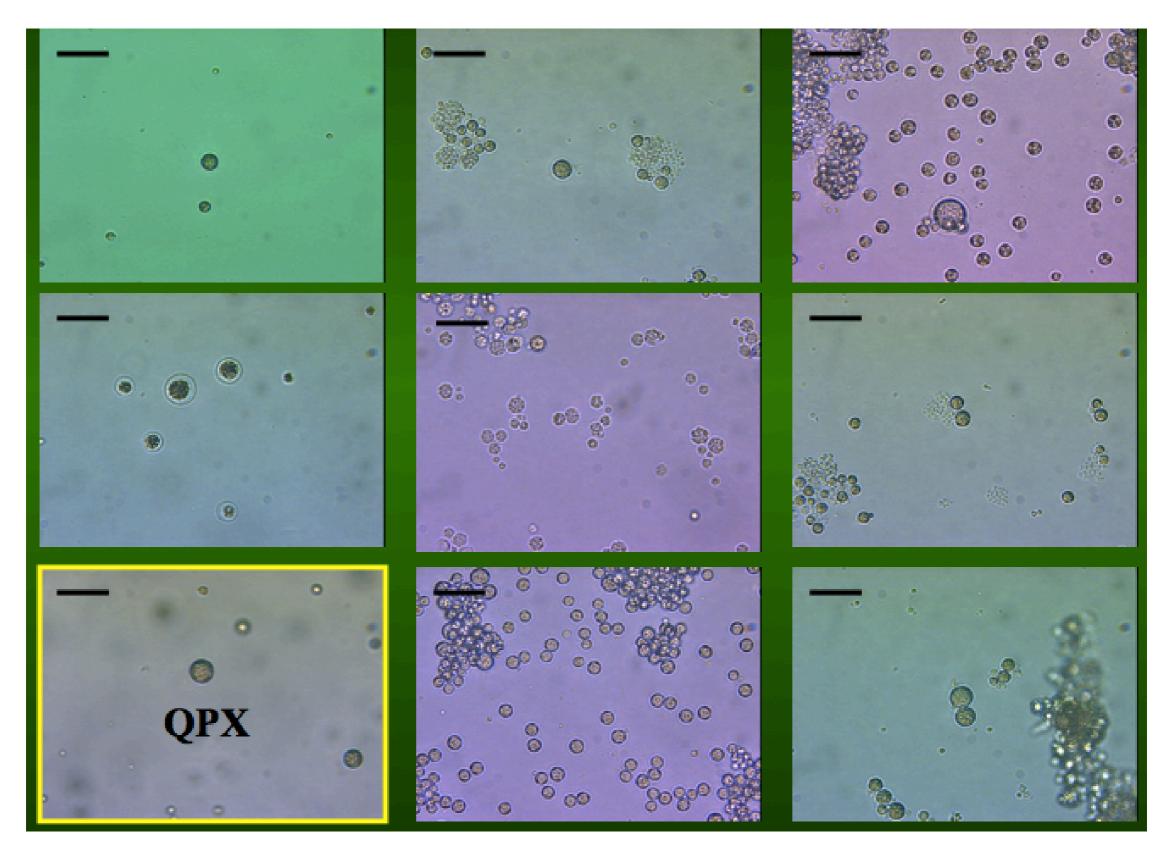
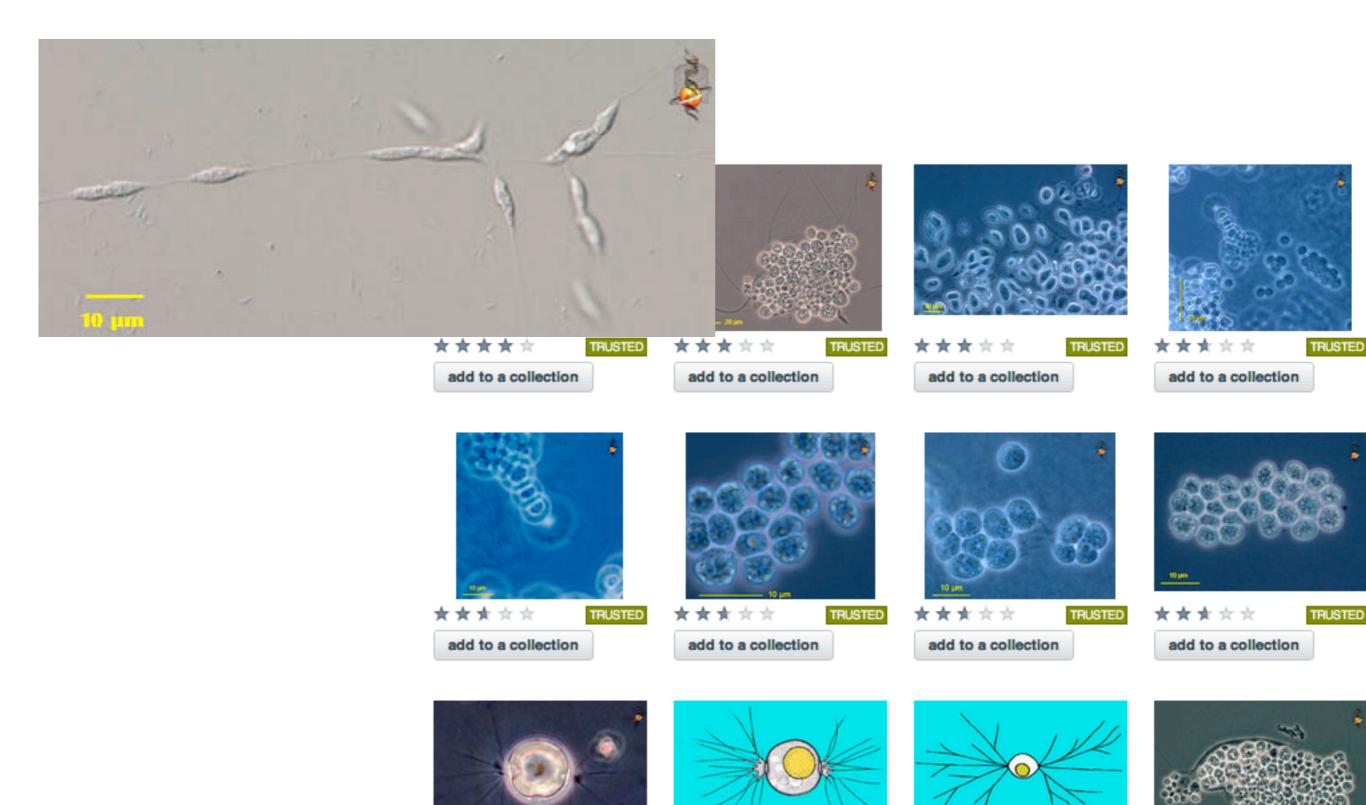
### Labyrinthulids





10 μm

## Labyrinthulids

Labyrinthulidae (synonym labyrinthulid) is a family of mainly marine unicellular protists.

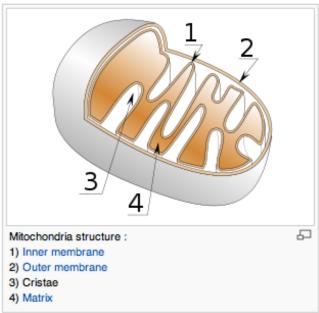
The labyrinthulids are characterized by tubulocristae mitochondria.

Members of the order Labyrinthulida were originally placed in the slime mould category, but their genetics demonstrate their relation to the stramenopiles.

## Labyrinthulids

Labyrinthulidae (synonym labyrinthulid) is a family of mainly marine unicellular protists.

The labyrinthulids are characterized by tubulocristae mitochondria.



Members of the order Labyrinthulida were originally placed in the slime mould category, but their genetics demonstrate their relation to the stramenopiles.

#### ORDER Labyrinthulida

#### UniProtKB (114) | Branch (372) | Caxonomy help

Taxon identifier	35131
Scientific name	Labyrinthulida
Common name	-
Synonym	-
Other names	<ul> <li>Labyrinthomorpha</li> <li>Labyrinthomorphids</li> <li>Labyrinthulomorpha</li> <li>Labyrinthulomycota</li> <li>slime nets</li> </ul>
Rank	ORDER
Lineage	<ul> <li>cellular organisms</li> <li>Eukaryota</li> <li>stramenopiles</li> </ul>
See also	• NCBI

Taxonomy navigation			
Î	<ul> <li>stramenopiles</li> </ul>		
Ţ	<ul> <li>environmental samples</li> <li>Labyrinthulidae</li> </ul>		
	<ul> <li>Thraustochytriidae</li> </ul>		
	<ul> <li>unclassified Labyrinthulida</li> </ul>		

