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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.

Introduction

Animals in the wild are often simultaneously exposed to multiple stressors (Thoré et al., 2021; 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 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 – 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

75　in Tanzania (Manyilizu et al., 2017). Cypermethrin is strongly neurotoxic for insects (Shi et al.,

76　2011) and diffuses easily through the cell membrane of various organisms due to its high

77　lipophilicity, interfering with neurotransmission by blocking neuronal sodium channels (Tiwari et

78　al., 2019). Consequently, when the compound enters the natural environment, it can have

79　deleterious effects on non-target organisms (Kim et al., 2008) and potentially disrupt population-

80　and community dynamics through direct or indirect effects on survival and reproduction of biota

81　(Guedes et al., 2016).

82　　　The effect of stressors (such as pesticides and predation) on aquatic biota are usually

83　studied in isolation and on a single focal species under controlled laboratory conditions (Relyea,

84　2018). Researchers now increasingly recognize that stressors often occur simultaneously and that

85　studying their combined effect represents a more realistic scenario with higher ecological

86　relevance (Jansen et al., 2011). Accordingly, there are increasingly more studies on how concurrent

87　stressors affect single species (Jansen et al., 2011; Coors and De Meester, 2009). Still, how

88　combined stressors may affect entire aquatic communities of tropical temporary ponds remains

89　poorly understood. Such information is important because these systems host unique biodiversity

90　including specialized key groups (e.g. killifish), and deliver important ecosystem services

91　(including clean water for humans, cattle, and wildlife) that depend on the proper functioning of

92　these ecosystems (Brendonck et al., 2022).

93　　　As a step towards better understanding the impact of multiple stressors under semi-realistic

94　conditions, we conducted an outdoor mesocosm experiment to study how fish predation combined

95　with exposure to an environmentally relevant concentration of the insecticide cypermethrin affects

96　the structure of invertebrate communities of tropical temporary ponds. Experimental invertebrate

97　communities and killifish were collected from the Lake Manyara Basin in northern Tanzania,

98 where many temporary ponds are located in agricultural area where cypermethrin is commonly

99 used. We furthermore assessed the effects on leaf litter decomposition and invertebrate grazing

100 efficiency as measures of ecosystem functioning. With the expectation that both these stressors,

101 alone and combined, affect invertebrate communities and ecological functioning of tropical

102 temporary ponds, we aim to ultimately increase our understanding of these systems as a necessary

103 step towards their sustainable management.

104 **Materials and methods**

105 **Preparation of exposure media**

106 Cypermethrin (Sigma - C2237, 98.0% purity, CAS: 52315-07-8) was purchased from Sigma-

107 Aldrich (St. Louis, MO, USA). A stock solution of 100 mg/L was prepared by dissolving 100 mg

108 of cypermethrin in a litre of milli-Q grade water, stirred for 24 hours at 2000 rpm at 26 °C. The

109 homogenous solution was stored at -20 °C until use. Experimental medium for the mesocosms was

110 prepared by adding standardized salt (Instant Ocean Sea Salt, Instant Ocean-Aquarium Systems,

111 Fiji) to dechlorinated tap water to a conductivity of 450 µS/cm as described by Kafula et al.(2022).

112 **Experimental design**

113 The experiment was conducted, with some modifications, following Relyea and Hoverman (2008)

114 at the Tropical Pesticide Research Institute station in Manyara, Tanzania, and ran for 23 days

115 during the short rainy season of 2020. Four different experimental conditions were used: a control

116 (i.e., no pesticide exposure and no fish predation), a condition with fish predation, a condition with

117 pesticide exposure (cypermethrin) and a condition with both fish predation and pesticide exposure

118 (Figure 1). Three free-swimming adult killifish (*Nothobranchius neumanni*, approximately 4.5 cm

119 total length) were used per mesocosm, collected from local temporary ponds as described by

120 Reichard and colleagues (2009). Each of the four experimental conditions was replicated six times,

121   making a total of 24 mesocosms of 150 L each (90 cm diameter, 30 cm height, in high-density

122   polyethylene) (Figure 1). Mesocosms were either red, green, or blue (randomly distributed among

123   the experimental conditions), which did not affect any of the measured endpoints (water quality

124   parameters, invertebrate community and ecosystem functioning, see Table S1).

125   At the onset of the experiment, mesocosms were filled with 150 L experimental medium

126   and 20 g of rabbit chow was added as a nutrient source. Each mesocosm was inoculated with 1 L

127   of a homogenized mixture of water collected from 10 temporary ponds in the Lake Manyara Basin

128   to serve as an initial source of representative plankton. These ponds were located in settlement

129   areas where they may have already been exposed to cypermethrin. This approach allows us to

130   realistically estimate the potential effects of cypermethrin on these local communities. The

131   integrated plankton sample was obtained by filtering 100 L of water through a 64-µm mesh

132   collected from different locations in each of the 10 ponds: open water, locations with submerged

133   vegetation, and with floating vegetation. In addition, throughout the experiment, mesocosms were

134   open which allowed colonizing organisms (e.g., aquatic insects) to naturally settle so that

135   colonization preference could be assessed.

136   After two days, pesticide mesocosms were dosed with 0.8 ng/L of cypermethrin. This

137   concentration is not acutely lethal to invertebrates but has nevertheless been shown to have

138   deleterious chronic effects on ecologically relevant traits of invertebrates such as reproduction,

139   growth, and population recovery (Zhou et al., 2019; Rasmussen et al., 2013; Kim et al., 2008). As

140   a reference, a 2-week cypermethrin exposure impairs adult growth and offspring production of

141   freshwater zooplankton at concentrations as low as 20 ng/L (Gottardi et al., 2017), and 7 ng/L of

142   cypermethrin impairs feeding in calanoid copepods (*Acartia tonsa*) even when exposed for less

143   than five days (Barata et al., 2002). Further, the selected test concentration was well below the

144 peak concentration range in Malawian surface waters (8.12 – 15.46 mg/L) (Kanyika-Mbewe et al.,

145 2020). A next cypermethrin pulse was applied to the relevant mesocosms every six days, as in

