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An Initial Comparison of Pesticides and Amphibian Pathogens Between Natural and Created Wetlands in the New Jersey Pinelands, 2014–16

Open-File Report 2018–1077

U.S. Department of the Interior
U.S. Geological Survey

Cover. Composite Image. U.S. Geological Survey (USGS) scientist holding American bullfrog tadpoles (*Lithobates catesbeianus*) collected from a stormwater basin in the New Jersey Pinelands. Photograph by Kelly Smalling, USGS. Background photograph of water by Ryan Wilson, Unsplash on May 21, 2015.

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Contents

Figures

Tables

Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
hectare (ha)	2.471	acre
square kilometer (km2)	247.1	acre
hectare (ha)	0.003861	square mile (mi2)
square kilometer (km2)	0.3861	square mile (mi2)
	Volume	
liter (L)	33.81402	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32.$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as:

$$°C = (°F - 32) / 1.8.$$

Datum

Vertical coordinate information is referenced to the North American Vertical Datum of 1983 (NAVD 83).

Horizontal coordinate information is referenced to North American Datum of 1983 (NAD 83).

Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or nanograms per liter (ng/L).

Concentrations of chemical constituents in sediment and anuran food are given in micrograms per kilogram (µg/kg) dry weight.

Concentrations of chemical constituents in tissue are given in micrograms per kilogram (µg/kg) wet weight.

Batrachochytrium dendrobatidis (Bd) detections are given in zoospore equivalents/swab.

Acronyms

ACS	American Chemical Society
ANOVA	Analysis of variance
Bd	*Batrachochytrium dendrobatidis*
DCM	Dichloromethane
DOC	Dissolved organic carbon
DNA	Deoxyribonucleic acid
EI	Electron ionization
GC-MS	Gas chromatography-mass spectrometry
KNP	Potassium hydrogen phthalate
MDL	Method detection limit
MRM	Multiple reaction monitoring
NJDEP	New Jersey Department of Environmental Protection
NTU	Nephelometric Turbidity Units
PCR	Polymerase chain reaction
RT-PCR	Real time polymerase chain reaction
SPE	Solid-phase extraction
USGS	U.S. Geological Survey

An Initial Comparison of Pesticides and Amphibian Pathogens Between Natural and Created Wetlands in the New Jersey Pinelands, 2014–16

Kelly L. Smalling[1], John F. Bunnell[2], Jonathan Cohl[1], Kristin M. Romanok[1], Lisa Hazard[3], Kirsten Monsen[3], Denise M. Akob[1], Angela Hansen[1], Michelle L. Hladik[1], Nicole Abdallah[3], Quratulain Ahmed[1], Araba Assan[3], Matt De Parsia[1], Amaryl Griggs[1], Megan McWayne-Holmes[1], Naisargi Patel[3], Corey Sanders[1], Yesha Shrestha[1], Sean Stout[1] and Brianna Williams[1]

Abstract

A study conducted by the U.S. Geological Survey, in cooperation with the New Jersey Pinelands Commission and Montclair State University, was designed to compare pesticide concentrations and the presence and prevalence of amphibian pathogens between natural ponds and two types of created wetlands, excavated ponds and stormwater basins, throughout the New Jersey Pinelands. The study described herein is part of a larger study by the New Jersey Pinelands Commission designed to compare the functional equivalency of natural and created wetlands throughout the New Jersey Pinelands. Sites were selected on the basis of land-use classifications within a 500-meter radius around each wetland from a pool of natural ponds, excavated ponds, and stormwater basins determined by the New Jersey Pinelands Commission. Water, bed-sediment, anuran-food, and composite larval-anuran-tissue samples were collected from four reference (minimum land-use effects) and four degraded (maximum land-use effects) sites from each wetland type for a total of 24 ponds or basins throughout the New Jersey Pinelands during 2014–16. Prevalence of *Ranavirus* was determined on the basis of tail clips collected from 60 individual larval anurans in each wetland, and 10 animals from each wetland also were swabbed for the presence of *Batrachochytrium dendrobatidis* (Bd). Other constituents measured included turbidity, pH, specific conductance, dissolved oxygen, dissolved organic carbon, percent organic carbon in sediment, and composite larval-anuran lipid content.

The amount of altered land (percent agricultural plus percent developed) ranged from 0 to 62.4 percent for the natural ponds, 0 to 63.6 percent for the excavated ponds, and 23.3 to 80.2 percent for the stormwater basins. The herbicides atrazine and metolachlor were observed in 60 and 89 percent of the water samples, respectively. The insecticide bifenthrin was the most frequently detected current-use pesticide (greater than 25 percent of the samples) in bed-sediment, anuran-food, and composite larval-anuran-tissue samples. The legacy insecticide *p,p′*-DDT and its primary degradates *p,p′*-DDD and *p,p′*-DDE were the most frequently detected compounds in bed-sediment and anuran-food samples (32–76 percent in sediment samples and 24–72 percent in anuran-food samples). Significantly, greater numbers of pesticides and higher total pesticide concentrations were observed in stormwater basins than in natural and excavated ponds. Reference wetlands had fewer pesticides and lower total pesticide concentrations compared to degraded wetlands, indicating a positive relation between percent altered land and pesticides throughout the New Jersey Pinelands. *Ranavirus* was observed in larvae from 4 wetlands, including 1 reference natural pond, 1 degraded natural pond, and 2 degraded stormwater basins, with prevalence ranging from 3 to 43 percent. Bd was detected in swabs from 18 animals and in 4 natural ponds (1 reference and 3 degraded), 3 excavated ponds (all reference), and 2 stormwater basins (1 reference and 1 degraded); however, detection probability was low. In the wetlands with Bd detections, between 10 and 30 percent (between 1 and 3) of the animal's swabbed tested positive for Bd. Owing to the limited number of positive detections for both Bd and *Ranavirus*, no statistical comparisons between wetland types and land-use classifications were possible.

Introduction

The loss and degradation of habitat, exposure to contaminants (including pesticides), and emergent disease are among several notable stressors to amphibian populations worldwide (Collins and Storfer, 2003; Grant and others, 2016). Land-use changes, such as increased development or agricultural production, may not destroy habitat, but usually include landscape

[1]U.S. Geological Survey.

[2]New Jersey Pinelands Commission.

[3]Montclair State University.

alterations, such as the application of chemicals, that can threaten native species like amphibians.

The New Jersey (N.J.) Pinelands is a heavily forested area of the coastal plain that stretches across seven N.J. counties and includes a wide variety of ponds, natural and created, that have the potential to support amphibian populations (Bunnell and others, 2018). Created wetlands can provide the habitat necessary for wetland-dependent plants and animals, especially in human-dominated landscapes where many of the more natural wetlands may have been degraded or eliminated. Two types of created wetlands commonly found in the N.J. Pinelands are shallow excavations that intercept groundwater (excavated ponds) and larger excavated areas designed to receive stormwater (stormwater basins). On the basis of previous studies in protected forested landscapes of the N.J. Pinelands, the hydrology, vegetation, and amphibian assemblages were similar in excavated and natural ponds (Bunnell and Zampella, 1999; Zampella and Laidig, 2003; Bunnell and Zampella, 2008).

Urban stormwater basins are among the most common features of stormwater management plans and are heavily utilized in southern New Jersey. These basins are designed to retain stormwater runoff, reduce sediment transport to streams, and promote recharge and are known sinks for pollutants, such as heavy metals and nutrients (Brand and others, 2010; Gallagher and others, 2011). Urban stormwaters are also a potential source of contaminants, such as pesticides, to aquatic life, especially in suburban areas. A few studies have demonstrated that amphibian populations utilize urban stormwater basins for breeding and as a permanent habitat (Bascietto and Adams, 1983; Ostergaard and others, 2008; Simon and others, 2009; McCarthy and Lathrop, 2011). In the N.J. Pinelands, despite the potential for urban and agricultural land-use to affect wetland communities, little is known about the presence of pesticides in natural and created wetlands.

Pesticides and other contaminants have the potential to reduce survival in some amphibian species (Hecnar, 1995; Belden and others, 2010; Brühl and others, 2013). More notably, many commonly used pesticides can cause sublethal effects such as compromised reproduction; reduced growth and development, particularly at the larval stage; and immunomodulation (Johnson and others, 2007; Brodkin and others, 2007; Gahl and others, 2011; Hayes and others, 2003). Exposure to pesticides can have positive or negative effects on disease transmission, infection rates, and host susceptibility (Clements and Rohr, 2009; Blaustein and others, 2011).

Two emergent amphibian diseases prevalent in the Northeast are the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), and *Ranavirus*, a highly virulent pathogen. Both have been implicated in mortality events worldwide (Daszak and others, 2003; Lips and others, 2008; Vredenburg and others, 2010; Bollinger and others, 1999; Docherty and others, 2003; Jancovich and others, 1997). Amphibian's susceptibility to pathogens is affected by the host's cutaneous microbiome (Belden and Harris, 2007) and antimicrobial peptides (Rollins-Smith and Conlon, 2005) found within natural secretions in the skin of the host. Limited information is available on the effects of environmental stressors, such as pesticides or other contaminants, on the skin microbiome or antimicrobial peptides. It has been hypothesized that these stressors can cause reduction of antimicrobial peptide production (Davidson and others, 2007), with the result that amphibians become more susceptible to pathogens. However, there are few studies that have investigated the link between land use, water quality, pesticides, and pathogen prevalence in natural and created wetland habitats, particularly in the N.J. Pinelands.

