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THE EFFECTS OF ELEVATED TOTAL DISSOLVED SOLIDS

ON THE BEHAVIOR AND SURVIVAL OF THE ALLEGHENY MOUNTAIN

DUSKY SALAMANDER, *DESMOGNATHUS OCHROPHAEUS*

A Thesis

Submitted to the Bayer School of Natural and Environmental Sciences

Duquesne University

In partial fulfillment of the requirements for

the degree of Master of Science

By

Megan Margaret Morrissey

May 2014

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Megan Margaret Morrissey

2014

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By

Megan Margaret Morrissey

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ABSTRACT

THE EFFECTS OF ELEVATED TOTAL DISSOLVED SOLIDS
ON THE BEHAVIOR AND SURVIVAL OF THE ALLEGHENY MOUNTAIN
DUSKY SALAMANDER, *DESMOGNATHUS OCHROPHAEUS*

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Dissertation supervised by Dr. Sarah Woodley.

Elevated total dissolved solids (TDS) have been found in streams in Pennsylvania primarily due to unconventional natural gas development (UNGD) and mine drainage. TDS is the concentration of dissolved salts in water. To understand the effects of TDS on amphibians, a semi-aquatic salamander (*Desmognathus ochrophaeus*) was exposed to synthetic solutions containing TDS up to 5,000 ppm. In an initial study, animals exposed to a solution of approximately 2,000 ppm TDS containing elevated sulfate experienced reduced survival and decreased locomotory activity. Follow-up studies were consistent with the hypothesis that elevated TDS had adverse effects on survival, but effects were not seen unless the TDS was closer to 5,000 ppm. In all studies, larger animals were more prone to adverse effects of TDS. Results indicated that elevated TDS can have

adverse effects on *D. ochrophaeus,* but further research is needed to understand the doses and conditions at which effects are evident.

DEDICATION

I would like to dedicate my thesis to my family who has always encouraged me to pursue my education.

ACKNOWLEDGEMENT

I would like to thank my advisor, Dr. Woodley, for her guidance and encouragement throughout my time at Duquesne University. Thank you to my committee, Dr. Porter, Dr. Stolz, and Dr. Kabala for their advisement. I would also like to thank the entire Woodley lab group for their support. Thank you to Jessica Thomas and Chris Fonner for help with animal collection and animal care as well as their constructive feedback throughout the research process. A special thanks to Bianca Coleman for her assistance with animal care.

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Chapter 1 Background

1.1 Total Dissolved Solids

Elevated levels of TDS found in waterways are becoming a worldwide concern due to increasing levels of development, industrial activity, and resource extraction (Canedo-Arguelles et al., 2013). TDS refers to the concentration of dissolved inorganic salts in water, including major cations (K^+, Ca^{2+}, Mg^{2+}, Na^+) and anions (CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-). In a stream system, the bed load, suspended load, and dissolved load are constituents of the overall stream load. The dissolved load is the product of the ionic concentration of the water and discharge (Allan and Castillo, 2007). TDS is measured in the laboratory by evaporating water and weighing the remaining residue. TDS is the sum of the concentrations of major ions and can be calculated using ion chromatography. TDS is reported in units of mass (mg/l), which is equivalent to parts per million (ppm). In the field, electrical conductivity is used to approximate TDS. Conductivity (reported in units of µS/cm at 20 or 25°C) is a measure of the electrical conductance of water. In order to approximate TDS from conductivity, the specific conductance is multiplied by a constant between 0.55 and 0.75 (Allan and Castillo, 2007). The TDS of freshwater is less than 1,500 ppm and water between 1,500 ppm and 5,000 ppm is considered brackish (Hem, 1985). Water with a TDS greater than 5,000 ppm is considered saline while seawater is between 34,000 ppm to 38,000 ppm (Hem, 1985).

Mine drainage (Sams and Beer, 2000), unconventional natural gas development (UNGD); (Ferrar et al., 2013), and road deicing salts are the dominant sources of TDS in southwestern Pennsylvania. Other sources of TDS include industrial activities, sewage treatment plant effluent, irrigation, and suburban development (Canedo-Arguelles et al., 2013; Timpano et al., 2010). The ionic composition of TDS differs between sources. Through an analysis of anion ratios, scientists are able to determine the source of TDS in the water. For example, the sulfate to chloride ratio is higher in mine drainage related water than in UNGD water (Wilson et al., 2013). The U.S. Environmental Protection Agency set a secondary standard for TDS of 500 mg/l for the purpose of managing drinking water for aesthetics and taste (EPA, 2013). In Pennsylvania, the Pennsylvania Code sets a secondary water quality standard of 500 mg/l TDS monthly average with a maximum of 750 mg/l for Potable Water Sources (PWS) and Coldwater Fisheries (CWF); (Pennsylvania Code).

1.2 Unconventional Natural Gas Development (UNGD)

There is concern in Pennsylvania over the high levels of TDS in the wastewater created by unconventional natural gas development in the Marcellus Shale play. Analysis of produced water (a by-product of the oil and gas drilling that contains TDS, hydrocarbons, chemicals, and sediment) in Pennsylvania showed a median TDS of 200,000 ppm (Wilson et al., 2013). One of the disposal methods available for produced water is through brine treatment facilities that directly discharge effluent into streams. These brine treatment facilities do not remove salts; therefore, high levels of TDS may be discharged into streams.

Water analyzed downstream of several wastewater treatment facilities that treat UNGD wastewater in Pennsylvania revealed high levels of TDS including high levels of chloride and sulfate (Ferrar et al., 2013 ; Warner et al., 2013). Chloride concentrations measured 1.7 km downstream of the facilities were 2-10 times higher than background chloride concentrations in Pennsylvania streams (Warner et al., 2013). Effluent from the Pennsylvania Josephine Brine Treatment Facility in Indiana County, Pennsylvania (which discharges into Blacklick Creek) showed mean TDS levels at approximately 160,000 mg/l with a mean sulfate concentration of 708 mg/l and mean chloride at 68,375 mg/l (Ferrar et al., 2013). Although elevated sulfate is normally considered an indication of abandoned mine drainage, the elevated levels of sulfate detected in Blacklick Creek can be attributed to the wastewater treatment process (Ferrar et al., 2013, Warner et al., 2013). Sodium sulfate is commonly added to the treatment process to precipitate barium and strontium but there is no reduction of the final TDS released in the effluent (Ferrar et al., 2013).

There is a strong interest in Pennsylvania to use abandoned mine drainage (AMD) instead of freshwater for unconventional natural gas extraction (Kondash et al., 2013). The produced water taken to treatment facilities will therefore be a combination of AMD and UNGD wastewater and could contain elevated sulfate as well as chloride.

1.3 Mine Drainage

As noted, a major source of TDS in Pennsylvania is mine drainage. The major components of mine effluent are sulfate, aluminum, iron, and manganese (Cravotta, 2008; Sams and Beer, 2000). Sulfate is the best indicator of mine drainage because sulfate is soluble and chemically stable at the pH levels found in natural waterways (Sams and Beer, 2000). Treatment of abandoned mine effluent aims to lower pH and reduce aluminum but does not remove or reduce sulfate (Sams and Beer, 2000). A U.S. Geological Survey study of streams in Southwestern Pennsylvania has found levels of sulfate exceeding 1,000 mg/l in some areas (Sams and Beer, 2000).

1.4 Effects of Mine Drainage on Aquatic Organisms

Many studies have shown that mine drainage negatively effects aquatic life and some studies have focused on isolating the effects of TDS in mine drainage on aquatic life (Scannell and Jacobs, 2001; Timpano et al., 2010). In a review of the effects of TDS on aquatic organisms, growth and survival were significantly reduced at a TDS greater than 1500 mg/L (Scannell and Jacobs, 2001). A majority of studies focusing on the effects of TDS in mine effluent on aquatic organisms focused on toxicity, egg fertilization, and early development (Brix et al., 2010; Porter and Hakanson, 1976; Stekoll et al., 2009). When exposed to simulated mine effluent at a TDS of 750 mg/l, fertilization and hatching rates of coho and chum salmon were significantly reduced (Stekoll et al., 2009). Field studies of streams dominated by sulfate found a significant reduction in macroinvertebrate species richness (Timpano et al., 2010).

1.5 Effects of TDS on Amphibians

The health of global amphibian populations is in peril as over 40% of amphibian species are in decline (Stuart et al., 2004). This decline can be attributed to several causes, including habitat destruction, climate change, over-exploitation, pollution, pesticide use, and infectious diseases. Salamander skin is very permeable as it is used to regulate water uptake as well as respiration (Smith et al., 2007). The most significant exposure route of environmental contaminants to amphibians is through the skin, which makes them very susceptible to changes in the environment. For this reason, amphibians are commonly used as models for studying environmental change and the effects of environmental contaminants (Hopkins, 2007).

The most studied constituent of the effects of TDS on amphibians is sodium chloride that enters streams and ponds due to road deicing salt runoff. Many studies have focused on the lethal effects of chloride on amphibians in field and laboratory settings (Karraker, 2008; Maleek et al., 1999). *Ambystoma maculatum* (Spotted Salamander) embryos had a survival rate of 3% when exposed to a chloride concentration of 945 mg/L in a field mesocosm (Karraker, 2008). *Lithobates* (*Rana) clamitans* (Green Frog) embryos and larvae were also exposed to a chloride concentration of 945 mg/L and were found to have a survival rate 88% (Karraker, 2007). It is clear that there are wide differences in chloride tolerance in amphibians.

Many studies also observed negative behavioral and developmental effects of sodium chloride on anurans (Cross and Elinson., 1980; Hopkins et al., 2013; Denoël et al., 2010). Cumulative effects of road deicing salt on Common Frog tadpoles (*Rana temporaria*) include decreased speed and movement when exposed to 1,500 mg/l NaCl (Denoël et al., 2010). *L. clamitans* (Green Frog) embryos exposed to a chloride concentration of 945 mg/l hatched with 15% exhibiting malformations including spinal curvatures, bubbles in the gill chamber, and tumor-like growths (Karraker, 2008). Developmental deformities including spinal deformities, cysts, and shrunken and missing limbs and organs were also found in *Taricha granulosa*

(Rough-Skinned Newt) exposed to various concentrations of NaCl and $MgCl_2$ (Hopkins et al., 2013). These deformities in newt hatchlings increased in severity as well as frequency when exposed to higher levels of road salts (Hopkins et al., 2013).

In a review of studies on road deicing salts, Karraker (2008) concludes that more research must be completed on a range of amphibians in all life stages. Only one species of salamander (*A. maculatum*) and one newt (*T. granulosa*) have been studied with road salt and none have examined possible effect on adults (Karraker, 2008; Hopkins et al., 2013). These few studies show that salamanders may be more susceptible than frogs to salinity; however, more studies on salamanders at various life stages are needed to fully understand these observations (Hopkins et al., 2013).

