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Evaluation of three aquatic consumers as organophosphate pesticide bioindicators in Costa Rican lowland wet forest streams

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Abstract

Pesticide use can impact not only cultivated land, but also protected ecosystems that receive pesticide inputs due to aquatic connectivity or atmospheric transport from agricultural regions. In Costa Rica's Caribbean lowlands, pesticides applied to banana and pineapple plantations are a potential source of pollution to ecological reserves. Macroinvertebrates and fish are both potentially useful bioindicators of agrochemical pollution in aquatic systems, and our goal was to determine whether three common stream consumer species (one fish and two aquatic insect species) could serve as bioindicators for the organophosphate pesticide ethoprophos. We identified thresholds at which ethoprophos impacts the survival (LC_{50}) and observed behavior (LOEC – lowest observed effect concentration) for each species. The LC_{50} of the guppy *Priapichthys annectens* was 1530 μg/L, with observable behavioral changes occurring at 1000 μg/L. Insects were more sensitive: the mayfly *Traverella holzenthali* had an LC_{50} of 15 μg/L and an LOEC of 2.5 μg/L, and the caddisfly *Leptonema* sp. had an LC_{50} of approximately 30 μg/L and an LOEC of 5 μg/L. The LC_{50} values are notably higher than ambient concentrations recorded from polluted Costa Rican streams and suggest that these taxa are not ideal indicator species. However, the lower LOEC values (in the same order of magnitude as ambient concentrations) highlight the potential ecological importance of behavioral modification due to pesticides. Quantifying the thresholds at which common pesticides impact ecosystems is a key step in identifying bioindicator species and protecting tropical biodiversity.

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Introduction

Tropical agriculture involves the use of large quantities of biocides that contaminate not only cultivated land, but also protected areas and biological reserves via aquatic transport (Castillo et al. 2000; Echeverría-Sáenz et al. 2012) or atmospheric transport, sometimes to distant ecosystems (Daly et al. 2007). Quantification of environmental impacts of pesticides is therefore a critical component of protecting tropical ecological reserves and biodiversity.

Costa Rica is an ideal location for examining ecological effects of pesticides, as its pesticide imports rank among the highest in Central America (Bravo et al. 2011; de la Cruz et al. 2014). In northeastern Costa Rica, banana and pineapple plantations are an increasingly common land use that involves application of high concentrations of fungicides, nematicides, and insecticides, and the Caribbean slope has an average pesticide load of up to 7.7 kg ha^{-1} (de la Cruz et al. 2014). Pesticide bioaccumulation occurs in wildlife as diverse as crocodiles (Rainwater et al. 2007) and sloths (Branford et al. 2014), and exposure to sulfur-containing pesticides has been linked to changes in howler monkey coat pigmentation (Galván et al. 2019).

Pesticide application has detrimental effects on aquatic as well as terrestrial ecosystems. Streams draining Costa Rican crop plantations often exhibit elevated pesticide concentrations and depauperate macroinvertebrate assemblages, and several aquatic macroinvertebrate taxa are good bioindicators of pesticide runoff (Castillo et al. 2006; Rizo-Patrón et al. 2013). Pesticides also affect fish; for example, organophosphates inhibit acetylcholinesterase and cause oxidative stress in fish (Lushchak 2011).

Our research focused on the ecological effects of ethoprophos, an organophosphate insecticide commonly used in pineapple plantations. Ethoprophos ranks in the top ten most-imported pesticides in Costa Rica (de la Cruz et al. 2014) and causes cholinesterase inhibition in cladocerans (Diepens et al. 2014). Ethoprophos also negatively impacts aquatic vertebrates: it has been linked to decreased neurotransmitter activity in fish (Echeverría-Sáenz et al. 2012) and increased fish mortality due to inability to avoid predators (Sandoval-Herrera et al. 2019). Ethoprophos can affect amphibian activity at a concentration of 31 μg/L (Ghose et al. 2014), higher than – but potentially within range of – recorded ambient stream concentrations of 1 μg/L (e.g., Castillo et al. 2000; Echeverría-Sáenz et al. 2012; Diepens et al. 2014).

