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Krill feeding on sediment in the Gulf of Maine (North Atlantic)

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ABSTRACT: Krill are key members of many marine ecosystems, serving as a critical trophic link between microscopic organisms and large predators such as whales, fish, and seabirds. Krill feeding is thus important to ecosystem carbon cycling. Traditional approaches to determining *in-situ* krill feeding require *a priori* assumptions, and may have prey-type detection biases. We took a DNA-based approach to measuring *in-situ* feeding by northern krill *Meganyctiphanes norvegica*. The diversity of prey consumed by *M. norvegica in situ* was analyzed for 80 krill at 8 stations throughout the Gulf of Maine (North Atlantic) using peptide nucleic acid mediated polymerase chain reaction (PNA-PCR) clone library sequencing of 18S rDNA. Relative abundance of the 2 most common prey types was measured with quantitative PCR (qPCR) in the guts of 16 krill. The 245 prey sequences recovered from krill gut contents included copepods, salps, phytoplankton, and a poorly known organism found to be sediment associated. *Calanus finmarchicus* and the sediment-associated organism were found most commonly, at 7 and 8 stations, respectively, and their 18S rDNA was present in nearly equal quantities in individual krill guts. *M. norvegica*, like most krill, are typically considered planktivorous; thus krill feeding on sediment organisms represents an unrecognized pathway for carbon flow from the sediment to the pelagic. Calculations suggest that this unrecognized pathway could potentially bring over 100 000 t of carbon annually back into the Gulf of Maine pelagic ecosystem, equivalent to 4% of annual primary production, or the energy demands of 80% of the region's fin whale population.

KEY WORDS: *Meganyctiphanes norvegica* · Gut contents · 18S rDNA · Benthic–pelagic coupling · PNA-PCR

INTRODUCTION

Krill play a key role in trophic dynamics and carbon cycling in many marine systems, by feeding on microscopic prey such as phytoplankton and zooplankton, and efficiently linking them to large predators including whales, fish, and seabirds (Macdonald 1927, Fisher & Goldie 1959, Quetin & Ross 1991). Determining the nature of these lower trophic links is challenging; the small size of krill and their prey and the difficulties of underwater observations in the open ocean exclude the possibility of extensive direct *in situ* krill feeding observations. In lieu of direct observation, a variety of approaches are used to estimate krill *in situ* feeding, most frequently bottle incubation experiments and microscopic examination of gut contents (McClatchie 1985, Båmstedt & Karlson 1998, Lass et al. 2001, Kaartvedt et al. 2002).

Past research has shown that northern krill *Meganyctiphanes norvegica* consume mainly copepods, but are highly adaptable to different food environments both spatially and temporally (Båmstedt & Karlson 1998, Lass et al. 2001, Kaartvedt et al. 2002, Schmidt 2010). *Calanus finmarchicus* is the single species most commonly observed in *M. norvegica* gut contents, making up 64 to 100% of observed prey in 1 study (Båmstedt & Karlson 1998). Other copepods observed in *M. norvegica* gut contents include a variety of species of *Calanus*, *Paracalanus*, *Pseudocalanus*, *Pareucheata*, *Temora*, *Oithona*, and *Acartia*

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(Macdonald 1927, Lass et al. 2001, Kaartvedt et al. 2002, Dalpadado et al. 2008). Phytoplankton, including diatoms and dinoflagellates, can be important prey seasonally, particularly during the spring bloom (Macdonald 1927, Fisher & Goldie 1959, Virtue et al. 2000, Kaartvedt et al. 2002, Dalpadado et al. 2008). Marine detritus may be an important food source, but as it is difficult to visually identify, its importance is poorly quantified (Fisher & Goldie 1959, Mauchline & Fisher 1969, Lass et al. 2001, Dalpadado et al. 2008). Components of terrestrial organisms including fern sporangia, insect eggs, and pine pollen are also occasionally consumed in large amounts by *M. norvegica* (Macdonald 1927, Fisher & Goldie 1959, Lass et al. 2001). *M. norvegica* feeding mainly on *C. finmarchicus* and other herbivorous copepods means they efficiently package carbon from small primary consumers into a size large enough to support fish, seabirds, and whales. The extent to which *M. norvegica* consumes other prey could strongly impact carbon and energy transfer to higher trophic levels, and the amount of organic carbon generated by primary production which is subsequently lost to respiration.

Traditional methods used to study krill feeding, such as incubation experiments and microscopic examination of gut contents, are far from ideal, as they require assumptions about the type of prey consumed and where in the environment krill feed. They can also be biased towards morphologically distinct prey and prey with rigid exoskeletons, leaving the majority of gut contents unidentifiable (Lass et al. 2001). Additional approaches which often compliment microscopic examination of gut contents include the use of biomarkers such as fatty acids and stable isotopes, but interpretation is complex, relies again on *a priori* assumptions of prey type and feeding environment, and may have low temporal and prey type resolution (Lass et al. 2001, Schmidt et al. 2003, Rossi et al. 2008). Recent work with Antarctic krill *Euphausia superba* showed the potential for using prey DNA inside krill guts to detect recent feeding (Martin et al. 2006, Passmore et al. 2006).

Here we took a DNA-based approach, using a krill-specific peptide nucleic acid mediated polymerase chain reaction (PNA-PCR) to create 18S rDNA clone libraries of all eukaryotes in the foreguts of *Meganyctiphanes norvegica*. PNA is a synthetic DNA analogue which blocks polymerase elongation and hence PCR amplification when bound to complimentary DNA (Ørum 2000) and has previously been applied in metazoan systems (Troedsson et al. 2008). We designed a PNA probe specific to krill and located between established universal 18S rDNA primers (Gast et al. 2004). This PNA-PCR approach allowed us to amplify all recently consumed prey without amplifying the overwhelming quantities of krill 18S rDNA from the predator itself. Prey in krill guts were identified by comparing gut-content clone library sequences with reference sequences of known organisms. This approach minimizes prey type assumptions, detecting all recent feeding except bacteriophagy and cannibalism. Prey-type-specific quantitative PCR (qPCR), based on the results of PNA-PCR clone libraries, was used to measure the relative importance of 2 different common prey items in krill diets. Our study focuses on the Gulf of Maine, a productive and historically well-studied region. We used the PNA-PCR and qPCR approach to analyze *in-situ M. norvegica* feeding, with PNA-PCR results based on 80 krill individuals collected at 8 stations throughout the region in late summer and mid-winter, and qPCR results based on a subset of 16 of these krill.

MATERIALS AND METHODS

Field collection of samples

Meganyctiphanes norvegica krill were collected on National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Science Center (NEFSC) Ecosystem Monitoring cruises in the Gulf of Maine in August 2008 (5 stations) and February 2009 (3 stations) (Kane 2007) (Table 1). Krill were immediately preserved in 80% ethanol (Passmore et al. 2006). Live krill were collected from 8 stations in February 2009 with a modified bongo net with non-filtering cod ends. These live krill were used in laboratory feeding experiments to confirm that prey DNA was detectable in krill guts, and to serve as food-deprived controls. On board, live krill were maintained for less than 1 wk in 120 l opaque flowing seawater aquariums at <0.5 krill l^{-1}. Krill (n = 123) were then transferred to a 1240 l darkened flowing seawater tank at <0.1 krill l^{-1} in the lab; all tanks were maintained at near ambient temperatures of around 6°C. In all tanks, krill were fed 2 to 7 d old *Artemia salina* larvae to a final concentration of approximately 60 *A. salina* l^{-1} daily. After 10 d acclimating to the lab environment, selected krill were fed *A. salina*, *Rhodomonas* sp. (CCMP 768 from the Provasoli-Guillard National Center for Marine Algae and Microbiota), or *Thalassiosira weissflogii* (CCMP 1048) for 5 h and then preserved in 80% ethanol. Remaining krill were maintained under the same aquarium conditions. Only visually healthy and actively swimming krill were used in feeding experiments.

