Effects of a glyphosate-based herbicide on *Fucus virsoides* (Fucales, Ochrophyta) photosynthetic efficiency☆

Annalisa Falace [a, c], Laura Tamburello [b, c, *], Giuseppe Guarnieri [b, c], Sara Kaleb [a], Loredana Papa [b, c], Simonetta Fraschetti [b, c, d]

[a] *Department of Life Sciences, University of Trieste, 34127, Trieste, Italy*
[b] *Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100, Lecce, Italy*
[c] *CoNISMa, Piazzale Flaminio 9, 00196, Roma, Italy*
[d] *Stazione Zoologica Anton Dohrn, 80121, Napoli, Italy*

ARTICLE INFO

Article history:
Received 11 April 2018
Received in revised form
16 August 2018
Accepted 17 August 2018
Available online 18 August 2018

Keywords:
Glyphosate-based herbicide
Nutrients
Photosynthetic efficiency
Macroalgae
Fucus

ABSTRACT

Herbicides are increasingly recognised as sources of water pollution. Glyphosate-based herbicides (GBHs) are widely used because of their low cost and high effectiveness. By measuring the photosynthetic efficiency of *Fucus virsoides* fronds exposed to a GBH (Roundup® Power 2.0), we investigated the effect of a continuous exposure (6 days) and the potential of recovery after a short exposure (24 h). Both experiments were carried out combining GBH with and without nutrient enrichment, simulating a runoff event. A factorial experimental design allowed us to assess the potential of interactions between GBH and nutrients, which are likely to co-occur in coastal areas. Our results show deleterious effects of GBH at low concentration on *F. virsoides*, independently from the duration of exposure and the presence of nutrients.

1. Introduction

The understanding of land-sea interactions is a major challenge for the conservation and management of coastal marine systems (Halpern et al., 2008; Claudet and Fraschetti, 2010). Ecosystem Based Management (EBM) and Integrated Coastal Zone Management (ICZM) require the transition from the traditional management of threats in single ecosystem compartments to large-scale management strategies (Mercurio et al., 2014). Recognizing the effects of land-based activities is urgently needed to identify major drivers of change in coastal marine systems, to guide specific legislation instruments, and to set appropriate monitoring approaches (Friberg et al., 2011).

Herbicides are widely used in agriculture, forestry, invasive species management, and ready-to-use products for the home and garden (Annette et al., 2014; Castro et al., 2015). Glyphosate-based herbicides (GBHs) are the most widely used to eradicate terrestrial plants and aquatic weeds because of their low cost and high effectiveness (Gaupp-Berghausen et al., 2015). Glyphosate was developed in 1974 and GBHs are sold in more than 750 formulations according to the surfactant used to facilitate plant uptake (Newton, 2013). Surfactants can exacerbate the toxicity of the glyphosate on non-target species (Annette et al., 2014). The use of GBHs rose almost 15-fold since the introduction of "Roundup Ready" genetically engineered glyphosate-tolerant crops, in 1996 (Benbrook, 2016). Outfalls, wastewater treatments, washout from the ground, agriculture and urban runoffs, erosion and drainage are considered responsible for transporting GBHs from the land to the coastal marine system (Solomon and Thompson, 2003; Sasal et al., 2015). Although soluble in water, glyphosate is readily ionized and adsorbed into sediments and soils, and degrades in both terrestrial and aquatic systems, predominantly via microbial processes (Maycock et al., 2010). However, quantitative studies on these pathways and their spatio-temporal variability are scarce (Lefrancq et al., 2017). Once in the sea, information on patterns of GBHs' degradation in the water column and/or sediments is not conclusive: many factors such as light, pH, and nutrients may affect the concentration of GBHs (Barceló and Hennion, 2003; Solomon and Thompson, 2003). However, there is evidence that extensive applications of GBHs and their relatively long half-life (7–315 days,

☆ This paper has been recommended for acceptance by Dr. Chen Da.
* Corresponding author. CoNISMa, Piazzale Flaminio 9, 00196, Roma, Italy.
E-mail address: lauratamburello81@gmail.com (L. Tamburello).

most commonly 45–60 days) can lead to their constant presence in coastal waters (Mercurio et al., 2014; Wang et al., 2016). In addition, long-distance transport and persistence in the environment through sediment and particulate binding have been hypothesized (Solomon and Thompson, 2003).

Worldwide, some regulatory bodies approved the use of GBHs (Dill et al., 2010), but the assessment of their human and environmental toxicity has resulted in different policies (e.g. USA monitoring programs disregard GBHs, whereas El Salvador banned GBHs since 2013, and Sri Lanka, Bermuda, and Colombia since 2015). The European Chemicals Agency (ECHA) recognised GBHs as toxic to aquatic life, but these are not included in most monitoring programs and their concentrations remain poorly documented in marine systems (Stachowski-Haberkorn et al., 2008). On December 2017, the EU Commission renewed the approval of glyphosate use for five years (https://ec.europa.eu/food/plant/pesticides/glyphosate.en).

Up to 2% of herbicides of urban and agricultural origin are estimated to reach coastal waters (Kemp et al., 1983), and GBHs have been already shown to cause significant changes in biomass, leaf growth, chlorophyll content and photosynthetic yield in macrophytes such as *Ruppia maritima* (Castro et al., 2015; Kittle and McDermid, 2016). However, despite macrophytes and macroalgae are expected to be one of the most sensitive groups (Cedergreen and Streibig, 2005), data on GBHs toxicity are very limited (Burridge and Gorski, 1998; Pang et al., 2012; Kittle and McDermid, 2016), and their impact on algae with a complex morpho-anatomical structure has never been assessed.

