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Pesticide sensitivity of *Nothobranchius neumanni*, a temporary pond predator with a non-generic life-history

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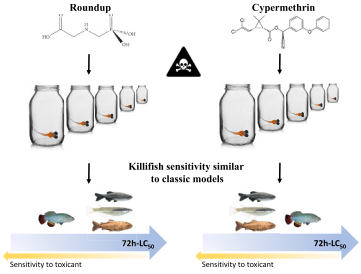
HIGHLIGHTS

- Fish with non-generic life-histories are seldom included in ecotoxicological tests.
- Sensitivity of *N. neumanni* to Roundup was higher than in classic fish models.
- Sensitivity to cypermethrin was lower compared to classic fish models.
- Overall, killifish sensitivity to pollutants is in line with that of other fish species.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticides are crucial to improve agricultural productivity, but often adversely affect surrounding aquatic systems and their fauna. To determine the environmental risk of pesticides, routine ecotoxicological tests are performed on several organisms, including standard fish models. However, these typically do not include fish species from variable habitats and with non-generic life-histories. In particular, inhabitants from temporary ponds such as annual killifish are conventionally understood to be resilient to natural stressors which could translate to higher pesticide resistance or, alternatively, trade-off with their resistance to pesticides and render them more sensitive than classic fish models. Using standard exposure tests, we assessed short-term toxicity effects of two commonly used pesticides, Roundup and cypermethrin, on the annual killifish *Nothobranchius neumanni,* and compared its sensitivity with that of classic fish models. For Roundup, we found a 72 h-LC_{50} of 1.79 ± 0.11 mg/L, which is lower than the values reported for zebrafish, medaka, fathead minnow and rainbow trout, suggesting that *N. neumanni* is more sensitive to the compound. The opposite was true for cypermethrin, with a 72 h-LC_{50} of 0.27 ± 0.03 mg/L. However, these LC_{50}-values do not deviate strongly from those reported

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for other fish species, supporting earlier findings in the congeneric *N. furzeri* that the sensitivity of annual killifish to pollutants is similar to that of classic fish models despite their assumed robustness to environmental stress.

1. Introduction

Agricultural pesticide use is critical to foster crop productivity, but their use often has undesired and far-reaching effects on adjacent freshwater ecosystems (Stoate et al., 2009). Through various mechanisms including runoff and wind drift, a wide variety of pesticides enter the natural environment (Bonmatin et al., 2015). These include, among others, insecticides such as cypermethrin and herbicides such as Roundup.

Cypermethrin is a pyrethroid insecticide that is commonly used in agriculture, forestry and horticulture around the world (Shi et al., 2011). This broad-spectrum pesticide is strongly neurotoxic for insects (Shi et al., 2011) and diffuses easily through the cell membrane of various organisms due to its high lipophilicity (Paravani et al., 2019). Besides targeting pest species, the compound is also highly toxic for non-target organisms. For instance, in fish, cypermethrin interferes with neurotransmission by blocking neuronal sodium channels (Velisek et al., 2006).

On the other hand, glyphosate-based herbicides such as Roundup are widely used in agriculture and for aquatic weed control (Ortiz-Ordoñez et al., 2011). Roundup consists of its main active ingredient (glyphosate) and a nonionic surfactant (polyethoxylene amine, POEA) to facilitate uptake in plants (Hued et al., 2012). It inhibits plant growth by interfering in the production of essential aromatic amino acids (Hued et al., 2012), but is also highly toxic for non-target aquatic animals as it causes oxidative stress through the production of reactive oxygen species (Ortiz-Ordoñez et al., 2011). Previous studies indicate that the toxicity of Roundup in fish may be mediated by glyphosate as well as POEA (Folmar et al., 1979; Ortiz-Ordoñez et al., 2011).

To assess the environmental risks of pesticides, routine ecotoxicological tests are used to determine the lethal effects of short-term (acute) exposure to a compound (Chapman et al., 1998). Typically, such ecotoxicological tests are performed on standard model organisms across trophic levels (Nienstedt et al., 2012). Fish-based tests are a fundamental component and are performed on standard model species including zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and rainbow trout (*Oncorhynchus mykiss*) (Thoré et al., 2021a). Mechanisms of toxicity are often evolutionary conserved in fish (Griffith, 2017), which generally allows to extrapolate results of toxicity across fish taxa.

