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8 Lipid Markers of Diet History and their Retention during Experimental Starvation
9 in the Bering Sea Euphausiid *Thysanoessa raschii*
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32 **Keywords:** euphausiid, lipids, starvation, Bering Sea, krill, fatty acid
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ABSTRACT

Two extended pulsed feeding experiments, following the spring bloom period, investigated lipid retention in the prominent Bering Sea euphausiid (krill) *Thysanoessa raschii*. These experiments occurred during late spring and early summer of 2010. Concurrent taxonomic analysis of the natural algal community allowed prey type to be linked to lipid composition of the natural communities. In late spring, experimental periods of feeding followed by starvation showed an overall decrease in total lipid for *T. raschii*. In early summer, no consistent trend was observed for total lipid with the visible presence of storage lipid in some animals. Polar lipids, as phospholipids, were the dominant krill lipid class in both experiments constituting $\geq 88\%$ of total lipid, and triacylglycerols reached a maximum of 5% of total lipid. The sterols cholesterol and brassicasterol+desmosterol comprised 98-99% of total sterol abundances in *T. raschii* throughout both experiments, even after feeding periods when alternative sterols (i.e. the algal sterol 24-methylenecholesterol) accounted for up to 39% of sterols in potential food particles. Cholesterol abundance and concentration increased during both incubations, likely due to the metabolism of dietary sterols. Major fatty acids observed in krill included C14:0n, C16:0n, C16:1(n-7), C18:1(n-7), C18:1(n-9), C20:5(n-3), and C22:6(n-3) with the diatom-attributed C16:1(n-7) decreasing in abundance and concentration during starvation. Low concentrations of the dinoflagellate-derived sterol and a novel C28:8 PUFA, typically found in dinoflagellates and prymnesiophytes, indicated predation on protozooplankton in early summer when diatom abundances were low. The stability of lipid distributions over periods of starvation and intermittent feeding suggest that fatty acid and sterol biomarkers present in this polar euphausiid principally reflect long-term diet history rather than short-term feeding episodes.

1. INTRODUCTION

Thysanoessaraschii are the most prominent euphausiid (also called krill) in the Bering Sea. Neritic and omnivorous, they typically reside in shallower and less saline waters (32.0-32.4) as compared to the other major regional krill, e.g. *Thysanoessa inermis*, and are widely distributed along the Bering Sea shelf (Pinchuk and Coyle, 2008; Bi et al., 2015). As such, *T. raschii* play a key role in the sub-Arctic food web, particularly as prey for juvenile pollock, marine seabirds, and other higher trophic organisms (Smith, 1991; Adams et al., 2007). Understanding how these krill adapt to seasonal changes in food concentrations in the Bering Sea, and how they utilize stored energy, can provide important insights into its quality as a food source for these predators and overwintering capabilities.

It is generally recognized that *T. raschii* progressively accumulate lipid from early spring through late autumn (Falk-Petersen et al., 1981); however, little information is available that addresses the longer-term changes in lipid concentration and distribution in *T. raschii* in response to a varied food supply. Specific lipid biomarkers have the potential to track dietary history through periods of feeding and starvation, but temporal information on their retention is critical. While few specific sterols or fatty acids can be solely linked to one algal group, multiple markers present among different algal groups can be used to discern feeding history (e.g. Volkman, 1986). Here we report the results of two *T. raschii* experimental incubations of short-term (i.e. pulsed) feeding followed by starvation using natural communities present in late spring and early summer. These incubations examined (1) the effects of starvation on *T. raschii* total lipid and lipid class composition over time and (2) the relative change among individual sterol and fatty acid biomarkers present in krill diets after a pulse of food from a natural algal community. The overall goals were to link lipid biomarker distributions in krill to

their natural diet and to determine how these distributions change during periods of starvation. To our knowledge, this is the first incubation experiment to examine lipid response in sub-Arctic krill using natural phytoplankton and protozooplankton communities followed by extended starvation under near natural conditions.

2. EXPERIMENTAL

2.1 Sampling and Experimental Design

Two *T. raschii* pulsed-feeding starvation and experiments were conducted in late spring (LSp) and early summer (ESu), 2010, using krill from two sites on the Bering Sea Shelf (Figure 1; Table 1) during two consecutive research cruises as part of the Bering Sea Project (see Harvey and Sigler, 2013). Krill were collected using a 60cm Bongo net with black 333µm mesh and solid cod ends, towed obliquely at night to catch animals in good condition during active periods of feeding. Seawater was collected from rosette-mounted Niskin bottles at the krill collection station and one additional station during each cruise for the food pulse in each experiment (four stations, in total). Both *T. raschii* experiments adhered to a similar design (Fig. 2). Healthy, actively swimming individual *T. raschii* with no visible damage were gently placed in filtered sea water (FSW) immediately after collection. Krill were transferred into a 10L carboy filled with FSW, placed in a temperature-controlled incubator (5°C), and allowed to clear their guts for 24 hours. After 24 hours, the krill first subset (T_0) was removed by pouring water from the carboy until the ten krill were obtained, and they were frozen at -80°C; the remaining *T. raschii* were starved for an additional five to six days. A second subset of six or eight krill were then frozen; the remaining animals were transferred into a carboy containing unfiltered Bering Sea water collected at the chlorophyll maximum from the food pulse

stations and allowed to feed. Following a three-day feeding period, a third subset of three krill was frozen, and the remaining animals were transferred into a carboy containing fresh FSW. This group of animals was maintained in FSW and subsampled three more times (three or four more krill per time point) during the subsequent three week (late spring experiment) or two week (early summer experiment) starvation incubations. A total of six subsets of animals were collected and sacrificed over the course of each incubation. This amounted to 30 and 32 animals analyzed for the late spring and early summer, respectively. Each time point is referred to by the experiment's abbreviation (either LSp or ESu) followed by a hyphen and the time point (e.g. LSp-T₀). At each time point, total length (TL) was measured for individual krill, observations on life stage and reproductive state were recorded, and animals composited and frozen at -80°C. It is possible that a small percentage of the animal's original wet weight was lost during the freezing process due to water exclusion. For information regarding krill sub-samples from each experiment, refer to Appendix 1. Seawater was collected at or near the chlorophyll max for all four stations using Niskin bottles; it was subsampled for chlorophyll, algal cell enumeration and identification, lipid analysis, and particulate organic carbon and particulate nitrogen (POC/PN) measurements. Seawater subsampled for lipids, hereafter referred to as water column particles, was filtered onto pre-combusted 47mm GF/F filters and stored at -80°C until processing at the lab. Any visible zooplankton were removed from the filter prior to freezing. For the late spring experiment, the krill and seawater were collected at station 111, and the seawater for the food pulse collected from station 168; the water column particle samples collected from these locations will hereafter be referred to as LSp-I (late spring, initial) and LSp-P (late spring, food pulse). Similarly, for the early summer experiment, *T. raschii* and plankton were collected from station 19

and fed with communities from station 46; the water column samples will be referred to as ESu-I (early summer, initial) and ESu-P (early summer, food pulse), respectively (Fig. 1).

2.2 Lipid extraction and analyses

Composited krill, collected at each experimental time point, were weighed and extracted wet via microwave-assisted solvent extraction (MASE; MARS-5 system) with 2:1 dichloromethane:methanol (DCM:MeOH) (see Harvey et al., 2012 and references therein). Resulting total lipid extract (TLE) was filtered through combusted, solvent-rinsed glass wool to remove particulates; evaporated to dryness; and redissolved in the original 2:1 DCM:MeOH solvent mixture. For krill samples, the TLE was split to accommodate total lipid and lipid class analysis, as well as hydrolysis for individual markers. Base hydrolysis of total lipid used 0.1N KOH with 5 α -cholestane and C19:0n fatty acid serving as the internal standards for the neutral and polar fractions, respectively. Water column particles, which include both living (algal) and non-living detritus, were similarly extracted. The entirety of the particle extract was hydrolyzed and used to measure individual markers.

Analysis of total lipid and lipid classes followed methods of Harvey et al. (2012), with an Iatroscan MK-V TLC-FID instrument with Agilent (ChemStation) integration and quantification. Lipid standards (Sigma Aldrich, Co.)— including dipalmitoylphosphatidylcholine (for polar lipids; PL), cholesterol (for sterols; ST), C19:0n fatty acid (for free fatty acids; FFA), glyceryl trioleate (for triacylglycerols; TAG), and palmityl stearate (for wax esters; WE)— were run in parallel and used to generate response curves to determine concentration.

Following base hydrolysis of total lipid, neutral lipids were partitioned with 9:1 hexane : diethylether. Following acidification with concentrated hydrochloric acid (aq), polar fatty acids

were similarly partitioned. Neutral components were derivatized using BSTFA to form their trimethylsilyl (TMS) products, and fatty acids were converted into their corresponding methyl esters using boron trifluoride (10% in methanol). Both fractions were quantified with an Agilent 6890N gas chromatograph with flame ionization detector (GC-FID) and identified with an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass spectrometer (GC-MS). Both instruments utilized a 60m DB5-MS column. Chromatographic details are described in detail in Belicka et al. (2002), with an inlet temperature of 250°C. Neutral lipids were analyzed in detail; however, for brevity, most individual components were categorized and summed as total alcohols, sterols, tocopherols, wax esters, or glycerol monoethers. For identification of double bond positions of fatty acids in krill, a portion of fatty acids were converted to methyl esters (Destailats and Angers, 2002) to provide confirmatory fragmentation information. Polyunsaturated fatty acids and fatty acids in low concentration were also validated by comparison of retention time and mass spectra of a 52-component fatty acid methyl ester standard (Nu-Chek Prep, Inc.).

