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PRIMARY RESEARCH PAPER

The long and short of it: temperature-dependent modifications of fatty acid chain length and unsaturation in the galactolipid profiles of the diatoms *Haslea ostrearia* and *Phaeodactylum tricornutum*

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Abstract The purpose of this study was to examine the effect of different growth temperatures on the fatty acid compositions of the photosynthetically important galactolipids, mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively), of the "blue" pennate diatom, *Haslea ostrearia,* and the model pennate diatom, *Phaeodactylum tricornutum*, with the hypothesis that their *sn*-2 fatty acids would be modulated in the same manner as for dinoflagellates. Positive-ion electrospray ionization/mass spectrometry/mass spectrometry was used to characterize the galactolipids of each diatom. At 20°C, *H. ostrearia* and *P. tricornutum* were rich in eicosapentaenoic acid (EPA; $C_{20:5}$) at the *sn*-1 position and in C_{16} fatty acids at the *sn*-2 position of MGDG and DGDG. At 30°C, however, *H. ostrearia* and *P. tricornutum* contained no EPA or other C_{20} fatty acids, but rather contained higher percentages of C_{18} fatty acids at *sn*-1. At 30°C, no galactolipid in either diatom contained more than three unsaturations on any of its fatty acids. While these two species differ in galactolipid composition, they both possess a similar method of acclimating their galactolipids to a higher growth temperature: reducing the numbers of the longest and shortest fatty acid chains, as well as decreasing the total number of unsaturations.

Keywords Chloroplast · Diatom · DGDG · Lipid · MGDG · Temperature

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Introduction

Diatoms are one of the largest and most varied groups of primary producers on the planet (Moustafa et al., 2009; Seckbach & Kociolek, 2011). They are responsible for over 40% of marine primary production and 20–25% of total primary production (Werner, 1977; Nelson et al., 1995). In all photosynthetic organisms (cyanobacteria, algae, plants), fatty acid composition is important for stabilizing the membrane proteins associated with photosynthesis, such as the light-harvesting complexes (LHCs) and photosystems (Dörmann & Benning, 2002; Mironov et al., 2012;

Mizusawa & Wada, 2012), and membrane fluidity is generally involved in the responses to temperature changes in the environment (Mikami & Murata, 2003; Los & Murata, 2004). Studies relating to the modulation of membrane fatty acids as a function of temperature have been performed on different photosynthetic microorganisms, such as the chlorophyte *Dunaliella salina* (Lynch & Thompson, 1982, 1984), the dinoflagellate *Gyrodinium aureolum* (Parrish et al., 1993), and the haptophyte *Pavlova lutheri* (Tatsuzawa & Takizawa, 1995). For instance, an increase in polyunsaturated fatty acids (PUFAs), a decrease in monogalactosyldiacylglycerol (MGDG), and almost no changes in digalactosyldiacylglycerol (DGDG) were observed in *P. lutheri* grown at 15 versus 25°C (Tatsuzawa & Takizawa, 1995). Specific studies dealing with the effect of temperature changes on the membrane fatty acid profile of diatoms in particular are lacking, and understanding the lipid biochemistry, with an emphasis on fatty acid regiochemistry, of these photosynthetically important organisms may provide insights into specific lipid-based survival strategies during changing environmental conditions.

Thompson et al. (1992) examined the fatty acid composition of eight marine phytoplankton, including the diatoms *Chaetoceros calcitrans, C. gracilis, C. simplex, Phaeodactylum tricornutum,* and *Thalassiosira pseudonana,* and saw a significant inverse relationship between the concentration of PUFAs and temperature, as did Renaud et al. (1995) in the diatoms *Nitzschia closterium* and *N. paleacea*. This could be considered a general trend, since in phylogenetically distinct groups of algae, total fatty acids and cellular PUFA content have been shown to be influenced by temperature changes. In the eustigmatophyte, *Nannochloropsis salina*, Hoffmann et al. (2010) showed that total PUFA proportion, and in particular the long chain eicosapentaenoic acid (EPA, $C_{20:5}$) content, which has an important value for aquaculture and human health, increased at low temperature (17°C, compared with 21 and 26°C), although, when compared with other results obtained using this species or others within the same genus (Olofsson et al., 2012 and references therein), these authors emphasized that the influence of temperature seemed to be complex and difficult to explain.

The major problem with these studies on total fatty acids is that it is unknown what fatty acids are attached to which lipid headgroups, let alone the regiochemistry of the intact lipids. Rousch et al. (2003) used gas chromatography/mass spectrometry (GC/MS) to examine the changes in fatty acid methyl esters (FAMEs) due to changes in growth temperatures in the diatoms *P. tricornutum* and *C. muelleri*, and found that certain fatty acids, such as EPA, $C_{16:1}$, and $C_{18:1}$, decreased as temperature increased, while others, such as $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and $C_{18:2}$, increased with temperature. Leblond et al. (2010) examined the galactolipid composition of six isolates from the dinoflagellate genus *Pyrocystis* grown under three temperatures (15, 20, and 25°C) using positive-ion electrospray ionization/mass spectrometry/mass spectrometry (ESI/MS/MS), which determined the intact lipid as well as the regiochemistry of the lipids. Leblond et al. (2010) found that DGDG tended to be more responsive to changes in temperatures (more unsaturated forms of the *sn*-2 fatty acid were found at lower temperatures) than did MGDG. There is, however, variation in other photosynthetic organisms, such as the red alga *Porphyridium cruentum*, as to which fatty acid (*sn*-1 and/or *sn*-2) and which lipid (MGDG and/or DGDG) was modified in response to temperature (Adlerstein et al., 1997).

