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Importance of ice algae and pelagic phytoplankton as food sources revealed by fatty acid trophic markers in a keystone species (*Mytilus trossulus*) from the High Arctic

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ABSTRACT: The Arctic is characterized by strong seasonality in sea ice extent and temperature. To survive seasonal changes, species have different coping mechanisms. However, knowledge of how intertidal species cope with seasonality remains limited. To study this problem, we analyzed the fatty acid composition in the hepatopancreas and in the gill tissue of an intertidal temperate keystone species (*Mytilus trossulus*) at its northernmost limit in Greenland (77° N). Fatty acid trophic markers (FATM) suggested that the diet mainly consisted of diatoms while the intertidal was covered by sea ice. During the following open-water period, food preferences shifted to pelagic dinoflagellates, and by the end of summer, food consisted of a diatom/dinoflagellates mixture. The contributions of macroalgae detritus, zooplankton and bacteria to the diet of *M. trossulus* were relatively low. We furthermore found that *M. trossulus* change membrane fatty acid composition in response to temperature changes in order to maintain functionality and avoid mortality. Membrane unsaturation significantly increased in response to decreasing temperatures as a result of selective retention of fatty acids from phytoplankton and from bacteria that on average constituted up to 24 % of the total fatty acids. Our results provide novel insight on how a temperate species survives in the Arctic and thereby strengthen the knowledge needed to understand the potential for non-arctic temperate species to expand into the Arctic region.

KEY WORDS: Arctic · Alternative food · Biomarkers · Fatty acid trophic markers · FATM · *Mytilus trossulus* · Temperature adaptation

INTRODUCTION

In the Arctic, marine species are affected by strong seasonality in temperatures and sea ice duration and extent. Sea ice influences Arctic marine ecosystems because light stimulating pelagic primary production is virtually absent while ice covers the ocean, and hence, pelagic primary production is limited to the ice-free period (Pabi et al. 2008). Consequently, biological activities such as feeding, growth, reproduction and larval development are linked to the ice-free period (Conover & Siferd 1993, Blicher et al. 2007, Sejr et al. 2009, Krause-Jensen et al. 2012). For instance, >80 % of the growth variability among populations of the sea urchins *Strongylocentrotus droebachiensis* in Greenland could be explained by the length of the pelagic productive season (Blicher et al. 2007), and the production and poleward distribution of kelp is linked to the duration of the ice-free period (Krause-Jensen et al. 2012). For intertidal species, sub-zero air temperature is another seasonal stressor in the Arctic. During winter, intertidal organisms are exposed to sub-zero temperatures, and associated freeze mortality is common (Blicher et al. 2013,

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Thyrring et al. 2015a). Marine coastal and intertidal species have therefore developed different adaptive coping mechanisms to survive the different Arctic seasons.

In response to food shortage during winter, some species rely on stored energy reserves, lowered metabolic rates (e.g. decreased protein synthesis), hibernation, reduced growth and/or swimming activity (Conover & Siferd 1993, Søreide et al. 2008). Other species (e.g. some amphipods, bivalves) utilize alternative food sources including ice algae, bacteria, macroalgae detritus or zooplankton as a nutritional supplement during winter (Gaillard et al. 2015, Renaud et al. 2015, Kohlbach et al. 2016). In this regard, the use of fatty acid trophic markers (FATM) has been widely applied to study the origin of ingested food (Dalsgaard et al. 2003, Kelly & Scheibling 2012) (Table 1). The method relies on the observation that neutral fatty acids (FA) are conservatively assimilated in the hepatopancreas tissue, thereby providing a reliable indication of the food ingested in the preceding days and/or weeks. Different classes of primary producers have distinguishable FA compositions, thus allowing detection of the food sources (Dalsgaard et al. 2003, Kelly & Scheibling 2012, Parrish 2013, Gaillard et al. 2015). For instance, pelagic diatoms are rich in palmitoleic (16:1ω7) and eicosapentanoic (20:5ω3) acid, dinoflagellates are characterized by high levels of stearidonic (18:4ω3) and docosahexaenoic (22:6ω3) acid (Reuss & Poulsen 2002, Kelly & Scheibling 2012, Gaillard et al. 2015), and bacteria contain high levels of methyltetradecanoic (*i*15:0) and heptadecenoic (17:1) acid (Parrish 2013). While studies using FATM to elucidate food web structure have been conducted in several Arctic regions, including Svalbard (Renaud et al. 2015), the central Arctic Ocean (Kohlbach et al. 2016), the Canadian Arctic (Gaillard et al. 2015) and Greenland (De Cesare et al. 2017), no studies have investigated food preferences in Arctic intertidal species.