2.3 Particulate organic carbon and particulate nitrogen

Water column samples for POC/PN were filtered through pre-combusted 25mm GF/F filters and frozen at -80°C. Filters were thawed, acidified drop-wise with HCl(aq) for an hour in a clean desiccator, and transferred to a 60°C oven to dry. Samples were repackaged in combusted foil packets for analysis by standard methods (Harvey et al., 2012).

2.4 Autotrophic and heterotrophic plankton abundance and biomass

The community taxonomy, size, and trophic composition of microplankton assemblages present where the krill were isolated and in food pulse additions were analyzed to determine the effect of prey type and quantity on the chemical composition of *T. raschii*. Results here, as well as results from other grazing experiments, suggest that *T. raschii* do not consume prey <5 µm in size (Agersted et al. 2010; Lessard, unpublished data). Picoplankton (eukaryotes and cyanobacteria) and some portion of the nanoflagellates (those <5 µm) are probably not part of the prey field available to krill; nevertheless, they do contribute to the particulate lipid pool and have been included here for completeness.

Samples for autotrophic and heterotrophic plankton identification, enumeration and sizing were collected with Niskin bottles and preserved with either acid Lugol's (5% final concentration) or with glutaraldehyde (0.5% final concentration). Glutaraldehyde fixed samples were stained with DAPI and proflavine, and then filtered onto 0.8µm or 0.2µm black polycarbonate membrane filters (Lessard and Murrell, 1996). Slides were stored frozen until analysis onshore.

Live samples were obtained at station LSp-I and analyzed with a benchtop FlowCAM (Fluid Imaging Technologies, Yarmouth, ME) running IMS software to image live plankton samples for semi-quantitative assessments of the > 5 µm plankton community. Samples were gently drawn through 1.4mm silicone tubing and a 2mm x 0.1mm flow cell with a peristaltic pump at a flow rate of 1ml/min for 10 min. Images were collected in the fluorescence/scatter trigger mode using a 10x objective.

2.4.1 Microscope Counts

Known volumes of Lugol's preserved samples were settled in counting chambers and examined with a Zeiss inverted microscope at 400x magnification for diatoms, ciliates and dinoflagellates

>40µm. Glutaraldehyde preserved samples were examined with a Zeiss standard epifluorescence microscope. Slides with 0.8µm filters were observed at 500x magnification for all other dinoflagellates and flagellates >10 µm. Slides with 0.2 µm filters were examined at 1250x magnification for cyanobacteria, picoeukaryotes and nanoflagellates. Autotrophs and heterotrophs were differentiated based on chlorophyll fluorescence.

2.4.2 Biomass Estimation

Picoplankton (cyanobacteria and picoeukaryotes) were sized using images taken with a QImaging Retiga EX charged couple device (CCD) camera and Image Pro Plus software. Measurements of all other cells were determined using a computer aided digitizing system (Roff and Hopcroft, 1986). Cell volumes were calculated using geometric equations. Carbon was calculated using the carbon to volume equations of Menden-Deuer and Lessard (2000) for diatoms, dinoflagellates and nanoplankton; the equations of Worden et al. (2004) were similarly used to calculate carbon for picoplankton.

2.5 Statistical Analyses

Non-parametric tests were used to compare *T. raschii* biomarkers to those of their food using Primer v5 software. A series of group cluster and multi-dimensional scaling (MDS) analyses were run using Bray-Curtis dissimilarity. Cluster analysis results were incorporated into the MDS plots to show groupings at the 30% dissimilarity level for sterols and fatty acids or the 20% dissimilarity level for total lipids. To compare the late spring and early summer krill, the wet weight concentrations of lipid classes were analyzed after a square root transformation. For sterols and fatty acids, each analysis utilized the relative abundance of markers to directly

compare water column particles to krill subsamples with no transformation. Due to the differing metabolic functions of sterols and fatty acids, the two categories of biomarkers were analyzed separately. Similarity percentage analysis (SIMPER) was used to determine the neutral and polar lipid components that differentiated krill from the water column samples in the data sets (70% cut-off). This cutoff was chosen because it included a majority of the overall abundance without including a large number of fatty acids that made little contribution to the overall total. Fatty acids and sterols were, again, analyzed separately.

3. RESULTS

3.1 Autotrophic and Heterotrophic Microplankton Community Structure

The abundance and estimated carbon biomass in each of five autotrophic categories (diatoms, dinoflagellates, nanoflagellates, picoeukaryotes and cyanobacteria) and three heterotrophic protist categories (dinoflagellates, ciliates and nanoflagellates) were counted and calculated for stations LSp-P, ESu-I, and ESu-P (Table 2). Despite the relatively short time difference of several weeks, the two experimental incubations began under differing conditions. Ice had recently retreated at the late spring LSp-I station, the water was cold (-0.9°C), and the phytoplankton community was characterized by ice-associated diatoms. By early summer, at ESu-I, the southeastern shelf system had transitioned to summer conditions and was dominated by small, non-diatom autotrophs and heterotrophic protists.

Late Spring Initial & Food Pulse Microplankton

FlowCAM data showed that the surface algae at station LSp-I, where krill for the late spring experiment were collected, was dominated by ice-associated chain forming pennate diatoms

(*Fragillariopsis* sp.) and the centric diatom, *Thalassiosira* sp.; *Pseudo-nitzschia* sp., *Chaetoceros* sp., and *Entomoneis* sp., were also present. Autotrophic and heterotrophic dinoflagellates and ciliates were rare.

At the food pulse station LSp-P, pelagic centric diatoms dominated the autotrophic biomass (Table 2). *Chaetoceros socialis*, the most abundant and largest phytoplankton biomass component (38% of total autotrophic biomass), was followed by the larger, but less numerous *Thalassiosira* sp. (26% of autotrophic biomass). Cyanobacteria were absent, but picoeukaryotes were present in low abundances. At this stage in the spring bloom, the heterotrophic protist grazers were relatively low in abundance and biomass (21% of the total carbon biomass); dinoflagellates and ciliates dominated the heterotrophic biomass (Table 2).

Early Summer Initial and Food Pulse Microplankton Composition

At station ESu-I, where the early summer experimental krill were collected, heterotrophic nanoflagellates dominated the plankton biomass. Heterotrophic and autotrophic nanoflagellate abundances were similar, but the heterotrophic nanoflagellates contributed the most biomass (49.5% of the total) due to their larger average size. The autotrophic flagellates included cryptophytes and the prymnesiophyte, *Phaeocystis pouchetii*. This station also had the lowest diatom abundance and biomass of the sampled stations, but the greatest abundance of heterotrophs of all stations (63% of the total). Picoeukaryotes and cyanobacteria were present in low numbers and contributed very little to total biomass.

The early summer food pulse station ESu-P was located in a diatom bloom dominated by *Chaetoceros* spp. While other autotrophs were present (picoeukaryotes were in relatively high abundance at ~3000 mL/L), the diatoms made up 95% of the autotrophic biomass, and 60% of

the total biomass. Heterotrophs only contributed 40% of the biomass at station ESu-P; however, of the three stations for which quantitative data was available, this station was characterized by the highest heterotrophic biomass (52 $\mu\text{gC/L}$), comprising large-sized heterotrophic dinoflagellates and ciliates, as well as nanoflagellates.

3.2 Characteristics of the Experimental Krill

The range of krill total lengths was similar for both experiments (17-26 mm), but the late spring experiment tended toward the 18-20mm range (avg. 19.75mm) while the slightly largerearly summer animals (avg. 22.71 mm) were generally in the 21-23 mm range (Appendix 1). All krill in both incubations were adults. In the late spring experiment, 53% of the krill showed evidence of reproductive activity, with developing ovaries and spermatophores in the females and developed sperm glands in the males. None of the females used for lipid analysis were carrying broods of mature eggs. Average total length of krill increased slightly between the initial collection and first subsample, but decreased slightly (an average of 0.09 mm/d) at each subsequent time point. In the early summer experiment there was no evidence of reproductive activity, but 28% of the animals had visible accumulation of clear lipids under the carapace, including the animals removed after the feeding pulse and all animals from the last two subsamples. Average total length increased between the initial collection and first subsample, decreased for the next two subsamples (an average of 0.3 mm/d), then increased for the last two subsamples (an average of 0.16 mm/d).

3.3 The lipid response by *Thysanoessa raschii*

Total lipid concentrations (mg lipid/g wet weight) in bulk krill composites showed different patterns between late spring and summer feeding and starvation regimes (Fig 3A). Late spring krill showed a 58% decrease in total lipid concentration between the beginning and end of the 31-day experiment. Early summer krill did not show any consistent increases or decreases in total lipid during the experiment regardless of the lipid metric. Both late spring and early summer krill showed an increase in lipid stores after the three-day feeding period, as compared to the previous time point with a much larger increase seen in the early summer incubations. After the T₂ time point, individuals in both experiments showed an overall decrease in total lipid at the final (T_f) time points.

