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Functional genomics resources for the North Atlantic copepod, *Calanus finmarchicus*: EST database and physiological microarray

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Abstract

The copepod, *Calanus finmarchicus* is a keystone species for the North Atlantic. Because of recent changes in the geographic distribution of this species, there are questions as to how this organism responds physiologically to environmental cues. Molecular techniques allow for examination and new understanding of these physiological changes. Here, we describe the development of a microarray for high-throughput studies of the physiological ecology of *C. finmarchicus*. An EST database was generated for this species using a normalized cDNA library derived from adult and sub-adult individuals. Sequence data were clustered into contigs and annotated using *Blastx*. Target transcripts were selected, and unique, 50 base-pair, oligomer probes were generated for 995 genes. *Blast2GO* processing provided detailed information on gene function. The selected targets included broad representation of biological processes, cellular components, and molecular functions. The microarray was tested in two sets of comparisons: adult females maintained at different food concentrations and field-caught sub-adults showing differences in lipid storage. Up-regulated and down-regulated transcripts were identified for both comparisons. Only a small subset of the genes up-regulated in low food individuals were also up-regulated in lipid-poor animals; no overlap was seen between the genes down-regulated in the two comparisons.

Keywords

Calanus finmarchicus; Copepoda; Crustacea; Microarray; cDNA library

1. Introduction

Zooplankton are critical links in oceanic pelagic food web which show rapid responses to climate variability, including shifts in species' distribution and abundance, timing of life history events, and trophic relationships. In spite of their ecological importance, genomic resources for marine planktonic animals are scarce (Bron et al., 2011). In fact, with the

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exception of the freshwater cladoceran *Daphnia pulex*, a model organism for studies of ecotoxicology and toxicogenomics (Colbourne et al., 2011), few planktonic species have been the targets of large-scale gene discovery. The goal of the project presented here was to develop functional genomic resources for the calanoid copepod, *Calanus finmarchicus*, a North Atlantic keystone species, which ranges from the Gulf of Maine to the Norwegian Sea (Fig. 1; Marshall and Orr, 1955; Meise and O'Reilly, 1996; Durbin et al., 2000; Head et al., 2000; Dale et al., 2001; Helaouët and Beaugrand, 2007; Gislason et al., 2008; Heath et al., 2008; Kimmel and Hameed, 2008; Madsen et al., 2008; Gaardsted et al., 2010). *C. finmarchicus* is an important prey for many commercially important fish, such as the cod *Gadus morhua* (Taggart et al., 1996; Kristiansen et al., 2009) and the herring *Clupea harengus* (Cohen and Lough, 1983; Kiørboe et al., 1988; Purcell and Grover, 1990), and shellfish, such as the shrimp *Pandalus borealis* (Savenkoff et al., 2006). Over its geographic range, *C. finmarchicus*' population dynamics can be variable (Speirs et al., 2006; Pepin and Head, 2009; Pepin et al., 2011; Helaouët et al., 2011; Petursdottir et al., 2010), which has raised questions about how this organism adapts to its environment physiologically (e.g. Hirche et al., 1997; Hansen et al., 2003).

A major factor driving population dynamics in all animals, including *C. finmarchicus* is the availability of food. For *Calanus*, it is likely that many individuals in any given population experience a 10 to 100-fold variation in food availability over the course of normal development (Durbin et al., 1995a,b; Meise and O'Reilly, 1996). Decreased food availability has been hypothesized to be one causal agent in increased mortality and decreased population growth and reproduction in *C. finmarchicus* (Plourde and Runge, 1993; Irigoien et al., 1998; Campbell et al., 2001a; Niehoff, 2004; Madsen et al., 2008; Plourde et al., 2009). In addition, it has been well documented that *C. finmarchicus* copepodites require lipid stores to successfully enter and complete diapause, and to initiate reproduction in the spring (Irigoien, 2004; Saumweber and Durbin, 2006; Johnson et al., 2008; Jónasdóttir et al., 2008; Madsen et al., 2008; Maps et al., 2010; 2011). However, pre-adult stages within any population vary in both size and lipid stores raising questions about timing and likelihood of diapause for some individuals (Pepin and Head, 2009; Pepin et al., 2011). During the summer and fall, lipid-poor and lipid-rich sub-adults are common (Miller et al., 2000; Pepin and Head, 2009), and there is evidence that these morphotypes differ physiologically (Hassett, 2006; Pepin et al., 2011).

Changes in the geographic distribution of *C. finmarchicus* have been recently documented, and in the North Sea, appear to be one source of large-scale declines in cod populations (Beaugrand et al., 2002; 2003). It is presumed that these changes in *C. finmarchicus* biogeography have occurred in response to global climate change, raising concerns about future changes in geographic range (Helaouët et al., 2011; Reygondeau and Beaugrand, 2011). However, the proximate causes for the decline in *C. finmarchicus* abundances remain unclear, and to fully understand them will require a better characterization of organism–environment interactions. In particular, physiological changes during critical periods of its life cycle, such as winter diapause, emergence from diapause and reproduction in the spring, and preparation for diapause in the summer and fall, need to be better understood (Fiksen, 2000; Maps et al., 2010; 2011). Molecular tools, such as transcriptomics, have the potential to greatly expand our ability to investigate the physiological ecology of organisms like *C. finmarchicus*. Differential regulation of genes from ecological samples has been demonstrated for *C. finmarchicus* using subtractive hybridization and real-time quantitative polymerase chain reaction (Tarrant et al., 2008; Aruda et al., 2011), suggesting that gene expression studies may be very informative for this species.

Here, we describe transcriptome-based resources recently developed for *C. finmarchicus*. We first generated an expressed sequence tag (EST) database from a normalized cDNA

library, which was submitted to GenBank (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov). The EST sequences were assembled into contigs, which in turn were annotated and used to generate a list of target transcripts. The selection process focused on obtaining good coverage of genes that annotated to functions and that might be regulated in response to environmental conditions, specifically biotic and abiotic stressors and food resources. Oligomer probes were designed for these transcripts for use in a species-specific microarray. The selected targets (995 in total) represent a wide range of both biological processes and involvement in important biochemical pathways (as shown by their Gene Ontologies [GO] and Kyoto Encyclopedia of Genes and Genomes [KEGG] pathway annotations). This platform, with its relatively small number of probes, allowed us to print four separate arrays on a single slide.

We used the microarray to investigate the effect of food availability on gene expression in adult females. In a laboratory experiment, we maintained groups of adults at two food levels for one week and compared their expression patterns. We also compared gene expression patterns in field-collected individuals that differed in their lipid stores to determine how these two morphotypes differed in expression/physiology. For the low vs. high food and lipid-poor vs. lipid-rich comparisons, both up-regulated and down-regulated genes were identified. Interestingly, a subset of the genes up-regulated in low food individuals were also up-regulated in the lipid-poor animals. No overlap was seen between the genes down-regulated under low food conditions and those down-regulated in lipid-rich individuals. Some of these data have appeared previously in abstract form (Christie et al., 2009; Hassett et al., 2010).

2. Materials and methods

2.1. Field collections

C. finmarchicus sub-adults (developmental stage: copepodite V [CV]) and adults were collected during the summer (June and July) in the Gulf of Maine (Lat: 44°2'N; Long: 68°3'W) by towing a 75 cm diameter (560 µm mesh) net vertically from 75 m depth. Plankton collections were immediately diluted into 10 L of subsurface seawater and individual animals were either directly sorted into *RNAlater* (Ambion) or placed into containers of filtered seawater for later experimental testing. The experimental containers were kept in coolers on ice and transferred into an incubator maintained at 8 °C upon return to the laboratory.