Fish species with non-generic life-histories (i.e. physiological attributes and life-history strategies that are rare or absent in standard fish models) from variable habitats like temporary wetlands are typically not included in standard ecotoxicological screening (Thoré et al., 2021a). Temporary wetlands are characterized by periodic drought and flooding. Resident organisms such as annual fish species are adapted to this seasonality by completing their life cycle in a very short time window and by producing drought-resistant eggs that bridge the dry period in the sediment (Nagy and Watters, 2019). *Nothobranchius* killifish, for instance, inhabit temporary freshwater ponds across central, eastern and southern Africa (Nagy and Watters, 2019). These fish typically mature in 3–4 weeks and have a short lifespan of about 6 months (Thoré et al., 2019a,b; Philippe et al., 2017). Because of their unique life-history, *Nothobranchius* killifish have recently become popular as study species in several fields of study, including gerontology (Reichwald et al., 2015), genomics (Cellerino et al., 2016), ecology (Grégoir et al., 2017, 2018), evolutionary biology (Blažek Radim and Reichard, 2013), behavioral biology (Thoré et al., 2018, 2019a) and ecotoxicology (Philippe et al., 2019). Their increasing popularity mainly derives from a fast generation time and short lifespan making full life-cycle and multigenerational tests possible in a relatively short time (Thoré et al., 2020a), 2021a). They also produce drought-resistant eggs that can be stored on the shelf and synchronously hatched on demand (Thoré et al., 2020b; Thoré et al., 2021a).

Nothobranchius killifish play a key role in the food web of temporary wetlands (Nagy and Watters, 2019; de Necker et al., 2020) through strong predation on the invertebrate community (Pinceel et al., 2021). Because these wetlands are home to unique biota and contribute significantly to biodiversity conservation (Waterkeyn et al., 2008), understanding how key species in these systems are affected by pesticide pollution is crucial to define tailored management practices – especially in developing regions where increasing intensification of agriculture threatens the integrity of wetlands.

Annual killifish are adapted to seasonal drying of their habitat and are conventionally understood to be resistant to strong daily fluctuations in environmental conditions (e.g., water temperature, dissolved oxygen) that are inherent to their habitat. Specifically, *Nothobranchius* killifish invest in fast development (Cellerino et al., 2016), high reproductive output (Polačik et al., 2016) and rapid acclimation to stress through general homeostatic mechanisms (e.g., cortisol production along the hypothalamic-pituitary-interrenal axis) (Henderson and Small, 2019). These mechanisms could also contribute to a higher resistance to pesticides or, alternatively, their robustness to natural stress may trade-off with their pesticide resistance (Lahr, 1997) and ultimately render them more sensitive than classic fish models. This is because limited energy budgets typically imply trade-offs that constrain energetic investment in other traits (Podrabsky et al., 2015; Thoré et al., 2019b) such as specific detoxification mechanisms which are needed to cope with the specific mode of action of pesticides (Ferreira et al., 2015). However, recent research with *N. furzeri* offered limited to no support for these hypotheses, showing that its sensitivity to acute pollutant exposure is comparable to that of classic fish models (Philippe et al., 2017; Philippe et al., 2019; Thoré et al., 2021b). Here, we aim to further explore this by testing the toxicity of different compounds in the congeneric *N. neumanni*, a dominant killifish species from temporary wetlands in northern and central Tanzania (Nagy and Watters, 2019). Specifically, we assessed how acute exposure to two common pesticides – cypermethrin and Roundup – affects the survival of juvenile fish and compared its sensitivity (LC_{50}) to that of classic fish models (including zebrafish, fathead minnow, medaka and rainbow trout). We chose cypermethrin and Roundup as both pesticides account for over 50% of used pesticides in the region (Manyilizu et al., 2017).

