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Effects of an experimental heat wave on fatty acid composition in two Mediterranean seagrass species

Pedro Beca-Carretero[a,*], Freddy Guihéneuf[a], Lázaro Marín-Guirao[b,c], Jaime Bernardeau-Esteller[b], Rocío García-Muñoz[b], Dagmar B. Stengel[a], Juan M. Ruiz[b]

[a] Botany and Plant Science, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland
[b] Seagrass Ecology Group, Oceanography Centre of Murcia, Spanish Oceanography Institute, Spain
[c] Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

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ABSTRACT

Global warming is emerging as one of the most critical threats to terrestrial and marine species worldwide. This study assessed the effects of simulated warming events in culture on two seagrass species, *Posidonia oceanica* and *Cymodocea nodosa*, which play a key role in coastal ecosystems of the Mediterranean Sea. Changes in fatty acids as key metabolic indicators were assessed in specimens from two geographical populations of each species adapted to different *in situ* temperature regimes. Total fatty acid (TFA) content and composition were compared in *C. nodosa* and *P. oceanica* from natural populations and following exposure to heat stress in culture. After heat exposure, individuals of *C. nodosa* and *P. oceanica* adapted to colder temperatures *in situ* accumulated significantly more TFA than controls. For both species, the proportion of polyunsaturated fatty acids (PUFA) decreased, and the percentage of saturated fatty acids (SFA) increased significantly after the heat treatment. These results highlight that populations of both species living at warmest temperatures *in situ* were more thermotolerant and exhibited a greater capacity to cope with heat stress by readjusting their lipid composition faster. Finally, exposure of seagrasses to warmer conditions may induce a decrease in PUFA/SFA ratio which could negatively affect their nutritional value and generate important consequences in the healthy state of next trophic levels.

1. Introduction

Anthropogenic activity has rapidly increased greenhouse gasses concentration in the atmosphere over the last decades. Global warming is a direct consequence of such gas emissions and has emerged as a threat for terrestrial and marine species worldwide (Cheung et al., 2009; Pounds et al., 2006). Over recent years, anomalous summer heat waves have been recorded from the Mediterranean Sea, with sea water temperatures exceeding 28 °C in 2003 and 2006 (Marbà and Duarte, 2010). Marine heat waves are generally defined as prolonged discrete anomalously warm water events that can be described by their duration, intensity, rate of evolution, and spatial extent (Hobday et al., 2016). Projected environmental changes predict an increase in such extreme events in number and intensity over this century, with anticipated increases in average summer seawater temperatures by 4–5 °C (IPCC, 2014). These potential scenarios may alter the metabolism, growth and life cycle of marine foundation species (Pörter and Farrell, 2008; Hoegh-Guldberg and Bruno, 2010) and also the ecological interactions among associated communities, including seagrass systems (Post and Pedersen, 2008). With some native primary producers already living at their thermal upper limit, future climate change may compromise their survival, with dramatic effects on Mediterranean ecosystem functioning (Meehl and Tebaldi, 2004). Future projections estimate that Mediterranean ecosystems will experience the largest change in biodiversity worldwide resulting in conditions less favourable for native seagrasses and but more favourable for tropical species (Parry, 2000; Sala, 2000; Moschella, 2008).

Seagrasses are clonal marine plants dominating in tropical and temperate marine coastal ecosystems, providing ecologically relevant goods and services such as food, nursery habitats, sediment stabilization enhancing water quality and shoreline protection, and sequestration of atmospheric CO_2 into sediments (Orth et al., 2006; Nordlund et al., 2017). Their distribution is experiencing a global regression due to human impacts (Waycott et al., 2009), in particular since they prefer

Abbreviations: FA, fatty acids; EFA, essential fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; FAME, fatty acid methyl esters; SST, sea surface temperature

* Corresponding author.
E-mail address: p.becacarretero2@nuigalway.ie (P. Beca-Carretero).

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shallow and sheltered coastal areas which are threatened by global change. *Posidonia oceanica* and *Cymodocea nodosa* are the dominant seagrass species in coastal Mediterranean ecosystems with contrasting biological attributes and ecological strategies. The endemic *P. oceanica* is the most dominant species in infralittoral bottoms to a maximum depth of 25–40 m (Procaccini et al., 2003). It is a large, long-lived (Arnaud-Haond, 2012) seagrass with low growth and recovery rates (Duarte et al., 2006), making it particularly vulnerable to environmental disturbances. Declines in the extent of *P. oceanica* meadows have been reported elsewhere mainly caused by anthropogenic impacts of coastal development (Marbá et al., 2005; Boudouresque et al., 2009; Díaz-Almela et al., 2009). *C. nodosa* is the second most abundant seagrass in Mediterranean Sea, and its distribution ranges from the Iberian Peninsula to Senegal and Cape Verde. It is a species adapted to a wide range of coastal habitats with contrasting regimes of salinity, temperature and nutrients, from the open sea to semi-enclosed coastal lagoons. Compared to *P. oceanica*, it is smaller in size, with higher growth rates, high phenotypic plasticity and rapid recovery such as from temperature disturbances (Olesen et al., 2002; Duarte et al., 2006; Sandoval-Gil et al., 2014). In the summer of 2003, Mediterranean seagrass meadows were exposed to an extreme heat wave episode leading to reduced recruitment rates and hence increased mortality (Balearic Islands, Marbà and Duarte, 2010; Jordà et al., 2012) and inducing massive flowering (Diaz-Almela et al., 2007). Responses of *C. nodosa* to heat stress are less well studied but are rather variable and less severe than those of *P. oceanica* (Olsen et al., 2012; Marín-Guirao et al., 2016; Tutar et al., 2017).

Primary producers play a key role biosynthesising the majority of essential fatty acids (Behrens and Kyle, 1996; Dalsgaard et al., 2003) which are subsequently transferred to higher trophic levels. Lipid metabolism and fatty acid synthesis play an important role in the membrane structure and energy storage in plants and algae (Rabbani et al., 1998; Mendoza et al., 1999; Klyachko-Gurvich et al., 1999). Polyunsaturated fatty acids (PUFA) are mainly partitioned into structural lipids (glycolipids and phospholipids) constituting the cellular membranes, in particular the thylakoid membranes of chloroplasts, promoting their fluidity, the electron transport, and therefore the photosynthetic activity (Gombos et al., 1994; Sanina et al., 2008). By contrast, saturated fatty acids (SFA) are mainly partitioned into triacylglycerols (TAG) as storage compounds. Lipids and fatty acids have been largely studied in microalgae, seaweeds, lichens and terrestrial plants, however, to a lesser extent in marine plants (Khotimchenko et al., 2002; Sanina et al., 2004). In aquatic plants, fatty acid metabolism is strongly regulated by environmental factors (Viso et al., 1993) and changes in sea surface temperature can be expected to affect fatty acid composition of seagrasses (Lee et al., 2007). To date, fatty acids in seagrasses have been mainly evaluated as qualitative markers for marine trophic relationships between species (Auel et al., 2002).