The abundance of the five major lipid classes in *T. raschii* varied little between experiments (Fig 3B). Phospholipids (PL) remained the dominant class throughout, accounting for 90-94% of *T. raschii* lipids over the late spring and 89-96% of early summer incubations. The greatest increase in PL abundance occurred in early summer animals after the 3-day grazing period. In contrast, no differences in lipid class abundance were seen for late spring animals fed under nearly identical conditions. Triacylglycerol (TAG), often considered to be the major storage lipid in *T. raschii*, was in low abundance relative to polar lipid, but was higher in larger, early summer animals than in the smaller, late spring krill (1.7-4.9% and 1.1-1.9%, respectively). Average free fatty acids were low (4.9%), but often greater than average TAG abundances (2.3%) in late spring krill. Wax esters, a minor storage lipid for *T. raschii*, averaged 1.1% (late spring) and 1.5% (early summer). Sterol abundances varied little between experiments (0.65 and 0.71%), which likely reflects their largely structural role in krill membranes.

3.4 Neutral Lipid Composition

307 Total neutral lipid concentrations, and relative abundances as a percent of total neutral lipids,
 308 were calculated for alcohols, sterols, tocopherols, waxes, and glycerol monoethers for all
 309 samples over both experimental incubations (Table 3). The dominant alcohol among all samples
 310 was phytol (92-97% of all alcohols), which increased in relative abundance in krill after feeding
 311 in both experiments (Table 3). Other alcohols that contributed at least 1% of the total alcohol
 312 content in samples included C20:1 (0.5-2.5%) and C22:1 (0.8-3%), which displayed no
 313 discernible trend over time. Glycerol monoethers (GME), or monoalkyl glycerol ethers, were in
 314 low abundance in both animal and particle compartments (Table 3). Two tocopherols were
 315 observed in krill at all experimental time points: α -tocopherol (vitamin E) and a tocomonoenol
 316 called marine-derived tocopherol (MDT). In both experiments, α -tocopherol represented 97-99%
 317 of tocopherols, and MDT comprised another 0.8-2.9% (late spring) to 0.4-1.2% (early summer).
 318 The only tocopherol observed in water column particles was α -tocopherol, which was present in
 319 trace quantities (<0.1% neutral lipids). For brevity, all tocopherols have been summed and shown
 320 in Table 3.

321 The relative abundances of sterols in krill and water column particles were measured for the late
 322 spring (Table 4) and early summer (Table 5) experiments. Six sterols comprised >80% of particle
 323 sample sterols at each of the four stations: 24-norcholesta-5,22-dien-3 β -ol (24-
 324 norsterol); cholesta-5,22-dien-3 β -ol (22-dehydrocholesterol); cholesta-5-en-3 β -ol (cholesterol); 24-
 325 methylcholesta-5,24(28)-dien-3 β -ol (24-methylenecholesterol); and the coeluting choelsta-5,24-
 326 dien-3 β -ol (desmosterol) + 23-methylcholesta-5,22-dien-3 β -ol (brassicasterol). In krill,
 327 brassicasterol was in low abundance or absent. Of these sterols, SIMPER analysis revealed
 328 cholesterol, 24-methylenecholesterol, and the combined desmosterol + brassicasterol were the
 329 most prominent contributors to differences among krill and water column particles.

Water column particle samples at both late spring stations LSp-I and LSp-P were dominated by the diatom derived sterols 24-methylenecholesterol (28.1% and 39%, respectively), followed by desmosterol + brassicasterol (26.1% and 17.4%). Together with cholesterol (10.2% for LSp-I and 13.8% for LSp-P) and 24-norsterol (11.8% and 9.8%), those four sterols alone were responsible for 76.2% and 80% of total sterols (Table 4). At the heterotroph-dominated early summer station ESu-I, cholesterol was the primary sterol, followed by brassicasterol+desmosterol (Table 5). Cholesta-5,22-dien-3 β -ol and 24-norsterol each contributed over 10% of the total sterols. The primary sterols found in ESu-P were the same as the two late spring stations; cholesterol abundance was approximately 2x higher than at LSp-I and LSp-P, and the combined brassicasterol + desmosterol about 2x lower in abundance. For all *T. raschii* subsamples, cholesterol and the combined brassicasterol+desmosterol were the most abundant sterols throughout the late spring experiment, comprising 98-99% of all sterols (Tables 4 and 5). Dietary phytosterols decreased in abundance during starvation, with an increase in cholesterol and 5 α -cholestan-3 β -ol (cholestanol) occurred. Cholest-5,7-dien-3 β -ol (7-dehydrocholesterol), also increased in abundance over time.

3.5 Fatty Acids

Among the large suite of observed fatty acids, major groups defined as saturated fatty acids (SFA), monounsaturates (MUFA) and polyunsaturates (PUFA) varied in particle samples reflecting the composition of the plankton community present at each station (Fig 4; Appendices 2 and 3). In contrast, fatty acid abundance and distribution in *T. raschii* remained relatively stable throughout both starvation experiments (Fig 4). Relative percentages of SFA, MUFA, and PUFA (as % SFA / % MUFA / % PUFA) for late spring krill averaged 30.6 / 39.6 / 29.8 over the

entirety of the experiment (31 / 41 / 28 at the beginning; 33 / 36 / 31 at the end). Average abundances shifted only slightly in early summer krill with relative abundances of SFAs increasing and PUFAs decreasing (37.3/ 38.3/ 24.4), with starting ratios of 37/ 39/ 24 and ending at 37 / 38 / 25. In particle samples, station LSp-I, the ice diatom station, ratios were 28.5/ 24.7 / 46.8, reflecting the high abundance of PUFAs, specifically C20:5(n-3) and C22:6(n-3). The two stations with high *Chaetoceros* abundances, LSp-P and ESu-P had ratios of 32.1/ 43.4/ 24.5 and 42.5/ 45.7/ 11.8, respectively. The heterotroph-dominated station LSu-I came in at 29 / 49 / 22.

A suite of seven fatty acids comprised the majority of total fatty acids in all krill and most particle samples: C14:0n, C16:0n, C16:1(n-7), C18:1(n-7), C18:1(n-9), C20:5(n-3), and C22:6(n-3) (Fig.4). These fatty acids comprised 91.3% of the total fatty acids at the beginning of the late spring experiment, decreasing to 88.4% at the end of the experimental period. A similar trend was seen in summer (87.9% at the start of the early summer incubation and 86.1% at the conclusion). SIMPER analyses revealed that these seven fatty acids were responsible for the dissimilarity between krill and particle samples. The heterotroph-dominated station ESu-I, maintained the highest relative abundances of C14:0n and C16:1(n-7) fatty acids of the four particle samples at 10% and 44%. Interestingly, the abundances of C20:5(n-3) and C22:6(n-3) at ESu-I were similar to those at diatom-dominated station LSp-P (10% and 2% versus 10% and 3%, respectively). *Chaetoceros* fatty acids (C14:0, C16:0, and C16:1(n-7)) become more prominent at the late June food pulse, station ESu-P, and *Chaetoceros* also account for even more of the estimated carbon than they did at station LSp-P (Table 2). The highest C20:5(n-3) and C22:6(n-3) PUFA abundances at 14.3% and 12.8%, respectively, occurred at the ice diatom station LSp-I.

By comparison, the next highest abundances for those PUFAs occurred at LSp-P with 10.4% and 3%, respectively.

While the relative abundances of most individual fatty acids did not change more than 3% between the initial and final time points for either *T. raschii* experimental incubation, the C16:1(n-7) fatty acid decreased over 8% between the beginning and end of the late spring experiment. The two SFAs (C14:0n and C16:0n) and C18:1(n-9) were more abundant in the larger, early summer krill than the smaller, early spring krill averaging 7%, 26%, and 11% versus 5%, 23%, and 9%. The opposite held true for the two main PUFAs, (C20:5(n-3) and C22:6(n-3)), and C16:1(n-7), which averaged 18%, 7% and 18% for the late spring krill and 16%, 5%, and 14% for the early summer animals. The C18:1(n-7) showed little change.

3.6 Statistical Analyses

The multidimensional scaling (MDS) plots generated for the krill lipid classes from both experiments (Fig 5A) showed a horizontal separation of the late spring and early summer krill, likely driven by the higher concentration of phospholipid in the summer animals. The sterol MDS plot (Fig 5B) showed three separate clusters: all subsampled *T. raschii*, particle sample ESu-I, and the other three particle samples (LSp-I, LSp-P, and ESu-P). In the fatty acid MDS plot (Fig 5C), there were also three distinct clusters: all subsamples animals, particle sample LSp-I, and the other three particle samples.

4. DISCUSSION

The distribution of individual lipid biomarkers in particle samples accurately reflected the seasonal algal transition from late spring when ice diatoms were prominent to early summer when the

heterotrophic population was steadily increasing. Yet, after three days of grazing on the natural algal community at *in situ* concentrations, the increased lipid stores seen in *T. raschii* in both incubations did not uniformly reflect their available diets. This suggests that lipid composition in krill reflects the longer-term interaction of diet and metabolism and is not rapidly altered by short-term feeding activities. Instead, lipid biomarkers may be more useful as long-term integrators of diet history.

