Population structure of *Pseudo-nitzschia australis* and its association to domoic acid production in the waters of Washington State

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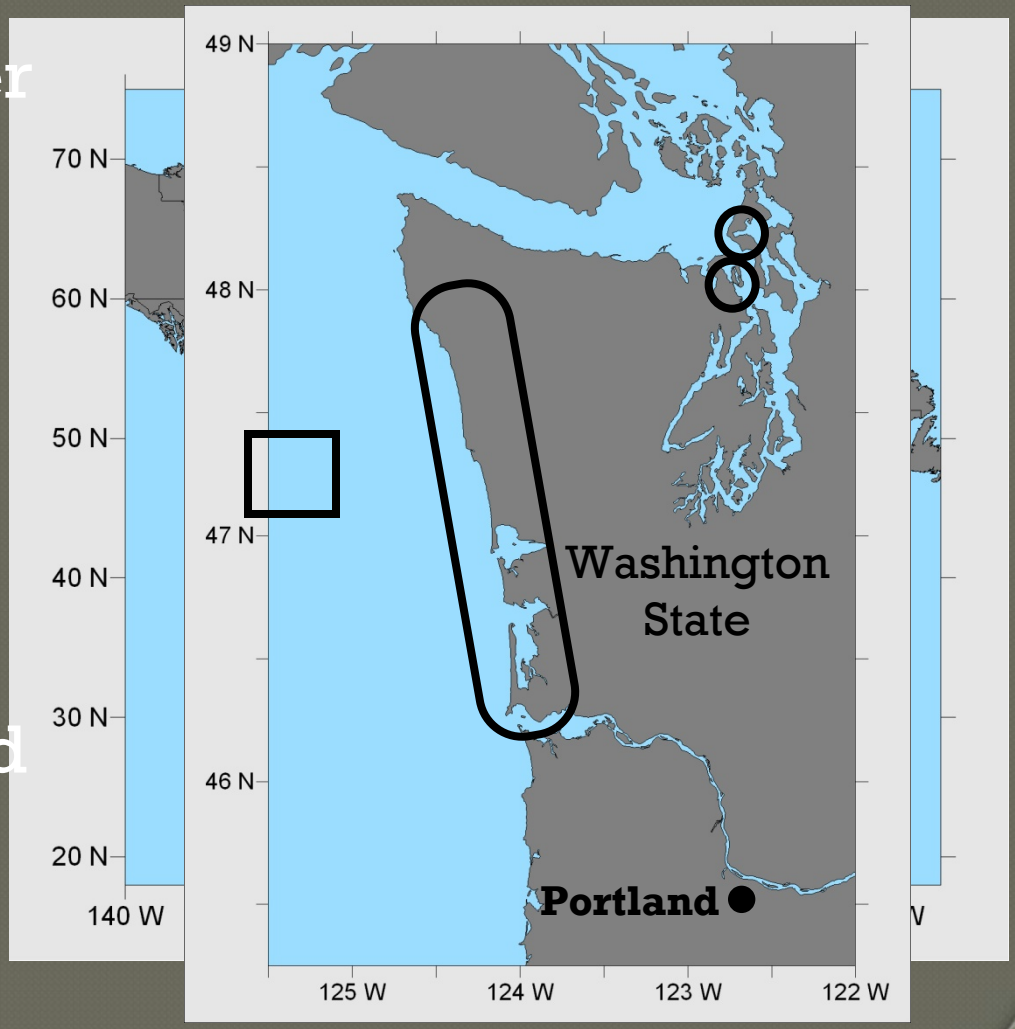
TOPICS

- Washington State domoic acid recap
- Project objectives
- Using microsatellites to analyze *Pseudo-nitzschia* populations (*P. pungens* as an example)
- Progress with *P. australis*