At the base of the marine food web, primary producers represent the main source of food, as well as essential PUFA (Iken et al., 1998). Since seagrasses constitute an essential part of herbivores diet, future warming may affect their nutritional value, and therefore the health of potential grazers *via* dietary intake (Havelange et al., 1997). Recent studies showed that future scenarios of climate change may induce changes in seagrass leaf composition, such as pigments concentration, and generate changes in the herbivore diet preferences (Hernán et al., 2017; Beca-Carretero et al., 2017). Previous studies in marine primary producers observed an increase of PUFA levels with a decrease of temperature (Gosch et al., 2015; Schmid et al., 2017a). Hence, decreases in PUFA levels and in PUFA/SFA ratios are anticipated under future warming. Therefore, further detailed analyses of the role of fatty acid metabolism as potential adaptive mechanism of marine plants to heat stress and hence, to future climate change scenarios, are required. However, potential effects of stress caused by heat waves on lipid metabolism in seagrasses have not been evaluated experimentally.

In this study, the effects of simulated warming events were investigated in a mesocosm system to assess changes in key metabolic indicators, such as fatty acids, in specimens from two geographical (northern and southern) populations of *P. oceanica* and *C. nodosa* adapted to different *in situ* temperature regimes. Total fatty acid content and composition were determined after exposure to heat treatment for six weeks, and again following six subsequent weeks of recovery. Moreover, the variation in the proportion of PUFA and PUFA/SFA ratios in seagrasses from across their geographical distribution range in relation to *in situ* annual seawater surface temperatures (SST) were assessed. Finally, the ecological implications of changes in fatty acid composition under potential future warming scenarios in higher trophic levels are discussed.

2. Methods

2.1. Experimental setup

In June 2016, large fragments of rooted *Posidonia oceanica* and (Linnaeus) Delile, and *Cymodocea nodosa* (Ucria) Ascherson bearing apical growth meristems and a large number of connected shoots were collected by divers in two Mediterranean regions of Spain at 5–7 m depth: the northern populations were located in Catalonia (42° 06′23″N, 3° 10′ 16″E), such as *P. oceanica* in Cala Montgó and *C. nodosa* in Ebro Delta, and the southern populations in the Murcia region (37°34′20.8″N, 1° 12′28.1″W). These two regions are about 700 km apart, displaying substantial differences in SST of about 6 °C in summer

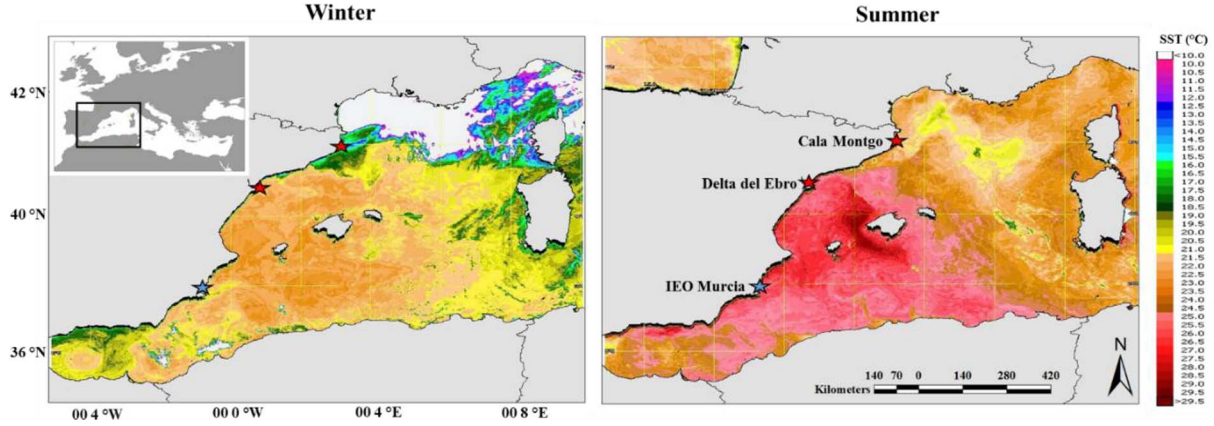

Fig. 1. Map of satellite-derived data of winter and summer sea surface temperatures (SST) (http://www.ieo-santander.net) in the western Mediterranean Sea with the location of the two sites where the samples were collected (red stars), two Catalonia, Cala Montgo and Delta del Ebro. IEO Murcia is the location where the experiments were conducted (blue star). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

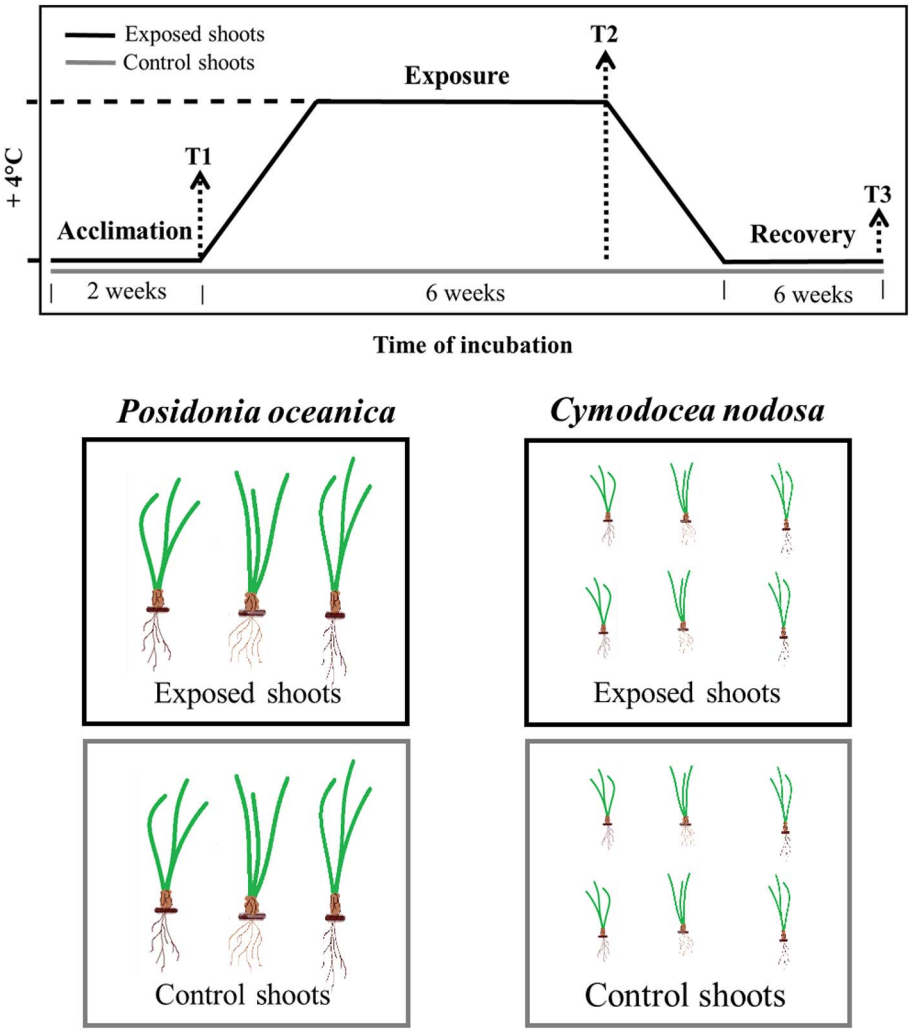

Fig. 2. Schematic of the experimental design. Exposed (black line) and control (grey line) of plants of *C. nodosa* and *P. oceanica*. T2 (exposed) and T3 (recovery) represent the time period when plants were collected for fatty acid (FA) analysis.

and up to 10 °C in autumn (Vargas-Yanez and Sabates, 2007, IEO data base, http://www.ieo-santander.net; pers. obs.; Fig. 1). Such temperature differences are sufficiently large to expect local adaptions of different populations. In the case of *P. oceanica*, the southern population was representing the warm-adapted population. By contrast, for *C. nodosa*, the (assumed) warm-adapted population was located in the northern region since the seagrass population was present at a shallower and more sheltered site (Ebro Delta) where temperatures in summer can reach 33 °C (southern population: 27–28 °C).

