TÜBİTAK

Turkish Journal of Zoology

Volume 47 | Number 2 Article 6

1-1-2023

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Turkish Journal of Zoology
http://journals.tubitak.gov.tr/zoology/
Research Article
Turk J Zool
(2023) 47: 103-110
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doi:10.55730/1300-0179.3120

Lethal and sublethal effects of exposure to Roundup 360 Plus for the *Chaoborus flavicans* larvae (Diptera: Chaoboridae)

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Received: 10.10.2022 • **Accepted/Published Online:** 31.01.2023 • **Final Version:** 01.03.2023

Abstract: The lethal and sublethal effects of Roundup (active substance glyphosate herbicide on phantom midge (*Chaoborus flavicans*, Meigen, 1830) larvae were checked in the range of concentrations from 20 to 2000 µg/L, in the five-day long tests. The herbicide caused the mortality of insects, with the LC_{50} values ranging from 357.2 to 120.9 µg of glyphosate/L, for one and five days, respectively. The correlation coefficient >0.9 indicated a strong positive correlation between the concentration of Roundup in water and the mortality of *C. flavicans*. Also, the LT_{50} values indicate the toxic activity of the herbicide for the larvae. Also, the reaction to mechanical stimuli was disturbed, in a dose-dependent manner. We also observed the altered vertical migration of midges within the water column. The microscopic observations revealed malformations in insects' morphology and anatomy. Among the most prominent changes were: altered body shape and lower density of chromatophores in air sacs. These changes are in tune with the microscopic observations. We conclude that Roundup 360 Plus herbicide may cause significant changes within *C. flavicans* populations, especially during long exposure. This, in consequence, may lead to a decline in the population of phantom midges, and next, affect the organisms at the other trophic levels.

Key words: Roundup, glyphosate, phantom midge, *Chaoborus flavicans*, lethal toxicity, sublethal effects

1. Introduction

Roundup belongs to the most popular broad-spectrum pesticides. It is used as a weed killer, both by farmers, as well as small farm holders, and homeowners. Its active substance-glyphosate (GLY)- is a systemic herbicide, active against various groups of autotrophs. Its biological activity focuses on the inhibition of EPSP synthetase which is not present in animals (Weber et al., 2016). Therefore, it is regarded as potentially nonharmful for animals. That, together with the introduction of Roundup Ready plants led to the massive use of Roundup. Some researchers postulated the toxicity of this herbicide for animals (Rzymski et al., 2013; Straw et al., 2020). In consequence, the debate concerning the toxicity of Roundup and glyphosate has begun. After many years of controversies and conflicting opinions, the International Agency for Research on Cancer classified glyphosate in 2015 and classified glyphosate as "possibly carcinogenic to humans" (Guyton et al., 2015). However, the European Union has approved the use of glyphosate as a plant protection product in agriculture until 2022 (Commission Implementing Regulation (EU) 2017/2324[1]). GLY's chemical and physical properties indicate that it should not bioaccumulate, nor biomagnify through the food chain to any considerable extent (USEPA 1993; WHO1994; Rzymski et al., 2013; Giesy et al., 2000). GLY is almost exclusively degraded by microbial-produced enzymes to sarcosine or aminomethylphosphonic acid (AMPA) (Weber et al., 2016, von Mérey et al., 2016).

Roundup is primarily used in terrestrial ecosystems and has a strong affinity for soil particles, and low mobility (Vereecken, 2005), it has been found in groundwater and aquatic ecosystems (Horth and Blackmore, 2009). GLY and its metabolites may be transferred to aquatic ecosystems mainly by surface runoff, leaching from soil particles to shallow groundwaters. It reaches surface waters by direct application (Rzymski et al., 2013; Matozzo et al., 2020). The presence of Roundup and GLY has been demonstrated in agricultural land and municipal sewage, rainwater, and tap water (Rendón-von Osten and Dzul–Caamal, 2017; Van Bruggen et al., 2018). In water ecosystems at pH > 3.5, GLY is easily ionized and strongly absorbed into

[1] Commission Implementing Regulation (EU) 2017/2324. Retrieved from: //eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R2324

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sediments. This makes it particularly toxic to organisms living permanently or periodically in the bottom zone of water bodies. Data concerning the impact of GLY and Roundup formulations on other water animals are scarce. GLY was reported to increase glutathione-S-transferase activity and malondialdehyde content in *Lymnea* snails (Fadhlaoui and Lavoie, 2021). Sublethal toxicity has been observed for picoplanktonic cyanobacteria (Zagarese et al., 2022), arthropods, echinoderms, and fish (Gill et al., 2018). The toxicity of GLY was reported for *Daphnia* sp. and *Artemia* sp. (Cuhra et al., 2013; de Brito Rodrigues et al., 2017; Suppa et al., 2020). These organisms provide a food base for other invertebrates and aquatic vertebrates. Differences in species sensitivity to glyphosate-based herbicide exposure can lead to a decrease in some species richness and affect ecosystem trophic chains (Relyea, 2005).

