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Region 2

Environmental Contaminants Program

WATER AND SEDIMENT QUALITY IN HABITAT SPRINGS OF EDWARDS AQUIFER SALAMANDERS

by

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ABSTRACT

Many springs associated with the Edwards Aquifer of Texas are inhabited by relict populations of neotenic salamanders in the genus *Eurycea*. This study was done to assess the water quality characteristics in habitat springs of *Eurycea* salamanders associated with various springs of the Edwards Aquifer. The goals of the study were to identify bioavailable contaminants associated with individual springs and to evaluate the effects of contaminants and sediment turbidity on the survival of spring-associated salamanders. Collections of samples and/or salamanders made from five habitat springs (Barrow Hollow Springs, Barton Springs, Collapsed Bridge Springs, San Marcos Springs, and Stillhouse Hollow Springs) from two counties in central Texas. Analyses were conducted to determine (1) the presence of organic contaminants in salamander habitat springs as determined with semipermeable membrane devices (SPMDs), (2) survival effects for salamanders under incremental increases in sediment loads, and (3) relative sensitivities of salamanders to selected contaminants under controlled conditions in a laboratory setting. Measurable concentrations were found by SPMD determinations for 13 organochlorines, 5 polycyclic aromatic hydrocarbons (PAHs), and 2 pyrethroid pesticides for the five habitat springs. Three types of sediment slurry tests (single 24-h pulse, three 8-h pulses, and continuous presence of sediment) did not produce large biomass changes in two salamander species. Polycyclic aromatic hydrocarbons (PAHs) in sediment at Barton Springs were sufficiently elevated to impact the amphipod *Hyalella azteca*, a food source of the endangered Barton Springs salamander (*Eurycea sosorum*). Although results from preliminary acute toxicity tests presented in this study would indicate that *Eurycea* salamander species are similar to or less sensitive to tested contaminants as compared to fish species, the test results have to be regarded as preliminary because of the small number of salamanders and contaminants used for testing. Due to the lack of existing information of sublethal effects of contaminants on neotenic salamanders, regulatory protective measures such as water quality criteria, pesticide use restrictions, etc. should be conservative with respect to protection of *Eurycea* species from water or sediment-borne contaminants. Additionally, considerations should be given to protection of the habitat including the salamander's prey base.

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Cover photo of Barton Springs salamander courtesy of Lisa O'Donnell

INTRODUCTION

Many springs associated with the Edwards Plateau in central Texas are inhabited by relict populations of neotenic salamanders belonging to the genus *Eurycea* (Sweet 1982). Distributions of these lungless salamanders are confined to certain springs, spring runs, and subterranean watercourses. Species within this group include the Austin blind salamander *(Eurycea waterlooensis),* Barton Springs salamander *(E. sosorum),* Georgetown salamander (*E. naufragia*), Jollyville Plateau salamander (*E. tonkawa*), Salado salamander (*E. chisholmensis*), San Marcos salamander *(E. nana),* Texas salamander *(E. neotenes),* and Texas blind salamander *(E. rathbuni)* (Chippindale *et al.* 2000). Some populations of these salamander species have declined or undergone extirpation as urban areas have expanded near salamander habitat. As a result, six of these salamander species are classified as either a candidate, endangered, or threatened species under the Endangered Species Act of 1973: (1) Austin blind salamander (candidate), (2) Barton Springs salamander (endangered), (3) Georgetown salamander (candidate), (4) Salado salamander (candidate), (5) San Marcos salamander (threatened), and (6) Texas blind salamander (endangered).