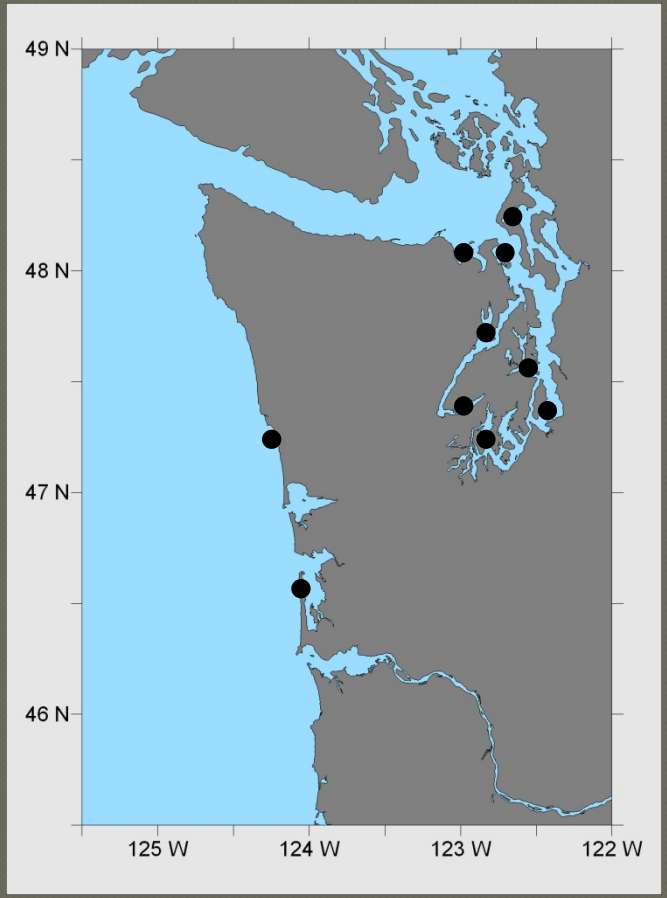
Washington State domoic acid recap

- 1991 to present: outer coast
- 2003: Kilisut Harbor, Puget Sound
- 2005: Penn Cove, Puget Sound
- *P. australis* implicated in Puget Sound and outer coast



Project Objectives

- Design microsatellite markers for *P. australis*
- Sample at various locations in Puget Sound and outer Coast
- Isolate and culture *P. australis* cells
- Measure DA concentrations
- Determine population structure



Some definitions

- Sample: discrete water sample from a single site
- Isolate (or individual): individual phytoplankton culture
- Locus (plural Loci): a specific location on a chromosome
- Allele: alternate forms of a gene (or microsatellite) that can occupy the same locus
- Heterozygote: 2 forms of an allele at a given locus
- Homozygote: the same form of an allele at a given locus
- Significance: $p<0.05$ for all tests

Why Microsatellite Markers?

Microsatellites are short, tandemly repeated sequences of DNA

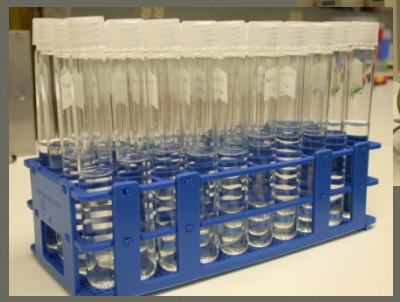
- Repeat unit: 2-5 base pairs
- Co-dominant
- Inherited in Mendelian fashion
- Often highly polymorphic
- Analyze multiple loci per individual
- Build genotypes or "genetic fingerprints"

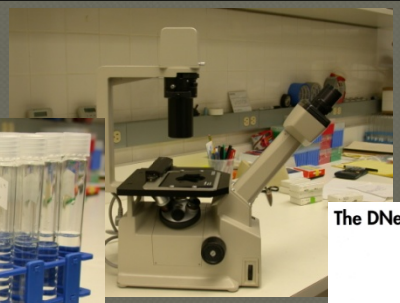
Types of repeats:		
	Pure:	CACACACACACACA
	Compound:	CACACACAGAGAGA
	Interrupted:	CACATTCACACATTCA

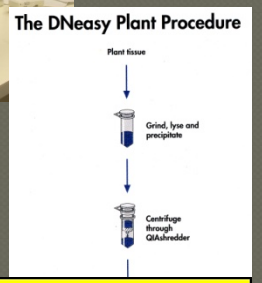
Jarne and Lagoda (1996)

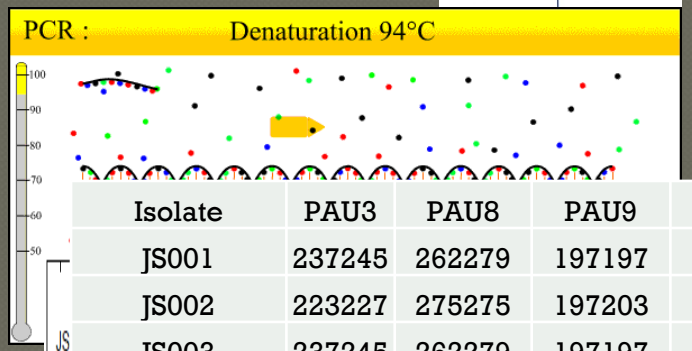
How you get there

- Isolate individual cells from a water sample
- Grow up cultures
- Extract DNA
- Amplify microsatellite loci with Polymerase Chain Reaction (PCR)
- Measure fragment size with genetic analyzer
- Use fragment sizes to construct genotypes
- Proceed with data analyses





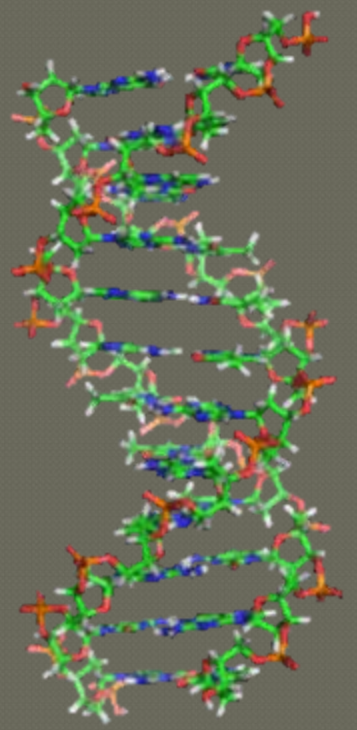




Isolate	PAU3	PAU8	PAU9	PAU12
JS001	237245	262279	197197	127129
JS002	223227	275275	197203	127129
JS003	237245	262279	197197	127129
JS004	227249	267275	197197	125127
JS005	227227	275275	197203	127129
NA865	227227	275275	197197	127129
NA867	223235	261279	197197	125127
NA869	227235	275279	197197	127127

