

Pesticide concentrations in frog tissue and wetland habitats in a landscape dominated by agriculture



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HIGHLIGHTS

- Habitat quality was similar between restored and reference wetlands in Iowa.
- Complex mixtures of pesticides are detected in frog tissues (liver and whole body).
- The number of fungicides (up to 8) in frog tissues is largest reported to date.
- Life history has the potential to impact pesticide bioaccumulation in frogs.

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ABSTRACT

Habitat loss and exposure to pesticides are likely primary factors contributing to amphibian decline in agricultural landscapes. Conservation efforts have attempted to restore wetlands lost through landscape modifications to reduce contaminant loads in surface waters and providing quality habitat to wildlife. The benefits of this increased wetland area, perhaps especially for amphibians, may be negated if habitat quality is insufficient to support persistent populations. We examined the presence of pesticides and nutrients in water and sediment as indicators of habitat quality and assessed the bioaccumulation of pesticides in the tissue of two native amphibian species *Pseudacris maculata* (chorus frogs) and *Lithobates pipiens* (leopard frogs) at six wetlands (3 restored and 3 reference) in Iowa, USA. Restored wetlands are positioned on the landscape to receive subsurface tile drainage water while reference wetlands receive water from overland run-off and shallow groundwater sources. Concentrations of the pesticides frequently detected in water and sediment samples were not different between wetland types. The median concentration of atrazine in surface water was 0.2 µg/L. Reproductive abnormalities in leopard frogs have been observed in other studies at these concentrations. Nutrient concentrations were higher in the restored wetlands but lower than concentrations thought lethal to frogs. Complex mixtures of pesticides including up to 8 fungicides, some previously unreported in tissue, were detected with concentrations ranging from 0.08 to 1500 µg/kg wet weight. No significant differences in pesticide concentrations were observed between species, although concentrations tended to be higher in leopard frogs compared to chorus frogs, possibly because of differences in life histories. Our results provide information on habitat quality in restored wetlands that will assist state and federal agencies, landowners, and resource managers in identifying and implementing conservation and management actions for these and similar wetlands in agriculturally dominated landscapes.

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1. Introduction

Degradation and loss of habitat are among the primary reasons amphibian populations have declined worldwide (Collins and Storfer, 2003). Between 1850 and 1950 the amount of farmland in the United States increased from less than 300 million to more than 1.1 billion

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acres (U.S. Department of Agriculture, 2013). However, in the last 5 decades the amount of total cropland in the United States has decreased as the amounts of urban, residential and special-use lands (mostly parks and wildlife areas) are increasing (U.S. Department of Agriculture, 2007). In agriculturally dominated states like Iowa, 75% of the acreage is cropland (U.S. Department of Agriculture, 2007). These land-use changes may not always destroy habitat but usually include alterations, such as the application of chemicals that can threaten amphibian survival. Amphibian declines and abnormalities have been attributed to contaminants, often with a focus on water quality, specifically water at breeding sites. In a recent survey 30–60% of shallow groundwater and 60–95% of streams across different land-use categories in the United States were contaminated with at least one pesticide (Gilliom, 2007).

Contaminants have the potential to cause lethal effects in amphibians, such as reduced survival or sub-lethal effects such as immunosuppression, malformations, compromised reproduction and reduction in growth and development (Hecnar, 1995; Taylor et al., 2005; Johnson et al., 2007; Gahl et al., 2011; Groner and Relyea, 2011). For example, the herbicide atrazine has the potential to cause immunosuppression in adult northern leopard frogs (Brodtkin et al., 2007) and impair sexual development of male frogs (Hayes et al., 2003). Glyphosate formulations (Howe et al., 2004; Relyea, 2005) as well as some fungicide formulations (Belden et al., 2010; Bruhl et al., 2003) are toxic to amphibians at environmentally relevant concentrations. Decreases in growth and development have also been observed after exposure to fungicide formulations containing pyraclostrobin (Hartman et al., 2014). Laboratory studies designed to identify acute and chronic effects frequently focus on a single compound or a specific class of compounds that are often conducted in simplified mesocosm settings (Relyea and Mills, 2001; Relyea, 2005; Boone et al., 2005; Boone, 2008). However, pesticides in the environment exist as mixtures and efforts in the field to elucidate some of these relationships in non-laboratory settings have been limited.

Exposure to pesticides can lead to suppression of the immune system, preventing amphibians from developing a normal response against pathogens (Mann et al., 2009). Christin et al. (2004) exposed frogs (*Xenopus laevis* and *Rana pipiens*) to a mixture of herbicides and insecticides and found that at environmentally relevant concentrations, combinations of these pesticides altered aspects of the immune system. However, Davidson et al. (2007) found no correlation between a common insecticide, carbaryl, and amphibians' susceptibility to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, Bd). Increased eutrophication due to nitrogen based fertilizers, coupled with pesticide application, may cause trophic cascades resulting in increased rates of parasitism in wetlands and has been linked to immunosuppression in amphibians (Brodtkin et al., 2007; Johnson et al., 2007). Although a direct link has been made between pesticide exposure and infection by trematodes (Rohr et al., 2008; Kiesecker, 2002), a general understanding of these interactions in the field is lacking because results vary by species and land-use (King et al., 2007).

Despite landscape alterations and the suite of potentially negative effects related to such alterations, there are examples of amphibians persisting in modified habitats. For example, in Eastern Europe amphibians breed successfully in man-made drainage ditches (Hartel et al., 2001), in the Midwest, certain species persist despite agrochemical inputs and habitat modifications (Kolozsvary and Swinhart, 1999; Gilliland et al., 2001) and in Florida not all species of anurans appeared to be adversely affected by development as long as permanent habitat was available for breeding (Delis et al., 1996). In areas of California where habitats have been altered by human activities many of the amphibian species (with the exception of *Ambystoma californiense*) have significantly declined (Davidson et al., 2002). Although animals can persist in altered landscapes, careful assessments of long-term persistence and population health are warranted. The presence of amphibians, or the appearance of population persistence can mask a host of problems that may manifest in the long-term such as increased

susceptibility to disease, reduced probability of survival and recruitment or other genetic issues (i.e. breeding) related to lack of habitat connectivity.

Much of Iowa illustrates the changes made to the land for crop production over the last two centuries; greater than 90% of the wetlands have been drained and replaced with row crop agriculture, primarily corn and soybeans (Whitney, 1994). Despite the dramatic changes, there is still a rich herpetofauna represented in Iowa and much of the Midwest. Although some amphibians persist, approximately 45% of the amphibian and reptile species in Iowa are imperiled because of habitat fragmentation and anthropogenic activities (Green, 2005; IDNR, 2006). Thus, we chose the Des Moines Lobe of central Iowa to assess the presence of pesticides and nutrients in water and sediment as indicators of habitat quality and to assess the bioaccumulation of pesticides in the tissue of two amphibian species (chorus frogs (*Pseudacris triseriata*) and leopard frogs (*Lithobates pipiens*)). Furthermore, in 2001, a major initiative between the state of Iowa and United States Department of Agriculture (USDA) Farm Service Agency enacted the Conservation Reserve Enhancement Program (CREP) to help identify and restore wetlands lost through landscape modifications as a means to reduce nitrogen concentrations and loads in surface waters. The Des Moines Lobe hosts 72 of these CREP sites in 29 counties. The resulting wetlands appear to provide additional ecosystem services such as habitat for migrating waterfowl (O'Neal et al., 2008), however, for many organisms, such as amphibians the related costs of living within a matrix of highly modified habitat have not been determined. For example, the value of increased habitat for species with low vagility (e.g., amphibians) is assumed to be high, but benefits may be negated if the quality of the habitat is insufficient to support amphibian populations' overtime.

Our objective was to determine if restored wetlands in an agricultural landscape provide similar quality habitat for amphibians as adjacent reference wetlands as determined by the occurrence and distribution of 1) pesticides and nutrients in water, 2) pesticides in bed sediment and 3) pesticides in tissues of leopard and chorus frogs, two amphibians found commonly in this area. Understanding the occurrence and distribution of contaminants provides information on habitat quality in restored wetlands that can assist state and federal agencies, landowners, and resource managers in identifying and implementing conservation and management actions for these and similar wetlands and their associated amphibian fauna. Our data also provides useful covariates (i.e. pesticide and nutrient concentrations) for assessing population demographics and the long-term trajectory of populations faced with the challenges of living in an altered landscape.

