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Microcosm investigations of stormwater pond sediment toxicity to embryonic and larval amphibians: Variation in sensitivity among species

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Variation among species in sensitivity to pollutants can influence stormwater pond amphibian assemblages.

Abstract

Stormwater ponds have become common features of modern development and often represent significant amounts of open space in urbanized areas. Although stormwater ponds may provide habitat for wildlife, factors responsible for producing variation in wildlife use of ponds have received limited attention. To investigate the role of variation in species tolerances of pollutants in structuring pond-breeding amphibian assemblages, we exposed species tolerant (*Bufo americanus*) and not tolerant (*Rana sylvatica*) of urbanization to pond sediments in laboratory microcosms. Pond microcosms had elevated sediment metal levels and chloride water concentrations. Among *R. sylvatica* embryos, exposure to pond sediments resulted in 100% mortality. In contrast, *B. americanus* embryos and larvae experienced only sublethal effects (i.e., reduced size at metamorphosis) due to pond sediment exposure. Our results suggest variation in pollutant tolerance among early developmental stages of amphibians may act in concert with terrestrial habitat availability to structure amphibian assemblages associated with stormwater ponds.

Keywords: Metals; Road salt; *Rana sylvatica*; *Bufo americanus*; Stormwater management

1. Introduction

One of the most pressing issues facing water resource planners and managers in rapidly urbanizing areas is the potential impact of stormwater runoff to natural bodies of water. The large amounts of impervious surface (e.g., roof tops, roads and parking lots) typical of urban and suburban areas collect a wide range of pollutants including metals, polycyclic aromatic hydrocarbons (PAHs), and salts (Councell et al., 2004; Davis et al., 2001; Marsalek, 2003; Pitt et al., 1995; Van Metre and Mahler, 2003). During precipitation events, impervious surfaces increase surface runoff (Dunne and Leopold, 1978; Hopkinson and Day, 1980), which transports accumulated pollutants to receiving waters.

Detention or retention pond structures (hereafter referred to as stormwater ponds) are among the most common features of stormwater management plans. Stormwater ponds are designed to detain or retain stormwater runoff, allowing pollutants such as sediments, heavy metals, and nutrients to be removed from runoff and reducing the impacts of flooding on natural water bodies by promoting infiltration (Novotny, 1995; US Environmental Protection Agency, 1991). Because stormwater ponds are often colonized by plants, they are significant elements of open space perceived by residents of urban and suburban areas and may provide habitat for wildlife (Stahre and Urbonas, 1990). However, stormwater ponds may serve to expose wildlife to the pollutants that they are designed to sequester (Bishop et al., 2000a,b; Campbell, 1994; Helfield and Diamond, 1997). There is a great deal of variation among ponds in their habitat quality. Although studies of toxicity of environmental conditions in ponds are limited, pollutants do not always exceed

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toxicity thresholds (Casey et al., 2005; Karouna-Renier and Sparling, 2001), and experimental exposure to pond sediments and water does not always produce toxic effects (Bishop et al., 2000b; Karouna-Renier and Sparling, 1997). Moreover, there is a great deal of variation in wildlife use among individual ponds (Bascietto and Adams, 1983; Bishop et al., 2000a; Massal et al., 2007; Ostergaard, 2001; Simon, 2006).

The potential role of stormwater ponds as wildlife habitat is particularly relevant for pond-breeding amphibians. Pond-breeding amphibians mate and deposit their eggs in ponds and wetlands where the embryonic and larval stages develop before metamorphosing into semi-aquatic or terrestrial juveniles. Use of stormwater ponds by pond-breeding amphibians has been documented in a number of areas (Bascietto and Adams, 1983; Bishop et al., 2000a; Ostergaard, 2001), but the role of these ponds in the disappearance or conservation of amphibians in urban and suburban environments remains unclear. Recently, links among the number of amphibian species utilizing a pond, metal levels in sediments, and land cover surrounding ponds have been developed (Simon, 2006). However, the relative roles of pond pollution and loss of upland habitat in restricting amphibian use of ponds have not received attention.

