



Transcription profiling: a multi-biomarker approach

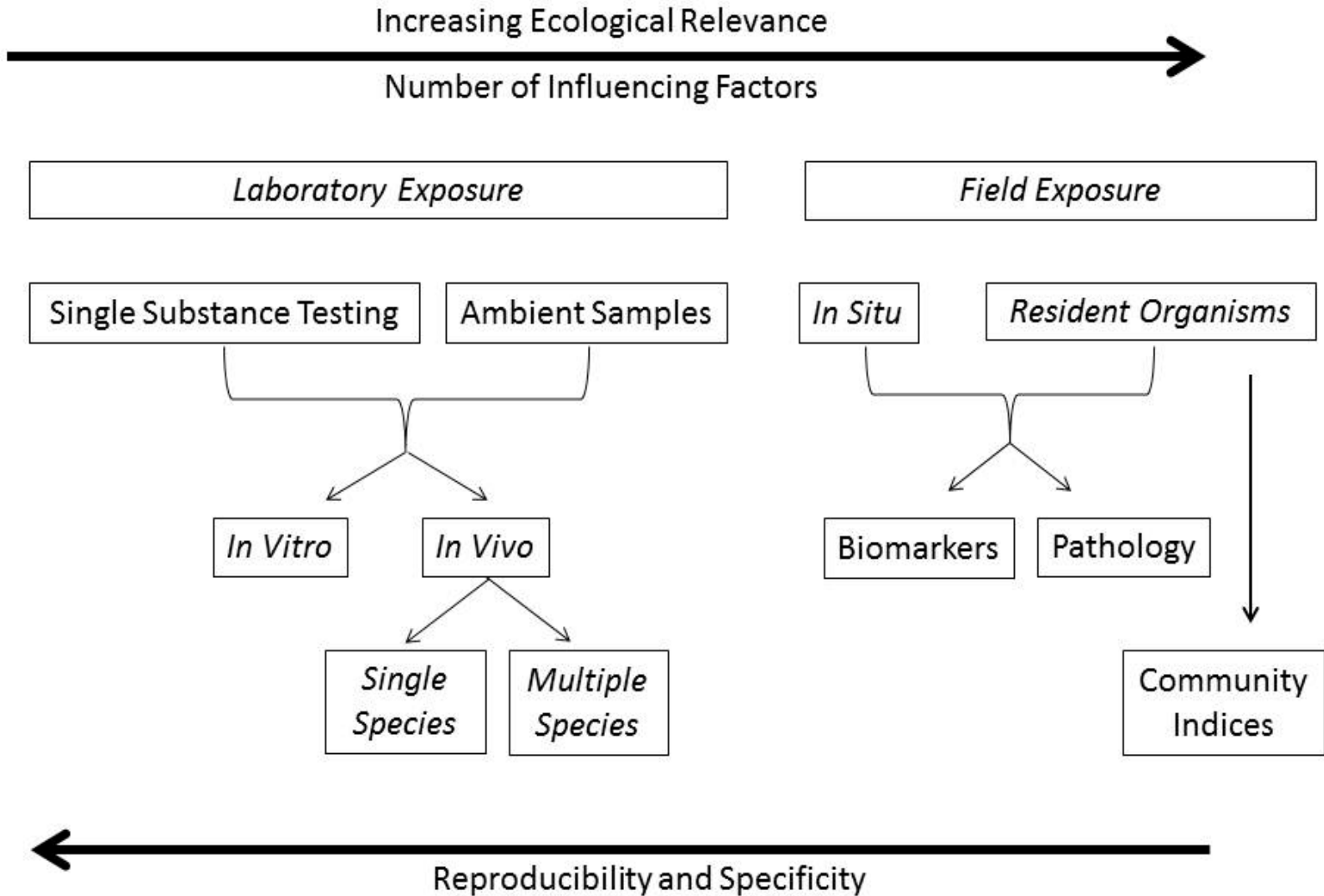


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Biomarker Workshop - October 24-25th, 2013

