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The Effects of Ditch Management in Agroecosystems on Embryonic and Tadpole Survival, Growth, and Development of Northern Leopard Frogs (*Lithobates pipiens*)

Amber Dyck[1] · Stacey A. Robinson[2] · Sarah D. Young[2] · Justin B. Renaud[3] · Lyne Sabourin[3] · David R. Lapen[4] · Frances R. Pick[1]

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Abstract

Agricultural drainage ditches help remove excess water from fields and provide habitat for wildlife. Drainage ditch management, which includes various forms of vegetation clearing and sediment dredging, can variably affect the ecological function of these systems. To determine whether ditch conditions following dredging/vegetation clearing management affected the survival, growth, and development of embryos and tadpoles of northern leopard frogs (*Lithobates pipiens*), we conducted three field studies using in situ cages over 2 years. We measured nutrients, pesticides, and other water quality properties in vegetated/unmanaged (i.e., no clearing or dredging) and newly cleared/dredged (i.e., treeless, then dredged), clay-bottomed drainage ditches in a river basin in Eastern Ontario, Canada. Nutrients, atrazine, and total neonicotinoid concentrations were generally lower at the cleared/dredged sites, whereas glyphosate was at higher concentrations. In contrast, water-quality variables measured in situ, particularly temperature, dissolved oxygen, and turbidity, tended to be higher in the cleared/dredged sites. Total phosphorous and total organic carbon concentrations at all sites were above the recommended limits for amphibian assays. No significant differences were detected in the survival, hatching success, or development of embryos among the ditch management treatments, but premature hatching was observed at one vegetated/unmanaged site where high specific conductivity may have been formative. We found the cleared/dredged sites supported earlier tadpole growth and development, likely as a result of the higher water temperatures. Increased temperature may have offset other growth/development stressors, such as those related to water chemistry. However, the long-term consequences of these differences on amphibian populations requires further study.

Agriculture drainage ditches are small, typically constructed waterways, designed to help drain excess water from agricultural fields to improve crop production. These ditches often receive artificial subsurface drainage (tile) from adjacent fields, and globally, it is estimated that 190 million hectares of agricultural land is artificially drained (Biggs et al. 2017). Ditches can provide a vast array of ecological services and can critically regulate the cycling, assimilation, and sequestration of agro-chemicals (Sunohara et al. 2012; Dollinger et al. 2015). Ditch bank and instream vegetation also can govern stream discharge and control mass export of sediment (Flora and Kröger 2014).

Ditches provide critical habitat for aquatic and terrestrial wildlife and support biodiversity in landscapes otherwise occupied by field crops (Herzon and Helenius 2008). In addition, ditches serve as migration corridors (Mazerolle 2005) and breeding zones (Hartel et al. 2011; Oda et al. 2016) for amphibians (and other taxa) by connecting isolated refuges. They also provide predatory fish-free zones (Herzon and Helenius 2008), which can support amphibian breeding.

Ditch management practices can impact ecosystem services and ecological functions to various degrees, depending

✉ Stacey A. Robinson
Stacey.Robinson@canada.ca

[1] Department of Biology, University of Ottawa, Ottawa, ON, Canada

[2] Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Science Directorate, Science and Technology Branch, National Wildlife Research Centre, Environment and Climate Change Canada, 1125 Colonel By Drive, Ottawa, ON K1A 0H3, Canada

[3] London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

[4] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada

on the nature and timing of management intervention (Needelman et al. 2007; Dollinger et al. 2015). Ditch management typically consists of dredging and various grades of shore and near-stream vegetation removal to augment dredging access and drainage potential. In some ditch systems, dredging can occur on a routine basis to reduce excessive sedimentation, which would otherwise have a negative impact on field drainage efficiency (Evans et al. 2007; Bracewell et al. 2019).

Dredging and vegetation removal have been shown to reduce a ditch's capacity to mitigate nutrient and pesticide levels in agricultural surface waters (Smith et al. 2006; Pappas and Smith 2007; Smith and Pappas 2007; Shigaki et al. 2009; Smith and Huang 2010). Vegetation removal and dredging have been shown to result in higher stream turbidity (depending on substrate type) and vegetation removal can decrease shading (increase insolation) of ditch waters, thereby potentially increasing water temperatures (Fiener and Auerswald 2003; Dollinger et al. 2015).

Amphibians use agriculture ditches for breeding and feeding and are sensitive to many stressors (Sparling et al. 2000; Herzon and Helenius 2008; Collins and Fahrig 2017; Bracewell et al. 2019). Exposure to agro-chemicals at critical environmental concentrations can cause lethal and sublethal effects to amphibians, 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). Amphibian health also is affected by many environmental factors, including (but not limited to) temperature, turbidity, water availability, population density, light, food availability, and salinity (Hecnar and M'Closkey 1996; Shi 2000). Conventional morphological and developmental endpoints for amphibians, which are often used as indicators for successful juvenile recruitment and adult fitness, include survival and life-history traits, such as growth (i.e., snout-to-vent length, body mass) and development (Wilbur and Collins 1973; Berven and Gill 1983; Semlitsch et al. 1988). Furthermore, the liver somatic index (LSI) can be an indicator of toxic stress (Edge 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 and applied ecosystem management.

The purpose of this study was to determine whether standard ditch management (bank vegetation removal and dredging) affects survival, growth, and development of the aquatic stages of a native anuran in Eastern Ontario: the northern leopard frog (*Lithobates [Rana] pipiens*). We hypothesized that "newly" cleared/dredged ditches (representing the most extreme successional state of such a practice) would negatively affect embryo and tadpole survival, growth, and development because of changes in water quality and differences in nutrient and pesticide exposure between sites. We anticipated negative effects on embryos and tadpoles at the cleared/dredged sites because of poorer water quality. Management, particularly dredging, was expected to increase turbidity and reduce the ditch's capacity to mitigate pesticide and nutrient concentrations, resulting in poorer water quality, as found in previous ditch management studies (Fiener and Auerswald 2003; Smith et al. 2006; Pappas and Smith 2007; Smith and Huang 2010; Dollinger et al. 2015). We used in situ cages (Harris et al. 2001) to test for effects of ditch management on both embryos and tadpoles. The use of cages provides control for genetic variation, predation, and food supply while allowing sufficient replication of treatments (de Solla et al. 2002).

Materials and Methods

Experimental Design and site Description

This study was undertaken in the ~ 4000 km^2 South Nation river basin in Eastern Ontario, Canada (Fig. S1). Approximately 60% of the watershed is used for intensive agriculture (Sunohara et al. 2012)—principally corn (*Zea mays*), soybean (*Glycine max*), and forage/hay production (Wilkes et al. 2019). Nutrient concentrations are elevated in South Nation streams along with herbicide concentrations (atrazine in particular) (Dalton et al. 2015a), as is common for streams draining agricultural lands. Dalton et al. (2015a) reported high in-stream concentrations of reactive phosphate (6–65 µg/L) and nitrate (3–3981 µg/L). Additionally, they reported time-weighted average concentrations of atrazine from 4–412 ng/L, with concentrations > 100 ng/L at more than half of their 24 study sites across the South Nation.

Specifically, we conducted our study within two paired experimental drainage ditch watersheds (see Sunohara et al. 2015 and Fig. 1in Wilkes et al. 2019). The total water catchment areas were 467 ha and 250 ha for the vegetated/unmanaged and cleared/dredged (i.e., managed) watersheds, respectively. Land use was characterized as per Wilkes et al. (2014, 2019) and Sunohara et al. (2015). Further details on the methods and results of the land use characterization are in Supplemental Information.