Here we report the results of experiments designed to assess the role of pollutants in limiting amphibian use of stormwater ponds as breeding sites. We specifically asked if the effects of exposure to polluted stormwater pond sediment varied between a species that is sensitive to urbanization (*Rana sylvatica*) and a species that is relatively insensitive to urbanization (*Bufo americanus*), where sensitive is defined by a negative correlation between urban land use and occurrence (Knutson et al., 1999). In our study area, *R. sylvatica* is rare and restricted to stormwater ponds surrounded by more than ~40% forest cover (i.e., relatively low cover of urban land use). In contrast, *B. americanus* is common, being reported from ~50% to 90% of the stormwater ponds in individual studies (Bascietto and Adams, 1983; Massal et al., 2007; Simon, 2006). Therefore, we expected *R. sylvatica* to be more sensitive to pond sediments than *B. americanus*. Alternatively, if both species exhibited similar effects of exposure to pond sediments, then other factors, such as the availability of upland habitat, might be limiting pond use by pond-breeding amphibians.

2. Methods

2.1. Experimental design

During the spring of 2005 we exposed embryo and larval *R. sylvatica* and *B. americanus* to sediments from one or two stormwater management ponds associated with heavily used roads. We chose two ponds from different regions as sources of contaminated sediment. The Owings Mills pond is located northwest of the city of Baltimore (southwest of the intersection of Owings Mills Boulevard and Red Run Boulevard, Baltimore County, MD) and the New Cut Road pond is located just southeast of Baltimore (northeast corner of the intersection of New Cut Road and Interstate 97, Anne Arundel County, MD). Both ponds primarily receive runoff from high-use roadways, have <~10% forest cover within 500 m of the pond, are contaminated with metals (primarily Cr, Cu, Ni, and Zn) and road salts, and are used for breeding by *B. americanus* but not *R. sylvatica* (Casey et al., 2005; 2007; Massal et al., 2007; Simon, 2006).

We conducted all experimental exposures in a greenhouse at Towson University and used a randomized block design with two treatments: (1) clean commercial sand as a control ($n = 20$); (2) sediment from a stormwater treatment pond ($n = 20$). Our overall goal in setting up microcosms was to mimic physical and chemical conditions of stormwater ponds during the breeding period of each species. For *R. sylvatica* we conducted a single exposure experiment using sediment from the Owings Mills pond. For *B. americanus*, we conducted two exposure experiments utilizing sediments from each pond separately. During the breeding period of each species we used pressure and acid-washed posthole diggers to collect sediments to a depth of ~10 cm. We transported and homogenized pond sediments in acid-washed plastic buckets before placement into experimental bins. We exposed developing embryos and larvae in 2 l acid-washed plastic bins containing 1 cm of clean sand or pond sediment and aged tap water (conductivity ~250 μS). Throughout each experiment we maintained water volumes at 1.75 l and replaced half the water volume of each bin every 4 days. We set up bins and allowed them to age for 3–4 days before starting experimental exposures.

Each experimental exposure consisted of two phases: (1) exposure of groups of developing embryos and hatchlings until they reached Gosner stage 25 (Gosner, 1960); (2) exposure of groups of five individual larvae through metamorphosis if they survived to that point. For both the *R. sylvatica* (21 March 2005) and the *B. americanus* experiments (5 May 2005) we collected 2–3 amplexed pairs from an uncontaminated local wetland, returned them to the laboratory, and allowed them to oviposit in 5 l plastic bins containing water from the collection wetland. We assigned groups of 14–20 eggs to each experimental bin. We separated eggs as a mass from individual clutches using a pair of sharp scissors, but did not attempt to combine eggs from different clutches because of the difficulty of separating individual eggs; we did assure that sediment treatment and clutch were not confounded. We monitored eggs in each bin daily until all eggs had died or hatched. When surviving larvae reached Gosner stage 25, we randomly selected five larvae to remain in the bin for the remainder of the experiment. Following reduction of larval numbers, we continued to monitor survival and development on a daily basis. We fed larvae equal amounts of rabbit food every 2–3 days during development, and adjusted feeding amounts to accommodate growth. Throughout each experiment we measured water pH, temperature, and specific conductance every 2–4 days.

