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Stress due to low nitrate availability reduces the biochemical acclimation potential of the giant kelp *Macrocystis pyrifera* to high temperature

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ABSTRACT

The performance and survival of macroalgae is largely determined by their ability to adjust to varying environmental conditions. In this study, we investigated the short-term response of the giant kelp *Macrocystis pyrifera* to varying temperatures (6, 17 and 24 °C) and low and high nitrate conditions (5 μM and 80 μM nitrate) on lipid and fatty acid levels. Results revealed that *M. pyrifera* was able to rapidly adjust to varying temperatures by changing the saturation level of the fatty acid composition at low and high nitrate conditions. On a lipid level, we observed interactive effects of temperature and nutrient conditions. Under high nitrate conditions, *M. pyrifera* maintained the same lipid profile. However, under low nitrate and high temperature conditions, an increase in free fatty acids (FFA) was observed, indicative of lipid degeneration at high temperatures. Results show that low nitrogen concentrations can magnify the negative effects of short term temperature stress in the giant kelp *M. pyrifera*. Our findings indicate that under rapid warming events, local nitrate availability might be a decisive factor for the acclimation potential of *M. pyrifera*.

1. Introduction

The seaweed habitat in the coastal inter- and subtidal zone is characterized by large and frequent fluctuations in abiotic conditions, including temperature, light and nutrient availability [1]. However, this natural environmental dynamic in coastal environments can be under strong anthropogenic influence, causing major changes at global (e.g., Ocean Warming, OW) and local (e.g., eutrophication) scales [2–4]. The alteration of local regimes of nutrient availability as a result of anthropogenic activities (e.g., agriculture, industry and sewage disposal) can negatively impact marine organisms such as corals [5]. For seaweeds, however, increased nutrient availability will play an important role in regulating responses to environmental stressors, such as OW [6–8]. Sufficient inorganic nutrient input, particularly nitrate (NO_3^-) and phosphate (PO_4^{3-}), are essential to support macroalgal health [9], and can counteract the negative effects of stressful conditions [7].

The current rates of OW, as well as the gradual increase in the frequency and intensity of marine heatwaves [10], are influencing the functioning, survival and local persistence of marine organisms [11,12]. Marine heat waves can be a significant stressor for marine life [7], especially in transition zones where organisms are at their upper temperature limits, and can lead to mass mortality of whole populations [13]. In recent years, seaweeds, especially large kelp species such as *Macrocystis pyrifera* (L.) C. Agardh *and Ecklonia radiata* (C. Agardh) J. Agardh (Order Laminariales) have exhibited a massive decline in various areas around the globe [11]. In most cases, these declines have been associated in part with marine heatwave events [14,15]. Kelps are ecosystem engineers [16] that create and support complex biogenic habitats, provide food for various marine organisms and ecosystem services in the form of carbon cycling and nutrient cycling [17]. In temperate regions of both Northern and Southern hemispheres, the

Abbreviations: DW, dry weight; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FFA, free fatty acids; Fx, fucoxanthin; GC-FID, gas chromatography-flame ionisation detector; MUFA, monounsaturated fatty acids; PL, polar lipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; ST, sterols; TAG, triacylglycerol; TFA, total fatty acids; TL, total lipids

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subtidal kelp *M. pyrifera* plays a crucial role to the functioning of the coastal ecosystem, as a foundation species, food source and habitat [18]. Therefore, the observed rapid declines in kelp forests can have multiple downstream implications for the whole marine ecosystem [19]. For *M. pyrifera*, nitrate has been identified as the main limiting nutrient [20,21], influencing its physiological and life-history responses during high thermal stress [22,23]. There is, therefore, a pressing need to better understand kelp responses to stressors like temperature and nutrient conditions and their interactions.

One major strategy of plants and algae to acclimate to changing environmental conditions is the adjustment of the composition and structure of cellular membranes [24]. Membrane lipids play a crucial role in both temperature-sensing and acclimation, which is achieved by altering the lipid and fatty acid profile [25–27]. High temperature can lead to a "hyper fluidity" or disintegration of the lipid bilayer of cellular membranes, with negative effects on cellular processes including photosynthesis [24]. However, organisms can counteract this by adjusting the fatty acid composition towards higher proportions of saturated fatty acids, helping to stabilize cellular membranes at high temperature [26–28]. This can be achieved by reducing the activity of various desaturases or alternatively by the up-regulation of the *de novo* production of saturated fatty acids (SFA) [29]. SFA, in comparison to mono- or polyunsaturated fatty acids (MUFA and PUFA, respectively), have higher melting points, and can sustain membrane stability at higher temperatures.

