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Exposure to herbicide Atrazine impairs growth and energy budget in African Catfish Clarias gariepinus fingerlings

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This study aims to determine, in a controlled environment, the impact of exposure to sub-lethal concentrations of the herbicide Atrazine on growth, feed utilization and energy budget in *Clarias gariepinus*. Fingerlings of *C. gariepinus* (2.54 ± 0.02 g mean weight, 7.32 ± 1.6 cm mean length) were exposed to concentrations of 0; 0.03165 and 0.3165 ppm Atrazine for 28 days. Biomass was assessed weekly and fish samples were taken from different aquaria to determine weight gain, specific growth rate, feed efficiency rate, protein efficiency ratio and the biochemical composition of fish. Results showed important behavioral reactions of the fish such as hyperexcitation, depth activity and surfacing activity during the first two weeks. Fish survival significanlty declined in exposed fish only during the first week. Atrazine inhibited the growth of *C. gariepinus* fingerlings throughout the exposure period without influencing feed utilization and protein efficiency compared with control fish. Carbohydrates were used primarily, followed by proteins and then lipids, as sources of energy in exposed fish, to meet energy demand generated by this chemical stressor.

Keywords: *Clarias gariepinus*, Chemical stressor, Growth parameters, Bioenergetic

INTRODUCTION

Fish is a highly nutritious source of protein, which is tasty and easily digested. It is a much sought after by a broad cross-section of the world's population, particularly in developing countries. Between 1961 and 2017, the average annual growth rate of total food fish consumption was 3.1%, exceeding that of 1.6% of the population (FAO, 2020). Per capita food fish consumption increased from 9.0 kg (live weight equivalent) in 1961 to 20.3 kg in 2017 (FAO, 2020). As of 2018, preliminary estimates of per capita fish consumption are 20.5 kg. But nowadays inland and marine fisheries are confronted with the contamination of pollutants of all kinds (Agbohessi et al., 2015a,b, 2020).

Among the pollutants, there are agricultural pesticides used extensively in northern Benin to increase cotton production and productivity (Agbohessi et al., 2012, 2015a). These products include herbicides sometimes called weedkillers (Naili, 2014). These are active ingredients or formulated products having the property of killing plants (Faure, 2000; Naili, 2014).

One of the molecules with phytocidal properties that are commonly used for farming in Africa,

particularly in its western part is Atrazine. Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-1-3, 5-triazine) is a synthetic herbicide belonging to the class of Triazines. It is a selective systemic herbicide, absorbed mainly by the roots but also by the leaves, with translocation towards the apex via the xylem and an accumulation in the apical meristem and the leaves.

Atrazine inhibits photosynthesis and interacts with other enzymatic processes (Tomlin, 2006). Atrazine is sparingly soluble in water (30 mg/l at 20 °C), its octanol/ water partition coefficient (log Kow = 2.75) close to 3 also indicates its low liposolubility (INERIS, 2007). Its vapor pressure is 2.89 x 10-7 mm Hg at 25 °C and its Henry's constant is 2.36 x 10-9 atm-m3/mol.

Atrazine is considered very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment (INERIS, 2007). Gbaguidi et al. (2011) reported levels of Atrazine of up to 5 ppb in the water of the Agbado River in Benin. Until recently, the adverse effects of pesticides and their residues on non-target organisms have not been seriously considered in Benin. This situation is worrying, especially with the phenomenon of climate change where the toxicity of several chemical pollutants is increasing as a result of global warming (Agbohessi et al., 2012).

As Atrazine is increasingly used for crop cultivation in Benin, its concentration in aquatic ecosystems could also increases. Studies have shown the harmful effects of pesticides on the survival, growth, reproductive and hepatic system of fish (Agbohessi et al., 2013 and 2014; Agbohessi, 2014; Agbohessi et al., 2015a and b), but few have discussed the impact of Atrazine on energy budget in fish.

The overall objective of this study is to determine, under local laboratory conditions, the effects of the sub-lethal concentrations of Atrazine in C. gariepinus, an important species of aquatic ecosystems receiving insecticides and herbicides from the cotton basin of northern Benin. This involves determining the effects of Atrazine on growth, feed utilization and nutrient use in C. gariepinus fingerlings.

MATERIALS AND METHODS

Animals and maintenance

Healthy fresh water teleost, C. gariepinus fingerlings were obtained from «Agro-Business le Paradi Plus», where they were produced artificially from parents reared in recirculating water conditions. They were transported to the Research Laboratory in Aquaculture and Aquatic Ecotoxicology (LaRAEAq) at the University of Parakou (Benin) in plastic bags with sufficient air in the day (7.00 am – 9.00 am). Laboratory stock of fish was maintained in glass aquarium containing dechlorinated and aerated tap water (capacity: 1000 l). The quality of water was assessed before exposure. Fish were acclimatized for 12 days (after an initial 48 h stabilization period as required by OECD Test 203 Guidelines) under laboratory conditions (ambient temperature 28.5 ± 0.5 °C; relative humidity 70 ± 5%; Light: Darkness, 12: 12 h). They were fed twice daily at 4 % of their biomass still 24 h before the beginning of the test with a commercial dry feed ("Le Gouessant" pellets of 2 mm, 46 % crude proteins). During this period, the stock water was changed once every other day to prevent the accumulation of decaying food particles and waste metabolites. The acclimatization tank was continuously aerated with 220 V air pumps and air stones throughout the period.

Chemicals

Commercial formulation of the Atrazine herbicide (Atraforce) was used for the studies. Atraforce containing 80% Atrazine was procured from a major pesticide market at Banikoara (Benin). The test solutions for this phytocide are obtained by mixing the products directly with dechlorinated tap water, as usually done in a farming environment. All working stock solutions were made immediately prior to the tests.

Experimental design and handling

The experiment was conducted according to OECD Guidelines 215 with some minor adaptations. Nine aquaria (Volume = 30 l) were used for three conditions with three replicates per treatment. Few minutes after the preparation of experiment solution, 14 fingerlings (2.54 ± 0.02 g mean weight, 7.32 ± 1.6 cm mean length), i.e. fish in their exponential growth phase were carefully counted, weighed and placed into each tank. Fish were fed at apparent satiation five times daily at 5 % of their biomass with a commercial dry feed "Le Gouessant" pellets of 2 mm during the experiment. According to the manufacturer's data, the feed contained 46 % crude proteins, 10 % crude fat and 8.6 % ash. The experiments were conducted in semi-static systems with renewal of 50 % of the aquarium solution each 48 h after checking of water quality parameters by standard methods. The experiments lasted 28 days (4 weeks). The food that was not served by aquarium was weighed at the end of each feeding day. Uneaten food and feces were siphoned upon renewal of experiment solutions. Temperature (28.2 ± 0.5 °C), pH (7.1 ± 0.1) and dissolved oxygen (5.5 ± 0.5 mg/l) were measured daily in each aquarium. During the experiment, the photoperiod was maintained at LD 12:12. The control and the herbicide treatments were run simultaneously. The behavioral pattern of fish was monitored regularly under above suggested treatment conditions. The nominal concentrations tested were as follows: C = Control, dilution water without any herbicide; T1 =1/100 LC50-96h = 0.03165 ppm; T2 = 1/10 LC50-96h = 0.3165 ppm. LC50-96h is the concentration of Atraforce that kills 50 % of the fish within 96 h. LC50-96h = 3.165 ppm in C. gariepinus fingerlings (Data not yet published).

In this study, nominal concentrations were not confirmed by chemical analyses. Precise measurement of the actual concentrations was considered to be of minor importance in these series of increasing concentrations. In addition, the half-lives of Atrazine (84 days at 20 °C, (INERIS, 2007)), in neutral environments are greater than the water-renewal times in our experiments. Furthermore, the active component is not very volatile (vapor pressure: 3 × 10-7 mm Hg (0.4 × 10-7 kPa) all at 20 °C, (INERIS, 2007). We therefore did not expect a significant quantity of this compound to be lost by volatilization during the study.

Growth and feed utilization data collection

At the beginning of the experiment, fish were individually weighed after 7 days (week 1), 14 days (week 2), 21 days (week 3) and at the termination (week 4) of the experiment. The biomass was always determined by weighing all fish from each aquarium. Collected data were used to calculate:

• Survival rate (%): S = 100 x Initial number of fingerlings / Final number of fingerlings

• Weight gain (g): W= Bw2 – Bw1

• Specific growth rate (%/d): SGR = 100 (ln Bw2 – ln Bw1) / Δt

• Feed efficiency: FE = TFS / (FB – IB)

• Protein efficiency ratio: PER = (FB – IB) / FPS

where B is biomass, n total number, Bw1,2 initial and final body weight (g); Δt, duration of the experiment (days); IB and FB, initial and final stock biomass (g), respectively; TFS, total feed served (g); FPS, feed protein served.

At the onset of the experiment, a sample of 10 fish (initial sample) was collected. Every 14 days until the end of the experiment, 9 fish per concentration (3 fish/aquaria) were sampled, anaesthetised with Clove oil (0.03 ml/l) and stored at -20 °C. At the end of the experiment, fish were separately mashed and homogenized. A portion of each sample of homogenate was frozen and brought back to Biochemistry laboratory at the Faculty of Agronomic Sciences of the University of

Abomey-Calavi (Benin) for analysis of protein and fat content and carbohydrate contents are determined by calculation (Rérat, 1956) to evaluate energy reserves.

Available energy reserves

Whole-body protein and lipid contents were measured in the Biochemistry laboratory at the Faculty of Agronomic Sciences of the University of Abomey-Calavi (Benin), by Kjeldahl and Folch et al. (1957) methods respectively. Whole-body carbohydrate content was determined by calculation (Dry matter – whole-body protein and lipid) according to Rérat (1956). Changes in body composition were expressed as changes in energy budget per day and were calculated using the formula:

with Tx being the time x and Yx the composition (carbohydrate, lipid, protein) at time x. This approach allows the quantification of changes in energy budget between different exposure regimes and periods. When calculating energetic values, an enthalpy of combustion of 17.3 kJ/g for carbohydrate (Rerat, 1956), 39.5 kJ/g for lipids, and 24 kJ/g for proteins (Jobling, 1994) was used. Using these values, the different energy sources may be summed up to give the changes in whole-body energy budget.

Statistical treatment

The experimental unit is the aquarium. The results were expressed as mean ± standard deviation of the mean. For each sampling time point and each parameter studied (S, W, SGR, FE, PER, total protein budget, total lipid budget, total carbohydrate budget, etc.), differences between means were evaluated by a one-way analysis of variance (ANOVA I) with concentration of herbicide as factor. Whenever significant differences were revealed, Turkey’s tests were applied. The statistical calculations were performed using Statistica Software® (StatSoft,Tulsa, OK, USA). P value of 0.05 or less was considered significant.

RESULTS

Behavioral responses of exposed fish

Fish exposed to sub-lethal concentrations of Atrazine expressed behavioral abnormalities, such as hyperexcitation, loss of balance, lethargy, surfacing activity, low rate of swimming and vertical position (Table 1). At the beginning of the exhibition, the fish were judged in good health and very active. During the experience, they tried to avoid Atrazine solution for a while quite swimming, jumping and other random movements. In these treated groups, the fish expressed hyperexcitation with a surfacing activity followed by a depth activity. There was a loss of balance, exhaustion and lethargy because of the respiratory incumbency. Finally, they took a vertical position with a mouth near the surface of the water and the direction of the tail to warn it air. Soon, they moved passively at the bottom of the reservoir. These signs appeared after each renewal of the test solutions and are more intense with the increase of the toxicant in the environment. Also note that the intensity of these sign has dropped from the beginning of the third week of exposure.

Survival rate

The impact of Atrazine concentrations tested on fish survival is shown in Figure 1. The analysis of this figure indicates that in the week 1 of exposure, the fish survival rates were lower in exposed fish, with the lowest rate in T2 ($p<0.05$). From the week 2, the fish survival rates in all treatments were statistically similar but with a downturn at the end of weeks 2 and 3.

Fish growth and feed utilization

Atrazine was found to significantly affect the growth of C. gariepinus fingerlings (Table 2). The

weight gain and the specific growth rate have been lower in the exposed fish throughout the period of the test compared to the controlled fish ($p<0.05$). The higher the concentrations of the pollutant in the environment, the lower the W and the SGR. However, the feed efficiency and the protein efficiency ratio are statistically similar for fish exposed to Atrazine and the control ($p>0.05$). As for the remnants of food, the higher the concentrations of the herbicide, the higher the amount of food ($p<0.05$). Which means that the more the pollutant is in the middle, the less food intake is high.

Changes in energy budget

At the start of the test, the average whole-body energy content of the fish based on the sum of lipid, protein and carbohydrate energy content was 369.4 KJ/g dry weight (DW). This whole-body energy consisted of 15.9 % lipids, 20.9 % protein and 63.1 % carbohydrate (DW). The total lipids content of the exposed fish was significantly low compared to the control ($p<0.05$) at the end of the week 2 and at the end of the test, especially at the highest concentration of Atrazine T2 (Table 3). In the same way, a significant effect of Atrazine exposure on the total protein content of fish was observed especially at the highest concentration at the end of the week 2 and at the end of week 4 ($p<0.05$). As for the carbohydrate content, the exposed fish have a higher content than the controlled fish at the end of the test ($p<0.05$). The mineral content did not vary from one treatment to another ($p>0.05$).

The effects of Atrazine on the absolute values of energy content are rather clear. However, if the changes in energy content are integrated over time the resulting energy budget provides clearer yet results. The lipids budget dropped after week 2 exposure to all Atrazine treatments, especially at the highest concentration of Atrazine T2 at the end of the test (Figure 2). But there is a tendency for lipids energy budget to increase at the end of week 4 for all treatments including controls. In the same way, in all Atrazine treatments the protein budget decreased after week 2 exposure and at the end of the experiment (Figure 3). But there is a tendency for protein energy budget to increase at the end of week 4 for all treatments including controls. The carbohydrate budget of the exposed fish fell at the end of week 2 and week 4, and remained deficit throughout the duration of the test, despite the high carbohydrate content of the fish at the start of the experimentation (Figure 4). The changes in individual energy budgets for lipids, proteins and carbohydrate were combined to determine the effects of Atrazine exposure on changes in the whole-body energy budget (Figure 5). After two weeks of exposure, whole-body energy of fish exposed to Atrazine was significantly lower than that in fish from the control groups. But there is a tendency for total energy to increase at the end of week 4 for all treatments including controls.

DISCUSSION

The purpose of this investigation was to determine under experimental conditions the effects of Atrazine on African catfish (C. gariepinus) survival, growth, feed utilization and change in whole-body energy budget.

Alterations of behavioral patterns and fish mortality

Slobodkin (1974) had indicated that faced with an environmental disturbance, behavioral responses constitute the first level of reaction before physiology. In this study, the fish exposed to Atrazine, just after renewal of the test solutions and therefore after each reintroduction of the herbicide into the environment, had unusual behaviors such as hyperexcitation, loss of balance, lethargy, surfaçing activity, depth activity, low rate of swimming and vertical position. Guedegba et al. (2019) and Houndji et al. (2020) had also observed hyperactivity or hyperexcitation reactions respectively in Nile tilapia Oreochromis niloticus exposed to Lambdacyhalothrin and in C. gariepinus subjected to Acetamiprid, Lambdacyhalothrin and Acer 35 EC. Hyperexcitation observed in this study had also been reported in C. gariepinus subjected to Endosulfan and Tihan 175 OTEQ (Agbohessi et al., 2013). Hyperexcitation is a reflex directly linked to the nervous system (Scott and Sloman, 2004). It

has been reported that under stress condition, fish become hyperactive, perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy demand (Alkahem et al., 2011). Surface activity and vertical position in fish can express hypoxic stress caused by toxicants (Graham, 1997; Val et al., 1998) or respiratory distress which is one of the first symptoms of pesticide poisoning (McDonald, 1983). Houndji et al. (2020) in the exposure of C. gariepinus juvenile to Acetamiprid, Lambdacyhalothrin and Acer also revealed the vertical position of some fish exposed 3 h later. The increase in surface activity was reported by Agbohessi et al. (2013) in certain juveniles of C. gariepinus exposed to Endosulfan. The loss of balance, depth activity and then lethargy are the last steps before the fish dies. The loss of equilibrium status, as recorded in our experiment, may have also been reported in fish exposed to heavy metal and pesticide contaminants (Saxena et al., 1981; Halappa and David, 2009). Moreover, loss of balance has also been reported in O. niloticus contaminated with Deltamethrin (Boateng et al., 2006), in C. gariepinus exposed to Diethyl phthalate (Obiezue et al., 2014) and in Spotted snakehead Channa punctatus subjected to Triazophos and Deltamethrin (Singh et al., 2018). Lethargy has been reported in O. niloticus contaminated with Acer and its active substances (Guedegba et al., 2019), in C. gariepinus exposed to the same pesticides (Houndji et al., 2020) and in C. gariepinus subjected to 2,4-D Amine (Makinde et al., 2015). The low rate of swimming is also associated with the possible nervous disorder (Obiezue et al., 2014). The increase in these different reactions when the toxicant increases in the environment, confirms that these behavioral changes are induced by Atrazine. According to several authors (Werner and Moran, 2008; Agbohessi et al., 2013; Guedegba et al., 2019; Houndji et al., 2020) these different reactions reveal the neurotoxicity of this pesticide.

After these successive behavioral responses, some fish die, especially much more at the high level of Atrazine concentrations and during the week 1. These mortalities may be linked to the combined effects of stress generated by taking biomass and chemical stress caused by the presence of Atrazine in the environment. Indeed, the stress induced by taking biomass and behavioral responses such as hyperexcitation, depth activity, surfacing activity, etc, generate metabolic costs that only fish in an adequate physiological state, therefore having an energy reserve to allocate to this activity can ensure (Domeneci et al., 2007; Péan, 2012). However, during this period the exposed fish consume less, so the death of these fish could be assimilated to the depletion of their energy reserves (Agbohessi et al., 2013). We will come back to this explanation later. In the present study, survival rates ranged from 90.47 to 100% throughout the test. Agbohessi et al. (2014) obtained 100% survival rates in C. gariepinus juveniles exposed to sub-lethal concentrations of Tihan 175 OTEQ and Thionex 350 EC for 28 days.

Growth and feed utilization

The higher the concentrations of Atrazine in the environment, the lower the Weight gain and the Specific growth rate. This means that this herbicide, as made to kill weeds, inhibits the growth of C. gariepinus fingerlings. Similar negative effects on fish growth were reported by McCarthy and Fuiman (2008) in Red drum Sciaenops ocellatus exposed to concentrations of 40 and 80 µg/l of Atrazine or 1 - 10 µg/l of Malathion. The reduction in growth was also obtained in the Australian catfish Tandanus tandanus contaminated with 2 or 10 µg/l of Chlorpyrifos (Huynh and Nugegoda, 2012), in O niloticus subjected to doses of 5 - 20 mg/l of Dimethoate or 0.5; 1 and 2 mg / l of Malathion (Sweilum, 2006). This same growth inhibition was found by Agbohessi et al. (2014) in C. gariepinus juveniles exposed to Endosulfan and Tihan. A single cause is at the base of this delay in growth: the chemical stress induced by the toxicant (Agbohessi et al., 2014; 2021a and b). Stress influences growth through modification of feeding behavior, energy metabolism, feed conversion efficiency and / or hormonal stimulation (Brett, 1979; Pickering et al., 1991; Gregory and Wood, 2010; Agbohessi et al., 2021a and b). In fact, in the present study, the herbicide Atrazine significantly influenced the food intake in fish throughout the test period, the rest of the food being more important in exposed fish. Several studies have already shown that the difference in food intake negatively influences the growth of fish (Lal et al., 2013; Agbohessi et al., 2014). However, Atrazine did not significantly influence feed efficiency and the protein efficiency ratio in this study. Indeed, the results of the present study indicate a statistical similarity between the feed efficiency

of the exposed fish and the controls and also a statistical similarity between their protein efficiency ratios. These results are contrary to those of Agbohessi et al. (2014) in the exposure of juveniles of C. gariepinus to Endosulfan and Tihan, and to those of Agbohessi et al. (2021a) in the exposure of juveniles of the same species to the insecticide Pyro FTE 472 EC. Furthermore, several studies (Jarvinen and Tanney, 1982; Cleveland and Hamilton, 1983; Huynh and Nugegoda, 2012) have also shown that the decrease in growth may be the result of inhibition of the acetylcholinesterase. However, studies have shown that Atrazine would disrupt the synthesis of acetylcholinesterase in zebrafish Dario rerio (Schmidel et al., 2014), other studies have rather indicated that this herbicide associated with Chlorpyrifos would have synergistic effects on the permanent inhibition of acetylcholinesterase in certain aquatic organisms including Common carp Cyprinus carpio (Xin et al., 2014; Mit et al., 2021). Lal et al. (2013) found a significant decline in plasma levels of GH and IGF-I in Malathion exposed Asian catfish Heteropneutes fossilis and showed that this decline was related to reductions in fish growth, also due to low food intake and influence of the pesticide on metabolization of feed into somatic growth. Other important factor explaining the delay in growth could be the transformation into energy of a portion of nutrients from digestion of food consumed to cope with chemical stress that constitutes the exposure to agricultural pesticides.

Changes in energy budgets

Significant effects of Atrazine exposure on the carbohydrate budget of C. gariepinus fingerlings were observed until the end of the experiment, suggesting that carbohydrate reserves were used quickly after chemical exposure to cope with energy demand. Vijayavel et al. (2006) explained this carbohydrate depletion by hypoxic conditions caused by the toxicant, which result in the extra expenditure of carbohydrate metabolism. Several other authors (Kharat et al., 2009; Tendulkar and Kulkarni, 2012; Agbohessi et al., 2014) have found that it is rather the glycogen which is used and its depletion is due to the increase of glycogenolysis induced by an elevation of the activities of phosphorylase, succinate and pyruvate dehydrogenase leading to anaerobic metabolism during anoxic stress conditions caused by the toxicant. Protein levels were significantly different in fish exposed to Atrazine compared to control. Even though protein is a prominent source of energy in fish, chemical stress in this study has preferably caused depletion of carbohydrate reserves instead of proteins. The decrease in protein level observed in the present study is contrary to the results of De Coen and Janssen (1997) in Daphnia magna when exposing them to different concentrations of Cadmium, Tributyltin, linear Alkyl sulfonic acid, Lindane and 2,4-Dichlorophenoxy acetic acid (2,4-D) and from Racotta and Hernandez-Herrera (2000) in white shrimp Penaeus vannamei exposed to 1.07 mmol / L of Ammonia-N. A decrease of protein levels observed in this study means hydrolysis and oxidation through tricarboxylic acid cycles to meet the increased demand for energy caused by the chemical stress (Mulet et al., 2007). The impact of pesticides on lipid content of exposed fish was also visible during the test. The reduction of fat content was observed in exposed fish during the test, much more marked the week 2 and less marked the week 4. This reduction in lipid level is relatively less compared to that in protein level. The depletion of lipid after pollutant exposure has been documented in different fish species chronically exposed to pollutants (Palackova et al., 1994; Sancho et al., 1998; Handy et al., 1999; Smolders et al., 2003; Agbohessi et al., 2014). Loss of lipids may be due to lipid synthesis inhibition and mobilization of the stored lipids, either through β-oxidation or through a gradual unsaturation of lipid molecules as suggested by Jha (1991).The fact that the individuals exposed to Atrazine are in carbohydrate deficit during the experiment, despite the high stock of this compound at the start, expresses that it is this compound which is used primarily by these fish to cope with chemical stress induced by Atrazine, followed by proteins and then after lipids. This observation is contrary to that reported by Agbohessi et al. (2014) in juveniles of C. gariepinus subjected to Endosulfan in which protein sparing was noted.

The changing in whole-body energy budget of fingerlings of C. gariepinus reveals a total drop in the energy level in exposed fish, especially in T2 during the first two weeks, which means that during this period the stress was at its height, and justifies the intensity of the behavioral modifications and the mortality of the fish recorded during this period, especially in T2. But the considerable

increase in whole-body energy budget of fingerlings in the last two weeks means that the fingerlings after a residence time have become accustomed to their new environment. This observation was also reported by Agbohessi et al. (2014) in their study mentioned above.

CONCLUSION

In conclusion, the results of the study showed that Atrazine induced chemical stress which caused behavioral reactions and some mortalities, much more noticeable in the first two weeks. This stress influenced feed intake and energy metabolism in this species and affected the weight growth of the fish. To cope with this stress, those are rather lipids which have been spared to the detriment of proteins. This study once again reveals the nuisances suffered by fish in their natural habitat exposed to multiple pollutants.

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