanders (*Ambystoma maculatum*). A companion study (Bommarito 2009) examined the effects of coal tar and asphalt sealants on eastern spotted newts (*Notophthalmus viridescens*).

The specific objectives of the current study were to determine:

- 1) If sediments contaminated with coal-tar sealants negatively affect spotted salamander larvae through mortality, growth, genotoxicity, behavior, or effects on the immune system.

- 2) If toxicity of coal-tar sealants is altered by ultraviolet radiation.

Materials and methods

Sediment preparation

We collected and homogenized 128 kg of sediment from Little Crab Orchard Creek, Carbondale, IL (UTM Zone 16, 0301377E, 4175002 N), a source of uncontaminated sediment used in previous toxicology studies conducted at Southern Illinois University. The coal-tar sealant was provided by chemists at Austin, Texas and came from commercial sources used by the city. The sealants were prepared by painting sheets of glass with the emulsion, allowing it to dry and the most volatile PAHs to dissipate, and then scraping the dried residue into chemically cleaned jars. This process of drying and flaking resembles the drying and chipping of sealant from paved surfaces. At the laboratory in Illinois coal-tar sealant was mixed with 800 g of sediment to create final concentrations of 0, 60, 280 and 1500 mg sealant/kg sediment. Most other studies that have examined effects of PAH's on aquatic organisms have relied on single or, at most, mixtures of two or three PAHs. In this study we used the mixture of PAHs that came from the coal tar sealant because this provided a more realistic field exposure of PAHs to amphibians inhabiting environments exposed to such sealants. However, because of this, determining the toxicity of single PAHs was not possible and comparisons with other published studies was difficult.

The mixtures of sealant and sediment were placed in 8 L aquaria and covered with 7 L of filtered, reconstituted tap water for 10 days to allow suspended flakes of sealant to sink. Water from each tank was completely drained and the sealant and sediment were mixed again. Sealants still were not thoroughly mixed into the sediments so the mixture was again covered with 7 L of filtered, reconstituted tap water and allowed to stabilize for two additional weeks. Tap water was filtered through gel ion-exchange columns and activated charcoal and reconstituted (ASTM 1988) to approximate the hardness and conductivity of natural waters around Austin, Texas. Water was aerated for at least 24 h prior to adding it to aquaria and gently aerated throughout the experiment.

Lighting conditions

Spotted salamander larvae were exposed to coal-tar sealants under either cool-white fluorescent lighting (Non-UV, 400-700 nm, Ecologic, Osram Sylvania, Danvers, MA, USA) alone or a combination of fluorescent lighting and UV

Table 1 Light sets in the non-UV and UV light phases that provided the required amount of light that larval spotted salamanders were exposed to, the length of time each light set was on, and the mean (SE) levels of UVB, UVA, and visible light

Level	Time	Light sets	UVB ($\mu\text{W}/\text{cm}^2$)	UVA ($\mu\text{W}/\text{cm}^2$)	Visible ($\mu\text{W}/\text{cm}^2$)
Non-UV					
A	0700–2300 (16 h)	Set A	0.04 (± 0.02)	0.04 (± 0.02)	8.5 (± 2.72)
B	0800–2200 (14 h)	Sets A & B	0.04 (± 0.0074)	0.04 (± 0.0072)	29.7 (± 1.39)
C	12:15–17:45 (5.5 h)	Sets A, B, & C	0.04 (± 0.009)	0.05 (± 0.006)	52.5 (± 4.0)
D	14:45–15:15 (30 min)	Sets A, B, C, & D	0.04 (± 0.008)	0.05 (± 0.005)	71.6 (± 3.8)
UV					
A	0700–2300 (16 h)	Set A	0.04 (± 0.01)	0.04 (± 0.02)	8.49 (± 2.72)
B	0800–2200 (14 h)	Sets A & B	0.5 (± 0.03)	4.14 (± 1.0)	29.6 (± 1.3)
C	12:15–17:45 (5.5 h)	Sets A, B, & C	1.3 (± 0.32)	7.9 (± 0.5)	52.5 (± 3.96)
D	14:45–15:15 (30 min)	Sets A, B, C, & D	1.8 (± 0.2)	14.4 (± 2.9)	71.6 (± 3.8)

radiation (UV, 290–400 nm, UVA–340, Q-Panel Company, Cleveland, OH, USA). The UV treatment was set to emit the approximate lighting found in south central Texas during summer months with a 16L:8D photoperiod (Table 1, Cummings, pers. comm.). The lamps were calibrated using a handheld radiometer (Macam UV 203; Macam Photometrics, Livingston, Scotland) sensor at 12 equally distributed locations for each lamp setup at the surface of the sediments.