Purpose and Scope

The overall objective of the study, conducted by the U.S. Geological Survey (USGS) in cooperation with the New Jersey Pinelands Commission and Montclair State University, was to compare current-use pesticides and amphibian pathogen presence and prevalence between natural ponds and two types of created wetlands, excavated ponds and stormwater basins, in the N.J. Pinelands. The specific objectives highlighted in this report were to

- Determine the pesticides present in wetland habitat (water and bed sediment), larval anuran food (periphyton), and composite larval-anuran tissues in samples collected from 24 wetlands;
- Compare pesticide occurrence/distribution and land use across all wetland types; and
- Understand the potential relation between pesticides and the presence of emerging amphibian pathogens.

This report discusses the methods used in the study and the results for the three objectives. Summary statistics for the results are given in tables. Differences in pesticide concentrations and the number of pesticides detected are shown in illustrations.

Methods

The overall approach of this study was focused on providing pesticide and pathogen information required to support the activities to assess the functional equivalency of natural ponds and two types of created wetlands (excavated ponds and stormwater basins) within the N.J. Pinelands (Bunnell and others, 2018).

Site Information

Twenty-four wetlands were sampled in the N.J. Pinelands during 2014–16 (fig. 1, table 1). Wetlands were selected on the basis of the surrounding land-use and availability of common amphibian species. Three types of wetlands—natural ponds, excavated ponds, and stormwater basins—were selected

from an inventory of wetlands mapped by the New Jersey Pinelands Commission (Bunnell and others, 2018). Wetlands were further classified by land use into reference (minimum land-use effect) and degraded (maximum land-use effect) sites. Four sampling locations were selected for each wetland type (natural ponds, excavated ponds, and stormwater basins) and land-use classification (reference and degraded) for a total of 24 sites (4 replicate wetlands × 2 wetland classifications × 3 wetland types). Eight sites were sampled per year from 2014 to 2016 during the summer when larval anurans are present. A mixture of wetland type and classification was sampled during the 3 years (table 1). Designation of each wetland classification was based on percent altered land (agricultural and developed land) in a 500-meter (m) radius around each wetland. The percent altered land surrounding the reference wetlands was less than 1 percent for natural and excavated ponds and less than 50 percent for stormwater basins, whereas the percent altered land surrounding degraded wetlands was greater than 30 percent for natural and excavated ponds and greater than 55 percent for stormwater basins (NJDEP, 2010).

Pesticide and Pathogen Sampling

Water, bed-sediment, and larval-anuran food (for example, algae, leaves, detritus) were collected for pesticide analysis from 24 wetlands by using the same procedures at all sites. Larval anurans also were collected for pesticide and pathogen analyses with dip nets at each location. All sampling equipment and field gear were disinfected between each site visit with an appropriate concentration of bleach solution and thoroughly rinsed with deionized water (Bryan and others, 2009; Phillott and others, 2010).

Water samples were collected by immersing a pre-cleaned, 1-liter (L), amber-glass bottle at one location at each site near a temporary staff gage (USGS, 2006; Hladik and others, 2009). Each bottle was filled at a depth of not less than 0.1-m below the water surface. The collection bottles were field rinsed three times with sample water prior to collection. All water samples were shipped on ice to the USGS Organic Chemistry Research Laboratory in Sacramento, California, for extraction and analysis for pesticides. At the time of sample collection, basic water-quality characteristics, including temperature, specific conductance, pH, dissolved oxygen concentration, and turbidity, were measured by using a multi-parameter meter (YSI model 6920V2-2). Water samples were also collected for dissolved organic carbon (DOC) in baked 125-milliliter (mL) amber-glass bottles and shipped on ice to the USGS California Water Science Center for analysis where they were filtered within approximately 24 hours after collection.

Bed-sediment samples were collected from the top 2-centimeters (cm) in areas of active sediment deposition using a stainless-steel scoop at multiple points within approximately a 1-square-meter (m^2) area. Sediment was passed through a pre-cleaned, 2-millimeter (mm) mesh stainless-steel sieve into a pre-cleaned, stainless-steel bowl, homogenized, and transferred to 250-mL, amber, baked-glass jars. Larval-anuran food sources, consisting of microorganisms and leaf litter, were collected by removing the material (greater than 2 mm) from the top of the sieve and placing it into a 250-mL, amber, baked-glass jar. Sediment and larval-anuran food samples were shipped on ice to the USGS Organic Chemistry Research Laboratory in Sacramento, Calif., where they were stored frozen at -20 degrees Celsius (°C) prior to analysis.

At each wetland, 60 larval anurans were collected and identified to the genus or species level when possible, and a tail clip was taken to be analyzed for *Ranavirus*; 50 individuals were then released back to the wetland. This sample size allowed for accurate measurement of disease prevalence (percent infected) at sites where disease existed. Tail clips (3 mm of tissue) were collected by using scissors disinfected with 95-percent ethanol. Tissue samples were stored at room temperature in 1.5-mL Eppendorf tubes filled with Drierite desiccant.

For Bd surveys, the ventral surface of the 10 remaining larval anurans were given a unique animal identification number then swabbed 30 times using pre-sterilized, 2-mm-diameter cotton swabs, turning the swab slightly after each pass. The individual swabs were stored in sterile vials and labeled with the site information and animal identification number. At each site, individual swabs were stored in a single Ziploc bag labeled with the site information, species name (if possible), date, and number of swabs collected. In the field, the samples were stored on ice in a cooler. Samples for Bd were shipped on ice to the USGS Reston Microbiology Laboratory (RML) in Reston, Virginia, and stored frozen at -20 °C until analysis. The 10 larval amphibians swabbed for Bd were then euthanized with a 0.2-percent benzocaine solution using standard procedures (Fellers and Freel, 1995), wrapped in aluminum foil, placed in Ziploc bags on ice, and returned to the USGS N.J. Water Science Center in Lawrenceville, N.J., where they were stored frozen (-20 °C). Frozen samples were shipped on ice to the USGS Organic Chemistry Research Laboratory in Sacramento, Calif., where they were composited by species (based on field identification), extracted, and analyzed for pesticides (table 2).

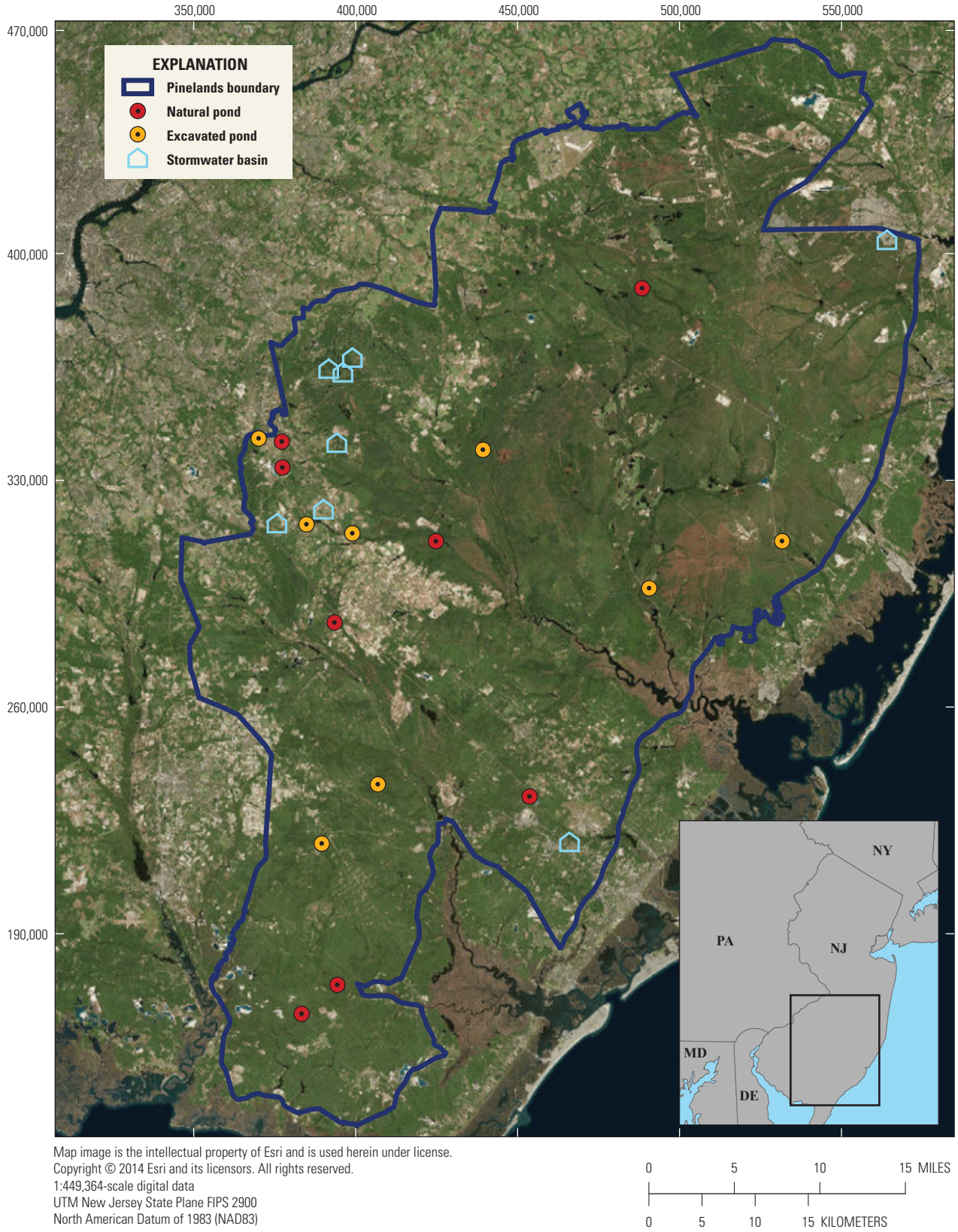

Figure 1. Twenty-four wetlands sampled in the New Jersey Pinelands, 2014–16.

