

Scanning electron micrograph (SEM) of a fish scale from *Salmo salar parr*. The scale exhibits a central core surrounded by numerous concentric, wavy growth rings, indicating the periodic deposition of calcium phosphate crystals. The rings are most distinct in the outer portion of the scale. The background shows other scales and the underlying skin structure.

Detecting Surface Water Contaminants Using Fish Scales

Penobscot Science Exchange
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Salmo salar parr scale

10 kV

50X

500 μm

Outline

- Project background
- Scale Architecture
- Biomarker: Cytochrome P450 1A (CYP1A)
 - Enzyme activity
 - Immunohistochemistry (IHC)
 - mRNA quantification
- Future work
- Project pitch

Project background

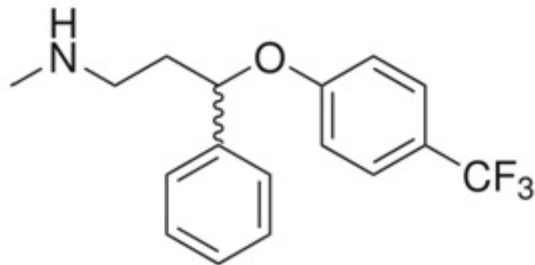
- Are field fish exposed to contaminants?
- Traditional contaminant screens are lethal
- Scales removal is non-lethal, non-invasive
- Regularly collected (NOAA, DMR, IFW)
- Rapid collection, long storage (field friendly)

Biomarkers in scales

- Biomarker proteins can be induced in fish scales:
 - Organic contaminants (PCBs, oil) → Cytochrome P450 1A (CYP1A) mRNA [1,2]
 - Estrogens (Endocrine Disruptors) → Estrogen receptor (ER) mRNA [3]
 - Toxic Metals (Mercury, Cadmium) → Metallothionein (MT) mRNA [4]
- Scales as biosensors (not models) is novel idea

Contaminants and Biomarkers of interest

Pharmaceutical



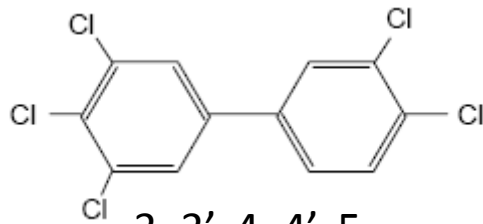
Fluoxetine (Prozac)

Considerations:

Metals may inhibit ER expression

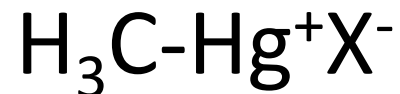
Estrogens may inhibit CYP1A expression

Industrial/Organic compound



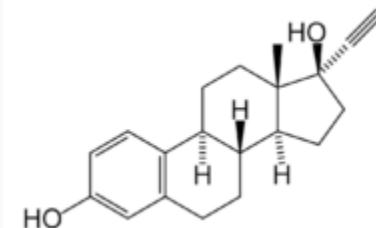
3, 3', 4, 4', 5-
pentachlorobiphenyl
(PCB-126)

Toxic metal



methyl mercury

Endocrine disrupter



17-α-Ethinylestradiol

CYP1A

MT mRNA

ER mRNA

Test Species: Atlantic Salmon (*Salmo salar*)

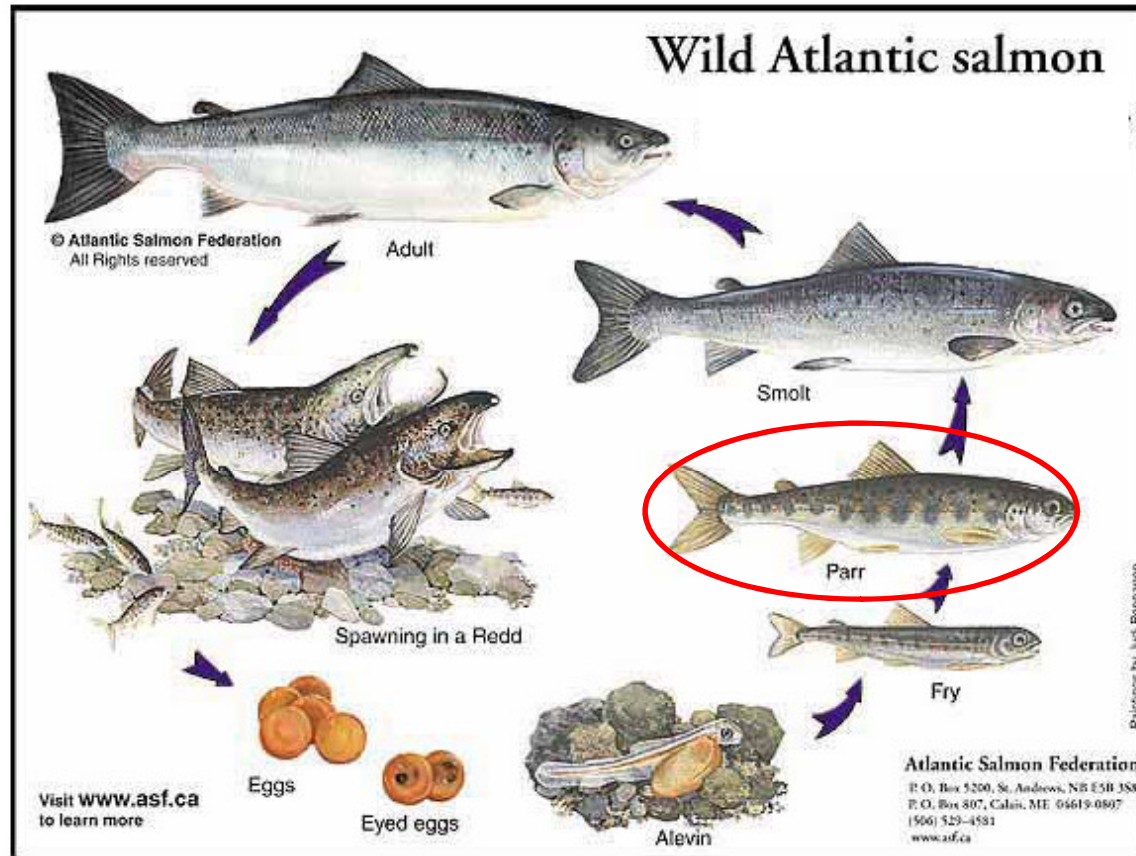


Figure 1.6. Life History of Atlantic salmon (*Salmo salar*). Taken from the Atlantic Salmon Federation (www.asf.ca)