Chemical analyses

Polycyclic aromatic hydrocarbon (PAH) analyses were conducted on sediment and water at Southern Illinois University, Carbondale, IL. Sample extraction, cleanup, and quantification followed EPA methods 3510C (USEPA 1996a), 3541 (USEPA 1994), 3630C (USEPA 1996b), and 8100 (USEPA 1986), respectively, using an Agilent 5975C Series Gas Chromatograph/Mass Spectrometer, Inert XL EI/CI MSD with Agilent 6850 network GC system and 6850 series autosampler (Agilent Technologies, Wilmington, Delaware, USA). Control sediment samples were spiked with 2-fluorobiphenyl (2-FBP) and PAH Mix (Ultra Scientific, Kingstown, RI, USA), and were extracted and analyzed to examine method extraction efficiency, with a recovery rate > 80%. Liquid–liquid extraction and quantification of PAHs in water followed EPA method 3510C (USEPA 1996a). One liter samples were collected from each treatment and spiked with 1.0 mL of 2-fluorobiphenyl.

Salamander husbandry and testing

Larval spotted salamanders were collected in the Shawnee National Forest (UTM 16S 0292409E 4128335 N) and given five days to acclimate to laboratory conditions. Ambient and water temperatures were held between 19–23°C. Water was gently aerated at all times to maintain a minimum dissolved oxygen concentration of 5 mg/L.

Seventy percent of the water was replaced twice a week with minimal disturbance of sediments to maintain water quality and clarity. Before the water in each tank was replaced, its pH, ammonia content, and hardness were recorded using a pH/mV/Ion meter (YSI 550DO, YSI Environmental, Yellow Springs, Ohio, USA).

Each salamander was fed approximately 0.5 ml of captive-raised blood worms (*Chironomus tentans*) every other day. A 10 cm long, 5 cm wide piece of PCV pipe cut lengthwise provided shelter. Salamander health and status were recorded daily through observing salamander activity, swimming ability, and food consumption. The condition of their skin was also examined daily for signs of irritation such as skin sloughing, ulcers, or decreased pigmentation. External surfaces of the salamanders were swabbed and a collective sample of swabs was sent to a commercial laboratory (Pisces Molecular, Boulder CO) to test for the presence of *Batrachochytrium dendrobatidis* through polymerase chain reaction (PCR) analysis; the sample proved negative for the fungus.

A baseline size and mass were obtained by randomly selecting a subsample of 10 salamanders from Non-UV and UV and weighing them (Fisher XT scale, Fisher Scientific, Hampton, New Hampshire, USA) to the nearest 0.001 g and measuring their snout vent lengths (SVL) and total lengths (TL) (Fisher Digital Caliper, Fisher Scientific, Hampton, New Hampshire, USA) to the nearest 0.1 mm. All salamanders were weighed and measured at the conclusion of the experiment. Salamanders were then humanely euthanized by adding 3.75 g of MS-222 to each aquarium and stored at -75°C until analyzed.

Swimming ability

Larval spotted salamanders were tested for speed and swimming distance. Each individual was placed in a 122 cm section of PVC pipe that was cut lengthwise and

capped on the ends. A tape measure was affixed to measure the distance swam and time was measured with a stopwatch. Each individual was gently prodded with a stainless steel probe to evoke swimming behavior. A swimming bout was defined as a period when probing either caused the individual to swim or did not produce an overt reaction. The distance (± 0.5 cm) and duration (± 1 s) of the initial bout for each trial were measured and rate was calculated as cm swam/sec. If no reaction occurred the animal was scored zero for time and distance for that bout. Each trial consisted of 10 bouts with a 10 s delay between bouts. The mean of the 10 bouts was used in further analyses.