To survive sub-zero temperatures, some intertidal algae and invertebrates can tolerate a certain degree of extracellular tissue freezing (Kanwisher 1957, Murphy 1983) where the biological membranes are cooled. When membranes are cooled below a threshold phase-transition temperature (T_m), they change from a liquid-crystalline phase to a dysfunctional gel phase, which may lead to mortality (Hazel & Williams 1990, Hazel 1995, Thyrring et al. 2015a). To avoid cold-induced mortality or injuries, an important mechanism, called remodeling, in terrestrial and marine organisms is modifying membrane FA composition to counteract the effects of decreasing temperatures (Hazel 1995). As each fatty acid has a different T_m, e.g. unsaturated FA have a lower T_m compared to saturated FA, membrane FA composition changes impact membrane fluidity (Hazel & Williams 1990). Thus, remodeling by increasing the proportion of unsaturated fatty acids in membranes can counteract the fluidity and potentially damaging effects of low temperatures via a process known as homeoviscous adaptation (Hazel 1995, Koštál 2010). While the FA composition of reserve lipids provides reliable information on food web structure, the FA composition of membranes can reveal how ectotherms physiologically adjust to survive sub-zero temperatures and tissue freezing (Hazel 1995, Pernet et al. 2006, 2007, Koštál 2010).

Fewer than 100 taxa have been described in the West Greenland intertidal (Hansen 1999, Høgslund et al. 2014). One commonly found bivalve genus is *Mytilus*, from which an isolated population of *M. trossulus* inhabit Northwest Greenland at 77° N (Mathiesen et al. 2017). *M. trossulus* is a temperate species capable of surviving large fluctuations in environmental stress (e.g. salinity, water and air temperatures) and displaying local adaptation and acclimatization among populations (Riginos & Cunningham 2005, Thyrring et al. 2015b, 2017a). However, little is known about this isolated population in Greenland, and it therefore provides an ideal population to study survival and adaptation of a temperate keystone species in the High Arctic.

In this study, we examine the composition of neutral FA in the hepatopancreas and polar (membrane lipids, mainly phospholipids) FA in the gill tissue of *M. trossulus* collected in the lower intertidal over the ice-free period from July to September 2014. We aim to answer the hypotheses (1) that *M. trossulus* feeds primarily on alternative food sources (e.g. macroalgae, bacteria or ice algae) while covered by intertidal ice, (2) that food source preferences would shift to pelagic phytoplankton during the open-water period, and (3) that *M. trossulus* would remodel gill membrane FA composition to maintain functionality and fluidity in response to changes in air temperatures.

MATERIALS AND METHODS

Animal collection and temperature measurements

Adult *Mytilus trossulus* was collected in the lower intertidal zone near Qaanaaq, Northwest Greenland (77° 28′ N; 69° 13′ W). Sea ice covers this region of Greenland from November to early July. In 2014, the

intertidal study site became ice-free on July 4, and adult mussels (shell length of 40–73 mm) were collected 5 times (July 6, July 27, August 11, August 27 and September 9) throughout the summer. Collected mussels was immediately frozen and kept frozen during transported to laboratory facilities until further analysis.

Air temperatures were measured every 60 min at the local weather station (no. 4205). The data were obtained for free from the Danish Meteorological Institute, at www.research.dmi.dk. A description of the data can be found in Boas & Wang (2011).

Fatty acid analysis

Fatty acid (FA) analyses were performed on hepatopancreas (average sample wet weight = 0.63 g) and gill (average sample wet weight = 0.36 g) tissues of *M. trossulus.* In both cases, total lipids were extracted by grinding in a dichloromethane:methanol (2:1, v/v) solution following a slightly modified Folch procedure described by Parrish (1999). Lipid extracts were separated into neutral and polar fractions by column chromatography on silica gel micro-columns (30 × 5 mm i.d., packed with Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) using chloroform:methanol (98:2, v/v) to elute neutral lipids, followed by methanol to elute polar lipids (Marty et al. 1992). FA profiles were determined on fatty acid methyl esters (FAMEs) using sulphuric acid:methanol (2:98, v/v) and toluene. FAMEs of neutral and polar fractions were concentrated in hexane, and the neutral fraction was purified on an activated silica gel with 1 ml of hexane:ethyl acetate (1:1 v/v) to eliminate free sterols. FAMEs were analyzed in the full scan mode (ionic range: 60–650 *m/z*) on a Polaris Q ion trap coupled multichannel gas chromatograph 'Trace GC ultra' (Thermo Scientific) equipped with an autosampler model Triplus, a PTV injector and a mass detector model ITQ900 (Thermo Scientific). The separation was performed with an Omegawax 250 (Supelco) capillary column with high-purity helium as a carrier gas. Data were treated using Xcalibur v.2.1 software (Thermo Scientific). Methyl nondecanoate (19:0) was used as an internal standard. FAMEs were identified and quantified using known standards (Supelco 37 Component FAME Mix and menhaden oil; Supleco) and were further confirmed by mass spectrometry (Xcalibur v.2.1 software). In all samples, unknown peaks were identified according to their mass spectra with emphasis on FA trophic markers. The unsaturation index (UI) is a measure of the number of double bonds calculated in polar lipids as the sum of the percentage of each unsaturated FA multiplied by the number of double bonds (Logue et al. 2000).

Statistical analysis

Multivariate statistical analyses on FA composition, expressed as percentages, were conducted using a non-parametric distance-based permutation multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities, using PRIMER v.7.0 (Clarke & Gorley 2015). Homogeneity was evaluated using the permutation analysis of multivariate dispersion (PERMDISP) (Anderson 2001). All FA were %arcsin square-root transformed and used as 2 variables: tissues (2 level factor) and sampling dates (5 level factor). Following significant PERMANOVA results, post hoc tests were carried out using multiple pair-wise comparison tests to identify differences among factors. To analyze similarity between FA, we used non-metric multidimensional scaling (n-MDS) plots based on Bray-Curtis dissimilarities, and a SIMPER analysis was employed to identify the FA explaining most of the dissimilarity between tissue and sampling dates.

