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Effects of Glyphosate-Based and Derived Products on Sea Urchin Larval Development

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Abstract: Despite the widespread use of herbicide glyphosate in cultivation, its extensive runoff into rivers and to coastal areas, and the persistence of this chemical and its main degradation product (aminomethylphosphonic acid, AMPA) in the environment, there is still little information on the potential negative effects of glyphosate, its commercial formulation Roundup® and AMPA on marine species. This study was conducted with the aim of providing a comparative evaluation of the effects of glyphosate-based and its derived chemicals on the larval development of the sea urchin *Paracentrotus lividus*, thus providing new data to describe the potential ecotoxicity of these contaminants. In particular, the effects on larval development, growth and metabolism were assessed during 48 h of exposure from the time of egg fertilization. The results confirm that AMPA and its parent compound, glyphosate have similar toxicity, as observed in other marine invertebrates. However, interestingly, the Roundup® formulation seemed to be less toxic than the glyphosate alone.

Keywords: herbicide; glyphosate; Roundup®; AMPA; sea urchin; larval development; *Paracentrotus lividus*

1. Introduction

Since the 1990s, the presence of emerging contaminants in the aquatic environments has been studied in increasing depth [1]. These molecules are ubiquitously distributed in aquatic ecosystems, where'they are continuously released and are found at medium–low (µg/L) and low levels (ng/L) [2,3]. The concentrations of pollutants are likely to increase due to the increasing number of new molecules with commercial relevance, and the inadequacy of wastewater treatment plants and waste management [4]. Therefore, a serious threat is posed to organisms that live in riverine, estuarine and coastal environments.

Numerous ecotoxicological studies have demonstrated that the first developmental stages (gametes, embryos and larvae) in the life cycle of marine invertebrates represent critical phases that are the most susceptible to significant and persistent alterations of environmental parameters and the presence of xenobiotics [2,5]. In fact, compared to adults, the early life stages have a greater surface/volume ratio and are less developed with regard to the ability to metabolise absorbed chemical substances. These characteristics facilitate the uptake of chemical products on the one hand, and result in the poor efficiency of the detoxification processes on the other [6]. As the larval stages represent a potential bottleneck in the maintenance of natural populations, they are commonly used to evaluate the toxicity of a large variety of contaminants and in the biomonitoring of marine pollution [5,7].

Glyphosate (N-[phosphonomethyl]-glycine) is a non-selective herbicide that is able to prevent the growth of plants through the inhibition of the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS),

a fundamental enzyme for the synthesis of aromatic amino acids [8,9]. Currently, glyphosate is among the most used chemical in agricultural practices, forestry and domestic weed control worldwide [10], peaking at more than 800,000 t globally in 2014 [11] with an expected increase in use of up to 920,000 t per year by 2025 [12]. It has been estimated that up to 2% of the herbicides used in agricultural fields reaches coastal waters [13]. The presence of glyphosate in marine coastal environments has been documented [14,15] at a level of several µg/L with a half-life of 47–315 days, depending on the light and temperature conditions [16]. Since the 1970s, glyphosate has been available on the market in the formulation known as Roundup. Roundup is one of the most widely used commercial formulations of glyphosate and consists of a combination of the active substance (glyphosate) with surfactant compounds, such as polyoxyethylene amine (POEA), and permeabilizing agents, which can increase the product toxicity [17]. It has been demonstrated that the presence of POEA contributes heavily to the toxicity of Roundup to aquatic animals [18,19]. Little is known about the toxicity of the degradation products of glyphosate. Aminomethylphosphonic acid (AMPA) is one of the main metabolites derived from the microbial degradation of glyphosate [20]. Giesy and colleagues [21] reported that the half-life and toxicity of AMPA are equivalent to those of the parent compound.

Many studies have been conducted in the last decade to assess the effects of glyphosate on several aspects of animal life in various species of aquatic organisms [3,22]. In fish and molluscs, this chemical has been shown to alter acetylcholinesterase (AChE) activity [23], to interrupt hormone synthesis and to increase oxidative stress, thus disrupting reproduction and metabolism [24–27]. Acute exposure to glyphosate can promote genotoxicity in fish [28–30]. These effects can strongly impact the development of animals in the early life stages, which is generally recognized as the period when animals are more susceptible to environmental changes as compared to adults [31].

The early life stages of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) have been widely used as biological model in ecotoxicological studies [32–38]. In the present work, they have been used to assess the effects of pure active glyphosate, the herbicide Roundup, and the main glyphosate degradation product, AMPA. To our knowledge, no previous studies have tested the effects of glyphosate-based products and glyphosate degradation products on sea urchin larvae.

Four environmentally relevant concentrations of the compounds (1, 10, 50 and 100 µg/L) were used to ascertain whether different toxic effects occurred, depending on the compound and concentration tested. The main endpoints considered were embryo/larval development at 24 and 48 hours post fertilization (hpf), and larval growth at 48 hpf. Respiration rate, as a proxy for metabolic expenditure, was also measured at the highest concentration of each compound.

2. Materials and Methods

2.1. Collection of Adult Specimens

P. lividus adult specimens (mean test diameter of 5.02 cm ± 0.31 s.d.) were collected by scuba divers at a 2–5 m depth in a coastal area of the North Adriatic Sea (45°13'42.9" N, 12°19'22.0" E). After collection, the organisms were immediately transported to the Hydrobiological Station, "Umberto D'Ancona" in Chioggia, kept for 5 days in tanks with a continuous flow of seawater and natural variation in the environmental parameters (mean temperature: 26.6 °C ± 1.8 s.d.; mean salinity: 33.8 ± 1.8 s.d.), and were fed ad libitum with *Ulva* sp.

2.2. Experimental Conditions Tested

Thirteen experimental conditions were tested: the control was represented by artificial sea water (ASW) (35 salinity, pH 8.2; Ocean Fish, Prodac International S.r.l, Padova, Italy) filtered with a 0.45 µm Sartorius filter, and four environmentally relevant concentrations (1, 10, 50, 100 µg/L) of glyphosate, AMPA and Roundup in ASW. For Roundup, the values refer to the concentration of the active substance, glyphosate. Glyphosate and AMPA were purchased from Sigma-Aldrich (Milano, Italy). Roundup (Roundup Power 2.0, composition: 360 g pure glyphosate/L, Monsanto Europe N.V.,

Antwerp, Belgium) was purchased from a local commercial vendor. ASW was chosen to prevent the interference of other contaminants that might be present in natural sea water.

The experiment was replicated three times, each time using a pool of eggs and sperm from three female and three male sea urchins.

2.3. Gamete Preparation and Larval Exposure

To induce gamete emission, each sea urchin was injected with 1 mL of 0.5 M KCl solution using a sterile syringe through the peristomal membrane. Sperm from three males was collected with a micropipette and maintained on ice until use. After KCl injection, the eggs of three females were collected in 250 mL beakers filled with ASW by placing each female upside down over the beaker mouth. Dams were allowed 30 min to release eggs, which were then filtered with a 200 µm nylon mesh to remove impurities (e.g., fragments of algae, prickles and pedicels), concentrated using a 20 µm nylon mesh and re-suspended in ASW. Egg density was assessed in subsamples under a Leica DM750 optical microscope. Eggs from the three females were pooled in a single beaker in equal proportions. Prior to preparing the sperm pool, the density of the ejaculate of each male was estimated in diluted subsamples using a Z2 Coulter Counter (Beckman Coulter Inc., Miami, FL, USA), in order to define an equal contribution of sperm from all males. Sperm were activated in ASW and activation was checked after one minute under optical microscope.

