



Western Washington University
Western CEDAR

Salish Sea Ecosystem Conference

2014 Salish Sea Ecosystem Conference
(Seattle, Wash.)

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

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

Jim Gawel
University of Washington, Tacoma, jimgawel@uw.edu

Julia Dolan
University of Washington, Tacoma

Jennifer Lanksbury
Washington (State). Department of Fish and Wildlife

James E. West
Washington (State). Department of Fish and Wildlife

Follow this and additional works at: <https://cedar.wwu.edu/ssec>



Part of the [Terrestrial and Aquatic Ecology Commons](#)

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

<https://cedar.wwu.edu/ssec/2014ssec/Day1/71>

This Event is brought to you for free and open access by the Conferences and Events at Western CEDAR. It has been accepted for inclusion in Salish Sea Ecosystem Conference by an authorized administrator of Western CEDAR. For more information, please contact westerncedar@wwu.edu.

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

Jim Gawel and Julia Dolan
University of Washington Tacoma

Jennifer Lanksbury and Jim West
Washington Dept. of Fish and Wildlife

Student Collaborators:

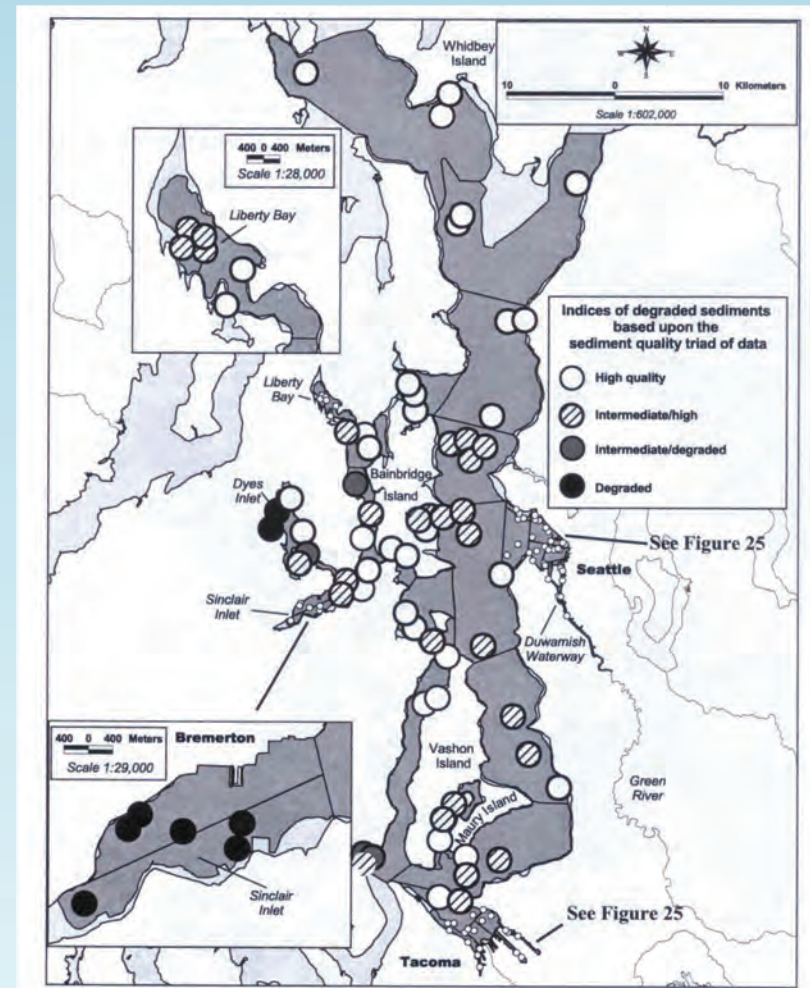
- **UW TACOMA UNDERGRADUATES**
 - 2003 Shannon Soto
 - 2004 LeeAnn Acker and Jeremiah McMahon
 - 2010-11 Joline Spencer, Vicky Karout and Kelly Doyle
- **HIGH SCHOOLS PARTICIPATING**
 - Science and Math Institute (SAMI)
 - Lincoln Center, Lincoln High School
 - School of the Arts (SOTA)

