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Annual cycle of lipid content and lipid class composition in zooplankton from the Beaufort Sea shelf, Canadian Arctic

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Abstract

We determined seasonal cycles of lipid content, lipid class composition, and carbon and nitrogen content of 7 taxa of zooplankton that were collected from the Beaufort Sea shelf, Canadian Arctic, over a 10 month period (September 2003 – August 2004). All taxa except the chaetognath *Parasagitta elegans* had substantial lipids stores (>50%), either seasonally (*Oikopleura* spp.), or throughout the year (*Calanus hyperboreus*, *C. glacialis*, *Paraeuchaeta glacialis*, *Metridia longa*, and *Eukrohnia hamata*). Wax esters were the dominant lipid class in the chaetognath *E. hamata* and in all copepods, including the carnivore *P. glacialis*. Seasonal trends in lipid content and composition varied among taxa; some taxa had little variation from winter through summer (e.g. *P. elegans*), other taxa showed little variation until summer (e.g. *C. glacialis*), and others showed increasing or decreasing trends during winter and spring (e.g. *C. hyperboreus*). Specifically, total lipid content of *C. hyperboreus* decreased from January through May at a rate of $\sim 450 \mu\text{g month}^{-1} \text{ ind}^{-1}$ in adult females, and $\sim 100 \mu\text{g month}^{-1} \text{ ind}^{-1}$ in juvenile copepodite IV, representing a 75-85% loss in lipid.

Keywords: overwinter, seasonality, lipids, carbon content, copepod, chaetognath, appendicularia

Introduction

Primary production in the Arctic Ocean is highly seasonal and episodic. Some zooplankton populations respond quickly to these ephemeral phytoplankton blooms with high rates of feeding. The energy obtained from the bloom may be used immediately to fuel oogenesis, vitellogenesis and spawning, or stored to sustain metabolic needs during winter or to fuel future egg production (Hagen et al. 1996; Hagen 1999; Niehoff et al. 2002; Lee et al. 2006; Hirche 2013; Daase et al. 2013). Other species appear to lack an immediate response to seasonal blooms (Hagen 1999; Parrish et al. 2009). Zooplankton commonly store energy as lipids, being ~2x more energy dense than carbohydrates or proteins. Life cycle events of zooplankton, such as reproduction, diapause and development, are directly linked to lipid accumulation and lipid class composition (Lee et al. 2006), which often reflect feeding mode. In the Arctic, storage of surplus energy is best known for copepods of the genus *Calanus*, which store primarily wax esters (WE) (Lee et al. 2006; Falk-Petersen et al. 2009). Carnivorous and omnivorous species, such as some amphipods, decapods, and chaetognaths, store primarily triacylglycerol (TG) or are lipid poor (Falk-Petersen et al. 2000; Connelly et al. 2012). However, some exceptions have been observed with some carnivorous species accumulating WE (Scott et al. 1999; Noyon et al. 2011; Connelly et al. 2012). Cold ocean larvacean tunicates, such as *Oikopleura vanhoeffeni*, have not been found to store lipids (Deibel et al. 1992) and therefore rely on a constant input of food to during winter and for reproduction (Choe and Deibel 2009). Lipid-rich zooplankton, in turn, supply higher trophic level organisms with a high-energy food source by concentrating dilute phytoplankton energy into lipid deposits. However, lipid content and lipid class composition of relatively few Arctic zooplankton species have been reported, and even less information on the annual cycle of lipid content are available, even for the best studied copepods.

The annual cycle of primary production in the Arctic Ocean and its marginal shelves is typified by a late spring or early summer bloom of diatoms in the water column. This water column bloom can

be preceded by an ice algae bloom, with ice algae becoming available to pelagic grazers during sea ice melt. Water column diatoms bloom earlier in polynyas due to increased light penetration compared to the surrounding ice-covered seas (Tremblay et al. 2006; Simpson et al. 2013). Our study area on the eastern Beaufort Sea shelf includes a flaw lead polynya, which opens into the Cape Bathurst polynya in the Amundsen Gulf, allowing us to examine the effect of early algal blooms on the lipid content of Arctic zooplankton species. Except for a companion study on hyperbenthic zooplankton, which primarily included species of mysids, amphipods and chaetognaths from fall and summer (Connelly et al. 2012), lipid class composition of zooplankton from the Beaufort Sea has not been studied. Further, even in other regions of the Arctic Ocean, temporal trends of lipid content and composition of zooplankton have only focused on a single season (e.g. Swalethorp et al. 2011).

In this paper we present lipid content and lipid class composition of 7 zooplankton taxa: the copepods *Calanus hyperboreus* (both CIV and CVI), *Calanus glacialis*, *Metridia longa* and *Paraeuchaeta glacialis*, the chaetognaths *Eukrohnna hamata* and *Parasagitta elegans*, and the larvacean tunicate *Oikopleura* spp. The study was conducted over a 10-month period (late September to early August) at several stations on the eastern Beaufort Sea shelf, but focused on an overwinter station in Franklin Bay, Amundsen Gulf. The 7 taxa collected include the 4 major feeding modalities of zooplankton, i.e. herbivory (*Calanus*), omnivory (*Metridia*), carnivory (*Paraeuchaeta*, chaetognaths), and non-selective, mucous filter-feeding (*Oikopleura*). As explained above, we predicted a decrease in storage lipids in order of the above continuum, as well as a transition from WE as the primary storage form to TG. Further, we predicted an increase in storage in late June and July, following ice melt across the shelf and the spring freshet of the Mackenzie River. Finally, we predicted little or no storage of neutral lipid by the mucous web filter feeder *Oikopleura* spp., based on the findings of a previous study where *O. vanhoeffeni* from boreal waters did not store lipids (Deibel et al. 1992). We examined the time series of lipid concentration to infer storage and spawning periods as well as taxa and times of

optimum lipid quantity and quality for higher trophic levels, such as larval fish.

