Assessment of the risk of death of *Clarias gariepinus* and *Oreochromis niloticus* pulse-exposed to selected agricultural pesticides

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Aquatic organisms are often exposed briefly to high pesticide concentration. Survival time model was used to study risk of death in *C. gariepinus* and *O. niloticus* fingerlings exposed to 24 mg/L atrazine, 42 mg/L mancozeb, 1 mg/L chlorpyrifos and 0.75 µg/L lambda cyhalothrin for 15, 30, 45 and 60 minutes and continuously for 96 hours. Mortality, time-to-death, weight, length, and condition factor of the fingerlings were recorded. Results obtained showed tilapia was more susceptible than catfish to continuous exposure but not pulse exposure. The survival probability of both species was similar when exposed for 15, 30 and 45 minutes (*p* > 0.05) but differed after 60 minutes (*p* < 0.05). Risk of death of catfish exposed briefly to atrazine, mancozeb and chlorpyrifos for 60 minutes was similar to 96 hours continuous exposure, same for tilapia exposed to 1 mg/L chlorpyrifos (*p* > 0.05). Survival probability of tilapia exposed to chlorpyrifos for 15, 30, 45 and 60 minutes was similar (*p* > 0.05) and was not influenced by pulse length. Pesticide hazard and risk of death decreased as fish size (weight, length, and condition factor) increased. Pulse toxicity assessment using survival models could make pesticides exposure assessment more realistic by studying factors that can influence the toxicity of pesticides.

Pesticides are major environmental concern due to their hazard to non-target aquatic organism. Among the different classes of pesticides, insecticides are generally the most toxic while fungicides and herbicides are considered second and third respectively. Atrazine, mancozeb, chlorpyrifos and lambda cyhalothrin are commonly used pesticides in Nigeria and globally. Atrazine is a selective and systemic herbicide used as pre and post emergence herbicide. Its half-life in surface water is greater than 100 days at 20 °C. Atrazine concentration in surface water varies from ppb to ppm with 0.48 mg/L reported in the runoff from Ohio River watershed shortly after application. Mancozeb is a fungicide used to control fungal pathogens affecting several crops. Its half-life in water is less than 2 days in which it breaks down to ethylene bis isothiocyanate sulfide (EBIS) and into ethylene bis isothiocyanate (EBI) by the action of UV light. Other metabolites are ethylene thiourea (ETU), and ethylene urea (EU). ETU concentration of 5.9–13.8 µg/L was reported in drainage and runnel water close to banana plantation in southeastern Mexico. Chlorpyrifos (O,O-diethyl O-[3,5,6-trichloro-2-pyridyl] phosphorothioate) is a broad-spectrum, organophosphate (OP) insecticide, acaricide and nematicide. The solubility of chlorpyrifos may range from 0.39 to 1.4 mg/L at temperature range of 19.5 to 25°C. In water, chlorpyrifos adsorb to suspended solids and sediment. Chlorpyrifos residue of 0.67 mg/L was reported in water samples from river Benue, in Adamawa State, Nigeria. Lambda-cyhalothrin (alpha-cyano-3-phenoxybenzyl (Z)-(1RS)—cis 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethyl cyclo propane carboxylate) is a pyrethroid insecticide and its solubility is 5 × 10⁻³ mg/L and 4 × 10⁻³ mg/L in purified and buffered water respectively. Lambda-cyhalothrin residue of 0.11–0.14 µg/L was detected in water samples from agricultural watersheds in Stanislaus County, California.

The pathway of pesticides into aquatic ecosystem include spray drift, run-off, dry and wet deposition as well as effluent discharge. Exposure of aquatic organisms to pesticides often occurs as pulse, repeated pulse or sequential exposure to high or fluctuating concentrations. Pulse exposure may involve one isolated brief exposure which may last from a couple of hours to a few days depending on the properties of the pesticide.
and the receiving water bodies. Aquatic organisms may be exposed during the brief period to high pesticide concentration capable of causing death or harm to early life stages. Standard testing procedures are at variance with environmentally relevant pesticide exposure scenarios in the aquatic environment. Standard toxicity tests usually involve maintaining constant exposure conditions to pesticides which limits their relevance to real situations. It does not evaluate survival chances of non-target organisms briefly exposed to high pesticide concentrations often encountered in real life scenarios. Also routine procedures often ignore the role of time in toxicity. Similarly, pesticide exposure assessment use constant exposure toxicity test set-ups to estimate the hazard of the pesticide even though aquatic organisms may be exposed to high or fluctuating pesticides concentrations in pulses of varying length. Pulse exposure testing differs from standardized testing, as the exposure are not constant and include the observation of post-exposure effects after the pulse exposure.

Despite increasing studies on pulse and repeated pulse exposure of aquatic organisms to pesticides or other toxicants spanning over a decade, little is known on the responses of catfish and Nile tilapia pulse exposed to pesticides and how species difference, pulse length, and size may influence pulse toxicity of pesticides. Most pesticide toxicity studies involving both species were performed using continuous exposure setups. Utilizing pulse exposure during exposure assessment may make the outcome of an ecological risk assessment more realistic. This could improve the ecological risk assessment of pesticides. It could be used to rank the relative susceptibility of aquatic organism to pesticide pulse exposure and enhance the selection of test organisms to provide broad level protection to other non-target organisms within the ecosystem.

Survival analysis is a relevant technique in eco-toxicological studies. Previous authors have shown how time-to-death analysis can be applied in ecotoxicology to study factors that influence the toxicity of toxicants in exposed aquatic organisms. But there are very limited research that have applied this technique to study pulsed toxicity and factors that may influence pulsed toxicity in exposed organisms. By noting time-to-death, post exposure observation period can be studied with more statistical power provided by survival analysis. Thus, this study applied survival analysis to provide insights on the response of fish briefly exposed to selected agricultural pesticide. The prognostic factors thought to be related to survival are species difference, pulse length, fish weight, length, and condition factor. Interest lies in elucidating the influence of these explanatory variables on the survival probability of fish. Commercially available pesticide products containing active ingredients of three pesticides classes were used in this study.

Materials and method

Experimental design. Survival of fingerlings exposed continuously and briefly to selected agricultural pesticides; atrazine (herbicide), mancozeb (fungicide), chlorpyrifos (organophosphate insecticide), and lambda cyhalothrin (pyrethroid insecticide) was investigated by noting time to death during and after exposure respectively. Survival analysis was used to estimate the influence of pulse length, weight, length, and condition factor on the survival of the fingerlings pulsed-exposed to pesticides. This study was carried out in compliance with the ARRIVE guidelines.

