

Geoduck as indicators of environmental change



**USING PROTEOMICS TO ASSESS GEODUCK PHYSIOLOGY
BETWEEN ENVIRONMENTS**



Laura H Spencer, UW SAFS

Micah Horwith, WA DNR

Alex Lowe, UW Biology

Emma Timmins-Schiffman & Brook L Nunn, UW Genome Sciences

Sean Bennet & Steven Roberts, UW SAFS

THE GEODUCK *Panopea generosa*

- Native to WA
- Largest burrowing clam
- Valuable & growing industry
- 27% WA shellfish aquaculture revenue, (\$24.5M in 2013), but only 7% by weight*

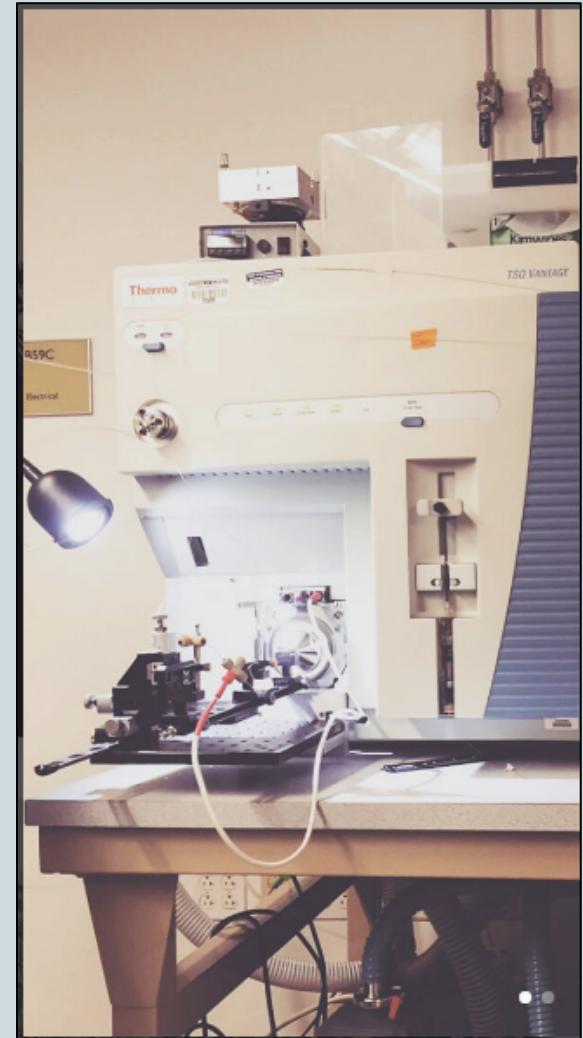


*WA Sea Grant report, 2015

WHY PROTEOMICS?

Proteins...

- carry out most cellular activity
- change based on environment
are dynamic
- provide direct measure of
physiology, energy allocation
- can indicate physiological health
status