Methods

Field

Zooplankton were collected from 30 September 2003 to 10 August 2004 from the Beaufort Sea shelf, Canadian Arctic, from the CCGS *Amundsen* as a part of the Canadian Arctic Shelf Exchange Study (CASES). The CASES study design incorporated two primary components, a regional-scale array of stations covering the Canadian Beaufort Sea shelf and an overwinter, time-series station in Franklin Bay, Amundsen Gulf (Fig. 1). The overwinter site (70°03'N, 126°18'W, St. 200 in Fig. 1) was in landfast ice 23 km northeast of the Horton River outflow, with a water column depth of 232 m. Zooplankton were collected ca. weekly for 25 weeks, from 10 December 2003 through 27 May 2004, at the overwinter station. Before and after the overwinter time series, zooplankton were collected at stations on the regional grid, from 30 September to 14 November 2003, and again from 7 June to 10 August 2004. During the regional sampling, we revisited the overwinter station once on 16 July 2004 and sampled a station that was only 16.5 km from the overwinter station twice, on 4 November 2003 and 7 August 2004. We determined zooplankton lipid content and lipid classes from 36 dates during the winter time series, and a total of 38 stations from the regional grid. Depth profiles of salinity, temperature and fluorescence were collected with a CTD (Seabird SBE-911+) deployed twice daily during the overwinter period and at each station on the regional grid.

Adult females of *Calanus hyperboreus*, *C. glacialis*, *Metridia longa* and *Paraeuchaeta glacialis*, juvenile *C. hyperboreus* CIV, *Eukrohnia hamata*, *Parasagitta elegans*, and *Oikopleura* spp. (*O. vanhoeffeni* + *O. labradoriensis*) were collected from each bottom-to-surface vertical net tow (1x1 m net frame with 200 µm mesh). When gut contents were present, animals were left in petri dishes containing filtered seawater for up to 12 h to depurate. At each station, up to 60 individuals of each taxon were collected for lipid analysis, depending upon availability and animal size. The number of

stations, samples, and total animals for each taxon are summarized in Table 1, for both the overwinter and spatial lipid study. A total of 276 samples were taken for zooplankton lipid analysis, consisting of ca. 9000 animals (Table 1). We also took one sample of *P. glacialis* eggs by removing and pooling six egg sacs from gravid females from February 2004 and three opportunistic samples of siphonophores during March 2004.

Animals were blotted dry onboard ship and placed in lipid-clean test tubes. Next, 2 ml of chloroform was added, the headspace was purged with N₂, and samples were sealed and stored at -20°C. When available, up to 20 additional animals were sorted, blotted dry, placed in microcentrifuge tubes, and stored at -20°C for carbon-nitrogen elemental analysis (CN).

Laboratory analyses

Frozen zooplankton samples were flown from the Arctic to our laboratory in Newfoundland, Canada in coolers filled with ice. Samples arrived frozen, and low levels of free fatty acid (mean across all samples: $1 \pm 2\%$ of total fatty acids) indicated little hydrolysis of lipids during transport (data not shown). In the laboratory, lipids were extracted in chloroform:methanol:water (2:1:0.5) following a modified version of Folch et al. (1957; Parrish 1999). Lipid classes were then determined by manually spotting extracts on silica-coated Chromarods (SIII), which were developed following Parrish (1987). Lipids were determined using flame-ionization detection with an Iatroscan MK V. Commercial standards were used to identify and quantify 9 lipid classes: hydrocarbons, steryl esters, ethyl ketones, TG, free fatty acids, fatty alcohols, sterols, acetone mobile polar lipids, and phospholipids (PL). This method does not separate WE and steryl esters. However, because WE have previously been confirmed to be a major lipid class in most zooplankton that we studied (Lee 1975; Stevens et al. 2004a) and steryl esters are usually only found at trace levels in zooplankton, we consider the WE-steryl ester peak to be WE. Total lipid content was calculated as $\mu\text{g-lipid ind}^{-1}$ by summing masses of all 10 lipid classes, and the relative amount of each lipid class was determined as a percent of total lipid classes.

Zooplankton samples for CN analysis were lyophilized and homogenized. Subsamples were then weighed to the nearest 10 μg before packing into tin capsules. Samples were analyzed on a Perkin Elmer 2400 CHN Analyzer with acetanilide standards. Duplicates were run for 10% of samples. C and N composition is reported as a percentage of dry weight and C:N as molar ratios. The coefficient of variation (c.v.) of C:N ratios for duplicates of the same sample was 2.9%.

Data analysis

The general linear model was used to test differences in total lipid content and in the ratio of storage lipids to PL (WE+TG:PL) among samples. All zooplankton samples (overwinter and regional grid) were used to test differences among taxa. When testing whether a taxon differed between the Amundsen Gulf and the Mackenzie shelf, only samples from the regional grid (September-November 2003 and June-August 2004) were used. The Amundsen Gulf and Mackenzie shelf were defined as stations east and west of Cape Bathurst, respectively. A rejection criteria of $p < 0.05$ was used to determine statistical significance in all analyses. Data were log transformed when residuals did not meet the assumptions of normality, independence or homoscedasticity. Temporal data were fitted with a locally weighted regression (LOESS) model. Confidence intervals for LOESS models are 95%. Other errors are reported as standard deviations unless stated otherwise.

Results

Seasonality of water column temperature, salinity and fluorescence

Throughout the entire study period water was relatively saline (≥ 30) and cold ($< 5^\circ\text{C}$), except in surface waters during summer (June – August) where we found salinities < 25 and temperatures up to 10°C (Fig. 2). The entire overwinter period was characterized by full ice-cover, whereas ice-cover varied on the regional grid depending on the station (data not shown). At the latitude of the overwinter station, there was complete darkness for ca. two months from 25 November until 18 January, and full sun from 15 May until 28 July. Chlorophyll *a* was low throughout the overwinter period ($< 0.6 \mu\text{g L}^{-1}$),

and increased from $<0.01 \mu\text{g L}^{-1}$ (prior to late March) to $>0.1 \mu\text{g L}^{-1}$ during April and May in surface waters ($<30 \text{ m}$) (Fig 2). Chlorophyll concentrations of $10\text{--}35 \mu\text{g L}^{-1}$ were found during June and July at depths $<50 \text{ m}$.

