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Lethal and sublethal effects of three mosquito larvicides on the endangered Romer's Tree Frog (Liuixalus romeri): implications for urban mosquito control

Yik-Hei Sung

h.sung@uos.ac.uk

University of Suffolk

Hiu Nam Kong

Lingnan University

Alex Hua Bing Liu

Lingnan University

Hiu Ching Yuen

Lingnan University

Franco Ka Wah Leung

James Cook University

Amy Wing Lam Fok

Lingnan University

Tin Yan Hui

Lingnan University

Michael Wai-neng Lau

Hong Kong Wetlands Conservation Association

Jia Huan Liew

University of Tasmania

Simon Yung Wa Sin

University of Hong Kong

Jonathan Fong

Lingnan University

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Abstract

Mosquito control is essential for public health, but the ecological impacts of larvicides on non-target organisms, particularly amphibians, remain poorly understood. In Hong Kong, three larvicides are commonly used: *Bacillus thuringiensis israelensis* (Bti), temephos, and larvicidal oil. This study evaluated the lethal and sublethal effects of these larvicides on the endangered Romer's Tree Frog (*Liuixalus romeri*), which occurs in peri-urban habitats in Hong Kong. We assessed the effects of prolonged exposure (up to 86 days) at recommended application rates on survival, growth, and movement behavior in tadpoles, and determined LC_{50} (lethal concentration that kills 50% of population) values. Under prolonged exposure, larvicidal oil caused near-complete mortality within 21 days and significantly reduced growth and movement. In contrast, Bti and temephos did not affect survival or growth, although both induced sublethal changes in movement behavior. These patterns were consistent with acute toxicity results: larvicidal oil exhibited the highest mortality, with a 96-hour LC_{50} only 4.6-fold higher than the recommended rate, whereas Bti and temephos showed larger safety margins, with 96-hour LC_{50} values 53–105-fold and 17–42-fold higher than recommended rates, respectively. Overall, larvicidal oil poses a high ecological risk and should be avoided in amphibian habitats, whereas Bti and temephos represent comparatively lower-risk alternatives. Furthermore, our results demonstrate that exposure duration strongly influences toxicity outcomes. Ecological risk assessments should incorporate both acute and prolonged exposure, as well as lethal and sublethal endpoints, when evaluating impacts of pesticides on non-target organisms.

Introduction

Mosquitoes are among the most significant vectors of human disease, responsible for over 600,000 deaths annually and imposing substantial economic costs globally (Roiz et al. 2024; World Health Organization 2024). In South China, including Hong Kong Special Administrative Region (hereafter referred to as Hong Kong), mosquito-borne diseases such as dengue fever, Japanese encephalitis, and chikungunya remain persistent public health threats (Wu et al. 2012; Yue et al. 2019). To mitigate these risks, integrated mosquito management strategies are widely implemented, including vector surveillance and population control (Ma et al. 2011). While mosquito control is essential for public health, it is equally important to minimize negative impacts on non-target organisms (World Health Organization 2013).

In Hong Kong, mosquito control primarily relies on two approaches: elimination of breeding sites and the application of insecticides targeting adult or larval stages (Food and Environmental Hygiene Department 2026). Larvicides are widely used to control mosquito populations at the aquatic stage, particularly in urban and peri-urban areas. The Hong Kong Government recommends six larvicides, three of which are most commonly used: *Bacillus thuringiensis israelensis* (Bti), larvicidal oil, and temephos (Food and Environmental Hygiene Department 2015; Legislative Council 2025). These agents represent different modes of action: Bti is a microbial insecticide that produces toxins targeting mosquito larvae (biological), temephos is an organophosphate insecticide that disrupts neural function (chemical), and larvicidal oil is a petroleum-based product that forms a surface film that suffocates larvae (physical).

While larvicides are applied to control mosquito larvae, they can also impact non-target aquatic animals, such as amphibian larvae. Amphibians are among the most threatened vertebrate groups, with 40% of species globally at risk of extinction (Luedtke et al. 2023). Species occupying aquatic habitats near urbanized areas are particularly vulnerable as their tadpoles can be directly exposed to mosquito larvicides applied to breeding sites. Among commonly used larvicides, temephos has been shown to be acutely toxic to amphibians, reducing egg survival and causing morphological abnormalities (Harischandra et al. 2012). Comparative studies further indicate that temephos is more toxic to tadpoles than Bti (Junges et al. 2017). In general, amphibians are more tolerant to Bti. Many studies have reported low acute toxicity at field-relevant concentrations (Allgeier et al. 2018; Gutierrez-Villagomez et al. 2021; Junges et al. 2017; Schweizer et al. 2019), while one documented acute toxicity at high concentration (Lajmanovich et al. 2015). Besides acute toxicity, indirect effects have been reported, including alterations to gut microbiota (Gutierrez-Villagomez et al. 2021) and broader ecosystem-level impacts mediated through reductions in chironomid populations (Allgeier et al. 2019). However, given species-specific differences in sensitivity to larvicides among amphibians, further empirical data are needed for species that have not been studied (Empey et al. 2021).

For larvicidal oil, it is widely used in Hong Kong despite its ecological impacts remaining poorly understood. This product consists primarily of aliphatic petroleum hydrocarbons (Yeung et al. 2020). Studies on related petroleum-based substances have reported mixed effects on amphibians, with some demonstrating reduced growth and survival (Lefcort et al. 1997; Mahaney 1994), while others found no significant effect on survival, growth, and development (Patterson et al. 2022). Evidence from other taxa indicates possible ecological risks, including moderate to high toxicity in marine organisms in Hong Kong (Yeung et al. 2020), highlighting the need for targeted assessment in amphibians.

