Ecotoxicological and metabolism-disrupting actions of *Bacillus thuringiensis israelensis* and deltamethrin insecticides in anuran tadpoles

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Abstract

Endocrine-disrupting chemicals are an increasing environmental concern due to their capacity to disrupt physiological processes, including growth, development, and metabolism. The insecticides VectoBac® 200G (a *Bacillus thuringiensis israelensis* (Bti) product) and deltamethrin (a pyrethroid) are widely used for mosquito control, but little research has examined their effects on amphibians. Here, we tested the hypothesis that VectoBac® 200G and deltamethrin adversely affect tadpole health and development via metabolic disruption. First, we assessed the toxicity of these insecticides on three North American species: the chorus frog (*Pseudacris maculata*), the leopard frog (*Lithobates pipiens*), and the wood frog (*Lithobates sylvaticus*). The 96 h median lethal concentration (LC_{50}) values were estimated to be 513,000 ± 1.15, 78,860 ± 1.10, and 525,363.4 ± 1.13 international toxic units (ITU)/L for chorus, leopard, and wood frog tadpoles exposed to VectoBac® 200G. The LC_{50} values for deltamethrin were estimated to be 2.69 ± 1.06, 7.30 ± 1.05, and 1.15 ± 1.06 μg active ingredient (a.i.)/L for chorus, leopard, and wood frog tadpoles, respectively. VectoBac® 200G and deltamethrin had varying effects on total length, and investigations on metabolic endpoints were pursued in the wood frog tadpole. Metabolic studies on tadpoles are sparse, and the biggest challenge was measuring blood glucose, as wood frog tadpoles are too small to collect blood from. We therefore designed and validated a novel assay that enables measurement of whole-body glucose in individual tadpoles. Following 30-day exposures, VectoBac® 200G significantly increased glucose uptake, whereas exposure to deltamethrin did not. Chronic exposure from the early larval stage through metamorphosis delayed time to complete metamorphosis in VectoBac® 200G-exposed tadpoles, and tadpoles exposed to both insecticides displayed altered hepatic lipid accumulation. These results further suggested that metabolic endpoints were altered from exposure, especially to VectoBac® 200G. To investigate pancreatic effects, a novel custom antibody targeting the frog insulin B-chain was generated and validated. Both insecticides increased the proportion and nuclear radius of pancreatic beta-cells in exposed wood frogs. Only VectoBac® 200G increased total insulin-positive staining per pancreas; however, insulin staining per beta-cell decreased. Increased beta-cell proliferation combined with reduced insulin staining per cell suggests altered insulin dynamics in VectoBac® 200G-exposed frogs. Collectively, these results address data gaps for both Bti and deltamethrin insecticides and provide insight into potential mechanisms by which these products may disrupt amphibian metabolism.

Résumé

Les perturbateurs endocriniens chimiques constituent une préoccupation environnementale croissante en raison de leur capacité à altérer les processus physiologiques, notamment la croissance, le développement et le métabolisme. Les insecticides VectoBac® 200G (un produit à base de *Bacillus thuringiensis israelensis* (Bti)) et la deltaméthrine (un pyréthroïde) sont largement utilisés pour lutter contre les moustiques, mais peu de recherches ont examiné leurs effets sur les amphibiens. Nous avons testé l'hypothèse que le VectoBac® 200G et la deltaméthrine ont des effets néfastes sur la santé et le développement des têtards en perturbant leur métabolisme. Nous avons d'abord évalué la toxicité de ces insecticides sur trois espèces nord-américaines : la rainette faux-grillon boréale (*Pseudacris maculata*), la grenouille léopard (*Lithobates pipiens*) et la grenouille des bois (*Lithobates sylvaticus*). Les valeurs médianes de concentration létale (CL_{50}) à 96 heures ont été estimées à 513 000 ± 1,15, 78 860 ± 1,10 et 525 363,4 ± 1,13 unités toxiques internationales (UTI)/L pour les têtards de rainette faux-grillon boréale, de grenouille léopard et de grenouille des bois exposés au VectoBac® 200G. Les valeurs CL_{50} pour la deltaméthrine ont été estimées à 2,69 ± 1,06, 7,30 ± 1,05 et 1,15 ± 1,06 µg de matière active (m.a.)/L pour les têtards de rainette faux-grillon boréale, de grenouille léopard et de grenouille des bois, respectivement. Le VectoBac® 200G et la deltaméthrine ont eu des effets variables sur la longueur totale, et des recherches sur les paramètres métaboliques ont été menées chez les têtards de grenouille des bois. Les études métaboliques sur les têtards sont rares, et le plus grand défi consistait à mesurer la glycémie, car les têtards de grenouille des bois sont trop petits pour prélever du sang. Nous avons donc conçu et validé un nouveau test permettant de mesurer la glycémie dans tout le corps de chaque têtard. Après 30 jours d'exposition, le VectoBac® 200G a considérablement augmenté l'absorption de glucose, contrairement à la deltaméthrine. Une exposition chronique depuis le stade larvaire précoce jusqu'à la métamorphose a retardé le temps à la métamorphose complète chez les têtards exposés au VectoBac® 200G, et les têtards exposés aux deux insecticides ont présenté une accumulation hépatique de lipides altérée. Ces résultats suggèrent également que les paramètres métaboliques ont été modifiés par l'exposition, notamment au VectoBac® 200G. Afin d'étudier les effets sur le pancréas, un nouvel anticorps spécifique ciblant la chaîne B de l'insuline de grenouille a été créé et validé. Les deux insecticides ont augmenté la proportion et le rayon nucléaire des cellules bêta pancréatiques chez les grenouilles des bois exposées. Seul le VectoBac® 200G a augmenté la

coloration totale positive à l'insuline par le pancréas ; cependant, la coloration à l'insuline par cellule bêta a diminué. L'augmentation de la prolifération des cellules bêta, combinée à une réduction de la coloration insulinique par cellule, suggère une modification de la dynamique de l'insuline chez les grenouilles exposées au VectoBac® 200G. Collectivement, ces résultats comblent les lacunes dans les données concernant les insecticides Bti et deltaméthrine et fournissent des informations sur les mécanismes potentiels par lesquels ces produits peuvent perturber le métabolisme des amphibiens.

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Abbreviations Used

AChE	Acetylcholinesterase
ACP	Acid phosphatase
a.i.	Active ingredients
AST	Aspartate aminotransferase
BaP	Benzo(a)pyrene
BS	*Bacillus sphaericus*
Bt	*Bacillus thuringiensis*
Bti	*Bacillus thuringiensis israelensis*
Btk	*Bacillus thuringiensis kurstaki*
Bw	Body weight
CAT	Catalase
CbE	Carboxylesterase
CFU	Colony-forming unit
D	Deltamethrin
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethyl sulfoxide
EDC	Endocrine-disrupting chemical
EEC	Expected environmental concentration
EPA	Environmental Protection Agency
G	Glucose
GLM	Generalized linear model
GR	Glutathione reductase
GS	Gosner stage
GST	Glutathione S-transferase
Ha	Hectare
HC5	Hazardous concentration (5%)
HSP 70	Heat shock protein 70

INS-IR	Insulin-immunoreactive
ITU	International toxic units
LC_{10}	Lethal concentration (10%)
LC_{15}	Lethal concentration (15%)
LC_{20}	Lethal concentration (20%)
LC_{50}	Median lethal concentration
LC_{100}	Absolute lethal concentration
LDH	Lactate dehydrogenase
LMM	Linear mixed models
LOEC	Lowest observed effect concentration
MATC	Maximum acceptable toxicant concentration
MDC	Metabolic disrupting chemical
MeOH	Methanol
N/A	Not applicable
ND	Not disclosed
NF	Nieuwkoop and Faber
NG	Non-glucose
NOEC	No observed effect concentration
PBS	Phosphate buffer
PBS-T	Phosphate buffer containing 0.1% Triton X-100
SSD	Species sensitivity distribution
SVL	Snout-vent length
TPP	Triphenyl phosphate

Chapter 1: General Introduction

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1.1 Thesis Rationale

Insecticides are essential for controlling crop pests and disease vectors, such as mosquitoes (Becker et al., 2010). Many, however, are endocrine-disrupting chemicals (EDCs) that contribute to global amphibian declines (Brühl et al., 2011). These chemicals disrupt hormone regulation, thereby affecting amphibian growth and metamorphosis. One widely used mosquito control agent is *Bacillus thuringiensis israelensis* (Bti). While there is little debate regarding the effectiveness of Bti, research on its impacts on amphibians remains controversial (Chapter 1; Empey et al., 2021). Even less research has examined whether Bt insecticides function as endocrine disruptors in amphibians. Another commonly used insecticide is deltamethrin, a neurotoxic pyrethroid used to kill various invertebrates (Pham et al., 1984). It is a suspected EDC (Işıldar et al., 2020), but its effects on the amphibian endocrine axis have not yet been studied.

1.2 Thesis Hypotheses and Objectives

I tested the hypothesis that the insecticides VectoBac® 200G (a Bti product) and deltamethrin negatively impact tadpole health and development through metabolic disruption. My objectives in addressing this hypothesis were to:

1. Determine the median lethal concentration of VectoBac® 200G and deltamethrin for chorus (*Pseudacris maculata*), leopard (*Lithobates pipiens*), and wood frog (*Lithobates sylvaticus*) tadpoles (Chapter 2).
2. Evaluate whether exposure to these insecticides alters glucose regulation in wood frog tadpoles and liver condition in wood frog metamorphs (Chapter 3).
3. Characterize pancreatic beta-cells and insulin immunostaining in exposed wood frog metamorphs (Chapter 4).

1.3 Endocrine Disruptors

Amphibians are particularly vulnerable to EDC exposure as both tadpoles and adults have permeable skin that is sensitive to pollutants (Trudeau et al., 2020). Many species have aquatic, terrestrial, or semi-terrestrial life stages, which may increase their susceptibility to contaminant exposure (Fort et al., 2007). Data suggest that EDCs can interfere with hormonal regulation, disrupting homeostasis and impairing metamorphosis and reproduction (Trudeau et al., 2020).

Consequently, endocrine disruption has been identified as a contributing factor to amphibian population decline as it can ultimately affect the fitness and fecundity of amphibian species (Hayes et al., 2006, 2010; Kloas & Lutz, 2006). A subset of EDCs, termed metabolic-disrupting chemicals (MDCs), impede metabolic processes and endocrine function (Zoeller et al., 2012). Some MDCs are classified as obesogens that affect glucose and lipid metabolism, promoting obesity by increasing the number of fat cells or the storage capacity of pre-existing adipocytes. These diverse compounds affect appetite regulation and energy expenditure, alter tissue sensitivity to neurotransmitters, and alter autonomic nervous system activity (Decherf & Demeneix, 2011; Heindel & Blumberg, 2019). Human studies further support the metabolic consequences of certain EDCs. Exposure to several chemicals, such as bisphenol A, dichlorodiphenyldichloroethylene (DDE), and hexachlorobenzene (among many others), is associated with increased risk of obesity and metabolic disruption (Glynn et al., 2003; Rubin et al., 2001).

Only a handful of studies have examined the effects of MDCs on amphibians. Recently, Regnault et al. (2018) observed that chemicals such as benzo(a)pyrene (BaP) and triclosan at concentrations of 50 ng/L induced a pre-diabetic state in female *Xenopus tropicalis*, including symptoms of glucose intolerance, liver steatosis, and pancreatic insulin hypersecretion. The progeny of exposed parents also experienced delayed metamorphosis and reduced reproductive success (Regnault et al., 2018). Usal et al. (2021) similarly reported that BaP and triclosan delayed metamorphosis and sexual maturity in F1 and F2 progeny of *X. Tropicalis.* Additionally, the F2 progeny developed nonalcoholic steatohepatitis (NASH), a common metabolic syndrome associated with pre-diabetes (Usal et al., 2021). Amphibian population declines have been associated with environmental exposure to EDCs, which can interfere with metabolic function, development, metamorphosis, and reproduction (Hayes et al., 2006). However, the precise roles of certain contaminants are not always clear and likely vary among species and environmental contexts (Hayes et al., 2006). Furthermore, knowledge of the effects of Bti and deltamethrin insecticides on the amphibian endocrine axis remains limited.

1.4 *Bacillus thuringiensis* insecticides

Insecticides to control mosquitoes and crop pests were first introduced in the 1910s (Becker & Ludwig, 1993; Stapleton, 2004). Controlling mosquito populations that transmit human diseases such as West Nile virus, Dengue fever, and malaria offers significant health benefits (Calba et al., 2017; Succo et al., 2016). In temperate regions, mosquito control may also

be used to reduce the nuisance of mosquito bites (Becker et al., 2010; Halasa et al., 2014). Despite these economic, health, and potential lifestyle benefits, insecticide use entails a range of risks, including environmental contamination, development of resistance, and sublethal effects and mortality on nontarget organisms (van den Berg et al., 2015; Coetzee & Koekemoer, 2013; Hemingway & Ranson, 2000). The potential harm that may result from the widespread use of mosquito control programs is exemplified by the insecticides Paris Green [copper(II) acetate triarsenite or copper(II) acetoarsenite] and dichlorodiphenyltrichloroethane (DDT) (Casida, 2012; National Pesticide Information Center, 1999), which were used in agriculture to control pests and reduce the incidence of mosquito-borne diseases. Despite their effectiveness, these products are highly persistent in the environment and are toxic to nontarget organisms, including humans. While DDT is banned in some regions, countries in South America, Africa, and Asia may continue to use it in malaria vector control strategies, as recommended by the World Health Organization (van den Berg et al., 2015).

The development of alternative insecticides with significantly less environmental and health impacts is of paramount importance. One such alternative is *Bacillus thuringiensis* (Bt) var. *israelensis* (Bti), a Gram-positive bacterium that naturally occurs in soil. It was discovered in 1976 and isolated from a stagnant pond in the Negev Desert in Israel (Goldberg & Margalit, 1977), then developed as a bioinsecticide targeting the order Diptera, predominantly mosquitoes and blackflies (Margalit, 1990). The bacteria produce insecticidal proteins as crystal inclusions during growth, known as Cry and Cyt toxins, which are effective for mosquito control programs (Bravo et al., 2011; Goldberg & Margalit, 1977). The insecticide contains three Cry toxins (Cry4Aa, Cry4Ba, and Cry11Aa) and one Cyt toxin (Cyt1Aa) (Ben-Dov, 2014), which, after being ingested by a target insect, dissolve in the alkaline conditions of the Dipteran gut, releasing protoxins that are activated by proteases (Rukmini et al., 2000; Vachon et al., 2012). Cry toxins bind to specific protein receptors in the gut, oligomerize, and form pores in the gut membrane of the insects, leading to death. Bacterial spores are then released into the hemolymph, where they germinate and can proliferate. Although mosquito resistance to individual Cry toxins has been reported, little resistance to Bti insecticide formulations has been recorded because they contain a mix of the four toxins (Goldberg & Margalit, 1977; Pardo-Lopez et al., 2013; Soberon et al., 2013). High-resolution structural analysis (Tetreau et al., 2020) has recently revealed the key steps in the Cyt1Aa bioactivation cascade, from in vivo crystallization in Bti cells to crystal

dissolution, proteolytic activation, and membrane insertion and perforation through oligomerization. Thus, the mechanisms of Cyt protein toxicity in insects are emerging.

Another widely used subspecies is Bt var. *kurstaki* (Btk). Commercial insecticide products containing Btk have been used in North America for more than 35 years. They are among the most widely used insecticides in Canada (Fuentealba et al., 2019) and are predominantly used in forestry and organic agriculture (Kreutzweiser et al., 1996). For example, more than 10 million hectares of Canadian forests were sprayed with Btk-based insecticides between 1985 and 2012 to control defoliator pests, such as the spruce budworm, spongy moth, and hemlock looper (Fuentealba et al., 2019). This formulation primarily targets more than 200 Lepidopteran larvae species and contains five Cry toxins (Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, and Cry2Ab) (Ben-Dov et al., 1999). The mechanism of action is similar to Bti, where the toxin crystals ingested by target larvae dissolve in the alkaline gut conditions and bind to the midgut epithelial cells, which then produce pores in the gut membrane through cell lysis, resulting in the death of the insect (Bravo et al., 2007; 2011).

In contrast to this mechanism of action in targeted pests, the effects of commercial products containing Bti and Btk on amphibians remain unknown. This is of concern as amphibians are important in food webs and ecosystem services. Some frog and salamander species inhabit wetlands or small ponds, which are typical sites for mosquito reproduction and may be treated with Bti (Becker & Lüthy, 2017). On the other hand, there is potential for Btk runoff, overspraying, and sediment deposition from agricultural sites to infiltrate wetland ecosystems (Hoffman et al., 2000). Exposure to Bti or Btk through insecticide applications may affect amphibian health across the larval (tadpole), juvenile (metamorphic), and adult phases, including survival, growth, metamorphic success rate, physiological functions, and behaviour. Research on the effects of Bti and Btk products on amphibians is in the early stages. Current studies report inconsistent findings, with some documenting sublethal effects and others reporting no observable impacts. Here, we critically assess the scientific literature to identify areas for future research on the effects of Bti and Btk on amphibians.

1.4.1 Bt-insecticide effects on tadpole survival

There are contrasting results regarding the acute toxicity effects of Bti insecticides. Lajmanovich et al. (2015) assessed the toxicity of Introban® (containing 1.2% Bti and 1,200 international toxic units (ITU)/mg potency; Valent BioSciences Corporation, USA) in South American frog (*Leptodactylus latrans*) tadpoles (see Table 1.1 for concentrations). Exposure to

2.5 mg/L produced a mortality rate of 3.5%, with a steady increase in mortality to approximately 20% at 20 mg/L. The highest tested concentration of 40 mg/L produced 100% tadpole mortality. The calculated 48 h acute median lethal concentration (LC_{50}) value for Introban® was 22.45 mg/L, and no mortality was observed in the water control group (Lajmanovich et al., 2015). In contrast to these findings, some studies reported minimal mortality following Bti exposure. Allgeier et al. (2018) exposed *Rana temporaria* tadpoles to a nominal, twofold, and tenfold field rate (see Table 1.1) of VectoBac® WG ice and sand formulations (both containing 37.4% Bti) and VectoBac® 12AS liquid formulation (11.6% of Bti) (Valent BioSciences Corporation, Illinois, USA) and repeated these applications three times. The authors reported 10% mortality following exposure to the nominal and doubled field rate of each formulation. Results did not show a dose-response pattern: the tenfold field rate of the ice and sand formulations yielded approximately 5% mortality, whereas no mortality was recorded at the tenfold field rate of the liquid formulation. The authors reported no significant differences in survival between the Bti formulations or application rates and the control group that received no Bti. Similarly, Schweizer et al. (2019) exposed *R. temporaria* tadpoles to VectoBac® WG (3,000 ITU/L; Valent BioSciences Corporation, Libertyville, IL) at 1, 10, and 100 mg/L. The lowest application rate produced 12% mortality, while the highest application rate resulted in 10% mortality. Schweizer et al. (2019) used a rice protein control and a negative control, which produced 2% mortality and none recorded, respectively. Overall, there was no significant difference between the treatment groups.

Differing study designs may account for the contrasting findings across these studies. Schweizer et al. (2019) exposed their tadpoles for 11 days, which may be short relative to likely exposure scenarios in a treated wetland over a season. Lajmanovich et al. (2015) used the study species *L. latrans*, whereas Allgeier et al. (2018) and Schweizer et al. (2019) used *R. temporaria*, suggesting differential sensitivities to Bti toxins across species. Other differences can be observed in the developmental stages of the tadpoles of these studies. Lajmanovich et al. (2015) used *L. latrans* tadpoles at Gosner Stage (GS) 26–30; Schweizer et al. (2019) used *R. temporaria* tadpoles at GS 23; and Allgeier et al. (2018) also used *R. temporaria*, but at GS 21–23. Different commercial formulations, such as VectoBac® and Introban®, may also yield different results, as Introban® contains 1,200 ITU/L and 1.2% Bti, and VectoBac® WG contains 3,000 ITU/L and 37.4% Bti. These commercial formulations also contain additives known only to their respective manufacturers, and data on how these additives affect amphibians and other nontarget organisms

are not available. Oxygen levels, water hardness, temperature, feeding regimen, and pH may also have varied across studies. Given differences in experimental design, carrier composition, unknown formulation components, and the limited number of anuran species tested, further acute toxicity testing is warranted.

Other studies have shown that applying Bti products under semi-natural conditions increases amphibian mortality. For example, Pauley et al. (2015) performed 300 L mesocosm studies to test the effects of bioinsecticides in the presence and absence of dragonfly larvae as predators on the performance of GS 25 *Hyla versicolor* tadpoles. Commercial formulations of MosquitoBits® (containing 2.86% of Bti) and MosquitoDunks® (containing 10.31% of Bti) (Summit Chemical Co., Baltimore, MD) were used, among other non-Bti insecticides (See Table 1.1 for concentrations of each formulation). MosquitoBits® consist of corn granules coated with Bti, whereas MosquitoDunks® are circular pucks that can be applied to bodies of water, with one puck considered one treatment (as described by the manufacturers). Pauley et al. (2015) applied one treatment of MosquitoDunks® every 30 days, one treatment of MosquitoBits® every 14 days, one treatment of Mosquito Torpedoes® every 60 days (which does not contain Bti and therefore will not be discussed), and a control, which received no insecticides, each allotted to three mesocosms replicated with and without predators. The total time of the mesocosm studies is not stated. The authors reported no significant difference in tadpole survival between predators and insecticide treatments ($p < 0.05$); however, biologically meaningful differences were observed. In control mesocosms, tadpole mortality rate without predators was approximately 20%, whereas with predators present, mortality increased to approximately 60%, suggesting that the stress response alone may have increased tadpole mortality. Mesocosms treated with MosquitoDunks® and predators produced a tadpole mortality rate of 91% ($p = 0.06$) compared with the control mesocosm group (no insecticide but with predators present) that yielded a mortality rate of 64%. In comparison, MosquitoBits® induced approximately 40% mortality in the absence of predators ($p = 0.26$) and 70% mortality with predators present ($p = 0.66$), suggesting an interaction between the natural predation stressor and the applied Bti bioinsecticide. The authors suggested MosquitoDunks® may be more toxic because it contains a higher percentage of Bti than MosquitoBits® (Pauley et al., 2015).

Allgeier et al. (2019) reported similar results in their mesocosm study. They tested 3,000 ITU/mg of VectoBac® WG in 90 L mesocosms (representing a high field rate) to evaluate the development of *Lissotriton vulgaris* and *Lissotriton helveticus* newts, and to compare food-web

communities. Authors observed that, with Bti treatments, newts were more susceptible to intraguild predation by dragonfly nymphs (a 27% increase relative to controls), indicating greater competition for food sources. A trophic niche expansion was also observed, with newts consuming fewer chironomids in Bti-treated mesocosms, particularly when predators were present. This finding may represent a suboptimal environment due to contaminants and limited or poor-quality food sources (Karlson et al., 2018). The findings by Pauley et al. (2015) and Allgeier et al. (2019) illustrate how interacting stressors can influence contaminant toxicity, and how outcomes may differ markedly between semi-natural conditions and controlled laboratory settings. Mesocosm studies are advantageous because they can more closely replicate environmental factors (e.g., temperature, rainfall, UV); however, controlling variables in a mesocosm factorial design is also challenging. Well-designed mesocosm studies are useful for examining interactions between Bti exposure and predation, yielding more ecologically realistic findings. Such studies also highlight indirect effects of food-web interactions on nontarget organisms. For instance, Brühl et al. (2020) emphasized concerns about trophic cascades, as mosquitoes and chironomids are primary food sources for many amphibian species (Becker & Ludwig, 1983; Gutierrez et al., 2017; Vinnersten et al., 2009). In this regard, mesocosm studies provide a useful framework to evaluate the influence of Bti applications on prey availability and food-web dynamics, and how these subsequently affect amphibian performance.

Derua et al. (2018) examined the effects of Bti on the diversity, richness, and abundance of amphibians in the Western Kenya Highlands. One application of either FourStar® (Central Life Sciences, Sag Harbour, NY) or LL3 (University of California, Irvine, CA) briquets (both containing 1% Bti—potency of 70 ITU/mg—and 6% *Bacillus sphaericus* (Bs)—potency of 60 ITU/mg—the only difference being that LL3 briquets are formulated to float) were applied and monitored after the first 24 h, 3 days, then every week for 5 months from January to June 2016. This study examined 289 sites comprising abandoned gold mines, ponds, canals, rock pools, and swamps, which were assigned to three treatments: LL3, FourStar®, or a control with no insecticide. The study reported no significant differences in amphibian diversity, richness, or abundance between insecticide-treated sites and control sites. However, the study did not specify which locations were assigned to each treatment, and given the diversity of sites, there was likely considerable variation in turbidity, UV exposure, and vegetation cover. This study also did not address potential changes in biodiversity variables that could influence the effectiveness of these insecticides. The briquets are designed to persist in the environment, slowly releasing active

ingredients into the water column (Derua et al., 2018). Because environmental factors can affect the toxicological actions and persistence of Bti and Bs, the effects of these briquets on amphibian health should be examined more rigorously in future controlled studies. It is difficult to compare this study with others because FourStar® and LL3 briquets contain not only Bti but also Bs. However, all commercial formulations also contain unknown additives that may influence solubility and bioavailability, and consequently the insecticide's ecotoxicological potential.

There are currently only two studies on the effect of Btk on amphibians. This insecticide warrants research into its potential effects on wetland amphibian populations, as these habitats can be exposed to Btk through runoff, overspraying, and sediment deposition from nearby agricultural operations (Hoffman et al., 2000). Weeks and Parris (2020) studied the effects of Monterrey® Btk (containing 98.35% of Btk) on Southern Leopard frog (*Lithobates sphenocephalus*) embryo and tadpole survival. In this laboratory experiment, embryos were exposed to a control with no insecticide and to 0.0042 and 2.73 ml/L of Btk, while premetamorphic tadpoles were exposed to 0.0042, 0.42, and 2.73 mL/L of Btk. The lowest concentration reflected the expected environmental concentration (EEC) that would occur in a shallow wetland sprayed with an application rate of 63 mL/100 m^2 (according to the Monterrey® product label), and the highest dose was derived by using half the maximum label concentration. The authors reported that 2.73 ml/L of Monterrey® Btk significantly increased embryo and tadpole mortality (86% and 62.5%, respectively) compared with the lower concentrations and the control. The 96 h LC_{50} of *L. sphenocephalus* was determined to be 1.81 ml/L. Although 2.73 ml/L exceeds the EEC for Btk, the authors emphasized that future research on sublethal effects at environmentally relevant exposure levels is essential to assess ecological risks accurately. In contrast, Raimondo et al. (2003) examined the effects of Btk on the abundance of salamander species in West Virginia. The authors established nine 200-hectare plots in the Monongahela National Forest, with three blocks each containing three plots. Each plot was treated through aerial application with fixed-wing aircraft with either 16 billion international units (BIU)/hectare of Forey48F® polyhedral inclusion bodies (PIB)/hectare of 8×10^{11} polyhedral inclusion bodies (PIB)/hectare of Gypchek® (which does not contain Btk, but the nucleopolyhedrosis virus to kill spongy moths), or a control of no insecticide. Plots were observed from May to September in 1997 and from May to October in 1998. The diet and abundance of the following five salamander species were analyzed: *Desmognathus fuscus, Desmognathus ochrophaeus, Desmognathus monitcola, Plethodon cinereus,* and *Plethodon glutinosus*. When comparing the

treated and control plots, the authors reported no significant difference in species abundance. However, prey abundance in treated and control plots was not assessed, which would have strengthened the study design and results. With only two papers identified to date, it is too early to draw firm conclusions regarding the potential toxicity of Btk, and further research is required.

1.4.2 Bt-insecticide effects on behaviour, hatching success, growth, and metamorphosis

Junges et al. (2017) is the only study we could identify on the effects of Bti on amphibian behaviour. Authors examined the effects of Introban® and two other non-Bti insecticides on *Rhinella arenarum*, *Rhinella fernandezae*, and *Physalaemus albonotatus* GS 33 tadpoles. Tadpoles were exposed to 1.5–40 mg/L for 48 h, and dechlorinated water served as the negative control (Table 1.1). It was reported that the Bti formulation was less toxic than the other tested insecticides and altered only behavioural endpoints in R. arenarum, with tadpoles moving significantly less than controls. These results emphasize that there are likely species-specific differences in sensitivity to Bti-based insecticides. Similarly, there is only one study to date on the hatching success of amphibians exposed to Bt insecticides. Weeks and Parris (2020) investigated whether 0.0042 and 2.73 mL/L of Monterrey® Btk affected the hatching success of *L. sphenocephalus* embryos. The authors reported no significant difference in hatching success between the no-treatment control and the low-dose exposure (77% vs 73%, respectively). However, the highest Btk concentration significantly reduced hatching success to 16%. The species *L. sphenocephalus* is known to be sensitive to chemicals during development (Hanlon et al., 2015) and could be less tolerant to pesticides than other amphibian species (Weeks & Parris, 2020). Further studies on hatching success following exposure to Bti and Btk formulations are required, as this is the only study currently available.

Critical information is also missing regarding the effects of Bti insecticides on amphibian development and metamorphosis. Allgeier et al. (2018; 2019) reported no significant differences in time to complete metamorphosis between Bti-treated and untreated control groups in *R. temporaria*, *L. helveticus*, and *L. vulgaris* (see Table 1.1 for doses and formulations). Only their 2019 study incorporated a predation stressor using dragonfly nymphs. Although this variable modestly affected newt body size, it did not alter the time to complete metamorphosis. Pauley et al. (2015) reported that *H. versicolor* tadpoles in mesocosms with predators took longer to complete metamorphosis, regardless of whether the tadpoles were treated with Bti formulations of MosquitoBits® or MosquitoDunks®, suggesting that stress induction was the variable affecting metamorphosis. In the absence of environmental stressors, insecticidal Bti formulations do not

appear to affect tadpole growth when nominal application rates are used (Allgeier et al., 2018; Pauley et al., 2015; Schweizer et al., 2019). Allgeier et al. (2018) reported no statistical differences in body mass of *R. temporaria* tadpoles exposed to ice, sand, and liquid Bti formulations (VectoBac® WG). Schweizer et al. (2019) similarly found no significant difference in body mass of *R. temporaria* tadpoles exposed to Bti formulations of VectoBac® WG when compared to the negative control group. To account for potential nutritional effects of increased protein content in the Bti formulation, Schweizer et al. (2019) also included a rice protein control. However, amphibians exposed to the rice protein control developed a smaller body mass compared to Bti-treated tadpoles.

1.4.3 Bt-insecticide effects on histopathology

Few studies have investigated the effects of Bti on gut morphology in amphibians. Lajmanovich et al. (2015) examined the intestinal tissues of *L. latrans* tadpoles treated with 2.5, 5, 10, 20, and 40 mg/L of Introban® (Table 1.1). The authors reported that Bti-exposed tadpoles exhibited signs of inflammation in intestinal connective tissue and dilated blood vessels compared to controls. The authors also observed malformed erythrocytes (i.e., nuclear buds, pycnotic, kidney-shaped, and lobed nuclei) in circulating blood and an increased frequency of micronuclei in erythrocytes, with 2.5 mg/L and 10 mg/L of Introban® producing micronuclei frequencies of 2.21% and 2.74%, respectively. Overall, this study did not show a clear dose–response pattern, as tadpoles exposed to 20 mg/L of Introban® exhibited a micronuclei frequency of 0.42%. In contrast, Schweizer et al. (2019) tested the effects of 1, 10, and 100 mg/L of VectoBac® WG in *R. temporaria* tadpoles and, following a histopathological assessment, found no impacts on the basal lamina or the muscular layers under the epithelium of the tadpole gut.

1.4.4 Bt-insecticide effects on biomarker status

Several classic toxicological biomarkers have been studied in relation to the potential effects of Bti formulations. Lajmanovich et al. (2015) reported that 48 h of exposure to Introban® significantly increased the antioxidant activity of glutathione S-transferase (GST; at 10 and 20 mg/L) and catalase (at 20 mg/L) in the intestinal tissues of GS 26–30 *L. latrans* tadpoles. These findings are consistent with those of Allgeier et al. (2018), who also observed increases in detoxification and antioxidant enzymatic activity in *R. temporaria* tadpoles following Bti exposure. Allgeier et al. (2018) examined the effects of ice, sand, and liquid Bti formulations on the activities of GST, glutathione reductase (GR), and acetylcholinesterase (AChE), as these are

common indicators of toxicity. Tadpoles were exposed at GS 21–23 for the first application, at GS 24–28 for the second application, and at GS 36–40 for the third application (see Table 1.1). The authors reported that all treatments induced significant increases in GST (37–550%), GR (5–140%), and AChE (38–137%), suggesting that detoxification, antioxidant activity, and alterations in neuronal activity occurred in Bti-treated animals. The authors also reported increases in both GR and AChE after the second round of Bti application, but no significant differences in enzymatic activity were observed after the third application compared with the control.

In contrast, Schweizer et al. (2019) analyzed the activity of heat shock protein 70, AChE, and carboxylesterase (CbE) in GS 23–29 *R. Temporaria* tadpoles following Bti exposure and reported no statistically significant differences between the treatment and the control groups. Although the biomarkers employed in this study are typically used in toxicology, they do not necessarily link mechanistically to the observed effects. For example, if inflammation is suspected, the selected biomarkers should directly reflect this endpoint. There is a rich biomedical literature (Almradi et al., 2020; Eugene et al., 2020) on inflammatory bowel disease, interleukin responses, and related topics that can be applied to amphibian ecotoxicology, particularly for assessing gut inflammation. Modern approaches, such as transcriptomic profiling, could be used to identify novel biomarkers, as has been reported for numerous other environmental contaminants affecting amphibians (Gutierrez-Villagomez et al., 2019; Trudeau et al., 2020).

Table 1.1. Summary of the studies of *Bacillus thuringiensis* (Bt) var. *israelensis* (Bti) and var. *kurstaki* (Btk) on amphibians

Species	Bt formulation	Concentration (ITU/L)	Number of applications	Development stage	Exposure time	Studied variables	Effects	References
Common frog (*Rana temporaria*)	VectoBac® WG (ice granules formulation), VectoBac® WG (sand granules formulation), VectoBac® 12AS (liquid formulation)	[3,900; 7,800; 39,000], [3,237; 6,494; 32,370], [6,494; 12,988; 64,940]	1 application	GS 19–23	5 days	Medium GST, GR, and AChE activity	↗ 37% (GST) ↗ 5% (GR) ↗ 38% (AChE)	Allgeier et al. (2018)
			2 applications	GS 23–25	11 days		↗ 150% (GST) ↗ 140% (GR) ↗ 137% (AChE)	
			3 applications	GS 25–39	43 days		↗ 550% (GST) ↗ 24% (GR) No effect (AChE)	
			Unique application	GS 19–25	11 days		↗ 48% (GST) ↗ 88% (GR) No effect (AChE)	
	VectoBac® WG (ice granule formulation)	[3,900; 7,800; 39,000]	3 applications	GS 19–25	11 days	Mortality, time to metamorphose,	No effects	

	VectoBac® WG (sand granule formulation)	[3,237; 6,494; 32,370]	3 applications	GS 19–25	11 days	size, weight, condition index		
	VectoBac® 12AS (liquid formulation)	[6,494; 12,988; 64,940]	3 applications	GS 19–25	11 days			
	VectoBac® WG	[3,000; 30,000; 300,000]	2 applications	GS 23–29	11 days	Mortality, weight, intestine histopathology, Hsp70 activity, AChE activity, CbE activity	No effects	Schweizer et al. (2019)
South American spotted grassfrog (*Leptodactylus latrans*)	Introban®	[3,000; 6,000; 12,000; 24,000; 48,000]	Unique application	GS 26–30	48 h	NOEC	3,000 ITU/L	Lajmanovich et al. (2015)
						LOEC	6,000 ITU/L	
						LC_{50}	26,940 ITU/L	
						LC_{100}	48,000 ITU/L	
						Medium GST activity	↗ to 12,000 and 24,000 ITU/L	
						Medium CAT activity	↗ to 24,000 ITU/L	

					Micronuclei frequency	↗169% to 3,000 ITU/L and ↗ 234% to 12,000 ITU/L	
					Frequency of pyknosis	↗ 4,345% to 3,000 ITU/L and ↗ 2,581% to 6,000 ITU/L	
					Frequency of kidney-shaped nuclei	↗ 74% to 6,000 ITU/L and ↗ 100% to 12,000 ITU/L	
					Frequency of lobed nuclei	↗ 180% to 12,000 ITU/L	
					Intestine histopathology	Inflammatory infiltration of connective tissues under the epidermis and dilation of blood vessels (all treatments)	
	Introban®	[1,800–48,000]	GS 33	24 h	LC_{50}	24,612 ITU/L	Junges et al. (2017)

				NOEC	22,656 ITU/L
				LOEC	18,516 ITU/L
Argentine toad (*Rhinella arenarum*)	22,656			Distance travelled	↘
				Time immobile	↗
				Global activity	↘
	[1,800–48,000]		48 h	LC_{50}	23,100 ITU/L
				NOEC	15,000 ITU/L
				LOEC	22,656 ITU/L
	[1,800–48,000]	Unique application	24 h	LC_{50}	12,876 ITU/L
				NOEC	3,600 ITU/L
				LOEC	6,000 ITU/L
Bella vista toad (*Rhinella fernandezae*)	3,600			Distance travelled	No effect
				Time immobile	
				Global activity	
	[1,800–48,000]		48 h	LC_{50}	12,876 ITU/L
				NOEC	3,600 ITU/L
				LOEC	6,000 ITU/L
	[1,800–48,000]		24 h	LC_{50}	14,244 ITU/L

Menwig frog (*Physalaemus albonatus*)						NOEC	6,000 ITU/L	
						LOEC	9,960 ITU/L	
						Distance travelled	No effect	
						Time immobile		
						Global activity		
					48 h	LC_{50}	14,244 ITU/L	
						NOEC	6,000 ITU/L	
						LOEC	9,960 ITU/L	
Palmate newt (*Lissotriton helveticus*) and smooth newt (*Lissotriton vulgarus*)	VectoBac® WG	1,491	Unique application	N/A	9 weeks	Chironomid consumption (no predator present)	↘ 22%	Allgeier et al. (2019)
						Chironomid consumption (presence of predator)	↘ 57%	
						Survival rate (presence of predator)	↘ 27%	
						Size of ecological niche	↗ 30%	

						(no predator present)		
						Size of ecological niche (presence of predator)	↗ 70%	
						Size	No effect	
						Diet composition		
Gray tree frog (*Hyla versicolor*)	Mosquito Dunks® and Mosquito Bits®	11,156	1 application every 30 days (Mosquito Dunks®) 1 application every 14 days (Mosquito Bits®)	GS 25–46	ND	Survival rate (presence of predator)	↘ from Mosquito Dunks®	Pauley et al. (2015)
						Survival rate (no predator present)	No effect	
						Size		
						Weight		
						Time to metamorphosis		
	Monterrey® (Btk)	[0.0042 – 2.73 ml/L]	2 applications	GS 19	7 days	Hatching success	↘ to 2.73 ml/L	Weeks & Parris (2020)

Southern leopard frog (*Lithobates sphenocephalus*)			GS 25		Survival Survival LC_{50}	↘ 37.5% to 2.73 ml/L 1.81 ml/L	
Northern dusky salamander (*Desmoghatnus fuscus*), seal salamander (*Desmognathus monticoloa*), Allegheny Mountain dusky salamander (*Desmognathus orchophaeus*), red-backed salamander (*Plethodon cinereus*), and Northern slimy	Foray 48F® (Btk)	16 billion International Units/hectare	1 application in May 1997 and 1998 (2 applications total)	N/A	May–September 1997 and May–October 1998	Abundance and diet analysis	No effect	Raimondo et al. (2003)

salamander (*Plethodon glutinosus*)	

The papers selected for this study were peer-reviewed publications on the effects of Bti or Btk on amphibians. Given the rather limited number of publications, an assessment of the data quality of these papers was not conducted.

ITU, international toxic units; *GS*, Gosner stage; *NOEC*, no observed effect concentration; *LOEC*, lowest-observed effect concentration; LC_{50}, median lethal concentration; LC_{100}, absolute lethal concentration; *AChE*, acetylcholinesterase; *CAT*, catalase; *CbE*, carboxylesterase; *GST*, glutathione s-transferase; *GR*, glutathione reductase; *Hsp70*, heat shock protein 70; *N/A*, not applicable; *ND*, not disclosed.

1.5 Deltamethrin

In contrast to Bt insecticides, deltamethrin is a type II neurotoxic pyrethroid synthetically derived from chrysanthemum flowers (Pham et al., 1984). Deltamethrin products are used in agriculture and aquaculture to control pests and in cities to reduce biting insects (Arnberg et al., 2023; Haverinen & Vornanen, 2016). When applied in these operations, deltamethrin can easily enter non-target ecosystems and affect organisms residing in these habitats (Pimpão et al., 2008). Although pyrethroids are generally thought to have relatively low toxicity to vertebrates, deltamethrin formulations have been shown to induce neurotoxicity in various organisms ranging from insects to mammals (Pham et al., 1984). As a type II pyrethroid, deltamethrin primarily targets the central nervous system by binding to voltage-gated sodium channels in neurons, preventing their inactivation. This binding results in prolonged sodium channel opening, depolarization, and neuronal overexcitation, which can ultimately lead to paralysis and death (Clark et al., 1989). In addition to its effects on sodium channels, deltamethrin can also bind and inhibit gamma-aminobutyric acid-gated chloride channels, reducing inhibitory signalling in the nervous system and contributing to tremors and seizures characteristic of type II pyrethroid poisoning (Bloomquist et al., 1986). Deltamethrin toxicity can be induced by ingestion or contact and poses a particular risk to amphibians due to their highly permeable skin relative to that of mammals (Materna et al., 1995).