2. Materials and methods

2.1. Site information

Six wetlands in the Des Moines Lobe landform of central Iowa (Fig. 1) were sampled in 2012 and 2013 (3 restored and 3 reference). The restored wetlands were developed through the CREP and were positioned on the landscape to receive substantial amounts of tile drainage water to reduce nitrate concentrations to surrounding surface water bodies. Approximately, 80% of the flow into the restored wetlands is from tile drains and all wetlands sampled had several tile lines and ditches leading directly into them. Two tile drains were observed at Marshall and Story while four large ditches with at least one outflow drain were observed at Greene. All drains were considered laterals and were about 20–25 cm in diameter. The reference wetlands are likely remnant wetlands but were restored from past agricultural use by landowners and are not typically positioned in the landscape to accept a significant amount of tile drainage from active agricultural fields and are not part of the CREP. Reference wetlands receive the majority of their water from overland flow (i.e. run-off) and, to a lesser extent, from shallow groundwater sources and tile drain outlets. Bjorkboda

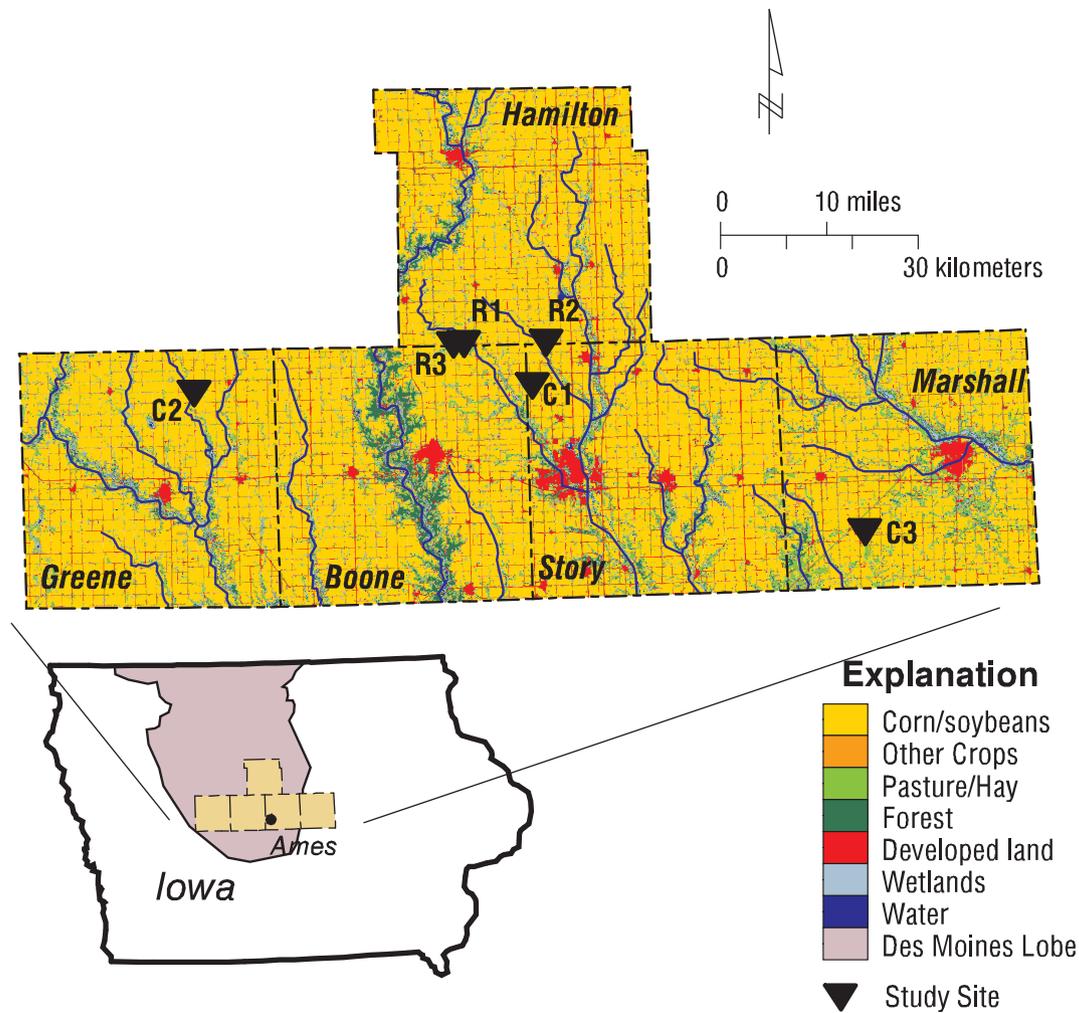


Fig. 1. Map of the six Iowa wetland sites sampled in 2012 and 2013 including land-cover information (Han et al., 2012). Study site labels beginning with a “C” are restored wetlands and “R” for reference wetlands. For corresponding site names see Table 1.

likely had at least 3 tile outlets into a constructed drainage ditch that runs into the wetland from the south. Boone appeared to have at least one small tile drain near the outlet of the wetland and there are at least 2 outlets on a small neighboring wetland to the north. No tile drains were observed at or around Bob Pyle. All wetlands were less than 2.5 ha in surface area (Table 1). Only restored wetlands greater than two years old were included to insure that vegetative buffers were well established. Average depths of the restored wetlands ranged from 56 to 70 cm with maximum depths between 158 and 240 cm. Average depths of the reference wetlands were less than 22 cm with maximum depths between 28 and 56 cm. Exact locations of the restored

wetlands are proprietary and written permission was obtained from the landowners and public land managers prior to the start of sampling.

2.2. County-level pesticide use data

Estimated county-level pesticide use data from 2011 was compiled for all compounds detected during the study (Table 2). To capture all potential pesticides applied to croplands in each county EPest-high estimates were used (Baker and Stone, 2013). If there was no pesticide use reported on a crop in a particular reporting district, EPest-high estimates report use as un-surveyed or in some cases will report use

Table 1

List of the three restored and three reference wetlands sampled in 2012 and 2013 for pesticides and nutrients in the Des Moines Lobe, IA, USA.

Number	USGS station name	Site name	Wetland type	County	Established	Wetland area (hectare)
C1	Wetlands nr Gilbert, IA	Story	Restored	Story	2005	1.5
C2	Wetlands nr Grand Junction, IA	Greene	Restored	Greene	2007	1.38
C3	Wetlands nr Melbourne, IA	Marshall	Restored	Marshall	2009	1.74
R1	Wetlands nr Bjork Boda, IA	Bjorkboda	Reference	Hamilton	Remnant	2.31
R2	Wetlands nr Story City, IA	Bob Pyle	Reference	Story	Remnant	0.607
R3	Wetlands nr Stratford, IA	Boone	Reference	Boone	Remnant	2.09

USGS = U.S. Geological Survey.

Table 2

2011 Estimated county level pesticide use (E-Pest high) data (kg) of the compounds detected during the study (Baker and Stone, 2013). Several compounds detected had no reported county level pesticide use and included azinphos methyl, EPTC, fenbuconazole, flusilazole, imazalil, resmethrin, pyrimethanil, prometon, triconazole, and zoxamide.

Compound	Boone	Greene	Hamilton	Marshall	Story
Alachlor	1072	1162	1323	1020	1107
Atrazine	33,669	30,547	41,517	32,030	34,745
Azoxystrobin	129	515	138	143	136
Bifenthrin	230	271	275	225	238
Captan	4	5	5	5	5
Carbaryl	–	865	–	–	–
Carbofuran	282	–	339	254	279
Chlorothalonil	21	–	2	3	23
Chlorpyrifos	352	1727	302	354	331
Clothianidin	1481	1480	1825	1412	1530
Fenhexamide	1	1	1	1	1
Fipronil	–	–	86	–	72
Fludioxinil	22	25	23	26	24
Fluoxastrobin	55	–	68	53	57
Glyphosate	135,396	132,439	155,337	140,429	142,045
Hexazinone	–	12	–	–	–
Imidacloprid	315	278	327	359	336
Metalaxyl	49	71	50	55	52
Metolachlor ^a	23,791	16,683	28,296	23,650	24,742
Pendimethalin	1944	1810	2101	2142	2064
Pyraclostrobin	1777	1003	2111	1771	1850
Tebuconazole	44	48	55	42	46
Thiamethoxam	540	832	620	559	566
Trifluralin	4585	6335	4768	5244	4905

^a Sum of metolachlor and S-metolachlor.

rates from neighboring counties as an estimate of the unreported use in a particular crop reporting district (Theelin and Stone, 2013).

2.3. Sample collection

Surface water samples were collected from the six wetlands at three times (early, mid and late) during the growing season in 2012 and 2013. Water samples from the six wetlands were collected over no more than a two day period during each of the three sampling events in both years. These samples were analyzed for pesticides and pesticide degradates as well as nutrients. Early samples were collected in April of 2012 and May of 2013, while mid and late samples were collected in June and July, respectively in both years. In 2012, the Midwest experienced a severe drought. Between July 2012 and February 2013, there were 30 weeks in which 25% or more of the Midwest was listed as experiencing “severe drought” (National Drought Mitigation Center (NDMC), 2014). For this reason, all three reference wetlands were dry by the July 2012 sampling event, and only water from the restored wetlands was collected and analyzed. Grab samples were collected from each site in pre-cleaned bottles from the outflow point of the wetland before being packed on ice and shipped to the laboratories for analysis. Water samples for nutrients and dissolved organic carbon were collected following standard USGS procedures (U.S. Geological Survey, 2006; Ward and Harr, 1990). Basic water quality parameters including specific conductance ($\mu\text{S}/\text{cm}@25\text{ }^\circ\text{C}$) and pH were also measured using a calibrated YSI probe (model 556) [YSI, Yellow Springs, Ohio, USA] at three points around the outflow of the wetland.

Bed sediment samples were collected (U.S. Geological Survey, 2006; Hladik et al., 2009) from the six wetlands in early August of 2012 (during the beginning of the drought) from several depositional areas near the inflow of water and were homogenized in the field. In 2013 bed sediment samples were collected from several depositional areas at each site twice, once in May and again in July. All bed sediments were collected within one to two weeks of their corresponding water sample. Distance from the inflow varied by wetland and sampling locations and sites were chosen based on the accumulation of fine grain

(depositional) sediment near an observable inflow at or near the edge of water.

