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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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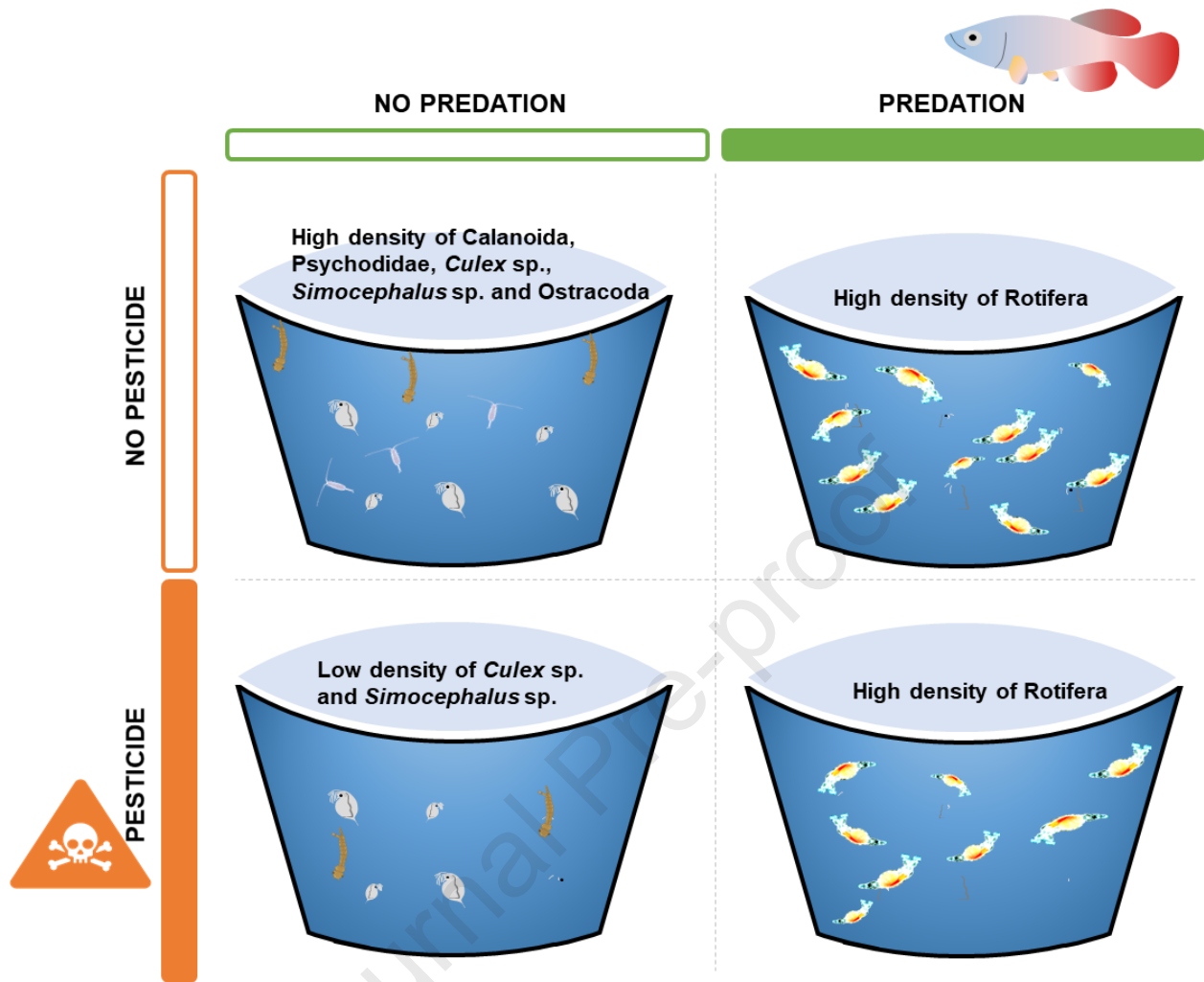
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1 **Fish predation affects invertebrate community structure of tropical temporary ponds, with**
2 **downstream effects on phytoplankton that are obscured by pesticide pollution**