2.2. Laboratory feeding experiments

C. finmarchicus adult females were maintained in the laboratory at 8 °C at 12:12 light:dark cycle at either 500 or 5000 cells mL⁻¹ of *Tetraselmis* sp. (Reed Mariculture paste). The incubation period was 7 days, and food was added daily at the two feeding levels. Groups of animals were kept at 7–10 individuals per liter in 3.5 L jars. At the end of the incubation period, individuals were staged and 10 to 15 individuals were preserved in *RNAlater* as a group. There were four replicates for each treatment (low food or high food). These samples were paired randomly and competitively hybridized on four separate microarrays, each representing a biological replicate for both conditions.

2.3. Lipid-rich vs. lipid-poor sub-adult *C. finmarchicus* (stage CV)

C. finmarchicus juveniles (CV) can be separated into either lipid-poor or lipid-rich morphotypes during the summer and the early fall depending on the size of their lipid store, *i.e.* oil sac (Miller et al., 2000; Hassett, 2006). CV individuals were collected from the Gulf of Maine in 2009 (June 5) and in July of 2010 (July 21) and separated into either lipid-rich (oil sac ≈ 20% by vol.) or lipid-poor (lipid sac ≈ 5% by vol.) individuals, and preserved in

RNA later in groups of 15 to 25 individuals. Two and three different pairs of RNA pools were prepared from the years, 2009 and 2010, respectively. In 2010, there were two additional technical replicates using a dye-swap.

2.4. Total RNA extraction

Two kits were used for extraction of total RNA. For the normalized cDNA library, total RNA was prepared using a Promega RNeasy Total RNA Isolation System. For the microarray hybridizations, a Qiagen RNeasy Mini Kit was used (in conjunction with the Qias shredder column [Qiagen]), following the instructions of the manufacturer, with a final elution volume of either 30 or 40 μ L. RNA samples were stored overnight at -20°C , or at -80°C for longer storage. Purified total RNA was checked for quality and quantity using an Agilent Model 2100 Bioanalyzer. For the microarray hybridizations, volumes were calculated to obtain at least 5 μ g of total RNA for the first strand synthesis reaction. Samples that were too dilute were concentrated at a low drying rate using a Savant ISS110 SpeedVac Concentrator (Thermo Scientific).

2.5. Normalized cDNA gene library and sequencing of ESTs

For the production of the normalized cDNA library, high quality total RNA was pooled from several *C. finmarchicus* samples. The pooled material included RNA from sub-adults and adults collected in June 2003, some of which had been subjected to thermal stress (Voznesensky et al., 2004). The pooled sample was subsequently sent to Invitrogen for the production of a normalized cDNA library. Reverse transcription of mRNA transcripts using an oligo-dT primer was followed by subtractive hybridization to enrich for rare transcripts using proprietary technology (Invitrogen). The cDNAs remaining after subtractive hybridization were incorporated into competent *Escherichia coli* (DH10B-T1) by directionally ligating them into PCMV Sport 6.1 vector, and then electroporating the insert-containing vector into the bacteria.

Expressed sequence tags (ESTs) were obtained from randomly selected clones grown from the cDNA library as described in Towle and Smith (2006). Briefly, aliquots of bacteria containing cDNA from the normalized library were diluted in $2\times$ Luria-Bertani (LB) broth and spread on 25 cm $\times$ 25 cm LB agar plus carbenicillin (100 mg L^{-1}) plates overnight at 37°C . Colonies were evaluated and picked randomly using a Genetix QPix2 robot and transferred into 96-well plates with $100\text{ }\mu\text{L}$ $2\times$ LB broth, 8% glycerol and carbenicillin (100 mg L^{-1}) and incubated overnight at 37°C . These plates were then stored at -80°C . Sample preparation for sequencing involved transferring a small sub-sample from each well into 96-deep-well plates containing 1 mL of LB broth and carbenicillin. The deep-well plates were incubated for 16 h at 37°C and then plasmids were isolated by alkaline lysis using a Beckmann-Coulter BioMek 2000 robot and Millipore miniprep reagents. Each insert was single-pass sequenced from the 5' end using an ABI 3100 16-capillary sequencer (Applied Biosystems) and a SP6 primer. Approximately 1000 clones were sequenced from each agar plate, with a total of 11,128 clones sequenced.

Sequences were prepared for submission to dbEST as described in Towle and Smith (2006). The *trace2dbest* component of the *PartiGene* software (University of Edinburgh; Parkinson et al., 2004) was used to process individual sequences: vector and low quality parts of the sequence were removed, and *BLASTx* analysis (Altschul et al., 1994) was performed for putative identification and preparation for submission to dbEST (National Center for Biotechnology Information, NCBI, database). All ESTs can be accessed through the NCBI database (www.ncbi.nlm.nih.gov).

2.6. Microarray design, probe characterization and construction

Prior to the selection of target transcripts for the microarray, the available ESTs in the NCBI database were further analyzed, first by clustering sequences using the CLOBB algorithm within the *PartiGene* software package (Parkinson et al., 2004). After clustering sequences, singletons and contigs were annotated again using *BLASTx* (Altschul et al., 1994), which was processed through a local (Mount Desert Island Biological Laboratory) TimeLogic DeCypher server (Active Motif, Inc); the ten top *BLASTx* hits were checked manually for consistency in annotation and degree of identity/similarity to the respective *C. finmarchicus* sequence.

1012 target transcripts were selected for the microarray from the list of annotated sequences. The selected transcripts were further annotated using *Blast2GO* (www.blast2go.org; A. Conesa and S. Goetz, Bioinformatics Dept., Centro de Investigación Príncipe Felipe, Valencia, Spain) in order to obtain GO terms for biological process, cellular component, and molecular function (www.geneontology.org; The Gene Ontology Consortium, 2000). Validation of GO terms was accomplished by running an InterPro Scan and Annex, followed by enzyme code mapping and KEGG pathway annotations (www.genome.ad.jp/kegg; Kanehisa and Goto, 2000; Kanehisa et al., 2006, 2010), all accessed through *Blast2GO*.

In preparation of the microarray, the sequences of the target transcripts were used to design unique, 50 base-pair oligomer probes using Array Designer 4 software (Premier Biosoft International). Of the 1012 target transcripts, 995 unique probes with similar annealing temperatures were designed. No unique probes could be obtained for the remaining sequences, and thus they were not included in the array. All oligomer probes were custom synthesized by Integrated DNA Technologies. Oligonucleotides were diluted into Pronto! Universal Spotting Solution (Corning), and subsequently spotted onto UltraGAPS Coated slides (Corning, Lot # 00509000) using a microarray printer (Gene Machines OmniGrid Accent Arrayer) with silica spotting pins (Parallel Synthesis Technologies Inc). Each oligomer probe was printed in duplicate, and four separate arrays of eight blocks each were printed on each slide. Printing quality was checked using a SpotQC kit (Integrated DNA Technologies, Inc.).

2.7. Microarray testing

The microarray was tested in two sets of comparisons. The first set of comparisons was between adult females kept at either high or low food conditions (see Section 2.2). In the second comparison, lipid-rich sub-adults were compared to lipid-poor ones (see Section 2.3). Total RNA was extracted from the samples and checked for quantity and quality as described above (see Section 2.4) prior to preparation for competitive hybridization.

2.7.1. First-strand synthesis and labeling—Fluorescently labeled cDNA was generated using the Invitrogen SuperScript™ Plus Direct cDNA Labeling System. Briefly, first-strand cDNA synthesis was performed on samples containing 5 to 15 µg of total RNA using SuperScript III RT and labeled using either Alexa Fluor 555-aha-dUTP or Alexa Fluor 647-aha-dUTP nucleotides. This was followed by hydrolysis and neutralization to degrade the original RNA, and purification of the labeled cDNA was accomplished using the Purification Module included in the kit.

2.7.2. Microarray hybridizations and scanning—Printed slides were pre-hybridized following the manufacturer's instructions (Pronto Microarray Hybridization Kit, Corning), and covered with a Maui coverslip (A4) that individually sealed the 4 separate arrays printed on each slide. For each sample, labeling yields were checked using a Nanodrop 2000 micro-

volume spectrophotometer (Thermo Scientific). Volumes were calculated for each pair of samples to provide equal amounts of labeled material, specifically, 4 and 9 pmoles of labeled cDNA. Samples were combined in a 1.5 mL centrifuge tube and dried in the SpeedVac Concentrator. 18 μ L of short oligomer hybridization solution (Pronto, Corning) was added to each microarray sample, and loaded and sealed into individual slide chambers. Slides were incubated in a Maui Hybridization Chamber overnight at 42 °C for 14 to 16 h. Slides were then processed following manufacturer's instructions, and dried using compressed air prior to scanning in a Genepix 4000 microarray scanner. Slides were scanned in their entirety and each microarray was stored in a separate file (.gpr file).

2.7.3. Data analysis—The scanned data were uploaded into Acuity 4.0 Microarray Informatics Software (Molecular Devices, Inc.). Normalization of individual slides was performed by print-tip (block-by-block) Lowess normalization with data centering and a smoothing factor of 0.4 for 4 iterations (Yang et al., 2002). The normalized median $\log_2$ ratios were used for all subsequent analyses. Following normalization, spot quality analysis was performed and those spots that did not meet the minimum quality control criteria (*i.e.*, spots that were flagged bad/absent, with non-uniform intensity and background, or were not detectable above background levels) were removed. Next, the genes that did not have intensity values in at least 75% of the replicate arrays per comparison were removed from the analysis. Technical replicates, if present, were averaged to minimize the effects of dye bias on fluorescent hybridization signals. Replicate arrays for each comparison were then scale normalized to adjust for differences in sample variances (Yang et al., 2002). A total of four biological replicates for high-low food comparison, and five biological replicates (with two technical replicates) for lipid-rich vs. lipid-poor copepod comparison was analyzed.

Two separate analyses were performed to identify differentially expressed transcripts. Using Acuity software, a *t*-test ($p < 0.05$) was carried out for each comparison along with a fold-change filter (set to mean $\log_2$ ratio=1 across replicate arrays). Principle component analysis was also carried out and the genes were plotted against the first principle component score *versus* *p*-value to generate a volcano plot for each comparison, and the significantly differentially expressed genes were identified according to both *t*-test ($p < 0.05$) and the fold-change criteria. In the second analysis, Significance Analysis of Microarrays (SAM; Tusher et al., 2001) was performed by using a one-class setting. This analysis includes a *t*-test statistic for each target transcript followed by a permutation analysis that generates a SAM score (*d*), including estimates for False Discovery Rate (FDR). Only those transcripts that had a FDR < 0.05 were considered significant. The final list of transcripts identified as significantly differentially expressed were those that were considered significant by both methods.

Functional analysis of the differentially expressed transcripts was done using (GO) as described above through *Blast2GO* Suite (Conesa et al., 2005; Götz et al., 2008). Gene enrichment analysis for the selected up- and down-regulated gene lists was then carried out to identify statistically overrepresented GO terms using Gene Ontology Enrichment Analysis Software Toolkit (GOEAST; Zheng and Wang, 2008). A hypergeometric test was used to test the GO term associations ($p < 0.05$) through GOEAST analysis (<http://omicslab.genetics.ac.cn/GOEAST/>). Area-proportional Venn diagrams were generated in order to compare and visualize selected lists by using BioVenn software (<http://www.cmbi.ru.nl/cdd/bioenn/index.php>; Hulsen et al., 2008).

The microarray platform and the data series were submitted to The Gene Expression Omnibus (GEO) (Edgar et al., 2002) with the series accession number GSE34322 and the microarray platform accession number GPL14742 (<http://www.ncbi.nlm.nih.gov/geo/>).

3. Results

3.1. EST database

As of June 14, 2011, 11,461 ESTs were available for *C. finmarchicus* in the NCBI database, with nearly all (11,128) originating from the cDNA library described here; the ESTs range in size from 153 to 931 base pairs, with a median length of 669 nucleotides. Clustering analysis of the 11,461 ESTs identified a total of 7287 clusters, with 5324 singletons and 1963 contigs with 2 sequences, which included three contigs with 15 or more sequences (Fig. 2). Using *BLASTx* we obtained putative annotations for 4745 (65%) of the singletons and contigs. The remaining sequences did not match known sequences, presumably representing unknown or highly modified genes. *Blastx* results indicated that *C. finmarchicus* sequences were most similar to those from other arthropods including *D. pulex*, *Drosophila melanogaster* and *Tribolium castaneum*.

3.2. Microarray target transcripts: Gene Ontologies and KEGG pathways

Initial selection of sequences for the microarray included transcripts that annotated to physiological functions that we hypothesized might be regulated in response to changing environmental conditions. The strategy for target selection included searching for genes that are differentially regulated in other organisms in response to changing environmental conditions, such as thermal stress (e.g. Buckley et al., 2006) or nutritional regimes (e.g. Gasch et al., 2000; Zinke et al., 2002). Temperature and nutrition were targeted because ecological studies on *C. finmarchicus* have shown that these two factors appear to drive much of its population cycling (Marshall and Orr, 1955; Meise and O'Reilly, 1996). In addition, *C. finmarchicus* and other high latitude calanoid copepods have been shown to exhibit physiological variability in lipid storage (Miller et al., 2000), citrate synthase activity, and fatty acid catabolism levels (Hassett, 2006). Transcripts annotating to these processes were searched for and included in the list of target sequences. Large differences in digestive enzyme activity and respiration rates have been found in *C. finmarchicus* individuals depending on their life stage (Saumweber and Durbin, 2006; Bonnet et al., 2007). Thus, the annotated sequences were searched for genes that annotated to both respiration and digestion for inclusion in the microarray. Likewise reproductive output is highly variable, and egg production changes rapidly in response to food availability (48 h; Runge, 1985; Niehoff, 2000). Therefore, singletons and contigs that annotated to reproduction and growth, including those involved in cell division and protein synthesis, were also searched for and included in the list of target transcripts, as were ones involved in lipid catabolism and biosynthesis. Lipids are known to play important roles in the life cycle of the *C. finmarchicus* and its congeners (Hagen and Auel, 2001). Lastly, we searched for sequences that annotated to neurological function and control of biological rhythms (e.g. circadian clock proteins and clock output signaling molecules); these transcripts were also included in the microarray. Table 1 provides examples of target transcripts, and their putative annotation and function, that were chosen based on these criteria.

Additional analysis of the final list of 1012 targets selected for the microarray showed that they represent broad ranges of biological processes, cellular components and molecular function. The majority of these sequences annotated to highly homologous sequences (766 had E-values of 1×10^{-20} or smaller) as indicated by follow-up annotation. Fewer than 20 annotated at E-values between 1×10^{-6} and 7×10^{-4} . The distribution of the target transcripts by biological process, cellular component and molecular function is shown in Fig. 3. Many targets are included in multiple categories within each pie chart, particularly at the lower levels of organization, such as levels 2 and 3. At the biological process level (level 2), the largest number of target sequences are involved in metabolic processes (555), biological regulation (348), and multicellular organism processes (242). At the cellular component

level (level 6), which is a higher level of organization, the largest number of target transcripts is contained within the category of cellular membrane-bound organelles (317). The molecular function ontology (level 3) shows that the microarray target sequences represent a wide array of functions including the following: transcription (transcription factor activity: 90), transport (substrate-specific transporter activity: 102; trans-membrane transporter activity: 94), protein binding (267) and lipid binding (17).

KEGG Pathway analysis identified 304 of the 1012 target transcripts as coding for enzymes involved in pathways annotated by this bioinformatic resource. The KEGG Pathway analysis did not identify all enzymes represented on the microarray, since some of them, such as “maltase 1” (ID: CFX04099) did not annotate to a pathway represented in KEGG. Examples of KEGG pathways represented on the microarray are summarized in Table 2. Six different KEGG pathways are listed with the number of target sequences and their associated enzyme codes. For example, the microarray includes probes that code for 10 enzymatic steps in the TCA cycle. Fig. 4 shows a schematic diagram of the TCA cycle (KEGG map:00020) with the *C. finmarchicus* targets highlighted. The probe identifications (CFX numbers), putative annotation and enzyme code (EC number) are summarized in Table 3. Note that in three cases, two probes annotated to the same enzyme.