Acute as well as long term chronic exposure studies are needed to determine developmental and behavioral effects of TDS on amphibians (Karraker, 2008). Salinity tolerances of amphibians (Hopkins et al., 2013), fish, (Canedo-Arguelles et al., 2013) and stream macroinvertebrates (Goetsch and Palmer., 1997) vary greatly between species and effects depend on the different ionic compositions of the TDS. It is important to gain a greater understanding of the effects of elevated TDS (with compositions likely to be found in the environment) on species at all life stages at both acute and chronic levels.

1.6 Sublethal Effects of Environmental Contaminants

Many studies on exposure of aquatic organisms to elevated TDS measure toxicity, however, the sublethal effects of environmental contaminants on organisms are needed to understand potential effects on the long term survival of a species.

Measuring the sublethal effects on organisms can involve the quantification of movement, feeding, reproduction, deformities, etc. Changes in locomotory activity in an organism can alter its ability to capture prey, avoid predators, and mate, all of which can eventually lead to population decline. It is important to measure the possible effects of locomotory behavior on salamanders because any alterations in their behavior may affect salamander populations and therefore stream communities. Common Frog tadpoles (*R. temporaria*) exposed to organochlorine pesticides had decreased locomotory behavior including travelling shorter distances and swimming less often than controls (Denoël et al., 2013).When exposed to mercurial chloride, mosquitofish exhibited changes in locomotor activity and decreased swimming speed (Burke et al., 2010).

Another important measure of understanding the chronic sublethal effects is a measurement of feeding activity. In addition to locomotory activity tracking, determining prey capture ability in salamanders with the use of flightless fruit flies was a successful method used to observe sublethal effects of mercury exposure on adult salamanders (Burke et al., 2010). Adult Northern Two-Lined Salamanders (*Eurycea bislineata*) from sites contaminated with mercury ate half as many flies as the control group (Burke et al., 2010). Consumption of black worms by *Ambystoma jeffersonianum (*Jefferson Salamander*)* larvae was significantly decreased when exposed to solutions containing increased conductivity at 4,000 ppb and 8,000 ppb (Chambers, 2011).

Chapter 2: Specific Aims

2.1 Background

The sublethal effects of elevated TDS on semi-terrestrial salamanders were first observed by Meghan Pascuzzi (Pascuzzi, 2012). Pascuzzi used water collected from Blacklick Creek (N40° 28’ 50.1”, W79° 10’ 8.8”), a tributary of the Conemaugh River in Western Pennsylvania, and Whiteley Creek (N39° 49’ 16.4”, W79° 57’ 6.3”), in the Ten Mile Creek system of Greene County, PA, in 2011 to test the behavioral effects of elevated TDS on *D.ochrophaeus* (Pascuzzi, 2012). In 2011, Blacklick Creek had a TDS of 1,120 ppm with elevated levels of sodium chloride. Blacklick Creek is influenced by UNGD from the Josephine Brine Treatment Facility. Whiteley Creek had elevated TDS of 2,010 ppm with high levels of sodium sulfate indicating impacts of mine drainage.

When testing for behavioral effects with Whiteley Creek water (2,000 ppm TDS), several salamanders became moribund and the experiment was halted (Pascuzzi, 2012). A subsequent study using synthetic Whiteley Creek water diluted to 1,000 ppm found reduced locomotory activity when animals were chronically exposed. In the same study, no effects on activity were observed when animals were chronically exposed to synthetic Blacklick Creek water at 1,000 ppm TDS (Pascuzzi, 2012). These results, however, were problematic because all animals across each treatment group lost body mass over the course of the experiment, with control animals losing the most body mass (an average of 12%). The drop in body mass was likely due to evaporative water loss because ambient humidity was not controlled, and salt water has a reduced vapor pressure compared to freshwater.

The primary objective of this thesis was to validate and expand upon the behavioral effects found in Pascuzzi, 2012. Testing was completed in the Animal Care Facility at a constant temperature and humidity to avoid prior problems of evaporative water loss. In response to the results of the behavioral tests, I expanded the study to include tests on the effects of elevated TDS on survival.

2.2 Aim 1: Behavioral Responses to Elevated TDS

The first aim of this study was to evaluate the effects of chronic elevation of TDS on locomotory activity and feeding of adult male *D.ochrophaeus*. The purpose of aim 1 was to understand the behavioral effects of elevated TDS (1,000-2,000 ppm TDS) on streamside salamanders with an emphasis on water with elevated sulfate and chloride. Two tests were completed that measured locomotory and feeding behaviors of salamanders after exposure to synthetic solutions that mimic field samples as well as a synthetic solution of spring water enriched with sodium sulfate.

Hypothesis: Adult male *D.ochrophaeus* chronically exposed to elevated TDS will have reduced locomotory activity and reduced feeding when compared with controls.

2.3 Aim 2: Survival in Response to Elevated TDS

Knowing the concentration at which TDS leads to a moribund state is important for assessing the effects of TDS and for making recommendations of acceptable levels. The purpose of the second aim was to systematically examine the effects of different concentrations of TDS on survival. Several studies have determined EC50's for aquatic organisms exposed to TDS (Scannell and Jacobs, 2001). The EC50 is the determination of the effective concentration of TDS that causes 50% of subjects to become moribund. Male *D. ochrophaeus* were exposed to doses of TDS ranging from 500 ppm to 5,000 ppm to determine whether animals become moribund. These levels of TDS are likely to be found in freshwater streams in Pennsylvania that have been impacted by mine drainage and natural gas development (Ferrar et al., 2013 ; Sams and Beer, 2000). Two toxicity tests were used to determine an EC50 value of TDS. An acute toxicity experiment was used to determine toxicity after 4 days of exposure. The acute toxicity test used animals that were exposed to TDS in previous experiments and were housed in the laboratory for greater than six months. The chronic toxicity experiment was used to determine toxicity after 14 days of exposure and used animals that were recently collected from the field and had no prior exposures to elevated TDS in the laboratory.

Hypothesis: At least 50% of adult male *D. ochrophaeus* exposed to synthetic Whiteley water at 4,557 ppm TDS will become moribund.

Chapter 3 Methods

All animals used in this study were collected using permits from the Pennsylvania Fish and Boat Commission. The Institutional Animal Care and Use Committee (IACUC) at Duquesne University approved all procedures described in this thesis.

3.1 Animal Care

All animals used in this study were adult male Allegheny Mountain Dusky Salamanders (*D. ochrophaeus*). Animals were collected from: Linn Run (40.138287, -79.204171) in Forbes State Forest ,Westmoreland County, Pennsylvania on 30 August 2010 ; Elk Rock Run (39°57'4.29", -79°34'40.15"), in Pennsylvania State Game Lands 51, Fayette County on 24 May 2011 and 16 April 2012 ; Brooks Run (41.409132, -78.055232) in Elk State Forest, Cameron County, Pennsylvania on 20 September 2013. After field collection and in between experimentation, animals were held individually in 15 x 15 cm plastic containers moistened with water and moistened crumpled unbleached paper towels. Containers were kept at 16° C with a 14:10 L:D cycle in incubators. Animals were fed one waxworm every two weeks. For exposures and testing, animals were moved to the Duquesne University Animal Care Facility at least one week prior to the experiment for acclimation. Animals were housed at a temperature of 19-21° C with an average room humidity of 30% and a 14:10 light-dark cycle in order to mimic summer conditions.

3.2 Solutions

Solutions were made to represent ion ratio compositions found in water samples collected from Western Pennsylvania streams in 2011 : Blacklick Creek (N40° 28' 50.1", W79° 10' 8.8") and Whiteley Creek (N39° 49' 16.4", W79° 57' 6.3") ; (Pascuzzi, 2012). Solutions were created by dissolving salts (Appendix A) into distilled deionized water using the compositions shown in Table 1. The pH of each solution was adjusted to approximately 8.00 by adding HCL or NaOH. A pH of 8.00 was used because field samples of Whiteley Creek and Blacklick Creek were approximately 8.00 when sampled. Solutions were dispensed into 50ml and 20ml propylene vials and were stored in the testing room at a temperature of around 19-20° C. Solutions were analyzed for anion concentrations using Dionex ICS-1100 Ion Chromatography System at Duquesne University and cations using Perkin-Elmer NexION 300x IC-ICP-MS at the University of Pittsburgh (Table 2). The TDS of each solution was determined by adding together the major ions greater than or equal to one milligram per liter.

3.3 TDS Exposure

A static renewal design was used to expose salamanders to elevated TDS. Animals were housed individually in petri dishes (14 cm diameter, 2 cm in height) lined with an unbleached paper towel moistened with 5 ml of water solution. The 5 ml of solution saturated the unbleached paper towel and no standing water occurred in the petri dish. Each morning, the petri dish bottom was replaced with a clean bottom containing a new paper towel moistened with 5 ml of solution. During the petri dish replacement, no transfer or accumulation of solution from the used petri dish to the new petri dish occurred. The observer was blind to the different treatments.

3.4 Locomotory Activity

Locomotory activity of subjects in their petri dishes was observed between the hours of 16:00 and 19:00. Visual barriers were placed on the outside edges of the petri dishes so that animals could not see one another. Gridlines containing 4 quadrants were placed under each petri dish so that they could be viewed by the observer from the top of the petri dish. Activity was recorded with a Sony 3CCD Progressive Scan, 48X Digital Zoom camcorder. Recording occurred for 65 minutes at intervals of 2 seconds every 30 seconds. The first 5 minutes of each recording was not scored and was considered an acclimation period. Movements were scored when half of the animal's body crossed into a new quadrant.

3.5 Feeding

To test for feeding behavior, animals were provided ten frozen fruit flies twice per week (a total of 20 flies per week). Fruit flies were scattered around the petri dish during the dark cycle and after any behavior observations and/or weighing. Uneaten flies were counted the following morning before the renewal of the substrate.

3.6 Behavioral Experiment 1: Exposure to sulfate enriched TDS (synthetic Whiteley water at 1,957 ppm) and chloride enriched TDS (synthetic Blacklick water at 891 ppm).

A static renewal design was used to determine behavioral effects of TDS. Forty-five male *D. ochrophaeus* were exposed to 5 ml of synthetic solution for 27 days. During the 27 day exposure, animals were housed and tested in an average temperature of 20.5°C and at an average humidity of 38%. Activity was observed once per week (total of 4 trials) and feeding was observed. Each treatment group had 15 salamanders of a similar mean body mass. Salamanders were exposed to one of three solutions: synthetic spring water (47 ppm TDS), synthetic Whiteley Creek water (1,957 ppm TDS) and synthetic Blacklick Creek water (891 ppm TDS) ; (Table 2). Synthetic spring water was the control. Salamanders were weighed before the experiment, once per week during the experiment, and at the end of the experiment. Salamanders were never weighed directly before recording activity. Animals were checked daily for signs of moribundity (no righting response, gaping behavior). Animals that became moribund were removed from the experiment and placed into new petri dishes for a few days to attempt to recover them; however, they did not recover and were euthanized with an overdose of MS-222.