The two pools of gametes thus constituted were brought together by adding an adequate volume of activated sperm to the egg suspension to obtain a sperm:egg ratio of 1250:1 [39]. After 15 min, the raising of the fertilization membrane in at least 90% of eggs was checked under the microscope. Fertilized eggs were collected with a 20 µm nylon mesh in order to eliminate sperm and then resuspended in ASW. Five 24 mL glass vials were prepared for each experimental condition. Equal volumes of the fertilized egg suspension were distributed into the vials at a final concentration of 60 eggs/mL. Exposures were carried out for 48 h at a temperature of 22 °C.

2.4. Larval Development and Growth Assessment

In each experiment, two replicates per experimental condition were fixed with 10% neutralized formalin at 24 hpf, and the other three were fixed at 48 hpf. Larval development was assessed at both 24 and 48 hpf on 100 larvae per replicate. The following developmental stages were assigned: blastula (the pre-hatching stages), gastrula, prism, early pluteus and pluteus. At 48 hpf, larval abnormalities (Figure 1) were also assessed in 100 echinoplutei per replicate, in accordance with the method proposed by His and colleagues [40]. The remaining larvae in the 48 hpf replicates were then filtered on a Sartorius acetate cellulose filter (25 mm in diameter and 8 µm porosity) and gently washed with deionized water to remove formalin and salts. The filter was placed on a histology slide in an oven at 60 °C for 1 h and then it was clarified with cedar oil and covered with a coverslip for preservation.

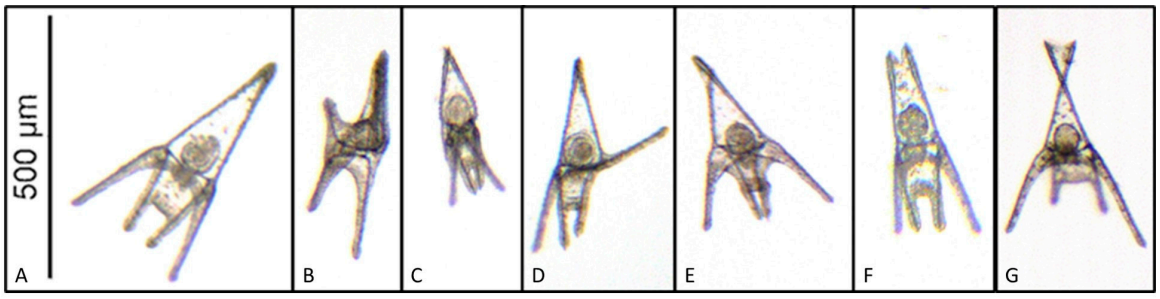


Figure 1. Various abnormalities observed in *P. lividus* larvae exposed for 48 h in control conditions and in four concentrations (1, 10, 50, 100 µg/L) of glyphosate, AMPA and Roundup. (**A**) Normal echinopluteus; (**B**,**C**) deformed echinopluteus; (**D**) twisted post-oral rod; (**E**) unequally long somatic rods; (**F**) apically spaced somatic rods; (**G**) apically crossed somatic rods.

Larval growth was evaluated by measuring the somatic and the post-oral skeletal rod of 100 plutei per replicate. All samples were observed using an optical microscope (Leica DM 750) provided with a Leica DFC 295 digital camera; and the computer acquisition of images was performed using Leica Application Suite 3.8 software. Larval skeletal rods were measured using the ImageJ image analysis program. Due to technical issues, in one out of three experiments the results from larvae exposed to 1 µg/L of AMPA were not available.

2.5. Respiration Rate

Oxygen consumption was measured in only one of the three experiments. Additional vials were prepared and equipped with an O_2 sensor spot (OXSP5, Pyro Science GmbH, Aachen, Germany): 3 replicates for each contaminant in a concentration equal to 100 µg/L, 3 replicates for ASW (control) and 2 replicates for the blank containing only ASW, i.e., in the absence of larvae. Fertilized eggs were added at the same final concentration reported above for the other bioassays. Before starting the measurements, all vials were completely filled and then sealed with a screw cap. To detect the concentration of O_2, the Fiber-Optic Oxygen Meter Piccolo2 (Pyro Science GmbH, Aachen, Germany) was used. This measurement was performed every 8 h, starting from 0 hpf up to 48 hpf. Oxygen saturation never fell below 80%. Values are calculated as the difference in O_2 concentration between 0 hpf and 48 hpf and expressed as the oxygen consumption rate in pmol/larva/h, after correcting the values with the blank.

2.6. Statistical Analysis

The differences in larval development among the exposure conditions were assessed using a non-parametric permutational multivariate analysis of variance, PERMANOVA [41], applying the Euclidean distance matrix on the raw data. First, a PERMANOVA was performed on the overall data set, then various PERMANOVA were used to perform pair-wise comparisons between exposure conditions ("adonis" function in "vegan" package [42]) with "experiment" as the random factor. To assess the differences in larval rod lengths, measurements were analysed through a linear mixed model fit by maximum likelihood ("lmer" function in the "lmerTest" package [43]) with "experiment" as the random factor, followed by a Tukey post-hoc test for the pairwise comparisons. For the oxygen consumption analysis, a one-way ANOVA followed by a Tukey multiple comparison post-hoc test was carried out, and the normality and homoscedasticity were checked in the residuals. Significant pair-wise comparisons were shown with respect to (i) control (ASW), (ii) different concentrations of the same contaminant, and (iii) different contaminants at the same concentration. All data were analyzed using R and RStudio software (version 3.5.3, [44]). The significance threshold was fixed at $p < 0.05$.

3. Results

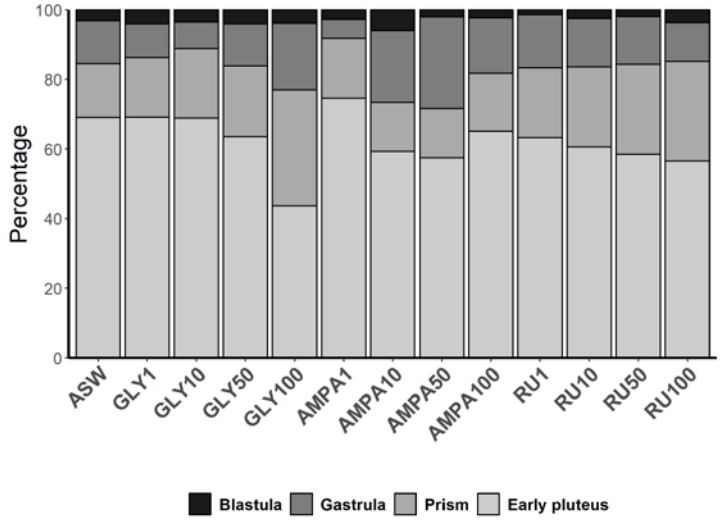
3.1. Larval Development, Abnormalities and Growth

The PERMANOVA and linear mixed model results highlighted significant differences among the experimental conditions tested at both 24 hpf and 48 hpf for all endpoints considered (Table 1).

Table 1. PERMANOVA results, with PseudoF values and *p*-values, for developmental stages at both 24 and 48 hpf and the linear mixed model (LMM) results, with Chi-square values and *p*-values, for the frequency of anomalies and rod lengths at 48 hpf in larvae exposed to the various concentrations of glyphosate, AMPA and Roundup.