Fundamental differences between *T. raschii* in the late spring incubation and the early summer incubation are apparent in the elevated concentrations of total lipid and increased abundance of TAG storage lipid class in early summer krill, as compared to late spring krill. Early summer krill also appeared to maintain tighter metabolic homeostasis with regard to sterols and fatty acid types (SFA, MUFA and PUFA) throughout the entire incubation versus those in the late spring incubation. That is not to say that dietary lipids are not informative; the diatom-derived C16:1(n-7) fatty acid abundance was nearly 9% higher in spring krill (21.3%) than in summer krill (12.7%), reflecting the decreased presence and consumption of diatom food at their catch stations (LSp-I and ESu-I, respectively). The greatest change in sterol abundances came not from an increase in phytosterols themselves, but from cholesterol as a likely metabolic product (+5.7% in late spring and +1.7% in early summer). Both of these changes are also noted in the wet weight concentrations, and are likely a product of the metabolic pathways associated with short-term starvation. The following subsections discuss physical differences between the *T. raschii* in both incubations, relate biomarkers found in krill to their known or suggested origins, and compare these results with other experiments.

4.1 Role of life stage and reproductive status on lipids

Krill physiology can have a confounding effect on studies that span an extended period of time. Prior studies have shown krill to shrink in size during periods of low food availability or intense reproductive activity (Marinovic and Mangel, 1999; Shaw et al., 2010). In the present study, both of these conditions were present during the late spring experiment. Many of the animals among the first three incubation time points showed clear signs of being reproductively active. Despite this, no krill in the last two time points showed signs of being reproductively active. The average length of individuals also declined steadily over the experimental period. It is possible that by the latter part of the experiment the animals were responding to the low food availability by abandoning reproductive activity and reducing their size as a survival strategy. Thus, although the average animal total length for the late spring experiment was smaller than for the early summer experiment, this may have been driven in part by animals shrinking over the course of the incubation. The food pulses for both experiments were dominated by the same prey item – the diatom *Chaetoceros* – and so, these results may reflect differential responses by the krill in relation to where they were in their seasonal cycle of spawning (late spring) and lipid accumulation (early summer). This is also supported by the visible lipid stores in the carapaces of *T. raschii* observed during the first two time points of the early summer experiment. MDS analysis of krill lipid class abundances showed clear separation of the late spring and early summer krill likely due to the enhanced presence of the TAG storage lipids and PL in the early summer krill.

4.2 Krill and Particle Biomarkers

Several lipid functional forms – sterols, alcohols and waxes, tocopherols and fatty acids – have the potential to provide information regarding the diet history of *T. raschii*. The chemical

signatures of potential krill planktonic prey items or alternative food sources for krill are linked to prey items.

Sterols

Sterols in particles reflected the changing algal communities throughout the late spring experiment. Diatom-associated sterols 24-methylenecholesterol and desmosterol + brassicasterol were the primary sterols present at stations LSp-I and LSp-P, in keeping with the observed phytoplankton distribution. Most notably, 24-methylenecholesterol had a large presence at station LSp-P where *Chaetoceros* sp. was the dominant diatom. Cholesterol and 24-methylenecholesterol are the dominant sterols in some species of *Chaetoceros* (Volkman, 1986 and references therein; Tsitsa-Tzardis et al., 1993). Cholesterol was the third most abundant sterol after the coeluting brassicasterol+desmosterol. Also prevalent were 24-norsterol, commonly attributed to dinoflagellates (Ghosh et al., 1998; Thompson et al., 2004), and 22-dehydrocholesterol, found in significant quantities in diatoms, and to a lesser degree, in numerous other phytoplankton groups (Volkman, 1986; Barrett et al., 1995).

It has been proposed that other crustaceans can convert desmosterol and 24-methylenecholesterol to cholesterol through dealkylation. The most notable change in animal sterols took place during the late spring starvation period: between day 0 and day 6, cholesterol increased in abundance from 90.5% to 94%, and brassicasterol+desmosterol decreased from 7.5% to 4.4%. Interestingly, no relative increases in dietary sterols were observed in the krill after completion of the three-day grazing period. If this metabolic pathway also applies to *T. raschii*, it would explain the gradual decrease in relative abundance of those two dietary sterols and the overall increase in cholesterol in krill during starvation periods in both experiments (Kanazawa, 2001 and references therein).

466 Some algal sterols, though significant contributors to the total available sterols in algal diets,
467 cannot be directly linked to consumption by grazers due to limited assimilation during digestion.
468 For example, the 4α sterols are commonly attributed to dinoflagellates and much less so to
469 diatoms (Dahl et al., 2004 and references therein; Rampen et al., 2010), but are not absorbed
470 by grazing calanoid copepods (Harvey et al., 1987) and likely other crustaceans. This constrains
471 the tracking of specific consumption for some algal groups. In the present study, while
472 dinoflagellates were observed as part of the phytoplankton community, no 4α sterols were
473 observed in any of the krill in either experiment.

474 Cholesterol, which assists in maintaining membrane fluidity, is the most abundant sterol in all *T.*
475 *raschii* subsamples. In order to obtain cholesterol, krill must either consume it directly or modify
476 suitable dietary sterols. Cholesterol is found in prey items like dinoflagellates and diatoms, and
477 may constitute 50% or more of total sterols in some of these microplankton species (Volkman,
478 1986); however, its high concentrations in krill make it difficult to separate the dietary
479 cholesterol contribution from the pre-existing krill cholesterol.

480 Other sterols may be more useful indicators of which plankton group was consumed. For
481 example, cholest-5,7,22-trien-3 β -ol, present at the heterotrophic station ESu-I in abundance of
482 <0.1% and present in all summer krill, holds the potential to be a marker for predation on
483 heterotrophic protists in a marine environment. No autotrophic organisms have been found to
484 contain this sterol, to date, but it has been detected in some strains of yeast, some ciliates, and
485 other heterotrophic protists, such as the heterotrophic dinoflagellate *Lessardia elongata* (Engel et
486 al., unpublished data). In feeding experiments, this triene was not detected in prey items of *L.*
487 *elongata*, but was seen in the predator, suggesting that it is not a dietary constituent, but a
488 product of biotransformation within the heterotrophic dinoflagellate itself. Another potentially

useful sterol marker is 24-methylenecholesterol, which was highly abundant in the water column particles when diatoms, either ice-associated or pelagic, dominated the system. Its abundance in krill decreased throughout the starvation period in both experiments.

The three clusters revealed by the MDS plot of sterol relative abundance for all samples can likely be explained largely by cholesterol and 24-methylenecholesterol (Fig 4B). Separation of krill subsamples from the particle samples, and their tight clustering, may be attributed to the high abundance of cholesterol and the lack of a number of sterols found in the natural community samples. The particle samples from stations LSp-I, LSp-P, and ESu-P all contained large concentrations of diatoms, particularly *Chaetoceros*, and correspondingly 24-methylenecholesterol comprised 17-26% of their total sterols.

Throughout the course of the starvation experiments, most sterols, with cholesterol being a primary exception, decreased in abundance, but did not disappear completely. If sterols are being used as a proxy for grazing on particular taxa and the signatures do not change rapidly over time as seen here, then the sterols can be more reflective of long-term dietary history. Therefore, it is likely more effective to utilize chemical markers to focus on seasonal and spatial changes in phytoplankton composition and diet.

Other neutral lipids

A number of other neutral lipids observed might serve as tracers for dietary history once the time frame for metabolism is established. Wax esters are a minor storage lipid for *T. raschii* (Sargent and Falk-Petersen, 1981). Small amounts of phytol-derived wax esters were seen in *T. raschii*, generally with C14:0 or C16:0 fatty acids comprising the acyl portion of the chain, as determined by mass spectra. Phytol-derived waxes have been documented in a variety of sources ranging

from marine bacteria and dinoflagellates to sediment (Withers and Nevenzel, 1977; Rontani et al., 1999). Determining which wax ester moieties are produced by *T. raschii* versus consumed could provide options for tracking diet history.

The marine-derived tocopherol (MDT) was present in very low concentrations in krill, but absent in particle samples. This tocomonoenol, found in cold-water organisms, has been documented at up to 8% of total tocopherols in the Antarctic *E. superba* (Dunlap et al., 2002), which is much higher than the abundance found here (1-3% of tocopherols in late spring and 0.4-1.2% in early summer). Its origin still remains uncertain, though it is presumed that MDT bioaccumulates through dietary consumption. If an algal group is responsible for its production, MDT could serve as a biomarker for its consumption among higher trophic levels, unlike vitamin E, which is somewhat ubiquitous amongst algal groups (Engel et al., unpublished data).

