Chapter 6

Fatty Acids' Profiles of Aquatic Organisms: Revealing the Impacts of Environmental and Anthropogenic Stressors

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Abstract

There is a great concern about the impacts of climate changes namely due to salinity sea-water and temperature alterations in aquatic organisms with the estuarine and coastal environments being the major affected areas. The intensive usage of chemicals in an indiscriminate way in agriculture practices, achieving, in some cases, values above the limits of contamination authorized by the European legislation, also drastically affects the surrounded estuarine areas with profound consequences to the water quality and the aquatic communities. It is known that stressors affect organisms' physiological conditions with recent works concerning alterations in the fatty acid (FA) profiles associated with environmental and contamination events that become more frequent. FA plays a key role in immune and physiological functions and is associated with the prevention of some diseases, shown to be good bio-indicators to assess the organisms' impacts under stress conditions. Thus, this chapter proposes to address natural (salinity and temperature) and chemical (herbicide and metal) stressors' impacts in the FA profiles of *Thalassiosira weissflogii* and *Cerastoderma edule* and infers about the effects on organisms' physiological processes and along the food web. Consequences in food resources and to healthier and nutritious food consumption with benefits to human beings are also assessed.

Keywords: fatty acids, bio-indicator, stressors, climate changes, salinity, temperature, pollutants, herbicide, metal, aquatic organisms, food quality, estuaries

1. Introduction

Fatty acids (FAs) are essential molecules with a crucial role in the maintenance of physiological functions of many organisms. These carboxylic acids provide fuel for the brain and the tissues at the metabolic level and are a major constituent in the cellular wall as part of phospholipids. Once transferred across the food chain, FAs perform the connection between primary producers and secondary consumers [1]. Greatly abundant in the brain tissues, these molecules represent almost half of the brain weight [2]. Fatty acid nomenclature is represented by X:YωZ, where X represents the number of carbon atoms in the chain, Y represents the number of double bonds and Z gives information about the position of the first double bond counting from the methyl group [3]. There are two major groups of FA: saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs), differing between them by the existence of double bonds in UFA. The position of the double bonds is determined by the desaturase enzyme activity, which performs the double bond in different positions accordingly to the type of enzyme present in different organisms (e.g. Δ3 in animals, Δ6 in animals and plants and Δ15 in algae and plants with chlorophyll) [3]. Saturated FAs are metabolized mainly as a source of energy and also as lipid storage. UFA can be identified by the number of double bonds: Monounsaturated FA (MUFA) have one double bond and can be synthesized *de novo* almost by all organisms [4]. Polyunsaturated FAs (PUFAs) are fatty acids with two or more double bonds that play an essential role in the brain development [5] and many physiological functions such as down regulating inflammation, cellular signalling [6] and regulation of transcription factors [7]. Essential fatty acids (EFAs) are some PUFAs that play major important functions in physiological and biochemical processes and that must be acquired externally, through dietary input, once the majority of the animals cannot synthesize them *de novo* [8], due to the lack of the desaturase enzyme [9]. Although some animals are able to synthetize EFA from linolenic precursors such as α-linolenic acid C18:3 (n-3), C18:4 (n-3) and C18:5 (n-3), found almost exclusively in plants [3] via elongation and desaturation, the rate this conversion succeeds is residual to supply the necessary amount of EFA required for an optimal growth and development. Thus, EFAs must be obtained by direct feeding on phytoplanktonic, plants or bacteria species [10], or by ingesting lipid emulsions with high content of EFAs [11]. Since EFA are later transferred along the food web, aquatic species like fish and other organisms from higher trophic levels are an important food sources of such molecules [4]. EFAs are represented mainly by some PUFAs. Highly unsaturated fatty acids (HUFAs) are a subset of PUFAs with a chain of 20 carbon atoms or more and with three or more double bonds that play an important role in cellular growth, with special relevance in tissue growth, energy storage, neural development and also reproductive fitness [4, 12]. The physiological activities of EFAs in animals are mainly represented by eicosapentaenoic acid (EPA—20:5n3) and docosahexaenoic acid (DHA—22:6n3), two of the most important HUFAs that are synthesized *de novo* by phytoplankton and bio-accumulated by animals. The arachidonic acid (ARA—20:4n-6) is also a representative EFA with functions as a precursor of animal hormones such as prostaglandins and leukotrienes amongst others [3]. EPA and DHA play a major role in brain development and maintenance of brain structure and function [13]. EPA intake influences many physiological processes such as reproduction, immunity efficiency and osmoregulation [3]. DHA is important for the health and developing

of neurons and for neurotransmission, with strong influence on cognition and behavior, and it is also proved to be important in the protection against oxidative stress [14]. Fatty acids are considered to be an accurate tool in trophic interaction studies [15], mainly due to their importance in the health/stability of the ecosystem, and because they are transferred conservatively to higher trophic levels along the trophic food web [4, 16]. Furthermore, FA profiles can reflect structural changes in species' biochemical composition in response to stressors [17–20]. Lipid components are also very sensitive to environmental changes, which make them an efficient assessment tool to monitor toxicological effects on the marine biota as bio-indicators of ecosystem health [19].

Environmental pollution worldwide is an undesirable by-product of the increased demand for natural resources in the modern civilization. However, since the advent of human societies, there have always been foci of environmental contamination, though nothing on the scale we see today. Practically, whole environment suffers from some degree of contamination in concentrations above those expected for the region. The pollutants that damage the ecosystem are the pollutants from industry and mining that release toxic substances such as metals and organic pollutants. Some pesticides and mainly metals (e.g. Cd, Cr, Pb, Hg, Ni, Cu) are non-degradable and therefore accumulate in nature, where they continue to affect ecosystem's function over the course of decades or even centuries. These chemicals can distress several biological organization levels affecting flora and fauna aquatic organisms, interfering with the metabolic and physiological processes and thus compromising the structure and physicochemical properties of the membrane, damage cells, tissues and organs. Long-term effects may lead to higher mortality among population, changing the diversity and structure of the communities. Furthermore, due to global climate changes, environmental conditions are expected to change considerably in several areas. In some regions, it is expected, seasonal differences become more notorious than they used to be exposing the organisms to a wide physiological stress. These changes not only act as additional stress factors but may also considerably modify the toxicity of pollutants in aquatic ecosystems.

Estuaries are coastal ecosystems, which are biologically highly productive and having great importance in the ecological and at socioeconomic contexts, providing exceptional natural resources and services to human beings, mainly to local populations. Some of these systems are located near farmlands, industrial and residential areas being under anthropogenic pressures that affect water quality and the aquatic communities. Estuarine systems are very useful model systems to study the ecological and evolutionary responses of organisms to highly variable, discontinuous habitats due to the extreme daily variations that occur in these transitional areas exposing the organisms to a widely physiological stress [21]. The transition between the freshwater and marine environments creates a gradient of physical and chemical conditions that determine the amount and distribution of the species and communities that live at these ecosystems [22], with salinity being one of the major controlling factor of species' distribution in estuarine systems. Aquatic organisms from these ecosystems are exposed to physical and chemical environmental conditions that vary greatly, on both seasonally and daily basis. Because planktonic species are strongly influenced by climatic factors, and particularly sensitive to changes in hydrological conditions [23], the

increase in frequency of flooding episodes has a strong impact on macrobenthic communities and is proven to be result into a significant decline in the diversity of suspension-feeder species, such as microcrustaceans (e.g. copepods) [24]. The temperature of the water is also linked with changes in salinity, and although some studies have tested the two parameters separately, both should also be studied in a bi-dimensional approach, to best simulate natural conditions [25]. According to Kinne [26] and Williams and Geddes [27], temperature can alter the physiological tolerance of an organism to salinity changes, and in turn, salinity can influence the impact of environmental temperature on the same organism. Salinity is indeed of major importance in the distribution of aquatic organisms because the ability of osmoregulation affects ecological tolerances and the type of ecosystem (marine, coastal, estuarine, freshwater) [28].

