Ditch management in agroecosystems: from water quality to frog health

Amber Dyck

A thesis submitted to the University of Ottawa in
partial fulfillment of the requirements for a degree of:
Master of Science in Biology

Department of Biology
Faculty of Science
University of Ottawa

Thesis advisors:
Frances Pick, University of Ottawa, Department of Biology
Stacey Robinson, Environment and Climate Change Canada, National Wildlife Research Centre
David Lapen, Agriculture and Agri-Foods Canada

Abstract

Agriculture ditch management (i.e. removal of vegetation and/or sediments) may disturb native wildlife, such as amphibian bioindicator species. The objective of this thesis was to determine whether ditch management affected northern leopard frogs (*Lithobates pipiens*). Twelve embryo and tadpole health endpoints were compared in vegetated and managed (treeless or dredged) ditches using *in situ* cages in 2018 and 2019, while water quality was monitored. The managed treatment did not negatively affect frog health compared to the vegetated treatment. The significantly faster development and larger body size due to warmer water temperatures at the managed treatment were likely advantageous. The water quality was typical of the region regardless of the ditch treatment and the pesticide mixture detected had limited toxicity. The main effects of the studied ditch managements on resident *L. pipiens* (and potentially other amphibians) appeared sub-lethal, likely beneficial, and mainly the result of temperature differences. Although poorer embryo health was observed at the vegetated treatment, it was likely unrelated to ditch management and possibly due to high specific conductivity causing premature hatching and mortality. Ditch management may be less important for frog health than the surrounding land use, and efforts should be directed at increasing suitable habitat for amphibians in agroecosystems.

Résumé

La gestion des fossés agricoles (plus spécifiquement l'élimination de la végétation des bandes riveraines et /ou le dragage des sédiments) peut perturber la faune indigène, comme les espèces bio-indicateurs d'amphibiens. L'objectif de cette thèse était de déterminer si la gestion des fossés affectait la grenouille léopard du Nord (*Lithobates pipiens*). Douze paramètres d'évaluation de la santé des embryons et des têtards ont été comparés dans des fossés végétalisés et gérés (sans arbres ou dragués) à l'aide de cages expérimentales *in situ* en 2018 et 2019, pendant que la qualité de l'eau a été suivie. Le traitement géré n'a pas eu d'effets négatifs sur la santé des grenouilles par rapport au traitement végétalisé. Le développement beaucoup plus rapide et la taille corporelle plus grande en raison des températures plus chaudes de l'eau lors du traitement géré étaient probablement avantageux. La qualité de l'eau était typique de la région quel que soit le traitement du fossé et le mélange de pesticides détecté avait une toxicité limitée. Les principaux effets de la gestion des fossés étudiés sur *L. pipiens* résidents (et potentiellement d'autres amphibiens) semblaient sublétaux, probablement bénéfiques, et résultaient principalement de différences de température. Bien que la santé des embryons ait été moins bonne lors du traitement végétalisé, elle n'était probablement pas liée à la gestion des fossés et pourrait être due à une conductivité spécifique élevée entraînant une éclosion prématurée et la mortalité. La gestion des fossés peut être moins importante pour la santé des grenouilles que l'utilisation des terres environnantes, et les efforts de protection devraient être dirigés vers une augmentation générale de l'habitat approprié pour les amphibiens dans les agroécosystèmes.

Acknowledgements

I recognize the unceded and surrendered (occupied and stolen) Algonquin Anishinaabe territory where I've lived and worked while in Ottawa, the St-Lawrence Iroquoians, Anishinabewaki, and Haudenosaunee territory where my research took place, and Tyendinaga and Ojibway territories where I spent time while writing (Native Land Digital 2015). I honour the land and water defenders of these territories and across Turtle Island, for their stewardship, and ways of knowing and being. I hold myself, the institutions I am a part of and the governments I am a citizen to, responsible for unkept treaties, the absence of treaties on stolen and occupied lands, and the ongoing genocide of Indigenous peoples inflicted by settler-colonial systems (Residential schools (TRC 2015), Sixties Scoop (Kimelman 1985), Missing and Murdered Indigenous Women, Girls and Two Spirit people (MMIWG 2019), and systemic racism in policing and the RCMP (Morin 2020)).

Although not the focus of this thesis, I am determined to speak up in what can feel like a silo of scientific pursuits, that does not prioritize addressing racism, inequity, and oppression. I come as a white, settler-colonial descendent, academically trained biologist and researcher who is acquiring a lens of intersectionality, seeking to unlearn oppressive mechanisms of colonialism and relearn how to be a land based person, in positive relations with Indigenous Peoples, Black people and people of colour; and for these positive relations to extend to all other-than human relations as well. I feel it necessary to call us all to situate ourselves in the broader context and find our responsibilities to the land and stewardship of life.

I recognize the intersectionality of oppressions caused by colonialism, patriarchy, capitalism and agriculture (particularly industrial agriculture). Beyond environmental problems associated with agriculture, I wish to recognize the social, political, economic, and spiritual problems associated with it. I have learned that extractive Eurocentric worldviews have informed federal and international policies focused on export-oriented agriculture and economies, that prioritize financial wealth over ecosystem and human wellbeing and sustainability. I do not wish to blame farmers for the extractive methods of industrial agriculture, I do however encourage all of us to find our role in shifting our systems towards principals of responsibility, stewardship, care, and sacredness. Food sovereignty and agroecology, as defined by La Via Campesina, are processes and tools that can guide us in this transformation (Rosset and Martinez-Torres).

I also make an urgent call for attention towards advocating for, legislating and implementing climate solutions that meet the rate and scale the climate crisis demands. This must be done in ways that do not further entrench colonial and capitalist power, but respect Indigenous rights, sovereignty, and solutions, and address systemic oppression and inequity. With less than 10 years to cut greenhouse gas emissions to safe levels (IPCC 2018), the urgency of our collective response to climate change and social inequity must go far beyond conducting more scientific studies of specific taxa.

And now, time for the gratitude.

I want to thank my three fantastic thesis supervisors. Thank you, Frances Pick for your extensive experience researching limnology in the South Nation watershed (and beyond), for your keen eye and enthusiasm for the health of aquatic systems, for your advise in all things academic, and thorough and thoughtful editing. Thank you, Stacey Robinson for guiding me with your frog knowledge, for the skills and tools of your research team shared with me, and for your supportive and affirming mentorship. Thank you, David Lapen for being the scientist whose riparian research drew me back into academics (from a beloved farm), for believing in the value of this work, and for your humour and spunk.

Thanks to the three research teams who I had the honour to work and become friends with. My research family is big: (Pick's Lab) Mary Ann Perron, Hebah Mejbel, Will Dodsworth, Charlotte Heller, Isabella Richmond, Meaghan MacIntyre-Newell, Courney Irwin, Jesse Fortier; (Robinson's Lab) Sarah Young, Melody Gavel, Jillian Rohonczy, Justin Marchand, David Carpenter, André Morill; (Lapen's team) Emilia Craiovan, Mark Sunohara, and the many summer field students. Thanks to Kimberly Bray, an Honour's student I had the opportunity to work with, and who contributed largely to calculating the Pesticide Toxicity Indices. Thanks also to Niloofar Alavi at Environment and Climate Change Canada for assistance with GIS mapping.

Thanks to Justin Renaud, Lyne Sabourin and Mark Sumarah at the London Research and Development Centre for collaborating with me to develop a method to measure pesticide mixture residues in tadpole tissue samples, and for attempting to answer my millions of questions about analytical chemistry. Thanks to all the students and researchers in their labs who assisted me along the way, with tea and/or answers.

Thanks to Vance Trudeau and his lab for supplying the embryo clutches. Special thanks go to the embryo clutches themselves, to the ditch waters, to the farmers for allowing research to take place in their fields. Thanks to Eric Lemieux, Drainage Superintendent for the Nation Municipality, for assisting me to find suitable research sites and procuring land access.

Thanks to my peers at the BGSA and GSAED for the community and sense of greater purpose. Deep thanks to my friends at Climate Justice Ottawa for journeying with me as I learn about intersectionality, and for a community to take action with on social and ecological justice issues. Deep thanks to the group of Indigenous youth and allies whose actions I had the honour to support (especially Wet'suwet'en solidarity) and practice using my privilege in justice-oriented ways. I honour Ryan, for the memories made, and the learning we did together and apart. Special thanks to my friends and family, for the fun, learning, love and adventures throughout my master's journey.

Table of Contents

List of Tables

List of Figures

Abbreviations

AAFC	Agriculture and Agri-Foods Canada
AIC	Akaike Information Criterion
CC	Coefficient of conservatism
Chl *a*	Chlorophyll *a*
df	Degrees of freedom
DO	Dissolved oxygen
DOC	Dissolved organic carbon
GLM	Generalized Linear Model
GLMM	General(ized) Linear Mixed-effect Model
GS	Gosner Stage
HPLC	High performance liquid chromatography
LC50	Median lethal dose
LCMS-MS	Liquid chromatography mass spectrometry
LOQ	Limit of quantification
LSI	Liver Somatic Index
M #	Managed treatment site number
MDL	Minimum detection limit
MRL	Minimum reporting limit
NeonicTot	Total neonicotinoid
NH3-NH4	Ammonia-ammonium
NO2	Nitrite
NO3	Nitrate
NTU	Nephelometric Turbidity Unit
NWRC	National Wildlife Research Centre
ORP	Redox potential
PCA	Principal component Analysis
PTI	Pesticide Toxicity Index
RP	Reactive phosphorus
SC	Specific conductivity
SD	Standard deviation
SE	Standard error
SVL	Snout-to-vent length
TDS	Total dissolved solids
Temp	Temperature
TIN	Total inorganic nitrogen
TKN	Total Kjeldahl Nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TSS	Total suspended solids
V #	Vegetated treatment site number

1. Introduction

1.1 Modern agriculture and impacts

Modern intensive agriculture has profoundly affected landscapes through land-use changes, that have cascading effects on terrestrial and aquatic environments (Foley et al., 2005; Stoate et al., 2001). The shift from smaller, family run, low-input, diverse growing systems, to large, high intensity corporate farms has accelerated in the last half of the 20th century and has led to many environmental (and other) problems (Klimek et al., 2014). Landscapes with intensive agriculture have lower proportions of uncropped habitat, such as field margins, fallow lands, hedgerows (Stoate et al. 2001), wetlands and riparian areas (Ducks Unlimited Canada 2010; SNCA 2018). This intensification of agriculture has led to habitat loss and fragmentation, the deterioration of remaining habitat quality (Robinson & Sutherland, 2002) and loss of biodiversity (Stoate et al. 2001). In addition, industrial agriculture production systems are highly dependent on external inputs of fossil fuels, machinery, fertilizers, pesticides, and pharmaceutical products. Agricultural intensification contributes to soil erosion and loss of organic matter, air and water pollution, and climate change (Stoate et al. 2001; Reeves 2014; Reid et al. 2018).

Industrial agriculture is currently responsible for much of the non-point source pollutant loading in most “developed” countries. Nutrients, in particular nitrogen and phosphorus, are higher in agriculture waterways compared to other natural water courses because of synthetic fertilizer and manure applications (Needelman et al., 2007; Skaggs et al., 2005). Nitrogen and phosphorus are key nutrients for primary production, and can lead to eutrophication when in

excess (Hasler and Swenson 1967; Lund 1967; Schindler 1974; Allan and Castillo 2007; Figueiredo and Rodrigues 2014). At least half of the 12 identified emerging or persistent threats to freshwater biodiversity (climate change, infectious diseases, invasive species, harmful algal blooms, contamination, freshwater salinization, and cumulative stresses) can be associated with industrial agriculture practices (Reid et al. 2018). Pesticide use is widespread across industrial agroecosystems, with mixtures of pesticides reaching waterways. For example, 50 different pesticides were detected in agricultural streams in central Germany (Shahid et al., 2018), and between 28 - 85 pesticides were detected across 100 streams in the Midwest US (Van Metre et al. 2017).

1.2 Ditches and ditch management

Agriculture ditches are relic streams or constructed waterways modified to transport water away from crop fields to downstream aquatic environments. In areas with significant precipitation (e.g., temperate climates), ditches are often associated with tile drainage and subsurface drains from cultivated fields. The advent of tile drainage allowed early settlers to drain wetlands and create cultivatable fields (King et al. 2015), and now allows field access by heavy farm machinery earlier in the growing season (Rosenzweig et al., 2002). Globally, it is estimated that 190 million hectares of agricultural lands are artificially drained (Biggs et al., 2017). As a result, there has been a concomitant extensive loss of natural wetlands in Europe and parts of Eastern Canada since European settlement, with over 50 % loss in many European countries (Hoffmann and Baattrup-Pedersen 2007), and 85 % loss in parts of south and eastern Ontario (Ducks Unlimited Canada 2010).

Tile drainage is widespread in temperate regions: over 80 % of some North American watersheds (Blann et al., 2009) and approximately 50 % of all Denmark is tile drained (Hoffmann and Baattrup-Pedersen 2007). In intensive agriculture watersheds, natural streams and open ditches can be almost indistinguishable, as both are typically channelized and fed by subsurface tile drainage systems (Needelman et al. 2007). This transfers agrochemicals to downstream aquatic environments, though vegetated ditches have the potential to mitigate this contamination (Needelman et al. 2007; Strock et al. 2007).

The primary purpose of ditches is to reduce water logging in soils, but they can provide other hydraulic functions including runoff collection, erosion control, groundwater recharge, and improving water quality (Needelman et al. 2007). Ditches reduce soil waterlogging by lowering the groundwater table, collecting surface runoff and exporting water downstream. This can increase crop yields by limiting anoxic conditions and decreasing plant disease (Rosenzweig et al., 2002). Less waterlogging also reduces the intensity of surface runoff from surrounding fields, and decreases soil erosion (Dollinger et al., 2015). Ditch groundwater recharge capacity requires the water table level to be below the ditch surface level; this is most significant for intermittent waterways following a drought (Dages et al. 2009). In addition to improving soil conditions for agricultural land-use, ditches also provide a valuable, but often overlooked ecosystem service, as especially vegetated ditches have a strong capacity to mitigate water contamination problems (Needelman et al., 2007).

Ditches uniquely integrate stream and wetland features and are able to fulfill many water purification processes. Vegetated ditches can be classified as wetland environments as they have an inundated period, hydric soils and support hydrophytes (obligate and facultative

wetland plants) (Kröger et al. 2009). Ditch morphology, water depth and velocity can be important factors for retaining sediments, nutrients, and pesticides, but vegetation (type and density), and bed sediment (type and texture) are even more important for chemical retention (Dollinger et al. 2015). Riparian buffers and ditch bed vegetation slow the flow of water causing sedimentation, infiltration of particle-loaded water, and sieving by vegetation and litter, and can remove 72 – 94 % of the total suspended solid load (Flora and Kröger 2014). Nutrient and pesticide retention are maximized when sorption, sedimentation, degradation (biotic and abiotic), plant uptake, and transformation (nutrients only) are optimized. However, nutrient and pesticide retentions vary greatly, from 3 to 92 %, and 3 to 99 %, respectively, depending on local conditions (Dollinger et al. 2015). For this reason, vegetated ditches can be critical for mitigating agrochemical contamination in downstream aquatic systems.

Ditches also provide critical habitat for wildlife (flora and fauna) as they are areas of higher biodiversity in intensive agriculture landscapes. In a review of the biological functions of agriculture ditches, Herzon and Helenius (2008) report that ditches support a wide diversity of species, including plants (wetland obligate, facultative, and upland species), invertebrates, insects (pollinators), amphibians, birds, and mammals. Although most ditches support species that are common elsewhere, some harbour species not found in other farmland habitats (Boutin et al. 2003). In a study of the conservation value of secondary habitats for orthopterans (grasshopper, locusts and crickets) Torma et al. (2017) concluded that ditch banks are a suitable habitat for the majority of taxa, including rare and endangered species. The viability of a ditch as a refuge is likely dependent on the complexity and biodiversity of submerged and emergent vegetation (Chester and Robson 2013). The aquatic and wetland plants supported by ditches

have greater diversity than the surrounding cropland, thereby supplying food resources otherwise lacking (Herzon and Helenius 2008). Ditches also serve as migration corridors (Mazerolle 2005) and breeding zones (Hartel et al. 2011; Oda et al. 2016) for amphibians (and other taxa) by connecting otherwise isolated refuges and often provide predatory fish-free zones (Herzon and Helenius 2008).

The riparian vegetation in ditches affects the resident fauna, including amphibians. Vegetation community composition impacts the abundance and species composition of Odonata (dragonflies and damselflies), an important food source for amphibians (McDiarmid and Altig 1999; Perron and Pick 2020). Furthermore there is a significant link between the presence of specific native obligate wetland plant species and Odonata abundance, which highlights the importance of native species in amphibian food webs (Perron and Pick 2020). Vegetation in riparian buffer strips can also affect the abundance of small mammals and herptiles (amphibians and reptiles), as Maisonneuve and Rioux (2001) found a positive relationship between complexity of vegetation structure and abundance of these herptiles. Additionally, they found that *Lithobates [Rana] pipiens* and wood frogs (*L. sylvaticus)* were associated mostly with wooded and shrubby riparian strips (Maisonneuve and Rioux 2001). Riparian vegetation is important for providing shelter and calling sites for anurans (frogs and toads), and anuran species composition was positively affected by the habitat type and vegetation cover (Oda et al. 2016).

Ditch management practices impact the potential ecosystem services and functions of ditches (Needelman et al. 2007; Dollinger et al. 2015). Historically, ditch management has focused on the hydrological functions (increasing ditch hydraulic capacity and reducing

flooding), while disregarding biological and chemical functions (Evans et al. 2007). In North America, ditch management regimes have been based on straightening and deepening channels, and removing vegetation and sediment to prevent potential or perceived clogging, waterlogging, or weed dispersal (Maisonneuve and Rioux 2001; Evans et al. 2007; Smith and Pappas 2007). Vegetation removal is done by cutting woody vegetation (to prevent blockages, or shading), and mowing, spraying herbicides, or burning non-woody vegetation (Needelman et al. 2007). Sediment removal (known as "dredging") requires mechanical excavation of roughly 30 cm depth to remove sediment, vegetation and debris from the ditch banks and channel bed. The removed material is left along the bank ridge, often where vegetation used to be. Depending on the region, dredging frequency varies from yearly to every 50 years; for example in France the average dredging frequency was found to be once every 10 years (Levavasseur et al. 2014; Smith & Pappas, 2007; Twisk et al., 2000). Dredging can have cascading negative effects on water quality due to the removal of vegetation and changes in bed sediment composition (Dollinger et al., 2015; Moore et al., 2001; Needelman et al., 2007; Pappas & Smith, 2007; Smith & Pappas, 2007; Smith et al., 2006; Zhang et al., 2010).

Dredging has been shown to reduce the ditch's capacity to mitigate nutrient and pesticide levels in agricultural surface waters (Smith and Pappas 2006; Smith et al. 2006; Pappas and Smith 2007; Shigaki et al. 2009; Smith and Huang 2010). Vegetation removal and dredging are both known to result in higher turbidity, due to reduced infiltration of 1) particle-loaded water fluxes and 2) sieving of particles by vegetation and litter (Fiener and Auerswald 2003; Dollinger et al. 2015). Vegetation removal and dredging can also result in faster water flow over destabilized sediments, which reduces sedimentation and can support resuspension of

particulates, thereby increasing turbidity (Royer et al. 1999; Dollinger et al. 2015). Ditch management is important because it can influence crop yields (water logging lowers yield), while also affecting water and habitat quality. As humans continue to stretch environmental boundaries (Raworth 2012), the need for identifying and implementing agricultural best management practices to optimize ditch functions is becoming even more pressing.

1.3 Frogs as bioindicators

Amphibians, especially frogs, can be found in agricultural ditches (Mazerolle 2005; Piha et al. 2007; Herzon and Helenius 2008), and use them to complete their life cycles. They are often used as bioindicators species, which are taxa that are highly sensitive to environmental stress and are the first to show signs of stress before the entire community is impacted (Welsh and Ollivier 1998). Amphibians are ideal bioindicators because their biphasic life cycle (both aquatic and terrestrial) and permeable skin make them sensitive to contaminants as well as other environmental conditions (Simon et al., 2011). Frogs are intermediate in the food web, being both predator and prey, with their trophic status changing with metamorphosis (Arribas et al., 2015). Additionally, they are philopatric, remaining close to where they hatched. For example, Swanson et al., (2018) found *L. pipiens* had a median annual range of 0.4 - 0.6 km, hence impacts in the expected habitat region can be identified with some certainty. Monitoring amphibians as indicators of ecosystem stress can serve as an early warning of habitat deterioration, and allow for more effective mitigation (Welsh and Ollivier 1998). Sievers et al. (2018) found amphibians to be the among the most sensitive taxon to changes in wetland environments, making them valuable bioindicators of wetland quality. Many researchers have used amphibians as bioindicators of environmental health (Welsh and Ollivier 1998; McDiarmid

and Mitchell 2000; Simon et al. 2011), and sensitive toxicological test species for contaminant associated risk assessments (OECD 2009). Further interest in studying (and protecting) amphibians stems from observed global declines in populations; the IUCN reported 41 % of amphibian species as threatened, endangered or extinct (Stuart et al. 2004; IUCN 2020).

Frogs use agriculture ditches for breeding and feeding, and are sensitive to several stressors that may be present there (Collins & Fahrig, 2017; Herzon & Helenius, 2008; Sparling et al., 2000). Exposure to agrochemicals at environmental concentrations can cause lethal and sub-lethal effects to frogs such as growth and developmental abnormalities, endocrine disruption, reproductive disorder, and immunosuppression (Howe et al. 2004; Hayes et al. 2006; Langlois et al. 2010; Christin et al. 2013; Bernabò et al. 2016; Collins and Fahrig 2017). However, the chemical toxicity of agrochemicals is tested on individual substances before approval by regulatory agencies (Pesticide Management Regulatory Agency, in Canada), leaving uncertainty with respect to the effects of agrochemical mixtures that occur *in situ* in agricultural waterways. Toxicologists and regulatory agencies are working to expand understandings of mixture toxicity but many questions remain in assessing compounds and their interactions (Rodney et al., 2013). Amphibian health is also affected by many environmental factors including (but not limited to) temperature, turbidity, water availability, population density, light, food availability, and salinity (Mg, Cl) (Hecnar and M'Closkey 1996; Shi 2000). Additional stressors such as habitat loss and modification, pollution, ultraviolet radiation, disease, predation, and climate change are all contributing to the global decline of these sensitive creatures (Alford and Richards 1999; Simon et al. 2011). Understanding the interactions and

relative importance of multiple stressors on amphibian success in agroecosystems continues to be the focus of scientific research.

Several studies have reported observations of wild amphibian species in ditches (Mazerolle 2005; Piha et al. 2007; Herzon and Helenius 2008) and other artificial habitats (Brand and Snodgrass 2010). Most relevant to the present thesis, Collins & Fahrig (2007) observed eight species of anurans in agriculture ditches of Eastern Ontario, in a study that found forest cover and habitat heterogeneity (i.e. smaller field sizes) were beneficial for conserving anuran diversity. Maisonneuve et al. (2001) found nine species of amphibians in ditches of southern Quebec, with the most frequently detected species (*Bufo americanus*, *Rana (Lithobates) pipiens* and *Rana sylvatica)* all found predominantly in wooded and shrubby areas.

1.4 *In situ* biomonitoring

Field biologists have made use of *in situ* cages to study environmental effects on sentinel or representative taxa since the 1970's if not earlier (Cooke 1973). *In situ* cages provide ambient environmental exposure that cannot be replicated in a laboratory or mesocosm and enable researchers to monitor native aquatic species under more natural conditions. Herpetologists use *in situ* cages to study embryonic and larval amphibian health and investigate life history effects of exposures to contaminants (Harris et al., 1998). To estimate sublethal endpoints and mortality on populations with known genetic origins, caged individuals can be observed through time, with sufficient replication for statistical analyses, under actual environmental conditions and contaminant mixture exposures (Crane et al. 2007). Using cages can also allows for control over potential predation losses and for sufficient animal nutrition.

By using *in situ* cages, the effects of agrochemical mixtures including nutrients and pesticides, and physiochemical conditions impinging on frog health can be directly tested in the field (De Solla et al. 2002). *In situ* cages could help determine the lethal and sub-lethal effects of environmental exposures to agriculture ditch waters on the aquatic stages of amphibians. In the current study, Northern Leopard Frogs (*Lithobates [Rana] pipiens*) were chosen as the test species of interest because they are native and common within the study region of Eastern Canada. In addition, they are listed as Not at Risk by the Committee on the Status of Endangered Wildlife in Canada making research on them less restricted.

Aquatic stages (embryos and larvae) are often more sensitive to toxicants and other stressors than the adult stage, making the former more conservative bioindicators (Harris et al., 1998). *L. pipiens* larvae transform from aquatic, omnivorous filter- and microphagous- feeders on bacteria, plankton and periphyton, breathing through external gills and skin, to terrestrial carnivorous juveniles and adults respiring through lungs and skin. Amphibians are susceptible to environmental stressors at different life stages, which can cause carryover and latent effects to perpetuate or become apparent at later stages, affecting growth, survival and reproduction in unpredictable ways (Rumrill et al. 2018). Because of this, many researchers investigate how environmental factors affect amphibian health outcomes beyond simply measuring survival. These studies focus on health outcomes such as embryo hatching success (McDaniel et al. 2004), growth and development (Jones et al. 2017; Robinson et al. 2019), responses to stressors (such as predation or parasite stress; Koprivnikar 2010; Robinson and Lee-Jenkins 2018), and successful sexual development and reproduction (Hogan et al. 2008a; Langlois et al. 2010).

1.5 Thesis objectives

The objective of this thesis was to determine whether ditch management affects northern leopard frog (*Lithobates pipiens)* health in agroecosystems. This was done by comparing sites along managed ditches (treeless, then dredged) with sites along more "natural" vegetated ditches (mature vegetation, undredged) in neighbouring watersheds. I hypothesized that ditch management (vegetation removal and dredging) would affect frog health outcomes because of changes in water quality. I predicted there would be higher turbidity, nutrient and pesticide concentrations at the managed ditch sites compared to the vegetated ditch sites. Further, I predicted poorer embryo development, hatching success, and survival, and poorer tadpole survival, growth, development, sex ratios, and greater bioaccumulation of pesticides at the managed ditch sites compared to the vegetated ditch sites.

By using *in situ* cages, I was able to study novel sublethal health end points in aquatic stage frogs under field conditions. I observed numerous health outcomes of aquatic stage *L. pipiens* exposed to environmental conditions, monitoring a mixture of pesticides, nutrients (fertilizers), and physiochemical water chemistry conditions along with ditch management variables. A strength of this field study was the high number of independent and dependent variables I was able to monitor.

2. Methods

2.1 Experimental design and site description

This study was undertaken in St-Lawrence Iroquoians, Anishinabewaki, and Haudenosaunee territories, in the South Nation Watershed, in Eastern Ontario, Canada (45.2616, -75.1669; Native Land Digital 2015). The land is relatively flat, with soils dominated by Bainsville silt loams of Gleysolic order (Wicklund and Richards 1962). Approximately 60 % of the watershed is used for intensive agriculture (Sunohara et al. 2012), principally in crop monocultures of corn (*Zea mays*), soybean (*Glycine max*) and forage/hay (Wilkes et al. 2019). Wetland cover has dropped to 17 % (SNCA 2018), compared to the 40 – 60 % wetland cover that existed before the arrival of European settlers (Ducks Unlimited Canada 2010). There is 22 % riparian forestry cover along waterways (SNCA 2018), well below the recommended minimum of 75 % (ECCC 2013).

This study utilized two small neighbouring agricultural watersheds that have been investigated since 2003 by researchers affiliated with Agriculture and Agri-Foods Canada (AAFC; Wilkes et al. 2019). The hydrology in each watershed is modified by sub-surface tile drainage systems that feed water into two parallel agriculture ditches (Appendix 1, Figure 8). These two ditches (and associated tile-drained fields) were considered to be paired watersheds that were hydraulically distinct, but had common topography, weather, soils and land uses (Wilkes et al. 2019). The paired watersheds approach enabled a comparison of ditch managements at a watershed scale between a vegetated (control) treatment and a managed treatment. The total surface catchment areas were 467 ha and 250 ha respectively for the vegetated and managed watersheds (Wilkes et al. 2019).

In this study, the paired watersheds were studied in 2018 - 2019, during which different ditch management practices occurred. The vegetated (V) watershed (Figure 1A) has had no dredging, and no recorded vegetation removal since the ditches were constructed in the early 1980's. The managed (M) watershed had all riparian woody vegetation removed in 2017, referred to subsequently as the treeless treatment (Figure 1B). This same ditch was dredged in the fall of 2018, referred to subsequently as the dredged treatment (Figure 1C). Both the treeless and dredged treatment collectively represent the managed treatment. In situations where a response was only relevant in one year at the managed treatment, it will be referred to by its specific management (dredged or treeless). Sites in the paired watersheds were named according to the direction of water flow. Upstream sites have lower numbers, and downstream sites have higher numbers; the additional sites outside the paired watersheds have the highest numbers (Figure 1D).

In 2018, a downstream site along each ditch (Vegetated 3 (V3) and Managed 3 (M3); Figure 1D) was used to carry out an *in situ* tadpole experiment with nine replicate cages used at each of these two sites (18 cages total). In early May 2019, four sites (Vegetated 3 (V3), Vegetated 4 (V4), Managed 3 (M3), Managed 4 (M4)) were used for an embryo hatching experiment, with three *in situ* embryo cages at each site (12 cages total). Two of the sites, V3 and M3 were in the paired watersheds (see red outline in Figure 1D), and two additional sites (V4 and M4) were within 4 km of the paired watersheds (Figure 1D). These additional sites were used in the second year of the study (2019) to increase the sample size of ditches under the desired ditch treatments. Six sites (three of each treatment) were originally selected however due to extensive spring flooding two had to be excluded. The additional sites were selected

based on the following criteria: ditch management was either maturely vegetated for dredged in 2018, ditches were fed by tile drained fields, ditches were located within logistically possible proximity to the paired watersheds, and land permission had been granted. In late May 2019, six sites in the paired watersheds (Vegetated 1 (V1), Vegetated 2 (V2), Vegetated 3 (V3), Managed 1 (M1), Managed 2 (M2), and Managed (3)) were used for a second tadpole experiment. Unfortunately, the upstream sites in both ditches (V1 and M1) dried out within two weeks of the start of the experiment, so had to be prematurely terminated, and are not considered further. The 2019 tadpole experiment thus used four sites (V2, V3, M2, M3), with three replicate *in situ* cages at each (12 cages total). In summary, the embryo experiment used four sites (V3, V4, M3, M4), the 2018 tadpole experiment used two sites (V3, M3), and the 2019 tadpole experiment used four sites (V2, V3, M2, M3).

Vegetation surveys and land use characterization were conducted at all sites in the paired watersheds to investigate potential confounding factors that could affect tadpole health outcomes beyond ditch management. To characterize and compare the riparian vegetation in each watershed, plant surveys were conducted in June and August 2019, at each of the four tadpole study sites, to catch early and late blooming species, following Perron & Pick (2020). At each site, three interrupted belt transects were used, each containing three – five quadrats, for a minimum of 12 1x1 m quadrats per site. Transects were spaced 10 m apart, spanning from the ditch channel to include submerged aquatic species, up the side of one bank (transects alternated ditch sides). In each quadrat every plant species was identified (or preserved for identification), the percent cover was estimated, as well as the percent water, canopy, bare

ground, and leaf litter estimated. All quadrats were sampled in the early summer (June) and resampled later in the summer (August) for a minimum 18 quadrats per site.

After all vegetation surveys and identification were completed, a vegetation list for each ditch treatment was compiled. Oldham's (1995) Floristic Quality Assessment System was used to calculate the total coefficients of conservatism, total weediness indices, and mean wetness indices in order to compare the vegetation communities by ditch treatment. The total coefficients of conservatism rank each native species in terms of rareness (0 being the most common through 10 being the rarest), and then sums these values across each treatment. The total weediness index ranks each non-native species according to its invasiveness (0 being less invasive to -3 being most invasive), and then sums these values across each treatment. The mean wetness index ranks each species by its preferences for moisture and wetland habitat (-5 being an obligate wetland species to +5 being an obligate upland species), and the mean was calculated across each treatment.

To characterize the surrounding land use and crop cover, remotely sensed AAFC Annual Crop Inventory for 2018 and 2019 (AAFC 2019) was analyzed in ArcGIS (version 10.6). The land use cover within a 2 km radius area from the center of each site was tabulated in order to compare forest cover and cropping systems between the paired watersheds. The total ditch length from start to end of the study watershed was measured to be 6915 m and 4403 m for the vegetated and managed ditches respectively.

