# Report as of FY2007 for 2006MD135B: "Chemical and Biological Impacts of Zinc and Road Salt from Road Runoff Entering Stormwater Retention Ponds"

## Publications

Project 2006MD135B has resulted in no reported publications as of FY2007.

## Report Follows

**Problem and Research Objective**

Highway runoff has the potential to negatively impact receiving systems due to elevated levels of constituents such as deicing salts, metals, organic compounds and nutrients. As part of the Federal Highway Administration - U.S. Geological Survey (USGS) National Highway Runoff Data and Methodology Synthesis, Buckler and Granato (1999) reviewed assessment strategies for the biological effects of highway runoff constituents and indicated that changes in individual organisms and in community structure have been reported. Recent work by Greenstein et al. (2004) showed that dissolved Zn likely caused observed toxicity (assessed using USEPA's marine toxicity, sea urchin fertilization test) in parking lot runoff after simulated rainfall, with tire wear particles and motor oil being suspected Zn sources. Previous estimates of Zn loading from motor oil leakage by Davis et al. (2001) suggested contributions of only 1-2% of the total Zn load in urban runoff. In contrast, tire wear particles, which contain about 1% Zn, make up approximately one-third of the vehicle derived particulates in highway runoff (Breault and Granato 2000). Davis et al. (2001) estimated that about 15 to 60% of the Zn in urban stormwater runoff comes from tire wear. Recent work from the USGS (Councell et al. 2004) has shown that tire wear particles constitute a significant source of Zn to the environment, with release inventories similar to waste incineration; during 1999 approximately 10,000 tons of Zn was released to roadways in the U.S.

Because these and other metal-bearing vehicular wear particles continuously form and collect on roadway surfaces, stormwater retention ponds become a focusing environment for their deposition. Retention ponds attract and are utilized by a wide range of wildlife species (Campbell 1994; Bishop et al. 2000), therefore deposition of metal-bearing vehicular wear particles may result in significant exposures of biota to elevated levels of Zn. Additionally, accumulation of Zn by biota inhabiting ponds (e.g., larval amphibians and fish) may result in trophic transfer of Zn out of ponds as semiaquatic wildlife (e.g., wading birds, waterfowl) feed on pond organisms that accumulate significant quantities of Zn. At this point, neither the magnitude nor the effects of such exposures are clearly known.

In addition to trace element loading, the transportation infrastructure in Maryland contributes large quantities of road salt into receiving waters. A study recently conducted in Central Maryland indicated that the increasing salinity of streams in the northeastern United States poses a threat to potable water sources and habitat for freshwater wildlife (Kaushal et al. 2005). At our study site in Owings Mills, Maryland, we have observed chloride concentrations exceeding the chloride level of seawater persisting in a stormwater retention pond through mid-summer (July 2005). While levels in the pond decreased after August, shallow groundwater immediately adjacent to the pond discharges into an adjacent wetland resulting in surface water chloride levels approximately 10% that of seawater. Shallow groundwater remains at this level year round. While trace elements such as Zn or Cu are effectively retained in stormwater pond sediments, road salt has little to no affinity for sediment particulate matter and can be easily leached into groundwater and subsequently transported into adjacent surface waters. Little is known about the magnitude of this contamination or the contribution of stormwater ponds to long-term salinization of surface waters via shallow groundwater inputs.

*Study Objectives*

In this study we pursued three objectives.

1. We assessed the magnitude of long-term Zn storage in sediments from multiple retention ponds and related that storage to the magnitude of Zn transport in the surface water of a tributary

of Red Run. We used soil cores from retention ponds to determine the storage of Zn in pond sediments. Cores above the high water line of the ponds were used to determine Zn background in these soils. Zinc background was subtracted from the total Zn in the cores to provide an estimate of anthropogenic Zn that had been stored in the ponds. Zinc storage in the subwatershed was compared to the load of Zn that was measured in surface water flow. We used these data to determine whether the scale of Zn storage in pond sediments was significant in comparison with the magnitude of Zn transport out of the watershed through surface water flow.

2. We used these same retention pond sites to quantify the annual pattern of salt concentrations in two retention ponds and in adjacent shallow groundwater to determine whether stormwater retention ponds were contributing to the long-term increases in salinization previously observed in this region (Kaushal et al. 2005). We monitored surface water and shallow groundwater inside two adjacent retention ponds and in a network of shallow groundwater wells immediately downgradient of the ponds. We also measured salt levels in first order streams originating in this floodplain to determine the spatial and temporal extent of elevated salinity resulting from proximity to the retention ponds.

3. Because roadway-derived Zn originates in large part from tire wear particles, we conducted a bioassay to measure the impacts of tire debris on the development of larval amphibians. We determined the toxicological effects of Zn and tire debris on amphibian eggs and larvae in saturated sediments simulating the sediment environment of stormwater ponds and wetlands where many of these particulates accumulate due to roadway runoff

## **Materials and Methods**

*Metal Storage in Retention Pond Cores*

Sediment cores were collected from within stormwater ponds using a grid system of approximately 10 x 10 m. Cores were obtained by inserting a soil probe to refusal which generally occurred near 30 cm depth. At least three samples representing background soil conditions were taken above the high water line at each pond. Sediment cores were capped and remained in their plastic sleeves for transport and storage.