146 Medina and coworkers (2004). Each time, pesticide aliquots were first dissolved into 1-L glass

147 jars with experimental medium and then added to the mesocosm. In doing so, the pesticide-

148 containing medium was distributed evenly over the surface of the mesocosm to simulate surface

149 runoff, aerial drift, or aerial overspray as in Relyea (2018). The achieved concentrations were

150 verified using Gas Chromatography Mass Spectrometry (see Supplementary Material for more

151 details). The achieved pesticide concentration was 0.812 ± 0.02 ng/l after 1 hour; 0.529 ± 0.05 ng/l

152 after two days; 0.137 ± 0.06 ng/l after four days and 0.0275 ± 0.01 ng/l after six days of mesocosm

153 dosing (Supplementary Material, Figure S7).
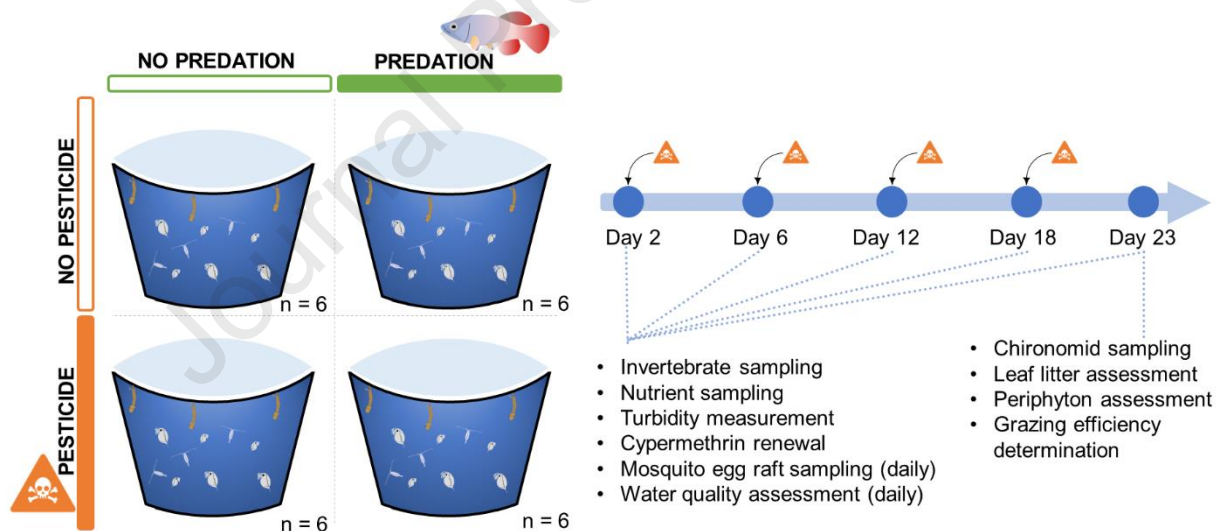
154
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156 **Figure 1. Schematic overview of experimental setup**. Four experimental mesocosm conditions
157 were tested to assess the impact of fish predation and cypermethrin exposure on invertebrate
158 communities of tropical temporary ponds: a control, a condition with three free-swimming
159 killifish, a condition with 0.8 ng/L cypermethrin applied every six days, and a condition with both
160 killifish and cypermethrin exposure. Each condition was replicated six times, and the experiment
161 ran for a total of 23 days.

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163 *Determination of physicochemical parameters and chlorophyll a*

164 Physicochemical parameters (dissolved oxygen, pH, salinity, electrical conductivity, and water

165 temperature) were measure *in situ* every day at 08:00am using a portable HANNA multi-parameter

166 probe (Model-HI 9829). Turbidity was measured once every week using a handheld turbidimeter

167 (HANNA-Model-HI93703). After every six days, a 1-L water sample was taken from each

168 mesocosm for the assessment of nutrient levels. Nutrient concentrations (nitrate and

169 orthophosphate) were spectrophotometrically assessed with a Hach DR2800 (Hach Company,

170 Loveland, CO). Orthophosphate concentrations were measured using the ascorbic acid (PhosVer

171 3) method (low range: 0.02 to 2.50 mg/L PO_4), while nitrate concentrations were assessed using a

172 cadmium reduction method (low range: 0.01 to 30.0 mg/L NO_3–N). All chemical analyses

173 followed the standard methods for the examination of water and wastewater by APHA (1999).

174 Phytoplankton biomass was indirectly determined as Chlorophyll a concentration using a handheld

175 AquaFluor Fluorometer (Turner Designs, USA, Model-8000-010).

176 *Invertebrate sampling, identification, and quantification*

177 Invertebrate samples were taken on day 2, 6, 12, 18, and 23. At each occasion, 10 L of water from

178 each mesocosm (sampled from different sections of the mesocosm) was filtered through a 64-µm

179 net. The filtrate was then concentrated to 100 ml and preserved in 70% ethanol. We found a total

180 of 7 taxa, identified to different taxonomic levels using standard identification guides (Dang et al.,

181 2015; Conway et al., 2003). Counting of invertebrate subsamples in the laboratory was done as

182 described by Goswami (2004). Subsamples of 30 ml were examined for counting the taxa under a

183 stereo microscope (Motic K-400L). Mosquito egg rafts were collected once every day at 08:00am,

184 visually counted, and returned to the respective mesocosms. Chironomids, however, occurred in

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185   all mesocosms and because of their bottom dwelling and tube-living behavior (Hershey, 1987),

186   they were counted at the end of the experiment when water was discarded and all medium filtered.

187   *Ecosystem functioning: leaf litter decomposition and grazing efficiency*

188   Tree leaf litter decomposition was assessed as described by Mitchell and coworkers (2014).

189   Briefly, 5 g of dried leaf litter was placed in 1-mm mesh 10 x 10 cm nylon bags. Each bag also

190   had eight 5-mm holes to allow entry of microfauna. Two litterbags were placed at the bottom in

191   each mesocosm throughout the experiment. At the end of the experiment, litterbags were collected

192   and frozen (at -21 °C) until analysis. For this, leftover litter was removed from each bag, oven-

193   dried overnight at 55 °C (BINDER D78532 Tuttlingen, Germany) and then ashed at 360 °C and

194   weighed to obtain the ash-free dry mass. The percentage mass lost was then calculated for each

195   bag, representing total decomposition (physical breakdown and mineralization). Grazing

196   efficiency was analyzed at the end of the experiment by sampling 1 L of water with invertebrates

197   and 1 L of water without invertebrates (filtered through a 64-µm net) from each mesocosm, as

198   described by Ká and colleagues (2012). The initial chlorophyll a concentration was measured in

199   both samples using a fluorometer (Turner Designs, USA, Model-8000-010). Then, both samples

200   were left in sealed bottles for 24 hours inside the mesocosms after which chlorophyll concentration

201   of both samples was measured again.

