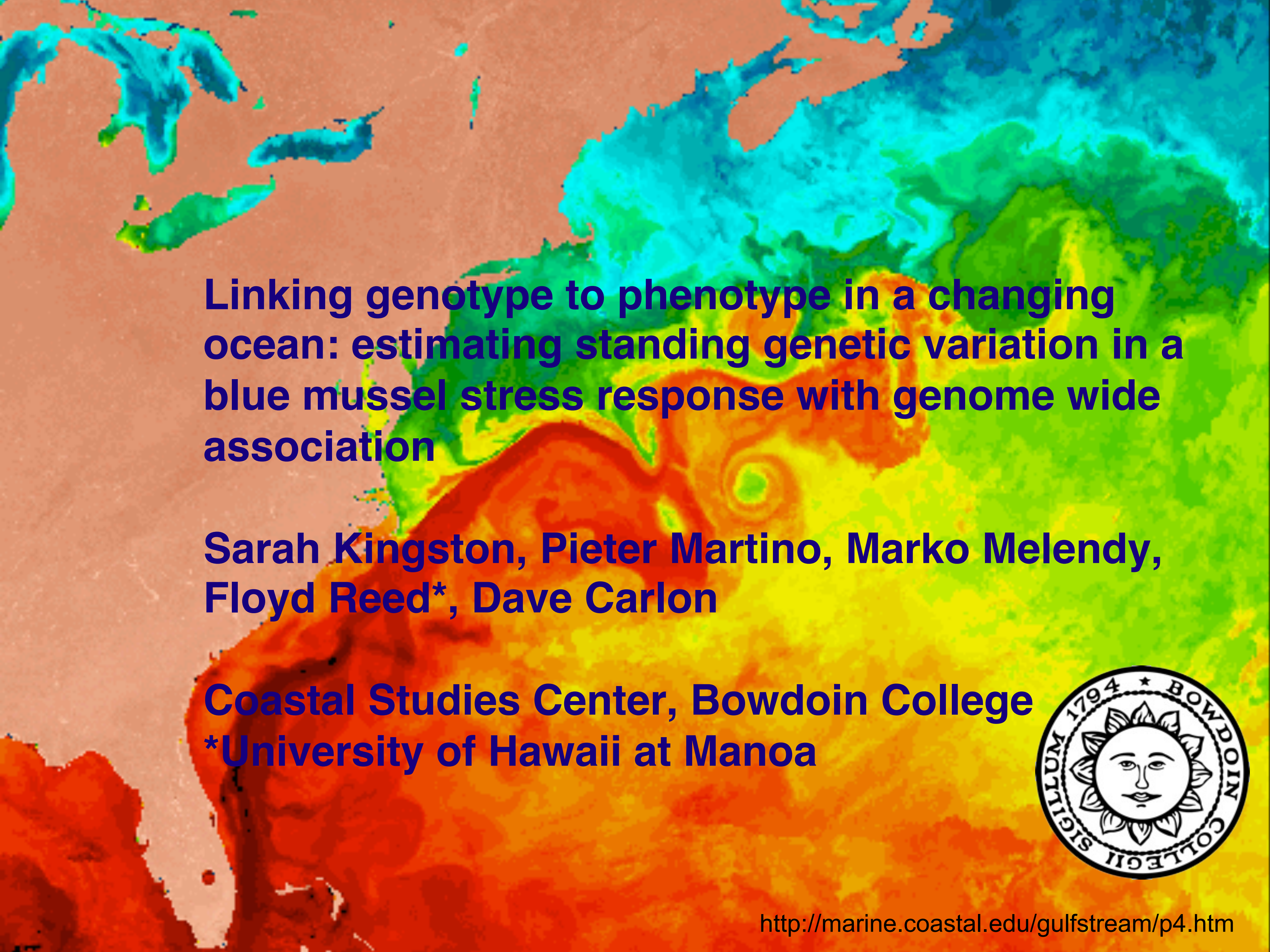

Linking genotype to phenotype in a changing ocean: estimating standing genetic variation in a blue mussel stress response with genome wide association

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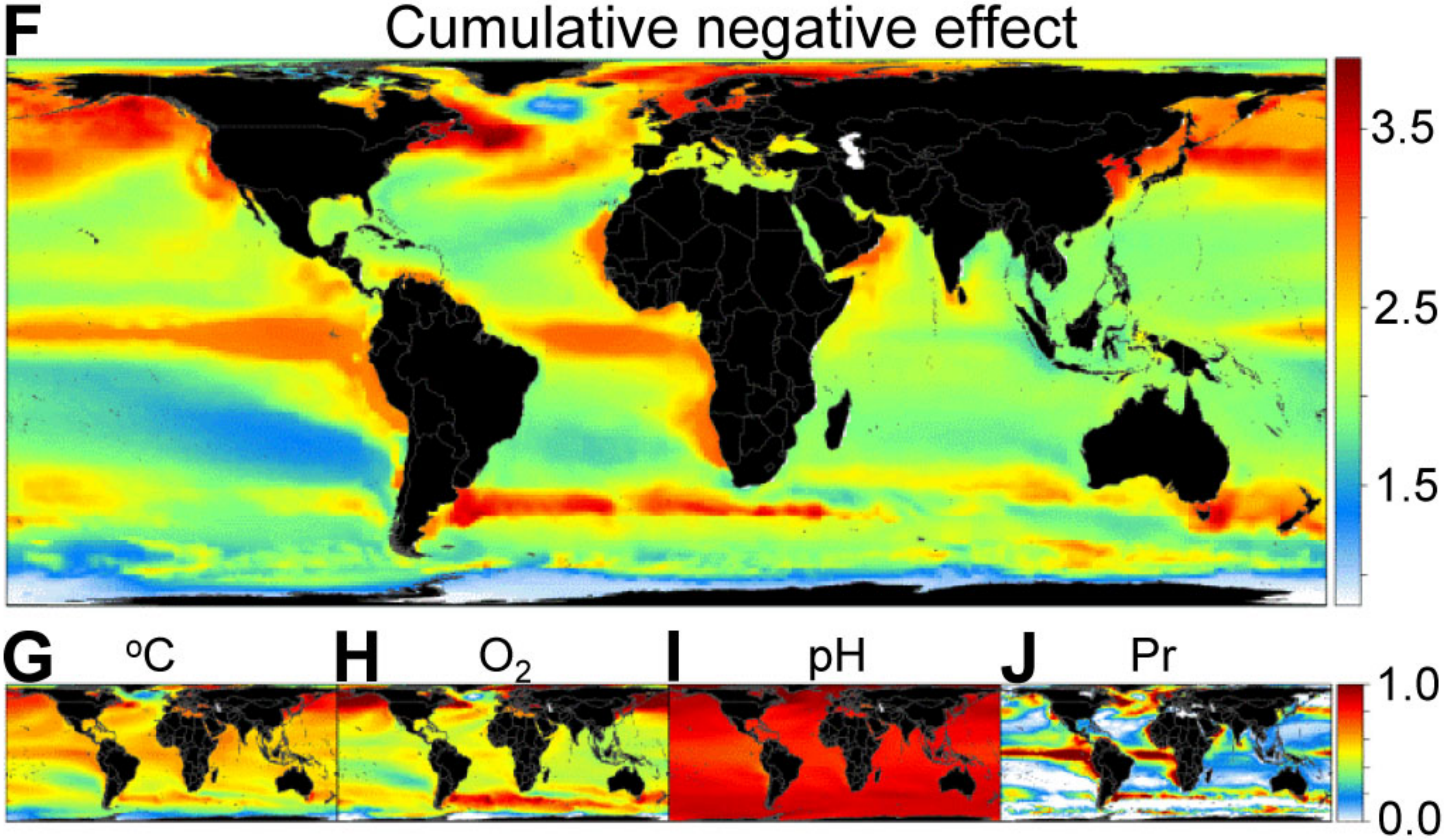
*University of Hawaii at Manoa