Using ESI/MS/MS to study diatom galactolipid composition, it was previously found that the "blue" diatom *Haslea ostrearia* contained primarily C_{18}/C_{16} forms of both MGDG and DGDG, with minor amounts of C_{20}/C_{16}, and was similar to another pennate diatom, *Navicula perminuta* (Dodson et al., 2013). The pennate diatom *P. tricornutum*, conversely, contained primarily C_{20}/C_{16} forms of MGDG and DGDG, with minor amounts of C_{16}/C_{16} and C_{18}/C_{16}, which was similar to the centric diatoms *Skeletonema marinoi* and *Thalassiosira weissflogii* (Dodson et al., 2013).

Haslea ostrearia is well known in France for the production of the water-soluble, non-photosynthetic blue pigment marennine, which causes the greening of oyster ponds and oysters (Nassiri et al., 1998; Gastineau et al., 2012a). *Phaeodactylum tricornutum* has become a model organism for diatom research since it is one of only two diatoms to have its genome sequenced and published, the other being *T. pseudonana* (Maheswari et al., 2005). *Phaeodactylum tricornutum* has since been used to study carbohydrate metabolism (Kroth et al., 2008), growth rate (Peréz et al., 2008), and lipid accumulation (Valenzuela et al., 2012). Since *H. ostrearia* and *P. tricornutum* differ in their galactolipid composition at 20°C (Dodson et al.,

2013), this work was done to determine the method of temperature acclimation to 30°C as it relates to these two pennate diatoms' galactolipids. *Phaeodactylum tricornutum* is usually considered a pelagic organism, whereas *Haslea ostrearia* is a tychopelagic diatom (Robert, 1983), an organism that can be not only benthic or epiphytic, but also planktonic (Round et al., 1990). *Haslea ostrearia* is euryhaline (Neuville & Daste, 1978; Wraige et al., 1998), and can develop in high light environments (Mouget et al., 1999), thus it seems well adapted to oyster ponds, characterized by shallow and nutrient-rich water, where it mainly proliferates in spring and autumn (Maestrini & Robert, 1981; Robert, 1983). According to the literature, *H. ostrearia* lives mainly in temperate to tropical waters (for a review, see Gastineau et al., 2012b), thus acclimation to 30°C could represent a stress relevant enough to study algal exacerbated responses to a water temperature rise.

Unlike the previous studies on diatom lipids, this study uses ESI/MS/MS to examine just what modifications are made to specific lipids, and to what positions (*sn*-1 or *sn*-2) those modifications are made. Indeed, what seems to be the case is that both species of diatoms displayed a similar response to temperature. Both *H. ostrearia* and *P. tricornutum* contained C_{20} fatty acids in their forms of MGDG and DGDG when grown at 20°C; however, when grown at 30°C these C_{20} fatty acids were replaced by C_{18} fatty acids at the *sn*-1 position.

Materials and methods

Cultures and growth conditions

Phaeodactylum tricornutum Bohlin CCMP 1327 was obtained from the National Center for Marine Algae and Microbiota (NCMA, East Boothbay, ME, USA). *Haslea ostrearia* (Gaillon) Simonsen NCC 344 was obtained from the Nantes Culture Collection (NCC, Université de Nantes, 2 Rue de La Houssiniere, 44322 Nantes, France). Each strain was grown in triplicate in 2L of L1 medium in separate incubators at 20 and 30°C, an irradiance of $\sim$50 µmol photons m^{-2} s^{-1}, and a light:dark cycle of 14:10 h; they were harvested according to the methodology of Leblond & Chapman (2000).

Lipid extraction and fractionation

Total lipids were extracted and galactolipids separated from other lipid classes according to the techniques described by Leblond & Chapman (2000). Briefly, the total lipid extracts were separated into five component lipid fractions on columns of activated Unisil silica (1.0 g, 100–200 mesh, activated at 120°C; Clarkson Chromatography, South Williamsport, PA, USA). The following solvent regime was used to separate lipids according to polarity, with fraction 5 eluting the most polar lipids (Leblond & Chapman, 2000): (1) 12 ml methylene chloride (sterol esters), (2) 15 ml 5% acetone in methylene chloride with 0.05% acetic acid (free sterols, tri- and di-acylglycerols, and free fatty acids), (3) 10 ml 20% acetone in methylene chloride (monoacylglycerols), (4) 45 ml acetone [chloroplast glycolipids, including MGDG, DGDG, and sulfoquinovosyldiacylglycerol (SQDG)], and (5) 15 ml methanol with 0.1% acetic acid (polar lipids, including phosphorous- and non-phosphorous-containing lipids).

Mass spectrometry of lipids

Following the procedure of Gray et al. (2009), the lipids of fraction 4 were suspended in methanol, chloroform, and 50 mM sodium acetate (Welti et al., 2002) prior to examination via mass spectrometry. The resulting MGDG and DGDG sodium adducts were analyzed using positive-ion ESI/MS full scans from 100 to 2,000 Da through direct injection (5 µl sample volume into a methylene chloride carrier solvent at 0.5 ml min^{-1}) into a Finnigan DecaXP ion trap mass spectrometer (Waltham, MA, USA). The abundance of each lipid was determined by calculating relative percent composition based on peak height of raw data in the positive-ion ESI/MS full-scan mode. Subsequent positive-ion ESI/MS/MS was performed using collision energy between 37.5 and 48.0%, and major cleaved fatty acids were identified by the differences between the masses of the original ions and their fragments. Regiochemistry was determined by positive ESI/MS/MS according to the methodology of Guella et al. (2003). Statistical significance between treatments was determined using ANOVA and Tukey's test ($P < 0.05$) in SigmaPlot version 12.3 (Systat Software, San Jose, CA, USA).