2.2. Sediment, water and tissue analyses

To document exposure conditions in experimental bins, at the termination of each experiment we collected sediment and water samples from five randomly selected bins within each experimental treatment. We collected two water samples; one was refrigerated (2 °C) until determination of Cl^- concentration using ion chromatography (IC; Dionex IC25), and the other was acidified to pH <2, filtered (0.45 μm PTFE syringe filter), and frozen (−20 °C) until analysis for metal concentrations (Cr, Ni, Cu, Zn, As, Cd, and Pb) using an inductively coupled plasma mass spectrometer (ICP-MS; Thermo Elemental Plasma Quad ExCell). We froze (−20 °C) sediment samples until they were processed for metal analysis. Sediment samples were dried at 110 °C then homogenized before digestion in Teflon digestion vessels using 6 M trace metal grade HNO_3 at 150 °C for 24 h. All IC and ICP-MS calibration standards were prepared from NIST-traceable commercial stock solutions (SPEX Certiprep).

2.3. Statistical analyses

To compare pH, temperature, and specific conductance between controls and pond sediment treatments within each experiment, we used repeated measures ANOVA as implemented in the PROC MIXED procedure of SAS with a compound symmetry co-variance structure. To compare pond sediment metal concentrations among experiments, and water concentrations of Cu, Zn, and Cl^- among treatments and experiments, we used a MANOVA model. We $\log(x + 1)$ transformed metal and Cl^- concentrations to meet the normality and homogeneity of variance assumptions of the MANOVA model. Because 93% of the Cr, Ni, As, Se, Cd, and Pb water concentrations were below detection limits (bdl; 1 μg l^{-1} for all elements), we made no attempt to statistically compare these concentrations among experiments or treatments within experiments. Similarly, because of a large number of bdl values (0.2 mg kg^{-1} for all elements) among sand metal concentrations in all experiments (70–72% in all experiments), we did not statistically compare sand metal concentrations among experiments or between controls and sediment treatments within experiments. Because there was no difference in water or sediment metal concentrations between experiments for sand or sediments from the Owing Mills pond (MANOVA; $p > 0.890$), we only report mean values calculated across both experiments for sand and Owings Mill pond treatments.

We used survival analyses as implemented in the LIFETEST procedure of SAS to compare survival between sand and pond sediment treatments during the first and second phases of each experiment. We treated embryos reaching Gosner stage 25 and larvae successfully completing metamorphosis as censored data. Because the survival functions were not exponential in nature, we used nonparametric log-rank tests to compare survival functions between sand and pond sediment treatments.

As measures of sublethal effects we determined the days to and size at metamorphosis for all larvae completing metamorphosis. To compare days to metamorphosis between control and pond sediments in each experiment, we used an ANOVA model. To compare size at metamorphosis we used an ANCOVA model including days to metamorphosis as a covariate in the model. Preliminary analyses indicated no interaction between days to metamorphosis and sediment type, so we did not include the interaction term in the final ANCOVA models. To meet the homogeneity of variance assumptions of both models, we $\log(x+1)$ transformed both dependent variables. Because observations of larvae from the same bin were not independent, we used means for individual bins as input for both models.

3. Results

3.1. Exposure conditions

The *R. sylvatica* experiment was terminated after 13 days when all embryos and hatchlings were dead in the pond sediment treatments. The *B. americanus* experiments lasted from 40 days (New Cut Road) to 48 days (Owings Mills Road) during which time most toads completed metamorphosis. During the *R. sylvatica* experiment, water temperature in bins ranged from 14 to 25 °C ($\bar{x} = 19.4$ °C) but did not differ significantly ($F_{1,18} < 0.01$; $p = 0.978$) among sediment and control treatments. Water temperatures during the *B. americanus* experiments ranged from 18 to 27 °C ($\bar{x} = 26.1$ °C), but differed significantly among treatments ($F_{1,18} = 13.36$; $p = 0.002$ for the New Cut Road and $F_{1,18} = 17.89$; $p < 0.001$ for the Owings Mills experiment). The difference among treatments in mean temperature was <0.4 °C in both cases, and was lower in sand treatments. In contrast to temperature, pH differed significantly between sand and pond sediment treatments in all three experiments ($F_{10,163} > 2.71$; $p < 0.016$ for the interaction term in all models). However, the slightly higher water pH in sand bins at some points during all experiments was probably not of biological significance as means for individual treatments ranged from 8.3 to 8.6 with extremely low variation (range in SD from 0.03 to 0.14). Of more biological consequence was the significantly ($F_{1,18} > 18.89$; $p < 0.001$ for the main effect of sediment type in all models) elevated conductivity of water in bins containing pond sediments in all experiments. While bins containing clean sands had an average conductivity of 354 μS (SD = 38), bins containing Owings Mills Road and New Cut Road pond sediments had an average conductivity of 4548 μS (SD = 2165) and 3686 μS (SD = 1430), respectively.