1.5.1 Deltamethrin affects tadpole survival

Research indicates that low concentrations of deltamethrin can induce mortality in various amphibian species. Macagnan et al. (2017) exposed *Physalaemis gracilis* GS 17–18 embryos and GS 24–25 larvae to Decis® 25 EC (containing 25 g/L of active ingredients; Bayer CropScience AG, Monheim am Rhein, Germany) or Cypermethrin (250 g/L of active ingredient; company not specified) for 96 h (See Table 1.2 for concentrations). The authors observed that both pyrethroids were more toxic to larvae than embryos. For deltamethrin, the 96 h LC_{50} was 76 µg a.i./L for embryos compared to 12.5 µg a.i./L for larvae. Similarly, cypermethrin exhibited a 96 h LC_{50} of 29,352.5 µg a.i./L for embryos and 1,252.5 µg a.i./L for larvae. The authors concluded that exposure to insecticides via the gills may increase direct uptake into the vascular system, thereby increasing mortality rates compared with embryos protected by a gelatinous coating. Vanzetto et al. (2019) also reported the effects of pyrethroids on *P. gracilis* tadpoles

across two experiments. In the first experiment, GS 25 tadpoles were exposed to 0.025–0.225 µg a.i./L of Decis® 25 EC for 168 h, and data were collected at 24 h intervals (Table 1.2). Although insecticide concentration did not significantly affect mortality, exposure time was significant. By the end of the exposure period, a mortality rate of 76% was observed in tadpoles, compared with 10% in controls. In the second experiment, the authors conducted a chronic exposure of 168 h with GS 20–25 hatchlings exposed to 12.5–500.0 µg a.i./L of Cipertrin® EC (250 g/L cypermethrin; Copyr, Milano, Italy). Similarly to deltamethrin, exposure time to cypermethrin significantly influenced mortality rather than insecticide concentration. By the end of the experiment, the hatchling mortality rate was significantly higher than that of controls at 27%. These findings are similar to those of Macagnan et al. (2017), in which both studies observed lower larval survival following pyrethroid exposure than embryos, likely due to their protective physical barrier. In Chapter 2, I further examine differences in LC_{50} estimates across deltamethrin products and species, elaborating on how developmental stages, product potencies, and study designs contribute to these differences in toxicity.

1.5.2 Deltamethrin affects behaviour, hatching success, growth, and metamorphosis

As with Bt insecticides, critical information is missing on the effects of deltamethrin on developmental endpoints in amphibians, and most studies to date have documented only swimming behaviour. Macagnan et al. (2017) tested acute concentrations of Decis® 25 EC and cypermethrin on *P. gracilis* embryos (from GS 17–18 until GS 19–20). The mobility of deltamethrin-exposed embryos was reduced at concentrations of 62.5 and 75 µg a.i./L within 24 h, and spasmodic contractions were observed at 96 h following exposure to 37.5 and 75 µg a.i./L. In cypermethrin-exposed embryos, all tested concentrations reduced mobility at 48 h, and 1,250 µg a.i./L induced spasmodic contraction in 60% of embryos at 96 h. The spasmodic contractions suggest that deltamethrin affected the nervous system, as similar effects have been recorded in mice, rats, rabbits, and guinea pigs exposed to deltamethrin (Soderlund et al., 2002). Vanzetto et al. (2019) also reported the effects of pyrethroids on the mortality, swimming activity, and oral morphology of *P. gracilis* tadpoles. They exposed GS 25 tadpoles to Decis® 25 EC or Cypertrin® EC, as well as hatchlings from GS 20–25 to Cypertrin® EC only, for 168 h (see Table 1.2 for concentrations). In exposed tadpoles, both pyrethroids significantly reduced swimming activity by 24 h of exposure, and spasmodic contractions were observed at 48 h of exposure. Similarly,

hatchlings also displayed reduced mobility within hours of exposure, and spasmodic contractions occurred at 48 h. While 74% of tadpoles exposed to deltamethrin developed oral deformities (such as malformations of the lower and upper jaw and denticles), this was insignificant at the $\alpha = 0.05$ level. In contrast, tadpoles and hatchlings exposed to cypermethrin displayed significant alterations in the mandible and denticles. As reported by Macagnan et al. (2017), deltamethrin likely produced neurotoxic effects, thereby decreasing swimming activity and inducing spasmodic contractions.

Interestingly, one study reports a stimulatory effect from the pyrethroid, permethrin. Permethrin is a type I pyrethroid and is generally considered less toxic than type II pyrethroids (such as deltamethrin and cypermethrin) because they lack a cyano group (Ahamad & Kumar, 2023). Junges et al. (2017) exposed *R. arenaum, R. fernandezae* and *P. albonatus* tadpoles for 48 h to the permethrin product Depe® (10% permethrin; Chemotecnica, Argentina) (see Table 1.2 for concentrations). The authors reported that *R. arenarum* and *P. albonatus* tadpoles exposed to no-observable-effect concentrations (NOEC) exhibited reduced overall swimming activity (consistent with the previously discussed studies), whereas the total global activity and distance moved of *R. fernandezae* significantly increased relative to controls. These contrasting behavioural results suggest that permethrin may induce species-specific responses. Collectively, these findings highlight the importance of investigating contaminant effects across multiple species to develop a robust understanding of lethal and sublethal impacts on nontarget organisms.

To my knowledge, no research has examined the impacts of deltamethrin on amphibian development or metamorphosis; however, two studies are available on permethrin. Boone (2008) exposed *Lithobates clamitans* and *Anaxyrus americanus* tadpoles to two permethrin insecticides via mesocosms. *A. americanus* tadpoles were exposed to 0–9 μg a.i./L of Cutter's Bug Free Back Yard® (2.5% permethrin; United Industries Incorporated, Missouri, USA), and *L. clamitans* tadpoles were exposed to 0–0.353 μg a.i./L of pure grade permethrin (98% purity; Bellefonte, PA, USA). Concentrations were chosen based on field levels, and tadpoles were exposed from GS 25 for 49 days (*A. americanus*) or 74 days (*L. clamitans*). Exposure to Cutter's Bug Free Back Yard® resulted in 97.5% mortality of *A. americanus* tadpoles before metamorphosis; therefore, other endpoints could not be analyzed. In contrast, survival of *L. Clamitans* tadpoles

was not affected by exposure to pure-grade permethrin, but tadpoles displayed significantly greater rates of development and larger mass by the end of the study. These findings further demonstrate that different formulations can produce varying biological responses. Although Cutter's Bug Free Back Yard® contains only 2.5% permethrin, achieving 9 µg a.i./L required a greater volume of product compared to the 98% pure grade permethrin. Despite its lower potency, this commercial formulation induced nearly 100% mortality in *A. americanus* tadpoles. This disparity may reflect species-specific sensitivity or the effect of undisclosed ingredients that influence toxicity. Given that permethrin and deltamethrin are both pyrethroids with similar modes of action, their toxicological effects are likely similar, though different formulations and exposure conditions may produce different outcomes.

1.5.3 Effects of deltamethrin on histopathology

Limited information is available on the effects of deltamethrin on amphibian gastrointestinal, liver, and kidney tissues. A study by Alnoaimi et al. (2020) orally administered 0.625, 1.25, and 2.5 µg a.i./kg of body weight of DEMOND® EC 2.5 (containing 25 g/L of deltamethrin; Safa Tarim, Konya, Turkey) to *Pelophylax ridibundus* adult frogs. After 96 h of exposure, frogs exhibited histopathological abnormalities in the liver, stomach, intestines, and kidneys, including abnormal cell morphology, edema, tissue disorganization, and necrosis. The liver also displayed pathological damage at all concentrations, including epithelial degeneration and congestion. The authors noted that liver tissue damage is particularly indicative of contaminant-induced toxicity, given the role of the liver in xenobiotic metabolism. The observed histopathological abnormalities were attributed to deltamethrin-induced oxidative stress, lipid peroxidation, and reduced antioxidant enzyme activity. Based on these findings, the authors concluded that the organ damage from deltamethrin was detrimental and irreversible. It is important to note that dosing was calculated based on body weight and was administered orally. Although this exposure route does not reflect typical environmental exposure, such applications provide accurate dosing and strengthen interpretations of toxic effects.

1.5.4 Effects of deltamethrin on biomarker status

Limited research has examined the effects of deltamethrin on biomarkers in amphibians, despite this insecticide's well-established neurotoxic properties. Lajmanovich et al. (2018) exposed GS 26–30 *R. arenarum* tadpoles to the TRISADA® insecticide (containing 1% (w/v)

deltamethrin and 0.33% (w/v) tetramethrin; Insumas, Argentina) for 48 h (specific concentrations of each pyrethroid are outlined in Table 1.2). At all exposure concentrations, the authors reported significant inhibition of up to 68% in AChE activity and 84% in CbE activity relative to controls. Because AChE hydrolizes acetylcholine to terminate nerve signals and transmissions at cholinergic synapses, its inhibition can disrupt neurotransmission and impair neuromuscular function (Colović et al., 2013; McHardy et al., 2017). CbEs function as detoxification enzymes with a high affinity for pyrethroids, protecting organisms through binding and sequestering these compounds (Denton, 2003). Consequently, inhibiting CbE activity may exacerbate toxic effects by reducing pyrethroid binding and detoxification (Lajmanovich et al., 2018).

In contrast, Aydin-Sinan et al. (2012) exposed Nieuwkoop and Faber stage 46 *Xenopus laevis* tadpoles to 1.1–27.4 µg a.i./L of Decis® EW 2.5 (25 g/L of deltamethrin; Bayer Crop Science, Leverkusen, Germany) for 24 h and observed a different pattern. At 4, 7.8, and 14 µg a.i./L, AChE activity was not inhibited but rather increased relative to controls. AChE is often used in toxicology studies, and inhibition is often documented in response to other insecticides such as organophosphates and carbamates (Fulton & Key, 2001). The authors suggested that because AChE is not the primary molecular target of deltamethrin insecticides, short-term exposure may not have been sufficient to suppress its activity (Aydin-Sinan et al., 2012). However, the authors observed a significant inhibition of acid phosphatase and aspartate aminotransferase, enzymes involved in cellular metabolism and anaerobic respiration (Vijayavel & Balasubramanian, 2007). These results indicate that, although cholinergic disruption was not observed, metabolic processes were altered.

Table 1.2. Summary of studies of the effects of deltamethrin and other pyrethroids on amphibians

Species	Product formulation	Concentration (µg a.i./L)	Number of applications	Development stage	Exposure time	Studied variables	Effects	References
Graceful dwarf frog (*Physalaemus gracilis*)	Decis® 25 EC	[12.5–125]	Unique application	GS 17–18	96 h	LC_{50}	76 µg a.i./L	Macagnan et al. (2017)
	Cypermethrin	[6,250–50,000]				LC_{50}	29,352.5 µg a.i./L	
	Decis® 25 EC	[0.25–75.0]		GS 24–25		LC_{50}	12.5 µg a.i./L	
	Cypermethrin	[500–2,000]				LC_{50}	1,252.5 µg a.i./L	
	Decis® 25 EC	[12.5–75]		GS 17–18	Until embryos reached GS 19–20	Mobility	↘ at 62.5 µg a.i./L within 24 h	
						Spasmodic contractions	↗ at 37.5 µg a.i./L	
	Cypermethrin	[1,250–3,000]				Mobility	↘ at 1,250 µg a.i./L	
						Spasmodic contractions	↗ at 1,250 µg a.i./L	
	Decis® 25 EC	[0.025–0.225]	Unique application	GS 25	168 h	NOEC	0.100 µg a.i./L	Vanzetto et al. (2019)
						LOEC	0.150 µg a.i./L	
						MATC	0.125 µg a.i./L	
						Swimming activity	↘ by 24 h	

			Spasmodic contractions	↗ by 48 h
			Oral morphology	No effects
			NOEC	12.5 µg a.i./L
			LOEC	17.5 µg a.i./L
			MATC	15.0 µg a.i./L
Cypertrin® EC (cypermethrin)	[2.5–25]	GS 25	Swimming activity	↘ by 24 h
			Spasmodic contractions	↗ by 48 h
			Oral morphology	Alterations to the mandible and denticles
			NOEC	25 µg a.i./L
			LOEC	125 µg a.i./L
			MATC	75 µg a.i./L
Cypertrin® EC (cypermethrin)	[12.5–500]	GS 17–18	Swimming activity	↘ within 24 h
			Spasmodic contractions	↗ by 48 h
			Oral morphology	↗ at 250 µg a.i./L

Common frog (*Rhinella arenarum*)	TRISADA® (1% deltamethrin and 0.33% tetramethrin)	C1 = 0.0003125% v/v [3.125 µg a.i./L deltamethrin; 10.31 µg a.i./L tetramethrin] C2 = 0.000625% v/v [62.5 µg a.i./L deltamethrin; 20.63 µg a.i./L tetramethrin C3 = 0.00125% v/v [125 µg a.i./L deltamethrin; 41.25 µg a.i./L tetramethrin]	Unique application	GS 26–30	48 h	NOEC	Deltamethrin = 3.1 µg a.i./L Tetramethrin = 1.0 µg a.i./L	Lajmanovich et al. (2018)
						LOEC	Deltamethrin = 6.2 µg a.i./L Tetramethrin = 2.0 µg a.i./L	
						LC_{50}	Deltamethrin = 12.5 µg a.i./L Tetramethrin = 4.1 µg a.i./L	
						AChE	↘ at C3	
						CbE	↘ at C2	
						Swimming activity	↘ at C1	
					24 h	NOEC	Deltamethrin = 12.5 µg a.i./L Tetramethrin = 4.1 µg a.i./L	

		C4 = 0.0025% v/v [250 µg a.i./L deltamethrin; 82.5 µg a.i./L tetramethrin] C5 = 0.005% v/v [500 µg a.i. deltamethrin; 165 µg a.i./L tetramethrin]				LOEC	Deltamethrin = 25 µg a.i./L Tetramethrin = 8.2 µg a.i./L
						LC_{50}	Deltamethrin = 28 µg a.i./L Tetramethrin = 9.2 µg a.i./L
Argentine toad (*Rhinella arenarum*)	Depe® (permethrin)	[0.3–50]	Unique application	GS 33	24 h	LC_{50}	4.7 µg a.i./L
						NOEC	0.6 µg a.i./L
						LOEC	1.2 µg a.i./L
		0.6				Distance travelled	↘
						Time immobile	↗
						Global activity	↘
		[0.3–50]			48 h	LC_{50}	3.5 µg a.i./L
						NOEC	0.3 µg a.i./L
						LOEC	0.6 µg a.i./L

Bella vista toad (*Rhinella fernandezae*)		[0.3–50]			24 h	LC_{50}	5.6 µg a.i./L	
						NOEC	1.2 µg a.i./L	
						LOEC	2.5 µg a.i./L	
		1.2				Distance travelled	↗	
						Time immobile	No effect	
						Global activity	↗	
		[0.3–50]			48 h	LC_{50}	2.8 µg a.i./L	
						NOEC	0.6 µg a.i./L	
						LOEC	1.2 µg a.i./L	
Menwig frog (*Physalaemus albonatus*)		[0.3–50]			24 h	LC_{50}	0.9 µg a.i./L	
						NOEC	0.3 µg a.i./L	
						LOEC	0.6 µg a.i./L	
		0.3				Distance travelled	No effect	
						Time immobile		
						Global activity		
		[0.3–50]			48 h	LC_{50}	0.7 µg a.i./L	
						NOEC	0.6 µg a.i./L	
						LOEC	1.2 µg a.i./L	
	Permethrin	0.353	Unique application	GS 25	49 days	Survival	No effect	Boone (2008)
						Weight	↗	

Green frog (*Lithobates clamitans*)						Development rate	↗	
American toad (*Anaxyrus americanus*)	Cutter's Bug Free Back Yard® (permethrin)	9.0			74 days	Survival	Survival rate of 2.5%	
Marsh frog (*Pelophylax ridibundus*)	DEMOND® EC 2.5	0.625, 1.25, and 2.5 µg a.i./g of bw	Unique application (oral gavage)	Adult	96 h	Survival	No effects	Alnoaimi et al. (2020)
						Liver	↗ pathological damage at 0.625 µg a.i./kg bw	
						Gastrointestinal tract	Tissue damage in the esophagus, stomach, and intestine	
						Kidney	↗ pathological damage at 0.625 µg a.i./kg bw	
						Genotoxicity	↗ erythrocytic nuclear abnormalities at 0.625 µg a.i./kg bw	

African clawed frog (*Xenopus laevis*)	Decis® EW 2.5	[1.1–27.4]	Unique application	NF 46	24 h	Biomarker status	↘ GST ↘ ACP ↘ AST ↗ AChE No effect on CbE and LDH	Aydin-Sinan et al. (2012)
		[1.1–38.4]	Renewal every 24 h		168 h	LC_{50}	6.26 µg a.i./L	

The papers selected for this study met the criterion of being peer-reviewed and published and addressed the effects of deltamethrin and other pyrethroids on amphibians. Given the rather limited number of publications, an assessment of the data quality of these papers was not conducted.

a.i., active ingredients; *GS*, Gosner stage; *NF*, Nieuwkoop and Faber; *bw*, body weight; *NOEC*, no observed effect concentration; *LOEC*, lowest-observed effect concentration; *LC_{50}*, median lethal concentration; *MATC*, maximum acceptable toxicant concentration, *AChE*, acetylcholinesterase; *ACP*, acid phosphatase; *AST*, aspartate aminotransferase; *CbE*, carboxylesterase, *GST*, glutathione s-transferase; *LDH*, lactate dehydrogenase.

1.6 General conclusions on Bt and deltamethrin insecticides

Data on the effects of Bti, Btk, and deltamethrin on amphibians are critically lacking. Only a few studies have assessed their effects on survival, growth, hatching success, metamorphosis, histopathology, and biomarker status. The diversity in Bti and Btk formulations and exposure regimes, species, and developmental stages studied means that consensus views cannot yet be proposed. Although more data are available on the effects of deltamethrin in vertebrates, conclusions about amphibians are often drawn from other pyrethroids, and the results are contradictory. Nevertheless, across the studies examined here, these insecticides have both lethal and sublethal effects. Chronic, environmentally relevant exposures that assess hatching success, development, and metamorphosis are of immediate importance. Mesocosm studies rigorously testing both direct and indirect food web interactions at environmentally relevant levels of Bti, Btk, and deltamethrin have yet to be conducted. As with numerous other pesticide formulations, those containing the described Bti and Btk toxins contain a host of other compounds, such as mixtures of proteins, spores, and proprietary additives. Deltamethrin products also have undisclosed formulations that extend beyond the percentage of active ingredients. It is challenging but necessary to develop appropriate controls to determine which effects on amphibians are attributable solely to the active ingredient, rather than to additives in commercial products. The establishment of physiologically relevant biomarkers and standardized analytical methods to quantify Cry and Cyt proteins is of paramount importance for advancing collective risk assessment of Bti and Btk insecticides. This information will mitigate potential effects on amphibians in wetland ecosystems. To reduce these knowledge gaps, I determined LC_{50} values for three North American species (Chapter 2) and assessed sublethal effects on metabolic and physiological endpoints (Chapters 3 and 4), providing possible mechanistic insights into how these insecticides may affect amphibian health.

1.7 Ethics statement

All experiments were approved by the University of Ottawa Animal Care Protocol Review Committee and performed in accordance with the guidelines established by the Canadian Council on Animal Care for the ethical use of animals in research.

Chapter 2: Toxicity of *Bacillus thuringiensis israelensis* and deltamethrin in three anuran species

This chapter was adapted from:

Empey, M.A., Reyes, Y.M., Ethier, J.P., Rosa, C.G.T., & Trudeau, V.L. (2025). Toxicity of *Bacillus thuringiensis israelensis* and deltamethrin in three anuran species. *Environmental Pollution*, *382*, 126702. https://doi.org/10.1016/j.envpol.2025.126702

Study contributions: M.A.E. conceived of and conducted experiments, analyzed data, and wrote the manuscript; Y.M.R. helped design and conduct experiments, helped with analysis, and reviewed the manuscript; J.P.E provided research animals and reviewed the manuscript; C.G.T. aided with experiments and reviewed the manuscript; V.L.T. conceived of and helped design experiments, revised the manuscript, and acquired funding for the study.

Abstract

Insecticides aid in eliminating crop pests and mosquito-borne diseases but can harm nontarget organisms. The insecticidal Gram-positive soil bacterium *Bacillus thuringiensis israelensis* (Bti) produces toxic proteins that are effective against dipteran insects, such as mosquitoes and black flies. Deltamethrin is an insecticide used to eliminate various invertebrates due to its neurotoxic properties. Despite their widespread use, there is limited toxicity data for these insecticides on nontarget organisms, especially amphibians. Median lethal concentrations (LC_{50}) of Bti and deltamethrin products for three North American amphibian species were determined. Chorus frog (*Pseudacris maculata*), leopard frog (*Lithobates pipiens*), and wood frog (*Lithobates sylvaticus*) tadpoles were exposed to VectoBac® 200G and deltamethrin for 96 h. The LC_{50} values for VectoBac® 200G were estimated to be 513,000 ± 1.15, 78,860 ± 1.10, and 525,363.4 ± 1.13 ITU/L for chorus, leopard, and wood frog tadpoles, respectively. The LC_{50} values for deltamethrin were estimated to be 2.69 ± 1.06, 7.30 ± 1.05, and 1.15 ± 1.06 µg a.i./L for chorus, leopard, and wood frog tadpoles, respectively. High concentrations of VectoBac® 200G may cause mortality, whereas low concentrations of deltamethrin induced mortality in all species. Effects on tadpole growth were variable. High concentrations of VectoBac® 200G increased total length in leopard and wood frog tadpoles, while deltamethrin often reduced total tadpole length. These LC_{50} values and data from literature were used to construct species sensitivity distributions (SSDs) of Bti and deltamethrin products. Analysis of SSDs revealed that amphibian species were relatively tolerant of Bti products and susceptible to deltamethrin exposure.

2.1 Introduction

The impact of insecticides on amphibians includes direct toxic effects and indirect effects through the elimination of invertebrate food sources (Brühl et al., 2020; Trudeau et al., 2020). Few studies have investigated the impact of *Bacillus thuringiensis* var. *israelensis* (Bti) and deltamethrin insecticides on amphibians. The Bti bioinsecticide controls Diptera populations, such as mosquitoes and black flies (Margalit, 1990). It is a Gram-positive bacterium that eliminates insects through Cry4Aa, Cry4Ba, Cry11Aa, and Cyt1Aa toxins (Ben-Dov, 2014). Some formulations, such as VectoBac® 200G, use granulated corn as a carrier to spread over water for larvae to ingest (World Health Organization [WHO], 2012). Once ingested, proteins are solubilized in the gut, releasing Cry and Cyt toxins (Rukmini et al., 2000; Vachon et al., 2012). These toxins activate, oligomerize, and form pores in the gut wall, disrupting digestion and causing death. Studies have shown that Bti can be lethal to nontarget dipterans, such as chironomids, which are essential to wetland food webs (Brühl et al., 2020). Data on the effects of Bti formulations on amphibians are unclear, with contrasting results. Some studies found no impact on amphibians, while others reported mortality, developmental effects, decreased hatching success, and histopathology (Empey et al., 2021). For example, Allgeier et al. (2018) found no mortality difference in *Rana temporaria* tadpoles exposed to VectoBac® WG and VectoBac® 12AS, whereas Lajmanovich et al. (2015) reported approximately 20% mortality in *Leptodactylus latrans* tadpoles exposed to Introban® at 24,000 ITU/L. Deltamethrin is a neurotoxic pyrethroid used in agriculture and aquaculture to eliminate pests (Arnberg et al., 2023; Brühl et al., 2013). It targets the central nervous system, binding to sodium channels and preventing them from returning to an inactive state, leading to paralysis and death (Clark et al., 1989). Deltamethrin can also inhibit gamma-aminobutyric acid-gated chloride channels, causing seizures, a hallmark of type II pyrethroid poisoning (Bloomquist et al., 1986). Deltamethrin toxicity can occur via ingestion or contact, posing a hazard to amphibians due to their permeable skin (Materna et al., 1995). Fewer than a dozen studies have tested the effects of deltamethrin on amphibians. Macagnan et al. (2017) exposed *Physalaemus gracilis* embryos and larvae to Decis® 25 EC, finding significant mortality and reduced mobility. Vanzetto et al. (2019) observed a 76% mortality rate in tadpoles exposed to deltamethrin, which significantly affected swimming activity. In this study, we address data gaps on the effects of Bti and deltamethrin products on North American amphibians. We determined the 96 h median lethal concentration (LC_{50}) of the

Bti product, VectoBac® 200G and deltamethrin for chorus frog (*Pseudacris maculata*), leopard frog (*Lithobates pipiens*), and wood frog (*Lithobates sylvaticus*) tadpoles. The potential effect of these short exposures on total body length was also examined. Species sensitivity distributions (SSDs) for Bti and deltamethrin products were constructed using LC50 values calculated in this study and those reported in the literature.

2.2 Materials and Methods

2.2.1 Animals

Chorus, leopard, and wood frog tadpoles (Gosner Stage 25) were used for median lethal concentration acute assays. In April 2021–2023, leopard and wood frog eggs were collected near Kemptville, Ontario, in a location with no direct insecticide application. Adults were also collected for breeding using the AMPHIPLEX method (Trudeau et al., 2010) for additional egg clutches. Chorus frog tadpoles were obtained from a colony established at the University of Ottawa (Ethier et al., 2024). Tadpoles were raised in a temperature- and light-controlled (12L:12D) room. Tadpoles were housed in 20 L glass aquariums with a maximum density of fifty tadpoles per tank. The average water temperature ranged from 18 to 20ºC. Starting at GS 20, tadpoles were fed a quarter teaspoon of Sera® Micron Nature powdered fish fry daily. All experiments were approved by the University of Ottawa Animal Care Committee (Protocol # BL-2206).

2.2.2 Bti and deltamethrin insecticides

The effects of Bti were tested using the commercial formulation VectoBac® 200G (Valent Canada, Guelph, ON). This product consists of corn granules coated with Bti bacterial crystals and spores. Bti-free corn granules were used as negative controls. Treatments were supplemented with Bti-free corn granule controls to ensure uniform exposure to the corn vehicle, with the only difference being the microbial agent concentration. Unopened bags of VectoBac® 200G (batch number 356–320–N830) and blank corn (batch number 297–171– W900) were obtained from GDG Environment (Trois-Rivières, Québec). Deltamethrin (95% purity) was purchased from AK Scientific Inc. (Union City, California; CAS# 52918–63–5). Dimethylsulfoxide (DMSO), the vehicle for deltamethrin, was used as a negative control. Concentrations are expressed in international toxic units (ITU)/L for Bti products and µg of active ingredient (a.i.)/L for deltamethrin products. For example, 50 mg/L of VectoBac® 200G (potency of 200 ITU/mg)

results in a total concentration of 10,000 ITU/L. Similarly, 1 µg/L of deltamethrin (95% purity) corresponds to 0.95 µg a.i./L in the product.

2.2.3 Validation of VectoBac® 200G and deltamethrin

Water samples inoculated with deltamethrin were analyzed to determine measured concentrations. Samples (n = 3) consisted of 25 ml of 250,000 µg a.i./L stock solution and water controls from tadpole facilities. Samples were immediately frozen at -20°C after inoculation to prevent deltamethrin degradation. Frozen samples were sent to MB Laboratories Ltd. (Sidney, BC) for analysis by liquid chromatography mass spectrometry, SOP# S11-LC/MS/MS Pesticides. See S1 for the method of analysis and Table S1.1 for water analysis results. To prepare solutions for Bti validations, VectoBac® 200G and blank corn were weighed, added to 1 L glass water bottles, and shaken for 24 h at room temperature. Bottles were inverted ten times, diluted 20-fold, and 100 µl aliquots of 1,400, 3,000, 5,000, and 10,000 ITU/L of VectoBac® 200G were plated (n = 3 per concentration) on Luria Broth Agar plates. Controls included non-Bti-coated granulated corn and a water control from our aquatic facility. Colony forming units (CFUs) were counted after 48 h of incubation at 37ºC. Insecticide effectiveness was also tested against target organisms. Larval mosquitoes (instar 1 to 4) were collected from a 72 L Tupperware bin containing pond water, hay, and manure (Trois-Rivières, Québec, July 2022) and exposed to graded concentrations of VectoBac® 200G and deltamethrin in transparent 10-well plates (n = 10). VectoBac® 200G exposure consisted of 700, 1,400, 3,000, 5,000, 10,000, and 20,000 ITU/L, pond water control, facilities water control, and blank corn control. Wells were inoculated with Bti treatments by adding VectoBac® 200G concentrations and a negative corn control to 1 L glass water bottles and placed on a shaker for 12 h at room temperature. Deltamethrin exposures comprised 0.24, 0.48, 0.95, 1.9, 2.85, and 3.8 µg a.i./L, pond water control, facilities water control, and DMSO solvent control. Mortality was observed after 24 h of exposure. Statistical analysis was performed by ANOVA with Tukey's post-hoc test to determine differences between treatments.

2.2.4 Acute toxicity tests of VectoBac® 200G and deltamethrin to chorus, leopard, and wood frog tadpoles

The LC_{50} estimates were determined through 96 h acute toxicity tests measuring the survival of chorus, leopard, and wood frog tadpoles exposed to VectoBac® 200G or deltamethrin. Tadpoles were placed in a 1 L glass mason jar containing 800 mL of water mixed with each treatment. Each jar contained ten tadpoles, and each treatment was replicated five times. Dead

tadpoles were removed every 24 h. Tested concentration ranges are based on pilot trials, in which some species tolerated higher insecticide concentrations than others. Chorus frog tadpoles were exposed to 20,000, 50,000, 100,000, 200,000, 300,000, 400,000, 500,000, and 600,000 ITU/L of VectoBac® 200G. Leopard frog tadpoles were exposed to 10,000, 20,000, 50,000, 100,000, 200,000, and 400,000 ITU/L of VectoBac® 200G. Wood frog tadpoles were exposed to 51,200, 102,400, 204,800, 409,600, and 819,200 ITU/L of VectoBac® 200G. Negative controls consisted of facility water and non-Bti-contaminated granulated corn. Chorus frog tadpoles were exposed to 0.95, 1.9, 2.85, 3.8, 4.75, and 5.7 µg a.i./L of deltamethrin. Leopard frog tadpoles were exposed to 0.48, 0.95, 1.43, 1.9, 2.85, 4.75, 7.13, and 9.5 µg a.i./L of deltamethrin. Wood frog tadpoles were exposed to 0.95, 1.19, 1.43, 2.4, and 4.75 µg a.i./L of deltamethrin. Negative controls consisted of facility water and DMSO. The total length (snout to tail) of surviving tadpoles was measured at the end of the exposure period. Surviving tadpoles were euthanized by immersion in 5 g/L of tricaine (MS-222) buffered with sodium bicarbonate to obtain a neutral pH, placed on a 1-cm grid paper, and photographed from above. The total length was measured with ImageJ (Schneider et al., 2012) using graph paper as a standardized ruler.

2.2.5 Statistical analysis

To calculate survival and LC_{50} estimates for tadpoles exposed to VectoBac® 200G and deltamethrin, the results were log-transformed and analyzed using non-linear regression in R statistical software (version 4.3.1). Survival was assessed using a Generalized Linear Models (GLM) with a binomial distribution and Tukey's post hoc test, with an α-value of 0.05. A GLM with a Gaussian distribution and Tukey's post hoc test was used to assess differences in tadpole total length between groups. The model fit was determined by plotting residuals against fitted values to confirm homogeneity and using a histogram of residuals to check normality.

2.2.6 Construction of species sensitivity distributions for VectoBac® 200G and deltamethrin insecticidal products

A comprehensive literature search was conducted to identify published LC_{50} values for organisms exposed to Bti and deltamethrin products. Data from articles were accepted if the product name, test species, and duration of insecticide exposure were clearly stated. While temperature can potentially influence insecticide toxicity, relevant details about the temperatures used in acute experiments were often unavailable in the surveyed literature. In many cases, we were unable to find this specific information, precluding any informative comparisons of

temperature. To be included in our analysis, the LC_{50} values must have been obtained from a single product and not mixed with other insecticides. Accepted reported values were those that could be converted to ITU/L for Bti products or µg a.i./L for deltamethrin products. To enable direct comparisons across insecticide products and formulations, we standardized all LC_{50} values to the active-ingredient concentration of each commercial product. Specifically, LC_{50} values reported in literature were multiplied by the proportion of active ingredients in the product. This conversion allowed toxicity to be expressed in terms of active ingredients alone, providing a more accurate comparison of insecticide potency across compounds with varying formulations. The SSDs were constructed using R software (version 4.3.1) "ssdtools" package (Delgarno, 2021). Distributions such as normal, logistic, Weibull, and burr were fitted to the datasets, and a goodness-of-fit test was used to select the appropriate distributions. The hazard concentration at the 5th percentile (HC5) was calculated from each SSD using the "ssdtools" package. The HC5 is a method for estimating the sensitivity of various species to chemical contaminants (Spurgeon et al., 2020). This value represents the hazardous concentration that would affect 5% of the species in a dataset. See Table S1.2 and S1.3 for accepted publications and unit conversions of Bti and deltamethrin products, respectively.

2.3 Results

2.3.1. Validation of VectoBac® 200G and deltamethrin viability

Water samples from tadpole facilities were used as blank controls or were inoculated with deltamethrin for analytical testing. No deltamethrin was detected in tadpole facility water samples (Table S1.1). The average measured deltamethrin concentration in the stock solution samples was 1.04% higher than the nominal concentration (See Table S1.1). This difference was taken into consideration in calculating LC_{50} values. Agar plates were inoculated with concentrations of VectoBac® 200G or controls to validate bacterial viability. No bacteria grew in the negative control plates (Fig. 2.1A–B). Concentrations of 1,400, 3,000, 5,000, and 10,000 ITU/L of VectoBac® 200G produced 89, 150, 139, and 307 CFU, respectively (Fig. 2.1C–F), and the total cell concentrations were calculated to be 17,867, 27,800, 30,000, and 61,467 cells/ml, respectively (Fig. 2.1G). Concentrations that encompassed environmentally relevant applications of VectoBac® 200G and deltamethrin were tested against mosquito larvae. Concentrations of 1,400, 3,000, 5,000, 10,000, and 20,000 ITU/L of VectoBac® 200G significantly affected survival ($p < 0.001$), where less than 50% of larvae survived after 24 h of exposure (Fig. 2.2A).

Pond water, facility water, blank corn, and 700 ITU/L of VectoBac® 200G had no effects on larval survival. Concentrations of 0.48, 0.95, 1.9, 2.85, and 3.8 µg a.i./L of deltamethrin significantly decreased survival ($p < 0.05$), while pond water, facility water, DMSO, and 0.24 µg a.i./L deltamethrin had no effects (Fig. 2.2B).

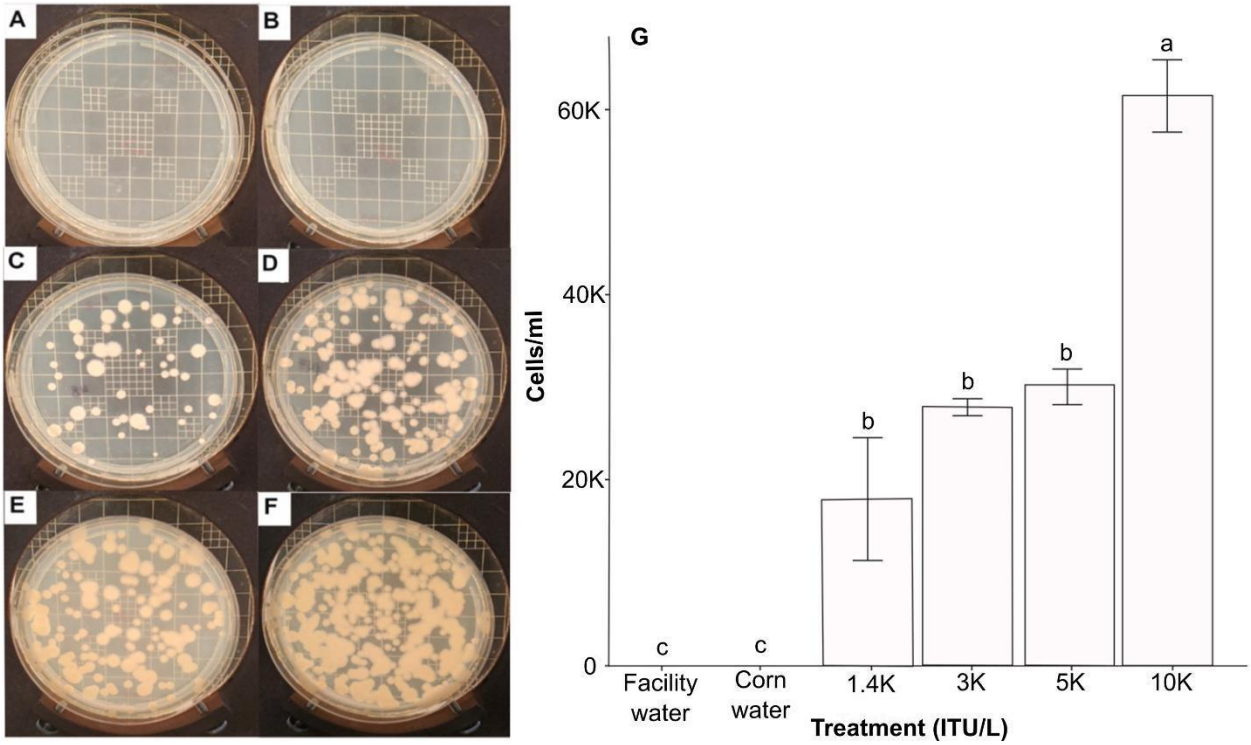

Figure 2.1. Colony-forming units of *Bacillus thuringiensis israelensis* on LB agar medium. **(A)** Facilities water control; **(B)** Blank corn control; **(C)** 1,400 ITU/L VectoBac® 200G; **(D)** 3,000 ITU/L VectoBac® 200G; **(E)** 5,000 ITU/L VectoBac® 200 G; **(F)** 10,000 ITU/L VectoBac® 200G; **(G)** Number of colony-forming units of facility water control, blank corn control, and concentrations of 1,400 (1.4K), 3,000 (3K), 5,000 (5K), and 10,000 (10K) ITU/L of VectoBac® 200G on LB agar medium. Values are presented as mean ± SEM (n = 3). Statistical analysis by ANOVA with Tukey's post-hoc test. Letters (a, b, c) denote significant differences between groups ($p < 0.05$).

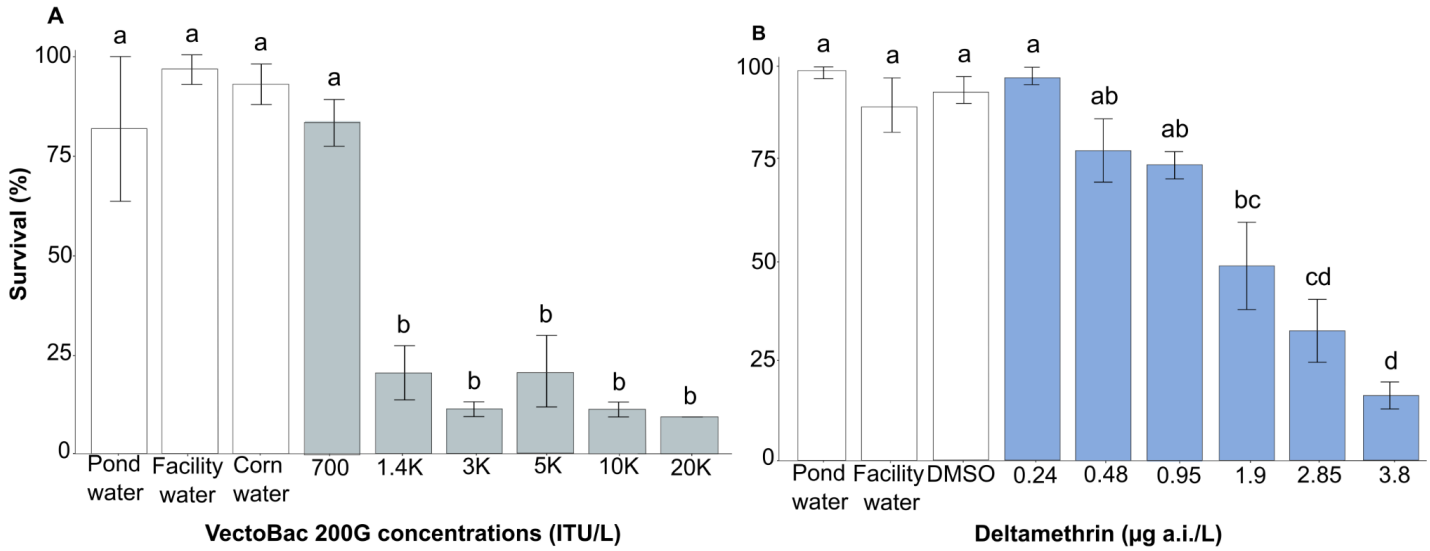

Figure 2.2. The percent survival of instar 1–4 mosquito larvae exposed for 24 h to negative controls and VectoBac® 200G or deltamethrin. (A) Mosquito larvae exposed to pond water, facility water, blank corn, and 700, 1,400 (1.4K), 3,000 (3K), 5,000 (5K),10,000 (10K), and 20,000 (20K) ITU/L of VectoBac® 200G. **(B)** Mosquito larvae exposed for 24 h to pond water, facility water, DMSO, and 0.24, 0.48, 0.95, 1.9, 2.85, and 3.8 µg a.i./L of deltamethrin. A GLM and Tukey's post-hoc test was used to determine differences in survival between groups. Letters (a, b, c,d) denote significant differences between groups ($p < 0.05$). Data are presented as the mean ± SEM (n = 10).

