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# Importance of sympagic production to Bering Sea zooplankton as revealed from fatty acid-carbon stable isotope analyses

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ABSTRACT: We analyzed the fatty acid (FA) composition and carbon stable isotope ratios of individual FAs ($\delta^{13}C_{FA}$) of 3 zooplankton species (*Themisto libellula*, *Calanus marshallae/glacialis*, and *Thysanoessa raschii*) sampled from the Bering Sea during winter maximum ice extent, spring ice melt, and summer ice-free conditions in 2009 and 2010. Our goal was to assess diets of these ecologically important species and estimate the proportional contribution of pelagic and sympagic carbon sources to their diets. FA profiles showed little variation in diet within species between ice conditions or years but revealed differences in diet among species. FA biomarkers confirmed that *T. libellula* was predominately carnivorous and that *C. marshallae/glacialis* and *T. raschii* were primarily herbivorous. Estimates from 4 stable isotope mixing models using combinations of $\delta^{13}C_{FA}$ values of diatom FA markers (16:1n-7, 20:5n-3), and a flagellate FA marker (22:6n-3) showed that substantial, albeit highly variable, proportions of these FAs originated from organic matter originating from sea ice algae (*T. libellula* 36 to 72%, *C. marshallae/glacialis* 27 to 63%, and *T. raschii* 39 to 71%). Our results suggest that ice algae may be an important food source for zooplankton when water column phytoplankton are not available during critical periods in their life history. Predicted increases in water column phytoplankton production in the Bering Sea may help offset the expected reduction in ice algal production and any detrimental effects that this might have on consumers such as zooplankton.

KEY WORDS: Sea ice algae · Phytoplankton · Compound-specific stable isotope analysis · Fatty acid biomarkers · Food web ecology · Climate change



## INTRODUCTION

Climate warming in the Arctic and the associated loss of sea ice is predicted to alter the timing and quantity of primary production by pelagic phytoplankton and phytoplankton associated with sea ice (Bluhm & Gradinger 2008, Stabeno et al. 2010, Brown & Arrigo 2012). Such changes in the food base may affect grazing zooplankton populations and upper trophic level species that depend on them for food (e.g. Søreide et al. 2006, 2013, Bluhm & Gradinger 2008). Understanding zooplankton foraging behavior and estimating the proportional contribution to zooplankton of carbon from sea ice algae versus pelagic phytoplankton will help predict how potential changes in primary production may affect the pelagic food web in the Bering Sea.

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Zooplankton, particularly crustaceans such as hyperiid amphipods, calanoid copepods, and euphausiids, are important prey for fishes, seabirds, and marine mammals in many marine ecosystems and are thus key trophic links in the transfer of carbon and energy from primary producers to higher trophic levels. In the Bering Sea, prominent species include the hyperiid amphipod *Themisto libellula*, the copepods *Calanus glacialis* and *C. marshallae*, and the euphausiid *Thysanoessa raschii* (e.g. Frost & Lowry 1981, Springer & Roseneau 1985, Baier & Napp 2003, Pinchuk et al. 2013). The food sources of these species in the Bering Sea are not well known, but are thought to be similar to those in other high latitude seas (e.g. Pinchuk et al. 2013). *T. libellula* is considered to be predatory, with a diet in the North Atlantic that mainly consists of *Calanus* spp. copepodites (Marion et al. 2008, Noyon et al. 2009). *C. marshallae* and *C. glacialis* are predominately herbivorous, but also can be omnivorous (e.g. Smith 1990, Hobson et al. 2002, Baier & Napp 2003, Stevens et al. 2004a), and the degree of omnivory appears to depend upon the availability of phytoplankton (Tamelander et al. 2008). *T. raschii* is also primarily herbivorous but also can be carnivorous, and may switch to detrital feeding during the winter (Mauchline & Fischer 1969, Sargent & Falk-Petersen 1981, Smith 1991, Hagen & Auel 2001, Hop et al. 2006).

All of these species are known to be coupled to sea ice algae in seasonally ice-covered seas. For instance, fatty acid (FA) data from *T. libellula* indicate a strong linkage with sea ice algal production in the northern Fram Strait (Auel et al. 2002), and *C. glacialis* females graze on sea ice algae to support reproduction during the spring in the Canadian and European Arctic (e.g. Tourangeau & Runge 1991, Søreide et al. 2008). In the Bering Sea, *C. glacialis* feeds on ice algal diatoms (*Fragilariopsis cynlindrus, Fragilaria* sp., and *Pseudonitzschia* sp.), and the release of ice algae in the water column supports the early reproduction of *C. glacialis* (Durbin & Casas 2014). Additionally, *C. marshallae* copepodite abundance was greatest in years of most southerly sea ice extent in the Bering Sea (Baier & Napp 2003), and *T. raschii* can be very abundant under sea ice in the Bering Sea and feeds on ice algae (R. R. Gradinger et al. unpubl. data). In the European Arctic, the contribution of carbon derived from sea ice algae to some zooplankton species (e.g. *C. finmarchicus, C. glacialis, Thysanoessa inermis, T. longicaudata, Themisto abyssorum*), depending on the age class, season, and trophic level, was estimated to be as high as 100% (Søreide et al. 2006, 2008, 2013). In the Chukchi region off Barrow, Alaska, USA, the contribution of sea ice algal carbon to *T. raschii* was estimated to be between 20 and 74% (Budge et al. 2008).

FA biomarkers have been used to determine sources of primary production (diatoms versus dinoflagellates) to zooplankton in Arctic Seas (Scott et al. 1999, 2001, Falk-Petersen et al. 2000, 2009, Hop et al. 2006, Søreide et al. 2008, 2013) and also to indicate bacterial or protozoan lipids in Arctic copepod diets (Stevens et al. 2004b). Diatoms are high in the FA 16:1n-7, C16 polyunsaturated FAs (PUFA), and 20:5n-3, while dinoflagellates are high in C18 and C22 PUFA (e.g. Dalsgaard et al. 2003). C16 and C18 FAs are found in elevated levels in diatoms and dinoflagellates, respectively (e.g. Reuss & Poulsen 2002); however, the ratios of C16/C18 FAs and 16:1/16:0 FAs are high in diatoms compared to dinoflagellates (Claustre et al. 1988/1989, Viso & Marty 1993) and can be used to scale the relative importance of diatoms versus dinoflagellates to zooplankton (e.g. Søreide et al. 2008).

FA biomarkers have also been used to detect the presence of sea ice algae in the diets of zooplankton (Scott et al. 1999, 2001). However, because the same FAs can characterize diatoms from sea ice and the water column, using FA biomarkers alone to distinguish between these carbon sources in zooplankton is often not sufficient (i.e. Søreide et al. 2008). The carbon stable isotope values of specific FAs (expressed as $\delta^{13}C_{FA}$) in, e.g. 16:4n-1 and 20:5n-3, have been found to be relatively higher in particulate organic matter (POM) from sea ice (i-POM, assumed to consist primarily of ice algae) compared with pelagic POM (p-POM, assumed to consist primarily of pelagic phytoplankton) (Wang et al. 2014). The $\delta^{13}C_{FA}$ values of sea ice algae were also found to be higher than in pelagic phytoplankton (Budge et al. 2008). These isotopic differences have been used to estimate the proportional contribution of sympagic (ice associated) and pelagic primary production to consumers in the Arctic (Budge et al. 2008, Graham et al. 2014).

Our goals in this study were to (1) use FA biomarkers to describe the foraging strategies of the zooplankters *T. libellula, C. marshallae, C. glacialis*, and *T. raschii* in the Bering Sea, and (2) compare $\delta^{13}C_{FA}$ values from these species with $\delta^{13}C_{FA}$ values of FAs from i-POM and p-POM in the Bering Sea (Wang et al. 2014) to estimate the proportional contribution of i-POM and p-POM to them. We hypothesized that previously documented differences in the diet between *T. libellula*, *C. marshallae/glacialis*, and *T. raschii* would be supported by different FA profiles

and biomarker compositions. We also hypothesized that the proportional contribution of sea ice-derived FAs would be highest in the primarily herbivorous *C. marshallae/glacialis* and *T. raschii* during maximum ice extent and decrease with the onset of phytoplankton blooms in the water column as the ice melted. Similarly, the contribution of sea ice-derived FAs to *T. libellula* would stem from their primary prey, *Calanus* copepods, and the estimates would vary in accordance with copepod availability.

## MATERIALS AND METHODS

### Sample collection

Zooplankton were collected from the Bering Sea shelf as part of the Bering Sea Ecosystem Study/Bering Sea Integrated Ecosystem Research Program during 3 major seasonal ice regimes (winter maximum ice extent, spring ice melt, and summer ice-free conditions) in 2009 and 2010 in the northern and central portions of the Bering Sea (Fig. 1). In 2009, maximum ice extent occurred by 28 February and remained close to the maximum level through most of March (NSIDC 2009). In 2010, maximum ice extent occurred on 31 March (Richter-Menge & Overland 2010). Details for sampling stations are provided in the Appendix. We examined 3 species: *Themisto libellula*, *Calanus marshallae/glacialis*, and *Thysanoessa raschii*. *C. marshallae and C. glacialis* co-occur in the Bering Sea (Nelson et al. 2009) and could not be distinguished from one another on board; therefore, we refer to them as *C. marshallae/glacialis*. Size and age class information were not recorded for these samples. Zooplankton were collected using a ring net (mesh size 333 µm) hauled vertically from 10 m above the bottom, or a maximum of 150 m depth, to the surface. During ice-free conditions (cruises KNORR195 and TN250), zooplankton were collected from a 1 $m^2$ Multiple Opening Closing Net and Environmental Sensing System (MOCNESS) fitted with

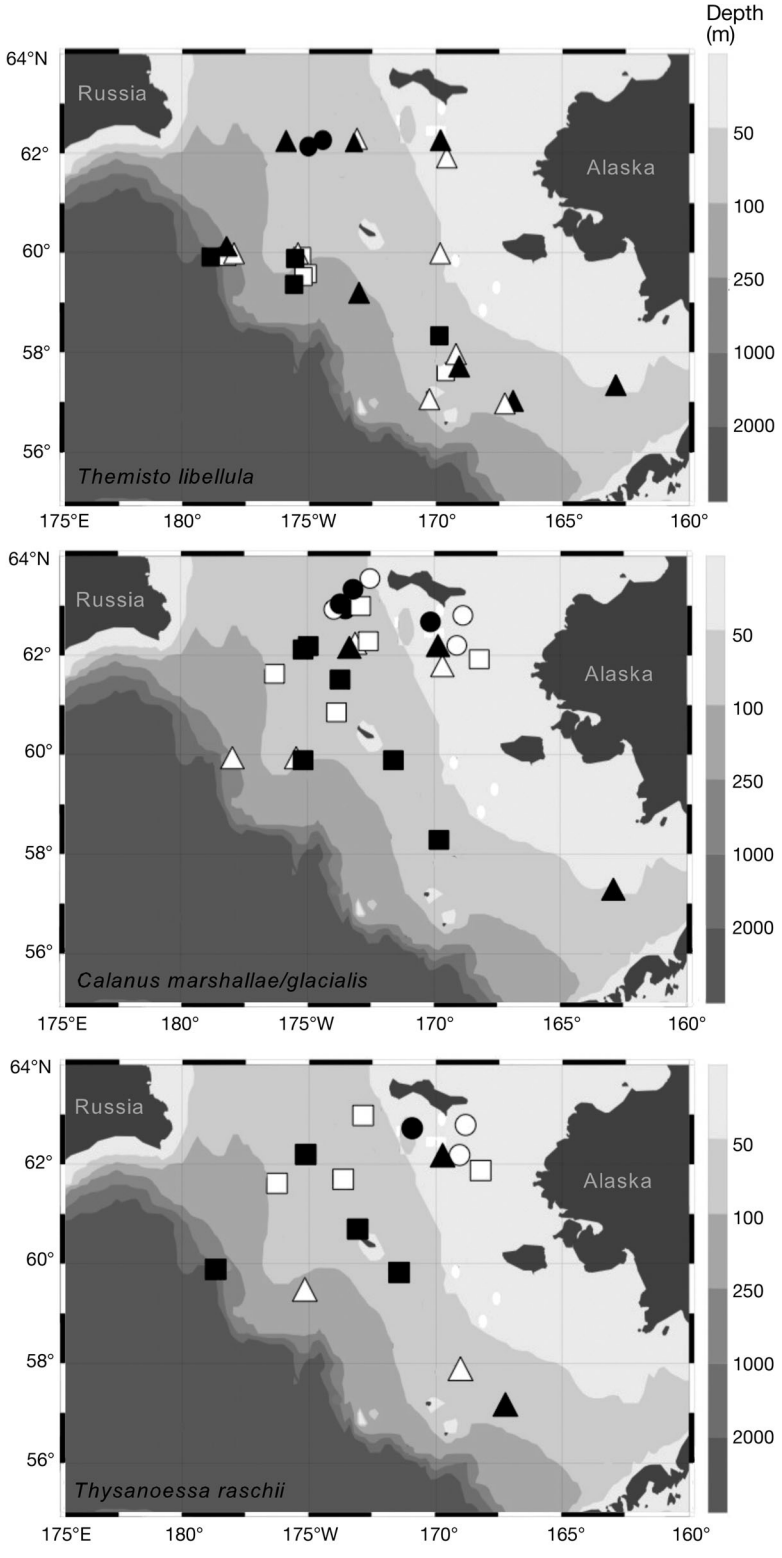


Fig. 1. Sampling stations for *Themisto libellula*, *Calanus marshallae/glacialis*, and *Thysanoessa raschii* during maximum ice extent (circles), ice melt (squares), and ice-free conditions (triangles) in 2009 (open symbols) and 2010 (shaded symbols). *T. libellula* samples were not collected during maximum ice extent in 2009. Details for sampling stations and sample sizes are given in the Appendix

500 µm mesh and fished obliquely to a maximum of 150 m. Samples were stored in plastic microcentrifuge vials at −20°C until arrival at University of Alaska Fairbanks. Samples from 2009 were stored at −20°C until laboratory analysis approximately 9 mo later. Samples from 2010 were stored at −80°C until laboratory analysis approximately 6 mo later.

