processes

MDPI

Article

Biochemical Effects of Two Pesticides in Three Different Temperature Scenarios on the Diatom *Thalassiosira weissflogii*

Andreia F. Mesquita [1], Fernando J. M. Gonçalves [1], Carolina P. Rocha [2], João C. Marques [2] and Ana M. M. Gonçalves [1,2,*]

[1] Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal; filipamesquita@ua.pt (A.F.M.); fjmg@ua.pt (F.J.M.G.)
[2] University of Coimbra, MARE—Marine and Environmental Sciences Centre, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; carolina.vpr@gmail.com (C.P.R.); jcmimar@ci.uc.pt (J.C.M.)
* Correspondence: amgoncalves@uc.pt; Tel.: +351-239-240-700 (ext. 262-286)

Abstract: The exponential increase of the human population demands the overuse of fertilizers and pesticides in agriculture practices to suppress food production needs. The excessive use of these chemicals (fertilizers and pesticides) can comport deleterious effects to the ecosystems, including aquatic systems and communities. Oxyfluorfen is a fluorine-based herbicide, and its application has increased, since it is seen as an alternative to control glyphosate-resistant weeds. Copper sulfate is an inorganic pesticide based on copper which is being used in several chemical formulations, and it is the second main constituent of fungicides. Besides the known effects of such products in organisms, climatic changes pose an additional issue, being a main concern among scientists and politicians worldwide, since these alterations may worsen ecosystems' and organisms' sensitivity to stress conditions, such as the exposure to pollutants. *Thalassiosira weissflogii* (Grunow) G. A. Fryxell & Hasle, 1977 plays an important role in aquatic food webs as a primary producer and an essential food source to zooplankton. Thus, alterations on the diatom's abundance and nutritional value may lead to consequences along the trophic chain. However, few studies have evaluated the biochemical impacts of oxyfluorfen and copper sulfate exposure on diatoms. This study intends to (1) evaluate the effects on the growth rate of both contaminants on *T. weissfloggi* at three temperatures, considering the actual scenario of climatic changes, and (2) assess biochemical changes on the diatom when exposed to the chemicals at different temperatures. To achieve these aims, the marine diatom was exposed to the two chemicals individually at different temperatures. The results showed an increase in the growth rate with increasing temperatures. Oxyfluorfen exhibited higher toxicity than copper sulfate. At the biochemical level, the microalgae were greatly affected when exposed to oxyfluorfen at 20 °C and 25 °C and when exposed to copper sulfate at 15 °C. Moreover, a general increase was observed for the polysaccharide content along the copper sulfate and oxyfluorfen concentrations. Therefore, the contaminants show the ability to interfere with the diatom growth and the nutritive value, with their effects dependent on the temperature.

Keywords: marine diatoms; pesticides; ecotoxicological effects; biochemical effects; climatic changes

Citation: Mesquita, A.F.; Gonçalves, F.J.M.; Rocha, C.P.; Marques, J.C.; Gonçalves, A.M.M. Biochemical Effects of Two Pesticides in Three Different Temperature Scenarios on the Diatom *Thalassiosira weissflogii*. *Processes* **2021**, *9*, 1247. https://doi.org/10.3390/pr9071247

Academic Editors: Cristiano V. M. Araújo, M. Dolores Basallote and Araceli Rodríguez Romero

Received: 15 May 2021
Accepted: 15 July 2021
Published: 19 July 2021

1. Introduction

The human population is constantly increasing, with a rise of about 30% in the next few decades predicted, increasing from 7 billion to 9.6 billion in 2050 [1], making it necessary to have great food production to feed the growing population. This is only possible with intensive agriculture practices and pesticide usage. However, intensive agriculture and the overuse of pesticides are known to cause dangerous effects on the biosphere, hydrosphere and atmosphere [2–6]. Therefore, these pesticides become significant pollutants not only to agricultural systems but also to the surrounding systems [7], including marine coastal ecosystems, causing negative effects on its structure and function [8]. Extensive research

has been conducted to assess the effects of these chemicals on non-target species, and effects have been reported ranging from sub-lethal to lethal levels, such as changes in enzymatic activity [9–16], changes in fatty acid profiles (and consequently in the nutritive value) [11,17–23], inhibition of the photosynthetic pigments and activity [21,24–26], and changes in behavior [22,27,28], growth inhibition [25,29], and mortality [16–18,27].

Pesticides comprise several chemical formulations and are classified according to their targets (e.g., herbicides, fungicides, and insecticides), the chemical compositions of the active ingredients (e.g., pyretroids, carbamates, organophosphorus, organochlorine, and pyretrine), and according to the risk (slightly, moderately, highly, and extremely dangerous). Another prominent form of classification includes organic (as the herbicide oxyfluorfen) or inorganic compounds (as copper sulfate, a copper-based pesticide). Oxyfluorfen is an organic persistent herbicide, being a diphenyl ether and one of the most used herbicides in the world [30]. The use of this herbicide has been increasing since it was seen as an alternative to control glyphosate-resistant weeds [31], but oxyfluorfen has been reported to be carcinogenic and possibly mutagenic, neurotoxic, and lethal by some researchers [9,11,12,32]. On the other side, copper sulfate is an inorganic pesticide, the second main constituent of fungicides [33] with the potential to accumulate in aquatic organisms [18,34] and reported effects on the metabolism of lipids and proteins, in addition to their possibly being lethal [13,18,20,35].

Alongside pesticides, nowadays, climate change is also one of the main concerns among scientists and politicians. Global warming is effectively occurring, as proven by the increase of air and ocean water mean temperatures, snow and ice melting in polar regions and high heights, and the increase in sea level [36]. The previsions about warming rates are diverse, from super slow (over 0.05 °C per decade) to super fast (over 0.45 °C per decade) [37]. Independent of the previsions, this phenomenon comports changes to the ecosystems and species' life cycles [36]. In marine systems, phytoplankton is a key element to many biogeochemical cycles and trophic food webs [38]. Several researchers reported the potential effects on this group as consequence of climatic changes, such as changes in biomass, communities' structures, and diversity [39–42]. Moreover, phytoplankton is responsible for about 50% of the global primary production [43], making coastal systems, where phytoplankton is abundant, the most productive systems [44]. Diatoms are the most abundant and ecologically successful phytoplankton group [45], playing a key role in the carbon cycle, organic matter production, nutrient production, lipids, and carbohydrates via CO_2 fixation [46,47]. Some researchers have reported changes at a large scale in fatty acid production, including the essential fatty acid generation by phytoplankton, as a consequence of eutrophication [48] and climate changes [49], with significant effects on fish communities [50]. *Thalassiosira weissfloggi* is a diatom, a marine, unicellular microalgae with a relevant ecological role since it is a primary producer and consumed as a food source by several zooplankton species. The diatom is used in waste treatment, ecotoxicological tests, and biodiesel production [51].

Fatty acids and carbohydrates are considered good biomarkers to evaluate stress conditions, such as organic and inorganic pollutants, the ashes of wildfires, or climate changes [18–20,52,53]. Fatty acids are the main lipid constituent and have a key role on biological processes, being essential constituents of the cell membrane and taking part in physiological functions. These biomolecules are used as fuel in metabolic processes at all trophic levels [54]. On the other hand, carbohydrates are the main and fastest energy source to answer to stress conditions. These molecules have a key role in biological functions such as the maintenance of cell homeostasis in the immunity defense process [55]. Polysaccharides are carbohydrate polymers composed of long-chain monosaccharides, and these biomolecules are reported as able to protect bacterial cells against environmental or chemical stress conditions [56–59].

Considering these two great current problems (pesticide overuse and climate change) and the key role of the diatoms, this work's purpose is to (1) evaluate the effects of oxyfluorfen and copper sulfate on the diatom *T. weissflogii* at a lethal level through cell

count, growth, and inhibition rate determination, (2) assess the effects of these chemicals at the biochemical level on the diatom through the analysis of fatty acids and carbohydrates profiles, and (3) determine the changes in the nutritive value of this primary producer.

2. Materials and Methods

2.1. Culture Maintenance

T. weissflogii was maintained with Guillard's f/2 medium (adapted from [59]) without EDTA and a salinity of 30 psu. The cultures were renewed with new medium every week and maintained at controlled laboratory conditions (temperature of 20 ± 2 °C, photoperiod 16hL:8hD).

2.2. Inhibition Growth Bioassays

T. weissflogii was exposed to three different temperatures (15 °C, 20 °C, and 25 °C). Two weeks before the bioassay at each temperature, an inoculum was acclimated to the respective temperature, and three days before the test, an inoculum was prepared from the acclimated culture and incubated with a photoperiod of 16hL:8hD. At the beginning of the bioassay, the inoculum cell density was determined using a Neubauer hemocytometer and then was rectified to an initial test cell density of 1×10^4 cell/mL. To evaluate the effects of the compounds on the growth rate, the diatoms were exposed to 9 treatments of oxyfluorfen (PESTANAL® analytical standard by Sigma Aldrich, Lisbon, Portugal; CAS: 42874-03-3) (0.00–0.018 mg/L) plus a solvent control and to 11 treatments of copper (II) sulfate pentahydrate (using EMSURE® ACS, ISO, Reag. Ph Eur, by Merck; CAS: 7758-99-8) (0.00–1.00 mg/L), with each treatment composed of three replicates over 96 h in the same photoperiod cited above. At the end of the bioassay, the cell density of each replicate was determined through microscopic counting in a Neubauer hemocytometer to determine the effective concentration of each chemical at the different temperatures.

2.3. Biochemical Bioassays

Considering the effective concentrations, mainly the EC_{10} and EC_{20} values, from the inhibition growth bioassays, biochemical bioassays were performed for 7 days using sub-lethal concentrations to evaluate the biochemical changes, namely in the fatty acids and carbohydrate profiles, in the organisms when exposed to oxyfluorfen and copper sulfate and the cited temperatures. The diatoms were exposed to 6 treatments of oxyfluorfen (at 15 °C: 0.000–0.0015 mg/L; at 20 °C: 0.000–0.0015 mg/L and at 25 °C: 0.000–0.006 mg/L) plus a solvent control and to 6 treatments of copper sulfate (at 15 °C: 0.00–0.125 mg/L; at 20 °C: 0.00–0.625 mg/L and at 25 °C: 0.00–0.750 mg/L). At the end of the bioassays, each inoculum was filtered through a GF/F Whatman filter using a vacuum pump, and the filters were stored at −80 °C for about 2 weeks for posterior analysis.

2.4. Biochemical Analysis

2.4.1. Fatty Acids Analysis

The fatty acid methyl ester (FAME) extraction was performed following the protocol [60]. The samples were incubated with methanol for the extraction of the lipids. Methyl nonadecanoate (C19:0, Fluka 74208) was used as the internal standard, being added to the samples during the extraction process. Then, sodium chloride was added, and the samples were centrifuged for the separation of FAMEs. At the end of the extraction process, the samples were centrifuged, and the supernatant was stored at −80 °C for posterior quantification. Additionally, the inferior phase was also stored for the sugar analysis.

The FAMEs were separated, identified, and quantified by gas chromatography coupled with mass spectrometry (GC-MS) using a Thermo Scientific Trace 1310 Network (Waltham, MA) equipped with a TR-FFAP column (0.32 mm internal diameter, 0.25 µm film thickness, and 30 m long). The carrier gas was helium with a flow rate of 1.4 mL min^{-1}. The sample (1.00 µL) was injected into the injector in splitless mode at a temperature of 250 °C, lined with a split glass liner of 4.0 mm i.d. The oven temperature increased linearly from

80 °C to 160 °C (25 °C min^{-1}), followed by another temperature increase until to 210 °C (2 °C min^{-1}) and, at the end, an increase of 40 °C min^{-1} to the final temperature of 230 °C, which was maintained for 10 min. A Thermo Scientific ISQ 7000 Network mass selective detector single quadrupole was used for scanning the m/z range of 50–500 in full scan mode for acquisition. The detector started operating 3.5 min after injection, corresponding to the solvent delay. The injector ion source and transfer line were maintained at 240 °C and 230 °C, respectively. Peak's identification was performed through the mass spectrum and the retention time of each FAME compared with the Supelco®37 component FAME mix (Sigma-Aldrich, Steinheim, Germany). FAME quantification was achieved following the method described in [60].

2.4.2. Sugar Analysis

The samples were subjected to a hydrolysis, acetylation, and reduction process, converting the neutral sugars (NS) in their alditol acetates following the method described in [61]. Before adding distilled water, the samples were incubated with sulfuric acid. Acetylation was performed with methylimidazole, acetic acid, and acetic acid anhydride after a reduction with sodium borohydride and ammonia as described by Coimbra et al. [61]. Moreover, 2-desoxiglucose was used as the internal standard being added to each sample. The composition of the monosaccharides was determined through gas chromatography equipped with a flame ionization detector (GC-FID), namely a Thermo Scientific Trace 1310. A TG-WAXMS A GC column with a length of 30 m, i.d. of 0.32 mm, and film thickness of 0.25 µm was used. The oven had an initial temperature of 180 °C, increasing linearly until reaching 230 °C (5 °C min^{-1}) and being maintained for 12 min. Helium was used as the carrier gas at a flow rate of 2.5 mL min^{-1}. Monosaccharide identification and quantification were conducted in comparison with the standards.

2.5. Data Analysis

The 3-parameter logistic model was applied to determine the effect concentration (EC) values, namely EC_{10}, EC_{20}, and EC_{50} with corresponding 95% confidence intervals for each compound at the different temperatures. ANOVA was also performed, followed by Dunnet's test to verify significant differences between each concentration and the control treatment using SigmaPlot 12.5 software. The fatty acid (FA) profiles and monosaccharide compositions were defined by quantification of the total FA concentrations (µg/10^4 cell) and sugar concentrations (µg/10^4 cell).

