

The importance of riparian vegetation for water quality and tadpole health in agricultural streams

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Abstract

Amphibians in agricultural drainage ditches may be exposed to contaminants through runoff.

The goal of this study was to determine how riparian vegetation affects water quality and amphibian health metrics in drainage ditches in the South Nation River Watershed, Ontario.

Cages containing northern leopard frog (*Rana [Lithobates] pipiens*) tadpoles were placed within nine agricultural ditches differing in their vegetation height and percentage of forest cover for 8 weeks (May-July). Physiochemical water quality measurements, contaminant concentrations and tadpole fitness-relevant metrics were assessed. Sites higher in forest cover tended to have lower specific conductance, neonicotinoids, atrazine, nitrate, and potassium concentrations.

Tadpole size (snout-to-vent length, tail length, mass) was positively associated with forest cover, but all tested tadpoles were negative for *Batrachochytrium dendrobatidis* and Frog Virus 3, suggesting these pathogens are not present in these systems. Overall, forest cover appears to play an important role for both water quality and tadpole growth.

Résumé

Les amphibiens vivant dans les fossés agricoles peuvent être exposés à des contaminants par ruissellement. Le but de cette étude était de déterminer si et comment la végétation riveraine affecte la qualité de l'eau et les paramètres de santé des amphibiens dans les fossés de drainage du bassin versant de la Rivière Nation Sud dans l'est ontarien. Pour ce faire, des cages contenant des têtards de grenouille léopard (*Rana [Lithobates] pipiens*) ont été placées, pendant 8 semaines (mai - juillet), dans neuf fossés agricoles ayant différentes tailles de végétation et pourcentage de couverture forestière. Les mesures physicochimiques de la qualité de l'eau, les concentrations de contaminants et les paramètres pertinents pour la condition physique des têtards ont été évalués. Suite à cette analyse, nous avons pu constater que les sites ayant un couvert de forêt plus important avaient tendance à avoir la conductance spécifique, et des taux de néonicotinoïdes, atrazine, nitrate, et potassium plus basses. De plus, la taille des têtards (longueur du corps, longueur de la queue, masse) était positivement associée au couvert forestier, mais tous les têtards testés étaient négatifs pour *Batrachochytrium dendrobatidis* et Frog Virus 3, ce qui suggère que ces agents pathogènes ne sont pas présents dans ces systèmes. Dans l'ensemble, le couvert forestier semble jouer un rôle important à la fois dans la qualité de l'eau et dans la croissance des têtards.

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Abbreviations

AIC	Akaike Information Criterion
Bd	<i>Batrachochytrium dendrobatidis</i>
BW	Body Width
CCAC	Canadian Council for Animal Care
CCME	Canadian Council for the Ministers of the Environment
Cl	Chloride
DL	Detection Limit
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EDC	Endocrine Disrupting Compound
FV3	Frog Virus 3
GLMM	Generalized Linear Mixed-Effects Model
GS	Gosner Stage
HDPE	High-Density Polyethylene
HPLC-ESI-MS/MS	High-Performance Liquid Chromatography-Electrospray Ionisation Tandem Mass Spectrometry
HSI	Hepatosomatic Index
iiPCR	Insulated Isothermal Polymerase Chain Reaction
K	Potassium
MLE	Maximum-Likelihood Estimate
N	Nitrogen
Na	Sodium
NH ₄ NO ₃	Ammonium Nitrate
NO ₂	Nitrite
NO ₃	Nitrate
NPK	Nitrogen Phosphorus Potassium
NWRC	National Wildlife Research Centre
OECD	Organisation for Economic Co-operation and Development
P	Phosphorus
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDF	Probability Density Function
RDA	Redundancy Analysis
ROPEC	Robert O. Pickard Environmental Centre
RT-qPCR	TaqMan Probe Real-Time Quantitative Polymerase Chain Reaction
SD	Standard Deviation
SN	South Nation
SPC	Specific Conductance
SPE	Solid Phase Extraction

SVL	Snout-to-Vent Length
TL	Tail Length
TNeo	Total Neonicotinoids
TSS	Total Suspended Solid

Chapter 1: Introduction

1.1 Wetland loss

Humans have been altering natural landscapes for at least 5000 years through urbanization and agriculture (Hooke, 2000). Modern agricultural practices have led to major alterations in natural landscapes which have the potential to affect wildlife and biodiversity. It is currently estimated that 7% of Canadian land is used for agriculture (Statistics Canada, 2014). In Ontario alone over 35 000 hectares of land are for crop use (Statistics Canada, 2017). The demand for food production and the expansion of agriculture with a greater reliance on chemical fertilizers and pesticides has caused the loss and degradation of natural landscapes, including wetland habitats.

Wetlands are highly productive ecosystems and are widely considered one of the most important resources for supporting biodiversity (Lillo et al., 2019; Zedler & Kercher, 2005). In Canada, approximately 1.29 million km² of landscape is wetland, 25% of which is in Ontario (ECCC, 2016; Ontario Biodiversity Council, 2021). Wetland loss is increasing at an alarming rate in Ontario (approximately 1825 ha/yr between 2011-2015), with less than 30% of natural wetlands now remaining in southern Ontario alone (Biodiv Canada, 2010; Environment and Climate Change Canada, 1994; Ontario Biodiversity Council, 2021). In Southern Ontario, over 70% of wetlands have been lost and much of this wetland loss is associated with urbanization and agriculture (Duck Unlimited Canada (DUC), 2006; Environment and Climate Change Canada, 1994; Environment and Climate Change Canada, 2010; Ontario Biodiversity Council, 2021; Snell, 1987). Wetland loss from agriculture has led to the use of agricultural land as habitat by many wetland species.

1.2 Drainage ditches as habitat

In areas with poor drainage, low slope, or high precipitation, canals or “ditches” are often created along the perimeter of cropland as a channel for water flow from subsurface tile drainage systems. A typical drainage system is composed of subsurface tile drains which allow water to flow along the slope of a field into surrounding ditches (Moore et al., 2004). Artificial drainage of crop fields prevents erosion, removes excess water from irrigation, prevents flooding, maximizes cultivatable land, and allows farmers to access fields earlier in the growing season (Blann et al., 2009; Herzon & Helenius, 2008). Worldwide, agricultural drainage is used in 11% of cropland (ICID, 2018; Kokulan, 2019; Suislepp et al., 2011). Tile drainage has been used in Canadian agriculture since the 19th century with approximately 45% of Ontario crop fields now being artificially drained (ICID, 2018; Kokulan, 2019). Habitat loss from agriculture has made drainage ditches one of the few habitats for wetland species in agricultural landscapes. In Canada, 7.6%, or more than 4.8 million hectares, of wetland and woodland are found within agricultural landscapes (Jeswiet & Hermsen, 2015).

Agricultural drainage ditches serve many other hydrological functions such as water purification, nutrient cycling and erosion control (Herzon & Helenius, 2008). Agricultural ditches also possess the characteristics of typical wetlands, which include ephemeral water levels and underlying hydrosols (Herzon & Helenius, 2008; Kröger et al., 2009). Additionally, ditches provide several ecosystem functions such as providing non-crop plants for pollinators and habitat for terrestrial and aquatic species (Herzon & Helenius, 2008; Kröger et al., 2009). Biodiversity in these ditches is attributable to the cool moist conditions, high productivity, habitat complexity, and low disturbances (Herzon & Helenius, 2008; Tews et al., 2004). Ditches

contain a variety of plant types and the species richness and composition rely largely on habitat structure and physical characteristics of ditches such as soil composition, landscape features, and the effects of agrochemicals (Boutin et al., 2003). Most ditches contain species that are tolerant to herbicides and are indicative of high soil/sediment nutrient concentrations such as weeds (Boutin et al., 2003; Herzon & Helenius, 2008) and many non-native species (Dalton et al., 2015b). Additionally, both obligate and facultative wetland plant species can often be found in agricultural drainage ditches (Boutin et al., 2003). Ditches also provide habitat for many invertebrate and vertebrate species such as gastropods, insects, fish, birds, and amphibians (Herzon & Helenius, 2008).

1.3 Amphibians as indicators of environmental health

Amphibians are of particular interest for the study of the effects of agriculture on aquatic ecosystems because they are a major component of wetland biodiversity (Hecnar, 2004). Their reliance on wetlands for breeding, development, foraging and refuge results in their increased use of agricultural drainage ditches as natural wetland habitats are destroyed (Collins & Fahrig, 2017; Herzon & Helenius, 2008; Li et al., 2019; Mazerolle, 2005; Piha et al., 2007). In fact, amphibian populations are declining globally, with habitat loss - particularly of wetlands -, global warming, pesticides, and emerging infectious diseases among the most cited causes for these declines (Alford et al., 2007; Alford & Richards, 1999; Collins & Storfer, 2003; Suislepp et al., 2011; Welsh & Ollivier, 1998). Amphibians are commonly used as indicator species of ecosystem health (Alford et al., 2007; Alford & Richards, 1999; Pollet & Bendell-Young, 2000). Their permeable skin and biphasic life cycle make them an important model

organism when studying the effects of environmental contaminants (Alford & Richards, 1999; Welsh & Ollivier, 1998). Amphibians are also generally abundant in local ecosystems.

Frogs use drainage ditches for breeding, foraging, and hibernation (Collins & Fahrig, 2017; Herzon & Helenius, 2008; Li et al., 2019; Mazerolle, 2005; Piha et al., 2007). Water levels and runoff vary seasonally in agricultural drainage ditches, with higher water levels in the spring and fall with drying in the summer. Frogs may benefit from shallow ditches that dry later in the summer, as this reduces their risk of predation by predatory fish (Herzon & Helenius, 2008). Additionally, ditches may act as corridors in hostile environments and increase the survival of amphibians when travelling to breeding sites (Mazerolle, 2005). However, due to their proximity to cropland, drainage ditches receive an influx of contaminants and nutrients from precipitation and snow melt runoff. Pesticides and nutrients entering these systems from runoff can contaminate the water and lead to eutrophic conditions (Blann et al., 2009; Chrétien et al., 2017; Herzon & Helenius, 2008; Janse & Van Puijenbroek, 1998). Consequently, the amphibians using these ditches as habitat may be at risk.

1.4 Contaminant runoff in drainage ditches

Agrochemicals are widely used in crop agriculture. Pesticides protect crops and greatly improve production yield and reduce crop loss (Aktar et al., 2009). Additionally, pesticides such as insecticides can reduce the spread of vector-borne diseases by eliminating insect vectors (Aktar et al., 2009). However, pesticides can also have many negative impacts on ecosystems. Pesticides can contaminate soil, surface and ground water, and can directly affect wildlife and non-target organisms (Aktar et al., 2009).

Herbicides, such as atrazine, and insecticides, such as neonicotinoids, are among the most commonly used pesticides in Ontario (Byer et al., 2011; Farm and Food Care Ontario, 2016). Atrazine enters aquatic ecosystems through runoff and has been shown to cause numerous developmental effects on frogs (Byer et al., 2011; Hoskins & Boone, 2018; Koprivnikar et al., 2007; McDaniel et al., 2008; Rimayi et al., 2018). Atrazine is an endocrine disrupting compound (EDC) that can cause female biased sex ratios, delayed development, and increased mortality in frogs exposed to environmentally relevant concentrations (Byer et al., 2011; Hoskins & Boone, 2018; Rimayi et al., 2018). Additionally, atrazine may increase susceptibility of wood frogs to parasitic infection (Koprivnikar et al., 2007).

Neonicotinoids are a widely used class of insecticide that function by interfering with insect nervous systems (Anderson et al., 2015; Sparks, 2013). Specifically, neonicotinoids bind insect nicotinic acetylcholine receptors and interfere with neurotransmission (Anderson et al., 2015). However, there is growing concern for the effect of neonicotinoid insecticides on amphibian health. Recent studies have demonstrated that the three most common formulations of neonicotinoids (imidacloprid, clothianidin, thiamethoxam) are associated with behavioural and physiological effects in frogs (Gavel et al., 2019; Holtswarth et al., 2019; Lee-Jenkins & Robinson, 2018; Sievers et al., 2018; Sweeney et al., 2021). Studies have shown that clothianidin and thiamethoxam can cause anemia, increased physiological stress, and reduced corticosterone levels in northern leopard frog (*Rana [Lithobates] pipiens*) tadpoles (Gavel et al., 2019). Neonicotinoids may also have indirect effects on tadpole or juvenile frog survival by reducing swimming speed and predator avoidance behaviours (Holtswarth et al., 2019; Lee-Jenkins & Robinson, 2018; Sievers et al., 2018; Sweeney et al., 2021).

In addition to pesticides, amphibians in drainage ditches are exposed to a variety of nutrients that can alter water quality and have negative effects on growth, development and survival. The application of synthetic and natural fertilizers in cropland drives the levels of nutrients up in surrounding aquatic habitats. Nitrogen (N), and phosphorus (P) are generally in excess in agricultural ecosystems and surface runoff from tile drains can lead to eutrophic conditions (Blann et al., 2009; Herzon & Helenius, 2008; Janse & Van Puijenbroek, 1998; Madramootoo et al., 2007). Eutrophication of aquatic ecosystems can cause toxic algal blooms and anoxic conditions which can be harmful to aquatic and semi-aquatic species (Blann et al., 2009; Herzon & Helenius, 2008; Janse & Van Puijenbroek, 1998). Eutrophic conditions can negatively affect the growth, development and survival of frogs (Harris et al., 1998; Peltzer et al., 2008). Additionally, common derivatives of synthetic fertilizers such as ammonium nitrate (NH_4NO_3), nitrate (NO_3), nitrite (NO_2) are associated with decreased hatching success, increased mortality and delayed growth in frogs at environmentally relevant concentrations (De Solla et al., 2002; Garriga et al., 2017; Marco et al., 1999; Oldham et al., 1997).

In addition to the use of agrochemicals, the use of de-icing salts – which can enter ditches through runoff – can increase the salinity of agricultural ditches due to their proximity to roads. High salinity has been associated with reduced survival and development in embryonic and larval frogs (Dananay et al., 2015; Karraker et al., 2008; Kearney et al., 2016; Langhans et al., 2009). Other studies have demonstrated a greater stress response upon exposure of larval southern leopard frogs (*Lithobates sphenocéphala*) to a combination of salts, nutrients, and pesticides, suggesting synergistic effects of these contaminants (Adelizzi et al., 2019). Overall, runoff from agricultural cropland contains pesticides, excess nutrients, and

elevated salts, meaning amphibians in drainage ditches are at risk of synergistic effects from these contaminants.

1.5 Emerging infectious diseases

Emerging infectious diseases are a threat to amphibian populations worldwide. Ranaviruses are large double-stranded DNA viruses of the family *Iridoviridae* that are known to infect ectothermic vertebrates such as fish, amphibians and reptiles (Chinchar, 2002; Lesbarrères et al., 2012). A strain of *Ranavirus*, Frog Virus 3 (FV3), has been associated with mass mortality events in wood frogs (*Lithobates sylvaticus*) and northern leopard frogs (*R. pipiens*) in southern Ontario (Duffus & Andrews, 2013; Greer et al., 2005). The pathology of FV3 can be seen in larval and adult stages and includes hemorrhaging, swelling, lesions, skin ulceration, erratic swimming, and mass mortality in infected populations (Lesbarrères et al., 2012). However, the virulence of FV3 appears to be dependent on developmental stage and number of infections. Studies have shown that individuals infected at both the hatchling and the tadpole stage experience higher mortality than individuals infected at only one life stage (Echaubard et al., 2016). Studies have also demonstrated that the pathogenicity of FV3 is temperature dependent and that at higher temperatures, FV3 is associated with increased transmission and mortality in *L. sylvaticus* (Brand et al., 2016). The detection of FV3 in southern Ontario suggests amphibians in agricultural ditches may be at risk.

Chytridiomycosis is an infectious disease that affects amphibians. Caused by the aquatic chytrid fungus *Batrachochytrium dendrobatidis* (Bd), chytridiomycosis is considered one of the deadliest wildlife pathogens worldwide (Monzon et al., 2020). Many amphibians depend on the

exchange of ions and gases at the water-skin interface for osmoregulation (Voyles et al., 2011). Chytrid fungus interferes with the exchange of electrolytes across the water-skin interface and can lead to lethargy, thickening of the skin and even death in infected amphibians (Voyles et al., 2011). Chytridiomycosis has also been shown to alter skin microbiome in amphibians, which can reduce host resistance to further infection (Jani & Briggs, 2014). Temperature can also affect the virulence of Bd. Bradley et al. (2019) found that western toad (*Anaxyrus boreas*) and northern red-legged frog (*Rana aurora*) exposed to fluctuating temperatures above and below thermal optimum experienced higher infection loads of Bd compared to larvae exposed to constant higher temperatures. Currently, Bd has been detected in Europe, Australia, Central America, South America, and North America (Olson et al., 2021). More specifically, Bd has been detected in Ontario but may have limited impact on native amphibians (Crawshaw et al., 2022).

1.6 Importance of riparian vegetation

Riparian buffer strips are generally used to reduce runoff entering drainage ditches. They function as vegetated dams that allow water to pool and deposit sediments before continuing into the drainage ditch (Vymazal & Březinová, 2015). Studies have shown that woody vegetation along the banks of agricultural drainage ditches may increase the sorption of many pesticides, thereby improving water quality (Aguar et al., 2015). Additionally, vegetated riparian strips are efficient at intercepting aerial drift of spray application of pesticides (Hancock et al., 2019). A buffer's ability to retain a pesticide is dependent on the physical and chemical properties of the pesticide. For example, the transport and fate of a pesticide is largely dependent on its organic carbon sorption coefficient (K_{oc}) or the ratio of pesticide adsorbed to

soil (more specifically, soil organic carbon) to pesticides dissolved in water (Arora et al., 2010). Pesticides with higher K_{oc} are less mobile than pesticides with low K_{oc} (FAO, 2000). Once retained in riparian soil, a pesticide's persistence in the environment is largely dependent on its half-life but it can also be degraded by photolysis or by microorganisms, which largely occur in the surface layer of soil (Arora et al., 2010).

The buffering capacity of riparian zones at filtering runoff against nutrient contamination has also been extensively studied. Riparian vegetation is efficient at removing N and P sediments from runoff (Gottschall et al., 2007; Mankin et al., 2007; Mayer et al., 2007). Root depth of riparian vegetation can also be important for the retention of excess nitrogen (Mayer et al., 2007).

Riparian vegetation may also help regulate the temperature within drainage ditches by providing shade (Broadmeadow et al., 2011; Wondzell et al., 2019). Lower water temperatures may be important for multiple reasons. Higher temperatures are associated with increased susceptibility to FV3 infection in frogs (Brand et al., 2016). Additionally, frogs develop faster at increased temperature, which may also lead to increased senescence rate and earlier death in developing frogs (Cayuela et al., 2021).

Current agricultural practices remove much or all of the riparian vegetation through clearing and dredging. Ditch clearing and dredging may reduce the ability of ditch bed material to remove nutrients and lead to lower water quality in these ditches (Smith & Pappas, 2007). Root removal from dredging may also reduce the stability of ditch soils and lead to increased erosion (Aviles et al., 2020). In addition, removal of riparian vegetation may decrease shading in agricultural ditches thereby increasing temperature (Broadmeadow et al., 2011; Wondzell et al.,

2019). Hence, removal of riparian vegetation may reduce habitat quality and have serious consequences for the health, development, and survival of frogs using these ditches as habitat.

1.7 Objectives and predictions

The goals of this research are two-fold: 1) Determine the importance of riparian vegetation on the water chemistry in agricultural drainage ditches in the South Nation (SN) River watershed, Ontario and 2) Determine the importance of riparian vegetation on amphibian development and survival in the SN.

The research questions guiding this study are: i) Can riparian vegetation effectively filter runoff entering agricultural drainage ditches, thereby affecting water chemistry and reducing contaminant levels? I predict that with decreasing riparian cover, water chemistry will be altered (i.e., increased temperature, increased conductivity, higher turbidity, lower dissolved oxygen) and contaminant levels will increase (i.e., higher pesticide concentrations, higher nutrient levels).

ii) Can riparian vegetation affect amphibian health metrics (i.e., body size, mass, developmental stage, sex ratios, stress, survival, FV3 and Bd infection)? I predict that given riparian cover likely plays an important role in filtering contaminants and moderating temperature in ditches, decreasing riparian cover will affect amphibian health outcomes (i.e. increased growth/development, uneven sex ratios, increased stress levels, decreased survival, and increased pathogen infection).

Chapter 2: Methods

2.1 Site description and experimental design

The experiments took place at ditch or stream sites within the South Nation (SN) River watershed, which spans much of Eastern Ontario (close to 4000 km²) (Dalton et al 2014, SNCA 2014; Figure 1). The South Nation River is approximately 113 km long with headwaters starting near Spencerville, close to the St Lawrence River (44°40'41"N, 75°41'58"W) to the south and outflows near Plantagenet into the Ottawa River to the north and east of the City of Ottawa (45°34'24"N, 75°06'00"W). Approximately 60% of the SN watershed land is comprised of agricultural fields with crops of corn (*Zea mays L.*) and soybean (*Glycine max L. (Merr.)*), with some forested area (approx. 29%), and some urban areas (Sunohara et al, 2012; SNC 2014, Dalton et al 2014). Soils in the SN watershed are comprised of Bainsville silt loams of Gleyosic order (Wicklund and Richards 1962). The SN watershed is a relatively flat plain that is prone to flooding, especially after early spring rains. Consequently, many of crop fields are artificially drained via tile drainage (Sunohara et al., 2015).