## Thraustochytrid-like isolates from marine bivalve mollusks

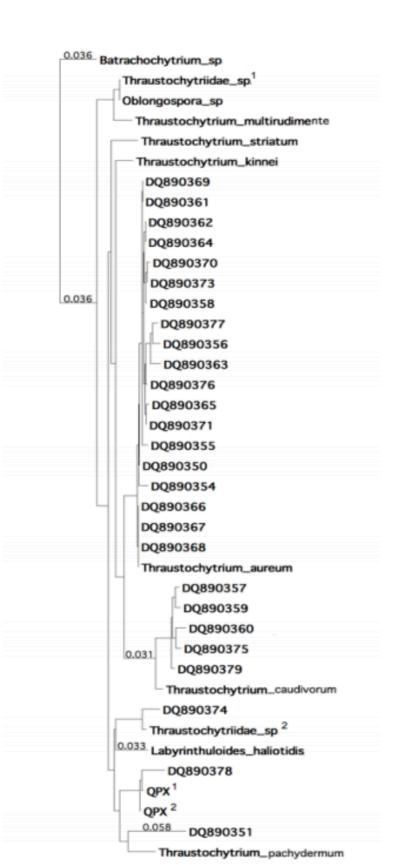
DQ890358	Crassostrea ariakensis	China	visceral mass	T. aureum
DQ890359	Mercenaria mercenaria	Virginia	mantle	T. caudivorum
DQ890360	Mercenaria mercenaria	Virginia	mantle	T. caudivorum
DQ890361 <sup>1</sup>	Mercenaria mercenaria	Virginia	mantle	T. aureum
DQ890362 <sup>2</sup>	Mya arenaria	Oregon	labial palps and gills	T. aureum
DQ890363	Mya arenaria	Oregon	labial palps and gills	T. aureum
DQ890364 <sup>2</sup>	Mercenaria mercenaria	Virginia	mantle	T. aureum
DQ890365	Mercenaria mercenaria	Virginia	mantle	T. aureum
DQ890366	Mya arenaria	Oregon	labial palps and gills	T. aureum **
DQ890367	Mercenaria mercenaria	Virginia	mantle	T. aureum **
DQ890368	Crassostrea virginica	Chesapeake Ba	y hemolymph	T. aureum **
DQ890369 <sup>1</sup>	Crassostrea virginica	Chesapeake Ba	y hemolymph	T. aureum
DQ890370	Mercenaria mercenaria	Virginia	mantle	T. aureum
DQ890371	Mya arenaria	Oregon	labial palps and gills	T. aureum
DQ890373	Mercenaria mercenaria	Virginia	mantle	T. aureum
DQ890374	Mercenaria mercenaria	Virginia	mantle	L. haliotidis
DQ890375	Mercenaria mercenaria	Virginia	mantle	T. caudivorum
DQ890376	Mya arenaria	Oregon	labial palps and gills	T. aureum
DQ890377	Mya arenaria	Oregon	labial palps and	T. aureum

## Thraustochytrid-like isolates from marine bivalve mollusks

	rachochytrium_sp  Thraustochytriidae_sp. <sup>1</sup>		
- f	Oblongospora_sp		
L	Thraustochytrium_multirudimente		
	Thraustochytrium_striatum		
	Thraustochytrium_kinnei		
	DQ890369		
	DQ890361		
	DQ890362		
	DQ890364		
	DQ890370		
	DQ890373		
0.036	DQ890358		
	DQ890377		
	DQ890356		
	DQ890363		
	DQ890376		
	DQ890365		
	DQ890371		
- 11	DQ890355 DQ890350		
	- DQ890354		
- 41	DQ890366		
	DQ890367		
	DQ890368		
	Thraustochytrium_aureum		
	DQ890357		
	DQ890359		
	DQ890360		
	0.031 DQ890375		
	DQ890379		
	Thraustochytrium_caudivorum		
	DQ890374		
1	Thraustochytriidae_sp <sup>2</sup>		
	0.033 Labyrinthuloides_haliotidis		
1	DQ890378		
	OPX 2		
	0.058 DQ890351		
	0000001		

DQ890358	Crassostrea ariakensis	China	visceral mass	T. aureum
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## Thraustochytrid-like isolates from marine bivalve mollusks



#### Thraustochytrid-like isolates from marine bivalve mollusks

Maille Lyons, Christopher Dungan, Steven Roberts

#### INTRODUCTION

Interest in thraustochytrid biology, ecology, and systematics has resurfaced in retheir elevated production of omega-3 fatty acids (Miller et al. 2007, Jain et al. 2007, Fan importance in marine microbial communities (Ramaiah et al. 2005, Bongiorni et al. 2005 2008), and their role as pathogens of aquatic invertebrates (Anderson et al. 2003, Kvinge Lyons et al. 2007, Scharer et al. 2007). As a group, thraustochytrids are unicellular, eulike protists whose classification is not clear. They may be classified in either the kingdo following Dick 2001 and Ragukumar 2002) or Chromista (if following Cavalier-Smith 1 Smith et al. 1994). The corresponding phylum, class, order, and family designations are between the two classification systems. There are at least five genera of thraustochytrids

#### http://goo.gl/a9x81

## Dynamics

Limnol. Oceanogr., 50(6), 2005, 1983-1988 © 2005, by the American Society of Limnology and Oceanography, Inc.

#### Lethal marine snow: Pathogen of bivalve mollusc concealed in marine aggregates

M. Maille Lyons and J. Evan Ward Department of Marine Science, University of Connecticut, 1080 Shennecossett Rd., Groton, Connecticut 06340

Roxanna Smolowitz and Kevin R. Uhlinger Marine Biological Laboratory, 7 MBL St., Woods Hole, Massachuse

Rebecca J. Gast Biology Department, Woods Hole Oceanographic Institution, Woods



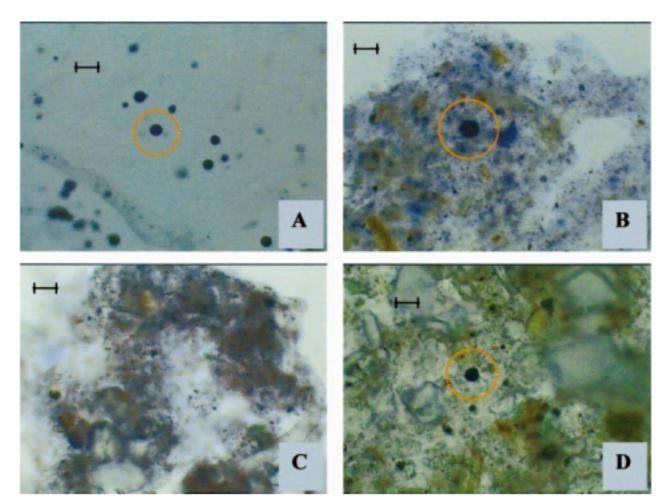
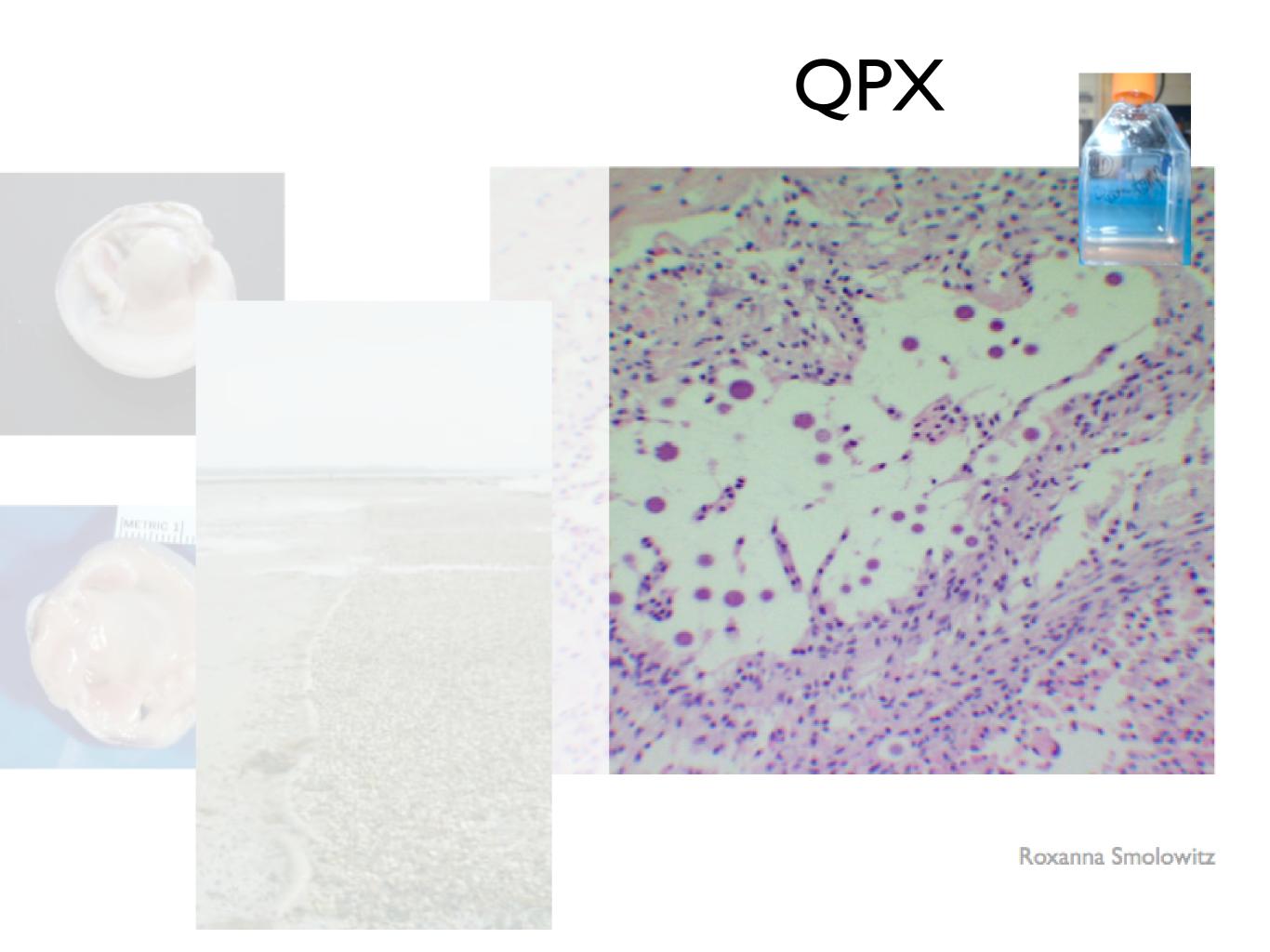


Fig. 2. Examples of in situ hybridization results for Quahog Parasite Unknown (QPX). (A) Positive results for QPX culture. (B) Positive results for laboratory-generated aggregates made with seawater and QPX culture (positive control). (C) Negative results for laboratory-generated aggregates made with seawater with no culture added (negative control; no dark round spheres present). (D) Positive results for natural aggregates collected near quahog beds infected with QPX. Each darkly stained sphere within a red circle is one QPX thallus. Scale bars, 25  $\mu$ m.