The relationship between treatments based on fatty acid profiles and on monosaccharides compositions was determined through multivariate analysis, carried out using PRIMER 6 software [62,63]. The original data were converted into similarity matrices by Bray–Curtis resemblance measures, and a cluster model based on the group average distance was applied to obtain a non-metric multidimensional scaling (n-MDS) plot, as well as a dendrogram obtained by hierarchical clustering. Moreover, differences between the tested conditions (treatments, temperatures, and compounds) were determined by one-way analysis of similarity (ANOSIM). Furthermore, through similarity percentage (SIMPER) analysis, it was possible to identify the main fatty acids and monosaccharides that contributed to the similarities and dissimilarities within and between the sample groups.

3. Results

3.1. Acute Bioassays

The results from the acute bioassays are exhibited in Figure 1. The graphs show a general decrease in the cell density and growth rate for both chemical concentrations for all exposure temperatures. Moreover, *T. weissflogii* showed inhibition on the growth rate at the lowest temperatures. Comparing the exposure to both pollutants, oxyfluorfen showed itself to be more dangerous than copper sulfate to the diatoms (Table 1).

Table 1. EC values and confidence intervals (95%) for *T. weissflogii* when exposed to oxyfluorfen and to copper sulfate (μg/L) for 96 h, considering the growth rate data.

	Oxyfluorfen (μg/L)			Copper Sulfate (μg/L)		
	15 °C	**20 °C**	**25 °C**	**15 °C**	**20 °C**	**25 °C**
EC10	0.57 (0.03–1.20)	0.90 (0.52–1.28)	4.41 (3.48–5.34)	77 (36–119)	479 (388–569)	689 (534–844)
EC20	0.96 (0.28–1.64)	1.44(0.99–1.89)	5.67 (4.81–6.54)	104 (61–147)	547 (472–622)	936 (822–1049)
EC50	2.31 (1.44–3.17)	3.22 (2.60–3.84)	8.74 (7.98–9.49)	173 (132–215)	687 (635–738)	1580 (1017–2142)

3.2. Biochemical Bioassays

Fatty Acid Content

Biochemical analyses showed changes in the fatty acid content of the microalga after exposure to the contaminants at the different temperatures. *T. weissflogii* showed itself to be more sensitive to exposure to oxyfluorfen at 20 °C and 25 °C, with its fatty acid content decreasing (Table 2). An opposite trend was observed when the diatom species was exposed to copper sulfate at the same temperatures, with the fatty acid content increasing. Considering the organisms' exposure to the organic herbicide (Table 2), the results showed a dominance of the saturated fatty acids on the organisms' profiles to the exposure at the three temperatures. Despite this, the saturated fatty acid (SFA) content decreased along with the concentrations in regard to the control treatment for all temperatures tested, as well as the monounsaturated fatty acid (MUFA) and highly unsaturated fatty acid (HUFA) content at 20 °C and 25 °C and the polyunsaturated fatty acid (PUFA) content at 25 °C. Moreover, an increase in the MUFA, HUFA, and of PUFA contents at 15 °C was observed (except at the highest concentrations for the last one). On the other hand, considering the organisms exposed to copper sulfate (Table 3), different trends were observed. The MUFAs assumed dominance of the organisms' profiles at 20 °C and 25 °C, whereas the HUFAs dominated the organisms' profiles when exposed at 15 °C at the CTL at 25 μg/L and 125 μg/L, while the SFAs dominated in the intermediate treatments. When analyzing the profiles, an increase in the SFA content was observed along with the concentrations in regard to the control at all temperatures, except for the organisms exposed to the highest concentrations at 20 °C. An increase of the PUFA and HUFA contents at 20 °C and 25 °C and of the MUFA content at 20 °C was also observed, with 15 °C being notorious for displaying a decrease in the amounts of all unsaturated fatty acids.

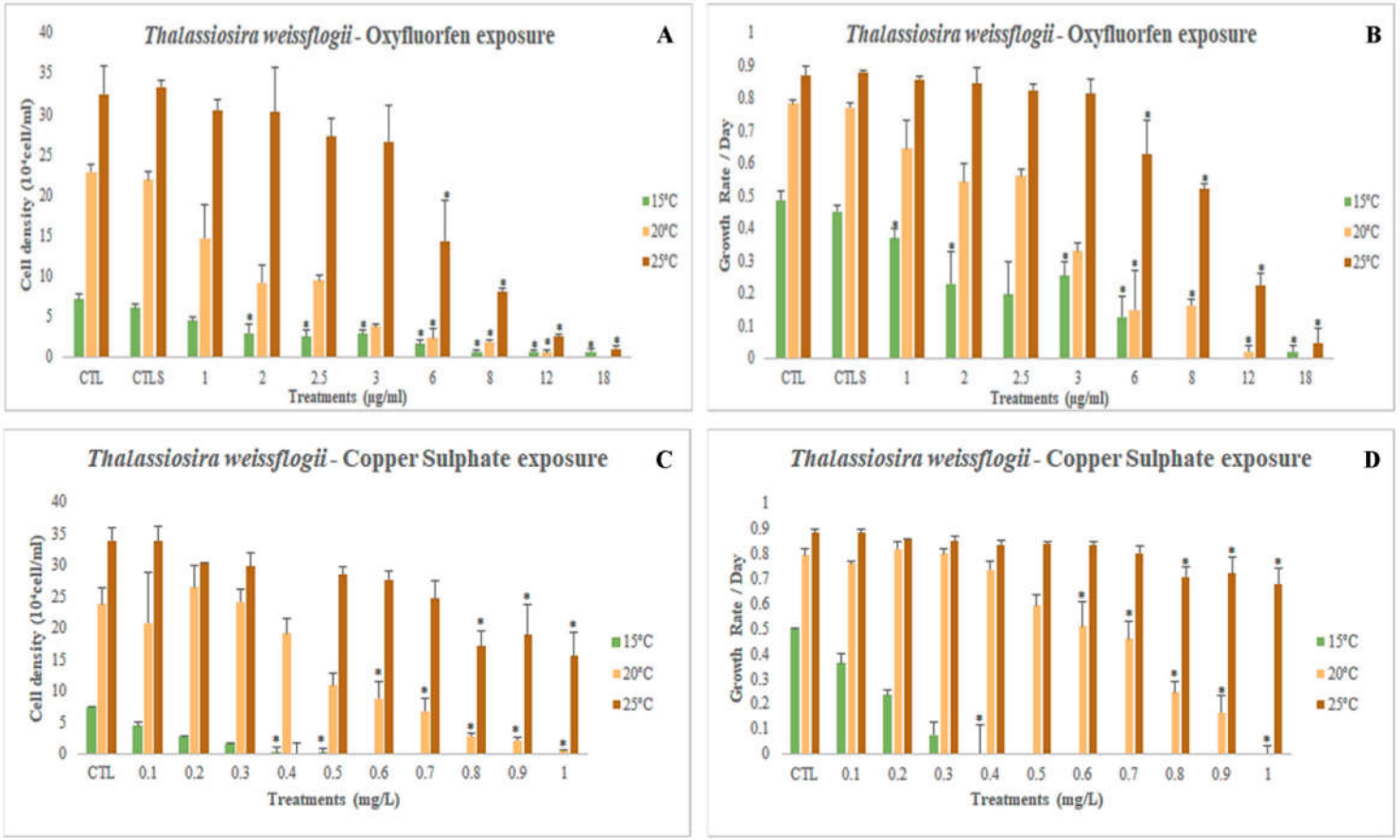

Figure 1. (**A**,**C**) The diatom cell density (10^4 cell/mL) along with the exposure to oxyfluorfen and to copper sulfate, respectively, at the three different temperatures (15 °C, 20 °C, and 25 °C). (**B**,**D**) The growth rate per day. The symbol * indicates the treatments with significant differences compared with the control situation.

Table 2. Quantification of the fatty acid profiles (SFA, MUFA, PUFA, and HUFA at μg/10^4 cell and expressed in %) of *Thalassiosira weissflogii*, determined in organisms after 7 days of exposure to 5 concentrations of oxyfluorfen (μg/L) and at 3 different temperatures (15 °C (white); 20 °C (light gray); 25 °C (dark gray)).

	15 °C						20 °C						25 °C					
FA	**CTL**	**CTLS**	**0.5**	**1.00**	**1.25**	**1.50**	**CTL**	**CTLS**	**0.5**	**1.00**	**1.25**	**1.50**	**CTL**	**CTLS**	**1.00**	**2.00**	**4.00**	**6.00**
C10:0	8.53	20.17		4.25				25.15	9.86				0.00					
C11:0			2.83	61.95			4.48	8.06	2.12	3.43			2.86	3.53	8.90	8.22	15.03	18.28
C12:0				2.00			0.56	31.05					2.60	1.60	1.18	0.50	0.71	0.67
C13:0	8.95	13.33	10.25	19.46	5.94				1.20	2.18			69.56	58.47	49.03	15.53	25.60	20.22
C14:0					2.32	2.82	22.81	26.14	1.18	0.00	0.72	0.55	1.05	1.03	0.95	0.41	0.43	0.00
C15:0				1.68			1.95	2.47	2.02	3.21	0.00	0.32	3.34	3.05	3.00	1.06	1.61	1.64
C16:0				29.40			33.20	40.89				0.27	112.57	108.29	72.23	32.19	39.78	35.86
C17:0	47.94	67.40	53.40	0.71	47.06	50.89			31.10	17.15	19.27	27.99	41.99				0.49	
C18:0	1.08	5.01					2.37	2.44	28.95	19.29	22.51	20.55	6.71	32.51				
C20:0													2.74	2.67				
C22:0													0.00	1.64				
∑SFA	**66.49**	**105.90**	**66.48**	**119.45**	**55.32**	**53.70**	**65.38**	**136.19**	**76.43**	**45.27**	**42.50**	**49.67**	**243.43**	**212.81**	**135.29**	**57.91**	**83.65**	**76.67**
C15:1			0.49												0.52	0.37		
C16:1			6.67	1.73	5.68	12.16	25.67	30.80					71.70	63.67	61.84	19.91	24.10	21.06
C17:1	0.72	0.59	44.31	17.50	19.03	23.09			1.08	2.97			14.54	3.30	5.49	2.38	2.24	2.99
C18:1n6													6.72	55.78	1.09	0.53	0.35	0.00
C20:1n9									1.75	1.72	2.12	2.19						
C22:1														10.85	5.23	2.76		
C24:1n9							7.24	12.42					13.20	12.46	9.93	3.88	3.97	
∑MUFA	**0.72**	**0.59**	**51.47**	**19.23**	**24.71**	**35.25**	**32.91**	**43.22**	**2.83**	**4.69**	**2.12**	**2.19**	**106.17**	**146.07**	**84.11**	**29.82**	**30.66**	**24.04**
C18:2n6c	2.17						1.97			59.85								
C18:2n6t	1.97	3.48	5.47		25.99	6.60			0.31	0.13								
C18:2n9													17.84	8.91	2.16	0.66	1.34	1.85
C18:3n6							3.06		2.76									
C20:2	3.62	1.71	6.17		35.14	14.68		1.55	3.32	64.03								
C22:2	27.32	32.13	52.84				3.80	9.07					6.20					
∑PUFA	**35.08**	**37.31**	**64.48**		**61.13**	**21.28**	**8.83**	**10.62**	**6.39**	**124.01**			**24.04**	**8.91**	**2.16**	**0.66**	**1.34**	**1.85**
EPA	0.87			25.66	4.28	17.82	32.68	46.37					78.72	61.91	59.26	23.64	21.95	22.55
DHA			15.88	34.39									55.59		4.09	6.74	8.72	7.99
∑HUFA	**0.87**	**0.00**	**15.88**	**60.06**	**4.28**	**17.82**	**32.68**	**46.37**					**134.31**	**61.91**	**63.35**	**30.38**	**30.68**	**30.54**

Table 2. *Cont.*

	15 °C						20 °C						25 °C					
∑ FA	**103.15**	**143.81**	**198.31**	**198.73**	**145.44**	**128.05**	**139.80**	**236.40**	**85.65**	**173.96**	**44.62**	**51.85**	**507.95**	**429.69**	**284.90**	**118.76**	**146.33**	**133.11**
%SFA	64.46	73.64	33.52	60.11	38.04	41.94	46.77	57.61	89.24	26.02	95.24	95.79	47.92	49.53	47.49	48.76	57.17	57.60
%MUFA	0.69	0.41	25.96	9.68	16.99	27.53	23.54	18.28	3.30	2.69	4.76	4.21	20.90	33.99	29.52	25.11	20.95	18.06
%PUFA	34.01	25.95	32.51		42.03	16.62	6.32	4.49	7.46	71.29			4.73	2.07	0.76	0.55	0.92	1.39
%HUFA	0.84		8.01	30.22	2.94	13.92	23.38	19.61					26.44	14.41	22.24	25.58	20.96	22.94

Table 3. Quantification of fatty acid profiles (SFA, MUFA, PUFA, and HUFA at μg/10^4 cell and expressed in %) of *Thalassiosira weissflogii*, determined in organisms after 7 days of exposure to different concentrations of copper sulfate (μg/L) at three different temperatures (15 °C (white); 20 °C (light gray); 25 °C (dark gray)).

