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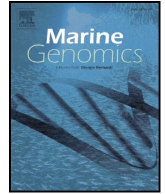
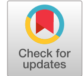


Genomics/technical resources

A transcriptomic resource for the northern krill *Meganyctiphanes norvegica* based on a short-term temperature exposure experiment



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ABSTRACT

The northern krill, *Meganyctiphanes norvegica*, is an important component of the pelagic food web across the North Atlantic. Widespread from the Mediterranean to the Subarctic Atlantic, populations appear to be strongly adapted to local temperatures, and seem to have very little plasticity. The goal of this study was to create and annotate a de novo transcriptome assembly to allow for comparative and physiological studies and to explore the gene expression response of *M. norvegica* from the Gulf of Maine to two different temperature conditions. Our Trinity assembly produced 405,497 transcripts with ~16% annotation success versus *nr* with a stringent cutoff ($>1e^{-10}$), and substantial cross-annotation versus FlyBase and other published pelagic crustacean transcriptomes. There were 122 transcripts that were differentially expressed based on our 2-day 9 versus 12 °C temperature exposure, and their annotation suggested changes in energetic metabolism and molting. These results generate a useful molecular resource for further more directed studies as well as provide initial insight into the physiological processes that may shape the temperature response of the northern krill.

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1. Introduction

Meganyctiphanes norvegica is distributed across the North Atlantic Ocean, from approximately 30° N to 80° N, including the Mediterranean Sea. It is present from shelf-break areas to deep basins, with preference for areas with a depth >100 m. It is one of the largest euphausiids and may be locally dominant, in terms of both number and biomass, in shelf zooplankton assemblages, where it is often found in dense aggregations (Mauchline and Fisher, 1969; Tarling et al., 2010). This species is a strong swimmer, capable of maintaining its position relative to mesoscale water masses, and it exhibits diel vertical migration (DVM), moving to depths > 100 m during the day time (Kaartvedt, 2010; Tarling et al., 2010). Very dense swarms of euphausiids have been observed to maintain their geographical position in the Bay of Fundy despite swift tidal currents (Brown et al., 1979), while single-species swarms in the canyons bordering Georges Bank are persistent despite currents flow along the slope (Greene et al., 1988). However, passive dispersal (drifting) can be significant for early developmental stages, which lack swimming behavior (Tarling, 2010). Spawning happens once per year, but since the life span of individuals may exceed a single year, multiple generations can co-occur at the same location (Tarling, 2010).

Meganyctiphanes norvegica prey spectrum includes phytoplankton, a very wide variety of zooplankton – including other euphausiids (Schmidt, 2010), floating debris (fragments of terrestrial plants, pollen), as well as sediments (Youngbluth et al., 1989; Cleary et al., 2012; Pond et al., 2012). Due to its size, migratory lifestyle, and wide range of prey, the contribution of *M. norvegica* to carbon flux and nutrient cycling is significant in those regions where the species is very abundant. Carbon transfer from surface waters is facilitated by the fast sinking fecal strings produced by *M. norvegica* that can represent up to the 6% of the daily primary production (Youngbluth et al., 1989). Furthermore, the unusual consumption of benthic food particles may constitute an important mechanism for carbon recirculation from the benthos into the pelagic environment; in the Gulf of Maine, this may be equivalent to up to 4% of the annual primary production (Cleary et al., 2012).

Due to its DVM behavior, *Meganyctiphanes norvegica* is a common prey item across the epi- and mesopelagic, epibenthic and demersal communities, and it has been shown to be consumed by a range of other invertebrates, birds, whales and fish (Youngbluth et al., 1989; Jaworski and Ragnarsson, 2006; Simard and Harvey, 2010; Tarling et al., 2010; Hirai and Jones, 2011). Among these predators, there are a large number of species of commercial interest, such as salmon, herring, cod, Atlantic bluefin tuna, for which *M. norvegica* sometimes makes up almost the entire prey composition (Simard and Harvey, 2010; Logan et al., 2011; Renkawitz and Sheehan, 2011; Varela et al., 2013). Furthermore, in the Mediterranean, *M. norvegica* is the main prey item for the

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endangered Mediterranean subpopulation of fin whale *Balaenoptera physalus* (Boucher and Thiriot, 1972; Forcada et al., 1996). Thus, changes in the distribution or abundance of *M. norvegica* could have substantial implications for carbon flux and food webs, with direct impacts on fisheries and endangered species populations. As a consequence there has been recent increased interest in identifying the sensitivities of this species to anthropogenic change.

In the marine environment, temperature is one of the main stressors to which ecosystems are exposed (Halpern et al., 2008; Doney et al., 2012; Byrne and Przeslawski, 2013), and among marine systems, the North Atlantic is one of the most heavily impacted by anthropogenic warming (Halpern et al., 2008). Across the range of *M. norvegica* there is a projected increase of between 2 °C and 5 °C, depending upon latitude, by the end of the century (IPCC, 2007). Within its distributional area, *M. norvegica* shows clear evidence of genetic differentiation, with four proposed major genetic groups: 'northern' NE Atlantic, 'southern' NE Atlantic, Ligurian Sea (likely a Mediterranean subpopulation), and the NW Atlantic (Patarnello et al., 2010). Individuals show physiological adaptation to local temperatures and a strong response to temperature with a $Q_{10} = 2$ (Einarsson, 1945; Mauchline and Fisher, 1969; Saborowski et al., 2002; Spicer and Saborowski, 2010; Tarling et al., 2010; Plourde et al., 2014). In general, 2 °C appears to be the lower limit for the species, while temperatures above 16 °C are lethal for adult stages (Fowler et al., 1971; Saborowski et al., 2002). The strong adaptation of sub-populations to local thermal conditions is of concern as environmental conditions begin to shift due to global warming.

Beyond adaptation and acclimation, however, migration of resilient genotypes among populations could serve as an alternative way for species to resist anthropogenic change. As a consequence of their broad distribution and local adaptation to regional temperature regimes, *M. norvegica* could serve as a useful model for assessing this sort of response. Research on this topic is currently limited by a lack of appropriate molecular tools to adequately track population structure and detail physiological response. A recent study has estimated the haploid genome size of *M. norvegica* to be ~18 Gb (Gigabases) with a haploid chromosome number of 19 (Jeffery, 2012). Despite the large size of the genome, transcriptomic resources can be made available for further directed molecular studies as has been demonstrated with the Antarctic krill, *Euphausia superba* (Meyer et al., 2015).

The objective of this study was thus to 1) create a transcriptome assembly for *Meganyctiphanes norvegica* 2) to annotate this assembly in the context of other pelagic crustacean transcriptomes and important biological functions, and 3) to explore the differential expression patterns of genes associated with a moderate, short-term temperature laboratory exposure (comparing 9 °C and 12 °C). These thermal treatments are representative of the upper range that is experienced by *M. norvegica* during the evening (surface) distribution of individuals of the Gulf of Maine population (Fig. 1; Bigelow, 1924). During the daytime, at depths ranging from 150 to 250 m, *M. norvegica* experience cooler temperatures year round in the Gulf of Maine (5.5–8.5 °C, Maas http://www.bco-dmo.org/dataset/491411/data). Should this region warm as is expected (~2.5 °C in the next 100 years; Pershing et al., 2015), these warmer temperatures will become a larger portion of the seasonal and vertical thermal norm. This resource is intended to provide a basis for further advancements in the knowledge of this key species for the North Atlantic Ocean ecosystem, with a particular focus on the processes of circadian rhythm, molt cycle, metabolism, and thermal sensitivity.

2. Materials and methods

2.1. Krill capture and laboratory exposure

Meganyctiphanes norvegica individuals were collected from multiple depths (100–250 m; temperatures 5.8 to 7.3 °C) from Wilkinson Basin in the Gulf of Maine (42° 21.18 N, 69° 46.99 W; cruise TI787) using a Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS; Wiebe et al., 1985) with 150-μm mesh on November 4th 2014 during the mid-day (Fig. 1; Table 1). Prior to the net tow, water had been collected from ~30 m depth on site using a submersible pump, coarsely filtered with a 64 μm mesh, and stored in large clean trash bins. Upon retrieval, cod ends were quickly sorted through and healthy live adults were placed in 1 L glass jars filled with in situ water with a density of ~10 individuals jar^{-1}. Jars were placed into coolers for transport back to the lab. In the lab, in situ water was transferred into large black barrels (~40 gal) inside an environmental room at 8 °C (one barrel per temperature treatment).

Once in the lab, adult krill were visually examined for mortality, and healthy individuals (actively swimming) were randomly sorted into

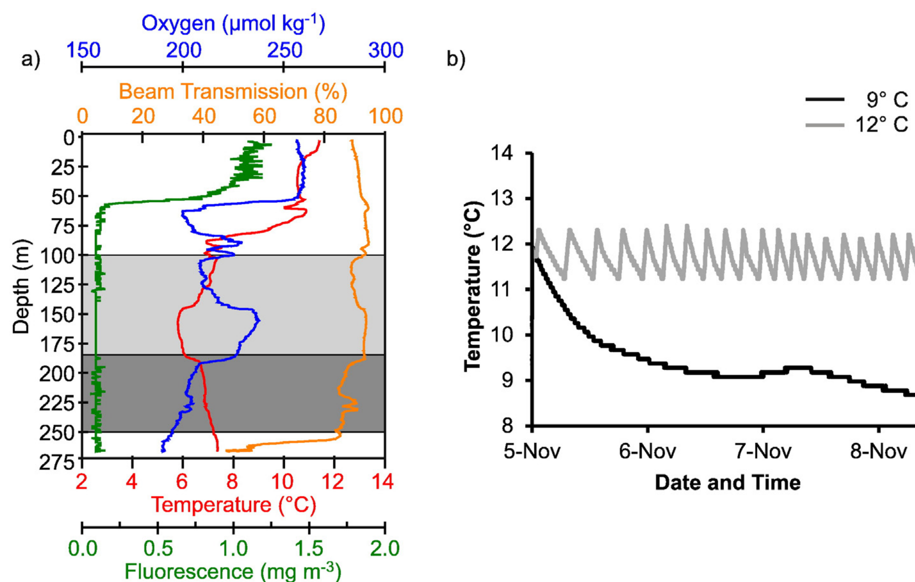

Fig. 1. In situ and laboratory conditions experienced by the krill prior to RNA-Seq analyses. The in situ environment (A) was sampled with a CTD with a SBE3/SBR4 sensor set. Krill were found abundantly in the 180–250 m nets (dark grey) and were present up to 100 m (light grey). Temperature in the laboratory exposure (B) was measured via hobo loggers. In black, temperature recorded in the lower treatment (9 °C). In grey, temperature recorded in the 12 °C treatment. Both treatments started at 12 °C due to the increase of water temperature during transit from the field to the laboratory.

Table 1
Characteristics of the *Meganyctiphanes norvegica* transcriptome sequencing project, compliant with the MIGS standards.

Item	Description
Investigation_type	Eukaryote
Species	*Meganyctiphanes norvegica*
Project_name	Transcriptome sequencing and differential expression of *Meganyctiphanes norvegica*
Geographic location (country and/or sea, region)	Gulf of Maine, USA
Collected_by	L. Blanco Bercial
Collection_date	November 4th 2014
Lat_lon	42° 21.18 N, 69° 46.99 W
Environment (biome)	Marine – pelagic
Rel_to_oxygen	Aerobe
Motility	Yes
Transcriptome assembly data	
Sequencing_meth	Illumina
Sequencing technology	HiSeq2000
Assembly method	De novo
Assembly name	GETT00000000 (Genbank)
Assembly	Trinity v.2.1.1
Finishing_strategy	Draft
Annot_source	BLASTX vs. nr (local)

one of the two barrels (Fig. 2). After equal allocation (40 and 39 individuals for the 9 °C and 12 °C respectively) the barrels were covered with vertical rotating paddles, two opaque lids and a shading net covering all the system to guarantee darkness. The paddles were set to their lowest setting (6 rpm), a heater set to 12 °C was placed in one barrel, and hobo temperatures loggers were added to both barrels at the bottom of the barrel (on the opposite side of the heater in the high temperature treatment). Soft aeration was placed near the bottom of the barrel to guarantee oxygenation but avoiding strong turbulence. Krill were maintained in these conditions for 3.5 days. They were fed 0.75 g of Golden Pearls (300–500 μm; Artemia International) per day, distributed in 3 feeding times. The bottom of each barrel was cleaned by siphoning at the end of day 2 to eliminate fecal residuals, dead individuals and unused food. Less than 3 L of water were removed in this procedure. At the end of the experiment individuals were gently siphoned into a smaller bucket and captured with a soft dip net. Those that were actively swimming were immediately preserved in RNAlater. They were stored frozen for ~4 mo.

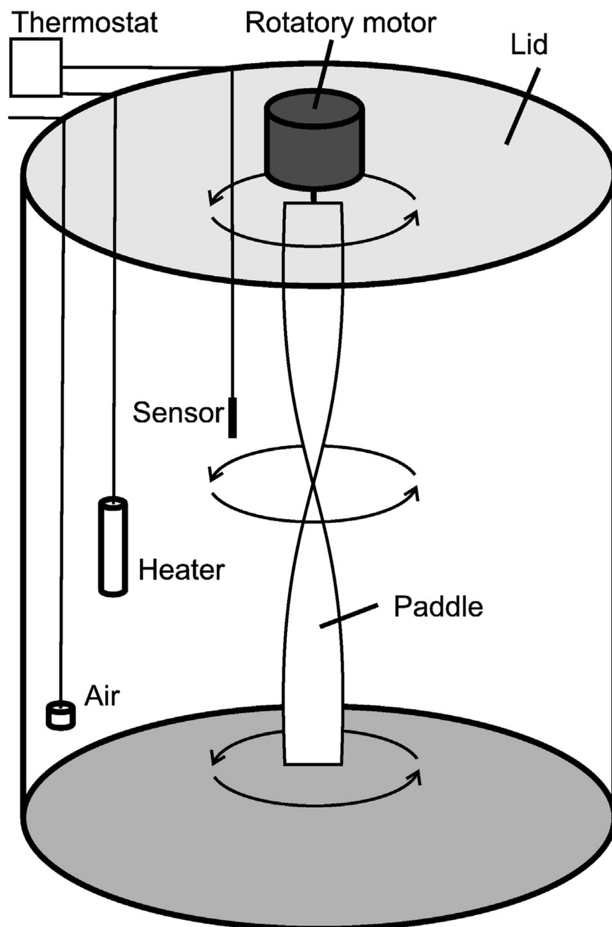

Fig. 2. Culture system. The water inside the 40 gallon barrel was kept in gentle movement by a twisted rotatory paddle hanging from the lid. Rotation was provided with a 12 V electric motor. Soft aeration kept oxygenation levels, without causing turbulent regimes inside the barrel. When placed (12 °C treatment), the heater was located ahead of the aeration (in terms of water circulation) to improve mixing.

2.2. RNA extraction and sequencing

Tail tissue was chosen for the DE experiment to avoid sex-specific differences. Artificial diet should have also prevented from any possibility of gut content contamination, reinforced by using fourth segments. Tails are mostly muscle tissue, responsible for the swimming activity (Kils, 1981). Therefore changes in temperature would likely have an effect of the metabolic signature of this tissue. Tail segments, each weighing 10 mg were excised from individual krill, pooled and extracted in batches of five individuals using the Universal Mini Kit RNA kit (QIAGEN). Five extractions of five individuals each were made for each treatment. Separate RNA extractions were also done with one whole adult female and one whole adult male from the 9 °C treatment. After thorough homogenization in buffer solution (QIAzol), an aliquot of ~1/3 of these whole animal lysates was used for the continuation of the extraction protocol to prevent overloading of the spin column. RNA purity and quantity were checked using a spectrophotometer (Agilent 8453; 260/280 ratios ranging 2.05–2.15). Extracted RNA was sent to the University of Rochester, Genomics Research Center where libraries were constructed using TruSeq library prep kits. One lane, containing the male and female, and one lane containing the ten temperature experiment extractions, was run on an Illumina HiSeq as a 125 bp paired end project.

2.3. Transcriptome assembly and annotation

Raw sequences were quality filtered using the program Trimmomatic (v.0.32; Bolger et al., 2014) to eliminate adapter sequences (settings: 2:30:10) and to discard low quality Phred scores using a sliding window (4:20) that removed both trailing and leading sequences (13) and left only sequences with a minimum length of 15 for downstream use. Using these cleaned sequences, a Trinity assembly (v.2.1.1; Grabherr et al., 2011) was made with the male, the female and one of the pooled high temperature tail samples using default parameters (k-mer = 25; cutoff of 200 bp; total of 7 individuals). The inclusion of the single extraction of 5 pooled tails from the high temperature treatment was to explicitly ensure that genes found in this condition were well represented in the transcriptome. The 9 °C treatment should be thoroughly represented by the single male and female individuals which were both from the 9 °C treatment. To explore the likely proportion of errors due to sequence or assembly error, we clustered the transcripts with CD-HIT EST (v.4.6.1; Li and Godzik, 2006), with a number of similarity cutoffs (Fig. 3). After visually inspecting the graphical representation of the decrease of sequences, we proceeded with downstream analyses of differential expression using the Trinity set of "genes", which is close in transcript number to the 97% similarity cutoff (Fig. 3). Likely a clustering of transcripts at the 92% or 95% similarity is closer to the "real" assembly, but use of these assemblies substantially reduced the read mapping using RSEM (see below), which is not ideal if mapping

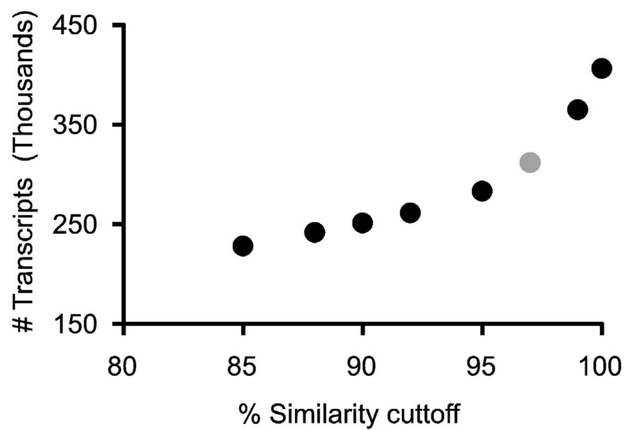

Fig. 3. Number of transcripts in the assembly based on similarity clustering using CD-HIT. The 97% clustering (grey dot) primarily grouped to isoform level (311,761 transcripts, compared to Trinity's report of 319,377 total genes).

Table 2
RNA-Seq and transcriptome assembly statistics.

Metric	Statistic
Total reads	389,536,386 paired
Reads post-trimming	379,719,040 paired
Reads for assembly	215,003,935 paired
Reads mapped (proper pairs)	98.18%
Total number of genes	319,012
Total number of transcripts	405,497
N50	728
Average contig length	549
Median contig length	307
Max contig length	26,644
Min contig length	201
% GC content	38
% annotated (nr > $1e^{-10}$)	16

ambiguity is prevalent among isoforms and for de novo assembled transcripts (as per RSEM readme).

Annotation of the full assembly was done locally using BLASTX vs. nr (database downloaded November 8th, 2015), with an e-value of $1e^{-10}$. Interpro scan and GO annotation were then run in Blast2GO (Conesa et al., 2005). To identify genes of physiological interest for the community, we compared our assembly to the assembly of *Euphausia superba* (Meyer et al., 2015), the most closely related species for which transcriptomic data is available. To do so we converted our transcriptome to a custom BLAST database and searched their 'master' assembly (available from EBI as study PRJEB6147, HACF01000001-HACF01058581) using TBLASTX and an e-value cutoff of $1e^{-5}$. We also reciprocally searched for similar sequences from *Calanus finmarchicus* using a TBLASTX and an e-value cutoff of $1e^{-5}$ and the assemblies of Lenz et al. (Genbank accession GAXK00000000.1; 2014) and Tarrant et al. (Genbank accession GBFB01000001.1; 2014). Finally, we BLASTed the sequences of interest from our transcript against those from the well annotated *Drosophila melanogaster* using a BLASTX search of the translated sequences database of Flybase (FB2016_03).

2.4. Differential expression analyses

Differential expression (DE) analyses were run with the Trinity pipeline (v.2.1.1; Haas et al., 2013). Briefly, samples were aligned to the transcriptome using Bowtie2 (v.2.2.3; Langmead and Salzberg, 2012) and estimates of abundances were made with RSEM (Li and Dewey, 2011). An edgeR analysis of DE was performed with R v.3.0.1 (Robinson et al., 2010), comparing the five 9 °C replicates to the five 12 °C replicates. Differentially expressed transcripts were tested for significance using the (RSEM derived) TMM (trimmed mean of *M*-values) normalized counts and with cutoffs of a >2-fold change and a p-value and false discovery rate < 0.05.

3. Results and discussion

3.1. Transcriptome assembly

Sequencing resulted in ~380 million paired-end 125 bp reads post-trimming (Table 2). The full transcriptome assembly, which was built from one female (84 million trimmed reads), one male (53 million trimmed reads), and one replicate from the 12 °C treatment (14 million trimmed reads) resulted in 405,497 transcripts that were clustered into 319,012 genes. Of the reads that contributed to the assembly (a total of 151 million) 98.18% mapped back. The N50 of the assembly was 728 with a median transcript size of 307 and an average size of 549. There was a 37.69% GC content. The number of transcripts was ~10× larger than those of other published krill transcriptomes. Meyer et al. (2015) reported 57,343 transcripts with an N50 = 691, although they used a different sequencing platform, different assemblers, a lower number of initial reads (2.6 million) and a more stringent approach (85% similarity clustering). Using a similar assembly method and a 95% similarity clustering approach Toullec et al. (2013) assembled only 36,345 transcripts from 14.4 million reads with an N50 = 698. Both of these assemblies chose 300 bp as a lower size threshold. If we remove all transcripts 200–299 bp in length from our assembly it is reduced to 210,334 transcripts without further clustering. This still remains a much larger number of transcripts than the available krill assemblies and likely reflects advances in sequencing technology as well as differences in initial read number, assembly and clustering parameters. The two copepod assemblies to which we compared our annotation did, however, have a similar number of transcripts (Lenz = 206,041 transcripts; Tarrant = 241,140 transcripts; Lenz et al., 2014; Tarrant et al., 2014) to what we found for *M. norvegica*. The large size of our transcriptome, paired with the high mapping success (~98%), suggests that this assembly does not only contain protein coding genes, but a number of lowly expressed transcripts, regulatory elements and non-coding regions that are unavailable in previous krill transcriptomes.

3.2. Functional annotation

Searches for homologous sequences using BLASTX versus nr resulted in relatively low annotation success for this assembly (16%; Table 3). This is likely because we chose a rather stringent cut-off for this search (>$1e^{-10}$), explicitly for the purposes of avoiding miss-annotation. Alternately this may just be due to the consistent difficulty found when attempting to annotate transcriptomes of non-model organisms that have minimal genomic resources available from closely related species (e.g. Lenz et al., 2014; Tarrant et al., 2014). The most abundant species represented in the top blast hits were all arthropods (*Zootermopsis, Daphnia and Limulus*) and represent 15% of the annotations.

To further increase the serviceability of this annotation, a number of cross-annotations were run with a less stringent cut-off (>$1e^{-5}$; Supplementary File 1A). Comparisons with FlyBase had a similar low level of annotation success (15%), as did comparisons with the two *C. finmarchicus* transcriptomes (~14%; Lenz et al., 2014; Tarrant et al., 2014). In contrast, comparison with the closest available pelagic crustacean relative, *E. superba*, resulted in ~20% annotation success (Meyer et al., 2015). Considering that our assembly contains seven times as many transcripts as that of Meyer et al. (2015), this annotation success exceeds the number of transcripts supplied by the *E. superba* transcriptome. This is likely partially a function of the higher clustering percentage (85%) in the Meyer et al. (2015) pipeline, with multiple of our transcripts having good hits with sequences found in *E. superba*. When the inverse TBLASTX function is performed (blasting *E. superba* with our *M. norvegica* transcripts with an e-value of −5) there is a 52.5% annotation success (Supplementary File 2A). This indicates that

Table 3
Annotation success statistics versus NCBI's non-redundant database (nr), FlyBase (FB), the *E. superba* transcriptome of Meyer et al. (2015), and the *C. finmarchicus* transcriptomes of Tarrant et al. (2014) and Lenz et al. (2014). Note that the e-value differed among database homology searches.

	nr (11-8-15)	FB (FB2016_03)	*E. superba* (Meyer)	*C. finmarchicus* (Tarrant)	*C. finmarchicus* (Lenz)
e-Value	e^{-10}	e^{-5}	e^{-5}	e^{-5}	e^{-5}
Total hits	66,220	59,769	84,252	54,543	55,944
% total	16.3%	14.7%	20.8%	13.5%	13.8%
Total unique	21,701	9432	17,334	11,726	12,493
% unique	32.8%	15.8%	20.6%	21.5%	22.3%

over half of the sequences identified by Meyer et al. (2015) have a similar transcript in our assembly, but that there remain transcripts within the Meyer et al. (2015) master assembly which are not represented, or have weak similarity, to those found in our *M. norvegica* assembly. All of our individuals were sampled at one time point and were exposed to temperatures on the higher end of their typical range, and as such it is very likely that some relevant transcripts may not yet have been sequenced. Interestingly, however, of the 15,347 sequences with annotation in the Meyer et al. (2015) assembly only 433 (~3%) did not have a match with one of our *M. norvegica* transcripts, while of the 7472 Meyer et al. (2015) transcripts with GO annotation, only 156 (2%) were without a close *M. norvegica* hit (Supplementary File 2A–B).