Plant fragments of similar size (20–25 cm length) with 15–20 interconnected shoots were transplanted into the mesocosm system based at the Oceanography Centre of Murcia (see Marín-Guirao et al., 2011 and Sandoval-Gil et al., 2014 for details) within 36 h after up-rooting. For each species, plant fragments were placed in plastic pots filled with coarse, cleaned of sediment and six plants from each of the two regions (Fig. 2) were randomly placed in one of twelve 500 L tanks: for each population (colder *vs* warmer site), three of the six tanks were randomly assigned to heat treatment (H) and the other three remained as controls (C). Four pots were positioned in each individual tank (n = 3).

Plants were acclimated for 2 weeks with the mean ambient conditions recorded at the sampling sites: salinity of 37.5 PSU and irradiance of 300–340 μmol photons $m^{-2} s^{-1}$ under a 14 h:10 h light:dark photoperiod using 500 W metal halide lamps. Mean ambient temperature was 23 °C for *P. oceanica* from the northern sites and 25 °C for southern *P. oceanica* and *C. nodosa* populations. After this acclimation period (T1), temperature was gradually increased (by 0.5 °C per day) in tanks with H treatment, to reach 4 °C above the control temperature. The duration of this exposition phase (T2) was six weeks, after which temperatures in H treatments were gradually returned to control values to allow plants to recover from heat stress for another six weeks (T3, recovery phase). The temperature treatment simulated the heat wave reported in Mediterranean Sea in 2003 (Marbá et al., 2010) and a heat wave recorded during the same year of the experiment using underwater sensors (data not available, pers. obs.). At the end of T2 and T3, samples were collected for fatty acid analysis.

2.2. Fatty acid analysis

On each sampling occasion (T2 and T3), fresh leaf biomass was collected from mature and healthy leaves of *P. oceanica* and *C. nodosa* for fatty acid analysis. Selected leaves were manually cleaned in distilled water and epiphytes or grazing marks removed. Fresh biomass was hand-dried on blotting paper before freezing for 48 h at − 30 °C. Subsequently, leaves were freeze-dried for 24 to 48 h using a freeze-dryer system (Labconco Freezone, Kansas City, MO, USA). Samples were kept at − 30 °C prior to analysis. Seagrass fatty acids were analysed using the protocol described below, which was previously applied to microalgae, algae and terrestrial plants (Miquel and Browse, 1992; Ventura et al., 2011; Schmid et al., 2014). Briefly, fatty acid methyl esters (FAME) were obtained by direct transmethylation of ~20–50 mg of the freeze-dried ground seagrass leaf biomass with dry methanol containing 2% (v/v) H_2SO_4. To avoid oxidation, vials with samples were closed under nitrogen gas and heated at 80 °C during 2 h with constant stirring. After transmethylation, 1 mL of mili-Q water was

Table 1
Total fatty acid content (% DW) and composition (% TFA) in northern and southern populations of *C. nodosa* exposed to control and heat wave temperatures, and their recovery. Results are expressed as mean ± SD (n = 5–6).

Cymodocea nodosa								
Location		Northern	Population			Southern	population	
Treatment	Exposure		Recovery		Exposure		Recovery	
Time	CNT	HW	CNT	HW	CNT	HW	CNT	HW
Fatty acids (% TFA)								
Saturated fatty acids								
14:0	1.1 ± 0.1	1.1 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	1.1 ± 0.04	1.2 ± 0.1	0.4 ± 0.03	0.4 ± 0.02
16:0	20.3 ± 0.6	23.4 ± 0.3	21.2 ± 0.4	21.7 ± 0.6	19.7 ± 0.3	22.0 ± 0.5	21.2 ± 0.4	21.2 ± 0.4
18:0	1.0 ± 0.04	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.1	1.0 ± 0.04	1.2 ± 0.1	1.2 ± 0.04
Sum of SFA	22.4 ± 0.5	25.4 ± 0.2	22.7 ± 0.5	23.4 ± 0.8	22.0 ± 0.3	24.2 ± 0.4	22.9 ± 0.5	22.6 ± 0.4
Monounsaturated fatty acids								
14.1	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.02	1.0 ± 0.1	0.8 ± 0.4	0.4 ± 0.1	0.9 ± 0.04	0.9 ± 0.04
16:1 n-7	0.4 ± 0.02	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.1	0.4 ± 0.03	0.2 ± 0.02	0.4 ± 0.04	0.4 ± 0.1
18:1 n-7	0.2 ± 0.03	0.3 ± 0.02	0.3 ± 0.04	0.3 ± 0.02	0.3 ± 0.02	0.2 ± 0.03	0.3 ± 0.04	0.3 ± 0.03
18:1 n-9	2.0 ± 0.3	2.6 ± 0.3	1.6 ± 0.1	1.9 ± 0.2	2.9 ± 1.1	2.4 ± 0.3	2.0 ± 0.1	2.2 ± 0.1
Sum of MUFA	3.2 ± 0.3	3.9 ± 0.3	3.1 ± 0.2	3.4 ± 0.3	4.4 ± 1.6	3.3 ± 0.3	2.9 ± 0.2	3.1 ± 0.3
Polyunsaturated fatty acids								
18:2 n-6	18.8 ± 0.3	19.5 ± 1.5	21.2 ± 0.4	18.0 ± 0.9	19.5 ± 0.9	21.4 ± 1.3	22.3 ± 0.9	22.1 ± 0.6
18:3 n-3	47.4 ± 0.5	41.8 ± 1.7	46.2 ± 1.6	47.2 ± 0.4	45.4 ± 2.0	43.0 ± 0.9	43.4 ± 0.9	43.4 ± 1.2
20:4 n-6	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.6	0.02 ± 0.1	0.1 ± 0.1	0.05 ± 0.1
20:5 n-3	1.2 ± 0.1	1.8 ± 0.7	1.3 ± 0.5	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.5	1.0 ± 0.1	1.1 ± 0.1
Sum of PUFA	67.6 ± 0.2	64.0.0 ± 0.4	67.6 ± 0.3	66.6 ± 0.5	66.5 ± 1.5	65.4 ± 0.6	66.8 ± 0.7	66.8 ± 0.6
n-3/n-6	2.6 ± 0.2	2.2 ± 0.1	2.2 ± 0.1	2.7 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.2
PUFA/SFA	3.0 ± 0.2	2.5 ± 0.3	3.0 ± 0.3	2.8 ± 0.2	3.0 ± 0.3	2.7 ± 0.2	2.9 ± 0.2	3.0 ± 0.3
Others	6.7 ± 0.3	6.7 ± 0.2	6.6 ± 0.3	6.6 ± 0.2	7.2 ± 0.2	7.2 ± 0.2	7.4 ± 0.3	7.5 ± 0.1
Fatty acids (%DW)	2.52 ± 0.2	2.60 ± 0.1	2.29 ± 0.2	2.16 ± 0.1	2.68 ± 0.1	2.85 ± 0.1	2.24 ± 0.05	2.13 ± 0.03