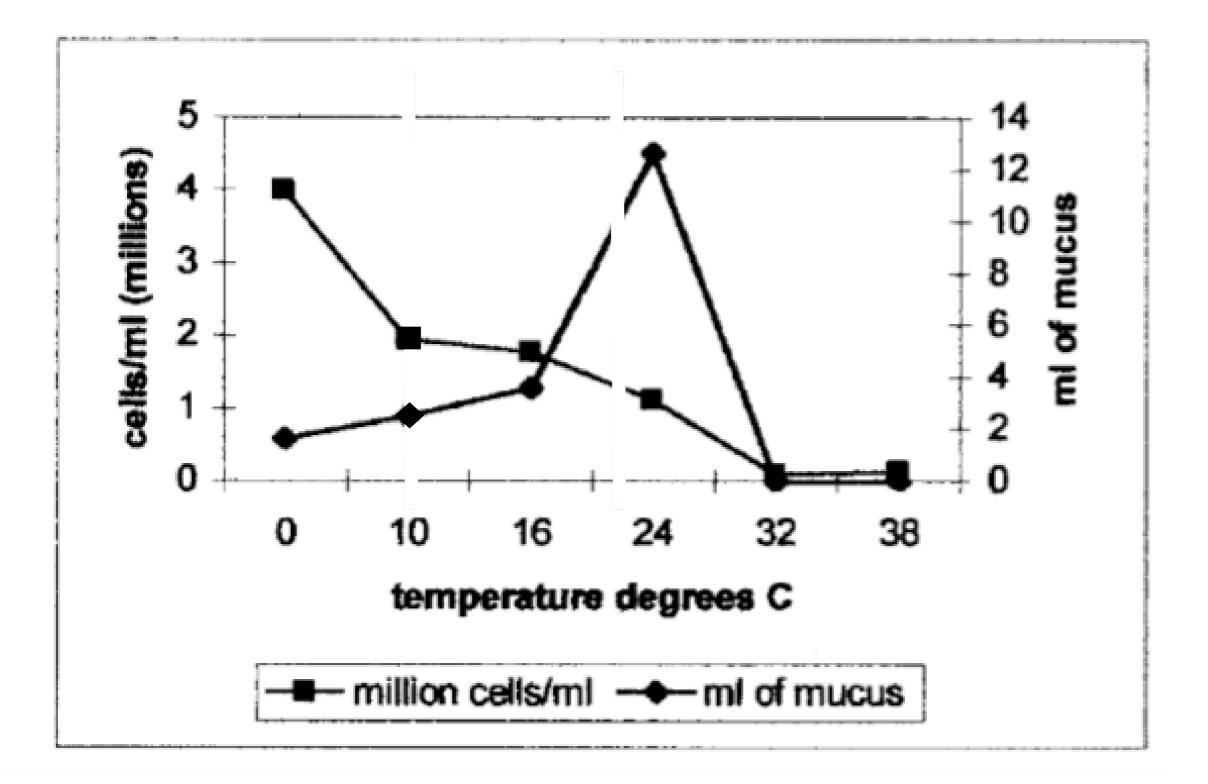
# QPX

- Single-celled relative of slime mold that has both animal and fungal characteristics
- QPX secretes a thick layer of mucus to ward off the clam's immune response
- Killing nine out of every ten clams in some plots when it first hit the Massachusetts coast in 1993



Captain Andrew Cummings stands atop clams he and others dug out of their Wellfleet plots to quell a QPX outbreak last winter. Cummings estimated the removal claimed nearly 2 million clams. (Photo courtesy of Captain Andrew Cummings)

#### **QPX** Biology



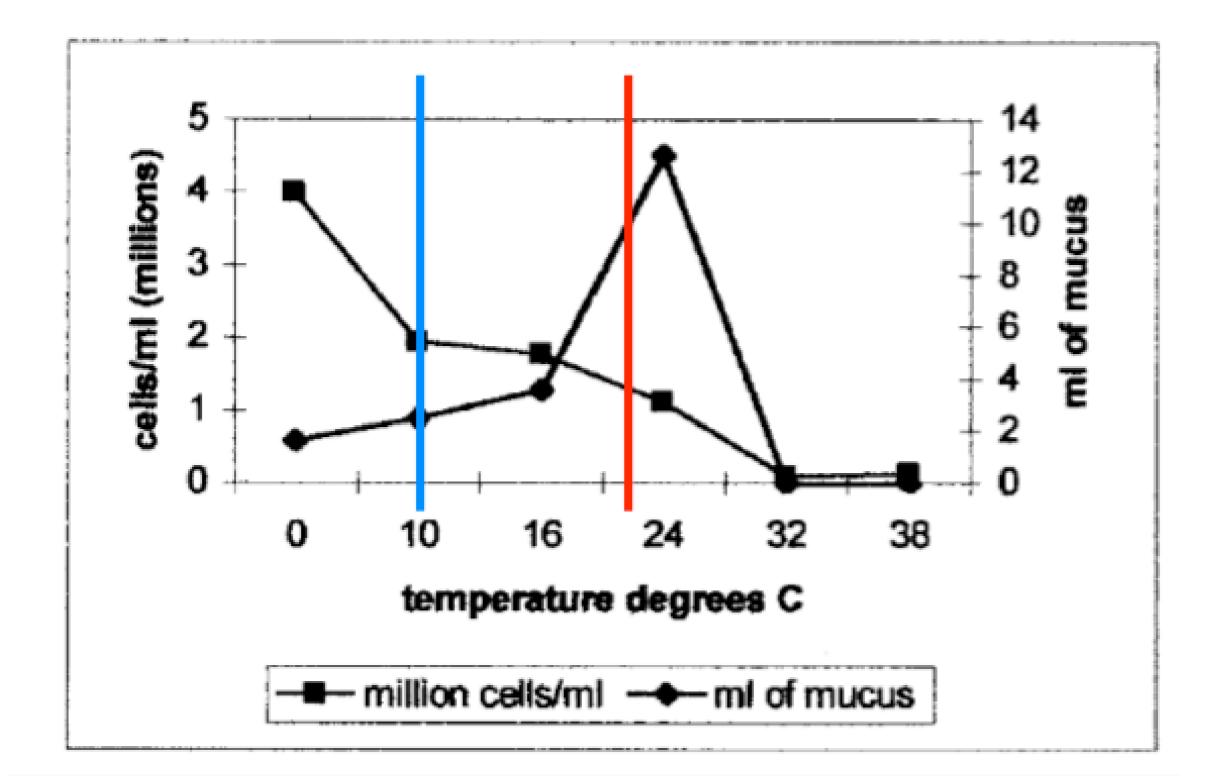
## An Experiment

# Rationale

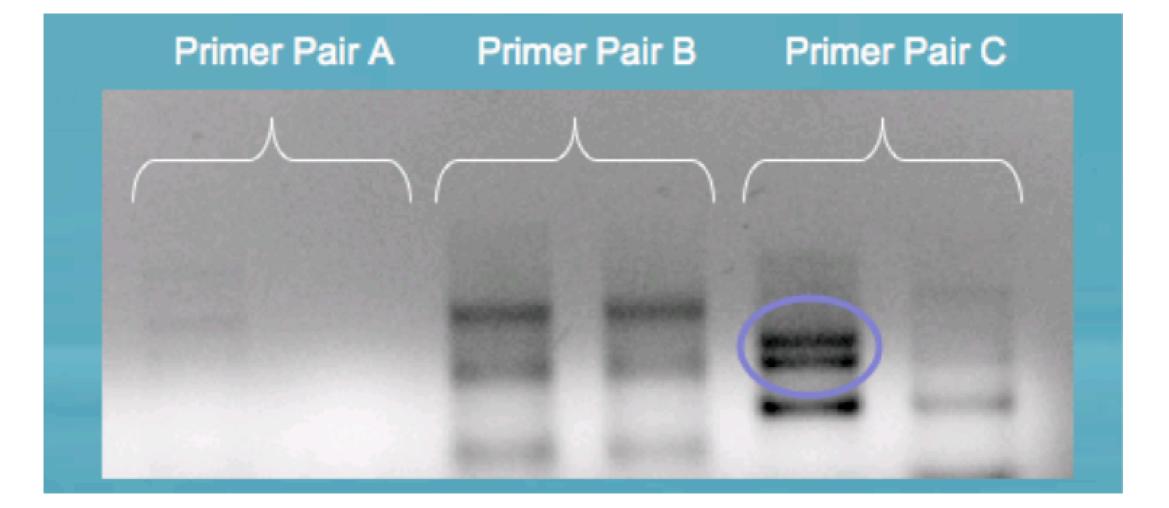
- Understand more concerning QPX physiology
  - Environmental influence
  - Differences across strain
  - Pathogenecity