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



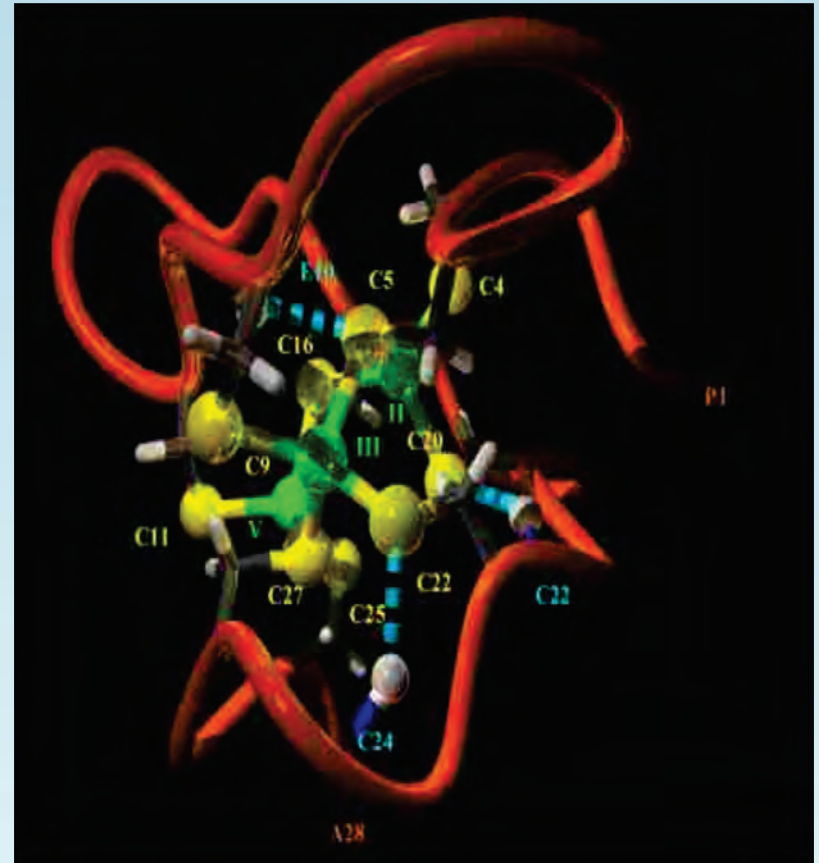
Mussels as Bioindicators

- 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



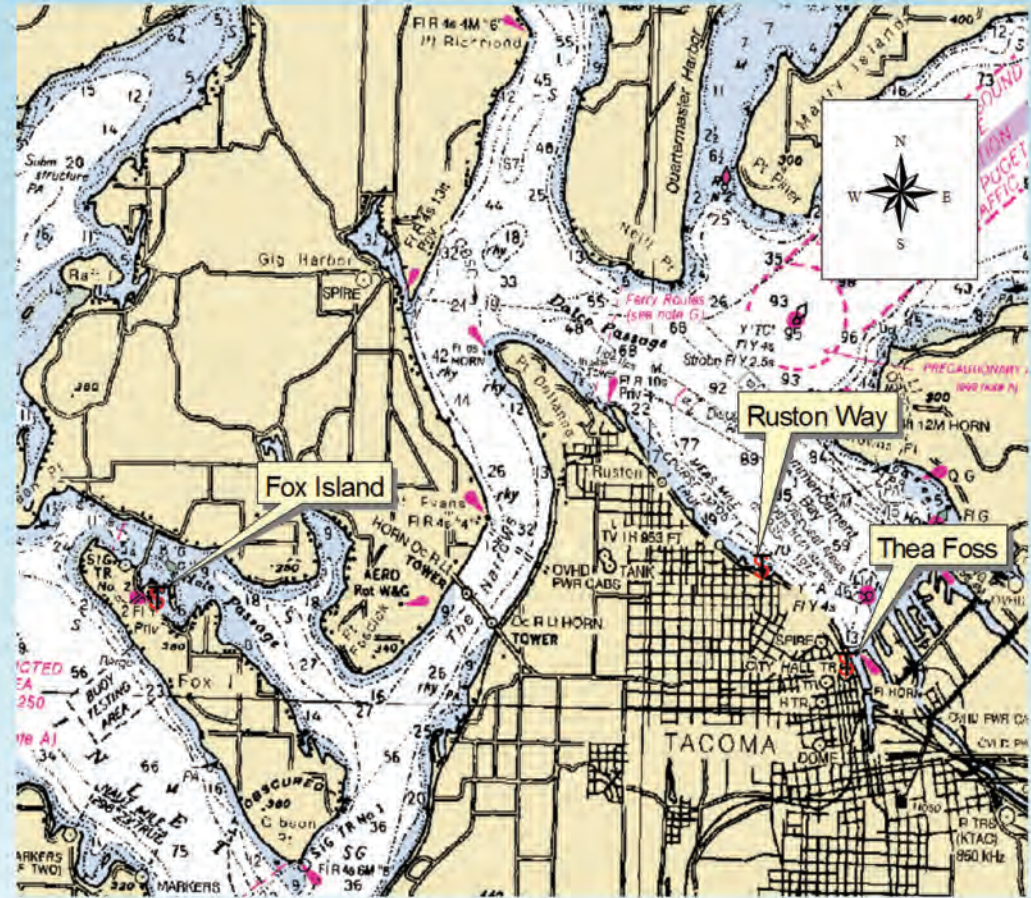
Metallothioneins

- 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)