The in situ anuran (i.e., embryo and tadpole) cage experiments were conducted on the paired watersheds during 2018 and 2019. The vegetated/unmanaged (*V*) watershed had no dredging or in-stream or riparian zone vegetation clearing since the ditch was constructed in the early 1980s. The adjacent cleared/dredged (CD) drainage ditch had all riparian woody vegetation removed in the winter of 2017 (i.e., woody vegetation removal along the banks) and was dredged in the fall of 2018 (i.e., removal of approximately 30 cm of sediment from the ditch bed). Overall, we selected six

ditch sites for the embryo and tadpole experiments, three in the vegetated/unmanaged ditches, and three in the cleared/dredged ditches. Three in situ anuran exposures were conducted in 2018 and 2019 (Table 1). The first tadpole experiment (2018) was conducted at two sites (i.e., V3 and CD3; Fig. S1a) with nine replicate cages at each site ($N = 18$ cages total). In 2019, a second tadpole experiment was conducted at four sites (i.e., V2, V3, CD2, and CD3; Fig. S1b) with three replicate cages at each site ($N = 12$ cages total). For the embryo experiment (2019), we used two of the same sites as the tadpole experiments (i.e., V3 and CD3) but included two additional sites (i.e., V4 and CD4; Fig. S1c). Here, the two additional sites (V4 and CD4) were selected based on proximity to the paired experiment watersheds and matched our ditch management needs (vegetated/unmanaged or cleared/dredged).

Anuran Collection and Husbandry

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, SR05-2019). Northern leopard frogs were chosen as the model species, because they are native and common within the study region (Collins and Fahrig 2017). Several studies have reported observations of wild amphibians, including Northern leopard frogs in agriculture ditches in Eastern Ontario and Southern Quebec (Maisonneuve and Rioux 2001; Collins and Fahrig 2017), and their presence has been recorded in our study watersheds by acoustic monitoring (unpublished data).

Adult male and female frogs were collected in late April 2018 (6 males and 6 females) and 2019 (3 males and 3 females), from nonagriculture-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 (Ottawa, ON, Canada) animal care facility, where they were paired and bred following an artificial breeding protocol (Trudeau et al. 2013).

For the tadpole experiment (2018), the tadpoles originated from six egg masses laid over May 3–4 at 16 °C at the University of Ottawa animal care facility. Full methods on the husbandry of stock tanks and rearing of stock tadpoles are in Robinson et al. (2020). Briefly, within 12 h of laying, we collected six egg masses from the University of Ottawa and transported them to Carleton University (Ottawa, ON, Canada). The egg masses were evenly distributed into four aerated 60-L stock tanks in a climate controlled Conviron environmental chamber (temperature: 22 °C; humidity: 70% humidity; diurnal cycle: 16:8 h light: dark cycle), where they developed to Gosner stage 25 tadpoles [i.e., GS25, larval/premetamorphic stage; (Gosner 1960)].

For the embryo and tadpole experiments (2019), three egg masses were laid over April 27–28 at 16 °C at the University of Ottawa animal care facility. We collected the egg masses and transported them to Carleton University, where they were held in a climate controlled Conviron environmental chamber at 13 °C for ~ 12 h (humidity: 70% humidity; diurnal cycle: 16:8 h light: dark cycle), then reduced to 10 °C to acclimatize to field site conditions (i.e., ditch water temperature of 4–8 °C) for the embryo experiment. The remaining eggs not used in the embryo experiment were held in the environmental chamber for 5 d, and the temperature was increased from 10 to 16 °C, before being evenly distributed into four 60-L aerated stock tanks in a greenhouse on the campus of Carleton University (Ottawa, ON, Canada). Conditions in the greenhouse fluctuated with natural daily rhythms (mean temperature ranged from 21.7 ± 1.4 to 42.4 ± 2.7 °C, mean humidity ranged from 18.0 ± 4.1 to 65.2 ± 10.3%, photoperiod 14–15:10–9 h light: dark cycle). Stock tank husbandry, water changes, and rearing of stock tadpoles followed similar methods described in Robinson et al. (2020) (but under greenhouse and not environmental chamber conditions), and once tadpoles reached GS25, they were collected for the tadpole experiment (as below).

Table 1 Experimental design for the three in situ anuran cage experiments using Northern leopard frogs (*Lithobates pipiens*) on paired watershed in the South Nation river basin in Eastern Ontario, Canada

Experiment	Exposure duration	Sites/treatment	Site names	Egg clutches/experiment	Replicate cages/treatment	Individuals/replicate cage	Individuals/treatment	Individuals/experiment
Embryo (2019)	16 days	2	V3, V4, CD3, CD4	3	6	60–70	384–397	781
Tadpole (2018)	28 days	1	V3, CD3	6	9	15	135	270
Tadpole (2019)	42 days	2	V2, V3, CD2, CD3	3	6	15	90	180

The length of exposure, numbers of egg clutches per experiment, number of replicate cages per treatment, number of sites per treatment, and the total number of individuals within replicate cages, treatments, and experiments in each case are included

V3, vegetated/unmanaged 3 site; V4, vegetated/unmanaged 4 site; CD2, clear/dredged 2 site; CD3, clear/dredged 3 site; CD4, clear/dredged 4 site

Nutrient and Pesticide Analysis

To monitor the agricultural chemical inputs at each ditch over the experimental periods, we collected water samples and analyzed them for nine nutrient and nine pesticide concentrations (Table 2; Table S12). We also conducted suspect screening to detect the presence of 418 pesticides, pharmaceuticals, and environmental contaminants by LC–MS/MS in both 2018 and 2019 tadpole experiments (further methods and results provided in Supplemental Information). For the tadpole experiment (2018), 1-L composite water samples (described in Sunohara et al. 2015) were collected once every 2 weeks using an ISCO 6712 portable sampler (Teledyne Isco Inc.) and analyzed for nutrient concentrations. For the embryo and tadpole experiments (2019), three grab samples (250–1000 mL) were collected once per week and analyzed for nutrient and pesticide concentrations. Amber borosilicate glass bottles with polytetrafluoroethylene (PTFE) lids were used for neonicotinoids and atrazine samples. Samples for nutrients and glyphosate were collected in high-density polyethylene (HDPE) sample bottles. Immediately before collecting water samples, all sample bottles were triple rinsed with ditch water and then filled by submerging them midway in the water column at the respective sampling site. The water samples were stored on ice during transport and samples of atrazine, glyphosate, and neonicotinoids were stored at 4 °C until analysis in July 2019. Subsamples of dissolved nutrients were filtered using 0.45-µm Whatman Uniflow Syringe Filters (GE Healthcare) and frozen until analysis 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^+), nitrate (NO_3^-), nitrite (NO_2^-), reactive phosphorus (RP), total phosphorus (TP), dissolved organic carbon (DOC), total organic carbon (TOC), and total Kjeldahl Nitrogen (TKN). Total inorganic nitrogen (TIN) was calculated by summing NH_3–NH_4^+, NO_2^-, and NO_3^- in each water sample. Total suspended solids (TSS) also were measured. The water chemistry analyses were conducted at the Robert O. Pickard Centre (Ottawa, ON, Canada), a certified laboratory, following standard protocols for the analysis of surface waters.

The pesticide water samples were collected in 2019 and analyzed for nine pesticide concentrations at the National Wildlife Research Centre (NWRC; Ottawa, ON, Canada) of Environment and Climate Change Canada (ECCC) using high-performance liquid chromatograph (1200 Series; Agilent Technologies) with tandem mass spectrometer (API 5000 Triple Quadrupole Mass Spectrometer and Turbo V™ Ion Source; AB Sciex). Specifically, analyses were conducted to determine concentrations of the more common pesticides likely to be used in the area: atrazine, glyphosate, six neonicotinoids (clothianidin, imidacloprid, thiamethoxam, acetamiprid, thiacloprid, and dinotenfuran) and a butenolide (flypyradifurone). Concentrations were determined using methods described in Collins et al. (2019) and Robinson et al. (2017) with minor modifications described in the Supplemental Information. The total concentration of neonicotinoids was calculated by summing the values of all individual neonicotinoids within each water sample, because these compounds have similar chemical structure and predicted additive toxicity (Morrissey et al. 2015).