added and FAME were extracted from the cooled samples using 1 mL of *n*-hexane. Analysis of FAME was performed on an Agilent 7890A GC/5975C mass selective detector (MSD). Sample volumes of 2 µL were injected in split mode (split ratio 20:1). Hydrogen was used as a carrier gas. The injector and detector temperatures were 250 and 300 °C, respectively. The temperature was programmed at 140 °C for 1 min, raised from 140 to 200 °C by a rate of 15 °C min^{-1} and then from 200 to 250 °C at a rate of 2 °C min^{-1}. Identification of FAME was obtained by co-chromatography with authentic commercially available FAME standards (Supelco™ 37 Component FAME Mix, catalogue no. 47885-U, Supelco, USA) and FAME of fish oil (Menhaden Oil, catalogue no. 47116, Supelco). Total and individual fatty acid contents were quantified by comparison with a known amount of added pentadecanoic acid 15:0 (99%, catalogue no. A14664-09, Alfa Aesar, UK) as internal standard. The internal standard (20 µL, 5 mg mL^{-1}) was added prior direct transmethylation to the ground seagrass powder. Results are expressed as the mean values of 5–6 replicates (n = 5–6) for each treatment combination.

2.3. Latitudinal and in situ annual SST comparison

Data obtained from plants incubated at control conditions (T2) were used to compare % PUFA and PUFA/SFA ratios with previous findings of seagrasses across their latitudinal distribution range in relation to site-specific annual seawater surface temperatures. SST data for the latitudinal comparison were derived from the Bio-ORACLE database (http://www.bio-oracle.org) (Tyberghein et al., 2012) with a resolution of 0.5 km^2.

2.4. Statistical analyses

Prior to performing statistical analyses data were tested for normal distribution using the Kolmogorov-Smirnov test (Sokal and Rohlf, 1995) and homogeneity using Levene's test. Data that did not pass the test were transformed into Ln to ensure the assumption of normality (Draper and Smith, 1981), and homogeneity. To report responses to different temperature treatments *t*-test was applied to assess significant effects ($p < 0.05$). A one-way ANOVA and *post hoc* Tukey's pairwise test was conducted to evaluate differences between northern and southern populations, and between *C. nodosa* and *P. oceanica*. All data treatments and statistical analyses were performed using IBM SPSS Inc., v.13.

3. Results and discussion

3.1. TFA content and composition in Cymodocea nodosa and Posidonia oceanica

Overall, the average total fatty acid (TFA) content of *C. nodosa* (2.45 ± 0.3 mg g^{-1} DW) was always significantly higher than the one observed in *P. oceanica* (1.80 ± 0.3 mg g^{-1} DW) (*t*-test, $p < 0.001$). This could be explained by the ability of different species to store excess energy in the form of carbon, such as fatty acids partitioned and accumulated into TAG, as observed in some seaweed species. For example, species-specific acclimation potential was observed between two important Irish intertidal macroalgae, summer-acclimated *Fucus serratus* diverted excess energy into storage lipids (TAG), while *Palmaria palmata* was more sensitive and susceptible to degradation of its chloroplast membranes, resulting in a decrease in TFA, PUFA-rich polar lipids and pigments, and a release of free fatty acids (Schmid et al., 2017b). Of interest, in previous studies, Sandoval-Gil et al. (2014) reported that *C. nodosa* had higher concentrations of soluble sugars than *P. oceanica* when exposed to an increase in salinity; this could be interpreted as an adaptive trait of *C. nodosa* to cope with environmental stress. The higher carbohydrate content supports the very high capacity of *C. nodosa* to uptake carbon *via* photosynthesis, as indicated by the ^{13}C isotopic signal (Sandoval-Gil et al., 2014). Indeed, *C. nodosa* appears to be more tolerant than *P. oceanica* to environmental stress, in

Table 2
Total fatty acid content (% DW) and composition (% TFA) in northern and southern populations of *P. oceanica* exposed to control and heat wave temperatures and their recovery. Results are expressed as mean ± SD (n = 5–6).

Posidonia oceanica								
Location		Northern	population			Southern	population	
Treatment	Exposure		Recovery		Exposure		Recovery	
Time	CNT	HW	CNT	HW	CNT	HW	CNT	HW
Fatty acids (% TFA)								
Saturated fatty acids								
14:0	0.6 ± 0.1	0.7 ± 0.04	0.6 ± 0.03	0.6 ± 0.2	0.5 ± 0.04	0.6 ± 0.03	0.6 ± 0.1	0.6 ± 0.1
16:0	19.8 ± 0.6	20.4 ± 0.6	20.2 ± 0.1	20.3 ± 0.8	19.8 ± 0.4	21.3 ± 0.7	19.6 ± 0.05	19.7 ± 0.5
18:0	2.4 ± 0.1	2.6 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	2.4 ± 0.2	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.3
Sum of SFA	22.7 ± 0.8	23.8 ± 0.8	22.8 ± 0.7	22.9 ± 0.8	22.7 ± 0.6	23.9 ± 0.7	22.2 ± 0.6	22.2 ± 0.8
Monounsaturated fatty acids								
14:1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.04	0.4 ± 0.1	0.5 ± 0.04	0.5 ± 0.1	0.4 ± 0.05	0.4 ± 0.1
16:1 n-7	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.03	0.3 ± 0.04	0.4 ± 0.1
18:1 n-7	0.2 ± 0.01	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.02	0.1 ± 0.1	0.3 ± 0.03	0.3 ± 0.05	0.3 ± 0.02
18:1 n-9	4.4 ± 0.4	5.5 ± 0.4	4.7 ± 0.3	4.7 ± 0.5	4.4 ± 1.1	6.0 ± 1.1	5.8 ± 0.7	7.0 ± 1.9
Sum of MUFA	5.5 ± 0.3	6.5 ± 0.5	5.6 ± 0.4	5.7 ± 0.5	5.3 ± 1.1	7.0 ± 1.1	6.8 ± 0.8	8.0 ± 1.9
Polyunsaturated fatty acids								
18:2 n-6	18.3 ± 1.0	15.0 ± 0.7	18.6 ± 0.5	19.3 ± 1.3	17.7 ± 1.5	24.9 ± 7.8	20.1 ± 1.1	22.5 ± 1.9
18:3 n-3	47.4 ± 0.7	47.9 ± 1.7	47.2 ± 0.8	46.4 ± 1.4	48.3 ± 2.0	38.8 ± 8.0	45.0 ± 1.2	41.8 ± 1.3
Sum of PUFA	65.7 ± 0.7	62.9 ± 1.2	65.8 ± 0.9	65.7 ± 0.7	66.0 ± 0.6	63.7 ± 1.3	65.2 ± 0.6	64.3 ± 1.5
n-3/n-6	2.6 ± 0.1	3.2 ± 0.3	2.5 ± 0.1	2.4 ± 0.1	2.7 ± 0.2	1.6 ± 0.1	2.2 ± 0.1	1.0.9 ± 0.1
PUFA/SFA	2.9 ± 0.1	2.6 ± 0.1	2.9 ± 0.1	2.9 ± 0.2	2.9 ± 0.1	2.7 ± 0.2	2.9 ± 0.3	3.2 ± 0.2
Others	6.0 ± 0.2	6.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	6.0 ± 0.3	5.4 ± 0.5	5.8 ± 0.2	5.5 ± 0.2
Fatty acids (%DW)	1.87 ± 0.05	2.07 ± 0.1	1.85 ± 0.1	1.76 ± 0.1	1.78 ± 0.1	1.69 ± 0.2	1.74 ± 0.1	1.69 ± 0.1

