ANIMAL SCIENCE AND GENETICS
Published by the Polish Society of Animal Production
vol. 18 (2022), no 3, 1-10
DOI: 10.5604/01.3001.0015.9691
Review Article *Open Access*

Roundup in the aquatic environment and fish reproduction

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SUMMARY

This review paper aims to present the effects of Roundup in the aquatic environment on fish reproduction. It describes the mechanism of action and toxic effects of Roundup and its active ingredient glyphosate on non-target organisms — flatworms, amphibians, and mammals. Commercial formulations based on glyphosate are dangerous for living organisms, as they lead to behavioural disorders, reduce reproductive parameters, and may cause metabolic disorders. In fish, Roundup causes damage to the liver, brain, and gills, and triggers degenerative changes in the kidneys. Sub-lethal doses of this pesticide impair locomotor activity and damage DNA through oxidative stress. Many experiments show toxic effects of Roundup on fish reproduction. Reported anomalies include disturbances in embryogenesis (e.g. pericardial oedema or tail bend), reduced embryo survival, and an increase in the number of deformed larvae. Roundup and glyphosate inhibit ovarian follicular growth in adult females. Adult males exposed to the pesticide have inferior semen quality, with limited sperm concentration and motility. Roundup represents a serious threat to land and water organisms, and therefore its use must be limited.

KEY WORDS: **pesticides, fish, reproduction, Roundup**

INTRODUCTION

Roundup, a non-selective herbicide containing glyphosate as its active ingredient, is among the most commonly used pesticides globally (Spinaci et al., 2000; Annett et al., 2014). Popularized in the early 1970s, it was long perceived as a product with few adverse effects. Roundup is considered a 'total herbicide', as its active ingredient, glyphosate, is toxic to both monocotyledons and dicotyledons. Active translocation of glyphosate throughout the plant is supported by the surfactants contained in Roundup, including polyoxyethylene amine (POEA) (Van Bruggen et al., 2018).

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Received: 27.04.2022 Received in revised form: 25.07.2022
Accepted: 18.08.2022 Published online: 22.08.2022

Glyphosate inhibits the activity of many enzymes involved in the synthesis of amino acids (including tyrosine) (Panetto et al., 2019). The global dominance of this herbicide, especially in transgenic crops, is due to its high effectiveness and the low cost of the technology involved.

Long-term, intensive use of glyphosate has caused some weeds and microorganisms to become resistant to it (Van Bruggen et al., 2018). Research from the last decade confirms numerous adverse effects of Roundup, including accumulation in soil (Kwiatkowska et al., 2013) and in animal organs and urine (Cai et al., 2020), as well as increased oxidative stress in animals (Santo et al., 2018). Roundup is increasingly thought to cause direct harm to human health as well — in 2015, the WHO reclassified glyphosate as probably carcinogenic to humans. Glyphosate has been shown to contribute to necrosis and apoptosis in various types of cells. Notably, commercial preparations containing glyphosate (including Roundup) are considerably more toxic than pure glyphosate (Kwiatkowska et al., 2013).

Glyphosate-containing products are classified as practically nontoxic to birds and mammals, possibly slightly toxic for aquatic invertebrates and fish, and moderately toxic to amphibians (Annett et al., 2014). In mammals, glyphosate is excreted mostly unchanged in urine (Sulukan et al., 2017) and has been found at low concentrations in human blood (Kwiatkowska et al., 2013). The compound and its metabolite aminomethylphosphonic acid (AMPA) are also detectable in foods such as sugar, maize, and soy (Cai et al., 2020).

Impact of commercial glyphosate preparations on non-target organisms

Research on glyphosate toxicity consistently demonstrates its harmfulness. The toxicity of glyphosate-based herbicides to non-target species seems to result from the multiple surfactants added (Annett et al., 2014). Exposure to even very low doses of glyphosate and its metabolites, resulting from environmental accumulation, seem to be especially dangerous to animal health (Van Bruggen et al., 2018). Tree frog tadpoles exposed to sublethal concentrations of Roundup exhibited impaired perception, resulting in greater vulnerability to predator attacks, which indirectly adversely affected the entire population (Moore et al., 2015). Similar findings were reported in an experiment on the flatworm *Girardia tigrina*, in which chronic exposure to glyphosate resulted in behavioural disorders and reduced fertility parameters. The LC50 of glyphosate for the species (the concentration leading to the death of 50% of the population), after 48 h of exposure, was 35.94 mg/L. Similar concentrations are commonly recorded in surface waters (López Córdova et al., 2019).

Roundup significantly affects invertebrate and vertebrate reproduction. Roundup® GT Max and glyphosate caused delayed and spontaneous maturation of oocytes of the frog *Xenopus laevis*, as well as meiotic spindle abnormalities (especially Roundup at a concentration of 1480 μM). All specific oocyte development disorders occurred only after direct exposure to Roundup or to glyphosate alone (Slaby et al., 2020). Analysis of porcine oocyte maturation in vitro showed no impact of glyphosate or Roundup on cell nucleus and embryo cleavage, but it did demonstrate impaired oocyte development in terms of blastocyst development rate and cell number. The study also confirmed the higher toxicity of Roundup compared to glyphosate alone, highlighting the role of adjuvants used in commercial preparations in the compound's toxicity (Spinaci et al., 2020). An experiment using bovine embryos also led to alarming conclusions, demonstrating that Roundup concentrations commonly found in agricultural areas lead to rapid developmental arrest and the death of the embryos (with increased numbers of apoptotic blastomeres). Teratogenic effects were observed even at very low doses of the herbicide: 0.9 parts per million (ppm). Abnormal calcium levels in the bovine embryos indirectly increased oxidative stress (Cai et al., 2020). Roundup and glyphosate can significantly disturb spermatogenesis. Delocalization of the signal from the membrane to the cytoplasm was observed in Sertoli cells from 20-day-old rats exposed to glyphosate or Roundup concentrations of 10 or 100 ppm. Although cell viability was maintained, their integrity was affected (Gorga et al., 2020). As the sustentacular cells of the testis form a part of the blood-testis barrier, any structural abnormality may significantly disrupt sperm maturation.

Adverse impact of Roundup in the aquatic environment, as exemplified by its impact on fish

Glyphosate-containing preparations and glyphosate metabolites, such as aminomethylphosphonic acid (AMPA), are currently detected in surface waters (Annet et al., 2014; Poiger et al., 2017; Smith et al., 2019). One reason is that these herbicides may be used in home gardens by untrained individuals without the necessary precautions. This leads to groundwater and surface water pollution. The amount of glyphosate acid equivalents detected in natural water bodies ranges from 0.01 to 5.2 mg/L (Annet et al., 2014; Smith et al., 2019), but in more contaminated aquatic ecosystems, glyphosate-based herbicide concentrations reach hundreds of micrograms per litre; the highest on record is 328 mg/L (Geyer et al., 2016). Glyphosate is degraded in the environment mainly by soil bacteria, but its half-life in soil varies widely and reaches up to 142 days (Annett et al., 2014). The estimated half-life of Roundup in water is between 45 and 60 days (Bridi et al., 2017). The accumulation of glyphosate and its adjuvants in aquatic environments poses a direct risk to organisms inhabiting them, including fish. Since Roundup is known to be toxic to terrestrial animals, similar effects are likely to occur in aquatic animals as well.

When *Jenynsia multidentata* fish were exposed to Roundup concentrations of 0.5 mg/L for 7 and 28 days, morphological changes were reported in the gills and liver. In another study, the same doses of Roundup introduced into an aquatic environment for 24 h or 96 h caused liver, gill, and brain damage in the same species (Sánchez Albañil et al., 2019). Three-month exposure of the Nile tilapia (*Oreochromis niloticus*) to the same herbicide at concentrations of 2.4 and 7.2 mg/L caused not only gill and liver damage, but also kidney abnormalities and highly elevated liver enzyme levels in the plasma (Annett et al., 2014). A study of the short-term effects of Roundup on *Clarias albopunctatus* fish demonstrated significant increases in biochemical parameters — total and protein-bound bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, creatinine, and urea. In addition, histomorphological analysis of the gills and liver revealed severe degeneration of these organs (Okonkwo et al., 2013). Given the findings on the short-term effects of the herbicide, the conclusions reported from an experiment on the impact of glyphosate on fish over a much longer period were surprising. Chronic exposure to an environmentally-representative dose of glyphosate (1 mg/L) for 10 months caused no change in reproductive parameters or mortality in rainbow trout (*Oncorhynchus mykiss*). Acetylcholinesterase and carbonic anhydrase activity remained at similar levels over time (Du-Carrée et al., 2021).

In the aquatic environment, predator recognition is key to survival, and in most cases, this function relies on a variety of olfactory stimuli. Any chemical, e.g. a pesticide, entering a body of water reduces animals' sensitivity to danger by modifying the chemical properties of the water. Common spiny loach (*Lepidocephalichthys thermalis*) exposed to a sublethal dose of Roundup (0.5 mg/L) were unable to detect alarm signals for 2 days. The herbicide also impaired the ability of the fish to learn to escape from danger. Anthropogenic stressors, including glyphosate, interfere with the sensitive respiratory surfaces of fish and amphibians, potentially leading to failure to identify threats (Tapkir et al., 2019). Exposure to Roundup or pure glyphosate (at doses of 0.01, 0.065, and 0.5 mg/L) for 96 hours impaired locomotor activity in 3-day-old zebrafish (*Danio rerio*) larvae. In addition, young zebrafish exposed to glyphosate at a concentration of 0.5 mg/L had decreased interocular distance. In adult zebrafish, Roundup at 0.5 mg/L caused severe memory impairment and significantly reduced aggressive behaviour (Bridi et al., 2017). The study suggests that Roundup and pure glyphosate may have very similar toxic effects in terms of behavioural and morphological changes in zebrafish at all stages of life. Behavioural disorders in fish exposed to glyphosate were confirmed in a study on *Piaractus mesopotamicus*, fish from the piranha family. Fish exposed to glyphosate concentrations of 0.2 ppm and 0.6 ppm for 15 days showed reduced food intake on the 13th day, although it had returned to normal by the end of the experiment. Glyphosate at a sublethal dose of 1.8 ppm drastically reduced food intake, which did not normalize during the experiment. A persistent reduction in food intake may have a number of negative consequences, disrupting the homeostasis of the body and significantly inhibiting growth (Giaquinto et al., 2017).

The metabolism of xenobiotics leads to the accumulation of reactive oxygen species (ROS) in the tissues of exposed organisms. The consequences include oxidative-stress-induced DNA damage and protein oxidation. In young tambaqui (*Colossoma macropomum*) fish exposed to Roundup, in addition to histological alterations in the gills, researchers reported ROS production, DNA damage in red blood cells, and reduced cholinesterase activity in the brain (Braz-Mota et al., 2015). In hybrid catfish (*Pseudoplatystoma reticulatum* × *Leiarius marmoratus*), exposure to sublethal Roundup concentrations (1.357 mg/L) for 6–96 h reduced the enzymatic antioxidant response in the brain and liver after 48 h, increased glutathione-S-transferase (GST) levels in the brain and gills, and reduced acetylcholinesterase (AChE) activity in the muscle at all times analysed. There were interesting findings concerning changes in non-protein thiol (GSH) levels in the liver, with a drop after 6 and 24 hours of exposure followed by an increase at 48 and 96 hours (De Moura et al., 2017). Short-term exposure to glyphosate preparations affects stress markers in fish, causing a response involving increased expression of the genes responsible for oxidative stress regulation (Velasques et al., 2016; Dos Santos Teixeira et al., 2018). Adult zebrafish were exposed to glyphosate and Roundup at a concentration of 0.1 mg/L, after which their ability to detoxify through protein activity was analysed. The analysis indicated that the brain was the most vulnerable tissue, as exposure to the herbicide did not trigger the detoxification mechanism in this tissue (Moraes et al., 2020).

The studies on terrestrial and aquatic organisms cited above clearly demonstrate the harmful effects of glyphosate and its commercial preparations. The broad range of adverse effects of these substances, from neurological disorders to permanent tissue and organ degeneration, is notable. Roundup has a destructive impact on organisms as a whole. As harmful substances accumulate in water, organisms living in such ecosystems are in constant danger. Not only do the resulting disorders affect the welfare of individual animals, but they also threaten entire populations.

Reproductive disorders in fish caused by Roundup accumulation in the environment

The acute toxicity and teratogenic effects of glyphosate were first observed in amphibians, due to their dependence on aquatic environments (Moore et al., 2015). Later, experiments on zebrafish embryos confirmed its toxicity to fish as well. Exposure to glyphosate at a dose of 50 μg/mL caused a number of abnormalities, including smaller eyes and heads as well as loss of delineated brain ventricles in 24-hour-old embryos. Glyphosate was found to be toxic to the fore- and midbrain, but not to the hindbrain (Roy et al., 2016). Roundup also has a negative impact on embryogenesis in zebrafish. At a sublethal dose of 58.3 mg/L, it leads to dysregulation of energy metabolism during embryogenesis, preventing inflation of the swim bladder (Panetto et al., 2019). Roundup and glyphosate alone can restrict egg production, increase early embryo mortality, and accelerate hatching in zebrafish. A 10 mg/L concentration of Roundup affects the expression of the genes *cyp19a1* and *esr1* in the ovaries and *hsd3b2*, *cat*, and *sod1* in the testicles of adult zebrafish (Uren Webster et al., 2014). Another experiment on zebrafish larvae did not demonstrate clear neurotoxic effects, but a dose of 5 µg/L did affect motor coordination and the response to aversive stimuli (Lanzarin et al., 2020). Doses of 8.5 µg/L increased mortality, lowered the heart rate, and increased the incidence of developmental defects in embryos, including pericardial and yolk sac oedema and tail bending (Gaur and Bhargava, 2019; Lanzarin et al., 2020). The cardiotoxicity of glyphosate results directly from altered expression of genes involved in calcium signalling and reduced generation of nitric oxide (NO), which regulates vascular smooth muscle tone (Gaur and Bhargava, 2019). Embryos exposed to glyphosate have reduced carbonic anhydrase activity, causing oxidative stress. This indirectly affects the rate of apoptosis and thus the incidence of a variety of developmental defects (Sulukan et al., 2017).

Research on the harmful effects of glyphosate in young fish has demonstrated significant increases in early mortality. It is likely that the main toxic effects do not involve embryos, but earlier processes associated with fertilization (Fiorino et al., 2018).

The teratogenicity of Roundup was confirmed in an experiment on Japanese medaka (*Oryzias latipes*). Exposure to glyphosate (0.5 mg/L) and Roundup (at doses of 0.5 mg/L and 5 mg/L) for the first 15 days of embryonic development reduced hatching parameters and caused a range of developmental defects (including spinal deformation and yolk sac enlargement). In addition, in adult female medaka exposed to glyphosate in early life, expression of the kisspeptin receptor (*Gpr54-1*), key to hormonal regulation of fish reproduction, was reduced (Smith et al., 2019). An experiment to investigate the impact of Roundup on embryonic development and hatching in common carp (*Cyprinus carpio L.*) confirmed that it inhibited hatching and increased the number of deformed larvae. This provided further evidence of the genotoxicity of Roundup, which resulted in altered transcription of the *foxr1* gene, necessary in early embryogenesis and gametogenesis (Socha et al., 2021).

Female zebrafish were exposed to low doses of Roundup for 15 days, after which their ovaries were analysed. The findings included increased numbers of initial ovarian follicles and reduced numbers of intermediate and late follicles. The follicles showed increased perivitelline space, with decreased diameter and doubled vitelline content (following exposure to concentrations of 0.065 and 6.5 mg/L) (Davico et al. 2021). In another experiment on female zebrafish, the authors reported an increased tendency towards excessive accumulation of connective and hematopoietic tissue in the ovaries (Velasques et al., 2016).

In male carp, Roundup slightly reduced sperm motility but significantly increased the mortality of newly hatched larvae (Ługowska, 2018). Glyphosate in commercial preparations reduces sperm motility and concentrations in male *Jenynsia multidentata* fish (Sánchez Albañil et al., 2019), as well as reducing their mating activity and number of copulations (Hued et al., 2012). Male guppies (*Poecilia vivipara*) exposed to Roundup for 96 hours at concentrations of 130 and 700 µg/L produced semen with significantly poorer sperm parameters than in controls: impaired plasma membrane integrity, reduced motility, and lower sperm concentration (Harayashiki et al., 2013). An analysis of eggs and semen of grass carp (*Ctenopharyngodon idella*) exposed to Roundup (at concentrations of 0.1–10 mg/L for the eggs and 0.1–50 mg/L for the semen) demonstrated increased swelling of the eggs and reduced sperm motility. Alterations occurring directly in the eggs may be due to disturbed electrolyte balance or altered physical properties of the eggs resulting from exposure to the pesticide (Ługowska, 2020). In male zebrafish exposed to glyphosate at concentrations of 5 and 10 mg/L, there was no change in sperm concentration, but sperm motility was strongly limited. Other common findings included reduced mitochondrial membrane integrity and DNA damage in the sperm (Lopes et al., 2014).

Over the course of centuries, many aquatic organisms, including *Austrolebias nigrofasciatus* fish, have adapted to difficult environmental conditions (periodic drying of water bodies), through mechanisms such as increased tolerance for higher temperatures and embryonic diapause. Exposure of adult fish of this species to Roundup during the spawning period caused females to lay significantly fewer eggs and reduced the temperature tolerance of the embryos by more than 2.6°C (Zebral et al., 2018). Continued widespread use of this herbicide may lead to the extinction of many species that face adverse environmental conditions on a daily basis, given their limited adaptive capacities, which take years of evolution to develop.

Chronic exposure of fish to the toxic effects of glyphosate results in decreased immunity, inhibited growth, lower survival rates, and a reduced hepatosomatic index (HSI) and gonadosomatic index (GSI). In red hybrid tilapia (*O. niloticus* × *O. mossambicus*) exposed to glyphosate for 7 weeks, weight reduction was observed at a dose of just 25 mg/L. There was also a negative correlation between glyphosate concentration and both HSI and GSI. In fish, effective vitellogenesis is closely associated with these parameters, and thus their reduction consistently limits the reproductive potential of the exposed individuals (Muhammad et al., 2021).

SUMMARY

The cited research on the impact of Roundup and its active ingredient, glyphosate, on non-target organisms, including humans, constitutes a clear warning. Substances purported to support agricultural development and increase yields have caused the death of many species that were not targeted.

Roundup seems to have harmful effects on virtually the whole spectrum of life. It disrupts natural behaviours required for survival, damages organs, interferes with or even prevents reproductive success, and ultimately causes death. Its presence in the environment has resulted in the collapse of many populations and forced numerous species to adapt quickly under unfavourable conditions.

The present review was primarily aimed at presenting the destructive impact of this popular herbicide on the aquatic environment, where organisms have limited opportunities for migration. Many fish species with high adaptability to extreme conditions have proven to be very vulnerable to glyphosate and its commercial preparations. Whether the herbicide affected eggs or adult animals, the consequences were dramatic for the entire population.

The accumulation of Roundup in soil and water is particularly dangerous. The example of fish clearly shows that both low and high concentrations of the compound have an adverse impact on multiple species. The real-life consequences of introducing this popular herbicide into the environment are difficult to predict. The similarities in the adverse effects of Roundup in fish and terrestrial mammals raise even more concerns, suggesting that a direct threat to humans exists as well. Complete discontinuation of Roundup use by farmers and households seems to be the only adequate solution. This process could take many years, however, thus sealing the fate of numerous terrestrial and aquatic animals. There is no doubt that the consequences of the popularization of this herbicide will be felt for decades to come.

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Funding information: Activation of scientific activity accounting records number: A 201 and A 255.