## Factors

- Temperature
- Strain



# **Differential Display**



# **QPX** Serine Protease

Top Blastx Hit	Species associated with	e-value	% similar
Glutathione-dependent formaldehvde-activating.	Ralstonia metallidurans	1.00E-04	45
Subtilisin-like serine protease pepC precursor	Aspergillus niger	2.00E-16	63
Permeases of the major facilitator superfamily	Yersinia frederiksenii	0.66	47
40s ribosomal protein s23	Theileria annulata	9.00E-38	96
ATP-binding cassette / transporter, sub-family G	, Toxoplasma gondii	4.00E-25	60
mitogen-activated protein kinase	Pan troglodytes	3.30E+00	46
Multidrug Resistance Protein family member (mr	<i>Caenorhabditis elegans</i>	3.00E-14	66
mitogen-activated protein kinase 2	Toxoplasma gondii	1.00E-12	84
haemagluttinin repeat protein	Pseudomonas fluoresens	2.70E+00	52
polysaccharide biosythesis protein	Methylococcus capsulatus	4.30E+00	47
Potassium inwardly-rectifying channel	Homo sapiens	1.00E-07	55

# Proteases

- Critical factor in disease pathogenesis
- Serine proteases produced by pathogen
  - Facilitate penetration
  - Stimulate degradation of host proteins involved in immune defense

Alkaline Serine Protease Is an Exotoxin of Vibrio alginolyticus in Kuruma Prawn, Penaeus japonicus

#### EFFECT OF HOMOGENATE FROM DIFFERENT OYSTER SPECIES ON <u>PERKINSUS</u> <u>MARINUS</u> PROLIFERATION AND SUBTILISIN GENE TRANSCRIPTION

GWYNNE D. BROWN, STEPHEN L. KAATTARI, and KIMBERLY S. REECE\*

Production of proteinase during experimental infection of Ostrea edulis L. larvae with Vibrio alginolyticus NCMB 1339 and the antigenic relationship between proteinases produced by marine vibrios pathogenic for fish and shellfish

A. S. NOTTAGE<sup>1</sup>T. H. BIRKBECK<sup>1</sup>

<sup>1</sup>Department of Microbiology, University of Glasgow, Scotland

# Complete Protein

		30	
M <mark>DVVKR</mark> IQKA	RPRLEVSGTH	MY <mark>H</mark> VAAVAVP	KPWGLNRVDQ
			▃▋▋▖▋▃▁▋▃▖
PDLPLDHTSF	KTSYTGKNVT	VYILDTGIWE	SHGDYGSRVR
Pep	tidase S8 Region		
90	100	110	120
PGVSFVKGED		HCAGTAVGTR	YGVAKDAQVV
Peptidase S8 Reg		_	
130	140	150	160
GIKILSAEGY Peptidase S8 Reg	GNTVDIIKGI	EWAVNDARSR	NTPGVLSLSL
170 GGSADPVLDA	180 GVDAAADAGM	190 LVAVAAGNDN	GDACKKSPAR
Peptidase S8 Reg			
210	220	230	240
AAQVITVGAT	NIADHRSYFS	NWGTCVNVFA	PGTNIISSWI
Peptidase S8 Reg	ion		
250	260	270	280
	GTSMATPLVA	GVLATLLEKH	DGDAEAAKMD
Peptidase S8 Reg			
290		306	
MFTLVARDKL	TDVKASSPNW	LVQTAR	

Length: 306 Frequencies: A: 38 (12.4%) 3 (01.0%) C: D: 23 (07.5%) E: 8 (02.6%) F: 5 (01.68)G: 32 (10.5%) (02.6%) 8 H: I: 14 (04.6%) 15 (04.9%) K: L: 18 (05.9%) М: 8 (02.6%) N: 14 (04.6%) 12 (03.9%) **P**: Q: 5 (01.6%) 14 (04.6%) R: s: 20 (06.5%) 21 (06.9%) T: V: 34 (11.1%) (02.0%) W: 6 (02.6%) Y: 8

# Sequence Analysis

Putative conserved domains have been detected, click on the image below for detailed results.

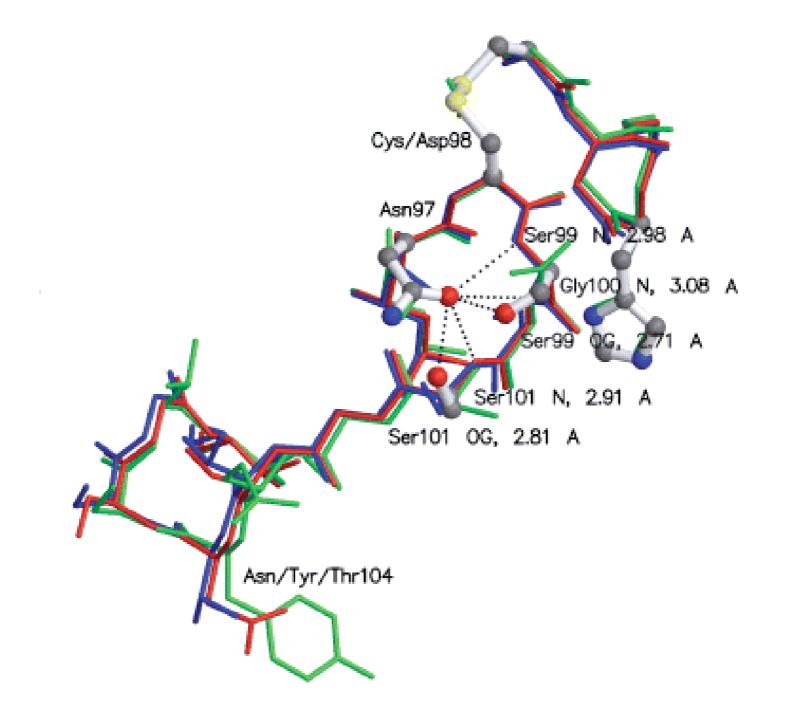


- •Subtilase family.
- •Subtilases are a family of serine proteases.
- •They appear to have independently and convergently evolved a catalytic triad

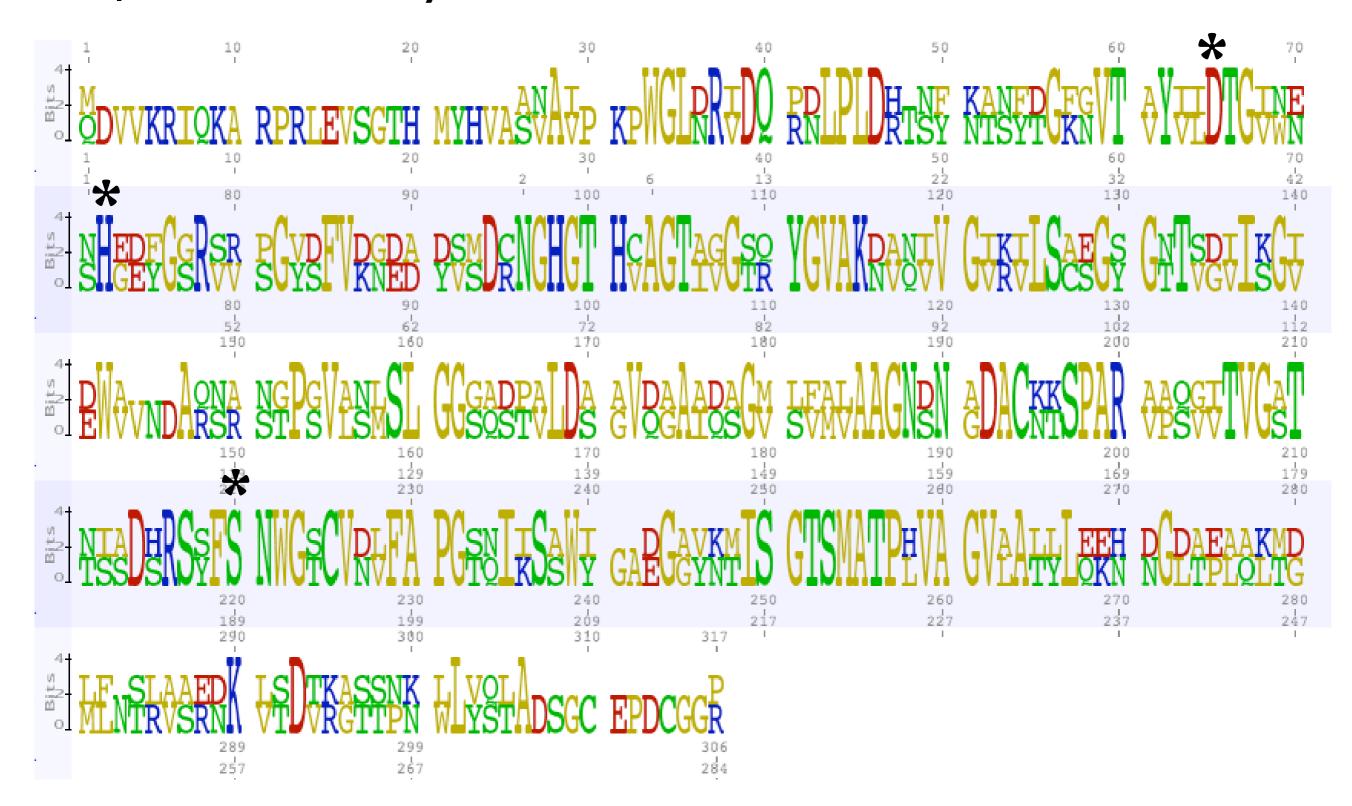
the three amino acid residues found inside the active site of certain protease enzymes: serine (S), aspartate (D) and histidine (H). They work together to break peptide bonds on polypeptides.
Structure is an alpha/beta fold containing a 7-stranded parallel beta sheet.