	15 °C						20 °C						25 °C				
FA	**CTL**	**25**	**50**	**75**	**100**	**125**	**CTL**	**125**	**250**	**375**	**500**	**625**	**CTL**	**250**	**500**	**625**	**750**
C14:0							0.97	0.22	0.47	0.32	0.49	0.34	0.76	0.18	0.18	0.43	0.24
C16:0	8.28	7.20	8.19	7.88	7.49	173.63	10.58	9.62	12.46	13.80	9.57	10.28	11.64	9.28	13.01	18.32	12.64
∑SFA	**8.28**	**7.20**	**8.19**	**7.88**	**7.49**	**17.36**	**11.55**	**9.85**	**12.93**	**14.12**	**10.06**	**10.62**	**12.39**	**9.45**	**13.19**	**18.75**	**12.87**
C13:1	6.41	6.24	4.77	6.17	5.28		9.56	8.52	12.17	12.14	10.96	12.81	16.46	13.78	18.68	22.86	15.02
C14:1				0.34	0.46		0.87	0.71	0.64	0.57	0.54	0.55	1.09	0.46	0.56	0.83	0.51
C16:1n9							0.66	0.57	0.76	0.75	0.63	0.78	1.09	0.66	0.54	0.90	0.72
C17:1							0.47	0.55	0.65	1.46	0.10	0.30	0.55	0.65		0.40	0.83
C18:1n9c							0.53		5.89	1.12	0.21	0.40	0.84				0.49
C18:1n9t							0.54				0.47	0.47		0.20		1.13	0.75
∑ MUFA	**6.41**	**6.24**	**4.77**	**6.51**	**5.73**		**12.63**	**10.36**	**20.12**	**16.04**	**12.90**	**15.32**	**20.04**	**15.75**	**19.78**	**26.12**	**18.33**
C16:2	1.27			1.42	0.72		2.48	2.43	3.00	3.70	2.96	3.59	3.29	2.61	3.42	3.82	2.54
C18:2n6							0.79	0.43	1.11	0.93	0.96	1.04		0.51		0.60	1.11
∑PUFA	**1.27**			**1.42**	**0.72**		**3.27**	**2.86**	**4.11**	**4.62**	**3.91**	**4.63**	**3.29**	**3.13**	**3.42**	**4.42**	**3.65**
EPA	89.49	39.12	4.31	4.22	3.49	20.51	11.53	11.04	13.85	13.56	12.84	14.69	9.31	7.78	11.58	13.87	10.04
∑FA	**105.46**	**52.57**	**17.27**	**20.04**	**17.43**	**37.87**	**38.99**	**34.11**	**51.01**	**48.34**	**39.71**	**45.26**	**45.02**	**36.10**	**47.97**	**63.16**	**44.89**
%SFA	7.85	13.70	47.43	39.34	42.99	45.85	29.63	28.88	25.35	29.21	25.32	23.47	27.53	26.19	27.50	29.68	28.68
%MUFA	6.08	11.88	27.60	32.50	32.89		32.40	30.36	39.44	33.18	32.50	33.84	44.50	43.61	41.24	41.36	40.83
%PUFA	1.21			7.10	4.12		8.40	8.38	8.06	9.56	9.85	10.23	7.30	8.67	7.13	7.00	8.13
%EPA	84.86	74.42	24.96	21.06	20.00	54.15	29.57	32.38	27.15	28.04	32.33	32.46	20.67	21.54	24.13	21.96	22.36

3.3. Multivariate Analysis

In the first analysis, considering both chemicals and the three temperatures (Figure 2), it was possible to observe a clear separation between the organisms exposed to copper sulfate and the organisms exposed to oxyfluorfen. Moreover, the organisms exposed to copper sulfate (group B) exhibited fatty acid profiles more similar among them than the organisms exposed to oxyfluorfen (group A), as demonstrated by the samples' aggregation.

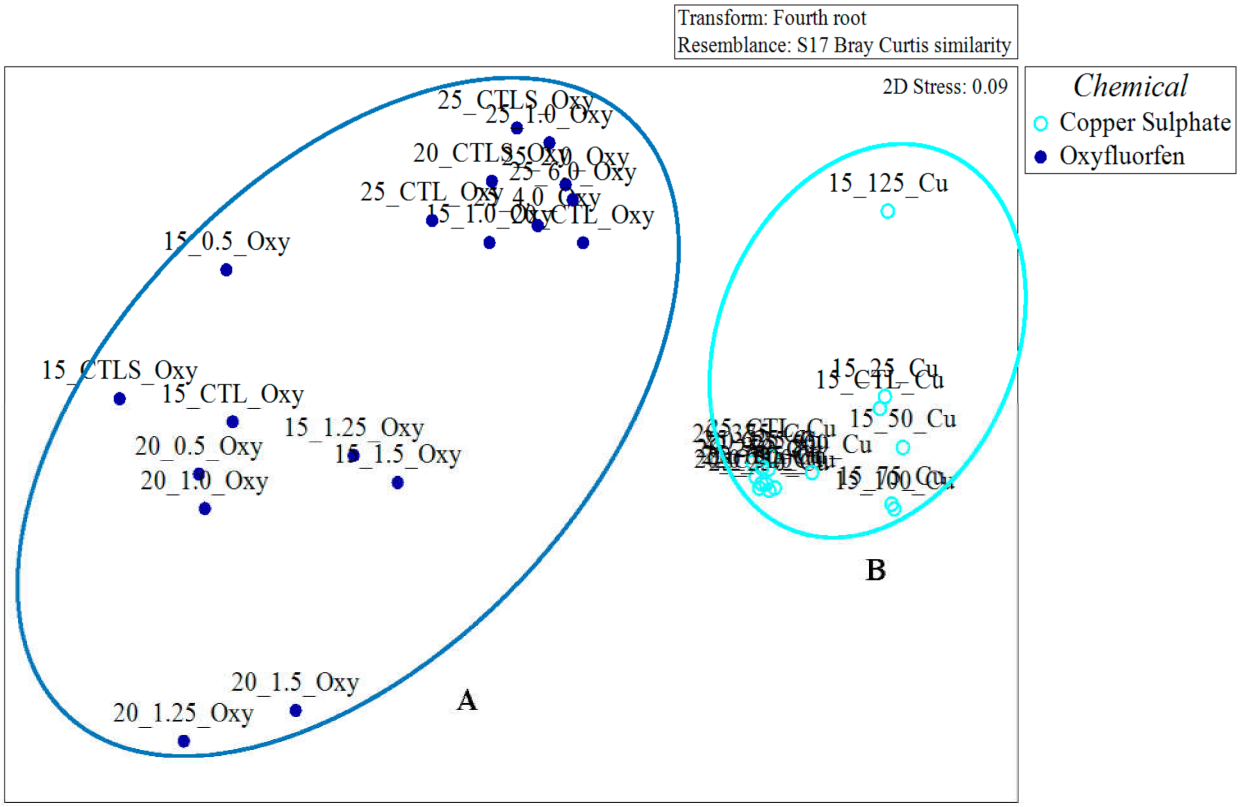

Figure 2. Two-dimensional non-metric MDS ordination plot of the FA contents of the organisms exposed to both chemicals (oxyfluorfen and copper sulfate) at the three temperatures (15 °C, 20 °C, and 25 °C). The full gray circles represent the organisms exposed to oxyfluorfen (Group A: 15 °C and 20 °C—CTL, CTLS, 0.50, 1.00, 1.25, and 1.50 µg/L; 25 °C—CTL, CTLS, 1.00, 2.00, 4.00, and 6.00 µg/L), and the empty circles represent the organisms exposed to copper sulfate (Group B: 15 °C—CTL, 25, 50, 75, 100, and 15 µg/L; 20 °C—CTL, 125, 250, 375, 500, and 625 µg/L; 25 °C—CTL, 250, 500, 625, and 750 µg/L).

3.4. Oxyfluorfen Exposure

Considering the organisms exposed to oxyfluorfen, it was observed by the cluster analysis that the organisms were grouped considering the abundance and diversity of the FAs (Figure 3), with a similarity of 70%. Eight groups were formed (Group I: composed of the organisms exposed to the control treatments at 20 °C; Group II: composed of the organisms exposed to the control treatments at 25 °C; Group III: organisms exposed to all concentrations at 25 °C and 1.0 µg/L of oxyfluorfen at 15 °C; Group IV: organisms exposed to the highest concentrations (1.25 and 1.5 µg/L) of the chemical at 20 °C; Group V: only composed of the organisms exposed to 0.5 µg/L of oxyfluorfen at 15 °C; Group VI: organisms exposed to the highest concentrations (1.25 and 1.5 µg/L) at 15 °C; Group VII: organisms exposed to the control treatments at 15 °C; and Group VIII: organisms exposed to the lowest concentrations (0.5 and 1.0 µg/L) at 20 °C). The n-MDS (Figure 4) grouped the organisms according to their FA contents (stress = 0.08). This analysis confirms the group formation on the cluster and gives us information about the spatial organization of the samples.

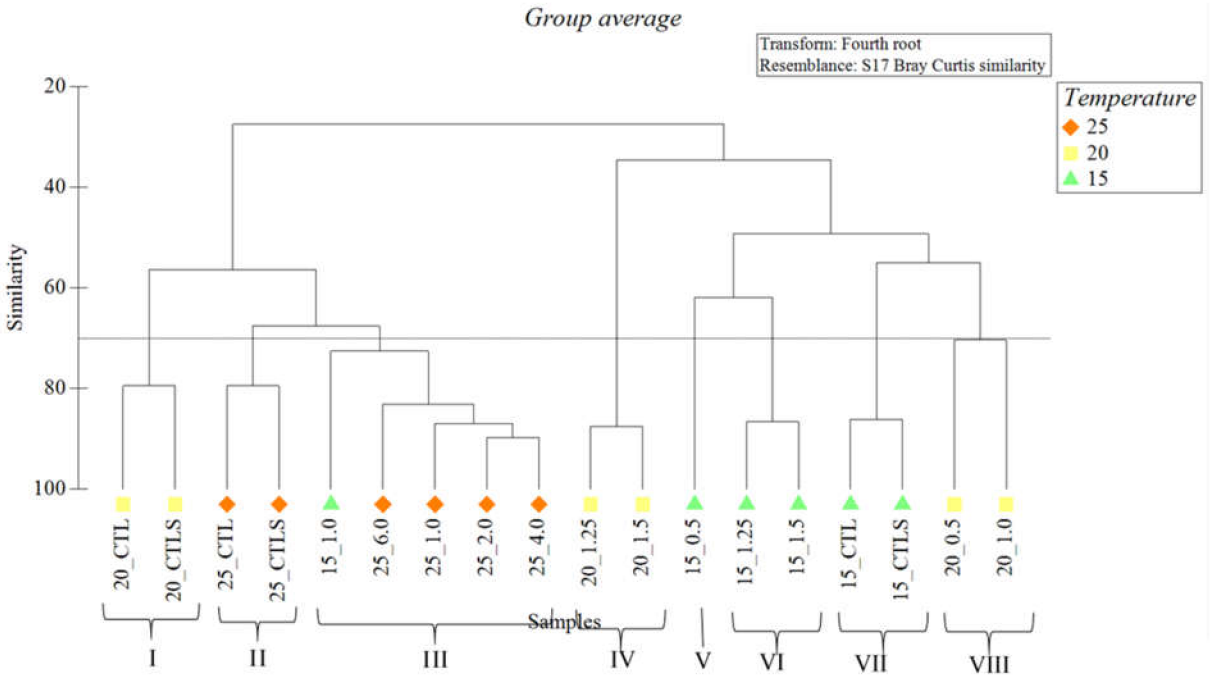

Figure 3. Cladogram (cluster analysis) grouping the diatom treatments related with the FA contents after exposure to oxyfluorfen at 15 °C, 20 °C, and 25 °C, respectively.

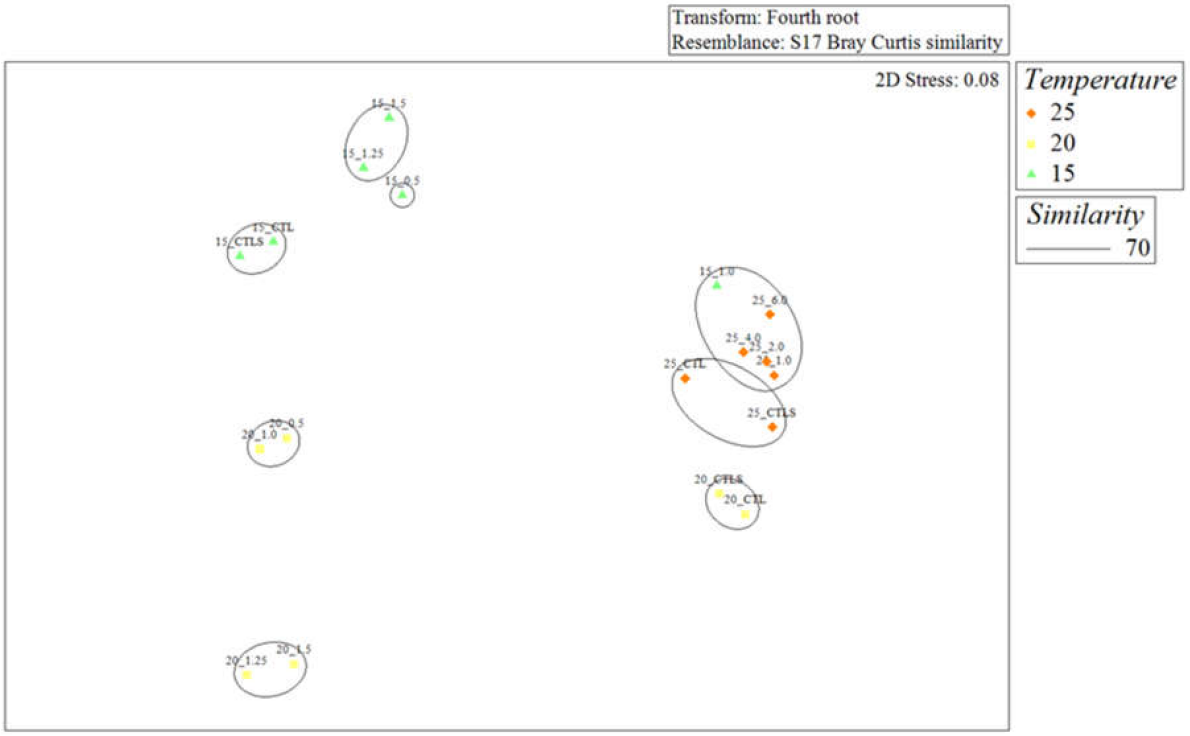

Figure 4. Two-dimensional non-metric MDS ordination plot of the FA contents of *T. weissflogii* exposed to oxyfluorfen at the three temperatures (15 °C, 20 °C, and 25 °C).

The ANOSIM suggested the presence of distinct groups (R = 0.581; $p = 0.001$). There were significant differences ($p < 0.05$) between all temperatures. However, the temperatures exhibited poor segregation (15 °C vs. 20 °C—R = 0.417, $p = 0.006$; 15 °C vs. 25 °C—R = 0.739, $p = 0.004$; 20 °C vs. 25 °C—R = 0.604, $p = 0.002$). Table 4 shows the main fatty acids that contributed to the similarity inside each temperature group (15 °C, 20 °C, and 25 °C), as well as the main FAs that contributed to the dissimilarities between the temperatures.