In this study, nine agricultural drainage ditch sites were selected to form a gradient of riparian vegetation height and percent forest cover. Vegetation heights were determined using a digital surface model (Lidar DRAPE 2014 point cloud data). Percent forest cover was determined from a 1 Km aerial buffer around each site. Ditch riparian vegetation height varied from 0.13 to 6.4 m while ditch percent forest cover varied from 1.2 to 60.4 % (Table 1). The nine agricultural ditch sites were used to conduct an *in situ* exposure experiment in the early spring of 2022.

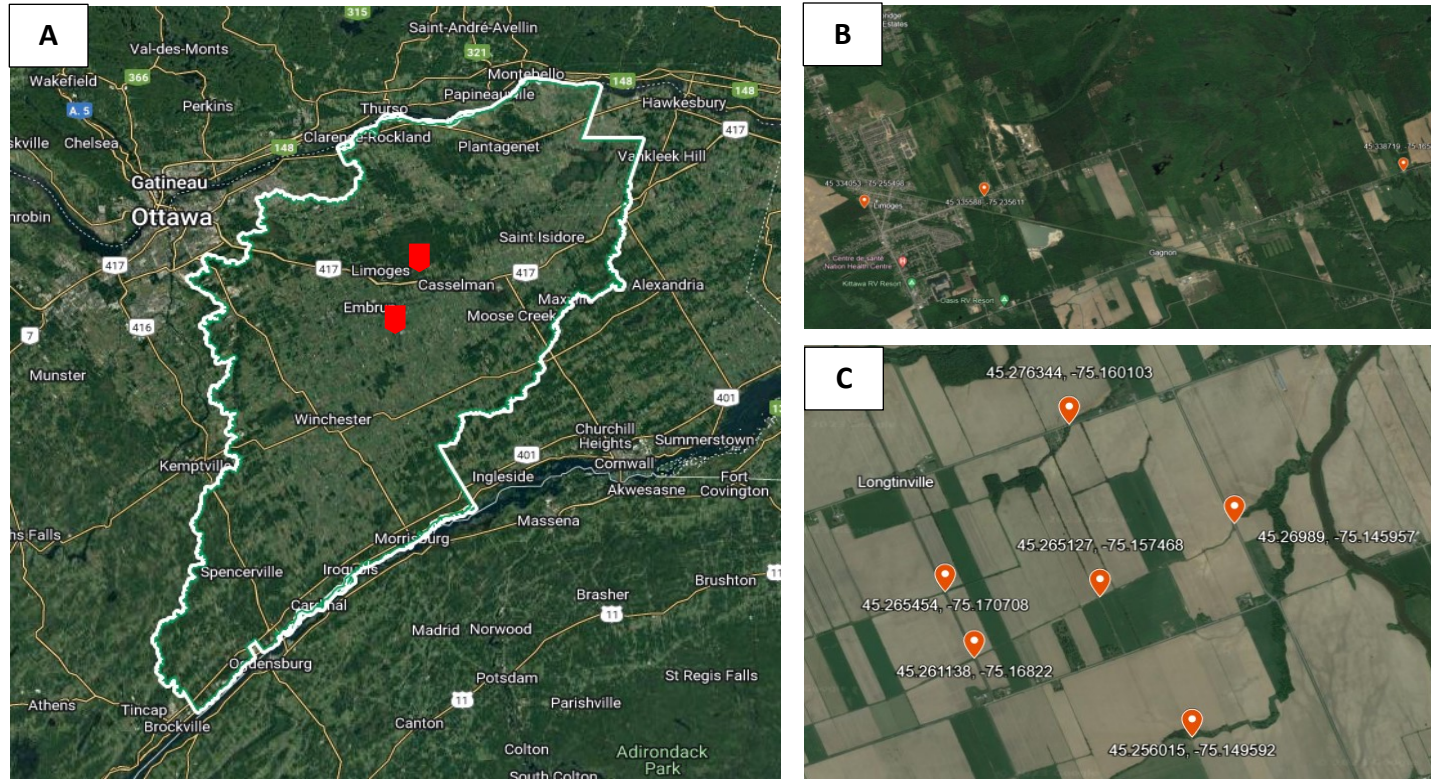


Figure 1. Map of the South Nation River Watershed (A) in southeastern Ontario and associated site locations (B – C). The nine experimental sites were located in agricultural drainage ditches near Limoges, Crysler, and St. Albert Ontario. Sites varied in their riparian vegetation height (D – E) and their percentage of forest cover (F). *Rana [Lithobates] pipiens* tadpoles were housed in Nytex mesh cages within ditch waters at all nine agricultural ditch sites with a total of three cages per site and 15 tadpoles per cage (G).

2.2 Water sampling and monitoring

From May 2 to July 13, 2022, water physiochemical variables were monitored thrice-weekly at all nine sites using a YSI Professional Plus meter (YSI Inc., Yellow Springs, OH, USA). The YSI probe recorded temperature (°C), specific conductance (SC, $\mu\text{S}/\text{cm}$), dissolved oxygen (DO as percent saturation), and pH. Additionally, Hobo pendant data loggers were placed approximately 10 cm below the water surface (attached to a cage) at each site and recorded temperature (°C) each hour for the duration of the experiment. Hobo loggers from sites 333 and 337 were lost during the experiment, no data could be collected from these pendants and therefore these sites were not included in any Hobo temperature analyses. Triplicate measurements of stream flow were also taken at each site 2-3 times weekly. Stream current velocity was measured by placing a wiffle ball in the ditch water and measuring the time it took for the wiffle ball to travel the length of a meter stick or measuring the distance the wiffle ball travelled in one minute using a stopwatch (whichever came first). Occasionally, the wind was too strong and the current could not be recorded on those days. Finally, water depth was recorded at each site by placing a meter stick on the substrate next to each cage and at the center of the stream. Mean depth at each site was calculated as the average depth of all of the cages and the depth at the center of the stream.

Surface water grab samples were collected 1-2 times weekly for pesticide and nutrient analyses. Specifically, water was sampled for the analysis of glyphosate (250 mL high-density polyethylene (HDPE) plastic bottles), neonicotinoids + atrazine (250 mL amber borosilicate glass bottles, triple rinsed with methanol and air dried before use), and nutrients + ions (HDPE plastic bottles). Immediately before sampling, each bottle was triple rinsed with ditch water. Water

was then collected by submerging the sample bottles upside-down to the middle of the water column, bottles were then flipped right-side-up to collect water. All samples were held in a cooler until the end of the sampling day.

2.3 Water sample analysis

Water samples were analyzed for the presence of the following pesticides: atrazine, glyphosate, and seven neonicotinoids (acetamiprid, clothianidin, dinotefuran, flupyradifurone, imidacloprid, thiacloprid, thiamethoxam). These pesticides were chosen as they are all commonly used in agriculture throughout Ontario and Canada. Water samples were analyzed for pesticide concentrations at the National Wildlife Research Centre (NWRC) in Ottawa, Ontario via high-performance liquid chromatography-electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS/MS) post-solid phase extraction (SPE) following standard methods outlined in Collins et al (2019) and Robinson et al. (2017) with minor modifications. Method detection limits are indicated in (Table A1).

For pesticide quality assurance, accuracy and precision values are as follows. For total neonicotinoids, no neonicotinoids were found in the sample blanks, indicating no cross-contamination. The percent recovery ranged from 85 - 115 % demonstrating good method accuracy. The relative percent difference was 1.09 – 23.9 %, demonstrating good precision (<15 % is considered good, this was only exceeded once). For total atrazine, no atrazine was found in the sample blanks, percent recovery ranged from 85 - 115 %, and the relative percent difference was 0.0 – 10.3 %. Finally, for glyphosate, no glyphosate was found in the sample

blanks, percent recovery ranged from 85 - 115 % and the relative percent difference was 2.76 – 9.59 %.

Water samples were analyzed for the presence of nutrients and ions at the Robert O. Pickard Environmental Centre (ROPEC), a certified laboratory of the City of Ottawa, following standard protocols for the analysis of surface waters (OMOE, 2007; OMOE, 2017; Standard Methods, 2004). Nutrients and major ions analyzed included Nitrate (NO₃), total phosphorus (P), total suspended solids (TSS), and dissolved organic carbon (DOC) while major ions analyzed included chloride (Cl) and dissolved potassium (K).

2.4 Amphibian husbandry

Free-ranging adult northern leopard frogs (*Rana [Lithobates] pipiens*) were collected in non-agricultural wetlands near Kemptville and Bishop Mills (ON; 44°52'25N, 75°42'16"W). The frogs were then transported to the University of Ottawa animal care facility, and artificially bred following established protocols (Trudeau et al., 2013; Vu et al., 2017).

Five egg masses were transported to Carleton University in Ottawa, Ontario, Canada. Here, the egg masses were placed in an environmentally-controlled Conviron chamber and maintained at 22°C, 70% humidity and a 16 h light:8 h dark photoperiod following Robinson et al. (2020). Egg masses were evenly distributed between four 60 L plastic stock tanks filled with City of Ottawa tap water that was dechloraminated using Prime® water conditioner, aerated and allowed to equilibrate with chamber temperatures for at least 24 hrs. Partial water changes (50-75%) occurred every 48-72 h for the duration of the experiment. Once egg masses hatched and were free-swimming they were fed President's Choice® frozen kale and Wards *Xenopus*

tadpole food *ad libitum*; tadpoles were also provided 1-3 Hikari KYORIN Co Inc. algae wafers once weekly (after Robinson et al, 2020). Stock tank water quality was monitored in order to ensure stock tadpoles were maintained within the Organisation for Economic Co-operation and Development (OECD) guidelines (OECD 2009; See Yable 2). Here, a YSI Professional Plus meter (YSI Inc., Yellow Springs, OH, USA) was used to measure temperature, pH, dissolved oxygen, and specific conductance. Ammonia, nitrate, nitrite and water hardness were assessed using API® Freshwater Master Test Kits and API® GH and KH Test Kits (Mars Fishcare North America Inc.).

All animal handling and sampling for this project was done in accordance with the Canadian Council for Animal Care (CCAC) Guidelines approved by the Wildlife East Animal Care Committee of Environment and Climate Change Canada (22SR01) and the University of Ottawa Animal Care Committee approved animal use protocol (BL-2206).

2.5 Embryo deployment and monitoring

On May 2, 2022, embryos from the above-mentioned stock tanks were manually separated using disposable pipettes and haphazardly selected from the five egg masses and assigned into groups of 80 embryos to be placed into one of three cages at each of the nine experimental sites. Additionally, two control groups, a driving control and an acclimatization control, were used in order to ensure that the deployment protocols did not cause increased mortality (n = 80 embryos per cage; 3 cages per site; 2 control groups; total embryos used = 2320).

Embryos were deployed into their cages on May 2, 2022 and monitored thrice weekly for two weeks (Until May 16th). On May 16, 2022, a flooding event occurred in the SN that

damaged the majority of the experimental cages at all sites. More than 30 mm of rainfall occurred between May 16 and 17 that raised the water depth at each site to the point that it was unsafe to enter the ditch (i.e. higher than 1.8 m at some sites) (Environment and Climate Change Canada, 2022). Due to the damage caused by the flooding event, no data could be collected from the embryo experiment.

2.6 Tadpole deployment and monitoring

Tadpole cages were deployed one week prior to tadpole deployment on May 27, 2022. Tadpole cages were cylindrical (60 cm deep, 35 cm diameter) and made of 500 μm Nytex mesh (Dynamic Aqua-Supply Ltd. Surrey BC; Figure 1). Each cage was secured to a wooden floatation frame using eyebolts and zip ties (see Harris et al 2001). The floatation frame ensured the bottom of the cages remained at least 15 cm below the water surface and kept the tadpoles near the water-air interface (after Harris et al 1998; Harris and Bogart 1997). Plastic snow fencing was placed around the cages to protect tadpoles from large predators such as muskrats and to protect cages from damage (Harris et al. 2001). Tadpole cages were also anchored to stakes using rope in order to prevent them from drifting in the event of flooding. On the morning of deployment, tadpoles were haphazardly selected from the four previously mentioned lab stock tanks and placed into replicate groups of 15 individuals. Twenty-seven replicate groups (three replicate cages per site) were created comprised of individuals that were similar in size and Gosner stage (Gosner 1960). An additional two control groups of 15 individuals each were also created; a transport control and an acclimatization control. All individual tadpoles were placed into 2 L Ziploc® bags containing 1 L of stock tank water and

transported to the experimental sites in coolers. Tadpoles acclimatized to ditch waters by slowly adding 250 mL of ditch water to the Ziploc® bags every 15 min for a total of one hour. Once tadpoles were acclimatized to ditch waters, they were placed into their respective cages by slowly pouring the contents of the bag into the cages. Tadpoles were fed President's Choice frozen kale and Ward's *Xenopus* tadpole food *ad libitum* throughout the experiment to ensure that any between-site tadpole differences were not due to lack of nutrition. Acclimatization control tadpoles received the same acclimatization treatment as experimental tadpoles but were then transported back to the lab at NWRC along with the transport control tadpoles. Control tadpoles were then placed into two aquaria filled with stock water and monitored for four weeks to ensure no mortality occurred due to the effects of acclimatization or transportation.

Experimental tadpoles were monitored twice weekly from May 27 to July 12, 2022 (8 weeks). Tadpoles were photographed at each site visit and assessed for survival, signs of stress (e.g. odd/erratic swimming, loss of equilibrium), and malformations. In order to photograph tadpoles, cages were gently lifted out of the water and allowed to drain. If any dirt or silt remained in the cage, the cage was re-submerged and gently rocked back and forth to allow any silt to dissolve into the water. A coin (a Canadian quarter) was then placed in the cage for scale and a photograph was taken of the tadpoles, ensuring no tadpoles overlapped in the photograph. On May 31st, 2022, a cage from site 346 was found damaged with no tadpoles; this cage was omitted from statistical analyses. Between sites, all equipment was decontaminated following standard protocols (CHHWG, 2017) to ensure no contaminants or pathogens were transported between sites.

2.7 Tadpole dissections and endpoint collection

The experiment ended on July 12, 2022, as ditch water depths were dropping below cutoff levels (less than 10 cm). Between July 12 and 14, 2022, tadpoles were collected from cages, placed in plastic sandwich containers (15 x 15 x15 cm) with some ditch water, and transported in coolers back to NWRC. Upon arrival at NWRC, a subset of three tadpoles were randomly selected from each cage and sampled for corticosterone levels. Following similar methods outlined in Gavel et al. (2019), tadpoles were placed into (250 mL) mason jars filled with (40 mL) of Prime[®] conditioned tap water at room temperature and allowed to sit for one hour. Once tadpoles had sat in the mason jar for one hour, the mason jar was tipped lightly and the water was placed into 50 mL falcon tubes and frozen at -80°C for future corticosterone analysis. Tadpoles were then removed from the mason jars and placed back into their respective container. The collected water was analyzed by the lab services at NWRC following methods outlined in Gavel et al. (2019).

For endpoint collections, tadpoles were first anesthetized in 0.015% buffered MS-222 solution following approved animal care protocols (ECCC: 22SR01). Once tadpoles were fully anesthetized (tadpoles move more slowly or stop moving altogether), individuals were blotted dry using a paper towel, weighed (Mettler Toledo; ± 0.01 g), then placed on grid paper and photographed for later measurements; tadpoles were also assessed for other abnormalities (e.g. tail kink, tail fungus) at this stage. Tadpoles were then euthanized by immersion in 0.2% buffered MS-222 solution and subsequent cervical dislocation using a scalpel. Any pooling blood from cervical dislocation was then used to determine glucose concentrations. Here, an Ascensia Contour Next Strip (Glucose strip) was lightly dipped in any residual blood and the

glucose concentration measured using the Ascensia Contour Next One Blood Glucose Monitor. The release of glucose as glucocorticoids is a known and measurable stress response in fish (Costas, 2008; Lankford et al., 2005; Sapolsky et al., 2000).

In order to determine if experimental tadpoles were infected with Ranavirus, “tail clips” were collected from euthanized tadpoles for DNA extraction (following Lung et al., 2017). Here, tadpoles were placed on a petri dish that was previously sterilized with 95% ethanol and a small (approx. 1 cm²) piece of tail tissue was incised using a scalpel. Tissue samples were then placed into a 2 mL cryovial and snap-frozen in liquid nitrogen. All tail clips were stored at -80°C until use in DNA extractions. All equipment (scalpels, tweezers, countertop, petri dishes) were sterilized between each tadpole to ensure no cross-contamination between samples.

Additionally, in order to determine if tadpoles were infected with *Batrachochytrium dendrobatidis* (Bd), tadpole mouthparts were removed using a scalpel and placed into a 2 mL cryovial and snap-frozen in liquid nitrogen and later shipped to the University of Guelph Animal Health Lab for determination of Bd infection status.

Tadpole heart smears were prepared as described in Gavel et al., (2019) for potential leukocyte profiling in a separate study. Individual tadpole livers were then removed and weighed (± 0.01 g); liver weight was also used to calculate liver hepatosomatic index (HSI; see Eq. 1). Liver HSI can be used as a proxy for toxic stress in frogs (Edge et al., 2011). To ensure the derived ratio of liver mass to body mass was appropriate, a scatterplot of these data was created (Figure 2; following Curran-Everett, 2013).

$$[\text{Equation 1}] \text{ Hepatosomatic Index} = \frac{\text{liver mass (g)}}{\text{body mass (g)} - \text{liver mass (g)}}$$

Tadpoles were then placed under a dissection microscope and their stage of development (Gosner 1960) and morphological sex were determined (Appendix 3: Figure 13; Figure 14). Only individuals at least GS36 or above were included in sex ratio calculations, as sexual differentiation is generally only complete by this stage (Hogan et al 2008). Tadpole sex ratio (or proportion female) is calculated by dividing the number of females by the total number of surviving male and female tadpoles.

2.8 Ranavirus and chytrid fungus detection

For determination of FV3 infection status, DNA was extracted from tail clip tissue samples using a Qiagen DNeasy Blood and Tissue kit (see Qiagen DNeasy Blood and Tissue Kit Handbook, 2011). The Qiagen kit uses ATL buffer in combination with Proteinase K to lyse cells and obtain mitochondrial, nuclear, and viral DNA. Here, tail clip tissue was first allowed to thaw then a small segment (<25 mg) was removed using a scalpel and cut into small pieces. Again, all equipment (scalpel, tweezers, counter top, petri dish) were sterilized using 95% ethanol between each sample to prevent cross-contamination. The tail tissue was then placed into a 2 mL microcentrifuge tube with a Proteinase K-buffer solution and left on a thermomixer at 56°C overnight. In order to confirm no cross-contamination occurred, a “bench” negative was obtained after the processing of every fourth sample. After the equipment and surfaces were sterilized, bench negatives were obtained by rubbing tweezers on the petri dish then placing them into a 2 mL microcentrifuge tube containing Proteinase K-buffer solution and swirled for 2-3 seconds (after Lung et al., 2017) then left in a thermomixer (Fisherbrand™ Temp Master;

SN: N08573) at 56°C overnight. If sterilization was effective, no evidence of cross-contamination should be present in the bench negative samples (i.e. all bench negatives should test negative for FV3). The next day, if the tissue was not fully digested, it was left to agitate for another 4 hours before DNA was eluted from the sample. In order to elute DNA, a 600 µL aliquot of extracted DNA solution was placed onto a filter and washed of any contaminants and unwanted material before being eluted into a 2 mL microcentrifuge tube. Eluate was then stored at 4°C for immediate use or kept at -80°C for long-term storage.

All samples were analyzed for the presence of Ranavirus using a POCKIT™ nucleic acid analyzer. POCKIT™ analyzers follow a principle known as conductive polymerase chain reaction (PCR), which is a method of nucleic acid amplification that makes use of a constant convective heat source below the reaction cell (Lung et al., 2017). When a liquid is heated from below, the warmer layer of reaction liquid will rise while cooler liquid descends, allowing the different stages of the PCR reaction to occur at different stages of convection (i.e. denaturation, primer annealing, extension; Krishnan et al., 2002; Lung et al., 2017). More specifically, POCKIT™ analyzers use thermal baffles to reduce heating and cooling times during reactions (Tsai et al., 2012); a technique that has been termed insulated isothermal polymerase chain reaction (iiPCR). This technique was later combined with a TaqMan probe fluorescence in POCKIT™ analyzers, allowing for quick, real-time results. For this study, the primers and probes used for the POCKIT™ iiPCR assays were supplied by GeneReach Biotechnology Corporation. The Frog Virus 3 Reagent Set contains primers and probes that target the major capsid protein-coding region (MCP4 and MCP5) common among many iridoviruses (2012; Lung et al., 2017). For shelf

stability and ease of use, reagents (primers, probes, dNTP's and enzyme) are all contained within a lyophilized pellet.