Physiochemical Monitoring—in situ water quality

To monitor the physiochemical conditions of the water at each ditch site over the experimental periods, we measured the following variables in situ: water temperature (°C), specific conductivity (SC; µS/cm), dissolved oxygen (DO; mg/L), pH, oxidation–reduction potential (ORP; mV), water turbidity (NTU), water depth (cm), water velocity (m/s) Chlorophyll-*a* (Chl-*a*; µg/L), and total dissolved solids (TDS; g/L). For the embryo experiment (2019), a handheld YSI 6600 multiparameter water quality sonde (YSI Incorporated) was used to record temperature, SC, DO, pH, ORP, and turbidity in each cage, and 30 cm upstream and downstream during each visit (every 2 days). For the tadpole experiments (2018, 2019), we installed a multiparameter water quality probe at each site. Specifically, in 2018, a YSI 6600 sonde (YSI Incorporated) was used to record temperature, SC, DO, pH, ORP, and turbidity. In 2019, a YSI EXO2 sonde (YSI Inc.) was used to record Chl-*a* and TDS, in addition to the parameters measured in 2018. These probes were calibrated biweekly and recorded measurements every 15 min. Parameters collected between years and experiments differ slightly due to the availability of equipment in 2018 and 2019.

Embryo Experiment

For the 2019 in situ embryo experiment, the 3.5-L cylindrical cages used followed the design in Harris et al. 2001 and were made of 500-µm Nitec Mesh (Dynamic Aqua-Supply Ltd., Surrey BC) with a Velcro lid (Fig. S2). The mesh cylinder was attached to a wooden frame for floatation (Harris et al. 2001) to ensure that the bottom of the cage was less than 15-cm deep resulting in embryos close to the water–air interface (Harris and Bogart 1997; Harris et al. 1998). The cages were deployed and anchored in position using wooden dowels and bricks, 1 week before embryo addition to allow flushing by natural waters.

At the beginning of the embryo experiment (i.e., May 2, 2019), 12 replicates of 60–70 embryos (~ 20 from each of the 3 clutches) were placed into a 2-L Ziploc bag, with 1 L of stock tank water, and placed in a cooler for transportation

Table 2 Embryo (2019) and tadpole in situ experiments (2018; 2019)

Variable	MDL	Embryo experiment (2019)		Tadpole experiment (2018)		Tadpole experiment (2019)	
		Vegetated	Cleared/dredged	Vegetated	Cleared/dredged	Vegetated	Cleared/dredged
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water quality variables (mg/L)[e]							
NH_3–NH_4^+	0.005	0.10 ± 0.10 (4)	0.02 ± 0.01 (4)	0.08 ± 0.04 (4)	0.03 ± 0.01 (4)	0.21 ± 0.46 (12)	0.02 ± 0.02 (12)
NO_3^-	0.02	7.00 ± 2.10 (4)	5.22 ± 1.79 (4)	1.23 ± 1.26 (4)	0.16 ± 0.23 (4)	2.61 ± 2.95 (12)	2.37 ± 2.04 (12)
NO_2^-	0.02	0.02 ± 0.00 (4)	0.02 ± 0.00 (4)	0.03 ± 0.01 (4)	0.02 ± 0.00 (4)	0.03 ± 0.01 (12)	0.02 ± 0.00 (12)
TIN	0.02	7.20 ± 2.10 (4)	5.26 ± 1.79 (4)	1.33 ± 1.23 (4)	0.22 ± 0.24 (4)	2.85 ± 2.85 (12)	2.41 ± 2.04 (12)
RP	0.004	0.004 ± 0.00 (4)	0.004 ± 0.00 (4)	0.05 ± 0.04 (4)	0.02 ± 0.01 (4)	0.05 ± 0.04 (12)	0.004 ± 0.00 (12)
TP	0.01	0.10 ± 0.00[d] (4)	0.02 ± 0.01[d] (4)	0.16 ± 0.11[d] (4)	0.05 ± 0.03[d] (4)	0.15 ± 0.09[d] (7)	0.03 ± 0.02[d] (7)
DOC	0.5	4.9 ± 0.3 (4)	3.5 ± 0.2 (4)	6.3 ± 1.8 (4)	6.3 ± 1.7 (4)	7.0 ± 3.2 (7)	4.7 ± 1.6 (7)
TOC	0.5	4.5 ± 0.3[d] (4)	3.3 ± 0.2[d] (4)	6.4 ± 1.9[d] (4)	6.4 ± 1.8[d] (4)	6.7 ± 3.2[d] (7)	4.8 ± 1.7[d] (7)
TKN	0.05	0.8 ± 0.2 (4)	0.45 ± 0.09 (4)	0.85 ± 0.20 (4)	0.62 ± 0.10 (4)	0.90 ± 0.32 (7)	0.58 ± 0.15 (7)
TSS	2.0	112.8 ± 22.2 (4)	130.0 ± 12.5 (4)	128.0 ± 22.0 (4)	122.5 ± 11.1 (4)	129.1 ± 28.1 (7)	120.0 ± 38.4 (7)
Pesticides (ng/L)[e]							
Glyphosate	25.0	ND (6)	ND (6)	[b]	[b]	56.3 ± 3.1 (4/12)	488.0 ± 329.8 (4/12)
Atrazine	0.4	14.0 ± 7.7 (6)	8.8 ± 3.3 (6)	[b]	[b]	19.5 ± 14.5 (14)	18.8 ± 17.8 (14)
Total neonics[c]		28.1 ± 14.5 (6)	8.1 ± 1.8 (6)	[b]	[b]	17.5 ± 9.5 (14)	10.0 ± 7.5* (14)
Physiochemical in situ water quality							
Temperature (C)		9.7 ± 3.2	11.3 ± 3.2	16.8 ± 3.0	17.4 ± 2.4*	15.9 ± 3.4	18.3 ± 5.8*
SC (µS/cm)		800 ± 400	500 ± 200	732.0 ± 28.3	732.9 ± 46.1	798.1 ± 30.6	607.5 ± 100.9*
DO (mg/L)		15.2 ± 3.6	17.9 ± 4.6	6.3 ± 2.0	2.4 ± 4.3[d]*	6.9 ± 4.1	10.4 ± 4.1
pH		7.6 ± 0.3	7.6 ± 0.3	7.6 ± 0.1	7.3 ± 0.1*	7.5 ± 0.2	7.9 ± 0.3
ORP (mV)		167.8 ± 30.0	186.9 ± 25.0*	407.8 ± 11.6	187.7 ± 327.4*	415.6 ± 42.9	298.5 ± 42.2
Turbidity (NTU)		6.6 ± 6.3	13.0 ± 10.0	12.0 ± 10.6	3.0 ± 3.2*	4.5 ± 7.5	20.6 ± 24.7
Depth (cm)		26.7 ± 10.0	24.4 ± 8.5	31.9 ± 8.2	30.5 ± 9.2	28.1 ± 6.9	24.2 ± 2.1*
Velocity (m/s)		0.2 ± 0.1	0.1 ± 0.1	[b]	[b]	[b]	[b]
Chl-*a* (µg/L)		[b]	[b]	[b]	[b]	7.1 ± 4.2	5.0 ± 2.7*
TDS (g/L)		[b]	[b]	[b]	[b]	518.8 ± 19.9	394.9 ± 65.6*

Mean and standard deviation (± SD) of nutrient [ammonium–ammonia (NH_3–NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total inorganic nitrogen (TIN), reactive phosphorus (RP), total phosphorus (TP), dissolved organic carbon (DOC), total organic carbon (TOC), total Kjeldahl nitrogen (TKN)], total suspended solids (TSS), and pesticide concentrations. Physiochemical in situ water quality (i.e., temperature, specific conductivity (SC), dissolved oxygen (DO), pH, oxidation–reduction potential (ORP), water turbidity, water velocity, water depth, Chlorophyll-*a* (Chl-*a*), total dissolved solids (TDS). Northern leopard frog (*Lithobates pipiens*) embryos or tadpoles were reared in vegetated/unmanaged (i.e., no clearing or dredging) or cleared/dredged ditch sites (i.e., cleared in 2018, dredged in 2019). Ditches were located in the South Nation river basin in Eastern Ontario, Canada, with one vegetated/unmanaged and one cleared/dredged site in 2018 (i.e., total of two ditch sites) and two vegetated/unmanaged and two cleared/dredged sites in 2019 (i.e., total of four ditch sites). Sample sizes are included in parenthesis or in Tables S8 and S9