In terrestrial plants high nitrate availability can alleviate negative impacts of high temperature stress on a lipid level by reducing lipid peroxidation ([30] and citations therein). For microalgae, there is an interaction between nitrogen availability and lipids, whereby nitrogen starvation results in increased lipid production [31]. Studies using transcriptome sequencing in the red alga *Pyropia* have identified fatty acids as important response for stress resistance [32]. But to the best of our knowledge, for seaweeds there are no published studies on how nitrate availability will affect such lipid based temperature responses and acclimation. However, kelps have been shown to perform better in response to heat stress under high nitrate conditions [23,33,34]. For terrestrial plants, increased nitrogen availability can increase thermal tolerance by facilitating an improved production of heat shock proteins and increased levels of antioxidants [35,36]. We therefore hypothesize that due to the facilitation of other temperature compensating mechanisms, under high nitrate availability, *M. pyrifera* will perform better at high temperature conditions by adjusting membrane lipids and fatty acids.

This study is builds on that of Fernández, Gaitán-Espitia, Leal, Schmid, Revill and Hurd [34], which examined the thermal response of *M. pyrifera* under high and low nitrate conditions, revealing that high nitrate conditions can ameliorate the negative effects of high temperature and support enhanced photosynthetic and growth rates [34]. The aim of this study was to determine if and how changes in the nitrogen status of *M. pyrifera* (deplete vs replete) can support biochemical changes in the algae, which allow for an improved response to temperature stress. To investigate this, using *M. pyrifera* that had been incubated under two nitrogen conditions (5 μM and 80 μM nitrate) and three temperatures treatments (6, 17 and 24 C), we examined the response on the fatty acid and lipid levels. The temperature conditions were chosen based on results of Fernández, Gaitán-Espitia, Leal, Schmid, Revill and Hurd [34], covering a maximum temperature range from 6 to 24 °C, where previous results showed positive growth and other physiological parameters still showed good health of the samples. We hypothesize that an exposure to different environmental conditions for only three days will result in biochemical changes in *M. pyrifera* on a lipid and fatty acid level. We also hypothesize that low nitrogen stress will enhance potential detrimental effects of high temperature in *M. pyrifera*. Results will help understand the short-term (~3 days) physiological responses and adjustments at a cellular level to temperature, and the mediation by nitrate in the ecologically important giant kelp *M. pyrifera*.

2. Material and methods

2.1. Seaweed collection and experimental design

Young blades (of a size of ca. 15–18 cm) of *M. pyrifera* samples were collected in the shallow subtidal (0–1.5 m) on Bruny Island (45°47′S, 170°43′E), Tasmania, Australia, in March 2016 and transported to the laboratory in an insulated container filled with ambient seawater. At the time of collection, temperature ranged from and 18.7–19.9 °C [34], which was ca. 3–4 °C above average, caused by longest recorded marine heat wave in Tasman Sea [37]. NO_{3-} concentrations in surface waters ranged from 0.14–0.53 μM NO_{3-} [34].

