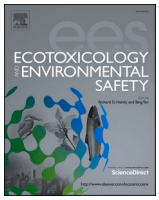
Ecotoxicology and Environmental Safety 213 (2021) 112068

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Effects of commercial formulations of glyphosate on marine crustaceans and implications for risk assessment under temperature changes

Isabella Parlapiano [a], Francesca Biandolino [a], Asia Grattagliano [b], Andrea Ruscito [b], Giovanni Libralato [c], Ermelinda Prato [a,*]

[a] National Research Council, Water Research Institute (IRSA-CNR), Via Roma, 3, 74123 Taranto, Italy
[b] Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Via della Ricerca Scientifica, 1, 00133 Roma, Italy
[c] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126 Naples, Italy

ARTICLE INFO

Edited by Dr. R. Pereira

Keywords:
Acute toxicity
Marine crustaceans
Glyphosate-based herbicides
Temperature

ABSTRACT

Glyphosate-based formulations are the most commonly used herbicides worldwide with the risk of potential contamination of aquatic bodies. The present study assessed the response of four marine crustaceans to three different brands of herbicides Roundup®Platinum, Efesto® and Taifun® MK CL.T, under two selected temperatures of 20 °C and 30 °C. The harpacticoid copepod *Tigriopus fulvus*, the anostracan *Artemia franciscana*, the amphipod *Corophium insidiosum* and the isopod *Sphaeroma serratum* were chosen as testing organisms. Effects of herbicides and temperatures were assessed by estimating lethal concentrations. The results showed that the high temperature rises the toxicity of glyphosate with an increase of mortality of all the tested species. This is an important aspect for future risk assessments of pesticides under global climate change scenarios. Efesto® resulted the most toxic brand, showing *C. insidiosum* the most sensitive with 96 h-LC50 values of 3.25 mg/L acid equivalent (a.e.) at 30 °C and 7.94 mg/L a.e. at 20 °C followed by *T. fulvus* while *A. franciscana* and *S. serratum* were the less sensitive. This study provides important information for assessing the toxic effects of three different brands of glyphosate-based herbicides on non-target marine organisms suggesting that they should be carefully managed to minimize any negative impact on marine organisms.

1. Introduction

Glyphosate (N-(phosphonomethyl) glycine) is a compound belonging to the chemical group of substituted glycine. It was originally developed and patented as a broad-spectrum herbicide. It inhibits the enzyme 5-enolpiruvilshikimato-3-phosphate (EPSP) synthase, the key enzyme involved in aromatic aminoacid synthesis in plants and many microorganisms (Cerdeira and Duke, 2006). Currently, it is approved in the EU, but the approval must be periodically reviewed being an active substance. The current approval of glyphosate expires on December 2022, so the renewal process has started in December 2019, i.e. 3 years before the deadline. Actually, glyphosate-based herbicides (GBH) are used in agriculture and horticulture primarily to control weeds that compete with cultivated crops. They are generally applied before sowing and as a pre-harvest drying treatment to speed up and standardize the ripening process (Carvalho, 2006, 2017).

In countries where genetically modified (GM) crops glyphosate-resistant (GR) are cultivated, the use of glyphosate has risen considerably. It can be considered one of the most used herbicides and its use has increased more than 12 times from 67 million kg in 1995 up to 826 million kg in 2014 (Benbrook, 2016), resulting the most widely herbicides used around the world for weeds control in both agricultural and non-agricultural settings (i.e. forestry, municipalities and private gardens).

Considering the great number of GR crops that are authorized worldwide, it is expected an increase of glyphosate use up to approximately 1000 million kg in 2023 (Mertens et al., 2018).

When debating about the toxicity of pesticides there is a tendency to focus on the effects of the active ingredients, but this might underestimate the potential toxicity of the wide variety of GBH commercial formulations currently available. Indeed, in commercial formulations the glyphosate is not present alone but in combination with other additives (i.e. surfactants and co-formulants). Co-formulants and surfactants are added to improve the efficacy by increasing herbicide adhesion to the leaf surface, as well as favoring transport across the waxy cuticle membrane to reach the action site. This fact makes the GBH toxic to

* Corresponding author.
E-mail address: linda.prato@iamc.cnr.it (E. Prato).

https://doi.org/10.1016/j.ecoenv.2021.112068
Received 6 August 2020; Received in revised form 4 February 2021; Accepted 14 February 2021
Available online 24 February 2021

animals as showed by several studies (Giesy et al., 2000; Tsui and Chu, 2003; Edginton et al., 2004; Costa et al., 2008; Contaro-Jara et al., 2009; Lushchak et al., 2009; Modesto and Martinez, 2010; Cuhra et al., 2013). Each formulation reports the concentration of glyphosate but often the identity of the co-formulants is not shown on the label, remaining secret proprietary of the company (Howe et al., 2004).

Because of their overuse, GBH constitutes a substantial source of contamination for aquatic ecosystems adjacent to intensive agricultural areas, where they can enter through spray drift or direct overspray applications or by runoff or leaching from terrestrial applications raising serious concerns (Abrantes et al., 2009). Measured concentrations of GBH in surface freshwater ranged from 2.7 to 10.3 mg acid equivalent (a.e.)/L (Ronco et al., 2008; Córdova López et al., 2019), while in seawater up to 1690 ng/L in the Western Pacific (Wang et al., 2016), and 13–1377 μg/L in the Baltic Sea (Skeff et al., 2015). Several studies on GBH effects were carried out in freshwater ecosystem but still little information is available about marine organisms (Zaller et al., 2014).

In the ecotoxicological context there is a growing concern due to climatic changes (Moe et al., 2013). One of the most relevant factors promoting global climate change is the increase of the temperature (IPCC, 2014; Cuco et al., 2016). Temperature is a critical factor for many marine organisms inhabiting coastal shallow waters and estuaries, because it can affect physiological and biochemical processes (Kinne, 1963). Generally, as temperature increases, the rate of metabolic processes increases consequently resulting in enhanced uptake rates of several substances including pesticides (Wilson and Parker, 1996; Prato et al., 2009). To date, there is limited knowledge on how the interaction of temperature and pesticide exposure will affect aquatic ecosystems (Cuco et al., 2016).

Marine invertebrates are ectothermic organisms, so temperature is a key factor for all its life biological functions (i.e. growth, reproduction and survival).

Crustaceans are extensively used in ecotoxicological tests because of their ecological relevance and even because they are easy to maintain under laboratory conditions all the year round (Sanchez-Fortùn et al., 1995; Nunes et al., 2006; Garaventa et al., 2010; Piazza et al., 2012).

In this study, four different species of marine crustaceans were used as target species: nauplii (24 h) of the anostracod *Artemia franciscana* and of the harparticoid *Tigriopus fulvus* and juveniles (2–4 mm) of amphipod *Corophium insidiosum* and the isopod *Sphaeroma serratum*. Since these crustaceans live in sand, on the rocky substrate or among algae in shallow water of coastal areas, they can be easily exposed to agrochemicals removed by runoff (Córdova López et al., 2019; Matozzo et al., 2020). The brine shrimp, *A. franciscana*, is considered a common model organism in acute bioassays for toxicity assessments (Manfra et al., 2015; Libralato et al., 2016). Copepods, as the harpacticoid *T. fulvus*, are widely distributed in the Mediterranean Sea and has been successfully used in ecotoxicological studies and ecological risk assessments (Faraponova et al., 2005, 2007, 2016; Manfra et al., 2010; Mariani et al., 2006; Prato et al., 2012, 2013, 2015; Tornambè et al., 2012), due to the ease of use, cost-effectiveness of tests, good sensitivity to different toxicants and reproducibility of tests (Faraponova et al., 2005).

As regards amphipods and isopods, several species are successfully used in whole-sediment toxicity evaluation, because they represent one of the most sensitive taxa among benthic animals, abundant and ecologically important component of soft-bottom estuarine and marine benthic communities. In particular, *C. insidiosum* was selected, because previous studies suggested their tolerance to non-contaminant variables (biotic and abiotic) and sensitivity to toxicants (Annicchiarico et al., 2007; Prato and Biandolino, 2006; Prato et al., 2009, 2012, 2015). *Sphaeroma serratum* are considered suitable species for aquatic bio-monitoring, responds to many pollutants, easy to culture and has short life cycle (Lee and Bang, 2000).