In order to start a reaction, 50 μ L of Premix Buffer B was added to tubes containing reagents (following methods outlined in GeneReach Inc. 2012; Lung et al., 2017). Five μ L of template DNA was then added to each tube and vortexed for 10 seconds. Fifty μ L of reaction solution was then transferred to an R-tube and spun down in a microcentrifuge for 10 seconds. R-tubes were then placed in an eight-well POCKIT™ analyzer using the 520 nm setting; reactions take approximately 1 hr to complete. After a full-cycle, results are indicated as either positive or negative for each sample. POCKIT™ analyzers have a detection limit of approximately 10 copies per reaction (GeneReach Inc. 2012; Lung et al., 2017). Positive controls were provided by the manufacturer and consisted of dried plasmid containing a partial sequence of Frog Virus 3. Negative controls consisted of lab-reared tadpole tail clips. A total of 247 tail clips were analyzed, six of which were controls.

The quantity and purity of eluted DNA was tested using a NanoDrop™ 2000 spectrophotometer. Ten voucher samples were randomly selected (Random.org; one sample from each site – except site 346 – and one control) to be tested via nanodrop. Each sample was tested twice for quality assurance. Here a 1 μ L aliquot of eluate was placed on the measurement pedestal and measured (Dodsworth, 2020). DNA is considered of good quality when the absorbance ratios of 260:280 nm is between 1.8 and 2.0 (Dodsworth, 2020). All absorbance ratios were above 1.9 and all nucleic acid concentrations were above 150 ng/ μ L for this study (absorbance and concentration values are reported in Table A2).

Tadpole mouthclips were sent to the University of Guelph Animal Health Lab for molecular analyses for determination of infection by Bd. DNA was first extracted from mouthclips by following standard protocols outlined in Boyle et al. (2004). Extracted DNA was then analyzed via TaqMan probe real-time quantitative PCR (RT-qPCR) designed to target highly conserved region 5.8, 18 and 28S DNA (Boyle et al., 2004). The target 5.8, 18 and 28S rRNA is separated by internal transcribed spacers (ITS-1 and ITS-2) and an intergenic spacer (Boyle et al., 2004). A total of 142 mouthclips were analyzed, 129 of which were experimental field tadpoles and 13 of which were lab-reared control tadpoles.

2.9 Statistical analyses

All statistical analyses were performed in Rstudio (Version 2023.06.1+524 "Mountain Hydrangea" for Windows) using R version 4.3.1 (R core team 2023). Statistical assumptions for all models were first assessed visually following methods outlined in Zuur et al. (2010). More specifically, Cleveland dotplots and boxplots were used to assess the data and for the presence of outliers.

Any samples that fell below detection limit (DL) were converted to maximum-likelihood estimates (MLE) for use in statistical analyses. This was done for all pesticides (neonicotinoids, atrazine, glyphosate), and corticosterone. Additionally, some current velocity measurements were estimated via maximum likelihood estimation, as velocity was too slow to measure at times. The estimates produced from MLE were then used instead of DL values in subsequent analyses. Maximum-likelihood estimates were determined following methods outlined in (Office of Pesticide Programs, 2005). Here, a probability density function (PDF) that maximizes

the likelihood of observing the collected data is produced. This PDF is then used to estimate values most consistent with the observed data.

The assumption of independence could not always be met for the models used in this study. The use of generalized linear mixed-effects models (GLMM) allows for the inclusion of random effects in the model to control for non-independence. For example, having many cages within the same site violates the assumption of independence of cages, including cage as a random effect in the models allows for the control of this assumption violation. The addition of random effects to the models also allows for the control of other potential confounding variables that were not included as fixed effects in the model. For example, any site effects that were not considered by the model can be controlled by including 'Site' as a random effect in the model. Finally, GLMM's allow for the use of non-normal distributions such as Poisson distributions which are necessary for some of the response variables analyzed in this study. All GLMM models with a Gaussian distribution were constructed with a logit-link function using the lme4 and lmerTest packages (Bates et al., 2015; Kuznetsova et al., 2017). Some models did not converge, meaning that the random effects had little to no variance, and therefore the full model could not fit the data. In these cases, the variance on the random effects was assessed and all random effects with no variance were removed from the models. In some cases, data were skewed; in these cases, data were log-transformed to better fit the model.

To assess whether vegetation height and percent forest cover had an effect on water chemistry, contaminant levels, and site physical characteristics GLMM's with a Gaussian distribution were used. Water chemistry variables (i.e., temperature, pH, specific conductance (SPC), and percent dissolved oxygen (DO)), pesticide concentrations (i.e. total neonicotinoids,

atrazine, or glyphosate), nutrient and ion concentrations, water depth, and current velocity were used as response variables in the models. For all water quality models, vegetation height and/or percent forest cover were included as fixed effects. For nutrient and ion models, a Pearson cross-correlation table was constructed (Table 3) to determine which nutrients and ions were most highly correlated. The Pearson correlations were then used to narrow down which water chemistry variables to analyze in subsequent models (i.e., nitrate (NO₃), total phosphorus (TP), dissolved organic carbon (DOC), and total suspended solids (TSS), while ions analyzed were chloride (Cl) and dissolved potassium (K)). Some sites (Sites 326, 327, 337) were within the same ditch and many samples were taken throughout the experiment. To account for this, site and date were used as random effects in the water chemistry models to account for the non-independence of sites and repeated measurements through time.

Principal component analysis (PCA) was used to visually compare water quality characteristics between sites and outline any potential relationships between water chemistry and sites.

To determine whether vegetation height and percent forest cover had an effect on tadpole health metrics, a series of distribution-specific GLMM's were used. Site and cage were included as random effects in the models where possible, to control for the non-independence of tadpoles from the same cage and multiple cages at the same site.

GLMMs with a Gaussian distribution were used to determine the effects of vegetation height and forest cover on body size (SVL, body width, tail length, mass), and measures of stress (HSI, corticosterone, glucose). Survival and GS were included as fixed effects (but were removed if they did not improve model fit) for all above-mentioned developmental endpoints to control

for the density effects of survival (Johnson et al., 2017) and to control for the effects of GS on development. However, GS was not used for the mass model, because including GS in the body mass model assumes a linear relationship between body mass and GS when the relationship is in fact not linear (Figure 3A). Instead, SVL was included as a fixed effect in the body mass model as body mass and SVL are linearly related (Figure 3B).

A GLMM with a binomial distribution and a logit link function (from Bates et al., 2015) was used for the survival and proportion female models following methods in Zuur et al. (2015). The developmental endpoint of GS used count data, therefore a penalized quasi-likelihood GLMM was fit with a Poisson distribution using the `glmmPQL` function from the MASS package in R (Venables & Ripley, 2002). Here, GS was the dependent variable with vegetation height, percent forest cover, and survival as fixed effects in the model with site and cage as a random effects. Dispersion was also assessed to ensure that the appropriate distribution was used. Here, a dispersion value of ~ 1 means the appropriate distribution was used (Zuur et al, 2015).

Redundancy analysis (RDA) was used to visually compare water chemistry variables, tadpole endpoints, and sites and to outline any potential relationships between these variables. Variables for RDA were selected using PCA results (i.e. variables that explained most of the site water chemistry variation). Variables were further selected using forward stepwise selection procedures, eliminating water chemistry variables that explained the least variation in tadpole health metrics.

Multi-model inference was used when possible to determine the best fitting model for the water chemistry and contaminant models and the tadpole response models. The Akaike Information Criterion (AIC; Burnham & Anderson, 2004; Nakagawa & Cuthill, 2007) was used to

select the best-fitting models by comparing their AIC scores and selecting the model with the lowest score. A backwards stepwise regression selection procedure was used to determine the best-fitting model. Here, the full model was first assessed, and then predictor variables were removed from the model and compared to the full model until a reduced model that best fit the data was found. Parameters for the best-fit model were reported following methods outlined in Zuur et al., (2009 and 2015). Model comparison with AIC is not possible with the glmmPQL package, so only the full model was used for GS.

All models were validated visually using residual vs fitted value plots to determine homogeneity of variance, quantile-quantile plots to determine if residuals were normally distributed, and density plots (following Zuur et al., 2010).

Descriptive summary statistics are provided for water chemistry, contaminant levels and tadpole health metrics such that mean \pm standard deviation are presented for normally distributed data and medians with 25 and 75% interquartile ranges are presented for non-normal data.

Chapter 3: Results

3.1 Site characteristics

Across the nine sites, riparian vegetation height varied from 0.13 m to 6.4 m while percent forest cover varied from 1% to 60% (Table 1). Vegetation height and percent forest cover have a correlation coefficient of $R^2 = 0.57$ (Figure 4). Ditch water depth varied from 0.093 m to 0.98 m with a mean depth of 0.37 m (SD ± 0.18 m) across sites and current velocity within the stream channel varied from 0 m/s to 0.33 m/s with a median velocity of 0.002 m/s (0.0003 – 0.04 m/s) across sites (Table 1).

Vegetation height was not correlated with mean ditch depth. However, percent forest cover was positively and significantly correlated with mean ditch depth across sites (GLMM: $t = 4.25$, $p = 0.002$; Table 4). In contrast, vegetation height and percent forest cover were not correlated with mean current velocity across sites.

3.2 Water chemistry

Temperature was highly variable over time but did not show any consistent differences between sites. Temperature measured via YSI probe varied from 11.9 °C to 29.9 °C with a mean of 18.2 °C (± 2.7 °C) across sites while temperature measured via Hobo pendant varied from 9.28 °C to 32.9 °C with a mean of 18.4 °C (± 2.67 °C) across sites (Table 5). Dissolved oxygen (DO) ranged from 17.7% to 195% saturation across sites with a mean of 91.3% ($\pm 35.2\%$; Table 5). Dissolved oxygen was highly variable within sites and at times did fall below OECD (2009) guideline levels of 40% air saturation at certain sites during the experiment, but would quickly rebound above guideline levels (Table 2). Specific conductance (SPC) ranged from 93.8 $\mu\text{S}/\text{cm}$

to 1084 $\mu\text{S}/\text{cm}$ across sites with a mean of 643 $\mu\text{S}/\text{cm}$ ($\pm 206 \mu\text{S}/\text{cm}$; Table 5). Finally, pH showed little variation across sites with values ranging from 7.11 to 8.28 and a mean of 7.73 (± 0.23 ; Table 5).

As mentioned, temperature varied highly across sites for both YSI probe and Hobo pendant measurements but no statistically significant relationship was detected between vegetation height or percent forest cover and temperature using either measurement (Table 6). Although DO varied by up to an order of magnitude across sites, no statistical relationship was detected between DO and vegetation height or percent forest cover (Table 6). A significant negative relationship between forest cover and SPC was found, with sites higher in percent forest cover having lower SPC (GLMM: $t = -2.26$, $p = 0.03$; Table 6). No relationship between pH and vegetation height or percent forest cover was found.

Significant differences in nutrient and ion concentrations were seen across the nine experimental sites (Table 5). Total phosphorus (P) concentrations varied from 0.009 mg/L to 0.5 mg/L with a median concentration of 0.04 mg/L (0.02 – 0.07 mg/L) across sites (Table 5). Nitrate (NO_3) concentrations varied from 0.02 mg/L to 20.5 mg/L with a median of 0.97 mg/L (0.42 – 4.01 mg/L; Table 5). Chloride (Cl) concentrations varied across sites from 6.4 mg/L to 156 mg/L with a mean concentration of 34.4 mg/L ($\pm 29.4 \text{ mg/L}$; Table 5). Dissolved potassium (K) concentrations ranged from 0.3 mg/L to 7.5 mg/L with a mean concentration of 3.2 mg/L ($\pm 1.8 \text{ mg/L}$) across sites (Table 5). Dissolved organic carbon (DOC) ranged from 2.8 mg/L to 28.8 mg/L with a mean of 9.2 mg/L ($\pm 6.6 \text{ mg/L}$) across sites (Table 5). Finally, total suspended solids (TSS) varied from 2 mg/L to 578 mg/L with a median of 7 mg/L (5 – 15 mg/L; Table 5).

Notably, percent forest cover was significantly correlated with most nutrient concentrations while vegetation height was only significantly correlated to Cl. More specifically, vegetation height had a significant positive effect on Cl concentrations (GLMM: $t = 4.55$, $p = 0.001$; Table 7). Higher forest cover was associated with significantly higher DOC concentrations (GLMM: $t = 3.76$, $p = 0.005$) and significantly higher P concentrations (GLMM: $t = 2.93$, $p = 0.017$; Table 7). No effect of vegetation height or percent forest cover was found for TSS. Conversely, percent forest cover was significantly negatively correlated with NO_3 (GLMM: $t = -4.31$, $p = 0.002$) and non-significantly negatively correlated with K (GLMM: $t = -2.08$, $p = 0.07$) with sites with higher forest cover tending to have lower concentrations of both NO_3 and K (Table 7).

Atrazine, glyphosate, and seven neonicotinoids (acetamiprid, clothianidin, dinotefuran, flupyradifurone, imidacloprid, thiacloprid and thiamethoxam) concentrations were analyzed from water samples (atrazine and total neonicotinoids $n = 117$, glyphosate $n = 72$). Of the seven neonicotinoids, only five had samples with concentrations above detection limits (DL): acetamiprid, clothianidin, flupyradifurone, imidacloprid and thiamethoxam. More specifically, acetamiprid was detected in two samples (0.02%), clothianidin was detected in all but five water samples (96%), flupyradifurone was only detected in two water samples (0.02%), imidacloprid was detected in 38 samples (32%) and thiamethoxam was detected in 33 samples (28%); all other samples were below the method detection limit (See Table A1 for MDL values). In total, neonicotinoids were detected in 113 samples (97%) and total neonicotinoid

concentrations varied from 0.00002 (DL¹; acetamiprid) to 0.021 µg/L with a mean concentration of 0.006 µg/L (\pm 0.004 µg/L; Table 5). Atrazine was detected in all water samples and ranged from 0.0008 to 1.03 µg/L across sites and time with a median concentration of 0.03 µg/L (0.01 – 0.07 µg/L; Table 5). Glyphosate was detected in 28 water samples (39%) and concentrations varied from 0.025 (DL) to 0.568 µg/L with a median concentration of 0.008 µg/L (0.002 – 0.03 µg/L; Table 5). Pesticide concentrations never exceeded Canadian Council for the Ministers of the Environment (CCME) guideline levels for the protection of aquatic life (Table 2). Peak concentrations for all pesticides occurred on the first sampling day of May 27, 2022 (Julian date: 147; Figure 5). The following peak for atrazine and glyphosate occurred on June 14, 2022 (Julian date: 165; Figure 5), while peak concentrations for total neonicotinoids occurred on June 24, 2022 (Julian date: 175; Figure 5).

Vegetation height had a significant positive effect on total neonicotinoid concentrations (GLMM: $t = 3.98$, $p = 0.004$; Table 8) while percent forest cover had a negative effect on total neonicotinoid concentrations across sites (GLMM: $t = -4.81$, $p = 0.001$; Table 8). Percent forest cover had a significant negative effect on atrazine concentrations across sites (GLMM: $t = -4.84$, $p = 0.001$; Table 8). No significant relationship between vegetation height or percent forest cover and glyphosate concentrations was found.

A PCA of water quality variables (9 in total) revealed some separation of the sites based on vegetation height along PCA Dim1, which explained 40.1% of the variation (Figure 6). Higher NO₃ and K were drivers separating sites with lower vegetation heights (326, 337) from sites

¹ DL = Detection limit. Pesticide concentrations below detection limit were later converted to maximum-likelihood estimates for use in statistical models.

with higher vegetation height along Dim1. Higher DOC and depth were drivers separating higher vegetation sites (340, 346) and site 333 from lower vegetation sites along Dim1. Finally, higher TP and TSS were associated with site 324 while high temperature and DO were associated with sites 341 and 325 along Dim2 which explained 19.1% of the variation.

3.3 Tadpole survival, development and pathogen infection

Tadpole survival was generally high with within-site survival ranging from 64% to 100% with a mean survival of 85% ($\pm 12\%$; Table 9). Of the nine experimental sites, only two sites dropped below 80% survival. Sex ratios varied highly with sites ranging from 31% to 59% female with a mean of 49% female ($\pm 9\%$; Table 9). All measures of body size (SVL, BW, TL) varied greatly across sites. More specifically, tadpole SVL ranged from 15 mm to 29.2 mm with a mean of 22.5 mm (± 2.5 mm) across sites (Table 9). Tadpole body width ranged from 8.3 mm to 17.4 mm with a mean of 13 mm (± 1.6 mm; Table 9). Tadpole tail length ranged from 27 mm to 62.7 mm with a mean of 43.8 mm (± 6.3 mm). Tadpole mass ranged from 0.82 g to 4.58 g with a mean of 2.41 g (± 0.73 g; Table 9). Tadpole Gosner stage ranged from 30 to 43 with a median Gosner stage of 38 (37 - 39; Table 9). Measures of tadpole stress (hepatosomatic index [HSI], glucose, and corticosterone) were also quite variable across sites. Tadpole HSI varied from 0.01 to 0.058 with a mean of 0.027 (± 0.008 ; Table 9). Tadpole blood glucose levels ranged from 0.6 mg/L to 8.3 mg/L with a mean concentration of 1.2 mg/L (± 0.9 mg/L; Table 10). Finally, corticosterone levels ranged from 1.3 pg/mL (DL) to 46 pg/mL with a mean concentration of 2.9 pg/mL (± 7.2 pg/mL; Table 9).

Vegetation height and percent forest cover had no effect on either tadpole survival or sex ratios across all sites (Table 10). Additionally, vegetation height had no significant effect on any measures of body size (Table 10). However, percent cover was positively associated with changes in body size across the sites. Specifically, percent forest had a positive effect on tadpole SVL (GLMM: $t = 2.55$, $p = 0.03$; Figure 7A; Table 10) and tail length (GLMM: $t = 3.13$, $p = 0.01$; Figure 7B; Table 10), meaning tadpoles tended to be larger at sites higher in percent forest cover compared to sites lower in forest cover (Figure 7). Moreover, vegetation height and percent forest cover both had a positive effect on tadpole mass, with increasing percent forest cover (GLMM: $t = 3.21$, $p = 0.003$; Figure 8A; Table 10) and vegetation height (GLMM: $t = 2.28$, $p = 0.03$; Figure 8B; Table 11) being associated with higher mass. Vegetation height also had a significant positive effect on Gosner stage (GLMM: $t = 2.29$, $p = 0.03$ Figure 9; Table 10). Vegetation height and percent forest cover had no effect on either HSI or corticosterone levels (Table 10). However, forest cover did have a positive effect on glucose levels (GLMM: $t = 3.52$, $p = 0.002$; Figure 10; Table 10).

Redundancy analysis (RDA) of tadpole health endpoints and significant water quality variables revealed that sites with lower vegetation height (325, 326, 337) were separated from sites with higher vegetation height along RDA1 (which explained 60.96% of the variation), this separation was driven mainly by higher NO₃ (Figure 10). Higher Cl separated sites with higher vegetation height (340, 346) and site 333 from sites with lower vegetation height along RDA1. High K separated site 325, 326, 337 and 341 from other sites along RDA1. Potassium was also strongly tied to mass along RA2. Additionally, clear relationships between three nutrients and four measures of tadpole size across the first axis of the RDA, which explained of the total

variation. Nitrate and Cl were drivers of tadpole SVL, BW, TL and mass across the nine experimental sites along RDA1.

All samples analyzed for the presence of *Batrachochytrium dendrobatidis* (Bd) and Frog Virus 3 (FV3) were negative, including all controls and bench negatives. All positive controls were positive for the FV3 analyses.

Table 1. Site numbers, coordinates, and associated riparian characteristics. Forest cover is the percentage woody vegetation and tree cover within a 1 km buffer around the site. Depth and current velocity measurements were collected between May 27 – July 13, 2022. Depth is presented with mean and standard deviation. Current velocity data were non-normal and therefore are presented median and 25% and 75% interquartile range. Sample sizes for depth and current velocity were n = 8.

Site ID	X	Y	Vegetation Height (m)	Forest cover (%)	Depth mean (SD; cm)	Current median (Q1 – Q3; cm/s)
324	45°16'34"	75°09'36"	0.35	11.7	29.6 (12.8)	2.2e ⁻⁰⁵ (1.0e ⁻⁰⁵ – 3.7e ⁻⁰⁵)
325	45°15'55"	75°10'14"	0.13	2.5	18.0 (9.4)	1.2e ⁻⁰⁴ (9.2e ⁻⁰⁵ – 1.6e ⁻⁰⁴)
326	45°15'54"	75°09'26"	0.20	2.3	27.3 (17.5)	3.1e ⁻⁰⁴ (2.4e ⁻⁰⁴ – 3.8e ⁻⁰⁴)
327	45°15'40"	75°10'05"	0.50	1.2	34.3 (21.9)	6.6e ⁻⁰⁴ (5.4e ⁻⁰⁴ – 8.2e ⁻⁰⁴)
333	45°20'19"	75°09'57"	0.35	60.5	63.4 (11.9)	0.05 (0.001 – 0.06)
337	45°16'11"	75°08'44"	0.20	3.9	37.8 (13.8)	0.0018 (0.0015 – 0.015)
340	45°20'02"	75°15'19"	5.1	31.3	37.1 (10.1)	0.0034 (0.0028 – 0.0041)
341	45°15'21"	75°08'58"	0.42	2.2	38.8 (11.1)	0.11 (0.08 – 0.15)
346	45°20'08"	75°14'08"	6.4	53.8	49.8 (13.6)	0.007 (0.006 – 0.008)

Table 2. Water quality guidelines for amphibian assays and the protection of aquatic life.