Micronucleus and white blood cell test

After euthanasia, salamanders were decapitated and blood was collected in heparinized capillary tubes. Blood smears were fixed and stained by the Wright-Giemsa method. The frequencies of micronucleated erythrocytes and leukocyte types were determined by randomly moving the field of view and counting 1000 erythrocytes on each slide using $1000\times$ magnification under oil immersion. A micronucleus was identified as a small body that was adjacent but not connected to the principle nucleus (Meintieres et al. 2001, Fig. 1).

Data analysis

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA) with $\alpha = 0.05$. All dependent variables were examined for meeting the assumptions of parametric statistics; normality was checked with the Shapiro-Wilks statistic and heteroscedasticity by examining the relationship between means and standard deviations. Initially, end point results were regressed against aqueous PAH concentrations to determine if dose/response relationships occurred. To test whether UV radiation and PAH concentrations interacted on end points; regressions

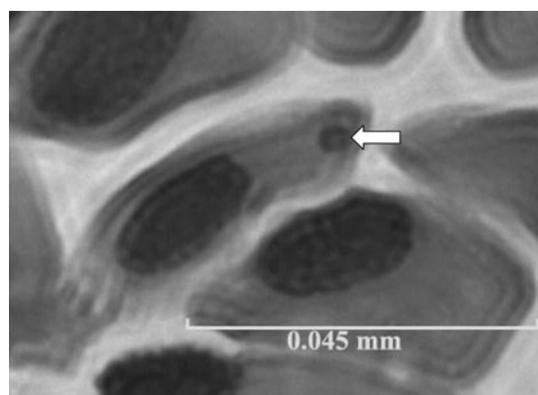


Fig. 1 A micronucleated erythrocyte (arrow) in a larval spotted salamander exposed to coal-tar sealant and UV light

were followed by analyses of variance (ANOVA) on TPAH concentrations, radiation exposure, and their interaction. Except for body mass and cell counts we used split plot, repeated measures ANOVAs that incorporated individual salamanders as repeated measures within aquaria which then served as experimental units. If the inter-aquarium effect was non-significant we repeated the analysis with individuals serving as experimental units. Although F statistics varied due to the difference in sample sizes, the two methods of analyses consistently produced the same statistical interpretations. Body mass was measured on the entire group of salamanders within an aquarium and was analyzed with a conventional two-way ANOVA. In all ANOVAs a fully developed model employing all explanatory variables was used first. When the interaction term was statistically significant but one main effect was not we followed the full model with separate one-way ANOVAs for each type of radiation exposure. Differences in micronucleated erythrocytes and white blood cells among treatments were evaluated with a MANOVA using cell frequencies after it was determined that no correlations existed among cell type frequencies.

Results

Sediment and water chemistry

Water conditions were within acceptable levels (ASTM 1988) throughout the study. Mean values were: water temperature = 19.6°C , pH = 7.4, hardness = $53.7 \mu\text{S}/\text{cm}$, dissolved oxygen = $5.8 \text{ mg}/\text{L}$, and ammonia = $10 \text{ ng}/\text{L}$. No differences were found between UV and Non-UV for these measurements. The sediment was composed of 60% sand, 35% silt, 5% clay and had 2% organic matter.

Initial analysis of the dried coal tar sealant revealed that it contained 17 different PAHs with phenanthrene, fluoranthene and pyrene having the highest concentrations, and it had a TPAH concentration of 84.3% dry weight. TPAH concentrations in sediments and water increased with sealant concentrations (Table 2). Overall, mean sediment TPAH concentrations were 74% of sealant concentrations. Water TPAHs averaged 0.056% of sealants and 0.08% of TPAH in sediments. The PAHs with the highest concentrations in water included anthracene, naphthalene, phenanthrene, fluorene and acenaphthene (Table 3).

Survival and growth

Percent mortality was less than 1% (1/120) under Non-UV and 2.5% (3/120) under UV after 28 days of exposure. This low mortality negated any need for detailed statistical analysis. For Non-UV and UV combined, body mass, SVL,

Table 2 Total PAHs measured within sediments and water of aquaria spiked with coal-tar sealants

Sealant concentration (mg/kg)	Sediment TPAH (mg/kg)	Percent of sealant	Water TPAH ($\mu\text{g/l}$)	Percent of sediment TPAH
0	0.89	na	65.6	7.37
60	47.5	79.2	72.2	0.15
280	148.3	53.0	88.4	0.06
1500	1360	90.7	263	0.02