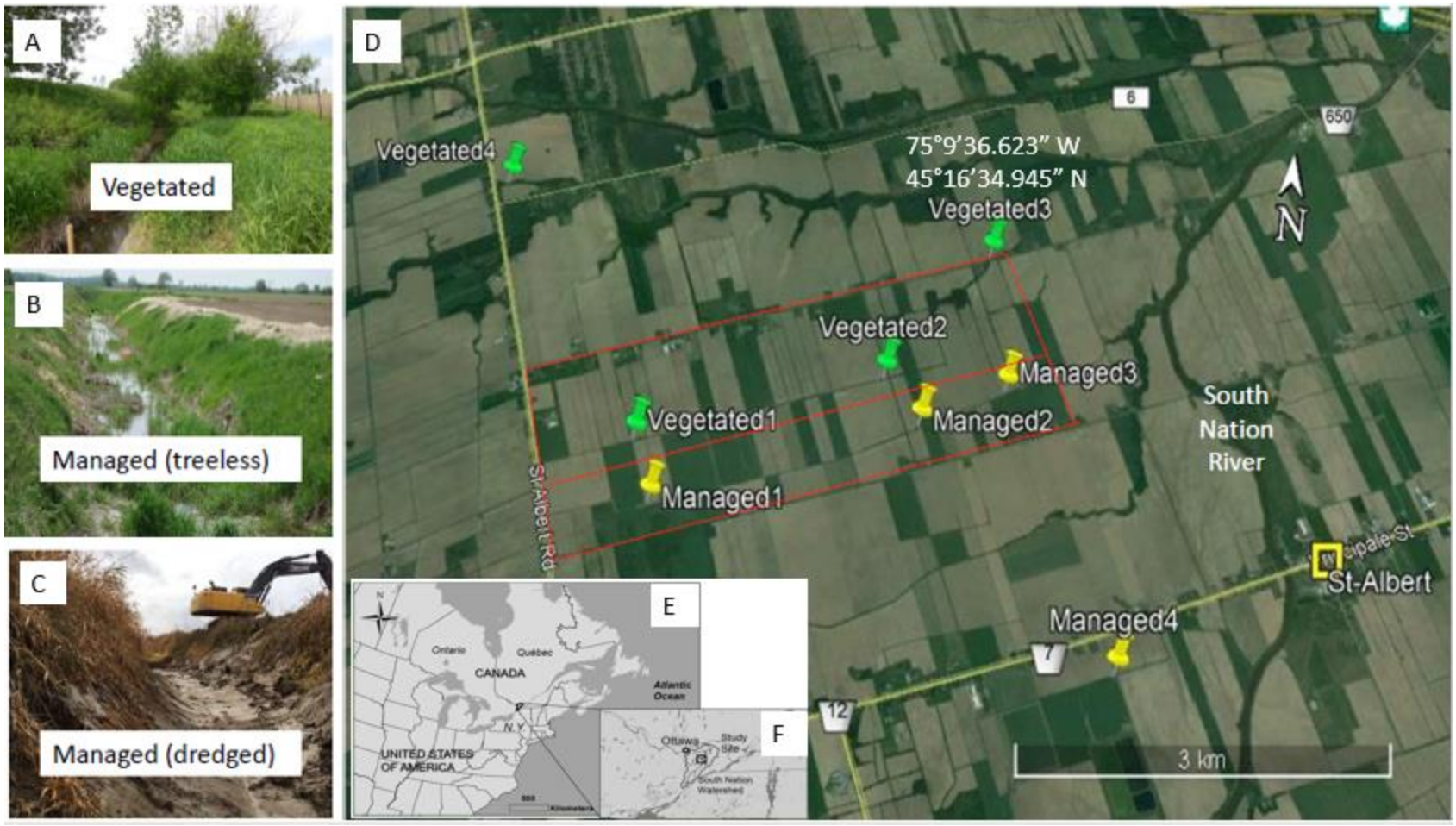

Figure 1: Vegetated (A) and managed (B: treeless, C: dredged) treatment sites on St-Lawrence Iroquoians, Anishinabewaki, and Haudenosaunee territory in the South Nation Watershed, Ontario, Canada. D) Map of the paired watersheds and all the experimental sites, near St-Albert, Ontario. Inset: location of the paired watershed in the South Nation Watershed (F), in eastern Canada (E). The paired watersheds are outlined in red, with the vegetated treatment (green pins) on the north side and the managed (yellow pins) on the south side; refer to Wilkes et al. (2019) for exact delineation of the watersheds' boundaries. Managed treatments were treeless in 2018 and dredged in 2019. Map inset credits to Wilkes et al. (2019).

2.2 Amphibian husbandry

Amphibian collection from wild populations and care in the laboratory was carried out by the Trudeau lab at the University of Ottawa, and the Robinson lab at the National Wildlife Research Centre (NWRC) in Ottawa, Ontario, Canada. Northern leopard frogs (*Lithobates pipiens*) were used in all the embryo and tadpole experiments. Adult male and female frogs were collected in late April 2018 (six male and six female) and 2019 (three male and three female), from non-agriculture–associated wetlands within a 20-km radius around Bishops Mills (ON, Canada; 44.87366, –75.70455). The frogs were transported to the University of Ottawa animal care facility, where they were paired and bred following the AMPHIPLEX artificial breeding protocol (Trudeau et al. 2013).

In 2018, a tadpole experiment was conducted. The tadpoles used in this study came from six embryo clutches laid over May 3 - 4 at 16 °C at the University of Ottawa. The embryos were transported to the NWRC and placed in an environmental chamber, set to 22 °C, 70 % humidity, with a photoperiod of 16 hr light: 8 hr dark, to develop to GS 25, following Young et al. (2020). After hatching, tadpoles were fed Presidents Choice frozen kale and Wards *Xenopus* tadpole food *ad libitum* and were provided 1 - 3 Hikari KYORIN Co Inc. algae wafers once per week (Young et al. 2020). Stock tanks were filled with City of Ottawa tap water aged >24 hr and dechloraminated by treating with Prime water conditioner. Partial water changes (50 - 75 %) occurred every 48 - 72 hrs. The six embryo masses were evenly distributed into four aerated 60 L stock tanks.

In 2019, three embryo clutches (each containing ~3000 - 6000 embryos; COSEWIC 2009) were used for both an embryo and a tadpole experiment. These embryo clutches were laid over

April 27 - 28, 2019 at 16 °C at the University of Ottawa. To prepare them for deployment to natural ditch waters (4 - 8 °C), they were held at 13 °C for two days, transported to the NWRC, where they were held at 10 °C for two more days. Approximately 840 embryos were used in the *in situ* embryo experiment (Section 2.4). The remaining embryos were divided evenly between four 67 L plastic tubs and allowed to develop to GS 25 for use in the tadpole experiment (Section 2.5) in a greenhouse at the NWRC. Conditions in the greenhouse fluctuated with natural daily rhythms; the mean temperature was 23 °C (range 17 – 50 °C), the humidity ranged from (10 – 72 %) and the photoperiod was 14 - 15 hr light: 10 - 9 hr dark.

In each year of the study, control tadpoles were used to ensure driving to the field and acclimatizing to ditch waters did not cause mortality in the first 24 hours. For each experiment there were two control replicates (n = 15 individuals) for an acclimatization control and driving control. All tadpoles (except driving controls) were acclimatized to ditch water conditions by adding 250 mL of water every 15 minutes, over the course on 1 hr. All control tadpoles (n = 30) returned to the lab and total survival was confirmed the next day. In 2019, the control tadpoles were raised in an environmental chamber at the NWRC, in two 10.6 L aerated tanks which had 50 % water changes three times each week with aged dechloraminated water. The tadpoles were fed frozen kale and Wards *Xenopus* tadpole food *ad libitum*. The control tadpoles were raised for the same duration as the field tadpoles, with experiment termination and final processing occurring simultaneously with the field tadpoles (Section 2.5). The physiochemical water conditions are reported in Appendix 3, Table 22. These lab control tadpoles serve as genetic reference specimens for the tadpole field study in 2019 since they came from the same three embryo clutches. Their tissues were also used during method development for pesticide

bioconcentration (Section 2.6). Sampling and handling of animals followed the Canadian Council for Animal Care guidelines and approved protocols by the University of Ottawa Animal Care Committee (BL-2206) and Environment and Climate Change Canada's Wildlife East Animal Care Committee (SR05-2018, ST05-2019).

2.3 Water Quality

2.3.1 Physical and chemical monitoring

In both 2018 and 2019, a multiparameter water quality probe was installed at each site used in the tadpole experiments. In 2018, a YSI 6600 multiparameter sonde (YSI Inc.) at sites V3 and M3, recorded temperature (°C), specific conductivity (SC) (µS/cm), dissolved oxygen (DO) (mg/L), pH, oxidation-reduction potential (ORP) (mV), and turbidity (NTU). In 2019, a YSI EXO2 multiparameter sonde (YSI Inc.) at sites V2, V3, M2, and M3 recorded Chlorophyll *a* (µg/L; Chl *a*) and total dissolved solids (g; TDS), in addition to the parameters measured in 2018. These probes were calibrated bi-weekly and recorded measurements every 15 minutes. Additionally, in 2019, a handheld YSI 6600 multiparameter water quality sonde (YSI Inc.) was used in both the embryo and tadpole experiments to record temperature, SC, DO, pH, ORP, and turbidity in each amphibian cage, and 30 cm upstream and downstream during each visit (every 2 days for embryos, and twice a week for tadpoles).

In 2018, 1 L composite water samples (as described in Sunohara et al. (2015)) were collected once every two weeks using an ISCO 6712 portable sampler (Teledyne Isco, Inc.) for nutrient analyses. In 2019, three grab samples were collected once a week specifically for analyses of glyphosate (250 mL), neonicotinoids + atrazine (250 mL), and nutrients (1 L). All sample bottles were washed using phosphate-free soap, tripled rinsed with City of Ottawa tap

water, and triple rinsed with deionized water. Amber borosilicate glass bottles with Polytetrafluoroethylene (PTFE) lids were used for neonicotinoids and atrazine samples; these bottles were additionally triple rinsed with methanol. Nutrient and glyphosate samples were collected in plastic sample bottles. Immediately prior to collecting water samples, all sample bottles were triple rinsed with ditch water, then filled by submerging midway in the water column. Samples were stored on ice until returning to the laboratory. Subsamples for dissolved nutrients were filtered using 0.45µm Whatman Uniflow Syringe Filters (GE Healthcare)**,** and frozen until analyzed along with separate subsamples for whole water analyses (total phosphorus, total Kjeldahl nitrogen, and total organic carbon). Nutrients measured included ammonium-ammonia (NH_3-NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), Total Kjeldahl Nitrogen (TKN), reactive phosphorus (RP, an estimate of phosphate), total phosphorus (TP), dissolved organic carbon (DOC), total organic carbon (TOC), and total suspended solids (TSS). These water samples were analyzed at the Robert O. Pickard Center, a certified laboratory of the City of Ottawa, following standard protocols for the analysis of surface waters (Standard Methods, 2004; EPA, 2007; OMOE, 2017). The atrazine, glyphosate, and neonicotinoids samples were stored at 4°C until analysis. Total inorganic nitrogen (TIN) was calculated by summing NH_3-NH_4^+, NO_2^-, and NO_3^- in each water sample.

2.3.2 Pesticide analyses

Water samples were analyzed for pesticides at the NWRC using high-performance liquid chromatography and tandem mass spectrometer (LCMS-MS) to determine concentrations of atrazine, glyphosate, and seven neonicotinoids (clothianidin, imidacloprid, thiamethoxam, acetamiprid, thiacloprid, dinotenfuran and flypyradinfurone). Concentrations were determined

using methods described in Collins et al. (2019) with minor modifications described below. Multiple reaction monitoring (MRM) transitions and mass spectrometer parameters used for each compound are provided in Appendix 2 (Table 18, 19). The method detection limits (MDL) ranged from 0.00002 – 0.025 µg/L and method reporting limits (MRL) ranged from 0.00005 – 0.08 µg/L for each pesticide (Table 1).

For determining concentrations of atrazine in water samples, the following method adjustments were made: Oasis HLB Solid phase extraction cartridges and 250 µL glass inserts were used, 20 µL aliquots were injected into the HPLC, and calibration curves were built with 8 levels, ranging from 0.005 to 5 ppb. No atrazine was found in the sample blanks, indicating no cross-contamination. The percent recovery ranged from 85 - 115 % demonstrating good accuracy and stability from start to end of daily injections sequence. The relative percent difference was 0.00 – 2.60 %, demonstrating good accuracy (<15 % is considered good).

For the determination of glyphosate concentration in water samples, the method was the same as Collins et al. (2019), with the following modifications: the water samples were pH adjusted between 7 and 8 (when necessary), the isocratic mobile phase was at pH 8.1, and calibration curves were built with 8 levels, ranging from 1 to 100 ppb. Glyphosate was not detected in the sample blanks, indicating no cross-contamination. The percent recovery ranged from 94 - 107 % indicating good accuracy and stability. The relative percent difference was 2.60 %, demonstrating good accuracy (<15 %).

For the determination of neonicotinoid concentrations in water samples, the method was the same as Collins et al. (2019), with the following modifications: three additional

neonicotinoids were screened (thiacloprid, dinotenfuran and flypyradinfurone), 250 µL glass inserts were used, and calibration curves were built with 7 levels, ranging from 0.10 to 20 ppb. No neonicotinoids were detected in the sample blanks. The percent recovery ranged from 85 - 115 % indicating good accuracy and stability. The relative percent difference ranged from 3.1 – 11.7 %, indicating good accuracy (<15 %). The total neonicotinoid concentration was calculated by summing the values of all individual neonicotinoids within each water sample. These compounds have similar structure and predicted additive toxicity (Morrissey et al. 2015b).

Table 1: Method detection limit (MDL) and method reporting limit (MRL) for the 9 pesticides screened in surface water samples, units are µg/L.

Compound	**MDL**	**MRL**
Acetamiprid	0.00002	0.00005
Atrazine	0.0004	0.002
Clothianidin	0.0001	0.0003
Dinotefuran	0.0004	0.001
Flupyradifurone	0.00006	0.0002
Glyphosate	0.025	0.08
Imidacloprid	0.00004	0.0001
Thiacloprid	0.00004	0.0001
Thiamethoxam	0.00009	0.0003

2.3.3 Pesticide Toxicity Index

The toxicity of pesticide mixtures in ditch surface waters on *L. pipiens* tadpoles was compared using a pesticide toxicity index (PTI) or toxic unit approach (Battaglin and Fairchild 2002; Nowell et al. 2014). The “concentration addition” model that was employed for this study assumes that all chemicals in consideration act as dilutions of each other, and as such the mixture’s toxicity is the sum of the individual potencies (Rodney et al. 2013). The concentration

addition model has been used extensively, and is recognized as a slightly conservative but broadly applicable model, even when toxicity modes of action differ for constituent compounds in a mixture (Belden et al. 2007; Rodney et al. 2013). The present PTI makes use of standard toxicity data – LC_{50}, the median lethal concentration at which 50% of individuals die. LC_{50} were selected as the acute concentration endpoint because it is the most commonly available toxicity measurement for a broad range of pesticides and species. The LC_{50} data were compiled through a literature search according to the following criteria. For each pesticide of interest (acetamiprid, atrazine, clothianidin, glyphosate, imidacloprid, thiacloprid, thiamethoxam), 96-hour LC_{50} were found for *L. pipiens* larval stages (GS 25 – 42), and when not available, surrogate aquatic species were accepted (Appendix 2, Table 21). The PTI was calculated according to the following equation:

[Equation 1] $$PTI = \sum_{i=1}^{n} \frac{c_i}{LC_{50}}$$

The PTI quotient is equal to the sum of all environmental concentrations for the pesticides of interest divided by the LC_{50} for that pesticide; where "*n*" is the total number of pesticides, "*i*" is the compound of interest, "c_i" is the observed concentration of a pesticide, and the "LC_{50}" is the concentration at which 50% of individuals experience mortality. The resulting quotient can be used to interpret the toxicity hazard of a mixture, where values over 1 indicate probable toxicity, values between 0.5 and 1.0 indicate potential toxicity, and values between 0.1 and 0.5 indicate limited toxicity (Battaglin and Fairchild 2002). Using the surface water pesticide data from 2019, a PTI was calculated for each water sample by date, then a seasonal mean PTI and seasonal maximum PTI for each treatment were determined.

2.4 Embryo experiment

In 2019, *in situ* embryo cages were used for an embryo hatching and survival study. The cages were cylindrical (15 cm diameter x 20 cm depth) and made of 500 µm Nitec Mesh (Dynamic Aqua-Supply Ltd., Surrey BC) with a Velcro lid (Appendix 3, Figure 9). The mesh cylinder was attached to a wooden frame (for floatation; Harris et al. 2001). The floatation frame ensured that the bottom of the cage was less than 15 cm deep, so embryos were close to the water-air interface (Harris et al., 1998; Harris & Bogart, 1997). The cages were deployed and anchored in position using wooden dowels and bricks, one week prior to embryo addition to allow flushing by natural waters.

On embryo deployment day (May 2, 2019), 14 replicates (two for driving and acclimatization controls) of 60 - 70 embryos (~20 from each of the three clutches) were placed into a 2 L Ziploc bag, with 1 L of stock tank water, and placed in a cooler for transportation to the field. Three replicate cages were used at each of the four sites. The embryos were acclimatized to ditch water conditions, by adding 250 mL of water to the Ziplocs bag every 15 minutes, over the course on 1 hr. Driving control and acclimatization control embryos were also used (each with approximately 70 embryos) to ensure driving and acclimatization were not cause for mortality. The controls returned to the laboratory and total survival was confirmed the following day. Embryo survival and hatching were monitored every other day for 16 days until all surviving embryos had hatched. At the end of the study, the hatchlings were brought back to the lab at the NWRC. There they were photographed using a Canon EOS 40D camera to confirm final hatching and survival counts. Gosner Stage (GS) was determined using a dissecting

microscope (Gosner 1960), and hatchlings then euthanized by immersion in 0.2% buffered MS-222 (tricaine methane sulfonate; Sigma-Aldrich).

2.5 Tadpole experiments

In 2018 and 2019, tadpole exposures were conducted using *in situ* cages as above. The tadpole cages were identical to the embryo cages except for their larger dimensions (60 cm depth, 35 cm diameter), and additional plastic fencing to prevent predation from raccoon or muskrat. Cages were anchored as described previously and deployed in the ditches one week prior to the addition of tadpoles to allow flushing by natural waters.

On tadpole deployment day (May 25, 2018, and May 22, 2019), replicates of 15 tadpoles (GS 25) were haphazardly selected from stock tanks, photographed with 5 mm grid paper in the background for scale, then placed in 2 L Ziploc bags with 1 L of tank water, and placed in coolers for transportation to the field. In 2018 there were 20 replicates (18 replicates for the 18 *in situ* cages, plus 2 controls), and in 2019 there were 14 replicates (12 replicates for the 12 *in situ* cages plus 2 controls). The sample sizes differ between years due to the exclusion of the two sites in 2019 that dried up prematurely. Tadpoles were acclimatized to the ditch water at each site by adding 250 mL of ditch water to the Ziploc bags every 15 minutes over 1 hour. After the acclimatization period, a tadpole replicate bag (2018: n = 18, 2019: n = 12) was added to each cage, and frozen kale and Ward's *Xenopus* tadpole food was provided *ad libitum* to ensure food supply would not be a confounding factor (Crane et al. 2007).

Each field site was visited twice a week (Mondays and Thursdays). At these times, all the tadpoles from a cage were captured (using a turkey baster or small fish net) and placed in a

clear container containing ditch water and photographed with 5 mm grid paper in the background for scale. Photographing was done for subsequent body measurements using ImageJ software (version 1.52a). Digital body measurements were made by importing each photo into ImageJ, setting the scale using the grid paper, and using the straight and/or segmented line tools to measure snout-to-vent length, body width and tail length for all clear photographs. Tadpole survival and water depth was recorded at each cage. Cages were cleaned as required to ensure all cages were at a similar state of cleanliness (surplus kale removed; brushes used to remove periphyton build-up). Frozen kale and Wards were provided *ad libitum*. General observations of wild anurans, and the planting of crops as well as pesticide spraying were noted. These observations were useful for interpreting the relevance of the study to wild *L. pipiens* and seasonal pesticide peaks in surface waters.

On June 21, 2018 (4 weeks) and July 2, 2019 (6 weeks), the tadpole experiments were terminated due to ditch waters approaching low levels (10 cm water depth was the cut-off). The tadpoles were collected from each cage using two plastic containers (15 x 15 x 5 cm) and transported in coolers to the lab at the NWRC. Individual tadpoles were then anesthetized by immersion in 0.015 % buffered MS-222. They were blotted dry, weighed (± 0.01 g) and photographed against grid paper for subsequent body measurements. Individuals were then euthanized by immersion in 0.2 % buffered MS-222. Developmental GS was determined (Gosner 1960). Deformities (tail kink) were ranked as none, mild, medium, or severe (Appendix 3, Figure 10). Notes on tail fungus presence and other abnormal observations were recorded. Tadpoles were dissected to remove the intestines (in 2019 only), to ensure bioconcentration analysis would not include their bowel contents. The heart was used to make a U-shaped blood

smear on a glass slide, allowed to dry, and then fixed with methanol, for leukocyte analysis for a separate future study. The liver was weighed (± 0.01 g) for calculation of Liver Somatic Index (LSI; Equation 2) and put back into the body. The LSI can be an indicator of toxic stress (Edge et al., 2011). To ensure this derived ratio was appropriate, a scatter plot of tadpole body mass and liver mass showed these data had a linear relationship through the origin (Curran-Everett 2013; Appendix 5, Figure 13). The sex was visually determined as male, female, or undifferentiated using a dissecting microscope (Appendix 3, Figure 11). The proportion male was calculated as the count of the number of males divided by the total number of surviving tadpoles, and was converted to a percent. Only tadpoles at GS 36 and above were included as this is when sexual differention is complete in *L. pipiens* (Hogan et al. 2008). All tissues (except the intestines) were wrapped in methanol-rinsed tinfoil and placed in the -80°C freezer until shipping to the London Research and Development Centre (AAFC) for analysis of pesticide bioconcentration. Tadpole processing took place over two days, each year, and was carried out by a team of 2-5 people.

[Equation 2] $$Liver\ Somatic\ Index = \frac{liver\ mass\ (g)}{body\ mass\ (g) - liver\ mass\ (g)}$$

2.6. Pesticide bioconcentration in tadpole tissues

In total, 66 pesticides were screened within tadpole tissue samples by high resolution LC-MS/MS at the London Research and Development Centre (Appendix 2, Table 20). A new protocol for extracting multiple pesticide residues from amphibian tissues, using LCMC-MS was developed. The preliminary method development was conducted using earthworm tissue (from a local bait shop), and then continued with tadpole tissue once confidence in recoveries was gained. Only the final methods are reported below which used a QuEChERS method (quick, easy, cheap, effective, rugged and safe) modified from Schenck & Hobbs (2004).

Tadpole tissue was freeze dried and dry masses were weighed. Six or ten individuals were randomly chosen from each field cage replicate, in 2018 and 2019 respectively, to make a pooled tissue sample for each cage. The difference in number of individuals used was due to sample size constraints; however, the number was kept consistent within each year, and the method was scaled to dry mass used. For simplicity in explaining this scaled method, the 2018 material amounts are included in brackets, though the proportions are the same except for the volume of acetonitrile used. Pooled samples ~1 g in 2019 (~0.5 g in 2018) were weighed (± 0.1 mg) and placed in 15 mL Falcon tubes (Life Science Ltd.). Solutions of 2 (1) mL water + 2 (2) mL 1 % acetic acid in acetonitrile + 800 (400) mg anhydrous magnesium sulfate + 200 (100) mg sodium acetate were added and shaken vigorously for 15 minutes on a mechanical wrist shaker. Samples were centrifuged at 3000 rpm for 3 minutes, then from the organic layer, 1 mL was transferred into 1.8 mL microcentrifuge tubes containing 185 (94.8) mg of dispersive solid-phase extraction salt (Agilent, PN: 5982-5156) (containing $MgSO_4$, C18, and PSA). Samples were shaken for 45 seconds and centrifuged at 3000 rpm for 3 minutes. A 500 µL of the top extract and 500 µL water was filtered using 0.45 µm Whatman filters into 1 mL glass amber HPLC vials, and stored in the freezer until analysis by LCMS-MS.

All tadpole extracts were analyzed using an identical LC-MS-MS method of 66 pesticides, acquired with a Thermo Q-Exactive Orbitrap mass spectrometer coupled to an Agilent 1290 HPLC system. The analytes were resolved using a C18 HPLC column (Agilent; 2.1 x 50mm, 1.8 µm). Mobile phase B was held at 5% for 0.5 min prior to being increased to 94% over 7.5 min. Mobile phase B was increased again to 97% over 4 minutes and returned to 5% over 0.5 min. The settings used were as follows: heated electrospray ionization (HESI) capillary temperature,

400 C; sheath gas, 17.00 units; auxiliary gas, 8.00 units; probe heater temperature, 450°C; S-Lens RF level, 45.00 and capillary voltage, 3.9. The MS/MS acquisition scans used the following settings: isolation 1.2 m/z; resolution, 17,500; automatic gain control (AGC), 3×10^6 and maximum injection time (max IT), 64 ms. The retention times, precursor ions, and fragment ions used for quantification are listed in Appendix 2, Table 20.

Processing methods were built for all compounds using XCalibur software version 3.0 (Thermo Fisher Scientific 2013); extracted ion chromatograms for precursor and product ions were generated using a ± 5 ppm mass accuracy threshold following (Renaud et al. 2017). The ICIS peak integration algorithm used 5 smoothing points, and a baseline window of 70 was used for all compounds, following (Renaud et al. 2017).

Pesticide detection was confirmed if all the following criteria were met, both the precursor and products ions (Appendix 2, Table 20) were detected, with a retention time ±0.03 min, in both the sample and the "neat", and the detection peak was smooth. Detection frequency was calculated as the number of samples in which a pesticide was detected divided by the total number of samples (n = 30). As no true baseline noise levels exist in data generated on high resolution Q-Exactive Orbitrap, the limit of quantification (LOQ) was defined as the lowest concentration achievable in the calibration curve whereupon the ion is detected in five consecutive injections, following (Renaud et al. 2017).

Analyte recoveries (Re) were determined by the ratio of measured concentrations between a composite tadpole sample spiked with 0.1 µg of pesticide standards before extraction with an equivalent composite sample spiked following extraction. Signal suppression

enhancement effects (SSE) were also determined by comparing the measured concentration of the tadpole extract fortified with the target analytes following extraction, with an equivalent amount of pesticide analytes in 1 mL of acetonitrile: water: acetic acid (50:49:1) (called a "neat"). To get final concentrations in units of ng/g wet mass, dry mass concentrations (ng/g dry mass) were multiplied by the average percent dry mass (7 %); this allowed comparisons with other studies.

2.7 Statistical analyses

As a first step all raw data were visualized and examined for violations of statistical assumptions, using R statistical software (version 3.6.2), following the data exploration methods suggested by Zuur et al. (2010). Specifically, Cleveland plots and boxplots were used to visualize the data and perform quality controls (Appendix 5, Figure 14). The data used in the water quality models came from the probes installed at each site, where measurements were recorded every 15 minutes, and from the water samples that were analyzed for nutrients and pesticides (2019 only). Some attention was required to correct the probe data. For instance, turbidity values were identified as outliers and removed if values more than doubled and did not maintain this magnitude for at least 45 minutes (3 data points), likely due to human disturbance of the water or floating debris hitting the probe.

A main consideration in selecting the appropriate statistical tests was meeting the assumption of independence. This was challenging due to the experimental design for the tadpole experiment that included two sites on the same ditch in each watershed in 2019, which meant sites were not independent. Additionally, there was the non-independence of multiple tadpoles within the same cage, which needed to be considered for in the tadpole models. To

correct for this, general(ized) mixed-effect models (GLMM) were used wherever possible, with site and cage included as random effects when relevant, to control for non-independence.

Gaussian GLMM were used to determine if ditch treatment (i.e. vegetated or managed) affected each of the following physiochemical water quality factors, in each year of the tadpole study: temperature, DO, SC, turbidity, pH, chlorophyll *a* (2019 only), and water depth. Each water variable was the response variable and ditch treatment was a fixed effect (dependent variable). For the physiochemical GLMMs, site (in 2019) and date (in both years) were included as random effects to control for the non-independence of multiple sites within a treatment (only an issue in 2019) and repeated measures through time (in both years).

Gaussian GLMM were also used to determine if ditch treatment (i.e. vegetated or managed) affected concentrations of pesticides (total neonicotinoids, atrazine, glyphosate) in 2019 and nutrients (TIN, TP, and TSS) in both years of the study. Each water quality variable was the response variable and ditch treatment was a fixed effect. Date was included as a factorial fixed effect in these GLMMs to assess how concentrations changed throughout time. This meant that the concentrations for each date were compared to the concentrations of the initial date (reference). Again, site was included as a random effect in all 2019 GLMM to control for non-independence of sites within a treatment.

All the water quality models were validated following Zuur et al. (2013), assessing homogeneity of variance and model fit by plotting residuals versus fitted values, quantile-quantile plots, and density plots. There were two GLMM that did not converge (2019 SC, and 2019 turbidity). This means the full models (which included site as a random effect to control

for non-independence) could not fit the data. To manage this, site was removed from the models, and simplified Gaussian GLM were run instead. Only the final models are reported as they converged and were validated.

To visually compare the water quality characteristics at each site and ditch treatments in 2019, the means for each water quality factor were calculated by treatment and a Principal Component Analysis (PCA) was performed. This approach was employed for purely descriptive purposes and intended to linearly combine correlated variables to show clustering of more similar and dissimilar sites in terms of water quality.

A similar approach for the frog health models was used, starting with data exploration to visualize the data, detect outliers and perform quality controls following Zuur et al. (2013). Following data exploration of the frog health endpoints, no outliers were removed, and subsequent analyses used the complete data sets. Generalized linear models (GLM) and GLMM were used to determine if ditch treatment (i.e. vegetated or managed) affected the survival and health outcomes of *L. pipiens* embryos and tadpole*s*. A GLM(M) was fit for each frog health endpoint, for each year of the study. The life-history traits tested were embryo development by Gosner Stage (GS), proportion hatching success, proportion embryo survival, tadpole survival, development (GS), tadpole body size (snout-to-vent length, body width, tail length), body mass, Liver Somatic Index, and proportion male.

GLMs with binomial distribution and logit link function, and the lme4 package (Bates et al. 2015) were used to determine whether treatment affected embryo survival, embryo hatching success, tadpole survival, and proportion male, following statistical approaches in Zuur

et al. (2015). The health outcome was the dependent variable, and the treatment was the fixed effect. Site was not included as a random effect in these binomial final models, because of a lack of convergence in the models. GLMMs with Gaussian distributions and log link function with package lme4 and lmerTest (Kuznetsova et al. 2015) were used to examine whether ditch treatment affected the life-history traits of tadpole body size (snout-to-vent length, body width, tail length), body mass, and liver somatic index. Response variables were the life-history traits of interest, and survival (to help control for density (Johnson et al. 2017)) and GS (to control for development) were included as fixed effects. This allowed the models to detect any significant differences due to ditch treatment, while controlling for GS and density effects. *In situ* cage identity was included as a random effect in each GLMM to account for the non-independence of tadpoles raised in the same cage.

For the developmental endpoints (GS) which are count data, GLMM were fit using the Poisson distribution using glmmPQL function in the MASS package (Venables and Ripley 2002). Gosner stage was the dependent variable, treatment and survival were fixed effects, and cage was a random effect. Dispersion was assessed to confirm the appropriate distribution was used (i.e. dispersion value ~1 (Zuur et al. 2015)). The embryo and tadpole health outcome models were validated using the same methods described above.

Multimodel inference (comparing 2+ competing models) was used to select and report the 'best-fit' models for the tadpole health endpoints. The 'best-fit' models were selected using Akaike Information Criterion (AIC; Burnham and Anderson 2004; Nakagawa and Cuthill 2007) model selection procedures, where the model with the lowest AIC score was selected. Model parameters for the best-fit model were reported following Zuur et al. (2009 and 2015).

3. Results

3.1 Site description: riparian vegetation and adjacent land-use

Vegetation and adjacent land-use were characterized to examine any potential confounding factors with ditch management. From the two vegetation surveys conducted in June and August of 2019, the total number of plant species at the vegetated treatment was 88 species while 59 species were identified at the managed treatment (Appendix 4, Table 24). The Floral Quality Assessment System (Oldham et al. 1995) was used to quantitatively compare the vegetation between the vegetated and managed ditches. The total coefficients of conservatism were 135 and 72 for vegetated and managed treatments respectively. Since rarer species receive higher scores (0 to 10), sites with more rare native species have higher total coefficient of conservatism. Therefore, the managed treatment hosted fewer rare native species compared to the vegetated, suggesting plant communities associated with a higher degree of disturbance (Oldham et al. 1995). The total weediness indices were almost identical at -31 and -32 for the vegetated and managed treatments respectively (non-native species got a more negative score the more invasive they were (0 to -3)). This indicates that the weedy species found at both treatments were similar, even if the plant communities were different, as indicated by the coefficient of conservatism. The mean wetness indices were -0.36 and -0.16 (on a scale from -5 at obligate wetland to +5 obligate upland) respectively for the vegetated and managed treatments, which placed both the treatments in the “facultative” wetness classification and indicates that the vegetated treatment was slightly more favorable for some wetland species, but only marginally so (Oldham et al. 1995).

The remotely sensed land-use data (AAFC 2019) for the 2 km radius around each site used in the tadpole experiments (V2, V3, M2, M3) revealed similar crop types and land cover (differed by 0.1 - 9 %) between treatments in both years of the study (Table 2, Figure 2, Figure 3). There was more than double the forest cover (5.2 - 11.4 %) at the vegetated sites compared to the managed sites (1.4 – 3.9 %). The remotely sensed land-use data did not detect the riparian tree cover along the vegetated ditch (except for the dense forest patch around Vegetated 3: V3), indicating an underestimation of forest cover. Corn and soybeans each covered 24 - 43 % of the area, with pasture and forage covering 14 - 21 % of the area (Table 2). These three crop types cumulatively covered 80 - 92% of the area, similar to previous analyses in earlier periods (Sunohara et al. 2012; Wilkes et al. 2019).

