Committee Meeting 1

Emma Timmins-Schiffman

May 21, 2010

Outline

- Guiding Themes of Research
- Title & Objectives
- Objective 1 Approaches
- Objective 2 Approaches
- Objective 3 Approaches
- Accomplishments to Date
- Future Perspectives

Guiding Themes of Research

- How do environmental changes affect populations?
  - Organism perspective: changes in physiology
  - Long-term perspective: changes in population dynamics & genetics
- Focus on the model organism Pacific oyster, *Crassostrea gigas*

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Title

The Effects of Human-mediated Environmental Change on the Physiology of the Pacific Oyster, *Crassostrea gigas*: Immediate & Long-term Perspectives

Objective 1

- Characterize the physiological response of bivalves to stressors of environmental change.
  - Laboratory trials

Objective 2

- Assay the effects of local environmental parameters and contaminants on wild sets of *C. gigas*.
  - Environmental sampling
  - Apply results from 1 + gene discovery

Objective 3

- Determine the population genetic resources available for potential adaptation to environmental change.
  - Discover adaption-correlated SNPs

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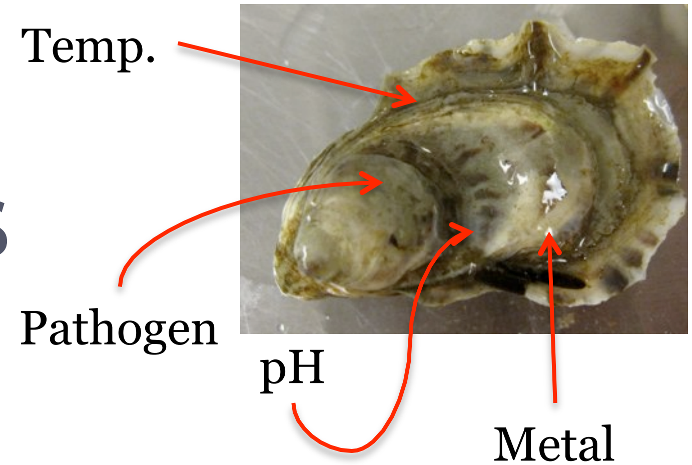
Objective 1: Characterize the physiological response of bivalves to stressors of environmental change.

Objective 1 Approaches

- What causes hatchery mortality events?
- How do oysters respond physiologically to environmental stress?
- How do changes in the environment affect the host-pathogen relationship?
- Develop a predictive assay of markers indicative of individual and multiple stressors.

Objective 1 Approaches



- Laboratory trials
  - Single -> dual -> multiple stressors
  - Host only (larvae) & host + pathogen (*Vibrio tubiashii*)
- pH: 380 & 840 pCO2
- Temperature: 12C & 25C
- Stages of development: D-hinge, veliger, pediveliger

Objective 1 Approaches

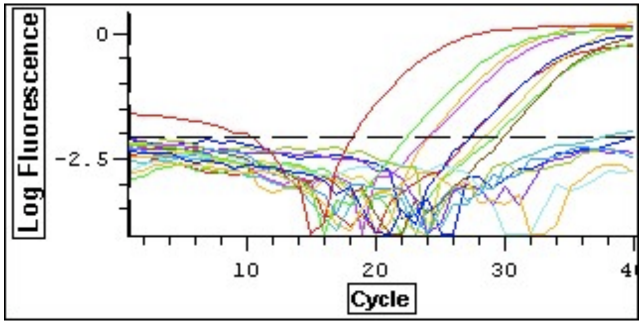
- Challenges
  - 2 weeks each – get larvae & algae from hatchery before each trial period
- 5/31: grow larvae with algae – sample for baseline
- 6/7: CO_2 challenges
- 6/21: CO_2 + Vt challenges
- 7/5: metal bioavailability
- FHL: pathogen + CO_2 + temperature

Objective 1 Approaches

- Sampling protocol
  - 4-8 chambers per treatment
  - 2 draws of larvae each day from each chamber
  - ~50-200 larvae for each draw (sample 100-400/ day per chamber)
- Sample 11,200-44,800 larvae per treatment period
- Need at least 50,000 larvae every 2 weeks

Objective 1 Approaches

- Measuring the effects of the treatments on oyster larvae
  - Gene expression
  - Mortality
  - Behavior
- Results will provide information on lethal and non-lethal effects of stressors