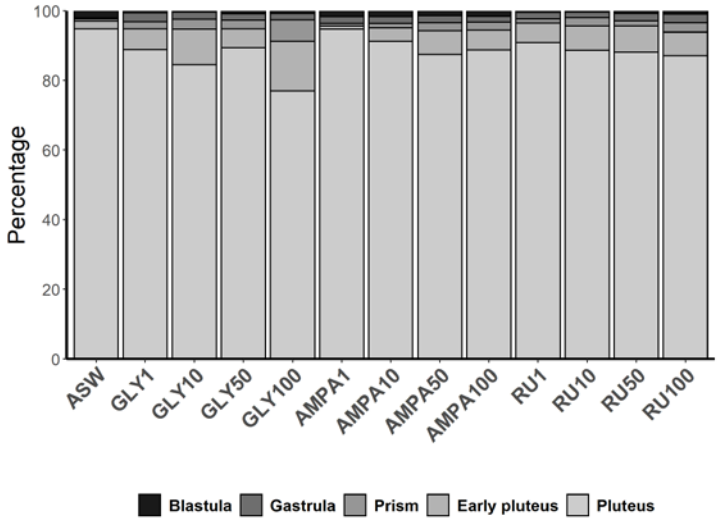
Developmental Stages 24 hpf	$PseudoF_{(12,60)} = 0.671$	<0.050
Developmental Stages 48 hpf	$PseudoF_{(12,98)} = 0.978$	<0.050
Anomalies 48 hpf	$\chi^2_{(12)} = 37.519$	<0.001
Somatic Rod Length 48 hpf	$\chi^2_{(12)} = 29.263$	<0.010
Post-oral Rod Length 48 hpf	$\chi^2_{(12)} = 20.810$	<0.050

In detail, at 24 hpf, the developmental stage was significantly affected in AMPA 50 µg/L and Roundup 100 µg/L with respect to the control ($p < 0.05$) (Figure 2). Although not significant, a dose-dependent pattern of delay in development can also be observed in glyphosate exposure. At 48 hpf, differences in development between treatments and control were always significant, except for AMPA 1 and 10 µg/L (Figure 3). With regard to exposures to the same contaminant, significant differences between concentrations were found for glyphosate and Roundup (Figure 2) at 24 hpf, for'AMPA at 48 hpf (Figure 3).



Pairwise comparison	*p* value
ASW - AMPA50	0.046
ASW - RU100	0.045
GLY1 - GLY100	0.020
GLY10 - GLY50	0.009
GLY10 - GLY100	0.019
GLY10 - AMPA10	0.043
RU1 - RU100	0.010

Figure 2. Percentage of developmental stages at 24 hpf in larvae reared in control conditions (ASW) and in four concentrations (1, 10, 50, 100 µg/L) of three contaminants: glyphosate (GLY), AMPA and Roundup (RU). Statistically significant pairwise comparisons are reported in the table on the right.

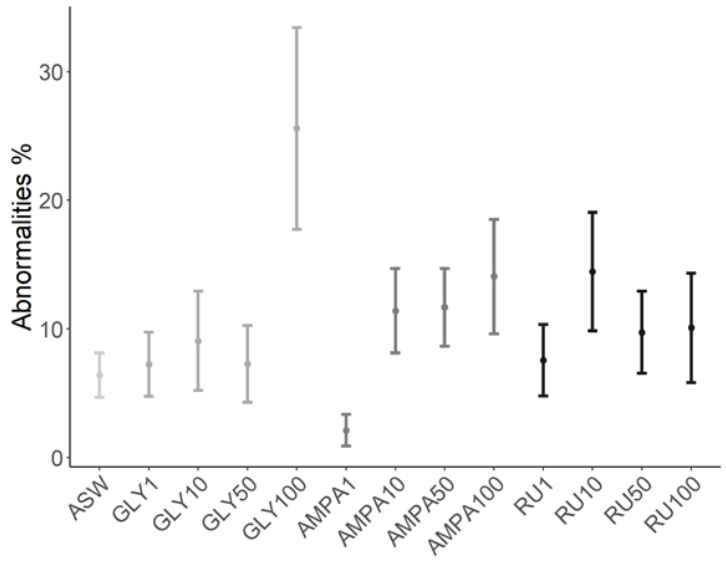


Pairwise comparison	*p* value
ASW - GLY1	0.018
ASW - GLY10	0.025
ASW - GLY50	0.026
ASW - GLY100	0.048
ASW - AMPA50	0.014
ASW - AMPA100	0.014
ASW - RU1	0.034
ASW - RU10	0.039
ASW - RU50	0.006
ASW - RU100	0.028
AMPA1 - AMPA100	0.047

Figure 3. Percentage of developmental stages at 48 hpf in larvae reared in control conditions (ASW) and in four concentrations (1, 10, 50, 100 µg/L) of three contaminants: glyphosate (GLY), AMPA and Roundup (RU). Statistically significant pairwise comparisons are reported in the table on the right.

Overall, the percentage of the pluteus stage decreased with the increase in contaminant concentration for all contaminants tested (Figure 3). When comparing contaminants at the same concentration, the only significant difference was between glyphosate and AMPA 10 µg/L, at 24 hpf (Figure 2), with AMPA showing a greater percentage of blastulae and gastrulae.

As shown in Figure 4, the exposure to increasing concentration of glyphosate and AMPA was positively related to the percentage of abnormal echinoplutei. This value reached its maximum (~26%) in larvae exposed to 100 µg/L of glyphosate, with significant differences compared to the control and all the other experimental conditions tested ($p < 0.01$). Conversely, the exposure to an increasing concentration of Roundup did not cause significant changes in the percentage of abnormalities compared to the control, except for a significant increase at 10 µg/L ($p < 0.05$).



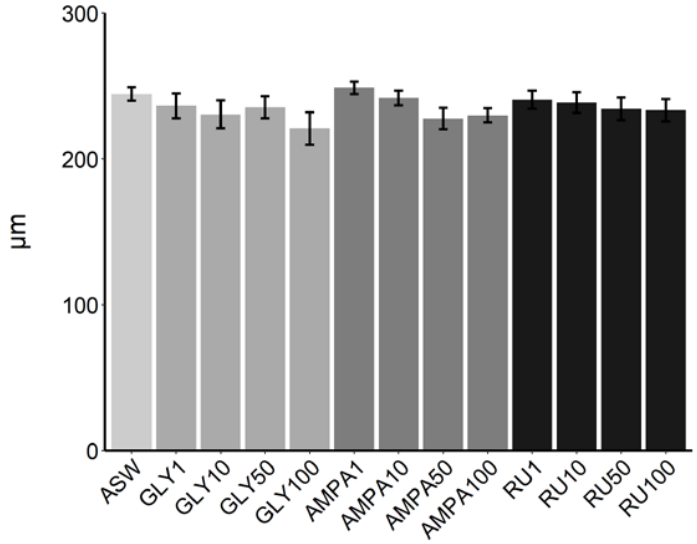
Pairwise comparison	*p* value
ASW - RU10	0.048
GLY100 - ASW	< 0.001
GLY100 - GLY1	< 0.001
GLY100 - GLY10	< 0.001
GLY100 - GLY50	< 0.001
GLY100 - AMPA100	0.001
GLY100 - AMPA10	0.001
GLY100 - AMPA50	0.001
GLY100 - AMPA100	0.005
GLY100 - RU1	< 0.001
GLY100 - RU10	0.006
GLY100 - RU50	< 0.001
GLY100 - RU100	< 0.001

Figure 4. Percentage of abnormalities at 48 hpf in larvae reared in control conditions (ASW) and in four concentrations (1, 10, 50, 100 µg/L) of three contaminants: glyphosate (GLY), AMPA and Roundup (RU). Values are means ± s.e., n = 6–9. Statistically significant pairwise comparisons are reported in the table on the right.