202   *Periphyton production*

203   To assess periphyton production, a small tile of 100 cm^2 was added in each mesocosm as described

204   by Stelzer and Lamberti (2001). At the end of the experiment, periphyton was collected from the

205   tile using a scraper (sharp knife) and was added in a small vial with 5 ml of distilled water. Then,

206   the mixture was stirred and chlorophyll *a* was measured using a fluorometer (Turner Designs,

207   USA, Model-8000-010).

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).

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.

Biotic response variables and water quality

We used linear mixed-effects models (lme4 package; Bates et al., 2017) 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:

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231   pH, electrical conductivity, nitrate content, orthophosphate content, dissolved oxygen level, water

232   turbidity, and water temperature. For these parameters, we assumed a Gaussian error distribution.

233   While all above parameters were assessed repeatedly throughout the experiment, number of

234   chironomids and periphyton production were assessed only once, at the end of the experiment.

235   Therefore, these were analyzed using linear mixed-effects models with predation treatment,

236   pesticide treatment, and their interaction as fixed factors, and with mesocosm color as a random

237   effect. We assumed a Poisson and Gaussian error distribution for number of chironomids and

238   periphyton production, respectively.

239   Type 3 Wald chi-square tests were used to test the significance of the fixed factors. Post hoc

240   differences between experimental groups were assessed by means of Tukey-corrected pairwise

241   comparisons (lsmeans package; Lenth, 2016). To confirm that the color of the mesocosm tanks

242   did not affect the outcomes of our experiment, likelihood-ratio tests were used to compare each

243   model with and without the mesocosm color random effect (Supplementary Material, Table S1).

244   *Biotic response variables and water quality per sampling campaign*

245   For further scrutiny, all parameters that were measured repeatedly throughout the experiment were

246   also analyzed for each of the five sampling campaigns separately (Supplementary Material, Table

247   S2–6). For this, we used linear models with predation treatment, pesticide treatment, and their

248   interaction as predictor variables.

249   *Community structure*

250   To assess how killifish presence and pesticide exposure ultimately affect the structure of the

251   invertebrate community, we ran a permutational PERMANOVA (Adonis function, with Bray

252   Curtis dissimilarity method) using data on the density of invertebrates per taxon during the last

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253 sampling campaign (i.e., at the end of the experiment). Predation treatment, pesticide treatment,

254 and their interaction were included as predictors.

255 *Measures of ecosystem functioning*

256 Leaf litter decomposition and invertebrate grazing efficiency were only measured at the end of the

257 experiment. Both were analyzed using a general linear model (Gaussian error distribution) with

258 predation treatment, pesticide treatment, and their interaction as predictor variables.

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272 **Results**

273 **Physicochemical parameters and invertebrate taxa**

274 Mesocosms with killifish were the least turbid and had a significantly lower phytoplankton

275 biomass compared mesocosms without killifish. However, this effect was conditional on

276 cypermethrin exposure (Table 1). Specifically, phytoplankton density was only lower in presence

277 of killifish when the mesocosm was not simultaneously exposed to cypermethrin (Table 1; Figure

278 2). Water temperature, dissolved oxygen, pH, conductivity, and nitrate and orthophosphate content

279 did not differ among experimental conditions (Table 1; Figure 2).

280   A total of seven invertebrate taxa were recorded throughout the experiment: Calanoida,

281 Chironomidae, *Culex* spp., Ostracoda, Psychodidae, Rotifera, and *Simocephalus* spp. Generally,

282 the overall abundance and richness of invertebrate taxa differed significantly among experimental

283 conditions driven by the presence *vs*. absence of killifish rather than by exposure to cypermethrin

284 (Table 1). Specifically, overall invertebrate abundance was higher in the presence of killifish,

285 regardless of cypermethrin exposure, which was mainly due to dominance of rotifers (Figure 3).

286 In contrast, richness of invertebrate taxa was lower in the presence of killifish (Table 1; Figure 3).

287 Likewise, the abundance of calanoids, ostracods, psychodids, and cladocerans (*Simocephalus* spp.)

288 was lower in the presence of killifish but was not affected by exposure to cypermethrin (Table 1;

289 Figure 3). Similar observations were made for mosquito oviposition and, subsequently, abundance

290 of *Culex* sp. (Table 1; Figure 3). Except for rotifers, abundance of all reported invertebrate taxa in

291 killifish and pesticide + killifish conditions rarely exceeded two individuals per liter. In contrast,

292 rotifers were almost entirely absent in conditions with large-sized invertebrate taxa such as

293 calanoids and cladocerans.