Test organisms. Catfish (Clarias gariepinus) and Nile tilapia (Oreochromis niloticus) fingerlings were selected because they are abundant in natural waters and commonly cultivated in fish farms in Nigeria. This facilitates their use in toxicity tests. The fishes were purchased from a fish farm and transported to the laboratory in the morning (7–8 am). They were acclimated to laboratory condition (ambient temperature 27 °C ± 0.5; relative humidity 72 ± 5%; Light: Darkness, 12:12 h) for seven days in plastic aquarium containing borehole water. The water was renewed daily to eliminate excess feed and metabolic byproducts, while the fishes were fed with commercially available fish feed Coppens twice daily ad libitum. They were not fed during the experiment.

Test pesticides. The formulated pesticides used in the study are commonly used by farmers in Nigeria. They include; Atraz 50FW, a herbicide containing 500 g/L atrazine active ingredient, Z-force, a fungicide containing 80% mancozeb active ingredients, Attacke, a pyrethroid insecticide containing 2.5% lambda cyhalothrin active ingredient and Chlorview an organophosphate insecticide containing 40% chlorpyrifos active ingredient.

Pesticide stock solution. Atrazine, chlorpyrifos and lambda cyhalothrin stock solutions were prepared by making up one milliliter (1 ml) of the pesticides to one litre (1 L), while mancozeb stock solution was prepared by mixing one gram (1 g) with one litre (1 L) of distilled water. Stock solutions were subsequently diluted to the appropriate test concentrations used in the study.

Pulse toxicity test. All toxicity tests were performed following established acute toxicity testing procedure, except in the manner of exposure. Ten C. gariepinus and O. niloticus fingerlings were exposed in separate duplicate experiments briefly and continuously to pesticide solutions containing 24 mg/L atrazine, 42 mg/L mancozeb, 1 mg/L chlorpyrifos and 0.75 µg/L lambda cyhalothrin nominal concentrations. These concentrations were selected because they were the maximal concentration used in a previous acute toxicity study of the pesticides using C. gariepinus which caused maximal effect i.e. 100% mortality (0% survival) for all the pesticides except atrazine which was 85% mortality (15% survival) after continuous exposure for 96 hours.

Different sets of fingerlings were exposed briefly for 15, 30, 45 and 60 minutes with slight modification and continuously for 96 hours similar to standard toxicity test. After the brief exposure, the exposed fishes were removed, rinsed with clean water, and transferred to plastic aquaria containing clean water and observed for 96 hours. Water was renewed daily. The 96-hours post-exposure observation period allowed the comparison of pulse exposure toxicity with standard 96-hours toxicity. Mortality and time-to-death were checked and recorded every hour for both the pulsed and continuously exposed fishes. Mortality was determined by lack of movement.
and response to gentle prodding. Weight and length of dead fish were recorded and fingerlings still alive after 96-hours post observation period were right censored while their length and weight were also measured. Fingerlings condition factor (CF) was calculated using the Eq. 32:

\[
CF = \frac{W}{L^3} \times 100
\]

W is fish wet weight (g), L is fish total length (cm).

**Statistical analysis.** Student T-test was used to test the equality of the weight, length and condition factor between Nile tilapia and catfish exposed to the pesticides. Pearson’s correlation was used to measure the strength of association between weight, length and condition factor. Student T-test and Pearson’s correlation was performed using SPSS version 22. Survival probability- survival time response curve was plotted using Statistica version 10. The equality of survival distribution of fish exposed continuously and briefly for 15, 30, 45 and 60 minutes to each pesticides was tested using the log rank test. Cox proportional hazard model was used to model the influence of species, pulse length, weight, fish length, and condition factor on the survival of fishes exposed to pesticides. The SAS procedure LIFETEST and PHREG was used to perform log rank test and fit the Cox PH model respectively. Continuous and pulse exposure were modelled as categorical variables in the LIFETEST procedure, while fish weight, length and condition factor were fitted as continuous variables in the PHEG procedure using SAS version 9.13.

**Ethics approval and consent to participate.** Approval was obtained from the Ethical Committee at the College of Medicine, University of Lagos to use the fish species for pesticide toxicity studies (CMUL/ACUREC/10/20/827). All the fish were handled humanely in compliance with Directive 2010/63/EU on the protection of animals used for scientific purposes and the International Council for Laboratory Animal Science (ICLAS) ethical guidelines.

**Results**

**Descriptive and correlation analysis of covariates.** Table 1 shows the mean weight, length and condition factor of catfish and Nile tilapia fingerlings used in the study. The weight and length of catfish exposed to the pesticides were significantly different (p < 0.05) from Nile tilapia except length of fish exposed to mancozeb for 15, 30 and 60 minutes (p > 0.05) and weight of fish exposed continuously for 30, 45 and 60 minutes to chlorpyrifos (p > 0.05) as well as fish exposed for 60 minutes to lambda cyhalothrin.

Table 2 shows the strength of the relationship between the intrinsic predictor variables. The weight and length of fish exposed to the pesticides was strongly positively correlated. Weight and condition factor of catfish exposed to atrazine and lambda cyhalothrin were moderately positively correlated, while those exposed to mancozeb had low correlation. There was no correlation between weight and condition factor of catfish exposed to chlorpyrifos. Weight and condition factor of Nile tilapia exposed to atrazine and lambda cyhalothrin had strong negative correlation, while the negative correlations were moderate in tilapia exposed to mancozeb and chlorpyrifos. The length and condition of fishes exposed to the pesticides was strongly negatively correlated.

**Survival analysis.** Survival probability- survival time response. Figure 1 shows survival time and probability of survival decreased as pulse length increased. Probability of survival decreased as exposure time increased. Both species were more sensitive to insecticides (Fig. 1c,d,g,h) than herbicides (Fig. 1a,e) and fungicides (Fig. 1b,f). Survival probability was significantly higher for Nile tilapia than catfish following pulse exposure to atrazine, mancozeb and lambda cyhalothrin, however catfish exposed to chlorpyrifos had a lower risk to death than Nile tilapia.

The log rank test indicate that the risk of death from continuous exposure was higher than pulse exposure for both catfish and Nile tilapia fingerlings (p < 0.01). Be that as it may, the risk of death of catfish continuously exposed to atrazine, mancozeb and chlorpyrifos were similar (p > 0.05) to those pulse exposed to the pesticides for 60 minutes. Similarly, the risk of death of tilapia continuously exposed to chlorpyrifos was also similar (p > 0.05) to 60 minutes pulse exposure. This suggests pulse exposure of 60 minutes was as hazardous as continuous exposure for 96 hours. On the other hand, risk of death of pulse exposure for 15, 30, 45 minutes differed significantly from 60 minutes pulse exposure for catfish exposed to atrazine, mancozeb, and lambda cyhalothrin; and both species exposed to chlorpyrifos (p < 0.05). This indicates increase in risk of death at longer pulse duration.