Beyond mortality, sublethal effects of contaminants are increasingly recognized as ecologically important, as they can influence individual physiology, behavior, and survival, and ultimately population viability (Baker et al. 2013; Sievers et al. 2019; Willson et al. 2012). Behavioral responses, such as movement activity, are sensitive indicators of contaminant exposure (Sievers et al. 2019). Tadpoles may exhibit either increased or decreased activity following exposure, both of which may carry ecological consequences by altering predator avoidance, foraging efficiency, and energy allocation, thereby influencing fitness (Brunelli et al. 2009; Cheron et al. 2023; Henao et al. 2024; Junges et al. 2017). Despite their importance, few studies have simultaneously assessed both lethal and sublethal endpoints (Empey et al. 2021).

The Romer's Tree Frog (*Liuixalus romeri*) is classified as endangered on the IUCN Red List (IUCN SSC Amphibian Specialist Group 2019). This species has a highly restricted distribution, naturally occurring on a few islands in and near Hong Kong, with an estimated area of occupancy of 140 km^2 (AmphibiaWeb 2022). It breeds in shallow stream pools, isolated temporary pools, man-made water containers, seepages, and ditches (Chan et al. 2005; Lau 1998). These habitats are commonly found near urban areas in Hong Kong, overlapping with mosquito breeding sites where larvicides are routinely applied.

In this study, we investigated the lethal and sublethal effects of the three most commonly used mosquito larvicides on *L. romeri*. Specifically, we (1) determined the 48- and 96-hour LC_{50} (lethal concentration that kills 50% of population) for each larvicide, and (2) examined the sublethal effects of prolonged exposure on growth rate and movement behavior in tadpoles. We used recommended application rates to reflect realistic exposure scenarios (Wolf and Segner 2023). Our findings aim to inform larvicide selection in mosquito control programs to minimize unintended impacts on this endangered species.

Methods

Study organisms

To assess the impacts of mosquito larvicides on *L. romeri* tadpoles, we obtained eggs either from captive breeding of wild-caught adults or by collecting eggs from the wild between August 2024 and July 2025. For captive breeding, adult frogs were temporarily maintained under laboratory conditions at Lingnan University and were released at the capture locations following egg collection, to minimize impacts on wild populations. All sampling was conducted in Tai Tam Country Park and Lantau Island, where the species is relatively abundant.

Eggs and tadpoles were reared in plastic containers (20 × 30 cm) under controlled conditions (22–25°C; 12 h light: 12 h dark photoperiod). Water depth was maintained at 3–5 cm using dechlorinated tap water (aged > 24 h). Leaves of longan (*Dimocarpus longan*) or Chinese mulberry (*Morus alba*) were provided as food and cover for tadpoles.

Larvicide

We evaluated three commonly used mosquito larvicides: *Bacillus thuringiensis israelensis* (Bti), larvicidal oil, and temephos. For Bti, a commercial granular formulation was used (200 ITU mg^{-1}; Hong Kong registration number: 2P12(GR); manufacturer: Joytime International Ltd.). According to manufacturer recommendations, this product is applied directly to water and reapplied every 7–14 days, with a recommended field application rate of 4.5–9 kg $acre^{-1}$. Larvicidal oil (Rasse anti-mosquito larvicide oil; Hong Kong registration number: 2P276; Pro-Link Asia Co., Ltd.) was applied to the water surface at a recommended rate of 2200 mL per 100 m^2. The specific composition of the oil is not provided by the manufacturer, but has been reported to consist primarily of aliphatic petroleum hydrocarbons (Yeung et al. 2020). Temephos was applied using a commercial granular formulation containing 1% (w/w) active ingredient (Hong Kong registration no. 2P179 (SG); Pro-Link Asia Co., Ltd.). According to manufacturer recommendations, it is applied directly to water and typically reapplied after 30 days, with a recommended rate of 2–5 g per 18 L of water. Chemical concentrations were not analytically verified; therefore, all concentrations are reported as nominal values.

Acute toxicity assays

Acute toxicity was assessed using 48-h and 96-h static exposure tests. Tadpoles (8–11 days post-hatching; Gosner stage 25) were exposed in glass tanks (15 × 15 × 15 cm) containing 1 L of stagnant dechlorinated water. Only tadpoles with > 8 days post-hatching were used to minimise mortality in ambient conditions (survival = 100% in preliminary trials). Preliminary range-finding tests were conducted to determine appropriate concentration ranges, which were selected ranging from the minimum recommended application rate up to concentrations producing complete mortality, with intermediate values chosen to characterise dose–response relationships. Final test concentrations were: Bti: 5,000 (minimum recommended rate), 50,000, 250,000, 500,000, 1,000,000, and 1,500,000 ITU L^{-1}; larvicidal oil: 0.22, 2.22 (recommended rate), 3.11, 5.56, 11.02, and 22.22 µL cm^{-2}; and temephos: 111 (minimum recommended rate), 1,110, 4,440, 7,770, and 11,100 mg L^{-1}. Each concentration was replicated in at least three tanks for each larvicide, with 6–8 tadpoles per tank, a density comparable to that observed in the field. A negative control (dechlorinated water only) was included in each experimental run, and tank positions were randomised. Tadpoles were not fed during the experiment (Junges et al. 2017). Mortality was recorded at 24 h intervals, and dead individuals were removed. Individuals were considered dead when no movement was observed following gentle stimulation. Surviving tadpoles were humanly euthanised at the end of the experiment using an overdose of MS-222 (Galex et al. 2020).