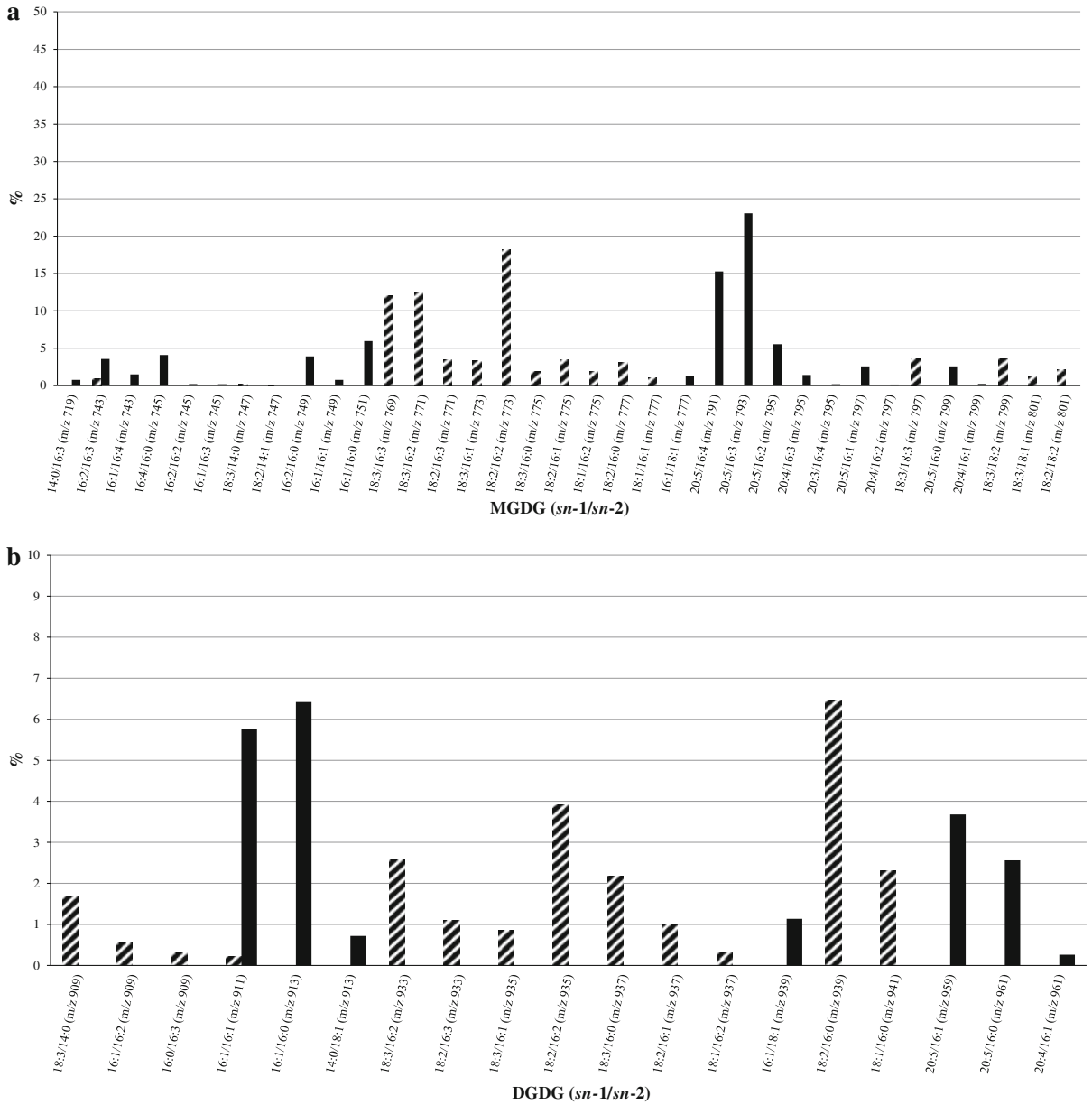


Fig. 1 Relative abundances of significantly different galactolipids with regiochemistry (*sn*-1/*sn*-2) and *m*/*z* ratio of **a** MGDG and **b** DGDG forms of *P. tricornutum* when grown at 20°C (*solid bars*) and 30°C (*hashed bars*) (standard deviations can be found in Online Resource 1)

Results

The most obvious response to temperature between the galactolipid profiles of both *H. ostrearia* and *P. tricornutum* was the complete lack of any C_{20} fatty acids, EPA or arachidonic acid (AA, $C_{20:4}$), in the cultures grown at 30°C. The MGDG and DGDG forms in *P. tricornutum* that showed significant differences between growth temperatures can be seen in Fig. 1. *Phaeodactylum tricornutum*'s most abundant form of MGDG at 20°C was a $C_{20:5}/C_{16:3}$ form (*sn*-1/*sn*-2, *m*/*z* 793) at an average of 23% relative abundance, however, forms of MGDG with C_{20} fatty acids at *sn*-1 seen at 20°C were completely absent at 30°C. Instead,