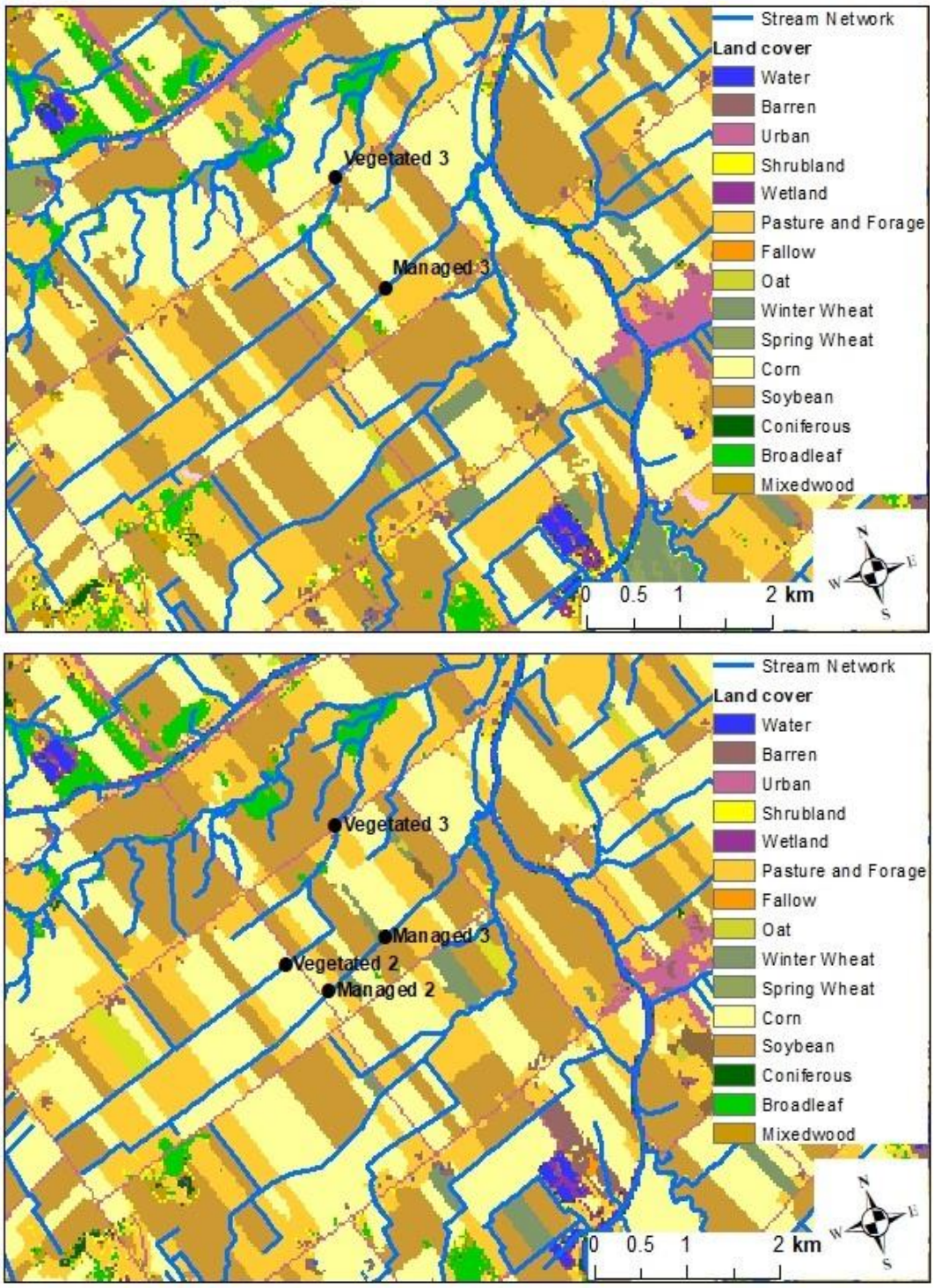

Figure 2: Maps showing crop and land use cover surrounding the sites where *L. pipiens* tadpoles were raised in situ. The top panel shows land use and sites for 2018, the bottom panel shows the same for 2019. Managed ditches were treeless (2018) and dredged (2019). AAFC Annual Crop Inventory was used for land cover data in each year (AAFC 2019).

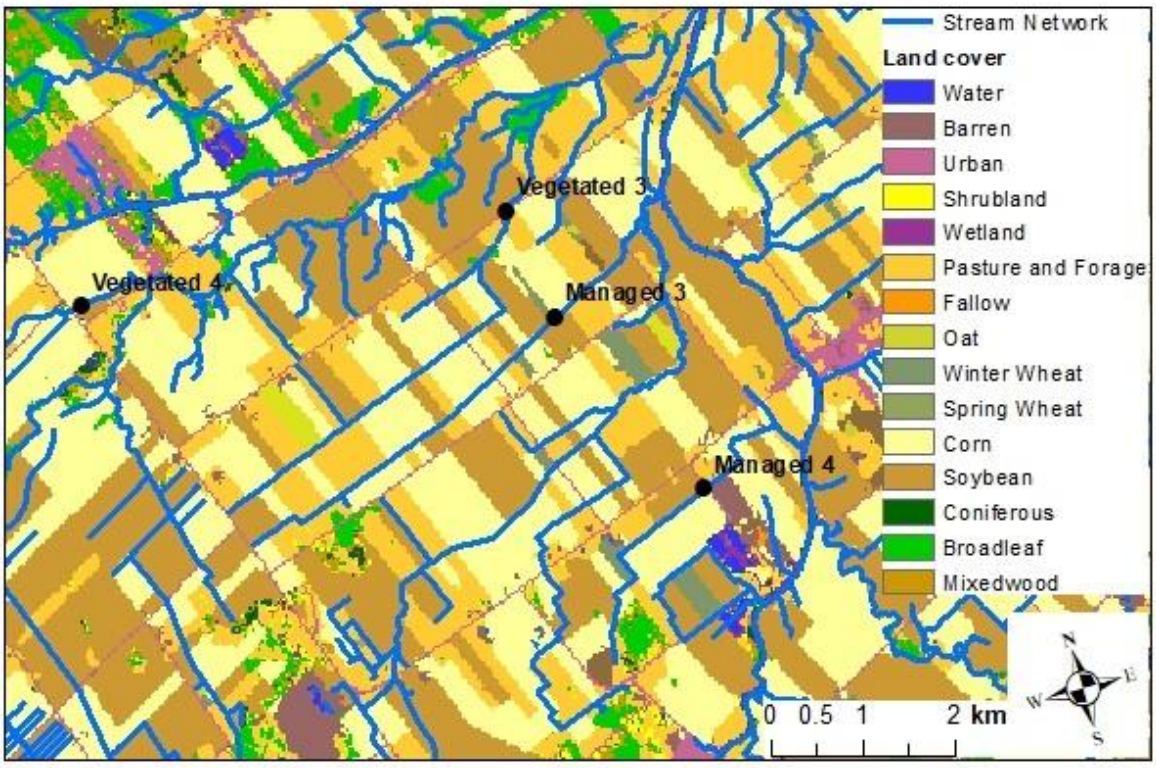

Figure 3: Map of crop and land use surrounding the sites where *L. pipiens* embryos were raised in situ in 2019. Managed ditches were dredged. AAFC Annual Crop Inventory for 2019 was used for land cover data (AAFC 2019).

Table 2: Land cover (%) classification within 2 km radius of sites where *L. pipiens* were raised *in situ*. Managed treatments were treeless in 2018 and dredged in 2019.

	2018		**2019**	
Land Cover [a]	**Vegetated**	**Managed**	**Vegetated**	**Managed**
Forest cover	11.4	3.9	5.2	1.4
Grains [b]	1.9	2.0	2.4	3.1
Corn	41.7	43.4	31.5	37.7
Soybeans	24.2	33.0	35.6	34.9
Pasture and forage	15.0	14.3	20.9	19.9
Other [c]	0.06	0.03	4.3	3.1

[a] The total area for each 2 km radius survey is 1256 ± 1 ha in 2018, and 2513 ±1 ha in 2019. Mapping accuracy for crop classes are 91.99% and 85.64% for 2018 and 2019, respectively, and for non-crop classes 76.02% and 74.89% for 2018 and 2019, respectively (AAFC 2019).

[b] "Grains" include barley, oat, winter wheat, and spring wheat.

[c] "Other" includes wetland, barren, fallow, urban, water, sod and peas.

3.2 Environmental conditions and water quality during embryo experiment

In 2019, water quality was monitored every other day during the 15-day embryo experiment that took place at four sites (2 vegetated, 2 managed; Figure 2). Water quality varied across all the sites and did not show consistent differences by ditch treatment (Table 3). The mean temperatures ranged from 7.7 – 12.6 °C across all sites (Table 3). Specific conductivity (SC) was higher at the vegetated sites, and at Vegetated 4 (V4), the mean was 1026 µS/cm, nearly double the means of the managed sites (503 and 542 µS/cm; Table 3). Mean dissolved oxygen ranged from 14.1 – 17.9 mg/L, with no measured values below the OECD guideline for amphibian larval studies (3.5 mg/L; OECD, 2015; Table 4). Mean turbidity values ranged from 5.8 – 16.5 NTU, with no consistent differences by ditch treatment (Table 3). Lowest temperatures and highest SC were observed at the V4, which made it stand out from the other sites. Within-treatment differences between sites were high enough to largely preclude finding between-treatment differences. In other words, there was so much variation in the environmental conditions and water quality parameters between sites that statistical differences by treatment were not analyzed because they would hide between-site variability.

Nutrient concentrations also varied more by site than by treatment with some exceptions (Table 5). Phosphorus species (RP and TP) were notably higher at the vegetated sites with mean TP values of 0.1 ± 0.01 mg/L at the vegetated sites compared to 0.02 ± 0.01 mg/L at the managed sites (Table 5). Nitrogen species (NH_3/NH_4^+, NO_3, and TIN) and TSS varied across all sites. Site V4 stood out with the highest values for every nutrient measured. Atrazine concentrations did not exceed the Canadian guideline for the protection of aquatic life (1.8 µg/L; CCME, 1999; Table 4). The mean atrazine concentration at vegetated treatments were

double those at managed treatments (Table 6), however atrazine concentrations varied more by site than ditch treatment (Appendix 3, Table 23). The mean total neonicotinoid concentrations were three times higher at the vegetated sites, compared to managed sites (Table 5), but all measurements were well below the Canadian guideline for the protection of aquatic life for imidacloprid (0.23 µg/L, CCME, 2007b; Table 4). The main contributing neonicotinoid was clothianidin. Glyphosate, thiacloprid and dinotefuran were not detected in any water samples during the embryo study.

3.3 Embryo health

In 2019, hatching success, survival and development of embryos were monitored in 3 *in situ* cages, at each of the four sites, with each cage containing ~60 embryos. Hatching success was not statistically different between treatments (Table 7), with mean hatching success averaging 62 ± 11 % and 68 ± 15 % for vegetated and managed treatments respectively (Table 8). In contrast, embryo survival was significantly higher at the managed treatment sites (GLM: z = -1.691, p-value = 6.1e-05; Table 7 and Table 8). There was 66 ± 15 % mean survival at the managed treatment compared to 51 ± 14 % mean survival at the vegetated treatment (Table 8). The lab controls (embryos used as driving and acclimatization controls, n = 138, raised at ~ 20 °C in an environmental chamber at NWRC) had 94.2 % hatching success and 94.9 % survival (Table 8). All embryo health outcomes were noticeably poor at the V4 site, compared to the other three sites (Table 8). Premature hatching, where embryos emerged from their casings before reaching Gosner Stage (GS) 20 (Appendix 3, Figure 12), was also observed at both vegetated sites, and at M3 to a lesser extent. The difference in GS between treatments was also statistically significant (GLMM: t = -2.611, p-value = 0.026; Table 7). GS Hatching began on Day 4

at the vegetated treatments and continued at a similar linear rate for four - six days, at which point all embryos had hatched or died (Figure 4). At the managed treatments, hatching began more slowly, starting in earnest on Day 8, and continuing for four more days at one site, and for eight more days at the other (Figure 4). The highest embryo survival and hatching success occurred at M4, where hatching occurred latest but with an accelerated hatching rate (Figure 4). The lab controls at the NWRC began hatching on Day 3 after field deployment, and hatching was complete by Day 7.

Table 3: *In situ* physiochemical water measurements obtained during *L. pipiens* embryo exposures in vegetated and managed (dredged*)* ditches in 2019.

	Managed 3 (n = 24) [a]			Managed 4 (n = 24)			Vegetated 3 (n = 24)			Vegetated 4 (n = 24)		
Variable	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Temperature (°C)	12.6	3.2	8.0 - 19.0	10.0	2.5	7.2 - 14.4	11.6	3.0	6.8 - 16.9	7.7	1.8	5.3 - 11.4
SC (µS/cm)	542	158	304 - 758	503	137	318 - 697	603	180	391 - 811	1026	362	539 - 1595
DO (mg/L)	16.3	3.6	9.9 - 21.6	18.0	3.7	13.0 - 23.6	16.3	4.1	8.6 - 22.8	14.1	2.2	10.2 - 18.6
pH	7.7	0.3	7.3 - 8.1	7.5	0.3	7.0 - 8.0	7.7	0.3	7.3 - 8.2	7.3	0.3	6.7 - 7.7
Redox (mV)	189.4	23.7	137.7 - 236.0	184.5	25.5	146.6 - 245.5	169.4	28.2	118.0 - 207.8	159.7	42.9	18.3 - 218.3
Turbidity (NTU)	15.5	9.6	5.6 - 39.0	8.8	4.2	4.5 - 19.8	5.7	2.3	2.1 - 10.9	7.4	8.5	1.1 - 32.3
Depth (cm)	18	5	12 - 32	30.4	6.6	21 - 43	30.9	9.7	13 - 50	22.5	8.0	10 - 35

[a] Sample size for DO was n = 21, due to instrument error. SD = standard deviation; n = sample size.

Table 4: Environmental and water quality guidelines for amphibian assays and the protection of aquatic life.

Variable	Guideline	Notes	Reference
Dissolved Oxygen	>40% or >3.5 mg/L	Characteristic for acceptable lab water for amphibian study	(OECD 2015)
pH	6.5 - 8.5	Characteristic for acceptable lab water for amphibian study	(OECD 2015)
Total organic carbon	2 mg/L	Characteristic for acceptable lab water for amphibian study	(OECD 2015)
Un-ionized ammonia	1 ug/L	Characteristic for acceptable lab water for amphibian study	(OECD 2015)
Particulate matter	5 mg/L	Characteristic for acceptable lab water for amphibian study	(OECD 2015)
Chloride ion	120 mg Cl-/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	(CCME 2011)
TN enrichment	>1.0 mg/L	Criteria for evaluating degree of nutrient enrichment. If >1 mg/L, highly enriched	(CCME 2007a)
TP enrichment	> 0.1 mg/L	Criteria for evaluating degree of nutrient enrichment. If >0.1 mg/L, highly enriched	(CCME, 2007b)
Total phosphorus	0.02 mg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life, summer	(CCME, 2014)
Nitrate ion	13 mg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life, long term	(CCME 2012a)
Imidacloprid	0.23 µg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	(CCME 2007b)
Glyphosate	800 µg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	(CCME 2012b)
Atrazine	1.8 µg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	(CCME 1999)

Table 5: Surface water nutrient concentrations (mg/L) during *L. pipiens* embryo in situ experiment in vegetated and managed (dredged) treatment ditches in 2019.

		Managed 3 (n = 2)		Managed 4 (n = 2)		Vegetated 3 (n = 2)		Vegetated 4 (n = 2)	
Variable	**DL**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
NH_3-NH_4^+	0.005	0.011	(0.010 - 0.012)	0.018	(0.017 - 0.019)	0.087	(0.027 - 0.146)	0.181	(0.068 - 0.293)
NO_3^-	0.02	3.73	(3.68 - 3.77)	6.72	(6.14 - 7.29)	5.31	(5.06 - 5.55)	8.75	(7.88 - 9.61)
TIN [a]	0.02	3.76	(3.71 - 3.80)	6.75	(6.18 - 7.33)	5.42	(5.25 - 5.60)	8.95	(8.193 - 9.698)
RP	0.004	DL	(DL)	DL	(DL)	0.01	(0.008 - 0.012)	0.04	(0.028 - 0.052)
TP	0.01	0.02	(DL - 0.02)	0.03	(0.02- 0.03)	0.10	(0.06 - 0.14)	0.11	(0.08 - 0.15)
DOC	0.5	3.55	(3.3 - 3.8)	3.35	(3.3 - 3.4)	4.75	(4.7 - 4.8)	5.10	(4.8 - 5.4)
TOC	0.5	3.40	(3.25 - 3.55)	3.14	(3.10 - 3.18)	4.31	(4.18 - 4.44)	4.63	(4.36 - 4.90)
TKN	0.05	0.39	(0.34 - 0.43)	0.52	(0.52 - 0.52)	0.83	(0.61 - 1.04)	0.86	(0.69 - 1.02)
TSS	2	130	(122 - 138)	130	(117 - 143)	129	(128 - 130)	97	(82 - 111)

[a] NO_2^- was at or below the detection limit (0.02 mg/L).

DL = detection limit; n = sample size; NH3-NH4+ = ammonia-ammonium; NO_3^- = nitrate; TIN = total inorganic nitrogen; RP = reactive phosphorus; TP = total phosphorus; DOC = dissolved organic carbon; TOC = total organic carbon; TKN = Total Kjeldahl Nitrogen; TSS = total suspended solids.

Table 6: Pesticide concentrations (µg/L) at each site during embryo *in situ* exposures in vegetated and managed (dredged) ditches in 2019.

	Managed 3 (n = 3)			**Managed 4 (n = 3)**			**Vegetated 3 (n = 3)**			**Vegetated 4 (n = 3)**		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Atrazine [a]	0.006	0.000	0.006 - 0.006	0.012	0.001	0.010 - 0.013	0.010	0.000	0.010 - 0.011	0.018	0.008	0.006 - 0.026
Total neonicotinoids [b,c]	0.010	0.001	0.009 - 0.011	0.012	0.002	0.010 - 0.014	0.022	0.002	0.019 - 0.024	0.040	0.013	0.022 - 0.051

[a] Method reporting limit for atrazine was 0.002 µg/L.

[b] Method reporting limit for total neonicotinoids was 0.0002 µg/L.

[c] Total neonicotinoids is the sum of Acetamiprid, Clothianidin, Thiamethoxam, Imidacloprid, and Flupyradifurone in each water sample. Glyphosate, Thiacloprid and Dinotefuran were not detected in any sample.

SD = standard deviation; n = sample size

Table 7: Generalized Linear (and Mixed-effect) Model summary results for *L. pipiens* embryo health outcomes.

Factor	β [d]	SE	z or t [a]	df	p-value	Significance [b]	SD [c]
Embryo Survival ~ Treatment							
Fixed effects (Intercept)	0.641	0.106	6.069		1.3E-09	***	
Treatment	-0.589	0.147	-4.008		6.1E-05	***	
Embryo Hatching Success ~ Treatment							
Fixed effects (Intercept)	0.754	0.108	7.010		2.4E-12	***	
Treatment	-0.255	0.151	-1.691		0.091	.	
Embryo Stage ~ Treatment + ~1|Cage							
Fixed effects (Intercept)	3.070	0.017	181.236	393	< 2e-16	***	
Treatment	-0.063	0.024	-2.611	10	0.026	*	
Random effects							
Cage (Intercept)							0.041
Residual							0.112

[a] z value for binomial distribution models (survival and hatching success) and t value for Poisson distribution model (Stage).

[b] Significance codes *** is <0.0001, ** is <0.001, * is <0.01, and . is >0.05<0.1.

[c] Random effects standard deviation value, using the glmmPQL package in R.

[d] β is the difference and direction (positive, negative) in the Managed treatment mean compared to the mean of the Vegetated treatment.

SE = standard error; SD = standard deviation; df = degrees of freedom.

Table 8: Mean survival, mean hatching success, median Gosner Stage, and occurrence of premature hatching for *L. pipiens* embryos at the end of the *in situ* experiment in 2019.

		Survival (%)			Hatching Success (%)			Gosner Stage			Premature
Site / Treatment [a]	**n**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Median**	**SD**	**Range**	**Hatching**
Managed 3	200	53.6	10.8	41.5 - 67.7	56.1	12.1	43.1 - 72.3	22	0.5	21 - 22	X
Managed 4	197	77.6	3.4	73.4 - 81.8	80.2	2.8	76.6 - 83.3	22	0.5	20 - 22	
Managed Treatment	397	65.6	14.5	41.5 - 81.8	68.1	14.9	43.1 - 83.3	22	0.5	20 - 22	1/2
Vegetated 3	196	56.5	11.0	46.2 - 71.6	69.3	3.1	65.6 - 73.1	22	0.6	19 - 22	X
Vegetated 4	188	45.8	14.7	25.4 - 59.7	54.8	11.1	39.7 - 66.1	19	0.5	17 - 20	X
Vegetated Treatment	384	51.1	14.0	25.4 - 71.6	62.1	10.9	39.7 - 73.1	21	1.3	17 - 22	2/2
Lab Controls [b]	138	94.2	na	na	94.9	na	na	nd	nd	nd	nd

[a] The managed treatment was dredged, the vegetated treatment was a reference treatment. Site means were calculated from the three *in situ* cages at each site; treatment means include all six cages at each treatment.

[b] Standard deviations (SD) and ranges were not applicable (na) because all individuals were raised in one tank; Gosner stage and premature hatching were not determined (nd).

n = sample size; X indicates observance of premature hatching; 1/2 indicates half of the sites of that treatment had premature hatching; 2/2 indicates all sites of that treatment had premature hatching.

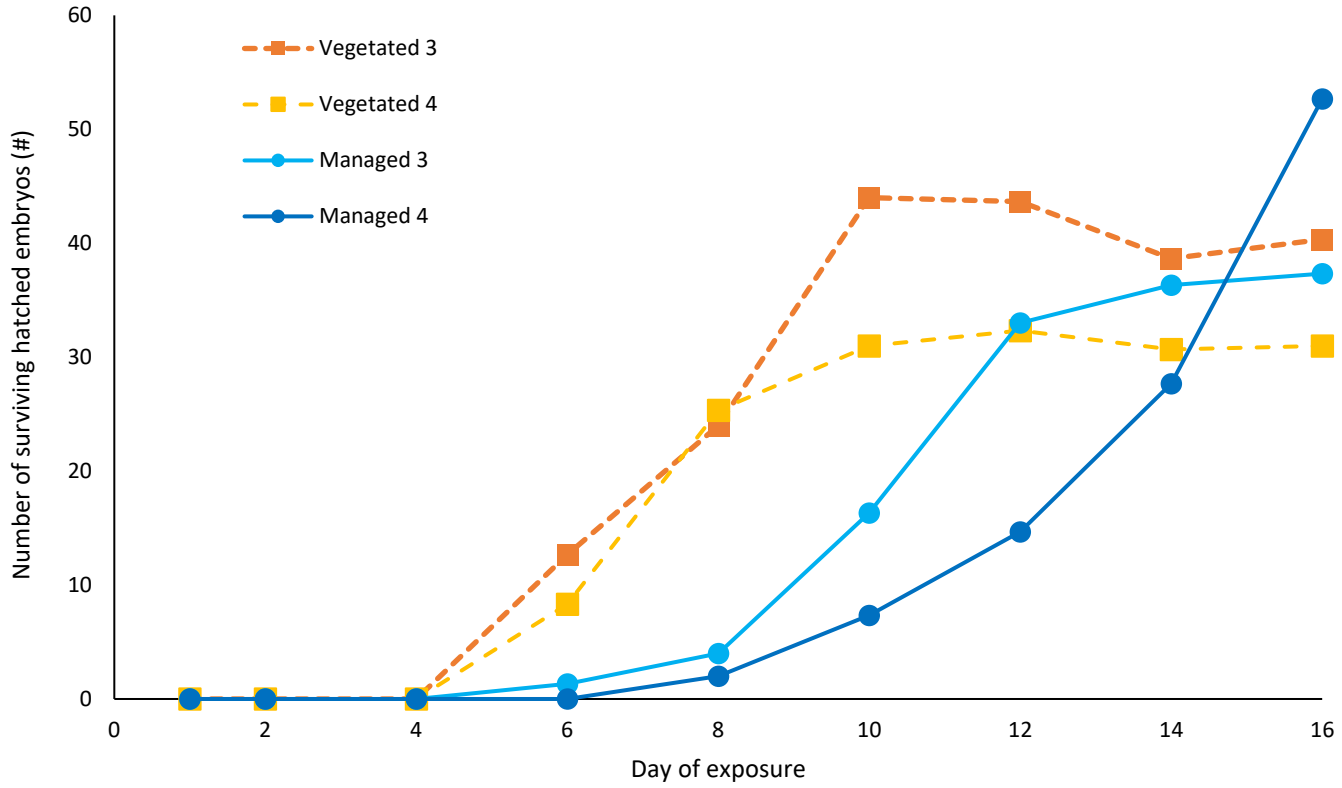

Figure 4: Mean number of living hatched embryos to show the hatching timeline of the *in situ* experiment of embryos in vegetated and managed (dredged) ditches. Initial embryo count was ~ 60 embryos in each cage. Embryo exposure began on May 2, 2019 and ran until all embryos either hatched or died (May 17, 2019).

3.4 Environmental conditions and water quality during tadpole experiments

Throughout the tadpole experiments, water quality and environmental variables were monitored twice a week in 2018 at the two treatment sites, and in 2019 at the four treatment sites (Figure 1). Temperature was significantly higher at the managed treatment in both years of the study (GLMM: t = -6.228 and 11.940, p-values = 0.025 and <2e-16; Table 9). The mean temperatures were 16.8 ± 2.96 °C and 17.4 ± 2.44 °C at the vegetated and managed treatments in 2018, and 15.9 ± 3.38 °C and 18.3 ± 5.77 °C respectively in 2019 (Table 10). The differences in mean temperatures were 0.6 °C and 2.4 °C higher at the managed treatment in 2018 and 2019 respectively (Table 10). There was a significant difference in specific conductivity between treatments in 2019 only (GLMM: t = 196.700, p-value = <2e-16; Table 9), with the vegetated treatment having a higher mean by 190 µS/cm (Table 9). Dissolved oxygen (DO) was highly significantly different by treatment in 2018, and marginally different in 2019 (GLMM: t = -60.180 and -3.015, p-values = <2e-16 and 0.095; Table 9). The mean DO was higher by ~3.7 mg/L at the vegetated treatment in 2018, and similarly also higher in 2019 (by ~3.5 mg/L) (Table 10). The mean pH was significantly different in 2018, but not in 2019 (GLMM: t = -97.000, p-value = <2e-16; Table 9). The mean pH values were fairly neutral across all treatments, ranging from 7.3 - 7.9, with more variation observed at the managed treatment (Table 10). Mean turbidity was significantly higher at the vegetated site in 2018 and significantly higher at the managed treatment in 2019 (GLMM: t = -47.120 and -58.070, p-value = <2e-16 and <2e-16; Table 10). There was no statistical difference in Chl *a* (an indicator of primary productivity) between treatments in 2019 (it was not measured in 2018; Table 9), but the mean was 2.1 µg/L

higher at the vegetated treatment and reached much higher maximum peaks within these treatments (Table 10).

Nutrient concentrations were generally higher at the vegetated treatment in both years (Table 11). In 2018, NH_4^+/NH_3, NO_3^-, TIN, RP and TP were all two to three times higher at the vegetated treatment compared to the managed treatment. In 2019, NH_4^+/NH_3, RP and TP were 4 - 13x higher at the vegetated treatment, while the rest of the parameters were relatively similar. Total phosphorus was significantly higher in both years for the vegetated treatment (GLMM: t = 3.982 and 4.333, p-value = 2.2e-03 and 1.9e-03; Table 9), and TIN was significantly higher in 2018 only (GLMM: t = 2.829, p-value = 0.016; Table 9). All mean TP values (Table 11) exceeded the Canadian water quality guideline for the protection of aquatic life of 0.02 mg/L (Table 4; CCME, 2014). Nitrate concentrations did not exceed 13 mg/L, the Canadian water quality guidelines for the protection of aquatic life (Table 4; CCME, 2012b), although they did reach a maximum of 9.40 mg/L at the vegetated treatment in 2019.

In 2019, atrazine, four neonicotinoids, and glyphosate were detected in water samples during the tadpole experiment. Atrazine was detected in all water samples (Table 12), none of which exceeded the maximum level of the Canadian water quality guidelines for the protection of aquatic life (Table 4). There was no significant difference in atrazine concentrations between treatments (Table 9), mean atrazine values were 0.018 ± 0.017 µg/L and 0.020 ± 0.015 µg/L for managed and vegetated treatments respectively and the range across both treatments was 0.006 – 0.059 µg/L (Table 12). Atrazine concentrations peaked to significant levels above the baseline (May 23, 2019) on June 24, 2019 at the vegetated treatment (GLMM: t = 5.361, p-value = 3.0e-5; Table 9) and continued to increase significantly above baseline on July 2, 2019 at

the managed treatment (GLMM: t = 4.144, p-value = 5.0e-4; Table 9, Figure 5). Among the neonicotinoids, clothianidin was detected in all samples (n = 28), thiamethoxam was detected in all samples except nine (both treatments), imidacloprid was detected in seven samples (both treatments), and acetamiprid was detected in only one sample (vegetated treatment only). None of the total neonicotinoid levels detected in the water samples exceeded the imidacloprid guideline for the protection of freshwater life (0.23 µg/L, Table 4; CCME, 2007a). The seasonal maximum in total neonicotinoid concentrations occurred on June 17th, 2019 and was significantly higher than baseline levels (GLMM: t = 2.270, p-value = 0.034; Table 9, Figure 5). The mean total neonicotinoid levels were significantly higher at the vegetated treatment compared to managed treatment (GLMM: t = 4.127, p-value = 5.2e-4; Table 9). Total neonicotinoid concentrations at the vegetated treatment were doubled those of the managed treatment with means of 0.012 ± 0.008 µg/L and 0.022 ± 0.007 µg/L and a total range from 0.005 – 0.037 µg/L (Table 12). Thiacloprid, dinotefuran and flupyradifurone were not detected in any water samples during the tadpole exposure. Glyphosate was detected in eight samples (four at each treatment) and was significantly higher at the managed (dredged) treatment compared to vegetated treatment (GLMM: t = -2.125, p-value = 3.6e-3, Table 9). The mean glyphosate concentration at the vegetated treatment was below the minimum detection limit (0.025 µg/L), and the mean at the managed treatment was 0.163 ± 0.283 µg/L with a range from <MDL – 0.929 µg/L (Table 12). None of the samples exceeded the glyphosate guideline for the protection of freshwater life (800 µg/L; Table 4, CCME, 2012). There was a clear seasonal maximum at the managed (dredged) treatment on June 10th, 2019 when the concentration was significantly higher than baseline levels (GLMM: t = 3.375, p-value = 3.6e-3; Table 9, Figure 5).

Based on climate data for 2019 (Figure 6) the maximum atrazine concentration detected by grab samples occurred on a day of light rain (5 mm, June 24). The total neonicotinoid maximum concentration occurred within six days following the largest rainfall event of the spring (17 mm, June 11). The glyphosate maximum occurred six days after four consecutive days of moderate rain (13 mm total, June 1-4). Pesticide spraying and planting of pesticide-coated seeds was observed in the field, alongside both treatment ditches on June 10 - 13, 2019.

The 2019 mean and maximum Pesticide Toxicity Indices (PTIs) were calculated using the concentration addition method (assumes toxicity is additive), according to Equation 1. The mean PTI's were 0.01 ± 0.01 and 0.04 ± 0.02, and the maximum PTI's were 0.09 ± 0.17 and 0.6 ± 0.21 for vegetated and managed treatment respectively. This revealed that the risks posed by most of the measured pesticide mixture concentrations in ditch waters were below the threshold considered potentially toxic (PTI>0.5) (Battaglin and Fairchild 2002). At the managed treatment, there was one date (June 10, 2019) when the maximum PTI indicated potential toxicity (PTI quotient was 0.6). This date coincides with large peaks in glyphosate concentrations at the managed treatment (Figure 5). All other PTI values ranged between 0.1 – 0.5, and indicate limited potential for toxicity (Battaglin and Fairchild 2002).

The 2019 water quality PCA showed clear separation of the sites by management treatment along PC1, which explained 61.9 % of variation (Figure 7). Higher Temperature, pH, and glyphosate were drivers of PC1 separating the managed treatment; while higher SC, ORP, TP, Depth, NH3, NO2, RP, TOC, DOC, and total neonicotinoids were drivers separating the vegetated treatment. PC2 explained 22.5 % of variance, with TIN, NO3, DO, turbidity, atrazine

and TSS being drivers of this separation; these parameters were more variable by site than treatment.