**Influence of species on fish survival with weight, length and condition factor held constant.** In Table 3, positive parameter estimate indicates that Nile tilapia had lower risk of death (higher survival) than catfish exposed to atrazine, mancozeb and lambda cyhalothrin, while negative parameter estimate indicates that Nile tilapia had higher risk of death (lower survival) than catfish pulse-exposed to chlorpyrifos (p < 0.05). Catfish pulse-exposed to atrazine, mancozeb, chlorpyrifos, and lambda cyhalothrin were 2.020, 1.875, 0.010, and 1.722 times more likely to die than Nile tilapia respectively. Thus risk of death for Nile tilapia exposed to atrazine, mancozeb and lambda cyhalothrin decreased by 102%, 87.5% and 72.2% compared to catfish respectively, while risk of death of catfish exposed to chlorpyrifos was 99% lower than Nile tilapia respectively.

However in Table 4, the risk of death for Nile tilapia and catfish pulse-exposed to atrazine, mancozeb and lambda cyhalothrin differed significantly only in the group exposed for 60 minutes (p < 0.05). Risk of death for both species were similar in the groups exposed for 15, 30 and 45 minutes (p > 0.05). On the other hand, for each pulse length, risk of death from chlorpyrifos exposure differed significantly between Nile tilapia and catfish.
Risk of death of 15, 30, 45 and 60 minutes pulse exposure to chlorpyrifos decreased by a factor of 104.94, 87.64, 117.72, and 83.52 respectively in catfish compared with tilapia fingerlings.

Influence of pulse length on Survival of fingerlings with weight, length and condition factor held constant. Table 5 shows the mortality count and estimated median survival time for groups in which 50% mortality had occurred after pesticide pulse exposure.

| Exposure duration | Parameter     | Fish species | Pesticides                  |
|-------------------|---------------|--------------|------------------------------|
|                   |               |              | Atrazine | Mancozeb | Chlorpyrifos | Lambda cyhalothrin |
| Continuous        | Weight (g)    | Catfish      | 0.55 ± 0.02 | 0.5 ± 0.02 | 0.49 ± 0.02* | 0.54 ± 0.02 |
|                   |               | Tilapia      | 0.23 ± 0.01 | 0.36 ± 0.05 | 0.37 ± 0.06* | 0.17 ± 0.01 |
|                   | Length (cm)   | Catfish      | 4.25 ± 0.04 | 3.85 ± 0.04 | 3.66 ± 0.05 | 4.13 ± 0.04 |
|                   |               | Tilapia      | 2.11 ± 0.05 | 2.64 ± 14  | 2.51 ± 16  | 1.89 ± 0.04 |
|                   | Condition factor | Catfish | 0.72 ± 0.04 | 0.88 ± 0.05 | 1 ± 0.06   | 0.78 ± 0.04 |
|                   |               | Tilapia      | 2.46 ± 0.1  | 1.85 ± 0.1  | 2.25 ± 0.11 | 2.51 ± 0.19 |
| 15 Minutes        | Weight (g)    | Catfish      | 0.55 ± 0.03 | 0.47 ± 0.03 | 0.47 ± 0.02 | 1.01 ± 0.08 |
|                   |               | Tilapia      | 0.4 ± 0.02  | 0.95 ± 0.13 | 0.83 ± 0.17 | 0.63 ± 0.07 |
|                   | Length (cm)   | Catfish      | 3.87 ± 0.07 | 4.05 ± 0.09* | 4.08 ± 0.07 | 5.11 ± 0.16 |
|                   |               | Tilapia      | 3 ± 0.08    | 3.71 ± 0.21* | 3.09 ± 0.29 | 3.2 ± 0.2 |
|                   | Condition factor | Catfish | 0.96 ± 0.05 | 0.73 ± 0.05 | 0.7 ± 0.05  | 0.76 ± 0.05 |
|                   |               | Tilapia      | 1.55 ± 0.1  | 1.8 ± 0.12  | 2.41 ± 0.15 | 2.1 ± 0.23 |
| 30 Minutes        | Weight (g)    | Catfish      | 0.46 ± 0.02 | 0.47 ± 0.02 | 0.44 ± 0.03* | 1 ± 0.05 |
|                   |               | Tilapia      | 0.29 ± 0.02 | 1.03 ± 0.14 | 0.41 ± 0.07* | 0.57 ± 0.07 |
|                   | Length (cm)   | Catfish      | 3.84 ± 0.09 | 3.88 ± 0.08* | 3.8 ± 0.1 | 5.03 ± 0.12 |
|                   |               | Tilapia      | 2.31 ± 0.07 | 4.02 ± 0.25* | 2.62 ± 0.22 | 3.05 ± 0.21 |
|                   | Condition factor | Catfish | 0.84 ± 0.05 | 0.82 ± 0.05 | 0.83 ± 0.03 | 0.83 ± 0.06 |
|                   |               | Tilapia      | 2.31 ± 0.09 | 1.55 ± 0.12 | 2.34 ± 0.19 | 2.18 ± 0.2 |
| 45 Minutes        | Weight (g)    | Catfish      | 0.43 ± 0.02 | 0.48 ± 0.03 | 0.42 ± 0.02* | 0.94 ± 0.08 |
|                   |               | Tilapia      | 0.34 ± 0.02 | 0.69 ± 0.06 | 0.55 ± 0.17* | 0.55 ± 0.07 |
|                   | Length (cm)   | Catfish      | 3.56 ± 0.06 | 3.93 ± 0.11 | 3.75 ± 0.08 | 5.11 ± 0.18 |
|                   |               | Tilapia      | 2.72 ± 0.07 | 3.46 ± 0.11 | 2.77 ± 0.28 | 3.12 ± 0.19 |
|                   | Condition factor | Catfish | 0.99 ± 0.07 | 0.83 ± 0.06 | 0.82 ± 0.05 | 0.71 ± 0.05 |
|                   |               | Tilapia      | 1.71 ± 0.08 | 1.63 ± 0.07 | 1.85 ± 0.17 | 1.9 ± 0.19 |
| 60 Minutes        | Weight (g)    | Catfish      | 0.48 ± 0.04 | 0.47 ± 0.02 | 0.41 ± 0.02* | 0.56 ± 0.03* |
|                   |               | Tilapia      | 0.34 ± 0.02 | 0.83 ± 0.06 | 0.33 ± 0.08* | 0.53 ± 0.07* |
|                   | Length (cm)   | Catfish      | 3.79 ± 0.09 | 3.79 ± 0.08* | 3.79 ± 0.14 | 4.86 ± 0.11 |
|                   |               | Tilapia      | 2.68 ± 0.06 | 3.69 ± 0.12* | 2.42 ± 0.22 | 2.9 ± 0.22 |
|                   | Condition factor | Catfish | 0.89 ± 0.05 | 0.87 ± 0.05 | 0.81 ± 0.07 | 0.5 ± 0.03 |
|                   |               | Tilapia      | 1.76 ± 0.07 | 1.63 ± 0.05 | 1.97 ± 0.15 | 2.27 ± 0.2 |
exposed for 60 minutes. Chlorpyrifos hazard however increased by a factor of 2.307 in fingerlings exposed for 45 minutes compared with those exposed for 60 minutes ($p < 0.05$) probably due to stochastic deaths.