Total lipid concentration and lipid class composition among taxa

On average PL, WE and TG made up $91\pm6\%$ of total lipids across all samples. Other lipid classes were generally minor components, each contributing on average $\leq 3\%$ to total lipids. Due to differences in diet, life history and size, mean total lipid content ind^{-1} and storage lipid:PL ratios differed among the 7 taxa (including the two stages of *Calanus hyperboreus*) across the entire study period ($p<0.001$, Fig. 3a-b). Total lipid content and storage lipid:PL ratios also varied considerably within taxa across the large temporal and spatial scales of our study, with mean c.v. of ca 65% and 120%, respectively. However, for a given taxa on the regional grid (not including overwinter samples), there was no distinct spatial pattern in total lipid content ind^{-1} or storage lipid:PL ratios, as samples collected from the Amundsen Gulf and the Mackenzie shelf were similar ($p>0.05$, data not shown).

Paraeuchaeta glacialis and adult *C. hyperboreus* had the most lipid (2.2 ± 0.6 and $1.5\pm1.0 \text{ mg ind}^{-1}$, respectively) and *Oikopleura* spp. and *Metridia longa* had the least (0.03 ± 0.03 and $0.15\pm0.08 \text{ mg ind}^{-1}$, respectively). Adult copepods had higher ratios of storage lipids to PL than juvenile *C. hyperboreus*, *Oikopleura* spp. and chaetognaths (Fig. 3b). This result includes the carnivorous copepod *P. glacialis*, which, in addition to having the highest total lipid content of all taxa, had a mean storage:PL ratio similar to that of *C. glacialis* (Fig. 3b). WE were the dominant lipid class in all taxa ($\geq 60\%$, Fig. 3c), except *Oikopleura* spp. and *Parasagitta elegans*, which both predominantly had PL ($>60\%$). Mean TG, in contrast, were $\leq 20\%$ of total lipids in all taxa, with only three taxa having proportions $>10\%$: *P. glacialis*, *M. longa* and *Oikopleura* spp. (Fig. 3c). Lipids of the unidentified siphonophore were predominantly WE (48-63%) and TG (16-26%).

Seasonality of total lipid content and composition

171 Patterns of lipid content and lipid class composition throughout the year varied among taxa
172 (Fig. 4-6). Depending on the variable (i.e., lipid content, %WE, %TG or %PL), some taxa had little
173 variation from winter through summer (e.g., %PL for *M. longa* and *P. glacialis*, Fig. 5), other taxa
174 showed little variation until summer (e.g., %TG for *C. hyperboreus* CVI and %WE for *C. glacialis*, Fig.
175 4), and others showed increasing or decreasing trends during winter and spring (e.g., total lipid content
176 for *C. hyperboreus* CVI and CIV, Fig. 4). Generally, however, there was greater variability in total
177 lipid content and lipid class proportions across all taxa during the months of June, July and August,
178 when samples were collected from station on the regional grid with variable environmental conditions
179 (e.g., temperature, fluorescence, Fig. 2). However, there was no correlation between depth integrated
180 chlorophyll *a* levels (≤ 100 m) or maximum chlorophyll *a* value at a station and total lipid content or
181 storage lipid levels for any taxon during summer ($p \geq 0.2$).

182 Total lipid content of both adult and juvenile *C. hyperboreus* decreased between January and
183 May (Fig. 4). Total lipid content of adult females decreased from a maximum of $2400 \mu\text{g ind}^{-1}$ at the
184 end of January, to $\sim 350 \mu\text{g ind}^{-1}$ at the end of May. This decrease represents a rate of lipid loss of ca.
185 $450 \mu\text{g month}^{-1} \text{ ind}^{-1}$, or an 80-85% loss from January values. Juvenile *C. hyperboreus* CIV lost
186 roughly $100 \mu\text{g-lipid month}^{-1} \text{ ind}^{-1}$ or about 75% from the beginning of January to the end of April.
187 Lipid content of adult female *C. hyperboreus* was generally higher during June-August than during
188 April-May, although there was high variability during summer (10-fold range, Fig. 4). We did not
189 collect *C. hyperboreus* CIV from the regional grid.

190 The decrease in total lipids during winter and spring was accompanied by changes in lipid class
191 proportions in both stages of *C. hyperboreus*. In adult females at the overwinter station, %WE was
192 highest in early winter and gradually decreased from January to August, although WE remained the
193 dominant lipid class in all samples, including summer ($>75\%$, Fig. 4). Concentrations of WE ($\mu\text{g-WE}$
194 ind^{-1}) in adult females showed similar seasonal patterns as total lipid content, with a rate of WE loss of

195 ca. 400 $\mu\text{g month}^{-1} \text{ ind}^{-1}$ from January through May (data not shown). %TG of adult and juvenile *C.*
196 *hyperboreus* was relatively constant and low throughout winter, although %TG in adult copepods was
197 slightly higher with greater variation prior to March than from March to May (Fig. 4). From the end of
198 February through May, TG was only detected in 3 of 18 samples of adult females. In contrast, TG was
199 detected in 15 of 26 samples from June, July and August, months in which %TG in adult *C.*
200 *hyperboreus* had greater variability (range 0-10%). %PL generally increased in both stages from
201 January until March, increasing by >2-fold during this time period, and then decreased again from
202 March through May (Fig. 4). This trend, however, is not as robust in juvenile *C. hyperboreus* due to
203 low *n* from April and May. Unlike %PL, concentrations of PL ($\mu\text{g-PL ind}^{-1}$) in adult female *C.*
204 *hyperboreus* began decreasing in late January (data not shown) concurrent with decreases in total lipid
205 content, roughly corresponding to a loss in PL of ca. 20 $\mu\text{g month}^{-1} \text{ ind}^{-1}$ from the end of January to the
206 end of April. Likewise, the range of %PL of adult female *C. hyperboreus* was greater during June, July
207 and August than during winter (Fig. 4).