In general, all metals that we measured were elevated in sediments from ponds when compared to sand (Table 1). Metal concentrations differed significantly ($F_{8,1} = 1412.71$; $p = 0.021$) between the Owings Mills and the New Cut Road pond sediments. While concentrations of Zn, Pb, and Se were similar between the two pond sediments, concentrations of Cr, Ni, and Cu were higher in the Owings Mills Pond sediments, and concentrations of As and Cd were higher in the New Cut Road sediments (Table 1).

Although water concentrations of metals were relatively low compared to sediment concentrations (Table 1), water concentrations of Cu, Zn and Cl^- differed significantly ($F_{3,14} = 82.32$; $p < 0.001$) between microcosms with sand and those with pond sediments. There was no difference for water concentrations of Cu, Zn and Cl^- between the two pond sediments used in the *B. americanus* experiments ($F_{3,14} = 0.84$; $p = 0.493$).

3.2. Rana sylvatica toxic effects

During the *R. sylvatica* experiment, hatchlings (i.e., Gosner stage 20–24) suffered high levels of mortality in the bins containing Owings Mills pond sediments, with no individuals

Table 1
Median and range of metal and chloride (Cl^-) concentrations in water and sediment samples from microcosms used to expose *R. sylvatica* and *B. americanus* embryos and larvae to stormwater retention pond sediments

Element	Sand (control)		Owings Mills		New cut road	
	Water	Sediment	Water	Sediment	Water	Sediment
Cr	bdl	0.75 (bdl–2.35)	bdl	14.90 (13.51–23.31)	bdl	9.37 (6.27–13.55)
Ni	bdl (bdl-1.73)	Bdl	bdl	14.16 (12.09–18.84)	0.69 (0.6–0.72)	4.34 (2.83–6.34)
Cu	6.14 (4.61–9.11)	0.50 (bdl–1.30)	2.75 (2.17–3.53)	13.73 (11.41–20.04)	2.93 (2.72–3.47)	7.03 (4.09–9.58)
Zn	4.71 (1.95–5.57)	0.14 (bdl–0.28)	3.50 (2.85–13.85)	56.52 (43.68–80.84)	5.21 (2.97–6.35)	62.68 (19.75–86.07)
As	bdl	0.18 (bld–0.20)	bdl	0.29 (bdl-0.47)	1.10 (1.03–1.28)	0.63 (0.42–0.69)
Cd	bdl	0.12 (bdl–0.13)	bdl	0.32 (0.25–0.40)	0.35 (0.35–0.39)	0.56 (0.27–0.74)
Pb	bdl	bdl	bdl	8.41 (6.92–10.35)	0.11 (0.06–0.23)	10.55 (6.57–14.94)
Cl	77 (69–87)		535 (224–1055)		404 (334–486)	

Values for sand and Owing Mills pond sediments did not differ among experiments and were combined across the *R. sylvatica* and *B. americanus* experiments to estimate medians and ranges. Chloride was not measured in sediments. All metal values for water and sediment are in μg l^{-1} or μg g^{-1}, respectively; Cl^- is in mg l^{-1}. bdl, below detection limits.

