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Fish predation affects invertebrate community structure of tropical temporary ponds, with downstream effects on phytoplankton that are obscured by pesticide pollution[☆]

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ABSTRACT

Aquatic biota of tropical temporary ponds typically experience a wide range of stressors that can drive the structure and dynamics of natural communities. Particularly in regions with intense agricultural activity, aquatic biota may not only experience predation pressure but also stress from pesticides that inadvertently enter the ponds. We increasingly understand how these different sources of stress affect classic model taxa under controlled laboratory conditions, but how predators and pesticides may jointly affect pond invertebrate communities is still unclear, particularly for tropical systems. Here, we conducted an outdoor mesocosm experiment to study how fish predation combined with exposure to an environmentally relevant concentration of the commonly used insecticide cypermethrin (0.8 ng/L) affects the structure of invertebrate communities, and its potential effects on leaf litter decomposition and invertebrate grazing efficiency as measures of ecosystem functioning. A total of seven invertebrate taxa were recorded in the mesocosm communities. Fish predation effectively lowered the number of invertebrate taxa, with fish mesocosms being dominated by high densities of rotifers, associated with lower phytoplankton levels, but only when communities were not simultaneously exposed to cypermethrin. In contrast, cypermethrin exposure did not affect invertebrate community structure, and neither fish predation nor cypermethrin exposure affected our measures of ecosystem functioning. These findings suggest that predation by killifish can strongly affect invertebrate community structure of tropical temporary ponds, and that downstream effects on phytoplankton biomass can be mediated by exposure to cypermethrin. More broadly, we contend that a deeper understanding of (tropical) temporary pond ecology is necessary to effectively manage these increasingly polluted systems.

1. Introduction

Animals in the wild are often simultaneously exposed to multiple

stressors (Thoré et al., 2021a, 2021b; Beermann et al., 2018; Gunderson et al., 2016). In freshwater systems, such stressors are regularly related to temporal fluctuations in abiotic conditions such as pH, water

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temperature, dissolved oxygen levels, turbidity or salinity (Chapman et al., 2011; Ross et al., 2001). In addition, animals may also experience stress from predation, amongst other natural stressors. In contrast to permanent freshwater systems, temporary ponds are usually devoid of predatory fish. However, some killifish genera of (sub)tropical regions are adapted to live in temporary ponds ('annual killifish') and may prey heavily on the invertebrate communities (Pinceel et al., 2021). While the ecology of annual killifish is increasingly studied (Pinceel et al., 2021; Thoré et al., 2019; Grégoir et al., 2018, 2017), it is not yet fully understood how killifish predation affects the structure and dynamics of invertebrate communities in tropical temporary ponds. A recent pioneering study offered valuable first insights, showing that *Nothobranchius furzeri* killifish from temporary ponds in Southern Africa do not prey on clam shrimps while they effectively reduce fairy shrimp populations by 90–100% (Pinceel et al., 2021).

Besides natural sources of stress, invertebrate communities of tropical temporary ponds may also face stress related to rapid agricultural intensification, including pesticide pollution. Temporary pond ecosystems and associated floodplains in Sub-Saharan Africa, are increasingly contaminated with agricultural pesticides (Brendonck et al., 2022; Lahr, 1998). For example, cypermethrin has been detected at concentrations between 8.12 and 15.46 mg/L in Malawian streams (Kanyika-Mbewe et al., 2020). Cypermethrin is currently the most commonly used pyrethroid insecticide globally (Shi et al., 2011; Kumar & Chandra, 2003) owing to its broad spectrum activity and, more specifically, accounts for ~20% of insecticides used in the lake Manyara Basin in Tanzania (Manyilizu et al., 2017). Cypermethrin is strongly neurotoxic for insects (Shi et al., 2011) and diffuses easily through the cell membrane of various organisms due to its high lipophilicity, interfering with neurotransmission by blocking neuronal sodium channels (Tiwari et al., 2019). Consequently, when the compound enters the natural environment, it can have deleterious effects on non-target organisms (Kim et al., 2008) and potentially disrupt population- and community dynamics through direct or indirect effects on survival and reproduction of biota (Guedes et al., 2016).

The effect of stressors (such as pesticides and predation) on aquatic biota are usually studied in isolation and on a single focal species under controlled laboratory conditions (Relyea, 2018). Researchers now increasingly recognize that stressors often occur simultaneously and that studying their combined effect represents a more realistic scenario with higher ecological relevance (Jansen et al., 2011). Accordingly, there are increasingly more studies on how concurrent stressors affect single species (Jansen et al., 2011; Coors and De Meester, 2009). Still, how combined stressors may affect entire aquatic communities of tropical temporary ponds remains poorly understood. Such information is important because these systems host unique biodiversity including specialized key groups (e.g. killifish), and deliver important ecosystem services (including clean water for humans, cattle, and wildlife) that depend on the proper functioning of these ecosystems (Brendonck et al., 2022).

As a step towards better understanding the impact of multiple stressors under semi-realistic conditions, we conducted an outdoor mesocosm experiment to study how fish predation combined with exposure to an environmentally relevant concentration of the insecticide cypermethrin affects the structure of invertebrate communities of tropical temporary ponds. Experimental invertebrate communities and killifish were collected from the Lake Manyara Basin in northern Tanzania, where many temporary ponds are located in agricultural area where cypermethrin is commonly used. We furthermore assessed the effects on leaf litter decomposition and invertebrate grazing efficiency as measures of ecosystem functioning. With the expectation that both these stressors, alone and combined, affect invertebrate communities and ecological functioning of tropical temporary ponds, we aim to ultimately increase our understanding of these systems as a necessary step towards their sustainable management.