208 In contrast to *C. hyperboreus*, total lipid content in *C. glacialis* was variable throughout the year
209 and did not show obvious trends during the overwinter period (Fig. 4). Proportions of WE, TG and PL
210 in *C. glacialis* were relatively constant during the overwinter period, with slight increases and
211 decreases in %PL and %WE, respectively, at the winter-time series station in summer (Fig. 4). The
212 increase in %PL for two sampling dates at the overwinter station in summer corresponds to ca. 5-fold
213 mean increase in the concentration of PL from December/early January (5-13 $\mu\text{g-PL ind}^{-1}$ versus 46-53
214 $\mu\text{g-PL ind}^{-1}$; data not shown). WE was the dominant lipid class in all *C. glacialis* samples and was
215 $\geq 75\%$ throughout the sampling period at the overwinter station (Fig. 4).

216 For *M. longa*, %WE generally increased from late March through summer, while %TG
217 decreased during the same time span and %PL remained relatively constant (Fig. 5). However, WE
218 were the dominant lipid class throughout the study period at the overwinter station. Data from two

samples collected in early/mid-February at the overwinter station suggest that total lipid content and %WE decreased and %TG increased from early/mid-February to mid-March in *M. longa* (Fig. 5).

There was little seasonal trend in %WE, %TG or %PL for all three carnivores: *P. glacialis*, *P. elegans* and *Eukrohnia hamata*, although these species had very different lipid class profiles (Fig. 3, Fig. 5-6). However, *n* was <10 for samples collected from the overwinter station for *P. elegans* and *P. glacialis*, which limits our ability to detect seasonal trends. For example, two samples of *P. elegans* in June-July suggest increased proportions of TG compared with the rest of the year, but more samples would be needed to confirm this observation (Fig. 6). In contrast, *E. hamata* was sampled regularly throughout the year, with consistently low %TG (<4%), and highly variable %WE (20-80%) and %PL (7-60%) at the overwinter station (Fig. 6). Data for *E. hamata* from June, July and August was also variable for all three lipid classes (Fig. 6). In contrast to both chaetognath species, total lipid content of *P. glacialis* showed seasonality, with highest levels in February and March (Fig. 5). *P. glacialis* egg sacs, with a mean length x width of 4.6 x 2.3 mm, contained 1900 µg-lipid egg sac⁻¹, and were 95% TG and 3% PL. WE were not detected. Prior to May, *Oikopleura* spp. was rarely present at abundances sufficient for lipid class analysis. Thus, most of our *Oikopleura* spp. samples were collected from May-August (25 of 29 samples). Within this three month timeframe, several trends across all samples (regional grid and winter-time series) were apparent: increasing lipid content, %WE and %TG, and decreasing %PL (Fig. 6). Most of these changes began to take place about mid-June.

Carbon and nitrogen content and ratios

Similar to lipid content and lipid class composition, C and N content and ratios varied among taxa across the entire study period ($p < .001$, Fig. 7). Ranking among taxa for mean C content was almost identical to storage lipids:PL ratio (Fig. 3b, 7a), where adult *C. hyperboreus* was highest ($56.7 \pm 5.2\%$ of dry weight (DW)) and *P. elegans* was lowest ($42.0 \pm 8.8\%$ DW). Mean N content ranged from 6.3% DW in *Oikopleura* spp. to 8.3% DW in *P. elegans* (Fig. 7b). Consistently, mean C:N ratios

were greatest in adult *C. hyperboreus* (10.6 ± 2.2) and lowest for *P. elegans* (6.0 ± 1.7 , Fig. 7c). The c.v. among taxa and within taxa were similar for both C and N content (10% among taxa and 14% within taxa), and variability within a taxon was lower for C and N content than for total lipid content and storage lipids:PL ratios.

We have fewer samples for C and N analyses than for lipid analyses, especially from the overwintering period, because our primary objective was collecting enough biomass for lipid analyses when constrained by the availability of animals. Thus, we focus our presentation of temporal pattern in C and N content and ratios to four taxa: adult female *C. hyperboreus*, *C. glacialis*, *M. longa*, and *E. hamata*. Consistent with lipid data, elemental content of the three copepod taxa showed temporal patterns, while those of the chaetognath *E. hamata* did not (Fig. 8). %C of both *C. hyperboreus* and *C. glacialis* decreased in winter (Fig. 8). However, decreasing %C continued into summer for *C. glacialis*, whereas it increased in *C. hyperboreus* after May. This pattern is consistent with patterns of total lipid content for *C. hyperboreus*, but not for *C. glacialis*, which had variable lipid content throughout the study period (Fig. 4). Likewise, %N was greater in summer than winter for *C. glacialis* and tended to decreased from late December through summer at the overwintering station for *C. hyperboreus*, although %N was variable throughout the study period for both taxa (Fig. 8). For *M. longa*, elemental content remained relatively unchanged from February until late May, after which there were decreases in C:N and increases in %N in June and July (Fig. 8).

Discussion

Zooplankton as a lipid conduit from autotrophs to higher level consumers

Because of the strong seasonality in light and persistently cold water temperatures in high latitude marine ecosystems, polar marine animals across trophic levels depend on lipids for energy storage during periods of low food availability and, in the case of marine mammals, for insulation. Thus, the production and transfer of lipids through the food web is essential for the overall health of

polar marine ecosystems (Falk-Petersen et al. 2007). Herbivorous and omnivorous polar zooplankton play a pivotal role in this lipid flux, by converting and concentrating organic matter from algae into high energetic lipid stores. *C. hyperboreus* and *C. glacialis* are perhaps the most remarkable taxa in this capacity because of their large lipid stores (Lee et al. 2006; Falk-Petersen et al. 2009) and their key role in Arctic food webs (Dahl et al. 2003; Wold et al. 2011b; Vogedes et al. 2014). However, only a few studies have quantified the lipid content of *Calanus* from the Beaufort Sea (Wold et al. 2011a; Connelly et al. 2012), although this genus dominates the biomass of zooplankton (Forest et al. 2008; Darnis and Fortier 2012). Specifically, *C. hyperboreus* and *C. glacialis* contributed nearly 80% of zooplankton biomass in Franklin Bay during our study (Forest et al. 2008), with >50% of the biomass of these two lipid-rich taxa being carbon (our data).

