

Effects of high temperature and marine heat waves on seagrasses: Is warming affecting the nutritional value of *Posidonia oceanica*?

Patrizia Stipcich^{a,*}, Pedro Beca-Carretero^{b,c}, Xosé Antón Álvarez-Salgado^b,
Eugenia T. Apostolaki^d, Niki Chartosia^e, Pavlos Theofilos Efthymiadis^d, Carlos E. Jimenez^{f,g},
Gabriella La Manna^{h,i}, Arianna Pansini^a, Elena Principato^j, Vasilis Resaikos^f,
Dagmar B. Stengel^d, Giulia Ceccherelli^h

^a Dipartimento di Architettura, Design e Urbanistica, Università degli Studi di Sassari, Via Piandanna 4, 07100, Sassari, Italy

^b Department of Oceanography, Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

^c Botany and Plant Science, School of Natural Sciences, University of Galway, Galway, Ireland

^d Institute of Oceanography, Hellenic Centre for Marine Research, PO Box 2214, 71003, Heraklion, Crete, Greece

^e Department of Biological Sciences, University of Cyprus, Nicosia, 1678, Cyprus

^f Enalia Physis Environmental Research Centre (ENALIA), Acropoleos St. 2, Aglanjia 101, Nicosia, Cyprus

^g Energy, Environment and Water Research Center (EEWRC) of the Cyprus Institute, Nicosia, Cyprus

^h Dipartimento di Scienze Chimiche Fisiche Matematiche e Naturali, Università degli Studi di Sassari, Via Piandanna 4, 07100, Sassari, Italy

ⁱ MareTerra Onlus, Environmental Research and Conservation, 07041, Alghero, SS, Italy

^j Area Marina Protetta "Isole Pelagie", Via Camerani, s.n.c., 92031, Lampedusa, Italy

ARTICLE INFO

Keywords:

Bioindicators
Carbon
Climate change
Fatty acids
Mediterranean sea
Nitrogen
Nutrients

ABSTRACT

Primary producers nutritional content affects the entire food web. Here, changes in nutritional value associated with temperature rise and the occurrence of marine heat waves (MHWs) were explored in the endemic Mediterranean seagrass *Posidonia oceanica*. The variability of fatty acids (FAs) composition and carbon (C) and nitrogen (N) content were examined during summer 2021 from five Mediterranean sites located at the same latitude but under different thermal environments. The results highlighted a decrease in unsaturated FAs and C/N ratio and an increase of monounsaturated FA (MUFA) and N content when a MHW occurred. By contrast, the leaf biochemical composition seems to be adapted to local water temperature since only few significant changes in MUFA were found and N and C/N had an opposite pattern compared to when a MHW occurs. The projected increase in temperature and frequency of MHW suggest future changes in the nutritional value and palatability of leaves.

1. Introduction

Due to the global warming, sea surface temperature (SST) has risen globally in the last decades (Pastor et al., 2019; Bulgin et al., 2020) and marine heat waves (MHWs) have increased in duration, frequency and intensity (Hulme, 2014; Oliver et al., 2018; Darmaraki et al., 2019). The SST rising and intensification of MHW events are affecting not only the distribution and abundance of species and therefore ecosystem functions (Orth et al., 2006; Ruckelshaus et al., 2011; Frölicher and Laufkötter, 2018; Lotze et al., 2019; Smale et al., 2019; Garrabou et al., 2022), but also physiological and biochemical processes at the species level (Newell and Branch, 1980; Palumbi et al., 2019).

Primary producers are at the base of the food chain and their nutritional content may affect the functionality of the entire food web (Malzahn et al., 2010). Changes in biochemical properties and thus nutritional composition of primary producers with temperature have been evidenced in terrestrial plants (Friend, 2010; Lin et al., 2010; Leisner, 2020), marine algae (Teoh et al., 2010; Brown et al., 2014; Lowman et al., 2021) and plankton (Ramlee et al., 2021), indicating that climate alterations can also affect their palatability and can potentially induce a shift in the herbivory feeding preferences (Van Alstyne et al., 2009; Hernán et al., 2019; Zhang et al., 2019). However, despite all the efforts, consistent evidence about changes in the nutritional value, if not for the unsaturation level of fatty acids (FA), has not

* Corresponding author.

E-mail address: patriziastipcich@libero.it (P. Stipcich).

<https://doi.org/10.1016/j.marenvres.2022.105854>

Received 22 August 2022; Received in revised form 17 November 2022; Accepted 18 December 2022

Available online 22 December 2022

0141-1136/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

been gained across species, likely because biochemical responses are highly species-specific (Teoh et al., 2010). Seagrasses are among the most important coastal habitats due to the multiple ecosystem services they provide, including oxygen production, nutrient cycling, carbon sequestration, provision of nursery grounds, sediment stabilization, and protection from erosion (Hemminga and Duarte, 2000; Larkum et al., 2006). The seagrass *P. oceanica* (L.) Delile is endemic to the Mediterranean Sea and forms extended meadows widely distributed in the whole basin (Telesca et al., 2015). *P. oceanica* leaves are consumed by few species, such as the fish *Sarpa salpa*, the sea urchin *Paracentrotus lividus* and the isopod *Idotea baltica* which have a very variable impact on the plant itself (Alcoverro et al., 1997; Tomas et al., 2005; Buñuel et al., 2021). This seagrass represents also an important source of organic matter thanks to its detritus (Coull, 1990), which is considered a good carbon (C) source and a nitrogen (N) reservoir for small pelagic and benthic invertebrates (Vizzini et al., 2002).

In the Mediterranean Sea, due to its conformation and the limited hydrological regimes, climate change effects are more exacerbated than in the global ocean (Diffenbaugh et al., 2007), and a more rapid increase in mean SST and in the frequency and duration of MHWs is also expected for the near future (Giorgi and Lionello, 2008). The thermal environment induces phenological changes in *P. oceanica* as indicated by recent studies demonstrating the spatio-temporal variability (Peirano et al., 2011; Pansini et al., 2021; Stipcich et al., 2022a) and this seagrass is seemingly threatened by both the increasing SST and MHWs (Marbà and Duarte, 2010; Stipcich et al., 2022b). MHWs, depending on the intensity, may trigger relevant *P. oceanica* shoot mortality (Marbà and Duarte, 2010) or may decrease the number of leaves per shoot and the leaf length and increase leaf necrosis (Stipcich et al., 2022b). MHWs in *P. oceanica* seem to induce a decrease in lipid content and increase in carbohydrates (Stipcich et al., 2022b), but specific changes in biochemical properties, especially leaf FA composition and C and N content, deserve further attention since they likely affect the nutritional values of the plant and thus they might have an influence on the trophic web.

The N and C elements are key basic component of leaf cells (Lourenço et al., 1998; Chikaraishi et al., 2009). Under thermal stress conditions primary producers increase metabolic costs by increasing the respiration rates, leading to reductions in C content in photosynthetic tissues relative to increases in N contents (Kepkay et al., 1997; Sardans et al., 2012). Besides, increased nutrient concentrations in the water column can also drive to an increased seagrass tissue nutrient content; specifically, a N-enrichment in the external environment can directly affect the physiology of the plant (Lee et al., 2004; Unsworth et al., 2015; Pazzaglia et al., 2020). Further, the ratio C/N is known to change according to the environmental conditions – low nutrient environments induce significantly higher C/N (Atkinson and Smith, 1983; Duarte, 1990) – even though C normally shows low variability (Duarte, 1990). Global warming can also play an important role in the nutrient distribution by enhancing the eutrophication (Zhou et al., 2016). Also, the abundance of C and N has been reported to influence food selection by herbivores since their variation may change leaves palatability and may reduce food quality (de los Santos et al., 2012). A change in the C and N cycle related to increasing temperature has been already found in terrestrial plants (Yuan and Chen, 2015), and in seagrasses (reviewed in Beca-Carretero et al., 2021), but to the best of our knowledge, in marine plants, this information under natural anomalous warming conditions is still lacking. Also, in *P. oceanica* changes in C and N leaf content have been described through seasons (Pirc and Wollenweber, 1988), but differences due to temperature throughout a whole summer period have never been investigated.