Variable	Guideline	Notes	Source
Temperature	22 ± 1°C	Characteristics for acceptable lab water for amphibians	OECD, 2009
Dissolved Oxygen	>40%	Characteristics for acceptable lab water for amphibians	OECD 2009
pH	6.5 - 8.5	Characteristics for acceptable lab water for amphibians	OECD 2009
Chloride	120 mg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	CCME, 2011
Nitrate	13 mg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	CCME, 2012b
Phosphorus	0.02 mg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	CCME, 2004
Imidacloprid	0.23 µg /L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	CCME, 2007
Atrazine	1.8 µg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	CCME, 1999
Glyphosate	800 µg/L	Canadian Water Quality Guidelines for the Protection of Aquatic Life	CCME, 2012a

Table 3. Pearson cross-correlation of water chemistry variables. Blue indicates positive correlations while red indicates negative correlations (with darker shades meaning a stronger correlation). DOC = Dissolved Organic Carbon; RP = Reactive Phosphorus; TKN = Total Kjeldahl Nitrogen; TOC = Total Organic Carbon; TP = Total Phosphorus; DO% = Dissolved Oxygen Percent Saturation; ORP = Oxidation-Reduction Potential; TN = Total Nitrogen; WQI = Water Quality Index.

	Ammonia	Calcium	Chloride	DOC	Magnesium	Nitrate	Potassium	RP	Silicon	Sodium	TKN	TOC	TP	Suspended sol	Temp	DO%	conductance	pH	ORP	Depth (cm)	TN	WQI	
Ammonia	1.00																						
Dissolved Calcium	-0.60	1.00																					
Chloride	0.66	-0.28	1.00																				
Organic dissolved carbon	0.57	-0.93	0.19	1.00																			
Dissolved magnesium	-0.21	0.76	-0.18	-0.74	1.00																		
Nitrate	-0.54	0.81	-0.32	-0.83	0.65	1.00																	
Dissolved potassium	-0.02	0.54	0.02	-0.65	0.85	0.66	1.00																
Reactive Phosphorus	0.61	-0.31	0.10	0.30	0.15	-0.49	0.18	1.00															
Dissolved silicon	0.24	0.02	-0.33	0.05	0.43	-0.10	0.18	0.64	1.00														
Dissolved sodium	0.73	-0.34	0.99	0.24	-0.16	-0.37	0.04	0.19	-0.22	1.00													
Total Kjeldahl nitrogen	0.65	-0.58	0.22	0.79	-0.47	-0.60	-0.56	0.32	0.28	0.25	1.00												
Total organic carbon	0.57	-0.93	0.18	1.00	-0.74	-0.83	-0.66	0.30	0.06	0.23	0.79	1.00											
Total phosphorus	0.59	-0.22	0.02	0.31	0.08	-0.45	-0.03	0.89	0.72	0.09	0.56	0.31	1.00										
Total suspended solid	-0.36	0.61	-0.47	-0.51	0.66	0.69	0.40	-0.24	0.52	-0.45	-0.17	-0.50	-0.08	1.00									
Temperature in °C	-0.43	0.31	0.01	-0.40	0.40	0.52	0.45	-0.55	-0.27	-0.01	-0.60	-0.41	-0.78	0.38	1.00								
DO%	-0.02	-0.10	0.09	-0.13	0.30	0.18	0.59	0.09	-0.04	0.13	-0.53	-0.14	-0.32	0.01	0.68	1.00							
Specific conductance (uS/cm)	-0.25	0.89	0.10	-0.88	0.86	0.71	0.76	-0.09	0.06	0.07	-0.55	-0.89	-0.11	0.49	0.37	0.12	1.00						
pH	-0.23	0.43	0.27	-0.65	0.54	0.41	0.69	-0.06	-0.17	0.27	-0.80	-0.66	-0.36	0.08	0.64	0.70	0.65	1.00					
ORP	-0.06	0.02	0.13	-0.10	-0.04	-0.40	-0.26	0.24	0.27	0.18	-0.14	-0.10	0.25	-0.14	-0.19	-0.11	0.05	0.27	1.00				
Depth (cm)	0.27	-0.69	0.25	0.55	-0.80	-0.71	-0.68	0.17	-0.11	0.27	0.29	0.55	0.19	-0.62	-0.47	-0.13	-0.69	-0.21	0.52	1.00			
TN	-0.49	0.79	-0.31	-0.79	0.63	1.00	0.64	-0.48	-0.07	-0.37	-0.52	-0.79	-0.41	0.72	0.48	0.13	0.69	0.34	-0.45	-0.72	1.00		
WQI	-0.77	0.31	-0.46	-0.36	0.20	0.35	0.20	-0.45	-0.40	-0.50	-0.71	-0.36	-0.68	0.04	0.63	0.43	0.13	0.37	-0.16	-0.36	0.28	1.00	

Table 4. General Linear Mixed-effect Model outputs for drainage ditch water depth and current velocity (n = 8).

Factor	β^a	SE	df	t	p-value	Variance	SD
Depth ~ Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)		28.5	4.18	19.7	6.83	1.34e ⁻⁰⁶	
Forest Cover		0.45	0.11	8.74	4.25	0.002	
Random effects							
Date						122	11.1
Site						47.4	6.89
Residual						61.8	7.86
Log(Current) ~ 1 + ~1 Date + ~1 Site							
Fixed effects (Intercept)		-6.05	0.83	10.6	-7.33	1.81e ⁻⁰⁵	
Random effects							
Date						0.81	0.90
Site						5.42	2.33
Residual						2.19	1.48

^a β is the direction and magnitude of the relationship between the response variable and the predictor variable (riparian vegetation height or percent forest cover).

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

Table 5. Water chemistry variable means and standard deviations. Water chemistry and contaminant variable means and standard deviations (or medians with 25% and 75% interquartile ranges for non-normal data) for sample measurements from May 27 to July 13, 2022. Sample sizes for atrazine and total neonicotinoids were n = 13 and n = 8 for glyphosate. Sample sizes for nutrients was n = 8. Sample sizes for YSI data was n = 16. Sample size for Hobo data was n = 1263.

Water chemistry variable	Site ^a								
	325	326	337	324	333	341	327	340	346
<i>YSI and Hobo data</i>									
Temperature (Hobo; °C)	16.7 (5.16)	16.9 (4.27)	NA ^b	16.8 (4.30)	NA ^b	17.1 (4.06)	16.5 (3.85)	17.1 (2.74)	19.1 (2.08)
Temperature (YSI; °C)	19.8 (4.58)	17.8 (1.89)	19.5 (2.60)	16.7 (2.46)	17.3 (1.95)	18.8 (2.81)	17.6 (2.35)	17.8 (1.74)	18.6 (1.93)
Dissolved Oxygen (%)	121.4 (37.6)	62.3 (32.9)	106.5 (34.1)	88.8 (23.3)	102.3 (7.60)	105.7 (36.5)	56.8 (49.0)	88.1 (5.86)	92.0 (10.9)
Specific Conductance (µS/cm)	788.8 (43.0)	728.6 (51.9)	691.1 (52.0)	852.7 (139.0)	255.4 (136.5)	659.4 (28.0)	731.1 (51.4)	366.4 (68.9)	715.9 (98.7)
pH	7.88 (0.23)	7.60 (0.13)	7.90 (0.19)	7.84 (0.19)	7.64 (0.17)	7.84 (0.27)	7.55 (0.16)	7.52 (0.23)	7.82 (0.1)
<i>Nutrients and ions</i>									
Ammonia (mg/L)	0.042 (0.036)	0.032 (0.02)	0.016 (0.01)	0.056 (0.038)	0.052 (0.021)	0.034 (0.027)	0.037 (0.025)	0.0550 (0.027)	0.064 (0.043)
Chloride (mg/L)	29.5 (6.59)	15.6 (3.54)	15.5 (3.53)	36.7 (15.8)	17.6 (13.4)	35.0 (17.4)	15.3 (3.46)	40.3 (7.35)	103.8 (27.3)
Dissolved Calcium (mg/L)	95.5 (9.46)	109 (6.91)	102.2 (8.08)	89.0 (16.7)	15.5 (1.53)	85.9 (15.4)	112 (6.39)	32.2 (5.35)	60.7 (11.6)
Dissolved Organic Carbon (mg/L)	4.88 (0.92)	5.34 (1.15)	5.42 (1.53)	6.68 (2.10)	18.4 (4.90)	4.52 (2.72)	5.34 (1.69)	21.5 (3.71)	11.0 (3.05)
Dissolved Magnesium (mg/L)	36.7 (4.48)	23.4 (1.93)	24.1 (1.89)	35.8 (6.64)	4.91 (0.66)	16.3 (1.59)	22.5 (3.11)	8.47 (2.09)	14.2 (0.86)
Dissolved Potassium (mg/L)	5.95 (0.93)	2.50 (1.01)	2.71 (0.97)	5.13 (1.47)	1.28 (0.50)	4.35 (1.01)	2.88 (1.35)	1.63 (0.24)	2.68 (0.54)
Dissolved Silicon (mg/L) ^c	7.00 (5.48 – 8.23)	6.10 (2.03 – 10.60)	5.90 (4.28 – 8.53)	8.45 (6.28 – 11.58)	6.00 (5.38 – 7.23)	2.70 (1.85 – 2.88)	6.20 (3.90 – 11.90)	4.80 (4.48 – 5.38)	4.85 (3.73 – 5.23)
Dissolved Sodium (mg/L)	25.0 (4.03)	13.9 (1.66)	13.5 (1.58)	29.7 (12.04)	20.5 (13.77)	23.7 (10.23)	12.7 (2.35)	28.3 (5.06)	67.3 (16.38)
Nitrate (mg/L) ^c	4.45 (0.71 – 10.26)	0.46 (0.09 – 4.65)	0.37 (0.08 – 4.66)	0.54 (0.32 – 4.12)	0.34 (0.21 – 0.40)	3.22 (2.52 – 5.81)	1.50 (0.62 – 7.34)	0.73 (0.63 – 0.79)	0.95 (0.56 – 1.36)
Reactive Phosphorus (mg/L) ^c	0.01 (0.004 – 0.02)	0.01 (0.004 – 0.02)	0.006 (0.004 – 0.01)	0.12 (0.05 – 0.19)	0.05 (0.03 – 0.08)	0.009 (0.005 – 0.02)	0.01 (0.004 – 0.01)	0.039 (0.036 – 0.042)	0.028 (0.025 – 0.032)
Total Kjeldahl Nitrogen (mg/L)	0.67 (0.18)	0.76 (0.26)	0.62 (0.18)	0.82 (0.21)	0.94 (0.20)	0.55 (0.19)	0.95 (0.74)	1.17 (0.13)	0.92 (0.18)
Total Organic Carbon (mg/L)	4.88 (0.99)	5.66 (1.82)	5.32 (1.55)	6.68 (2.09)	18.81 (5.31)	4.36 (2.89)	5.35 (1.81)	21.59 (3.53)	11.02 (2.96)
Total Phosphorus (mg/L) ^c	0.025 (0.018 – 0.036)	0.033 (0.015 – 0.049)	0.024 (0.014 – 0.056)	0.16 (0.10 – 0.18)	0.098 (0.07 – 0.11)	0.025 (0.02 – 0.036)	0.026 (0.015 – 0.038)	0.078 (0.073 – 0.084)	0.059 (0.051 – 0.071)
Total Suspended Solids (mg/L) ^c	8 (5 - 14)	5 (2 - 13)	11 (6 - 31)	26 (5 - 58)	7 (5 - 10)	3 (2 - 7)	5 (4.5 - 25)	11 (9 - 14)	9 (7 - 12)
<i>Pesticides</i>									
Total Neonicotinoids (µg/L)	0.0057 (0.004)	0.0053 (0.0024)	0.0081 (0.0043)	0.007 (0.0024)	0.0006 (0.0004)	0.0076 (0.0054)	0.0059 (0.0048)	0.007 (0.0043)	0.0074 (0.0046)
Atrazine (µg/L) ^c	0.06 (0.03 – 0.86)	0.06 (0.05 – 0.08)	0.07 (0.05 – 0.08)	0.08 (0.07 – 0.13)	0.01 (0.003 – 0.01)	0.08 (0.03 – 0.17)	0.07 (0.05 – 0.1)	0.01 (0.01 – 0.02)	0.02 (0.02 – 0.04)
Glyphosate (µg/L) ^c	0.04 (0.01 – 0.1)	0.04 (0.01 – 0.11)	0.01 (0.01 – 0.02)	0.1 (0.02 – 0.1)	0.01 (0.01 – 0.02)	0.2 (0.06 – 0.24)	0.01 (0.01 – 0.02)	0.01 (0.01 – 0.02)	0.08 (0.07 – 0.11)

^a Sites are ordered by increasing riparian vegetation height

^b Site 333 and site 337 Hobo data loggers were lost during field exposures, no data could be salvaged from them

^c Variables marked with an asterisk were non-normal. Instead, the median is presented with 25% and 75% interquartile range
SD = Standard Deviation

Table 6. General Linear Mixed-effect Model outputs for drainage ditch water physiochemical measurements. Sample sizes for YSI temperature, DO, SPC, pH was n = 16. Sample size for Hobo data was n = 1263. DO = Dissolved Oxygen; SPC = Specific Conductance.

Factor	β^a	SE	df	t	p-value	Variance	SD
YSI Temperature ~ 1 + ~1 Date + ~1 Site							
Fixed effects (Intercept)	18.2	0.56	19.4	32.7	<2e ⁻¹⁶		
Random effects							
Date						3.04	1.74
Site						0.72	0.85
Residual						3.56	1.89
Hobo Temperature ~ Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	16.7	0.39	82.6	42.6	<2e ⁻¹⁶		
Forest Cover	0.01	0.005	6.47	1.88	0.11		
Random effects							
Date						10.8	3.29
Site						0.06	0.24
Residual						6.56	2.56
DO ~ 1 + ~1 Date + ~1 Site							
Fixed effects (Intercept)	91.6	7.87	14.5	11.6	9.28e ⁻⁰⁹		
Random effects							
Date						252	15.9
Site						363	19.0
Residual						638	25.3
SPC ~ Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	747	62.4	9.59	12.0	4.44e ⁻⁰⁷		
Forest Cover	-5.51	2.10	9	-2.62	0.03		
Random effects							

Date						1871	43.3
Site						19556	140
Residual						5203	72.13
pH ~ Vegetation Height + Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	7.73	0.06	15.5	132	<2e ⁻¹⁶		
Random effects							
Date						0.02	0.12
Site						0.02	0.14
Residual						0.02	0.14

^a β is the direction and magnitude of the relationship between the response variable and the predictor variable (riparian vegetation height or percent forest cover).

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

Table 7. General Linear Mixed-effect Model outputs for drainage ditch water nutrient and ion concentrations (n = 8). DOC = Dissolved Organic Carbon; TP = Total Phosphorus; TSS = Total Suspended Solids.

Factor	β^a	SE	df	t	p-value	Variance	SD
Log(Chloride) ~ Vegetation Height + ~1 Site							
Fixed effects (Intercept)	2.95	0.14	9	19.9	9.71e ⁻⁰⁹		
Vegetation Height	0.21	0.05	9	3.92	0.004		
Random effects							
Site						0.13	0.36
Residual						0.11	0.33
DOC ~ Vegetation Height + Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	5.25	1.65	9.01	3.18	0.01		
Forest Cover	0.21	0.06	9	3.76	0.005		
Random effects							
Date						0.04	0.19
Site						13.8	3.71
Residual						7.33	2.71
Log(TP) ~ Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	-3.49	0.16	12.7	-21.9	1.74e ⁻¹¹		
Forest Cover	0.01	0.005	8.86	2.93	0.02		
Random effects							
Date						0.08	0.29
Site						0.07	0.25
Residual						0.46	0.68
Log(TSS) ~ 1 + ~1 Date							
Fixed effects (Intercept)	2.26	0.11	13	20.9	2.15e ⁻¹¹		
Random effects							
Date						0.003	0.05
Residual						1.34	1.16

Nitrate ~ Forest Cover + ~1|Date + ~1|Site

Fixed effects (Intercept)	3.69	0.65	17.3	5.68	2.56e ⁻⁰⁵		
Forest Cover	-0.06	0.01	8.45	-4.31	0.002		
Random effects							
Date						3.44	1.85
Site						0.48	0.69
Residual						4.69	2.17

Potassium ~ Forest Cover + ~1|Date + ~1|Site

Fixed effects (Intercept)	3.95	0.54	9.50	7.30	3.43e ⁻⁰⁵		
Forest Cover	-0.038	0.018	9	-2.08	0.067		
Random effects							
Date						0.11	0.33
Site						1.44	1.20
Residual						0.83	0.91

^a β is the direction and magnitude of the relationship between the response variable and the predictor variable (riparian vegetation height or percent forest cover).

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

Table 8. General Linear Mixed-effect Model outputs for drainage ditch water pesticide concentrations ($n_{\text{neo+atrazine}} = 13$, $n_{\text{glyphosate}} = 8$).

Factor	β^a	SE	df	t	p-value	Variance	SD
Total Neonicotinoids ~ Vegetation Height + Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	0.007	9.22e ⁻⁰⁴	17.2	7.20	1.37e ⁻⁰⁶		
Vegetation Height	8.30e ⁻⁰⁴	1.99e ⁻⁰⁴	8.32	4.16	0.003		
Forest Cover	-9.76e ⁻⁰⁵	2.04e ⁻⁰⁵	8.32	-4.79	0.001		
Random effects							
Site						6.92e ⁻⁰⁷	8.32e ⁻⁰⁴
Date						8.02e ⁻⁰⁶	0.003
Residual						6.67e ⁻⁰⁶	0.003
Log(Atrazine) ~ Forest Cover + ~1 Date + ~1 Site							
Fixed effects (Intercept)	-2.52	0.27	16	-9.27	7.84e ⁻⁰⁸		
Forest Cover	-0.04	0.008	8.79	-4.87	9.50e ⁻⁰⁴		
Random effects							
Site						0.24	0.49
Date						0.32	0.56
Residual						0.28	0.53
Log(Glyphosate) ~ 1 + ~1 Date + ~1 Site							
Fixed effects (Intercept)	-3.72	0.36	14.0	-10.5	5.48e ⁻⁰⁸		
Random effects							
Site						0.58	0.76
Date						0.43	0.66
Residual						0.63	0.80

^a β is the direction and magnitude of the relationship between the response variable and the predictor variable (riparian vegetation height or percent forest cover).

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

Table 9. Mean and standard deviations (median with 25% and 75% interquartile range for Gosner stage) of northern leopard frog (*Rana [Lithobates] pipiens*) tadpole endpoints in relation to ditch sites of the South Nation River ($n_{\text{cage}} = 2 - 3$, $n_{\text{tadpole}} = 20 - 45$). Site 346 lost a cage during tadpole exposures; survival is calculated based on two cages for this site.

Tadpole endpoint	Site ^a								
	325	326	337	324	333	341	327	340	346
<i>Survival</i>									
Survival (%)	93.33 (0)	91.11 (15.40)	93.33 (6.67)	100 (0)	84.44 (13.88)	84.44 (3.85)	64.44 (32.89)	84.44 (26.94)	66.67 (18.86)
<i>Development</i>									
Percent female (%)	58.98 (6.82)	46.51 (8.03)	40.90 (23.06)	57.22 (6.93)	37.11 (15.47)	43.72 (9.38)	56.74 (6.08)	53.56 (10.16)	50.93 (28.12)
Snout-to-vent length (mm)	20.24 (2.18)	22.18 (1.78)	22.80 (2.87)	22.77 (1.80)	23.78 (2.68)	21.57 (2.35)	22.61 (1.99)	23.51 (2.35)	23.98 (2.25)
Body width (mm)	11.59 (1.15)	12.90 (1.01)	13.00 (1.89)	13.18 (1.10)	13.46 (1.77)	12.46 (1.29)	13.06 (1.40)	13.74 (1.34)	14.26 (1.58)
Tail length (mm)	37.01 (4.81)	43.11 (4.54)	42.15 (5.37)	44.82 (4.34)	47.95 (6.51)	41.91 (5.49)	43.53 (4.94)	47.34 (6.07)	49.84 (5.80)
Mass (g)	1.66 (0.41)	2.15 (0.50)	2.48 (0.77)	2.43 (0.42)	2.90 (0.69)	2.18 (0.66)	2.27 (0.52)	2.86 (0.70)	3.13 (0.72)
Gosner Stage ^a	38 (36 - 40)	38 (36 - 39)	38 (37 - 39)	40 (39 - 40)	37 (36 - 38)	38 (36 - 39)	38 (37 - 39)	39 (38 - 40)	39 (39 - 40)
<i>Stress</i>									
Hepatosomatic index	0.03 (0.0061)	0.027 (0.0055)	0.019 (0.0045)	0.031 (0.0061)	0.026 (0.0057)	0.024 (0.0075)	0.026 (0.0065)	0.033 (0.0095)	0.034 (0.0068)
Glucose (mg/L)	0.87 (0.31)	0.85 (0.16)	1.17 (0.78)	0.96 (0.22)	2.36 (2.16)	0.92 (0.28)	1.09 (0.37)	1.34 (0.46)	1.10 (0.36)
Corticosterone (pg/mL)	1.37 (1.29)	0.48 (0.40)	15.60 (17.43)	1.19 (1.95)	2.53 (3.04)	1.56 (1.27)	1.25 (1.24)	1.70 (1.92)	4.08 (3.05)

^a Sites are ordered by increasing riparian vegetation height

^b Variables marked with an asterisk were non-normal. Instead, the median is presented with 25% and 75% interquartile range
SD = Standard Deviation; n_{cage} = cage sample size range across sites; n_{tadpole} = range of sample size of tadpoles across sites.