2.3.2. Median lethal concentrations of chorus, leopard, and wood frog tadpoles exposed to VectoBac® 200G and deltamethrin

The $LC_{50–96h}$ of chorus, leopard, and wood frog tadpoles exposed to VectoBac® 200G was estimated to be 513,000 ± 1.15, 78,860 ± 1.10, and 525,363.4 ± 1.13 ITU/L, respectively (Table 1). The $LC_{50–96h}$ of chorus, leopard, and wood frog tadpoles exposed to deltamethrin was calculated to be 2.69 ± 1.06, 7.30 ± 1.05, and 1.15 ± 1.06 µg a.i./L, respectively (see Table 2.1).

Table 2.1. The median lethal concentration of GS 25 chorus, leopard, and wood frog tadpoles exposed to VectoBac® 200G and deltamethrin insecticides for 96 h.

INSECTICIDE	Chorus frog tadpole	Leopard frog tadpole	Wood frog tadpole	Recommended application rate
VectoBac® 200G	513,000 ± 1.15 ITU/L	78,860 ± 1.10 ITU/L	525,363.4 ± 1.13 ITU/L	2,000-6,600 ITU/L

Deltamethrin	2.69 ± 1.06 µg a.i./L	7.30 ± 1.05 µg a.i./L	1.15 ± 1.06 µg a.i./L
			0.5–1.5 g deltamethrin/ha

a.i., active ingredient; ha, hectare; *ITU,* international toxic units

2.3.3. The effects of VectoBac® 200G and deltamethrin on the total body length of chorus, leopard, and wood frog tadpoles

Facility water, blank corn, and DMSO controls did not affect total tadpole length (Fig. 2.3 and 2.4). No concentrations of VectoBac® 200G affected the total length of chorus frog tadpoles (Fig. 2.3A). Chorus frog tadpoles exposed to 0.95 µg a.i./L of deltamethrin were significantly shorter, while 5.7 µg a.i./L produced significantly longer tadpoles ($p < 0.05$) (Fig. 2.4A). Leopard frog tadpoles exposed to 10,000, 20,000, and 50,000 ITU/L of VectoBac® 200G were significantly longer than controls ($p < 0.05$) (Fig. 2.3B). Leopard frog tadpoles exposed to deltamethrin concentrations of 0.95, 1.43, 1.9, 2.85, 4.75, and 7.13 µg a.i./L were significantly shorter ($p < 0.05$) (Fig. 2.4B). Wood frog tadpoles exposed to VectoBac® 200G concentrations of 51,200, 102,400, 204,800, and 409,600 ITU/L were significantly longer ($p < 0.01$) (Fig. 2.3C). Wood frog tadpoles exposed to 0.95 µg a.i./L were significantly longer, while the remaining concentrations produced significantly shorter tadpoles ($p < 0.01$) (Fig. 2.4C).

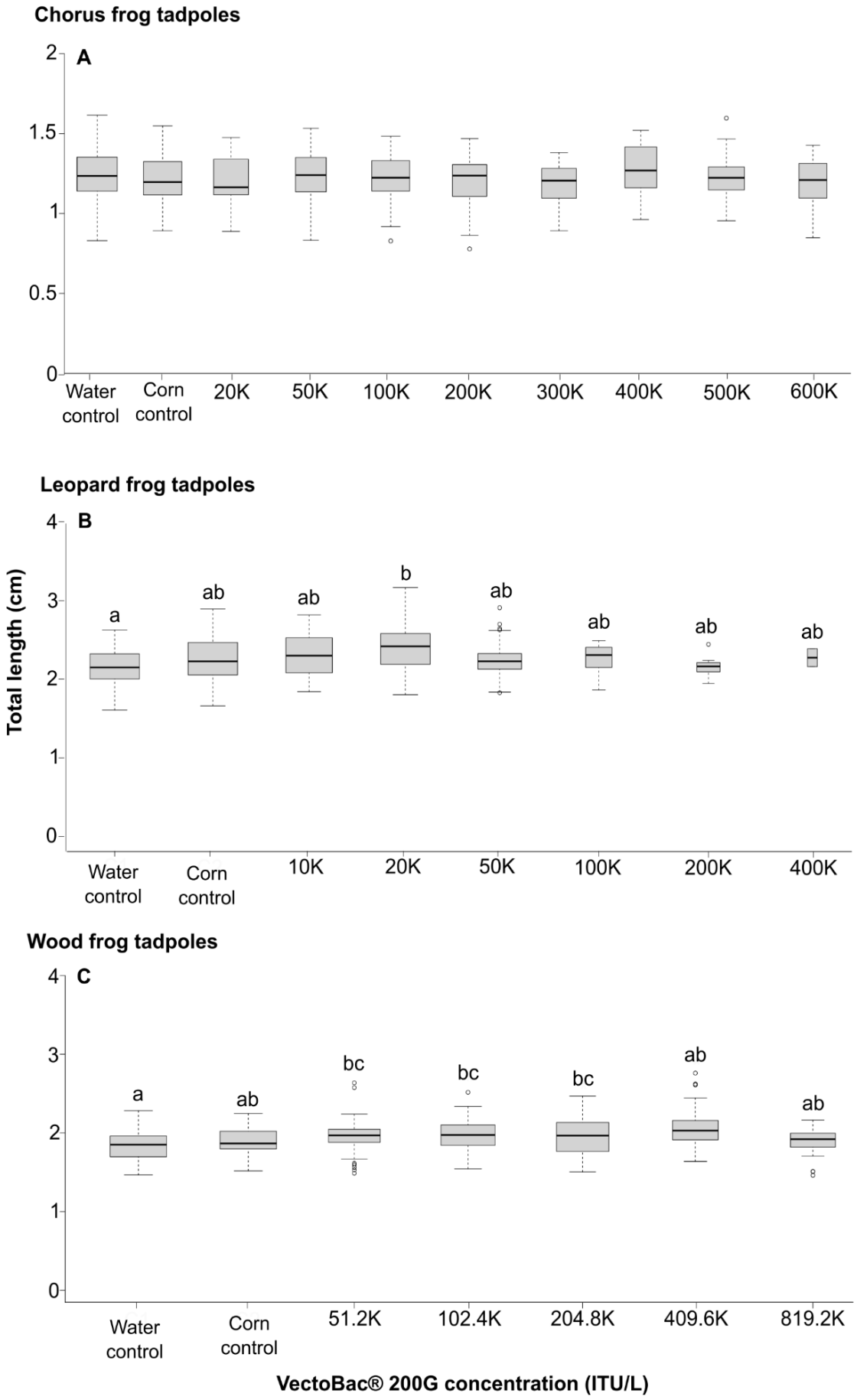
Chorus frog tadpoles
A
2
1.5
1
0.5
0
Water control
Corn control
20K
50K
100K
200K
300K
400K
500K
600K
Leopard frog tadpoles
B
Total length (cm)
4
3
2
1
0
a
ab
ab
b
ab
ab
ab
ab
Water control
Corn control
10K
20K
50K
100K
200K
400K
Wood frog tadpoles
C
4
3
2
1
0
a
ab
bc
bc
bc
ab
ab
Water control
Corn control
51.2K
102.4K
204.8K
409.6K
819.2K
VectoBac® 200G concentration (ITU/L)

Figure 2.3. Effect of 96 h acute exposure to controls and VectoBac® 200G on the total length of GS 25 chorus, leopard, and wood frog tadpoles. (A) Chorus frog tadpoles exposed to water control, corn control, 20,000 (20K), 50,000 (50K), 100,000 (100K), 200,000 (200K), 300,000 (300K), 400,000 (400K), 500,000 (500K), and 600,000 (600K) of VectoBac® 200G; (B) Leopard frog tadpoles exposed to water control, corn control, 10,000 (10K), 20,000 (20K), 50,000 (50K), 100,000 (100K), 200,000 (200K), and 400,000 (400K) of VectoBac® 200G; (C) Wood frog tadpoles exposed to water control, corn control, 51,200 (51.2K), 102,400 (102.4K), 204,800 (204.8K), 409,600 (409.6K), 819,200 (819.2K) of VectoBac® 200G. The bolded bar represents the mean; the box represents the lower and upper quartiles; the whiskers depict the minimum and maximum data values; the thickness of the box reflects the remaining sample size at 96 h; and circles represent outliers. A GLM and Tukey's post-hoc test was used to determine differences between groups. Letters (a, b, c) denote significant differences between groups ($p < 0.05$). Note that the X- and Y-axis scales differ between species.

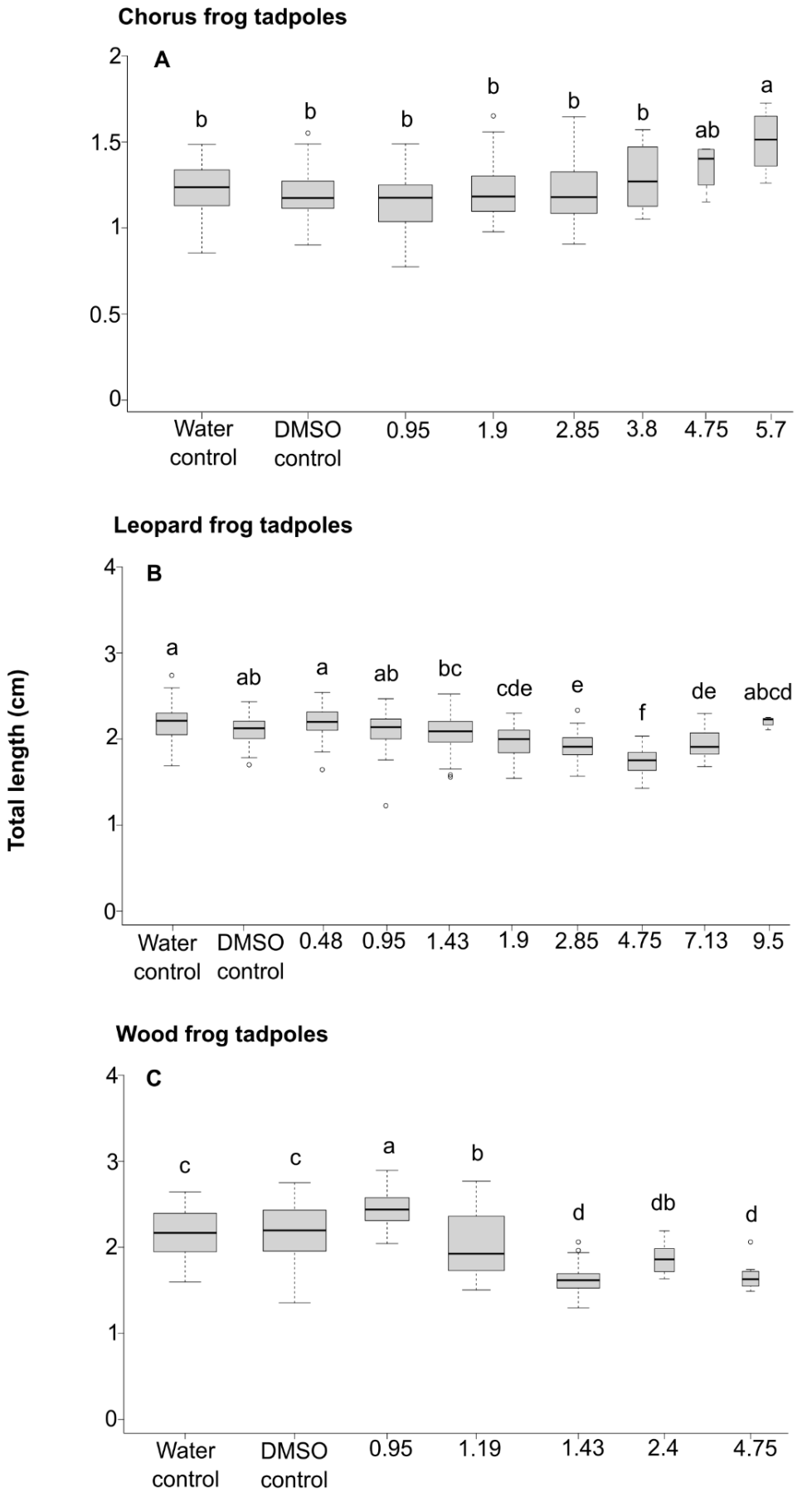
Chorus frog tadpoles
A
2
1.5
1
0.5
0
b
b
b
b
b
b
ab
a
Water control
DMSO control
0.95
1.9
2.85
3.8
4.75
5.7
Leopard frog tadpoles
B
4
3
2
1
0
a
ab
a
ab
bc
cde
e
f
de
abcd
Water control
DMSO control
0.48
0.95
1.43
1.9
2.85
4.75
7.13
9.5
Wood frog tadpoles
C
4
3
2
1
0
c
c
a
b
d
db
d
Water control
DMSO control
0.95
1.19
1.43
2.4
4.75
Total length (cm)
Deltamethrin concentration (µg a.i./L)

Figure 2.4 Effect of 96 h acute exposure to controls and deltamethrin on the total length of GS 25 chorus, leopard, and wood frog tadpoles. (A) Chorus frog tadpoles; **(B)** Leopard frog tadpoles; **(C)** Wood frog tadpoles. The bolded bar represents the mean; the box represents the lower and upper quartiles; the whiskers depict the minimum and maximum data values; the thickness of the box reflects the remaining sample size at 96 h; and circles represent outliers. A GLM and Tukey's post-hoc test was used to determine differences between groups. Letters (a, b, c, d, e, f) denote significant differences between groups ($p < 0.05$). Note that the X- and Y-axis scales differ between species

2.3.4. Species sensitivity distributions for Bti insecticides

The 2nd instar yellow fever mosquito, *Aedes aegypti*, was the most sensitive organism with an $LC_{50–24h}$ of 1.22 ITU/L (exposed to VectoBac® 12AS) (Valtierra-de-Luis et al., 2020) (Fig. 2.5). The mummichog fish, *Fundulus heteroclitus*, were the most tolerant, exhibiting an $LC_{50–96h}$ value of 1.18×10^6 ITU/L from exposure to VectoBac® (Lee & Scott, 1989). The mouse, *Mus musculus*, was the only mammal with available data and was exposed to Bti strain AM65–52, with $LC_{50–4h}$ of 8,520 ITU/L (WHO, 2012). The LC_{50}s of four amphibian species were available including from *Rhinella fernandezae* (LC_{50} of 24 and 48 h of 12,876 ITU/L), *Rhinella arenarum* ($LC_{50–24h}$ of 24,612 ITU/L and $LC_{50–48h}$ of 23,100 ITU/L), *Physalaemus albonatus* (LC_{50} of 24 and 48 h of 14,244 ITU/L), and *Leptodactylus latrans* ($LC_{50–48h}$ of 26,940 ITU/L), all exposed to Introban® (Junges et al., 2017; Lajmanovich et al., 2015). The most sensitive anuran thus far is *R. fernandezae*, with a calculated $LC_{50–48h}$ of 12,876 ITU/L, while the most tolerant is the wood frog tadpole, with an $LC_{50–96h}$ of 525,363.4 ± 1.13 ITU/L (See Fig. 2.5). The HC5 was calculated to be 14.0 ± 16.0 ITU/L.

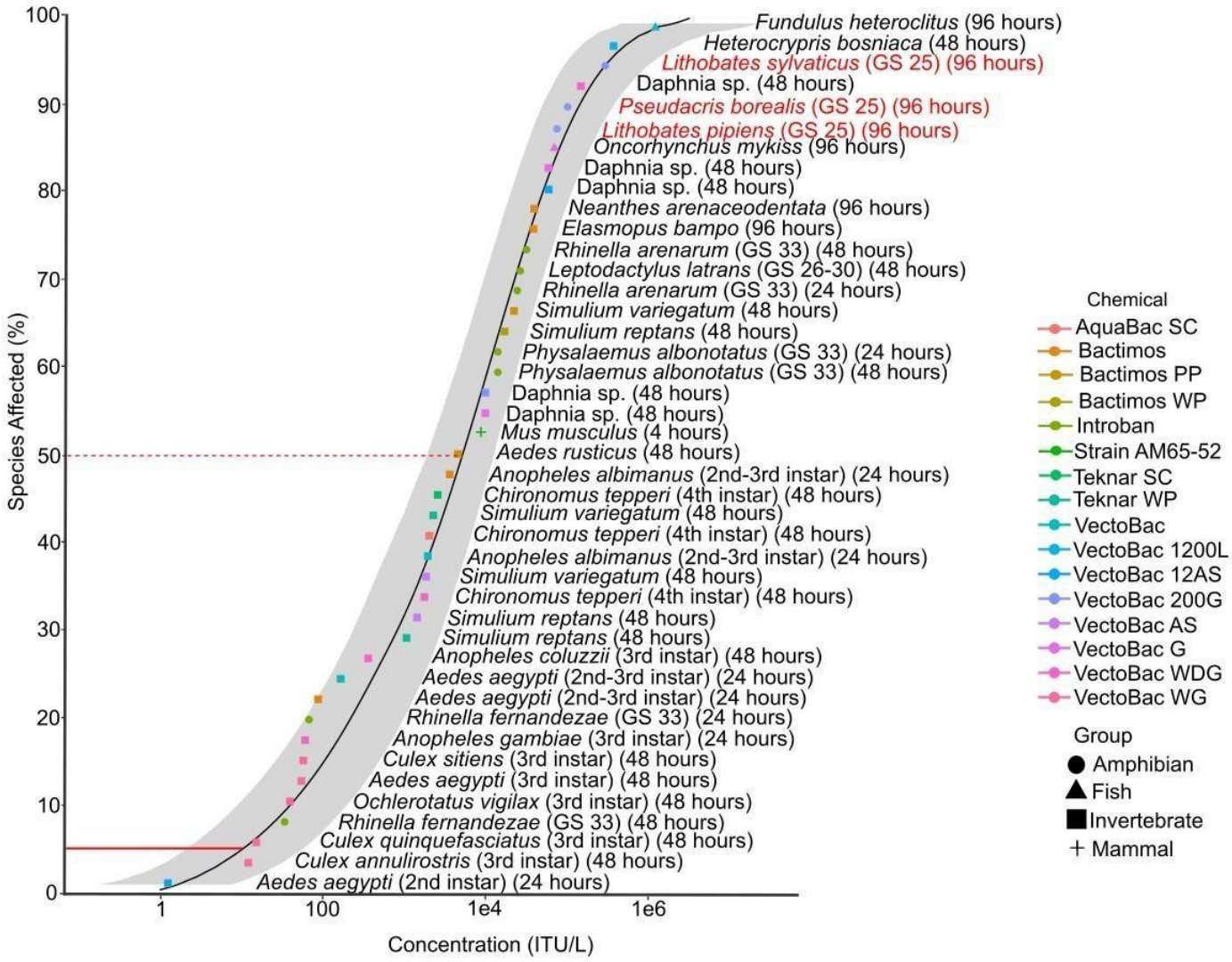

Figure 2.5. The species sensitivity distributions of median lethal concentration estimates of organisms exposed to Bti products. The colours denote the various Bti-containing products, while shapes denote different groups of organisms included. The species names highlighted in red are those from the current study. The dotted red line represents the concentration at which 50% of the species are affected. The solid red line denotes the calculated HC5 value.

The second larval stage of the American lobster, *Homarus americanus*, was the most sensitive organism with an $LC_{50–24h}$ of 6×10^{-6} µg a.i./L (exposed to AlphaMax®) (Burridge et al., 2014) (Fig. 2.6). The Japanese quail (*Coturnix coturnix japonica*) was the most tolerant with $LC_{50–5\ days}$ of 4.96×10^{6} µg a.i./L (deltamethrin formulation of 99.2%) (WHO, 2023). The most tolerant fish was *Clarius gariepinus*, which had an LC_{50} of 1,000.25 µg a.i./L to K–Obiol® 2.5 WP for 48, 72, and 96 h of exposure (Datta & Kaviraj, 2003). The most sensitive fish species was larval *Cyprinus carpio*, which had an $LC_{50–48h}$ of 0.00185 µg a.i./L to Decis® EC (Köprücü

& Aydin, 2004). The LC_{50} values for mice ranged from 20 µg a.i./L (2 h exposure to 2.5% deltamethrin formulation) to 3,075.2 µg a.i./L (4 h exposure to deltamethrin formulation 99.2%) (MERCK, 2020; WHO, 2023). The wood frog tadpole was the most sensitive amphibian species, while the most tolerant anuran was *P. gracilis* embryos (Macagnan et al., 2017) (See Fig. 2.6). The HC5 was determined to be $6.74 \times 10^{-4} \pm 5.28 \times 10^{-5}$ µg a.i./L.

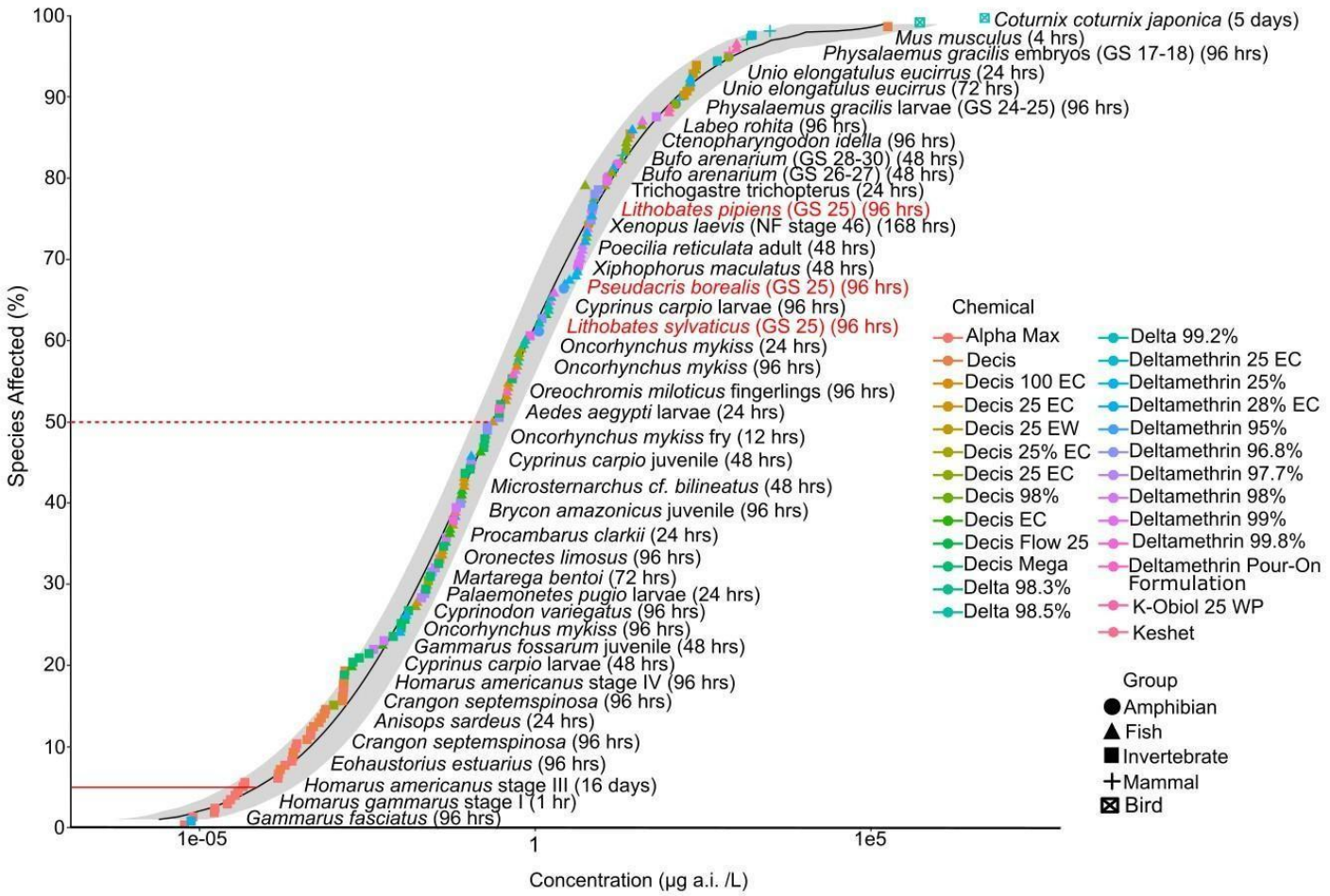

Figure 2.6. The species sensitivity distributions of median lethal concentration estimates of organisms exposed to deltamethrin products. Colours denote various deltamethrin products, while shapes denote different groups of organisms included. The species names highlighted in red are those from the current study. The dotted red line represents the concentration at which 50% of the species are affected. The solid red line denotes the calculated HC5 value.

2.4 Discussion

VectoBac® 200G was plated to confirm the Bti source for toxicological assessments. The plated concentrations encompassed the recommended application rate, showing an expected

increase in bacterial load. This product was also tested against mosquito larvae, resulting in significant mortality at the minimum recommended rate, confirming its viability. Mosquito larvae were also exposed to environmentally relevant concentrations of deltamethrin. Though primarily an adulticide, deltamethrin was effective against larvae, causing rapid mortality. The ecotoxicological effects of Bti products remain debated, particularly regarding proliferation from viable spores. Concerns about the persistence and recycling of Bti in wetlands are growing (Poulin et al., 2022). Germany remains the only country to gamma-irradiate Bti products to sterilize bacteria and prevent new sporulation (Becker, 2002). Environmental persistence of deltamethrin is also a concern. Deltamethrin degrades quickly in water via oxidation and ester hydrolysis (half-life 8–48 h) (Aiello et al., 2021; Erstfeld, 1999) but persists in soil for 11–72 days (Elliott, 1989). Deltamethrin may also accumulate in vertebrate and plant tissues (Erstfeld, 1999), raising concerns about long-term ecological impacts. The VectoBac® 200G LC_{50} values for chorus, leopard, and wood frog tadpoles were higher than the recommended application rate of VectoBac® 200G in Canada (refer to Table 2.1). These results imply that it is unlikely a single application of VectoBac® 200G would induce acute tadpole mortality. Determining the effects of multiple applications, chronic use, and sublethal effects in these species is an important next step to evaluate environmental risk. Chorus, leopard, and wood frog tadpoles were less sensitive to Bti than other amphibian species, where data are available. Before this study, the LC_{50} values of the Bti product Introban® had only been determined in four South American amphibian species: *R. fernandezae* ($LC_{50–48h}$ of 12,876 ITU/L), *P. albonatus* ($LC_{50–48h}$ of 14,244 ITU/L), *R. arenarum* ($LC_{50–48h}$ of 23,100 ITU/L), and *L. latrans* ($LC_{50–48h}$ of 26,940 ITU/L) (Junges et al., 2017; Lajmanovich et al., 2015). Introban® is used in Argentina and is not a registered product under Canada's Pest Regulatory Management Agency. Introban® is usually applied at concentrations of 0.5–75 mg/L, equivalent to 600–90,000 ITU/L, approximately 13 times the maximum application rate for VectoBac® 200G.

The LC_{50} of deltamethrin to chorus, leopard, and wood frog tadpoles were variable. Chorus and wood frog tadpoles had LC_{50} values below 3 µg a.i./L, while the leopard frog was above 7 µg a.i./L. It is difficult to compare results to the mosquito control agent, DeltaGard® 20 EW, as it is applied at a rate of 0.5–1.5 g of deltamethrin per hectare. However, the water quality guideline set by the Canadian Council of Ministers of the Environment to protect freshwater organisms is 0.0004 µg/L (Canadian Council of Ministers of the Environment, 1991). This limit

is significantly lower than that of other countries, for example, the United States EPA sets the water limit at 0.2 μg/L (Environmental Protection Agency, 2023). The limit of deltamethrin in water in European countries is not strictly defined; however, the maximum residue limit is 0.01–0.05 mg/kg for vegetables, fruits, and spices (European Commission, 2024). The LC_{50} values for other amphibians from available literature were similar to or higher than those of the leopard frog tadpole, demonstrating that the chorus and wood frog are susceptible in comparison. The embryos of the species *P. gracilis* had the highest LC_{50} estimate of 85.12 μg a.i./L, while larvae had a value of 14 μg a.i./L (Macagnan et al., 2017).

We tested the sublethal effects of VectoBac® 200G and deltamethrin on total tadpole length. Contaminant-induced changes in tadpole growth can negatively affect adult size and fecundity. Smaller body size is often linked to accelerated metamorphosis, which typically results in reduced adult size and is associated with reduced egg and clutch size, limited dispersal ability, increased desiccation risk, and decreased predator avoidance (Denver & Middlemis Maher, 2010). In contrast, larger tadpoles tend to undergo delayed metamorphosis, which can promote greater adult body size but also increases the risk of predation and exposure to harsh environmental conditions during the extended larval period (Székely et al., 2020). Wood and leopard frog tadpoles exposed to high VectoBac® 200G concentrations grew longer than controls, but this trend was not observed in chorus frog tadpoles. Some contaminants disrupt metabolism, forcing animals to expend energy on detoxification, tissue repair, and energy balance (Snyder et al., 2017). However, the mode of action of Bti in vertebrates remains unclear. Gutierrez-Villagomez et al. (2021) exposed *L. sylvaticus* and *A. americanus* tadpoles to VectoBac® 200G and VectoBac® 1200L from GS 25 to metamorphosis. While neither species showed size changes, VectoBac® 200G delayed metamorphosis in *L. sylvaticus* at concentrations up to 4,000 ITU/L but accelerated it at 20,000 ITU/L. The opposite effect was observed in *A. americanus*, where lower concentrations accelerated metamorphosis, whereas 20,000 ITU/L delayed it. VectoBac® 1200L prolonged metamorphosis in all tested concentrations for both species. The study also found that chronic exposure altered the gut microbiota, potentially affecting physiology, fitness, and development.

Unlike VectoBac® 200G, deltamethrin exposure reduced total length in leopard and wood frog tadpoles, though chorus frog tadpoles grew longer at the highest concentration. The effects of deltamethrin on amphibian growth remain unstudied, and our findings provide insight into

potential impacts. Macagnan et al. (2017) exposed *P. gracilis* embryos to 14–84 µg a.i./L of Decis® 25 EC from GS 17–20, and reported reduced mobility and spasmodic contractions in 38% of embryos. Vanzetto et al. (2019) studied Decis® 25 EC exposure (0.028–0.25 µg a.i./L) in *P. gracilis* tadpoles over seven days, observing spasmodic contractions at 48 h and oral deformities in 74% of tadpoles, including malformed jaws and denticles. These impairments could hinder feeding and growth. Other research has examined the effects of deltamethrin on the gastrointestinal system of larval amphibians. Alnoaimi et al. (2020) orally administered 0.625–2.50 mg/kg body weight of DEMOND® EC 2.5 (25 g/L deltamethrin) to *Pelophylax ridibundus* tadpoles. After 96 h, exposed tadpoles exhibited abnormal cells, edemas, tissue disorganization, and necrosis in the liver, stomach, intestines, and kidneys. Such damage may reduce nutrient absorption. Although our study did not assess histological effects, reduced tadpole size may have resulted from similar impacts.

It is curious that chorus frog tadpoles showed different or no effects on total length compared to leopard and wood frog tadpoles. These differences may reflect differences in life history traits. Leopard and wood frogs (*Lithobates* spp.) share characteristics absent in chorus frogs (*Pseudacris* spp.), which are smaller (20–40 mm at maturity) than wood (31–40 mm) and leopard frogs (up to 110 mm). Chorus frogs are primarily terrestrial, feeding on small invertebrates, while leopard and wood frogs inhabit diverse environments, including wetlands, meadows, and bogs. Wood frogs, with the broadest range, occupy tundra and temperate woodlands. Their larger size allows them to consume a wider range of prey, including conspecifics in wood frogs. Habitat differences may also influence stomach contents and microbiomes based on prey availability. Using multiple amphibian species to monitor ecosystem health is beneficial, as life-history traits such as size, habitat, and diet influence contaminant responses. Smalling et al. (2021) found that amphibian larvae sensitivity to metals (e.g., mercury, arsenic, lead) varied by species. Analyzing 17 larval species, they observed that metal concentrations decreased with increasing weight and development. They concluded that life history traits influence metal uptake through diet, habitat, and biochemical changes that limit accumulation and dilute metals via growth. Pesticide dynamics may differ from metals, requiring further research on how species traits affect pollutant susceptibility (Brühl et al., 2011).

This study provides SSDs for Bti products on invertebrates and vertebrates, offering key insights for ecological assessments (Boeckman & Layton, 2017; Maltby et al., 2005). SSDs help

mitigate the impact of insecticides on non-target species, identify data gaps, and guide future research. The LC_{50} values for chorus, leopard, and wood frog tadpoles exposed to VectoBac® 200G exceed 80% of affected species (Fig. 2.5). Other amphibians, including *L. latrans*, *R. arenarum*, and *P. albonatus*, are less tolerant than North American species. The Argentinian toad, *R. fernandezae*, may be especially susceptible to Introban®. SSDs are most reliable with large datasets, and relying on limited species, especially from a single life stage, is inadvisable.

Few studies have examined the effects of Bti on vertebrates. The $LC_{50–4h}$ for the mouse *Mus musculus* is 8,520 ITU/L for the Bti strain AM65–52 (WHO, 2012). In comparison, the average $LC_{50–96h}$ for the rainbow trout *O. mykiss* is 37,185 ITU/L when exposed to VectoBac® 12AS, WDG, 200G, and G (Valent Biosciences, 2015, 2020a, 2020b, 2024). The mummichog fish, *F. heteroclitus*, had an estimated $LC_{50–96h}$ of 1.17×10^6 ITU/L to VectoBac® (Lee & Scott, 1989). LC_{50} data for other fish and birds are lacking, despite evidence suggesting insectivorous birds may be at risk from Bti exposure (Poulin et al., 2010; Poulin & Lefebvre, 2018). Poulin et al. (2010) observed *Delichon urbicum* in Bti-treated areas of the Camargue, France, over three years. Mosquitoes, midges, spiders, and dragonflies declined in the diets of birds at treated sites, while flying ants increased. Clutch size and fledgling survival also significantly decreased in treated areas compared to controls.

Expectedly, target organisms, such as black flies and disease vectors like *A. aegypti*, *Anopheles* spp., and *Culex* spp., were lower on the SSD curve. The range and distribution of disease-carrying mosquitoes are expected to fluctuate due to climate change (Wang et al., 2024), and countries may consider further investing in targeted insecticide use. The constructed SSDs may help determine which products and concentrations are suitable for reducing the population of specific mosquitoes while considering the potential impact on non-target species. Applications of Bti products have sparked controversial debates about food web effects, primarily due to concerns about Chironomidae populations (Brühl et al., 2020; Poulin & Lefebvre, 2018). These include non-biting midges that are essential to food webs but are affected by Bti insecticides. We found that 4th instar *Chironomus tepperi* have an average LC_{50} of 2,130 ITU/L (Stevens et al., 2005), which is much higher than the $LC_{50–48h}$ of 28 ITU/L for *Culex* species (Russell et al., 2003). Thus, Bti applications targeting *Culex* species may not directly affect Chironomidae, but those aimed at malaria-carrying *Anopheles* species (LC_{50} of 1,685.5 ITU/L for 2nd–3rd instars) may pose a risk (Fillinger et al., 2003; Garza-Almanza et al., 2020; Gowelo et al., 2020).

The SSD for deltamethrin products on invertebrates and vertebrates is also provided. Of the three North American species tested, leopard frog tadpoles were most tolerant to deltamethrin. Chorus and wood frog tadpoles were the most susceptible to deltamethrin among other amphibians. Embryos of *P. gracilis* were the most tolerant amphibian species. Leopard frog tadpoles had LC_{50} values similar to those of *Bufo arenarum* tadpoles (Salibián, 1992), but were slightly more tolerant to deltamethrin exposure. Although these species have higher LC_{50} values, studies suggest that adverse sublethal health effects would likely occur in response to deltamethrin exposure, as reported by Alnoaimi et al. (2020), Macagnan et al. (2017), and Vanzetto et al. (2019).

Compared to Bti, more data exists on the effects of deltamethrin on vertebrates, as it is used for delousing in aquaculture (Čolak et al., 2019; Yadav et al., 2023). Fish tolerance to deltamethrin varies by species, product, and life stage. *Cyprinus carpio* larvae are highly susceptible (average $LC_{50–48–96h}$ of 0.73 μg a.i./L to Decis® products) (Köprücü & Aydin, 2004; Svobodová et al., 2003), while adults are more tolerant (average $LC_{50–28–96h}$ of 9.26 μg a.i./L to Decis® products) (Lakota et al., 1989; Rao et al., 1983; Velíšek et al., 2006). Studies on other vertebrates are limited. The only mammal for which we found data was *Mus musculus*, with LC_{50} values for a 2.5% deltamethrin formulation ranging from 20 μg a.i./L (2 h exposure) to 140.25 μg a.i./L (4 h exposure) (MERCK, 2020). A more potent 99.2% formulation had an $LC_{50–4h}$ of 3,075.2 μg a.i./L (WHO, 2023). Only two studies on *Coturnix coturnix japonica* met our criteria. Exposure to a 99.2% deltamethrin product for five days resulted in an LC_{50} of 4.96×10^6 μg a.i./L, while an 8 day exposure to a 98.5% formulation had an LC_{50} of 5.37×10^5 μg a.i./L (WHO, 2023). Although large concentrations are required to induce mortality, feed contaminated with 0.25–0.50 mg deltamethrin/kg body weight for 21 days induced aggressiveness, nervousness, and anorexia (Hamidipoor et al., 2015). Markers of liver and tissue damage, including aspartate aminotransferase, lactate dehydrogenase, and glucose, also increased significantly.

Non-target invertebrates have variable LC_{50} values in response to deltamethrin. Crustacean species have low LC_{50} estimates, as shown in Fig. 2.6. As deltamethrin is used to control sea lice, nontarget crustaceans are at risk of exposure to lethal concentrations. For example, the average LC_{50} of the larval American lobster, *Homarus americanus*, is 3.2×10^{-4} μg a.i./L (stages I to IV, exposed for 24 h to 16 days to AlphaMax® and Decis®) (Burridge et al.,

2014; Fairchild et al., 2010; Kumar et al., 1999). The 3rd instar of the fall armyworm, *Spodoptera frugiperda*, has the highest invertebrate LC_{50} value of 1.79×10^5 µg a.i./L, exposed to Decis® over 5 days. The fall armyworm moth consumes soybean, maize, and cotton crops, and its populations are often controlled with insecticides (Matova et al., 2020). Deltamethrin may not be an appropriate pesticide for the armyworm, as many non-target species on the curve would be affected if exposed to concentrations equal to or greater than the LC_{50} of *S. frugiperda*.

The HC5 is a widely used tool for chemical applications, but values should be interpreted cautiously as SSD predictions may underestimate contaminant effects (Dhond & Barron, 2022; Spurgeon et al., 2020). A robust HC5 estimate requires taxonomic diversity. SSDs with insufficient data or low sample sizes can misrepresent chemical toxicity. The HC5 of Bti products, based on current literature, is estimated at 14.0 ± 16.0 ITU/L, primarily affecting invertebrates like *A. aegypti*. The HC5 estimate for deltamethrin is $6.74 \times 10^{-4} \pm 5.28 \times 10^{-5}$ µg a.i./L, affecting amphipods and crustaceans. As more research emerges, HC5 values may change. Reviewing the effects of Bti and deltamethrin on nontarget organisms reveals critical data gaps for amphibians, mammals, fish, and birds. Further studies on their impacts, especially in operational and over-application scenarios, are needed. More research on indirect effects, such as changes in food webs, is also essential.

2.5 Conclusions

High concentrations of VectoBac® 200G insecticide caused significant mortality in chorus, leopard, and wood frog GS 25 tadpoles. However, the maximum application rate of this Bti product is below the LC_{50} values for these species, making acute mortality unlikely. In contrast, deltamethrin can induce significant mortality in these tadpoles at minimal concentrations. Insecticide effects on growth were species- and concentration-dependent. High concentrations of VectoBac® 200G increased total length in leopard and wood frog tadpoles, while deltamethrin often reduced total length in these species. Because effects on growth and development can reflect disruptions in metabolic homeostasis, these findings prompted further investigation into metabolic endpoints in Chapter 3. SSDs facilitate assessment of sublethal effects and ecosystem-wide impacts. Further research is needed to understand long-term and sublethal effects of Bti and deltamethrin on amphibian populations, and how these insecticides may influence habitats where they are applied.

Chapter 3: Exposure to Bti and deltamethrin insecticides disrupts glucose uptake and hepatic lipid accumulation in wood frog tadpoles

Study contributions: Madelaine A. Empey conceived of and conducted experiments, analyzed data, and wrote the manuscript; Yol Monica Reyes helped design and conduct experiments; Ayesha Iqbal helped conduct experiments; and Vance L. Trudeau conceived of and helped design experiments, revised the manuscript, and acquired funding for the study.

Abstract

Endocrine-disrupting chemicals are a growing concern, as mounting evidence indicates they can disrupt fundamental physiological processes. Specifically, some of these chemicals can induce metabolic disorders by impairing glucose metabolism and altering insulin sensitivity. While the metabolic effects of the bioinsecticide, *Bacillus thuringiensis israelensis* (Bti), remain largely unexplored, the insecticide deltamethrin is known to disrupt metabolic processes. To investigate potential metabolic effects, wood frog tadpoles (*Lithobates sylvaticus*) were chronically exposed to these insecticides. Tadpoles exposed to 40,000, 100,000, and 200,000 ITU/L of VectoBac® 200G for 30 days (from Gosner stage (GS) 25–30) exhibited significantly higher glucose uptake than controls. The average hourly glucose uptake rate of the corn control, 40,000, 100,000, and 200,000 ITU/L of VectoBac® 200G was 6.0 ± 1.0, 9.9 ± 1.4, 11.2 ± 2.0, and 11.0 ± 2.4 mmol/L, respectively. Conversely, tadpoles exposed to 0.068, 0.140, and 0.325 µg a.i./L of deltamethrin for 30 days had no significant difference in glucose uptake. Wood frog tadpoles were exposed to lower concentrations (10,000, 20,000, and 40,000 ITU/L) of VectoBac® 200G from GS 25 to metamorphosis (approximately 56 days). Deltamethrin exposures were conducted at the same concentrations as the 30-day glucose uptake experiment. Hepatic lipid content was affected in tadpoles exposed to these insecticides from GS 25 to metamorphosis. Concentrations of 20,000 ITU/L of VectoBac® 200G and 0.325 µg a.i./L of deltamethrin increased the number of hepatic lipid droplets. Conversely, high concentrations of VectoBac® 200G and 0.068 µg a.i./L of deltamethrin decreased the area of individual lipid droplets. Although snout-vent length at metamorphosis was unaffected, wood frogs exposed to 20,000 ITU/L of VectoBac® 200G completed metamorphosis 11 days longer on average. The results address data gaps for both Bti and deltamethrin insecticides, whose impacts on amphibians remain critically understudied.

3.1 Introduction

Endocrine-disrupting chemicals (EDCs) are natural or synthetic compounds that interfere with hormonal signalling pathways, leading to adverse health effects (Mnif et al., 2011). Due to their chemical stability and widespread use, pesticides often persist in the environment by accumulating in soil and vegetation, leaching into aquatic systems, and volatilizing into the atmosphere (Bédos et al., 2002; Scholtz & Bidleman, 2007; Sharma et al., 2020). A subclass of EDCs, known as metabolic-disrupting chemicals (MDCs), specifically impairs metabolic processes, including glucose and lipid regulation, and has been linked to metabolic disorders such as diabetes. Several widely used pesticide classes, including pyrethroids, organophosphates, and organochlorines, fall under this category (Wei et al., 2023). As MDCs can impact pathways involved in glucose metabolism, species that rely on rapid glucose mobilization may be more susceptible. Amphibians, such as the wood frog, rapidly draw glucose from hepatic glycogen stores to use as a cryoprotectant in subzero temperatures (Storey & Storey, 1986). Disruption to glucose regulation in these species may therefore have important consequences on survival.

There are multiple mechanisms by which pesticides promote metabolic disorders, such as inducing lipotoxicity, altering acetylcholine neurotransmission, elevating reactive oxygen species levels, altering insulin signalling pathways, and inducing dysbiosis of the gut microbiome (Slawik & Vidal-Puig, 2006; Sule et al., 2022; Wei et al., 2023). Although the bioinsecticide *Bacillus thuringiensis israelensis* (Bti) is not classified as an MDC, early studies provide evidence that Bti may affect the gastrointestinal tract of vertebrates (Gutierrez-Villagomez et al., 2021; Snarski, 1990; Wilcks et al., 2006), which may then affect metabolism. Perturbations of the gastrointestinal tract may be linked to the Cry and Cyt crystal proteins produced by the bacterium, which bind to specific receptors in the gut wall of dipterans, leading to gut leakage and death (Vachon et al., 2012). Although the effects of *Bacillus thuringiensis* (Bt) insecticides on amphibian metabolism remain poorly studied, some research has examined their effects on other vertebrates. Mariano et al. (2019; 2021) found that the Bt *kurstaki* (Btk) product Dipel® WP significantly decreased plasma glucose levels in *Piaractus mesopotamicus* and *Arapaima gigas* fish 24 and 48 h after its addition to feed and tank water. Fish exposed to Btk-contaminated water showed a decrease in goblet cell number and villi height in the intestinal tract, whereas fish fed Btk-coated feed showed an increase in villi height and mucus production. These symptoms

suggest that Bt exposure may have adversely affected the gastrointestinal tract and, consequently, metabolism by hindering nutrient uptake.

In contrast to Bti, deltamethrin is a known MDC and a neurotoxic insecticide with broadspectrum applications, including household, agriculture, and aquaculture pest control (Arnberg et al., 2023; Clark et al., 1989). While pyrethroids primarily target voltage-gated sodium channels, studies also suggest they can influence calcium channel function (Hildebrand et al., 2004; Neal et al., 2010; Symington et al., 1999). This is particularly relevant, as pancreatic β-cells are electrically excitable, and insulin secretion is regulated by calcium influx (Fu et al., 2013). Therefore, pyrethroid interference with ion channel activity may alter metabolism through both neurotoxic and insulin pathways (Fu et al., 2013). Studies on the effects of deltamethrin on metabolism are also lacking in amphibians, but studies are available on other vertebrates. In a study assessing the metabolic effects of Bt insecticides and deltamethrin, rats were exposed throughout pregnancy and lactation to either 1 mg/100g of Bt *aizawai*, 1 mg/100g of Btk, or 2 mg/kg of deltamethrin (Alves et al., 2021). Markers of liver disease, such as alanine transaminase and aspartate transferase activity, increased significantly in offspring across all insecticide exposures. It was found that Btk induced oxidative stress, lipid peroxidation, and an inflammatory response in rat hepatic cells. An increase in liver congestion and a reduction in glycogen were also observed in both Bt and deltamethrin treatments. In another study, pregnant mice administered deltamethrin (1 mg/kg body weight) throughout gestation and lactation produced offspring with decreased expression of insulin-response, lipogenic, and glucosetransport genes (Armstrong et al., 2013). Although body weight and blood glucose were not significantly affected, these results suggest that deltamethrin may indirectly influence metabolic regulation (Armstrong et al., 2013).

We have determined that the Bti formulation, VectoBac® 200G, and deltamethrin may act as MDCs in the wood frog *(Lithobates sylvaticus*). In Chapter 2, we identified effects on growth, prompting further investigation into metabolic endpoints. We found that exposure to VectoBac® 200G significantly increased glucose uptake, whereas deltamethrin had no significant effects. Both insecticides increased the number of hepatic lipid droplets and altered lipid droplet distribution in metamorphs, suggesting hepatotoxicity. Growth was generally unaffected; however, VectoBac® 200G significantly delayed metamorphosis by 11 days.

3.2 Materials and Methods

3.2.1 Animals and Insecticides

Wood frog (*Lithobates sylvaticus*) tadpoles were used for all experiments. The effects of Bti were tested using VectoBac® 200G (potency of 200 ITU/mg) and blank corn as a control. Deltamethrin of 95% purity, and dimethyl sulfoxide (DMSO) as a negative control, were used for deltamethrin exposure experiments. For information regarding insecticide product number and acquisitions, and animal breeding and housing, please refer to the Materials and Methods section of Chapter 2. The University of Ottawa Animal Care Committee approved all experiments.

3.2.2 Glucose Quantification in Tadpoles

As wood frog tadpoles are too small to extract blood, an assay was designed and validated to measure glucose levels in the whole body (Fig. 3.1). In experiments involving waterborne glucose administration, both non-glucose controls and glucose-treated tadpoles were rinsed with facility water for approximately 10 seconds, and then weighed and euthanized by rapid freezing on dry ice. Samples were processed immediately or stored at –80°C for subsequent analysis. Each tadpole was homogenized on ice using a Kontes® Micro Ultrasonic Cell Disruptor (20 kHz) for approximately 30–60 seconds. Homogenates were centrifuged at 16,100 x g for 10 minutes at 4 °C using an Eppendorf® 5415 R centrifuge. A 2 µL aliquot of the supernatant was applied to a Contour® Next Gen test strip, and glucose levels were measured using a Contour® Next Gen glucometer (Ascensia® Diabetes Care Canada).

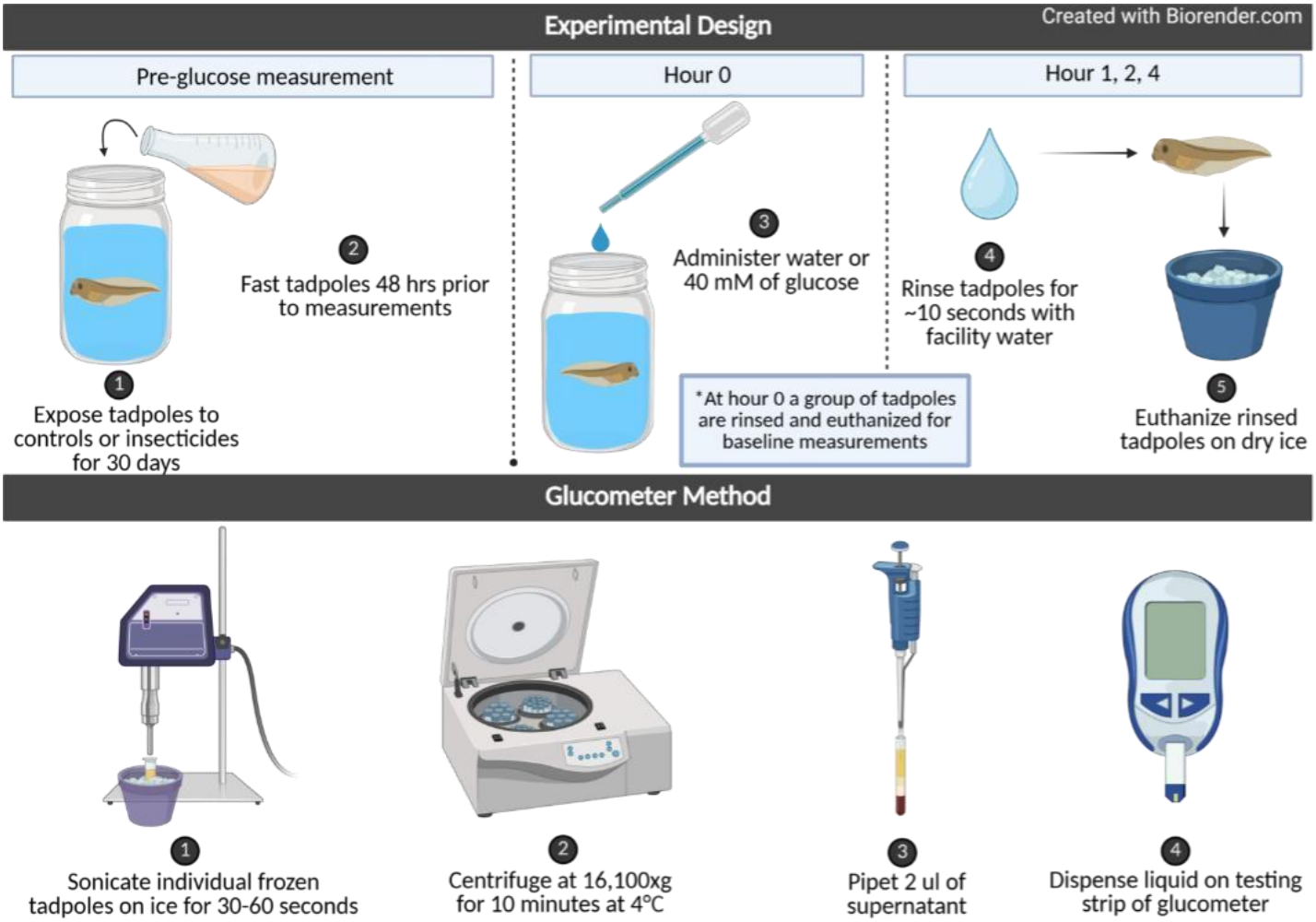

Figure 3.1. Experimental design and method for whole body glucose measurements via a glucometer in individual wood frog tadpoles.

3.2.3 Validation of Glucometer Readings

3.2.3.1 Insulin Test

Gosner stage 38–39 tadpoles (n = 10) were weighed (OHAUS® Analytical Standard) and then anesthetized by immersion (30–60 seconds) in MS-222 (250 mg/L) buffered with sodium bicarbonate (Letcher, 1992). When the reflex response was absent, tadpoles were placed dorsally on a wet sponge. Tadpoles were injected intraperitoneally in the lower right abdomen with a saline control (0.7% NaCl) or bovine insulin (Sigma-Aldrich, CAS# 11070-73-8) at a volume of 20 µl/g of body weight using a 32-gauge needle attached to a 5 µl Hamilton syringe (PN# 87931). The insulin solution was prepared to deliver a dose of 5 milli-international units per gram of body weight (mU/g bw) (Brahim et al., 1987). After injection, tadpoles were placed in fresh facility water to recover from the anesthetic. Two hours post-injection, tadpoles were

sacrificed by rapid freezing on dry ice and processed for glucose measurements. Differences between the saline-control and insulin-injected groups were assessed using an independent t-test ($\alpha = 0.05$). This and all subsequent analyses verified whether weight was a confounder of glucose measurements, and data were evaluated for normality and homogeneity of variance. All analyses were performed with GraphPad Prism (v10.4.1) unless otherwise stated.

3.2.3.2 Fasting and Fed Tadpole Glucose Levels Over Time

Glucose levels of GS 30–33 tadpoles were measured at 10:00 h each day for four consecutive days in fasted or fed conditions (n = 10 per day). Fasted tadpoles were not fed during the entire experimental period, while fed tadpoles were given food daily at 16:00 h. Glucose concentrations were measured as described in section 3.2 (see Fig. 3.1). A two-way ANOVA with Tukey's post-hoc test ($\alpha = 0.05$) was used to evaluate the effects of treatment (fasting vs fed), time (days), and their interaction on glucose levels.

3.2.4 Experimental Design of the Glucose Uptake Assay

GS 25 wood frog tadpoles were exposed for 30 days to (1) a corn control, 40,000, 100,000, and 200,000 ITU/L VectoBac® 200G, or (2) a DMSO control, 0.068, 0.140, and 0.325 µg a.i./L deltamethrin. At the end of the exposure period, tadpoles developed to approximately GS 30. Tested concentrations represent the calculated LC_{10}, LC_{15}, and LC_{20} values based on previous toxicological assessments (see Chapter 2; Empey et al., 2025). Tadpoles were fasted for 48 h before glucose uptake trials (Fig. 3.1). To reduce the effects of circadian rhythm on metabolism, all glucose uptake trials began at 10:00 h (hour 0). At this timepoint, non-glucose (NG) control tadpoles were placed in water, and glucose-treated (G) tadpoles were placed in a solution with 40 mM of glucose (Sigma-Aldrich, CAS# 50-99-7) (n = 6). This concentration was selected based on preliminary trials that demonstrated rapid and measurable glucose uptake (data not shown). In parallel, at hour 0, an additional group of tadpoles (n = 6) were set aside and immediately sampled to determine basal glucose levels for normalization. Subsequent glucose measurements of NG and G tadpoles were taken at 11:00 h (hour 1), 12:00 h (hour 2), and 14:00 h (hour 4). At each timepoint, tadpoles were weighed, rinsed, and then euthanized on dry ice, after which glucose concentrations were measured. Glucose concentrations were normalized to baseline (hour 0) levels by subtracting the mean basal glucose concentration from subsequent timepoints. The hourly glucose uptake rate was calculated as the mean glucose concentration divided by the corresponding time, and the three time points were averaged to yield the overall

hourly glucose uptake rate (mmol/L/h). Effects of glucose, time, and interaction were assessed using a two-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$). Comparison of hourly glucose uptake rates between treatments was assessed using a one-way ANOVA with Tukey's post-hoc test ($\alpha = 0.05$).

3.2.5 Experimental Design of Growth, Days to Complete Metamorphosis, and Hepatic Lipid Content Experiments

Wood frog tadpoles were chronically exposed to VectoBac® 200G or deltamethrin from GS 25 to metamorphic climax (GS 46; approximately 56 days). Lower concentrations of VectoBac® 200G (10,000, 20,000, and 40,000 ITU/L) were used for chronic exposures because the high concentrations used in the 30-day glucose uptake assays led to significant mortality before tadpoles reached metamorphic climax. This effect was not observed in deltamethrin exposed tadpoles, therefore exposure concentrations (0.068, 0.140, and 0.325 µg a.i./L) did not change for this experiment. At metamorphic climax, animals were euthanized with 5 g/L of MS222 buffered with sodium bicarbonate (Torreilles et al., 2009). They were then placed on 1-cm grid paper and photographed from above to obtain snout-to-vent length (SVL) measurements using ImageJ (v1.51) (n = 8). Limbs were cut off, and a superficial vertical abdominal incision was made, followed by fixation in 10% non-buffered formalin for 72 h. Animals were transferred to 70% ethanol, then embedded in paraffin, sectioned, and stained at the Louise Pelletier Histology Core (Ottawa, ON). Transverse sections corresponding to approximately the midregion of the liver were selected and stained with hematoxylin and eosin (H&E) to visualize lipid droplets (n = 3). Slides were photographed at 10x magnification using an Olympus CX41 light microscope. Hepatic lipid droplet number, total lipid droplet area, and mean area of individual lipid droplets were measured in Fiji software (v2.9.0) (Schindelin et al., 2012). Differences in SVL between treatments were assessed using a GLM with a Gaussian distribution in R statistical software (version 4.5.2). Differences in days to complete metamorphosis, lipid droplet number, total area of lipid droplets, and mean area of individual lipid droplets across treatments were determined using a one-way ANOVA with Tukey's post-hoc test ($\alpha = 0.05$).

3.3 Results

3.3.1 Glucometer Validation

A 56% reduction in glucose was observed in wood frog tadpoles injected with insulin compared to tadpoles injected with saline ($p = 0.014$; Fig. 3.2A). Two hours following injections,

glucose levels in tadpoles injected with saline averaged 3.7 ± 0.4 mmol/L, whereas insulininjected tadpoles averaged 1.6 ± 0.2 mmol/L.

Glucose levels also differed between fasting and fed tadpoles over the four-day sampling period (Fig. 3.2B). The main effect of fasting was significant (Treatment: $F(1, 72) = 71.37$, $p < 0.0001$), but glucose levels did not vary significantly over the test days (Time: ($F(3, 72) = 2.24$, $p = 0.091$), and fasting and time interactions were also not significant ($F(3, 72) = 0.27$, $p = 0.847$). Fasting tadpoles showed significantly lower glucose levels (ranging from 0.5 ± 0.1 to 2.4 ± 0.2 mmol/L and averaging 1.4 ± 0.2 mmol/L) than fed tadpoles (ranging from 5.1 ± 1.0 to 6.3 ± 1.1 mmol/L and averaging 5.7 ± 0.5 mmol/L).

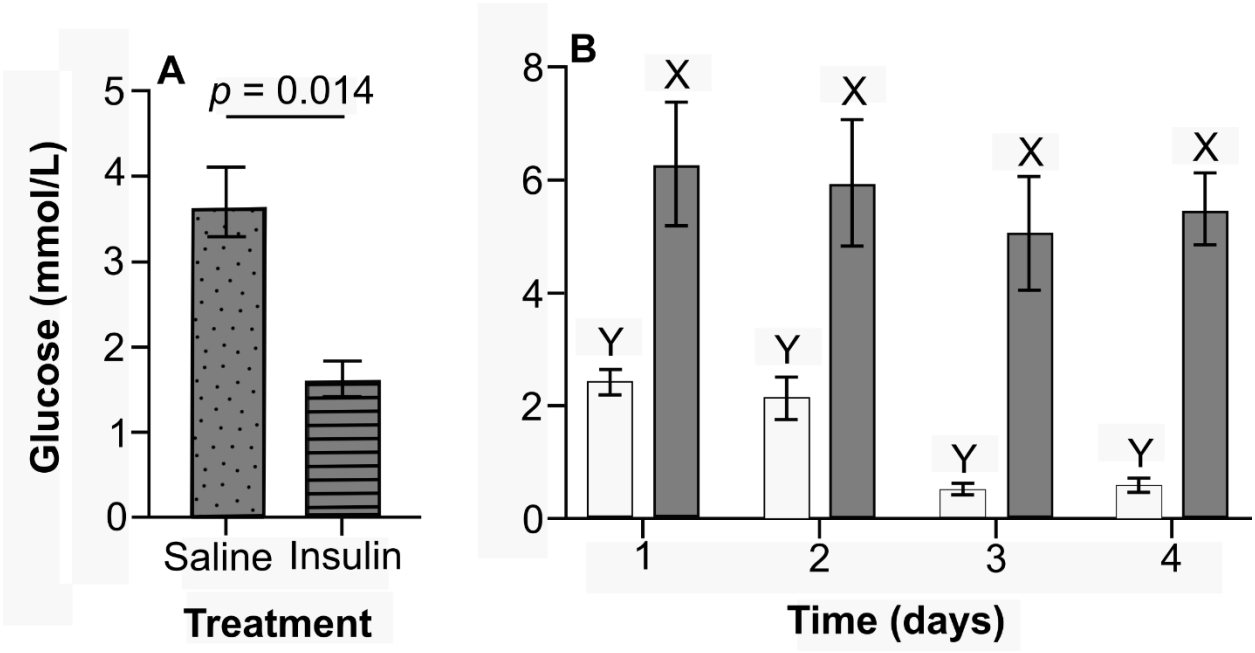

Figure 3.2. Validation of glucometer readings in wood frog tadpoles. (A) Glucose levels in fed GS 38–39 tadpoles measured 2 h after intraperitoneal injection with either 0.7% saline (control) or 5 mU/g bw of bovine insulin (n = 10); a significant effect of insulin treatment was observed (independent t-test, $p = 0.014$); gray bars with dots represent fed tadpoles injected with saline and grey bars with lines indicate fed tadpoles injected with insulin. **(B)** Glucose levels of GS 30 tadpoles under fasted and fed conditions measured daily over four consecutive days (n = 10 per day); different letters (X, Y) indicate a main effect of fasting vs fed treatment (two-way ANOVA; $p < 0.0001$); open bars represent fasting tadpoles and gray bars represent fed tadpoles. Data are presented as the mean ± SEM.

3.3.2 Glucose Uptake

A two-way ANOVA revealed a significant main effect of glucose treatment in the corn control and across all VectoBac® 200G concentrations (Table 3.1). Time significantly affected glucose uptake across all VectoBac® 200G concentrations, but not in the corn control. A significant interaction between glucose and time was observed in the corn control and all VectoBac® 200G exposures (Table 3.1).

There was no significant difference between NG and G tadpoles at hour 1 in the corn control group ($p > 0.05$; Fig. 3.3A). Glucose levels of NG tadpoles were significantly lower than G tadpoles at both hours 2 ($p < 0.0001$) and 4 ($p = 0.006$). Glucose uptake in G tadpoles increased significantly from 7.6 ± 1.6 mmol/L at hour 1 to 16.0 ± 3.6 mmol/L at hour 2 ($p = 0.034$), followed by a non-significant decline to 9.8 ± 2.1 mmol/L at hour 4 ($p = 0.195$). In contrast, exposure to VectoBac® 200G resulted in sustained elevated glucose uptake following glucose administration. Glucose levels in NG tadpoles exposed to all VectoBac® 200G concentrations remained stable and significantly lower than G tadpoles at all timepoints ($p < 0.05$; Fig. 3.3B–D). At 40,000 ITU/L, glucose levels in G tadpoles rose from 12.3 ± 1.0 mmol/L at hour 1 to 19.8 ± 6.3 mmol/L at hour 2 ($p = 0.023$), and further increased to 29.5 ± 1.5 mmol/L at hour 4 ($p < 0.0001$; Fig. 3.3B). At 100,000 ITU/L, glucose levels of G tadpoles increased from 12.6 ± 2.0 mmol/L at hour 1 to 27.4 ± 2.4 mmol/L at hour 2 ($p < 0.0001$) and remained elevated at 29.0 ± 2.6 mmol/L at hour 4 ($p < 0.0001$), with no significant differences between the later timepoints ($p = 0.650$; Fig. 3.3C). A similar pattern was observed at 200,000 ITU/L, where glucose levels in G tadpoles increased from 12.8 ± 1.8 mmol/L at hour 1 to 27.8 ± 6.5 mmol/L at hour 2 ($p < 0.0001$) and further to 25.2 ± 5.8 mmol/L at hour 4 ($p = 0.0002$) with no significant differences between hours 2 and 4 ($p = 0.871$; Fig. 3.3D).

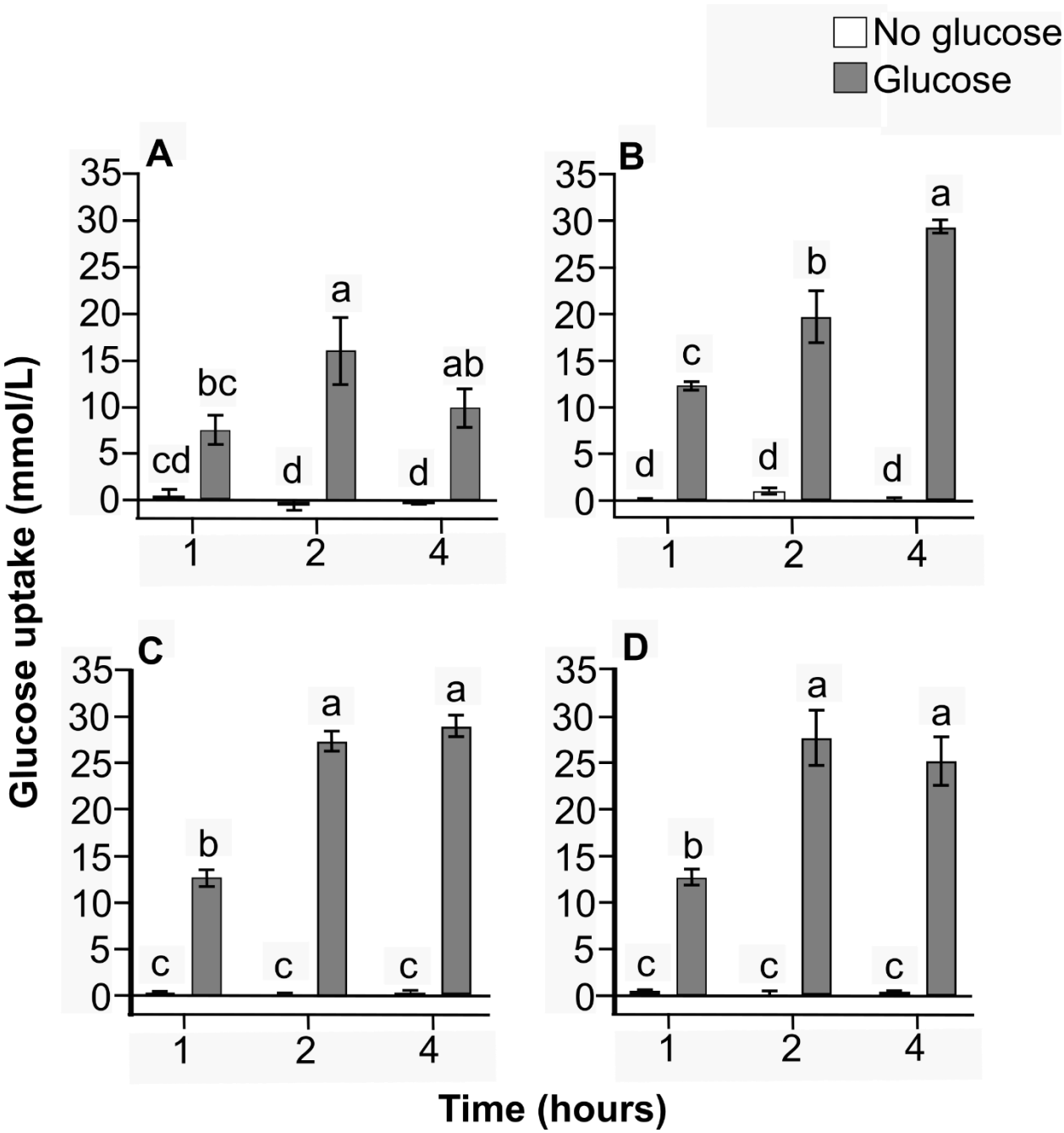

Figure 3.3.Glucose uptake measurements of wood frog tadpoles exposed to VectoBac® 200G. Tadpoles (n = 6) were exposed for 30 days (from GS 25–30) to (A) blank corn control, (B) 40,000 ITU/L, (C) 100,000 ITU/L, or (D) 200,000 ITU/L of VectoBac® 200G. Open bars represent non-glucose-administered tadpoles (placed in water only), and gray bars represent tadpoles that were placed in a 40 mM glucose solution. Different letters (a, b, c, d) denote significant differences between groups (Tukey's post-hoc test; $p < 0.05$). Values are adjusted for basal glucose levels measured at hour 0. Data are presented as the mean ± SEM.

Table 3.1. Two-way ANOVA results for glucose uptake measurements over time following glucose treatment in wood frog tadpoles exposed to VectoBac® 200G. Tadpoles (n = 6) were exposed for 30 days (from GS 25–30) to a corn control, 40,000, 100,000, or 200,000 ITU/L of VectoBac® 200G. Shown are the main effects of treatment (no glucose or 40 mM glucose), time (hours 1–4), and treatment × time interaction within each concentration. Asterisks denote statistically significant effects.

Treatment	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	*p*-value
Corn control	Treatment	966.2	1	966.2	<0.0001*
	Time	78.2	2	39.1	0.119
	Interaction	118.1	2	59.1	0.046*
40,000 ITU/L	Treatment	3021.0	1	3021.0	<0.0001*
	Time	369.5	2	184.8	<0.0001*
	Interaction	372.3	2	186.2	<0.0001*
100,000 ITU/L	Treatment	3893.0	1	3893	<0.0001*
	Time	408.5	2	204.2	<0.0001*
	Interaction	404.3	2	202.1	<0.0001*
200,000 ITU/L	Treatment	3498.0	1	3498.0	<0.0001*
	Time	308.9	2	154.4	0.0003*
	Interaction	335.6	2	167.8	0.0002*

Two-way ANOVA revealed a significant main effect of glucose treatment on glucose uptake in tadpoles exposed to the DMSO control and all deltamethrin concentrations (Table 3.2). In contrast, the main effect of time was not significant at any DMSO or deltamethrin exposure, and a significant treatment × time interaction was only observed in the DMSO control ($F(2, 24) = 7.30$, $p = 0.003$) (Table 3.2).

In the DMSO control, NG tadpoles maintained low glucose concentrations across all timepoints and did not differ from each other ($p > 0.05$; Fig. 3.4A). In G tadpoles, glucose levels rose from 2.5 ± 2.4 mmol/L at hour 1 insignificantly to 3.7 ± 1.5 mmol/L at hour 2 ($p = 0.747$), and further to 6.1 ± 1.4 mmol/L at hour 4 ($p = 0.096$). Glucose uptake was only significant

between hours 1 and 4 (p = 0.004). Only the main effect of glucose treatment was significant for 0.068, 0.140, and 0.325 ug a.i./L of deltamethrin treatments (Table 3.2). At these concentrations, G tadpoles exhibited higher glucose uptake than NG tadpoles, but glucose levels did not change significantly over time (Fig. 3.4B–D).

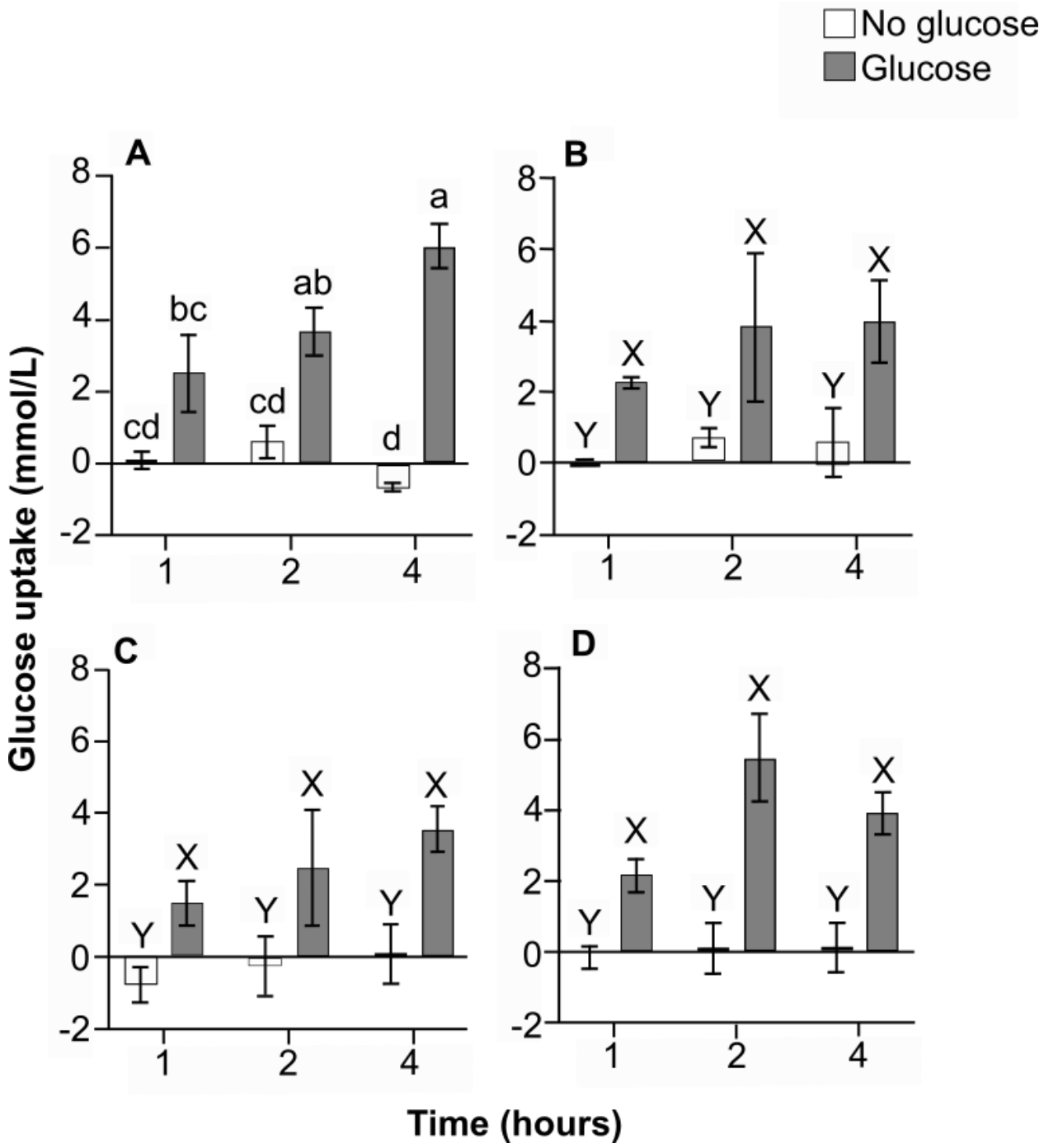

Figure 3.4. Glucose uptake measurements of wood frog tadpoles exposed to deltamethrin. Tadpoles (n = 6) were exposed for 30 days (from GS 25–30) to (A) DMSO control, (B) 0.068 µg a.i./L, (C) 0.140 µg a.i./L, or (D) 0.325 µg a.i./L of deltamethrin. Open bars represent nonglucose-administered tadpoles (placed in water only), and gray bars represent tadpoles that were placed in a 40 mM glucose solution. In (A), means with different letters (a,b,c,d) are significantly different (Tukey's post-hoc test; $p < 0.05$). In (B–D), means with different letters (X, Y) indicate a main effect of glucose treatment (Two-way ANOVA; $p < 0.05$). Values are adjusted for basal glucose levels measured at hour 0. Data are presented as the mean ± SEM.

Table 3.2. Two-way ANOVA results for glucose uptake measurements over time following glucose treatment in wood frog tadpoles exposed to deltamethrin. Tadpoles (n = 6) were exposed for 30 days (from GS 25–30) to DMSO control, 0.068, 0.140, or 0.325 µg a.i./L of deltamethrin. Shown are the main effects of treatment (no glucose or 40 mM glucose), time (hours 1–4), and the treatment × time interaction within each concentration. Asterisks denote statistical significance.

Treatment	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	*p*-value
DMSO control	Treatment	123.9	1	123.9	<0.0001*
	Time	9.9	2	5.0	0.088
	Interaction	26.8	2	13.4	0.003*
0.068 µg a.i./L	Treatment	37.9	1	37.9	0.006*
	Time	5.1	2	2.6	0.489
	Interaction	1.1	2	0.6	0.847
0.140 µg a.i./L	Treatment	36.0	1	36.0	0.002*
	Time	6.4	2	3.2	0.311
	Interaction	1.1	2	0.5	0.806

	Treatment	88.3	1	88.3	<0.0001*
0.325 µg a.i./L	Time	13.2	2	6.6	0.069
	Interaction	9.5	2	4.7	0.135

To test the suitability of the vehicle controls, hourly glucose uptake rates in tadpoles exposed to the corn and DMSO controls were compared with those of tadpoles exposed to a water control (data not shown). The glucose uptake rate of tadpoles exposed to the water control was 2.0 ± 0.4 mmol/L/h, which was significantly lower than the corn control (6.0 ± 1.0 mmol/L/h; $p = 0.0009$), but did not significantly differ from the DMSO control (2.1 ± 0.3 mmol/L/h; $p = 0.913$). These results indicate that the corn vehicle affects glucose uptake, whereas DMSO has no significant effect. The corn control was used to compare VectoBac® 200G concentrations to ensure that observed effects on glucose uptake were attributable to the insecticide rather than the vehicle.

The mean hourly glucose uptake rate differed significantly among the corn control and VectoBac® 200G-exposed tadpoles ($F(3, 56) = 6.95$, $p = 0.0005$; Fig. 3.5A). Exposure to VectoBac® 200G resulted in significantly increased glucose uptake rates compared to the corn control (6.0 ± 1.0 mmol/L/h), including 40,000 ITU/L (9.9 ± 1.4 mmol/L/h; $p = 0.022$), 100,000 ITU/L (11.2 ± 2.0; $p = 0.001$), and 200,000 ITU/L (11.0 ± 2.4 mmol/L/h; $p = 0.002$). No significant differences were detected between the three VectoBac® 200G exposure concentrations ($p > 0.05$ for all comparisons). In contrast, the mean hourly glucose uptake rate did not differ among deltamethrin treatments ($F(3, 41) = 1.06$, $p = 0.378$) (Fig. 3.5B).

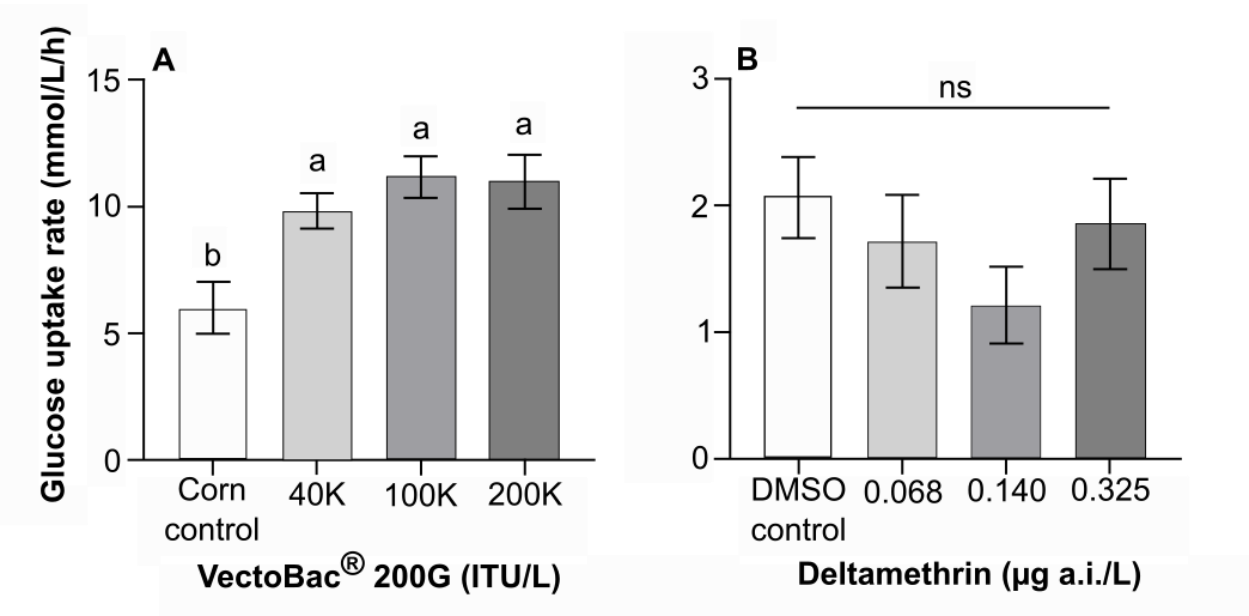

Figure 3.5. The hourly glucose uptake rate in wood frog tadpoles exposed to VectoBac® 200G or deltamethrin. Tadpoles (n = 6) were exposed for 30 days (from GS 25–30) to **(A)** corn control, 40,000 (40K), 100,000 (100K), and 200,000 (200K) ITU/L of VectoBac® 200G, or **(B)** DMSO control, 0.068, 0.140, or 0.325 µg a.i./L of deltamethrin. In **(A)**, means with different letters (a, b) are significantly different (Tukey's post-hoc test; $p < 0.05$). In **(B)**, no significant (ns; $p > 0.05$) effects of treatment were observed. Note that the Y-axis scales differ between treatment groups. Data are presented as the mean ± SEM.