Rationale: The synthetic solutions used were made to represent the ion ratios found in Blacklick Creek and Whiteley Creek when they were sampled in 2010. The TDS levels used in this experiment are also similar to those collected in the field. Whiteley Creek water was approximately 2,000 ppm TDS when sampled in 2010, and Blacklick Creek was approximately 1,000 ppm TDS when collected in 2010. This original aim of this experiment was to test behavioral effects of both the synthetic Whiteley Creek and synthetic Blacklick Creek water at 1,000 ppm. However, due to error, the synthetic Whiteley water used in this experiment was not diluted and the behavioral tests were conducted with synthetic Whiteley water at approximately 2,000 ppm TDS.

3.7 Behavioral Experiment 2: Exposure to TDS enriched in sulfate (synthetic Whiteley water at 1,479 ppm and synthetic spring water with elevated sodium sulfate at 1,332 ppm)

Forty-one male *D. ochrophaeus* salamanders were exposed to 5 ml of synthetic solution for 25 days. During the 25 day exposure, animals were housed and tested in an average temperature of 21°C and at an average humidity of 44%. Animals were exposed to 1 of 3 treatments: synthetic spring water (47 ppm TDS), synthetic Whiteley water (1,479 ppm TDS) and synthetic spring water with elevated sodium sulfate (1,332 ppm TDS). The same methods for feeding, weighing, and recording were followed as described in Behavioral Experiment 1.

Rationale: The purpose of the second behavioral experiment was to further understand the sublethal effects of the TDS in the synthetic Whiteley water. The synthetic Whiteley water was diluted in order to reduce the effect on survival so that behavioral effects could be observed. A second synthetic solution was created to mimic the ion ratios and TDS of the synthetic Whiteley water while removing chloride. This solution was created by adding sodium sulfate to synthetic spring water. This was done in order to understand if the sulfate anion that is dominant in synthetic Whiteley water is the anion of concern by removing most of the chloride. The synthetic Whiteley water used in this experiment had 13% chloride and 51% sulfate. The synthetic spring water with elevated sodium sulfate solution had 1% chloride and 65% sulfate.

3.8 Acute Toxicity Experiment

Thirty male *D. ochrophaeus* were used in this experiment. The experiment was completed in two sets. Each set had a total of 15 animals with 5 animals in each treatment group with body mass evenly distributed among treatments. Animals were exposed for 4 days to 1 of 3 solutions: synthetic Whiteley water at 4,557 ppm TDS, 2,276 ppm TDS, and 569 ppm TDS (Table 2). Animals were monitored daily for signs of moribundity. Animals were considered moribund if they had no righting response when placed on their backs. Animals were not fed or weighed during this study.

Rationale: Synthetic Whiteley water was used in this experiment because of the effect on survival observed in the behavioral experiments. Because significant effects on survival were observed in synthetic Whiteley water at approximately 2,000 ppm, I expected that doubling the TDS would provide an EC50. Synthetic Whiteley water was tested at three concentrations in order to develop an EC50. The effect on survival occurred quickly in the behavioral experiments; therefore, an acute toxicity test was performed.

3.9 Chronic Toxicity Experiment

Thirty- nine male *D. ochrophaeus* were used in this study. The animals used in this study were not used in any previous study and were collected from the field approximately one month prior to laboratory testing. Animals were exposed for 14 days to the same solutions as the acute toxicity experiment: synthetic Whiteley water at 4,557 ppm TDS, 2,276 ppm TDS, and 569 ppm TDS. Body mass was evenly distributed among treatments. Animals were monitored daily for signs of moribundity. Body mass was measured before and after the experiment. Animals were not fed or weighed during this study.

Rationale: The purpose of this experiment was to determine an EC50 of TDS using animals not used in prior tests. The solutions used in this test were the same solutions used in the previous acute toxicity experiment. The exposure time was expanded to understand the toxicity of chronic exposure.

3.10 Statistical Analysis

Statistical analyses were done using PAWS Statistics 18 (SPSS Inc.). Differences in survival were analyzed using a Log Rank (Mantel-Cox) Test. Data for the moribund animals were excluded from analysis of locomotory activity, feeding, and body mass. T-tests were used to analyze locomotory activity because the previous study indicated a significant effect of treatment on locomotory activity. The sum of flies eaten was analyzed with a Kruskal-Wallis Test and followed with a Mann-Whitney Test. Relationships between body mass, feeding, and activity were analyzed with Pearson correlations. Changes in body mass were assessed with repeated measures ANOVA. Data that were analyzed with parametric statistics were normally distributed with homoscedastic variance.

Synthetic Spring water	Na_2SiO_3 x $9H_2O$	NaCl	Na_2SO_4	$CaCL_2$	$MgCl_2$	$FeCl_3$ x $6H_2O$	Expected TDS
g/l	0.0349	0.012	0.006	0.0065	0.0035	0.0066	70 ppm
Synthetic Whiteley water	Na_2SO_4	NaCl	$MgSO_4$	$CaCL_2$	KCl	$SrCl_2$ x $6H_2$	Expected TDS
g/l	1.52	0.184	0.198	0.276	0.0082	0.0053	2191 ppm
Synthetic Blacklick water	$BaBr_2$	$SrBr_2$	$CaCl_2$	$SrCl_2$	$MgSO_4$	$MgCl_2$	Expected TDS
g/l	0.0059	0.475	15.4	3.624	0.468	0.633	61000 ppm
Synthetic Blacklick water (Continued)	KCl	LiCl	$FeCl_3$ x $6H_2O$	NaCL			
g/l	0.297	0.279	0.0675	39.83			
Synthetic Spring water with elevated Na_2SO_4	Na_2SiO_3 x $9H_2O$	NaCl	Na_2SO_4	$CaCL_2$	$MgCl_2$	$FeCl_3$ x $6H_2O$	Expected TDS
g/l	0.0349	0.012	2.904	0.0065	0.0035	0.0066	3000 ppm

Table 1: Recipes for synthetic solutions used in all experiments. Synthetic solutions were created by dissolving salts (g/l) into distilled and deionized water. The recipe for synthetic spring water is derived from (Presnell, 1997). Synthetic Whiteley water and Synthetic Blacklick water recipes were created to represent ion compositions found in field samples (Pascuzzi, 2012). Expected TDS is the sum of the masses of the ions. The pH of each solution was adjusted to approximately 8.00 by adding HCL or NaOH. Solutions were diluted to desired TDS using distilled water.

Behavioral Experiment 1	**TDS**	**CL^-**	**SO_4^{2-}**	**Na**	**Ca**	**Mg**	**Sr**	**Br^-**	**K**	**Si**	**F^-**	**NO_3^-**
Synthetic Whiteley water (ppm)	1957	262	928	611	104	43	2	n/d	6	n/d	n/d	n/d
% Composition		13	47	31	5	2	0	n/a	0	n/a	n/a	n/a
Synthetic Blacklick water (ppm)	891	461	4	295	116	5	1	5	3	n/d	n/d	n/d
% Composition		52	0	33	13	1	0	1	0	n/a	n/a	n/a
Behavioral Experiment 2	**TDS**	**CL^-**	**SO_4^{2-}**	**Na**	**Ca**	**Mg**	**Sr**	**Br^-**	**K**	**Si**	**F^-**	**NO_3^-**
Synthetic Whiteley water (ppm)	1479	194	755	430	68	26	n/d	n/d	n/d	n/d	n/d	n/d
% Composition		13	51	29	5	2	n/a	n/a	n/a	n/a	n/a	n/a
Synthetic elevated Sodium Sulfate water (ppm)	1332	18	872	421	13	n/d	n/d	n/d	n/d	9	n/d	n/d
% Composition		1	65	32	1	n/a	n/a	n/a	n/a	1	n/a	n/a
Toxicity Experiments	**TDS**	**CL^-**	**SO_4^{2-}**	**Na**	**Ca**	**Mg**	**Sr**	**Br^-**	**K**	**Si**	**F^-**	**NO_3^-**
Synthetic Whiteley water (ppm)	4557	761	2518	959	166	68	4	n/d	81	n/d	n/d	n/d
% Composition		17	55	21	4	1	0	n/a	2	n/a	n/a	n/a
Control	**TDS**	**CL^-**	**SO_4^{2-}**	**Na**	**Ca**	**Mg**	**Sr**	**Br^-**	**K**	**Si**	**F^-**	**NO_3^-**
Synthetic Spring water (ppm)	42	n/d	7	14	3	n/d	n/d	n/d	n/d	6	5	7
% Composition		n/a	16	34	8	n/a	n/a	n/a	n/a	14	12	16

Table 2: Ion analysis of synthetic solutions used in all experiments. Synthetic solutions were created to represent TDS levels found in the field. The synthetic spring water derived from (Presnell, 1997) was used as a control in all experiments and is representative of the controls used in (Pascuzzi, 2012). Solutions were analyzed for anion concentrations using Dionex ICS-1100 Ion Chromatography System and cations using Perkin-Elmer NexION 300x IC-ICP-MS. The percent composition is listed to highlight the differences in chloride and sulfate content of each solution. All synthetic Whiteley solutions are at least 47% sulfate and between 13-17% chloride. The synthetic Blacklick solution is 0% sulfate and 52% chloride.

Chapter 4: Results

4.1 Behavioral Experiment 1: Exposure to sulfate enriched TDS (synthetic Whiteley water at 1,957 ppm) and chloride enriched TDS (synthetic Blacklick water at 891 ppm).

4.12 Survival

Survival of animals exposed to synthetic Whiteley water at 1,957 ppm TDS was significantly reduced compared to the other treatments ($\chi^2_2 = 11.45$, P = 0.003) ; (Figure 1). 33% of animals exposed to synthetic Whiteley water became moribund in the first 6 days of exposure. Despite removal from the study, moribund animals did not recover and were euthanized. A correlation between body mass and survival was observed (r = 0.785, P = 0.001). All but one animal exposed to synthetic Whiteley water with an initial body mass over 1.2g became moribund (Figure 2). All animals exposed to synthetic Blacklick water (891 ppm TDS) and synthetic spring water (49 ppm TDS) survived.

4.13 Locomotory Activity

Animals chronically exposed to Whiteley water moved less than the control animals ($t_{23} = 2.748$, P =0.011). Activity levels of animals exposed to Blacklick water were no different than the activity in control animals ($t_{28} = 0.723$, P = 0.476) ; (Figure 3). No correlation between initial mass and activity was observed (r = 0.106, P = 0.513).

4.14 Feeding

Treatment had an effect on the number of flies eaten ($\chi^2_2 = 7.914$, P = 0.019) ; (Figure 4). Animals exposed to Blacklick water ate fewer flies than did controls (U = 44.00, P = 0.005). In contrast, there was no difference between Whiteley treatment and the control treatment (U = 66.00, P = 0.643). Interestingly, of the surviving animals exposed to Whiteley water, larger animals ate fewer flies (r = -0.928, P < 0.001) ; (Figure 5). Similar relationships between body size and feeding were not observed in the other treatment groups (Blacklick: r = 0.431, P = 0.108, n = 15).

