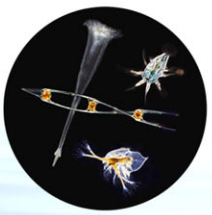
Journal of
Plankton Research

academic.oup.com/plankt



J. Plankton Res. (2017) 39(4): 664–674. *First published online June 23, 2017 doi:10.1093/plankt/fbx030*

Transfer of ice algae carbon to ice-associated amphipods in the high-Arctic pack ice environment

THOMAS A. BROWN[1,2]*, PHILIPP ASSMY[3], HAAKON HOP[3,4], ANETTE WOLD[3] AND SIMON T. BELT[2]

[1]MARINE ECOLOGY AND CHEMISTRY, SCOTTISH ASSOCIATION FOR MARINE SCIENCE, OBAN, ARGYLL PA37 1QA, UK, [2]SCHOOL OF GEOGRAPHY, EARTH AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF PLYMOUTH, PLYMOUTH PL4 8AA, UK, [3]NORWEGIAN POLAR INSTITUTE, FRAM CENTRE, N-9296 TROMSØ, NORWAY AND [4]DEPARTMENT OF ARCTIC AND MARINE BIOLOGY, FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS, UIT THE ARCTIC UNIVERSITY OF NORWAY, N-9037 TROMSØ, NORWAY

*CORRESPONDING AUTHOR: Thomas.Brown@sams.ac.uk

Received February 1, 2017; editorial decision May 9, 2017; accepted May 15, 2017

Corresponding Editor: Marja Koski

Sympagic (ice-associated) amphipods channel carbon into the marine ecosystem. With Arctic sea ice extent in decline, it is becoming increasingly important to quantify this transfer of sympagic energy. Recently, a method for quantifying sympagic particulate organic carbon (iPOC) in filtered water samples was proposed based on the abundances of the Arctic sea ice biomarker IP_{25}. Here, we tested the hypothesis that adoption of this method could also provide quantitative estimates of iPOC transfer within Arctic amphipods. We analysed five amphipod species collected north of Svalbard and compared findings to some previous studies. Estimates showed that *Onisimus glacialis* and *Apherusa glacialis* contained the most iPOC, relative to dry mass (23.5 ± 4.5 and 9.8 ± 1.9 mg C g^{-1}, respectively), while *Gammarus wilkitzkii* had the highest grazing impact on the available ice algae (0.48 mg C m^{-2}, for an estimated 24 h), equating to 73% of algal standing stock. Our findings are also broadly consistent with those obtained by applying the H-Print biomarker approach to the same samples. The ability to obtain realistic quantitative estimates of iPOC transfer into sympagic and pelagic fauna will likely have important implications for modelling energy flow in Arctic food webs during future climate scenarios.

KEYWORDS: Arctic amphipods; organic carbon; IP_{25}; H-Print; Nansen Basin

INTRODUCTION

Arctic sea ice provides a unique habitat for ice-associated algae, in particular diatoms (Dieckmann and Hellmer, 2010; Leu *et al.*, 2015), which offer food for a wide range of heterotrophic organisms. Some of the most important of these consumers are crustaceans

available online at academic.oup.com/plankt

(Arrigo, 2014) such as copepods, decapods, euphausiids and amphipods (Arndt and Swadling, 2006). In Arctic waters, analyses of baited traps and sediment traps have demonstrated that amphipods can dominate biomass in such settings (Nygård *et al.*, 2009; Kraft *et al.*, 2010). Consequently, amphipods provide an important link between sea ice algae and intermediary, as well as higher trophic level, consumers, including fish, seabirds and marine mammals (Lønne and Gulliksen, 1989; Lønne and Gabrielsen, 1992; Dalpadado *et al.*, 2016). With Arctic sea ice extent receding (Serreze *et al.*, 2016), there is a growing need to understand the impact of potential changes in the timing, magnitude and composition of ice algal blooms on sympagic (ice-associated), pelagic and benthic grazers (Søreide *et al.*, 2013; Leu *et al.*, 2015). Ice-associated amphipods are particularly sensitive to changes in sea ice conditions related to climate change (Kraft *et al.*, 2010; Barber *et al.*, 2015).

Direct coupling between sympagic and pelagic communities has been demonstrated recently following the identification of the Arctic sea ice diatom biomarker IP_{25} (Fig. 1; Belt *et al.*, 2007) in ice-associated zooplankton during springtime (Brown and Belt, 2012a). IP_{25} is a highly branched isoprenoid (HBI) lipid that serves as a selective tracer of ice-derived organic matter since it is only biosynthesized by certain Arctic sympagic diatoms (Belt *et al.*, 2007; Brown *et al.*, 2014c). Although these particular diatoms are generally the minority species, they are, nonetheless, pan-Arctic in distribution (Brown *et al.*, 2014c). The presence and abundance of IP_{25} in Arctic sea ice correlate well with spring sea ice diatom biomass (Brown *et al.*, 2011), which has led to the use of this lipid as a qualitative biomarker for sea ice particulate organic carbon (iPOC) (Brown *et al.*, 2016). Consistent with this, IP_{25} has been identified in sinking iPOC (Belt *et al.*, 2008; Brown, 2011; Brown *et al.*, 2016), sediments (Belt and Müller, 2013) and animals (Brown and Belt, 2012b; Brown *et al.*, 2014a, 2015) across the Arctic.

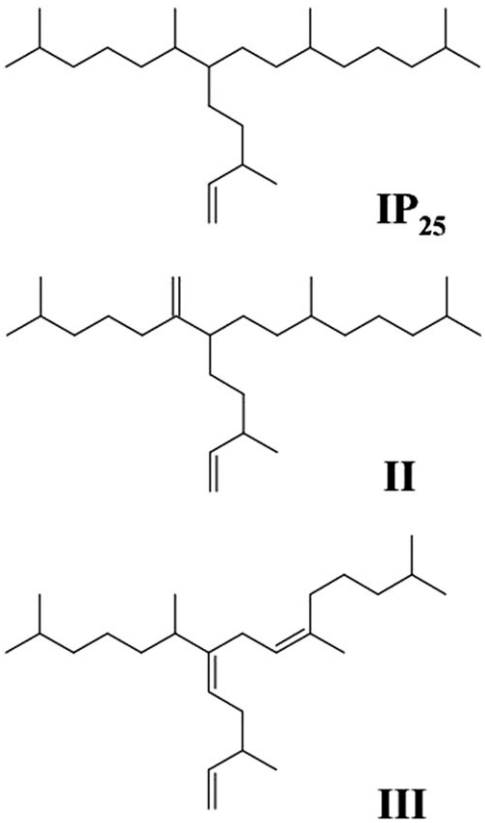


Fig. 1. Structures of sea ice diatom (IP_{25}, II) and phytoplanktic diatom (III) HBIs measured in amphipods.