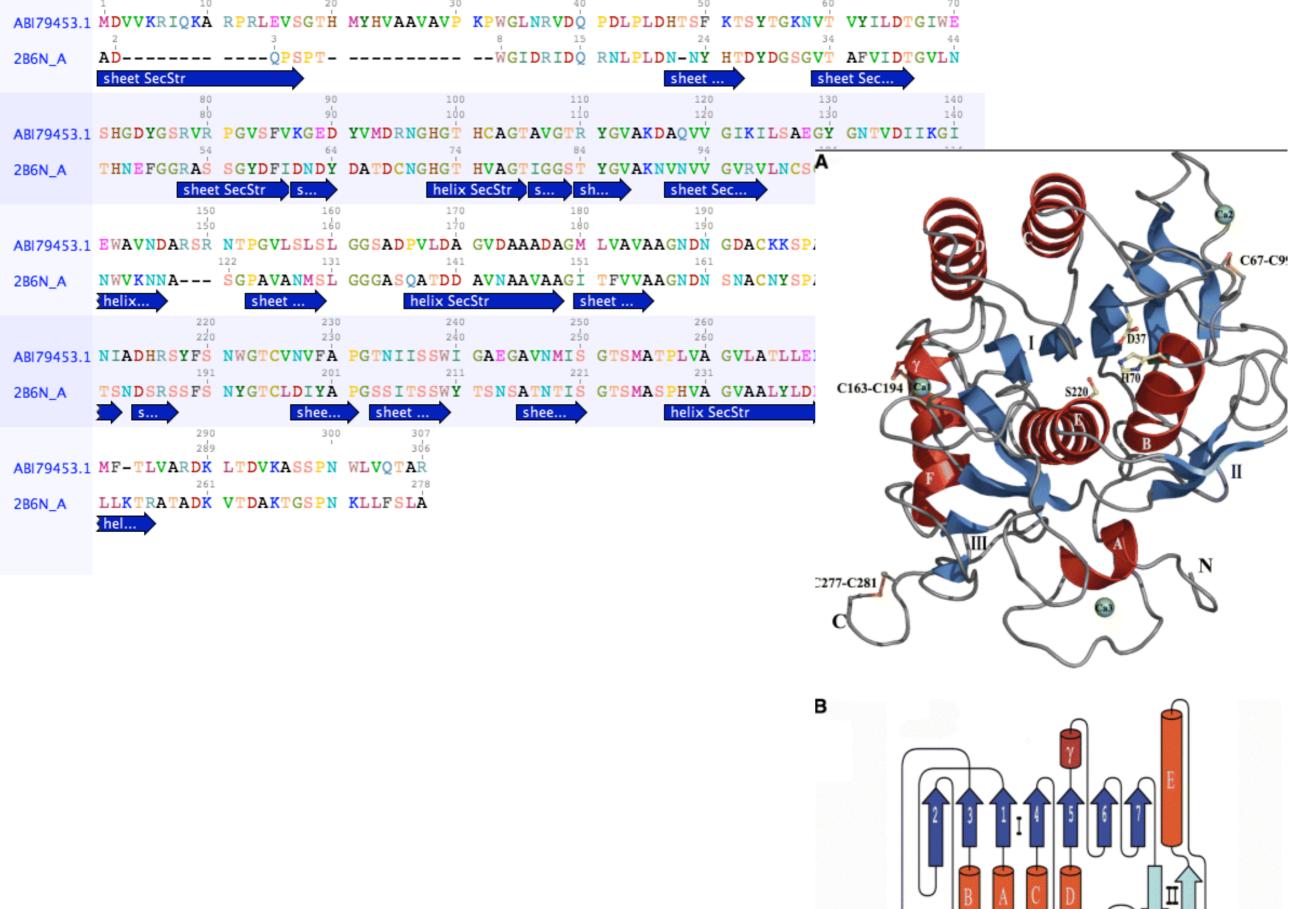
#### The 1.8 Å crystal structure of a proteinase K-like enzyme from a psychrotroph Serratia species

Ronny Helland<sup>1</sup>, Atle Noralf Larsen<sup>2</sup>, Arne Oskar Smalås<sup>1,3</sup> and Nils Peder Willassen<sup>1,2</sup>