Four to five adult male frogs of each species were collected in 2012 from 4 of the 6 wetlands by hand at night during peak calling events. Males were selected over breeding females to limit the potential impact collection might have on the population. In May, four chorus frogs were collected from one restored wetland (Greene) and five chorus frogs were collected from one reference wetland (Boone). In June, a total of twenty leopard frogs (five from each wetland) were collected from two restored (Greene, Story) and two reference wetlands (Bjorkboda, Boone). Individuals were shipped alive to the USGS National Wildlife Health Center (NWHC) in Madison, WI and assessed for parasites, Bd and abnormalities, and cultured for *Ranavirus*. In the laboratory, individuals were euthanized using a dilute buffered solution of Tricaine methanesulfonate (MS222; 0.5 g/l L water) and the livers removed, wrapped in clean aluminum foil and frozen on dry ice prior to pesticide analysis. In 2013, five adult male chorus frogs were collected from each of six wetlands, euthanized in the field using a dilute buffered solution of MS222 (Fellers and Freel, 1995). Individual mass was measured in the laboratory at Iowa State University in Ames, IA and the individuals were wrapped in aluminum foil, and frozen for pesticide analysis. All individuals were captured at night in May, within a three-day period.

2.4. Extraction and analysis

2.4.1. Surface water samples for pesticides and nutrients

Filtered water samples were analyzed for a suite of 98 pesticides and pesticide degradates by gas chromatography mass spectrometry using previously published methods (Hladik et al., 2008; Reilly et al., 2012). Briefly, 1 L of sample water was extracted onto an Oasis HLB solid-phase extraction (SPE) cartridges (6 cm³, 500 mg, 60 μm , Waters Corporation, Milford, Massachusetts, USA). All samples were spiked with ¹³C₃-atrazine (Cambridge Isotope, Andover, Massachusetts, USA) as a recovery surrogate. SPE cartridges were eluted with ethyl acetate. Additionally, the empty bottle was rinsed with dichloromethane (DCM) to remove any pyrethroids that may have sorbed to the bottle during sample processing, this fraction was added to the SPE eluent. All sample extracts were analyzed on an Agilent (Santa Clara, California, USA) 7890 gas chromatograph coupled to an Agilent 5975 (Folsom, CA) mass spectrometer (GC-MS) operating in electron ionization (EI) mode. Data was collected in selective ion monitoring (SIM) mode with each compound having one quantifier ion and 1–2 qualifier ions. Method detection limits (MDLs) for all compounds ranged from 0.001 to 0.01 $\mu\text{g}/\text{L}$ (Reilly et al., 2012).

Six neonicotinoids, diuron and three diuron degradates were measured in the water samples using a previously published method (Hladik and Calhoun, 2012). Briefly, a 1-L water sample was extracted onto an Oasis HLB SPE cartridge and eluted with 10 mL of 50:50 DCM:acetone. Samples were spiked with d₄-imidacloprid (Cambridge Isotope) and monuron (USEPA Pesticide Repository, Ft. Meade, Maryland, USA) as recovery surrogates prior to extraction. Extracts were analyzed on an Agilent 1260 bio-inert liquid chromatograph (LC) coupled to an Agilent 6430 tandem mass spectrometer (MS/MS). The MS/MS was operated under electrospray ionization (ESI) in positive mode, data were collected in multiple reaction monitoring (MRM) modes. The MDLs ranged from 0.003 to 0.006 $\mu\text{g}/\text{L}$ (Hladik and Calhoun, 2012).

Water samples were also analyzed for glyphosate, aminomethylphosphonic acid (AMPA) and glufosinate by LC/MS/MS with ESI in negative-ion mode using MRM. Filtered water samples were stored at 4 °C then derivatized within 5 days after collection using a 5-percent borate buffer to adjust the pH to 9.0, followed by the addition of 2.5 millimolar (mM) 9 fluorenylmethylchloroformate in acetonitrile. Derivatization was carried out in the dark in a water bath at 40 °C for approximately 24 h. Following derivatization, the samples were extracted onto SPE cartridges, and the SPE cartridges were rinsed with

500 µL of deionized water. MDLs for AMPA, glyphosate, and glufosinate in surface water were 20 ng/L (Meyer et al., 2009).

Nutrients, including total nitrogen, total phosphorous, orthophosphate, and nitrate/nitrite, were analyzed in filtered and unfiltered water samples by the National Water Quality Laboratory using approved methods outlined in Patton and Kryskalla (2003). MDLs for total nitrogen, total phosphorous, and nitrate/nitrite as nitrogen were 0.05, 0.003, and 0.008 mg/L, respectively. Filtered water samples were analyzed for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) by high-temperature catalytic combustion using a Shimadzu TOC-VCNS total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, Maryland) according to a modified version of United States Environmental Protection Agency (USEPA) Method 415.3 (Bird et al., 2003; Potter and Wimsatt, 2005).

2.4.2. Bed sediment samples for pesticides

Bed sediment samples were extracted and analyzed for 94 pesticides and pesticide degradates based on previously published methods (Smalling et al., 2013a; Hladik and McWayne, 2012). Briefly, wet sediments (10 g) were spiked with trifluralin-d₁₀, ring-¹³C-*p,p'*-DDE and phenoxy-¹³C-*cis*-permethrin (Cambridge Isotopes, Andover, MA, USA) as recovery surrogates, homogenized with sodium sulfate and extracted using pressurized liquid extraction. Following extraction, extracts were dried over sodium sulfate, reduced and sulfur was removed by gel permeation chromatography. Samples were again reduced to 0.5 mL, split in half and subjected to two different clean-up methods: 1) 6% deactivated Florisil for all fungicides and 2) carbon/alumina stacked SPE cartridges for all herbicides and insecticides. Sample extracts were analyzed on an Agilent 5975 GC/MS (Santa Clara, California, USA) in EI mode. Data were collected in SIM mode with each compound having one quantifier ion and 1–2 qualifier ions. MDLs for all compounds in ranged from 0.6 to 3.8 µg/kg sediment dry weight (Smalling et al., 2013a; Hladik and McWayne, 2012).

Bed-sediment samples were analyzed for organic carbon and nitrogen content by combustion and thermal conductivity using a Perkin Elmer CHNS/O elemental analyzer (Perkin Elmer Corporation, Waltham, Mass.) according to a modified version of USEPA 440.0 (Zimmerman et al., 2007). Dry, homogenized sediments were combusted at 925 °C in silver boats after being exposed to concentrated hydrochloric acid (HCl) fumes in a desiccator for 24 h to remove inorganic carbon. Before analysis, sediments were dried at 100 °C for 3 h. Acetanilide was used for instrument calibration. MDLs for carbon and nitrogen were 0.01%.

2.4.3. Tissue samples for pesticides

Whole bodies and livers were extracted and analyzed for 98 pesticides and pesticide degradates based on previously published methods (Smalling et al., 2013b). Briefly, tissue samples (whole frogs or livers) were thawed and homogenized with Na₂SO₄ using a clean, solvent-rinsed mortar and pestle. Samples were spiked with trifluralin-d₁₀, ring-¹³C-*p,p'*-DDE and phenoxy-¹³C-*cis*-permethrin as recovery surrogates and extracted with DCM using pressurized liquid extraction. Following extraction, sample extracts were dried over Na₂SO₄, reduced to 1 mL and 10% by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.001 g using a microbalance. A majority of the lipid was removed using gel permeation chromatography followed by 6% deactivated Florisil previously activated at 550 °C for 16 h. Prior to analysis, samples were reduced to 0.2 mL, and a deuterated internal standard was added to each extract. Sample extracts were analyzed on an Agilent 7890 GC coupled to an Agilent 5975 MS operating in EI mode. Data for all pesticides were collected in SIM mode with each compound having one quantifier ion and 1 to 2 qualifier ions. MDLs for all compounds ranged from 0.5 to 4.2 µg/kg wet weight (Smalling et al., 2013b).

2.5. Quality control

All sample glassware was hand-washed and rinsed with tap water followed by acetone and hexane prior to use. All solvents and other reagents were American Chemical Society (ACS) grade or better (Thermo Fisher Scientific). Pesticide standard materials were donated by the USEPA National Pesticide Repository. Purities ranged from 95% to 99%.

Performance-based quality assurance and quality control included the parallel analysis of procedural blanks, matrix spikes, and replicates in 10% of the samples analyzed for each matrix (water, sediment and tissue). Procedural blanks run with each batch of samples did not contain detectable levels of pesticides or nutrients. Mean (± standard deviation) of ¹³C-atrazine, imidacloprid-d₄ and monuron added to each water sample as recovery surrogates prior to extraction was 105 ± 15%, 78 ± 6%, and 84 ± 8%, respectively. Mean (± standard deviation) of trifluralin-d₁₀, ring-¹³C-*p,p'*-DDE and phenoxy-¹³C-*cis*-permethrin added prior to sediment samples extraction as recovery surrogates was 83 ± 16%, 85 ± 14%, 89 ± 12%, respectively. Mean (± standard deviation) of trifluralin-d₁₀, ring-¹³C-*p,p'*-DDE and phenoxy-¹³C-*cis*-permethrin added prior to tissue sample extraction as recovery surrogates was 92 ± 15%, 101 ± 14%, and 100 ± 18%, respectively. Matrix spikes were analyzed in 10% of the water, sediment and tissue samples and the recoveries ranged from 70% to 131% (median of 92%), 61%–129% (median of 93%) and 60% to 129% (median of 85%), respectively. Relative percent difference of all replicate samples was less than 25% for both water (pesticides and nutrients) and sediment (pesticides). Water samples were held for no longer than 48 h at 4 °C prior to extraction for all pesticides except glyphosate and AMPA. Water samples for glyphosate and AMPA were stored frozen at –20 °C and held for no longer than 1 week prior to derivatization. Water samples for nutrients and DOC were held for no longer than 1 week at 4 °C prior to analysis. Sediment and tissue for pesticides were stored frozen at –20 °C and held for no longer than 1 year prior to extraction.