MDL method detection limit, *ND* not detected

*Significantly different from vegetated/unmanaged ditches (i.e. cleared/dredged ditches compared to vegetated/unmanaged ditches) at $p \leq 0.05$ (see Tables S8 and S9)

[a]For the pesticide concentrations, sample size denoted as a fraction (i.e., 4/12) indicates of the 12 samples collected, 4 had detectable concentrations and 8 were ND

[b]Variables not assessed due to logistical constraints and equipment availability

[c]Total neonics = combined concentration of 6 neonicotinoids; acetamiprid, clothianidin, thiamethoxam, imidacloprid, thiacloprid, and dinotefuran

[d]Exceeds environmental and water quality guidelines for amphibian assays and the protection of aquatic life (see Supplemental Information; Table S7)

[e]Statistical analyses not performed on variables with low sample sizes of $N \leq 12$

to the field. Three replicate cages were used at each of the four sites: V3, V4, CD3, and CD4 ($N = 188–200$ embryos per site; $N = 384–397$ embryos per ditch). The embryos were acclimatized to ditch water conditions, by adding 250 mL of ditch water to the Ziploc bags every 15 min, over the course of 1 h (to minimize temperature and osmotic shock). Embryo survival was monitored every second day for 16 days, and dead embryos were recorded and removed until all surviving embryos had hatched. At the end of the experimental period (i.e., 2 weeks; May 17, 2019), all hatchlings were removed from cages, transported in individually labeled plastic containers in coolers to the NWRC of ECCC, and photographed using a Canon EOS 40D digital camera to determine survival, hatching success, and GS of development (Gosner 1960). GS was determined for all tadpoles (dead and/or alive) collected from each cage at the end of the experiment (i.e., any tadpoles that had died within 24–48 h before the end of the experiment and were not degraded had their GS assessed) using photographs and a dissecting microscope. Hatchlings were euthanized by immersion in 0.2% buffered MS-222 (tricaine methane sulfonate; Sigma-Aldrich).

Tadpole Experiments

In 2018 and 2019, the tadpole experiments were conducted using larger (58 L) cylindrical in situ cages with the same 500-µm Nitec Mesh (as described in; Harris et al. 2001; Fig. S2) with additional plastic fencing to prevent predation. Cages were anchored as described in the embryo experiment (as above) and similarly deployed in the ditches 1 week before the addition of tadpoles.

At the beginning of the tadpole experiments (i.e., May 25, 2018 and May 22, 2019), replicates of 15 tadpoles (GS25; Gosner 1960) of similar size were haphazardly selected from stock tanks, added to 2-L Ziploc bags containing 1 L of stock tank water, and placed in coolers for transportation to the field sites. In 2018, there were 18 replicates at sites V3 and CD3 (i.e., 9 cages per site; Table 1). In 2019, there were 12 replicates at sites V2, V3, CD2, CD3 (i.e., 3 cages per site; Table 1). Tadpoles were acclimatized to the ditch water following the same protocol as for the embryos (as above). After the acclimatization period, tadpoles were added to each cage, and frozen kale (Presidents Choice®) and Ward's *Xenopus* tadpole food was provided ad libitum to ensure food supply would not be a confounding factor (Crane et al. 2007). Sites were visited twice per week (Mondays and Thursdays), where all tadpoles from a cage were captured (using a turkey baster or small fish net) and placed in a clear container containing site-specific ditch water and placed in a shaded area. For each cage, tadpole survival and water depth were recorded, uneaten/surplus kale was removed, and cages were lightly cleaned with brushes to remove excessive periphyton build up to ensure all cages were at a similar state of cleanliness and thus water exchange. Once cages were clean, tadpoles were returned to their respective cages, and frozen kale and Wards *Xenopus* tadpole food were provided ad libitum.

On June 21, 2018 (4 weeks) and July 2, 2019 (6 weeks), the tadpole experiments were terminated due to low ditch water levels (≤ 10 cm water depth). Tadpoles were collected from each cage using two individually labeled plastic containers and transported in coolers to the NWRC of ECCC. Individual tadpoles were then anesthetized by immersion in 0.015% buffered MS-222, blotted dry, weighed (± 0.01 g; Mettler Toledo analytical balance), and photographed for later body measurements (i.e., snout-to-vent length, body width, and tail length) using ImageJ image analysis software [Version 1.52a; U.S. National Institutes of Health (Schneider et al. 2012)]. To confirm inter- and intra-observer variability in measurements, 3–6 tadpoles were randomly selected and measured 2–20 times without reference to previous measurements. Acceptable levels of precision were found, where the mean coefficients of variation ranged from 0.01 to 0.04% (Hayek et al. 2001). Individuals were then euthanized by immersion in 0.2% buffered MS-222, and the Gosner stage of development for each tadpole was determined (Gosner 1960). In 2019, tadpoles were dissected, and the liver was weighed (± 0.01 g) to calculate the liver somatic index by dividing liver mass by body mass minus liver mass [i.e., LSI = (liver mass/(body mass − liver mass)]. We confirmed the LSI was appropriate using a scatter plot of tadpole body mass and liver mass, showing the data had a linear relationship through the origin (Curran-Everett 2013) (Fig. S3). In 2019, tadpole sex was visually determined by inspecting external gonad morphology as male, female, or undifferentiated using a dissecting microscope (Witschi 1931; Robinson et al. 2019). Only tadpoles at $\geq$ GS36 were included, as this is when sexual differention is visually identifiable in *L. pipiens* (Hogan et al. 2008).

Statistical Analyses

All data were visualized and examined using R-statistical software (version 3.5.1; R Core Team 2017) following the data exploration methods suggested by Zuur et al. (2010). We used general linear models (GLMs) and general(ized) linear mixed models (GLMMs) in R statistical software with packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017). We compared the cleared/dredged ditches to the vegetated/unmanaged ditches (i.e., treatments) for determining differences in pesticide concentrations, physiochemical water quality, and effects on the survival, growth, and development of embryos and tadpoles. Best-fit models were selected using Akaike Information Criterion (AIC; Burnham and Anderson 2004; Nakagawa and Cuthill 2007), where the

model with the lowest AIC was selected, specifically for when models failed to converge or issues of singularity occurred with the random effects (Table S6).

First, we used general linear mixed models with Gaussian distributions and default (identity) links to determine if cleared/dredged (compared to vegetated/unmanaged) ditches affected the pesticide concentrations during the 2019 tadpole experiments and physiochemical water quality during the embryo (2019) and tadpole (2018, 2019) experiments. Models were selected based on the lowest AIC values for including the random effects of site (to account for the variation among multiple sites per ditch treatment) and date (to account for the variation over time; Table S6). For the tadpole 2018 experiment, site was not included as a random effect, because there was only one site per ditch treatment. Statistical models were not used for nutrient concentrations and some pesticide concentrations because of small sample sizes ($n \leq 12$), where only samples with detections were included (nondetects were not included in statistical analyses or mean calculations; Williams and Sweetman 2019).