Macroalgal communities (Ochrophyta: Laminariales and Fucales) can thrive in both intertidal and subtidal rocky habitats. These valuable marine habitats (Thibaut et al., 2005) are showing a significant regression on a global scale (Strain et al., 2014). Due to their role in supporting biodiversity and food webs, their depletion is leading to a decline in ecosystem services, such as fisheries (Cheminée et al., 2013). The observed loss of macroalgal canopies has been linked with sediment runoff (Gorman et al., 2013) and metals pollution (Mayer-Pinto et al., 2010) while nutrient enrichment seems to exert either positive (Falkenberg et al., 2012) or negative effects (Gorman et al., 2009).

In this study, we addressed this issue with two laboratory experiments to test the effects of a GBH (i.e. Roundup® Power 2.0) on the photosynthetic performance of *Fucus virsoides* J. Agardh (Fucales, Ochrophyta), a glacial relict endemic of the Adriatic Sea living in the intertidal fringe, potentially exposed to herbicides carried at sea by runoff, erosion, and drainage from land. In the last five years, *F. virsoides* almost disappeared from the Adriatic Sea (Falace et al., 2010; Orlando-Bonaca et al., 2013; Battelli, 2016). Currently, it is no longer present along the Slovenian coasts (Battelli, 2016) and just one small patchy population persists in the Gulf of Trieste (Italy). By assessing the chlorophyll-fluorescence of photosystem II, we tested: 1) the toxic effect of a continuous exposure (6 days) to GBH in factorial combination with nutrient enrichment; 2) the potential of recovery after a short exposure (24 h) to GBH simulating a runoff event, with and without nutrient enrichment. A factorial experimental design allowed us to assess the potential of interactions between GBH and nutrients, which are likely to co-occur in coastal areas.

2. Material and methods

2.1. Laboratory experimental setting

Healthy apical fronds of *F. virsoides* were collected in October 2017 during the low tide in the Gulf of Trieste-Northern Adriatic (45° 46′ 25.67″ N, 13° 31′ 52.31″ E) and transported in insulated containers under dark, cold conditions to the laboratory, within 1 h of collection. Specimens were quickly cleaned with a brush and rinsed with filtered ambient seawater, to remove epibionts and sediment. Temperature (19 °C) and photoperiod (11 L:13 D) were selected to reflect natural conditions at the sampling site. Light irradiance, provided by LED lamps (AM366 Sicce USA Inc., Knoxville, USA), was set at 100 μmol photons m^{-2} s^{-1} and measured with a LI-COR LI-190/R Photometer (LICOR-Biosciences, Lincoln, NE, USA).

Two experiments were carried out to test the effect of a 6 days exposure to GBH, with and without nutrient enrichment (Experiment 1); and the recovery of *F. virsoides* after a short exposure event (24 h) to GBH, with and without nutrient enrichment (Experiment 2).

In Experiment 1, the following conditions were considered: GBH-NU- (control medium), GBH+NU+ (GBH and nutrient addition), GBH+NU- (GBH addition only), GBH-NU+ (nutrient enrichment only). In Experiment 2, since the aim was to investigate the recovery potential of *F. virsoides* from GBH exposure, the same experimental conditions were included, without the condition GBH-NU+ (nutrient enrichment only). After 24 h of exposure to GBH (day 0), culture mediums containing GBH (i.e. GBH+NU+ and GBH+NU-) were renewed with media without GBH (i.e. GBH-NU+ and GBH-NU-). In both experiments, each condition was replicated in three aquaria (5L), each containing 40 apical fronds. The position of the aquaria in the temperature and light regulated room was randomized. Constant aeration in the aquaria was provided using air pumps to keep conditions homogeneous.

To guarantee controlled conditions during the whole experiment and in each aquarium, we used filtered seawater (0.22 μm filters membrane) with known concentration of nutrients and GBHs. Filtering seawater did not remove the organic matter (DOM), but eliminated its particulate component (POM), together with phytoplankton, zooplankton and bacterial communities, which could have affected the nutrient and GBH concentration during the experiments. The control medium (GBH-NU) was filtered seawater collected at the sampling site with a nutrient concentration of 17.5 μg l^{-1} N-NO_3 and 0.63 μg l^{-1} P-PO_4. For the nutrient enriched medium (NU+), $NaNO_3$ and $Na_2HPO_4 \bullet 12H_2O$ (Sigma-Aldrich) were added to the control medium to attain a final concentration of 364 μg l^{-1} N-NO_3 and 1.395 μg l^{-1} P-PO_4. These concentrations correspond to the higher mean annual values registered on the coasts of the Gulf of Trieste (data provided by ARPA FVG, 2009–2014). In GBH addition medium (GBH+), the commercial formulation Roundup® Power 2.0 (MONSANTO Europe S.A.) was mixed in a volumetric flask with the control medium to a final concentration of 2.5 mg l^{-1}. The concentration of glyphosate potassium salt in the solution was 882.5 μg l^{-1} (i.e. 35.3% of the Roundup® formulation). The remaining was surfactant (i.e. 6% of the formulation) and water. Glyphosate concentration used in Experiment 1 and 2 was identified after a pilot study exposing *F. virsoides* to 1.76, 17.6, 176.5 and 1765 μg l^{-1} of glyphosate. Deleterious effects were observed at 176.5 μg l^{-1}, while 1765 μg l^{-1} concentration resulted in the mortality of all the fronds after 3 days. The GBH and nutrient addition medium (GBH + NU+) was obtained by mixing the Roundup® Power 2.0 with the nutrient enriched seawater in a volumetric flask. In each aquarium the medium was renewed every day to minimize the effect of nutrient and herbicide limitation or degradation.