3
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29 Abstract

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

51 **Keywords:** Insecticide, *Nothobranchius*, killifish, ecotoxicology, mixed stressors

52 **Introduction**

53 Animals in the wild are often simultaneously exposed to multiple stressors (Thoré et al., 2021;
54 Beermann et al., 2018; Gunderson et al., 2016). In freshwater systems, such stressors are regularly
55 related to temporal fluctuations in abiotic conditions such as pH, water temperature, dissolved
56 oxygen levels, turbidity or salinity (Chapman et al., 2011; Ross et al., 2001). In addition, animals
57 may also experience stress from predation, amongst other natural stressors. In contrast to
58 permanent freshwater systems, temporary ponds are usually devoid of predatory fish. However,
59 some killifish genera of (sub)tropical regions are adapted to live in temporary ponds ('annual
60 killifish') and may prey heavily on the invertebrate communities (Pinceel et al., 2021). While the
61 ecology of annual killifish is increasingly studied (Pinceel et al., 2021; Thoré et al., 2019; Grégoir
62 et al., 2018; 2017), it is not yet fully understood how killifish predation affects the structure and
63 dynamics of invertebrate communities in tropical temporary ponds. A recent pioneering study
64 offered valuable first insights, showing that *Nothobranchius furzeri* killifish from temporary ponds
65 in Southern Africa do not prey on clam shrimps while they effectively reduce fairy shrimp
66 populations by 90–100% (Pinceel et al., 2021).

67 Besides natural sources of stress, invertebrate communities of tropical temporary ponds
68 may also face stress related to rapid agricultural intensification, including pesticide pollution.
69 Temporary pond ecosystems and associated floodplains in Sub-Saharan Africa, are increasingly
70 contaminated with agricultural pesticides (Brendonck et al., 2022; Lahr, 1998). For example,
71 cypermethrin has been detected at concentrations between 8.12 – 15.46 mg/L in Malawian streams
72 (Kanyika-Mbewe et al., 2020). Cypermethrin is currently the most commonly used pyrethroid
73 insecticide globally (Shi et al., 2011; Kumar & Chandra, 2003) owing to its broad spectrum
74 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 $\mu\text{S}/\text{cm}$ as described by Kafula et al.(2022).

112 **Experimental design**

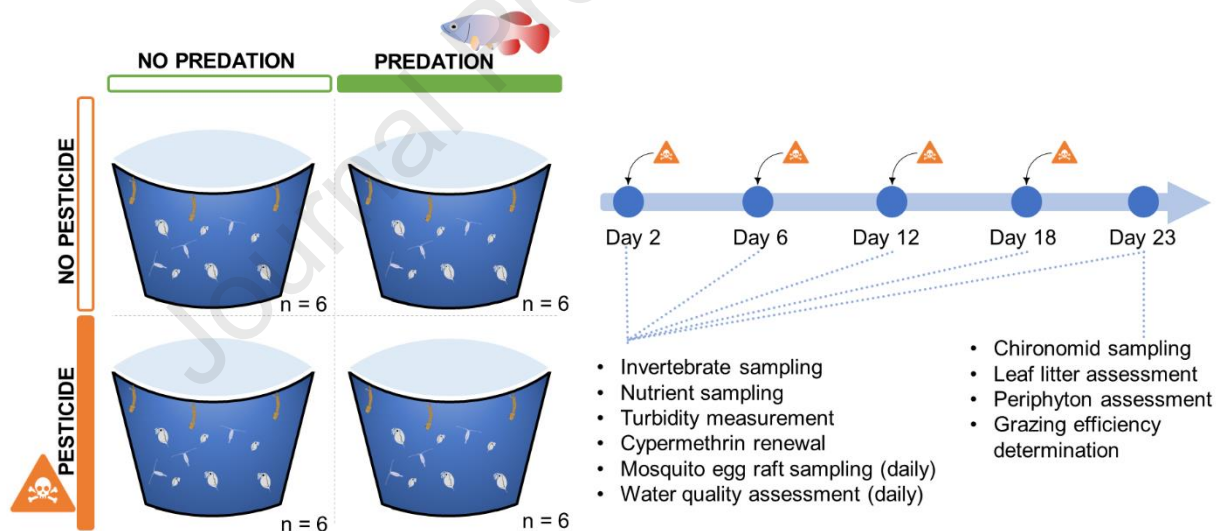
113 The experiment was conducted, with some modifications, following Relyea and Hoverman (2008)
114 at the Tropical Pesticide Research Institute station in Manyara, Tanzania, and ran for 23 days
115 during the short rainy season of 2020. Four different experimental conditions were used: a control
116 (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- μ m 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
 155
 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.