Lipid content in adult *C. hyperboreus* (2000 $\mu\text{g ind}^{-1}$) and *C. glacialis* (430 $\mu\text{g ind}^{-1}$) during summer on the Beaufort Sea shelf were comparable to those found in waters off Svalbard and in the North Water Polynya (Scott et al. 2000; Stevens et al. 2004a), where the links between *Calanus* and higher level consumers have been well studied (Falk-Petersen et al. 2002; Dahl et al. 2003; Wold et al. 2011b; Vogedes et al. 2014). However, the magnitude of maximum lipid levels for *C. hyperboreus*, and the timing of minimum lipid levels for *C. glacialis*, differ between Disko Bay, Greenland (Swalethorp et al. 2011) and the Beaufort Sea shelf. *C. glacialis* on the Beaufort Sea shelf was more lipid rich during March – June (>400 $\mu\text{g ind}^{-1}$) than *C. glacialis* from Disko Bay during the same time period (Swalethorp et al. 2011). Also, *C. hyperboreus* on the Beaufort Sea shelf reached minimum lipid levels ~1 month later during spring than *C. hyperboreus* from Disko Bay, although absolute minimum (<400 $\mu\text{g ind}^{-1}$) and maximum (~2000 $\mu\text{g ind}^{-1}$) lipid levels during spring were similar between the two locations (Swalethorp et al. 2011). The difference in timing likely reflects earlier ice break-up and an earlier phytoplankton bloom in Disko Bay.

In our study area, the carnivorous copepod *P. glacialis* was as lipid rich as *C. hyperboreus*, and

had storage lipid:PL ratios similar to that of *C. glacialis*. These results are consistent with previous work elsewhere in the Arctic on *P. glacialis* that showed high lipid content in this taxon (Auel and Hagen 2005). But, the temporal pattern in lipid content in *P. glacialis* in our study differs from that observed in sediment traps in the Canada Basin, where relative lipid sac size for individuals was variable throughout the year, with no obvious temporal trends (Matsuno et al. 2014). Although Auel and Hagen (2005) had previously determined that *P. glacialis* from the Greenland Sea are ~47% lipid (DW) with ~2000 $\mu\text{g-lipid ind}^{-1}$ (estimated from their DW and %lipid data), we consider the similar lipid content between *P. glacialis* and *C. hyperboreus* striking because body length of *P. glacialis* and *C. hyperboreus* were similar and we expected lipid stores to be less important for carnivorous copepods compared to herbivorous copepods. High lipid stores in *P. glacialis* may reflect constant feeding on lipid rich prey, even during winter, as has been observed for other carnivorous zooplankton (i.e., *Themisto libellula*, Scott et al. 1999). Accordingly, *P. glacialis* may be a high quality prey, in terms of energy, for consumers that usually feed on *C. hyperboreus*, especially during February-April, when lipid content of *C. hyperboreus* decreased below levels of *P. glacialis*. However, because *P. glacialis* was much less abundant than *C. hyperboreus* throughout the year (> 10x less abundant, unpublished data), *P. glacialis* likely contributes substantially less to diets of consumers than *C. hyperboreus*. For example, at the overwintering station, 6-9% of Arctic cod sized >14 cm contained *P. glacialis* but 62-66% contained *C. hyperboreus* (Benoit et al. 2010).

In contrast, *M. longa*, the smallest copepod analyzed, had less than half the lipid content of *C. glacialis* and *C. hyperboreus* CIV, but was the most abundant taxon of those analyzed, only less abundant than *Oithona* and *Oncaea* during our study period (Forest et al. 2008). Compared to *Calanus*, less is known about the contribution of *M. longa* to diets of higher trophic level animals, but their numerical density and bioluminescence may make them easy prey, offsetting their lower individual energy content. For example, at the same overwinter site, *Metridia* spp. was found in >70% of Arctic

cod sized >14 cm (Benoit et al. 2010) and the number of *M. longa* in a bowhead whale caught in the Gulf of Boothia (Canadian Arctic) was 2 orders of magnitude greater than the number of *Calanus* (Pomerleau et al. 2011).

Time of year would strongly influence the quantity of energy a consumer receives from its non-carnivorous prey. For example, a predator feeding on *C. hyperboreus* CVI or CIV would obtain at least 75-80% less lipid per copepod (CVI and CIV) and 15-20% less carbon per unit biomass consumed (CVI only), in April compared to January. Although we found no indication of seasonal variation in lipid content or composition in either taxon of chaetognath, their prey items may become less energy dense. Thus, these taxa would have to feed more, change prey type, or reduce their metabolism to maintain homeostasis. Recent work on *P. elegans* points to the latter explanation, as this taxon has lower growth rates, minimal or non-existent reproduction, and reduced feeding activity during winter in waters near Svalbard (Grigor et al. 2014, 2015). Likewise, *P. glacialis* may be food limited during winter because of a mismatch between its primary depth and that of diapausing copepods as seen for *Paraeuchaeta norvegica* in the Norway Sea (Fleddum et al. 2001). These results argue against the notion that zooplankton predators are insensitive to seasonality (c.f. Hagen 1999). Even when prey are available all year round, predators may need to adapt to seasonal changes in the energy content and biochemical composition of their prey. Considering only bulk energy and lipid content, however, overlooks the availability of essential nutrients such as amino acids and fatty acids that must be obtained from the diet to meet nutritional requirements. Seasonal variations in lipid composition likely influence the lipid quality of prey (i.e., fatty acid composition) as different lipid classes often have different fatty acid profiles within a taxon (Sargent and Falk-Petersen 1981; Scott et al. 1999; Wold et al. 2011a).