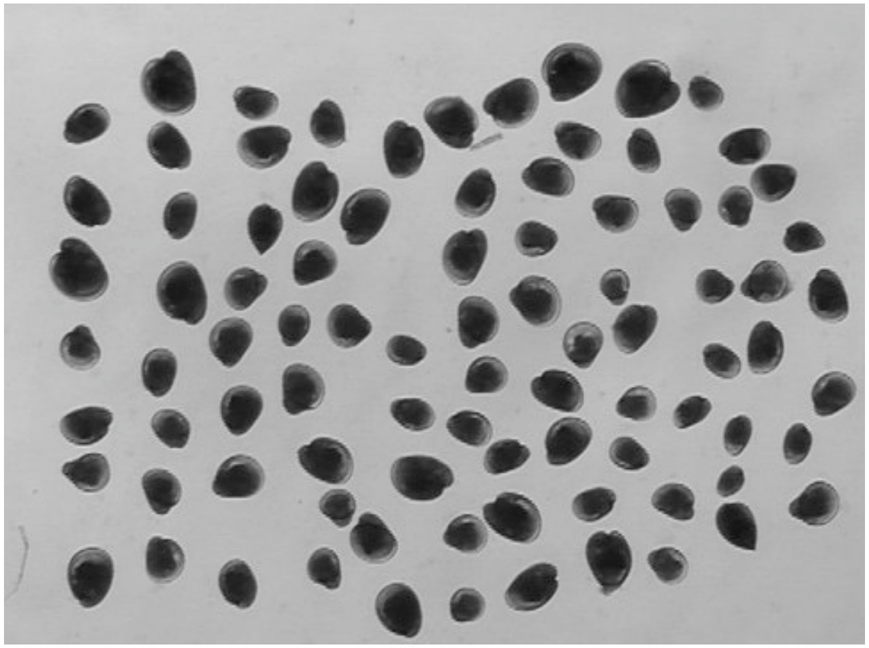


Objective 1 Approaches

- Gene expression
  - Determine impacted physiological pathways
  - Develop assays for future applications
- Methods
  - Targeted genes
  - Differential display
  - General stress: Western blot

Objective 1 Approaches

- Behavior
  - Settlement – shell in bottom of chamber
  - Development time
  - Shell dissolution
  - Lipid content – effects on nutrition



Miller et al. 2009

Objective 1 Approaches

- Intra-system microbial community
  - 1 mL water from each chamber each day
  - Filter out larvae
  - Vacuum filter sample
- Extract DNA & PCR for specific pathogens

Objective 2: Assay the effects of local environmental parameters and contaminants on wild sets of *C. gigas*.

Objective 2 Approaches

- How is larval response different inside the hatchery?
- Are there different stresses and responses around Puget Sound?
  - How does this affect oyster phenotype?

Objective 2 Approaches

- Hatchery sampling
  - Oyster larvae (*C. gigas*)
  - Microbial community
- Puget Sound sampling
  - Sibling juveniles (*C. gigas*)
  - Passive sampling devices

Objective 2 Approaches

- Hatchery sampling – what are the oyster larvae experiencing now?
  - Early spring-summer, 2 years
  - Netarts, OR & Quilcene, WA (hatcheries)
  - Netarts Bay & Dabob Bay (field)
  - Inside vs. outside hatchery
- Metrics
  - Candidate genes (assay) & Western blot
  - Environment: salinity, temperature, alkalinity & DIC, *V. tubiashii*
  - Settlement (spat collectors at Taylor?)

Objective 2 Approaches

- Details of hatchery oyster sampling
  - Outside (weekly)
    - Pump near water intake
    - Salinity, T, pCO_2, pH, plankton, *V. tubiashii*
  - Inside (weekly)
    - Oyster larvae tanks (2 reps per tank, 3 tanks)
    - T, alkalinity, DIC, salinity, *V. tubiashii*
- Complementary data from Netarts for comparison?