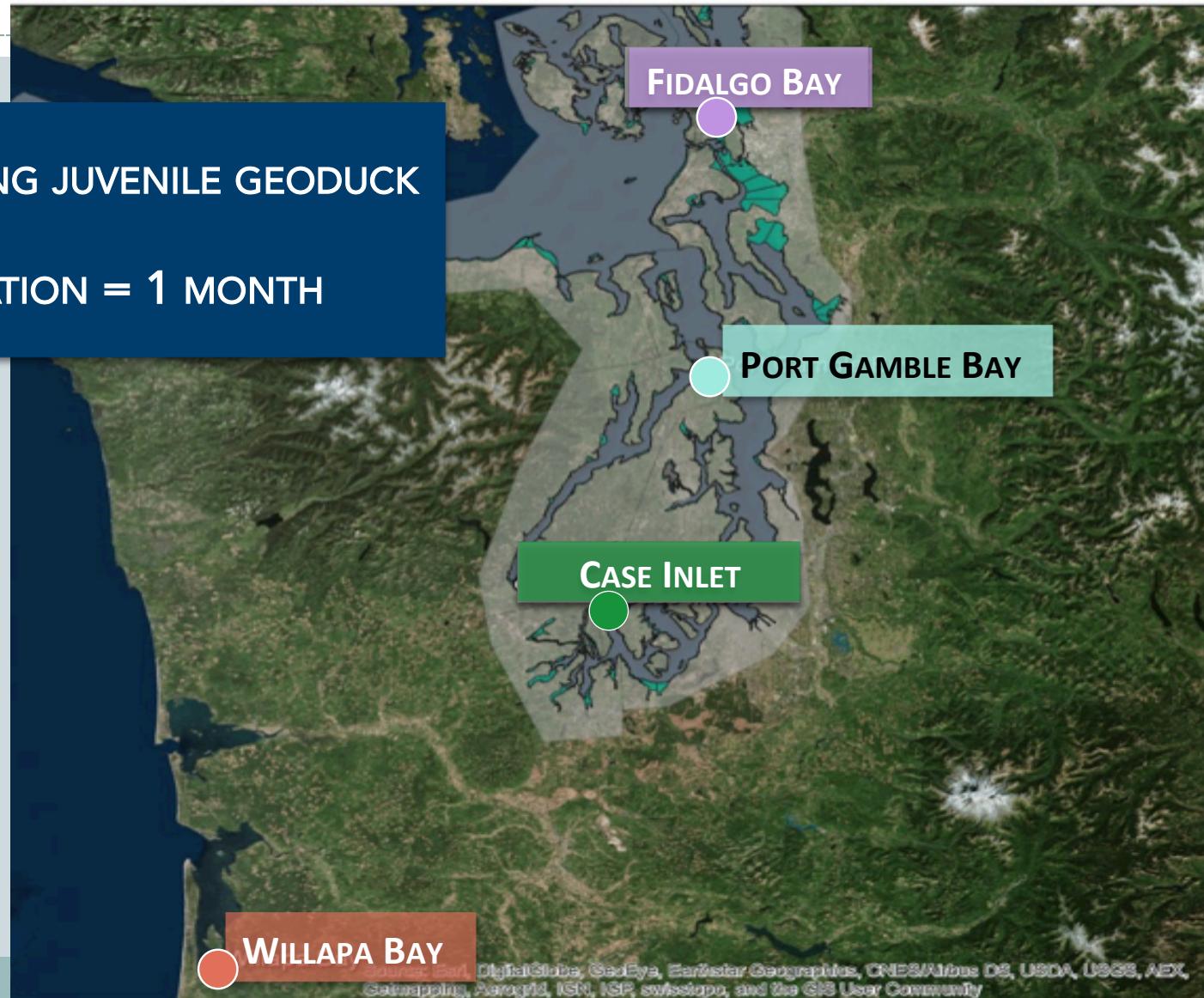
THE QUESTIONS

- How does protein abundance differ between geoduck outplanted in different sites?
 - How do environmental differences correspond to physiological differences?
 - Which proteins can we use to monitor physiology, assess site suitability in geoduck?

OUTPLANT: 4 sites, Puget Sound & Willapa Bay

SIBLING JUVENILE GEODUCK

DURATION = 1 MONTH



DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Protein analysis in 2-phases