Springs serving as habitat (i.e., habitat springs) of these aquatic salamanders derive their source water from the Edwards Aquifer. This karst aquifer is the dominant regional hydrologic feature of the Edwards Plateau in Texas and is typified by caves, sinkholes, and conduits that are interspersed throughout highly porous limestone. Groundwater in the aquifer is recharged primarily during rainstorm events. Since the aquifer is rapidly replenished from surface runoff into highly fractured and permeable limestone outcroppings, contaminants in surface runoff are readily transported during the recharge process. The Barton Springs/Edwards Aquifer Conservation District (1994) found above-normal concentrations of petroleum hydrocarbons, sediment, bacteria, pesticides, and metals in wells and springs near highly developed portions of the Edwards Aquifer. The U.S. Geological Survey (USGS 1990) found a direct relationship between urbanization (as defined by impervious cover such as roads, parking lots, roof tops, etc.) and various indicators of aquifer water quality including suspended solids, nutrients, fecal coliform bacteria, biological oxygen demand, total organic carbon, and pesticides. Urbanization has expanded within the recharge zone overlying the aquifer, and this development is projected to continue indefinitely thereby threatening the aquifer's water quality. Habitat springs of these salamanders are therefore vulnerable to degradation from urbanization.

This study was done to assess water quality in habitat springs of *Eurycea* salamanders associated with the Edwards Aquifer. The goals of the study were to identify bioavailable contaminants associated with the springs and to evaluate the effects of contaminants and sediment turbidity on these salamanders. Objectives of the study were:

1. Determine the presence of organic contaminants in salamander habitat springs of the Edwards Aquifer with semipermeable membrane devices (SPMDs).

2. Determine effects of various pulses of sediment turbidity on two species of the genus *Eurycea*.

3. Determine the relative sensitivity of salamanders to selected contaminants under controlled conditions in a lab setting.

To fulfill objective 1, bioavailable contaminants at the springs were identified through the use of SPMDs. These devices are lipid-containing membrane tubes that mimic key aspects of the bioconcentration process and passively sample bioavailable aqueous organic contaminants. The SPMDs consist of thin-walled polyethylene tubing or tubing of other nonpolar dense polymers that contain a thin film of triolein, a neutral lipid with a large molecular mass (Huckins *et al*. 1993). The devices concentrate trace levels of organic contaminants including persistent polychlorinated biphenyls, dioxins, and organochlorine pesticides and other organic contaminants such as polycyclic aromatic hydrocarbons and pyrethroid insecticides. A detailed description of SPMDs is provided by Petty *et al*. (2000). The preparation and deployment of SPMDs is described by Lebo *et al*. (1992).