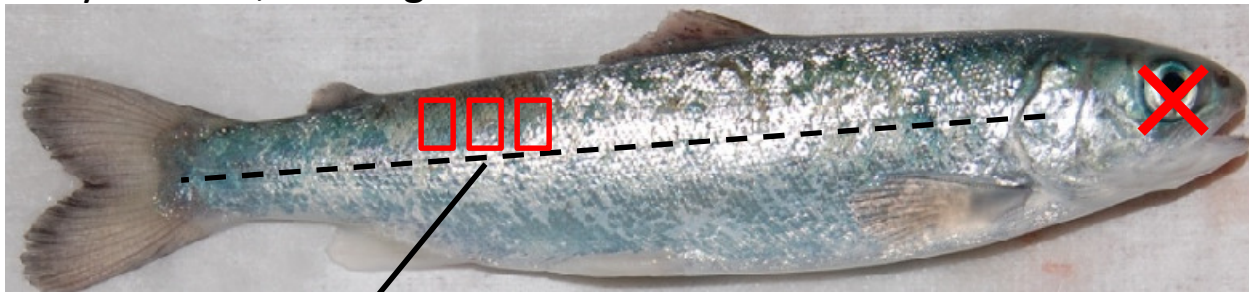
HYPOTHESES

- Can measure biochemical response of fish to surface water contaminants using scales
- Our biomarkers are reliable in contaminant mixtures

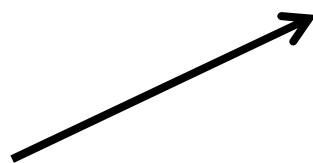
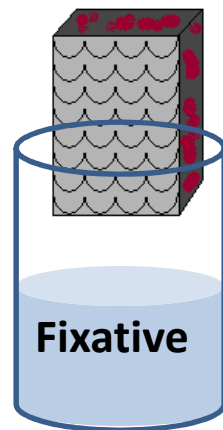
Scale Architecture

Schematic of imaging process

< 2 years-old, 60-80 g



I am dead.

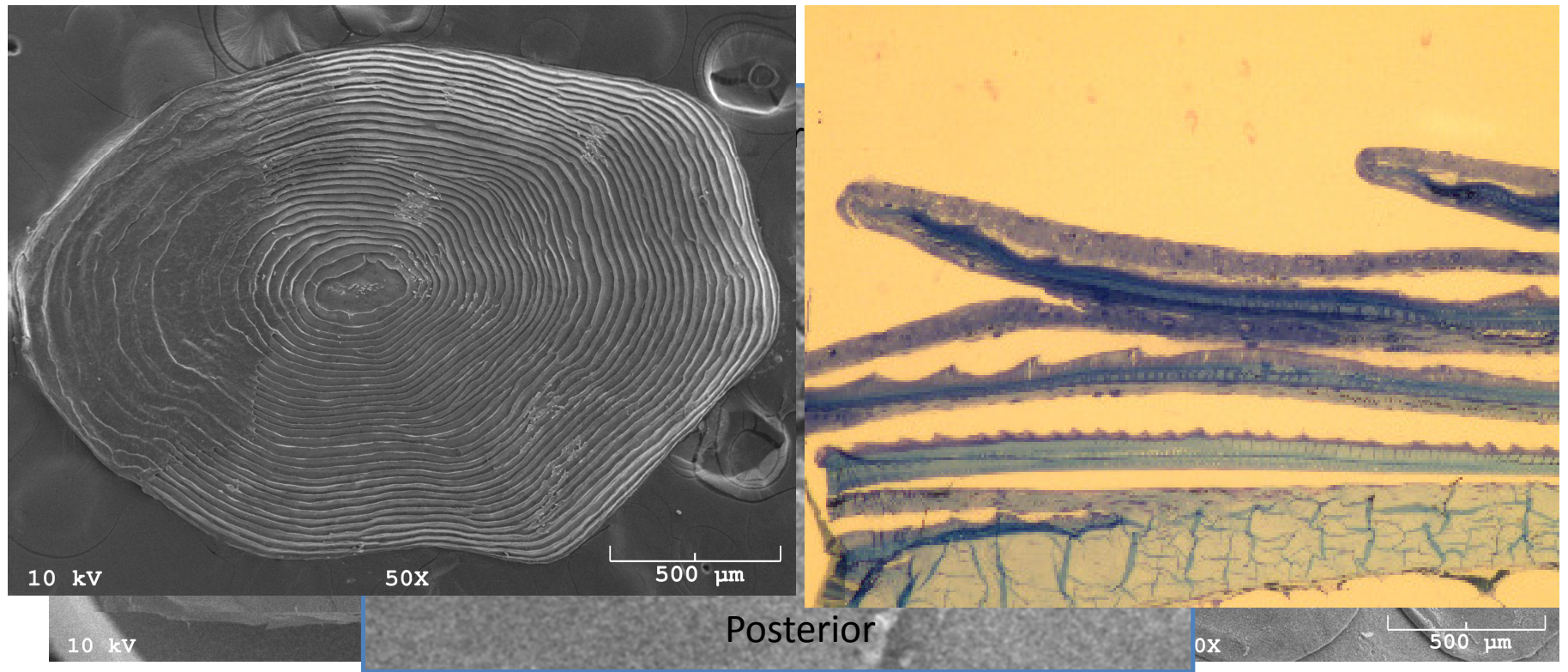


Scanning Electron Microscope (SEM)