POM samples in 2009 were collected on the Bering Sea shelf during ice melt conditions (Table 1). i-POM samples were obtained at varying depths from ice cores ranging from the bottom 1 cm section of the core to the bottom 4 cm of the core. Ice core samples were completely melted in the dark and filtered on pre-combusted GF/F filters. p-POM samples were collected from a single Niskin bottle closed at depths of 10 or 15 m below the surface. Samples were filtered using a GF/F filter (pore size 0.2 µm; Nayar & Chou 2003) that had been pre-combusted to remove any organic traces that would interfere with the isotope signature of the samples. Filtered samples were then stored in chloroform at −20°C until laboratory analysis. Details for the collection of POM samples from 2010 were previously described by Wang et al. (2014).

Table 1. Sample information for sea ice and pelagic particulate organic matter (i-POM and p-POM) collected from the Bering Sea shelf in 2009. Samples were taken on the cruise HLY0902 during ice melt conditions. Details for i-POM and p-POM collected in 2010 are described in Wang et al. (2014)

| POM type | Date (m/dd/yyyy) | Station no. | Station name | Latitude (°N) | Longitude (°W) | No. of samples |
|---|---|---|---|---|---|---|
| i-POM (N = 6) | 4/14/2009 | 29 | St. 29 | 61.792 | 176.802 | 2 |
| | 4/16/2009 | 35 | SL 9 | 63.094 | 173.291 | 1 |
| | 5/01/2009 | 45 | St. 92 | 61.589 | 173.709 | 2 |
| | 5/02/2009 | 93 | BN1 | 62.333 | 172.703 | 1 |
| p-POM (N = 6) | 4/27/2009 | 73 | BL4 | 60.537 | 176.205 | 2 |
| | 4/29/2009 | 85 | BL15 | 59.550 | 175.096 | 1 |
| | 4/30/2009 | 90 | BL20 | 59.555 | 175.150 | 1 |
| | 5/06/2009 | 115 | BL21 | 59.444 | 174.082 | 1 |
| | 5/06/2009 | 116 | BL15/2 | 59.552 | 175.150 | 1 |

### Fatty acid analysis

Total lipids were extracted from all samples using 2:1 chloroform/methanol (Folch et al. 1957, Parrish 1999). While some samples of *T. libellula* and *T. raschii* consisted of single individuals, most individual plankters were pooled by species and station so that the combined samples contained on average 3.3 ± 3.4 (*T. libellula*), 17.6 ± 10.4 (*C. marshallae/glacialis*), and 4.1 ± 2.6 (*T. raschii*) ind. per sample (mean ± 1 SD; see Appendix for no. ind. per sample). Fatty acid methyl esters (FAME) were prepared using sulfuric acidic transesterification (Budge et al. 2006). Because fatty alcohols resulting from the transesterification of wax esters in zooplankton may co-elute with FAME when analyzed by gas chromatography (GC), they were identified and removed from the samples using thin layer chromatography. FAME were quantified using temperature-programmed GC on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm internal diameter column coated with 50% cyanopropylmethylpolysiloxane (DB-23) and linked to a computerized integration system (Varian Star software). Shorthand nomenclature of A:Bn-X was used to describe each FAME, where A represents the number of carbon atoms, B the number of double bonds, n represents the terminal methyl group, and X the position of the double bond closest to the terminal methyl group. Approximately 70 FAME were identified by comparison of retention times with known standards (Nu Check Prep, Elysian), or using GC-mass spectrometry. All FA data are available upon request.

### Carbon stable isotope analysis of individual fatty acids

Carbon stable isotope ratios of FAME samples (expressed as $\delta^{13}C$ values in per mille, ‰) were analyzed by routing the effluent from a GC (Trace GC Ultra) through a combustion interface (Finnigan GC combustion III) to an isotope ratio mass spectrometer (IRMS) (Thermo Finnigan Delta V) at the Alaska Stable Isotope Facility (ASIF), University of Alaska Fairbanks (UAF). The same GC column and method described above for FID analyses of FAME were used to separate the FAME for analysis using GC-IRMS (Budge et al. 2008, 2011, Wang et al. 2014). The correction for the addition of carbon during methylation was performed according to Wang et al. (2014). The average $\delta^{13}C$ value for the methyl-derived carbon was −48.8 ± 1.3‰ (mean ± 1 SD). The $\delta^{13}C$ values from the individual FAMEs were calibrated using a standard mixture consisting of ethyl and methyl esters of 14:0, 16:0, 18:0, and 20:0 (supplied by Indiana University Stable Isotope Reference Materials), where the coefficient of determination ($r^2$) of the

measured versus expected relationship was >0.99. 16:0 and 18:0 FAME laboratory standards were analyzed after every 10 samples to track analytical error of the GC-IRMS system, which was ≤0.3‰ (representing the 1 SD of 23 analyses of the 16:0 and 18:0 standards interspersed during the samples runs). All $\delta^{13}C$ values are reported relative to Vienna Pee Dee Belemnite (VPDB) using standard notation, where $\delta^{13}C$ (‰) = $[(R_{sample} / R_{standard}) - 1] \times 1000$, and $R$ is the corresponding ratio of $^{13}C/^{12}C$.

### Data analysis

Bray-Curtis similarity matrices and permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) were used to investigate the variation in FA compositions of zooplankton, based on the 63 FAs present in proportions >0.1 %, among species, and between years and ice conditions within species. A principal component analysis (PCA) was performed to visualize differences between FA compositions among and within species. Specific FA markers reviewed in Dalsgaard et al. (2003) were displayed on the same plot to show similarities and dissimilarities in FA composition in zooplankton samples analyzed. FA data were standardized to 100 % and log(1 + $x$) transformed prior to analysis to downweigh the FAs present in higher proportions and increase the weighting of FAs present in lower proportions.

We examined the proportions of FA biomarkers to assess the relative presence of different algal taxonomic groups, relative levels of carnivory, and the presence of bacteria in the 3 zooplankton species examined (e.g. Søreide et al. 2008, 2013). The relative proportions of diatoms to flagellates in zooplankton diet were determined using the ratios of 16:1/16:0 and ΣC16/ΣC18 FAs (Claustre et al. 1988/1989, Viso & Marty 1993). The ratio of 22:6n-3/20:5n-3 has been used in the past as a dinoflagellate indicator because 22:6n-3 can be produced in large amounts by dinoflagellates (Budge & Parrish 1998, Dalsgaard et al. 2003). However, 22:6n-3 can also be biosynthesized by heterotrophic flagellates (e.g. Desvilettes & Bec 2009, Bec et al. 2010). Therefore, we used the ratio of FAs 20:5n-3/22:6n-3 to determine the proportion of diatoms to flagellates in zooplankton diets. Relative levels of carnivory were determined from the ratios of 18:1n-9/18:1n-7 (Falk-Petersen et al. 1990, 2009, Graeve et al. 1997, Auel et al. 2002) and PUFA/saturated FAs (SFA) (Cripps & Atkinson 2000). The sum of C20 and C22 monounsaturated FAs (MUFA) was used to determine the presence of *Calanus* copepods in the diet of zooplankton (Falk-Petersen et al. 1987, 2002, Søreide et al. 2013). The sum of 15:0 and 17:0, and the iso- and anteiso FAs (i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, and ai-17:0) were used to determine the proportion of bacterial FAs in the samples (Budge & Parrish 1998). A Kruskal-Wallis ANOVA was used followed by Bonferroni adjustment for multiple comparisons to test for differences in FA biomarkers among zooplankton species. ANOVA was performed using Statistica v.12 (StatSoft).

Not all FAs were present in sufficient quantities to determine their respective $\delta^{13}C_{FA}$ values. $\delta^{13}C_{FA}$ values were determined for 16:0, 16:1n-7, 18:1n-11/9, 20:1n-11/9, 20:5n-3, and 22:6n-3 in all samples. The $\delta^{13}C$ values for these 6 FAs were transformed into Euclidean distances, and a PERMANOVA was used to investigate the variation in the $\delta^{13}C_{FA}$ values among species and inter-annual and seasonal variation within species. PERMANOVA and PCA were performed in PRIMER v.6 (Primer-E).

We used Bayesian multi-source stable isotope mixing models (SIAR, Parnell et al. 2010) to estimate the proportional contribution of i-POM relative to p-POM in zooplankton. The SIAR model incorporates the isotope values of consumers and representative sources of diet (end member sources) as well as trophic enrichment factors and concentration dependencies to produce estimates of the proportion of given sources in consumers' diets (Parnell et al. 2010). The model employs Bayesian statistics, which allows for incorporation of uncertainty and variation of isotope data to give a 95 % credibility interval that includes the probability distribution of the estimates (Parnell et al. 2010). Although it is ideal to add trophic enrichment factors into the model to account for tissue specific isotopic turnover rates of consumers, no data exist for isotopic turnover of $\delta^{13}C_{FA}$ in zooplankton. Thus, trophic enrichment factors were assumed to be zero (Budge et al. 2008, 2011), and the consequences are explored in the 'Discussion'. Models were run with and without concentration dependencies for comparison. The $\delta^{13}C_{FA}$ values for i-POM and p-POM were generated from many of the same sampling locations from which zooplankton were taken in 2009 (Fig. 1) and 2010 (Wang et al. 2014) and were used as the end member sources in our mixing models. Specifically, for zooplankton in 2009, average i-POM and p-POM $\delta^{13}C_{FA}$ values from samples collected during ice melt in 2009 were used as sources in the mixing models for zooplankton collected in all ice conditions in 2009. For zooplankton collected in 2010, the average i-POM $\delta^{13}C_{FA}$ value from samples collected during maximum ice extent in 2010 was used

as the i-POM source, and p-POM was collected from all 3 ice conditions in 2010, and their $\delta^{13}C_{FA}$ values were used to model their respective zooplankton samples (i.e. p-POM from ice-free conditions used as the p-POM source for zooplankton collected during ice-free conditions in 2010). The $\delta^{13}C_{FA}$ values for i-POM and p-POM are given in Table 2. A non-parametric Mann-Whitney *U*-test was performed using Statistica v.12 to assess differences in $\delta^{13}C_{FA}$ values of FAs 16:1n-7, 20:5n-3, and 22:6n-3 between i-POM and p-POM in 2009. We used the diatom marker FAs 16:1n-7 and 20:5n-3 in the model because the algal composition in i-POM is typically dominated by diatoms (Horner 1985, Gradinger 2002, Arrigo et al. 2010). The presence of diatoms was also found in p-POM in 2010 (Wang et al. 2014). We used the flagellate marker 22:6n-3 in the model because flagellates also may dominate the algal biomass in ice cores, particularly during ice melt (Tamelander et al. 2009). Additionally, the water column can also contain non-diatom phytoplankton such as dinoflagellates and flagellates (Moran et al. 2012). To test the use of these different FA markers as indicators of i-POM and p-POM, we ran 4 models using combinations of the FA markers: (1) 16:1n-7, 20:5n-3, and 22:6n-3, (2) 16:1n-7 and 20:5n-3, (3) 20:5n-3 and 22:6n-3, and (4) 20:5n-3. Results are presented as means and 95% credibility intervals (Bayesian confidence interval).