3.3.3 Snout-Vent Length, Days to Complete Metamorphosis, and Hepatic Lipid Content

Neither VectoBac® 200G nor deltamethrin significantly affected the SVL of metamorphs ($p > 0.05$ for all comparisons). However, the number of days required for tadpoles to complete metamorphosis differed across VectoBac® 200G treatments ($F(3, 201) = 4.71$, $p = 0.003$; Fig. 3.6A). Post-hoc analysis indicates that tadpoles exposed to 20,000 ITU/L metamorphosed significantly later than the corn control and the 10,000 ITU/L treatment, with a mean delay of 11 days ($p < 0.0001$ for both comparisons). The mean time to metamorphosis was longest at 20,000 ITU/L (55.9 ± 1.8 days), followed by 40,000 ITU/L (51.8 ± 2.4 days), 10,000 ITU/L (47.2 ± 1.7 days), and the corn control (47.1 ± 1.6 days). No significant effects on metamorphosis were observed in tadpoles exposed to deltamethrin ($F(3, 178) = 1.196$, $p = 0.313$) (Fig. 3.6B).

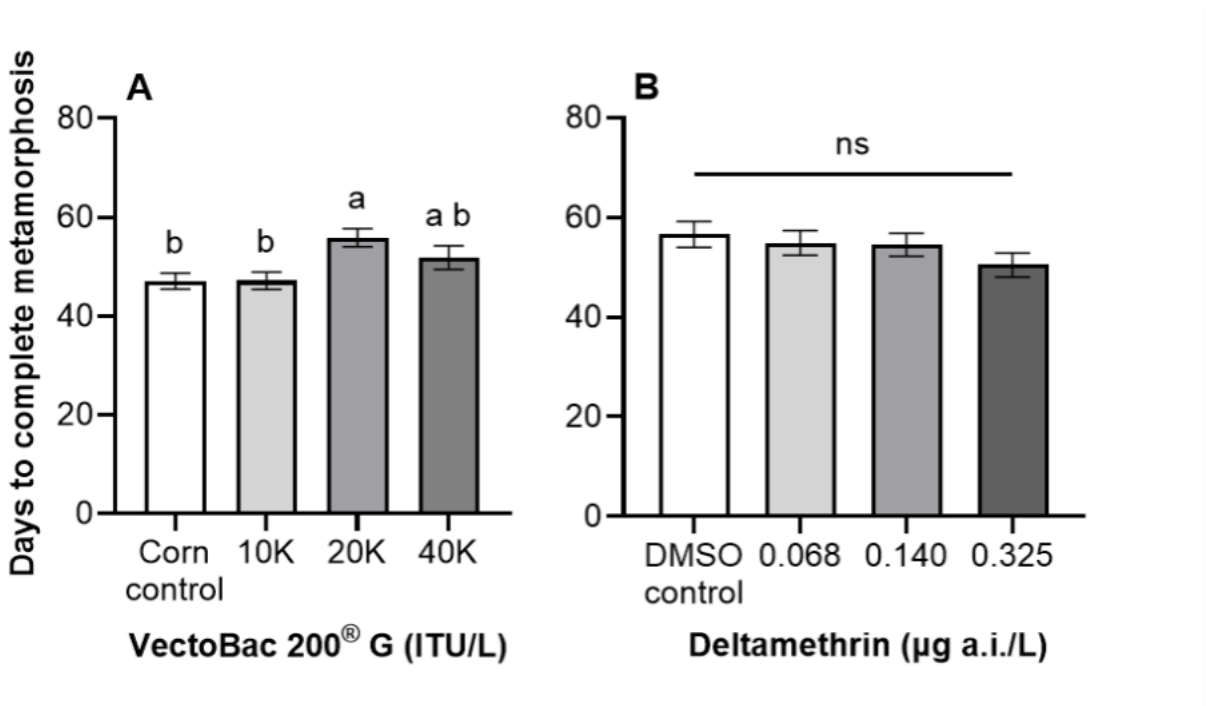

Figure 3.6. Time to complete metamorphosis in wood frogs chronically exposed to VectoBac® 200G or deltamethrin. Tadpoles (n = 10) were exposed from GS 25–46 (approximately 56 days) to **(A)** blank corn, 10,000 (10K), 20,000 (20K), or 40,000 (40K) ITU/L of VectoBac® 200G, or **(B)** DMSO control, 0.068, 0.140, and 0.325 µg a.i./L of deltamethrin. In **(A)**, different letters (a, b) denote significant differences between groups (Tukey's post-hoc test; $p < 0.05$). In **(B)**, no significant (ns; $p > 0.05$) effects of treatment were evident. Data are presented as the mean ± SEM.

A significant difference in the number of hepatic lipid droplets was observed in tadpoles exposed to VectoBac® 200G ($F(3, 48) = 8.26$, $p < 0.0002$; Fig. 3.7A). Post-hoc comparisons indicate that exposure to 20,000 ITU/L resulted in a significantly higher number of hepatic lipid droplets compared to the corn control and 10,000 ITU/L ($p = 0.031$ and $p = 0.0002$, respectively). The highest mean lipid droplet counts were in tadpoles exposed to 20,000 ITU/L (171.8 ± 43.5) and 40,000 ITU/L (96.0 ± 11.7), followed by the corn control (36.2 ± 14.5), and 10,000 ITU/L (23.5 ± 4.7). No significant difference in total lipid droplet area was observed between treatments ($F(3, 8) = 0.425$, $p = 0.741$; Fig. 3.7B); however, the average area of individual lipid droplets significantly varied ($F(3, 466) = 37.63$, $p < 0.0001$; Fig. 3.7C). Tadpoles exposed to 20,000 and 40,000 ITU/L had significantly smaller individual lipid droplet areas than all other treatments ($p < 0.0001$ for all comparisons), though they did not differ from each other

($p = 0.568$). The largest individual droplets were observed in the corn control (466.8 ± 15.9 µm²), followed by 10,000 ITU/L (437.1 ± 15.8 µm²), 40,000 ITU/L (303.5 ± 14.3 µm²), and 20,000 ITU/L (260.6 ± 11.6 µm²).

Significant differences were observed in the number of hepatic lipid droplets in tadpoles exposed to deltamethrin treatments ($F(3, 56) = 18.34$, $p < 0.0001$; Fig. 3.7D). Tadpoles exposed to 0.325 µg a.i./L had a significantly greater number of lipid droplets compared to all other treatments ($p < 0.0001$ for all comparisons). The mean highest droplet accumulation was observed at 0.325 µg a.i./L (598.6 ± 60.1), followed by 0.140 µg a.i./L (290.3 ± 42.0), the DMSO control (258.4 ± 48.1), and 0.068 µg a.i./L (148.7 ± 20.7). No significant difference in total lipid droplet area was observed between treatments ($F(3, 7) = 0.165$, $p = 0.917$; Fig. 3.7E); however, the mean area of individual lipid droplets differed ($F(3, 553) = 2.98$, $p = 0.031$; Fig. 3.7F). The individual mean droplet area of tadpoles exposed to 0.068 µg a.i./L was significantly smaller than that of tadpoles exposed to 0.325 µg a.i./L ($p = 0.016$), but did not differ significantly from other treatments ($p > 0.05$ for all comparisons). The average individual droplet area was greatest in the 0.325 µg a.i./L treatment (415.6 ± 15.2 µm²), followed by 0.140 µg a.i./L (375.9 ± 13.3 µm²), the DMSO control (373.3 ± 18.3 µm²), and 0.068 µg a.i./L (338.0 ± 24.0 µm²).

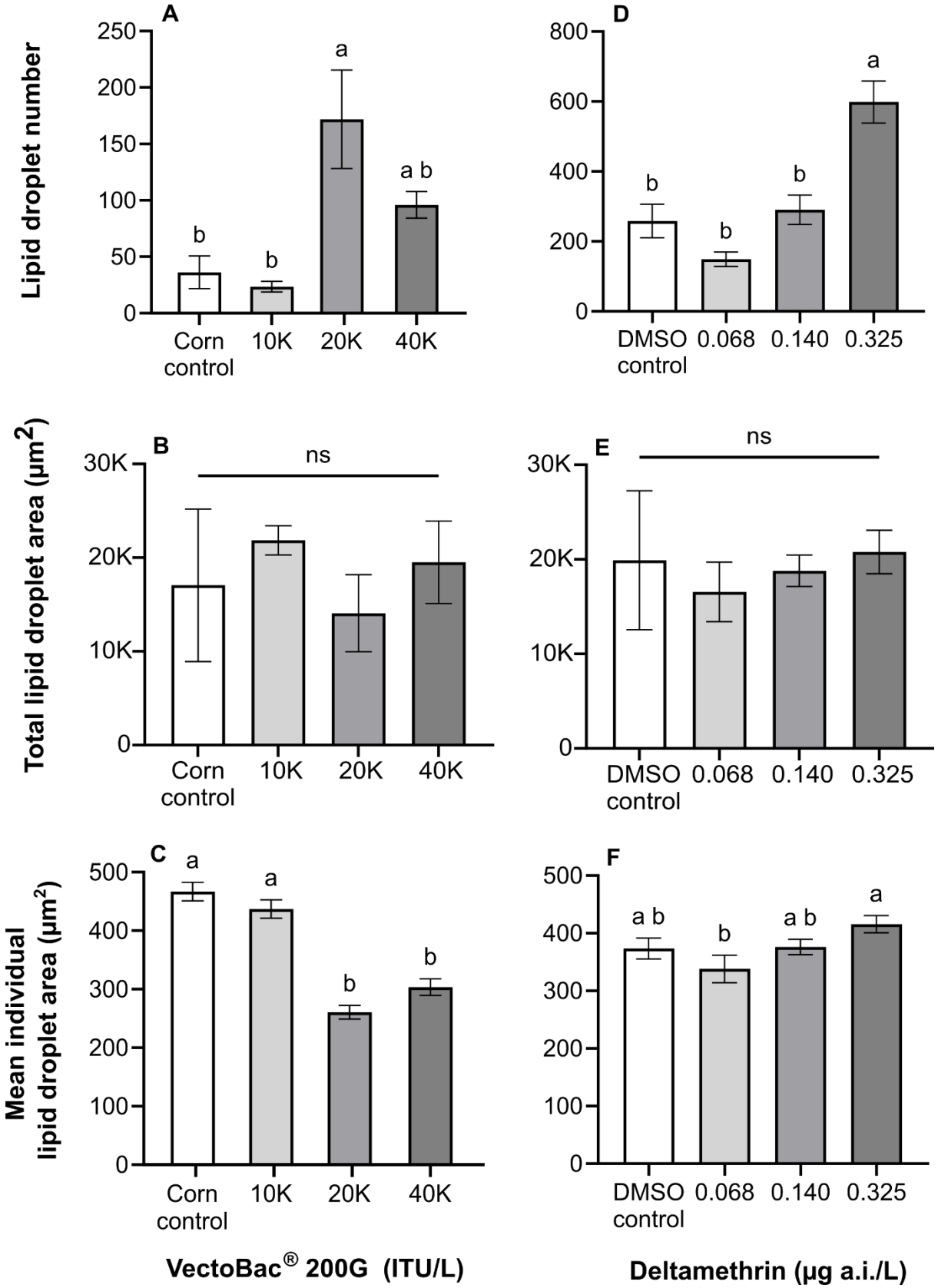
A
Lipid droplet number
250
200
150
100
50
0
b
b
a
a b
Corn control
10K
20K
40K
D
800
600
400
200
0
b
b
b
a
DMSO control
0.068
0.140
0.325
B
Total lipid droplet area (µm2)
30K
20K
10K
0
ns
Corn control
10K
20K
40K
E
30K
20K
10K
0
ns
DMSO control
0.068
0.140
0.325
C
Mean individual lipid droplet area (µm2)
500
400
300
200
100
0
a
a
b
b
Corn control
10K
20K
40K
VectoBac® 200G (ITU/L)
F
500
400
300
200
100
0
a b
b
a b
a
DMSO control
0.068
0.140
0.325
Deltamethrin (µg a.i./L)

Figure 3.7. Hepatic lipid droplet number, total lipid droplet area, and mean individual lipid droplet area in GS 46 wood frog metamorphs chronically exposed to VectoBac® 200G or deltamethrin. Tadpoles (n = 3) were exposed from GS 25–46 (approximately 56 days) to **(A–C)** blank corn, 10,000 (10K), 20,000 (20K), and 40,000 (40K) ITU/L of VectoBac® 200G, or **(D–F)** DMSO control, 0.068, 0.140, and 0.325 µg a.i./L of deltamethrin. Different letters (a, b) denote significant differences between groups (Tukey's post-hoc test; $p < 0.05$). In panels **(B-E)**, no significant (ns; $p > 0.05$) effects were observed. The Y-axis scales differ between the number of lipid droplets for **(A)** VectoBac® 200G and **(D)** deltamethrin-exposed metamorphs. Data are presented as the mean ± SEM.

3.4 Discussion

We provide evidence that the Bti insecticide VectoBac® 200G significantly increases glucose uptake in wood frog tadpoles. In the case of VectoBac® 200G, a moderate delay in metamorphosis was also observed. Both VectoBac® 200G and deltamethrin increased hepatic lipid droplet numbers, while VectoBac® 200G and low concentrations of deltamethrin significantly reduced the area of individual lipid droplets. These data suggest a disruption of metabolic processes in tadpoles exposed to these widely used insecticides.

Research on the chemical pollutants benzo(a)pyrene (BaP) or triclosan established that metabolic disruption in *Silurana* (*Xenopus*) *tropicalis* detrimentally affected metamorphosis, growth, and metabolic endpoints (Regnault et al., 2018; Usal et al., 2019; Veyrenc et al., 2022), with some effects that were irreversible and persisted in subsequent generations (Regnault et al., 2018; Usal et al., 2019). In these studies, blood glucose measurements in adult frogs indicated the development of a pre-diabetic state following exposure to these endocrine disruptors. As blood collection is not feasible in wood frog tadpoles due to their small size, we developed and validated a novel whole-body glucose assay to examine glucose dynamics during early tadpole development. Using this method, we demonstrated a classical physiological response to bovine insulin injections and fasting in wood frog tadpoles, confirming the assay's sensitivity. For example, fed tadpoles typically displayed whole body glucose levels of 5–6 mmol/L, whereas levels were less than 1 mmol/L after 3 days of fasting. We found that tadpoles could take up waterborne glucose within 1 h, and in some insecticide-treated groups, this uptake increased significantly over the 4 h test period. To our knowledge, this represents the first validated method for quantifying glucose levels in tadpoles using a glucometer. This assay provides a new tool for

assessing the effects of environmental contaminants on glucose regulation, and may be broadly applicable to other tadpole species or small organisms.

The product VectoBac® 200G consists of Bti-coated corn granules, and in our glucose uptake experiments, we observed a glucose uptake response in corn control tadpoles. It is important to note that corn is primarily composed of starch, some proteins and lipids, and micronutrients (Hasjim et al., 2009), which could contribute to glucose levels. We observed a marked increase in glucose uptake in tadpoles exposed to VectoBac® 200G compared to the corn control. This prolonged glucose uptake is a hallmark of metabolic syndrome and pre-diabetic states, such as observed in frogs exposed to BaP or triclosan, or fed high-fat diets (Usal et al., 2019; Veyrenc et al., 2022). While few studies have examined glucose regulation in amphibians, the available data suggest that various contaminants impact glucose metabolism. For example, Regnault et al. (2018) observed glucose intolerance in *S.* (*X*). *tropicalis* frogs exposed to BaP or triclosan. Although their test conditions differed from ours, where glucose was injected into the dorsal lymph sac of adults, the glucose response patterns were comparable. In control frogs, blood glucose peaked at 1 h after injection, and gradually declined thereafter, whereas blood glucose in contaminant-exposed frogs increased 1.8–2.4 fold compared to controls (Regnault et al., 2018). Similarly, tadpoles exposed to VectoBac® 200G experienced a glucose uptake rate that was almost double relative to the corn control. No studies to date have directly examined the effects of Bt insecticides on glucose uptake in amphibians, and comparisons must be drawn from studies on other vertebrates. In contrast to our findings, Mariano et al. (2019; 2021) reported that exposure to the Btk product Dipel® WP (via feed and tank water) significantly decreased plasma glucose levels in *P. mesopotamicus* and *A. gigas* fish. However, the authors stated that the results may partly be attributable to reduced food intake in their fish. Moreover, Mariano et al. (2019; 2021) measured circulating glucose, whereas our study assessed glucose uptake, which are two different metabolic endpoints. These differences reflect a large gap in the literature and highlight the novelty of our results.

Several mechanisms may account for the elevated glucose uptake we observed in VectoBac® 200G-exposed tadpoles. Bti-insecticides are effective against invertebrate larvae because the alkaline conditions of their gut (pH > 10) dissolve ingested Bti crystals and release the Cry and Cyt toxins (Cyt1Aa, Cry4Aa, Cry4Ba, and Cry11Aa) (Bravo et al., 2007). These toxins then bind to receptors on the gut wall, such as cadherin-like receptors (Pigott & Ellar,

2007), oligomerize, and insert into the membrane to form pores. These pores allow the passage of water and ions into cells, inducing swelling, lysis, and death (Knowles & Ellar, 1987). Although the vertebrate gut differs from insect larvae, it is reasonable to consider that this bioinsecticide could induce gut dysbiosis. Gutierrez-Villagomez et al. (2021) reported shifts in gut bacterial composition in *Anaxyrus americanus* metamorphs after exposure to Bti insecticides. Additionally, vegetative Btk spores were detected in rats fed Btk-contaminated food, suggesting that spores are resistant to low pH, can survive gastric passage, and may adhere to and germinate on the gut epithelium (Wilcks et al., 2006). While the health outcomes in these studies remain unclear, microbiome perturbations can influence host metabolism by altering lipopolysaccharide dynamics, short-chain fatty acid production, amino acid availability, and gut hormone signalling (Utzschneider et al., 2016).

Another likely possibility is that Bt insecticides may disrupt insulin production in pancreatic beta (β)-cells or alter insulin signalling pathways. Chronic hyperglycemia, accompanied by excess lipid accumulation, can elicit an inflammatory and oxidative stress response that promotes β-cell dysfunction and death (Cnop et al., 2005). These mechanisms are further exacerbated by changes in the expression of genes responsible for insulin secretion and βcell apoptosis (Fu et al., 2013). As glucose is the principal regulator in β-cell activity, chronic exposure to abnormal glucose levels can impair insulin synthesis, storage, and secretion (Ling et al., 1996). In conditions of insulin resistance, β-cells may compensate for insulin production through increasing in mass and number. Although adaptive, prolonged and increased insulin secretion induces cellular stress, ultimately leading to β-cell dysfunction and demise (Cerf, 2013). Evidence of Bti effects on insulin regulation in vertebrates remains limited, but the observed increases in glucose uptake and hepatic lipid accumulation in metamorphs from our study are consistent with insulin resistance, highlighting the need to further explore β-cell responses.

Although the total area of lipid droplets did not change in either treatment, the increase in lipid droplet number and decrease in mean individual droplet area from VectoBac® 200G and deltamethrin exposure is consistent with pesticide-induced hepatotoxicity, in which altered lipid accumulation and distribution are common endpoints (Yang & Park, 2018). This redistribution of lipid droplets is characteristic of microvesicular steatosis (Wei et al., 2008) and is often associated with contaminant-induced impairment of fatty acid oxidation in the liver (Wahlang et

al., 2016). Studies on the effects of Bt-products on vertebrates further support these results. Mariano et al. (2019; 2021) reported changes in liver histopathology, including lipid accumulation, without concurrent changes in body weight. These findings are consistent with our results that Bti products can alter liver conditions without necessarily altering body size. Similarly, Li et al. (2025) reported that *Paralichthys olivaceus* fish exposed to 0.07–0.28 µg/L of deltamethrin exhibited lipid accumulation, fibrosis, cell congestion, and necrosis in liver cells. Consistent with our study, fish exposed to the highest deltamethrin concentration exhibited the greatest amount of hepatic lipid accumulation. Fish also exhibited elevated oxidative stress and inflammatory markers, as well as disruptions to metabolic pathways, including steroid biosynthesis (Li et al., 2025). Similar cellular stress responses may have occurred in the metamorphs in our study, thereby altering lipid droplet dynamics.

We did not observe changes in the SVL in metamorphs chronically exposed to VectoBac® 200G or deltamethrin; however, exposure to the Bti insecticide delayed metamorphosis. Our results are largely consistent with those of Gutierrez-Villagomez et al. (2021), who reported no differences in body length at metamorphosis in *L. sylvaticus* metamorphs chronically exposed to VectoBac® 200G or 1200L, but observed differences in the number of days to complete metamorphosis. However, some discrepancies between results were observed. In the present study, *L. sylvaticus* tadpoles exposed to VectoBac® 200G required an average of 51.6 days to complete metamorphosis, while Gutierrez-Villagomez et al. (2021) reported a mean time to metamorphosis of approximately nine days shorter, despite using lower exposure concentrations. Furthermore, their finding that exposure to 20,000 ITU/L accelerated metamorphosis to 39 days directly contrasts with our result of 55.9 days at the same concentration. This discrepancy may reflect differences in experimental design, population sensitivity, or environmental factors such as diet, density, or water chemistry. Nevertheless, these results indicate that VectoBac® products may impact amphibian development. Although mechanisms vary by contaminant, pesticides can interfere with critical aspects of metamorphosis, especially thyroid hormone pathways. The mechanism by which Bti insecticides influence metamorphosis is unknown; however, the delayed development observed in our study is consistent with effects expected from disrupting endocrine pathways that regulate metamorphosis.

Although concentrations used in this study exceed the label application rates of VectoBac® 200G, comparable exposures may occur under repeated applications, the use of highpotency products, or long-term environmental persistence. For example, products such as Introban® have varying application rates under different environmental conditions and deliver a higher toxic units per application (potency of 1,200 ITU/mL). In addition, climate warming is expected to affect mosquito populations and breeding seasons (Wang et al., 2024), potentially increasing the frequency and intensity of mosquito control product use. Species-specific sensitivity further increases the relevance of our exposure range. For instance, we calculated the 96 h LC_{50} of the leopard frog (*Lithobates pipiens*) to be 78,860 ITU/L (See Chapter 2; Empey et al., 2025), indicating greater sensitivity relative to the wood frog (96 h LC_{50} of 525,363 ITU/L). Exposures overlapping with our study have also been used in other amphibian studies. Lajmanovich et al. (2015) exposed *Leptodactylus latrans* tadpoles to 48,000 ITU/L (comparable to our 40,000 ITU/L concentration) to Introban® for 48 h, and observed oxidative stress, genotoxicity, and intestinal histopathology in tadpoles. The environmental persistence of Btinsecticides may also reflect our exposure rates. The spore density of Bti has been reported to increase up to 500-fold in treated sites relative to controls, with concentrations remaining elevated 3–4 years after the cessation of applications (Poulin et al., 2022). Persistence is influenced by environmental conditions, including vegetation, soil pH, light availability, temperature, and water turbidity, all of which affect spore survival and bacterial germination (Duchet et al., 2014). Moreover, Bti is unlikely to be uniformly distributed throughout the water column and may accumulate in some areas more than others. Indeed, the application rates in our study are higher than typically used, but these rates provide insight into potential effects on more sensitive amphibian populations that are exposed to higher-potency product formulations, subjected to repeated treatments, or inhabiting environments where Bti persists.

3.5 Conclusion

VectoBac® 200G significantly increased glucose uptake in wood frog tadpoles, whereas deltamethrin had minimal effects at tested concentrations; however, both insecticides induced traits consistent with contaminant-induced hepatotoxicity. The mechanisms by which Btinsecticides may affect glucose metabolism remain unclear, but disruption of insulin signalling and β-cell function may contribute to the insulin-resistant phenotype observed in our results. Deltamethrin is often applied at concentrations higher than those used in this study, warranting further investigation into its metabolic effects at environmentally relevant levels. Our results

suggest that Bt and deltamethrin insecticides can affect metabolic processes, with downstream effects on developmental endpoints. These findings carry important implications for population health and underscore the complexity of insecticide effects on non-target organisms, highlighting the need to assess the ecological safety of these agents.

Chapter 4. Effects of *Bacillus thuringiensis israelensis* and deltamethrin on insulin regulation in wood frog (*Lithobates sylvaticus*) metamorphs

Study contributions: Madelaine A. Empey conceived of, designed, and conducted experiments, analyzed data, and wrote the manuscript; Brianna H. Raven provided training and guidance for methods and imaging; Vance L. Trudeau conceived of and designed experiments, revised the manuscript, and acquired funding for the study.

Abstract

Contaminant-induced disruption of metabolic regulation may affect growth, development, and cryoprotectant adaptations in amphibians, such as rapid glucose mobilization to cells. Many insecticides have been reported to disrupt metabolic processes; however, little research has examined the effects of *Bacillus thuringiensis israelensis* (Bti) and deltamethrin. In Chapter 3, we demonstrated that exposure to the Bti insecticide, VectoBac® 200G, significantly increased glucose uptake. Exposure to both VectoBac® 200G and deltamethrin altered hepatic lipid dynamics in the wood frog (*Lithobates sylvaticus*). In the present study, we used a custom rabbit polyclonal anti-frog insulin B-chain antibody to examine the effects of these insecticides on insulin in the pancreas of wood frog metamorphs. Animals were exposed from a young tadpole stage to metamorphic climax (GS 25–46; average of 56 days) to 40,000 ITU/L of VectoBac® 200G or 0.325 μg a.i./L of deltamethrin. Metamorphs exposed to VectoBac® 200G exhibited a 76.3% increase in the number of insulin-immunoreactive (INS-IR) cells and a 21.3% increase in total area of insulin-positive staining. However, staining per INS-IR cell decreased by 57%, suggesting that although beta (β)-cell number increased, insulin content per cell was reduced. This reduction could reflect decreased insulin production, increased release, or altered insulin storage. In contrast, deltamethrin did not affect the number of INS-IR cells or the area of insulin staining, although the proportion of INS-IR cells relative to other cell types increased significantly. Both insecticides increased the nuclear radius in INS-IR and non-INS-IR cells. Overall, VectoBac® 200G altered pancreatic insulin regulation in metamorphs by increasing βcell number and total insulin staining per pancreas, while reducing cellular insulin content, suggesting compensatory changes consistent with insulin resistance. These findings provide novel insight into the mechanistic effects of VectoBac® 200G and deltamethrin on endocrine metabolic endpoints in the wood frog.

4.1 Introduction

Glucose homeostasis is essential for maintaining energy and supporting normal physiological function, growth, and development in vertebrates (Mergenthaler et al., 2014). This process is primarily regulated by insulin and glucagon, which respectively decrease and increase blood glucose levels (Karpińska & Czauderna, 2022). Disruption of these hormonal pathways impairs glucose regulation, causes insulin resistance, and may lead to metabolic disorders such as diabetes (Bouwens & Roomen, 2005). While the pancreas is primarily composed of exocrine tissue, the islets of Langerhans contain endocrine cells that are essential for glucose regulation (Karpińska & Czauderna, 2022). These islets contain alpha (α), beta (β), and delta (δ) cells, as well as pancreatic polypeptide cells, which have been identified in amphibian species including *Xenopus, Bufo orentialis*, and *Rana temporaria* (El-Salhy et al., 1982; Pearl et al., 2009). In *Xenopus,* the islets primarily contain the insulin-secreting β-cells; whereas other cell types are dispersed throughout the pancreas or loosely arranged around the islets (Horb & Slack, 2002; Maake et al., 1998).

In humans, insulin is a 51-amino acid peptide composed of an A-chain (21 amino acids) and a B-chain (30 amino acids) (Ward & Lawrence, 2011), with a molecular weight of approximately 5.8 kDa (Fu et al., 2013). Two disulphide bonds connect the two chains, and the A-chain possesses an additional internal disulphide bond (Abel, 1926). Although insulin is a highly conserved peptide, differences in amino acid sequences have been reported in numerous amphibian species (Conlon et al., 1998a; Conlon et al., 1998b). Insulin secretion is primarily stimulated by glucose but can also be induced by fatty acids, amino acids, and monosaccharides (Fu et al., 2013). In pancreatic β-cells , glucose metabolism increases the ATP:ADP ratio, leading to the closure of ATP-sensitive potassium channels (Sekine et al., 1994). This depolaizes the β-cell membrane and opens voltage-gated calcium channels, resulting in calcium influx that triggers the exocytosis of insulin-containing secretory granules (Rorsman & Ashcroft, 2018). After release, insulin circulates in the blood and acts on insulin receptors on cells in target tissues such as skeletal muscle, adipose tissue, and the liver. Insulin reduces blood glucose by stimulating glycogen synthesis and suppressing hepatic glucose production (Fritsche et al., 2018; Taneera et al., 2019).

Growing evidence shows that environmental contaminants can disrupt metabolic pathways, but relatively little is known about their effects on insulin and glucose regulation in amphibians. Regnault et al. (2014) demonstrated that exposure of female *Xenopus tropicalis* to

10 µg/L of benzo(a)pyrene (BaP) for 24 hours induced hyperglycemia and hepatic lipid accumulation. While acute exposure led to rapid disruptions in glucose metabolism, long-term exposure produced more persistent metabolic dysfunction. In a subsequent study, Regnault et al. (2018) chronically exposed female *X. tropicalis* to 50 ng/L of BaP or triclosan from the tadpole stage through adulthood. Exposed frogs displayed symptoms consistent with prediabetes, including glucose intolerance, liver steatosis, and insulin hypersecretion. Although insulin production in insecticide-exposed frogs increased twofold, glucose tolerance was reduced, with a 1.8- to 2.4-fold increase in blood glucose levels during glucose tolerance tests. In both studies, gene pathway analysis revealed associations between the insecticides and alterations in metabolic pathways, steroid hormone synthesis, and xenobiotic metabolism. Exposed frogs also exhibited an increase in transcription of enzymes involved in gluconeogenesis. Together, these changes are consistent with the diagnosis of insulin resistance and prediabetes.

The purpose of the current study was to determine whether VectoBac® 200G and deltamethrin affect β-cells in wood frog metamorphs, potentially providing a mechanistic link to the alterations in glucose regulation and hepatic lipid dynamics observed in Chapter 3. We found that VectoBac® 200G and deltamethrin increased the proportion and nuclear radius of insulinimmunoreactive (INS-IR) cells. An increase in the total number of INS-IR cells and total area of pancreatic insulin staining was significant only in VectoBac® 200G-exposed metamorphs. Insulin content per INS-IR cell was reduced, suggesting altered insulin production, release, or storage. These findings suggest that VectoBac® 200G can disrupt both glucose and insulin homeostasis in wood frog metamorphs.

4.2 Materials and Methods

4.2.1 Animals and insecticide exposure

Wood frogs (*Lithobates sylvaticus*) were used for all experiments. Tadpoles were exposed to insecticides from GS 25 to metamorphic climax (GS 46; approximately 56 days). Exposure concentrations consisted of (1) a corn control (n = 5) and 40,000 ITU/L VectoBac® 200G (n = 4); or (2) a DMSO control (n = 6) and 0.325 µg a.i./L deltamethrin (n = 5). Sublethal exposure concentrations were calculated based on previous LC_{50} values (Chapter 2) and glucose uptake results (Chapter 3). For information on breeding, housing, and insecticide acquisition, refer to the Materials and Methods section of Chapter 2.

4.2.2 Immunofluorescence staining and imaging

At GS 46, frogs were euthanized using 5 g/L MS-222 buffered with sodium bicarbonate (Torreilles et al., 2009). Limbs were removed, and a superficial abdominal incision was made to facilitate the fixation of internal organs. Whole frog bodies were fixed in 10% non-buffered formalin for 72 h on a shaker (set at a speed of 4, The Belly Dancer™, Stoval Life Science), then transferred to 70% ethanol. Samples were sent to the Louise Pelletier Histology Core (University of Ottawa) for paraffin embedding and sectioning. Animals were embedded in a prone orientation, sectioned at 4 μm, and mounted on Superfrost Plus™ microscope slides (25 × 75 × 1 mm; Fisher Scientific, Cat# 1255015) with cover slips (24 × 60 × 1.5 mm; Corning, Cat# 2980246). Slides were stored at 4℃ for up to 1 week prior to immunofluorescence staining. Due to sectioning artifacts such as tissue loss during processing, torn sections, and instances where the pancreas was not captured in the sectioning pane, the sample size differed between treatments.

Slides were heated (Fisher Scientific 12-594-5V3 Slide Warmer) at 60℃ for 30 minutes to melt the paraffin and dehydrate samples. Using Coplin jars, slides were then deparaffinized in xylene twice, each for 20 minutes. Samples were rehydrated through a graded ethanol series (100% and 95% twice for 10 minutes each, followed by 70% for 10 minutes) and rinsed once with 1x phosphate-buffered saline (PBS) for 10 minutes. Antigen retrieval was performed by immersing slides in 0.01 M sodium citrate buffer (pH 6.0) in an 85–95℃ water bath (Haake Model D1 Heated Water Bath Circulator W13) for 25 minutes. Slides were cooled at room temperature for 10 minutes, then rinsed twice in 1x-PBS containing 0.1% Triton X-100 (PBS-T) for 3 minutes each. Endogenous peroxidase activity was quenched by incubating samples in 3% hydrogen peroxide containing 0.1% Triton X-100 for 15 minutes. Slides were rinsed twice in PBS-T for 5 minutes each and incubated in blocker buffer for 1 h at room temperature. Samples were then incubated overnight at 4℃ with 150 μL of the primary antibody diluted to 1:200 in blocking buffer. Because no insulin antibodies specific to wood frogs are commercially available, the primary antibody used was a custom-made rabbit anti-frog insulin polyclonal antibody (Biomatik Corporation, product ID AB016031; 98% purity). This antibody was raised against a conserved domain in the insulin B-chain from the Northern leopard frog (*Lithobates pipiens*), a species closely related to the wood frog and for which a genome assembly is available (Table 4.1). Following primary antibody incubation, slides were washed in PBS-T twice for 10

minutes each. During the washes, the secondary antibody was centrifuged at 2,000 × g for 10 minutes (Benchmark MyFuge™ Mini, Benchmark Scientific) while covered from light to reduce precipitate formation. Samples were then incubated in the dark for 2 h at room temperature with 150 µL goat anti-rabbit Alexa Fluor 647 secondary antibody (Invitrogen, catalogue# A-21245) diluted to 1:500. While kept in the dark, slides were washed twice in 1x-PBS for 10 minutes each and incubated with 60 µl of TrueVIEW® Autofluorescence Quenching Kit (Vector Laboratories, product# SP-8400-15) for 8 minutes. Slides were rinsed in 1x-PBS for 5 minutes, and excess buffer was gently removed around the samples using KimWipes™. Samples were mounted in 40 µl of VECTASHIELD® Antifade Mounting Medium with DAPI (Vector Laboratories, product# H-1200-10). Samples were allowed to cure for 1–2 h in the dark before coverslips were sealed with clear nail polish. Slides were imaged using a Zeiss Axio Imager Z.2 Upright Fluorescence Microscope. Serial images spanning the entire liver were acquired as Z-stacks and processed using the Extended Depth of Focus function in ZEN 3.2.0.

Preliminary analyses in non-insecticide-exposed metamorphs indicated that the average nuclear diameter of INS-IR cells is approximately 4 µm; therefore, whole cells were counted on every third section to reduce the likelihood of counting the same cell twice, yielding three series (S1, S2, S3) (Fig. 4.1). Counts from the three series were averaged for each individual prior to statistical analysis. Cells with nuclei intersecting the edge of sections were not counted. CellProfiler (version 4.2.8) was used for automated individual cell segmentation based on DAPIstained nuclei. As cytoplasmic staining was not used, nuclear measurements were performed rather than estimating whole-cell dimensions. An intensity threshold was established to classify pixels as positive for immunoreactivity. The software was used to quantify the total number of cells, the number of INS-IR cells, the mean nuclear radius, the total area of insulin staining per pancreas, and the average insulin staining per INS-IR cell.

All statistical analyses were performed using R (version 4.5.2), and figures were created in GraphPad Prism (version 10.6.1). To account for the division of the pancreas into three series, linear mixed-effects models (LMMs) were used with series treated as a random effect nested within individual frogs to avoid pseudoreplication. A separate LMM was used to analyze nuclear radius, with treatment (control or insecticide) and cell type (non-INS-IR cells vs INS-IR cells) as fixed effects, and series nested within individual frogs as a random effect. Comparisons between treatments and cell types were performed using Tukey's post-hoc test ($\alpha = 0.05$).

Table 4.1. Comparison of the B-chain sequence of multiple amphibian species and insulin antibody epitope. Highlighted regions indicate similarities between the B-chain amino acid sequence and the antibody epitope. In parentheses is the substitution of Cysteine (C) with Serine (S).

	Amino acid sequence	**Reference**
Leopard frog, *Lithobates pipiens*	FDNOYL**CGSHLVEALYMV**C**GDRG**FFYSPRS	Conlon et al., 1998a; The Uniprot Consortium, 2025
Wood frog, *Lithobates sylvaticus*	FPNQHL**CGSHLV**D**ALYMV**C**GDRG**FFYSPRS	Conlon et al., 1998a
Bullfrog, *Lithobates catesbeiana*	FPNQYL**CGSHLVEALYMV**C**G**E**RG**FFYSPRS	Conlon et al., 1998a; The Uniprot Consortium, 2025
African clawed frog, *Xenopus laevis*	LANQHL**CGSHLVEALY**L**VC**G**DRG**FFYYPKI	The Uniprot Consortium, 2025
Antibody epitope	CGSHLVEALYMV(S)GDRG	

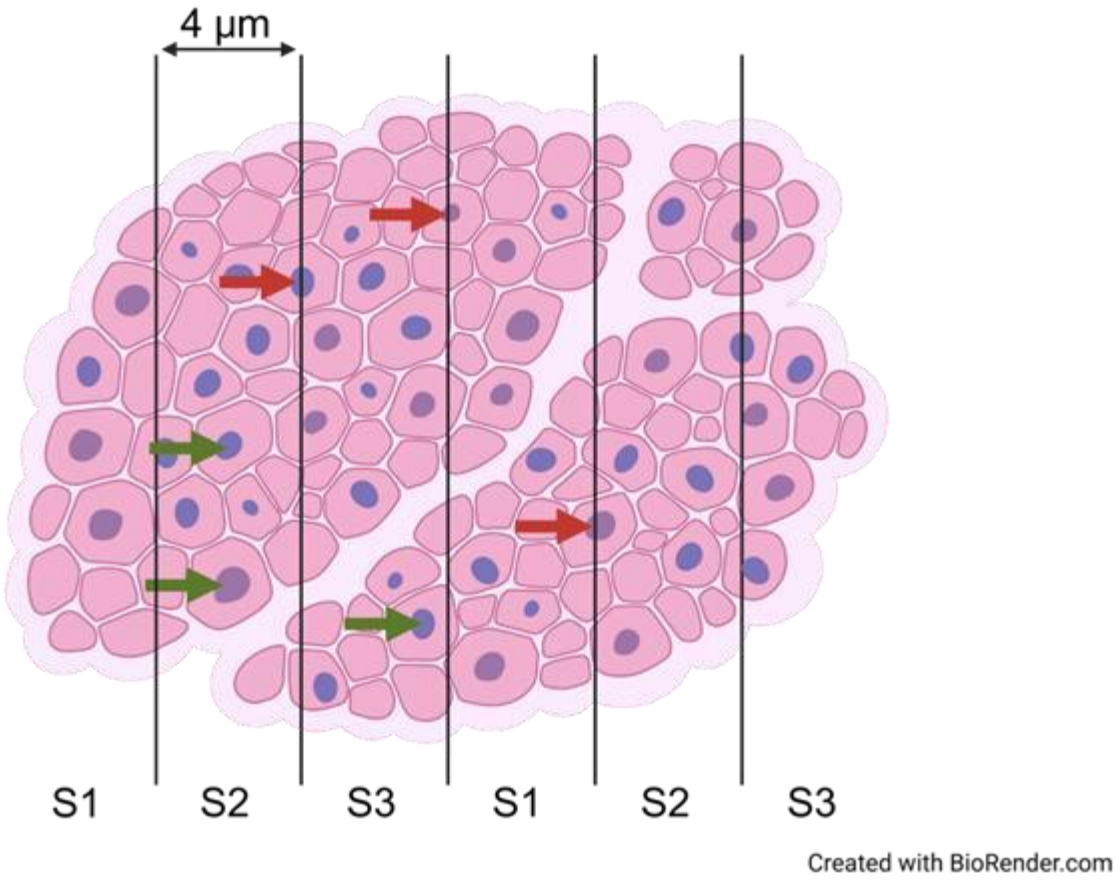

Figure 4.1. Schematic representation of the pancreatic sectioning series used for wood frog metamorphs. Serial transverse sections were cut at a thickness of 4 µm, with S indicating the sequence number. Green arrows denote representative cells that met the inclusion criterion for counting. Red arrows indicate nuclei that intersected section boundaries that were not counted.