Light Microscope

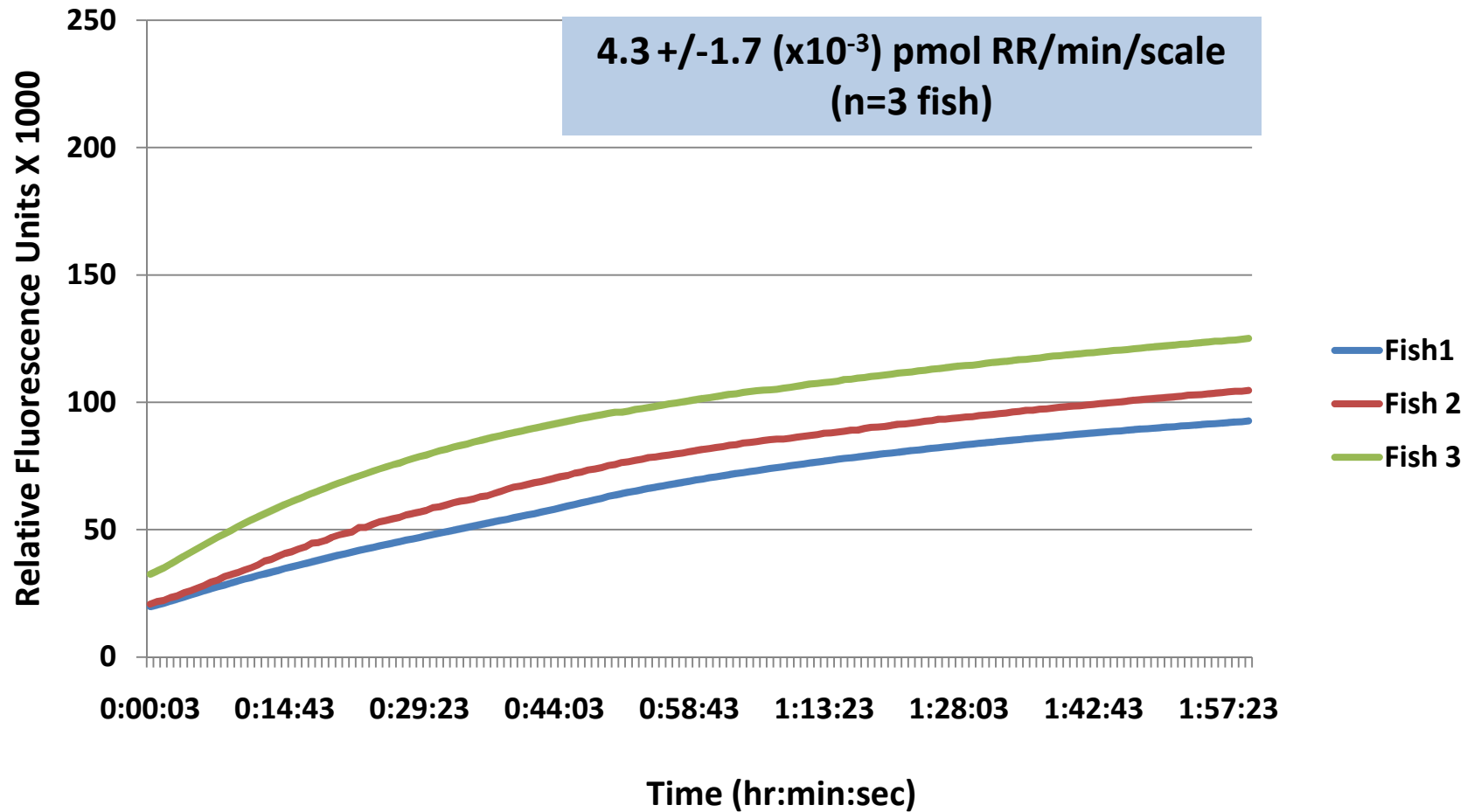
Scale Architecture



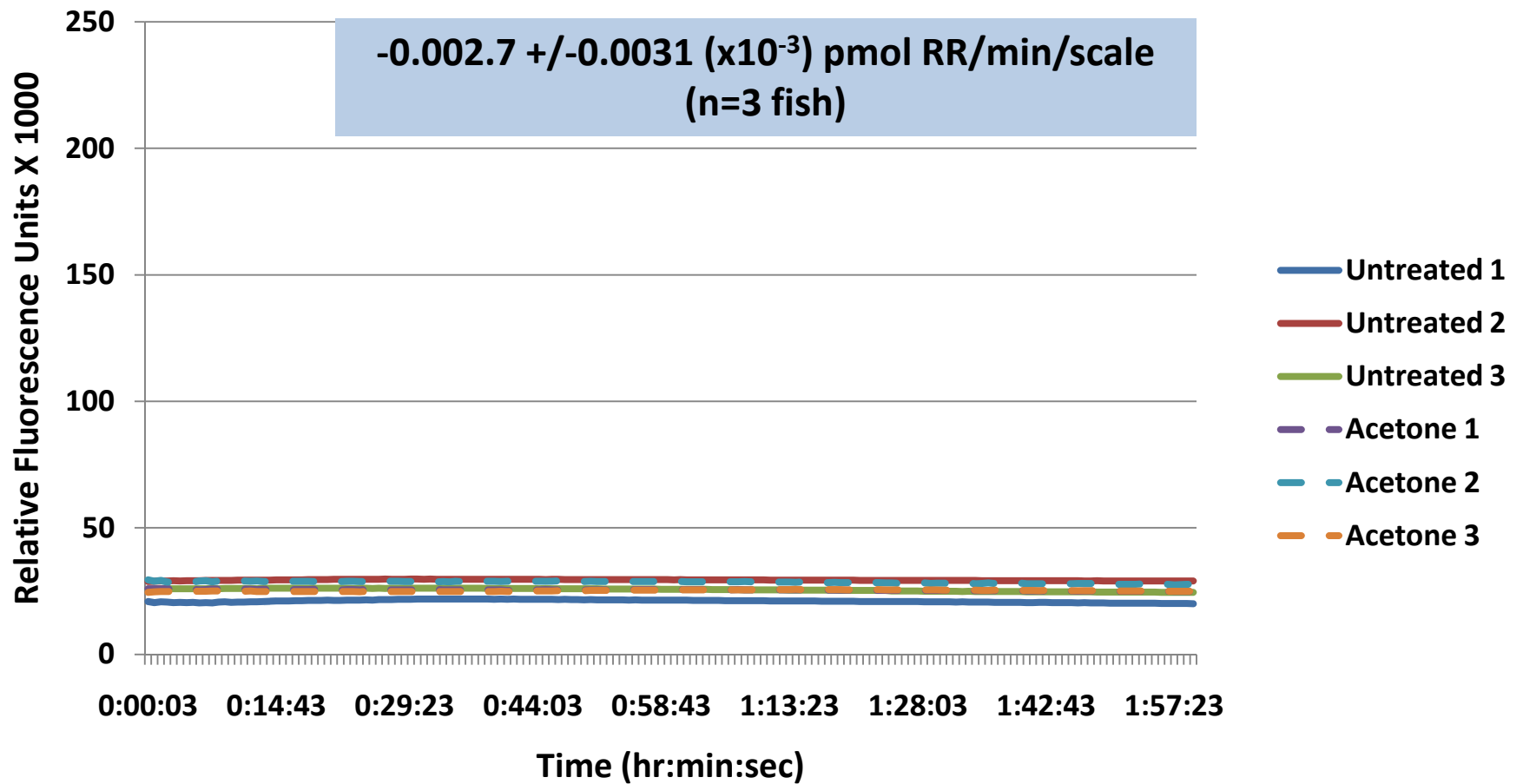
Images courtesy of Kelly Edwards

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CYP1A activity: 32.7 ppb PCB126-Treated Fish



CYP1A activity: Control Fish