Table 1. *Meganyctiphanes norvegica.* Station (Stn) information for individuals (ind.) analyzed for *in situ* feeding

Stn	Date sampled (ddmmyy)	Eastern Standard Time (h)	Latitude	Longitude	Bottom depth (m)	No. of ind.
1	26 08 08	02:00	41° 58.6′	69° 37.0′	207	8
2	26 08 08	00:00	42° 16.3′	69° 28.0′	222	8
3	23 08 08	17:45	42° 06.5′	68° 00.6′	228	8
4	24 08 08	02:10	42° 43.6′	67° 28.7′	201	8
5	24 08 08	23:10	44° 13.4′	66° 54.8′	174	24
6	12 02 09	16:00	41° 53.8′	69° 36.8′	200	8
7	06 02 09	20:15	40° 40.1′	66° 53.1′	199	8
8	10 02 09	17:15	43° 21.2′	67° 34.7′	219	8

Zooplankton hypothesized to be potential krill prey were collected in the Great South Channel in June 2008 on the RV 'Endeavor,' and from the pier at the University of Rhode Island, Graduate School of Oceanography, in Narragansett Bay (41° 29.5′ N, 71° 25.1′ W) in January 2010. In the Great South Channel, potential prey zooplankton were collected using an *in situ* plankton pump and preserved in 95% ethanol. In Narragansett Bay, potential prey were collected with a 1 m ring net and anesthetized with MS222 for identification, sorting, and immediate DNA extraction. Potential phytoplankton prey (*Heterocapsa triquetra*: CCMP 448; *Thalassiosira weissflogii*: CCMP 1048; *Rhodomonas* sp.: CCMP 768; *Isochrysis* sp.; and *Tetraselmis* sp.) were cultured in f/2 (Guillard 1975) in continuous light (15 µmol photons m^{-2} s^{-1}) at 20°C, filtered onto 0.4 µm membrane filters (Nucleopore), and frozen at −20°C.

Sediment and water samples were collected to detect potential prey on NOAA Ecosystem Monitoring cruises in August 2009 and June 2010 from areas in the Gulf of Maine where *Meganyctiphanes norvegica* is typically abundant (Wilkinson Basin: 42° 30′ N, 69° 40′ W, 253 m deep; and Jordan Basin: 43° 25′ N, 67° 42′ W, 259 m deep). Sediment was collected using a standard Ponar grab (23 cm by 23 cm mouth opening), and benthic diatoms, epifauna, and/or benthic organism tracks confirmed that the sediment–water interface was captured. The sediment surface was sub-sampled by gentle scraping with sterile wooden popsicle sticks to a depth of 1 to 5 mm, and approximately 0.25 ml sediment sub-samples were frozen. Water samples of 200 to 500 ml collected from surface waters and near bottom waters in Niskin bottles were gently vacuum filtered (<75 mm Hg) onto 0.4 µm membrane filters (Nucleopore) and frozen at −20°C.

Sample preparation and DNA extraction

Three replicate sets of 8 krill from 1 net tow in the Bay of Fundy, 1 set of 8 krill from 1 net tow at each of the remaining 7 stations, and 3 lab-maintained krill (1 from each feeding condition) were dissected and their foreguts isolated under a stereo microscope at 6 to 20× magnification under sterile conditions. The foregut is the first step of the digestive process, in which prey is further macerated by the gastric mill. Hence, it is the area in which prey DNA is least digested (Suh & Nemoto 1988). Total DNA was immediately extracted from these foreguts using the DNeasy Blood and Tissue kit (Qiagen) with mechanical disruption and overnight lysis. The same procedure was followed to extract DNA from potential zooplankton prey (*Pseudocalanus* sp., *Microcalanus pusillus*, *Metridia lucens*, *Calanus finmarchicus*, *Centropages typicus*, *Oithona* sp., *Acartia tonsa*, *Artemia salina*, and hyperid amphipod sp.), dinoflagellate prey (*Heterocapsa triquetra* CCMP 448), and *Meganyctiphanes norvegica* eyeballs (to prevent contamination from gut contents). DNA was extracted from other potential prey phytoplankton (*Thalassiosira weissflogii*: CCMP 1048; *Rhodomonas* sp.: CCMP 768; *Isochrysis* sp.; and *Tetraselmis* sp.) using the DNeasy plant kit (Qiagen) as per the manufacturer's instructions. Whole-community DNA was extracted from water filters using the DNeasy Blood and Tissue kit (Qiagen) and doubled volumes of lysis buffers to ensure that the entire filter was submerged and evenly lysed. DNA was extracted from 0.25 g sediment samples using the PowerSoil DNA kit (MoBio) as per the manufacturer's instructions.

PNA-PCR method testing

We designed a PNA probe that annealed to the 18S rDNA between universal primers (Gast et al. 2004) and that was complimentary to krill while being highly dissimilar to all other organisms, using an alignment of 18 phylogenetically diverse 18S rDNA sequences covering major planktonic phyla (MegAlign; Clewley & Arnold 1997; Table 2). Quantitative PCR was used to test the efficiency of this probe in reducing amplification of krill DNA, and to measure the non-specific effect of the PNA probe on potential prey DNA. *Meganyctiphanes norvegica* and *Thalassiosira weissflogii* full-length 18S rDNA amplicons were created and purified as described below for potential prey sequencing, and quantified spectrophotometrically (Nanodrop). *M. norvegica* and *T.*

Table 2. Peptide nucleic acid (PNA) probe and selective PCR primer information. Position on 18S rDNA is relative to *Meganyctiphanes norvegica* sequence GU595169. OTU: operational taxonomic unit; -sp: specific

Name	Sequence 5′ to 3′	Species targeted	Position on 18S rDNA	Annealing temperature (°C)
Krill PNA	CGTCGGGTTGTCTTG	*Meganyctiphanes norvegica*	1338–1352	67
OTU A-sp forward	CGGGAAACCTTACTAGGGTAAG	Sediment-associated unknown lineage	1184–1205	56
OTU A-sp reverse	TCACAGACCTGATTTAGCCCG		1423–1443	56
OTU A-sp reverse 2	AACGCGGCCAACTAAACAGCTCG		1321–1345	59
OTU A-sp reverse 3	ATTCGTGATTCACCCCAC		1514–1532	65
Calanus-type forward	GTCCTGCTAAATAGTGTCTGC	*Calanus* spp., *Microcalanus* spp., *Pseudocalanus* spp.	1323–1344	61
Calanus-type reverse	TACCACGAATAGGGTTCAGC		1527–1546	61
Rhodomonas-sp forward	ATGTCCGGGCCTTTCTGC	*Rhodomonas* spp.	654–675	65
Rhodomonas-sp reverse	GGAGTCGCAAATTGACATCCACTG		1031–1055	65
Thalassiosira weissflogii -sp forward	CACACCCTGTGTGAGAACTTGTG	*Thalassiosira* spp.	637–659	67
T. weissflogii-sp reverse	CGGAGTCAAAAAACAACCGCCAATCCT		1026–1056	67

weissflogii 18S rDNA at 10 to 10^5 copies μl^{-1} were used as templates with and without krill-targeted PNA probe at 20 µM final concentration. Reactions were run in 25 µl volumes, with 1× Brilliant II SYBR-Green Master Mix (Agilent Technologies), 0.1 µM each forward and reverse primer (Gast et al. 2004), 1× ROX reference dye, and 5 µl DNA template (2 to 20 000 18S rDNA copies μl^{-1} final concentration), with and without 20 µM krill PNA in 0.02% final concentration trifluoroacetic acid, on an MX3005p optically sensing thermocycler (Stratagene) with reactions without PNA serving as a 5-point standard curve. Thermal cycling consisted of 40 cycles of 94°C for 30 s (denaturing), 67°C for 30 s (PNA binding), 58°C for 30 s (primer binding), and 60°C for 45 s (polymerase extension) (end point fluorescence detection). A low extension temperature prevented disassociation of the PNA during polymerase extension.