We then determined if embryonic and tadpole survival, growth, and development were affected (positive or negative) by ditch management. Depending on the lowest AIC, models included the random effects of site (to account for the variation among multiple sites per ditch treatment) and cage (to account for the nonindependence of tadpoles within a cage; Table S6). For the embryo experiment (2019), we modeled effects on embryonic survival and hatchling success, using GLMMs with binomial distributions and logit link functions. GS was assessed for all tadpoles (dead or alive) removed from cages at the end of the embryo experiment. For both tadpole experiments (2018, 2019), we modeled effects on tadpole survival and the proportion of males to females (2019 only) using GLMs with binomial distributions and logit link functions. For the remaining growth and development endpoints [i.e., snout-to-vent length, body width, tail length, body mass, liver somatic index (2019 only)], we used GLMMs with Gaussian distributions and default (identity) links. Survival and Gosner stage of development were included as fixed effects to account for density effects (Johnson et al. 2017) and differences in size with developmental age (Gosner 1960). For GS of development, we used the multiquantal Jonckheere–Terpstra test (MQJT) for ordered differences between classes—where we calculated and compared the percentiles (20%, 30%, 40%, 50%, 60%, 70%, and 80%) of Gosner stage for each cage of each ditch and the overall effect was determined using the median p value (Green et al. 2018). Model validation followed Zuur et al. (2013), and outliers were removed if determined to be from equipment malfunction or error.

Results

Nutrient and Pesticide Concentrations

Although small sample sizes precluded statistical comparisons for nutrient concentrations between treatments across all the studies, a few differences emerged between treatments. For all three experiments, nutrient concentrations were generally similar or lower at the cleared/dredged ditches relative to the vegetated ditches (Table 2). NH_3–NH_4^+, RP, and TP were all 2 × to 13 × greater at the vegetated/unmanaged ditch, whereas the remaining nutrient concentrations were relatively similar in both ditches (Table 2). TP and TOC concentrations were both above the recommended limits for amphibian assays and for the protection of aquatic life (> 0.02 mg/L for TP; > 2 mg/L for TOC; OECD 2015; Table S7) at both ditch types for all experiments.

Again, due to the small number of pesticide samples, statistical comparisons were only possible for some in the tadpole 2019 study. As such, we simply highlight the main apparent differences during the embryo 2019 study. Glyphosate was not detected at either the vegetated/unmanaged or the cleared/dredged ditches during the embryo study (Table 2). Concentrations of atrazine and total neonicotinoids tended to be elevated at the vegetated/unmanaged treatment (Table 2), and five of the seven screened neonicotinoids were detected at the vegetated/unmanaged treatment compared with only two of seven at the cleared/dredged treatment (Table S12). Clothianidin was the neonicotinoid with the highest concentrations at both treatments. The total neonicotinoid concentration was 28.1 ± 14.5 ng/L at the vegetated/unmanaged ditches and 8.1 ± 1.8 ng/L at the cleared/dredged ditches, both of which are well below the Canadian guideline of 230 ng/L for imidacloprid (CCME 2007; Table 2, S7). We used imidacloprid as a surrogate for the toxicity of total neonicotinoids, because guideline toxicity information is not available for each individual neonicotinoid. They have the same mode of action, suggesting additive toxicity (Rodney et al. 2013; Anderson et al. 2015).

For the tadpole experiment (2019), glyphosate was detected at both the vegetated/unmanaged ditches and cleared/dredged ditches at 18.8 ± 27.7 ng/L and 162.7 ± 295.6 ng/L, respectively (Table 2; Table S9). Atrazine also was detected at both ditches, at concentrations of 19.5 ± 14.5 ng/L at the vegetated/unmanaged ditches and 18.8 ± 17.8 at the cleared/dredged ditches (Table 2; Table S9). Of the seven neonicotinoids, three were detected at the vegetated/unmanaged ditches, with clothianidin having the highest detected concentration of 16.2 ± 9.1 ng/L (Table S12). Six neonicotinoids were

detected at the cleared/dredged ditches, with clothianidin also having the highest detected concentration of 9.1 ± 6.8 ng/L. The total neonicotinoid concentration at both ditches was below the Canadian guideline for imidacloprid at 17.5 ± 9.5 ng/L and 10.0 ± 7.5 ng/L for vegetated/unmanaged and cleared/dredged ditches respectively (Table 2; Table S7). Detection frequencies for a wide range of other pesticide compounds were determined for both of the tadpole experiments (2018 and 2019). Overall, 25 of the 35 compounds with positive detections in 2018 and 38 of the 62 compounds with positive detections in 2019 were found in both ditch treatments (see Supplemental Information Table S11).

Physiochemical In Situ Water Quality

For the embryo experiment (2019), there were no significant differences detected in physiochemical in situ water quality variables when comparing the cleared/dredged ditches to the vegetated/unmanaged ditches (Table 2; Table S8). However, there was a small but significant difference in the oxidative-reduction potential (ORP) between the cleared/dredged ditches compared to the vegetated/unmanaged ditches, where the cleared/dredged ditches had a higher mean ORP of 186.9 ± 25.0 mV compared with 164.7 ± 36.9 mV at the vegetated/unmanaged ditch sites (Table 2; Table S8). The concentrations of physiochemical variables were compared with the recommended limits for amphibian assays and the protection of aquatic life, where the dissolved oxygen and pH levels for both ditches were within recommended limits (DO > 3.5 mg/L; pH 6.5–8.5; (OECD 2009, 2015); Table S7).

The in situ water quality was significantly different for multiple variables in the cleared/dredged ditches compared with the vegetated/unmanaged ditches during both tadpole experiments (2018, 2019). For the tadpole experiment (2018) specifically, temperature was significantly higher at the cleared/dredged ditch with a mean of 17.4 ± 2.4 °C compared with 16.8 ± 3.0 °C at the vegetated/unmanaged ditch (Table 2; Table S9). However, dissolved oxygen (CD = 2.4 ± 4.3; V = 6.3 ± 2.0 mg/L), pH (CD = 7.3 ± 0.1; V = 7.6 ± 0.1), oxidative-reduction potential (CD = 187.7 ± 327.4; V = 407.8 ± 11.6 mV), and turbidity (CD = 3.0 ± 3.2; V = 12.0 ± 10.6 NTU) were all significantly lower at the cleared/dredged ditch compared with the vegetated/unmanaged ditch during time of study (Table 2; Table S9). Specific conductivity and water depth were not significantly different. Dissolved oxygen at the cleared/dredged ditch (2.4 ± 4.3 mg/L) was below the OECD recommended limit, and pH was within the recommended limits for amphibian assays (DO > 3.5 mg/L; pH 6.5–8.5; (OECD 2009, 2015); Table S7).

For the tadpole experiment (2019), water temperature also was significantly higher in the cleared/dredged ditches (18.3 °C ± 5.8) compared with the vegetated/unmanaged ditches (15.9 °C ± 3.4; Table 1; Table S9). Specific conductivity (CD = 607.5 ± 100.9; V = 798.1 ± 30.6 µS/cm), water depth (CD = 24.2 ± 2.1; V = 28.1 ± 6.9 cm), chlorophyll-*a* (CD = 5.0 ± 2.7; V = 7.1 ± 4.2 µg/L), and total dissolved solids (CD = 394.9 ± 65.6; V = 518.8 ± 19.9 g) were all significantly lower in the cleared/dredged ditches than in the vegetated/unmanaged ditches (Table 2; Table S9). Dissolved oxygen, pH, oxidative-reduction potential, and turbidity were not significantly different. Dissolved oxygen and pH were within the OECD recommended limits (OECD 2015; Table S7).