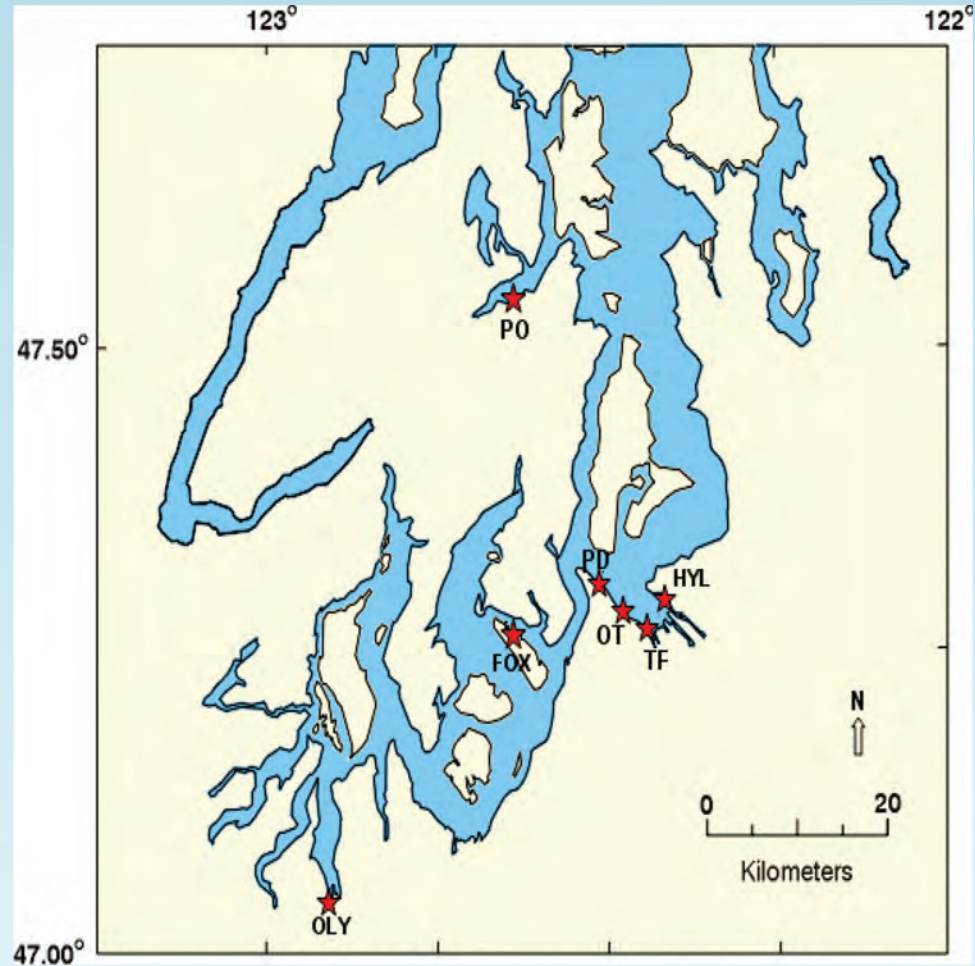
Metallothioneins – 2003 study

- **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



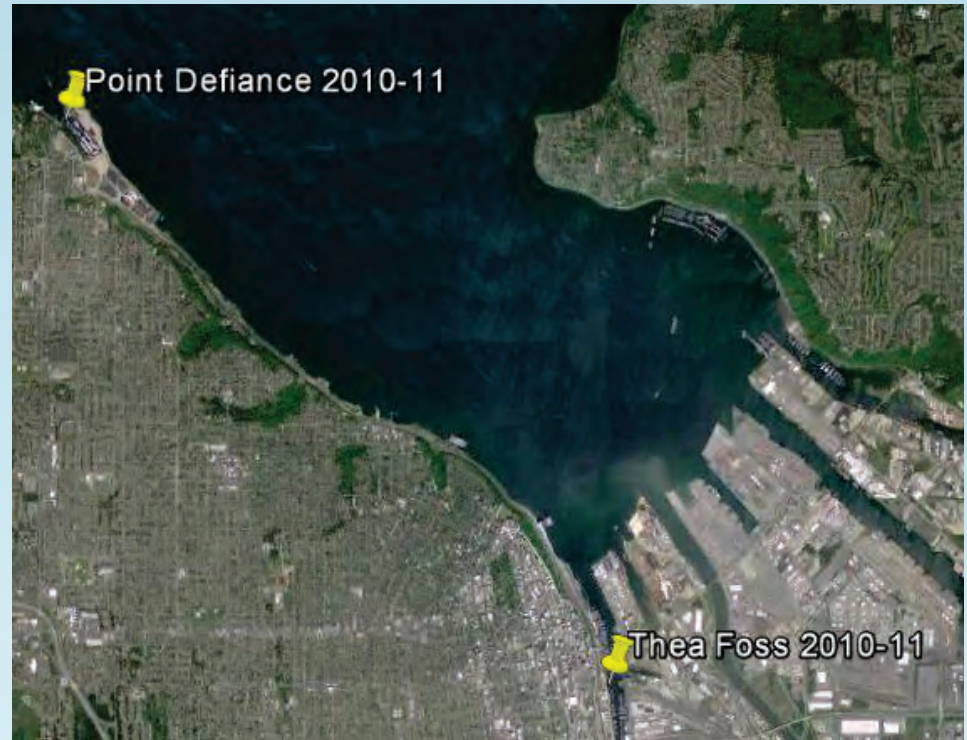
Metallothioneins – 2004 study

- **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