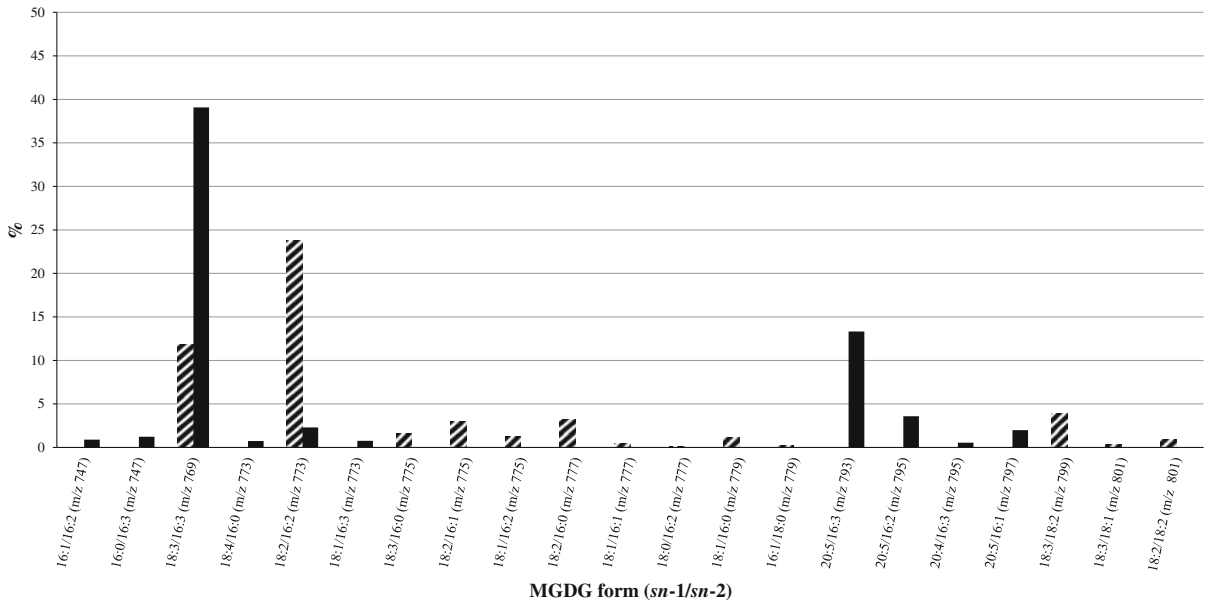
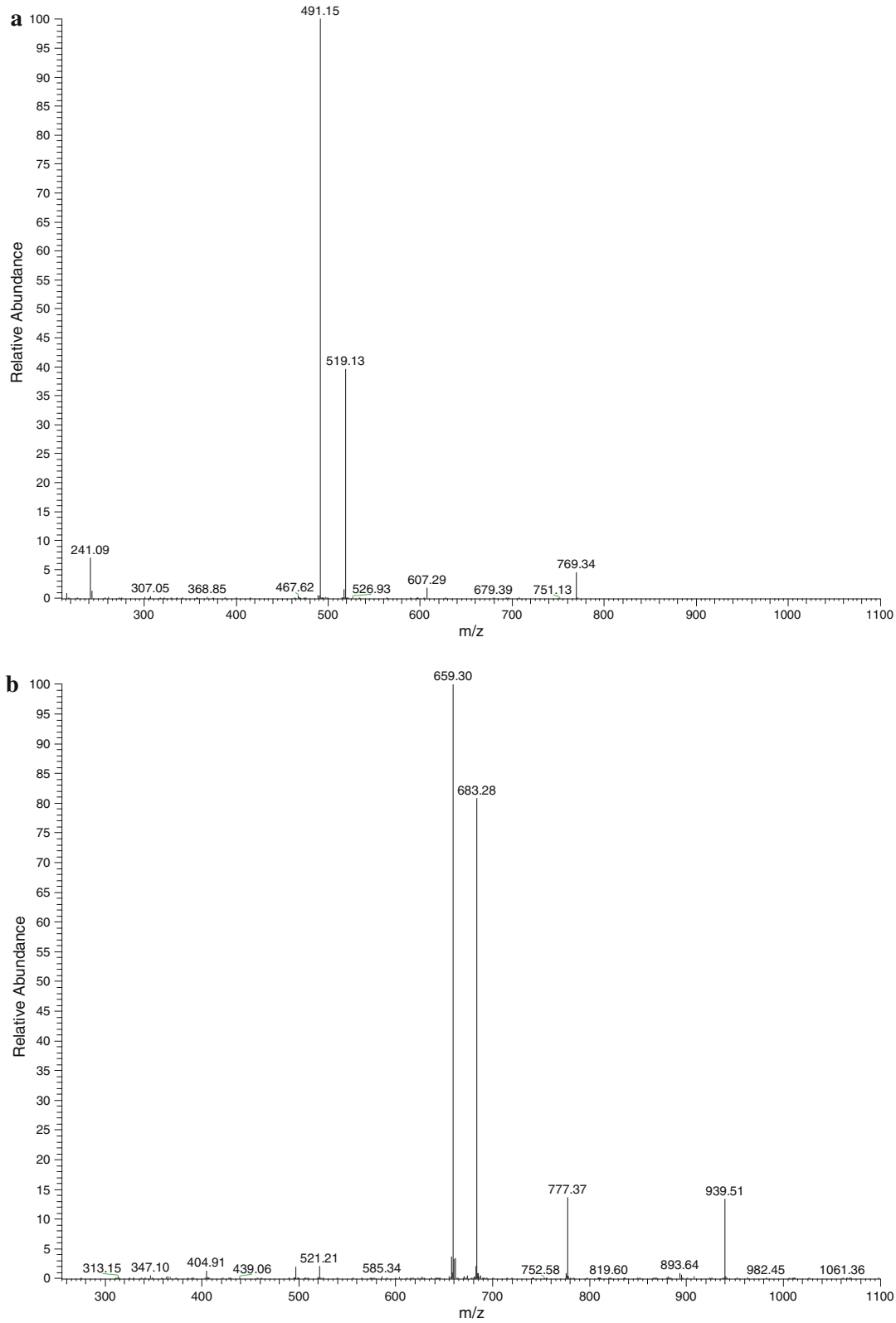


Fig. 2 Relative abundances of significantly different MGDG forms with regiochemistry and *m*/*z* ratios of *H. ostrearia* when grown at 20°C (*solid bars*) and 30°C (*hashed bars*) (standard deviations can be found in Online Resource 2)

the most abundant form of MGDG at 30°C became a $C_{18:2}/C_{16:2}$ form of MGDG at *m*/*z* 773 at 18.2% relative abundance. There were also a number of other C_{18}/C_{16} forms of MGDG that were present in the 30°C cultures, but not the 20°C cultures. There was also a noticeable difference in the DGDG forms present in *P. tricornutum*. The two most abundant forms of DGDG in the 20°C cultures were $C_{16:1}/C_{16:0}$ (*m*/*z* 913) and $C_{16:1}/C_{16:1}$ (*m*/*z* 911) forms at 6.4 and 5.8% relative abundance, respectively. The two most abundant forms of DGDG in the 30°C cultures, however, were a $C_{18:2}/C_{16:0}$ form (*m*/*z* 939; seen in Fig. 3b) at 6.52% relative abundance and an $C_{18:2}/C_{16:2}$ form (*m*/*z* 935) at 3.9% relative abundance. There were also four forms of DGDG with C_{20} fatty acids at *sn*-1 in *P. tricornutum* at 20°C that were not seen in the cultures grown at 30°C.