Quantification of IP_{25} in bulk zooplankton from the Amundsen Gulf (Beaufort Sea) between February and June 2008, demonstrated that analysis of IP_{25} represents a potentially useful method for confirming the link between ice algae and heterotrophs (Brown and Belt, 2012a). During the sampling period, increases in the grazing impact of zooplankton on ice algae were inferred based on higher IP_{25} concentrations within zooplankton. However, a more thorough understanding of the effects of declining sea ice thickness and extent, and therefore sympagic algae, on Arctic animals requires quantification of sympagic carbon consumption, which, until recently, has not been achievable from IP_{25} concentration data alone. However, a more recent study demonstrated that concentrations of IP_{25} measured in seawater beneath sea ice during the spring melt can be used to obtain quantitative estimates of sinking iPOC (Brown *et al.*, 2016). In essence, quantitative estimates of iPOC in the water column were obtained by combining respective IP_{25} concentrations with the iPOC/IP_{25} ratio derived from analysis of the overlying sea ice. Using this approach, Brown *et al.* (2016) showed that iPOC accounted for up to 100% of the total organic carbon available to consumers in the upper water column at the time when sympagic algae were being released from the ice matrix.

Combined, the previous identification of IP_{25} in zooplankton (Brown and Belt, 2012a) and the recent demonstration that iPOC could be quantified in the water column (Brown *et al.*, 2016), led us to hypothesize that a similar approach could be used to quantify iPOC in Arctic primary consumers, such as some amphipod species that are known to graze on ice algae. Here, we tested this hypothesis by (i) determining the iPOC/IP_{25} ratio within ice algal aggregates collected beneath sea ice, north of Svalbard in the Nansen Basin, (ii) quantifying IP_{25} in amphipods sampled from beneath sea ice,

which were observed feeding on ice algae aggregates and (iii) combining these findings to quantify iPOC in amphipods. Having established a means of quantifying iPOC in amphipods, our aim was to provide the first quantitative estimates of iPOC consumption for *in situ* "autochthonous" (permanently inhabiting sea ice) amphipod species; *Gammarus wilkitzkii* Fabricius, 1775, *Apherusa glacialis* Hansen, 1887, *Onisimus nanseni* Sars, 1900, *Onisimus glacialis* Sars, 1900 and the "allochthonous" (partly ice-associated) amphipod, *Eusirus holmi* Hansen, 1887. To complement the iPOC/IP_{25} approach, we also calculated the so-called H-Print (Brown *et al.*, 2014d; Brown and Belt, 2017) for each sample, a method that combines the relative abundances of a variety of diatom-derived HBIs, and has been adopted previously to provide semi-quantitative estimates of the proportion of sympagic versus pelagic carbon in zooplankton (Brown and Belt, 2017) fish, seals and marine mammals (Brown *et al.*, 2017).

METHOD

Site description

Samples were collected in the Nansen Basin north of Nordaustlandet, Svalbard (Fig. 2). Ice algal aggregates and ice-associated amphipods were collected at an ice-station during the ICE12 expedition in July 2012, where the Norwegian Polar Institute research vessel *Lance* was moored to a large drifting ice floe at starting point 82.5°N, 21°E. The drift was southward towards the outer margins of the marginal ice zone (Fig. 2). The sea ice comprised mainly first-year ice and extended over a region where the water depth was up to 2500 m.

Sampling of ice algal aggregates

Floating ice algal aggregates were collected with a coarse-meshed sieve through a specially drilled ice hole (3.2 m^2 in size) at 12 h intervals from 29 July to 1 August 2012 (for more details see Assmy *et al.*, 2013). Upon return to the ship, ice algal aggregates were transferred into 50 mL centrifuge tubes and frozen at −20°C.

Sampling of amphipods

Samples of amphipods were collected on 24 separate occasions from 28 July to 1 August 2012 below the ice by scuba divers using an electrical suction pump with a 500 µm mesh net (Lønne, 1988). Qualitative sampling of amphipods for IP_{25} analysis was carried out by sampling as many organisms as possible during 40–60 min of diving. The amphipods were sorted by species and frozen at −80°C in zip-lock plastic bags.

Quantitative amphipod sampling was carried out by scuba divers using 50 × 50 cm standard frames (Hop *et al.*, 2000). Electrical suction pumps were used to collect samples from a set area of flat or ridged sea ice by placing these frames 10 times (one replicate sample) in a direction from the dive hole where ice amphipods occurred and exhaled bubbles were absent. Replicates (5 per flat or ridged sea ice) were taken by a single diver in different directions from the dive hole to avoid repeated sampling of the same under-ice area. The samples were preserved in buffered formaldehyde solution at a final concentration of 4% and were subsequently analysed for species composition, abundance and biomass at the Institute of Oceanology, Sopot, Poland. The total length of amphipods was determined from formaldehyde-preserved organisms blotted on filter paper. Abundance estimates (per m^2) were made based on the area covered for each replicate sample (2.5 m^2).

Total organic carbon

Sub-samples (~50 mg) of freeze-dried algae were decarbonated (10% HCl; 10 mL), washed (3 × 10 mL Milli-Q water) and freeze-dried prior to analysis using a Thermoquest EA1110 CHN analyser. L-Cystine was used as a calibration standard.

Lipid analysis

Extraction of HBI lipids from freeze-dried algae and amphipods was carried out using established techniques (Belt *et al.*, 2012; Brown *et al.*, 2014d). An internal standard (9-octylheptadec-8-ene (9-OHD); 10 µL; 2 µg mL^{-1}) was added to enable the quantification of IP_{25} (Belt *et al.*, 2012). Samples were covered in methanol (4 mL) and amphipods were mechanically crushed using a glass rod. Samples were then sonicated for 10 min. Milli-Q water (1 mL) and hexane (3 × 4 mL) were added, and then solutions were vortexed (1 min) and centrifuged (2 min; 2500 rpm). Supernatant solutions containing lipids were transferred to clean vials with glass pipettes and dried (N_2 stream). Extracts were then re-suspended in hexane (1 mL) and fractionated, providing non-polar lipids (IP_{25} and other HBIs) using column chromatography (5 mL hexane; SiO_2; 0.5 g).

Analyses of purified non-polar lipid extracts containing IP_{25} and other HBIs were carried out using gas chromatography–mass spectrometry (GC–MS) (Belt *et al.*, 2012). Total ion current chromatograms were used to determine the retention times and mass spectra of HBIs, and these were compared with those of