162

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 PO₄), while nitrate concentrations were assessed using a
172 cadmium reduction method (low range: 0.01 to 30.0 mg/L NO₃-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- μ m
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

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 cm² 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).

208 Animal welfare statement

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

216 Data analysis

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

221 Biotic response variables and water quality

222 We used linear mixed-effects models (lme4 package; Bates et al., 2017) to analyze the density of
223 invertebrates per taxon, the total invertebrate density (i.e., all taxa pooled together), number of
224 taxa (i.e., taxon richness), and the number of mosquito egg rafts. For all these models, we assumed
225 a Poisson error distribution as appropriate for count data, with predation treatment (killifish vs. no
226 killifish), pesticide treatment (cypermethrin vs. no cypermethrin), and their interaction, as fixed
227 factors. Mesocosm identity, mesocosm color, and sampling campaign (referring to the repeated
228 measures: day 2, 6, 12, 18, and 23) were added as random effects. For rotifer density and total
229 invertebrate density, we additionally added an observation-level random effect to account for
230 overdispersion. Similar mixed-effects models were used to analyze physicochemical parameters:

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

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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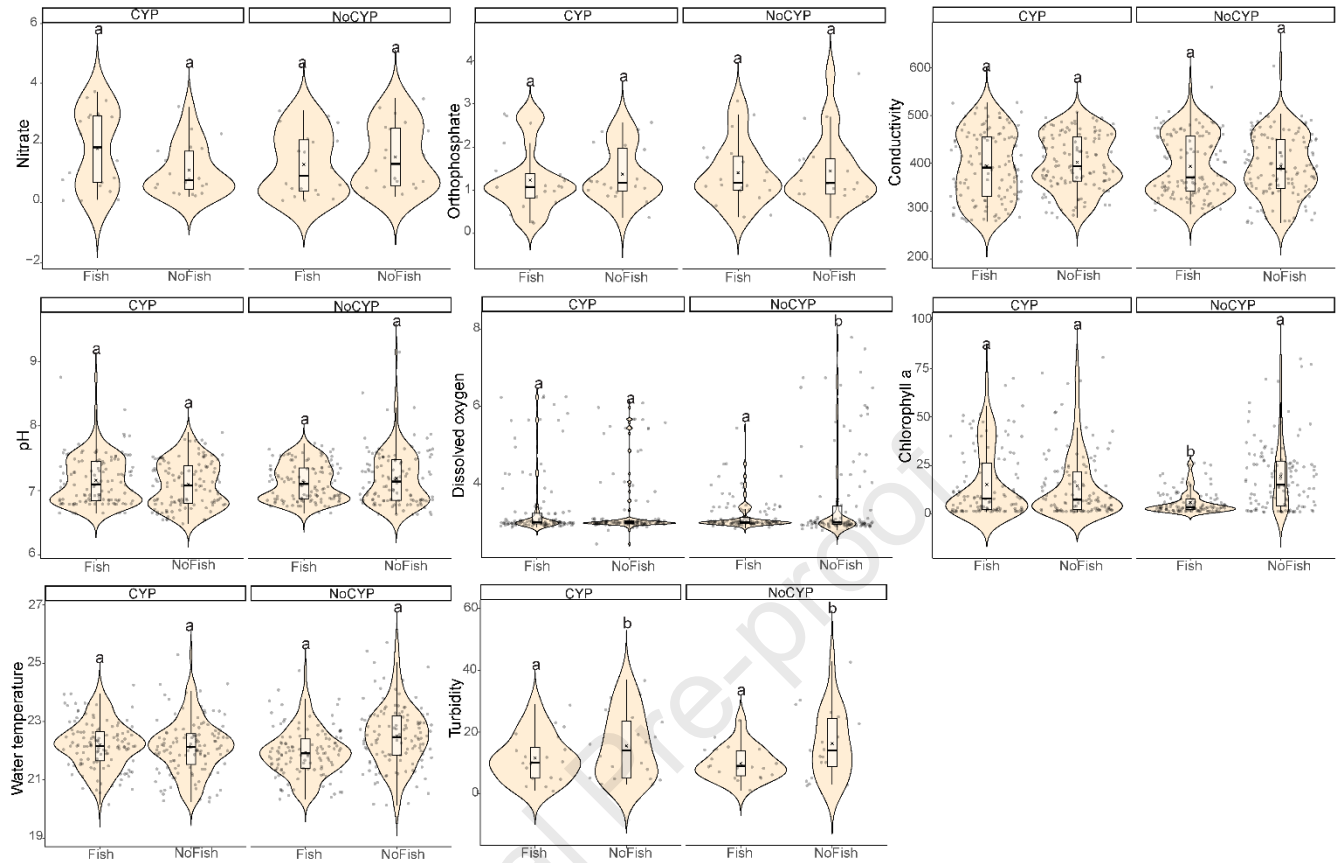
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272 **Results**