These crustaceans occupy an important position in the food chain, providing a major source of food for predatory fish and other invertebrates (Marsden and Rainbow, 2004; Prato et al., 2006).

Temperature is an important controlling factor that can affect the physiology of organisms, as well as the toxicity of contaminants. However, while several studies showed either glyphosate-based herbicides or temperature separately affect the survival of the organisms, very little is known on possible effects of the interaction of the two.

Because traditional pesticide risk assessments are based on tests conducted at one standard temperature, they have a limited relevance in more realistic risk assessments, especially those taking into consideration climate change scenarios.

This study evaluated the potential harmful effects of three GBH commercial brands in short-term laboratory experiments. The main novelty of the present study is relate to (i) new toxicity data of three brands of commercial glyphosate on four marine crustaceans; (i) investigation of two temperatures on GBHs effects in controlled laboratory conditions.

2. Materials and methods

2.1. Glyphosate and test solutions

Three glyphosate formulations have been considered: Roundup®Platinum, Efesto® and Taifun® MK CL purchased in the garden centers. Roundup®Platinum contained 480 g/L a.e. glyphosate (potassium salt, 43.8%) and other ingredients (56.2%). Efesto® and Taifun® MK CL contained 360 g/L a.e. glyphosate (isopropylamine salt, 41%) and other ingredients (59%).

Individual stock solutions of the three commercial glyphosate were prepared in deionized water and stored in the dark at 4 °C. Test suspensions were prepared from stock solutions immediately prior the use in toxicity tests using 0.22 μm and 0.45 μm-filtered natural sea water (FNSW) (8.2 ± 0.1 pH, 8.0 ± 0.1 mg/L dissolved oxygen and 36‰ salinity).

The concentrations used for each trial were calculated using the concentration (g/L) stated on the product's label. Nominal glyphosate concentrations in experimental solutions were verified using high-performance liquid chromatography (HPLC) (1100 system, Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector and an auto-sampler. A reversed phase with a C18 column (5 μm, 4.6 mm internal diameter, and 250 mm length) and a mobile phase made up of 10% acetonitrile (vol/vol) and 90% water were used. The flow rate was 1.0 mL/min and the injection volume was 10 μL. Under these analytical conditions, the detection limit and recovery were 0.08–1 μg/L and 90–111%, respectively (Table 1).

2.2. Ecotoxicity tests

The median lethal concentration (LC50) of the GBH formulations to the selected marine crustaceans were determined during a 6 months period between January 2019 and June 2019. Toxicity tests were chosen to assess lethal toxicity on four consumer species belonging to different trophic levels: *A. franciscana*, *T. fulvus*, *C. insidiosum* and *S. serratum* (Table 2). Tests were conducted at two temperatures (20 °C and 30 °C) and repeated three times with three replicates each. The exposure temperatures were kept according to temperature-controlled incubators (±0.5 °C) according to their range recorded in the central Mediterranean Sea where their wild populations live. Screening tests were carried out with reference toxicants under standard conditions according to the relative protocols for each species (Table 2). to verify their relative sensitivity.

2.3. Experimental materials

Certified dehydrated cysts of *A. franciscana* were purchased from the Laboratory for Biological Research in Aquatic Pollution, University of Ghent (Belgium). *Artemia* nauplii of less than 48 h old (Instar II or III)

Table 1
Glyphosate concentrations measured in water (mg/L, mean ± SD).

EFESTO				TAIFUN				ROUNDUP			
20 °C		30 °C		20 °C		30 °C		20 °C		30 °C	
Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Nominal concentrations (mg/L)	Measured concentrations (mg/L)
Tigriopus fulvus											
20 °C		30 °C		20 °C		30 °C		20 °C		30 °C	
4	3.9 ± 0.3	2	1.8 ± 0.05	25	24.5 ± 0.2	25	24.5 ± 0.2	60	58.7 ± 5.8	60	58.7 ± 5.8
6	6.1 ± 0.2	4	3.9 ± 0.3	50	52.5 ± 4.3	50	52.5 ± 4.3	100	108.0 ± 3.1	100	108.0 ± 3.1
8	7.8 ± 0.4	6	6.1 ± 0.2	75	74.5 ± 1.8	75	74.5 ± 1.8	140	147.6 ± 9.7	140	147.6 ± 9.7
10	10.2 ± 0.3	8	7.8 ± 0.4	100	111.5 ± 12.5	100	111.5 ± 12.5	180	185.5 ± 9.4	180	185.5 ± 9.4
12	12.7 ± 1.2	10	10.2 ± 0.3	125	124.3 ± 8.7	125	124.3 ± 8.7	220	227.0 ± 9.5	220	227.0 ± 9.5
Artemia franciscana											
100	97.6 ± 4.2	100	97.6 ± 4.2	80	77.5 ± 4.5	80	77.5 ± 4.5	100	102.3 ± 7.5	100	102.3 ± 7.5
300	295.5 ± 3.8	300	295.5 ± 3.8	120	117.5 ± 7.2	100	105.5 ± 2.5	300	289.5 ± 6.2	300	289.5 ± 6.2
500	507.7 ± 1.6	500	507.7 ± 1.6	160	168.5 ± 4.4	120	117.5 ± 7.2	500	479.3 ± 8.5	500	479.3 ± 8.5
700	710.5 ± 6.8	700	710.5 ± 6.8	200	185.2 ± 5.7	140	147.4 ± 2.8	700	714.8 ± 10.5	700	714.8 ± 10.5
900	895.7 ± 7.7	900	895.7 ± 7.7	240	244.7 ± 6.0	160	168.5 ± 4.4	900	922.3 ± 18.8	900	922.3 ± 18.8
Corophium insidiosum											
2	1.8 ± 0.05	1	0.8 ± 0.05	30	27 ± 4.5	7.5	8.12 ± 0.3	50	50.6 ± 2.2	25	24.5 ± 0.2
4	3.9 ± 0.3	2	1.9 ± 0.3	60	61.0 ± 2.2	15	14.6 ± 0.5	100	108.0 ± 3.1	50	50.6 ± 2.2
8	7.8 ± 0.4	4	3.9 ± 0.3	90	92.3 ± 0.5	30	27.2 ± 4.5	150	146.8 ± 2.6	75	77.0 ± 1.5
16	16.5 ± 0.6	8	7.8 ± 0.4	120	119 ± 2.3	60	61.0 ± 2.2	200	185.0 ± 5.7	100	108.0 ± 3.1
32	29.5 ± 1.2	16	16.5 ± 0.6	150	152.3 ± 4.1	120	119.0 ± 2.3	250	250.5 ± 1.8	125	123.7 ± 5.5
Sphaeroma serratum											
100	105.0 ± 2.5	25	24.5 ± 0.2	100	111.5 ± 12.5	50	52.5 ± 4.3	100	102.3 ± 7.5	100	102.3 ± 7.5
200	185.2 ± 5.7	50	52.5 ± 4.3	150	152.3 ± 4.1	100	111.5 ± 12.5	300	289.5 ± 6.2	300	289.5 ± 6.2
300	288.0 ± 7.4	75	67.7 ± 2.5	200	185 ± 5.7	150	152.3 ± 4.1	500	479.3 ± 8.5	500	479.3 ± 8.5
400	410.5 ± 9.5	100	105 ± 2.5	250	248. 7 ± 8.0	200	185.0 ± 5.7	700	714.8 ± 10.5	700	714.8 ± 10.5
500	516.8 ± 15.5	125	122.5 ± 1.4	300	288.0 ± 7.4	250	248. 7 ± 8.0	900	922.3 ± 18.8	900	922.3 ± 18.8

Table 2
Summary of the test conditions utilized for the four selected testing species.