Table 4. SIMPER analyses showing the average similarity and dissimilarity among groups of samples, related with n-MDS analysis.

Temperatures	Similarity	Fatty Acids	Av. Abund		Av. Sim	Sim/SD	Contrib %	Cum %
15 °C	54.09	C17:0	2.40		13.29	2.22	24.57	24.57
		C17:1	1.78		8.48	2.41	15.67	40.24
		C13:0	1.52		6.85	1.35	12.66	52.90
20 °C	43.33	C18:0	1.87		12.06	1.77	27.84	27.84
		C17:0	1.47		7.51	0.74	17.33	45.16
		C14:0	1.21		4.89	1.23	11.29	56.45
25 °C	77.06	C16:0	2.79		10.36	8.55	13.45	13.45
		EPA	2.52		9.33	8.41	12.11	25.56
		C16:1n9	2.50		9.24	8.63	11.99	37.55
	Dissimilarity				**Av. Dis**	**Dis/SD**		
15 °C/20 °C	65.75	C18:0	0.42	1.87	5.09	1.76	7.75	7.75
		C17:1	1.78	0.39	4.92	1.68	7.49	15.24
		C13:0	1.52	0.38	4.25	1.58	6.47	21.71
15 °C/25 °C	63.01	C16:0	0.39	2.79	5.96	2.56	9.46	9.46
		C17:0	2.40	0.56	5.00	1.78	7.94	17.40
		C20:2	1.42	0.00	3.60	1.75	5.71	23.11
20 °C/25 °C	65.91	C13:0	0.38	2.44	5.36	2.72	8.13	8.13
		C16:0	0.94	2.79	5.04	1.55	7.64	15.77
		EPA	0.83	2.52	4.90	1.57	7.44	23.21

3.5. Copper Sulfate Exposure

According to the cluster analysis, the organisms exposed to copper sulfate were grouped considering the abundance and diversity of the FA compositions (Figure 5) and to a similarity of 75%. Three groups were formed (Group I: only composed of the organisms exposed to the highest concentration of copper sulfate—125 ug/L—at 15 °C; Group II: composed of the organisms exposed to 20 °C and 25 °C; and Group III: composed of the organisms exposed at 15 °C). The n-MDS (Figure 6) showed the organisms grouped according to the FA contents (stress = 0.02). In group A, the organisms exposed to two temperatures (20 °C and 25 °C) are presented, whereas group B shows the organisms exposed at 15 °C. As observed in the cluster graph, the organisms exposed to 125 ug/L at 15 °C appeared in an independent position (group C), revealing a distinct content of FAs.

The ANOSIM suggests the presence of distinct groups (R = 0.515; $p = 0.001$). There were significant differences ($p < 0.05$) between 15 °C vs. 20 °C and 15 °C vs. 25 °C, but not between 20 °C and 25 °C ($p = 0.104$). The groups with significant differences also exhibited good segregation (R = 0.783 and R = 0.709, respectively). the temperatures of 20 °C and 25 °C, as expected, presented low segregation (R = 0.163; $p = 0.104$). Table 5 shows the main fatty acids (EPA, C16:0 and C13:1) that contributed to the similarity inside each group of temperatures, as well as the main FAs that contributed to the dissimilarities between the temperatures.

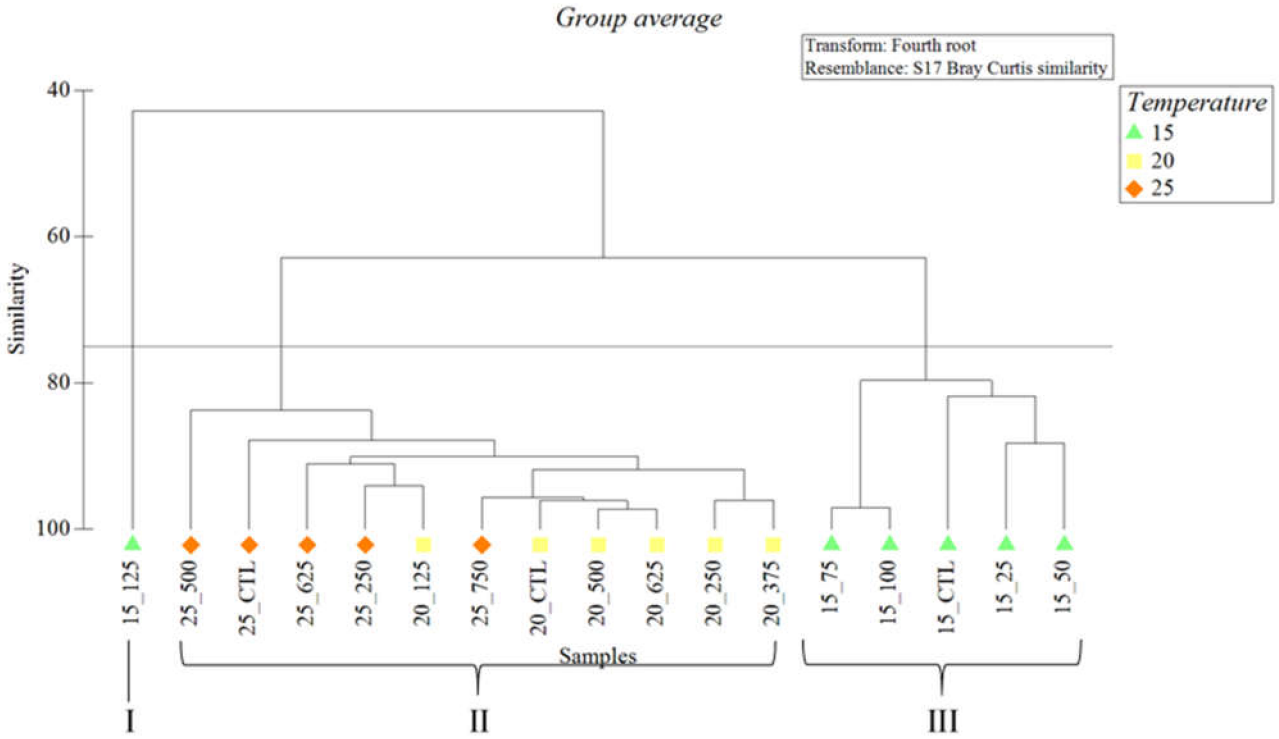

Figure 5. Cladogram (cluster analysis) grouping the diatoms related with FA content after the exposure to copper sulfate at 15 °C, 20 °C, and 25 °C.

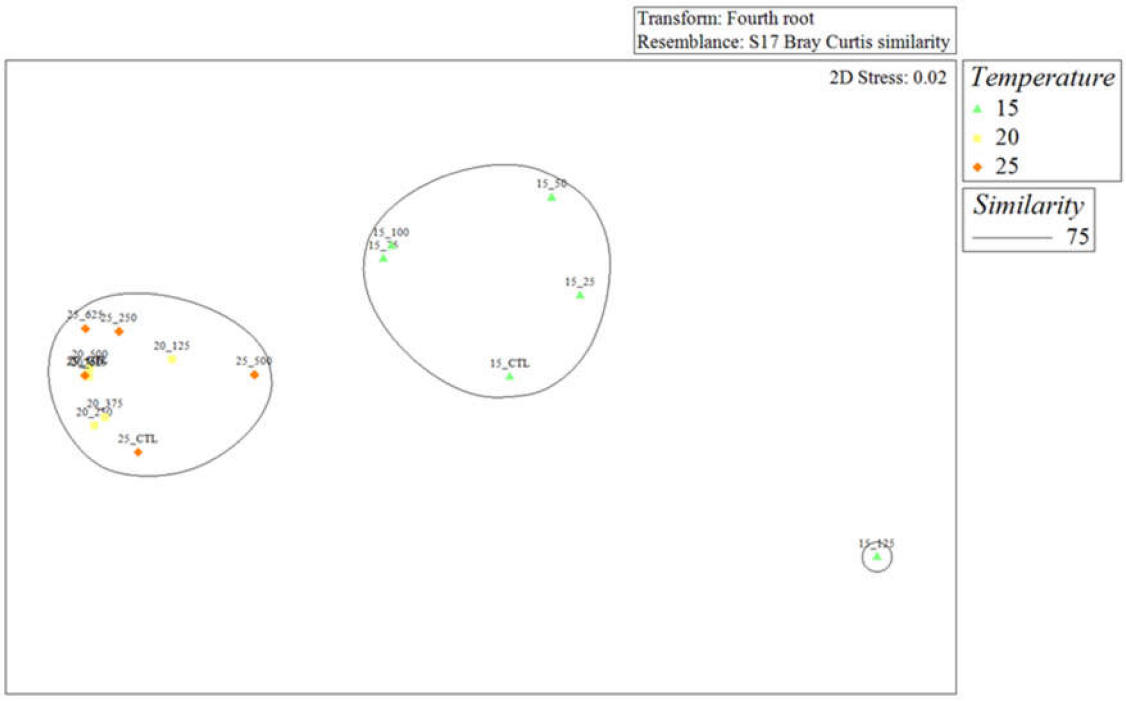

Figure 6. Two-dimensional non-metric MDS ordination plot of the FA content of the *T. weissflogii* exposed to copper sulfate at three temperatures (15 °C, 20 °C, and 25 °C).

Table 5. SIMPER analyses showing average similarity and dissimilarity among groups of samples, related with n-MDS analysis.

Temperatures	Similarity	Fatty Acids	Av. Abund		Av. Sim	Sim/SD	Contrib %	Cum %
15 °C	73.00	C16:0	2.00		26.49	9.77	36.28	36.28
		EPA	2.27		26.18	3.66	35.86	72.14
		C13:1	1.29		16.68	1.35	22.85	94.98
20 °C	92.30	EPA	1.89		14.92	29.64	16.17	16.17
		C16:0	1.82		14.27	26.12	15.46	31.63
		C13:1	1.82		14.22	35.28	15.41	47.04
25 °C	87.42	C13:1	2.04		16.84	12.50	19.26	19.26
		C16:0	1.89		15.53	12.98	17.76	37.02
		EPA	1.79		14.79	12.68	16.92	53.94
	Dissimilarity				**Av. Dis**	**Dis/SD**		
15 °C/20 °C	42.27	C18:2n6	0.00	0.96	5.11	14.82	12.09	12.09
		C16:1	0.00	0.91	4.86	16.21	11.50	23.59
		EPA	2.27	1.89	4.45	1.70	10.52	34.11
15 °C/25 °C	40.17	C16:1	0.00	0.93	5.19	11.84	12.93	12.93
		EPA	2.27	1.79	4.62	1.45	11.51	24.44
		C16:2	0.51	1.33	4.62	1.49	11.50	35.94
20 °C/25 °C	10.82	C18:1n9c	0.82	0.36	2.60	1.30	24.05	24.05
		C18:1n9t	0.42	0.53	1.96	1.17	18.11	42.16
		C18:2n6	0.96	0.55	1.90	1.01	17.55	59.71

Carbohydrate Content

Regarding the carbohydrate content, different temperatures and chemicals led to different responses. After exposure to the contaminants at the different temperatures, *T. weissflogii* showed a great decrease in carbohydrates when exposed to oxyfluorfen at 25 °C (Table 6) and when exposed to copper sulfate at 15 °C (Table 7), with an opposite trend to the remaining exposure conditions (i.e., the neutral sugars increasing) being observed. Considering the organisms' exposure to the organic herbicide (Table 6), the results showed an increase in the sugar content along with the concentrations when the organisms were exposed to temperatures of 15 °C and 25 °C, verifying an opposite trend when exposed at 20 °C. Considering the three temperatures, a decrease of the galactose and an increase of the glucose content with the temperature increase was observed. Moreover, a gradual increase of the glucose content along with the concentrations was notorious at the different temperatures. On the other hand, considering the organisms exposed to copper sulfate (Table 7), an increase in the amount of sugars, mainly of glucose, along with the concentrations was observed, except for the organisms exposed at 15 °C. Changes in the sugar diversity was also observed in the exposed temperatures, namely for the organisms exposed at 15 °C, the ones with lower varieties of neutral sugars, and glucose showed to be the dominant sugar regardless of the temperature.

Table 6. Neutral sugar quantification (μg/10^4 cell) of *Thalassiosira weissflogii*, determined in organisms after 7 days of exposure to five concentrations of oxyfluorfen (μg/L) at three different temperatures (15 °C (white); 20 °C (light gray); and 25 °C (dark gray)).

	15 °C						20 °C						25 °C					
Neutral sugars	**CTL**	**CTLS**	**0.50**	**1.00**	**1.25**	**1.50**	**CTL**	**CTLS**	**0.50**	**1.00**	**1.25**	**1.50**	**CTL**	**CTLS**	**1.00**	**2.00**	**4.00**	**6.00**
Rhamnose	0.784	0.567	0.795	0.565	0.824	1.479	1.606	0.511	12.362	0.889	0.625	0.320	0.484	0.561	0.182	0.133	0.167	0.310
Fucose	0.151	0.238	0.352	0.283	0.262	0.362	0.739	0.698	1.549	0.800	0.399	0.393	0.278	0.554	0.190	0.154	0.171	0.223
Ribose	0.192	0.193	0.226	0.192	0.175	0.205	0.028	0.518	2.522	0.843	0.570	0.355	0.427	0.457	0.236	0.126	0.179	0.512
Arabinose	0.449	0.736	0.915	0.815	0.734	0.888	0.837	1.018	0.726	0.408			0.278	0.335	0.263	0.171	0.287	0.307
Xylose	0.643	1.144	1.461	1.279	1.166	1.444	0.990	2.225	1.610	1.280	0.180	0.299	0.122	0.232	0.454	0.248	0.487	0.528
Manose	1.281	1.370	0.839	0.720	1.241	1.171	0.598	10.588	2.329	3.320	4.740	2.478	3.685	4.876	0.021	0.024	0.025	7.322
Galactose	2.737	5.956	8.280	7.899	6.912	7.490	0.965	0.324	1.767	1.086	0.801	0.438	0.767	1.069	0.176	0.086	0.178	0.433
Glucose	0.537	0.849	1.162	1.193	1.303	1.302	0.097	2.081	1.036	1.469	2.538	2.399	0.595	1.621	3.843	2.110	3.266	4.237

Table 7. Neutral sugar quantification (μg/10^4 cell) of *Thalassiosira weissflogii*, determined in organisms after 7 days of exposure to different copper sulfate concentrations (μg/L) and at three different temperatures (15 °C (white); 20 °C (light gray); and 25 °C (dark gray)).