Examination of the sequences that were annotated among all of these cross-searches reveals that most of the typical genes of interest (i.e. metabolic cycles, molting, stress response) are well represented and cross-annotated with the closely related *E. superba* reference transcriptome. Of those 360 genes explicitly identified by Meyer et al. (2015) as being associated with important metabolic processes, response to stress, and biomineralization, all but four were cross annotated with a transcript from our assembly, and for each of those four transcripts, alternate isoforms had a TBLASTX hit (Supplementary File 2C). GO annotation further supports the supposition that the transcripts that are annotated comprehensively covered most of the basic metabolic processes (Fig. 4; Supplementary File 1B–D). The remaining unannotated sequences in this, and other pelagic crustacean transcriptomes, represent an important line of further research. Generation and annotation of the large genome, which Jeffery (2012) suggested to be on the order of ~18 Gb, is not likely to occur in the near future, leaving transcriptomic datasets, such as the one provided here, the best resource for further directed studies for processes of interest.

3.3. Differential expression response to laboratory temperature experiment

Analyses of in situ temperatures via CTD data collected on site suggest that prior to collection krill had been experiencing ~5.8–7.3 °C in their daytime distribution (Fig. 1). They were maintained in 8–9 °C water during transit and were then placed into the experimental chambers directly. Review of the hobo loggers, however, revealed that

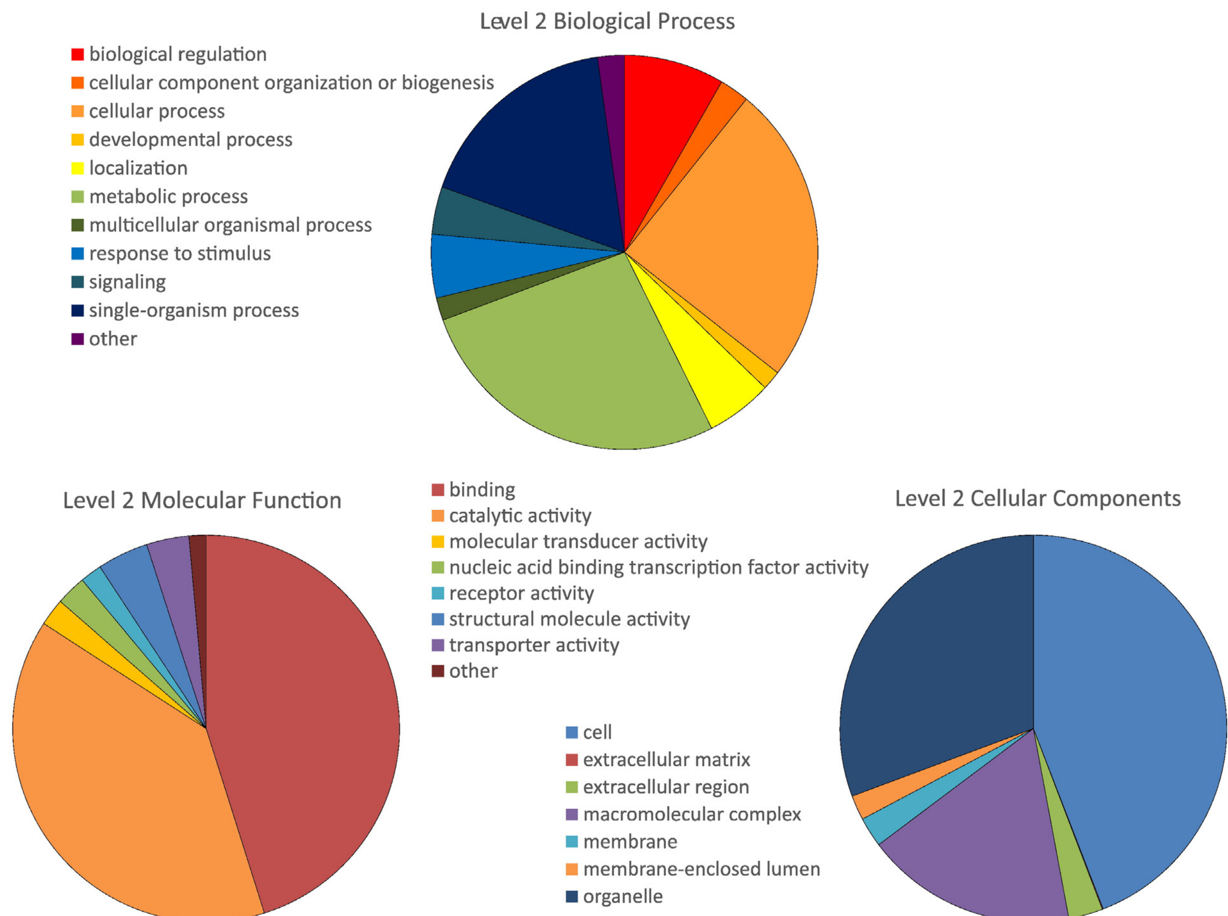

Fig. 4. Level 2 GO annotation for the *M. norvegica* transcriptome based on the e-value cutoff of $1e^{-10}$.

temperature of the in situ water in the experimental barrels had reached ~12 °C in transit to the laboratory (Fig. 1), so all animals experienced a slight heat shock. Although no sampling occurred at night during this cruise, based on the typical DVM pattern of the species, it is assumed that during the night these individuals were within the upper water column, and 12 °C is within a reasonable temperature range for these organisms. Thereafter, the "ambient" treatment took ~36 h to reach ~9 °C, where it remained for the next 2 d. The organisms thus had sufficient time to gently acclimatize to their temperature conditions and ~2 d of consistent exposure to either 9 or 12 °C, providing a 3 °C temperature contrast for the differential expression analysis. Mortality was lower in the 9 °C treatment (8% versus 26%), suggesting that there were organism-level differences in sensitivity to temperature, but the lack of experimental replicates (barrels) prevents conclusive analyses.