Rzymski et al. (2013) found a decrease in biodiversity and biomass of the benthic invertebrate community after direct Roundup application to the lake ecosystem. Some experiments showed the toxicity of GLY and its commercial formulations on different levels of biological organizations of mollusks, like altered gametogenesis, disrupted larval development, a decrease in weight and shell length, and mal-activity of digestive glands of marine bivalve *Crassostrea gigas* (Akcha et al., 2012; Séguin et al., 2017). Also, disruption of biochemical markers for *Ruditapes*, *Mytilus*, and *Unio* mussels exposed to GLY was stated (Hanana et al., 2012; Malecot et al., 2013; Matozzo et al., 2018; Milan et al., 2018; Matozzo et al., 2019). Roundup formulations may also alter the behavior of many aquatic organisms. Amphibians and fish may avoid zones contaminated with Roundup formulations (Tirney et al., 2011). Also, Bengtson et al. (2004) found a decrease in the feeding activity of *Daphnia pulex*.

However, the effects of Roundup compounds on aquatic ecosystems are still not clearly elucidated. For these reasons, it was decided to investigate the effects of the Roundup 360 Plus herbicide on the larvae of a phantom midge *Chaoborus flavicans* (Diptera, Chaoboridae). This is a cosmopolitan species, common in various types of water reservoirs and rivers. The larvae are transparent, which enables observation of internal organs, like air sacs or gut. Next, it is regarded as a species resistant to various stressors. Altogether, these features make phantom midges an interesting, and not yet sufficiently recognised species, in terms of their ecological and ecotoxicological significance. The prospective toxic effects observed in this species may suggest that also the other aquatic invertebrates, less resistant to stressors, may be affected by Roundup. Conversely, if *C. flavicans* were not affected by the herbicide, there would be a high probability that the pesticide was not harmful to other invertebrates, too. The next point supporting the need for the research is that the data describing the effect of Roundup and glyphosate on aquatic invertebrate fauna is scanty. Therefore, we decided to test the selected toxic effects of the popular herbicide – Roundup – on this arthropod species. The set of tests, including lethality, reactivity to stimuli, behavioral responses, and morphological malformations may, in our opinion, bring a set of interesting data concerning the toxic effects of Roundup herbicide to water ecosystems. In this paper, we present the results of our preliminary research on the toxicity of Roundup 360 Plus for *C. flavicans* larvae. We focused on the lethal and sublethal effects of this herbicide, within the 5-day long term of exposure.

2. Materials and methods

2.1. Used herbicide

We used commercially acquired Roundup 360 Plus (Monsanto Europe S.A.). That strategy imitated a real case of environmental pollution. However, all concentrations were recalculated to show the amount of glyphosate in solutions. We used the range of concentrations in water, from 20 to 2000 μg of glyphosate/L, dissolved in water obtained from the local lake (Strzeszynskie Lake, Wielkopolska, Poland).

2.2. Animals and mortality tests

Larvae of *C. flavicans* were obtained from the commercial supplier. The larvae were placed in plastic Falcon Tissue Culture Flask bottles of 250 mL, in the following concentrations of GLY: 0 (control), 20, 50, 100, 200, 400, 1000, and 2000 μg/L. There is no data concerning the lethality of GLY to *C. flavicans*. Therefore, we selected the range of concentrations that have already been tested for aquatic invertebrates, where LC_{50} for Roundup was estimated as 220 μg/L/24 h and 0.8-μg/L/48 h (Husak et al., 2022). Having in mind, that *C. flavicans* is larger than daphnids, we also increased the range over the concentrations used by Husak and colleagues. In each of the trials, 15 *C. flavicans* larvae were used. The mortality was checked every 24 h, until 120 h, and the mortality-concentration curves, the correlation between survival and herbicide concentration (R^2), LC_{50}, and LC_{95} values were determined using probit analysis (Finney 1971). Next, the time of exposure that led to the mortality of 50% of exposed insects (LT_{50}) was calculated, for each tested concentration of GLY. All the tests in this study were duplicated.