Samples were taken from adult sporophytes, sampling the second and third blades below the apical scimitar. In the laboratory, blades were cleaned of visible epibionts by washing with 0.5 μm filtered natural seawater (NSW). Each individual blade was cut to a similar size of ca. 6 cm length and 6 cm width with an initial fresh weight of 1.0 ± 0.2 g, at 2 cm from the neumatocyst/blade junction (meristematic zone). Using small cuttings of the meristematic area of kelp is a common approach for physiological experiments to understand kelp responses to different environmental conditions [38]. After a healing period of 12 h, blade sections were incubated for 3 days under low (5 μM) and enriched-NO_3^- concentrations (80 μM) at 17 °C to obtain *M. pyrifera* blades with different nitrogen status, i.e. deplete and replete, respectively. A 20 mM $NaNO_3$ solution was utilized to provide the desired NO_3^- concentrations in each culture tank, and 100 mM PO_4^- was used to avoid P limitation during the experiment (5:1 N:P). Six blade sections were placed into each of twelve 2 l-culture tanks, six containing low-NO_{3-}NSW and one containing enriched-NO_{3-}NSW. Water was bubbled with air and a saturating light intensity of 120–130 μmol m^{-2} s^{-1} was provided overhead by florescent white tubes (Envirolux CE F28T5/4100K-120477 240V) set on a 12L:12D photoperiod. Incident light was measured using a Li-Cor LI-1400 (LiCor Biosciences, Lincoln, NE USA) data logger equipped with a flat underwater radiation sensor LI-192.

After the incubation period, *M. pyrifera* blades previously incubated under low and enriched NSW (i.e. N-deplete and N-replete blades, respectively) were further incubated under seven different temperatures: 6-10-14-17-20-24-27 °C, and two nitrate concentrations: 5 μM and 80 μM. Of the 72 blades, 56 (28 of each treatment) were randomly selected and placed into each of 56 Erlenmeyer flasks (volume 250 ml), containing either low or enriched SW (blades from low N culture were put into low N treatment and blades from high N treatment into high N experimental vessels). Each 250 ml culture flask was randomly assigned to one of the seven temperature treatments, with four replicates ($n = 4$) for each temperature x N treatment. The culture flasks under each temperature treatment were maintained in a controlled temperature water bath for three days and subjected to a 12 L:12D photoperiod under a saturating light intensity of 120–130 μmol m^{-2} s^{-1} provided and measured as described in the pre-experimental incubations. After a 3-day incubation, *M. pyrifera* blades were harvested. Individual specimens exhibited growth under all culture conditions apart from the 27 °C temperature treatment [34]. Based on these findings and due to logistic constraints preventing analysis of all samples, three temperature treatments were selected (6, 17 and 24 °C) and three independent replicate samples ($n = 3$) out of the four samples per treatment from high and low NO_3^- concentrations were randomly selected for analysis of lipids and fatty acids in this experiment. The temperatures represent a low winter (6 °C) and summer temperature (17 °C) encountered in the natural habitat of the giant kelp samples in Tasmania. The high temperature (24 °C) scenario was chosen because it was the maximum temperature where positive growth was observed [34]. The harvested biomass was frozen immediately and freeze-dried using a Labconco® Freezone 4.5 freeze-drier unit.

2.2. Lipid and fatty acid analysis

Total lipids were extracted from three independent replicates ($n = 3$) per treatment, following a modified version of Bligh and Dyer [39] from the dried and weighed seaweed tissue. Lipids were extracted overnight using a one-phase methanol (MeOH): dichloromethane (DCM): Milli-Q (2:1:08 v/v/v) solvent mixture. Phase separation was achieved by addition of 10 ml of DCM and 10 ml of saline Mill-Q water. The lower lipid-containing layer was drained into a round bottom flask and solvent was removed using a rotary evaporator (ca. 40 °C). The lipid extracts were transferred to pre-weighed vials and solvents were evaporated under a constant stream of nitrogen gas. Total lipid contents were determined gravimetrically.

For lipid class composition, an exact aliquot of the total lipid extract was spotted on SIII chromarods (5 μm particle size). Samples were co-eluted with a lipid class standard mix to determine the lipid class composition. The mobile phase consisted of hexane:diethyl ether:glacial acetic acid (70:10:0.1 v/v/v). Chromarods were developed for 25 min and then dried at 50 °C for 10 min. The dried samples were analysed using an Iatroscan Mark V TH10 (NTS instruments, USA) thin layer chromatograph (TLC) with a flame ionisation detector (FID). Peak identification was achieved by comparison with retention factors of co-eluted standards. For quantification, the SIC480II IatroscanTM integrating software (System Instruments, Mitsubishi Chemical Instruments) was used. The peak areas were transformed to mass per μl spotted using pre-determined linear regression calculations.