Table 10. General(ized) Linear Mixed-Effects Model output table of northern leopard frog tadpole endpoints ($n_{\text{cage}} = 2 - 3$, $n_{\text{tadpole}} = 20 - 45$).

Factor	β^a	SE	df	t/z ^b	p-value	Variance	SD
Survival ~ 1 + ~1 Cage + ~1 Site							
Fixed effects (Intercept)	2.33	0.40	-	<u>5.89</u>	3.93e ⁻⁰⁹		
Random effects							
Cage						1.59	1.26
Site						0.30	0.55
Proportion Female ~ 1 + ~1 Cage							
Fixed effects (Intercept)	-0.001	0.13	-	<u>-0.01</u>	0.99		
Random effects							
Cage						0.03	0.18
SVL ~ Vegetation Height + Forest Cover + Survival + Stage + ~1 Cage + ~1 Site							
Fixed effects (Intercept)	-11.69	2.38	190	-4.91	1.98e ⁻⁰⁶		
Vegetation Height	-0.12	0.15	10.47	-0.81	0.44		
Forest Cover	0.04	0.02	8.75	2.55	0.03		
Survival	-0.20	0.10	29.15	-2.10	0.05		
Stage	0.95	0.05	320	18.74	<2e ⁻¹⁶		
Random effects							
Cage						1.14	1.07
Site						0.21	0.46
Residual						1.83	1.35
Body Width ~ Vegetation Height + Forest Cover + Survival + Stage + ~1 Cage							
Fixed effects (Intercept)	-2.35	1.54	161	-1.53	0.13		
Vegetation Height	0.02	0.1	26.44	0.25	0.80		
Forest Cover	0.02	0.01	26.07	1.84	0.08		
Survival	-0.17	0.07	30	-2.43	0.02		
Stage	0.45	0.03	318	14.19	<2e ⁻¹⁶		

Random effects							
Cage						0.67	0.82
Residual						0.71	0.84
Tail Length ~ Vegetation Height + Forest Cover + Survival + Stage + ~1 Cage + ~1 Site							
Fixed effects (Intercept)	-41.51	5.18	238	-8.01	5.27e ⁻¹⁴		
Vegetation Height	-0.16	0.38	9.92	-0.42	0.69		
Forest Cover	0.11	0.04	8.92	3.13	0.01		
Survival	-0.64	0.18	25.84	-3.64	0.001		
Stage	2.41	0.12	326	20.79	<2e ⁻¹⁶		
Random effects							
Cage						2.66	1.63
Site						2.91	1.71
Residual						9.67	3.11
Mass ~ Vegetation Height + Forest Cover + SVL + ~1 Cage							
Fixed effects (Intercept)	-2.93	0.18	278	-16.04	<2e ⁻¹⁶		
Vegetation Height	0.04	0.02	28.26	2.28	0.03		
Forest Cover	0.005	0.002	28.40	3.21	0.003		
SVL	0.23	0.008	305	28.31	<2e ⁻¹⁶		
Random effects							
Cage						0.02	0.12
Residual						0.1	0.31
Gosner Stage ~ Vegetation Height + Forest Cover + Survival + ~1 Cage + ~1 Site							
Fixed effects (Intercept)	3.65	0.024	307	152	0		
Vegetation Height	0.0052	0.0023	22	2.29	0.032		
Forest Cover	-1.91e ⁻⁰⁴	2.16 e ⁻⁰⁴	22	-0.88	0.39		
Survival	-5.80e ⁻⁰⁴	0.0017	22	-0.33	0.74		
Random effects							
Cage						-	0.012

Site						-	0.012
Residual						-	0.24
HSI ~ Vegetation Height + Forest Cover + Stage + ~1 Cage + ~1 Site							
Fixed effects (Intercept)	-0.06	0.01	231	-7.78	2.33e ⁻¹³		
Vegetation Height	9.00e ⁻⁰⁴	6.01e ⁻⁰⁴	9.84	1.48	0.17		
Forest Cover	1.40e ⁻⁰⁵	5.96 e ⁻⁰⁵	8.86	0.24	0.82		
Stage	0.003	1.76 e ⁻⁰⁴	321	13.93	<2e ⁻¹⁶		
Random effects							
Cage						6.59e ⁻⁰⁶	0.003
Site						7.40e ⁻⁰⁶	0.003
Residual						2.44 e ⁻⁰⁵	0.005
Log(Glucose) ~ Forest Cover + ~1 Cage							
Fixed effects (Intercept)	-0.12	0.074	25.81	-1.63	0.11		
Forest Cover	0.0093	0.0026	25.92	3.52	0.002		
Random effects							
Cage						0.1	0.28
Residual						0.1	0.30
Corticosterone ~ 1 + ~1 Cage + ~1 Site							
Fixed effects (Intercept)	3.32	1.54	8.96	2.16	0.06		
Random effects							
Cage						14.6	3.82
Site						13.4	3.66
Residual						23.6	4.85

^a β is the direction and magnitude of the relationship between the response variable and the predictor variable (riparian vegetation height or percent forest cover)

^b Model t-values or z-scores. Z-scores are underlined

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

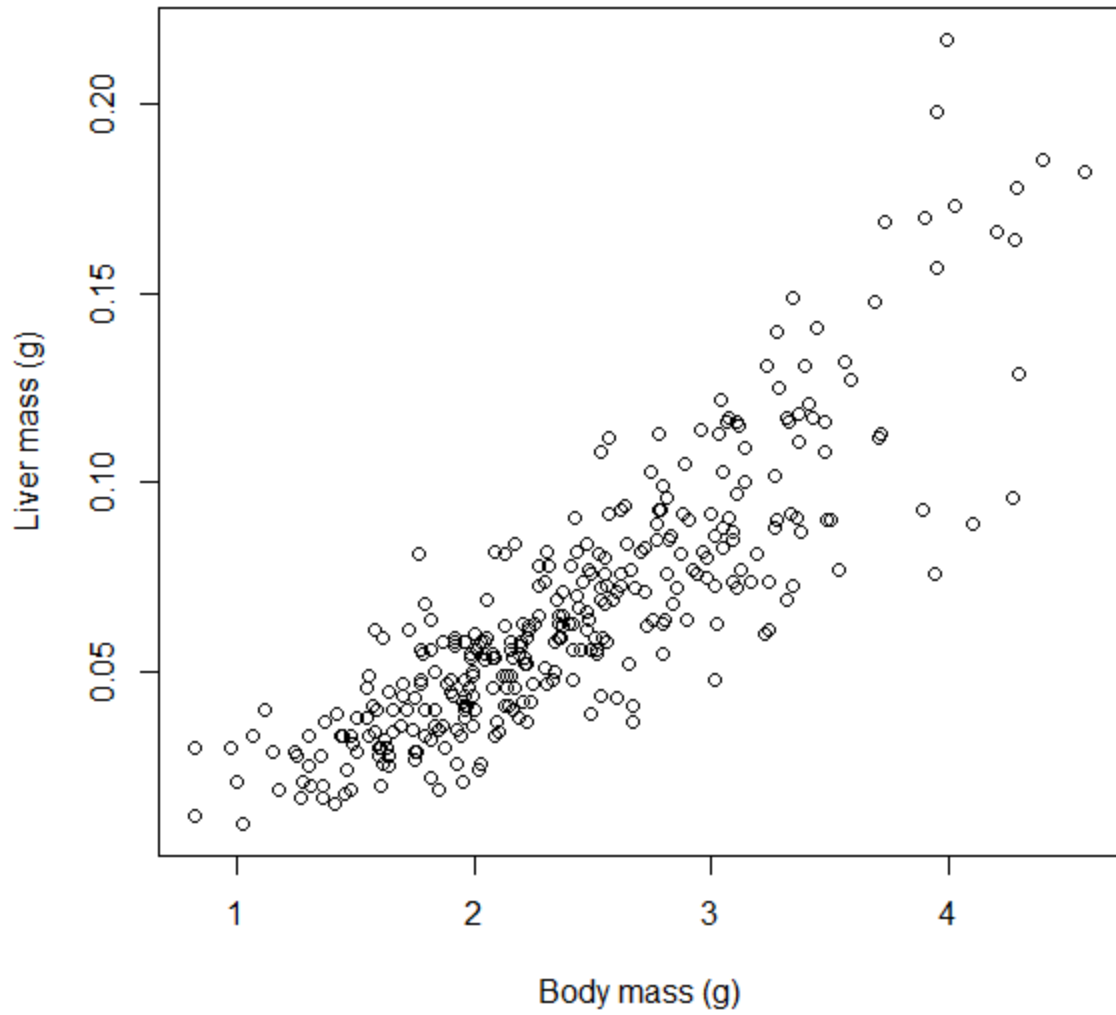


Figure 2. *Rana [Lithobates] pipiens* tadpole liver vs body mass. A clear linear relationship can be seen for liver hepatosomatic index. All tadpoles from the experiment are represented as a dot (n = 333).

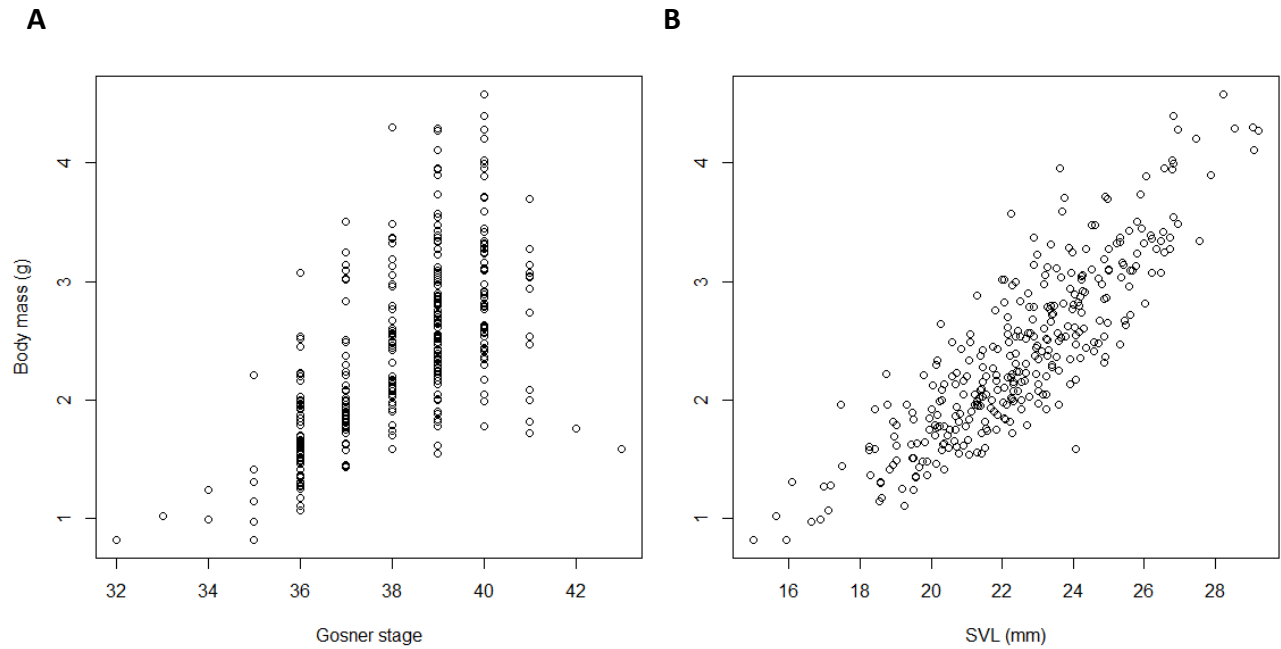


Figure 3. Body mass vs Gosner stage (**A**) and snout-to-vent length (SVL; **B**) of experimental *Rana [Lithobates] pipiens* tadpoles. Since mass and stage do not have a linear relationship, stage is not used in body mass model construction. However, mass and SVL have a clear linear relationship. Therefore, SVL is used for mass model construction with Generalized Linear Mixed-Effect models. Each dot represents a tadpole (n = 333).

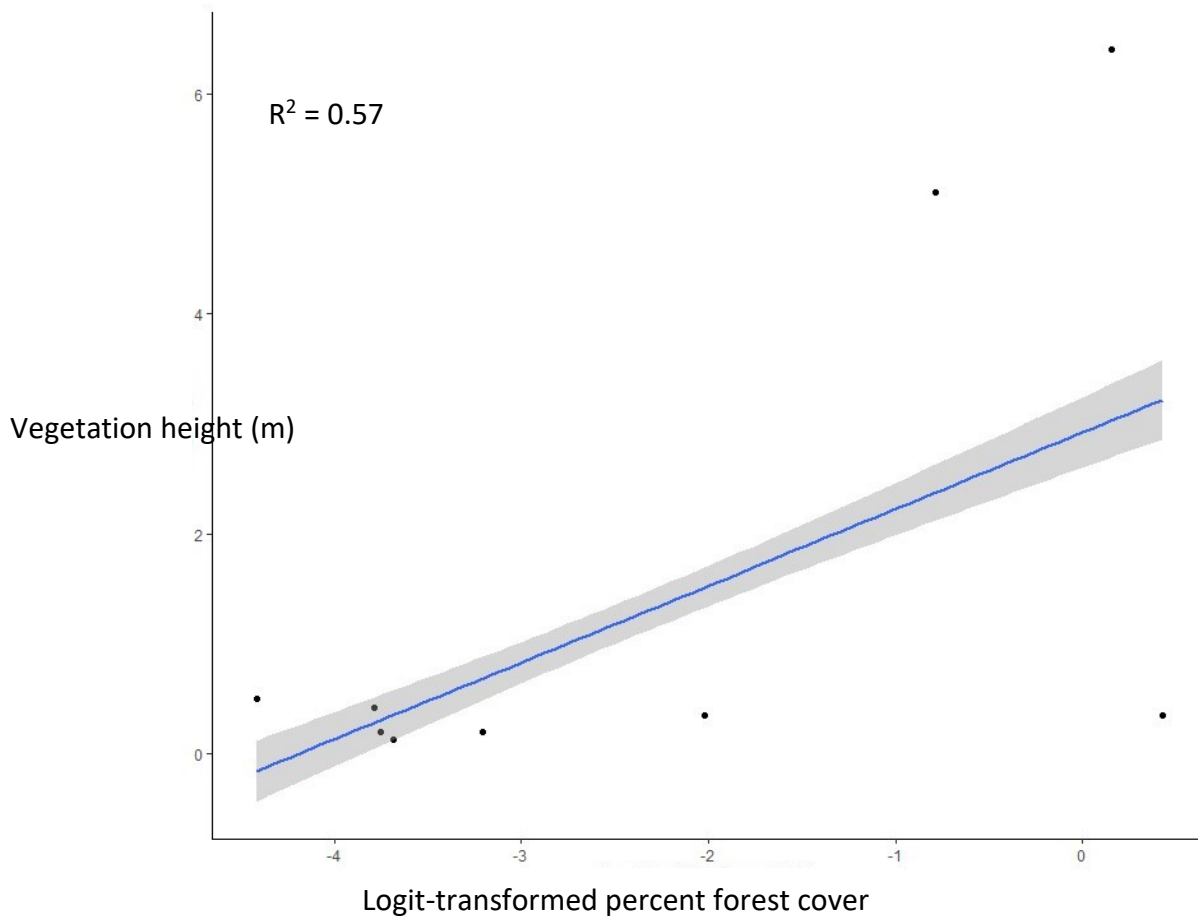


Figure 4. Scatterplot of correlation of vegetation height and logit-transformed percent forest cover. Blue line is the best-fitted line.

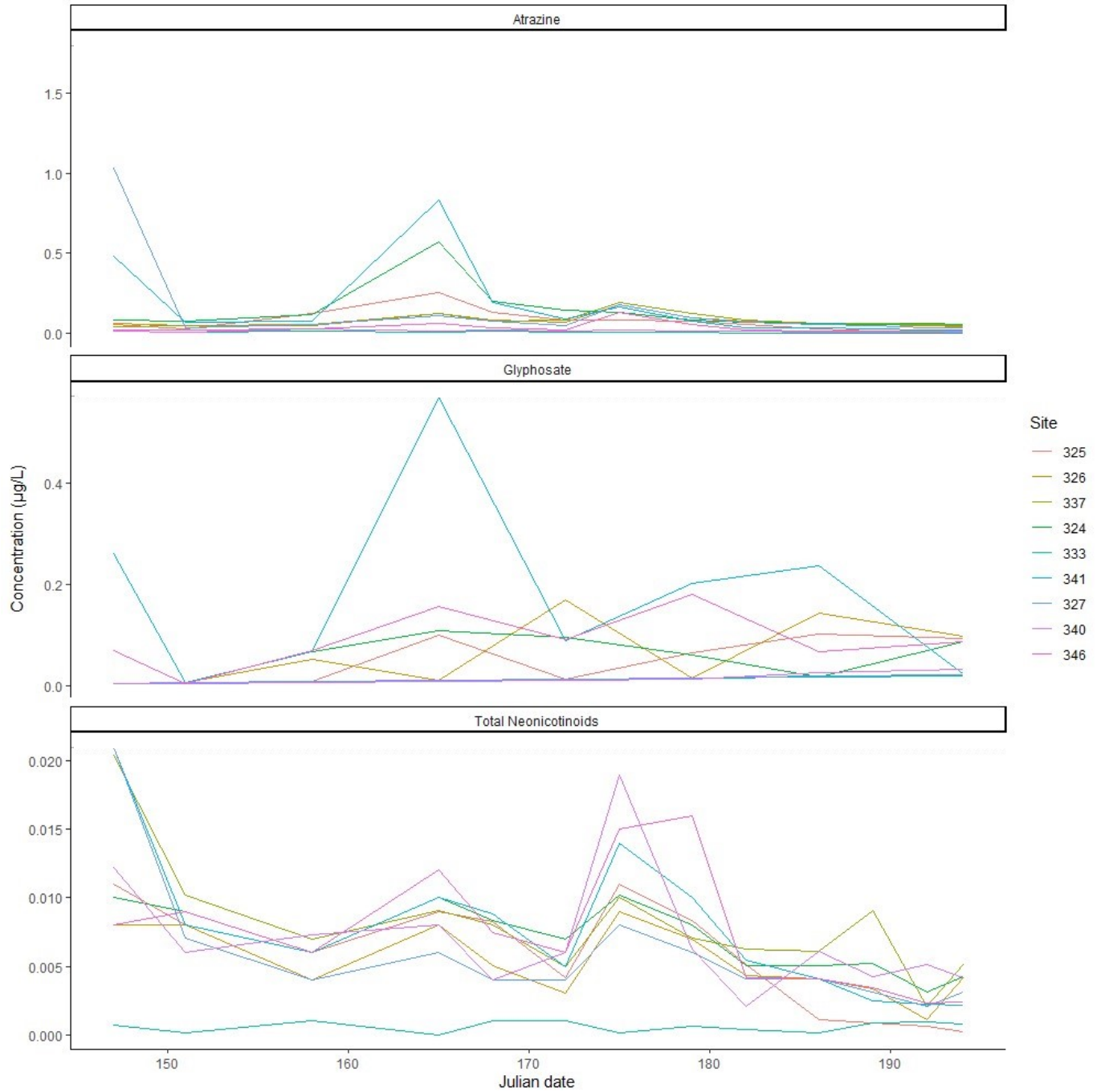


Figure 5. Atrazine, glyphosate and total neonicotinoid levels in surface water of agricultural ditch sites. Samples were taken weekly (n = 13 samples per site for atrazine and total neonicotinoids; n = 8 samples per site for glyphosate) from May 27, 2022 – July 13, 2022.