The skeletal rod lengths of the *P. lividus* echinoplutei, measured at 48 hpf in the various experimental conditions are shown in Figures 5 and 6. A reduction in the length of the rods can be observed, and therefore the larval size is reduced with a decreasing trend in variation with increasing contaminant concentration (1, 10, 50, 100 µg/L). This trend was not so evident in the post-oral rod length of larvae exposed to glyphosate, where values dropped at 10 µg/L and remained stable in the higher concentrations.

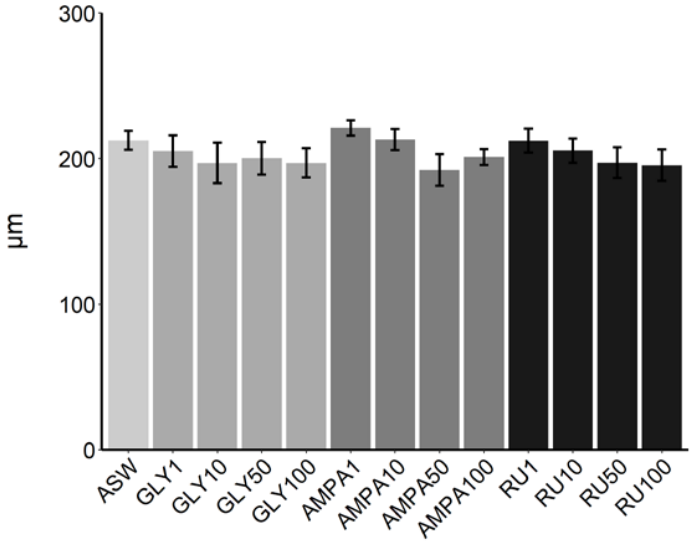
In particular, the somatic rod was significantly different from the control in the presence of 10, and 100 µg/L of glyphosate ($p < 0.05$ and $p < 0.001$, respectively), and 50 and 100 µg/L of AMPA ($p < 0.01$ and $p < 0.05$, respectively) (Figure 5). In addition, the somatic rod in larvae exposed to 100 µg/L of glyphosate was significantly shorter than in larvae exposed to 1 and 50 µg/L of glyphosate ($p < 0.01$ and $p < 0.05$, respectively), and to 100 µg/L of Roundup ($p < 0.05$). A significant decrease in somatic rod length was also found in larvae exposed to 50 µg/L of AMPA compared to those exposed at 10 µg/L of the same chemical ($p < 0.05$).

With respect to the control, the post-oral rod length was significantly affected by the presence of 10 and 100 µg/L of glyphosate ($p < 0.05$), 50 µg/L of AMPA ($p < 0.01$), and 50 and 100 µg/L of Roundup ($p < 0.05$) (Figure 6). Moreover, significant decreases in post-oral rod length were observed after exposure to both glyphosate 10 µg/L and AMPA 50 µg/L compared to AMPA 10 µg/L treatment ($p < 0.05$ and $p < 0.01$, respectively), and after exposure to Roundup 50 and 100 µg/L compared to Roundup 1 µg/L treatment ($p < 0.05$).



Pairwise comparison	*p* value
ASW - GLY10	0.016
ASW - GLY100	< 0.001
ASW - AMPA50	0.004
ASW - AMPA100	0.012
GLY1 - GLY100	0.008
GLY50 - GLY100	0.012
GLY100 - RU100	0.031
AMPA10 - AMPA50	0.015
AMPA10 - AMPA100	0.039

Figure 5. Somatic rod length in sea urchin larvae reared in control conditions (ASW) and in four concentrations (1, 10, 50, 100 µg/L) of three contaminants: glyphosate (GLY), AMPA and Roundup (RU). Values are means ± s.e., n = 6–9. Statistically significant pairwise comparisons are reported in the table on the right.

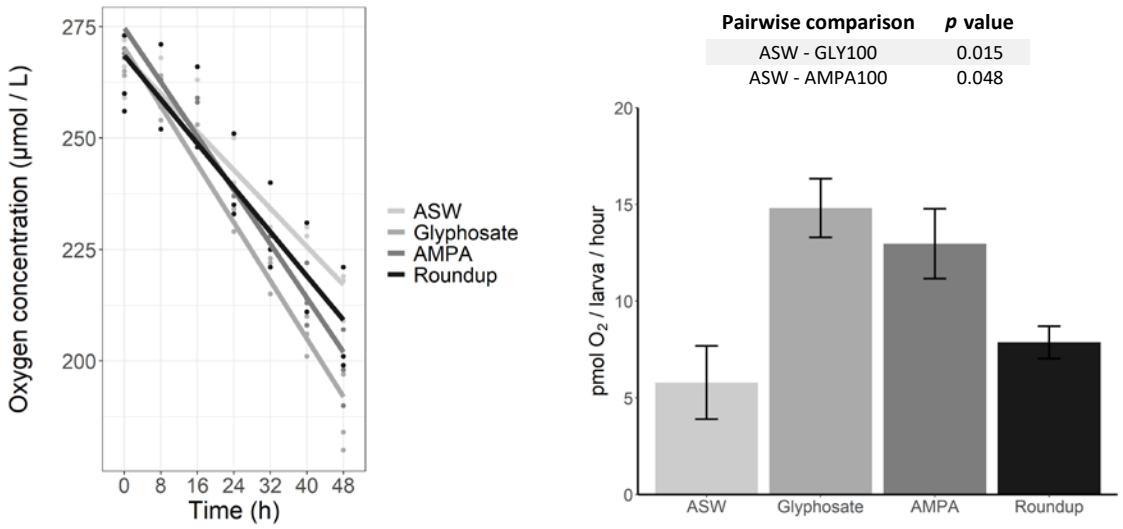


Pairwise comparison	*p* value
ASW - GLY10	0.040
ASW - GLY100	0.040
ASW - AMPA50	0.007
ASW - RU50	0.041
ASW - RU100	0.023
GLY10 - AMPA10	0.033
AMPA10 - AMPA50	0.006
RU1 - RU50	0.045
RU1 - RU100	0.025

Figure 6. Post-oral rod length in sea urchin larvae reared in control conditions (ASW) and in four concentrations (1, 10, 50, 100 µg/L) of three contaminants: glyphosate (GLY), AMPA and Roundup (RU). Values are means ± s.e., n = 6–9. Statistically significant pairwise comparisons are reported in the table on the right.

3.2. Respiration Rate

The oxygen consumption in *P. lividus* larvae exposed to four experimental conditions (control and 100 µg/L of glyphosate, AMPA and Roundup), measured for 48 h from fertilization, is shown in Figure 7. Exposure to the tested contaminants caused a statistically significant alteration in the oxygen consumption rate ($F_{(3,8)} = 7.27$, $p = 0.011$). The respiration rate of the larvae was significantly higher in glyphosate and AMPA treatments than in the control. No significant variations in oxygen consumption were found in larvae exposed to Roundup with respect to the control.



(**A**) (**B**)

Figure 7. (**A**) Oxygen concentration throughout the larval exposure (48 h) in the four experimental conditions tested: control (ASW) and 100 µg/L of the three contaminants, glyphosate (GLY), AMPA and Roundup (RU). Regression lines are shown. (**B**) Oxygen consumption (pmol O_2/larva/hour) in larvae exposed to the four experimental conditions for 48 h. Values are means ± s.e., n = 3. Statistically significant pairwise comparisons are reported in the table above the plot.

4. Discussion

Although some previous studies have assessed the effects of glyphosate and Roundup on the very early life stages of sea urchins [40,45], this is the first that compares three glyphosate-related substances in 48 hpf exposures of *P. lividus* embryos. In addition, a standard "one male/female pair" toxicological test was not chosen [39,46] in order to enhance the ecotoxicological relevance of the study. In particular, the use of pools of three females and three males provides an opportunity to investigate biological responses at the population level [47,48], thus removing the possible bias of inter-individual incompatibility [49]. As suggested in a recent review [5], the multiple parent approach should be preferred when the goal of the research is to make future predictions using an external spawner as a model species.

The concentrations tested in this study were selected based on the information available on the levels of glyphosate and AMPA in coastal waters [14,15,50]. Although the concentration of most emerging contaminants is relatively low [51,52], the potential impacts of these substances on exposed biota represent a global concern, owing to their continuous release into marine and estuarine areas worldwide [53].

Since the 1980s, echinoderm early life stages have been used to test the toxicity of pollutants [54,55]. In particular, several sea urchin species have been used as useful bioindicators to evaluate the harmful effects of pollutants and other environmental stressors [36,46,56–58].

In the present study, alterations in larval development were revealed by focusing on various endpoints: larval development, larval growth, morphological anomalies and metabolic variations measured as oxygen consumption. Based on the frequencies of the various developmental stages, a delay in larval development was mainly evident at 48 hpf, with a dose-dependent trend and statistically significant differences with respect to the control in most of the cases, even at the lowest concentrations tested for glyphosate and Roundup. Similarly, the measurement of the skeletal rods proved to be a sensitive tool for assessing the various exposure conditions. Skeletal rod growth was significantly reduced by all contaminants at the highest concentration tested. However, the somatic rod length was most affected in glyphosate and AMPA treatments, which also caused a significant

decrease at lower concentrations (10 and 50 µg/L, respectively). Conversely, the post-oral rod length showed a more pronounced effect in Roundup treatment, with a significant reduction starting from 50 µg/L. Since the post-oral rod originates later from the somatic rod [59], some differences in the mode of action of Roundup can be hypothesized. Interestingly, similar results were obtained in *P. lividus* larvae exposed to the surfactant linear alkylbenzene sulfonate (LAS) [57]. This suggests a key role for the surfactant component in the Roundup formulation.

Significant alterations in larval morphology can have direct effects on larval performance. In several sea urchin species (*P. lividus*, *Heliocidaris tuberculata*, *Centrostephanus rodgersii*, *Dendraster excentricus*), it has been demonstrated that survival of larvae depends on their ability to swim and feed, which is determined by the length of the arms and therefore of the skeletal spicules that support them [60–62]. Reductions in the post-oral rod can result in less efficient food assimilation [63] or even lead to a reduction of the pluteus stomach and swimming ability, thus exposing larvae to higher predation risks [64].

His and colleagues [40] used experimental conditions similar to those used in our work, but no significant increase in larval abnormalities was found up to 200 µg/L of glyphosate. Unlike those results, in our study 100 µg/L of the same contaminant caused a significant increase in the percentage of abnormalities, mostly at the skeletal level, with incomplete, deformed or apically crossed skeletal rods. The higher frequency of abnormal larvae could be related to a reduction in acetylcholinesterase (AChE) activity [65] since it has a central role in primary mesenchyme cell movement beginning with gastrulation, and thus, in the spiculae formation [66]. In fact, glyphosate and AMPA significantly inhibited AChE activity in the gills of *Mytilus galloprovincialis* exposed in vivo to the contaminants for 7 and 21 days [67]. Similar results were obtained in other bivalve species, crustaceans and fish for glyphosate [68–70]. Surprisingly, in our study, an increased presence of larval abnormalities was not found in the Roundup 100 µg/L exposure, even if the concentration of the active substance was the same.

Other substances in the Roundup formulation probably acted antagonistically to the active substance.

In fact, the oxygen consumption results suggested that Roundup was less toxic than the glyphosate alone. Both glyphosate- and AMPA-exposed larvae, but not those exposed to Roundup, showed a significantly increased oxygen consumption rate, which highlights increased energy expenditure to support larval development. Alterations in respiration rates have been widely reported in various life stages and taxa after exposure to anthropogenic stressors, such as pollutants and seawater acidification [71–75]. To our knowledge, no data regarding respiratory alterations due to pollutants in the larval stages of sea urchins are available and a limited number of studies have been carried out in larvae of marine invertebrates [76]. Nonetheless, in a recent study analyzing the effect of ocean acidification on larvae of the sea urchin *Heliocidaris crassispina*, a decrease in pH caused a gradual increase in oxygen consumption, doubling the value at pH 7.3 compared to the control at pH 8.0 [77]. Similar increases in respiratory rates at reduced pH have also been observed in other studies on larvae of different sea urchin species, *Strongylocentrotus droebachiensis* and *Strongylocentrotus purpuratus* [78,79]. In sea urchin larvae exposed to reduced pH, increased metabolic rates are likely responsible for the slower development and the decreased body length. Similarly, in larvae exposed to glyphosate and AMPA increased respiration rate can be explained by increased energy demand necessary to maintain homeostasis in stressful conditions, to the detriment of growth. Although oxygen consumption rate was not affected in Roundup treated larvae, a significant reduction in larval size compared to the control was observed, as found in both glyphosate and AMPA treatments.

The secrecy behind the ingredients that compose Roundup makes difficult to understand if these compounds act antagonistically or synergistically. A recent study showed that Roundup causes renal damage in rats, while the glyphosate active substance alone does not [80]. In some studies, the synergistic activity of the other ingredients in the Roundup formulation and the main component, glyphosate, has been revealed. Additive substances, such as POEA, were shown to boost glyphosate

toxicity or to cause per se lethal [81] or sublethal effects [82,83], namely, disruption of the very first cell divisions in sea urchin embryos and a delay in the cell cycle.

Very little is known about the effects of AMPA on invertebrates, and even less on marine invertebrates. It has been recently demonstrated that both glyphosate and AMPA can disrupt the bacterial community in the digestive apparatus of the mussel *M. galloprovincialis* [84]. These contaminants caused changes in the expression of genes related to the immune defense system, which enhances the propagation and growth of pathogens. Moreover, exposure to AMPA affected the hemocyte parameters of *M. galloprovincialis* [67,85], and the response was even greater when animals were exposed to a mixture of glyphosate and AMPA. These findings match our results and suggest that AMPA has a similar toxicity to glyphosate.

To summarise, glyphosate-related chemicals are harmful to sea urchin populations as smaller larvae are produced, which have a higher frequency of abnormalities, and reach the pluteus stages in lesser amounts and with higher energy requirements.