One-way analysis of variance (ANOVA) was used to test for differences in total fatty acids concentration (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturation index (UI), and the MUFA/PUFA-ratio among sampling dates. Tukey HSD post hoc pair-wise tests were used to compare significant treatment effects ($p < 0.05$). Prior to any analysis, data exploration was carried out, and TFA, SFA, PUFA, MUFA, UI and the MUFA/PUFA-ratio data showed homogeneity and normality of distribution. Once we had identified valid models, we re-examined the residuals to ensure model assumptions were acceptable (Thyrring et al. 2015a).

RESULTS

Temperatures

Air temperatures decreased significantly during the study period (linear regression; $p < 0.0001$; Fig. 1). The average air temperature was 5.8°C during the sampling period from July 6 to September 9. Average air temperatures between sampling dates were 6.88°C (July 7 to 27), 7.38°C (July 28 to August 11), 5.25°C (August 12 to 27) and 2.48°C (August 28

to September 9). There was a significant increase in temperatures between samplings 1 and 2 (July 7 and 27; linear regression slope = 0.005; $p < 0.0001$) and samplings 2 and 3 (July 27 and August 11; linear regression slope = 0.01; $p < 0.0001$) before temperatures significantly decreased between samplings 3 and 4 (August 11 and 27, linear regression slope = −0.016; $p < 0.0001$) and between samplings 4 and 5 (August 28 and September 9, linear regression slope = 0.007; $p < 0.0001$). We observed minimum temperature near or below 0°C only before samplings 4 and 5.

Table 1. Fatty acids used as dietary biomarkers

Fatty acids (FA)	Indicator for	Source
14:0; 16:1ω7; 20:5ω3	Diatoms	Reuss & Poulsen 2002, Dalsgaard et al. 2003
18:4ω3; 22:6ω3	Dinoflagellates	Kelly & Scheibling 2012
18:2ω6; 18:3ω3	Macroalgae	Kelly & Scheibling 2012
20:1ω9; 22:1ω9	Zooplankton	Parrish 2013
i-15:0; 15:1; *i*-17:0; 17:0; 17:1	Bacteria	Dalsgaard et al. 2003, Kelly & Scheibling 2012, George & Parrish 2015

Fatty acids

We included FA that contributed >0.5% of total FA. This filtering resulted in 19 FA in the hepatopancreas and gills of *M. trossulus* (detailed profiles of the 19 FA can be found in Table S1 in the Supplement at www.int-res.com/articles/suppl/m572p155_supp.pdf). Comparison of FA composition of neutral and polar FA between the 2 tissues (gills and hepatopancreas) and 5 sampling dates (July to September) showed a significant interaction between tissues and dates (PERMANOVA; p(perm) < 0.0001; Fig. 2a, Table 2). Differences in FA composition between tissues were significant at each sampling time (pair-wise tests; all p(perm) < 0.01; Fig. 2b, Table S2 in the Supplement). SIMPER pair-wise analysis showed that FA composition between gills and hepatopancreas had a level of dissimilarity of 45%. The 8 FA presented in Table 3 explained ~80% of the average Bray-Curtis dissimilarity between FA profiles among tissues. Because the same 8 FA were also involved in the temporal variability among sampling dates (Table S3 in the Supplement displays the SIMPER results of the relative contribution of all 19 FA), we focused our further analysis only on these 8 FA.

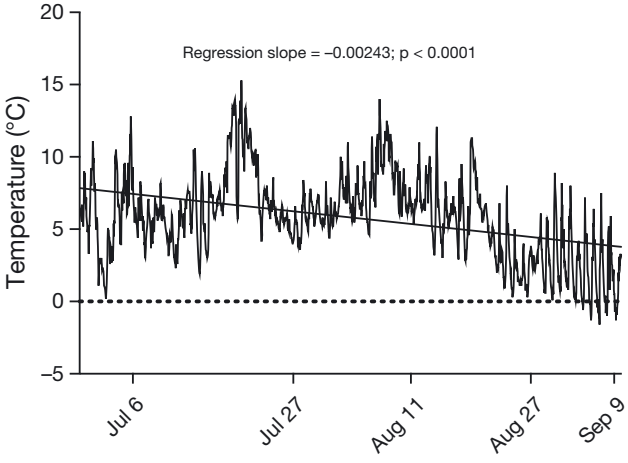

Fig. 1. Atmospheric temperatures measured every 60 min at the nearest weather station (DMI station no. 4205) from July to September 2014. The significantly declining linear regression slope is indicated