Objective 2 Approaches

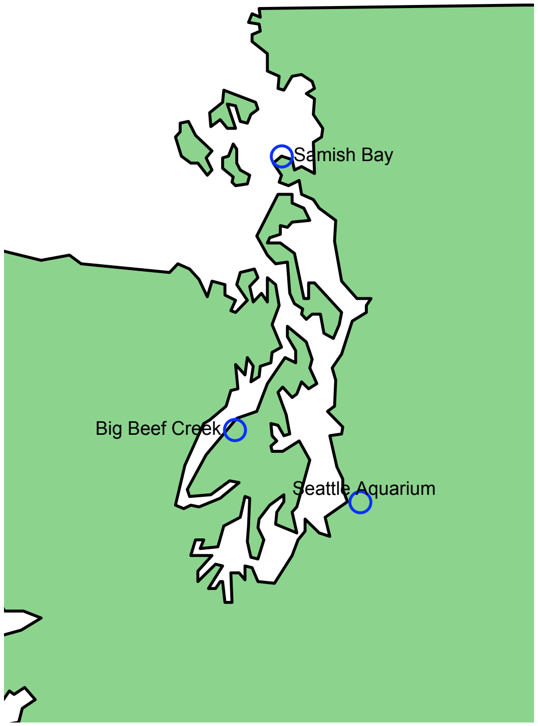
- Environmental sampling of microbial communities at Quilcene hatchery (weekly)
  - Sample at locations inside and outside of hatchery
- Use ARISAs to characterize microbial community structure
  - How is it affected by different environmental conditions?
  - Are there predictors of mortality events?

Objective 2 Approaches

- Lab data + environment data -> oyster response to environmental stress
- Specific impacts – which stressors are responsible?
- Oyster as proxy for ecosystem health

Objective 2 Approaches

- Semi-monthly sampling of 3 sites in Puget Sound with different human impact levels
  - *C. gigas* sibling juveniles
- Sampling scheme
  - Gills – gene expression & epigenetics
  - Hemocytes – immune response
  - Growth & mortality



Objective 2 Approaches

- Monitor contaminants at sampling sites
- Use passive sampling devices (2 kinds that collect complementary data)
- Measure:
  - Pharmaceuticals
  - Personal care products
  - POPs
- Deploy at sampling sites
  - Change devices every month
- Results: Which contaminants are the most problematic for oyster & ecosystem health?

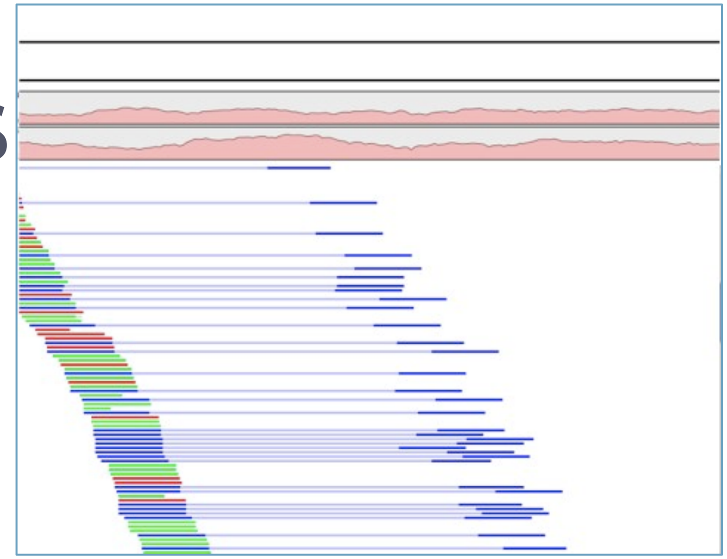
Objective 3: Determine the population genetic resources available for potential adaptation to environmental change.

Objective 3 Approaches

- Are there genotypes linked to potential for adaptation in climate change?
- How will current and future change affect Ne and other population genetic parameters?
- How does environmental change modify the genetic profile of populations?
- Are there inter-species differences in potential for adaptation?

Objective 3 Approaches

- During laboratory stress trials, sample pre- and post-trial larvae
- Find SNPs using existing NGS data
  - Which SNPs are consistently different between mortalities & survivors?
  - Are the SNPs associated with any genes?



Objective 3 Approaches

- Links to Objective 1
  - Same larvae used for physiology analysis – genotype-phenotype link
- Genetic diversity in survivors
  - Does environmental change create a bottleneck?
- Do different SNPs have a functional significance at the population level?