4.15 Body Mass

Due to the fact that larger animals in the Whiteley treatment group did not survive, body mass of survivors was marginally significantly less than the other groups ($F_{2, 37} = 2.612$, P = 0.087). Otherwise, body mass remained relatively similar throughout the study until the last measurement, when body mass dropped in the control animals relative to Blacklick and Whiteley animals (interaction between treatment and body mass: $F_{2, 37} = 6.606$, P = 0.004); (Figure 6).

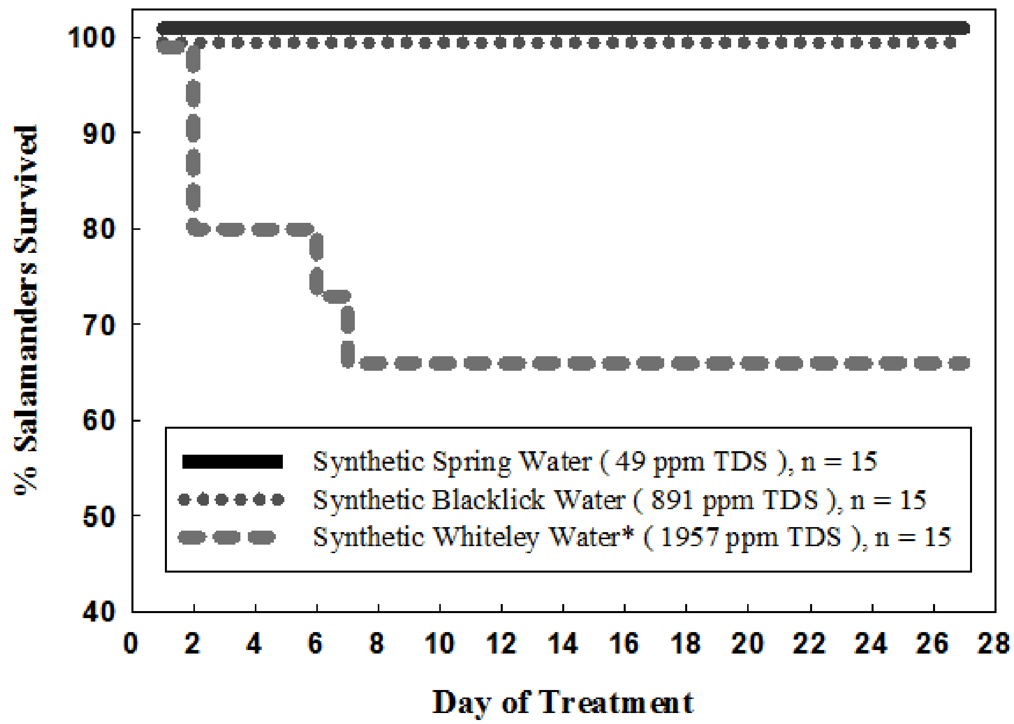

Figure 1: Percent of salamanders that survived after exposure for 27 days to water of different TDS composition and concentration. Within 6 days of exposure, 33% of animals exposed to synthetic Whiteley water became moribund. All animals exposed to synthetic Blacklick water and synthetic spring water survived. *Animals exposed to synthetic Whiteley water had reduced survival compared to the other treatments (χ^2_2 = 11.45, P = 0.003).

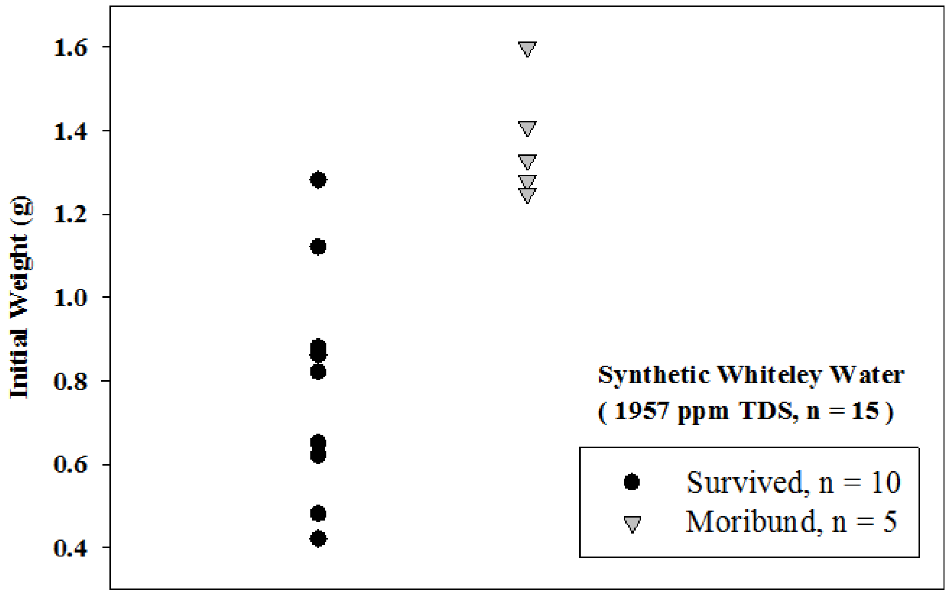

Figure 2: Body mass of animals exposed to synthetic Whiteley water (1,957 ppm TDS). A negative correlation between body mass and survival was observed ($r = -0.785$, $P = 0.001$). Animals that became moribund were the larger animals.

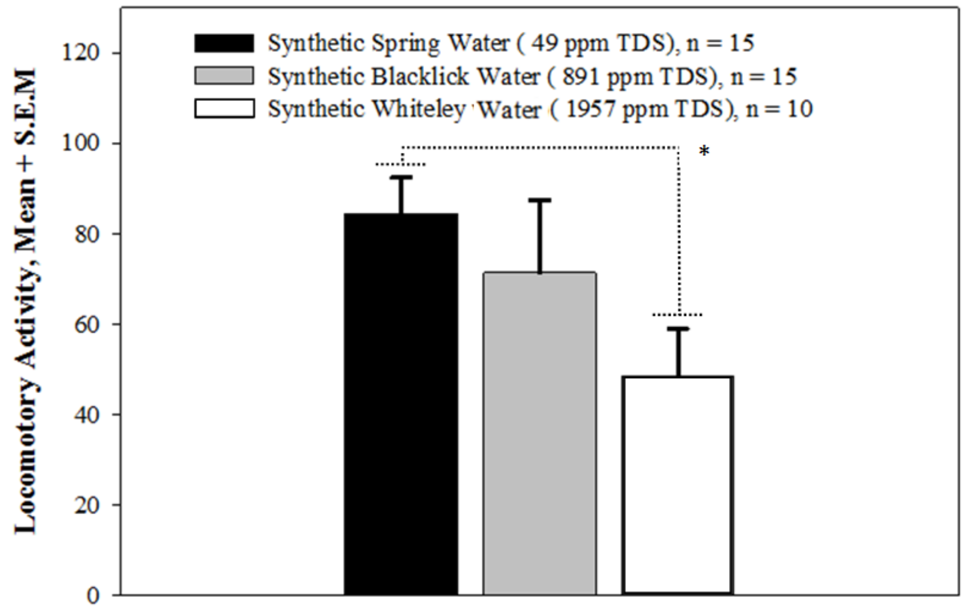

Figure 3: Locomotory activity of salamanders exposed to water of different TDS. * Male salamanders exposed for 27 days to synthetic Whiteley water (1,957 ppm TDS) moved less than controls exposed to synthetic spring water (49 ppm TDS); (t_{23} = 2.748, P = 0.011). Activity levels of animals exposed to Blacklick water were no different than activity in control animals (t_{28} = 0.723, P = 0.476).

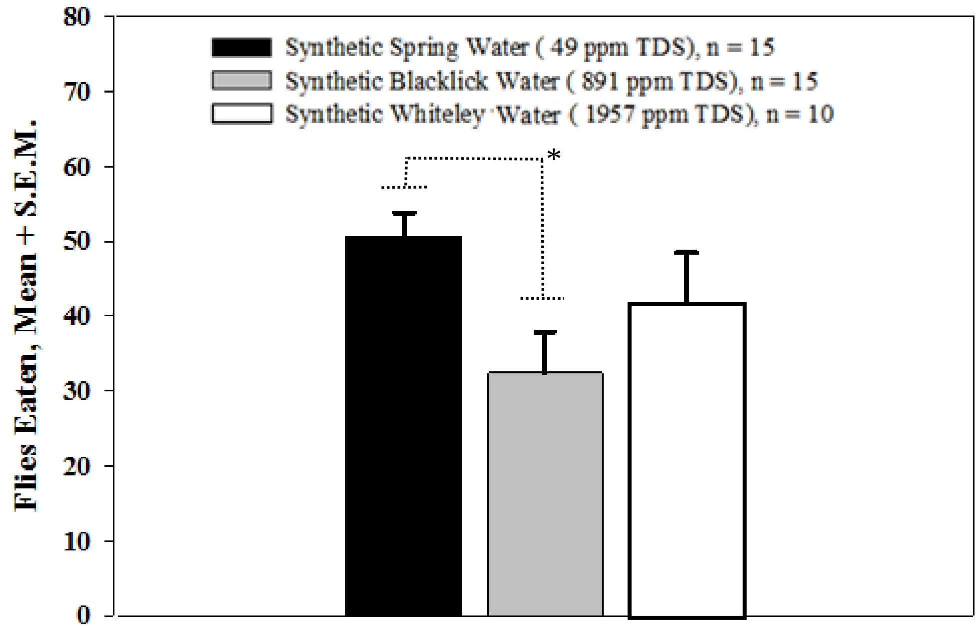

Figure 4: Sum of flies eaten after exposure to water of different TDS over the course of 6 feedings (10 flies per feeding). *Males exposed to synthetic Blacklick water (891 ppm TDS) ate fewer flies than control animals exposed to synthetic spring water (49 ppm TDS); (U = 44.00, P = 0.005). There was no difference in feeding between animals exposed to synthetic Whiteley water and the control (U = 66.00, P = 0.643).