Anuran Response—Embryo Experiment

Embryonic survival was not significantly different between cleared/dredged and vegetated/unmanaged ditches. Cleared/dredged ditches had a mean survival of 65.6 ± 15.8% compared with the vegetated/unmanaged ditches of 51.1 ± 15.4% (Table 3; Table S8). Hatching success also was not significantly different between ditches, with mean hatching success of 68.5 ± 16.3% and 62 ± 12.0% for the cleared/dredged ditches compared with the vegetated/unmanaged ditches, respectively (Table 3; Table S8). We observed hatching beginning on Day 4 at the vegetated/unmanaged ditches where it continued at a similar linear rate for 4 to 6 days, at which point all embryos had hatched or died (Fig. 1). At the cleared/dredged ditches, hatching began more slowly, starting in earnest on Day 8 and continued for 4 more days at one site and for 8 more days at the other (Fig. 1). We observed premature hatching, where hatchlings emerged from their casings before reaching GS20, at the vegetated/unmanaged ditches (Fig. 2). Although we did not find a statistical difference in Gosner stage between the two ditch treatments (Table 3; Table S10), the proportion of premature hatching (i.e., hatchlings < GS20) was 76% at Vegetated 4 site and 0% at both the Cleared/Dredged sites (Fig. 2). Lastly, Gosner stage of tadpoles was not significantly different at the cleared/dredged (GS22 ± 1) compared with the vegetated/unmanaged ditches (GS20 ± 1; Table 2; Table S10).

Anuran Response—Tadpole Experiments

For the tadpole experiment in 2018, survival was not significantly different between cleared/dredged ditches and vegetated/unmanaged ditches, with a mean of 95 ± 5.8% for cleared/dredged compared with 89.6 ± 6.8% in vegetated/unmanaged ditches (Table 3; Table S9). Similarly, we found no significant differences in the growth and development endpoints of body width, tail length, or body mass (Table 3; Table S9). However, there was

Table 3 Summary statistics [mean ± standard deviation (SD)] for northern leopard frog (*Lithobates pipiens*) embryo and tadpole experiments using in situ cages in vegetated/unmanaged (i.e., no clearing or dredging) and cleared/dredged ditch sites (i.e., cleared in 2018, dredged in 2019) in the South Nation river basin in Eastern Ontario, Canada

Variable	Vegetated/unmanaged ditches	Cleared/dredged ditches
	Mean ± SD	Mean ± SD
Embryo experiment (2019)		
Survival	51.1 ± 15.4	65.6 ± 15.8
Hatching success	62.1 ± 12.0	68.1 ± 16.3
GS	20 ± 1	22 ± 1
Tadpole experiment (2018)[a]		
Survival	89.6 ± 6.8	95.6 ± 5.8
SVL	13.1 ± 1.8	15.4 ± 1.6*
Body width	7.5 ± 1.0	9.1 ± 1.0
Tail length	23.8 ± 3.8	29.6 ± 3.5
Body mass	1.0 ± 0.3	1.6 ± 0.4
GS	31 ± 2	35 ± 1*
Tadpole experiment (2019)		
Survival	85.6 ± 6.6	93.3 ± 8.4
Male[b]	91.7 ± 20.4	58.6 ± 12.9*
SVL	16.6 ± 2.2	19.1 ± 1.8*
Body width	9.5 ± 1.2	11.1 ± 1.1
Tail length	32.4 ± 4.8	37.4 ± 3.8*
Body mass	2.0 ± 0.6	2.8 ± 0.6
GS	35 ± 2	39 ± 2*
LSI	0.02 ± 0.01	0.02 ± 0.01

For the embryo experiment (2019), variables are percent survival (%), hatching success (%), and Gosner stage of development of hatchlings (GS). For the tadpole experiments (2018, 2019), variables include percent survival (%), proportion of males to females (Male), and tadpole growth and development: snout-to-vent length (SVL; mm), body width (mm), tail length (mm), body mass (g), liver somatic index (LSI), and GS of surviving tadpoles. Sample sizes (N) are included in Tables S9 and S10

*Significantly different from vegetated/unmanaged ditches (i.e., cleared/dredged ditches compared with vegetated/unmanaged ditches) at $p \leq 0.05$ (see Tables S8 to S10)

[a]Proportion male and LSI were not assessed in the tadpole experiment (2018), because tadpoles were not of sufficient age and size to collect measurements

[b]For proportion male, the sample size is the total number of male tadpoles from the total of ≥ GS36

a significant difference in Gosner stage of development, where tadpoles in the cleared/dredged ditches were more developed at GS35 ± 1 compared with GS31 ± 2 in the vegetated/unmanaged ditches (Table 3; Table S10). As such, we found that snout-to-vent length also was significantly different in the cleared/dredged ditches, where tadpoles were significantly longer with a mean of 15.4 ± 1.6 mm compared with tadpoles in the vegetated/unmanaged ditches of 13.1 ± 1.8 mm (Table 3; Table S9).

For the tadpole experiment in 2019, survival also was not significantly different in the cleared/dredged ditches (93.3 ± 8.4%) compared with the vegetated/unmanaged ditch (85.6 ± 6.6%; Table 3; Table S9). However, the proportion of male to female tadpoles was significantly lower in the cleared/dredged ditch, with a mean of 58.6 ± 12.9% (cleared/dredged) compared with 91.7 ± 20.4% (vegetated/unmanaged; Table 3; Table S9). In addition, Gosner stage of development was significantly higher in the cleared/dredged ditch (GS39 ± 2) compared with the vegetated/unmanaged ditch (GS35 ± 2), and tadpoles had significantly longer snout-to-vent length and tail lengths at the cleared/dredged ditch (Table 3; Tables S9 and S10). However, we did not detect significant differences between cleared/dredged and vegetated/unmanaged ditch in the body mass, body width, or liver somatic indices of tadpoles (Table 3; Table S9).

Discussion

Drainage ditch treatment had an effect on tadpole growth and development, but the direction of change was opposite to our initial predictions. Specifically, we had predicted that the cleared/dredged ditch treatment would decrease tadpole survival, growth, development, and produce unbalanced sex ratios compared with the vegetated/unmanaged ditch treatment because of reduced water quality (i.e., higher concentrations of nutrients and pesticides) at the cleared/dredged sites. However, we found tadpoles from both years were larger and more developmentally advanced at the cleared/dredged ditches where management effects on ditch morphology, bed exposure and slope, and vegetation impacts were most strongly expressed.

These ditch systems invariably dry up by mid-July, eliminating the aquatic habitat and posing a threat to frog survival if terrestrial life stages have not yet been attained. Amphibians have been known to accelerate metamorphic development due to risk of desiccation (Székely et al. 2017), and although it was outside the scope of our study, this could be an important factor at play in both of our ditch treatments and warrants future research. There also may be interplay between elevated temperature and desiccation risk, which are both known to expedite metamorphic development (Székely et al. 2017; Ruthsatz et al. 2018). Completing metamorphosis at a larger size can increase the likelihood to survive to the adult terrestrial stage (Altwegg and Reyer 2003) and potentially increase reproductive success (Wilbur 1977; Smith 1987; Rudolf and Rödel 2007). To assess this, further research that encompasses the entire lifecycle from aquatic to terrestrial stages is required. Additionally, in this experiment food was not limiting across the treatments, because

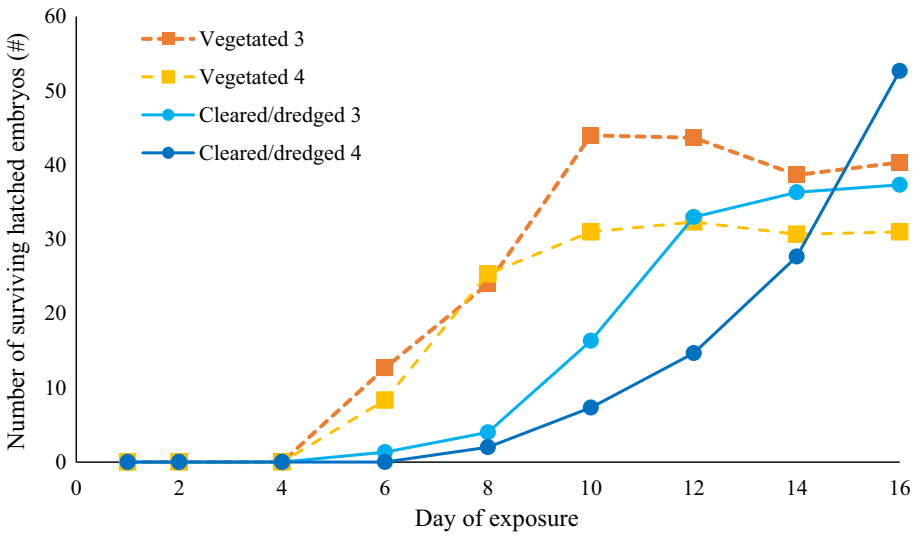

Fig. 1 Mean number of living hatched embryos to show the hatching timeline of the in situ experiment of embryos in vegetated/unmanaged and cleared/dredged (managed) ditches. Initial embryo count was ~ 60 embryos in each cage. Embryo exposure began on May 2, 2019 and ran until all embryos either hatched or died (May 17, 2019)