Table 3 Concentrations ($\mu\text{g/l}$) of specific PAHs in water overlying sediments treated with coal tar sealants along with their water solubilities and Log K_{ow} values

Analyte	Sealant concentration				Solubility (mg/L) ^a	Log K_{ow} ^a
	0 mg/kg	60 mg/kg	280 mg/kg	1500 mg/kg		
Acenaphthene	7	9	10	13	4.08	3.92
Acenaphthylene	ND	ND	3	13	N/A	3.89
Anthracene	22	22.5	28	55	0.058	4.61
Benzene(a)anthracene	<DL	ND	1	ND	0.011	5.91
Benzo(a)pyrene	ND	ND	ND	17	0.001	6.13
Benzo(g,h,i)perylene	2.6	3.4	<DL	10	0.0001	6.22
Chrysene	<DL	<DL	3.4	24	0.002	5.81
Dibenzo(a,h)anthracene	ND	<DL	<DL	10	0.002	6.86
Fluoranthene	<DL	<DL	<DL	12	0.220	5.20
Fluorene	7	8.3	9	14	1.84	4.18
Ideno(1,2,3-cd)pyrene	ND	<DL	<DL	<DL	N/A	7.09
Naphthalene	21	22	22	23	30.7	3.35
Phenanthrene	6	7	12	61	1.11	4.55
Pyrene	<DL	<DL	<DL	11	0.129	5.14
Total PAHs	65.6	72.2	88.4	263		

^a Low K_{ow} values from Crunkilton and DeVita (1997) and van Noort (2009), solubility data from van Noort (2009)

and TL at the end of the experiment were significantly and negatively related to sediment TPAH concentration but the respective regression coefficients were low. The relationships were: body mass ($r^2 = 0.091$, $p = 0.006$), SVL ($r^2 = 0.071$, $p = 0.017$), and TL ($r^2 = 0.063$, $p = 0.025$). In ANOVAs there were no significant differences in body mass (Fig. 2a) or SVL (Fig. 2b) by TPAH concentration, radiation exposure, or their interaction. There were differences in TL due to UV radiation ($F_{1,72} = 12.68$; $p = 0.007$) and marginally across TPAH concentrations ($F_{3,72} = 2.63$; $p = 0.056$) but not in their interaction (Fig. 2c). Salamanders exposed to Non-UV radiation were shorter than those exposed to UV radiation and those at 1500 mg/kg sealant were shorter than those at 280 mg/kg sealant.

Speed trials

There was a significant difference in the swimming rate (distance/time) among TPAH concentrations and between radiation types. Salamanders under UV radiation were

slower ($F_{1,224} = 8.08$; $p = 0.005$) than those under Non-UV (Fig. 3). Animals exposed to 60 and 1500 mg/kg sealant under UV radiation were slower than controls ($F_{3,224} = 3.69$; $p = 0.013$). In the Non-UV radiation animals exposed to 280 and 1500 mg/kg sealant were slower than controls. There was no significant difference for the interaction of radiation and TPAH concentration.

Both components of rate (duration and distance) were also affected by exposure to coal-tar and UV radiation. The duration of swimming differed significantly between UV and Non-UV ($F_{1,224} = 79.27$; $p = 0.001$) and in the interaction of TPAH concentration and radiation ($F_{3,224} = 2.98$; $p = 0.032$), but not by TPAH alone. The distance salamanders swam differed significantly between radiation types ($F_{1,224} = 39.54$; $p < 0.001$), across TPAH concentrations ($F_{3,224} = 2.74$; $p = 0.044$), and their interaction ($F_{3,224} = 2.69$; $p = 0.047$). Those under Non-UV swam further and longer than those under UV. Those under UV and exposed from 60 to 1500 mg/kg sealant swam shorter distances and shorter times than similar controls.