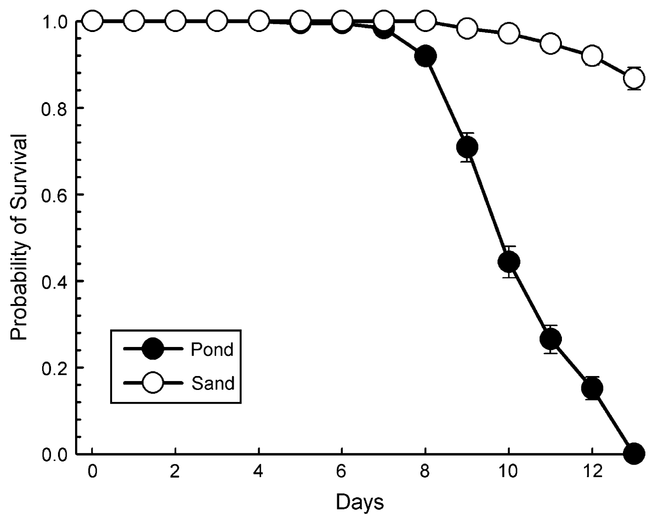

Fig. 1. Probability of survival of *Rana sylvatica* embryos as a function of days of exposure to clean sand or sediments from a roadway-associated stormwater management pond during the spring of 2005. Error bars are 1 SE.

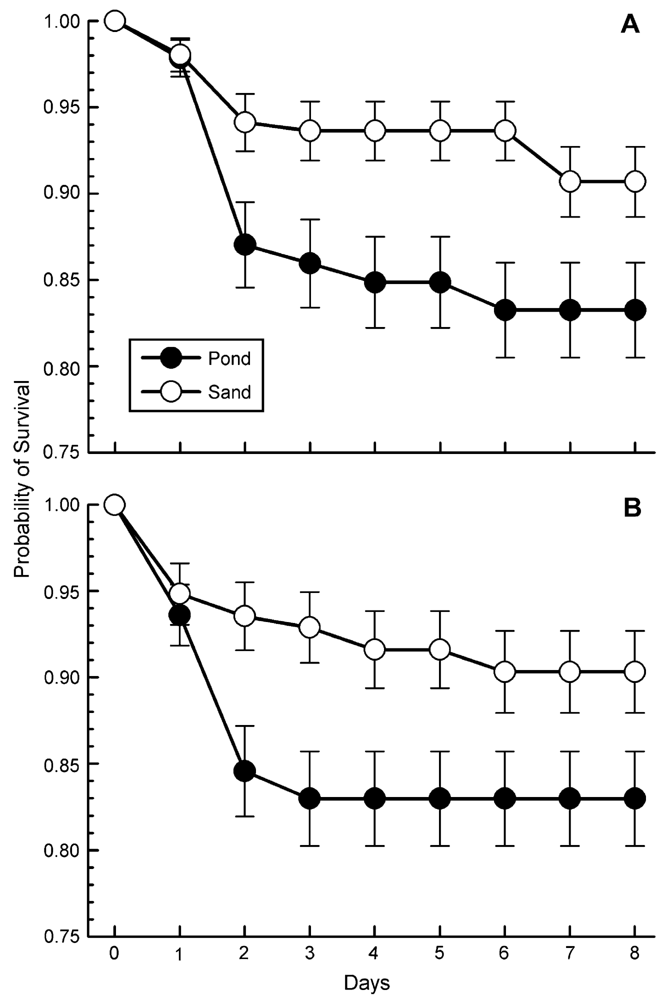

Fig. 2. Probability of survival of *Bufo americanus* embryos as a function of days of exposure to clean sand or sediments from two roadway-associated stormwater management ponds during the spring of 2005. Error bars are 1 SE.

surviving to Gosner stage 25 (Fig. 1). In contrast, survival of embryos in bins containing sand was 85% over the first 13 days of the experiment, and trends in survival differed significantly between sand and pond sediments during this period ($\chi^2 = 320.6$; DF = 1; $p < 0.001$). The experiment was terminated at day 13 because all embryos exposed to pond sediments were dead. Survival was 100% over the first 5 days of the experiment in both treatments, but many of the eggs in the bins containing pond sediments showed signs of stress, including a reduction in the size of egg capsules, failure of the perivitelline chamber to expand, lack of embryo movement, bent or kinked tails, and a lack of activity of embryos following hatching (~Gosner stage 19–20). The majority of mortality among individuals exposed to pond sediments occurred during the hatchling phase of development (Gosner stage 20–24), after embryos hatched but before they began feeding (Fig. 1).