Species	*T. fulvus*	*A. franciscana*	*C. insidiosum*	*S. serratum*
Test type	Static	Static	Static	Static
Stage of development	Nauplii (I-II)	Nauplii (II-III)	Juveniles (2–4 mm)	Juveniles (2–4 mm)
Test chambers	12-well plates	24-well plates	Glass beakers	Glass beakers
Luminosity	500–1200 lx cool light	0	500–1200 lx cool light	500–1200 lx cool light
Light/dark photoperiod	16 h:8 h	Absent	16 h:8 h	16 h:8 h
Dilution water	Filtered sea water (0.22 μm)	Filtered sea water (0.22 μm)	Filtered sea water (0.45 μm)	Filtered sea water (0.45 μm)
Salinity ‰	38 ± 2	35 ± 1	36 ± 2	36 ± 2
Temperature	20−30 ± 0.5 °C	20−30 ± 0.5 °C	20−30 ± 0.5 °C	20−30 ± 0.5 °C
pH	8 ± 0.5	8 ± 0.5	8 ± 0.5	8 ± 0.5
Aeration	Absent	Absent	Present	Present
Reference toxicant	CuSO4·5H2 O	$CuSO_4{\cdot}5H_2$ O	$CdCl_2$	$CdCl_2$
Concentrations of Ref. Tox.	0.03–0.06–0.12–0.25–0.5 (Cu mg/L)	1.0–2.0–4.0–8.0–16.0 mg/L	0.4–0.8–1.6.3.2–6.4 (Cd mg/L)	0.8–1.6.3.2–6.4–12.8 (Cd mg/L)
Testing volume	3 mL	1 mL	700 mL	700 mL
Bioassay duration	96 h	48 h	96 h	96 h
N° organisms/ replicate	10	10	20	20
N° replicates	3	3	3	3
N° run	3	3	3	3
End-point (LC50)	Mortality rate	Mortality rate	Mortality rate	Mortality rate
Validity criteria	Control mortality ≤ 10%	Control mortality ≤ 10%	Control mortality ≤ 15%	Control mortality ≤ 15%
Reference protocols	ISO/FDSI 14669 (1999), Faraponova et al. (2016)	Artoxkit Manfra et al. (2014, 2015)	American Society for Testing and Materials (1993), USEPA (1994)	American Society for Testing and Materials (1993), USEPA (1994)

were obtained from newly hatched eggs according to Manfra et al. (2015). Adults of the harpacticoid copepod *T. fulvus*, collected in coastal microenvironments of the Ligurian Sea, have been kept in laboratory acclimatized culture for several generations; nauplii (< 24 h-old) were obtained according to ISO/FDSI 14669 (1999) and Faraponova et al. (2005).

The amphipod *C. insidiosum* and the isopod *S. serratum* were collected in sediment and on macroalgae, from an unpolluted area of the Mar Piccolo of Taranto (Ionian Sea) using a 0.5 mm sieve. Organisms were stored in polyethylene buckets containing seawater, immediately transported to the laboratory and placed in aerated glass containers with their native sediment. Experimental organisms were acclimated for 3–4 days before the beginning of toxicity tests.

2.4. Test procedures

Nauplii of *A. franciscana* and *T. fulvus* and young adult of *C. insidiosum* and *S. serratum* were exposed to increasing concentrations of the three selected commercial GBH and two experimental temperatures (20 °C and 30 °C) in static non-renewal condition. Prior to the definitive test, preliminary screening with a wide concentration series was carried out to determine the definitive exposure ranges to test the herbicide. The main procedural aspects, including concentrations and references to protocols for all toxicity tests, are provided in Tables 1 and 2.

Tests with *A. franciscana* and *T. fulvus* were performed in multi-well plates (24 and 12 wells, respectively) containing 1 mL and 3 mL of test solution, respectively. Ten nauplii were randomly transferred to each well/replicate using a pipette. After the exposure period (96 h for *T. fulvus* and 48 h for *A. franciscana*) larvae that do not move any appendage after gentle mechanical stimulation for approximately 10–20 s were considered dead. Because of the short duration of the test, no food and no aeration were supplied during the experiments.

Tests were rejected when the control mortality exceeded 10%.

For amphipods and isopods, twenty young-adults (2–4 mm) were selected randomly and allocated in 1 L beakers containing 700 mL of test solutions. The exposure time of the organisms to each glyphosate formulation was 96 h. Animals were not fed throughout the experiment. At the end of the test, the survivors were counted. Organisms with no response at all were considered dead. Apparently dead individuals were considered living if movement was exhibited after gentle stimulation. Missing animals were assumed to be dead. Tests were rejected when the control mortality exceeded 15%.

All toxicity tests consisted of five concentrations, plus one control with three replicates per treatment. The negative control and dilution water used in the experiments consisted of natural seawater collected in an unpolluted area and filtered through GF/C Wathman filters with 0.22 μm and 0.45 μm porosity depending on the test species (Table 2). Positive controls (reference toxicant) were performed to determine the sensitivity of the testing organisms over time.

2.5. Data analysis

Data were presented as mean percentage of effect and the relative standard deviation (S.D.), Data were analyzed for normality and variance homogeneity through Kolmogorov–Smirnov and Bartlett's tests, respectively. When either assumption was met, data were examined by analysis of variance (one-way ANOVA) to find significant variations ($p < 0.05$) among treatments. When requirements for normality and homogeneity were not met, the non-parametric Kruskal–Wallis test on ranks was applied ($p \leq 0.05$).

Differences of toxicity were tested using three-way ANOVA, with factor Temperature (fixed, two levels and orthogonal), factor Toxicants (fixed, three levels and orthogonal) and factor species (fixed, five levels and orthogonal). When factors or interactions among factors were found to be statistically significant (p-value < 0.05), *post-hoc* comparisons were performed using the Student–Newman–Keuls (SNK) tests (Underwood, 1997). Statistical tests were performed using the GMAV5 software package (University of Sydney, Australia).

The LC50 values and 95% confidence limits after 48 h exposure were calculated using the Spearman-Karber method (USEPA, 1994 - ToxStat software package). A smooth function was used to fit dose-response curves with KaleidaGraph 4.5.4 program.

3. Results and discussion

The results obtained showed that the mean mortality in all concentration groups, for all crustacean species, exposed to the three GBH formulations was significantly higher than the control treatments that showed a survival rate > 98%. All toxicity tests negative controls were in

line according to OECD, Organization for Economic Cooperation and Development (2004). Positive controls results were within the range of previously reported values for the testing species (Prato et al., 2012; Annicchiarico et al., 2007; Manfra et al., 2015).

In all exposure scenarios, mortality showed a concentration-response relationship reaching about 100% effect at the highest exposure concentrations (Figs. 1 and 2). The Efesto® treatment showed that, already with 4 mg/L at 20 °C and 2 mg/L at 30 °C, the mortality rate of *C. insidiosum* was significantly different from the control ($p < 0.05$), similarly *T. fulvus* showed a mortality percentage significantly higher than the control with 4 mg/L at 20 °C and 30 °C ($p < 0.05$). While with Taifun® and Roundup® treatments significant mortality differences from the control at very high concentrations in all tested species were observed (Figs. 1 and 2).

The obtained LC50 values are shown in Table 3. The LC50 for each glyphosate formulation at both tested temperatures showed that juveniles of *C. insidiosum* and nauplii of *T. fulvus* were significantly more sensitive than the other species (Figs. 3 and 4), therefore the different life stage did not affect the sensitivity of species. For all species, Efesto® exhibited a toxicity greater or equal than the other GBH formulations at both temperatures ($p < 0.05$). In particular, for *C. insidiosum* and *T. fulvus*, the Efesto® has a toxicity that is about 7 times greater than Taifun and this latter showed a toxicity almost double than Roundup®. *S. serratum* showed a low dead number at very high Roundup® concentrations, similarly *A. franciscana* exhibited a high number of survivor at very high Efesto® and Roundup® concentrations (Fig. 3), for all these tests, infact, LC50 resulted > 500 mg/L (Table 3, Fig. 3).

High temperature increased the sensitivity of all tested species. The lowest LC50 was shown by *C. insidiosum* exposed to Efesto® at 30 °C (3.25 ± 0.15 mg/L; CV = 4.7%) (Table 3). For *T. fulvus*, *C. insidiosum* and *S. serratum*, the acute exposure to Roundup resulted in LC50 values higher than Taifun® and Efesto® at both temperature ($p < 0.05$). Only for *A. franciscana*, Roundup® and Efesto® showed similar toxicity at both temperatures ($p > 0.05$) (Fig. 4).