Lipids are major sources of metabolic energy and essential materials for the formation of cell and tissue membranes (Sargent et al., 1995; Bergé and Barnathan, 2005). In particular, FAs are considered useful tracers of diets and marine trophic chain for different reasons: i) organisms are able to biosynthesize and modify chain-length depending on

the phylogenetic group they belong: only primary producers are able to biosynthesize essential FAs; ii) FAs are generally not degraded during digestion and they are accumulated by tissues in their basic form so they can be traced back to specific food web origins; iii) lipids are stored in animal bodies in reservoirs and these reservoirs can later be mobilized to provide fuel for short or long-term energy demands (Pond, 1998). Thus, the study of FA accumulation over time in *P. oceanica* systems needs attention as it reflects the organism energy intake and storage rates (Parrish, 2009). Overall, the change in FAs composition in seagrasses due to temperature rise seems to manifest by decreases in polyunsaturated FAs (PUFA) and increases in saturated FAs (SFA) (Beca-Carretero et al., 2018, 2021; Franzitta et al., 2021). Generally, the decrease in PUFA due to high temperature is accompanied by an increase of SFA, even though the relationship between temperature and SFA is more variable and more species-specific than the strong relationship between temperature and PUFA (Hixson and Arts, 2016). Sometimes, the monounsaturated FAs (MUFA) increase with temperature rather than the SFA (Hixson and Arts, 2016). Although FA remodeling in seagrasses has been demonstrated in principle (Parrish, 2009; Beca-Carretero et al., 2018, 2020; Franzitta et al., 2021), only limited effort was focused on *P. oceanica* (Beca-Carretero et al., 2018; Stipcich et al., 2022c). This significant gap of knowledge needs to be filled as FA composition is considered not only as a good indicator of the physiological status of the plant (Rabbani et al., 1998; Sanina et al., 2008; Beca-Carretero et al., 2018, 2022), but also a proxy of the food quality of the plant (Tan et al., 2022). Moreover, in marine ecosystems, some of the essential FAs (e.g. n-3) are synthesized mostly by primary producers and then they are transferred through the trophic chain by the consumers (Nichols, 2003; Dalsgaard et al., 2003; Twining et al., 2016). This study aimed to investigate whether the thermal environment affects the nutritional values of *P. oceanica* through a short-term spatio-temporal variability field study. The hypothesis was that high temperature and MHW occurrence decrease unsaturation levels in photosynthetic structures of seagrasses to optimize membrane functionality, and particularly lead to a reduction in essential n-3 FA. In addition, we expect decreases in the C/N ratio in responses to stress warming conditions since respirations rates generate higher CO₂ productions and therefore N contents increases relative to C contents. To test these hypotheses, the variability of FA composition and C and N content of *P. oceanica* leaves were examined during summer 2021 at five Mediterranean sites located at the same latitude (same photoperiod) and same depth, but under different thermal environments in terms of both mean temperature and MHW occurrence.

2. Materials and methods

2.1. Study sites

In order to ensure a temperature gradient, five sites, at different annual and summer natural thermal conditions, were selected nearly at the same latitude across a wide longitudinal transect in the Mediterranean Sea (between 10 and 35° E, centered around 35° N) (Fig. 1): Konnos Bay (C1) and Akrotiri Cape (C2) in Cyprus (south-eastern coast and south of Cyprus, respectively), Pigadia (KA) and Gourmes (CR) in Greece (south-eastern coast of Karpathos, and northern Crete, respectively), and Cala Pulcino (LA) in Italy (south of Lampedusa Island). All selected sites were far from direct anthropogenic stressors such as urbanization or ports. At each site, a *P. oceanica* meadow (always between 400 and 700 shoots/m² and therefore “dense” according to the classification of Giraud, 1977) on sandy bottom at approximately 10m of depth was haphazardly selected.

2.2. Data collection

During summer 2021, three or four sampling times (T1 = end of June, T2 = end of July, T3 = end of August and T4 = end of September)

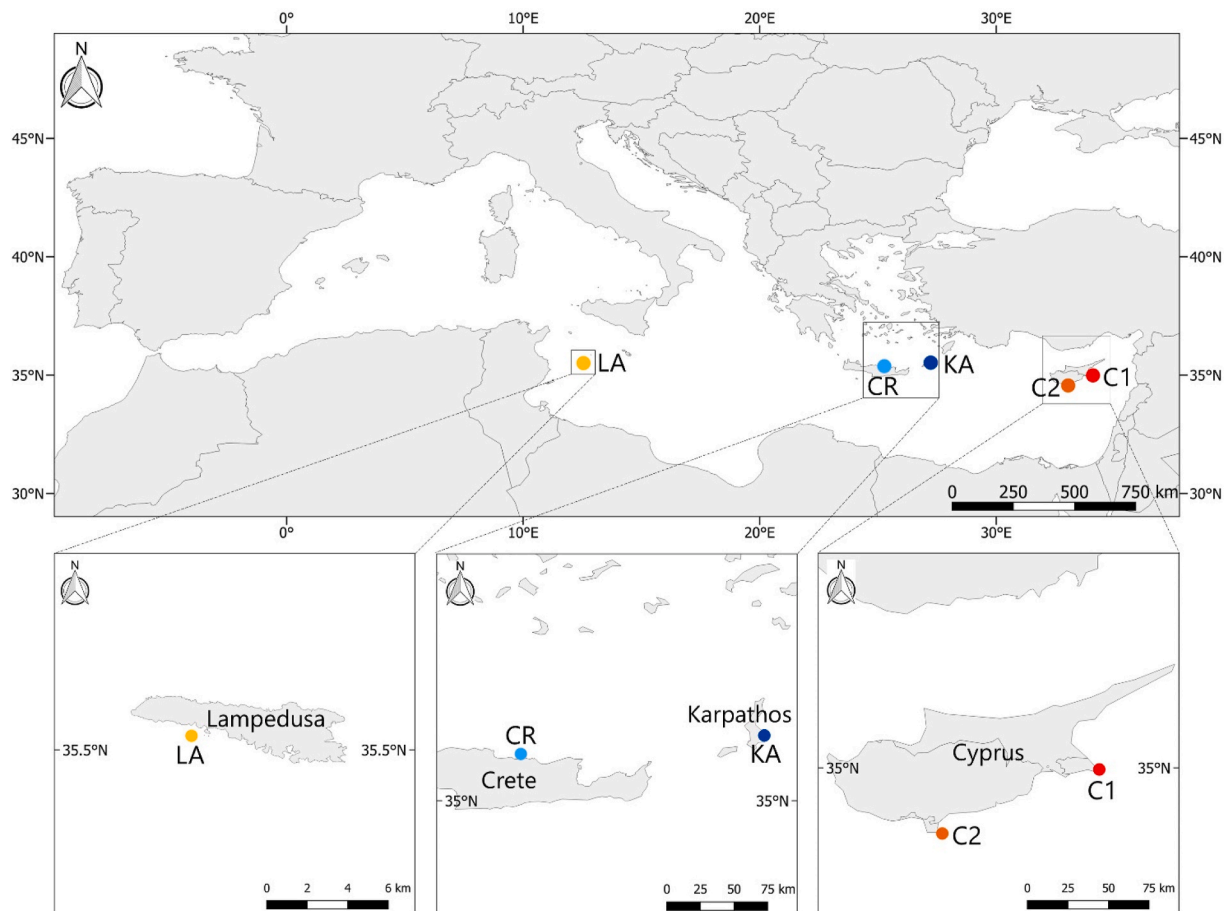


Fig. 1. Location of the study sites: LA = Cala Pulcino; CR = Gournes; KA=Pigadia; C1=Konnos Bay; C2 = Akrotiri Cape.

about 30 days apart were considered (Table S1). On each sampling time, six *P. oceanica* orthotropic shoots per site were randomly chosen; from each of them, the second intermediate leaf was manually collected, transported to the laboratory and frozen at -80°C for 48h before freeze-drying.

The daily SST of the summer 2021 period (June–September) was acquired for each site by Advanced Very High Resolution Radiometer (AVHRR) instruments aboard NOAA polar-orbiting satellites that contains global, twice-daily (Day and Night), 4 km SST derived from measurements captured (<https://coastwatch.pfeg.noaa.gov/erddap/index.html>, dataset ncdcOisst21Agg) (Fig. S1). Then, the average thermal condition during the 10 days preceding each sampling time (SS10 temperature from hereafter), in each site, was estimated and attributed to a category: Low = L, Medium = M and High = H temperature (ranges are given in the Results section as they were *a posteriori* decided). Furthermore, all the MHWs that occurred in each site during summer 2021 were identified following Hobday et al. (2016), using the heatwaveR (Schlegel and Smit, 2018) and the rerdap (Scott, 2021) package in R (Core Team, 2013).