Since past decades, extreme weather episodes are frequent worldwide, and Portugal is not an exception. At the Mondego estuary, located in the western coast of Portugal, near Figueira da Foz city, episodes of drought and flood have been registered and are well documented in the literature revealing ecological impacts on aquatic communities [24, 29–31]. This temperate estuarine system surrounded by agriculture fields, the commercial port, beach and industries, and with a high marine exploitation of resources, suffers high anthropogenic pressures similar to many other estuarine systems mainly from the Mediterranean region. Rice and corn fields are the main agriculture production in the Mondego valley, being Viper and Primextra® Gold TZ the most used pesticides in agriculture practices, respectively, according to information from the cooperatives of the region. Furthermore, copper is one of the main constituents of pesticides formulations, with application in agricultural activities. It is an essential metal, with vital importance in low concentrations to organisms, acting as a co-factor of many enzymes, i.e. it is a component of superoxide dismutase, an enzyme defending living organisms against reactive oxygen species [32]. Still becomes toxic at high concentrations affecting several biochemical and metabolic processes such as FA metabolism, cell division, photosynthesis, respiration and synthesis of carbohydrates, pigments and chlorophyll [33, 34]. In 1998, and similarly to other estuarine systems near intensive agriculture practices with wide usage of pollutants, a pesticide-monitoring program was implemented in Mondego estuary to recover the system [35].

Stressors affect organisms' growth and biochemical processes and also their performance and healthy status. To compensate extreme conditions, or at least conditions that are far from the optimal, some organisms developed strategic and adaptive mechanisms to compensate physiological requirements. Nevertheless, it is apparent to have the occurrence of significant losses and large alterations on the FA contents. Some studies allow to recognize and assess the response of specific markers in order to identify and validate precise bio-indicators that are able to capture the impact of disturbances resulted at extreme conditions or at the presence of stressors, which might be used as early warning signals of stressing conditions and, eventually, be associated to strategic prevention of stress-associated diseases. Therefore, it is crucial to determine and assess lethal effects and physiological responses of aquatic organisms under the influence of environmental (e.g. salinity, temperature) and chemical (e.g. pollutants) stressors in order to predict the impacts on

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communities and thus on aquatic ecosystems and food quality. Still, there are some characteristics that must be taken into account when choosing the species to be tested, such as (1) the species' sensitiveness to the studied parameter/substance; (2) well-known nutrient requirements; (3) low genetic and phenotypic variability among strains/organisms; and (4) fast and cost efficient maintenance in the laboratory [36]. The marine phytoplankton species, *Thalassiosira weissflogii*, is often used as a bio-indicator in several studies, becoming increasingly relevant in environmental monitoring studies [36]. The marine diatom value is closely related to its many applications in 1) ecotoxicological studies; 2) biodiesel production; 3) prey for zooplankton (rotifers, copepods, brine shrimp) and 4) production of metabolites such as lipids, proteins, carbohydrates, pigments and vitamins [37]. *Cerastoderma edule* plays a key role between primary producers and consumers. They live in intertidal shallow areas, presenting a suspension-feeder behavior [38]. This bivalve species lives worldwide occurring from the Northern Norway to the North Africa, on the east coast of the Atlantic and in Murmansk in the Arctic [39]. Due to its sessile life style, easy sampling collection, maintenance, handling and sensitivity to chemicals, *C. edule* is widely used as standard species in ecotoxicological bioassays [19, 20]. Due to its high ability to filtrate and accumulate large amount of pollutants, this species is also used as bio-indicator in ecological studies [39–43]. Furthermore, *C. edule* is very much appreciated as food source mainly by local populations, which highlights its importance to the socioeconomic sector.

In this chapter, it is proposed to determine and assess the effects of environmental (salinity and temperature) and chemical (Primextra® Gold TZ and Copper) stressors, individually and combined, in the fatty acid profiles of a marine phytoplankton species (*T. weissflogii*) and an estuarine bivalve species (*C. edule*). The impacts of global stressors to the quality of aquatic food resources and thus to a healthy and nutritive food consumption are also assessed.

2. Material and methods

2.1. Study area and sampling procedure

The Mondego estuary is a small mesotidal system in the West Atlantic coast of Portugal (40°08′N, 8°50′W). The estuary is divided into two arms, north and south (**Figure 1**). The northern arm is characterized by a salt-wedge during low tide, which changes to a partially mixed water column at high tide. It is characterized by a partially mixed water column at low tide and a well-mixed one at high tide at spring tides [44]. The southern arm is shallower and its water circulation is mostly dependent on tides and on freshwater input from a small tributary system, the Pranto River. Freshwater discharges of this river are controlled by a sluice according to the water needs by the rice fields of Mondego valley [29].

C. edule was sampled at the south arm of the estuary (**Figure 1**). Organisms were transported from the field in cold boxes with brackish water.

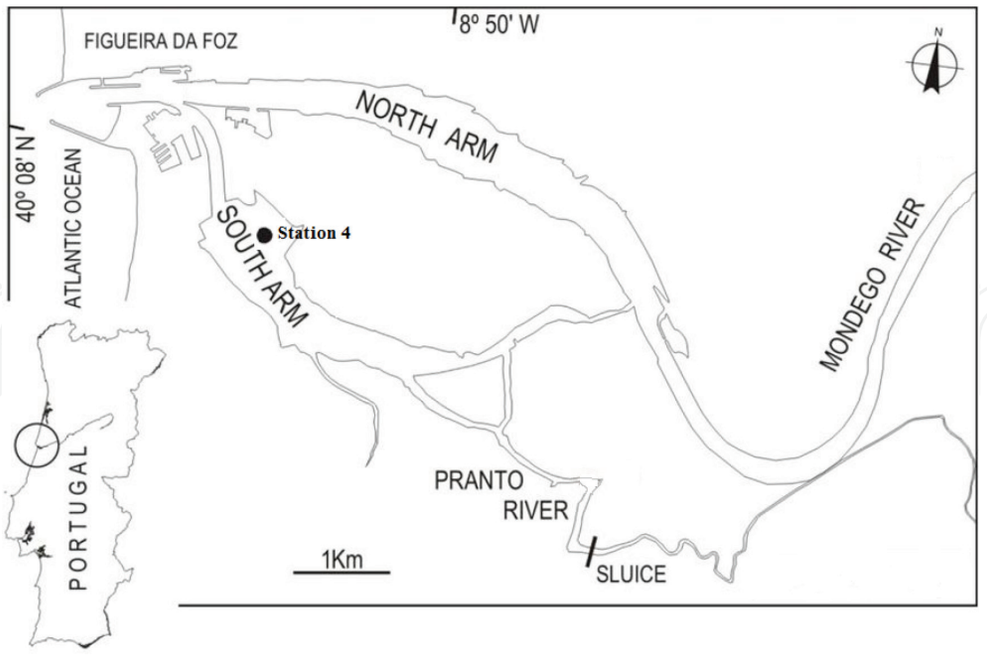

Figure 1. The Mondego estuary location and the sampling site within the estuary.

