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Food resources of the bivalve *Astarte elliptica* in a sub-Arctic fjord: a multi-biomarker approach

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ABSTRACT: It is generally agreed that pelagic–benthic coupling is tight on Arctic shelves, i.e. that organic matter produced in the surface layers supports the seafloor and benthos. However, this paradigm is mainly based on the assumption that phytoplankton and ice algae are the main sources of carbon for the benthic communities. Climate change is expected to alter the relative contribution of food sources for benthic organisms. Macroalgal biomass is predicted to increase in near-shore systems in response to increased temperature and reduced sea ice cover. Thus, a better understanding of the relative contribution of benthic and pelagic components in benthic food webs in the Arctic is needed. In this study, a multi-biomarker approach (stable isotopes, fatty acid trophic markers, and compound-specific stable isotope analysis) was applied to link potential sources of carbon, including particulate organic matter from subsurface and bottom waters, sediment organic matter, and 6 macroalgal species to the diet of the bivalve *Astarte elliptica* collected below the euphotic zone in a sub-Arctic fjord (Kobbefjord, Greenland). Results showed that *A. elliptica* feeds on particulate and sediment organic matter and that brown macroalgae significantly support the Arctic benthic food web. Multi-biomarker approaches can be used to determine the diet of benthic organisms and track temporal variability in sources of food. It therefore appears to be an interesting method to study food regime strategies in response to changing primary production dynamics.

KEY WORDS: Trophic ecology · Stable isotopes · Fatty acid trophic markers · Compound-specific stable isotope analysis · Sub-Arctic fjord · Bivalve

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INTRODUCTION

Unprecedented changes of natural systems have been taking place since the mid-20th century in response to observed global warming, with the high-latitudes being the most affected regions (IPCC 2013). Changes in physical conditions, including warming of the sea surface, decreases in Arctic sea ice cover, and changes in water column stratification are expected to affect primary production (Wassmann 2011). Timing, magnitude, and spatial distribution of primary production may be modified and conse-

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quently may have effects on the trophic pathways supporting pelagic and benthic consumers (Wassmann & Reigstad 2011). In Arctic environments, benthic communities can be sustained by direct sedimentation of ice algae and phytoplankton cells and/or through the sinking of organic material processed by pelagic secondary consumers (pelagic–benthic coupling; Piepenburg 2005). Ice algae, largely dominated by pennate diatoms (Hsiao 1980, Poulin et al. 2011), live both attached to the bottom of sea ice and within the ice column and bloom during spring, while phytoplankton live in the water column and bloom after the ice melts in early summer (Hsiao 1992). Ice algae may be important as early season food sources for the benthos when reduced grazing pressure in the water column increases the amount of organic matter reaching the seafloor (Ambrose & Renaud 1995). Based on the assumption that phytoplankton and ice algae are the 2 main primary producers in Arctic systems, a future scenario for pelagic–benthic coupling is largely accepted. The paradigm is that, in response to the decline in sea ice extent and thickness, a shift from the present ice algae–benthos dominance to a predicted phytoplankton–zooplankton prevalence could lead to a weaker pelagic–benthic coupling (Carroll & Carroll 2003, Carmack & Wassmann 2006).

To date, macroalgae have been sparsely considered in Arctic food web studies. However, it has been shown that they can substantially contribute to the total primary production and even exceed pelagic primary production in shallow depths in the Arctic (Glud & Rysgaard 2007, Krause-Jensen et al. 2007). Recent studies (McMeans et al. 2013, Renaud et al. 2015) indicate that macroalgae-derived material can substantively supply the diet of Arctic benthic organisms in coastal areas and also filter-feeders traditionally considered relying completely on phytoplankton. This macroalgal primary production enters food webs through direct grazing (Steneck et al. 2002, Wessels et al. 2012), exudation of dissolved organic carbon, and as detritus, and could be especially beneficial to growth of benthic suspension feeders (Duggins et al. 1989, Perez et al. 2013). The role of macroalgae as potential sources of carbon for higher trophic levels is important to determine since warming of the Arctic is expected to favor some macroalgal communities (Krause-Jensen & Duarte 2014). Cold temperate and sub-Arctic macroalgae are predicted to extend their distribution to the Arctic Ocean with global change (Hop et al. 2012, Krause-Jensen et al. 2012, Krause-Jensen & Duarte 2014). Changes in macroalgal community composition have already been noted in fjords of the Svalbard archipelago. The dominance of calcareous algae, well adapted to low-light and cold-water regimes, has been replaced by a rapid expansion of erect boreal macroalgae in response to increased sea surface temperature and improved light availability (Kortsch et al. 2012). In addition, warming of the Arctic will likely allow macroalgal growth to start earlier and extend the growing season in response to the longer ice-free period (Krause-Jensen & Duarte 2014). Yet, it remains unclear how benthic communities will respond to those changes in food sources. There are few studies on changes in Arctic benthic systems (e.g. Wassmann et al. 2011), and no shifts in benthic macrofaunal communities have been reported on west Greenlandic coasts in response to shifts in primary producers.

Carbon (C) and nitrogen (N) stable isotopes are particularly useful to trace pathways of organic matter in food webs and to determine the contribution of various food items to diets of organisms (Fry 2007). $\delta^{15}N$ is used to assess the mean trophic position of organisms in a food web (DeNiro & Epstein 1981, Peterson & Fry 1987, Hobson & Welch 1992), while $\delta^{13}C$ values can provide information on major carbon sources of consumers (Peterson & Fry 1987, Vander Zanden & Rasmussen 2001, Post 2002). The transfer of carbon isotopic compositions (<1‰) to the animal from its diet can be useful in tracing food webs in systems where food sources show large differences in $\delta^{13}C$ values, such as marine versus terrestrial systems, or coastal versus oceanic systems (DeNiro & Epstein 1978, Peterson & Fry 1987, Vander Zanden & Rasmussen 2001).

The use of fatty acid (FA) trophic markers (FATMs) is based on the observation that marine primary producers lay down certain FA patterns that may be conserved and transferred through aquatic food webs. Thus, they can be recognized in lipids of their primary consumers (Dalsgaard et al. 2003, Bergé & Barnathan 2005). Marine animal cells can synthetize de novo saturated FAs (SFAs) and monounsaturated FAs (MUFAs), but biosynthesis of major polyunsaturated FAs (PUFAs) is considered very limited due to the low or non-existent activity of the specific enzymes required (Glencross 2009). Consequently, trophic resources must provide the needed PUFAs and can be traced to the primary consumers. The presence and combinations of particular FAs can be therefore used as taxonomic signature to differentiate primary producers, such as diatoms from dinoflagellates (Ackman et al. 1968, Viso & Marty 1993, Zhukova & Aizdaicher 1995). Diatoms are rich in eicosapen-

taenoic acid (20:5ω3) and unsaturated FAs (UFAs) with 16 carbons, especially 16:4ω1, while dinoflagellates are often dominated by PUFAs with 18 carbons (especially 18:4ω3), and docosahexaenoic acid (22:6ω3) (Budge & Parrish 1998, Mansour et al. 1999, Dalsgaard et al. 2003). Dominance of FAs 14:0, 16:0 and 18:0 are also characteristic of Prymnesiophyceae such as *Phaeocystis* spp. (Claustre et al. 1990, Nichols et al. 1991). Macroalgae are, in general, rich in PUFAs (Dalsgaard et al. 2003). Major PUFAs characterizing Phaeophyceae (brown macroalgae) are 18:4ω3, 20:4ω6, and 20:5ω3, while Chlorophyta (green macroalgae) have FA compositions dominated by 18:3ω3 and 18:2ω6 (Graeve et al. 2002, Kelly & Scheibling 2012). Kelly & Scheibling (2012) showed that the FAs contributing to differentiate brown and green macroalgae are 18:3ω3 (more abundant in green algae) and 18:1ω9 (more abundant in brown algae). Odd-numbered and branched FAs, such as 15:0, 17:0, *iso*- and *anteiso*-SFA, are typically dominant in bacterial FA composition and are used as tracers for the contribution of heterotrophic bacteria to sediments, suspended organic material, and animal diets (Meziane & Tsuchiya 2002, Dalsgaard et al. 2003). Long-chain MUFAs (20:1 and 22:1) are typically accumulated in zooplankton, especially calanoid copepods, and have been used as tracers to identify them in consumers (Dalsgaard et al. 2003, Kelly & Scheibling 2012).

Compound specific stable isotope analysis (CSIA) is expected to avoid limits encountered when using common trophic markers such as bulk stable isotope and FA analyses. Especially CSIA appeared to be more successful than the other trophic markers when different food sources have similar bulk carbon isotope and FA signatures (Gladyshev et al. 2012). Isotope analysis on specific FAs has been particularly investigated to distinguish diatoms from the water column from diatoms associated with the sea ice and assess their relative contribution to the Arctic food web (Budge et al. 2008, Wang et al. 2014). Budge et al. (2008) investigated 2 specific FA markers of diatoms, 16:4ω1 and 20:5ω3, and conclude that their $\delta^{13}C$ values are clearly different according to whether they are of sea ice or pelagic origin. $\delta^{13}C_{FA}$ values of sea ice diatoms are 7 to 9‰ higher than those of pelagic diatoms (compared to a difference of 4 to 5‰ for bulk carbon isotope analysis; e.g. Hobson et al. 2002, Tamelander et al. 2006). Increased seasonal light variability (Gradinger 2009) and the carbon-limiting environment of the closed sea ice system (Schubert & Calvert 2001, Kennedy et al. 2002) explain differences in $\delta^{13}C$ signatures.

To examine importance of these different Arctic primary producers in the diet of benthic consumers, we chose to examine the diet of *Astarte* spp., which are among the most common mollusks in marine Arctic shelves (Piepenburg et al. 2011, Roy et al. 2014). Astartids are well adapted to cold, boreal-Arctic waters (Gordillo & Aitken 2000) and inhabit depths ranging from 2 to >100 m, preferring mixed sediments. As endobenthic suspension-feeders, they filter food particles through a siphon from the bottom water and sediment–water interface (Khim et al. 2001, Hobson et al. 2002, Simstich et al. 2005). *Astarte* spp. are generally slow-growing species (maximum 3.8 mm yr^{-1}; Mueller-Lupp et al. 2003, Simstich et al. 2005) characterized by low metabolic rates. The *A. elliptica*-complex as defined and used by Petersen (2001) includes 5 forms (or species), which are difficult to distinguish on the basis of morphological study. Therefore, we did not differentiate among *Astarte* species, but collected a number of individuals within the *A. elliptica*-complex.

Our study combined FAs, stable isotopes and CSIA to characterize and trace sources of food and their use by the benthos in a sub-Arctic fjord (Kobbefjord, SW Greenland). Although *A. elliptica* is not a keystone species at this site, we used this long-lived species as a valuable indicator for ecosystem processes. More specifically, the objectives were to: (1) characterize sources of food using their FA composition, (2) determine dominant source(s) of food and their relative contribution to the diet of a bivalve (*A. elliptica*-complex) and (3) show seasonal variability in the diet of this bivalve. We hypothesized that (1) particulate organic matter (POM), sediment organic matter (SOM) and major macroalgal taxa are distinguishable from each other because of their different FA profiles and isotopic signatures, (2) as a suspension-feeder, *A. elliptica* mainly feeds on microalgae and (3) the diet of *A. elliptica* changes with seasonal phytoplankton patterns, showing an opportunistic feeding strategy in the context of changing primary production.

MATERIALS AND METHODS

Study site

The study was conducted in the sub-Arctic Kobbefjord, in southwest Greenland (Fig. 1). This sill fjord is a part of the Godthåbsfjord system and is ca. 17 km long, 0.8 to 2 km wide and has a maximum depth of 150 m. It is subject to important exchange of coastal

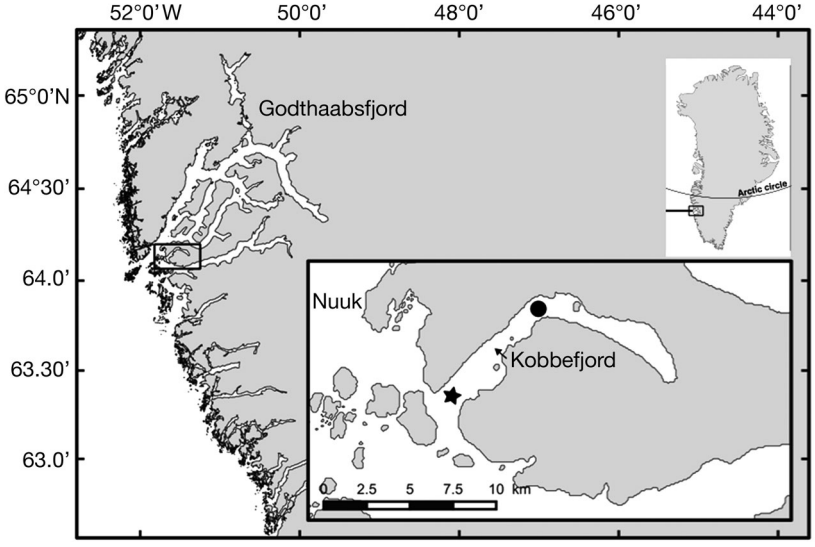

Fig. 1. Study site and sampling stations in Kobbefjord, southwest Greenland for investigation of food resources of *Astarte elliptica*. Samples were collected in May and September 2013 from the outer region of the fjord (★), except for sediment samples, which were collected in the central area (●)

water driven by a tidal range of 1 to 4.5 m (Richter et al. 2011) and receives freshwater from a river in the innermost part of the fjord, leading to a salinity gradient in the surface water. The outer part of the fjord is permanently ice free, whereas sea ice usually covers the innermost part of the Kobbefjord, with extensive interannual variation (Mikkelsen et al. 2008). Sea surface temperatures range from ca. −1.5°C during winter to ca. 8°C in late summer (Blicher et al. 2009). The stratification of the water column begins in late spring and lasts throughout the summer, when atmospheric heating and freshwater inputs from melting glaciers increase (Sejr et al. 2014, Sørensen et al. 2015). Phytoplankton is the main primary producer in the central parts of the fjord (Sejr et al. 2014). Generally, diatoms (particularly the genera *Chaetoceros* and *Thalassiosira*) dominate the microplankton assemblage during the spring bloom in late April/early May in the Godthåbsfjord system and are complemented by haptophytes (Juul-Pedersen et al. 2015, Krawczyk et al. 2015). Large brown macroalgae (*Agarum clathratum* and *Laminaria* spp.) form subtidal productive benthic habitats along the shores to water depths of ca. 40 m (Krause-Jensen et al. 2012). Communities of intertidal macroalgae are dominated by *Ascophyllum nodosum* but also with high abundance of *Fucus* spp. (Krause-Jensen et al. 2015). Subtidal and intertidal macroalgal communities form important habitats for sea urchins and bivalves (e.g. blue mussel *Mytilus edulis*) (Blicher et al. 2009, 2013, Krause-Jensen et al. 2012).

Field sampling

Sampling of the bivalve *Astarte elliptica*-complex and its potential sources of food were carried out on 15 May and 2 September 2013. Live *A. elliptica* were collected using a triangular dredge at a depth of 50 to 60 m in the outer regions of Kobbefjord (64° 07′ N, 51° 38′ W; Fig. 1) in a shell substrate bottom with high densities of scallops *Chlamys islandica* and sea urchins *Strongylocentrotus droebachiensis*. Specimens were frozen at −80°C shortly after being collected and kept at −80°C until further analysis. In total, 50 and 49 specimens were collected in May and in September, respectively. For each sampling period, 17 individuals were randomly selected and were dissected on ice to separate soft tissues (digestive gland and foot) from shells. As different tissues show different isotopic enrichment from food source to consumer, depending on the tissue turnover rate (e.g. Lorrain et al. 2002, Deudero et al. 2009), we used the digestive gland (high metabolic activity) to elucidate recent bivalve diet and foot (a tissue with lower metabolic activity) to provide an 'average' of dietary source over a longer period of time (Paulet et al. 2006).

To characterize suspended particulate organic matter (POM), both from the subsurface waters at 5 m depth (s-POM) and from 5 m above the bottom (b-POM), we collected 10 l water samples (n = 3 during both sampling periods) at the study site using Niskin bottles. Three sub-samples for each type of analysis (FAs, stable carbon isotopes of specific FAs and bulk stable isotopes) were made from the initial 10 l samples. On average, 2 l sub-samples were filtered onto pre-combusted (450°C for 4 h) 47 mm diameter Whatman GF/F glass-fiber filters (pore size 0.7 µm). All POM filters were kept at −80°C until analyses.

Because sediment sampling was impossible using a hand corer on *A. elliptica* habitat, we collected sediment cores (n = 3 for both sampling periods) at a depth of 110 m in a sedimentation basin in the middle section of the fjord (64° 10′ N, 51° 31′ W; Fig. 1) using a Kajak sampler (KC-Denmark). The upper 1 cm was retained and frozen at −80°C until further analysis. Given the large tidal amplitude and the strong mixing in the entire outer Godthåbsfjord area (Mortensen et al. 2011), we expected that small particles

would be transported over large distances and thus similar food source conditions are expected at the bivalve location and the nearby sediment station (J. Mortensen pers. comm.). Since bivalves and sediment were collected below the euphotic zone, we did not specifically sample microphytobenthos.

Lastly, the most abundant macroalgae were collected in May using a grab hook from the boat for subtidal species (brown macroalgae: *Agarum clathratum*, *Laminaria* spp.) or by hand on the rocky shores for intertidal communities (brown macroalgae: *Ascophyllum nodosum*, *Dictyosiphon foeniculaceus*, *Fucus vesiculosus*; green macroalgae: *Ulva lactuca*). All these species were fixed on hard substratum. Three specimens were collected for each species. Salt and epibionts were then removed with Milli-Q and samples were frozen at −80°C until further analysis.

As sea ice cover was very limited in Kobbefjord in winter 2013 (Jensen & Christensen 2014), biomass of sea ice algae was nearly zero and sampling was difficult. We did not have enough material to characterize this source of food.

Analysis of stable carbon and nitrogen isotopes

Bulk stable isotope analyses were performed on potential sources of food available at the time of sampling (i.e. pelagic s-POM and b-POM, sediment and macroalgae) and on the digestive gland of *A. elliptica*. All samples were freeze-dried before treatment and analysis. Digestive glands were then ground to a homogeneous powder and ca. 1 mg weighed in tin capsules for analyses. POM filters, sediments and macroalgal samples were duplicated: one untreated, and one acid-fumed or acid-washed to remove carbonate (Harris et al. 2001). POM filters were treated following the acid fume method, while sediment and macroalgal samples were treated by the acid washing method. Both acidified and non-acidified samples were analyzed for C and N. Samples were then dried at 60°C for 24 h and weighed in tin capsules before analyses. $\delta^{13}C$ and $\delta^{15}N$ composition were determined at the UC Davis Stable Isotope Facility (Department of Plant Sciences, University of California at Davis, CA) using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). Isotopic ratios for carbon and nitrogen were expressed using the standard δ notation according to the following equation:

$$\delta X = [(R_{sample}/R_{reference}) - 1] \times 1000 \quad (1)$$

where X is the ^{13}C or ^{15}N of the sample, and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The reference for C was Vienna Pee Dee Belemnite (VPDB), and atmospheric N_2 was the reference for N. The analytical precision was 0.2 and 0.3‰ for C and N, respectively.

FA analysis

Table 1 is a summary of the FAs used as dietary tracers in our study. FA analyses were performed on potential sources of food and on digestive gland and foot of *A. elliptica* following the method of Bligh & Dyer (1959), as slightly modified in Meziane & Tsuchiya (2002). Briefly, samples were freeze-dried overnight and lipids extracted via ultrasonication for 20 min in distilled water:$CHCl_3$:MeOH (1:1:2 v:v:v) after addition of an internal standard (23:0). Lipids were concentrated under N_2 flux and saponified with a mixture of NaOH:MeOH (1:2 v:v) at 90°C during 90 min to separate FAs. Saponification was stopped with HCl (35%) and $CHCl_3$ was added to recover

Table 1. Selected fatty acid trophic markers (FATMs) used as dietary tracers in the study of food resources of *Astarte elliptica* in Kobbefjord, southwest Greenland

Source	FATM	References
Bacteria	Σ*i*-FA + *ai*-FA, 18:1ω7, 16:1ω7	Viso & Marty (1993), Budge & Parrish (1998), Stevens et al. (2004)
Diatoms	16:4ω1, 16:1ω7, 20:5ω3	Viso & Marty (1993), Napolitano et al. (1997), Reuss & Poulsen (2002), Kelly & Scheibling (2012)
Dinoflagellates	22:6ω3, 18:4ω3, 18:1ω9	Napolitano et al. (1997), Mansour et al. (1999), Kelly & Scheibling (2012)
Copepods	Σ20:1ω9 + 22:1ω11	Dalsgaard et al. (2003), Lee et al. (2006)
Chlorophyta (green macroalgae)	18:3ω3, 18:4ω3, 16:4ω3	Graeve et al. (2002), Khotimchenko et al. (2002), Kelly & Scheibling (2012), this study
Phaeophyceae (brown macroalgae)	18:2ω6, 20:4ω6, 20:5ω3, 18:1ω9	Graeve et al. (2002), Kelly & Scheibling (2012), Wessels et al. (2012), this study

FAs. FAs were concentrated under N_2 flux and converted to methyl esters by incubation with BF_3-MeOH at 90°C for 10 min. FA methyl esters (FAMEs) were extracted with addition of mixture distilled water:$CHCl_3$ (1:1 v:v) and concentrated under N_2 flux to transfer in hexane. 1 µl of the sample was injected to a gas chromatograph (GC, Varian CP-3800 equipped with flame ionization detector) equipped with a Supelco OMEGAWAX 320 column (30 m × 0.32 mm inside diameter, 0.25 µm film thickness) and helium as carrier gas to separate and quantify FAs. FAs were identified by comparison of the retention time with analytical standards (Supelco® 37 Component FAME Mix) and analysis of the sample in a gas chromatograph coupled to a mass spectrometer (GC–MS, Varian 450GC with Varian 220-MS). Each FA concentration was given as a percentage of total FAs (TFAs).

Stable carbon isotope analysis of specific FAs

Carbon stable isotope ratios of FAME samples (expressed as $\delta^{13}C_{FA}$ values in ‰) were analyzed at the UC Davis Stable Isotope Facility. Isotopic analyses of individual FAMEs were carried out under a continuous helium flow using a GC (Agilent 6890N) equipped with a BPX70 column (60 m × 0.25 mm outside diameter, 0.25 µm film thickness) coupled to an isotope ratio mass spectrometer (IRMS) (Thermo Finnegan MAT 253). Once separated, samples were converted to CO_2 in an oxidation reactor at 950°C, and CO_2 entered the IRMS. FAME $\delta^{13}C$ were corrected for the methyl group addition during methylation according to the following formula:

$$\delta^{13}C_{FA} = \frac{(x+1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}}{x} \quad (2)$$

where $\delta^{13}C_{FAME}$ and $\delta^{13}C_{MeOH}$ are the $\delta^{13}C$ values of the measured FAME and methanol used during methylation, respectively. $\delta^{13}C_{FA}$ represents the $\delta^{13}C$ of the given FA prior to methylation, and x is the number of carbon atoms in the (non-methylated) FA. In this study, the $\delta^{13}C$ value of the methanol used for the FAME preparation was equal to −38.43‰. All $\delta^{13}C_{FA}$ values are reported relative to VPDB using standard δ notation.

Data analysis

The effect of acid treatment on bulk carbon isotopic ratios of sources of food was tested using paired Student's *t*-test. Effects of 'Season' (fixed with 2 levels) and 'Depth' (fixed with 2 levels) on $\delta^{13}C$ and $\delta^{15}N$ in POM were assessed with 2-way crossed ANOVA. Effects of 'Season' (fixed with 2 levels) on $\delta^{13}C$ and $\delta^{15}N$ in sediment samples and in digestive glands were tested with 1-way ANOVA. Differences in isotopic signatures between the 6 macroalgal 'Species' (fixed with 6 levels) were identified using 1-way ANOVA followed by Tukey's HSD post hoc tests. $\delta^{13}C_{FA}$ values of 7 FAs in tissues of *A. elliptica* were analyzed with 3-way crossed ANOVA to assess the effect of 'Fatty acid', 'Tissue', 'Season', and their interactions. Normality and homogeneity of variance of the residuals were assessed using the Shapiro-Wilk test and explanatory checks of plots of residuals against predicted values as suggested by Quinn & Keough (2002), respectively. When required, a logarithmic transformation was applied to the data.

Sampled sources of food were analyzed to determine discriminant FAs for each source in order to trace them in tissues of *A. elliptica* afterwards. To investigate FA composition between the 2 sampling periods and among the different samples, permutational multivariate ANOVA (PERMANOVA, 9999 permutations) was conducted using PRIMER 6 (Clarke 1993, Clarke & Gorley 2006) and PERMANOVA+ (Anderson et al. 2008). Multivariate homogeneity of group dispersion was verified before each analysis (PERMDISP, 9999 permutations) and data were transformed when necessary. In case of significant PERMANOVA tests, *a posteriori* pairwise multiple comparison tests were used to identify differences among levels of source of variation. Multivariate analyses on TFA composition of *A. elliptica* tissues, POM, sediments and macroalgal species, including *a posteriori* pairwise comparisons, were performed using a distance-based PERMANOVA (9999 permutations) based on Bray-Curtis dissimilarities. The number of replicates varied between 3 and 7. Variations in FA composition, expressed in percentages, were visualized using non-metric multidimensional scaling ordination, based on Bray-Curtis dissimilarities between samples after standardization of data. The similarity percentages (SIMPER) procedure (Clarke 1993) was performed on untransformed data to identify FAs explaining the most dissimilarity between significant different levels. We grouped brown macroalgae in order to seek FA markers of major taxa (i.e. brown macroalgae versus green macroalgae) through the SIMPER analysis. To seek differentiation in FATM contents between the 2 seasons and among the

digestive gland samples and the potential sources of food, a 2-way PERMANOVA with 'Sample' (fixed with 4 levels) and 'Season' (fixed with 2 levels) as sources of variation was performed on 12 selected FATMs (see Table 1, all FAs were used except for 16:4ω3). A significance threshold of $\alpha = 0.05$ was adopted for all statistical tests.

RESULTS

Seasonal $\delta^{13}C$ and $\delta^{15}N$ variability

No significant difference in $\delta^{13}C$ was observed between acid-treated and untreated samples of sediment and macroalgae (paired *t*-test, $p > 0.05$). However, paired *t*-tests showed significant differences in $\delta^{13}C$ between acidified and non-acidified samples of POM (both s-POM and b-POM, $p < 0.05$) collected in September. Statistical tests were therefore performed on acid-treated samples for $\delta^{13}C$ and on untreated samples for $\delta^{15}N$.

A significant interaction between season and depth was observed in POM $\delta^{13}C$ (2-way ANOVA, $p < 0.01$; Fig. 2). $\delta^{13}C$ values were similar in May between subsurface and bottom samples (–22.00 ± 0.06‰, mean ± SE), while s-POM was more enriched in ^{13}C (–24.26 ± 0.11‰) than b-POM (–25.49 ± 0.21‰) in September. No significant effect of season and depth or interaction was observed in POM $\delta^{15}N$ (2-way ANOVA, $p > 0.05$; Fig. 2). Surface sediment $\delta^{13}C$ and $\delta^{15}N$ values were similar between May and September (1-way ANOVA, $p = 0.36$ and $p = 0.82$, respectively; Fig. 2). Mean $\delta^{13}C$ and $\delta^{15}N$ values were –20.87 ± 0.04‰ and 6.08 ± 0.03‰, respectively. $\delta^{13}C$ values of 6 macroalgal species were determined and these significantly differed (1-way ANOVA, $p < 0.001$; Fig. 2) from one another. *Ulva lactuca* was the macroalga most enriched in ^{13}C ($\delta^{13}C$ = –16.44 ± 0.05‰), while *Dictyosiphon foeniculaceus* was the most depleted in ^{13}C ($\delta^{13}C$ = –22.44 ± 0.37‰). $\delta^{15}N$ in macroalgae also differed significantly among species (1-way ANOVA, $p < 0.001$; Fig. 2). $\delta^{15}N$ values were highest in *Laminaria* spp. and *U. lactuca* (7.12 ± 0.30‰), lowest in *Ascophyllum nodosum* and *Fucus vesiculosus* (3.73 ± 0.12‰), and intermediate in *Agarum clathratum* and *D. foeniculaceus* (5.56 ± 0.59‰). Isotopic signatures of the digestive glands of *Astarte elliptica* were not significantly different between May and September (1-way ANOVA, $p = 0.48$ and $p = 0.13$ for $\delta^{13}C$ and $\delta^{15}N$, respectively; Fig. 2). $\delta^{13}C$ of *A. elliptica* digestive gland was –21.23 ± 0.17‰ and $\delta^{15}N$ was 5.54 ± 0.13‰.

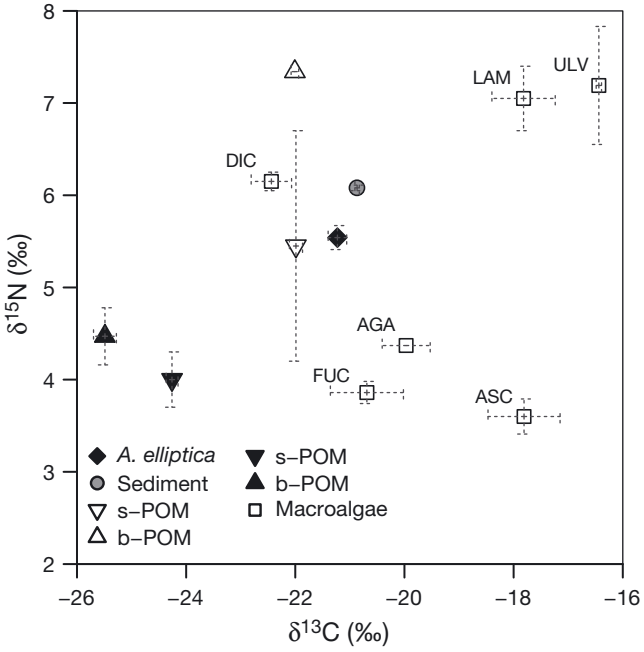

Fig. 2. Carbon and nitrogen isotopic composition of digestive glands of *Astarte elliptica* and of its potential sources of food in Kobbefjord, southwest Greenland. Particulate organic matter was collected from subsurface (s-POM) and bottom waters (b-POM) in May (open triangles) and September 2103 (black triangles). Macroalgae were sampled in May (AGA = *Agarum clathratum*; ASC = *Ascophyllum nodosum*; DIC = *Dictyosiphon foeniculaceus*; FUC = *Fucus vesiculosus*; LAM = *Laminaria* spp.; ULV = *Ulva lactuca*). *A. elliptica* and sediments were sampled in May and September, but data were pooled since no significant difference was found between seasons (1-way ANOVA)

FA analysis

Sources of food

FA profiles of macroalgae were significantly different (PERMANOVA, $p < 0.001$; Table 2, Fig. 3). Each of the 6 species had a specific FA composition (pairwise tests, $p < 0.05$). Table 3 presents detailed FA compositions for each species and the 5 major FAs contributing to explain more than 60% of the average similarity within each species group. SIMPER analysis on major taxa indicated that 16:4ω3, 18:3ω3, 18:4ω3 were more abundant in the green alga *U. lactuca*, whereas 14:0, 18:2ω6, 20:4ω6, 20:5ω3 were present in higher proportion in brown macroalgae (Table 3). These 7 FAs explained more than 60% of average similarity between the 2 taxa.

Detailed FA compositions in POM are given in Table S1A in the Supplement at www.int-res.com/articles/suppl/m567p139_supp.pdf. SFAs were largely dominant in POM samples (>85% of TFAs), but sig-

nificant differences in FA compositions were observed between s-POM and b-POM, and between May and September (PERMANOVA, $p < 0.01$; Table 2). SIMPER analysis showed that the same set of FAs explained the differences among depths and among seasons. Levels of SFAs (16:0 and 18:0) were more important in b-POM and in September, while UFAs (especially 16:1ω7, 16:4ω1, 18:4ω3, 20:5ω3, and 22:6ω3) showed higher proportions in s-POM and in May (Table S1A).

No significant differences were found in sediment TFA composition between the 2 sampling periods (PERMANOVA, $p = 0.07$; Table 2), with 16:1ω7 (17.2 ± 0.7%), 16:0 (14.1 ± 0.3%), 20:5ω3 (10.2 ± 0.4%), and 18:1ω7 (7.2 ± 0.5%) as major FAs (Table S1B).

SIMPER analysis on all sampled sources of food indicated the large influence of 18 carbon UFAs (18:1ω7, 18:3ω3 and 18:4ω3) in *U. lactuca* and the importance of SFAs (16:0 and 18:0) in POM, and the isolation of sediment samples was based primarily on the presence of 16:1ω7 and 20:5ω3 (Fig. 3). The other macroalgae (brown macroalgae) were divided into 3 groups. The first one included *A. nodosum* and *F. vesiculosus*, mainly due to higher proportions of 18:1ω7 and 18:2ω6 compared to all the other groups. The second cluster included *D. foeniculaceus* and *Laminaria* spp., presumably due to their higher 20:4ω6 and 20:5ω3 contents. The third group included *A. clathratum*, with appears to be distinct based on its higher 16:0, 16:1ω7 and 18:1ω9 contents (Fig. 3).

A. elliptica tissues

Complete FA compositions of the digestive gland and feet of *A. elliptica* (see Table S1C) showed significant differences between tissues and seasons (PERMANOVA, $p < 0.01$; Table 2, Fig. 4) but no interaction between tissues and seasons (Table 2). SIMPER analysis showed the influence of long-chain PUFAs (20 and 22 carbons) in the feet of *A. elliptica*, while UFAs with 16

Table 2. Results of PERMANOVA on total fatty acid (FA) composition of *Astarte elliptica* tissues and potential sources of food (i.e. POM, sediment, and macroalgae) collected in Kobbefjord, southwest Greenland in May and September 2013, based on the Bray-Curtis dissimilarity matrix. Data for *A. elliptica* were log(x + 1) transformed prior to analysis. Significant values ($p < 0.05$) are in **bold**: $^{**}p < 0.01$, $^{***}p < 0.001$. Analyses were based on 48 FAs with 2 sources of variation: 'Tissue' (fixed with 2 levels: digestive gland and foot) and 'Season' (fixed with 2 levels: May and September) for *A. elliptica*; 27 FA with 2 sources of variation: 'Depth' (fixed with 2 levels: subsurface and bottom) and 'Season' (fixed with 2 levels) for pelagic POM; 52 FAs with 1 source of variation: 'Season' (fixed with 2 levels) for sediments; 45 FAs with 1 source of variation: 'Species' (fixed with 6 levels) for macroalgae

Source of variation	df	*A. elliptica* MS	*A. elliptica* Pseudo-*F*	Source of variation	df	POM MS	POM Pseudo-*F*
Tissue (T)	1	1488.300	**48.856*****	Depth (D)	1	361.680	**10.667****
Season (S)	1	299.030	**9.816****	Season (S)	1	504.760	**14.886*****
T × S	1	39.719	1.304	D × S	1	28.428	0.838
Residual	22	30.463		Residual	8	33.908	
		Sediment				**Macroalgae**	
	df	MS	Pseudo-*F*		df	MS	Pseudo-*F*
Season	1	136.130	3.241	Species	5	2829.200	**43.228*****
Residual	4	42.001		Residual	12	65.449	

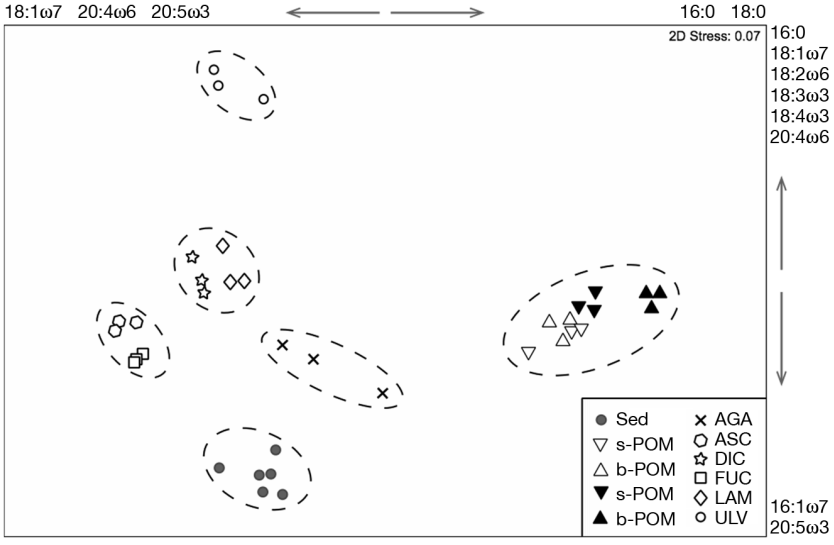

Fig. 3. Non-metric multidimensional scaling ordination based on a Bray-Curtis dissimilarity matrix calculated on untransformed data for potential sources of food of *Astarte elliptica* in Kobbefjord, southwest Greenland: particulate organic matter from subsurface (s-POM) and bottom waters (b-POM) collected in May (open triangles) and September (black triangles), sediment (Sed), and 6 macroalgae species (see Fig. 2 for abbreviations). The factor 'Season' is not shown for sediment data since no significant difference was found (PERMANOVA, Table 2). Arrows and fatty acids indicate the main component explaining the group dissimilarities (SIMPER outputs). Dashed ellipses represent samples similar at 70%

Table 3. Fatty acid composition, with concentrations expressed as percentage of mass of total fatty acids (MTFA), of 6 macroalgal species (see Fig. 2 for abbreviations) collected in Kobbefjord, southwest Greenland in May 2013. Values are means (SE). Only values >3% are shown. MFTA content, expressed in mg g^{-1}, is shown at the foot of the table, along with average similarity within each species group. The main FAs responsible for similarities (SIMPER outputs) are given in bold in the table. SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA. '–' indicates not determined

	AGA	ASC	DIC	FUC	LAM	ULV
14:0	5.0 (0.6)	**12.7 (0.3)**	7.0 (0.5)	**12.9 (0.3)**	**10.3 (0.4)**	1.0 (0.1)
16:0	**28.7 (3.0)**	**13.3 (0.6)**	**18.0 (0.4)**	**11.5 (0.0)**	**20.6 (1.2)**	**20.1 (1.4)**
Σ SFA	36.4 (3.4)	28.1 (1.0)	27.1 (1.0)	26.0 (0.3)	33.2 (0.5)	24.0 (1.6)
16:1ω7	**11.9 (3.2)**	1.3 (0.0)	1 .7 (0.1)	1.6 (0.1)	2.2 (0.2)	2.1 (0.3)
17:1ω9	0.1 (0.0)	0.2 (0.0)	0.1 (0.0)	0.3 (0.0)	–	5.7 (0.5)
18:1ω9	**8.2 (1.1)**	–	8.9 (0.5)	**14.8 (0.8)**	–	0.8 (0.1)
18:1ω7	0.6 (0.1)	**28.2 (0.7)**	0.2 (0.1)	7.0 (0.4)	**11.0 (0.6)**	**8.0 (0.3)**
Σ MUFA	24.0 (2.7)	32.7 (0.8)	13.4 (0.7)	28.3 (0.4)	15.0 (0.6)	19.2 (0.9)
16:4ω3	0.3 (0.0)	–	0.2 (0.1)	0.2 (0.0)	–	**15.7 (0.5)**
16:4ω1	3.8 (0.9)	–	0.0 (0.0)	0.1 (0.0)	–	0.3 (0.0)
18:2ω6	5.7 (2.1)	**8.7 (0.2)**	**8.6 (0.1)**	**12.2 (0.3)**	7.2 (0.6)	2.1 (0.2)
18:3ω3	2.9 (0.9)	3.9 (0.3)	**9.2 (0.6)**	6.0 (0.2)	6.3 (1.1)	**17.8 (2.3)**
18:4ω3	1.3 (0.4)	3.3 (0.2)	9.6 (1.2)	4.3 (0.2)	9.5 (2.8)	**13.1 (0.8)**
20:4ω6	**7.1 (0.6)**	9.0 (0.6)	**17.8 (0.7)**	**9.8 (0.2)**	**11.0 (2.6)**	0.2 (0.0)
20:5ω3	**7.2 (2.4)**	**9.7 (0.6)**	**12.0 (0.6)**	9.2 (0.1)	**15.2 (0.8)**	2.6 (0.2)
22:5ω3	3.1 (0.5)	–	–	–	–	2.7 (0.2)
Σ PUFA	39.6 (6.1)	39.2 (1.5)	59.6 (1.7)	45.8 (0.2)	51.8 (1.1)	56.8 (2.4)
MTFA	6.5 (1.9)	3.0 (0.4)	9.1 (0.8)	7.6 (1.2)	7.5 (0.9)	9.4 (2.4)
Average similarity	80%	95%	94%	96%	88%	92%

and 18 carbons were more abundant in digestive glands. Higher proportions of 18:4ω3, 16:4ω1, and 16:1ω7 were observed in May, while 18:2ω3, 20:4ω6, and 20:1ω9 showed higher levels in September (Fig. 4).

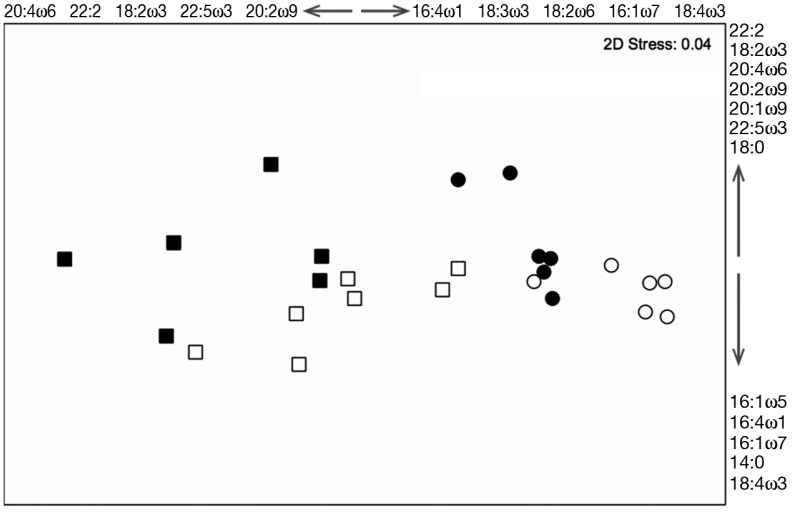

Fig. 4. Non-metric multidimensional scaling ordination based on Bray-Curtis dissimilarity matrix calculated on log(x + 1)-transformed data for *Astarte elliptica* tissues from Kobbefjord, southwest Greenland: digestive glands collected in (□) May and (■) September, and feet collected in (○) May and (●) September. PERMANOVA results are given in Table 2. Arrows and fatty acids indicate the main component explaining the group dissimilarities (SIMPER outputs)

We looked at variations of the 12 FATMs between seasons and between the digestive gland of *A. elliptica* and its potential sources of food (Table 4). Proportions of 18:1ω7 and 18:3ω3 were similar between May and September for all groups. Sums of 20:1ω9 and 22:1ω11 and levels of 16:4ω1, 18:2ω6, 20:4ω6, and 20:5ω3 significantly differed between May and September for all groups (PERMANOVA, $p < 0.05$; Table 4). Proportions of 20:1ω9, 22:1ω11, and 20:4ω6 significantly increased between May and September, while levels of the 3 other FATMs (16:4ω1, 18:2ω6, and 20:5ω3) decreased (Table 4). Proportions of 16:1ω7 significantly decreased between May and September in POM (both s-POM and b-POM, from 4.9 ± 0.7 to 1.4 ± 0.2% and from 4.4 ± 0.3 to 0.5 ± 0.1%, respectively) and in sediment (from 18.5 ± 0.5 to 15.9 ± 0.8%) (Table 4). Proportions of 18:4ω3 in b-POM, sediment and digestive glands of *A. elliptica* were significantly lower in September compared to May, while they were similar in s-POM (Table 4). Levels of 18:1ω9 were the same in

Table 4. Values (%) of the 12 fatty acid (FA) trophic markers used as dietary tracers for comparison between the digestive gland of *Astarte elliptica* and its potential sources of food, and between seasonal values. Values are means (SE). Results were analysed by 2-way PERMANOVA with 2 sources of variation: 'Sample' (fixed with 4 levels: particulate organic matter from subsurface (s-POM) and bottom waters (b-POM), sediment and digestive gland (DG) of *A. elliptica*) and 'Season' (fixed with 2 levels: May and September). Superscript letters indicate significant differences between groups (samples) in May and September; and '*' indicates significant differences among seasons (pairwise tests, $p < 0.05$). '–' indicates not determined

FA	May				September			
	s-POM	b-POM	Sediment	DG	s-POM	b-POM	Sediment	DG
Σ *i*-FA + *ai*-FA	0.6 (0.2)^{a}	0.5 (0.1)^{a}	6.3 (0.1)^{b}	2.0 (0.1)^{c}	0.5 (0.1)^{a}	0.1 (0.0)^{b*}	7.3 (0.7)^{c}	2.4 (0.1)^{d}
16:1ω7	4.9 (0.7)^{a}	4.4 (0.3)^{a}	18.5 (0.5)^{b}	5.2 (0.2)^{a}	1.4 (0.2)^{a*}	0.5 (0.1)^{b*}	15.9 (0.8)^{c*}	4.4 (0.3)^{d}
18:1ω9	1.0 (0.1)^{a}	1.1 (0.0)^{a}	5.7 (0.2)^{b}	1.4 (0.0)^{c}	1.0 (0.2)^{a}	1.1 (0.2)^{a}	3.8 (0.0)^{b*}	1.7 (0.0)^{c*}
18:1ω7	0.6 (0.1)^{a}	0.9 (0.1)^{a}	6.7 (0.2)^{b}	4.0 (0.2)^{c}	0.6 (0.1)^{a}	0.3 (0.1)^{a}	7.6 (1.1)^{b}	4.5 (0.2)^{c}
Σ 20:1ω9 + 22:1ω11	–	–	0.8 (0.0)^{a}	2.3 (0.1)^{b}	–	–	4.3 (0.7)^{a*}	3.5 (0.4)^{a*}
16:4ω1	0.9 (0.2)^{a}	1.0 (0.2)^{a}	1.5 (0.2)^{b}	1.4 (0.1)^{c}	0.3 (0.0)^{a*}	0.0 (0.0)^{a*}	1.1 (0.2)^{b*}	0.6 (0.0)^{c*}
18:2ω6	0.7 (0.1)^{a}	0.4 (0.0)^{b}	0.5 (0.0)^{b}	1.4 (0.0)^{c}	0.5 (0.1)^{a*}	0.3 (0.1)^{b*}	0.3 (0.1)^{b*}	1.5 (0.1)^{c*}
18:3ω3	0.2 (0.1)^{a}	0.1 (0.0)^{b}	0.4 (0.1)^{c}	0.8 (0.0)^{d}	0.4 (0.0)^{a}	0.1 (0.0)^{b}	0.5 (0.1)^{c}	0.7 (0.1)^{d}
18:4ω3	0.8 (0.2)^{ab}	0.7 (0.0)^{a}	1.5 (0.2)^{b}	10.0 (0.7)^{c}	1.1 (0.1)^{a}	0.1 (0.0)^{b*}	0.9 (0.1)^{a*}	6.1 (0.5)^{c*}
20:4ω6	–	–	1.9 (0.1)^{a}	1.0 (0.1)^{b}	–	–	4.4 (2.0)^{a*}	1.4 (0.1)^{b*}
20:5ω3	1.8 (0.3)^{a}	2.8 (0.5)^{a}	10.7 (0.3)^{b}	24.7 (0.5)^{c}	1.6 (0.1)^{a*}	0.4 (0.2)^{a*}	9.8 (0.8)^{b*}	23.1 (0.2)^{c*}
22:6ω3	0.6 (0.1)^{a}	0.6 (0.0)^{a}	2.9 (0.3)^{b}	9.5 (0.1)^{c}	1.3 (0.1)^{a*}	0.2 (0.1)^{b*}	2.7 (0.3)^{c}	10.7 (0.5)^{d*}

POM (both s-POM and b-POM) for the 2 periods, while sediment and digestive glands of *A. elliptica* showed reverse trends with a lower proportion in sediment and a higher proportion in digestive glands in May compared to September (Table 4). Levels of 22:6ω3 were significantly higher in s-POM and digestive gland of *A. elliptica* in September compared to May (Table 4). Levels of 22:6ω3 decreased in b-POM between May and September and remained constant in sediment. Seasonal differences in proportions of branched FAs, used as markers of bacteria, were only found in b-POM (Pairwise test, $t = 3.82$, $p = 0.02$), with higher proportions in May (0.5 ± 0.1%) compared to September (0.1 ± 0.0%) (Table 4).

As result of significant interactions between the 2 factors 'Season' and 'Sample' (PERMANOVA, $p < 0.5$), branched FAs, sums of 20:1ω9 and 22:2ω11, 16:1ω7, 18:4ω3, and 22:6ω3 varied differently between b-POM, s-POM, sediment, and the digestive glands of *A. elliptica* in May and in September (Table 4). A higher variability was observed in September compared to May since the 4 groups were often significantly different from one another, with the exception of the sum of 20:1ω9 and 22:2ω11 which was significantly higher in digestive gland than in sediment in May. In September, the percentages of 20:1ω9 and 22:2ω11 were similar in sediment and in digestive glands, but significantly higher than percentages found in May (Table 4). Relative proportions of 18:1ω9, 18:1ω7 and 16:4ω1 remained the same among the 4 groups (s-POM, b-POM, sediment and digestive glands) in May and September, with the lowest proportions in POM, intermediate proportions in digestive glands and the highest proportions in sediment (Table 4). Levels of 20:5ω3 remained low in POM, intermediate in sediment and higher in the digestive glands. Levels of 18:2ω6 were intermediate in s-POM, lower in b-POM and sediment and higher in the digestive glands, and proportions of 20:4ω6 were significantly higher in sediment than in the digestive glands of *A. elliptica* (Table 4).

Carbon stable isotope of individual FA

Isotopic signatures of 7 FAs in tissues (digestive glands and feet) of *A. elliptica* are shown in Fig. 5. A significant effect of the interaction between the 2 factors 'Fatty acid' and 'Tissue' was observed on $\delta^{13}C$ values, while no effect of the factor 'Season' was detected (3-way ANOVA, $p < 0.01$; Table 5). The highest $\delta^{13}C$ values (–25.0 ± 0.4‰) were found for the FA 22:6ω3 and the lowest $\delta^{13}C$ values (–37.2 ± 0.8‰) were obtained for 18:3ω3, both in foot samples (Fig. 5). The range of $\delta^{13}C$ values in digestive glands was smaller, from –25.4 ± 1.0‰ for 22:6ω3 to –31.0 ± 0.6‰ for 18:2ω6 (Fig. 5). 18:2ω6 and 18:3ω3 were significantly more depleted in ^{13}C than the other FAs in the foot, while they had $\delta^{13}C$ values about equal to the other FAs in the digestive gland (Fig. 5). Whether in digestive gland or in foot, $\delta^{13}C$ values of 20:4ω6, 20:5ω3 and 22:6ω3 tended to decrease with unsatura-

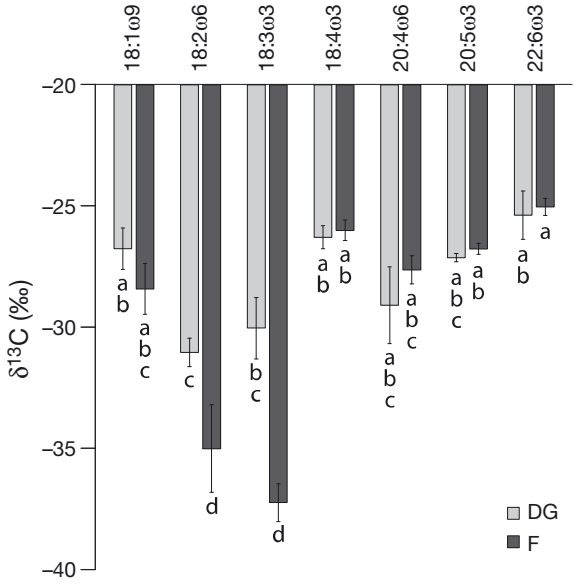

Fig. 5. Carbon isotope ratios ($\delta^{13}C$) of fatty acids (mean ± SE) in *Astarte elliptica* digestive gland (DG) and foot (F) tissue collected from Kobbefjord, southwest Greenland. Different letters indicate significant differences for the interaction between tissue and fatty acid (Tukey's HSD post hoc test, $p < 0.05$)

tion, although no significant difference was observed (Fig. 5).

$\delta^{13}C$ values of 10 FAs used as FATMs are shown in Fig. 6. In *A. elliptica*, mean $\delta^{13}C$ values in feet widely varied, from −37.2 ± 0.8‰ for 18:3ω3 to −25.0 ± 0.4‰ for 22:6ω3. Among all sources of food, $\delta^{13}C$ values ranged between −32.2‰ for 20:4ω6 in POM and −19.1 ± 1.5‰ for 20:5ω3 in the green macroalgae *U. lactuca*.

DISCUSSION

In accordance with our first hypothesis, trophic resources (organic matter from the water column and the sediment, and macroalgae) were distinguishable in terms of FA compositions and isotopic signatures (bulk stable isotopes, and carbon isotopic ratio on individual FAs). Thus, these biological and chemical segregations allowed us to trace these resources in the benthic food web and to accurately identify the diet of the bivalve *Astarte elliptica* in a sub-Arctic fjord. However, contrary to our second hypothesis, the multiple biomarker approach showed that *A. elliptica* did not feed only on microalgae, but rather on a mixture of POM, including microalgae and macroalgal (mainly brown algae) material. Moreover, $\delta^{13}C$ signatures in *A. elliptica* tissues suggest that isotopic composition was not the most appropriate tool to track seasonal variability in its diet, whereas TFA profiles and FATMs showed marked differences between the 2 sampling periods. Thus, a combination of these 2 tools seems to be a useful method to understand trophic strategy in response to

Table 5. Results of 3-way ANOVA testing the effect of 'Fatty acid' (fixed with 7 levels: 18:1ω9, 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, 20:5ω3, and 22:6ω3), 'Tissue' (fixed with 2 levels: digestive gland and foot), and 'Season' (fixed with 2 levels: May and September) and their interactions on the carbon isotopic ratio of 7 fatty acids in *Astarte elliptica*. Significant values are in **bold**: $^{**}p < 0.01$; $^{***}p < 0.001$

Sources of variation	df	MS	*F*
Fatty acid (FA)	6	161.139	**22.416*****
Tissue (T)	1	72.212	**10.046****
Season (S)	1	14.205	1.976
FA × T	6	27.919	**3.884****
FA × S	6	3.210	0.447
T × S	1	27.289	3.796
FA × T × S	6	7.355	1.023
Residual	105	7.188	

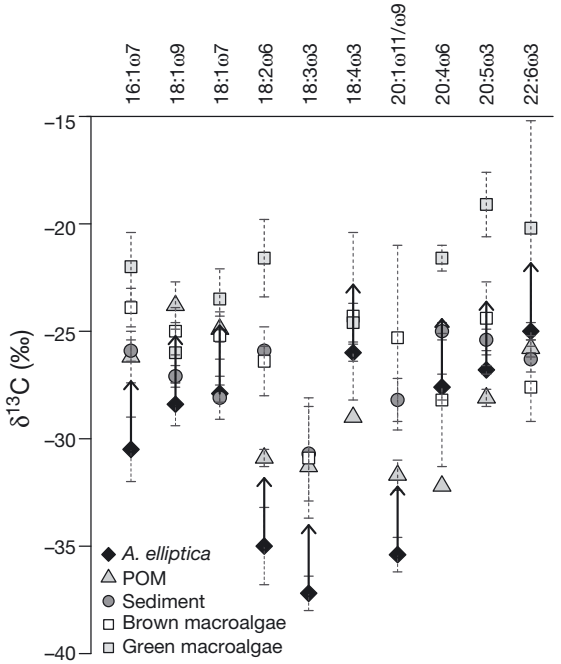

Fig. 6. Stable carbon isotopic values of 10 fatty acids ($\delta^{13}C_{FA}$) in feet of *Astarte elliptica* and in its potential sources of food (POM, sediment, and green and brown macroalgae) collected in Kobbefjord, southwest Greenland in May and September 2013. Data from different depths (for POM) and from the 2 seasons are pooled. Values are means, errors bars show SE. Arrows indicate a fractionation constant (3.17‰) applied on $\delta^{13}C_{FA}$ in *A. elliptica* tissue to take account of the decrease in the isotope ratio of the consumer's tissue compared to that of the food resource, as described by Gladyshev et al. (2014)

changing primary production dynamics as proposed in our third hypothesis.

Characterization of trophic resources

Our study included 9 potential sources of food available at the time of samplings (i.e. pelagic POM, including s-POM and b-POM, surface sediment and 6 macroalgal species) that showed distinct FA compositions and isotopic signatures. As observed by Khotimchenko et al. (2002) and Li et al. (2002), the green macroalgae (*Ulva lactuca*) showed typical FA composition containing higher concentrations of PUFAs with 18 carbons (especially 18:3ω3 and 18:4ω3) and lower levels of PUFAs with 20 carbons than brown macroalgae. Furthermore, we found high levels of 16:4ω3 (up to 15%), which are characteristic in the order Ulvales (Fleurence et al. 1994, Khotimchenko et al. 2002), making this FA a relevant biomarker of *U. lactuca* in the diet of primary consumers. Brown algae were distinguishable from the green macroalgae by relatively high levels of 20:4ω6, 20:5ω3, 18:2ω3 and 18:1ω9, as already shown in previous studies (Graeve et al. 2002, Khotimchenko et al. 2002, Li et al. 2002). The clear differences between the 2 macroalgal groups (brown vs. green) and also between species, since the 6 algae species had distinct FA compositions, make these lipids a suitable tracer of algal resources in studies of benthic food webs. However, considerable variability in FA composition has been shown in macroalgae among species, sites and seasons (Nelson et al. 2002, Dethier et al. 2013). Sampling for FA characterization ideally should span all seasons to capture this variation and how it propagates up through consumers in food webs. Despite these variations, FATMs could readily distinguish macrophyte phyla (Dethier et al. 2013).

Stable isotope compositions of POM were in the range of values observed in other Arctic studies (Hobson et al. 1995, Renaud et al. 2011, Oxtoby et al. 2013). Although POM isotopic composition is affected by the type of settling material (e.g. phytodetritus, faeces of pelagic grazers or marine snow; Mintenbeck et al. 2007), we found no significant depth effect on $\delta^{15}N$. In particular, increased POM $\delta^{15}N$ values with depth may be a consequence of zooplankton grazing and microbial degradation (Ostrom et al. 1997). POM also showed variations in $\delta^{13}C$ and more ^{13}C-depleted values in September than in May (see 'Seasonal variability' below). Although microalgal species were not identified in POM samples, the algal class composition can be partially deduced using FATMs (e.g. reviewed in Dalsgaard et al. 2003, Kelly & Scheibling 2012). POM FA composition was largely dominated by SFAs. This dominance of SFAs, markers of *Phaeocystis* spp., is consistent with observations conducted during the monitoring program of Godthåbsfjord, since *Phaeocystis* spp. dominated the phytoplankton community from March to June (86% on average) and in September (78%) 2013 (Jensen & Christensen 2014). Alternatively, it has already been shown that marine detritus contains significant quantities of SFAs with between 14 and 18 carbons (Fahl & Kattner 1993). Higher levels of SFA and lower levels of UFAs in b-POM compared to s-POM could explain depth-related differences in FA composition of POM samples related to degradation processes (Reemtsma et al. 1990, Fileman et al. 1998). The more rapid degradation of PUFAs with increasing depth compared to SFAs and MUFAs is well established, although lower degradation rates have been shown in cold environments (Fileman et al. 1998). Lower levels of PUFAs likely denoted degradation of pelagic organic matter during transfer through the water column.

Sediment had higher $\delta^{13}C$ values than POM, likely related to sinking phytodetritus that may be slightly ^{13}C-enriched due to microbial degradation (Hobson et al. 1995). The relatively high $\delta^{13}C$ values in sediment may also result from the influence of macroalgae (especially *Fucus vesiculosus* and *Agarum chlathratum*), which were mostly enriched in ^{13}C. The ^{13}C composition of the sediment indicates therefore that phytoplankton and material from brown algae both contribute to the organic material buried in the sediment of the Kobbefjord. Alternatively, with regard to FA composition, relatively high levels of 16:1ω7 (17.2 ± 0.7%), 16:4ω1 (1.3 ± 0.1%) and 20:5ω3 (10.2 ± 0.4%) in sediment may reflect the presence of diatoms, which are major taxa of microplankton during the blooms (Krawczyk et al. 2015), and in microphytobenthos (Wulff et al. 2009 and references therein, but see discussion about 20:5ω3 origin below), although we did not isolate this trophic source. Microphytobenthos can be assumed to be of minor importance since the sampling depth of *A. elliptica* is below the euphotic zone (ca. 40 m; Jensen & Christensen 2014). Two other potential sources may contribute to Arctic food webs: terrestrial carbon and ice algae. Since the POM analysed clearly had a marine $\delta^{13}C$ signature (marine $\delta^{13}C$ from −22 to −25‰, terrestrial $\delta^{13}C$ from −27 to −31‰; Dunton et al. 2006) and ice algae contributes less than 1% of total (sympagic plus pelagic) primary production annually in Kobbefjord (Mikkelsen et al. 2008), ter-

restrial and sea ice associated sources can be excluded as food significantly contributing to the diet of *A. elliptica*.

Diet of *A. elliptica*

Coupled approaches of bulk isotopic, FA and CSIA showed that *A. elliptica* feeds on a mixture of suspended POM and brown macroalgal material. Based on bulk isotope analysis, 3 sampled sources of food (suspended POM, in May, and the 2 brown algae *Dictyosiphon foeniculaceus* and *F. vesiculosus*) are likely to support the bivalve *A. elliptica* in Kobbefjord. FA composition and FATM analyses supported these results since digestive glands of *A. elliptica* contained high levels of FA markers of microalgae, both diatoms and dinoflagellates (16:1ω7, 18:4ω3, 20:5ω3 and 22:6ω3), and brown macroalgae (PUFAs with 18 carbons, 20:4ω6 and 20:5ω3).

The use of FATMs is sometimes limiting since a unique FA tracer assigned to a given taxonomic group is a relatively rare situation. Compound-specific isotope analysis appears to be a more successful method than the use of other trophic markers when different food sources have similar bulk carbon isotope and FA signatures (Gladyshev et al. 2012). In our study, we analyzed $\delta^{13}C$ in 7 FAs, both in digestive glands and feet of *A. elliptica*. As also observed by Gladyshev et al. (2012) in organisms of a 4-link food chain from the Yenisei River, a parabolic dependence of $\delta^{13}C$ values of FAs on their degree of unsaturation/chain length occurred, with 18:2ω6 and 18:3ω3 at their lowest point (Fig. 5). However, our results showed that the parabolic pattern of $\delta^{13}C_{FA}$ was less pronounced in the digestive glands than in feet of *A. elliptica*. Since bivalves have a very limited or no ability to synthesize PUFAs (especially essential FAs, such as 20:4ω6, 20:5ω3 and 22:6ω3) from the precursors 18:2ω6 and 18:3ω3 contained in their food (De Moreno et al. 1976, Waldock & Holland 1984, Fernández-Reiriz et al. 1998), we suggest that dissimilarities in $\delta^{13}C$ values between the tissues of *A. elliptica* is likely caused by a differential isotopic fractionation, as is the case for bulk stable isotopes. Since $\delta^{13}C$ values on individual FAs from feet of *A. elliptica* showed a larger range, CSIA in this tissue seems to be more appropriate to determine and distinguish the trophic source of the FAs. FAs in *A. elliptica* lipids were generally ^{13}C-depleted compared with their counterpart in trophic resources. This depletion in ^{13}C content of FAs, especially EFAs, appears to be a widespread and common phenomenon (Bec et al. 2011, Gladyshev et al. 2012). Gladyshev et al. (2014) have therefore proposed a fractionation constant equal to 3.17‰ in $\delta^{13}C$ values of PUFA as a result of a decrease in the isotope ratio of the consumer tissues compared to that of the trophic resource. After applying this fractionation constant, our compound-specific isotope data reinforce results from bulk isotope analysis and FA analysis and indicate that the diet of *A. elliptica* is comprised of POM and SOM including macroalgal material. *A. elliptica* probably feeds unselectively on macroalgae and the dominance of brown algal signature in their tissues is a consequence of the huge biomass and abundance of brown algae species in Kobbefjord (Krause-Jensen et al. 2012, Jensen & Christensen 2014). For example, *Ascophyllum nodosum* completely covers the rocky shore with mean estimated abundance and biomass of 134 ± 13 ind. m^{-2} and 15.8 ± 3 kg fresh weight m^{-2}, respectively (Jensen & Christensen 2014). This supports the notion that even suspension feeders, generally presumed to feed mainly on phytodetritus, incorporate significant amounts of resuspended macroalgal material.

In Arctic food web studies, isotope analysis on individual FAs has been mainly investigated to distinguish diatoms originating from the pelagic and sympagic habitats and to assess their relative contribution in consumer diet (Budge et al. 2008, Wang et al. 2014). It has been established that ice algae $\delta^{13}C_{FA}$ (especially 16:4ω1 and 20:5ω3, which are common FAs used as diatom markers) values were higher than in phytoplankton (Budge et al. 2008, Wang et al. 2014). In our study, however, high levels of 20:5ω3 in brown macroalgae (>7%, compared to 0.4 to 2.8% in POM) combined with $\delta^{13}C_{20:5\omega3}$ values in tissues of *A. elliptica* (following application of the fractionation constant) indicated that the bivalve incorporates 20:5ω3 from brown algae rather than diatom-derived organic matter from sediment or pelagic primary production (POM). Sampling and characterization of all trophic resources in a given study site seems to be essential since $\delta^{13}C_{FA}$ can be similar between 2 resources. For instance, our results showed that the green algae *U. lactuca* had a carbon isotopic ratio of the FA 20:5ω3 (−19.1 ± 1.5‰) within the range of that in ice algae (−18.3 ± 2.0‰, Budge et al. 2008).

Seasonal variability

POM showed high seasonal $\delta^{13}C$ and $\delta^{15}N$ variability. The large range of POM $\delta^{13}C$ values has been related to several factors, such as phytoplankton growth conditions, changes in species composition,

temperature, water masses and aqueous CO_2 limitation (Rau et al. 1992, Ostrom et al. 1997, Michener & Kaufman 2007). In our study, POM $\delta^{13}C$ variability is related to the spring phytoplankton bloom and may directly reflect variations in dissolved inorganic carbon (DIC) availability. During the spring bloom, intense phytoplankton growth leads to reduce DIC concentrations. As concentrations of DIC decrease, phytoplankton cells are forced to incorporate ^{13}C-enriched $CO_{2(aq)}$ resulting in higher particulate organic carbon isotopic ratios (Rau et al. 1992, Ostrom et al. 1997). Depletion in ^{13}C in September could, hence, be explained by the replenishment of DIC as a consequence of the decreased primary production combined with a weakening of stratification at the end of the summer (Jensen & Christensen 2014). A seasonal variability has also been observed in POM FA composition despite the dominance of SFAs in this study, likely explained by the high occurrence of *Phaeocystis* spp. in phytoplankton communities during the studied year (Jensen & Christensen 2014). Slightly higher levels of FA markers of microalgae, both diatoms (16:1ω7, 16:4ω1 and 20:5ω3; Viso & Marty 1993, Reuss & Poulsen 2002, Kelly & Scheibling 2012) and dinoflagellates (18:4ω3 and 22:6ω3; Napolitano et al. 1997, Mansour et al. 1999, Kelly & Scheibling 2012), as well as markers of brown macroalgae (18:4ω3 and 20:5ω3; Graeve et al. 2002, Kelly & Scheibling 2012, Wessels et al. 2012) were found in May. POM contained more diatoms, dinoflagellates and/or macroalgae-derived organic matter in May, while higher levels of SFAs in September suggest a more degraded material (De Baar et al. 1983, Fahl & Kattner 1993). The lower concentration of TFAs (almost 30%) and the decreased level of UFAs in September compared to May, as well as the $\delta^{13}C$ variability, well depict bloom and post-bloom conditions in Kobbefjord.

By contrast, SOM showed similar $\delta^{13}C$ and $\delta^{15}N$ values and FA composition in May and September. Stability in isotopic composition of SOM may reflect heterogeneity of carbon sources reaching the seafloor and their long-time persistence. This accumulation of phytodetritus can create a sediment 'food bank' (Mincks et al. 2005), that is available and can sustain benthic organisms with continuous supplies of food that offset the effects of the strong seasonality of primary production in the Arctic marine system (McMahon et al. 2006, Norkko et al. 2007). As *A. elliptica* were collected in a relatively high-current habitat where a 'food bank' is unlikely, stability in isotopic and FA composition of sediment could reflect residual organic matter after a consumption of a large amount of fresh organic matter by pelagic and benthic fauna (including filter-feeders). In areas where macroalgae biomass is relatively important, the pool of phytodetritus can be supplemented by macroalgae-derived material, which can then be exploited by filter-feeders (Hop et al. 2002, Quijón et al. 2008, Legeżyńska et al. 2012).

Digestive glands of *A. elliptica* showed very similar $\delta^{13}C$ and $\delta^{15}N$ values between the 2 sampling periods. Bulk stabe isotope analysis in bivalve tissues therefore integrates the isotopic signature of a very specific source of food with a similar carbon isotopic ratio to the bivalve. Alternatively, the carbon isotopic ratio of *A. elliptica*, as a non-selective feeder, is an average of isotopic signature of food and reflects heterogeneity of available sources of food. This temporal stability of $\delta^{13}C$ values in *A. elliptica* suggests, however, that bulk isotopes are not an appropriate approach to characterize seasonal variability in food supply to bivalves. The low (<1‰) or non-existent seasonal variability in benthic organism $\delta^{13}C$ has been already recorded in previous Arctic studies (e.g. Dunton et al. 1989, Kędra et al. 2012, Roy et al. 2015). This may be related to slow metabolic rates, slow growth and/or low temperatures (e.g. Kaufman et al. 2008). By contrast, FA profiles and FATMs showed differences between the 2 sampling periods and seem therefore to represent a useful method to look into food regime strategies in response to changing primary production dynamics. More specifically, we tracked changes in relative levels of FATMs between May and September in the digestive gland, which is a tissue with relatively high turnover rates, leading to rapid changes in biochemistry in response to changes in diet quality (Shin et al. 2008, Stead et al. 2013). Overall, seasonal variability in the diet of *A. elliptica* is related to a smaller contribution of microalgae, especially diatoms (lower levels of 16:1ω7 and 16:4ω1), and macroalgae (general decreased levels of PUFAs with 18 carbons) in September compared with May. In addition, *A. elliptica* showed a higher consumption of zooplankton, more specifically calanoid copepods (associated with increased proportion of 20:1ω9 and 22:1ω11) and bacteria (due to higher levels of branched FAs) in September. Benthic organisms depend on processes that take place in the water column and affect the organic matter (in terms of quantity and quality) sinking to the seafloor. Exported organic matter from the euphotic zone is strongly dependent upon the timing and the diversity of the primary producers, consumption (grazing by heterotrophs) and biological degradation by bacteria in the water column (Forest et al. 2010, Wassmann &

Reigstad 2011). Hence, *A. elliptica* seems to rather quickly respond to changes in organic matter reaching the benthos by behaving opportunistically to get its food.

Higher biomass and northward-expanded distribution of macroalgal communities may already be occurring in some Arctic areas in response to increased temperatures and reduced sea ice cover (e.g. Weslawski et al. 2010, Kortsch et al. 2012) and could be beneficial to benthic macrofaunal communities. Nevertheless, further investigations are needed to determine relative contributions of benthic and pelagic components (i.e. macroalgae, microphytobenthos, phytoplankton, ice algae and zooplankton) in benthic coastal food webs and how the diet of suspension feeders could be affected by a changing Arctic environment.

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LITERATURE CITED

Ackman RG, Tocher CS, McLachlan J (1968) Marine phytoplankter fatty acids. J Fish Res Board Can 25: 1603–1620

Ambrose WG, Renaud PE (1995) Benthic response to water column productivity patterns: evidence for benthic-pelagic coupling in the Northeast Water Polynya. J Geophys Res 100:4411–4421

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Bec A, Perga ME, Koussoroplis A, Bardoux G, Desvilettes C, Bourdier G, Mariotti A (2011) Assessing the reliability of fatty acid-specific stable isotope analysis for trophic studies. Methods Ecol Evol 2:651–659

Bergé JP, Barnathan G (2005) Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. In: Ulber R, Le Gal Y (eds) Marine biotechnology I. Springer-Verlag, Berlin, p 49–125

Blicher ME, Sejr MK, Rysgaard S (2009) High carbon demand of dominant macrozoobenthic species indicates their central role in ecosystem carbon flow in a sub-Arctic fjord. Mar Ecol Prog Ser 383:127–140

Blicher ME, Sejr MK, Høgslund S (2013) Population structure of *Mytilus edulis* in the intertidal zone in a sub-Arctic fjord, SW Greenland. Mar Ecol Prog Ser 487:89–100

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29: 1547–1559

Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ (2008) Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia 157:117–129

Carmack E, Wassmann P (2006) Food webs and physical–biological coupling on pan-Arctic shelves: unifying concepts and comprehensive perspectives. Prog Oceanogr 71:446–477

Carroll ML, Carroll J (2003) The Arctic seas. In: Black KD, Shimmield GB (eds) Biogeochemistry of marine systems. Blackwell, Oxford, p 127–156

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143

Clarke KR, Gorley RN (2006) PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth

Claustre H, Poulet SA, Williams R, Marty JC and others (1990) A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. J Mar Biol Assoc UK 70:197–207

Dalsgaard J, St John M, Kattner G, Muller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

De Baar HJW, Farrington JW, Wakeham SG (1983) Vertical flux of fatty acids in the North Atlantic Ocean. J Mar Res 41:19–41

De Moreno JEA, Moreno VJ, Brenner RR (1976) Lipid metabolism of the yellow clam, *Mesodesma mactroides*: 2-polyunsaturated fatty acid metabolism. Lipids 11: 561–566

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Dethier MN, Sosik E, Galloway AWE, Duggins DO, Simenstad CA (2013) Addressing assumptions: variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. Mar Ecol Prog Ser 478:1–14

Deudero S, Cabanellas M, Blanco A, Tejada S (2009) Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel *Mytilus galloprovincialis*. J Exp Mar Biol Ecol 368:181–188

Duggins DO, Simenstad CA, Estes JA (1989) Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science 245:170–173

Dunton KH, Saupe SM, Golikov AN, Schell DM, Schonberg SV (1989) Trophic relationships and isotopic gradients among arctic and subarctic marine fauna. Mar Ecol Prog Ser 56:89–97

Dunton KH, Weingartner T, Carmack EC (2006) The nearshore western Beaufort Sea ecosystem: circulation and importance of terrestrial carbon in arctic coastal food webs. Prog Oceanogr 71:362–378

Fahl K, Kattner G (1993) Lipid content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). Polar Biol 13:405–409

Fernández-Reiriz MJ, Labarta U, Albentosa M, Pérez-Camacho A (1998) Effect of microalgal diets and commercial wheatgerm flours on the lipid profile of *Ruditapes decussatus* spat. Comp Biochem Physiol A 119: 369–377

Fileman TW, Pond DW, Barlow RG, Mantoura RFC (1998) Vertical profiles of pigments, fatty acids and amino acids: evidence for undegraded diatomaceous material sedimenting to the deep ocean in the Bellingshausen Sea, Antarctica. Deep-Sea Res I 45:333–346

Fleurence J, Gutbier G, Mabeau S, Leray C (1994) Fatty acids from 11 marine macroalgae of the French Brittany coast. J Appl Phycol 6:527–532

Forest A, Bélanger S, Sampei M, Sasaki H, Lalande C, Fortier L (2010) Three-year assessment of particulate organic carbon fluxes in Amundsen Gulf (Beaufort Sea): satellite observations and sediment trap measurements. Deep-Sea Res I 57:125–142

Fry B (2007) Stable isotope ecology. Springer, New York, NY

Gladyshev MI, Sushchik NN, Kalachova GS, Makhutova ON (2012) Stable isotope composition of fatty acids in organisms of different trophic levels in the Yenisei River. PLOS ONE 7:e34059

Gladyshev MI, Sushchik NN, Makhutova ON, Kalachova GS (2014) Trophic fractionation of isotope composition of polyunsaturated fatty acids in the trophic chain of a river ecosystem. Dokl Biochem Biophys 454:4–5

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev Aquacult 1:71–124

Glud RN, Rysgaard S (2007) The annual organic carbon budget of Young Sound, NE Greenland. In: Rysgaard S, Glud RN (eds) Carbon cycling in Arctic marine ecosystems: case study Young Sound. Vol 58, Meddelelser om Grønland, Bioscience, Copenhagen, p 194–203

Gordillo S, Aitken AE (2000) Palaeoenvironmental interpretation of late quaternary marine molluscan assemblages, Canadian Arctic Archipelago. Geogr Phys Quat 54: 301–315

Gradinger R (2009) Sea-ice algae: major contributors to primary production and algal biomass in the Chukchi and Beaufort Seas during May/June 2002. Deep-Sea Res II 56:1201–1212

Graeve M, Kattner G, Wiencke C, Karsten U (2002) Fatty acid composition of Arctic and Antarctic macroalgae: indicators for phylogenetic and trophic relationships. Mar Ecol Prog Ser 231:67–74

Harris D, Horwáth WR, van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci Soc Am J 65: 1853–1856

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84:9–18

Hobson KA, Ambrose WG Jr, Renaud PE (1995) Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: insights from $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 128:1–10

Hobson KA, Fisk A, Karnovsky N, Holst M, Gagnon JM, Fortier M (2002) A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep-Sea Res II 49:5131–5150

Hop H, Pearson T, Hegseth EN, Kovacs KM and others (2002) The marine ecosystem of Kongsfjorden, Svalbard. Polar Res 21:167–208

Hop H, Wiencke C, Vögele B, Kovaltchouk Nikolaj A (2012) Species composition, zonation, and biomass of marine benthic macroalgae in Kongsfjorden, Svalbard. Bot Mar 55:399–414

Hsiao SIC (1980) Quantitative composition, distribution, community structure and standing stock of sea ice microalgae in the Canadian Arctic. Arctic 33:768–793

Hsiao SIC (1992) Dynamics of ice algae and phytoplankton in Frobisher Bay. Polar Biol 12:645–651

IPCC (2013) Climate change 2013: the physical science basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jensen LM, Christensen TR (2014) Nuuk ecological research operations: 7th annual report, 2013. Danish Centre for Environment and Energy, Aarhus University

Juul-Pedersen T, Arendt KE, Mortensen J, Blicher ME, Søgaard DH, Rysgaard S (2015) Seasonal and interannual phytoplankton production in a sub-Arctic tidewater outlet glacier fjord, SW Greenland. Mar Ecol Prog Ser 524:27–38

Kaufman MR, Gradinger RR, Bluhm BA, O'Brien DM (2008) Using stable isotopes to assess carbon and nitrogen turnover in the Arctic sympagic amphipod *Onisimus litoralis*. Oecologia 158:11–22

Kędra M, Kuliński K, Walkusz W, Legeżyńska J (2012) The shallow benthic food web structure in the high Arctic does not follow seasonal changes in the surrounding environment. Estuar Coast Shelf Sci 114:183–191

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Mar Ecol Prog Ser 446:1–22

Kennedy H, Thomas DN, Kattner G, Haas C, Dieckmann GS (2002) Particulate organic matter in Antarctic summer sea ice: concentration and stable isotopic composition. Mar Ecol Prog Ser 238:1–13

Khim BK, Krantz DE, Brigham-Grette J (2001) Stable isotope profiles of Last Interglacial (Pelukian Transgression) mollusks and paleoclimate implications in the Bering Strait Region. Quat Sci Rev 20:463–483

Khotimchenko SV, Vaskovsky VE, Titlyanova TV (2002) Fatty acids of marine algae from the Pacific coast of North California. Bot Mar 45:17–22

Kortsch S, Primicerio R, Beuchel F, Renaud PE, Rodrigues J, Lønne OJ, Gulliksen B (2012) Climate-driven regime shifts in Arctic marine benthos. Proc Natl Acad Sci USA 109:14052–14057

Krause-Jensen D, Duarte CM (2014) Expansion of vegetated coastal ecosystems in the future Arctic. Front Mar Sci 1:77

Krause-Jensen D, Kühl M, Christensen PB, Borum J (2007) Benthic primary production in Young Sound, northeast Greenland. In: Rysgaard S, Glud RN (eds) Carbon cycling in Arctic marine ecosystems: case study Young

Sound. Vol 58, Meddelelser om Grønland, Bioscience, Copenhagen, p 160–173
Krause-Jensen D, Marbà N, Olesen B, Sejr MK and others (2012) Seasonal sea ice cover as principal driver of spatial and temporal variation in depth extension and annual production of kelp in Greenland. Glob Change Biol 18:2981–2994
Krause-Jensen D, Duarte CM, Hendriks IE, Meire L, Blicher ME, Marbà N, Sejr MK (2015) Macroalgae contribute to nested mosaics of pH variability in a sub-Arctic fjord. Biogeosciences Discuss 12:4907–4945
Krawczyk DW, Witkowski A, Juul-Pedersen T, Arendt KE, Mortensen J, Rysgaard S (2015) Microplankton succession in a SW Greenland tidewater glacial fjord influenced by coastal inflows and run-off from the Greenland Ice Sheet. Polar Biol 38:1515–1533
Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306
Legeżyńska J, Kędra M, Walkusz W (2012) When season does not matter: summer and winter trophic ecology of Arctic amphipods. Hydrobiologia 684:189–214
Li X, Fan X, Han L, Lou Q (2002) Fatty acids of some algae from the Bohai Sea. Phytochemistry 59:157–161
Lorrain A, Paulet YM, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: implications for ecology and physiology. J Exp Mar Biol Ecol 275:47–61
Mansour MP, Volkman JK, Jackson AE, Blackburn SI (1999) The fatty acid and sterol composition of five marine dinoflagellates. J Phycol 35:710–720
McMahon KW, Ambrose WG, Johnson BJ, Sun MY, Lopez GR, Clough LM, Carroll ML (2006) Benthic community response to ice algae and phytoplankton in Ny Ålesund, Svalbard. Mar Ecol Prog Ser 310:1–14
McMeans B, Rooney N, Arts M, Fisk A (2013) Food web structure of a coastal Arctic marine ecosystem and implications for stability. Mar Ecol Prog Ser 482:17–28
Meziane T, Tsuchiya M (2002) Organic matter in a subtropical mangrove-estuary subjected to wastewater discharge: origin and utilisation by two macrozoobenthic species. J Sea Res 47:1–11
Michener RH, Kaufman L (2007) Stable isotope ratios as tracers in marine foodwebs: an update. In: Michener RH, Lajtha K (eds) Stable isotopes in ecology and environmental sciences. Blackwell, Oxford, p 238–282
Mikkelsen DM, Rysgaard S, Glud RN (2008) Microalgal composition and primary production in Arctic sea ice: a seasonal study from Kobbefjord (Kangerluarsunnguaq), West Greenland. Mar Ecol Prog Ser 368:65–74
Mincks SL, Smith CR, DeMaster DJ (2005) Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment 'food bank'. Mar Ecol Prog Ser 300:3–19
Mintenbeck K, Jacob U, Knust R, Arntz WE, Brey T (2007) Depth-dependence in stable isotope ratio $\delta^{15}N$ of benthic POM consumers: the role of particle dynamics and organism trophic guild. Deep-Sea Res I 54:1015–1023
Mortensen J, Lennert K, Bendtsen J, Rysgaard S (2011) Heat sources for glacial melt in a sub-Arctic fjord (Godthåbsfjord) in contact with the Greenland Ice Sheet. J Geophys Res 116:C01013
Mueller-Lupp T, Erlenkeuser H, Bauch HA (2003) Seasonal and interannual variability of Siberian river discharge in the Laptev Sea inferred from stable isotopes in modern bivalves. Boreas 32:292–303
Napolitano GE, Pollero RJ, Gayoso AM, Macdonald BA, Thompson RJ (1997) Fatty acids as trophic markers of phytoplankton blooms in the Bahía Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). Biochem Syst Ecol 25:739–755
Nelson MM, Phleger CF, Nichols PD (2002) Seasonal lipid composition in macroalgae of the northeastern Pacific Ocean. Bot Mar 45:58–65
Nichols PD, Skerratt JH, Davidson A, Burton H, McMeekin TA (1991) Lipids of cultured *Phaeocystis pouchetii*: signatures for food-web, biogeochemical and environmental studies in Antarctica and the Southern Ocean. Phytochemistry 30:3209–3214
Norkko A, Thrush SF, Cummings VJ, Gibbs MM, Andrew NL, Norkko J, Schwarz AM (2007) Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. Ecology 88:2810–2820
Ostrom NE, Macko SA, Deibel D, Thompson RJ (1997) Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean environment. Geochim Cosmochim Acta 61:2929–2942
Oxtoby LE, Mathis JT, Juranek LW, Wooller MJ (2013) Technical note: constraining stable carbon isotope values of microphytobenthos (C_3 photosynthesis) in the Arctic for application to food web studies. Biogeosciences Discuss 10:18151–18174
Paulet YM, Lorrain A, Richard J, Pouvreau S (2006) Experimental shift in diet $\delta^{13}C$: a potential tool for ecophysiological studies in marine bivalves. Org Geochem 37: 1359–1370
Perez V, Olivier F, Tremblay R, Neumeier U, Thébault J, Chauvaud L, Meziane T (2013) Trophic resources of the bivalve, *Venus verrucosa*, in the Chausey archipelago (Normandy, France) determined by stable isotopes and fatty acids. Aquat Living Resour 26:229–239
Petersen GH (2001) Studies on some Arctic and Baltic *Astarte* species (Bivalvia, Mollusca). Danish Polar Center, Copenhagen
Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320
Piepenburg D (2005) Recent research on Arctic benthos: common notions need to be revised. Polar Biol 28: 733–755
Piepenburg D, Archambault P, Ambrose WG, Blanchard AL and others (2011) Towards a pan-Arctic inventory of the species diversity of the macro- and megabenthic fauna of the Arctic shelf seas. Mar Biodiv 41:51–70
Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718
Poulin M, Daugbjerg N, Gradinger R, Ilyash L, Ratkova T, von Quillfeldt C (2011) The pan-Arctic biodiversity of marine pelagic and sea-ice unicellular eukaryotes: a first-attempt assessment. Mar Biodiv 41:13–28
Quijón PA, Kelly MC, Snelgrove PVR (2008) The role of sinking phytodetritus in structuring shallow-water benthic communities. J Exp Mar Biol Ecol 366:134–145
Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge
Rau GH, Takahashi T, Des Marais DJ, Repeta DJ, Martin JH (1992) The relationship between $\delta^{13}C$ of organic matter and $[CO_2(aq)]$ in ocean surface water: data from a JGOFS site in the northeast Atlantic Ocean and a model. Geochim Cosmochim Acta 56:1413–1419

Reemtsma T, Haake B, Ittekkot V, Nair RR, Brockmann UH (1990) Downward flux of particulate fatty acids in the Central Arabian Sea. Mar Chem 29:183–202

Renaud PE, Tessmann M, Evenset A, Christensen GN (2011) Benthic food-web structure of an Arctic fjord (Kongsfjorden, Svalbard). Mar Biol Res 7:13–26

Renaud PE, Løkken TS, Jørgensen LL, Berge J, Johnson BJ (2015) Macroalgal detritus and food-web subsidies along an Arctic fjord depth-gradient. Front Mar Sci 2:31

Reuss N, Poulsen LK (2002) Evaluation of fatty acids as biomarkers for a natural plankton community. a field study of a spring bloom and a post-bloom period off West Greenland. Mar Biol 141:423–434

Richter A, Rysgaard S, Dietrich R, Mortensen J, Petersen D (2011) Coastal tides in West Greenland derived from tide gauge records. Ocean Dyn 61:39–49

Roy V, Iken K, Archambault P (2014) Environmental drivers of the Canadian Arctic megabenthic communities. PLOS ONE 9:e100900

Roy V, Iken K, Gosselin M, Tremblay JÉ, Bélanger S, Archambault P (2015) Benthic faunal assimilation pathways and depth-related changes in food-web structure across the Canadian Arctic. Deep-Sea Res I 102:55–71

Schubert CJ, Calvert SE (2001) Nitrogen and carbon isotopic composition of marine and terrestrial organic matter in Arctic Ocean sediments: implications for nutrient utilization and organic matter composition. Deep-Sea Res I 48: 789–810

Sejr MK, Krause-Jensen D, Dalsgaard T, Ruiz-Halpern S and others (2014) Seasonal dynamics of autotrophic and heterotrophic plankton metabolism and P_{CO2} in a subarctic Greenland fjord. Limnol Oceanogr 59:1764–1778

Shin PKS, Yip KM, Xu WZ, Wong WH, Cheung SG (2008) Fatty acid as markers to demonstrating trophic relationships among diatoms, rotifers and green-lipped mussels. J Exp Mar Biol Ecol 357:75–84

Simstich J, Harms I, Karcher MJ, Erlenkeuser H and others (2005) Recent freshening in the Kara Sea (Siberia) recorded by stable isotopes in Arctic bivalve shells. J Geophys Res 110:C08006

Sørensen HL, Meire L, Juul-Pedersen T, de Stigter HC and others (2015) Seasonal carbon cycling in a Greenlandic fjord: an integrated pelagic and benthic study. Mar Ecol Prog Ser 539:1–17

Stead RA, Richoux NB, Pereda SV, Thompson RJ (2013) Influence of an intermittent food supply on energy storage by the subpolar deposit feeder *Yoldia hyperborea* (Bivalvia: Nuculanidae). Polar Biol 36:1333–1345

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ Conserv 29:436–459

Stevens CJ, Deibel D, Parrish CC (2004) Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis* Jaschnov. J Exp Mar Biol Ecol 303:135–156

Tamelander T, Renaud PE, Hop H, Carroll ML, Ambrose WG Jr, Hobson KA (2006) Trophic relationships and pelagic–benthic coupling during summer in the Barents Sea Marginal Ice Zone, revealed by stable carbon and nitrogen isotope measurements. Mar Ecol Prog Ser 310: 33–46

Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Viso AC, Marty JC (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521–1533

Waldock MJ, Holland DL (1984) Fatty acid metabolism in young oysters, *Crassostrea gigas*: polyunsaturated fatty acids. Lipids 19:332–336

Wang SW, Budge SM, Gradinger RR, Iken K, Wooller MJ (2014) Fatty acid and stable isotope characteristics of sea ice and pelagic particulate organic matter in the Bering Sea: tools for estimating sea ice algal contribution to Arctic food web production. Oecologia 174:699–712

Wassmann P (2011) Arctic marine ecosystems in an era of rapid climate change. Prog Oceanogr 90:1–17

Wassmann P, Reigstad M (2011) Future Arctic Ocean seasonal ice zones and implications for pelagic-benthic coupling. Oceanography (Wash DC) 24:220–231

Wassmann P, Duarte CM, Agust ÍS, Sejr MK (2011) Footprints of climate change in the Arctic marine ecosystem. Glob Change Biol 17:1235–1249

Weslawski JM, Wiktor J Jr, Kotwicki L (2010) Increase in biodiversity in the arctic rocky littoral, Sorkappland, Svalbard, after 20 years of climate warming. Marine Biodiversity 40:123–130

Wessels H, Karsten U, Wiencke C, Hagen W (2012) On the potential of fatty acids as trophic markers in Arctic grazers: feeding experiments with sea urchins and amphipods fed nine diets of macroalgae. Polar Biol 35: 555–565

Wulff A, Iken K, Quartino María L, Al-Handal A, Wiencke C, Clayton Margaret N (2009) Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. Bot Mar 52:491–507

Zhukova NV, Aizdaicher NA (1995) Fatty acid composition of 15 species of marine microalgae. Phytochemistry 39: 351–356

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