The goal of this project was to identify thresholds at which ethoprophos impacts three common species of stream consumers (a leptophlebiid mayfly, hydropsychid caddisfly, and poeciliid fish) and to compare these thresholds to ambient ethoprophos concentrations from Costa Rican streams to determine whether any of these taxa could be useful bioindicators.

Materials and Methods

Study site and focal taxa

This research was conducted at La Selva Biological Station in Costa Rica (10°25′19"N 84°00′54"W). La Selva Biological Station encompasses 1,536 hectares of lowland tropical wet rainforest and includes 13 streams that feed the Puerto Viejo and Sarapiquí Rivers (McDade et al. 1994). We focused on three common stream species: (1) the mayfly *Traverella holzenthali* (Ephemeroptera: Leptophlebiidae), a grazer (feeds on algae); (2) the caddisfly *Leptonema* spp. (Trichoptera: Hydropsychidae), a filter-feeder (consumes particles in the water), and (3) the guppy *Priapichthys annectens* (Cyprinodontiformes: Poeciliidae), a small insectivore.

Methods

We conducted laboratory mesocosm experiments in June-August 2018 and June-August 2019 in the La Selva laboratory to determine the effect of ethoprophos on LC_{50} (lethal concentration for 50% of individuals) and LOEC (lowest observed effect concentration) of each taxon. A stock solution of ethoprophos was prepared from analytical-grade ethoprophos (Sigma Aldrich) dissolved in water, and serial dilutions were performed to reach desired concentrations.

Fish were collected in the field using a hand net, weighed on an analytical balance, measured using millimeter graph paper, and maintained in the laboratory in 1-L plastic containers of aerated water from their native stream (one fish per container). Fish were acclimated for 48 hours prior to toxicity trials. Based on initial range-finding trials conducted at concentrations of 100, 1000, and 5000 μg/L, nine experimental concentrations ranging from 100 to 3000 μg/L were selected (n = 4 fish per concentration). Survivorship and behavioral data were collected at the start of the experiment and every 24 hours for four days after pesticide addition to the container. Behavior was scored using an ethogram (Table 1) developed from preliminary observations of fish behavior in the lab. Fish were not fed during the trials.

Table 1 *Priapichthys annectens* ethogram categories.

Behavior	Code	Criteria
Activity Level	0	Dead
	1	Moving fins but staying still.
	2	Moving the equivalent of one body length in five seconds.
	3	Moving more than one body length in five seconds.
Responsiveness	0	Dead
	1	Does not move when touched with a probe.
	2	Moves in response to being touched with a probe.
Orientation	0	Dead
	1	Fish was dorsal side down when observed.
	2	Fish was dorsal side up when observed.
Location	0	Dead
	1	Fish is at the water's surface (may be trying to gulp air).
	2	Fish is below the water surface.

Insects were collected in the field by flipping rocks and sorting natural leafpacks and maintained in the laboratory in individual 200 mL plastic containers of aerated water from their native stream. Before and after the trial, mayfly length was measured using millimeter graph paper, and caddisflies were weighed on an analytical balance. Insects were acclimated for 24 hours before pesticide trials. After initial range-finding trials with concentrations ranging from 1 to 5000 μg/L, 12 concentrations ranging from 2.5 to 100 μg/L were selected for five-day trials (n = 5 insects per taxon per concentration). Survivorship and behavioral data were collected at the start of the experiment and every 24 hours for five days after pesticide addition to the container. Behavior was scored using ethograms (Tables 2 and 3) developed from preliminary observations of insect behavior in the lab. Insects were not fed during the trials, and only intact and apparently healthy insects (i.e., those with six legs and swimming upright prior to trials) were used.