Objective 3 Approaches

- Olympia oysters, *Ostrea lurida*
  - Wild population
  - Inter-species difference in environmental response
- Distribution of SNPs throughout Puget Sound area
  - Sample wild larvae: 2 locations, twice during season
  - Same challenge trials as *C. gigas*
  - Use NGS to find SNPs

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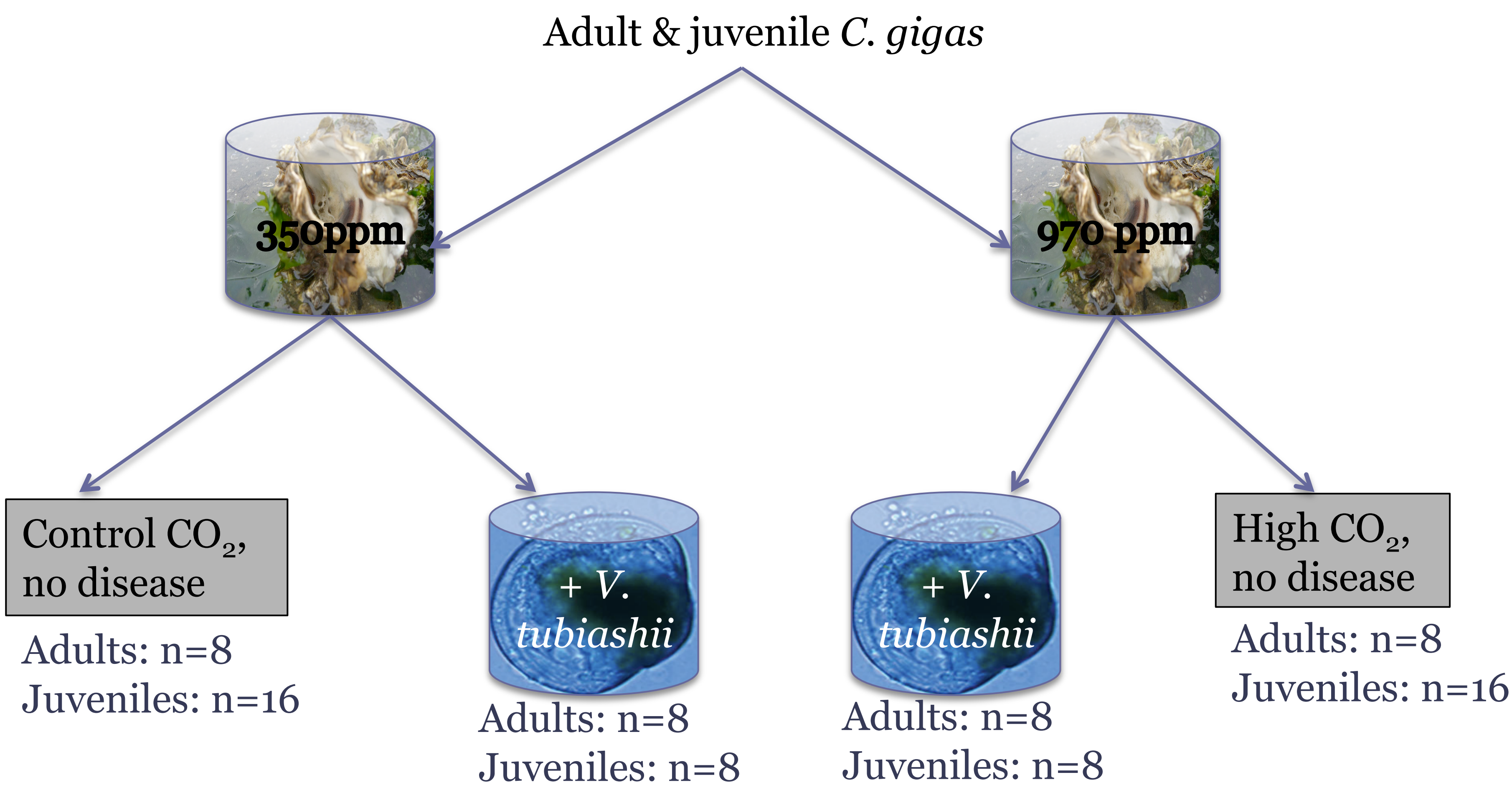
Accomplishments to Date