MATERIALS AND METHODS

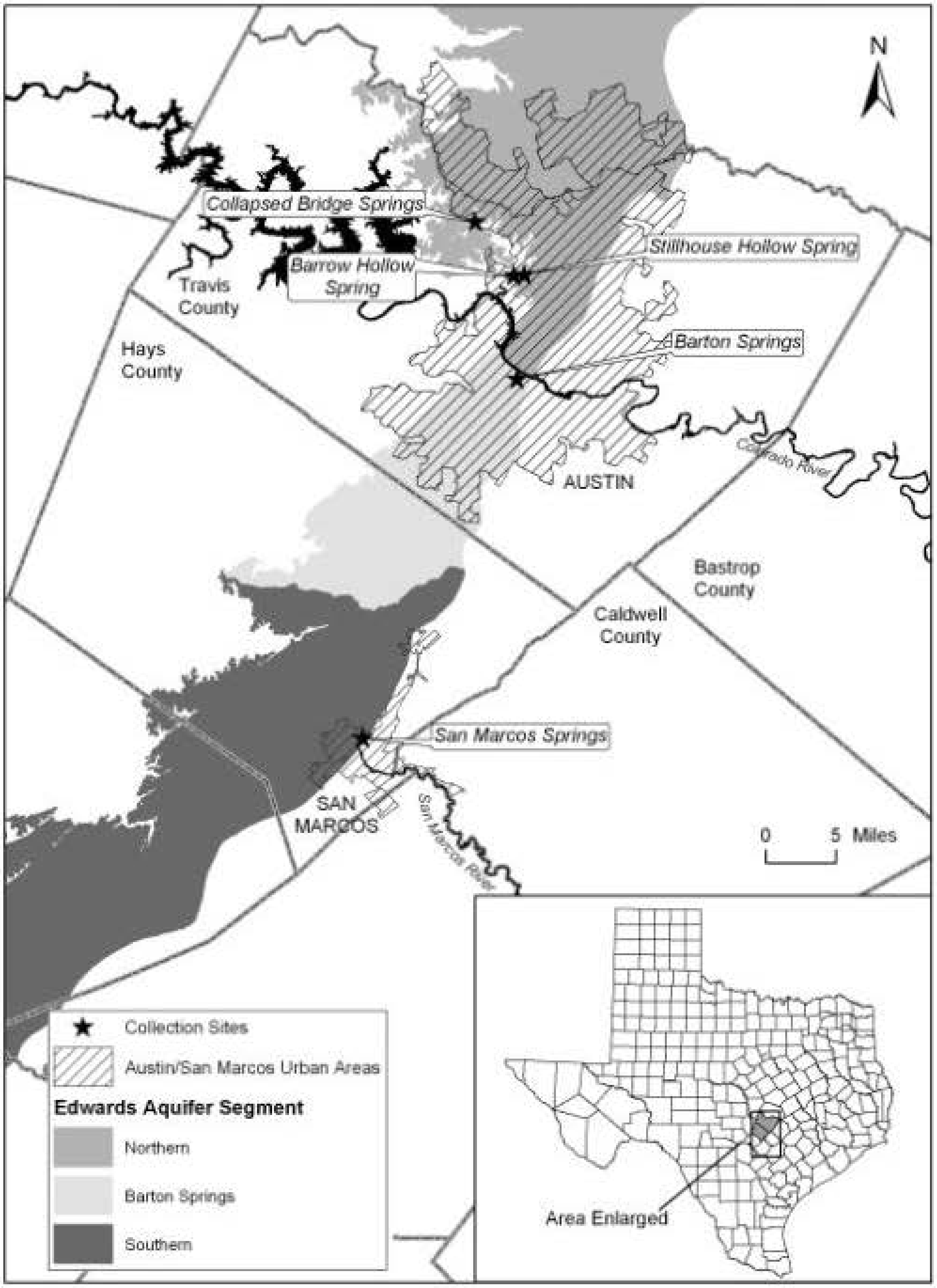
Habitat Springs of Edwards Aquifer Salamanders

Locations of habitat springs in Texas that were sampled during April to May1997 are shown in Figure 1. Sampled habitat springs and associated salamander species are summarized in Table 1. Barton Springs salamanders were collected for the study in 1995 either by dip net or by hand from Barton Springs in downtown Austin, Texas. San Marcos Springs and Texas salamanders were obtained from captive-reared populations at the San Marcos National Fish Hatchery & Technology Center in San Marcos, Texas.

Table 1. Summary of sampled habitat springs, associated salamander species, aquifer segments, and Texas counties.

Sampled Habitat Springs	Associated Salamander Species	Edwards Aquifer Segment	County
Barrow Hollow Springs, Collapsed Bridge Springs, and Stillhouse Hollow Springs	Jollyville Plateau salamanders	Northern Segment	Travis
Barton Springs	Barton Springs salamander	Barton Springs Segment	Travis
San Marcos Springs	San Marcos salamander	Southern Segment	Hays

Figure 1. Locations of sampled habitat springs of Edwards Aquifer salamanders.



Sediment Data Collection and Analysis

Collection, storage, and shipment of samples followed standard operating procedures of the U.S. Fish and Wildlife Service and U.S. Geological Survey. Samples for sediment turbidity tests and chemical analyses were collected with a shovel from the main Barton Springs Municipal Pool in Austin, Texas which contains Barton Springs. Sediment not in direct contact with the shovel was transferred to a 10-L high density polyethylene jar and shipped overnight on ice to the USGS Environmental Research Center at Columbia, Missouri for sediment turbidity tests. Separate aliquots were transferred to chemically-clean sample jars and shipped for chemical analysis to contract laboratories (Mississippi State Chemical Laboratory, Trace Element Research Laboratory) of the Patuxent Analytical Control Facility of the U.S. Fish and Wildlife Service in Laurel, Maryland. Analyses of sediments collected from the springs included grain-size, organic carbon, nutrients, inorganic contaminants, acid volatile sulfides, organochlorines (OCs), organophosphate insecticides (OPs), triazine herbicides, and polycyclic aromatic hydrocarbons (PAHs).