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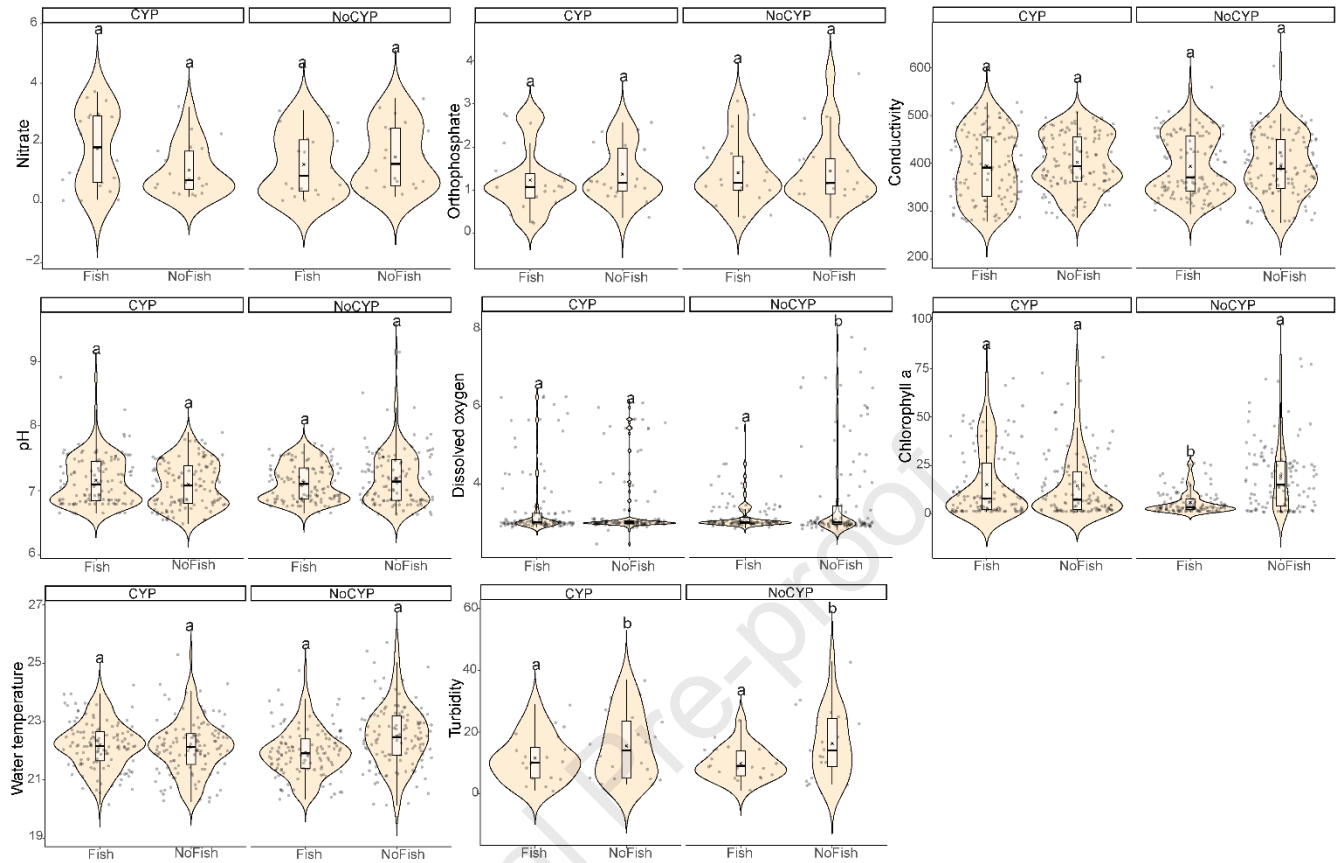
Figure 2: Water quality variables in four experimental conditions (control, predation, pesticide, and predation + pesticide) in outdoor mesocosms. Boxplots show the 25th, 50th (median), and 75th quartiles. The mean is indicated by 'x'. Different letters indicate significant differences. (CYP = cypermethrin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

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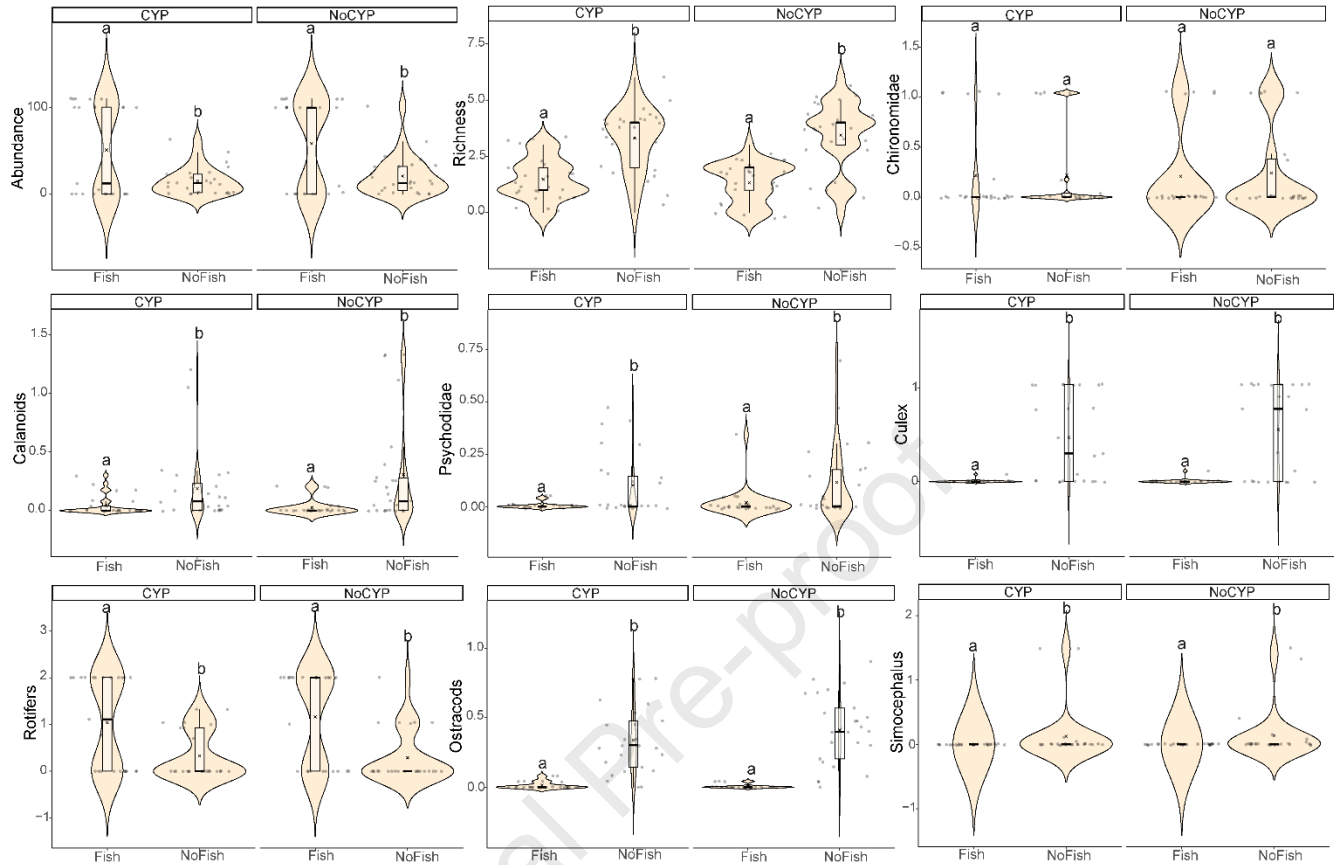
Figure 3: Invertebrate density per taxon in four experimental conditions (control, predation, pesticide, and predation + pesticide) in outdoor mesocosms. Boxplots show the 25th, 50th (median), and 75th quartiles. The mean is indicated by 'x'. Different letters indicate significant differences. (CYP =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