Stress effects on *F. virsoides* fronds were evaluated by measuring the chlorophyll-fluorescence of photosystem II (Maxwell and Johnson, 2000; Roháèek, 2002; Ritchie, 2006), using a Handy PEA chlorophyll fluorimeter (Hansatech Instruments ltd, Norfolk, UK). The time required for dark adaptation and irradiance were determined experimentally. To estimate the maximum fluorescence yield Fv/Fm = (Fm - Fo)/Fm, the initial fluorescence Fo after 20 min dark adaptation was measured, followed by a saturating pulse of actinic light (3500 μmol m^{-2} s^{-1}) to induce maximal fluorescence (Fm). Standard values of Fv/Fm in unstressed thalli are usually between 0.7 and 0.8 (Maxwell and Johnson, 2000; Ritchie, 2006). Thus *F. virsoides* was considered to be in a healthy ecophysiological status if the ratio Fv/Fm exceeded 0.70 (Young et al., 2007); a decrease in this variable was considered as a measure for induced stress (Huppertz et al., 1990; Maxwell and Johnson, 2000). The Fv/Fm measured on 50 fronds after collection was consistent with a healthy, unstressed status for *Fucus* (i.e. Fv/Fm = 0.7−0.8).

Fv/Fm was measured 2 cm from the apex of each frond. To guarantee independency among replicates, at each sampling time, 7 randomly chosen apical tips were sampled in each of the three aquaria according to the experimental conditions. Different tips were sampled at each time.

In Experiment 1, Fv/Fm was measured after 1, 3 and 6 days of exposure. In Experiment 2, to assess trajectories of recovery Fv/Fm was measured at day 0 when exposed to GBH, and at days 1, 2, 3 and 6 after the short exposure.

2.2. Statistical analyses

Effects of a continuous exposure to GBH and nutrients on Fv/Fm (Experiment 1) were tested by means of analysis of variance (ANOVA). The model included four factors: GBH (fixed, with two levels), nutrients (fixed, with two levels, orthogonal to GBH), time (random, with three levels, orthogonal to GBH and nutrients), and aquarium (random, with three levels, nested in the interaction GBH × nutrients and orthogonal to time).

The recovery potential of Fv/Fm after short exposure to GBH (Experiment 2) in combination with nutrient enrichment was assessed with a three-way ANOVA, including factor treatment (fixed, with three levels), time (random, with 5 levels, orthogonal to treatment), and aquarium (random, with three levels, nested in treatment and orthogonal to time).

As no variability was associated to the term aquarium in isolation or in interaction with time, it was removed from the analysis and the model was simplified to the factorial combination of all other factors in both analyses. Cochran's C-test was used to check for homogeneity of variances. If heterogeneity of variances could not be removed by transforming the data, the level of significance was fixed at $\alpha = 0.01$, to minimize the probability of making a type II error. Student-Newman-Keuls (SNK) tests were used for the ranking of the means. Analyses were performed in R v3.4.3 (R Core Team, 2017) using the GAD package (Sandrini-Neto and Camargo, 2014).

3. Results

Mean Fv/Fm of *F. virsoides* before exposure to GBH, after 24 h of acclimatization, was 0.759 (SE ± 0.002, n = 50). In Experiment 1, exposure to GBH significantly reduced the Fv/Fm of *F. virsoides* (Table 1, Fig. 1). The deleterious effect of GBH varied through time, with a reduction of Fv/Fm from 62% (day 1) up to 95% (day 6). Nutrient enrichment did not affect Fv/Fm, either in isolation or in combination with GBH (Table 1).

Experiment 2 revealed no potential of recovery of *F. virsoides* despite the short exposure to GBH. Yet, Fv/Fm of *F. virsoides* fronds exposed to GBH significantly varied through time (Table 2, Fig. 2). Fv/Fm decreased to 62% of control condition immediately after exposure to GBH (day 0). On day 1, Fv/Fm increased 23% compared to the values measured at day 0. At day 3, Fv/Fm decreased again to mean values comparable to those observed immediately after exposure to GBH (Fig. 2). Similarly to Experiment 1, at the end of Experiment 2, Fv/Fm decreased of 97%. Also in this case, no additional effect on recovery patterns was observed in thalli exposed to the combination of GBH and nutrient enrichment (Table 2). During both the experiments, starting from the first days, a chlorosis (i.e. chloroplast disruption) was also observed in the distal section of the fronds exposed to GBH (with and without nutrients) (Fig. 3). At the end of the experiment, we also recorded the exudation of brownish metabolites probably due to GBH-induced cell damage.

Table 1
Analysis of variance (ANOVA) of the effects of GBH and nutrients on *F. virsoides* in terms of Fv/Fm during 6-day exposure to GBH in Experiment 1. $^{***}P < 0.0001$, *ns* = not significant.

Source of variation	df	MS	F
GBH (G)	1	22.50	63.53 ns
Nutrients (N)	1	0.01	8.57ns
Time (T)	2	0.33	30.93***
G × N	1	0.01	9.00 ns
G × T	2	0.35	33.02 ***
N × T	2	<0.01	0.02 ns
G × N × T	2	<0.01	0.05 ns
Residual	240	0.01	
Cochran test	C = 0.26, *P* < 0.001		

4. Discussion

This is the first study documenting the effects of GBHs on an Ochrophyta with complex morpho-anatomical structure. Previous investigations tested the effects of GBHs on macroalgae with simplified morphologies, as ephemeral epiphytic filamentous (i.e. *Neosiphonia savatieri*, *Rhizoclonium riparium*) or laminar thalli (i.e. *Gayralia oxysperma*, *Ulva intestinalis*) belonging to Rhodophyta and Chlorophyta (Pang et al., 2012; Kittle and McDermid, 2016). Only two larger fleshy Rhodophyta (i.e. *Pterocladiella capillacea* and *Kappaphycus alvarezii*) have been examined (Pang et al., 2012).