3.3. Bufo americanus toxic effects

In contrast to *R. sylvatica*, survival of *B. americanus* embryos and larvae was high, with 85% of embryos successfully reaching Gosner stage 25 and 86% of larvae completing metamorphosis. Survival among embryos exposed to sediments from the Owings Mills pond was 83% and differed significantly from survival among the respective controls (91%; $\chi^2 = 4.8$; DF = 1; $p = 0.028$). Survival among embryos exposed to New Cut pond sediments was 83%, but did not differ significantly from controls (90%) for this experiment ($\chi^2 = 3.8$; DF = 1; $p = 0.051$). Among all embryos in the *B. americanus* experiment, mortality was highest just after eggs hatched (days 2 and 3 of the experiments) and was relatively low during hatchling stages (Fig. 2). Larval survival was high in all treatments (> 90%) and did not differ significantly between pond sediments and controls in the Owings Mills pond ($\chi^2 = 0.9$; DF = 1; $p = 0.330$) or the New Cut pond experiments ($\chi^2 = 2.4$; DF = 1; $p = 0.281$).

Larval *B. americanus* also suffered sublethal effects when exposed to pond sediment. Exposure to pond sediments slowed larval development slightly and decreased the size of metamorphs. Although larvae took 1.6 days (New Cut) and 1.9 days (Owings Mills) longer, on average, to complete metamorphosis when exposed to pond sediments (Fig. 3), the difference in developmental rates was not significant in either the New Cut ($F_{1,16} = 0.4$; $p = 0.512$) or Owings Mills pond experiments ($F_{1,16} = 0.5$; $p = 0.501$). More substantial were the effects of exposure to pond sediments on size at metamorphosis; larvae from controls were on average 62% and 78% larger than larvae exposed in the New Cut and Owings Mills pond experiments, respectively (Fig. 3). Size of metamorphs was significantly lower among individuals exposed to pond sediments in both the New Cut ($F_{1,17} = 56.6$; $p < 0.001$) and Owings Mills pond experiments ($F_{1,17} = 101.41$; $p < 0.001$). However, there was no effect of days to metamorphosis on size at metamorphosis in either experiment (New Cut, $F_{1,17} < 0.1$, $p = 0.932$; Owings Mills, $F_{1,17} = 0.6$, $p = 0.443$).

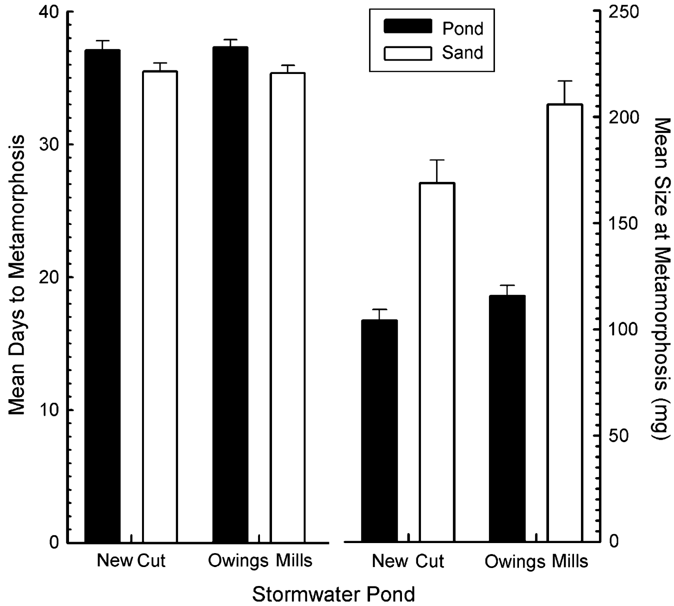

Fig. 3. Mean days to and size at metamorphosis for *Bufo americanus* larvae successfully complete metamorphosis after exposure to clean sand or sediments from two roadway-associated stormwater management ponds during the spring of 2005. Error bars are 1 SE.

