Links to a microarray and transcriptome for C. finmarchicus in the Gulf of Maine from 2008-2011 (CFINTRANSCRIPT project)

Website: https://www.bco-dmo.org/dataset/528312 Version: Version Date: 2017-12-06

Project

» <u>Application of transcriptomics to investigate organism-environment relationships in marine zooplankton</u> (CFINTRANSCRIPT)

Contributors	Affiliation	Role
<u>Lenz, Petra H.</u>	University of Hawai'i at Mānoa (PBRC)	Principal Investigator
Christie, Andrew E.	University of Hawai'i at Mānoa (PBRC)	Scientist
<u>Roncalli, Vittoria</u>	University of Hawai'i at Mānoa (PBRC)	Student
<u>Copley, Nancy</u>	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

- Dataset Description
 - Methods & Sampling
 - Data Processing Description
- Data Files
- Parameters
- Instruments
- Deployments
- Project Information
- Funding

Dataset Description

This project focused on developing molecular tools based on relative gene expression to investigate physiological ecology of *Calanus finmarchicus* (Calanoida: Copepoda) in the Gulf of Maine. For this project, a **custom microarray** was developed for this species and tested on field and experimental data. Using *RNA-seq* technology, a *de novo* transcriptome was obtained from RNA extracted from different developmental stages of *C. finmarchicus* from embryo to adult female.

Datasets generated through this project were deposited at NCBI (<u>www.ncbi.nlm.nih.gov</u>) in 5 separate NCBI BioProjects:

- 1. BioProject PRJNA151477 (<u>www.ncbi.nlm.nih.gov/bioproject/PRJNA151477</u>): Microarray platform information and relative expression data for lipid-rich vs. lipid-poor pre-adults (stage CV) and adult females maintained at high- and low-food levels.
- BioProject PRJNA149523 (<u>www.ncbi.nlm.nih.gov/bioproject/PRJNA1495230</u>): Microarray platform information and relative expression data for pre-adults (stage CV) and adult females collected from depth (130-170 m) and from surface waters (0-30 m).
- 3. BioProject PRJNA236528 (<u>www.ncbi.nlm.nih.gov/bioproject/PRJNA236528</u>): RNA-Seq data. Data includes biosample description, raw sequence data for each sample, and transcriptome shotgun assembly of sequences. *Calanus finmarchicus* transcriptome from 6 developmental stages (embryo, early nauplius, late nauplius, early copepodite, late copepodite and adult females).
- 4. BioProject PRJNA312028 (<u>http://www.ncbi.nlm.nih.gov/bioproject/PRJNA312028</u>): RNA-Seq data. Data includes biosample description, raw sequence data for each sample, and transcriptome shotgun assembly of sequences. *Calanus finmarchicus* adult female transcriptome of Alexandrium fundyense response.
- Bioproject: PRJNA356331(<u>http://www.ncbi.nlm.nih.gov/bioproject/PRJNA356331</u>): RNA-Seq data. Data includes biosample description, raw sequence data for each sample, and transcriptome shotgun assembly of sequences *Calanus finmarchicus* late nauplii (NV-NVI) transcriptome of *Alexandrium*

Dataset Related References:

Lenz, P.H., Roncalli, V., Hassett, R.P., Wu, L.-S., Cieslak, M.C., Hartline, D.K. and Christie, A.E. (2014) De novo assembly of a transcriptome for *Calanus finmarchicus* (Crustacea, Copepoda) – the dominant zooplankter of the North Atlantic Ocean. Plos One 9: e885389 <u>DOI: 10.1371/journal.pone.0088589</u>)

Roncalli, V., Cieslak, M.C., Lenz, P.H. Transcriptomic responses of the calanoid copepod *Calanus finmarchicus* to the saxitoxin producing dinoflagellate Alexandrium fundyense. Scientific Reports 6, Article number: 25708 (2016) <u>doi:10.1038/srep25708</u>.

Roncalli V., Cieslak M.C., Lenz P.H. (2016) Data from: Transcriptomic responses of the calanoid copepod *Calanus finmarchicus* to the saxitoxin producing dinoflagellate Alexandrium fundyense. Dryad Digital Repository. <u>http://dx.doi.org/10.5061/dryad.11978</u>

Roncalli, V., Jungbluth, M.J., Lenz, P.H. (2016). Glutathione S-transferase regulation in *Calanus finmarchicus* feeding on the toxic dinoflagellate Alexandrium fundyense. PloS One, 11(7): e0159563.