2.2. Laboratory procedures and bioassays

2.2.1. Microalga species

2.2.1.1. Culture maintenance and laboratory bioassays

T. weissflogii was obtain from the Scottish Marine Institute, Dunbeg, PA37 1QA, UK (strain number 1085/18). The microalga was maintained under laboratory conditions by renewing the alga medium once a week, maintaining a cell density of 2×10^4 cells/mL. f/2 medium was prepared accordingly to Guillard and Reyther [45] with water collected from the Mondego estuary with a salinity of 30 psu, previously filtered with Whatman glass microfiber filters with 1.2 μm pores and stored at 4°C. By the experiments with copper, the medium was prepared without EDTA, adapted after Rippingale and Payne [46]. A renew of algae culture was done weekly. All assays and organism cultures were maintained under artificial light with photoperiod of $16h^L$:$8h^D$. Different cultures were maintained in 15, 20 and 25 ± 2°C, respectively.

Before the beginning of bioassays, an inoculum of *T. weissflogii* was harvested from the bulk culture and incubated for 3 days in a chamber with photoperiod ($16h^L$: $8h^D$) at 20°C [18]. This procedure was repeated from other two temperatures (15 and 25°C) to the salinity experiments where a set of different temperatures (15, 20 and 25°C) were assessed to a range of salinity concentrations. The cellular concentration was then adjusted to 10^4 cells/mL after cellular density determination at a Neubauer haemocytometer, and the microalgae was then exposed to a range of salt and chemical concentrations, respectively. Eight salinity concentrations (0, 5, 10, 15, 20, 25, 33) plus the control were performed at three distinct temperatures (15, 20 and 25°C). The experiments with the both toxicants were conducted to a range of concentrations from 0.200 to 0.800 mg/L of copper(II) sulphate pentahydrate and 0.005 to 0.040 mg/L of the herbicide Primextra® Gold TZ, plus the control. Glass beakers were used

to saline and herbicide experiments, whereas plastic beakers were used in the experiments with the metal. Three replicas per treatment were prepared in each bioassay. The experiments were conducted under photoperiod ($16h^{L}$: $8h^{D}$), with a duration of 96 h. At the end of the experiment, the cellular density was determined to each treatment using a Neubauer haemocytometer.

2.2.1.2. Microcosm bioassays

Microcosm bioassays had a duration of 7 days and has been conducted under the same conditions of the laboratory bioassays previously described. Three treatments corresponding to 96h-EC_{10}, EC_{20} and EC_{50} calculated from the bioassays described in the subsection above plus the control treatment were employed whenever possible due to the duration of the microcosm experiments. According to the results of the microalga growth obtained at the previous experimental bioassays, the control treatments of the salinity microcosm bioassays were performed to a salinity concentration of 33, 33 and 25 to the temperature of 15, 20 and 25°C, respectively. Although at 20°C the microalga presented a similar growth at the saline treatments of 30 (CTL) and 33, and being considered the optimal growth to *T. weissflogii* at the salinity of 30, the control treatment to the microcosm bioassay was performed to a salinity concentration of 33 in order to compare the results with the microcosm experiments conducted at 15°C. Three replicas per treatment were conducted. At the end of the microcosm bioassays, a final concentration of 7.2×10^6 cells/mL was measured in each Erlenmeyer flask and then filtered through a GF/F Whatman filter and frozen at −80°C for FA analysis.

2.2.2. Bivalve species

2.2.2.1. Culture maintenance and laboratory bioassays

In the lab, organisms were divided in aquaria with aeration and filtrated sea water at the salinity of 20. On 10 selected organisms, collected in the field and not under any laboratorial process, a set of measurements (shell length, total weight, tissue weight and foot weight) were assessed to determine the condition indices. After the measurements, the muscle (foot) of each organism was removed and stored at −80°C for fatty acid analysis. The remaining organisms collected in the field were maintained in the aquaria, under photoperiod conditions ($12h^{L}$:$12h^{D}$) and control temperature (20 ± 2°C), without food, during a depuration period of 48 h, previously to the experiments.

Salinity bioassay was performed on organisms exposed to a range of saline concentrations from 0 to 35 plus the control. The salinity concentrations were obtained from successive dilutions of filtrated seawater at the salinity of 35 in distilled water. Experiments with the contaminants were conducted on individuals under six concentrations of copper(II) sulphate pentahydrate ranging from 0.6 to 2.1 mg/L and a set of eight concentrations from 0.5 to 60 mg/L of the herbicide Primextra® Gold TZ plus the control, respectively. The test medium was used as negative control. Bioassays were conducted under control temperature (20 ± 2°C), $12h^{L}$:$12h^{D}$photoperiod, with filtrated sea water medium at the salinity of 20, during 120 h. Tests were carried out in glass (to saline and herbicide experiments) and plastic (to the metal) vials, 10 per treatment, containing a final test volume of 1000 mL per replicate. Organisms were fed daily with a commercial mixture of microalgae and rotifers. Organisms were transferred to newly prepared test

solutions every next day. Bivalves were checked daily for mortality and behavioural conditions (to evaluate the conditions of the valves, organism behavior during feeding and the activity of the siphon). After the exposure period, all survival organisms were dissected, measured the weight and the body length and evaluated the condition indices of each individual. After the measurements, the muscle tissue (foot) was stored at −80°C for further fatty acid analysis.

2.2.3. Fatty acid analysis

The sample extraction for the FA analysis was obtained accordingly to the method described in Gonçalves et al. [19], substituting BF3-methanol by H_2S0_4, due to reported deficiency in PUFA detection [47]. A differentiated phase was extracted, and an internal standard (fatty acid Methylnonadecanoate (C19) Fluka 74208) was added to the quantification of FA. The samples were later analyzed using a gas chromatograph with a mass spectrometer and an HP88 column (60 m × 25 mm × 0.20 μm). It was conducted in splitless mode, with a 1 μL injector per run. The column temperature was set to increase from 75 to 230°C at a rate of 2°C/min. The carrier gas was helium at a flow rate of 1.3 mL/min. The results of the GC analyses were obtained, and fatty acid methyl esters (FAMEs) were identified by comparison of their retention times with those of individual purified standards. FAMEs can also be quantified by determining the area of the peaks of each fatty acid with the help of calibration factors [47].

2.2.4. Statistical analysis

The cellular density of *T. weissflogii* measured with a Neubauer Haemocytometer, at the end of the bioassays, was used to estimate the concentrations which induced *x*% growth inhibition (EC_x values, with $x = 10, 20, 50$) and the corresponding 95% confidence intervals by non-linear regression, using the least-squares method to fit the data to the logistic equation.

The LC10, LC20 and LC50 values with corresponding 95% confidence intervals for *C. edule* were determined using Probit analysis [48].

To determine significant differences between treatments, one-way analysis of variance (ANOVA) was performed, followed by Dunnett's multiple comparison test to identify significant differences between salinity treatments and the control treatment, considering a level of significance of 0.05.

The FA profiles were assessed by determining total (mg/ind) or relative (%) FA concentrations.