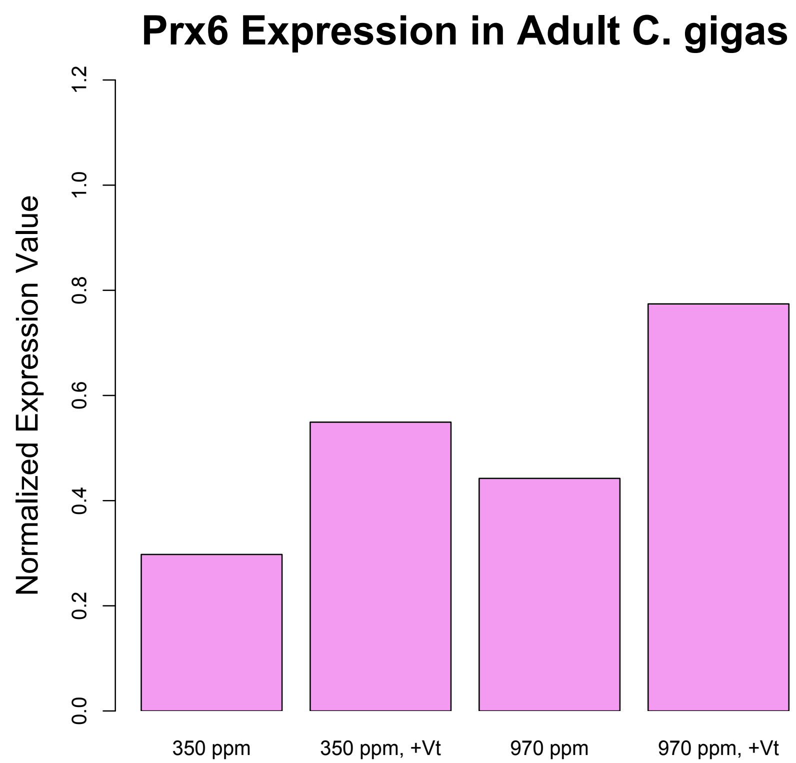
- Stress trials of *C. gigas*
  - Juvenile & adult *C. gigas* and elevated CO_2 and *V. tubiashii* presence
  - Juveniles challenged with *V. tubiashii*
  - Juveniles challenged with copper nitrate
  - Maintain larvae in experimental larval chambers