Table 1. Site information, including site number, official station name, site name, year sampled, wetland type, and land-use classification for the natural ponds, excavated ponds, and stormwater basins sampled throughout the New Jersey Pinelands, 2014–16.

[nr, near; mi, mile; NJ, New Jersey; XPond, excavated pond]

Wetland site name	Site number	Official station name	Year sampled	Wetland type	Land-use classification
Burnt Pond	395410074304701	Burnt pond near Mount Misery NJ	2014	Natural	Reference
AlbertsonBog Pond	394116074442201	Unnamed pond near Albertson Brook near Atsion NJ	2016	Natural	Reference
Button Pond	391843074504301	Unnamed pond nr Tuckahoe River nr Hunters Mill NJ	2016	Natural	Reference
Plumegrass Pond	391714074530401	Plumegrass pond near Belleplain NJ	2016	Natural	Reference
Cooper Pond	394500074542901	Cooper Folly pond near West Atco NJ	2014	Natural	Degraded
Hearthstone Pond	394608074542101	Ivystone Farms pond near Tansboro NJ	2014	Natural	Degraded
Pennypot Pond	393707074510101	Unnamed pond near Penny Pot Stream near Folsom NJ	2015	Natural	Degraded
Camera Pond	392816074380901	Unnamed pond near Leipzig Ave near McKee City NJ	2016	Natural	Degraded
Hampton XPond	394557074411501	Excavated pond 0.3 mi south of Hampton Furnace NJ	2014	Excavated	Reference
Scrape XPond	393855074301901	Excavated pond 1 mi southeast of Harrisville NJ	2014	Excavated	Reference
Forge XPond	394117074213601	Excavated pond 2.4 mi north of Stafford Forge NJ	2014	Excavated	Reference
Third XPond	392906074481101	Excavated pond 1.5 mi east of Mizpah NJ	2016	Excavated	Reference
Arrowwood XPond	394205074525201	Arrowwood pond near Braddock NJ	2015	Excavated	Degraded
Duckweed XPond	394627074560201	Excavated pond 1.3 mi south of Berlin NJ	2015	Excavated	Degraded
MacDonald XPond	392555074514701	Excavated pond near MacDonald Ave near Milmay NJ	2016	Excavated	Degraded
Pump XPond	394140074495301	Excavated pond near Wharton Ave nr Ancora NJ	2015	Excavated	Degraded
Ashford Basin	394615074505501	Ashford stormwater basin near Atco NJ	2014	Stormwater basin	Reference
Wilderness Basin	394945074552301	Wilderness stormwater basin near Taunton Lake NJ	2014	Stormwater basin	Reference
Jackson Basin	395034074495401	Jackson stormwater basin near Taunton Lake NJ	2015	Stormwater basin	Reference
Slab Basin	395000074512801	Slab Branch stormwater basin near Taunton Lake NJ	2015	Stormwater basin	Reference
Charles Basin	395635074143701	Lambert Way stormwater basin at Holiday Heights NJ	2015, 2016	Stormwater basin	Degraded
Seth Basin	394252074514601	Seth Drive stormwater basin nr Waterford Works NJ	2015	Stormwater basin	Degraded
Cardinal Basin	394209074545001	Stormwater basin nr Cardinal Ln nr Cedar Grove NJ	2016	Stormwater basin	Degraded
Windsor Basin	392559074353301	Stormwater basin nr Windsor Dr near McKee City NJ	2016	Stormwater basin	Degraded

Table 2. Larval anuran species collected from natural ponds, excavated ponds, and stormwater basins throughout the New Jersey Pinelands, 2014–16.

[Number in parentheses represent the number of larval anurans collected per species in each wetland; Xpond, excavated pond]

Wetland name	Species[1]	Common name
AlbertsonBog Pond	*Lithobates clamitans*	Green frog (6)
	Lithobates sphenocephalus	Southern leopard frog (4)
Burnt Pond	*Lithobates sphenocephalus*	Southern leopard frog (10)
Button Pond	*Lithobates sphenocephalus*	Southern leopard frog (10)
Plumegrass Pond	*Lithobates sphenocephalus*	Southern leopard frog (10)
Camera Pond	*Lithobates clamitans*	Green frog (5)
	Hyla versicolor	Northern gray treefrog (5)
Pennypot Pond	*Lithobates sylvaticus*	Wood frog (5)
	Pseudacris crucifer	Spring peeper (5)
Cooper Pond	*Lithobates sylvaticus*	Wood frog (5)
	Pseudacris crucifer	Spring peeper (5)
Hearthstone Pond	*Lithobates sylvaticus*	Wood frog (5)
	Lithobates clamitans	Green frog (5)
Hampton XPond	Unknown	Unknown (10)
Scrape XPond	*Lithobates sphenocephalus*	Southern leopard frog (10)
Third XPond	*Lithobates clamitans*	Green frog (10)
Forge XPond	*Lithobates sphenocephalus*	Southern leopard frog (10)
MacDonald XPond	*Lithobates clamitans*	Green frog (5)
	Hyla versicolor	Northern grey treefrog (5)
Pump XPond	*Lithobates catesbeianus*	American bullfrog (10)
Arrowwood XPond	*Lithobates catesbeianus*	American bullfrog (4)
	Lithobates clamitans	Green frog (1)
	Pseudacris crucifer	Spring peeper (5)
Duckweed XPond	*Lithobates clamitans*	Green frog (5)
	Pseudacris crucifer	Spring peeper (5)
Slab Basin	*Lithobates clamitans*	Green frog (5)
	Hyla versicolor	Northern gray treefrog (5)
Jackson Basin	*Hyla versicolor*	Northern gray treefrog (10)
Ashford Basin	*Anaxyrus fowleri*	Fowler's toad (10)
Wilderness Basin	*Hyla versicolor*	Northern gray treefrog (10)
Charles Basin	*Hyla versicolor*	Northern gray treefrog (5)
	Lithobates catesbeianus	American bullfrog (11)
Seth Basin	*Hyla versicolor*	Northern gray treefrog (10)
Windsor Basin	*Lithobates catesbeianus*	American bullfrog (10)
Cardinal Basin	*Lithobates catesbeianus*	American bullfrog (10)

[1]Nomenclature obtained from the Integrated Taxonomic Information System (https://www.itis.gov/).

Pesticide Extraction and Analysis

Water samples were analyzed for pesticides by extracting 1L of water onto Oasis® HLB solid-phase extraction (SPE) cartridges by following previously published methods (Hladik and others, 2008). Prior to extraction, all water samples were spiked with $^{13}C_3$-atrazine and d_{14}-trifluralin as the recovery surrogates. For samples collected in 2016, $^{13}C_4$-fipronil was added to the method as another recovery surrogate. The SPE cartridges were dried with carbon dioxide and eluted with 12 mL of ethyl acetate; deuterated internal standards were added to the eluant. All sample extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). Method detection limits (MDLs) ranged from 0.9 to 10.5 nanograms per liter (ng/L) (table 3 in Romanok and others, 2018).

Prior to extraction, larval-anuran samples from each site were composited by species. Bed-sediment and anuran-food samples were homogenized individually, and their masses were recorded prior to extraction. The median mass of bed sediment and anuran food extracted was 5.01 grams (g; range, 1.4–11g) and 5.04 (range, 2.0–6.8g), respectively. All samples were homogenized with sodium sulfate using a solvent-rinsed mortar and pestle and extracted three times by pressurized liquid extraction with dichloromethane (DCM) at 100 °C at 1,500 pounds per square inch. Prior to extraction, samples were spiked with d_{14}-trifluralin, ring-$^{13}C_{12}$-*p,p'*-DDE, and phenoxy-$^{13}C_6$-*cis*-permethrin as recovery surrogates. For bed-sediment and anuran-food samples, sulfur was removed using a gel permeation chromatograph/high performance liquid chromatography system with ethyl acetate as the carrier solvent, and the sample matrix was removed using column chromatography with either packed Florisil or pre-packed Alumina/Carbon SPE cartridges, depending on the compound class (Hladik and McWayne, 2012). In addition, moisture content and percent organic carbon were measured for each bed-sediment sample. Following extraction of composite larval-anuran tissue samples, 10 percent 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. Most of the lipid content was removed by GPC with DCM and methanol (98:2 volume/volume) as the carrier solvent at a flow rate of approximately 4 mL per minute. Following GPC clean-up, the sample extracts were subjected to further clean-up using packed Florisil (Smalling and others, 2013).

Bed-sediment and anuran-food extracts were analyzed on an Agilent 5975 (Folsom, Calif., USA) GC-MS operating in electron ionization mode (EI) (Hladik and McWayne, 2012). Anuran-tissue extracts were analyzed on an Agilent 7890 GC coupled to an Agilent 7000 MS/MS operating in EI mode (Hladik and others, 2016). Analyte separation was achieved by using a 30 m × 0.25 mm inner-diameter × 0.25 micrometer DB-5ms fused silica column (Agilent Technologies, Folsom, Calif., USA) with helium as the carrier gas. The temperature of the splitless injector was held constant at 275 °C. The transfer line, quadrupole, and source temperatures were 280 °C,

150 °C and 230 °C, respectively. Data for all pesticides were collected in either selective ion monitoring mode or multiple reaction monitoring with each compound having one quantifier ion and 1 to 2 qualifier ions. MDLs for bed-sediment and anuran-food samples ranged from 0.5 to 3.1 micrograms per kilogram (µg/kg), dry weight, and anuran-tissue MDLs ranged from 0.5 to 4.2 µg/kg, wet weight (see table 3 in Romanok and others, 2018).