## RESULTS

### Fatty acid profiles

FA profiles differed among zooplankton species (3-factor PERMANOVA with pairwise comparison, $p = 0.001$). Overall, the proportions of 20:1n-11, 20:1n-9, 22:1n-11, and 22:6n-3 were higher in *Themisto libellula* compared with *Thysanoessa raschii* (Kruskal-Wallis ANOVA, $p < 0.02$, Table 3). Additionally, the proportions of 18:1n-7 were higher in *T. raschii* than in *Calanus marshallae/glacialis* (Kruskal-Wallis ANOVA, $p < 0.002$, Table 3), while the levels of 16:1n-7 and the longer chain MUFA 20:1n-9, 22:1n-11, and 24:1 were higher in *C. marshallae/glacialis* compared with levels found in *T. raschii* (Kruskal-Wallis ANOVA, $p < 0.01$, Table 3). *C. marshallae/glacialis* contained the highest amount of total MUFA (38 to 52%, Table 3; Kruskal-Wallis ANOVA, $p < 0.02$). The *T. raschii* samples from maximum ice extent and ice melt in 2009 contained the highest amount of PUFA (52%, Table 3). PCA showed grouping by species (Fig. 2). PC1 and PC2 explained 33.2 and 20.5% of the total variation, respectively with loading plots supporting the results from individual Kruskal-Wallis ANOVAs. PC1 was influenced by the *Calanus* copepod markers C20 and C22 MUFA while PC2

Table 2. Carbon stable isotope values ($\delta^{13}C_{FA}$) for fatty acids 16:1n-7, 20:5n-3, and 22:6n-3 from sea ice and pelagic particulate organic matter (i-POM and p-POM), *Themisto libellula*, *Calanus marshallae/glacialis* (*Calanus* spp.), and *Thysanoessa raschii* collected in the Bering Sea in 2009 and 2010. i-POM and p-POM data are from Wang et al. (2014). N = sample sizes: some are given as ranges, as not all $\delta^{13}C_{FA}$ values could be determined from all samples (Wang et al. 2014). na: samples not collected. Values are given as means ±1 SD

| | 2009 | | | | 2010 | | | |
|---|---|---|---|---|---|---|---|---|
| | N | 16:1n-7 | 20:5n-3 | 22:6n-3 | N | 16:1n-7 | 20:5n-3 | 22:6n-3 |
| **Maximum ice** | | | | | | | | |
| i-POM | 0 | na | na | na | 12 | −25.2 ± 4.5 | −26.5 ± 2.8 | −23.8 ± 3.2 |
| *T. libellula* | 0 | na | na | na | 2 | −26.0 ± 0.5 | −27.3 ± 0.7 | −25.6 ± 0.0 |
| *Calanus* spp. | 4 | −27.6 ± 0.3 | −27.0 ± 0.6 | −26.2 ± 0.3 | 4 | −25.9 ± 1.1 | −27.0 ± 0.4 | −25.5 ± 0.4 |
| *T. raschii* | 2 | −26.2 ± 0.1 | −27.7 ± 0.3 | −25.8 ± 0.2 | 1 | −28.4 | −30.5 | −26.0 |
| p-POM | 0 | na | na | na | 11–12 | −28.4 ± 1.2 | −29.7 ± 1.6 | −27.0 ± 2.1 |
| **Ice melt** | | | | | | | | |
| i-POM | 4–6 | −21.0 ± 6.8 | −26.5 ± 3.0 | −26.2 ± 2.9 | 0 | na | na | na |
| *T. libellula* | 5 | −26.0 ± 1.2 | −27.8 ± 0.9 | −27.0 ± 0.9 | 4 | −26.1 ± 0.7 | −27.1 ± 0.7 | −26.2 ± 0.8 |
| *Calanus* spp. | 6 | −26.8 ± 1.4 | −27.1 ± 0.7 | −26.6 ± 0.9 | 6 | −28.2 ± 0.8 | −28.4 ± 1.4 | −27.3 ± 1.4 |
| *T. raschii* | 4 | −24.7 ± 3.0 | −26.6 ± 1.1 | −25.6 ± 1.0 | 4 | −26.7 ± 1.3 | −26.9 ± 0.8 | −26.3 ± 1.5 |
| p-POM | 6 | −28.8 ± 1.5 | −29.7 ± 1.3 | −30.4 ± 0.8 | 20 | −29.7 ± 1.7 | −29.3 ± 1.6 | −27.3 ± 2.5 |
| **Ice-free** | | | | | | | | |
| i-POM | 0 | na | na | na | 0 | na | na | na |
| *T. libellula* | 8 | −26.2 ± 1.5 | −27.2 ± 1.7 | −26.2 ± 1.9 | 8 | −26.0 ± 1.3 | −27.4 ± 2.0 | −26.3 ± 2.0 |
| *Calanus* spp. | 2 | −28.9 ± 0.7 | −28.6 ± 0.7 | −26.9 ± 0.6 | 2 | −28.1 ± 0.4 | −27.1 ± 1.4 | −27.2 ± 0.1 |
| *T. raschii* | 2 | −26.8 ± 1.8 | −29.6 ± 3.7 | −26.8 ± 0.5 | 2 | −25.6 ± 0.5 | −26.0 ± 0.5 | −25.6 ± 0.6 |
| p-POM | 0 | na | na | na | 14 | −29.5 ± 1.6 | −30.2 ± 1.9 | −28.3 ± 2.3 |

Table 3. Fatty acid (FA) proportions and markers for (a) *Themisto libellula*, (b) *Calanus marshallae/glacialis* (*Calanus* spp.), and (c) *Thysanoessa raschii* collected in 2009 and 2010 from the Bering Sea during maximum ice, ice melt, and ice-free periods. The bacteria marker is the sum of 15:0, 17:0, and the iso and anteiso FA (i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, and ai-17:0). The ratios of 16:1/16:0 and ΣC16/ΣC18 FA represent diatom to dinoflagellate ratios, and 20:5n-3/22:6n-3 represent diatom to flagellate ratios. The diatom marker is the sum of 16:1n-7, C16 polyunsaturated FA (PUFA), and 20:5n-3. The *Calanus* marker is the sum of C20 and C22 monounsaturated FA (MUFA). The ratios of 18:1n-9/18:1n-7 and of PUFA/saturated FA (SFA) represent the degree of carnivory (the greater the ratios, the greater the degree of carnivory). SFA, MUFA, PUFA, and means for each biomarker across species are also reported. *T. libellula* samples were not collected during maximum ice extent in 2009. Values are given as means ±1 SD (Table 3b,c on following pages)

| | Overall average | 2009 | | | 2010 | | |
|---|---|---|---|---|---|---|---|
| | | Max. ice | Ice melt | Ice-free | Max. ice | Ice melt | Ice-free |
| **(a) *T. libellula*** | | | | | | | |
| N | 27 | 0 | 5 | 8 | 2 | 4 | 8 |
| 14:0 | 3.7 ± 1.8 | | 3.5 ± 1.6 | 4.3 ± 1.8 | 2.7 ± 2.3 | 5.3 ± 2.2 | 2.7 ± 1.4 |
| 16:0 | 15.5 ± 2.2 | | 16.4 ± 1.7 | 14.5 ± 1.6 | 14.1 ± 1.1 | 17.8 ± 3.3 | 15.2 ± 1.8 |
| 16:1n-7 | 7.5 ± 4.8 | | 3.2 ± 1.2 | 8.9 ± 4.6 | 4.6 ± 2.2 | 10.0 ± 2.5 | 8.2 ± 6.1 |
| 16:1n-5 | 0.4 ± 0.1 | | 0.3 ± 0.2 | 0.4 ± 0.1 | 0.4 ± 0.2 | 0.4 ± 0.1 | 0.4 ± 0.1 |
| 16:4n-1 | 0.3 ± 0.6 | | 0.1 ± 0.1 | 0.7 ± 0.9 | 0.1 ± 0.1 | 0.4 ± 0.4 | 0.1 ± 0.2 |
| 18:0 | 1.8 ± 1.1 | | 2.6 ± 1.8 | 1.4 ± 0.4 | 1.0 ± 0.3 | 1.2 ± 0.4 | 2.3 ± 1.1 |
| 18:1n-9 | 10.9 ± 3.7 | | 11.7 ± 3.6 | 8.4 ± 2.0 | 15.1 ± 4.9 | 12.0 ± 3.4 | 11.1 ± 4.2 |
| 18:1n-7 | 3.9 ± 1.4 | | 3.7 ± 1.4 | 3.7 ± 1.3 | 3.3 ± 0.4 | 5.4 ± 1.8 | 3.6 ± 1.3 |
| 18:1n-5 | 1.2 ± 0.4 | | 1.4 ± 0.6 | 1.0 ± 0.4 | 1.0 ± 0.5 | 1.0 ± 0.6 | 1.3 ± 0.3 |
| 18:2n-6 | 1.6 ± 1.3 | | 0.9 ± 0.2 | 1.0 ± 0.2 | 1.4 ± 0.0 | 0.9 ± 0.3 | 3.0 ± 1.6 |
| 18:3n-3 | 0.3 ± 0.1 | | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.2 | 0.1 ± 0.0 | 0.4 ± 0.2 |
| 18:4n-3 | 1.2 ± 0.9 | | 0.6 ± 0.2 | 1.8 ± 1.1 | 1.0 ± 0.8 | 1.1 ± 0.7 | 1.1 ± 0.8 |
| 20:1n-11 | 3.6 ± 4.1 | | 4.3 ± 3.8 | 3.5 ± 4.3 | 2.3 ± 2.7 | 3.8 ± 4.9 | 3.4 ± 4.8 |
| 20:1n-9 | 3.4 ± 2.3 | | 3.7 ± 1.9 | 3.8 ± 2.6 | 3.6 ± 2.5 | 2.2 ± 1.4 | 3.3 ± 2.8 |
| 20:1n-7 | 0.8 ± 0.3 | | 0.7 ± 0.2 | 0.7 ± 0.2 | 1.0 ± 0.5 | 0.7 ± 0.2 | 0.9 ± 0.4 |
| 20:4n-6 | 0.5 ± 0.3 | | 0.9 ± 0.1 | 0.3 ± 0.0 | 0.8 ± 0.4 | 0.4 ± 0.4 | 0.3 ± 0.1 |
| 20:5n-3 | 14.3 ± 3.6 | | 13.2 ± 2.4 | 16.4 ± 3.8 | 13.6 ± 5.5 | 15.8 ± 4.2 | 12.3 ± 2.6 |
| 22:1n-11 | 2.2 ± 1.8 | | 2.7 ± 1.6 | 2.6 ± 2.1 | 2.1 ± 2.5 | 1.8 ± 1.6 | 1.8 ± 1.8 |
| 22:1n-9 | 0.8 ± 0.3 | | 1.3 ± 0.3 | 0.7 ± 0.2 | 0.6 ± 0.7 | 0.5 ± 0.2 | 0.7 ± 0.2 |
| 22:5n-3 | 0.6 ± 0.1 | | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.2 | 0.5 ± 0.1 |
| 22:6n-3 | 16.9 ± 7.3 | | 19.7 ± 2.8 | 16.8 ± 9.1 | 22.5 ± 14.0 | 11.7 ± 6.5 | 16.6 ± 6.0 |
| 24:1 | 0.6 ± 0.4 | | 1.1 ± 0.5 | 0.6 ± 0.2 | 0.9 ± 0.4 | 0.4 ± 0.4 | 0.4 ± 0.1 |
| SFA | 23.1 ± 4.0 | | 24.0 ± 5.6 | 21.4 ± 1.4 | 19.3 ± 2.1 | 25.0 ± 1.5 | 24.4 ± 5.2 |
| MUFA | 37.3 ± 8.7 | | 36.3 ± 3.3 | 36.4 ± 9.8 | 36.7 ± 16.6 | 40.3 ± 4.7 | 37.4 ± 11.1 |
| PUFA | 38.3 ± 7.9 | | 38.5 ± 4.7 | 41.0 ± 10.1 | 42.5 ± 18.4 | 34.2 ± 4.0 | 36.6 ± 6.2 |
| Bacteria | 1.1 ± 0.1 | | 1.1 ± 0.2 | 1.0 ± 0.2 | 1.1 ± 0.3 | 0.7 ± 0.3 | 1.3 ± 0.5 |
| 16:1/16:0 | 0.6 ± 0.4 | | 0.2 ± 0.7 | 0.7 ± 0.4 | 0.4 ± 0.1 | 0.6 ± 0.3 | 0.6 ± 0.5 |
| ΣC16/ΣC18 | 1.2 ± 0.4 | | 1.0 ± 0.1 | 1.4 ± 0.3 | 0.9 ± 0.1 | 1.3 ± 0.3 | 1.2 ± 0.6 |
| 20:5n-3/22:6n-3 | 1.0 ± 0.7 | | 0.7 ± 0.1 | 1.2 ± 0.7 | 0.7 ± 0.2 | 1.8 ± 1.1 | 0.8 ± 0.4 |
| Diatom | 23.0 ± 7.3 | | 17.1 ± 3.2 | 27.1 ± 6.5 | 18.9 ± 3.0 | 27.4 ± 6.0 | 21.3 ± 8.4 |
| *Calanus* | 11.0 ± 6.8 | | 13.0 ± 5.6 | 11.6 ± 7.8 | 9.8 ± 8.9 | 9.1 ± 7.1 | 10.4 ± 7.5 |
| Carnivory | 3.1 ± 1.5 | | 3.3 ± 0.7 | 2.4 ± 0.9 | 4.5 ± 0.9 | 2.2 ± 0.3 | 3.6 ± 2.4 |
| PUFA/SFA | 1.7 ± 0.5 | | 1.7 ± 0.5 | 1.9 ± 0.6 | 2.3 ± 1.2 | 1.4 ± 0.2 | 1.5 ± 0.2 |