The protocol was modeled after that of Ghose et al. (2014) and was approved by the MWSU IACUC. After each trial, fish exposed to pesticide were euthanized

Table 2 *Leptonema* sp. activity categories.

Code	Criteria
0	Dead
1	Does not swim at all
2	Swims less than twice its body length when touched
3	Swims more than twice its body length when touched

Table 3 *Traverella holzenthali* righting response categories.

Code	Criteria
0	Cannot right itself.
1	Rights itself in <1 minute
2	No issues with righting response; moves easily

with benzocaine and then preserved in 70% ethanol, while control specimens that did not come into contact with pesticide were released to their sites of capture. Insects were preserved in 70% ethanol.

Statistical analysis

Binary logistic regressions were used to test the effects of ethoprophos concentration and initial size on survival for each taxon. Multiple linear regressions were performed to test (1) the effect of ethoprophos concentration and initial size on percent change in fish biomass and (2) the effects of pesticide concentration and initial size on mean behavioral scores for all taxa. All analyses were conducted in IBM SPSS v. 27 (IBM Corp 2020).

Results

There was a strong negative correlation between survivorship of *P. annectens* and ethoprophos concentration ($p = 0.02'$ PAC 90.7, Nagelkerke $R^2 = 0.83$; Fig. 1a). There was 100% survival in fish exposed to pesticide concentrations of 750 µg/L or less, while fish exposed to concentrations of 2000 µg/L or above had 100% mortality rates. The LC_{50} was approximately 1530 µg/L. Initial mass of fish used in trials ranged from 0.033 g to 0.751 g, and initial mass did not significantly affect survivorship ($p = 0.21$).