Pesticide 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 obtained from the U.S. Environmental Protection Agency (EPA) National Pesticide Repository. Purities ranged from 95 percent to 99 percent. Performance-based quality assurance and quality control included the parallel analysis of procedural blanks, matrix spikes, and replicates in 10 percent of the samples analyzed for each matrix (water, bed sediment, anuran food, and anuran tissue). No pesticides were detected in any blanks analyzed with the water, bed-sediment, anuran-food, and composite anuran-tissue samples. Means (±standard deviation) of $^{13}C_3$-atrazine, d_{14}-trifluralin, and $^{13}C_4$-fipronil (2016 samples only) added to each water sample as recovery surrogates prior to extraction were 104 ± 14 percent, 100 ± 10 percent, and 108 ± 8 percent, respectively. Means (±standard deviation) of d_{14}-trifluralin, ring-$^{13}C_{12}$ *p,p′*-DDE, and phenoxy-$^{13}C_6$-*cis*-permethrin added prior to each bed-sediment and anuran-food sample as recovery surrogates prior to extraction were 101 ± 15 percent, 92 ± 13 percent, and 91 ± 14 percent, respectively. Means (±standard deviation) of d_{14}-trifluralin, ring-$^{13}C_{12}$ *p,p′*-DDE, and phenoxy-$^{13}C_6$-*cis*-permethrin added to each tissue sample as recovery surrogates prior to extraction were 101 ± 16 percent, 87 ± 11 percent, and 85 ± 10 percent, respectively. Matrix spikes were analyzed in at least 10 percent of the water-quality, bed-sediment, anuran-food, and composite anuran-tissue samples, and the recoveries ranged from 7 to 144 percent (median of 98 percent), 54 to 123 percent (median of 90 percent), 49 to 144 percent (median of 90 percent), and 43 to 140 percent (median of 85 percent), respectively. Field replicates were collected for water samples only, and no laboratory replicates were analyzed. Relative percent difference of the water field replicates ranged from 2 to 23 percent. Relative percent difference of all laboratory replicate samples was less than 30 percent for bed sediment, anuran food, and anuran tissue (where available). Water samples for pesticides and DOC were held for no longer than 48 hours at 4 °C prior to analysis. Bed-sediment, anuran-food, and anuran-tissue samples were stored frozen at -20 °C and held for no longer than 6 months prior to extraction and analysis. Detailed information on individual surrogate and matrix spike recoveries can be found in tables 5 and 6 in Romanok and others (2018). All environmental data were considered to be of acceptable quality on the basis of the quality assurance/quality control results.

Organic Carbon Analysis

Filtered-water samples were analyzed for DOC by high temperature catalytic combustion using a Shimadzu TOC-V_{CNS} total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, Maryland), according to a modified version of EPA Method 415.3 (Potter and Wimsatt, 2005). The instrument was calibrated by using potassium hydrogen phthalate (KNP) standards prepared in organic-free water with concentrations ranging from 0.0 to 4.0 milligrams per liter (mg/L). All standards, blanks, and water samples were acidified prior to analysis with approximately four drops of concentrated sulfuric acid to lower the pH to approximately 1.9. Results are reported in mg/L. The accuracy and precision of the measurements were within 5 percent, as indicated by internal standards (KNP and caffeine), laboratory replicates, and matrix spike samples (Bird and others, 2003, Potter and Wimsatt, 2005). The relative percent difference for the field replicates was less than 10 percent.

Bed-sediment samples were analyzed for organic carbon content by combustion and thermal conductivity by using a Perkin Elmer CHNS/O elemental analyzer (Perkin Elmer Corporation, Waltham, Mass.), according to a modified version of EPA 440.0 (Zimmerman and others, 2007). Dry, homogenized sediments were combusted at 925 °C in silver boats after being exposed to concentrated hydrochloric acid fumes in a desiccator for 24 hours to remove inorganic carbon. Before analysis, sediments were dried at 100 °C for 3 hours. Acetanilide was used for instrument calibration. The instrument was calibrated with blanks and standards prior to sample analysis. Standards were required to be within 98 percent of the nominal value. Blanks, replicates, and standards were analyzed every 10 samples to assess instrument stability. Replicate samples were re-run if the relative percent difference between the two was greater than 20 percent. The method detection limit for carbon was 0.01 percent.

Larval Pathogen Analysis

Deoxyribonucleic acid (DNA) was extracted from Bd swabs using the PrepMan Ultra Sample Preparation Reagent (Applied Biosystems®, Carlsbad, Calif.), according to the method described in Hyatt and others (2007). Bd was detected using quantitative polymerase chain reaction (qPCR), according to the following methods established by Kirshtein and others (2007). A SYBR based assay was utilized with Boyle primers (5.8Schytr and ITS1-3 chytr) as described in Kirshtein and others (2007). A standard curve was constructed using eight serial dilutions of Bd zoospore clone extracts of a known origin. The output Cq (quantification cycle) data were used to calculate gene copies per microliter (µL) (from a 50 µL DNA

extract). The DNA extract from each swab was analyzed in triplicate with an additional sample run as a spike control to test for PCR inhibition (total=4). If the standard deviation of the three Cq values was higher than 0.40, that sample was re-run. Cq averages of 10 Bd gene copies per µL were considered to be positive for Bd. However, if the average Cq value was equal to or greater than the highest Non Template Control value, then the sample was considered to be below the detection limit and negative for Bd even if the gene copies per µL were greater than 10. Any sample average of less than 10 gene copies per µL was scored as negative for Bd.

For *Ranavirus*, DNA was extracted from 25 milligrams (mg) of all tail clip samples by using the Qiagen QIAamp DNA Mini Kit and following manufacturer's instructions. The presence of *Ranavirus* was detected with SYBR Green fluorescence in real time polymerase chain reaction (RT-PCR). A 530 base pair piece of the *Ranavirus* Major Capsid Protein Gene was amplified in RT-PCR reactions using *Ranavirus*-specific primers designed by Mao and others (1997). RT-PCR was used to detect the presence of the pathogen; to quantify the amount of pathogen present in the individual, a standard curve was established with known concentrations of pathogen DNA. All extracts from a single DNA extraction were analyzed in triplicate. If some replicates were positive and others negative, samples were re-run. After re-running, if only one of the replicates was positive, then the sample was considered negative for *Ranavirus*.

Statistical Analysis

A Shapiro-Wilk test was used to determine that the data were not normally distributed. For this reason, data were log-transformed prior to analysis. Three Way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison, was performed to assess the interactions between wetland type (natural pond, excavated pond, and stormwater basins), land-use classification (reference, degraded), and the matrix sampled (water, bed-sediment, anuran-food, and composite larval-anuran-tissue). In addition, the multi-factor ANOVA was performed to assess potential interactions between each variable (wetland type × land-use classification, wetland type × matrix, land-use classification × matrix, wetland type × land-use classification × matrix). A Kruskal-Wallis ANOVA also was used to assess differences in turbidity, pH, specific conductance, dissolved oxygen, DOC, percent organic carbon in bed sediment, and composite larval-anuran lipid content among the three wetland types. Pathogen results did not yield enough detections for statistical comparison among wetland types. Results were considered to be statistically significant if the P-value was less than or equal to 0.05. Pesticide non-detections were given a value of one-half the reported MDL. These values were used in the calculations of total pesticide concentrations for each wetland and in all statistical analyses. SigmaStat was used for all statistical analyses.

Results

The information provided in this report is a summary of the data collected. Detailed information on the individual pesticides detected and their concentrations in each wetland, as well as detailed pathogen information, are available in a data release that is companion to this report (Romanok and others, 2018).

Current-Use Pesticides in Water

Twenty-two individual pesticides were detected in one or more of the 24 water samples collected during 2014–16, including 7 fungicides, 7 herbicides, 3 insecticides, and 5 pesticide degradates. The herbicides metalochlor, atrazine, and dithiopyr were detected most frequently in 89 percent, 60 percent, and 44 percent of the water samples, respectively. The maximum number of pesticides was 13 compounds in a single sample collected in 2015 from one stormwater basin. Total pesticide concentrations (sum of all pesticides detected) in all 24 wetlands sampled ranged from less than the MDL to 912 nanograms per liter (ng/L).

For natural ponds, 6 pesticides were detected in 1 or more of the 8 water samples collected, including 2 fungicides and 4 herbicides. Metolachlor was detected in 100 percent of the natural ponds, atrazine in 63 percent, and dithiopyr in 38 percent. Total pesticide concentrations in water samples from natural ponds ranged from 11.6 to 235 ng/L with a median of 93 ng/L.

For excavated ponds, 6 pesticides were detected in 1 or more of the 8 water samples collected, including 3 fungicides, 2 herbicides, and 1 insecticide. Metolachlor was detected in 75 percent of the excavated pond samples, the fungicide azoxystrobin in 38 percent, and atrazine in 25 percent. Total pesticide concentrations in water samples from excavated ponds ranged from not detected (ND) to 176 ng/L with a median of 59 ng/L.

For stormwater basins, 18 pesticides were detected in 1 or more of the 8 water samples collected, including 4 fungicides, 6 herbicides, 3 insecticides, and 5 pesticide degradates. Atrazine and dithiopyr were detected in 89 percent of the stormwater basin samples, metolachlor in 78 percent, and fipronil desulfinyl in 78 percent. The insecticide fipronil and its degradates (fipronil desulfinyl, fipronil desulfinyl amide, fipronil sulfide, and fipronil sulfone) were observed only in samples collected from the stormwater basins. Total pesticide concentrations in water samples from stormwater basins ranged from 41.4 to 912 ng/L with a median of 281 ng/L.