Our results show a prompt response of *F. virsoides* to GBH exposure: after 24 h a 62% reduction in the photosynthetic performance and after 6 days exposure up to a 95% of reduction, accompanied by extensive chlorosis and harm to the tissues. Our findings also document the high vulnerability of *F. virsoides*, as the species was unable to recover from short term exposure to GBH (24 h). However, since we used a Roundup formulation, it is not possible to discriminate if the glyphosate, the surfactant or their combination are responsible for the observed effects on *Fucus* fronds.

Surfactants are declared inert diluents because they are not held to be directly responsible for the herbicide activity and are classified as confidential for regulatory purposes (Mesnage et al., 2015). At the regulatory level, glyphosate is tested alone, although it is always used as part of a mixture with adjuvants in commercial formulations. Surfactants and adjuvants may in some cases be more toxic than the glyphosate active ingredient itself, and eventually interact synergistically with glyphosate, thus enhancing its toxicity (Hanana et al., 2002; de Liz Oliveira Cavalli et al., 2013; Wagner et al., 2013 and references therein; Cattani et al., 2014; de Brito Rodrigues et al., 2016). However, surfactants' toxicity greatly varies among different compounds, and detrimental effects have been exhibited also for marine microalgae (Hampel et al., 2001). As a result of this variability in the use of adjuvants, and since most of them are not compulsorily declared, GBH effects are complex and not always comparable (Mesnage et al., 2015). Because analyses are expensive and challenging, the result is a long-term deficiency in global datasets on environmental concentrations of surfactants and adjuvants used in GBHs. Nevertheless, the risk of agricultural

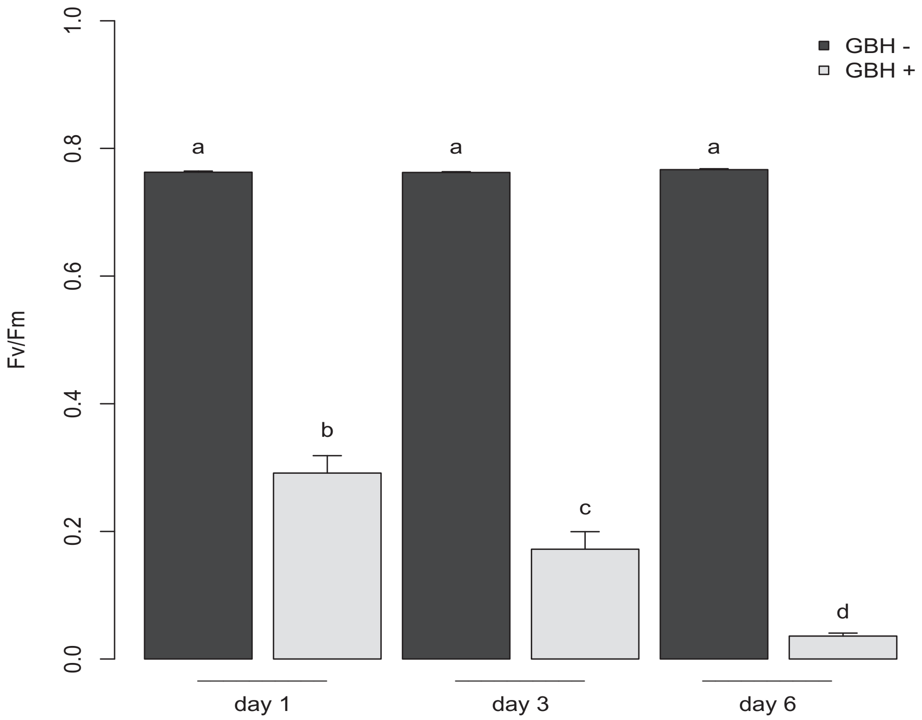

Fig. 1. Mean Fv/Fm (+SE, $n = 42$) of *F. virsoides* thalli in presence and absence of GBH during 6-day exposure to GBH in Experiment 1. Letters above the columns illustrate the ranking of the means from SNK test.

Table 2
Experiment 2. analysis of variance (ANOVA) on the recovery capability of *F. virsoides* in terms of Fv/Fm across treatment conditions (GBH- NU-, GBH+NU-, GBH+NU+) and time (at day 0 when exposed to GBH, and at days 1, 2, 3 and 6 after) in Experiment 2. ***$P < 0.001$, *ns* = not significant.

Source of variation	df	MS	F
Treatment	2	7.30	236.61 ***
Time	4	0.98	31.88 ***
Treatment × Time	8	0.26	8.33 ***
Residual	300	0.03	
Cochran test	C = 0.12, *ns*		

practices using glyphosate, surfactants or their combination to aquatic biota is increasingly considered an issue (Bento et al., 2018), and despite the paucity of studies estimating their concentration in the marine environment, it has been suggested that seas could act as sink for these compounds, which have been detected in the water column and in marine sediments, where they can persist up to several weeks (NRA, 1996; Mann and Bidwell, 1999; Jackson et al., 2016).

The concentration of glyphosate tested (883 μg l^{-1}) is low if compared with those of previous studies on macroalgae (121–300 mg l^{-1}) (Burridge and Gorski, 1998; Pang et al., 2012; Kittle and McDermid, 2016). Yet, direct comparison of our results with the literature is unpractical, since experiments were conducted on different taxa and with different GBH formulations, concentrations, and time of exposure. There is also evidence that algae with a diverse morpho-anatomy can have a different response to the same concentration of GBH, ranging from negligible changes to visible harm (Pang et al., 2012; Kittle and McDermid, 2016). Algae featured by simplified thalli (i.e. filamentous or monostratified) show a higher vulnerability to GBHs than algae with thick cortical layers perhaps because the herbicides cannot efficiently penetrate without longer exposure times and concentrations. In particular, thalli with smaller surface area to volume ratios may be less susceptible to the toxicant at lower concentration (Burridge and Gorski, 1998). Toxicological studies on the marine flora showed that unicellular stages of growth (i.e. spores, gametes) are more sensitive than larger embryos and juveniles to exogenous coumpounds. *F. virsoides* showed significant responses at a concentration of glyphosate lower than the LC_{50} (i.e. concentration of a substance that kills 50% of cells exposed to it) of a kelp gametophyte (4.56–84.4 mg l^{-1}) (R.J. Lewis, in Kittle and McDermid, 2016). Effects of the glyphosate tested in two Laminariales as inhibitor of zoospore germination and gametophyte mortality were observed at concentration >10 mg l^{-1} (Burridge and Gorski, 1998). Our experiments were carried out on adult specimens, more resistant to stress exposure than early life-history stages. Thus, if contamination peaks coincided with fertilization or germination times, recruitment could be adversely affected (Thursby and Steele, 1986; Burridge et al., 1995).

Unfortunately, in Italy systematic marine monitoring of GBHs is not carried out, so that quantitative data on herbicides degradation and concentration in the coastal seawater are not available. To set experimental concentration, GBH concentrations reported in the literature were taken into account. Wang et al. (2016) measured concentrations of glyphosate in seawater samples ranging from 13 μg l^{-1} to 1377 μg l^{-1}. Milan et al. (2018) consider as "environmentally realistic" for the Northern Adriatic Sea glyphosate concentrations from 10 to 1000 μg l^{-1}. The concentration we used falls within these ranges. Seawater contamination can also have an urban origin: storm waters may be contaminated by herbicides by the leaching of polluted urban surfaces (Botta et al., 2009; Zgheib et al., 2012). When heavy rain events or rivers overflow move large amounts of GBHs bounded to terrigenous sediments, little degradation of glyphosate is expected before sediments reach the marine environment.

In our experiments nutrients did not play any role in affecting the photosynthetic efficiency either alone or in combination with

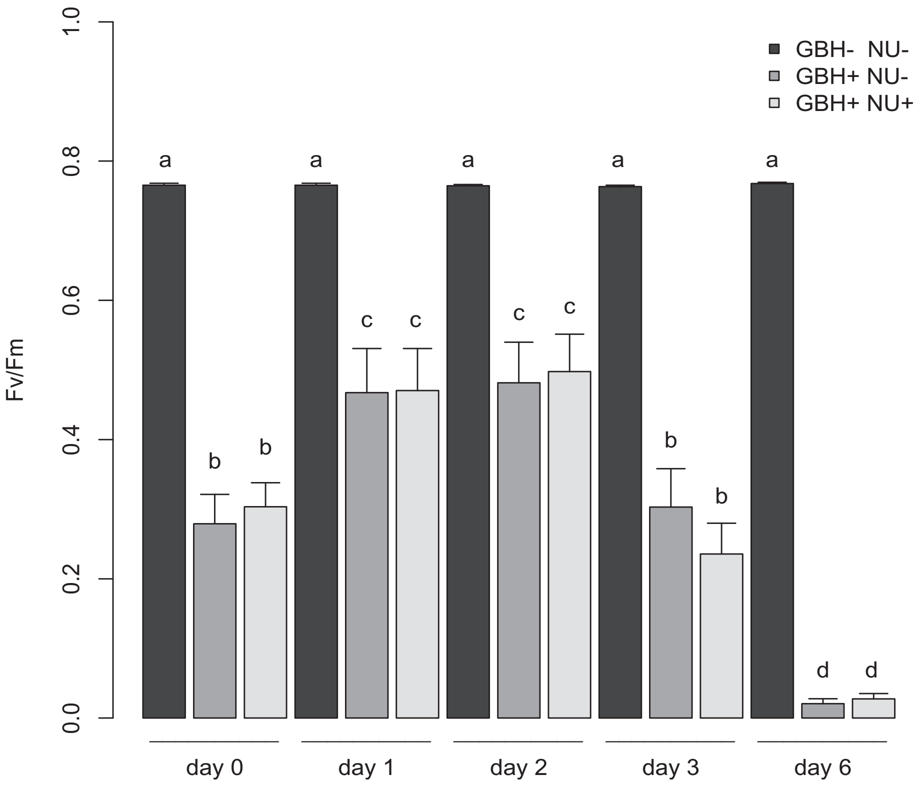

Fig. 2. Mean Fv/Fm (+SE, $n = 21$) of *F. virsoides* thalli in presence or absence of GBH and nutrients at day 0 when exposed to GBH, and at days 1, 2, 3 and 6 after the short exposure in Experiment 2. Letters above the columns illustrate the ranking of the means from SNK test.

