Apr 30th, 1:30 PM - 3:00 PM

Monitoring metal stress in Puget Sound using metallothionein production in mussels in the nearshore

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Gawel, James; Dolan, Julia; Lanksbury, Jennifer; and West, James, "Monitoring metal stress in Puget Sound using metallothionein production in mussels in the nearshore" (2014). Salish Sea Ecosystem Conference. 71.
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Monitoring metal stress in Puget Sound using metallothionein production in mussels in the nearshore

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Washington Dept. of Fish and Wildlife
Student Collaborators:

• UW TACOMA UNDERGRADUATES
  • 2003 Shannon Soto
  • 2004 LeeAnn Acker and Jeremiah McMahon
  • 2010-11 Joline Spencer, Vicky Karout and Kelly Doyle

• HIGH SCHOOLS PARTICIPATING
  • Science and Math Institute (SAMI)
  • Lincoln Center, Lincoln High School
  • School of the Arts (SOTA)
Importance of Monitoring the Nearshore Environment

• Direct impact from stormwater runoff
• Habitat for commercially and culturally important species
• Greater potential for human exposure from harvested organisms, especially for low-income populations
• Critical indicator of the health of Puget Sound
Sediment Toxicity in Puget Sound

- May not be indicative of nearshore
- Environmental variables (sediment characteristics, temperature, salinity, etc.) affect toxicity
Why Use Bioindicators?

- Difficult to monitor aqueous contaminant concentrations in changing system
- Aquatic organisms are temporal integrative sampling devices with direct implications for environmental health
- Provide a metric for bioavailability, not just concentration
- Account for micro-scale and macro-scale deposition barriers and enhancements
Measuring Metal Stress in “Natural” Systems

- “Stress” – sub-lethal effects
- Metals really only toxic inside cell
- Want widely available measure – does no good if only found in left-handed Norwegian coots
- Non-specific health indicators are difficult to use to find a culprit
Mussels as Bioindicators

- Local mussel species make suitable biological indicators of dissolved metals
- Filter feeding allows uptake of dissolved and particulate metals
- Sessile and ubiquitous habitat ideal for monitoring (esp. floating docks)
- However, spawning cycle affects tissue normalization and pollutant uptake
Metallothioneins

- Cysteine-rich (20-30%), Low MW (<8000 D), Produced by all animals
- Induced by As, Cd, Cu, Zn, and Hg
- Role in detoxification (Amiard et al. 2006)
- MT production coupled to cell toxicity (Bolognesi et al. 1999)
- Seasonal variations in MT, but correlate with metals in tissues (Ivanković et al. 2005)
Metallothioneins – 2003 study

- **NATIVE** mussels in Commencement Bay and South Puget Sound
  - Only duplicates per date, only Cd analyzed, June-Sept., n = 26
  - Spectrophotometric method from Viarengo et al. 1997
  - No sig. difference in MT levels between *M. trossulus* and hybrid of *M. galloprovincialis* and *M. trossulus*
  - No correlation between Cd and MT
Metallothioneins – 2004 study

- **CAGED/NATIVE** mussels in Commencement Bay, South Puget Sound and Western Puget Sound
  - Only duplicate samples, one date for caged (Oct), four dates for native (all Aug)
  - No correlation between Cd and MT in **NATIVE** or **CAGED** mussels
Metallothioneins – 2010-11 study

- **NATIVE** mussels in Commencement Bay from Oct 2010 – Apr 2011
  - n = 10 per date per site, metals analysis on separate mussels than MT
  - Significant correlation \( (p < 0.05) \) between MT and As and Zn, but not Cd, Cu, Pb in mussels
  - Significant difference \( (p < 0.05) \) between sites for Cd, Cu, Pb in mussels
MusselWatch Pilot Expansion 2012-13 study

• **CAGED** mussels throughout Puget Sound in intertidal from Nov 2012 – Jan 2013

• All from common stock, same age, deployed same week by volunteers

• Safe-guarded from predators and above sediments
Metallothioneins 2012-13 methods

- Homogenized whole mussel tissue received from WDFW and stored at -80°C prior to analysis
- Tissues weighed and homogenized in 3 mL/g solution:
  - 0.1 M Tris-HCl, 1 mM DTT, 50 µM PMSF, 6 µM leupeptin
  - Centrifuge 20 min 35,000g, 4°C
  - Supernatant heated 95°C for 10 min
  - Centrifuge 15 min 14,000g, 4°C
- Calibrated using rabbit liver MT
- Reduced and denatured using 100 mM DTT, 0.1 M EDTA, 10% SDS and hot water bath at 70°C for 20 min
- Tagged with 50 mM mBBr in the dark for 15 min
- Analyzed by RP-HPLC with fluorescence detection (method modified from Alhama et al. 2006)
Metallothioneins – 2012-13 study

- Weak ($P = 0.06$) positive correlation ($r = 0.2$) between MT levels and Hg concentrations in CAGED mussels
Metallothioneins – 2012-13 study

- MT levels significantly (p < 0.05) elevated in Hood Canal and W Puget Sound compared to reference
- Hg (wet wt) levels significantly higher in Hood Canal and W Puget Sound than all other sites except Central Puget Sound
Possibilities for Future MusselWatch...

• More significant difference between contaminated/uncontaminated sites during spring, winter difference often non-existent (Geffard et al. 2005)

• Digestive gland better than gills or whole organism (Amiard et al. 2006)

• May be nice to investigate significant metal sources with intensive transect

• Measure native and caged mussels simultaneously
This wouldn’t happen without students!!!
In Loving Memory
of
LeeAnn Acker