**Combined influence of species, pulse length, weight, length and condition factor on survival of fingerlings.** In Table 7, taking all predictors—species, pulse length, weight, length and condition factor into account, the probability of surviving atrazine pulse toxicity was about 9 times higher in tilapia compared with catfish. Hazard ratio < 1 and a significant negative parameter estimate indicates risk of death (hazard) of atrazine decreased as pulse length decreased and fish weight increased. The likelihood of death after 15, 30 and 45 minutes exposure decreased by a factor of 0.454, 0.309 and 0.457 respectively compared to 60 minutes ($p < 0.05$), while risk of death decreased by 100% in fishes weighing 0.1 gram more than another fish. Furthermore, significant positive parameter estimate and hazard ratio > 1 indicates increased risk of death for longer fingerlings and fishes with higher condition factor. The risk of death was 27.648 and 31.071 times higher in fishes longer than another by 1 cm and condition factor higher by 0.1. Longer fishes have more surface area which can facilitate uptake of pesticides during the pulse exposure than shorter fishes.

After accounting for species, pulse length, weight, length and condition factor, mancozeb pulse toxicity was only significantly associated with pulse length and fish length. Hazard ratio < 1 and a significant negative parameter estimate indicates the likelihood of death after 15, 30 and 45 minutes exposure decreased by a factor of 0.206, 0.194 and 0.229 respectively compared to 60 minutes ($p < 0.05$), while the risk of death decreased by 89.2% in fishes longer by 1 cm.

Chlorpyrifos pulse toxicity was associated with species alone. The probability of surviving chlorpyrifos toxicity decreased by 100% in catfish compared to Nile tilapia.

Lambda cyhalothrin was associated with species, pulse length, and fish weight. The probability of survival was 38.32 times higher in tilapia compared with catfish. Hazard ratio < 1 and a significant negative parameter estimate indicates the likelihood of death after 15, 30 and 45 minutes exposure to lambda cyhalothrin decreased by a factor of 0.253, 0.521 and 0.436 respectively compared to 60 minutes exposure ($p < 0.05$). Risk of death decreased by 86.9% in fishes weighing 0.1 gram more than another fish.

**Discussion**

The pesticide concentrations used in this study may be considerably higher than background concentrations in the environment. The use of high concentration in pulse assessment enables effects to be adequately characterized and provides a basis for estimating impacts at the predicted environmental concentration\(^3\). Moreover, in aquatic ecosystems adjacent to agricultural lands, peak pesticide concentration may be reached during rainfall events shortly after field application and could be at least 20-fold higher than the background concentrations\(^2\).
Figure 1. Survival probability-Time curve of catfish exposed to atrazine (A), mancozeb (B), chlorpyrifos (C), lambda cyhalothrin (D), and Nile tilapia exposed to atrazine (E), mancozeb (F), chlorpyrifos (G) and lambda cyhalothrin (H). Unit of pesticide concentration is mg/L, except lambda cyhalothrin which is µg/L.

Table 3. Cox PH model summary of species effect on survival of fingerlings. *Hazard ratio constant with time. aStatistical significant (p<0.05). bNot significant (p>0.05).
with some categorized as “more efficient metabolizers” than others. Mancozeb and lambda cyhalothrin could probably lie in quicker toxico-dynamic recovery suggesting a more efficient biotransformation of pesticide in Nile tilapia. Previous authors have shown that phase I and II biotransformation efficiency varies among finfish species, and the differences could explain the differences in the two species detected in this study. The similarity in the risk to death of fingerlings exposed to atrazine, mancozeb, and lambda cyhalothrin for 15, 30, and 45 minutes suggest that the difference between the two fish species after 60 minutes pulse-exposure probably lie in quicker toxico-dynamic recovery suggesting a more efficient biotransformation of pesticide in Nile tilapia. Previous authors have shown that phase I and II biotransformation efficiency varies among finfish species, and the differences could explain the differences in the two species detected in this study. The similarity in the risk to death of fingerlings exposed to atrazine, mancozeb, and lambda cyhalothrin for 15, 30, and 45 minutes suggest that the difference between the two fish species after 60 minutes pulse-exposure probably lie in quicker toxico-dynamic recovery suggesting a more efficient biotransformation of pesticide in Nile tilapia. Previous authors have shown that phase I and II biotransformation efficiency varies among finfish species, and the differences could explain the differences in the two species detected in this study.

As a herbicide, atrazine causes the reversible inhibition of photosynthesis in photosystem II in plants. However in fish and other animals, it might be genotoxic, clastogenic, and affect hormones, and biochemical processes. Its solubility in water is about 30 mg/L at 20 °C and also readily metabolized in fish, excreted and does not bio-concentrate in tissues. On the other hand, mancozeb (manganese–zinc ethylenebis dithiocarbamate) inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those that are involved in the production of ATP. Its chelating properties possibly interfere with a number of enzyme systems that contain metals, such as zinc, copper, and iron (e.g., dopamine b-hydro xylase). Its solubility in water is about 6.4 mg/L at 25°C. Lambda cyhalothrin, blocks voltage-gated sodium channels present on neuronal axons in brain and muscles causing swift paralysis and death to insects. It has solubility of 5 × 10−3 mg/L in purified water. Chlorpyrifos has low water solubility and can bio-concentrate in the liver, intestine and gills. It has a specific mode of action which involves preventing the breakdown of acetylcholine by inhibiting acetylcholinesterase activity. The resulting accumulation of acetylcholine in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death.

Catfish was expected to have higher survival time (lower risk of death) than Nile tilapia given that catfish is considered sturdy and more tolerant to stressors than Nile tilapia. On the other hand, mancozeb (manganese–zinc ethylenebis dithiocarbamate) inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those that are involved in the production of ATP. Its chelating properties possibly interfere with a number of enzyme systems that contain metals, such as zinc, copper, and iron (e.g., dopamine b-hydro xylase). Its solubility in water is about 6.4 mg/L at 25°C. Lambda cyhalothrin, blocks voltage-gated sodium channels present on neuronal axons in brain and muscles causing swift paralysis and death to insects. It has solubility of 5 × 10−3 mg/L in purified water. Chlorpyrifos has low water solubility and can bio-concentrate in the liver, intestine and gills. It has a specific mode of action which involves preventing the breakdown of acetylcholine by inhibiting acetylcholinesterase activity. The resulting accumulation of acetylcholine in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death.