For fatty acid analysis, an aliquot of the lipid extract was methylated using MeOH:DCM:conc. HCl (10:1:1, v/v/v), with heating for 1 h at 80 °C. After cooling, 1 ml H_2O was added and the resulting fatty acid methyl esters (FAME) were extracted three times into hexane:DCM, (4:1, v/v). The samples were analysed using gas chromatography (GC) on an Agilent Technologies 7890 (Palo Alto, California, USA) GC coupled with a flame ionisation detector (FID). Fatty acids were separated on a non-polar EquityTM-1 fused silica capillary column (15 m × 0.1 mm internal diameter, 0.1 μm film thickness). Agilent ChemStation software was used for quantification of FAME peaks. The identification of individual fatty acids were confirmed using a Finnigan ThermoQuest GCQ (Thermo Finnigan LLC, San Jose, California, USA) GC-mass spectrometer (MS) (GC–MS) system fitted with an on-column injector and using Thermoquest Xcalibur software.

2.3. Statistical analysis

All the analyses were carried out in R version 3.5.3 using the GLM (general linear model) function (stats package) with nitrate treatment and temperature as the two fixed factors. Analysis of residuals using diagnostic plots (residuals v. fitted plot and Normal QQ plot) indicated that all models met statistical assumptions of independence, normality, linearity and homoscedasticity. Data in the graphs and table are displayed as mean ± standard error, based on 3 replicates per treatment combination. Results of the statistical analysis are displayed in S1.

3. Results

3.1. Lipid class composition

Results of the lipid class composition analysis (Fig. 1) showed significant effects of both temperature and nitrate concentration as well as interactive effects on the partitioning of lipids into different lipid classes. Total lipid (TL) concentrations in the algae tissue ranged from 3.61–5.15% of DW (Table 1). As a temperature effect, there was a significant increase of sterols (ST) observed with increasing temperature, almost doubling in its proportion of the TL, 3.6 to 6.9% at 6 and 24 °C, respectively. There was a significant interactive effect of temperature and nitrate concentration on the proportions of polar lipids (PL, includes phospholipids and glycolipids) (GLM, $F_{2,12} = 5.335$, $p < 0.05$). Generally, PL decreased with increasing temperature (GLM, $F_{2,12} = 8.443$, $p < 0.01$). This trend was much more pronounced in the low nutrient treatment where proportions of PL decreased from 92.9% of TL to 86.8% of TL. Furthermore, there was a significant interactive effect on proportions of free fatty acids (FFA) (GLM, $F_{2,12} = 4.747$, $p < 0.05$) observed with increasing temperature treatments under low nitrate concentrations: Proportions of FFA increased from 1.4% to 3.3% (Fig. 1).

3.2. Fatty acid composition

The fatty acid profile of *M. pyrifera* (Table 1) consisted of ca. 15–27% SFA, 13–16% MUFA and 54–67% PUFA, with 16:0, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6 and 20:5n-3 being the main fatty acids present. No significant effects were detected for nitrate and temperature x nitrate treatments of total lipids, SFA, MUFA or PUFA. However, temperature had significant effects on the proportions of both SFA (GLM, $F_{2,12} = 10.166$, $p < 0.01$) and PUFA (GLM, $F_{2,12} = 7.864$, $p < 0.01$). SFA increased ca. two-fold under both nitrate concentrations with increasing temperatures (Fig. 2). PUFA decreased from around 66–67% of TFA to only around 54–55% of TFA at the highest temperature treatment (Fig. 2 and Table 1).

4. Discussion

This study reveals that the nitrogen status of *M. pyrifera* modulates the remodelling of membrane lipids in response to temperature, and provides a cellular mechanism to explain the findings of Fernández, Gaitán-Espitia, Leal, Schmid, Revill and Hurd [34] that high-temperature tolerance is improved under replete compared to deplete nitrogen conditions. Under low nitrate concentrations, the decrease in polar lipids (PL) with increasing temperatures was much more pronounced, with a concomitant increase in free fatty acids (FFA). Under high nitrate concentrations, *M. pyrifera* maintains high proportions of PL and shows no increase in FFA. Polar lipids are mainly incorporated into cellular membranes [40], which implies that under high nitrate concentrations, *M. pyrifera* maintained its lipid membrane and was able to support high photosynthetic activity at increased temperatures [34]. Under low nitrate concentrations, *M. pyrifera* exhibited a potential destabilization of those membrane components, which also explains the peak in FFA under low nitrate and high temperature conditions.