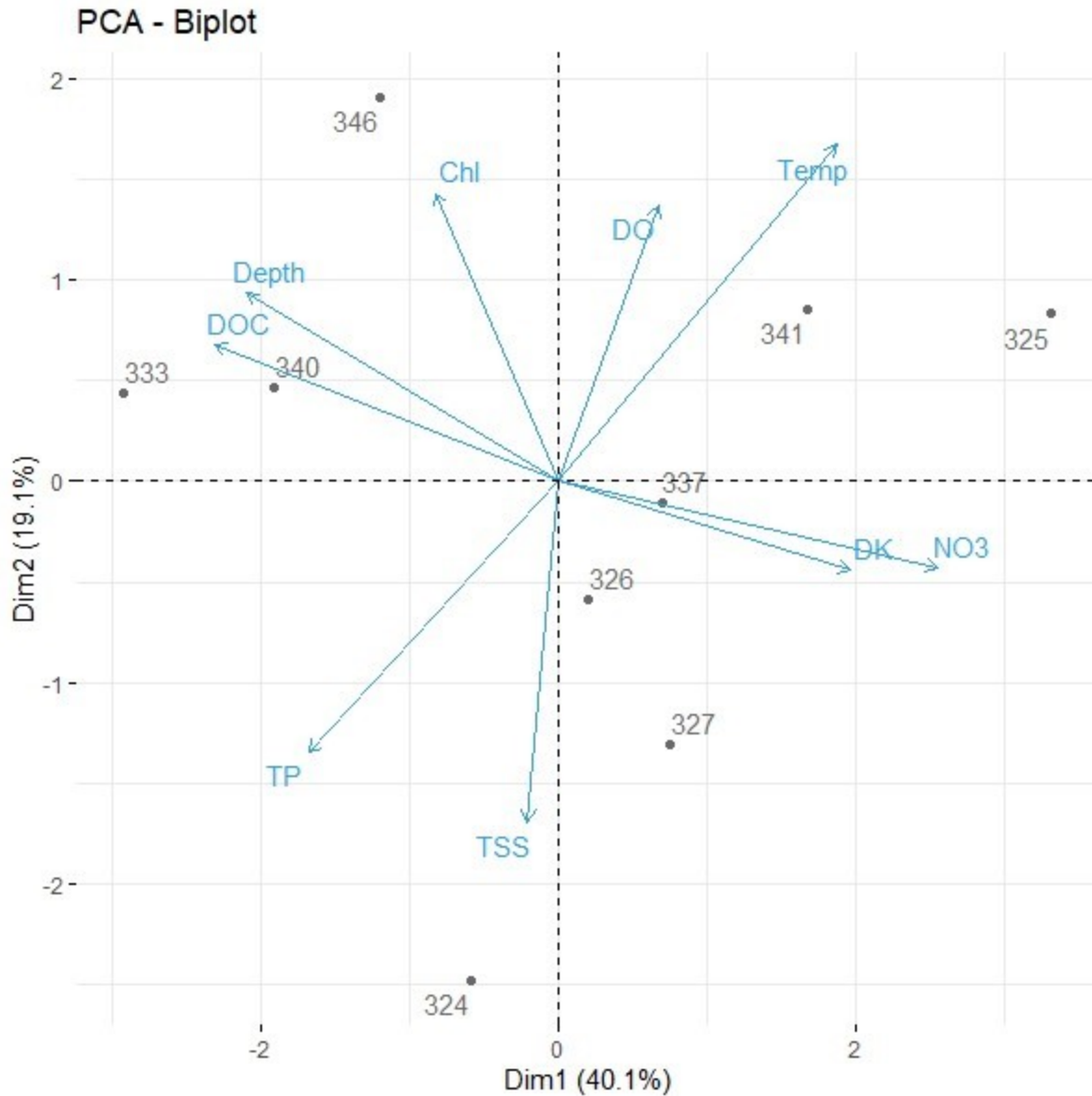


Figure 6. Principal component analysis (PCA) of nine water chemistry variables in relation to the nine ditch sites across the South Nation River watershed. Water samples were collected between May 27 to July 13 2022. Sample size is $n = 8$ for nutrients (Total phosphorus, dissolved organic carbon, chloride, dissolved potassium and total suspended solids). Sample size is $n = 16$ for temperature, depth and dissolved oxygen. DO = Dissolved oxygen (%), Temp = Temperature ($^{\circ}\text{C}$), NO_3 = Nitrate (mg/L), DK = Dissolved Potassium (mg/L), TSS = Total Suspended Solids (mg/L), TP = Total Phosphorus (mg/L), DOC = Dissolved Organic Carbon (mg/L), Chl = Chloride (mg/L).

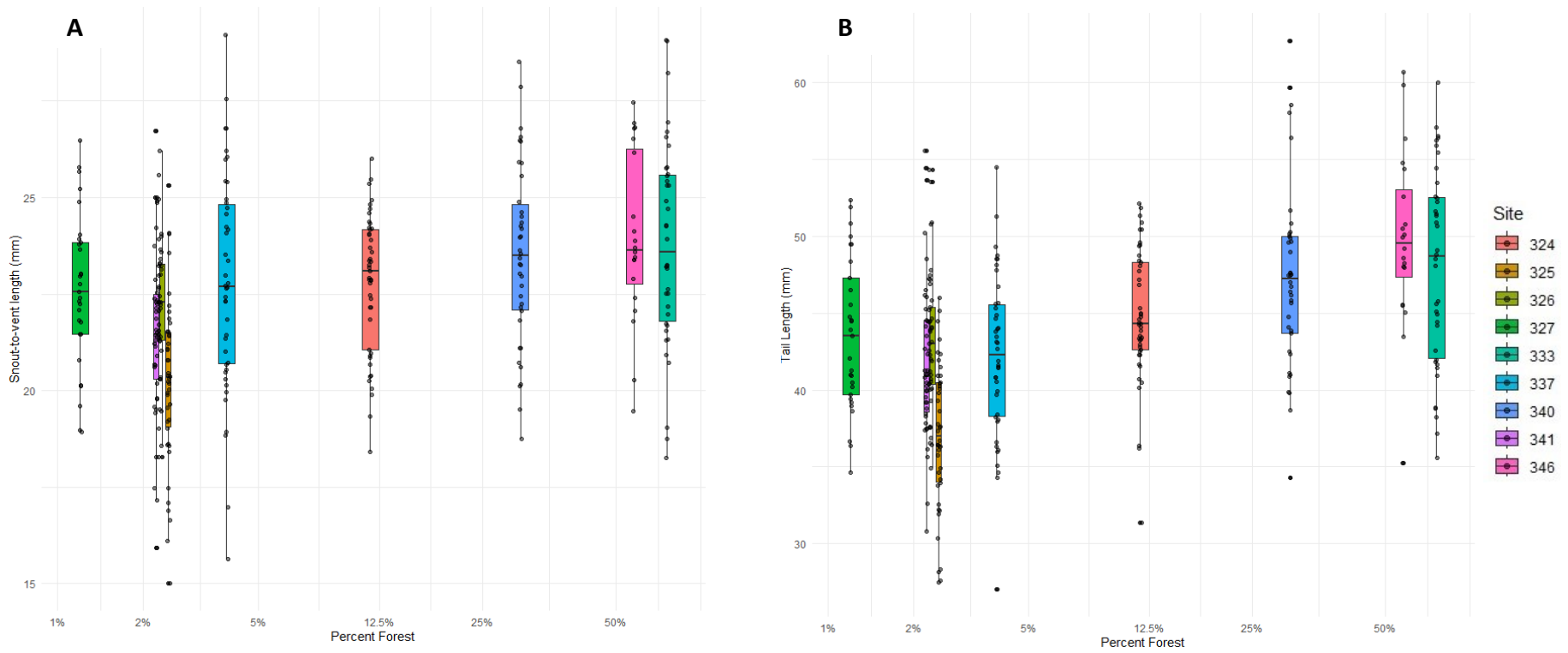


Figure 7. Boxplot of snout-to-vent length (**A**) and tail length (**B**) of tadpoles in mm ($n = 4 - 15$ tadpoles per cage, 3 replicate cages per site) against percent forest cover at all nine experimental sites. Percent forest has been logit-transformed for better visualization. Each dot represents a tadpole.

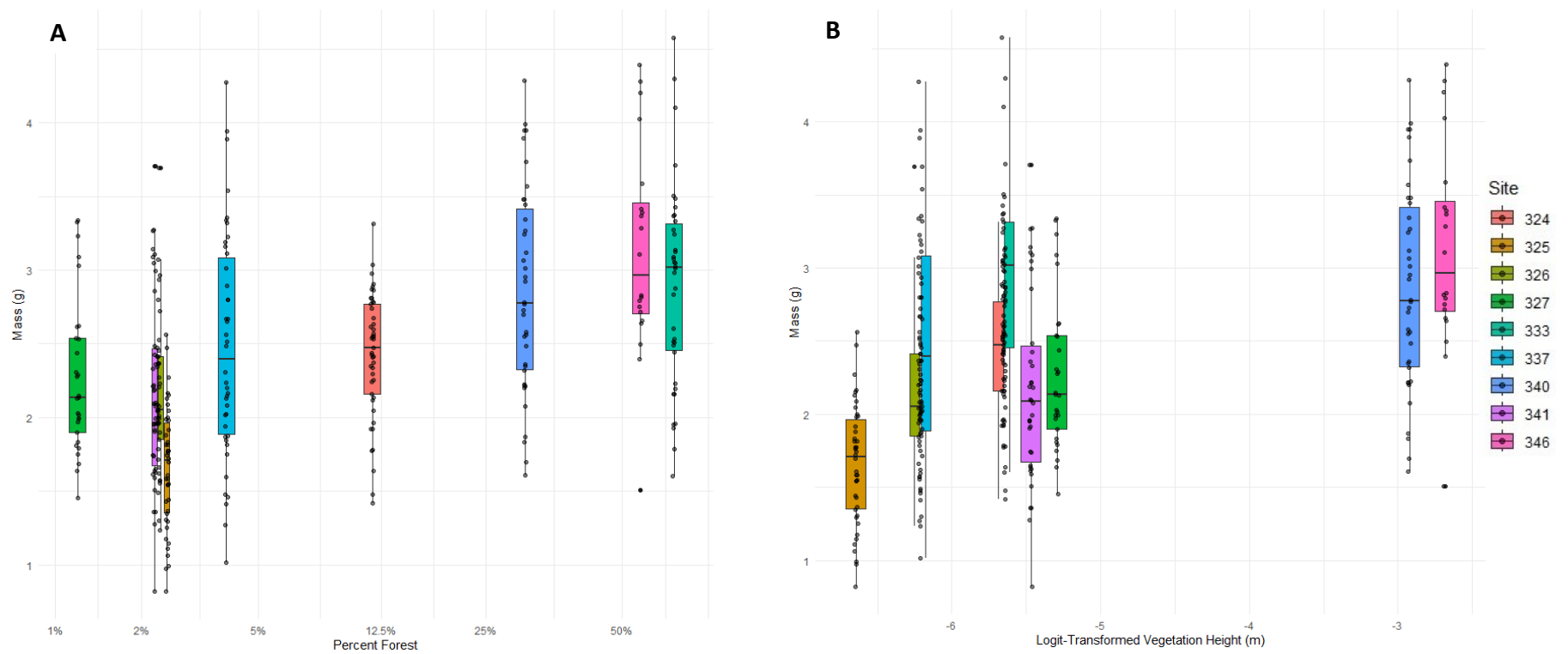


Figure 8. Boxplot of tadpole mass against percent forest cover (**A**) and tadpole mass against vegetation height (**B**; $n = 4 - 15$ tadpoles per cage, 3 replicate cages per site) at all nine experimental sites. Percent forest and vegetation height have been logit-transformed for better visualization. Each dot represents a tadpole.

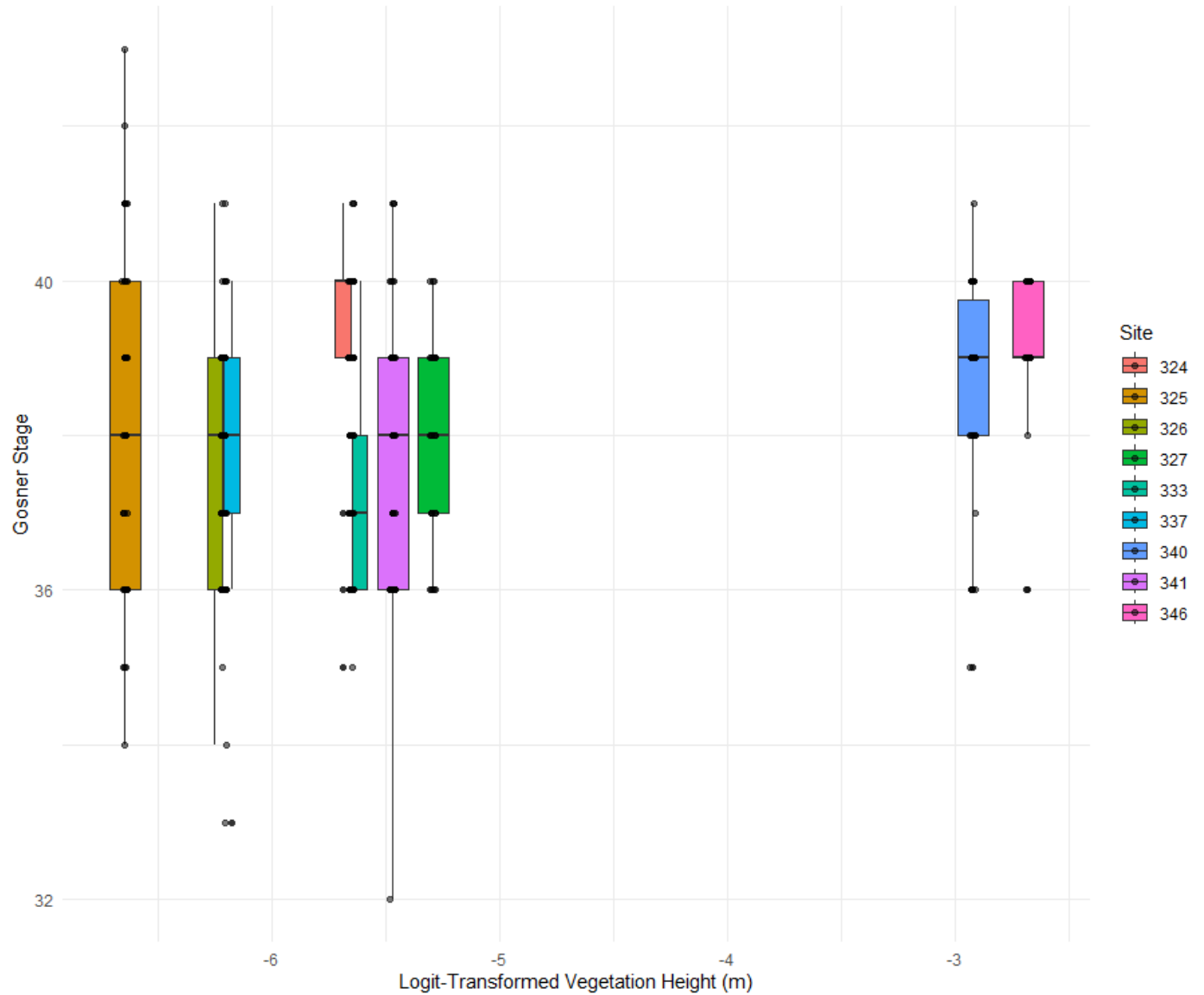


Figure 9. Boxplot of Gosner stage against logit-transformed vegetation height (n = 4 - 15 tadpoles per cage, 3 replicate cages per site). Vegetation height has been logit-transformed for better visualization. Each dot represents a tadpole.

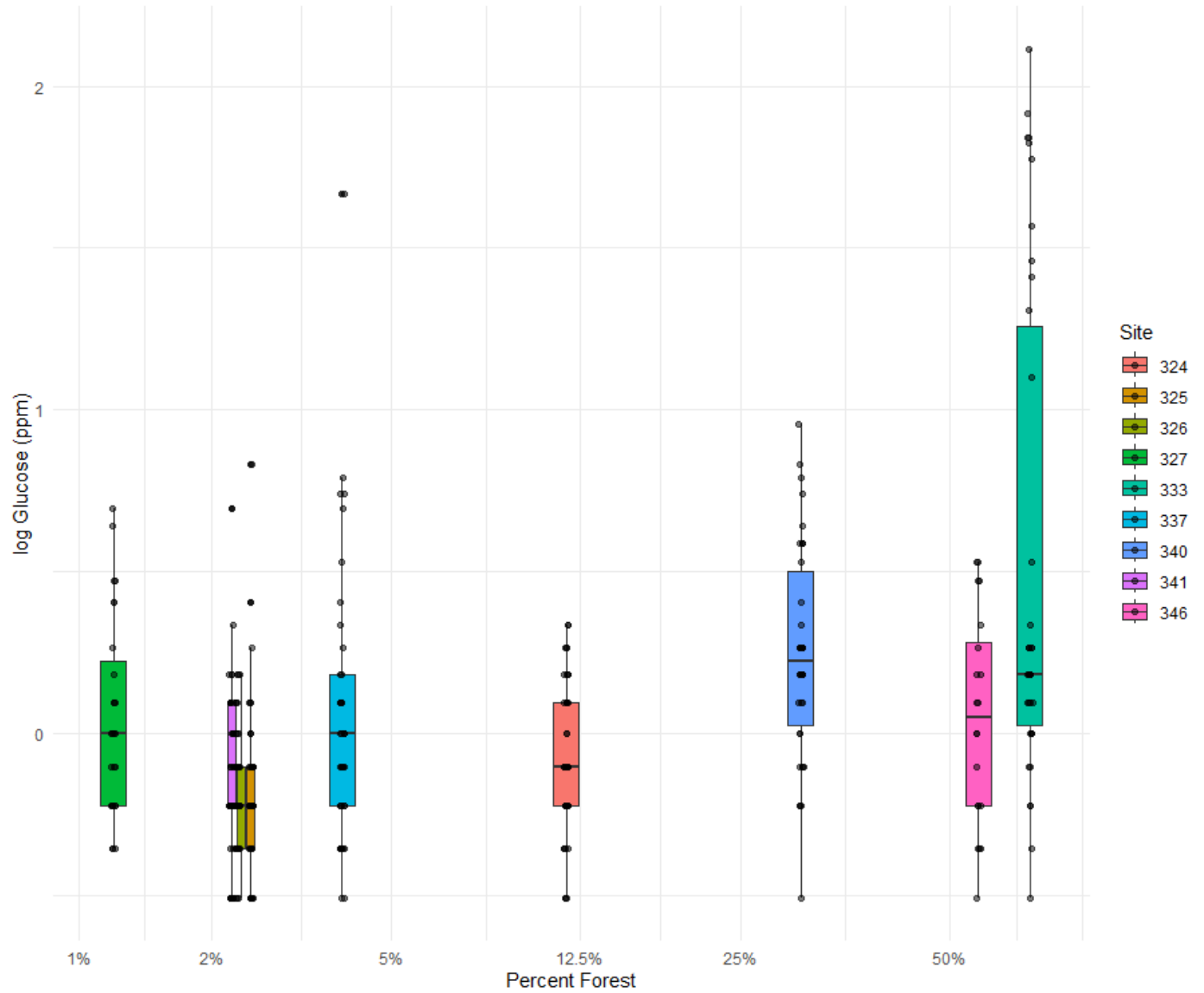


Figure 10. Boxplot of log-transformed tadpole blood glucose concentration against percent forest cover (n = 4 - 15 tadpoles per cage, 3 replicate cages per site). Percent forest has been logit-transformed for better visualization. Each dot represents a tadpole.

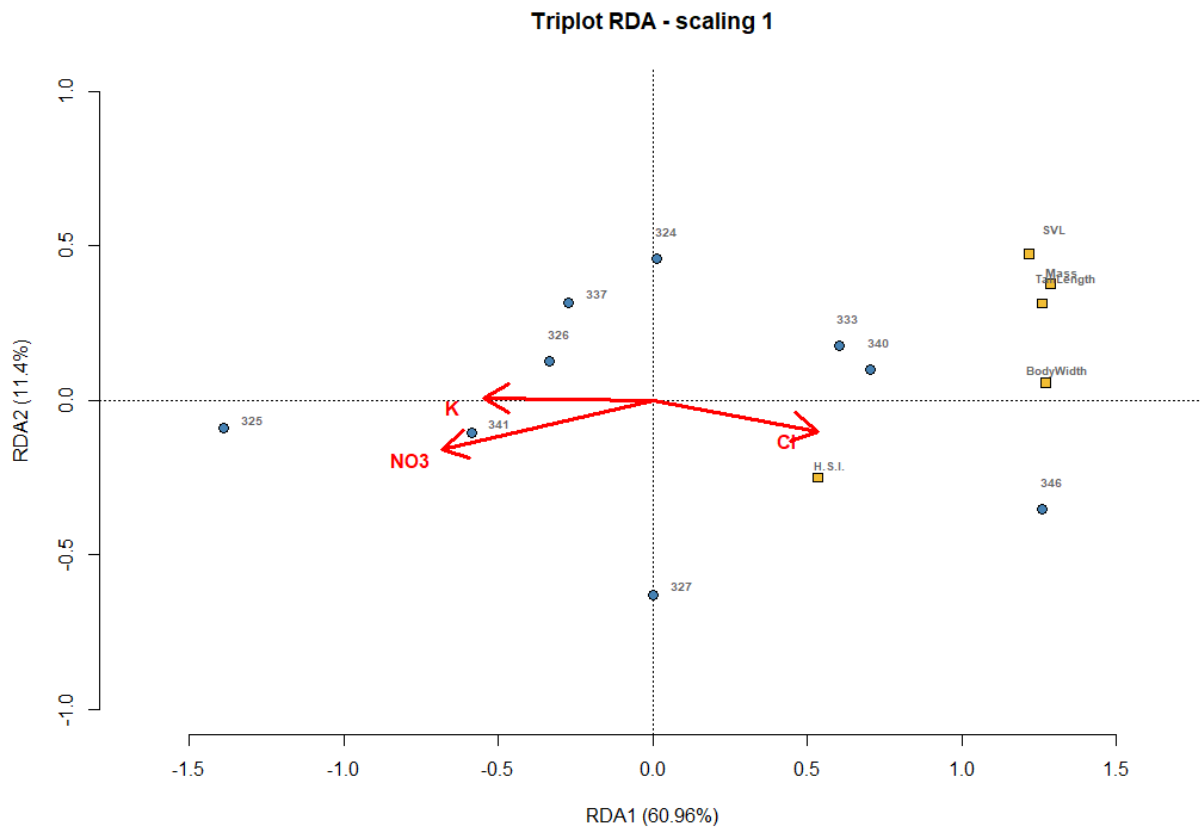


Figure 11. Redundancy analysis (RDA) triplot of tadpole health endpoints (yellow squares) and significant water quality variables (red vectors) across the nine experimental sites (blue dots). Sample size is $n = 8$ for nutrients (dissolved potassium, chloride, nitrate). Water samples were taken between May 27 to July 13, 2022. Sample size for tadpoles is $n = 45$ (15 tadpoles per cage, 3 replicate cages per site). SVL = Snout-to-Vent Length, H.S.I. = Hepatosomatic Index, Cl = Chloride, NO3 = Nitrate, K = Potassium.