| Pesticide         | Exposure duration (minutes) | Df | Parameter estimate | SE  | Chi-square | Pr > ChiSq | Hazard ratio |
|-------------------|-----------------------------|----|--------------------|-----|------------|------------|-------------|
| Atrazine          | 15*                         | 1  | − .373             | .646| .334       | .563       | .689        |
|                   | 30*                         | 1  | − .539             | .510| 1.120      | .290       | .583        |
|                   | 45*                         | 1  | − .373             | .541| .476       | .490       | .689        |
|                   | 60*                         | 1  | − 1.319            | .486| 7.364      | .007       | .268        |
| Mancozeb          | 15*                         | 1  | − .117             | .671| .031       | .861       | .889        |
|                   | 30*                         | 1  | − .498             | .628| .629       | .428       | .608        |
|                   | 45*                         | 1  | − .106             | .557| .037       | .848       | .899        |
|                   | 60*                         | 1  | − 1.514            | .485| 9.745      | .002       | .220        |
| Chlorpyrifos      | 15*                         | 1  | 4.653              | 1.900| 5.999     | .014       | 104.944     |
|                   | 30*                         | 1  | 4.473              | 1.864| 5.760     | .016       | 87.638      |
|                   | 45*                         | 1  | 4.768              | 1.842| 6.700     | .010       | 117.715     |
|                   | 60*                         | 1  | 4.425              | 1.855| 5.688     | .017       | 83.515      |
| Lambda cyhalothrin| 15*                         | 1  | .015               | .519| .001      | .977       | 1.015       |
|                   | 30*                         | 1  | − .827             | .440| 3.524     | .060       | .437        |
|                   | 45*                         | 1  | .208               | .495| .177      | .674       | 1.231       |
|                   | 60*                         | 1  | − 2.295            | .739| 9.636     | .002       | .101        |

Table 4. Cox PH model summary of species effect on survival of fingerlings at different exposure duration. *Hazard ratio constant with time. **Statistical significant (p < 0.05). ***Not significant (p > 0.05).
are widely distributed\textsuperscript{50}. Catfish may experience delayed chlorpyrifos toxicity due to the bigger cerebellum size and clearly defined strata.

The effect of pulse length rather than pulse concentration was the focus in this study. Earlier study\textsuperscript{51} showed that pulse exposure to pesticides may cause more hazard than continuous exposure. This is consistent with the results of this study in which the risk of death after 60 minutes pulse exposure was similar to continuous exposure for 96 hours. Time is an important variable in toxic response. Critical threshold dose resulting in adverse effect could occur after a "short" time\textsuperscript{52}. Data from this study suggests 60 minutes pulse may be a critical exposure time for pulse exposure to high pesticide concentration below which toxicity is minimal. Previous study provides support for this. For instance, 50% mortality was only reached for \textit{C. californica} exposed to 1,730 mg/L carbaryl for 60 minutes but not after 15 or 30 minutes pulse exposure\textsuperscript{31}. Survival time presents a way to express individual/species tolerance of toxicants. Survival time after pulse exposure reflects "individual tolerance" as it represent

Table 5. Mortality count and median survival time of fingerlings pulse exposed to pesticides. C.I, Confidence interval. Median survival time and confidence interval was only estimated when 50% mortality occur; m-minutes, h-hours *- continuous exposure.

| Fish species | Pesticide | Exposure duration | Total | Failed | Censored | Median survival time (h) | 95% C.I Lower | 95% C.I Upper |
|--------------|-----------|-------------------|-------|--------|----------|--------------------------|---------------|---------------|
| Catfish      | Atrazine  | 15-m              | 20    | 6      | 14       | 60                       |               |               |
|              |           | 30-m              | 20    | 11     | 9        | 77.5 33                  |               |               |
|              |           | 45-m              | 20    | 8      | 12       | 50                        |               |               |
|              |           | 60-m              | 20    | 15     | 5        | 53  27  96               |               |               |
|              |           | 96-h\*            | 20    | 17     | 3        | 9  5  60                 |               |               |
|              | Mancozeb  | 15-m              | 20    | 5      | 15       |                         |               |               |
|              |           | 30-m              | 20    | 7      | 13       | 92                       |               |               |
|              |           | 45-m              | 20    | 7      | 13       | 75                       |               |               |
|              |           | 60-m              | 20    | 16     | 4        | 7.5  2  51               |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 9  8  15                 |               |               |
|              | Chlorpyrifos | 15-m            | 20    | 4      | 16       |                         |               |               |
|              |           | 30-m              | 20    | 6      | 14       | 35                       |               |               |
|              |           | 45-m              | 20    | 8      | 12       | 9                        |               |               |
|              |           | 60-m              | 20    | 15     | 5        | 9  7  13                 |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 9  7  13                 |               |               |
|              | Lambda cyhalothrin | 15-m        | 20    | 7      | 13       | 3                        |               |               |
|              |           | 30-m              | 20    | 13     | 7        | 2  2                     |               |               |
|              |           | 45-m              | 20    | 7      | 13       | 1                        |               |               |
|              |           | 60-m              | 20    | 20     | 0        | 1                        |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 1                        |               |               |
| Nile tilapia | Atrazine  | 15-m              | 20    | 4      | 16       |                         |               |               |
|              |           | 30-m              | 20    | 6      | 14       | 11                       |               |               |
|              |           | 45-m              | 20    | 6      | 14       | 70                       |               |               |
|              |           | 60-m              | 20    | 6      | 14       | 75                       |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 3  2  4                  |               |               |
|              | Mancozeb  | 15-m              | 20    | 4      | 16       |                         |               |               |
|              |           | 30-m              | 20    | 4      | 16       |                         |               |               |
|              |           | 45-m              | 20    | 6      | 14       | 65                       |               |               |
|              |           | 60-m              | 20    | 6      | 14       | 61                       |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 6  5  8                  |               |               |
|              | Chlorpyrifos | 15-m            | 20    | 20     | 0        | 1.5  1  2                |               |               |
|              |           | 30-m              | 20    | 20     | 0        | 2  1  2                  |               |               |
|              |           | 45-m              | 20    | 20     | 0        | 2  1  2                  |               |               |
|              |           | 60-m              | 20    | 20     | 0        | 2                        |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 2                        |               |               |
|              | Lambda cyhalothrin | 15-m        | 20    | 8      | 12       | 8                        |               |               |
|              |           | 30-m              | 20    | 9      | 11       | 7                        |               |               |
|              |           | 45-m              | 20    | 10     | 10       | 4                        |               |               |
|              |           | 60-m              | 20    | 11     | 9        | 15.5  2                  |               |               |
|              |           | 96-h\*            | 20    | 20     | 0        | 2  1  2                  |               |               |
the time for which biological processes like, absorption, distribution, bioaccumulation, and onset of cellular/physiological impairment have occurred leading to either death or recovery for each fingerlings. Fish weight, length and condition factor are intrinsic variables that may influence the survival of pulsed-exposed fishes. Risk of death in fingerlings exposed to pesticide was anticipated to decrease as fish weight increased. In line with our expectations, risk of death decreased as weight of fingerlings increased particularly in the groups exposed to atrazine and lambda cyhalothrin given the slight differences in the weight of fishes exposed to different pulse lengths of each pesticides. Chlorpyrifos and mancozeb pulse toxicity were not associated with fish weight due to similar fish weights exposed to each pesticide for different duration. Also, results from this study suggests risk of death of pulse exposure to pesticides may decrease as length and condition factor of fingerlings increase. The condition factor (CF) of a fish is often used to depict the health condition of a fish53, so the higher the condition factor, the healthier or fit the fish. Risk of death from pulse exposure to atrazine decreased as fish length and condition factor increased. Length of fish species exposed to atrazine differed slightly. Survival probability may have increased as weight, length and condition factor increased because bigger and fitter fingerlings may have higher tolerance for stress than smaller fingerlings. This is consistent with previous study29 where the sensitivity of fishes exposed to benzocaine where higher for smaller fishes than bigger fishes. Decrease in risk of death with increasing size is consistent with individual tolerance concept54.