Multiple transcripts involved in fatty acid metabolism are represented on the microarray (Fig. 5; KEGG Pathway map:00071). Because annotations are based on degree of similarity, any individual probe might have multiple enzyme codes (Table 4). In many cases, this occurs because the KEGG pathway lists more than one enzyme code for a single enzymatic step and the target sequence might annotate to any one of those enzymes. For example, the probe CFX02954 was annotated as either “long-chain-enoyl-CoA hydratase” (EC:4.2.1.74) or “enoyl-CoA hydratase” (EC:4.2.1.17). In addition, this probe also annotated to a second closely related enzymatic step involving the enzymes “long-chain-3-hydroxyacyl-CoA dehydrogenase” (EC: 1.1.1.211) or “3-hydroxyacyl-CoA dehydrogenase” (EC:1.1.1.35). A second probe (CFX02200) annotated to this enzymatic step as “3-hydroxyacyl-CoA dehydrogenase” (EC: 1.1.1.35) as well. Multiple probes annotated to the pathway step involving the enzymes acyl-CoA oxidase (EC:1.3.3.6; probe: CFX02263) and acyl-CoA dehydrogenase (EC:1.3.99.3; probes: CFX02803, CFX02569, CFX02263, CFX00958).

3.3. Microarray testing on ecological samples

3.3.1. High vs. low food conditions—To assess the effects of food abundance on gene transcription, adult females under one of two feeding regimes were compared: low food treatment consisted of a daily algal paste ration of 500 cells/mL for one week, while the high food treatment were received 5000 cells/mL for the seven day period. At the end of the experiment, the culture containers with the females under high food conditions were characterized by an abundance of fecal pellets on the bottom. In contrast the containers with the low food treatment were clear and with few fecal pellets on the bottom. The data from the one-sample *t*-test ($p < 0.05$) and SAM analysis ($FDR < 0.05$) were compared and 67 up-regulated and 19 down-regulated features showed significant differences in expression in both analyses. Regulated genes were organized by function and are listed in Tables 5 and 6. However, it should be noted that many of the transcripts annotated to proteins with multiple functions, and could thus have been placed in more than one category. In general, expression differences were modest with the majority of significant Log_2 ratios less than 2 (<4-fold difference). Only one heat shock protein (hsp 70, CFX00426) was significantly up-regulated in the low food females. None of the other heat shock proteins/factors showed significant differences in expression. Six transcripts coding for enzymes involved in lipid metabolism were up-regulated in the low food females. These included endoclyceramidase (CFX00129), oxysterol binding protein (CFX00191), lysosomal phospholipase (CFX00212),

lysophosphatidylcholine acyltransferase (CFX02017), lipase (CFX02590) and phytanoyl-CoA hydroxylase (CFX02697; Table 5). Seven transcripts involved with cytoskeleton or contractile elements were significantly up-regulated in the low food females. In addition to these seven, GOEAST analysis identified one more regulated transcript as “cytoskeleton part” (GO: 0044430; probe ID: CFX00833) indicating a significant enrichment in the regulated transcripts annotating to this cellular component (GOEAST analysis, $p=0.0031$). Two proteins in this group, tropomyosin (CFX00674) and troponin (CFX02278) are actin-binding proteins involved in muscle contraction. Fifteen transcripts involved in energy and homeostasis were up-regulated in the low food females and three of these (CFX00567, CFX01090 and CFX01211) code for enzymes in the tricarboxylic acid cycle (GO: 0006099), indicating a significant enrichment for this biological process (GOEAST; $p=0.03627$). Enrichment in proton transport (GO:0015992) was also identified using GOEAST analysis ($p=0.01842$; transcripts: CFX00397, CFX00567, CFX00623 and CFX01282). A number of transcripts involved in protein synthesis were up-regulated as well, and these included two translation elongation factors, a transcription initiation factor, a transcription factor and a premRNA processing factor (Table 5). Several transcripts involved in neuronal signaling pathways were up-regulated in the low food females, including the enzyme involved in the rate-limiting step for dopamine synthesis (CFX00280: tyrosine hydroxylase; Table 5). Three enzymes, carbonic anhydrase (CFX02388), catalase (CFX03773) and FAD-linked oxidase domain protein (CFX01897) that are important in cellular homeostasis were up-regulated.

Nineteen transcripts were significantly down-regulated in females maintained under low food conditions (Table 6). Among the down-regulated transcripts were hexokinase (CFX04635), which is involved in glycolysis, as well as delta-6 fatty acid desaturase (CFX01484), which is involved in lipid biosynthesis. A transcript associated with oxygen transport (CFX01167: hemocyanin, Table 6) was down-regulated in the low food females, as were two transcripts that may be involved in innate immunity (CFX01094: prophenol oxidase activating enzyme precursor and CFX01290: cytosolic IMP-GMP specific 5'-nucleotidase). Five transcripts involved with neuronal function were down-regulated in low food females. GOEAST analysis indicated significant enrichment in positive regulation of cell communication (GO:0010647, $p=0.0151$).

3.3.2. Lipid-rich vs. lipid-poor pre-adult *C. finmarchicus*—In the lipid-rich vs. lipid-poor comparisons, 35 transcripts were significantly up-regulated, and 11 transcripts were significantly down-regulated in lipid-poor stage CV individuals (Tables 7 and 8). Expression differences between lipid-poor and lipid-rich individuals were modest with most $\log_2$ ratios less than $|2|$ (4-fold difference in expression). Expression differences were similar in magnitude to the low food–high food comparison. The comparison between lipid-rich and lipid-poor pre-adult *C. finmarchicus* showed some similarities with the comparison between females on either high or low food diets as shown in Fig. 6. The transcripts that were up-regulated in both females subjected to low food conditions and lipid-poor sub-adults included lipase (CFX02590), oxysterol binding protein (CFX00191), SPT transcription factor (CFX00565), melanopsin (CFX02386), laminin receptor (CFX00787), hemolectin (CFX00358) and ubiquinol-cytochrome c reductase (CFX00550). However, most of the transcripts that were up-regulated in lipid-poor sub-adults were different from the ones that were up-regulated in the low food females. There was no overlap among the down-regulated transcripts in lipid-poor sub-adults and the low food females (Fig. 6B).

SNF4-AMP-activated protein kinase gamma transcript, which is an enzyme related to energy homeostasis was up-regulated in lipid poor individuals (Table 7). Two other transcripts (CFX01880 and CFX02214) involved in chemical homeostasis (GO: 0048878) were up-regulated in lipid-poor sub-adults, suggesting enrichment (GOEAST, $p=0.0397$). In

addition to the melanopsin transcript, the lipid-poor individuals also showed up-regulation of rhodopsin and the pre-prohormone of the neuropeptide allatostatin A (Table 7). Other up-regulated transcripts in the lipid-poor individuals included three members of the cytochrome P450 (*cyp*) family. These three transcripts are grouped with five other transcripts (CFX00565, CFX00567, CFX01002, CFX01008 and CFX02214) under oxidoreductase activity (GO:0016491), which was a significantly enriched molecular function (GOEAST, $p=0.0315$). Two transcripts involved in sodium transport were up-regulated in the lipid-poor individuals (CFX01757: sodium–potassium dependent ATPase and CFX02214: NADH dehydrogenase (ubiquinone) Fe–S protein; Table 7).

Down-regulated transcripts in lipid-poor CV included two enzymes involved in glycolysis (GO:0006096), indicating enrichment (GOEAST, $p=0.00790$) (steps 6 and 7, Table 8). Other down-regulated transcripts included proteins involved in apoptosis (CFX00414), copper homeostasis (CFX00284) and one cytochrome P450 member (CFX04230, Table 8).