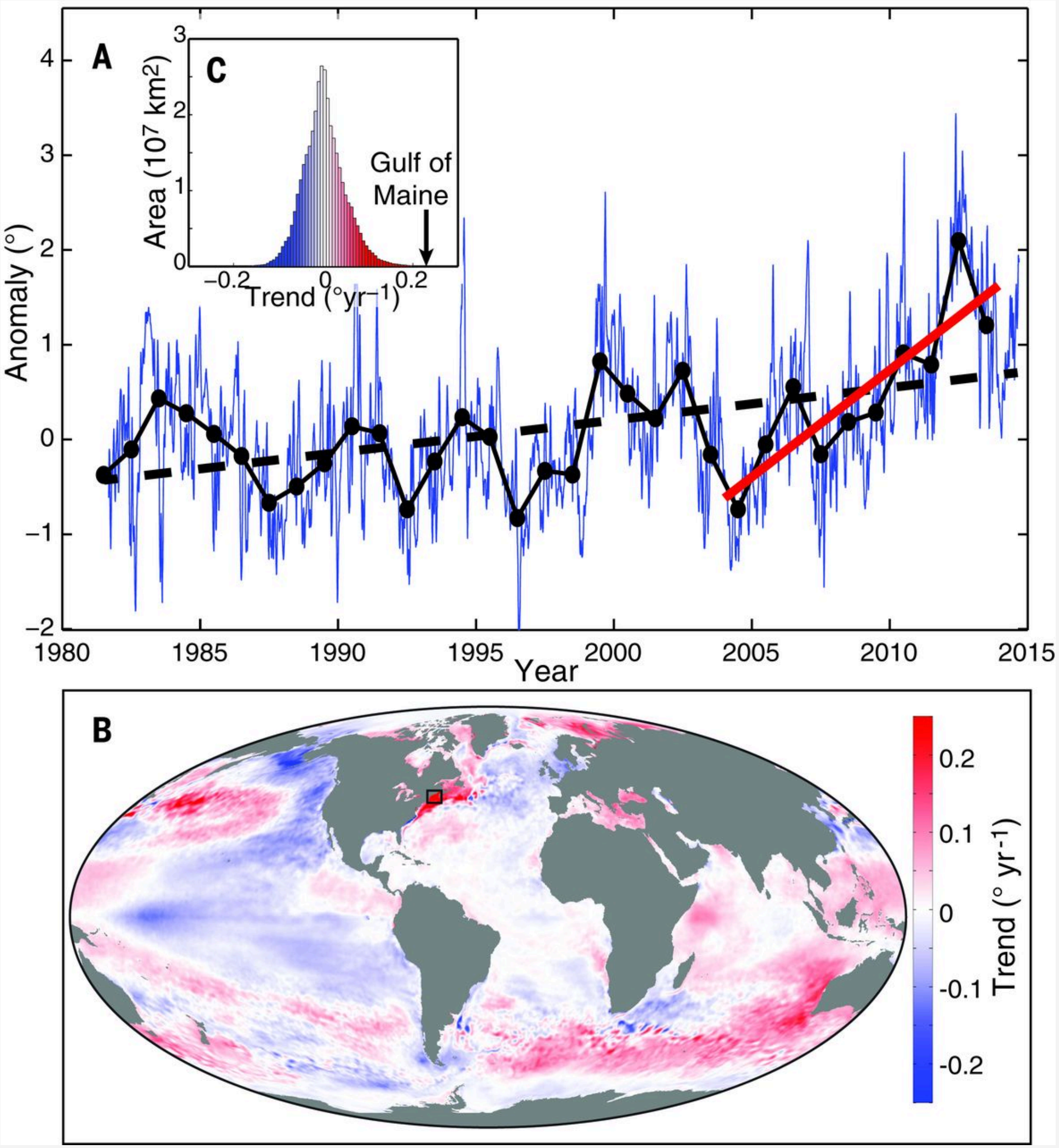
http://marine.coastal.edu/gulfstream/p4.htm

The Oceans in 2100



Mora, C. *et al.* 2013. Biotic and Human Vulnerability to Projected Changes in Ocean Biogeochemistry over the 21st Century. *PLoS Biology* 11: e1001682.

The Gulf of Maine now



A. Pershing *et al*. 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. *Science* (350, Issue 6262) pp. 809-812. DOI:10.1126/science.aac9819

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Evolutionary Applications

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GUEST EDITORS
Juha Merilä & Andrew Hendry

SPECIAL ISSUE: Climate change, adaptation and phenotypic plasticity

Blue Mussels – genus *Mytilus*

- Growing aquaculture species in Maine
- Calcification model - effects of stress on calcification and metabolic pathways beginning to be worked out
- How much genetic variation in stress response exists in natural populations?
  - Ecosystem - Will natural selection “rescue” natural populations from climate change?
  - Aquaculture - Can we identify genetic strains that resist climate change?