2. Material and methods

2.1. Preparation of exposure media

Cypermethrin (Sigma - C2237, 98.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of 100 mg/L was prepared with Milli-Q grade water and stored at −20 °C. Roundup was purchased from a local pesticide shop in Arusha, Tanzania available as Roundup 360 SL (Monsanto, Bayer Agriculture BVBA, Belgium), which has pure N-phosphonomethylglycine – glyphosate (74.70%) as active ingredient and POEA (25.30%) as surfactant. A stock solution of 1 g/L Roundup was prepared and stored at −20 °C. Experimental medium was produced by adding pesticide stock solution to reconstituted water (see below for concentrations). Reconstituted water was prepared by adding standardized salt (Instant Ocean Sea Salt, Instant Ocean-Aquarium Systems, Fiji) to distilled water to a conductivity of 490 μS/cm.

2.2. Collection and maintenance of experimental fish

2.2.1. Paternal generation

Wild *N. neumanni* adult fish were initially collected from temporary ponds (pooled sample from a total of 4 ponds) in the Lake Manyara basin (Northern Tanzania) in April 2020, under permit number TWRI/RS-331/VOL.IV/2013/39 issued collaboratively by the Tanzania Commission for Science and Technology and the Wildlife Research Institute. These fish were transferred and kept in the laboratory at optimal breeding conditions: fish were kept in 100-L aerated static reconstituted water tanks in social groups of 40 individuals per tank, at a water temperature of 27 ± 1 °C, 490 μS/cm conductivity (as observed in their natural habitat) and a 14:10 h light:dark regime. Fish were fed twice daily (morning and evening) to satiation with live brine shrimp nauplii and frozen *Chironomus* larvae (Polačik et al., 2016). Adult fish (n = 80) were allowed to spawn in 8-L tanks provided with fine sand as spawning substrate. For this, three mature females were coupled with one male for three days (i.e. 20 spawning groups of 4 fish each). Afterwards, sand was sieved (~1 mm mesh size) to collect eggs. After collection, eggs from all spawning groups were pooled and stored on moist peat at 17 °C (Polačik et al., 2016).

2.2.2. Experimental fish

Prior to hatching, about 350 eggs were incubated at 28 °C for three weeks to stimulate embryo development (Polačik et al., 2016). Following the protocol by Philippe et al. (2018a), *N. neumanni* fry were hatched by inundating the eggs with reconstituted water at a temperature of 15 °C. Afterwards, water temperature gradually converged to room temperature (27 °C). Forty-eight hours after hatching (i.e. at an age of two days), 264 healthy and buoyant *N. neumanni* hatchlings were randomly assigned to an experimental treatment and transferred individually to a transparent 0.5-L glass jar for individual monitoring. Starting 24 h post hatching and throughout the experiment, fish were fed to satiation with freshly hatched *Artemia* nauplii (Ocean Nutrition, Essen, Belgium) twice a day. The experimental jars were subjected to a 14 h:10 h light:dark regime. Experimental medium was refreshed every other day. Throughout the experiment, dissolved oxygen levels were kept above 80% by refreshing the experimental medium every 48 h to maintain good water quality. Conductivity ranged between 490 ± 10 μS/cm, and pH was 7.8 ± 0.4. Water temperature in all jars was maintained at 27 ± 1 °C using thermostats in water tubs.

3. Experimental protocol

The acute exposure tests (Fig. 1) with cypermethrin and Roundup were carried out following the protocol by Philippe et al. (2018a). For both products, five nominal concentrations (cypermethrin: 0.05, 0.1, 0.2, 0.4, and 0.8 mg/L; Roundup: 0.3, 0.6, 1.2, 2.4, and 4.8 mg/L) and a control (i.e. reconstituted water as described above with no added pesticide) were used. Achieved concentrations were 0.043; 0.06; 0.15, 0.31 and 0.58 (60–86.5% of the nominal concentrations) for cypermethrin, measured using GC-MS (Model QP 2010, Shimadzu corporation, Japan). For Roundup, achieved concentrations were 0.21, 0.69, 0.92, 2.43, and 2.73 mg/L (57–115% of the nominal concentrations), measured using High performance Liquid Chromatography (Model Wufeng LC 100, China) with a UV detector. Due to lack of literature on acute effects of pesticides on *N. neumanni*, nominal concentrations for all pesticides were selected based on prior range finding test results (Supplementary Material). For each pesticide, treatments were replicated 22 times. Throughout the experiment (total duration of 96 h), mortality was monitored daily at 24, 48, 72 and 96 h after the start of exposure.