Prolonged exposure experiment

To assess sublethal effects, tadpoles were exposed to the minimum recommended rate of each larvicide (Bti: 5,000 ITU L^{-1}; larvicidal oil: 2.22 µL cm^{-2}; and temephos: 111 mg L^{-1}), resulting in four treatments (three larvicides and a control) until all tadpoles died or emerged, for up to 86 days. Each treatment was replicated in three randomised tanks, with eight tadpoles (10 days post-hatching; Gosner stage 25) per tank. Tadpoles were maintained in 1 L of dechlorinated water in laboratory conditions and fed mulberry leaves, supplemented with fish pellets (0.02 g per tank) once per week. Half of the water volume was replaced twice weekly for all tanks. Following each water change, 50% of the larvicide amount was reapplied to maintain the original concentration over time.

We assessed three aspects: prolonged exposure survival rate, growth rate, and movement behavior. For prolonged exposure survival rate, the number of live and dead tadpoles in each tank was recorded every one to two days. Growth rate was quantified as change in total body length. All tadpoles came from the same egg clutch and were randomly assigned to treatment tanks. Body size was not measured at the start of the experiment. Measurements were taken after 96 h of exposure and subsequently at weekly intervals. Three tadpoles were randomly selected from each tank, photographed individually in a Petri dish (9 cm diameter), and measured using *ImageJ* software (Rasband 2012).

Movement behavior was assessed concurrently with the growth measurements. Three tadpoles per tank were randomly selected, then placed individually in a Petri dish filled with dechlorinated water and allowed to acclimatize for 5 min before recording. Behavior was recorded for 5 min using a digital camera (Model: TG-7, Brand: Olympus). Videos were analyzed using ToxTrac software to quantify movement parameters (Rodriguez et al. 2018). Tracking quality was assessed, and only videos with a

visibility rate > 80% were included in the analysis. Movement parameters directly obtained from ToxTrac output were selected to reflect activity, space use, and inactivity, including average speed (mm s^{-1}), average acceleration (mm s^{-2}), mobile average speed (mm/s; mean speed during active movement), mobility rate (%; proportion of time in motion), exploration rate (%; proportion of arena area visited), and number of freezing events (discrete episodes of immobility) (Rodriguez et al. 2018).

Due to high tadpole mortality in the larvicidal oil treatment during later time stages of the prolonged exposure experiment, we separated the analysis into two phases: an early phase and a full phase. For the early phase, we included measurements from Days 4 and 11, with all treatments retained. For the full phase, the larvicidal oil treatment was excluded due to high mortality after Day 11; only the Bti and temephos treatments were analyzed across all time points.

Statistical analysis

We conducted all statistical analyses in R (R Core Team 2024). For acute toxicity, 48-h and 96-h LC_{50} values (concentration at which 50% of mortality occurred after 48 h or 96 h of exposure) were estimated by fitting dose-response models using two-parameter log-logistic functions (LL.2) using the *drc* package (Ritz and Streibig 2005). For prolonged exposure, survival was visualised using a Kaplan–Meier estimator (Kaplan and Meier 1985), and the treatment effects on survival were tested using Cox proportional hazards models (Cox 1972), using the s*urvival* package (Therneau and Lumley 2015).

Growth and continuous movement parameters (average speed, average acceleration, mobile average speed, and number of freezing events) were log-transformed and analysed using linear mixed-effects models fitted with the *lme4* and *lmerTest* packages (Bates et al. 2014; Kuznetsova et al. 2017). Proportion parameters (exploration rate and mobility rate) were analysed using beta regression with a logit link in the *glmmTMB* package (Brooks et al. 2017). For all models, fixed effects were treatment, time (days since treatment), and their interaction. For growth and movement analyses, each tadpole measurement was treated as a separate observation. Tadpoles were not individually marked, therefore, repeated measurements represented randomly sampled individuals within tanks across time. Tank identity was included as a random effect to account for non-independence among tadpoles from the same tank. Model assumptions were assessed using residual diagnostics implemented in the *DHARMa* package (Hartig 2017). Post hoc comparisons were conducted using estimated marginal means using the e*mmeans* package (Lenth 2023).

Ethics declaration

As *L. romeri* is a protected species in Hong Kong, all procedures were conducted under a research permit issued by the Agriculture, Fisheries and Conservation Department [permit no.: (110) in AF GR CON 09/51 Pt.8]. The use of live animals in experiments was approved by the Department of Health, Hong Kong SAR Government [permit no.: (23–50) in DH/HT&A/8/2/8 Pt.5] under Animals (Control of Experiments) Ordinance, CAP. 340. All experimental protocols were conducted in accordance with the conditions of

these permits and Guidelines for use of live amphibians and reptile in field and laboratory research (Herpetological Animal Care and Use Committee 2004).