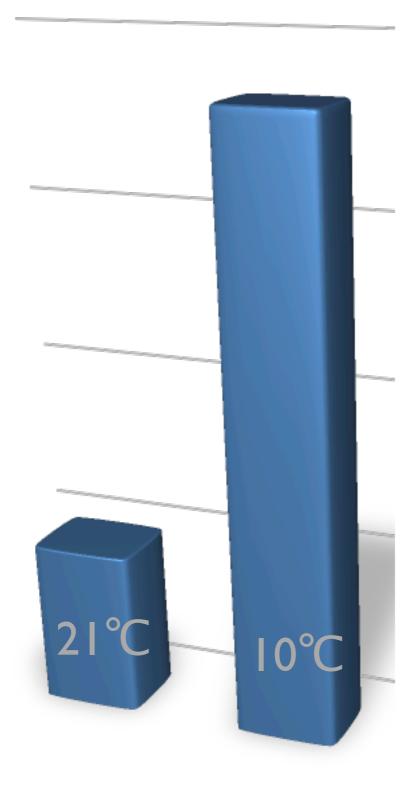


#### Asp/Ser/His Catalytic Triad



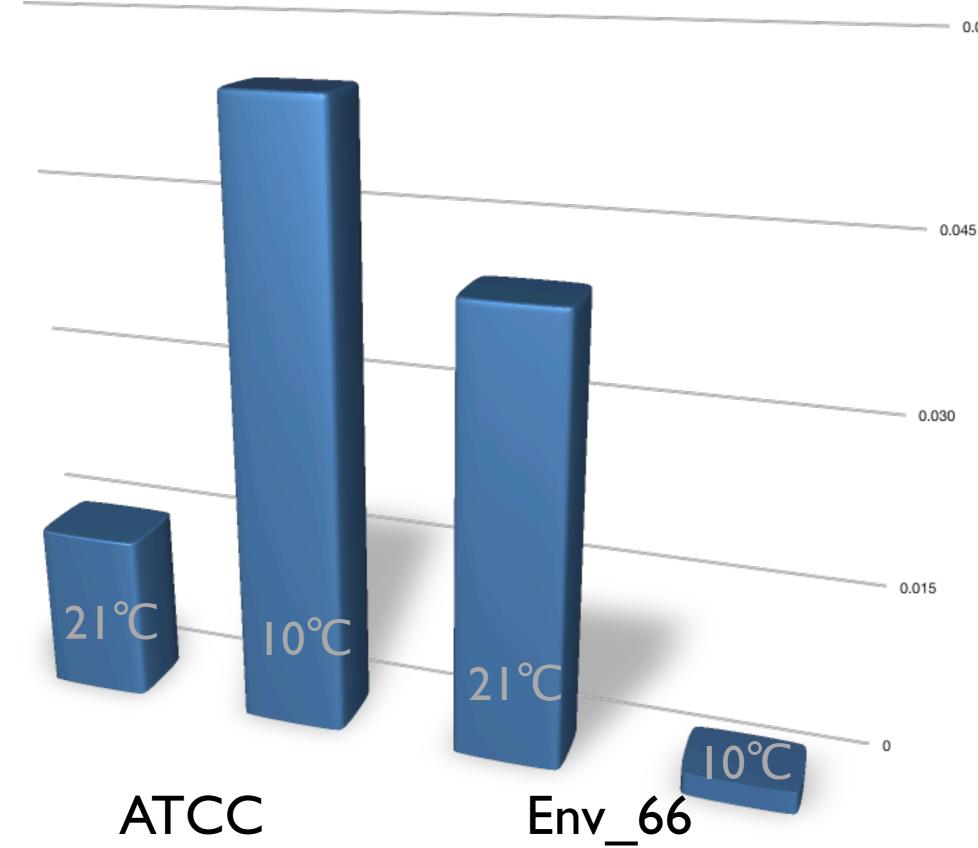


# Serine Protease gene expression

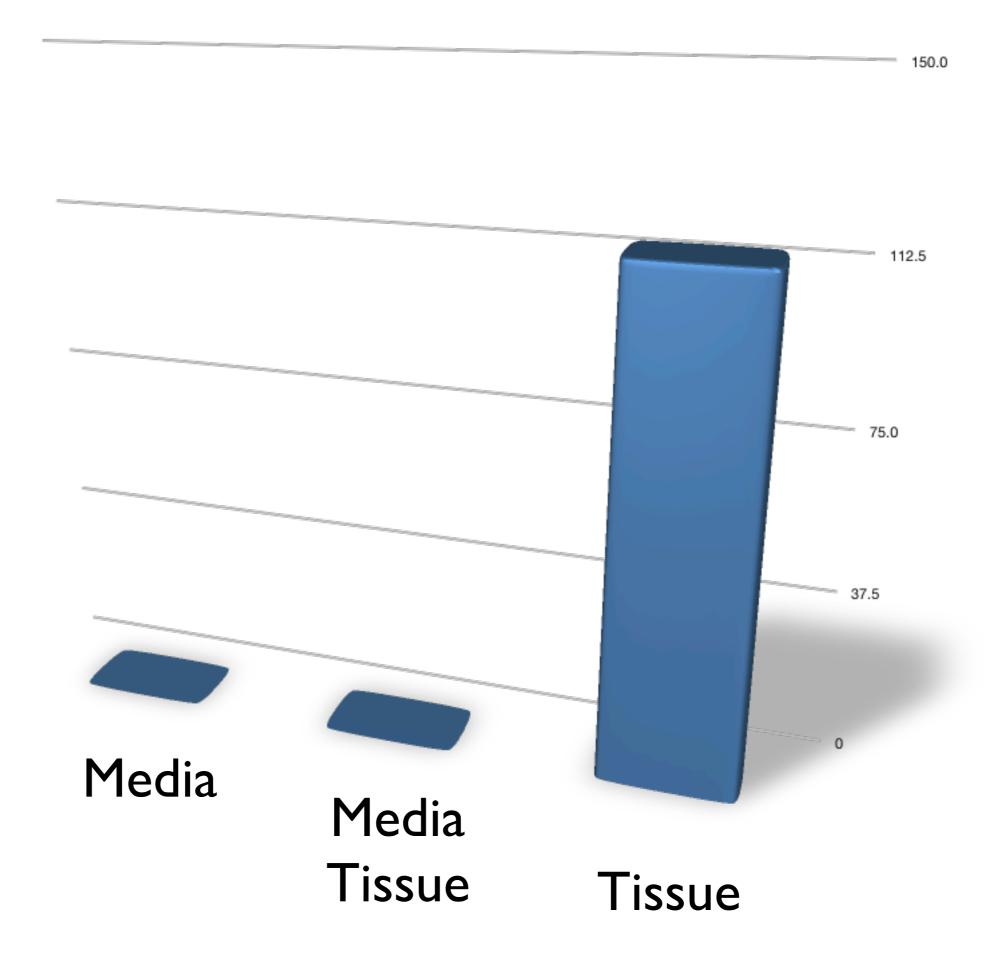


#### ATCC





Serine Protease gene expression



# Summary

- Complete CDS of Subtilisin-like serine protease
  - Catalytic Triad
- Expression varies across strains
  - temperature dependent
- Significantly upregulated when in contact with host tissue

## Another experiment





# Rationale

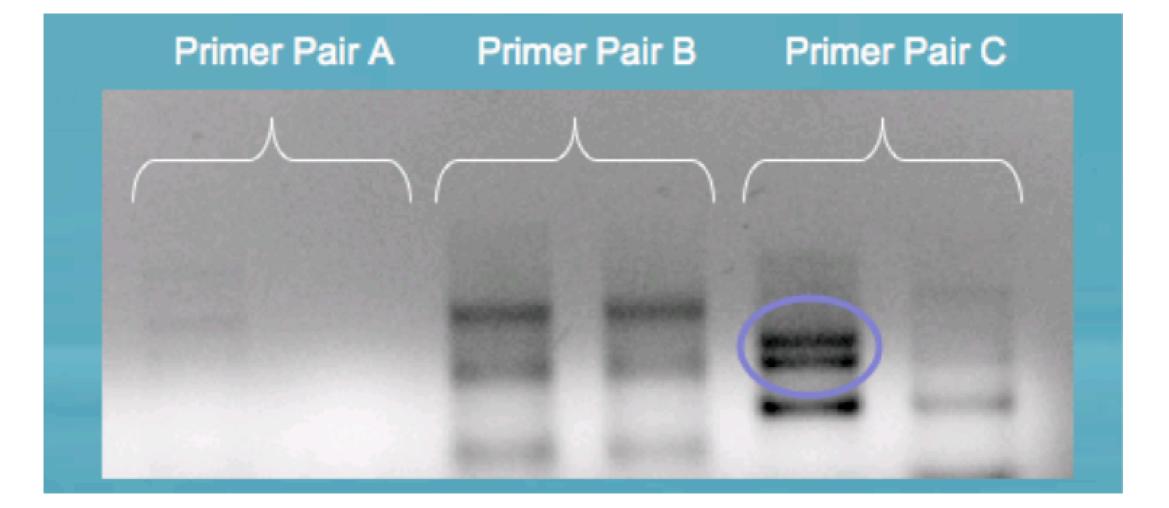
- Understand more concerning Clam physiology
  - Environmental Influence
  - Differences in Strain
  - Immune Response



# Approach

- Differential Display Analysis
- Construction of cDNA library from hemocytes
  - Gene Discovery

## **Differential Display**



## Differential Expression

- Infected versus uninfected
  - 7 upregulated
  - 6 downregulated
- Comparison across strains
  - I0 upregulated FL (June 7:Aug 3)
  - 8 upregulated NJ (June 2: Aug 6)
  - I upregulated MA (June)

## Results

- Few ribosomal proteins
- Hemocyte receptors
  - Important signaling cascade
- Unexpected difficulty identifying products
  - ????
- Serine Protease Inhibitor

## Serine Protease Inhibitor

• Critical factor in disease pathogenesis

- Serine proteases produced by pathogen
  - Facilitate penetration
  - Stimulate degradation of host proteins involved in immune defense



Current Microbiology

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A. S. NOTTAGE<sup>I</sup>T. H. BIRKBECK<sup>1</sup>

<sup>1</sup>Department of Microbiology, University of Glasgow, Scotland

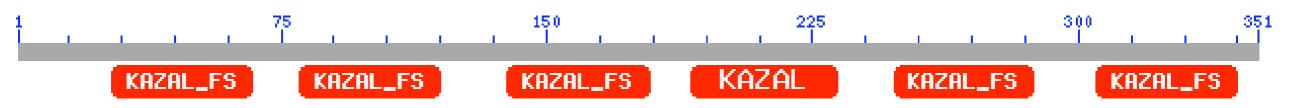
### **Inhibitors of proteases**

#### Protease Inhibitory Activity in Selectively Bred Families of Eastern Oysters

J. L. Oliver<sup>a</sup>, P. M. Gaffney<sup>b</sup>, S. K. Allen, Jr<sup>2</sup>, M. Faisal<sup>d</sup>, and S. L. Kaattari<sup>d</sup>

## Hard Clam Serine Protease Inhibitor

Putative conserved domains have been detected, click on the image below for detailed results.



#### Organism Report

Dipetalogaster maximus [bugs] taxid 72496 <u>sp 096790 DPGN_DIPMA</u> Serine protease inhibitor dipetalogas <u>emb CAA10384.1 </u> dipetalogastin [Dipetalogaster maximus]	<u>83</u>	5e-15 5e-15
Triatoma brasiliensis [bugs] taxid 65344 <u>qb ABI96910.1 </u> brasiliensin precursor [Triatoma brasiliensis] Chlamys farreri [bivalves] taxid 202578	82	le-14
qbABB58758.1serine protease inhibitor-1L [Chlamys farreri]qbABB58759.1serine protease inhibitor-1S [Chlamys farreri]qbABB89133.1serine protease inhibitor CFSPI3 [Chlamys fqbABC02759.1serine protease inhibitor [Chlamys farreri]	80	3e-14 4e-14 2e-10 2e-09

Domain indicating that protein is able to inhibit subtilisin-like serine protease

CRP-LCACPRIYKPVCGQDGKTYPNQCELNCAGVALFEEGPCIATSPQFDFAPEAPCICT CRP C CP IY PVCG DGKTY N C C V + C C+CT CRPEQCVCPSIYSPVCGYDGKTYSNACSAGCDNVKIRCNRKCPCKG-----IGCVCT \* \* \* \* \* \* \* \*

Contains cysteine residues necessary for tertiary structure

# Inhibitor Expression

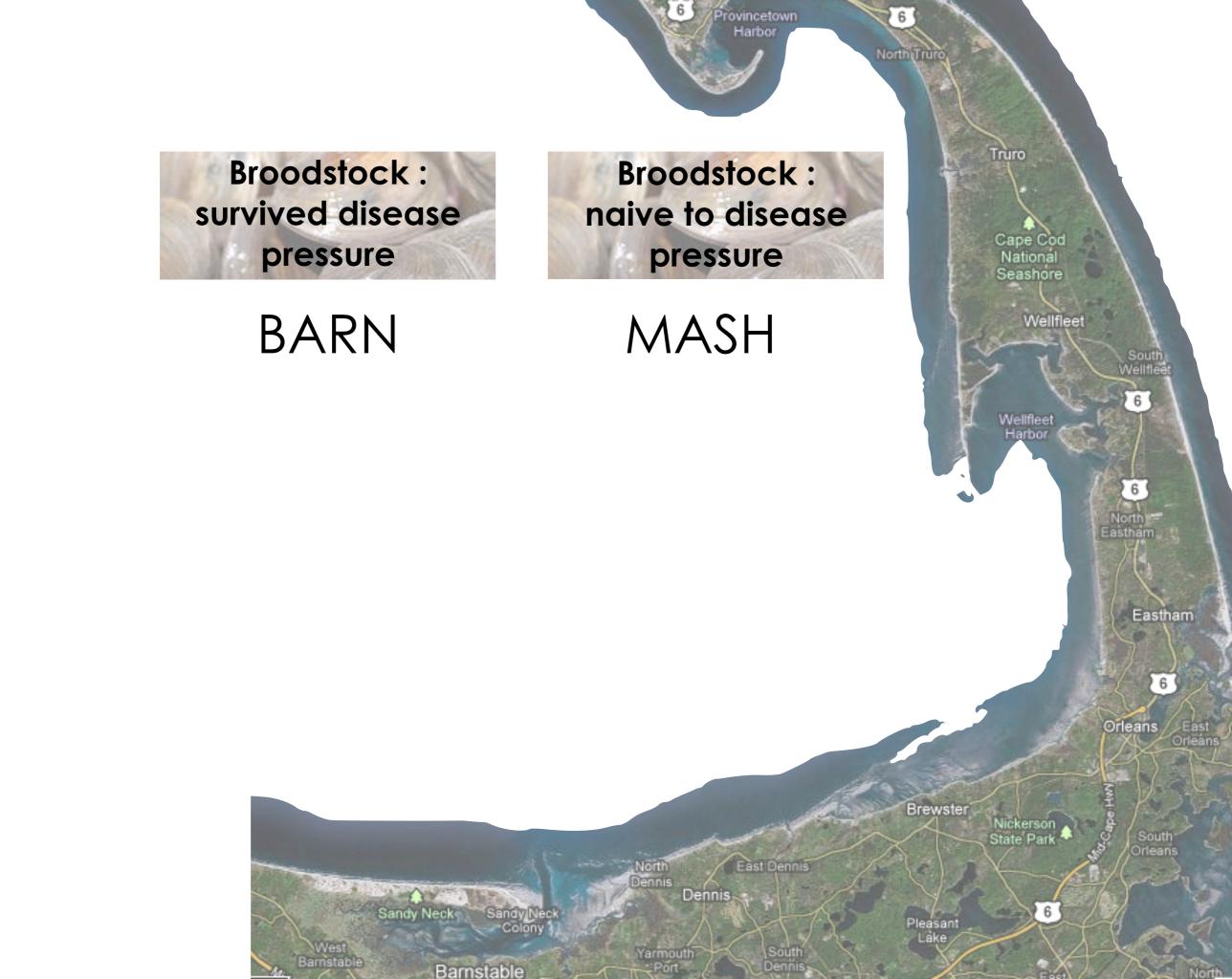
- Currently do not have a complete picture
  - Different across strains
- Is it induced upon recognition of QPX?
  - Upon QPX expression of protease?
- Difference in sequence?
- What is the role temperature?

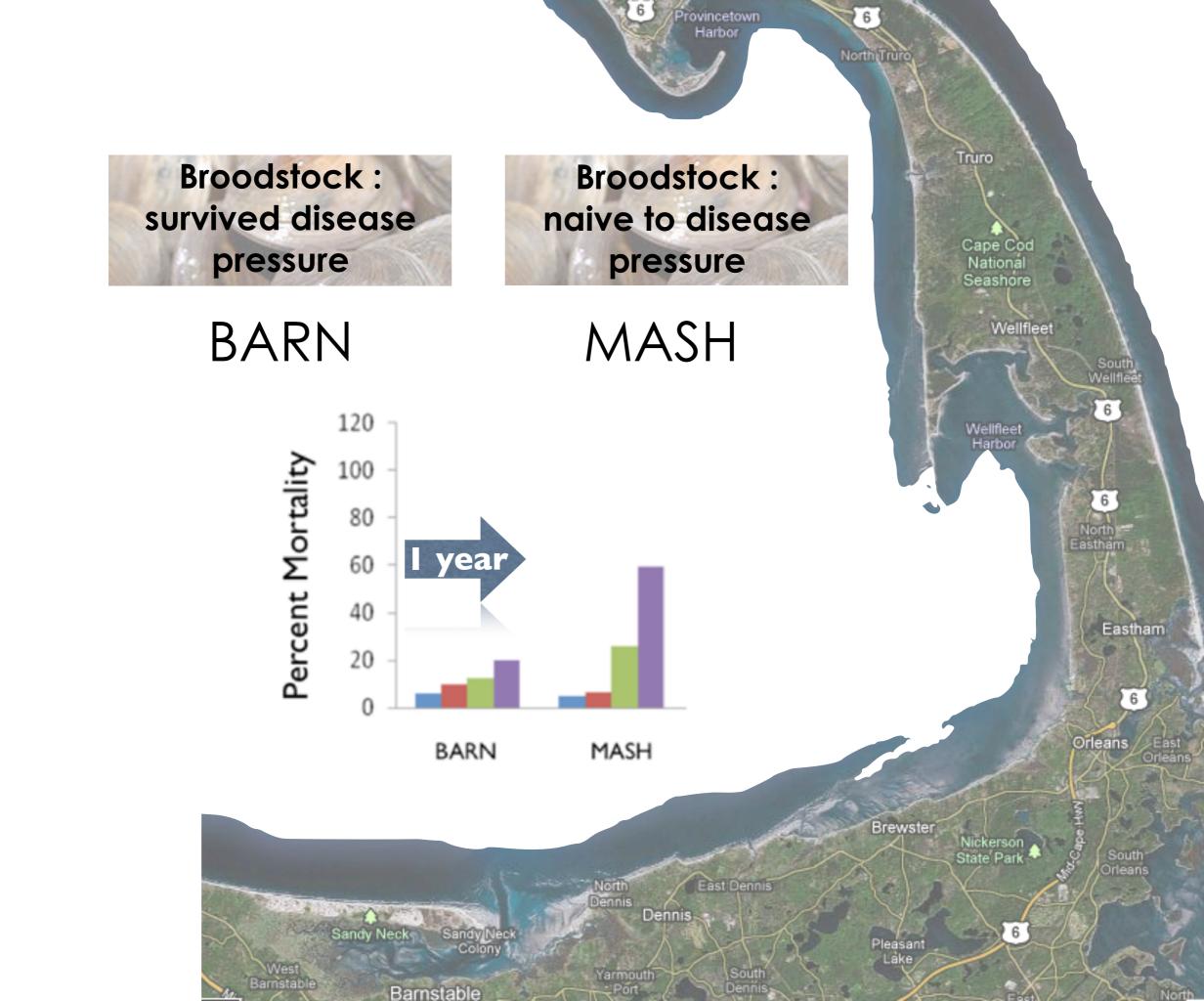
# Summary

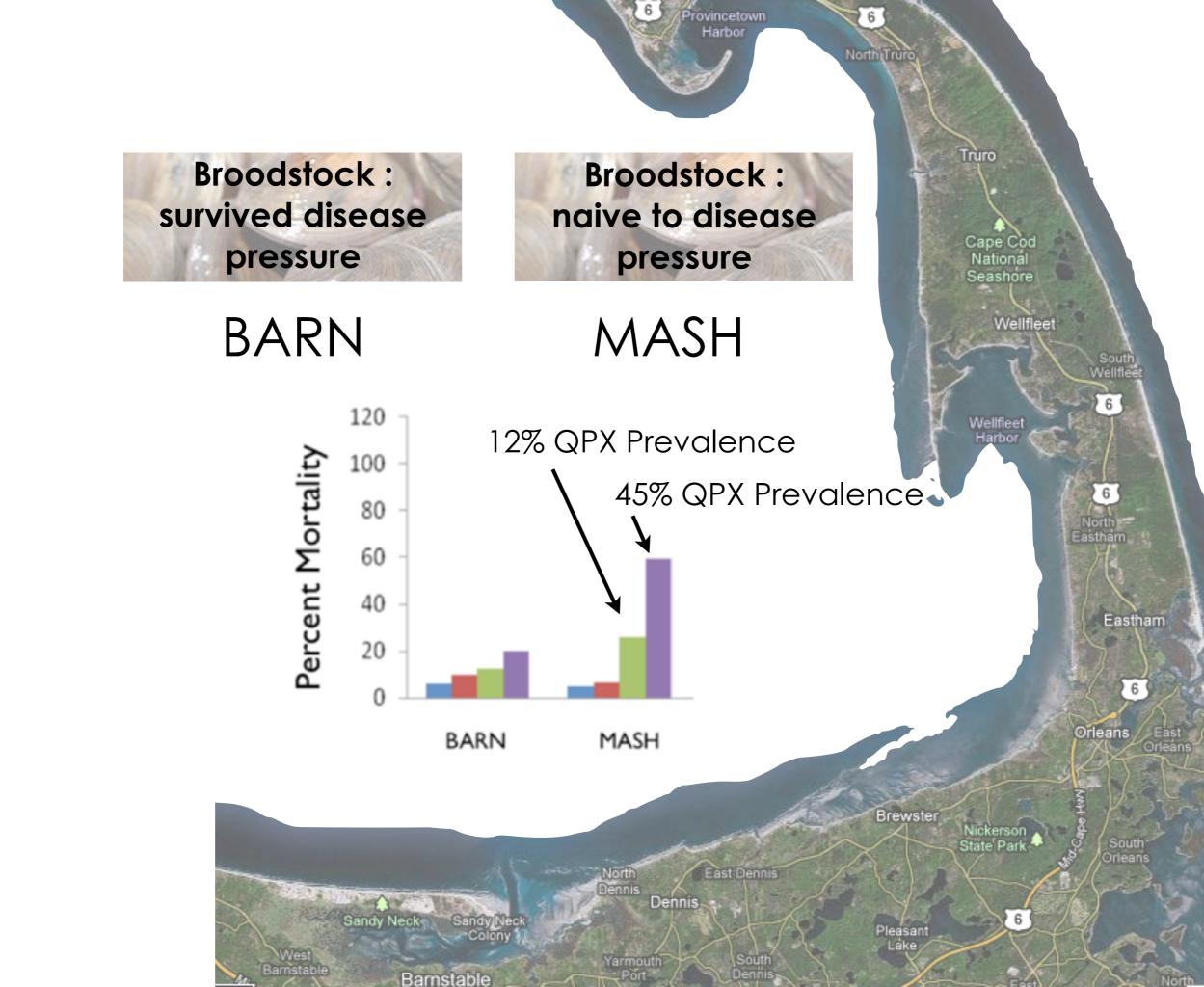
- Difficulty in identifying homologus sequences
- Serine Protease Inhibitor is highly regulated and likely associated with proper immune function
  - Expression pattern could be developed as a marker

### Disease tolerance

QPX and Mercenaria mercenaria



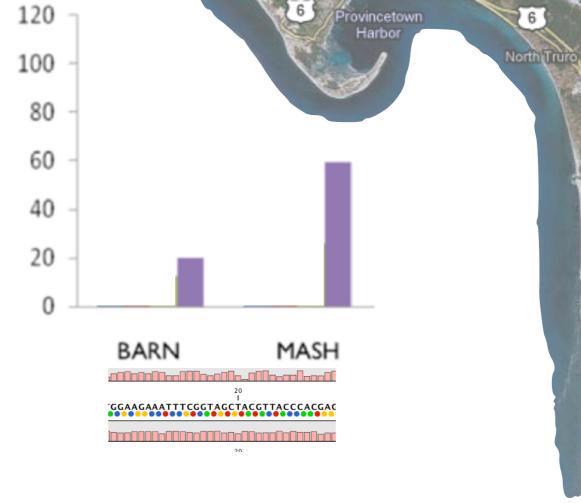




## RNA-Seq

8482 contigs

Percent Mortality 100 million reads



Truro

Cape Cod National

Seashore

Wellfleet Harbor

Wellfleet

South Wellfleet

6

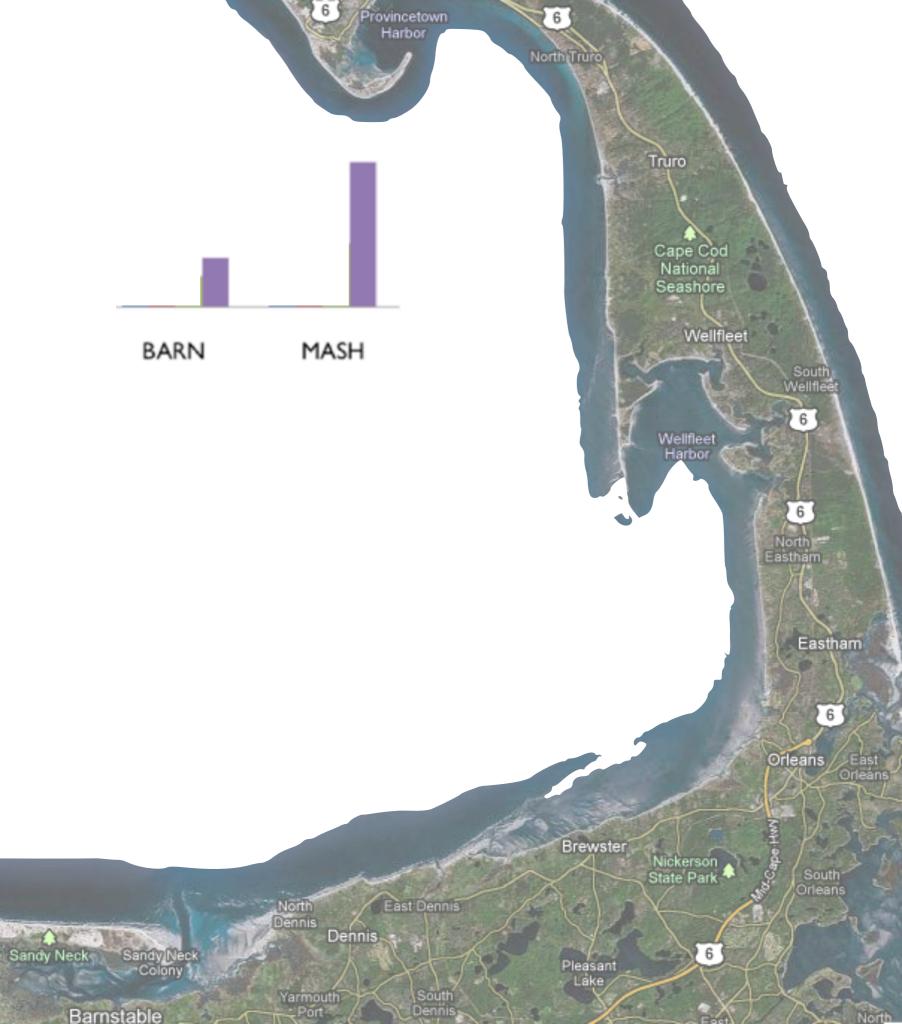
6

North Eastham

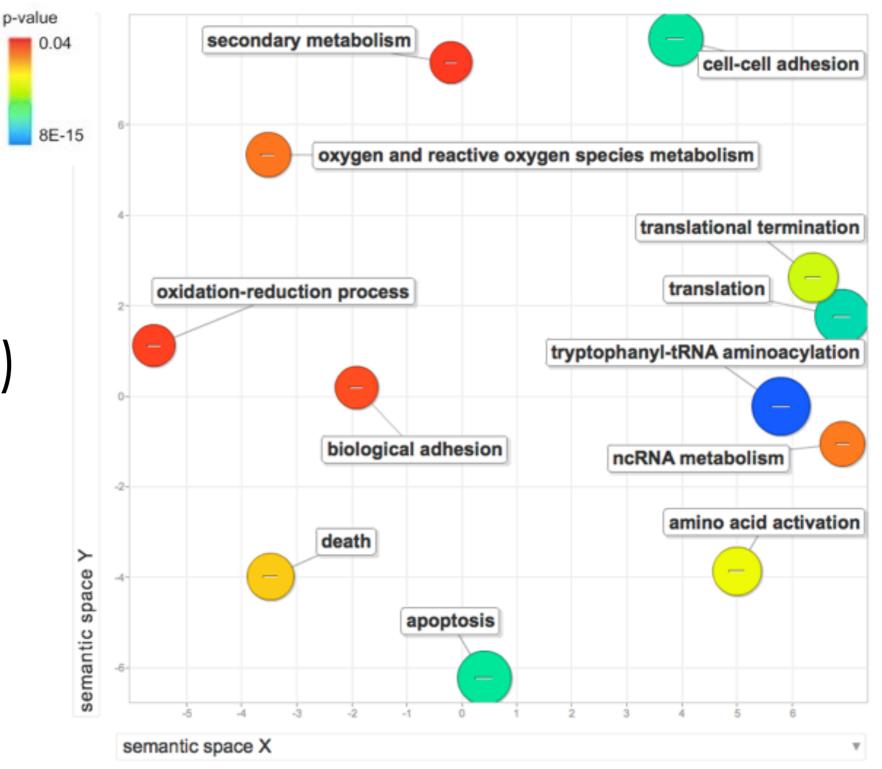


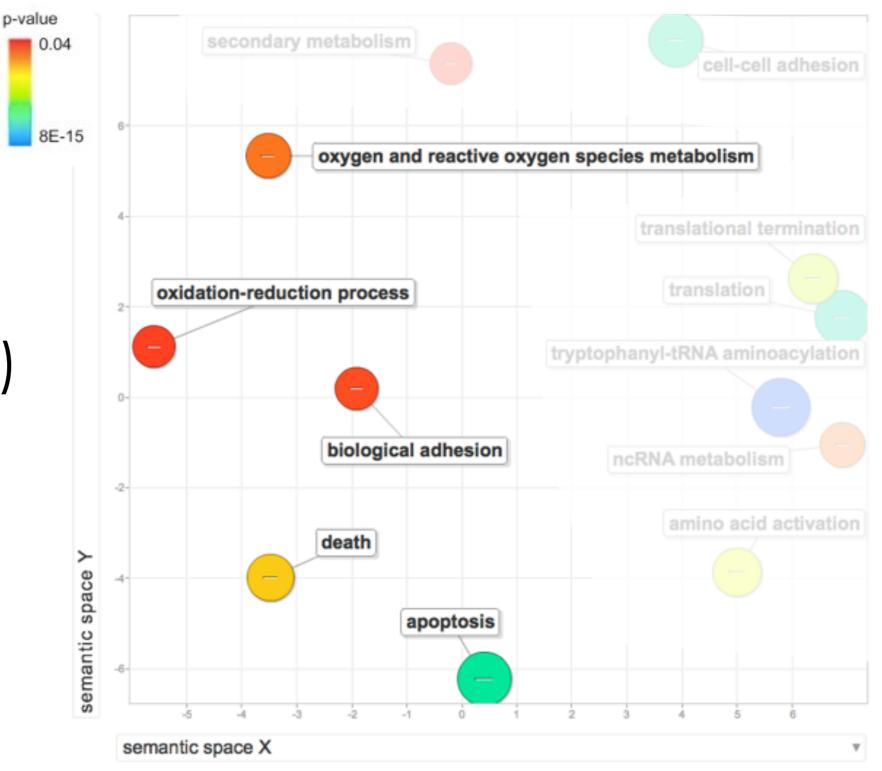
### RNA-Seq

684 DEGs 459 higher in BARN (superior)









#### apoptosis

baculoviral IAP repeat-containing protein, apoptosis 1 inhibitor, TNF receptorassociated factor 3, protein FADD **adhesion** 

neuroglian, protocadherin **oxidation reduction processes** 

peroxidasin, thyroid peroxidase, epidermis-type lipoxygenase, hydroxysteroid 11-beta-dehydrogenase, carbonyl reductase, cytochrome P450, superoxide dismutase, sorbitol dehydrogenase

#### translation

asparaginyl-tRNA synthetase, eukaryotic initiation factor, tryptophanyl-tRNA synthetase, eukaryotic peptide chain release factor subunit



Broodstock : naive to disease pressure

BARN



- Restriction Enzyme Assisted Digestion
- -Sequencing



Broodstock : naive to disease pressure

BARN

### MASH

Restriction Enzyme Reduce Representation Assisted Digestion

Sequencing



Broodstock : naive to disease pressure

BARN



Restriction Enzyme Reduce Representation Assisted Digestion

Sequencing

Sequence multiple individuals



Broodstock : naive to disease pressure

BARN



Restriction Enzyme Reduce Representation Assisted Digestion

Sequencing Sequence multiple individuals

### 145 Diagnostic Markers