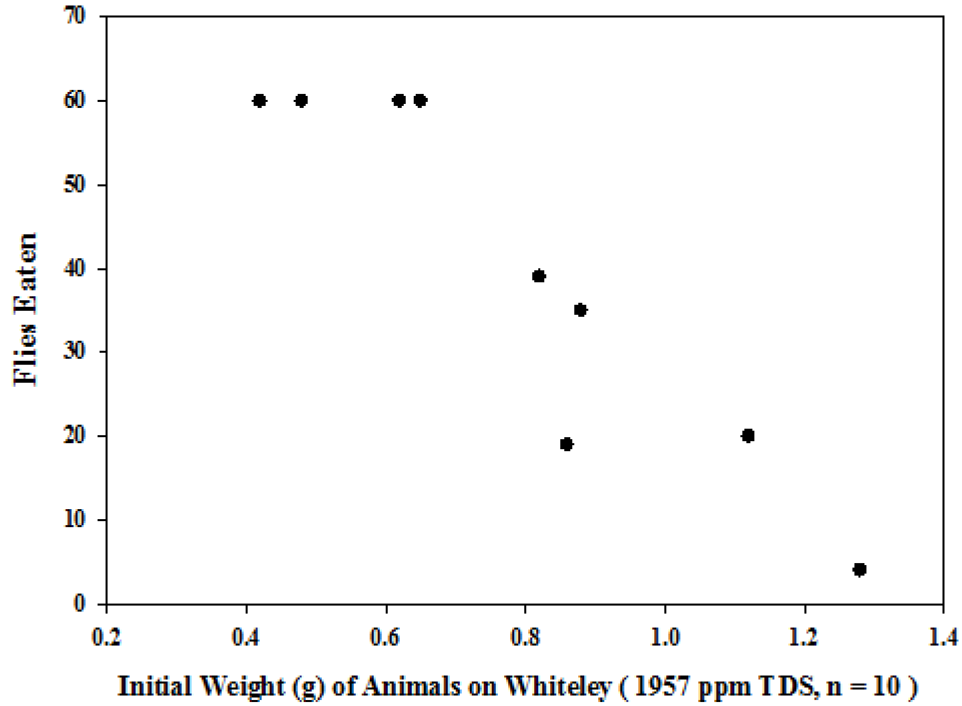

Figure 5: Relationship of body mass and flies eaten in the Whiteley treatment. Of the surviving animals within the Whiteley treatment, initial body mass was negatively correlated with amount of flies eaten. Salamanders with a higher initial body mass ate fewer flies ($r = -0.928$, $P < 0.001$, $n = 10$).

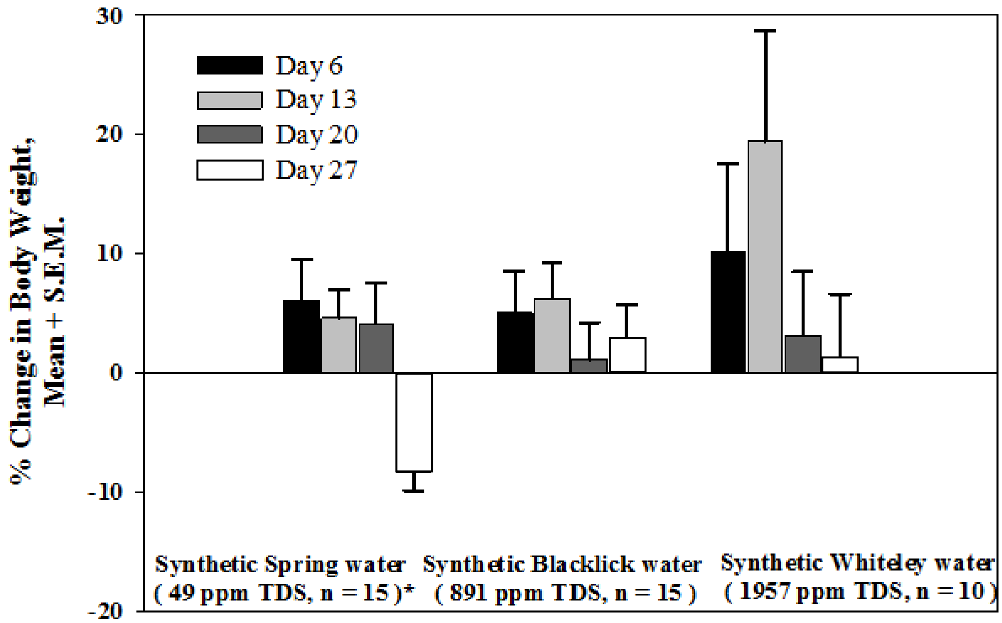

Figure 6: Body mass changes over time. Body mass remained relatively similar throughout the study until the last measurement, when body mass dropped in the control animals relative to Blacklick and Whiteley animals (interaction between treatment and body mass: $F_{2, 37} = 6.606$, $P = 0.004$*).

4.2 Behavioral Experiment 2: Exposure to TDS enriched in sulfate (synthetic Whiteley water at 1,479 ppm and synthetic spring water with elevated sodium sulfate at 1,332 ppm)

4.21 Survival

No significant effect of TDS on survival was observed when animals were exposed to synthetic solutions with elevated TDS; (χ^2_1 = 1.553, P = 0.21). However, some animals did become moribund within the first 10 days of exposure: 7% of animals exposed to synthetic Whiteley water (1,479 ppm TDS) and 13% of animals exposed to synthetic spring water with elevated sodium sulfate (1,332 ppm TDS) became moribund (Figure 7).

4.22 Locomotory Activity

No effect of treatment on locomotory activity was observed (χ^2_5 = 4.20, P = 0.52) ; (Figure 8).

4.23 Feeding

No effect of treatment on feeding activity was observed (t_{23} = 0.736, P = 0.468) (Figure 9). A correlation between body mass and feeding was observed in animals exposed to synthetic spring water with elevated sulfate (1,332 ppm TDS); Larger animals ate less flies over the course of 6 feedings (r = -0.731, P = 0.004).

4.24 Body Mass

No effect of treatment on body mass was observed ($F_{2,39}$ = 0.135, P = 0.874) ; (Figure 10).

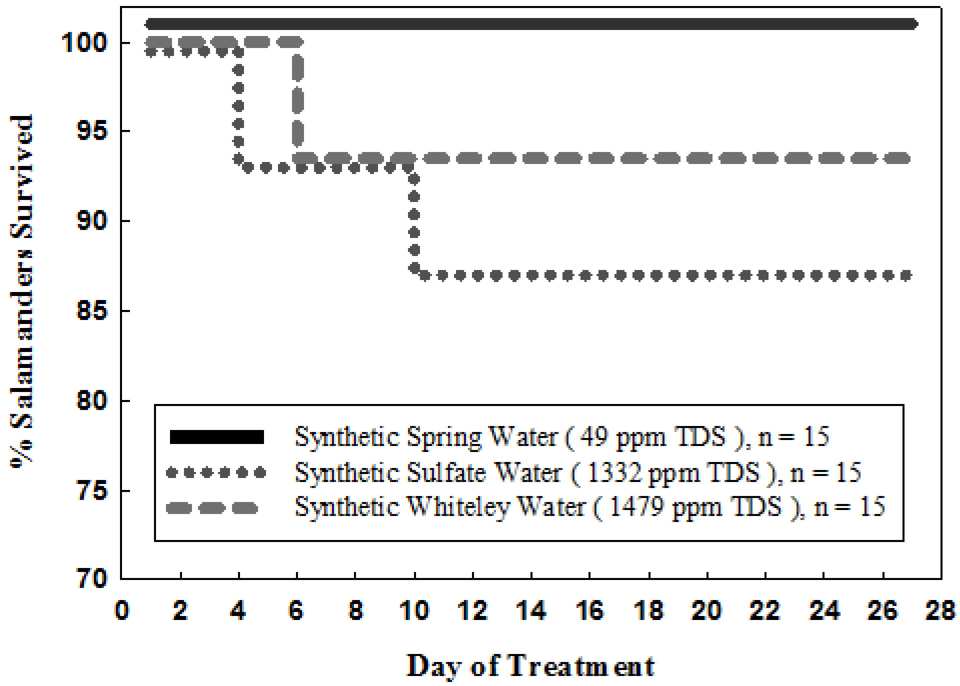

Figure 7: Percent survival after exposure for 27 days to water of different TDS composition and amount. No significant effect of TDS on survival was observed when animals were exposed to waters with elevated TDS; (χ^2_1 = 1.553, P = 0.21). Of the animals exposed to synthetic sulfate water (1,332 ppm TDS), 13% became moribund within 6 days of exposure. Of the animals exposed to synthetic Whiteley water (1,479 ppm TDS), 7% became moribund within 10 days of exposure.

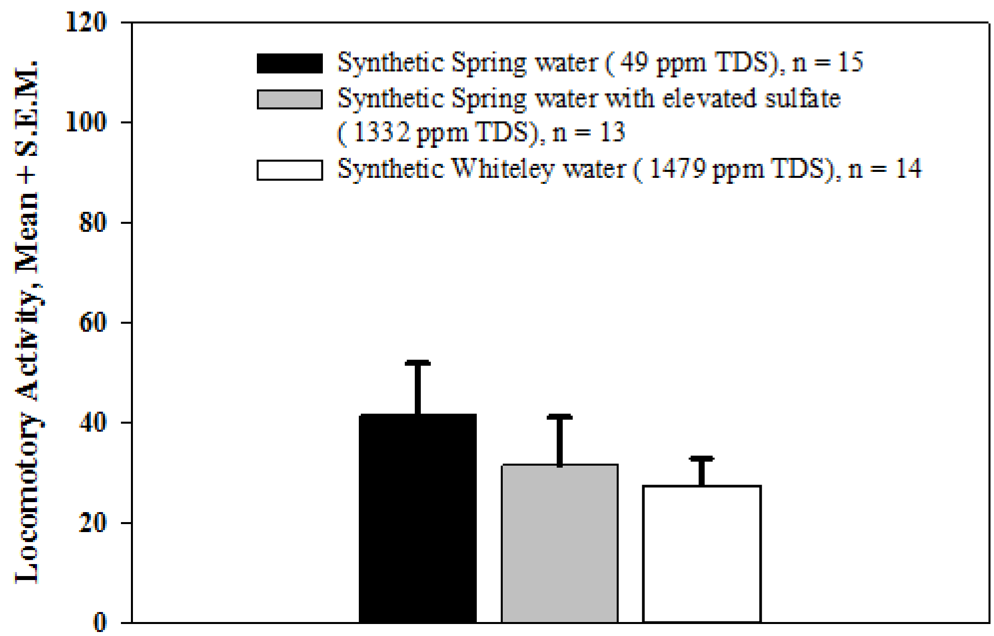

Figure 8: Locomotory activity of salamanders exposed to water of different TDS. No effect of treatment on activity was observed in animals chronically exposed to synthetic Whiteley water (1,479 ppm TDS), synthetic spring water with elevated sodium sulfate (1,332 ppm TDS), and synthetic spring water (49 ppm TDS).