4. Discussion

Microarrays provide a cost-effective and accurate approach for targeted, yet broad-spectrum, analysis of gene expression. By focusing on transcripts with known physiological function, the *C. finmarchicus* microarray can determine expression levels of genes across a broad range of biological processes and diverse biochemical pathways. In ecological studies, a broad-based assessment of physiological state can contribute important information on individuals in a population. Specific examples include determining whether individuals found near geographic boundaries show up-regulation in genes involved in the environmental stress response. Individuals experiencing food limitation may show up-regulation in genes involved in energy conservation, and down-regulation of genes involved in growth and reproduction. Likewise, the presence of toxic algae or pollutants may result in the up-regulation of genes involved in detoxification. These types of applications of molecular tools to answer ecological questions are still uncommon, but initial studies have been promising (review: Kammenga et al., 2007).

Ecological studies often focus on non-model organisms with limited genomic resources. This is changing rapidly, and transcriptome sequence data (EST database) are increasing for a variety of species. This has opened opportunities to build species-specific microarrays to investigate the physiological ecology of organisms of interest (Gracey and Cossins, 2003). *C. finmarchicus* falls into this category given its importance as a keystone species in the North Atlantic. Life history and ecological studies of this species suggest a high degree of physiological regulation in response to environmental conditions, as well as physiological changes associated with season (Hirche, 1996; Hirche et al., 1997; Miller et al., 2000; Campbell et al., 2001a; Irigoien, 2004; Madsen et al., 2008; Pepin and Head, 2009; Petursdottir et al., 2010).

The microarray described here was tested in two sets of comparisons designed to start asking physiological questions of ecological interest. *C. finmarchicus* individuals can experience large changes in food quantity and quality. In our study, we compared adult females maintained for seven days at two food concentrations that differed by an order of magnitude. Previous studies investigating the effect of food abundance on *C. finmarchicus* biology found that growth and egg production rates decline with food level (Hirche et al., 1997; Campbell et al., 2001b). RNA/DNA ratios are also much lower at low food abundances (Wagner et al., 2001). The microarray results indicate that under low food conditions there are multiple changes in gene expression relating to lipid metabolism, protein turnover, neural function, metabolism and structural elements. However, the magnitude of gene expression differences and the transcripts showing significant differences

in expression do not correspond to an “environmental stress response”, which typically involves changes in expression in 500 or more genes, including up-regulation of multiple heat shock proteins (Gasch et al., 2000). Our microarray included multiple probes encoding heat shock proteins (Table 1), however, only heat shock protein 70 was significantly up-regulated in females maintained at low food. In contrast, Aruda et al. (2011) found up-regulation of three heat shock proteins (hsp70A, hsp 21 and hsp22) in response to handling stress in *C. finmarchicus*.

Up-regulation of a variety of cytoskeletal elements was similar to expression changes observed in killifish (*Austrofundulus limnaeus*) subjected to high and fluctuating temperatures (Podrabsky and Somero, 2004). Down-regulation of hexokinase (first step in the glycolytic pathway) and up-regulation of a lipase are consistent with a switch to alternate sources of energy under low food conditions. Down-regulation of delta-6 fatty acid desaturase, an enzyme involved in fatty acid elongation, would also be predicted under conditions of low food. Starvation studies in larval *D. melanogaster* also indicated upregulation in the expression of genes involved in transcription and translation, and lipid and energy metabolism (Zinke et al., 2002). However, there seemed to be little overlap in the down-regulated genes. In contrast to *C. finmarchicus*, *D. melanogaster* larvae are not resistant to starvation (Zinke et al., 2002), so differences in gene expression patterns, particularly among the down-regulated transcripts would be expected.

The co-occurrence of sub-adults ranging in lipid stores is characteristic of *C. finmarchicus* populations during the summer and early fall (Miller et al., 2000; Pepin and Head, 2009). The microarray results suggest that lipid-poor individuals differ from the lipid-rich ones physiologically. Furthermore, lipid-poor sub-adults are also physiologically different from *C. finmarchicus* subjected to a period of low food conditions, with few shared probes showing significant differences in expression. The nine probes that were up-regulated in both lipid-poor and low-food individuals included five involved in metabolism, two in neuronal function, one in transcription and one in wound healing (hemolectin). Interestingly, none of the transcripts encoding cytoskeletal elements or involved in amino acid metabolism showed differential expression in lipid-poor relative to lipid-rich. On the other hand, the down-regulation of two enzymes involved in glycolysis (adjacent steps in the pathway) and the up-regulation of AMPK (AMP-activated protein kinase) suggest that the lipid-poor individuals are conserving energy.

The microarray contains eleven transcripts that annotated to members of the cytochrome P450 family, which is a large group of enzymes that are involved in the oxidation of organic substances, including xenobiotics. None of these showed significant regulation in the high-low food comparisons. However, in the lipid-rich vs. lipid-poor comparison, three transcripts were up-regulated and one was down-regulated in lipid-poor individuals. Using a target gene approach, Hansen et al. (2008; 2009) found down-regulation of cytochrome P450 CYP330A1, but not of CYP1A2 mRNA when *C. finmarchicus* was subjected to pollutants (naphthalene, or dispersed oil). Our microarray includes a probe for CYP1A2, but not for CYP330A1. The former did not show significant regulation in our two comparisons. Two other target genes, catalase and heat shock protein 70 were up-regulated both under naphthalene exposure (Hansen et al., 2008) and in low-food females (this study).

The *C. finmarchicus* microarray aims at broad physiological coverage while staying small (995 features), in order to include multiple hybridizations per slide. It was specifically designed to allow good replication and multiple comparisons between ecological and/or experimental samples. The current microarray can be used to select target genes that seem to be responsive to environmental factors and study them in more detail using quantitative real-time PCR. High throughput analysis of expression patterns such as is possible using the

microarray described here, allows changes in expression patterns to be put into a broader physiological context. Future applications of the *Calanus* physiology microarray will include increasing the number of genes and retargeting of genes and metabolic pathways shown to be of particular interest and importance in studies of individual responses to temperature, food availability, and other potential environmental stressors.

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Fig. 1.

A. Light micrograph of a sub-adult (stage CV) *Calanus finmarchicus*. The oil sac is clearly visible along the central axis of the prosome (arrow head). First antennae are approximately the length of the prosome plus the urosome, and they extend beyond the micrograph. B. Approximate geographic range of *C. finmarchicus* based on distribution information in Helauouët et al. (2011) and the Global Biodiversity Information Facility web site ([data.gbif.org/species/species/Calanus+ finmarchicus](http://data.gbif.org/species/species/Calanus+finmarchicus)).

Fig. 2. Frequency distribution of the number of singletons and contigs derived from the *Calanus finmarchicus* expressed sequence tag (ESTs) database. For the contigs composed of high numbers of ESTs, the total number of contigs contained within the bar is printed above it.

Fig. 3.

Distribution of target genes by biological process (A), cellular component (B) and molecular function (C) based on gene ontology annotations. Level of organization shown is listed for each chart. Categories are listed and include the number of probes in parenthesis in each category.

Fig. 4. Tricarboxylic acid cycle (TCA) diagram from KEGG Pathway analysis. Color coded enzymes indicate target genes represented on the microarray. Specific targets and their respective annotation, enzyme code and color-coding are given in Table 3.

Fig. 5. Lipid (fatty acid) metabolic pathway diagram from KEGG Pathway analysis. Color coded enzymes indicate target genes represented on the microarray. Specific targets and their respective annotation, enzyme code and color-coding are given in Table 4.

Fig. 6.

Area-proportional Venn diagrams comparing differences in expression in the two sets of microarray experiments: low food vs. high food females (pink) and the lipid-poor vs. lipid rich sub-adults (green). A) Up-regulated probes. B) Down-regulated probes. Identification numbers (CFX numbers) for the probes showing significant differences ($p < 0.05$) in expression are listed.