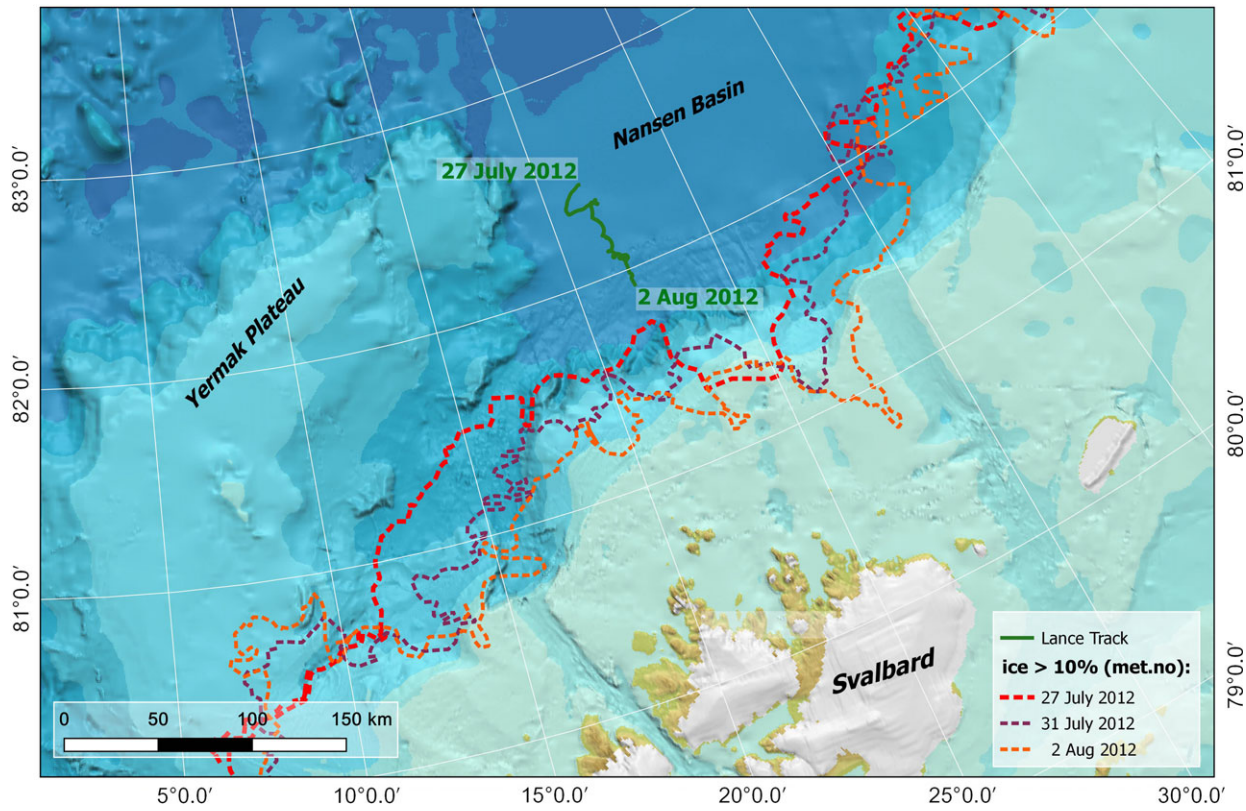


Fig. 2. Study location north of Svalbard with bathymetry. Green line is the drift trajectory of the ice floe that the RV *Lance* was moored to, with start and end dates. The ice edge positions for 27 and 31 July and 2 August are indicated by the broken lines and are representative for the drift period. Map created by the Norwegian Polar Institute, Max König. Bathymetry with permission from IBACO (Jakobsson *et al.*, 2012).

authentic standards (Belt *et al.*, 2012) and published literature (Brown, 2011 and references therein) for identification purposes.

For gravimetric quantification of IP_{25}, GC–MS responses were obtained in selective ion monitoring mode (m/z 350.3) and normalized using an instrumental response factor and the masses of internal standard and sample (Belt *et al.*, 2012).

iPOC quantification

Based on a previous method for estimating iPOC in seawater (Brown *et al.*, 2016), range and mean estimates of the iPOC content of amphipods ($iPOC_{amph}$) were obtained by combining amphipod IP_{25} concentrations with $iPOC/IP_{25}$ ratios derived from an ice algal aggregate sampled on 29 June 2012 (equation (1)). $iPOC_{amph}$ concentration estimates were obtained for the five amphipod species sampled.

$$iPOC_{amph} = IP_{25\,(amphipod)} \times \frac{iPOC_{(aggregate)}}{IP_{25\,(aggregate)}}. \quad (1)$$

HBI biomarker H-Print

H-Prints (%) were calculated using the abundance of pelagic (III) and sympagic (IP_{25} and II) HBIs according to equation (2).

$$H - Print\,\% = \frac{(III)}{(IP_{25} + II + III)} \times 100. \quad (2)$$

In addition to quantifying sympagic carbon contribution to the amphipod diet using equation (1), estimates of the proportion of sympagic carbon (with 99% confidence intervals), relative to total marine carbon (i.e. sympagic plus pelagic), were also derived by converting H-Prints using a previously modelled regression curve (Brown and Belt, 2017).

Statistical analysis

Statistical analysis was carried out in R-Studio version 1.0.136 (R-Core-Team, 2016). ANOVA, with post hoc Least Significant Difference mean separation tests (pairwise comparisons), was used to compare $iPOC_{amph}$

between amphipod species. All data are reported as mean ± standard error (SE) unless stated otherwise with tests considered significant at $\alpha = 0.05$.

RESULTS

Ice algae aggregates

Taxonomic analysis of floating ice algal aggregates (1–15 cm in diameter) sampled within the meltwater layer during the ICE12 cruise showed an assemblage of densely packed diatoms, with a dominance of the ice-associated pennate diatoms *Navicula pelagica*, *Hantzschia weyprechtii*, *Entomoneis paludosa* and *Cylindrotheca closterium* (Assmy *et al.*, 2013). Analysis of one of these ice algal aggregates, which was sampled alongside amphipods, showed a total organic carbon (TOC) content (261 ± 5 mg g^{-1}; 26%) consistent with previous data from diatom cultures (e.g. *Berkeleya rutilans* 30%; Brown *et al.*, 2014b) and floating ice algal aggregates (27–31%; Brown *et al.*, 2014c). The IP_{25} content was 1.14 ± 0.02 μg g^{-1}, giving an iPOC/IP_{25} ratio of 2.29 ± 0.04 × 10^5; $n = 6$. The dominance of sea ice diatom species within the aggregate was also reflected in the H-Print (<1%; $n = 5$).

iPOC in amphipods

Mean abundances of individual species, derived from 24 separate sampling operations (Table I, Fig. 3), showed that *A. glacialis* (mean 7.7 ind. m^{-2}) and *O. nanseni* (0.1 ind. m^{-2}) were the most and least abundant species, respectively. The largest species, *G. wilkitzkii*, was the second most abundant (0.7 ind. m^{-2}), while *E. holmi* and *O. glacialis* were comparable (0.3 ind. m^{-2}).

Our $iPOC_{amph}$ estimates show that *G. wilkitzkii* contained the most iPOC, with between 96 and 2052 μg C ind^{-1} for specimens ranging in length from 15 to 40 mm (Fig. 4a,b). Such specimens contained significantly more $iPOC_{amph}$ than any other species, including *E. holmi*, despite being of similar size ($F = 15.8$, df = 4, $P \leqslant 0.001$; Table I). Normalization of $iPOC_{amph}$ estimates to account for amphipod mass (dry) revealed that *O. glacialis* had the highest dry mass (DM) normalized $iPOC_{amph}$, with more than twice as much $iPOC_{amph}$ as any other species ($F = 13.6$, df = 4, $P \leqslant 0.001$; Table I). In contrast to absolute $iPOC_{amph}$ estimates, the DM normalized $iPOC_{amph}$ content of *G. wilkitzkii* was similar to the much smaller *A. glacialis* ($F = 13.6$, df = 4, $P \leqslant 0.001$). Finally, DM normalized data revealed that *E. holmi* and *O. nanseni* had similar $iPOC_{amph}$ content, both being significantly less than other species (Table I), despite their difference in size (28 ± 1.5 and 14 ± 4 mm, respectively).