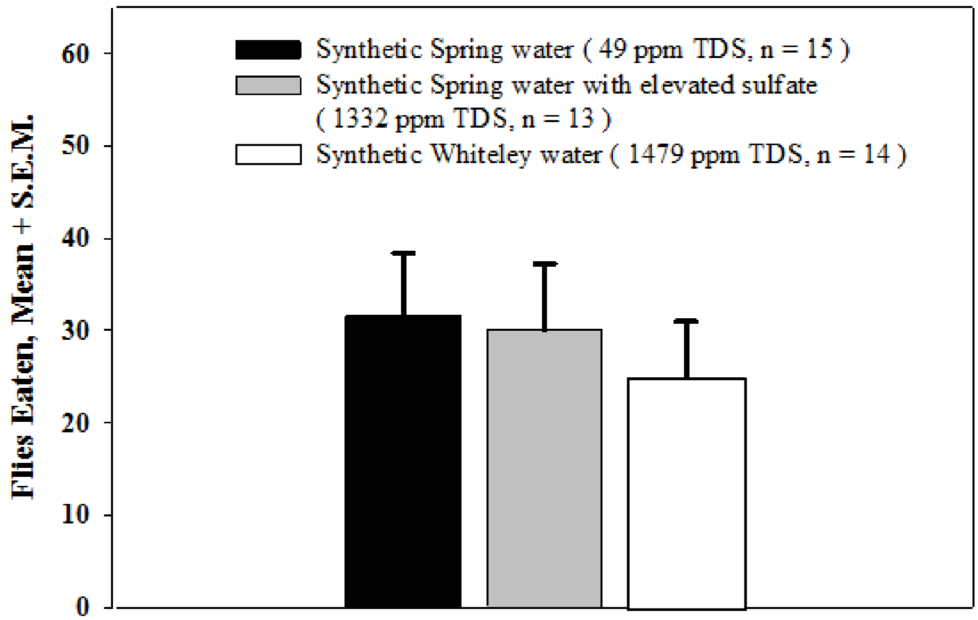

Figure 9: Sum of flies eaten after exposure to water of different TDS over the course of 6 feedings (10 flies per feeding). No effect of treatment on feeding activity was observed when animals were chronically exposed to synthetic Whiteley water (1,479 ppm TDS), synthetic spring water with elevated sodium sulfate (1,332 ppm TDS), and synthetic spring water (49 ppm TDS).

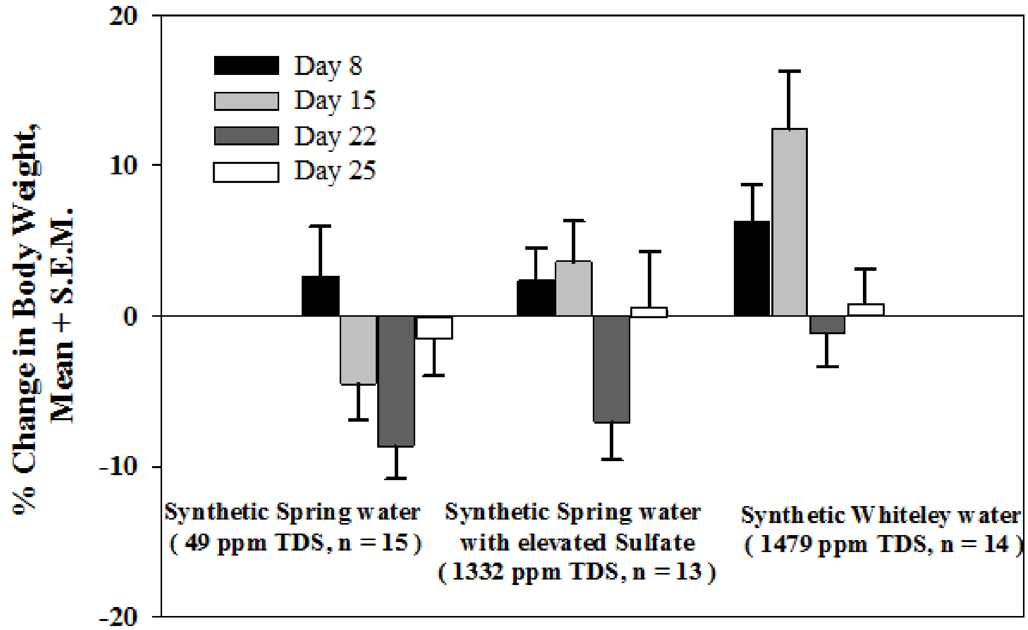

Figure 10: Animals were exposed to synthetic Whiteley water (1,479 ppm TDS), synthetic spring water with elevated sulfate (1,332 ppm TDS), and synthetic spring water (49 ppm TDS) for 26 days. No significant changes in body mass occurred across treatment groups ($F_{2, 39}$ = 0.135, P = 0.874).

4.3 Acute Toxicity Experiment

4.31 Survival

A significant effect of treatment on survival was observed ($\chi^2_2 = 6.622$, $P = 0.036$) (Figure 11). Three animals exposed to synthetic Whiteley water containing 4,557 ppm TDS became moribund within 4 days and were euthanized. A Pearson Correlation test showed a significant effect of mass on survival ($r = 0.908$, $P < 0.01$). Animals with a higher initial body mass were more likely to become moribund when exposed to synthetic Whiteley water at 4,557 ppm TDS (Figure 12).

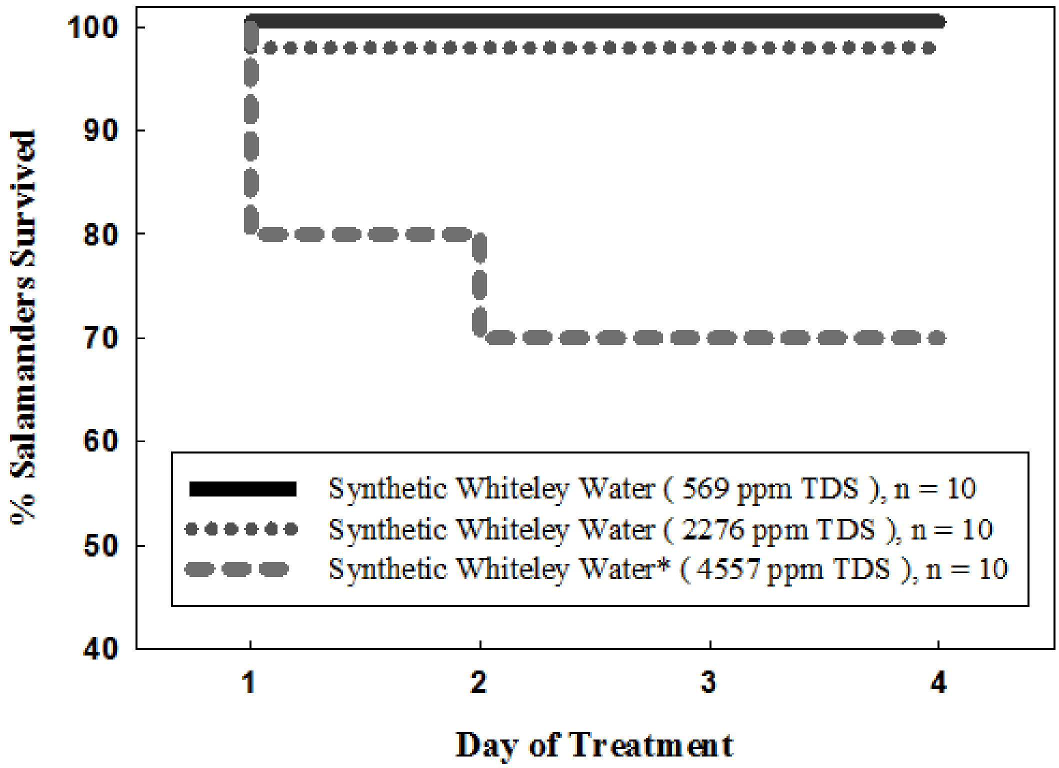

Figure 11: Percent survived after acute exposure to different TDS. *Survival of male salamanders exposed synthetic Whiteley water at 4,557 ppm TDS was decreased compared to the control ($\chi^2_2 = 6.622$, $P = 0.036$). Within 2 days of exposure, 30% of animals exposed to synthetic Whiteley water (4,557 ppm TDS) became moribund and were euthanized.

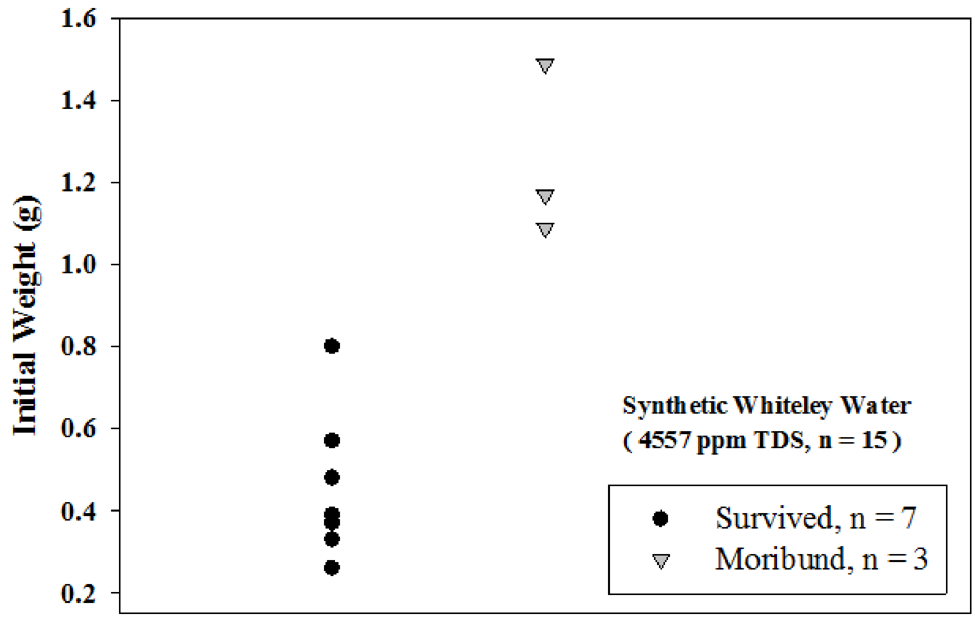

Figure 12: Body mass compared to survival in sodium sulfate enriched TDS. *Animals with a higher initial body mass (over 1 g) were more likely to become moribund when exposed to synthetic Whiteley water at 4,557 ppm TDS ($P < 0.01$, $n = 10$).

4.4 Chronic Toxicity Experiment

4.41 Survival

Within 3 days, 17% of animals exposed to synthetic Whiteley water at 4,557 ppm TDS became moribund; however, no significant effect of treatment on survival was observed ($\chi^2_2 = 3.707$, $P = 0.157$); (Figure 13). Thus, an EC50 could not be determined for animals exposed to synthetic Whiteley water at various TDS levels.