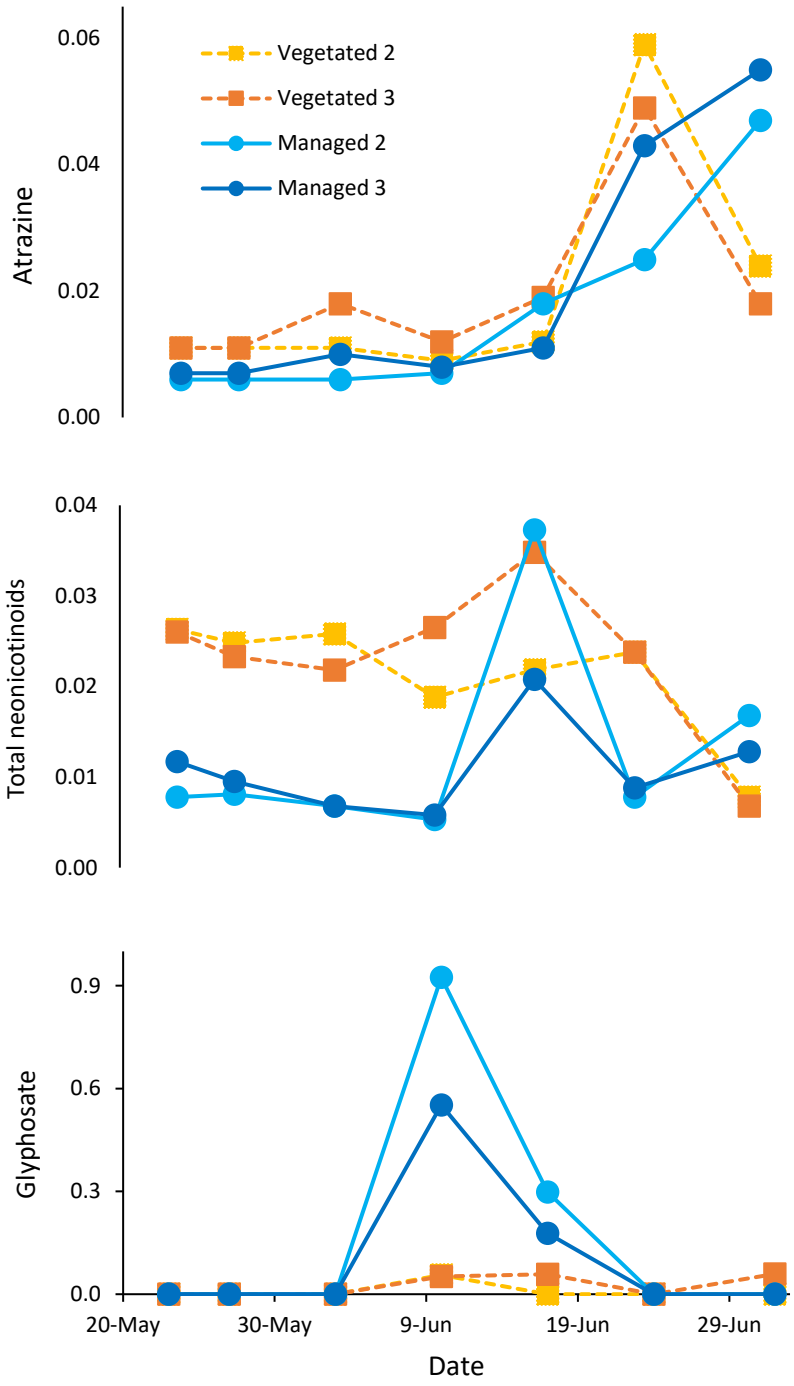

Figure 5: Seasonal surface water pesticide concentrations (µg/L) of atrazine, total neonicotinoids, and glyphosate during *in situ* tadpole exposure in vegetated and managed (dredged) ditches in the South Nation watershed, Ontario in 2019. Total Neonicotinoids is the sum of Acetamiprid, Clothianidin, Thiamethoxam, Thiacloprid, Dinotefuran, Imidacloprid, and Flupyradifurone in each water sample. The sample size is 7 for each site.

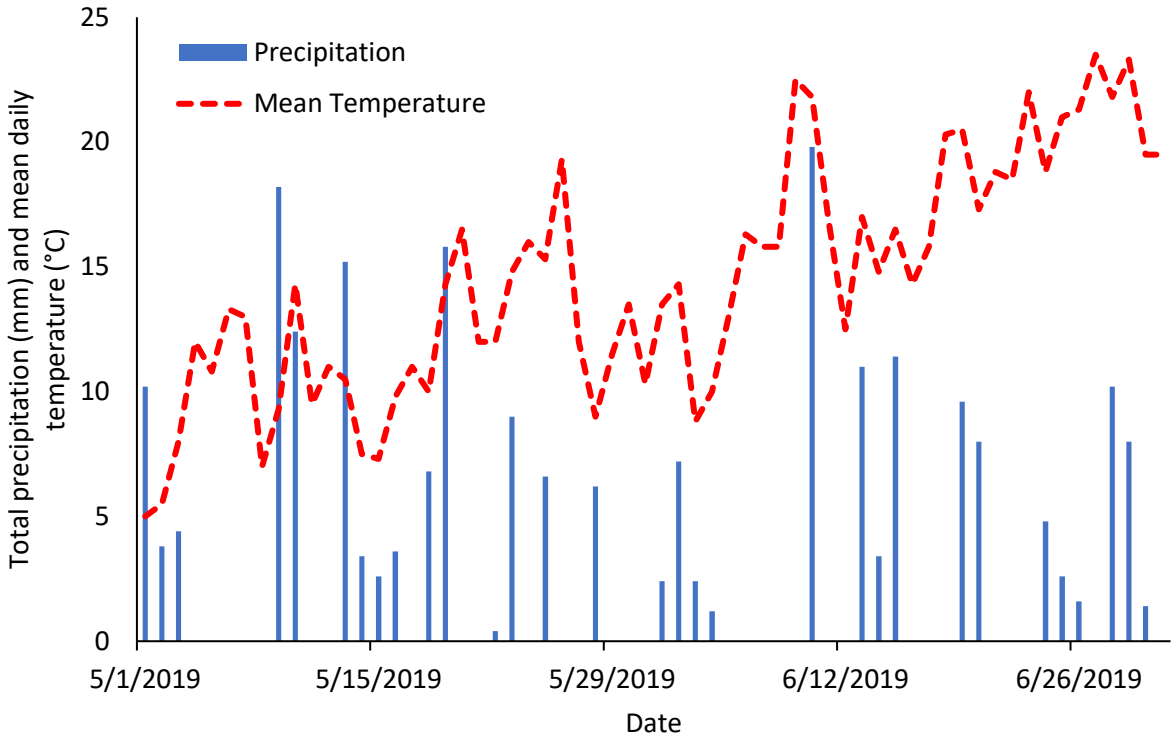

Figure 6: Daily total precipitation (mm) and mean temperature (°C) during frog experiments in May-July 2019. Historical climate data were collected from the closest weather station to the sites (St. Albert, ON.; ECCC, 2020).

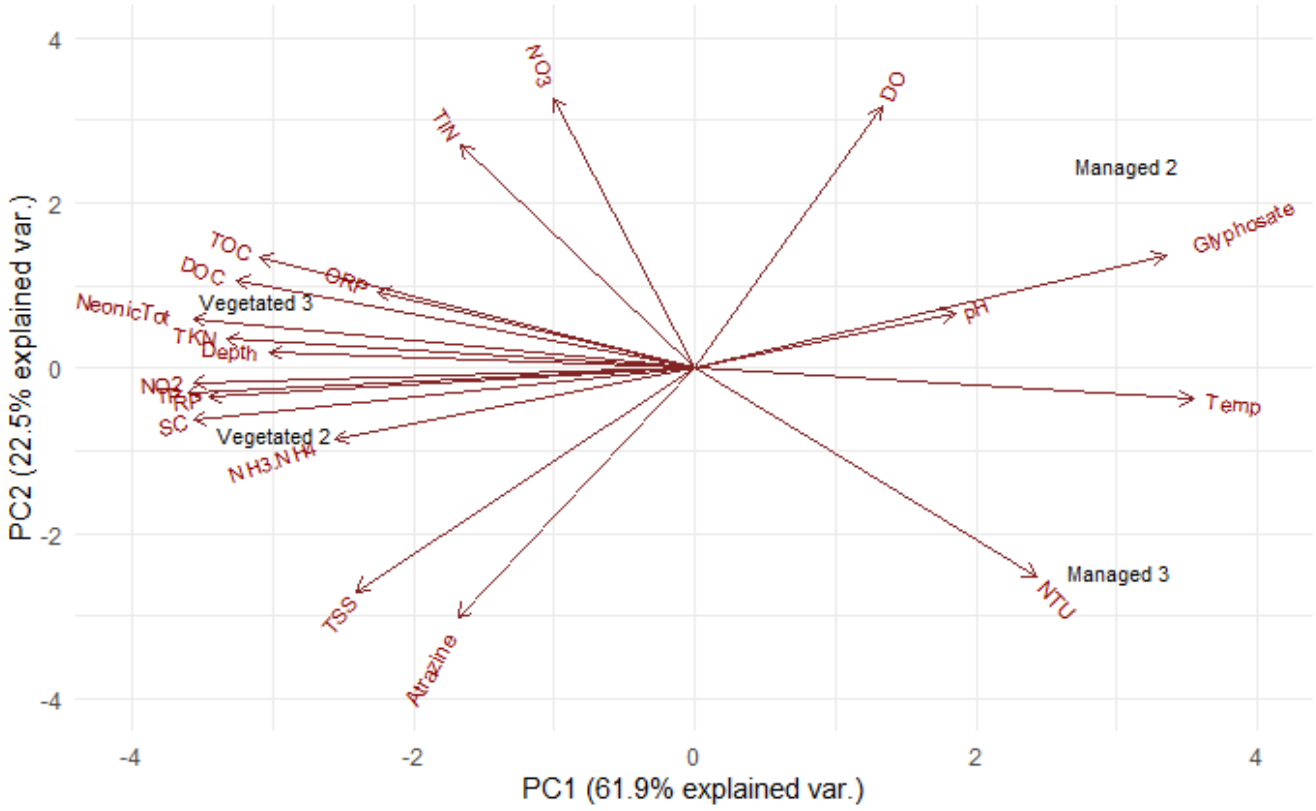

Figure 7: Principal component analysis (PCA) of the mean for all water quality parameters during the *in situ* tadpole exposure at 2 vegetated and 2 managed treatment ditch sites. Sample sizes per site: nutrients (n = 6), pesticides (n = 7), physical water quality (n = 24). Parameter full names are: temperature (Temp), dissolved oxygen (DO), specific conductivity (SC), redox potential (ORP), turbidity (NTU), ammonia/ammonium (NH3/NH4), Nitrate (NO3), Nitrite (NO2), Total Inorganic Nitrogen (TIN), Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), Reactive phosphorus (RP), dissolved organic carbon (DOC), total organic carbon (TOC), total suspended solids (TSS), glyphosate, atrazine, total neonicotinoids (NeonicTot), and depth.

Table 9: General Linear Mixed-effect Model summary results for water quality parameters during tadpole exposures.

Factor	β [a]	SE	df	t	p-value	Significance [b]	Variance	SD
2019 Temperature ~ Treatment + ~1|Site + ~ 1|Date								
Fixed effects (Intercept)	18.3452	0.2775	2	66.102	8.4E-05	***		
Treatment	-2.364	0.3796	2	-6.228	0.025	*		
Random Effects								
Date (Intercept)							19.561	4.423
Site (Intercept)							0.143	0.379
Residual							2.962	1.721
2018 Temperature ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	16.822	0.344	31	48.890	<2e-16	***		
Treatment	0.601	0.050	5828	11.940	<2e-16	***		
Random Effects								
Date (Intercept)							3.744	1.935
Residual							3.523	1.877
2019 Specific Conductivity ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	607.200	0.978	6632	621.000	<2E-16	***		
Treatment	190.600	0.969	11640	196.700	<2E-16	***		
Random Effects								
Date (Intercept)							1906.000	43.660
Residual							3656.000	60.460
2018 Specific Conductivity ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	733.308	4.723	32	155.269	<2E-16	***		
Treatment	-0.616	0.802	5827	-0.768	0.442			
Random Effects								
Date (Intercept)							6.310	2.512
Residual							5.239	2.289
2019 Dissolved Oxygen ~ Treatment + ~1|Site + ~ 1|Date								
Fixed effects (Intercept)	10.396	0.823	2	12.634	0.006	**		
Treatment	-3.501	1.161	2	-3.015	0.095			

Random Effects								
Date (Intercept)							11.170	3.342
Site (Intercept)							1.346	1.160
Residual							5.047	2.247
2018 Dissolved Oxygen ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	6.082	0.446	31	13.630	1.0E-14	***		
Treatment	-3.691	0.061	5827	-60.180	<2E-16	***		
Random Effects								
Date (Intercept)							6.310	2.512
Residual							5.239	2.289
2019 Turbidity ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	20.646	0.217	9472	94.950	<2E-16	***		
Treatment	-16.078	0.277	11568	-58.070	<2E-16	***		
Random Effects								
Date (Intercept)							34.930	5.910
Residual							298.180	17.270
2018 Turbidity ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	11.893	0.648	33	18.350	<2E-16	***		
Treatment	-8.804	0.187	5626	-47.120	<2E-16	***		
Random Effects								
Date (Intercept)							12.850	3.584
Residual							46.510	6.820
2019 pH ~ Treatment + ~1|Site + ~ 1|Date								
Fixed effects (Intercept)	7.871	0.099	2	79.394	1.6E-04	***		
Treatment	-0.367	0.140	2	-2.616	0.120			
Random Effects								
Date (Intercept)							0.031	0.176
Site (Intercept)							0.020	0.140
Residual							0.028	0.166
2018 pH ~ Treatment + ~ 1|Date								
Fixed effects (Intercept)	7.594	0.017	32	458.300	<2E-16	***		

Treatment	-0.274	0.003	5830	-97.000	<2E-16	**		
Random Effects								
Date (Intercept)							0.009	0.093
Residual							0.011	0.105
2019 Chlorophyll a ~ Treatment + ~1|Site + ~ 1|Date								
Fixed effects (Intercept)	4.942	0.580	2	8.519	0.014	*		
Treatment	2.102	0.820	2	2.563	0.124			
Random Effects								
Date (Intercept)							0.633	0.796
Site (Intercept)							0.670	0.819
Residual							11.598	3.406
Atrazine ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	0.008	0.005	20	1.624	0.120			
Treatment	0.001	0.004	20	0.386	0.703			
Date5/27/2019	0.000	0.007	20	0.000	1.000			
Date6/10/2019	0.000	0.007	20	0.038	0.970			
Date6/17/2019	0.006	0.007	20	0.951	0.353			
Date6/24/2019	0.035	0.007	20	5.361	3.0E-05	***		
Date6/3/2019	0.003	0.007	20	0.380	0.708			
Date7/2/2019	0.027	0.007	20	4.144	5.0E-04	***		
Random Effects								
Site (Intercept)							0.00	0.00
Residual							8.65E-05	0.009
Total Neonicotinoids ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	0.013	0.004	20	3.562	2.0E-03	**		
Treatment	0.010	0.003	20	4.127	5.2E-04	***		
Date5/27/2019	-0.002	0.005	20	-0.322	0.750			
Date6/10/2019	-0.004	0.005	20	-0.814	0.425			
Date6/17/2019	0.011	0.005	20	2.270	0.034	*		
Date6/24/2019	-0.002	0.005	20	-0.402	0.692			
Date6/3/2019	-0.003	0.005	20	-0.560	0.581			

Date7/2/2019	-0.007	0.005	20	-1.459	0.160			
Random Effects								
Site (Intercept)							0.00	0.00
Residual							4.47E-05	0.007
Glyphosate ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	0.072	0.090	17	0.803	0.433			
Treatment	-0.144	0.068	17	-2.125	0.049	*		
Date5/27/2019	0.000	0.117	17	0.000	1.000			
Date6/10/2019	0.396	0.117	17	3.375	3.6E-03	**		
Date6/17/2019	0.134	0.117	17	1.138	0.271			
Date6/3/2019	0.000	0.117	17	0.000	1.000			
Date7/2/2019	0.015	0.117	17	0.126	0.901			
Random Effects								
Site (Intercept)							0.00	0.00
Residual							0.028	0.166
2019 TIN ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	3.905	0.599	16	6.518	7.1E-06	***		
Treatment	-0.143	0.464	16	-0.308	0.762			
Date5/27/2019	-0.432	0.781	16	-0.552	0.588			
Date6/13/2019	-1.853	0.781	16	-2.372	0.031	*		
Date6/17/2019	3.479	0.847	16	4.106	8.3E-04	***		
Date6/24/2019	-3.177	0.781	16	-4.067	9.0E-04	***		
Date6/26/2019	-3.477	1.257	16	-2.767	0.014	*		
Date7/2/2019	-3.499	0.781	16	-4.479	3.8E-04	***		
Random Effects								
Site (Intercept)							0.00	0.00
Residual							1.220	1.105
2018 TIN ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	1.042	0.443	11	2.350	0.039	*		
Treatment	1.122	0.397	11	2.829	0.016	*		
Date5/28/2018	-1.382	0.561	11	-2.465	0.031	*		

Date6/11/2018	0.471	0.561	11	0.840	0.419			
Date6/25/2018	-1.393	0.561	11	-2.484	0.030	*		
Random Effects								
Site (Intercept)							0.00	0.00
Residual							0.629	0.793
2019 TP ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	-0.025	0.039	9	-0.649	0.533			
Treatment	0.120	0.028	9	4.333	1.9E-03	**		
Date5/27/2019	0.023	0.045	9	0.503	0.627			
Date6/13/2019	0.101	0.045	9	2.245	0.051	.		
Date6/24/2019	0.082	0.045	9	1.826	0.101			
Random Effects								
Site (Intercept)							0.000	0.000
Residual							0.003	0.052
2018 TP ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	0.015	0.026	11	0.594	0.565			
Treatment	0.091	0.023	11	3.982	2.2E-03	**		
Date5/28/2018	0.139	0.032	11	4.280	1.3E-03	**		
Date6/11/2018	-0.031	0.032	11	-0.950	0.362			
Date6/25/2018	0.071	0.032	11	2.179	0.052	.		
Random Effects								
Site (Intercept)							0.000	0.000
Residual							0.002	0.046
2019 TSS ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	128.429	21.338	9	6.019	2.0E-04	***		
Treatment	9.143	15.088	9	0.606	0.560			
Date5/27/2019	22.250	24.445	9	0.910	0.386			
Date6/13/2019	-28.000	24.445	9	-1.145	0.282			
Date6/24/2019	-23.750	24.445	9	-0.972	0.357			
Random Effects								
Site (Intercept)							0.000	0.000

Residual							796.800	28.230
2018 TSS ~ Treatment + Date + ~1|Site								
Fixed effects (Intercept)	117.875	9.844	11	11.974	1.2E-07	***		
Treatment	11.250	8.805	11	1.278	0.228			
Date5/28/2018	-12.250	12.452	11	-0.984	0.346			
Date6/11/2018	3.750	12.452	11	0.301	0.769			
Date6/25/2018	2.000	12.452	11	0.161	0.875			
Random Effects								
Site (Intercept)							0.000	0.000
Residual							310.100	17.610
2019 Depth ~ Treatment + ~1|Site + ~1|Date								
Fixed effects (Intercept)	24.189	1.839	4	13.150	1.4E-04	***		
Treatment	3.947	2.129	2	1.854	0.205			
Random Effects								
Date (Intercept)							12.295	3.506
Site (Intercept)							4.142	2.035
Residual							12.864	3.587
2018 Depth ~ Treatment + ~1|Date								
Fixed effects (Intercept)	30.469	2.047	9	14.881	9.3E-08	***		
Treatment	1.395	1.074	152	1.299	0.196			
Random Effects								
Date (Intercept)							32.540	5.704
Residual							46.730	6.836

[a] β is the difference and direction (positive, negative) in the Managed (treeless in 2018 and dredged in 2019) treatment mean compared to the mean of the Vegetated treatment.

[b] Significance codes *** is <0.0001, ** is <0.001, * is <0.01, and . is >0.05<0.1.

SE = standard error; SD = standard deviation; df = degrees of freedom.

Table 10: Physiochemical water quality during tadpole *in situ* experiments in vegetated and managed ditches. The sample size (n) provided in the table represent total number of timepoints that data were collected during the exposure.

	2018						**2019**					
	Vegetated (n = 2776)			**Managed [b] (n = 2780)**			**Vegetated (n = 7787)**			**Managed (n = 7787)**		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Temperature (°C)	16.8	2.96	(10.9 - 23.8)	17.4	2.44	(11.6 - 24.5)	15.9	3.38	(8.1 - 24.7)	18.3	5.77	(5.8 - 34.9)
SC (µS/cm)	732	28	(575 - 878)	732	48	(592 - 820)	798	31	(696 - 887)	608	101	(360 - 829)
DO (mg/L)	6.32	1.99	(2.30 - 12.16)	2.62	4.25	(-0.03 - 20.35)	6.91	4.08	(0.07 - 20.42)	10.42	4.14	(1.11 - 27.08)
pH	7.6	0.15	(7.27 - 7.92)	7.33	0.13	(7.11 - 7.91)	7.5	0.20	(7.09 - 8.04)	7.87	0.31	(7.3 - 9.05)
Turbidity (NTU)	8.6	10.6	(0.2 - 78.9)	8.6	3.2	(0.6 - 136.1)	4.6	7.5	(0.54 - 227.92)	20.6	24.7	(2.71 - 173.67)
Depth [a] (cm)	32	8.11	(21 – 52)	30	9.19	(17 – 56)	28	6.89	(14 – 45)	24	2.08	(18 – 28)
ORP (mV)							415.61	42.87	(318.12 - 498.42)	298.53	42.18	(189 - 397.95)
TDS (mg/L)							518.76	19.88	(452.35 - 576.7)	394.88	65.58	(233.78 - 539.11)
Chlorophyll *a* (µg/L)							7.07	4.22	(1.94 - 114.59)	4.95	2.73	(1.09 - 43.57)

[a] Depth sample sizes were n = 80 in 2018, and n = 65 in 2019 for each treatment.

[b] Managed treatments were treeless in 2018 and dredged in 2019.

SD = Standard deviation; n = sample size.

Table 11: Surface water nutrient concentrations (mg/L) during tadpole *in situ* experiments in vegetated and managed watersheds.

		2018						2019					
		Vegetated (n = 8)			Managed [b] (n = 8)			Vegetated (n = 12) [a]			Managed (n = 12) [a]		
Variable	**DL**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
NH_3-NH_4^+	0.005	0.08	0.05	(0.01 - 0.18)	0.04	0.02	(0.02 - 0.08)	0.21	0.44	(0.01 - 1.63)	0.02	0.01	(0.01 - 0.06)
NO_3^-	0.02	1.48	1.45	(0.06 - 3.93)	0.41	0.48	(DL - 1.21)	2.61	2.82	(DL - 9.40)	2.37	1.95	(DL - 6.78)
NO_2^-	0.02	0.03	0.01	(DL - 0.05)	DL	0.00	(DL - 0.03)	0.03	0.01	(DL - 0.05)	DL	0.00	(DL - 0.03)
TIN	0.02	1.59	1.42	(0.15 - 3.96)	0.47	0.47	(0.08 - 1.25)	2.85	2.73	(0.05 - 9.46)	2.41	1.95	(0.05 - 6.8)
RP	0.004	0.04	0.03	(DL - 0.09)	0.01	0.01	(DL - 0.03)	0.05	0.04	(0.01 - 0.13)	DL	DL	(DL - 0.01)
TP	0.01	0.15	0.10	(0.04 - 0.31)	0.06	0.04	(0.02 - 0.13)	0.15	0.08	(0.05 - 0.26)	0.03	0.02	(0.01 - 0.06)
DOC	0.5	6.2	1.6	(4.3 - 8.4)	6.4	1.7	(4.5 - 8.8)	7.0	2.9	(4.7 - 13.7)	4.7	1.5	(3.2 - 7.3)
TOC	0.5	6.17	1.67	(4.15 - 8.25)	6.40	1.68	(4.61 - 8.68)	6.70	3.00	(4.18 - 13.72)	4.76	1.74	(3.19 - 7.92)
TKN	0.05	0.84	0.17	(0.55 - 1.12)	0.65	0.11	(0.48 - 0.84)	0.90	0.30	(0.61 - 1.38)	0.58	0.14	(0.34 - 0.80)
TSS	2	128	17	(100 - 150)	116	15	(96 - 137)	129	26	(86 - 156)	120	36	(59 - 169)

[a] Due to funding restraints, only TP, DOC, TOC, TKN, TSS were analyzed in n = 6 samples.

[b] Managed treatments were treeless in 2018 and dredged in 2019.

SD = Standard deviations; n = sample size; DL = detection limit.

Table 12: Pesticide concentrations (µg/L) during tadpole *in situ* cage exposures in vegetated and managed ditches in 2019.

	Atrazine (n = 14)			**Total Neonicotinoids [a] (n = 14)**			**Glyphosate (n = 12)**		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Vegetated	0.020	0.015	0.009 - 0.059	0.022	0.007	0.007 - 0.035	<MRL [b]	0.027	<MRL - 0.059
Managed	0.018	0.017	0.006 - 0.055	0.012	0.008	0.005 - 0.037	0.163	0.283	<MRL - 0.924

[a] Total Neonicotinoids were the sum of Acetamiprid, Clothianidin, Thiamethoxam, Imidacloprid, Thiacloprid, Dinotefuran and Flupyradifurone in each water sample.

[b] Method reporting limits (MRL) were 0.002 ug/L for atrazine, 0.0002 ug/L for total neonicotinoids, and 0.025 µg/L for glyphosate.

SD = standard deviation; n = sample size.

3.5 Tadpole health

The overall tadpole survival was high, with 90 % and 96 % in 2018, and 86 % and 93 % in 2019 for vegetated and managed treatments respectively (Table 13). Tadpole survival was not significantly affected by ditch treatment in either year (GLM: z = 1.808 and -1.660, p-value = 0.071 and 0.097; Table 14). Tadpoles reached significantly higher metamorphic GS in the managed ditch in both years (GLMM: t = 9.600 and -11.419, p-value = 8.5e-8 and 1.1e-6; Table 13 and Table 14). For context, GS are used to place an individual along its metamorphic path from a fertilized embryo (GS 1), to a hatchling (GS 20), to a free swimming larvae (GS 25), through hind limb development (tiny foot paddles, differentiating toes, becoming functional legs (GS 26-41), to forelimb emergence (GS 42), to tail reabsorption and metamorphosis completion (GS 46; see Gosner Stage Chart in Appendix 3, Figure 12). In both years, most of the tadpoles reached the hind limb development stages (GS 31-39) at the end of the experiments, based on the median GS. There was a median five-stage difference between treatments in 2018, with more complete toe indentation at the managed treatment compared to the vegetated treatment (GS 31 vs GS 36). In 2019, the median four-stage difference meant the utility of hind legs at the managed treatment (GS 39) but only a small foot paddle at the vegetated treatment (GS 34). In both years, at the vegetated treatment, no metamorphic development occurred for a small portion of individuals (<1% remained at GS 25), while all individuals at the managed treatments advanced to at least GS 27. Tadpole size (snout-to-vent length: SVL) also was significantly higher at the managed treatment compared to the vegetated treatment in both years (GLMM: t = -2.277 and 2.588, p-value = 0.030 and 0.018; Table 14). The median differences in SVL were 2.1 mm and 2.3 mm larger at the managed treatment in 2018

and 2019 respectively (Table 13). Tail length was significantly larger at the managed treatment compared to vegetated treatment in 2019, but not in 2018 (GLMM: t = 3.780, p-value = 0.001; Table 14). Other tadpole health outcomes (body width and mass, liver mass, and Liver Somatic Index) were not significantly different by treatment in either year (Table 13 and Table 14). In 2019, a significant difference in sex ratio was observed between treatments (GLM: z = -4.300, p-value = 1.7e-5; Table 14). Almost complete masculinization (97 % male) occurred at the vegetated treatment of all tadpoles GS 36 and above (n = 31), compared to 59 % male at the managed treatment (n = 81; Table 13). The sex ratio of the control tadpoles raised at the NWRC from the same embryo clutches was 58 % male (n = 26). All the results from the tadpole health GLMM and GLM's are presented in Table 13, with the multimodel selection procedure using AIC in Table 15.

3.6 Pesticide residues in tadpole tissues

Imidacloprid (neonicotinoid) and tebuconazole (fungicide) were detected at quantifiable levels in tadpole tissue samples (Table 16). The maximum concentrations were 123.80 ng/g wet weight and 86.31 ng/g wet weight for imidacloprid and tebuconazole respectively. Imidacloprid was found in tissues from both treatments in 2019 and was detected in 33 - 40 % of the samples. Tebuconazole was detected in 2018 in 100 % of the managed treatment tissue samples, but none of the vegetated treatment tissues samples. Pesticide detection recoveries for these two pesticides ranged from 62 - 80 % (Table 17). Signal suppression enhancement ranged from 65 - 79 % (Table 17). Limits of quantification (LOQ) were 8.03 ng/g for imidacloprid and 10.52 ng/g for tebuconazole (Table 17). Additional pesticides thiabendazole, azoxystrobin, pyraclostrobin, and diazinon were detected in trace amounts (<LOQ); while metolochlor,

triazophos and DEET were detected but not quantified. These include three fungicides (pyraclostrobin, azoxystrobin and thiabendazole), three insecticides (triazophos, diazinon and DEET), and one herbicide (metolochlor; Table 17). Further analysis may be done to quantify these compounds for a future publication.

Table 13: *L. pipiens* tadpole health outcomes by ditch treatment (vegetated or managed) each year of the *in situ* study.

2018					**2019**				
Treatment	**Mean/Median**	**SEM**	**Range**	**p-value**	**Treatment**	**Mean/Median**	**SEM**	**Range**	**p-value**
Gosner Stage									
Managed	35.4/36	0.16	(27-37)	**8.50E-08**	Managed	39.0/39	0.21	(34-41)	**1.10E-06**
Vegetated	31.4/31		(25-36)		Vegetated	34.9/35		(25-37)	
Snout-to-vent length (mm)									
Managed	15.5/15.5	0.14	(9.2 - 19.3)	**0.0298**	Managed	19.0/19.3	0.19	(11.5 - 21.8)	**0.0184**
Vegetated	13.2/13.4		(6.2 - 16.8)		Vegetated	16.7/16.9		(8.2 - 21.7)	
Survival (initial count 15)									
Managed	14.4/15	0.06	(13 - 15)	0.0706	Managed	14.1/15	0.09	(12 - 15)	0.0969
Vegetated	13.5/14		(12 - 15)		Vegetated	12.9/13		(12 -14)	
Body Width (mm)									
Managed	9.1/9.1	0.09	(5.5 - 10.9)	0.724	Managed	11.1/11.1	0.11	(7.2 - 13.0)	0.3338
Vegetated	7.5/7.6		(3.5 - 9.0)		Vegetated	9.5/9.6		(4.9 - 11.6)	
Tail length (mm)									
Managed	29.6/29.6	0.32	(15.5 - 38.1)	0.195	Managed	37.4/37.6	0.4	(23.7 - 46.2)	**7.84E-04**
Vegetated	24.2/24.2		(9.3 - 33.6)		Vegetated	32.4/32.8		(13.0 - 39.9)	
Body Mass (g)									
Managed	1.62/1.66	0.03	(0.31 - 2.79)	0.993	Managed	2.78/2.78	0.06	(0.77 - 4.24)	0.288
Vegetated	0.96/0.99		(0.08 - 1.79)		Vegetated	1.97/2.06		(0.20 - 3.31)	
Liver somatic index (2019 only)									
					Managed	2.32/2.21	0.07	(0.76 - 3.96)	0.624
					Vegetated	2.22/2.10		(0.79 - 10.09)	
Proportion Male (2019 only)									
					Managed	63.4/ 63 %	21 %	(53 – 80 %)	**1.71E-05**
					Vegetated	97 / 96.5 %		(93 – 100 %)	

Standard error of the mean (SEM) is included. Tadpole samples sizes: 2018: Managed (n = 129), Vegetated (n = 121); 2019: Managed (n = 84), Vegetated (n = 77). Boldfaced values indicate significant differences by treatment within a year. Managed treatments were treeless in 2018 and dredged in 2019.

Table 14: General(ized)* Linear (and Mixed-effect) Model summary results for *L. pipiens* tadpole health outcomes.