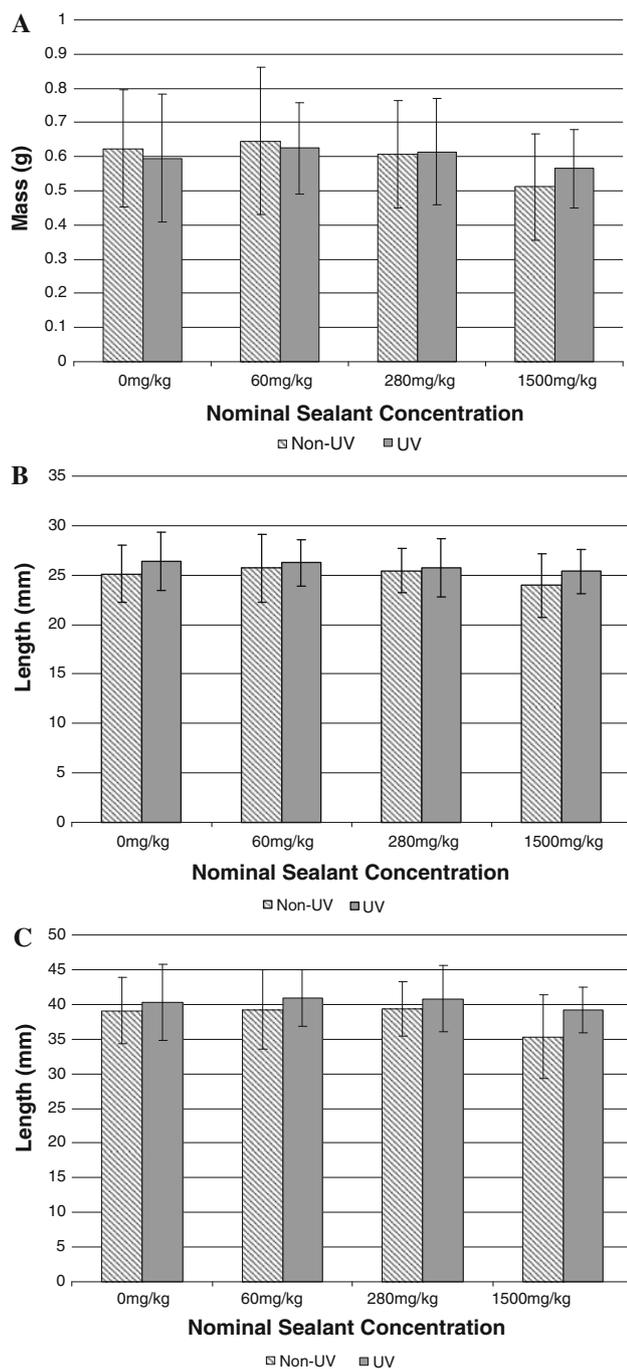


Fig. 2 Mass (a), SVL (b), and TL (c) of larval spotted salamanders exposed to coal-tar sealant under UV and non-UV light

Micronucleus and white blood cell test

In the MANOVA on square root transformed leukocyte counts most cell types differed between radiation exposures but not among TPAH concentrations or the interaction between radiation and sealant. Across radiation types ANOVA results ($df = 1,79$ in all cases) were: erythroblasts