Although FFAs can indicate an increased amount of signalling lipids, it is mainly considered an indicator of lipid degradation [41,42]. In seaweeds, high amounts of FFA have been, for example, detected in the red alga *Palmaria palmata* towards the end of its growing season under high summer temperatures, indicative of lipid degradation [42]. Under nitrogen-replete conditions, *M. pyrifera* most likely was able to facilitate other temperature protective responses, such as increased expression of heat shock proteins and compounds with anti-oxidative properties, which supported stability of cellular membranes [27,35,36]. The changes in the lipid profiles also showed a general increase of sterols with increasing temperatures. Sterols, mainly known through studies on land plants, can act to reinforce membrane structure and are usually produced in increased quantities under high temperature stress [43]: here we reveal for the first time that *M. pyrifera* responds in a similar way with sterols being increasingly metabolised to support membrane stability under high temperature.

From seasonal field studies, it has been observed that macroalgae adjust their fatty acid composition to the ambient temperature conditions by adjusting the level of unsaturation of the fatty acids [27,44,45]. *Macrocystis pyrifera* exhibited a fatty acid profile distinctive of brown algae with 16:0, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6 and 20:5n-3 as the main components [27,46,47], but exhibited an increase in the level of saturation with increasing temperatures. The fatty acid profile exhibited a decrease in the percentage of PUFAs with increasing temperatures, which was balanced by an increase in SFA. A higher level of