The wet mass of each core was determined to allow for subsequent estimates of metal storage in each area of the pond and a sub-sample was then used for dry weight determination. For ICP-MS analysis, approximately 60 mg dry sample was placed into a clean Teflon vial. Samples were acidified using 1 ml of HF and 3 ml of HNO3 and placed on a hot plate overnight. Both acids were of trace metal grade. The samples were dried and 3 mL of hydrogen peroxide was added to the vessels and placed onto the hot plate. The samples were again dried and HF and HNO3 was added in the same proportions as before and placed onto the hot plate. This extra digestion step was to ensure that the samples were completely digested. Finally samples were dried and an internal standard solution of 2% HNO3 containing 10 ppb of germanium and 1 ppb of indium was added to each vial and samples were put back onto the hotplate for re-digestion. Samples were then analyzed by ICP-MS for total metal concentrations. The NIST standard reference material 2709 (San Joaquin Soil) was also analyzed with the sediment samples to monitor external reproducibility.

For samples analyzed by X-Ray fluorescence spectrometry (XRF), a portion of each dried soil sample was ground using a SPECS Mixer/Mill 2500. The mixer/mill was cleaned between each sample using DI water and methanol. Ground samples were pressed into pellets using the SPEC X-Press.  Approximately 7 g of sample and 0.7 g of a cellulose binder were mixed together and placed into the pressing die.  Each pellet was then kept in a desiccator until analyzed by the XRF.  NIST SRM 2709 (San Joaquin Soil) was used during XRF analysis for QA/QC.  Using the mass of the sediment cores, the surface area of the sampler, and the area of the sampling grid, total pond storage was estimated for Cu and Zn.

*Stream Discharge and Load Determinations*

Stream discharge measurements of a second order tributary of Red Run were made periodically over 10 months in 2006-2007 just above the confluence with Red Run at the bottom of the watershed in this investigation to establish a rating curve.  The monitoring location was selected in order to characterize the total suspended and dissolved load leaving the watershed. USGS protocols for current meter measurement of discharge were followed and an average discharge was calculated for each sampling event.  Over the same time period a levelogger  was placed in the stream at the same location and depth measurements were collected every 3 minutes.  A curve was fitted to the field discharge vs. depth measurements and a rating curve was established ($r^2 = 0.75$).  Based on the rating curve equation, depth measurements from the levelogger were converted to discharge.

In order to make accurate estimates of both the particulate and dissolved Zn load leaving the watershed, a series of equations were developed to estimate the Zn load from the levelogger data using the relationship between field measurements for discharge and suspended sediment, Zn particulate concentration and dissolved Zn.  While there are a series of assumptions that must be made in order to relate these parameters to one another, this is the only reasonable approach to estimate the elemental load from a small watershed without continuously sampling dissolved and suspended sediment from the stream.

*Monitoring of Salinization Due to Retention Ponds*

Two stormwater retention ponds were chosen for detailed monitoring of chloride and conductivity to determine the temporal and spatial extent of elevated salinity due to road salt application. Surface water in each stormwater pond was periodically monitored, along with shallow groundwater approximately 1 m below ground surface. A network of shallow groundwater wells (~ 1 m below ground surface) was deployed in the floodplain between the retention ponds and an unnamed second order tributary of Red Run. Three first order streams originate in this floodplain between the ponds and the tributary. Conductivity and chloride levels were determined for the first order streams, the second order tributary and the groundwater in the floodplain. Conductivity was determined using a hand-held field probe (YSI, Inc.) and chloride was determined using a Dionex IC 20 ion chromatograph.

*Tire Debris Bioassay*

The bioassay was performed using soils obtained adjacent to a retention pond. These soils represent the background material in pond sediments prior to mixture with road debris. Soil was collected and placed into three 5 gallon plastic containers. One treatment contained soil amended with approximately 8380 mg $kg^{-1}$ of tire material, the second contained soil that had been amended with approximately 1000 mg $kg^{-1}$ of $Zn^{2+}$ in the form of $ZnCl_2$, while the final treatment was unamended soil. The tire material was obtained from a scrap tire facility in Baltimore, MD and contained 1.26% Zn by mass (Councell et al. 2005). Particle size was less than 590 μm and steel belt debris had been removed. Enough $ZnCl_2$ and tire debris were added to their respective containers to yield approximately equal amounts of total Zn at a target concentration of 1000 mg $kg^{-1}$, with the $ZnCl_2$ serving as a positive control representing completely bioavailable Zn. Enough aged tap water was mixed with each treatment to saturate the soils and soils were homogenized mechanically using a mortar mixing attachment on a hand-held drill. All treatments were sealed and allowed to age for 8 months to simulate conditions occurring after deposition of roadway debris in a stormwater retention pond. Although continual mixing of surface sediments in retention ponds with standing water can occur, this usually takes place most often in the flow path of runoff entering the pond and leaving the pond over the spillway. In deeper sections of retention ponds sediments can settle for longer periods of time, similar to the conditions that are represented in this study.