The use of the tail segment for DE analysis reflected our interest of characterizing the effect of temperature on this strong and active swimmer. Tails are mostly muscle tissue, responsible for the swimming activity (Kils, 1981). Therefore changes in temperature would likely have an effect of the metabolic signature of this tissue. The use of the tail also avoids sex-specific differences which were not specifically controlled for in the experimental design of the DE analysis. Gut contamination would be unlikely due to the homogeneous diet given to all individuals during the experiment, the nature of the diet (dry pellets), and the fact that the same segment (also a relatively distant one, the fourth) of the tail was always chosen. The election of tail tissue for the experiment does not affect however the reference transcriptome, built using two whole (male and female) individuals. The mapping of the experimental replicates, which ranged in size between 10 and 14 million trimmed reads, resulted in 100% alignment with an average of 84.5% of all reads mapping uniquely to a gene (Supplementary File 3), supporting this choice.

Differential gene expression analysis resulted in identification of 122 genes that were significantly affected, 19 of which (16%) were annotated either via BLAST or GO terms (Table 4, Supplementary File 4). There was some variation in expression among replicates, particularly in genes upregulated in the 9 °C treatment (Fig. 5). Of those genes in the 9 °C treatment which have a more similar expression to the 12 °C treatment, those which were identified with BLASTX searches (three) are associated with cuticle proteins. This may be a result of random differences in gender, age or other phenotypic traits within the pooled samples. The statistical analyses indicate, however, that despite the downregulation within these replicates in the 9 °C treatment, there is still a substantial difference among treatments with regards to these genes to suggest that they are influenced by temperature.

Functional annotation of the differential expression identified 12 of the 86 upregulated transcripts in the 9 °C treatment. Half of these (six) were associated with the cuticle and/or calcification, while two were associated with nucleic acid binding. Of the 36 transcripts upregulated in the 12 °C treatment, only seven were annotated and three of these were associated with the mitochondria and ribosomes. GO enrichment analysis done in Blast2GO did not, however, reveal significant changes in categories of terms. This is not terribly surprising due to their small number and overall low annotation success, and it suggests that the changes in overall gene expression are not substantial between 9 and 12 °C.

These results do suggest a small overall shift to higher protein metabolism and a potential slight increase in either energetic metabolism or oxidative stress response (based on one transcript) under higher temperatures, as well as a possible decrease in molting or shell production. It is known generally that increased temperature produces a rise in the pace of life of endothermic organisms (Hochachka and Somero, 2002), which may be what is reflected in the increased mitochondrial and ribosomal transcripts. Temperature has previously been shown to substantially influence the intermolt time of *M. norvegica*, but it tends to shorten the periods between molts (Cuzin-Roudy and Buchholz, 1999; Tarling and Cuzin-Roudy, 2003). It has, however, been previously found in wild caught samples that a greater proportion of the population that remained in deep (and cooler) waters were engaged in molting (Tarling et al., 1999), whereas surface caught individuals were more engaged in reproductive activity. In other studies it was shown that reproductive activity and molt cycle are also linked (Cuzin-Roudy and Buchholz, 1999). The measured increase in biomineralization transcripts at our colder temperature may thus be associated with this observed vertical partitioning of molt behavior to deeper/cooler waters where individuals are less susceptible to visual predation or cannibalism. There was no apparent link between temperature, molting, and reproduction in our dataset, but this could be a consequence of our sampling design, which sampled only tail tissue and pooled individuals of both genders. Thus the upregulation of cuticle and biomineralization associated transcripts is interesting as it implies that there may be some energetic trade-offs between molt speed, mineralization, and growth that are measurable in a 3 °C thermal exposure.

Although *Meganyctiphanes norvegica* populations experience a wide range of temperatures across their geographical range, there appears to be strong population-specific acclimation or adaptation to local temperatures (Tarling et al., 2010; Plourde et al., 2013; Plourde et al., 2014) and an upper thermal threshold of ≥16 °C (Fowler et al., 1971; Saborowski et al., 2002; Spicer and Saborowski, 2010). Understanding the physiological mechanisms which enable this broad distribution and lead to such clear local thermal adaptation/acclimation is important in an increasingly heated ocean system. For the of *M. norvegica* population in the Gulf of Maine, coping with the fast warming of the water temperatures in this region (Pershing et al., 2015) might result in physiological trade-

Table 4
Differentially expressed genes (change > 2-fold, p-value and FDR < 0.05) for individuals exposed to the lower (~9 °C) and higher (~12 °C) temperature treatments. The identity of all DE genes was based on BLASTX versus nr and GO Annotation (e-value > $1e^{-5}$). Full details of the DE genes (ID, length, e-value, etc.) are contained in Supplementary File 4.