2.3. The effect of Roundup 360 Plus on the reactivity of *C. flavicans* to mechanical stimuli

Groups of 5 larvae were placed in 150 mL Petri dishes, in the following five concentrations of the glyphosate + control: 20, 50, 100, 200, and 400 μg/L. Every 24 h, the insects were touched gently with a needle. The reaction was classified as 0- no activity, 1-low activity (slow, fine moves of the body), and 2-high activity (rapid, vigorous movements). The results were averaged.

2.4. Effect of Roundup 360 Plus on vertical migrations of *C. flavicans*

Diel Vertical Migration (DVM) is behavior typical for, among others, zooplankton, including phantom midges (Brierley 2014). To test the effect of Roundup 360 Plus on the migrations of *C. flavicans* larvae, the groups of 5 larvae were placed in plastic Falcon Tissue Culture Flask bottles of 250 mL, in the following concentrations of glyphosate: 0 (control), 20, 50, 100, 200, and 400 µg/L. The location of the larvae had been observed every 24 h, for five days. The number of larvae found at the surface, in the water column, and near the bottom, was recorded and classified as 0-bottom of the reservoir, 1-water column, and 2-water surface, and was applied, to calculate the average position of individuals.

2.5. Morphology and anatomy of *C. flavicans* larvae

Morphological malformations were documented using the SteREO Lumar.V12 (Zeiss) stereoscopic microscope. The measurements of the larval size, as well as the size of the air sacs, were done.

2.6. Statistical analysis

The significance of the statistical differences of *C. flavicans* larvae distribution within water column in different GLY concentrations was checked with one-way ANOVA and post-hoc Tukey test. Statistica 13.3, Tibco Software Inc. was used.

3. Results

3.1. Mortality

The larval mortality revealed a concentration-dependent relationship, with a very high correlation coefficient (>0.9). Also, the LC_{50} values decreased over time (Table 1). The two highest concentrations: 1000 and 2000 µg GLY/L, caused acute lethal effects and indicated that very high concentrations of this herbicide can be lethal, even for organisms resistant to various stressors, like phantom midge larvae. The LC_{50} values were relatively high. The first three concentrations did not reach 50% mortality. For the other concentrations, the typical relationship-the higher the concentration, the shorter the LT_{50} time-was noted (Table 2).

3.2. Effect of Roundup 360 Plus on the reactivity of *C. flavicans* to mechanical stimuli

After 24 h, the highest activity was observed for 50, and 100 µg/L, and then at 20, and 200 µg/L. The mobility of the larvae in the water was the earliest trial attempt made in the 400 µg/L control. In the next days, the most vigorous reaction was seen in the control group and the group exposed to 50 µg/L of glyphosate. For the higher concentrations, as the exposure time and the concentration of GLY increased, the reactions were weaker (Table 3).

3.3. Effect of Roundup 360 Plus on DVM of *C. flavicans*

It was found that larvae exposed to higher concentrations of Roundup revealed a very weak ability for horizontal migrations. In the control experiment, it was observed that the *C. flavicans* larvae mainly occupy the middle of the water column. Some individuals migrated to the subsurface zone, but usually, they returned to deeper water (Figure 1). A similar pattern of behavior was found in the experiment where larvae were exposed to 20 µg GLY/L. At higher concentrations, a change in the occupied area was observed, the higher the dose applied, the faster the response was over time. At 50 µg GLY/L, after the first 24 h, the larvae like in the control stayed in the middle of the water column, but after 48 h 50% of the individuals remained permanently near the surface. From 96 h of the experiment, all larvae stayed below the surface. The changes in the larvae distribution between time points of this experiment were statistically significant (Figure 1). Migration toward the surface was even more rapid when larvae were exposed to 100 µg GLY/L, and at concentrations of 200 µg GLY/L and higher, all larvae stayed close to the surface already after 24 h (Figure 1).

Table 1. Probit analysis. LC_{50}, LC_{95}, equation of the function, and correlation coefficient (R^2) values for *C. flavicans* larvae exposed to Roundup 360 Plus, in the following days of exposure.

Exposure time [h]	LC_{50} [µg glyphosate/L]	LC_{95} [µg glyphosate/L]	Equation Y = ax + b	R^2
24	357.523	1676.123	y = 2.1837 x – 0.6734	0.9737
48	225.942	934.952	y = 2.295 x – 0.5046	0.9673
72	169.623	718.409	y = 2.3687 x – 0.2697	0.9762
96	146.391	782.242	y = 2.2773 x + 0.0663	0.9824
120	120.944	598.902	y = 2.3692 x + 0.0655	0.9951

Table 2. LT_{50} values for *C. flavicans* larvae exposed to various concentrations of Roundup 360 Plus.