2. Materials and methods

2.1. Preparation of exposure media

Cypermethrin (Sigma - C2237, 98.0% purity, CAS: 52315-07-8) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of 100 mg/L was prepared by dissolving 100 mg of cypermethrin in a liter of Milli-Q grade water, stirred for 24 h at 2000 rpm at 26 °C. The homogenous solution was stored at –20 °C until use. Experimental medium for the mesocosms was prepared by adding standardized salt (Instant Ocean Sea Salt, Instant Ocean-Aquarium Systems, Fiji) to dechlorinated tap water to a conductivity of 450 µS/cm as described by Kafula et al. (2022).

2.2. Experimental design

The experiment was conducted, with some modifications, following Relyea and Hoverman (2008) at the Tropical Pesticide Research Institute station in Manyara, Tanzania, and ran for 23 days during the short rainy season of 2020. Four different experimental conditions were used: a control (i.e., no pesticide exposure and no fish predation), a condition with fish predation, a condition with pesticide exposure (cypermethrin) and a condition with both fish predation and pesticide exposure (Fig. 1). Three free-swimming adult killifish (*Nothobranchius neumanni*, approximately 4.5 cm total length) were used per mesocosm, collected from local temporary ponds as described by Reichard and colleagues (2009). Each of the four experimental conditions was replicated six times, making a total of 24 mesocosms of 150 L each (90 cm diameter, 30 cm height, in high-density polyethylene) (Fig. 1). Mesocosms were either red, green, or blue (randomly distributed among the experimental conditions), which did not affect any of the measured endpoints (water quality parameters, invertebrate community and ecosystem functioning, see Table S1).

At the onset of the experiment, mesocosms were filled with 150 L experimental medium and 20 g of rabbit chow was added as a nutrient source. Each mesocosm was inoculated with 1 L of a homogenized mixture of water collected from 10 temporary ponds in the Lake Manyara Basin to serve as an initial source of representative plankton. These ponds were located in settlement areas where they may have already been exposed to cypermethrin. This approach allows us to realistically estimate the potential effects of cypermethrin on these local communities. The integrated plankton sample was obtained by filtering 100 L of water through a 64-µm mesh collected from different locations in each of the 10 ponds: open water, locations with submerged vegetation, and with floating vegetation. In addition, throughout the experiment, mesocosms were open which allowed colonizing organisms (e.g., aquatic insects) to naturally settle so that colonization preference could be assessed.

After two days, pesticide mesocosms were dosed with 0.8 ng/L of cypermethrin. This concentration is not acutely lethal to invertebrates but has nevertheless been shown to have deleterious chronic effects on ecologically relevant traits of invertebrates such as reproduction, growth, and population recovery (Zhou et al., 2019; Rasmussen et al., 2013; Kim et al., 2008). As a reference, a 2-week cypermethrin exposure impairs adult growth and offspring production of freshwater zooplankton at concentrations as low as 20 ng/L (Gottardi et al., 2017), and 7 ng/L of cypermethrin impairs feeding in calanoid copepods (*Acartia tonsa*) even when exposed for less than five days (Barata et al., 2002). Further, the selected test concentration was well below the peak concentration range in Malawian surface waters (8.12–15.46 mg/L) (Kanyika-Mbewe et al., 2020). A next cypermethrin pulse was applied to the relevant mesocosms every six days, as in Medina and coworkers (2004). Each time, pesticide aliquots were first dissolved into 1-L glass jars with experimental medium and then added to the mesocosm. In doing so, the pesticide-containing medium was distributed evenly over the surface of the mesocosm to simulate surface runoff, aerial drift, or