Lipids for energy storage, buoyancy and reproduction

Large lipid reserves help zooplankton survive times when food is limited. WE are thought to be

stored in taxa that need to endure long periods without food and TG in taxa that need to sustain themselves over shorter periods without food (Lee et al. 2006). In both cases, substantial portions of either WE or TG generally indicate animals that are tightly linked to annual cycles of primary production, with WE indicating greater reliance on seasonal production. In contrast, high proportions of PL are found in taxa that do not rely on energy stores, and thus, feed continuously throughout the year. As expected for zooplankton from the Arctic, most taxa analyzed here had high WE proportions, indicating strong dependence on short intense blooms of phytoplankton and the need to withstand relatively long periods of food deprivation. During October – January in Franklin Bay, WE reached $\geq 90\%$ in more than half the samples of adult *C. hyperboreus* and *C. glacialis*, which is in the high end of the range of recorded values, especially for *C. glacialis* (Scott et al. 2000; Stevens et al. 2004a; Swalethorp et al. 2011). Lower WE stores and proportions of TG $\geq 10\%$ in *M. longa* is consistent with previous work on this taxon suggesting that *M. longa* is more omnivorous than *C. hyperboreus*, and feeds continuously throughout the year with reliance on episodic pulses of primary production (Falk-Petersen et al. 1987; Stevens et al. 2004b; Seuthe et al. 2007). The seasonal pattern of decreasing %WE until March-April and increasing again through summer mirrors seasonal trends seen for *M. longa* in a sub-Arctic fjord (Falk-Petersen et al. 1987). However, for a given month, %WE in our data was always higher than in the sub-Arctic fjord. If the apparent decrease in total lipid between February and late March represents catabolism, then it seems that *M. longa* preferentially catabolizes WE during winter. In contrast, increasing %WE and decreasing %TG from late March through summer may reflect greater consumption and access to wax-ester rich eggs and nauplii of *C. hyperboreus* (Darnis 2013), as proposed by Darnis and Fortier (2014) for *M. longa* during 2008 in the Amundsen Sea.

Even the filter-feeding appendicularian *Oikopleura* spp. contained surprisingly high levels of storage lipids in summer (mean TG + WE was 22% of total lipids), which contrasts to results from a previous study on lipid composition in *O. vanhoeffeni* before and after a spring bloom in boreal waters,

where WE was not detected and only one sample had TG >5% (Deibel et al. 1992). Histological studies have shown that tissues of *Oikopleura albicans* and *Oikopleura dioica* from the Mediterranean Sea frequently contain lipid drops (Fenaux 1963; Burighel et al. 2001; Cima et al. 2002). However, lipid content was not quantified and the type of lipid was not determined in these studies. As far as we know, the current study is the first to quantify lipid content and lipid class composition in pelagic tunicates in the Arctic Ocean. Thus, we currently only have a rudimentary understanding of energy storage capabilities and benefits to polar appendicularians. Our data indicate that storage lipids do play a role in the life history of polar *Oikopleura* spp. (*O. vanhoeffeni*/*O. labradoriensis*), especially in summer.

The only taxon that seemed to lack any appreciable storage lipids was the chaetognath *P. elegans*. This result sharply contrasts with data from the other chaetognath we studied, *E. hamata*, which sometimes had 75% or more of their lipids as WE. This difference in lipid composition between *P. elegans* and *E. hamata* is consistent with lipid class profiles of these two taxa from the hyperbenthos of the Beaufort Sea shelf, where storage lipids contributed 75% of total lipid in *E. hamata*, but <3% in *P. elegans* (Connelly et al. 2012). *E. hamata* from the hyperbenthos also occupied a lower trophic level than *P. elegans* (trophic level 2.7 versus 3.3; Connelly et al. 2014), suggesting that greater storage lipids in *E. hamata* may reflect its lower trophic position and stronger ties to seasonal production compared to *P. elegans*.

WE are also implicated in regulating buoyancy for vertically migrating zooplankton such as copepods that undertake seasonal diapause (Visser and Jónasdóttir 1999; Campbell and Dower 2003). Recent work (Pond and Tarling 2011; Clark et al. 2012; Pond 2012) proposes that the composition of WE in diapausing copepods, specifically the degree of unsaturation, controls timing of descent and ascent, and helps copepods maintain neutral buoyancy at depth. Attaining neutral buoyancy at the depth of diapause greatly reduces metabolic cost, suggesting that WE may contribute more to the life history of *Calanus* beyond serving as an energy reserve. Buoyancy regulation may also explain why WE were

lacking in *P. elegans* but were often > 50% in *E. hamata*. Lipid vacuoles (Lee et al. 2006; Pond 2012) and WE stores in *Eukrohnia* is not uncommon (Lee et al. 1971; Lee and Hirota 1973; Lee 1975; Connelly et al. 2012) and the depth distribution of *Eukrohnia* is generally thought to be deeper than that of *Parasagitta*. Thus, Pond (2012) hypothesizes that lipid vacuoles likely play a role in buoyancy control and trim in this deeper residing chaetognath genus. The lack of a defined seasonal trend in WE and %C in *E. hamata* hints at a function of WE beyond energy storage or reflect asynchronous reproduction (see below). These discoveries shed new light on the role of lipids in the life history of zooplankton, while raising new questions on interactions between buoyancy and WE composition in copepods and other marine zooplankton.

C. hyperboreus egg production during our study period (Darnis 2013) coincided with decreases in total lipid content (our data), %C (our data) and total C content (Darnis 2013) from January to May. *C. hyperboreus* is a capital breeder, so reproduction is fuelled by internal energy stores and is independent of concurrent feeding (Hirche and Niehoff 1996). The clear decrease in total lipid content during the period of egg production, before the onset of any measurable primary production, confirms the reliance on internal lipid stores for reproduction in *C. hyperboreus* on the Beaufort Sea shelf. Based on the decrease in lipid content female⁻¹ from January to May, we estimate up to 2.0 mg-lipids female⁻¹ was allocated to eggs throughout winter, assuming all lipid loss from female *C. hyperboreus* was due to spawning. This assumption may be valid as ingestion at the overwinter site covered metabolic costs from respiration for *C. hyperboreus* (Seuthe et al. 2007). Furthermore, the simultaneous decrease in the concentration of PL and WE indicate that both were directed to egg production. Based on the rate of decrease of these two lipid classes during winter, WE accounted for 95% of the total lipid lost and PL for 5%. These proportions are remarkably similar to data from Jung-Madsen et al. (2013), who measured 89% WE and 8% PL in eggs of *C. hyperboreus* from Disko Bay, especially considering our proportions do not consider modification among lipid classes and disregard TG.