PNA-PCR clone library analysis

DNA extracts from the foreguts of 8 krill from a single tow were pooled (2 µl aliquots of each krill gut DNA extract, diluted 10× to a final concentration of ~3 ng μl^{-1}) and amplified in a 20 µl PNA-PCR containing 1× GoTaq Green Master Mix (Promega), 0.5 µM each forward and reverse primer (Gast et al. 2004), 20 µM PNA probe in 0.02% triflouroacetic acid, and 0.5 ng μl^{-1} template DNA. Thermocycling consisted of initial denaturing at 95°C for 30 s, followed by 25 cycles of the PNA-PCR cycling conditions described above and by a final extension at 60°C for 5 min. PNA-PCR amplicons were agarose gel extracted using the Wizard SV gel and a PCR clean-up kit (Promega).

PNA-PCR amplicon clone libraries were created using the pGEM-T Easy Vector system (Promega) as per the manufacturer's instructions. Plasmid inserts from 30 to 50 *Escherichia coli* colonies for each cloning reaction were amplified with 1× GoTaq Green Master Mix, and 0.5 µM each M13 forward and reverse primer (Sambrook et al. 1989) with thermocycling of 95°C for 30 s, then 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s followed by 1 cycle of 72°C for 5 min. Insert amplicons were purified by ethanol precipitation or Qiaquick (Qiagen), quantified (Nanodrop), and sequenced in the forward direction on a 3130xl genetic analyzer (ABI).

Prey sequencing

Reference zooplankton and phytoplankton 18S rDNA genes were sequenced using universal primers (Medlin et al. 1988; GenBank accessions FJ422281, GU595169 and GU594637 – GU594648). Operational taxonomic unit (OTU) A, the most common prey item found in krill guts, was sequenced for full length 18S rDNA using universal primers (Medlin et al. 1988, Rueckert et al. 2011) and OTU A-specific primers (Table 2) from individual krill gut contents and from sediment samples. OTU A-specific primers were designed based on an alignment of 18 sequences of varying divergence from OTU A (MegAlign, IDT Oligo Analyzer). Species-specific

phytoplankton primers were similarly designed and were used to amplify and sequence gut contents of krill from phytoplankton feeding incubations (Table 2). Primer specificity for OTU A-specific sequencing primers (OTU A reverse 2 and 3) and phytoplankton-specific primers (*Rhodomonas*-sp and *Thalasiossira weissflogii*-sp forward and reverse) was tested against DNA extracts of 10 to 15 diverse planktonic organisms. PCR amplification reactions for all krill potential prey, OTU A, and feeding incubation krill gut contents contained 1× GoTaq Green Master Mix, 0.5 µM each forward and reverse primer, and ~1 ng μl^{-1} DNA template. Thermocycling consisted of 95°C for 30 s, 35 cycles of 94°C for 30 s, annealing temperature (Table 2) for 1 min, 72°C for 2 min, followed by 72°C for 10 min. Amplicons were sequenced following established protocols on a 3130xl genetic analyzer.

Sequence data analysis

High-quality clone library sequence reads from krill gut contents were trimmed, aligned (MegAlign and EditSeq – DNAstar), and classified into OTUs based on a 3% sequence divergence cut-off using Kimura 2-parameter distances (Kimura 1980; GenBank accession numbers GU569078 – GU569090). All OTUs, all closest GenBank BLAST hits, previously identified krill prey (Båmstedt & Karlson 1998, Lass et al. 2001, Schmidt 2010), and representatives from diverse eukaryotic lineages (Keeling et al. 2005) from GenBank were aligned using Clustal W (Thompson et al. 1994) with MegAlign (Clewley & Arnold 1997) and Mega4 (Tamura et al. 2007). Minimum evolution, neighbor-joining, and unweighted pair-group method with arithmetic mean (UPGMA) trees were constructed with 5000 bootstraps run from a random seed in Mega4. All trees had similar topology, so only the minimum evolution tree is shown. From this tree, sub-trees were constructed of each group of related krill gut contents and reference prey sequences. Each gut content OTU was classified to the lowest taxonomic group in which it consistently clustered in trees made with different methods. Potential prey zooplankton and phytoplankton sequences were assembled in MegAlign, and OTU A primer walking sequences were assembled in Geneious 5.5.2 (Drummond et al. 2010). The taxonomic position of OTU A was investigated using a MUSCLE alignment (Edgar 2004) and Bayesian tree (Huelsenbeck & Ronquist 2001) of the 830 base pair region for which data are available from a range of other studies, with 1 100 000 iterations, 10% burn-in, 6 gamma categories, and all other parameters at default values in Geneious 5.5.2.

Quantitative PCR (qPCR)

Quantitative PCR was used to quantify the abundance of OTU A and *Calanus*-type copepod 18S rDNA in individual krill guts using 2 primer sets. qPCR conditions were optimized prior to analysis of samples using PCR-amplified plasmid standards, which were included in reactions as described above at 2×10^3 copies μl^{-1} final concentration. Plasmid standards were amplified with M13 forward and reverse primers, and quantified by comparison with 4- or 5-point standard curves. Group-specific qPCR primers were designed using NCBI's primer-BLAST design tool (Rozen & Skaletsky 2000) and following the recommendations of Innis & Gelfand (1990). One primer set was designed to amplify a 237 base pair region of only OTU A and closely-related unidentified sequences (Table 2). OTU A-specific primers were tested against OTU A plasmid inserts, *Meganyctiphanes norvegica*, *Thalassiosira weissflogii*, *Rhodomonas* sp., *Isochrysis* sp., *Tetraselmis* sp., *Artemia salina*, *Acartia tonsa*, and *Calanus finmarchicus*. Amplification efficiency for target sequences was 84.2%. A second set of qPCR primers was designed to amplify a 202 base pair region of only *Calanus* spp., *Pseudocalanus* spp., and *Microcalanus* spp. (Table 2). *Calanus*-type primer specificity was tested against *C. finmarchicus*, *M. norvegica*, *Rhodomonas* sp., *T. weissflogii*, *A. salina*, *Microcalanus pusillus*, *Metridia lucens*, *Oithona* sp., hyperid amphipod sp., *Pseudocalanus* sp., *Centropages* sp., and OTU A plasmid inserts. Calanus-specific primers amplification efficiency was 79.6%. All qPCR runs included melt curve analysis. From each of 4 stations, 4 krill individuals (16 total krill) were each run in duplicate in 2 qPCR runs: 1 with OTU A primers, and 1 with *Calanus* primers. Additionally, the abundance of OTU A 18S rDNA was quantified in the guts of 3 krill which had been maintained in captivity for 10 d on a diet of cultured organisms prior to preservation. The abundance of OTU A in the Gulf of Maine environment was measured with qPCR. Four sediment samples and 16 water samples from the same sampling locations and times were run simultaneously, with all reactions run in duplicate.

All qPCR were run on an Mx3005p including no template controls and 4- or 5-point standard curves of purified 18S rDNA amplicons covering the range

of sample values. Reactions were run in 25 µl volumes, with 1× Brilliant II SYBRGreen Master Mix, 0.1 µM each forward and reverse primer, 1× ROX reference dye, and 5 µl DNA template. All krill gut samples were diluted 100× and all sediment and water column samples diluted 10× in deionized water prior to analysis to minimize effects of inhibitors. Thermal cycling was as follows: 95°C for 30 s, followed by 35 cycles of 94°C for 30 s, 56°C (OTU A) or 61°C (*Calanus*) or 58°C (plasmid standards) for 30 s, 72°C for 45 s (end point fluorescence detection), with melt curve analysis from 55 to 95°C (continuous fluorescence detection). 18S rDNA copy numbers for unknown samples were calculated by linear regression against the standard curve. For measuring OTU A in the guts of lab-maintained krill, 45 cycles were run to maximize sensitivity to potentially very low levels of OTU A.

Carbon flux estimate calculations

Carbon transport by krill benthic feeding from the sediment into the pelagic food web in the Gulf of Maine region was estimated based on the abundance of *Meganyctiphanes norvegica* in the Gulf of Maine using data from NOAA and United States Geological Survey, the proportion of krill diet made up of sediment-associated organisms from qPCR results, and literature values for assimilation efficiency, carbon content, daily ration, and wet weight (WW):dry weight (DW). For the purpose of these calculations, the southern boundary of the Gulf of Maine was defined as the northern edge of Georges Bank at approximately the 100 m isobath, and the eastern boundary was taken as a north–south line from the southern-most point of Nova Scotia, Canada.