Accomplishments to Date

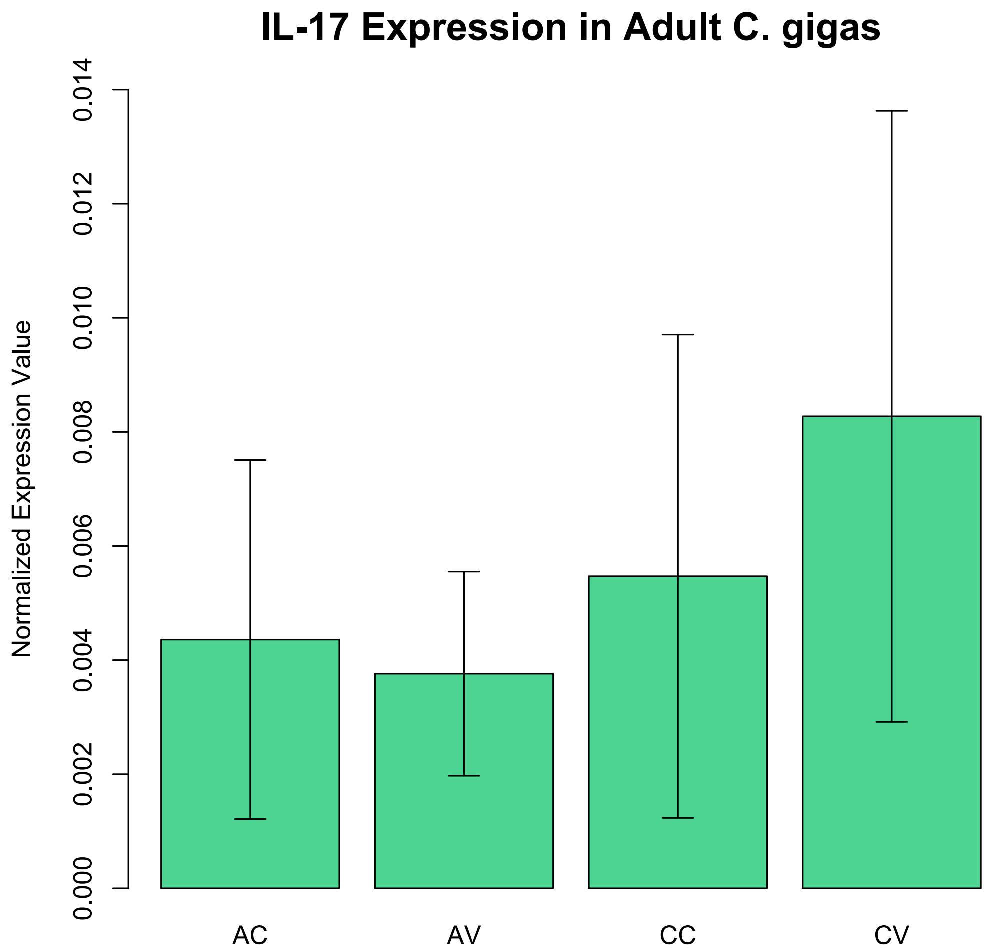


Preliminary Results

Up-regulation of Prx6 to elevated CO_2 and to secondary stress of *V. tubiashii*.



Preliminary Results



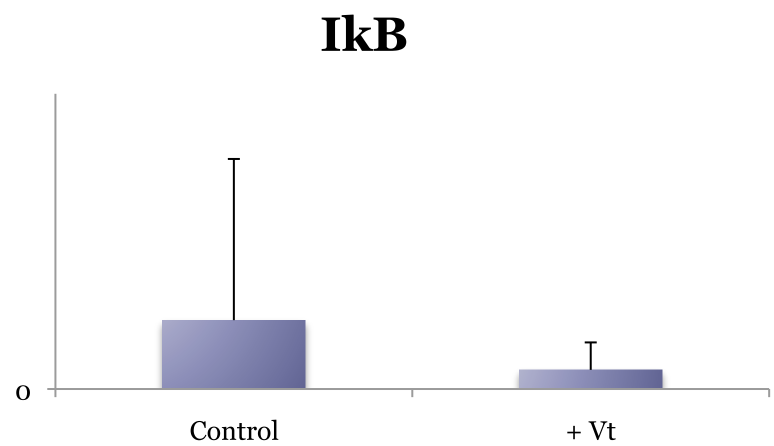
Up-regulation of IL-17 in exposure to elevated CO2 and secondary stressor *V. tubiashii*.