Chapter 4: Discussion

4.1 Effects of riparian vegetation on water chemistry

I predicted that sites with higher levels of riparian vegetation would have lower mean temperature. However, neither vegetation height nor percent forest cover had an effect on surface water temperature for either method used in this study (YSI and Hobo pendant readers). This result is contrary to many other studies that have suggested that riparian shading can affect water temperature (Dugdale et al., 2018; Kaandorp et al., 2019; Sweeney et al., 2004). In another study using ditches in the same region as the present study, Dyck et al. (2021) found that ditch management practices such as vegetation clearing and ditch dredging had a significant impact on temperature. It was found that managed ditches that had been cleared of all riparian vegetation had higher mean temperatures than ditches that remained relatively unmanaged (Dyck et al., 2021). A possible explanation for the results of the present study is that water temperature in these systems is largely regulated by groundwater input that bypasses any effects that riparian shade may have on stream temperature. Previous work has shown that riparian canopy cover plays a role in temperature regulation to certain extent but groundwater sources may play a more significant role in regulating stream temperature, particularly during low or baseflow periods. McPhaden (2013) found that ditches that were recently cleared of vegetation experienced a significant increase in temperature that eventually stabilized due to inputs from groundwater, suggesting that groundwater plays an important role in regulating temperature in agricultural streams, especially in areas that have been cleared of riparian vegetation. Taken together, it is possible that no significant differences were

found in water temperature across the experimental ditches in my study because ditches with less riparian and canopy shading were buffered against heating by groundwater inputs.

Dissolved oxygen (DO) percent saturation was also not affected by vegetation height or percent forest cover. This was not what I predicted for this study as previous work has shown that riparian vegetation can play an important role in DO levels in stream systems. Dissolved oxygen saturation in stream systems is dependent on a multitude of factors including temperature, solar radiation, presence of microorganisms and organic matter, among others; all of which can be affected by riparian vegetation (US EPA, 2015a).

Specific conductance (SPC) was not affected by vegetation height but was negatively affected by forest cover. Previous work has suggested that SPC in ditch streams adjacent to public roads could be driven by road salt inputs after spring snowmelts (Corsi et al., 2010; Novotny et al., 2007; Szklarek et al., 2022). In the present study, Pearson correlations showed a strong positive relationship between chloride (Cl) and sodium (Na) ($R^2 = 0.99$; Table 4). The presence of both Cl and Na is suggestive of road salt inputs (Godwin et al., 2003; Kelly et al., 2008). However, SPC did not have a strong correlation with either Na ($R^2 = 0.07$) nor Cl ($R^2 = 0.1$) suggesting that Na and Cl are not strong drivers of SPC in these systems. However, nitrate (NO₃) and potassium (K) were strongly correlated to SPC ($R^2 = 0.71$ and $R^2 = 0.76$ respectively) across sites (Table 4). Both NO₃ and K were also negatively affected by forest cover (Table 8). Taken together, it is likely that sites with lower forest cover and adjacent to agricultural fields receive a larger influx of NO₃ and K ions through fertilizer and manure applications suggesting that higher SPC may be driven by agricultural contaminants in these systems. Farmers in these regions do use Nitrogen Phosphorus Potassium (NPK) - based fertilizers (personal

communications) and previous studies have shown that NPK-based fertilizers can increase both water and soil SPC (Garg et al., 2022; Onwe et al., 2020; Tenório Marinho Da Rocha et al., 2023).

pH remained relatively stable across sites and was not affected by vegetation height or forest cover and never exceeded guideline levels of 8.7 at any of the sites throughout the experiment (OECD 2009). This result was unexpected as surface water pH levels can be affected by acid rain, leaf litter, agricultural contaminants, and sequestering of airborne sulfur and nitrogen pollutants by tree canopies (Nisbet & Evans, 2014; US EPA, 2015b). However, other studies have found similar results, suggesting that landscape features such as riparian vegetation do not play a significant role in regulating pH in stream systems (Dyck et al., 2021; S. Li et al., 2009). It is also possible that pH is largely regulated by rain and/or groundwater inputs that bypass the riparian cover of these streams. Another explanation is that other upstream factors that were not captured within the scale of this project.

Vegetation height did not have an effect on depth at any of the sites. However, percent forest cover had significant positive effect on mean ditch depth across the experimental sites. This result supports my original prediction that riparian vegetation would help reduce water loss in agricultural streams. Studies have shown that wooded riparian strips can provide a matrix of deep roots that not only prevent erosion but also increase soil porosity and promote water retention (Cole et al., 2020).

The complex structure of standing vegetation and fallen debris left by wooded riparian areas can help reduce flow velocity in adjacent streams (Cole et al., 2020). Deadwood and other debris can also cause the formation of pools that help regulate erosion and reduce the effects

of flooding (Cole et al., 2020; Sykes, 2015; Thomas & Nisbet, 2007). Mean current velocity was not affected by either vegetation height or percent forest cover across the sites in the present study. Tabacchi et al. (2000) suggest that a riparian buffer's ability to reduce flow velocity is dependent on the composition and structure of ground-level material. Wooded riparian with poorly developed understories are less likely to reduce flow than areas with more densely packed and developed vegetation. The majority of the sites used in this study were composed of relatively small, homogenous riparian zones with sparse vegetation, which could help explain why no difference was found in current velocity across sites.

Vegetation height had a significant positive effect on Cl levels but was unaffected by forest cover. A possible explanation for this result is that sites with higher riparian such as sites 333, 340, 346 were closer to public roads and were positively associated to Cl concentrations (Figure 7). Studies have demonstrated that wetlands closer to public roads tend to have higher inputs of road salts, which could help explain the results of the present study (Walker et al., 2021).

Percent forest cover had a positive effect on dissolved organic carbon (DOC) levels but vegetation height had no effect. This result is in support of my original hypothesis and has also been documented in the literature. A likely explanation is that sites higher in forested riparian receive larger inputs of leaf litter and other organic matter that contribute to in-stream DOC. Leaf litter has been documented as a significant source of DOC in adjacent streams. Researchers have found that streams with forested riparian tend to have higher DOC than non-forested streams (Smiley & Rumora, 2015; Mulholland, 2003).

Percent forest cover had a significant positive effect on total phosphorus (P) while vegetation height had no effect. This result is somewhat counterintuitive; however, in streams receiving large inputs of agricultural contaminants, riparian strips can become saturated, limiting their ability to assimilate these contaminants (Sabater et al., 2003; Stutter et al., 2012; Valkama et al., 2019; Vidon et al., 2010). As a result, over-saturated buffers can act as a source of nutrient inputs rather than a sink (Dosskey et al., 2010; Vidon et al., 2010). Additionally, decaying vegetation can produce additional sources of P in adjacent streams (Uusi-Kämppe et al., 2012). It is possible that the forested riparian buffers in the present study are contributing to the concentration of P in these streams, which could help explain why forest cover has a significant effect on P in these systems. Again, another possible explanation is that other upstream factors may be affecting P inputs into these systems, and these effects were not captured within the scope of this study. Overall, P concentrations in the present study were comparable to those found in other studies within the same region (Dalton et al., 2015b, 2015a; Dyck et al., 2021).

Neither vegetation height nor percent forest cover had a significant effect on total suspended solids (TSS). Stream TSS is largely dictated by sediment size and stream velocity, and the level of disturbances in the water (Allan, 1995). Additionally, spring runoff and rainfall events can have strong effects on in-stream TSS, and therefore concentrations of TSS can be highly variable across time (Langland & Cronin, 2003). However, throughout the time of the experiment, sediments were quite fine at all of the sites and stream velocity was usually quite low. It is likely that due to the low current velocity and lack of disturbances in the water (i.e. movement of fish or other aquatic organisms) TSS remained low across sites.

Nitrate was not affected by vegetation height but was negatively affected by forest cover; a result supported by the literature. The concentration of soluble and highly mobile ions such as NO₃ can be reduced by riparian vegetation through infiltration or assimilation and degradation by plants (Cole et al., 2020). Additionally, the organic debris caused by forested buffers promotes increased microbial activity, which can also increase denitrification in agricultural streams (Osborne & Kovacic, 1993). Higher concentrations of DOC have been shown to promote increased rates of denitrification by providing more energy resources for denitrifying microorganisms (Bradley et al., 1992; Greenan et al., 2006). In this study, sites higher in percent forest were also associated with higher concentrations of DOC. Therefore, it is likely that sites higher in percent forest had greater inputs of organic material, which increased bioavailable DOC. Consequently, sites with greater wooded riparian cover promoted higher denitrification, as they were richer in energetic resources for denitrifying bacteria (i.e. greater DOC concentrations). Other studies within the same region have also found similar NO₃ concentrations in surface waters (Dalton et al., 2015a; Dalton et al., 2015b; Dyck et al., 2021).

Dissolved potassium was not affected by riparian vegetation height but was negatively affected by percent forest cover. Studies have demonstrated that dissolved ions such as K can increase dramatically for years after deforestation or removal of woody vegetation (Likens et al., 1969). Dredging practices remove much or all of the riparian vegetation from the banks of agricultural streams. Removal of riparian vegetation can mobilize sediment through erosion which can cause an increase of nutrients such as K (Likens et al., 1970). This could help explain why sites higher in forest cover were associated with lower dissolved K concentrations compared to sites lower in riparian cover. Lower forested riparian is suggestive of more recent

dredging, which could help explain the higher K concentrations in these sites. Another possible explanation is that the greater plant biomass in forested riparian buffers is more efficiently sequestering agricultural contaminants. Sites higher in forest cover are associated with lower NO₃ and K concentrations. Additionally, NO₃ and K are fairly correlated across sites ($R^2 = 0.66$), suggesting a potential NPK fertilizer signal, as previously explained.

4.2 Effects of riparian vegetation on pesticide concentrations

In the present study, higher riparian vegetation was associated with higher total neonicotinoid concentrations (TNeo) while sites higher in percent forest cover were associated with lower TNeo concentrations (Table 9). Satkowski et al. (2018) demonstrated that riparian buffer strips were more efficient at retaining imidacloprid than grass buffers or cropland. Soils of forested buffers tend to have higher concentrations of soil organic carbon, which increases the sorption capacity of the soil (Aguiar et al., 2015; Novak et al., 2015; Satkowski et al., 2018). Sites higher in percent forest cover were also associated with lower atrazine concentrations (Table 9). Aguiar et al., (2015) demonstrated that large woody riparian buffers were effective at filtering pesticides. This is because the sorption of hydrophobic pesticides such as atrazine is related to the amount of soil organic matter in the buffer zone (Laabs et al., 2002; Ramsey et al., 2005). Forested buffers have more fallen organic material that increases the sorption capacity of the soil (Aguiar et al., 2015; Laabs et al., 2002; Ramsey et al., 2005). In the present study, sites higher in percent forest were also associated with higher DOC, which likely contributed to the organic matter and therefore the sorption capacity of the soil in forested sites. It is likely that in the present study, sites higher in woody vegetation retain higher

concentrations of neonicotinoids and atrazine in their soil due to their high DOC concentrations, thereby reducing the amount of these pesticides entering surface waters. Plants can also directly absorb atrazine as well as systemic insecticides such as neonicotinoids; therefore, sites higher in woody vegetation can absorb higher concentrations of these pesticides due to their deeper and denser root systems (Aguiar et al., 2015; Botías et al., 2016; Hall et al., 2022). Measured concentrations of both TNeo and atrazine were comparable to other studies within the same region under similar crop rotations (Dalton et al., 2014; Dyck et al. 2021).

In contrast to TNeo and atrazine, glyphosate concentrations were not affected by vegetation height or forest cover. Glyphosate has a very high K_{oc} (28,000 L/Kg), which could help to explain this result (CCME, 2012). Glyphosate readily adsorbs to soils and therefore effects of leaching into groundwater are generally negligible (Lupi et al., 2019). Research has suggested that the highest proportion of glyphosate loss occurs from aerial spray drift and runoff events (Hancock et al., 2019; Lupi et al., 2019; Reichenberger et al., 2007). Therefore, riparian buffer strips may have little effect on surface water concentrations of glyphosate, when compared to triazine herbicides and neonicotinoids. Concentrations of all pesticides analyzed in the present study were comparable to other studies within the same region under similar crop rotations (Dalton et al., 2014; Dyck et al., 2021).

Collectively, these results suggest that percent forest cover plays a more important role than vegetation height in reducing the concentration of pesticides entering surface water in agricultural streams. Sites with greater forest cover tended to be higher in DOC, which increases the sorption capacity of soils (Aguiar et al., 2015; Inoue et al., 2006; Laabs et al., 2002;

Novak et al., 2015; Ramsey et al., 2005; Satkowski et al., 2018). Additionally, the denser root systems of forested buffers are more efficient at capturing certain pesticides (Aguiar et al., 2015; Foster et al., 2002). Regardless of the effects of riparian, no pesticides exceeded guideline levels throughout this study (Table 2; Figure 6).

4.3 Effects of riparian on tadpole survival, development and pathogen infection

This study revealed that vegetation height did not have any significant effects on tadpole survival. Additionally, riparian vegetation height did not have any significant effects on tadpole sex ratios or size and development. However, this study did reveal that percent forest cover may play a more important role with respect to tadpole growth. Specifically, tadpole snout-to-vent length (SVL), body width (BW), tail length (TL), and mass were all positively affected by increasing forest cover across all sites. Although, vegetation height did have a positive effect on tadpole mass and developmental stage. Additionally, vegetation height did not have any significant effects on metrics of tadpole stress (HSI, glucose, corticosterone). However, percent forest cover did have a significant positive effect on tadpole-blood-glucose levels. Finally, Frog Virus 3 (FV3) and *Batrachochytrium dendrobatidis* (Bd) were not detected in the tissue of any of the tadpoles exposed to agricultural ditch waters.

4.4 Survival

Survival of tadpoles was relatively high at all sites (64 – 100%) and was not significantly affected by ditch riparian vegetation height or forest canopy cover percentage. Other studies using *in situ* cage monitoring observed similar survival rates. Dyck et al. (2021) observed

survival rates of 86 – 96% in *L. pipiens* caged within the same watershed as the present study. Using in situ cages, Harris et al. (2001) found 79 – 93% survivorship in tadpoles of the same species (*L. pipiens*) within sites of the Akwesasne Mohawk territory along the St. Lawrence River that were potentially impacted by pulp and paper mill effluent, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons; this survival was different from that at a reference wetland (96%). McDaniel et al. (2004) conducted a study in the same region and found tadpole survival to range from 86 – 91%. Finally, in a study conducted in eastern England, Cooke (1981) found 40 – 90% survival in *Rana temporaria* tadpoles in agricultural ditches compared to 100% survival in tadpoles in a control wetland.

Although survival was high overall, sites 327 and 340 had survival below 80% (Table 10). Multiple stressors in agricultural streams can affect tadpole survival such as low DO, excess nutrients, and pesticides (Navarro-Martín et al., 2014; Ortiz-Santaliestra & Marco, 2015; Rimayi et al., 2018; Smith et al., 2005). Site 327 had the lowest mean DO, at times DO dropped well below the OECD (2009) guideline levels of 40% saturation at times during the last three weeks of the study. Low oxygen saturation has been shown to cause increased mortality in *Bufo calamita* tadpoles, especially in regions that receive influxes of nutrients from fertilizer, where additive effects can also occur (Ortiz-Santaliestra & Marco, 2015). Collectively, site 327 and 346 had some of the highest concentrations of atrazine, glyphosate, and NO₃, all of which have been associated with increased mortality in amphibians (Navarro-Martín et al., 2014; Ortiz-Santaliestra & Marco, 2015; Rimayi et al., 2018; Smith et al., 2005). Although the design of this study makes it difficult to determine exactly what caused high mortality at these sites, it is likely a combination of factors including any synergistic effects from the contaminants at these sites.

4.5 Tadpole development and growth

My original hypothesis proposed that riparian vegetation height would have indirect positive effects on tadpole development and growth through its effects on water chemistry. However, the results from this study clearly demonstrate that percent forest may play a more important role for tadpole growth. Sites higher in percent forest were associated with larger tadpoles overall: tadpoles caged at sites higher in percent forest cover tended to be larger (i.e. higher SVL, TL, mass). I originally hypothesized that sites higher in riparian cover would have lower mean temperatures, which would positively affect tadpole development. Previous work has suggested that temperature can have a positive effect on amphibian development (Goldstein et al., 2017; Kuan & Lin, 2011; Marian & Pandian, 1985). However, this was not supported by the results of this study. The results from the present study suggest that the main benefits of riparian strips on tadpole development is from their ability to reduce surface water contaminant loads, thereby providing better conditions for tadpole development. Sites higher in forest cover tended to have lower concentrations of both NO₃ and K (Table 8). RDA results from this study suggest that NO₃ is one of the strongest drivers of tadpole development in these systems and sites higher in NO₃ tended to have smaller frogs overall (i.e. lower SVL, BW, TL and mass). Nitrate has been shown to negatively affect tadpole development (Ortiz-Santaliestra & Marco, 2015; Smith et al., 2005). More specifically, NO₃ has been shown to delay the development of both American bullfrog (*Rana catesbeiana*) and southern leopard frog (*Rana sphenoccephala*) tadpoles exposed to NO₃ concentrations comparable to those detected in the present study (Ortiz-Santaliestra & Sparling, 2007; Smith et al., 2005). Sites higher in forest cover also tended to have lower concentrations atrazine, which has been shown to

negatively affect tadpole development (Rimayi et al., 2018; Zaya et al., 2011). Atrazine is also a known endocrine disrupting compound and has been shown to alter sex ratios in amphibians. Hoskins & Boone (2018) showed that Blanchard's cricket frogs (*Arcis blanchardi*) exposed to atrazine at concentrations comparable to those found in the present study showed feminized sex ratios. Although atrazine was higher at sites with lower forest cover, I found no effect of vegetation height or percent forest cover on tadpole sex ratios in this study. Taken together, these results highlight the buffering capacity of wooded riparian strips against increased nutrient loads from agriculture and their importance at mitigating the negative effects of agricultural contaminants on tadpole development.

4.6 Tadpole stress

Tadpoles from sites higher in forest cover tended to have higher blood glucose concentrations (Table 11). Higher glucose in the form of glucocorticoids is a known stress response in fish (Costas, 2008; Lankford et al., 2005; Sapolsky et al., 2000). Multiple stressors exist in agricultural landscapes that could cause a glucose response in frogs. For example, RDA results showed that sites higher in in forest cover tended to be higher in CI levels, a known stressor for frogs (Adelizzi et al., 2019; Corsi et al., 2010; Dananay et al., 2015; Kearney et al., 2016). Riparian vegetation height and percent forest cover had no significant effect on hepatosomatic index (HSI) or corticosterone (Table 11). Overall, based on the stress metrics used in this study, it does not appear that tadpoles are sublethally compromised by stress at these sites. However, it would be prudent to investigate other biomarkers to determine if frogs produced from these systems are in optimal health. Agricultural contaminants such as

pesticides can have significant effects on other stress metrics such as blood cell profiles (Gavel et al., 2019). Tadpoles exhibiting a stress or immune response are at risk of increased susceptibility to disease (Buck et al., 2015; Pochini & Hoverman, 2017).