Factor	β [d]	SE	df	t or z[a]	p-value	Significance [b]	Variance	SD
2019 Stage ~ Treatment + Survival + ~1|Cage								
Fixed effects (Intercept)	3.532	0.052	149	68.349	2.1E-114	***		
Treatment	-0.099	0.009	9	-11.419	1.1E-06	***		
Survival	0.009	0.004	9	2.565	0.031	*		
Random effects								
Cage (Intercept)								0.005 [c]
Residual								0.267 [c]
2018 Stage ~ Treatment + Survival + ~1|Cage								
Fixed effects (Intercept)	3.576	0.091	232	39.112	4.1E-104	***		
Treatment	0.129	0.013	15	9.600	8.5E-08	***		
Survival	-0.010	0.007	15	-1.420	0.176			
Random effects								
Cage (Intercept)								0.023 [c]
Residual								0.245 [c]
2019 SVL ~ Treatment + Survival + Stage + ~1 |Cage								
Fixed effects (Intercept)	11.521	2.694	36	-4.276	1.3E-04	***		
Treatment	0.961	0.371	18	2.588	0.018	*		
Survival	-0.542	0.132	10	-4.121	0.002	**		
Stage	0.979	0.056	142	17.420	<2e-16	***		
Random effects								
Cage (Intercept)							0.109	0.331
Residual							1.145	1.070
2018 SVL ~ Treatment + Survival + Stage + ~1 |Cage								
Fixed effects (Intercept)	12.060	1.816	206	-6.641	2.7E-10	***		
Treatment	-0.825	0.362	31	-2.277	0.030	*		
Stage	0.801	0.057	210	13.970	<2e-16	***		
Random effects								
Cage (Intercept)							0.237	0.487

Residual							1.53	1.237
2019 Survival ~ Treatment								
Fixed effects (Intercept)		2.639	0	6.245	4.2E-10	***		
Treatment		-0.860	1	-1.660	0.097	.		
2018 Survival ~ Treatment								
Fixed effects (Intercept)		2.157	0	7.640	2.2E-14	***		
Treatment		0.911	1	1.808	0.071	.		
2019 Sex Ratio ~ Treatment								
Fixed effects (Intercept)		-0.455	0	-2.043	0.041	*		
Treatment		-2.709	1	-4.300	1.7E-05	***		
2019 Width ~ Treatment + Survival + Stage + ~1|Cage								
Fixed effects (Intercept)	-5.712	1.774	34	-3.221	0.003	**		
Treatment	0.246	0.248	18	0.994	0.334			
Survival	-0.309	0.089	10	-3.468	0.006	**		
Stage	0.542	0.036	142	15.058	<2e-16	***		
Random effects								
Cage (Intercept)							0.056	0.236
Residual							0.467	0.683
2018 Width ~ Treatment + Stage + ~1|Cage								
Fixed effects (Intercept)	-6.220	1.067	209	-5.831	2.1E-08	***		
Treatment	-0.077	0.216	32	-0.357	0.724			
Stage	0.435	0.034	212	12.915	< 2e-16	***		
Random effects								
Cage (Intercept)							0.088	0.297
Residual							0.524	0.724
2019 Tail ~ Treatment + Survival + Stage + ~1|Cage								
Fixed effects (Intercept)	31.264	5.195	56	-6.019	1.4E-07	***		
Treatment	2.527	0.668	27	3.780	0.001	***		
Survival	-1.095	0.216	12	-5.079	2.9E-04	***		
Stage	2.155	0.121	141	17.743	<2e-16	***		
Random effects								

Cage (Intercept)							0.061	0.247
Residual							5.586	2.364
2018 Tail ~ Treatment + Stage + ~1|Cage								
Fixed effects (Intercept)	31.597	3.821	216	-8.269	1.4E-14	***		
Treatment	-1.114	0.839	29	-1.327	0.195			
Stage	1.760	0.120	218	14.623	< 2e-16	***		
Random effects								
Cage (Intercept)							1.626	1.275
Residual							6.509	2.551
2019 Body Mass ~ Treatment + Survival + Stage + ~1|Cage								
Fixed effects (Intercept)	-5.373	0.718	54	-7.484	7.1E-10	***		
Treatment	0.103	0.095	27	1.085	0.288			
Survival	-0.205	0.031	11	-6.709	4.1E-05	***		
Stage	0.283	0.017	156	16.625	<2e-16	***		
Random effects								
Cage (Intercept)							0.002	0.047
Residual							0.121	0.348
2018 Body Mass ~ Treatment + Survival + Stage + ~1|Cage								
Fixed effects (Intercept)	-4.209	3.7E-01	245	1.2E+01	<2e-16	***		
Treatment	0.001	9.2E-02	26	0.009	0.993			
Stage	0.165	1.1E-02	247	14.348	<2e-16	***		
Random effects								
Cage (Intercept)							0.024	0.154
Residual							0.064	0.252
2019 Liver Mass ~ Treatment + Body Mass + 1|Cage								
Fixed effects (Intercept)	-0.026	0.005	71	-4.798	8.5E-06	***		
Treatment	0.004	0.004	11	0.993	0.341			
Body Mass	0.033	0.001	152	20.087	< 2e-16	***		
Random effects								
Cage (Intercept)							4.6E-05	0.007
Residual							1.3E-04	0.011

2019 Liver Somatic Index ~ Treatment + ~1|Cage								
Fixed effects (Intercept)	2.311	0.142	9.275	16.240	4.0E-08	***		
Treatment	-0.083	0.203	9.613	-0.410	0.691			
Random effects								
Cage (Intercept)							0.073	0.270
Residual							0.675	0.822

[a] Wald-z for binomial models and t-statistic for all other life-history models.

[b] Significance codes *** is <0.0001, ** is <0.001, * is <0.01, and . is >0.05<0.1.

[c] Random effects standard deviation value, using the glmmPQL package in R.

[d] β is the difference and direction (positive, negative) in the Managed (treeless in 2018 and dredged in 2019) treatment mean compared to the mean of the Vegetated treatment.

SE = standard error; SD = standard deviation; df = degrees of freedom.

* Stage models used Poisson distribution in the GLMMs; SVL, Width, Tail, Body Mass, Liver Mass, Liver Somatic Index models used Gaussian distribution in the GLMMs; survival and sex ratio used binomial distribution in the GLMs.

Table 15: Multimodel comparisons of General(ized) Linear (and Mixed-effect) Models for tadpole health outcomes and associated AIC values used for model selection.

Model [a]	AIC [b]	ΔAIC [c]
2019 Stage		
Model selection not possible with glmmPQL package, presented full model		
2018 Stage		
Model selection not possible with glmmPQL package, presented full model		
2019 Snout-to-vent length		
1. Tmt + Survival + Stage + 1|Cage	**463.5**	-
2. Tmt + 1|Cage	619.5	155.9
2018 Snout-to-vent length		
1. Tmt + Survival + Stage + 1|Cage	760.9	3.7
2. Tmt + Stage + 1|Cage	**757.2**	-
3. Tmt + 1|Cage	887.9	130.8
2019 Survival		
Model selection not possible with binomial glm, presented full model		
2018 Survival		
Model selection not possible with binomial glm, presented full model		
2019 Sex Ratio		
Model selection not possible with binomial glm, presented full model		
2019 Body Width		
1. Tmt + Survival + Stage + 1|Cage	**336.3**	-
2. Tmt + 1|Cage	461.8	125.6
2018 Body Width		
1. Tmt + Survival + Stage + 1|Cage	527.7	4.3
2. Tmt + Stage + 1|Cage	**523.4**	-
3. Tmt + 1|Cage	637.2	113.8
2019 Tail length		
1. Tmt + Survival + Stage + 1|Cage	**684.4**	-
2. Tmt + 1|Cage	846.1	161.7
2018 Tail length		
1. Tmt + Survival + Stage + 1|Cage	1081.6	1.9
2. Tmt + Stage + 1|Cage	**1079.7**	-
3. Tmt + 1|Cage	1218.8	139.1
2019 Body Mass		
1. Tmt + Survival + Stage + 1|Cage	**148.0**	-
2. Tmt + 1|Cage	298.3	150.3
2018 Body Mass		
1. Tmt + Survival + Stage + 1|Cage	81.5	6.1
2. Tmt + Stage + 1|Cage	**75.4**	-
3. Tmt + 1|Cage	212.6	137.2
2019 Liver Mass		

1. Tmt + Body Mass + Survival + Stage + 1|Cage	-895.1	25.4
2. Tmt + Body Mass + Survival + 1|Cage	-909.1	11.4
3. Tmt + Body Mass + Stage + 1|Cage	-906.6	13.9
4. Tmt + Body Mass + 1|Cage	**-920.5**	-
5. Tmt + 1|Cage	-738.3	182.3
2019 Liver Somatic Index		
1. Tmt + Survival + Stage + 1|Cage	423.3	8.8
2. Tmt + Survival + 1|Cage	419.3	4.7
3. Tmt + Stage + 1|Cage	418.7	4.2
4. Tmt + 1|Cage	**414.5**	-

[a] Predictor variables are ditch treatment (Tmt: managed and vegetated), Survival and Gosner stage (Stage); Body Mass was a predictor variable for liver mass model only. Cage was included as a random effect to account for the non-independence of individuals raised in the same in situ cage.

[b] The best-fit models have the lowest AIC value and are shown in bold.

[c] ΔAIC values are the difference in AIC compared to the best-fit model.

Table 16: Pesticides concentrations* (ng/g wet weight) in tadpole tissues that were raised in agriculture ditches under different ditch managements.

	Imidacloprid	**Tebuconazole**
LOQ [a]	8.03	10.52
Vegetated Mean	29.95	<LOD
Vegetated Max	115.40	<LOD
Vegetated SD	42.21	<LOD
Detection frequency (n=15)	40 %	NA
Managed Mean	48.78	13.16
Managed Max	123.80	86.31
Managed SD	49.48	20.81
Detection frequency (n=15)	33 %	100 %
Year(s) detected [b]	2019	2018

*Additional pesticides thiabendazole, azoxystrobin, pyraclostrobin, and diazinon were detected in trace amounts (<LOQ); metolochlor, triazophos and DEET were detected but not quantified.

[a] Limit of quantification (LOQ) reported here is the highest limit of quantification measured for each pesticide (for all see Table 17).

[b] Ditch treatments were vegetated (2018, 2019), and managed (treeless (2018) and dredged (2019)).

Table 17: Average recovery (Re) and signal suppression enhancement (SSE) for pesticide detection in tadpole tissues using LCMS-MS.

	Thiabendazole	**Imidacloprid**	**Azoxystrobin**	**Tebuconazole**	**Pyraclostrobin**	**Diazinon**	**Metolochlor**	**Triazophos**	**DEET**
2018 (n=4)									
Re	0.70	0.80	0.76	0.77	0.76	0.77	NA	NA	NA
SSE	0.70	0.73	0.43	0.65	0.38	0.40	NA	NA	NA
Re * SSE	0.49	0.59	0.33	0.51	0.29	0.30	NA	NA	NA
LOQ [a]	0.52	8.03	2.10	10.52	0.38	0.70	NA	NA	NA
2019 (n = 6)									
Re	0.38	0.68	0.58	0.62	0.54	0.63	NA	NA	NA
SSE	1.11	0.79	0.58	0.75	0.55	0.36	NA	NA	NA
Re * SSE	0.42	0.54	0.34	0.46	0.30	0.23	NA	NA	NA
LOQ	0.30	4.64	1.21	6.08	0.22	0.40	NA	NA	NA

[a] Limit of detection (LOD) concentrations are included in ng/g.

4. Discussion

4.1. Frog health in relation to ditch management

The goal of this thesis was to determine whether ditch management (vegetation removal and dredging compared to maturely vegetated) impacted *Lithobates pipiens* health outcomes. The hypothesis was that ditch management would impact frog health outcomes due to changes in water quality. The prediction that the managed watershed (treeless and dredged) would have poorer frog health outcomes compared to the vegetated watershed was not supported.

4.1.1 Tadpole survival

Tadpole survival was high (86 – 96 %) and not significantly different by ditch treatment in either year of study (Table 13, Table 14). Similar survival rates were observed by Harris et al. (2001) using *in situ* cages, with 96% survival of *L. pipiens* tadpoles in a reference wetland, and 79-93% survival in wetlands of concern with historical industrial contamination from polycyclic aromatic hydrocarbons and polychlorinated biphenyls near Akwesasne Mohawk Territory along the St. Lawrence River. A later study in the same region using *L. pipiens* in *in situ* cages in creeks found tadpole survival to range from 86-91% (McDaniel et al. 2004). Cooke et al. (1981) observed 100 % survival of caged *Rana temporaria* tadpoles in a control wetland and 40-90% survival in agriculture ditches in Eastern England. The survival rates observed in the present study are therefore similar tof those reported in the literature for *in situ* tadpole experiments.

4.1.2 Embryo hatching, survival and development

The embryo hatching, survival, and developmental success tells a more nuanced story. Embryo health outcomes were not better at the vegetated treatment compared to the

managed treatment (Table 8). This does not support the initial prediction that managed treatments would negatively impact embryo health outcomes. Average embryo hatching success ranged from 54 – 80 % and was not significantly different by ditch treatment (Table 8, Table 8). In a study comparing *L. pipiens* hatching success in orchard-associated wetlands with reference wetlands, Harris et al. (1998) reported average hatching success between 70 - 100 %, which is higher than the current study. Harris et al. (1998) and the current study both found that hatching success was approximately 10 % higher than embryo survival. The current study found that embryo survival ranged from 46 – 77 % (Table 8) and Harris et al. (1998) reported embryo survival range of 50 – 85 %. Lower embryo survival than hatching success may be related to the fact that hatching success does not guarantee the health of the hatchling, merely that the individual emerged from its casing. In the current study, premature hatching (emergence prior to GS 20) was observed at both vegetated treatment sites (V3, V4), and to a lesser extent at one managed site (M3). Consequently, embryo hatchling survival at the vegetated treatment was significantly lower than the to managed, with a vegetated treatment mean survival of 51 ± 14.0 % compared to 65 ± 14.5 % mean survival at the managed treatment (Table 7, Table 8). The differences in survival by treatment also affected development (GS) for the embryo study as many of the premature hatchlings died. The mortality of the premature hatchlings (which had lower GS) resulted in significantly higher GS at the managed treatment compared to the vegetated treatment (Table 7). Although no studies on premature hatching in *L. pipiens* could be found, previous studies on other amphibians have found premature embryo hatching occurs in response to hypoxia (Valls and Mills 2007; Warkentin 2011), desiccation (Warkentin 2011), water pollution (Pohl et al. 2015), pathogen

presence (e.g. water mold; Smith 2006; Warkentin 2011), and predation (Gomez-Mestre et al. 2008). The potential contributions of these various factors are discussed below in Section 4.2 Explanations for differences in amphibian health responses. The overall time to hatching was no different from other studies that used wild *L. pipiens* and *in situ* cages (Eddy, 1976; Harris et al., 1998). Embryo hatching in the current study took place over two weeks, which is a similar time range to the 10-11 days reported for wild *L. pipiens* hatching (Eddy 1976) and two to three weeks using *in situ* cages (Harris et al. 1998).

4.1.3 Tadpole development and growth

The tadpole endpoints that were most sensitive to differences in ditch management were metamorphic development (GS) and body size (SVL). Both GS and SVL were significantly different between treatments in all years of the study (Table 14). The median GS was four to five stages more advanced at the managed treatment compared to the mature treatment. Differences in developmental stage and rate have the potential to be either beneficial or harmful, sometimes representing a biological trade-off (Coyle and Karasov 2010; Pujol-Buxó et al. 2013; Ruthsatz et al. 2018). Many environmental factors can influence the timing of amphibian metamorphosis including temperature, water quality, habitat desiccation, density, predation cues, food availability, and photoperiod (Ruthsatz et al. 2018). Thus amphibians exhibit dynamic responses in growth and developmental rates to environmental conditions, which can influence survival and population dynamics (Walters and Hassall 2006; Rudolf and Rödel 2007; Ruthsatz et al. 2018). A shorter larval period and larger size at metamorphosis are traits that are broadly recognized to confer greater fitness in amphibians (Wilbur and Collins 1973; Beck and Congdon 2000; Egea-Serrano et al. 2012; Ruthsatz et al. 2018).

Because caged specimens were studied (rather than free-ranging specimens), and the experiments were ended before ditch desiccation, evidence informed inference is helpful to interpret the biological significance of the higher GS and larger size observed at the managed treatment compared to the vegetated treatment. In ephemeral waters, individuals experience a strong reduction in fitness if they have not metamorphosed into the terrestrial stage before the habitat dries (Rudolf and Rödel 2007). In the current study, the more advanced GS reached at the managed treatment were likely advantageous. The ditches dry up completely by early to mid-July, which would result in mortality of wild tadpoles that had not completed metamorphosis (GS 46). In support of this interpretation, wild GS 40 - 43 tadpoles (with tails not yet reabsorbed: Appendix 3, Figure 12) were observed struggling to move in water 1 - 5 cm deep, in the field in July. Desiccation may be a significant threat in ditch systems. Individuals with more rapid development (at the managed treatment) have an advantage compared to the slower development observed at the vegetated treatment. Further studies are needed to determine how the hydroperiod, along with other environmental factors (such as differences in temperature, which is related to ditch management) affect the success of wild *L. pipiens* reaching metamorphosis before desiccation.

The larger size (SVL) at the managed treatment may also indicate better health outcomes compared to the vegetated treatment. Sometimes a reduced development time can come at the cost of reaching a smaller size at metamorphosis, exhibiting a trade-off between body size and surviving the larval period (Rudolf and Rödel 2007). A trade-off in size was not observed in the current study as SVL was significantly higher at the managed site (and the statistical model accounted for differences in GS). The SVL was higher not due to a faster development time

alone, but because of better growth at the managed treatment. Altwegg & Reyer (2003) found individuals (*Rana lessonae* and *R. esculenta*) that metamorphosed at a larger size, had increased survival during terrestrial stages, and were larger at maturity. There is debate regarding the impact of size at metamorphosis on reproductive success. Some studies have attributed greater reproductive success to larger size at metamorphosis (Wilbur 1977; Smith 1987; Rudolf and Rödel 2007), while others report a lack of relationship between larval characteristics and survival to first reproduction (Beck and Congdon 2000; Green and Bailey 2015). The larger SVL observed at the managed treatment could have a negligible or beneficial population level affect compared to the mature treatment; future studies would be required to follow the life history and reproductive success of ditch-raised individuals to determine if the size effect was biologically significant. The differences in development (GS) and size (SVL) support the hypothesis that ditch management affects frog health outcomes, however in the opposite direction as predicted since better health outcomes were observed at the managed treatment than the vegetated treatment.

4.1.4. Tadpole sex ratios

The sex ratio difference by ditch treatment appeared strong (Table 13, Table 14); however, this is likely due to developmental differences that made the sex ratio simply appear male biased. In 2019, when gonad identification was undertaken, the sex ratios were identified as 97% male, 59% male and 58% male for the vegetated treatment, managed treatment and lab controls respectively. Only individuals that reached GS 36 and above by the end of the experiment were included because only at this stage do gonads appear morphologically distinct in *L. pipiens* (Hogan et al. 2008). Unfortunately, this meant the exclusion of 53 individuals (3

Managed, 46 Vegetated, and 4 controls) who were GS 35 or below (49 of these individuals were GS 34 or GS 35). Consequently, the delayed development at the vegetated treatment caused a much smaller sample size at the vegetated treatment (n = 31) compared to the managed treatment (n = 81). Almost all the individuals at the vegetated treatment were between GS 34 – 35 (91%), based on the degree of toe indentation and were thus on the cusp of sexual development (Hogan et al. 2008). Therefore, these observed sex ratios should be interpreted with caution and are more likely the result of the developmental delay and small sample size. Ideally, the tadpoles could have been left *in situ*, or brought back to the lab to complete development to metamorphosis to alleviate this ambiguity. However, this was not logistically possible as the ditches were rapidly drying up and the experimental design was to end the exposure when water depth dropped to 10 cm (desiccation risk). Future studies could employ gonadal histology for definitive sex identification and detection of gonad abnormalities such as oocytes in testes (Hogan et al., 2008).

The sex ratios of both the managed treatment and lab controls were skewed slightly towards male, since a 50 M : 50 F ratio is considered typical (Hayes, 1998). However a 60 : 40 sex ratio (M:F or F:M) is within an acceptable range for amphibians as it has often been reported in controls (Schwaiger et al. 2003; Orton et al. 2006). Some of the lab control replicates from other *L. pipiens* experiments conducted at the NWRC from the same embryo clutches as the *in situ* study showed a 60 F: 40 M sex ratio. The average sex ratio observed in six NWRC lab controls in 2019 was 47 M: 53 F with a standard deviation of 11 and a range of 25 – 57 M: 43 – 75 F (Robinson et al., preliminary, unpublished). Because the perceived male bias in the sex ratio is attributed to differences in development, which is associated with differences in

treatment, the sex ratio may indirectly support the hypothesis. However, the impact is in the opposite direct than predicted, since the sex ratio was heavily male biased at the vegetated treatment and similar to reported species relevant sex ratios at the managed treatment. The other tadpole endpoints of body width, tail length, body mass, and Liver Somatic Index had too much variability and/or were not as sensitive to environmental changes as GS and SVL so no differences in tadpole health were detected by ditch treatment; none of these health endpoints supported the current study's hypothesis.

4.1.5 Bioconcentration of pesticides

Of the nine pesticide residues detected in tadpole tissue samples, only two had concentrations above the limits of quantification: the neonicotinoid imidacloprid (max 123.8 ng/g wet weight) and the fungicide tebuconazole (max 86.31 ng/g wet weight). There are only a handful of studies that have measured pesticide concentrations in tadpoles raised in the environment (Reeves, 2014; Smalling et al., 2012, 2013; 2015). Reeves (2014) detected a higher number of pesticides bioaccumulated in tadpole tissues; 18 pesticides and pesticide degradation products (of 98 that were screened) were found in tissues of boreal chorus frog (*Pseudacris maculata*) raised in agricultural-associated wetlands. They did not detect imidacloprid in any tissue samples, likely because water concentrations were low (max 2.8 ng/L; Reeves, 2014). The current study detected much higher concentrations of imidacloprid and total neonicotinoids in surface waters, with the maximum imidacloprid concentration at 800 ng/L, and the maximum total neonicotinoid concentration 0.037 µg/L, which were still below 0.23 µg/L, the imidacloprid guideline for the protection of aquatic life (Table 4 and Table 122). The higher surface water concentrations in the current study may explain why bioconcentration

of imidacloprid was found. There is only one available study to date that has reported imidacloprid uptake in amphibians: Van Meter et al. (2014) exposed seven species of terrestrial stage anurans to contaminated soil, and measured an imidacloprid body burden of 0.019 µg/g. The first study of imidacloprid uptake in aquatic vertebrates (fish) found that although imidacloprid is highly soluble in water it had lower bioconcentration potential, and there was uptake across all tissues monitored (except blood), with internal concentrations on par with those of the exposure medium (Iturburu et al. 2017). Relatively large fluxes of toxic compounds in aquatic systems may cause acute or chronic toxicity even without bioconcentration or bioaccumulation (e.g. Iturburu et al. 2017). Bioaccumulation of imidacloprid in the freshwater crustacean *Gammarus pulex* was reported by Shahid et al. (2018) and furthermore they identified imidacloprid as the most toxic compound in a mixture of 50 pesticides that were detected in agriculture stream waters using a toxic units approach. The current study, to my knowledge, is the first to report bioconcentration of imidacloprid in tadpole tissues of amphibians raised in agriculture waterways.

Various studies have investigated the effects of neonicotinoids on tadpole health under laboratory conditions, and have found that they are not generally toxic to amphibians at environmentally relevant concentrations (Smalling et al. 2015; Morrissey et al. 2015a; Anderson et al. 2015). For instance the 48h LC_{50}'s for imidacloprid ranged from 165 - 219 mg/L for two different *Rana* species (Feng et al. 2004). Robinson et al. (2017) found that *L. sylvaticus* chronically exposed to environmentally relevant concentrations (10 – 100 µg/L) of imidacloprid throughout larval development had increased survival and a minor delay in completing metamorphosis. This suggests neonicotinoids are a minor concern for larval amphibian

mortality. Sublethal effects on behaviour are a concern, as a later study found that juvenile *L. sylvaticus* exposed to 10 – 100 µg/L were less like to respond to a simulated predator attack, potentially increasing their vulnerability to predation (Lee-Jenkins and Robinson 2018). The maximum total neonicotinoid concentration detected in the present study (0.037 µg/L) was 2-3 orders of magnitude below the concentration at which behavior effects are observed (10 – 100 µg/L; Robinson et al., 2017). Hence, the body burden of imidacloprid does not likely cause toxic effects to individuals, and the total neonicotinoid concentration is far below those shown to increase risk of predation. Therefore, the levels of neonicotinoids assessed in the current study are unlikely to produce negative effects on tadpole health outcomes.

In addition to imidacloprid, the other pesticide that had detectable concentrations in the tadpole tissues was the fungicide tebuconazole. The maximum concentration of tebuconazole bioconcentrated in tadpole tissues in the current study was 86.31 ng/g wet weight. The bioconcentration of the tebuconazole in tadpoles (*Pseudacris regilla*) raised in agriculture wetlands was reported by Smalling et al. (2013). They reported a median tebuconazole tissue concentration of 74 ng/g wet weight (Smalling et al., 2013), quite similar to the current study. Smalling et al. (2013) reported that the biological effects of tebuconazole bioaccumulation were unknown. Hansen et al. (2014) found that *Xenopus laevis* exposed to 10 µg/L of tebuconazole for 4 weeks bioaccumulated 182 ng/mg in adipose tissue and 36 ng/mg in liver tissue, but still did not know the biological consequences. In a laboratory toxicity study, Bernabò et al. (2016) reported only 36 % survival of larval Italian tree frogs (*Hyla intermedia*) exposed to 5 µg/L tebuconazole for 78 days. They also found strong correlations between fungicide exposure and incidence of morphological abnormalities (malformations and edema;

Bernabò et al., 2016). The concentrations detected in the current study are well below these harmful concentrations, and with the high tadpole survival rate coupled with the lack of observed malformations, the toxicity risk due to tebuconazole was likely quite low. However, fungicide exposure can increase susceptibility to disease, such as the amphibian chytrid fungus (*Batrachochytrium dendrobatidis)* (Battaglin et al. 2016), so fungicide exposure may still pose threats to the long term health of wild amphibian populations.

4.1.6 Pesticide toxicity index

Pesticide Toxicity Indexes (PTI) were calculated in this study to quantify and compare the mixture toxicity of pesticides detected in ditch surface waters (Battaglin and Fairchild 2002; Nowell et al. 2014). All except one PTI indicated limited toxicity (index values <0.5), the one exception occurred on a single date at the managed treatment (index 0.6). Given that tadpole survival was high across both treatments, it seems the PTIs did a reasonable job of reflecting the limited toxicity of these water samples. The limited toxicity of agricultural waters on *L. pipiens* is similar to the results of Battaglin & Fairchild (2002) who found that only 1% of their water samples from agriculture waterways had indexes above 0.5, also indicating limited acute toxicity to chorus frogs (*Pseudacris triseriata*). They reported limitations in the utility of the PTI approach for amphibians due to the lack of toxicity studies, compared to other aquatic species such as duckweed (*Lemna gibba)* and bluegill sunfish (*Lepomis macrochirusa*) that are more commonly used in toxicity assessments (Battaglin and Fairchild 2002). Since the current study detected only sublethal effects, had the toxicity data been available, it would have been more informative for the PTI to use a sublethal endpoint such as LOEC (lowest observed effective concentration), rather than LC_{50} (median lethal dose). Although the approach here was

somewhat limited, overall PTIs have value in integrating quantitative measurements of mixture toxicity which is too often overlooked (Battaglin and Fairchild 2002; Zwart 2005; von der Ohe and de Zwart 2013; Nowell et al. 2014; Battaglin et al. 2016). Future studies could apply safety factors, as done in risk assessments, when using surrogate species, in order to use sublethal toxicity endpoints (i.e. LOEC).

4.2 Explanations for differences in amphibian health responses

4.2.1 Differences in water quality between the paired watersheds

The prediction that water quality at the managed watershed was poorer compared to the vegetated watershed was not supported. It was predicted that turbidity, nutrient (TIN and TP) and pesticide concentrations would be higher at the managed treatment compared to the vegetated treatment due to reduced retention. The turbidity was four times higher after dredging (managed 2019) compared to the vegetated watershed (Table 10), but there was no difference between the treeless (managed 2018) and vegetated watersheds. The increase in turbidity after dredging was predicted (Dollinger et al. 2015). The 2018 mean turbidity values for both watersheds were 8.5 ± 0.15 NTU, and ranged from 0.2 - 136.1 NTU, which is similar to mean turbidity 9.1 NTU and range 0 - 185 NTU reported in agriculture-influenced ponds in southwestern Ontario where amphibians breed (Hecnar and M'Closkey 1996). With the exception of the dredged treatment (managed 2019), the mean turbidity observed in both watersheds was fairly low (4.55 – 8.62 NTU) and similar to the mean (14.1 NTU) and median (5.42 NTU) values reported across 29 sites in the South Nation watershed in 2003 (Allaway 2006). This places only the mean turbidity value from the dredged watershed (20.63 NTU) above the range normally encountered in the region. However, regardless of ditch

management, maximum turbidity increases of 70-220 NTU were observed in both study years, which is well above the guideline for the protection of aquatic life of 8 NTU above baseline values (CCME 2002). This suggests that despite normally low mean turbidity, turbidity spikes caused by storm events have the potential to harm aquatic life in both watersheds. It is interesting to note the lack of difference in turbidity between treeless and vegetated treatments, as this does not support the prediction that ditch management would cause differences in water quality. However, large differences in turbidity were observed between the dredged and vegetated treatments, with higher turbidity at the managed treatment, as predicted. The ditch managements (tree removal and dredging) caused different effects on water quality, with only dredging causing a deterioration compared to the vegetated reference treatment; therefore, the prediction that ditch management would cause poorer water quality was only partially supported.

The nutrient levels measured in the current study are similar to those reported across the South Nation River and tributaries of the watershed (Allaway 2006). The means (0.46 – 2.85 mg/L) and ranges (0.046 – 9.45 mg/L) for TIN in both ditch treatments were similar to those reported by Allaway (2006): mean 1.67 mg/L, and range of 0.46 – 8.67 mg/L. Collins et al., (2019) also reported very similar nitrogen concentrations, with mean TIN of 2.68 mg/L and a range from detection limit to 9 mg/L, in 25 agriculture ditches across Eastern Ontario. The means and ranges for TP in the current study (mean 0.033 – 0.153 mg/L, range 0.004 – 0.308 mg/L) were also similar to those reported in the region (mean 0.069 mg/L, range 0.005-0.233 mg/L; Allaway, 2006). Nutrient levels were similar (TIN in 2019) or higher (TIN in 2018 and TP in both years) at the vegetated watershed compared to the managed watershed, which is

contrary to the prediction that water quality would be poorer (with higher nutrient levels) at the managed watershed compared to the vegetated watershed. An increase in TIN was observed at the managed treatment following dredging compared to the previous year (Table 11), which may be due to decreases in nitrogen retention following dredging, as observed by Shigaki et al. (2009) and Smith & Pappas (2006). The decrease in nitrogen retention was not substantial enough to cause higher nitrogen concentrations to be observed in the managed treatment compared to the vegetated treatment. Phosphorus (TP and RP) retention was observed following dredging at the managed treatment with the mean TP concentration dropping in half in 2019, while phosphorus concentrations remained consistent at the vegetated treatment in both years. This indicates that dredging may have improved P retention, which was also reported by (Smith and Huang 2010) where TP loads decreased by 5.4 kg over 12 months after dredging. The apparently improved TP retention, and reduced TIN retention following dredging are interesting results as they add to the few studies that explore the effects of ditch management on nutrient dynamics. Further studies are needed to confirm that these results are not associated with confounding factors such as differences in the watershed catchment areas and nutrient loading; such work is underway by AAFC researchers and collaborators. The current study found nutrient levels were typical to the region, and similar or higher at the vegetated watershed compared to the managed watershed, which does not support the prediction that ditch management would lead to poorer water quality in terms of nutrient concentrations.

The surface water pesticide concentrations were not consistently higher (as was predicted) at the managed watershed compared to the vegetated watershed, and differences in

concentrations were dependent on the type of pesticide. During the tadpole experiment in 2019, atrazine concentrations were similar across treatments (means 0.018 and 0.020 µg/L), total neonicotinoids were significantly higher at the vegetated watershed (means 0.012 and 0.022 µg/L), and glyphosate was significantly higher at the managed watershed (means 0.163 µg/L and <MDL; Table 12). These results indicate that pesticide concentrations do not depend on ditch management alone. Furthermore, none of the pesticide concentrations exceeded the guidelines for the protection of aquatic life (Table 4). This leads me to reject the prediction that water quality is poorer at the managed watershed compared to the vegetated watershed.

The mean concentrations of all three of these pesticide classes were on the lower end of ranges reported in the literature for ditches in Eastern Ontario. Collins et al. (2019) report mean atrazine of 0.17 µg/L (range 0.005 – 2.76 µg/L), mean total neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) of 0.04 µg/L (range <LOD - 0.61 µg/L), and mean glyphosate of 0.38 µg/L (range <LOD – 6.18 µg/L; (Collins et al. 2019). The 56d time-weighted-average concentrations of atrazine across 24 stream/river sites in the South Nation watershed using polar organic chemical integrative samples (POCIS) ranged from 4 - 412 ng/L (Dalton 2014). Our sampling may have missed peak pesticide concentrations, due to using grab samples rather than a temporally integrated sampling method like POCIS (Dalton, 2014; Morrissey et al., 2015). Grab samples can underestimate average concentrations of pesticides by 50 %, and maximum concentrations by 1-3 orders of magnitude (Xing et al., 2013). This suggests that our reported measurements likely underestimate surface water concentrations; however even if we doubled our average concentrations, the mean concentrations would be within normal ranges for the region.

Overall, the water quality was not degraded at the managed treatment compared to the vegetated treatment as was initially anticipated. There was indeed higher turbidity after dredging, but not after vegetation removal; both treatments exhibited turbidity peaks that exceeded the guidelines for the protection of aquatic life. There were higher nutrient concentrations at the vegetated treatment than the managed treatment; but all values were typical for the region. There were pesticide-specific differences by treatment, with higher glyphosate concentrations at the managed treatment, higher total neonicotinoids at the vegetated treatment, and no differences in atrazine concentrations by treatment. All the pesticide concentrations were on the lower end of typical for the region and were below the guidelines for the protection of aquatic life. All these elements together did not support the prediction that water quality was poorer at the managed treatment, rather that water quality across the paired watersheds were typical of the surrounding area.