The present study fills a gap in the knowledge about the effects of glyphosate-based and derived chemicals as single stressors on sea urchin early life stages, even though the still unknown composition of Roundup impedes a definite interpretation of its adverse effects. The results highlight that the selected pollutants, in environmentally relevant concentrations, induce significant effects on larval development and metabolic consumption in the sea urchin *P. lividus*, raising further concerns about the presence of glyphosate-related chemicals in marine ecosystems. Future studies could focus on the effects of mixtures of these emerging contaminants or combinations of contaminants and other ecologically relevant human-driven stressors.

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References

1. Tijani, J.O.; Fatoba, O.O.; Babajide, O.O.; Petrik, L.F. Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: A review. *Environ. Chem. Lett.* **2016**, *14*, 27–49. [CrossRef]
2. Di Poi, C.; Evariste, L.; Serpentini, A.; Halm-Lemeille, M.P.; Lebel, J.M.; Costil, K. Toxicity of five antidepressant drugs on embryo–larval development and metamorphosis success in the Pacific oyster, *Crassostrea gigas*. *Environ. Sci. Pollut. Res.* **2014**, *21*, 13302–13314. [CrossRef]
3. Matozzo, V.; Fabrello, J.; Marin, M.G. The effects of glyphosate and its commercial formulations to marine invertebrates: A review. *J. Mar. Sci. Eng.* **2020**, *8*, 399. [CrossRef]
4. Murray, K.E.; Thomas, S.M.; Bodour, A.A. Prioritizing research for trace pollutants and emerging contaminants in the freshwater environment. *Environ. Pollut.* **2010**, *158*, 3462–3471. [CrossRef]
5. Byrne, M. Global change ecotoxicology: Identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Mar. Environ. Res.* **2012**, *76*, 3–15. [CrossRef]
6. Mohammed, A. Why are Early Life Stages of Aquatic Organisms More Sensitive to Toxicants than Adults? In *New Insights into Toxicity and Drug Testing*; InTech Open: London, UK, 2013.
7. Gosselin, L.A.; Qian, P.Y. Juvenile mortality in benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* **1997**, *146*, 265–282. [CrossRef]
8. Shilo, T.; Zygier, L.; Rubin, B.; Wolf, S.; Eizenberg, H. Mechanism of glyphosate control of *Phelipanche aegyptiaca*. *Planta* **2016**, *244*, 1095–1107. [CrossRef]
9. Schönbrunn, E.; Eschenburg, S.; Shuttleworth, W.A.; Schloss, J.V.; Amrhein, N.; Evans, J.N.S.; Kabsch, W. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1376–1380. [CrossRef]

10. Myers, J.P.; Antoniou, M.N.; Blumberg, B.; Carroll, L.; Colborn, T.; Everett, L.G.; Hansen, M.; Landrigan, P.J.; Lanphear, B.P.; Mesnage, R.; et al. Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environ. Health* **2016**, *15*, 19. [CrossRef]
11. Benbrook, C.M. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* **2016**, *28*, 1–15. [CrossRef]
12. Maggi, F.; la Cecilia, D.; Tang, F.H.M.; McBratney, A. The global environmental hazard of glyphosate use. *Sci. Total Environ.* **2020**, *717*, 137167. [CrossRef]
13. Kemp, M.W.; Twilley, R.R.; Stevenson, J.C.; Boynton, W.R.; Means, J.C. The decline of submerged vascular plants in Upper Chesapeake Bay: Summary of results concerning possible causes. *Mar. Technol. Soc. J.* **1983**, *17*, 78–89.
14. Wang, S.; Liu, B.; Yuan, D.; Ma, J. A simple method for the determination of glyphosate and aminomethylphosphonic acid in seawater matrix with high performance liquid chromatography and fluorescence detection. *Talanta* **2016**, *161*, 700–706. [CrossRef]
15. Amri, S.; Samar, M.F.; Sellem, F.; Ouali, K. Seasonal antioxidant responses in the sea urchin *Paracentrotus lividus* (Lamarck 1816) used as a bioindicator of the environmental contamination in the South-East Mediterranean. *Mar. Pollut. Bull.* **2017**, *122*, 392–402. [CrossRef]
16. Mercurio, P.; Flores, F.; Mueller, J.F.; Carter, S.; Negri, A.P. Glyphosate persistence in seawater. *Mar. Pollut. Bull.* **2014**, *85*, 385–390. [CrossRef]
17. Williams, G.M.; Kroes, R.; Munro, I.C. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharmacol.* **2000**, *31*, 117–165. [CrossRef]
18. Moore, L.J.; Fuentes, L.; Rodgers, J.H.; Bowerman, W.W.; Yarrow, G.K.; Chao, W.Y.; Bridges, W.C. Relative toxicity of the components of the original formulation of Roundup® to five North American anurans. *Ecotoxicol. Environ. Saf.* **2012**, *78*, 128–133. [CrossRef]
19. Folmar, L.C.; Sanders, H.O.; Julin, A.M. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* **1979**, *8*, 269–278. [CrossRef]
20. Borggaard, O.K.; Gimsing, A.L. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. *Pest Manag. Sci.* **2008**, *64*, 441–456. [CrossRef]
21. Giesy, J.P.; Dobson, S.; Solomon, K.R. Ecotoxicological Risk Assessment for Roundup® Herbicide. In *Reviews of Enviromental Contamination and Toxicology*; Springer-Verlag: New York, NY, USA, 2000; Volume 167, pp. 35–120.
22. Annett, R.; Habibi, H.R.; Hontela, A. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *J. Appl. Toxicol.* **2014**, *34*, 458–479. [CrossRef]
23. Modesto, K.A.; Martinez, C.B.R. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere* **2010**. [CrossRef]
24. Uren Webster, T.M.; Santos, E.M. Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup. *BMC Genom.* **2015**, *16*, 32. [CrossRef]
25. Uren Webster, T.M.; Laing, L.V.; Florance, H.; Santos, E.M. Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (*Danio rerio*). *Environ. Sci. Technol.* **2014**, *48*, 1271–1279. [CrossRef]
26. Iummato, M.M.; Di Fiori, E.; Sabatini, S.E.; Cacciatore, L.C.; Cochón, A.C.; Ríos de Molina, M.d.C.; Juárez, Á.B. Evaluation of biochemical markers in the golden mussel *Limnoperna fortunei* exposed to glyphosate acid in outdoor microcosms. *Ecotoxicol. Environ. Saf.* **2013**, *95*, 123–129. [CrossRef]
27. Epelboin, Y.; Quéré, C.; Pernet, F.; Pichereau, V.; Corporeau, C. Energy and antioxidant responses of pacific oyster exposed to trace levels of pesticides. *Chem. Res. Toxicol.* **2015**, *28*, 1831–1841. [CrossRef]
28. Cavalcante, D.G.S.M.; Martinez, C.B.R.; Sofia, S.H. Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. *Mutat. Res. Toxicol. Environ. Mutagen.* **2008**, *655*, 41–46. [CrossRef]
29. Guilherme, S.; Gaivao, I.; Santos, M.A.; Pacheco, M. European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®—A glyphosate-based herbicide. *Mutagenesis* **2010**, *25*, 523–530. [CrossRef]
30. Guilherme, S.; Gaivão, I.; Santos, M.A.; Pacheco, M. Tissue specific DNA damage in the European eel (*Anguilla anguilla*) following a short-term exposure to a glyphosate-based herbicide. *Toxicol. Lett.* **2009**, *189*, S212. [CrossRef]