Three pairs of wood frogs (*Rana sylvatica)* in amplexus were collected in the beginning of March 2006 from a reference site in Baltimore County, MD and allowed to deposit eggs in the laboratory. Approximately 1 cm of the aged sediment treatments was placed into the bottom of clean acid-washed plastic containers with approximately 3 L of aerated tap water. Exposure bins were set up in a randomized block design with a total of ten replicates for each treatment. Eggs were placed into exposure bins 3 days after set-up of the bins to allow sediments to settle. Between 5 and 6 eggs from each clutch were placed into each bin, and the hatching success of each clutch was recorded. Once all tadpoles had hatched, all but one from each container was removed and euthanized using MS-222. The one that remained in each bin was randomly selected and these remaining organisms were maintained until metamorphosis.

Each tadpole was fed Tetramin® fish food every 1-2 days. The starting mass of food was 10 mg per ration; however every 2-3 feedings the ration was increased to account for increased growth in the tadpoles with a final ration of 65 mg per feeding. Half of the water in each container was removed and replaced with fresh aerated tap water once per week. Temperature, pH, and conductivity were also measured once per week. Filtered water samples were collected randomly from 3 bins in each treatment once per week. Water samples were acidified to 0.2 N with trace metal $HNO_3$ and stored until analysis. The first water sample was collected 2 days after exposure bin set-up, prior to egg addition. The mass of each organism was measured on day 36 when organisms were between Gosner stages 33-35. Tadpoles were removed from the water, blotted to decrease the transfer of water, then place into a tared beaker of water to obtain their mass.

Once organisms developed front limbs, they were removed from the treatment bins and placed into a separate clean container with moist towels until metamorphosis was completed. Once organisms had developed to Gosner stage 46 they were euthanized using MS-222 and frozen until further analysis. Time to metamorphosis was recorded for all organisms.

Acidified water samples were spiked with an internal standard solution containing indium and germanium and analyzed using inductively coupled mass spectrometry (ICP-MS). A previously digested NIST SRM 2976 (mussel tissue) was analyzed along with the water samples for quality assurance. All samples were analyzed for Cu, Zn, Ni, Cr, As, Se, Cd and Pb. Average recovery for each of the metals was: Cr = 125%, Ni = 103%, Cu = 90%, Zn = 105%, As = 88%, Se = 80%, Cd = 137% and Pb = 97%. All elements except for Cd were within in the certified confidence ranges. Cd recovery was 115% of the upper limit of the certified confidence range.

The froglets were placed into a drying oven at 70ºC and then weighed to obtain dry mass. Organisms were then placed into clean Teflon vessels digested overnight with 5 mL of 6N $HNO_3$

on a hotplate at 150ºC.  Samples were dried and 3 mL of trace metal grade $H_2O_2$ (Ultrex II, J.T. Baker) was added to each vial then digested overnight on the hotplate again.  Samples were diluted to 40 mL using 0.2N $HNO_3$ and analyzed on the ICP-MS for the same analytes as the water samples.

Sediment samples collected from the treatment bins were leached overnight using 6 M $HNO_3$ at 150ºC then analyzed using ICP-MS. All results are reported in terms of dry weight.

An ANCOVA was used to assess significance of size at metamorphosis between treatments with time to metamorphosis as a covariate.  A repeated measure ANOVA was used to compare pH, conductivity, Zn concentrations in water samples, and temperature over the course of the experiment for each treatment.  An ANOVA followed by Tukey's HSD test for *post hoc* determination was used to assess significance between treatments for Zn in sediments, Zn concentrations in tissues, size at mid experiment, and time to metamorphosis.  All data were log transformed in order to meet the assumptions of a normal distribution.

## Results

*Storage and Transport of Zn in a Small Watershed*

The results presented here are preliminary. Data acquisition is still under way, as per the no cost extension agreement that we obtained.

Of the ten retention ponds in the watershed being investigated, we have sampled and determined Zn storage for three. Subtracting soil background Zn from the sediments in the retention ponds these three ponds stored 3.4, 8.6 and 0.8 kg of anthropogenic Zn, respectively. If we consider these ponds to be typical of others in the watershed and extrapolate this storage throughout the watershed, we estimate that the ten retention ponds are storing a total of 42.6 kg of anthropogenic Zn. This storage has occurred over approximately 6 years in these ponds.

The annual background corrected Zn transport out of the catchment area is estimated at 6.9 kg $yr^{-1}$. This estimate is based on the calculated particulate and dissolved Zn load derived from field measurement. This annual Zn load estimate is approximately equal to the annualized total anthropogenic Zn storage in storm water retention ponds from the watershed being investigated. Based on this comparison, the storm water retention basins in this catchment are storing approximately 50% of the total anthropogenic Zn load and are in fact acting as a significant sink for roadway derived Zn.

*Salinization in Stormwater Ponds and Surroundings*

The results presented here are preliminary. Data acquisition is still under way, as per the no cost extension agreement that we obtained.

Conductivity measurements from three ponds within the Red Run watershed are presented in figures 1 and 2. The data in figure 1 was collected every 12 hours continuously between March, 2005 and June, 2007. The points in black represent conductivity measurements from shallow groundwater directly beneath the deepest portion of the retention pond. Open symbols are bottom water conductivity measurements from the deepest point in the pond. Also plotted are a field, marking the observed range of down-gradient groundwater conductivities and dashed lines that represent the conductivity of seawater and the adjacent portion of Red Run which will ultimately receive groundwater discharged from the retention pond.