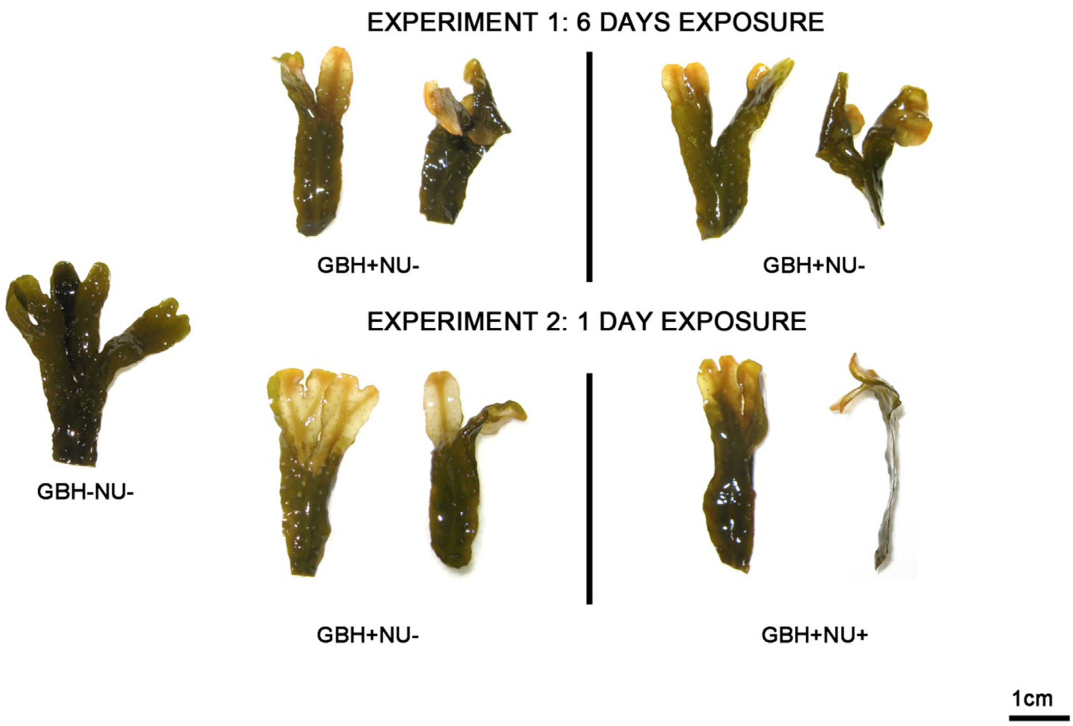

Fig. 3. Apices of *F. virsoides* with or without GBH and nutrients addition at day 6, in Experiments 1 and 2.

GBH, probably because *F. virsoides* shows high potential to withstand trophic changes, which are frequent in eutrophic waters (i.e. Lagoon of Venice). However, fertilization treatments are known to exacerbate glyphosate bioavailability in the soil solution and in the eventual runoff, as $PO4^{3-}$ and glyphosate compete for soil adsorbing sites because of their chemical similarities (Gomes et al., 2015). Very few researches have investigated the effects of GBHs in combination with other factors (Doddaiah et al., 2013; Wang et al., 2016; Amid et al., 2017), but never on macroalgae. Yet, effects of $PO4^{3-}$ addition on glyphosate roots uptake have been observed in terrestrial plants (Clua et al., 2012; Gomes et al., 2015).

Our results highlight critical gaps in knowledge about the effects of GBHs on marine-coastal systems and call for the monitoring of underestimated threats originating on land and affecting the sea. A combination of laboratory experiments and field studies are needed to understand ecologically relevant limits to the indiscriminate loading of toxic agricultural compounds into the marine ecosystem. Existing management/monitoring instruments (e.g. Marine Strategy Framework Directive) and international directives (Ecosystem Based Management, Maritime Spatial Planning) could benefit from information about glyphosate impacts on seaweeds.

5. Conclusions

Our study highlights the detrimental impact that GBHs can exert in the marine environment on *Fucus virsoides*. This recognition is an opportunity to reconsider the importance of concretely incorporating land-sea connections in present environmental monitoring. Current gaps in knowledge about the environmental risks of GBHs demand an in-depth investigation of patterns of degradation, thresholds of change, species-specific responses and potential of interaction with other processes.

Declaration of interest

None.

Acknowledgment

We are sincerely grateful to four anonymous referees for their useful comments. Research funded by the EU Interreg MED AMAre Project (http://msp-platform.eu/projects/amare-actions-marine-protected-areas) and the project MERCES of the European Union's Horizon 2020 research (Grant agreement No. 689518, http://www.merces-project.eu).

References

Amid, C., Olstedt, M., Gunnarsson, J.S., Le Lan, H., Tran Thi Minh, H., Van den Brink, P.J., Hellostrӧm, M., Tedengren, M., 2017. 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. https://doi.org/10.1007/s11356-016-8320-7.

Annette, R., Habibi, H.R., Hontela, A., 2014. Impacts of glyphosate and glyphosate-based herbicides on the freshwater environment. J. Appl. Toxicol. 34, 458–479.

Barceló, D., Hennion, M., 2003. Trace Determination of Pesticides and Their Degradation Products in Water. ISBN 9780444818423, p. 556.

Battelli, C., 2016. Disappearance of *Fucus virsoides* J. Agardh from the Slovenian coast (Gulf of Trieste, northern Adriatic). Ann. Ser Hist Nat 26, 1–9.

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

Bento, C.P.M., Commelin, M.C., Baartman, J.E.M., Yang, X., Peters, P., Mol, H.G.J., Ritsema, C.J., Geissen, V., 2018. Spatial glyphosate and AMPA redistribution on the soil surface driven by sediment transport process - a flume experiment. Environ. Pollut. 234, 1011–1020.

Botta, F., Lavison, G., Couturier, G., Alliot, F., Moreau-Guigon, E., Fauchon, N., Guery, B., Chevreuil, M., Blanchoud, H., 2009. Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems. Chemosphere 77, 133–139.

Burridge, T.R., Lavery, T., Lam, P.K.S., 1995. Acute toxicity tests using *Phyllospora comosa* (Phaeophyta: Fucales) and *Allorchestes compressa* Dana (Crustacea: Amphipoda). Bull. Environ. Contam. Toxicol. 55, 621–628. https://doi.org/10.1007/BF00196045.