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.

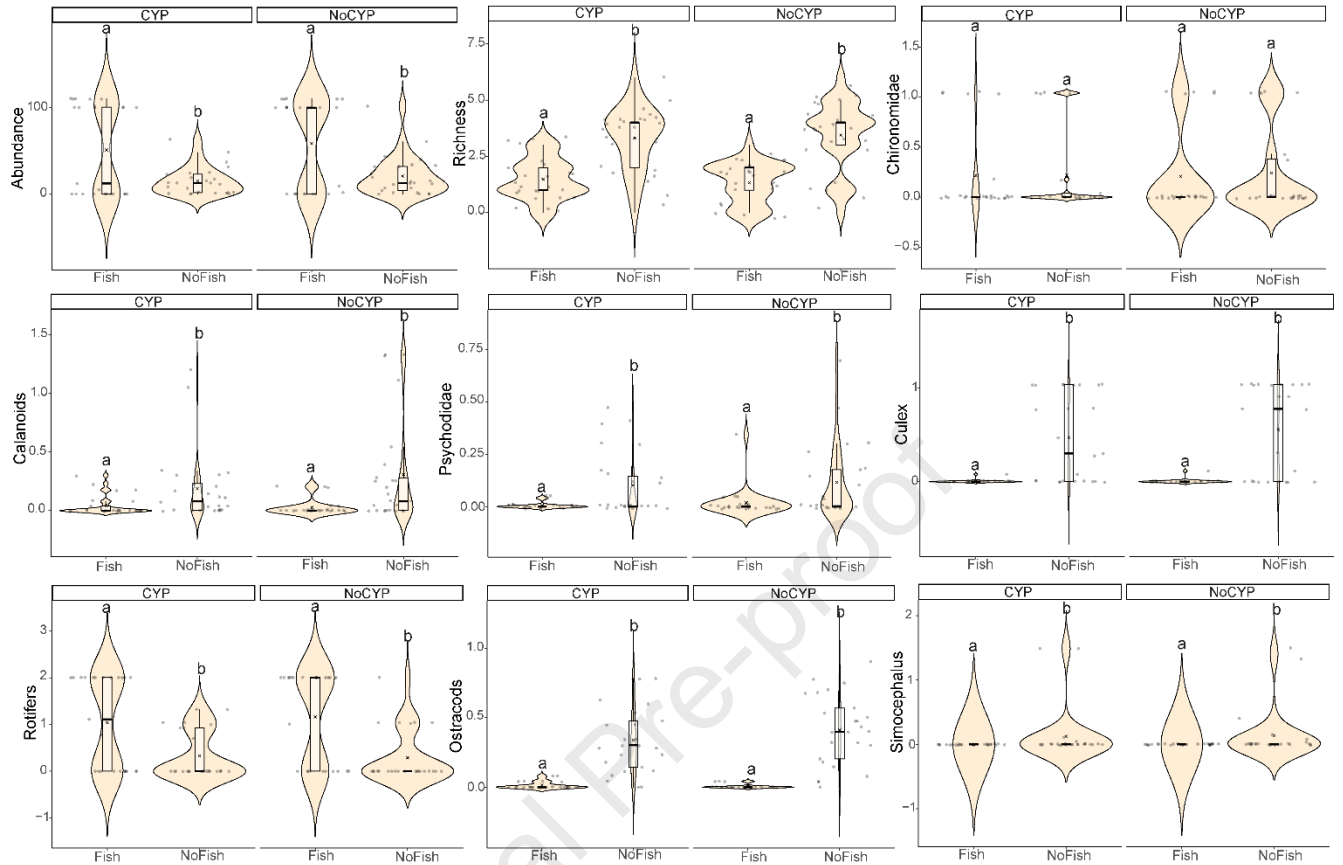


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295 **Figure 2: Water quality variables in four experimental conditions (control, predation,**
 296 **pesticide, and predation + pesticide) in outdoor mesocosms.** Boxplots show the 25th, 50th
 297 (median), and 75th quartiles. The mean is indicated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

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

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317 **Table 1: Output of linear mixed-effects models using all invertebrate taxa, physicochemical**
 318 **parameters, and mosquito egg rafts to show the effect of predation (killifish presence),**
 319 **pesticide exposure (cypermethrin), and their interaction.**
 320

Responses	Predictors					
	Predation		Pesticide		Predation* Pesticide	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
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

321

322 Results per time point (presented in Supplementary Material, Table S2) reveal no
 323 significant effect of killifish presence and cypermethrin exposure on both invertebrate densities
 324 and water quality variables on the second day of exposure. However, from the sixth day onwards,
 325 the presence of killifish significantly affected invertebrate density and number of taxa

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 ($F = 38.9$).

330 **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

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 on 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,

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