Leaf N and C (%) were determined in samples of ~ 7.0 mg of freeze-dried, finely grounded and homogenized material from each sample. Leaf N and C contents were analyzed by using a high temperature catalytic oxidation at 900°C with an elemental analyzer PerkinElmer 2400.

To estimate the FA content and composition on *P. oceanica* leaves, the collected material was dried for 24–48 h at 40°C to obtain constant weight, and later kept frozen at -20°C in hermetically sealed bags containing silica gel (see protocol in Beca-Carretero et al., 2020). Before analyses, biomass was grounded into a fine powder using a bead mill homogenizer Beadmill 4 (Fisher Scientific, USA) machine at 5 m s^{-1} for 3 min. The content and composition of FAs in leaves were determined by

modifying a protocol previously applied in seagrass species (Beca-Carretero et al., 2018): extraction of FAs was done using ~ 15 mg of powdered leaf material per replicate. To quantify total and individual FA contents, a known quantity of 0.1 mL of the saturated fatty acids (SFAs; $15:0$ in 0.4 mg mL^{-1}) of hexane (99%, Sigma Aldrich, catalog no. 89680) was added as an internal standard before starting the direct transmethylation. Fatty acid methyl esters (FAMES) were obtained by direct transmethylation with 2 mL dry methanol containing 2% (v/v) H_2SO_4 . Extraction of FAMES was conducted at 78°C for 2 h under continuous stirring conditions. To prevent oxidation, vials were sealed with nitrogen gas before heating. After transmethylation, 1 mL of Milli-Q water was added and later the FAMES were extracted using 0.25 mL of n-hexane. Identification of FAMES was achieved by co-chromatography with authentic commercially available FAME standards of fish oil (Menhaden Oil, catalog no. 47116; Supelco). FAMES analysis was conducted using an Agilent 7890A/5975C gas chromatography/mass selective detector (GC/MSD) Series (Agilent Technologies, USA) equipped with a flame ionization detector and a fused silica capillary column (DB-WAXETR, $0.25\text{ mm} \times 30\text{ m} \times 0.25\text{ }\mu\text{m}$, Agilent Technologies, Catalogue no.122–7332).

2.3. Data analyses

The C and N content and the FA composition grouped as SFA, MUFA and PUFA and some ratios (PUFA/SFA; MUFA/PUFA) were considered as variables. Based on the SS10 temperature and the presence/absence of MHW in each site, data gained for each sampling time were attributed to a temperature and MHW level, producing two datasets only partially overlapped. Two-way permutational analyses of variance (PERMANOVA; Anderson, 2001) were run for each variable using a similarity

matrix based on the Euclidean distance of untransformed data, testing the effect of SS10 temperature (L = low, M = medium, and H = high) and MHW (Yes and No) as fixed factors. Significant differences were investigated using a *posteriori* pair-wise tests. Then, a SIMPER test (90% of cut off) was run to identify the single FAs most contributing to the dissimilarities. Once those were identified, PERMANOVAs were run to test the effect of SS10 temperature and MHW (using the same models described above) on the FAs most contributing to the dissimilarities.

3. Results

3.1. Temperature conditions and MHWs

Between the end of June until end of September, SS10 temperature in the five sites ranged from 23.8 °C to 28.9 °C (Fig. S1). Based on the SS10 temperature, three categories of 1.6 °C SS10 temperature ranges were defined (23.9–25.5 °C as L temperature, 25.6–27.2 °C as M temperature and 27.3–28.9 °C as H temperature) and each sample was attribute to one of them (Table S1). Across the sampling times, a single MHW (minimum five days of temperature above the threshold before the sampling time) occurred in four sites (C1, C2, KA, LA), while two MHWs were only found in CR (Tables S1 and S2).

3.2. Biochemical leaf content

P. oceanica leaf elemental composition was affected by the natural thermal regime as the N was lower at H ($1.10 \pm 0.05\%$ DW) and C/N was higher at H (42.16 ± 1.99) compared to M and L temperature (Table 1, Table S3 and Fig. 2). Opposite results were found when the effect of MHW was tested (Table S3). Leaf N content was higher when the MHW occurred ($1.43 \pm 0.06\%$ DW), but C/N was higher in the absence of MHWs ($38.11 \pm 0.83\%$ DW) (Table 1 and Fig. 3).

A significant influence of the average SS10 temperature and MHW occurrence was also found on FA composition (Table 2). MUFA composition of *P. oceanica* leaves changed depending on the SS10 temperature: it was similar in H and M temperature ($7.34 \pm 0.61\%$ of total fatty acids, TFA and $7.01 \pm 0.36\%$ of TFA), higher than in L conditions ($5.68 \pm 0.35\%$ of TFA) (Table S4 and Fig. 4). The specific FAs which gave a higher contribution to the dissimilarities between treatments were identified as n-3 PUFA α -linolenic acid (C18:3n-3), the n-6 PUFA linolenic acid (C18:2n-6), and the SFA palmitic acid (C16:0) (Table 3). C18:3n-3, C18:2n-6, within all the FAs that contributed the most, and C18:3n-3/C16:0 and n-3/n-6 ratios changed depending on SS10 temperature (Table 3 and Fig. S2).

Furthermore, MHW affected the FA composition of *P. oceanica* leaves by not only increasing the MUFA content ($8.16 \pm 0.39\%$ of TFA), but also by decreasing PUFA content ($50.57 \pm 1.43\%$ of TFA) (Table 2 and Fig. 5). The MUFA/PUFA ratio was also significantly affected by the MHW (Table 2). The SIMPER test identified the same FAs (C18:3n-3, C18:2n-6, C16:0) that responded to SS10 temperature: C18:3n-3 was higher when there was no MHW, but C18:2n-6 followed the opposite

Table 1

PERMANOVA results testing the effect of SS10 temperature (High, Medium, and Low) and Marine Heat Wave (MHW) occurrence (Yes and No) on C and N content (C, N, and C/N) of *P. oceanica* leaves; pairwise tests for the significant results. L = low; M = medium; H = high. Bold refers to significant F-values.

PERMANOVAs	Temperature	MHW
variables	<i>Pseudo-F</i> _{2,99}	<i>Pseudo-F</i> _{1,99}
C	0.46	0.65
N	6.06*	15.27*
C/N	7.90*	11.52*
PAIR-WISE TESTS		
N	L = M > H	YES > NO
C/N	L = M < H	YES < NO

pattern. Nevertheless, both C18:3n-3/C16:0 and n-3/n-6 ratio increased with no MHW (Table 4 and Fig. S3).

4. Discussion

Changes in the nutritional value of *P. oceanica* leaves related to temperature and extreme events like MHWs were investigated by considering the C and N content and FA composition. This study, which was entirely carried out in the field on unmanipulated plants and tested natural thermal conditions, confirms trends previously observed in laboratory experiments (Beca-Carretero et al., 2018, 2021; Britton et al., 2020; Franzitta et al., 2021); PUFA, and particularly n-3 PUFA, contents and the ratio C/N decreased, while MUFA content increased when a MHW occurred (Hernán et al., 2017; Pazzaglia et al., 2020). On the other hand, interesting results provide new evidence of a possible biochemical acclimation to the SS10 temperature.

The primary producer *P. oceanica* plays an important role in the food chain by being a nutritional food source (Jiang et al., 2020), from the very early stage of its development (Hernán et al., 2017), and changes at any level (morphological, physiological, and biochemical) can cascade throughout the food web and affect the functioning of the whole ecosystem (Alcoverro et al., 1997; Duarte, 2002; Beca-Carretero et al., 2018). An alteration in leaf nutritional value can be suggested by a change of N and C content and C/N ratio and in this study the sudden change of temperature occurring during each MHW produced higher values of N and lower C/N ratio; this can probably be explained by stressful thermal conditions increasing respiration rates, with consequent release of CO₂ and therefore a reduction in C relative to N contents in photosynthetic structures with MHW favoring the accumulation of leaf N for a higher production of amino acids, accompanied by a reduction in C availability for leaf carbohydrate production. Such trends were already observed in manipulative experiments on *P. oceanica* and *Halophila stipulacea* when multiple stressors were tested (Pazzaglia et al., 2020; Beca-Carretero et al., 2022). However, the effects of SS10 temperature in our study produced opposite patterns to those due to the effect of the MHW but similar to those obtained in giant kelps and macrophytes even though this change is highly species-specific (Viana et al., 2020; Lowman et al., 2021). N content was lower with the increased temperature while C/N ratio was higher in H temperature. These results may suggest an adaptation of the C and N content leaves to the local temperature indicating the existence of an optimal temperature range that can be exceeded during a MHW event when the temperature is changing faster and the leaves cannot adapt as fast to the new thermal conditions. As the quality of food (in terms of palatability) of seagrass can be expressed in terms of C/N ratio (Mazzella et al., 1992), the higher C/N ratio in *P. oceanica* leaves, that was found at H temperature, suggests the presence of specific nutrient-balancing strategies (Pazzaglia et al., 2020). The change in element composition induced by the high temperature in our experiment, at the light of the expected SST increase in the future decades (Soto-Navarro et al., 2020), suggests that this primary producer will have a different nutritional value in the future. Nevertheless, it seems noteworthy to highlight that C was not affected either by the SS10 temperature, nor by MHW.