Conclusion

In this study, intrinsic (specie difference, weight, length and condition factor) and extrinsic factors (pulse length) influenced risk of death of fingerlings exposed to pesticides. Generally, the hazard of pesticide pulse exposure decreased as fingerling size (weight, length and condition factor) increased but increased as pulse length increased. Nile tilapia fingerlings were more susceptible to continuous pesticides exposure than catfish, but appeared to be less susceptible to pulse exposure. Brief exposure of fingerlings to pesticides 60 minutes was as

| Pesticide      | Species | df | Parameter estimate | SE   | Chi-square | Hazard ratio |
|----------------|---------|----|--------------------|------|------------|--------------|
| Atrazine       | Catfish*| 15 min| 0.1300*            | .484 | 7.221      | .272*        |
|                |         | 30 min| −.5122             | .397 | 1.663      | .599*        |
|                |         | 45 min| −.928*             | .438 | 4.483      | .395*        |
|                |         | 60 min| .9001              |      |            |              |
|                | Tilapia | 15 min| −.3615             | .646 | .313       | .692*        |
|                |         | 30 min| .1411              | .578 | .060       | 1.152*       |
|                |         | 45 min| .0511              | .577 | .008       | 1.052*       |
|                |         | 60 min| .654               |      |            |              |
| Mancozeb       | Catfish | 15 min| −1.945*            | .518 | 14.123     | .143*        |
|                |         | 30 min| −1.577*            | .459 | 11.826     | .206*        |
|                |         | 45 min| −1.519*            | .458 | 11.007     | .219*        |
|                |         | 60 min| 23.341             |      |            |              |
|                | Tilapia*| 15 min| −.3515             | .646 | .296       | .704*        |
|                |         | 30 min| −.4123             | .646 | .407       | .662*        |
|                |         | 45 min| .0375              | .577 | .004       | 1.037*       |
|                |         | 60 min| 16.070             |      |            |              |
| Chlorpyrifos   | Catfish*| 15 min| −1.864*            | .567 | 10.818     | .155*        |
|                |         | 30 min| −1.418*            | .488 | 8.444      | .242*        |
|                |         | 45 min| −.999*             | .441 | 5.130      | .368*        |
|                |         | 60 min| .779               |      |            |              |
|                | Tilapia*| 15 min| .2460*             | .317 | .601       | 1.279*       |
|                |         | 30 min| .0455              | .316 | .020       | 1.046*       |
|                |         | 45 min| .836*              | .334 | 6.429      | 2.307*       |
|                |         | 60 min| 7.694              |      |            |              |
| Lambda cyhalothrin | Catfish | 15 min| −1.994*            | .499 | 15.958     | .136*        |
|                |         | 30 min| −1.179*            | .411 | 8.227      | .308*        |
|                |         | 45 min| −1.710*            | .489 | 12.202     | .181*        |
|                |         | 60 min| 20.231             |      |            |              |
|                | Tilapia*| 15 min| −.5475             | .465 | 1.385      | .579*        |
|                |         | 30 min| −.4100             | .450 | .832       | .663*        |
|                |         | 45 min| −.2340             | .437 | .286       | .791*        |
|                |         | 60 min| 1.604              |      |            |              |

Table 6. Cox PH model summary of effect of pulse duration on hazard of single pesticide. *Hazard ratio constant with time. Statistical significant (p < 0.05). bNot significant (p > 0.05).
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TABLE 7. Cox PH model summary of all predictors on survival of fingerlings. *Increase in hazard ratio, while b indicates decrease in hazard ratio compared with when other predictors were held constant. *Significant when other predators were held constant but no longer significant. *Not significant when other predators were held constant and not significant now. *Not significant when other predators were held constant but now significant.

Table 7.

| Pesticide          | Parameter estimate | SE    | Chi-square | Df | Pr > ChiSq | Hazard ratio | 95.0% CI for Hazard ratio |
|--------------------|--------------------|-------|------------|----|------------|--------------|---------------------------|
|                    |                    |       |            |    |            |              |                           |
| Atrazine           |                    |       |            |    |            |              |                           |
| Species            | 2.197              | .565  | 15.146     | 1  | .000       | 9.002         | 2.977 – 27.223            |
| 15 min             | −.789              | .394  | 4.007      | 1  | .045       | .454          | .210 – .984               |
| 30 min             | −1.175             | .391  | 9.035      | 1  | .003       | .309          | .144 – .664               |
| 45 min             | −.784              | .353  | 4.936      | 1  | .026       | .457          | .229 – .912               |
| 60 min             |                    |       | 11.018     | 3  | .012       |               |                           |
| Weight             | −12.315            | 2.027 | 36.898     | 1  | .000       | .000          | .000 – .000               |
| Length             | 3.320              | .706  | 22.128     | 1  | .000       | 27.648        | 6.934 – 110.244           |
| Condition factor   | 3.436              | .660  | 27.081     | 1  | .000       | 31.071        | 8.517 – 113.351           |
| Mancozeb           |                    |       |            |    |            |              |                           |
| Species            | .859               | .541  | 2.520      | 1  | .112       | 2.360         | .817 – 6.816              |
| 15 min             | −1.581             | .416  | 14.464     | 1  | .000       | .206          | .091 – .465               |
| 30 min             | −1.639             | .395  | 17.235     | 1  | .000       | .194          | .090 – .421               |
| 45 min             | −1.474             | .368  | 16.017     | 1  | .000       | .229          | .111 – .471               |
| 60 min             |                    |       | 27.527     | 3  | .000       |               |                           |
| Weight             | −2.856             | 1.918 | 2.216      | 1  | .137       | .058          | .001 – 2.469             |
| Length             | −2.223             | .876  | 4.333      | 1  | .011       | .108          | .019 – .604               |
| Condition factor   | −.697              | .850  | .673       | 1  | .412       | .498          | .094 – 2.634              |
| Chlorpyrifos       |                    |       |            |    |            |              |                           |
| Species            | −12.725            | 51.934| .060       | 1  | .806       | .000          | .000 – 4.8E+38            |
| 15 min             | −.428              | .290  | 2.180      | 1  | .140       | .652          | .369 – 1.151             |
| 30 min             | −.406              | .395  | 2.266      | 1  | .132       | .666          | .393 – 1.130             |
| 45 min             | .216               | .271  | .634       | 1  | .426       | 1.241         | .729 – 2.113             |
| 60 min             |                    |       | 6.404      | 3  | .094       |               |                           |
| Weight             | .107               | .525  | .042       | 1  | .838       | 1.113         | .398 – 3.116             |
| Length             | .061               | .327  | .035       | 1  | .852       | 1.063         | .560 – 2.016             |
| Condition factor   | .066               | .220  | .089       | 1  | .765       | 1.068         | .694 – 1.642             |
| Lambda cyhalothrin |                    |       |            |    |            |              |                           |
| Species            | 3.646              | .574  | 40.291     | 1  | .000       | 38.32         | 12.431 – 118.125         |
| 15 min             | −3.175             | .356  | 14.925     | 1  | .000       | .253          | .126 – .508              |
| 30 min             | −.653              | .321  | 4.124      | 1  | .042       | .521          | .278 – .978              |
| 45 min             | −.380              | .328  | 6.412      | 1  | .011       | .436          | .229 – .829              |
| 60 min             |                    |       | 15.783     | 3  | .001       |               |                           |
| Weight             | −2.033             | .863  | 5.543      | 1  | .019       | .131          | .024 – .711              |
| Length             | −.652              | .364  | 3.216      | 1  | .073       | .521          | .255 – 1.063             |
| Condition factor   | .342               | .281  | 1.486      | 1  | .223       | 1.408         | .812 – 2.442             |