We searched the ECOTOX database (US Environmental Protection Agency), Google Scholar, Web of Science and PubMed (keywords included 'cypermethrin', 'Roundup', 'glyphosate', 'fish toxicity', 'LC_{50}') to find comparable acute exposure tests in other fish for interspecies comparison.

3.1. Animal welfare statement

Throughout the experiment, procedures and methods were in accordance with the animal welfare commission requirements of Tanzania. Experimental fish were checked twice daily by the researcher. To reduce stress and discomfort on exposed fish, disturbance and handling were kept to a minimum. At the end of the experiment, fish from the control condition were reassigned as breeding fish, while surviving fish that were exposed to the pesticide were euthanized by means of an overdose of MS-222 (250 mg/L of tricaine).

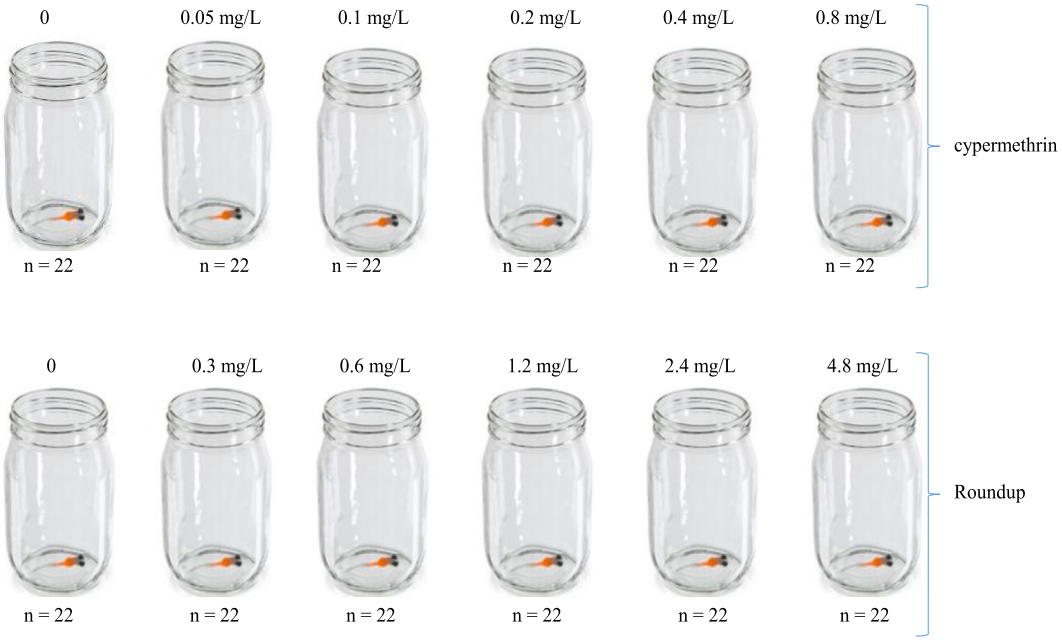

Fig. 1. Experimental design to assess the sensitivity of *Nothobranchius neumanni* to cypermethrin and Roundup exposure. Fish were kept in individual jars, and each treatment was replicated 22 times.

3.2. Data analysis

Data analysis was performed in R v3.2.1 (R Development Core Team, 2016). In all treatment jars, a binary outcome was assigned as either 0 (dead) or 1 (alive). LC_{50} values were calculated from achieved pesticide dose-response curves at 24, 48, 72, and 96 h in which mortality was used as response variable (Ritz et al., 2015), using the 'drm' function in the *drc* package (version 3.5.0). The reported standard error (SE) for LC_{50} values is a measure for the reliability of the LC_{50} values (Ritz et al., 2015). We set the maximum cut-off at SE < 15% for reliable LC_{50} values. This criterion was reached for 72 h-LC_{50} values, which are therefore focused on in the Results section.