Therefore, changes on FAs due to MHW and SS10 temperature during summer can also potentially shape the herbivore and detritivore activity and nutrition. The outcomes of this study indicate that sharp increases in temperature reduce in few days the unsaturation levels of *P. oceanica* photosynthetic structures, as it was previously reported in other primary producers, enabling optimal membrane fluidity and functionality (Beca-Carretero et al., 2018, 2021; Britton et al., 2020; Franzitta et al., 2021). Reductions in unsaturation levels were explained by decreases in PUFA, and particularly reductions in n-3 PUFA (C18:3n-3) relative to n-6 PUFA (C18:2n-6). PUFAs can be stored in any organism but accumulation will change according to ambient environmental conditions: their requirement in the diet will occur only if there is not a sufficient quantity already stored, if they cannot be produced from

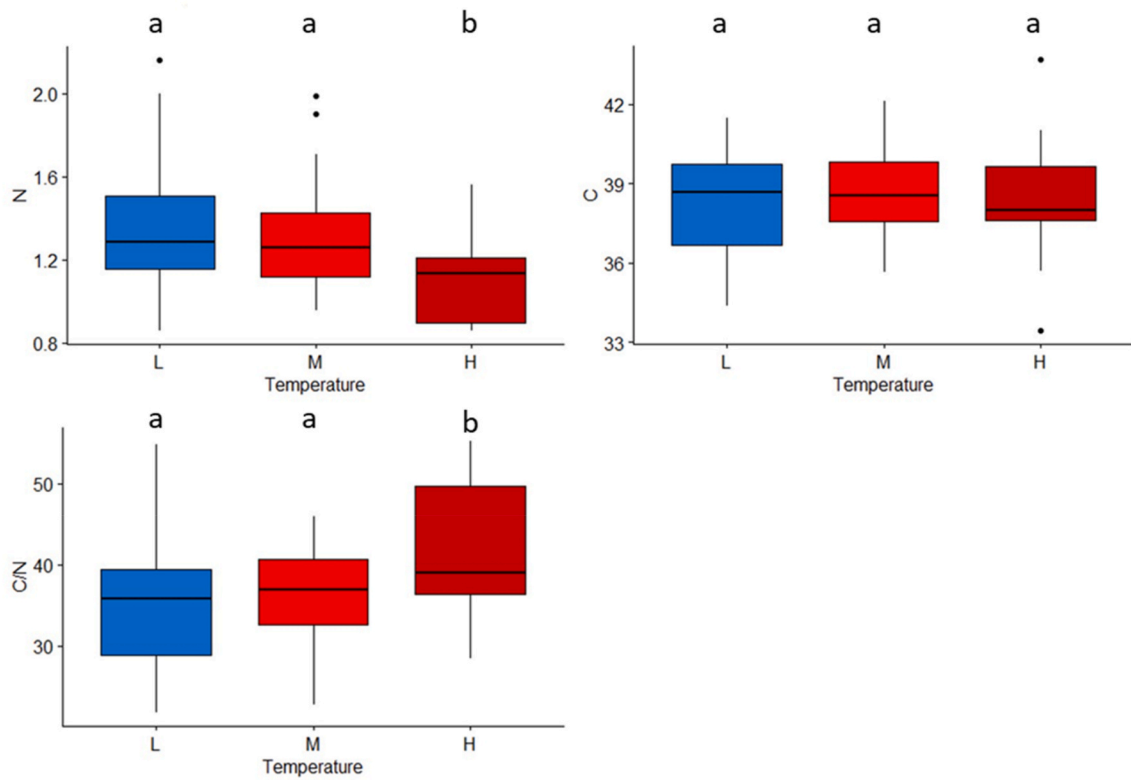


Fig. 2. Boxplots depicting the effect of SS10 temperature (L = low, M = medium and H = high) on the N, C and C/N *P. oceanica* leaf content. C and N are expressed as percentage.

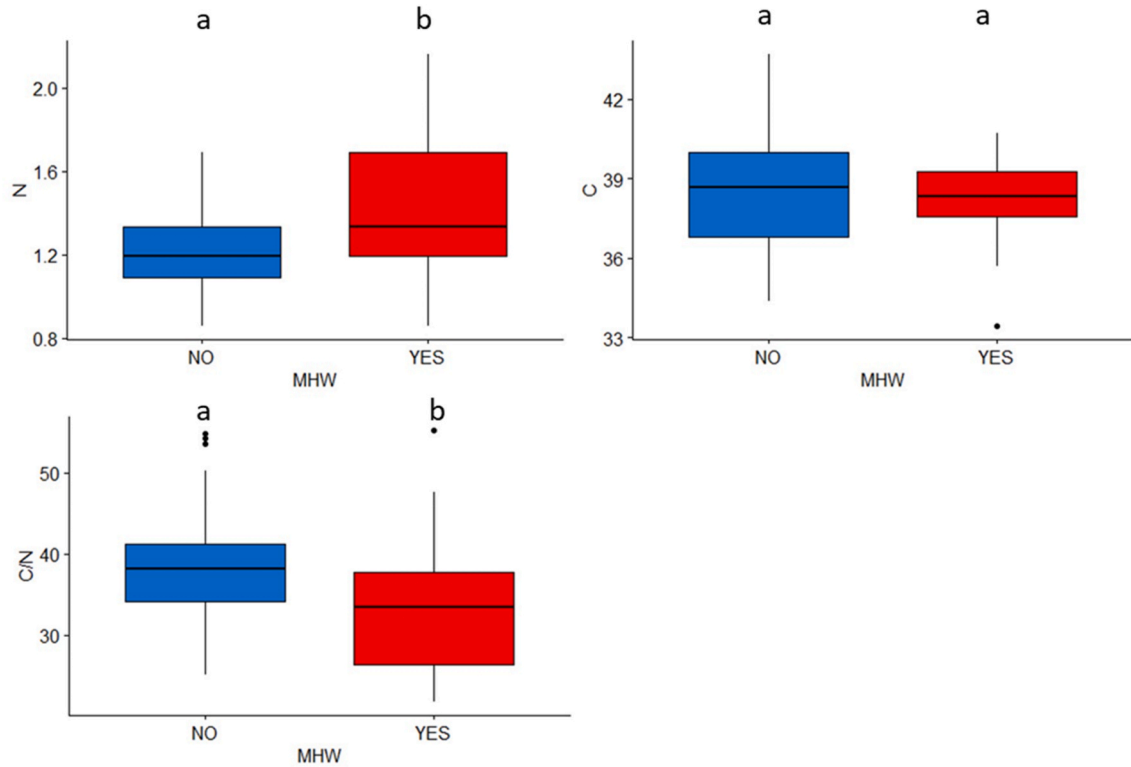


Fig. 3. Boxplots depicting the effect of MHW occurrence (YES or NO) on the N, C and C/N *P. oceanica* leaf content. C and N are expressed as percentage.

Table 2

PERMANOVA results testing the effect of SS10 temperature (High, Medium, and Low) and Marine Heat Wave (MHW) occurrence (Yes and No) on fatty acid content (SFA, MUFA, PUFA, PUFA/SFA, MUFA/SFA) of *P. oceanica* leaves; pairwise tests for the significant results. L = low; M = medium; H = high. Bold refers to significant F-values.