487 **Conflicts of Interest**

488 The authors declare no conflicting interest.

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509 **Supplementary Materials**

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

Responses	χ^2	<i>P</i>
Community Structure		
Overall invertebrate density	1.81	1
Species richness	0	1
Rotifera abundance	12.94	1
<i>Culex</i> abundance	18.88	1
Ostracoda abundance	6.499	1
Calanoida abundance	4.69	1
<i>Simocephalus</i> 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.**

Responses	Predation			Predictors Pesticide			Predation*Pesticide		
	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.**

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

Responses	Predation			Predictors			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

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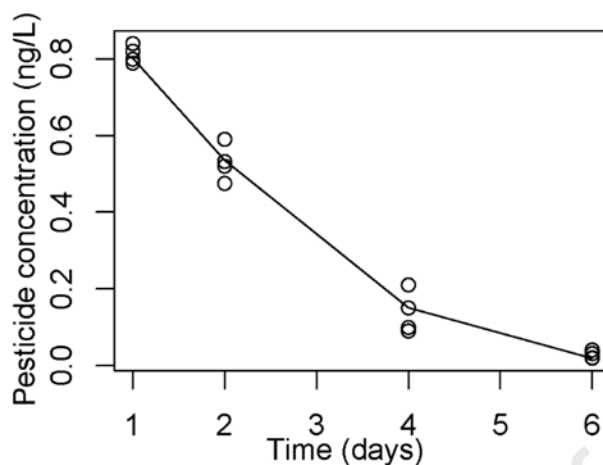
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592 **Table S6. Outputs of the linear models showing the significance of predation, pesticide**
 593 **exposure, and their interaction on invertebrate communities, turbidity, and nutrients in**
 594 **outdoor mesocosms. Results are for the fifth mesocosm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

Responses	Predation			Predictors Pesticide			Predation*Pesticide		
	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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Figure S7. Achieved cypermethrin concentration after 1 hour, 2 days, 4, days, and 6 days of the mesocosm dosing.

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

Journal Pre-proof

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:

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