was influenced by the diatom markers 16:1n-7, 16:4n-1, and 20:5n-3 (Fig. 2).

We found little inter-annual variation in FA profiles within each zooplankton species (Fig. 2). FA profiles of *T. libellula* differed between years only during ice melt (2-factor PERMANOVA with pairwise comparisons, p = 0.01). Similarly, FA profiles of *C. marshallae/glacialis* also differed between years during ice melt (2-factor PERMANOVA with pairwise comparisons, p = 0.002). FA profiles of *T. raschii* from all ice conditions were not different between years (p = 0.34). In contrast, some variation in FA profiles among ice conditions was evident within all zooplankton species (Fig. 2). In 2009, the FA profiles of *T. libellula* differed between ice melt and ice-free conditions (PERMANOVA pairwise comparisons, p = 0.04). Similarly, the FA profiles of *C. marshallae/glacialis* in 2009 were different between ice melt and ice-free conditions in 2009 (PERMANOVA pairwise comparisons, p = 0.04). In 2010, *C. marshallae/*

Table 3b

| | Overall average | 2009 | | | 2010 | | |
|---|---|---|---|---|---|---|---|
| | | Max. ice | Ice melt | Ice-free | Max. ice | Ice melt | Ice-free |
| **(b) *Calanus* spp.** | | | | | | | |
| N | 24 | 4 | 6 | 2 | 4 | 6 | 2 |
| 14:0 | 6.0 ± 2.1 | 8.4 ± 2.7 | 5.3 ± 2.2 | 6.9 ± 1.0 | 5.3 ± 2.2 | 5.4 ± 1.2 | 5.2 ± 1.7 |
| 16:0 | 13.9 ± 3.9 | 12.6 ± 3.9 | 15.8 ± 4.2 | 9.6 ± 1.0 | 17.4 ± 2.7 | 11.7 ± 2.6 | 13.9 ± 3.1 |
| 16:1n-7 | 16.6 ± 8.9 | 14.3 ± 4.3 | 9.2 ± 5.6 | 21.6 ± 0.0 | 9.4 ± 3.9 | 25.0 ± 6.9 | 28.1 ± 3.5 |
| 16:1n-5 | 0.9 ± 0.9 | 0.8 ± 0.2 | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.9 ± 0.5 | 3.4 ± 1.5 |
| 16:4n-1 | 0.4 ± 0.7 | 0.0 ± 0.0 | 0.1 ± 0.1 | 2.1 ± 1.5 | 0.0 ± 0.0 | 0.6 ± 0.6 | 0.3 ± 0.1 |
| 18:0 | 2.8 ± 2.5 | 3.7 ± 2.4 | 5.0 ± 3.4 | 0.9 ± 0.1 | 2.6 ± 1.2 | 0.7 ± 0.2 | 2.9 ± 1.2 |
| 18:1n-9 | 4.8 ± 1.8 | 6.8 ± 0.8 | 4.2 ± 1.0 | 3.6 ± 0.2 | 6.6 ± 1.0 | 3.1 ± 0.6 | 5.8 ± 2.4 |
| 18:1n-7 | 2.3 ± 0.7 | 2.1 ± 0.4 | 2.6 ± 0.6 | 1.4 ± 0.1 | 3.0 ± 0.2 | 2.3 ± 0.9 | 1.3 ± 0.1 |
| 18:1n-5 | 1.7 ± 0.6 | 1.8 ± 0.5 | 1.9 ± 0.6 | 0.7 ± 0.1 | 2.3 ± 0.3 | 1.3 ± 0.5 | 2.0 ± 0.1 |
| 18:2n-6 | 0.8 ± 0.4 | 1.0 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.1 | 1.1 ± 0.1 | 0.5 ± 0.1 | 1.4 ± 0.8 |
| 18:3n-3 | 0.2 ± 0.2 | 0.5 ± 0.3 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.0 |
| 18:4n-3 | 0.8 ± 0.8 | 0.3 ± 0.3 | 0.2 ± 0.1 | 2.7 ± 0.5 | 0.5 ± 0.4 | 1.3 ± 0.7 | 1.2 ± 0.1 |
| 20:1n-11 | 0.5 ± 0.3 | 0.8 ± 0.2 | 0.6 ± 0.3 | 0.5 ± 0.1 | 0.7 ± 0.3 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| 20:1n-9 | 6.6 ± 3.1 | 8.4 ± 3.2 | 6.4 ± 3.5 | 10.6 ± 0.9 | 6.1 ± 3.6 | 5.6 ± 1.6 | 3.7 ± 1.1 |
| 20:1n-7 | 1.2 ± 0.7 | 2.1 ± 1.2 | 1.0 ± 0.5 | 1.2 ± 0.2 | 1.3 ± 0.5 | 0.9 ± 0.3 | 0.6 ± 0.2 |
| 20:4n-6 | 0.2 ± 0.1 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.2 |
| 20:5n-3 | 11.2 ± 4.6 | 5.8 ± 2.4 | 12.1 ± 3.1 | 15.3 ± 0.8 | 10.7 ± 3.9 | 14.5 ± 1.2 | 6.8 ± 4.3 |
| 22:1n-11 | 3.2 ± 1.6 | 4.4 ± 1.9 | 3.3 ± 1.7 | 3.4 ± 0.4 | 2.8 ± 1.7 | 2.7 ± 1.5 | 1.9 ± 0.8 |
| 22:1n-9 | 1.4 ± 0.7 | 2.0 ± 0.6 | 2.1 ± 0.7 | 1.1 ± 0.2 | 1.0 ± 0.7 | 1.0 ± 0.1 | 0.9 ± 0.2 |
| 22:5n-3 | 0.5 ± 0.3 | 0.1 ± 0.1 | 0.6 ± 0.3 | 0.5 ± 0.0 | 0.3 ± 0.2 | 0.6 ± 0.1 | 0.4 ± 0.1 |
| 22:6n-3 | 12.5 ± 5.3 | 9.3 ± 3.8 | 15.7 ± 6.4 | 6.1 ± 1.3 | 16.0 ± 1.3 | 12.5 ± 4.7 | 9.2 ± 6.1 |
| 24:1 | 1.9 ± 1.0 | 2.6 ± 0.8 | 2.8 ± 1.0 | 1.1 ± 0.1 | 1.7 ± 0.6 | 1.0 ± 0.3 | 1.1 ± 0.0 |
| SFA | 24.4 ± 6.0 | 27.4 ± 5.0 | 28.1 ± 6.7 | 18.2 ± 2.0 | 27.8 ± 2.8 | 18.7 ± 1.8 | 24.1 ± 7.2 |
| MUFA | 44.1 ± 8.8 | 50.2 ± 7.8 | 37.5 ± 10.2 | 48.4 ± 1.2 | 38.6 ± 7.0 | 46.2 ± 6.5 | 51.6 ± 3.2 |
| PUFA | 29.8 ± 8.5 | 19.7 ± 6.2 | 32.5 ± 8.6 | 32.5 ± 0.7 | 31.4 ± 9.1 | 34.5 ± 4.6 | 22.6 ± 10.7 |
| Bacteria | 1.6 ± 0.7 | 2.5 ± 0.3 | 1.7 ± 0.5 | 1.0 ± 0.0 | 2.1 ± 0.4 | 0.9 ± 0.2 | 1.2 ± 0.5 |
| 16:1/16:0 | 1.5 ± 1.1 | 1.4 ± 0.6 | 0.7 ± 0.5 | 2.4 ± 0.2 | 0.6 ± 0.3 | 2.5 ± 1.3 | 2.4 ± 0.7 |
| ΣC16/ΣC18 | 2.6 ± 1.2 | 1.7 ± 0.2 | 1.8 ± 0.5 | 3.3 ± 0.1 | 1.7 ± 0.3 | 4.1 ± 1.2 | 3.2 ± 0.7 |
| 20:5n-3/22:6n-3 | 1.1 ± 0.7 | 0.6 ± 0.1 | 0.8 ± 0.3 | 2.6 ± 0.4 | 0.7 ± 0.4 | 1.4 ± 0.9 | 0.8 ± 0.0 |
| Diatom | 29.8 ± 11.2 | 21.3 ± 3.3 | 22.6 ± 5.5 | 41.8 ± 1.5 | 21.1 ± 8.6 | 42.1 ± 6.8 | 36.6 ± 8.0 |
| *Calanus* | 13.2 ± 5.2 | 18.1 ± 5.6 | 13.7 ± 5.4 | 17.2 ± 1.6 | 12.2 ± 6.0 | 10.6 ± 2.8 | 7.7 ± 2.1 |
| Carnivory | 2.3 ± 1.2 | 3.4 ± 0.9 | 1.7 ± 0.7 | 2.6 ± 0.1 | 2.2 ± 0.4 | 1.5 ± 0.5 | 4.6 ± 2.2 |
| PUFA/SFA | 1.3 ± 0.5 | 0.7 ± 0.3 | 1.2 ± 0.5 | 1.8 ± 0.2 | 1.2 ± 0.4 | 1.8 ± 0.1 | 1.1 ± 0.8 |

*glacialis* FA profiles from ice melt conditions were different from both maximum ice extent and ice-free conditions (2-factor PERMANOVA with pairwise comparison, $p < 0.04$). With years combined, *T. raschii* FA profiles were different between maximum ice extent and ice-free conditions (PERMANOVA pairwise comparison, $p < 0.02$).

### Fatty acid biomarkers

With years and ice conditions combined, some of the FA biomarkers differed between species. For example, the diatom/flagellate marker ratio 20:5n-3/22:6n-3 was higher in *T. raschii* than the other 2 species (Kruskal-Wallis ANOVA, $p < 0.001$; Table 3). In contrast, the diatom/dinoflagellate marker ratios 16:1/16:0 and ΣC16/ΣC18 were higher in *C. marshallae/glacialis* than in *T. libellula* and *T. raschii* (Kruskal-Wallis ANOVA, $p < 0.01$; Table 3). The *Calanus* marker (sum of C20 and C22 MUFA) and the carnivory marker (18:1n-9/18:1n-7) were significantly lower in *T. raschii* than in the other 2 species (Kruskal-Wallis ANOVA, $p < 0.005$; Table 3). These biomarkers reveal different but overlapping levels of omnivory as described by the carnivory and diatom/dinoflagellate marker ratios (18:1n-9/18:1n-7 and 16:1/16:0). This indicates the highest relative level of carnivory in *T. libellula* and the lowest in *T. raschii* (Table 3). The bacterial marker (sum of 15:0, 17:0, and the iso- and anteiso FA i-14:0, i-15:0, ai-15:0, i-16:0, i-17:0, and ai-17:0) in all zooplankton was less than 2.5% (Table 3).

Table 3c

| | Overall average | 2009 | | | 2010 | | |
|---|---|---|---|---|---|---|---|
| | | Max. ice | Ice melt | Ice-free | Max. ice | Ice melt | Ice-free |
| **(c) *T. raschii*** | | | | | | | |
| N | 15 | 2 | 4 | 2 | 1 | 4 | 2 |
| 14:0 | 3.9 ± 2.7 | 1.5 ± 0.1 | 2.3 ± 1.0 | 8.9 ± 1.1 | 2.1 | 3.2 ± 1.4 | 6.6 ± 0.8 |
| 16:0 | 20.0 ± 4.4 | 17.3 ± 1.2 | 17.3 ± 0.6 | 27.0 ± 4.9 | 17.0 | 18.7 ± 3.2 | 25.3 ± 0.8 |
| 16:1n-7 | 10.7 ± 7.8 | 3.2 ± 0.8 | 4.4 ± 1.7 | 15.9 ± 9.6 | 3.9 | 15.1 ± 6.6 | 20.2 ± 0.8 |
| 16:1n-5 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.2 | 0.2 ± 0.0 | 0.2 ± 0.0 |
| 16:4n-1 | 0.4 ± 0.3 | 0.1 ± 0.0 | 0.6 ± 0.4 | 0.5 ± 0.3 | 0.1 | 0.4 ± 0.4 | 0.2 ± 0.0 |
| 18:0 | 2.0 ± 1.3 | 1.4 ± 0.1 | 1.6 ± 0.2 | 2.2 ± 0.1 | 1.3 | 2.7 ± 2.6 | 2.3 ± 0.5 |
| 18:1n-9 | 8.9 ± 3.9 | 9.8 ± 2.0 | 7.6 ± 2.3 | 9.6 ± 4.1 | 18.9 | 6.3 ± 3.3 | 10.2 ± 1.2 |
| 18:1n-7 | 7.4 ± 1.1 | 8.1 ± 0.1 | 7.5 ± 0.5 | 7.0 ± 0.9 | 5.9 | 7.5 ± 2.0 | 7.4 ± 0.3 |
| 18:1n-5 | 0.4 ± 0.5 | 0.4 ± 0.0 | 0.4 ± 0.1 | 0.2 ± 0.0 | 1.6 | 0.5 ± 0.6 | 0.2 ± 0.0 |
| 18:2n-6 | 1.0 ± 0.6 | 0.8 ± 0.0 | 0.8 ± 0.2 | 1.8 ± 1.7 | 1.6 | 1.0 ± 0.4 | 0.7 ± 0.2 |
| 18:3n-3 | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.6 | 0.2 ± 0.1 | 0.1 ± 0.0 |
| 18:4n-3 | 0.8 ± 0.5 | 0.3 ± 0.0 | 0.8 ± 0.3 | 1.0 ± 0.3 | 0.6 | 0.9 ± 0.8 | 0.7 ± 0.0 |
| 20:1n-11 | 0.9 ± 2.5 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.3 | 0.9 | 2.6 ± 4.8 | 0.0 ± 0.0 |
| 20:1n-9 | 0.5 ± 0.3 | 0.6 ± 0.1 | 0.7 ± 0.3 | 0.4 ± 0.1 | 1.1 | 0.3 ± 0.1 | 0.3 ± 0.1 |
| 20:1n-7 | 1.0 ± 0.7 | 1.0 ± 0.4 | 1.0 ± 0.4 | 0.6 ± 0.0 | 0.8 | 1.2 ± 1.4 | 0.8 ± 0.1 |
| 20:4n-6 | 1.2 ± 1.6 | 1.6 ± 0.1 | 1.2 ± 0.2 | 0.2 ± 0.1 | 1.1 | 2.2 ± 2.9 | 0.3 ± 0.1 |
| 20:5n-3 | 21.5 ± 6.3 | 25.9 ± 1.9 | 29.0 ± 2.0 | 13.5 ± 0.6 | 16.3 | 20.3 ± 2.6 | 14.9 ± 1.6 |
| 22:1n-11 | 0.2 ± 0.3 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.7 ± 0.6 | 0.6 | 0.1 ± 0.1 | 0.1 ± 0.0 |
| 22:1n-9 | 0.3 ± 0.2 | 0.4 ± 0.0 | 0.6 ± 0.1 | 0.2 ± 0.1 | 0.5 | 0.1 ± 0.0 | 0.2 ± 0.0 |
| 22:5n-3 | 0.6 ± 0.4 | 0.9 ± 0.3 | 0.7 ± 0.2 | 0.2 ± 0.0 | 1.3 | 0.6 ± 0.6 | 0.2 ± 0.0 |
| 22:6n-3 | 10.8 ± 6.1 | 19.6 ± 0.5 | 14.9 ± 5.0 | 4.2 ± 0.2 | 12.5 | 8.7 ± 1.3 | 3.5 ± 0.3 |
| 24:1 | 0.2 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.6 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| SFA | 27.2 ± 6.6 | 21.2 ± 1.4 | 22.5 ± 0.9 | 39.2 ± 3.4 | 23.8 | 25.9 ± 1.5 | 35.1 ± 1.1 |
| MUFA | 32.4 ± 6.6 | 25.9 ± 1.9 | 25.0 ± 3.2 | 36.5 ± 2.9 | 37.4 | 35.5 ± 2.7 | 41.1 ± 0.5 |
| PUFA | 39.5 ± 12.0 | 51.8 ± 3.2 | 51.9 ± 3.1 | 23.9 ± 0.5 | 36.5 | 37.4 ± 2.5 | 23.4 ± 1.7 |
| Bacteria | 0.7 ± 0.4 | 0.7 ± 0.1 | 0.6 ± 0.0 | 0.6 ± 0.2 | 1.4 | 0.7 ± 0.6 | 0.4 ± 0.0 |
| 16:1/16:0 | 0.5 ± 0.3 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.6 ± 2.0 | 0.3 | 0.8 ± 0.3 | 0.8 ± 0.1 |
| ΣC16/ΣC18 | 1.6 ± 0.6 | 1.0 ± 0.0 | 1.2 ± 0.3 | 2.0 ± 2.1 | 0.7 | 1.9 ± 0.7 | 2.2 ± 0.2 |
| 20:5n-3/22:6n-3 | 2.4 ± 1.0 | 1.3 ± 0.1 | 2.1 ± 0.6 | 3.2 ± 0.3 | 1.3 | 2.4 ± 0.3 | 4.3 ± 0.1 |
| Diatom | 33.6 ± 6.7 | 29.7 ± 1.2 | 35.3 ± 4.3 | 30.9 ± 8.3 | 20.8 | 36.9 ± 8.5 | 36.7 ± 2.4 |
| *Calanus* | 3.0 ± 3.2 | 2.5 ± 0.7 | 2.8 ± 0.5 | 2.3 ± 1.2 | 4.0 | 4.4 ± 6.6 | 1.6 ± 0.0 |
| Carnivory | 1.3 ± 0.6 | 1.2 ± 0.3 | 1.0 ± 0.3 | 1.3 ± 0.4 | 3.2 | 1.3 ± 0.4 | 0.7 ± 0.1 |
| PUFA/SFA | 1.6 ± 0.7 | 2.4 ± 0.3 | 2.3 ± 0.2 | 0.6 ± 0.1 | 1.5 | 1.4 ± 0.2 | 0.7 ± 0.1 |

There was little variation in biomarker levels within species between 2009 and 2010 and among ice conditions. For *C. marshallae/glacialis*, both of the diatom/dinoflagellate marker ratios (16:1/16:0 and ΣC16/ΣC18) exceeded a value of 1 (which indicates higher amounts of diatom markers relative to dinoflagellate markers), except for 2009 ice melt and 2010 maximum ice extent (Table 3). In contrast, for both *T. libellula* and *T. raschii* only ΣC16/ΣC18 was >1 (except for 2010 maximum ice extent), and 16:1/16:0 was <1 (Table 3). The diatom to flagellate marker (20:5n-3/22:6n-3) did not show any differences between years and ice conditions for all zooplankton (Kruskal-Wallis ANOVA, $p > 0.07$). The carnivory marker ratio PUFA/SFA differed only in *C. marshallae/glacialis* between maximum ice extent and ice melt conditions in 2010 (Kruskal-Wallis ANOVA, $p = 0.04$).

### Carbon stable isotopes of fatty acids

Differences in the $\delta^{13}C_{FA}$ values among species were observed, but there were little annual or seasonal differences within each species (Table 2). The $\delta^{13}C_{FA}$ values of *C. marshallae/glacialis* were higher than those of both *T. libellula* and *T. raschii* (3-factor PERMANOVA with pairwise comparisons, $p < 0.04$). $\delta^{13}C_{FA}$ values were not different between *T. libellula* and *T. raschii* ($p = 0.26$). For both *T. libellula* and *T. raschii*, the $\delta^{13}C_{FA}$ values did not differ between years or between ice conditions (2-factor PERMANOVA, $p > 0.12$). Similarly, the $\delta^{13}C_{FA}$ values of *C. marshallae/glacialis* did not differ between years within ice conditions (i.e. maximum ice extent in 2009 and 2010) or between ice conditions in 2009 (2-factor PERMANOVA with pairwise comparisons, $p > 0.10$).

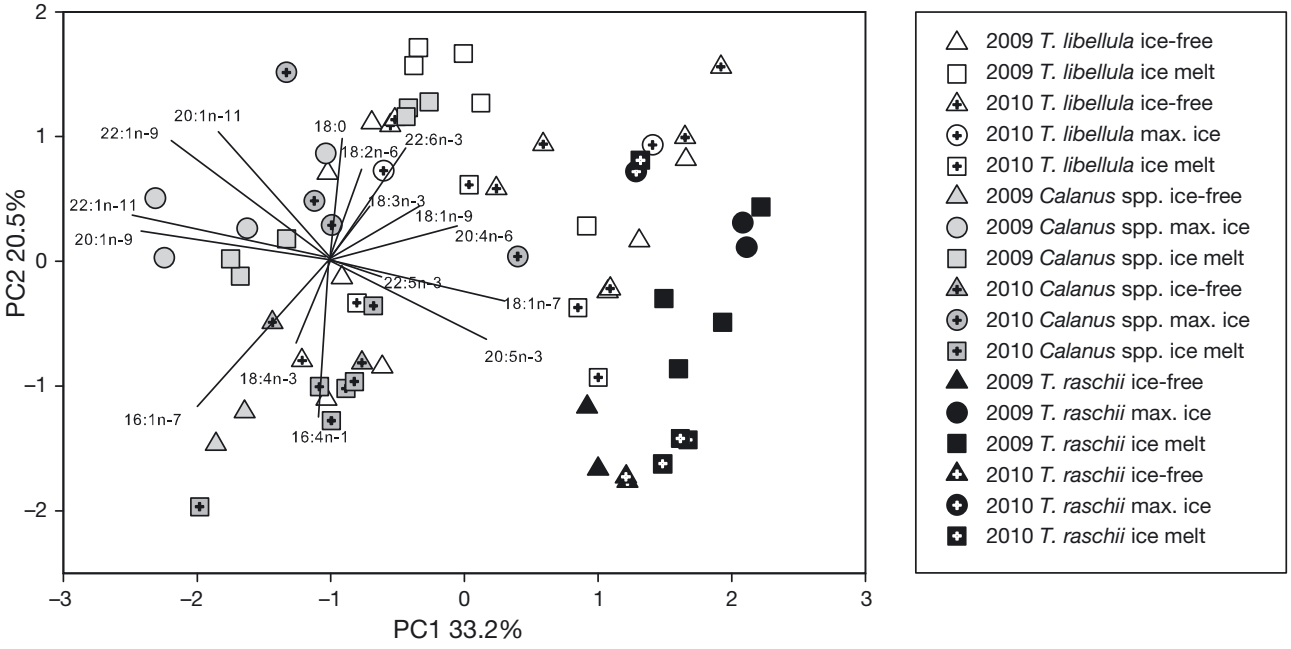


Fig. 2. Principal components analysis of *Themisto libellula*, *Calanus marshallae/glacialis* (*Calanus* spp.), and *Thysanoessa raschii* using 63 fatty acids (FAs) present in proportions >0.1% in samples collected in 2009 and 2010 during maximum ice extent, ice melt, and ice-free conditions. *T. libellula* samples were not collected during maximum ice extent in 2009. FA compositions are given in Table 3

In 2010, the $\delta^{13}C_{FA}$ values of *C. marshallae/glacialis* differed only between maximum ice extent and ice melt conditions (2-factor PERMANOVA with pairwise comparisons, $p = 0.04$).

i-POM $\delta^{13}C_{FA}$ values of 16:1n-7, 20:5n-3, and 22:6n-3 were significantly higher than those from p-POM during ice melt conditions in 2009 (Mann-Whitney *U*-test, $p < 0.04$; Table 2). i-POM collected in 2010 during maximum ice conditions also had significantly higher $\delta^{13}C_{FA}$ values of 16:1n-7, 20:5n-3, and 22:6n-3 than p-POM. p-POM $\delta^{13}C_{FA}$ values of 16:1n-7, 20:5n-3, and 22:6n-3 did not vary between ice conditions (Wang et al. 2014, Table 2). The $\delta^{13}C_{FA}$ values of 16:1n-7, 20:5n-3, and 22:6n-3 from *T. libellula, C. marshallae/glacialis,* and *T. raschii* fall within the range of values between i-POM and p-POM (Table 2).