The results of the three-way ANOVA showed that all three tested factors, i.e. toxicants (glyphosate formulations), temperature and species, individually affect the LC50 values ($p < 0.05$). Similarly, a significant two-way interaction occurred between the factors ($p < 0.05$) (Table 4).

Glyphosate is a persistent compound in marine and freshwater sediments that tend to be adsorbed by the suspended particulate matter and bottom sediments in aquatic ecosystems (Major et al., 2003; Widenfalk et al., 2008). So, invertebrates living in water bodies located near or around the agricultural areas may represent an ecological risk, indirectly affecting the trophic dynamics of the water bodies.

In light of these statements, it is important to have a correct view of

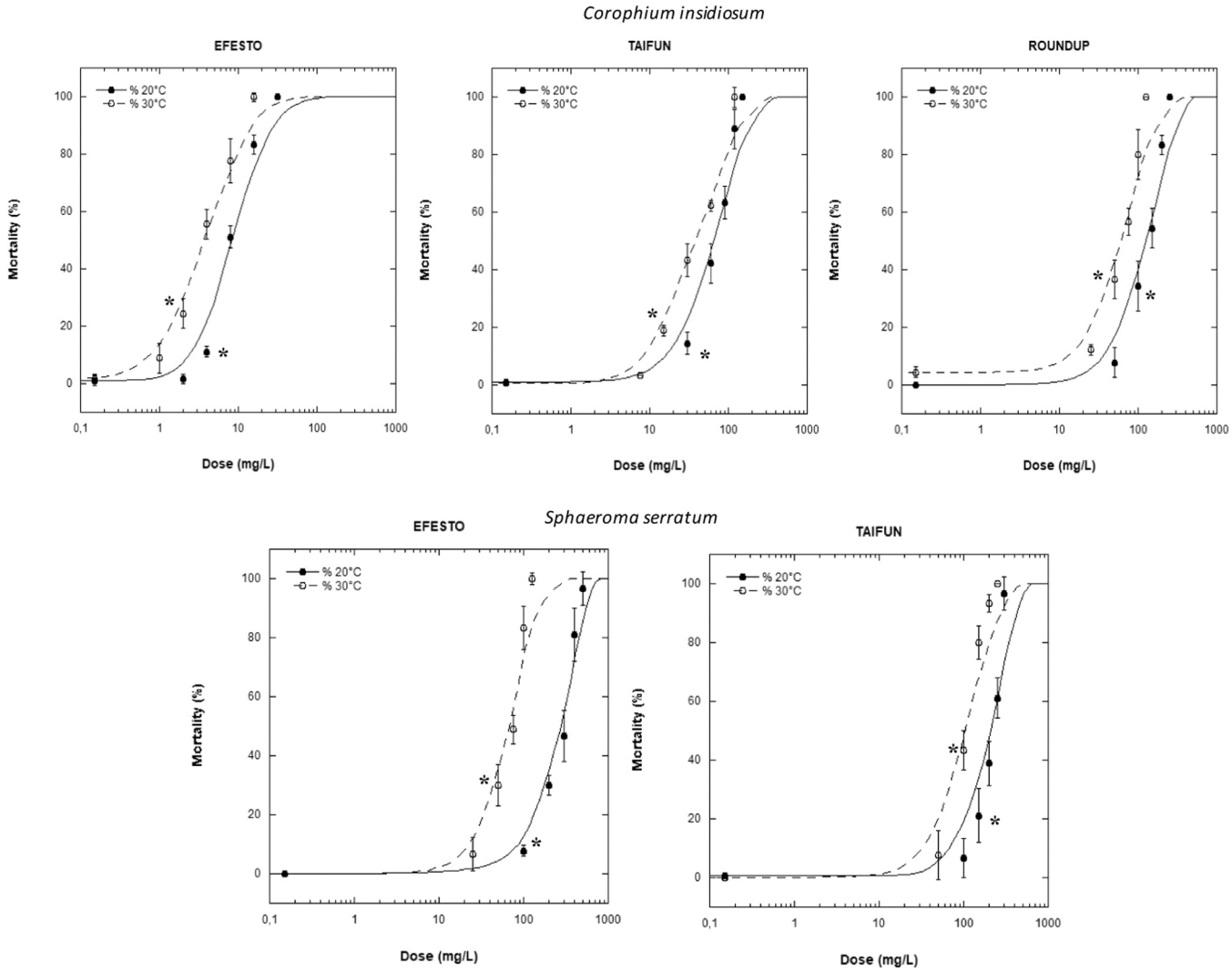


Fig. 1. Dose-response curves (fitted by Smooth function with a logaritmic scale) based on the mean (±S.D.) mortality rates of *C. insidiosum* and *S. serratum* exposed to all glyphosate formulations tested and at the two temperature scenarios (20 and 30 °C). Asterisks indicate the lowest concentrations which shows significant differences with the controls.

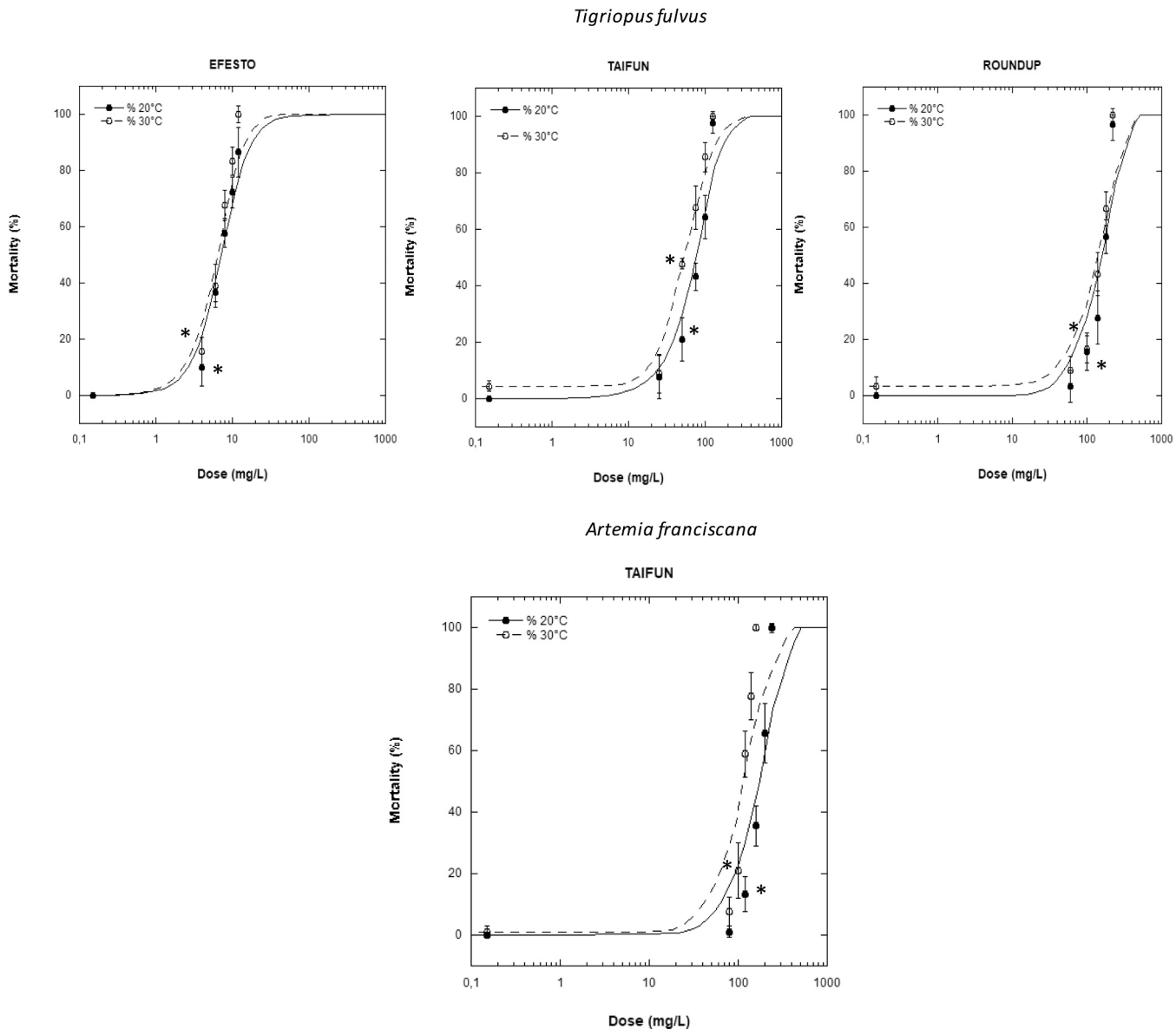


Fig. 2. Dose-response curves (fitted by a Smooth function with a logaritmic scale) based on the mean (±S.D.) mortality rates of *T. fulvus* and *A. franciscana* exposed to all glyphosate formulations tested and at the two temperature scenarios (20 and 30 °C). Asterisks indicate the lowest concentrations which shows significant differences with the controls.