Glyphosate concentration [µg/L]	LT_{50} [h]	Glyphosate concentration [µg/L]	LT_{50} [h]
0	–	200	64
20	–	400	22
50	–	1000	14
100	–	2000	12

„-„ no mortality ≥ 50% obtained.

Table 3. Intensity of the reaction to the mechanical stimuli of *C. flavicans* larvae exposed to Roundup 360 Plus, in various concentrations.

Exposure time [h]	Glyphosate concentration [µg/L]					
	Control	20	50	100	200	400
24	0.6 ± 0.80	0.2 ± 0.40	1.2 ± 0.91	1.0 ± 0.77	0.2 ± 0.40	0.5 ± 0.67
48	2.0 ± 0.00	0.3 ± 0.46	1.0 ± 1.00	1.0 ± 0.89	0.0 ± 0.0	0.0 ± 0.0
72	0.8 ± 0.40	0.0 ± 0.0	1.0 ± 0.82	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
96	2.0 ± 0.00	0.7 ± 0.64	2.0 ± 0.00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
120	2.0 ± 0.00	1.0 ± 0.77	2.0 ± 0.00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

The table shows mean ± SD, n = 10. Values close to 0 indicate no reaction, 1-weak reaction, and 2-vigorous reaction.

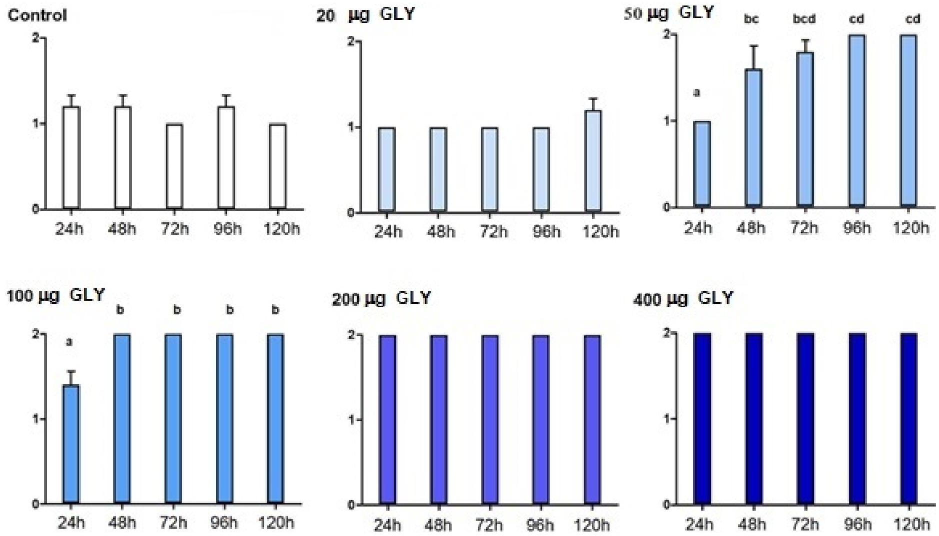

Figure 1. The distribution of *C. flavicans* larvae within the water column under different concentrations of GLY. Box-mean, whisker-SD, n = 10, 0-close to the bottom, 1-in the water column, 2-close to the surface of the water. The letters above boxes indicate significant differences between time points (Tukey test, $p < 0.05$)

3.4. Morphology and anatomy of *C. flavicans* larvae

We did not find any significant differences between the control and tested species, in terms of their body size as well as size of their air sacs. The two pairs of air sacs- the anterior pair and the posterior one (Figure 2)- are clearly visible within the *C. flavicans* larvae. The ones