This pond is perennially wet and is receiving direct highway runoff from an adjacent 6 lane primary roadway. The conductivity of the surface water in the pond is highly seasonal with the highest conductivities directly related to road salt events during winter storms. Over the monitoring period, there were only 2 major snow fall events one in December 2005 and the second in January 2007. As the snow from the winter storms melted salt applied to the roadway before, during and after the snow fall was washed into the retention pond increasing the conductivity by 2 orders of magnitude. This elevated conductivity remains for up to 10 months before returning to background surface water levels. The conductivity of groundwater directly beneath the pond lags behind surface water demonstrating the connection between the surface water and groundwater. This slow infiltration rate dramatically limits this systems ability to flush roadway derived salt and creates a continuing source of salt to groundwater and ultimately surface waters. Groundwater directly beneath the pond is constantly elevated and fluctuates, depending on the amount of salt free recharge, between 10 and 200 times the conductivity of fresh water in Red Run. Groundwater down-gradient of the pond, between the pond and the main branch of Red Run, is elevated year round and has a relatively constant conductivity that is 2 – 20 times the conductivity of Red Run. This large plume of salt is migrating towards Red Run and will provide a significant continuing source of salt to Red Run once it begins to discharge.

In order to evaluate the relevance this perennially wet pond, 2 adjacent rapid infiltration ponds in a more suburban portion of the watershed were also investigated. The results of this part of the investigation are on-going however; it has become clear that rapid infiltration ponds can also act as a continuing source of salt groundwater and ultimately Red Run. Figure 2 is a plot of conductivity along a transect from the input of one of the ponds through the flood plain to a 1st order tributary of Red Run which represents a discharge point for groundwater in this system. This pond is representative of the behavior of both ponds. Results from 4 sampling events beginning in January 2007 immediately following a major storm event are plotted at each point along the transect. Note the movement of roadway salt through the system over time and more significantly the sustained elevated conductivities at the point labeled "well" which represents groundwater beneath the retention pond. Despite the rapid infiltration design of this pond, groundwater conductivities remain up to 100 times that of surface water for more than 5 months after a major salting event. These ponds are demonstrating the same dynamics as the perennially wet highway pond and are similar with respect to the range of conductivities found in groundwater year round. These ponds will be continually monitored until spring 2008 in order to document range of conductivities over a calendar year.

*Toxicity of Tire Debris Amended Sediments to Larval Amphibians*

Conductivity, pH, and temperature values were similar through all treatments (Table 1). Conductivity and pH were significantly different between treatments, but in terms of environmental conditions they were most likely not a controlling factor in the experiment. Aqueous Zn concentrations decreased dramatically after the first two weeks of the experiment for both the $ZnCl_2$ and tire treatments (Figure 3). During the first week the $ZnCl_2$ treatment had a concentration of 300 µg $L^{-1}$ Zn which dropped to 80 µg $L^{-1}$ by the second week and 21 µg $L^{-1}$ by the third week. A similar trend was seen in the tire treatment with week one concentrations approximately 25 µg/L Zn and week three concentrations approximately 7 µg/L. These changes reflect dilution of water column Zn by the weekly water changes in the bioassay containers.

The presence of Zn in the water column of the tire treatment indicated that the aging period did in fact leach some of the Zn out of the tire material, making it potentially available for uptake by larvae. Sediment Zn concentrations between treatments were significant ($F_{3, 15}$=154.11, $P < 0.0001$) with the tire and $ZnCl_2$ treatments having significantly higher Zn concentrations than the soil treatment as measured by 6M $HNO_3$ leach. However, sediment Zn levels were statistically similar between the $ZnCl_2$ and tire treatments. All other metal concentrations were substantially lower than Zn in both the water and sediment (Table 2) and were generally similar between all three treatments.

Eggs from three clutches were placed into separate corners of each exposure bin in order to determine hatching success of each clutch. Differences in hatching success were significant between clutches ($F_{1, 27} = 24.74$, $P < 0.0001$) and moderately significant between treatments ($F_{2, 27} = 3.51$, $P = 0.0442$). Of the three clutches, one had a 0% success rate due to lack of fertilization of the eggs; this clutch was not included in the statistical analyses. The mean success rate for eggs from the soil, $ZnCl_2$ and tire treatments were 86%, 68% and 80%, respectively.

Zn concentrations in anuran tissues were significantly different between treatments ($F_{3, 33} = 99.23$, $P < 0.0001$). Organisms in the $ZnCl_2$ and tire treatments had statistically higher accumulations of Zn than those from the unamended soil. Additionally, tissue Zn accumulation in the $ZnCl_2$ treatment was statistically higher than the accumulation in organisms from the tire treatment (Figure 4). There was little accumulation of other metals in the organisms (Table 2). Organisms weighed at day 37 of the experiment showed no significant difference between the size of the organism and the exposure treatment ($F_{3, 36}$=2.00, p=0.1322).

The number of days to complete metamorphosis was significantly different depending on the treatment ($F_{3, 27}$ = 4.83, P = 0.0081). The tire treatment and the $ZnCl_2$ treatment were significantly slower to reach metamorphosis than the control soil treatment. There was no significant difference in time to metamorphosis between the tire and $ZnCl_2$ treatments.

An ANCOVA with day as the covariate showed that exposure to Zn had no effect on the mass of the organisms at metamorphosis ($F_{5, 15}$ =1.41, P = 0.4344). Although the differences were not significant, there was a relationship between days to metamorphosis and size at metamorphosis. Larvae that were exposed to control soils had a positive relationship between days to metamorphosis and size at metamorphosis, however larvae exposed to tire treatments had a negative relationship (Figure 5).

Only one larval amphibian died during the exposure period. The organism died 15 days after hatching and was in the $ZnCl_2$ treatment. Since there were no other mortalities during this time, the single mortality cannot be attributed to the exposure conditions. After organisms developed front legs they were removed from exposure bins and placed into containers with only wet paper towels to ensure high moisture. Eight organisms died during metamorphosis, but mortality during metamorphosis was not related to Zn exposure. Out of the eight organisms that died, 2 were from the tire treatment, 3 from the soil treatment, and 3 from the Zn treatment. Even though these organisms did not complete metamorphosis, they were saved and analyzed for total metal accumulation along with all other organisms. They were not used for time to metamorphosis or size at metamorphosis calculations.

The most substantial difference between exposure conditions was the concentration of Zn in the sediment as well as the water column. Zn was present in the water column in tire exposures showing that a portion of the total Zn in the tire rubber leached out of the tire material and entered the water column. Decreases in water column Zn were due to the water changes that occurred once per week. During the first week, substantial Zn that was dissolved in the pore water or loosely attached to the sediment surface was released into the water column. The first water change and subsequent water changes removed approximately half of the Zn in the water column. The highest concentrations of Zn were present during the hatching period and may have affected the hatching success of eggs. This pulse of high aqueous Zn is similar to what is seen during a storm event with the first half of the stormwater runoff having higher concentrations of metals. This indicates that it is possible for organisms to be exposed to very high concentrations of metals for a very short amount of time. Different life stages of anurans could exhibit varying susceptibility to metals, so the stage at which these organisms are exposed to these high metal pulses in the environment could affect the magnitude of their response.

Accumulation of Zn in the tissues of the adult anurans was seen in both the tire and $ZnCl_2$ amended treatments in comparison with the unamended soil treatment; however no significant larval mortality occurred in any of these treatments. Tissue Zn concentrations in the tire-exposed organisms are similar to concentrations in organisms found in a stormwater management pond (Casey et al. 2005) but lower then tissue concentrations found in organisms from a river system in Greece (Loumbourdis and Wray 1998). A study by Snodgrass et al. (2003) indicated that Zn is one of several elements that are retained in anuran tissues through metamorphosis.

Water column data as well as accumulation data show that Zn is leaching out of the tire material and becoming available to the larvae. Both the tire and $ZnCl_2$ amended soils showed slowed rates of development when compared to the unamended soil treatment, however there was no difference in the metamorphosis rate between the $ZnCl_2$ treatment and the tire treatment. The delayed metamorphosis of organisms exposed to Zn indicates that there is a sublethal effect of the metal on *R. sylvatica* larvae. The $ZnCl_2$ treatment should have more available Zn than the tire treatments and it would have been expected that the tire treatment would have reached metamorphosis faster based solely on Zn concentrations. However, Zn is not the only component of tire material that could be causing a sublethal effect. In addition to Zn, tires may also contain a variety of other pollutants including PAHs, various organic chemicals, and other metals (Wik and Dave 2005) which could have contributed to the slowed time to metamorphosis. The amended soils reached metamorphosis approximately 7 days after those from the unamended soil treatment. It may be that this 7 day difference is not large enough to have a major impact on survival in the environment; however, this is only one measure of stress and may be indicative of other sublethal stresses occurring as a result of the exposure. The negative relationship of days to metamorphosis and size at metamorphosis for tire treatments indicates the length of time that larvae are exposed to tire debris adversely affects their size. This negative relationship has also been observed in larvae exposed to coal combustion wastes (Snodgrass et al. 2004).

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| | Soil | $ZnCl_2$ | Tire | P values | | |
|---|---|---|---|---|---|---|
| | | | | Treatment | Day | T x D |
| Zn (µg/L) | bdl (bdl-3.7) | 65.89 (2.5-374.3) | 10.06 (1.15-32.93) | <0.001 | <0.001 | <0.001 |
| pH | 8.51 (7.90-9.49) | 8.14 (7.43-9.79) | 8.17 (7.60-9.24) | <0.001 | <0.001 | <0.001 |
| Conductivity (µS/cm) | 373 (338-386) | 491 (364-627) | 387 (369-488) | 0.0265 | 0.6822 | 0.0793 |
| Temperature (Celsius) | 18.4 (14.9-21.9) | 18.4 (15.1-21.9) | 18.2 (15.0-22.4) | 0.3168 | <0.001 | 0.2343 |

Table 1: Arithmetic means of experimental water conditions (ranges in parentheses) and repeated measure ANOVA results.

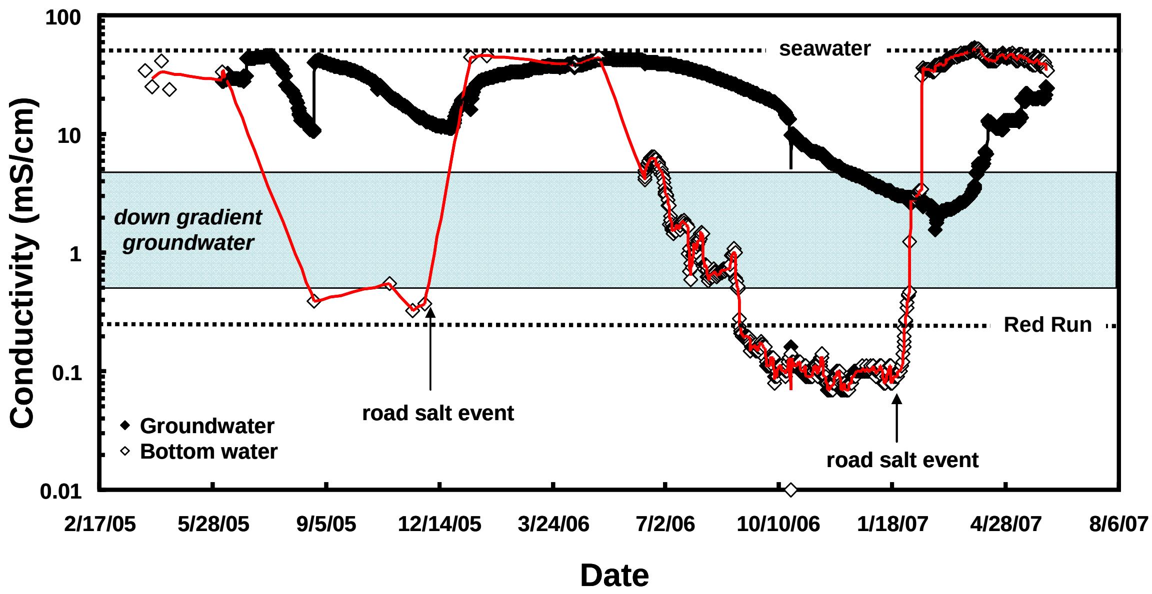


Figure 1. Continuous record of surface and ground water conductivity from a perennially wet highway pond in the Red Run watershed.

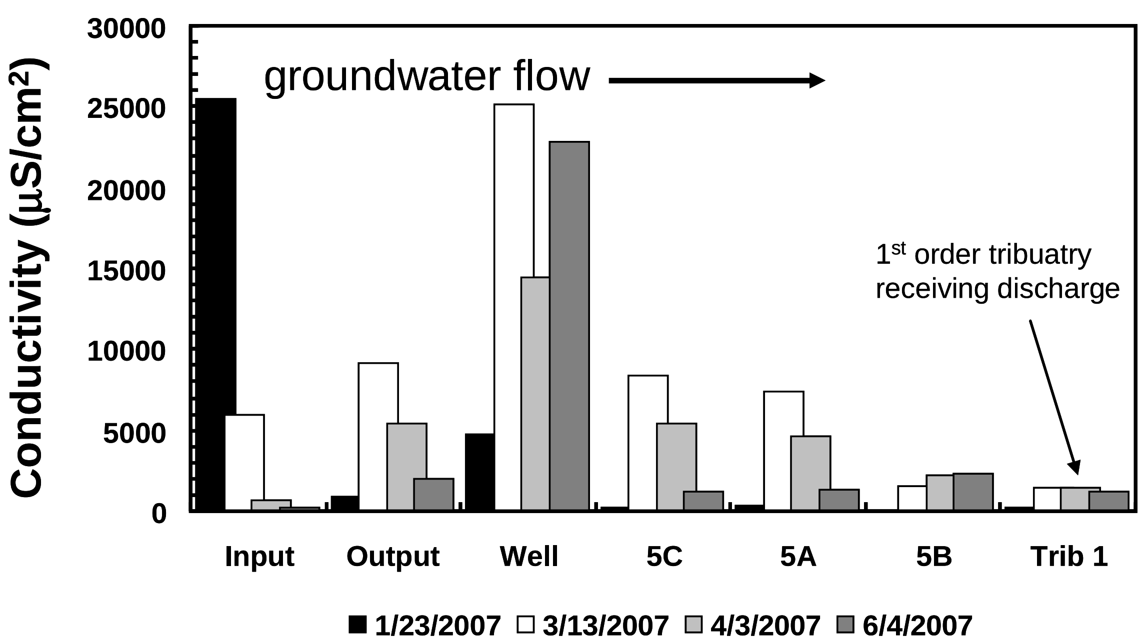


Figure 2. Plot of conductivity along a transect between the storm water input point of a rapid drainage suburban pond and a 1st order tributary of Red Run receiving discharge for groundwater connected to the pond. Input and Output are surface water measurements while Well (inside the pond), 5C, 5A and 5B (in the flood plain) are shallow groundwater wells in the pond adjacent flood plain.

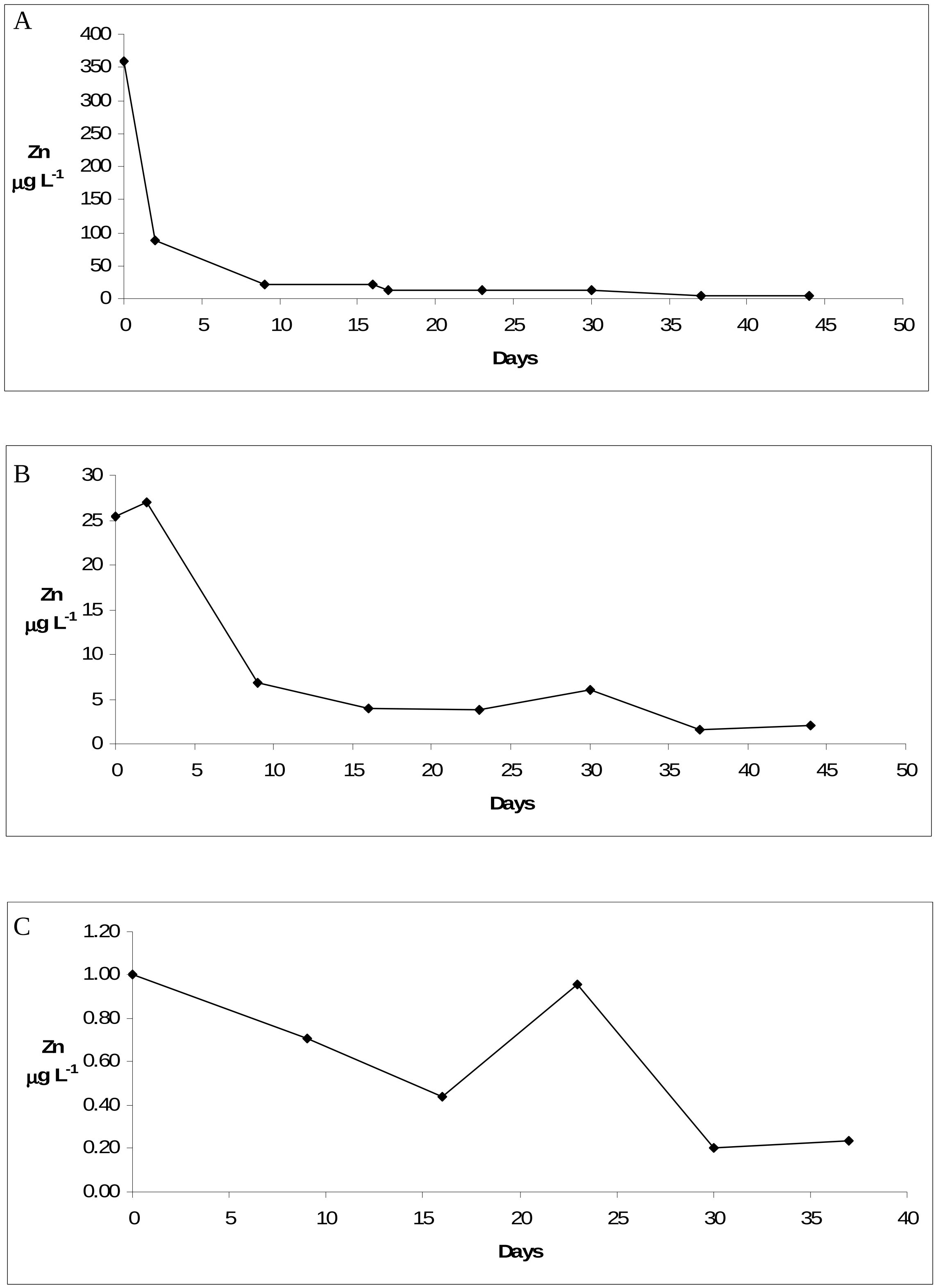


Figure 3: Mean Zn concentration in the water column over the course of larval exposure (day 0 is day eggs were added) for the treatments; A) $ZnCl_2$ B) Tire C) Soil

| | Sediment (mg/kg) | | | Tissues (mg/kg) | | | Water (µg/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Soil | Tire | ZnCl2 | Soil | Tire | ZnCl2 | Soil | Tire | ZnCl2 |
| Cr | 52.5 | 37.2 | 40.1 | 0.40 | 0.29 | 0.41 | bdl | bdl | bdl |
| Ni | 45.4 | 35.4 | 42.0 | 0.96 | 0.59 | 0.86 | bdl | bdl | bdl |
| Cu | 19.1 | 19.4 | 22.9 | 4.53 | 8.9 | 3.9 | 3.5 | 3.2 | 3.4 |
| Zn | 62.0 | 1155 | 1187 | 81.7 | 212 | 324 | bdl | 12.6 | 32.8 |
| As | 0.13 | bdl | bdl | bdl | bdl | Bdl | bdl | bdl | bdl |
| Se | bdl | bdl | bdl | 1.0 | 0.76 | 1.2 | bdl | 2.1 | bdl |
| Cd | 0.15 | 0.43 | bdl | 0.15 | 0.11 | 0.11 | bdl | bdl | bdl |
| Pb | 26.1 | 41.5 | 24.8 | 0.04 | 0.38 | 0.04 | bdl | bdl | bdl |

Table 2: Arithmetic means of metal concentrations in sediment, tissues, and water. The detection limit (bdl) for water is 1 µg $L^{-1}$.