4.2.2 Water quality explanations

Of all the frog health endpoints monitored, ditch management had the strongest effect on metamorphic development (measured by GS). Median hatchling and tadpole GS were significantly higher at the managed treatment compared to the vegetated treatment. There are many factors that can affect amphibian development as mentioned previously (temperature, water quality, habitat desiccation, density, predation cues, food availability, and photoperiod (Ruthsatz et al. 2018)). However, the reasoning for this differential development may be different for tadpoles and embryos as explained in more detail below. Briefly, the most compelling argument for the tadpoles faster development at the managed treatment compared to vegetated treatment is the higher temperature under the latter treatment (with perhaps

confounding stressors of higher neonicotinoids and higher nutrients). However, the higher embryo development at managed sites is more likely due to differences in survival: hatchlings that reached higher GS had better survival, while premature hatchlings died (and were not counted in the final GS). Embryo hatching and survival was more variable by site than treatment, and likely linked to several physiochemical water characteristics.

4.2.2.1 Temperature

The significantly higher development and size (snout-to-vent length) goes in hand with significantly higher mean seasonal temperatures (by 0.6 and 2.36 °C) at the managed treatment compared to mature treatment, with all other water quality parameters being fairly comparable. Canopy cover causes shading, which can lower water temperatures (Barton et al. 1985). Canopy cover was one of the main differences between the vegetated and managed treatment, since all woody vegetation capable of shading was removed from the managed treatment before the experiments. Canopy cover has been shown to cause slower growth rates in anuran species, which was primarily attributed to reduced temperature (Skelly and Freidenburg 2002). Skelly et al (2002) observed an average of 5 °C warmer water temperatures at open canopy (<25 % cover) vs. closed canopy (>75 % cover) ponds. These temperatures and canopy cover differences are more extreme than in the current study, which found a 0.6 - 2.4 °C average warmer water temperature at the managed watershed (0 % cover) compared to the vegetated watershed (13 – 15 % cover). Skelly et al. (2002) found that tadpoles in the open canopy ponds (5 °C warmer) grew 80 – 93 % faster (measured by mg/day) than tadpoles in the closed canopy ponds. In laboratory conditions, a 5 °C water temperature difference led to doubling of larval growth rate of *L. pipiens* (Smith-Gill and Berven 1979; Skelly and Freidenburg

2002). No other studies could be found that directly compare GS with temperature differences of 1 – 3 °C, but the faster development and growth rates at higher temperatures observed in previous studies is consistent with the current study. Based on Riha & Berven (1991), a 10 day difference in larval periods for *L. sylvaticus* can be estimated with a 2 °C difference in temperature (20 - 22 °C), such as seen in the present study. The slight differences in water temperature were biologically significant; the warmer temperatures at the managed treatment were likely the main factor driving more rapid tadpole development compared to the vegetated treatment.

4.2.2.2 Canopy cover

Vegetative cover and land use in the surrounding area may also have played a role in the differences observed in frog health outcomes. Different anuran species favour full canopy cover while others prefer open; for instance wood frogs (*L. sylvaticus*) developed faster under canopy, and spring peepers (*Pseudacris crucifer*) developed faster in the open, while controlling for temperature differences (Skelly and Freidenburg 2002). The faster development at the managed treatment could be a species-specific response, if *L. pipiens* are more similar to spring peepers than wood frogs in their preference for low canopy cover. In the absence of studies that indicate differences in *L. pipiens* development by canopy cover, it seems reasonable that *L. pipiens* may be more similar to *L. sylvaticus* than *P. crucifer* as they are phylogenetically more closely related. Maisonneuve & Rioux (2001) found that *L. [Rana] pipiens* was associated more closely to wooded riparian strips and *L. [Rana] sylvaticus* was captured mostly in shrubby riparian strips. This indicates that the differences in tadpole development were not likely due to

species specific preferences for open vs closed canopy cover, but this is relevant to consider for species selection in future studies.

4.2.2.3 Specific conductivity

In the embryo study hatching rates were not significantly different by ditch treatment or site, suggesting that hatching success was robust to differences in conditions. However, the premature hatching, and significantly lower survival and development at the vegetated treatment showed that these endpoints were more sensitive to variations in sites and ditch treatments. It became apparent that poor survival rates occurred where premature hatching was most abundant, therefore identifying the factor(s) leading to premature hatching were of great interest.

Several factors have been associated with premature hatching in amphibians (hypoxia, desiccation, water pollution, pathogens, and predation). Of these, hypoxia, desiccation and predation can be eliminated as dissolved oxygen concentrations did not go below the OECD guidelines of 3 mg/L (even at night time), desiccation is only a risk for embryos in the terrestrial environment (Warkentin 2011), and the risk of predation was controlled by using *in situ* cages. Water chemistry was the main potential factor causing premature hatching that the present study was able to evaluate. However, pathogen exposure was not assessed in the present study; further studies would be needed to explore this potential stressor.

There was a gradient of lowest specific conductivity (SC; 503 µS/cm) where survival was highest (77.8 %) and no premature hatching occurred, to the highest SC (1026 µS/cm) where survival was lowest (45.8 %) and premature hatching most abundant (Table 3; Table 8). Effects

of high SC on premature hatching have been reported by Pohl et al. (2015) who found premature hatching in *Xenopus tropicalis* above 750 µS/cm. In a study of the impacts of anthropogenic road salt in vernal-pool breeding amphibians, Karraker et al. (2008) found *L. sylvaticus* embryo survival dropped from 91 % to 77 % above 500 µS/cm. Beyond simply affecting hatching and survival, high SC from road salt was also negatively correlated with amphibian species richness (Hecnar and M'Closkey 1996). However, Karraker & Ruthig (2009) found that increased SC due to road salt did not increase the susceptibility of three amphibian species embryos to infection by pathogenic water molds (aquatic oomycetes). Although SC alone may not be responsible for the differences in embryo premature hatching and survival, it is likely the main contributor and should be studied further. Since SC is not directly associated with tree removal or dredging (Dollinger et al. 2015), the differences observed in SC by treatment is likely due to surrounding land use and road salt rather than ditch management.

4.2.2.4 Agrochemicals

Agrochemicals (pesticides, fertilizers, and pharmaceuticals) can impact amphibian development and survival individually but also can have additive or synergistic toxic effects in mixtures that occur in the environment (Hayes et al. 2006; Mann et al. 2009). It is possible the slower development (lower GS) and smaller size (SVL) at the vegetated treatment could also be associated with a higher presence of pollutants there than at the managed treatment. For instance, in a meta-analysis of the impacts of chemicals on amphibians, Egea-Serrano et al. (2012) found a moderate negative effect of pollutant exposure on body size. Further they identified that size (quantified by mass, no body measurements were done) was lower when the pollutant contained nitrogenous compounds (Egea-Serrano et al. 2012). The smaller size

and slower development at the vegetated treatment could be simply due to cooler water temperatures. Alternatively, confounding factors of elevated nitrogen and neonicotinoid concentrations (and any other pollutants that were not measured or detected such as pharmaceuticals) could have caused the potentially poorer health outcomes at the vegetated treatment. Mixture toxicity has greater potential to cause inhibition of larval growth and development than individual pollutants, and have been shown to negate the typical positive correlation between time to metamorphosis and size, with exposed amphibian larvae being smaller at metamorphosis, which confers poorer fitness (Hayes et al. 2006). This draws attention to the health outcomes at the vegetated treatment where the larval period was significantly longer (measured by lower GS), tadpole sizes were significantly smaller (measured by SVL), and although not statistically significant, survival was lower in both years. It is conceivable that all of these poorer health outcomes at the vegetated treatment, are not only due to slightly lower temperatures, but confounded by higher mixture toxicity.

In addition to the temperature differences, pollutant exposure (especially as a mixture or interacting with other environmental factors) could be driving differences in development and even sexual differentiation at the vegetated and managed watersheds. For instance, NO_3^-, at environmentally relevant concentrations of 5 - 10 mg/L was found to interact with UV-B radiation and dramatically decrease larval mass and survival in *Ambystoma macrodactylum* and *Hyla regilla* (Hatch and Blaustein 2003). Perhaps more importantly, NO_3^- at 10 mg/L was found to cause 100 % mortality to *L. pipiens* tadpoles (Denton and Bernot 2011). However, Orton et al. (2006) found no effect of 10 mg/L NO_3^- on survival or growth in *L. pipiens*. While the mechanism of NO_3^- toxicity has not been clearly identified and different studies have found

inconsistent NO_3^- toxicity (Mann et al. 2009). Hecnar (1995) determined a 96-hr LC_{50} of 100 mg/L for *L. pipiens*. Although all the mean NO_3^- concentrations in the current study were between 0.41 – 2.61 mg/L, the max NO_3^- at the vegetated treatment reached 9.4 mg/L in 2019 which some may argue could have exerted toxic pressure, especially in combination with other factors (Hatch and Blaustein 2003; Mann et al. 2009; Denton and Bernot 2011).

Estrogenic exposure may also be particularly relevant in the current study because there is a dairy operation upstream along the vegetated watershed (but not on the managed watershed) which may leach various hormones. Estrogens, androgens and progestogens are commonly detected downstream of dairy operations, and were shown to exceed the 2 ng/L predicted-no-effect-concentration for fish in 39 % of measured run off events in other watersheds (Havens et al. 2020). The effects of exogenous steroids on amphibian development and sexual differentiation have long been studied (Padoa 1936). Hogan et al. (2008) found that estrogenic exposure from ethinylestradiol (EE2) on mid-metamorphic *L. pipiens* tadpoles (GS 30-36) caused immediate developmental delays resulting in a two-week delay to complete metamorphosis (GS 46) compared to controls. Further, they reported that tadpoles exposed to estrogens in early stages (GS 27-30) displayed a strong sex bias (albeit female dominated) at metamorphic climax 2-3 months after exposure. This is consistent with many studies that have found short exposures to pollutants during larval stages can have lasting effects on development and sexual differentiation (Tavera-Mendoza et al. 2002, and the references therein; Mann et al. 2009). There is potential that the higher nutrient concentrations at the vegetated treatment along with any other unmeasured pollutants contributed to the slower development and smaller size of tadpoles compared to the managed treatment.

In the current study, the perceived sex bias was male, which is fairly uncommon in the literature, as most pollutant associated sex biases have caused feminization (Hayes et al. 2002, 2003; Tavera-Mendoza et al. 2002; Hogan et al. 2008b). There is evidence that high temperatures can cause masculinization in amphibians, although typically when above environmentally relevant temperatures (Witschi 1929, 1930; Pickford et al. 2015; Lambert et al. 2018). For instance, Lambert et al. (2018) observed increasing male dominance in *Rana sylvaticus*, from a 50:50 sex ratio at 19 °C to 100:0 (M:F) at 34 °C (also coinciding with 80% mortality). In a meta-analysis, Hayes (1998) interprets the sex effects occurring at high temperatures to be artefacts of growth outside ideal temperature ranges, and that temperature is not likely an important factor for sex determination under more natural conditions. Although sex determination is based on the temperature of embryo incubation in some species of fish, turtles, lizards and crocodiles, Hayes (1998) suggested that sex determination in amphibians is controlled exclusively by genetics under environmental conditions. The masculinization (96% male) that was observed in the current study could not have been due to higher female die-off, since the survival was high (between 86-96%). Further studies are necessary to determine whether the perceived male bias in the present study is arising in wild populations in these agricultural systems.

4.2.2.5 Other possible factors

Beyond the most likely factors contributing to differences in frog health outcomes (temperature, SC and agrochemicals), there are several factors that were likely minor in the current study because they were controlled for but might be significant for wild populations. These factors are ditch desiccation, food availability, and vegetation community. Agriculture

ditches in the South Nation watershed are largely ephemeral, drying up in early- to mid-July in recent years. Wild larval stage anurans are at high risk of mortality if they do not reach terrestrial stages by this time. The experimental design of this study removed this risk, selecting sites with ample water depth, and terminating sites with insufficient depth to ensure ethical care of the frogs. There were no significant differences in water depth between treatments, which indicates desiccation pressure should not have been a driver of differential development in this study. However, in the wild a species' plasticity in response to desiccation will determine its ability to survive and reproduce in these agricultural ditches. Some species may be more adapted to accelerate development due to desiccation pressure, which would impact amphibian species composition and distribution in agriculture waterways (Mann et al. 2009). Although accelerated development in response to desiccation can be necessary for survival, it has been shown to come at a cost to immune responses (Gervasi 2007). This can make metamorphs more susceptible to parasites and pathogens such has *Batrachocytrium dendrobatidis* (chytrid fungus), which pesticide exposure can also worsen, collectively leading to higher amphibian declines (Kleinhenz et al. 2012; Hanlon and Parris 2012, 2014; Buck et al. 2015). Climate change, with its more extreme weather patterns, is also increasing the occurrence of droughts and heightens the risks of desiccation, which may affect wild anuran abundance and community composition (Piha et al. 2007; Mann et al. 2009). Although accelerated development in response to desiccation can be necessary for survival, it has been shown to come at a cost to immune responses, which may leave populations more susceptible to parasites and disease (Gervasi 2007). Climate change, with its more extreme weather

patterns and droughts heighten the risks of desiccation, which may affect wild anuran abundance and community composition (Piha et al. 2007).

Food availability was the other factor that was controlled in the study but is environmentally relevant for amphibian health. Tadpoles in the *in situ* cages were fed similarly to tadpoles in other laboratory and *in situ* studies (Crane et al. 2007). Although it may not have been necessary since periphyton (algae growing on substrates) built up inside the cages, and feeding removed a degree of naturalness, it ensured that any differences in tadpole mortality and development were not due to lack of food, since cages limit foraging opportunities. This helped ensure that the study was able to accomplish its objectives of determining the effects of ditch management on frog health. In the wild, tadpoles browse on a variety of food sources, including periphyton, detritus, and sediment (McDiarmid and Altig 1999). Periphyton and other algal food sources can be found in abundance in the South Nation watershed due to nutrient enriched waters (Contant and Pick 2013). Chlorophyll *a* (Chl *a*) is an indicator of primary productivity (and therefore tadpole food availability) that was monitored in 2019 so inferences could be made about food availability for wild frog populations. The mean Chl *a* concentrations were higher at the vegetated treatment compared to the managed treatment, with respective values of 7.07 µg/L and 4.95 µg/L, and a total range of 1.09 - 114.59 µg/L. These values are typical of the South Nation watershed (mean 9.2 µg/L, median 5.8 µg/L, and range of 2-149.3 µg/L; Allaway 2006). The slightly higher Chl *a* (corresponding with higher periphyton biomass) at the vegetated treatment was likely due to higher nitrogen levels at this watershed compared to the managed treatment, as Dalton et al. (2015) found periphyton biomass increased along a nitrate gradient in the South Nation watershed. There was no significant difference between

Chl *a* concentrations between ditch treatments, so food availability in the form of periphyton for wild anurans was likely similar across the paired watersheds.

Vegetation communities may also impact food availability for wild amphibians through food web interactions. Native plant species have been shown to be extremely important for insects, which are a main food source for adult anurans. Perron & Pick (2020) found Odonata (dragonflies and damselflies) abundance was largely explained by plant communities and significantly linked to the presence of specific obligate wetland species. A number of these obligate wetland species (such as *Lemna minor*, *Galium palustre*, and *Alnus incana*) were observed at the vegetated treatment. Ditch management had a large effect on the coefficients of conservatism (CC) in the present study, as the total CC at the vegetated treatment (135) was doubled that of the managed treatment (72). This indicates that the less disturbed sites at the vegetated treatment had plant communities with higher conservation value that may provide more food sources (such as Odonata) for wild anurans. Additionally, the South Nation Conservation Authority (2018) found riparian forest cover to be 22 % across the watershed, which is considerably lower than the Environment and Climate Change guideline of 75 % riparian forest cover (ECCC 2013). The poor riparian forest cover in the region, along with the reduction of native plant species associated with management (which may affect food web interactions), suggests that conservation efforts should be directed to increasing the amount of habitat for amphibians in agroecosystems.

4.3 Methodological considerations

This study examined both tadpoles and embryos to assess frog health outcomes and found tadpoles were more informative than embryos because of the sensitivity of tadpole

development and growth to differences in temperature. However, there are a number of benefits to studying embryos. For instance, the embryo study required a shorter (~ two weeks) but more intense field season (monitoring every two days) compared to the tadpole studies that lasted longer (one - two months) but require less frequent monitoring (twice a week). Sites suitable for *in situ* embryo cages are less restricted by depth than for *in situ* tadpole cages, since they are much smaller in size (30 cm instead of 60 cm) and embryo exposures occur during spring peak flow. Embryo studies are also less expensive to run because cages are smaller and require less material to make. A final benefit of embryo studies is that it is easier to have a large sample size, since a single embryo clutch can have thousands of eggs. Disadvantages of embryo studies are that fewer health endpoints are possible, and that embryos appear less sensitive than tadpoles to many agrochemicals. Embryo hatching occurs early in the spring, so requires an early field season which can be unpredictable.

The main benefits of studying tadpoles over embryos are the extensive possible health endpoints that can be studied. For instance, beyond what was measured in the current study, immune responses using leukocyte profiles and cortisol, and effects of parasites and pathogens can be studied in tadpoles. Of course, more health endpoints require greater financial input. A main drawback to using *in situ* cages to study tadpoles in agriculture ditches was the issue of inadequate water depth. To reduce potential impacts of high density, it is necessary to have smaller sample sizes per cage, which often means a higher number of replication to achieve the desired statistical inferences. This requires that statistical models account for non-independence of experimental cages and sites, which can be accomplished using general(ized) linear mixed-effect models. *In situ* cage experiments provide different health endpoint than

wild frog call or sampling surveys can provide. Although they come with some limitation, they are particularly valuable for investigating the sub-lethal effects of various environmental conditions on amphibians.

Another consideration for interpreting this study is site variability, and particularly within-treatment variability compared to between-treatment variability. A challenge of the current study was the small sample size of different ditches that were logistically possible to use for *in situ* caging experiments. Due to the amount of time involved in driving and animal care, and the requirements of adequate water depth, ditch treatment, and permission to access the land, the current study was limited to using sites within the paired watersheds for the tadpole experiments. For the embryo experiment it was possible to include an additional site for each ditch treatment which were outside the paired watersheds, for the added benefit of increased site variability. The notable within-treatment variability of water quality factors measured during the embryo study indicated that within-treatment differences may be comparable to between-treatment differences. Specifically, during the embryo study, water quality varied more by site, than by ditch treatment (Table 3). Ideally, more sites under the desired treatments would have been used to better determine within-treatment site variability. However, due to the constraints of deploying *in situ* caging, this was not possible. Other studies, for instance, those investigating wild amphibian surveys can complement the findings of the current study and provide the wider context of ditch variability. Despite its limitations, the present study was novel in raising tadpoles in agriculture ditches in *in situ* cages, which allowed many health outcomes to be measured (beyond wild frog abundance or species richness) which are not typical in field studies. By doing so, this study provided evidence that

ditch management can affect metamorphic development and growth of *L. pipiens* due to differences in water temperatures.

5. Conclusion

This study found that the managed (treeless/dredged) ditch treatment compared to the vegetated treatment did not negatively affect *L. pipiens* embryo or tadpole health outcomes, contrary to the hypothesis and predictions. In fact, the significantly faster development (higher GS) and larger body size (SVL) due to warmer water temperatures at the managed treatment was advantageous compared to the vegetated treatment. The water quality was typical of the region in both paired watersheds regardless of ditch treatment and the measured pesticides had limited toxicity according to the calculated Pesticide Toxicity Indices. This research indicates that the main effects of the studied ditch managements on resident wild *L. pipiens* (and potentially other anurans) are sub-lethal, likely beneficial, and mainly due to differences in temperature. Although poorer average embryo survival was observed at vegetated treatment sites, it was thought to be unrelated to ditch management and more likely due to high specific conductivity at these sites.

Given that ditch management in the current study caused no apparent detrimental health effects to *L. pipiens*, it seems that the management of these small riparian buffers may not be as important as the surrounding land management for wild amphibian health. For instance, smaller field size (farmland heterogeneity) and regional forest cover were the strongest predictors of anuran species richness and abundance in the agricultural landscapes of Eastern Ontario (Collins and Fahrig 2017). Further, amphibian community and population structure were more strongly correlated with water chemistry than instream habitat, and post-metamorphic community responses were negatively correlated to nitrogenous compound concentrations (Jordan et al. 2016). This indicates that agricultural best management practices

for amphibian conservation efforts should focus on increasing forested habitat and reducing nutrient concentrations in the surface waters. Future studies should investigate the effects of larval size on life-history traits such as reproductive success, sex ratio abnormalities in wild amphibians exposed to agrochemicals (particularly pharmaceuticals related to dairy operations), direct or mixture effects of SC on premature embryo hatching and survival in *L. pipiens*, and the effects of climate change (particularly increased drought and temperatures) on wild amphibian populations.

Appendix 1: Study Region

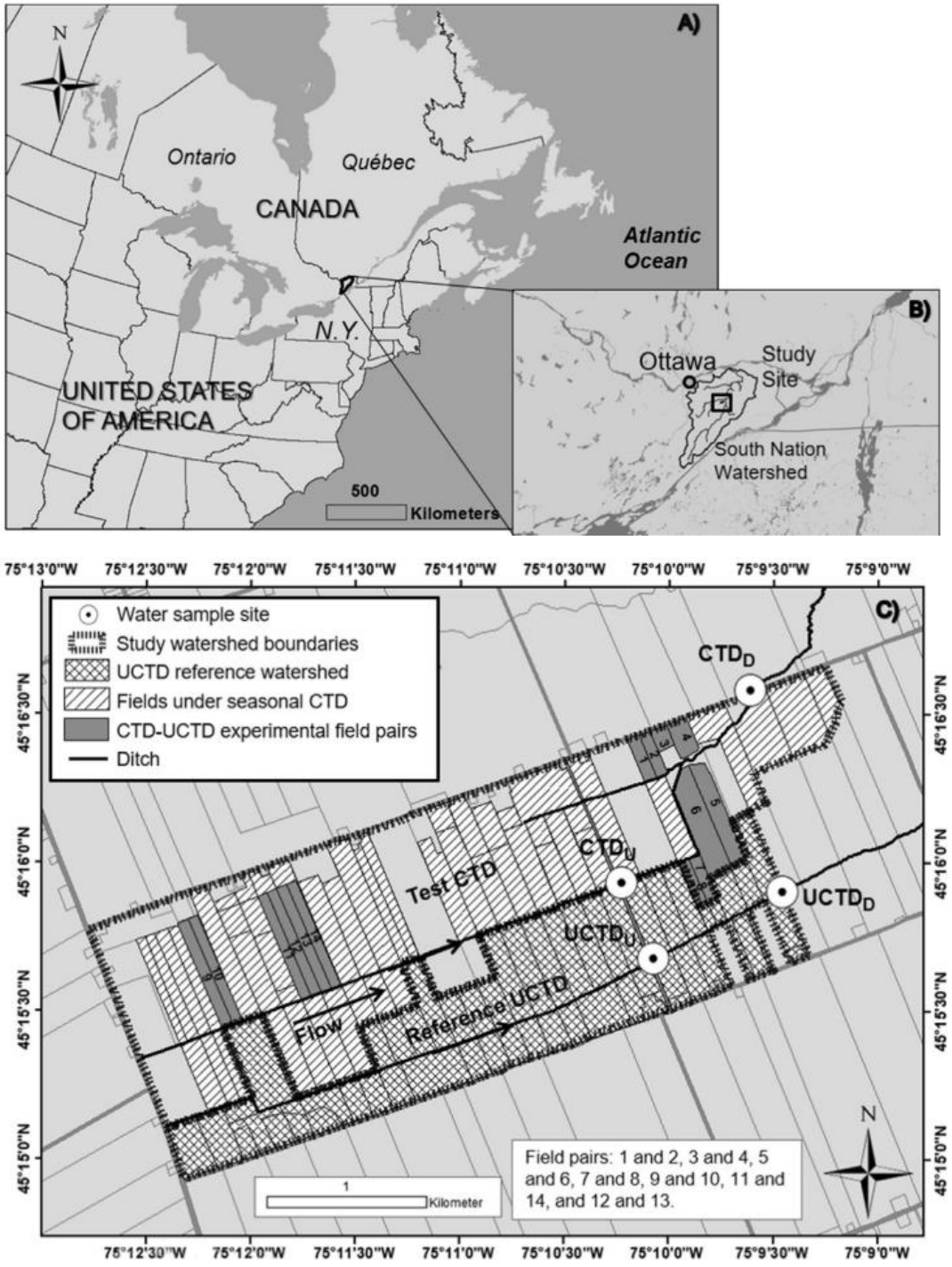

Figure 8: Map of study site. A) The South Nation River basin in eastern North America. B) The South Nation River basin in eastern Ontario, Canada and the location of the experimental paired-watersheds. C) The vegetated ("Test CTD") watershed and the managed ("reference UCTD") watershed. This figure is taken from Figure 1 from Wilkes et al. (2019) to show the tile-drainage defined paired-watersheds.

Appendix 2: Pesticides

Table 18: Multiple reaction monitoring (MRM) transitions and mass spectrometer parameters used for the analysis of atrazine and glyphosate and the internal standards (IS).

Compound	Parent ion (Da)	Daughter ion (Da)	Collision energy (V)	Cell exit potential (V)	Dwell Time (msec)
Atrazine	215	174	25	12	150
Atrazine-d5 (IS)	220	179	27	14	150
Glyphosate	168	63	-30	-9	200
Glyphosate-d2 (IS)	170	63	-30	-9	200

Table 19: Multiple reaction monitoring (MRM) transitions and mass spectrometer parameters used for the analysis of seven neonicotinoid insecticides and their internal standards (IS).

Compound	Parent ion (Da)	Daughter ion (Da)	De-clustering potential (DP) (V)	Collision energy (CE) (V)	Cell exit potential (CXP) (V)	Dwell Time (msec)
Acetamiprid	222	126	96	29	22	150
Acetamirpid-d3 (IS)	225	126	96	29	22	150
Clothianidin	249	169	66	19	22	150
Clothianidin-d3 (IS)	252	172	66	19	22	150
Dinotefuran	202	129	21	19	8	150
Dinotefuran-d3 (IS)	205	132	21	19	8	150
Imidacloprid	255	209	96	21	28	150
Imidacloprid-d4 (IS)	259	213	96	21	28	150
Flupyradifurone	289	126	31	31	20	150
Flupyradifurone-d5 (IS)	294	126	31	31	20	150
Thiamethoxam	291	211	76	19	18	150
Thiamethoxam-d3 (IS)	294	214	76	19	18	150
Thiacloprid	253	126	21	31	28	150
Thiacloprid-d4 (IS)	295	214	76	19	18	150

Table 20: Pesticide specifications used in bioconcentration of pesticides in tadpole tissues using LCMS-MS analysis. The pesticide chemical formula, class, Retention time (RT), precursor ion mass to charge ratio, and product ion mass to charge ratio are provided.

Compound	Chemical Formula	Class	RT (min)	Precursor ion (m/z)	Product ion (m/z)
Acetamiprid	C10H11ClN4	neonicotinoid	3.71	223.0745	126.0101
Ametryn	C9H17N5S	herbicide	4.28	228.1277	186.0798
Atrazine	C8H14ClN5	herbicide	5.07	216.1011	174.0533
Azoxystrobin	C22H17N3O5	fungicide	6.09	404.1241	372.0961
Benoxacor	C11H11Cl2NO2	herbicide	6.02	260.024	260.024
Bupirimate	C13H24N4O3S	fungicide	5.61	317.1642	166.0966
Buprofezin	C16H23N3OS	insecticide	6.82	306.1635	116.0524
Carbaryl	C12H11NO2	insecticide	5.05	145.0641	117.0701
Carbofuran	C12H15NO3	insecticide	4.87	222.1125	165.0908
Carpropamid	C15H18Cl3NO	fungicide	6.98	334.0527	196.0299
Chloroxuron	C15H15ClN2O2	herbicide	5.99	291.0895	72.04482
Chlorpyrifos	C9H11Cl3NO3PS	insecticide	8.08	349.9336	197.9284
Clothianidin	C6N5H8SO2Cl	neonicotinoid	3.37	250.016	169.0534
Cyazofamid	C13H13ClN4O2S	oomycete fungicide	6.85	325.0521	108.0114
Cyprodinil	C14H15N3	fungicide	5.5	226.1339	226.1326
DEET	C12H17NO	insecticide	5.13	192.1383	119.0488
Diazinon	C12H21N2O3PS	insecticide	7.17	305.1083	169.0785
Difenoconazole	C19H17Cl2N3O3	fungicide	6.89	406.072	251.0034
Dimethametryn	C11H21N5S	herbicide	5.29	256.159	186.0815
Dimethoate	C5H12NO3PS2	insecticide	3.61	230.0069	198.9654
Dimethomorph	C21H22ClNO4	fungicide	5.74	388.131	301.0625
Epoxiconazole	C17H13ClFN3O	fungicide	6.15	330.0804	121.0431

Fenamidone	C17H17N3OS	fungicide	6.16	312.1165	236.1171
Fenhexamid	C14H17Cl2NO2	fungicide	6.23	302.0709	97.10128
Fenpropathrin	C22H23NO3	insecticide	6.36	181.096	123.0798
fenpropimorph	C20H33NO	fungicide	4.75	304.2635	304.2635
Fenpyroximate	C24H27N3O4	insecticide	8.17	422.2074	366.1433
Flonicamid	C9H6F3N3O	aphicide	3.04	230.0536	230.0536
Flufenoxuron	C21H11ClF6N2O3	insecticide	7.99	489.0435	158.042
Hexythiazox	C17H21ClN2O2S	ovicide	8.12	353.1085	228.0253
Imazalil	C14H14Cl2N2O	fungicide	4.14	297.0556	158.9769
imazamox	C15H19N3O4	herbicide	3.5	306.1448	261.1243
Imazaquin	C17H17N3O3	herbicide	4.49	312.1343	267.1115
Imidacloprid	C9H10ClN5O2	neonicotinoid	3.52	256.0596	209.0597
Linuron	C9H10Cl2N2O2	herbicide	5.95	249.0192	159.9722
Malathion	C10H19O6PS2	insecticide	6.47	331.0433	127.0383
Mefluidide	C11H13F3N2O3S	herbicide	4.81	311.0672	178.1104
Metalaxyl	C15H21NO4	fungicide	5.19	280.1543	220.134
Methoxyfenozide	C22H28N2O3	insecticide	6.42	313.1547	149.0603
Metolochlor	C15H22ClNO2	herbicide	6.54	284.1412	252.1137
Metrabuzine	C8H14N4OS	herbicide	4.6	215.0961	187.1003
Metrafenone	C19H21BrO5	fungicide	7.53	409.0645	228.969
Pirimiphos-methyl	C11H20N3O3PS	insecticide	7.11	306.1036	164.1171
Propazine	C9H16ClN5	herbicide	5.67	230.1167	146.0221
propiconazole	C15H17Cl2N3O2	fungicide	6.62	342.0771	342.0753
Pyraclostrobin	C19H18ClN3O4	fungicide	7.17	388.1059	194.082
Pyrimethanil	C12H13N3	fungicide	4.69	200.1182	200.1172

Quinclorac	C10H5Cl2NO2	herbicide	4.42	241.977	223.9673
Quinoxyfen	C15H8Cl2FNO	fungicide	7.52	308.004	272.0259
Rotenone	C23H22O6	insecticide	6.61	395.1489	213.09
Simazine	C7H12ClN5	herbicide	5.01	202.0854	175.0312
Spinetoram	C42H69NO10	insecticide	5.7	748.4994	142.1226
Spinosyn A	C41H65NO10	insecticide	5.42	732.4681	142.122
Spinosyn D	C42H67NO10	insecticide	5.57	746.4838	142.1221
Spirodiclofen	C21H24Cl2O4	insecticide	8.64	411.1124	313.0393
Tebuconazole	C16H22ClN3O	fungicide	6.31	308.1524	70.04083
Tebuthiuron	C9H16N4OS	herbicide	4.12	229.1118	172.0894
Thiabendazole	C10H7N3S	fungicide	2.46	202.0433	175.0316
Thiacloprid	C10H9ClN4S	neonicotinoid	4.11	253.0309	126.0101
Thiamethoxam	C8H10ClN5O3S	neonicotinoid	3.38	292.0266	211.0649
Thiazopyr	C16H17F5N2O2S	herbicide	7.17	397.1004	377.0923
Triazophos	C12H16N3O3PS	insecticide	6.5	314.0723	162.0654
Tricyclazole	C9H7N3S	fungicide	3.74	190.0433	163.0316
Trifloxystrobin	C20H19F3N2O4	fungicide	7.54	409.137	186.0516
Trifluralin	C13H16F3N3O4	herbicide	8.18	336.1165	336.1166
Triticonazole	C17H20ClN3O	fungicide	5.8	318.1368	75.22921

Table 21: Pesticide toxicity information (96h LC_{50} for *Lithobates pipiens* and surrogate species) for atrazine, glyphosate and a suite of neonicotinoid pesticides detected in ditch surface waters in the South Nation Watershed.