Table 3
The mean (±s.d.) LC50 values mg/L of the tested organisms to the three herbicides at the two temperature.

	EFESTO				TAIFUN				ROUNDUP			
	20 °C		30 °C		20 °C		30 °C		20 °C		30 °C	
Test species	LC50	CV %	LC50	CV%	LC50	CV%	LC50	CV%	LC50	CV%	LC50	CV%
T. fulvus	7.40 ± 0.6	8.5	6.57 ± 0.9	14.6	69.70 ± 10.6	15.2	51.20 ± 2.02	4.0	151.56 ± 20.1	13.2	122.96 ± 11.2	9.1
A. franciscana	> 500	–	> 500	–	155.26 ± 12.6	8.1	126.57 ± 7.66	6.0	> 500	–	> 500	–
C. insidiosum	7.94 ± 0.5	6.1	3.25 ± 0.1	4.7	59.04 ± 4.9	8.3	29.49 ± 1.47	5.0	111.17 ± 17.5	15.7	53.12 ± 6.87	13.0
S. serratum	224.70 ± 39.7	17.7	54.82 ± 6.5	12.0	195.63 ± 37.9	19.3	101.0 ± 16.51	16.3	> 500	–	> 500	–

how temperature can interact with contaminants to correctly assess the risks of contaminants in the environment.

The intentions of this study were to highlight both the impact of different commercial brands of GBH on marine crustaceans and the additional impact that a change of temperature can exert in concert with a pollutant. The joint effect of increasing temperature and toxic effects of pesticides on aquatic organisms is important to understand and predict, as the combination of stressors might be more noxious when compared to their individual effects (Barnett et al., 2005; Schiedek et al., 2007).

Temperature-dependent chemical toxicity studies evaluating the influence of temperature on toxicity of pesticides show that toxicity varies depending on the chemical, species, and life-stage tested (Weston et al., 2009; Seeland et al., 2013), but few studies have been carried out to identify the potential risk for marine organisms. In a previous study,

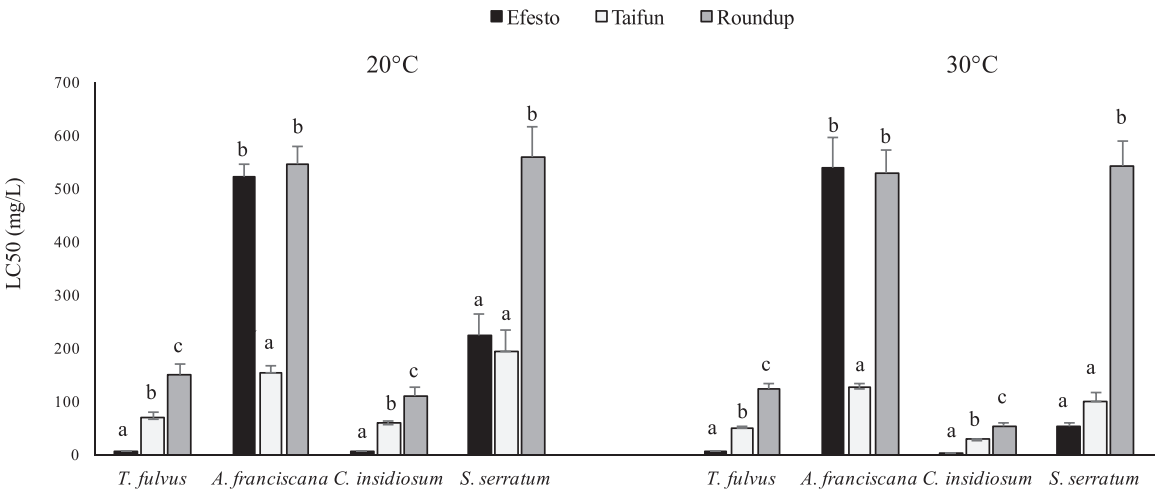


Fig. 3. Mean and standard deviation of LC50 (mg/L) determined by Efesto®, Taifun® and Roundup® for each tested species at 20° and 30 °C. For each species, data with different superscript letters significantly differ ($p < 0.05$) among glyphosate-based formulations tested.

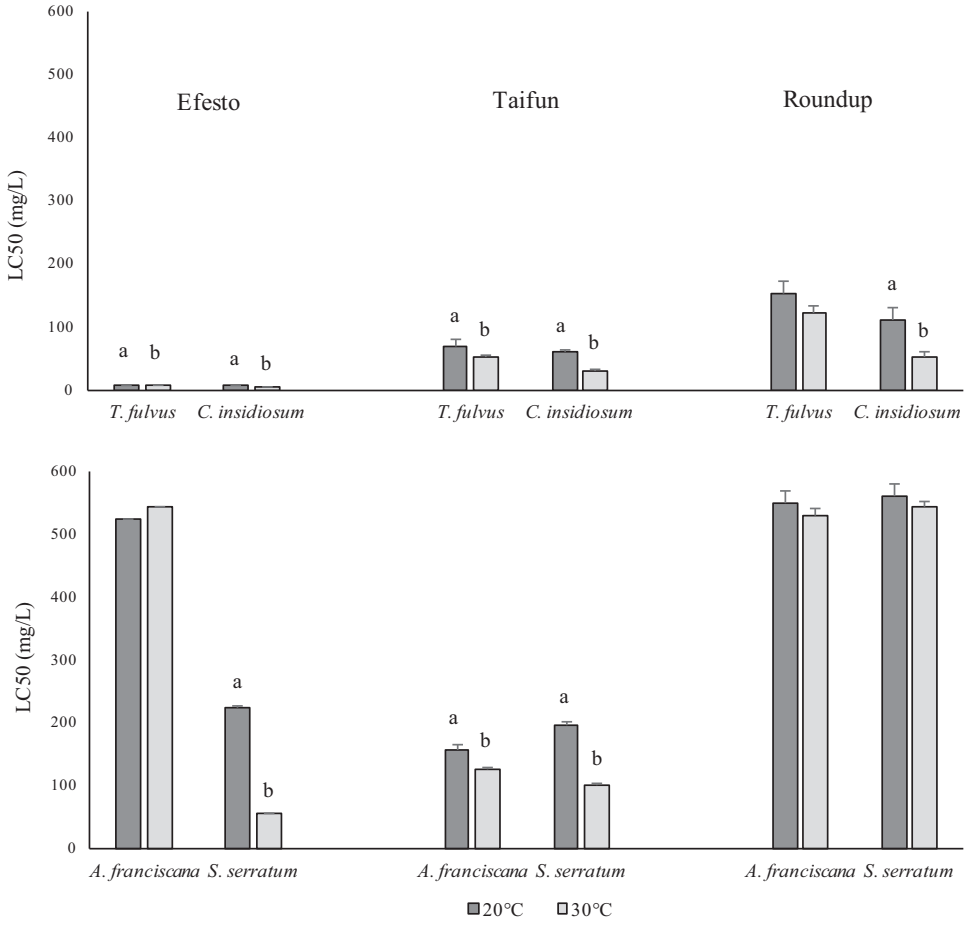


Fig. 4. Comparison of mean (±S.D.)LC50 (mg/L) at 20 °C and 30 °C determined by Efesto®, Taifun® and Roundup® for each tested species. For each species, data with different superscript letters significantly differ ($p < 0.05$) between temperatures tested.