Table 1

Examples of contigs included in the microarray organized by protein function or class, contig identification code and putative annotation. Annotation information includes the NCBI accession number and E-value of *Blasts* result.

Function/Class	I.D.	Annotation	Accession number	E-value
Wax ester biosynthesis	CFX03948	Fatty acyl CoA synthetase	NP_652034	4.8E-46
	CFX00170	Acyl-CoA desaturase (Stearoyl-CoA desaturase) (Fatty acid desaturase) (Delta(9)-desaturase)	Q92038	1.3E-54
	CFX03858	Fatty acid elongase	XP_569369	5.4E-33
Triacylglycerol biosynthesis	CFX02970	Diacylglycerol kinase family member (dgc-3)	NP_499031	2.7E-20
Lipid catabolism	CFX00958	Acyl-coenzyme A dehydrogenase, C-4 to C-12 straight chain	NP_999204	5.3E-56
	CFX02803	Acyl-coenzyme A dehydrogenase, very long chain	NP_997776	1.6E-54
	CFX02954	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl Coenzyme A hydratase (trifunctional protein), beta subunit	NP_001080077	9.2E-82
	CFX00246	Acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl Coenzyme A thiolase)	NP_001006571	1.5E-33
	CFX01995	Palmitoyltransferase ZDHHC2 (Zinc finger DHHC domain containing protein 2)	Q9JKR5	1.2E-42
Carbohydrases	CFX04099	Alpha-amylase	XP_001656069	1.8E-61
	CFX00393	Beta-glucosidase (Gentiobiase) (Cellobiase) (Beta-D-glucoside glucoside glucohydrolase) (Amygdalase)	Q03506	5.2E-58
	CFX02125	Maltase-like protein Agm2	CAA60858	4.5E-80
	CFX02615	Maltase-glucoamylase	XP_001496710	4.9E-27
Proteases	CFX04358	Trypsin	CAA75309	1.9E-38
	CFX02951	Chymotrypsin BII precursor	P36178	6.2E-29
Lipases	CFX02590	Lipase 1 precursor (DmLip1)	XP_972874	1.5E-47
	CFX03367	Lipase A, lysosomal acid, cholesterol esterase	NP_001096793	5.1E-47
Heat shock proteins	CFX03522	Heat shock protein 71	ABU41013	6.6E-81
	CFX00326	Heat shock protein cognate 71	AAA28628	2.0E-95
	CFX00426	Heat shock protein 70 kDa	XP_535180	1.3E-42
	CFX04161	Heat shock protein 40	ABC84495	4.6E-11
	CFX03814	Chaperone protein DnaJ	XP_001661333	2.6E-23
	CFX02718	DnaJ homolog subfamily C member 13	XP_542783	1.0E-54
	CFX01986	DnaJ (Hsp40) homolog, subfamily A, member 1	NP_001011012	5.3E-63
	CFX03765	DnaJ-like protein	ABD76373	5.9E-43
	CFX01166	DnaJ (Hsp40) homolog, subfamily C, member 7 isoform 1	XP_393522	7.7E-50
	CFX01928	DnaJ domain containing protein	EDP33391	1.3E-32
	CFX04499	Heat shock protein 90	ABR66910	8.9E-21
	CFX02210	Heat shock protein gp96	NP_999808	6.0E-63
	CFX00874	Heat shock protein 22	XP_001847194	6.0E-11
	CFX00018	Small heat shock protein 19.7	BAE94664	2.2E-14
	CFX01546	Heat shock protein 19.7	BAF03558	2.4E-11
	CFX02411	Heat shock factor protein (HSF) (Heat shock transcription factor-HSTF)	XP_395321	8.6E-54
	CFX04122	HIF (hypoxia inducible factor) homolog family member (hif-1)	NP_001023894	1.0E-07

Function/Class	I.D.	Annotation	Accession number	E-value
Signaling peptides	CFX02388	Carbonic anhydrase II	NP_954685	1.5E-26
	CFX03461	Carbonic anhydrase 4	AAC47449	1.1E-19
	CFX03424	Oxidative stress protein	AAX09928	6.0E-27
	CFX04633	Prepro-A-type allatostatin	ABS29318	3.7E-72
	CFX03884	Juvenile hormone esterase	XP_001663023	1.4E-49
Circadian rhythms	CFX04266	Timeless	AAM77468	4.5E-65
	CFX03856	Cryptochrome 2 protein	ABO3112	9.5E-75

Table 2

Examples of KEGG pathways and the number of probes that annotated to that specific pathway on the microarray. Selected KEGG pathways and their map number are listed with enzyme codes represented on the microarray.

KEGG Pathway	Number of sequences	Enzyme codes	KEGG map
Glycolysis/gluconeogenesis	12	ec:1.2.4.1, ec:2.7.1.1, ec:1.2.1.12, ec:1.2.1.3, ec:2.7.2.3, ec:2.7.1.40, ec:1.8.1.4. ec:2.3.1.12. ec:2.7.1.11	Map:00010
Alanine, aspartate and glutamate metabolism	10	ec:1.2.1.24. ec:6.3.1.2, ec:2.6.1.2. ec:2.6.1.1, ec:2.6.1.16, ec:6.3.4.4, ec:3.5.1.2. ec:3.5.1.1, ec:1.4.1.14	Map:00250
Pyrimidine metabolism	9	ec:2.7.7.7 ec:2.7.7.6, ec:2.4.2.4. ec:2.7.4.6, ec:2.7.1.48, ec:3.1.3.5, ec:1.8.1.9, ec:1.17.4.1	Map:00240
Pyruvate metabolism	11	ec:1.2.4.1, ec:1.1.2.4, ec:1.2.1.3, ec:2.7.1.40. ec:1.1.1.40. ec:1.1.1.37. ec:1.8.1.4. ec:2.3.1.12, ec:1.1.1.28. ec:6.4.1.2	Map:00620
Nitrogen metabolism	8	ec:6.3.1.2, ec:4.3.1.3, ec:4.2.1.1, ec:3.5.1.2. ec:3.5.1.1, ec:1.4.1.14	Map:00910
Aminoacyl-tRNA biosynthesis	5	ec:6.1.1.17. ec:6.1.1.16. ec:6.1.1.14, ec:6.1.1.9, ec:6.1.1.5, ec:6.1.1.4. ec:6.1.1.1	Map:00970

Table 3

Annotated microarray contigs involved in the tricarboxylic acid cycle using KEGG Pathway analysis. Color refers to the color-coding in Fig. 4 (electronic version only).

I.D.	Annotation	Enzyme code	Color
CFX01032	Oxoglutarate dehydrogenase (succinyl-transferring)	ec:1.2.4.2	Red
CFX01549	Pyruvate dehydrogenase (acetyl-transferring) ^a	ec:1.2.4.1	Dark yellow
CFX00988	Pyruvate dehydrogenase (acetyl-transferring)	ec:1.2.4.1	Dark yellow
CFX00003	Citrate (Si)-synthase	ec:2.3.3.1	Orange
CFX01211	Succinate — CoA ligase (ADP-forming)	ec:6.2.1.5	Green
CFX00011	Dihydrolipoyllysine-residue succinyltransferase	ec:2.3. 1.61	Blue
CFX01178	Aconitate hydratase	ec:4.2.1.3	Violet
CFX01090	Aconitate hydratase	ec:4.2.1.3	Violet
CFX02052	Fumarate hydratase	ec:4.2.1.2	Purple
CFX04612	Malate dehydrogenase	ec:1.1.1.37	Pink
CFX00974	Malate dehydrogenase	ec:1.1.1.37	Pink
CFX00269	Dihydrolipoyl dehydrogenase	ec:1.8.1.4	Light green
CFX01354	Dihydrolipoyllysine-residue acetyltransferase	ec:2.3.1.12	Light yellow

^a Annotated as “Branched chain keto acid dehydrogenase” by *Blastx* (see Table 5).

Table 4

Annotated microarray contigs involved in fatty acid metabolism using KEGG Pathway analysis. Color refers to the color-coding in Fig. 5 (electronic version only).