31. Foo, S.A.; Byrne, M. Acclimatization and Adaptive Capacity of Marine Species in a Changing Ocean. In *Advances in Marine Biology*; Elsevier: Amsterdam, The Netherlands, 2016; Volume 74, pp. 69–116.
32. Tato, T.; Salgueiro-González, N.; León, V.M.; González, S.; Beiras, R. Ecotoxicological evaluation of the risk posed by bisphenol A, triclosan, and 4-nonylphenol in coastal waters using early life stages of marine organisms (*Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Acartia clausi*). *Environ. Pollut.* **2018**, *232*, 173–182. [CrossRef]
33. Chiarore, A.; Musco, L.; Bertocci, I.; Gallo, A.; Cannavacciuolo, A.; Mutalipassi, M.; Caramiello, D.; Giomi, F.; Fusi, M.; Danovaro, R.; et al. Sea urchin chronicles. The effect of oxygen super-saturation and marine polluted sediments from Bagnoli-Coroglio Bay on different life stages of the sea urchin *Paracentrotus lividus*. *Mar. Environ. Res.* **2020**, *159*, 104967. [CrossRef]
34. Manzo, S.; Buono, S.; Cremisini, C. Toxic effects of irgarol and diuron on sea urchin *Paracentrotus lividus* early development, fertilization, and offspring quality. *Arch. Environ. Contam. Toxicol.* **2006**, *51*, 61–68. [CrossRef]
35. Morroni, L.; Pinsino, A.; Pellegrini, D.; Regoli, F.; Matranga, V. Development of a new integrative toxicity index based on an improvement of the sea urchin embryo toxicity test. *Ecotoxicol. Environ. Saf.* **2016**, *123*, 2–7. [CrossRef]
36. Morroni, L.; Giuliani, S.; Pellegrini, D.; Sartori, D. In situ embryo toxicity test with sea urchin: Development of exposure chamber for test execution. *Chemosphere* **2018**, *196*, 354–360. [CrossRef]
37. Bellas, J.; Granmo, Å.; Beiras, R. Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus edulis*). *Mar. Pollut. Bull.* **2005**, *50*, 1382–1385. [CrossRef]
38. Bellas, J.; Nieto, Ó.; Beiras, R. Integrative assessment of coastal pollution: Development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data. *Cont. Shelf Res.* **2011**, *31*, 448–456. [CrossRef]
39. Moschino, V.; Marin, M.G. Spermiotoxicity and embryotoxicity of triphenyltin in the sea urchin *Paracentrotus lividus* Lmk. *Appl. Organomet. Chem.* **2002**, *16*, 175–181. [CrossRef]
40. His, E.; Heyvang, I.; Geffard, O.; de Montaudouin, X. A comparison between oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) larval bioassays for toxicological studies. *Water Res.* **1999**, *33*, 1706–1718. [CrossRef]
41. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46. [CrossRef]
42. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. Vegan: Community Ecology Package, Version 2.5-2. CRAN R. 2019. Available online: https://CRAN.R-project.org/package=vegan (accessed on 1 September 2019).
43. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **2017**, *82*. [CrossRef]
44. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019. Available online: https://www.R-project.org (accessed on 22 June 2020).
45. Favret, K.P.; Lynn, J.W. Flow-cytometric analyses of viability biomarkers in pesticide-exposed sperm of three aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* **2010**, *58*, 973–984. [CrossRef]
46. Marin, M.G.; Moschino, V.; Cima, F.; Celli, C. Embryotoxicity of butyltin compounds to the sea urchin *Paracentrotus lividus*. *Mar. Environ. Res.* **2000**, *50*, 231–235. [CrossRef]
47. Byrne, M.; Soars, N.A.; Ho, M.A.; Wong, E.; McElroy, D.; Selvakumaraswamy, P.; Dworjanyn, S.A.; Davis, A.R. Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. *Mar. Biol.* **2010**, *157*, 2061–2069. [CrossRef]
48. Byrne, M.; Ho, M.; Selvakumaraswamy, P.; Nguyen, H.D.; Dworjanyn, S.A.; Davis, A.R. Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proc. R. Soc. B Biol. Sci.* **2009**, *276*, 1883–1888. [CrossRef]
49. Evans, J.P.; Marshall, D.J. Male-by-female interactions influence fertilization success and mediate the benefits of polyandry in the sea urchin *Heliocidaris erythrogramma*. *Evolution* **2005**, *59*, 106–112. [CrossRef]
50. Skeff, W.; Neumann, C.; Schulz-Bull, D.E. Glyphosate and AMPA in the estuaries of the Baltic Sea method optimization and field study. *Mar. Pollut. Bull.* **2015**, *100*, 577–585. [CrossRef]
51. Sauvé, S.; Desrosiers, M. A review of what is an emerging contaminant. *Chem. Cent. J.* **2014**, *8*, 15. [CrossRef]
52. Wilkinson, J.; Hooda, P.S.; Barker, J.; Barton, S.; Swinden, J. Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. *Environ. Pollut.* **2017**, *231*, 954–970. [CrossRef]