particular to changes in temperature and salinity (Marbà et al., 1996; Sandoval-Gil et al., 2014; Marín-Guirao et al., 2016). Differences in TFA could also be associated with variation in leave structure and morphology of both species, with *P. oceanica* having large and thick leaves with high contents of cellulose and lignin (Romero et al., 1992), low nutrient content (Enríquez et al., 1993; Sandoval-Gil et al., 2014), and slow leaf turnover. By contrast, *C. nodosa* has smaller and thinner leaves with fast turnover rates (Perez and Romero, 1994). Therefore, a greater leaf surface per biomass in *C. nodosa* may be associated with higher photosynthetic activity in comparison to *P. oceanica* (Olsen et al., 2012), inducing a greater carbon fixation capacity and therefore a higher TFA content.

The TFA composition of both seagrasses is displayed in Tables 1 and 2, indicating only slight variations in profiles. The main, but only slight, difference was observed regarding the overall level of polyunsaturated fatty acids (PUFA), which was 66.4 ± 1.1% of TFA in *C. nodosa* and 64.9 ± 1.1% in *P. oceanica* (*t*-test, $p < 0.05$). For both species, the most abundant PUFA in leaves was α-linolenic acid (ALA, 18:3 n-3). Previous studies also reported high levels of ALA in *P. oceanica*, with significantly higher percentages in leaves than in rhizomes (Viso et al., 1993), supporting the role of this n-3 PUFA in photosynthetic performance. In addition, PUFA in *C. nodosa* and *P. oceanica* contained substantial levels of linoleic acid (LA, 18:2 n-6) ranging, respectively, from 18.8 to 22.3% and 15.0 to 24.9% of TFA. Significant differences in the overall percentages of monounsaturated fatty acids (MUFA) were also observed between both species, which ranged from 3.4 ± 0.5% in *C. nodosa* to 6.3 ± 0.9% in *P. oceanica* (*t*-test, $p < 0.001$). The main difference was observed in the levels of oleic acid (OLE, 18:1 n-9) which was 55% higher in *P. oceanica* than in *C. nodosa*. No significant differences in overall saturated fatty acid (SFA) levels were observed between each species (23.0 ± 1.0% of TFA in *C. nodosa* and 22.9 ± 0.6% in *P. oceanica*), palmitic acid (PAL, 16:0) being the main SFA. Also, noteworthy, *C. nodosa* contained low levels of long-chain (LC)-PUFA, such as arachidonic (ARA, 20:4 n-6) and eicosapentaenoic (EPA, 20:5 n-3) acids, which never exceeded 2% of TFA (Table 1). The presence of such LC-PUFA was also previously reported from some other seagrasses, as represented in Table 3, including *Posidonia sinuosa*, *Zostera* spp., *Thalassia hemprichii* and *Halphila ovalis*.

LA and ALA are the most abundant PUFA previously reported from leaves of a range of seagrass species (Nichols and John, 1985; Khotimchenko, 1993). Both PUFA are precursors for synthetizing essential fatty acids for the next trophic levels (Veloza et al., 2006; Richoux and Froneman, 2008). Essential fatty acids (EFA) are fatty acids not biosynthesized effectively by animals (Arts et al., 2001), such as ALA and LA, which are both PUFA, and precursors for the synthesis of biologically important LC-PUFA (*e.g.* EPA and DHA). The four main fatty acids (PAL, OLE, LA and ALA) accounted for 80–90% of the TFA composition in both species analysed here. In the case of *P. oceanica,* these findings are in accordance with previous studies conducted in different regions of the Mediterranean Sea (Viso et al., 1993), but our study represents the first detailed investigation of the fatty acid composition of *C. nodosa*. Moreover, as shown in Table 3, previous analyses of seagrass leaves have demonstrated similar fatty acid profiles similar to results here with the four mentioned major fatty acids ranging from 62 to 90% of TFA (*i.e.* Jeffries, 1972; Viso et al., 1993; Kharlamenko et al., 2001). The presence of n-6 and n-3 LC-PUFA (> 20 carbon) such as ARA, EPA and docosahexaenoic (DHA, 22:6 n-3) acids as traces reported for some seagrass species (Table 3) are potentially due to algal epiphytes colonizing their leaf surfaces which are able to synthetize and accumulate LC-PUFA (Khozin-Goldberg and Boussiba, 2011; Schmid et al., 2014).

3.2. Regulation of FA metabolism exposed to experimental warming

The result indicated a species-specific metabolic response of fatty acids to experimental warming. After exposure to heat stress for 6 weeks, *C. nodosa* from the southern population, considered to be adapted to colder temperatures *in situ*, demonstrated a significantly increase of 9% in TFA content in comparison to control plants (*t*-test, $p < 0.001$) (Fig. 3B). A similar pattern was observed for the cold-adapted (northern) population of *P. oceanica*, where plants exposed to warmer temperatures produced 4.7% more TFA content than controls

Table 3
Comparison of the fatty acid profiles (% TFA) of *C. nodosa* and *P. oceanica* determined here in comparison with data from other studies. Data are expressed as ranges in % TFA. References 1 to 9 represent data compiled from published literature shown in Table 4.