2.6. Statistical analyses

An alpha level of 0.05 and a 95% confidence interval was used for all statistical analyses. Because the data was not normally distributed, a Kruskal–Wallis one-way analysis of variance by ranks was used to determine if there were significant differences in pesticide, nutrient and DOC concentrations in water between wetland types and years. The same test was used to determine if there were significant differences in pesticide concentrations between, species, wetland type and years. No statistical tests were conducted on the sediment data because sample size was small. All non-detections were assigned a value of 1/2 the method detection limit for all statistical tests. Statistical analyses were performed using R software (R Development Core Team 2013, Vienna Austria).

3. Results and discussion

3.1. Water and sediment quality

Thirty-two pesticides and pesticide degradates were detected in water samples collected in both 2012 and 2013 from the 6 wetlands with concentrations ranging from 0.1 to 19 µg/L (Table A1). Atrazine was detected in all water samples collected in 2012 and 2013 and at the highest concentration (19 µg/L) relative to the other pesticides detected (Fig. 2A–B). The most frequently detected pesticides in water samples were atrazine, metolachlor, and glyphosate (all herbicides), and AMPA (glyphosate's primary degradate). These three herbicides were the most heavily used in the study area (Table 2) and have historically been among the most frequently detected pesticides in surface and groundwater in the Midwest (Battaglin et al., 2014; Battaglin et al., 2005; Kolpin et al., 1995). Two neonicotinoid insecticides,

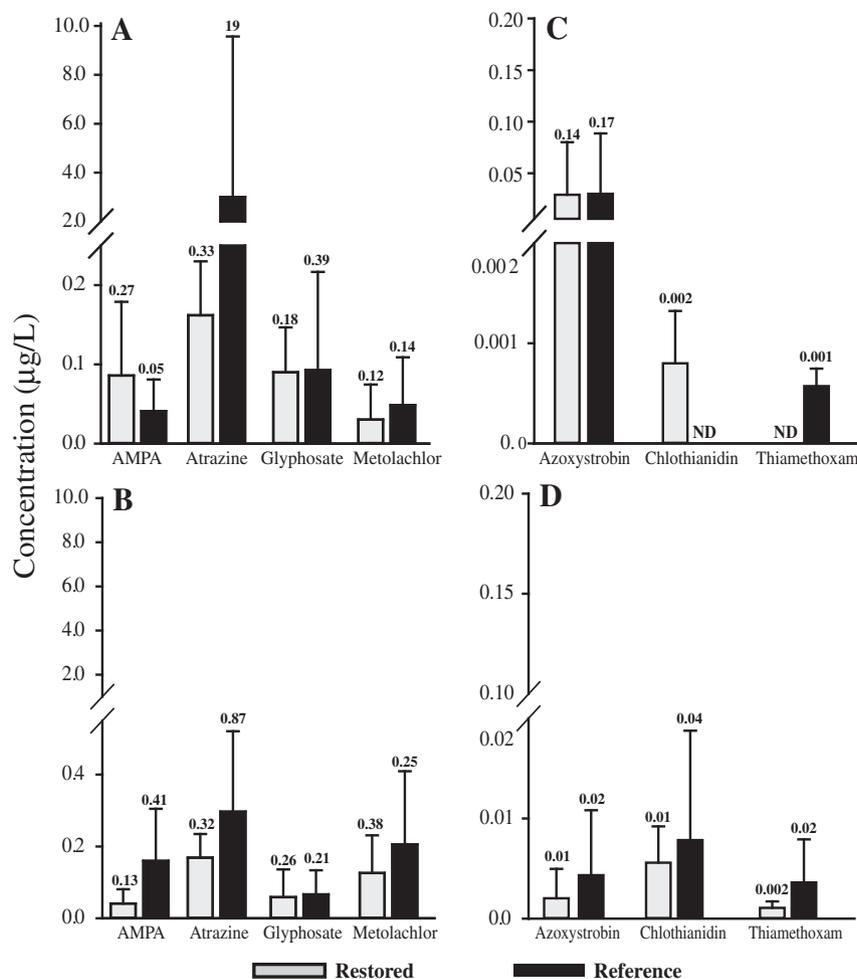


Fig. 2. Average dissolved surface water concentrations ($\mu\text{g/L}$) of AMPA, atrazine, glyphosate and metolachlor (herbicides) were collected in A) 2012 and B) 2013 and azoxystrobin (fungicide), clothianidin and thiamethoxam ((insecticides) were collected in C) 2012 and D) 2013 from the restored and reference wetlands. Plot includes both detects and non-detects assigned a value of 1/2 the limit of detection. The maximum concentration detected for each compound is reported above each error bar.

clothianidin and thiamethoxam, were detected infrequently in 2012 and at concentrations near the MDL (Table A1, Fig. 2C), however in 2013 detection frequencies for clothianidin and thiamethoxam were 45% and 44% respectively, with concentration ranging from 0.002 to 0.04 $\mu\text{g/L}$ (Fig. 2D). Only two studies to date have reported the occurrence of neonicotinoids in agricultural wetlands; one in the Canadian prairie pothole region (Main et al., 2014) and another in playa lakes in the Southern High Plains of the United States (Anderson et al., 2013). Although, maximum concentrations reported previously were an order of magnitude higher than our results, neonicotinoids were detected approximately 45% of the time throughout the spring/summer. Similarly, in a recent study by Hladik et al. (2014), neonicotinoids were reported frequently (40–100%) in Iowa surface water samples collected between April and July of 2013. Neonicotinoids are receiving increased scrutiny because they have been implicated in adversely affecting pollinators and linked to colony collapse disorder in bees (Spivak et al., 2011; vanEngelsdorp et al., 2009) but currently potential effects on amphibians are largely unknown.

To understand if the restored wetlands provided similar habitat as the reference wetlands and to determine if habitat quality changed temporally, pesticide concentrations in water samples were compared by type and by year. Average total pesticide concentrations in water samples in both 2012 and 2013 were higher in the reference compared to the restored wetlands but these results were not significant by wetland type or by year (data not shown). Furthermore, total concentrations of fungicides, herbicides and insecticides were compared and

no significant differences in wetland type or year was observed. To attempt to tease out differences in pesticide occurrence between wetland type and year, data from the seven compounds detected in greater than 25% of the samples were further compared. There were no statistically significant differences in concentrations between wetland types for these seven compounds, even though average concentrations for all compounds except AMPA and clothianidin were higher in the reference wetlands (Fig. 2). The higher concentration observed in the reference wetlands, although not significant, could be due to differences in source water (surface run-off vs. subsurface tile drainage) as well as depth. Concentrations of the neonicotinoid insecticides, clothianidin and thiamethoxam, and the herbicide metolachlor were higher in 2013 compared to 2012 ($p < 0.05$) (Fig. 2C–D). These temporal differences observed for these three water soluble compounds were likely due to the lack of rain early in the growing season in 2012 compared to 2013.

Fourteen pesticides and three pesticide degradates were detected in sediment samples with concentrations ranging from 0.05 to 47.5 $\mu\text{g/kg}$ dry weight (Table A2). Prometon (herbicide) and metalaxyl (fungicide) were detected most frequently in sediment (Table 3). Due to the limited number of sediment samples and the high frequency of non-detections, there was not enough information to statistically compare wetlands and years. All water and sediment data were used as a base of comparison for the frog tissue samples.

County-level pesticide use information for 2011 (Table 2) was the most recent data available to compare occurrence information with

Table 3
Detection frequency (%) for all pesticides detected in water, sediment and tissue (liver and whole body) in 2012 and 2013. Compound type and log K_{ow} (Pesticide Properties Database, 2014) are also reported.

Compound	Type	log K_{ow} ^a	Detection frequency (%)		
			Water (N = 33)	Sediment (N = 18)	Tissue (N = 59)
3,4-DCA	D	NA	6	nd	2
3,5-DCA	D	NA	6	nd	nd
Alachlor	H	3.09	nd	11	8
AMPA	D	na	64	na	na
Atrazine	H	2.7	100	nd	nd
Azinphos methyl oxon	D	na	nd	nd	8
Azoxystrobin	F	2.5	27	nd	nd
Bifenthrin	I	7.3	nd	39	20
Captan	F	2.5	3	nd	10
Carbaryl	I	2.39	6	nd	nd
Carbofuran	I	1.8	nd	11	12
Chlorothalonil	F	2.94	6	nd	2
Chlorpyrifos	I	4.7	3	nd	nd
Chlorpyrifos oxon	D	na	nd	28	2
Clothianidin	I	0.905	45	na	na
S-ethyl dipropylthiocarbamate (EPTC)	H	3.2	3	6	nd
Fenbuconazole	F	3.79	3	nd	7
Fenhexamide	F	3.51	9	nd	5
Fipronil	I	3.75	nd	nd	12
Fipronil desulfanyl	D	na	3	nd	10
Fludioxinil	F	4.12	nd	22	nd
Fluoxastrobin	F	2.86	3	6	24
Flusilazole	F	3.87	12	nd	nd
Glyphosate	H	-3.2	64	na	na
Hexazinone	H	1.17	3	nd	nd
Imazalil	F	2.56	9	nd	12
Imidacloprid	I	0.57	6	na	na
Metalaxyl	F	1.65	21	44	19
Metolachlor	H	3.4	73	28	24
Pentachloronitrobenzene	D	na	3	nd	nd
Pendimethalin	H	5.2	6	nd	5
Prometon	H	2.91	3	61	nd
Pyraclostrobin	F	3.99	3	22	41
Pyrimethanil	F	2.84	21	6	nd
Resmethrin	I	5.43	24	nd	nd
Tebuconazole	F	3.7	3	nd	nd
Thiamethoxam	I	-0.13	27	na	na
Trifluralin	H	5.27	6	nd	17
Triticonazole	F	3.29	nd	6	nd
Zoxamide	F	3.76	6	11	nd
<i>p,p'</i> -DDD	D	6.0	nd	28	3
<i>p,p'</i> -DDE	D	6.51	nd	61	36
<i>p,p'</i> -DDT	I	6.91	nd	nd	29

D = degradate; F = fungicide, H = herbicide; I = insecticide.
na = not applicable, nd = not detected, DCA = dichloroaniline.

^a Pesticide Properties Database, 2014; <http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm>.

use within the study area (Baker and Stone, 2013). Twenty-four of the 34 pesticides detected in water and sediment had county level reported agricultural use in 2011. The discrepancy between occurrence and use data could be a result of: 1) changes in pesticide use between 2011 and 2012–2013, 2) limitations to the use data with regard to the agricultural use of some fungicides as seed treatments, and 3) use of pesticides for non-agricultural purposes (mosquito control and/or homeowner use).

Because trends in nutrient concentrations between years and by wetland type were similar for all constituents and the fact that the restored wetlands were established to reduce nitrate concentrations to surface waters, only the results for nitrate ($\text{NO}_3\text{-N}$) are discussed (see Table A3 for concentrations of all nutrients measured). Although, nitrate concentrations varied by year in the restored and reference wetlands we observed no significant temporal differences in nutrient concentrations among the wetlands even though rainfall differed between the two sample years (Fig. 3A). Maximum nutrient

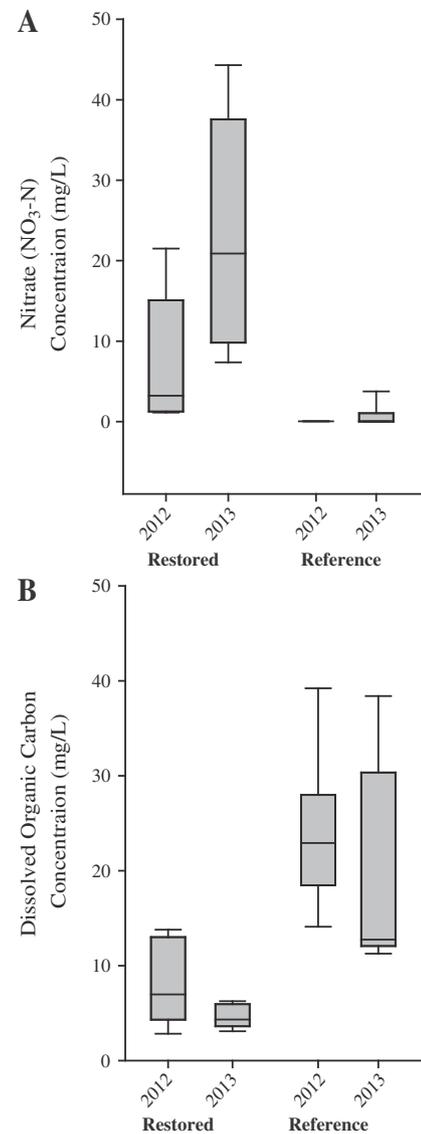


Fig. 3. Box plots of A) nitrate ($\text{NO}_3\text{-N}$) and B) dissolved organic carbon (DOC) concentrations (mg/L) in water samples from restored and reference wetlands collected in 2012 and 2013. Boxes depict interquartile ranges, thick horizontal lines indicate medians, vertical lines extend to 5th and 95th percentiles, and dots are individual observations below 5th and above 95th percentiles.

concentrations in both wetland types were observed in the spring (April and May) of both years. In Iowa, 45–85% of the annual nitrate loss through subsurface drains typically occurred in the spring and fall when crops were not growing (Bjorneberg et al., 1996) indicating a potentially higher exposure to both chorus frogs (early breeders) and leopard frogs that overwinter in the wetlands. With the exception of one sample, nitrate values in restored wetlands made up between 86 and 105% of the total dissolved nitrogen in the system. On the other hand nitrate in the reference wetlands was less than 5% of the total dissolved nitrogen measured with the exception of 2 samples collected from Bjorkboda in 2013 where nitrate made up 56 and 79% of the dissolved nitrogen present.

Despite the lack of temporal differences, restored wetlands consistently had higher nutrient concentrations compared to the reference wetlands ($p < 0.05$) and average nitrate concentrations in the restored wetlands were an order of magnitude higher than those observed in the reference wetlands (Fig. 3A). Furthermore, restored wetlands had significantly lower concentrations of DOC compared to reference

wetlands (Fig. 3B). This outcome is expected and reflects the differences in water sources (surface run-off vs. subsurface tile drainage) between the two wetland types. The restored wetlands were designed to receive tile drainage water with high nutrient concentrations from surrounding agricultural fields to reduce nutrient exports to surface water. In the Midwest, tile drainage tends to have lower DOC compared to run-off (Royer et al., 2007) which mirrors the observed differences in DOC between the restored and reference wetlands.

3.2. Occurrence of pesticides in amphibians

Seventeen pesticides including eight fungicides, four herbicides and five insecticides as well as four pesticide degradates (Table 3) were detected in tissue samples (liver and whole frogs) collected in 2012 and 2013. Concentrations in the liver samples collected in 2012 ranged from 0.1 to 1500 $\mu\text{g}/\text{kg}$ wet weight (Table A4) while concentrations in whole frogs collected in 2013 ranged from 0.08 to 470 $\mu\text{g}/\text{kg}$ wet weight (Table A5). Of the compounds detected in both tissue types, four pesticides including two fungicides (fluoxastrobin and pyraclostrobin), one herbicide (metolachlor) and one insecticide (bifenthrin) were detected frequently (>20% of the time) with median detectable concentrations ranging from 0.5 to 163 $\mu\text{g}/\text{kg}$ wet weight and maximum concentrations ranging from 10 to 1500 $\mu\text{g}/\text{kg}$ wet weight. Two fungicides, pyraclostrobin and tebuconazole, were first reported in adult chorus frogs collected from remote locations in California's Sierra Nevada Mountains (Smalling et al., 2013b) but this is the first study to document up to eight different fungicides in tissues collected in a single study. The detection of these compounds in the environment corresponds with county level use data; however this is the first study to date to document their occurrence in tissue. Currently, limited information is available on the bioaccumulation and effects of many of these compounds, particularly fungicides, in amphibians. The legacy pesticide *p,p'*-DDT and its highly persistent and bioaccumulative metabolite *p,p'*-DDE were detected in 36% and 29% of all samples collected (Table 3). Although banned in the United States in 1972, DDT and its degradates persist in the environment and are biologically available for uptake by wildlife (Pereira et al., 1996). Median concentrations of DDT and DDE were 5.3 and 16.7 $\mu\text{g}/\text{kg}$ wet weight, respectively and were similar to those observed in Blanchard cricket frogs from Ohio in the late 1990s (Russell et al., 2002). On the other hand, DDE concentrations observed in the current study were twice as high as total organochlorine concentrations in green frogs from southwestern Michigan where limited deformities were observed (Gilliland et al., 2001).

Thirty two compounds were detected in water, 17 in sediment and 22 in tissue (Table 3). Of the 22 compounds detected in tissue, 80% of the compounds were also detected in either water and/or sediment but in only a few cases did the compounds detected in the habitat at a particular site correspond to the compounds observed in the tissue. From a landscape perspective, many of the water soluble herbicides (atrazine) were detected frequently in water and less frequently in sediment and tissues, whereas several more hydrophobic compounds (DDE, pyraclostrobin) were detected predominantly in sediment and more frequently in tissues (Table 3). Wetland sediment can be a sink for hydrophobic contaminants and our results support previous work documenting the bioaccumulation of agrochemicals in amphibians (Smalling et al., 2013b; Fellers et al., 2004). Frogs are exposed to pesticides in both the aquatic and terrestrial environments and in many cases wetland habitats where they are captured do not completely explain their contaminant body burdens. However, in an agricultural landscape, habitat does seem to be one indicator of exposure to amphibian populations compared to more remote locations where there is no direct source of pesticides to the habitat (Smalling et al., 2013b). More complete knowledge on the movement of frogs throughout the landscape as well as the uptake and bioaccumulation of pesticides is necessary to help further prioritize research on the effects of frequently detected pesticides at environmentally relevant concentrations.

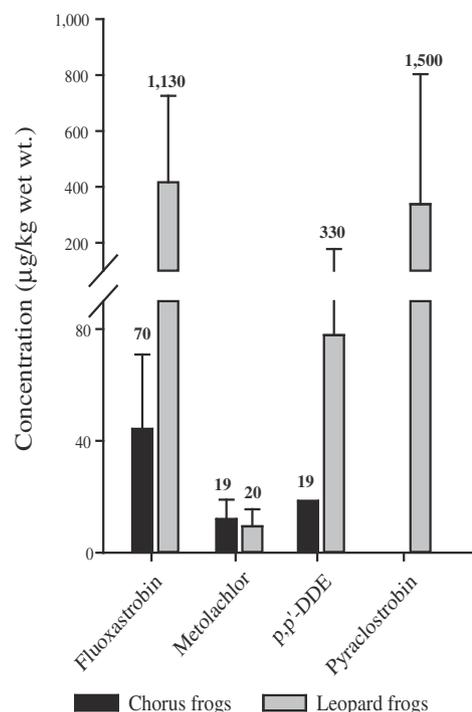


Fig. 4. Average concentrations ($\mu\text{g}/\text{kg}$ wet weight) of the 4 most frequently detected pesticides in chorus (*Pseudacris maculata*) and leopard (*Lithobates pipiens*) frog liver samples collected in 2012 from all sites. The maximum concentration detected for each compound is reported above each error bar.

In 2012, both chorus and leopard frogs were collected from 2 and 4 of the wetlands, respectively. In 2013, chorus frogs were collected from all 6 wetlands. The liver was originally selected as the tissue to analyze because it has the potential to retain pesticides and other contaminants taken up through feeding. However, because amphibian skin is particularly permeable, and because their skin is consistently exposed to either water or the substrate, we used whole bodies for tissue analyses in 2013 to incorporate dermal diffusion as another possible bioaccumulation pathway. Due to the nature of the frog tissue data, species differences were investigated using 2012 results and wetland types were investigated using 2013 results.

Eleven pesticides were detected in the livers from chorus frogs in 2012 and concentrations ranged from 5.0 to 327 $\mu\text{g}/\text{kg}$ wet weight (Table A4). Twelve pesticides were detected in the livers from leopard frogs in 2012, and concentrations ranged from 0.1 to 1500 $\mu\text{g}/\text{kg}$ (Table A4). Of the livers sampled in 2012, only 2 of the 4 sites had corresponding liver samples from both species. To qualitatively compare species, we chose the four compounds measured in greater than 20% of the samples. Concentrations of several more persistent compounds, fluoxastrobin, pyraclostrobin and *p,p'*-DDE, occurred at higher concentrations in leopard frogs compared to chorus frogs while, average concentrations of metolachlor, a relatively hydrophilic herbicide detected frequently in the water samples, were similar between the two species (Fig. 4). For example, DDE was detected in 53% of the leopard frog livers analyzed but in only 10% of the chorus frog livers. DDE is hydrophobic, known to persist in agricultural sediments (Ding et al., 2010; Pereira et al., 1996) and was also detected in 61% of the sediment samples. Differences in concentrations between the two species could be a function of their life histories (i.e. overwintering behavior, home range and dietary preferences). Leopard frogs may be more susceptible to contaminants since they tend to overwinter within the wetland buried in shallow sediment deep enough to avoid freezing, whereas chorus frogs overwinter in logs and other burrows away from the wetland (Dodd, 2013). Many of the fungicides detected in frog tissues are considered to be moderately persistent in sediment with half-lives of up

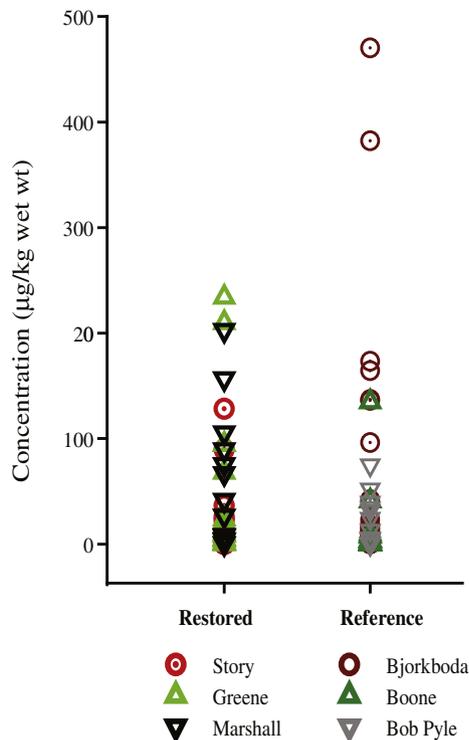


Fig. 5. Whole body concentrations ($\mu\text{g}/\text{kg}$ wet weight) of all pesticides detected in chorus frogs (*Pseudacris maculata*) collected from the three restored and three reference wetlands in 2013. The names and symbols under the x-axis correspond to the wetlands sampled (Table 1).

to 184 days (Pesticide Properties Database, 2014) increasing the potential for exposure to leopard frogs during overwintering. To assess species level impacts at the field scale, more studies are needed to better understand susceptibility to pesticide mixtures and how different life histories relate to an animals' exposure to these contaminants.

There was no significant difference in whole body pesticide concentrations between the two wetland types in adult chorus frogs collected in 2013. Sixteen pesticides were detected at least once in chorus frogs from the restored wetlands with concentrations ranging from 0.1 to 234 $\mu\text{g}/\text{kg}$ wet weight (Fig. 5, Table A5). Fourteen pesticides were detected at least once in chorus frogs from the reference wetlands with concentrations ranging from 0.1 to 470 $\mu\text{g}/\text{kg}$ wet weight (Fig. 5, Table A5). In an agricultural landscape, amphibians are breeding during the crop growing season, are likely moving between the wetlands, are present in agricultural fields during planting and pesticide application, and thus may be bioaccumulating a variety of pesticides in their tissues. The wetlands are a source of pesticides to amphibians but it is unclear if they are the only source, particularly for adults. Chorus frogs tend to remain relatively close to their natal wetland, with home-ranges of only 200 m (Dodd, 2013) but still have the potential to bioaccumulate pesticides from limited terrestrial sources, while leopard frogs generally travel further afield. Contaminants persisting in amphibian habitats can impact amphibians and their prey beyond the growing season and potentially throughout the year. To better understand bioaccumulation of pesticides in frogs from wetlands, more targeted sampling is necessary paying close attention to larval amphibians whose only source of pesticides is the wetland itself. Although laboratory mesocosm studies are a useful tool for understanding of acute and chronic effects of pesticides (single compounds or simple mixtures) and other stressors (nutrients and disease) on test organisms, it is very difficult to replicate complex field conditions in the laboratory with native species. In addition, information on the movements of adult amphibians, especially during the spring and summer when pesticide use is highest, would be useful.

3.3. Potential impacts to amphibians

Amphibians are of particular toxicological and ecological importance because they bridge the gap between the aquatic and terrestrial landscape and are sensitive to both pesticides and environmental changes (Hopkins, 2007). A wide variety of pesticides were detected in water, sediment and tissues collected (Tables A1 and A3–A5) including several fungicides (some previously unreported). Previous studies have focused on the effects of the herbicides glyphosate and atrazine (Mann et al., 2009; Hayes et al., 2003; Relyea, 2005); the insecticides carbaryl, chlorpyrifos, diazinon and endosulfan (Relyea and Mills, 2001; Sparling and Fellers, 2009) and the fungicides azoxystrobin, propiconazole, pyraclostrobin and trifloxystrobin (Belden et al., 2010; Hartman et al., 2014). In most cases, agrochemicals in high doses have direct lethal effects on amphibians but these concentrations are not typically measured in the environment. More often, sub-lethal concentrations of pesticide are detected in complex mixtures in wetlands (i.e., breeding sites), but even these sub-lethal concentrations can cause changes in reproduction, immune response, physiology, morphology and behavior (Hayes et al., 2006; Buck et al., 2012). Atrazine, for example, has the potential to act as an endocrine disruptor in amphibians causing immunosuppression at 21 $\mu\text{g}/\text{L}$ (Brodtkin et al., 2007) and reproductive abnormalities at concentrations ranging from 0.1 to 6.7 $\mu\text{g}/\text{L}$ (Hayes et al., 2003). Northern leopard frogs across the upper Midwestern United States in areas of intense row crop agriculture may experience varying degrees of sexual disruption due to exposure to a combination of pesticides and nutrients (Hayes et al., 2003; McDaniel et al., 2008). Atrazine concentrations in all wetlands ranged from 0.07 to 19 $\mu\text{g}/\text{L}$ (Fig. 2A–B), and the median concentrations in 2012 and 2013 were 0.2 and 0.1 $\mu\text{g}/\text{L}$, respectively. In 2012, up to 25 amphibians (5 from each site) were submitted to the USGS NWHC for gross histopathology. One male leopard frog collected from the reference wetland, Bjorkboda (maximum atrazine concentration 19 $\mu\text{g}/\text{L}$), showed incidence of intersex (data not shown). Atrazine acting alone or in combination with other pesticides as well as nutrients has the potential to affect wetland breeding amphibians negatively in agricultural areas like Iowa's Des Moines Lobe.

Although, a causal link between pesticide exposure and disease outbreaks has not been identified in the laboratory or in the field (Paetow et al., 2012; Rohr et al., 2013), it is still an intriguing relationship worth continued investigations. Pesticides have the potential to increase susceptibility (Mann et al., 2009) while elevated nutrient concentrations have the potential to increase the incidence of disease in amphibian populations (Johnson et al., 2007). On the other hand, studies have documented the relationship between pesticides and susceptibility to infections by trematodes (Rohr et al., 2008). These parasitic infections have the potential to negatively impact populations causing malformations (Reeves et al., 2013) which can lead to impaired mobility, decreased food intake, and an increased susceptibility to predators (Blaustein and Johnson, 2003). These studies suggest that through suppression of the immune system, agrochemicals can also indirectly contribute to population declines by facilitating mortality or altering adult fitness (i.e. increasing infection rates or reducing growth and development). Amphibian populations may be at an increased risk of parasitic infections in restored wetlands that are receiving water from tile drainage systems, and therefore experiencing higher nitrogen concentrations than in the reference wetlands. Maximum dissolved nitrogen concentrations in our study were approximately 40 mg/L in the restored wetlands, composed primarily of nitrate (Fig. 3), however, these concentrations were below the chronic toxicity reported by Hecnar (1995) of approximately 100 mg/L $\text{NO}_3\text{-N}$ for leopard and chorus frogs.

In conclusion, except for nitrogen, we observed no differences in water or sediment quality between restored and reference wetlands indicating that, from a pesticide standpoint, restored wetlands provide similar habitat for amphibians as the reference wetlands. Pesticides and pesticide degradates occurred frequently in water and sediment and were bioaccumulated by the adult frogs sampled in this study.

Amphibians residing and breeding in both restored and reference wetlands in an agricultural landscape are exposed to a wide variety of contaminants throughout their lifecycle and the potential impacts of these environmental mixtures are unknown. Also, quantifying the relationship between habitat quality (such as pesticide and nutrient levels) and the prevalence of disease and parasites in both restored and reference wetlands are needed to manage quality habitat for amphibians. Furthermore, understanding and documenting the quality of the habitat available for amphibians will help prioritize research necessary to decipher the effects of pesticides, nutrients, habitat loss/degradation and other potential stressors on the long term viability and management of native amphibian populations in an agricultural landscape.

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References

Anderson TA, Salice CJ, Erickson RA, McMurray ST, Cox SB, Smith LM. Effects of land-use and precipitation on pesticides and water quality in playa lakes in the southern high plains. *Chemosphere* 2013;92:84–90.

Baker NT, Stone WW. Preliminary estimates of annual agricultural pesticide use for counties of the conterminous United States, 2010–11: U.S. Geological Survey Open-File Report 2013-1295. [2-p. pamphlet, 14 tables] <http://dx.doi.org/10.3133/ofr20131295>, 2013.

Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW. Glyphosate, other herbicides and transformation products in Midwestern streams, 2002. *JAWRA* 2005;41:323–32.

Battaglin WA, Meyer MT, Kuivila KM, Dietze JE. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *JAWRA* 2014;50(2):275–90. <http://dx.doi.org/10.1111/jawr.12159>.

Belden J, McMurray S, Smith L, Reilly P. Acute toxicity of fungicide formulations to amphibians and environmentally relevant concentrations. *Environ Toxicol Chem* 2010;29:2477–80.

Bird SM, Fram MS, Crepeau KL. Method of analysis by the U.S. Geological Survey California District Sacramento Laboratory—determination of dissolved organic carbon in water by high temperature catalytic oxidation, method validation and quality control practices: U.S. Geological Survey Open-File Report 2003-366; 2003 [14 pp.].

Bjorneberg DL, Kanwar RS, Melvin SW. Seasonal changes in flow and nitrate N loss from subsurface drains. *Trans ASAE* 1996;36:961–76.

Blaustein AR, Johnson PT. The complexity of deformed amphibians. *Front Ecol Environ* 2003;1(2):87–94.

Boone MD. Examining the single and interactive effects of three insecticides on amphibian metamorphosis. *Environ Toxicol Chem* 2008;27:1561–8.

Boone MD, Bridges CM, Fairchild JF, Little EE. Multiple sublethal chemicals negatively affect tadpoles of the green frog, *Rana clamitans*. *Environ Toxicol Chem* 2005;24:1267–72.

Brodtkin MA, Madhoun H, Rameswaran M, Vatnick I. Atrazine is an immune disruptor in adult northern leopard frogs (*Rana pipiens*). *Environ Toxicol Chem* 2007;26(1):80–4.

Bruhl CA, Schmidt T, Pieper S, Alscher A. Terrestrial pesticide exposure of amphibians: an underestimated global decline? *Sci Rep* 2003;3:1135. <http://dx.doi.org/10.1038/srep01135>.

Buck JC, Scheessele EA, Relyea RA, Blaustein AR. The effects of multiple stressors on wetland communities: pesticides, pathogens and competing amphibians. *Freshwater Biol* 2012;57(1):61–73.

Christin MS, Ménard L, Gendron AD, Ruby S, Cyr D, Marcogliese DJ, et al. Effects of agricultural pesticides on the immune system of *Xenopus laevis* and *Rana pipiens*. *Aquat Toxicol* 2004;67(1):33–43.

Collins JP, Storfer A. Global amphibian declines: sorting the hypotheses. *Divers Distrib* 2003;9:89–98.

Davidson C, Shafer HB, Jennings MR. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conserv Biol* 2002;16:1588–601.

Davidson C, Benard MF, Shaffer HB, Parker JM, O'Leary C, Conlon JM, et al. Effects of chytrid and carbaryl exposure on survival, growth and skin peptide defenses in foothill yellow-legged frogs. *Environ Sci Technol* 2007;41:1771–6.

Delis PR, Mushinsky HR, McCoy ED. Declines of some west-central Florida anuran populations in response to habitat degradation. *Biodivers Conserv* 1996;5:1579–95.

Ding Y, Harwood AD, Foslund HM, Lydy MJ. Distribution and toxicity of sediment-associated pesticides in urban and agricultural waterways from Illinois, USA. *Environ Toxicol Chem* 2010;29(1):149–57.

Dodd CK. *Frogs of the United States and Canada*, vol. 1. Johns Hopkins University Press; 2013.

Fellers GM, Freel KL. A standardized protocol for surveying aquatic amphibians. NPS/WRUC/NRTR-95-001. Technical Report. Davis, CA, USA: National Biological Service, Cooperative Park Studies Unit, University of California; 1995.

Fellers GM, McConnell LL, Pratt D, Datta S. Pesticides in mountain yellow-legged frogs (*Rana muscosa*) from the Sierra Nevada Mountains of California, USA. *Environ Toxicol Chem* 2004;23:2170–7.

Gahl MK, Pauli DB, Houlahan JF. Effects of chytrid fungus and a glyphosate-based fungicides on survival and growth of wood frogs (*Lithobates sylvaticus*). *Ecol App* 2011; 21:2521–9.

Gilliland CD, Summer CL, Gilliland MG, Kannan K, Villeneuve DL, Coady K, et al. Organochlorine insecticides, polychlorinated biphenyls, and metals in water, sediment and green frogs from Southwestern Michigan. *Chemosphere* 2001;44:327–39.

Gilliom RJ. Pesticides in the nation's streams and ground water. *Environ Sci Technol* 2007; 41(10):3408–14.

Green DM. *Bufo americanus*. In: Lannoo M, editor. Amphibian declines. Berkeley, CA USA: University of California Press; 2005.

Groner ML, Relyea RA. A tale of two pesticides: how common insecticides affect aquatic communities. *Freshwater Biol* 2011;56:2391–404.

Han W, Yang Z, Di L, Mueller R. CropScape: A Web service based application for exploring and disseminating US conterminous geospatial cropland data products for decision support. *Comput Electron Agr* 2012;84:111–23.

Hartel T, Bancila R, Cogalniceanu D. Spatial and temporal variability of aquatic habitat use by amphibians in a hydrologically modified landscape. *Freshwater Biol* 2001;56: 2288–98.

Hartman EAH, Belden JB, Smith LM, McMurry ST. Chronic effects of strobilurin fungicides on development, growth and mortality of larval Great Plains toads (*Bufo cognatus*). *Ecotoxicology* 2014;23:396–403.

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. *Environ Health Persp* 2003;111(4):568–75.

Hayes TB, Case P, Chui S, Chung D, Haeffele C, Haston K, et al. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? *Environ Health Persp* 2006;114:40–50.

Hecnar SJ. Acute and chronic toxicity of ammonium-nitrate fertilizer to amphibians from southern Ontario. *Environ Toxicol Chem* 1995;14(12):2131–7.

Hladik ML, Calhoun DL. Analysis of the herbicide diuron, three diuron degradates, and six neonicotinoid insecticides in water—method details and application to two Georgia streams: U.S. Geological Survey Scientific Investigations Report 2012-5206. <http://pubs.usgs.gov/sir/2012/5206>, 2012. [10 pp.].

Hladik ML, McWayne MM. Methods of analysis—determination of pesticides in sediment using gas chromatography/mass spectrometry: U.S. Geological Survey Techniques and Methods 5-C3. <http://pubs.usgs.gov/tm/tm5c3>, 2012. [18 pp.].

Hladik ML, Smalling KL, Kuivila KM. A multi-residue method for the analysis and pesticides and pesticide degradates in water using Oasis HLB solid phase extraction and gas chromatography-ion trap mass spectrometry. *Bull Environ Contam Toxicol* 2008;80:139–44.

Hladik ML, Orlando JL, Kuivila KM. Collection of pyrethroids in water and sediment matrices: development and validation of a standard operating procedure: U.S. Geological Survey Scientific Investigations Report 2009-5012. <http://pubs.usgs.gov/sir/2009/5012/>, 2009. [22 pp.].

Hladik ML, Kolpin DW, Kuivila KM. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ Pollut* 2014; 193:189–96.

Hopkins WA. Amphibians as models for studying environmental change. *ILAR J* 2007;48: 270–7.

- Howe CM, Berrill M, Pauli BD, Helbing CC, Werry K, Veldhoen N. Toxicity of glyphosate-based pesticides to four North American frog species. *Environ Toxicol Chem* 2004; 23:1928–38.
- IDNR. Iowa Wildlife Action Plan. Iowa Department of Natural Resources; 2006.
- Johnson PT, Chase JM, Dosch KL, Hartson RB, Gross JA, Larson DJ, et al. Aquatic eutrophication promotes pathogenic infection in amphibians. *Proc Natl Acad Sci U S A* 2007; 104(40):15781–6.
- Kiesecker JM. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proc Natl Acad Sci U S A* 2002;99:9900–4.
- King KC, McLaughlin JD, Gendron AD, Pauli BD, Giroux I, Rondeau B, et al. Impacts of agriculture on the parasite communities of northern leopard frogs (*Rana pipiens*) in southern Quebec, Canada. *Parasitology* 2007;134:2063–80.
- Kolozsvary MB, Swinhart RK. Habitat fragmentations and the distribution of amphibians: patch and landscape correlates in farmland. *Can J Zool* 1999;77(8):1288–99.
- Kolpin DW, Kalkhoff SJ, Goolsby DA, Sneek-Fahrer DA, Thurman EM. Occurrence of selected herbicides and herbicide degradation products in Iowa's ground water, 1995. *Ground Water* 1995;35(4):679–88.
- Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ, Morrisey CA. Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pot-hole Region. *PLoS One* 2014;9(3):e92821. <http://dx.doi.org/10.1371/journal.pone.0092821>.
- Mann RM, Hynes RV, Choung CB, Wilson SP. Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environ Pollut* 2009;157:2903–27.
- McDaniel TV, Martin PA, Struger J, Marvin CH, McMaster ME, Clarence S, et al. Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) from areas of intensive row crop agriculture. *Aquatic Toxicol* 2008;88(4):230–42.
- Meyer MT, Loftin KA, Lee EA, Hinshaw GH, Dietze JE, Scribner EA. Determination of glyphosate, its degradation product aminomethylphosphonic acid, and glufosinate. Water by isotope dilution and online solid-phase extraction and liquid chromatography/tandem mass spectrometry: U.S. Geological Survey Techniques and Methods; 2009 [book 5, chap. A10, 32 pp.].
- National Drought Mitigation Center (NDMC). United States drought monitor archives. <http://droughtmonitor.unl.edu/>, 2014.
- O'Neal BJ, Heske EJ, Stafford JD. Waterbird response to wetlands restored through the Conservation Reserve Enhancement Program. *J Wildlife Manage* 2008;72:654–64.
- Paetow LJ, McLaughlin JD, Cue RI, Pauli BD, Marcogliese DJ. Effects of herbicides and the chytrid fungus *Batrachochytrium dendrobatidis* on the health of post-metamorphic northern leopard frogs (*Lithobates pipiens*). *Ecotox Environ Safe* 2012;80:372–80.
- Patton CJ, Kryskalla JR. Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—evaluation of alkaline persulfate digestion as an alternative to Kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in Water: U.S. Geological Survey Water-Resources Investigations Report 03–4174; 2003. <http://nwql.usgs.gov/pubs/WRIR/WRIR-03-4174.pdf> [33 pp.].
- Pereira W, Domagalski JL, Hostettler F, Brown LR, Rapp JB. Occurrence and accumulation of pesticides and organic contaminants in river sediment, water and clam tissues from the San Joaquin River and tributaries, California. *Environ Toxicol Chem* 1996; 15:172–80.
- Pesticide Properties Database <http://sitem.herts.ac.uk/aeru/footprint/index2.htm>, 2014.
- Potter BB, Wimsatt JC. Measurement of total organic carbon, dissolved organic carbon and specific UV absorbance at 254 nm in source water and drinking water: U.S. Washington, DC: Environmental Protection Agency; 2005 [EPA/600/R-05/055, Method 415.3].
- Development Core R, Team R. A language and environment for statistical computing. R Foundation for Statistical Computing. Austria: Vienna; 2013 [ISBN 3-900051-07-0, URL <http://www.R-project.org/>].
- Reeves MK, Medley KA, Pinkney AE, Holyoak M, Johnson PTJ, Lanoo MJ. Localized hotspots drive continental geography of abnormal amphibians on U.S. Wildlife Refuges. *PLoS One* 2013;8(11):e77467.
- Reilly TJ, Smalling KL, Orlando JL, Kuivila KM. Occurrence of boscalid and other selected fungicides in surface water and groundwater in three targeted use areas in the United States. *Chemosphere* 2012;89:228–34.
- Relyea RA. The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Arch Environ Con Tox* 2005;48:351–7.
- Relyea R, Mills NN. Predator-induced stress makes the pesticide carbaryl more deadly to gray tree frog tadpoles (*Hyla versicolor*). *Proc Natl Acad Sci U S A* 2001;98:2491–6.
- Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT, et al. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* 2008; 455:1235–9.
- Rohr JR, Raffel TR, Halstead NT, McMahon TA, Johnson SA, Boughton RK, et al. Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. *Proc R Soc B-Biol Sci* 2013;280:20131502.
- Royer E, Anders DA, Chantigny MH, Simard RR, Cluis D. Dissolved organic carbon in runoff and tile-drain water under corn and forage fertilized with hog manure. *J Environ Qual* 2007;36(3):855–63.
- Russell RW, Lipps GJ, Hecnar SJ, Haffner GD. Persistent organic pollutants in Blanchard's cricket frogs (*Acris crepitans* Blanchardi) from Ohio. *Ohio J Sci* 2002;102(5):119–22.
- Smalling KL, Reilly TJ, Sandstrom MW, Kuivila KM. Occurrence and persistence of fungicides in bed sediments and suspended solids from three targeted uses areas in the United States. *Sci Total Environ* 2013a;447:179–85.
- Smalling KL, Fellers GM, Kleeman PM, Kuivila KM. Accumulation of pesticides in pacific chorus frogs (*Pseudacris regilla*) from California's Sierra Nevada Mountains, USA. *Environ Toxicol Chem* 2013b;32(9):2026–34.
- Sparling DW, Fellers GM. Toxicity of two insecticides to California, USA anurans and its relevance to declining amphibian populations. *Environ Toxicol Chem* 2009;28: 1696–703.
- Spivak M, Mader E, Vaughan M, Euliss NH. The plight of the bees. *Environ Sci Technol* 2011;45:34–8.
- Taylor B, Skelly D, Demarchis LK, Slade MD, Galusha D, Rabinowitz PM. Proximity to pollution sources and risk of amphibian limb malformation. *Environ Health Persp* 2005; 113:1497–501.
- Thelin GP, Stone WW. Estimation of annual agricultural pesticide use for counties of the conterminous United States, 1992–2009: U.S. Geological Survey Scientific Investigations Report 2013–5009; 2013 [54 pp.; <http://pubs.usgs.gov/sir/2013/5009/>].
- U.S. Department of Agriculture. Economic Research Service. Major uses of land in the United States; 2007 [http://www.ers.usda.gov/data-products/major-land-uses.aspx#U39Xx_IdXg8].
- U.S. Department of Agriculture. Census of Agriculture Historical Census Publications. Accessed 7/2013 at http://www.agcensus.usda.gov/Publications/Historical_Publications/index.php, 2013.
- U.S. Geological Survey. The national field manual for the collection of water quality data. Collection of water samples (ver 2.0): U.S. Geological Survey Techniques of Water Resources Investigations; 2006 [book 9, chap A4; <http://pubs.water.usgs.gov/twri9A4/>].
- vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, et al. Colony collapse disorder: a descriptive study. *PLoS One* 2009;8:e6481.
- Ward JR, Harr CA, editors. Methods for collection and processing of surface-water and bed-material samples for physical and chemical analyses: U.S. Geological Survey Open-File Report 90–140; 1990. [71 pp., Available at <http://pubs.er.usgs.gov/usgspubs/ofr/ofr90140>].
- Whitney GC. From coastal wilderness to fruited plain: a history of environmental change in temperate North America, 1500 to the present. Cambridge, UK: Cambridge University Press; 1994.
- Zimmerman CF, Keefe CW, Bashe J. Determination of carbon and nitrogen in sediments and particulates of estuarine/coastal waters using elemental analysis: U.S. Environmental Protection Agency Method 440.0. 2007. <http://www.caslab.com/EPA-Methods/PDF/EPA-Method-440.pdf>, 2007.