Fatty Acids

Changes in several fatty acids from water column particles reflected the transition from ice diatoms (LSp-I) to pelagic diatoms (LSp-P) at late spring sampling stations, and then from a heterotroph-dominated station (ESu-I) and back to pelagic diatoms (ESu-P), again, in early summer stations (Fig 4). Cell counts or carbon estimates were not available for ice diatom station LSp-I, but it is known that *Fragillariopsis* sp. was prominent with some *Thalassiosira* sp. also present. These ice diatoms often differ in fatty acid composition versus pelagic diatoms; for example, some species of *Thalassiosira* attribute 19% of total fatty acids to C20:5(n-3), whereas *Chaetoceros*, the pelagic diatom dominant at LSp-P, distributes the majority of its fatty acids between C14:0, C16:0, and C16:1(n-7) (Volkman et al., 1989; Viso and Marty, 1993; Vega et al., 2010). The C22:6(n-3) PUFA, was highest in abundance at the ice diatom-dominated station LSp-

I (12.8%) and much lower (0.4-3.0%) for all other particles samples. Similarly, C20:5(n-3) was also highest at the ice diatom station, but lower in both the water column particles and the krill. While they are not solely markers for ice diatoms, the shift in PUFA relative abundance between particle samples does highlight the change in diet throughout the period of ice retreat. Overall, for this Bering Sea system, higher abundances of C20:5(n-3) and C22:6(n-3) seem to indicate a large ice diatom presence whereas higher amounts of the SFAs C14:0n and C16:0n in addition to C16:1(n-7) convey the transition to more pelagic diatoms (e.g. *Chaetoceros* sp.) and perhaps heterotrophic plankton. Among the 70 different fatty acids identified in both incubation experiments, an unusual C28:8 fatty acid was observed in both the water column particles from station ESp-I and the Day 0 *T. raschii* feeding incubation (Appendices 2 and 3). The (n-3) moiety of this fatty acid has been observed in autotrophic and heterotrophic dinoflagellates, and prymnesiophytes (Mansour et al., 1999; Van Pelt et al., 1999; Mansour et al., 2005). Interestingly, Antarctic krill have also been known to store this unusual C28 fatty acid (Phleger et al., 2002). Small amounts of C24:1 (n-9), a known copepod marker, was also found in all of the early summer animals, but only in some of the late spring animals. The variation in markers between the two krill experiments may indicate differential feeding patterns for these animals that extend outside of the algal regime.

4.3 Comparisons with Antarctic Krill Starvation Experiments

Incubation experiments that analyze changes in krill lipids over time are more labor-intensive, and thus there are fewer of these types of studies with which to compare our results. There are a number of commonalities between the species in this study, *Thysanoessa raschii*, and the Antarctic krill *Euphausia superba*. Both krill exhibit omnivorous tendencies (Falk-Petersen et

al., 2000). Phospholipids make up the majority of their lipid class distribution; TAG is their primary storage lipid, though phospholipids are also likely contributors; and both contain low abundances of wax esters (Sargent and Falk-Petersen, 1981 and references therein; Auerswald et al., 2009; Ju et al., 2009; O'Brien et al., 2011; Harvey et al., 2012). Maximum, summer, and autumn total lipid concentrations, as a percent dry weight, from previous studies were very similar (Falk-Petersen et al., 2000). On that basis, a comparison of general trends in lipid retention and fatty acid distribution between these krill may be reasonable. One short-term starvation experiment (18 days) involving *Euphausiia superba*, which were collected near the end of austral winter before krill begin to build energy stores, found that total lipid concentrations based on dry weight decreased by 40% within the first 5 days, largely attributed to decreases in PL and TAG (Auerswald et al., 2009). These results are similar to the present study in which phospholipids (PL) dropped to 43% of the original concentration for the late spring experiment. While some adjustment is needed for the differences in units (wet versus dry weights) it appears there is commonality in lipid utilization for omnivorous, polar krill during periods of starvation. In a long-term starvation experiment by O'Brien et al. (2011), *E. superba* were monitored for 207 days, subsampling frequently for the first 47 days and then only at day 207. Over the first 26 days, individual total lipid decreased by 54%. On a bulk average basis ($\text{mg lipid} \cdot \text{ind}^{-1}$), there was a 35% decrease in total lipid for the late spring *T. raschii* over by the end of the 31-day period, suggesting that *T. raschii* and *E. superba* have similar responses to starvation conditions when neither species has had a chance to build up lipid stores. As both of these Antarctic *E. superba* experiments by Auerswald et al (2009) and O'Brien et al (2011) showed, PL was always the dominant lipid, and it gradually increased throughout the course of the experiment as TAG

stores were depleted, a trend similar to what was observed for *T. raschii* in the late spring experiment.

CONCLUSIONS

Small, reproductively active *Thysanoessa raschii* exhibit considerable lipid loss under starvation conditions within a short period of time. Early summer adults fare better than the smaller, late spring adults under similar conditions. During conditions of spring and summer phytoplankton communities, most *T. raschii* sterol and fatty acid biomarkers maintain a fairly constant relative abundance, despite fluctuations in total lipid after brief periods of feeding. Unless brought upon by long periods of starvation, subtle changes in lipid compositions appear more reflective of life stage than responses to short-term feeding or transient phytoplankton food sources.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation through the Arctic Natural Sciences program to HRH (ARC-0732667) and EJL (ARC-073289) as part of the Bering Sea Project, with instrumentation support through an MRI award to HRH (OCE-1229197). We would like to thank Jessica Faux and the crew of the R/V *Thomas G. Thompson* for sampling assistance, Carin Ashjian for assistance with the map figure, Calvin Mordy at NOAA for chlorophyll measurements, and Edward Durbin at the University of Rhode Island for the loan of his Bongo net. This is OEASMOGEL Contribution #15-002 of Old Dominion University and BEST-BSIERP Bering Sea Project Contribution #163.

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Figure & Table Legends

Figure 1

Stations sampled for late spring (LSp) and early summer (ESu) pulsed-feeding experiments (map from ETOPO1). Locations at which *T. raschii* and water column particle samples were collected for the initiation of each experiment (-I); locations at which whole water was collected for feeding pulses and particle samples (-P).

Figure 2

Experimental scheme for both krill incubations. Day x (y) indicates the days on which the krill were subsampled where x corresponds to the late spring experiment and (y) to the early summer experiment. The removal of krill for each subsample is indicated by the downward arrow to the krill timepoint. Shaded box indicates the addition of the water column food pulse.

Figure 3

Total lipid normalized to wet weight (●) during late spring (top) and early summer (bottom) *T. raschii* pulsed feeding experiments (A). Corresponding lipid class abundances for each time point are also shown (B). In 'A', numbers in parentheses indicate the number of krill in each composite. The arrows above the graph indicate periods of starvation (FSW = filtered sea water) or feeding (WC = water column pulse). The area between the two dashed lines corresponds to the pulse food period. In 'B', lipid classes are denoted as follows: PL = phospholipid; ST = sterols; FFA = free fatty acids; TAG = triacylglycerols; WE = wax esters.

Figure 4

Fatty acid relative abundance in krill and particle samples throughout each feeding experiment. Particle columns are noted by their sampling station name; krill are indicated by the numeric day they were sampled. Fatty acids present at <5% in krill are excluded for brevity. Pie charts above each bar indicate the percentage of SFA (white), MUFA (black), and PUFA (gray) of total FA in each sample. Note: C18:4(n-3) accounted for 6% of FA at LSp-I, but was ≤2% in all other krill and particle samples, and was therefore excluded from the graph.*Krill after 3 days of grazing on water column (WC) particle additions.

□ C14:0n ■ C16:0n ▣ C16:1(n-7) ■ C18:1(n-9) ▣ C18:1(n-7) □ C20:5(n-3) ■ C22:6(n-3)

Figure 5

Multi-dimensional scaling (MDS) plots of relative abundance of lipid classes for all *T. raschii* subsamples (A), sterols abundances in *T. raschii* and particle samples (B), and fatty acids abundances for krill and particle samples (C). Krill from late spring (LSp-) and early summer (ESu-) have labels appended with their subsampled time point (T₀ through T_f; 6 subsamples in total for each experiment). Water column particles from late spring (LSp) and early summer (ESu) are appended with -I for the initial sampling time or -P indicating it was a food pulse. The ellipses represent the groupings that fall into a dissimilarity level of 5% in A, and 30% in B and C.

Table 1

Summary information for the four sites used for animal and particle incubation experiments. Chl refers to surface chlorophyll. POC and PN numbers correspond to measurements taken at the particle collection depths.

Table 2

Algal taxa abundance and estimated biomass for the four sites at which qualitative and quantitative data were available. (Only qualitative FlowCAM data was available from station LSP-I.) *Ciliates include mixotrophs.

Table 3

Relative abundances of neutral lipids for both particle and *T. raschii* samples throughout both the late spring (LSp) and early summer (ESu) experiments. Total neutral concentrations for all samples are listed. “\” indicates below detection limits; tr=Trace (Abundance <0.1% of neutral lipids); *Particle samples only contained α -tocopherol.

Table 4

The relative abundances of sterols in water column particles (shaded columns) and each krill sub-sample during the late spring (LSp) experiment. Bolded sterols are observed in both krill and water column particles. “\” indicates absent or below detection limits; tr = Trace (Abundance <0.1%); ¹sterols coelute; ²isomers coelute, but the unknown only in particle samples; *sterols that accounted for <0.5% relative abundance in krill and particles (cholest-7-en-3 β -ol; cholest-5,7-dien-3 β -ol; and 24-methylcholest-7-en-3 α -ol) were omitted for brevity.

Table 5

The relative abundances of sterols in water column particles (shaded columns) and each krill sub-sample during the early summer (ESu) experiment. Bolded sterols are observed in both krill and water column particles. “\” indicates absent or below detection limits; tr = Trace (Abundance <0.1%); ¹sterols coelute; ²isomers coelute, but the unknown only in particle samples; *sterols that accounted for <0.5% relative abundance in krill and particles (cholest-7-en-3 β -ol; cholest-5,7,22-trien-3 β -ol; 24-methylcholest-7-en-3 α -ol; 24-methylcholest-24(28)-en-3 β -ol; 23,24-dimethylcholest-5,22-dien-3 β -ol; 24-ethylcholest-7-en-3 β -ol; and 24-propylcholest-5,24-dien-3 β -ol,) were omitted for brevity.