Amid et al. (2018) evaluated the combined effects of glyphosate and increased temperature to the tropical staghorn coral *Acropora formosa*, the results had been evidenced a significant effect on loss of color and also on chlorophyll *a* content, mainly at the joint effect of high temperatures and glyphosate levels.

Marked evidences showed that temperature modifies the physiology and ecology of aquatic organisms, e.g. high temperature increases the rate of uptake of pollutants via changes in ventilation rate, in response to an increased metabolic rate and decrease in oxygen solubility (Kennedy and Walsh, 1997). Previous studies reported LC50 values obtained for Roundup® formulations of 1.5 mg/L a.e. for the amphipod *Hyalella azteca* and 1.77 mg/L a.e. for the calanoid *Acartia tonsa*, 3.7–10.6 mg/L

Table 4
Summary of the effects of pesticides and temperature/species treatments on LC50 values *F values* for the three-way ANOVA on the effect of GBHs (glyphosate-based herbicides), temperature (T°) and species (Sp) on LC50 values, d.f.-degree of freedom, p-value = 0.05.

Source	SS	DF	MS	F	p-value
T°	97,903.13	1	97,903.13	336.25	0.00
GBHs	1,287,591.03	2	643,795.51	2211.1	0.00
Sp	72,228.40	3	24,076.13	82.69	0.00
T°XTo	36,468.19	2	18,234.10	62.62	0.00
T°XSp	130,140.89	3	43,380.30	148.99	0.00
ToXSp	1,173,600.68	6	195,600.11	671.78	0.00
T°XToXSp	237,477.98	6	39,579.66	135.94	0.00
RES	13,975.91	48	291.16		
TOT	3,049,386.20	71			

a.e. for the cladoceran *Daphnia magna* to 62.0 mg/L a.e.for the amphipod *Gammarus pseudolimnaeus*, 81.5 mg/L a.e. for the cladoceran *Ceriodaphnia dubia* and 251.5 mg/L a.e. to freshwater midge *C. xanthus* (Folmar et al., 1979; Tsui and Chu, 2003; Cuhra et al., 2013; Ferreira-Junior et al., 2017).

It should be emphasized that, even though showing different toxicities, the GBHs used in this study reported in the labels the same composition of the active ingredient (glyphosate) without specifying which were the other ingredients. This is an important issue since the greater toxicity of commercial products depends by the presence of glyphosate with surfactants or co-formulants. Formulations vary between different brands and between different countries. These are cocktails of chemicals composed by glyphosate as active principle (36–48%), water, salts, and co-formulants such as polyoxyethylene tallow amine (POEA). Formulations with POEA are relatively toxic compared to other formulations (Mesnage et al., 2013; Mesnage and Antoniou, 2018). For example, formulations with POEA were more toxic to *A. salina* and *Danio rerio* than formulations without POEA at both 360 g glyphosate a.e. /L water (Rodrigues et al., 2017). However, experimentation on health effects of co-formulants by independent entities has been quite limited due to the proprietary nature of these chemicals (Diamond and Durkin, 1997; Durkin, 2011).

Glyphosate is never used without co-formulants, which allow and enhance its herbicidal activity by promoting its toxicity. However, co-formulants are considered and declared as inert diluents because they are not considered to be directly responsible for the toxic effects to non-target species, even though Mesnage and Antoniou (2018) stated that the classification as inert or active has no scientific basis. The lack of consistency in reporting the exact formula used and the relative proportion of individual constituents can lead to incorrect herbicide applications and to an over or underestimation of toxicity. With large differences in toxicity between the individual formulations, it is essential to include the full name and description of the product concerned on the labels.

Also, the literature is quite heterogeneous because not all authors clearly indicate which GBH formulation have used (Chan et al., 2007; Hokanson et al., 2007; Sivikova and Dianovsky, 2006; Mesnage et al., 2015), confusing the products or the co-formulants (Contaro-Jara et al., 2009; Gehin et al., 2005). Glyphosate is often written for “Roundup” (George et al., 2010; Cavusoglu et al., 2011), or “Roundup (glyphosate)” is written as if Roundup were equivalent to glyphosate alone (Stachowski-Haberkorn et al., 2008). Thus, it is not even clear if the authors are assessing glyphosate or its formulations. The median lethal doses vary considerably for different formulations, especially compared to the surfactants (Diamond and Durkin, 1997; Durkin, 2011).

Overall, data generated from this study provide important new information for assessing the toxic effects of different brands of glyphosate-based herbicides on non-target marine organisms under two temperatures. It was highlighted that Efesto® causes higher toxicity than Taifun® and Roundup®.

4. Conclusions

This study demonstrated that increasing temperature can result in increased toxicity for all glyphosate formulations being of extreme importance in a climate change scenario. The health risk assessment of pesticides in the European Union and in the United States focuses almost exclusively on the stated active principle, ignoring co-formulants that can be toxic ton non-target marine species.

Urgent actions must be taken to characterize the presence of co-formulants as well as their ecotoxicological properties. The labels of commercialized products must be fully transparent stating the exact list of ingredients present.

Although information concerning the levels of glyphosate in the marine environment is limited, our results clearly indicated that this substance can cause undesirable effects on marine organisms at different trophic levels even though acute toxicity tests (few hours or few days) indicate that glyphosate and its commercial formulations can be lethal at high concentrations being not environmentally realistic (apart relevant spill out or industrial accident).

Further research is needed investigating long-term low-concentration chronic exposure, comparing commercial formulations with their active principle to measure adverse outcomes stemming from the co-formulants. It is also necessary to focus knowledge and the consequent uncertainties in risk assessment related to the toxicity of chemical mixtures, including co-formulants.

CRediT authorship contribution statement

Ermelinda Prato: Conceptualization, Writing - original draft, Supervision, Project administration. **Francesca Biandolino**: Conceptualization, Investigation, Data curation, Writing - original draft. **Isabella Parlapiano**: Methodology, Investigation, Data curation, Writing - review & editing. **Andrea Ruscito**: Methodology, Data curation, Writing - review & editing. **Asia Grattagliano**: Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Giovanni Libralato**: Methodology, Data curation, Writing - review & editing. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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

References

Abrantes, N., Pereira, R., de Figueiredo, D.R., Marques, C.R., Pereira, M.J., Goncalves, F., 2009. A whole sample toxicity assessment to evaluate the sub-lethal toxicity of water and sediment elutriates from a lake exposed to diffuse pollution. Environ. Toxicol. 24 (3), 259–270.

American Society for Testing and Materials, 1993. Standard guide for conducting 10-d static sediment toxicity tests with marine and estuarine amphipods. ASTM Standards, E. 1367–92, Vol. 11.4. Philadelphia, PA, p. 1138–1164.

Amid, C., Olstedt, M., Gunnarsson, J.S., LeLan, H., TranThiMinh, H., VandenBrink, P.J., Hellström, M., Tedengren, M., 2018. Additive effects of the herbicide glyphosate and elevated temperature on the branched coral *Acropora formosa* in Nha Trang, Vietnam. Environ. Sci. Pollut. Res. 25, 13360–13372.

Annicchiarico, C., Biandolino, F., Cardellicchio, N., Di Leo, A., Giandomenico, S., Prato, E., 2007. Predicting toxicity in marine sediments in Taranto Gulf (Ionian Sea, Southern Italy) using Sediment Quality Guidelines and a battery of bioassay. Ecotoxicology 16, 239–246.

Artoxkit, M., 2014. Artemia Toxicity Screening Test for Estuarine and Marine Waters. Standard Operational Procedure. Mariakerke-Gent, Microbiotest.

Barnett, T.P., Pierce, D.W., AchutaRao, K.M., Gleckler, P.L., Santer, B.D., Gregory, J.M., Washington, W.M., 2005. Penetration of human-induced warming into the world's oceans. Science 309, 284–287.