Go to schematic 3

Images of IHC slides

Non-specific

Architecture:

(-) Ab:

CYP1A (+) Ab:

Epidermal layer

Scale layer

Dermal layer

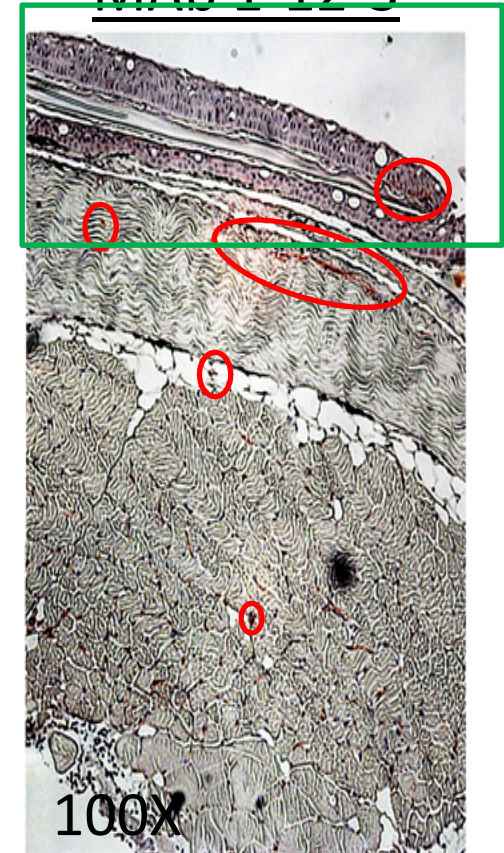
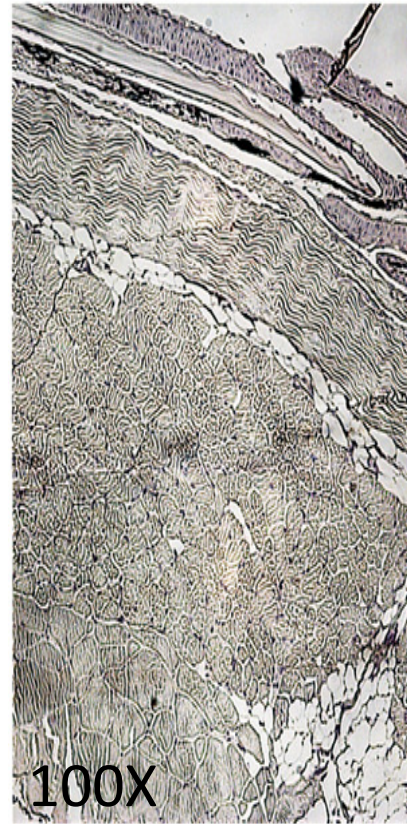
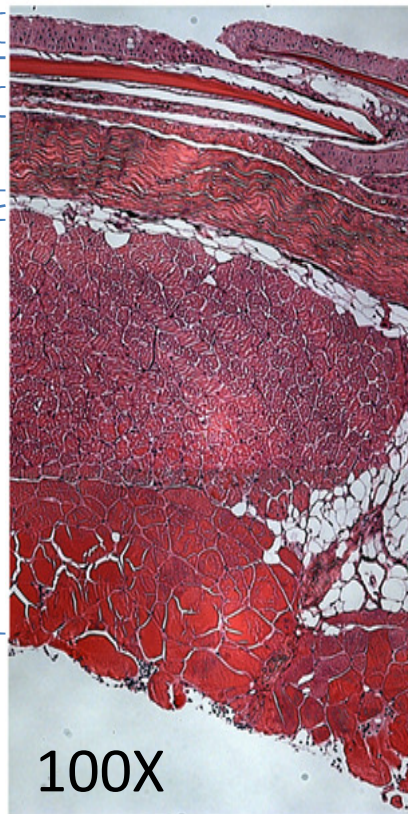
Fat layer

Muscle layer

H&E

MOPC 31

MAb 1-12-3

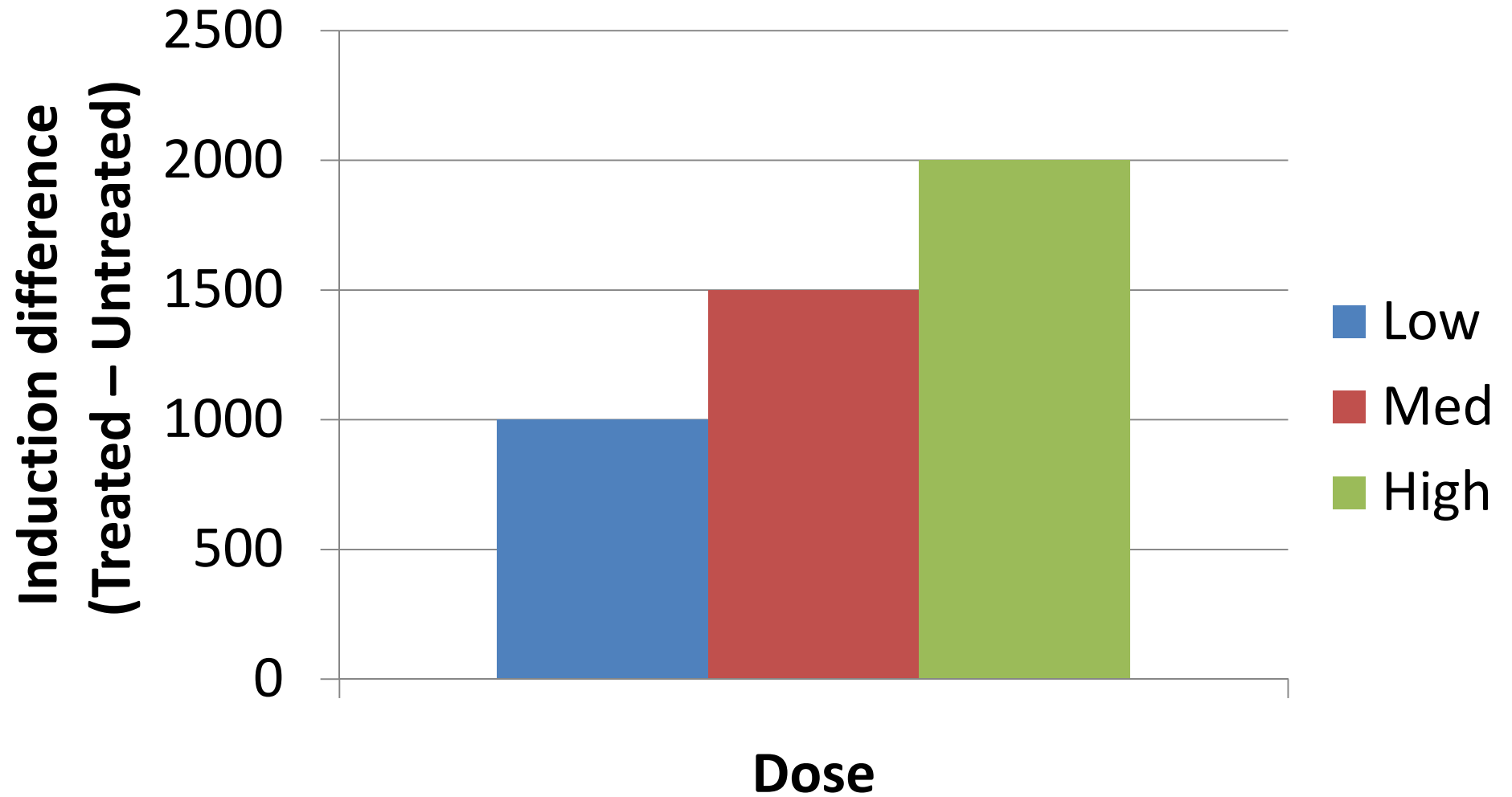


Go to schematic 4

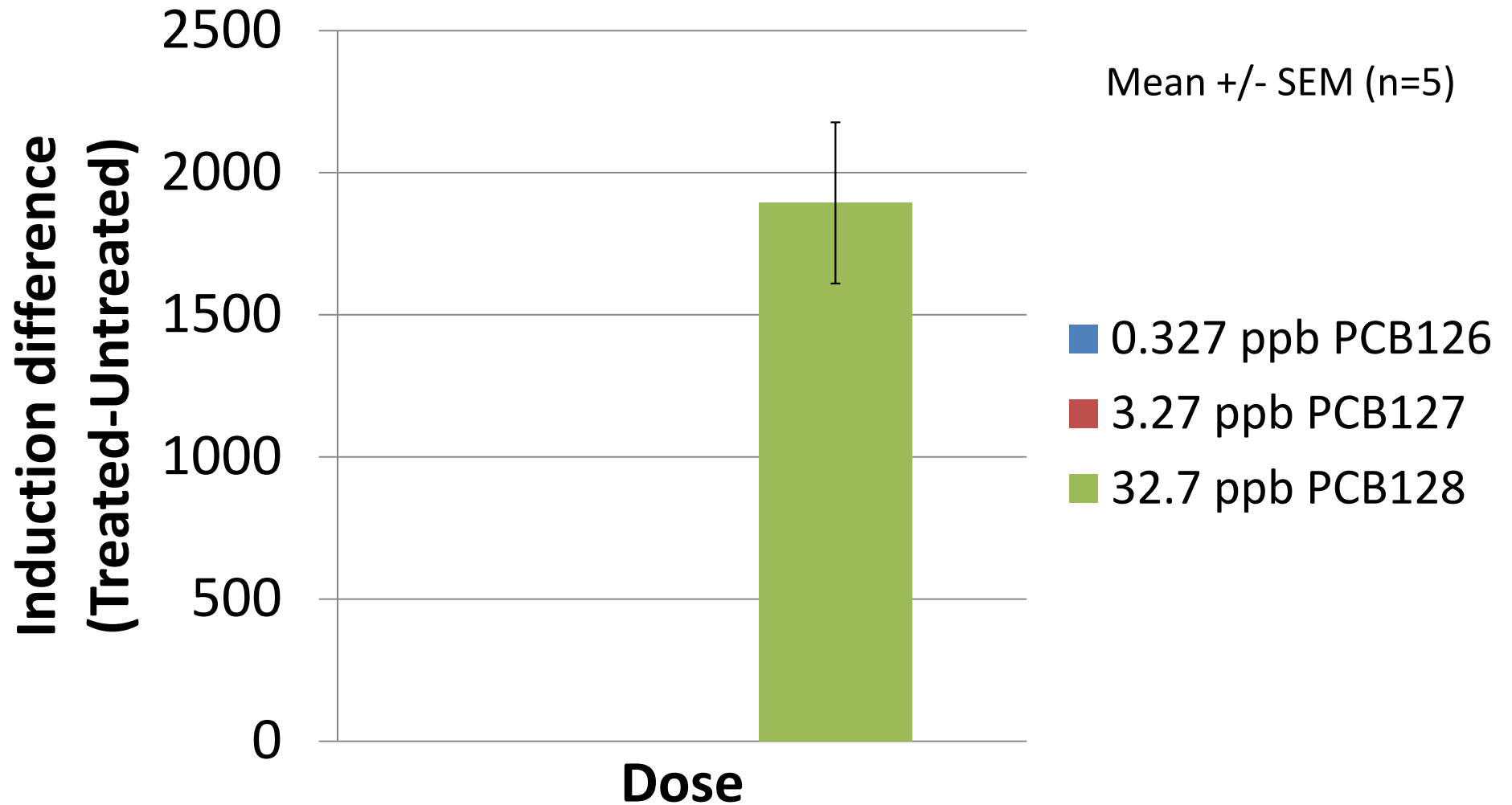
CYP1A mRNA results to date

- High quantity and good quality RNA
- CYP1A and B-actin primer testing successful
- qPCR Optimization:
 - Primer and template concentrations determined
 - B-actin effective housekeeping gene
- Began quantification process

Expected Dose-Response



CYP1A mRNA in PCB exposed fish



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Project pitch