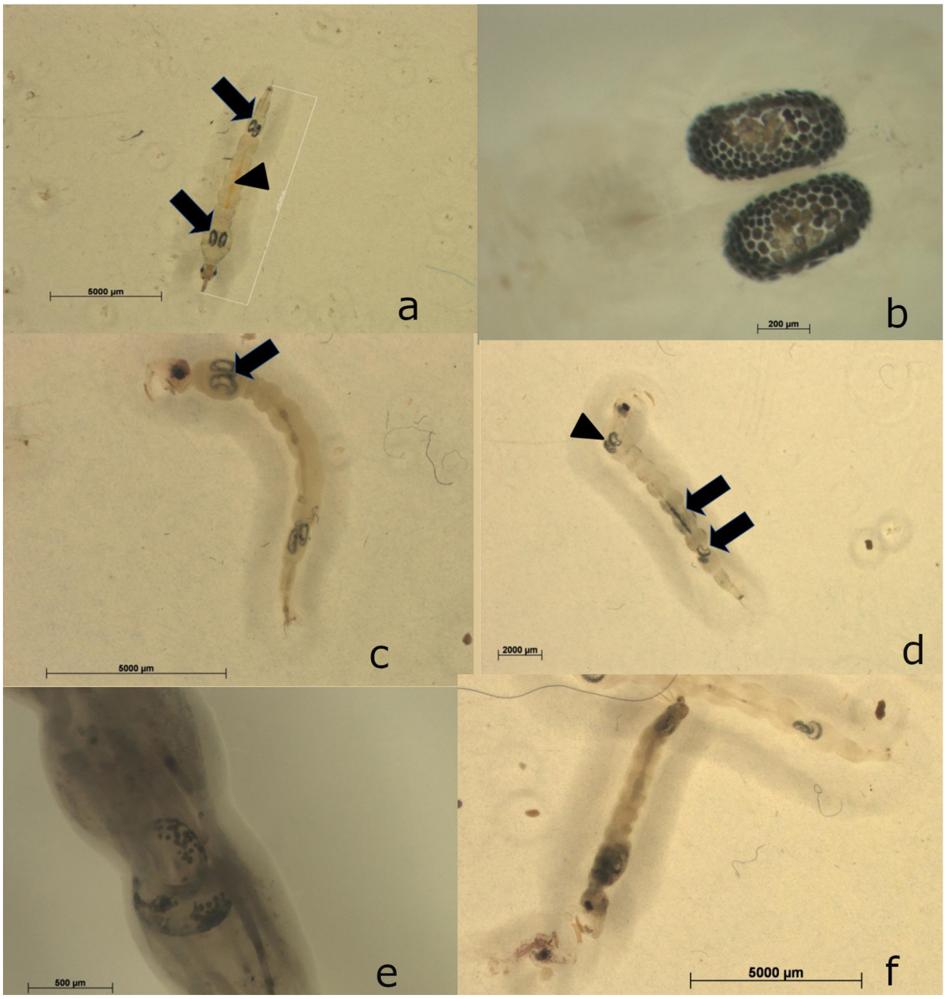

Figure 2. Morphological malformations of *C. flavicans* larvae. a– control organisms. Note two pairs of air sacs (arrows) and the digestive system (arrowhead). b– magnified picture of a pair of air sacs of a control insect. Note the regular distribution of chromatophores over the sacs. c– *C. flavicans* larva exposed to 20 µg/L GLY. Note altered pigmentation of the air sacs (arrow) and contortion of the body. d– *C. flavicans* larva exposed to 100 µg/L GLY. Note altered pigmentation, a contortion of the body that is close to the front air sacs (arrowhead), and darkening of the body. e, f– *C. flavicans* larva exposed to 400 µg/L GLY. Note the disappearance of pigmentation of air sacs, and the darkening of the body.

that were treated with selected concentrations of the herbicide showed significant malformations, compared to the control ones: body contortions close to the air sacs, changes in their pigmentation, as well narrowing of the entire body and its darkening (Figure 2).

4. Discussion

The herbicide caused a concentration-dependent relationship. However, the LC_{50} values were relatively high and the acute toxicity for the majority of the population would be rather difficult to obtain. Nevertheless, the decreasing LC_{50} value suggests that bioconcentration may play an important role in the toxicity of Roundup.

Bioconcentration was reported as an important process in case of cadmium toxicity, to *C. flavicans* (Croteau et al., 2001). On the other hand, Munger and Hare (1997) claimed that the amount of toxins that enter the body of phantom midges is low. Hence, the diffusion of the toxins through the midges' cuticle is still disputable. However, the observed increased mortality, as well as decreasing LC_{50} values, suggest that in the case of Roundup, contact toxicity may play an important role.

The larval mobility did not show a clear linear relationship. For some days, the most vigorous reaction was seen in the control group and the group exposed to 50 µg/L of glyphosate. Perhaps, there is a hormetic range of glyphosate action on *C. flavicans*-low concentrations and low doses of toxic substances may increase the vigor of exposed organisms (Berry III and Martinez, 2020). The weaker reactions in higher concentrations of GLY can be linked with the gradually decreasing vigor of larvae and it may support the previously stated hypothesis, that the toxic effects may be caused not only by the chemicals present in Roundup but also by their breakdown products. Cuhra and coworkers (2013) described immobilization of *Daphnia magna*, after 24 and 48 h of exposure to GLY and Roundup, at the level of 1.4–10.6 µg GLY/L. This suggests that lethal effects may be obtained by higher concentrations. One must have in mind the differences in the body size and body mass of arthropods belonging to both genera. The midges are several times bigger than daphnids. Phantom midges' larvae are ambush predators-after the detection of the smaller specimen, *Chaoborus* strikes and captures the prey-so the reaction is immediate. Next, *Chaoborus* is very readily eaten by fish (Peckan-Heikim et al., 2006), and the ability to respond to predation is crucial (von Ende, 1979). Therefore, the decreased reaction to mechanical stimuli may negatively affect the *Chaoborus* population, either by decreasing their ability to find food or by limiting their ability to escape.