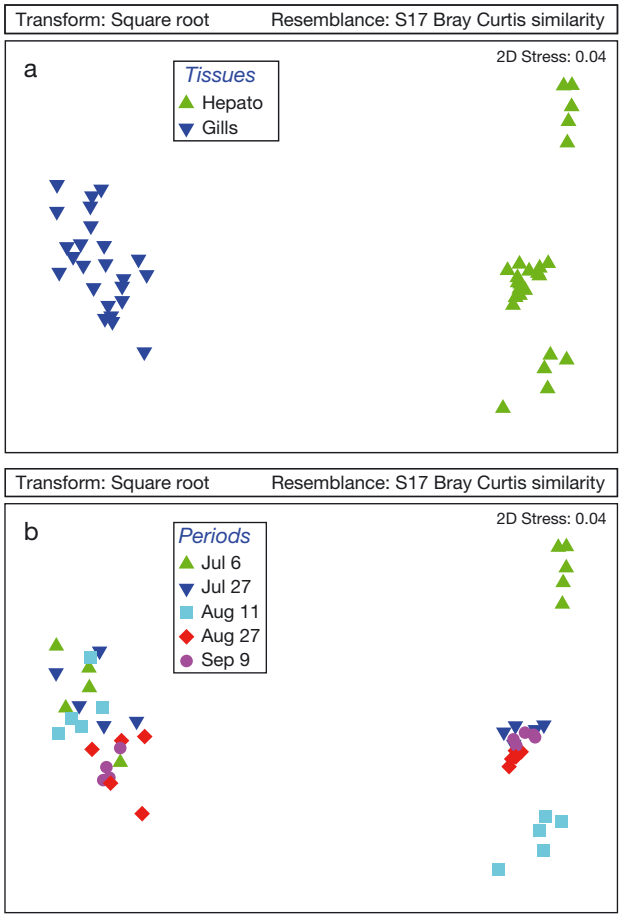

Fig. 2. n-MDS plots based on Bray-Curtis dissimilarities between (a) the total fatty acid composition in the hepatopancreas and gill tissue and (b) the 5 sampling dates

Table 2. Results of PERMANOVA testing the effects of tissue (hepatopancreas and gills) and sampling date on fatty acid composition. Significant pseudo-*F* values are in **bold.** p-values are calculated from the Monte Carlo method

Source of variation	df	MS	Pseudo-*F*	p(perm)
Tissue	1	9837	**654.47**	<0.0001
Sampling date	4	256.53	**16.833**	<0.0001
Tissue × Sampling date	4	206.86	**13.573**	<0.0001
Residuals	39	15.24		

Hepatopancreas FA

There was a significant temporal variation in the total concentration of FA (ANOVA; $F_{4,20}$ = 7.49, p = 0.0007; Fig. 3a) with the highest concentration found on July 6 with an average of 119 ± 39 mg g^{-1} (Tukey HSD; p < 0.01; Fig. 3a). Saturated fatty acids (SFA) significantly increased over the summer (ANOVA; $F_{4,20}$ = 7.86, p = 0.0001; Fig. 3b). No significant differences were found in monounsaturated FA (MUFA) (ANOVA; $F_{4,20}$ = 1.628, p = 0.206; Fig. 3c), unsaturation index (UI) (ANOVA; $F_{4,20}$ = 2.44 p = 0.0803; Fig. 3d), polyunsaturated FA (PUFA) (ANOVA; $F_{4,20}$ = 1.082, p = 0.392; Fig. 3e) or the MUFA/PUFA ratio (ANOVA; $F_{4,20}$ = 1.33 p = 0.294; Fig. 3f).

FA composition was significantly different between the 5 sampling dates (pair-wise test, all p(perm) < 0.05) except between July 27 and September 9 (pair-wise test, p(perm) = 0.219). Differences in FA composition between sampling dates were attributed to a high content of FA biomarkers for diatoms (16:1ω7 and 20:5ω3), with 16:1ω7 on average constituting 9.2–21.5% and 20:5ω3 8.4–40.5% of total FA depending on sampling date (Fig. 4a, Table 3). Tukey HSD post-hoc testing indicated a significantly higher content of diatoms FATM in July 6 samples compared to August and September (Tukey HSD; p < 0.0001, Fig. 4a). The lowest content of diatoms FATM was found in mussels collected August 11 (Tukey HSD; p < 0.0001; Fig. 4a). Dinoflagellates FATM (18:4ω3 and 22:6ω3) increased significantly over the summer (18:4ω3: ANOVA; $F_{4,20}$ = 51.83, p < 0.0001; 22:6ω3: ANOVA; $F_{4,20}$ = 300.2, p < 0.0001). Both 18:4ω3 and 22:6ω3 increased from July 6 to August 11 before a decline in late August and September (Tukey HSD; p < 0.001; Fig. 4a). Macroalgae FATM (18:2ω6 and 18:3ω3) were relatively low during the summer, with mean values of 3.2 ± 0.2% for 18:2ω6 and 2.2±0.1% for 18:3ω3, respectively (Table S1 in the Supplement). The amount of both 18:2ω6 and 18:3ω3 increased significantly over the summer (18:2ω6: ANOVA; $F_{4,20}$ = 67.47, p < 0.0001; 18:3ω3: ANOVA; $F_{4,20}$ = 301.2, p < 0.0001). Finally, FATM for zooplankton (e.g. 20:1ω9 and 22:1ω9) and bacteria (e.g. 17:0 and 17:1) were very low (0.2 to ~4%) and did not contribute significantly to temporal changes in FA composition in the hepatopancreas (Table S1 in the Supplement).