4. Results

During the entire exposure duration, survival was 100% in the control condition of both pesticides. The LC_{50} values for cypermethrin and Roundup at different time points during the exposure duration are presented in Table 1, with their respective standard errors. For both pesticides, LC_{50} values decreased with increasing exposure duration. The LC_{50} values ranged from 0.44 mg/L at 24 h to 0.25 mg/L at 96 h for cypermethrin, and from 2.36 mg/L at 48 h to 1.67 mg/L at 96 h for Roundup. The dose – response curves after 72 h of exposure for both pesticides are shown in Fig. 2. Because no mortality was recorded at 24 h of Roundup exposure, this 24 h-LC_{50} value could not be calculated. LC_{50} values for cypermethrin and Roundup for interspecies comparison are given in Table 2.

5. Discussion

We assessed the short-term toxicity of two commonly used pesticides, cypermethrin and Roundup, on the annual killifish *Nothobranchius neumanni* and compared its sensitivity to that of classic fish models. *Nothobranchius neumanni* was more sensitive to Roundup than classic fish models, while the opposite was observed for cypermethrin. However, the observed LC_{50}-values do not deviate strongly from those reported for other fish species, suggesting that the sensitivity of *N. neumanni* to pollutants is in line with that of other species despite their assumed robustness to environmental stress.

Following the hypothesis that high resilience to natural stressors may trade off with pesticide resistance in temporary pond organisms due to limited energy budgets that constrain energetic investment in detoxification mechanisms (Lahr, 1997; Thoré et al., 2021b), we expected *N. neumanni* to be more sensitive to pesticides than classic fish models. In line with this hypothesis, the sensitivity of *N. neumanni* to Roundup (72 h-LC_{50} = 1.79 mg/L) was ~10x higher than that of zebrafish (96 h-LC_{50} = 10.17 mg/L), medaka (96 h-LC_{50} = 8.5 mg/L for glyphosate specifically) and rainbow trout (96 h-LC_{50} = 8.3 mg/L), suggesting that the resistance of *N. neumanni* to the specific mode of action of Roundup is lower than that other fish species. Although the mode of action of Roundup toxicity in non-target species is not fully understood (Mottier et al., 2014), several studies have reported neurotoxic effects that are related to a lowered acetylcholinesterase activity (Menéndez-Helman et al., 2012; Abdelghani et al., 1997; de Brito Rodrigues et al., 2017; Gholami-Seyedkolaei et al., 2013; Kreutz et al., 2008). Moreover, reactive oxygen species are produced that lead to oxidative damage (Van Bruggen et al., 2018). Alternatively and non-mutually exclusive, the high pace-of-life of *N. neumanni* compared to classic fish models may result in a higher uptake of the compound and lead to rapid accumulation in tissues, but this hypothesis remains to be tested. It is worth noting that we compared a 72-h LC_{50} value for *N. neumanni* to a 96-h LC_{50} value for zebrafish, medaka and rainbow trout. The lower LC_{50} of *N. neumanni*, despite shorter exposure time, corroborates our observation that *N. neumanni* may be more sensitive to this compound than classic fish models. Despite these observations, the difference in LC_{50} for Roundup between *N. neumanni* and fathead minnow (*Pimephales promelas*) is small (96 h-LC_{50} = 2.3 mg/L), showing that *N. neumanni* sensitivity to Roundup does not necessarily deviate strongly from that of other fish.

Sensitivity of *N. neumanni* to cypermethrin could be linked to the lipophilic nature of cypermethrin, which binds and gets absorbed easily via fish gills. The compound is metabolized slowly which increases the time in the body (Velisek et al., 2006). In the body, cypermethrin inhibits acetylcholinesterase, resulting in a higher level of acetylcholine in the synaptic cleft (Ullah et al., 2018). Cypermethrin also causes hyperexcitability by interacting with Na^+ channels, and prolongs the depolarization phase in synaptic clefts (Ullah et al., 2018). Moreover, cypermethrin forms toxic metabolites (cyanohydrin, cyanides and aldehydes) that cause oxidative damage through production of reactive oxygen species (Ullah et al., 2018). The sensitivity of *N. neumanni* was ~100x lower than that of traditional fish models such as zebrafish, medaka and rainbow trout, but still in line with values reported for other fish species. Consistent with this finding, earlier studies using the congeneric *N. furzeri* showed that its sensitivity to reference pesticides is comparable to that of commonly studied fish species rather than being higher (Philippe et al., 2018a,b). This suggests that the assumed robustness of annual killifish to natural environmental stress may not significantly mediate killifish sensitivity to pesticides.