One-way analysis of similarity (ANOSIM) was applied to determine differences in FA profiles of each species across the different treatments.

2.2.5. Fatty acid trophic markers

FA ratios of bacteria, algae or animal were assessed at the extracts of lipids of *C. edule*. The FA ratios determined and respective food sources are described in **Table 1**.

Marker	Source	Reference
DHA/EPA	Dinoflagellates/diatoms, carnivory	[16, 64, 67]
EPA	Diatoms	[16, 64–65]
DHA	Carnivory, dinoflagellates	[16, 64, 66–67, 70]
18:1n9	Carnivory	[66, 70]
18:2n6	Carnivory	[66, 70]
Σiso and anteiso C15 and C17	Bacteria	[68, 69]

Table 1. Dietary and trophic fatty acid markers used in the present study.

3. Results

3.1. Bioassays

3.1.1. Thalassiosira weissflogii

After 96 h of exposure to a range of salinity concentrations and three different temperatures, significant statistical differences were observed between the control and the lower salinity concentrations with an exception to the bioassay conducted at 20°C where a significant statistical difference was also observed to the highest salinity treatment (**Figure 2**). At the lowest temperature (15°C), the microalga presented the lowest growth than at higher temperatures. Still, to salinities near the optimal value of salinity to this microalga (30), the growth was high at all temperatures tested, not observing statistical significant differences between those concentrations and the control (**Figure 2**).

Considering the exposure to both contaminants (the herbicide and the metal), *T. weissflogii* showed to be more sensitive to Primextra®Gold TZ than to copper (**Figure 3**).

A significant growth inhibition was detected after the exposure to both contaminants with the herbicide revealing to be more toxic than the metal. In fact, all treatments of the herbicide showed statistical significant differences with the control, whereas to copper, only the three higher concentrations presented statistical significant differences with the control.

3.1.2. Cerastoderma edule

Considering the optimality of salinity for the activity of *C. edule* is 20–25, the species revealed to be mostly affected by low salinities (LC_{50} = 11.01 (10.66–11.54) mg/L) with 100% of mortality at salinity concentrations below 10. Although the growth inhibition of *T. weissflogii* to lower salinity concentrations, the microalga demonstrated to be more tolerant than *C. edule*.

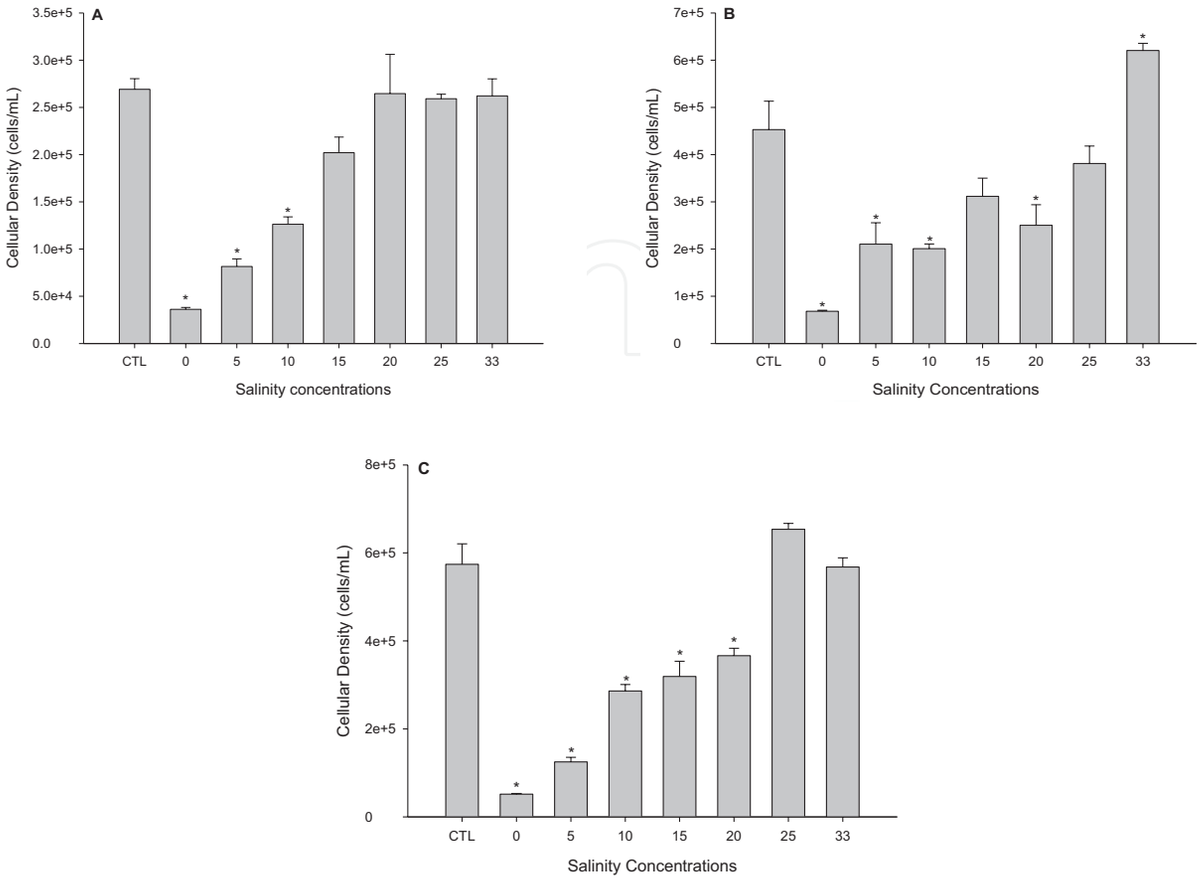

Figure 2. Cell density of *T. weissflogii* at (a) 15, (b) 20 and (c) 25°C after 96 h of exposure to salinity treatments, where CTL refers to the negative control treatment. Symbol '*' indicates a significant ($P < 0.05$) difference of the treatments compared to the CTL.

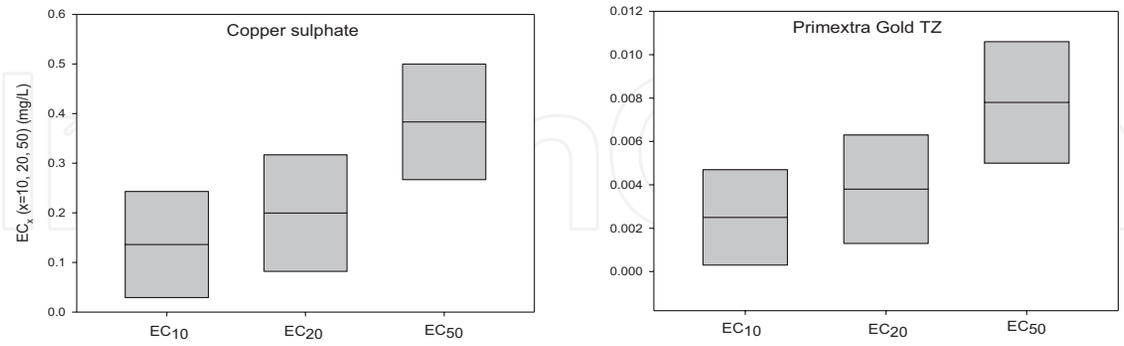

Figure 3. Selected EC_x (x = 10, 20, 50) values (mg/L^{-1}) estimated for *Thalassiosira weissflogii*. The central band of each box denotes the EC_X (x=10, 20, 50) value and the bottom and top of the box represent the lower and upper 95% confidence limits.