Up	BLAST annotation	GO annotation
9	2106164acuticle protein	C:cytoplasmic membrane bounded vesicle
9	AGAP006931-PA	F:structural constituent of cuticle
9	Calcification-associated peptide-2	F:structural constituent of cuticle
9	Calcification-associated peptide-2	F:structural constituent of cuticle
9	Cuticle protein 6	F:structural constituent of cuticle
9	Cuticle protein	F:chitin binding; P:chitin metabolic process; C:extracellular region
9	TcasGA2_TC010287	F:nucleic acid binding; F:zinc ion binding
9	Laccase	F:metal ion binding; P:oxidationreduction process; F:oxidoreductase activity; F:copper ion binding; F:hydroquinone:oxygen oxidoreductase activity
9	Lectin	-NONE-
9	Oligoribonuclease, mitochondrial-like	F:nucleic acid binding; F:exonuclease activity; P:nucleic acid phosphodiester bond hydrolysis
9	Serine threonine-protein kinase tricorner isoform x2	-NONE-
9	trna:m x modification enzyme trm13 homolog	-NONE-
9	74 unannotated	
12	40s ribosomal protein s5	F:structural constituent of ribosome; F:RNA binding; P:translation; C:small ribosomal subunit
12	anntoxin	P:negative regulation of endopeptidase activity; F:serinetype endopeptidase inhibitor activity
12	mytiLec 3	-NONE-
12	Exocyst complex component 2	-NONE-
12	GLOINDRAFT_21476	F:nucleic acid binding; F:DNA binding
12	Mitochondrial cytochrome c oxidase subunit via precursor	-NONE-
12	60s ribosomal protein l15	C:ribosome; F:structural constituent of ribosome; P:translation
12	29 unannotated	

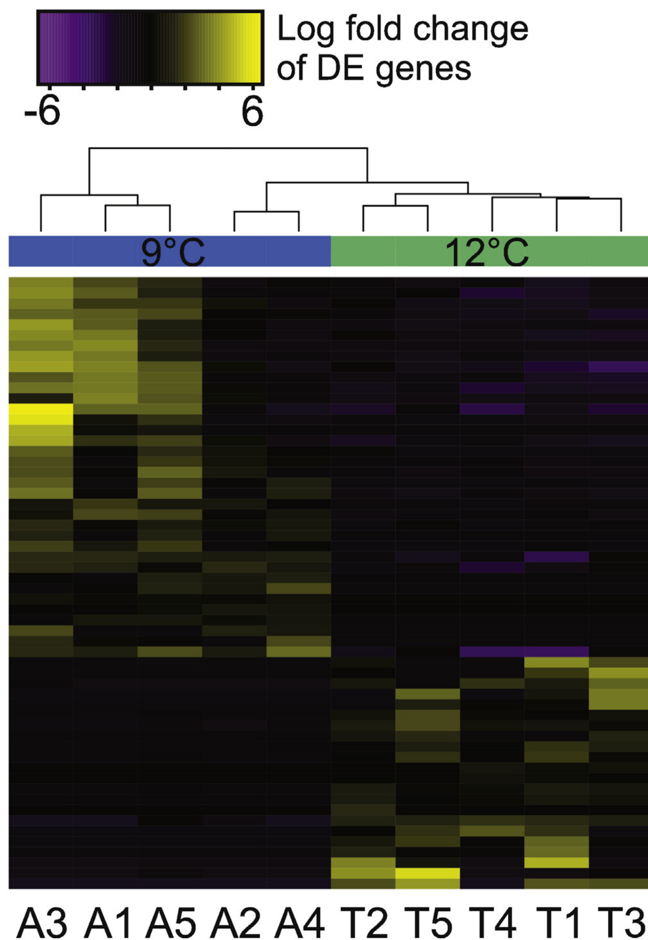

Fig. 5. Heat map of the log fold change (log_2) of the differentially expressed genes (change > 2-fold, p-value and FDR < 0.05) superimposed on a Pearson correlation matrix dendrogram depicting the similarity of gene expression patterns for individuals exposed to the lower (~9 °C) and higher (~12 °C) treatments. Replicate number is indicated below each column.

offs, affecting processes like the intermolt period or growth (Cuzin-Roudy and Buchholz, 1999; Tarling and Cuzin-Roudy, 2003) in the long term and might therefore have serious impacts on this population under future conditions. Considering the pivotal role of *M. norvegica* in Gulf of Maine food webs (Lacroix and Knox, 2005; Simard and Harvey, 2010; Cleary et al., 2012), this might have significant effect on a system already endangered by global change (Pershing et al., 2015; Waller et al., 2016). Further analyses using much colder treatments (reflecting the daytime depth distribution temperatures) could help resolve how these processes are influenced on both diel and seasonal cycles, while experiments at slightly higher temperatures will provide insight into how future anthropogenically influenced warming will impact this particular population. Expanded analyses, using other potentially sensitive tissue types (such as the brain) are also warranted.

4. Conclusion

The transcriptome assembly presented here gives a base for future research on this key species for the North Atlantic pelagic ecosystem, and together with other available krill and crustacean transcriptomes, opens the door to comparative studies across species or higher ranks on adaptation and gene evolution. In groups with giant and likely highly duplicated genomes (Jeffery, 2012; Deagle et al., 2015), transcriptomes are likely the best resource for such approaches.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2017.05.013.

Data accessibility

Sequence and assembly data associated with this project are archived in Genbank under BioProject PRJNA324094. The Transcriptome Shotgun Assembly used for differential expression and comparative annotation can be accessed as GETT00000000. Cruise report and hydrographic data for cruise TI787 available at BCO-DMO http://www.bco-dmo.org/deployment/562792.

Author contributions

LBB designed the laboratory experiment, while both authors collaborated on organismal collection, maintenance, sampling and RNA extraction. Downstream bioinformatics was run by AM, with intellectual input by LBB. Both authors contributed to manuscript preparation.

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