Just as was seen in *P. tricornutum*, forms of MGDG and DGDG that contained C_{20} fatty acids seen in the 20°C *H. ostrearia* cultures were also absent from the 30°C cultures. Significantly different MGDG forms of *H. ostrearia* can be seen in Fig. 2. The most abundant form of MGDG in *H. ostrearia* when grown at 20°C was a $C_{18:3}/C_{16:3}$ form (*m*/*z* 769; seen in Fig. 3a) at 39.1% relative abundance. When grown at 30°C, however, the most abundant MGDG form became $C_{18:2}/C_{16:2}$ (*m*/*z* 773) at 23.8% relative abundance. There were also a number of MGDG forms containing C_{18}/C_{16} fatty acids that were present when grown at 30°C, but not present when grown at 20°C. Regarding *H. ostrearia*'s DGDG profile, there was a decrease in the amount of $C_{18:2}/C_{16:0}$ (*m*/*z* 939), $C_{18:3}/C_{18:1}$ (*m*/*z* 963), and $C_{18:2}/C_{18:2}$ (*m*/z 963) in cells grown at 20°C compared to 30°C.

Figures 4 and 5 show total carbons and unsaturations for all fatty acids of MGDG and DGDG forms detected at both growth temperatures for *P. tricornutum* and *H. ostrearia*, respectively. It is apparent in both figures that both species tended to possess longer carbon chains and more unsaturations in their MGDG and DGDG forms when grown at 20°C than when grown at 30°C. It is also evident that at 20°C, both species of diatoms contained galactolipids with shorter chain fatty acids. In fact, at the higher growth temperature, both diatom species were rich in both MGDG and DGDG forms with 34 carbons in both fatty acids combined. At the lower growth temperature, however, *P. tricornutum* was rich in MGDG and DGDG with 36 carbons, while *H. ostrearia* only possessed MGDG with 36 carbons. No galactolipid in either species possessed more than six combined unsaturations in both fatty acids when grown at 30°C, yet reached up to 8 or 9 unsaturations when grown at lower temperatures.


a
Relative Abundance
m/z
491.15
519.13
241.09
307.05
368.85
467.62
526.93
607.29
679.39
751.13
769.34
b
Relative Abundance
m/z
659.30
683.28
777.37
939.51
313.15
347.10
404.91
439.06
521.21
585.34
752.58
819.60
893.64
982.45
1061.36

◀ **Fig. 3** Positive-ion electrospray ionization/mass spectrometry/mass spectrometry (ESI/MS/MS) spectra of two galactolipids: **a** $C_{18:3}/C_{16:3}$ monogalactosyldiacylglycerol (MGDG) (molecular ion of *m*/*z* 769) from *H. ostrearia* grown 20°C, also seen at 30°C. The *m*/*z* 491 fragment represents the lipid minus the $C_{18:3}$ fatty acid from the *sn*-1 position, and the *m*/*z* 519 fragment represents the lipid minus the $C_{16:3}$ fatty acid from the *sn*-2 position; **b** $C_{18:2}/C_{16:0}$ digalactosyldiacylglycerol (DGDG) (*m*/*z* 939) from *P. tricornutum* grown at 30°C. The *m*/*z* 659 fragment represents the lipid minus the $C_{18:2}$ fatty acid from the *sn*-1 position, and the *m*/*z* 683 fragment represents the lipid minus the $C_{16:0}$ fatty acid from *sn-2* position. The minor *m*/*z* 777 fragment represents the loss of one of the galactose molecules from the *sn*-3 head group

Significant differences between experimental treatments were seen in a majority of both species' galactolipid forms. Only three forms of MGDG and three forms of DGDG were not statistically significant in the *P. tricornutum* samples—$C_{14:0}/C_{16:1}$ MGDG, $C_{16:1}/C_{16:2}$ MGDG, $C_{16:0}/C_{16:3}$ MGDG, $C_{18:2}/C_{14:0}$ DGDG, $C_{16:0}/C_{16:2}$ DGDG, and $C_{20:5}/C_{16:2}$ DGDG—and of these six galactolipids, only $C_{16:1}/C_{16:2}$ MGDG and $C_{16:0}/C_{16:3}$ MGDG were present at both growth temperatures. The galactolipids without significant differences that were only present at 30°C were $C_{14:0}/C_{16:1}$ DGDG and $C_{20:5}/C_{16:2}$ DGDG, while $C_{18:2}/C_{14:0}$ DGDG and $C_{16:0}/C_{16:2}$ DGDG were only present at 20°C. There were 19 galactolipid forms found in *H. ostrearia* that were not significantly different between the two growth temperatures, 6 MGDG forms and 13 DGDG forms, and of these molecular forms 10 were present at both growth temperatures. Only three forms of DGDG were significantly different in *H. ostrearia* between the two growth temperatures—$C_{18:2}/C_{16:3}$, $C_{18:3}/C_{18:1}$, and $C_{18:2}/C_{18:2}$—with all of them present at less than 2% at 30°C and not present at all at 20°C. The statistical analyses of each lipid including standard deviations as well as individual relative percent abundances for each replicate of *P. tricornutum* and *H. ostrearia* had been included as Online Resources 1 and 2, respectively.