Biological Approaches for Measuring Ecotoxicity of Chemicals



Molecules

to

Higher Levels of Biological Organization

Endocrinology

Histopathology

Immunology

Physiology

Detoxification

Cells

Development and
Reproduction

Population and
Community



230 m

345 m

1 280 m

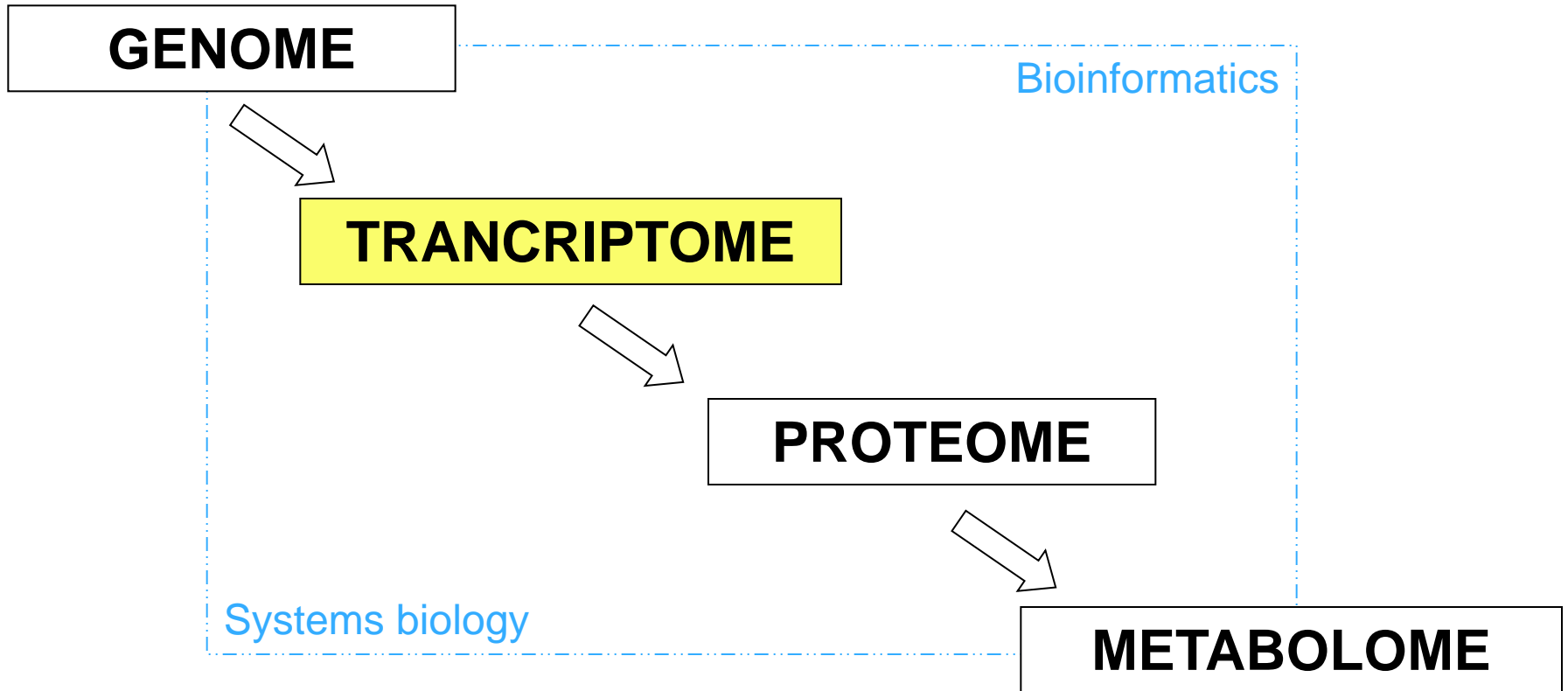
345 m

1 970 m

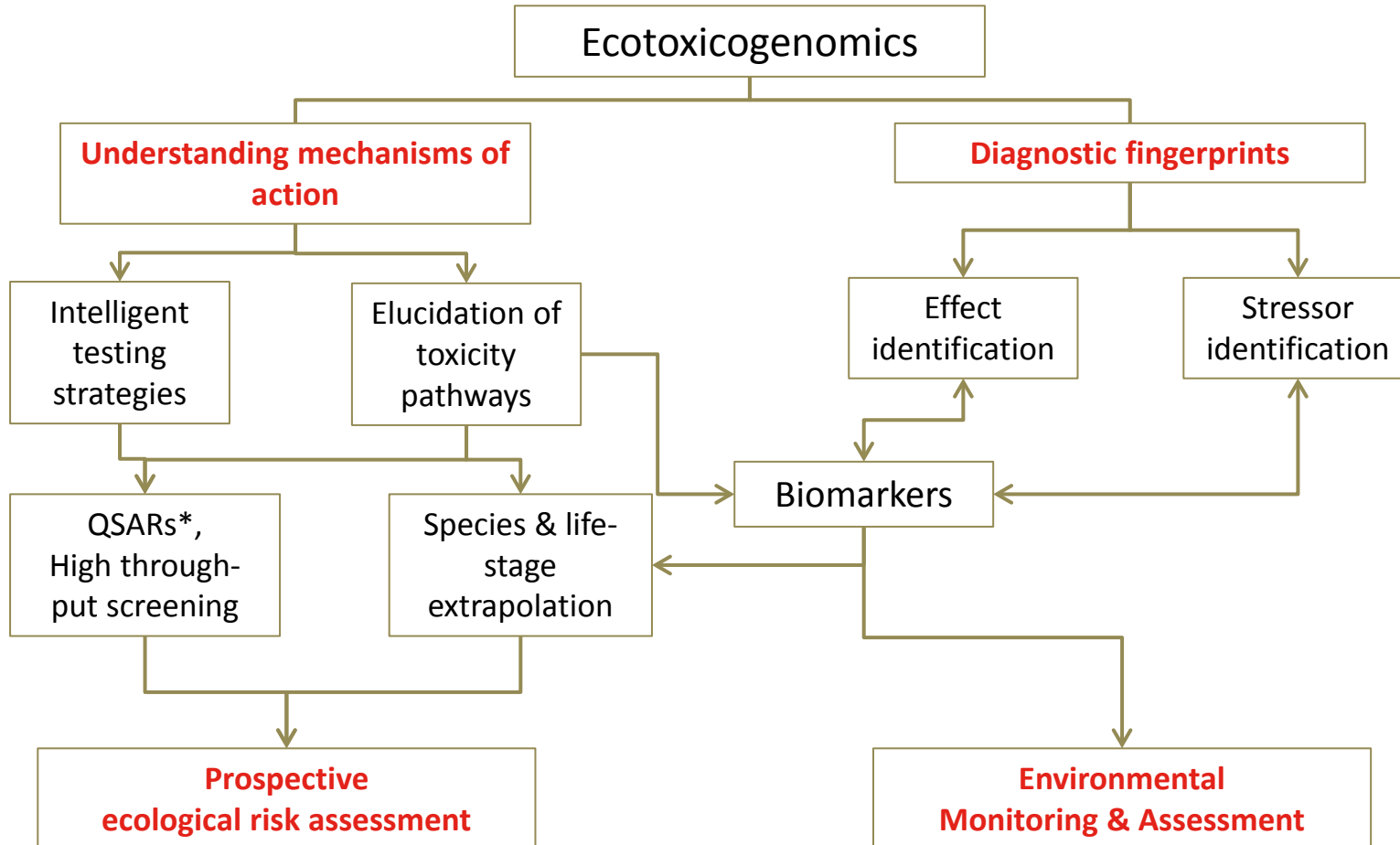
Molecules



“omics”



The Promise of Ecotoxicogenomics



*quantitative structure activity relationships

Based on: Villeneuve et al. 2011, in 'Applications of Toxicogenomics in Safety Evaluation and Risk Assessment' (Boverhof, Gollapudi, eds), John Wiley & Sons.

Ecotoxicogenomics for Environmental Monitoring

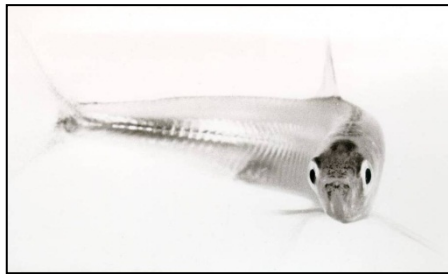
From:

- Multiple physiological biomarkers with individual protocols, sample, skill and equipment needs
- Resource intensive when adapting to non-model species

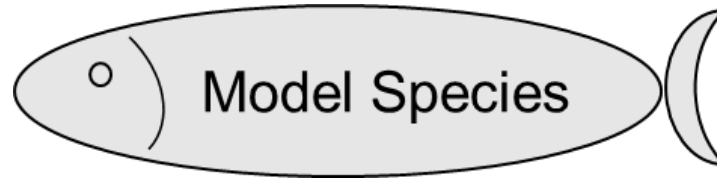
To:

- Multiple molecular biomarkers with one protocol; very small sample volume
- Application in non-model species relatively easy