Roncalli, V., Turner, J.T., Kulis, D., Anderson, D.M., Lenz, P.H. (2016). The effect of the toxic dinoflagellate Alexandrium fundyense on the fitness of the calanoid copepod *Calanus finmarchicus*. Harmful Algae, 51: 56-66

Roncalli, V., Lenz, P.H., Cieslak, M.C., Hartline, D.K. (2017). Complementary mechanisms for neurotoxin resistance in a copepod. Scientific Reports, 2017; 7: 14201, doi: 10.1038/s41598-017-14545-z

Methodology References:

<u>Microarray</u>

Lenz, P.H., Unal, E., Hassett, R.P., Smith, C.M., Bucklin, A., Christie, A.E. and Towle, D.W. (2012) Functional genomics resources for the North Atlantic copepod, *Calanus finmarchicus*: EST database and physiological microarray. Comparative Biochemistry Physiology Part D, Genomics & Proteomics, 7:110-123

Unal, E., Bucklin, A., Lenz, P.H. and Towle, D.W. (2013) Gene expression of the marine copepod *Calanus finmarchicus*: Responses to small-scale environmental variation in the Gulf of Maine (NW Atlantic Ocean). Journal of Experimental and Marine Biology and Ecology, 446:76-85

Gene discovery studies using C. finmarchicus transcriptome:

Christie, A.E., Fontanilla, T.M., Nesbit, K.T. and Lenz, P.H. (2013) Prediction of the protein components of a putative *Calanus finmarchicus* (Crustacea, Copepoda) circadian signaling system using a de novo assembled transcriptome. Comparative Biochemistry and Physiology Part D, Genomics & Proteomics, 8:165-193

Christie, A.E., Roncalli, V., Wu, L.-S., Garrote, C.L., Doak, T. and Lenz, P.H. (2013) Peptidergic signaling in *Calanus finmarchicus* (Crustacea: Copepoda): in silico identification of putative peptide hormones and their receptors using a de novo assembled transcriptome. General and Comparative Endocrinology, 187:117-135

Christie, A.E., Roncalli, V., Batta Lona, P., McCoole, M.D., King, B.L., Bucklin, A., Hartline, D.K. and Lenz, P.H. (2013) In silico characterization of the insect diapause-associated protein couch potato (CPO) in *Calanus finmarchicus* (Crustacea: Copepoda). Comparative Biochemistry and Physiology. Part D, Genomics & Proteomics, 8:45-57.

Christie, A.E., Fontanilla, T.M., Roncalli, V., Cieslak, M.C. and Lenz, P.H. (2014) Diffusible gas transmitter signaling in the copepod crustacean *Calanus finmarchicus*: Identification of the biosynthetic enzymes of nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H2S) using a de novo assembled transcriptome. General and Comparative Endocrinology 202: 76-86

Christie, A.E., Fontanilla, T.M., Roncalli, V., Cieslak, M.C. and Lenz, P.H. (2014) Identification and developmental expression of the enzymes responsible for dopamine, histamine, octopamine and serotonin biosynthesis in the copepod crustacean *Calanus finmarchicus*. General and Comparative Endocrinology 195: 28-39.

Roncalli, V., Cieslak, M.C., Passamaneck, Y., Christie, A.E., & Lenz, P.H. (2015). Glutathione S-Transferase (GST) Gene diversity in the crustacean *Calanus finmarchicus* - contributors to cellular detoxification. PloS One, 10(5): e012332.

Methods & Sampling

See publications for detailed information on sample acquisition, experimental treatment and methodology.

Source of *C. finmarchicus*:

- Mid- and late copepodites and adults were collected during the summer (June and July, 2009, 2010, 2011, 2012) 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. These individuals were used in microarray (2009, 2010) and *RNA-Seq* (2011, 2012) studies.
- 2. Embryos, nauplii and early copepodites were obtained from offspring of field-collected adult females raised in laboratory cultures. These individuals were used in *RNA-Seq* studies.
- 3. Late stage copepodites (CV) for microarray analysis (Unal et al. 2013) were collected in April 17, 2008 from Wilkinson Basin in the Gulf of Maine as part of a multi-year time-series study by the Center for Coastal Ocean Observation and Analysis (COOA) at the University of New Hampshire, USA. Vertically-stratified plankton tows (0-30 m and 130-170 m) were taken using a 1/4-m MOCNESS equipped with 150 µm mesh nets.

Data Processing Description

Brief summary of methods

RNA was extracted from fresh and preserved samples. Samples were preserved either in RNALater or in liquid nitrogen. RNA was extracted using the Qiagen RNeasy Mini or Mini Plus kits for both microarray and RNASeq. Quality and quantity of RNA was checked in an Agilent 2100 Bioanalyzer prior to further processing of samples as described in the references. Microarray hybridization incubations were performed in a Micro Array User Interface (MAUI) Hybridization Chamber (BioMicro Systems). For the RNASeq, high quality total RNA samples were sent to the Georgia Genomics Facility at U. of Georgia. Multiplex cDNA gene libraries were prepared from the samples, and these were sent to Alpha Hudson Institute for Biotechnology for paired 100-base-pair sequencing on an Illumina HISeq 2000 instrument.

The RNASeq data were used to assemble and annotate a de novo transcriptome. Raw reads were analyzed for quality: low quality and over-represented sequences were removed, and sequences were trimmed to remove the random primer sequences (first 9 bases) prior to assembly using Trinity (Trinity 2012-03-17-IU_ZIH_TUNED software, on the National Center for Genome Analysis Support's [NCGAS; Indiana University, Bloomington, IN, USA] Mason Linux cluster; each node of this computer system is composed of four Intel Xeon L7555 8-core processors running at 1.87 GHz with 512 GB of memory. Assembled contigs were annotated using Blast2GO software and targeted gene discovery workflows.

[table of contents | back to top]

Data Files

 File

 CfinTrans_access_nums.csv(Comma Separated Values (.csv), 858 bytes)

 MD5:0cefa6adf1864a904be1b5a212f60ac1

 Primary data file for dataset ID 528312

[table of contents | back to top]

Parameters

Parameter	Description	Units
BioProject	NCBI BioProject number	unitless
description	type of analysis	unitless
species	specimen species	unitless
date	For microarrays, this is the date specimens were collected; for transcriptomes, adult and CV were collected on Jul 14, 2011; embryo-CII were raised in the lab, and were produced by females collected both June 26 and July 14, 2011.	уууу- month
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
comment	comments	unitless

[table of contents | back to top]

Instruments

Dataset- specific Instrument Name	Automated Sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset- specific Description	HiSeq2000 Illumina sequencer, in conjunction with Trinity software. Located at the Alpha Hudson Institute for Biotechnology, Huntsville, Alabama.
	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Electrophoresis Chamber
Dataset-specific Description	2100 Bioanalyzer, Agilent Technologies, to check the quality and quantity of RNA.
Generic Instrument Description	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

[table of contents | back to top]

Deployments

GoME_2008-2011

Website	https://www.bco-dmo.org/deployment/528502
Platform	Lenz_lab
Start Date	2008-04-17
End Date	2011-07-14
Description	Genetic analysis of copepods

[table of contents | back to top]

Project Information

Application of transcriptomics to investigate organism-environment relationships in marine zooplankton (CFINTRANSCRIPT)

Coverage: Gulf of Maine, North Atlantic

This project focused on developing molecular tools based on relative gene expression to investigate physiological ecology of *Calanus finmarchicus* (Calanoida: Copepoda). For this project, a **custom microarray** was developed and tested for this species. In addition, using *RNA-seq* technology, a *de novo* transcriptome was obtained from RNA extracted from different developmental stages of *C. finmarchicus* from embryo to adult female.

Description from NSF award abstract:

This project will develop transcriptomics approaches to investigate gene regulation as a function of environmental cycles and in response to experimental manipulation. Currently, there are few tools to establish physiological state of marine zooplankton, in particular for oceanic species. Molecular approaches based on quantifying the transcriptome could serve as powerful tools to obtain a physiological profile for individuals and groups of individuals collected in the field. In combination with laboratory experiments, transcriptome analysis will provide a new approach to understanding organism-environment interactions in the pelagic zone.

The PI will focus on a model planktonic crustacean, *Calanus finmarchicus*, to develop the molecular tools. *C. finmarchicus*, a calanoid copepod, is highly abundant in the North Atlantic, with populations extending from the Gulf of Maine and Labrador Sea to the North Sea. Pyrosequencing and microarray technologies will be used to develop a diagnostic tool to determine physiological state in *C. finmarchicus*. The goal of having a measurement of physiological state is to determine if individuals in the population are growing, are synthesizing or catabolizing storage lipids, and are metabolically active and/or experiencing environmental stress. Specific objectives of this project include:

1. High throughput sequencing of *C. finmarchicus* transcriptome from pre-adult (copepodid stage V [CV]) individuals representing distinct phases of the annual cycle (late spring-early summer, early fall, diapausing individuals).

2. Analysis of the sequence data for discovery of seasonally regulated genes for the development of an ecologically relevant microarray. Probes for this microarray will include seasonally regulated genes, genes involved in the environmental stress response and control genes.

3. Preliminary testing of microarray on existing samples collected from the Gulf of Maine and stored in liquid nitrogen, as well as on experimentally manipulated animals.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1040597</u>

[table of contents | back to top]