	15 °C						20 °C						25 °C				
Neutral Sugars	**CTL**	**25**	**50**	**75**	**100**	**125**	**CTL**	**125**	**205**	**375**	**500**	**625**	**CTL**	**250**	**500**	**625**	**750**
Rhamnose							0.105	0.186	0.606	0.258	0.321	0.571					
Fucose	0.072	0.127	0.194	0.162	0.269	0.102	0.034	0.051	0.155				0.063	0.146	0.133	0.199	0.148
Ribose	0.177	0.222	0.140	0.146	0.170	0.222	0.095	0.093	0.394	0.150	0.127	0.102	0.056	0.083	0.105	0.107	0.060
Xylose	0.129	0.103	0.055	0.043	0.031	0.057	0.045	0.020	0.156	0.391	0.454	0.282	0.051	0.069	0.080	0.095	0.028
Manose								2.621	5.542	2.506	3.253	2.967	0.011	0.028			
Galactose													0.080	0.092	0.127	0.062	
Glucose	3.958	4.498	3.047	2.626	3.373	3.857	0.304	1.252	2.707	1.593	1.294	1.273	4.618	5.489	6.402	5.816	2.298

3.6. Multivariate Analysis

Considering both chemicals and the three temperatures (Figure 7), we observed a separation between the organisms exposed to copper sulfate and the organisms exposed to oxyfluorfen. Moreover, a larger dispersion was observed between the organisms exposed to copper sulfate, being clustered into three different groups.

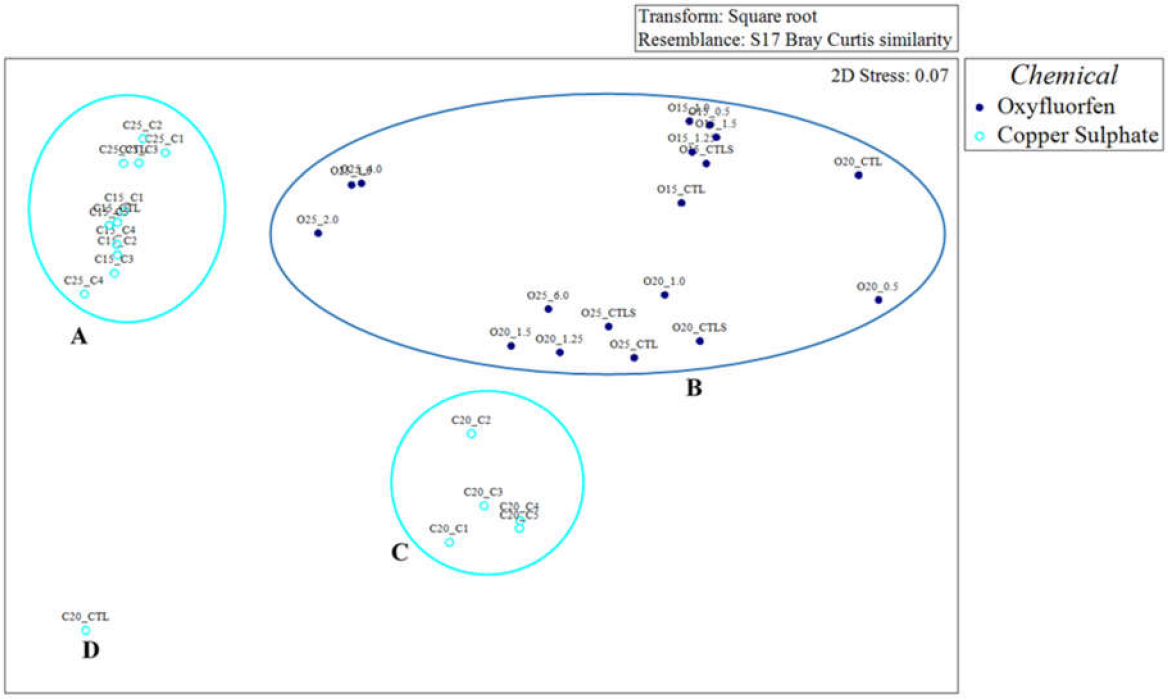

Figure 7. Two-dimensional non-metric MDS ordination plot of the polysaccharide content of *T. weissflogii* exposed to both contaminants at the three temperatures (15 °C, 20 °C, and 25 °C). Empty circles represent the organisms exposed to copper sulfate (Groups A, C, and D), and the full gray circles represent the organisms exposed to oxyfluorfen (Group B).

3.7. Oxyfluorfen Exposure

A cluster analysis grouped the organisms, considering the abundance and diversity of the neutral sugars (Figure 8) to a similarity of 70%. Four groups were formed (Group I: composed of the organisms exposed to oxyfluorfen concentrations of 1.0, 2.0, and 4.0 µg/L at 25 °C; Group II: composed of the organisms exposed to the control treatments and 6.0 µg/L of oxyfluorfen at 25 °C and exposed to CTLS and the highest oxyfluorfen concentrations (1.0, 1.25, and 1.5 µg/L) at 20 °C; Group III: organisms exposed at 15 °C and the control treatment at 20 °C; and Group IV: organisms exposed to the lowest concentrations of oxyfluorfen (0.5 µg/L) at 20 °C). The n-MDS (Figure 9) showed the organisms grouped according to the neutral sugar content (stress = 0.08). This analysis confirmed the group formation on the cluster and gave us information about the spatial organization of the samples.

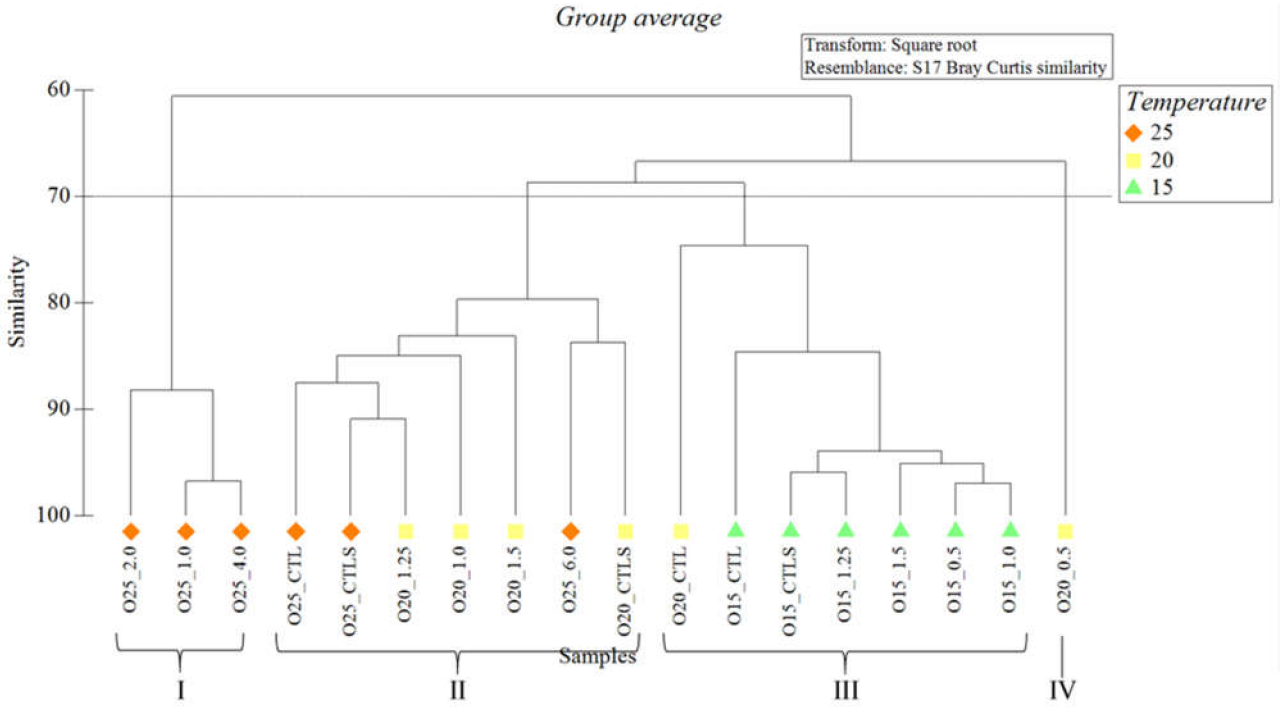

Figure 8. Cladogram (cluster analysis) grouping the diatom's treatments, related with the neutral sugar content after the exposure to oxyfluorfen at 15 °C, 20 °C, and 25 °C.

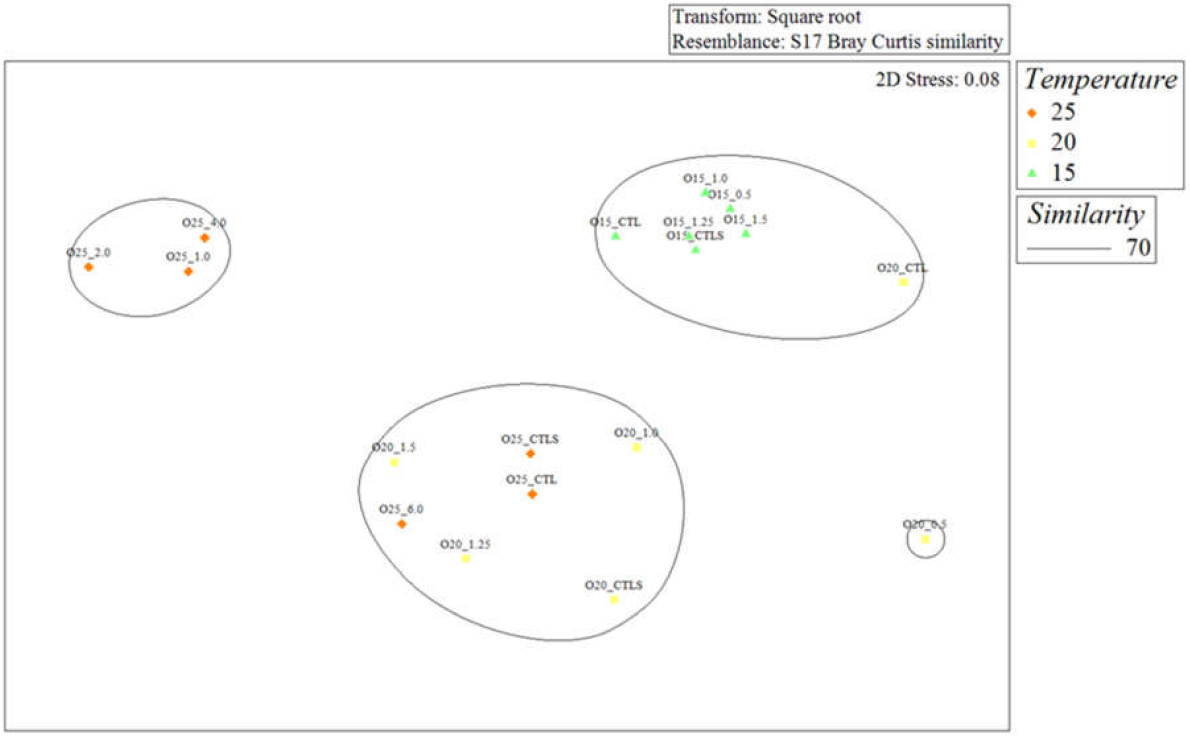

Figure 9. Two-dimensional non-metric MDS ordination plot of the neutral sugar content of the *T. weissflogii* exposed to oxyfluorfen at the three temperatures.

The ANOSIM suggested the presence of distinct groups (R = 0.49; $p = 0.001$). There were significant differences ($p < 0.05$) between 15 °C vs. 20 °C and 15 °C vs. 25 °C. However, only the condition of 15 °C vs. 25 °C exhibited good segregation (R = 0.752, $p = 0.002$), while the temperatures of 20 °C vs. 15 °C presented low segregation (R = 0.531, $p = 0.002$) and 20 °C vs. 25 °C exhibited no significant differences and, consequently, poor segregation (R = 0.233, $p = 0.063$). Table 8 shows the main neutral sugars that most contributed to the similarity inside each temperature group (galactose, glucose, and mannose), as well as the main ones that contributed to the dissimilarities between the temperatures (galactose, mannose, glucose, and rhamnose).

Table 8. SIMPER analyses showing the average similarity and dissimilarity among temperatures, related with n-MDS analysis.