In contrast to *C. hyperboreus*, *C. glacialis* is primarily an income breeder in Franklin Bay and in the rest of the Amundsen Gulf, where successful reproduction depends on concurrent consumption of ice algae or phytoplankton (Daase et al. 2013). In other regions of the Arctic Ocean, *C. glacialis* begins reproduction without food, but egg production dramatically increases when females begin to feed (Hirche and Kattner 1993; Daase et al. 2013). However, egg production prior to feeding seems minor for *C. glacialis* on the Beaufort Sea shelf (Daase et al. 2013). The constancy of lipid class proportions, especially of WE, until April/May supports this view. Specifically, lipid class composition began to change post-April/May when ice algae bloomed (Rózanska et al. 2009), numbers of *C. glacialis* in surface waters increased (Daase et al. 2013), and fecal pellet production began (Seuthe et al. 2007). Further, lower %C and C:N, and higher %N in June compared to January-March likely reflect egg production that started after *C. glacialis* began to feed on ice algae in April/May.

Unlike *C. hyperboreus*, there was no clear trend during winter in total lipid content of *C. glacialis*, which varied highly throughout the year. At first glance, this seems to contrast with data from 2008 when neutral lipids of *C. glacialis* decreased from February through March in the Amundsen Gulf (Wold et al. 2011a). However, when considering a similar time period in 2004 (mid-January to early March) there was a consistent decrease in total lipid content and concentrations of WE. In our data, WE decreased from 438 $\mu\text{g-WE ind}^{-1}$ in mid-January (16 January: 481 $\mu\text{g-total lipid ind}^{-1} \times 91\%\text{-WE}$) to 110 $\mu\text{g-WE ind}^{-1}$ in early March (9 March: 114 $\mu\text{g-total lipid ind}^{-1} \times 95\%\text{-WE}$). This decrease is similar to a decrease from 413 to <170 $\mu\text{g-neutral lipid ind}^{-1}$ from early February to late March in the Amundsen Gulf in 2008 (Wold et al. 2011a). However, lower WE concentrations in December and early January and high variability from late March until June in our data masks this two month trend. Unfortunately, there is no time series data from 2008 prior to the first week of February or from April through May in the Amundsen Gulf (except for one value on 11 May of 94 $\mu\text{g-neutral lipid ind}^{-1}$ from Franklin Bay; Wold et al. 2011a) to resolve whether our observations prior to mid-January and post-

March were limited to 2004 or reflect a more general pattern. Because lipid class composition remained relatively constant (%WE, %TG, and %PL) from December to April, if lipids were catabolized for energy or allocated for developing gonads during this time period, the 3 major lipid classes were used in similar proportions.

Less is known about the seasonal reproductive cycles of zooplankton predators and *Oikopleura* spp. in the Arctic Ocean and how they relate to lipid content and composition. We did not observe any clear seasonal pattern in lipid content or lipid class composition in either chaetognath species to speculate on periods of highest reproduction. Nor did we observe any obvious seasonal pattern in %C, %N or C:N in *E. hamata*. These results may indicate that reproduction could be constant for these zooplankton predators throughout the year on the Beaufort Sea shelf, although this is unlikely (Samemoto 1987; Grigor et al. 2014). Some of the seasonal trend in lipid content in *P. glacialis* may have been due to various stages of ovary development, because total lipid content was highest in February-April and because we observed females with egg sacs in February. In addition to high energetic investment into eggs (80%), *P. glacialis* also has large egg sacs that contain more lipid than the female (Auel 2004). We also found that lipid content was lower in the bodies of egg-carrying females than in eggs ($1200 \mu\text{g female}^{-1}$ versus $1900 \mu\text{g-lipids egg sac}^{-1}$).

We do not know if the increase in total lipid content and %TG + %WE in late June for *Oikopleura* spp. relates to energy storage for catabolism, for reproduction, or for something else. We suggest that increased lipid stores of *Oikopleura* spp., which coincide with greater chlorophyll *a* levels, may be destined for reproductive investment. If true, these lipids stores may be ephemeral, as oocyte formation and spawning in *Oikopleura dioica* occurs within a few days of encountering food (Troedsson et al. 2002; Lombard et al. 2009). Similarly, *Oikopleura vanhoeffeni* responds relatively rapidly to phytoplankton production in the Northwater polynya, beginning to reproduce in open water as early as late April and May (Deibel et al. in prep). Low levels of total lipids and storage lipids in

459 January and May to mid-June in our data seem to preclude an adaptation against starvation/low food
460 conditions. This data suggests that *Oikopleura* spp. meets its energy requirements with constant feeding
461 during winter on the Beaufort Sea shelf, as implied by the presence of their fecal pellets in sediments
462 traps in Franklin Bay during winter (Sampei et al. 2009) and as noted for populations in boreal waters
463 (Urban et al. 1993; Choe and Deibel 2010).

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Figure 1. Station location for zooplankton collected from September 2003 to August 2004 from the Beaufort Sea shelf as part of the Canadian Arctic Shelf Exchange Study (CASES). The overwinter station (St. 200), which was occupied from 10 December 2003 to 27 May 2004, is marked with an asterisk (70°03'N, 126°18'W).

Figure 2. Time-depth profiles of temperature (Temp, a-c), salinity (d-f) and chlorophyll a (Chl, h-j) for stations sampled across the Beaufort Sea shelf from September 2003 – August 2004. The middle section of each panel, from 10 December to 27 May, is a time series from the overwinter station in Franklin Bay, Amundsen Gulf (70°03'N, 126°18'W) (b, e). The left and right sections of each panel are a composite of stations from the regional grid, incorporating stations from the Mackenzie shelf and Amundsen Gulf. For chlorophyll a, the left scale bar is for the time series at the overwinter station (middle section, i) and the right scale bar is for the regional grid (left and right section, h, j). Note the log scale for chlorophyll a (h-j).