SPMD tests (Objective 1)

To determine the presence of organic contaminants in salamander habitat springs, SPMDs were placed within four different spring systems with plethodontid salamanders (three replicates per deployment). SPMD deployment included (1) Barrow Hollow Springs and Collapsed Bridge in the Northern segment of the Edwards Aquifer, (2) Barton Springs in the Barton Springs segment, and (3) San Marcos Springs in the Southern segment. The SPMDs were deployed during a 40 day period in May into June 1997 to involve one or more storm events. SPMDs used for purposes of quality assurance/quality control (QA/QC) were concurrently deployed including two SPMDs for performance evaluation, three SPMDs as process blanks, and three SPMDs as trip blanks. These SPMDs were used to minimize the identification of false positives and are not considered separate samples of other media such as air. SPMDs utilized for QA/QC were involved during the entire process from deployment through sample preparation and analysis.

Procedures for deployment and processing of SPMDS followed those developed by the USGS Environmental Research Center at Columbia Mo. The SPMDs were suspended in cages constructed of aluminum. Cages were approximately 61 x 30.5 x 15.3 cm (L x W x H) and had aluminum mesh screen covering all sides. The SPMDs were double-sided (61 x 2.5 cm) and were suspended between aluminum rods welded inside the cages. Three replicates were made for each site. Processing involved (1) removal of surface lipid by organic solvent dialysis with hexane for 48 hours and (2) gel permeation and Florisil chromatography to remove co-dialyzed interfering components. Analyses were conducted at Mississippi State Chemical Laboratory using gas chromatography/mass spectrometry. Purified dialysates were analyzed for OCs, OPs, PAHs, and pyrethroid pesticides. Spiked samples were used for quality control. Results from the three replicates were averaged to provide an average contaminant concentration accumulated for each site.

Sediment pulse tests (Objective 2)

Sediment pulse studies were conducted using artificial springs (upwellings) similar to a design used by the Dallas Zoo Aquarium. The upwellings were made by mounting an aquarium (61 x 30.5 x 30.5 cm) at the top of a 124.5 cm long tube (15.25 cm diam.). The bottom of the tube (approximately 62 cm) was covered with greenhouse shade cloth. Both the aquarium and tube were made from 1.3 cm plexiglass. Water flowed from the bottom of the tube with a standpipe into the aquarium (approximately 35 L water). Water flow was initially calibrated at about 5.5 L/min; however, flow varied somewhat over time. Commercially available lava rocks were used as habitat substrate in the tube. A single hole was drilled through each lava rock, and then the rocks were stacked along the entire length of a stainless steel threaded rod (1.3 cm). Lava rocks were also provided in the aquarium. Amphipod (*Hyallela azteca*) and bloodworm species (*Lumbriculus variegates*) were provided *ad libitum* to salamanders as a food source. Artificial light was provided at a light to dark ratio of 16:8. Sediment slurries were mixed in a 4000-L fiberglass tank containing two circulation pumps that mixed both vertically and horizontally. A hose was connected from the bottom of the fiberglass tank to a rheostat-controlled peristaltic pump and then to the bottom of the upwelling. Sediment pulses were initiated by pumping sediment slurry into the water delivery pipe at the bottom of the upwelling. Sediment then rose through the upwelling tube and into the aquaria.