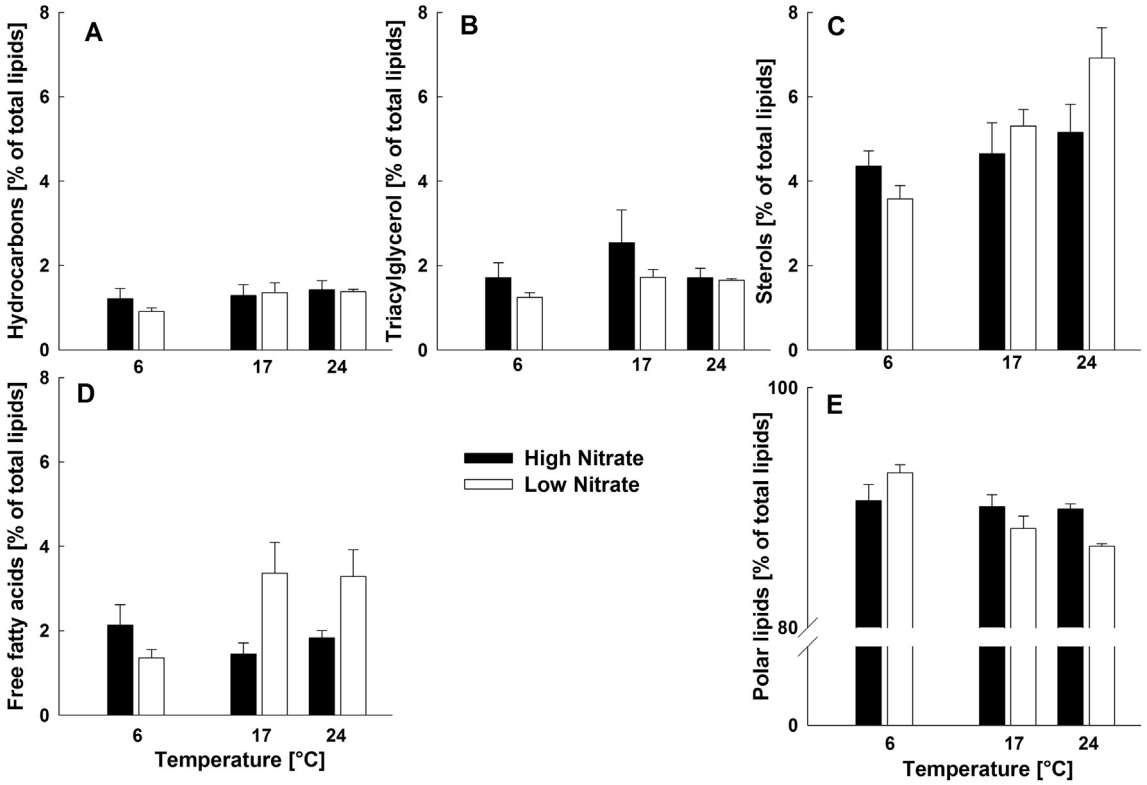

Fig. 1. Lipid class composition [as % of total lipids] of *Macrocystis pyrifera* at 6, 17 and 24 °C and under low (5 μM NO_3^-) and high (80 μM NO_3^-) nitrate concentrations. Data displayed as mean (n = 3) for hydrocarbons (A), triacylglycerol (B), sterols (C), free fatty acids (D), and polar lipids (E) ± standard error.

Table 1
Fatty acid contents and profile of *Macrocystis pyrifera* cultured at 6, 17 and 24 °C and under low (5 μM NO_3^-) and high (80 μM NO_3^-) nitrate concentrations. TFA and lipid contents are expressed as % of DW. Fatty acids are expressed as % of total fatty acids (TFA). All values are given as mean (n = 3) ± standard error. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. DW, dry weight.

Temperature	6		17		24	
	Low	High	Low	High	Low	High
Nitrate						
Lipids % of DW	5.15 ± 0.34	4.77 ± 0.72	3.78 ± 0.29	3.61 ± 0.63	4.07 ± 0.47	4.63 ± 0.54
TFA % of DW	1.14 ± 0.19	0.86 ± 0.16	0.66 ± 0.06	0.90 ± 0.28	0.95 ± 0.17	1.00 ± 0.09
14:0	0.57 ± 0.32	0.18 ± 0.12	1.11 ± 1.04	2.34 ± 1.04	2.75 ± 0.16	2.64 ± 0.27
16:0	13.73 ± 0.88	12.50 ± 0.31	18.26 ± 3.16	17.36 ± 2.34	22.58 ± 1.85	22.05 ± 2.56
18:0	1.35 ± 0.01	1.29 ± 0.23	1.08 ± 0.15	1.11 ± 0.04	1.10 ± 0.11	1.20 ± 0.28
20:0	0.54 ± 0.03	0.91 ± 0.39	1.46 ± 0.30	1.15 ± 0.38	1.26 ± 0.54	0.85 ± 0.10
22:0	0.11 ± 0.02	0.12 ± 0.01	0.26 ± 0.07	0.10 ± 0.05	0.18 ± 0.04	0.12 ± 0.07
SFA	16.30 ± 1.05	15.00 ± 0.16	22.16 ± 4.43	22.05 ± 2.96	27.87 ± 1.88	26.86 ± 3.05
16:1n-5c	0.13 ± 0.02	0.26 ± 0.11	0.07 ± 0.02	0.10 ± 0.02	0.13 ± 0.00	0.22 ± 0.09
16:1n-7c	0.55 ± 0.04	0.54 ± 0.03	0.70 ± 0.17	0.69 ± 0.11	0.66 ± 0.02	1.38 ± 0.52
16:1n-9c	0.18 ± 0.05	0.05 ± 0.04	0.19 ± 0.12	0.33 ± 0.05	0.31 ± 0.09	0.50 ± 0.09
16:1n-13 t	0.82 ± 0.18	0.57 ± 0.10	0.79 ± 0.37	0.94 ± 0.09	1.14 ± 0.17	1.61 ± 0.38
18:1n-7c	0.10 ± 0.02	0.16 ± 0.02	0.15 ± 0.03	0.14 ± 0.02	0.17 ± 0.05	1.08 ± 0.78
18:1n-9c	13.53 ± 1.37	14.65 ± 1.21	14.16 ± 1.68	11.06 ± 1.1	13.06 ± 0.81	11.05 ± 1.87
MUFA	15.30 ± 1.11	16.24 ± 1.03	16.05 ± 0.99	13.28 ± 1.12	15.48 ± 0.79	15.84 ± 1.81
18:2n-6	6.57 ± 0.59	7.23 ± 0.27	5.64 ± 0.42	7.03 ± 0.70	5.00 ± 0.32	6.80 ± 0.82
18:3n-3	5.22 ± 0.43	4.28 ± 0.54	3.60 ± 0.66	4.00 ± 0.62	3.36 ± 0.56	4.13 ± 1.00
18:3n-6	1.88 ± 0.28	1.37 ± 0.36	0.62 ± 0.05	1.33 ± 0.05	0.52 ± 0.05	0.68 ± 0.14
18:4n-3	8.79 ± 0.84	6.21 ± 1.13	5.80 ± 1.38	8.69 ± 1.19	6.17 ± 0.93	6.90 ± 1.86
20:2n-6	0.14 ± 0.03	0.21 ± 0.04	0.23 ± 0.05	0.19 ± 0.02	0.19 ± 0.03	0.16 ± 0.06
20:3n-6	1.68 ± 0.06	1.67 ± 0.10	0.89 ± 0.15	1.28 ± 0.11	0.61 ± 0.04	0.56 ± 0.14
20:4n-3	0.88 ± 0.01	1.28 ± 0.19	1.41 ± 0.06	1.07 ± 0.09	1.66 ± 0.22	1.32 ± 0.17
20:4n-6	29.46 ± 1.16	31.12 ± 0.51	28.58 ± 3.87	26.34 ± 1.11	26.50 ± 1.07	23.84 ± 2.46
20:5n-3	12.20 ± 0.94	13.89 ± 0.30	12.58 ± 1.77	12.56 ± 1.36	10.11 ± 0.81	10.56 ± 0.59
PUFA	66.83 ± 0.28	67.26 ± 1.11	59.35 ± 3.89	62.49 ± 3.90	54.11 ± 2.12	54.93 ± 4.87
n-3 PUFA	27.10 ± 0.84	25.67 ± 1.65	23.39 ± 3.19	26.32 ± 3.14	21.29 ± 2.24	22.90 ± 3.56
n-6 PUFA	39.73 ± 0.86	41.59 ± 0.58	35.96 ± 3.69	36.17 ± 1.65	32.82 ± 0.85	32.03 ± 3.13
Other	1.57 ± 0.25	1.51 ± 0.12	2.44 ± 0.40	2.18 ± 0.13	2.54 ± 0.18	2.37 ± 0.26