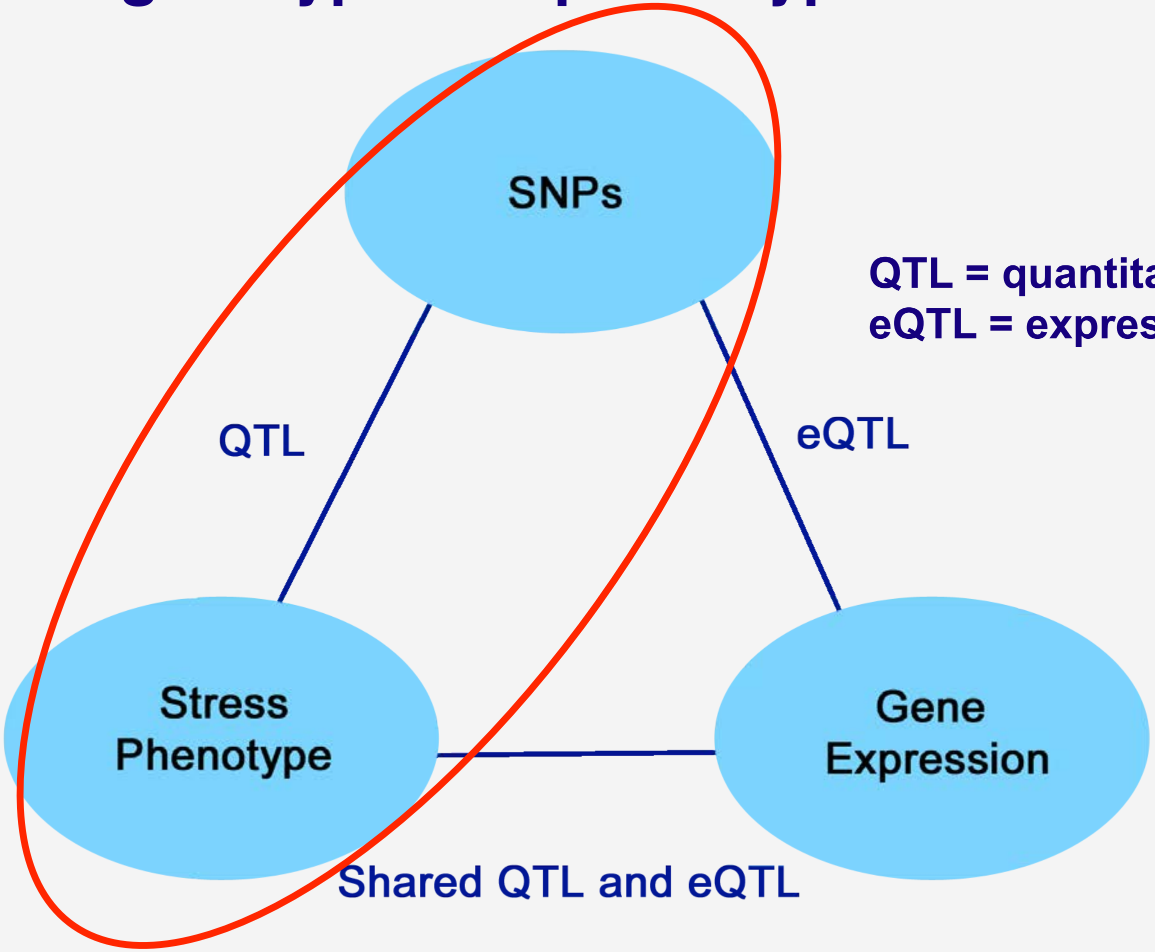
Genotype and Phenotype

How much genetic variation in stress response exists in natural populations?

$$V_p = V_g + V_e + (V_g \times V_e)$$

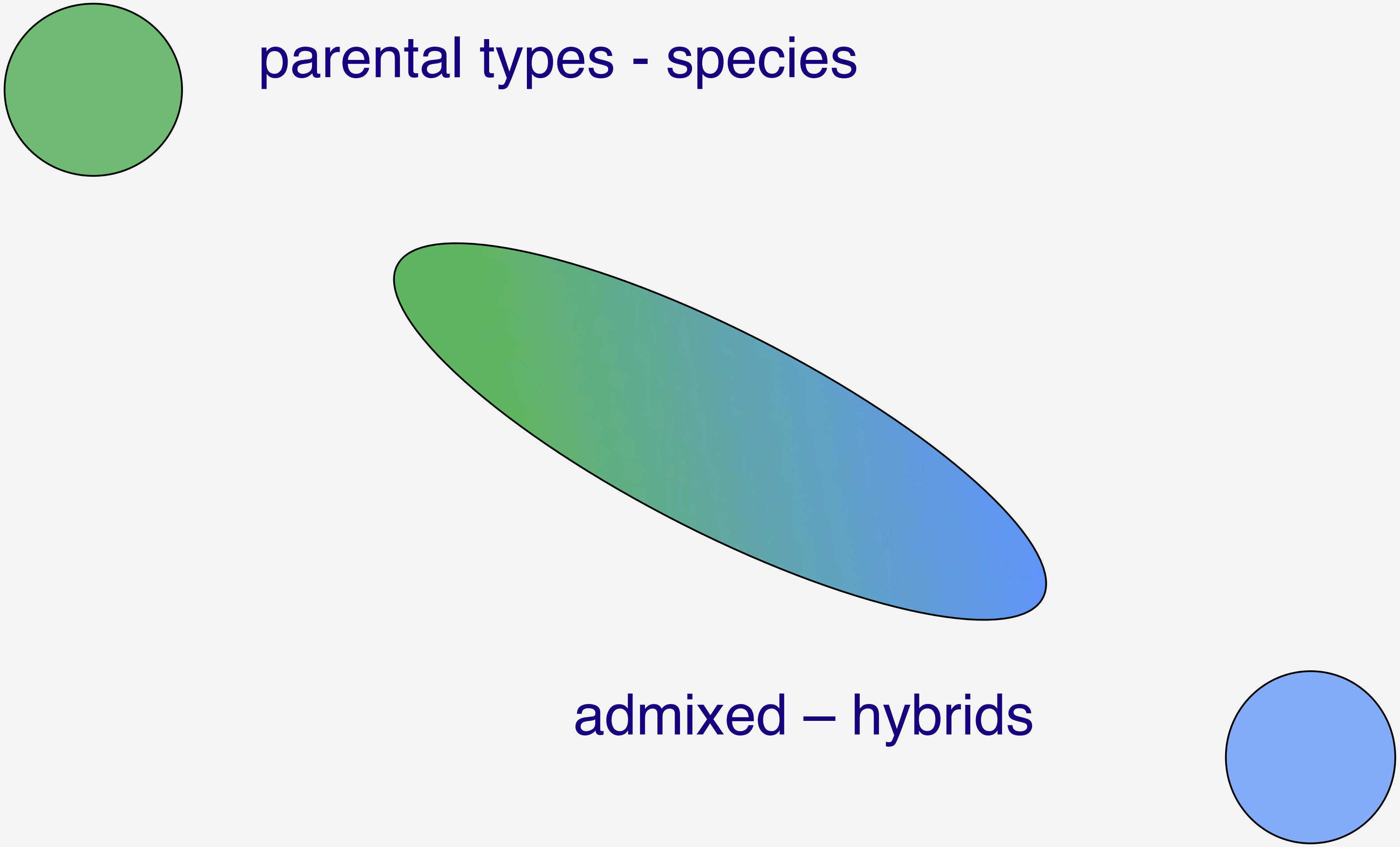
Estimating V_g

Utilize standing population variation and highly multilocus sequencing to infer associations between genotype and phenotype



QTL = quantitative trait locus
eQTL = expression QTL

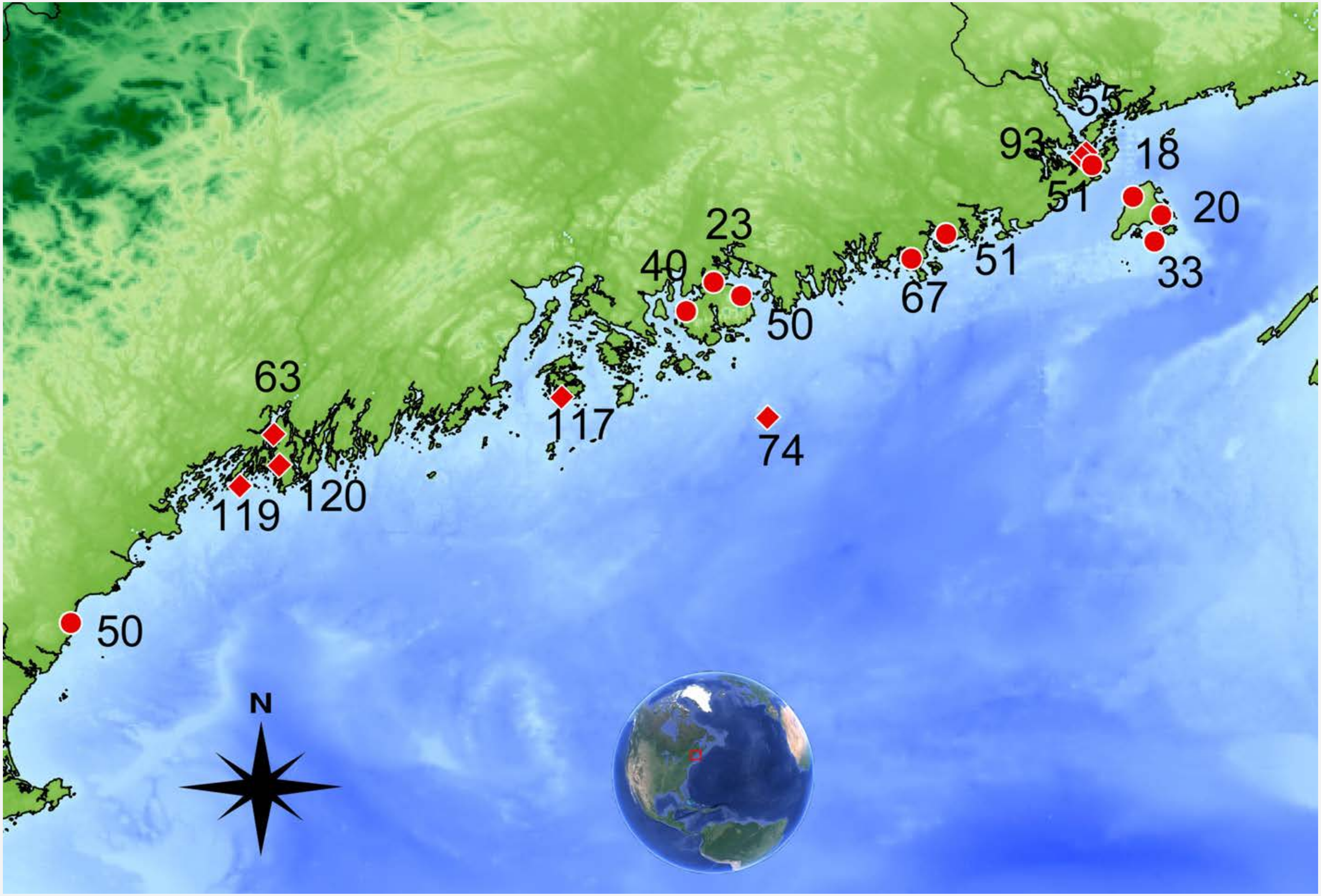
Power of the *M. edulis* x *M. trossolus* hybrid zone



parental types - species

admixed – hybrids

Sampling



Experimental Design

☐ Measure phenotype (V_p): shell calcification rate under physiological stress

☐ Common garden experiment: 14-day exposure, multifactor climate stress treatment

☐ Sequence highly multilocus genotype (V_g): next gen SNP assay

☐ Utilize genome-wide association survey (GWAS) to link genotype and phenotype

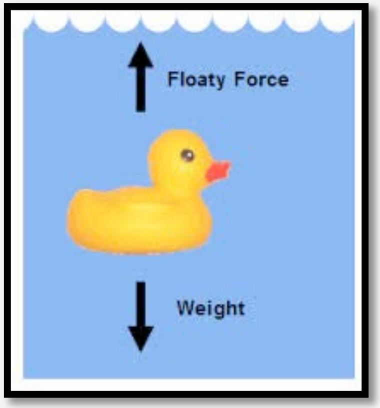
Methods – Common Garden
Stress Exposure

CONTROL: high food availability, ambient summer temp (17C), ambient pH (~8.0) n = 256

