Gene transcript profiles in mussels from Prince William Sound, 2012 & 2013, as an indicator of ecosystem health

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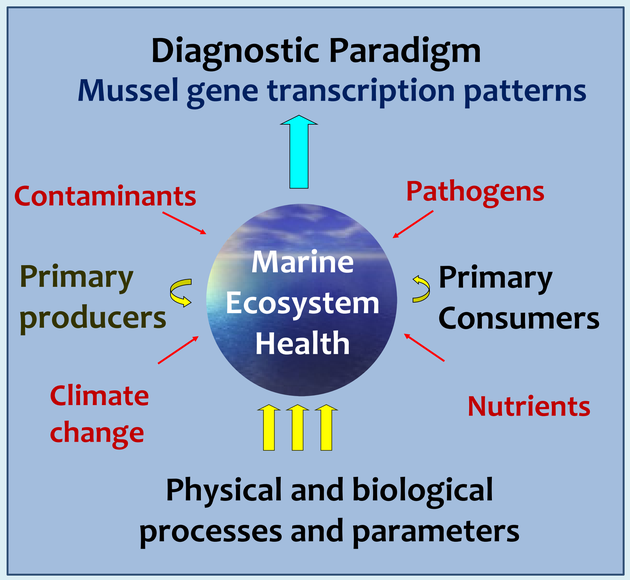
The Challenge: Evaluating the Health of the Nearshore Marine Ecosystem

Introduction:

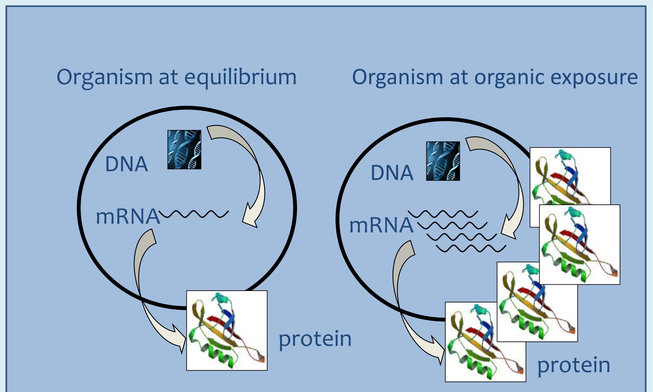
- Nearshore marine ecosystems are at risk from continued development, pollution, climate change
- Mussels can serve as a sentinel species to assess health and environmental change in the nearshore
- Quantification of gene transcription and mRNA can provide insight into the real world of changing environmental conditions, anthropogenic stressors, and population dynamics



Methods:

- 3 mussels (*Mytilus trossolus*) collected from each of 5 sites in western PWS in 2012 & 2013 (total of 30 individual mussels)
- Adductor muscle sampled from each mussel and preserved for RNA assays
- Standard RNA extraction and cDNA creation
- Real-time PCR diagnostic assays for 13 genes of interest

What is gene transcription?



The quantity of mRNA produced (transcribed) increases with exposure to stressor. Gene transcription measures the quantity of mRNA present and thus the cellular and physiologic response to stressor(s).

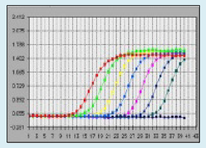
Genes selected for use in initial diagnostic panel

Gene	Function	Indicates
Myticin B	Antimicrobial	Pathogen presence
MIF	Immune function	Pathogen presence
Mytilin	Antimicrobial	Pathogen presence
HSP 70	Immune function	Thermal stress
HSP 90	Immune function	Thermal stress
Caspace 8	Inflammatory	Contaminant and/or pathogen stress
HIFa	Homeostasis	hypoxia
COX4	Homeostasis	hypoxia
CaM	Shell formation	Ocean acidification
Chitinase	Growth and remodeling	Ocean acidification
Calponin	Calcium binding	Ocean acidification
Cyp 3	Detoxification	Contaminant exposure
MT20	Detoxification	Metal exposure

Example of printout from R-T PCR analysis

Real-Time PCR detection

The automatic detection of PCR product growth throughout the amplification process



A measure of WHEN = C_T value (threshold cycle)

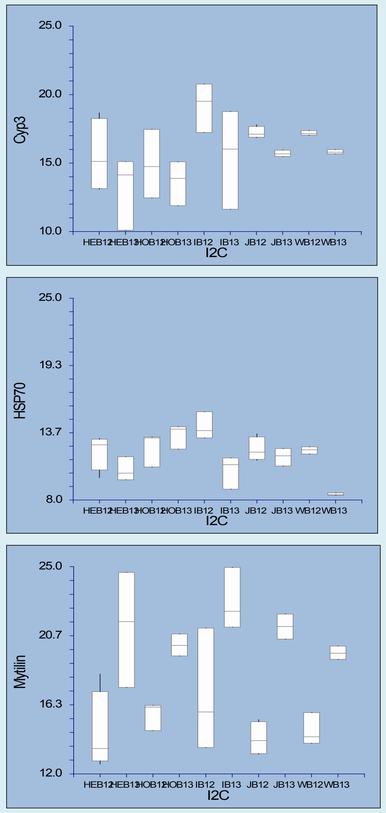
The lower the C_T value, the greater the transcription

Initial Results:

Significant differences among sites and between years for several genes:

- CYP3 – higher transcription in 2013; higher at Herring Bay and Hogan Bay – suggest s higher contaminant exposure in 2013
- HSP70 - higher transcription in 2013; higher at all sites except Johnson Bay – suggests heat stress in 2013
- MYT – lower transcription in 2013 at all sites – suggests pathogen exposure in 2012

Mussels gaping following exposure to high temperatures in June 2013



Box plots for mean C_t values for 3 genes, 2012 and 2013: CYP3, HSP70, and Mytilin, by site and area. HEB = Herring Bay, HOB = Hogan Bay, IKB = Iktua Bay, JB = Johnson Bay, and WB = Whale Bay.

Continuing Work:

- Increase sample size to better characterize baseline variation
- Conduct controlled laboratory studies to identify gene transcription profiles unique to specific stressors
- Collect mussels from broader geographical area and characterize environmental conditions
- Apply gene transcription assays in a broader ecological context