PERMANOVAs	Temperature	MHW
variables	<i>Pseudo-F</i> _{2,78}	<i>Pseudo-F</i> _{1,78}
SFA	1.61	1.93
MUFA	4.78*	31.67*
PUFA	1.43	6.55*
PUFA/SFA	1.90	3.64
MUFA/PUFA	2.44	26.81*
PAIR-WISE TESTS		
MUFA	L < M = H	YES > NO
PUFA		YES < NO
MUFA/PUFA		YES > NO

other compounds, or if they cannot be replaced by other compounds (Parrish, 2009). Reductions in unsaturation levels were also supported by the reductions in the thermal biomarker C18:3n-3/C16:0, as it was already found in the first stages of development of *P. oceanica* (Stipcich et al., 2022c). This ratio can detect changes in unsaturation/saturation levels of photosynthetic structures since C18:3n-3 is the most abundant PUFA with the highest unsaturation level and C16:0 is the most abundant SFA present in seagrasses (Parrish, 2009). The opposite pattern between n-3 and n-6 depends on the same enzyme used for the synthesis of n-3 and n-6 which, under high temperature conditions, tends to favor n-6 over n-3 production (Hixson and Arts, 2016; Beca-Carretero et al., 2022). Disproportions in the n-3/n-6 can be detrimental to the immune response and cardiovascular health of vertebrates (Hixson and Arts, 2016), since the n-6 is a precursor to inflammatory eicosanoids and the n-3 is a precursor to anti-inflammatory eicosanoids (Arts and Kohler, 2009).

Based on information derived from previous manipulative experiments, SFA content was expected to increase with MHW occurrence (Beca-Carretero et al., 2018; Britton et al., 2020; Franzitta et al., 2021); however, in the present study we did not observe any significant change in SFA nor in the 16:0 FA. Usually, SFA in primary producers increases with increasing temperature to make the membranes more rigid, and thus maintaining the required fluidity and functionality (Gounaris and

Barber, 1985; Duarte et al., 2018). The lack of SFA changes in this study opens several questions regarding the sensitivity of this FA group and whether detecting SFA changes would depend on the magnitude of temperature variation that happen in the field rather than in mesocosms. Nevertheless, the lack of a significant effect of MHWs on SFA could also be due to the plant material analyzed (i.e. the second leaf): a significant change in SFA content was in fact found between the second and the fifth leaf of *P. oceanica* shoot (Pansini et al., submitted), suggesting that the sensitivity of SFA to thermal changes increases with the age of the leaf. However, in this study we observed an increase of MUFA rather than SFA. MUFA may increase in cell membranes in response to temperature (Fuschino et al., 2011) depending on the FA composition during the time of temperature adaptation, which is subject to nutrient availability and other environmental conditions (Hixson and Arts, 2016).

The MUFA/PUFA ratio, here tested for the first time in a seagrass, highlighted a significant response to thermal conditions, supporting the use of this ratio as a qualitative biomarker for marine trophic relationship (Daza et al., 2005). Most of the MUFAs and PUFAs are structural lipids, forming the membranes of the cellular organelles and membranes of the thylakoid that change with temperature (Kumari et al., 2013), but many PUFAs, including n-3 PUFA, are also essential dietary constituents for many marine animals which cannot synthesize PUFA *de novo* from MUFA (Harwood and Moore, 1989; Parrish, 2009; Beca-Carretero et al., 2020). Thus, it is important to consider that a large decrease in PUFA in *P. oceanica* leaves due to the temperature increase in the future, would reduce the nutritional value and likely bring a change in the herbivory preferences, affecting also their biological and physiological processes such as cognitive or reproductive functions (Burri et al., 2012; Kumar et al., 2022).

Furthermore, an acclimation to temperature of *P. oceanica* leaf FAs composition was suggested since no significant differences were found in PUFA and SFA across the temperature gradient (H, M and L temperature). The differences were found only in MUFA, where at M and H temperature, they exhibited similar values which were higher compared to those in L. The reported increases in MUFA after the MHW, and its higher values at H temperature, can be explained by the role of this group as precursor of PUFA (Wallis et al., 2002) and it seems the fastest to be converted as soon as the conditions change.

In conclusion, the results of this study suggest that the biochemical composition (FAs composition and C and N content) of *P. oceanica* leaves can be expected to change with ocean warming, and such changes likely

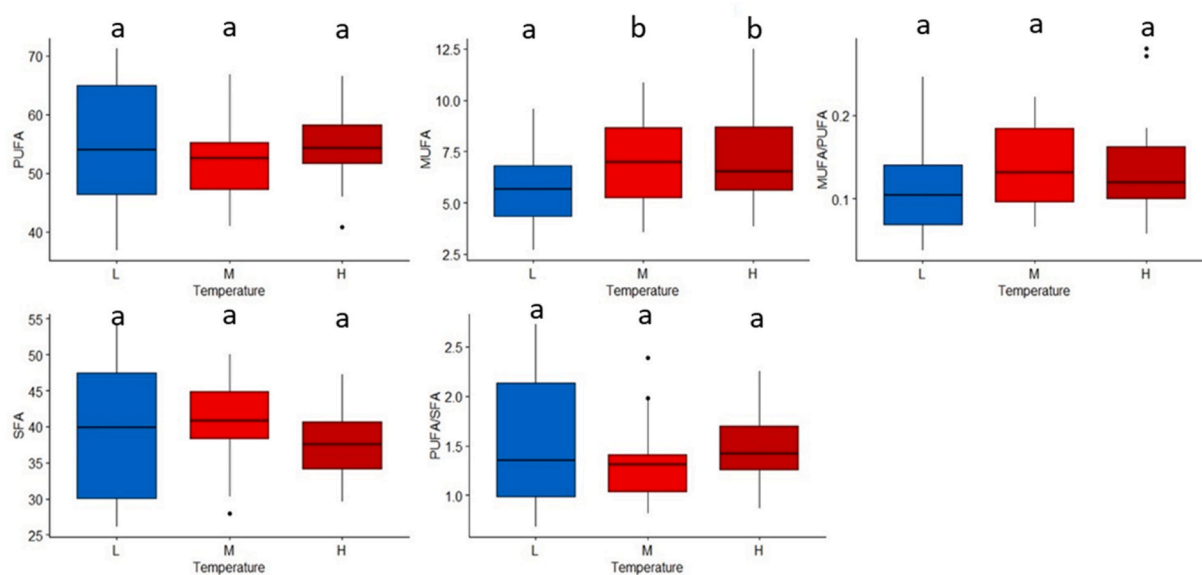


Fig. 4. Boxplots depicting the effect of SS10 temperature (L = low, M = medium and H = high) on the PUFA, MUFA, SFA, MUFA/PUFA and PUFA/SFA *P. oceanica* leaf content. PUFA, MUFA and SFA are expressed as percentage.

Table 3

SIMPER test results on the effect of SS10 temperature (L = low, M = medium, and H = high) on *P. oceanica* leaves, which have identified the most contributing FAs to dissimilarities (in parenthesis the percentage contribution, 90% cut off). PERMANOVA results testing the effect of Temperature (High, Medium, and Low) on single FA content (C 16:0; C 18:2 n-6; C 18:3 n-3; C18:3 n-3/C16:0; n-3/n-6) of *P. oceanica* leaves; pairwise tests for the significant results. Bold refers to significant F-values.

SIMPER test	contribution of variables
H vs L	C 18:3 n-3 (68.19%); C18:2 n-6 (17.06%); C16:0 (10.22%)
H vs M	C 18:3 n-3 (61.44%); C18:2 n-6 (16.52%); C16:0 (12.82%)
L vs M	C 18:3 n-3 (69.97%); C18:2 n-6 (15.71%); C16:0 (10.52%)
PERMANOVAs	
variables	Temperature
C18:3 n-3	<i>Pseudo-F</i> _{2,78} 3.52*
C18:2 n-6	5.89*
C16:0	0.53
C18:3 n-3/C16:0	3.81*
n-3/n-6	6.28*
PAIR-WISE TESTS	
C18:3 n-3	L = M = H
C18:2 n-6	L = M = H
C18:3 n-3/C16:0	L = M = H
n-3/n-6	L = M = H

modify the nutritional value of these primary producers with anticipated effects on the food chain. Further studies should investigate leaf epiphytes (here disregarded) which represent an important component of the nutritional quality (Alcoverro et al., 1997; Vizzini et al., 2002; Hernán et al., 2019) and likely palatability. Furthermore, the association of epiphyte community and *P. oceanica* leaf biochemistry should be investigated, as changes in leaf metabolites could correspond to changes in epibiota through direct or indirect mechanisms. In turn, leaf epibionts will also be impacted by temperature, nutrient and pH change in the ambient seawater, suggesting complex effects due to multiple climate change and local stressors. Overall, this study provides a first insight into the spatio-temporal variability at biochemical level in response to ambient temperature regimes of the most important seagrass of the Mediterranean Sea highlighting several remaining knowledge gaps and suggesting directions of future research.