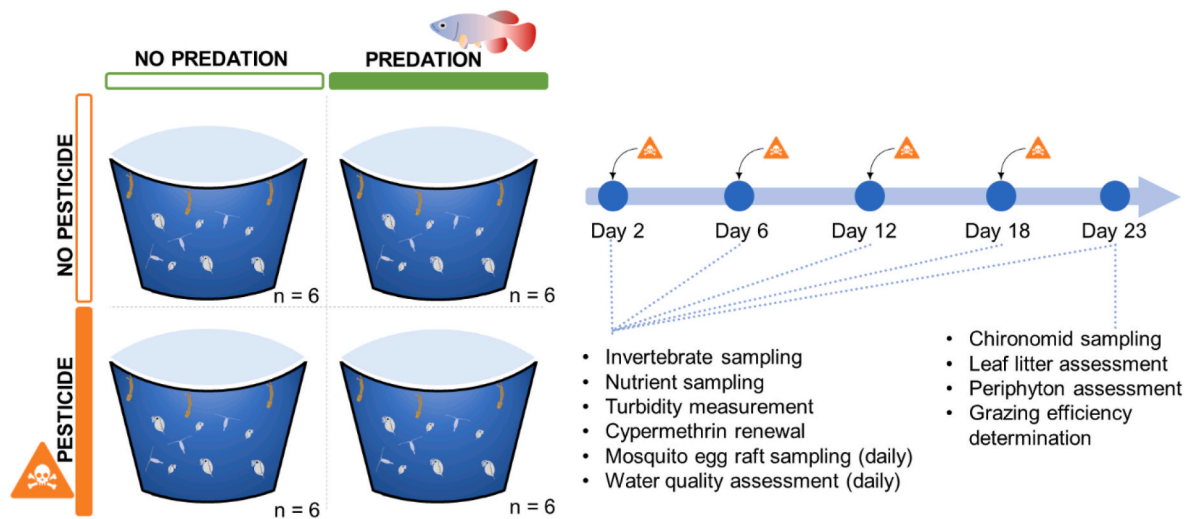


Fig. 1. Schematic overview of experimental setup. Four experimental mesocosm conditions were tested to assess the impact of fish predation and cypermethrin exposure on invertebrate communities of tropical temporary ponds: a control, a condition with three free-swimming killifish, a condition with 0.8 ng/L cypermethrin applied every six days, and a condition with both killifish and cypermethrin exposure. Each condition was replicated six times, and the experiment ran for a total of 23 days.

aerial overspray as in Relyea (2018). The achieved concentrations were verified using Gas Chromatography Mass Spectrometry (see Supplementary Material for more details). The achieved pesticide concentration was 0.812 ± 0.02 ng/L after 1 h; 0.529 ± 0.05 ng/L after two days; 0.137 ± 0.06 ng/L after four days and 0.0275 ± 0.01 ng/L after six days of mesocosm dosing (Supplementary Material, Figure S7).

2.2.1. Determination of physicochemical parameters and chlorophyll *a*

Physicochemical parameters (dissolved oxygen, pH, salinity, electrical conductivity, and water temperature) were measured *in situ* every day at 08:00am using a portable HANNA multi-parameter probe (Model-HI 9829). Turbidity was measured once every week using a handheld turbidimeter (HANNA-Model-HI93703). After every six days, a 1-L water sample was taken from each mesocosm for the assessment of nutrient levels. Nutrient concentrations (nitrate and orthophosphate) were spectrophotometrically assessed with a Hach DR2800 (Hach Company, Loveland, CO). Orthophosphate concentrations were measured using the ascorbic acid (PhosVer 3) method (low range: 0.02–2.50 mg/L PO_4), while nitrate concentrations were assessed using a cadmium reduction method (low range: 0.01–30.0 mg/L $\text{NO}_3\text{-N}$). All chemical analyses followed the standard methods for the examination of water and wastewater by APHA (1999). Phytoplankton biomass was indirectly determined as Chlorophyll *a* concentration using a handheld AquaFluor Fluorometer (Turner Designs, USA, Model-8000-010).

2.2.2. Invertebrate sampling, identification, and quantification

Invertebrate samples were taken on day 2, 6, 12, 18, and 23. At each occasion, 10 L of water from each mesocosm (sampled from different sections of the mesocosm) was filtered through a 64- μm net. The filtrate was then concentrated to 100 ml and preserved in 70% ethanol. We found a total of 7 taxa, identified to different taxonomic levels using standard identification guides (Dang et al., 2015; Conway et al., 2003). Counting of invertebrate subsamples in the laboratory was done as described by Goswami (2004). Subsamples of 30 ml were examined for counting the taxa under a stereo microscope (Motic K-400 L). Mosquito egg rafts were collected once every day at 08:00am, visually counted, and returned to the respective mesocosms. Chironomids, however, occurred in all mesocosms and because of their bottom dwelling and tube-living behavior (Hershey, 1987), they were counted at the end of the experiment when water was discarded and all medium filtered.

2.2.3. Ecosystem functioning: leaf litter decomposition and grazing efficiency

Tree leaf litter decomposition was assessed as described by Mitchell and coworkers (2014). Briefly, 5 g of dried leaf litter was placed in 1-mm mesh 10×10 cm nylon bags. Each bag also had eight 5-mm holes to allow entry of microfauna. Two litterbags were placed at the bottom in each mesocosm throughout the experiment. At the end of the experiment, litterbags were collected and frozen (at -21°C) until analysis. For this, leftover litter was removed from each bag, oven-dried overnight at 55°C (BINDER D78532 Tuttlingen, Germany) and then ashed at 360°C and weighed to obtain the ash-free dry mass. The percentage mass lost was then calculated for each bag, representing total decomposition (physical breakdown and mineralization). Grazing efficiency was analyzed at the end of the experiment by sampling 1 L of water with invertebrates and 1 L of water without invertebrates (filtered through a 64- μm net) from each mesocosm, as described by Ká et al. (2012). The initial chlorophyll *a* concentration was measured in both samples using a fluorometer (Turner Designs, USA, Model-8000-010). Then, both samples were left in sealed bottles for 24 h inside the mesocosms after which chlorophyll concentration of both samples was measured again.

2.2.4. Periphyton production

To assess periphyton production, a small tile of 100 cm^2 was added in each mesocosm as described by Stelzer and Lamberti (2001). At the end of the experiment, periphyton was collected from the tile using a scraper (sharp knife) and was added in a small vial with 5 ml of distilled water. Then, the mixture was stirred and chlorophyll *a* was measured using a fluorometer (Turner Designs, USA, Model-8000-010).

2.3. Animal welfare statement

All procedures and methods were in accordance with the animal welfare commission requirements of Tanzania. Health and condition of all fish was checked daily by Y. A. Kafula. To reduce stress and discomfort of fish, any disturbance and handling was kept to the minimum. At the end of the experiment, fish from conditions without pesticide exposure were reassigned as breeding fish in the fish culturing facility at the School of Life Sciences and Bio-engineering at the Nelson Mandela African Institution of Science and Technology, while fish that were exposed to the pesticide were euthanized by means of an overdose of MS-222 (250 mg/L of tricaine).