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 3. https://doi.org/10.1186/s12302-016-0070-0.

Carvalho, F.P., 2006. Agriculture, pesticides, food security and food safety. Environ. Sci. Policy 9, 685–692.

Carvalho, F.P., 2017. Pesticides, environment, and food safety. Food Energy Secur. 6 (2), 48–60. https://doi.org/10.1002/fes3.108.
Cavusoglu, K., Yapar, K., Oruc, E., Yalcin, E., 2011. Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. J. Med. Food 14, 1263–1272.
Cerdeira, A.L., Duke, S.O., 2006. The current status and environmental impacts of glyphosate-resistant crop: a review. J. Environ. Qual. 35, 1633–1658.
Chan, Y.C., Chang, S.C., Hsuan, S.L., Chien, M.S., Lee, W.C., Kang, J.J., Wang, S.C., Liao, J.W., 2007. Cardiovascular effects of herbicides and formulated adjuvants on isolated rat aorta and heart. Toxicol. Vitro 21, 595–603.
Contaro-Jara, V., Klingelmann, E., Wiegand, C., 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. Environ. Pollut. 157, 57–63.
Córdova López, A.M., Almeida Sarmento, R., de Souza Saraiva, A., Ramos Pereira, R., Soares, A.M.V.M., Pestana, J.L.T., 2019. Exposure to Roundup® affects behaviour, head regeneration and reproduction of the freshwater planarian *Girardia tigrina*. Sci. Total Environ. 675, 453–461.
Costa, M.J., Monteiro, D.A., Oliveira-Neto, A.L., Rantin, F.T., Kalinin, A.L., 2008. Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. Ecotoxicology 17 (3), 153–163.
Cuco, A.P., Abrantes, N., Gonçalves, F., Wolinska, J., Castro, B.B., 2016. Toxicity of two fungicides in Daphnia: is it always temperature-dependent? Ecotoxicology 25, 1376–1389.
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 (2), 251–262. https://doi.org/10.1007/s10646-012-1021-1.
Diamond, G.L., Durkin, P.R., 1997. Effects of Surfactants on the Toxicitiy of Glyphosate, With Specific Reference to RODEO. Animal and Plant Health Inspection Service (APHIS), Biotechnology, Biologics and Environmental Protection, Environmental Analysis and Documentation, United States Department of Agriculture, Riverdale, MD 20737, USA, 28 pp. https://www.fs.fed.us/foresthealth/pesticide/pdfs/Surfactants.pdf.
Durkin, P.R., 2011. Glyphosate: Human Health and Ecological Risk Assessment. Final Report Submitted to the USDA Forest Service, SERA TR-052-22-03b. Syracuse Environ- mental Research Associates, Inc., Manlius, New York, USA. https://www.fs.fed.us/foresthealth/pesticide/pdfs/Glyphosate_SERA_TR-052-22-03b.pdf.
Edginton, A.N., Sheridan, P.M., Stephenson, G.R., Thompson, D.G., Boermans, H.J., 2004. Comparative effects of pH and Vision® herbicide on two life stages of four anuran amphibian species. Environ. Toxicol. Chem. 23 (4), 815–822.
Faraponova, O., De Pascale, D., Onorati, F., Finoia, M.G., 2005. Tigriopus fulvus (Copepoda, Harpacticoida) as a target species in biological assays. Meiofauna Mar. 14, 91–95.
Faraponova, O., Giacco, E., Biandolino, F., Prato, E., Del Prete, F., Valenti, A., Sarcina, S., Pasteris, A., Montecavalli, A., Comin, S., Cesca, C., Francese, M., Cigar, M., Piazza, V., Falleni, F., Lacchetti, I., 2016. *Tigriopus fulvus*: the interlaboratory comparison of the acute toxicity test. Ecotoxicol. Environ. Saf. 124, 309–314.
Faraponova, O., Lera, S., Savorelli, F., Palazzi, D., Onorati, F., Cicero, A.M., Magaletti, E., 2007. Valutazione della tossicità acuta di un prodotto disperdente per gli stadi giovanili di quattro specie di crostacei (caso studio). Biol. Mar. Mediterr. 14 (1), 58–63.
Ferreira-Junior, D.F., Sarmento, R.A., Saraiva A de, S., Ramos Pereira, R., Coutinho Picanço, M., Pestana, J.L.T., Soares Amadeu, M.V.M., 2017. Low concentrations of glyphosate-based herbicide affects the development of *Chironomus xanthus*. Water Air Soil Pollut. 228, 390–398.
Folmar, L.C., Sanders, H.O., Julin, A.M., 1979. Toxicity of the herbicide glyphosate and sev- eral of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol. 8, 269–278. https://doi.org/10.1007/BF01056243.
Garaventa, F., Gambardella, C., Di Fino, A., Pittore, M., Faimali, M., 2010. Swimming speed alteration of *Artemia* sp. and *Brachionus plicatilis* as a sub- lethal behavioural end-point for ecotoxicological surveys. Ecotoxicology 19, 512–519.
Gehin, A., Guillaume, Y.C., Millet, J., Guyon, C., Nicod, L., 2005. Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. Int. J. Pharm. 288, 219–226.
George, J., Prasad, S., Mahmood, Z., Shukla, Y., 2010. Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. J. Proteom. 73, 951–964.
Giesy, J.P., Dobson, S., Solomon, K.R., 2000. Ecotoxicological risk assessment for Roundup® herbicide. Rev. Environ. Contam. Toxicol. 167, 35–120.
Hokanson, R., Fudge, R., Chowdhary, R., Busbee, D., 2007. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Hum. Exp. Toxicol. 26, 747–752.
Howe, C.M., Berrill, M., Pauli, B.D., Helbing, C.C., Werry, K., Veldhoen, N., 2004. Toxicity of glyphosate-based pesticides to four North American frog species. Environ. Toxicol. Chem. 23, 1928–1938.
IPCC, 2014. Climate Change 2014: Synthesis Report. Pachauri, R., Meyer, L. (Eds), Geneva, Switzerland, p. 151.
ISO/FDSI 14669, 1999. Water quality–determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea), p. 16.
Kennedy, C.J., Walsh, P.J., 1997. Effects of temperature on xenobiotic metabolism. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming – Implications for Freshwater and Marine Fish. Cambridge University Press, pp. 303–324.
Kinne, O., 1963. The effects of temperature and salinity in marine and brackish water animals. J. Temp. Oceanogr. Mar. Biol. Annu. Rev. 1, 301–340.
Lee, J.H., Bang, K.W., 2000. Characterization of urban stormwater runoff. Water Res. 34, 1773–1780.
Libralato, G., Prato, E., Migliore, L., Cicero, A.M., Manfra, L., 2016. A review of toxicity testing protocols and endpoints with Artemia spp. Ecol. Indic. 69, 35–49.
Lushchak, O.V., Kubrak, O.I., Storey, J.M., Storey, K.B., Lushchak, V.I., 2009. Low toxic herbicide Roundup induces mild oxidative stress in gold fish tissues. Chemosphere 76 (7), 932–937.
Major, W.W., Grue, C.E., Gardne, S.C., Grassley, J.M., 2003. Concentrations of glyphosate and AMPA in sediment following operational applications of Rodeo to control smooth cordgrass in Willapa bay Washington, USA. Bull. Environ. Contam. Toxicol. 71, 912–918.
Manfra, L., Maggi, C., Bianchi, J., Mannozzi, M., Faraponova, O., Mariani, L., Onorati, F., Tornabè, A., Virno Lamnerti, C., Magaletti, E., 2010. Toxicity evaluation of produced formation waters after filtration treatment. Nat. Sci. 2 (1), 33–40.
Manfra, L., Savorelli, F., Di Lorenzo, B., Libralato, G., Comin, S., Conti, D., Floris, B., Francese, M., Gallo, M.L., Gartner, I., Guida, M., Leoni, T., Marino, G., Martelli, F., Palazzi, D., Prato, E., Righini, P., Rossi, E., Volpi, Ghirardini, A., Migliore, L., 2015. Intercalibration of ecotoxicity testing protocols with *Artemia franciscana*. Ecol. Indic. 57, 41–47.
Mariani, L., De Pascale, D., Faraponova, O., Tornambè, A., Sarni, A., Giuliani, S., Ruggiero, G., Onorati, F., Magaletti, E., 2006. The use of a battery test in marine ecotoxicology: the acute toxicity of sodium dodecyl sulfate. Environ. Toxicol. 21, 373–379.
Marsden, D., Rainbow, P.S., 2004. Does the accumulation of trace metals in Crustaceans affect their ecology the Amphipods example? J. Exp. Mar. Biol. Ecol. 300, 373–408.
Matozzo, V., Fabrello, J., Marin, M.G., 2020. The effects of glyphosate and its commercial formulations to marine invertebrates: a review. J. Mar. Sci. Eng. 8, 399. https://doi.org/10.3390/jmse8060399.
Mertens, M., Höss, S., Neumann, G., Afzal, J., Reichenbecher, W., 2018. Glyphosate, a chelating agent—relevant for ecological risk assessment? Environ. Sci. Pollut. Res. 25, 5298–5317.
Mesnage, R., Antoniou, M.N., 2018. Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. Front. Public Health 5, 361. https://doi.org/10.3389/fpubh.2017.00361.
Mesnage, R., Bernay, B., Seralini, G., 2013. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology 313, 122–128.
Mesnage, R., Defarge, N., Spiroux de Vendomois, J., Seralini, G.E., 2015. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem. Toxicol. 84, 133–153. https://doi.org/10.1016/j.fct.2015.08.012.
Modesto, K.A., Martinez, C.B., 2010. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. Chemosphere 78, 294–299. https://doi.org/10.1016/j.chemosphere.2009.10.047.
Moe, S.J., De Schamphelaere, K., Clements, W.H., Sorensen, M.T., Van den Brink, P.J., Liess, M., 2013. Combined and interactive effects of global climate change and toxicants on populations and communities. Environ. Toxicol. Chem. 32, 49–61.
Nunes, B.S., Carvalho, F.D., Guilhermino, L.M., Van Stappen, G., 2006. Use of the genus *Artemia* in ecotoxicity testing. Environ. Pollut. 144, 453–462.
OECD, Organization for Economic Cooperation and Development, 2004. Guideline for the testing of chemicals 202: *Daphnia* sp., acute immobilisation test, 13 April 2004, Paris, France.
Piazza, V., Ferioli, A., Giacco, E., Melchiorre, N., Valenti, A., Del Prete, F., Biandolino, F., Dentone, L., Frisenda, P., Faimali, M., 2012. A standardization of *Amphibalanus (Balanus) amphitrite* (Crustacea, Cirripedia) larval bioassay for ecotoxicological studies. Ecotoxicol. Environ. Saf. 79, 134–138.
Prato, E., Biandolino, F., 2006. *Monocorophium insidiosum* (Crustacea, Amphipoda) as a Candidate species in sediment toxicity testing. Bull. Environ. Contam. Toxicol. 77 (1), 1–8.
Prato, E., Biandolino, F., Scardicchio, C., 2009. Effects of temperature on the sensitivity of *Gammarus aequicauda* (Martynov, 1931) to. Cadmium. Bull. Environ. Contam. Toxicol. 83, 469–473.
Prato, E., Parlapiano, I., Biandolino, F., 2012. Evaluation of a bioassays battery for eco-toxicological screening of marine sediments. Environ. Monit. Assess. 9, 5225–5238.
Prato, E., Parlapiano, I., Biandolino, F., 2013. Assessment of individual and combined toxicities of three metals (Cu, Cd and Hg) by using *Tigriopus fulvus*. Chem. Ecol. 29 (7), 635–642.
Prato, E., Parlapiano, I., Biandolino, F., 2015. Ecotoxicological evaluation of sediments by battery bioassays: application and comparison of two integrated classification systems. Chem. Ecol. 31 (7), 6612–6678.
Rodrigues, L., de, B., de Oliveira, R., Abe, F.R., Barroso Brito, R., Dousa Moura, D., Campos Valadares, M., Koppe Grisolia, C., Palma de Oliveira, D., Rodrigues de Oliveira, G.A., 2017. Ecotoxicological assessment of glyphosate-based herbicides: effects on different organisms. Environ. Toxicol. Chem. 36, 1755–1763.
Ronco, A.E., Carriquiriborde, P., Natale, G., Martin, M.L., Mugni, H., Bonetto, C., 2008. Integrated approach for the assessment of biotech soybean pesticides impact on low order stream ecosystems of the Pampasic region. In: Columbus, F. (Ed.), Ecosystem Ecology Research Developments. NOVA Publishers, New York, NY, pp. 209–239.
Sanchez-Fortùn, S., Barrera, F., Barahona-Gomariz, M.V., 1995. Acute toxicities of selected insecticides to the aquatic arthropod Artemia salina. Bull. Environ. Contam. Toxicol. 54, 76–82.
Schiedek, D., Sundelin, B., Readman, J.W., Macdonald, R.W., 2007. Interactions between climate change and contaminants. Mar. Pollut. Bull. 54, 1845–1856.
Seeland, A., Albrand, J., Oehlmann, J., Müller, R., 2013. Life stage-specific effects of the fungicide pyrimethanil and temperature on the snail *Physella acuta* (Draparnaud, 1805) disclose the pitfalls for the aquatic risk assessment under global climate change. Environ. Pollut. 174, 1–9.
Sivikova, K., Dianovsky, J., 2006. Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes. Int. J. Hyg. Environ. Health 209, 15–20.
Skeff, W., Neumann, C., Schulz-Bull, D.E., 2015. Glyphosate and AMPA in the estuaries of the Baltic Sea method optimization and field study. Mar. Pollut. Bull. 2015 (100), 577–585.