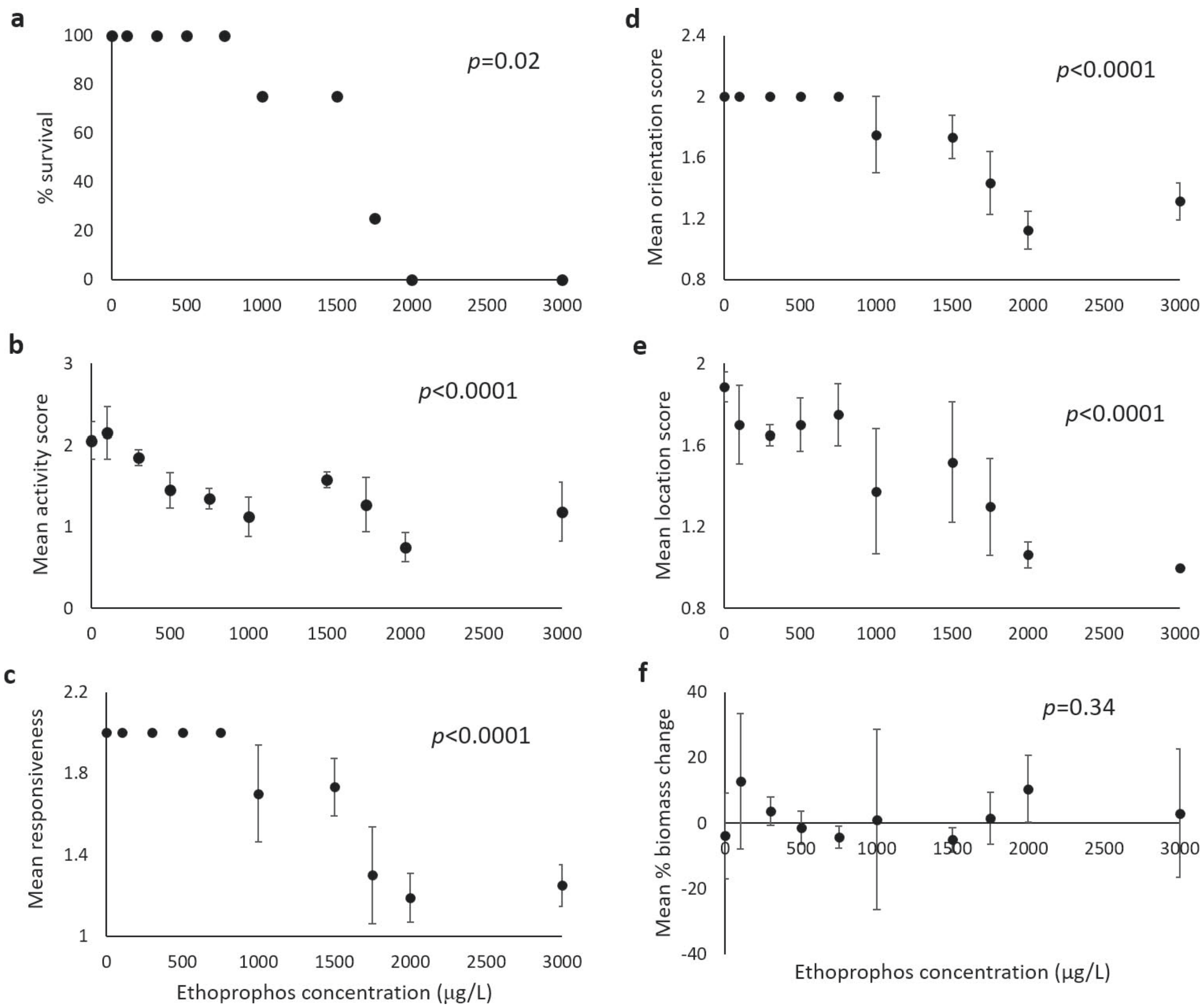

Figure 1 Effects of ethoprophos exposure on the guppy *Priapichthys annectens* in 96-hour trials. Increasing ethoprophos concentrations led to significant decreases in (a) survival, with an LC_{50} of approximately 1530 µg/L, (b) activity score (mean ± SE), (c) responsiveness (mean ± SE), (d) orientation (mean ± SE), and (e) location in the tank (mean ± SE). Scoring of the latter four parameters is explained in Table 2. (f) There was no significant effect of ethoprophos concentration on change in biomass (mean ± SE). n = 4-7.

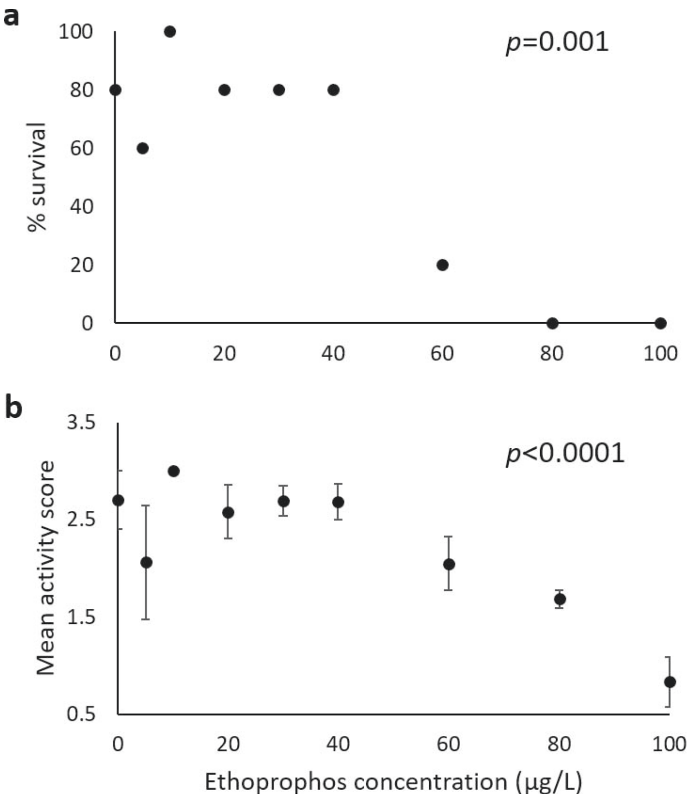

Figure 2 Effects of ethoprophos exposure on case-building caddisfly larvae (*Leptonema* sp.) in 120-hour trials. There was a significant decrease in (a) survival and (b) activity score (mean ± SE) with increasing ethoprophos concentration. Activity scoring is explained in Table 2. The LC_{50} is approximately 30 μg/L, and the LOEC is approximately 5 μg/L. n = 5.