2.4. Data analysis

Data analysis was performed in R version 4.3.1 (R Core Team, 2023). Model assumptions, including homoscedasticity and fit of the error distribution, were verified graphically for all analyses. In addition, we used a Shapiro-Wilk test to verify the normality assumption in case of a Gaussian error distribution.

2.4.1. Biotic response variables and water quality

We used linear mixed-effects models (lme4 package; Bates et al., 2015) to analyze the density of invertebrates per taxon, the total invertebrate density (i.e., all taxa pooled together), number of taxa (i.e., taxon richness), and the number of mosquito egg rafts. For all these models, we assumed a Poisson error distribution as appropriate for count data, with predation treatment (killifish vs. no killifish), pesticide treatment (cypermethrin vs. no cypermethrin), and their interaction, as fixed factors. Mesocosm identity, mesocosm color, and sampling campaign (referring to the repeated measures: day 2, 6, 12, 18, and 23) were added as random effects. For rotifer density and total invertebrate density, we additionally added an observation-level random effect to account for overdispersion. Similar mixed-effects models were used to analyze physicochemical parameters: pH, electrical conductivity, nitrate content, orthophosphate content, dissolved oxygen level, water turbidity, and water temperature. For these parameters, we assumed a Gaussian error distribution. While all above parameters were assessed repeatedly throughout the experiment, number of chironomids and periphyton production were assessed only once, at the end of the experiment. Therefore, these were analyzed using linear mixed-effects models with predation treatment, pesticide treatment, and their interaction as fixed factors, and with mesocosm color as a random effect. We assumed a Poisson and Gaussian error distribution for number of chironomids and periphyton production, respectively.

Type 3 Wald chi-square tests were used to test the significance of the fixed factors. Post hoc differences between experimental groups were assessed by means of Tukey-corrected pairwise comparisons (lsmeans package; Lenth, 2016). To confirm that the color of the mesocosm tanks did not affect the outcomes of our experiment, likelihood-ratio tests were used to compare each model with and without the mesocosm color random effect (Supplementary Material, Table S1).

2.4.2. Biotic response variables and water quality per sampling campaign

For further scrutiny, all parameters that were measured repeatedly throughout the experiment were also analyzed for each of the five sampling campaigns separately (Supplementary Material, Table S2–6). For this, we used linear models with predation treatment, pesticide treatment, and their interaction as predictor variables.

2.4.3. Community structure

To assess how killifish presence and pesticide exposure ultimately affect the structure of the invertebrate community, we ran a permutational PERMANOVA (Adonis function, with Bray Curtis dissimilarity method) using data on the density of invertebrates per taxon during the last sampling campaign (i.e., at the end of the experiment). Predation treatment, pesticide treatment, and their interaction were included as predictors.

2.4.4. Measures of ecosystem functioning

Leaf litter decomposition and invertebrate grazing efficiency were only measured at the end of the experiment. Both were analyzed using a general linear model (Gaussian error distribution) with predation treatment, pesticide treatment, and their interaction as predictor variables.

3. Results

3.1. Physicochemical parameters and invertebrate taxa

Mesocosms with killifish were the least turbid and had a significantly lower phytoplankton biomass compared mesocosms without killifish. However, this effect was conditional on cypermethrin exposure (Table 1). Specifically, phytoplankton density was only lower in presence of killifish when the mesocosm was not simultaneously exposed to cypermethrin (Table 1; Fig. 2). Water temperature, dissolved oxygen, pH, conductivity, and nitrate and orthophosphate content did not differ among experimental conditions (Table 1; Fig. 2).

A total of seven invertebrate taxa were recorded throughout the experiment: Calanoida, Chironomidae, *Culex* spp., Ostracoda, Psychodidae, Rotifera, and *Simocephalus* spp. Generally, the overall abundance and richness of invertebrate taxa differed significantly among experimental conditions driven by the presence vs. absence of killifish rather than by exposure to cypermethrin (Table 1). Specifically, overall invertebrate abundance was higher in the presence of killifish, regardless of cypermethrin exposure, which was mainly due to dominance of rotifers (Fig. 3). In contrast, richness of invertebrate taxa was lower in the presence of killifish (Table 1; Fig. 3). Likewise, the abundance of calanoids, ostracods, psychodids, and cladocerans (*Simocephalus* spp.) was lower in the presence of killifish but was not affected by exposure to cypermethrin (Table 1; Fig. 3). Similar observations were made for mosquito oviposition and, subsequently, abundance of *Culex* sp. (Table 1; Fig. 3). Except for rotifers, abundance of all reported invertebrate taxa in killifish and pesticide + killifish conditions rarely exceeded two individuals per liter. In contrast, rotifers were almost entirely absent in conditions with large-sized invertebrate taxa such as calanoids and cladocerans.