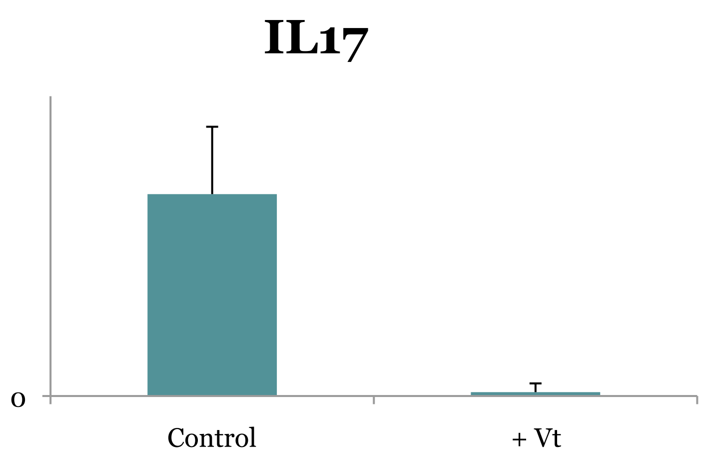
Differential Display: Response to CO_2

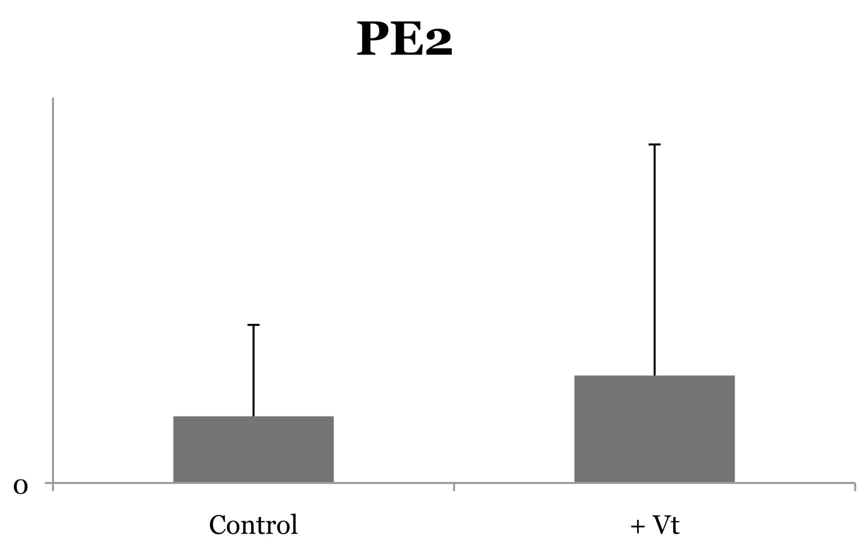
Primer Set	blastx	EST
10	TGF-B-inducible nuclear protein	
12 (200 bp)	Chaperonin subunit	Cg in temp. stress
38	NADH dehydrogenase	Cg
18	Beta-tubulin	Oyster stress
9	Matrilin (ECM)	Oyster stress

Response to *V. tubiashii*

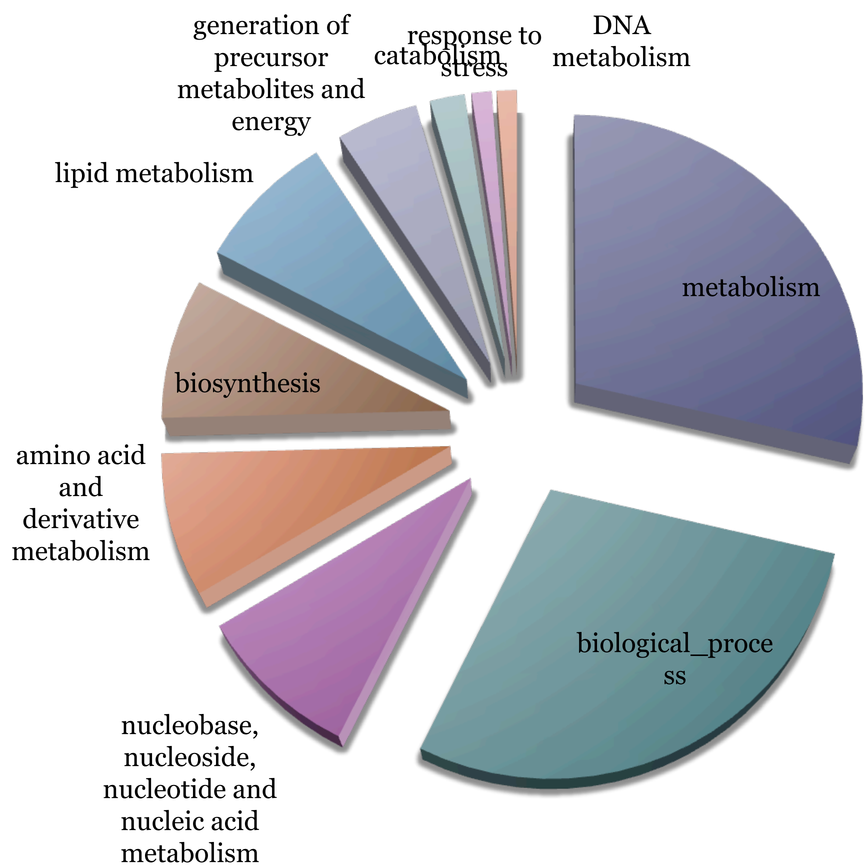
Juvenile *C. gigas* challenged with bacteria for 3 hours.







GO from Sigenae & SwissProt: metal binding



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Genes of Interest

- Thioredoxin reductase: thioredoxin reductase, glutaredoxin and gluathione reductase activities. Catalyzes disulfide bond isomerization.
- Fatty acid desaturase: catalyzes biosynthesis of highly unsaturated fatty acids.
- mRNA decapping enzyme: degradation of mRNA in turnover and decay.
- Hematopoietic prostaglandin D synthase: implicated in smooth muscle function & exhibits low glutathione peroxidase activity.
- Copper-transporting ATPase: in copper excess, functions in efflux of copper from cells.

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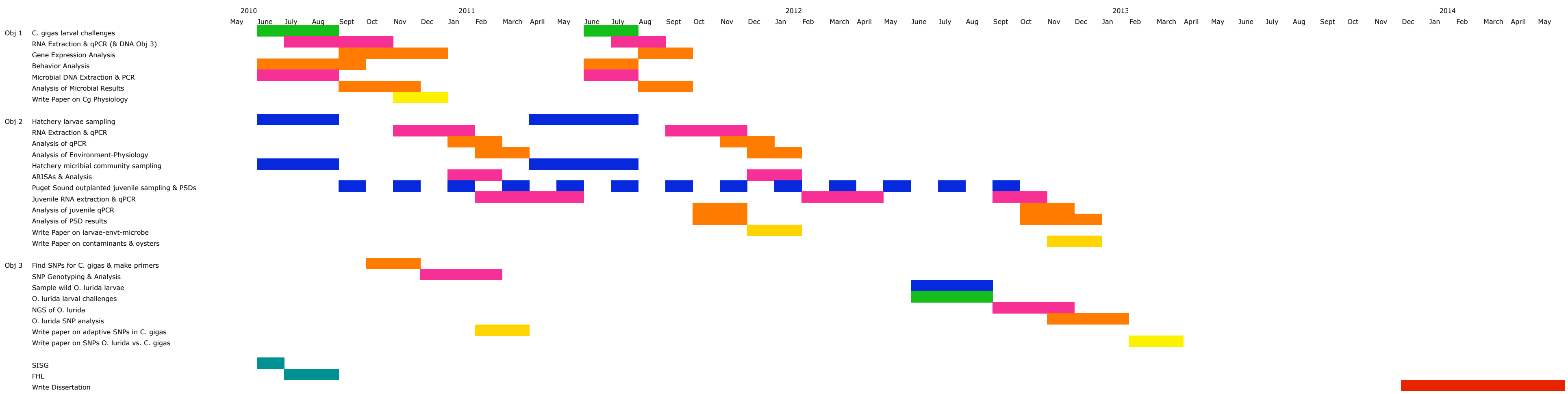
Timeline


2010
2011
2012
2013
2014
May June July Aug Sept Oct Nov Dec Jan Feb March April May June July Aug Sept Oct Nov Dec Jan Feb March April May June July Aug Sept Oct Nov Dec Jan Feb March April May June July Aug Sept Oct Nov Dec Jan Feb March April May
Obj 1
C. gigas larval challenges
RNA Extraction & qPCR (& DNA Obj 3)
Gene Expression Analysis
Behavior Analysis
Microbial DNA Extraction & PCR
Analysis of Microbial Results
Write Paper on Cg Physiology
Obj 2
Hatchery larvae sampling
RNA Extraction & qPCR
Analysis of qPCR
Analysis of Environment-Physiology
Hatchery micribial community sampling
ARISAs & Analysis
Puget Sound outplanted juvenile sampling & PSDs
Juvenile RNA extraction & qPCR
Analysis of juvenile qPCR
Analysis of PSD results
Write Paper on larvae-envt-microbe
Write Paper on contaminants & oysters
Obj 3
Find SNPs for C. gigas & make primers
SNP Genotyping & Analysis
Sample wild O. lurida larvae
O. lurida larval challenges
NGS of O. lurida
O. lurida SNP analysis
Write paper on adaptive SNPs in C. gigas
Write paper on SNPs O. lurida vs. C. gigas
SISG
FHL
Write Dissertation

Short-term Goals

- Establish regular sampling procedure
  - Outplant juvenile sibling *C. gigas* week of September 6, 2010
- Begin stress trials
  - Trouble-shoot system
  - Feasibility of multiple stressors
  - Get larvae, grow food
- Design project for FHL
- Grants!!

Plan of Study

- Completed courses
  - Stats
  - Histology of Disease
  - Integrative Environmental Physiology
  - Proposal Writing
  - Literature seminar
  - R Programming
- Intended courses
  - Climate Science seminar
  - Multivariate statistics
  - FHL
  - SISG
  - Genomics
  - Population genetics
  - Statistics for genome sciences