Results

Acute toxicity

LC_{50} values of all larvicides were higher than their recommended rates (Table 1). Larvicidal oil, however, showed the smallest margin between LC_{50} and the recommended rate, whereas Bti exhibited the largest margin (Fig. 1; Table 1). Larvicidal oil had a 96-h LC_{50} of 10.1 µL cm^{-2}, 4.6-fold higher than the recommended rate (2.2 µL cm^{-2}). Bti had a 96-h LC_{50} of 525,866.9 ITU L^{-1}, 53 to 105-fold higher than its recommended rate (5,000–10,000 ITU L^{-1}). Temephos had a 96-h LC_{50} of 4,615.9 mg L^{-1}, 17 to 42-fold higher than its recommended rate (111–278 mg L^{-1}).

Table 1
Estimated LC_{50} values of *Liuixalus romeri* tadpoles exposed to three mosquito larvicides (*Bacillus thuringiensis israelensis* [Bti], larvicidal oil, and temephos) over different exposure durations. Values are presented with 95% confidence intervals. *Confidence intervals could not be estimated by the model.

Larvicide	**Exposure duration (hours)**	**Estimated LC_{50} (95% confidence intervals)**
Bti	48	758179 (616790–899568) ITU L^{-1}
	96	525866 (523061–528671) ITU L^{-1}
Larvicidal oil	48	11 (4.8–17.2) µL cm^{-2}
	96	10.1 (3.6–16.6) µL cm^{-2}
Temephos	48	7424 (5266–9582) mg L^{-1}
	96	4616* mg L^{-1}

Prolonged exposure - survival

At the recommended rate, larvicidal oil significantly increased mortality compared to the control, with an approximately eight-fold increase in hazard (hazard ratio = 8.44, $P < 0.001$; Fig. 2; Table S1). In contrast, survival under Bti (hazard ratio = 0.53, $P = 0.252$) and temephos (hazard ratio = 0.56, $P = 0.276$) did not differ significantly from the control.

Prolonged exposure – growth

Tadpole body length increased significantly over time ($P < 0.001$; Fig. 3; Table S2). Growth trajectories differed significantly among treatments, as indicated by a significant treatment × time interaction ($P = 0.002$). Pairwise comparisons of daily proportional growth rates indicated that tadpoles exposed to

larvicidal oil had significantly lower growth rates than those in the control, Bti, and temephos treatments (log-scale differences = 0.010–0.014, all P ≤ 0.048), corresponding to 1.0–1.4% lower daily proportional growth (Table S3). No significant differences were detected among the control, Bti, and temephos treatments (P ≥ 0.529).

Prolonged exposure – movement

Movement parameters were affected by the larvicide treatment during the early exposure period (Days 4–11; Table S4). Significant treatment × time interactions were detected for average speed (P = 0.005), mobile average speed (P = 0.005), average acceleration (P = 0.008), and exploration rate (P = 0.001), indicating that treatment effects varied over time. No significant interactions were detected for mobility rate or freezing events (P ≥ 0.058).

Post hoc comparisons indicated that treatment differences were absent on Day 4 but emerged on Day 11 (Table S5). On Day 11, the larvicidal oil treatment reduced movement activity compared to the Bti treatment, with average speed, average acceleration, and mobile average speed being 89–90% lower in the larvicidal oil treatment (log-scale estimate = -2.19 to -2.31, all P < 0.001; Fig. 4; Table S5). Similarly, the temephos treatment showed higher movement activity than the larvicidal oil treatment, with average speed, average acceleration, and mobile average speed being 331–410% higher (log-scale estimate = 1.46 to 1.63, all P ≤ 0.013). Compared with the control, the larvicidal oil treatment reduced mobile average speed by 76% (log-scale estimate = -1.43; P = 0.048). For proportional parameters, exploration rate was lower in the larvicidal oil treatment than in the Bti treatment (odds ratio = 0.124, P < 0.001). The exploration rate wase also higher in the temephos treatment than in the larvicidal oil treatments (odds ratio = 4.59, P = 0.001). The Bti and temephos treatments increased the mobility rate compared with the control (Bti: odds ratio = 2.34, P = 0.027; temephos: odds ratio = 2.58, P = 0.008).

Over the full period, all movement parameters were significantly affected by time (all P ≤ 0.001), except mobility rate (P = 0.088; Table S5). Significant treatment × time interactions were detected for average speed (P = 0.035), mobile average speed (P = 0.023), average acceleration (P = 0.020), and exploration rate (P < 0.001), indicating that treatment effects depended on exposure duration. In contrast, interactions were not significant for mobility rate or freezing events (P ≥ 0.060).

Post hoc comparisons revealed that on Day 18, the temephos treatment resulted in higher movement than Bti treatment, with average acceleration and mobile average speed 263–282% higher in temephos treatment (log-scale estimate = 1.29–1.34, P ≤ 0.043; Table S5). The exploration rate was higher in the temephos treatment than in the control (odds ratio = 3.20, P = 0.014) and the Bti treatment (odds ratio = 3.16, P = 0.011). In contrast, the Bti treatment showed lower odds of exploration than the control (odds ratio = 0.36, P = 0.028). On Day 25, the Bti treatment reduced mobile average speed by 76% compared with the control (log-scale estimate = -1.44; P = 0.034).

Discussion

In this study, we evaluated the lethal and sublethal effects of three commonly used mosquito larvicides on the endangered *L. romeri*. Larvicidal oil exhibited the strongest negative effects, with the smallest margin between the LC_{50} and the recommended application rate, and high mortality under prolonged exposure at the recommended application rate. In contrast, Bti and temephos showed larger safety margins based on LC_{50}. However, both Bti and temephos still induced sublethal effects, significantly altering tadpole movement under prolonged exposure. We discuss the implications of these findings for environmental management.