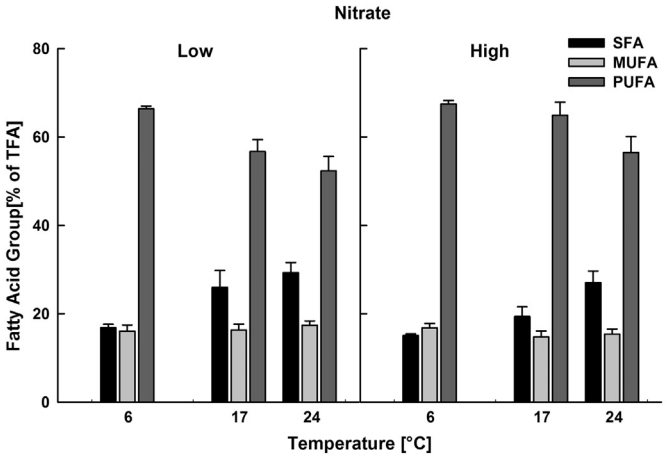

Fig. 2. Fatty acid composition (as % of total FA) of *Macrocystis pyrifera* at 6, 17 and 24 °C and under low (5 μM NO_3^-) and high (80 μM NO_3^-) nitrate concentrations. Data displayed as mean (n = 3) ± standard error.

unsaturation at low temperatures supports the maintenance of membrane fluidity under reduced temperature conditions [48,49]. At high temperatures, saturated fatty acids can stabilize the membranes and increase thermal tolerance [50]. Both processes, the desaturation of fatty acids and the reverse process, involve the use of energy to support the oxidation-reduction processes occurring [51]. Results from this study show for the first time in seaweeds that such changes can be implemented over a relatively short time frame of 3 days.