Sediment slurries were conducted with both San Marcos and Texas salamanders. Each study lasted a total of 14 days but had a different type of sediment exposure during the 14 day period. For each study, two salamanders were stocked in an upwelling. In addition to observations of survival, the biomass of each salamander was determined for each study. To calculate change in biomass, the initial weight of each salamander was recorded just prior to stocking into an upwelling and at the end of each of the 14 day studies. All sediment studies were conducted in well water (alkalinity, 258 mg/L as $CaCO_3$; hardness, 286 mg/L as $CaCO_3$; pH 7.8; 18 ^{o}C). [a]

Three different types of turbidity pulse studies were conducted. The first study consisted of a single pulse event conducted over a 24-h period. Salamanders were held in an upwelling chamber for seven days prior to the pulse event. The pulse event consisted of an initial pulse of sediment that provided a turbidity measurement of approximately 40 Nessler Turbidity Units (NTU) in the upwelling aquaria. The initial turbidity of 40 NTU used in this study was similar to the maximum turbidity measured in Barton Springs pool following a storm event (City of Austin, personal communication). After six hours, the pump was adjusted such that sediment slurry was pumped at a rate about half the previous rate. This process was followed at 6-h intervals until the pump was turned off after a total of 24 hours. The approach provided a 24-h sediment hydrograph simulation that included an initial spike of sediment into the upwelling followed by a fading sediment pulse. Observations on salamander survival were made each day prior to the pulse event. Due to the limited number of available salamanders, the same salamanders were used in all three pulse studies.

[a] Concentration units are mg/L, parts per million; µg/L or µg/kg, parts per billion; and ng/L, parts per trillion.

The second type of pulse study consisted of three sediment pulses with one pulse per day for three consecutive days. In this pulse study, a turbidity of approximately 40 NTU was used on the day of a sediment pulse with readjustment of the peristaltic pump every two hours. Adjustment of the pump provided an 8-h pulse event that was repeated over the next two consecutive days. The overall study format was the same as the first pulse study. Salamanders were observed for seven days prior to the pulse events. The salamanders were then observed during (1) three days of pulses with observations made just before the pulse event and ten minutes after a pump adjustment, and (2) four days following the pulse event (total study length - 14 days).

The final type of sediment pulse consisted of a single large dose of sediment introduced into the upwelling without salamanders present. The sediment was allowed to settle naturally in the system. Salamanders were then introduced into the system and observed for 14 days.

Toxicity tests (Objective 3)

To evaluate the sensitivity of the salamanders to contaminant exposure, chemicals selected for use in the toxicity assessments were representative of classes of chemicals identified in the springs after evaluation of contaminant data obtained from sediments, SPMDs, or other monitoring information. Methods for conducting static acute lethality toxicity tests (96-h LC_{50}) were similar to those of the American Society for Testing and Materials (ASTM 1996). Static acute toxicity tests were conducted for 96 hours using 19.6 L glass jars with 15 L of test solution. Toxicity tests were conducted with Texas and Barton Springs salamanders. Chemicals used in testing included carbaryl, copper, diazinon, fluoranthene, and permethrin. Tests were conducted in either ASTM hard water (alkalinity, 110-120 mg/L as $CaCO_3$; hardness 160 mg/L as $CaCO_3$) or well water at 17 ^{o}C. For each exposure concentration, only one replicate with three to four individuals was tested. Controls included both water-only and acetone which was used as a carrier solvent for the organic contaminants.

RESULTS AND DISCUSSION

SPMD test results

SPMD dialysates in the five habitat springs were analyzed for 77 different contaminants including OCs, PAHs, OPs, and pyrethroid pesticides. Of the 77 different contaminants, only 20 compounds were detected that met QA/QC screening requirements and were considered contaminants present in salamander habitat springs (Table 2). In addition, not all five springs had measurable concentrations for each of the 20 compounds. Measurable concentrations were found for 13 OCs, 5 PAHs, and 2 pyrethroid pesticides. The site with the greatest number of contaminants identified was Collapsed Bridge Springs (16 contaminants), followed by Barrow Hollow Springs (13), Barton Springs (6), and Stillhouse Hollow Springs (2). San Marcos Springs had no detectable contaminant concentrations. PAHs were detected only at Barrow Hollow Springs (3) and Collapsed Bridge Springs (2). Cis-permethrin and trans-permethrin were detected only at Collapsed Bridge Springs. There were 9 OCs common to both Collapsed Bridge Springs and Barrow Hollow Springs, and these two sites accounted for most of the OCs measured. Collapsed Bridge Springs had 12 OCs, and Barrow Hollow Springs had 10 OCs. The rest of the habitat spring sites had 6 OCs or fewer (Barton Springs, 6; Stillhouse Hollow Springs, 2; and San Marcos Springs, 0).

Table 2. Contaminants measured in semipermeable membrane devices (SPMDs) from salamander habitat springs. Concentrations are the average of the chemical accumulated (total micrograms) in each of three SPMDs deployed at each spring.

Contaminant	Barrow Hollow Springs	Barton Springs	Collapsed Bridge Springs	San Marcos Springs	Stillhouse Hollow Springs	Detection Limit	% Recovery
	µg	µg	µg	µg	µg	µg	
Organochlorines							
alpha-chlordane	0.19	0.02	0.03	n.d.*	n.d.	0.01	107%
gamma-chlordane	0.14	0.02	0.04	n.d.	n.d.	0.01	102%
oxychlordane	0.05	n.d.	0.01	n.d.	n.d.	0.01	95%
p,p-DDD	n.d.	n.d.	0.03	n.d.	n.d.	0.01	92%
p,p-DDE	0.01	0.02	0.01	n.d.	n.d.	0.01	99%
o,p-DDT	n.d.	n.d.	0.01	n.d.	n.d.	0.01	90%
p,p-DDT	n.d.	n.d.	0.03	n.d.	n.d.	0.01	93%
dieldrin	1.13	0.02	0.01	n.d.	n.d.	0.01	n.m. †
endrin	0.01	n.d.	n.d	n.d.	n.d.	0.01	113%
heptachlor epoxide	0.23	0.03	0.01	n.d.	0.03	0.01	99%
hexachlorobenzene	0.01	n.d.	0.01	n.d.	n.d.	0.01	103%
cis-nonachlor	0.09	n.d.	0.02	n.d.	n.d.	0.01	104%
trans-nonachlor	0.29	0.03	0.05	n.d.	0.01	0.01	119%
PAHs							
fluoranthene	0.57	n.d.	n.d.	n.d.	n.d.	0.25	98%
1-methyl phenanthrene	n.d.	n.d.	0.57	n.d.	n.d.	0.25	n.m.
C1-phenanthrene	n.d.	n.d.	0.57	n.d.	n.d.	0.25	n.m.
phenanthrene	0.35	n.d.	n.d.	n.d.	n.d.	0.25	88%
pyrene	0.3	n.d.	n.d.	n.d.	n.d.	0.25	102%
Pyrethroids							
cis-permetrhin	n.d.	n.d.	0.07	n.d.	n.d.	0.04	n.m.
trans-permethrin	n.d.	n.d.	0.04	n.d.	n.d.	0.04	n.m.

* not detected, † not measured.

In general, contaminants measured by SPMDs inside salamander habitat springs were relatively low with contaminants at each spring only somewhat greater than detection limits. One exception was the amount of the pesticide dieldrin measured in SPMDs from Barrow Hollow Springs. Dieldrin measured in SPMDs from this spring averaged 1.13 µg. The corresponding concentration for dieldrin in water flowing through Barrow Hollow Springs would be 3.5 ng/L according to the model in Huckins *et al*. (1993). This computed concentration of dieldrin in

Barrow Hollow Springs is a higher concentration than EPA's chronic criterion of 1.9 ng/L for dieldrin in freshwater. Although measured amounts of organic contaminants in SPMDs can be used to approximate average concentrations of contaminants in water, factors such as spatial variability in the water column may affect contaminant concentrations indicated by SPMDs (Huckins *et al.* 1993, Louch *et al.* 2003).

Results for sediment turbidity tests

The three types of sediment slurry tests (single 24-h pulse, three 8-h pulses, and continuous presence of sediment) did not produce large changes in biomass of the two salamander species (Table 3). Texas salamanders generally had a slight loss of biomass during slurry trials with three 8-h pulses over a period of 72 hours. Changes in survivability of salamanders were not observed for either salamander species during the 14 day period. High loads of sediment into salamander habitat may have other effects on salamanders such as (1) burial of eggs, (2) filling voids in cobble and gravel that serve salamanders as habitat, or (3) impacting food organisms.

Table 3. Biomass changes (wet wt.) of two salamander species under sediment pulse tests.

Species	Study	Trial	Biomass gain or loss in grams	Mean ± S.E.
Texas salamander	Single 24-h pulse	1	-0.114	0.02 ± 0.09
		2	-0.014	
		3	0.196	
	Three 8-h pulses	1	-0.004	-0.05 ± 0.03
		2	-0.110	
		3	-0.026	
	Continuous presence	1	0.168	0.04 ± 0.07
		2	-0.082	
		3	0.025	
	Water only	1	-0.072	-0.01 ± 0.06
		2	-0.064	
		3	0.117	
San Marcos salamander	Single 24-h pulse	1	0.199	0.02 ± 0.1
		2	-0.147	
		3	0.01	
	Three 8-h pulses	1	-0.002	0.01 ± 0.01
		2	0.023	
	Continuous presence	1	0.011	0.07 ± 0.05
		2	0.119	

Toxicity test results

Due to low numbers of individual salamanders available for testing, standard acute toxicity tests for Edwards Aquifer salamanders were not conducted. Only three to four individuals could be tested at each concentration, and exposure concentrations were less than a standard 60% dilution series. Therefore, toxicity test results from this study must be regarded as preliminary. However, the relative sensitivity of *Eurycea* species to contaminants (with mortality as an endpoint) may possibly be inferred from toxicity test results on two *Eurycea* species as shown in Table 4.

Table 4. Summary of acute toxicity tests using selected contaminants on two species of salamanders (Barton Springs and Texas).

Chemical	Salamander species	Exposure water	Individuals per replicate	Salamander 96-h LC_{50}	Bluegill 96-h LC_{50}*	Rainbow trout 96-h LC_{50}*
carbaryl	Barton Springs	ASTM	3	4.3 - 30.0 mg/L	1.8 - 39.0 mg/L	0.8 - 4.5 mg/L
carbaryl	Texas	ASTM	3	11.8 - 29.5 mg/L	1.8 - 39.0 mg/L	0.8 - 4.5 mg/L
copper	Texas	ASTM	3	> 500 µg/L	236 – 620 µg/L †	20 - 30 µg/L †
diazinon	Barton Springs	Well	4	> 343 µg/L	168 µg/L	90 µg/L
fluoranthene	Barton Springs	Well	4	> 490 µg/L	n.a. ‡	n.a. ‡
permethrin	Texas (adult, 0.38g) §	ASTM	3	> 20.6 µg/L	2.3 - 13.0 µg/L	2.9 - 8.2 µg/L
permethrin	Texas (juvenile, 0.19 g) §	Well	3	> 20.7 µg/L	2.3 - 13.0 µg/L	2.9 - 8.2 µg/L

* Mayer and Ellersieck (1986), † Eisler (1997), ‡ not available, § average weight of three individuals.

Results from the acute static exposures indicate that the sensitivity of *Eurycea* salamander species to tested contaminants (carbaryl, copper, diazinon, fluoranthene, and permethrin) was similar to or less than other aquatic vertebrate species such as fish. With the possible exception of carbaryl, the LC_{50}s for salamander species for the 5 chemicals tested are generally higher than LC_{50}s for bluegill (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*). Carbaryl exposures were conducted in the same water for both salamander species, and results indicate that the two species may have similar sensitivity to those chemicals. Also, adult and juvenile Texas salamanders both had an LC_{50} > 20.6 µg/L for permethrin. Differences in sensitivity between age groups cannot be determined since the lethal concentrations are greater than the highest concentration tested.