Lethal effects

Larvicidal oil showed the lowest safety margin relative to its recommended dosage. More importantly, under prolonged exposure at the recommended application rate, mortality increased substantially, with 23 out of 24 tadpoles dead within 21 days and the last one dead by Day 48. Given that the tadpole stage of *L. romeri* exceeds four weeks (Karsen et al. 1998), our results indicate that at recommended application rates, larvicidal oil can result in near-complete mortality in *L. romeri* tadpoles. As such, larvicidal oil should be avoided in habitats supporting *L. romeri*. Although our study was based on only one species, the high mortality observed suggests that similar risks may extend to other amphibian species, warranting cautious application in amphibian breeding habitats until further targeted studies are conducted.

For temephos, previous studies have demonstrated acute toxicity in some amphibian species at recommended application rates (Harischandra et al. 2012). However, *L. romeri* appeared tolerant, with LC_{50} values 17–42 times higher than the recommended rates. Similarly, Bti showed a wide safety margin (53–105-fold), consistent with previous studies indicating that amphibians are generally tolerant to Bti (Empey et al. 2025; Junges et al. 2017; Thierry et al. 2023). Notably, *L. romeri* is less sensitive to Bti than most previously tested amphibian species, with a 96-h LC_{50} of 525,867 ITU L^{-1}, which is comparable to that for *Lithobates sylvaticus* (525,363.4 ITU L^{-1}), and represents the highest LC_{50} documented in amphibians to date (Empey et al. 2025). Therefore, the safety margins observed in this study should be assumed to apply to other amphibian species, which may be more sensitive to these larvicides.

Sublethal effects

At the minimum recommended application rate, larvicidal oil caused significant effects on both behavior and growth rate. Tadpoles exposed to larvicidal oil had a reduction in average swimming speed, exploration behavior, and growth rate. Mortality was high, with most tadpoles not surviving beyond 21 days. Even among survivors, reduced growth and impaired movement may delay development and increase vulnerability to predation, with potential consequences for long-term survival (Lavorato et al. 2013; Relyea and Hoverman 2006).

Bti and temephos did not affect growth but showed variable impacts on movement. On Day 11, both treatments showed increased activity. At later time points, responses diverged, with temephos increasing exploration on Day 18. Similar stimulatory effects on movement have been reported in other

studies, where exposure to insecticides increased swimming activity and distance travelled (Junges et al. 2017). On Day 25, Bti reduced movement compared to the control. These behavioral changes may affect individual fitness and population persistence through decreased predator avoidance, decreased foraging efficiency, and increased energy expenditure (Weis et al. 2001). More research is needed to determine the consequences of these altered behaviors.

Movement behavior can vary with tadpole body size (Cheron et al. 2021). Because larvicide treatment affected growth, differences in movement behavior could potentially be influenced by differences in body size among treatments. As such, we conducted additional analyses with body length included as an additional fixed factor, which produced results similar to those of the original models (Table S6). This suggests that larvicide treatments affected tadpole behavior even after accounting for body size differences.

Mechanisms underlying larvicidal oil toxicity

The high mortality observed in larvicidal oil treatment is likely related to both its physical and chemical modes of action (Goncharuk et al. 2022; Oparaji et al. 2017). The formation of a surface film may impede gas exchange at the air–water interface, leading to hypoxic conditions that can inhibit tadpole growth (Schofield and White 2025). In addition, larvicidal oil consists mainly of aliphatic petroleum hydrocarbons (Yeung et al. 2020), which may also cause toxic effects on tadpoles (Mahaney 1994). The observed reduction in movement and delayed mortality further suggest that toxicity may not be solely due to suffocation, but may also involve physiological stress or chemical toxicity (Hagstrum and Mulla 1968). However, as dissolved oxygen was not measured in this study, we cannot distinguish between the relative contributions of suffocation and toxicity. Nonetheless, *Liuixalus romeri* inhabits shallow water bodies (Lau 1998), which may be particularly vulnerable to water evaporation. Evaporation could increase larvicide concentrations and exacerbate both hypoxic and chemical toxic effects.

Importance of exposure duration

Our results demonstrate that exposure duration is a critical determinant of ecological risk. Although all larvicides appeared relatively safe based on acute LC_{50} values (≤ 4 days), prolonged exposure led to markedly different outcomes: larvicidal oil caused complete mortality, while Bti and temephos induced sublethal behavioral changes. These findings highlight a key limitation of standard acute toxicity assessments and underscore the need to incorporate longer exposure durations and sublethal endpoints into ecological risk evaluations.

Limitations and future work

This study has several limitations. First, we used tadpoles that were at least eight days old, whereas earlier life stages may be more sensitive to larvicide exposure (Harischandra et al. 2012). As such, the sensitivity of *L. romeri* may be underestimated, particularly for younger tadpoles and eggs. Future studies should therefore include eggs and younger tadpoles. Second, environmental concentrations of these larvicides in natural habitats remain unknown, so future studies should quantify field

concentrations (Smetanova et al. 2014; Wolf and Segner 2023). Third, although *L. romeri* may be among the more vulnerable species due to its breeding habitat overlapping with mosquito-prone environments, it is important to extend testing to a wider range of amphibian species, especially Asian amphibians, which remain understudied with respect to the impacts of mosquito larvicides.