4. Discussion

4.1. Stormwater pond pollutants as factors shaping amphibian assemblages

Our goal in conducting the microcosm exposures reported here was to investigate the potential selective effects of variation in pollution levels in shaping the amphibian assemblages associated with stormwater ponds. In all surveys of amphibian use of stormwater ponds to date, the number of species that utilized individual ponds ranged from 0 to 11, with a nested pattern of species occurrence such that rare species were found mainly at species rich sites and species poor assemblages contained only common species (Bascietto and Adams, 1983; Bishop et al., 2000a; Massal et al., 2007; Ostergaard, 2001; Simon, 2006). A nested pattern of species occurrence suggests a common environmental axis along which species that are intolerant of severe conditions are eliminated from the community (Hecnar and M'Closkey, 1997). Because field studies have found co-variation among land use adjacent to ponds, pollutant levels in ponds, and pond use by amphibians (Karouna-Renier and Sparling, 2001 Simon, 2006), it is difficult to distinguish the direct and indirect effects of land use change on amphibians. Direct effects of adjacent land use include the provisioning of upland habitats for juveniles and adults and sources of mortality such as roads. Indirect effects include contributions of pollutants from different land use types to pond sediments, which could ultimately determine pond habitat quality for embryos and larvae.

Our results indicate a noteworthy role for accumulation of upland derived pollutants from adjacent land use in controlling stormwater pond habitat quality for embryos and larvae. *Rana sylvatica* can be classified as a species that is intolerant of urbanization, as indicated by negative correlations with urban land use and positive correlations with forest cover adjacent to wetlands (Houlahan and Findlay, 2003; Knutson et al., 1999). In contrast, *B. americanus* often exhibits a positive correlation with urban land use (Houlahan and Findlay, 2003), and remains abundant even in highly urbanized areas (Bascietto and Adams, 1983; Massal et al., 2007). Congruent with these patterns of sensitivity to urbanization, we found *R. sylvatica* embryos and hatchlings to be highly sensitive to exposure to polluted pond sediments from a roadway-associated stormwater management pond, suffering 100% mortality over a 13 day period. On the other hand, *B. americanus* suffered relative minor lethal effects and smaller size following metamorphosis as a result of exposure to the same pond sediments. Although individual frogs that are smaller in size at metamorphosis can suffer lower survival and recruitment to adult populations (Berven, 1990; Smith, 1987), it remains unclear if these effects would be realized in urban settings where predator and competitor populations have been greatly reduced or eliminated.

While our results indicate that pollution of breeding sites could be responsible for a decline of sensitive species in urbanized settings, other factors may also contribute to or be responsible for declines. Roads, which can account for as much as 50% of the land cover surrounding ponds (Simon, 2006), can be significant sources of mortality for juvenile and adult frogs and toads (Fahrig et al., 1995; Gibbs and Shiver, 2005). For species that spend the majority of their juvenile and adult lives in terrestrial habitat, upland habitat loss may be responsible for declines and result in a negative correlation between amphibian occurrence and forest cover surrounding ponds that is often observed in many systems (Gibbs, 1998; Homan et al., 2004). Finally, other factors can be responsible for declines in breeding-site quality including introduction of non-native amphibians and fishes (Kats and Ferrer, 2003). Therefore, because upland habitat loss, pollution of breeding sites, and introduction of non-native species are all positively correlated within land use changes accompanying urbanization, more mechanistic studies are needed to evaluate the relative roles of these factors in producing amphibian declines in urban landscapes.

4.2. Toxic elements of stormwater pond sediment

A number of pollutants may have contributed to the toxic effects that we observed among *R. sylvatica* and *B. americanus* embryos and larvae. We monitored Cl^- and metal levels in pond water and sediment during our experiment because road de-icing agents (primarily NaCl) and metals derived from the deterioration of car parts have been documented as accumulating on road surfaces and ultimately being transported to stormwater ponds via runoff (Bishop et al., 2000b; Casey et al., 2007; Marsalek, 2003). Although significantly elevated above control conditions, in no case did water or sediment metal levels exceed the US Environmental