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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.

| | Predictors | | | | | |
| | Predation | | Pesticide | | Predation* Pesticide | |
Responses	$\chi 2$	*P*	$\chi 2$	*P*	$\chi 2$	*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
Culex 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
Simocephalus 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

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 onwards, the presence of killifish significantly affected invertebrate density and number of taxa

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326 (Supplementary Material, Table S3 –6). The PERMANOVA model revealed that killifish presence

327 explains 71.7% ($r^2 = 0.7165$, $p = 0.001$), cypermethrin exposure explains 1.3% ($r^2 = 0.01285$, $p = $

328 0.43), while their interaction explains 1.3% ($r^2 = 0.013$, $p = 0.39$) of the observed variation in

329 invertebrate community structure ($F = 38.9$).

Ecosystem functioning and periphyton production

331 Decomposition rate and periphyton concentration did not differ significantly among experimental

332 conditions. Specifically, neither the presence of killifish ($z = 2.36$, $p = 0.06$), cypermethrin

333 exposure ($z = 1.29$, $p = 0.22$) nor their interaction ($z = -1.58$, $p = 0.13$) affected decomposition

334 rate. Likewise, neither the presence of killifish ($z = 0.09$, $p = 0.92$), cypermethrin exposure ($z = $

335 1.64, $p = 0.12$) nor their interaction ($z = -0.44$, $p = 0.67$) affected periphyton concentration. Neither

336 the presence of killifish ($z = -0.89$, $p = 0.83$), cypermethrin exposure ($z = -0.81$, $p = 0.43$) nor their

337 combination ($z = 1.399$, $p = 0.1810$) affected grazing efficiency.

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347 **Discussion**

348 We assessed how fish predation combined with exposure to the insecticide cypermethrin affects

349 the structure of invertebrate communities of tropical temporary ponds, and its potential effects on

350 leaf litter decomposition and invertebrate grazing efficiency as measures of ecosystem functioning.

351 Overall, when killifish were present, invertebrate taxon diversity decreased while invertebrate

352 density increased. This observation was mostly driven by a sharp increase in the number of rotifers

353 in the presence of killifish. However, this change in community structure did not translate to

354 changes in leaf litter decomposition and invertebrate grazing efficiency. In contrast to the effects

355 of fish predation, exposure to 0.8 ng/L of cypermethrin did not strongly affect community

356 structure, nor did it affect measures of ecosystem functioning. Still, the influence of killifish

357 presence on phytoplankton biomass was contingent upon cypermethrin exposure. Specifically,

358 when communities were simultaneously exposed to cypermethrin, the presence of fish had no

359 discernible impact on phytoplankton biomass.

360 *Impact of killifish predation on invertebrate community structure and ecosystem functioning*

361 As expected, killifish predation strongly affected invertebrate community structure. Specifically,

362 when killifish were present, the overall number of invertebrate taxa was lower compared to

363 killifish-free conditions. This finding aligns with results of previous studies in permanent pond

364 communities where macro- and microinvertebrate community composition, richness, and densities

365 were significantly shaped by the presence of bluegill (*Lepomis macrochirus*) (Gilinsky, 2010;

366 Shurin, 2001). However, despite the decrease in invertebrate richness, the overall invertebrate

367 density increased in the presence of killifish. This increase in invertebrate density seems mostly

368 driven by a sharp increase in the number of rotifers, which exceeded a density of 100 individuals

369 per liter by the end of the experiment. Similar observations, where a reduction in the density and

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370 diversity of larger invertebrates was associated with a concomitant increase in rotifer density, have

371 been made previously (Medina et al., 2004). In contrast to larger zooplankton, the small rotifers

372 were probably not detected or preyed upon by the killifish and could therefore proliferate freely.

373 Size-selective predation and its impact on invertebrate community structure has already been

374 documented for various fish species (Hessen, 1985; Greene, 1983; Hall et al., 1976; Dodson,

375 1974), including *Nothobranchius* killifish (Pinceel et al., 2021). The observation that rotifer

376 density remained low in the control condition is likely due to competition with (or predation by)

377 larger zooplankton species (such as *Simocephalus* spp. and copepods).

378   As we expected, colonization by flying insects was limited when killifish were present, and

379 *Culex* sp. mosquito egg rafts were almost entirely absent from fish mesocosms throughout the

380 experiment. Impact of fish predation and their cues on colonization by insects has been shown

381 previously. For example, habitat selection based on the presence of fish predators explained up to

382 43% of the observed variation in colonizing bug community structure (Trekels and

383 Vanschoenwinkel, 2017). Similarly, mosquitos seem to preferentially colonize habitats that are

384 devoid of predators (Trekels and Vanschoenwinkel, 2019). In contrast to *Culex* sp., Chironomidae

385 density was high regardless of the presence or absence of killifish. This is likely because killifish

386 may not be efficient predators of Chironomidae. Instead, *Nothobranchius* fish have a superior

387 mouth (i.e. an upward-directed mouth) that allows to efficiently prey on invertebrates on or near

388 the water surface (Keppeler et al., 2015). However, they are far less efficient to forage on

389 organisms with a benthic or burrowing lifestyle, such as chironomids (Hershey, 1987). Other

390 organisms with a benthic lifestyle, such as clam shrimps, were previously also shown to be

391 relatively unaffected by *Nothobranchius* predation (Pinceel et al., 2021).