4.2.3 Validation of the specificity of the insulin primary antibody

The specificity of the primary insulin antibody was validated using a series of blocking and control treatments to distinguish immunoreactivity from potential nonspecific binding. For this experiment, GS 46 wood frog metamorphs that were not exposed to any insecticide treatments were used. Consecutive pancreatic tissue sections were mounted sequentially across multiple slides. This method ensures visual confirmation that cells identified as immunoreactive in the positive control show a loss of signal in the blocking and negative control treatments. Slides were treated with: (a) primary antibody incubated with the secondary antibody (positive control); (b) pre-absorbed primary antibody with 20 µM of the custom insulin B-chain antigenic peptide (BioMatik Corporation, peptide ID 1145993); (c) pre-absorbed primary antibody with 20 µM bovine insulin (Sigma-Aldrich, product # I0516); (d) deletion of primary antibody to test for

potential secondary antibody binding; (e) blocking buffer only with no primary or secondary antibody (negative control).

4.3 Results

4.3.1 Verification of insulin antibody in wood frog pancreas

As the insulin B-chain antibody developed for this study is novel, it was necessary to first determine if immunoreactivity was detectable in the wood frog pancreas. It was determined that the insulin antibody produced the strongest signal at a 1:200 dilution, and immunoreaction was present across all treatment exposures. INS-IR cells were shown to be present throughout the pancreas, often clustering centrally within the tissue. Figure 4.2 presents photographs of immunoreactions in the pancreas of frogs treated with the corn control (25 INS-IR cells, Fig. 4.2A), 40,000 ITU/L of VectoBac® 200G (99 INS-IR cells, Fig. 4.2B), the DMSO control (28 INS-IR cells, Fig. 4.2C), and 0.325 µg a.i./L deltamethrin (56 INS-IR cells, Fig. 4.2D).

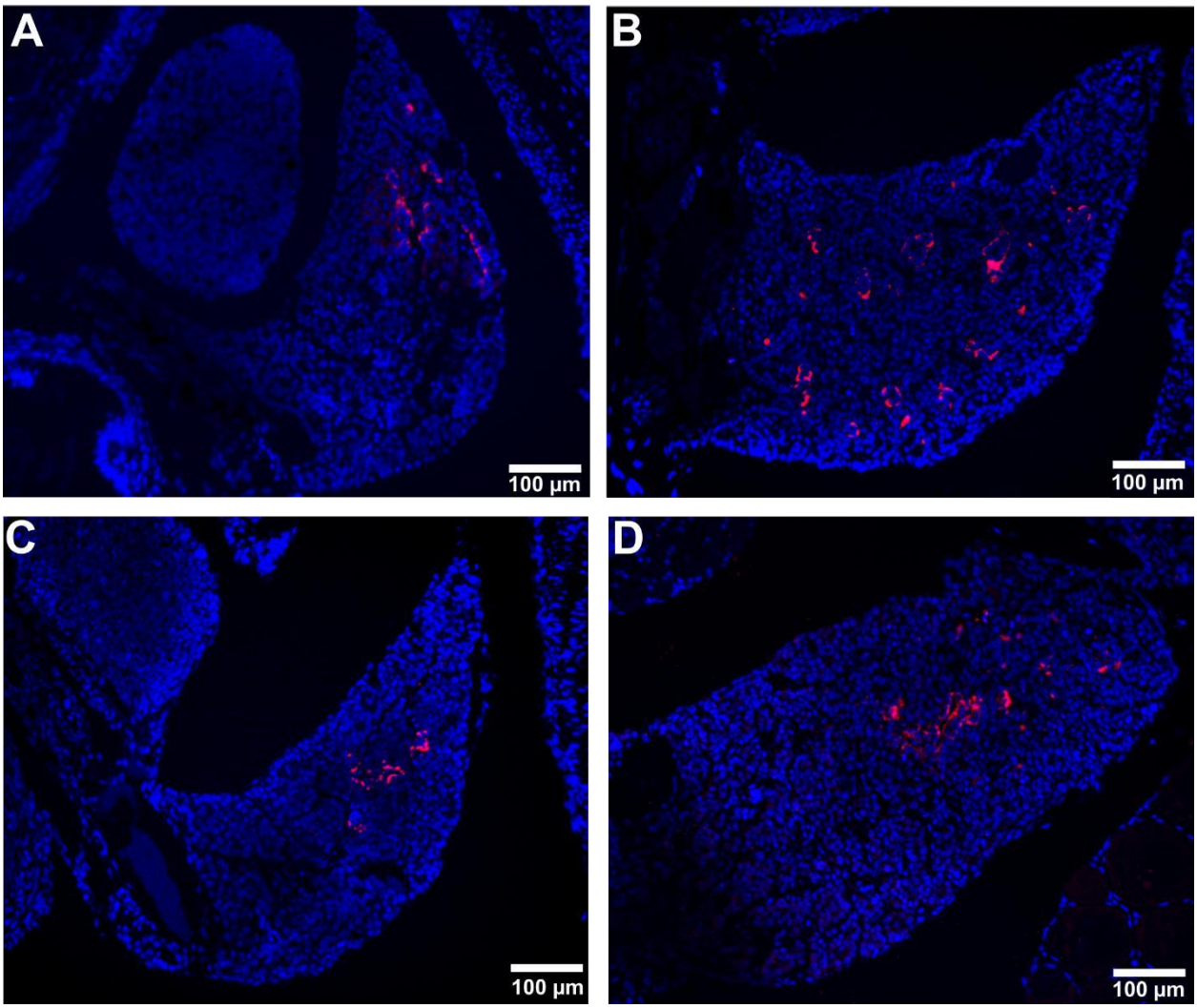

Figure 4.2. Representative photos of the pancreas of wood frog metamorphs exposed to controls, VectoBac® 200G, and deltamethrin incubated with the insulin antibody. Frogs were exposed from GS 25 to metamorphic climax (GS 46; approximately 56 days) to **(A)** corn control, **(B)** 40,000 ITU/L of VectoBac® 200G, **(C)** DMSO control, and **(D)** 0.325 µg a.i./L of deltamethrin. Red fluorescence indicates B-chain insulin immunoreactivity, and blue indicates DAPI staining of cell nuclei. Scale bars represent 100 µm. Samples were imaged using a Zeiss Axio Imager Upright Fluorescence Microscope at 10X magnification.

To test antibody specificity, blocking agents were used. Normal reactivity was observed in response to the positive control (17 INS-IR cells, Fig. 4.3A). No reaction was observed in tissue blocked with 20 µM insulin antigenic peptide (0 INS-IR cells, Fig. 4.3B) or 20 µM bovine insulin (0 INS-IR cells, Fig. 4.3C). No reaction was observed in the pancreas that was incubated with the secondary antibody (0 INS-IR cells, Fig. 4.3D) or in the negative control with blocking

buffer only (0 INS-IR cells, Fig. 4.3E). As the validation of the antibody was successful, INS-IR cells are deemed to be pancreatic β-cells.

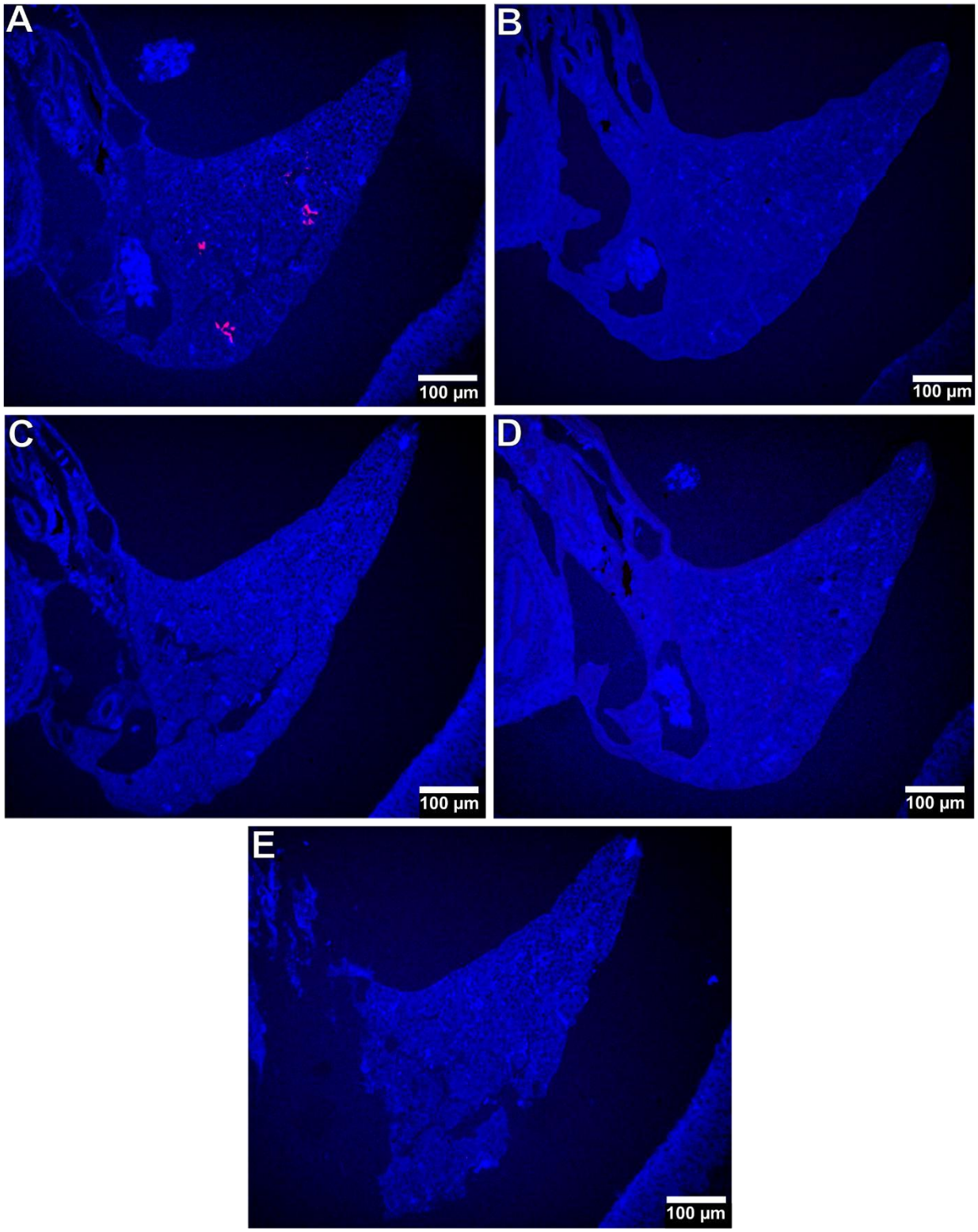

Figure 4.3. Blocking immunoreactivity confirmed the specificity of the primary insulin antibody in the pancreas of GS 46 wood frog metamorphs. (A) 1:200 primary antibody

incubates with 1:500 secondary antibody; **(B)** 1:200 primary antibody pre-absorbed with 20 µM insulin antigen peptide; **(C)** 1:200 primary antibody pre-absorbed with 20 µM bovine insulin; **(D)** Pancreas incubated with 1:500 secondary antibody only; **(E)** Pancreas incubated with blocking buffer only. Red fluorescence indicates B-chain insulin immunoreactivity, and blue indicates DAPI staining of cell nuclei. Samples were imaged using a Zeiss Axio Imager Upright Fluorescence Microscope at 10X magnification.

4.3.2 Effects of insecticide treatments on pancreatic cells and insulin staining

A difference in the number of INS-IR cells was observed in the pancreas between metamorphs exposed to the corn control and VectoBac® 200G treatments ($F(1, 22) = 12.69$, $p = 0.0018$; Fig. 4.4A). Metamorphs exposed to the corn control had an average of 16.9 ± 2.6 INS-IR cells, while metamorphs exposed to VectoBac® 200G had an average of 37.8 ± 8.3 INS-IR cells. This difference represents a 76.3% increase in the number of β-cells in metamorphs exposed to VectoBac® 200G relative to the corn control. In contrast, the number of INS-IR cells did not differ significantly between the DMSO control and deltamethrin treatment ($F(1, 28) = 2.54$, $p = 0.122$; Fig. 4.4B). The number of INS-IR cells averaged 24.7 ± 2.5 in the DMSO control and 31.8 ± 4.4 in the deltamethrin treatment.

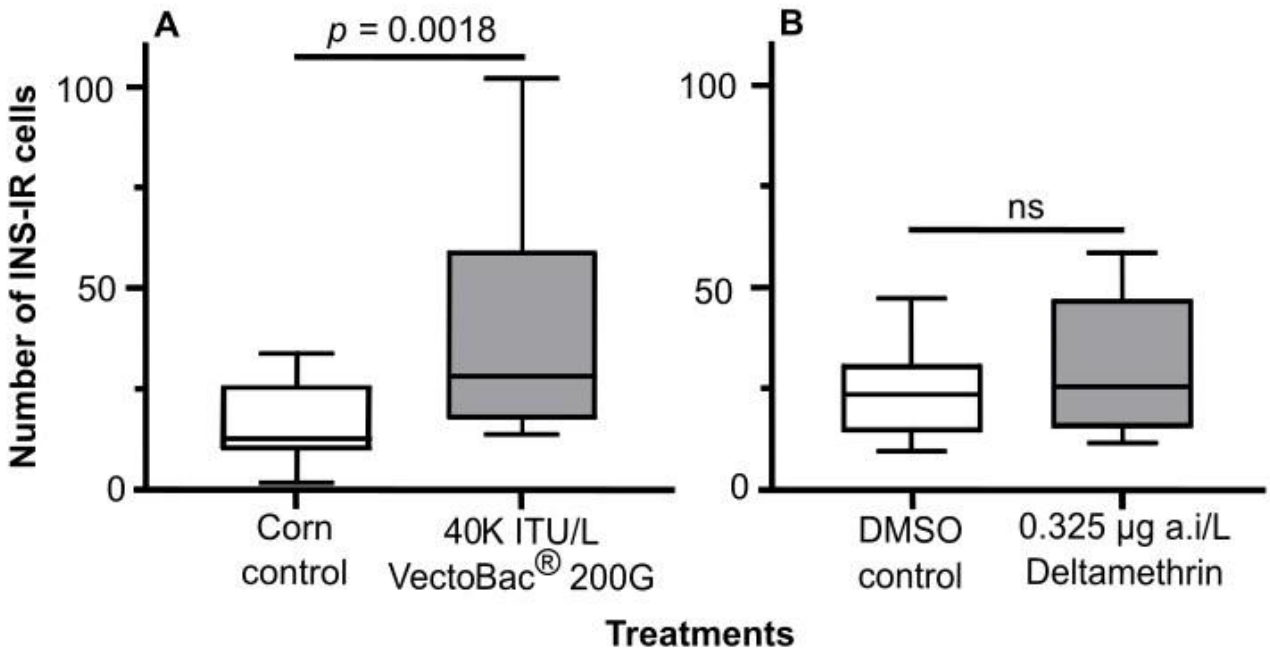

Figure 4.4. The number of INS-IR cells in wood frog metamorphs exposed to VectoBac® 200G and deltamethrin. Frogs were exposed from GS 25 to metamorphic climax (GS 46;

approximately 56 days) to VectoBac® 200G or deltamethrin, and their respective controls. (**A)** Number of INS-IR cells following exposure to the corn control (n = 5) or 40,000 (40K) ITU/L of VectoBac® 200G (n = 4); a significant difference between treatments was detected (linear mixed models, $p = 0.0018$). **(B)** Number of INS-IR cells following exposure to the DMSO control (n = 6) or 0.325 µg a.i./L (n = 5); no significant (ns, $p > 0.05$) differences between treatments were observed. Data are presented as boxplots showing the minimum, first quartile, median, third quartile, and maximum values.

A difference in the percentage of INS-IR cells was detected between the corn control and VectoBac® 200G treatment ($F(1, 22) = 16.19$, $p = 0.0006$; Fig. 4.5A). The percent of INS-IR cells in the insecticide treatment averaged 2.1 ± 0.3%, which was twice as high as the percent of INS-IR cells in the corn control (average of 1.0 ± 0.1%). A difference in the percentage of INS-IR cells was also detected between the DMSO control and deltamethrin treatment ($F(1, 26.4) = 6.38$, $p = 0.018$; Fig. 4.5B). The percent of INS-IR cells in the deltamethrin treatment averaged 1.95 ± 0.18%, which was 21.4% higher than in the DMSO control (1.6 ± 0.2%).

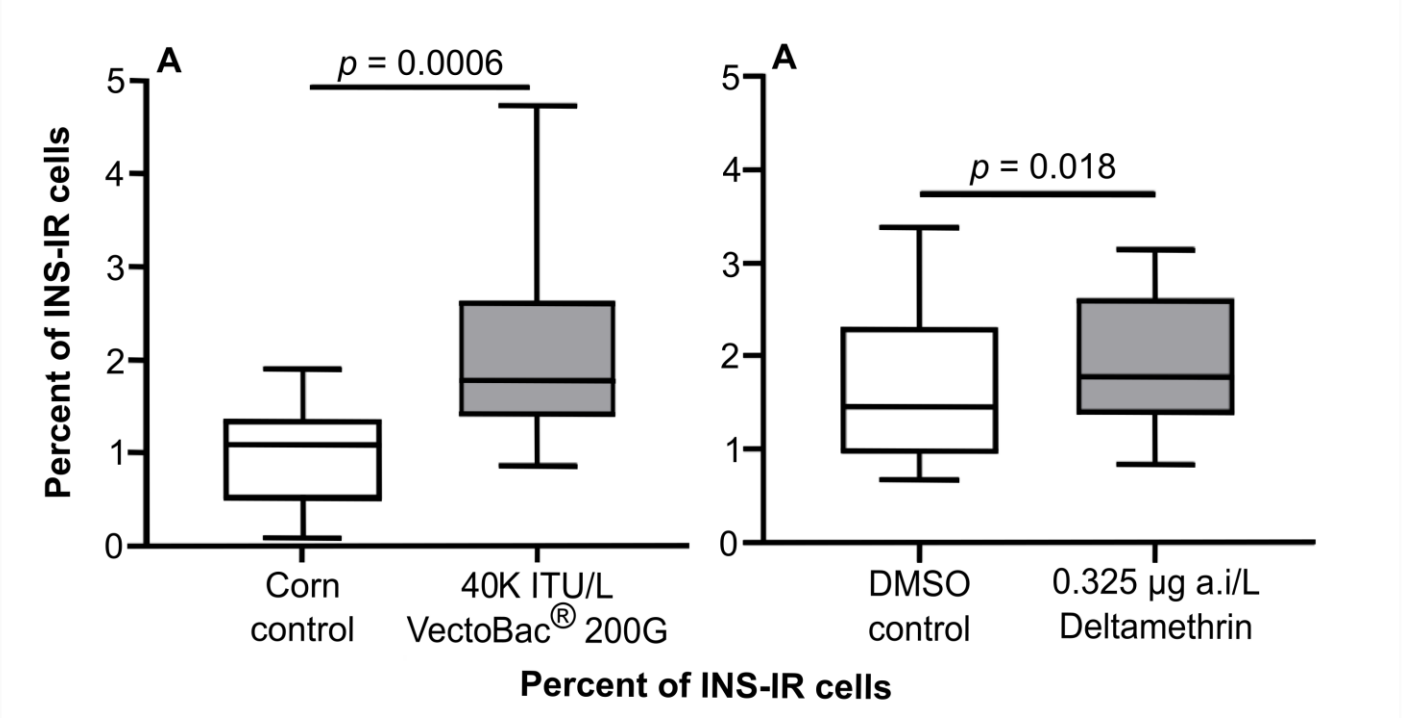

Figure 4.5. The percentage of INS-IR cells in wood frog metamorphs exposed to VectoBac® 200G or deltamethrin. Frogs were exposed from GS 25 to metamorphic climax (GS 46; approximately 56 days) to VectoBac® 200G or deltamethrin, and to their respective controls. (**A)** Percentage of INS-IR cells following exposure to the corn control (n = 5) or 40,000 (40K) ITU/L

of VectoBac® 200G (n = 4); a significant difference was observed between treatments (linear mixed models, $p = 0.0006$). **(B)** The percentage of INS-IR cells following exposure to the DMSO control (n = 6) or 0.325 µg a.i./L of deltamethrin (n = 5); a significance between treatments was observed (linear mixed models, $p = 0.018$). Data are presented as boxplots showing the minimum, first quartile, median, third quartile, and maximum values.

Significant main effects of treatment (corn control vs VectoBac® 200G; $F(1, 46) = 91.23$, $p < 0.0001$) and cell type (non-INS-IR and INS-IR cells; $F(1, 46) = 78.28$, $p < 0.0001$) on the nuclear radius of pancreatic cells were evident. A significant treatment × cell-type interaction was also observed ($F(1, 46) = 6.15$, $p = 0.017$). Within corn controls, INS-IR cells exhibited significantly larger nuclei than non-INS-IR cells (2.00 ± 0.04 µm vs 1.79 ± 0.04 µm; $p < 0.0001$; Fig. 4.6A). Within the VectoBac® 200G treatment, the nuclei of INS-IR cells were also larger than those of non-INS-IR cells (2.12 ± 0.02 µm vs 2.00 ± 0.04 µm, $p = 0.0007$). Compared to the corn control, VectoBac® 200G exposure increased the nuclear radius in both non-INS-IR cells (11.2%; $p < 0.0001$) and INS-IR cells (5.9%; $p < 0.0001$).

Significant main effects of treatment (DMSO control vs deltamethrin; $F(1, 57) = 50.23$, $p < 0.0001$) and cell-type (non-INS-IR vs INS-IR; $F(1, 57) = 76.39$, $p < 0.0001$) on the nuclear radius of pancreatic cells were observed. However, no interaction between treatment and cell type was observed ($F(1, 57) = 1.15$, $p = 0.288$). Within the DMSO control, the nuclei of INS-IR cells were significantly larger than those of non-INS-IR cells (1.99 ± 0.05 µm vs 1.79 ± 0.06 µm; $p < 0.0001$; Fig. 4.6B). In the deltamethrin treatment, INS-IR cells were also significantly larger than non-INS-IR cells (2.14 ± 0.05 µm vs 1.98 ± 0.06 µm; $p < 0.0001$). Deltamethrin increased the nuclear radius in both non-INS-IR cells (10.2%; $p < 0.0001$) and INS-IR cells (7.2%; $p = 0.0004$) relative to the DMSO control.

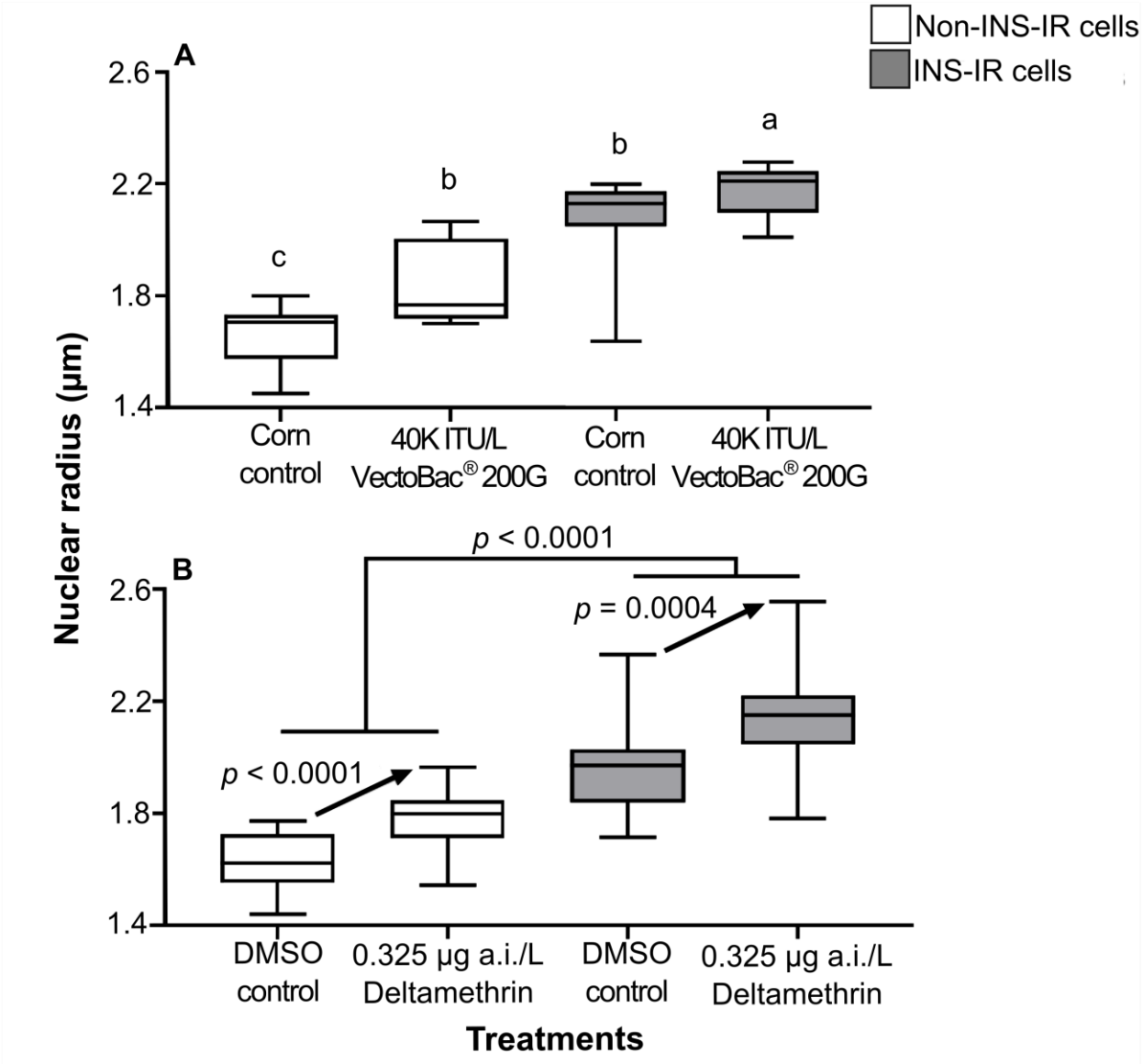

Figure 4.6. Nuclear radius of non-INS-IR and INS-IR cells in the pancreas of wood frog metamorphs exposed to VectoBac® 200G or deltamethrin. Tadpoles were exposed from GS 25 to metamorphic climax (GS 46; approximately 56 days) to VectoBac® 200G or deltamethrin, and their respective controls. **(A)** Nuclear radius of pancreatic cells following exposure to corn control (n = 5) or 40,000 (40K) ITU/L VectoBac® 200G (n = 4); different letters (a, b, c) denote significant differences between groups (Tukey's post-hoc test; $p < 0.05$). **(B)** Nuclear radius of pancreatic cells following exposure to DMSO control (n = 6) or 0.325 µg a.i./L deltamethrin (n = 5); significant main effects of treatment and cell-type were detected (linear mixed models, $p < 0.0001$ for both effects). Data are presented as boxplots showing the minimum, first quartile, median, third quartile, and maximum values. Note that the Y-axis does not reach zero.

A difference in the total insulin staining area per pancreas was detected between frogs exposed to the corn control and the VectoBac® 200G treatment ($F(1, 21) = 8.84$, $p = 0.007$; Fig. 4.7A). The total area of insulin staining in the pancreas of corn control frogs averaged 32.3 ± 3.0 μm^2 and was 21.3% less than that of frogs exposed to VectoBac® 200G (40.0 ± 3.6 μm^2). In contrast, no significant differences in total insulin staining area were observed between frogs exposed to the DMSO control (40.8 ± 2.2 μm^2) and deltamethrin treatment (41.6 ± 3.1 μm^2) ($F(1, 27) = 0.30$, $p = 0.588$; Fig. 4.7B).

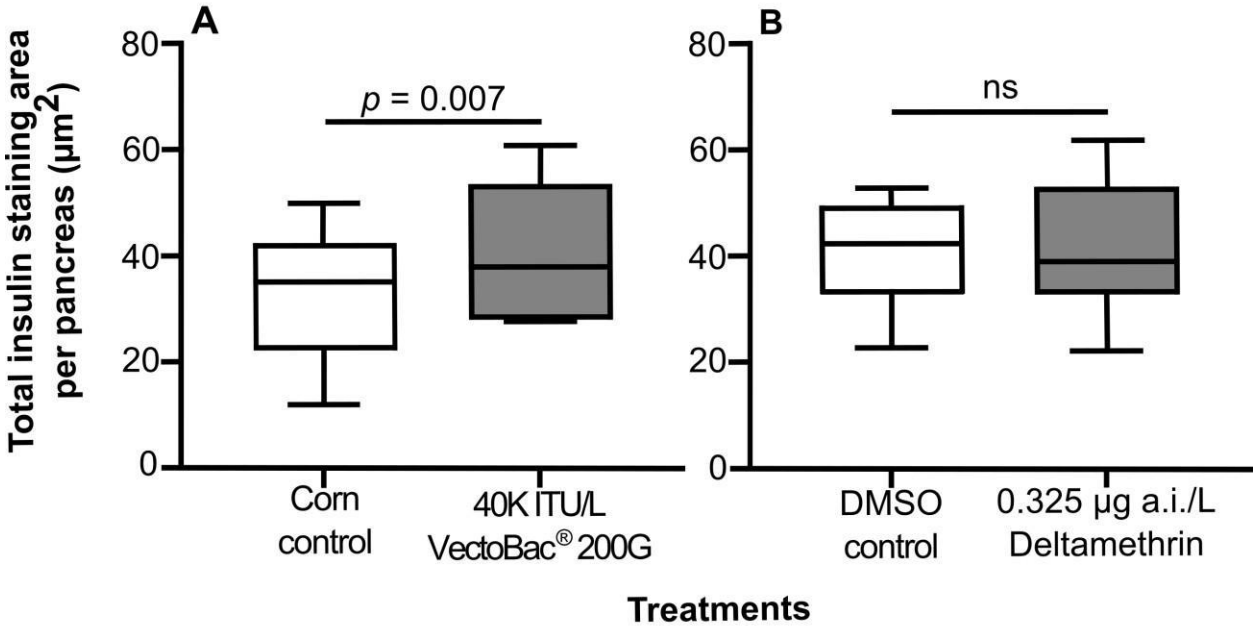

Figure 4.7. The total insulin staining area in the pancreas of wood frog metamorphs exposed to VectoBac® 200G or deltamethrin. Frogs were exposed from GS 25 to metamorphic climax (GS 46; approximately 56 days) to VectoBac® 200G or deltamethrin, and their respective controls. **(A)** Total insulin staining area following exposure to the corn control (n = 5) or 40,000 (40K) ITU/L VectoBac® 200G (n = 4); a significant difference between treatments was detected (linear mixed models, $p = 0.007$). **(B)** Total insulin staining area following exposure to the DMSO control (n = 6) or 0.325 µg a.i./L deltamethrin (n = 5); no significant (ns, $p > 0.05$) difference was observed between treatments. Data are presented as boxplots showing the minimum, first quartile, median, third quartile, and maximum values.

The average insulin staining area per INS-IR cell significantly differed between frogs exposed to the corn control and VectoBac® 200G treatments ($F(1, 22) = 4.91$, $p = 0.038$; Fig. 4.8A). Metamorphs exposed to the corn control exhibited an average staining area per INS-IR cell of 4.2 ± 0.6 µm^2, which was 57% greater than frogs exposed to VectoBac® 200G (2.4 ± 0.4 µm^2). No significant difference was observed in the insulin staining area per INS-IR cell between the DMSO control (3.4 ± 0.5 µm^2) and the deltamethrin treatment (2.7 ± 0.7 µm^2) ($F(1, 28) = 1.38$, $p = 0.250$; Fig. 4.8B).

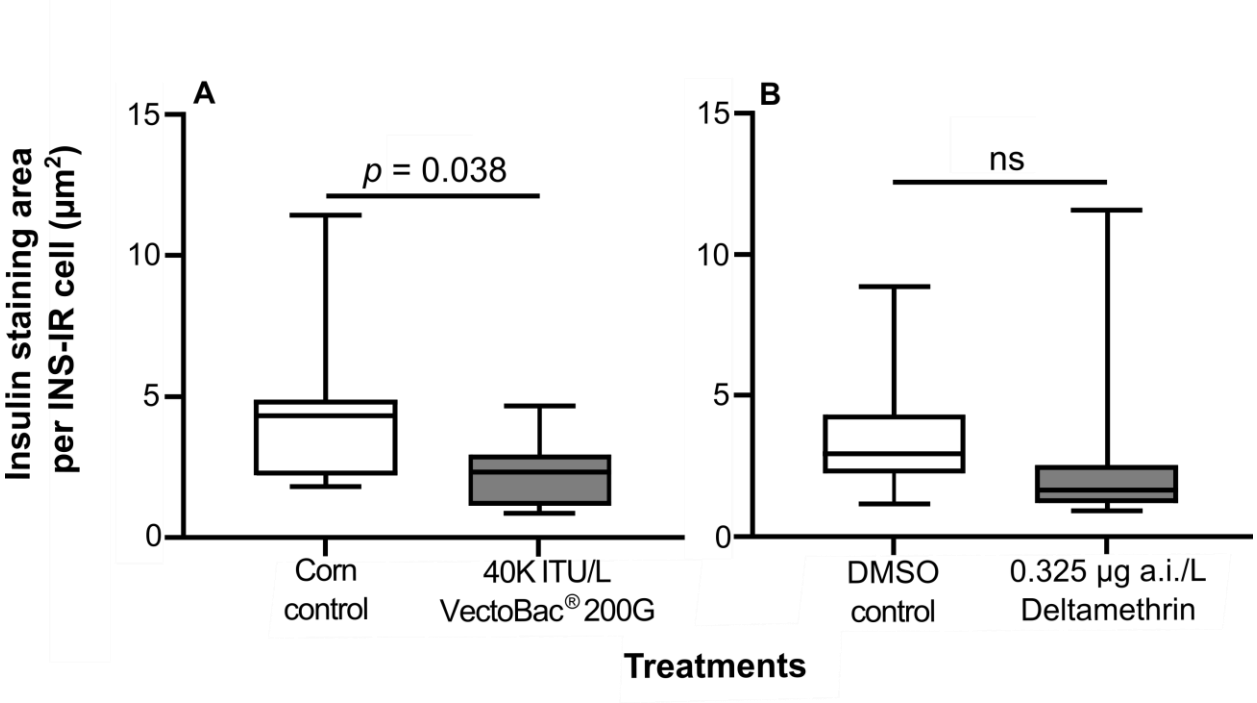

Figure 4.8. Average insulin staining area per INS-IR cell in the pancreas of wood frog metamorphs exposed to VectoBac® 200G or deltamethrin. Frogs were exposed from GS 25 to metamorphic climax (GS 46; approximately 56 days) to VectoBac® 200G or deltamethrin, and their respective controls. **(A)** Insulin staining area per INS-IR cell in metamorphs exposed to corn control (n = 5) or 40,000 (40K) ITU/L of VectoBac® 200G (n = 4); significance between treatments was detected (linear mixed models, $p = 0.038$). **(B)** Insulin staining area per INS-IR cell in metamorphs exposed to DMSO control (n = 6) or 0.325 µg a.i./L deltamethrin (n = 5); no significant (ns, $p > 0.05$) difference was observed between treatments. Data are presented as boxplots showing the minimum, first quartile, median, third quartile, and maximum values.

4.4 Discussion

We provide evidence that the Bti formulation, VectoBac® 200G, affects insulin regulation in chronically exposed wood frog metamorphs. These data are significant for three reasons: (1) the number and percentage of INS-IR cells significantly increased, (2) the nuclear radius of pancreatic cells significantly increased, and (3) the total insulin staining area per pancreas increased, but the insulin staining area per INS-IR cell decreased relative to controls. Effects on INS-IR cells were less pronounced following deltamethrin exposure, with only increases in the percentage of INS-IR cells and in nuclear radius observed, and no changes in insulin-stained area detected.

To our knowledge, no commercially available antibodies specifically target amphibian insulin. The antibody used in this study was custom-designed based on the insulin B-chain sequence of the Northern leopard frog. The leopard and wood frog are separate species, but both are closely related, and the B-chain of the insulin peptide is highly conserved across taxa (see Table 4.1; Chrudinová et al., 2026). The wood frog insulin B-chain sequence was reported in a publication (Conlon et al., 1998a), in which the amino acid sequence was validated, but the biological activity was not assessed due to insufficient quantities obtained. The sequence from *L. pipiens* was chosen for this study because it is available in widely accepted gene databases, such as the National Center for Biotechnology Information and UniProt. Antibodies raised against mammalian insulin have been used in amphibians (Regnault et al., 2018; Usal et al., 2021), but without appropriate validation, the specificity and interpretation of those results are unclear. Generating an antibody raised against a defined peptide epitope increases specificity (MuñozPrieto et al., 2017) and reduces the likelihood of cross-reactivity with structurally similar peptides (Scott et al., 1987). Insulin and insulin-like growth factor I and II are highly similar in structure, and studies indicate that the genes encoding these peptides share a common evolutionary history (Bell et al., 1980; Gauguin et al., 2008; Rinderknecht & Humbel, 1978). Although insulin is highly conserved, sequence differences have been identified in frog species such as the wood frog, bullfrog (*Lithobates catesbeiana*), and the marsh frog (*Pelophylax ridibundus*) relative to humans (See table 4.1; Conlon et al., 1998a). The custom insulin antibody developed in this study may therefore be a valuable tool for studying insulin regulation in anurans.

We observed an increase in the number of INS-IR cells in metamorphs exposed to VectoBac® 200G, whereas no changes were observed in deltamethrin-exposed metamorphs. In contrast, the percentage of INS-IR cells increased following exposure to both insecticides. Both parameters provide important insights into β-cell function (Fedchenko & Reifenrath, 2014). Specifically, the number of INS-IR cells is associated with absolute changes in β-cell numbers, and the percentage indicates β-cell abundance relative to other pancreatic cell types, allowing us to assess whether the proportion of β-cells changes. To date, there are no published studies on the effects of these insecticides on β-cell proliferation, particularly in amphibian species. Comparisons, therefore, must be made with other contaminants and vertebrate models. The capacity for insulin secretion depends on β-cell number and function (Bouwens & Rooman, 2005); however, increased β-cell number does not necessarily indicate regenerative growth, nor is it generally beneficial (Cerf, 2013). For example, rats chronically exposed to the organophosphate monocrotophos experienced hyperglycemia and hyperinsulinemia, while exhibiting enlarged pancreatic islets and a doubling of β-cells (Nagaraju & Rajini, 2016). Such traits are consistent with a compensatory response to insulin resistance, in which reduced insulin sensitivity in peripheral tissues stimulates β-cell proliferation and insulin secretion (Cerf et al., 2013; Sachdeva & Stoffers, 2009). These findings are consistent with our previous observations of hyperglycemia, in which glucose levels in tadpoles exposed to 40,000 ITU/L increased by approximately 140% between 1 and 4 h following waterborne glucose administration (see Fig. 3.3B; Chapter 3). The concurrent increase in INS-IR cells may therefore indicate a compensatory upregulation of β-cell production to enhance insulin secretion in response to reduced insulin sensitivity from insecticide exposure.

The nuclear radius of pancreatic cells also increased significantly following exposure to both insecticides. Nuclear radius was specifically assessed as a proxy for cellular radius, because a cytoplasmic fluorescent marker to estimate total cell size was not used due to variations in βcell shape. No comparable studies are available on the effects of Bti or deltamethrin insecticides on cell dimensions. Increased β-cell nuclear diameter has been observed in obese (Saisho et al., 2013) and insulin-resistant humans (Mezza et al., 2014). Although the relationship between nuclear size and insulin secretion remains to be elucidated, the nuclear size of endocrine cells is often correlated with secretory activity (Meier et al., 2006; Norman et al., 1988; Studer & Dewahl, 1995). The enlarged nuclear radius observed in insecticide-exposed metamorphs

therefore suggests increased functional demand of β-cells, consistent with early onset insulin resistance. Prolonged metabolic stress can ultimately reduced β-cell number and size due to cell exhaustion, as is often observed in individuals with type II diabetes (Sasaki et al., 2021).