Pesticide	Species	Gosner/life stage	96h-LC_{50} (mg/L)	Reference
Acetamiprid	Zebra fish, *Danio rerio*	Larvae	15.52	(Wang et al. 2018)
Atrazine	Northern leopard frog, *L. pipiens*	29, 40	30.1	(Howe et al. 1998)
Clothianidin	Bluegill sunfish, *Lepomis macrochirus* Sheepshead minnow, *Cyprinodon variegatus*	Adult	111 [a]	(Howard et al. 2003)
Glyphosate	Northern leopard frog, *L. pipiens*	25	1.55	(Relyea and Jones 2009)
Imidacloprid	Green frog, *Rana clamitans*	25	75	(Puglis and Boone 2011)
Thiacloprid	Zebrafish, *Danio rerio*	Larvae	72.27	(Wang et al. 2020)
Thiamethoxam	Nile tilapia, *Oreochromis niloticus*	Juvenile	322.07	(Albinati et al. 2016)a

This table was compiled by Kimberly Bray, University of Ottawa, 2020.

[a] The average LC50 was taken from both surrogate species (117 mg/L and 105 mg/L for sunfish and minnow respectively)

Appendix 3: Frogs

Figure 9: *In situ* amphibian cages: embryo cages (left) and tadpole cages (right) installed in agriculture ditches in the South Nation Watershed.

Figure 10: Axial tail deformities increasing from none (left) to mild, moderate, and severe (right) in *Lithobates pipiens* tadpoles (Gosner Stage 36-41). Scale grid is 5x5 mm.

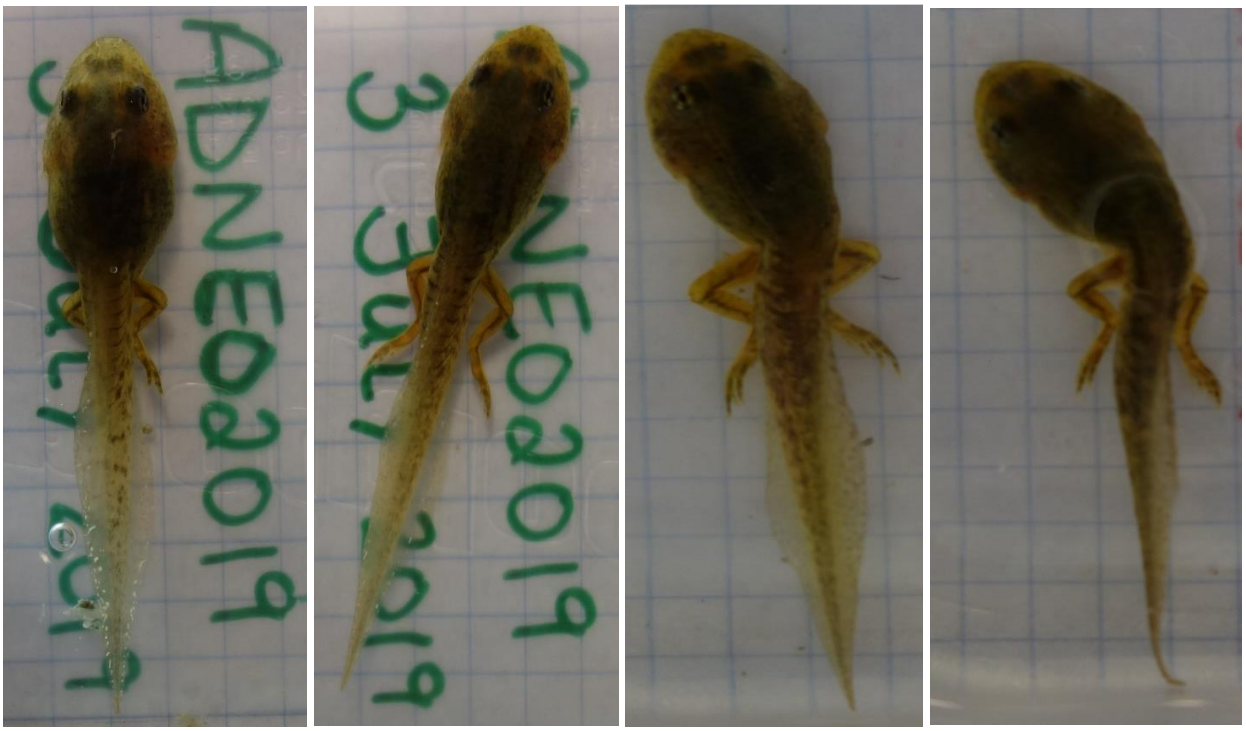

Figure 11: Gonad identification examples of female (top), male (middle), intersex/undifferentiated (bottom) *Lithobates pipiens*. Gonads appear a white clouds (ovaries) or balls (testes) over yellow kidneys. Original source: Metamorph Endpoints-Euthanization Protocol SR04-2016, Robinson Lab, NWRC.

Figure 12: Gosner Stages used to characterize *Lithobates pipiens* metamorphic development (Gosner 1960).

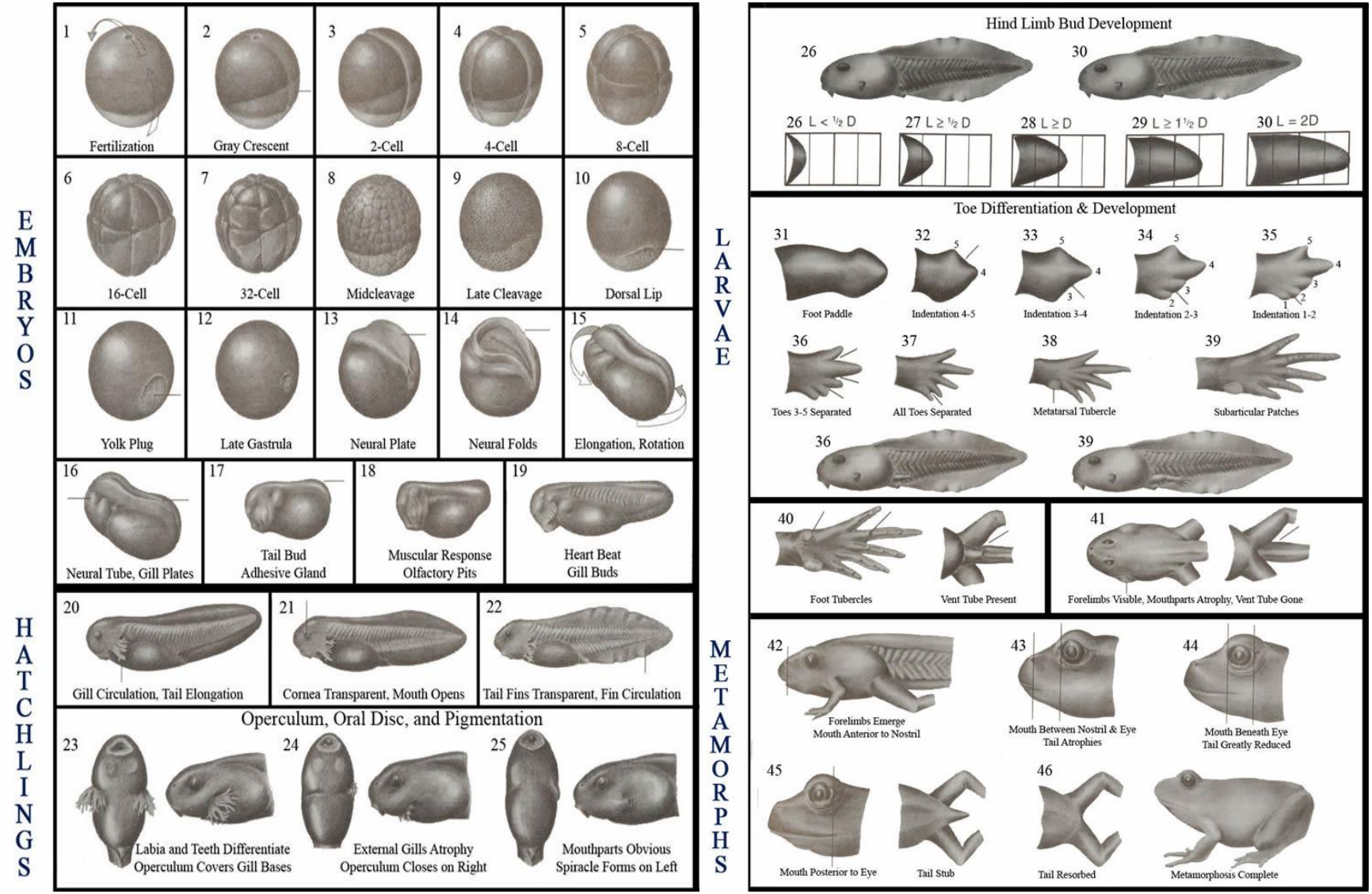

Table 22: Physiochemical water conditions in the growth chamber that control tadpoles were raised in at the National Wildlife Research Centre.

	Mean	Range
Temperature (°C)	22.7	(22.1 - 23.4)
Humidity (%)	62.7	(53.7 - 71.6)
DO (mg/L)	7.6	(4.4 - 8.6)
SC (µS/cm)	198.6	(148.2 - 239.9)
pH	7.7	(7.2 - 8.1)
TIN [a]	4.1	(0.3 - 8.6)

[a] TIN is the Total Inorganic Nitrogen.

Table 23: Mean values for water quality variables during *in situ L. pipiens* embryo experiment between vegetated and managed (dredged) treatments in 2019 (rather than by site). The mean and standard deviation (SD) for two sites at each treatment are presented, along with sample size (n) for the variable. The detection limit (DL) for RP was 0.004 mg/L. Na indicates not applicable.

		Managed		Vegetated	
Variable	**n**	**Mean**	**SD**	**Mean**	**SD**
Temperature (°C)	n = 48	11.260	3.126	9.710	3.158
SC (µS/cm)	n = 48	521.979	148.878	810.213	354.307
DO (mg/L)	n = 48	17.936	4.530	15.212	3.509
pH	n = 48	7.647	0.303	7.550	0.342
Redox (mV)	n = 48	186.885	24.754	164.657	36.469
Turbidity (NTU)	n = 48	12.954	9.943	6.555	6.221
Depth (cm)	n = 48	24.385	8.396	26.656	9.858
NH_3-NH_4^+ (mg/L)	n = 4	0.015	0.004	0.134	0.102
NO_3^- (mg/L)	n = 4	5.220	1.550	7.030	1.830
TIN (mg/L)	n = 4	5.250	1.550	7.180	1.840
RP (mg/L)	n = 4	<DL	Na	0.030	0.020
TP (mg/L)	n = 4	0.020	0.010	0.110	0.040
DOC (mg/L)	n = 4	3.450	0.210	4.930	0.280
TOC (mg/L)	n = 4	3.270	0.170	4.470	0.270
TKN (mg/L)	n = 4	0.450	0.070	0.840	0.190
TSS (mg/L)	n = 4	130	11	113	19
Atrazine (ug/L)	n = 6	0.009	0.003	0.014	0.007
Total Neonicotinoids (ug/L)	n = 6	0.011	0.002	0.031	0.013

Appendix 4: Plants

Table 24: Master list containing all plant species identified at the vegetated and managed (dredged) ditches in Eastern Ontario, in 2019. Two surveys were conducted (in June and August) to catch early and late blooming species. Coefficient of conservatism (CC), wetness index, total weediness Index, and physiognomy were determined from Oldham 1995. * indicates adventives (non-native species). Species that were not identified to species level are included in this table for an indication of species richness, but are not included in the summary information provided in Chapter 3.6.

Common name	Scientific name and authority	CC	Wetness	Weediness	Physiognomy
Yellow Avens	*Geum aleppicum* Jacq.	2	FAC+	NA	N Forb
Algae	*Cladophora* spp. Kütz.				
Algae	*Aulacoseira* spp. Thwaites				
Algae	*Euglenoids* spp.				
Algae	Diatoms				
Algae	*Spirogyra* spp. Link In C. G. Nees, 1820				
Alternate leaved dogwood	*Cornus alternifolia* L. f.	6	UPL	NA	N Shrub
Marsh bedstraw	*Galium palustre* L.	5	OBL	NA	N Forb
Rough bedstraw	*Galium asprellum* Michx.	6	OBL	NA	N Forb
Black cherry	*Prunus serotina* Ehrh.	3	FACU	NA	N Tree
Canada goldenrod	*Solidago canadensis* L.	1	FACU	NA	N Forb
Canada thistle	*Cirsium arvense* (L.) Scop.	*	FACU	-1	A Forb
Choke cherry	*Prunus virginiana* L.	2	FAC-	NA	N Shrub
Colts foot	*Tussilago farfara* L.	*	FACU	-2	A Forb
Common burdock	*Arctium minus* (Hill) Bernh.	*	UPL	-2	A Forb
Broad-Leaved cattail	*Typha latifolia* L.	3	OBL	NA	N Forb
Common milkweed	*Asclepias syriaca* L.	0	UPL	NA	N Forb
Cow vetch	*Vicia cracca* L.	*	UPL	-1	A Forb
Common dandelion	*Taraxacum* sp. F.H. Wigg.	*	FACU	-2	A Forb
Climbing nightshade	*Solanum dulcamara* L.	*	FAC	-2	A Forb
Small duckweed	*Lemna minor* L.	2	OBL	NA	N Forb
Common waterweed	*Elodea canadensis* Michx.	4	OBL	NA	N Forb
Field horsetail	*Equisetum arvense* L.	0	FAC	NA	N Forb
Marsh fleabane	*Erigeron philadelphicus* L.	1	FACW	NA	N Forb
Fowl meadow grass	*Poa palustris* L.	5	FACW+	NA	N Grass

Smooth brome	*Bromus inermis* Leyss.	*	UPL	-3	A Grass
Glossy buckthorn	*Frangula alnus* Mill.	*	FAC+	-3	A Shrub
Grape-woodvine	*Parthenocissus quinquefolia* (L.) Planch.	3	FACU	NA	N Vine
Green ash	*Fraxinus pennsylvanica* Marshall	3	FACW	NA	N Tree
Spotted jewelweed	*Impatiens capensis* Meerb.	4	FACW	NA	N Forb
Lady's sorel	*Oxalis stricta* L.	0	FACU	NA	N Forb
Lamb's quarters	*Chenopodium album* L.	*	FAC-	-1	A Forb
Manitoba maple	*Acer negundo* L.	0	FACW-	NA	N Tree
Marsh st-johns wort	*Hypericum fraseri* Steud.	7	OBL	NA	N Forb
Mouse ear chickweed	*Cerastium fontanum* Baumg.	*	FACU	NA	A Forb
Narrow leaved cattail	*Typha angustifolia* L.	3	OBL	NA	N Forb
Grass-leaved goldenrod	*Euthamia graminifolia* (L.) Nutt.	2	FACW-	NA	N Forb
Perenial sow thistle	*Sonchus arvensis* L.	*	FAC-	-1	A Forb
Pin cherry shrub	*Prunus pensylvanica* L. f.	3	FACU-	NA	N Tree
Kentucky bluegrass	*Poa pratensis* L.	0	FAC-	NA	N Grass
Purple loosestrife	*Lythrum salicaria* L.	*	OBL	-3	A Forb
Queen Ann's Lace	*Daucus carota* L.	*	UPL	-2	A Forb
Red maple	*Acer rubrum* L.	4	FAC	NA	N Tree
Red-oiser dogwood	*Cornus sericea* L.	2	FACW	NA	N Shrub
Reed canary grass	*Phalaris arundinacea* L.	0	FACW+	NA	N Grass
Rough goldenrod	*Solidago rugosa* Mill.	4	FAC+	NA	N Forb
Sensitive fern	*Onoclea sensibilis* L.	4	FACW	NA	N Fern
False nettle	*Boehmeria cylindrica* (L.) Sw.	4	OBL	NA	N Forb
Stinging nettle	*Urtica dioica* L. subsp. *dioica*	*	FAC+	-1	A Forb
Tall meadow-rue	*Thalictrum pubescens* Pursh	5	FACW-	NA	N Forb
Trailing arbutus	*Epigaea repens* L.	9	UPL	NA	N Shrub
UNK 11	-				
UNK 12	-				
UNK 15	-				
UNK 16	-				
Curly dock	*Rumex crispus* L.	*	FAC+	-2	A Forb
UNK 21	-				
UNK 22	-				

UNK 24	-				
UNK 26	-				
UNK 27	-				
UNK 28	-				
UNK 30	-				
UNK 31	-				
UNK 32	-				
UNK 33	-				
UNK 34	-				
UNK 55	-				
UNK 57	-				
UNK 58	-				
Speckled alder	*Alnus incana* (L.) Moench	6	OBL	NA	N Shrub
UNK 61	-				
UNK 62	-				
UNK 63	-				
UNK 64	-				
UNK 7	-				
Wild Currant	*Ribes glandulosum* Grauer	6	FACW	NA	N Shrub
UNK Z	-				
UNK 60	-				
Violet	*Viola cucullata* Aiton	5	OBL	NA	N Forb
Water plantain	*Alisma gramineum* Lej.	6	OBL	NA	N Forb
White willow	*Salix alba* L.	*	FACW	-2	A Tree
Wild cucumber	*Echinocystis lobata* (Michx.) Torr. & A. Gray	3	FACW-	NA	N Forb
Wild parsnip	*Pastinaca sativa* L.	*	UPL	-3	A Vine
Wild red raspberry	*Rubus idaeus* L.	0	FACW-	NA	N Shrub
Wild strawberry	*Fragaria virginiana* Mill.	2	FAC-	NA	N Forb
Woolgrass	*Scirpus cyperinus* (L.) Kunth	4	OBL	NA	N sedge
Yellow birch	*Betula alleghaniensis* Britton	6	FAC	NA	N Tree
Garlic mustard	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	0	FAC	-3	A Forb

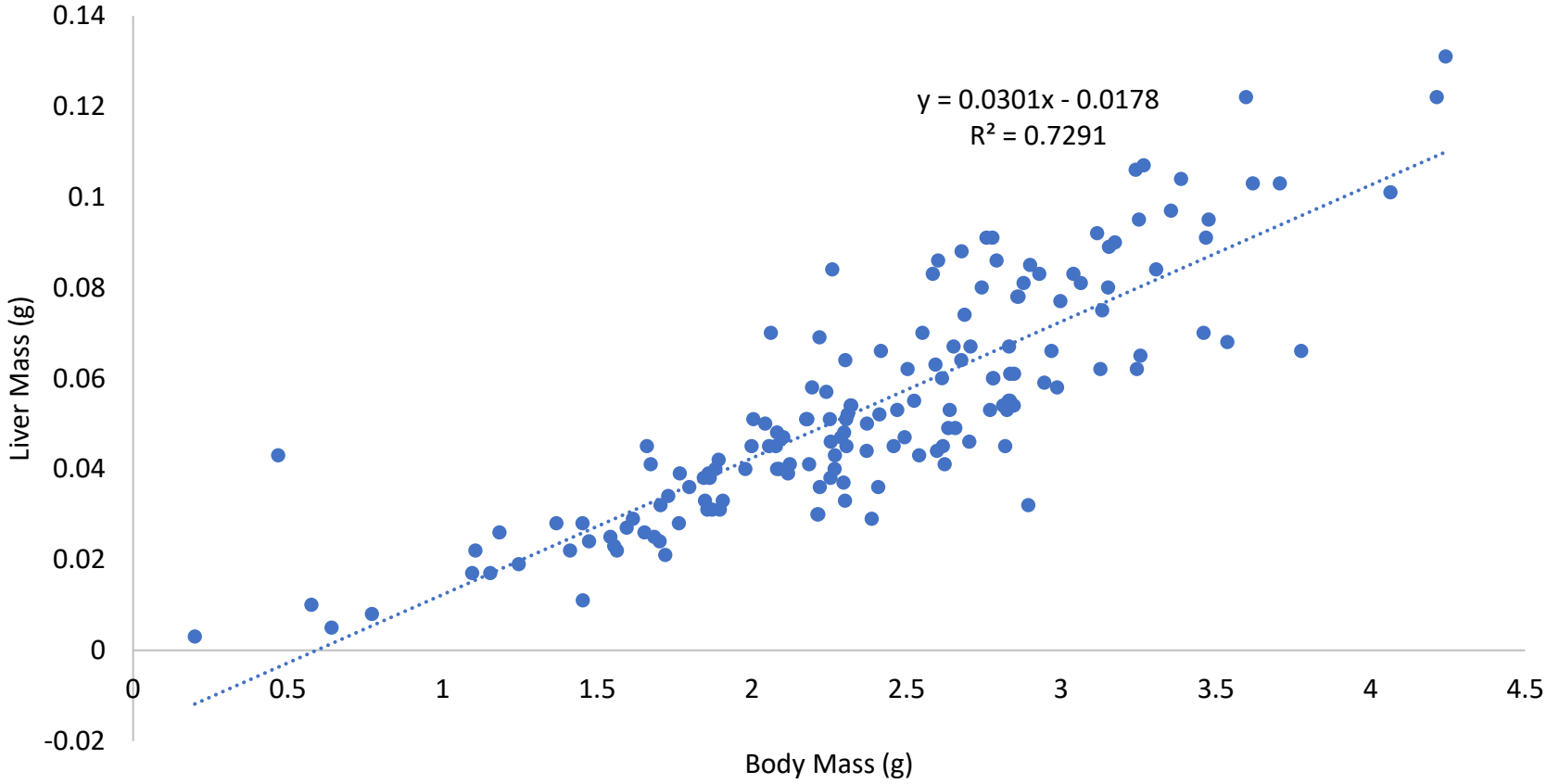

Figure 13: *L. pipiens* tadpole liver (numerator) and body mass (denominator) of the Liver Somatic Index (LSI) shows this is a linear relationship that runs through the origin. Each of the individual tadpoles from all four sites in 2019 are presented as a dot.

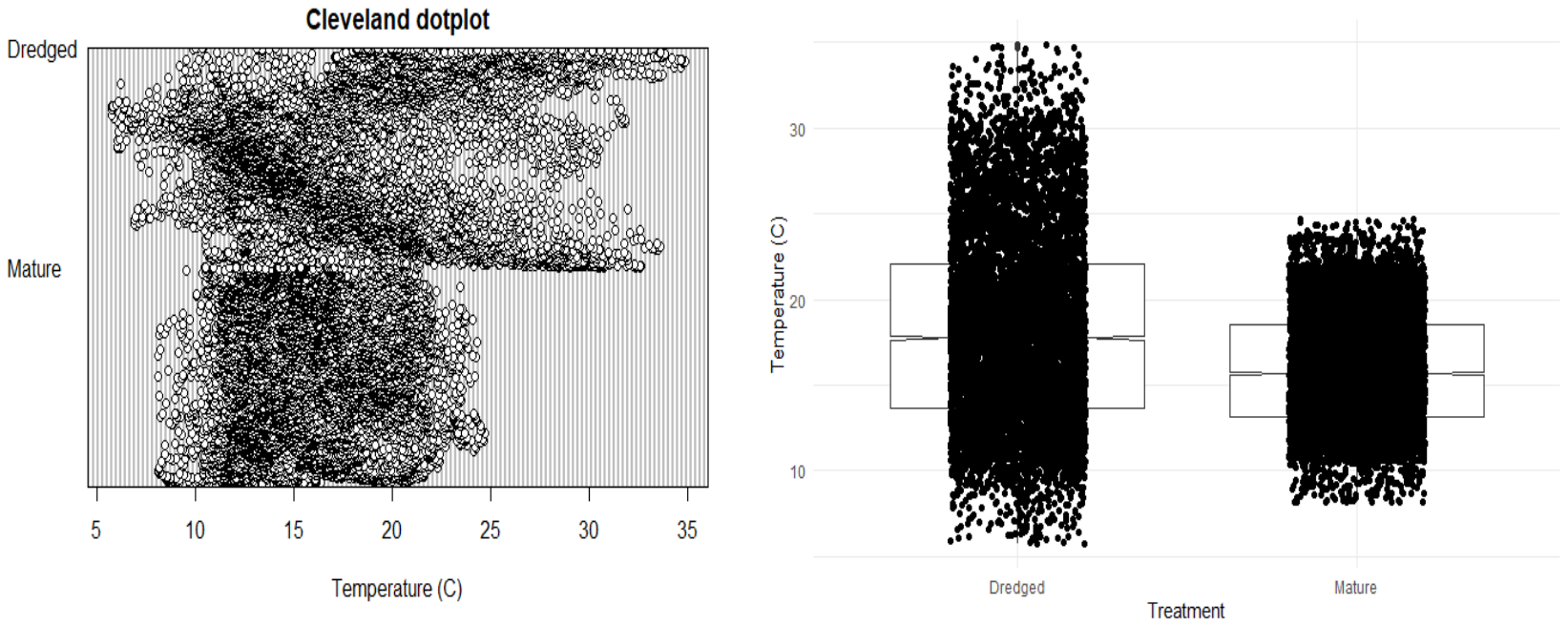

Figure 14: Cleveland dotplot (left) and boxplot (right) were used to visualize datasets before running statistical analyses. An example of temperature in 2019 is shown here at the managed (dredged) and vegetated (mature) treatments. In the dotplot, each dot represents one measurement. In the boxplot, each dot represents one measurement, the median is shown within the 25 % - 75 % inter-quantile range (appearing as boxes), and whiskers indicate 95 % confidence intervals. The samples size (n) was 7788 measurements for each treatment.

References

AAFC (2019) Annual Crop Inventory. In: Agric. Agri-Food Canada; Sci. Technol. Branch; Gov. Canada. https://open.canada.ca/data/en/dataset/ba2645d5-4458-414d-b196-6303ac06c1c9. Accessed 8 May 2020

Albinati AC, Albinati RC, Lira AD, Soares PC (2016) Toxicidade aguda e risco ecotoxicológico do inseticida tiametoxam para alevinos de tilápia-do-nilo. Arq. bras. med. vet. zootec, 68(5), 1343-1350

Alford RA, Richards SJ (1999) Global Amphibian Declines: A Problem in Applied Ecology. Source Annu Rev Ecol Syst 3095102:133–165. https://doi.org/10.1146/annurev.ecolsys.30.1.133

Allan J, Castillo M (2007) Stream Ecology: structure and function of running waters, 2nd edn. SpringerNetherlands, Dordrecht, Netherlands

Allaway C (2006) Combined nitrogen retention in an agricultural river system: MSc Thesis. University of Ottawa

Altwegg R, Reyer HU (2003) Patterns of natural selection on size at metamorphosis in water frogs. Evolution (N Y) 57:872–882. https://doi.org/10.1111/j.0014-3820.2003.tb00298.x

Anderson JC, Dubetz C, Palace VP (2015) Neonicotinoids in the Canadian aquatic environment: A literature review on current use products with a focus on fate, exposure, and biological effects. Sci Total Environ 505:409–422. https://doi.org/10.1016/J.SCITOTENV.2014.09.090

Arribas R, Díaz-Paniagua C, Caut S, Gomez-Mestre I (2015) Stable isotopes reveal trophic partitioning and trophic plasticity of a larval amphibian guild. PLoS One 10:. https://doi.org/10.1371/journal.pone.0130897

Barton DR, Taylor WD, Biette RM (1985) Dimensions of Riparian Buffer Strips Required to Maintain Trout Habitat in Southern Ontario Streams. North Am J Fish Manag 5:364–378. https://doi.org/10.1577/1548-8659(1985)5<364:dorbsr>2.0.co;2

Bates DM, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.1177/009286150103500418

Battaglin W, Fairchild J (2002) Potential toxicity of pesticides measured in midwestern streams to aquatic organisms. Water Sci Technol 45:95–102. https://doi.org/10.2166/wst.2002.0213

Battaglin WA, Smalling KL, Anderson C, et al (2016) Potential interactions among disease, pesticides, water quality and adjacent land cover in amphibian habitats in the United States. Sci Total Environ 566–567:320–332. https://doi.org/10.1016/j.scitotenv.2016.05.062

Beck CW, Congdon JD (2000) Effects of age and size at metamorphosis on performance and metabolic rates of Southern Toad, Bufo terrestris, metamorphs. Funct Ecol 14:32–38. https://doi.org/10.1046/j.1365-2435.2000.00386.x

Belden JB, Gilliom RJ, Lydy MJ (2007) How well can we predict the toxicity of pesticide mixtures to aquatic life? Integr Environ Assess Manag 3:364–372. https://doi.org/10.1897/1551-3793(2007)3[364:HWCWPT]2.0.CO;2

Bernabò I, Guardia A, Macirella R, et al (2016) Effects of long-term exposure to two fungicides, pyrimethanil and tebuconazole, on survival and life history traits of Italian tree frog (Hyla intermedia). Aquat Toxicol 172:56–66. https://doi.org/10.1016/j.aquatox.2015.12.017

Biggs J, von Fumetti S, Kelly-Quinn M (2017) The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. Hydrobiologia 793:3–39. https://doi.org/10.1007/s10750-016-3007-0

Blann KL, Anderson JL, Sands GR, Vondracek B (2009) Effects of Agricultural Drainage on Aquatic Ecosystems: A Review. Crit Rev Environ Sci Technol 39:909–1001. https://doi.org/10.1080/10643380801977966

Boutin C, Jobin B, Belanger L (2003) Importance of riparian habitats to flora conservation in farming landscapes of southern Quebec, Canada. Agric Ecosyst Environ 94:73–87. https://doi.org/10.4103/1673-5374.172311

Brand AB, Snodgrass JW (2010) Value of artificial habitats for amphibian reproduction in altered landscapes: Contributed paper. Conserv Biol 24:295–301. https://doi.org/10.1111/j.1523-1739.2009.01301.x

Buck JC, Hua J, Brogan WR, et al (2015) Effects of pesticide mixtures on host-pathogen dynamics of the amphibian chytrid fungus. PLoS One 10:. https://doi.org/10.1371/journal.pone.0132832

Burnham KP, Anderson DR (2004) Multimodel inference: Understanding AIC and BIC in model selection. Sociol Methods Res 33:261–304. https://doi.org/10.1177/0049124104268644

CCME (1999) Canadian Water Quality Guidelines for the Protection of Aquatic Life: Atrazine. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment. Winnipeg, Canada

CCME (2011) Canadian Water Quality Guidelines for the Protection of Aquatic Life: Chloride. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment. Winnipeg, Canada

CCME (2007a) Canadian Water Quality Guidelines for the Protection of Aquatic Life: Nutrients. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment. Winnipeg, Canada

CCME (2012a) Canadian Water Quality Guidelines for the Protection of Aquatic Life: Nitrate. Canadian water quality guidelines for the protection of aquatic life: Nitrate. In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment. Winnipeg, Canada

CCME (2007b) Canadian Water Quality Guidelines for the Protection of Aquatic Life:

Imidacloprid. In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment. Winnipeg, Canada

CCME (2012b) Canadian water quality guidelines for the protection of aquatic life: Glyphosate. In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment. Winnipeg, Canada

CCME (2014) Water Quality Index: WQI Calculator 1.2. In: Canadian Environmental Quality Guidelines. http://ceqg-rcqe.ccme.ca/en/index.html#void. Accessed 21 May 2020

CCME (2002) Canadian Water Quality Guidelines for the Protection of Aquatic Life: Total Particulate Matter. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment. Winnipeg, Canada

Chester ET, Robson BJ (2013) Anthropogenic refuges for freshwater biodiversity: Their ecological characteristics and management. Biol Conserv 166:64–75. https://doi.org/10.1016/j.biocon.2013.06.016

Christin MS, Ménard L, Giroux I, et al (2013) Effects of agricultural pesticides on the health of Rana pipiens frogs sampled from the field. Environ Sci Pollut Res 20:601–611. https://doi.org/10.1007/s11356-012-1160-1

Collins SJ, Bellingham L, Mitchell GW, Fahrig L (2019) Life in the slow drain: Landscape structure affects farm ditch water quality. Sci Total Environ 656:1157–1167. https://doi.org/10.1016/j.scitotenv.2018.11.400

Collins SJ, Fahrig L (2017) Responses of anurans to composition and configuration of agricultural landscapes. Agric Ecosyst Environ 239:399–409. https://doi.org/10.1016/j.agee.2016.12.038

Contant J, Pick FR (2013) Picophytoplankton during the ice-free season in five temperate-zone rivers. J Plankton Res 35:553–565. https://doi.org/10.1093/plankt/fbt013

Cooke AS (1973) The effects of DDT, when used as a mosquito larvicide, on tadpoles of the frog Rana temporaria. Environ Pollut 5:259–273. https://doi.org/10.1016/0013-9327(73)90003-7

Cooke AS (1981) Tadpoles as indicators of harmful levels of pollution in the field. Environ Pollution Ser A, Ecol Biol 25:123–133. https://doi.org/10.1016/0143-1471(81)90012-X

Coyle TLC, Karasov WH (2010) Chronic, dietary polybrominated diphenyl ether exposure affects survival, growth, and development of Rana pipiens tadpoles. Environ Toxicol Chem 29:133–141. https://doi.org/10.1002/etc.21

Crane M, Burton GA, Culp JM, et al (2007) Review of aquatic in situ approaches for stressor and effect diagnosis. Integr Environ Assess Manag 3:234–245. https://doi.org/10.1897/IEAM_2006-027.1

Curran-Everett D (2013) Explorations in statistics: The analysis of ratios and normalized data. Am J Physiol - Adv Physiol Educ 37:213–219. https://doi.org/10.1152/advan.00053.2013

Dages C, Voltz M, Bsaibes A, et al (2009) Estimating the role of a ditch network in groundwater recharge in a Mediterranean catchment using a water balance approach. J Hydrol 375:498–512. https://doi.org/10.1016/j.jhydrol.2009.07.002

Dalton RL (2014) Effects of agrochemicals on riparian and aquatic primary producers in an agricultural watershed. University of Ottawa, Ottawa, Canada

Dalton RL, Boutin C, Pick FR (2015) Determining in situ periphyton community responses to nutrient and atrazine gradients via pigment analysis. Sci Total Environ 515–516:70–82. https://doi.org/10.1016/j.scitotenv.2015.01.023