Temperatures	Similarity	Sugars	Av. Abund		Av. Sim	Sim/SD	Contrib %	Cum %
15 °C	91.37	Gal	2.52		26.94	6.49	29.48	29.48
		Xyl	1.08		11.85	10.65	12.97	42.45
		Man	1.04		11.60	6.33	12.69	55.14
20 °C	71.66	Man	1.85		16.30	2.99	22.74	22.74
		Glc	1.19		10.91	1.80	15.22	37.96
		Gal	0.91		9.06	3.60	12.64	50.60
25 °C	74.59	Glc	1.55		21.53	2.55	28.86	28.86
		Ara	0.52		8.19	7.50	10.98	39.84
		Xyl	0.57		8.18	3.16	10.97	50.81
	Dissimilarity				**Av. Diss**	**Diss/SD**		
15 °C/20 °C	29.93	Gal	2.52	0.91	9.71	3.12	32.42	32.42
		Man	1.04	1.85	5.28	1.47	17.63	50.05
		Rha	0.90	1.30	3.35	0.77	11.18	61.23
15 °C/25 °C	35.44	Gal	2.52	0.62	13.40	3.35	37.81	37.81
		Man	1.04	1.22	7.30	4.55	20.60	58.41
		Glc	1.02	1.55	4.40	1.68	12.41	70.82
20 °C/25 °C	32.13	Man	1.85	1.22	8.98	1.49	27.96	27.96
		Rha	1.30	0.53	5.00	0.89	15.55	43.51
		Glc	1.19	1.55	4.23	1.20	13.15	56.66

3.8. Copper Sulfate Exposure

Regarding the organisms exposed to copper sulfate, cluster analysis showed the organisms grouped into three groups, considering the abundance and diversity of the neutral sugars (Figure 10) and a similarity of 70%. Group I was composed of the organisms exposed at concentrations of copper sulfate at 20 °C. Group II was composed of the organisms exposed to the control treatment at 20 °C, and in Group III were all the organisms exposed at 15 °C and 25 °C. The n-MDS (Figure 11) shows the organisms grouped according to the neutral sugar content (stress = 0). This analysis also shows the group formation on the cluster and gives us information about the spatial organization of the samples, observed a great distance between the groups.

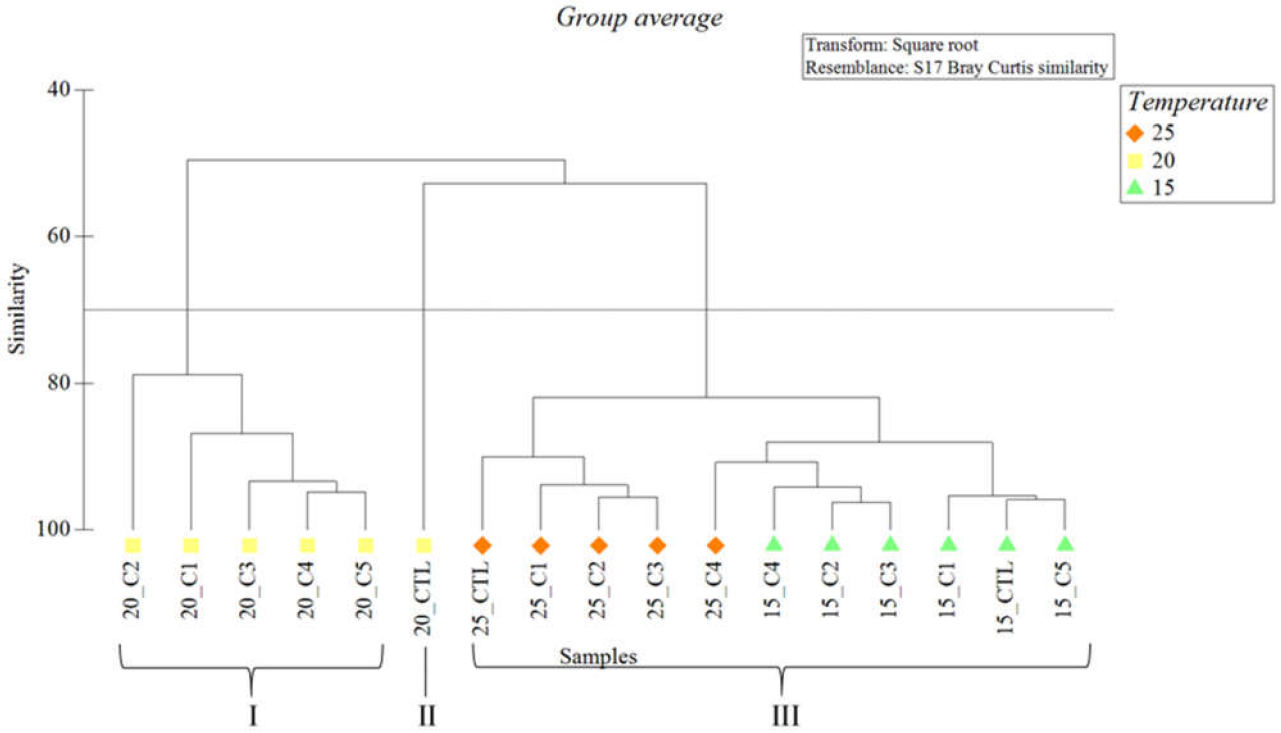

Figure 10. Cladogram (cluster analysis) grouping the diatoms, related with the neutral sugar content after exposure to copper sulfate at 15 °C, 20 °C, and 25 °C.

Figure 11. Two-dimensional non-metric MDS ordination plot of the neutral sugar content of *T. weissflogii* exposed to copper sulfate at the three temperatures.

The ANOSIM suggested the presence of distinct groups (R = 0.667; $p = 0.001$). There were significant differences ($p < 0.05$) between all temperatures. Moreover, all of them exhibited good segregation (20 °C vs. 15 °C—R = 0.748, $p = 0.002$; 20 °C vs. 25 °C—R = 0.776, $p = 0.004$; 15 °C vs. 25 °C—R = 0.608, $p = 0.011$). Table 9 shows the neutral sugars, major contributors (glucose, fucose, and ribose among others) to the similarity inside each temperature group, as well as the main contributors (mannose, glucose, and rhamnose) to the dissimilarities between the temperatures.

Table 9. SIMPER analyses showing the average similarity and dissimilarity among temperatures, related with n-MDS analysis.

Temperatures	Similarity	Sugars	Av. Abund		Av. Sim	Sim/SD	Contrib %	Cum %
15 °C	92.00	Glc	1.88		60.04	23.15	65.25	65.25
		Rib	0.42		13.47	21.09	14.64	79.90
		Fuc	0.38		11.23	5.09	12.21	92.10
20 °C	72.85	Man	1.51		23.61	1.35	32.42	32.42
		Glc	1.14		22.34	5.65	30.67	63.09
		Rha	0.56		10.81	7.40	14.85	77.93
25 °C	85.86	Glc	2.19		57.48	9.84	66.95	66.98
		Fuc	0.37		9.69	4.44	11.29	78.24
		Rib	0.28		7.66	10.83	8.92	87.16
	Dissimilarity				**Av. Diss**	**Diss/SD**		
15 °C/20 °C	48.06	Man	0.00	1.51	19.80	2.18	41.20	41.20
		Glc	1.88	1.14	11.90	1.38	24.76	65.96
		Rha	0.00	0.56	7.81	5.98	16.24	82.20
15 °C/25 °C	16.34	Glc	1.88	2.19	7.19	2.17	44.00	44.00
		Gal	0.00	0.24	3.62	1.89	22.13	66.13
		Rib	0.42	0.28	2.23	2.24	13.63	79.76
20 °C/25 °C	51.85	Man	1.51	0.05	18.42	2.23	35.53	35.53
		Glc	1.14	2.19	15.18	1.64	29.28	64.81
		Rha	0.56	0.00	7.38	5.22	14.24	79.05

4. Discussion

This study highlights the highly toxic action of organic and inorganic chemicals on the diatom *T. weissflogii*. Considering the toxicological effects, the organisms exhibited higher sensitivity to the oxyfluorfen's action than to the copper sulfate's action. The highly toxic action of oxyfluorfen on the diatom compared with copper sulfate may be justified by the fact that herbicides are designed to control plants; therefore, the sensitivity of vegetal cells such as algae to these chemicals is extremely improved [64].

The optimal growth conditions, such as the temperature, vary for each species. In the present study, the greatest growth rate and cell density was observed for the organisms exposed to the highest temperatures (20 °C and 25 °C), as was already observed by other authors for *Thalassiosira* sp. [65,66]. The temperature of 25 °C was suggested to be the optimal temperature for diatom growth in [67]. As expected, a decrease in the growth rate along with the concentration range for both contaminants was exhibited upon exposure at the three temperature conditions. Few studies evaluated the effects of oxyfluorfen. Nevertheless, several studies that assessed the herbicide's exposure to *Thalassiosira* sp. observed the same pattern of growth inhibition with the increase of the toxic concentration [68–72]. Furthermore, considering the copper exposure, some recent studies also reported a trend of growth inhibition, mostly at higher concentrations of *T. weissflogii* [68,69] and *T. pseudonana* [73]. Regardless, the growth stimulation at the highest temperature (25 °C) could suggest a benefit of the temperature increase for the diatom. At the biochemical level, such a clear trend was not observed.

Changes in the fatty acid profiles have been reported as an adaptative response to fight against stressful conditions, such as contaminants, through the activation of defense

and reparation mechanisms by the organisms' cells [74]. Regarding the different changes observed in the FA profiles dependent on the exposure temperature, few studies have focused on this interaction, but the authors of [75] reported an increase in the lipid peroxidation with the increase in the temperature of *Peridinium gatunense*. Moreover, the temperature presents a key role in biochemical and physiological processes [76–78], and structural adaptations when the algae cells were submitted to warmer water to adjust the membrane's fluidity, resulting in reduced amounts of PUFAs, were observed [79].

In the present study, a decrease in the PUFA percentage with an increase in temperature was observed in the diatoms exposed to both chemicals, also suggesting larger lipid peroxidation at higher temperatures. Moreover, when lipid peroxidation occurs, PUFAs act on free radical generation [74], with decreases in the PUFA and HUFA contents. That justifies the decrease in the PUFA and HUFA percentages in the diatom profiles with the temperature increase. When the organisms were exposed to oxyfluorfen, they exhibited a decrease in their fatty acid contents, with the lipid profile being dominated by SFAs. The dominance of the SFAs and the decrease in unsaturated fatty acids with the exposure to the herbicide may also be explained by the action mode, since oxyfluorfen acts by inhibiting protoporphyrinogen oxidase, an enzyme involved in chlorophyll synthesis and cytochrome. When the enzyme is inhibited, an accumulation of protoporphyrinogen in the cytoplasm occurs and the consequent generation of protoporphyrin X [80–82]. The reaction of this molecule with light and oxygen causes free radical production with consequent dangerous impacts on the cells, such as the production of ROS or membrane damage [83]. Considering the action mode of the herbicide and the formation of free radicals, namely singlet oxygen, oxyfluorfen has been reported to be an inductor of lipid peroxidation [84]. As mentioned above, lipid peroxidation is suggested as being responsible for the PUFA decrease observed in the exposed organisms.

Considering the biochemical response to the copper sulfate exposure, a decrease in the unsaturated FAs at 15 °C and an increase of these at 20 °C and 25 °C was observed. This difference of responses when compared with oxyfluorfen exposure may be due to the copper's action mode. Despite the lack of information about the copper mechanisms and the fatty acid profiles, it may include the effect of desaturation [85], leading to a decrease in SFAs and an increase in unsaturated fatty acids. Moreover, copper, like other metals, may be responsible for ROS generation via a Fenton reaction, inducing oxidative stress to the cells and compromising the metabolism [86].

The carbohydrate profiles also changed with the exposure to different temperatures and contaminants. It is known that macroalgae use different carbohydrates as energy sources and storage for carbon molecules [87]. Moreover, intracellular carbon flux is an important process for cell energy production and the other molecular composites needed for microalgae growth. Therefore, changes to the carbohydrate type and storage may affect several cell processes, such as carbon fixation, photosynthetic ability, carbon storage, or lipid accumulation [88]. There is a lack of studies evaluating the contaminants' effects on the carbohydrate compositions of marine algae. However, it is known that carbohydrate production by marine algae is variable, depending on factors such as the species, growth stage, or environmental conditions [89]. In this study, an increase in the glucose content with the increase in temperature was observed, the same trend observed by the authors of [90], who reported an increase in D-glucose production by *T. weissflogii* with the temperature's increase. Moreover, a dominance of glucose when the organisms were exposed to copper sulfate was observed for all the temperatures tested. When the organisms were exposed to oxyfluorfen, an increase in glucose with the increase of the temperature was also observed, whereas the galactose content decreased with the increase in temperature.

5. Conclusions

This study highlights the response of a marine diatom under scenarios of climatic events and chemical contaminations, revealing changes in the FA and carbohydrate profiles

of the primary producer species *T. weissflogii* when exposed to oxyfluorfen and copper sulfate under different temperature conditions. These variations in the FAs and carbohydrates may comprise and cause sever impacts at the nutritive value of this primary producer species and thus have consequences along the trophic food web. This work also underlines the importance of FAs and carbohydrates as relevant biomarker tools and early warning indicators to detect the presence of combined stressors, namely those induced by temperature change and contaminants in marine systems. With the increase in temperature, there is an increase in the diatom growth rate. However, this pattern is not so clear at the biochemical level. This study intends to also be an alert to call attention to the excessive growth of the primary producers and the reduction of their nutritive value. As a consequence of the temperature increase, the consumers will be negatively affected, with dangerous consequences to the communities and thus to the ecosystem and to human health, as some essential molecules like PUFAs are produced de novo by primary producers and further transferred to consumers at the trophic levels of the marine food chains.