In the pancreas of tadpoles exposed to VectoBac® 200G, the total insulin staining area per pancreas increased, but staining per INS-IR cell decreased. These results imply that the pancreas has increased β-cell production, rather than individual β-cells increasing insulin secretion. This remodelling of pancreatic tissue is common in early-onset insulin resistance (Sasaki et al., 2021). For example, in insulin receptor knockout zebrafish, β-cell hyperplasia was observed, indicating that the pancreas attempted to compensate through increased β-cell production (Yang et al., 2017). Although β-cell hyperplasia may initially compensate for metabolic demand, excessive or sustained cell production can induce endoplasmic reticulum stress, promote apoptosis, and ultimately contribute to tissue dysfunction (Bonner-Weir, 2000; Weir et al., 2020). These findings suggest that VectoBac® 200G promotes β-cell proliferation to compensate for metabolic demand, while the reduced insulin staining per cell may reflect altered insulin dynamics, possibly due to decreased insulin stores and/or increased insulin release.

In our study, we observed minimal effects on glucose uptake, insulin-positive staining, and metabolic disruption in wood frogs exposed to deltamethrin, except for altered hepatic lipid dynamics (see Fig. 3.7, Chapter 3). Similar findings have been reported in other organisms exposed to deltamethrin. For example, mice given 0.01–1 mg/kg bw/day of deltamethrin for 16 weeks exhibited increased insulin sensitivity and glucose uptake (Tsakiridis et al., 2023). Specifically, the lowest exposure dose of deltamethrin in these mice improved glucose tolerance without affecting insulin action, consistent with our observations of minimal effects on these metabolic parameters in wood frog metamorphs. In contrast to Tsakiridis et al. (2023), we observed an increase in the number of lipid droplets (although total lipid area was unaffected), whereas their study found a decrease in overall fat accumulation. The authors attributed these effects to increased physical activity at low doses of deltamethrin, which was associated with increased oxygen consumption. Although the mechanism underlying the observed increase in physical activity is unknown, a similar response in tadpoles could have promoted metabolic regulation. This interpretation is somewhat controversial, as studies have reported conflicting results regarding amphibian locomotion in response to pyrethroid exposure. For example, *Rhinella arenarum* tadpoles exposed to nominal concentrations of TRISADA® (an insecticide

containing deltamethrin and tetramethrin) exhibited a significant decrease in swimming movement (Lajmanovich et al., 2018). Similarly, reduced swimming was reported in *R. arenarum* tadpoles exposed to the no-observed-effect concentration (NOEC) of permethrin; however, *Rhinella fernandezae* tadpoles exposed to the same NOEC significantly increased swimming activity (Junges et al., 2019). Notably, the decreased locomotion observed by Lajmanovich et al. (2018) was correlated with altered acetylcholinesterase activity, a biomarker generally associated with motor activity. These findings suggest that the metabolic effects of deltamethrin are complex and that low-dose exposure may differ from higher, neurotoxic levels.

4.5 Conclusion

Exposure to VectoBac® 200G significantly increased the number and nuclear radius of INS-IR cells, and total insulin staining in the pancreas of wood frog metamorphs (although insulin staining per INS-IR cell was reduced). In contrast, deltamethrin induced modest effects, affecting only the percentage of INS-IR cells and pancreatic nuclear radius. These findings suggest that Bti-based products may alter pancreatic insulin dynamics in frogs, potentially disrupting metabolic regulation. Given the central role of insulin and glucose homeostasis in freeze tolerant in species such as the wood frog, such sublethal effects could have important physiological consequences. More broadly, this study highlights the limited understanding of the sublethal effects of widely used insecticides on non-target organisms.

Chapter 5: General Discussion and Conclusions

5.1 Thesis summary

The goal of my thesis was to elucidate the lethal and sublethal effects of *Bacillus thuringiensis israelensis* (Bti) and deltamethrin insecticides on several North American amphibians. Before this project, there was limited research on these insecticides, and no data was available regarding their effects on my study species. I present the first comprehensive comparisons of Bti and deltamethrin products across taxa, providing valuable information on species sensitivities. My findings demonstrate that Bti insecticides, and to a lesser extent deltamethrin, alter metabolic processes in the wood frog. Three primary objectives to fill critical data gaps in the literature are illustrated in Fig. 5.1.

The first objective was to establish foundational toxicological information for VectoBac® 200G (200 international toxic units (ITU)/mg of Bti) and deltamethrin (98% purity) in wood, leopard, and chorus frog tadpoles (Chapter 2), as the impacts of exposure to these insecticides were previously unknown. The median lethal concentration (LC_{50}) estimates indicated that all three species were highly tolerant to VectoBac® 200G exposure, with LC_{50} values several hundred times higher than the label application rate. In contrast, deltamethrin induced mortality at low concentrations (LC_{50} range of 1.15–7.30 µg active ingredient (a.i.)/L). These results also highlighted species-specific differences. The leopard frog tadpole was the most sensitive species to VectoBac® 200G (LC_{50} of 78,860 ITU/L), but the most tolerant to deltamethrin (LC_{50} of 7.30 µg a.i./L). Species sensitivity distribution (SSDs) curves of published LC_{50}s of amphibian species and other taxa exposed to Bti and deltamethrin insecticides were constructed. These curves enable the comparison among species, developmental stages, exposure durations, and product formulations. Results indicate that most amphibian species are tolerant to Bti insecticides but susceptible to deltamethrin formulations. Although Bti-insecticides are generally regarded as target-specific, high concentrations of VectoBac® 200G increased the snout-vent length (SVL) of tadpoles, while varying concentrations of deltamethrin decreased tadpole SVL. These findings provided the foundation for investigating sublethal metabolic effects on development, glucose regulation, and hepatotoxicity in Chapter 3.

The second thesis objective was to determine whether these insecticides disrupt glucose regulation in wood frog tadpoles (Chapter 3). Wood frogs were selected for their ability to

rapidly utilize glucose for cryoprotection without invoking tissue damage. Glucose measurements using a glucometer have not previously been conducted with wood frog tadpoles due to their small size; therefore, we designed and validated a glucometer assay suitable for amphibian larvae. This assay enabled the measurement of whole-body glucose in tadpoles, allowing us to quantify glucose uptake over time. All VectoBac® 200G exposure concentrations significantly increased the hourly glucose uptake rates in tadpoles relative to the corn control (~40–60% increase). In contrast, glucose uptake did not differ between the DMSO control and deltamethrin treatments. At the highest exposure level, tadpoles exposed to VectoBac® 200G exhibited hourly glucose uptake rates 141% greater than tadpoles exposed to the highest concentration of deltamethrin (Fig. 3.5; Chapter 3). Both insecticides altered hepatic lipid dynamics, indicating metabolic disruption beyond glucose regulation. This is the first study to suggest that Bti insecticides may possess metabolic-disrupting properties in amphibians and to present a novel glucose assay that can be readily used across amphibian species.

As the previous chapter demonstrated significant glucose uptake from VectoBac® 200G and altered hepatic lipid dynamics from both Bti- and deltamethrin insecticide exposure, the final objective was to determine whether these insecticides impacted insulin dynamics in the wood frog metamorph (Chapter 4). To improve specificity over mammalian-derived antibodies, a custom antibody was developed based on the insulin B-chain sequence of *Lithobates pipiens*, a close relative of the wood frog. The antibody was successfully validated and represents a valuable tool for research on North American amphibians. Tadpoles exposed to VectoBac® 200G exhibited a significant increase in pancreatic beta (β)-cell number, nuclear radius, and total insulin staining. However, insulin production per immunoreactive cell was reduced, suggesting pancreatic compensation for elevated glucose levels and potential onset of insulin resistance. In contrast, tadpoles exposed to deltamethrin exhibited comparatively minor changes consistent with limited effects on glucose uptake, where only the proportion of β-cells and the nuclear radius of cells increased. These findings provide mechanistic insight into how exposure to these insecticides may disrupt metabolism.

Collectively, these findings advance knowledge of the lethal and sublethal effects of these widely used insecticides in North American amphibians (Fig. 5.1). While this work addresses critical knowledge gaps, it also raises additional questions for future research.

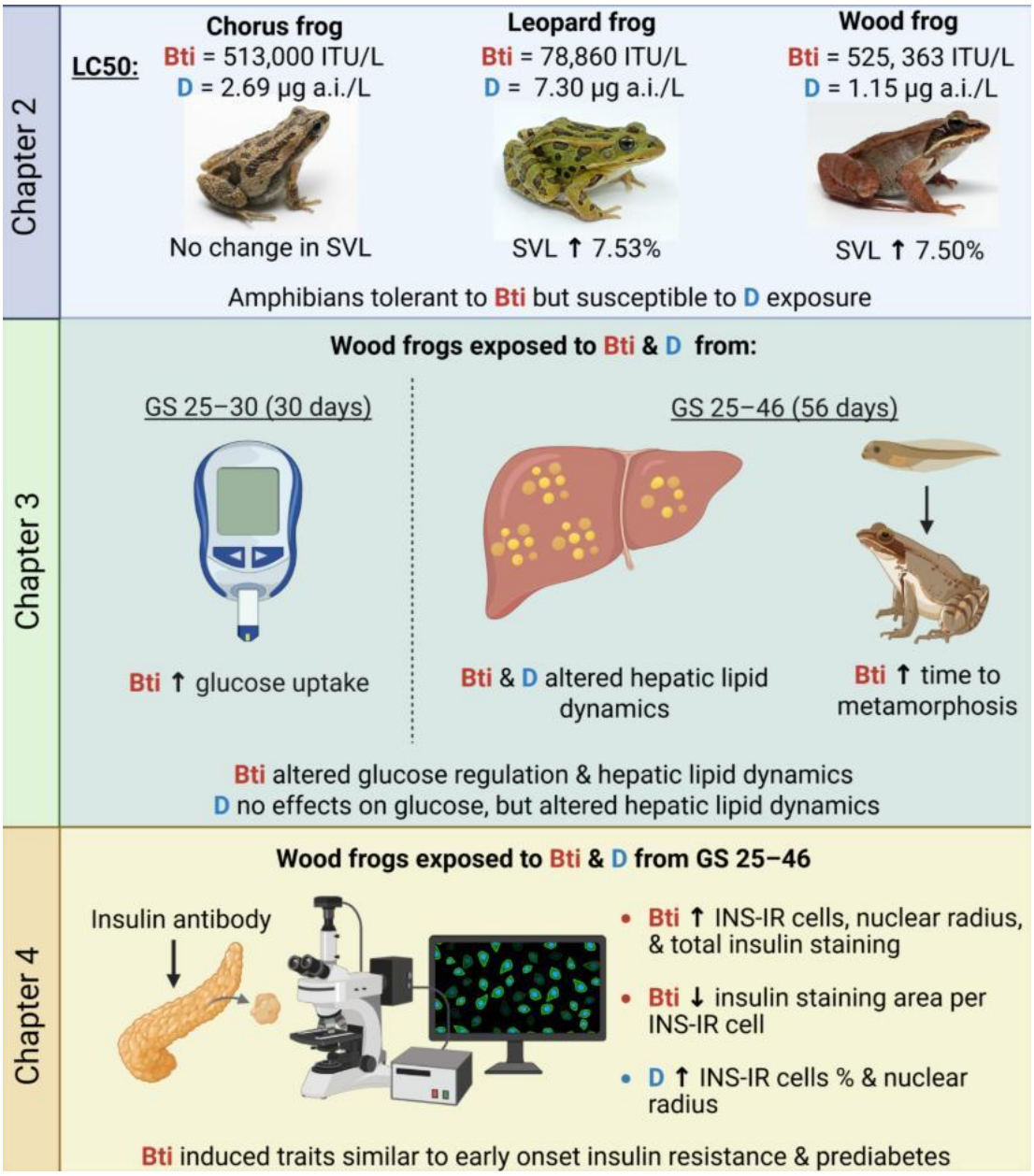

Figure 5.1. Schematic overview of thesis data chapters. Chapter 2 presents the median lethal concentration of the chorus, leopard, and wood frog species to VectoBac® 200G and deltamethrin, as well as associated effects on growth. Chapter 3 summarizes metabolic endpoints in wood frogs exposed to VectoBac® 200G and deltamethrin, including glucose uptake (exposure from GS 25–30; 30 days), and hepatic lipid dynamics and time to complete metamorphosis (exposure from GS 25–46, ~56 days). Chapter 4 presents immunohistochemical analyses of the pancreas in wood frogs exposed to VectoBac® 200G and deltamethrin, quantifying insulin immunoreactive cells using an amphibian-derived anti-insulin B-chain antibody. a.i., active ingredient; Bti, *Bacillus thuringiensis israelensis* (derived from VectoBac® 200G exposure); D,

deltamethrin; GS, Gosner Stage; INS-IR, insulin immunoreactive; ITU, international toxic units; LC_{50}, median lethal concentration; SVL, snout-vent length. Chorus frog photograph courtesy of Chris Callaghan; leopard and wood frog photographs courtesy of the New Brunswick Museum. Schematic created in BioRender.com.

5.2 Future directions

5.2.1 General research directions

We determined the lethal concentrations and sublethal effects of VectoBac® 200G and deltamethrin in the wood, leopard, and chorus frog tadpole, providing an important foundation for understanding insecticide sensitivity of North American amphibians. Additional species should be evaluated to enhance the risk assessments of insecticide applications. As discussed in Chapter 2, amphibians exhibit diverse life histories which can influence toxicological sensitivity. Further research should also examine other Bti and deltamethrin products, as differences in application vehicles, formulation potency, and inert ingredients may alter toxicity. Because these insecticides are rarely applied alone, experiments that incorporate co-exposure to other contaminants, such as fertilizers, metals, and other pesticides, are important for interpreting possible modulatory effects in natural settings.

This research also provides the framework for investigating other *Bacillus thurigiensis* (Bt)–based insecticides, such as Bt *kurstaki* and Bt *aizawai*, which differ in toxin composition and target specificity. Evaluating the effects of individual Cry and Cyt toxins would provide a clearer understanding of whether the observed effects are attributable to specific toxins or to possible inactive ingredients in the applied formulations. Because Bt insecticides were designed to primarily target the gut of insect larvae, examining amphibian gut microbial composition following exposure may provide further mechanistic insight related to the metabolic alterations observed in this study. Disruptions to microbial communities may influence nutrient absorption, metabolic regulation, and immune function, potentially contributing to toxic effects.

Sublethal test concentrations were based on calculated LC_{10}, LC_{15}, and LC_{20} estimates. Bti exposure concentrations exceeded the label application rates, whereas deltamethrin exposure concentrations were below typical field rates. The selected Bti test concentrations may reflect conditions of repeated applications, over-application, run-off events, and localized accumulation. Concentrations may also reflect application ranges in other regions of the world where intensive insecticide application is required to control mosquito populations. As climate change is

expected to alter mosquito distribution and increase insecticide use, ecological risk assessments that reflect intensive applications are needed. Extending these experiments to mesocosm and field-based studies would improve the ecological relevance of the effects of these insecticides. Incorporating temperature variability, predator presence, and natural food webs would provide more accurate insight into effects under field conditions.

5.2.2 Future research on glucose regulation

Findings on glucose uptake were novel and provided important insight into the metabolic regulation in insecticide-exposed tadpoles. The use of whole-organism glucose measurements provides a foundation for metabolic regulation studies in amphibian larvae. While this study focused on short-term glucose uptake, incorporating long-term assessments on glucose clearance would better characterize glucose regulation following insecticide exposure. Conducting these measurements throughout development, especially during the metabolically demanding periods of metamorphosis, would delineate the shifts in metabolic sensitivity between developmental stages. Comparative studies among species with varying development rates, body sizes, habitats, and breeding strategies could further elucidate glucose regulation across these life-history traits.

The novel glucometer method established in this study enables metabolic studies to include larval stages more easily. Glucose regulation could be examined in response to environmental stressors, including temperature fluctuations, hypoxia, drought, increased density, and predation. During infection, glucose dynamics can be evaluated in relation to immune responses to determine whether metabolic dysregulation predicts disease susceptibility or mortality. In a toxicological context, long-term glucose monitoring could also provide insight into whether embryonic or larval contaminant exposure induces alterations to metabolic regulation into adulthood or subsequent generations. By applying these approaches across various developmental stages and environmental contexts, the interpretation of physiological and ecological consequences of contaminant exposure may be better understood.

5.2.3 Advancements in insulin dynamics

The development of a novel insulin antibody represents an important advancement in amphibian endocrine research. A future direction of this project would be to incorporate immunohistochemical analyses of glucagon using an amphibian-specific antibody. Assessing both insulin-producing β-cells and glucagon-producing alpha (α)-cells would allow the analysis

of changes in islet composition and elucidate if insecticide exposure influences the insulin glucagon axis.

In a broader context, the frog-derived insulin antibody provides a powerful tool for comparative analyses of pancreatic structure and β-cell organization across amphibian species and in relation to other vertebrates. It enables the investigation of pancreatic development, β-cell differentiation, and islet composition from the embryonic and larval stages through metamorphic climax and into adulthood. Because amphibian development involves major metabolic reorganization, this approach may help elucidate how insulin production and β-cell dynamics shift in response to tissue remodeling and energetic demands. The antibody can also be used to study insulin regulation under extreme physiological conditions, including freeze tolerance during overwintering, prolonged fasting, and brumation. This method may also be applied to studies on energy-demanding life-history events such as calling, migration, and reproduction. The opportunity to explore insulin dynamics in the presence of immune challenges, such as infection with *Batrachochytrium dendrobatidis* (chytrid fungus), a disease that has contributed to global amphibian declines, may provide insight into the metabolic costs of this disease and host resilience. These research directions can be integrated with contaminant exposure to investigate disruptions to insulin regulation and β-cell composition across these developmental, physiological, and environmental contexts.

5.3 Concluding remarks

This research addresses critical knowledge gaps regarding the effects of widely used insecticides on understudied North American amphibian species (Fig. 5.1). By characterizing the lethal, sublethal, and metabolic effects following exposure to Bti and deltamethrin, this work provides data useful for environmental risk assessments. These findings are directly relevant to municipalities and insecticide applicators operating in habitats where these species occur, supporting evidence-based decision-making. Improving our understanding of how these products affect non-target amphibian species is essential for balancing mosquito-borne disease control and biodiversity conservation.

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Appendix A: Supplementary Material

Supplemental File S1

Analysis of water control and deltamethrin samples

To analyze the water control, 0.250 mL of the sample was pipetted into a Robo vial containing 0.728 ml MeOH and 22.2 µL of the internal standard of 0.765 ppm triphenyl phosphate (TPP). Samples containing the measured stock solutions were diluted in series because of their expected high concentrations. Subsequently, 100 µL of the sample was mixed with methanol (MeOH) (10 mL). Then, 20 µL of this solution was added to 958 µL of MeOH and 22.2 µL of the internal standard (0.765 ppm TPP) in a Robo vial and mixed. All solutions were filtered through a 0.2 µm filter into a Robo vial. Duplicate water and stock solutions were prepared and subjected to liquid chromatography-mass spectrometry (SOP# S11-LC/MS/MS Pesticides; MB Laboratories Ltd., Sidney, BC).

Table S1.1. Water analysis results of control and deltamethrin samples.

Sample description	Measured deltamethrin concentration (µg/L)	LOQ (µg/L)
Water control - A	None detected	0.1
Water control - B	None detected	0.1
Water control - C	None detected	0.1
Deltamethrin sample - 250,000 µg/L - A	266,819	0.1
Deltamethrin sample - 250,000 µg/L - B	235,743	0.1
Deltamethrin sample - 250,000 µg/L - C	261,722	0.1

LOQ, Limit of quantitation

Table S1.2. Summary of the studies including species, chemicals, and LC_{50} of organisms exposed to *Bacillus thuringiensis* var. *israelensis* products

Species	Chemical	Chemical potency (ITU/L)	LC_{50} of organism (mg/L)	LC_{50} of organism (ITU/L)	Group	Reference
Aedes aegypti, 3rd instar (48 h)	VectoBac® WG	3,000	0.018	54	Invertebrate	Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., Kay, & B.H. (2003). Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J. Econ. Entomol*. *96*(6), 1786–1791. https://doi.org/10.1093/jee/96.6.1786
Aedes aegypti, 2nd instar (24 h)	VectoBac® 12 AS	1,200	0.00102	1.224	Invertebrate	Valtierra-de-Luis, D., Villanueva, M., Lai, L., Williams, T., & Caballero, P. (2020). Potential of Cry10Aa and Cyt2Ba, Two Minority δ-endotoxins Produced by *Bacillus thuringiensis ser. Israelensis*, for the Control of *Aedes aegypti* Larvae. *Toxins*, *12*(6), 355. https://doi.org/10.3390/toxins12060355
Aedes aegypti, 2nd-3rd instar (24 h)	Bactimos®	3,000	0.029	87	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570v1
Aedes aegypti, 2nd-3rd instar (24 h)	Bactimos®	3,000	0.031	93	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera:

						Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570v1
Aedes aegypti, 2nd-3rd instar (24 h)	VectoBac®	3,000	0.055	165	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570v1
Aedes aegypti, 2nd-3rd instar (24 h)	VectoBac®	3,000	0.056	168	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570
Anopheles albimanus, 2nd-3rd instar (24 h)	VectoBac®	3,000	0.651	1,953	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570v1
Anopheles albimanus, 2nd-3rd instar (24 h)	VectoBac®	3,000	0.691	2,073	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570.v1

Anopheles albimanus, 2nd-3rd instar (24 h)	Bactimos®	3,000	1.216	3,648	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570.v1
Anopheles albimanus, 2nd-3rd instar(24 h)	Bactimos®	3,000	1.216	3,648	Invertebrate	Garza-Almanza, V., Ulíbarri, G., & Sanchez-Yañez, J.M. (2020). Efficacy of 2 Commercial Formulations of *Bacillus thuringiensis* H-14 in Larvae of *Anopheles albimanus W* and *Aedes aegypti L* (Diptera: Culicidae). *Preprints*. https://doi.org/10.20944/preprints202010.0570.v1
Anopheles coluzzii, 3rd instar (48 h)	VectoBac® WDG	3,000	0.12	360	Invertebrate	Gowelo, S., Chirombo, J., Spitzen, J., Koenraadt, C. J.M., Mzilahowa, T., van den Berg, H., Takken, W., & McCann, R. (2020). Effects of larval exposure to sublethal doses of *Bacillus thuringiensis* var. *Israelensis* on body size, oviposition and survival of adult *Anopheles coluzzii* mosquitoes. *Parasit. Vectors*. *13*(1), 259. https://doi.org/10.1186/s13071-020-04132-z
Anopheles gambiae, 3rd instar (24 h)	VectoBac® WDG	2,700	0.021	56.7	Invertebrate	Fillinger, U., Knols, B.G.J., & Becker, N. (2003). Efficacy and efficiency of new *Bacillus thuringiensis* var. *Israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. TM & IH. *8*(1), 37–47. https://doi.org/10.1046/j.1365-3156.2003.00979.x

Anopheles gambiae, 3rd instar (24 h)	Bactimos® PP	10,000	0.006	60	Invertebrate	Fillinger, U., Knols, B.G.J., & Becker, N. (2003). Efficacy and efficiency of new *Bacillus thuringiensis* var. *Israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. *TM & IH*. *8*(1), 37–47. https://doi.org/10.1046/j.1365-3156.2003.00979.x
Chironomus tepperi, 4th instar (48 h)	VectoBac® WDG	3,000	0.59	1,770	Invertebrate	Stevens, M.M., Helliwell, S., & Hughes, P.A. (2005). TOXICITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FORMULATIONS, SPINOSAD, AND SELECTED SYNTHETIC INSECTICIDES TO *CHIRONOMUS TEPPERI* LARVAE. *JAMCA*. *21*(4), 446–450. https://doi.org/10.2987/8756-971X(2006)21[446:TOBTVI]2.0.CO;2
Chironomus tepperi, 4th instar (48 h)	AquaBac® SC	1,200	1.7	2,040	Invertebrate	Stevens, M.M., Helliwell, S., & Hughes, P.A. (2005). TOXICITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FORMULATIONS, SPINOSAD, AND SELECTED SYNTHETIC INSECTICIDES TO *CHIRONOMUS TEPPERI* LARVAE. *JAMCA*. *21*(4), 446–450. https://doi.org/10.2987/8756-971X(2006)21[446:TOBTVI]2.0.CO;2
Chironomus tepperi, 4th instar (48 h)	Teknar® SC	1,200	2.15	2,580	Invertebrate	Stevens, M.M., Helliwell, S., & Hughes, P.A. (2005). TOXICITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FORMULATIONS, SPINOSAD, AND SELECTED SYNTHETIC INSECTICIDES TO *CHIRONOMUS TEPPERI* LARVAE. *JAMCA*.

						21(4), 446–450. https://doi.org/10.2987/8756-971X(2006)21[446:TOBTVI]2.0.CO;2
Culex annulirostris, 3rd instar (48 h)	VectoBac® WG	3,000	0.004	12	Invertebrate	Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., & Kay, B.H. (2003). Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J. Econ. Entomol*. *96*(6), 1786–1791. https://doi.org/10.1093/jee/96.6.1786
Culex quinquefasciatus, 3rd instar (48 h)	VectoBac® WG	3,000	0.005	15	Invertebrate	Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., & Kay, B.H. (2003). Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J. Econ. Entomol*. *96*(6), 1786–1791. https://doi.org/10.1093/jee/96.6.1786
Culex sitiens, 3rd instar (48 h)	VectoBac® WG	3,000	0.019	57	Invertebrate	Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., & Kay, B.H. (2003). Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J. Econ. Entomol. 96*(6), 1786–1791. https://doi.org/10.1093/jee/96.6.1786
Daphnia (48 h)	VectoBac® 200g	200	50	10,000	Invertebrate	Valent BioSciences. (2015). Safety data sheet for VectoBac® 200G. https://gardexinc.com/MSDS/5168Vectobac_EN_MSDS_2015_06.pdf (accessed May 27 2024).
Daphnia (48 h)	VectoBac® G	200	50	10,000	Invertebrate	Valent BioSciences. (2020). Safety data sheet for VectoBac® G Biological Larvicide Granules. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/SDS-VBC-0020R5-VectoBac-G-01-03-20.pdf (Accessed May 27 2024).

Daphnia (48 h)	VectoBac® 1200L	1,200	50	60,000	Invertebrate	Valent BioSciences. 2020. Safety data sheet for VectoBac® G Biological Larvicide Granules. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/SDS-VBC-0020R5-VectoBac-G-01-03-20.pdf (Accessed May 27 2024).
Daphnia (48 h)	VectoBac® 12 AS	1,200	50	60,000	Invertebrate	Valent BioSciences. (2020). Safety data sheet for VectoBac® G Biological Larvicide Granules. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/SDS-VBC-0020R5-VectoBac-G-01-03-20.pdf (Accessed May 27 2024).
Daphnia (48 h)	VectoBac® WDG	3,000	50	150,000	Invertebrate	Valent BioSciences. (2024). Safety data sheet for VectoBac® WDG Biological Larvicide. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2024/04/SDS-VBC-0019R6-VectoBac-WDG-03-25-2024-.pdf (accessed May 27 2024).
Fundulus heteroclitus, adult (96 h)	VectoBac®	1,200	980	1,176,000	Fish	Lee, B.M. & Scott, G.I. (1989). Acute Toxicity of Temephos, Fenoxycarb, Diflubenzuron, and Methoprene and *Bacillus thuringiensis* var. *Israelensis* to the Mummichog (*Fundulus heteroclitus*). *Bull. Environ. Contam. Toxicol*. *43*, 827-832.
Heterocypris bosniaca, 3rd-4th instar (48 h)	VectoBac® 12AS	1,200	298.75	358,500	Invertebrate	Aguilar-Alberola, J.A. & Mesquita-Joanes, F. (2012). Acute Toxicity Tests with Cadmium, Lead, Sodium Dodecyl Sulfate, and *Bacillus*

						thuringiensis on a Temporary Pond Ostracod. *Int. Rev. Hydrobiol*. *97*(4), 375–388. https://doi.org/10.1002/iroh.201211497
Leptodactylus latrans, (GS - 26-30) (48 h)	Introban®	1,200	22.45	26,940	Amphibian	Lajmanovich, R.C., Junges, C.M., Cabagna-Zenklusen, M.C., Attademo, A.M., Peltzer, P.M., Maglianese, M., Márquez, V.E., & Beccaria, A.J. (2015). Toxicity of *Bacillus thuringiensis* var. *israelensis* in aqueous suspension on the South American common frog *Leptodactylus latrans* (Anura: Leptodactylidae) tadpoles. *Environ. Res. 136*, 205–212. https://doi.org/10.1016/j.envres.2014.10.022
Lithobates pipiens, (GS-25) (96 h)	VectoBac® 200G	200	394.3	78,860	Amphibian	Current thesis.
Lithobates sylvaticus, (GS-25) (96 h)	VectoBac® 200G	200	2,626.81	52,5363.4	Amphibian	Current thesis.
Mus musculus (4 h)	Strain AM65-52	3,000	2.84	8,520	Mammal	World Health Organization. (2012). WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES *Bacillus thuringiensis* subspecies *israelensis* strain AM65-52. https://extranet.who.int/prequal/sites/default/files/vcp-documents/WHOVC-SP_Bti_strain_AM65-52_2012.pdf (accessed May 16, 2024).

Ochlerotatus vigilax, 3rd instar (48 h)	VectoBac® WG	3,000	0.013	39	Invertebrate	Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., & Kay, B.H. (2003). Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J. Econ. Entomol*. *96*(6), 1786–1791. https://doi.org/10.1093/jee/96.6.1786
Oncorhynchus mykiss (96 h)	VectoBac® 200G	200	370	74,000	Fish	Valent BioSciences. (2015). Safety data sheet for VectoBac® 200G. https://gardexinc.com/MSDS/5168Vectobac_EN_MSDS_2015_06.pdf (accessed May 27, 2024).
Oncorhynchus mykiss (96 h)	VectoBac® 12 AS	1,200	0.31	370	Fish	Valent BioSciences. (2020). Safety data sheet for VectoBac® G Biological Larvicide Granules. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/SDS-VBC-0020R5-VectoBac-G-01-03-20.pdf (Accessed May 27, 2024).
Oncorhynchus mykiss (96 h)	VectoBac® G	200	370	74,000	Fish	Valent BioSciences. (2020). Safety data sheet for VectoBac® G Biological Larvicide Granules. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2017/02/SDS-VBC-0020R5-VectoBac-G-01-03-20.pdf (Accessed May 27, 2024).
Oncorhynchus mykiss (96 h)	VectoBac® WDG	3,000	0.12	370	Fish	Valent BioSciences. (2024). Safety data sheet for VectoBac® WDG Biological Larvicide. https://www.valentbiosciences.com/publichealth/wp-content/uploads/sites/4/2024/04/SDS-VBC-0019R6-VectoBac-WDG-03-25-2024-.pdf (accessed May 27, 2024).

Physalaemus albonotatus (GS-33) (48 h)	Introban®	1,200	11.87	14,244	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll*. *228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Physalaemus albonotatus (GS-33) (24 h)	Introban®	1,200	11.87	14,244	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll*. *228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Pseudacris borealis (GS-25) (96 h)	VectoBac® 200G	200	2,565	513,000	Amphibian	Current thesis.
Rhinella arenarum (GS-33) (24 h)	Introban®	1,200	20.51	24,612	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll*. *228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Rhinella arenarum (GS-33) (48 h)	Introban®	1,200	19.25	23,100	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat.*

						Air and Soil Poll. 228(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Rhinella fernandezae (GS-33) (24 h)	Introban®	1,200	10.73	12,876	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll. 228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Rhinella arenarum (GS-33) (24 h)	Introban®	1,200	20.51	24,612	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll. 228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Rhinella arenarum (GS-33) (48 h)	Introban®	1,200	19.25	23,100	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll. 228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6
Rhinella fernandezae (GS-33) (24 h)	Introban®	1,200	10.73	12,876	Amphibian	Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., & Attademo, A.M. (2017). Acute Toxicity and Etho-toxicity of Three Insecticides Used for Mosquito Control on Amphibian Tadpoles. *Wat. Air and Soil Poll. 228*(4), 143. https://doi.org/10.1007/s11270-017-3324-6

Simulium reptans larvae (48 h)	VectoBac® AS	1,200	1.2	1,440	Invertebrate	Coupland, J.B. (1993). Factors affecting the efficacy of three commercial formulations of *Bacillus thuringiensis* var. *Israelensis* against species of European black flies. *Biocontrol Sci. Technol*. *3*(2), 199–210. https://doi.org/10.1080/09583159309355276
Simulium variegatum larvae (48 h)	VectoBac® AS	1,200	1.55	1,860	Invertebrate	Coupland, J.B. (1993). Factors affecting the efficacy of three commercial formulations of *Bacillus thuringiensis* var. *Israelensis* against species of European black flies. *Biocontrol Sci. Technol*. *3*(2), 199–210. https://doi.org/10.1080/09583159309355276
Simulium variegatum larvae (48 h)	Teknar® WDC	1,500	1.52	2,280	Invertebrate	Coupland, J.B. (1993). Factors affecting the efficacy of three commercial formulations of *Bacillus thuringiensis* var. *Israelensis* against species of European black flies. *Biocontrol Sci. Technol*. *3*(2), 199–210. https://doi.org/10.1080/09583159309355276
Simulium variegatum larvae (48 h)	Bactimos® WP	3,500	6.43	22,505	Invertebrate	Coupland, J.B. (1993). Factors affecting the efficacy of three commercial formulations of *Bacillus thuringiensis* var. *Israelensis* against species of European black flies. *Biocontrol Sci. Technol*. *3*(2), 199–210. https://doi.org/10.1080/09583159309355276

GS, Gosner stage; *ITU*, international toxic units; $LC_{50,}$ median lethal concentration

Table S1.3 Summary of the studies including species, chemicals, and LC_{50} of organisms exposed to deltamethrin products

Species	Chemical	Chemical potency (% of active ingredient)	LC_{50} of organism (µg/L)	LC_{50} of organism (µg a.i./L)	Group	Reference
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	0.03	0.02904	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	0.08	0.07744	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	0.2	0.1936	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1

Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	0.2	0.1936	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	0.3	0.2904	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	1.3	1.2584	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	7	6.776	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1

Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	8	7.744	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Aedes aegypti larvae (24 h)	Deltamethrin 96.8%	96.8	9	8.712	Invertebrate	Rodríguez, M.M., Bisset, J.A., & Fernández, D. (2007). LEVELS OF INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *AEDES AEGYPTI* FROM SOME LATIN AMERICAN COUNTRIES. *JAMCA*. *23*(4), 420–429. https://doi.org/10.2987/5588.1
Americamysis bahia juvenile (24 h)	Deltamethrin 97.7%	97.7	0.1133	0.1106941	Invertebrate	DeLorenzo, M.E., Key, P.B., Chung, K.W., Sapozhnikova, Y., & Fulton, M.H. (2014). Comparative toxicity of pyrethroid insecticides to two estuarine crustacean species, *Americamysis bahia* and *Palaemonetes pugio*. *Environ. Toxicol*. *29*(10), 1099–1106. https://doi.org/10.1002/tox.21840
Anisops sardeus (24 h)	Decis®	5	0.013	0.00065	Invertebrate	Lahr, J., Badji, A., Marquenie, S., Schuiling, E., Ndour, K.B., Diallo, A.O., & Everts, J.W. (2001). Acute Toxicity of Locust Insecticides to Two Indigenous Invertebrates from Sahelian Temporary Ponds. *Ecotoxicol. Environ. Saf*. *48*(1), 66–75. https://doi.org/10.1006/eesa.2000.1995

Anisops sardeus (48 h)	Decis®	5	0.012	0.0006	Invertebrate	Lahr, J., Badji, A., Marquenie, S., Schuiling, E., Ndour, K.B., Diallo, A.O., & Everts, J.W. (2001). Acute Toxicity of Locust Insecticides to Two Indigenous Invertebrates from Sahelian Temporary Ponds. *Ecotoxicol. Environ. Saf. 48*(1), 66–75. https://doi.org/10.1006/eesa.2000.1995
Apis cerana (24 h)	Deltamethrin 99.2%	99.2	520	515.84	Invertebrate	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27 2024).
Apis mellifera ligustica (24 h)	Decis® 2.5 EC	2.5	60,800	1,520	Invertebrate	Dai, P.-L., Wang, Q., Sun, J.-H., Liu, F., Wang, X., Wu, Y.-Y., & Zhou, T. (2010). Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee *Apis mellifera ligustica*. *Environ. Toxicol. Chem. 29*(3), 644–649. https://doi.org/10.1002/etc.67
Brachydanio rerio, larvae (35 days)	Deltamethrin 98%	98	0.52	0.5096	Fish	Görge, G., Nagel, R. (1990). Toxicity of lindane, atrazine, and deltamethrin to early life stages of zebrafish (*Brachydanio rerio*). *Ecotoxicol. Environ. Saf. 20*(3), 246–255. https://doi.org/10.1016/0147-6513(90)90004-O
Brycon amazonicus,	Keshet®	2.5	2.6	0.065	Fish	Moraes, F., Venturini, F., Cortella, L., Rossi, P. (2013). Acute toxicity of pyrethroid-based insecticides in the Neotropical

juvenile (96 h)						freshwater fish *Brycon amazonicus*. Ecotoxicol. Environ. Contam. 8, 59–64. https://doi.org/10.5132/eec.2013.02.009
Buenoa tarsalis (72 h)	Decis® 25 EC	25	0.004	0.001	Invertebrate	Gutiérrez, Y., Tomé, H.V.V., Guedes, R.N.C., & Oliveira, E.E. (2017). Deltamethrin toxicity and impaired swimming behavior of two backswimmer species. *Environ. Toxicol. Chem. 36*(5), 1235–1242. https://doi.org/10.1002/etc.3645
Bufo arenarium (GS 26-27) (48 h)	Deltamethrin 99%	99	11.93	11.8107	Amphibian	Salibián, A. (1992). Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ. Contam. Toxicol*. *48*(4), 616–621. https://doi.org/10.1007/BF00199082
Bufo arenarium (GS 26-27) (72 h)	Deltamethrin 99%	99	7.09	7.0191	Amphibian	Salibián, A. (1992). Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ. Contam. Toxicol*. *48*(4), 616–621. https://doi.org/10.1007/BF00199082
Bufo arenarium (GS 26-27) (96 h)	Deltamethrin 99%	99	4.37	4.3263	Amphibian	Salibián, A. (1992). Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ. Contam. Toxicol*. *48*(4), 616–621. https://doi.org/10.1007/BF00199082
Bufo arenarium	Deltamethrin 99%	99	16.84	16.6716	Amphibian	Salibián, A. (1992). Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ.*

(GS 28-30) (48 h)						*Contam. Toxicol*. *48*(4), 616–621. https://doi.org/10.1007/BF00199082
Bufo arenarium (GS 28-30) (72 h)	Deltamethrin 99%	99	12.04	11.9196	Amphibian	Salibián, A. (1992). Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ. Contam. Toxicol*. *48*(4), 616–621. https://doi.org/10.1007/BF00199082
Bufo arenarium (GS 28-30) (96 h)	Deltamethrin 99%	99	4.5	4.455	Amphibian	Salibián, A. (1992). Effects of deltamethrin on the South American toad, *Bufo arenarum*, tadpoles. *Bull. Environ. Contam. Toxicol*. *48*(4), 616–621. https://doi.org/10.1007/BF00199082
Caenis miliaria, larvae (96 h)	Decis®	5	0.0091	0.000455	Invertebrate	Beketov, M.A. (2004). Comparative Sensitivity to the Insecticides Deltamethrin and Esfenvalerate of Some Aquatic Insect Larvae (Ephemeroptera and Odonata) and *Daphnia magna*. *Russ. J. Ecol*. *35*(3), 200–204. https://doi.org/10.1023/B:RUSE.0000025972.29638.46
Carassius auratus gibelio (96 h)	Deltamethrin 98%	98	6.194	6.07012	Fish	Wu, H., Gao, J., Xie, M., Xiang, J., Zuo, Z., Tian, X., Song, R., Yuan, X., Wu, Y., & Ou, D. (2022). Histopathology and transcriptome analysis reveals the gills injury and immunotoxicity in gibel carp following acute deltamethrin exposure. *Ecotoxicol. Environ. Saf. 234*, 113421. https://doi.org/10.1016/j.ecoenv.2022.113421

Catla catla, fingerling (96 h)	Deltamethrin 98%	98	4.84	4.7432	Fish	Vani, T., Saharan, N., Mukherjee, S.C., Ranjan, R., Kumar, R., & Brahmchari, R.K. (2011). Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. *Pest. Biochem. Phys. 101*(1), 16–20. https://doi.org/10.1016/j.pestbp.2011.05.007
Ceriodaphnia dubia (24 h)	Deltamethrin 99.8%	99.8	0.06	0.05988	Invertebrate	Shen, M.-F., Kumar, A., Ding, S.-Y., & Grocke, S. (2012). Comparative study on the toxicity of pyrethroids, α-cypermethrin and deltamethrin to *Ceriodaphnia dubia*. *Ecotoxicol. Environ. Saf. 78*, 9–13. https://doi.org/10.1016/j.ecoenv.2011.07.018
Ceriodaphnia dubia (24 h)	Deltamethrin 99.8%	99.8	0.84	0.83832	Invertebrate	Shen, M.-F., Kumar, A., Ding, S.-Y., & Grocke, S. (2012). Comparative study on the toxicity of pyrethroids, α-cypermethrin and deltamethrin to *Ceriodaphnia dubia*. *Ecotoxicol. Environ. Saf. 78*, 9–13. https://doi.org/10.1016/j.ecoenv.2011.07.018
Channa argus (96 h)	Deltamethrin 99%	99	1.94	1.9206	Fish	Kong, Y., Li, M., Shan, X., Wang, G., & Han, G. (2021). Effects of deltamethrin subacute exposure in snakehead fish, *Channa argus*: Biochemicals, antioxidants and immune responses. *Ecotoxicol. Environ. Saf. 209*, 111821. https://doi.org/10.1016/j.ecoenv.2020.111821

Channa punctatus (96 h)	Decis® 2.5% EC	2.5	7.33	0.18325	Fish	Singh, S., Tiwari, R.K., & Pandey, R.S. (2018). Evaluation of acute toxicity of triazophos and deltamethrin and their inhibitory effect on AChE activity in *Channa punctatus*. *Toxicol. Rep. 5*, 85–89. https://doi.org/10.1016/j.toxrep.2017.12.006
Cherax destructor (24 h)	Decis® Mega	4.8	3.57	0.17136	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Cherax destructor (48 h)	Decis® Mega	4.8	1.88	0.09024	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Cherax destructor (72 h)	Decis® Mega	4.8	0.49	0.02352	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Cherax destructor (96 h)	Decis® Mega	4.8	0.27	0.01296	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED

Clarias gariepinus (24 h)	K-Obiol® 2.5 WP	2.5	3,930	98.25	Fish	Datta, M. & Kaviraj, A. (2003). Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Freshwater Catfish *Clarias gariepinus*. *Bull. Environ. Contam. Toxicol. 70*(2), 296–299. https://doi.org/10.1007/s00128-002-0190-7
Clarias gariepinus (48 h)	K-Obiol® 2.5 WP	2.5	40,010	1,000.25	Fish	Datta, M. & Kaviraj, A. (2003). Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Freshwater Catfish *Clarias gariepinus*. *Bull. Environ. Contam. Toxicol. 70*(2), 296–299. https://doi.org/10.1007/s00128-002-0190-7
Clarias gariepinus (72 h)	K-Obiol® 2.5 WP	2.5	40,010	1,000.25	Fish	Datta, M. & Kaviraj, A. (2003). Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Freshwater Catfish *Clarias gariepinus*. *Bull. Environ. Contam. Toxicol. 70*(2), 296–299. https://doi.org/10.1007/s00128-002-0190-7
Clarias gariepinus (96 h)	K-Obiol® 2.5 WP	2.5	40,010	1,000.25	Fish	Datta, M. & Kaviraj, A. (2003). Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Freshwater Catfish *Clarias gariepinus*. *Bull. Environ. Contam. Toxicol. 70*(2), 296–299. https://doi.org/10.1007/s00128-002-0190-7
Cleon dipterum, larvae (96 h)	Decis®	5	0.005	0.00025	Invertebrate	Beketov, M.A. (2004). Comparative Sensitivity to the Insecticides Deltamethrin and Esfenvalerate of Some Aquatic Insect Larvae (Ephemeroptera and Odonata) and *Daphnia magna*. *Russ. J. Ecol. 35*(3), 200–204. https://doi.org/10.1023/B:RUSE.0000025972.29638.46

Colossoma macropomum, fingerling (96 h)	Decis 25 EC	25	55.6	13.9	Fish	Cunha, F.D.S., Sousa, N.D.C., Santos, R.F.B., Meneses, J.O., Do Couto, M.V.S., De Almeida, F.T.C., De Sena Filho, J.G., Carneiro, P.C.F., Maria, A.N., & Fujimoto, R.Y. (2018). Deltamethrin-induced nuclear erythrocyte alteration and damage to the gills and liver of *Colossoma macropomum*. *Environ. Science Poll. Res. 25*(15), 15102–15110. https://doi.org/10.1007/s11356-018-1622-1
Cordulia aenea, larvae (96 h)	Decis®	5	0.76	0.038	Invertebrate	Beketov, M.A. (2004). Comparative Sensitivity to the Insecticides Deltamethrin and Esfenvalerate of Some Aquatic Insect Larvae (Ephemeroptera and Odonata) and *Daphnia magna*. *Russ. J. Ecol. 35*(3), 200–204. https://doi.org/10.1023/B:RUSE.0000025972.29638.46
Coturnix coturnix japonica (5 days)	Deltamethrin 99.2%	99.2	5,000,000	4,960,000	Bird	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Coturnix coturnix japonica (8 days)	Deltamethrin 98.5%	98.5	545,510	537,327.35	Bird	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/W

						HOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Crangon septemspinosa (14 days)	Decis®	5	0.0151	0.000755	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Crangon septemspinosa (14 days)	AlphaMax®	1	0.0238	0.000238	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)

Crangon septemspinosa (24 h)	AlphaMax®	1	0.027	0.00027	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Crangon septemspinosa (96 h)	AlphaMax®	1	0.142	0.00142	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Crangon septemspinosa (96 h)	AlphaMax®	1	0.0453	0.000453	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876.

						https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Crangon septemspinosa (96 h)	Decis®	5	0.027	0.00135	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Crangon septemspinosa (96 h)	Decis®	5	0.027	0.00135	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)

Ctenopharyngodon idella (24 h)	Decis® 25 EC	25	155	38.75	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Ctenopharyngodon idella (48 h)	Decis® 25 EC	25	96	24	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Ctenopharyngodon idella (96 h)	Decis® 25 EC	25	91	22.75	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Cyprinodon variegatus (96 h)	Deltamethrin 2.5%	2.5	0.48	0.012	Fish	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).
Cyprinodon variegatus (96 h)	Deltamethrin Pour-On Formulation	98	0.48	0.4704	Fish	MERCK. (2023). Material safety data sheet - deltamethrin pour-on formulation. https://www.msd.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20Pour-On%20Formulation_AH_MX_EN.pdf (accessed May 27, 2024).