Table 3. The 8 fatty acids that contributed 80% of the SIMPER dissimilarity between the 2 tissue types and the 5 sampling dates. Data presented as mean ± SE, n = 5 (n = 4 for gill tissue sampled on September 5). **Bold** p-values indicate significant differences in FA between sampling dates

Fatty acids	Sampling dates					p
	July 6	July 27	August 11	August 27	September 9	
Gill tissue						
14:0	0.39 ± 0.03	0.60 ± 0.10	0.67 ± 0.07	0.72 ± 0.13	0.68 ± 0.05	0.125
16:1ω7	2.38 ± 0.18	3.53 ± 0.28	2.59 ± 0.21	2.57 ± 0.36	2.31 ± 0.47	0.081
17:1	20.50 ± 2.94	24.18 ± 3.37	21.29 ± 2.21	12.95 ± 1.44	11.42 ± 1.44	**0.0063**
18:1ω9	1.56 ± 0.17	1.50 ± 0.06	1.79 ± 0.07	2.01 ± 0.22	1.65 ± 0.18	0.153
18:4ω3	1.57 ± 0.27	1.58 ± 0.21	0.98 ± 0.23	0.77 ± 0.10	0.70 ± 0.09	**0.0099**
20:5ω3	14.88 ± 1.28	13.08 ± 1.78	12.97 ± 0.53	17.54 ± 0.52	16.60 ± 0.56	**0.0264**
22:2	14.14 ± 1.26	10.63 ± 0.62	13.13 ± 1.28	8.47 ± 1.04	10.04 ± 1.28	**0.0196**
22:6ω3	14.20 ± 1.96	17.06 ± 1.43	17.98 ± 0.35	21.60 ± 0.94	21.12 ± 0.81	**0.0031**
Hepatopancreas tissue						
14:0	5.43 ± 0.11	4.60 ± 0.12	6.69 ± 0.20	5.08 ± 0.12	5.03 ± 0.12	**<0.0001**
16:1ω7	21.54 ± 1.15	18.01 ± 1.47	9.15 ± 0.69	13.64 ± 0.62	16.15 ± 1.14	**<0.0001**
17:1	0.28 ± 0.05	0.45 ± 0.04	0.65 ± 0.36	0.39 ± 0.06	0.50 ± 0.05	0.604
18:1ω9	3.80 ± 0.19	7.59 ± 0.30	11.33 ± 0.47	9.43 ± 0.95	8.01 ± 0.46	**<0.0001**
18:4ω3	2.53 ± 0.17	5.51 ± 0.33	11.13 ± 0.32	6.32 ± 0.76	6.45 ± 0.32	**0.0099**
20:5ω3	40.48 ± 1.33	17.75 ± 0.86	8.43 ± 0.26	17.59 ± 0.83	18.62 ± 0.84	**<0.0001**
22:2	0.43 ± 0.04	0.63 ± 0.10	0.82 ± 0.14	0.60 ± 0.05	0.65 ± 0.04	0.0736
22:6ω3	5.79 ± 0.41	17.15 ± 0.58	25.61 ± 0.49	17.90 ± 0.13	17.35 ± 0.26	**<0.0001**