Although cypermethrin and Roundup account for over 50% of the used pesticides in East Africa (Manyilizu et al., 2017), very few studies exist on their environmental concentrations in surface waters in the region, where temporary wetlands are a dominant type of aquatic ecosystems. Cypermethrin was detected at concentrations between 0.0007 and 40.7 μg/L in surface waters in South Africa (Ansara-Ross et al., 2012), and at concentrations ranging from 8.115 to 15.460 mg/L in Southern Malawi (Kanyika-Mbewe et al., 2020). To date only one study is available on the presence of glyphosate in surface waters of South Africa and reports the levels of glyphosate to be below detection limit (0.2 μg/L) (Horn et al., 2019).

The reported environmental concentrations for cypermethrin and Roundup to date seem to be below the reported lowest observable effect concentrations (LOEC) for *N. neumanni.* Still, this does not mean that there is no risk for surface waters. In this study, the LOEC was 0.1 mg/L for cypermethrin and 0.6 mg/L for Roundup. Based on short- and long-term toxicity tests with eight different aquatic species from five different taxonomic groups, Mensah et al. (2013) recommended safe concentrations of 0.250 (0.106–0.589) mg/L and 0.002 (0.000–0.021) mg/L for Roundup, respectively. If these standards are maintained, direct mortality effects of Roundup on *N. neumanni* in the field seem unlikely. Although LC_{50}-values are good parameters to estimate the relative toxicity of chemicals, it is important to consider that sub-lethal concentrations of pesticides may also adversely affect organisms, for instance by interfering with life-history traits (e.g. fecundity, growth and maturation time), physiology (e.g. stress levels, energy reserves), and behavior (e.g. anti-predator and foraging behavior, swimming activity, mating). An additional factor that should be considered is that, in the field, *N. neumanni* are likely exposed over longer time periods rather than acutely. Cypermethrin is indeed used all year round in Northern

Table 1
LC_{50} values at 24, 48, 72 and 96 h after the start of exposure to cypermethrin and Roundup, including the corresponding standard errors and p-values.

	Duration (h)	LC_{50} (mg/L)	Standard error	p-value
Cypermethrin	**24**	0.44	0.08	<0.001
	48	0.35	0.24	0.1391
	72	0.27	0.03	<0.001[a]
	96	0.25	0.18	0.1679
Roundup	**24**	/	/	/
	48	2.36	0.32	<0.001
	72	1.79	0.11	<0.001[a]
	96	1.67	0.19	<0.001

[a] Reliable LC_{50} values.

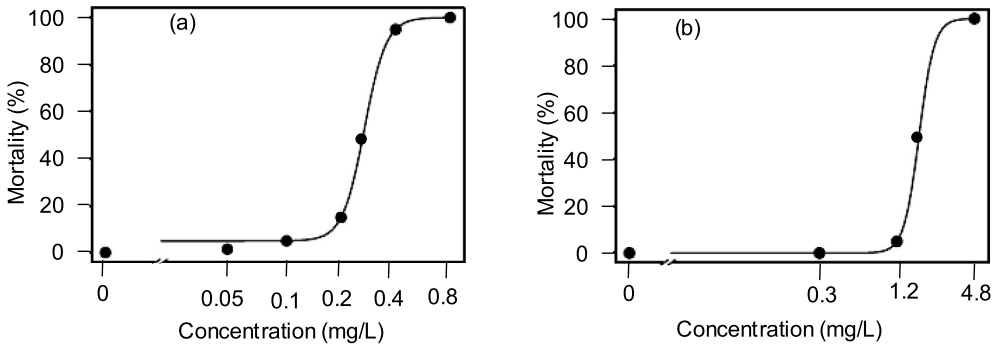

Figure 2. Dose-response curves showing cumulative mortality after 72 h of exposure of *Nothobranchius neumanni* to (a) cypermethrin and (b) Roundup.

Table 2
Acute sensitivity of different fish species to cypermethrin, Roundup and glyphosate.