Protection Agency (1999) National Recommended Water Quality Criteria or consensus-based sediment quality guidelines (MacDonald et al., 2000). Levels of Cl^- did suggest substantial pollution of sediments and pore water by road salts. We assume that salt accumulated in the sediments and pore water from the pond diffused into the water column of our microcosms after they were set up. Even though water was changed frequently during each experiment, there was no decline in Cl^- concentrations with time (as measured by conductivity), and Cl^- concentrations were within the range of those reported from stormwater ponds and wetlands in northern latitudes of North America (Benbow and Merritt, 2004; Bishop et al., 2000b; Karraker, 2007; Sanzo and Hecnar, 2006). For example, Bishop et al. (2000b) reported an upper and lower mean Cl^- concentration of 37 and 449 mg l^{-1}, respectively, and a maximum of 1230 mg l^{-1} for 15 stormwater ponds in the Guelph and Greater Toronto Area, Ontario. However, the levels of Cl^- in our pond sediment microcosms were below those associated with the degree of adverse effects that we observed among *R. sylvatica* hatchlings. Sanzo and Hecnar (2006) reported 96 h LC50 values of 1599 mg l^{-1} Cl^- and chronic sublethal effects at 625 mg l^{-1} Cl^- for larval *R. sylvatica* (Gosner stage 25 and greater). We observed 100% and 17% mortality among *R. sylvatica* and *B. americanus* hatchlings at Cl^- concentrations in the range of ~224–243 mg l^{-1}.

Other factors that may be responsible for the lethal effects we observed among *R. sylvatica* hatchlings include exposure to polycyclic aromatic hydrocarbons that accumulate in stormwater ponds (Bishop et al., 2000b), interactions among pollutants, and elevated Cl^- levels at the sediment–water interface. Although there is little toxicity information available for native North American species (Sparling, 2000), low levels of PAH can be toxic to *Xenopus laevis* (Bryer et al., 2006; Hatch and Burton, 1998; Sadinski et al., 1995) and *R. sylvatica* has been shown to be more sensitive to organic pollutants than other ranids (Hogan et al., 2006). Synergistic interactions among pollutants are also possible in stormwater ponds. For example, metals have been shown to disrupt Na^+ ion regulation in freshwater fishes and invertebrates (e.g., Brooks and Mills, 2003; Sola et al., 1995; Wilson and Taylor, 1993) and therefore, metals accumulated in pond sediments may compromise the ability of developing hatchlings to deal with hypertonic conditions typical of stormwater ponds contaminated by road de-icing agents. Finally, stormwater ponds contaminated by road de-icing compounds often exhibit a strong vertical gradient in Cl^- concentrations, with lowest levels occurring at the surface and highest levels at the bottom of ponds (Marsalek, 2003). Because we collected water samples from mid-depth of our microcosms, and eggs were placed on the bottom, it is possible that eggs experienced exposure to higher Cl^- concentrations than indicated by our water samples. Moreover, because amphibians may place eggs directly on the bottom or attach them to sticks or vegetation in the water column, future studies should document and strive to incorporate egg placement and vertical profiles of Cl^- concentration into field and laboratory experiments.

5. Conclusions

Our results indicate that the influence of stormwater ponds on amphibian populations will vary among species and depend on individual species' tolerances for pollutants that accumulate in ponds. For species that persist in urbanized areas and are tolerant of pollutants such as metals, road salts, and PAHs, stormwater ponds may provide additional or alternative breeding sites that ultimately enhance population sizes in urban settings. In contrast, for species such as *R. sylvatica* that are not tolerant of pollutants that accumulate in stormwater ponds, ponds will provide no additional habitat and could act as ecological traps if frogs are not eliminated by upland habitat loss and choose to use ponds for breeding purposes. An ecological trap occurs when environmental cues used by wildlife to select habitats do not provide an accurate depiction of the suitability of a habitat for reproduction and survival (e.g., Battin, 2004; Robertson and Hutto, 2006; Schlaepfer et al., 2002). When both the quality of the cue is increased and the quality of the habitat decreases, a "severe trap" is created (Robertson and Hutto, 2006). Traps can result from rapid changes in the environment, similar to those occurring during urbanization, which preclude an organism's ability to adapt or modify behavior as the habitat changes (Battin, 2004; Schlaepfer et al., 2002). More importantly, drastic environmental changes have the potential to produce severe traps that can cause population crashes leading to extirpation even when natural habitats remain. Because stormwater ponds present cues that might be attractive to pond-breeding amphibians (i.e., they are often colonized by vegetation and contain surface waters) and accumulate pollutants that may prove toxic, they have the potential to act as severe ecological traps. Consequently, for intolerant species stormwater ponds could contribute to population declines observed in urban areas; but a more complete understanding of the effects of land use change on juvenile and adult amphibians is needed to fully assess the role of stormwater ponds as ecological traps.

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