I.D.	Annotation	Enzyme code	Color
CFX02954	Enoyl-CoA hydratase	ec:4.2.1.17	Red
CFX02263	Acyl-CoA oxidase	ec:1.3.3.6	Yellow
CFX01002	Aldehyde dehydrogenase (NAD ⁺)	ec:1.2.1.3	Orange
CFX02954	Long-chain-acyl-CoA hydratase ^a	ec:4.2.1.74	Green
CFX02954	Long-chain-3-hydroxyacyl-CoA dehydrogenase ^a	ec:1.1.1.211	Blue
CFX02954	3-Hydroxyacyl-CoA dehydrogenase ^a	ec:1.1.1.35	Pink
CFX02200	3-Hydroxyacyl-CoA dehydrogenase	ec:1.1.1.35	Pink
CFX02954	Acetyl-CoA C-acyltransferase ^a	ec:2.3.1.16	Purple
CFX00268	Acetyl-CoA C-acyltransferase	ec:2.3.1.16	Purple
CFX01016	Glutaryl-CoA dehydrogenase	ec:1.3.99.7	Salmon
CFX02803	Acyl-CoA dehydrogenase	ec:1.3.99.3	Light green
CFX02569	Acyl-CoA dehydrogenase	ec:1.3.99.3	Light green
CFX02263	Acyl-CoA dehydrogenase	ec:1.3.99.3	Light green
CFX00958	Acyl-CoA dehydrogenase	ec:1.3.99.3	Light green

^aAnnotated as "Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit (see Table 1).

Table 5

Microarray probes that were significantly up-regulated in *Calanus finmarchicus* adult females maintained under low food conditions relative to high food.

Probe I.D.	Annotation	Log ₂ (ratio) ± S.D.	Probability
<i>Amino acid transfer, repair and breakdown</i>			
CFX00563	Aspartate aminotransferase	1.75±0.23	0.0058
CFX01055	Homogentisate 1,2-dioxygenase	1.61±0.49	0.0070
CFX01364	Peptide methionine sulfoxide reductase	1.11±0.37	0.0093
CFX01549	Branched chain keto acid dehydrogenase	4.76±0.37	0.0001
CFX02117	Solute carrier family 7 (cationic amino acid transporter)	2.35±0.28	0.0005
CFX02548	Transmembrane amino acid transporter protein	2.18±0.71	0.0086
<i>Cytoskeletal and contractile elements</i>			
CFX00044	Dynactin p62 subunit	1.65±0.46	0.0057
CFX00362	Alpha tubulin	2.00±0.77	0.0455
CFX00403	Uncoordinated family member (Unc-76)	3.01±0.48	0.0011
CFX00667	Myosin heavy chain	1.95±0.57	0.0065
CFX00674	Tropomyosin 1, isoform A	1.37±0.17	0.0006
CFX00981	Actin-related protein 3	1.00±0.61	0.0459
CFX02278	Troponin 1	1.09±0.51	0.0237
<i>Energy, homeostasis, mitochondrial genes</i>			
CFX00393	Beta-glucosidase (Gentiobiase) (Cellobiase)	1.76±0.96	0.0350
CFX00397	ATP-synthase, H ⁺ transporting	1.52±0.79	0.0313
CFX00529	Cytochrome oxidase biogenesis protein	2.14±0.50	0.0034
CFX00550	Ubiquinol-cytochrome c reductase	1.98±0.74	0.0129
CFX00567	Nicotinamide nucleotide transhydrogenase	1.65±0.70	0.0182
CFX00623	Vacuolar AIP synthase	2.02±0.66	0.0087
CFX00947	Pyruvate dehydrogenase phosphatase regulatory subunit	2.67±0.84	0.0080
CFX00997	NADH dehydrogenase	1.31±0.68	0.0309
CFX01090	Aconitate hydratase	1.19±0.28	0.0036
CFX01211	Succinyl-CoA synthase	2.21±0.43	0.0020
CFX01282	H ⁺ transporting synthase	2.70±0.49	0.0016
CFX01354	Dihydrolipoamide acetyltransferase	2.95±0.82	0.0055
CFX01897	FAD linked oxidase domain protein	1.32±0.70	0.0328
CFX02388	Carbonic anhydrase	1.96±0.45	0.0032
CFX03773	Catalase	1.17±0.20	0.0013
<i>Lipid metabolism and other lipid-related functions</i>			
CFX00129	Endoglycoceramidase	1.23±0.24	0.0019
CFX00191	Oxysterol binding protein	1.61±0.59	0.0121
CFX00212	Lysosomal phospholipase	1.69±0.76	0.0214
CFX02017	Lysophosphatidylcholine acyltransferase	1.10±0.58	0.0320
CFX02590	Lipase	2.58±0.71	0.0054
CFX02697	Phytanoyl-CoA 2 hydroxylase	2.21±0.77	0.0106

Probe I.D.	Annotation	Log ₂ (ratio) ± S.D.	Probability
<i>Neuronal involvement</i>			
CFX00023	Defective proboscis extension response	1.67±0.41	0.0040
CFX00089	Choline cotransporter	2.11±0.54	0.0044
CFX00280	Tyrosine hydroxylase	1.33±0.76	0.0392
CFX00332	Nicotinic acetylcholine receptor	1.66±0.33	0.0022
CFX00527	N-methyl-D-aspartate receptor associated protein	1.10±0.66	0.0444
CFX00787	Laminin receptor	1.74±0.14	0.0020
CFX00813	Cholinergic receptor	1.26±0.37	0.0064
CFX02386	Melanopsin	1.12±0.42	0.0125
<i>Transcription and translation</i>			
CFX00278	Translation elongation factor 1 gamma	1.92±0.46	0.0188
CFX00418	DNA-directed RNA polymerase	1.09±0.27	0.0044
CFX00565	SPI transcription factor	1.96±1.00	0.0295
CFX00833	Mitotic checkpoint protein and poly(a)+ RNA export	2.20±0.40	0.0016
CFX00927	Transcription initiation factor IIa gamma chain	1.33±0.39	0.0063
CFX01159	Pre-mRNA processing factor (PRP-39)	1.39±0.79	0.0387
CFX01803	Translation elongation factor g	1.42±0.23	0.0012
<i>Transcription and translation</i>			
CFX01918	Enhancer of mRNA decapping	2.35±0.91	0.0139
CFX02579	Thyrotrophic embryonic factor	1.10±0.24	0.0026
CFX02869	Peroxisome proliferator-activated receptor binding	1.92±0.21	0.0003
<i>Other</i>			
CFX00024	Importin 9	2.20±0.50	0.0030
CFX00277	Blastula protease-10	1.45±0.45	0.0312
CFX00358	Hemolysin	2.51±1.30	0.0303
CFX00426	Heat shock 70 kDa	2.11±0.27	0.0006
CFX00431	Nuclear autoantigenic sperm protein (histone binding)	1.47±0.34	0.0034
CFX00770	Vacuolar protein sorting	2.33±1.11	0.0246
CFX01188	Serine carboxypeptidase	1.65±0.51	0.0073
CFX01318	Transport protein Sec61 alpha subunit	1.83±0.79	0.0190
CFX01358	DNA polymerase	1.20±0.57	0.0251
CFX01902	Cell cycle progression 2 protein	1.54±0.75	0.0263
CFX02065	Vesicular-fusion protein nsf	1.10±0.20	0.0017
CFX02402	Beta-1, 4-endoglucanase	1.14±0.51	0.0207
CFX02413	WD40-repeat containing protein (notchless)	1.98±0.39	0.0021
CFX02419	Tyrosine kinase receptor (torso-like)	1.56±0.13	0.0025
CFX02611	Nicastrin	1.49±0.50	0.0095

Transcripts are grouped by function, annotation details are provided at NCBI Gene Expression Omnibus (GEO) with the microarray platform accession number GPL14742 (<http://www.ncbi.nlm.nih.gov/geo/>).