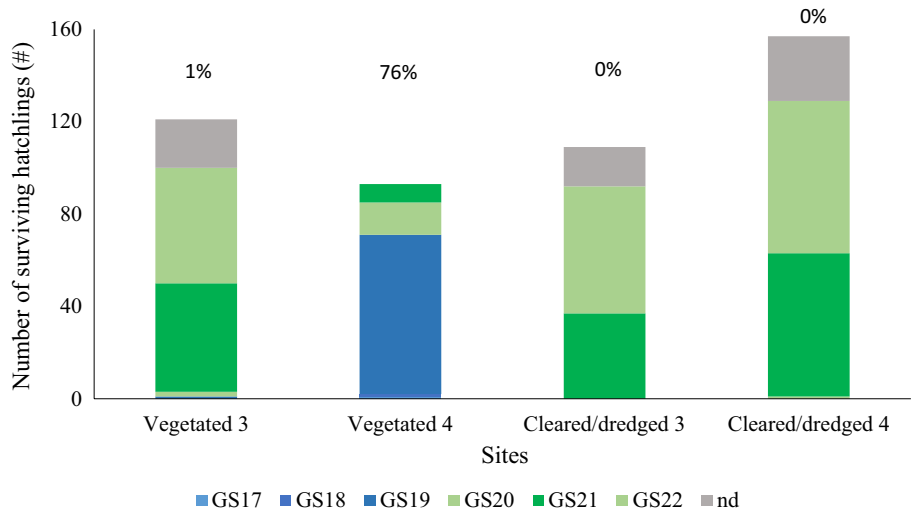

Fig. 2 Number of surviving *L. pipiens* embryo hatchlings at each Gosner Stage (GS) by site at the end of the embryo exposure (Day 16). The experiment began with ~ 180 embryos at each site across three in situ cages. A premature hatchling is an embryo that emerges from its casing prior to GS20, as prevalent at Vegetated 4. Shades of blue indicate < GS20, shades of green indicate ≥ 20, and gray indicates not determined. Proportions of premature hatchlings at each site are indicated by the percentage over the stacked bar

tadpoles were regularly fed. This was to ensure that this was not a confounding factor, because food is critical for tadpole growth. However, food availability or quality might in fact vary between managed and unmanaged ditches and influence development in the field.

Interestingly, the sex ratio was biased toward males—a result that has been rarely reported in the literature relative to the occurrence of female biases (Hayes et al. 2002, 2003; Tavera-Mendoza et al. 2002; Hogan et al. 2008). Incidents of endocrine disruption causing gonadal development delay have been reported for *L. pipiens* at atrazine concentrations > 100 ng/L (> 0.1 ppb). Our mean atrazine concentration was an order of magnitude lower at 19.5 ng/L (0.0195 ppb; Table 2), suggesting low concern. However, Dalton et al. (2015a) reported time-weighted atrazine averages up to 412 ng/L in the South Nation watershed. In the present study, because there was no significant difference between atrazine concentrations between treatments, we cannot attribute any potential atrazine associated endocrine disruption associated with ditch management per se.

Furthermore, this sex bias was most evident at the vegetated/unmanaged sites. However, although the proportions appear striking at 91% male in the vegetated/unmanaged ditch and 58% male in the cleared/dredged ditch, this may be an artefact of sample size and the significantly reduced Gosner stage of development at the vegetated/unmanaged sites. Only GS ≥ 36 were used for the sex ratio analyses, which resulted in much smaller sample sizes for the vegetated/unmanaged treatment ($n = 31$) compared with the cleared/dredged treatment ($n = 81$).

Contrary to our predictions with respect to the embryos, there were no significant differences in survival, hatching success, or GS between ditch treatments. However, we did observe premature hatching, where there was a higher occurrence of this at the Vegetated 4 site and minor occurrences at the other vegetated/unmanaged site (Fig. 2). Premature

hatching occurs when larvae emerge from their jelly egg coating prior to reaching Gosner stage 20 (Gosner 1960). At GS < 20, larvae are not fully developed and are likely at a greater risk of mortality. In the caged environment, the premature hatchlings were protected from predators, siltation, water flow, etc., which could artificially elevate their survival compared with when premature hatching occurs for free-ranging animals. Premature hatching has been reported in other studies and associated with hypoxia (Valls and Mills 2007; Warkentin 2011), desiccation (Warkentin 2011), water pollution from road salt resulting in high specific conductivity (Pohl et al. 2015), pathogen presence (e.g., water mold; Touchon et al. 2006; Warkentin 2011), and predation (Gomez-Mestre et al. 2008).

The reasons for premature hatching are unclear in the embryo study as the ditch waters were not hypoxic (consistently > 3.5 mg/L dissolved oxygen even at night), there was sufficient water throughout the experiment in the ditches, and the cages prevented access by predators. Although not significantly different between ditch treatments during the embryo experiment (Table 1), specific conductivity (SC) should be investigated further regarding premature hatching. The SC was double at the Vegetated 4 site (1026 µS/cm) where premature hatching was most prevalent compared with the other sites (503–603 µS/cm), which had little to no premature hatching (Table S13). Previous studies have documented premature hatching and associated reduced survival in *Rana sylvatica* and *Xenopus tropicalis* with elevated SC above 500 µS/cm and 750 µS/cm (Karraker et al. 2008; Pohl et al. 2015). Haramura (2016) observed nearly immediate hatching of *Buergeria japonica* embryos upon immersion in waters with salinity 5‰ or higher, and mortality increased with salinity levels (17% mortality at 1‰ to 79% mortality at 30‰). Further work is required to determine whether conditions at the vegetated/unmanaged ditches, such as pathogen presence or specific conductivity/salinity, promoted premature hatching more so than at cleared/dredged sites. However, such explanations are not likely related to drainage ditch management, but rather surrounding land uses, such as crop specific fertilizer applications, residual N, pesticide applications, or winter road salt.

Although we did find significant differences in some of the pesticide concentrations and physiochemical properties between the two ditch treatments (Table S9), statistical inferences were limited by small sample sizes. However, we were able to detect a strong effect of temperature, which was both statistically different between treatments and biologically significant in the tadpole experiments. The other detected significant differences were not those anticipated. Specifically, we expected that nutrient and pesticide concentrations would be higher at the cleared/dredged ditch treatment because of reduced retention by vegetation and sediment/detritus in the ditch (Dollinger et al. 2015). However, we found many of the nutrient variables, such as NH_3-NH_4, TIN, and TP, were all consistently higher in the vegetated/unmanaged ditches compared with the cleared/dredged ditch treatments for all three experiments (i.e., 2019 embryo, 2018 and 2019 tadpole). This may have been a result of unknown tile drainage/surface runoff factors influencing water quality/quantity at the sampling sites (i.e., dilution, greater agrochemical inputs, etc.) as found by Sunohara et al. (2015). Furthermore, atrazine and total neonicotinoids were also consistently higher during the 2019 experiments for both embryo and tadpole at the vegetated/unmanaged ditches compared with the cleared/dredged ditch treatments. However, glyphosate was measured at higher concentrations at the cleared/dredged ditch treatment as we had expected. Such differences in concentrations may be more related to surrounding land use, tile drainage factors, and pesticide chemical characteristics than ditch treatment.