Species	*Posidonia oceanica*	*Posidonia sinuaosa*	*Cymodocea nodosa*	*Zostera marina*	*Zostera noltii*	*Zostera capensis*	*Phyllospadix iwatensis*	*Thallassia hemprichii*	*Halophila ovalis*	*Ruppia maritima*
References	Our study, 9	6	Our study	1, 2 and 5	3	7	2	4	6	8
Fatty acids (% TFA)										
Saturated fatty acids										
14:0	[0.5–0.7]	0.2	[0.4–1.1]	0.4	0.8			0.3	0.89	
16:0	[19.7–21.3]	22.5	[19.7–23.4]	[16.5–19.9]	12.99	31	12.4	25	32.42	18.1
17:0		0.3		0.2	0.51	0.9		0.5	0.26	
18:0	[2.0–2.6]	6.7	[1.0–1.3]	[1–1.5]	2.64	2.1	0.8	2.9	4	2
20:0		0.4		[0.7–0.9]	2.65		1.0	0.6		
22:0		0.9		[1.9–2.1]	4.86	0.3	2.5	1.0	0.04	
24:0		1.6		[0.6–0.7]	5.71		1.6	1.5	1.59	
28:0		7.7						0.6	0.61	
30:0		10.0						0.4	0.03	
Sum of SFA	[22.0–25.4]	50.2	[22.0–25.4]	[17.5–25.7]	30.2	34.3	18.3	32.8	39.8	20.1
Monounsaturated fatty acids										
14:1	[0.4–0.6]		[0.5–1.0]	0.1						
16:1										3.4
16:1 n-7	[0.3–0.5]	2.1	[0.2–0.4]	[1–2.4]	0.24	1.8	1.2	1.7	1.94	
17:1										4.1
18:1										5.3
18:1 n-7	[0.1–0.3]		[0.2–0.3]	0.5	1.17	0.8		0.2		
18:1 n-9	[4.4–7.0]	4.1	[1.6–2.9]	[1.5–1.8]	0.62	1.6	0.7	10.1	3.04	
20:1 n-9		0.1						0.4		
22:1 n-9									0.42	
24:1 n-9		0.1			1.03				0.06	
Sum of MUFA	[2.9–4.4]	6.4	[2.9–4.4]	[2.6–4.3]	3.1	4.2	1.9	12.4	5.5	12.8
Polyunsaturated fatty acids										
16:2 n-4		0.09								
16:3 n-3				[3.5–7.1]		2.9	8.4		0.05	
16:3 n-4					2.28					
18:2 n-6	[15.0–22.5]	12.23	[18.0–22.3]	[15.7–21.2]	14.82	28	3.0		13.96	16.6
18:3								39.4		41.9
18:3 n-3	[38.8–48.3]	23.02	[41.8–47.4]	[41.3–49.3]	46.38	28.8	60.6		31.26	
18:3 n-6		0.08							0.56	
18:4 n-6		0.26								
20:4 n-6		0.33	[0.02–0.5]		0.49	0.3		0.3	0.38	
20:5 n-3		0.4	[0.9–1.8]	[0.1–0.3]	0.17		0.2	0.4	1.88	
22:2 n-6				0.4						
22:4 n-6								0.2		
22:5 n-3								0.2		
22:6 n-3		0.05			0.7				0.03	
Sum of PUFA	[62.9–66.0]	36.5	[64.0–67.6]	[60.6–78.3]	64.8	60.0	72.2	40.5	48.1	58.5
Others	[6.6–7.5]	7.3	[6.6–7.5]	[1.8–3.2]	1.96	1.5	7.6	14.3	6.6	8.6

(*t*-test, $p < 0.001$). By contrast, seagrass populations adapted to warmer temperatures, *i.e.* northern population of *C. nodosa* and southern population from *P. oceanica*, did not reveal any significant differences in TFA contents after heat exposure.

For both species, the proportion of PUFA (*i.e.*, LA and ALA) decreased, while SFA (*i.e.*, PAL) increased after the heat treatment (Fig. 4). As PUFA are mainly partitioned to structural lipids promoting membrane fluidity, electron transport rate and photosynthetic activity, our results may be explained by the reduced requirement for PUFA to maintain membrane fluidity under elevated temperatures (Marr and Ingraham, 1962). These findings support recent evidence regarding the transcriptomic modulation of lipids biosynthesis in experimentally heat-stressed *P. oceanica* plants (Marín-Guirao et al., 2017); they are thus in accordance with previous studies on seagrasses, and unicellular algae, seaweeds and terrestrial plants, where a decrease in PUFA was related to an increase in SFA when exposed to higher temperatures *in situ* or during lab-experiments (Harwood and Russel, 1984; Cohen et al., 1988; Sanina et al., 2004).

Heat-stressed seaweeds and terrestrial plants typically respond to abiotic stress by remodelling membrane fluidity by realising PUFA (*i.e.* ALA) from membrane lipids (Gosch et al., 2015; Upchurch, 2008), inducing therefore an increase in the proportion of SFA. In parallel, fatty acid desaturases activities regulate the unsaturated fatty acid levels and mediated changes in membrane fluidity. In algae, accumulation of storage lipids, such as TAG containing mostly SFA, is also a common mechanism to store excess of energy generated by photosynthesis, and to cope with stressors such as increased temperatures, light or nutrient deficiency (Goncharova et al., 2004; Pal et al., 2011; Solovchenko, 2012). Moreover, under heat stress conditions (T2), % of MUFA increased significantly in populations of *P. oceanica* adapted to lower temperatures *in situ*. These results are in agreement with previous studies on other marine primary producers where, under temperature stress, the % of MUFA increased more in thermo-intolerant than in thermo-tolerant species (Rousch et al., 2003). Such changes may be associated with a reduced synthesis of PUFA under heat stress (Sukenik et al., 1993). Overall, our results highlight that seagrasses living at warmest temperatures *in situ* were more thermo-tolerant, with a higher capacity to adjust their fatty acid composition, than populations inhabiting colder regions.

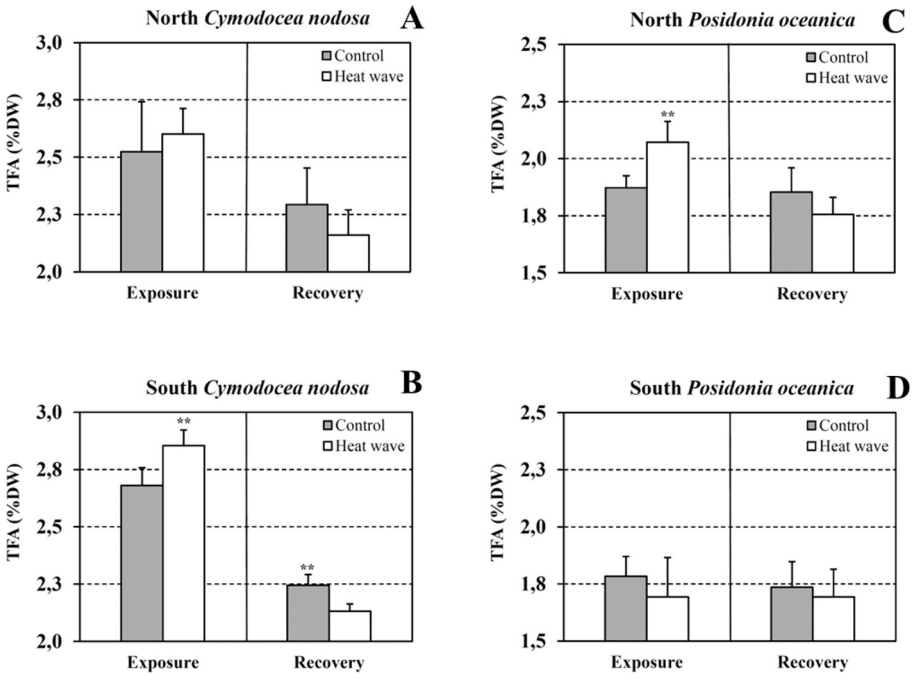

Fig. 3. Total fatty acid contents (% DW) of northern and southern populations of *C. nodosa* and *P. oceanica* exposed to control and heat wave temperatures, and their recovery. Differences between temperature treatments were evaluated performing t-student (** represent $p < 0.01$ and * represent $p < 0.05$–0.01).