Compound	Species	Time (h)	LC_{50} (mg/L)	Life stage	T (°C)	Reference
Cypermethrin	***Nothobranchius neumanni***	**96**	**0.25**	**Juvenile**	**27 ± 1**	[a]
	Channa punctatus	96	0.4	Adult	27 ± 1	Kumar et al. (2007)
	Clarias batrachus	96	0.21	Adult	–	Begum (2005)
	Cyprinus carpio	96	0.0026	Juvenile	20	Saha and Kaviraj (2008)
	Danio rerio	96	0.0021	Juvenile	25	Uddin et al. (2018)
	Labeo rohita	96	0.139	Juvenile	26.5	Das and Mukherjee (2003)
	Oncorhynchus mykiss	96	0.0031	Juvenile	15.8	Velisek et al. (2006)
	Oreochromis niloticus	96	0.006	Adult	24 ± 1	Sarikaya (2009)
	Oryzias latipes	48	0.0385	Juvenile	25 ± 1	Kim et al. (2008)
	Oryzias latipes	96	0.1114	Embryo	25 ± 1	Kim et al. (2008)
	Poecilia reticulata	48	0.0214	Adult	22 ± 1	Polat et al. (2002)
	Rhamdia quelen	96	1.17	Juvenile	24 ± 1	Montanha et al. (2012)
Roundup (glyphosate + POEA)	***Nothobranchius neumanni***	**72**	**1.79**	**Juvenile**	**27 ± 1**	[a]
	Cyprinus carpio	96	22.19	Juvenile	20 ± 1	Gholami-Seyedkolaei et al. (2013)
	Danio rerio	96	10.17	Juvenile	26 ± 1	de Brito Rodrigues et al. (2017)
	Ictalurus punctatus	96	13	Juvenile	22	Folmar et al. (1979)
	Jenynsia multidentata	96	19.02	Adult	21 ± 1	Hued et al. (2012)
	Lepomis microchirus	96	13.0	Juvenile	19.5	Abdelghani et al. (1997)
	Oncorhynchus mykiss	96	8.3	Juvenile	12	Folmar et al. (1979)
	Oreochromis niloticus	96	16.8	Juvenile	26.0	Jiraungkoorskul et al. (2003)
	Pimephales promelas	96	2.3	Juvenile	22	Folmar et al. (1979)
	Piaractus mesopotamicus	48	3.74	Adult	26 ± 1	Shiogiri et al. (2012)
	Prochilodus lineatus	96	13.69	Juveniles	22	Langiano and Martinez (2008)
	Pseudoplatystoma sp.	96	15	Juvenile	–	Sinhorin et al. (2014)
	Rhamdia quelen	96	7.3	Juvenile	22 ± 2	Kreutz et al. (2008)
Glyphosate	*Oncorhynchus mykiss*	96	140	Juvenile	22	Folmar et al. (1979)
	Oryzias latipes	96	160	Juvenile	25 ± 1	Uchida et al. (2012)
	Pimephales promelas	96	97	Juvenile	22	Folmar et al. (1979)

[a] This study.

Tanzania with an application frequency that varies depending on targeted crops (Manyilizu et al., 2017). In onion farming, for instance, cypermethrin can be sprayed 8–12 times until harvest (Manyilizu et al., 2017). As the half-life of cypermethrin in water is 28 days (Laskowski, 2002), it is likely that wildlife is chronically exposed to this compound. Similarly, glyphosate has a half-life of 30 days in water and has been detected in surface waters even after 60 days of application (de Brito Rodrigues et al., 2019). Consequently, organisms are likely to experience effects of long-term exposure, even when exposed to concentrations that do not elicit observable effects on the short term. For instance, a recent study showed that the neuroactive chemical fluoxetine did not affect fish somatic growth in the first generation of exposed fish, while an inhibitory effect emerged only after two generations of exposure (Thoré et al., 2021c). Furthermore, it is important to note that organisms may be able to adapt to pesticide exposure resulting in a higher pesticide resistance (Christie et al., 2019), which should be taken into account in follow-up studies.