Author Contributions: Conceptualization, A.F.M., F.J.M.G. and A.M.M.G.; methodology, A.F.M., F.J.M.G. and A.M.M.G.; practical work and data curation, A.F.M.; biochemical samples running in GC-FID and GC-MS, C.P.R.; writing—original draft preparation, A.F.M.; writing—review and editing, A.F.M., J.C.M., F.J.M.G. and A.M.M.G.; supervision, F.J.M.G. and A.M.M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Fundação para a Ciência e a Tecnologia (FCT) through the strategic projects UIDB/04292/2020 granted to the Marine and Environmental Sciences Centre (MARE) and UIDP/50017/2020+UIDB/50017/2020 granted to the Centre for Environmental and Marine Studies (CESAM). This research was also partially supported by PORBIOTA, E-Infrastructure Portuguese Information and Research in Biodiversity (POCI-01-0145-FEDER-022127), supported by the Competitiveness and Internationalization Operational Programme and Regional Operational Programme of Lisbon through FEDER, and by the Portuguese Foundation for Science and Technology (FCT) through national funds (OE). A. F. Mesquita and C. P. Rocha thank FCT for the financial support provided through doctoral grants SFRH/BD/139831/2018 and SFRH/BD/140922/2018, respectively, co-funded by the Human Potential Operational Programme (National Strategic Reference Framework 2007–2013), European Social Fund (EU), and the program POPH/FSE. A. M. M. Gonçalves also acknowledges the University of Coimbra for contract IT057-18-7253.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data reported in the present study is compared and discussed in relation to other published works on the subject, identified and cited throughout the manuscript. There is no resort to publicly available datasets.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fujiwara, T.; O'Hagan, D. Successful Fluorine-Containing Herbicide Agrochemicals. *J. Fluor. Chem.* **2014**, *167*, 16–29. [CrossRef]
2. Rio, B.; Parent-Massin, D.; Lautraite, S.; Hoellinger, H. Effects of a Diphenyl-Ether Herbicide, Oxyfluorfen, on Human BFU-E/CFU-E Development and Haemoglobin Synthesis. *Hum. Exp. Toxicol.* **1997**, *16*, 115–122. [CrossRef] [PubMed]
3. Rodrigo, M.A.; Oturan, M.A.; Oturan, N. Electrochemically Assisted Remediation of Pesticides in Soils and Water: A Review. Chemical Reviews. *Am. Chem. Soc. Sept.* **2014**, *10*, 8720–8745. [CrossRef]
4. Özkara, A.; Akyil, D.; Konuk, M. Pesticides, Environmental Pollution, and Health. In *Environmental Health Risk—Hazardous Factors to Living Species*; IntechOpen: London, UK, 2016. [CrossRef]
5. Verma, J.P.; Jaiswal, D.K.; Sagar, R. Pesticide Relevance and Their Microbial Degradation: A-State-of-Art. *Rev. Environ. Sci. Biotechnol.* **2014**, *13*, 429–466. [CrossRef]
6. Zhao, H.; Xu, J.; Dong, F.; Liu, X.; Wu, Y.; Wu, X.; Zheng, Y. Characterization of a Novel Oxyfluorfen-Degrading Bacterial Strain *Chryseobacterium* aquifrigidense and Its Biochemical Degradation Pathway. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 6837–6845. [CrossRef]
7. Hackenberger, D.K.; Stjepanović, N.; Lončarić, Ž.; Hackenberger, B.K. Acute and Subchronic Effects of Three Herbicides on Biomarkers and Reproduction in Earthworm *Dendrobaena veneta*. *Chemosphere* **2018**, *208*, 722–730. [CrossRef]

8. Vinebrooke, R.D.; Cottingham, K.L.; Norberg, J.; Scheffer, M.; Dodson, S.I.; Maberly, S.C.; Sommer, U. Impacts of Multiple Stressors on Biodiversity and Ecosystem Functioning: The Role of Species Co-Tolerance. *Oikos* **2004**, *104*, 451–457. [CrossRef]
9. Geoffroy, L.; Teisseire, H.; Couderchet, M.; Vernet, G. Effect of Oxyfluorfen and Diuron Alone and in Mixture on Antioxidative Enzymes of *Scenedesmus obliquus*. *Pestic. Biochem. Physiol.* **2002**, *72*, 178–185. [CrossRef]
10. Hassanein, H.M.A. Toxicological Effects of the Herbicide Oxyfluorfen on Acetylcholinesterase in Two Fish Species: *Oreochromis niloticus* and *Gambusia affinis*. *J. Environ. Sci. Health Part A* **2002**, *37*, 521–527. [CrossRef]
11. Peixoto, F.; Alves-Fernandes, D.; Santos, D.; Fontaínhas-Fernandes, A. Toxicological Effects of Oxyfluorfen on Oxidative Stress Enzymes in Tilapia *Oreochromis niloticus*. *Pestic. Biochem. Physiol.* **2006**, *85*, 91–96. [CrossRef]
12. Hassanein, H.M.A.; Banhawy, M.A.; Soliman, F.M.; Abdel-Rehim, S.A.; Müller, W.E.G.; Schröder, H.C. Induction of Hsp70 by the Herbicide Oxyfluorfen (Goal) in the Egyptian Nile Fish, *Oreochromis niloticus*. *Arch. Environ. Contam. Toxicol.* **1999**, *37*, 78–84. [CrossRef]
13. Mesquita, A.F.; Marques, S.M.; Marques, J.C.; Gonçalves, F.J.M.; Gonçalves, A.M.M. Copper Sulphate Impact on the Antioxidant Defence System of the Marine Bivalves *Cerastoderma edule* and *Scrobicularia plana*. *Sci. Rep.* **2019**, *9*, 1–11. [CrossRef] [PubMed]
14. Lozano, P.; Trombini, C.; Crespo, E.; Blasco, J.; Moreno-Garrido, I. ROI-Scavenging Enzyme Activities as Toxicity Biomarkers in Three Species of Marine Microalgae Exposed to Model Contaminants (Copper, Irgarol and Atrazine). *Ecotoxicol. Environ. Saf.* **2014**, *104*, 294–301. [CrossRef]
15. Matozzo, V.; Ballarin, L.; Pampanin, D.M.; Marin, M.G. Effects of Copper and Cadmium Exposure on Functional Responses of Hemocytes in the Clam, *Tapes philippinarum*. *Arch. Environ. Contam. Toxicol.* **2001**, *41*, 163–170. [CrossRef] [PubMed]
16. Gutiérrez, I.B.; Mesquita, A.F.; Gonçalves, F.J.M.; Marques, J.C.; Gonçalves, A.M.M. Biomarkers' Responses of the Benthic Clam *Scrobicularia plana* to the Main Active Ingredients (S-Metolachlor and Terbuthylazine) of a Common Herbicide. *Ecol. Indic.* **2019**, *96*, 611–619. [CrossRef]
17. Gonçalves, A.M.M.; Mesquita, A.F.; Verdelhos, T.; Coutinho, J.A.P.; Marques, J.C.; Gonçalves, F. Fatty Acids' Profiles as Indicators of Stress Induced by of a Common Herbicide on Two Marine Bivalves Species: *Cerastoderma edule* (Linnaeus, 1758) and *Scrobicularia plana* (Da Costa, 1778). *Ecol. Indic.* **2016**, *63*, 209–218. [CrossRef]
18. Mesquita, A.F.; Gonçalves, F.; Verdelhos, T.; Marques, J.C.; Gonçalves, A.M.M. Fatty Acids Profiles Modifications in the Bivalves *Cerastoderma edule* and *Scrobicularia plana* in Response to Copper Sulphate. *Ecol. Indic.* **2018**, *85*, 318–328. [CrossRef]
19. Gutiérrez, I.B.; Mesquita, A.F.C.; Nunes, C.; Coimbra, M.A.; Gonçalves, F.J.M.; Marques, J.C.; Gonçalves, A.M.M. Impacts of S-Metolachlor and Terbuthylazine in Fatty Acid and Carbohydrate Composition of the Benthic Clam *Scrobicularia plana*. *Ecotoxicol. Environ. Saf.* **2019**, *173*, 293–304. [CrossRef]
20. Filimonova, V.; Gonçalves, F.; Marques, J.C.; De Troch, M.; Gonçalves, A.M.M. Fatty Acid Profiling as Bioindicator of Chemical Stress in Marine Organisms: A Review. *Ecol. Indic.* **2016**, *67*, 657–672. [CrossRef]
21. Pinto, E.; Carvalho, A.P.; Cardozo, K.H.M.; Malcata, F.X.; dos Anjos, F.M.; Colepicolo, P. Effects of Heavy Metals and Light Levels on the Biosynthesis of Carotenoids and Fatty Acids in the Macroalgae *Gracilaria tenuistipitata* (Var. Liui Zhang & Xia). *Rev. Bras. Farmacogn.* **2011**, *21*, 349–354. [CrossRef]
22. Mayor, D.J.; Gray, N.B.; Elver-Evans, J.; Midwood, A.J.; Thornton, B. Metal-Macrofauna Interactions Determine Microbial Community Structure and Function in Copper Contaminated Sediments. *PLoS ONE* **2013**, *8*, e64940. [CrossRef]
23. De Carvalho, C.C.C.R. Adaptation of Rhodococcus Erythropolis Cells for Growth and Bioremediation under Extreme Conditions. *Res. Microbiol.* **2012**, *163*, 125–136. [CrossRef] [PubMed]
24. Sheeba, V.P.S.; Kumar, P.S.; Prasad, S.M. Differential Physiological and Biochemical Responses of Two Cyanobacteria *Nostoc muscorum* and *Phormidium foveolarum* against Oxyfluorfen and UV-B Radiation. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 1981–1993. [CrossRef] [PubMed]
25. Anu, P.R.; Bijoy Nandan, S.; Jayachandran, P.R.; Don Xavier, N.D. Toxicity Effects of Copper on the Marine Diatom, *Chaetoceros calcitrans*. *Reg. Stud. Mar. Sci.* **2016**, *8*, 498–504. [CrossRef]
26. Nguyen, T.H.L.; Janssen, C. Embryo-Larval Toxicity Tests with the African Catfish (*Clarias gariepinus*): Comparative Sensitivity of Endpoints. *Arch. Environ. Contam. Toxicol.* **2002**, *42*, 256–262. [CrossRef]
27. Johnson, A.; Carew, E.; Sloman, K.A. The Effects of Copper on the Morphological and Functional Development of Zebrafish Embryos. *Aquat. Toxicol.* **2007**, *84*, 431–438. [CrossRef]
28. Nekoubin, H.; Gharedaashi, E.; Hatefi, S.; Sudagar, M.; Shahriari, R.; Asgharimoghadam, A. Determination of LC 50 of Copper Sulfate and Lead (II) Nitrate and Behavioral Responses of Grass Carp (*Ctenopharyngodon idella*). *Walailak J. Sci. Technol. WJST* **2012**, *9*, 333–340.
29. Gharedaashi, E.; Nekoubin, H.; Imanpoor, M.R.; Taghizadeh, V. Effect of Copper Sulfate on the Survival and Growth Performance of *Caspian sea* Kutum, *Rutilus frisii* Kutum. *Springerplus* **2013**, *2*, 498. [CrossRef]
30. Dos Santos, E.V.; Sáez, C.; Cañizares, P.; Martínez-Huitle, C.A.; Rodrigo, M.A. UV Assisted Electrochemical Technologies for the Removal of Oxyfluorfen from Soil Washing Wastes. *Chem. Eng. J.* **2017**, *318*, 2–9. [CrossRef]
31. Fernández, P.; Alcántara, R.; Osuna, M.D.; Vila-Aiub, M.M.; De Prado, R. Forward Selection for Multiple Resistance across the Non-Selective Glyphosate, Glufosinate and Oxyfluorfen Herbicides in Lolium Weed Species. *Pest Manag. Sci.* **2017**, *73*, 936–944. [CrossRef]
32. DataBase, P.P.P. General Information for Oxyfluorfen. 2021. Available online: https://www.nature.com/articles/nclimate1507#citeas (accessed on 16 March 2021).

