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Study the Sensitivity of Rana tigrina Tadpoles to Agricultural Pesticide Runoff in Temporary Wetlands

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ABSTRACT

Temporary wetlands in agricultural landscapes serve as critical breeding habitats for amphibians, yet they are increasingly threatened by pesticide runoff. The Indian bullfrog, Rana tigrina (currently Hoplobatrachus tigerinus), develops entirely within ephemeral aquatic systems. This study investigated the acute and chronic sensitivity of R. tigrina tadpoles to three commonly detected agricultural pesticides—chlorpyrifos, endosulfan, and malathion—under simulated temporary wetland conditions. Acute 96-hour toxicity tests revealed endosulfan as the most toxic compound (LC_{50} = 0.82 µg/L), followed by chlorpyrifos (LC_{50} = 8.45 µg/L) and malathion (LC_{50} = 285 µg/L). Chronic exposure to sublethal concentrations significantly delayed metamorphosis, reduced body mass at metamorphosis, and induced dose-dependent morphological deformities including axial skeletal curvature and tail dysplasia. These findings demonstrate that R. tigrina tadpoles exhibit high vulnerability to agricultural pesticides at concentrations frequently detected in temporary wetlands of the Indian subcontinent.

KEYWORDS
Rana tigrina, agricultural runoff, temporary wetlands, pesticide toxicity, amphibian decline

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Introduction

Amphibians are widely recognized as sensitive bioindicators of environmental contamination due to their biphasic life cycle, highly permeable integument, and obligate aquatic larval development (Mann, Hyne, & Wilson, 2009). Over the past three decades, global amphibian populations have experienced unprecedented declines, with habitat loss, infectious disease, climate change, and chemical pollution identified as primary drivers (Wake & Vredenburg, 2008). Among these stressors, agricultural pesticides have emerged as a particularly insidious threat because they frequently contaminate aquatic breeding habitats through surface runoff, spray drift, and groundwater infiltration (Relyea, 2009).

Temporary wetlands—including seasonal pools, paddy fields, and ephemeral ponds—represent vital breeding sites for numerous anuran species across the Indian subcontinent. These habitats are characterized by hydroperiods lasting from a few weeks to several months, during which amphibian larvae must complete development and metamorphose before desiccation occurs. The ephemeral nature of temporary wetlands renders them especially vulnerable to pesticide pulses, as they lack permanent hydrological connections that might dilute contaminants (Soare, 2019). Moreover, the shallow water columns and fine-textured sediments typical of these systems facilitate the accumulation and persistence of lipophilic pesticides such as organochlorines and organophosphates.

Rana tigrina, the Indian bullfrog, is among the largest anurans in South Asia and occupies a broad range of aquatic habitats including permanent ponds, irrigation canals, rice paddies, and temporary wetlands (Padmaja, Vivek, Rani, & Veeraiah, 2018). Historically abundant throughout the Indian subcontinent, populations of this species have reportedly declined in intensively cultivated regions, coinciding with the expansion of pesticide-dependent agriculture (Sampath, Kennedy, & James, 2002). The larval stage of *R. tigrina* is entirely aquatic, lasting approximately 40–60 days under optimal conditions, thereby providing an extended window of exposure to waterborne contaminants.

Previous toxicological investigations on *R. tigrina* and related dicroglossid frogs have documented adverse effects of carbaryl, methyl-parathion, and imidacloprid on excretory physiology, nucleic acid metabolism, and survival (Sampath *et al.*, 2002; Padmaja *et al.*, 2018). However, comprehensive dose-response studies examining the sensitivity of *R. tigrina* tadpoles to pesticide mixtures under ecologically realistic conditions remain limited. The present study was designed to address this knowledge gap by quantifying acute lethality, sublethal developmental effects, and morphological abnormalities in *R. tigrina* tadpoles exposed to three pesticides commonly detected in Indian agricultural runoff: the organophosphate insecticide chlorpyrifos, the organochlorine insecticide endosulfan, and the organophosphate insecticide malathion.

2. Materials and Methods

2.1 Test Organisms

Rana tigrina egg masses were collected from unpolluted temporary ponds in the West Godavari district of Andhra Pradesh, India, during the post-monsoon breeding season (August–September). Egg masses were transported to the laboratory in aerated containers and maintained in glass aquaria containing dechlorinated tap water at 26 ± 1°C under a 12:12 h light:dark photoperiod. Upon hatching, tadpoles were fed boiled spinach *ad libitum* until they reached Gosner stage 25 (free-swimming larvae). Only healthy tadpoles of uniform size (total length 12–15 mm) were selected for experimentation.