hazardous as continuous exposure for 96 hours to some pesticides. Data from this study suggests 60 minutes pulse may be a critical exposure time for pulse exposure to high pesticide concentration.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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References

1. Aktar, M. W., Sengupta, D. & Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.*, 2, 1–12 (2009).
2. Mahmood I, Imadi S.R, Shazadi K, Gul A, & Hakeem K. R. Effects of pesticides on environment in plant, soil and microbes (ed. Hakeem K., Akhtar M., Abdullah S.) (Springer, Cham. 2016) https://doi.org/10.1007/978-3-319-27455-3_13
3. West Africa Agricultural Productivity Programme (WAAPP-NIGERIA). Report of a baseline study on status of use, registration and regulation of pesticides in Nigeria. Available online at: http://waapp.gov.ng/images/finalversionofbaselinestudiesonpesticideusesubmittedtowapp2013.pdf (2013).
4. Wang, Y. et al. Joint acute toxicity of the herbicide butachlor and three insecticides to the terrestrial earthworm, *Eisenia fetida*. *Environ. Sci. Pollut. Res.*, 23, 11766–11776 (2016).
5. Tomlin, C. Pesticide Manual 10th edn. (The Royal Society of Chemistry, 1994).
6. World Health Organization. Atrazine in drinking-water: background document for development of WHO guidelines for drinking-water quality (No. WHO/SEDEWS/3/03.04/32). World Health Organization. Available from Microsoft Word - GDWQ2ndEdit. Atrazine.doc (who.int) (2003).
7. Ghosh, P. K. & Philip, L. J. G. Y. Environmental significance of atrazine in aqueous systems and its removal by biological processes: An overview. Global NEST J, 8(2), 159–178 (2006).
8. Kumar, M., Chand, R., & Shah, K. Mycotoxins and pesticides: toxicity and applications in food and feed. In Microbial Biotechnology 207–252. (Springer, Singapore, 2018).
9. Raghavendra, S. N., Raghu, H. S., Chaitra, C., & Rajeshwara, A. N. Potency of mancozeb conjugated silver nanoparticles synthesized from goat, cow and buffalo urine against Colletotrichum gloeosporioides causing anthracnose disease. Nat. Environ. Pollut. Technol. 19(3) (2020).
10. Melgar, C. et al. Pollutants in drainage channels following long-term application of Mancozeb to banana plantations in southeastern Mexico. J. Plant. Nutr. Soil. Sci. 171(4), 597–604 (2008).
11. National Center for Biotechnology Information. PubChem Compound Summary for CID 2730, Chlorpyrifos. Retrieved May 24, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Chlorpyrifos (2021).
12. Akam, J. C., Battah, N., Waziri, M. & Mahmud, M. M. Organochlorine, organophosphorus and pyrethroid pesticides residues in water and sediment samples from river benue in vinkilang, yola, adamawa state, nigeria using gas chromatography-mass spec- tommetry equipped with electron capture detector. Am. J. Environ. Prot. 3, 164–173 (2015).
13. Oeil, M. J. The Merck Index-An Encyclopedia of Chemicals, Drugs, and Biologicals 462 (Merck and Co., Inc., 2006).
14. He, L. M., Troiano, J., Wang, A., & Goh, K. Environmental chemistry, ecotoxicity, and fate of lambda-cyhalothrin. Rev. Environ. Contam. Toxicol. 71–91 (2008).
15. Sumon, K. A., Rashid, H., Peeters, E. T., Bosma, R. H. & Van den Brink, P. J. Environmental monitoring and risk assessment of organophosphate pesticides in aquatic ecosystems of north-west Bangladesh. Chemosphere 206, 90–120 (2018).
16. Ashauer, R., Boxall, A. & Brown, C. Predicting effects on aquatic organisms from fluctuating or pulsed exposure to pesticides. Environ. Toxicol. Chem. 25(7), 1899–1912 (2006).
17. Ashauer, R., Boxall, A. B. & Brown, C. D. Modeling combined effects of pulsed exposure to carbaryl and chlorpyrifos on Gammarus pulex. Environ. Sci. Technol. 41(15), 5535–5541 (2007).
18. Rasmussen, J. I., Wiberg-Larsen, P., Kristensen, E. A., Cedergreen, N. & Friberg, N. Pyrethroid effects on freshwater invertebrates: a meta-analysis of pulse exposures. Environ. Pollut. 182, 479–485 (2013).
19. Dennis N, Tiede K, & Thompson H. Repeated and multiple stress (exposure to pesticides) on aquatic organisms. Supporting information. J. Environ. Qual. 41(5), 1487–1490 (2012).
20. Andersen, T. H., Tjørnhøj, R., Wollenberger, L., Slothuus, T. & Baun, A. Acute and chronic effects of pulse exposure of Daphnia magna to dimethoate and pirimicarb. Environ. Toxicol. Chem. 25(5), 1187–1195 (2006).
21. Ewing, R. Diminishing returns: Salmon decline and pesticides. J. Pesticide Res. 21, 917–925 (Springer, 2013).
22. Zhao, Y. & Newman, M. C. Effects of exposure duration and recovery time during pulsed exposures. Environ. Toxicol. Chem. 25(5), 1298–1304 (2006).
23. Stoughton, S. J., Liber, K., Culp, I. & Cessna, A. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates Chironomus tentans and Hyalella azteca under constant-and pulse-exposure conditions. Arch. Environ. Contam. Toxicol. 54(4), 662–673 (2008).
24. Jegede, O. O., Hale, B. A. & Siciliano, S. D. Multigenerational exposure of populations of Oppia nitens to zinc under pulse and continuous exposure scenarios. Environ. Toxicol. Chem. 36(4), 896–904 (2017).
25. Reintert, K. H., Giddings, J. M. & Judd, I. Effects analysis of time-varying or repeated exposures in aquatic ecological risk assessment of agrochemicals. Environ. Toxicol. Chem. 21(9), 1977–1992 (2002).
26. Dixon, P. M., & Newman, M. C. Analyzing toxicity data using statistical models for time-to-death: An introduction in Metal Ecotoxicology, Concepts and Applications (ed. Newman M. C. & McIntosh A. W.) 203–242 (Lewis Publishers, Inc. 1991).