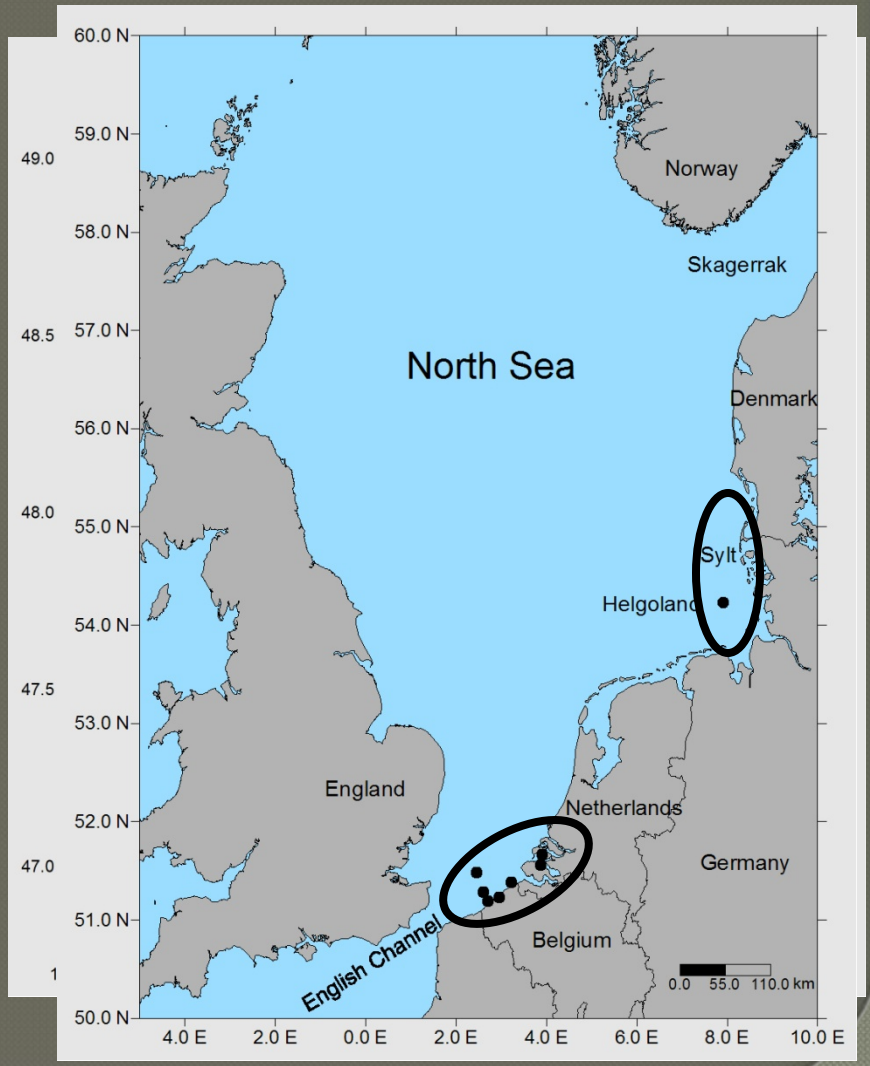
Genetic Analyses

- Identify/differentiate individuals
- Hardy-Weinberg equilibrium (HWE)
  - Expected allele frequencies with random mating, no mutation, no migration, no selection, large population size
- Linkage equilibrium
  - Random association of alleles at different loci
- *"F"* Statistics
  - F_{IS} (heterozygote deficit/excess)
  - F_{ST} (population differentiation)



Pseudo-nitzschia pungens

- Pacific Northwest
  - 2004 and 2005
  - 5 samples (20, 20, 24, 33, 75)
  - 172 isolates
  - Genotyped at 4 loci
  - 147 of 172 unique
- North Sea
  - 25 isolates
  - 2002, 2003, and 2004
  - Genotyped at 4 loci
  - All unique



Genetic testing and results

Test	Individual PNW	Pooled PNW	North Sea
Hardy-Weinberg equilibrium			
Linkage (dis-)equilibrium			

*Also had significant heterozygote deficiency

	Individual PNW vs. each other	Individual PNW vs. North Sea	Pooled PNW vs. North Sea
Population Differentiaton			

F_{ST}: range 0-1, 0=similar, 1=completely diverged

Reasons for deviations and low F_{ST} in the PNW sample

- HWE
  - Migration, mutation, selection
  - Population admixture (no sex)
- Linkage (dis-)equilibrium
  - Clonal selection
  - Population admixture (no sex)
- Low F_{ST}
  - High migration
  - Population/species admixture (no sex)