To estimate *Meganyctiphanes norvegica* population size and biomass in the Gulf of Maine, we used abundance data from >6000 points of plankton survey data collected as per Kane (2007) from 1977 to 2010 throughout the Gulf of Maine region as part of the NOAA NEFSC Ecosystem Monitoring program (J. Hare pers. comm.). Only krill catches from 17:00 to 05:00 h Eastern Standard Time were used since daylight tows are less likely to catch krill due to net avoidance and near-bottom aggregation behaviors (Sameoto et al.1993). However, this estimate is still conservative, as net catch abundance may be orders of magnitude lower than true abundance due to active net avoidance by krill (Tarling et al. 2010). Average *M. norvegica* abundance per 100 m^3 was calculated for 50 m bottom depth bins. These averages were applied to the area of the Gulf of Maine in each bottom depth bin (Roworth & Signell 1999) to calculate total *M. norvegica* abundance in the Gulf of Maine region. Total *M. norvegica* biomass in the Gulf of Maine was calculated based on the abundance estimates described above and an average krill WW of 0.12 g (Sameoto et al. 1993).

The proportion of *Meganyctiphanes norvegica* diet which consists of carbon from sediment-associated organisms was estimated based on qPCR results. The proportion of sediment carbon was calculated as the mean OTU A 18S rDNA divided by the total measured mean 18S rDNA (mean OTU A + mean *Calanus*). In order to be conservative with respect to 18S rDNA copy number:carbon variations between organisms, and because so little is known about OTU A, we assumed a 10-fold greater 18S rDNA copy number:carbon for OTU A as compared to *Calanus* copepods. Using a 10-fold greater 18S rDNA:carbon for OTU A results in much lower estimates of benthopelagic fluxes than a simple 1:1 ratio.

To estimate total biomass of sediment-associated organisms consumed by *Meganyctiphanes norvegica* in the Gulf of Maine region, we used a daily krill ration of 10% body DW (Båmstedt & Karlson 1998). This total sediment-associated organism DW biomass consumed was converted to carbon based on a 40% prey carbon content for all prey (Tyler 1973) and a 0.2 DW:WW of krill (Tyler 1973). In order to account for metabolic losses of this carbon through respiration by the krill themselves, a 30% krill growth efficiency (Iguchi & Ikeda 1995) was included to give a final estimate of the amount of carbon from the sediments available to higher predators due to krill benthic feeding.

RESULTS

PNA-PCR as a gut content analysis method

By including the krill PNA probe in PCR with universal primers, amplification of krill DNA was reduced to negligible levels, with very little effect on non-target sequences (Fig. 1). When PNA was included in the reaction, the amplification of krill 18S rDNA was less than that of a no PNA control, which had 1000-fold lower 18S rDNA initial template concentration, and eventual amplification was linear rather than exponential. In the presence of krill PNA, amplification of *Thalassiosira weissflogii* 18S rDNA was slightly reduced, with the increase in 18S rDNA to detectable levels delayed from 25 to 28 cycles,

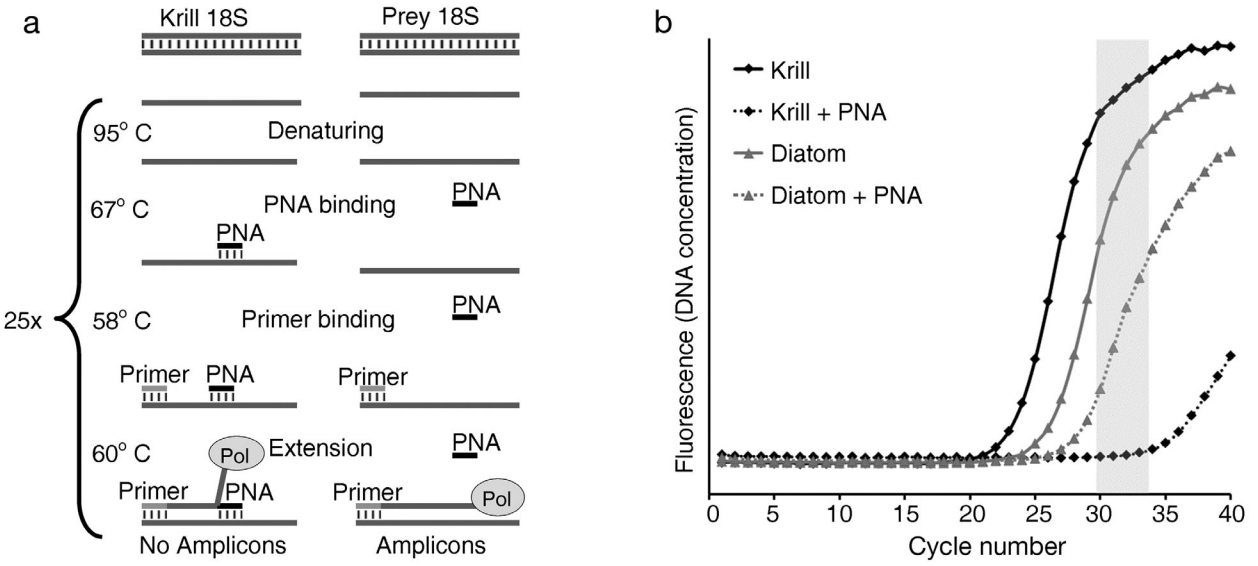

Fig. 1. Effect of a krill-specific peptide nucleic acid (PNA) on DNA amplification of krill and their prey. (a) Schematic of PNA-PCR with krill 18S rDNA and prey 18S rDNA as templates, Pol: DNA polymerase. (b) Effect of krill PNA on amplification of 18S rDNA from the krill *Meganyctiphanes norvegica* and the diatom *Thalassiosira weissflogii*, using qPCR. The shaded bar shows cycle numbers where krill amplification is suppressed by the PNA but where there is ample amplification of diatom DNA in the presence of PNA. Krill and diatom amplification without PNA are shown for comparison

similar to the effect of a 10-fold reduction in initial template concentration (Fig. 1).

Cloning and sequencing of 80 krill guts from 8 stations yielded 308 krill gut clone library sequences that were classified into 33 OTUs. All sequences obtained were eukaryotic and all differed from krill at the PNA site by at least 1 base pair. Twenty OTUs, containing 63 sequences, clustered most closely with krill and were excluded from further analysis. The remaining 13 OTUs, 245 sequences of 261 to 268 base pairs, came from a variety of prey that krill had consumed.

In situ feeding

Krill gut contents clone libraries contained a taxonomically diverse range of prey items (Fig. 2). Differences in the availabilities of reference sequences meant that different prey could be categorized with varying degrees of specificity. Metazoan prey sequences found in krill guts included *Calanus finmarchicus* (OTU B, 100 total clone library sequences), *Centropages* sp. (OTU D, 34 sequences), another unidentified copepod species (OTU C, 3 sequences), and the gelatinous salp *Thalia democratica* (OTU M, 3 sequences). Protist sequences found in krill guts included a dinoflagellate, *Prorocentrum* sp. (OTU K, 1 sequence), a prasinophyte green alga (OTU L, 1 sequence), 2 unidentified alveolate species (OTUs I and J, 1 sequence each), and an unidentified ophistokont (OTU H, 4 sequences). Highly divergent from all taxonomically described lineages, krill gut contents OTUs A (93 sequences), E (2 sequences), F (1 sequence), and G (1 sequence), cluster with sequences found in previous studies of sediment micro-