PHASE I

Protein Discovery

**8 SAMPLES
2 PER SITE**

CAPTURED ALL PROTEINS
MEASURED 8,076

IDENTIFIED 5,690 PROTEINS VIA
TRANSCRIPTOME

SELECTED PROTEINS BASED ON:
DETECTABILITY
QUALITY
BIOLOGICAL FUNCTION

PHASE II

Targeted Protein Quantification

**48 SAMPLES
12 PER SITE**

TARGETED 13 PROTEINS

QUANTIFIED ABUNDANCE

ASSESSED SIMILARITY
BETWEEN SITES

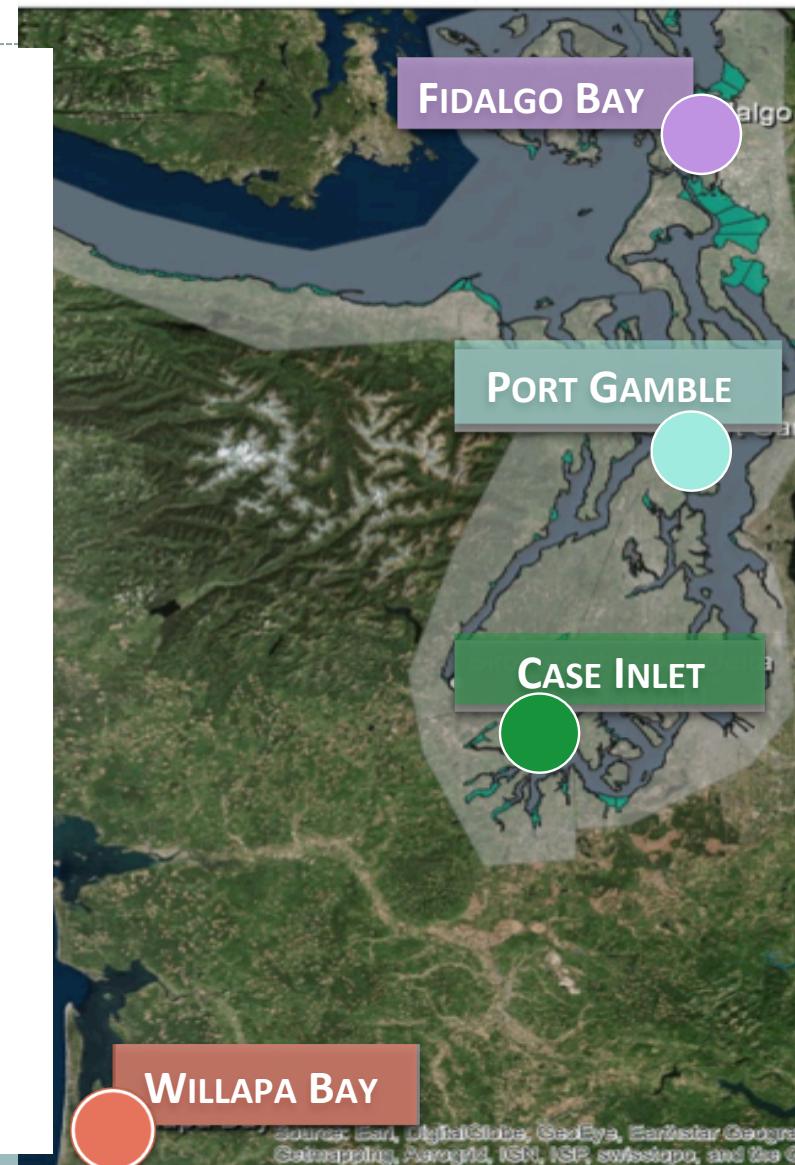
Proteins selected for Phase 2



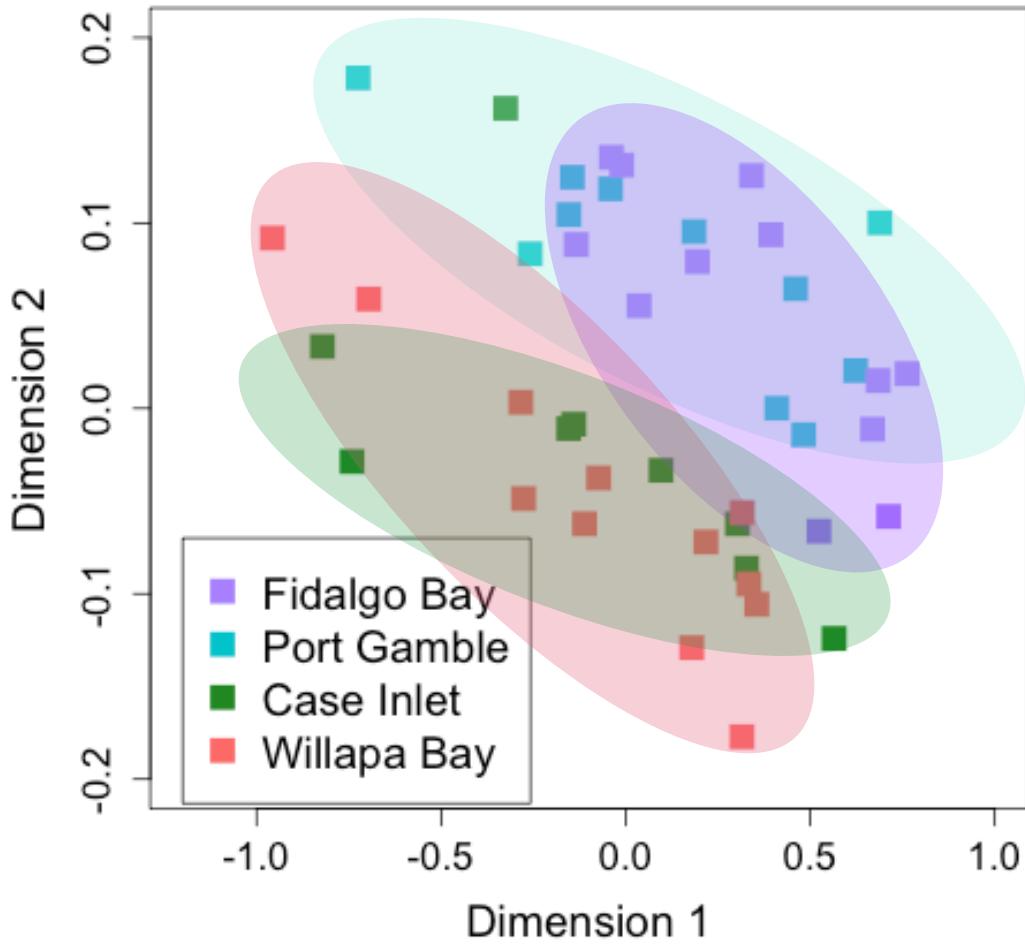
PROTEIN	Response to...
Heat Shock Protein 90-alpha	General Stress
Heat Shock Protein 70	General Stress
Superoxide Dismutase	Oxidative stress
Catalase	Oxidative stress
Peroxiredoxin-1	Oxidative stress
Puromycin-sensitive aminopeptidase	Oxidative stress
Protein disulfide-isomerase	Oxidative stress
Ras-related protein Rab-11B	Acidic pH
Sodium/Potassium-transporting ATPase subunit alpha	pH changes
Glycogen Phosphorylase (muscle form)	Carbohydrate metabolism
Trifunctional enzyme subunit beta (mitochondrial)	Lipid metabolism
Cytochrome P450	Toxins
Arachidonate 5-lipoxygenase	Inflammation

Results

*Site Difference, all proteins
ANOSIM, P=0.03*



Geoduck Gill NMDS, Similarity Plot



Sources: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the USGS.

Results *Site Differences, per protein*



PROTEIN	Response to...	ANOSIM P-Value
Heat Shock Protein 90-alpha	General Stress	0.023
Heat Shock Protein 70	General Stress	0.000050
Superoxide Dismutase	Oxidative stress	0.89
Catalase	Oxidative stress	0.75
Peroxiredoxin-1	Oxidative stress	0.41
Puromycin-sensitive aminopeptidase	Oxidative stress	0.56
Protein disulfide-isomerase	Oxidative stress	0.029
Ras-related protein Rab-11B	Acidic pH	0.82
Sodium/Potassium-transporting ATPase subunit alpha	pH changes	0.64
Glycogen Phosphorylase (muscle form)	Carbohydrate metabolism	0.70
Trifunctional enzyme subunit beta	Lipid metabolism	0.089
Cytochrome P450	Toxins	0.81
Arachidonate 5-lipoxygenase	Inflammation	0.42