27. Newman, M. C. & Aplin, S. M. Enhancing toxicity data interpretation and prediction of ecological risk with survival time modeling: an illustration using sodium chloride toxicity to mosquitofish (Gambusia holbrooki). Aquat. Toxicol. 23(2), 85–96 (1992).
28. Newman, M. C. & McCloskey, J. T. Time-to-event analyses of ecotoxicity data. Ecotoxicology 5(3), 187–196 (1996).
29. American Public Health Association, APHA. Standard methods for the examination of water and wastewater, (16th edition) 800–819 (Washington DC, 1985).
30. Peterson, J. L., Jepson, P. C. & Jenkins, J. J. Effect of varying pesticide exposure duration and concentration on the toxicity of carbaryl to two field-collected stream invertebrates, Calineura californica (Plecoptera: Perlidae) and Cinygna sp. (Ephemeroptera: Heptageniidae). Environ. Toxicol. Chem. 20(10), 2215–2223 (2001).
31. Busacker, G. P., Adelman, I. R., & Goolish, E. M. Growth in Methods for Fish Biology (eds Schreck, C. B., Moyle, P. B.) 363–387 (American Fisheries Society, 1999).
32. Nwani, C. D. et al. Toxicity of the herbicide atrazine: Effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus Bloch. Int. J. Environ. Res. Public Health 7(8), 3298–3312 (2010).
33. Solomon, K. R. & et al. Effects of atrazine on fish, amphibians, and aquatic reptiles: A critical review. Crit. Rev. Toxicol. 38, 721–772 (2008).
34. Thiruchelvam, M. Mancozeb. In Encyclopedia of Toxicology (eds Wesler, P. et al.) (Academic Press, London, 2005). doi.org/ 10.1016/B0-12-369400-0/00575-5.
35. National Center for Biotechnology Information. PubChem Compound Summary for CID 3034368, Mancozeb. Retrieved May 24, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Mancozeb (2021).
36. Kostich, M. S. et al. Multigene biomarkers of pyrethroid exposure: Exploratory experiments. Environ. Toxicol. Chem. 38, 2436–2446 (2019).
37. Bonansea, R. L., Marino, D. J., Bertrand, L., Wunderlin, D. A. & Amé, M. V. Tissue-specific bio-concentration and biotransformation of cypermethrin and chlorpyrifos in a native fish (Jenynsia multifilis) exposed to these insecticides singly and in mixtures. Environ. Toxicol. Chem. 36(7), 1764–1774 (2017).
38. Kwong, T. C. Organophosphate pesticides: Biochemistry and clinical toxicity. Ther. Drug Monit. 24, 144–149 (2002).
39. Ezeonyejiaku, C. D., Obiakor, M. O. & Ezenwelu, C. O. Toxicity of copper sulphate and lambda-cyhalothrin to the fish Crinotrichum sp. (Ephemeroptera: Heptageniidae). J. Aquatic. Res. Dev. 1(1), 1–15 (2018).
40. Osman, A. G. et al. Blood biomarkers in Nile tilapia Oreochromis niloticus and African catfish Clarias gariepinus to evaluate water quality of the river Nile. J. FisheriesSciencescom. 12(1), 1–15 (2018).
41. Ogwej, O. E. Comparative acute toxicity of chlorpyrifos-ethyl (organophosphate) and lambda-cyhalothrin (pyrethroid) to the African Catfish (Clarias gariepinus) using some biochemical parameters. Global J. Pure Appl. Sci. 14(3), 263–269 (2008).
42. Marzouk, M. S. et al. Effect of atrazine exposure on behavioral, haematological and biochemical aspects of female African catfish (Clarias gariepinus). J. Sci. Res. 9, 290–299 (2012).
45. Guedegba, N. L. et al. Comparative acute toxicity of two phytosanitary molecules, lambda-cyhalothrin and acetamiprid, on Nile Tilapia (Oreochromis Niloticus) juveniles. J. Environ. Sci. Health Part B. 54(7), 580–589 (2019).
46. Kanu, K. C., Ogbonna, O. A. & Mpamah, I. C. Acute toxicity and biological responses of Clarias gariepinus to environmentally realistic chlorpyrifos concentrations. Pollution 5(4), 839–846 (2019).
47. Majumder, R. & Kaviraj, A. Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish Oreochromis niloticus. Drug Chem. Toxicol. 42(5), 487–495 (2019).
48. González, J. F., Reimenschuessel, R., Shaikh, B. & Kane, A. S. Kinetics of hepatic phase I and II biotransformation reactions in eight fish species. Mar. Environ. Res. 67(4–5), 183–188 (2009).
49. Zayed, A. E. & Mohamed, S. A. Morphological study on the gills of two species of freshwater fishes: Oreochromis niloticus and Clarias gariepinus. Ann. Anat. 186(4), 295–304 (2004).
50. Danmaigoro, A., Hena, S. A., Ibrahim, A. A., Shehu, S. A., & Mahmud, M. A. Comparative morphometry and histological studies of the cerebellum of catfish (Clarias gariepinus) and tilapia (Oreochromis niloticus). J. Appl. Life Sci. Int. 1–6 (2016).
51. Tucker, K. A. & Burton, G. A. Assessment of nonpoint-source runoff in a stream using in situ and laboratory approaches. Environ. Toxicol. Chem. 18, 2797–2803 (1999).
52. Naddy, R. B., Johnson, K. A. & Klaine, S. J. Response of Daphnia magna to pulsed exposures of chlorpyrifos. Environ. Toxicol. Chem. 19, 423–431 (2000).
53. Kanu, K. C. & Idowu, E. T. Health status of Chrysichthys nigrodigitatus in response to aquatic pollution in Epe, Lagos and Ologe Lagoons, Southwest Nigeria. Environ. Exp. Biol. 15(2), 151–159 (2017).
54. Ashauer, R. & Escher, B. I. Advantages of toxicokinetic and toxicodynamic modelling in aquatic ecotoxicology and risk assessment. J. Environ. Monit. 12(11), 2056–2061 (2010).

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Author contributions
K.K.C. conceptualized, performed the experiment and statistical analysis and wrote the original draft. O.A.A. supervised, and reviewed and edited the initial draft. A.N.H provided resources, supervised, and edited. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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