Funding

This research was financially supported by Botany and Plant Science at University of Galway, by the project Marine Habitats Restoration in a Climate Change-impaired Mediterranean Sea [MAHRES] funded by the Italian Ministry of Research under the PRIN 2017 Program (Project N. 2017MHHWBN; CUP: 74119001320001), by “Fondo di Ateneo per la Ricerca 2019” by the University of Sassari, by PON - National Operational Programme - Research and Innovation 2014–2020, PhDs and research contracts on innovation-related topics and by the project DRESSAGE (MIS5045792) (through the Operational Program ‘Competitiveness, Entrepreneurship and Innovation’ (EPAnEK 2014–2020).

Author contributions

Patrizia Stipcich: Conceptualization, Data curation, Formal analysis, Writing - original draft, review & editing; Funding acquisition

Table 4

SIMPER test results on the effect of Marine Heat Waves (Yes or No) on *P. oceanica* leaves, which have identified the most contributing FAs to dissimilarities (in parenthesis the percentage contribution, 90% cut off). PERMANOVA results on raw data in single FA content (C 16:0; C 18:2 n-6; C 18:3 n-3; C18:3 n-3/C16:0; n-3/n-6) of *P. oceanica* leaves; pairwise tests for the significant results. Bold refers to significant F-values.

SIMPER test	contribution of variables
NO vs YES	C 18:3 n-3 (68.42%); C18:2 n-6 (15.95%); C16:0 (10.79%)
PERMANOVAs	
variables	MHW
C18:3 n-3	<i>Pseudo-F</i> _{1,78} 10.55*
C18:2 n-6	10.10*
C16:0	2.34
C18:3 n-3/C16:0	7.81*
n-3/n-6	8.39*
PAIR-WISE TESTS	
C18:3 n-3	YES < NO
C18:2 n-6	YES > NO
C18:3 n-3/C16:0	YES < NO
n-3/n-6	YES < NO

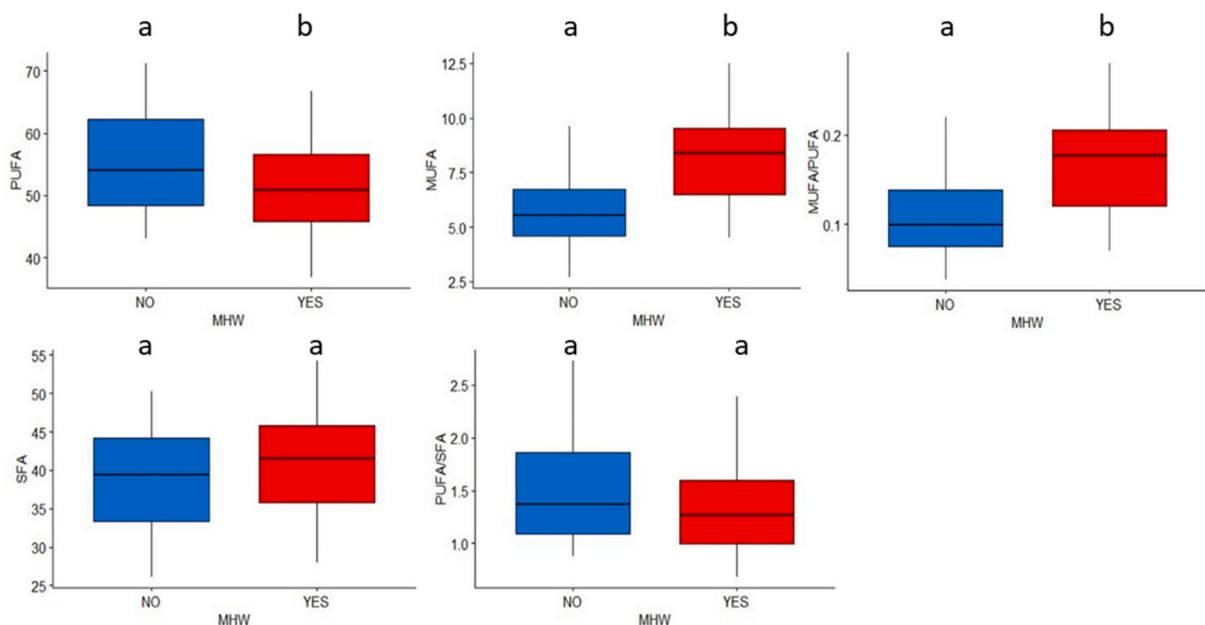


Fig. 5. Boxplots depicting the effect of MHW occurrence (YES or NO) on PUFA, MUFA, SFA, MUFA/PUFA and PUFA/SFA *P. oceanica* leaf content. PUFA, MUFA and SFA are expressed as percentage.

Pedro Beca-Carretero: Data curation, Writing - original draft; **Xosé Antón Álvarez-Salgado, Dagmar B. Stengel:** Funding acquisition; **Eugenia T. Apostolaki, Niki Chartosia, Pavlos Theofilos Efthymiadis, Carlos E. Jimenez, Gabriella La Manna, Arianna Pansini, Elena Principato, Vasilis Resaikos:** Data curation; **Giulia Ceccherelli:** Conceptualization, Writing - original draft, review & editing; Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank the Department of Fisheries and Marine Research (DFMR) and the Department of Environment of Cyprus for giving us on time, all the appropriate permits to study *Posidonia oceanica* meadows in Cyprus. We also sincerely thank Daniel and Tamara from Cyprus Diving Center, George from Blue Instinct, Dino from Karpathos Diving Center and Simone from Pelagos 2.0 Lampedusa for helping and supporting us in the field.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2022.105854>.