Phantom midge DVM is a behavior that, either allows one to avoid visual predators or ensures active prey. The individuals use to spend the day in the deeper aphotic zone of the lake and migrate up during the night (Rudstam and Johannsson, 2009, Brierley, 2014). The ability to vertical migration within the water column is thus a crucial feature for *Chaoborus* larvae (McQueen et al., 1999, Wissel et al., 2003, Farrell et al, 2017). In the control experiment, the larvae were mainly concentrated in the middle of the water column. Such behavior is typical for phantom midges in fishless ecosystems where predation pressure is negligible (Dawidowicz et al., 1990). These results are in tune with the low reactivity to stimuli and suggest that the herbicide may affect the movement abilities of the midges. Low midges' mobility and a tendency to stay in the clear zone below the water surface suggests that the insects are less able to find prey and also became easy to be hunted. Then, the population of *C. flavicans* may collapse, despite the low lethal effects.

Phantom midge larvae are transparent, slim, and straight. The two pairs of air sacs play an important role in vertical movement and keeping the position in the water well. The sacs are equipped with chromatophores that may contract or expand, changing their color from pale light to darker one. The insects that are closer to the surface of the water have less pigmented air sacs (Weber and Grosmann 1988; Brokent and Brokent 2008). The air sacs alter their color due to their altered pigmentation. Weak pigmentation suggests that the larvae live close to the water surface. These results are in tune with our observations of the mean location of larvae. This suggests that Roundup may affect the ability to move in the water well, as well as affects body shape. Perhaps, the inability to regulate the volume of gas in the sacks is a major cause of the presence of insects close to the surface of the water. The inability of performing successful DVM may decrease the ability to find food and avoid predators. The darkening of the body and the gut is probably a sign of the putrefying of the insect's tissues.

We conclude that Roundup 360 Plus herbicide affects mortality, response to mechanical stimuli, and DVM of aquatic larvae. The observed effects depend on the concentration, but even the small doses affect aquatic organisms if exposure is longer than acute. Considering the environmental effects, one must pay attention to the possible cause-and-effect activity of Roundup: the herbicide may reduce the biomass of producers. This may decrease the number of small herbivorous organisms, on which *C. flavicans* feeds (Moore 1986; Baker et al., 2014). Consequently, this will decline in the population of midges, and next, the abundance of organisms at higher trophic levels.

References

Akcha F, Spagnol C, Rouxel J. (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquatic Toxicology 106-107: 104-113. https://doi.org/10.1016/j.aquatox.2011.10.018

Baker LF, Mudge JF, Houlahan E, Thompson DG, Kidd KA (2014). The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands. Environmental Toxicology and Chemistry 33 (9): 2076-2085. https://doi.org/10.1002/etc.2657

Bengtsson G, Hansson L-A, Mnontenegro K (2004). Reduced grazing rates in *Daphnia pulex* caused by contaminants: Implications for trophic cascades. Environmental Toxicology and Chemistry 23 (11): 2641-2648. doi.org/10.1897/03-432

Berry III R, López-Martínez G (2020). A dose of experimental hormesis: When mild stress protects and improves animal performance. Comparative Biochemistry and Physiology. Part A 242: 110658. https://doi.org/10.1016/j.cbpa.2020.110658

Borggaard OK, Gimsing AL (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. Pest Management Science 64 (4): 441-456. https://doi.org/10.1002/ps.1512

Borkent CJ, Borkent A (2008). Description and phylogenetic interpretation of chromatophore migration from larval air sacs to adult structures in some Chaoboridae (Diptera). The Canadian Entomologist 140 (6): 630-640. doi.org/10.4039/N08-049

Brierley AS (2014). Diel vertical migration. Current Biology 24 (22): R1074-R1076. doi.org/10.1016/j.cub.2014.08.054

van Bruggen AHC, He MM, Schin K, Mai V, Jeong KC, Finckh MR, et al. (2018). Environmental and health effects of the herbicide glyphosate. Science of the Total Environment 616-617: 225-268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Calabrese EJ, Baldwin LA (2002). Defining hormesis. Human and Experimental Toxicology 21 (2): 91-97. https://doi.org/10.1191/0960327102ht217oa