Appendix 1

Physiological information regarding the *T. raschii* krill composites for the late spring and early summer experiments.

Appendix 2

Total fatty acid concentration and percent composition of individual fatty acids in particle samples (shaded columns) and all krill sub-samples taken throughout the late spring experiment. Fatty acids seen in both the water column and krill samples are in bold. \ = absent or below detection limits; tr = Trace (Relative abundance <0.1%); *inclusion of multiple isomers. Fatty acids present in trace abundance in particles only were excluded for brevity and include C12:0n, C13:0i, C13:0a, C13:0n, C14:0a, C23:0n, and C26:0n.

Appendix 3

861 Total fatty acid concentration and percent composition of individual fatty acids in particle
862 samples (shaded columns) and all krill sub-samples taken throughout the early summer
863 experiment. Fatty acids seen in both the water column and krill samples are in bold. \ = absent or
864 below detection limits; tr = Trace (Relative abundance <0.1%); *inclusion of multiple isomers.
865 Fatty acids present in trace abundance in particles only were excluded for brevity and include
866 C12:0n, C13:0i, C14:0a, C26:0n, and C26:1.

Late
Spring

Total Lipid Concentration
(mg Total Lipid / g Wet Weight)

Early
Summer

(B)

Relative Abundance of Lipid Classes
(Percent of Total Lipid)

Relative Abundance of Fatty Acids

Krill subsamples (days) and Associated Particle Samples

(A)

(B)

(C)

Particle Sample Name	Sampling Stations			
	LSp-I	LSp-P	ESu-I	ESu-P
Cruise Name	TN249	TN249	TN250	TN250
Date	6/1/2010	6/8/2010	6/20/2010	6/25/2010
Station Name, No.	70M16, 111	EV-7, 168	CN-6, 19	NP-8, 46
Latitude (°)	57.5001	59.6867	56.8921	57.6650
Longitude (°)	167.6651	177.2954	164.0591	169.5217
Station Depth (m)	72	185	122	71
Tow Depth (m)	46	N/A	57	N/A
Particle Collection Depth (m)	20	23	30	23
Temp (°C)	-0.9	0.1	3.0	2.1
Chl (µg/L)	5.52	4.41	0.74	3.99
POC, n=2 (µg/L)	286 ± 5	287 ± 16	170 ± 97	659 ± 75
PN, n=2 (µg/L)	60 ± 1	56 ± 3	44 ± 22	131 ± 23
POC/PN	4.77	5.13	3.86	5.03

	Abundance (cell·mL ⁻¹)				Estimated Biomass (µg C·L ⁻¹)				Percent Estimated Biomass (%)			
Plankton Group	LSp-I	LSp-P	ESu-I	ESu-P	LSp-I	LSp-P	ESu-I	ESu-P	LSp-I	LSp-P	ESu-I	ESu-P
Autotrophs												
Diatoms	-	853	9	2308	-	64	0.5	85.4	-	76.5	2.6	59.7
Dinoflagellates	-	8	28	2	-	0.7	1.0	0.7	-	0.8	5.3	0.5
Nanoflagellates	-	278	3174	618	-	0.8	5.3	0.9	-	1.0	27.9	0.6
Picoeukaryotes	-	168	264	3037	-	0.2	0.3	3.1	-	0.2	1.6	2.2
Cyanobacteria	-	0	86	391	-	0	0	0.1	-	0	0	0.1
Heterotrophs												
Dinoflagellates	-	39	51	27	-	5.7	1.6	26.4	-	6.8	8.4	18.5
Ciliates*	-	7	3	3	-	9.4	0.9	21.2	-	11.2	4.7	14.9
Nanoflagellates	-	587	3820	2074	-	2.9	9.4	5.0	-	3.5	49.5	3.5
Total	-	1940	7438	8760	-	84	19	143	-	100	100	100

Pleuthner et al. Table 2

Neutral Lipid Composition								
Late Spring (LSp)	LSp-I	LSp-T ₀	LSp-T ₁	LSp-P	LSp-T ₂	LSp-T ₃	LSp-T ₄	LSp-T _f
	Day 0	Day 0	Day 6	Day 6 (post T ₁)	Day 10	Day 17	Day 24	Day 31
Alcohols	34.5	1.5	1.9	61.4	1.5	2.7	1.3	3.2
Phytol	45.6	46.9	35.8	20.0	31.1	43.5	46.2	48.4
Sterols	19.1	49.1	58.4	18.3	62.9	50.0	49.2	44.9
Tocopherols*	tr	1.7	3.3	tr	3.2	3.1	2.6	2.7
Waxes	\	0.3	0.1	\	tr	tr	tr	0.1
Glycerol Monoethers	0.8	0.5	0.5	0.2	1.3	0.7	0.7	0.8
Summed Relative Abundance	100	100	100	100	100	100	100	100
Krill neutral lipid conc. (µg lipid/g WW)	14.6	1888.7	1666.5	21.7	1201.8	1963.5	1975.1	2027.5
Particle neutral lipid conc. (mg lipid/g OC)								

Early Summer (ESu)	ESu-I	ESu-T ₀	ESu-T ₁	ESu-P	ESu-T ₂	ESu-T ₃	ESu-T ₄	ESu-T _f
	Day 0	Day 0	Day 5	Day 5 (post T ₁)	Day 11	Day 18	Day 25	Day 32
Alcohols	70.2	2.5	4.3	23.9	1.9	2.9	3.9	3.0
Phytol	6.6	54.5	54.4	24.6	63.3	60.8	54.2	62.9
Sterols	21.2	33.1	36.3	50.2	31.7	29.6	32.4	30.6
Tocopherols*	tr	2.5	2.7	tr	2.3	2.5	2.1	2.3
Waxes	\	6.5	1.5	\	0.3	3.6	6.6	0.7
Glycerol Monoethers	1.9	0.9	0.8	1.3	0.5	0.7	0.8	0.4
Summed Relative Abundance	100	100	100	100	100	100	100	100
Krill neutral lipid conc. (µg lipid/g WW)	24.4	3109.4	2655.9	7.1	2787.8	3560.1	3094.7	3482.7
Particle neutral lipid conc. (mg lipid/g OC)								

Sterols (Late Spring)	LSp-I	LSp-T ₀	LSp-T ₁	LSp-P	LSp-T ₂	LSp-T ₃	LSp-T ₄	LSp-T _f
	Day 0	Day 0	Day 6	Day 6 (post T ₁)	Day 10	Day 17	Day 24	Day 31
24-norcholest-5,22-dien-3β-ol	11.8	0.2	0.1	9.8	tr	tr	tr	tr
27-nor-24-cholest-5,22-dien-3β-ol	0.7	0.7	0.6	0.7	0.4	0.3	0.1	0.2
cholest-5,22-dien-3β-ol	6.3	tr	tr	5.3	tr	tr	tr	tr
cholest-5-en-3β-ol	10.2	90.5	94.0	13.8	95.8	94.5	96.2	96.2
5α-cholestan-3β-ol	0.7	0.2	0.2	0.5	0.3	0.3	0.3	0.3
cholest-5,24-dien-3β-ol¹	26.1	7.5	4.4	17.4	2.9	4.2	2.8	2.6
24-methylcholest-5,22-dien-3β-ol¹								
24-methylcholest-22-en-3 β -ol	0.6	\	\	0.3	\	\	\	\
24-methylcholest-5,7,22-triene-3 β -ol	0.8	\	\	0.4	\	\	\	\
4 α -methylcholest-8(14)-en-3 β -ol	0.7	\	\	1.0	\	\	\	\
24-methylcholest-5,24(28)-dien-3β-ol	28.1	0.4	0.2	39.0	0.2	0.2	0.1	0.1
24-methylcholest-5-en-3β-ol	2.2	0.1	0.1	1.3	tr	0.1	tr	0.1
24-methylcholest-24(28)-en-3 β -ol	0.4	\	\	1.1	\	\	\	\
23,24-dimethylcholest-5,22-dien-3 β -ol	1.1	\	\	0.5	\	\	\	\
24-ethylcholest-5,22-dien-3 β -ol	0.6	\	\	0.6	\	\	\	\
24-ethylcholest-5-en-3β-ol²	2.8	0.1	0.1	1.8	tr	tr	tr	tr
Unidentified sterol ²								
24-ethylcholestan-3 β -ol	1.3	\	\	0.6	\	\	\	\
24-ethylcholest-5,24(28)-dien-3 β -ol	2.4	\	\	2.2	\	\	\	\
4 α ,23,24-trimethylcholest-22-en-3 β -ol	0.6	\	\	1.0	\	\	\	\
24-ethylcholest-7-en-3 β -ol	0.3	\	\	0.5	\	\	\	\
9,19-cyclo-9 β -lanost-24-en-3 β -ol	0.7	\	\	0.6	\	\	\	\
4 α ,23,24-trimethylcholest-8(14)-en-3 β -ol	1.2	\	\	1.0	\	\	\	\
Other*	0.4	0.3	0.3	0.6	0.3	0.3	0.4	0.4
SUM:	100	100	100	100	100	100	100	100