References

- Alcoverro, T., Duarte, C.M., Romero, J., 1997. The influence of herbivores on *Posidonia oceanica* epiphytes. *Aquat. Bot.* 56 (2), 93–104.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* 26 (1), 32–46.
- Arts, M.T., Kohler, C.C., 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. *Lipids in Aquatic Ecosystems* 237–256.
- Atkinson, M.J., Smith, S.V., 1983. C: N: P ratios of benthic marine plants 1. *Limnol. Oceanogr.* 28 (3), 568–574.
- Beca-Carretero, P., Guihéneuf, F., Marín-Guirao, L., Bernardeau-Esteller, J., García-Munoz, R., Stengel, D.B., Ruiz, J.M., 2018. Effects of an experimental heat wave on fatty acid composition in two Mediterranean seagrass species. *Mar. Pollut. Bull.* 134, 27–37.
- Beca-Carretero, P., Guihéneuf, F., Krause-Jensen, D., Stengel, D.B., 2020. Seagrass fatty acid profiles as a sensitive indicator of climate settings across seasons and latitudes. *Mar. Environ. Res.* 161, 105075.
- Beca-Carretero, P., Azcárate-García, T., Julia-Miralles, M., Stanschewski, C.S., Guihéneuf, F., Stengel, D.B., 2021. Seasonal acclimation modulates the impacts of simulated warming and light reduction on temperate seagrass productivity and biochemical composition. *Front. Mar. Sci.* 8, 731152.
- Beca-Carretero, P., Azcárate-García, T., Teichberg, M., Patra, P., Feroze, F., González, M. J., et al., 2022. Predicted warming intensifies the negative effects of nutrient increase on tropical seagrass: a physiological and fatty acid approach. *Ecol. Indic.* 142, 109184.
- Bergé, J.P., Barnathan, G., 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Mar. Biotechnol.* 1, 49–125.
- Britton, D., Schmid, M., Noisette, F., Havenhand, J.N., Paine, E.R., McGraw, C.M., et al., 2020. Adjustments in fatty acid composition is a mechanism that can explain resilience to marine heatwaves and future ocean conditions in the habitat-forming seaweed *Phyllospora comosa* (Labillardière) C. Agardh. *Global Change Biol.* 26 (6), 3512–3524.
- Brown, M.B., Edwards, M.S., Kim, K.Y., 2014. Effects of climate change on the physiology of giant kelp, *Macrocystis pyrifera*, and grazing by purple urchin, *Strongylocentrotus purpuratus*. *ALGAE* 29 (3), 203–215.
- Buñuel, X., Alcoverro, T., Romero, J., Arthur, R., Ruiz, J.M., Pérez, M., et al., 2021. Warming intensifies the interaction between the temperate seagrass *Posidonia oceanica* and its dominant fish herbivore *Sarpa salpa*. *Mar. Environ. Res.* 165, 105237.
- Bulgin, C.E., Merchant, C.J., Ferreira, D., 2020. Tendencies, variability and persistence of sea surface temperature anomalies. *Sci. Rep.* 10 (1), 1–13.
- Burri, L., Hoem, N., Banni, S., Berge, K., 2012. Marine omega-3 phospholipids: metabolism and biological activities. *Int. J. Mol. Sci.* 13 (11), 15401–15419.
- Chikaraishi, Y., Ogawa, N.O., Kashiya, Y., Takano, Y., Suga, H., Tomitani, A., et al., 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol. Oceanogr. Methods* 7 (11), 740–750.
- Core Team, R.C.T.R., 2013. R: A Language and Environment for Statistical Computing. R Foundation for statistical computing, Vienna.
- Coull, B.C., 1990. Are members of the meiofauna food for higher trophic levels? *Trans. Am. Microsc. Soc.* 233–246.
- Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment-1 introduction. *Adv. Mar. Biol.* 46, 227–237.
- Darmaraki, S., Somot, S., Sevault, F., Nabat, P., 2019. Past variability of Mediterranean Sea marine heatwaves. *Geophys. Res. Lett.* 46, 9813–9823.
- Daza, A., Rey, A.I., Ruiz, J., Lopez-Bote, C.J., 2005. Effects of feeding in free-range conditions or in confinement with different dietary MUFA/PUFA ratios and α -tocopheryl acetate, on antioxidants accumulation and oxidative stability in Iberian pigs. *Meat Sci.* 69 (1), 151–163.
- de los Santos, C., Brun, F.G., Onoda, Y., Cambridge, M.L., Bouma, T.J., Vergara, J.J., Pérez-Lloréns, J.L., 2012. Leaf-fracture properties correlated with nutritional traits in nine Australian seagrass species: implications for susceptibility to herbivory. *Mar. Ecol. Prog. Ser.* 458, 89–102.
- Diffenbaugh, N.S., Pal, J.S., Giorgi, F., Gao, X., 2007. Heat stress intensification in the Mediterranean climate change hotspot. *Geophys. Res. Lett.* 34 (11).
- Duarte, C.M., 1990. Seagrass nutrient content. *Marine Ecol. Progress Ser. Oldendorf.* 6 (2), 201–207.
- Duarte, C.M., 2002. The future of seagrass meadows. *Environ. Conserv.* 29 (2), 192–206.
- Duarte, B., Carreiras, J., Pérez-Romero, J.A., Mateos-Naranjo, E., Redondo-Gomez, S., Matos, A.R., et al., 2018. Halophyte fatty acids as biomarkers of anthropogenic-driven contamination in Mediterranean marshes: sentinel species survey and development of an integrated biomarker response (IBR) index. *Ecol. Indic.* 87, 86–96.
- Franzitta, M., Repolho, T., Paula, J.R., Cacador, I., Matos, A.R., Rosa, R., Duarte, B., 2021. Dwarf eelgrass (*Zostera noltii*) fatty acid remodelling induced by climate change. *Estuar. Coast Shelf Sci.* 261, 107546.
- Friend, A.D., 2010. Terrestrial plant production and climate change. *J. Exp. Bot.* 61 (5), 1293–1309.
- Frölicher, T.L., Laufkötter, C., 2018. Emerging risks from marine heat waves. *Nat. Commun.* 9 (1), 1–4.
- Fuschino, J.R., Guschina, I.A., Dobson, G., Yan, N.D., Harwood, J.L., Arts, M.T., 2011. Rising water temperatures alter lipid dynamics and reduce n-3 essential fatty acid concentrations in *Scenedesmus obliquus* (Chlorophyta). *J. Phycol.* 47 (4), 763–774.
- Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., et al., 2022. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Global Change Biol.* 28 (19), 5708–5725.
- Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. *Global Planet. Change* 63 (2–3), 90–104.
- Giraud, G., 1977. Contribution à la description et à la phenologie quantitative des herbiers de *Posidonia oceanica* (L.) Delile. In: Doctorat de spécialité en oceanologie, Université Aix-Marseille II, Fac. Des Sciences de Lumy, Marseille, pp. 1–50.
- Gounaris, K., Barber, J., 1985. Isolation and characterisation of a photosystem II reaction centre lipoprotein complex. *FEBS Lett.* 188 (1), 68–72.
- Harwood, J., Moore Jr., T.S., 1989. Lipid metabolism in plants. *Crit. Rev. Plant Sci.* 8 (1), 1–43.
- Hemminga, M.A., Duarte, C.M., 2000. *Seagrass Ecology*. Cambridge University Press.
- Hernán, G., Ortega, M.J., Gándara, A.M., Castejón, I., Terrados, J., Tomas, F., 2017. Future warmer seas: increased stress and susceptibility to grazing in seedlings of a marine habitat-forming species. *Global Change Biol.* 23 (11), 4530–4543.
- Hernán, G., Castejón, I., Terrados, J., Tomas, F., 2019. Herbivory and resource availability shift plant defense and herbivore feeding choice in a seagrass system. *Oecologia* 189 (3), 719–732.
- Hixson, S.M., Arts, M.T., 2016. Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Global Change Biol.* 22 (8), 2744–2755.
- Hobday, A.J., Alexander, L.V., Perkins, S.E., Smale, D.A., Straub, S.C., Oliver, E.C., et al., 2016. A hierarchical approach to defining marine heatwaves. *Prog. Oceanogr.* 141, 227–238.
- Hulme, M., 2014. Attributing weather extremes to ‘climate change’ A review. *Prog. Phys. Geogr.* 38 (4), 499–511.
- Jiang, Z., Huang, D., Fang, Y., Cui, L., Zhao, C., Liu, S., et al., 2020. Home for Marine species: seagrass leaves as vital spawning grounds and food source. *Front. Mar. Sci.* 7, 194.
- Keppay, P.E., Jellett, J.F., Niven, S.E.H., 1997. Respiration and the carbon-to-nitrogen ratio of a phytoplankton bloom. *Mar. Ecol. Prog. Ser.* 150, 249–261.
- Kumar, N., Chandan, N.K., Gupta, S.K., Bhushan, S., Patole, P.B., 2022. Omega-3 fatty acids effectively modulate growth performance, immune response, and disease resistance in fish against multiple stresses. *Aquaculture* 547, 737506.
- Kumari, P., Kumar, M., Reddy, C.R.K., Jha, B., 2013. Algal lipids, fatty acids and sterols. In: *Functional Ingredients from Algae for Foods and Nutraceuticals*. Woodhead Publishing, pp. 87–134.
- Larkum, A.W., Orth, R.J., Duarte, C.M., 2006. Seagrasses: biology, ecology and conservation. *Phycologia* 45 (5), 5.