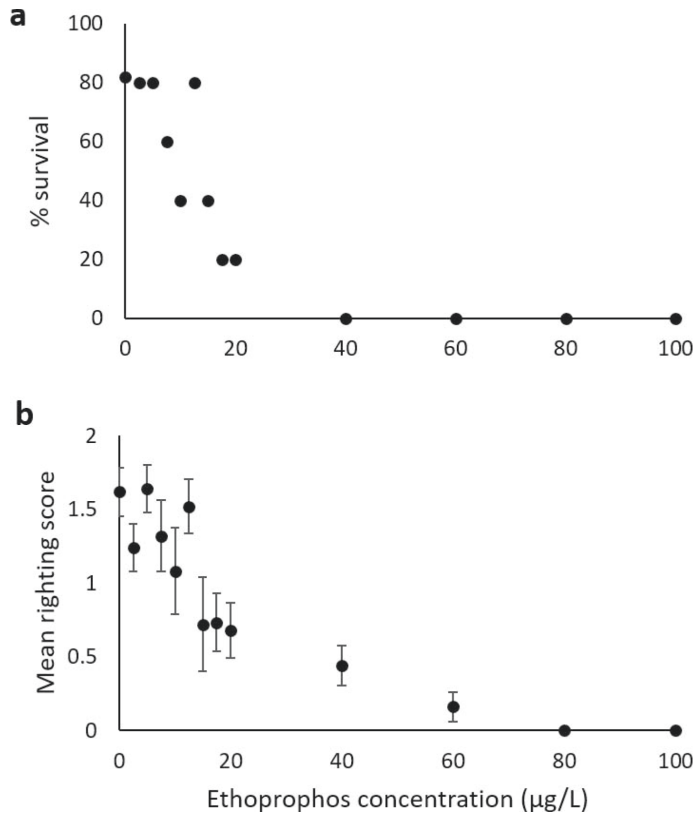

Figure 3 Effects of ethoprophos exposure on mayfly naiads (*Traverella holzenthali*) in 120-hour trials. There was a significant decrease in (a) survival and (b) righting response score (mean ± SE) with increasing ethoprophos concentration. Righting response scoring is explained in Table 3. The LC_{50} is approximately 15 μg/L, and the LOEC is approximately 2.5 μg/L. n = 5 mayflies except for control n = 11.

Mean *P. annectens* activity was negatively correlated with pesticide concentration ($p < 0.0001$, $R^2 = 0.28$; Fig. 1b) and was not affected by initial mass ($p = 0.61$). Mean responsiveness and pesticide concentration were strongly correlated ($p < 0.0001$, $R^2 = 0.61$; Fig. 1c), with the first decrease in responsiveness score happening at 1000 μg/L; there was no effect of initial mass ($p = 0.10$). Mean orientation score and pesticide concentration were also strongly correlated ($p < 0.0001$, $R^2 = 0.58$; Fig. 1d), with the first decrease in average orientation score taking place at 1500 μg/L and no effect of initial mass ($p = 0.12$). Finally, mean location score was also strongly correlated with pesticide concentration ($p < 0.0001$, $R^2 = 0.42$; Fig. 1e) with no effect of initial mass ($p = 0.49$). Percent change in biomass was not significantly affected by ethoprophos concentration ($p = 0.64$) or initial mass ($p = 0.15$; overall $p = 0.34$, $R^2 = 0.05$; Fig. 1f).