6. Conclusion

Despite the fact that annual killifish are typically assumed to be robust to environmental stress, our results suggest that the sensitivity of *N. neumanni* to the tested compounds cypermethrin and Roundup is in line with that of other fish species. Future research should assess the sensitivity of other ecologically relevant traits (fecundity, age at maturation, behavior) as not all traits are equally affected by chemical exposure (Thoré et al., 2019b). To better understand the long-term impact of exposure to pesticides, we suggest to also include embryo sensitivity and multigenerational effects of pesticide exposure. Furthermore, with an understanding that in nature organisms are concurrently exposed to a cocktail of stressors (Bonifacio and Hued, 2019; Bonifacio et al., 2020), dose response relationships should ideally be evaluated under scenarios of several co-occurring chemical and natural stressors.

Authorship contribution statement

Yusuph A. Kafula: Conceptualization, Methodology, Formal analysis,

Writing – original draft. Eli Thoré: Conceptualization, Methodology, validation, Writing – review and editing – original draft. Charlotte Philippe: Methodology, validation, Formal analysis, review and editing. Tom Pinceel: Methodology, validation, review and editing. Linus K. Munishi: Supervision, review and editing. Francis Moyo: Supervision, review and editing. Bram Vanschoenwinkel: Supervision, review and editing. Luc Brendonck: Funding acquisition, supervision, review and editing.

Declaration of competing interest

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

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Appendix

24 h range finding results for Roundup

In range finding trials, we used five concentrations and a control. Each treatment was replicated five times. The nominal concentrations used were 0.25, 0.5, 1, 2 and 4 mg/L of Roundup.

Parameter estimates:
Estimate Std. Error t-value p-value.
Slope:(Intercept) −1.66127 1.03092–1.6114 0.24840.
Lower Limit:(Intercept) −2.84468 17.86008–0.1593 0.88808.
Upper Limit:(Intercept) 110.35710 30.85290 3.5769 0.07005.
LC50:(Intercept) 1.58082 0.92267 1.7133 0.22879.
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1.

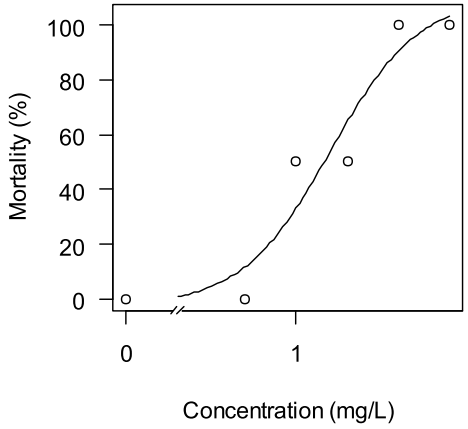

Fig. 2. Dose-response curve showing cumulative mortality after 24 h of exposure of *Nothobranchius neumanni* to Roundup concentration gradients.

24 h range finding results for Cypermethrin

In range finding trials, we used five concentrations and a control. Each treatment was replicated five times. The nominal concentrations used were 0.05, 0.1, 0.2, 0.4 and 0.8 mg/L of cypermethrin.

Parameter estimates:
Estimate Std. Error t-value p-value.
Slope:(Intercept) −1.4480e+01 4.3148e-01 -33.5594 4.66e-08 ***
Lower Limit:(Intercept) −1.8799e-04 2.7152e-04 -0.6924 0.5146.
Upper Limit:(Intercept) 1.0000e+02 3.6719e-04 272341.4378 < 2.2e-16 ***
LC_{50}:(Intercept) 2.2989e-01 5.4593e-03 42.1095 1.20e-08 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1.

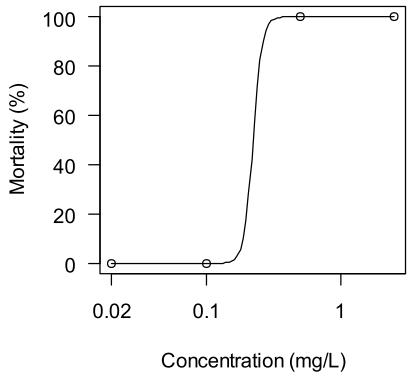

Fig. 3. Dose-response curve showing cumulative mortality after 24 h of exposure of *Nothobranchius neumanni* to Cypermethrin concentration gradients.

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