The results from 4 $\delta^{13}C_{FA}$ mixing models gave varying estimates of the proportional contribution of i-POM to the diets of each of the zooplankton species. The estimated amount of FA from i-POM (from models without concentration dependencies in Table 4) for *T. libellula* ranged from 36% (16 to 56%) (mean, 95% credibility interval) to 72% (50 to 95%), for *C. marshallae/glacialis* from 27% (8 to 47%) to 63% (36 to 96%), and for *T. raschii* from 39% (0 to 78%) to 71% (47 to 99%) (Table 5). Estimates from models without concentration dependencies were similar to concentration dependent models (Table 5).

## DISCUSSION

### Fatty acid profiles

FA profiles showed that diets differed between *Themisto libellula*, *Calanus marshallae/glacialis*, and *Thysanoessa raschii*. The variability in FA profiles within each of the zooplankton species was much less than the variability among species. The FA profiles indicated that there was little variation between 2009 and 2010 in the foraging patterns of all 3 species, which suggests a similar food base in the

Table 4. Percentages of 16:1n-7, 20:5n-3, 22:6n-3 in sea ice and pelagic particulate organic matter (i-POM and p-POM) from 2009 and 2010 used as concentration dependencies in the SIAR stable isotope mixing models using diatom markers 16:1n-7, 20:5n-3, and flagellate marker 22:6n-3 as sources. Values are given as means ±1 SD

| Source | 16:1n-7 | | 20:5n-3 | | 22:6n-3 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| i-POM 2009 | 18.7 | 6.1 | 20.3 | 7.6 | 2.2 | 0.8 |
| p-POM 2009 | 16.5 | 6.2 | 23.2 | 4.4 | 3.8 | 0.5 |
| i-POM 2010 | 12.7 | 5.2 | 19.6 | 6.6 | 2.5 | 0.8 |
| p-POM 2010 Max. | 5.6 | 4.0 | 5.5 | 5.4 | 2.5 | 1.0 |
| p-POM 2010 Melt | 28.1 | 12.5 | 13.7 | 5.0 | 3.7 | 2.0 |
| p-POM 2010 Free | 10.0 | 6.6 | 10.2 | 4.0 | 9.0 | 2.6 |

Table 5. Estimates of sea ice particulate organic matter (i-POM, %) in *Themisto libellula*, *Calanus marshallae/glacialis* (*Calanus* spp.), and *Thysanoessa raschii* collected from the Bering Sea in 2009 and 2010 during maximum ice, ice melt, and ice-free conditions from 4 SIAR mixing models (a) without and (b) with concentration dependencies (see Table 4). *T. libellula* samples were not collected during maximum ice extent in 2009. Samples sizes are shown in Table 1. Values are means (95% credibility interval)

| | *T. libellula* | | | *Calanus* spp. | | | *T. raschii* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Max. ice | Ice melt | Ice-free | Max. ice | Ice melt | Ice-free | Max. ice | Ice melt | Ice-free |
| **(a) Without** | | | | | | | | | |
| 2009 | | | | | | | | | |
| 16:1n-7, 20:5n-3, 22:6n-3 | | 46 (25–65) | 51 (27–74) | 48 (7–82) | 50 (28–71) | 39 (0–74) | 48 (11–87) | 64 (39–92) | 46 (4–84) |
| 16:1n-7, 20:5n-3 | | 36 (16–56) | 38 (18–58) | 30 (1–61) | 36 (14–59) | 31 (0 –69) | 42 (5–78) | 54 (29–85) | 39 (0–78) |
| 20:5n-3, 22:6n-3 | | 55 (34–78) | 72 (50–95) | 63 (36–96) | 63 (42–85) | 50 (10–91) | 56 (19–97) | 71 (47–99) | 55 (13–97) |
| 20:5n-3 | | 47 (16–80) | 59 (32–90) | 56 (25–94) | 55 (29–86) | 44 (2–85) | 49 (8–93) | 60 (28–98) | 48 (3–91) |
| 2010 | | | | | | | | | |
| 16:1n-7, 20:5n-3, 22:6n-3 | 47 (10–82) | 49 (27–71) | 54 (38–72) | 49 (22–77) | 27 (8–47) | 42 (5–79) | 45 (1–88) | 49 (26–72) | 57 (25–96) |
| 16:1n-7, 20:5n-3 | 49 (11–90) | 53 (28–81) | 59 (39–80) | 52 (26–82) | 30 (7–53) | 47 (5–87) | 46 (1–91) | 53 (21–81) | 58 (21–99) |
| 20:5n-3, 22:6n-3 | 49 (9–88) | 47 (18–77) | 55 (32–78) | 51 (17–85) | 31 (4–56) | 48 (5–87) | 47 (2–91) | 50 (19–82) | 57 (20–98) |
| 20:5n-3 | 51 (10–95) | 53 (19–93) | 63 (34–96) | 57 (25–96) | 39 (2–71) | 54 (12–98) | 48 (3–93) | 56 (23–96) | 56 (12–99) |
| **(b) With** | | | | | | | | | |
| 2009 | | | | | | | | | |
| 16:1n-7, 20:5n-3, 22:6n-3 | | 47 (21–72) | 51 (22–81) | 48 (4–87) | 53 (23–81) | 39 (0–77) | 49 (10–91) | 68 (40–97) | 46 (3–86) |
| 16:1n-7, 20:5n-3 | | 36 (15–58) | 36 (16–58) | 28 (1–60) | 36 (11–60) | 32 (0–70) | 42 (4–80) | 55 (26–87) | 39 (0–79) |
| 20:5n-3, 22:6n-3 | | 62 (38–86) | 79 (57–99) | 68 (39–98) | 70 (49–92) | 53 (10–95) | 58 (19–99) | 76 (50–100) | 56 (13–98) |
| 20:5n-3 | | 49 (17–81) | 61 (34–92) | 57 (25–94) | 56 (30–88) | 44 (2–85) | 50 (9–94) | 61 (30–99) | 48 (2–92) |
| 2010 | | | | | | | | | |
| 16:1n-7, 20:5n-3, 22:6n-3 | 41 (5–80) | 54 (30–79) | 56 (37–77) | 40 (12–69) | 34 (12–56) | 45 (7–81) | 46 (1–90) | 54 (29–78) | 59 (26–97) |
| 16:1n-7, 20:5n-3 | 43 (4–83) | 58 (29–87) | 52 (32–74) | 39 (10–73) | 37 (9–60) | 44 (4–85) | 48 (2–93) | 56 (29–86) | 56 (21–98) |
| 20:5n-3, 22:6n-3 | 45 (4–86) | 48 (15–80) | 60 (39–82) | 45 (11–80) | 30 (2–56) | 50 (8–88) | 48 (2–92) | 50 (15–84) | 59 (21–99) |
| 20:5n-3 | 48 (6–94) | 50 (15–91) | 63 (35–96) | 47 (11–88) | 36 (1–69) | 54 (13–98) | 49 (3–94) | 53 (19–93) | 57 (15–99) |

2 years. Samples from this study were collected during a 'cold' period in the Bering Sea when there was extensive sea ice, cold water temperatures, spring ice-edge blooms, and low inter-annual variability in spring sea ice conditions (Stabeno et al. 2012). Furthermore, a qualitative assessment of phytoplankton communities in the Bering Sea revealed similar diatom species present in spring and summer from 2008 to 2010 (Sherr et al. 2013). Sampling cruises during the winter (maximum ice extent) and summer (ice-free conditions) occurred during the same time in 2009 and 2010. However, sampling during spring (ice melt) conditions was later in the season in 2010 than in 2009. There was little ice remaining in 2010 during what is referred to as the 'ice melt' period, and the diatom communities were dominated by planktonic instead of sympagic species (Sherr et al. 2013). Some of the variability in FA profiles of *T. libellula* and *C. marshallae/glacialis* between years during ice melt conditions can likely be attributed to the differences in plankton communities during the time of sampling between years.

Although there was little inter-annual variation in the diets of all 3 species, FA profiles indicated that there was some seasonal variability in their diets. This seasonal change in diet may reflect the seasonal changes in phytoplankton composition on the Bering Sea shelf, where algal biomass in the spring is dominated by sea ice and planktonic diatoms (Sukhanova et al. 1999, Lomas et al. 2012, Moran et al. 2012) and can be succeeded in the summer by non-diatom phytoplankton such as dinoflagellates, cryoptophytes, *Phaeocystis* spp., and *Synechococcos* spp. (Moran et al. 2012). Sporadic blooms of the diatoms *Chaetoceros* spp. and *Thalassiosira* spp. also occur in the summer in the eastern Bering Sea (Sukhanova et al. 1999, Sambrotto et al. 2008). Analysis of FA profiles from p-POM samples indicated a seasonal change in algal taxa in the water column in 2010, with an increase in diatoms as the ice melted in the spring followed by a decrease in the diatom signal as the ice disappeared (Wang et al. 2014). This suggests that diatoms were more abundant during ice melt, and the level of herbivory would be expected to be higher during this time relative to maximum ice extent and ice-free conditions when omnivory increases. In addition, sampling during phytoplankton bloom events would explain the predominance of herbivorous feeding, while sampling outside of bloom events would result in higher degrees of omnivory (Tamelander et al. 2008).

Seasonal differences in diet might also be due to changes in dietary preferences with different age classes in zooplankton. For instance, early juvenile *T. libellula* feed on both phytoplankton and small zooplankton (Søreide et al. 2006, Tamelander et al. 2006, Noyon et al. 2009), while older stages feed extensively on *Calanus* copepods (e.g. Scott et al. 1999, Auel et al. 2002, Dalpadado et al. 2008). Thus, the stronger presence of older *T. libellula* relative to juvenile stages during ice melt than ice-free conditions in 2009 may explain the difference in FA profiles between the 2 ice seasons. The age of individuals in the study is unknown, but results from Pinchuk & Coyle (2012) showed that the age structure of *C. marshallae/glacialis* populations in the Bering Sea was different between seasons in 2009, with adult females dominating during ice melt and copepodites dominating during ice-free conditions. The amount and type of lipid stored varies with developmental stage and season in zooplankton (Sargent & Henderson 1986, Lee et al. 2006). Thus, dietary preferences and differences in lipid storage could contribute to the observed differences in *C. marshallae/glacialis* FA profiles between ice melt and ice-free conditions in 2009. In 2010, the populations during ice melt and ice-free conditions along the middle shelf of the Bering Sea were both dominated by copepodite stages (Pinchuk & Coyle 2012), and accordingly there was no seasonal difference between FA profiles in our samples in 2010. The seasonal decrease in 20:5n-3 and 22:6n-3 in the total FA profiles of *T. raschii* in 2009 and 2010 might be explained by these FAs being stored preferentially in polar lipids later in the summer due to an increase in growth (Falk-Petersen et al. 2000). Both 20:5n-3 and 22:6n-3 are likely more concentrated in polar lipids, so that their proportions in total lipids decrease as neutral lipid stores increase.

Variability in FA profiles could also stem from effects of spatial variability in copepod distribution. In 2009 and 2010 during ice-free conditions, younger copepodite stages occurred in the north, while older stages dominated in the south along the 70 m isobath in the middle shelf of the Bering Sea (Pinchuk & Coyle 2012). *C. marshallae/glacialis* samples in this study were collected mostly from the northern part of the middle shelf and south of St. Lawrence Island, and the effect of sampling location on variability of FA profiles is unknown. Samples were collected widely across the Bering Sea shelf; however, too few samples were collected at each station to examine the effect of spatial variability.