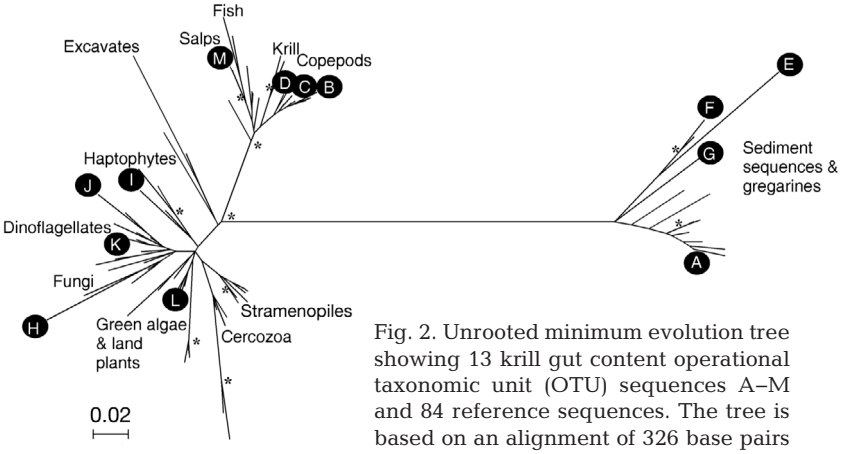

Fig. 2. Unrooted minimum evolution tree showing 13 krill gut content operational taxonomic unit (OTU) sequences A–M and 84 reference sequences. The tree is based on an alignment of 326 base pairs with 5000 bootstraps (Mega4). Labels indicate broad groupings of reference sequences. OTUs are shown as letters in black circles, *: bootstrap values >90%, scale bar = 2% sequence divergence

eukaryotes and with some aquatic gregarines based on both clone library sequences and OTU A full length 18S sequences from primer walking. (Fig. 3).

Of these 13 prey items, different assemblages of prey had been consumed by krill at different locations among the 8 stations sampled (Fig. 4). *Calanus finmarchicus* was absent from krill guts only at Stn 7, where the concentration of *C. finmarchicus* in the water column was <2 ind. m^{-3} (J. Hare pers. comm.; Fig. 4B). OTU A was the only prey item found in krill guts at every station sampled (Fig. 4C). OTU A and *C. finmarchicus* were the only 2 prey items in krill guts at Stns 1 and 6 in the Great South Channel. The unidentified ophistokont was found at Stns 4 and 5 (Fig. 4D). All other prey items were found in krill guts at only a single station. In the Bay of Fundy (Stn 5) krill consumed a prasinophyte, an alveolate, and *Thalia democratica* in addition to the unidentified ophistokont, *C. finmarchicus*, and OTU A. A second unknown alveolate species was found at Stn 4. Just north of Georges Bank at Stn 3, krill gut contents included the copepod *Centropages* sp. Another copepod species was found in krill guts at Stn 2 to the west. Off the southern edge of Georges Bank at Stn 7, *Prorocentrum* sp. and OTUs F and G were found in krill guts in addition to OTU A. No clear seasonal trends were observed in krill gut contents.

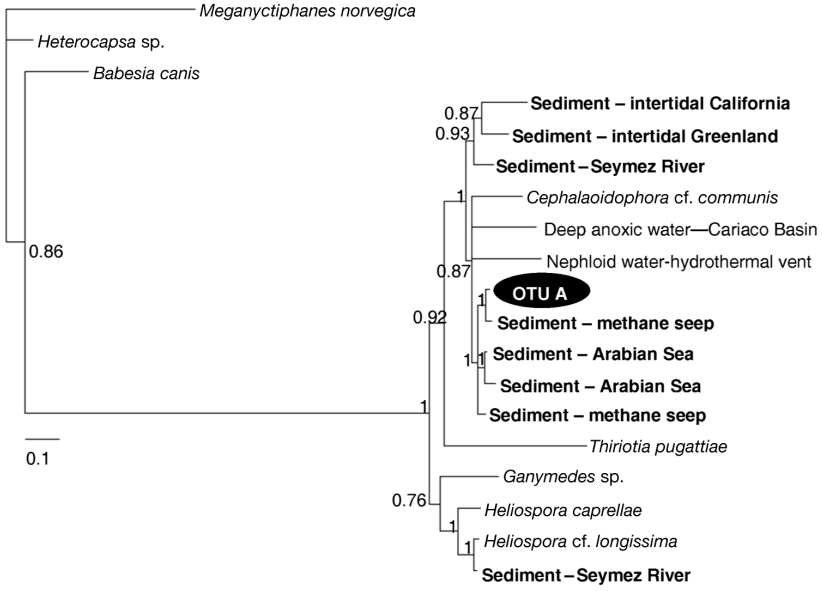

Fig. 3. Bayesian tree of operational taxonomic unit (OTU) A (dark oval), sediment organisms, and gregarine parasites (*Cephalaoidophora*, *Thiriotia*, *Heliospora*, and *Ganymedes* spp.). *Babesia canis* is included as a comparison apicomplexan parasite from mammals. *Meganyctiphanes norvegica* and *Heterocapsa* sp. are included as outgroups. Tree is based on 830 aligned base pairs present in every sequence. Nodes are labeled with posterior probabilities, scale bar indicates 0.1 substitutions per site

In individual krill guts, the abundance of OTU A 18S rDNA rivaled that of *Calanus finmarchicus* 18S rDNA (Fig. 5). Using sequence-specific qPCR, there was no significant difference between the number of 18S rDNA copies per krill of OTU A and *Calanus*-type copepods (OTU A mean 18S rDNA gut^{-1}: $5.15\times10^6 \pm 1.64\times10^6$; *Calanus* mean 18S rDNA gut^{-1}: $3.20\times10^6 \pm 1.36\times10^6$, paired *t*-test, p = 0.4, n = 16). Because these same individual krill had been analyzed with PNA-PCR clone libraries and did not contain other *Calanus*-type copepods, qPCR measurements of *Calanus*-type 18S rDNA in their guts can be attributed entirely to predation on *C. finmarchicus*. Melt curve analysis in qPCR runs showed a single consistent peak, confirming that only the target sequences were amplified. Internal plasmid standards included in initial reactions confirmed that PCR inhibition due to impurities in the DNA extracts was below 10× for all sample types analyzed. All no-template controls had no amplification. OTU A 18S rDNA quantity was close to the mean in every krill analyzed, whereas copepod 18S rDNA was more variable, ranging from undetectable (<10) to 10^7 copies per krill gut.

Three krill individuals which had been maintained in the lab for 10 d contained prey DNA sequences identical to pure culture of the phytoplankton (*Rhodomonas* sp. or *Thalassiosira weissflogii*) they were feeding on immediately before preservation. These same krill did not contain detectable levels of OTU A. In qPCR, lab-maintained krill showed no amplification of OTU A through 45 amplification cycles, whereas standards at 10 copies μl^{-1} increased exponentially in concentration after 33 cycles. All captive krill were collected on the same cruise and in similar areas as krill analyzed for *in situ* feeding. Captive krill individuals not used in the feeding trials survived an additional 6 wk following the feeding experiments and exhibited active swimming and bioluminescence behaviors.

OTU A was abundant in sediments of the Gulf of Maine. In surface sediments, qPCR yielded concentrations of $2\times10^6 \pm 1.5\times10^6$ copies g^{-1} WW (mean ± SD, n = 4). In the overlying water column, both sur-