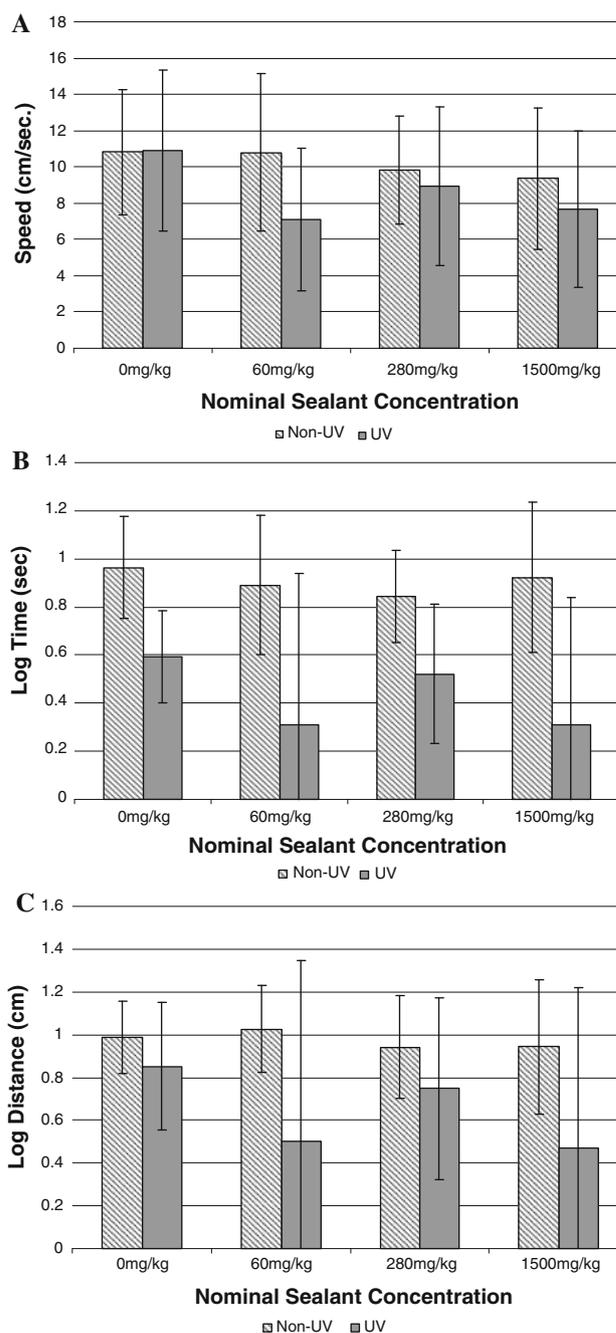


Fig. 3 Swimming rate (a), duration (b), and distance (c) of larval spotted salamanders exposed to coal-tar sealant under UV and non-UV light

($F = 7.06$, $p = 0.0097$); monocytes ($F = 29.85$, $p < 0.0001$); basophils ($F = 9.45$, $p = 0.0030$); eosinophils ($F = 7.98$, $p = 0.0061$); and neutrophils ($F = 4.62$, $p = 0.0349$). Lymphocytes did not differ between radiation types, across sealant concentrations or in the interaction of the two factors. The combined test, using Wilks Lambda as the test statistic, revealed that leukocytes differed between radiation exposures ($F_{6,67} = 8.47$, $p < 0.0001$)

Table 4 Mean (SE) of micronucleated erythrocytes and white blood cells of salamanders exposed to coal-tar sealant in the UV and non-UV phase

	MN RBC ^a	Lymphocyte	Erythroblast	Monocyte	Basophil	Eosinophil	Neutrophil
UV	0.23 (0.42)	23.2 (23.7)	12.8 (13.2)	3.15 (2.23)	0.78 (1.07)	5.45 (6.40)	1.25 (1.85)
Non-UV	0.05 (0.22)	22.7 (17.4)	6.60 (5.83)	1.05 (1.58)	0.20 (0.51)	2.83 (3.60)	2.45 (3.28)

^a Mean \pm SE of micronucleated erythrocytes and white blood cells per 1000 erythrocytes

but not across TPAH concentration ($F_{18,189.99} = 1.00$, $p = 0.4649$) or their interaction ($F_{18,189.99} = 1.37$, $p = 0.1525$). The incidence of micronucleated erythrocytes was greater under UV radiation than under non-UV ($F_{1,79} = 5.44$, $p = 0.0224$) but did not differ among concentrations or the interaction of radiation and TPAH concentration. Similarly, total white blood cells differed between UV and non-UV exposures ($F_{7,66} = 5.97$; $p < 0.001$), but not among TPAH concentrations or the interaction of exposure and TPAH. The number of micronucleated erythrocytes, erythroblasts, monocytes, basophils, and eosinophils were statistically higher in salamanders exposed to UV radiation than those exposed only to visible light (Table 4).

Discussion

Sediment and water chemistry

Whereas measured TPAH concentration corresponded with sealant concentrations in sediment, the concentrations of TPAH and specific PAH analytes in water were only a small fraction of what was in the respective sediments. PAHs occur naturally and are ubiquitous in the environment so finding small concentrations of TPAH in untreated sediments and overlying water of controls is not unexpected. Low aqueous concentrations are most likely due to the high log K_{ow} s (range 3.35 to 7.09) and generally low solubilities (0.0001 to 30.7 mg/L) of the individual PAHs (Crunkilton and DeVita 1997, van Noort 2009). High log K_{ow} values indicate high tendency to bind with sediments and organic matter and low solubilities further reduce the likelihood that unbound PAHs would be found in solution. Aqueous PAHs, whether in the water column or in pore water, are more bioavailable than those in sediments (Garrigues et al. 2004) so salamanders were probably exposed to only a fraction of the TPAHs in their environment. The PAHs with the highest concentrations in the water column included naphthalene, anthracene, acenaphthene, fluorene, and phenanthrene. Naphthalene had the highest solubility of all the PAHs present and may have reached saturation concentrations in even the lowest sediment treatments and controls.