Figure 3. Total lipid content (a) and lipid composition (b-c) among zooplankton taxa collected from September 2003 – August 2004 across the Beaufort Sea shelf. Taxa are ordered by decreasing storage lipids:phospholipid (PL) ratios, where storage lipids are the sum of wax esters and triacylglycerol (b). Specific contributions of wax esters (white boxes) and triacylglycerol (grey boxes) to total lipid content are shown in (c). Full taxonomic names are given in Table 1. Horizontal black bar shows the median, boxes encompass the interquartile range, whiskers are 1.5 times the interquartile range, and grey dots are outliers.

Figure 4. Total lipid content, and proportions of wax esters (%WE), triacylglycerol (%TG), and phospholipids (%PL) in *Calanus hyperboreus* adult females (left panel), stage CIV juveniles (middle

panel), and *Calanus glacialis* (right panel) from the Amundsen Gulf (circles) and the Mackenzie Shelf (triangles) on the Beaufort Sea shelf in 2003-2004. Grey circles are from the overwinter time series in Franklin Bay. Grey line and shading depicts temporal trends ($\pm 95\%$ confidence interval) predicted from a LOESS regression model. *C. hyperboreus* CIV was only collected from the overwinter station. Note difference in date range (x-axes) and scaling for lipid variables (y-axes) among plots.

Figure 5. Total lipid content, and proportions of wax esters (%WE), triacylglycerol (%TG), and phospholipids (%PL) in the copepod *Metridia longa* (left panel) and *Paraeuchaeta glacialis* (right panel) from the Amundsen Gulf (circles) and the Mackenzie Shelf (triangles) in the Beaufort Sea in 2003-2004. See Figure 4 for definition of point and line shading. Note difference in date range (x-axes) and scaling for lipid variables (y-axes) between plots for both taxa.

Figure 6. Total lipid content, and proportions of wax esters (%WE), triacylglycerol (%TG), and phospholipids (%PL) in the chaetognaths *Eukrohnia hamata* (left panel) and *Parasagitta elegans* (middle panel), and the filter feeding pelagic tunicate *Oikopleura* spp. (*O. vanhoeffeni* + *O. laboradensis*) (right panel) from the Amundsen Gulf (circles) and the Mackenzie Shelf (triangles) in the Beaufort Sea in 2003-2004. See Figure 4 for definition of point and line shading. Note difference in date range (x-axes) and scaling for lipid variables (y-axes) among plots for all taxa.

Figure 7. Carbon content (A) and nitrogen content (B) (% dry weight), and C:N molar ratios (C) among zooplankton taxa collected from 2003 –2004 on the Beaufort Sea shelf, ordered by decreasing %C. Full taxonomic names are given in Table 1. See Figure 3 for definitions of box and whiskers.

Figure 8. Carbon (%C) and nitrogen (%N) content (% dry weight), and C:N molar ratios in the

705 copepods *Calanus hyperboreus* (left panel), *Calanus glacialis* (second panel) and *Metridia longa* (third
706 panel), and the chaetognath *Eukrohnia hamata* (right panel) from the Amundsen Gulf (circles) and the
707 Mackenzie Shelf (triangles) in the Beaufort Sea in 2003-2004. See Figure 4 for meaning of point and
708 line shading. Note different date range on x-axis among taxa.

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Table 1. Summary of stations and lipid samples as a function of zooplankton taxon collected from the regional grid or during the overwintering period as part of the CASES program from September 2003 to August 2004. The regional grid of stations and the overwintering time series station in Franklin Bay (70°03'N, 126°18'W) are indicated in Fig. 1. Total animals taxon⁻¹ is the product of the number of samples taxon⁻¹ and the mean number of animals sample⁻¹. Length (mean ± standard deviation (SD)) is prosome length for copepods, trunk length for *Oikopleura* spp., and total length for the chaetognaths.

	Regional Grid		Overwintering Time Series		Length ^c (mm ± SD)
	38 stations		36 stations		
	samples taxon ⁻¹	total animals taxon ⁻¹	samples taxon ⁻¹	total animals taxon ⁻¹	
<i>Calanus hyperboreus</i> CVI	35	1268	33	1221	6.5 ± 0.14
<i>Calanus hyperboreus</i> CIV ^a			15	556	3.4 ± 0.15
<i>Calanus glacialis</i>	26	919	27	833	3.4 ± 0.30
<i>Metridia longa</i>	18	717	14	494	2.7 ± 0.04
<i>Paraeuchaeta glacialis</i>	5	77	8	49	6.6 ± 0.16
<i>Oikopleura</i> spp. ^b	24	993	5	65	1.5 ± 0.42
<i>Eukrohnia hamata</i>	26	756	24	756	23.3 ± 3.88
<i>Parasagitta elegans</i>	10	104	6	134	27.3 ± 5.36
Grand Total	144	4834	132	4108	

^a *C. hyperboreus* CIV not collected from the regional grid.

^b *O. vanhoeffeni* is likely the dominant or only species of *Oikopleura* in our study area, but *O. labradoriensis* could be present.

^cFor copepods, length includes samples collected prior to October 10, December 10 – January 3, February 20 – March 27, and post-May 10; length for other taxa includes all samples.

Figure 2. Time-depth profiles of temperature (Temp, a-c), salinity (d-f) and chlorophyll a (Chl, h-j) for stations sampled across the Beaufort Sea shelf from September 2003 – August 2004. The middle section of each panel, from 10 December to 27 May, is a time series from the overwinter station in Franklin Bay, Amundsen Gulf (70°03'N, 126°18'W) (b, e). The left and right sections of each panel are a composite of stations from the regional grid, incorporating stations from the Mackenzie shelf and Amundsen Gulf. For chlorophyll a, the left scale bar is for the time series at the overwinter station (middle section, i) and the right scale bar is for the regional grid (left and right section, h, j). Note the log scale for chlorophyll a (h-j).
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