392   While the presence of fish had a notable impact on the structure of the invertebrate

393   community within our mesocosms, this influence did not extend to leaf litter decomposition rate

394   and invertebrate grazing efficiency (i.e., despite having lower phytoplankton levels in the presence

395   of killifish which coincided with an increase in rotifer density). Several factors may explain this

396   apparent lack of impact. First, it is possible that the change in species composition of the

397   invertebrate community was not accompanied by a change in functional trait composition strong

398   enough to affect our measures of ecosystem functioning (i.e., functional redundancy). However, it

399   is also important to consider that the groups that were mainly affected by killifish presence may

400   not be key contributors to the functioning of the temporary pond ecosystem. For example, rotifers

401   primarily feed on bacterioplankton and small phytoplankton species and may therefore not

402   efficiently graze on all species of phytoplankton (Gilbert, 2022). Lastly, it is worth considering

403   that the resolution of our species identification and the relatively short duration of our study,

404   spanning only 23 days, may limit the strength of our conclusions concerning the link between the

405   observed shifts in invertebrate community structure and their ultimate effects on ecosystem

406   functioning.

407   *Impact of pesticide exposure on community structure and ecosystem functioning*

408   Contrary to our predictions, cypermethrin exposure did not affect the density nor diversity of

409   invertebrate taxa. This is surprising, given that cypermethrin is known to be highly toxic and

410   impact various invertebrate species, even at low concentrations (Ranatunga et al., 2023). Possibly,

411   our applied concentration (0.8 ng/L) may have been too low to cause any toxic effects within the

412   given timeframe. Furthermore, it may be important to consider that we applied cypermethrin in

413   pulses, which is more ecologically relevant compared to chronic pesticide exposure at a constant

414   concentration but can influence the overall impact of the pesticide, including so-called "latent"

415   effects that may only emerge later on (Schäfer et al., 2023; Liess & Gröning, 2024). For example,

416   cypermethrin rapidly degraded between pulses (Figure S7 in Supplementary Material) which may

417   have dampened the toxic impact of cypermethrin on the invertebrate community. Similar to our

418   observations, 19 days of exposure to 5 µg/L of cypermethrin only had limited effect on cladoceran

419   density and allowed cladocerans to recover (Medina et al., 2004). Moreover, plankton used to

420   inoculate the mesocosms were collected from local ponds in settlement areas which can get

421   contaminated by cypermethrin, among others by spraying for mosquito control. Given that

422   organisms, including zooplankton (Almeida et al., 2021), are sometimes able to adapt to pesticide

423   exposure, it is possible that the sampled biota were already relatively resistant to cypermethrin

424   exposure. Indeed, organisms can sometimes adapt relatively quickly, as was for example the case

425   with aphids (*Aphis gossypii*) that were exposed to cypermethrin and became less sensitive to the

426   compound after as little as one generation of exposure (Ullah et al., 2020). Therefore, follow-up

427   research should take into account the community's history of exposure to pesticides, as well as its

428   evolutionary responses, to get a deeper insight into how concurrent pesticide and predation stress

429   affects the community structure and ecosystem functioning of tropical temporary ponds.

430   *Mixed stressors – interactive effects between predation and pesticide exposure on community*

431   *structure and ecosystem functioning*

432   We hypothesized that joint effects of multiple stressors could shape the invertebrate community

433   structure and, consequently, impact ecosystem functions. Although we did not observe any

434   interactive effects of killifish presence and cypermethrin exposure on invertebrate community

435   structure, we did observe that the negative impact of killifish presence of phytoplankton biomass

436   only emerged when communities were not simultaneously exposed to cypermethrin. Likely, the

437   decrease in phytoplankton biomass in the presence of killifish and while not simultaneously

438   exposed to cypermethrin is due to the high observed density of rotifers under these conditions.

439   Gosselain et al. (1998) made similar observations on the Belgian side of River Meuse of a decline

440   in phytoplankton biomass linked to a peak in rotifer density. At this moment, it is difficult to

441   explain why this observed decrease in phytoplankton biomass in the presence of killifish no longer

442   emerged when communities were simultaneously exposed to cypermethrin, particularly given that

443   cypermethrin exposure had otherwise no discernible impact on the abundance of the various

444   invertebrate taxa. It is conceivable that cypermethrin exposure may have had various sub-lethal

445   impacts, such as inhibitory or stimulatory changes in behavior (e.g. changes in foraging), that were

446   not measured in the current study but may nevertheless have contributed to the observed pattern.

447   For example, feeding efficiency of *Daphnia magna* was already reduced by over 50% after as little

448   as 6h of exposure to 0.1 μg/L cypermethrin (Christensen et al., 2005). Such sub-lethal effects in

449   response to low pesticide exposure, and which may potentially translate to reduced fitness after

450   longer-term exposure (Liess & Gröning, 2024), can nevertheless lead to important shifts at the

451   population and/or community level. Ultimately, pesticide exposure can influence zooplankton

452   communities through a complex interplay of various direct and indirect (sub)lethal effects

453   (Almeida et al., 2023).

454   **Conclusion**

455   Our results show that fish predation can strongly affect invertebrate community structure of

456   tropical temporary ponds, a finding that contributes to our understanding of these habitats. In

457   contrast, exposure to 0.8 ng/L of cypermethrin did not affect invertebrate community structure

458   even though we did observe interactive effects with killifish presence on the abundance of

459   phytoplankton. Because pesticides are expected to increasingly reach tropical temporary ponds, in

460   particular in developing regions where agriculture is rapidly intensifying, future research should

461 study how higher doses of pesticides may affect structure and functioning of temporary pond

462 ecosystems. A proper understanding of these systems, and how different factors affect community

463 structure and functioning is critical to develop efficient and sustainable management practices to

464 protect or restore these vulnerable systems.

465 **Data availability**

466 The data and scripts that support the findings of this study are openly available in figshare at

467 https://doi.org/10.6084/m9.figshare.25186355

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478 **Author Contributions**

479 **Yusuph A. Kafula:** Conceptualization, Methodology, Visualization, Investigation, Formal

480 analysis, Writing – original draft. **Gordian R. Mataba:** Methodology, Formal analysis, Writing.