4.7 Pathogens

Baseline levels of both Frog Virus 3 (FV3) and *Batrachochytrium dendrobatidis* (Bd) were unknown in the South Nation (SN) river watershed prior to the present study. Generally, investigations for the presence of these pathogens begin after reports of mass mortality events (Duffus & Andrews, 2013; Greer et al., 2005). Research has also shown that amphibians can be more susceptible to these pathogens in anthropogenically altered environments such as constructed ponds (Youker-Smith et al., 2018). However, such events can go largely unnoticed in rural/isolated areas such as agricultural drainage ditches. There is evidence for low prevalence of both FV3 and Bd in Ontario (Carstairs, 2019; Crawshaw et al., 2022). Based on the results of this study, it does not appear that FV3 or Bd are present in these systems. One possible explanation for the lack of detection of either pathogen is possible protective effects of the cages. However, this is unlikely, as these pathogens are spread between amphibians and reptiles through the water (Brenes et al., 2014). The mesh of the cages used in this study is 500 μm and both water and sediment pass freely through it. Prevalence of both FV3 and Bd vary seasonally, with FV3 being most prevalent in the summer and Bd being most prevalent in the winter (Garnham et al., 2022; Herath et al., 2023). This could help explain why Bd was not detected, as tadpoles were exposed to ditch waters in the spring and summer months for the present study, when Bd prevalence is generally low. All tadpoles for the present study were

exposed to agricultural ditch waters from May 27 to July 13 2022. Taken together, these results suggest that FV3 and Bd are not yet present in these systems. However, further monitoring is required to confirm this result.

4.8 Conclusions

The present study has demonstrated the importance of riparian vegetation for both water chemistry and tadpole health metrics. Overall percentage of forest cover appears to play a key role in reducing contaminant levels in these systems. Further, sites higher in forest cover tended to have larger tadpoles overall, likely attributable to lower contaminant levels at these sites. Surprisingly, vegetation height and forest cover did not have any significant effects on surface water temperatures, a result opposite to what was expected based on previous studies (Dugdale et al., 2018; Dyck et al., 2021; Kaandorp et al., 2019; Sweeney et al., 2004). It is likely that temperatures in these streams are largely regulated by cooler groundwater inputs. Sites higher in forest cover tended to have more favorable conditions for tadpoles overall. Sites higher in forest cover tended to have lower SPC likely due to lower inputs from NPK-based fertilizers (Garg et al., 2022; Onwe et al., 2020; Tenório Marinho Da Rocha et al., 2023). High forest sites also tended to have lower NO₃, K, atrazine and TNeo (Table 8). This is likely attributable to higher inputs of organic matter that promotes higher denitrification and higher soil sorption (Aguiar et al., 2015; Inoue et al., 2006; Laabs et al., 2002; Novak et al., 2015; Ramsey et al., 2005; Satkowski et al., 2018).

Tadpole survival was high overall and appears to be largely unaffected by riparian buffers (Table 8). Therefore, any effects to amphibians in these systems are likely sublethal. The

results of this study suggest that NO₃ is strongly affects multiple measures of tadpole size (SVL, TL, mass). Nitrate can delay amphibian development in agricultural systems (Ortiz-Santaliestra & Marco, 2015; Ortiz-Santaliestra & Sparling, 2007; G. R. Smith et al., 2005). Additionally, atrazine concentrations were negatively associated with forest cover. Atrazine is a known endocrine disrupter that can have negative effects on tadpole development (Hoskins & Boone, 2018; Rimayi et al., 2018; Zaya et al., 2011).

Both FV3 and Bd were undetected across all sites from this experiment. Previous work has suggested that prevalence of both FV3 and Bd are relatively low in Ontario (Carstairs, 2019; Crawshaw et al., 2022). However, seasonal variation in Bd prevalence could help explain why it was not detected in the present study, as samples were taken during the spring and summer months, when prevalence is generally low (Garnham et al., 2022).

The complexity of these systems can make it difficult to truly understand the mechanisms affecting surface water quality. Altogether, the results of the present study suggest that forest cover plays an important role in mitigating contaminant concentrations in these systems. This could also be indicative of overall reduction in agriculture in regions higher in forest cover. It should be noted that contaminant levels in these streams is likely dictated by upstream factors that could not be captured in the scale of this study, which has been demonstrated in this region (Noteboom et al., 2021).

Overall, the tadpoles from this study appeared healthy. However, studies like this are important to assess the overall health of amphibians in these systems to better understand if agricultural ditches can support amphibian populations in these areas over the long-term. Amphibians are declining worldwide. This study occurred over a single season, using non-free-

ranging tadpoles. The design of this study could not elucidate the overall trends in amphibian declines in these systems at the population scale. Monitoring studies have shown that anthropogenic change to natural landscapes as well as climate change can contribute to population declines (Canova & Balestrieri, 2018; Rebouças et al., 2021). Longer-term studies at the population scale on natural amphibian populations in these regions may reveal steady declines. Key long-term indicators of population decline such as reduction in egg mass depositions or reductions in population size may be affected in these regions. Future work should aim to monitor amphibian populations in these regions. Land managers should aim to preserve natural habitats such as forested buffers, as these areas appear to provide the most protection to amphibians and aquatic life.

Appendices

Appendix 1: Nanodrop quality assurance

Table A1. Absorbance ratio (260:280) and nucleic acid concentrations from eluted tadpole DNA (n = 10). An absorbance ratio of 1.8 to 2.0 is considered of good quality. Each sample was tested twice for quality assurance.

Site	Nucleic acid (ng/μL)	Absorbance ratio (260:280)
324a	221.1	1.96
324b	221.7	1.97
325a	169.6	1.95
325b	169.9	1.95
326a	152.3	1.97
326b	153.6	1.95
327a	206.2	1.97
327b	206.5	1.97
333a	209.8	1.98
333b	247.2	1.98
337a	210.9	1.96
337b	248.2	1.97
340a	158.0	2.00
340b	157.3	2.03
341a	200.4	2.02
341b	200.7	2.01
341a	198.8	1.95
341b	199.0	1.94
Controla	223.1	1.97
Controlb	223.7	1.98

Appendix 2: Multi-model Comparisons

Table A2. Multi-model comparisons of General Linear Mixed-Effects Models for ditch depth and current velocity with associated AIC scores. AIC values were used for model selection

Model ^a	AIC ^b	Δ AIC ^c
Depth		
1. VH + PF + 1 Date + 1 Site	1016.5	0.1
2. VH + 1 Date + 1 Site	1025.4	9.0
3. PF + 1 Date + 1 Site	1016.4	-
4. 1 + 1 Date + 1 Site	1024.2	7.8
Current		
1. VH + PF + 1 Date + 1 Site	457.7	2.8
2. VH + 1 Date + 1 Site	456.3	1.4
3. PF + 1 Date + 1 Site	455.8	0.9
4. 1 + 1 Date + 1 Site	454.9	-

^a Predictor variables are vegetation height (VH) and percent forest (PF). Date and site were both included as random effects in the models

^b Models with the best fit have the lowest AIC (shown in bold)

^c Δ AIC is the difference in AIC value compared to the best-fit model.

Table A3. Multi-model comparisons of General Linear Mixed-effect Models for water physiochemical measurements and their associated AIC values used to select best-fit models.

Model ^a	AIC ^b	Δ AIC ^c
YSI temperature		
1. VH + PF + 1 Date + 1 Site	600.9	2.9
2. VH + 1 Date + 1 Site	600.0	2.0
3. PF + 1 Date + 1 Site	599.3	1.3
4. 1 + 1 Date + 1 Site	598.0	-
Hobo temperature		
1. VH + PF + 1 Date + 1 Site	57294.0	1.0
2. VH + 1 Date + 1 Site	57294.0	1.0
3. PF + 1 Date + 1 Site	57293.0	-
4. 1 + 1 Date + 1 Site	57293.8	0.8
Dissolved oxygen		
1. VH + PF + 1 Date + 1 Site	1289.5	3.5
2. VH + 1 Date + 1 Site	1288.0	2.0
3. PF + 1 Date + 1 Site	1287.8	1.8
4. 1 + 1 Date + 1 Site	1286.0	-
Specific conductance		
1. VH + PF + 1 Date + 1 Site	1583.3	1.4
2. VH + 1 Date + 1 Site	1586.5	4.6
3. PF + 1 Date + 1 Site	1581.9	-
4. 1 + 1 Date + 1 Site	1585.0	3.1

pH

1. VH + PF + 1 Date + 1 Site	-72.5	3.7
2. VH + 1 Date + 1 Site	-74.5	1.7
3. PF + 1 Date + 1 Site	-74.5	1.7
4. 1 + 1 Date + 1 Site	-76.2	-

^a Predictor variables are vegetation height (VH) and percent forest (PF). Date and site were both included as random effects in the models

^b Models with the best fit have the lowest AIC (shown in bold)

^c Δ AIC is the difference in AIC value compared to the best-fit model.

Table A4. Multimodel comparisons of General(ized) Linear (Mixed-effect) Models for water nutrient concentrations measurements and their associated AIC values used to select best-fit models.

Model ^a	AIC ^b	Δ AIC ^c
Chloride		
1. VH + PF + 1 Site	110.6	1.6
2. VH + 1 Site	109.0	-
3. PF + 1 Site	116.6	7.6
4. 1 + 1 Site	116.0	7.0
Dissolved organic carbon		
1. VH + PF + 1 Date + 1 Site	606.3	1.6
2. VH + 1 Date + 1 Site	609.7	5.0
3. PF + 1 Date + 1 Site	604.7	-
4. 1 + 1 Date + 1 Site	611.2	6.7
Total phosphorus		
1. VH + PF + 1 Date + 1 Site	274.0	2.0
2. VH + 1 Date + 1 Site	276.3	4.3
3. PF + 1 Date + 1 Site	272.0	-
4. 1 + 1 Date + 1 Site	275.9	3.9
Total suspended solids		
1. VH + PF + 1 Date + 1 Site	376.4	3.9
2. VH + 1 Date + 1 Site	374.3	1.8
3. PF + 1 Date + 1 Site	374.4	1.9
4. 1 + 1 Date + 1 Site	372.5	-
Nitrate		
1. VH + PF + 1 Date + 1 Site	558.0	1.9
2. VH + 1 Date + 1 Site	563.1	7.0
3. PF + 1 Date + 1 Site	556.1	-
4. 1 + 1 Date + 1 Site	564.0	7.9
Dissolved potassium		
1. VH + PF + 1 Date + 1 Site	360.0	2.0
2. VH + 1 Date + 1 Site	360.2	2.2

3. PF + 1 Date + 1 Site	358.0	-
4. 1 + 1 Date + 1 Site	359.6	1.6

^a Predictor variables are vegetation height (VH) and percent forest (PF). Date and site were both included as random effects in the models

^b Models with the best fit have the lowest AIC (shown in bold)

^c ΔAIC is the difference in AIC value compared to the best-fit model.

Table A5. Multimodel comparisons of General(ized) Linear Mixed-effect Models for water pesticide concentrations and their associated AIC values used to select best-fit models.

Model ^a	AIC ^b	ΔAIC ^c
Total neonicotinoids		
1. VH + PF + 1 Date + 1 Site	-1011.4	-
2. VH + 1 Date + 1 Site	-1002.3	9.1
3. PF + 1 Date + 1 Site	-1004.0	7.4
4. 1 + 1 Date + 1 Site	-1003.6	7.8
Atrazine		
1. VH + PF + 1 Date + 1 Site	246.9	1.4
2. VH + 1 Date + 1 Site	255.4	9.9
3. PF + 1 Date + 1 Site	245.5	-
4. 1 + 1 Date + 1 Site	255.0	9.5
Glyphosate		
1. VH + PF + 1 Date + 1 Site	216.0	3.2
2. VH + 1 Date + 1 Site	214.6	1.8
3. PF + 1 Date + 1 Site	214.7	1.9
4. 1 + 1 Date + 1 Site	212.8	-

^a Predictor variables are vegetation height (VH) and percent forest (PF). Date and site were both included as random effects in the models

^b Models with the best fit have the lowest AIC (shown in bold)

^c ΔAIC is the difference in AIC value compared to the best-fit model.

Table A6. Multi-model comparisons of General(ized) Linear (Mixed-effect) Models for tadpole health metrics with associated AIC values

Model ^a	AIC ^b	ΔAIC ^c
Survival		
1. VH + PF + 1 Site + 1 Cage	111.7	2.6
2. VH + 1 Site + 1 Cage	109.8	0.7
3. PF + 1 Site + 1 Cage	110.4	1.3
4. 1 + 1 Site + 1 Cage	109.1	-
Proportion female		
1. VH + PF + 1 Cage	105.3	2.5
2. VH + 1 Cage	104.8	2.0
3. PF + 1 Cage	103.9	1.1
4. 1 + 1 Cage	102.8	-
SVL		
1. VH + PF + Survival + Stage + 1 Cage + 1 Site	1222.7	-
2. VH + PF + Stage + 1 Cage + 1 Site	1224.7	2.0
3. VH + PF + Survival + 1 Cage + 1 Site	1457.2	234.5
4. VH + PF + 1 Cage + 1 Site	1459.0	236.3
5. VH + 1 Cage + 1 Site	1461.0	238.3
6. PF + 1 Cage + 1 Site	1457.8	235.1
7. 1 + 1 Cage + 1 Site	1462.8	240.1
Body width		
1. VH + PF + Survival + Stage + 1 Cage	911.6	-
2. VH + PF + Stage + 1 Cage	915.0	3.4
3. VH + PF + Survival + 1 Cage	1066.5	154.9
4. VH + PF + 1 Cage	1069.2	157.6
5. VH + 1 Cage	1068.8	157.2
6. PF + 1 Cage	1069.3	157.7
7. 1 + 1 Cage	1072.4	160.8
Tail length		
1. VH + PF + Survival + Stage + 1 Cage + 1 Site	1766.3	-
2. VH + PF + Stage + 1 Cage + 1 Site	1774.5	8.2
3. VH + PF + Survival + 1 Cage + 1 Site	2038.7	272.4
4. VH + PF + 1 Cage + 1 Site	2044.9	278.6
5. VH + 1 Cage + 1 Site	2048.7	282.4
6. PF + 1 Cage + 1 Site	2045.0	278.7
7. 1 + 1 Cage + 1 Site	2052.4	286.1
Mass		
1. VH + PF + Survival + Stage + 1 Cage	204.1	1.9
2. VH + PF + Stage + 1 Cage	202.2	-
3. VH + PF + Survival + 1 Cage	602.0	397.9
4. VH + PF + 1 Cage	603.9	401.7
5. VH + 1 Cage	611.2	409.0

6. PF + 1 Cage	605.0	402.8
7. 1 + 1 Cage	619.7	417.5

Gosner stage

Model selection not possible with glmmPQL package. Full model is presented

HSI

1. VH + PF + Survival + Stage + 1 Cage + 1 Site	-2510.9	-
2. VH + PF + Stage + 1 Cage + 1 Site	-2361.6	149.3
3. VH + PF + Survival + 1 Cage + 1 Site	-2510.8	0.1
4. VH + PF + 1 Cage + 1 Site	-2360.0	150.9
5. VH + 1 Cage + 1 Site	-2362.0	148.9
6. PF + 1 Cage + 1 Site	-2357.5	153.4
7. 1 + 1 Cage + 1 Site	-2357.8	153.1

Glucose

1. VH + PF + Survival + Stage + 1 Cage	205.8	4.5
2. VH + PF + Stage + 1 Cage	204.0	2.7
3. VH + PF + Survival + 1 Cage	204.3	3
4. VH + PF + 1 Cage	202.5	1.2
5. VH + 1 Cage	210.5	9.2
6. PF + 1 Cage	201.3	-
7. 1 + 1 Cage	209.4	8.1

Corticosterone

1. VH + PF + Survival + Stage + 1 Cage + 1 Site	513.2	7.4
2. VH + PF + Stage + 1 Cage + 1 Site	511.3	5.5
3. VH + PF + Survival + 1 Cage + 1 Site	511.7	5.9
4. VH + PF + 1 Cage + 1 Site	509.8	4.0
5. VH + 1 Cage + 1 Site	507.8	2.0
6. PF + 1 Cage + 1 Site	507.8	2.0
7. 1 + 1 Cage + 1 Site	505.8	-

^a Predictor variables are vegetation height (VH) and percent forest (PF). Cage and site were both included as random effects in the models

^b Models with the best fit have the lowest AIC (shown in bold)

^c ΔAIC is the difference in AIC value compared to the best-fit model.

Appendix 3: Pesticides

Table A7. Method detection limit (MDL) and method reporting limit (MRL) for the nine pesticides screened from surface water samples. All units are in 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

Appendix 4: Frogs

Figure A1. Gonadal identification of *Rana [Lithobates] pipiens*. Female ovaries (top), male testes (middle), intersex/undifferentiated (bottom). Gonads appear as white clouds (ovaries) or balls (testes) over yellow kidneys. Pictures taken from: Metamorph Endpoints-Euthanization Protocol SR04-2016, Robinson Lab, NWRC.

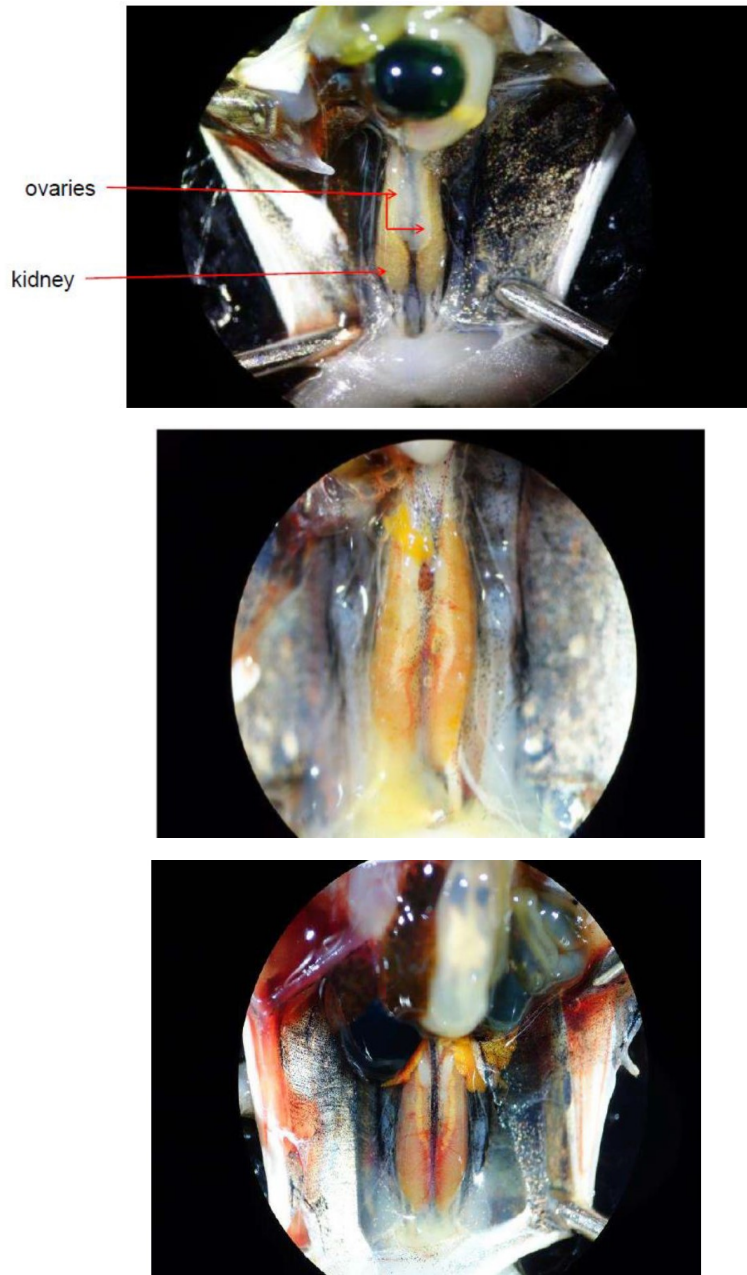
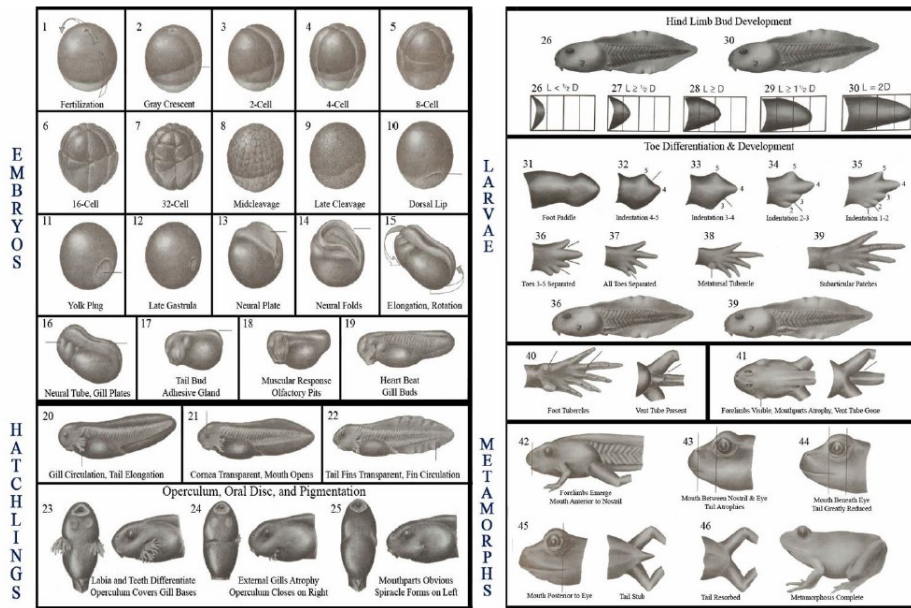


Figure A2. Gosner stage identification chart for *Rana [Lithobates] pipiens* tadpoles used to characterize metamorphic development (from Gosner, 1960)



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