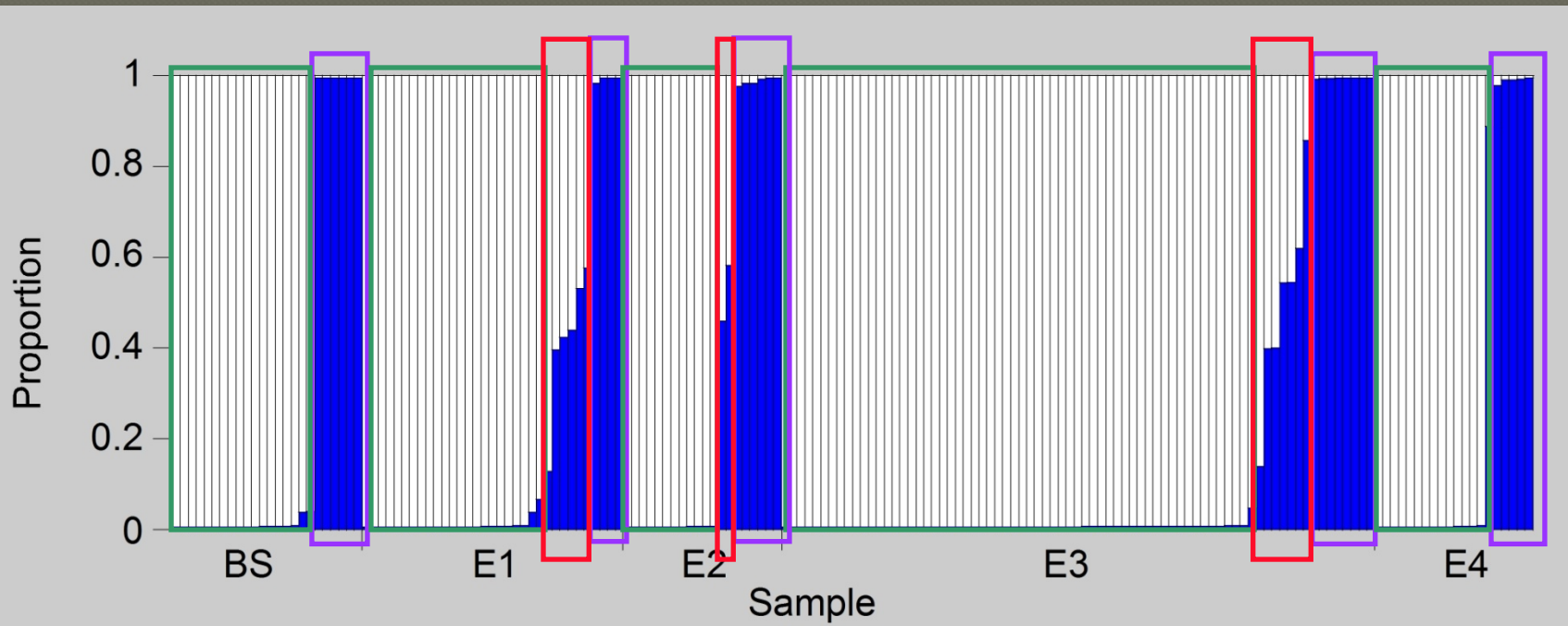
PNW Population Structure

- Wahlund effect
  - F_{IS} → heterozygote deficiency
- STRUCTURE software
- Attempts to find populations in:
  - Hardy-Weinberg equilibrium
  - Linkage equilibrium

- RESULT: 2 populations likely in PNW

PNW isolates

Bar color = proportion originating from each population



<u>Further analyses:</u>
Individuals with
>90% membership

Blue (PNW1) = 29 individuals
White (PNW2) = 127 individuals
16 intermediates (excluded)