Cyprinus carpio (96 h)	Deltamethrin 2.8% EC	2.8	2.3	0.0644	Fish	Sun, F. (1987). Evaluating Acute Toxicity of Pesticides to Aquatic Organisms: Carp, Mosquito Fish and Daphnids. *Plant Prot. Bull. 29*(4), 385-396.
Cyprinus carpio (24 h)	Decis® 2.5 EC	2.5	3.5	0.0875	Fish	Lakota, S., Raszka, A. Utracki, T., & Chmiel, Z. (1989). Side-Effect of Deltamethrin and Cypermethrin in the Environment of Water Biocenoses. *Organika*. 71-77.
Cyprinus carpio (24 h)	Decis® 25 EC	25	91	22.75	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Cyprinus carpio (48 h)	Decis® 2.5 EC	2.5	3.5	0.0875	Fish	Lakota, S., Raszka, A. Utracki, T., & Chmiel, Z. (1989). Side-Effect of Deltamethrin and Cypermethrin in the Environment of Water Biocenoses. *Organika*. 71-77.
Cyprinus carpio (48 h)	Decis® 25 EC	25	89	22.25	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Cyprinus carpio (48 h)	Deltamethrin 2.8% EC	2.8	4	0.112	Fish	Sun, F. (1987). Evaluating Acute Toxicity of Pesticides to Aquatic Organisms: Carp, Mosquito Fish and Daphnids. *Plant Prot. Bull. 29*(4), 385-396.
Cyprinus carpio (96 h)	Decis® 2.5 EC	2.5	3.5	0.0875	Fish	Lakota, S., Raszka, A. Utracki, T., & Chmiel, Z. (1989). Side-Effect of Deltamethrin and Cypermethrin in the Environment of Water Biocenoses. *Organika*. 71-77.

Cyprinus carpio (96 h)	Decis® 25 EC	25	78	19.5	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Cyprinus carpio (96 h)	Decis® Flow 2.5	2.5	3.25	0.08125	Fish	Velíšek, J., Dobšíková, R., Svobodová, Z., Modrá, H., & Lusková, V. (2006). Effect of Deltamethrin on the Biochemical Profile of Common Carp (*Cyprinus carpio L.*). *Bull. Environ. Contam. Toxicol. 76*(6), 992–998. https://doi.org/10.1007/s00128-006-1016-9
Cyprinus carpio, embryos (48 h)	Decis® EC	2.5	0.213	0.005325	Fish	Köprücü, K. & Aydın, R. (2004). The toxic effects of pyrethroid deltamethrin on the common carp (*Cyprinus carpio L.*) embryos and larvae. *Pest. Biochem. Phys. 80*(1), 47–53. https://doi.org/10.1016/j.pestbp.2004.05.004
Cyprinus carpio, juvenile (72 h)	Decis® 2.5 EC	2.5	2.37	0.05925	Fish	Calta, M. & Ural, M. (2004). Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, *Cyprinus Carpio*. *Fres. Environ. Bull. 13*, 1179–1183.
Cyprinus carpio, juvenile (96 h)	Decis® 2.5 EC	2.5	1.65	0.04125	Fish	Calta, M. & Ural, M. (2004). Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, *Cyprinus Carpio*. *Fres. Environ. Bull. 13*, 1179–1183.

Cyprinus carpio, juvenile (24 h)	Decis® 2.5 EC	2.5	9.41	0.23525	Fish	Calta, M. & Ural, M. (2004). Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, *Cyprinus Carpio*. *Fres. Environ. Bull. 13*, 1179–1183.
Cyprinus carpio, juvenile (48 h)	Decis® 2.5 EC	2.5	4.47	0.11175	Fish	Calta, M. & Ural, M. (2004). Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, *Cyprinus Carpio*. *Fres. Environ. Bull. 13*, 1179–1183.
Cyprinus carpio, larvae (48 h)	Decis® EC	2.5	0.074	0.00185	Fish	Köprücü, K. & Aydın, R. (2004). The toxic effects of pyrethroid deltamethrin on the common carp (*Cyprinus carpio L.*) embryos and larvae. *Pest. Biochem. Phys. 80*(1), 47–53. https://doi.org/10.1016/j.pestbp.2004.05.004
Cyprinus carpio, larvae (96 h)	Decis® Flow 2.5	2.5	58	1.45	Fish	Svobodová, Z., Lusková, V., Drastichová, J., Svoboda, M., & Žlábek, V. (2003). Effect of Deltamethrin on Haematological Indices of Common Carp (*Cyprinus carpio L.*). *Acta Vet. Brno. 72*(1), 79–85. https://doi.org/10.2754/avb200372010079
Danio rerio, embryo (6 days)	Deltamethrin 99%	99	40	39.6	Fish	DeMicco, A., Cooper, K.R., Richardson, J.R., & White, L.A. (2010). Developmental Neurotoxicity of Pyrethroid Insecticides in Zebrafish Embryos. *Toxicol. Sciences*. *113*(1), 177–186. https://doi.org/10.1093/toxsci/kfp258

Daphnia magna (48 h)	Deltamethrin 98%	98	65	63.7	Invertebrate	Rodrigues, S., Teixeira, M.I., Diogo, B.S., & Antunes, S.C. (2023). Assessment of the ecotoxicological effects of deltamethrin to *Daphnia magna*: Linking sub-individual and supra-individual parameters. *Water. Ecol. Environ. 5*, 231–240. https://doi.org/10.1016/j.wsee.2023.10.002
Daphnia magna, juvenile (96 h)	Decis®	5	0.0293	0.001465	Invertebrate	Beketov, M.A. (2004). Comparative Sensitivity to the Insecticides Deltamethrin and Esfenvalerate of Some Aquatic Insect Larvae (Ephemeroptera and Odonata) and *Daphnia magna*. *Russ. J. Ecol. 35*(3), 200–204. https://doi.org/10.1023/B:RUSE.0000025972.29638.46
Daphnia magnia, juvenile (24 h)	Decis®	5	520	26	Invertebrate	Xiu, R., Xu, Y., & Gao, S. (1989). Toxicity of the new pyrethroid insecticide, deltamethrin, to *Daphnia magna. Hydro. 188*, 411-413. https://doi-org.proxy.bib.uottawa.ca/10.1007/BF00027808
Daphnia magnia, neonate (96 h)	Decis®	5	0.01	0.0005	Invertebrate	Xiu, R., Xu, Y., & Gao, S. (1989). Toxicity of the new pyrethroid insecticide, deltamethrin, to *Daphnia magna. Hydro. 188*, 411-413. https://doi-org.proxy.bib.uottawa.ca/10.1007/BF00027808
Eohaustorius estuarius (96 h)	AlphaMax®	1	0.00166	0.0000166	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE

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Eohaustorius estuarius (96 h)	Decis®	5	0.0032	0.00016	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Eohaustorius estuarius (96 h)	Decis®	5	0.00799	0.0003995	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences

						2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Esox lucius (48 h)	Decis® 25 EC	25	30	7.5	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Esox lucius (24 h)	Decis® 25 EC	25	44	11	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Esox lucius (96 h)	Decis® 25 EC	25	23	5.75	Fish	Rao, K.J., Madhu, C., & Murthy, V.S.R. (1983). Histopathology of Malathion on Gills of a Freshwater Teleost, *Tilapia mossambica* (Peters). *J. Environ.Biol. 4*(1), 9-13.
Gammarus fasciatus (96 h)	Deltamethrin 2.5%	2.5	0.0003	0.0000075	Invertebrate	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).
Gammarus fasciatus (96 h)	Deltamethrin Pour-On Formulation	98	0.3	0.294	Invertebrate	MERCK. (2023). Material safety data sheet - deltamethrin pour-on formulation. https://www.msd.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20Pour-

						On%20Formulation_AH_MX_EN.pdf (accessed May 27, 2024).
Gammarus fossarum, adult (48 h)	Deltamethrin 98%	98	0.0332	0.032536	Invertebrate	Adam, O., Degiorgi, F., Crini, G., & Badot, P.-M. (2010). High sensitivity of Gammarus sp. juveniles to deltamethrin: Outcomes for risk assessment. *Ecotoxicol. Environ. Saf. 73*(6), 1402–1407. https://doi.org/10.1016/j.ecoenv.2010.02.011
Gammarus fossarum, juvenile (48 h)	Deltamethrin 98%	98	0.004	0.00392	Invertebrate	Adam, O., Degiorgi, F., Crini, G., & Badot, P.-M. (2010). High sensitivity of Gammarus sp. juveniles to deltamethrin: Outcomes for risk assessment. *Ecotoxicol. Environ. Saf. 73*(6), 1402–1407. https://doi.org/10.1016/j.ecoenv.2010.02.011
Gammarus pulex, adult (48 h)	Deltamethrin 98%	98	0.068	0.06664	Invertebrate	Adam, O., Degiorgi, F., Crini, G., & Badot, P.-M. (2010). High sensitivity of Gammarus sp. juveniles to deltamethrin: Outcomes for risk assessment. *Ecotoxicol. Environ. Saf. 73*(6), 1402–1407. https://doi.org/10.1016/j.ecoenv.2010.02.011
Gammarus pulex, juvenile (48 h)	Deltamethrin 98%	98	0.0057	0.005586	Invertebrate	Adam, O., Degiorgi, F., Crini, G., & Badot, P.-M. (2010). High sensitivity of Gammarus sp. juveniles to deltamethrin: Outcomes for risk assessment. *Ecotoxicol. Environ. Saf. 73*(6), 1402–1407. https://doi.org/10.1016/j.ecoenv.2010.02.011
Hepteropneustes fossilis (96 h)	Deltamethrin 2.8% EC	2.8	520	14.56	Fish	Kumar, S., Lata, S., & Gopal, K. (1999). Deltamethrin Induced Physiological Changes in Freshwater Cat Fish *Heteropneustes*

						fossilis. *Bull. Environ. Contam. Toxicol. 62*(3), 254–258. https://doi.org/10.1007/s001289900867
Hepteropneustes fossilis (96 h)	Decis® EC	2.5	1.86	0.0465	Fish	Lyons, M.C., Burridge, D.K.H., & MacKeigan, K.G. (2017). The lethality of the anti-sea lice formulation AlphaMax (deltamethrin) to adult American lobster (*Homarus americanus*) during chronic or pulse dose exposures. Canadian Technical Report of Fisheries and Aquatic Sciences 3217. https://publications.gc.ca/collections/collection_2018/mpo-dfo/Fs97-6-3217-eng.pdf (Accessed February 16, 2024)
Homarus americanus (10 days)	AlphaMax®	1	0.0147	0.000147	Invertebrate	Kumar, S., Lata, S., & Gopal, K. (1999). Deltamethrin Induced Physiological Changes in Freshwater Cat Fish *Heteropneustes fossilis*. *Bull. Environ. Contam. Toxicol. 62*(3), 254–258. https://doi.org/10.1007/s001289900867
Homarus americanus, adult (24 h)	AlphaMax®	1	0.015	0.00015	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Homarus americanus, adult (96 h)	AlphaMax®	1	0.0188	0.000188	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and

						Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Homarus americanus, stage I (24 h)	AlphaMax®	1	0.0008	0.000008	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Homarus americanus, stage I (96 h)	AlphaMax®	1	0.0034	0.000034	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Homarus americanus, stage II (24 h)	AlphaMax®	1	0.0006	0.000006	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Homarus americanus, stage III (16 days)	AlphaMax®	1	0.00445	0.0000445	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN

						AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus americanus, stage III (96 h)	AlphaMax®	1	0.00374	0.0000374	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus americanus, stage III (96 h)	AlphaMax®	1	0.00474	0.0000474	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876.

						https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus americanus, stage III (96 h)	Decis®	5	0.00492	0.000246	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus americanus, stage IV (24 h)	AlphaMax®	1	0.0017	0.000017	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Homarus americanus, stage IV (96 h)	AlphaMax®	1	0.0282	0.000282	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE.

						Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus americanus, stage IV (96 h)	Decis®	5	0.028	0.0014	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876. https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus americanus, stage IV (96 h)	Decis®	5	0.028	0.0014	Invertebrate	Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., & Cook, A.M. (2010). ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE. Canadian Technical Report of Fisheries and Aquatic Sciences 2876.

						https://publications.gc.ca/collections/collection_2010/mpo-dfo/Fs97-6-2876-eng.pdf (Accessed February 16, 2024)
Homarus Gammarus, *stage* I (1 h)	AlphaMax®	1	0.0026	0.000026	Invertebrate	Parsons, A.E., Escobar-Lux, R.H., Sævik, P.N., Samuelsen, O.B., & Agnalt, A.-L. (2020). The impact of anti-sea lice pesticides, azamethiphos and deltamethrin, on European lobster (*Homarus gammarus*) larvae in the Norwegian marine environment. *Environ. Poll. 264*, 114725. https://doi.org/10.1016/j.envpol.2020.114725
Homarus Gammarus, stage II (1 h)	AlphaMax®	1	0.0029	0.000029	Invertebrate	Parsons, A.E., Escobar-Lux, R.H., Sævik, P.N., Samuelsen, O.B., & Agnalt, A.-L. (2020). The impact of anti-sea lice pesticides, azamethiphos and deltamethrin, on European lobster (*Homarus gammarus*) larvae in the Norwegian marine environment. *Environ. Poll. 264*, 114725. https://doi.org/10.1016/j.envpol.2020.114725
Labeo rohita (96 h)	Deltamethrin 2.8% EC	2.8	1,000	28	Fish	Rathnamma, V.V., Kumar, M.V., & Philip, G.H. (2007). Effect of Deltamethrin on glycogen phosphorylase and glucose-6-phosphatase activity in freshwater fish *Labeo rohita. BPAS-Z. 26*(2), 1–1.
Lepomis macrochirus (96 h)	Deltamethrin 98.5%	98.5	0.727	0.716095	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/W

						HOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Lestes sponsa, larvae (96 h)	Decis®	5	0.0145	0.000725	Invertebrate	Beketov, M.A. (2004). Comparative Sensitivity to the Insecticides Deltamethrin and Esfenvalerate of Some Aquatic Insect Larvae (Ephemeroptera and Odonata) and *Daphnia magna*. *Russ. J. Ecol. 35*(3), 200–204. https://doi.org/10.1023/B:RUSE.0000025972.29638.46
Lithobates pipiens (GS 25) (96 h)	Deltamethrin 95%	95	7.7	7.3	Amphibian	Current thesis.
Lithobates sylvaticus (GS 25) (96 h)	Deltamethrin 95%	95	1.212	1.15	Amphibian	Current thesis.
Martarega bentoi (72 h)	Decis® 25 EC	25	0.1025	0.025625	Invertebrate	Gutiérrez, Y., Tomé, H.V.V., Guedes, R.N.C., & Oliveira, E.E. (2017). Deltamethrin toxicity and impaired swimming behavior of two backswimmer species. *Environ. Toxicol. Chem. 36*(5), 1235–1242. https://doi.org/10.1002/etc.3645
Microsternarchus cf.	Decis® EC	2.5	6.12	0.153	Fish	Chaves, V.D.S., Marcon, J.L., Duncan, W.P., & Alves-Gomes, J.A. (2020). Acute toxicity of a deltamethrin based pesticide (DBP) to the Neotropical electric fish *Microsternarchus cf.*

bilineatus (24 h)						*Bilineatus* (Gymnotiformes). *Acta. Amazon. 50*(4), 355–362. https://doi.org/10.1590/1809-4392201904001
Microsternarchus cf. bilineatus (48 h)	Decis® EC	2.5	3.21	0.08025	Fish	Chaves, V.D.S., Marcon, J.L., Duncan, W.P., & Alves-Gomes, J.A. (2020). Acute toxicity of a deltamethrin based pesticide (DBP) to the Neotropical electric fish *Microsternarchus cf. Bilineatus* (Gymnotiformes). *Acta. Amazon. 50*(4), 355–362. https://doi.org/10.1590/1809-4392201904001
Microsternarchus cf. bilineatus (72 h)	Decis® EC	2.5	2.14	0.0535	Fish	Chaves, V.D.S., Marcon, J.L., Duncan, W.P., & Alves-Gomes, J.A. 2020. Acute toxicity of a deltamethrin based pesticide (DBP) to the Neotropical electric fish *Microsternarchus cf. Bilineatus* (Gymnotiformes). *Acta. Amazon. 50*(4), 355–362. https://doi.org/10.1590/1809-4392201904001
Microsternarchus cf. bilineatus (96 h)	Decis® EC	2.5	2.15	0.05375	Fish	Chaves, V.D.S., Marcon, J.L., Duncan, W.P., & Alves-Gomes, J.A. 2020. Acute toxicity of a deltamethrin based pesticide (DBP) to the Neotropical electric fish *Microsternarchus cf. Bilineatus* (Gymnotiformes). *Acta. Amazon. 50*(4), 355–362. https://doi.org/10.1590/1809-4392201904001
Mus musculus (2 h)	Deltamethrin 2.5%	2.5	800	20	Mammal	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-

						sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).
Mus musculus (2 h)	Deltamethrin Pour-On Formulation	98	800	784	Mammal	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Mus musculus (4 h)	Deltamethrin 2.5%	2.5	5,610	140.25	Mammal	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).
Mus musculus (4 h)	Deltamethrin 98%	98	232	227.36	Mammal	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Mus musculus (4 h)	Deltamethrin 98.3%	98.3	1,451.9	1,427.2177	Mammal	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/W

						HOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Mus musculus (4 h)	Deltamethrin 99.2%	99.2	3,100	3,075.2	Mammal	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Mysid sp. (96 h)	AlphaMax®	1	0.139	0.00139	Invertebrate	Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., & VanGeest, J.L. (2014). The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox®Paramove™50 to lobster and shrimp. *Aquac*. 180–186. https://doi.org/10.1016/j.aquaculture.2013.10.041
Oncorhynchus mykiss (48 h)	Decis® 25 EC	25	2.3	0.575	Fish	Lakota, S., Raszka, A. Utracki, T., & Chmiel, Z. (1989). Side-Effect of Deltamethrin and Cypermethrin in the Environment of Water Biocenoses. *Organika*. 71-77.
Oncorhynchus mykiss (24 h)	Decis® 25 EC	25	2.5	0.625	Fish	Lakota, S., Raszka, A. Utracki, T., & Chmiel, Z. (1989). Side-Effect of Deltamethrin and Cypermethrin in the Environment of Water Biocenoses. *Organika*. 71-77.
Oncorhynchus mykiss (96 h)	Decis® 25 EC	25	2.3	0.575	Fish	Lakota, S., Raszka, A. Utracki, T., & Chmiel, Z. (1989). Side-Effect of Deltamethrin and Cypermethrin in the Environment of Water Biocenoses. *Organika*. 71-77.

Oncorhynchus mykiss fry (24 h)	Decis® 2.5 EC	2.5	3.1856	0.07964	Fish	Ural, M.Ş. & Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Pest. Biochem. Phys.* *83*(2), 124–131. https://doi.org/10.1016/j.pestbp.2005.04.004
Oncorhynchus mykiss fry (1 h)	Decis® 2.5 EC	2.5	15.8708	0.39677	Fish	Ural, M.Ş. & Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Pest. Biochem. Phys.* *83*(2), 124–131. https://doi.org/10.1016/j.pestbp.2005.04.004
Oncorhynchus mykiss fry (12 h)	Decis® 2.5 EC	2.5	7.0014	0.175035	Fish	Ural, M.Ş. & Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Pest. Biochem. Phys.* *83*(2), 124–131. https://doi.org/10.1016/j.pestbp.2005.04.004
Oncorhynchus mykiss fry (48 h)	Decis® 2.5 EC	2.5	1.6568	0.04142	Fish	Ural, M.Ş. & Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Pest. Biochem. Phys.* *83*(2), 124–131. https://doi.org/10.1016/j.pestbp.2005.04.004
Oncorhynchus mykiss fry (72 h)	Decis® 2.5 EC	2.5	0.98	0.0245	Fish	Ural, M.Ş. & Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Pest. Biochem. Phys.* *83*(2), 124–131. https://doi.org/10.1016/j.pestbp.2005.04.004

Oncorhynchus mykiss fry (96 h)	Decis® 2.5 EC	2.5	0.6961	0.0174025	Fish	Ural, M.Ş. & Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss Walbaum*, 1792). *Pest. Biochem. Phys. 83*(2), 124–131. https://doi.org/10.1016/j.pestbp.2005.04.004
Oncorhyncus mykiss (96 h)	Decis® 100 EC	10.5	5.1	0.5355	Fish	Bayer Crop Science. (2021). Safety data sheet - DECIS® 100 EC INSECTICIDE. https://www.cropscience.bayer.ca/-/media/Bayer-CropScience/Country-Canada-Internet/Products/Decis-Prairies/DECIS_100_EC_INSECTICIDE-EN_04-29-21_ac.ashx (accessed May 27, 2024).
Oncorhyncus mykiss (96 h)	Deltamethrin 2.5%	2.5	0.39	0.00975	Fish	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).
Oncorhyncus mykiss (96 h)	Deltamethrin Pour-On Formulation	98	0.39	0.3822	Fish	MERCK. (2023). Material safety data sheet - deltamethrin pour-on formulation. https://www.msd.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20Pour-On%20Formulation_AH_MX_EN.pdf (accessed May 27, 2024).

Oncorhyncus mykiss (96 h)	Deltamethrin 98.5%	98.5	0.688	0.67768	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Orconectes limosus (24 h)	Decis® Mega	4.8	9.48	0.45504	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Orconectes limosus (48 h)	Decis® Mega	4.8	6.35	0.3048	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Orconectes limosus (72 h)	Decis® Mega	4.8	2.24	0.10752	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Orconectes limosus (96 h)	Decis® Mega	4.8	0.76	0.03648	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED

Oreochromis miloticus fingerlings (24 h)	Decis® 2.5 EC	2.5	16	0.4	Fish	Golow, A.A. & Godzi, T.A. (1994). Acute toxicity of deltamethrin and dieldrin to *Oreochromis niloticus* (LIN). *Bull. Environ. Contam. Toxicol. 52*(3). https://doi.org/10.1007/BF00197820
Oreochromis miloticus fingerlings (48 h)	Decis® 2.5 EC	2.5	15	0.375	Fish	Golow, A.A. & Godzi, T.A. (1994). Acute toxicity of deltamethrin and dieldrin to *Oreochromis niloticus* (LIN). *Bull. Environ. Contam. Toxicol. 52*(3). https://doi.org/10.1007/BF00197820
Oreochromis miloticus fingerlings (96 h)	Decis® 2.5 EC	2.5	14.5	0.3625	Fish	Golow, A.A. & Godzi, T.A. (1994). Acute toxicity of deltamethrin and dieldrin to *Oreochromis niloticus* (LIN). *Bull. Environ. Contam. Toxicol. 52*(3). https://doi.org/10.1007/BF00197820
Oreochromis mossambicus juvenile (96 h)	Decis® 98%	98	250	245	Fish	Vijayavel, K. & Balasubramanian, M.P. (2007). Interaction of potash and decis in the ecophysiology of a freshwater fish *Oreochromis mossambicus*. *Ecotoxicol. Environ. Saf. 66*(2), 154–158. https://doi.org/10.1016/j.ecoenv.2005.12.005
Oreochromis niloticus fingerlings (24 h)	Deltamethrin 98%	98	5.14	5.0372	Fish	Yildirim, M.Z., Benlı, A.Ç.K., Selvı, M., Özkul, A., Erkoç, F., & Koçak, O. (2006). Acute toxicity, behavioral changes, and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile tilapia

						(*Oreochromis niloticus L.*) fingerlings. *Environ. Toxicol. 21*(6), 614–620. https://doi.org/10.1002/tox.20225
Oreochromis niloticus fingerlings (48 h)	Deltamethrin 98%	98	4.85	4.753	Fish	Yildirim, M.Z., Benlı, A.Ç.K., Selvı, M., Özkul, A., Erkoç, F., & Koçak, O. (2006). Acute toxicity, behavioral changes, and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile tilapia (*Oreochromis niloticus L.*) fingerlings. *Environ. Toxicol. 21*(6), 614–620. https://doi.org/10.1002/tox.20225
Oreochromis niloticus fry (48 h)	Deltamethrin 98%	98	1.7	1.666	Fish	Benli, A.Ç.K., Selvı, M., Sarikaya, R., Erkoç, F., & Koçak, O. (2009). Acute Toxicity of Deltamethrin on Nile Tilapia (*Oreochromis niloticus L*. 1758) Larvae and Fry. *Gazi U. J. Sci. 22*(1), 1–4.
Oreochromis niloticus larvae (48 h)	Deltamethrin 98%	98	1.17	1.1466	Fish	Benli, A.Ç.K., Selvı, M., Sarikaya, R., Erkoç, F., & Koçak, O. (2009). Acute Toxicity of Deltamethrin on Nile Tilapia (*Oreochromis niloticus L*. 1758) Larvae and Fry. *Gazi U. J. Sci. 22*(1), 1–4.
Oryzias latipes (96 h)	Deltamethrin 2.5%	2.5	8200	205	Fish	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).

Pacifastacus leniusculus (24 h)	Decis® Mega	4.8	0.07	0.00336	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Pacifastacus leniusculus (48 h)	Decis® Mega	4.8	0.05	0.0024	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Pacifastacus leniusculus (72 h)	Decis® Mega	4.8	0.04	0.00192	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Pacifastacus leniusculus (96 h)	Decis® Mega	4.8	0.03	0.00144	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Palaemon serratus (96 h)	Deltamethrin 98%	98	0.0484	0.047432	Invertebrate	Oliveira, C., Almeida, J., Guilhermino, L., Soares, A.M.V.M., & Gravato, C. (2012). Acute effects of deltamethrin on swimming velocity and biomarkers of the common prawn *Palaemon serratus*. *Aquat. Toxicol*. 209–216. https://doi.org/10.1016/j.aquatox.2012.08.010

Palaemonetes pugio (24 h)	Deltamethrin 97.7%	97.7	0.0232	0.0226664	Invertebrate	DeLorenzo, M.E., Key, P.B., Chung, K.W., Sapozhnikova, Y., & Fulton, M.H. (2014). Comparative toxicity of pyrethroid insecticides to two estuarine crustacean species, *Americamysis bahia* and *Palaemonetes pugio*. *Environ. Toxicol. 29*(10), 1099–1106. https://doi.org/10.1002/tox.21840
Palaemonetes pugio, larvae (24 h)	Deltamethrin 97.7%	97.7	0.0208	0.0203216	Invertebrate	DeLorenzo, M.E., Key, P.B., Chung, K.W., Sapozhnikova, Y., & Fulton, M.H. (2014). Comparative toxicity of pyrethroid insecticides to two estuarine crustacean species, *Americamysis bahia* and *Palaemonetes pugio*. *Environ. Toxicol. 29*(10), 1099–1106. https://doi.org/10.1002/tox.21840
Peocilia immobiliza (96 h)	Deltamethrin 99.2%	99.2	1.74	1.72608	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Physalaemus gracilis, embryos (GS 17-18) (96 h)	Decis® 25 EC	2.8	3,040	85.12	Amphibian	Macagnan, N., Rutkoski, C.F., Kolcenti, C., Vanzetto, G.V., Macagnan, L.P., Sturza, P.F., Hartmann, P.A., & Hartmann, M.T. (2017). Toxicity of cypermethrin and deltamethrin insecticides on embryos and larvae of *Physalaemus gracilis* (Anura: Leptodactylidae). *Environ. Science Poll. Res. Inter. Heid. 24*(25), 20699–20704.

						http://dx.doi.org.proxy.bib.uottawa.ca/10.1007/s11356-017-9727-5
Physalaemus gracilis, larvae (GS 24-25) (96 h)	Decis® 25 EC	2.8	500	14	Amphibian	Macagnan, N., Rutkoski, C.F., Kolcenti, C., Vanzetto, G.V., Macagnan, L.P., Sturza, P.F., Hartmann, P.A., & Hartmann, M.T. (2017). Toxicity of cypermethrin and deltamethrin insecticides on embryos and larvae of *Physalaemus gracilis* (Anura: Leptodactylidae). *Environ. Science Poll. Res. Inter. Heid. 24*(25), 20699–20704. http://dx.doi.org.proxy.bib.uottawa.ca/10.1007/s11356-017-9727-5
Pimephales promelas (96 h)	Deltamethrin 2.5%	2.5	8,200	205	Fish	MERCK. (2020). Material safety data sheet - deltamethrin (2.5%) formulation. https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Deltamethrin%20(2.5_pct)%20Formulation_AH_NO_6N.pdf (accessed May 27, 2024).
Poecilia reticulata (24 h)	Deltamethrin 98.5%	98.5	1.6	1.576	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).

Poecilia reticulata (48 h)	Deltamethrin 98.5%	98.5	1.6	1.576	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Poecilia reticulata (72 h)	Deltamethrin 98.5%	98.5	1.17	1.15245	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Poecilia reticulata (96 h)	Deltamethrin 98.5%	98.5	0.56	0.5516	Fish	World Health Organization. (2023). WHO specifications and evaluations for public health pesticides - deltamethrin. https://extranet.who.int/prequal/sites/default/files/doc_parts/WHOVC-SP_%20Deltamethrin%20_2023.pdf (accessed May 27, 2024).
Poecilia reticulata, adult male (48 h)	Deltamethrin 98%	98	5.1251	5.022598	Fish	Viran, R., Ünlü Erkoç, F., Polat, H., & Koçak, O. (2003). Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). *Ecotoxicol. Environ. Saf. 55*(1), 82–85. https://doi.org/10.1016/S0147-6513(02)00096-9
Procambarus clarkii (24 h)	Decis® Mega	4.8	0.9	0.0432	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous

						crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus clarkii (48 h)	Decis® Mega	4.8	0.16	0.00768	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus clarkii (48 h)	Decis® Mega	4.8	0.24	0.01152	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus clarkii (72 h)	Decis® Mega	4.8	0.21	0.01008	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus virginalis (24 h)	Decis® Mega	4.8	6.06	0.29088	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus virginalis (48 h)	Decis® Mega	4.8	3.7	0.1776	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous

						crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus virginalis (72 h)	Decis® Mega	4.8	0.57	0.02736	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Procambarus virginalis (96 h)	Decis® Mega	4.8	0.21	0.01008	Invertebrate	Lidova, J., Buric, M., Kouba, A., & Velisek, J. (2019). Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veter. Med. 64*(3), 125–133. https://doi.org/10.17221/136/2018-VETMED
Pseudacris maculata (96 h)	Deltamethrin 95%	95	2.828	2.69	Amphibian	Current thesis.
Salmo salar, juvenile (96 h)	Decis® 25 EC	2.8	0.59	0.01652	Fish	Zitko, V., McLeese, D.W., Metcalfe, C.D., & Carson, W.G. (1979). Toxicity of permethrin, decamethrin, and related pyrethroids to salmon and lobster. *Bull. Environ. Contam. Toxicol. 21*(1), 338–343. https://doi.org/10.1007/BF01685433
Spodoptera frugiperda, 3rd instar (5 days)	Decis®	5	3,580,000	179,000	Invertebrate	Vinha, G.L., Plata-Rueda, A., Soares, M.A., Zanuncio, J.C., Serrão, J.E., & Martínez, L.C. (2021). Deltamethrin-Mediated Effects on Locomotion, Respiration, Feeding, and Histological

						Changes in the Midgut of *Spodoptera frugiperda* Caterpillars. *Insects. 12*(6), 483. https://doi.org/10.3390/insects12060483
Trichogaster trichopterus (24 h)	Deltamethrin 2.5%	2.5	293	7.325	Fish	Hedayati, A., Tarkhani, R., & Shadi, A. (2012). Investigation of Acute Toxicity of Two Pesticides Diazinon and Deltamethrin, on Blue Gourami, *Trichogaster trichopterus* (Pallus). *Global Vet. 8*. https://doi.org/10.5829/idosi.gv.2012.9.2.6347
Trichogaster trichopterus (48 h)	Deltamethrin 2.5%	2.5	280	7	Fish	Hedayati, A., Tarkhani, R., & Shadi, A. (2012). Investigation of Acute Toxicity of Two Pesticides Diazinon and Deltamethrin, on Blue Gourami, *Trichogaster trichopterus* (Pallus). *Global Vet. 8*. https://doi.org/10.5829/idosi.gv.2012.9.2.6347
Trichogaster trichopterus (72 h)	Deltamethrin 2.5%	2.5	236	5.9	Fish	Hedayati, A., Tarkhani, R., & Shadi, A. (2012). Investigation of Acute Toxicity of Two Pesticides Diazinon and Deltamethrin, on Blue Gourami, *Trichogaster trichopterus* (Pallus). *Global Vet. 8*. https://doi.org/10.5829/idosi.gv.2012.9.2.6347
Trichogaster trichopterus (96h)	Deltamethrin 2.5%	2.5	223	5.575	Fish	Hedayati, A., Tarkhani, R., & Shadi, A. (2012). Investigation of Acute Toxicity of Two Pesticides Diazinon and Deltamethrin, on Blue Gourami, *Trichogaster trichopterus* (Pallus). *Global Vet. 8*. https://doi.org/10.5829/idosi.gv.2012.9.2.6347
Unio elongatulus	Decis® 2.5 EC	2.5	7,300	182.5	Invertebrate	Köprücü, K. & Seker, E. (2008). Acute Toxicity of Deltamethrin for Freshwater Mussel, *Unio elongatulus eucirrus*

eucirrus (72 h)						*Bourguignat*. *Bull. Environ. Contam. Toxicol. 80*(1), 1–4. https://doi.org/10.1007/s00128-007-9254-z
Unio elongatulus eucirrus (1 h)	Decis® 2.5 EC	2.5	10,070	251.75	Invertebrate	Köprücü, K. & Seker, E. (2008). Acute Toxicity of Deltamethrin for Freshwater Mussel, *Unio elongatulus eucirrus Bourguignat*. *Bull. Environ. Contam. Toxicol. 80*(1), 1–4. https://doi.org/10.1007/s00128-007-9254-z
Unio elongatulus eucirrus (24 h)	Decis® 2.5 EC	2.5	8,990	224.75	Invertebrate	Köprücü, K. & Seker, E. (2008). Acute Toxicity of Deltamethrin for Freshwater Mussel, *Unio elongatulus eucirrus Bourguignat*. *Bull. Environ. Contam. Toxicol. 80*(1), 1–4. https://doi.org/10.1007/s00128-007-9254-z
Unio elongatulus eucirrus (48 h)	Decis® 2.5 EC	2.5	8,090	202.25	Invertebrate	Köprücü, K. & Seker, E. (2008). Acute Toxicity of Deltamethrin for Freshwater Mussel, *Unio elongatulus eucirrus Bourguignat*. *Bull. Environ. Contam. Toxicol. 80*(1), 1–4. https://doi.org/10.1007/s00128-007-9254-z
Unio elongatulus eucirrus (96 h)	Decis® 2.5 EC	2.5	6,600	165	Invertebrate	Köprücü, K. & Seker, E. (2008). Acute Toxicity of Deltamethrin for Freshwater Mussel, *Unio elongatulus eucirrus Bourguignat*. *Bull. Environ. Contam. Toxicol. 80*(1), 1–4. https://doi.org/10.1007/s00128-007-9254-z

Xenopis laevis tadpoles (NF stage 46) (168 h)	Decis® 2.5 EW	2.5	6.26	6.26	Amphibian	Aydin-Sinan, H., Güngördü, A., & Ozmen, M. (2012). Toxic effects of deltamethrin and λ-cyhalothrin on *Xenopus laevis* tadpoles. *J. Environ. Sci. Health. 47*(5), 397–402. https://doi.org/10.1080/03601234.2012.648545

a.i., active ingredient; *GS*, Gosner Stage; LC_{50}, median lethal concentration; *NF*, Nieuwkoop and Faber