### Fatty acid biomarkers

FA biomarkers confirmed previously described diets for *T. libellula*, *C. marshallae/glacialis*, and *T. raschii*. Overall, the ratio of diatom FA markers to flagellate FA markers in *T. libellula* was smaller than in the other 2 species. The diatom/flagellate FA biomarker 20:5n-3/22:6n-3 in *T. raschii* was twice as high as that in the other 2 species. These results suggest that although both species consumed diatoms more than *T. libellula*, *T. raschii* could have fed more on diatoms relative to flagellates compared to *C. marshallae/glacialis*.

Although we have used FA markers to compare the feeding ecologies of these zooplankton species, the comparisons should be done with caution as they have different FA metabolic pathways and selective retention of long chain PUFA. For example, *Calanus* spp. are unique from euphausiids and amphipods in that they can elongate the FAs 14:0, 16:0, and 18:0 to 20:1, and FAs 16:1n-7 and 18:1n-9 to 22:1 (Sargent & Henderson 1986). This may influence a number of the biomarker ratios and, consequently, the interpretation of the biomarkers that include the diatom marker 16:1n-7 and carnivory marker 18:1n-9. In addition, differences in the relative proportions and selective retention of PUFA among species may reflect differences in physiological demands (Brett et al. 2009). Furthermore, the use of FA markers to track trophodynamic relationships is more complex for omnivorous and carnivorous species than for herbivores. For instance, the diatom and dinoflagellate FA markers we document in *T. libellula* may arise from ingestion of *Calanus* spp. that fed on diatoms (Scott et al. 1999) but could also indicate herbivory. Early juvenile *T. libellula* are known to feed on both phytoplankton and small zooplankton (Søreide et al. 2006, Tamelander et al. 2006, Noyon et al. 2009, 2012), and adults mainly prey on zooplankton. However, the position of *T. libellula* in the positive direction on PC2 suggests little influence of diatoms in their diet. In contrast, the location of most of *C. marshallae/glacialis* from 2010 and *T. raschii* from 2010 in the negative direction on PC2 suggests that diatoms, compared to flagellates, mostly supported their diets. Because the age class of these samples is unknown, it is difficult to interpret the FA markers to derive feeding behavior.

The range in the carnivory marker PUFA/SFA found in *T. libellula* (1.4 to 2.3) falls within the range reported for omnivorous and carnivorous zooplankton (Cripps & Atkinson 2000). Furthermore, another carnivory FA biomarker ratio (18:1n-

9/18:1n-7) from *T. libellula* was similar to ratios reported by Auel et al. (2002) for *T. libellula* and *T. abyssorum* in the European Arctic. The diets of *T. libellula* are mainly comprised of *Calanus* copepodites and may include other zooplankton such as other copepod species, euphausiids, amphipods, mysids, and chaetognaths (Marion et al. 2008, Noyon et al. 2009). Thus, as expected, there was a stronger presence of the *Calanus* copepod marker (sum of C20 and C22 MUFA) in *T. libellula* than in *T. raschii*. This is further supported by the position of individuals on PC1. *C. marshallae/glacialis* loaded more negatively, *T. raschii* loaded more positively, and *T. libellula*, which feeds on *Calanus* spp., positioned near the center and intermediate between the two. However, the existence of C20 and C22 MUFA in *T. raschii* implies that they were ingesting material from *Calanus* (Falk-Petersen et al. 2000), which could possibly be in the form of detritus, copepodites, or eggs. The low levels of the carnivory marker PUFA/SFA in *C. marshallae/glacialis* (0.7 and 1.8) indicated a mostly herbivorous and possibly omnivorous feeding strategy (Cripps & Atkinson 2000), which also is consistent with findings from previous studies (Smith 1990, Hobson et al. 2002, Baier & Napp 2003, Stevens et al. 2004b). The ratios for PUFA/SFA as a carnivory marker in *T. raschii* ranged widely (0.6 and 2.4) and overlap with the ratios found in Antarctic herbivorous zooplankton (0.7 to 1.4), and with the lower ranges found in Antarctic omnivorous (range 0.9 to 3.8) and predatory zooplankton (range 1.5 to 5.2) (Cripps & Atkinson 2000). This suggests a certain degree of omnivory in *T. raschii* in this study, which is consistent with reports that although *T. raschii* is primarily herbivorous, the species can be carnivorous and may switch to detrital feeding during the winter (Mauchline & Fischer 1969, Sargent & Falk-Petersen 1981, Smith 1991, Hagen & Auel 2001, Hop et al. 2006). Our results suggest that these *C. marshallae/glacialis* and *T. raschii* have a certain degree of omnivory, which likely varies depending on environmental conditions and age of the organisms.

The proportion of bacterial FA markers in all 3 species were low and comparable to those found by Søreide et al. (2013) for several zooplankton species (including *T. libellula*) in the European Arctic. Bacterial marker values for all 3 species were also similar in abundance to bacterial FAs in *C. glacialis* reported by Stevens et al. (2004c). Therefore, we assumed that bacteria did not substantially contribute to zooplankton diets in this study.

### Carbon stable isotopes of fatty acids

The $\delta^{13}C_{FA}$ data indicate that all 3 zooplankton species incorporated sympagic sources of FAs between March and July in 2009 and 2010. Our results are comparable to those found by Søreide et al. (2006, 2008, 2013), where the contribution of sympagic carbon to zooplankton was estimated to be as high as 50% and up to 100% in *Calanus* copepods in the European Arctic. Our estimated range of the contribution of i-POM to zooplankton in the Bering Sea (27 to 71%) is also within the range of estimates of sea ice algal contribution to *T. raschii* from the Chukchi region off of Barrow, Alaska (20 to 74%; Budge et al. 2008). The variability among our model estimates could stem from the different FAs used in the models. The FA 20:5n-3 originates mainly from diatoms, while 22:6n-3 is predominately derived from flagellates (Sargent et al. 1987, Volkman et al. 1989, Dunstan et al. 1993, Graeve et al. 1994, Reuss & Poulsen 2002). In addition to elevated proportions of FA 20:5n-3, feeding on diatoms by herbivorous consumers can be detected using FA 16:1n-7 (Graeve et al. 1994, Dalsgaard et al. 2003). Nevertheless, 16:1n-7 in consumers can also result from de novo synthesis and chain shortening of other FAs and might not be appropriate for estimating diatom sources in omnivorous and carnivorous animals. Therefore, the model that included the diatom FA marker 20:5n-3 alone may be the most accurate to estimate the proportional contribution of i-POM if diatoms dominated the POM communities. However, the use of the flagellate marker 22:6n-3 should be taken into consideration because non-diatom phytoplankton may contribute substantially to the algal composition of POM.

Spatial differences in the $\delta^{13}C_{FA}$ values of the i-POM and p-POM end members may have also contributed to the variability among model estimates. i-POM and p-POM samples were collected over a broad region of the Bering Sea shelf, and the standard deviations for the i-POM samples suggest that there is a large range of $\delta^{13}C_{FA}$ values for i-POM across the shelf (i.e. standard deviations for i-POM ranged between 2.9 and 6.8‰ in 2009, and between 3.2 and 4.5‰ in 2010). In addition to the high variability in i-POM values, the $\delta^{13}C_{FA}$ value of 20:5n-3 in pure ice algae from Budge et al. (2008) was isotopically heavier (−18.3‰) than the value for i-POM in this study (−26.5‰). If the value of i-POM was more negative than the true value of the sea ice carbon end member value, then the model estimates of the contribution of i-POM FAs to zooplankton might be over-

estimated. The i-POM values likely represent an upper limit on i-POM contribution and may not be reflective of values of pure sea ice algae.

The method of analyzing $\delta^{13}C_{FA}$ of specific FAs relies on the assumptions that these FAs cannot be synthesized or modified by the species in question and that the isotopic fractionation associated with the metabolism of FAs analyzed is negligible (Budge et al. 2008, 2011). Feeding experiments with herbivorous copepod species have shown that phytoplankton-derived FAs such as 16:1n-7, 20:5n-3, and 22:6n-3 are incorporated largely unchanged (reviewed by Dalsgaard et al. 2003). Additionally, Parrish et al. (2012) showed that there was little modification or sequestration of the PUFA 20:5n-3 and 22:6n-3 by *Calanus* copepods. Although 16:1n-7 can be used as a diatom marker, it can also result from biosynthesis in animals (e.g. Rangan & Smith 2002). If these FAs are incorporated from diet into zooplankton without modification, it is reasonable to assume that their $\delta^{13}C_{FA}$ values also remain unchanged. Very few studies have been published about isotope fractionation of FAs, and there are conflicting results (Bec et al. 2011, Budge et al. 2011, Gladyshev et al. 2014). We acknowledge that fractionation of $\delta^{13}C$ values for these FAs from POM to zooplankton would influence the estimates of the model. For instance, if the $\delta^{13}C_{FA}$ became isotopically heavier in the consumer relative to the source (as seen with bulk stable carbon isotopes), estimates would be skewed to favor the isotopically heavier source (i-POM), potentially overestimating the contribution of i-POM FAs. Future controlled feeding experiments of zooplankton species with different foraging strategies (i.e. herbivory, omnivory, carnivory) would be valuable to further examine the effect of dietary modification on $\delta^{13}C_{FA}$ values and isotopic fractionation of FAs.

Mixing models often assume that the proportional contribution of a diet source to a consumer is the same for all components included, in this case, FAs (Phillips & Koch 2002). However, the proportions of FAs can vary among diet sources; thus, the estimates of the proportional contribution of sources could be biased toward one source or another, depending on their relative proportions. The proportions of the FAs used in the models (16:1n-7, 20:5n-3, and 22:6n-3) only differed between i-POM and p-POM in 2009 by between 1.5 and 3%. In contrast, the proportions of these FAs in i-POM compared with p-POM in 2010 were much greater, especially for the diatom markers 16:1n-7 and 20:5n-3. For example, the percentage of 16:1n-7 in p-POM from ice melt in 2010 (28%) was over twice as high as the percentage in i-POM during maximum ice extent in 2010 (12%). Despite these differences in the proportions of FAs between POM sources used in the model, the concentration dependent model estimates were very similar to the results from the models that assumed that the proportions of FAs were the same between POM sources. We speculate that this could be due to the large variation in $\delta^{13}C_{FA}$ values of i-POM (e.g. Parnell et al. 2010) and also to the substantial variation in concentration data for each FA, which is reflected in the wide 95% Bayesian credibility intervals for each of the model estimates.

Our estimates of sea ice-derived FAs to zooplankton did not completely support our hypothesis that the predominately herbivorous *C. marshallae/glacialis* and *T. raschii* would have the highest contributions of sympagic FAs during maximum ice extent, and that it would decrease as the ice melted and progressed into ice-free conditions. The average contribution of i-POM estimated by all models was similar between the 2 species during maximum ice extent in both years but suggests that *T. raschii* consumed more sea ice-derived FAs than *Calanus* spp. did during ice melt and ice-free conditions in both years. For both species, the average estimates of i-POM contribution during ice-free conditions decreased from ice melt conditions in 2009 as predicted but increased in 2010. The contrast in seasonal patterns could be due to a larger pelagic summer diatom bloom in 2009 than in 2010. In other words, if the amount of phytoplankton FAs increased during the ice melt of 2009 relative to 2010, the signal from p-POM would be stronger during the ice melt of 2009 than 2010. In fact, the average chlorophyll *a* (chl *a*) concentration across all stations during the summer cruise in 2009 ($1.03 \pm 0.86$ µg $l^{-1}$) was significantly higher than the average during the summer cruise in 2010 ($0.70 \pm 0.85$ µg $l^{-1}$; Student's *t*-test, $p = 0.001$)[1]. However, chl *a* concentration alone does not necessarily confirm that pelagic primary production was higher in 2009 than in 2010 because different stations were sampled between years, and grazing pressure might also have differed between years. In addition, higher estimates of FAs from i-POM within species could be due to differences in age classes, as juveniles of some zooplankton species are more herbivorous than adults (e.g. Søreide et al. 2006).