Results per time point (presented in Supplementary Material, Table S2) reveal no significant effect of killifish presence and cypermethrin exposure on both invertebrate densities and water quality variables on the second day of exposure. However, from the sixth day

Table 1

Output of linear mixed-effects models using all invertebrate taxa, physicochemical parameters, and mosquito egg rafts to show the effect of predation (killifish presence), pesticide exposure (cypermethrin), and their interaction.

| Responses | Predictors | | | | | |
|-----------------------------------|------------|------------------|-----------|-------|-------------------------|--------------|
| | Predation | | Pesticide | | Predation* Pesticide | |
| | χ^2 | P | χ^2 | P | χ^2 | P |
| Community structure | | | | | | |
| Overall invertebrate density | 4.657 | 0.031 | 0.256 | 0.613 | 0.002 | 0.966 |
| Species richness | 38.63 | <0.001 | 0.004 | 0.949 | 0.279 | 0.597 |
| Rotifera abundance | 33.19 | <0.001 | 0.014 | 0.906 | 0.699 | 0.403 |
| <i>Culex</i> abundance | 12.46 | <0.001 | 0.779 | 0.378 | 0.312 | 0.577 |
| Ostracoda abundance | 11.56 | <0.001 | 0.123 | 0.726 | 0.499 | 0.479 |
| Calanoida abundance | 13.37 | <0.001 | 0.926 | 0.336 | 0.01 | 0.919 |
| <i>Simocephalus</i> abundance | 0 | 0.99 | 0.218 | 0.641 | 0 | 0.999 |
| Psychodidae abundance | 4.412 | 0.04 | 0.153 | 0.695 | 0.183 | 0.669 |
| Chironomidae abundance | 0.009 | 0.924 | 0.005 | 0.946 | 0.001 | 0.971 |
| Physicochemical parameters | | | | | | |
| Phytoplankton | 6.749 | 0.009 | 1.083 | 0.298 | 10.46 | 0.001 |
| Dissolved oxygen level | 3.677 | 0.06 | 0.452 | 0.501 | 0.108 | 0.742 |
| Electrical conductivity | 0.04 | 0.841 | 0.561 | 0.454 | 0.058 | 0.809 |
| pH | 0.737 | 0.391 | 0.907 | 0.341 | 0.038 | 0.845 |
| Water temperature | 3.113 | 0.08 | 0.353 | 0.552 | 1.734 | 0.188 |
| Turbidity | 17.9 | <0.001 | 1.513 | 0.219 | 10.489 | 0.001 |
| Orthophosphate | 0.169 | 0.68 | 0.318 | 0.573 | 0.049 | 0.825 |
| Nitrate | 0.938 | 0.333 | 0.03 | 0.85 | 3.95 | 0.05 |
| Mosquito egg rafts | 10.81 | 0.001 | 0.589 | 0.443 | 0.673 | 0.412 |