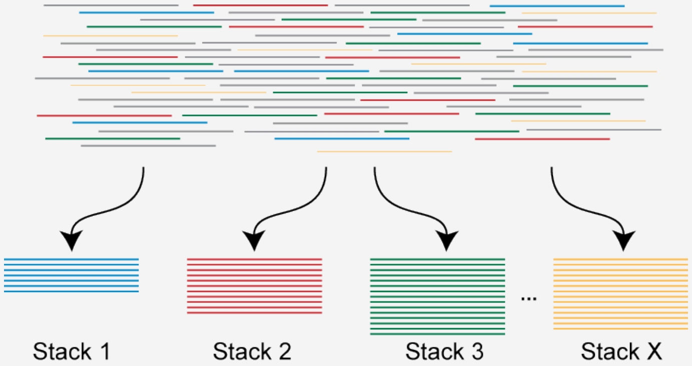
TREATMENT: low food availability, high temp (20C), lower pH (7.7) n = 648

Methods – Phenotype and Genotype

- V_p: measure shell calcification rate via change in buoyant weight



- V_g: SNP genotypes via GBS (genotype by sequencing)

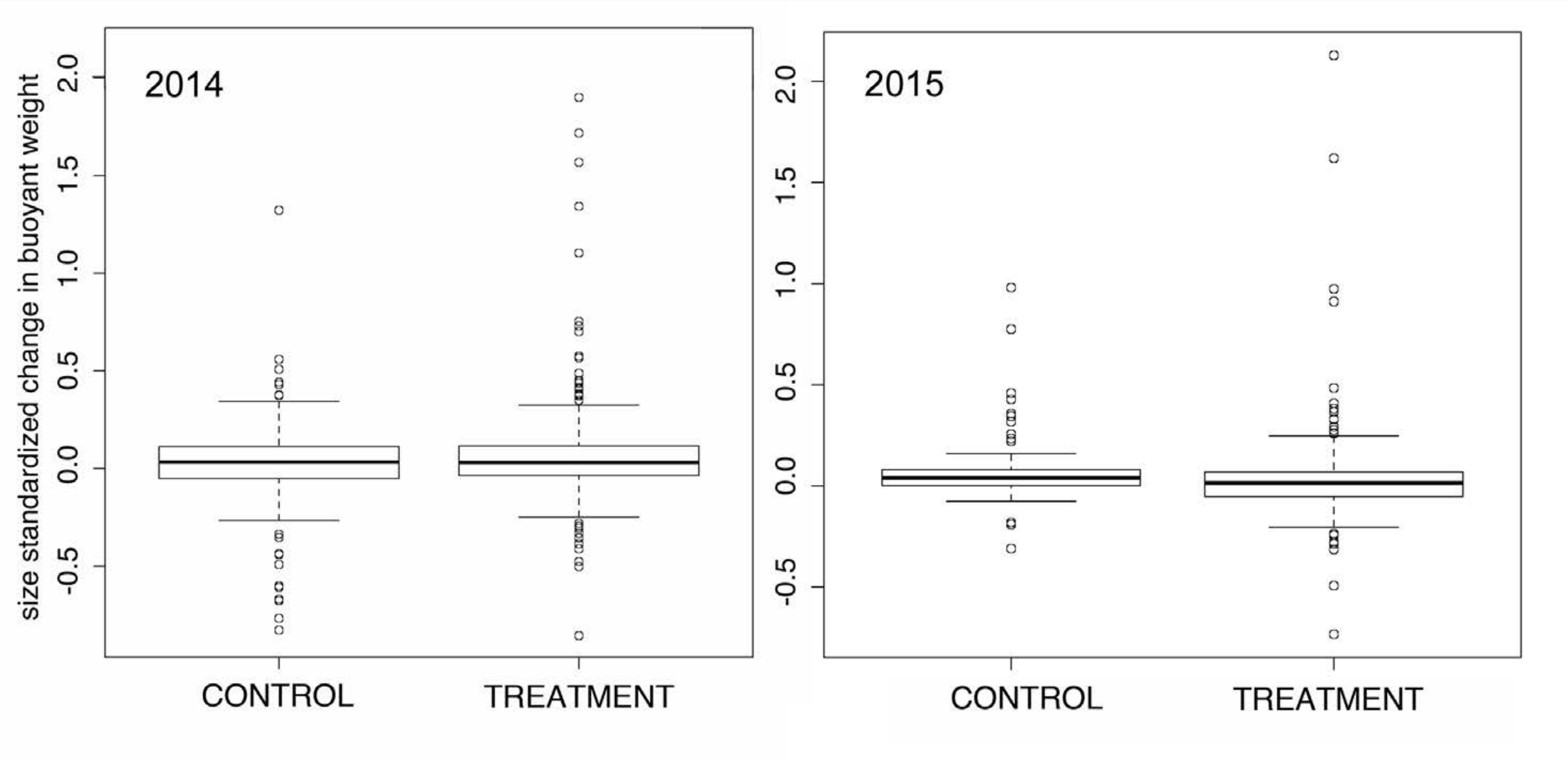


Methods – Linking Phenotype and Genotype

- *De novo* assembly of millions of short sequences to score highly multilocus genotypes
- Measure population parameters (genetic distance, individual ancestry)
- Statistically associate phenotype and genotype using two models:
  - Univariate Linear Mixed Model
  - Bayesian Variable Selection Regression