33. DGAV. *Vendas De Produtos Fitofarmacêuticos Em Portugal Em 2014*; DGAV: Lisboa, Portugal, 2016.
34. Al-Malki, A.L.; Moselhy, S.S. Impact of Pesticides Residue and Heavy Metals on Lipids and Fatty Acids Composition of Some Seafoods of Red Sea (KSA). *Hum. Exp. Toxicol.* **2011**, *30*, 1666–1673. [CrossRef]
35. Maria, V.L.; Bebianno, M.J. Antioxidant and Lipid Peroxidation Responses in *Mytilus galloprovincialis* Exposed to Mixtures of Benzo(a)Pyrene and Copper. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2011**, *154*, 56–63. [CrossRef] [PubMed]
36. U.S. Environmental Protection Agency. *Effects of Climate Change on Aquatic Invasive Species and Implications for Management and Research*; U.S. Environmental Protection Agency: Washington, DC, USA, 2008.
37. Belkin, I.M. Rapid Warming of Large Marine Ecosystems. *Prog. Oceanogr.* **2009**, *81*, 207–213. [CrossRef]
38. Falkowski, P.G.; Barber, R.T.; Smetacek, V. Biogeochemical Controls and Feedbacks on Ocean Primary Production. *Science* **1998**, *281*, 200–206. [CrossRef]
39. Barton, S.; Jenkins, J.; Buckling, A.; Schaum, C.-E.; Smirnoff, N.; Raven, J.A.; Yvon-Durocher, G. Evolutionary Temperature Compensation of Carbon Fixation in Marine Phytoplankton. *Ecol. Lett.* **2020**, *23*, 722–733. [CrossRef]
40. Gao, K.; Xu, J.; Gao, G.; Li, Y.; Hutchins, D.A.; Huang, B.; Wang, L.; Zheng, Y.; Jin, P.; Cai, X.; et al. Rising CO_2 and Increased Light Exposure Synergistically Reduce Marine Primary Productivity. *Nat. Clim. Change* **2012**, *2*, 519–523. [CrossRef]
41. Follows, M.J.; Dutkiewicz, S.; Grant, S.; Chisholm, S.W. Emergent Biogeography of Microbial Communities in a Model Ocean. *Science* **2007**, *315*, 1843–1846. [CrossRef]
42. Boyce, D.G.; Lewis, M.R.; Worm, B. Global Phytoplankton Decline over the Past Century. *Nature* **2010**, *466*, 591–596. [CrossRef]
43. Field, C.B.; Behrenfeld, M.J.; Randerson, J.T.; Falkowski, P. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science* **1998**, *281*, 237–240. [CrossRef]
44. Cloern, J.E.; Foster, S.Q.; Kleckner, A.E. Phytoplankton Primary Production in the World's Estuarine-Coastal Ecosystems. *Biogeosciences* **2014**, *11*, 2477–2501. [CrossRef]
45. Malviya, S.; Scalco, E.; Audic, S.; Vincent, F.; Veluchamy, A.; Poulain, J.; Wincker, P.; Iudicone, D.; De Vargas, C.; Bittner, L.; et al. Insights into Global Diatom Distribution and Diversity in the World's Ocean. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E1516–E1525. [CrossRef]
46. Zulu, N.N.; Zienkiewicz, K.; Vollheyde, K.; Feussner, I. Current Trends to Comprehend Lipid Metabolism in Diatoms. *Prog. Lipid Res.* **2018**, *70*, 1–16. [CrossRef]
47. Obata, T.; Fernie, A.R.; Nunes-Nesi, A. The Central Carbon and Energy Metabolism of Marine Diatoms. *Metabolites* **2013**, *3*, 325–346. [CrossRef] [PubMed]
48. Ahlgren, G.; Van Nieuwerburgh, L.; Wänstrand, I.; Pedersén, M.; Boberg, M.; Snoeijs, P. Imbalance of Fatty Acids in the Base of the Baltic Sea Food Web—A Mesocosm Study. *Can. J. Fish. Aquat. Sci.* **2005**, *62*, 2240–2253. [CrossRef]
49. Litzow, M.; Bailey, K.; Prahl, F.; Heintz, R. Climate Regime Shifts and Reorganization of Fish Communities: The Essential Fatty Acid Limitation Hypothesis. Mar. *Ecol. Prog. Ser.* **2006**, *315*, 1–11. [CrossRef]
50. Fuiman, L.A. Egg Boon Fatty Acids Reveal Effects of a Climatic Event on a Marine Food Web. *Ecol. Monogr.* **2018**, *88*, 585–599. [CrossRef]
51. Araújo, C.F.C.; Souza-Santos, L.P. Use of the Microalgae *Thalassiosira weissflogii* to Assess Water Toxicity in the Suape Industrial-Port Complex of Pernambuco, Brazil. *Ecotoxicol. Environ. Saf.* **2013**, *89*, 212–221. [CrossRef]
52. Gonçalves, A.M.M.; Barroso, D.V.; Serafim, T.L.; Verdelhos, T.; Marques, J.C.; Gonçalves, F. The Biochemical Response of Two Commercial Bivalve Species to Exposure to Strong Salinity Changes Illustrated by Selected Biomarkers. *Ecol. Indic.* **2017**, *77*, 59–66. [CrossRef]
53. Sánchez-Muros, M.J.; Villacreces, S.; Miranda-de la Lama, G.; de Haro, C.; García-Barroso, F. Effects of Chemical and Handling Exposure on Fatty Acids, Oxidative Stress and Morphological Welfare Indicators in Gilt-Head Sea Bream (*Sparus aurata*). *Fish Physiol. Biochem.* **2013**, *39*, 581–591. [CrossRef]
54. Neves, M.; Castro, B.B.; Vidal, T.; Vieira, R.; Marques, J.C.; Coutinho, J.A.P.; Gonçalves, F.; Gonçalves, A.M.M. Biochemical and Populational Responses of an Aquatic Bioindicator Species, *Daphnia longispina*, to a Commercial Formulation of a Herbicide (Primextra®Gold TZ) and Its Active Ingredient (S-Metolachlor). *Ecol. Indic.* **2015**, *53*, 220–230. [CrossRef]
55. Lochmiller, R.L.; Deerenberg, C. Trade-Offs in Evolutionary Immunology: Just What Is the Cost of Immunity? *Oikos* **2000**, *88*, 87–98. [CrossRef]
56. Davey, M.E.; O'toole, G.A. Microbial Biofilms: From Ecology to Molecular Genetics. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 847–867. [CrossRef] [PubMed]
57. Hung, C.-C.; Santschi, P.H.; Gillow, J.B. Isolation and Characterization of Extracellular Polysaccharides Produced by *Pseudomonas fluorescens* Biovar II. *Carbohydr. Polym.* **2005**, *61*, 141–147. [CrossRef]
58. Kazy, S.K.; Sar, P.; Singh, S.P.; Sen, A.K.; D'Souza, S.F.D. Extracellular Polysaccharides of Copper-Sensitive and Copper-Resintant Pseudomonas Aeruginosa Strain: Synthesis, Chemical Nature and Copper Binding. *World J. Microbiol. Biotechnol.* **2002**, *18*, 583–588. [CrossRef]
59. Sutherland, I.W. Microbial Polysaccharides from Gram-Negative Bacteria. *Int. Dairy J.* **2001**, *11*, 663–674. [CrossRef]
60. Payne, M.F.; Rippingale, R.J. Intensive Cultivation of the Calanoid Copepod *Gladioferens imparipes*. *Aquaculture* **2001**, *201*, 329–342. [CrossRef]
61. Gonçalves, A.M.M.; Azeiteiro, U.M.; Pardal, M.A.; De Troch, M. Fatty Acid Profiling Reveals Seasonal and Spatial Shifts in Zooplankton Diet in a Temperate Estuary. *Estuar. Coast. Shelf Sci.* **2012**, *109*, 70–80. [CrossRef]

62. Coimbra, M.A.; Waldron, K.W.; Selvendran, R.R. Isolation and Characterisation of Cell Wall Polymers from Olive Pulp (*Olea Europaea* L.). *Carbohydr. Res.* **1994**, *252*, 245–262. [CrossRef]
63. Clarke, K.R.; Gorley, K.N. *PRIMER v6: User Manual/Tutorial*; ScienceOpen: Burlington, MA, USA, 2006.
64. Prado, R.; García, R.; Rioboo, C.; Herrero, C.; Abalde, J.; Cid, A. Comparison of the Sensitivity of Different Toxicity Test Endpoints in a Microalga Exposed to the Herbicide Paraquat. *Environ. Int.* **2009**, *35*, 240–247. [CrossRef]
65. Sobrino, C.; Neale, P.J. Short-Term and Long-Term Effects of Temperature on Photosynthesis in the *Diatom Thalassiosira pseudonana* under UVR Exposures. *J. Phycol.* **2007**, *43*, 426–436. [CrossRef]
66. Jin, P.; Agustí, S. Fast Adaptation of Tropical Diatoms to Increased Warming with Trade-Offs. *Sci. Rep.* **2018**, *8*, 1–10. [CrossRef]
67. Tam, L.T.; Van Cong, N.; Thom, L.T.; Ha, N.C.; Hang, N.T.M.; Van Minh, C.; Vien, D.T.H.; Hong, D.D. Cultivation and Biomass Production of the Diatom *Thalassiosira weissflogii* as a Live Feed for White-Leg Shrimp in Hatcheries and Commercial Farms in Vietnam. *J. Appl. Phycol.* **2021**, *33*, 1559–1577. [CrossRef]
68. Filimonova, V.; Gonçalves, F.; Marques, J.C.; De Troch, M.; Gonçalves, A.M.M. Biochemical and Toxicological Effects of Organic (Herbicide Primextra®Gold TZ) and Inorganic (Copper) Compounds on Zooplankton and Phytoplankton Species. *Aquat. Toxicol.* **2016**, *177*, 33–43. [CrossRef]
69. Filimonova, V.; Nys, C.; De Schamphelaere, K.A.C.; Gonçalves, F.; Marques, J.C.; Gonçalves, A.M.M.; De Troch, M. Ecotoxicological and Biochemical Mixture Effects of an Herbicide and a Metal at the Marine Primary Producer Diatom *Thalassiosira weissflogii* and the Primary Consumer Copepod *Acartia tonsa*. *Environ. Sci. Pollut. Res.* **2018**, *25*, 22180–22195. [CrossRef] [PubMed]
70. Wang, C.; Lin, X.; Li, L.; Lin, S. Differential Growth Responses of Marine Phytoplankton to Herbicide Glyphosate. *PLoS ONE* **2016**, *11*, 1–20. [CrossRef] [PubMed]
71. Yi, A.X.; Leung, P.T.Y.; Leung, K.M.Y. Photosynthetic and Molecular Responses of the Marine Diatom *Thalassiosira pseudonana* to Triphenyltin Exposure. *Aquat. Toxicol.* **2014**, *154*, 48–57. [CrossRef]
72. Stillway, M.E.; Teh, S.J. The Effect of Herbicide Formulations and Herbicide—Adjuvant Mixtures on Aquatic Food Web Species of the Sacramento-San Joaquin Delta, California, USA. *Environ. Toxicol. Chem.* **2020**, *39*, 1375–1381. [CrossRef]
73. Leung, P.T.Y.; Yi, A.X.; Ip, J.C.H.; Mak, S.S.T.; Leung, K.M.Y. Photosynthetic and Transcriptional Responses of the Marine Diatom *Thalassiosira pseudonana* to the Combined Effect of Temperature Stress and Copper Exposure. *Mar. Pollut. Bull.* **2017**, *124*, 938–945. [CrossRef]
74. Rocchetta, I.; Mazzuca, M.; Conforti, V.; Ruiz, L.; Balzaretti, V.; De Molina, M.D.C.R. Effect of Chromium on the Fatty Acid Composition of Two Strains of *Euglena gracilis*. *Environ. Pollut.* **2006**, *141*, 353–358. [CrossRef]
75. Butow, B.; Wynne, D.; Sukenik, A.; Hadas, O.; Tel-Or, E. The Synergistic Effect of Carbon Concentration and High Temperature on Lipid Peroxidation in *Peridinium gatunense*. *J. Plankton Res.* **1998**, *20*, 355–369. [CrossRef]
76. Renaud, S.M.; Thinh, L.-V.; Lambrinidis, G.; Parry, D.L. Effect of Temperature on Growth, Chemical Composition and Fatty Acid Composition of Tropical Australian Microalgae Grown in Batch Cultures. *Aquaculture* **2002**, *211*, 195–214. [CrossRef]
77. Renaud, S.M.; Zhou, H.C.; Parry, D.L.; Thinh, L.-V.; Woo, K.C. Effect of Temperature on the Growth, Total Lipid Content and Fatty Acid Composition of Recently Isolated Tropical Microalgae *Isochrysis Sp., Nitzschia Closterium, Nitzschia Paleacea*, and Commercial Species *Isochrysis Sp.* (Clone T.ISO). *J. Appl. Phycol.* **1995**, *7*, 595–602. [CrossRef]
78. Jiang, H.; Gao, K. Effects of Lowering Temperature during Culture on the Production of Polyunsaturated Fatty Acids in the Marine Diatom *Phaeodactylum tricornutum* (Bacillariophyceae). *J. Phycol.* **2004**, *40*, 651–654. [CrossRef]
79. Fuschino, J.R.; Guschina, I.A.; Dobson, G.; Yan, N.D.; Harwood, J.L.; Arts, M.T. Rising Water Temperatures Alter Lipid Dynamics and Reduce N-3 Essential Fatty Acid Concentrations in *Scenedesmus obliquus* (Chlorophyta). *J. Phycol.* **2011**, *47*, 763–774. [CrossRef]
80. Camadro, J.M.; Matringe, M.; Scalla, R.; Labbe, P. Kinetic Studies on Protoporphyrinogen Oxidase Inhibition by Diphenyl Ether Herbicides. *Biochem. J.* **1991**, *277 Pt. 1*, 17–21. [CrossRef]
81. Duke, S.O.; Lydon, J.; Becerril, J.M.; Sherman, T.D.; Lehnen, L.P.; Matsumoto, H. Protoporphyrinogen Oxidase-Inhibiting Herbicides. *Weed Sci.* **1991**, *39*, 465–473. [CrossRef]
82. Hallahan, B.J.; Camilleri, P.; Smith, A.; Bowyer, J.R. Mode of Action Studies on a Chiral Diphenyl Ether Peroxidizing Herbicide: Correlation between Differential Inhibition of Protoporphyrinogen IX Oxidase Activity and Induction of Tetrapyrrole Accumulation by the Enantiomers. *Plant Physiol.* **1992**, *100*, 1211–1216. [CrossRef]
83. Bianchi, J.; Cabral-de-Mello, D.C.; Marin-Morales, M.A. Toxicogenetic Effects of Low Concentrations of the Pesticides Imidacloprid and Sulfentrazone Individually and in Combination in in Vitro Tests with HepG2 Cells and *Salmonella typhimurium*. *Ecotoxicol. Environ. Saf.* **2015**, *120*, 174–183. [CrossRef]
84. Park, J.; Ahn, Y.O.; Nam, J.-W.; Hong, M.-K.; Song, N.; Kim, T.; Yu, G.-H.; Sung, S.-K. Biochemical and Physiological Mode of Action of Tiafenacil, a New Protoporphyrinogen IX Oxidase-Inhibiting Herbicide. *Pestic. Biochem. Physiol.* **2018**, *152*, 38–44. [CrossRef]
85. Engle, T.E.; Fellner, V.; Spears, J.W. Copper Status, Serum Cholesterol, and Milk Fatty Acid Profile in Holstein Cows Fed Varying Concentrations of Copper. *J. Dairy Sci.* **2001**, *84*, 2308–2313. [CrossRef]
86. Kumar, M.; Kumari, P.; Gupta, V.; Anisha, P.A.; Reddy, C.R.K.; Jha, B. Differential Responses to Cadmium Induced Oxidative Stress in Marine Macroalga *Ulva lactuca* (Ulvales, Chlorophyta). *BioMetals* **2010**, *23*, 315–325. [CrossRef] [PubMed]
87. Raven, J.A. Cellular Location of Starch Synthesis and Evolutionary Origin of Starch Genes. *J. Phycol.* **2005**, *41*, 1070–1072. [CrossRef]

88. Hildebrand, M.; Manandhar-Shrestha, K.; Abbriano, R. Effects of Chrysolaminarin Synthase Knockdown in the Diatom *Thalassiosira pseudonana*: Implications of Reduced Carbohydrate Storage Relative to Green Algae. *Algal Res.* **2017**, *23*, 66–77. [CrossRef]
89. Urbani, R.; Magaletti, E.; Sist, P.; Cicero, A.M. Extracellular Carbohydrates Released by the Marine Diatoms Cylindrotheca Closterium, *Thalassiosira pseudonana* and *Skeletonema costatum*: Effect of P-Depletion and Growth Status. *Sci. Total Environ.* **2005**, *353*, 300–306. [CrossRef]
90. Chen, J.; Guo, K.; Thornton, D.C.O.; Wu, Y. Effect of Temperature on the Release of Transparent Exopolymer Particles (TEP) and Aggregation by Marine Diatoms (*Thalassiosira weissflogii* and *Skeletonema marinoi*). *J. Ocean Univ. China* **2021**, *20*, 56–66. [CrossRef]