Carles L, Gardon H, Joseph L, Sanchís J, Farré M, et al. (2019). Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms. Environment International 24: 284-293. https://doi.org/10.1016/j.envint.2018.12.064

Croteau MN, Hare L, Tessier A (2001). Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*. Canadian Journal of Fisheries and Aquatic Science 58 (9): 1737-1746. https://doi.org/10.1139/cjfas-58-9-1737

Cuhra M, Traavik T, Bøhn T (2013). Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. Ecotoxicology 22: 251-262. https://doi.org/10.1007/s10646-012-1021-1

Dawidowicz P, Pijanowska J, Ciechomski K (1990). Vertical migration of *Chaoborus* larvae is induced by the presence of fish. Limnology and Oceanography 35 (7): 1631-1637. https://doi.org/10.4319/lo.1990.35.7.1631

de Brito Rodrigues L, de Oliveira R, Abe FR, Brito LB, Sousa Moura D, et al. (2017). Ecotoxicological assessment of glyphosate-based herbicides: Effects on different organisms. Environmental Toxicology and Chemistry 36 (7): 1755-1763. https://doi.org/10.1002/etc.3580

von Ende CN (1979). Fish predation, interspecific predation, and the distribution of two *Chaoborus* species. Ecology 60 (1): 119-128. https://doi.org/10.2307/1936474

Fadhlaoui M, Lavoie I (2021). Effects of Temperature and Glyphosate on Fatty Acid Composition, Antioxidant Capacity, and Lipid Peroxidation in the Gastropod *Lymneae* sp. Water 13 (8): 1039. https://doi.org/10.3390/w13081039

Farrell JL, Siegfried CA, Daniels RA, Sutherland JW, Boylen CW et al. (2017). The dynamics of *Chaoborus americanus* in an Adirondack lake following the reintroduction of fish. Limnologica 65: 38-45. https://doi.org/10.1016/j.limno.2017.07.002

Finney DJ (1971). Probit analysis. New York: Cambridge University Press

Gill JPK, Sethi N, Mohan A, Datta S, Girdhar M (2018). Glyphosate toxicity for animals. Environmental Chemistry Letters 16: 401-426. https://doi.org/10.1007/s10311-017-0689-0

Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Tallaa–Benbrahim L et al. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. The Lancet Oncology 16 (5): 490-491. https://doi.org/10.1016/S1470-2045(15)70134-8

Hanana H, Simon G, Kervarec N, Mahammadou BA, Cérantola S (2012). HRMAS NMR as a tool to study metabolic responses in heart clam *Ruditapes decussatus* exposed to Roundup®. Talanta 97: 425-431. https://doi.org/10.1016/j.talanta.2012.04.057

Horth H, Blackmore K (2009). Survey of Glyphosate and AMPA in Groundwaters and Surface Waters in Europe. WRC Report No. UC8073.02; WRc plc: Swindon, UK.

Husak V, Strutynska T, Burdyliuk N, Pitukh A, Bubalo V et al. (2022). Low-toxic herbicides Roundup and Atrazine disturb free radical processes in *Daphnia* in environmentally relevant concentrations. EXCLI Journal 21: 595-609. https://doi.org/10.17179/excli2022-4690

Malecot M, Guével B, Pineau C, Holbech BF, Bormans M et al. (2013). Specific proteomic response of *Unio pictorum* missel to a mixture of glyphosate and microcystin–LR. Journal of Proteome Research 12 (11): 5281-5292. https://doi.org/10.1021/pr4006316

Matozzo V, Fabrello J, Masiero L, Ferraccioli F, Finos L et al. (2018). Ecotoxicological risk assessment for the herbicide glyphosate to non-target aquatic species: A case study with the mussel *Mytilus galloprovincialis*. Environmental Pollution 233: 623-632. doi.org/10.1016/j.envpol.2017.10.100

Matozzo V, Zampieri C, Munari M, Marin MG (2019). Glyphosate affects haemocyte parameters in the clam *Ruditapes philippinarum*. Marine Environmental Research 146: 66-70. https://doi.org/10.1016/j.marenvres.2019.03.008

McQueen DJ, Ramcharan CW, Demers E, Yan ND, Conforti LM et al. (1999). *Chaoborus* behavioural responses to changes in fish density. Archiv für Hydrobiologie 145 (2): 165-179. https://doi.org/10.1127/archiv-hydrobiol/145/1999/165

von Mérey G, Manson PS, Mehrsheihk A, Sutton P, Levine SL (2016). Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota. Environmental Toxicology 35 (11): 2742-2752. https://doi.org/10.1002/etc.3438

