Ocean Acidification/Collaborative Research: Effects of Ocean Acidification on Larval Competence, Metamorphosis, and Juvenile Performance in a Planktotrophic Gastropod

Jan A. Pechenik, P.I. Tufts University
Anthony Pires, P.I. Dickinson College

1. Research Products, Data Formats and Data Sharing: We propose to investigate the following aspects of Ocean Acidification (OA) for larvae of the marine gastropod *Crepidula fornicata*: 1) Impact of OA on rates of larval tissue growth and shell growth; 2) Latent effects of larval exposure to OA on juvenile survival and growth; 3) Influence of elevated OA on gene expression patterns in larvae; 4) impact of OA on duration of the pre-competent larval swimming period; 5) impact of OA on the response of competent larvae to a variety of metamorphic inducers; 6) impact of larval diet quality on vulnerability to OA, both in the larval stages and following metamorphosis (latent effects); 7) the influence of OA exposure in the larval stage on gene expression patterns in juveniles; and 8) the impact of OA on larval swimming ability, behavior, time to metamorphic competence, and ability to delay metamorphosis.

Data Products, Formats, Sharing		
RESEARCH DATA/PRODUCTS	**FORMAT OF DATA STORAGE**	**FORMAT OF DATA SHARING**
1. *C. fornicata*: Physiological/Morphological		
a. pH Larval Growth Rates	Microns per day	Excel Spreadsheet summaries
b. pH Larval Shell and Tissue Weight	Micrograms tissue, micrograms shell weight after ashing at 55 °C	Excel Spreadsheet summaries
c. Larval Metamorphosis	Percent metamorphosis	Excel Spreadsheet summaries
d. Larval Survival	Percent survival	Excel Spreadsheet summaries
e Juvenile Growth Rates	Microns shell length per day	Excel Spreadsheet summaries
f. Juvenile Feeding Rates	ml water cleared of food particles per hour	Excel Spreadsheet summaries
2. ***C. fornicata*: Behavioral**		
a. Ciliary Beat Frequency/Arrests	Video; raw data recorded in LabChart 7.2.4 data files (data acquisition software from AD Instruments); processed data Excel files (.xlx) or Graphpad Prism (.pzf)	Excel Spreadsheet summaries
b. Swimming speeds/positions	Video; raw data recorded as .avi video files and analyzed in ImageJ; processed data Excel files (.xlx) or Graphpad Prism (.pzf)	Excel Spreadsheet summaries
3. *C. fornicata*: Gene Expression		
a. MRNA Sequencing Libraries	.sra	NCBI SRA (Sequence Read Archive)
b. MRNA Transcripts	.fasta	NCBI TSA (Transcriptome Shotgun Assembly)

c. Differential Expression	.csv	Pechenik Laboratory Webpage http://ase.tufts.edu/biology/labs/pechenik/) Pires Laboratory Webpage http://www.dickinson.edu/academics/programs/biology/content/Dr--Anthony-Pires/
Bound laboratory notebooks	.txt; Stored in locked cabinets in the lab; scanned weekly and saved on pc hard drive, with a copy posted to the Q drive.	Upon request
Protocols and procedures	.pdf, .excel files on lab computers	Available through lab webpage as Readme file.

Physical Products of Research: The *C. fornicata* larvae are obtained from brooding adults that will be collected from Shelton, WA (just north of Olympia); Pires has collected these species at that site previously. All larvae, juveniles and adults are kept in seawater with phytoplankton as a food source (see first page of Proposal Methods). To ensure easy access to information about Dickinson College's data resulting from this project for outside parties at the time of award, a web page link is available (title: "Federally Funded Research Data") under Dickinson's public internet website where an active email address exists to request data or other products resulting from this project.

Metadata will be created to facilitate data interpretation and analysis. This metadata will be obtained from: (a) The software that interfaces with the lab instrumentation and generates the metadata. This includes information such as file name for the results, date, owner, runtime, units, sequencing, etc. (b) Document information about authors, dates and brief descriptions for scanned PDFs, notebooks and lab work for those data not collected by software. See table above for post-grant metadata dissemination.

The PI's will store and preserve the data generated on laboratory computers and lab notebooks; PI's web site(s) and NCBI repositories (see table above).Tufts University maintains a distributed information technology environment, with central as well as local aspects of overall planning and control. Tufts' Information security program is structured in a similar manner. Operationally, Tufts central IT organization (Tufts Technology Services, TTS) and each local IT group maintain standards of quality and professionalism regarding operational processes and procedures that enable effective operational security. All devices and users are subject to the Tufts Information Stewardship Policy.

Data will be shared through publication in traditional and open-access journals, where suitable, and presentations at conferences, workshops, seminars, public lectures and PI's laboratory websites.

2. Re-Use, Re-Distribution and Production of Derivatives: The data acquired and preserved as part of the proposed research will be governed by Tufts University's policies regarding intellectual property, record retention, and data management. This proposal will not involve human subjects. This research proposal will use invertebrate animals.

3. Data Archiving and Retention: The PI's will maintain a complete data archive on their computers and post a copy on the Tufts University shared drive (see above for further information). Each PI will have a copy of the complete datasets for all studies. All lab notebooks will be scanned weekly, and the pdf's stored on the computers of both PI's and on the University shared drive. The original data files will be archived on the Tufts University managed research drive. A Readme metadata file will be kept with the data files, protocols and procedures to ensure consistent data description. Files used by individual members of the research team for data analysis and manuscript preparation will be shared using Prism software. We will work with the DataDryad data repository (http://datadryad.org) to maintain complete data files and associated publications.