Stachowski-Haberkorn, S., Becker, B., Marie, D., Haberkorn, H., Coroller, L., de la Broise, D., 2008. Impact of Roundup on the marine microbial community, as shown by an in situ microcosm experiment. Aquat. Toxicol. 89, 232–241.

Tornambè, A., Manfra, L., Mariani, L., Faraponova, O., Onorati, F., Savorelli, F., Cicero, A.M., Virno Lamberti, C., Magaletti, E., 2012. Toxicity evaluation of diethylene glycol and its combined effects with produced waters of off-shore gas platforms in the Adriatic Sea (Italy): bioassays with marine/estuarine species. Mar. Environ. Res. 77, 141–149.

Tsui, M.T., Chu, L.M., 2003. Aquatic toxicity of glyphosate based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52, 1189–1197. https://doi.org/10.1016/S0045-6535(03)00306-0.

Underwood, A.J., 1997. Environmental decision-making and the precautionary principle: what does this principle mean in environmental sampling practice? Landsc. Urban Plan. 37, 137–146.

USEPA, 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. In: Klemm, D.J., Morrison, G.E., Norberg-Ring, J.J., Peltier. W.H., Herber, M.A., U.S. Environmental Protection Agency, Report EPA-600-4-91/003, Cincinnati, OH 483.

Wang, S., Liu, B., Yuan, D., Ma, J.A., 2016. A simple method for the determination of glyphosate and aminomethylphosphonic acid in seawater matrix with high performance liquid chromatography and fluorescence detection. Talanta 161, 700–706.

Weston, D.P., You, J., Harwood, A.D., Lydy, M.J., 2009. Whole sediment toxicity identification evaluation tools for pyrethroid insecticides: III. Temperature manipulation. Environ. Toxicol. Chem. 28, 173–180.

Widenfalk, A., Bertilsson, S., Sundh, I., Goedkoop, W., 2008. Effects of pesticides on community composition and activity of sediment microbe's e responses at various levels of microbial community organization. Environ. Pollut. 152, 576–584.

Wilson Jr., W.H., Parker, K., 1996. The life history of the amphipod, *Corophium volutator*: the effects of temperature and shorebird predation. J. Exp. Mar. Biol. Ecol. 196, 239–250, 239–25.

Zaller, J.G., Heigl, F., Ruess, L., Grabmaier, A., 2014. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. Sci. Rep. 4, 5634.