Sterols (Early Summer)	ESu-I	ESu-T ₀	ESu-T ₁	ESu-P	ESu-T ₂	ESu-T ₃	ESu-T ₄	ESu-T _f
	Day 0	Day 0	Day 5	Day 5 (post T1)	Day 9	Day 12	Day 16	Day 19
24-norcholest-5,22-dien-3β-ol	17.4	0.1	0.1	9.8	tr	tr	tr	tr
27-nor-24-cholest-5,22-dien-3β-ol	2.7	0.3	0.4	0.7	0.2	0.2	0.1	0.3
cholest-5,22-dien-3β-ol	14.7	tr	tr	4.6	0.1	tr	tr	tr
cholest-5-en-3β-ol	21.9	91.1	92.4	26.7	91.8	91.3	92.7	92.8
5α-cholestan-3β-ol	0.8	0.1	0.3	0.5	0.2	0.3	0.2	0.2
cholest-5,24-dien-3β-ol¹	20.6	7.4	5.9	7.3	6.8	7.2	6.2	6.0
24-methylcholest-5,22-dien-3β-ol¹								
cholest-5,7-dien-3β-ol	1.8	0.1	0.2	\	0.3	0.3	0.3	0.2
24-methylcholest-22-en-3 β -ol	0.4	\	\	0.9	\	\	\	\
24-methylcholest-5,7,22-triene-3 β -ol	2.2	\	\	0.7	\	\	\	\
4 α -methylcholest-8(14)-en-3 β -ol	2.6	\	\	1.3	\	\	\	\
24-methylcholest-5,24(28)-dien-3β-ol	6.2	0.2	0.2	38.3	0.1	0.2	0.1	0.1
24-methylcholest-5-en-3β-ol	0.8	0.3	0.2	1.0	0.2	0.2	0.2	0.1
24-ethylcholest-5,22-dien-3 β -ol	0.5	\	\	0.4	\	\	\	\
24-ethylcholest-5-en-3β-ol²	2.0	tr	tr	1.2	tr	tr	tr	tr
Unidentified sterol ²								
24-ethylcholestan-3 β -ol	0.4	\	\	0.6	\	\	\	\
24-ethylcholest-5,24(28)-dien-3 β -ol	2.4	\	\	3.7	\	\	\	\
4 α ,23,24-trimethylcholest-22-en-3 β -ol	0.6	\	\	0.4	\	\	\	\
9,19-cyclo-9 β -lanost-24-en-3 β -ol	0.6	\	\	0.4	\	\	\	\
4 α ,23,24-trimethylcholest-8(14)-en-3 β -ol	0.6	\	\	0.9	\	\	\	\
Other*	0.8	0.2	0.2	0.6	0.2	0.2	0.1	0.2
SUM:	100	100	100	100	100	100	100	100

Late Spring						
Krill Sample	LSp-T ₀	LSp-T ₁	LSp-T ₂	LSp-T ₃	LSp-T ₄	LSp-T _f
Time Point	Day 0	Day 6	Day 10	Day 17	Day 24	Day 31
Avg Total Length (mm)	19.21 ± 1.02	20.93 ± 2.55	20.80 ± 2.08	19.84 ± 0.75	19.18 ± 1.00	18.62 ± 1.94
No. composited krill	10	6	4	3	3	4
Avg Wet Weight (mg/ind)	65.8	96.1	91.5	68.3	64.7	54.9
Total Lipid Conc (mg lipid/g WW)	112.2	48.8	71.3	82.8	70.2	47.4

Early Summer						
Krill Sample	ESu-T ₀	ESu-T ₁	ESu-T ₂	ESu-T ₃	ESu-T ₄	ESu-T _f
Time Point	Day 0	Day 5	Day 9	Day 12	Day 16	Day 19
Avg Total Length (mm)	21.55 ± 1.75	24.06 ± 2.08	22.46 ± 1.52	21.94 ± 0.88	22.19 ± 0.48	23.04 ± 1.08
No. composited krill	10	8	4	3	3	4
Avg Wet Weight (mg/ind)	97.7	136.2	135.9	105.2	103.3	110.6
Total Lipid Conc (mg lipid/g WW)	139.6	115.5	189.5	170.8	142.9	166.8

Pleuthner et al. Appendix 1

Fatty Acids	(Late	LSp-	LSp-	LSp-P	LSp-	LSp-	LSp-	LSp-

Spring)	I	T ₀	T ₁	LSp-P	T ₂	T ₃	T ₄	T _f
	Day 0	Day 0	Day 6	Day 10	Day 10	Day 17	Day 24	Day 31
				(post T ₁)				
Saturated FA (SFA)								
C12:0n	0.1	\	\	tr	\	\	\	\
C14:0i	0.7	\	\	tr	\	\	\	tr
C14:0n	6.5	5.7	5.7	9.2	4.4	5.2	5.7	5.3
C15:0i	0.5	tr	tr	0.5	tr	tr	tr	tr
C15:0a	0.3	tr	tr	0.3	\	tr	tr	tr
C15:0n	0.5	0.2	0.2	0.7	0.2	0.2	0.2	0.3
C16:0i	0.3	tr	tr	0.3	tr	tr	tr	tr
C16:0n	13.3	23.4	21.8	18.4	21.4	22.7	24.3	25.1
C17:0i	0.1	tr	tr	0.2	tr	tr	tr	tr
C17:0a	tr	tr	tr	tr	tr	tr	tr	tr
C17:0n	0.3	tr	tr	0.1	tr	tr	tr	tr
C18:0i	\	\	\	\	\	tr	\	tr
C18:0n	4.8	1.7	1.4	1.4	1.7	1.5	1.7	2
C20:0 (methylphytanate)	0.2	\	\	tr	\	\	\	\
C20:0n	0.2	tr	tr	0.1	tr	tr	tr	tr
C22:0n	0.3	\	\	tr	\	\	\	\
C24:0n	tr	\	\	tr	\	\	tr	tr
Monounsaturated FA (MUFA)								
C14:1*	0.1	tr	tr	0.2	tr	tr	tr	tr
C15:1*	0.5	tr	tr	0.8	tr	tr	tr	tr
C16:1 (n-7)	12.0	21.3	23	33.3	20.7	18.7	19.4	12.6
C16:1*	1.5	0.4	0.4	1.5	0.4	0.4	0.4	0.4
C17:1*	0.2	0.1	0.1	0.4	0.1	0.2	0.2	0.2
C18:1 (n-9)	3.1	8.7	7.7	3.4	7.5	9.5	9	11.4
C18:1 (n-7)	3.1	9	8.4	2.6	9.8	9.4	9	8.4
C18:1*	\	0.2	0.2	\	0.3	0.3	0.2	0.2
C19:1*	0.1	tr	tr	0.1	tr	tr	tr	tr
C20:1 (n-5)	1.5	tr	tr	0.2	0.1	0.5	tr	0.9
C20:1*	0.9	0.8	0.8	0.6	0.8	0.5	1	0.6
C22:1*	1.3	0.2	0.2	0.4	\	0.5	0.1	0.4
C24:1 (n-9)	0.2	\	\	tr	\	\	\	tr
C24:1*	\	tr	tr	\	0.1	0.2	0.1	0.2
Polyunsaturated FA (PUFA)								
C16:2*	0.7	1.3	1.4	1.3	1.5	1.5	1.4	1.4
C16:3 (n-1)	0.5	tr	tr	0.9	\	tr	tr	tr
C16:3	0.1	\	\	tr	\	\	\	\
C16:4 (n-3)	0.7	0.2	0.2	1.2	0.1	0.2	0.2	0.3
C17:2	0.2	tr	tr	tr	\	tr	tr	tr

C18:2 (n-6)	2.0	0.4	0.4	1.2	0.5	0.5	0.4	0.5
C18:2*	0.7	0.4	0.7	0.4	0.7	0.4	0.6	0.4
C18:3 (n-6)	4.3	0.1	0.1	0.5	0.1	tr	0.1	tr
C18:3	2.2	\	\	2.6	\	\	\	\
C18:4 (n-3)	5.8	0.5	0.4	1.9	0.3	0.4	0.4	0.6
C18:4 (n-6)	\	tr	tr	\	tr	tr	tr	tr
C19:2	\	tr	tr	\	tr	tr	tr	tr
C20:2 (n-6)	0.3	\	\	tr	\	\	\	\
C20:2*	\	0.2	0.2	\	0.3	0.3	0.2	0.1
C20:3 (n-6)	0.4	tr	0.16	0.2	0.1	0.1	0.1	0.1
C20:4 (n-3)	0.6	0.2	0.3	0.5	0.2	0.2	0.2	0.2
C20:4 (n-6)	1.1	0.3	0.4	0.1	0.6	0.5	0.5	0.5
C20:5 (n-3)	14.3	17.7	18.2	10.4	19.5	17.4	17.4	18
C20:5	\	\	\	\	\	0.2	\	0.2
C21:4	\	tr	tr	\	tr	tr	tr	0.3
C21:5	0.2	0.3	0.3	0.1	0.3	0.3	0.3	tr
C22:5*	\	0.4	0.4	\	0.4	0.4	0.3	0.5
C22:6 (n-3)	12.8	5.5	6.1	3.0	7.6	7.2	5.8	7.6
C22:6	\	tr	tr	\	tr	tr	tr	0.3

SFA	28.5	31.3	29.4	32.1	27.9	29.9	32.2	33.0
MUFA	24.7	41.0	41.0	43.4	39.9	40.2	39.7	35.6
PUFA	46.8	27.7	29.6	24.5	32.2	29.9	28.1	31.4