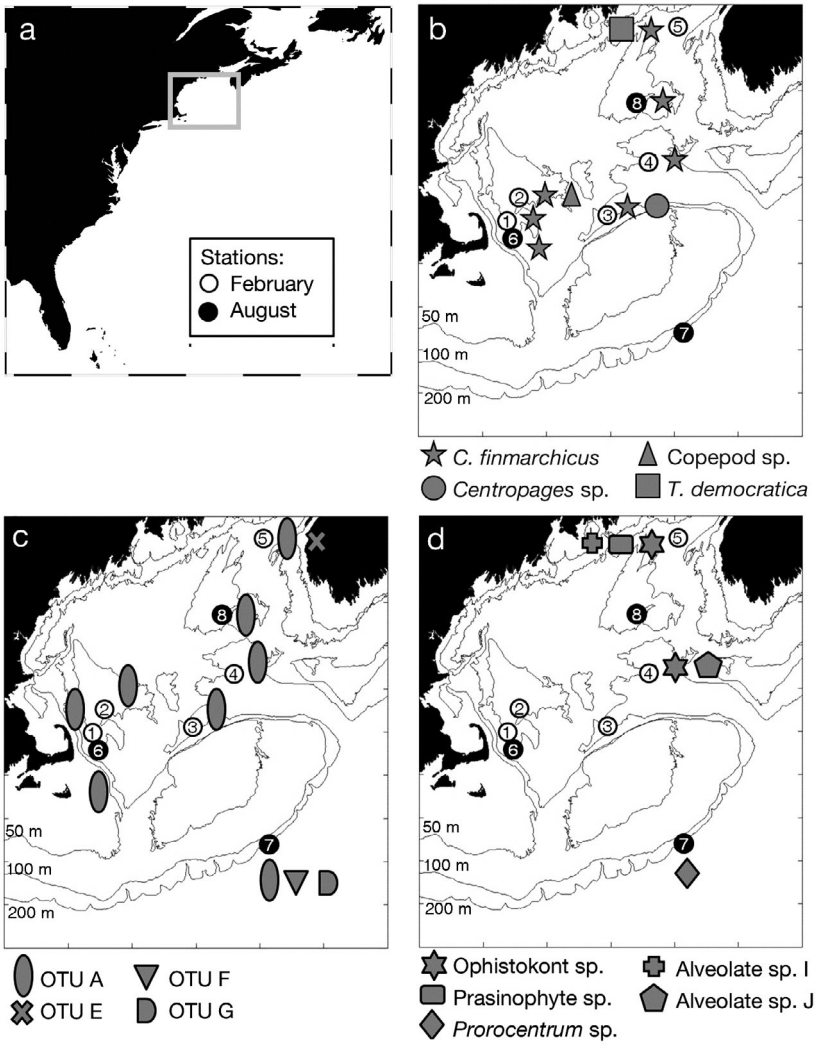

Fig. 4. Sampling locations where each prey item was found in krill guts. (a) US east coast, highlighting the Gulf of Maine (grey box) with station legend for panels b–d (inset); (b) zooplankton; (c) sediment-associated operational taxonomic units (OTUs) A, E, F, and G; (d) phytoplankton and protists. The 50, 100, and 200 m isobaths are shown

face and near bottom, this sequence was relatively rare, with overall mean concentrations 1000-fold less than in surface sediments with $4.5\times10^3 \pm 4.5\times10^3$ copies ml^{-1} (n = 16).

DISCUSSION

The PNA-PCR approach used in this study effectively identified a diverse range of prey items including organisms which might not have been detected with traditional microscopy and incubation methods. Soft-bodied organisms such as salps and prasinophytes are unlikely to be morphologically identified after maceration by krill, but were readily identified by DNA sequence. This approach could be applied to understanding the feeding of any relatively small animal, provided neither cannibalism nor bacteriophagy are potentially important food sources. Universal primers allow for the simultaneous detection of nearly any eukaryote present in the gut contents, minimizing assumptions about prey type or feeding environment, while the krill-specific PNA prevents krill DNA from overwhelming prey signals. In this study, only 20% of the sequences obtained clustered strongly with krill and were subsequently excluded from analysis. These krill-like sequences likely represented artifacts of the method and included truncated sequences, sequences with single nucleotide changes from *Meganyctiphanes norvegica*, and sequences containing insertions. These sequences may have resulted from single-stranded amplification of the region upstream of the PNA or of the unclamped strand, from pseudogenes, or from rare sequence variations between the many 18S rDNA copies within each krill individual (Vestheim & Jarman 2008). Because DNA molecules are sugar-phosphate rich they are subject to many of the same processes as the bulk nutritive component of a prey item, and are thus representative of the amount of biologically useful prey biomass consumed by the predator. The PNA-PCR gut contents analysis approach requires minimal *a priori* sequence knowledge in that only the predator 18S rDNA gene sequence is needed. Large and growing public sequence databases, such as GenBank, provide references allowing for gut contents sequences to be classified to species level in many cases, although for some groups sequence knowledge is still limited.

Meganyctiphanes norvegica diet in the Gulf of Maine consistently relied on *Calanus finmarchicus*, but also included a diverse range of other prey organisms. *C. finmarchicus* has been considered one of, if not the, most important prey for *M. norvegica* in many studies throughout the geographic range of *M.*

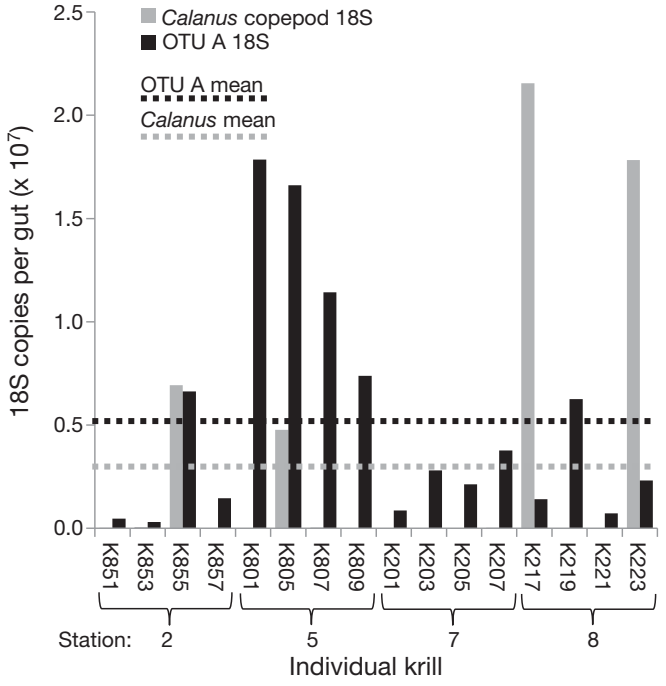

Fig. 5. Abundance of operational taxonomic unit (OTU) A and *Calanus* copepod 18S rDNA copies in guts of individual *Meganyctiphanes norvegica* from the Gulf of Maine measured with qPCR

norvegica using a variety of methodologies (Macdonald 1927, Båmstedt & Karlson 1998, Kaartvedt et al. 2002). In this study, the only station at which krill had not recently consumed *C. finmarchicus* was Stn 7, where *C. finmarchicus* was in very low abundance, suggesting that krill consume *C. finmarchicus* whenever it is available. In addition to *C. finmarchicus*, *Centropages* sp. and another copepod species were found in krill guts at a single station each, indicating that while *C. finmarchicus* is an important prey for *M. norvegica,* other copepods are also consumed by krill, as has been found previously (Macdonald 1927, Båmstedt & Karlson 1998, Kaartvedt et al. 2002).

The salp *Thalia democratica* is seasonally abundant in the Gulf of Maine, but has not previously been considered a prey of *Meganyctiphanes norvegica*. Krill and salps are often considered to feed on similar prey, filling similar trophic niches, and potentially competing for the same food resources (Loeb et al. 1997). The related krill *Euphausia superba* has been observed to feed on salps in captivity (Kawaguchi & Takahashi 1996), although salps are not considered a major prey item. *M. norvegica* feeding on salps adds an interesting dimension to our understanding of krill–salp dynamics in the Gulf of Maine, and could be investigated in other ecosystems.

Phytoplankton prey were relatively rare in krill guts in this study, and unlike previous studies of *Meganyctiphanes norvegica* (Macdonald 1927, Fisher & Goldie 1959, Dalpadado et al. 2008), we did not find diatoms as prey. The primers used in this study to examine krill guts were also used here to amplify the diatom *Thalassiosira weissflogii* (e.g. Fig. 1) and have previously been used to amplify diatom DNA under conditions similar to those employed here (Gast et al. 2004). Thus the absence of diatom DNA in krill guts is unlikely to be a methodological artifact, but more likely indicative of the absence of diatoms from krill diets during the seasons sampled. Krill were sampled here in August and February, whereas krill consumption of phytoplankton has previously been observed mainly in spring when large phytoplankton are most abundant (Kaartvedt et al. 2002). Thus the absence of diatoms in krill diets as measured in this study may be due to sample collection during periods of low diatom abundance.

OTU A: sediment prey or crustacean symbiont?

Krill guts also included a set of sequences (OTUs A, E, F, and G) that were highly divergent from most described organisms, of which OTU A (GenBank accession JQ004804) was by far the most common in krill guts. Database sequences showing similarity to our OTU A were mainly found in sediment samples; however, they also included a gut content sequence from *Euphausia superba*, and purported crustacean parasites (Table 3), raising the question of the source of OTU A in *Meganyctiphanes norvegica* gut contents.

Complete absence of OTU A from the guts of lab maintained-krill, krill foregut grinding morphology, the ubiquity of OTU A in krill guts even in the absence of metazoan prey, high concentrations of OTU A in Gulf of Maine sediments, and broadscale phylogeny all indicate that OTU A measured in *Meganyctiphanes norvegica* gut contents was a sediment-associated organism consumed by the krill and not a symbiont of the krill or their prey. No copies of OTU A were detected in foreguts of krill fed and maintained in captivity for 10 d prior to preservation, suggesting that OTU A was not symbiotic. The parasites previously found in amphipods which show some limited sequence similarity to OTU A have abundances correlated with host maturity stage (Takahashi et al. 2004, 2008, Prokopowicz et al. 2010), suggesting such parasites are accumulated

Table 3. Sequences showing similarity with operational taxonomic unit (OTU) A. SI = sequence identity with OTU A

SI %	Length of overlap (bp)	Accession no.	Source type	Region of origin	Identity	Reference
98	1565	AB275067	Sediment, methane cold seep	Sagami Bay, Japan	Anoxia tolerant taxa, not assignable to any major eukaryotic group	Takishita et al. (2007)
97	228	DQ201542.1	*Euphausia superba* gut contents	Anvers Island, Antarctica	Uncultured eukaryote	Martin et al. (2006)
94	817	GU072099.1	Sediment, anoxic	Arabian Sea	Uncultured eukaryote	Jebaraj et al. (2010)
94	754	AY605189.1	Sediment, river	Geneva, Switzerland	Potential novel high-level taxon	Berney et al. (2004)
91	750	FJ646763	Sediment, oceanic	Weddell Sea, Antarctica	Potential komokiacean foraminiferans	Lecroq et al. (2009)
87	1464	AF372808	Sediment, anoxic intertidal	Bolinas, California, USA	Novel kingdom-level group	Dawson & Pace (2002)
87	1546	AF290084.2	Deep water	Southern Ocean	Potential new lineage	López-Garcia et al. (2001)
87	1605	HQ876008.1	*Balanus glandula* barnacle guts	Vancouver Island, Canada	*Cephaloidophora* cf. *communis* gregarine parasite	Rueckert et al. (2011)
86	1595	AY046643	Nephloid water, hydrothermal vent field	Guaymas Basin, Pacific	Early branch in eukaryotic tree	Edgcomb et al. (2002)
84	1606	HQ891115.1	*Eulimnogammarus vittatus* amphipod guts	Lake Baikal, Russia	*Heliospora* cf. *longissima* gregarine parasite	Rueckert et al. (2011)
84	1602	HQ876007.1	*Caprella alaskana* skeleton shrimp guts	Vancouver Island, Canada	*Heliospora caprellae* gregarine parasite	Rueckert et al. (2011)
84	1324	GU823843.1	Deep water, anoxic	Cariaco Basin, Atlantic	Uncultured eukaryote	Orsi et al. (2011)
82	1613	FJ976721	*Themisto libellula* amphipod guts	Beaufort Sea, Canada	*Ganymedes* sp. gregarine parasite	Prokopowicz et al. (2010)
78	1604	HQ876006	*Pugettia gracilis* kelp crab guts	Vancouver Island, Canada	*Thiriotia pugettiae* gregarine parasite	Rueckert et al. (2011)

over time. Parasites accumulated over a crustacean's life time would be highly unlikely to have been completely eliminated in a short laboratory incubation.

We used only the foregut, the first portion of the krill's digestive system, to detect recently consumed prey. This organ contains the gastric mill and consists of teeth, spines, and armored areas and is used by the krill to further macerate prey (Mauchline & Fisher 1969, Suh & Nemoto 1988, Schmidt 2010); it is therefore not an environment conducive to soft-bodied symbionts, such as gregarines (Rueckert et al. 2011). OTU A was not in krill guts as a parasite of copepods or other metazoan prey since it and related prey sequences OTUs F and G were found in krill guts at Stn 7 where no metazoan prey were found in krill guts. Similarly, OTU A was found in krill guts in the absence of phytoplankton prey in both winter (Stns 6 and 8) and summer (Stns 1, 2, and 3), which would be highly unlikely if krill were filtering OTU A from the water column.

Environmental concentrations of OTU A are 1000-fold higher in sediment than in equivalent volumes of water. Assuming OTU A is a single-celled organism and based on previous estimates of 18S rDNA copy number per cell (Zhu et al. 2005), OTU A was present in the sediment at concentrations of 100 to 1000 organisms g^{-1} sediment but at ≤1 organism ml^{-1} seawater. These concentrations suggest that OTU A is a strongly sediment-associated organism, and that krill most likely consumed this sequence from the sediments. OTU A is much more similar to uncultured sediment organisms (98% identity) than it is to any gregarine sequences (87% identity). This 13% divergence suggests that OTU A is only distantly related to gregarine parasites. For comparison, *Meganyctiphanes norvegica* shows 87% sequence identity over the same length 18S amplicon with the dragonfly *Epiophlebia superstes* (Hovmoller et al. 2002), an organism which is clearly very different in terms of phylogeny, morphology, and lifestyle. Phylogenetic analysis indicates that OTU A clusters strongly with sediment organisms, while marine gregarines cluster more strongly with each other, distinct from sediment organisms (Fig. 3). Most gregarines still remain unknown (Leander 2008), and thus this group may include free-living organisms in addition to gut symbionts of larger animals. With their soft bodies and characteristic gliding motility (Rueckert et al. 2011), the marine sediment surface might be a likely environment for any such as yet

unknown free-living gregarines. The OTU A sequence found in krill foreguts could represent feeding on resting cysts of symbiotic gregarines, on free-living benthic gregarines, or on other poorly known sediment-associated organisms for which sequence data are unavailable.

Further evidence that the OTU A sequence was consumed in association with sediment particles comes from initial assessments of qPCR inhibition by krill gut DNA extracts. Humic acid is known to be a strong PCR inhibitor and is abundant in most sediments (Matheson et al. 2010). At high concentrations (~3 ng μl^{-1}), DNA extracts of krill feeding *in situ* contained OTU A and inhibited qPCR amplification of plasmid standards by 10-fold to 10^6-fold (initial plasmid template/measured plasmid template) (mean reduction 4×10^5-fold). DNA extracts of krill maintained in captivity with no sediment contained no OTU A and had a negligible effect on amplification of plasmid standards at similar krill and plasmid concentrations (mean reduction 4.5-fold). This difference in inhibition between wild krill and captive krill gut DNA extracts suggests that PCR inhibitors came from krill prey consumed *in situ* and were not found in the diets of captive krill, with sediment humic acid a probable explanation. In qPCR reactions used to quantify prey, krill gut DNA extracts were at 100-fold lower concentrations than the initial tests described above, eliminating the problem of inhibition.

Meganyctiphanes norvegica benthic feeding

OTU A is likely not the only sediment material consumed by krill in the Gulf of Maine, but may simply be the most easily detected by 18S rDNA sequencing of gut contents. Other sediment material not detected using our methods could potentially include detritus with degraded DNA, and bacteria. Additionally, the *Prorocentrum* sequence found in krill guts in February may represent feeding on epibenthic cells (Maranda et al. 1999) or resting cysts in the sediment (Matsuoka & Fukuyo 2000). Dinoflagellates are at very low abundance (0.0001–0.045 cells l^{-1}) in the Gulf of Maine water column in mid-winter (Head & Pepin 2010), but are highly abundant in the sediments, with densities commonly above 100 cells cm^{-3}, or 10^5 cells l^{-1} (Anderson et al. 2005). These concentrations suggest that krill most likely consumed this organism from the sediments.