Overall, when grown at 30°C, the MGDG to DGDG ratio decreased from what was seen at 20°C in both diatom species: from 3.3 to 2.8 in *P. tricornutum*, and from 7.3 to 3.6 in *H. ostrearia*. In both species, the ratios reflect a decrease in MGDG coupled to an increase in DGDG, when grown at the higher temperature. MGDG decreased from 76.7 to 74.0 and DGDG

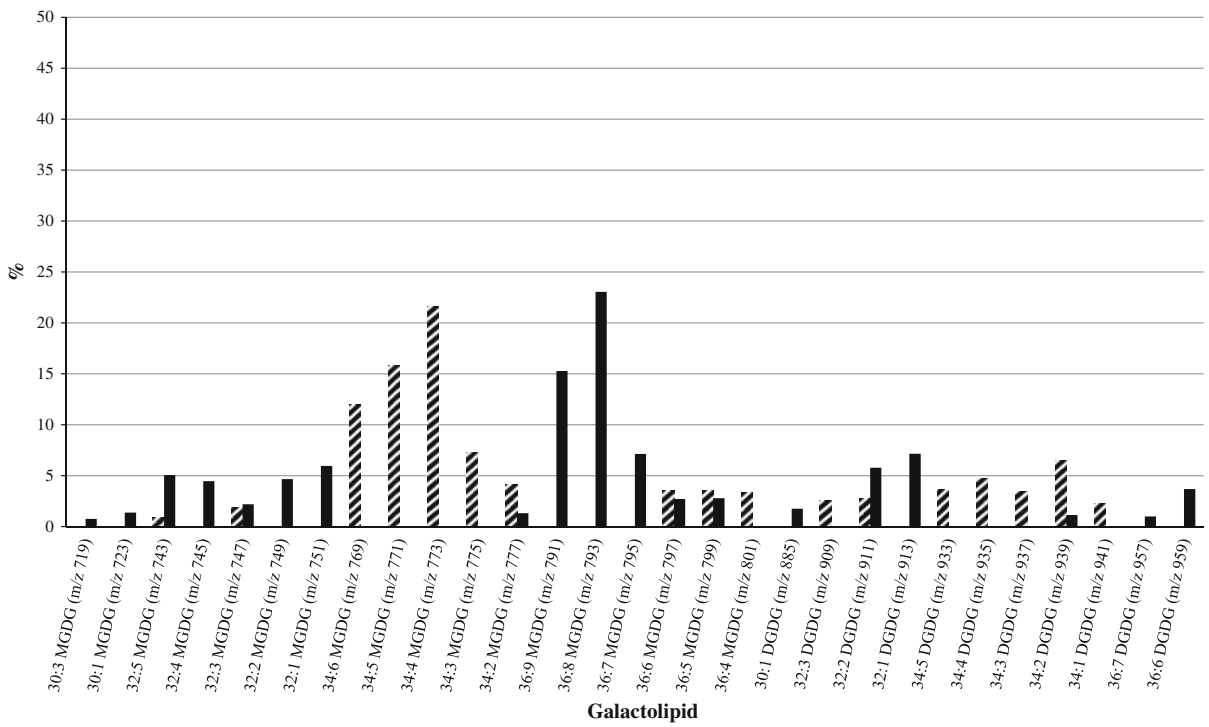


Fig. 4 Total number of carbons and unsaturations for all galactolipids of *P. tricornutum* when grown at 20°C (*solid bars*) and 30°C (*hashed bars*)

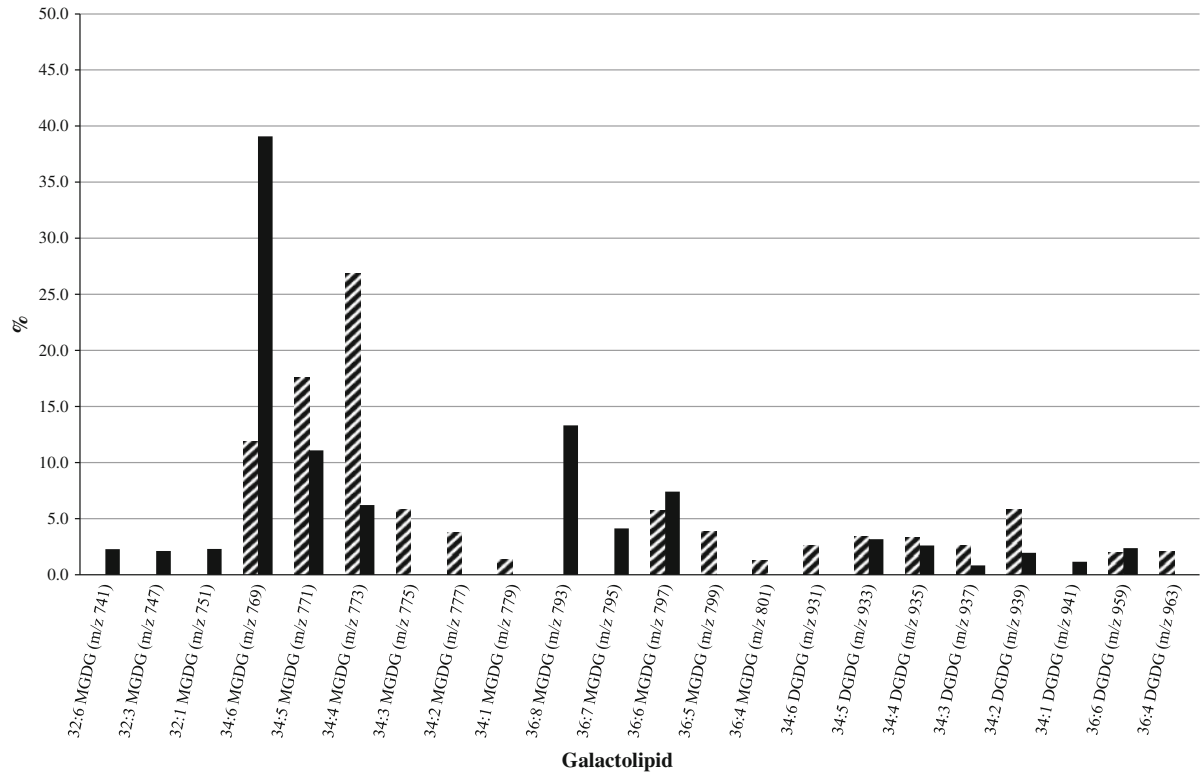


Fig. 5 Total number of carbons and unsaturations for all galactolipids of *H. ostrearia* when grown at 20°C (*solid bars*), and 30°C (*hashed bars*)

increased from 23.3 to 26.0 % of total galactolipids in *P. tricornutum*. In *H. ostrearia*, the corresponding values are 87.9 to 78.1 (MGDG) and 12.1 to 21.9 % of total galactolipids (DGDG), respectively.