Table I: Mean (± SE) and relative (%) amphipod abundance, $iPOC_{amph}$ and H-Print estimates (99% CI) of sympagic carbon, as a percentage of sympagic and pelagic marine carbon consumed, based on the regression model of Brown and Belt (2017)

	Abundance			$iPOC_{amph}$							iPOC % amphipod C	H-Print estimates (%) of sympagic carbon				
Species	*n*	ind. m^{-2}	% of all species	*n*	μg C ind^{-1}	% of all species	Significant difference[1]	mg C g^{-1} DM	% of all species	Significant difference[1]		Significant difference[1]	mg C m^{-2}	% of all species	Mean (99% CI)	Max (99% CI)	Min (99% CI)	Significant difference[1]
A. glacialis	367	7.7	85	15	16.3 ± 2.4	2	a	9.8 ± 1.9	21	a	2.5 ± 0.5	a	0.13	19	92 (78–106)	100 (86–115)	37 (24–52)	a
E. holmi	12	0.3	3	14	54.7 ± 8.3	6	a	1.6 ± 0.2	3	b	0.4 ± 0.1	b	0.01	2	100 (86–115)	100 (86–115)	100 (86–115)	b
O. nanseni	6	0.1	1	13	68.5 ± 18.9	7	a	5.1 ± 1.1	11	ab	1.3 ± 0.3	ab	0.01	1	100 (86–115)	100 (86–115)	99 (84–113)	b
O. glacialis	15	0.3	3	10	107.5 ± 14.5	12	a	23.5 ± 4.5	49	c	5.9 ± 1.1	c	0.03	5	100 (86–115)	100 (86–115)	100 (86–115)	b
G. wilkitzkii	34	0.7	8	34	673.6 ± 94.5	73	b	7.8 ± 1.3	16	a	2.0 ± 0.3	a	0.48	73	99 (84–113)	100 (86–115)	93 (79–108)	b
Total	434	9.14*		86	920.6*			47.8*					0.66*					

*Calculated from mean values.
[1]Least Significant Difference mean separation tests (pairwise comparisons), $\alpha = 0.05$.

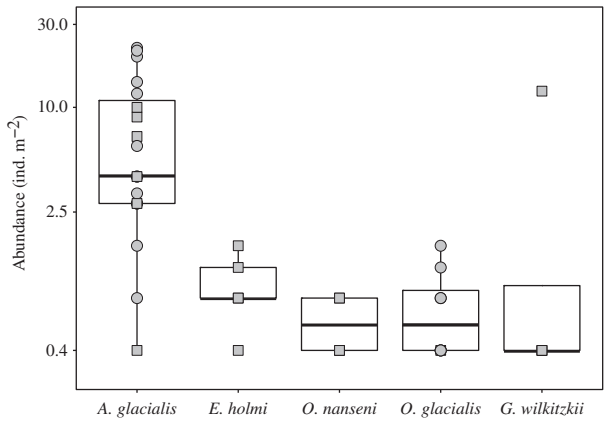


Fig. 3. Averaged amphipod abundance for each species beneath the ice floe during sampling (note: logged *y*-axis). Circles = flat under-ice surface, squares = ridged under-ice surface.

The quantity of $iPOC_{amph}$ per unit area of sea ice at the time of sampling was estimated by combining mean $iPOC_{amph}$ values with mean amphipod abundance derived from the 24 separate observations made during sampling. This showed that the amount of iPOC being retained by amphipods was ca. 0.66 mg iPOC m^{-2} (Table I). The majority of $iPOC_{amph}$ was found within *G. wilkitzkii* (73%; 0.48 mg m^{-2}), followed by *A. glacialis* (19%), with *O. glacialis*, *E. holmi* and *O. nanseni* containing the least (all <5%; 0.03 mg m^{-2}). When compared to the carbon standing stock of ice algal aggregates (0.74 mg C m^{-2}; from Assmy *et al.* (2013)), $iPOC_{amph}$ in the five amphipod species corresponded to approximately 89% of available ice algal aggregate carbon.

Source of amphipod POC

The majority (95%) of amphipod H-Prints for all species ranged from 0.1 to 7.2% (Fig. 4g,h), with only four individuals (all *A. glacialis*) having H-Prints >7.2% (12.0, 13.1, 40.1 and 62.2%). Using the regression model defined previously by Brown and Belt (2017), these H-Print values were re-expressed to estimate % sympagic carbon consumed by amphipods. In all cases, mean %sympagic consumption was estimated as >90% (Table I).

DISCUSSION

Organic carbon and IP_{25} content of sea ice algal aggregate

The $iPOC/IP_{25}$ ratio used in the current study (2.29 ± 0.04 × 10^5) is much higher than that reported previously for sea ice POC from Resolute Bay in the Canadian Arctic (ca. 2.6 × 10^3; Brown *et al.*, 2016) and we provide two explanations for this. Firstly, in contrast to the Resolute Bay sea ice samples, there were high amounts of extracellular polymeric substances in the ICE12 sea ice algal aggregates from the Arctic Ocean (Assmy *et al.*, 2013), consistent with a "stressed/old" community (Søreide *et al.*, 2006), and supported further by observations of a large number of empty diatom frustules (Assmy *et al.*, 2013). In addition, the percentage of IP_{25}-producing species in the ICE12 algae was lower (<0.1%) compared to Resolute Bay (0.3–3.6%; Brown *et al.*, 2014c). Since ICE12 aggregates were sampled from within the water column, rather than directly from within sea ice, this reduction could potentially be due to the *in situ* incorporation of non-IP_{25} producing phytoplankton species, although Assmy *et al.* (2013) showed that the aggregate composition was dominated by ice-associated diatoms and this is supported here by very low H-Prints (<1%). Instead, although *Haslea crucigeroides*, a known producer of IP_{25} (Brown *et al.*, 2014c), could be identified in the ICE12 aggregates (T. A. Brown, personal observation), it was not sufficiently abundant to be included in previous taxonomic reports (Assmy *et al.*, 2013). Indeed, the IP_{25}-producing species (*H. crucigeroides*, *H. spicula*, *H. kjellmanii* and *Pleurosigma stuxbergii* var *rhomboides*) are typically <1% of sea ice diatom assemblages from north-east Svalbard and west Greenland (von Quillfeldt, 2000), while the same species comprised >3% of the diatoms present in the Resolute Bay aggregates (Brown *et al.*, 2014c and references therein). In any case, regardless of the exact reasons for the differences in $iPOC/IP_{25}$, this study reinforces the importance of measuring this ratio on a case-by-case basis, as recommended by Brown *et al.* (2016). In contrast, adoption of a fixed value for $iPOC/IP_{25}$ will likely lead to anomalous estimates of iPOC within suspended/sinking POC and food-web constituents, as discussed in detail by Brown *et al.* (2016).