Conclusion

In this study, we demonstrate the importance of considering both lethal and sublethal effects, as well as exposure duration, when evaluating the ecological risk of pesticides. Among the three commonly used larvicides, larvicidal oil poses the greatest risk to amphibians and should be avoided in habitats that support *L. romeri* and other species of conservation concern. In contrast, Bti and temephos represent comparatively lower-risk alternatives in terms of acute toxicity and growth; however, their sublethal behavioral effects warrant further investigation. Overall, these findings provide a scientific basis for minimizing the risks of mosquito larvicides to non-target organisms while balancing effective mosquito control with biodiversity conservation.

Declarations

Statements and Declarations

The authors have no relevant financial or non-financial interests to disclose. All collection and laboratory procedures were conducted under a research permit issued by the Agriculture, Fisheries and Conservation Department [permit no.: (110) in AF GR CON 09/51 Pt.8] and an animal ethics permit by the Department of Health, Hong Kong SAR Government (permit no.: (23-50) in DH/HT&A/8/2/8 Pt.5).

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Author Contributions

Conceptualization: YHS, FKWL, MWL, SYWS, JJF

Funding Acquisition: YHS, MWL, SYWS, JJF

Methodology: YHS, HNK, AHBL, HCY, FKWL, AWLF, TYH

Investigation: YHS, HNK, AHBL, HCY, FKWL, AWLF, TYH

Data Curation: YHS

Formal Analysis: YHS, TYH

Writing – Original Draft: YHS

Writing – Review & Editing: all authors

Visualization: YHS

Data Availability

Raw data were deposited and are available at: https://doi.org/10.6084/m9.figshare.32681358

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Figures

Figure

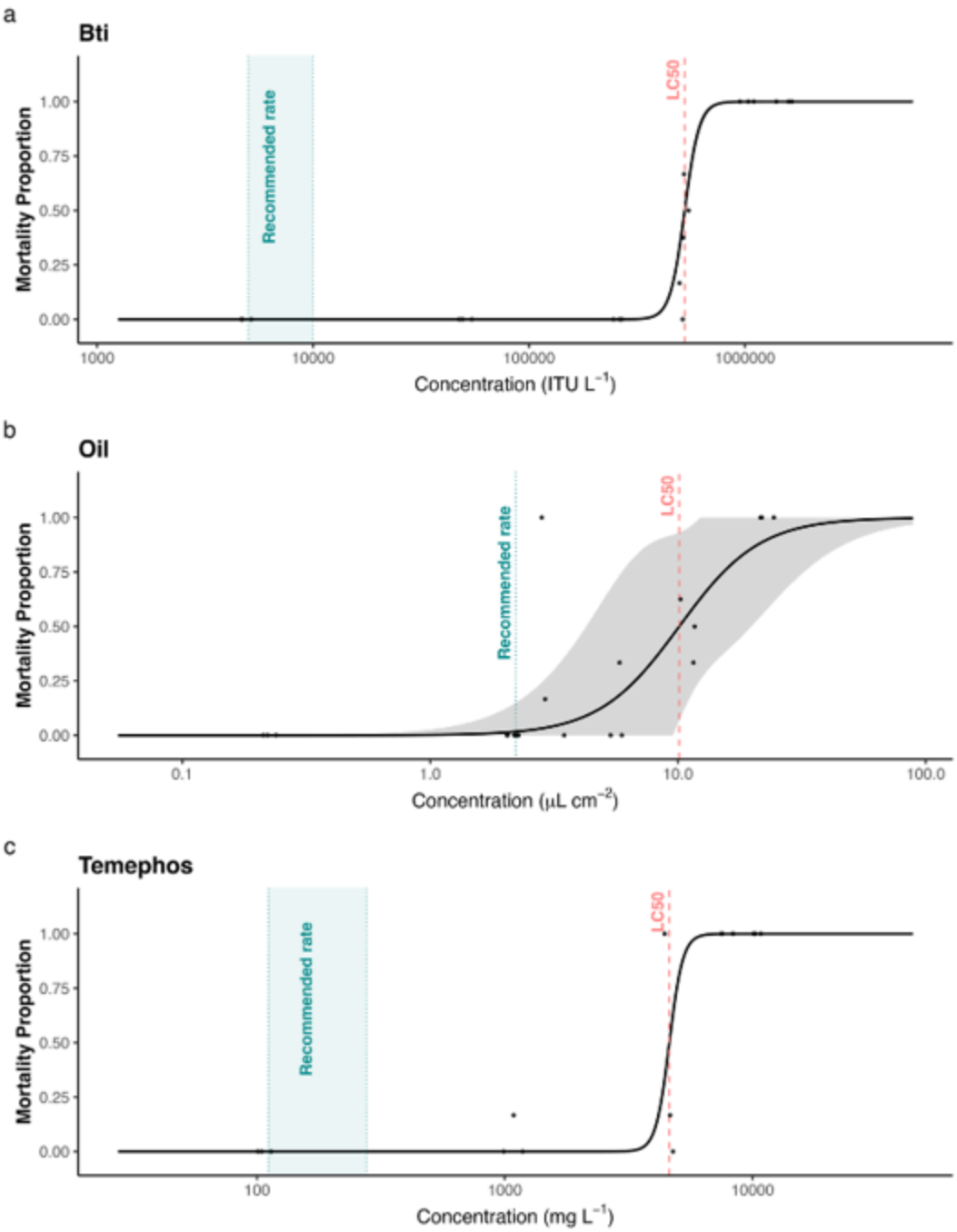


Figure 1

Proportion of mortality of *Liuixalus romeri* tadpoles exposed to three mosquito larvicides, including *Bacillus thuringiensis israelensis* (Bti) (a), larvicidal oil (b), and temephos (c), over 96 hours. The red dashed line indicates the estimated LC_{50}, shaded areas indicated 95% confidence intervals, and the green lines and areas show the recommended rates. Note: confidence intervals for Bti were too narrow to be visible, and those for temephos could not be estimated.