- Scale collection is easy, fast, and non-lethal
- Scales are easy to handle
- Scale storage is easy and long lasting!
- Repeated sampling from same individual

Acknowledgements

- WRRRI funding
- My thesis committee
- Neil Greenberg, ARC, UMaine
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- Ian Bricknell, Sally Malloy, Jennifer Fortier, UMaine
- Becky Van Beneden, Patrick Carlson, UMaine
- Christina Newman, Gregory Ballard, MIHGH

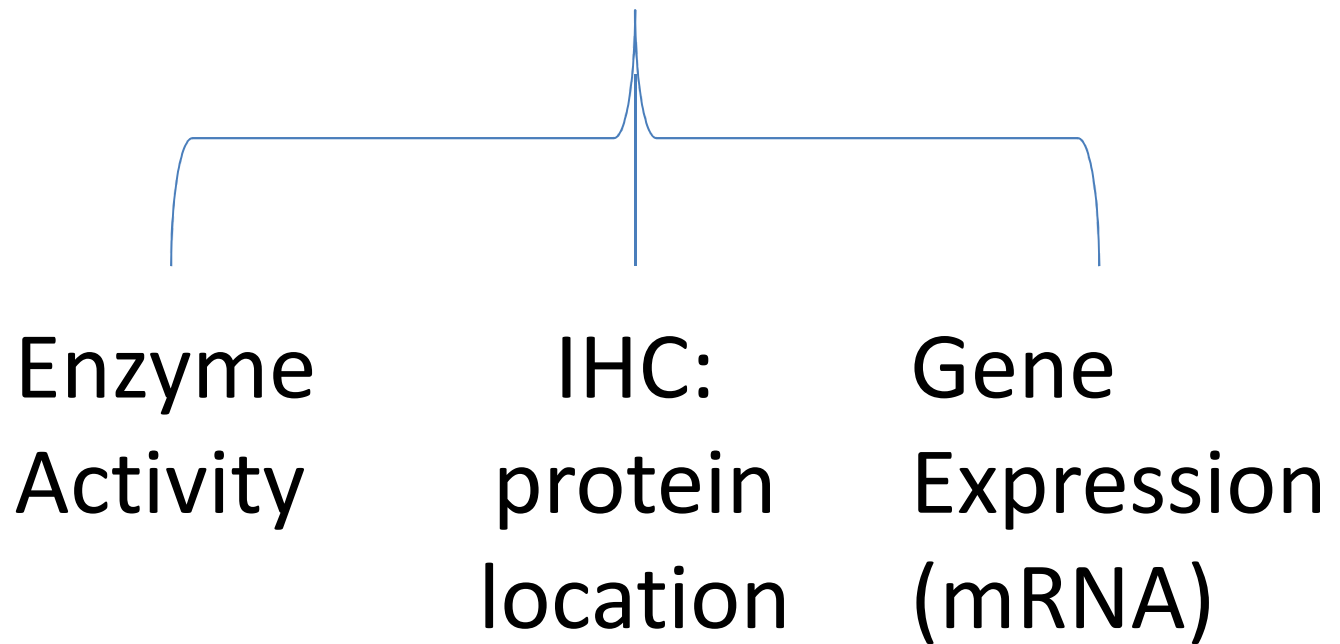
Questions?

Thank you!

Schematic 1: CYP1A overview

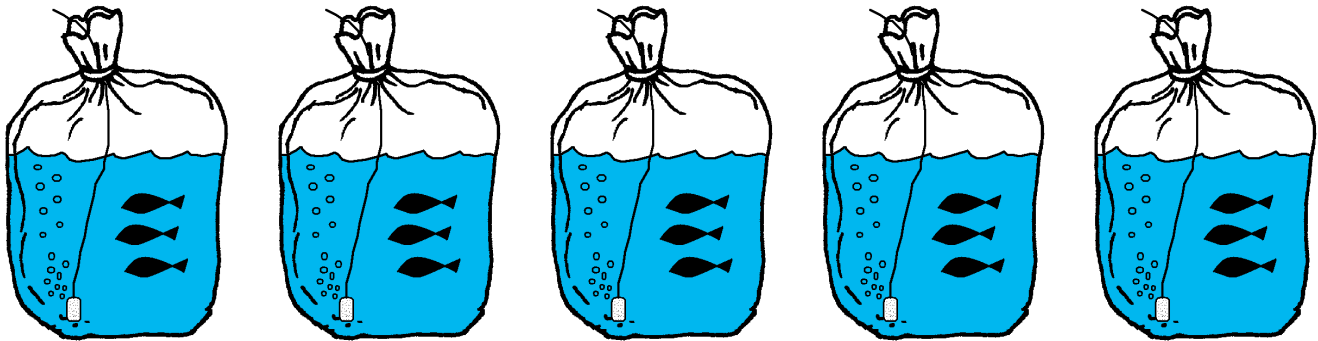


Fish: < 2 years-old, 60-80 g



Go to schematic 2

Schematic 2: CYP1A - Organic Contaminant Biomarker



untreated

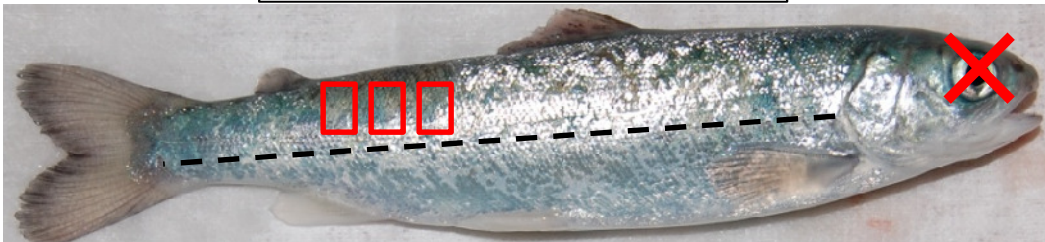
40 ppb acetone
(vehicle)

3.27 ppb
PCB126

32.7 ppb
PCB126

330 ppb
βNF

48 hr aqueous exposure



Fish: < 2 years-old, 60-80 g

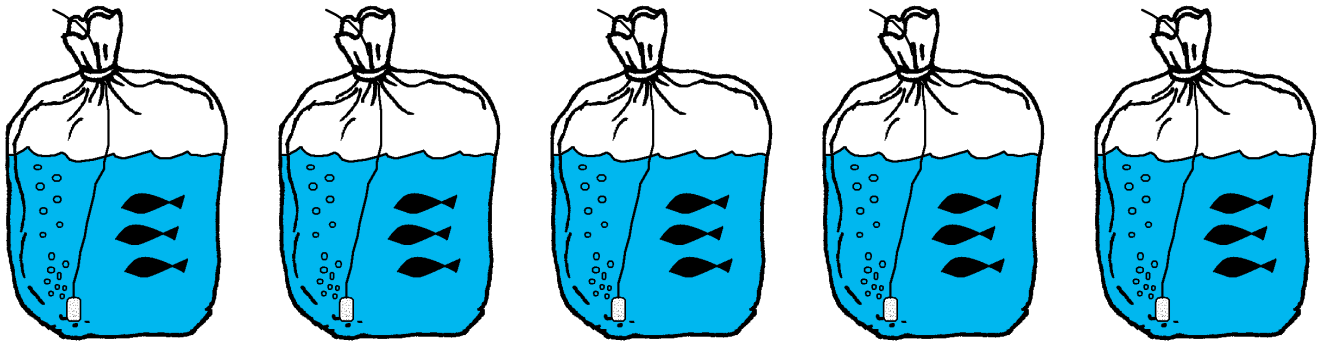
Enzyme
Activity

IHC:
protein
location

mRNA
expression

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Schematic 3: CYP1A - Organic Contaminant Biomarker



untreated

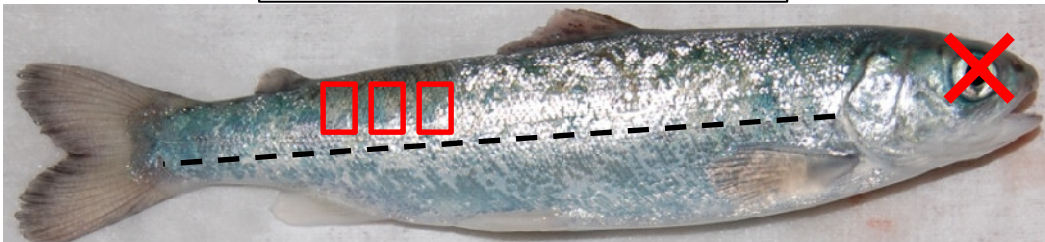
40 ppb acetone
(vehicle)

3.27 ppb
PCB126

32.7 ppb
PCB126

330 ppb
βNF

48 hr aqueous exposure



Fish: < 2 years-old, 60-80 g

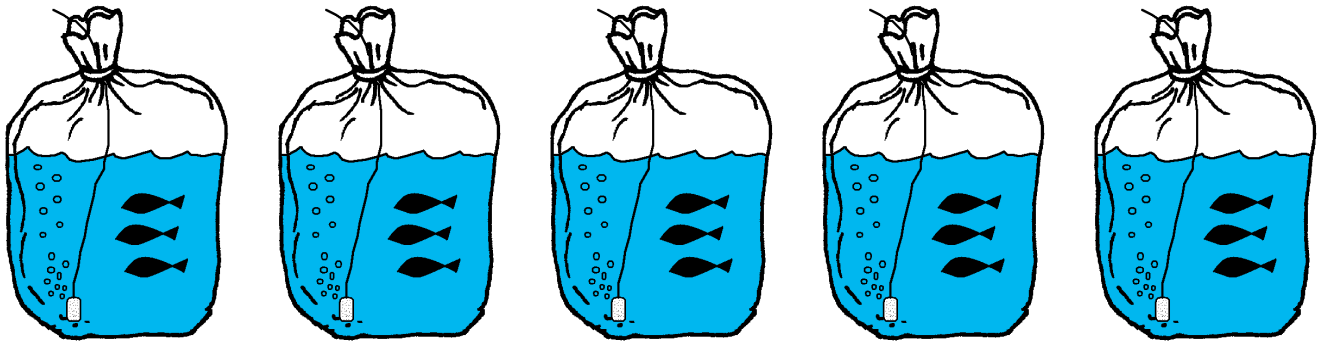
Enzyme
Activity

IHC:
protein
location

mRNA
expression

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Schematic 4: CYP1A - Organic Contaminant Biomarker



untreated

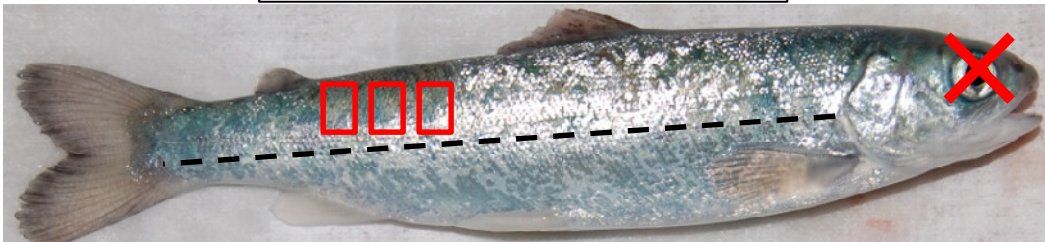
40 ppb acetone
(vehicle)

3.27 ppb
PCB126

32.7 ppb
PCB126

330 ppb
βNF

48 hr aqueous exposure



Fish: < 2 years-old, 60-80 g

Enzyme
Activity

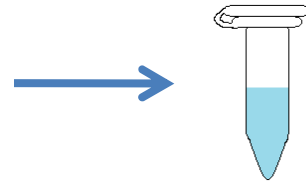
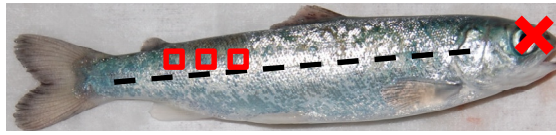
IHC:
protein
location

mRNA
expression

Go to schematic 5

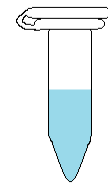
Schematic 5: CYP1A mRNA quantification

Remove scales



RNAlater
(indefinite storage)

Extract RNA

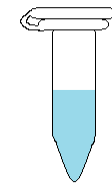


RNA

Reverse Transcribe
to cDNA



<http://www.sci-support.com/items/Applied-Biosystems-PCR-GeneAmp-9700-Thermocycler-1464.htm>



cDNA

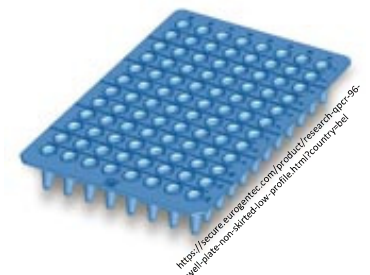
Design/test Primers:
CYP1A, B-actin

Optimize qPCR

Amplify cDNA
(CYP1A, B-actin)



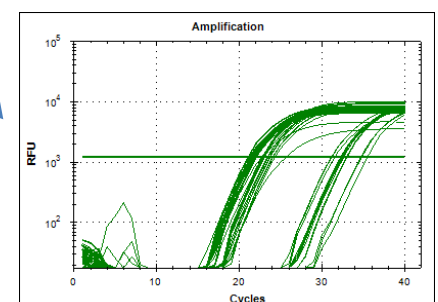
<http://www.gene-quantification.de/bio-rad/>



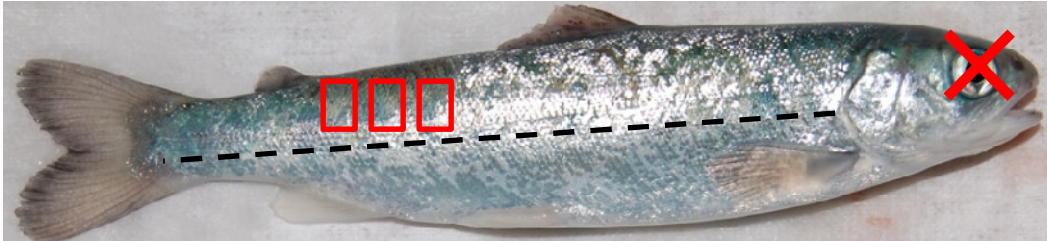
cDNA, primers,
qPCR mix

Quantify

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Schematic 6: Future work



Fish: < 2 years-old, 60-80 g

Enzyme
Activity

IHC

mRNA

Repeat

Prozac (CYP1A)

EE2 (ER)

Metal (MT)

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