There is limited data evaluating the effects of contaminants on aquatic salamanders. Rose and Harshbarger (1977) found skin lesions occurring on tiger salamanders (*Ambystoma tigrinum*) collected from a lagoon heavily polluted with secondary treated domestic sewage. Because of the relatively few contaminants tested in this study and inadequate replication, further testing is necessary to determine the sensitivity of neotenic salamanders to contaminants. Since these tests were acute toxicity tests, additional tests should be conducted to evaluate chronic exposures and sublethal effects. Sublethal effects in organisms (alterations in behavior, endocrine disruption, etc.) generally occur at lower concentrations of contaminants than lethal effects.

As shown in Table 5, six types of PAHs found in sediment at Barton Springs exceeded sediment ERMs (Effects Range Median) for the amphipod *Hyalella azteca* as developed by Ingersoll *et al*. (1996). Effects range medians in toxicity testing indicate the concentration of a chemical in sediment above which effects are frequently or always observed or predicted among most species (Ingersoll *et al.* 1996). A food source for the endangered Barton Springs salamander, the amphipod *Hyalella azteca*, may be affected by concentrations of these PAHs in sediment at the habitat spring.

Table 5. Concentrations (in dry wt.) of polycyclic aromatic hydrocarbons (PAHs) in sediment collected at Barton Springs.

PAH	Sediment PAH concentration in µg/kg	Effects Range Median (ERM) for *Hyalella azteca* in µg/kg*
benzo(a)pyrene	0.544	0.47
benzo(g,h,i,)perylene	0.524	0.28
chrysene	0.518	0.5
fluoranthene	0.865	0.18
indeno(1,2,3-c,d)pyrene	0.575	0.25
pyrene	0.692	0.35

* Ingersoll et al. (1996).

CONCLUSIONS AND RECOMMENDATIONS

Although the level of contaminants found in SPMD dialysates was not elevated in the five habitat springs of the Edwards Aquifer salamanders, contaminants in the water column and sediment of these springs should be continuously monitored. Contaminants can be carried by stormwater runoff into the Edwards Aquifer through subsurface features such as sinkholes and caves thereby reflecting sources of stormwater contaminants from residential and commercial areas in watersheds overlying the aquifer. As development increases in these watersheds, the potential for increased levels of contaminants in salamander habitat springs will also increase.

The water concentration of 3.5 ng/L for dieldrin at Barrow Hollow Springs that was calculated from SPMD data represents a single estimate only. Dieldrin (a persistent organochlorine) has not been commercially available as a pesticide in the U.S. since 1974, and the dieldrin residues found at Barrow Hollow Springs presumably originated from eroded soil or sediment that was carried off by surface runoff.

Although results from preliminary acute toxicity tests presented in this study would indicate that *Eurycea* salamander species are similar to or less sensitive to tested contaminants as compared to fish species, the test results have to be regarded as preliminary because of the small number of salamanders and contaminants used for testing. Because of the lack of existing information on sublethal contaminant effects on neotenic salamanders, regulatory protective measures such as water quality criteria, pesticide use restrictions, etc. should be conservative with respect to protection of *Eurycea* species from water or sediment-borne contaminants. Only the most sensitive surrogate species for Edwards Aquifer salamanders should be used to infer effects on *Eurycea* species by contaminants. In addition, the protection of important prey species of the salamanders should also be considered in devising appropriate regulatory approaches.

Recommendations for future research of contaminants in Edwards Aquifer salamanders include:

1. This study only evaluated the acute toxicity of five chemicals with toxicologically different modes of action. Further testing is necessary to determine the sensitivity of neotenic salamanders to other contaminants. Long-term chronic studies should be conducted to evaluate the effects of contaminants on reproduction, behavior, growth, or other important sublethal endpoints for salamander species. Since there are no current methodologies available to evaluate sublethal endpoints for these species, additional methodologies should also be developed.

2. SPMDs should be deployed in salamander habitat springs during various seasons in consecutive years to determine long-term trends in contaminant flows. Additional evaluations with dieldrin should also be made to determine actual concentrations in Barrow Hollow Springs and possible impacts to salamanders and the habitat.

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