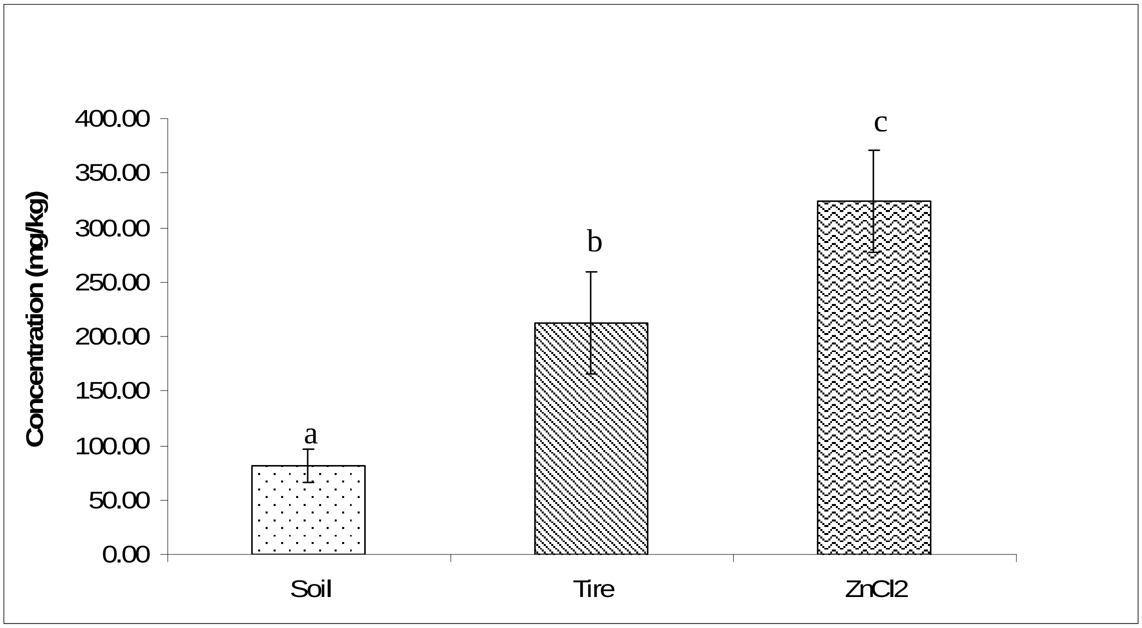


Figure 4: Concentration of Zn accumulation in tissues of *R. sylvatica* for the three treatments; soil, tire, and $ZnCl_2$.  Error bars are ± 1 S.D.  Letters indicate significant differences ($P < 0.05$) between treatments for Zn accumulation based on Tukey's post hoc test

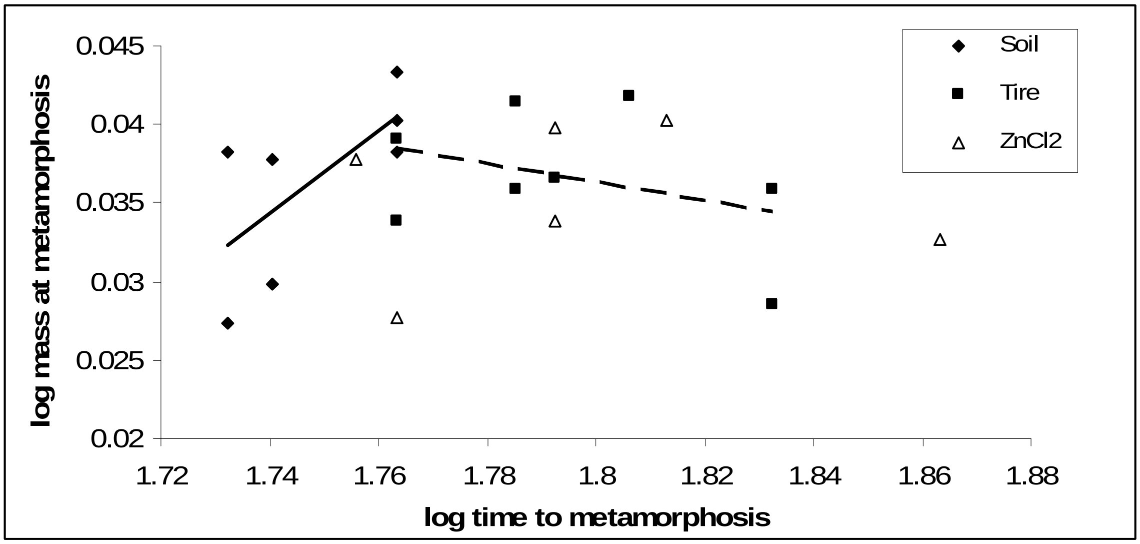


Figure 5: Relationship between days to metamorphosis and size at metamorphosis. Solid line is a linear regression for soil treatment ($y = 0.2631x - 0.4234$; $R^2 = 0.4624$; $P = 0.006$). The dotted line is a linear regression for the tire treatment ($y = -0.0585x + 0.1416$; $R^2 = 0.1358$; $P = 0.055$) No line is given for the $ZnCl_2$ treatment because the relationship was not significant ($P = 0.886$).