In **Table 2**, there are the lethal concentration (LC) values determined to *C. edule* exposed to the herbicide and the metal. The results clearly revealed that estuarine bivalve is more sensitive to the metal than to the herbicide, showing an opposite pattern of the one observed with *T. weissflogii*.

	Copper (II) sulfate pentahydrate (mg/L)	Primextra® Gold TZ (mg/L)
LC_{10}	0.341 (0.000; 0.571)	21.298 (11.008; 25.222)
LC_{20}	0.504 (0.083; 0.698)	23.868 (16.271; 27.422)
LC_{50}	0.818 (0.595; 0.987)	28.784 (24.731; 33.238)

Table 2. Lethal concentration (LC) values to *C. edule* exposed to copper sulfate and Primextra® Gold TZ. In brackets are indicated the 95% confidence limits.

3.2. Fatty acid profiles

3.2.1. Thalassiosira weissflogii

3.2.1.1. Salinity experiments

The fatty acid composition (mg/individual) of *T. weissflogii* exposed to three salinity treatments (10.46, 15.9, 18.5) plus the control is summarized in **Table 3** (15°C), **Table 4** (20°C) and **Table 5** (25°C). After the saline exposure to 15°C, the FA composition of *T. weissflogii* is varied in general with a significant decrease in MUFA and PUFA, especially at the salinity treatments of 10.46 and 15.9. The amount of SFAs in the control treatment is very low compared to other treatments. The saline treatment of 18.5 registered a drastic increase in the abundance of SFA compared to all treatments, with the control registering the lowest amount of saturated FA. MUFA was the dominant FA group in the FA profile of the marine diatom, with a decrease at the saline treatment of C2 (15.9), followed by C1 (18.5) and then C3 (10.46). In fact, the control at 15°C was the treatment that registered the highest amount in MUFA from the three temperatures tested. A dominance of LC-MUFAs is notorious, although C20:1n9(cis11) was absent at the control and at the concentration C1 (18.5). PUFA also displayed a high abundance at the control and at C1 (18.5), with a decrease at the concentrations C2 (15.9) and C3 (10.46). In fact the total amount of PUFA at C1 treatment was almost twofold of the quantity registered at the control. Important precursors of LC-PUFA such as C18:2n6c were presented only in the control and in the salinity concentration of 15.9, whereas C18:3n3 was presented in all treatments, with a residual quantity (closely zero) at the salinity treatment of 10.46. This FA group showed to be the most sensitive to salinity. HUFAs were presented at highest quantity at the extreme salinity treatments (C1 = 18.5; C3 = 10.46) with the control and the C2 treatment (15.9) registering the lowest values. All EFAs (ARA, EPA, DHA) had a similar pattern after saline exposure: their level remained very close in each concentration apart from EPA that registered the lowest value of the HUFA determined at all treatments. DHA was the most abundant EFA in the control treatment and at the salinity treatment of 18.5.

At 20°C, the FA profile of *T. weissflogii* exposed to the three saline treatments (15, 30.5 and 32) plus the control reported an increase in SFA content from the control to the lowest salinity concentration (15), although with relatively low diversity, had been notorious for higher amount of SFA of longer chain (**Table 4**). A significant rise was observed at the PUFA and HUFA contents at the lowest salinity treatment (15). An opposite trend was identified at the total amount of MUFA to all tested treatments when compared to the control. The salinity

	CTL	±Std. error	C1 (18.5)	±Std. error	C2 (15.9)	±Std. error	C3 (10.46)	±Std. error
C14:0			54.9161	21.8215				
C15:0								
C16:0	32.4438	7.8688			32.6365	13.7101		
C17:0								
C18:0								
C20:0			808.2884	321.1812			24.9692	10.8120
C21:0			803.2505	162.62326	467.5087	87.8701	195.8909	38.09074
C22:0								
C23:0			1207.2149	479.6986				
C24:0	75.8923	18.4066	395.4571	157.1387				
Total SFA	108.3361	26.2754	3269.1270	1142.4632	500.14525	101.5802	220.8601	48.9027
C14:1	52.5108	12.7357						
C15:1								
C16:1	34777.3500	1912.2145	15868.4131	3200.9993	9562.9247	3989.8029		
C17:1	49.0293	2.9871	1262.8013	339.8028	10046.7004	4146.2759	427.3974	99.6523
C18:1n9t	34.7431	1.4553	359.6901	93.3756	36.7104	8.8740	2.1760	0.9422
C18:1n9c	48.2467	0.3976	417.0501	117.3960	45.6232	4.9456	215.0315	84.0745
C20:1n9(cis-11)					28.5713	12.0023	255.5463	110.6548
C22:1n9	82.6656	20.0494	570.6772	172.9728	84.4090	10.3481	484.0046	109.6170
C24:1n9	316.5629	39.3603	425.4274	169.0477	139.9360	58.7849	116.9618	29.7734
Total MUFA	35361.1083	1989.1999	18904.0593	4093.5942	19944.8750	8231.0337	1501.1175	434.7142
C18:2n6t	212.8497	26.0495	400.9721	79.7362	19.2169	8.0727	6.9028	2.9890
C18:2n6c	21.1455	5.12853			73.1763	30.7402		

	CTL	±Std. error	C1 (18.5)	±Std. error	C2 (15.9)	±Std. error	C3 (10.46)	±Std. error
C20:2cis(11-14)	4.7466	1.15123	309.2416	110.9660	42.2756	6.8694	23.8036	10.3073
C22:2cis(13-16)			23.3495	9.2782			33.5178	14.5136
C18:3	111.1073	26.9475			22.6410	9.5111	32.1885	8.5154
C18:3n3	83.7401	20.3000	83.5544	33.2012	27.8042	11.6801		
C20:3n3			4462.2590	1751.2907	99.5302	37.7730	95.8512	23.9086
Total PUFA	433.5892	79.5867	817.1177	233.1816	185.1140	66.8735	96.4128	9.0813
C20:4n6 (ARA)	32.0602	7.7756	693.0573	265.71303	55.3162	7.4896	141.8455	27.7393
C20:5n3 (EPA)	42.1809	10.2304	175.5987	57.4274	56.2248	23.6191	64.45161	27.9084
C22:6n3 (DHA)	110.8065	13.1428	787.9480	313.0988			110.4819	47.8401
Total HUFA	185.0476	31.1488	1656.604	636.23923	111.541	31.1087	316.77901	103.4878
N	19		21		18		17	

Table 3. Abundance of fatty acids (saturated fatty acids—SFA, monounsaturated fatty acids—MUFA, polyunsaturated fatty acids—PUFA and highly unsaturated fatty acids—HUFA, in mg/ind) in the profile of *T. weissflogii* after exposure to salinity treatments (10.46; 15.9; 18.5) at 15°C.