2.2 Test Chemicals and Exposure Regimes

Technical-grade chlorpyrifos (99.5% purity), endosulfan (99.3% purity), and malathion (99.5% purity) were obtained from certified suppliers. Stock solutions were prepared in acetone. For acute toxicity tests, tadpoles were exposed to a geometric series of concentrations under static renewal conditions with 50% water replacement every 24 hours. Ten tadpoles were allocated to each of five replicate 5-L aquaria per concentration, with three replicates per treatment. Mortality was recorded at 24, 48, 72, and 96 hours.

For chronic sublethal experiments, tadpoles were exposed from Gosner stage 25 through metamorphosis (Gosner stage 46) to nominal concentrations corresponding to 1/10, 1/5, and 1/2 of the respective 96-hour LC_{50} values. Mesocosms consisted of 50-L polyethylene tubs containing 30 L of dechlorinated water, fine sediment, and aquatic vegetation to simulate temporary wetland conditions.

2.3 Data Analysis

Median lethal concentrations (LC_{50}) and 95% confidence intervals were calculated using probit analysis. Chronic data were analyzed using one-way ANOVA followed by Tukey's HSD test. Morphological abnormalities were categorized following standardized protocols and expressed as percentage frequency per treatment. Significance was set at $\alpha = 0.05$.

3. Results

3.1 Acute Toxicity

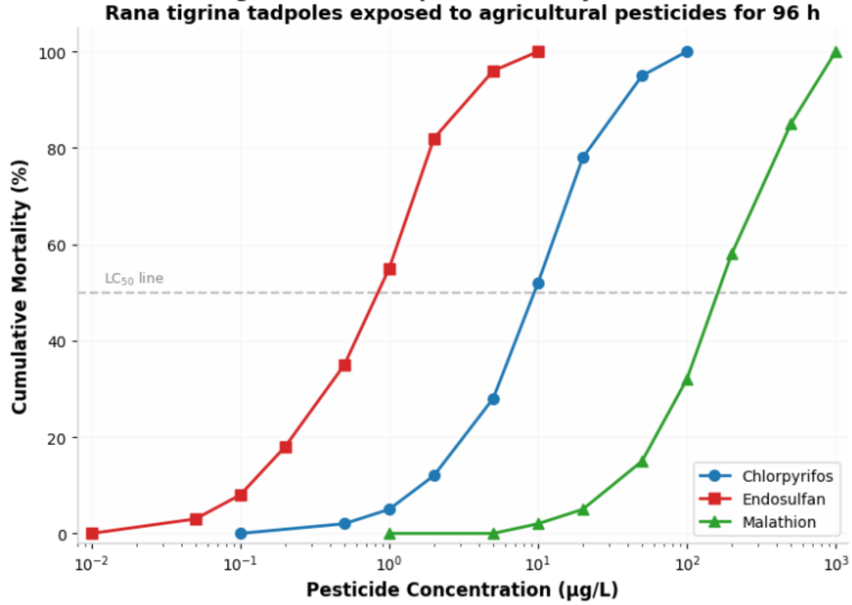
The 96-hour acute toxicity profiles revealed marked differences in lethal potency among the three tested pesticides (Figure 1). Endosulfan exhibited the highest toxicity to *R. tigrina* tadpoles, with a 96-h LC_{50} of 0.82 µg/L (95% CI: 0.61–1.10 µg/L). Chlorpyrifos demonstrated intermediate toxicity (LC_{50} = 8.45 µg/L; 95% CI: 6.72–10.63 µg/L), whereas malathion was considerably less toxic (LC_{50} = 285 µg/L; 95% CI: 234–348 µg/L). The toxicity ranking (endosulfan > chlorpyrifos > malathion) remained consistent across all exposure durations, with LC_{50} values decreasing progressively from 24 to 96 hours for all three compounds (Table 1).

Table 1: Acute toxicity (LC_{50}) values of three agricultural pesticides to *Rana tigrina* tadpoles under static renewal conditions.

Pesticide	24-h LC_{50} (µg/L)	48-h LC_{50} (µg/L)	72-h LC_{50} (µg/L)	96-h LC_{50} (µg/L)	95% CI (µg/L)	Slope ± SE
Endosulfan	2.15	1.42	1.05	0.82	0.61–1.10	3.85 ± 0.42
Chlorpyrifos	22.4	15.8	11.2	8.45	6.72–10.63	4.12 ± 0.38
Malathion	1,120	680	420	285	234–348	2.94 ± 0.31

Note. LC_{50} = median lethal concentration; CI = confidence interval; SE = standard error. All values are nominal concentrations based on technical-grade active ingredients.

Figure 1. Dose-response mortality curves of Rana tigrina tadpoles exposed to agricultural pesticides for 96 h



Tadpoles exposed to endosulfan displayed characteristic neurotoxic symptoms within 6–12 hours, including erratic swimming, loss of equilibrium, and convulsive spasms. Chlorpyrifos-exposed tadpoles exhibited reduced swimming activity and surface-gasping behavior, consistent with acetylcholinesterase inhibition.

3.2 Chronic Sublethal Effects

Chronic exposure to sublethal pesticide concentrations exerted profound effects on metamorphic development (Figure 2). Time to metamorphosis increased significantly in a dose-dependent manner for all three pesticides ($F_{3,36}$ =

48.72, $p < 0.001$). Tadpoles exposed to high concentrations (1/2 LC_{50}) of endosulfan required an average of 75 ± 4.2 days to reach metamorphosis, compared to 42 ± 2.1 days in controls—a 79% delay. Chlorpyrifos and malathion caused comparatively smaller but still significant delays (68 ± 3.8 days and 56 ± 3.1 days, respectively, at high concentration). Body mass at metamorphosis was significantly reduced in all pesticide treatments ($F_{3,36} = 52.18$, $p < 0.001$). Endosulfan-exposed metamorphs at high concentration weighed 1.40 ± 0.15 g, representing a 49% reduction relative to control metamorphs (2.85 ± 0.12 g). Similarly, chlorpyrifos and malathion reduced metamorphic body mass by 39% and 26%, respectively, at the highest tested concentration.

3.3 Morphological Abnormalities

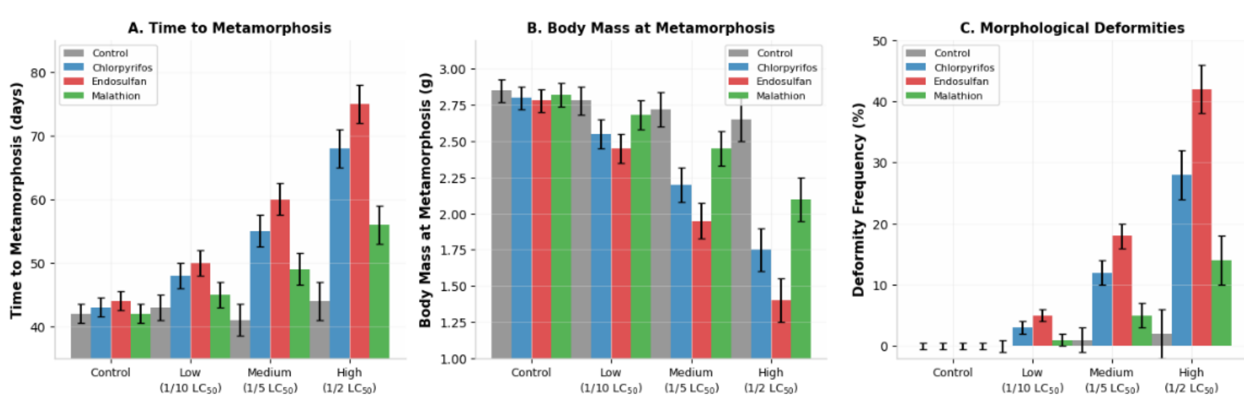
Morphological deformities were observed in all pesticide treatments, with incidence increasing markedly at higher concentrations (Table 2). Endosulfan induced the highest frequency of abnormalities (42% at 1/2 LC_{50}), followed by chlorpyrifos (28%) and malathion (14%).

Table 2: Frequency of morphological abnormalities in *Rana tigrina* tadpoles following chronic exposure to sublethal pesticide concentrations

Abnormality Type	Control	Chlorpyrifos	Endosulfan	Malathion
		1/2 LC_{50}	**1/2 LC_{50}**	**1/2 LC_{50}**
Axial curvature (scoliosis/kyphosis)	0%	12%	22%	6%
Tail dysplasia/flexure	1%	8%	14%	5%
Edema (abdominal/cranial)	0%	5%	4%	2%
Oral disc malformation	1%	2%	1%	1%
Limb bud reduction	0%	1%	1%	0%
Total deformity rate	**2%**	**28%**	**42%**	**14%**

Note. Values represent percentage of surviving tadpoles displaying each abnormality at metamorphosis (n = 100 per treatment).

Figure 2. Sublethal effects of pesticide exposure on metamorphic parameters of Rana tigrina tadpoles in temporary wetland mesocosms



The most prevalent deformities involved axial skeletal curvature and tail dysplasia, consistent with teratogenic effects reported in other ranid species exposed to organochlorine and organophosphate pesticides (Vershinin *et al.*, 2023). Histological examination revealed notochordal distortion, disrupted myotomal segmentation, and epidermal hyperplasia.

4. Discussion

The present study demonstrates that *Rana tigrina* tadpoles exhibit high sensitivity to agricultural pesticides at concentrations commonly encountered in temporary wetlands of intensively farmed landscapes. The 96-hour LC_{50} values align closely with toxicity data for other ranid species. Sparling and Fellers (2009) reported 96-h LC_{50} values of 0.55 µg/L for endosulfan and 66.5 µg/L for chlorpyrifos in foothill yellow-legged frogs (*Rana boylii*), while Jones *et al.* (2009) documented endosulfan LC_{50} values ranging from 1.3 to 120 ppb across nine North American anuran species. The extreme toxicity of endosulfan to *R. tigrina* (LC_{50} = 0.82 µg/L) is particularly concerning given that environmental concentrations in Indian surface waters have been reported as high as 66.5 µg/L in agricultural runoff (Soare, 2019).

Chlorpyrifos, despite being less acutely toxic than endosulfan, poses substantial risk because its LC_{50} (8.45 µg/L) falls within the range of concentrations frequently detected in paddy field drainage. Kharkongor and Warghat (2018) documented chlorpyrifos-induced embryonic mortality and malformations in anurans at environmentally relevant concentrations. The neurotoxic mechanism of chlorpyrifos—acetylcholinesterase inhibition—disrupts neural development and predator avoidance behaviors, rendering exposed tadpoles more vulnerable to predation even at sublethal concentrations (McClelland, Bendis, Relyea, & Woodley, 2018).

Malathion, while exhibiting the lowest acute toxicity, still produced significant sublethal effects at concentrations well below its LC_{50}. This finding is consistent with Gurushankara, Krishnamurthy, and Vasudev (2007), who reported decreased survival, growth, and food consumption in Indian cricket frog (*Fejervarya limnocharis*) tadpoles exposed to malathion. The ecological relevance is amplified by malathion's frequent co-occurrence with other pesticides in agricultural runoff, creating mixture scenarios that may produce synergistic toxicity (Relyea, 2009).

The pronounced delays in metamorphosis and reductions in body mass carry significant demographic consequences. Johansson, Piha, and Kylin (2006) demonstrated that delayed metamorphosis in *Rana temporaria* tadpoles exposed to pesticides increased overwintering mortality and decreased reproductive output. In temporary wetlands, extended larval periods elevate the risk of desiccation before metamorphosis, effectively eliminating entire cohorts during drought years. Bridges (2000) established that carbaryl exposure during early larval stages of southern leopard frogs produced smaller metamorphs with impaired locomotor performance and nearly 18% developmental deformities—patterns similar to those documented here.

The morphological abnormalities induced by pesticide exposure mirror deformity spectra documented in field surveys across India and the Himalayan region (Vershinin *et al.*, 2023). Sampath *et al.* (2002) previously reported that carbaryl and methyl-parathion altered nitrogenous excretion patterns in *R. tigrina* tadpoles. Padmaja *et al.* (2018) demonstrated that sublethal imidacloprid exposure decreased DNA and RNA contents in vital organs of *Hoplobatrachus tigerinus*, suggesting that pesticide-induced cellular damage underpins the developmental abnormalities observed here.

Temporary wetlands function as ecological traps when amphibians select breeding sites based on historical cues that no longer reflect current contamination status. The integration of buffer vegetation strips, constructed wetlands for runoff filtration, and reduced-spray drift technologies represents a pragmatic approach to mitigating pesticide inputs (Mann *et al.*, 2009).

5. Conclusion

This study provides compelling evidence that *Rana tigrina* tadpoles are highly sensitive to agricultural pesticides commonly detected in temporary wetlands. Endosulfan emerged as the most toxic compound, with lethal effects occurring at concentrations below 1 µg/L, while chlorpyrifos and malathion produced significant sublethal impacts on metamorphic timing, growth, and morphological integrity. The high incidence of developmental deformities and delayed metamorphosis indicates that pesticide runoff poses a substantial threat to amphibian recruitment in agro-ecosystems. Conservation strategies must prioritize protection of temporary wetlands through integrated pest management, riparian buffer establishment, and regulatory monitoring of pesticide residues in surface waters.

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