- Lee, K.S., Short, F.T., Burdick, D.M., 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. *Aquat. Bot.* 78 (3), 197–216.
- Leisner, C.P., 2020. Climate change impacts on food security-focus on perennial cropping systems and nutritional value. *Plant Sci.* 293, 110412.
- Lin, D., Xia, J., Wan, S., 2010. Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. *New Phytol.* 188 (1), 187–198.
- Lourenço, S.O., Barbarino, E., Marquez, U.M.L., Aidar, E., 1998. Distribution of intracellular nitrogen in marine microalgae: basis for the calculation of specific nitrogen-to-protein conversion factors. *J. Phycol.* 34 (5), 798–811.
- Lotze, H.K., Tittensor, D.P., Brydum-Buchholz, A., Eddy, T.D., Cheung, W.W., Galbraith, E.D., et al., 2019. Global ensemble projections reveal trophic amplification of ocean biomass declines with climate change. *Proc. Natl. Acad. Sci. USA* 116 (26), 12907–12912.
- Lowman, H.E., Emery, K.A., Dugan, J.E., Miller, R.J., 2021. Nutritional Quality of Giant Kelp Declines Due to Warming Ocean Temperatures (Oikos).
- Malzahn, A.M., Hantzsche, F., Schoo, K.L., Boersma, M., Aberle, N., 2010. Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. *Oecologia* 162 (1), 35–48.
- Marbà, N., Duarte, C.M., 2010. Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Global Change Biol.* 16 (8), 2366–2375.
- Mazzella, C., Durkin, K., Cerini, E., Buralli, P., 1992. Sex role stereotyping in Australian television advertisements. *Sex. Roles* 26 (7), 243–259.
- Newell, R.C., Branch, G.M., 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. In: *Advances in Marine Biology*, vol. 17. Academic Press, pp. 329–396.
- Nichols, D.S., 2003. Prokaryotes and the input of polyunsaturated fatty acids to the marine food web. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 219 (1), 1–7.
- Oliver, E.C., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L.V., et al., 2018. Longer and more frequent marine heatwaves over the past century. *Nat. Commun.* 9 (1), 1–12.
- Orth, R.J., Carruthers, T.J., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., et al., 2006. A global crisis for seagrass ecosystems. *Bioscience* 56 (12), 987–996.
- Palumbi, S.R., Evans, T.G., Pespeni, M.H., Somero, G.N., 2019. Present and future adaptation of marine species assemblages. *Oceanography* 32 (3), 82–93.
- Pansini, A., La Manna, G., Pinna, F., Stipcich, P., Ceccherelli, G., 2021. Trait gradients inform predictions of seagrass meadows changes to future warming. *Sci. Rep.* 11 (1), 1–12.
- Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: *Lipids in Aquatic Ecosystems*. Springer, New York, NY, pp. 309–326.
- Pastor, F., Valiente, J.A., Palau, J.L., 2019. Sea surface temperature in the Mediterranean: trends and spatial patterns (1982–2016). In: *Meteorology and Climatology of the Mediterranean and Black Seas*. Birkhäuser, Cham, pp. 297–309.
- Pazzaglia, J., Santillán-Sarmiento, A., Helber, S.B., Ruocco, M., Terlizzi, A., Marín-Guirao, L., Procaccini, G., 2020. Does warming enhance the effects of eutrophication in the seagrass *Posidonia oceanica*? *Front. Mar. Sci.* 7, 564805.
- Peirano, A., Cocito, S., Banfi, V., Cupido, R., Damasso, V., Farina, G., et al., 2011. Phenology of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile: medium and long-term cycles and climate inferences. *Aquat. Bot.* 94 (2), 77–92.
- Pirc, H., Wollenweber, B., 1988. Seasonal changes in nitrogen, free amino acids, and C/N ratio in Mediterranean seagrasses. *Mar. Ecol.* 9 (2), 167–179.
- Pond, C.M., 1998. *The Fats of Life*. Cambridge University Press.
- Rabbani, S., Beyer, P., Lintig, J.V., Huguency, P., Kleinig, H., 1998. Induced β -carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. *Plant Physiol.* 116 (4), 1239–1248.
- Ramlee, A., Chembarathy, M., Gunaseelan, H., Yatim, S.R.M., Taufek, H., Rasdi, N.W., 2021. Enhancement of nutritional value on zooplankton by alteration of algal media composition: a review. In: *IOP Conference Series: Earth and Environmental Science*, vol. 869. IOP Publishing, No. 1, p. 012006.
- Ruckelshaus, M., English, C.A., Chan, F., Grebmeier, J.M., Hollowed, A.B., Galindo, H. M., et al., 2011. Climate change impacts on marine ecosystems. *Ann. Rev. Mar. Sci.* 4 (11), 37.
- Sanina, N.M., Goncharova, S.N., Kostetsky, E.Y., 2008. Seasonal changes of fatty acid composition and thermotropic behavior of polar lipids from marine macrophytes. *Phytochemistry* 69 (7), 1517–1527.
- Sardans, J., Rivas-Ubach, A., Peñuelas, J., 2012. The C: N: P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. *Perspect. Plant Ecol. Evol. Systemat.* 14 (1), 33–47.
- Sargent, J.R., Bell, M.V., Bell, J.G., Henderson, R.J., Tocher, D.R., 1995. Origins and functions of n-3 polyunsaturated fatty acids in marine organisms. In: *Phospholipids: Characterization, Metabolism, and Novel Biological Applications: Proceedings of the 6th International Colloquium*. American Oil Chemists Society, Urbana, IL, pp. 248–259.
- Schlegel, R.W., Smit, A.J., 2018. heatwaveR: a central algorithm for the detection of heatwaves and cold-spells. *J. Open Source Software*. 3 (27), 821.
- Scott, Chamberlain, 2021. Rerddap: General Purpose Client for 'ERDDAP' Servers. R Package Version 0, vol. 7, p. 6.
- Smale, D.A., Wernberg, T., Oliver, E.C., Thomsen, M., Harvey, B.P., Straub, S.C., et al., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat. Clim. Change* 9 (4), 306–312.
- Soto-Navarro, J., Jordá, G., Amores, A., Cabos, W., Somot, S., Sevaut, F., et al., 2020. Evolution of Mediterranean Sea water properties under climate change scenarios in the Med-CORDEX ensemble. *Clim. Dynam.* 54 (3), 2135–2165.
- Stipcich, P., Apostolaki, E.T., Chartosia, N., Efthymiadis, P.T., Jimenez, C.E., La Manna, G., Pansini, A., Principato, E., Resaikos, V., Ceccherelli, G., 2022a. Assessment of *Posidonia oceanica* traits along a temperature gradient in the Mediterranean Sea shows impacts of marine warming and heat waves. *Front. Mar. Sci.* 9, 895354.
- Stipcich, P., Marín-Guirao, L., Pansini, A., Pinna, F., Procaccini, G., Pusceddu, A., et al., 2022b. Effects of current and future summer marine heat waves on *Posidonia oceanica*: plant origin matters? *Front. Climate*. 4, 844831.
- Stipcich, P., Pansini, A., Beca-Carretero, P., Stengel, D.B., Ceccherelli, G., 2022c. Field thermo acclimation increases the resilience of *Posidonia oceanica* seedlings to marine heat waves. *Mar. Pollut. Bull.* 184, 114230.
- Tan, K., Zhang, H., Zheng, H., 2022. Climate change and n-3 LC-PUFA availability. *Prog. Lipid Res.*, 101161.
- Telesca, L., Belluscio, A., Criscoli, A., Ardizzone, G., Apostolaki, E.T., Fraschetti, S., et al., 2015. Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of change. *Sci. Rep.* 5 (1), 1–14.
- Teoh, M.L., Chu, W.L., Phang, S.M., 2010. Effect of temperature change on physiology and biochemistry of algae: a review. *Malays. J. Sci.* 29 (2), 82–97.
- Tomas, F., Turon, X., Romero, J., 2005. Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Mar. Ecol. Prog. Ser.* 301, 95–107.
- Twining, C.W., Brenna, J.T., Hairston Jr., N.G., Flecker, A.S., 2016. Highly unsaturated fatty acids in nature: what we know and what we need to learn. *Oikos* 125 (6), 749–760.
- Unsworth, R.K., Collier, C.J., Waycott, M., McKenzie, L.J., Cullen-Unsworth, L.C., 2015. A framework for the resilience of seagrass ecosystems. *Mar. Pollut. Bull.* 100 (1), 34–46.
- Van Alstyne, K.L., Pelletreau, K.N., Kirby, A., 2009. Nutritional preferences override chemical defenses in determining food choice by a generalist herbivore, *Littorina sitkana*. *J. Exp. Mar. Biol. Ecol.* 379 (1–2), 85–91.
- Viana, I.G., Moreira-Saporiti, A., Teichberg, M., 2020. Species-specific trait responses of three tropical seagrasses to multiple stressors: the case of increasing temperature and nutrient enrichment. *Front. Plant Sci.* 11, 571363.
- Vizzini, S., Sara, G., Michener, R.H., Mazzola, A.J.A.O., 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta Oecol.* 23 (4), 277–285.
- Wallis, J.G., Watts, J.L., Browne, J., 2002. Polyunsaturated fatty acid synthesis: what will they think of next? *Trends Biochem. Sci.* 27 (9), 467–473.
- Yuan, Z.Y., Chen, H.Y., 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. *Nat. Clim. Change* 5 (5), 465–469.
- Zhang, P., Grutters, B.M., Van Leeuwen, C.H., Xu, J., Petruzzella, A., Van den Berg, R.F., Bakker, E.S., 2019. Effects of rising temperature on the growth, stoichiometry, and palatability of aquatic plants. *Front. Plant Sci.* 9, 1947.
- Zhou, X., Chen, N., Yan, Z., Duan, S., 2016. Warming increases nutrient mobilization and gaseous nitrogen removal from sediments across cascade reservoirs. *Environ. Pollut.* 219, 490–500.