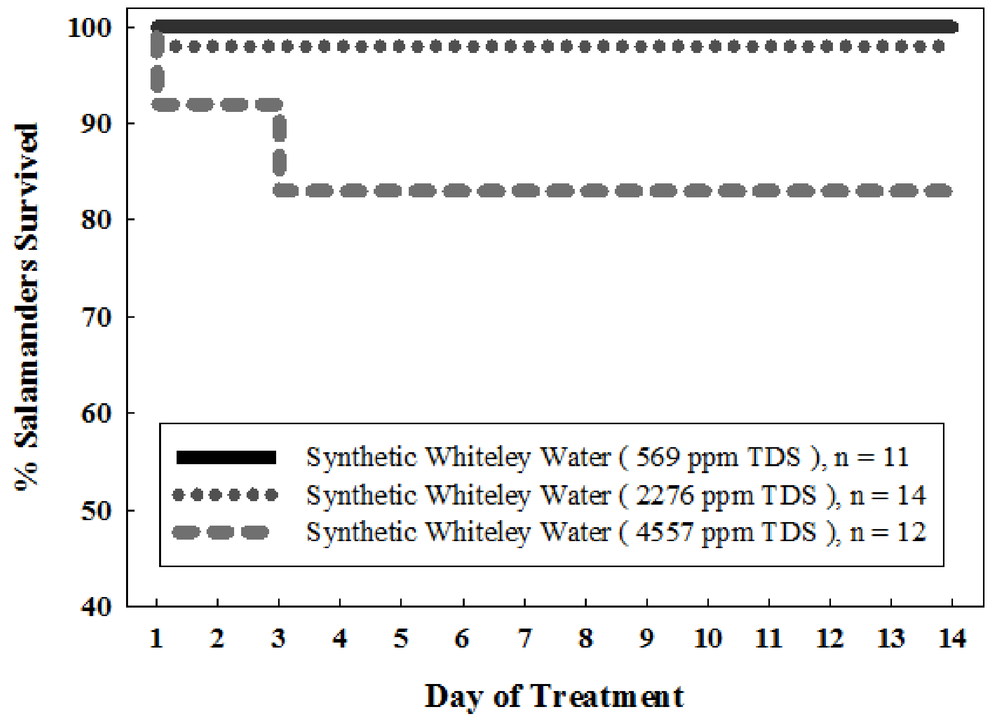

Figure 13: Survival of animals exposed to different TDS. Within 3 days of exposure, 17% of animals exposed to synthetic Whiteley water at 4,557 ppm TDS became moribund; however, no effect of treatment on survival was observed ($\chi^2_2 = 3.707$, P = 0.157).

Chapter 5 Discussion

5.1 Behavioral Responses

The results of behavioral experiment 1 support my hypothesis that elevated TDS has sublethal effects on the Allegheny Mountain Dusky Salamander. However, the behavioral effects differed due to the amount of TDS. Locomotory activity was reduced when animals were exposed to synthetic Whiteley water at around 2,000 ppm TDS and no effect of locomotory activity was observed in animals exposed to synthetic Blacklick water at around 1,000 ppm. The reduction in locomotory activity found in this study confirms the results of a previous study of *D. ochrophaeus* and synthetic Whiteley water at 1,000 ppm TDS. When exposed to synthetic Whiteley water at 1,000 ppm TDS, male *D.ochrophaeus* had decreased activity compared to controls (Pascuzzi, 2012).

I also found that feeding behavior was reduced in animals exposed to synthetic Blacklick water at 891 ppm TDS with chloride as the dominant anion (Figure 4). A reduction in feeding behavior may inhibit an animal to maintain proper ion balance. A previous study of Jefferson Salamander larvae found a significant reduction in feeding when animals were exposed to high conductivity (Chambers, 2011). In behavioral experiment 1, animals exposed to synthetic Blacklick water at around 1,000 ppm TDS ate fewer flies than did control animals (Figure 4). In contrast, no change in feeding was observed in animals exposed to synthetic Whiteley water at around 2,000 ppm TDS. However, of the surviving animals exposed to synthetic Whiteley water, the larger animals ate fewer flies (Figure 5). The larger animals within the Whiteley group became moribund and were removed from the analysis; therefore, the feeding results may be nonsignificant because larger bodied animals were not fully represented in the statistical analysis. Thus, it appears that feeding may be reduced in the presence of elevated TDS.

Body mass remained relatively constant throughout the experiment until the end of the experiment, when body mass dropped in the control animals. The decrease in body mass in the control group was probably a result of dehydration due to evaporative water loss. Salt water evaporates less than fresh water, so the moisture levels in the control chambers were likely drier than chambers with elevated TDS. A previous study from my laboratory also observed that body mass decreased in control animals more than in experimental animals (Pascuzzi, 2012). In order to avoid this, my experiments were conducted in a humidity controlled room. Despite my efforts to maintain humidity there was some evidence that by the end of the experiment, the control chambers were starting to dry out. Because the difference in body mass was not evident until the end of the experiment, behavioral results are not influenced by this confounding factor.

The purpose of behavioral experiment 2 was to further investigate the effect of the sulfate on salamander behavior. Sodium Sulfate was added to synthetic spring water to create a solution similar to the synthetic Whiteley water in overall sulfate while removing the chloride that was found in Whiteley water (Table 2). The purpose of this study was to compare the effects of the sodium sulfate solution with the Whiteley solution in order to understand if the sulfate anion is the anion of concern. The TDS of the synthetic Whiteley water used in this experiment (1,479 ppm TDS) was less than the synthetic Whiteley water used in behavioral experiment

1 (1,957 ppm TDS). No significant effects of treatment on locomotory activity (Figure 8), or feeding (Figure 9) were observed. The lack of a decrease in activity during the second behavioral experiment as well as the second survival experiment may be due to the low levels of activity in the controls. Control levels of activity were ½ those of the behavioral experiment 1, perhaps due to the fact that these experiments were done during the winter months, when activity tends to be lower (Woodley, personal observation) ; (Shealy, 1975). When activity is low to begin with, it is difficult to discern a further decrease due to treatment.

It is unclear from this study whether effects differ due to ion composition of the TDS or from the amount of TDS. Both sulfate and chloride are present in both solutions, however, their concentrations vary. The solution high in sulfate (Whiteley) had the strongest effect on survival. This effect was not seen in the solution with high levels of chloride (Blacklick), although this solution was half the amount of TDS of Whiteley. More research is needed to understand the impacts of ion composition of TDS on amphibian behavior.

This study provides evidence that TDS at relatively low levels (1,000 – 2,000 ppm) affects the behavior of male *D.ochrophaeus*. Locomotory activity has been used in studies to measure sublethal effects of an environmental contaminant on an organism (Burke et al., 2010; Denoël et al., 2013). Sublethal effects may be detrimental to the survival of the species; a decrease in locomotory activity may limit the animal's ability to capture prey, avoid predators, and reproduce. Reduced feeding may impact osmoregulation and nutrient uptake (Shoemaker and Nagy, 1977). Future studies are needed to provide the link between locomotory activity levels and mating in semi-terrestrial salamanders in order to understand the impacts of reduced locomotory activity on fitness.

5.2 Effects on Survival

The first set of experiments was designed to test for sublethal effects of TDS and not lethal effects. However, exposure to synthetic Whiteley water at 1,957 ppm TDS resulted in 33% of animals becoming moribund (Figure 1). My results are similar to results found in a study of Whiteley Creek water collected from the field at 2,000 ppm TDS ; Exposure to Whiteley Creek water for 8 days was lethal to 20% of male *D.ochrophaeus* (Pascuzzi, 2012).

Because of the significant effect of Whiteley water at 1,957 ppm TDS on survival seen in behavioral experiment 1, I expected to see reduced survival in both the 2,276 ppm TDS and the 4,557 ppm TDS treatments in the acute survival test. However, reduced survival was only observed in the animals exposed to 4,557 ppm TDS (Figure 10) precluding my ability to conduct an EC50. Several factors may have attributed to inability to determine an EC50 in the first test of survival. The animals used in the acute survival test were exposed to elevated TDS over the course of many experiments, therefore, may have developed a tolerance for the TDS. Also, because many of the larger animals had become moribund in the previous experiments, the sample size and the average body mass was smaller and could have had an effect on the results.

The toxicity study was repeated using animals more recently collected from the field that had not been exposed to TDS in any prior experiments. This ensured a more even body mass distribution based on what was found in the field and that animals would not have built a tolerance for the TDS solutions used in the laboratory. No significant reduction in survival was observed in the chronic survival test; however, statistical significance would have been reached if one more animal became moribund.

The failure to determine an EC50 for synthetic Whiteley water revealed that effects of TDS are variable between individuals and populations. A strong effect of TDS at 1,957 ppm TDS showed effects on survival within the largest males in the group. The same solution at 4,557 ppm TDS had no significant effect on survival within a different population of animals, however, the trend was in the right direction.

5.3 Body Mass Effects

A significant correlation between survival and body mass was found in the first behavioral study. Larger animals greater than 1.2 g were more likely to become moribund after exposure to TDS at 1,957 ppm. A significant body size effect was also observed in the first test of survival. The largest animals (> 1.0g) exposed to 4,557 ppm TDS became moribund (Figure 11). My results show that body mass has an effect on the individual's response to elevated TDS. Survival and reproduction is generally higher among amphibians with larger body size (Semlitsch et al., 1988). Thus, a disproportionate effect on survival to animals with higher body mass may have serious consequences to the demographic structure of a population and community. Future studies should include tests on other species in the genus *Desmognathus*. The salamanders used in this study, *D. ochrophaeus*, are the more terrestrial and smaller bodied salamanders in the genus (Bruce, 2013). It would be useful to determine the effects of elevated TDS on salamanders in the same genus that are generally larger bodied and more aquatic such as *D. quadramaculatus, D. monticola, and D. ocoee*.

The effect of body mass on survival was an interesting find of this study; however, there may be differences in the response of animals due to their percentage of body fat opposed to muscle mass. Future studies on impacts of elevated TDS should include these measurements in the analysis.

5. 4 Conclusions and Future Directions

The elevated levels of TDS in streams in Western Pennsylvania due to mine drainage and UNGD are of concern to aquatic and semi-aquatic species. The overall results of this study show that elevated levels of TDS attributed to UNGD and mine drainage affect the behavior and survival of the Allegheny Mountain Dusky Salamander. The effects observed, however, vary with the concentration of TDS as well as across individuals, with larger animals being more susceptible.

This study examined the effects of elevated TDS resulting from mine drainage and UNGD separately. The use of abandoned mine drainage (AMD) in UNGD will result in the mixing of the two waste streams and possibly increase the load of sulfate and chloride released into streams from wastewater treatment facilities. Future studies should investigate the effects of elevated TDS with both elevated sulfate and elevated chloride to determine any negative synergistic effects of combining UNGD wastewater and AMD.

Water quality standards that are developed for aquatic life utilize studies on toxicity in order to determine acceptable levels of various pollutants. However, toxicity may vary between species, populations, communities, and individuals. This study shows the variability in the individual and population level response to elevated TDS. This study may be expanded upon by finding the EC50 of water with elevated sulfate as well as elevated chloride for *D.ochrophaeus* and other species of Plethodontid salamander. Numerous studies on the effects of road salts on amphibians reveal vast differences in the effects on different species at various life stages (Hopkins et al., 2013). It would be useful to understand the differences in toxicity as well as sublethal effects between species in the same family, as well as among life stages. When determining pollutant levels that are safe for organisms, chronic and sublethal effects should also be considered along with toxicity. The sublethal effects of TDS observed in this study may have negative consequences to the overall survival and fitness of the species.