Discussion

In the present work, a positive-ion ESI/MS/MS technique was used to specifically study intact lipids and to identify the regiochemistry of the fatty acids attached to two galactolipids, MGDG and DGDG, in two different pennate diatoms, the model organism *P. tricornutum* and the marennine-producing *H. ostrearia*. Both *H. ostrearia* and *P. tricornutum* demonstrated a complete shift away from C_{20} fatty acids in both MGDG and DGDG when grown at 30°C, reducing the numbers of the longest and most unsaturated lipids from their galactolipid profiles. This supports the hypothesis that more saturated lipids will be more prevalent at higher temperatures due to the need for increased membrane melting temperature. It is evident that the loss of C_{20} fatty acids from the chains is offset by the correlated loss of many of the shorter chain fatty acids, such as many C_{14} and C_{16} fatty acids which are present at the *sn*-1 position of multiple MGDG forms and a single DGDG form only in the samples grown at 20°C. *H. ostrearia* and *P. tricornutum* did not produce fatty acids with four or more unsaturations when grown at 30°C.

When grown at lower temperatures, *H. ostrearia* and *P. tricornutum* exhibited longer fatty acid chains and more unsaturations. This observation is consistent with the interpretation that to maintain membrane fluidity at lower temperatures, unsaturations are needed to force spacing between the membrane lipids and decrease the melting temperature of the membrane. Fewer unsaturations and shorter fatty acid chains, seen at the higher temperatures, demonstrate the need for a more tightly packed membrane, reducing the fluidity of the membrane. These changes allow for the membrane to maintain the fluidity or order it requires for a particular growth temperature by maintaining, increasing, or decreasing its melting

temperature, a response observed in almost all photosynthetic organisms (e.g., Mikami & Murata, 2003; Mizusawa & Wada, 2012).

While the total amount of PUFAs, the fatty acid chain length, the level of unsaturation, and the ratio of lipid molecular forms have been observed as general possible lipid modifications in response to environmental change in microalgae, what seems to be especially prevalent with diatom species in response to changes in growth temperature is the alteration of fatty acid chain length and saturation (Chen, 2012; Jiang & Gao, 2004; Renaud et al., 1995; Thompson et al., 1992; this study), but see Boelen et al. (2013) for a mitigated view. Renaud et al. (2002) examined the lipid content of four tropical algae, the diatom *Chaetoceros* sp., the cryptomonads *Rhodomonas* sp. and *Crytpomonas*, and an unidentified prymnesiophyte, under increasing growth temperatures using GC/MS analysis of FAMEs. All four species examined showed an increase in saturated fatty acids and a decrease in the amount of EPA with increasing temperatures. The present study confirms that levels of EPA decreased in *P. tricornutum* grown under higher temperatures, a result in accordance with Jiang & Gao (2004), and demonstrates that this response is seen in *H. ostrearia* as well.

Contrary to what was seen by Rousch et al. (2003), increasing levels of $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$ fatty acids were not seen in *P. tricornutum* under the higher growth temperature, nor were decreasing levels of $C_{16:1}$ and $C_{18:1}$. These data also show a much greater variety of fatty acids detected compared to the nine that were found by Rousch et al. (2003). However, Rousch et al. (2003) examined total FAMEs using GC/MS, and it is possible that the changes seen in that study may occur in other lipids outside of the galactolipid fraction examined here. However, the experimental conditions were different between the two studies, for instance regarding the light regime: 30 µmol photons m^{-2} s^{-1}, and a light:dark cycle of 12:12 h, as compared to 50 µmol photons m^{-2} s^{-1}, and a light:dark cycle of 14:10 h in the present study. Moreover, Rousch et al. (2003) focused on the changes in FA profiles on the short-term (24 h) and the very short-term (2 h), and exposed their *P. tricornutum* cultures to temperature stress (up to 30 and 40°C, respectively), whereas the cultures used in the present study were acclimated to the two growth temperatures (20 and 30°C). These differences in experimental set up, especially the time lapse for temperature acclimation (see Lynch & Thompson, 1984) do not allow further comparison of the two studies. More generally, two recent studies illustrate the difficulties encountered when comparing data obtained using different experimental devices, parameters, and species. Chen (2012) studied the total lipids and FA composition of 12 diatom species grown under various combinations of temperatures and light intensities, corresponding to ambient conditions in summer and winter (for instance, temperature ranging from 23.5 to 38°C and 11.5 to 25°C, respectively), and in a constant incubator (24°C, 12:12 h light:dark cycle, 122 µmol photons m^{-2} s^{-1}). Chen (2012) showed that the FA composition could be affected by temperature only, light only, or by both temperature and light, and that responses were species-dependent. On the other hand, Boelen et al. (2013) compared EPA production in a polar (*Chaetoceros brevis*) and a temperate diatom (*T. weissflogii*) grown at control (3 and 16°C, respectively), and "high" temperature (7 and 20°C, respectively). Possibly due to the small range of temperature changes tested for each species, in contrast to Chen (2012), these authors observed no significant effect of temperature on EPA content (expressed on an algal biovolume basis), whatever the irradiance (10, 25, 75, and 150 µmol photons m^{-2} s^{-1}) .