Burridge, T.R., Gorski, J., 1998. The Use of Biocidal Agents as Potential Control Mechanism for the Exotic Kelp *Undaria pinnatifida*. Technical Report No. 16. Centre for Research on Introduced Marine Pests, CSIRO Marine Research, Hobart, Australia, pp. 1–27, 27 pp. ISBN 064306180 0.

Castro, A.V., Colares, I.G., Santos Franco, T.C.R., Cutrim, M.V.J., Luvizotto-Santos, R., 2015. Using a toxicity with *Ruppia maritima* (Linnaeus) to assess the effects of Roundup. Mar. Pollut. Bull. 91, 506–510.

Cattani, D., de Liz Oliveira Cavalli, V.L., Heinz Rieg, C.E., Domingues, J.T., Dal-Cim, T., Tasca, C.I., Mena Barreto Silva, F.R., Zamoner, A., 2014. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. Toxicology 320, 34–45.

Cedergreen, N., Streibig, J.C., 2005. The toxicology of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. Pest Manag. Sci. 62, 1152–1160.

Cheminée, A., Sala, E., Pastor, J., Bodilis, P., Thiriet, P., Mangialajo, L., Cottalorda, J.M., Francour, P., 2013. Nursery value of *Cystoseira* forests for Mediterranean rocky reef fishes. J. Exp. Mar. Biol. Ecol. 442, 70–79.

Claudet, J., Fraschetti, F., 2010. Human-driven impacts on marine habitats: a regional meta-analysis in the Mediterranean Sea. Biol. Conserv. 143, 2195–2206.

Clua, A., Conti, M., Beltrano, J., 2012. The effects of glyphosate on the growth of birdsfoot trefoil (*Lotus corniculatus*) and its interaction with different phosphorus contents in soil. J. Agric. Sci. 4, 208–218.

de Brito Rodrigues, L., de Oliveira, R., Abe, F.R., Brito, L.B., Moura, D.S., Valadares, M.C., Grisolia, C.K., de Oliveira, D.P., Rodrigues de Oliveira, G.A., 2016. Ecotoxicological assessment of glyphosate-based herbicides: effects on different organisms. Environ. Toxicol. Chem. 36, 1755–1763.

de Liz Oliveira Cavalli, V.L., Cattani, D., Elise Heinz Rieg, C., Pierozan, P., Zanatta, L., Benedetti Parisotto, E., Wilhelm Filho, D., Regina Mena Barreto Silva, F., Pessoa-Pureur, R., Zamoner, A., 2013. Roundup disrupted male reproductive functions by triggering calcium-mediated cell death in rat testis and sertoli cells. Free Radical Biol. Med. 65, 335–346.

Dill, G.M., Sammons, R.D., Feng, P.C.C., Kohn, F., Kretzmer, K., Mehrsheikh, A., Bleeke, M., Honegger, J.L., Farmer, D., Wright, D., Haupfear, E.A., 2010. Glyphosate: discovery, development, applications, and properties. In: Nandula, V.K. (Ed.), Glyphosate Resistance in Crops and Weeds. John Wiley and Sons, Hoboken, pp. 1–33.

Doddaiah, K.M., Narayan, A., Aswathanarayana, R.G., Ravi, S., 2013. Effect of metabolic inhibitors on growth and carotenoid production in *Dunaliella bardawil*. J. Food Sci. Technol. 50, 1130–1136. https://doi.org/10.1007/s13197-011-0429-6.

Falace, A., Alongi, G., Cormaci, M., Furnari, G., Curiel, D., Cecere, E., Petrocelli, A., 2010. Changes in the benthic algae along the Adriatic Sea in the last three decades. J. Chem. Ecol. 26, 77–90.

Falkenberg, L.J., Russell, B.D., Connell, S.D., 2012. Stability of strong species interactions resists the synergistic effects of local and global pollution in kelp forests. PLoS One 7 (3), e33841. https://doi.org/10.1371/journal.pone.0033841.

Friberg, N., Bonada, N., Bradley, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M., Woodward, G., 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. Adv. Ecol. Res. 44, 1–68.

Gaupp-Berghausen, M., Hofer, M., Rewald, B., Zaller, J.G., 2015. Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. Sci Rep-UK 5, 12886. https://doi.org/10.1038/srep12886.

Gomes, M.P., Maccario, S., Lucotte, M., Labrecque, M., Juneau, P., 2015. Consequences of phosphate application on glyphosate uptake by roots, of phosphate application on glyphosate uptake by roots: impacts for environmental management practices. Sci. Total Environ. 276, 97–104.

Gorman, D., Russell, B.D., Connell, S.D., 2009. Land-to-sea connectivity: linking human-derived terrestrial subsidies to subtidal habitat change on open rocky coasts. Ecol. Appl. 19, 1114–1126.

Gorman, D., Bajjoux, T., Populus, J., Vasquez, M., Ehrhold, A., 2013. Modelling kelp forest distribution and biomass along temperate rocky coastlines. Mar. Biol. 160, 309–325.

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. Science 319, 948–952.

Hampel, M., Moreno-Garrido, I., Sobrino, C., Lubian, M., Blasco, J., 2001. Acute toxicity of LAS homologues in marine microalgae: esterase activity and inhibition growth as endpoints of toxicity. Ecotoxicol. Environ. Saf. 48, 287–292.

Hanana, H., Simon, G., Kervarec, N., Mohammadou, B.A., Cérantola, S., 2002. HRMAS NMR as a tool to study metabolic responses in heart clam Ruditapes decussatus exposed to Roundup®. Talanta 97, 425–431.

Huppertz, K., Hanelt, D., Nultsch, W., 1990. Photoinhibition of photosynthesis in the marine brown alga *Fucus serratus* as studied in field experiments. Mar. Ecol. Prog. Ser. 66, 175–182.