Quantitative estimates of ice-derived organic carbon in amphipods

IP_{25} was present in each of the amphipod specimens analysed, enabling us to estimate $iPOC_{amph}$ in all cases. $iPOC_{amph}$ estimates varied by three orders of magnitude, broadly reflecting the range in amphipod size, with the smallest (*A. glacialis*) and largest (*G. wilkitzkii*) containing the lowest and highest $iPOC_{amph}$, respectively. Dry mass-normalized abundances showed the opposite trend, however, with smaller species (and smaller individuals of species) having relatively higher $iPOC_{amph}$, which aligns with smaller animals having to sustain higher weight-specific ingestion rates to offset their higher

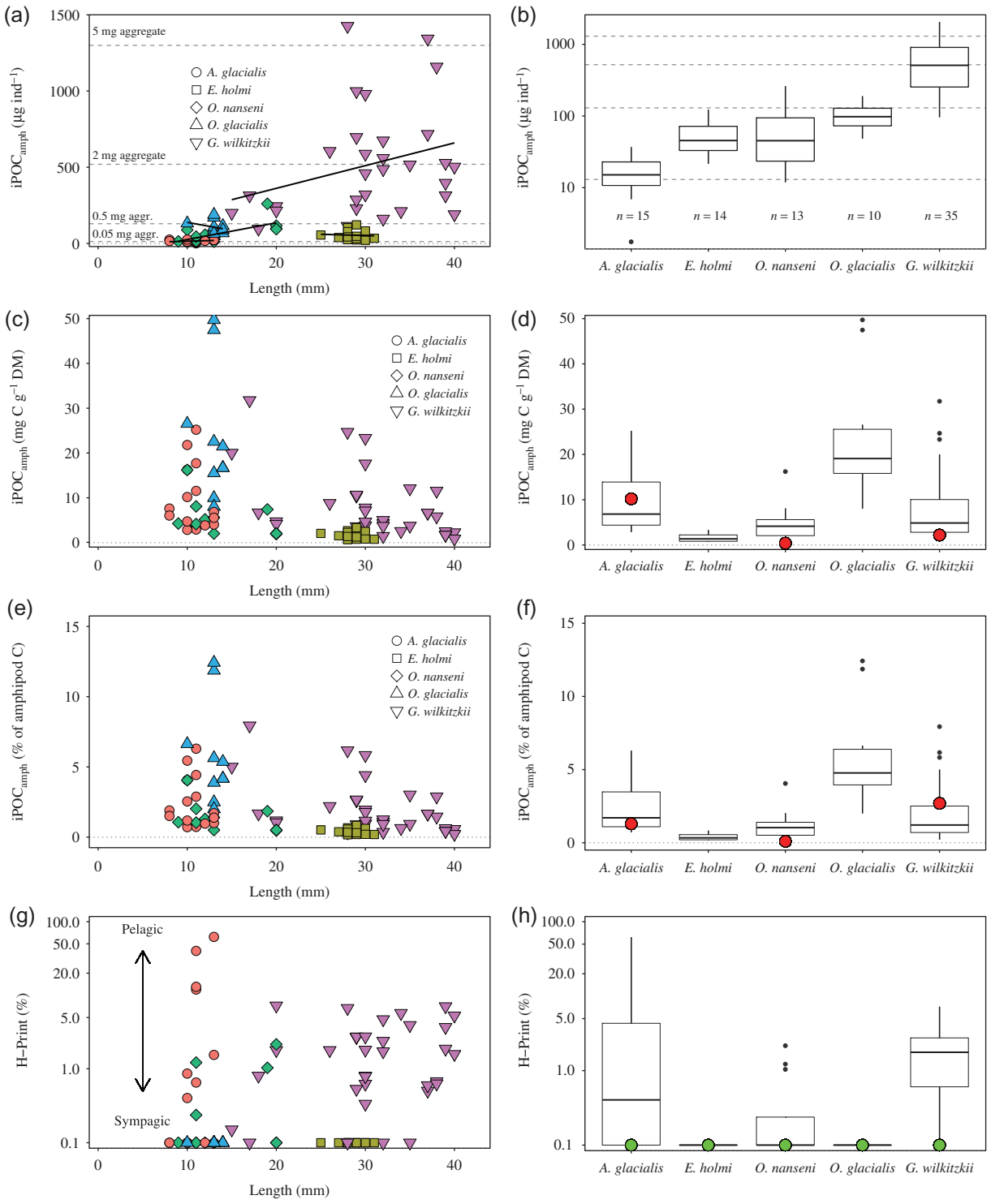


Fig. 4. Amphipod iPOC content (a–f) and H-Print (g-h; note logged scale) compared to amphipod length (left) and for species average (right). Horizontal dotted lines (a, b) represent equivalent total algal aggregate mass consumed for selected iPOC concentrations (note logged scale in b). Shaded circles (d and f) show data derived from laboratory experiments (Werner, 1997). Shaded circles (h) show the H-Print value derived from ice algal aggregates that amphipods were observed grazing upon in this study.

metabolic activity (cf. larger animals) (Werner, 1997). On the other hand, this size-dependant difference in $iPOC_{amph}$ may potentially reflect the variable dietary preference of amphipods, especially as the smaller *A. glacialis* are more herbivorous than the larger and mainly omnivorous/carnivorous *G. wilkitzkii* (Poltermann, 2001).

Next, by expressing the $iPOC_{amph}$ values as a percentage of estimated amphipod carbon content (ca. 40%; Werner, 1997; Yuichiro and Tsutomu, 2003; Kiørboe, 2013; Fig. 4e, f), our data show that <13% of amphipod body carbon comprised carbon derived from sea ice algal aggregates, in good agreement with data obtained from captive *G. wilkitzkii*, *O. nanseni* and *A. glacialis*, which consumed between 0.1 and 16% of body carbon during experiments carried out in fixed-volume vessels containing physical ice substrate (Werner, 1997).

We then combined $iPOC_{amph}$ data with the TOC content of ICE12 aggregates to obtain estimates of the total mass of ice algae consumed by amphipods. Our data indicate that individual *G. wilkitzkii* had consumed between 0.5 and 5 mg of ice algal aggregate leading up to their capture during our sampling campaign. Since our data represent *in situ* values, it is not possible to definitively report data as estimates of daily consumption rates that would facilitate direct comparisons with other studies. However, since our $iPOC_{amph}$ estimates fall within a similar range of daily consumption rates reported previously for *Gammarus* spp. feeding on macrophytes in the Baltic Sea (1–5 mg ind. d^{-1}; Orav-Kotta *et al.*, 2009) and captive *G. wilkitzkii* (0.08–0.14 mg algae ind. d^{-1}; Werner, 1997), we suggest that our estimates potentially reflect grazing rates over approximately 24 h. Further direct comparisons of $iPOC/IP_{25}$ derived values between *in situ* and captive zooplankters should improve such comparisons in the future.

Finally, by combining $iPOC_{amph}$ with amphipod abundance (i.e. number of individuals per unit area) for each of the five species, we estimate that $iPOC_{amph}$ accounted for ca. 89% of the available ice algal aggregate carbon during the course of sampling, which agrees well with previous estimates of 63% and 58–92% (Siferd *et al.*, 1997; Kohlbach *et al.*, 2016). Similarly, our consistent amphipod H-Prints indicate that most amphipod species appeared to be obtaining energy almost exclusively (>90%; Table I) from ice algal aggregates. On the other hand, based on a composite of field-measured abundances and laboratory-based grazing studies, Werner (1997) estimated the daily grazing impacts of *A. glacialis*, *Onisimus* spp. and *G. wilkitzkii* to be ca. 1.1% and 2.6% for the Laptev and Greenland Sea, respectively. One explanation for these different outcomes might be associated with the high degree of variability in amphipod abundance, which is strongly seasonally dependent in response to the development of sea ice (Siferd *et al.*, 1997; Werner and Auel, 2005). Thus, it is possible that our amphipod abundances (and therefore estimates of grazing impact) were influenced, to some extent, by the sea ice conditions during the late melt season, when the sea ice was becoming increasingly heterogeneous, with a growing number of melt ponds (Assmy *et al.*, 2013). At this time in the season, after much of the iPOC had likely already been exported (e.g. Brown *et al.*, 2016), it is also possible that ice algae aggregates represented an important and concentrated food source in an otherwise relatively oligotrophic period. In this case, amphipod diet at the time of sampling would likely be dominated by ice algae, rather than other sources, resulting in relatively high estimates of grazing impact.

A part of $iPOC_{amph}$ could have been acquired from other carbon sources since, for example, large *G. wilkitzkii* has an omnivorous diet, and consumes both zooplankton and ice amphipods (Werner, 1997; Søreide *et al.*, 2006). The most likely candidate of zooplankton prey is *Calanus glacialis*, which is known to utilize ice algal blooms to fuel early maturation and reproduction (Søreide *et al.*, 2010). Qualitative assessment of herbivory/carnivory in amphipods has been established based on faecal pellet colouration, where green-yellow and orange-red pigments indicate herbivory and carnivory, respectively (Werner, 2000). Accordingly, the observed orange colouration of *G. wilkitzkii* lipid extracts in this study likely indicates that this species incorporated at least some of the estimated $iPOC_{amph}$ through carnivory, probably from *Calanus* sp., rather than direct herbivory. Despite this, we note that our *G. wilkitzkii* $iPOC_{amph}$ data remain comparable to other captive grazing experiments where carnivory was absent (Fig. 4d,f; Werner, 1997). Indeed, it is well established that IP_{25} is transferred across trophic levels, and is readily identified in Arctic consumers, from fish (Brown and Belt, 2012b; Brown *et al.*, 2015) and seabirds (Megson *et al.*, 2014), right up to marine mammals (Brown *et al.*, 2013, 2014a).

$iPOC_{amph}$ in *E. holmi*

In contrast to the other amphipod species in this study, comparison of $iPOC_{amph}$ data with literature values was not possible for *E. holmi*, despite this species being pan-Arctic in distribution (Tencati and Geiger, 1968; Siferd, 2015). The limited reporting of *E. holmi* likely reflects its low abundance in the Arctic, rather than difficulties in identification, as it is easily recognized based on its light orange eyes, orange markings on coxa and pleopods, and four long antennae and long leg segments. *Eusirus holmi* accounted for ca. 3% of the amphipods in our

samples and was even lower (<1%) in a previous study from the same region (Macnaughton *et al.*, 2007). Our $iPOC_{amph}$ data therefore likely represent the only documented estimates of iPOC consumption for this species. Notably, absolute $iPOC_{amph}$ estimates in *E. holmi* were most similar to those for the smaller *A. glacialis* and *O. nanseni*, while normalized (dry body mass) values were also comparable to the much smaller *O. nanseni*. Although the paucity of literature data prevents us from assessing our $iPOC_{amph}$ estimate for *E. holmi* further, we note that, in contrast to the other species investigated here, a relatively low $iPOC_{amph}$ content for this species might imply that it obtained the majority of its organic carbon from sources other than sea ice algae. *Eusirus holmi* is frequently observed by divers in the water column, typically with legs spread out to suspend itself while slowly sinking. It occasionally propels itself upwards with its large telson and then repeats the slow sinking. This likely represents the feeding behaviour of *E. holmi* in the water column, but it can also be observed clinging to the underside of sea ice. However, the consistent H-Print values indicated that ca. 100% (86–115%; 99% CI) of marine carbon in amphipods was of sympagic origin (Table I). While there are a number of potential reasons for the low $iPOC_{amph}$ estimates, including, for example, selectivity during grazing, further analysis of this species will be necessary before firmer conclusions can be made. What is clear, however, is that the low field abundances of *E. holmi* in other studies and low $iPOC_{amph}$ content estimated here indicate that this species is currently of minor importance with respect to channelling the sympagic carbon component into the ecosystem, at least in comparison to other more abundant species in this study, particularly, *G. wilkitzkii* and *A. glacialis*.

Having focused here on a somewhat localized setting, we anticipate that further application of this technique to a wider range of Arctic ice fauna and zooplankters has the potential to improve our knowledge and understanding of the role that ice algae play in supporting the broader Arctic ecosystem. Concomitant with the long-term trend of decreasing sea ice extent and thickness (Barber *et al.*, 2015), a similar decline in ice amphipods, particularly *G. wilkitzkii* has occurred, with associated reduction of high-energy food to upper trophic consumers (Hop *et al.*, 2013). Reduction in sea ice extent in Antarctica has similarly been identified as one of the causes of the recent decline in Antarctic krill populations in the Southern Ocean (Flores *et al.*, 2012), with impacts on higher trophic level animals (Reiss *et al.*, 2017). In both polar areas, increased ridging in thinner ice may partly compensate for loss in sea ice extent by creating complex structures as enhanced habitat for sympagic fauna (Gradinger *et al.*, 2010; Melbourne-Thomas *et al.*, 2016). The more pelagic *E. holmi* may increase in abundance with changing ice conditions towards thinner first-year ice and more frequent open water in the Arctic Ocean. Under such a scenario, *E. holmi* may potentially represent an alternative energy source to that currently derived from ice algae (and associated amphipods) and, therefore, an important target for future research efforts. In any case, the data generated from this, and subsequent studies, will provide the necessary numerical input required to assist models in predicting the potential impact of declining Arctic sea ice extent on sea ice biota, and further evaluation of sea ice algae as an energy source for other Arctic consumers.

CONCLUSION

Combining sea ice-derived $iPOC/IP_{25}$ data with IP_{25} concentration data obtained from amphipods can provide realistic estimates of the amount of sympagic organic carbon within these primary consumers. Here, it was shown that 0.66 mg C m^{-2} was retained by the amphipods studied at the point of sampling, corresponding to 89% of the available ice algae standing stock. The data generated from this, and subsequent studies, will provide numerical input required to assist models in predicting the potential impact of declining Arctic sea ice extent on sea ice biota, and to further evaluate sea ice algae as an energy source for other Arctic consumers.

ACKNOWLEDGEMENTS

We are indebted to the captain and crew of RV *Lance*. We are also grateful to A Tonkin (University of Plymouth) for carrying out CHN analysis of algal aggregates. Taxonomic analyses were based on a contract with Institute of Oceanology, Sopot, Poland, with expertize administered by Slawomir Kwasniewski. We are also grateful to the Associate Editor and an anonymous reviewer for their helpful comments.

FUNDING

This work was supported by the award of a Research Project Grant from the Leverhulme Trust (UK). The ICE2012 cruise was funded and conducted as part of the Centre for Ice, Climate and Ecosystems (ICE) at the Norwegian Polar Institute.

REFERENCES

Arndt, C. E. and Swadling, K. M. (2006) Crustacea in Arctic and Antarctic Sea Ice: distribution, diet and life history strategies. *Adv. Mar. Biol.*, **51**, 197–315.

Arrigo, K. R. (2014) Sea ice ecosystems. *Ann. Rev. Mar. Sci.*, **6**, 439–467.

Assmy, P., Ehn, J. K., Fernández-Méndez, M., Hop, H., Katlein, C., Sundfjord, A., Bluhm, K., Daase, M. *et al.* (2013) Floating ice-algal aggregates below melting Arctic sea ice. *PLoS One*, **8**, e76599.

Barber, D. G., Hop, H., Mundy, C. J., Else, B., Dmitrenko, I. A., Tremblay, J.-E., Ehn, J. K., Assmy, P. *et al.* (2015) Selected physical, biological and biogeochemical implications of a rapidly changing Arctic Marginal Ice Zone. *Progr. Oceanogr.*, **139**, 122–150.

Belt, S. T., Brown, T. A., Navarro-Rodriguez, A., Cabedo-Sanz, P., Tonkin, A. and Ingle, R. (2012) A reproducible method for the extraction, identification and quantification of the Arctic sea ice proxy IP_{25} from marine sediments. *Anal. Methods*, **4**, 705–713.

Belt, S. T., Massé, G., Rowland, S. J., Poulin, M., Michel, C. and Leblanc, B. (2007) A novel chemical fossil of palaeo sea ice: IP_{25}. *Org. Geochem.*, **38**, 16–27.

Belt, S. T., Massé, G., Vare, L. L., Rowland, S. J., Poulin, M., Sicre, M.-A., Sampei, M. and Fortier, L. (2008) Distinctive ^{13}C isotopic signature distinguishes a novel sea ice biomarker in Arctic sediments and sediment traps. *Mar. Chem.*, **112**, 158–167.

Belt, S. T. and Müller, J. (2013) The Arctic sea ice biomarker IP_{25}: a review of current understanding, recommendations for future research and applications in palaeo sea ice reconstructions. *Quat. Sci. Rev.*, **79**, 9–25.

Brown, T. A. (2011) Production and preservation of the Arctic sea ice diatom biomarker IP_{25}. PhD Thesis. University of Plymouth.

Brown, T. A., Alexander, C., Yurkowski, D. J., Ferguson, S. and Belt, S. T. (2014a) Identifying variable sea ice carbon contributions to the Arctic ecosystem: a case study using highly branched isoprenoid lipid biomarkers in Cumberland Sound ringed seals. *Limnol. Oceanogr.*, **59**, 1581–1589.

Brown, T. A. and Belt, S. T. (2012a) Closely linked sea ice–pelagic coupling in the Amundsen Gulf revealed by the sea ice diatom biomarker IP_{25}. *J. Plankton Res.*, **34**, 647–654.

Brown, T. A. and Belt, S. T. (2012b) Identification of the sea ice diatom biomarker IP_{25} in Arctic benthic macrofauna: direct evidence for a sea ice diatom diet in Arctic heterotrophs. *Polar Biol.*, **35**, 131–137.

Brown, T. A. and Belt, S. T. (2017) Biomarker-based H-Print quantifies the composition of mixed sympagic and pelagic algae consumed by *Artemia* sp. *J. Exp. Mar. Biol. Ecol.*, **488**, 32–37.

Brown, T. A., Belt, S. T. and Cabedo-Sanz, P. (2014b) Identification of a novel di-unsaturated C_{25} highly branched isoprenoid in the marine tube-dwelling diatom *Berkeleya rutilans*. *Environ. Chem. Lett.*, **12**, 455–460.

Brown, T. A., Belt, S. T., Ferguson, S. H., Yurkowski, D. J., Davison, N. J., Barnett, J. E. F. and Jepson, P. D. (2013) Identification of the sea ice diatom biomarker IP_{25} and related lipids in marine mammals: a potential method for investigating regional variations in dietary sources within higher trophic level marine systems. *J. Exp. Mar. Biol. Ecol.*, **441**, 99–104.

Brown, T. A., Belt, S. T., Gosselin, M., Levasseur, M., Poulin, M. and Mundy, C. J. (2016) Quantitative estimates of sinking sea ice particulate organic carbon based on the biomarker IP_{25}. *Mar. Ecol. Prog. Ser.*, **546**, 17–29.

Brown, T. A., Belt, S. T., Philippe, B., Mundy, C. J., Massé, G., Poulin, M. and Gosselin, M. (2011) Temporal and vertical variations of lipid biomarkers during a bottom ice diatom bloom in the Canadian Beaufort Sea: further evidence for the use of the IP_{25} biomarker as a proxy for spring Arctic sea ice. *Polar Biol.*, **34**, 1857–1868.

Brown, T. A., Belt, S. T., Tatarek, A. and Mundy, C. J. (2014c) Source identification of the Arctic sea ice proxy IP_{25}. *Nat. Commun.*, **5**, 4197.

Brown, T. A., Chrystal, E., Ferguson, S. H., Yurkowski, D. J., Watt, C., Hussey, N. and Belt, S. T. (2017) Coupled changes in the sea ice carbon contribution to diet and trophic position of Cumberland Sound beluga whales identified by H-Print and $\delta^{15}N$ analysis. *Limnol. Oceanogr.* doi: 10.1002/lno.10520. (In press).

Brown, T. A., Hegseth, E. N. and Belt, S. T. (2015) A biomarker-based investigation of the mid-winter ecosystem in Rijpfjorden, Svalbard. *Polar Biol.*, **38**, 37–50.

Brown, T. A., Yurkowski, D. J., Ferguson, S. H., Alexander, C. and Belt, S. T. (2014d) H-Print: a new chemical fingerprinting approach for distinguishing primary production sources in Arctic ecosystems. *Environ. Chem. Lett.*, **12**, 387–392.

Dalpadado, P., Hop, H., Rønning, J., Pavlov, V., Sperfeld, E., Buchholz, F., Rey, A. and Wold, A. (2016) Distribution and abundance of euphausiids and pelagic amphipods in Kongsfjorden, Isfjorden and Rijpfjorden (Svalbard) and changes in their relative importance as key prey in a warming marine ecosystem. *Polar Biol.*, **39**, 1765–1784.

Dieckmann, G. S. and Hellmer, H. H. (2010) The importance of sea ice: an overview. In Thomas, D. and Dieckmann, S. (eds), *Sea Ice*, 2nd edn. Blackwell Publishing, Chichester, pp. 1–22.

Flores, H., Atkinson, A., Kawaguchi, S., Krafft, B. A., Milinevsky, G., Nicol, S., Reiss, C., Tarling, G. A. *et al.* (2012) Impact of climate change on Antarctic krill. *Mar. Ecol. Prog. Ser.*, **458**, 1–19.

Gradinger, R., Bluhm, B. and Iken, K. (2010) Arctic sea-ice ridges—safe heavens for sea-ice fauna during periods of extreme ice melt? *Deep-Sea Res. II*, **57**, 86–95.

Hop, H., Bluhm, B. A., Daase, M., Gradinger, R., Poulin, M. (2013) Arctic Sea Ice Biota. Arctic Report Cards, NOAA. http://www.arctic.noaa.gov/reportcard.

Hop, H., Poltermann, M., Lønne, O. J., Falk-Petersen, S., Korsnes, R. and Budgell, W. P. (2000) Ice amphipod distribution relative to ice density and under-ice topography in the northern Barents Sea. *Polar Biol.*, **23**, 367–367.

Jakobsson, M., Mayer, L., Coakley, B., Dowdeswell, J. A., Forbes, S., Fridman, B., Hodnesdal, H., Noormets, R. *et al.* (2012) The International Bathymetric Chart of the Arctic Ocean (IBCAO) Version 3.0. *Geophys. Res. Lett.*, **39**, L12609.

Kiørboe, T. (2013) Zooplankton body composition. *Limnol. Oceanogr.*, **58**, 1843–1850.

Kohlbach, D., Graeve, M., Lange, B., David, C., Peeken, I. and Flores, H. (2016) The importance of ice algae-produced carbon in the central Arctic Ocean ecosystem: Food web relationships revealed by lipid and stable isotope analyses. *Limnol. Oceanogr.*, **61**, 2027–2044.

Kraft, A., Bauerfeind, E. and Nöthig, E.-M. (2010) Amphipod abundance in sediment trap samples at the long-term observatory

HAUSGARTEN (Fram Strait, ~79°N/4°E). Variability in species community patterns. *Mar. Biodivers.*, **41**, 353–364.

Leu, E., Mundy, C. J., Assmy, P., Campbell, K., Gabrielsen, T. M., Gosselin, M., Juul-Pedersen, T. and Gradinger, R. (2015) Arctic spring awakening – steering principles behind the phenology of vernal ice algal blooms. *Progr. Oceanogr.*, **139**, 151–170.

Lønne, O. J. (1988) A diver-operated electric suction sampler for sympagic (=under-ice) invertebrates. *Polar Res.*, **6**, 135–136.

Lønne, O. J. and Gabrielsen, G. W. (1992) Summer diet of seabirds feeding in sea-ice-covered waters near Svalbard. *Polar Biol.*, **12**, 685–692.

Lønne, O. J. and Gulliksen, B. (1989) Size, age and diet of polar cod, *Boreogadus saida* (Lepechin 1773), in ice covered waters. *Polar Biol.*, **9**, 187–191.

Macnaughton, M., Thormar, J. and Berge, J. (2007) Sympagic amphipods in the Arctic pack ice: redescriptions of *Eusirus holmii* Hansen, 1887 and *Pleusymtes karstensi* Barnard, 1959. *Polar Biol.*, **30**, 1013–1025.

Megson, D., Brown, T. A., Johnson, G. W., O'Sullivan, G., Bicknell, A. W. J., Votier, S. C., Lohan, M. C., Comber, S. *et al.* (2014) Identifying the provenance of Leach's storm petrels in the North Atlantic using polychlorinated biphenyl signatures derived from comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *Chemosphere*, **114**, 195–202.

Melbourne-Thomas, J., Corney, S. P., Trebilco, R., Meiners, K. M., Stevens, R. P., Kawaguchi, S., Sumner, M. D. and Constable, A. J. (2016) Under ice habitats for Antarctic krill larvae: Could less mean more under climate warming? *Geophys. Res. Lett.*, **43**, 10322–10327.

Nygård, H., Vihtakari, M. and Berge, J. (2009) Life history of *Onisimus caricus* (Amphipoda: Lysianassoidea) in a high Arctic fjord. *Aquat. Biol.*, **5**, 63–74.

Orav-Kotta, H., Kotta, J., Herkül, K., Kotta, I. and Paalme, T. (2009) Seasonal variability in the grazing potential of the invasive amphipod *Gammarus tigrinus* and the native amphipod *Gammarus salinus* (Amphipoda: Crustacea) in the northern Baltic Sea. *Biol. Invasions*, **11**, 597–608.

Poltermann, M. (2001) Arctic sea ice as feeding ground for amphipods – food sources and strategies. *Polar Biol.*, **24**, 89–96.

R-Core-Team (2016) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Reiss, C. S., Cossio, A., Santora, J. A., Dietrich, K. S., Murray, A., Mitchell, B. G., Walsh, J., Weiss, E. L. *et al.* (2017) Overwinter habitat selection by Antarctic krill under varying sea-ice conditions: implications for top predators and fishery management. *Mar. Ecol. Prog. Ser.*, **568**, 1–16.

Serreze, M. C., Crawford, A. D., Stroeve, J. C., Barrett, A. P. and Woodgate, R. A. (2016) Variability, trends, and predictability of seasonal sea ice retreat and advance in the Chukchi Sea. *J. Geophys. Res. Ocean.*, **121**, 7308–7325.

Siferd, T. D. (2015) Central and Arctic Multi-Species Stock Assessment Surveys Version 5 In OBIS Canada Digital Collections. Bedford Institute of Oceanography, Dartmouth, NS, Canada. Published by OBIS, Digital http://www.iobis.org/. Accessed on 15-12-2016.

Siferd, T. D., Welch, H. E., Bergmann, M. A. and Curtis, M. F. (1997) Seasonal distribution of sympagic amphipods near Chesterfield Inlet, N.W.T., Canada. *Polar Biol.*, **18**, 16–22.

Søreide, J. E., Carroll, M. L., Hop, H., Ambrose, W. G.Jr, Hegseth, E. N. and Falk-Petersen, S. (2013) Sympagic-pelagic-benthic coupling in Arctic and Atlantic waters around Svalbard revealed by stable isotopic and fatty acid tracers. *Mar. Biol. Res.*, **9**, 831–850.

Søreide, J. E., Hop, H., Carroll, M. L., Falk-Petersen, S. and Hegseth, E. N. (2006) Seasonal food web structures and sympagic–pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. *Progr. Oceanogr.*, **71**, 59–87.

Søreide, J. E., Leu, E., Berge, J., Graeve, M. and Falk-Petersen, S. (2010) Timining in blooms, algal food quality and *Calanus glacialis* reproduction and growth in a changing Arctic. *Glob. Change Biol.*, **16**, 3154–3163.

Tencati, J. R. and Geiger, S. R. (1968) Pelagic amphipods of the slope waters of Northeast Greenland. *J. Fish. Res. Board Can.*, **25**, 1637–1650.

von Quillfeldt, C. H. (2000) Common diatom species in Arctic spring blooms: their distribution and abundance. *Bot. Mar.*, **43**, 499–516.

Werner, I. (1997) Grazing of Arctic under-ice amphipods on sea-ice algae. *Mar. Ecol. Prog. Ser.*, **160**, 93–99.

Werner, I. (2000) Faecal pellet production by Arctic under-ice amphipods – transfer of organic matter through the ice/water interface. *Hydrobiologia*, **426**, 89–96.

Werner, I. and Auel, H. (2005) Seasonal variability in abundance, respiration and lipid composition of Arctic under-ice amphipods. *Mar. Ecol. Prog. Ser.*, **292**, 251–262.

Yuichiro, Y. and Tsutomu, I. (2003) Metabolism and chemical composition of four pelagic amphipods in the Oyashio region, western subarctic Pacific Ocean. *Mar. Ecol. Prog. Ser.*, **253**, 233–241.