A new integrated approach to establish fish health as an indicator of ecological stressors: Part 1

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ECOSYSTEM RESTORATION PROGRAM

California Department of Fish & Wildlife
National Oceanic & Atmospheric Administration
US Fish & Wildlife Service

UCDAVIS School of Veterinary Medicine

Acknowledgement

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- **US Geological Survey**
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- **Moyle Lab:** James Hobbs, Eva Bush, Others
- **AHP:** Georgia Ramos, Andrew Pedersen, Christopher Tai, Gary Wu, Ching Teh, Others

Question

- What affects the health of fish?
  - Environment
    - Size
    - Water quality
    - Food quality
  - Organism
    - Condition
    - Nutrition
    - Reproduction
  - Xenobiotics
    - Contaminants
    - Pathogens

Environment

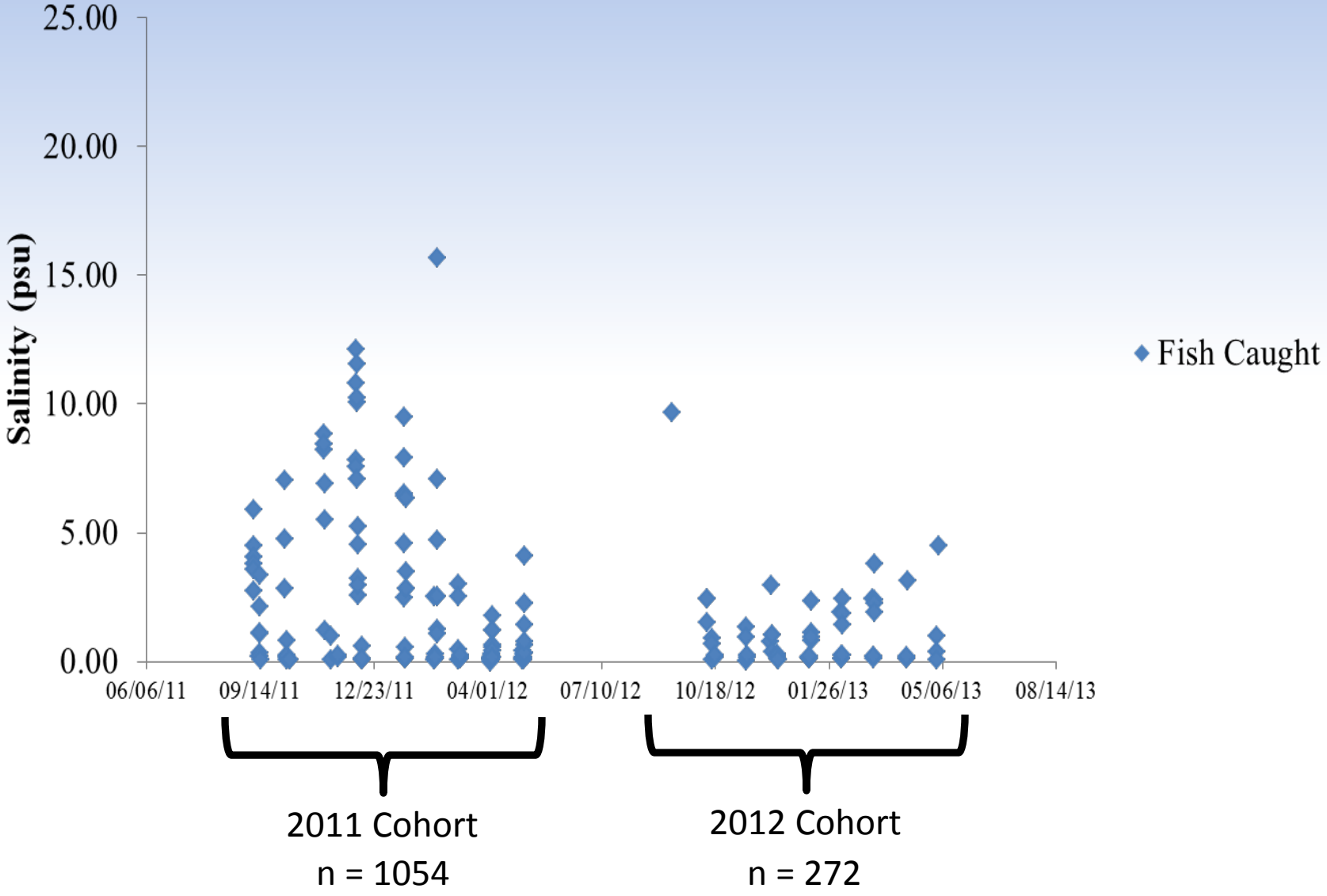
Organism

Xenobiotics

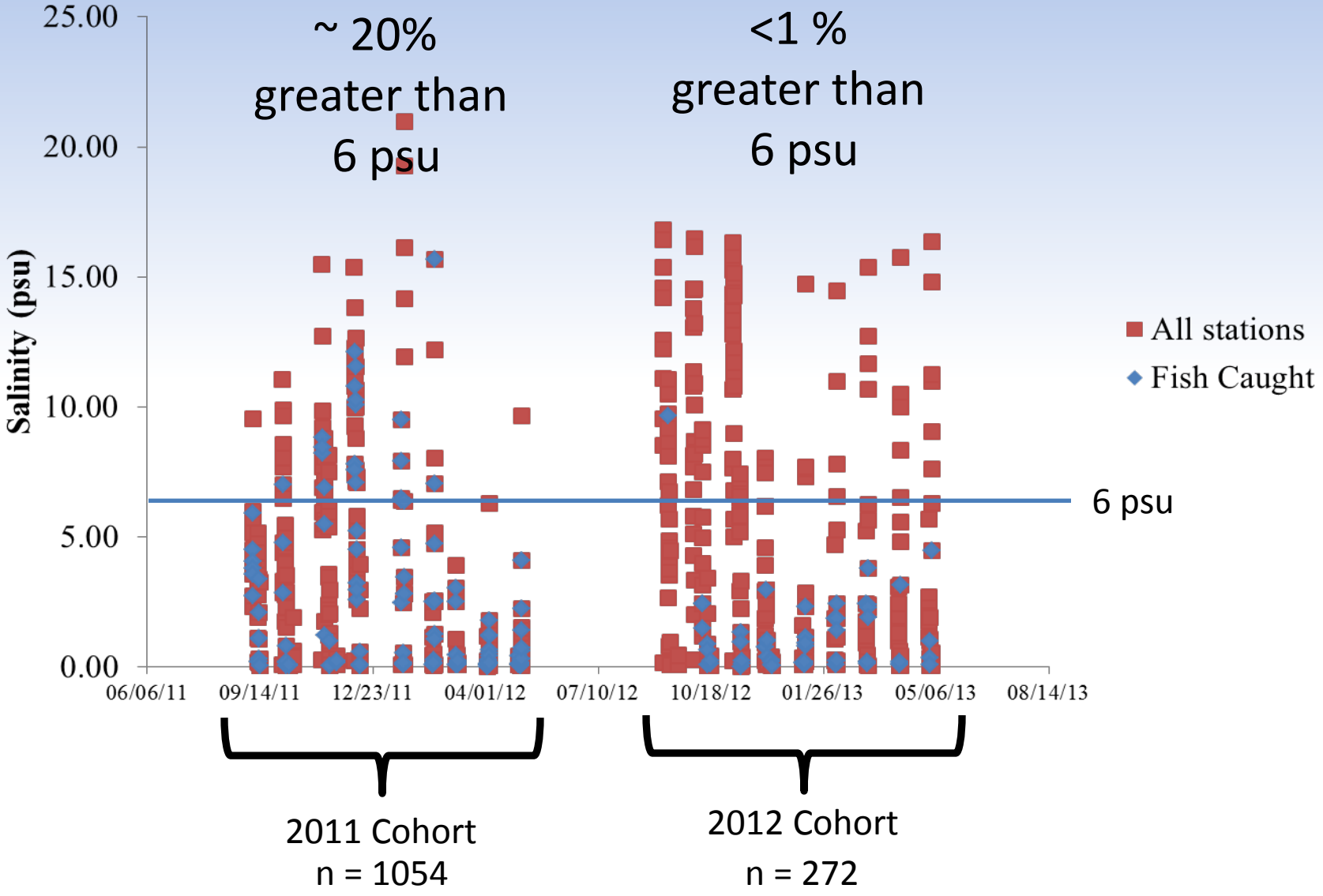
Environment

- Temporal
- Temperature
- Hydrology
- Turbidity
- Productivity
- Salinity
- Spatial

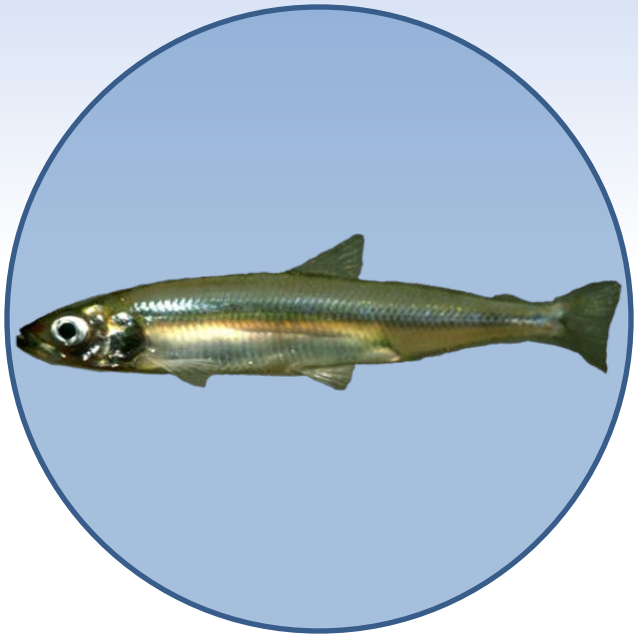
Salinity


25.00
20.00
15.00
10.00
5.00
0.00
Salinity (psu)
06/06/11
09/14/11
12/23/11
04/01/12
07/10/12
10/18/12
01/26/13
05/06/13
08/14/13
Fish Caught
2011 Cohort
n = 1054
2012 Cohort
n = 272

Salinity


~ 20% greater than 6 psu
<1 % greater than 6 psu
25.00
20.00
15.00
10.00
5.00
0.00
Salinity (psu)
06/06/11
09/14/11
12/23/11
04/01/12
07/10/12
10/18/12
01/26/13
05/06/13
08/14/13
All stations
Fish Caught
6 psu
2011 Cohort
n = 1054
2012 Cohort
n = 272

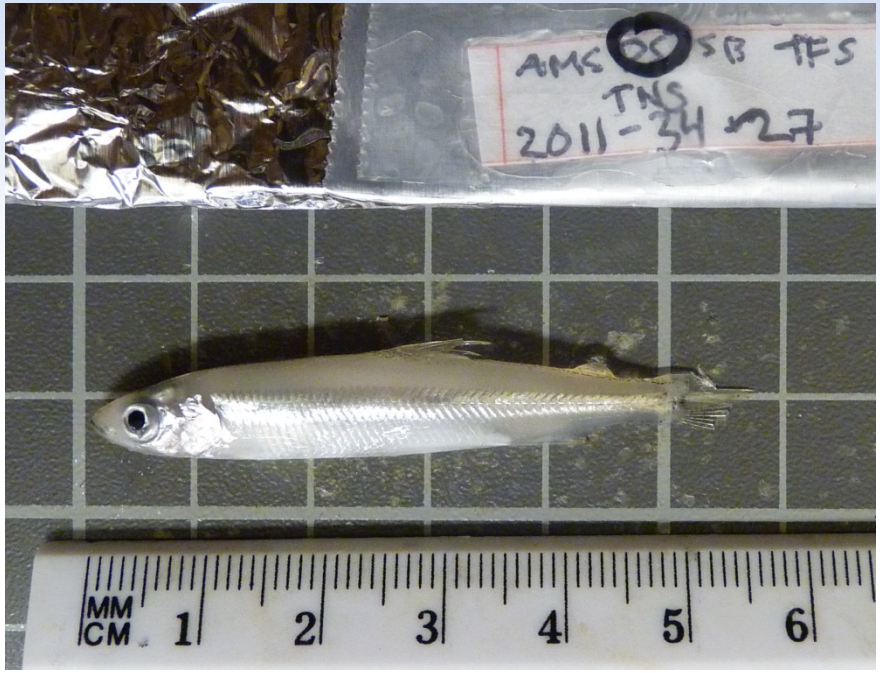
Organism



- Population
  - Presence
  - Abundance
- Age
  - Otolith
- Life history
  - Otoliths
- Condition
  - Condition factor (CF)
  - Hepatosomatic index
  - Histopathology
- Nutrition
  - Triglyceride (TAG)
  - RNA/DNA
- Reproduction
  - Sex
  - Gonadosomatic index (GSI)
  - Estradiol (E_2)
  - Egg stage

Images of a low & high condition

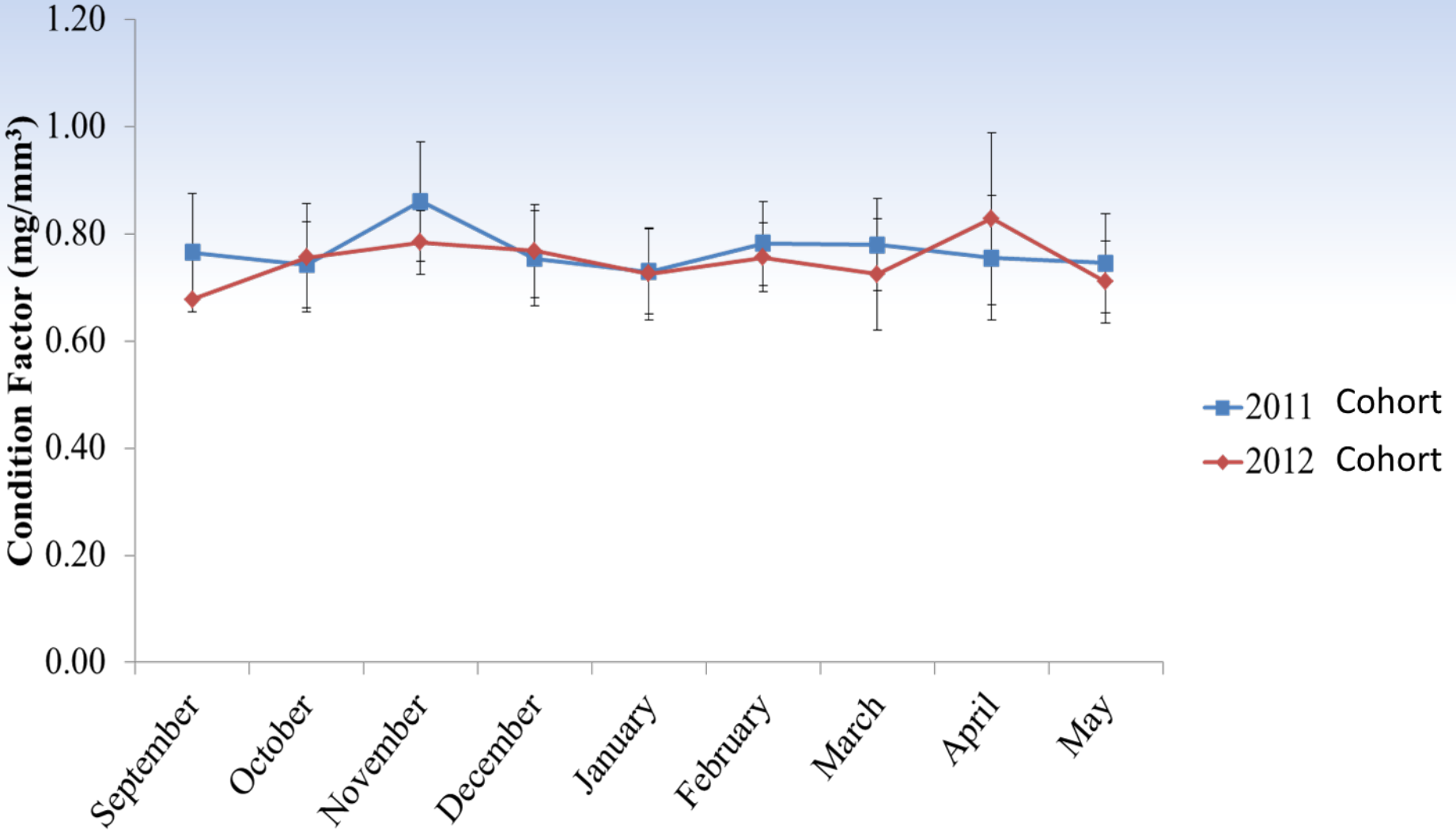
Low Condition Factor



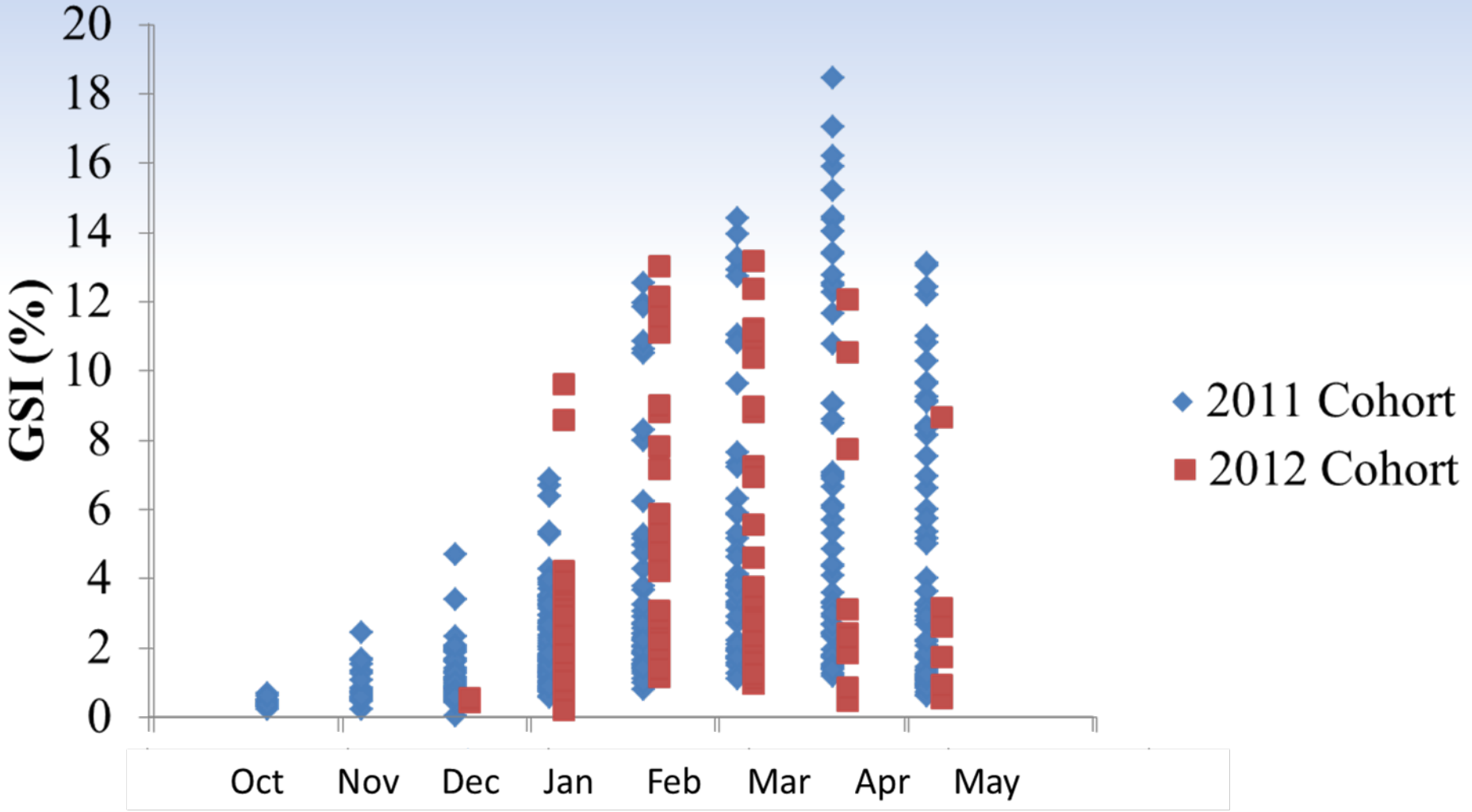
High Condition Factor

- Over 1300 fish have been processed
  - > 35 different indices measured
  - >15 different physical parameters

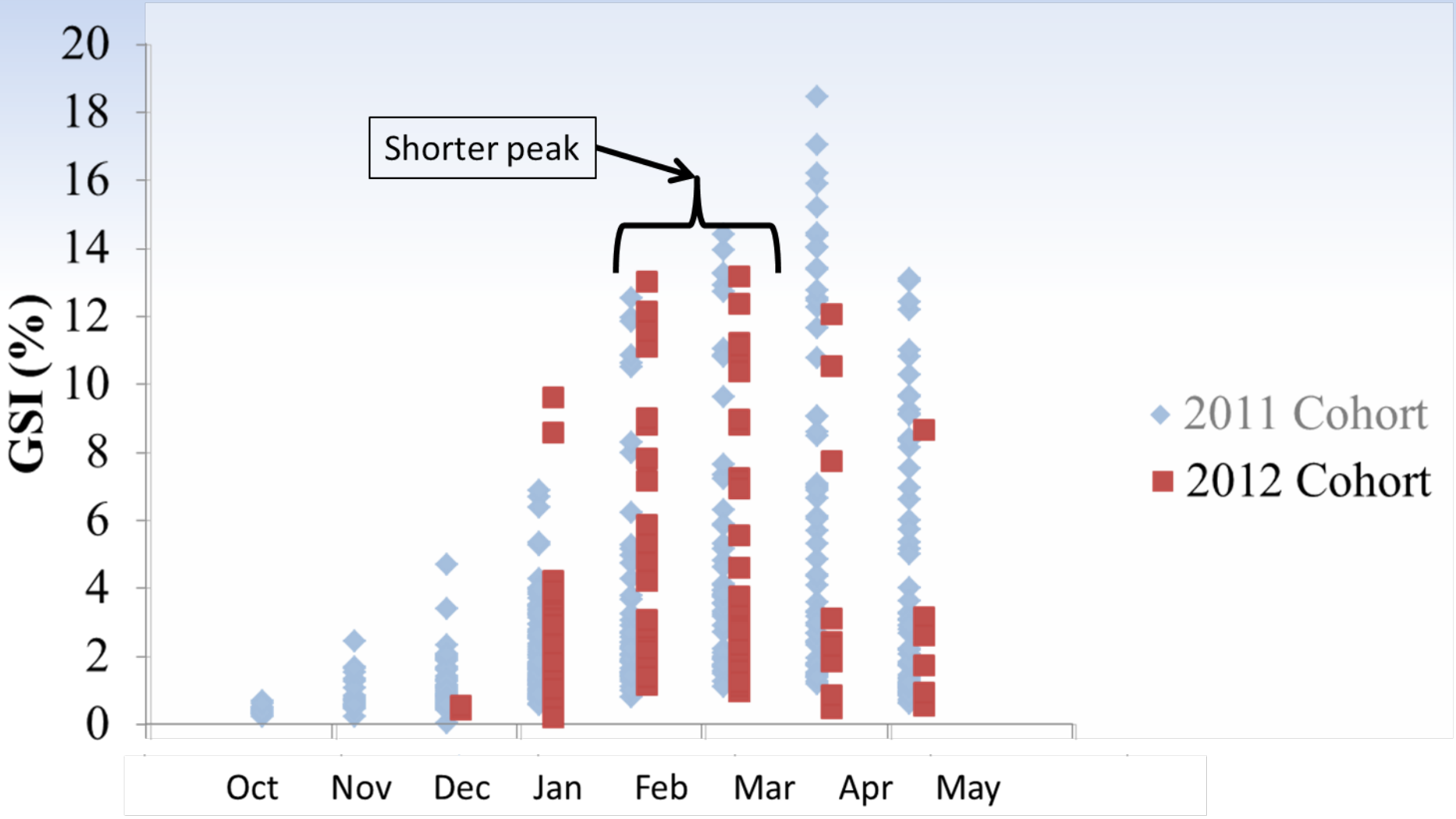
Condition Factor

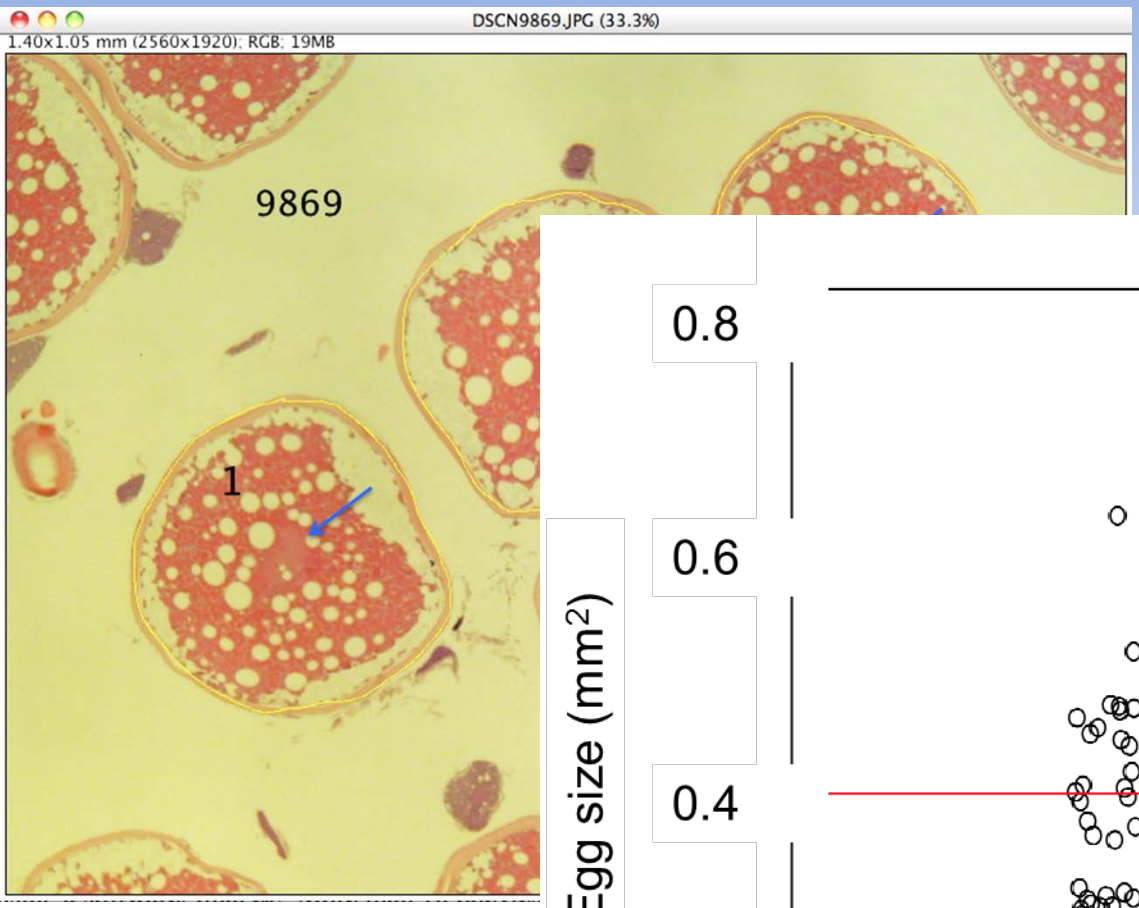


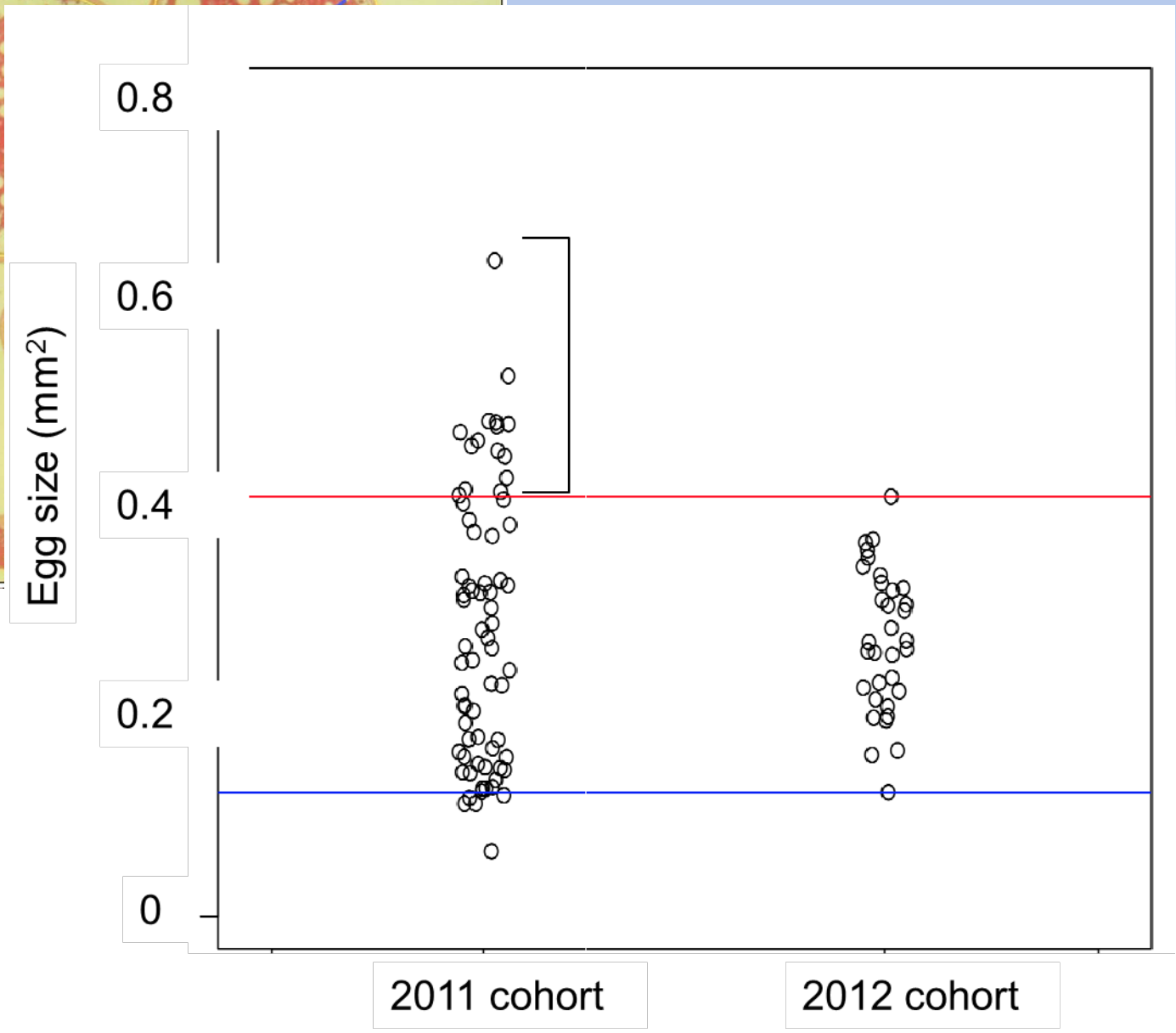
Gonadosomatic Index

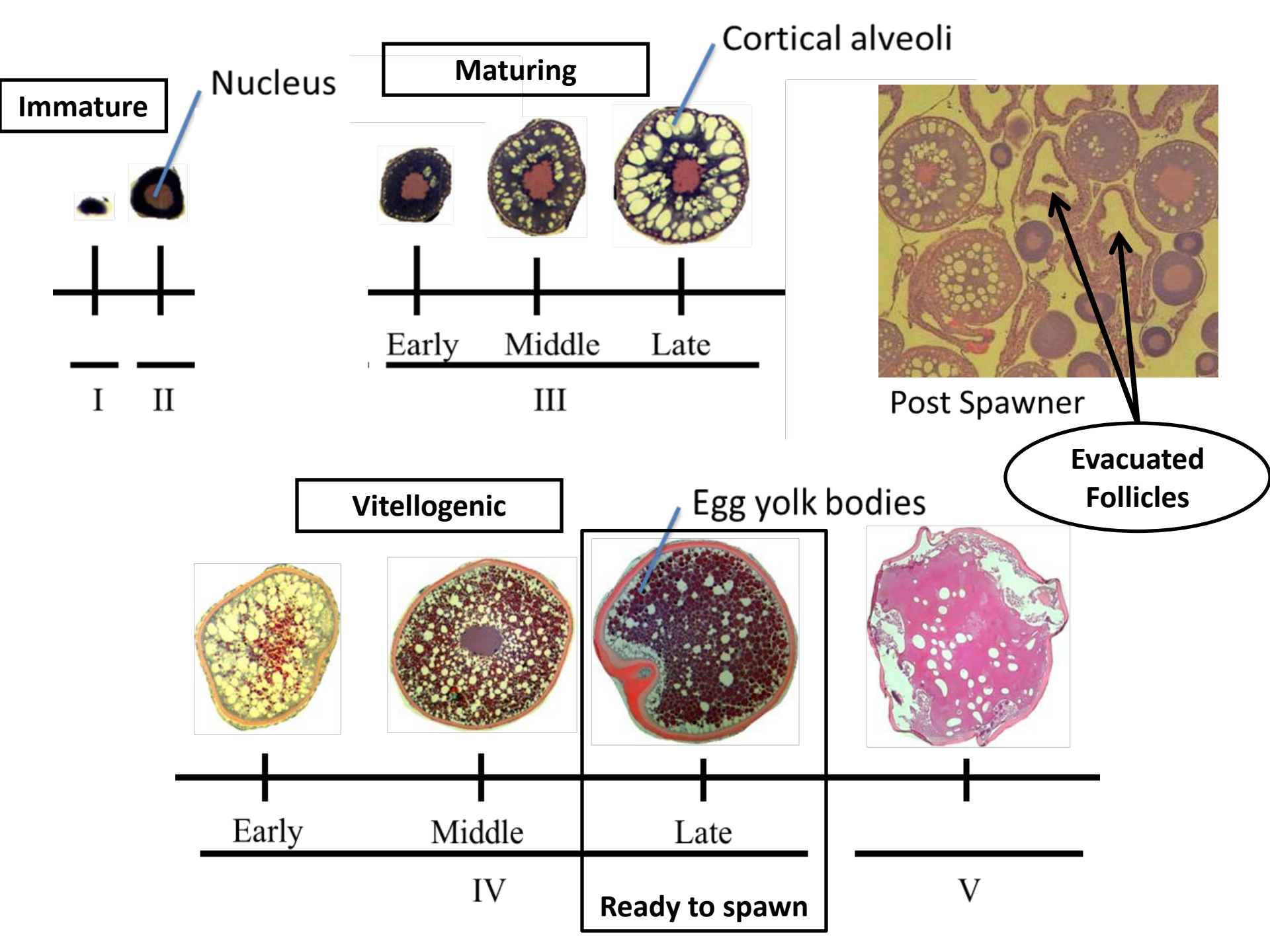


Gonadosomatic Index



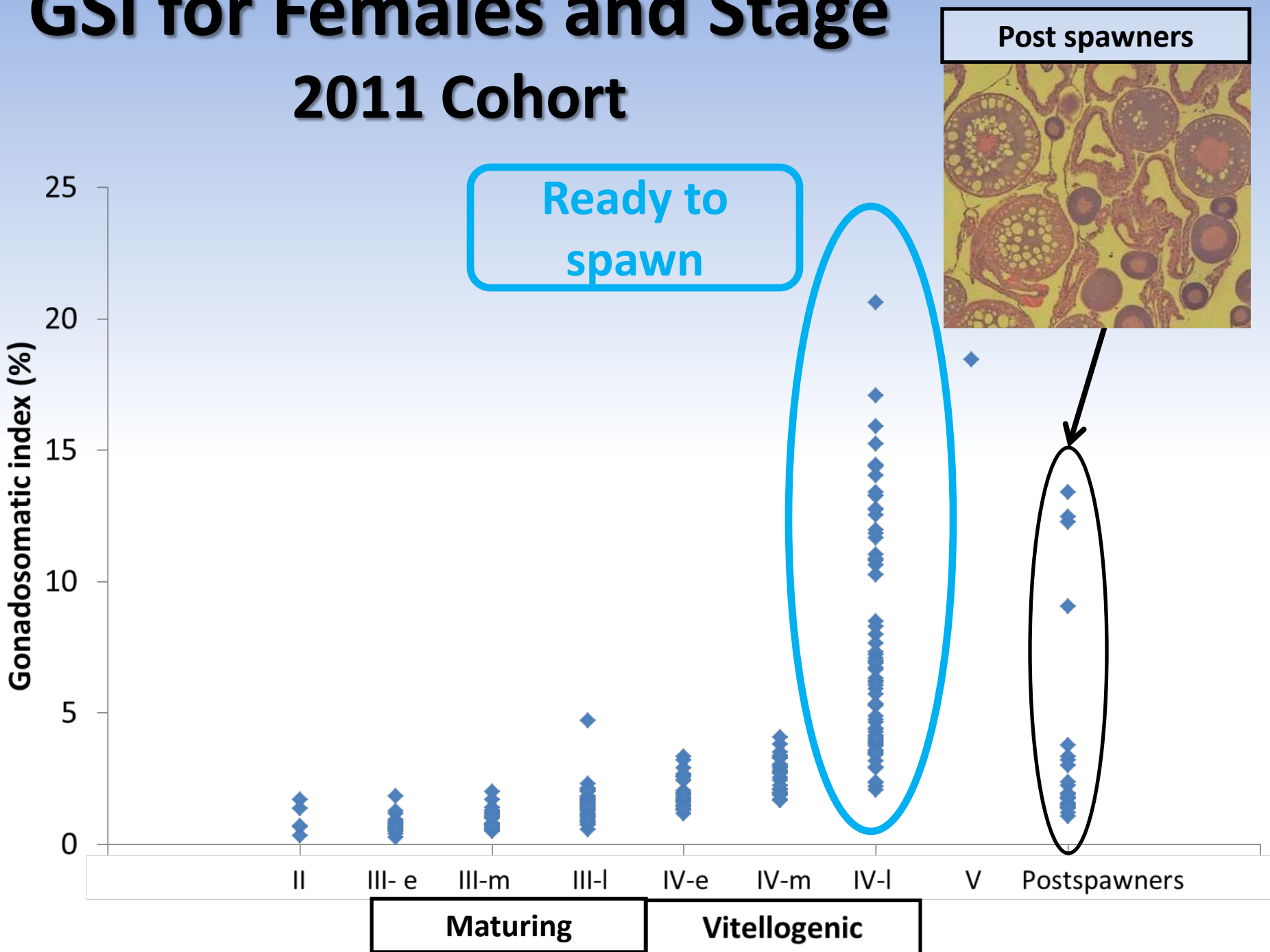

DSCN9869.JPG (33.3%)
1.40x1.05 mm (2560x1920); RGB; 19MB
9869
1


Egg size (mm²)
0.8
0.6
0.4
0.2
0
2011 cohort
2012 cohort


Immature
Nucleus
Maturing
Cortical alveoli
Early
Middle
Late
I
II
III
Post Spawner
Evacuated Follicles
Vitellogenic
Egg yolk bodies
Early
Middle
Late
IV
Ready to spawn
V

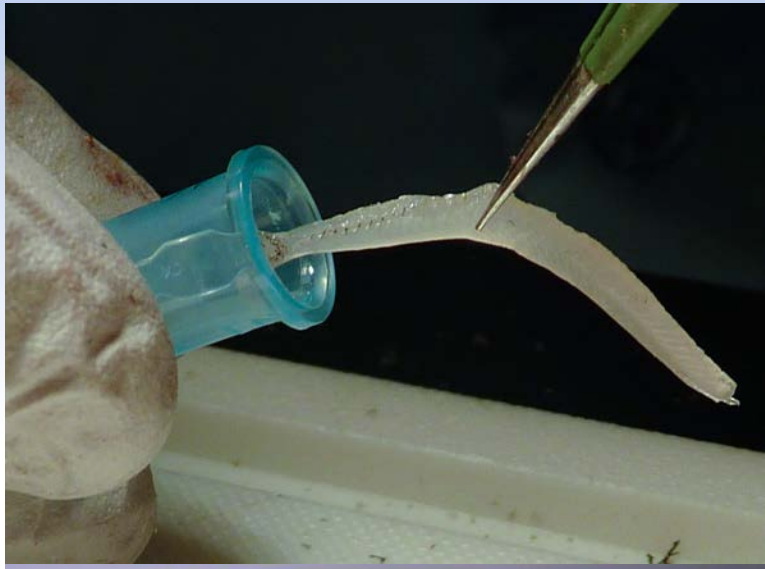
GSI for Females and Stage

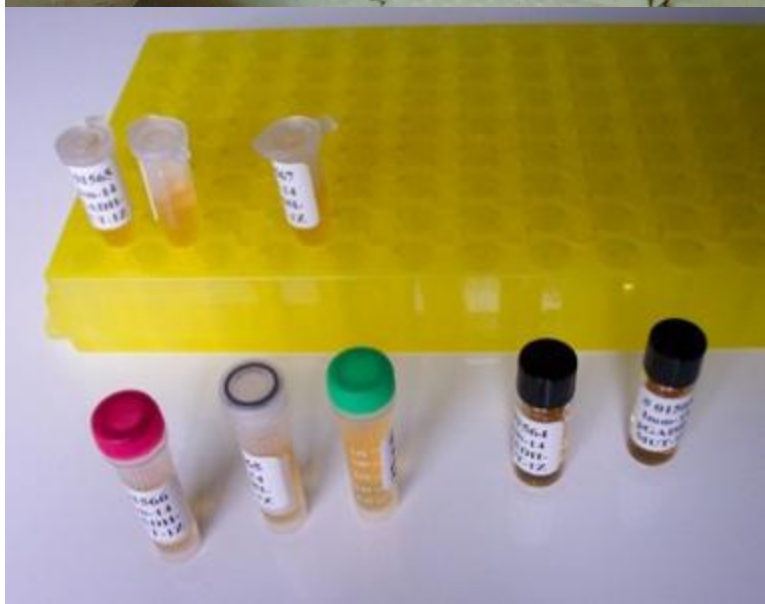
2011 Cohort



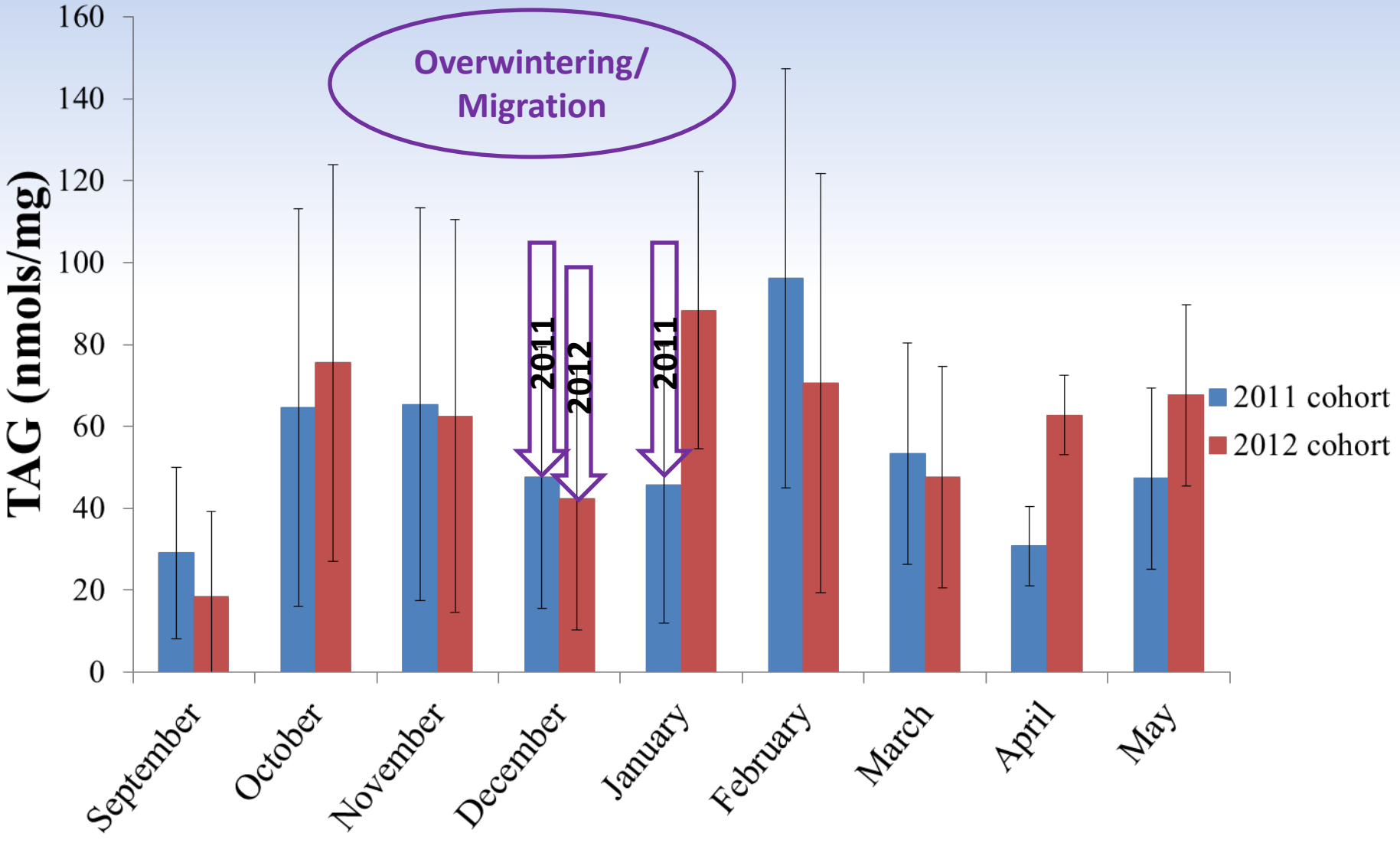
Nutritional data

- RNA/DNA
  - Muscle tissue
  - Short term nutritional status
  - Growth potential
  - Nutritional stress
- Triglycerides (TAG)
  - Muscle tissue
  - Long term nutritional status
  - Overwintering
  - Reproductive growth

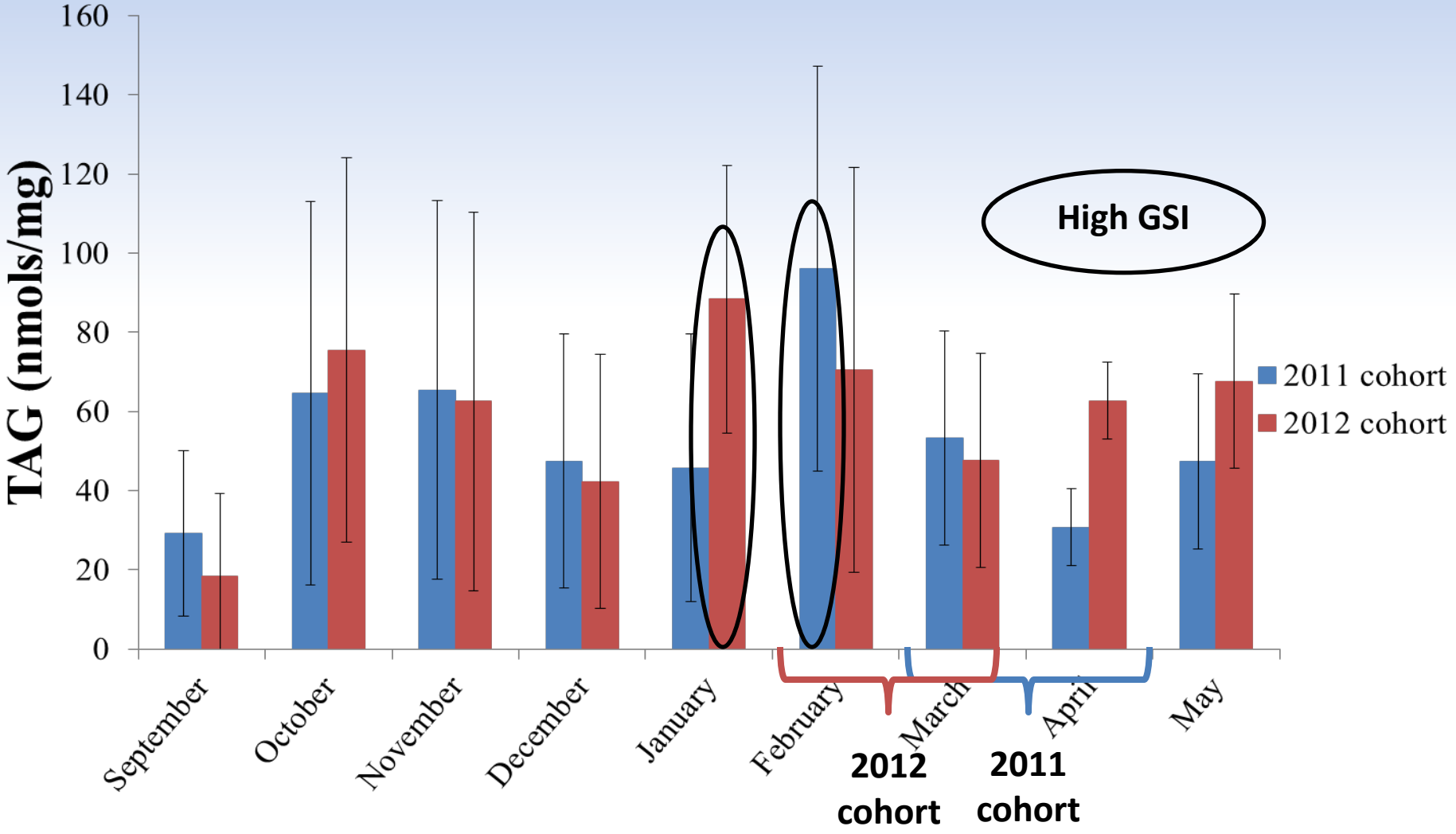




Triglyceride



Triglyceride



Xenobiotics

Pathogens

Metals

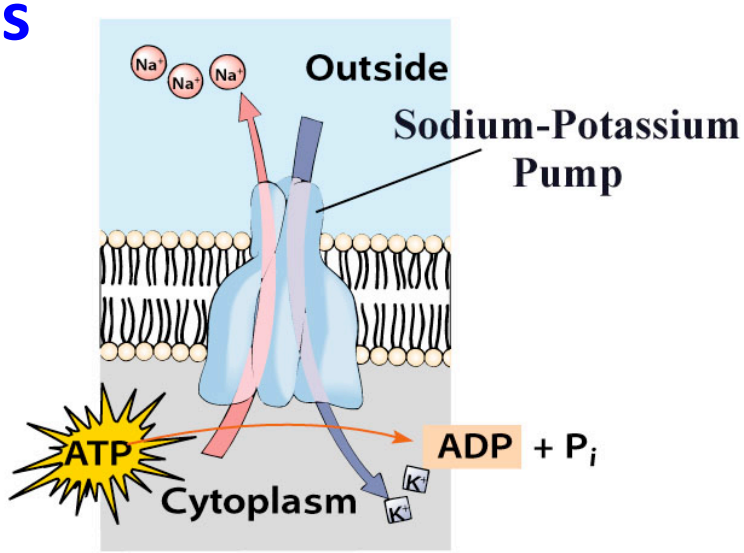
Runoff

Enzymatic biomarkers

Sodium Potassium ATPase (Na/K ATPase)
- **Salinity,** temperature and turbidity
- **Contaminants** and/ or **disease**

Acetylcholinesterase (AChE)
- **Metals**
- **Pesticides: Organophosphates**
- **Carbamates**

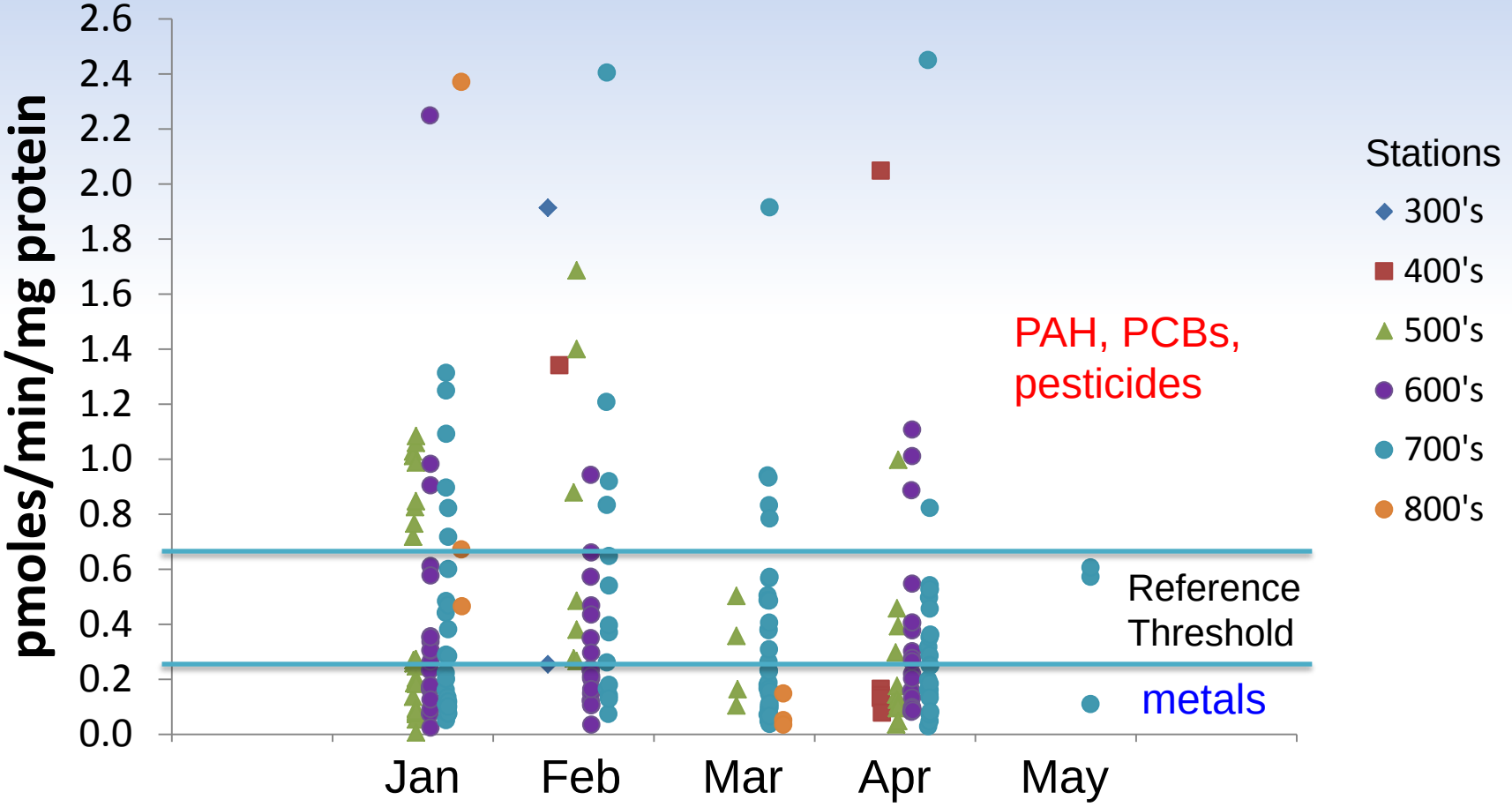
EROD (Cytochrome P450 1A)
- **PAH, PCBs, Biocides**
- **Metals**



http://iws.collin.edu/biopage/faculty/mcculloch

EROD Activity between Jan and May 2012

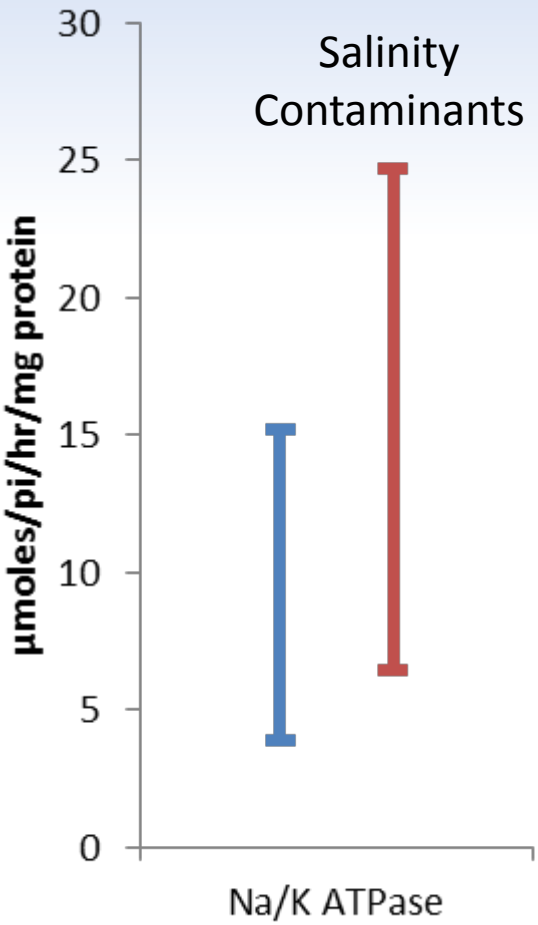
2011 Cohort

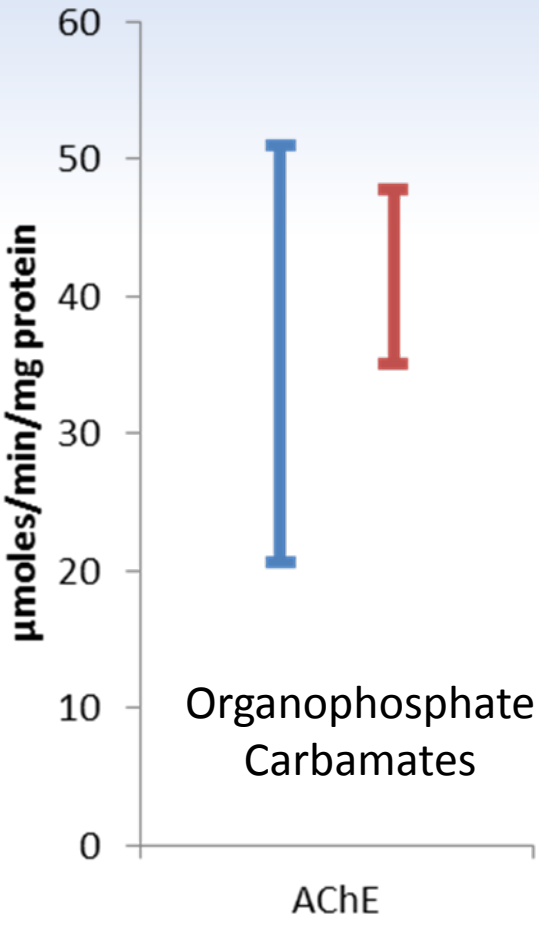


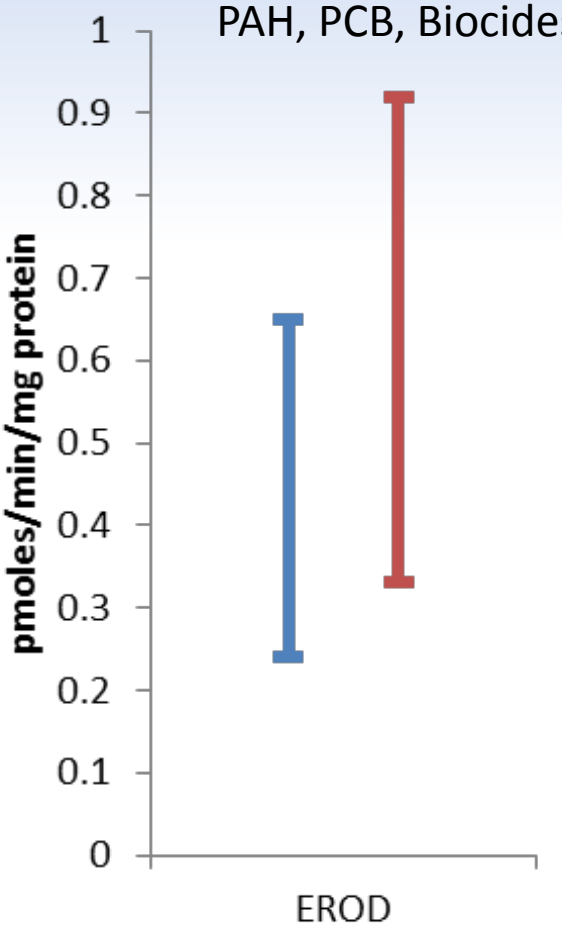
n = 230

Thresholds of Enzyme Activity

Sept-Dec 2011 and 2012 cohort







Pathogen Presence

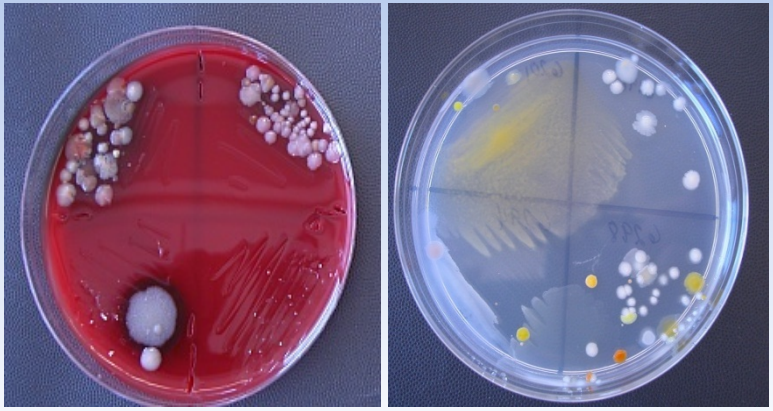
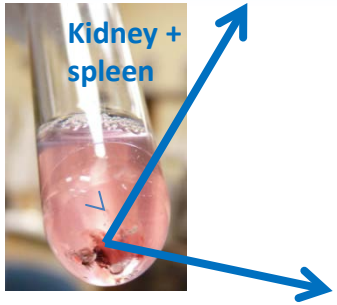
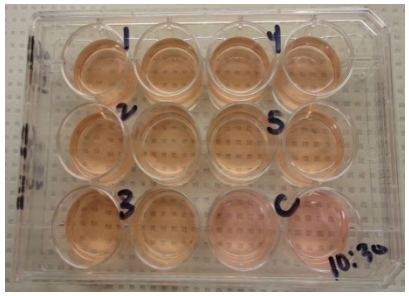
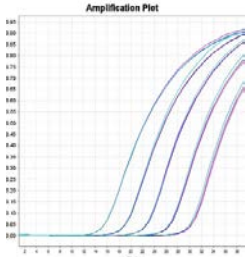


Plate culture – Pathogen score:0–4

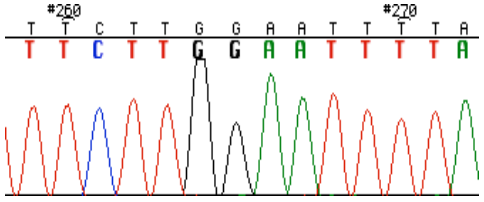




Tissue culture



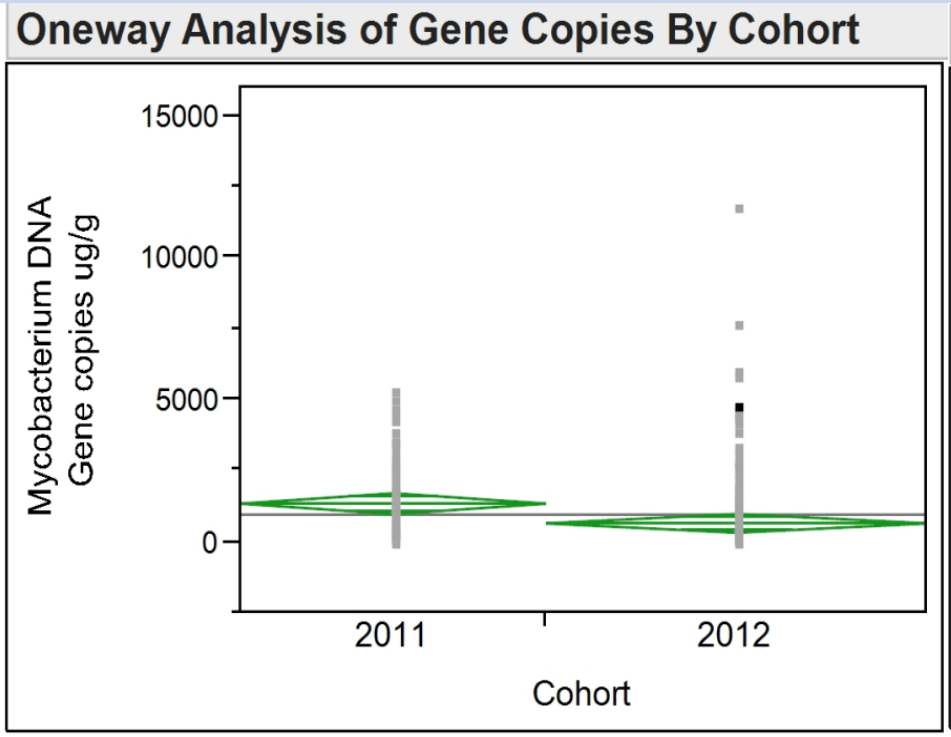
qPCR

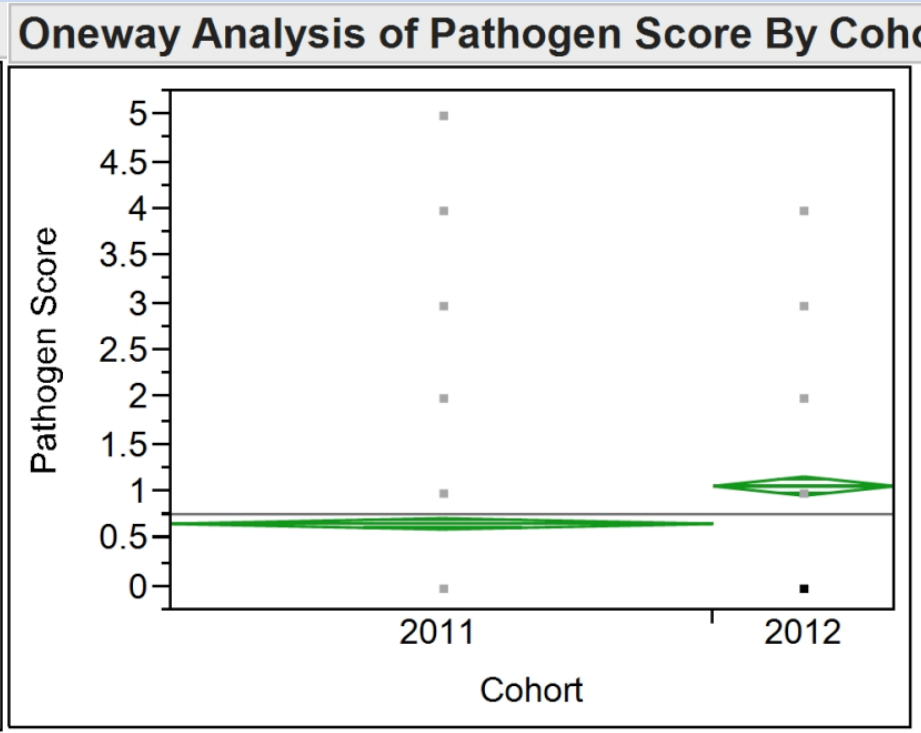


Gene sequencing

- Bacterial isolation
  - Colony number & identity
    - Phenotypic characteristics
    - 16SrDNA sequencing
  - *Mycobacterium* qPCR
- Virus testing
  - Tissue culture
    - Delta smelt cell line
    - Cell lines specific to striped bass & threadfin shad
- **Indicator of cumulative effects from stressors**

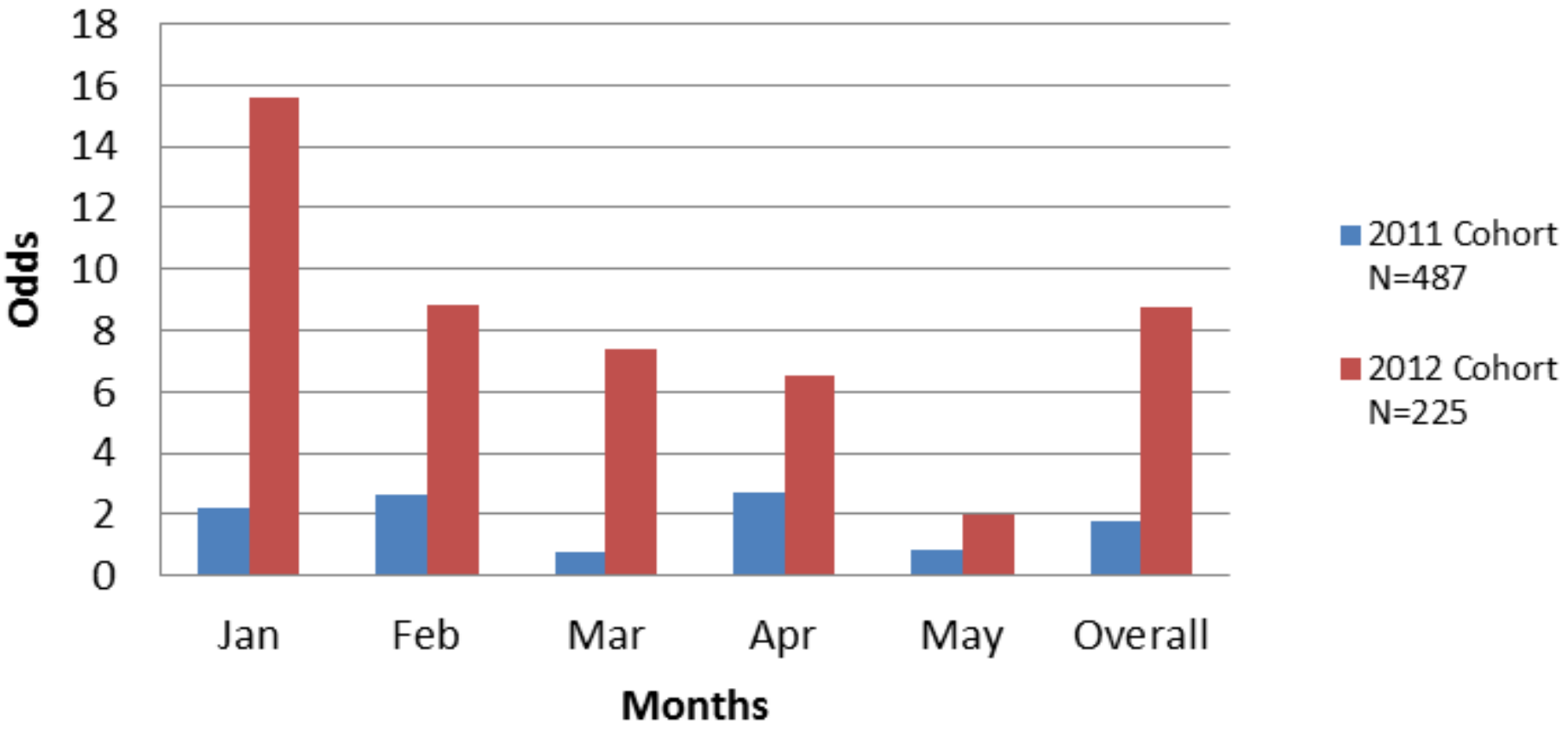
Disease Analysis: 2011 vs. 2012 cohorts





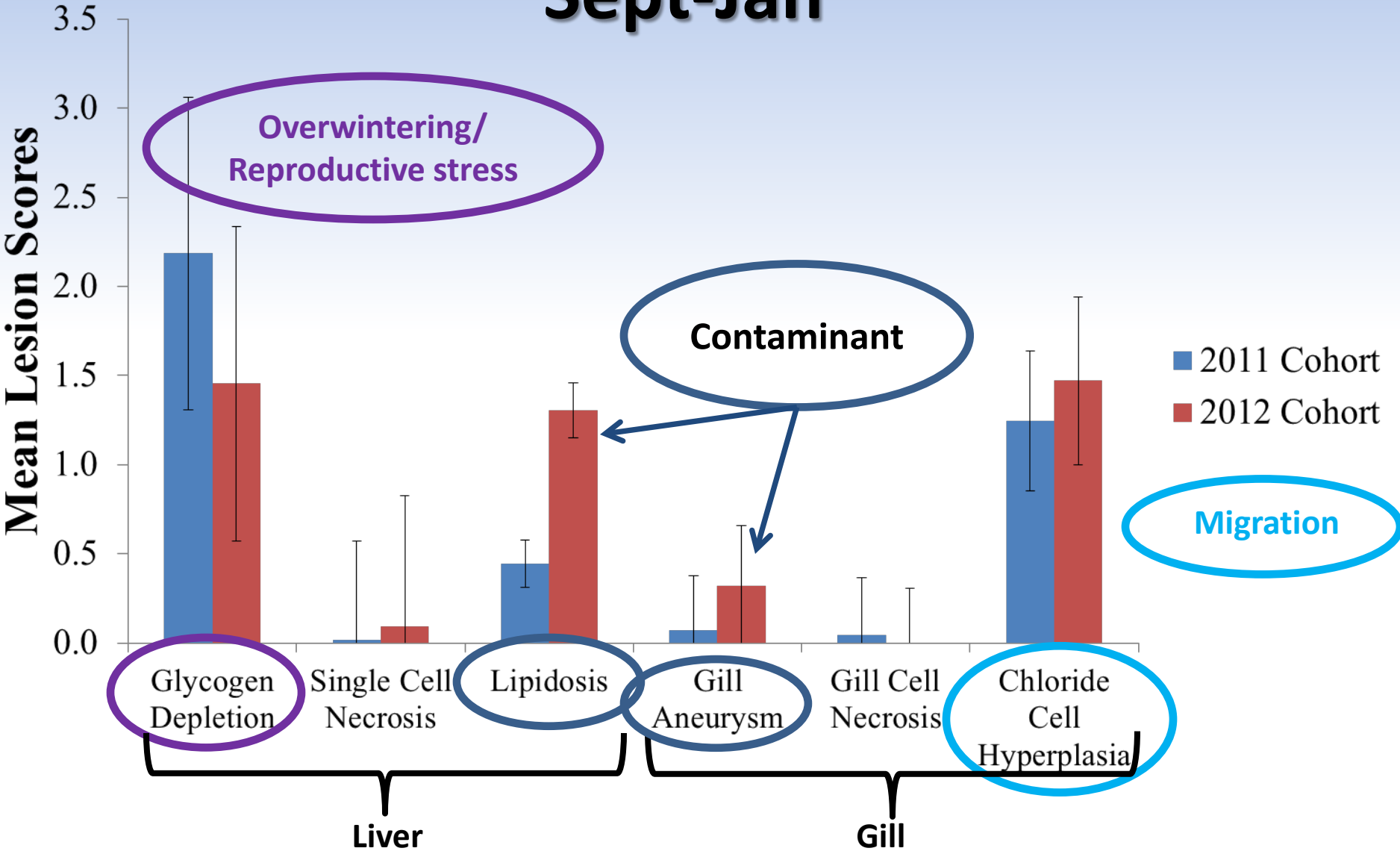
Index	2011	2012	P value
Pathogen Score, Mean	0.667 (n=805)	1.066 (n=270)	0.0001
Mycobacterium gene copies, Mean (μg/g DNA)	1348 (n=196)	654 (n=245)	0.0059

Disease Analysis: 2011 vs. 2012 cohorts



Odds: # Fish with Pathogens/ # Fish without Pathogens

Histopathology
Sept-Jan



Delta smelt

SFE

Xenobiotic

Venn Diagram

Delta smelt

Condition

Nutrition

Reproduction

Incorrect

Hea

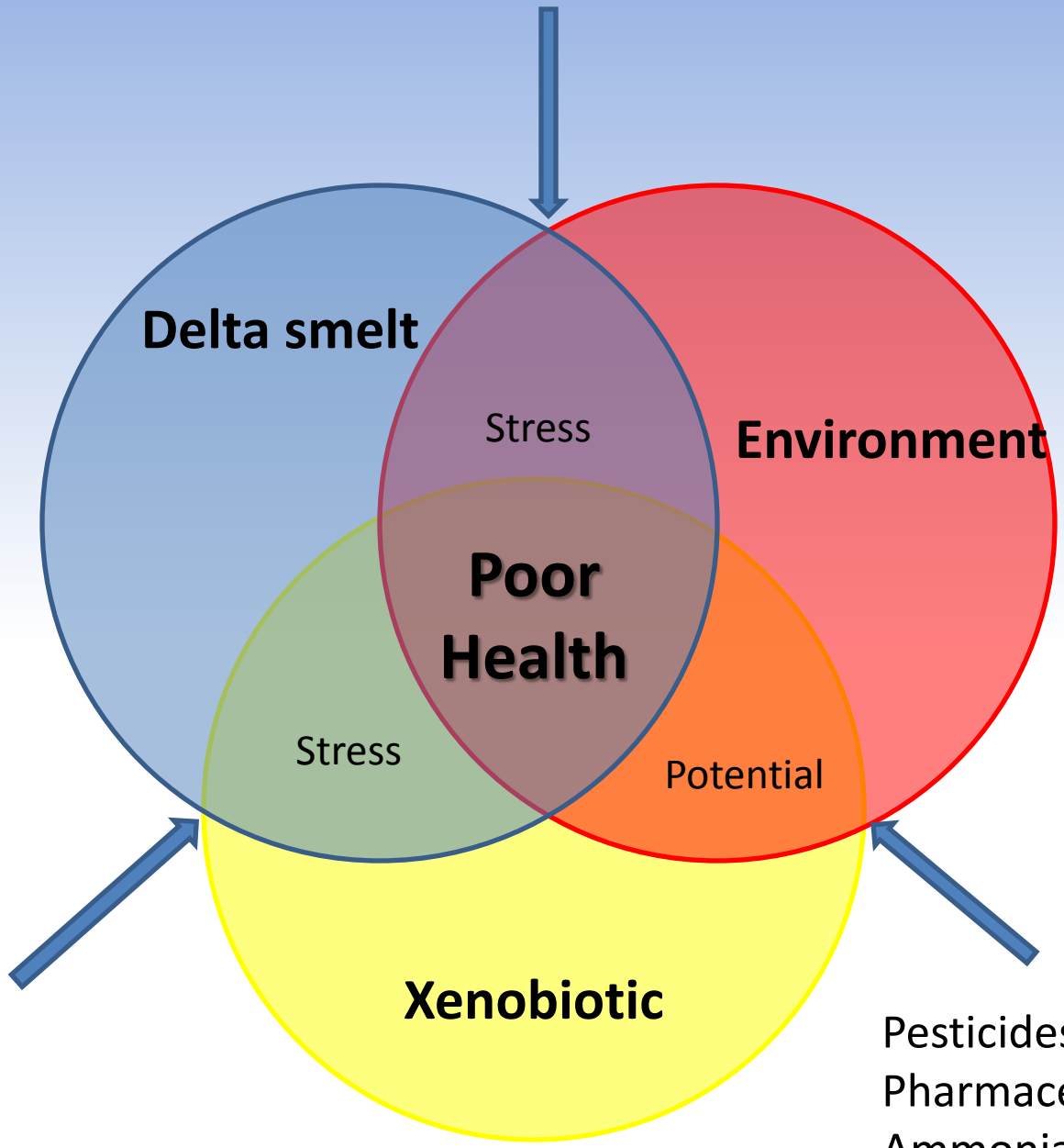
Environment

Turbidity

Temperatures

Salinity

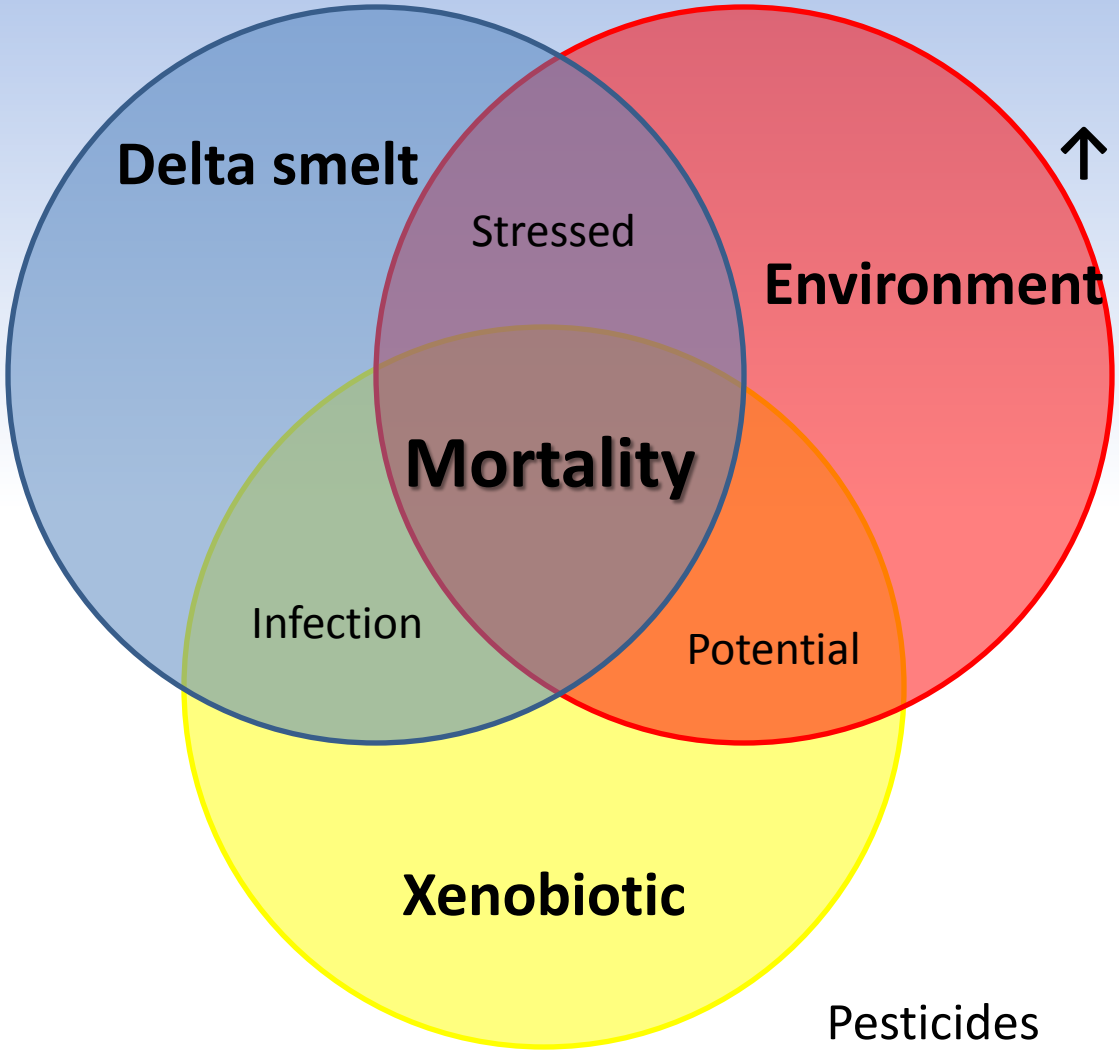
Xenobiotic



Condition
Nutrition
Reproduction

Turbidity
Temperatures
Salinity

Pesticides
Pharmaceuticals
Ammonia
Metals
Pathogens



Condition

↓ **Nutrition**

Reproduction

↑ **Temperatures**

Turbidity

Salinity

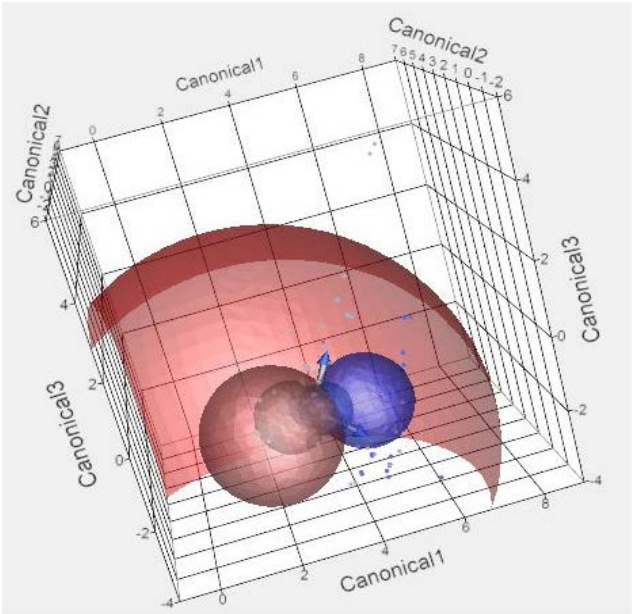
Pesticides

Metals

↑ **Pathogens**

Future Directions

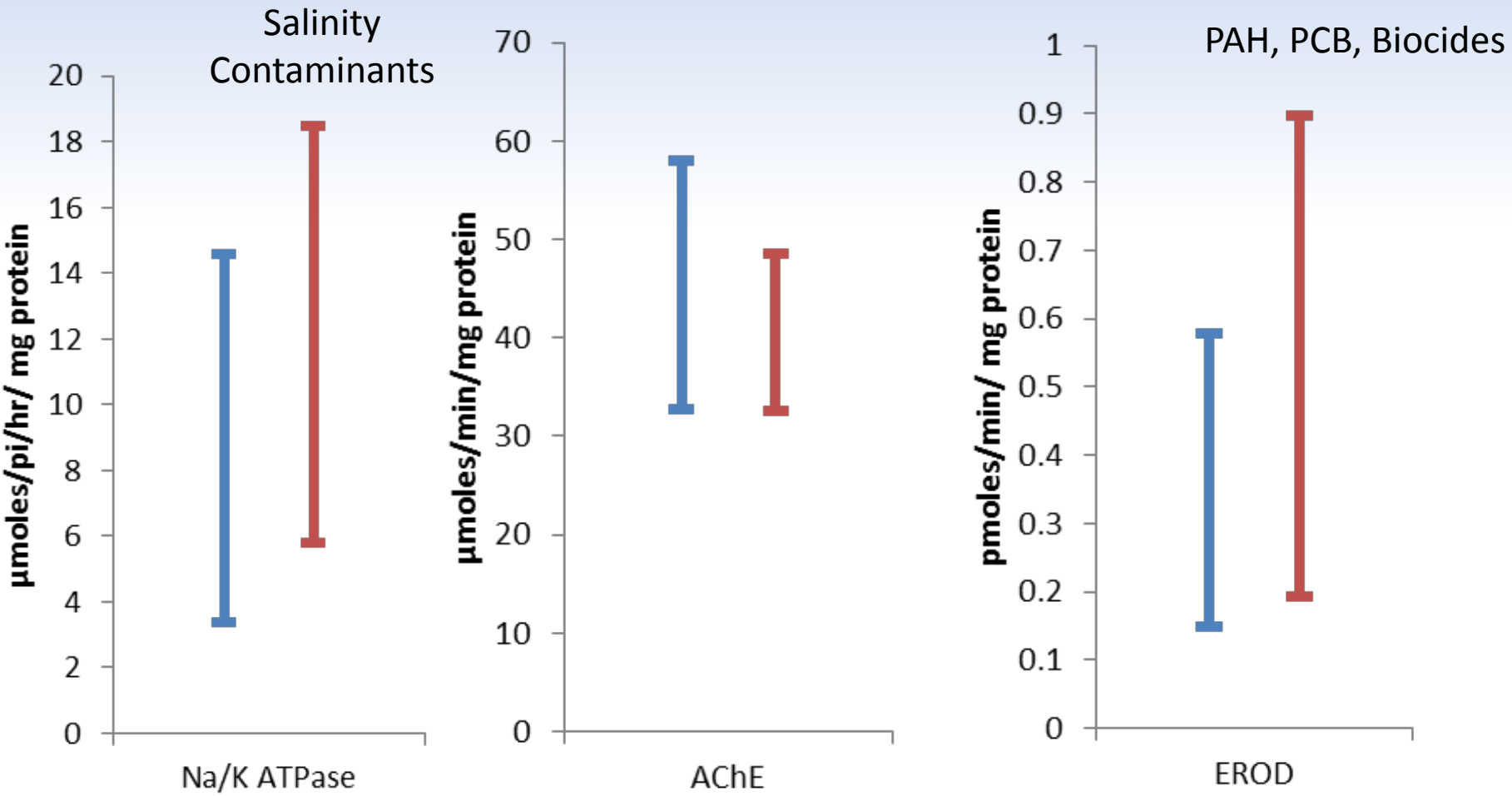
- **Continue analysis with ‘reference’ cohort**
  - Compare to 2013 cohorts
- **Collaboration**
  - DFW gut content analysis
  - USGS water quality data
- **Multivariate analysis**
  - Canonical analysis
  - Modeling
  - Establish health index

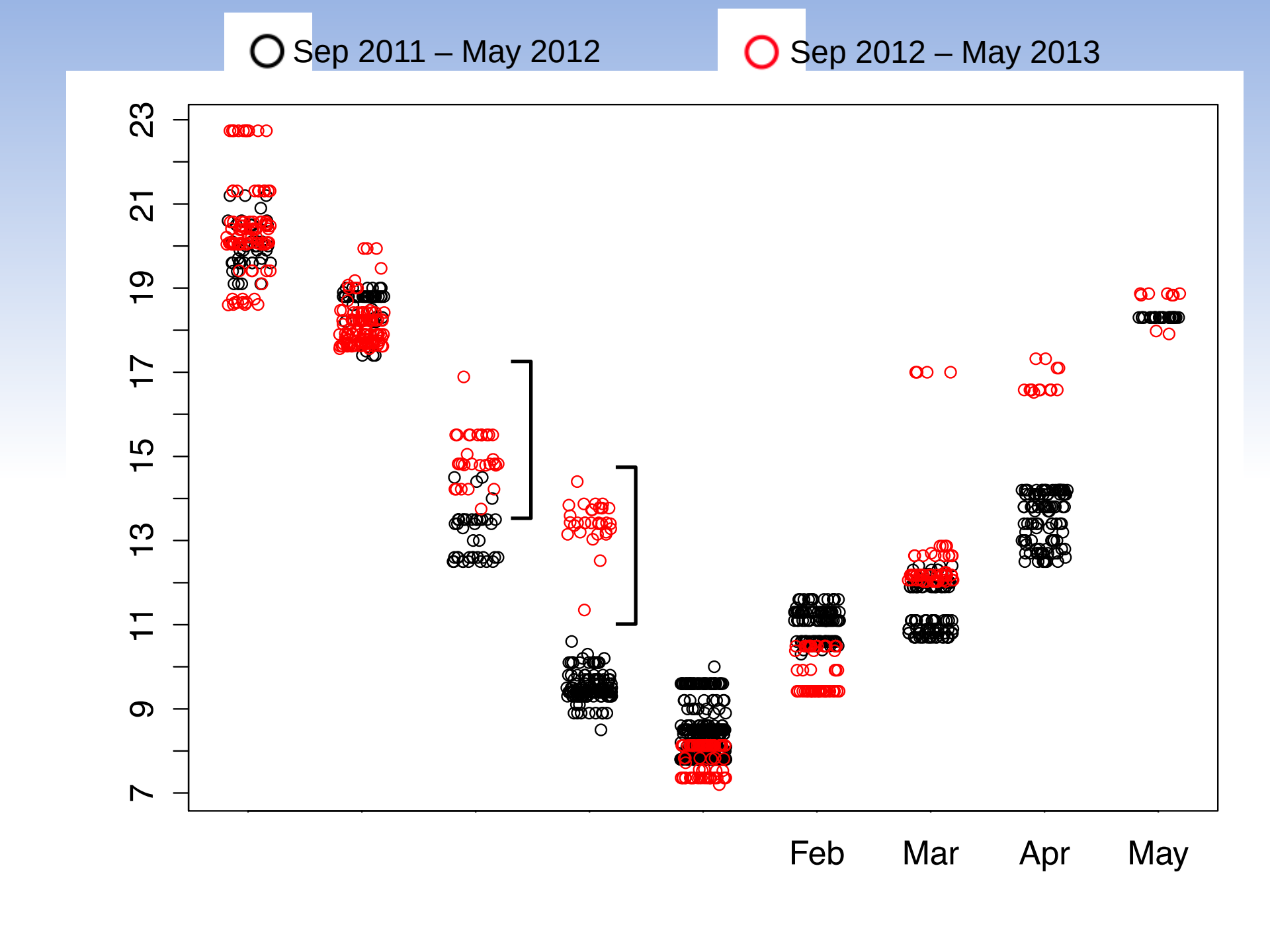


Questions

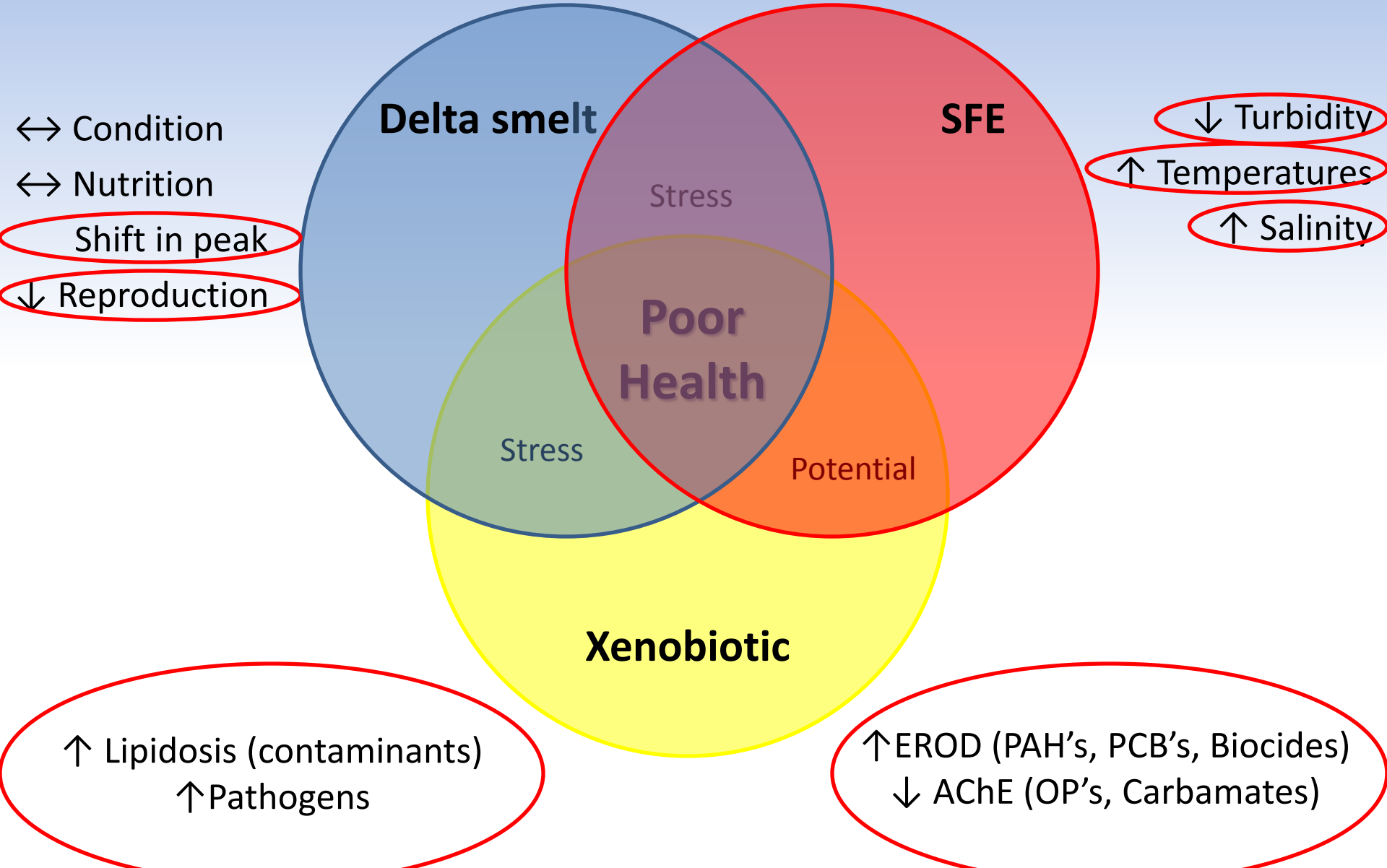
Thresholds of Enzyme Activity

2011 and **2012** cohort



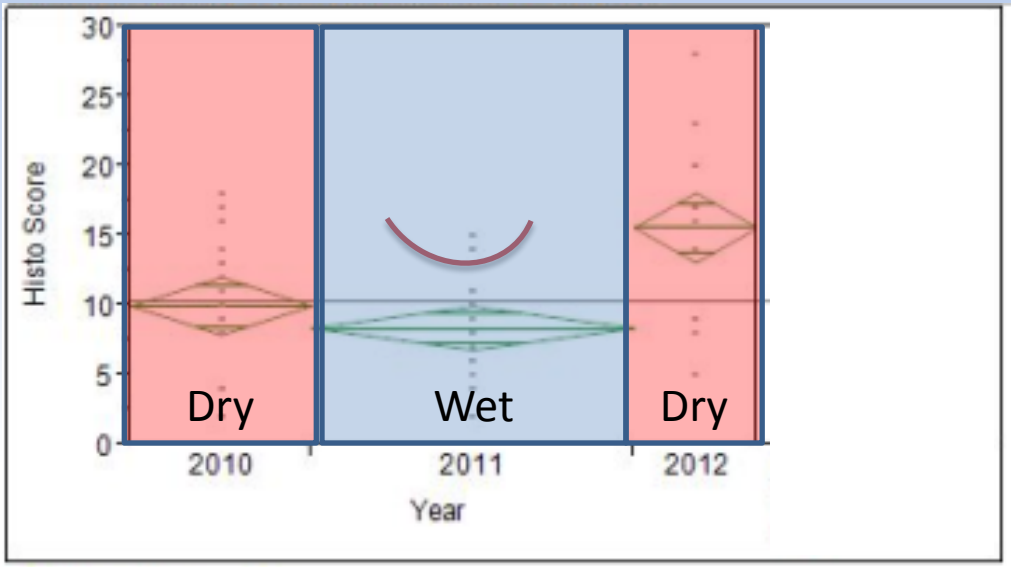

Sep 2011 – May 2012
Sep 2012 – May 2013
23
21
19
17
15
13
11
9
7
Feb
Mar
Apr
May

2011 vs 2012


↔ Condition
↔ Nutrition
Shift in peak
↓ Reproduction
Delta smelt
SFE
↓ Turbidity
↑ Temperatures
↑ Salinity
Stress
Poor Health
Stress
Potential
Xenobiotic
↑ Lipidosis (contaminants)
↑Pathogens
↑EROD (PAH's, PCB's, Biocides)
↓ AChE (OP's, Carbamates)

Histopathology Indices

- Comparison
  - Turbidity Study 2010
  - FLaSH 2011
  - Special Turbidity Study 2012
- Dry year
  - Higher lesion scores



Missing Rows 186

Oneway Anova

Summary of Fit

Rsquare	0.276097
Adj Rsquare	0.253116
Root Mean Square Error	4.516833
Mean of Response	10.28788
Observations (or Sum Wgts)	66

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Year	2	490.2181	245.109	12.0141	<.0001*
Error	63	1285.3122	20.402		
C. Total	65	1775.5303			