Studies on land plants (*Arabidopsis thaliana*) show that the initial acclimation in membrane fatty acid composition to higher temperatures occurs 60–100 h after the temperature change (from 22 °C to 29 °C), and changes in fatty acid composition were still observed after 250 h [52]. In our study, we also saw that changes in the fatty acid profile can be implemented in *M. pyrifera* after only three days (72 h). However, the fatty acid profile of *M. pyrifera* at the lower temperature (6 °C) treatment exhibited smaller within-replicate variation compared to the two higher temperature treatments (17 and 24 °C). The increase in desaturation of the fatty acids can be implemented by up-regulation of desaturase activity, but the reverse process usually involves *de novo* synthesis of saturated fatty acids and the suppression of desaturase activity [29]. Our 3 day experiment was most likely long enough for the adjustment to low temperatures (6 °C) by an increased activity of desaturases, but at higher temperatures (17 °C and 24 °C) the results most likely represent a transition period to further biochemical adjustments. Results from seasonal studies show that seaweeds generally exhibit changes in the composition of fatty acids, with higher concentrations of PUFA during winter and more SFA during the warmer months [53,54]. However, very little is known on how fast such changes can occur. Most studies which aim at investigating the effects of different environmental drivers on biochemical composition, culture seaweeds from weeks to years [55,56], although Al-Hasan, Hantash and Radwan [57] showed that a one week acclimation at different temperatures can result in changes in fatty acids in green, brown and red algae. Our study shows that young blades of *M. pyrifera* can implement such changes within 3 days, and potentially even faster, in order to maintain cellular membrane fluidity at different temperature and nutrient conditions.

Our results specifically aimed at understanding the short-term response of *M. pyrifera* to various temperature treatments under high and low N status of the seaweed tissue. We recognize that *M. pyrifera*, with the small blade pieces even showing positive growth under 24 °C in this experiment, would most likely not sustain longer exposure under such high temperatures. Experiments with larger blade sections and longer experimental exposure periods have shown that at lower temperatures of around 20 °C, detrimental effects on *M. pyrifera* can been observed [58]. Also in a natural setting, other stressors such as grazing and UV stress can additionally weaken seaweed response to high stress conditions [58,59]. Further studies, including larger algal parts and a longer experimental duration aiming at mimicking natural conditions, will be needed to further elucidate how the observed mechanistic differences will translate to responses in the natural habitat.

5. Conclusions

Macrocystis pyrifera has effective mechanisms to adjust its fatty acid profile rapidly (≤72 h) to a wide temperature range at both high and low nitrate availability. However, an increase in FFA indicated a detrimental effect on the seaweed under high temperature and low nitrate concentrations. Further, low nitrate concentrations can magnify the negative effects of short-term temperature stress. From a physiological perspective, the combination of two stressors - low nutrients and high temperature - seems to have a negative synergistic effect on *M. pyrifera* compared to only one stressor on its own. From an ecological perspective, this indicates that local impacts such as low nutrient conditions might enhance the impacts of high temperature on *M. pyrifera*, at least on a short time scale. Large assemblages of *M. pyrifera* have disappeared on the east coast of Tasmania after the 2015/2016 heat wave [37]. This marine heatwave lasted for a total of 251 days reaching a maximum intensity of 2.9 °C above previously reported daily mean sea surface temperatures with an maximum temperatures over 19 °C on the East Coast of Tasmania [37]. Increased incursions of the East Australian Current (EAC) during heat waves in eastern Tasmania not only influence temperature but have also affect nutrient loading, because the EAC is nutrient-poor (e.g. often ≤1 μM nitrate, particularly in summer) [60], which might have enhanced the rapid decline in kelps in this area. Our findings indicate that under rapid warming events such as marine heat waves, local nitrate availability might be a decisive factor for the acclimation potential and survival of *M. pyrifera*.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

CRediT authorship contribution statement

Matthias Schmid:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Pamela A. Fernández**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Juan Diego Gaitán-Espitia**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Patti Virtue**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Pablo P. Leal**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Andrew T. Revill**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Peter D. Nichols**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.**Catriona L. Hurd**:Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons whose contribution to the research merits co-authorship. We further confirm that the order of authors listed in the manuscript has been approved by all of us, and that the manuscript has not been previously published and is not under consideration for publication elsewhere. This work builds on that of Fernandez et al. (in review Journal of Experimental Botany), which we

are pleased to make available upon request.

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Appendix A. Supplementary data

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