Literature Cited

1. Allan, J.D. and M.M. Castillo. 2007. Stream Ecology: Structure and Function of Running Waters Second Edition. Springer.

2. Beebee, T.J.C. and R.A. Griffiths. 2005. Review: The amphibian decline crisis: A watershed for conservation biology? Biological Conservation 125:271-286.

3. Blaustein, A.R., and J.M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5: 597-608.

4. Brand, A.B., Snodgrass, J.W., Gallagher, M.T., Casey, R.E., Van Meter, R. 2010. Lethal and sublethal effects of embryonic and larval exposure of *hyla versicolor* to stormwater pond sediments. Archives of Environmental Contamination and Toxicology 58: 325-331.

5. Brix, K.V., Gerdes, R., Curry, N., Kasper, A., Grosell, M. 2010. The effects of total dissolved solids on egg fertilizations and water hardening in two salmonids-Arctic Grayling (*Thymallus arvticus*) and Dolly Varden (*Salvelinus malma*). Aquatic Toxicology 97:109-115.

6. Bruce, R. 2013. Size-mediated tradeoffs in life-history traits in dusky salamanders. Copeia 2013(2):262-267.

7. Burke, J.N., Bergeron, C.M., Todd,B.D., Hopkins, W.A. 2010. Effects of mercury on behavior and performance of northern two-lined salamanders (*Eurycea bislineata*). Environmental Pollution 158:3546-3551.

8. Canedo-Arguelles, M., Kefford, B.J., Piscart, C., Prat, N., Schafer, R.B., Schulz, C. 2013. Salinisation of rivers: An urgent ecological issue. Environmental Pollution 173:157-167.

9. Chambers, D.L. 2011. Increased conductivity affects corticosterone levels and prey consumption in larval amphibians. Journal of Herpetology 45(2):219-223.

10. Collins, S.J. and R.W. Russell. 2009. Toxicity of road salt to Nova Scotia amphibians. Environmental Pollution 157: 320-324.

11. Cravotta, C.A. 2008. Dissolved metals and associated constituents in abandoned coal-mine discharges, Pennsylvania, USA. Part: Constituent quantities and correlations. Applied Geochemistry 23(2):166-202.

12. Cross, N.L. and R.P. Elinson. 1980. A fast block to polyspermy in frogs mediated by changes in the membrane potential. Developmental Biology 75(1):187-198.

13. Denoël, M., Libon, S., Kestemont,P., Brasseur, C., Focant, J., De Pauw, E. 2013. Effects of a sublethal pesticide exposure on locomotor behavior: A video-tracking analysis in larval amphibians. Chemosphere 90:945-951.

14. Denoël, M., Bichota, M., Ficetolab, G.F., Delcourta, J., Ylieffa, M., Kestemontc,P., Poncin, P. 2010. Cumulative effects of road de-icing salt on amphibian behavior. Aquatic Toxicology 99:275-280.

15. Egea-Serrano, A., and M. Tejedo. 2014. Contrasting effects of nitrogenous pollution on fitness and swimming performance of Iberian waterfrog, *Pelophylax perezi* (Seoane, 1885), larvae in mesocosms and field enclosures. Aquatic Toxicology 146:144-153.

16. Ferrar, K.J., Michanowicz, D.R., Christen, C.L., Mulcahy, N., Malone, S.L., Sharma, R.K. 2013. Assessment of effluent contaminants from three facilities discharging Marcellus shale wastewater to surface waters in Pennsylvania. Environmental Science and Technology 47: 3472-3481.

17. Goetsch, P.A. and C.G. Palmer. 1997. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. Archives of Environmental Contamination and Toxicology 32: 32-41.

18. Goodfellow, W.L., Ausley,L.W., Burton, D.T., Denton,D.L., Dorn, P.B., Grothe, D.R., Heber, M.A., Norberg ‑ King, T.J., Rodgers, J.H. 2000. Annual Review: Major ion toxicity in effluents: A review with permitting recommendations. Environmental Toxicology and Chemistry 19:1:175-182.

19. Griffith, M.B., Norton, S.B., Alexander, L.C., Pollard, A.L., LeDuc, S.D. 2012. The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the central Appalachians: A Review. Science of the Total Environment: 417-418: 1-12.

20. Haluszczak, L.O., Rose, A.W., Kump, L.R. 2013. Geochemical evaluation of flowback brine from Marcellus gas wells in Pennsylvania, USA. Applied Geochemistry 28: 55-61.

21. Hamer, A. J., and M.J. McDonnell. 2008. Amphibian ecology and conservation in the urbanizing world: A review. Biological Conservation 141:4232-2449.

22. Hem, J.D. 1986. Study and interpretation of the chemical characteristics of natural water: U.S. Geological Survey Water- Supply Paper 2254.

23. Hopkins, G.R., French, S.S., Brodie Jr., E.D. 2013. Increased frequency and severity of developmental deformities in rough-skinned newt (*Taricha granulosa*) embryos exposed to road deicing salts (NaCL & $MgCl_2$). Environmental Pollution 173: 264-269.

24. Hopkins, W.A. 2007. Amphibians as models for studying environmental change. Institute of Laboratory Animal Research Journal 48 (3): 270-277.

25. Karraker, N.E. 2008. Impacts of road deicing salts on amphibians and their habitats. Society for the Study of Amphibians and Reptiles. Urban Herpetology. J.C. Mitchell, R.E. Jung Brown, and B. Bartholomew, editors. Herpetological Conservation 3:211-223.

26. Karraker, N.E. 2007. Are embryonic and larval Green Frogs (*Rana clamitans*) insensitive to road deicing salt? Herpetological Conservation and Biology 2(1):35-41.

27. Kondash, A.J., Warner, N.R., Lahav, O., Vengosh, A. 2013. Radium and barium removal through blending hydraulic fracturing fluids with acid mine drainage. Environmental Science and Technology 47:24.

28. Kaushal, S.S, Groffmann, P.M., Likens, G.E., Belt, K.T., Stack, W.P., Kelly, V.R., Band, L.E., Fisher, G.T. 2005. Increased salinization of fresh water in the northeastern United States. Proceedings of the National Academy of Sciences of the United States of America 102 (38): 13517-13520.

29. Maleek R., Sullivan, P., Hoff, K.v.S., Baula, B., Hillyard, S.D. 1999. Salt sensitivity and hydration behavior of the toad, *Bufo marinus*. Physiology and Behavior 67(5): 739-745.

30. Pascuzzi, M. 2012. The effects of total dissolved solids on locomotory behavior and body weight of streamside salamanders, and a baseline survey of salamander diversity and abundance. (MS Thesis Duquesne University). Retrieved from http://digital.library.duq.edu/u?/etd,154119

31. Pennsylvania Code, ch. 93, § 93.6. General Water Quality Standards. Accessed 02 Sept 2012. http://www.pacode.com/secure/data/025/chapter93/chap93toc.html

32. Porter, K.R. and D.E. Hakanson. 1976. Toxicity of mine drainage to embryonic and larval boreal toads (*Bufonidae:Bufo Boreas*). Copeia 2: 327-331.

33. Presnell, J. and M. Schreibman. 1997. Humanson's Animal Tissue Techniques 5th Edition. The Johns Hopkins University Press.

34. Rahm, B.G., Bates, J.T., Bertoia, L.R., Galford, A.E., Yoxtheimer, D.A., Riha, S.J. 2013. Wastewater management and marcellus shale gas development: trends, drivers, and planning. Journal of Environmental Management: 120: 105-113.

35. Sams III, J.I. and K.M. Beer. 2000. Effects of coal-mine drainage on stream water quality in the Allegheny and Monongahela River basins-sulfate transport and trends. U.S. Department of the Interior and U.S. Geological Survey. Water Resources Investigations Report 99-4208.

36. Scannell, P.W. and L.L. Jacobs. 2001. Effects of total dissolved solids on aquatic organisms: A literature review. Alaska Department of Fish and Game Division of Habitat Restoration. Juneau, AK: 01-06.

37. Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum.* Ecology 69 (1): 184-192.

38. Shealy, R.M. 1975. Factors influencing activity in the salamanders *Desmognathus ochrophaeus* and *D. monticola* (Plethodontidae). Herpetologica 31 (1):94-102.

39. Shoemaker, V.H. and K.A. Nagy. 1977. Osmoregulation in amphibians and reptiles. Annual Reviews of Physiology 39:449-471.

40. Smith, P.N., Cobb, G.P., Godard-Codding, C., Hoff, D., McMurry, S.T., Rainwater, T.R., Reynolds, K.D. 2007. Contaminant exposure in terrestrial vertebrates. Environmental Pollution 150:41-64.

41. Snodgrass, J.W. Casey, R.E., Joseph, D., Simon, J.A. 2008. Microcosm investigations of stormwater pond sediment toxicity to embryonic and larval amphibians: Variation in sensitivity among species. Environmental Pollution 154: 291-297.

42. Stekoll, M.S., Smoker, W.W., Failor-Rounds,B.J., Wang, I.A., Joyce, V.J. 2009. Response of the early developmental stages of hatchery reared salmonids to major ions in a simulated mine effluent. Aquaculture 298:172-181.

43. Timpano A.J., Schoenholtz, S., Soucek, D., Zipper, C. 2010. Isolating effects of total dissolved solids on aquatic life in central Appalachian coalfield

streams. Proceedings, National Meeting of the American Society of Mining and Reclamation: 1284-1307.

44. Warner, N.R., Christie, C.A., Jackson, R.B., Vengosh, A. 2013. Impacts of shale gas wastewater disposal on water quality in western Pennsylvania. Environmental Science and Technology 47(20) 11849-11857.

45. Wilson, J.M., Wang, Y., VanBriesen, J.M. 2013. Sources of high total dissolved solids to drinking water supply in southwestern Pennsylvania. Journal of Environmental Engineering 10.1061/(ASCE)EE.1943-7870.0000733.

Appendix A: Chemical Information

Chemical	Distributer	CAS #
$BaBr_2$	Strem Chemicals	10553-31-8
$CaCL_2$	Sigma - Aldrich	10043-52-4
$FeCl_3$ x $6H_20$	Sigma	10025-77-1
KCl	Sigma - Aldrich	7447-40-7
LiCl	Acros Organics	7447-41-8
$MgCl_2$	Aldrich	7786-30-3
$MgSO_4$	Sigma	7487-88-9
NaCl	Sigma	7647-14-5
Na_2SiO_3 x $9H_2o$	Aqua Solutions	6834-92-0
Na_2SO_4	Sigma - Aldrich	7757-82-6
$SrBr_2$	Strem Chemicals	10476-81-0
$SrCl_2$ x $6H_2$	Sigma - Aldrich	10025-70-4