481 **Grite N. Mwaijengo:** Formal analysis, review and editing. **Linus K. Munishi:** Supervision,

482 review and editing. **Francis Moyo:** Supervision, review and editing. **Bram Vanschoenwinkel:**

483 Supervision, Formal analysis, review and editing. **Luc Brendonck:** Funding acquisition,

484 Conceptualization, Supervision, review and editing. **Eli S. J. Thoré:** Conceptualization,

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485 Supervision, Methodology, Formal analysis, Visualization, Writing – original draft, review and

486 editing.

Conflicts of Interest

488 The authors declare no conflicting interest.

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Supplementary Materials

Table S1. Output of linear mixed-effects models using all invertebrate taxa, physicochemical parameters, and mosquito egg rafts showing the significance of mesocosm color as a random effect.

Responses	$\chi2$	P
Community Structure		
Overall invertebrate density	1.81	1
Species richness	0	1
Rotifera abundance	12.94	1
Culex abundance	18.88	1
Ostracoda abundance	6.499	1
Calanoida abundance	4.69	1
Simocephalus abundance	2.16	1
Psychodidae abundance	2.993	1
Chironomidae abundance	0.005	1
Physicochemical parameters		
Phytoplankton level	0	1
Dissolved oxygen level	3.08	0.07
Electrical conductivity	0.08	0.77
pH	1.28	0.25
Water temperature	0.27	0.6
Turbidity	0	1
Orthophosphate	0	1
Nitrate	0	1
Mosquito egg rafts	0	1

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534 **Table S2. Outputs of the linear models showing the significance of predation, pesticide**
535 **exposure, and their interaction on invertebrate communities in outdoor mesocosms. Results**
536 **are for the first mesocosm sampling campaign, 2 days after initial exposure.**

				Predictors					
	Predation			**Pesticide**			**Predation*Pesticide**		
Responses	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**
Overall species abundance	2.64	1.39	0.16	-1.09	-0.3	0.76	0.41	0.11	0.91
Species richness	0.98	1.45	0.15	-1.099	-0.95	0.34	0.406	0.31	0.76
Rotifera abundance	-0.0001	0	**1**	-0.0001	0	1	0.0001	0	1
Culex abundance	-0.0001	0	**1**	-0.0001	0	1	0.0001	0	1
Ostracoda abundance	2.12	1.09	0.27	-1.09	-0.30	0.76	0.92	0.25	0.81
Calanoida abundance	21.16	1.89	0.99	-0.0001	0	1	0.0001	-0.00	0.99
Simocephalus abundance	19.4	0	**1**	-0.0001	0	1	0.0001	0	1
Psychodidae abundance	-0.0001	0	**1**	-0.0001	0	1	0.0001	0	1
Chironomidae abundance	-0.0001	0	**1**	-0.0001	0	1	0.0001	0	1

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550 **Table S3. Outputs of the linear models showing the significance of predation, pesticide**
551 **exposure, and their interaction on invertebrate communities, turbidity, and nutrients in**
552 **outdoor mesocosms. Results are for the second mesocosm sampling campaign, 6 days after**
553 **initial exposure.**

	Predictors								
	Predation			**Pesticide**			**Predation*Pesticide**		
Responses	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**
Overall species abundance	-1.40	-9.19	**0.0001**	0.63	7.54	**0.0001**	0.44	2.48	**0.01**
Species richness	0.75377	1.76	0.08	0.12	0.24	0.81	0.09	0.16	0.87
Rotifera abundance	-3.07685	-9.51	**0.0001**	0.63	7.56	**0.0001**	1.76	5.17	**0.0001**
Culex abundance	21.25	0.01	0.99	0	0	1	0.002	0	1
Ostracoda abundance	2.657	1.62	0.1	-1.39	-0.39	0.69	1.67	0.47	0.64
Calanoida abundance	0.916	0.35	0.73	0	0	1	1.48	0.42	0.68
Simocephalus abundance	1.949	0	0.99	-2.22	0	1	-1.09	0	1
Psychodidae abundance	3.04	0.94	0.35	0	0	1	-0.48	-0.1	0.92
Chironomidae abundance	-0.0001	0	1	0	0	1	0	0	1
Turbidity	3.20	1.23	0.24	-2.60	-1.00	0.33	0.20	0.06	0.96
Orthophosphate	-0.05	-0.11	0.92	0.43	0.87	0.39	-0.32	-0.45	0.66
Nitrate	-0.42	-0.65	0.52	-1.80	-2.79	**0.01**	2.34	2.57	**0.023**

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Table S4. Outputs of the linear models showing the significance of predation, pesticide exposure, and their interaction on invertebrate communities, turbidity, and nutrients in outdoor mesocosms. Results are for the third mesocosm sampling campaign, 12 days after initial exposure.

Responses	Predation			Predictors Pesticide			Predation*Pesticide		
	Estimate	z value	P value	Estimate	z value	P value	Estimate	z value	P value
Overall species abundance	4.62	3.25	**0.001**	1.57	1.01	0.31	-1.92	-1.22	0.22
Species richness	1.45	2.60	**0.009**	0.41	0.63	0.53	-0.35	-0.48	0.63
Rotifera abundance	-0.0001	0	1	-0.0001	0	1	0.0001	0	1
Culex abundance	5.04	2.25	**0.02**	0.41	0.14	0.89	-0.61	-0.21	0.83
Ostracoda abundance	4.77	1.50	0.13	-0.0001	0	1	0.0001	-0.15	0.88
Calanoida abundance	19.57	0.003	0.99	18.18	0.003	0.99	-18.96	-0.03	0.99
Simocephalus abundance	-0.0001	0	1	-0.0001	0	1	0.0001	0	1
Psychodidae abundance	3.31	1.46	0.15	1.95	0.81	0.42	-2.46	-0.99	0.32
Chironomidae abundance	-0.0001	0	1	-0.0001	0	1	0.0001	0	1
Turbidity	1.00	1.05	0.31	1.20	1.27	0.22	-1.60	-1.19	0.25
Orthophosphate	-0.09	-0.6	0.55	-0.06	-0.40	0.69	0.18	0.83	0.42
Nitrate	-1.04	-1.59	0.13	0.14	0.21	0.83	0.36	0.39	0.7