3.3. Recovery from experimental heat wave events

After six weeks of exposure to the heat treatment, both species were incubated for a further six weeks at their original *in situ* temperatures, in an attempt to test their ability to recover after an anomalous heat wave event.

C. nodosa appeared to recover fully, but TFA of the two populations displayed two distinct patterns, with a slightly lower content in the southern population at T3 (end of recovery) which was equivalent to the control in the northern population (Fig. 1, A and B). Heat-exposed plants and controls from both populations had lower TFA contents at T2 (end of heat exposure) and T3 – with significant decreases of 15–20% (ANOVA, $p < 0.001$).

The TFA content of *P. oceanica* reached initial levels within the recovery period. In contrast to *C. nodosa*, TFA content of northern and southern populations of *P. oceanica* did not change significantly between exposure (T2) and recovery (T3) (Fig. 3, C and D). The fact that the sampling date of *C. nodosa* and *P. oceanica* shoots harvested at T3 occurred in late September may explain these different species-specific performances; *C. nodosa* typically can maintain high productivity rates until the end of the summer, while the productivity of *P. oceanica* may decrease in mid-summer (Buia et al., 1992; Marbà et al., 1996).

Mediterranean seagrasses are pre-adapted to seasonal temperature cycles and may be additionally influenced by their internal biological clock (Buia et al., 1992; Marbà et al., 1996). Rather than acclimating to short-term ambient environmental factors, the growth pattern of *P. oceanica* is relatively independent of seasonality. By contrast, growth of *C. nodosa* is highly sensitive to environmental conditions, displaying marked seasonal patterns (Marba et al., 1996). Here, after recovery (T3), the TFA compositions of both *C. nodosa* and *P. oceanica* populations were similar to controls (Fig. 4), suggesting full recovery from

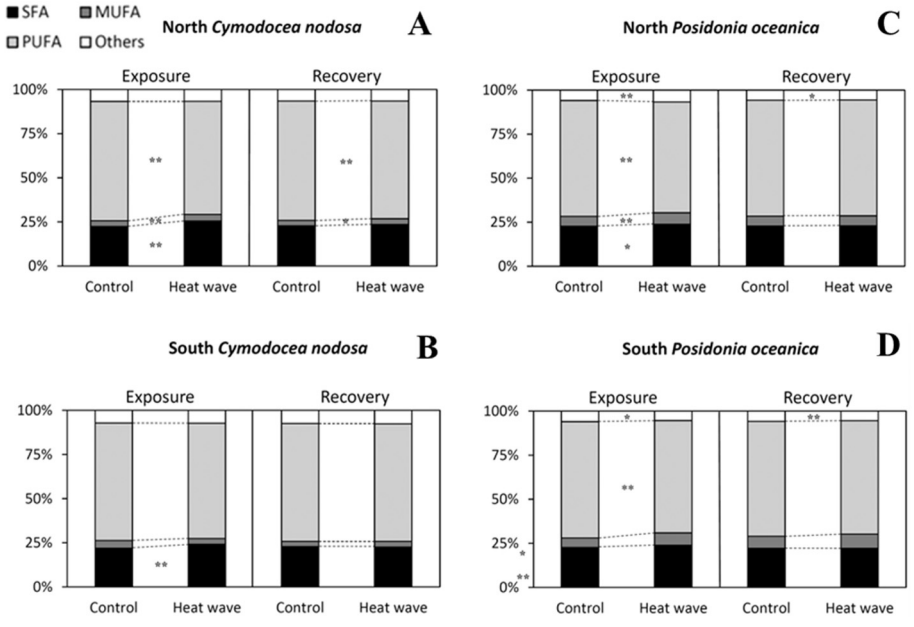

Fig. 4. Proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and non-identified (Others) fatty acids (% TFA). Differences between temperature treatments were evaluated performing t-student (** represent $p < 0.01$ and * represent $p < 0.05$–0.01).

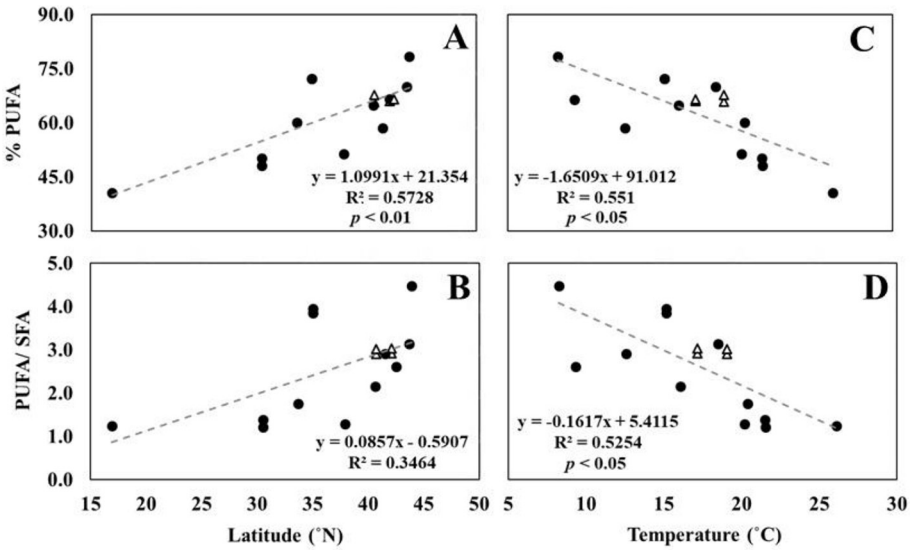

Fig. 5. Percentage of PUFA and ratio between PUFA: SFA and corresponding geographical latitudinal distribution range (Panel A and B) and annual sea surface water temperature (Panel C and D) with results from the literature (circles) and from the present study (triangles) (Tables 3 and 4). Grey lines represent the linear regression line.

heat stress by readjusting their lipid composition and membrane fluidity to control conditions.

Moreover, data displayed in Tables 2 and 3 suggest that plants adapted to warmer *in situ* conditions, which performed better after the heat treatment, showed higher ratio of n-3/n-6 PUFAs after the exposure (T2) than after the recovery (T3). As suggested by Sanina et al. (2004), these results may be related to the important role of n-3 PUFAs within lipids of photosynthetic membranes; on the other hand, n-6 PUFAs are mostly linked to polar lipids of extra-plastidial membranes (Schmid et al., 2017b). In general, *C. nodosa* displayed a greater plasticity with regard to fatty acid composition than *P. oceanica*, resulting in a fast adjustment in composition and lipid structure in response to environmental changes.