Similar, contrasting, or variable effects of temperature on lipid profiles have been observed in phylogenetically different algae. For instance, in the eustigmatophyte *Nannochloropsis* sp., the total EPA content decreased with increasing temperature (Hoffmann et al., 2010). Moreover, the ratio of FAs in the *sn*-1 and *sn*-2 positions of MGDG changed with temperature, mainly due to the percentage of the form $C_{20:5}/C_{20:5}$ MGDG (Sukenik et al., 1993) which significantly decreased with increasing temperature, as it did in the red alga *P. cruentum* (Adlerstein et al., 1997). Flaim et al. (2012) also observed a shift in the lipid forms produced by the cold-adapted dinoflagellate *Borghiella dodgei*. While *B. dodgei* possessed primarily $C_{18:5}$ and $C_{18:4}$ fatty acids in its MGDG and DGDG forms, these lipids decreased as growth temperature increased and were replaced by other less unsaturated fatty acids. The authors also posit that by reducing the activity of the desaturases required to make those PUFAs, *B. dodgei* was conserving energy at higher growth temperatures (Flaim et al., 2012). In other dinoflagellates, Leblond et al. (2010) examined temperature dependent

modifications of the galactolipid profile of members of the genus *Pyrocystis,* and observed that only the *sn*-2 fatty acid of DGDG was modified, while MGDG and trigalactosyldiacylglycerol (TGDG) remained mostly constant. In fact, only one form of MGDG, $C_{20:5}/C_{18:5}$, in *P. lunula* UTEX 2166 showed any response to temperature. Likewise, another study performed on the chromerid, *Chromera velia,* observed that the *sn*-2 fatty acid became less saturated when grown at higher temperatures (Dahmen et al., 2013). Across most galactolipids present in the two species, *H. ostrearia* and *P. tricornutum*, the largest modifications appear to occur at the *sn*-1 position of either MGDG or DGDG.

In the previous study, it was shown that *H. ostrearia* and *P. tricornutum* differed regarding their galactolipid profiles (Dodson et al., 2013). The former contained primarily C_{18}/C_{16} forms of both MGDG and DGDG, and the latter primarily C_{20}/C_{16} forms of MGDG and DGDG. The present work shows that these two pennate diatoms respond similarly to changes in growth temperature, not only an increase in FA unsaturation and chain length at 20°C in comparison with 30°C, but also an increase in the ratios of MGDG to DGDG. However, they differ significantly as to the amplitude of the ratio MGDG to DGDG, mostly due to the decrease in DGDG at low temperature, which is >50% in *H. ostrearia*, as compared to ca. 10% in *P. tricornutum*. These changes in galactolipid profiles could reflect differences in their respective strategies of acclimation of the photosynthetic apparatus to the growth temperature, and in their autecology—*P. tricornutum* is usually considered a pelagic organism, while *H. ostrearia* is considered a benthic or epiphytic diatom, although occasionally it is tychopelagic. In the laboratory, *P. tricornutum* can outcompete *H. ostrearia* (J.-L. Mouget, unpublished results), and the former displays two times more nonphotochemical chlorophyll *a* fluorescence quenching (NPQ) than the latter (Rech et al., 2005). This reveals a higher capacity to dissipate overexcitation at the PSII antenna that could result from differences in their galactolipid profiles. Moreover, galactolipids in thylakoid membranes, especially MGDG, and to a lesser extent DGDG, play important roles in the response to change in growth temperature in photosynthetic organisms. Their role is not only structural (increasing the unsaturation of fatty acids allows survival at low temperature, by decreasing the membrane's melting temperature), but also functional (for instance, regarding the operation of the xanthophyll cycle), as far as the two aspects can be dissociated (Goss et al., 2009). The xanthophyll cycle is located in the chloroplast and represents an important component of the photoprotection mechanisms displayed by photosynthetic organisms, by preventing an over excitation of the PSII reaction centers (Lavaud, 2007). In diatoms, it consists of the conversion in high light of diadinoxanthin (DD) to its de-epoxidized form, diatoxanthin (DT), due to the activation of a diadinoxanthin de-epoxidase (DDE) by the acidification of the thylakoid lumen (Lavaud, 2007). The de-epoxidation of DD to DT results in an increased ability to dissipate excess excitation energy in the antenna complex of PSII. This photoprotective capacity is closely related to the NPQ, one of the most important mechanism for rapid (seconds to minutes) regulation of photochemistry (Lavaud, 2007).

If in diatoms MGDG and SQDG are quantitatively the two most important lipids in the thylakoid membranes (Goss et al., 2009), the LHCs, the fucoxanthin chlorophyll protein complexes (FCPs), are especially enriched in MGDG (Goss et al., 2009). It has been shown in diatoms that MGDG facilitates DD de-epoxidation to DT by DDE (Goss et al., 2005). Furthermore, in vitro experimental evidence showed that MGDG molecules do not form lamellar bilayer membranes but specific three-dimensional domains in the thylakoids, which allow hypothesizing that MGDG influences the curvature of the membranes (Goss et al., 2005), and facilitates xanthophyll solubilization and accessibility to the de-epoxidase active sites in the lumen (Schaller et al., 2010), especially at low temperature (Vieler et al., 2008). Moreover, at low temperature, high rates of de-epoxidation were observed in the MGDG-forming domains of the thylakoid membranes (Latowski et al., 2003). Regarding other galactolipid molecules, DGDG is specifically bound to PSII and plays an important role for the stabilization of the oxygen-evolving complex, through the binding of extrinsic proteins on the donor side of PSII (Sakurai et al., 2007). DGDG could also participate in the organization of the thylakoid membrane domains and influence the functioning of PSI and the intersystem electron transport rate (Ivanov et al., 2006).

In this study, the data demonstrate that both *H. ostrearia* and *P. tricornutum* possess similar methods for acclimating their galactolipid profiles to a higher growth temperature. The reduction in number of the

longest, C_{20}, and shortest, C_{14} and C_{16}, fatty acids from the galactolipids at the higher growth temperature suggests a narrowing of the melting temperature range of these photosynthetically important membranes, which helps preserve the membrane's fluidity at various growth temperatures. The reduction in the number of unsaturations at the higher growth temperature induces a tighter packing of the galactolipids, which also increases the melting temperature of these membranes. Interesting questions raised by this study include whether or not this lipid modification strategy extends to other classes of membrane lipids, such as phospholipids, and if centric diatoms or other pennate diatoms use these strategies as well.

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