Caddisfly survival decreased significantly with increasing ethoprophos concentration ($p = 0.001$, PAC = 80.0, Nagelkerke $R^2 = 0.55$), and the LC_{50} of caddisflies was between 20 and 40 μg/L (Fig. 2a). Mean activity score was significantly correlated to ethoprophos concentration ($p < 0.0001$, $R^2 = 0.41$; Fig. 2b) and there was no effect of initial mass ($p = 0.16$). Percent biomass change was not significantly affected by ethoprophos concentration ($p = 0.86$) or initial size ($p = 0.68$). The estimated LOEC for caddisflies was 5 μg/L.

Mayfly survival also decreased significantly with increasing ethoprophos concentration ($p = 0.001$, PAC = 76.1, Nagelkerke $R^2 = 0.54$), and the LC_{50} value for *T. holzenthali* was approximately 15 μg/L (Fig. 3a). Initial size did not significantly affect survival ($p = 0.20$). There was a significant and fairly strong negative correlation between the mean righting response and ethoprophos concentration ($p < 0.0001$, $R^2 = 0.55$; Fig. 3b), with no significant effect of initial size ($p = 0.23$). The estimated LOEC for mayflies was 2.5 μg/L.

Discussion

Our results show a fairly high LC_{50} (1.5 mg/L) and LOEC (1 mg/L) for *P. annectens* (Fig. 1). Tests of the effects of ethoprophos on other Neotropical fish have reported LC_{50} values from 242 μg/L to 540 μg/L (Diepens et al, 2014; Mena, 2014). Our results therefore suggest that *P. annectens* is more resistant to ethoprophos effects than the other species tested from the tropics. Ambient ethoprophos concentrations in La Selva streams were not quantified in this project due to logistical constraints (time and funding), but ethoprophos concentrations in other lowland Caribbean Costa Rican waterways typically report concentrations of 1 μg/L or less (e.g., Echeverría-Sáenz et al. 2012; Diepens et al. 2014). These data suggest that *P. annectens* is unlikely to be a useful bioindicator in the field.

For aquatic insects, our results indicate LC_{50} values of 15-40 μg/L and LOEC values of 2.5-5 μg/L (Figs. 2 and 3). While the LC_{50} values are approximately three to 40 times higher than ambient concentrations recorded from Costa Rican streams (Echeverría-Sáenz et al. 2012; Diepens et al. 2014), the LOEC values are close to these recorded concentrations, suggesting that pesticide exposure at environmentally realistic levels could detrimentally impact the behavior of these insect species.

Future work in this system should focus on (1) quantifying *in situ* ethoprophos concentrations in La Selva streams, especially when occasional backflooding (uphill streamflow due to flooding of the main rivers) occurs. Further, (2) testing pesticide tolerance of the same taxa from collection sites at higher elevations (which do not backflood) would provide a comparison that could indicate whether the populations surviving in lower-elevation streams have been exposed to even occasional pesticide influx, which could potentially act as a selective factor.

Anthropogenic modification of tropical landscapes is increasing, and understanding and quantifying the impacts of land use on neighboring protected areas is critical to preserving tropical biodiversity. While the rate of tropical deforestation has increased in the past few decades (Kim et al. 2015), we have little evidence of how land-use changes and their accompanying pesticides and herbicides may be affecting biological reserves and little knowledge of which species might serve as bioindicators of pesticide exposure. Laboratory trials are a first step toward identifying potential bioindicator species. Future work should consider that ecosystems are often exposed to multiple pesticides simultaneously (e.g., Mena 2014), and possible synergistic effects of tropical aquatic organisms' prolonged exposure to various pesticides deserve further investigation.

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Author Contributions

All authors contributed to project design. CG acquired funding and provided oversight for the project. All authors collected data and analyzed the results. CK, RP, CT, and CG drafted the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest.