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Gene expression of the marine copepod *Calanus finmarchicus*: Responses to small-scale environmental variation in the Gulf of Maine (NW Atlantic Ocean)

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A B S T R A C T

The pelagic copepod *Calanus finmarchicus* is one of the most important zooplankton species in the North Atlantic Ocean. Despite its ecological importance and pivotal role in the food chain, the molecular mechanisms underlying this species' complex life history (ontogenetic development, reproduction, molting, and diapause) and physiology (digestion, neural processes, and membrane physiology) have remained poorly characterized. This study examined differential expression of nearly 1000 genes, selected based on physiological function and hypothesized ecological importance, for *C. finmarchicus* collected in the Gulf of Maine (NW Atlantic Ocean) using an oligonucleotide DNA microarray. Replicate analyses compared adult females and final-stage juveniles (Copepodite-V stages) collected from surface (0–30 m) and deep (130–170 m) layers during April, 2008. Differentially expressed genes were identified by statistical analysis of multiple replicates, including a control for False Discovery Rate (FDR). Functional relationships of selected genes and/or proteins were evaluated using the Gene Ontology Enrichment Analysis Software Toolkit (GOEAST). Genes involved in protein synthesis, cell-cycle and tissue buildup were shown to be up-regulated (i.e., significantly higher expression levels) in deep females and juveniles; genes related to protein turnover, cellular homeostasis, activity, and stress/immunity responses were up-regulated in surface females. Additional functional analyses using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis indicated up-regulation of genes encoding enzymes related to energy metabolism and osmoregulation in surface females, and citric acid cycle and anaerobic metabolism in deep females and juveniles. Overall, an active physiological state was pronounced in surface females, and a number of processes related with emergence from diapause were pronounced in deep females and juveniles.

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1. Introduction

1.1. Ecology and physiology of Calanus finmarchicus

The planktonic copepod *C. finmarchicus* (Crustacea, Copepoda) is an abundant and ecologically important species in the N Atlantic Ocean and the Barents Sea. The species plays a significant role in the pelagic food web as a secondary producer transferring energy from primary producers to higher trophic levels. It is an important prey of the juvenile stages of ecologically important fish, including mackerel and herring (Prokopchuk and Sentyabov, 2006). The species follows a Subarctic biogeographical distribution and is found from the New England shelf to the Greenland and Barents Seas, with a latitudinal range from 40°N to 80°N (Bryant et al., 1998).

C. finmarchicus is an especially important species in the pelagic assemblage of the Gulf of Maine, where it may contribute up to 70% of total copepod biomass in spring and early summer and dominate zooplankton secondary production during spring in most years (Planque and Batten, 2000). The Gulf of Maine is a semi-enclosed continental shelf sea bounded by Cape Cod, Georges Bank, and southwestern Nova Scotia (Fig. 1). The bathymetry of the Gulf of Maine is complex, with three deep basins, (Georges, Jordan, and Wilkinson) that exhibit highly-reduced exchange of waters deeper than 200 m (Townsend et al., 2005).

The complex life history of *C. finmarchicus* has been extensively studied in the Gulf of Maine region, most recently as a part of the US GLOBEC program (Wiebe et al., 2001). Individuals pass through six larval (naupliar) and six juvenile (copepodite) stages. Naupliar stages N1 and N2 do not feed, while later naupliar stages and all copepodite stages feed primarily on phytoplankton. In the southern Gulf of Maine, *C. finmarchicus* reproduction is closely tied to annual phytoplankton production cycle, beginning in early January (Durbin et al., 2000). After reaching CV stage, a portion of the population descends to deep waters in order to initiate diapause, while the remaining CV population

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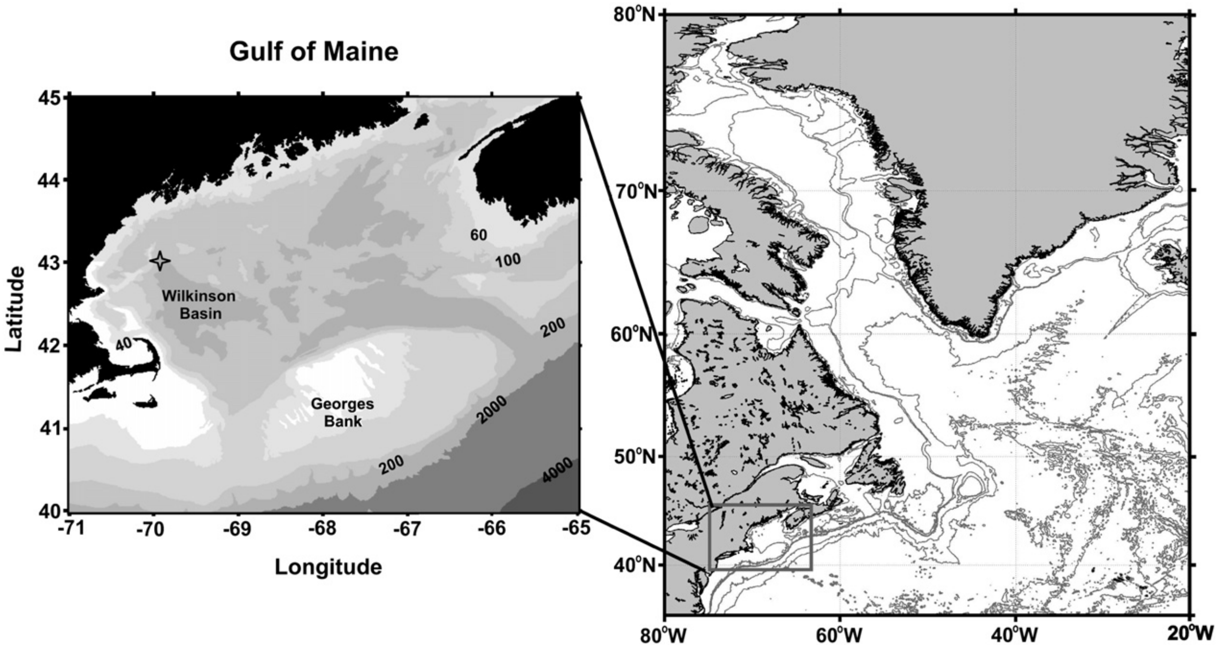

Fig. 1. Location of the Gulf of Maine in the Northwest Atlantic Ocean, showing the sampling station for the *Calanus finmarchicus* microarray analysis in Wilkinson Basin with a star. Vertically-stratified plankton tows were taken at station WB7 on April 17, 2008 (see Methods for more explanation).

molt into adults, reproduce and spawn another generation (Hirche, 1996). Diapause is an arrested state of development at the CV stage with reduced metabolism during the late summer and autumn, which may serve as a means of surviving periods of low food abundance (Hirche, 1996). Copepodites accumulate lipid reserves throughout the summer, which may enhance survival during diapause (Hirche, 1996; Miller and Ambrose, 2000) and provide energy to fuel gonad maturation, molting and reproduction in early spring (Rey-Rassat et al., 2002). More recently, a conceptual model of the possible role of lipids (in particular wax ester composition) in influencing diapause patterns, determining the overwintering depth, and population adaptations to the hydrological conditions have been proposed (Irigoien, 2004; Pond et al., 2012).

C. finmarchicus appears to initiate diapause in both neritic and oceanic waters of the North Atlantic, where diapausing populations can be found within 50–100 m of the bottom in neritic environments and 500–1500 m in oceanic waters (Heath et al., 2004; Miller et al., 1991).

In the Gulf of Maine, *C. finmarchicus* populations emerge from diapause during winter and early spring, molt into adults at depth, and ascend to surface waters. Two or three generations may be produced during January–June in the Gulf of Maine, with a considerable overlap of generations (Durbin et al., 2000; Miller et al., 2000). The vertical distribution of *C. finmarchicus* is known to be bimodal in the Gulf of Maine during fall and winter; the depth of highest abundance of diapausing individuals has been reported to be below the cold intermediate layer, close to 150 m depth (Durbin et al., 1997; Johnson et al., 2006).

Diapause includes a number of phases, with its initiation consisting of preparatory and induction phases, a refractory phase, and activation and termination phases (Hirche, 1996). In insects, the activation and termination phases are characterized by a reactivation of the endocrine system and its resultant increases in DNA/RNA synthesis and metabolism (Denlinger, 2002). During diapause, copepods are behaviorally inactive showing depressed respiration rates and limited swimming (Hirche, 1996). Diapausing copepods are thought to have reduced transcriptional/and translational activity indicated by low RNA/DNA ratios and reduced rates of protein synthesis (Wagner et al., 1998, 2001; Yebra et al., 2006). Much less is known about the physiological changes associated with the phases of diapause in *C. finmarchicus*, although it has been proposed that it follows an insect-like trajectory (Hirche, 1996).

The early maturation processes including mitotic divisions, cell growth and early yolk synthesis take place upon being triggered by internal lipid reserves, and the release of oocytes from the ovary occurs before moulting from CV to adult (Grigg and Bardwell, 1982; Niehoff et al., 2002). Gonad development of both diapausing and newly-moulted females were reported to take place concurrently while the females were residing at depth >150 m (Madsen and Nielsen, 2001; Niehoff et al., 2002). Males mature earlier than females, and adults may mate as they migrate to surface waters during which a spermatophore is deposited on the female and females begin reproducing in late winter to early spring (Durbin et al., 1997; Hirche, 1996). During final maturation processes, oocytes complete maturation when females are ready to spawn, which involves chromosome modifications, incorporation of lipids and late yolk synthesis (Niehoff, 1998; Niehoff and Hirche, 1996; Niehoff et al., 2002).

1.2. Analysis of gene expression using oligonucleotide microarrays

A DNA microarray was designed for *C. finmarchicus* to investigate gene expression consequences of major physiological functions (environmental stress, metabolism, molting, digestion, neural processes, and membrane physiology). The microarray has been used in a number of experiments analyzing field-collected specimens (Lenz et al., 2012).

Microarrays are particularly useful for examination of gene expression patterns in natural populations, which may exhibit substantially greater variation in gene expression than laboratory model species, which in turn may show less variation in expression of fewer genes (Colbourne et al., 2011). Differential gene expression may be used to infer variation in significant physiological processes affecting longevity, reproductive fitness and probability of survival (Oleksiak et al., 2002). However, high levels of variation among individuals may interfere with or prevent resolution of differences among populations or life stages.

In this study, we examined expression levels of ~1000 functionally important genes for *C. finmarchicus* collected from surface and deep waters of the Gulf of Maine using a custom-designed "*Calanus* physiology microarray". We hypothesized that surface and deep copepods are