578 **Table S5. Outputs of the linear models showing the significance of predation, pesticide**
579 **exposure, and their interaction on invertebrate communities, turbidity, and nutrients in**
580 **outdoor mesocosms. Results are for the fourth mesocosm sampling campaign, 18 days after**
581 **initial exposure.**

| Responses | Predictors | | | | | | | | |
| | **Predation** | | | **Pesticide** | | | **Predation*Pesticide** | | |
	Estimate	**z value**	**P value**	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**
Overall species abundance	-1.67	-14.9	**0.0001**	-0.003	-0.05	0.96	0.19	1.23	0.22
Species richness	0.64	1.64	0.1	-0.51	-0.99	0.32	0.56	0.93	0.36
Rotifera abundance	-2.76	-15	**0.0001**	0	0	1	-0.47	-1.59	0.11
Culex abundance	19.48	0.008	0.99	0	0	1	0.39	0	1
Ostracoda abundance	20.36	0.003	0.99	0	0	1	0.73	0	1
Calanoida abundance	1.8	2.24	0.03	-1.09	-0.74	0.46	2.22	1.45	0.15
Simocephalus abundance	19.1	0.012	0.99	0	0	1	0	0	1
Psychodidae abundance	1.74	0.003	0.99	0	0	1	1.94	0	1
Chironomidae abundance	0.18	0.09	0.92	-17	0	0.99	18.64	0.004	0.99
Turbidity	7.200	1.54	0.14	-0.2	-0.04	0.96	1.8	0.27	0.79
Orthophosphate	0.3600	0.71	0.49	0.14	0.28	0.77	0.04	0.06	0.96
Nitrate	-0.2200	-0.36	0.73	0.4	0.65	0.53	0.36	0.4	0.69

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592 **Table S6. Outputs of the linear models showing the significance of predation, pesticide**
593 **exposure, and their interaction on invertebrate communities, turbidity, and nutrients in**
594 **outdoor mesocosms. Results are for the fifth mesocosm sampling campaign, 23 days after**
595 **initial exposure.**

	Predictors								
	Predation			**Pesticide**			**Predation*Pesticide**		
Responses	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**	**Estimate**	**z value**	**P value**
Overall species abundance	-1.15	-13.2	**0.0001**	-0.001	-0.02	0.99	0.18	1.51	0.13
Species richness	0.61	1.69	0.09	-0.09	-0.21	0.8	0.13	0.26	0.79
Rotifera abundance	-2.39	-15.5	**0.0001**	0	0	1	0	-3.06	**0.002**
Culex abundance	19.53	0.008	0.994	0	0	1	0	0	1
Ostracoda abundance	22.28	0.002	0.998	0	0	1	0	0	1
Calanoida abundance	2.66	2.82	0.0048	-0.69	-0.44	0.66	1.63	1.02	0.31
Simocephalus abundance	19.09	0.012	0.99	0.04	0	1	0.04	0	1
Psychodidae abundance	17.69	0.004	0.99	0	0	1	1.5	0	1
Chironomidae abundance	-0.0001	0	1	0	0	1	0	0	1
Turbidity	4.20	0.73	0.47	-5.40	-0.94	0.36	9.8	1.2	0.24
Orthophosphate	0.34	1.41	0.18	0.17	0.72	0.49	-0.29	-0.86	0.4
Nitrate	-1.2	-1.7	0.11	-0.86	-1.22	0.24	0.82	0.82	0.42

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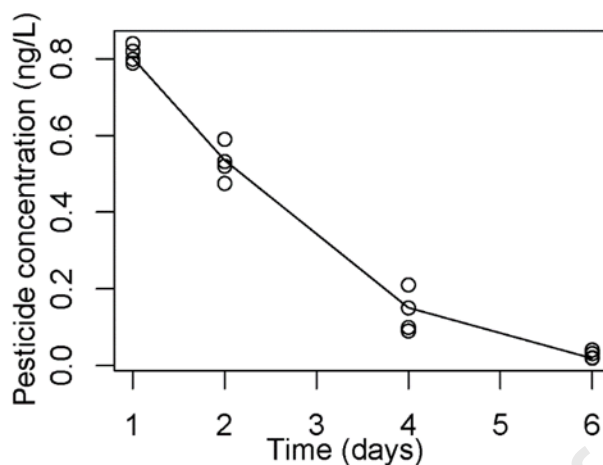
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607   **Figure S7. Achieved cypermethrin concentration after 1 hour, 2 days, 4, days, and 6 days of**
608   **the mesocosm dosing.**
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611   *Sampling and analysis of realized pesticide concentration*

612   Four water samples from randomly chosen mesocosms in each of the pesticide conditions (four

613   samples in pesticide condition and another four in pesticide + fish condition) were collected at the

614   start of the experiment after 1 hour, 2 days, 4 days, and 6 days of application, to determine the

615   actual achieved concentrations. Afterwards, samples were taken after every 6 days (before

616   renewing the pesticide medium) to determine pesticide concentrations. Each time, approximately

617   200 ml was collected in the middle of the water column and all samples for a given treatment were

618   pooled, frozen, and then shipped to the chemistry laboratory at the University of Dar es Salaam

619   for analysis. The achieved concentrations were measured using Gas Chromatography Mass

620   Spectrometry (Model QP 2010, Shimadzu corporation, Japan) as described by Kishimba and

621   coworkers (2004). The achieved pesticide concentration was 0.812 ± 0.02 ng/l after 1 hour; 0.529

622   ± 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

623   of mesocosm dosing (Supplementary Material, Figure S7).

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- It is unclear how combined predation and pesticides affect tropical pond communities
- We exposed invertebrate communities in a controlled mesocosm experiment
- Predation but not pesticide affected invertebrate community structure
- Downstream effects on phytoplankton biomass were mediated by pesticide exposure
- A deeper understanding of stressor interactions is necessary for effective management

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: