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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.

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

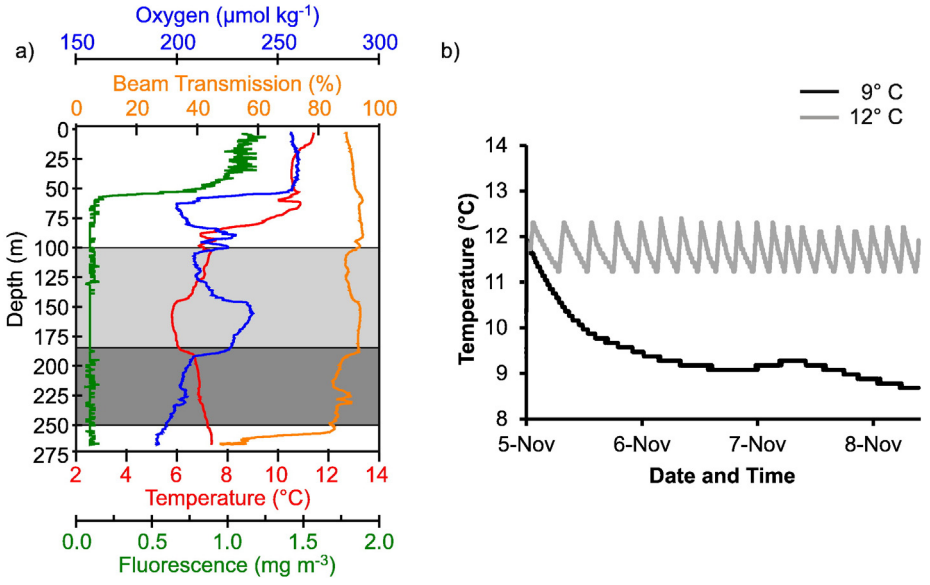

Fig. 1. In situ and laboratory conditions experienced by the krill prior to RNA-Seq analyses. The in situ environment (A) was sampled with a CTD with a SBE3/SBR4 sensor set. Krill were found abundantly in the 180–250 m nets (dark grey) and were present up to 100 m (light grey). Temperature in the laboratory 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.

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