The number of pesticides detected in reference wetlands varied between natural ponds, excavated ponds, and stormwater basins and ranged from 0 to 10 with total pesticide concentrations ranging from ND to 240 ng/L (table 3). Similarly, the number of pesticides detected in degraded natural ponds, excavated ponds, and stormwater basins varied and ranged from 2 to 13 with total pesticide concentrations ranging from 24.0 to 912 ng/L (table 3).

Table 3. Summary statistics, including the median and range, for the number of pesticides detected and total pesticide concentrations in water samples collected from reference and degraded natural ponds, excavated ponds, and stormwater basins in the New Jersey Pinelands, 2014–16.

[ND, not detected; ng/L, nanograms per liter]

Wetland type	Classification	Number of pesticides detected		Total pesticide concentration (ng/L)	
		Median	Range	Median	Range
Natural Pond	Reference	1	1–2	40	11.6–46.3
	Degraded	3	2–6	54.6	35.7–235
Excavated Pond	Reference	1	0–3	7.2	ND–10.3
	Degraded	3	2–3	83.9	24.0–176
Stormwater Basin	Reference	6	2–10	118	41.4–240
	Degraded	9	7–13	420	246–912

Current-Use Pesticides in Bed Sediment and Anuran Food

Twenty-two individual pesticides were detected in 1 or more of the 24 bed-sediment samples collected during 2014–16, including 3 fungicides, 5 herbicides, 7 insecticides, 6 pesticide degradates, and 1 insect growth regulator (methoprene). In the anuran-food samples, 23 pesticides were detected. With the exception of azoxystrobin (fungicide), all compounds observed in the bed-sediment samples were also detected in the anuran-food samples. In both bed-sediment and anuran-food samples, the legacy insecticide *p,p′*-DDT and its primary degradates, *p,p′*-DDD and *p,p′*-DDE were the most frequently detected compounds (sediment: 32 percent, 32 percent, and 76 percent, respectively, and food: 28 percent, 24 percent, and 72 percent, respectively). The pyrethroid insecticide bifenthrin and the herbicide dithiopyr were the only current-use pesticides observed in greater than 25 percent of the bed-sediment and anuran-food samples. The maximum number of pesticides was 15 compounds in both bed-sediment and anuran-food samples in 2015 from the same stormwater basin that had the most pesticides in water. Total pesticide concentrations in bed-sediment samples ranged from ND to 196 µg/kg, dry weight, and in anuran-food samples from ND to 178 µg/kg, dry weight. The pesticides detected and their concentrations in bed-sediment and anuran-food samples were highly correlated ($P<0.001$). Only 3 percent of the observations (17 out of 549) resulted in a compound being detected in one matrix and not the other.

The number of pesticides detected in bed-sediment and anuran-food samples from reference wetlands varied between natural ponds, excavated ponds, and stormwater basins and ranged from 0 to 9 (table 4). Total pesticide concentrations in bed-sediment and anuran-food samples from reference wetlands ranged from ND to 87.3 µg/kg, dry weight, and from ND to 92.8 µg/kg, dry weight, respectively (table 4). Similarly, the number of pesticides detected in degraded natural ponds, excavated ponds, and stormwater basins varied and ranged from 1 to 15 (table 4). Total pesticide concentrations in bed-sediment and anuran-food samples from degraded wetlands ranged from 1.4 to 196 µg/kg, dry weight, and from 0.3 to 178 µg/kg, dry weight, respectively (table 4).

Current-use Pesticides in Anuran Tissue

Larval-anuran-tissue samples collected from each site were composited by species (approximately 5 individuals per composite and 2 to 4 composites per site) and analyzed for pesticides and percent lipid content. Seven different species were captured during the study, and American bullfrogs were observed most frequently at the more degraded excavated ponds and stormwater basins (table 2). The percent lipid in the composite samples ranged from 0.4 to 3.8 with a median of 1.5 percent. Despite species differences, there was no difference in median percent lipid content on the basis of wetland type or land-use classification.

Nine individual pesticides were detected in one or more of the 52 composite larval-anuran-tissue samples collected during 2014–16, including 3 herbicides, 2 insecticides, and 4 pesticide degradates. Bifenthrin and *p,p′*-DDE were the most frequently detected pesticides and occurred in 37 percent and 31 percent of the samples, respectively. The maximum number of pesticides observed in a single sample was four compounds in 3 stormwater basins and 1 excavated pond. Total pesticide concentrations ranged from less than the MDL to 573 µg/kg, wet weight (table 5).

In natural ponds, the only pesticides detected in anuran tissue were *p,p′*-DDT and its degradates (*p,p′*-DDD and *p,p′*-DDE); detection frequencies for *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT were 38 percent, 44 percent, and 13 percent respectively. In excavated ponds, bifenthrin was observed in 24 percent of the samples; the fungicide degradate pentachloroanisole was detected in 12 percent of the samples. *p,p′*-DDD and *p,p′*-DDE were detected in 24 percent and 29 percent of the samples. No pesticides were detected in composite larval-anuran-tissue samples from reference excavated ponds. Bifenthrin and dithiopyr were detected most frequently and occurred in 79 percent and 47 percent of the samples collected from stormwater basins.

The number of pesticides detected in composite larval-anuran tissue samples from reference wetlands ranged from 0 to 2 with total pesticide concentrations ranging from ND to 14.0 µg/kg, wet weight (table 5). The number of pesticides detected in degraded wetlands varied and ranged from 0 to 4; total pesticide concentrations ranged from ND to 573 µg/kg, wet weight (table 5).

Table 4. Summary statistics, including the median and range, for the number of pesticides detected and total pesticide concentrations in bed-sediment and anuran-food samples collected from reference and degraded natural ponds, excavated ponds, and stormwater basins in the New Jersey Pinelands, 2014–16.

[ND, not detected; µg/kg, micrograms per kilogram dry weight]

Wetland type	Matrix	Classification	Number of pesticides detected		Total pesticide concentration (µg/kg)	
			Median	Range	Median	Range
Natural pond	Sediment	Reference	1	0–3	3.4	1.5–4.8
		Degraded	2	1–2	5.3	1.4–21.8
	Food	Reference	2	1–3	4.1	ND–4.5
		Degraded	2	1–2	7.4	0.3–12.5
Excavated pond	Sediment	Reference	0	0–2	0.5	ND–1.0
		Degraded	3	1–6	38.5	7.6–99.0
	Food	Reference	1	0–1	4.1	ND–7.7
		Degraded	4	3–6	8.8	1.0–41.8
Stormwater basin	Sediment	Reference	2	1–9	14	1.0–87.3
		Degraded	6	5–15	46.5	18.1–196
	Food	Reference	3	1–8	32.6	2.4–92.8
		Degraded	6	4–15	61.7	6.8–178

Table 5. Summary statistics, including the median and range, for the number of pesticides detected and total pesticide concentrations in larval anuran-tissue samples collected from reference and degraded natural ponds, excavated ponds, and stormwater basins in the New Jersey Pinelands, 2014–16.

[ND, not detected; µg/kg, micrograms per kilogram wet weight]

Wetland type	Classification	Number of pesticides detected		Total pesticide concentration (µg/kg)	
		Median	Range	Median	Range
Natural pond	Reference	1	0–2	5	ND–14.0
	Degraded	0	0–3	5	ND–5.5
Excavated pond	Reference	0	0	ND	ND
	Degraded	2	0–4	7.9	ND–45.2
Stormwater basin	Reference	1	0–1	4.8	ND–11.4
	Degraded	4	2–4	41.8	24.8–573

Ancillary Water and Sediment Measurements

Results for the water- and sediment-quality characteristics [dissolved oxygen (mg/L), pH (standard units), specific conductance (microsiemens per centimeter at 25 degrees Celsius; µS/cm at 25 °C), turbidity (Nephelometric Turbidity Unit, NTU), DOC (mg/L) and organic carbon in sediment (percent)] can be found in Romanok and others (2018). Organic carbon in sediment and DOC concentrations in water were higher in natural pond samples than in stormwater basins (P=0.005) with no other differences observed. As expected, pH was higher in the stormwater basins than in the natural and excavated ponds (P<0.001) with no differences observed between natural and excavated ponds. No differences were observed in the other water-quality characteristics (DO, specific conductance, and turbidity) by wetland type or classification.

Relations to Percent Altered Land

A summary of the total pesticide concentrations in water, bed-sediment, anuran-food, and composite larval-anuran tissue samples in relation to land use is presented in table 6. The percent altered land in a 500-m radius around each wetland ranged from 0 to 62.4 percent for the natural ponds, 0 to 63.6 percent for the excavated ponds, and from 23.3 to 80.2 percent for the stormwater basins (table 6). The percent altered land surrounding stormwater basins ranged from 23.3 to 41.9 percent (reference) and 56.6 to 80.2 percent (degraded). The percent altered land surrounding reference stormwater basins was similar to the percent altered land surrounding degraded natural and excavated ponds (table 6).

A multi-factor ANOVA was used to assess relations between pesticide occurrence, distribution, and land use among the natural and created wetlands in the N.J. Pinelands. More specifically, the total pesticide concentration and the number of pesticides detected in all matrices (water, bed-sediment, anuran-food, and composite larval-anuran-tissue) were compared among wetland types (natural ponds, excavated ponds, and stormwater basins) and land-use classifications (reference and degraded), as well as the interactions between these factors (table 7). For wetland types, the analysis indicated stormwater basins had significantly higher total pesticide

Table 6. Land-use information for a 500-meter radius around each wetland and total pesticide concentrations (sum of all pesticides detected) in water, sediment, anuran-food, and composite larval-anuran tissue samples collected from each wetland in the New Jersey Pinelands.

[2007 land-use data available at http://www.nj.gov/dep/gis/lulc07shp.html (New Jersey Department of Environmental Protection, 2010); sediment and anuran food concentrations reported as dry weight; tissue concentration reported as wet weight; tissue concentrations are reported as an average of the 2–4 composites analyzed per wetland; ng/L, nanograms per liter; µg/kg, micrograms per kilogram; ND, not detected; X, excavated]

Wetland name	Land-use classification	Land use (percent)			Concentration			
		Agricultural	Developed	Altered[1]	Water (ng/L)	Sediment (µg/kg)	Anuran food (µg/kg)	Tissue (µg/kg)
AlbertsonBog Pond	Reference	0	0	0	46.3	4.8	3.7	11.4
Burnt Pond	Reference	0	0	0	11.6	1.5	3.3	2.9
Button Pond	Reference	0	0	0	11.3	2	4.5	ND
Plumegrass Pond	Reference	0	0	0	33.5	ND	ND	ND
Camera Pond	Degraded	13.3	23	36.3	35.7	8	12.5	ND
PennyPot Pond	Degraded	22	24.8	46.8	235	21.8	9.1	1.4
Cooper Pond	Degraded	9.3	46.8	56.1	57.6	1.4	0.3	ND
Hearthstone Pond	Degraded	5.9	56.5	62.4	51.7	2.6	5.7	4.6
Hampton XPond	Reference	0	0	0	9.4	0.5	ND	ND
Scrape XPond	Reference	0	0	0	ND	ND	ND	ND
Third XPond	Reference	0	0	0	10.3	ND	ND	ND
Forge XPond	Reference	0	0.3	0.3	ND	1	7.7	ND
MacDonald XPond	Degraded	17.5	11.6	29.1	42.6	7.6	6.8	ND
Pump XPond	Degraded	24.8	8.4	33.2	24	28.8	1	ND
Arrowwood XPond	Degraded	33.5	25.3	58.8	125	48.2	10.9	9.1
Duckweed XPond	Degraded	27.1	36.5	63.6	176	99	41.8	33.4
Slab Basin	Reference	0	23.3	23.3	176	1.5	2.4	ND
Jackson Basin	Reference	0	23.5	23.5	59.5	87.4	92.8	4.1
Ashford Basin	Reference	1.4	35.3	36.8	41.4	1	2.6	ND
Wilderness Basin	Reference	0	41.9	41.9	240	26.5	62.7	8.5
Charles Basin[2]	Degraded	0	56.6	56.6	912 411	18.2 196	33.3 178	273 103
Windsor Basin	Degraded	1.7	67.5	69.2	603	62.3	61.7	41
Cardinal Basin	Degraded	9.2	61.4	70.6	246	46.5	132	26.9
Seth Basin	Degraded	45.3	35	80.2	420	24.4	6.8	30.2

[1]Percent altered land use is the sum of agricultural and developed lands.

[2]Sampled in 2015 (top) and 2016 (bottom).

concentrations and a significantly greater number of pesticides detected compared to the natural and excavated ponds. However, no significant differences were observed between natural and excavated ponds (fig. 2). For land-use classifications, the analysis indicated significant differences between degraded and reference wetlands. The degraded wetlands had higher total pesticide concentrations and a greater number of pesticides detected compared to the reference wetlands (fig. 3). When comparing the interactions among land-use classification and wetland types, the differences observed in total pesticide concentrations, as well as the number of pesticides detected, varied. For example, degraded excavated ponds and stormwater basins had significantly higher pesticide concentrations and a significantly greater number of pesticides detected than their reference counterparts. However, no significant differences were observed between degraded and reference natural ponds. When comparing among land-use classifications, reference stormwater basins had significantly higher total pesticide concentrations and a significantly greater number of pesticides detected compared to natural and excavated ponds (fig. 4). Significant differences in total pesticide concentrations also were observed between the three degraded wetland types; total pesticide concentrations were lowest in degraded natural ponds and highest in degraded stormwater basins (fig. 5). However, when comparing the number of pesticides detected among degraded wetlands, a significantly greater number of pesticides were detected in stormwater basins than in natural and excavated ponds with no differences between natural and excavated ponds (fig. 5). Total pesticide concentrations and the number of pesticides detected in reference stormwater basins also were compared to those in degraded natural and excavated ponds because of similarities in the percent altered land in a 500-m radius around each wetland (table 6). On the basis of these results, no significant differences were observed between these three wetland types.

Differences in total pesticide concentrations and the number of pesticides detected were observed between the four matrices (table 7). Total pesticide concentrations were significantly higher in water than in the other three matrices with no differences observed among the other matrices. Significantly fewer pesticides were detected in the composite larval-anuran tissue samples than in the other three matrices. When comparing the interaction between land-use classification and matrix, no differences were observed in the total pesticide concentrations or the number of pesticides detected between reference and degraded wetlands. Furthermore, no differences in wetland type by matrix were observed for total pesticide concentrations (table 7). However, a significant interaction between wetland type and matrix was observed for the number of pesticides detected. For water, bed-sediment, and anuran-food samples, stormwater basins were significantly different from other wetland types with a greater number of pesticides detected. However, the number of pesticides detected was not significantly different between natural and excavated ponds. The number of pesticides detected in composite-anuran-tissue samples was not different among wetland types.

Table 7. Results of Three-Way Analysis of Variance, including F-statistic and P-values, comparing total pesticide concentrations and the number of pesticides detected across all wetland types (natural ponds, excavated ponds, and stormwater basins), land-use classifications (reference and degraded) and matrices (water, bed-sediment, anuran-food, and composite larval-anuran tissues), as well as interactions between the factors.

[The "x" represents the interaction tested between factors. All statistical analyses were conducted in SigmaStat. <, less than]

Variable	F-statistic	P-value
Total pesticide concentration		
Wetland type	31.97	<0.001
Land-use classification	41.37	<0.001
Matrix	27.78	<0.001
Wetland type x Land-use classification	6.32	0.003
Wetland type x Matrix	1.32	0.255
Land-use classification x Matrix	2.23	0.089
Wetland type x Land-use classification x Matrix	1.81	0.105
Number of pesticides detected		
Wetland type	46.73	<0.001
Land-use classification	40.67	<0.001
Matrix	13.4	<0.001
Wetland type x Land-use classification	7.18	0.001
Wetland Type x Matrix	4.25	<0.001
Land-use classification x Matrix	0.753	0.523
Wetland type x Land-use classification x Matrix	0.49	0.814

Emerging Pathogens

Batrachochytrium dendrobatidis (Bd) Fungus

The 10 larval-anurans specimens from each wetland euthanized and composited for pesticide analysis were first individually swabbed for Bd. Of the 240 swabs analyzed, 18 were positive for Bd, and the number of zoospore equivalents per swab ranged from 11.6 to 1,735 with a median of 21.9. Bd was observed in 4 natural ponds (1 reference and 3 degraded), 3 excavated ponds (all reference), and 2 stormwater basins (1 reference and 1 degraded); however, detection probability was low. In the wetlands with Bd detections, between 10 and 30 percent (between 1 and 3) of the animals swabbed were positive for Bd. Owing to the limited number of positive detections, no statistical comparisons between wetland types or land-use classifications were possible.

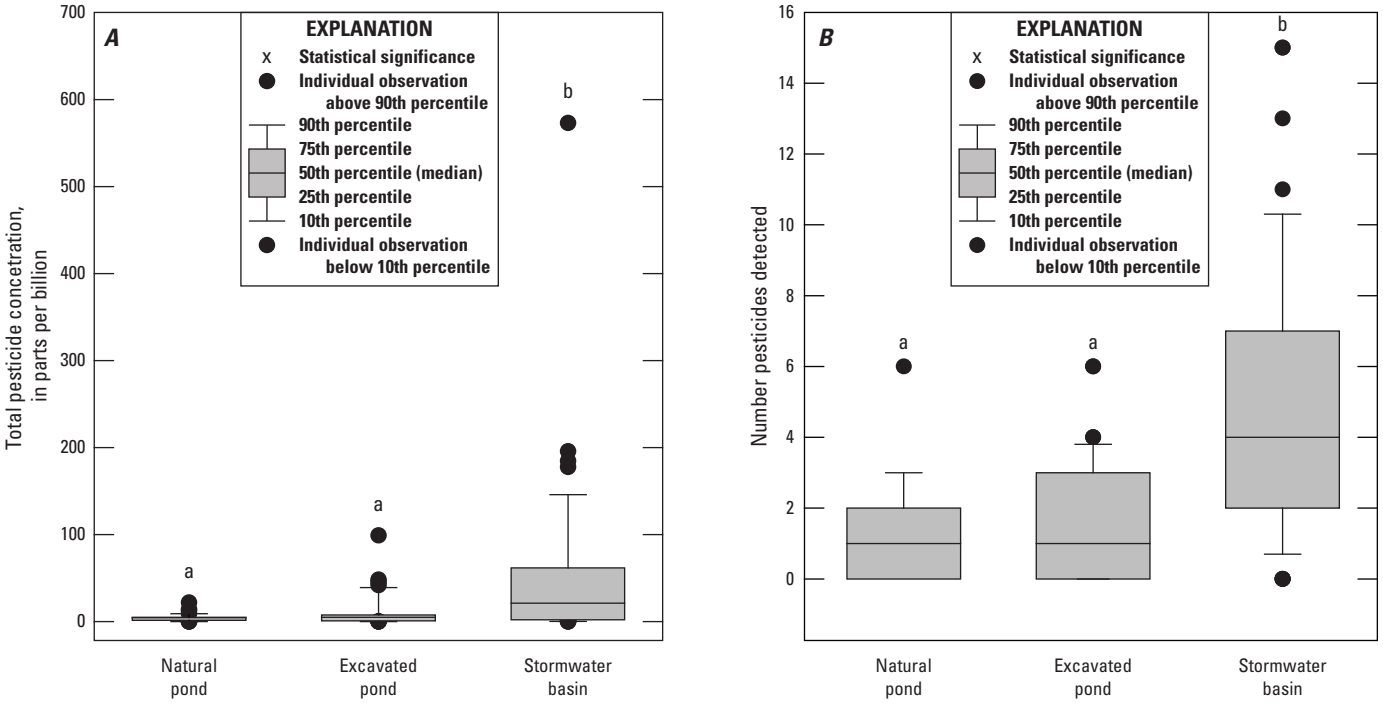

Figure 2. Differences in *A*, total pesticide concentrations and *B*, the number of pesticides detected in water, bed-sediment, anuran-food, and composite larval-anuran-tissue samples from natural ponds, excavated ponds, and stormwater basins collected throughout the New Jersey Pinelands, 2014–16. Letters above the box plot represent statistical significance, and areas with no letters in common are significantly different from one another (P< 0.05).

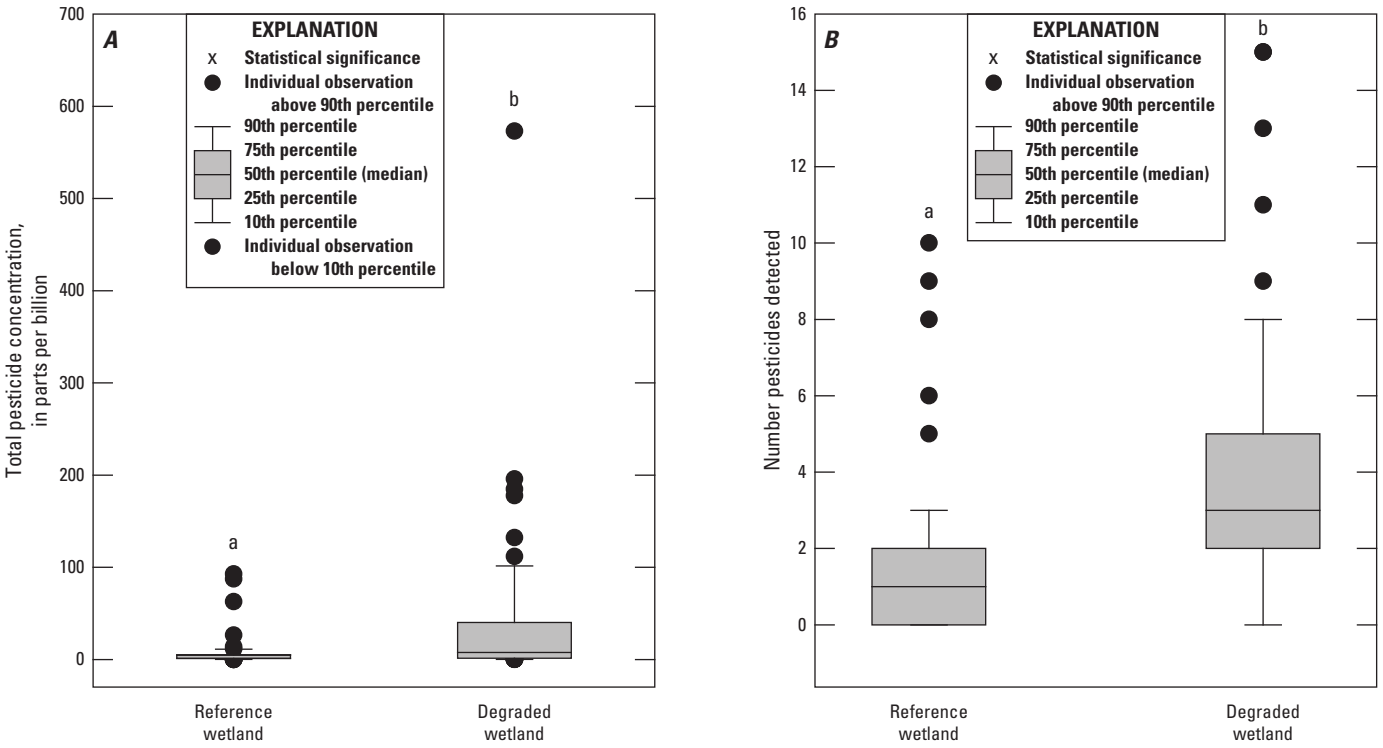

Figure 3. Differences in *A*, total pesticide concentrations and *B*, the number of pesticides detected in water, bed-sediment, anuran-food, and composite larval-anuran-tissue samples from reference and degraded wetlands collected throughout the New Jersey Pinelands, 2014–16. Letters above the box plot represent statistical significance, and areas with no letters in common are significantly different from one another (P< 0.05).

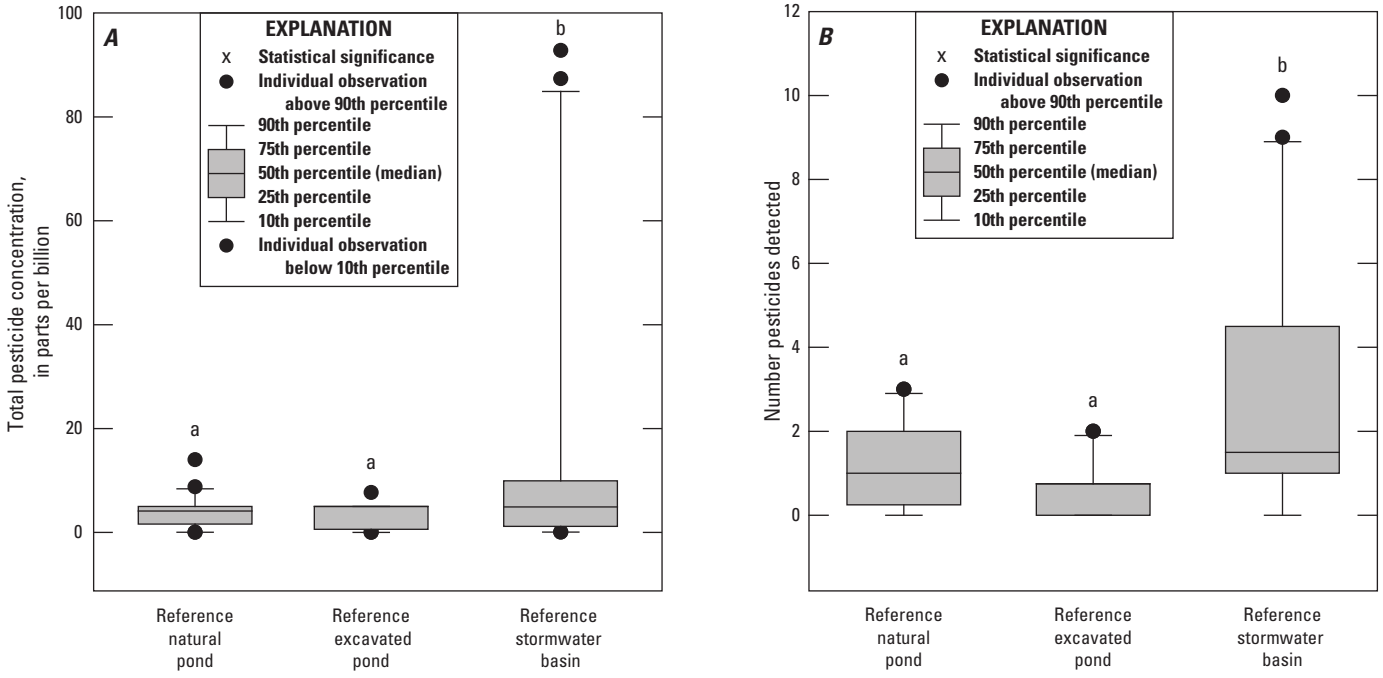

Figure 4. Differences in *A*, total pesticide concentrations and *B*, the number of pesticides detected in water, bed-sediment, anuran-food, and composite larval-anuran-tissue samples from reference natural ponds, excavated ponds, and stormwater basins collected throughout the New Jersey Pinelands, 2014–16. Letters above the box plot represent statistical significance, and areas with no letters in common are significantly different from one another (P< 0.05).

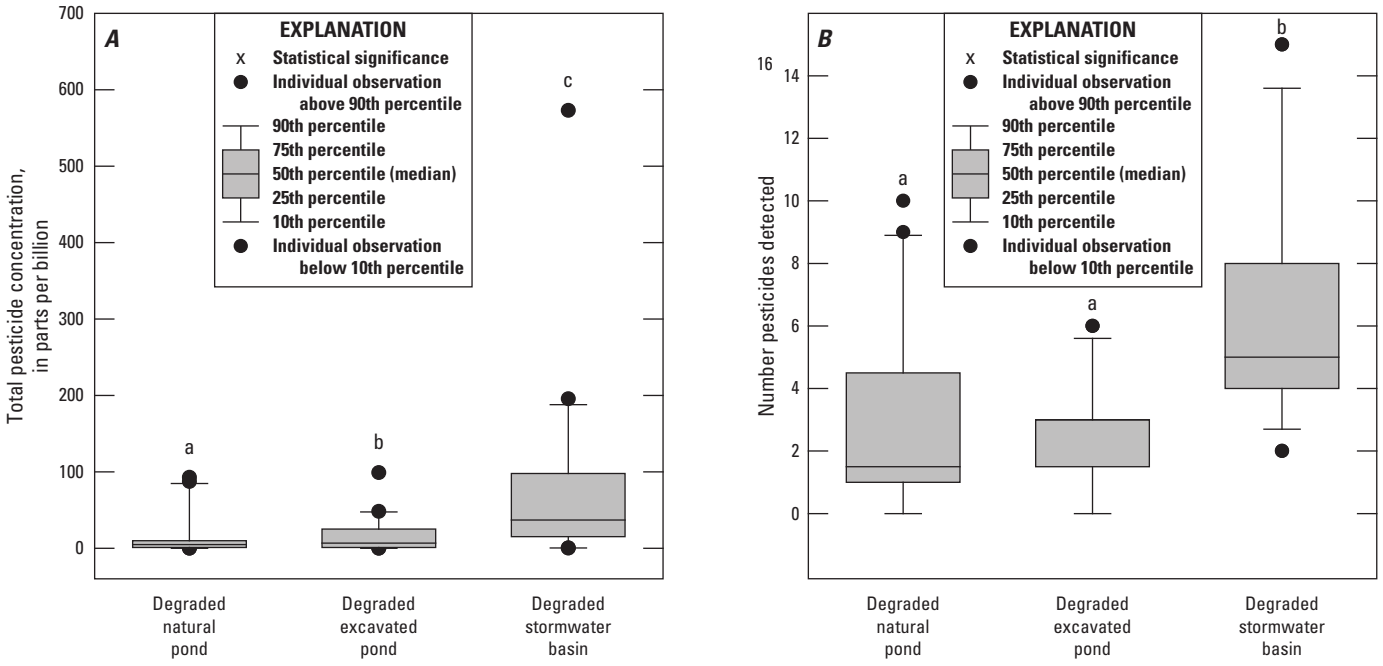

Figure 5. Differences in *A*, total pesticide concentration and *B*, the number of pesticides detected in water, bed-sediment, anuran-food, and composite larval-anuran-tissue samples from degraded natural ponds, excavated ponds, and stormwater basins collected throughout the New Jersey Pinelands, 2014–16. Letters above the box plot represent statistical significance, and areas with no letters in common are significantly different from one another (P< 0.05).

Ranavirus

The presence and prevalence of *Ranavirus* was determined using tail clips from 60 larval frogs from each of the 24 wetlands. *Ranavirus* was observed in larvae from 4 wetlands, including 1 reference natural pond, 1 degraded natural pond, and 2 degraded stormwater basins. Prevalence ranged from 3.4 percent to 42.9 percent, and the highest prevalence was observed in one of the degraded stormwater basins. *Ranavirus* was observed in larval wood frogs, American bullfrogs, and southern leopard frogs (table 2). Similar to Bd, no statistical comparison between wetland types or land-use classifications could be conducted because of the low presence and prevalence of *Ranavirus* in the sampled wetlands.

Summary

In a study conducted by the U.S. Geological Survey, in cooperation with the New Jersey Pinelands Commission and Montclair State University, a number of different pesticides were observed in wetland habitats (water and sediment), larval-anuran food, and composite larval-anuran-tissue samples collected from three types of wetlands—natural ponds, excavated ponds, and stormwater basins—throughout the New Jersey Pinelands. Wetlands were further classified by land use into reference (minimum land-use effect) and degraded (maximum land-use effect) sites based on the percent altered land in a 500-meter radius around each wetland. Three herbicides—metolachlor, atrazine, and dithiopyr—were detected frequently in water, whereas dithiopyr and the pyrethroid insecticide bifenthrin, along with *p,p′*-DDT and its degradates *p,p′*-DDD and *p,p′*-DDE, were detected in bed-sediment and anuran-food samples. In composite tissue samples, bifenthrin and DDE were detected most frequently. In stormwater basins, a significantly greater number of pesticides were detected, and total pesticide concentrations were significantly higher compared to natural and excavated ponds. In reference wetlands, the pesticides detected and the total pesticide concentrations were significantly lower compared to degraded wetlands. Interestingly, no relation was observed between total pesticide concentrations in a given matrix and wetland type or land-use classification. This could be due to the low detection frequencies in the natural and excavated ponds and the large variability in concentrations observed in wetlands surrounded by a higher percent altered land (stormwater basins). However, a significant interaction between wetland type and matrix was observed for the number of pesticides detected. A significantly greater number of pesticides were detected in water, bed-sediment, and anuran-food samples from stormwater basins than those from natural and excavated ponds, whereas the number of pesticides in composite-anuran-tissue samples was not different among wetland types.

The detection of emerging amphibian pathogens was low during the 3 years of wetland sampling. *Batrachochytrium dendrobatidis* (Bd) was observed in 9 of 24 wetlands, but detections were limited to between 1 and 3 animals per site (10–30 percent). *Ranavirus* was observed in 4 of 24 wetlands, with prevalence ranging from 3 to 43 percent. *Ranavirus* was observed in four wetlands (all three wetland types), with prevalence ranging from 3 to 43 percent, and was highest in one of the stormwater basins. We were unable to determine the relation between pathogens and pesticides owing to the limited observations of Bd and *Ranavirus* in the wetlands sampled.

Created wetlands have the potential to provide refugia for native anuran populations in the New Jersey Pinelands. However, amphibians residing and breeding in wetlands surrounded by a higher percent altered land (stormwater basins) are exposed to a variety of pesticides throughout their lifecycle, and the effects of these mixtures are relatively unknown. The occurrence and distribution of pesticides was more similar between excavated and natural ponds than other wetland types; however, stormwater basins had a greater number of pesticides and higher overall pesticide concentrations. Land use also played a significant role in the occurrence and distribution of pesticides throughout the Pinelands. As the percent-altered land increased around a wetland, the concentrations and number of pesticides detected also increased, especially for reference and degraded wetlands. Documenting the quality of the habitat available for amphibians and other aquatic Pinelands species will help prioritize research necessary to decipher the effects of pesticides, pathogens, habitat loss/degradation, and other potential stressors on the long-term viability and management of amphibian populations in a modified landscape.

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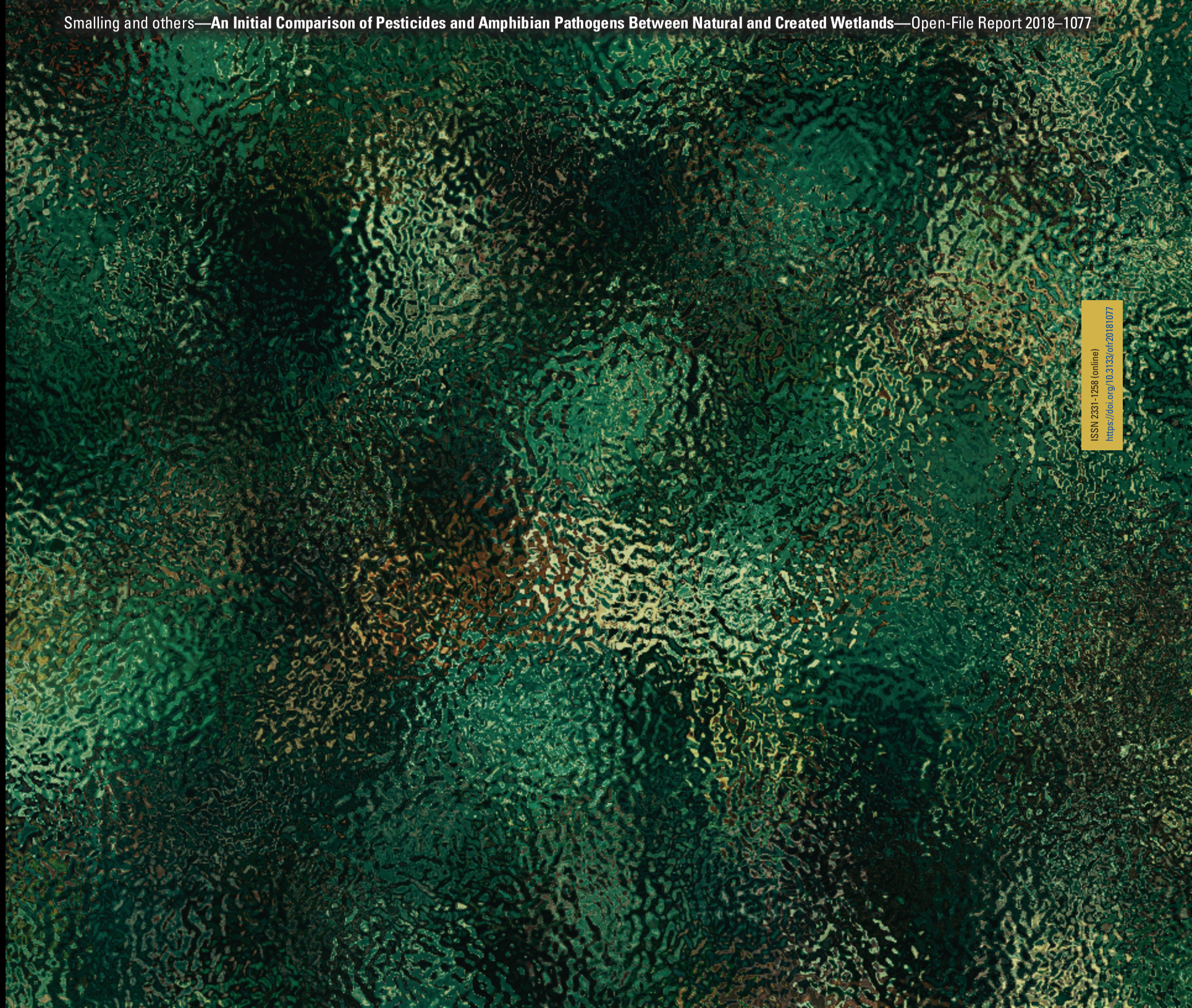

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