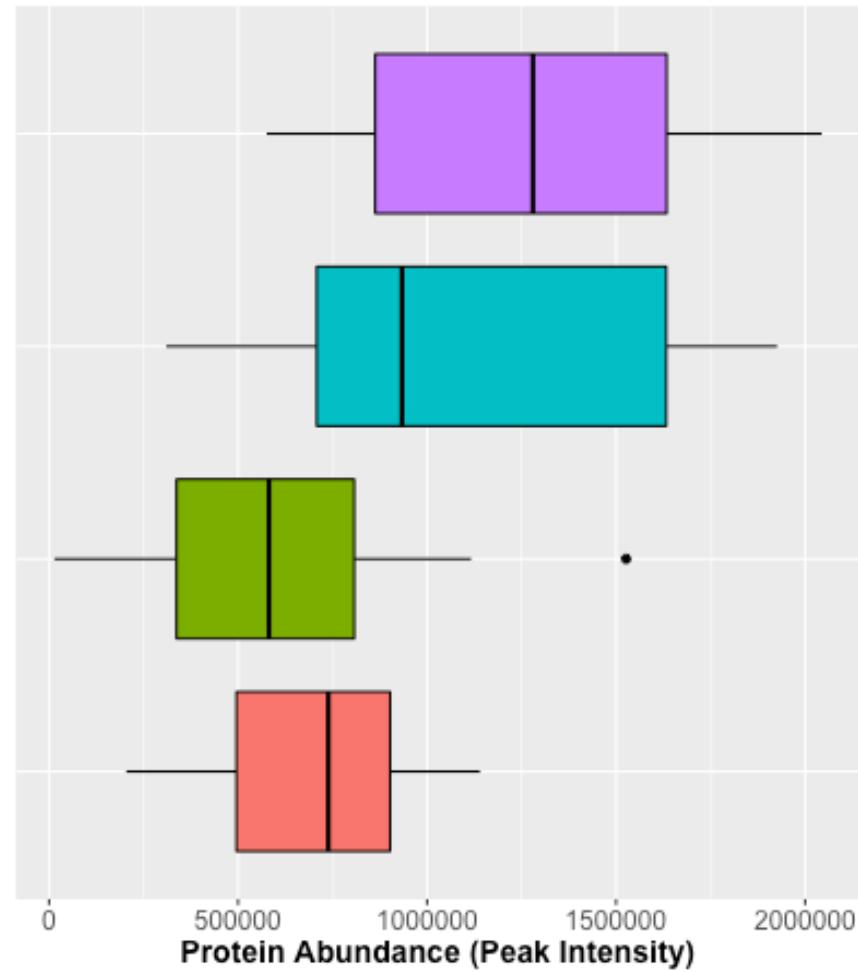
Results *Stress proteins and pH, DO*



N
↑
S

Fidalgo

Protein Disulfide Isomerase
abundance by site

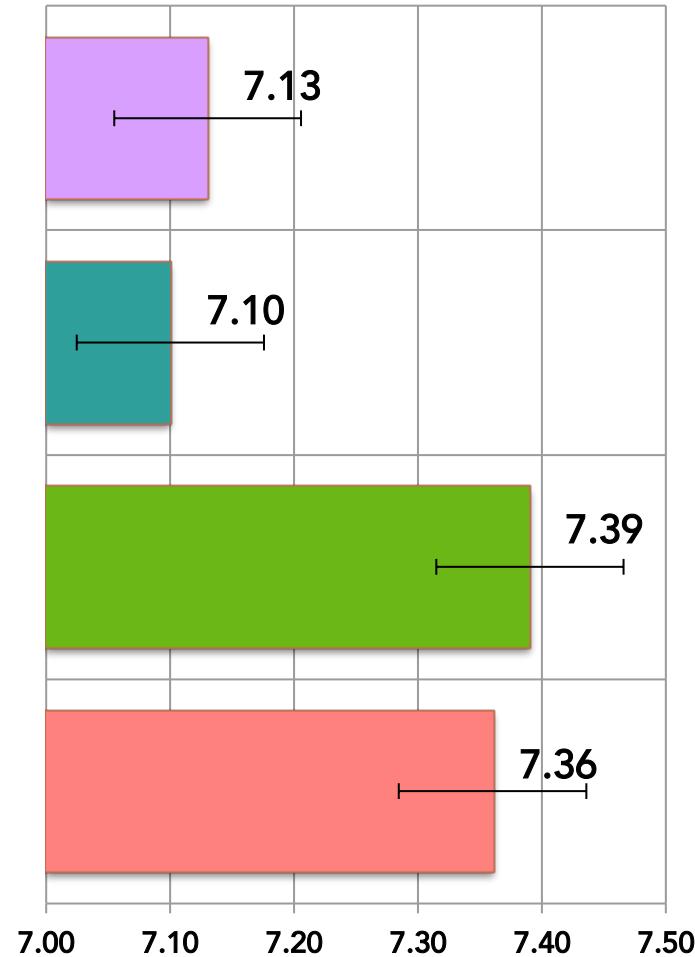


Port
Gamble

Case
Inlet

Willapa

Mean pH



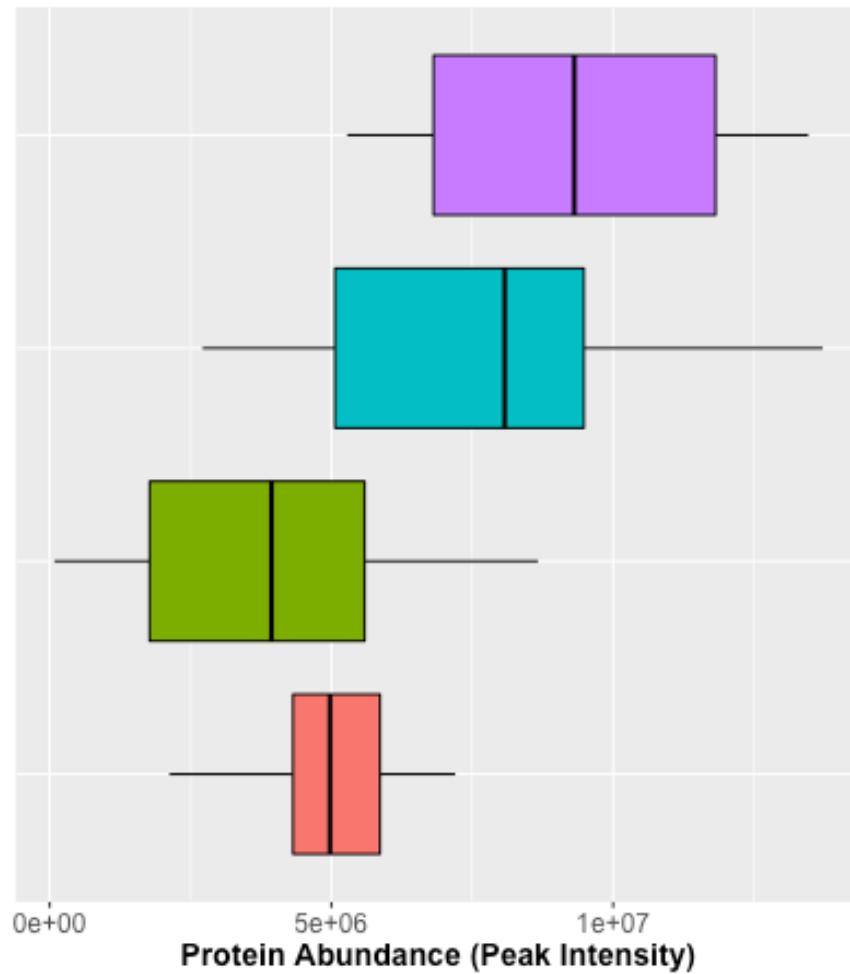
Results *Stress proteins and pH, DO*



N
↑
S

Fidalgo

Heat shock 70
abundance by site

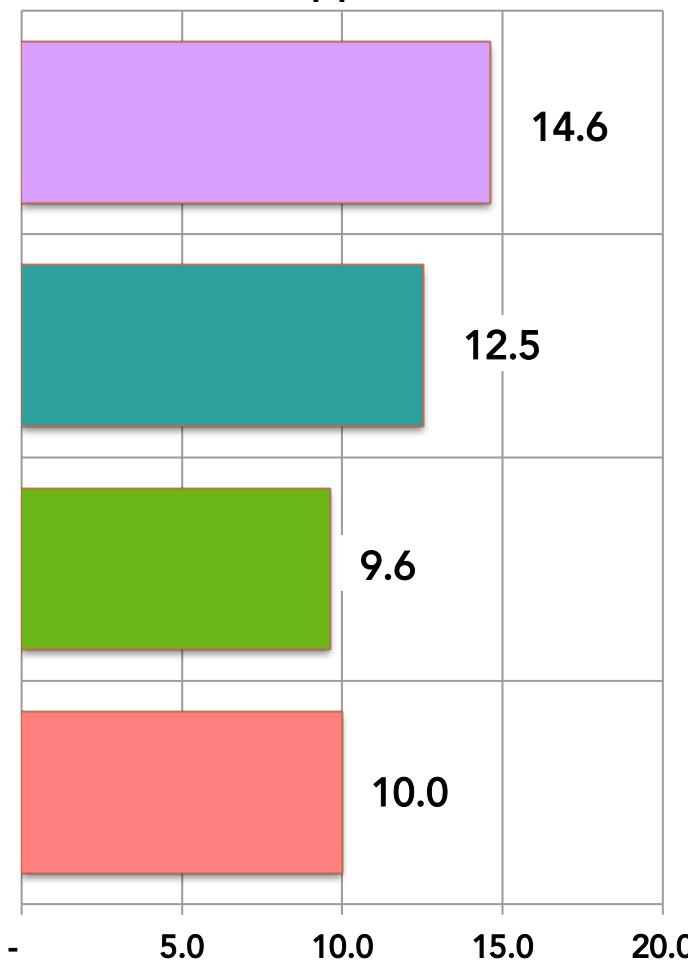


Port
Gamble

Case
Inlet

Willapa

Mean Dissolved Oxygen
(ppm)



Conclusions & Next Steps



Findings

- Clear physiological differences between sites
- Geographic pattern: more stress proteins in northern sites
- Correlation with low pH, high DO, possibly due to oxidative stress

Next steps

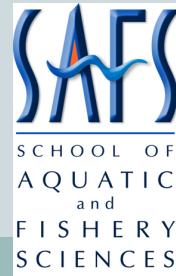
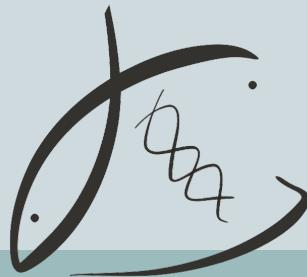
- Longer outplant with growth & reproduction data
- Re-run samples with different targets
- Assess proteins for use as site suitability assays



Thank you...



- Emma Timmins-Schiffman & the rest of the Brook Nunn lab, UW Genome Sciences
- Micah Horwith, WA Department of Natural Resources
- Alex Lowe, UW Biology
- Yaamini Venkataraman, Jose Guzman, Grace Crandall, Sean Bennet, Steven Roberts, UW SAFS
- This work is supported by NSF GRFP



Selected proteins have strong, clear signal