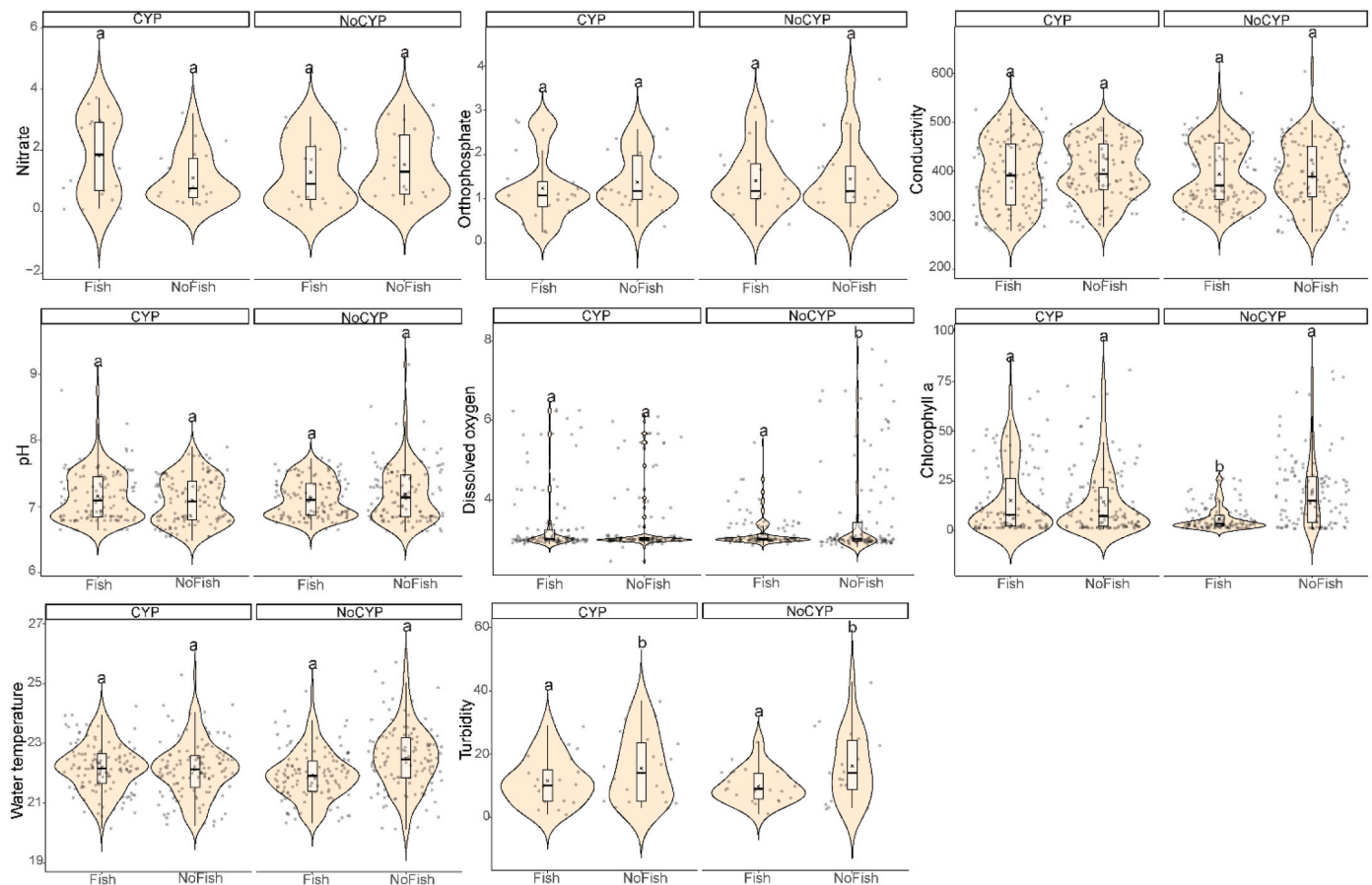


Fig. 2. Water quality variables in four experimental conditions (control, predation, pesticide, and predation + pesticide) in outdoor mesocosms. Boxplots show the 25th, 50th (median), and 75th quartiles. The mean is indicated by 'x'. Different letters indicate significant differences. (CYP = cypermethrin added; noCYP = no cypermethrin added).

onwards, the presence of killifish significantly affected invertebrate density and number of taxa (Supplementary Material, Table S3–6). The PERMANOVA model revealed that killifish presence explains 71.7% ($r^2 = 0.7165$, $p = 0.001$), cypermethrin exposure explains 1.3% ($r^2 = 0.01285$, $p = 0.43$), while their interaction explains 1.3% ($r^2 = 0.013$, $p = 0.39$) of the observed variation in invertebrate community structure ($F = 38.9$).

3.2. Ecosystem functioning and periphyton production

Decomposition rate and periphyton concentration did not differ significantly among experimental conditions. Specifically, neither the presence of killifish ($z = 2.36$, $p = 0.06$), cypermethrin exposure ($z = 1.29$, $p = 0.22$) nor their interaction ($z = -1.58$, $p = 0.13$) affected decomposition rate. Likewise, neither the presence of killifish ($z = 0.09$, $p = 0.92$), cypermethrin exposure ($z = 1.64$, $p = 0.12$) nor their interaction ($z = -0.44$, $p = 0.67$) affected periphyton concentration. Neither the presence of killifish ($z = -0.89$, $p = 0.83$), cypermethrin exposure ($z = -0.81$, $p = 0.43$) nor their combination ($z = 1.399$, $p = 0.1810$) affected grazing efficiency.

4. Discussion

We assessed how fish predation combined with exposure to the insecticide cypermethrin affects the structure of invertebrate communities of tropical temporary ponds, and its potential effects on leaf litter decomposition and invertebrate grazing efficiency as measures of ecosystem functioning. Overall, when killifish were present, invertebrate taxon diversity decreased while invertebrate density increased.

This observation was mostly driven by a sharp increase in the number of rotifers in the presence of killifish. However, this change in community structure did not translate to changes in leaf litter decomposition and invertebrate grazing efficiency. In contrast to the effects of fish predation, exposure to 0.8 ng/L of cypermethrin did not strongly affect community structure, nor did it affect measures of ecosystem functioning. Still, the influence of killifish presence on phytoplankton biomass was contingent upon cypermethrin exposure. Specifically, when communities were simultaneously exposed to cypermethrin, the presence of fish had no discernible impact on phytoplankton biomass.

4.1. Impact of killifish predation on invertebrate community structure and ecosystem functioning

As expected, killifish predation strongly affected invertebrate community structure. Specifically, when killifish were present, the overall number of invertebrate taxa was lower compared to killifish-free conditions. This finding aligns with results of previous studies in permanent pond communities where macro- and microinvertebrate community composition, richness, and densities were significantly shaped by the presence of bluegill (*Lepomis macrochirus*) (Gilinsky, 2010; Shurin, 2001). However, despite the decrease in invertebrate richness, the overall invertebrate density increased in the presence of killifish. This increase in invertebrate density seems mostly driven by a sharp increase in the number of rotifers, which exceeded a density of 100 individuals per liter by the end of the experiment. Similar observations, where a reduction in the density and diversity of larger invertebrates was associated with a concomitant increase in rotifer density, have been made previously (Medina et al., 2004). In contrast to larger zooplankton, the