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	CTL	±Std. error	C1 (32)	±Std. error	C2 (30.5)	±Sth. error	C3 (15)	±Std. error
C14:0							524.4776	198.2339
C15:0								
C16:0					185.5345	44.1380	1299.3503	491.1083
C17:0								
C18:0								
C20:0			10.7682				171.8261	64.9442
C21:0	43.9605	11.4958	51.9012	11.4958			143.5637	33.9657
C22:0	31.5296	15.7648	45.9347	15.7648	8.3243	2.9431	6.1552	2.3265
C23:0	67.8728	21.6031	45.6180	21.6031			35.3845	13.3741
C24:0			10.0689		12.5133	4.4241	60.0374	22.6920
Total SFA	143.3628	48.8636	164.2909	48.8636	206.3721	51.5052	2240.7950	826.6445
C14:1								
C15:1								
C16:1	32041.6830	480.9976	10230.4003	480.9976	28290.2142	1943.4874		
C17:1	89.0514	13.4534	27.1444	13.4534	8.4810	2.9985	21.0489	7.9558
C18:1n9t					58.9215	13.1667004	197.3032	74.5736
C18:1n9c	386.1857	112.0796	76.3323	112.0796	25.4705	1.9659	390.8133	131.4833
C20:1n9(cis-11)			95.0662				1704.3170	629.4505
C22:1n9	176.2199	7.9133	278.4564	7.9133	78.0943	2.8059	989.9304	324.5449
C24:1n9	14.1459	7.0729	238.0316	7.0729			156.5543	59.1720
Total MUFA	32707.2860	621.5169	10945.4313	621.5169	28461.1817	1964.4245	3459.9672	1227.1800
C18:2n6t	201.34907	58.6126	67.9254	58.61256	32.2866	1.7356	198.9214	59.3448
C18:2n6c								

	CTL	±Std. error	C1 (32)	±Std. error	C2 (30.5)	±Sth. error	C3 (15)	±Std. error
C22:2cis(13-16)			155.7379		31.9686	2.1246	116.8187	39.0555
C18:3					5.7043728	2.01680		
C18:3n3			79.5902				11.1572	4.2170
C20:2cis(11-14)	30.5477	15.2738	32.7481	15.2738	1.2155	0.4298	354.8936	131.3944
C20:3n3	181.6203	49.4586	108.7435	49.4586	47.9214	8.4011	20.5600	4.1581
C20:4n6	147.5854	21.7236	122.3678	20.5766	46.5139	5.8593	1731.6336	848.9185
Total PUFA	561.1024	145.0686	567.1130	143.9215	165.6103	20.5672	2433.9846	1087.0884
C20:4n6 (ARA)	147.5854	19.15840	122.3678	19.1584	46.5137	5.2815	1731.6336	632.7465
C20:5n3 (EPA)			182.5960		41.0818	6.0011	1318.1238	452.1425
C22:6n3 (DHA)					11.1902	3.9563	65.5965	24.7931
Total HUFA	147.5854	19.15840	304.9637	19.1584	98.7860	15.2390	3115.35390	1109.6821
N	15		22		20		25	

Table 4. Abundance of fatty acids (saturated fatty acids—SFA, monounsaturated fatty acids—MUFA, polyunsaturated fatty acids—PUFA and highly unsaturated fatty acids—HUFA, in mg/ind) in the profile of *T. weissflogii* after exposure to salinity treatments (15; 30.5; 32) at 20°C.

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	CTL	±Std. error	C1 (23.76)	±Std. error	C2 (22.59)	±Std. error	C3 (14.79)	±Std. error
C14:0			1170.3476	361.1771				
C15:0	15.8093	6.2820						
C16:0			265.4131	81.9083	91.3424	55.9356		
C17:0								
C18:0			56.2356	17.3547				
C20:0	14.7009	2.9211	51.8891	16.0133				
C21:0	34.1393	9.4712	58.1041	17.9313	52.5980	32.2000	15.8881	11.2346
C22:0	133.0046	21.8387	301.2867	92.9791				
C23:0	324.9469	59.3290	716.0168	156.4173	16.1034	4.9800		
C24:0	144.2169	32.1972						
Total SFA	666.8180	132.0393	2619.2930	743.78106	160.0438	93.1342	15.8881	11.23460
C14:1								
C15:1								
C16:1	3920.3316	1557.7818			10178.9007	3313.9257		
C17:1								
C18:1n9t			58.3992	18.0224				
C18:1n9c			106.4004	15.3800	37.1824	22.76945		
C20:1n9(cis-11)	23.9182	4.8087	56.05451	17.2988				
C22:1n9	283.4383	26.872	1218.6964	295.9116	31.2481	9.9562	77.1268	11.6267
C24:1n9	136.0564	41.155	440.7829	105.7048				
Total MUFA	4363.7445	1877.394	1880.3334	518.1971	3415.7770	5184.6099	77.1268	14.7068
C18:2n6t	89.4747	26.9962	261.2764	33.1211				
C18:2n6c								

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	CTL	±Std. error	C1 (23.76)	±Std. error	C2 (22.59)	±Std. error	C3 (14.79)	±Std. error
C20:2cis(11-14)	36.7421	10.5830	163.4404	33.0000				
C22:2cis(13-16)	78.7990	20.2553	383.4063	79.1263				
C18:3	9.3324	3.7083	39.3623	12.1475				
C18:3n3	38.6596	7.7462	106.3339	32.8154				
C20:3n3	252.3527	53.0700	1185.6409	323.0043				
Total PUFA	505.3605	122.3691	2139.4602	513.3264				
C20:4n6 (ARA)	344.5002	55.6143	640.6545	135.8612	12.2297	7.4891	18.2719	4.7866
C20:5n3 (EPA)	185.2005	7.6715	384.7258	88.5744				
C22:6n3 (DHA)	91.9278	5.9549	513.8760	103.9311				
Total HUFA	621.6286	69.2407	1539.2573	328.3666	12.2297	7.4891	18.2719	4.7866
N	19		21		7		3	

Table 5. Abundance of fatty acids (saturated fatty acids—SFA, monounsaturated fatty acids—MUFA, polyunsaturated fatty acids—PUFA and highly unsaturated fatty acids—HUFA, in mg/ind) in the profile of *T. weissflogii* after exposure to salinity treatments (14.79; 22.59; 23.76) at 25°C.

concentration C2 (30.5) registered a sharp decrease in the total amount of MUFA, PUFA and HUFA when compared to the control. MUFA constituted the most representative group with the highest amount of FA in each treatment.

Comparing to the salinity experiments conducted at 15°C, the control treatment at 20°C presented the highest content of SFA and PUFA, with the control at 15°C registered a higher amount of total MUFA and HUFA. Comparing the salinity treatments tested, the highest quantity of SFA, PUFA and HUFA was observed at the salinity concentrations C1 (18.5) and C3 (15) to the experiments performed at the temperature of 15 and 20°C, respectively. The total MUFA registered the highest values at the middle concentrations (C2 = 15.9; C2 = 30.5) of the experiments conducted at 15 and 20°C correspondingly.

At the FA profiles of *T. weissflogii* exposed to the salinity treatments at 25°C, a sharp decreased of total SFA from the control to the other treatments was observed with the exception of salinity concentration of 23.76, where a great increase was observed (**Table 5**). In general, MUFA constituted the most abundant group of FA, mainly represented by longer chain MUFAs, except at the highest salinity treatment (23.76). Still this salinity concentration registered the highest quantity of PUFA from all salinity experiments conducted at distinct temperatures (15, 20 and 25°C). PUFA content was only observed in the control and at salinity concentration C3 (23.76). C18:3n3 was present in the higher salinity treatment and in low amounts at the control, which may indicate that these LC-PUFA precursors were desaturated and elongated in the synthesis of HUFA. PUFA was characterized by a great diversity, still absent at the lower salinity treatments (14.79 and 22.59). HUFA registered the highest amount in the highest saline concentration (23.76) followed by the control treatment, decreased drastically at the other salinity treatments. Also, the control that registered the highest amount of HUFA was the one performed at 25°C. EFAs are mainly represented by ARA that were identified at all treatments. In the negative control, ARA is the most abundant EFA followed by EPA and lastly by DHA. Indeed, EPA and DHA were absent in the two lowest salinity treatments.

The FA content of *T. weissflogii* at the different salinity concentrations plus the control showed sharp changes among all treatments at 25°C than at the other temperatures (15 and 20°C). Furthermore, a great decrease in FA diversity was observed in C2 and C3 salinity treatments at the experiment conducted at 25°C, whereas the experiments conducted at 15 and 20°C presented higher or similar diversity of FA among the treatments, which indicate a detrimental effect of salinity in the FA content of the species.

3.2.1.2. Pollutants experiments

The results obtained demonstrated the fatty acid profiles of the microalga were affected by the presence of both toxicants, mainly by the metal (**Table 6**). Although it was not detected clear differences among the treatments, moderate changes were observed at the highest herbicide concentration compared to the other treatments. Small changes at the total amount of SFA and MUFA were also registered between the control and the highest concentration (0.0078 mg/L) of Primextra.

	Primextra® Gold TZ (mg/L)				Copper (mg/L)			
	CTL	**0.0025**	**0.0038**	**0.0078**	**CTL**	**0.1361**	**0.1995**	**0.3834**
% total SFA	42.51	41.72	41.18	44.51	48.85	42.16	43.30	54.44
% total MUFA	15.03	14.63	16.03	16.46	15.17	17.70	16.81	14.96
% total PUFA	25.95	29.03	27.48	26.70	21.48	23.21	23.14	18.02
% total HUFA	16.48	14.62	15.30	12.32	14.49	16.93	16.75	12.59
EPA	13.69	12.16	12.71	10.32	12.33	14.53	14.33	10.59
DHA	2.79	2.46	2.59	2.00	2.16	2.40	2.42	2.00
N	18	18	18	18	18	18	18	18

Table 6. Total fatty acid and EFA (%) content in *T. weissflogii* after the exposure to the herbicide Primextra® Gold TZ and the metal copper.

A marginal decrease of SFA from the CTL to the copper concentration of 0.1995 mg/L, with a rise at the highest copper treatment was observed. An opposite trend was verified to the unsaturated fatty acids (MUFA, PUFA and HUFA). A slightly rise in the total amount of the UFA was registered from the CTL to the metal concentration of 0.1995 mg/L followed by a decrease at the highest treatment. Although significant differences were not registered at the fatty acid content between the control and the lowest copper treatments, clear differences between the control and the highest copper concentrations were verified.

The amount of HUFA in herbicide and copper treatments was related to the presence of DHA and EPA, with ARA being absent at all treatments. EPA was the dominant EFA at all treatments (**Table 6**).

3.2.2. Cerastoderma edule

3.2.2.1. Salinity experiments

A considerable increase in the main FA groups (saturated fatty acids—SFA and unsaturated fatty acids—UFA) was clearly observed in the individuals from the field to the organisms exposed to a range of salinity concentrations, unless to polyunsaturated fatty acid (PUFA) where an opposite trend was verified (**Table 7**). Individuals from the field were mostly constituted by PUFA (95.773%), presented lower amounts of SFA (3.314%), HUFA (0.708%) and MUFA (0.205%). Under a range of salinity concentrations, a lacking of SFA of short chain was clearly observed in all organisms, with only the individuals exposed to the highest salinity treatment presented slightly amounts of C6:0, C8:0 and C10:0. Omega-6 was mainly represented by γ-linolenic acid, C18:3n6 and arachidonic acid (ARA), C20:4n6, whereas omega-3 occurred mainly at the forms of docosahexaenoic acid (DHA), C22:6n3, eicosapentaenoic acid (EPA), C20:5n3 and α-Linolenic acid (ALA), C18:3n3.

	Salinity concentrations					
	Field	**10**	**15**	**25**	**30**	**35**
% total SFA	3.314	26.561	31.434	19.843	59.711	30.511
% total MUFA	0.205	32.280	28.336	15.043	14.606	19.847
% total PUFA	95.773	25.130	16.162	44.712	4.095	42.821
% total HUFA	0.708	16.030	24.068	20.401	21.588	6.821
ARA	0.000	0.000	3.849	5.401	9.963	3.245
EPA	0.117	14.835	15.468	7.888	7.838	2.988
DHA	0.591	1.195	4.750	7.112	3.786	0.588
N	28	21	15	15	15	22

Table 7. Total fatty acid and EFA (%) content in *C. edule* in the field and after the exposure to a range of salinity treatments.

3.2.2.2. Pollutants experiments

A slightly increase at all FA groups was observed in the organisms exposed to the herbicide compared with the individuals from the field (**Table 8**). An opposite pattern was found in the organisms exposed to copper where a slender decrease in SFA (at the first metal treatment), PUFA and HUFA was observed compared to the individuals from the field. Comparing the HUFA content in the organisms exposed to both pollutants, a slightly increase was observed at the herbicide treatments with a reduction at the copper concentrations. DHA was the EFA occurring in higher amount after the exposure to commercial formulation of the herbicide. In the treatments exposed to the metal, a clear pattern was not observed. In general, there was a higher diversity in FA in the individuals exposed to Primextra compared to the organisms under copper treatments, with exception to the highest copper concentration that registered the highest diversity of FA (**Table 8**).

	Primextra® Gold TZ (mg/L)								**Copper (mg/L)**				
	Field	**CTL**	**0.5**	**2.5**	**5**	**10**	**20**	**30**	**Field**	**CTL**	**0.6**	**0.9**	**1.2**
% total SFA	0.074	0.129	0.204	0.0744	0.155	0.130	0.165	0.098	0.016	0.001	0.005	0.052	0.077
% total MUFA	0.019	0.041	0.057	0.023	0.039	0.036	0.039	0.023	0.025	0.030	0.029	0.029	0.031
% total PUFA	0.051	0.108	0.061	0.049	0.066	0.054	0.069	0.048	0.153	0.042	0.078	0.086	0.121
% total HUFA	0.113	0.187	0.258	0.110	0.221	0.196	0.224	0.150	0.116	0.084	0.159	0.144	0.075
EPA	0.047	0.084	0.095	0.050	0.069	0.075	0.070	0.062	0.084	0.042	0.094	0.074	0.034
DHA	0.067	0.102	0.164	0.061	0.156	0.121	0.154	0.088	0.032	0.041	0.065	0.070	0.040
N	11	12	15	12	11	13	13	12	11	9	12	12	16

Table 8. Total fatty acid and EFA (%) content in *C. edule* in the field and after the exposure to a set concentrations of the herbicide Primextra® Gold TZ and the metal copper.

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3.2.2.3. Fatty acid trophic markers (FATMs): Cerastoderma edule

In **Table 9**, the FA composition of the food (rotifers and microalgae) used daily to feed *C. edule* in the lab is represented. Microalgae food source presented a highest richness in PUFA whereas rotifers showed higher composition in SFA.