PNW 1 and PNW 2 analyses

Test	PNW 1	PNW 2
Hardy-Weinberg equilibrium		
Linkage (dis-)equilibrium		

*significant heterozygote deficiency at this locus

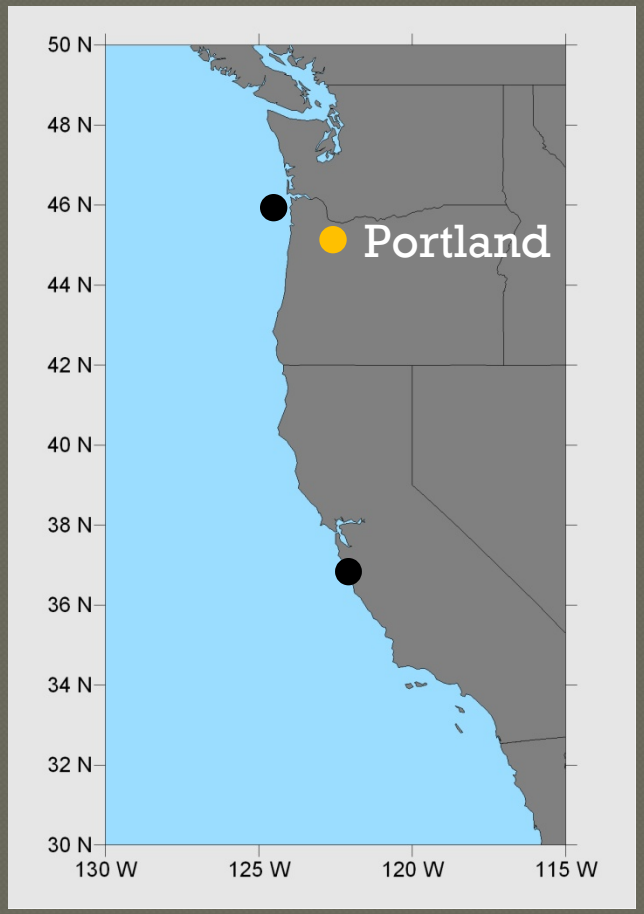
	PNW 1 vs. North Sea	PNW 2 vs. North Sea	PNW 1 vs. PNW2
Population Differentiaton			

Suggests:

- Admixture of *P. pungens* populations in original PNW sample
  - Casteleyn *et al.* (2008) 2 ITS types in PNW (ITS1–5.8S–ITS2 of rDNA)
- Cryptic species or 2 varieties
  - Schwenk *et al.* (2004), Casteleyn *et al*. (2009)

Progress on *P. australis* project

- 25 individuals
  - 20 Pacific Northwest
  - 5 Monterey Bay
- PCR primers for 7 loci
  - 4 loci with 3+ alleles
  - 3 loci with 2 alleles
- 5 loci to optimize



Preliminary *P. australis* results

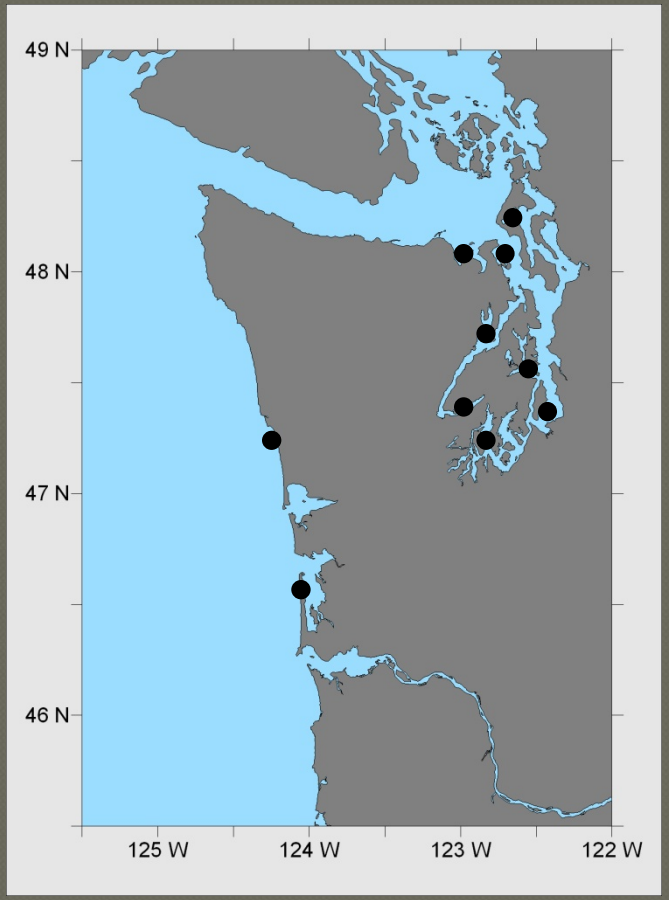
- Some genotypes incomplete
- All but 2 isolates are distinct

Test	*P. australis*
Hardy-Weinberg equilibrium	
Linkage (dis-)equilibrium	

- Preliminary data suggests a single population
- May have a sufficient number of loci but need more isolates

Further work

- Continue optimizing PCR primers
- Collect more *P. australis* individuals
- Perform population genetic analyses
- DA measurements



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