3.4. Latitudinal and in situ annual SST comparison

Both % of PUFA and PUFA/SFA ratio were correlated with latitude, with an average of 1.4 and 0.12 increase per degree latitude, respectively (Fig. 5, A and B). In addition, % of PUFA and PUFA/SFA ratio were correlated with *in situ* annual SST, with an average of 2.12 and 0.18 decrease per increase of 1 °C, respectively (Fig. 5, C and D). Lowest proportions of PUFA (40.5–48.12%) and of PUFA/SFA ratios (1.23–1.21) were observed in two tropical species *Thallassia hemprichii* and *Halophila ovalis* adapted to warmest annual SST (Tables 3 and 4). By contrast, larger proportions of PUFA (78.3%) and of PUFA/SFA ratios (4.47) were observed in the subarctic *Zostera marina* populations adapted to annual SST of 8.24 °C. In line with these results, previous studies highlighted that leaf production in eelgrass was also correlated with temperature, and productivity increased with temperature (Lee et al., 2007; Olesen et al., 2015; Beca-Carretero et al., under review). These findings demonstrate that fatty acid composition changes in accordance with *in situ* temperature, suggesting a physiological adaptation to large-scale factors such as annual seawater temperature. In macroalgae, changes in PUFA levels to changes in temperature (Viso et al., 1993; Gosch et al., 2015) were previously related with the capacity of acclimatization of photosynthetic organisms to adjust their membrane lipid structures to support adequate membrane fluidity tin response to different environmental conditions (Falkowski and Raven, 1997).

3.5. Implications of fatty acid responses to heat waves for the next trophic level

In marine ecosystems, the synthesis of essential fatty acids (EFA) is limited to primary producers, and then transferred conservatively through the marine trophic chain (Charpy-Roubaud and Sournia, 1990; Dalsgaard et al., 2003). As a response to environmental fluctuations, marine primary producers adjust their lipid and fatty acid composition (Guschina and Harwood, 2006; Gerasimenko et al., 2011). Changes in FA ratios (*i.e.* n-6/n-3 and PUFA/SFA) are important food quality

Table 4
Data compiled from published literature used to assess differences of fatty acids profiles of different seagrass species across world.

Identification	References	Species	Location	Latitude	Longitude	PUFA	PUFA/SFA	Mean SST
Our data	This study	*Posidonia oceanica*	Spain, Cala Montgo	40.73	0.87	65.70	2.89	19.05
Our data	This study	*Posidonia oceanica*	Spain, Delta del Ebro	42.11	3.17	66.00	2.91	17.18
Our data	This study	*Cymodocea nodosa*	Spain, Cala Montgo	40.73	0.87	67.60	3.02	19.05
Our data	This study	*Cymodocea nodosa*	Spain, Delta del Ebro	42.11	3.17	66.50	3.02	17.18
1	Sanina et al., 2004	*Zostera marina*	Russia, Sea of Japan	43.92	135.57	78.30	4.47	8.24
2	Vaskovsky et al., 1996	*Zostera marina*	China, Yellow Sea	35.03	125.81	72.20	3.84	15.17
2	Vaskovsky et al., 1996	*Phyllospadix iwatensis*	China, Yellow Sea	35.03	125.81	72.20	3.95	15.17
3	Coelho et al., 2011	*Zostera noltii*	Portugal, Ria Aveiro	40.63	− 8.75	64.84	2.15	16.08
4	Nichols and John, 1985	*Thallassia hemprichii*	USA, Lizard Island	− 16.90	145.77	40.50	1.23	26.10
5	Kharlamenko et al., 2001	*Zostera marina*	Russia, Sea of Japan	42.54	132.12	66.40	2.61	9.33
6	Hanson et al., 2010	*Halophila ovalis*	Australia, Bay Marine Park	− 30.51	115.04	48.12	1.21	21.53
6	Hanson et al., 2010	*Posidonia sinuosa*	Australia, Bay Marine Park	− 30.51	115.04	50.21	1.38	21.50
7	Richoux and Froneman, 2008	*Zostera capensis*	South Africa, Kariega estuary	− 33.69	26.68	60.00	1.75	20.38
8	Jeffries, 1972	*Ruppia maritima*	USA, Rodhe Island	41.51	71.10	58.50	2.91	12.60
9	Viso et al., 1993	*Posidonia oceanica*	Greece, Athens	37.94	23.68	51.40	1.28	20.19
9	Viso et al., 1993	*Posidonia oceanica*	France, Villefrnache-sur-mer	43.69	7.27	70.00	3.14	18.50

indicators in marine ecosystems, which may affect trophodynamic relationships (French et al., 2000; Pommier et al., 2012; Parrish, 2009). Our results suggest that production of PUFA in marine plants may is reduced, and synthesis of SFA increased, under future scenarios of global warming. The key physiological and structural roles that fatty acids play in the different life stages of marine herbivores, invertebrates and fishes is well documented (Rodríguez et al., 2004). For instance, ARA and EPA play a key role as the most important eicosanoid precursors in fish cells and also controlling important physiological functions such as reproduction (Bell et al., 1994; Sorbera et al., 1998). Variations in the FA ratios in the diet of primary producer consumers affect significantly their fatty acid composition including vitally important LC-PUFA (Graeve et al., 1994; Felline et al., 2014). Recent studies on microalgae have already predicted a decrease in the levels of PUFA in the pelagic food web in response to climate change (Hixson et al., 2015). Mediterranean seagrasses systems represent an important food source for keystone herbivores such as the fish *Salpa salpa* (Sparidae) and the sea urchin *Paracentrotus lividus* (Echinidae) and for other consumers such as *Idotea baltica* Pall. (Idoteidae) or herbivores belonging to Palaemonidae (Ackman et al., 1968; Kirkman and Young, 1981; Velimirov, 1984). Overall, these present findings suggest that future warming could significantly affect the nutritional value of seagrasses, with a remarkable depletion in the synthesis EFA (*i.e.*, ALA and LA), and thus potential consequences in the fatty acid composition of higher trophic consumers and in their physiological functions. Warming is also expected to increase the metabolic requirements of herbivores (Burnell et al., 2013), which may fulfil their nutritional requirements by increasing grazing pressure on seagrasses or changing preferences for other dominant macrophytes (Hernán et al., 2016; Pagès et al., 2017). Climate-driven changes in the strength of herbivory may have important consequences for Mediterranean coastal ecosystems due to the important role of grazing in altering seagrass biomass, productivity and in modulating species composition (Kirsch et al., 2002; Valentine and Duffy, 2006). Future warming is therefore expected to directly, and indirectly, modify productivity and distribution of seagrasses that will cascade throughout the food web and affect the overall functioning of Mediterranean coastal ecosystems.

4. Conclusions

In this study, for the first time, we highlight the importance of the role of fatty acids in assessing the physiological status of seagrasses. Our findings demonstrate the influence of simulated warming affecting the total fatty acid content and composition of *P. oceanica* and *C. nodosa*. Results suggest that plants exposed to experimental warming adjusted their fatty acid metabolism, by changing TFA levels, increasing proportions of SFA and reducing PUFA levels. This could be explained by a reduced requirement for PUFA to maintain membrane fluidity under experimental heat wave conditions; on the other hand, SFA are mainly partitioned to storage lipids. In general, *C. nodosa* displayed a greater fatty acid plasticity than *P. oceanica*, with a fast adjustment of fatty acid composition and lipid structures in response to heat stress. Results indicate also that exposure of seagrasses to warmer conditions may induce a decrease of the % of EFA (*i.e.* ALA and LA) and the PUFA/SFA ratio, and potentially affecting their nutritional value for higher trophic levels. Therefore, future studies on the specific FA composition of the major lipid classes will lead to a better understanding of the underlying metabolic processes causing the responses to experimental warming. Indeed, evaluation of the species-specific responses of seagrasses and their capacity to adjust to climate change is essential to support current efforts in seagrass monitoring and management in Europe.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2017.12.057.

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