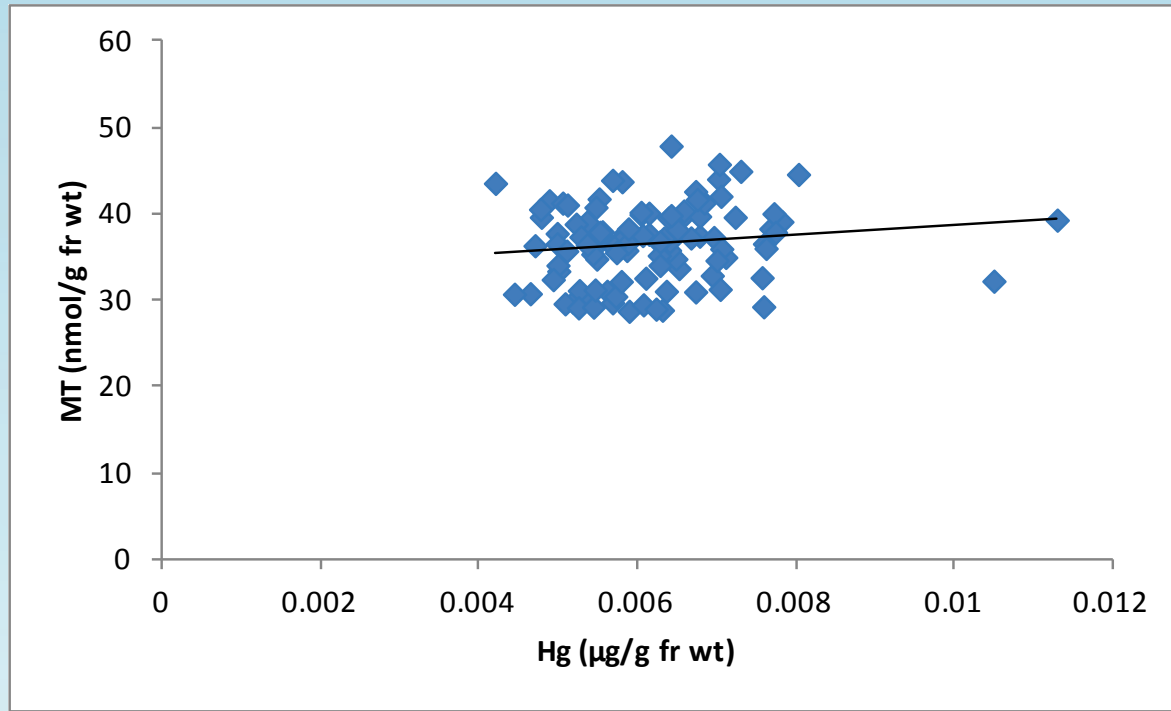
- **CAGED** mussels throughout Puget Sound in intertidal from Nov 2012 – Jan 2013
- All from common stock, same age, deployed same week by volunteers
- Safe-guarded from predators and above sediments