The average contribution of sea ice algal FAs estimated by all models was lower in *T. libellula* than the other 2 species only during ice melt in 2009 but was

[1]Data collected by M. W. Lomas as part of the Bering Sea Project under NSF project ANS-0732359

still substantial at 46%. Interestingly, the average estimate for *T. libellula* was ~10% higher than both predominantly herbivorous species during ice-free conditions in 2009, and *C. marshallae/glacialis* during ice melt and ice-free conditions in 2010. Estimates for *T. libellula* also increased in 2009 from maximum ice extent to ice melt conditions, and from ice melt to ice-free conditions in 2010. These patterns observed in *T. libellula* may be explained by a seasonal increase in abundance of its prey; *C. marshallae/glacialis*. *C. marshallae/glacialis* populations in the Bering Sea increased after a shift to cold years in 2006, with heavy spring sea ice cover compared to the warmer years of 2001 to 2005 (Baier & Napp 2003, Coyle et al. 2011) and also increased seasonally along the middle shelf in 2009 and 2010 (Pinchuk & Coyle 2012). Therefore, a seasonal increase in *C. marshallae/glacialis* populations could increase the i-POM contribution in consumers (such as *T. libellula*) that prey on them.

The variation in the estimated proportional contribution of i-POM FAs to zooplankton could also be due to a delay in carbon stable isotope or seasonal and species-specific lipid turnover in zooplankton. Differences in body size among species may also affect the estimated contribution of i-POM to zooplankton diets. For example, the larger body size of *T. raschii* relative to *C. glacialis/marshallae* suggests that *T. raschii* would retain the i-POM FAs and $\delta^{13}C_{FA}$ signatures for a longer time. Longer retention time of the i-POM signal within *T. raschii* could explain the apparently higher contribution of i-POM to their diet later in the season. Although dietary lipids are incorporated in copepods in as little as 24 h, only 40% of the lipids had turned over after 14 d in feeding experiments with *C. glacialis* females (Graeve et al. 2005). For the Arctic gammarid amphipod *Onisimus litoralis,* sufficient changes in carbon stable isotopes in tissues can be detected in animals collected in the spring under ice-cover and animals collected 4 wk later, whereas during ice-free conditions animals may integrate carbon from their diet over a period of months (Kaufman et al. 2008). Therefore, the estimates of i-POM contribution for samples we collected during ice-free conditions could reflect FA sources from maximum ice extent and ice melt conditions because of slower turnover of carbon in animals during ice-free conditions. For example, the average estimates from all models were higher during ice-free conditions in both years for *T. libellula*, and in 2010 for both predominant herbivores *Calanus* spp. and *T. raschii*. In 2009, samples collected during ice melt and ice-free conditions were collected ~4 and 15 wk after samples collected during maximum ice conditions. In 2010, samples collected during ice melt and ice-free conditions were collected ~11 and 16 wk, respectively, after samples were collected during maximum ice conditions. The possibility of slower turnover of carbon isotopes between seasons complicates the interpretation of our estimates, suggesting that model results during ice melt and ice-free conditions could reflect the proportional contribution of i-POM earlier during periods of ice cover. In addition to timing of sample collection, the variability in the amount of ice cover and timing of break up between years may also affect the variability in i-POM contribution to zooplankton between years. In fact, the number of days with ice cover after 15 March in the vicinity of Mooring 2 on the Bering Sea shelf was 46 d in 2009 and 38 d in 2010, and the ice cover index (relative to the 1981 to 2000 mean) was 3.54 in 2009 and 3.28 in 2010 (lower index indicates lower ice cover; NOAA 2013).

Regardless of which FAs were used, the different mixing models provided similar estimates and showed that *T. libellula*, *C. marshallae/glacialis,* and *T. raschii* in the Bering Sea in 2009 and 2010 consumed substantial (27 to 71%) amounts of sea ice-derived FAs and were possibly still consuming it as the ice retreated. The estimated contributions of i-POM FAs to zooplankton in this study are representative of heavy ice conditions in the Bering Sea as they occurred in 2009 and 2010 and may be used as a baseline for comparison with future studies conducted during warmer years with less sea ice cover. Predicted changes in timing of sea ice retreat could be detrimental to zooplankton species that are dependent on the spring bloom. For example, in the Bering Sea, *C. glacialis* grazes on ice algae before ice melt and pelagic production events that are triggered by the release of ice algae into the water column (e.g. Durbin & Casas 2014). *T. raschii* fuel spring and summer reproduction from the spring bloom (Harvey et al. 2012). Consequently, a mismatch between the timing of the spring bloom and reproduction and feeding could be unfavorable to these species and others that depend on them for food. In addition to changes in the timing of sea ice retreat, the predicted loss of sea ice, and subsequent decrease in sympagic primary productivity will lead to a reduction in available sea ice-derived FAs to zooplankton. Current sea ice loss has driven a 30% increase in net primary productivity in the Chirkov Basin and a 20% increase in the Arctic Ocean (Brown & Arrigo 2012). Future loss of sea ice is also predicted to increase net primary production on the Bering Sea shelf (Brown & Arrigo

2012). Such increases in phytoplankton production in the Bering Sea, should they occur, may help offset the expected reduction in ice algal production and any detrimental effects it may have on consumers such as zooplankton.

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**Appendix.** Sample information for (a) *Themisto libellula*, (b) *Calanus marshallae/glacialis*, and (c) *Thysanoessa raschii* collected from the Bering Sea in 2009 and 2010 during maximum ice, ice melt, and ice-free conditions. *T. libellula* samples were not collected during maximum ice conditions in 2009

| Cruise, ice conditions | Date (m/dd/yyyy) | Station no. | Station name | Latitude (°N) | Longitude (°W) | No. of ind. |
|---|---|---|---|---|---|---|
| **(a) *Themisto libellula*** | | | | | | |
| HLY0902, Ice melt (N = 5) | 4/10/2009 | 19 | MN13 | 59.876 | 175.215 | 1 |
| | 4/12/2009 | 25 | MN19 | 59.901 | 178.908 | 4 |
| | 4/22/2009 | 58 | NP9 | 57.445 | 169.754 | 1 |
| | 4/29/2009 | 69 | BL1 | 59.537 | 175.205 | 1 |
| | 4/29/2009 | 85 | BL15 | 59.55 | 175.096 | 1 |
| KNORR195-10, Ice-free (N = 8) | 6/20/2009 | 32 | CNN6 | 56.787 | 167.874 | 2 |
| | 6/22/2009 | 49 | NP11 | 56.983 | 170.288 | 3 |
| | 6/22/2009 | 45 | NP7 | 57.911 | 169.248 | 5 |
| | 7/01/2009 | 98 | MN4 | 59.910 | 169.803 | 2 |
| | 7/02/2009 | 108 | MN13 | 59.913 | 175.206 | 2 |
| | 7/03/2009 | 114 | MN18 | 59.910 | 178.206 | 3 |
| | 7/06/2009 | 133 | SLN2 | 61.868 | 169.876 | 6 |
| | 7/07/2009 | 140 | SL9 | 62.202 | 173.118 | 3 |
| PSEA10-01, Max. ice (N = 2) | 3/13/2010 | 1 | VNG-1 | 62.046 | 175.067 | 1 |
| | 3/14/2010 | 3 | NWC-4 | 62.418 | 174.696 | 1 |
| TN249, Ice melt (N = 4) | 5/17/2010 | 39 | IE1 | 59.337 | 175.611 | 15 |
| | 5/19/2010 | 49 | MN19 | 59.911 | 178.953 | 10 |
| | 5/27/2010 | 81 | 70m26 | 58.175 | 169.907 | 1 |
| | 6/08/2010 | 170 | MN13 | 59.901 | 175.202 | 3 |
| TN250, Ice-free (N = 8) | 6/15/2010 | 45 | NP7 | 57.889 | 169.223 | 1 |
| | 6/19/2010 | 18 | CN4 | 57.279 | 162.923 | 9 |
| | 6/23/2010 | 33 | CNN5 | 57.052 | 167.449 | 1 |
| | 6/29/2010 | 69 | P14-N4 | 58.960 | 173.873 | 6 |
| | 7/03/2010 | 99 | MN18 | 59.900 | 178.200 | 4 |
| | 7/06/2010 | 124 | SL1 | 62.200 | 169.849 | 1 |
| | 7/06/2010 | 132 | SL9 | 62.200 | 173.116 | 1 |
| | 7/07/2010 | 139 | SL16 | 62.200 | 175.905 | 1 |
| **(b) *Calanus marshallae/glacialis*** | | | | | | |
| HLY0901, Max. ice (N = 4) | 3/18/2009 | 8 | 08NWC1 | 63.705 | 172.540 | 15 |
| | 3/24/2009 | 24 | 24WAL12 | 62.125 | 169.250 | 10 |
| | 3/26/2009 | 36 | 36MK1B | 62.847 | 169.022 | 16 |
| | 3/28/2009 | 41 | 41NWC3 | 62.912 | 174.069 | 30 |
| HLY0902, Ice melt (N = 6) | 4/14/2009 | 29 | St. 29 | 61.792 | 176.802 | 8 |
| | 4/16/2009 | 35 | SL 9 | 63.094 | 173.291 | 8 |
| | 4/18/2009 | 45 | SL1 | 61.958 | 167.991 | 7 |
| | 4/28/2009 | 83 | St. 83 | 60.821 | 174.384 | 12 |
| | 5/01/2009 | 92 | St. 92 | 61.589 | 173.709 | 8 |
| | 5/02/2009 | 93 | BN1 | 62.333 | 172.703 | 8 |
| KNORR195-10, Ice-free (N = 2) | 7/06/2009 | 133 | SLN2 | 61.868 | 169.876 | 12 |
| | 7/07/2009 | 140 | SL9 | 62.202 | 173.118 | 15 |
| PSEA10-01, Max. ice (N = 4) | 3/16/2010 | 8 | VNG-4 | 62.946 | 173.461 | 20 |
| | 3/21/2010 | 26 | NEC2 | 62.612 | 170.166 | 20 |
| | 3/25/2010 | 41 | NWC-3 | 62.971 | 173.941 | 15 |
| | 3/28/2010 | 47 | NWC-2 | 63.354 | 173.221 | 8 |
| TN249, Ice melt (N = 6) | 5/27/2010 | 81 | 70m26 | 58.175 | 169.907 | 30 |
| | 6/03/2010 | 134 | 70m39 | 59.846 | 171.838 | 40 |
| | 6/04/2010 | 147 | 70m52 | 61.418 | 173.736 | 30 |
| | 6/05/2010 | 156 | SL12 | 62.193 | 175.155 | 20 |
| | 6/05/2010 | 158 | SL9 | 62.104 | 175.291 | 30 |
| | 6/08/2010 | 170 | MN13 | 59.901 | 175.202 | 40 |
| TN250, Ice-free (N = 2) | 7/06/2010 | 124 | SL1 | 62.200 | 169.849 | 10 |
| | 7/06/2010 | 132 | SL9 | 62.200 | 173.116 | 10 |

(continued on next page)

**Appendix** (cont.)

| Cruise, ice conditions | Date (m/dd/yyyy) | Station no. | Station name | Latitude (°N) | Longitude (°W) | No. of ind. |
|---|---|---|---|---|---|---|
| **(c) *Thysanoessa raschii*** | | | | | | |
| HLY0901, Max. ice (N = 2) | 3/23/2009 | 21 | 21MK1 | 62.969 | 169.156 | 8 |
| | 3/24/2009 | 24 | 24WAL12 | 62.125 | 169.250 | 10 |
| HLY0902, Ice melt (N = 4) | 4/14/2009 | 29 | St. 29 | 61.792 | 176.802 | 4 |
| | 4/16/2009 | 35 | SL 9 | 63.094 | 173.291 | 6 |
| | 4/18/2009 | 45 | SL1 | 61.958 | 167.991 | 4 |
| | 5/01/2009 | 92 | St. 92 | 61.589 | 173.709 | 2 |
| KNORR195-10, Ice-free (N = 2) | 6/22/2009 | 45 | NP7 | 57.911 | 169.248 | 1 |
| | 7/05/2009 | 122 | XB2-12 | 59.573 | 175.250 | 1 |
| PSEA10-01, Max. ice (N = 1) | 3/23/2010 | 37 | CD10-D | 62.832 | 171.42 | 3 |
| TN249, Ice melt (N = 4) | 5/19/2010 | 49 | MN19 | 59.911 | 178.953 | 3 |
| | 6/03/2010 | 134 | 70m39 | 59.846 | 171.838 | 6 |
| | 6/04/2010 | 147 | 70m52 | 61.418 | 173.736 | 4 |
| | 6/05/2010 | 156 | SL12 | 62.193 | 175.155 | 4 |
| TN250, Ice-free (N = 2) | 6/23/2010 | 33 | CNN5 | 57.052 | 167.449 | 2 |

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