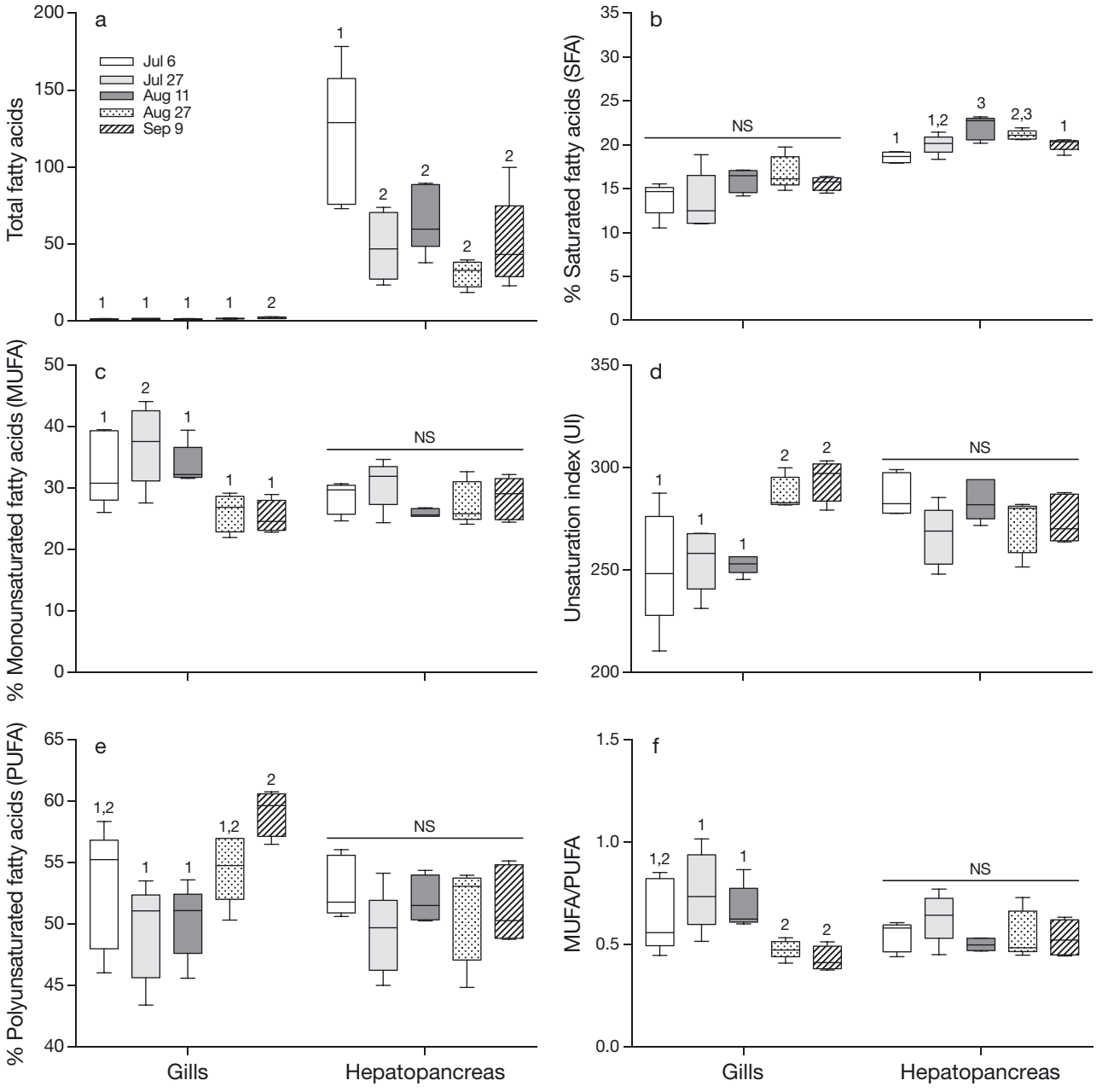

Fig. 3. Boxplots showing the temporal variation in (a) total fatty acids, (b) saturated fatty acids (SFA), (c) monounsaturated fatty acids (MUFA), (d) the unsaturation index (UI), (e) polyunsaturated fatty acids (PUFA), and (f) the MUFA/PUFA ratio in *Mytilus trossulus* between 5 sampling dates. Boxplot whiskers indicate minimal and maximal values, and different numbers above the boxes indicate significant differences among sampling dates ($p < 0.05$); NS: not significant

Gill tissue FA

In gills, FA composition were similar on July 6, July 27 and August 11 (pair-wise test, p(perm) > 0.05). The FA composition on August 27 and September 9 was significantly different compared to July 6, July 27 and August 11 (pair-wise test, p(perm) < 0.05). There was no temporal change in SFA (ANOVA; $F_{4,19}$ = 2.34, $p < 0.0922$; Fig. 3b), but we observed a significant change in the total FA concentration (ANOVA; $F_{4,19}$ = 3.77, p = 0.0202; Fig. 3a), MUFA (ANOVA; $F_{4,20}$ = 1.628, p = 0.003; Fig. 3c), UI (ANOVA; $F_{4,19}$ = 27.97 p = 0.0006; Fig. 3d), PUFA (ANOVA; $F_{4,19}$ = 5.37, p = 0.0046; Fig. 3e) and the MUFA/PUFA ratio (ANOVA; $F_{4,19}$ = 5.21 p = 0.0053; Fig. 3f). Five out of 8 FA changed significantly during the summer

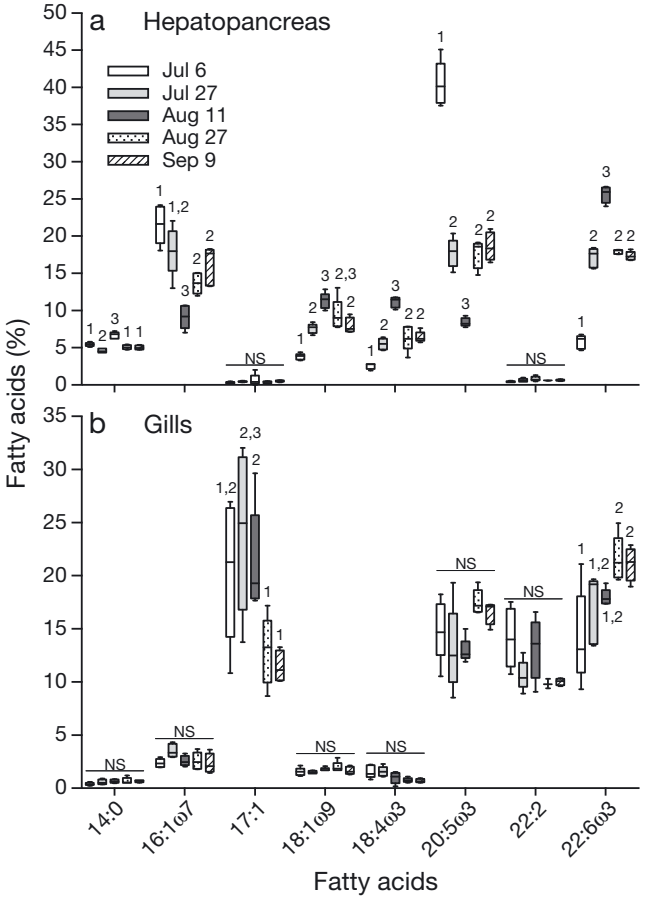

Fig. 4. Boxplot showing the temporal variation in 8 investigated fatty acids in (a) the hepatopancreas and (b) the gills of *Mytilus trossulus* on 5 sampling dates. Boxplot whiskers indicate minimal and maximal values, and different numbers above the boxes indicate significant differences among sampling dates ($p < 0.05$); NS: not significant

(ANOVA; all $p < 0.05$; Table 3). *M. trossulus* selectively retained high levels of bacteria FA in their gills (17:1) with an average between 24.2 ± 3.4% (July 27) and 11.4 ± 1.4% (September 9) (Fig. 4b, Table 3) compared to <0.5% in the hepatopancreas. There was a significant increase in 22:6ω3 (dinoflagellates biomarker) from July 6 to August 27 and September 9 (Tukey HSD; $p < 0.05$; Fig. 4b). Mussels retained low values of zooplankton (e.g. 20:1ω9 and 22:1ω9) and macroalgae (18:2ω6 and 18:3ω3) FATM in the gills (Fig. 4b, Table S1 in the Supplement).

DISCUSSION

For the first time, we present results on the dietary preferences over the ice-free season in an intertidal keystone species from the High Arctic. We found that the temperate bivalve *Mytilus trossulus* in North Greenland (77° N) fed preferentially on diatoms while covered by intertidal sea ice. During the open-water period after ice break-up, the food consisted mainly of pelagic dinoflagellates. We found also that macroalgae detritus, zooplankton and bacteria contributed to the mussels' diet, but FATM values suggest a limited contribution of these alternative food sources. Furthermore, we provide evidence that this bivalve adjusts its membrane FA composition by selectively retaining FA from bacteria and phytoplankton in response to changes in air temperatures. Thereby, *M. trossulus* likely maintains membrane functionality and avoids membrane damage during the fluctuating temperatures characterizing the Arctic seasons.

Hepatopancreas FA

Using fatty acid trophic markers (FATM), we found support for our first hypothesis that *M. trossulus* feed on alternative food sources such as ice algae or macroalgae detritus while ice covered. Ice algae consist mainly of diatoms that are rich in palmitoleic (16:1ω7) and eicosapentanoic (20:5ω3) acid (Falk-Petersen et al. 1998, Henderson et al. 1998). Neutral FA from the hepatopancreas in specimens collected just a few days after ice break up showed that *M. trossulus* had fed on food specifically rich in 16:1ω7 and 20:5ω3. These 2 FA combined constituted 62% (16:1ω7 = 21.5% and 20:5ω3 = 40.5%) of the total FA found on this sampling date (July 6). For macroalgae biomarkers, we observed the lowest values, <2%, in the beginning of July compared to >4% in September. Based on the high levels of diatom FATM, our results therefore indicate that ice algae are a central food source for ice-covered *M. trossulus* at their northernmost distribution in Arctic Greenland. This conclusion was further supported by the low values of docosahexaenoic (22:6ω3) (5.8% of total FA) and stearidonic (18:4ω3) (2.5% of total FA) acid found in the specimens collected on July 6, as diatoms are deficient in these FA (Falk-Petersen et al. 1998). It should be noted that we *per se* are not able to determine if the diatoms ingested originate from ice algae or pelagic diatoms as the FA signal from these is similar (Dalsgaard et al. 2003, Søreide et al. 2008). An alternative scenario is that ice-covered filter feeders consume phytoplankton advected under the ice from surrounding ice-free open-water areas. For instance, diatoms could originate from nearby ice-edge plankton blooms as the ice-free open-water area expands in the fjord prior to the intertidal ice break off (Perrette et al. 2011).

Dinoflagellates are commonly found in open-water plankton communities and are characterized by being rich in 18:4ω3 and 22:6ω3 and deficient in 16:1ω7 and 20:5ω3 (Dalsgaard et al. 2003, Kohlbach et al. 2016). This FATM signal corresponds with the observed significant decrease in 16:1ω7 and 20:5ω3 and the significant increase in 18:4ω3 and 22:6ω3 during the ice-free period from July 6 in the hepatopancreas. These results hereby support our second hypothesis that *M. trossulus* would shift to an open-water pelagic plankton diet after ice break up. At the end of the season from August 11, diatom FATM markers again increased significantly, thus indicating mussels fed on a mixture of open-water diatoms (16:1ω7 and 20:5ω3 = 31.2% of total ingested FA) and dinoflagellates (18:4ω3 and 22:6ω3 = 24.2% of total FA on August 27) during in this period. With the development of macroalgae biomass during the ice-free season (Krause-Jensen et al. 2012), FATM (18:2ω6 and 18:3ω3) increased in the mussels' hepatopancreas, showing use of this food source. However, as the values remained low (<5% for each FA), we suggest that macroalgae detritus contributed weakly to cover mussels' nutritional needs. Similarly, we found the *Calanus* biomarker 20:1ω9 present in small amounts on all sampling dates (Table S1 in the Supplement). This indicates that mussels may, in addition, ingest lipids originating from dead *Calanus* spp. associated with the sea ice community (Scott et al. 2002). *Calanus* spp. effectively convert low-energy carbon and proteins from ice algae into high-energy lipids (Lee et al. 2006), and the nutritional importance of the food supplement should be investigated further. The bacterial FATM heptadecenoic (17:1) acid was on average <1% throughout the study period and could most likely be related to the low nutritional quality of bacteria compared to the available ice algae. However, the gill data demonstrated the importance of this FA for the structure of polar lipids composition and suggest a rapid transfer from neutral lipids of the hepatopancreas to polar lipids of the gills. Because ice algae are an important food source for Arctic bivalves, the ongoing changes in sea ice distribution may have major implications for their ecology. The ecological consequences of changes in sea ice and its implications for food web structure and carbon turnover should therefore be studied further in the Arctic.