Milan M, Dalla Rovere G, Smits M, Ferraresso S, Pastore P, et al. (2018). Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: Transcriptional responses in the mussel *Mytilus galloprovincialis*. Environmental Pollution 237: 442-451. https://doi.org/10.1016/j.envpol.2018.02.049

Moore MV (1986). Method for Culturing the Phantom Midge, *Chaoborus* (Diptera: Chaoboridae), in the Laboratory. Aquaculture 56 (3-4): 307-31610. https://doi.org/10.1016/0044-8486(86)90345-5

Munger C, Hare L (1997). Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting bioaccumulation in nature. Environmental Science and Technology 31 (3): 891-895. https://doi.org/10.1021/es9606440

Pekcan-Hekim Z, Liljendahl-Nurminen A, Horppila J (2006). *Chaoborus flavicans* in the food web–competitor or resource for fish? Polish Journal of Ecology 54 (4): 701-707

Relyea RA (2005). The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. Archives of Environmental Contamination and Toxicology 48 (3): 351-357. https://doi.org/10.1007/s00244-004-0086-0

Rendón–von Osten J, Dzul–Caamal R (2017). Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchén, Campeche, Mexico. International Journal of Environmental Research and Public Health, 14 (6): 595. https://doi.org/10.3390/ijerph14060595

Rudstam LG, Johannsson OE (2009). Advances in the ecology of freshwater mysids. Aquatic Biology 5: 246-248. https://doi.org/10.3354/ab00167

Rzymski P, Klimaszyk P, Kubacki T, Poniedziałek B (2013). The effect of glyphosate-based herbicide on aquatic organisms-A case study. Limnological Review 13 (4): 215-220. https://doi.org/10.2478/limre-2013-0024

Séguin A, Mottier A, Perron C, Lebel JM, Serpentini A et al. (2017). Sub-lethal effects of a glyphosate-based commercial formulation and adjuvants on juvenile oysters (*Crassostrea gigas*) exposed for 35days. Marine Pollution Bulletin 117 (1-2): 348-358. https://doi.org/10.1016/j.marpolbul.2017.02.028

Straw EA, Carpentier EN, Brow MJF (2021). Roundup causes high levels of mortality following contact exposure in bumble bees. Journal of Applied Ecology 58 (6): 1167–1176. doi.org/10.1111/1365-2664.13867

Suppa A, Kvist J, Li X, Dhandapani V, Almulla H, et al. (2020). Roundup causes embryonic development failure and alters metabolic pathways and gut microbiota functionality in non-target species. Microbiome 8: 170. doi.org/10.1186/s40168-020-00943-5

Tierney KB, Sekela MA, Cobbler CE, Xhabija B, Gledhill M et al. (2011). Evidence for behavioral preference toward environmental concentrations of urban-use herbicides in a model adult fish. Environmental Toxicology 30 (9): 2046-2054. https://doi.org/10.1002/etc.588

USEPA. U. S. Environmental Protection Agency. R.E.D. Facts: Glyphosate, US EPA: Office of Pesticide Programs, Washington, 1993. p. 7

Vereecken H (2005). Mobility and leaching of glyphosate: A review. Pest Management Science 61 (12): 1139-1151. https://doi.org/10.1002/ps.1122

Weber W, Grosmann M (1988). Ultrastructure of the chromatophores system on the tracheal bladders of the phantom larva of *Chaoborus crystallinus* (Insecta, Diptera). Zoomorphology 108: 167-171. https://doi.org/10.1007/BF00363933

Weber R, Kita W, Pusz W (2016). Wpływ herbicydów zawierających glifosat na odporność i odżywianie roślin. The influence of herbicides containing glyphosate on plant resistance and nutrition. Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wrocławiu, Rolnictwo. Scientific Journals of the University of Life Sciences in Wrocław. Agriculture CXVIII, 620: 83-92 (in Polish, summary in English).

Wissel D, Yan ND, Ramcharan CW (2003). Predation and refugia: implications for *Chaoborus* abundance and species composition. Freshwater Biology 48 (8): 1421-1431. https://doi.org/10.1046/j.1365-2427.2003.01100.x

WHO. World Health Organization, Glyphosate. Environmental health criteria 159, WHO: IPCS, Geneva 1994, p. 177.

Zagarese HE, Diovisalvi NR, González Sagrario MdlÁ, Izaguirre I, Fermani P et al. (2022). Increases in Picocyanobacteria Abundance in Agriculturally Eutrophic Pampean Lakes Inferred from Historical Records of Secchi Depth and Chlorophyll-a. Water 14 (2): 159. https://doi.org/10.3390/w14020159