Both *Meganyctiphanes norvegica* and *Euphausia superba* krill have been anecdotally observed feeding on sediment (Macdonald 1927, Mauchline & Fisher 1969, Kawaguchi et al. 1986, Youngbluth et al. 1989, Clarke &Tyler 2008, Hirai & Jones 2012). Recent work in the Southern Ocean has shown that benthic feeding may be a common behavior among *E. superba*, with implications for biogeochemical cycling (Schmidt et al. 2011). Benthic materials such as detritus, lithogenic particles, and filamentous alga have been found in *M. norvegica* gut contents (Schmidt 2010). However, *M. norvegica* continues to be considered mainly a planktivore (Båmstedt & Karlson 1998, Torgersen 2001, Kaartvedt et al. 2002, Link et al. 2007, Schmidt 2010). Krill feeding morphology would appear to be adapted to planktivorous feeding and not to benthic grazing (McClatchie 1985, Schmidt 2010). However, krill appear to adapt their morphology to benthic feeding using behaviors such as nose-diving into the sediment, or lying on or near the sediment beating their pleiopods, and then filter feeding on the resulting cloud of resuspended particles (Macdonald 1927, Mauchline & Fisher 1969, Clarke & Tyler 2008, Schmidt 2010). At the other edge of the water column, *E. superba* are well known to feed on the solid surface of the water sea–ice interface during austral spring (Hamner & Hamner 2000). Thus, krill foraging on the solid interfaces at the edges of their environment is an established behavior, although the contributions of feeding on surfaces are poorly known.

Previous observations of krill benthic feeding raised the question of whether there are 2 separate populations of krill (1 benthic, 1 pelagic) or 1 migrating population of krill feeding in a range of habitats (Youngbluth et al. 1989, Brierley 2008). Our results suggest the latter explanation; all of the krill analyzed in this study were collected in oblique bongo tows, with maximum depths of 5 to 30 m, about 150 to 600 krill body lengths, above the seafloor (174–228 m depth), suggesting that the krill analyzed in this work were not actively engaged in benthic feeding at the moment of collection. Additionally, of the krill individuals analyzed with qPCR, more than half contained both planktonic copepod as well as sediment-associated OTU A 18S rDNA, indicating that the same krill individuals were feeding in both benthic and pelagic habitats.

Meganyctiphanes norvegica are known to exhibit pronounced diel vertical migration throughout their geographic range and to form both surface swarms and near-bottom aggregations under certain conditions (Nicol 1984, Greene et al. 1988, Lass et al. 2001, Hudson & Wigham 2003, Hirai & Jones 2012). In the western North Atlantic, *M. norvegica* form dense near-bottom aggregations which can exceed

2000 krill m^{-3} in the canyons south of Georges Bank (Greene et al. 1988, Youngbluth et al. 1989), as well as in the St. Lawrence estuary (Coté & Simard 2005). Krill diel vertical migration is suggested to represent a compromise between high concentrations of planktonic prey in surface waters and predation avoidance in high light environments (Nicol & Endo 1997, Lass et al. 2001). In the deep canyons south of Georges Bank, as well as in the lower Bay of Fundy and Maine coastal current, krill concentrate near the bottom during the day, and while most of the population ascends through the water column in darkness, some individuals remain consistently near the sediment (Greene et al. 1988, Youngbluth et al. 1989, Thal 2004, Hirai & Jones 2012). This group of krill individuals remaining at depth throughout the diel cycle may be substantial and has been estimated to make up one-third of the total population (Tarling et al. 1998, Hirai & Jones 2012). Krill analyzed in this study were collected from late afternoon through very early morning (Table 1) and represent a cross section of individuals including those recently ascended from depth as well as those who have been in surface waters for several hours. Given their diel vertical migration behavior, krill already at depth during the day for predator avoidance may gain additional nutrition by feeding on the sediment.

The opportunity for sediment feeding at daytime depths may also influence the spatial distribution of *Meganyctiphanes norvegica*. *M. norvegica* is broadly distributed over most continental shelves in the North Atlantic, yet unlike its common prey *Calanus finmarchicus* which is present throughout the basin, *M. norvegica* is rarely found in deep waters beyond the shelf break (Nicol & Endo 1997, Planque et al. 1997, Tarling et al. 2010). Rather, *M. norvegica* is typically most numerous in water depths between 100 and 500 m (Nicol & Endo 1997, Tarling et al. 2010). At these depths, daily vertical migrations could bring krill out of the photic zone and into contact with the seafloor, offering the potential for daytime sediment feeding out of sight of most visual predators. Thus the opportunity for daytime sediment feeding may be one of the factors limiting the distribution of *M. norvegica* to the shelf regions.

Our results suggest that benthic feeding may potentially form ca. 50% of *Meganyctiphanes norvegica in-situ* diets in the Gulf of Maine, with implications for carbon and energy cycling. This conservative estimate was derived from qPCR analysis using specific primers for OTU A and *Calanus* spp. Because the *Calanus* primers used here amplify a 15% shorter region of the 18S rDNA than their OTU A counterparts, our qPCR analysis most likely underestimates the importance of sediment-associated OTU A in krill diet. The length of the qPCR amplicon strongly influences the measured 18S rDNA copy number in zooplankton gut contents, with shorter amplicons more likely to survive preliminary digestion (Troedsson et al. 2009). The relatively constant quantities of OTU A 18S rDNA and the more variable quantities of *Calanus* 18S rDNA in krill guts are consistent with krill grazing on OTU A at a relatively constant rate, while large copepods were captured relatively infrequently. Although during the spring phytoplankton may make up a larger fraction of krill diet, after the spring bloom, sinking phytoplankton may enhance benthic food resources for krill. Thus our winter–summer average is likely to be representative of total fluxes over an annual cycle.

Krill benthic feeding on sediment-associated organisms in the dietary proportion we measured suggests an unrecognized aspect to krill ecology and gives krill an unexpected role in carbon cycling in the Gulf of Maine. Generally in marine ecosystems, organic carbon is fixed by phytoplankton in sunlit surface waters and slowly makes its way to the sediment through sinking phytoplankton and fecal pellets, where it can be sequestered and made unavailable to the pelagic ecosystem. Krill feeding on sediment organisms serves as a direct link between benthic and pelagic habitats, bringing organic carbon back into the pelagic food web and making it available to the wide range of krill predators. In the Gulf of Maine, this pathway may be important as *Meganyctiphanes norvegica* are numerically abundant, particularly in the deeper basins and canyons, and in coastal regions of the western Gulf (Sullivan & Meise 1996, Thal 2004).

There are an estimated 3.5 trillion (3.5×10^{12}) *Meganyctiphanes norvegica* in the Gulf of Maine, with an estimated total biomass of 425 000 t. While uncertainties about the biology of OTU A, and potential seasonal and diel variations in krill feeding behavior prevent an exact quantification of the benthic to pelagic flux, literature values and conservative assumptions allow for calculating a reasonable estimate. We estimate that benthic feeding by *M. norvegica* may potentially bring 108 000 t of carbon back into the pelagic ecosystem annually. This quantity of carbon is equivalent to 4% of the annual primary production in the Gulf of Maine region (O'Reilly et al. 1987) and is greater than the total commercial landings of fish, bivalves, gastropods, and crustaceans in the Gulf of Maine, which are ~150 000 t WW annually, equivalent to 12 000 t of carbon (Link et al. 2006).

Meganyctiphanes norvegica is taken as prey by all but the largest fish in the Gulf of Maine (Garrison & Link 2000) and forms a dietary staple for the resident fin and humpback whales (Brodie et al. 1978, Sourisseau et al. 2006, Waring et al. 2011). Our estimated transport of benthic carbon into the pelagic by krill feeding on sediment-associated organisms is enough to sustain 3200 fin whales (Brodie 1975), which is over 80% of the fin whale population in the western North Atlantic (Waring et al. 2011). Carbon flows through this ecosystem thus also have implications for fisheries management, as well as for the management and protection of marine mammal species. Both krill and OTU A-like sediment-associated organisms are globally distributed, with krill playing keystone roles in the trophic ecology of many marine ecosystems. Thus the return of carbon to the pelagic through krill benthic feeding may represent an unrecognized and important trophic link across many marine ecosystems and in the global cycling of carbon.

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