Jackson, M., Eadsforth, C., Schowanek, D., Delfosse, T., Riddle, A., Budgen, N., 2016. Comprehensive review of several surfactants in marine environments: fate and ecotoxicity. Environ. Toxicol. Chem. 35, 1077–1086.

Kemp, W.M., Boynton, W.R., Stevenson, J.C., Twilley, R.R., Means, J.C., 1983. The decline of submerged vascular plants in upper Chesapeake Bay: summary of results concerning possible causes. Mar. Technol. Soc. J. 17, 78–89.

Kittle, R.P., McDermid, K.J., 2016. Glyphosate herbicide toxicity to native Hawaiian macroalgal and seagrass species. J. Appl. Phycol. 28, 2597–2604. https://doi.org/10.1007/s10811-016-0790-y.

Lefrancq, M., Jadas-Hécart, A., La Jeunesse, I., Landry, D., Payraudeau, S., 2017. High frequency monitoring of pesticides in runoff water to improve understanding of their transport and environmental impacts. Sci. Total Environ. 587–588, 75–86.

Mann, R.M., Bidwell, J.R., 1999. The toxicity of glyphosate and several glyphosate

formulations to four species of Southwestern Australian frog. Arch Environ Con Tox 36, 193–199.
Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 51, 659–668.
Maycock, D., Crane, M., Atkinson, C., Johnson, I., 2010. Proposed EQS for Water Framework Directive Annex VIII Substances: Glyphosate. Water Framework Directive. United Kingdom Technical Advisory Group (WFD-UKTAG), Edinburgh, p. 120.
Mayer-Pinto, M., Underwood, A.J., Tolhurst, T., Coleman, R.A., 2010. Effects of metals on aquatic assemblages: what do we really know? J. Exp. Mar. Biol. Ecol. 391, 1–9.
Mercurio, P., Flores, F., Mueller, J.F., Carter, S., Negri, A.P., 2014. Glyphosate persistence in seawater. Mar. Pollut. Bull. 85, 385–390.
Mesnage, R., Defarge, N., Spiroux de Vendômois, J., Séralini, G.E., 2015. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem. Toxicol. 84, 133–153.
Milan, M., Dalla Rovere, G., Smits, M., Ferraresso, S., Pastore, P., Marin, M.G., Bogialli, S., Patarnello, T., Bargelloni, L., Matozzo, V., 2018. Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: transcriptional responses in the mussel *Mytilus galloprovincialis*. Environ. Pollut. 237, 442–451.
Newton, J., 2013. Glyphosate. Chemistry World. Royal Society of Chemistry. www.rsc.org/chemistryworld/2013/12/glyphosateweedkiller-roundup-gm-podcast. (Accessed 8 August 2014).
NRA, 1996. NRA special Review of Glyphosate. Chemical Review Section, National Registration Authority for Agricultural & Veterinary Chemicals. NRA Special Review Series 96.1, Canberra.
Orlando-Bonaca, M., Mannoni, P.A., Poloniato, D., Falace, A., 2013. Assessment of *Fucus virsoides* distribution in the Gulf of Trieste (Adriatic Sea) and its relation to environmental variables. Bot. Mar. 65, 451–459.
Pang, T., Liu, J., Liu, Q., Zhang, L., Lin, W., 2012. Impacts of glyphosate on photosinthetic behaviours in *Kappaphycus alvarezii* and *Neosiphona savatieri* detected by JIP-test. J. Appl. Phycol. 24, 467–473.
R Core Team, 2017. R: a Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.
Ritchie, R.J., 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynth. Res. 89, 27–41.
Roháèek, K., 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. Photosynthetica 40, 13–29.
Sandrini-Neto, L., Camargo, M.G., 2014. GAD: an R Package for ANOVA Designs from General Principles. Available on CRAN.
Sasal, M.C., Demonte, L., Cislaghi, A., Gabioud, E.A., Oszust, J.D., Wilson, M.G., Michligm, N., Bendomenico, H.R., Repetti, M.R., 2015. Glyphosate loss by runoff and its relationship with phosphorus fertilization. J. Agric. Food Chem. 63, 4444–4448.
Solomon, K., Thompson, D., 2003. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J. Toxicol. Environ. Health B 6, 289–324.
Stachowski-Haberkorn, S., Becker, B., Marie, D., Haberkorn, H., Coroller, L., Dela Broise, D., 2008. Impact of Roundup on the marine microbial community, as shown by an *in situ* microcosm experiment. Aquat. Toxicol. 89, 232–241.
Strain, E.M.A., Thomson, R.J., Micheli, F., Mancuso, F.P., Airoldi, L., 2014. Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. Global Change Biol. 20, 3300–3312.
Thibaut, T., Pinedo, S., Torras, X., Ballesteros, E., 2005. Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Albéres coast (France, North-western Mediterranean). Mar. Pollut. Bull. 50, 1472–1489.
Thursby, G.B., Steele, R.L., 1986. Comparison of short- and long-term sexual reproduction tests with the Maine red alga *Champia parvula*. Environ. Toxicol. Chem. 5, 1013–1018. https://doi.org/10.1002/etc.5620051109.
Wagner, N., Reichenbecher, W., Teichman, H., Tappeser, B., Lotters, S., 2013. Questions concerning the potential impact of glyphosate-based herbicides on amphibians. Environ Toxical Chem 32, 1688–1700.
Wang, S., Liu, B., Yuan, D., Ma, J., 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.
Young, E.B., Dring, M.J., Savidge, G., Birkett, D.A., Berges, J.A., 2007. Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant Cell Environ. 30, 764–774.
Zgheib, S., Moilleron, R., Chebbo, G., 2012. Priority pollutants in urban stormwater: Part 1 e case of separate storm sewers. Water Res. 46, 6683–6692.