De Solla SR, Pettit KE, Bishop CA, et al (2002) Effects of agricultural runoff on native amphibians in the lower Fraser River Valley, British Columbia, Canada. Environ Toxicol Chem 21:353–360. https://doi.org/10.1002/etc.5620210218

Denton RD, Bernot MJ (2011) Effects of Multiple Agricultural Chemicals on Northern Leopard Frog, Lithobates Pipiens, Larvae. Proc Indiana Acad Sci 120:39–44

Dollinger J, Dagès C, Bailly JS, et al (2015) Managing ditches for agroecological engineering of landscape. A review. Agron Sustain Dev 35:999–1020. https://doi.org/10.1007/s13593-015-0301-6

Ducks Unlimited Canada (2010) Final Report: Southern Ontario Wetland Conversion Analysis. Barrie

ECCC (2013) How Much Habitat is Enough? A Framework for Guiding Habitat Rehabilitation in Great Lakes Areas of Concern. Environment and Climate Change Canada. Toronto, Ontario

ECCC (2020) Daily Data Report for St Albert, ON. In: Historical Data. Environment and Climate Change Canada. https://climate.weather.gc.ca/climate_data/daily_data_e.html?hlyRange=%7C&dlyRange=1986-06-01%7C2020-04-15&mlyRange=1986-01-01%7C2006-12-01&StationID=4377&Prov=ON&urlExtension=_e.html&searchType=stnName&optLimit=yearRange&StartYear=1840&EndYear=2020&selRo. Accessed 22 May 2020

Eddy SB (1976) Population ecology of the leopard frog Rana pipiens Schreber at Delta Marsh, Manitoba. University of Manitoba, Winnipeg, Manitoba

Edge CB, Gahl MK, Pauli BD, et al (2011) Exposure of juvenile green frogs (Lithobates clamitans) in littoral enclosures to a glyphosate-based herbicide. Ecotoxicol Environ Saf 74:1363–1369. https://doi.org/10.1016/j.ecoenv.2011.04.020

Egea-Serrano A, Relyea RA, Tejedo M, Torralva M (2012) Understanding of the impact of chemicals on amphibians: a meta-analytic review. Ecol Evol 2:1382–1397. https://doi.org/10.1002/ece3.249

Evans RO, Bass KL, Burchell MR, et al (2007) Management alternatives to enhance water quality and ecological function of channelized streams and drainage canals. Soil Water Conserv 62:308–320

Feng S, Kong Z, Wang X, et al (2004) Acute toxicity and genotoxicity of two novel pesticides on amphibian, Rana N. Hallowell. Chemosphere 56:457–463. https://doi.org/10.1016/j.chemosphere.2004.02.010

Fiener P, Auerswald K (2003) Effectiveness of grassed waterways in reducing runoffand sediment delivery from agricultural watersheds. Environ Qual 32:927–936

Figueiredo J, Rodrigues D de J (2014) Effects of four types of pesticides on survival, time and size to metamorphosis of two species of tadpoles (Rhinella marina and Physalaemus centralis) from the southern Amazon, Brazil. Herpetol J 24:7–15

Flora C, Kröger R (2014) Use of vegetated drainage ditches and low-grade weirs for aquaculture effluent mitigation: II. Suspended sediment. Aquac Eng 60:68–72. https://doi.org/10.1016/j.aquaeng.2014.04.007

Foley JA, Defries R, Asner GP, et al (2005) Global Consequences of Land Use. Science (80-) 309:570–574. https://doi.org/10.1126/science.1111772

Gervasi SS (2007) Pond desiccation rate affects immune system responsiveness in a temporary pond breeding amphibian, the wood frog (Rana sylvatica). University of Michigan

Gomez-Mestre I, Wiens JJ, Warkentin KM (2008) Evolution of adaptive plasticity: Risk-sensitive hatching in neotropical leaf-breeding treefrogs. Ecol Monogr 78:205–224. https://doi.org/10.1890/07-0529.1

Gosner K (1960) A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. Herpetologica 16:183–190

Green AW, Bailey LL (2015) Reproductive strategy and carry-over effects for species with complex life histories. Popul Ecol 57:175–184. https://doi.org/10.1007/s10144-014-0461-2

Hanlon SM, Parris MJ (2012) The impact of pesticides on the pathogen batrachochytrium dendrobatidis independent of potential hosts. Arch Environ Contam Toxicol 63:137–143. https://doi.org/10.1007/s00244-011-9744-1

Hanlon SM, Parris MJ (2014) The interactive effects of chytrid fungus, pesticides, and exposure timing on gray treefrog (Hyla versicolor) larvae. Environ Toxicol Chem 33:216–222. https://doi.org/10.1002/etc.2419

Hansen M, Poulsen R, Luong X, et al (2014) Liquid chromatography tandem mass spectrometry method using solid-phase extraction and bead-beating-assisted matrix solid-phase dispersion to quantify the fungicide tebuconazole in controlled frog exposure study: Analysis of water and animal tissue. Anal Bioanal Chem 406:7677–7685. https://doi.org/10.1007/s00216-014-8207-8

Harris ML, Bishop CA, McDaniel T V. (2001) Assessment of rates of deformity in wild frog populations using in situ cages: A case study of leopard frogs (Rana pipiens) in Ontario, Canada. Biomarkers 6:52–63. https://doi.org/10.1080/135475001452797

Harris ML, Bishop CA, Struger J, et al (1998) The functional integrity of northern leopard frog

(Rana pipiens) and green frog (Rana clamitans) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. Environ Toxicol Chem 17:1351–1363. https://doi.org/10.1897/1551-5028(1998)017<1351:TFIONL>2.3.CO;2

Harris ML, Bogart JP (1997) A cage for evaluation of in situ water quality using frog eggs and tadpoles. Herpetol Rev 28:134–135

Hartel T, Băncilă R, Cogălniceanu D (2011) Spatial and temporal variability of aquatic habitat use by amphibians in a hydrologically modified landscape. Freshw Biol 56:2288–2298. https://doi.org/10.1111/j.1365-2427.2011.02655.x

Hasler A, Swenson M (1967) Eutrophication. Science, 158(3798), 278-282. www.jstor.org/stable/1722454

Hatch AC, Blaustein AR (2003) Combined effects of UV-B radiation and nitrate fertilizer on larval amphibians. Ecol Appl 13:1083–1093. https://doi.org/10.1890/1051-0761(2003)13[1083:CEOURA]2.0.CO;2

Havens SM, Hedman CJ, Hemming JDC, et al (2020) Occurrence of estrogens, androgens and progestogens and estrogenic activity in surface water runoff from beef and dairy manure amended crop fields. Sci Total Environ 710:136247. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.136247

Hayes T, Haston K, Tsui M, et al (2003) Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (Rana pipiens): Laboratory and field evidence. Environ Health Perspect 111:568–575. https://doi.org/10.1289/ehp.5932

Hayes T, Haston K, Tsui M, et al (2002) Feminization of male frogs in the wild. Nature 419:895–896. https://doi.org/10.1038/419895a

Hayes TB (1998) Sex determination and primary sex differentiation in amphibians: Genetic and developmental mechanisms. In: Journal of Experimental Zoology. pp 373–399

Hayes TB, Case P, Chui S, et al (2006) Pesticide mixtures, endocrine disruption, and amphibian declines: Are we underestimating the impact? Environ Health Perspect 114:40–50. https://doi.org/10.1289/ehp.8051

Hecnar S, M'Closkey R (1996) Amphibian species richness and distribution in relation to pond water chemistry in south-western Ontario, Canada. Freshw Biol 36:7–15. https://doi.org/10.1046/j.1365-2427.1996.00054.x

Hecnar SJ (1995) Acute and chronic toxicology of ammonium nitrate fertilizer to amphibians from southern ontario. Environ Toxicol Chem 12:2131–2137

Herzon I, Helenius J (2008) Agricultural drainage ditches, their biological importance and functioning. Biol Conserv 141:1171–1183. https://doi.org/10.1016/j.biocon.2008.03.005

Hoffmann CC, Baattrup-Pedersen A (2007) Re-establishing freshwater wetlands in Denmark. Ecol Eng 30:157–166. https://doi.org/10.1016/j.ecoleng.2006.09.022

Hogan NS, Duarte P, Wade MG, et al (2008a) Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (Rana pipiens): Identifying critically vulnerable periods of development. Gen Comp Endocrinol 156:515–523. https://doi.org/10.1016/j.ygcen.2008.03.011

Hogan NS, Duarte P, Wade MG, et al (2008b) Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (Rana pipiens): Identifying critically vulnerable periods of development. https://doi.org/10.1016/j.ygcen.2008.03.011

Howard J, Julian S, Ferrigan J (2003) Golf course maintenance: impact of pesticides on amphibians. Golf Course Mon September:94–101

Howe CM, Berrill M, Pauli BD, et al (2004) Toxicity of glyphosate based pesticides to four North American frog species. Env Toxicol Chem 23:1928–1938. https://doi.org/10.1897/03-71

Howe GE, Gillis R, Mowbray RC (1998) Effect of chemical synergy and larval stage on the toxicity of atrazine and alachlor to amphibian larvae. Environ Toxicol Chem 17:519–525. https://doi.org/10.1897/1551-5028(1998)017<0519:EOCSAL>2.3.CO;2

IPCC (2018) Summary for Policymakers. In: Global Warming of 1.5 °C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Geneva, Switzerland

Iturburu FG, Zömisch M, Panzeri AM, et al (2017) Uptake, distribution in different tissues, and genotoxicity of imidacloprid in the freshwater fish Australoheros facetus. Environ Toxicol Chem 36:699–708. https://doi.org/10.1002/etc.3574

IUCN (2020) Summary Statistics. In: IUCN Red List Threat. Species. https://www.iucnredlist.org/. Accessed 6 Jan 2020

Johnson MS, Aubee C, Salice CJ, et al (2017) A review of ecological risk assessment methods for amphibians: Comparative assessment of testing methodologies and available data. Integr Environ Assess Manag 13:601–613. https://doi.org/10.1002/ieam.1881

Jones DK, Dang TD, Urbina J, et al (2017) Effect of Simultaneous Amphibian Exposure to Pesticides and an Emerging Fungal Pathogen, Batrachochytrium dendrobatidis. Environ Sci Technol 51:671–679. https://doi.org/10.1021/acs.est.6b06055

Jordan MA, Castañeda AJ, Smiley PC, et al (2016) Influence of instream habitat and water chemistry on amphibians in channelized agricultural headwater streams. Agric Ecosyst Environ 230:87–97. https://doi.org/10.1016/j.agee.2016.05.028

Karraker NE, Gibbs JP, Vonesh JR (2008) Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecol Appl 18:724–734. https://doi.org/10.1890/07-1644.1

Karraker NE, Ruthig GR (2009) Effect of road deicing salt on the susceptibility of amphibian

embryos to infection by water molds. Environ Res 109:40–405. https://doi.org/10.1016/j.envres.2008.09.001

Kimelman EC (1985) No quiet place. In: Review Committee on Indian and Metis Adoptions and Placements. Winnipeg

King KW, Williams MR, Macrae ML, et al (2015) Phosphorus transport in agricultural subsurface drainage: A review. J Environ Qual 44:467–485. https://doi.org/10.2134/jeq2014.04.0163

Kleinhenz P, Boone M, Fellers G (2012) Effects of the Amphibian Chytrid Fungus and Four Insecticides on Pacific Treefrogs (Pseudacris regilla). J Herpetol 46:625–631. https://doi.org/10.1670/11-070

Klimek S, Lohss G, Gabriel D (2014) Modelling the spatial distribution of species-rich farmland to identify priority areas for conservation actions. Biol Conserv 174:65–74. https://doi.org/10.1016/j.biocon.2014.03.019

Koprivnikar J (2010) Interactions of environmental stressors impact survival and development of parasitized larval amphibians. Ecol Appl 20:2263–2272. https://doi.org/10.1890/09-1558.1

Kröger R, Moore MT, Locke MA, et al (2009) Evaluating the influence of wetland vegetation on chemical residence time in Mississippi Delta drainage ditches. Agric Water Manag 96:1175–1179. https://doi.org/10.1016/j.agwat.2009.03.002

Kuznetsova A, Brockhoff PB, Haubo Bojesen R (2015) lmerTest: Tests in linear mixed effects models. R Package Version 2.0–29.

Lambert MR, Smylie MS, Roman AJ, et al (2018) Sexual and somatic development of wood frog tadpoles along a thermal gradient. J Exp Zool Part A, Ecol Integr Physiol 329:72–79. https://doi.org/10.1002/jez.2172

Langlois VS, Carew AC, Pauli BD, et al (2010) Low levels of the herbicide atrazine alter sex ratios and reduce metamorphic success in Rana pipiens tadpoles raised in outdoor mesocosms. Environ Health Perspect 118:552–557. https://doi.org/10.1289/ehp.0901418

Lee-Jenkins SY, Robinson SA (2018) Effects of Neonicotinoids on Putative Escape Behavior of Juvenile Wood Frogs (Lithobates sylvaticus) Chronically Exposed as Tadpoles. Env Toxicol Chem Chem 37:3115–3123. https://doi.org/10.1002/etc.4284

Levavasseur F, Biarnès A, Bailly JS, Lagacherie P (2014) Time-varying impacts of different management regimes on vegetation cover in agricultural ditches. Agric Water Manag 140:14–19. https://doi.org/10.1016/j.agwat.2014.03.012

Lund J (1967) Eutrophication. Nature 214:557–558

Maisonneuve C, Rioux S (2001) Importance of riparian habitats for small mammal and herpetofaunal communities in agricultural landscapes of southern Québec. Ecosyst Environ 83:165–175

Mann RM, Hyne R V., Choung CB, Wilson SP (2009) Amphibians and agricultural chemicals: Review of the risks in a complex environment. Environ Pollut 157:2903–2927. https://doi.org/10.1016/j.envpol.2009.05.015

Mazerolle MJ (2005) Drainage Ditches Facilitate Frog Movements in a Hostile Landscape. Landsc Ecol 20:579–590. https://doi.org/10.1007/s10980-004-3977-6

McDaniel T V., Harris ML, Bishop CA, Struger J (2004) Development and survivorship of Northern Leopard frogs (Rana pipiens) and green frogs (Rana clamitans) exposed to contaminants in the water and sediments of the St. Lawrence River near Cornwall, Ontario. Water Qual Res J Canada 39:160–174. https://doi.org/10.2166/wqrj.2004.025

McDiarmid RW, Altig R (1999) Tadpoles: Biology of Anuran Larvae, 1st edn. University of Chicago Press, Chicago and London

McDiarmid RW, Mitchell JC (2000) Diversity and distribution of amphibians and reptiles. In: Sparling D, Linder G, Bishop C (eds) Ecotoxicology of Amphibians and Reptiles. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp 15–69

MMIWG (2019) Reclaiming power and place: The final report of the national inquiry into the missing and murdered indigenous women and girls. https://www.mmiwg-ffada.ca/. Accessed 18 Jun 2020

Moore M, Bennett E, Cooper C, et al (2001) Transport and fate of atrazine and lambda-cyhalothrin in an agricultural drainage ditch in the Mississippi Delta, USA. Agric Ecosyst Environ 87:309–314. https://doi.org/10.1016/S0167-8809(01)00148-7

Morin B (2020) As the RCMP deny systemic racism, here's the real history | The Star. Star

Morrissey CA, Mineau P, Devries JH, et al (2015a) Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environ. Int. 74:291–303

Morrissey CA, Mineau P, Devries JH, et al (2015b) Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review Environment International. Environ Int 74:291–303. https://doi.org/10.1016/j.envint.2014.10.024

Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: A practical guide for biologists. Biol Rev 82:591–605. https://doi.org/10.1111/j.1469-185X.2007.00027.x

Native Land Digital (2015) Native-Land.ca | Our home on native land. https://native-land.ca/about/. Accessed 18 Jun 2020

Needelman BA, Kleinman PJA, Allen AL (2007) Improved management of agricultural drainage ditches for water quality protection : An overview. J Soil Water Conserv 62:171–178

Nowell LH, Norman JE, Moran PW, et al (2014) Pesticide toxicity index-a tool for assessing potential toxicity of pesticide mixtures to freshwater aquatic organisms. Sci Total Environ 476–477:144–157. https://doi.org/10.1016/j.scitotenv.2013.12.088

Oda FH, Batista VG, Gambale PG, et al (2016) Anuran species richness, composition, and breeding habitat preferences: A comparison between forest remnants and agricultural landscapes in Southern Brazil. Zool Stud 55:1–14

OECD (2009) Test No. 231: Amphibian Metamorphosis Assay. In: OECD Guidelines for the Testing of Chemicals. OECD, Paris

OECD (2015) Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA). OECD iLibrary 227:1–19. https://doi.org/10.1787/9789264242340-en

Oldham M, Bakowsky W, Sutherland D (1995) Floristic quality assessment system for Southern Ontario, Ontario, Canada

Orton F, Carr JA, Handy RD (2006) Effects of nitrate and atrazine on larval development and sexual differentiation in the northern Leopard frog Rana pipiens. Environ Toxicol Chem 25:65–71. https://doi.org/10.1897/05-136R.1

Padoa E (1936) Effetto paradossale (mascolinizzazione) sulla differenziazione sessuale di girini Rana esculenta trattati con ormone follicolare. Mon Zool Ital 47:285–289

Pappas E, Smith D (2007) Effects of dredging an agricultural drainage ditch on water column herbicide concentration, as predicted by fluvarium techniques. J Soil Water Conserv 62:262–268

Perron MAC, Pick FR (2020) Stormwater ponds as habitat for Odonata in urban areas: the importance of obligate wetland plant species. Biodivers Conserv 29:913–931. https://doi.org/10.1007/s10531-019-01917-2

Pickford DB, Jones A, Velez-Pelez A, et al (2015) Screening breeding sites of the common toad (Bufo bufo) in England and Wales for evidence of endocrine disrupting activity. Ecotoxicol Environ Saf 117:7–19. https://doi.org/10.1016/j.ecoenv.2015.03.006

Piha H, Luoto M, Pihas M, Merila J (2007) Anuran abundance and persistence in agricultural landscapes during a climatic extreme. Glob Chang Biol 13:300–311. https://doi.org/10.1111/j.1365-2486.2006.01276.x

Pohl J, Örn S, Norrgren L, Carlsson G (2015) Toxicological evaluation of water from stormwater ponds using Xenopus tropicalis embryos. Wetl Ecol Manag 23:1091–1098. https://doi.org/10.1007/s11273-015-9444-0

Puglis HJ, Boone MD (2011) Effects of Technical-Grade Active Ingredient vs. Commercial Formulation of Seven Pesticides in the Presence or Absence of UV Radiation on Survival of Green Frog Tadpoles. Arch Env Contam Toxicol 60:145–155. https://doi.org/10.1007/s00244-010-9528-z

Pujol-Buxó E, San Sebastián O, Garriga NN, et al (2013) How does the invasive/native nature of species influence tadpoles' plastic responses to predators? OIKOS 122:19–29. https://doi.org/10.1111/j.1600-0706.2012.20617.x

Raworth K (2012) A safe and just space for humanity. Oxfam Discuss Pap 26

Reeves R (2014) Amphibian stress , survival , and habitat quality in restored agricultural wetlands in central Iowa

Reid AJ, Carlson AK, Creed IF, et al (2018) Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev. https://doi.org/10.1111/brv.12480

Relyea R, Jones D (2009) The Toxicity of Roundup Original Max To 13 Species of Larval Amphibians. Environ Toxicol Chem 28:2004–2008

Renaud JB, Sabourin L, Topp E, Sumarah MW (2017) Spectral Counting Approach to Measure Selectivity of High-Resolution LC−MS Methods for Environmental Analysis. Anal Chem 89:5. https://doi.org/10.1021/acs.analchem.6b03475

Riha VF, Berven KA (1991) An Analysis of Latitudinal Variation in the Larval Development of the Wood Frog (Rana sylvatica) Published by : American Society of Ichthyologists and Herpetologists (ASIH) Stable URL : http://www.jstor.org/stable/1446264 Accessed : 26-05-2016 20 : 18. Am Soc Ichthyol Herpetol 1991:209–221

Robinson RA, Sutherland WJ (2002) Post-war changes in arable farming and biodiversity in Great Britain

Robinson SA, Richardson SD, Dalton RL, et al (2019) Assessment of Sublethal Effects of Neonicotinoid Insecticides on the Life-History Traits of 2 Frog Species. Environ Toxicol Chem 38:1967–1977. https://doi.org/10.1002/etc.4511

Robinson SA, Richardson SD, Dalton RL, et al (2017) Sublethal effects on wood frogs chronically exposed to environmentally relevant concentrations of two neonicotinoid insecticides. Environ Toxicol Chem 36:1101–1109. https://doi.org/10.1002/etc.3739

Rockström J, Steffen WL, Noone K, et al (2009) Planetary Boundaries : Exploring the Safe Operating Space for Humanity. Nature 14:. https://doi.org/10.1038/461472a

Rodney SI, Teed RS, Moore DRJ (2013) Estimating the Toxicity of Pesticide Mixtures to Aquatic Organisms: A Review. Hum Ecol Risk Assess 19:1557–1575. https://doi.org/10.1080/10807039.2012.723180

Rosenzweig C, Tubiello FN, Goldberg R, et al (2002) Increased crop damage in the US from excess precipitation under climate change. Glob Environ Chang 12:197–202. https://doi.org/10.1016/S0959-3780(02)00008-0

Rosset PM, Martinez-Torres ME La Via Campesina and Agroecology

Royer T V., Prussian AM, Minshall GW (1999) Impact of suction dredging on water quality, benthic habitat, and biota in the Fortymile River and Resurrection Creek, Alaska. Pocatello, Idaho USA

Rudolf VHW, Rödel MO (2007) Phenotypic plasticity and optimal timing of metamorphosis under uncertain time constraints. Evol Ecol 21:121–142. https://doi.org/10.1007/s10682-006-0017-9

Rumrill CT, Scott DE, Lance SL (2018) Delayed Effects and Complex Life Cycles: How the Larval Aquatic Environment Influences Terrestrial Performance and Survival. Environ Toxicol Chem 37:2660–2669. https://doi.org/10.1002/etc.4228

Ruthsatz K, Peck MA, Dausmann KH, et al (2018) Patterns of temperature induced developmental plasticity in anuran larvae. J Therm Biol 74:123–132. https://doi.org/10.1016/j.jtherbio.2018.03.005

Schenck FJ, Hobbs JE (2004) Evaluation of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Approach to Pesticide Residue Analysis Environmental Contamination and Toxicology 24. Bull Environ Contam Toxicol 73:24–30. https://doi.org/10.1007/s00128-004-0388-y

Schindler DW (1974) Eutrophication and Recovery in Experimental Lakes : Implications for Lake Management. Science (80-) 184:897–899

Schwaiger J, Ferling H, Mallow U, et al (2003) Endocrine effects of environmental pollution on Xenopus laevis and Rana temporaria. Env Res 93:195–201

Shahid N, Becker JM, Krauss M, et al (2018) Pesticide body burden of the crustacean Gammarus pulex as a measure of toxic pressure in agricultural streams. Environ Sci Technol acs.est.8b01751. https://doi.org/10.1021/acs.est.8b01751

Shi Y-B (2000) Amphibian metamorphosis : from morphology to molecular biology. Wiley-Liss, Bethesda, MD

Shigaki F, Schmidt JP, Kleinman PJA, et al (2009) Nitrogen Fate in Drainage Ditches of the Coastal Plain after Dredging. J Environ Qual 38:2449. https://doi.org/10.2134/jeq2008.0268

Sievers M, Hale R, Swearer SE, Parris KM (2018) Contaminant mixtures interact to impair predator-avoidance behaviours and survival in a larval amphibian. Ecotoxicol Environ Saf 161:482–488. https://doi.org/10.1016/j.ecoenv.2018.06.028

Simon E, Puky M, Braunn M, Tothmeresz B (2011) Frogs and toads as biological indicators in enviromental assessment. In: Murray J (ed) Frogs: Biology, Ecology and Uses. Nova Science Publishers, Inc., Debrecen, Hungary, pp 141–150

Skaggs R, Youssef M, Chescheir G, Gilliam J (2005) Effect of drainage intensity on nitrogen losses from drained lands. Trans ASAE 48:2169–2177. https://doi.org/10.13031/2013.20103

Skelly D, Freidenburg LK (2002) Forest Canopy and the Performance of Larval Amphibians. Ecology 83:983–992. https://doi.org/10.1890/0012-9658(2002)083

Smalling K, Orlando J, Calhoun D, et al (2012) Occurrence of Pesticides in Water and Sediment Collected from Amphibian Habitats Located Throughout the United States , 2009 – 2010. Reston, Virginia

Smalling KL, Fellers GM, Kleeman PM, Kuivila KM (2013) Accumulation of pesticides in pacific chorus frogs (Pseudacris regilla) from California's Sierra Nevada Mountains, USA. Environ

Toxicol Chem 32:2026–2034. https://doi.org/10.1002/etc.2308

Smalling KL, Reeves R, Muths E, et al (2015) Pesticide concentrations in frog tissue and wetland habitats in a landscape dominated by agriculture. Sci Total Environ 502:80–90. https://doi.org/10.1016/j.scitotenv.2014.08.114

Smith-Gill SJ, Berven KA (1979) Predicting amphibian metamorphosis. Am Nat 113:563–585. https://doi.org/10.1086/283413

Smith (2006) Hatching plasticity in two temperate anurans: responses to a pathogen and predation cues. Can J Zool 84:556–563. https://doi.org/10.1139/Z06-058

Smith (1987) Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–350. https://doi.org/10.2307/1939265

Smith D, Huang C (2010) Assessing Nutrient Transport Following Dredging of Agricultural Drainage Ditches. Trans ASABE 53:429–436

Smith D, Pappas E (2007) Effect of ditch dredging on the fate of nutrients in deep drainage ditches of the Midwestern United States. Soil Water Conserv 62:252

Smith D, Pappas E (2006) Effect of ditch dredging on the fate of nutrients in deep drainage ditches of the Midwestern United States. J Soil Water Conserv 62:252–261

Smith DR, Warnemuende EA, Haggard BE, Huang C (2006) Dredging of Drainage Ditches Increases Short-Term Transport of Soluble Phosphorus. J Environ Qual 35:611. https://doi.org/10.2134/jeq2005.0301

SNCA (2018) State of the Nation Watershed Report Card 2018. Finch

Sparling D, Linder G, Bishop C (2000) Ecotoxicology of amphibians and reptiles, 1st edn. Society of Environmental Toxicology and Chemistry, Pensacola, FL

Stoate C, Boatman ND, Borralho RJ, et al (2001) Ecological impacts of arable intensification in Europe. J Environ Manage 63:337–365. https://doi.org/10.1006/jema.2001.0473

Strock J, Dell cJ, Schmidt J (2007) Managing natural processes in drainage ditches for non point source nitrogen control. Soil Water Conserv 62:188–196

Stuart SN, Chanson JS, Cox NA, et al (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–6. https://doi.org/10.1126/science.1103538

Sunohara MD, Gottschall N, Wilkes G, et al (2015) Long-term observations of nitrogen and phosphorus export in paired-agricultural watersheds under controlled and conventional tile drainage. J Environ Qual 44:1589–1604. https://doi.org/10.2134/jeq2015.01.0008

Sunohara MD, Topp E, Wilkes G, et al (2012) Impact of Riparian Zone Protection from Cattle on Nutrient, Bacteria, F-coliphage, , and Loading of an Intermittent Stream. J Environ Qual 41:1301. https://doi.org/10.2134/jeq2011.0407

Swanson JE, Muths E, Pierce CL, et al (2018) Exploring the amphibian exposome in an

agricultural landscape using telemetry and passive sampling. Sci Rep 8:1–10. https://doi.org/10.1038/s41598-018-28132-3

Tavera-Mendoza L, Ruby S, Brousseau P, et al (2002) Response of the amphibian tadpole (Xenopus laevis) to atrazine during sexual differentiation of the testis. Environ Toxicol Chem 21:527–531. https://doi.org/10.1002/etc.5620210309

Thermo Fisher Scientific (2013) Xcalibur 3.0 Data Acquisition and Processing User Guide Version A

Torma A, Bozsó M, Gallé R (2017) Secondary habitats are important in biodiversity conservation: A case study on orthopterans along ditch banks. Anim Biodivers Conserv 41:97–108. https://doi.org/10.32800/abc.2018.41.0097

TRC (2015) Truth and Reconciliation Commission of Canada. http://www.trc.ca/. Accessed 18 Jun 2020

Trudeau VL, Schueler FW, Navarro-Martin L, et al (2013) Efficient induction of spawning of Northern leopard frogs (Lithobates pipiens) during and outside the natural breeding season. Reprod Biol Endocrinol 11:14. https://doi.org/10.1186/1477-7827-11-14

Twisk W, Noordervliet MAW, ter Keurs WJ (2000) Effects of ditch management on caddisfly, dragonfly and amphibian larvae in intensively farmed peat areas. Aquat Ecol 34:397–411. https://doi.org/10.1023/A:1011430831180

Valls JH, Mills NE (2007) Intermittent hypoxia in eggs of Ambystoma maculatum: Embryonic development and egg capsule conductance. J Exp Biol 210:2430–2435. https://doi.org/10.1242/jeb.003541

Van Meter RJ, Glinski DA, Hong T, et al (2014) Estimating terrestrial amphibian pesticide body burden through dermal exposure. https://doi.org/10.1016/j.envpol.2014.07.003

Van Metre PC, Alvarez DA, Mahler BJ, et al (2017) Complex mixtures of Pesticides in Midwest U.S. streams indicated by POCIS time-integrating samplers. Environ Pollut 220:431–440. https://doi.org/10.1016/j.envpol.2016.09.085

Venables WN, Ripley BD (2002) Modern applied statistics with S, 4th edn. Springer, New York

von der Ohe P, de Zwart D (2013) Toxic Unit (TU) Indicators. Encycl. Aquat. Ecotoxicol. 1161–1170

Walters RJ, Hassall M (2006) The Temperature-Size Rule in Ectotherms: May a General Explanation Exist after All? Source Am Nat 167:510–523. https://doi.org/10.1086/501029

Wang Y, Li X, Yang G, et al (2020) Changes of enzyme activity and gene expression in embryonic zebrafish co-exposed to beta-cypermethrin and thiacloprid. Environ Pollut 113437

Wang Y, Wu S, Chen J, et al (2018) Single and joint toxicity assessment of four currently used pesticides to zebrafish (Danio rerio) using traditional and molecular endpoints. Chemosphere 192:14–23. https://doi.org/10.1016/j.chemosphere.2017.10.129

Warkentin KM (2011) Plasticity of hatching in amphibians: Evolution, trade-offs, cues and mechanisms. In: Integrative and Comparative Biology. pp 111–127

Welsh HH, Ollivier LM (1998) Stream Amphibians as Indicators of Ecosystem Stress : A Case Study from California ' s Redwoods STREAM AMPHIBIANS AS INDICATORS OF ECOSYSTEM STRESS : A CASE STUDY FROM CALIFORNIXS REDWOODS. Ecol Appl 8:1118–1132. https://doi.org/10.1890/1051-0761(1998)008[1118:SAAIOE]2.0.CO;2

Wicklund RE, Richards NR (1962) Soil survey of Russell and Prescott counties

Wilbur HM (1977) Propagule Size, Number, and Dispersion Pattern in Ambystoma and Asclepias. Am Nat 111:43–68. https://doi.org/10.1086/283137

Wilbur HM, Collins JP (1973) Ecological Aspects of Amphibian Metamorphosis

Wilkes G, Sunohara MD, Topp E, et al (2019) Do reductions in agricultural field drainage during the growing season impact bacterial densities and loads in small tile-fed watersheds? Water Res 151:423–438. https://doi.org/10.1016/j.watres.2018.11.074

Witschi E (1930) Studies on sex differentiation and sex determination in amphibians. IV. The geographical distribution of the sex races of the European grass frog (Rana temporaria, L.). A contribution to the problem of the evolution of sex. J Exp Zool 56:149–165. https://doi.org/10.1002/jez.1400560202

Witschi E (1929) Studies on sex differentiation and sex determination in amphibians. II. Sex reversal in female tadpoles of Rana sylvatica following the application of high temperature. J Exp Zool 52:267–291. https://doi.org/10.1002/jez.1400520203

Xing Z, Chow L, Rees H, et al (2013) Influences of sampling methodologies on pesticide-residue detection in stream water. Arch Environ Contam Toxicol 64:208–218. https://doi.org/10.1007/s00244-012-9833-9

Young SD, Gavel MJ, Gutierrez-Villagomez JM, et al (2020) Assessment of sublethal ecotoxicity of solvents on larvae of a model native amphibian (Lithobates pipiens). J Appl Toxicol 40:483–492. https://doi.org/10.1002/jat.3920

Zhang S, Zhou Q, Xu D, et al (2010) Effects of sediment dredging on water quality and zooplankton community structure in a shallow of eutrophic lake. J Environ Sci 22:218–224. https://doi.org/10.1016/S1001-0742(09)60096-6

Zuur A, Hilbe J, Leno E (2015) A Beginner's Guide to GLM and GLMM with R: A Frequentist and Bayesian Perspective for Ecologists. Highland Statistics Ltd, Newburgh, UK

Zuur AF (2009) Mixed effects models and extensions in ecology with R. Springer

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Zwart D De (2005) Impact of Toxicants on Species Composition of Aquatic Communities: Concordance of Predictions and Field Observations. Thesis Univ Amsterdam 224