53. Bowen, R.E.; Depledge, M.H. Rapid Assessment of Marine Pollution (RAMP). *Mar. Pollut. Bull.* **2006**, *53*, 631–639. [CrossRef]
54. Den Besten, P.J.; Herwig, H.J.; Zandee, D.I.; Voogt, P.A. Effects of cadmium and PCBs on reproduction of the sea star *Asterias rubens*: Aberrations in the early development. *Ecotoxicol. Environ. Saf.* **1989**, *18*, 173–180. [CrossRef]
55. Pagano, G.; Cipollaro, M.; Corsale, G.; Esposito, A.; Ragucci, E.; Giordano, G.G.; Trieff, N.M. Comparative toxicities of chlorinated biphenyls on sea urchin egg fertilisation and embryogenesis. *Mar. Environ. Res.* **1985**, *17*, 240–244. [CrossRef]
56. Bonaventura, R.; Matranga, V. Overview of the molecular defense systems used by sea urchin embryos to cope with UV radiation. *Mar. Environ. Res.* **2017**, *128*, 25–35. [CrossRef]
57. Bressan, M.; Marin, M.G.; Brunetti, R. Effects of linear alkylbenzene sulphonate (LAS) on skeletal development of sea urchin embryos (*Paracentrotus lividus* Lmk). *Water Res.* **1991**, *25*, 613–616. [CrossRef]
58. Byrne, M.; Lamare, M.; Winter, D.; Dworjanyn, S.A.; Uthicke, S. The stunting effect of a high CO_2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles. *Philos. Trans. R. Soc. B Biol. Sci.* **2013**, *368*, 20120439. [CrossRef]
59. Okazaki, K. Spicule formation by isolated micromeres of the sea urchin embryo. *Am. Zool.* **1975**, *15*, 567–581. [CrossRef]
60. Strathmann, R.R.; Fenaux, L.; Strathmann, M.F. Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. *Evolution* **1992**, *46*, 972. [CrossRef]
61. Allen, J.D. Size-specific predation on marine invertebrate larvae. *Biol. Bull.* **2008**, *214*, 42–49. [CrossRef]
62. Soars, N.A.; Prowse, T.A.A.; Byrne, M. Overview of phenotypic plasticity in echinoid larvae, 'Echinopluteus transversus' type vs. typical echinoplutei. *Mar. Ecol. Prog. Ser.* **2009**, *383*, 113–125. [CrossRef]
63. Hart, M.W. Particle captures and the method of suspension feeding by echinoderm larvae. *Biol. Bull.* **1991**, *180*, 12–27. [CrossRef]
64. Chan, K.Y.K.; Grunbaum, D.; O'Donnell, M.J. Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. *J. Exp. Biol.* **2011**, *214*, 3857–3867. [CrossRef]
65. Maisano, M.; Cappello, T.; Catanese, E.; Vitale, V.; Natalotto, A.; Giannetto, A.; Barreca, D.; Brunelli, E.; Mauceri, A.; Fasulo, S. Developmental abnormalities and neurotoxicological effects of CuO NPs on the black sea urchin *Arbacia lixula* by embryotoxicity assay. *Mar. Environ. Res.* **2015**, *111*, 121–127. [CrossRef]
66. Ohta, K.; Takahashi, C.; Tosuji, H. Inhibition of spicule elongation in sea urchin embryos by the acetylcholinesterase inhibitor eserine. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2009**, *153*, 310–316. [CrossRef] [PubMed]
67. Matozzo, V.; Munari, M.; Masiero, L.; Finos, L.; Marin, M.G. Ecotoxicological hazard of a mixture of glyphosate and aminomethylphosphonic acid to the mussel *Mytilus galloprovincialis* (Lamarck 1819). *Sci. Rep.* **2019**, *9*, 14302. [CrossRef] [PubMed]
68. Sandrini, J.Z.; Rola, R.C.; Lopes, F.M.; Buffon, H.F.; Freitas, M.M.; Martins, C.d.M.G.; da Rosa, C.E. Effects of glyphosate on cholinesterase activity of the mussel *Perna perna* and the fish *Danio rerio* and *Jenynsia multidentata*: In vitro studies. *Aquat. Toxicol.* **2013**, *130–131*, 171–173. [CrossRef] [PubMed]
69. Banaee, M.; Akhlaghi, M.; Soltanian, S.; Gholamhosseini, A.; Heidarieh, H.; Fereidouni, M.S. Acute exposure to chlorpyrifos and glyphosate induces changes in hemolymph biochemical parameters in the crayfish, *Astacus leptodactylus* (Eschscholtz, 1823). *Comp. Biochem. Physiol. Part C Toxicol. Pharm.* **2019**, *222*, 145–155. [CrossRef]
70. Pala, F. A survey on weed management in dry lentil fields. *Appl. Ecol. Environ. Res.* **2019**, *17*. [CrossRef]
71. Waldbusser, G.G.; Hales, B.; Langdon, C.J.; Haley, B.A.; Schrader, P.; Brunner, E.L.; Gray, M.W.; Miller, C.A.; Gimenez, I.; Hutchinson, G. Ocean acidification has multiple modes of action on bivalve larvae. *PLoS ONE* **2015**, *10*, e0128376. [CrossRef]
72. Zhang, L.; Zhang, L.; Shi, D.; Wei, J.; Chang, Y.; Zhao, C. Effects of long-term elevated temperature on covering, sheltering and righting behaviors of the sea urchin *Strongylocentrotus intermedius*. *PeerJ* **2017**, *5*, e3122. [CrossRef]
73. Batista de Melo, C.; Côa, F.; Alves, O.L.; Martinez, D.S.T.; Barbieri, E. Co-exposure of graphene oxide with trace elements: Effects on acute ecotoxicity and routine metabolism in *Palaemon pandaliformis* (shrimp). *Chemosphere* **2019**, *223*, 157–164. [CrossRef]

74. Falfushynska, H.; Sokolov, E.P.; Haider, F.; Oppermann, C.; Kragl, U.; Ruth, W.; Stock, M.; Glufke, S.; Winkel, E.J.; Sokolova, I.M. Effects of a common pharmaceutical, atorvastatin, on energy metabolism and detoxification mechanisms of a marine bivalve *Mytilus edulis*. *Aquat. Toxicol.* **2019**, *208*, 47–61. [CrossRef]
75. Rist, S.E.; Assidqi, K.; Zamani, N.P.; Appel, D.; Perschke, M.; Huhn, M.; Lenz, M. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*. *Mar. Pollut. Bull.* **2016**, *111*, 213–220. [CrossRef]
76. Goulletquer, P.; Wolowicz, M.; Latala, A.; Brown, C.; Cragg, S. Application of a micro-respirometric volumetric method to respiratory measurements of larvae of the Pacific oyster *Crassostrea gigas*. *Aquat. Living Resour.* **2004**, *17*, 195–200. [CrossRef]
77. Dorey, N.; Maboloc, E.; Chan, K.Y.K. Development of the sea urchin *Heliocidaris crassispina* from Hong Kong is robust to ocean acidification and copper contamination. *Aquat. Toxicol.* **2018**, *205*, 1–10. [CrossRef] [PubMed]
78. Stumpp, M.; Wren, J.; Melzner, F.; Thorndyke, M.C.; Dupont, S. CO2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2011**, *160*, 331–340. [CrossRef] [PubMed]
79. Dorey, N.; Lançon, P.; Thorndyke, M.; Dupont, S. Assessing physiological tipping point of sea urchin larvae exposed to a broad range of pH. *Glob. Chang. Biol.* **2013**, *19*, 3355–3367. [CrossRef]
80. Dedeke, G.A.; Owagboriaye, F.O.; Ademolu, K.O.; Olujimi, O.O.; Aladesida, A.A. Comparative assessment on mechanism underlying renal toxicity of commercial formulation of Roundup herbicide and glyphosate alone in male albino rat. *Int. J. Toxicol.* **2018**, *37*, 285–295. [CrossRef]
81. Marc, J.; Le Breton, M.; Cormier, P.; Morales, J.; Bellé, R.; Mulner-Lorillon, O. A glyphosate-based pesticide impinges on transcription. *Toxicol. Appl. Pharmacol.* **2005**. [CrossRef]
82. Marc, J.; Mulner-Lorillon, O.; Boulben, S.; Hureau, D.; Durand, G.; Bellé, R. Pesticide Roundup provokes cell division dysfunction at the level of CDK1/Cyclin B activation. *Chem. Res. Toxicol.* **2002**, *15*, 326–331. [CrossRef]
83. Marc, J.; Mulner-Lorillon, O.; Bellé, R. Glyphosate-based pesticides affect cell cycle regulation. *Biol. Cell* **2004**, *96*, 245–249. [CrossRef]
84. Iori, S.; Rovere, G.D.; Ezzat, L.; Smits, M.; Ferraresso, S.S.; Babbucci, M.; Marin, M.G.; Masiero, L.; Fabrello, J.; Garro, E.; et al. The effects of glyphosate and AMPA on the mediterranean mussel *Mytilus galloprovincialis* and its microbiota. *Environ. Res.* **2020**, *182*, 108984. [CrossRef]
85. Matozzo, V.; Marin, M.G.; Masiero, L.; Tremonti, M.; Biamonte, S.; Viale, S.; Finos, L.; Lovato, G.; Pastore, P.; Bogialli, S. Effects of aminomethylphosphonic acid, the main breakdown product of glyphosate, on cellular and biochemical parameters of the mussel *Mytilus galloprovincialis*. *Fish Shellfish Immunol.* **2018**, *83*, 321–329. [CrossRef]