Results – Stress Phenotypes

- Variance in growth *increases* under stress

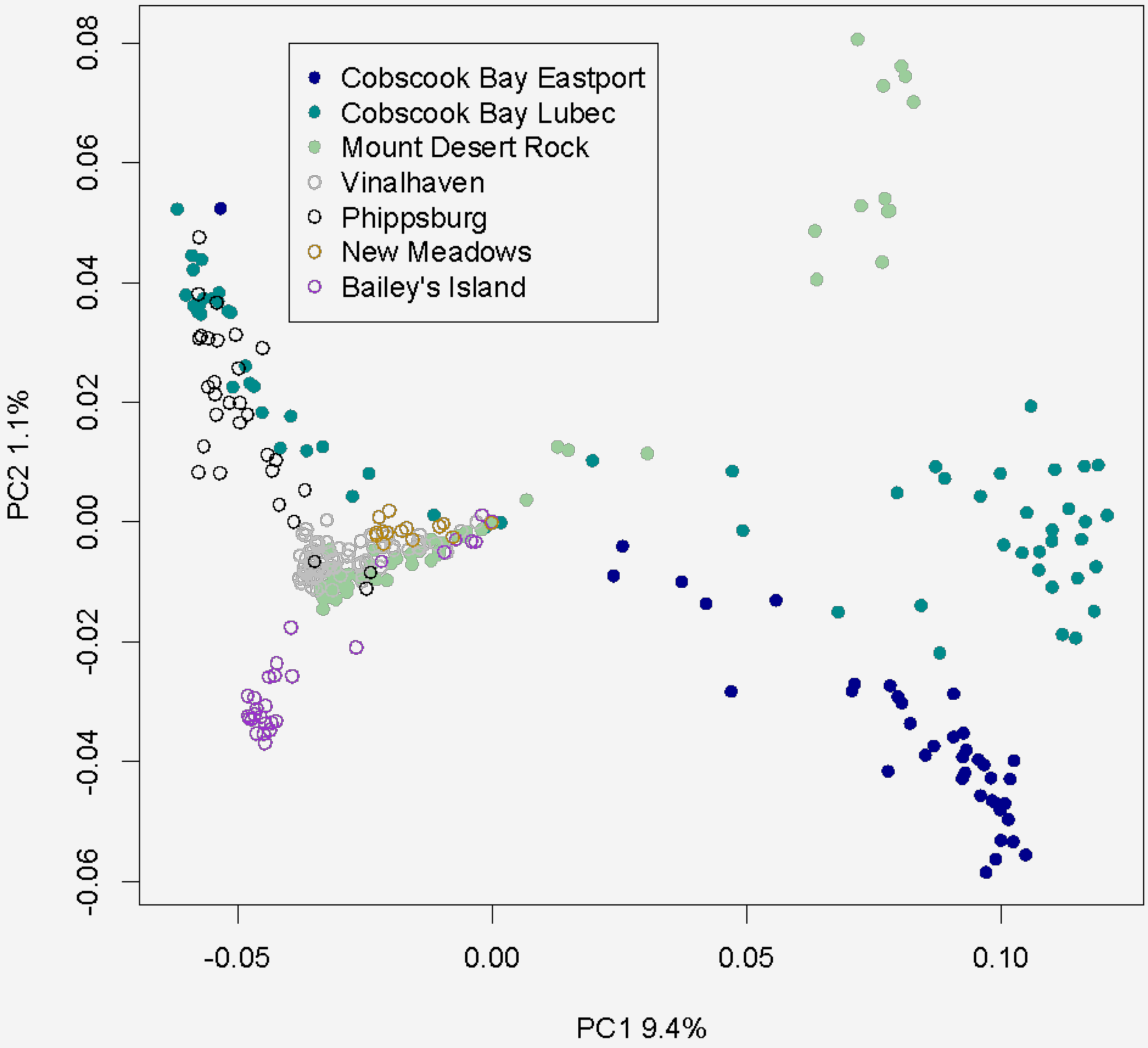


- Bartlett’s test for heteroskedasticity across treatments: $p < 0.0003$ for both years

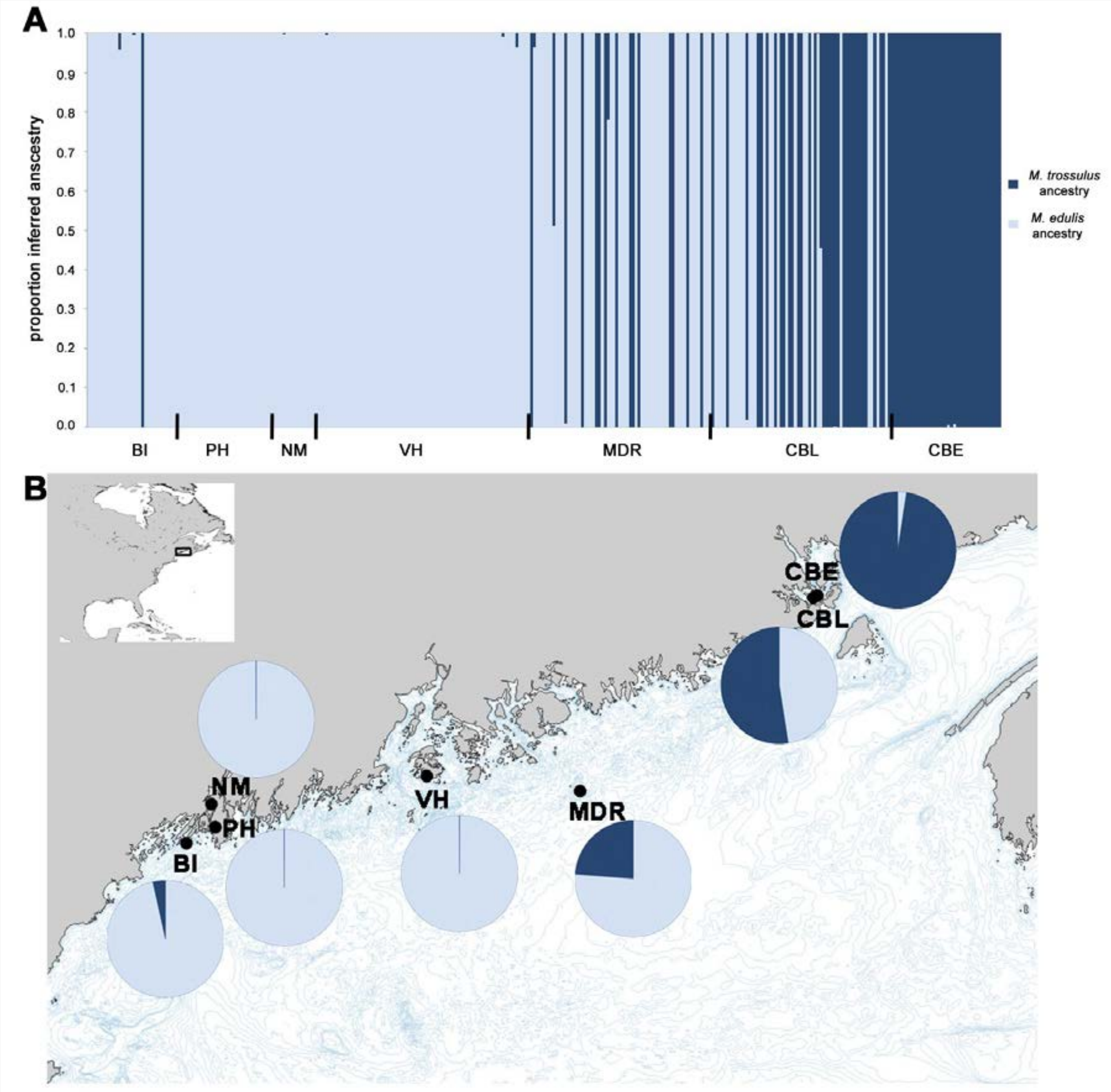
Results - Genotyping

- Genotype-by-sequencing SNP assay – 322 individuals (stress phenotype group 2014)
- 220,093,969 78bp reads (filtered)
- 171,645 SNP loci (after *de novo* assembly and locus filtering)

Results - Genotypes



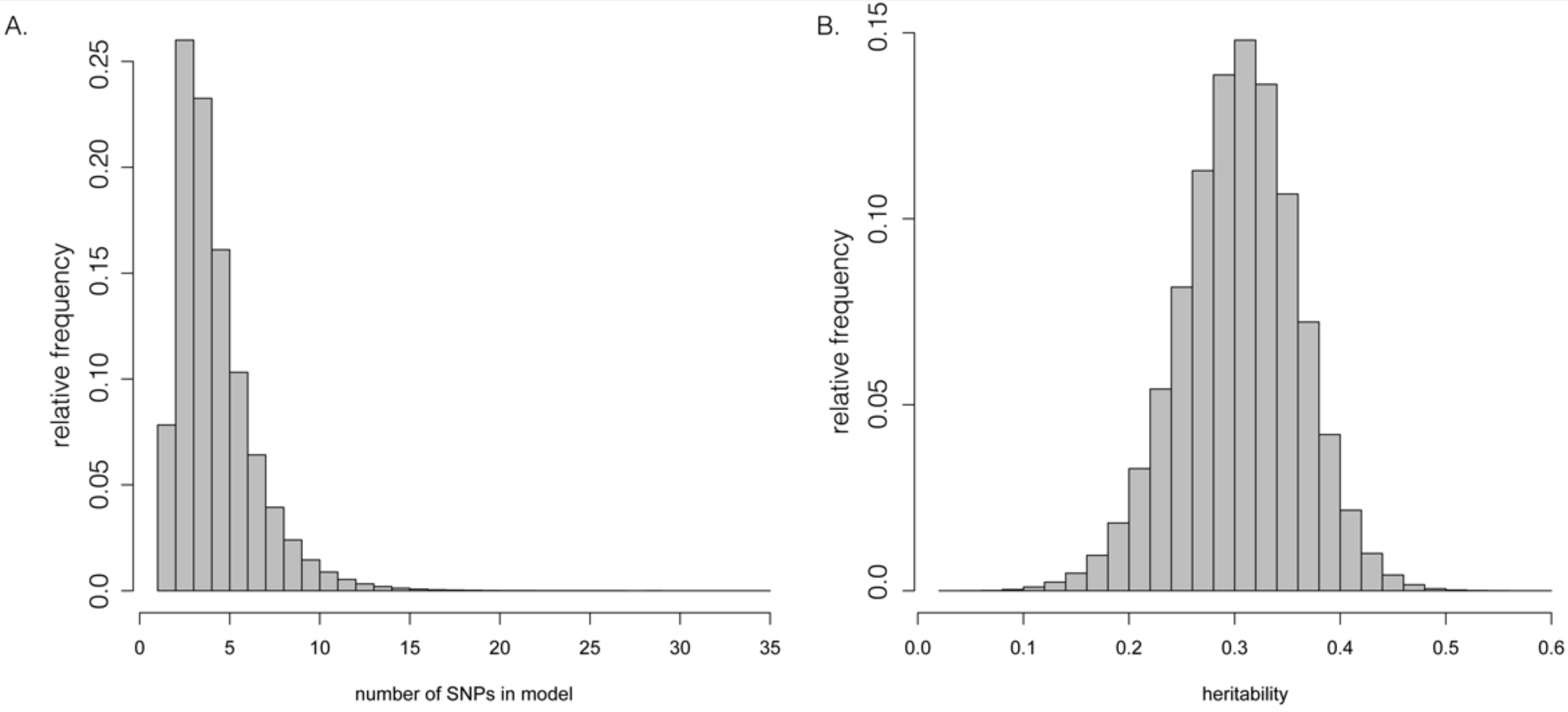
Results - Genotypes



STRUCTURE plot – population assignmen

Dark blue – *Mytilis trossulus,* Light blue – *Mytilius edulis*

Results - Association



posterior probability distributions

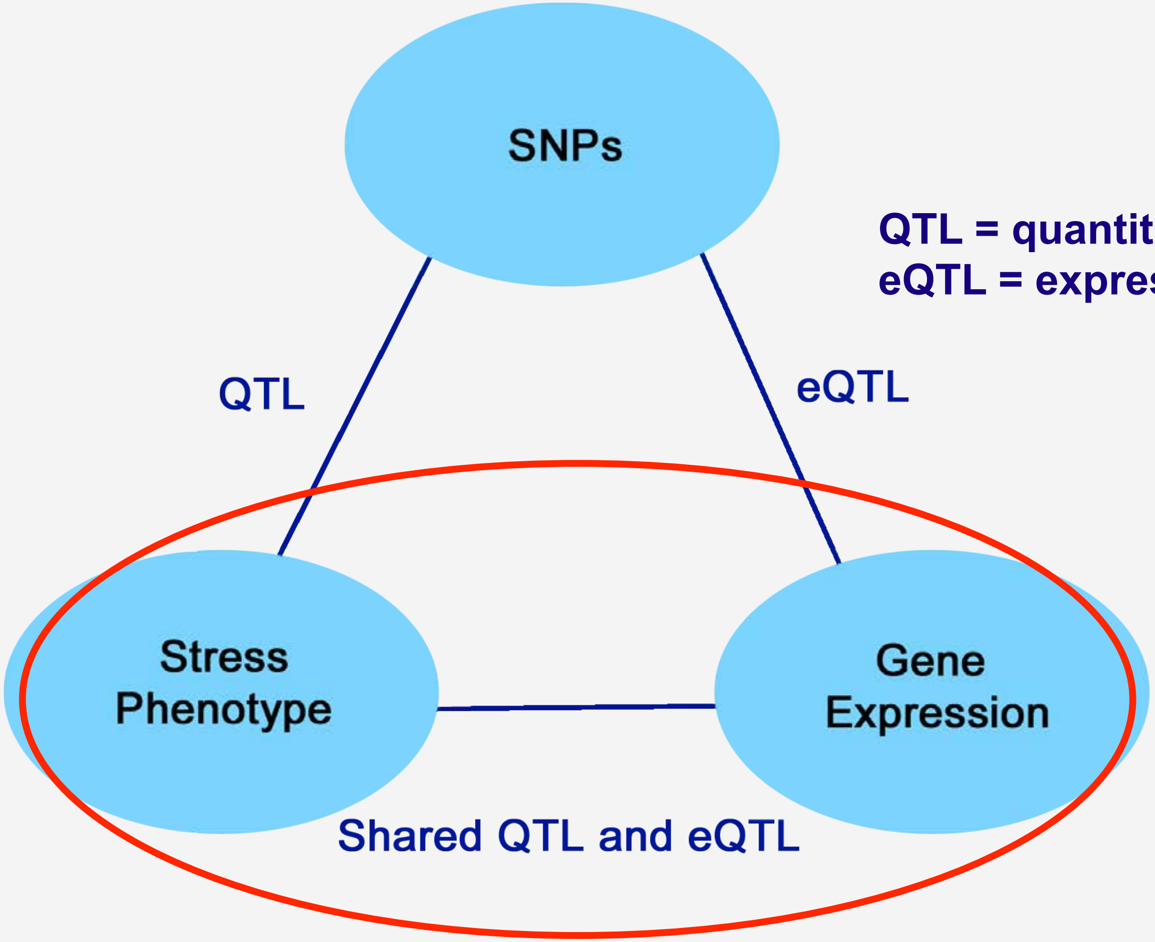
Results - Association

locus	effect size	posterior probability	linear p-value
104243	8.08	0.999	1.27E-13
115310	5.18	0.869	3.84E-07
71596	4.10	0.416	2.07E-09
72070	2.82	0.141	4.07E-08

- **GWAS – 4 loci associated with stress phenotype: calcification rate**
- **Anonymous loci; no hits from *M. galloprovincialis* genome nor public databases**


WANTED

Continuing Research



SNPs

QTL

eQTL

Stress Phenotype

Gene Expression

Shared QTL and eQTL

QTL = quantitative trait locus
eQTL = expression QTL

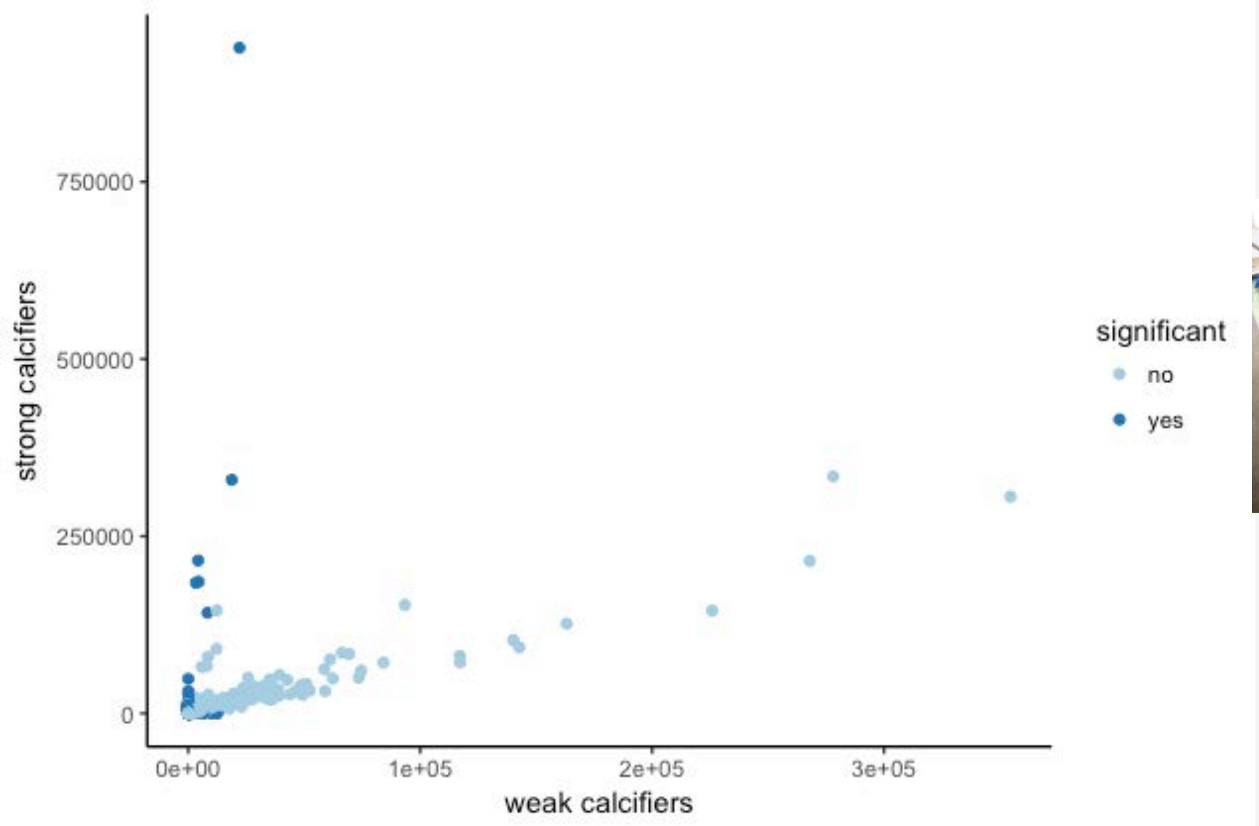
Gene Expression Data

- RNAseq mRNA library for 24 mussels
- selection of high and low calcifiers, geographic variation
- 298,514,080 150bp reads
- 77,565 transcripts (assembled to *M. galloprovincialis* genome)
- Test for differential expression under climate stress

Gene Expression Results

(Pieter Martino's Honors Thesis)

- ☐ Control vs. climate stress - 1527 significantly differentially expressed loci
- ☐ Strong vs. weak calcifiers - 1377 significantly differentially expressed loci



Pieter Martino

Implications

- We have discovered 4 QTL for climate stress tolerance in wild populations
- …and thousands of potential eQTL – genes that are differentially expressed under stress
- Our work provides a framework for understanding the gene regulatory network underlying calcification
- Future
  - Develop genetic assay for screening tolerant aquaculture strains
  - Larval and juvenile susceptibility

Acknowledgments

Co-authors

Bowdoin College

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Steven Allen

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