MacDonald et al. (2000) estimated that TPAH concentrations in sediment greater than 22.8 mg/kg exceeded their probable effects concentration (PEC) for aquatic organisms. While our control sediment had a substantially lower concentration than the PEC, sediments spiked with 60 mg/kg sealant exceeded that value. Unfortunately, no similar estimates of effects levels in water have been made for TPAH. The acute toxicities of specific PAHs to aquatic organisms may differ by three orders of magnitude (Eisler 2000). Of the PAHs found in the water of the current study, anthracene appears to be most acutely toxic with 96 h LC50 in bluegills (*Lepomis macrochirus*) in the 1 to 10 $\mu\text{g/L}$ range. The concentrations found in this study for anthracene exceeded that level at all sealant concentrations including controls. Based on data from fish, the other PAHs are over 100 times less acutely toxic than anthracene (Eisler 2000). However, acute toxicity is not a good predictor of chronic effects; some PAHs such as benzo(a)pyrene, which was found at the highest sealant concentration, have relatively low acute toxicity but are highly carcinogenic (Eisler 2000).

Survival and growth

After 28 days of exposure to coal-tar sealant under UV and Non-UV radiation, larval spotted salamanders did not experience any treatment-related mortality and the experimental conditions cannot be considered to be acutely or subchronically lethal. Fernandez and l'Haridan (1994) found that mortalities resulted from exposing larvae of the Spanish ribbed newt (*Pleurodeles waltl*) to 25 $\mu\text{g/L}$ of benzo(a)pyrene (BaP) in water under UVA radiation. Tadpoles of northern leopard frogs (*Rana pipiens*) experienced similar results when they were exposed to 30.6 $\mu\text{g/L}$ of fluoranthene and 250 $\mu\text{g/L}$ of anthracene. When tadpoles were exposed to UV radiation, mortalities increased (Kagan et al. 1984; Monson et al. 1999). In our experiment, maximal aqueous concentrations of benzo(a)pyrene (17 $\mu\text{g/L}$), fluoranthene (13 $\mu\text{g/L}$) and anthracene (65 $\mu\text{g/L}$) were below these toxic concentrations. Anuran tadpoles can suffer mortality when exposed to ultraviolet radiation in the absence of PAHs (Tietge et al. 2001).

There were important sublethal effects produced by PAH and UV radiation. Control salamanders experienced approximately a 68% increase in body mass and a 23%

increase in total length over the 28 d period. Because the spotted salamanders were larvae, growth during the 28 days of the experiment could be expected. The rate of growth was reduced with increasing PAH concentrations, regardless of radiation exposure. However, salamanders exposed to UV radiation were actually longer than those under Non-UV. The effects of PAH on growth has varied among studies. Hatch and Burton (1998) did not see changes in growth when larval spotted salamanders were exposed to fluoranthene and UV radiation, but Bryer et al. (2006) reported developmental and growth differences in tadpoles of the African clawed frog (*Xenopus laevis*) when exposed to 3 mg/kg of coal-tar sealant in water. Bommarito (2009) did not see any differences in body size or mass in eastern spotted newts that were exposed to similar conditions as the current study but he tested adult newts that would not be expected to demonstrate much growth in 28 days.

Speed trials

Salamanders exposed to coal-tar sealant and UV radiation experienced noticeable effects on speed, time, and the distance they swam. Control animals under both UV and Non-UV had essentially the same rate of swimming. As the concentrations of PAHs increased, rate of swimming decreased under both light exposures. This indicates that PAH exposure had some effect on salamander swimming ability. Reduced swimming ability may interfere with prey capture or escape from predators in natural environments. Within both Non-UV and UV exposures TPAH concentration affected the duration and distance that salamanders.

The results for swimming distance contrasted with those of Walker et al. (1998). They found that bullfrog larvae exposed to concentrations $\geq 37.97 \mu\text{g/L}$ of fluoranthene swam farther than larvae in controls and lower concentrations. The authors did not find any difference in speed.