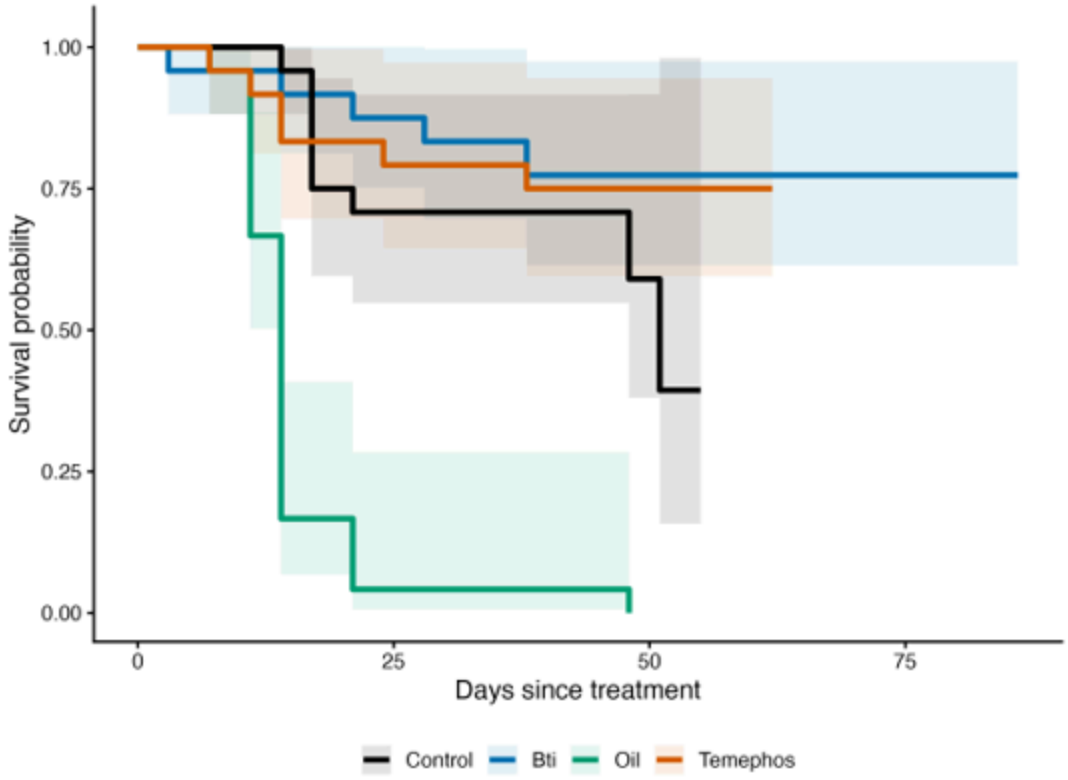


Figure 2

Survival probability of *Liuixalus romeri* tadpoles exposed to three mosquito larvicides (*Bacillus thuringiensis israelensis* [Bti], larvicidal oil, and temephos) and a control (dechlorinated water without larvicides) over time, as estimated using the Kaplan Meier method. Shaded areas represent 95% confidence intervals.