Krill FA Conc (mg FA/g WW)		23.6	21.3		14.1	23.3	21.6	20.9
Particle FA Conc (mg FA/g OC)	39.6			89.2				

Fatty Acids (Early Summer)	ESu-I	ESu-T ₀	ESu-T ₁	ESu-P	ESu-T ₂	ESu-T ₃	ESu-T ₄	ESu-T _f
	Day 0	Day 0	Day 5	Day 5 (post T ₁)	Day 9	Day 12	Day 16	Day 19
Saturated FA (SFA)								
C12:0n	tr	tr	tr	0.1	tr	tr	0.1	tr
C13:0n	tr	tr	tr	0.4	\	tr	tr	tr
C14:0i	0.1	tr	tr	0.3	tr	tr	tr	tr
C14:0n	10.1	6.6	6.5	14.7	6.2	6.0	6.4	6.3
C15:0i	0.2	tr	tr	0.4	tr	tr	tr	tr
C15:0a	0.2	tr	tr	0.3	tr	tr	tr	tr
C15:0n	0.5	0.3	0.3	0.8	0.2	0.3	0.3	0.3
C16:0i	0.4	tr	tr	0.3	tr	\	tr	tr
C16:0n	15.2	27.7	27.3	21.1	29.3	29.1	26.9	27.1
C17:0i	tr	tr	tr	0.2	tr	tr	tr	tr
C17:0a	tr	tr	tr	tr	tr	tr	tr	tr
C17:0n	tr	tr	tr	0.2	tr	tr	tr	tr
C18:0i	\	tr	0.1	\	0.1	0.1	0.1	0.1
C18:0n	1.1	1.9	1.9	3.1	2	2.1	2.3	2.2
C20:0 (methylphytanate)	tr	tr	tr	tr	tr	tr	tr	tr
C20:0n	0.1	tr	tr	\	tr	tr	0.1	tr
C22:0n	tr	tr	0.2	0.1	tr	tr	tr	tr
C23:0n	tr	tr	tr	tr	\	tr	tr	tr
C24:0n	tr	tr	tr	tr	tr	tr	tr	tr
Monounsaturated FA (MUFA)								
C14:1*	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1
C15:1*	0.4	tr	tr	0.9	tr	tr	tr	tr
C16:1 (n-7)	41.7	15.6	14.9	35.6	13.8	13.4	13.6	12.7
C16:1*	2.4	0.6	0.7	2.4	0.7	0.7	0.7	0.7
C17:1*	0.1	0.1	0.2	0.3	tr	0.1	0.2	0.2
C18:1 (n-9)	1.4	11.2	10.9	1.4	12	13	11.5	11.6
C18:1 (n-7)	2.2	8.6	8.4	3.3	9.3	9.3	9	9.6
C18:1*	\	0.3	0.3	\	0.4	0.4	0.3	0.4
C19:1*	tr	tr	tr	0.1	tr	tr	tr	tr
C20:1 (n-5)	0.2	tr	tr	0.2	tr	0.1	tr	0.1
C20:1*	0.5	1.1	1.4	0.5	1.2	1.2	1.3	1.5
C22:1*	0.2	0.6	tr	0.6	0.5	0.6	0.7	0.8
C23:1*	\	tr	tr	\	tr	tr	tr	tr
C24:1 (n-9)	tr	0.2	0.2	tr	0.1	0.2	0.2	0.2
C24:1*	\	tr	tr	\	0.1	tr	0.1	tr
Polyunsaturated FA (PUFA)								
C16:2*	1.4	1.9	2.2	2.7	2.2	2.4	2.3	2.3

C16:3 (n-1)	1.1	tr	tr	0.4	tr	tr	tr	tr
C16:3	tr	tr	tr	tr	\	tr	tr	tr
C16:4 (n-3)	2.5	0.2	0.3	0.7	0.1	0.1	0.2	0.2
C17:2*	0.1	tr	tr	tr	tr	tr	tr	tr
C17:3	\	tr	tr	\	tr	tr	tr	tr
C18:2 (n-6)	0.7	0.8	0.8	1.5	0.7	0.7	0.9	1
C18:2*	0.2	0.3	0.5	0.3	0.5	0.5	0.4	0.4
C18:3 (n-6)	0.5	0.1	0.1	0.3	0.1	0.1	0.1	0.1
C18:3	0.7	tr	tr	0.8	tr	tr	tr	tr
C18:4 (n-3)	1.9	0.7	0.7	0.9	0.6	0.5	0.6	0.6
C18:4 (n-6)	\	tr	tr	\	tr	tr	tr	tr
C19:2	\	tr	tr	\	\	\	\	\
C20:2 (n-6)	0.1	\	\	0.1	\	\	\	\
C20:2*	\	0.3	0.3	\	0.3	0.3	0.3	0.3
C20:3 (n-6)	0.2	0.3	0.3	tr	\	\	\	\
C20:4 (n-3)	0.4	0.4	0.3	\	0.3	0.3	0.4	0.4
C20:4 (n-6)	0.2	0.2	0.2	3.3	0.2	0.2	0.2	0.2
C20:4	\	tr	tr	\	tr	tr	tr	tr
C20:5 (n-3)	9.6	15.9	16.5	0.2	14.9	14.6	16.7	16
C20:5	\	tr	tr	\	tr	tr	tr	tr
C21:4	\	tr	tr	\	tr	tr	tr	tr
C21:5	tr	0.1	0.2	\	0.1	0.1	0.1	0.2
C22:2*	\	\	0.3	\	tr	tr	tr	tr
C22:3	\	tr	tr	\	0.1	tr	tr	tr
C22:5*	\	0.4	0.4	\	0.5	0.4	0.5	0.4
C22:6 (n-3)	2.4	2.3	2.5	0.4	2.2	2	2.2	2.7
C22:6	\	tr	tr	\	tr	tr	tr	tr
C28:8	tr	tr	tr	\	\	tr	tr	tr

SFA	28.5	37.2	36.8	42.4	38.4	38.1	36.8	36.7
MUFA	49.4	38.6	37.3	45.7	38.5	39.4	37.9	38.2
PUFA	22.1	24.2	25.9	11.8	23.1	22.5	25.3	25.1

Krill FA Conc (mg FA/g WW)		80.6	68.5		54	78.9	70.1	57.2
Particle FA Conc (mg FA/g OC)	202.2			84.8				

Table 1

Summary information for the four sites used for animal and particles incubation experiments. Chl refers to surface chlorophyll. POC and PN numbers correspond to measurements taken at the particle collection depths.

Table 2

Algal taxa abundance and estimated biomass for the four sites at which qualitative and quantitative data were available. (Only qualitative FlowCAM data was available from station LSP-I.) *Ciliates include mixotrophs.

Table 3

Relative abundances of neutral lipids for both particle and *T. raschii* samples throughout both the late spring (LSp) and early summer (ESu) experiments. Total neutral concentrations for all samples are listed. “\” indicates below detection limits; tr = Trace (Abundance <0.1% of neutral lipids); *Particle samples only contained α -tocopherol.

Table 4

The relative abundances of sterols in water column particles (shaded columns) and each krill sub-sample during the late spring (LSp) experiment. Bolded sterols are observed in both krill and water column particles. “\” indicates absent or below detection limits; tr = Trace (Abundance <0.1%); ¹ sterols coelute; ² isomers coelute, but the unknown only in particle samples; *sterols that accounted for <0.5% relative abundance in krill and particles (cholest-7-en-3 β -ol; cholest-5,7-dien-3 β -ol; and 24-methylcholest-7-en-3 α -ol) were omitted for brevity.

Table 5

The relative abundances of sterols in water column particles (shaded columns) and each krill sub-sample during the early summer (ESu) experiment. Bolded sterols are observed in both krill and water column particles. “\” indicates absent or below detection limits; tr = Trace (Abundance <0.1%); ¹ sterols coelute; ² isomers coelute, but the unknown only in particle samples; *sterols that accounted for <0.5% relative abundance in krill and particles (cholest-7-en-3 β -ol; cholest-5,7,22-trien-3 β -ol; 24-methylcholest-7-en-3 α -ol; 24-methylcholest-24(28)-en-3 β -ol; 23,24-dimethylcholest-5,22-dien-3 β -ol; 24-ethylcholest-7-en-3 β -ol; and 24-propylcholest-5,24-dien-3 β -ol,) were omitted for brevity.

Appendix 1

Physiological information regarding the *T. raschii* krill composites for the late spring and early summer experiments.

Appendix 2

Total fatty acid concentration and percent composition of individual fatty acids in particle samples (shaded columns) and all krill sub-samples taken throughout the late spring experiment. Fatty acids seen in both the water column and krill samples are in bold. \ = absent or below detection limits; tr = Trace (Relative abundance <0.1%); *inclusion of multiple isomers. Fatty acids present in trace abundance in particles only were excluded for brevity and include C12:0n, C13:0i, C13:0a, C13:0n, C14:0a, C23:0n, and C26:0n.

Appendix 3

Total fatty acid concentration and percent composition of individual fatty acids in particle samples (shaded columns) and all krill sub-samples taken throughout the early summer experiment. Fatty acids seen in both the water column and krill samples are in bold. \ = absent or below detection limits; tr = Trace (Relative abundance <0.1%); *inclusion of multiple isomers. Fatty acids present in trace abundance in particles only were excluded for brevity and include C12:0n, C13:0i, C14:0a, C26:0n, and C26:1.