Table 6

Microarray probes that were significantly down-regulated in *Calanus finmarchicus* adult females maintained at low food levels relative to high food levels.

Probe I.D.	Annotation	Log ₂ (ratio) ± S.D.	Probability
<i>Energy, homeostasis, mitochondrial genes</i>			
CFX00102	Alcohol dehydrogenase	-1.47±0.49	0.0357
CFX00679	D-lactate dehydrogenase 2	-1.20±0.40	0.0091
CFX04635	Hexokinase	-1.49±0.45	0.0071
<i>Lipid metabolism and other lipid-related functions</i>			
CFX01484	Delta-6 fatty acid desaturase	-1.44±0.08	0.0000
<i>Neuronal involvement</i>			
CFX00721	Choline transporter-like protein 2	-1.27±0.25	0.0020
CFX01355	Transient receptor potential cation channel	-1.75±0.61	0.0383
CFX01446	Glutamate receptor	-1.94±0.48	0.0196
CFX02066	Skeletal muscle receptor tyrosine kinase	-1.76±0.37	0.0145
CFX03281	Cyclic-AMP phosphodiesterase (dunce)	-1.32±0.29	0.0027
<i>Other</i>			
CFX00526	EPsiN (endocytic protein homolog)	-1.79±1.07	0.0438
CFX00554	Monocarboxylate transporter	-1.26±0.39	0.0074
CFX00576	Limkain bl	-1.70±0.96	0.0381
CFX01094	Prophenol oxidase activating enzyme	-1.86±1.12	0.0453
CFX01167	Hemocyanin D chain	-1.33±0.78	0.0425
CFX01290	Cytosolic IMP-GMP specific 5'-nucleotidase	-1.03±0.19	0.0016
CFX01329	Glycosyl-phosphatidyl-inositol-anchored protein	-1.46±0.41	0.0255
CFX01723	Dentin sialophosphoprotein	-1.22±0.35	0.0060
CFX01921	DNA-binding bromodomain-containing protein	-2.81±1.05	0.0435
CFX02434	Nodal modulator 1	-1.05±0.09	0.0002

Transcripts are grouped by function, annotation details are provided at NCBI Gene Expression Omnibus (GEO) with the microarray platform accession number GPL14742 (<http://www.ncbi.nlm.nih.gov/geo/>).

Table 7

List of transcripts that were significantly up-regulated in *Calanus finmarchicus* lipid-poor sub-adults (CV) relative to lipid-rich sub-adults.

Probe I.D.	Annotation	Log ₂ (Ratio) ± S.D.	Probability
<i>Cytochrome P450 family</i>			
CFX00103	Cytochrome P450, family 2, subfamily K, polypeptide 6	2.13±0.49	0.0006
CFX01610	Cytochrome P450 CYP2N	1.36±0.53	0.0047
CFX02044	Cytochrome P450, family 2, subfamily c, polypeptide 44	3.85±1.18	0.0019
<i>Cytoskeletal and contractile elements</i>			
CFX01616	Gelsolin precursor	1.18±0.53	0.0078
<i>Energy, homeostasis, mitochondrial genes</i>			
CFX00085	SNF4-AMP-activated protein kinase	1.52±0.72	0.0092
CFX00550	Ubiquinol-cytochrome c reductase	2.98±0.83	0.0013
CFX00567	Nicotinamide nucleotide transhydrogenase	2.47±1.06	0.0065
CFX00947	Pyruvate dehydrogenase phosphatase regulatory subunit	1.09±0.51	0.0088
CFX01002	Aldehyde dehydrogenase	1.59±0.65	0.0056
CFX02214	NADH dehydrogenase (ubiquinone) Fe-S protein	1.14±0.49	0.0067
CFX02829	Glucoamylase-glucan 1,4- α -glucosidase	1.67±0.57	0.0028
<i>Lipid metabolism and other lipid-related functions</i>			
CFX00191	Oxysterol binding protein	2.12±0.66	0.0019
CFX00639	N-acetyllactosaminide beta-1,3-n-acetylglucosaminyltransferase	1.74±0.69	0.0048
CFX02372	1-Acylglycerophosphocholine O-acyltransferase 1	2.25±0.65	0.0015
CFX02590	Lipase	1.96±0.53	0.0012
CFX03072	AMP dependent ligase	2.74±1.09	0.0049
<i>Neuronal involvement</i>			
CFX00787	Laminin receptor	1.05±0.41	0.0049
CFX00814	Alpha 5 type IV collagen isoform 1	1.43±0.23	0.0001
CFX01356	Poly(rC)-binding protein (mushroom-body expressed)	2.16±0.77	0.0033
CFX01426	Rhodopsin	2.47±0.85	0.0029
CFX01880	ATP-sensitive inward rectifier potassium channel	3.80±1.02	0.0050
CFX02386	Melanopsin	1.86±0.57	0.0019
CFX04633	Prepro-A-type allatostatin	1.52±0.27	0.0002
<i>Transcription and translation</i>			
CFX00549	C-terminal Src kinase	1.48±0.69	0.0084
CFX00565	SPT transcription factor	1.62±0.77	0.0092
CFX00775	Transcription initiation factor IIB	1.90±0.64	0.0026
CFX00977	Eukaryotic peptide chain release factor subunit 1 (eRF1)	2.63±0.25	0.0000
<i>Other</i>			
CFX00358	Hemolentin	2.21±0.41	0.0002
CFX00422	Permease, MFS superfamily	1.91±0.81	0.0061
CFX00553	Caspase	1.78±0.48	0.0011
CFX01008	Methylenetetrahydrofolate dehydrogenase NADP+ dependent)	2.01±0.27	0.0001

Probe I.D.	Annotation	Log ₂ (Ratio) ± S.D.	Probability
CFX01757	Sodium–potassium–transporting ATPase	2.99±0.79	0.0011
CFX02321	Pantothenate kinase (fumble)	2.22±0.40	0.0002
CFX02435	Butyrate response factor 1	1.24±0.44	0.0033
CFX04127	Glycosyl–phosphatidylinositol– linked carbonic anhydrase	2.58±0.99	0.0044

Transcripts are grouped by function, annotation details are provided at NCBI Gene Expression Omnibus (GEO) with the microarray platform accession number GPL14742 (<http://www.ncbi.nlm.nih.gov/geo/>).

Table 8

List of transcripts that were significantly down-regulated in *Calanus finmarchicus* lipid-poor sub-adults (CV) relative to lipid-rich sub-adults.

Probe I.D.	Annotation	Log ₂ (ratio) ± S.D.	Probability
<i>Cytochrome P450 family</i>			
CFX04230	(Cytochrome P450 6a17 (CYPVIA17))	-1.11±0.33	0.0017
<i>Energy, homeostasis, mitochondrial genes</i>			
CFX00570	Glyceraldehyde-3-phosphate dehydrogenase 3 (GAPDH 3)	-1.67±0.59	0.0031
CFX00930	Phosphoglycerate kinase	-1.11±0.36	0.0084
<i>Neuronal involvement</i>			
CFX00442	Ecto-nucleoside triphosphate diphosphohydrolase	-1.69±0.59	0.0031
CFX01514	Neuroserpin	-1.36±0.36	0.0011
<i>Other</i>			
CFX00012	Carboxylesterase	-1.20±0.54	0.0077
CFX00284	Copper resistance protein	-1.40±0.44	0.0021
CFX00414	Cellular apoptosis susceptibility protein	-1.75±0.55	0.0020
CFX01619	Spermatogenesis associated protein	-1.01±0.45	0.0072
CFX01939	Sorting nexin	-1.96±0.71	0.0036
CFX01957	Asparaginyl endopeptidase	-1.84±0.81	0.0071

Transcripts are grouped by function, annotation details are provided at NCBI Gene Expression Omnibus (GEO) with the microarray platform accession number GPL14742 (<http://www.ncbi.nlm.nih.gov/geo/>).