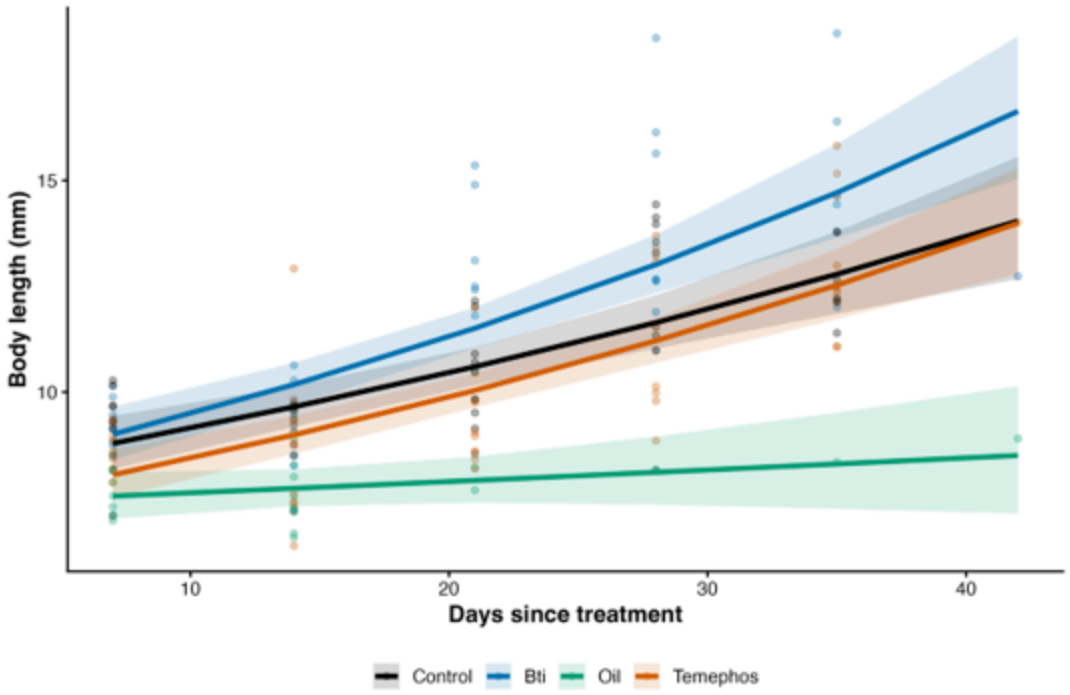


Figure 3

Total body length (mm) of *Liuixalus romeri* tadpoles exposed to three mosquito larvicides (*Bacillus thuringiensis israelensis* [Bti], larvicidal oil, and temephos) and a control (dechlorinated water without larvicides) over time. Lines represent model-predicted mean growth trajectories, and shaded areas indicate 95% confidence intervals estimated from the linear mixed-effects model.

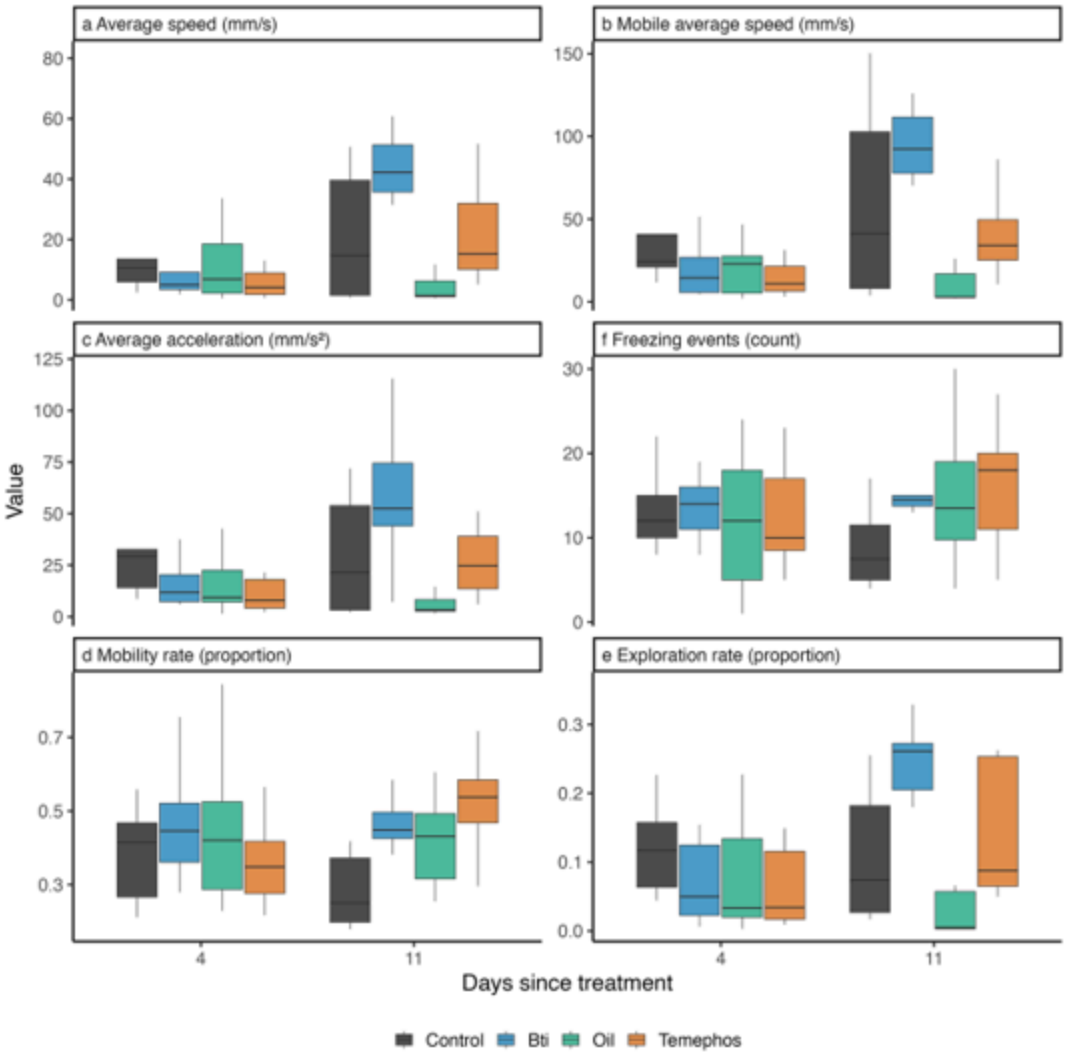


Figure 4

Impacts of mosquito larvicides at minimum recommended rates on movement parameters (average acceleration, average speed, exploration rate, freezing events, mobile average speed, and mobility rate) of *Liuixalus romeri* tadpoles at 4 and 11 days since exposure. Treatments include *Bacillus thuringiensis israelensis*(Bti), larvicidal oil, temephos, and control. Movement was analyzed using the ToxTrac software.

Supplementary Files

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