Although we observed some differences in concentrations for nutrients and pesticides, the measured concentrations were still within levels that are not overly toxic (Table S7).

Neonicotinoids are not generally toxic to amphibians at environmentally relevant concentrations (Smalling et al. 2015; Morrissey et al. 2015; Anderson et al. 2015). For instance, the 48 h LC_{50} for imidacloprid ranged from 165 to 219 mg/L for two different *Rana* species (Feng et al. 2004). In addition, 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 up to 100 µg/L. However, sublethal effects on behaviour have been observed in juvenile *L. sylvaticus* exposed as tadpoles to 10–100 µg/L, potentially increasing their vulnerability to predation (Lee-Jenkins and Robinson 2018). The maximum total neonicotinoid concentration detected in the present study (0.035 µg/L) was 2–3 orders of magnitude below the concentration at which behavior effects are observed (10–100 µg/L; Lee-Jenkins and Robinson 2018). With respect to glyphosate, the 96-h LC_{50} have been determined as 1.8 mg/L (Moore et al. 2012) and 1.5 mg/L (Relyea and Jones 2009) for *L. [Rana] pipiens* GS25 tadpoles. While our glyphosate concentrations were much lower with a mean of 488 ng/L (in the 2019 tadpole cleared/dredged; Table 2), we did not see reduced survival or other negative health effects at this site, indicating glyphosate was likely not affecting tadpole health. As mentioned previously, the atrazine levels in the water samples were well below those known to cause sublethal effects; however, grab samples are known to underestimate concentrations, which would apply to all pesticides reported (Dalton et al. 2014).

The nutrient and pesticide concentrations were within the ranges reported in other studies from the same watershed

(Allaway 2006; Dalton et al. 2015b; Collins et al. 2019). Hence, the difference in nutrients and pesticides between the vegetated/unmanaged and cleared/dredged sites, even if in line with our tadpole results (i.e., increase in growth and development at the lower contaminated cleared/dredged ditches), suggests these water quality metrics were not the driving force for the effects on tadpoles found in the present study. However, the toxicity assessment is in reference to toxic effects of single compound exposures, and the combined effects of nutrients and pesticides and other compounds can be additive or synergistic on amphibians in mixtures that occur in the environment (Hayes et al. 2006; Mann et al. 2009). Indeed, for all water quality metrics we measured for which relevant amphibian toxicity information was available the concentrations did not exceed any of the reported toxicity thresholds (with the exception of SC in the embryo study). With our suspect screening chemical analyses we detected up to 62 different pesticide compounds in these ditches, suggesting the tadpoles were exposed to complex mixtures in both ditch systems (see Supplemental Information, Table S11). This is particularly important to consider in future mixture toxicity research, because reduced development and size was found in the vegetated/unmanaged ditch that tended to have higher concentrations of both nutrients and pesticides.

Finally, we expected that physiochemical water quality would differ between the ditches. In particular, we expected the cleared/dredged ditches would have increased temperature and turbidity because of the lack of vegetation to provide shade (Barton et al. 1985) and trap sediments (Flora and Kröger 2014; Dollinger et al. 2015). As expected, we found consistent increases in temperature at the cleared/dredged ditches for all experiments (albeit the embryo experiment was not statistically significant likely because of minimal shading from vegetation in early spring). For turbidity and our other physiochemical measures (i.e., DO, pH, ORP), the results were more inconsistent. Mean turbidity values ranged between 3 and 20 NTU across the years, and although not significantly different, it was higher at the cleared/dredged sites after dredging as expected (Dollinger et al. 2015). However, it was significantly higher at the vegetated/unmanaged treatment in 2018, contrary to expectations, and as such, we cannot attribute any significant changes in turbidity to ditch management practices. The turbidity guideline for the protection of aquatic life (CCME 2002) is a maximum spike of 8 NTU above background levels for short-term exposure (24 h), which was observed routinely in all of the ditches. These spikes were particularly pronounced (as seen by higher SD in 2019) after dredging at the cleared/dredged sites, although they also occurred at the vegetated treatment in 2018. Given the high survival, increased growth, and development of tadpoles at the cleared/dredged ditches, there was likely no negative effect of turbidity at any of the ditches. These ditches are dredged into varved clays, and as such, turbidity resulting from the clay substrate proper was expected to be modest during the experimental time frame of this study. Yet for a time period (decreasing over time) following the dredging/clearing, it was expected that there would be increased potential for elevated turbidity resulting from sediment/soil slumping and greater rainfall-induced soil erosion along ditch banks.

Specific conductivity was significantly different only in the 2019 tadpole experiment, where higher specific conductivity was measured at the vegetated/unmanaged ditch treatment. Hence, temperature was likely the most influential in situ water quality measure on tadpole growth and development because of its consistent difference between vegetated/unmanaged and cleared/dredged treatments and its well-known effect on tadpole growth and development (Marian and Pandian 1985; Newman 1998). For example, an increase in temperature of 5 °C increased growth rate in wood frogs (*L. sylvatica*) and spring peepers (*Pseudacris crucifer*) by 83–90% (measured by mg/day; Skelly et al. 2002) and a 2 °C increase can advance larval development by 10 days in wood frogs (Riha and Berven 1991). In our study, the difference in mean temperatures between the ditch treatments ranged from 0.6 to 2.4 °C, which is within this established temperature inducing effects range (Riha and Berven 1991).

Indeed, a meta-analysis exploring the effect of temperature on the rate of metamorphosis in anurans found that across all studies every 1 °C increase in temperature resulted in tadpoles completing metamorphosis almost 1 day earlier (Ruthsatz et al. 2018). In fact, each individual study showed this advancement in development with increasing temperature but to different extents (Ruthsatz et al. 2018). Hence, temperature has a profound effect on rate of metamorphosis for a variety of anuran species, with temperate anurans, such as leopard frogs having some of the most plastic responses. In our study, the mean temperatures between ditch treatments ranged from 0.6 to 2.4 °C, which is within this established temperature inducing effects range (Ruthsatz et al. 2018; Riha and Berven 1991). Unfortunately, we were not able to continue our in situ exposure to the completion of metamorphosis because of insufficient water remaining in the ditches. Thus, we could not determine whether the size differences of tadpoles would have persisted in the metamorphs. However, the more advanced development in tadpoles at the cleared/dredged ditch treatment would increase their likelihood to escape desiccation and thus increase survival compared with the vegetated/unmanaged ditch. Further work is required to determine whether ditch management effects on temperature have fitness consequences on tadpoles that complete metamorphosis, but this requires ditches that retain enough water throughout the aquatic larval life stage.

Conclusions

We determined that freshly cleared and dredged ditch systems (representing the most extreme contrast with mature ditch systems in the context of flora and fauna, ditch bed sediment exposure, insolation potential, etc.) had no obvious detrimental effects on northern leopard frog embryo or tadpole survival, growth, or development. In fact, the increased growth and development at the cleared/dredged ditch may be beneficial for tadpoles to reach sufficient size and developmental stage to survive in the terrestrial environment before the ditches become intermittent and dry up. Furthermore, we found that temperature was likely the most influential physiochemical parameter that affected tadpole growth and development in these ditches. Further work is required to understand the conditions causing premature hatching in embryos and perceived male-biased sex ratios. Our study provides novel information on the effects of clearing and dredging practices for drainage ditch management on amphibian early life stages. However, further work is required to determine if ditch management has an effect or not on amphibian populations and should include a large sample size of ditches with varying degrees of management and include longer exposures to evaluate age at the onset of metamorphosis, size at metamorphosis, and sex ratios.

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Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by AD, SDY, SAR, JBR, LS, and FRP. AD, SDY, and SAR wrote the first draft of the manuscript. All authors contributed to the final manuscript. FRP, SAR, and DRL provided supervision. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analysed during the current study are available as Supplementary Information.

Code Availability Not applicable.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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