

Integrating ‘Omics, Aquaculture, and Parasitology to Investigate Eastern Oyster Immunity to Disease

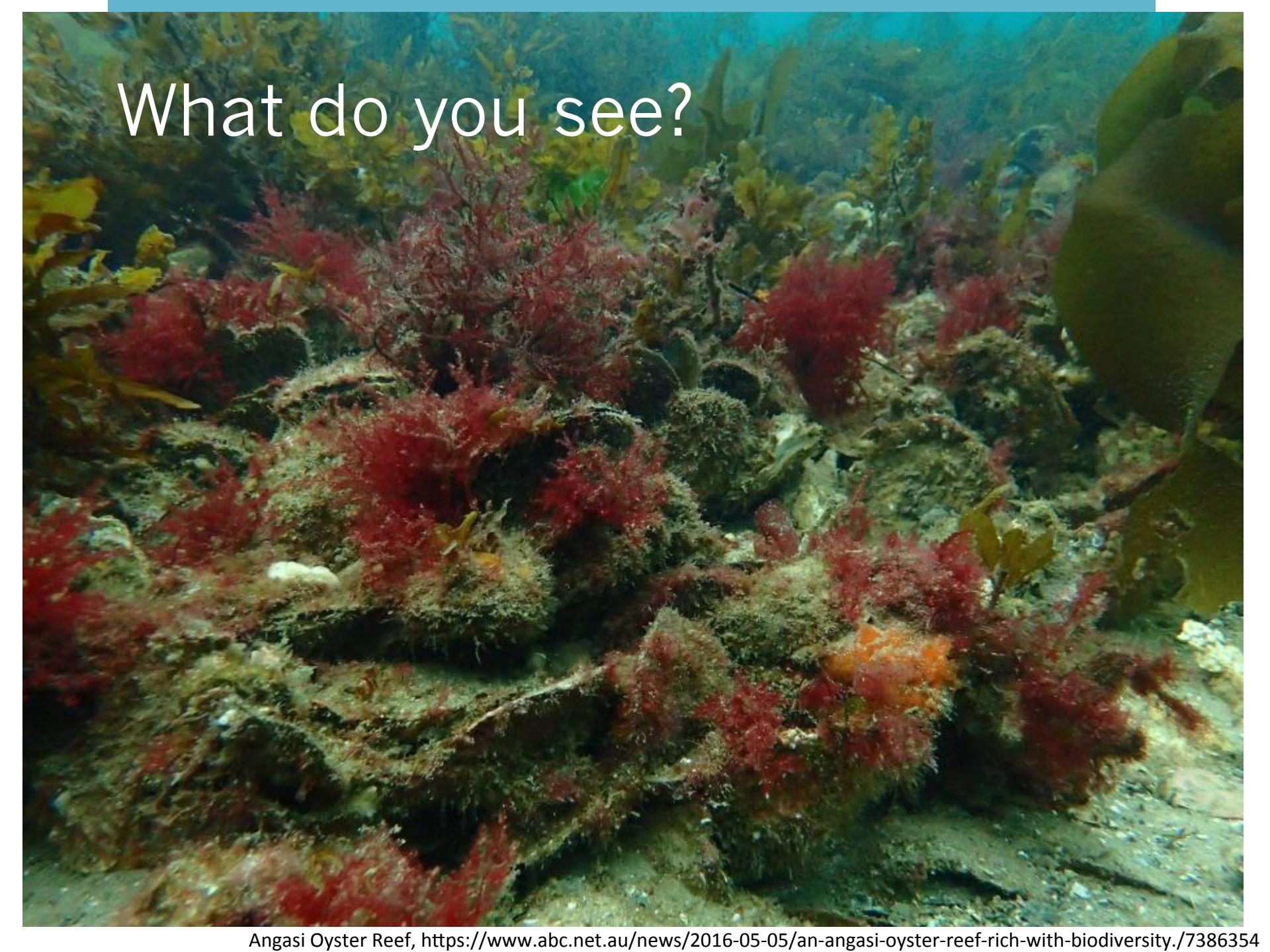
Erin M. Roberts, Ph.D. Candidate

Marta Gomez-Chiarri Lab, University of Rhode Island

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UNIVERSITY
OF RHODE ISLAND



USDA

The background image shows an underwater landscape of a rocky reef. The rocks are heavily covered in various types of marine life, including bright red, branching corals or anemones and patches of green, leafy algae. In the upper right corner, a large, dark, leafy seaweed plant is visible. The water is slightly hazy, typical of an underwater environment.

What do you see?

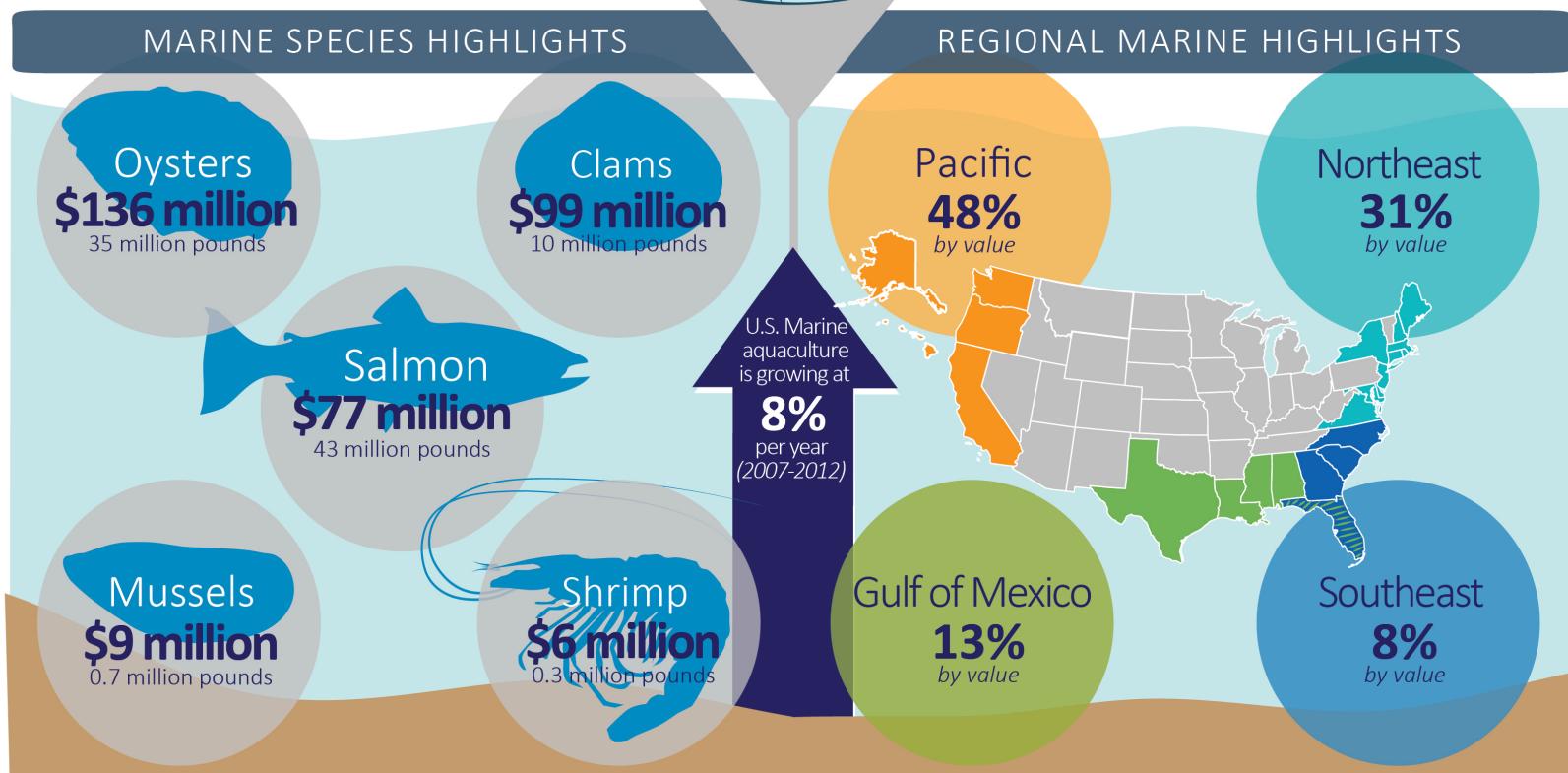
\$136 million production value in 2012



East Beach Oyster Co., Ninigret Pond, RI; East Beach Farm, Quonochontaug Pond, RI

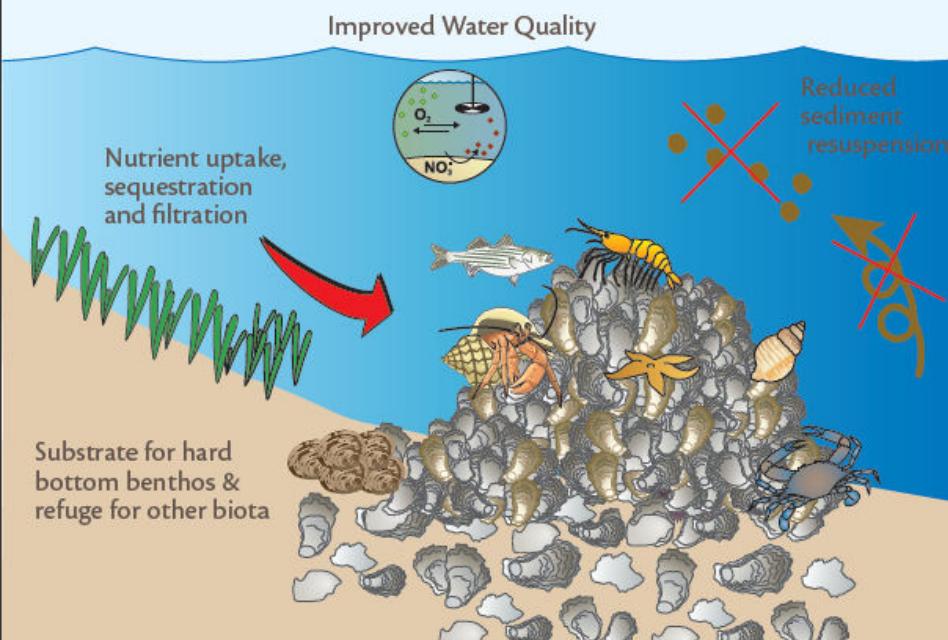
Largest aquaculture value in U.S.

2012 AQUACULTURE PRODUCTION VALUES

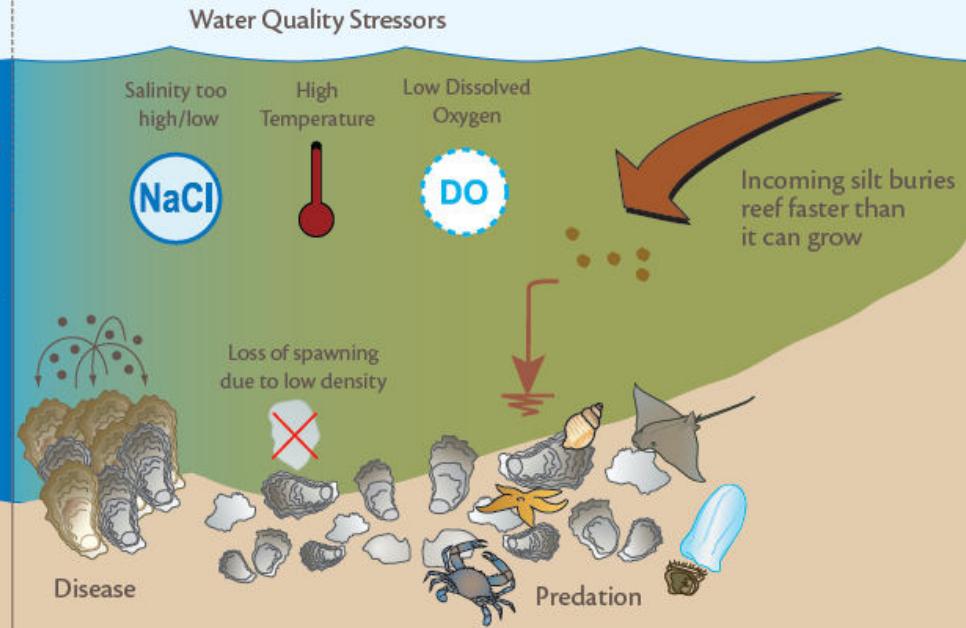


Ecosystem Benefits and Stressors

Ecosystem benefits provided by Oysters

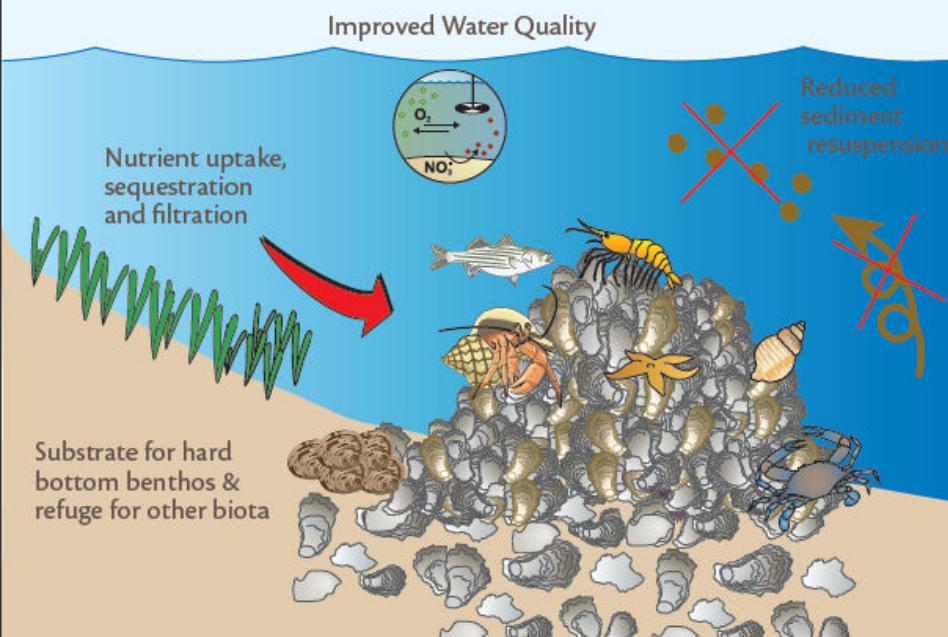


Ecosystem stressors to Oysters

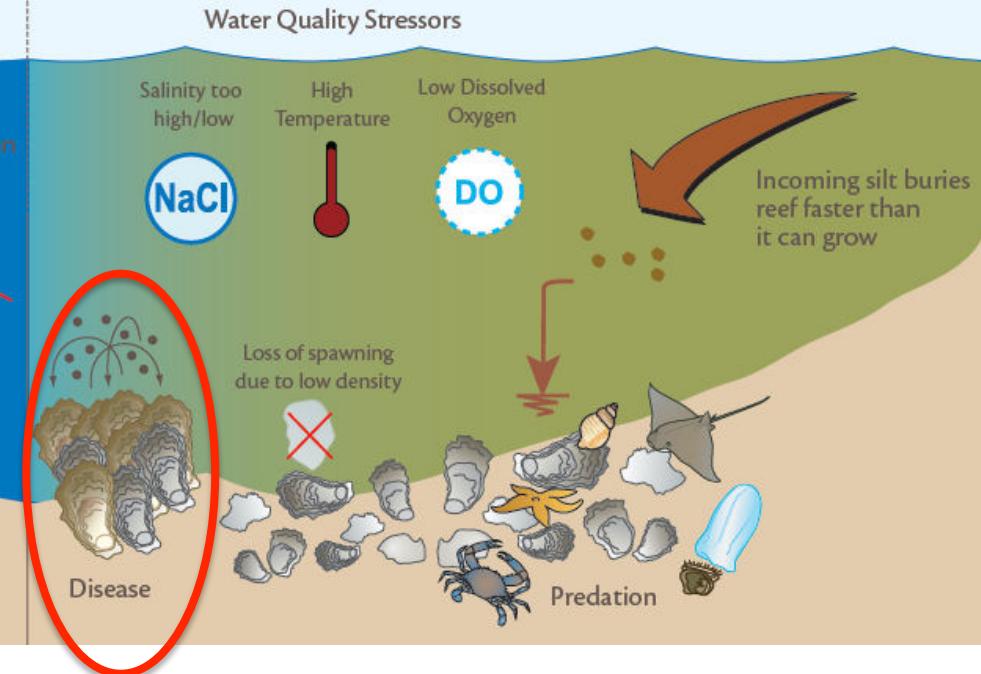


Ecosystem Benefits and Stressors

Ecosystem benefits provided by Oysters

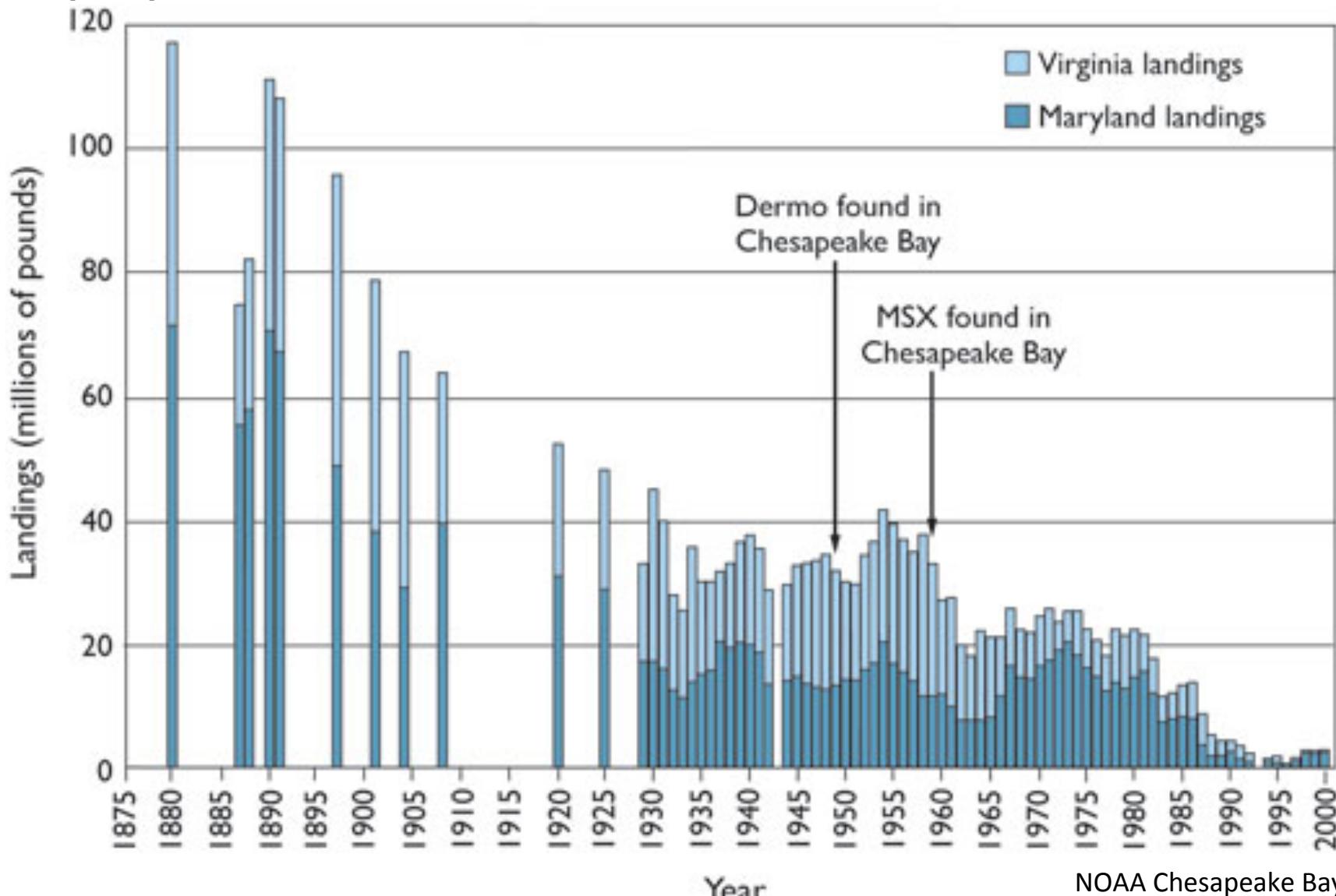


Ecosystem stressors to Oysters



Disease

Disease outbreaks have caused massive population declines



Gaps in Knowledge

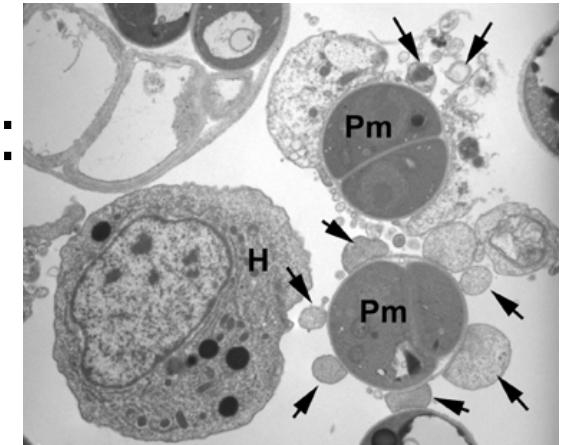
Oysters have evolved to survive in an environment surrounded by diverse bacterial and pathogens. How are they able to fight or adapt to these diverse stressors across their wide range?

Research applications

If we can understand what mechanisms oysters use to adapt to their dynamic and stressful environment we may be able to design better disease mitigation strategies (i.e. selective breeding)

Oysters Fight Disease Using Complex Innate Immune System

- Lack adaptive immunity of vertebrates
- Physical barriers: shell, mucus
- First responses: inflammation and humoral defenses
- Cellular defenses by Hemocytes: recognition, signaling, phagocytosis, killing

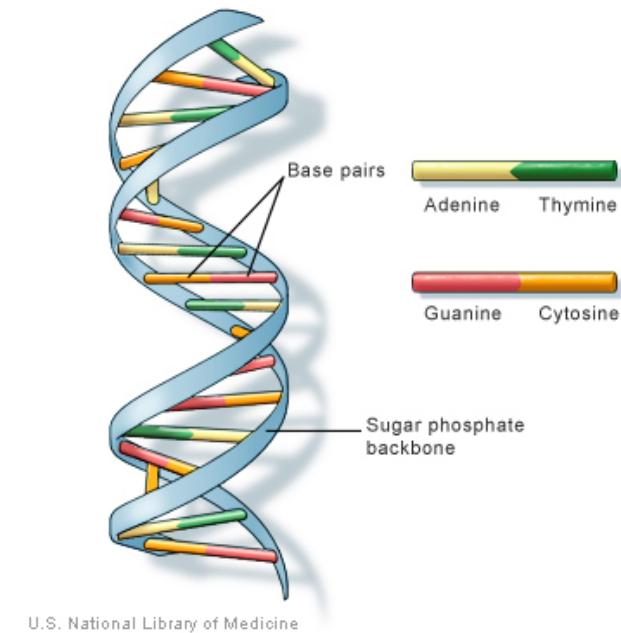
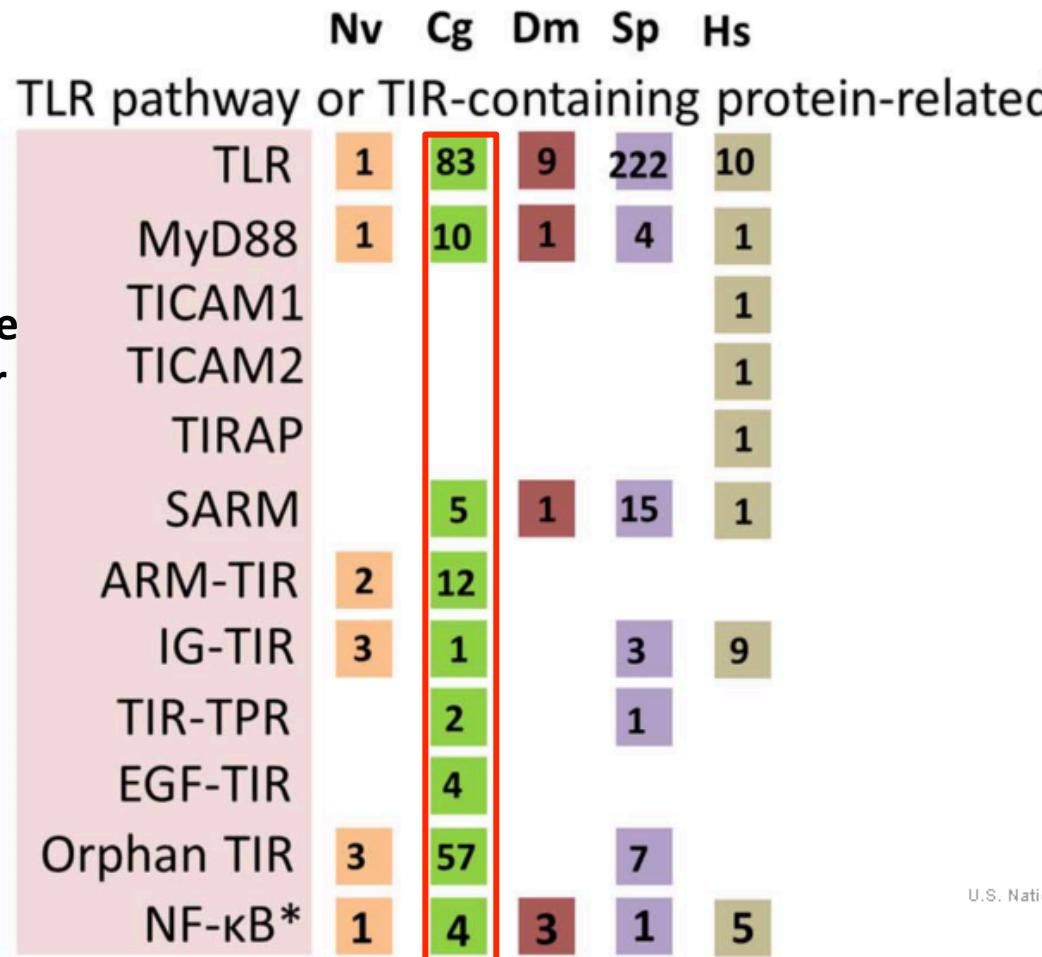


Apoptosis of *C. virginica* hemocytes infected with *Perkinsus marinus*¹

Innate Immunity is Expanded in Oysters

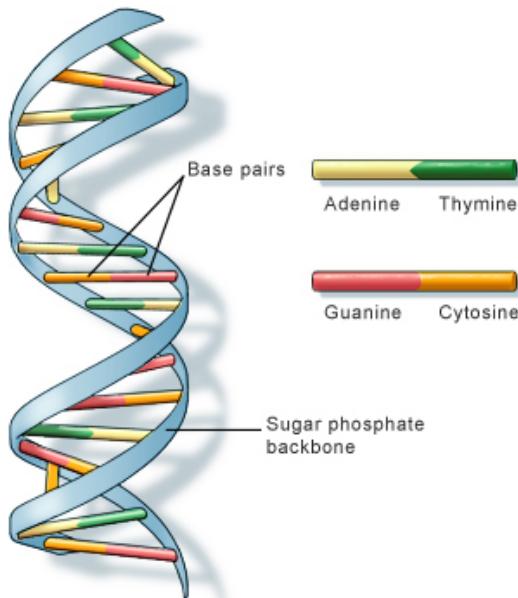
**Expansion =
more copies of
a gene in the
genome**

Nv= Sea anemone
Cg= Pacific oyster
Dm = fruit fly
Sp = Sea urchin
Hs= human



U.S. National Library of Medicine

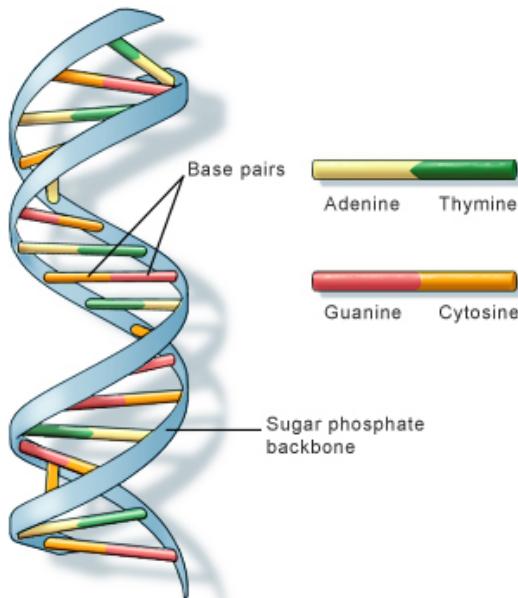
Innate Immunity is Expanded in Oysters



U.S. National Library of Medicine

What could happen if an organism has more copies of a gene?

Innate Immunity is Expanded in Oysters

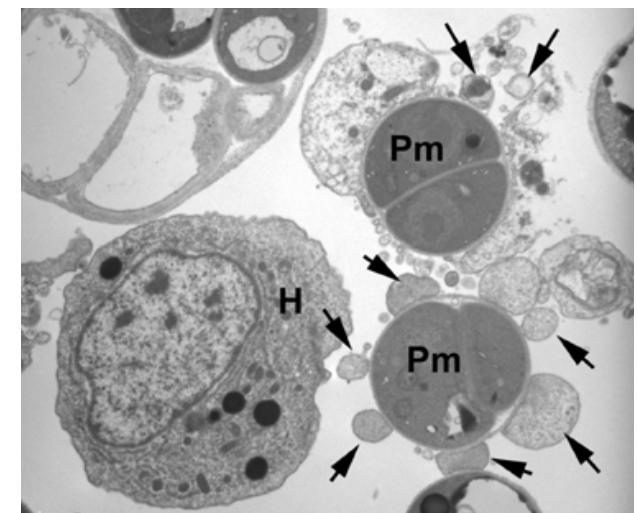


U.S. National Library of Medicine

What could happen if an organism has more copies of a gene?

Apoptosis is Critical in Disease Response and **Involves Expanded Genes**

- Apoptosis in oysters critical for tissue homeostasis and disease response¹
- Involves expanded gene families¹
GIMAP, IAP, TLR²



Apoptosis of *C. virginica* hemocytes infected with *Perkinsus marinus*³

¹Romero et al., 2015,

²McDowell et al., 2016,

³Sokolova, 2009

My targeted question to address larger knowledge gap

Does expansion of apoptosis immune gene families help oysters tailor their apoptotic response to diverse diseases to increase fitness?

Data I need in order to address this question

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Genetic data on the apoptosis gene families present in oysters

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What is the full list of apoptosis genes in oysters, which families are expanded, and how many copies do they have?

Genetic data on the apoptosis gene families present in oysters

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Gene expression data and apoptosis phenotype data demonstrating differing apoptotic responses following challenge with different types of pathogens

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Genetic data on the apoptosis gene families present in oysters

Investigation of connection between survival and specific apoptosis expression pattern

Gene expression data and apoptosis phenotype data demonstrating differing apoptotic responses following challenge with different types of pathogens

Experiment 1: Exploratory Study Design

Using Previously obtained data

Aim 1

Apoptosis Genes and
Transcripts in Pacific
and eastern oyster

- ↓
- 1. Apoptosis Literature Search
- 2. Find genomic location and number of annotated apoptosis pathway genes in genome¹
- 3. Manual annotation

Aim 2

Investigate patterns of apoptosis gene family expression following challenge

- ↓
- 1. Viral
- 2. Pathogenic bacterial
- 3. Probiotic

Aim 3

Analyze expression of expanded apoptosis gene families

¹Gomez-Chiarri et al., 2015

Aim 1: Using command line tools to find annotated apoptosis genes

- Identified gene names via literature search
- Found list of gene aliases using UniProt (<https://www.uniprot.org>)
- Performed text search in the genome annotation file (listing all annotated genes and proteins) for aliases
- Use Bash language and “grep” command, identifies lines in a file where the group of characters is found together

Aim 1: Using command line tools to find annotated apoptosis genes

```
NC_035788.1      Gnomon  exon    647219  649471 .       +     .       ID=id635586;Parent=rna56939;Dbxref=GeneID:111115463,Gen  
bank:XM_022454215.1;gbkey=mRNA;gene=LOC111115463;product=caspase-7-like;transcript_id=XM_022454215.1  
NC_035788.1      Gnomon  CDS     647219  647484 .       +     2       ID=cds51389;Parent=rna56939;Dbxref=GeneID:111115463,Gen  
bank:XP_022309923.1;Name=XP_022309923.1;gbkey=CDS;gene=LOC111115463;product=caspase-7-like;protein_id=XP_022309923.1  
NC_035788.1      Gnomon  mRNA    2488919 2498818 .       -     .       ID=rna57113;Parent=gene33136;Dbxref=GeneID:111110874,Ge  
nbank:XM_022447524.1;Name=XM_022447524.1;gbkey=mRNA;gene=LOC111110874;model_evidence=Supporting evidence includes similarity to  
: 2 Proteins%2C and 100%25 coverage of the annotated genomic feature by RNAseq alignments%2C including 19 samples with support  
for all annotated introns;product=caspase-7-like;transcript_id=XM_022447524.1  
NC_035788.1      Gnomon  exon    2488919 2489638 .       -     .       ID=id637364;Parent=rna57113;Dbxref=GeneID:111110874,Gen  
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bank:XP_022303232.1;Name=XP_022303232.1;gbkey=CDS;gene=LOC111110874;product=caspase-7-like;protein_id=XP_022303232.1
```

Aim 1: Manual Annotation of Apoptosis Genes

- Manual annotation involves “checking” annotation software correctly annotated a present gene or didn’t incorrectly not annotate
- Command line tools used:
 - **tblastn**¹: used to find sequence similarities to known sequences
 - **TCOFFEE**²: performed sequence alignment between conserved protein domains and proteins in question
 - **HMMER**³: used to find similarities in domain structure to identify protein families

The screenshot shows the NCBI BLAST homepage. At the top, there's a navigation bar with the NIH logo, U.S. National Library of Medicine, NCBI, and a 'Sign in to NCBI' link. Below this, the 'BLAST®' logo is prominently displayed. A 'NEWS' sidebar on the left has a 'QuickBLASTP webinar video' section with a link to YouTube and a date (Tue, 16 Jan 2018 09:00:00 EST). The main content area is titled 'Basic Local Alignment Search Tool' and describes BLAST as finding regions of similarity between biological sequences. It highlights 'Nucleotide BLAST' (nucleotide > nucleotide), 'blastx' (translated nucleotide > protein), 'tblastn' (protein > translated nucleotide), and 'Protein BLAST' (protein > protein).

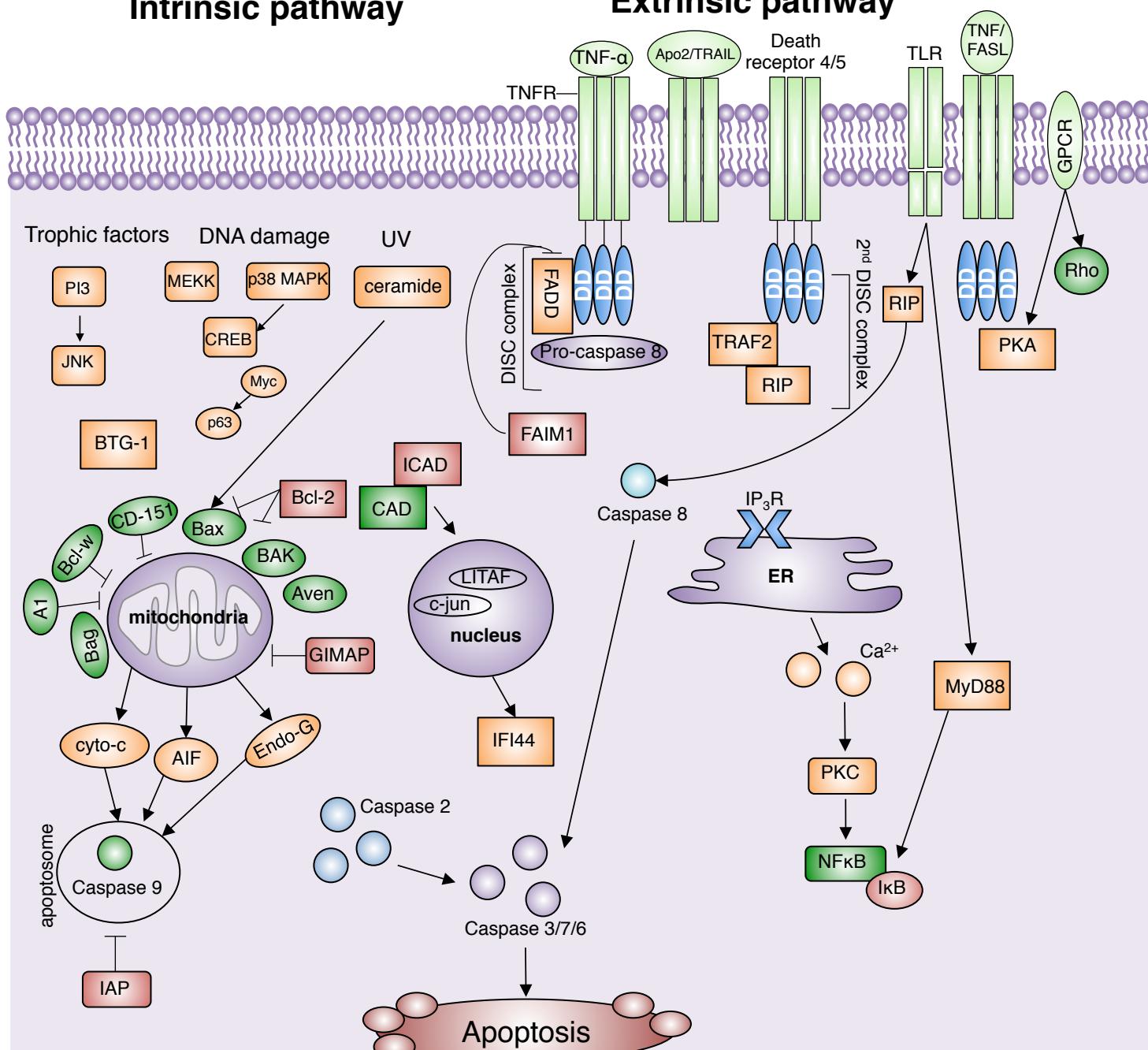
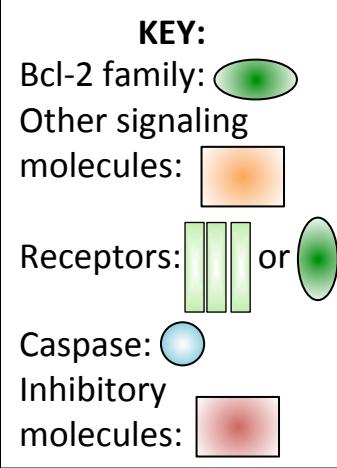


¹Altschul, 1990; ² Pais et al., 2014; ³Eddy 2010

Critical Apoptosis Molecules Annotated in *C. virginica* Genome

Intrinsic pathway

Extrinsic pathway



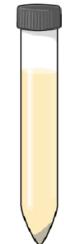
Manual Annotation

Protein Name	Apo2L/TRAIL (TNFSF10)	p63 (p53 homolog)	BAK (Bcl-2 antagonist killer)
Annotated in C. gigas?	NO	YES	NO
tblastn query seq. to C. vir genome	sp P50591.1 TNF10_HUMAN RecName: Full=Tumor necrosis factor ligand superfamily member 10	CAJ85664.2 p53-like protein [Crassostrea gigas]	>NP_001179.1 bcl-2 homologous antagonist/killer [Homo sapiens]
tblastn top hit	PREDICTED: Crassostrea virginica uncharacterized (LOC111133752), transcript variant X2	PREDICTED: Crassostrea virginica tumor protein 63-like (LOC111133472)	PREDICTED: Crassostrea virginica uncharacterized LOC111105030
tblastn Eval	9e-17	5e-50	5e-35
%identity	27%	40%	35%
SMARTBLAS T of C. vir. tophit	Top hit for all TNFSF10	Top hits for all p63	Bcl2 Antagonist Killer as top hit for Mus musculus (Eval=1e-35, %Iden=38%), Homo sapiens (Eval=3e-34, %Iden=40%)
HMMER tophit	Same as tblastn		XP_022291806.1 apoptosis regulator BAX-like isoform X2 [Crassostrea virginica]
TCOFFEE aln.	AVERAGE	GOOD	GOOD
Presence of Functional domains	TNF(tumor Necrosis Factor) family, absence of death domain	P53 DNA-binding domain, SAM domain of tumor-p63, P53 tetramerisation motif P53 transactivation motif	Bcl-2_like super family, missing BH-4
Presence	HIGHLY LIKELY	HIGHLY LIKELY	LIKELY

Aim 2: Obtain transcriptomes from challenge experiments

Pathogenic bacteria

LPS, *V. anguillarum*,
V. aestuarinus,
V. alginolyticus,
V. tubiashii,
*Micrococcus luteus*¹



C. gigas adult



Gill: 12h
→



A. crassostreae
CV919-312 (7.5×10^6 CFU ml⁻¹)²



C. virginica juvenile



Pooled
Tissue:
1,5,15d
→



GX: ROD Resistant⁵

F3L: ROD Susceptible⁵

Viral

OsHV-1μVar
(1.5×10^9 VGU)³



C. gigas juvenile

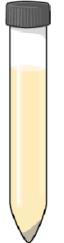


Gill:
6, 12, 24,
48,120hr
→



Probiotic

Bacillus pumilus
RI06-95 (10^4 colony forming
units (CFU)/ml)⁴



C. virginica larvae



Pooled
Larvae:
5, 12, 16d
→



¹Zhang L., 2015

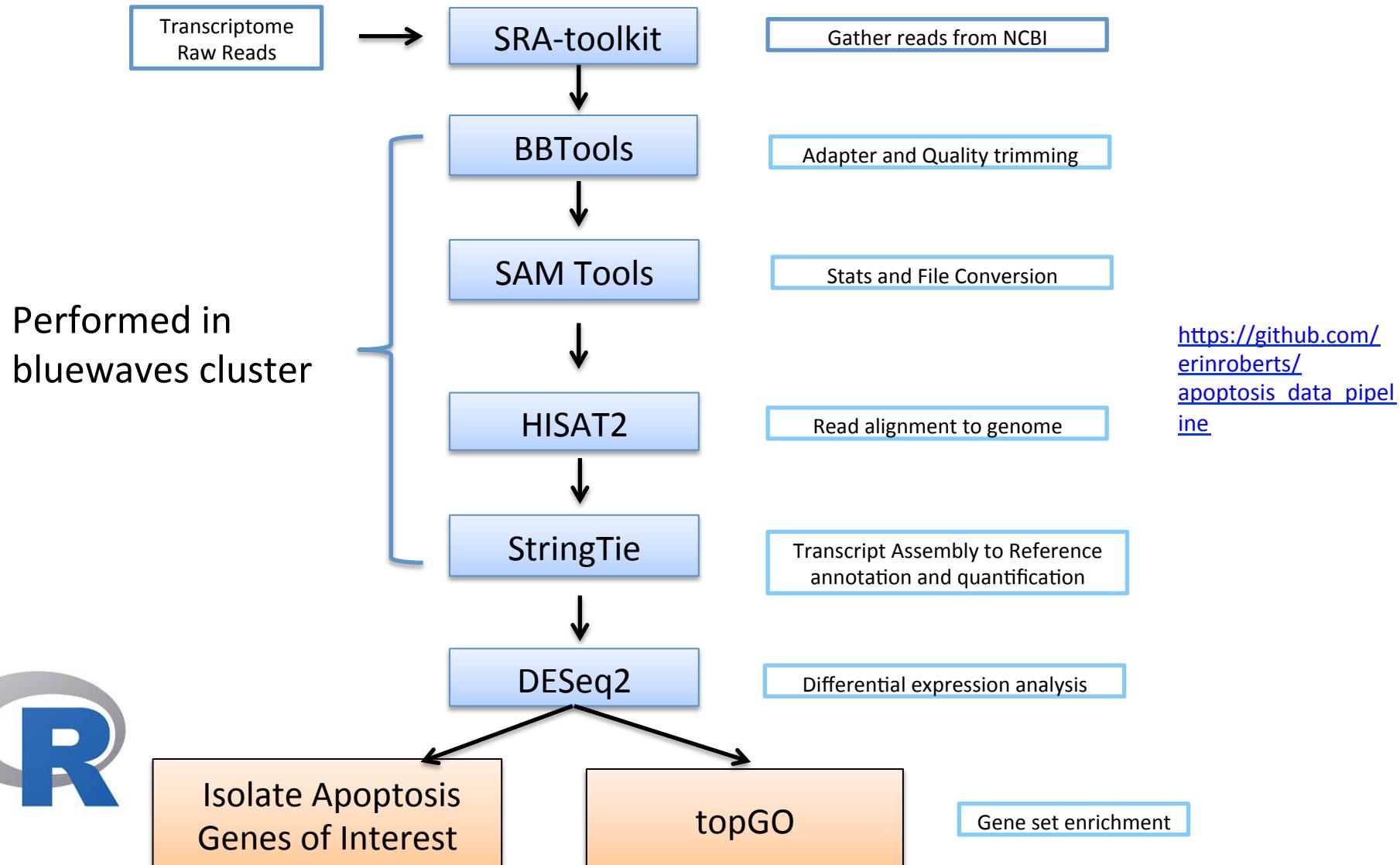
²McDowell et al., 2014

³He et al., 2015

⁴Tejasree Modak

⁵Haskins Shellfish Laboratory

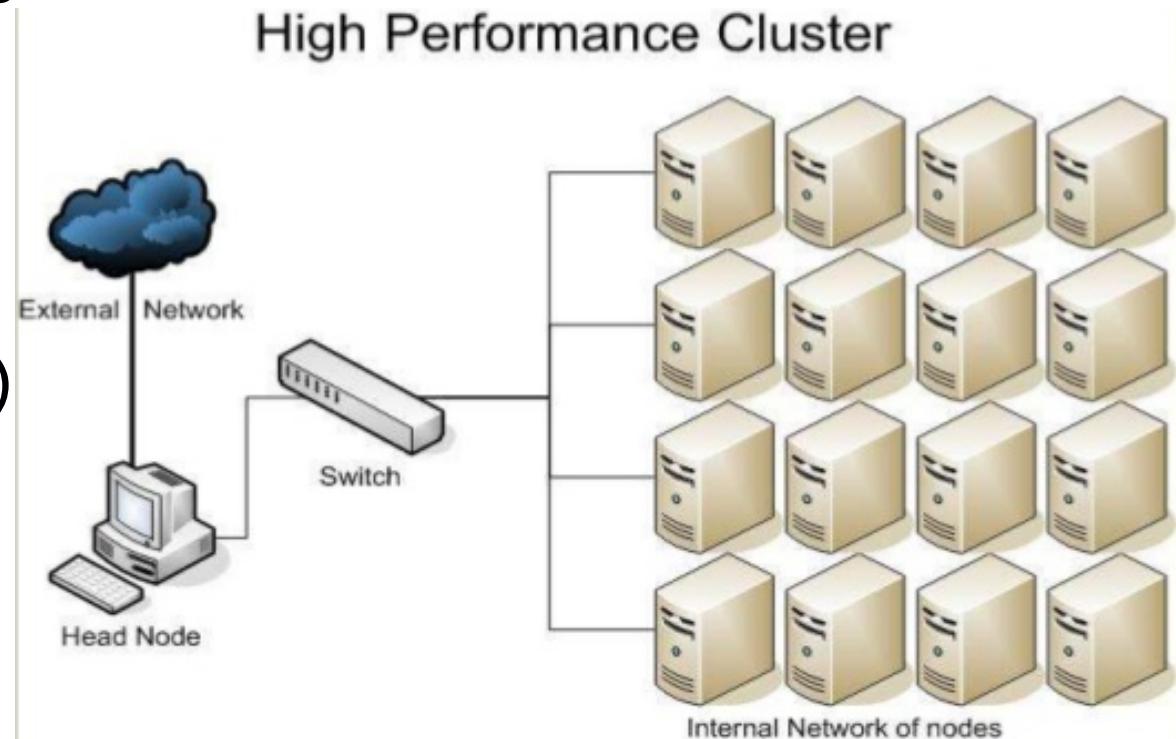
Aim 3: Analyze differential gene expression using command line and R tools



Using Cluster computing to wrangle big data



- 56 total transcriptomes processed
- Created bash scripts to run with SLURM (job scheduler) to complete transcriptome assembling processes





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erinroberts edited script 01

84e4a3d on Oct 3, 2017

1 contributor

Executable File 139 lines (110 sloc) | 5.89 KB

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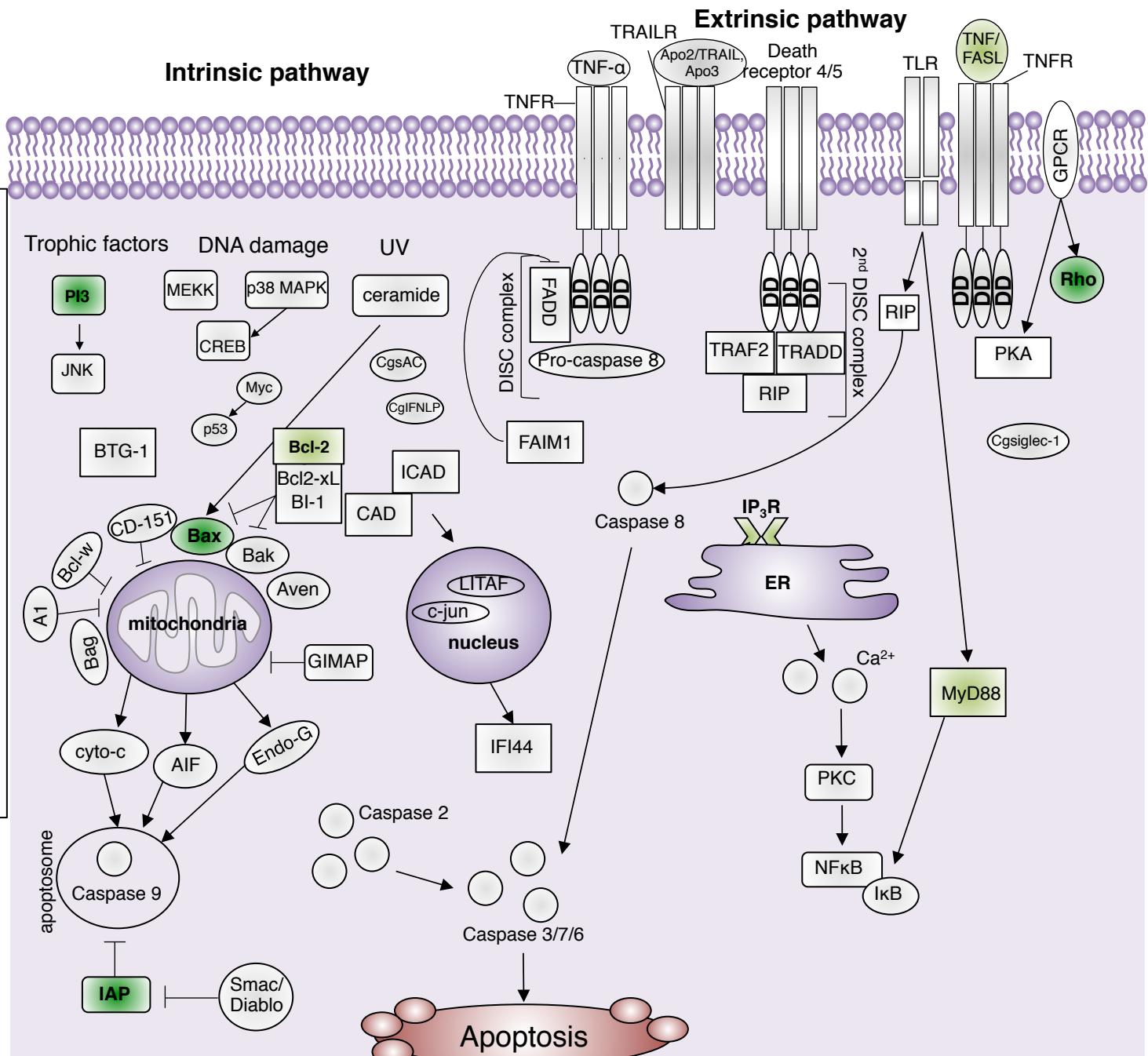
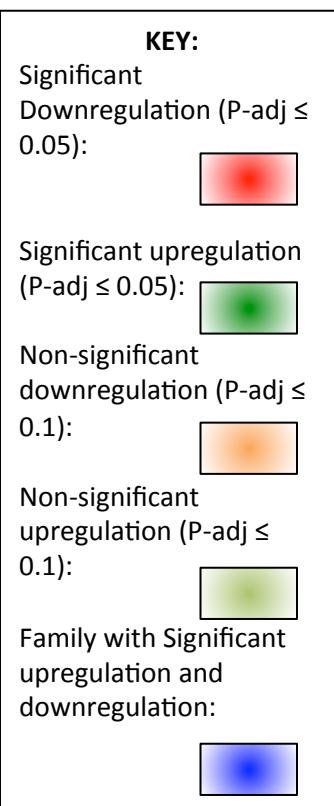
```
1 #!/bin/bash
2 #PBS-1 nodes=1
3 #PBS-1 walltime=1000:00:00
4 #PBS -j oe
5 #PBS -q default
6 #PBS -o out_BBtools_preprocessing2
7 #PBS -e err_BBtools_preprocessing2
8 #PBS -m ae -M erin_roberts@my.uri.edu
9
10
11 #01_C_Vir_Bac_Subset_Preprocessing. This script runs the preprocessing steps on raw SRA transcriptomes
12 # from GX3 and F3L Single end transcriptomes and UW transcriptomes with RI probiotic challenge. This script skips
13 # the step to use BBMerge to obtain the stats file about which adapters are present.
14
15 set -e
16 echo "START $(date)"
17 module load BBMap/37.36-foss-2016b-Java-1.8.0_131
18
```

Data Analysis using R language in R studio

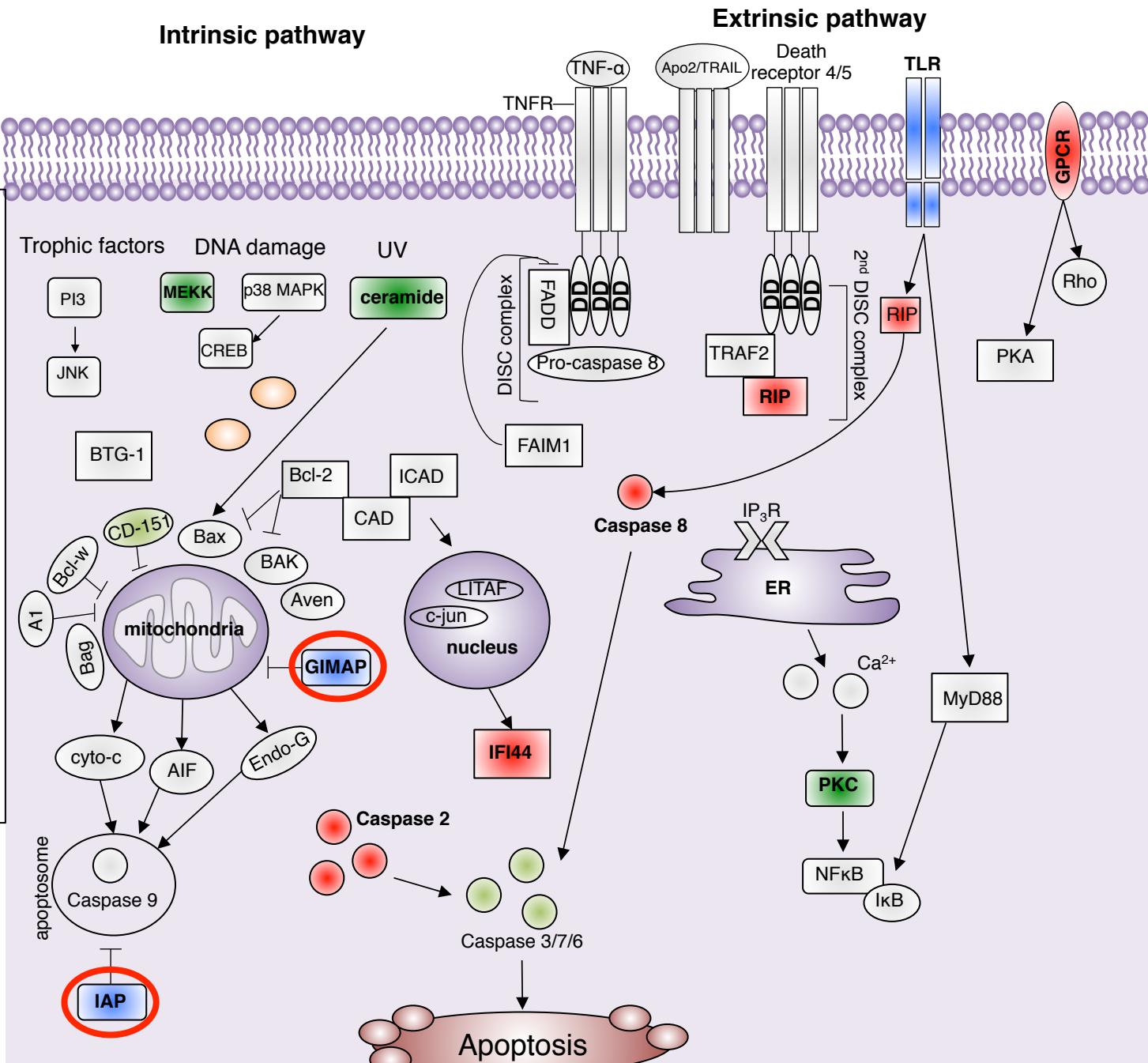
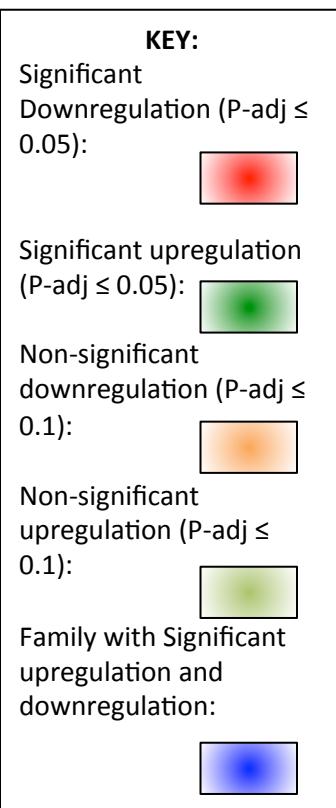
- Used the R computing language in R studio to perform all downstream processing
- Specialized R packages for analyzing differential gene expression and data visualization
 - DESeq2, topGO, ggplot2, pheatmap
- Identified apoptosis genes
- compared gene expression between challenges
- Graphing of data using “heatmaps”

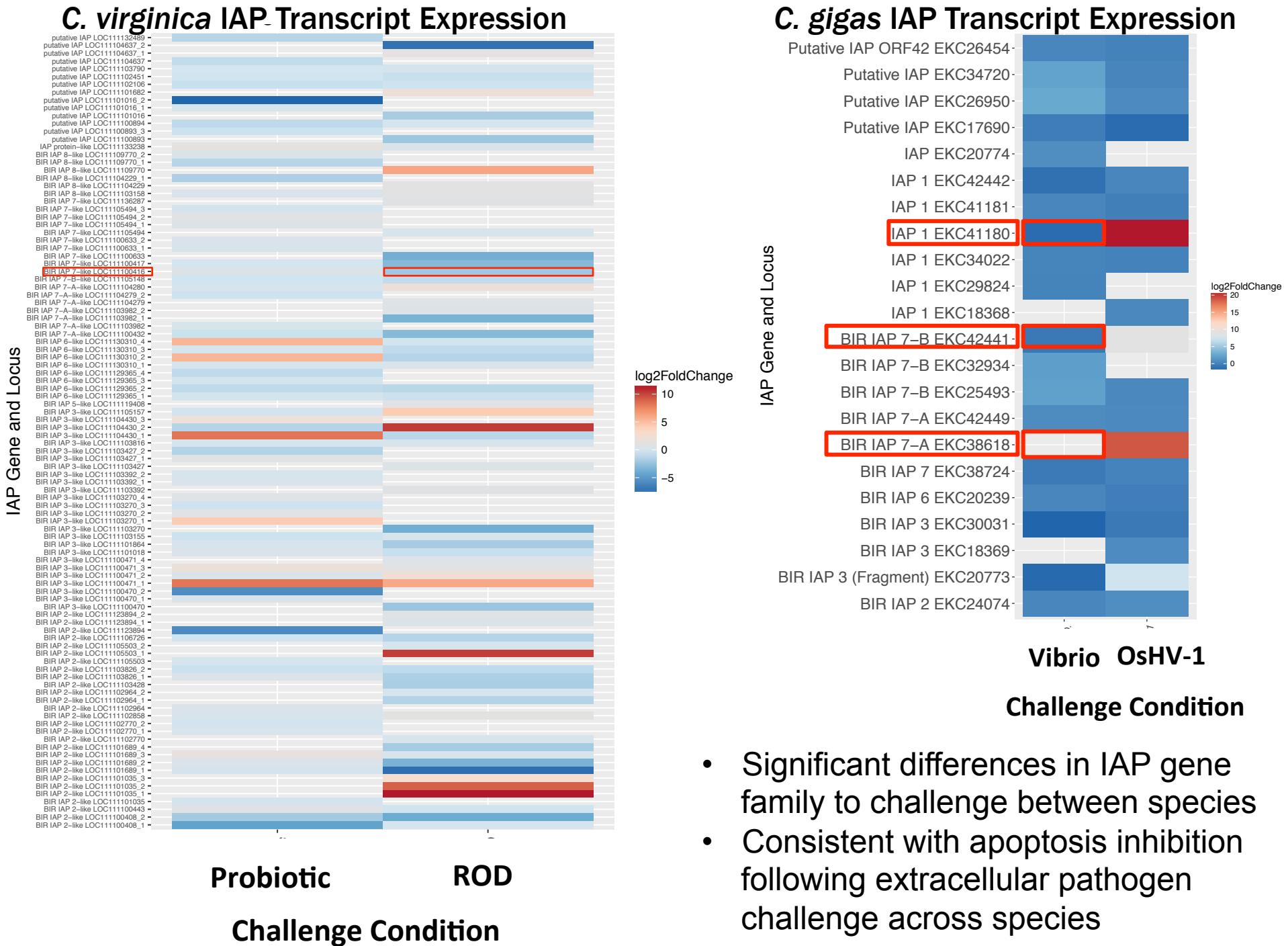


C. *gigas* Vibrio spp. challenge triggers inhibitory apoptotic response

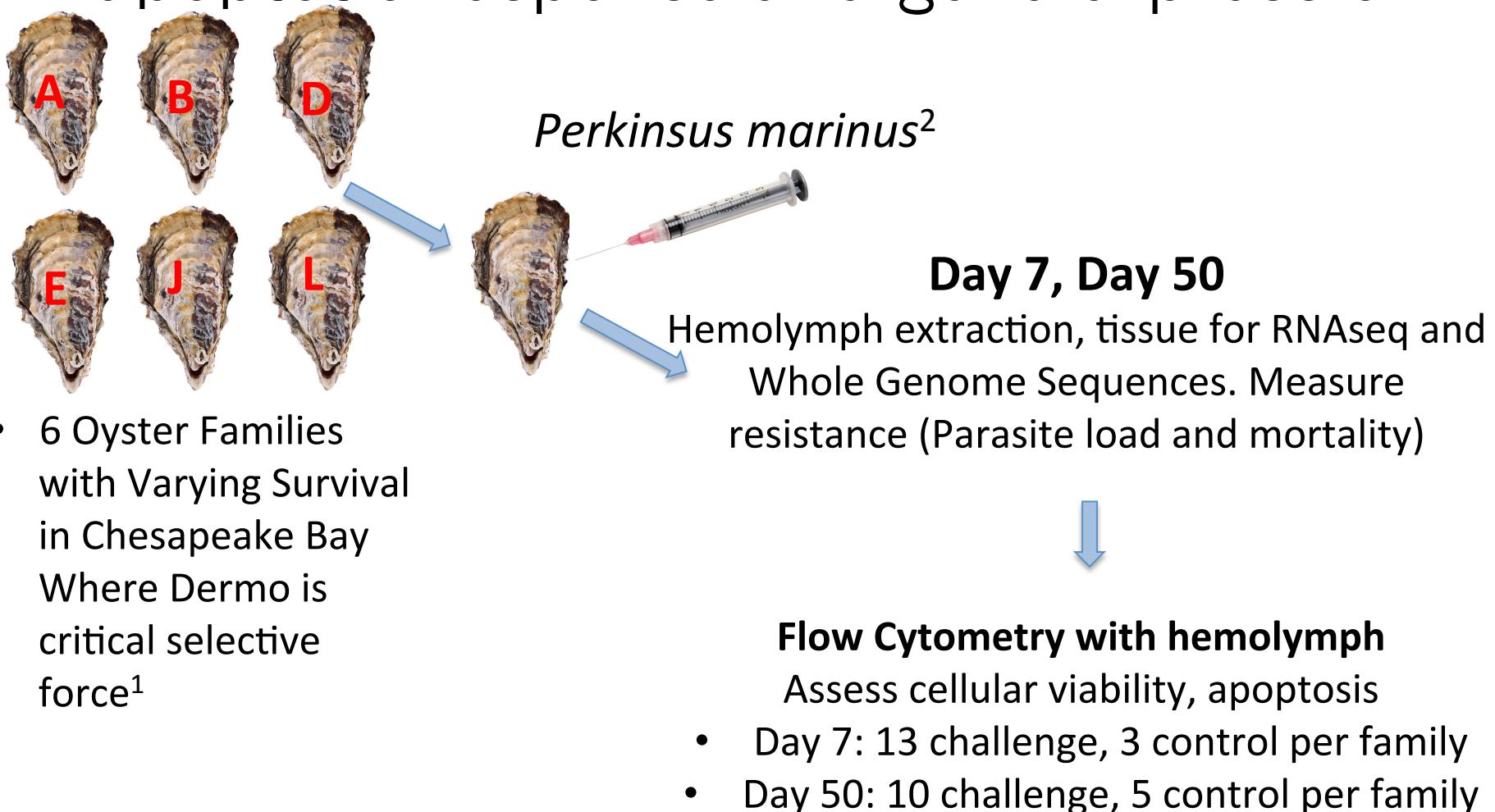


C. virginica ROD challenge triggers complex apoptotic response





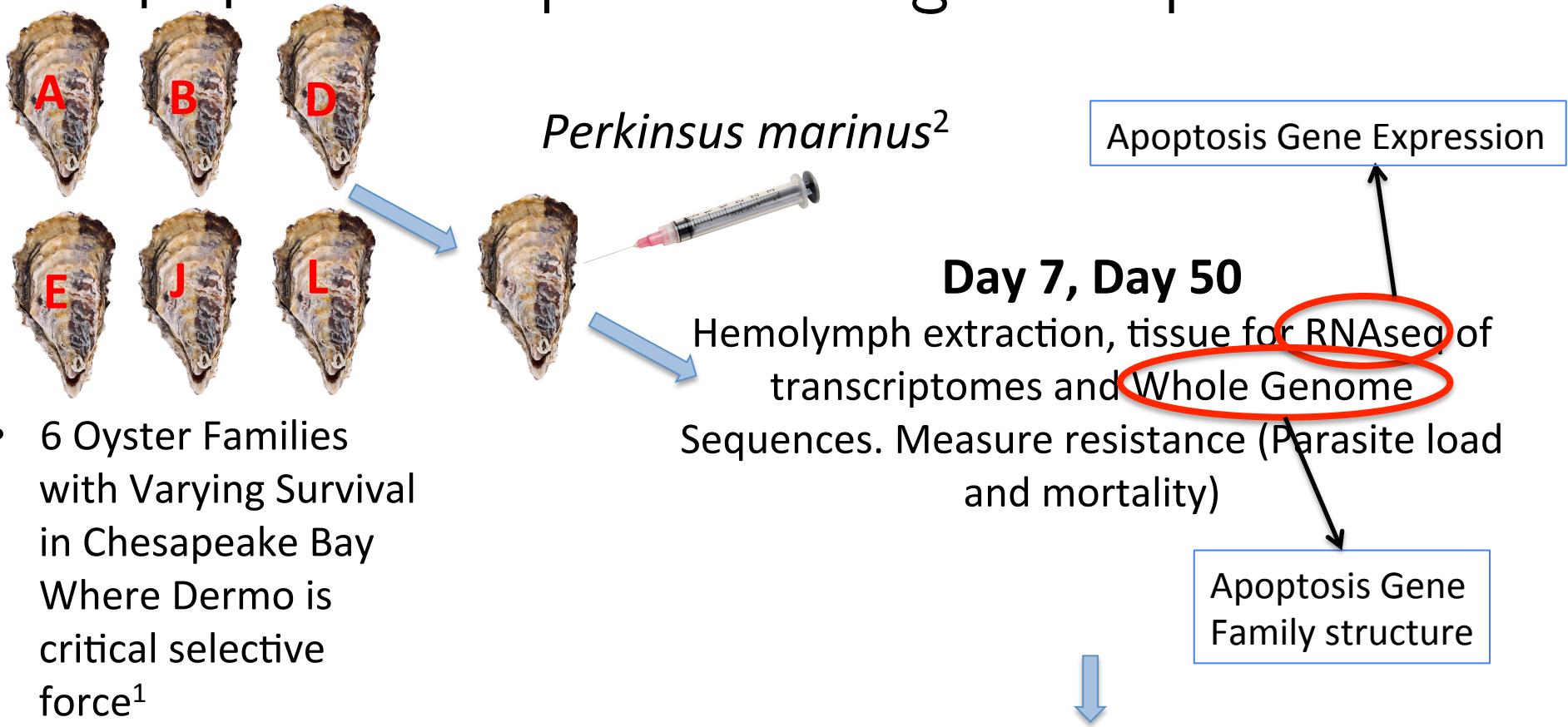
Experiment 2: Analysis *Perkinsus marinus* apoptotic response and gene expression



¹Stan Allen, VIMS ABC

²Bushek, David

Experiment 2: Analysis *Perkinsus marinus* apoptotic response and gene expression



¹Stan Allen, VIMS ABC

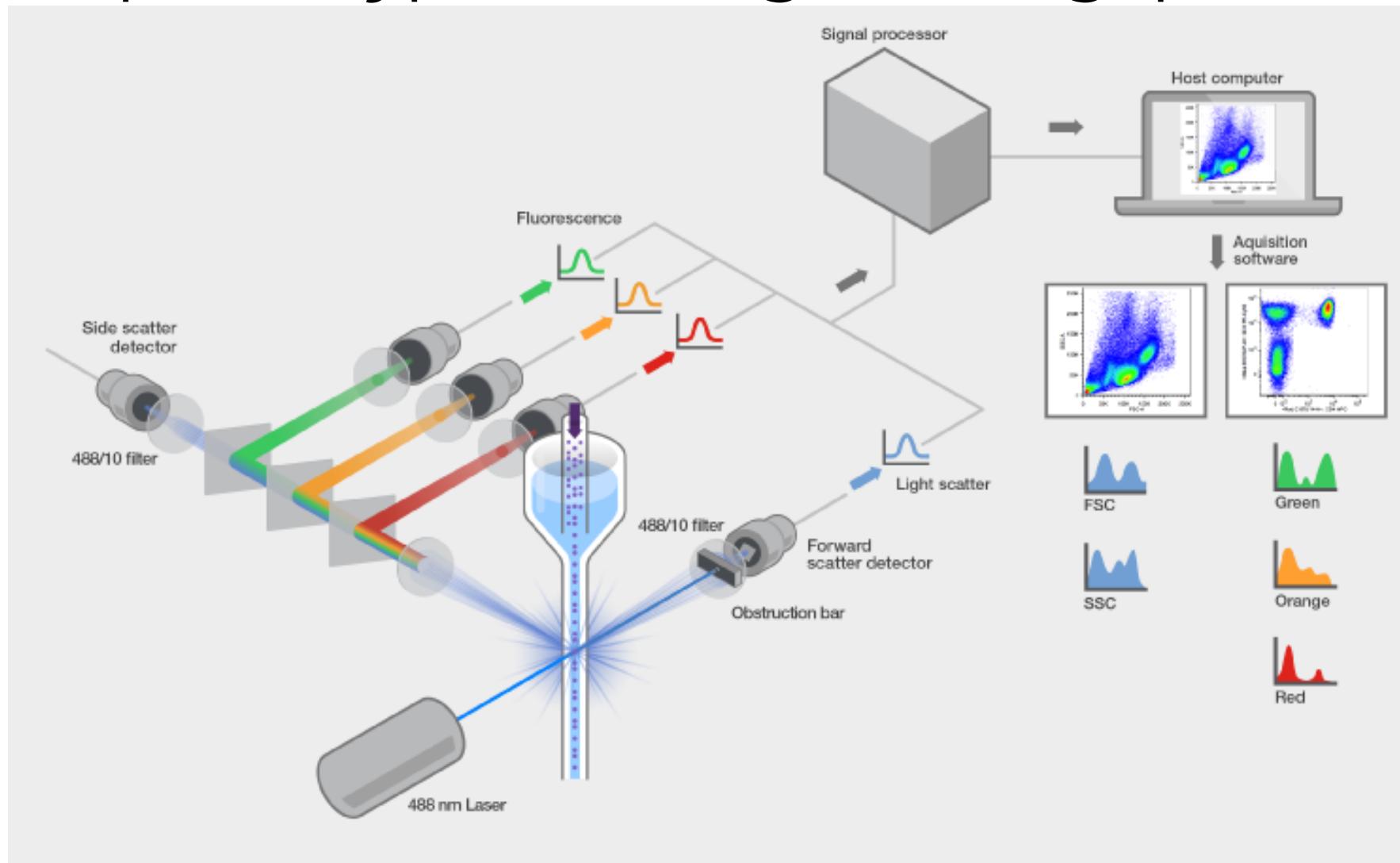
²Bushek, David

Project collaborators: Dina Proestou, Tal Ben Horin, Gary Wikfors, Kathryn Markey Lundgren Marta Gomez Chiarri

Flow Cytometry with hemolymph
Assess cellular viability, apoptosis

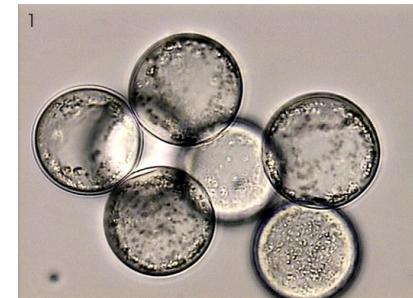
- Day 7: 13 challenge, 3 control per family
- Day 50: 10 challenge, 5 control per family

Flow Cytometry: Analyzing cellular phenotype with high throughput



Dermo Disease

- Exposure via filter feeding, parasite may use hemocytes to replicate and spread²
- Causes watery, thinned tissues, tissue necrosis, loss of hemocytes²



¹*P. marinus*



Left: Healthy *C. virginica*, Right: *C. virginica* with advanced Dermo⁴

¹Ifremer, B.Chollet, ²Smolowitz 2013, ³Ford and Smolowitz, 2007, ⁴Fisheries and Forestry,

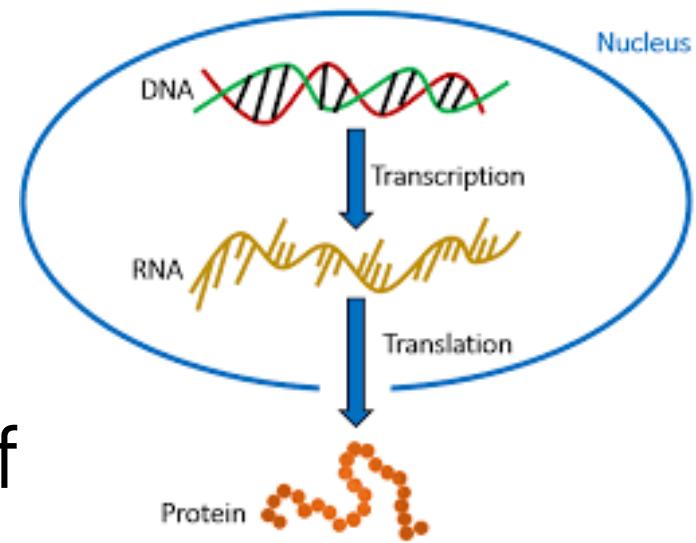
⁵Degremont et al. 2015, ⁶Ben-Horin et al. 2018

Assessing difference in disease susceptibility and apoptosis response (preliminary data)

- Parasite load significantly different between families (One-Way ANOVA P=0.04)
 - E > J (P= 0.07)
- % Apoptosis significantly different in hemocytes between exposed families at Day 7 (One-Way AOV P = 0.0004)
 - Family L < B,D, J
 - Family A < B

Data Analysis

- Transcriptome analysis of apoptosis immune gene expression using **command line** and **R pipeline**
- Genome Analysis of apoptosis genes using **automated scripts**
- Bayesian modeling in **R** of relationships between apoptosis and/resistance



The Big picture

- Specific apoptosis pathway genes that help oysters better respond to diverse stressors these could be targeted for **selective breeding**
- **Selectively bred oysters can limit losses to disease**



Nick Papa East Beach Oyster Co.,
Ocean State Shellfish Cooperative

How did I gain these skills and why do I know you can too?

- Taking offered classes
- Constantly googling errors and reading online forums
- R cheat sheets
- Asking for help from people who know better
- Perseverance!



Acknowledgements

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 - Tal Ben Horin
 - Kathryn Markey Lundgren
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 - Stephanie Spada
 - Katherine Brooks
- NOAA Fisheries NEFSC
 - Gary Wikfors
 - Magali Bazzano

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- USDA ARS Collaborative Project 58-8030-5-009
- USDA NRSP-8

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Thanks for your attention!