The highest amount of total fatty acids (TFA) in *M. trossulus* were found on July 6 before it significantly decreased on July 27. The decline in TFA was probably related to a decrease in 20:5ω3 from 40.5 ± 1.3% July 6 to 17.4 ± 0.3% July 27. The significant decline in hepatopancreas TFA can be caused by the transfer of FA to the gonads during gametogenesis (Zandee et al. 1980, Barber & Blake 1981). This agrees with previous work finding a 20:5ω3 decrease in the hepatopancreas of blue mussels *M. edulis* and eastern oysters *Crassostrea virginica* in summer (Pernet et al. 2007). Palacios et al. (2005) found the same pattern during the gametogeneic cycle of the giant lion's paw *Nodipecten subnodosus*. Because *M. trossulus* generally mature and spawn during summer in both Canada (Toro et al. 2002) and Greenland (Thyrring et al. 2017b), it seems likely that this species also transfers essential FA to the gonads for initiating and sustaining gametogenesis during summer.

Membrane FA composition and cold tolerance

We found the dominant polar FA in the gills to be 17:1, 20:5ω3 and 22:6ω3, in addition to the *de novo* biosynthesized non-methylene-interrupted (NMI) FA 22:2. These 4 FA contributed on average 62% of the total FA over the season from July 6 to September 9. However, only 17:1 and 22:6ω3 showed temporal variation. The significant increase in UI and relative amount of PUFA in the cell membrane from August 11 to September 9 is attributed to significant changes of 17:1 and 22:6ω3 in response to decreasing air temperatures. We observed a significant decrease of 17:1 in the mussels' gill related to a concomitant increase of 22:6ω3 and UI after mussels were first exposed to temperatures <0.5°C (after August 23; Fig. 1). The presented results therefore support the third hypothesis that *M. trossulus* would remodel gill membrane FA composition to maintain fluidity and counteract membrane dysfunctionality as a consequence of changes in environmental temperatures (Hazel 1995). Increased unsaturation lead to a more disorganized and fluid membrane with a lowered T_m. In this way, ectothermal organisms can avoid membrane damage during winter. Although we did not measure membrane fluidity, these changes are in agreement with homeoviscous adaptation and other studies on ectotherms showing how lowering environmental temperatures produces increases in membrane UI (Hazel 1995, Pernet et al. 2006, 2007, Parent et al. 2008, Koštál 2010).

Bacteria are characterized by odd-numbered short-chained and branched FA (Dalsgaard et al. 2003). There was a surprisingly high retention of the bacterial FATM 17:1 in the gills with values between 11 and 24% of the total FA. Ingested bacteria from the water column or re-suspended sediment may have been selectively retained in the gill membrane to

maintain proper unsaturation levels during summer when temperatures are mild. During late August and September, the decrease in MUFA and relative increase in PUFA was, among other factors, attributed to a significant decrease in 17:1. Increasing the amount of PUFA in the membrane counteracted the ordering effect of the decreasing temperatures. The final important FA in the membrane was NMI, which is commonly found in marine invertebrates as they can *de novo* synthesize it (Barnathan 2009). NMI 22:2 can therefore be used to replace certain FA that otherwise must be obtained from external food sources. Replacement with NMI has been suggested to occur in periods where feeding has stopped or PUFA-rich food is insufficiently available (Pernet et al. 2006), and an increased amount of NMI has been observed in response to decreasing temperatures (Pernet et al. 2006). We found no temporal NMI 22:2 variation and therefore no consistent pattern between temperature and NMI content. For instance, the average %FA of 22:2 was 10.6 ± 0.6% on July 27 and 10.0 ± 1.3% on September 9, despite the fact that the air temperatures were significantly lower in September compared to July (Fig. 1). The inconsistent temperature pattern agrees with a study that found no correlation between NMI and temperatures in the eastern oyster *C. virginica* (Pernet et al. 2007). Therefore, it seems that the biosynthesis of MNI may constitute a range of biological purposes depending on season and local conditions, and their functional and structural role remain to be well described (Barnathan 2009). In *M. trossulus* specifically, NMI FA could be utilized in combination with FA 17:1 to maintain proper membrane fluidity during summer, while energy-rich MUFA and PUFA assimilated from the food are utilized to conserve proteins and carbohydrates for energy in winter (Kluytmans et al. 1985), or alternatively allocated to physiological processes including growth and gonad development during gametogenesis.

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