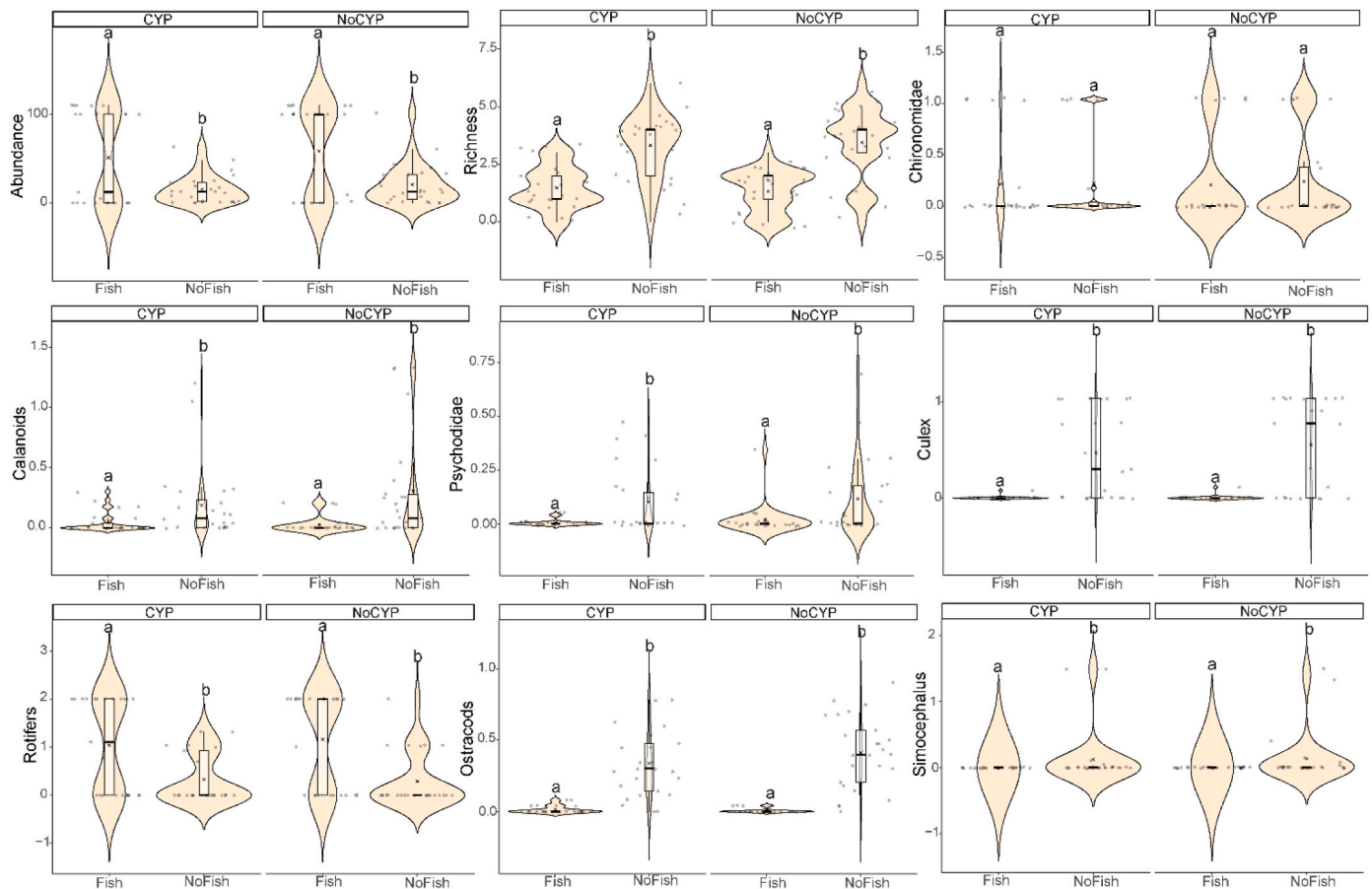


Fig. 3. Invertebrate density per taxon in four experimental conditions (control, predation, pesticide, and predation + pesticide) in outdoor mesocosms. Boxplots show the 25th, 50th (median), and 75th quartiles. The mean is indicated by 'x'. Different letters indicate significant differences. (CYP = cypermethrin added; noCYP = no cypermethrin added).

small rotifers were probably not detected or preyed upon by the killifish and could therefore proliferate freely. Size-selective predation and its impact on invertebrate community structure has already been documented for various fish species (Hessen, 1985; Greene, 1983; Hall et al., 1976; Dodson, 1974), including *Nothobranchius* killifish (Pinceel et al., 2021). The observation that rotifer density remained low in the control condition is likely due to competition with (or predation by) larger zooplankton species (such as *Simocephalus* spp. and copepods).

As we expected, colonization by flying insects was limited when killifish were present, and *Culex* sp. mosquito egg rafts were almost entirely absent from fish mesocosms throughout the experiment. Impact of fish predation and their cues on colonization by insects has been shown previously. For example, habitat selection based on the presence of fish predators explained up to 43% of the observed variation in colonizing bug community structure (Trekels and Vanschoenwinkel, 2017). Similarly, mosquitoes seem to preferentially colonize habitats that are devoid of predators (Trekels and Vanschoenwinkel, 2019). In contrast to *Culex* sp., Chironomidae density was high regardless of the presence or absence of killifish. This is likely because killifish may not be efficient predators of Chironomidae. Instead, *Nothobranchius* fish have a superior mouth (i.e. an upward-directed mouth) that allows to efficiently prey on invertebrates on or near the water surface (Keppeler et al., 2015). However, they are far less efficient to forage on organisms with a benthic or burrowing lifestyle, such as chironomids (Hershey, 1987). Other organisms with a benthic lifestyle, such as clam shrimps, were previously also shown to be relatively unaffected by *Nothobranchius* predation (Pinceel et al., 2021).