FATM ratios indicated an omnivorous diet with the organisms ingesting phytoplankton and zooplankton, with some of the individuals in the lab and at the field consuming higher amounts of phytoplankton (diatoms). Still some organisms from the field also showed a diet based mainly on zooplankton with few also feeding on bacteria.

	Food source	
	Microlagae	**Rotifera**
% total SFA	7.7	62.7
% total MUFA	3.0	23.0
% total PUFA	86.5	9.0
% total HUFA	3.0	5.4

Table 9. Fatty acid composition of food source used daily to feed *Cerastoderma edule* in the lab.

4. Conclusion

In general, an organism under stress conditions may change its physiological and biochemical responses as a strategic mechanism to compensate the organism's requirements [20]. In this study, the species from different trophic levels (a marine microalgae and estuarine bivalve species) exposed to a range of salinity concentrations under distinct temperature conditions and under different treatments of a metal (copper) and a herbicide (Primextra) revealed changes in its fatty acid content. The results obtained confirmed that the environmental and chemical stressors affect the fatty acid profile of aquatic species with sharp changes in the FA content of these species and reflecting then in lower quality food. It was clear that higher temperature had a great impact on the FA composition of the microalga, with the diatom not presenting several FA mainly PUFA, DHA and EPA in its profile, making the microalga more vulnerable to the effects of different salinities, mainly under salinity concentrations lower than 22.59. In the salinity treatments, an increase of SFA and MUFA was also observed to both studied species which can indicate that PUFA and HUFA are being metabolized so that the cells can obtain more energy necessary to maintain homeostatic ionic balance while maintaining basal functions of the body, such as respiration and excretion of ammonia. Since synthesizing of PUFAs and HUFAs becomes too energetically costly, the organism may not complete the elongation processes presenting higher concentrations of SFAs and MUFAs in the FA composition. The increase in saturation level can also be explained by a cellular response toward the osmotic shock, an attempt to maintain the stability of lipid membranes [49] and as such maintain the osmotic pressure from cell damaging. A low concentration of HUFA in general may also occur due to the high sensitiveness of this FA group to environmental fluctuations and to cell stress.

In literature studies, the most characteristic FA of diatoms are included in SFA and MUFA group and are characteristic of the plant domain: C14:0, C16:0, C16:1, C18:4 and C20:5, precursors for LC-PUFA such as EPA and DHA, while in turn most diatoms are reported to be very poor in C18:2 and C18:3 [50, 51]. In the present study, the absence of most of these FA at both species with a wide lacking of SFA is clearly shown. Fisher [52] stated *Thalassiosira pseudonana* Hasle & Heimdal was dominated by the FA reported above, although in different relative amounts. Fisher [52] also observed that the concentration of C16:0 varied with the culture age and that C16:1 declined drastically in the dark. The LC-PUFA content of microalgae can indeed depend not only on the species, but also on factors related to culture condition including composition of the medium, aeration, light intensity, temperature and age of culture [53], being crucial algae be maintained under its optimality of growth conditions.

In bivalve species, the reproductive success is related to the presence of great amount of lipids that are the second major constituent of bivalves' eggs [54, 55]. The maturation of the germ cell is closely related to the FA C18:3n3, C18:4n3, C20:1n9 and C20:2n6, while C14:0, C16:0, C16:1n7, C18:1n7 and C18:1n9 play a key role at the embryonic development of the bivalve eggs [54, 56]. Thus, it is crucial the presence of these FA during the breeding and thus in the FA profiles of the individuals.

The low concentration of HUFA reported in the lowest salinity treatments (22.59 and 14.79) at 25°C to *T. weissflogii* and to *C. edule* exposed to the metal may translate a metabolic response from the cells, which cannot efficiently maintain the cellular homeostatic ionic balance and cannot use energetic reservations in the elongation and synthesis of LC-PUFA such as EPA (C20:5n3) and DHA (C22:6n3). In the salinity treatments, EFAs of both studied species (the diatom and the bivalve) were mainly represented by ARA that is an important precursor of signalling molecules including prostaglandins, prostacyclins and thromboxanes [57]. DHA was the EFA that presented the lowest concentration or was absent in all salinity treatments at the highest temperature (25°C) and also under the exposure to both pollutants of the diatom species. A similar pattern was reported to the bivalve species exposed to saline and copper concentrations. This EFA is crucial in neurophysiologic processes and influences visual acuity once is abundant in retina cells, as well as a preventive role in cardiac diseases being also presented in high amount in the brain tissues [4, 16]. In fact, a high concentration of EFA such as the combination DHA + EPA is linked to the reduction of coronary heart diseases. These two FA molecules also show a key role in bipolar disorder and other neurological dysfunctions, cognitive functions and fetal development. They are associated with benefits in the treatment of rheumatoid arthritis and inflammatory bowel disease, as well as for Crohn's disease, which is related with the suppression of ARA-derived eicosanoids [4]. Once EPA and ARA play an important role in mediating immunological responses to infections and regulating ion and water flux, a low or absent content of such EFAs can translate into alterations in membrane phospholipids once ARA and DHA components can influence cellular signalling but also sharply alter many membrane physical properties such as fluidity and bilayer thickness, among others. In humans, a deficiency of DHA affects neurotransmission, membrane-bound enzyme and ion channel activities, intensity of inflammation and immunity, all of

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these associated with normal aging, Alzheimer disease, hyperactivity and schizophrenia [13]. These EFAs (EPA and ARA) are also associated to the further improvement of adaption of the individuals to anthropogenic and environmental stress conditions [58]. Furthermore, C18:3n3 (α LNA) is associated with the processes of elongation and desaturation at the synthesis of EPA and DHA in mammals, whereas C18:2n6, C20:4n6 and C18:3n6 are involved in the biosynthesis of PUFA of long chain [59].

The level of essential fatty acids (n-3 EFA) in algae can be highly variable, [60], including among major taxa [61], making it hard to compare between phylogenetic close species. PUFAs and HUFAs can reach their highest content during periods of rapid cell growth or bloom episodes [62] and are important components of the microalgae membranes that affect cell membrane fluidity [3] that promotes a rapid response to environmental changes, such as variations in temperature, light and pH. Thus, HUFAs are the most affected FA group in case of cellular damage due to failed osmoregulation. The reduction of EFA content, mainly in the base of the trophic food web, may have serious implications at higher levels in the food web, once the compromising of the nutritious value of the primary producers influences the uptake of EFAs and thus the fundamental processes of regulation of many species in the ecosystems. Some of these species are the food source of human beings, having the climate events in larger scale a great impact at the biochemical values of nutritional requirements of many aquatic species and thus at human health. Thus, a balanced fatty acid profile is essential, with a balanced amount of essential fatty acids (EFAs) and of other fatty acids with a key role in the regulation and functioning of the organisms.

Fatty acid proves to be a useful bio-indicator of ecological and healthy status of aquatic ecosystems, providing crucial information about the impacts of global stressors in the aquatic communities and thus in the trophic food web with severe repercussions to human beings and food quality. Recent reports predict the occurrence, at the next 100 years, of changes in salinity seawater, rise in temperature and water acidification [63]. In addition to these climate changes, an intensive agriculture production with an excessive usage of fertilizers and pesticides near coastal wetlands will have severe impacts to the aquatic communities and thus to the ecosystem. Therefore, it is of major importance and becomes a priority to determine and predict the effects of environmental and anthropogenic stressors in the aquatic systems in order to maintain the healthy status and the biodiversity and thus the food quality.

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