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)

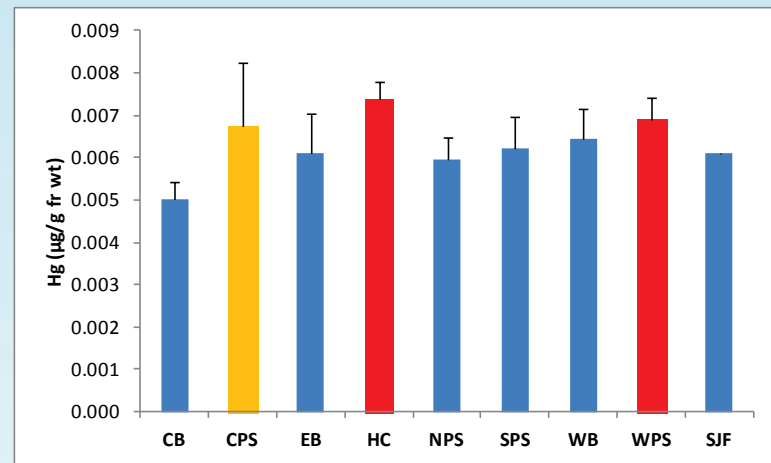
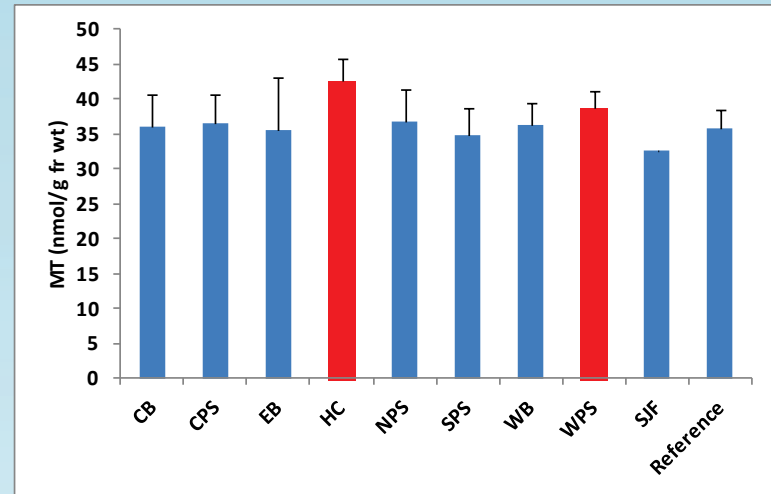
Metallothioneins – 2012-13 study



- 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**