While the presence of fish had a notable impact on the structure of the invertebrate community within our mesocosms, this influence did

not extend to leaf litter decomposition rate and invertebrate grazing efficiency (i.e., despite having lower phytoplankton levels in the presence of killifish which coincided with an increase in rotifer density). Several factors may explain this apparent lack of impact. First, it is possible that the change in species composition of the invertebrate community was not accompanied by a change in functional trait composition strong enough to affect our measures of ecosystem functioning (i.e., functional redundancy). However, it is also important to consider that the groups that were mainly affected by killifish presence may not be key contributors to the functioning of the temporary pond ecosystem. For example, rotifers primarily feed on bacterioplankton and small phytoplankton species and may therefore not efficiently graze on all species of phytoplankton (Gilbert, 2022). Lastly, it is worth considering that the resolution of our species identification and the relatively short duration of our study, spanning only 23 days, may limit the strength of our conclusions concerning the link between the observed shifts in invertebrate community structure and their ultimate effects on ecosystem functioning.

4.2. Impact of pesticide exposure on community structure and ecosystem functioning

Contrary to our predictions, cypermethrin exposure did not affect the density nor diversity of invertebrate taxa. This is surprising, given that cypermethrin is known to be highly toxic and impact various invertebrate species, even at low concentrations (Ranatunga et al., 2023). Possibly, our applied concentration (0.8 ng/L) may have been too low to cause any toxic effects within the given timeframe. Furthermore, it may be important to consider that we applied cypermethrin in pulses, which

is more ecologically relevant compared to chronic pesticide exposure at a constant concentration but can influence the overall impact of the pesticide, including so-called “latent” effects that may only emerge later on (Schäfer et al., 2023; Liess & Gröning, 2024). For example, cypermethrin rapidly degraded between pulses (Figure S7 in Supplementary Material) which may have dampened the toxic impact of cypermethrin on the invertebrate community. Similar to our observations, 19 days of exposure to 5 µg/L of cypermethrin only had limited effect on cladoceran density and allowed cladocerans to recover (Medina et al., 2004). Moreover, plankton used to inoculate the mesocosms were collected from local ponds in settlement areas which can get contaminated by cypermethrin, among others by spraying for mosquito control. Given that organisms, including zooplankton (Almeida et al., 2021), are sometimes able to adapt to pesticide exposure, it is possible that the sampled biota were already relatively resistant to cypermethrin exposure. Indeed, organisms can sometimes adapt relatively quickly, as was for example the case with aphids (*Aphis gossypii*) that were exposed to cypermethrin and became less sensitive to the compound after as little as one generation of exposure (Ullah et al., 2020). Therefore, follow-up research should take into account the community’s history of exposure to pesticides, as well as its evolutionary responses, to get a deeper insight into how concurrent pesticide and predation stress affects the community structure and ecosystem functioning of tropical temporary ponds.

4.3. Mixed stressors – interactive effects between predation and pesticide exposure on community structure and ecosystem functioning

We hypothesized that joint effects of multiple stressors could shape the invertebrate community structure and, consequently, impact ecosystem functions. Although we did not observe any interactive effects of killifish presence and cypermethrin exposure on invertebrate community structure, we did observe that the negative impact of killifish presence of phytoplankton biomass only emerged when communities were not simultaneously exposed to cypermethrin. Likely, the decrease in phytoplankton biomass in the presence of killifish and while not simultaneously exposed to cypermethrin is due to the high observed density of rotifers under these conditions. Gosselain et al. (1998) made similar observations on the Belgian side of River Meuse of a decline in phytoplankton biomass linked to a peak in rotifer density. At this moment, it is difficult to explain why this observed decrease in phytoplankton biomass in the presence of killifish no longer emerged when communities were simultaneously exposed to cypermethrin, particularly given that cypermethrin exposure had otherwise no discernible impact on the abundance of the various invertebrate taxa. It is conceivable that cypermethrin exposure may have had various sub-lethal impacts, such as inhibitory or stimulatory changes in behavior (e.g. changes in foraging), that were not measured in the current study but may nevertheless have contributed to the observed pattern. For example, feeding efficiency of *Daphnia magna* was already reduced by over 50% after as little as 6 h of exposure to 0.1 µg/L cypermethrin (Christensen et al., 2005). Such sub-lethal effects in response to low pesticide exposure, and which may potentially translate to reduced fitness after longer-term exposure (Liess & Gröning, 2024), can nevertheless lead to important shifts at the population and/or community level. Ultimately, pesticide exposure can influence zooplankton communities through a complex interplay of various direct and indirect (sub)lethal effects (Almeida et al., 2023).

5. Conclusion

Our results show that fish predation can strongly affect invertebrate community structure of tropical temporary ponds, a finding that contributes to our understanding of these habitats. In contrast, exposure to 0.8 ng/L of cypermethrin did not affect invertebrate community structure even though we did observe interactive effects with killifish presence on the abundance of phytoplankton. Because pesticides are

expected to increasingly reach tropical temporary ponds, in particular in developing regions where agriculture is rapidly intensifying, future research should study how higher doses of pesticides may affect structure and functioning of temporary pond ecosystems. A proper understanding of these systems, and how different factors affect community structure and functioning is critical to develop efficient and sustainable management practices to protect or restore these vulnerable systems.

CRedit authorship contribution statement

Yusuph A. Kafula: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gordian R. Mataba:** Writing – review & editing, Methodology, Formal analysis. **Grite N. Mwaijengo:** Writing – review & editing, Formal analysis. **Francis Moyo:** Writing – review & editing, Supervision. **Linus K. Munishi:** Writing – review & editing, Supervision. **Bram Vanschoenwinkel:** Writing – review & editing, Supervision, Formal analysis. **Luc Brendonck:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Eli S.J. Thoré:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data and scripts that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.25186355>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.123592>.

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