Micronucleus and white blood cell test

The frequency of micronucleated erythrocytes significantly increased with UV exposure in this study. Fernandez and l'Haridan (1994) reported a significant increase in micronucleated cells when larvae of the Spanish ribbed newt were simultaneously exposed to oil refinery effluent and UVA radiation. The frequency of micronucleated cells was only elevated when both UVA radiation and effluent were present. However, these newts were exposed to PAHs in experimental containers without any sediment.

Exposure to UV radiation resulted in elevated numbers of erythroblasts, monocytes, basophils, and eosinophils; neutrophils significantly increased under Non-UV, and lymphocytes remain unchanged. The total white blood cell

count was also significantly higher across treatments under UV than under Non-UV. While no external signs were observed, exposure of larval spotted salamanders to UV radiation may have resulted in tissue damage, resulting in an elevated immune response. Salo et al. (2007) reported a similar white blood cell response after rainbow trout (*Onchorhynchus mykiss*) were exposed to ultraviolet radiation. Tadpoles of the red-legged frog (*Rana aurora*) and the Pacific treefrog (*Pseudacris regilla*) experienced prominent lens opacities and substantial skin burns when exposed to UVB (Flamarique et al. 2000) but we know of no studies that examined the effects of UV radiation on amphibian immune systems.

Conclusions

In the current study only minimal mortality occurred when larval salamanders were exposed to coal-tar sealants, regardless of UV radiation exposure. Interspecific comparisons in toxicity can be problematic, but the absence of lethal toxicity in spotted salamanders in this study and on eastern newts (Bommarito 2009) suggests that coal tar sealants may not be acutely lethal to salamanders, including *Eurycea sosorum*. In a survey of PAHs in 14 sites around Austin, TX Scoggins et al. (2007) recorded a high concentration of TPAH in sediments of 32 mg/kg (mean = 1.16 mg/kg above parking lots and 12.28 mg/kg downstream from parking lots). Hayward et al. (2002) recorded TPAH concentrations as high as 500 mg/kg sediment in Barton Creek and Barton Springs pools. Compared to their studies, our exposures provide a stringent test on the acute effects of PAHs. However, Mahler et al. (2005) found that suspended sediments in runoff from sealed parking lots had a mean TPAH concentration of 3500 mg/kg so acute exposures to PAHs in small bodies of water could be higher than what we tested.

We found that exposure to PAHs can diminish growth in larval salamanders. They also affected the rate, distance, and duration at which salamanders swam. Thus, exposure to coal-tar sealant may influence predation on or food acquisition by salamanders. We can surmise that avian and mammalian predators would likely have little risk from PAH runoff. Thus salamanders including *E. sosorum* may be at greater risk from these predators when PAHs are present than when they are not. For aquatic predators, risk will vary with the comparative sensitivity of predators and salamanders. If predators are more sensitive than salamander species, predation risk may be reduced (Boone and Semlitsch 2001). However, if predators are less sensitive the added debility of PAHs may compound risk (Relyea 2004). Slower swimming may also negatively affect prey capture.

The elevated number of micronucleated erythrocytes and certain leukocytes suggests that salamanders such as *E. solorum* might encounter cellular damage under UV radiation. However, many salamanders, including *E. solorum*, seek shelter under boulders, cobblestones, and gravel during daylight (Chippendale et al. 1993), allowing them to avoid prolonged exposure to UV radiation.

Whereas we focused on the PAHs commonly associated with coal tar, there are other, more volatile, PAHs in coal tar that were not quantified. These often have higher acute toxicities than more stable forms (Eisler 2000). Under typical conditions of use these PAHs likely would volatilize before they could enter a waterway. However, if a rainfall event occurred soon after application of sealants, some of them could make their way into salamander habitats. Runoff from parking areas and roadways may also be a source for a variety of other contaminants including pesticides, oil and grease and heavy metals (Scoggins et al. 2007). In more northern climates salt used for de-icing may also pose hazards to nearby bodies of water (Sanzo and Hecnar 2006). The City of Austin has ceased using coal tar sealants on their parking areas but other municipalities still use the sealant and must decide if the environmental risks are worth the advantages. Methods to mitigate the flow of contaminants from paved surfaces such as the creation of berms or grassy borders can be implemented to reduce these risks.

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