... From Genes to Higher Level Effects ...



Delta Smelt
Biomarker Genes



Other
Information



**Linkages to Ecologically Relevant
Adverse Effects**

e.g. survival, growth, reproduction,
behavior

Gene Expression Omnibus



GEO is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles.



- [Danio rerio \(5620\)](#)
- [Oncorhynchus mykiss \(2012\)](#)
- [Salmo salar \(1366\)](#)
- [Pimephales promelas \(865\)](#)
- [Fundulus heteroclitus \(777\)](#)
- [Oncorhynchus nerka \(491\)](#)
- [Coregonus clupeaformis \(362\)](#)
- [Sparus aurata \(345\)](#)
- [Oncorhynchus tshawytscha \(243\)](#)
- [Salvelinus fontinalis \(235\)](#)
- [Micropterus salmoides \(230\)](#)
- [Gillichthys mirabilis \(199\)](#)
- [Perca flavescens \(178\)](#)
- [Oryzias latipes \(177\)](#)
- [Gasterosteus aculeatus \(125\)](#)
- [Haplochromis burtoni \(107\)](#)
- [Solea solea \(106\)](#)
- [Hypomesus transpacificus \(22\)](#)

...as of October 21st, 2013



California DFW-ERP Grant # E1183010



Interagency Ecological Program (IEP) Grant # 46-10040

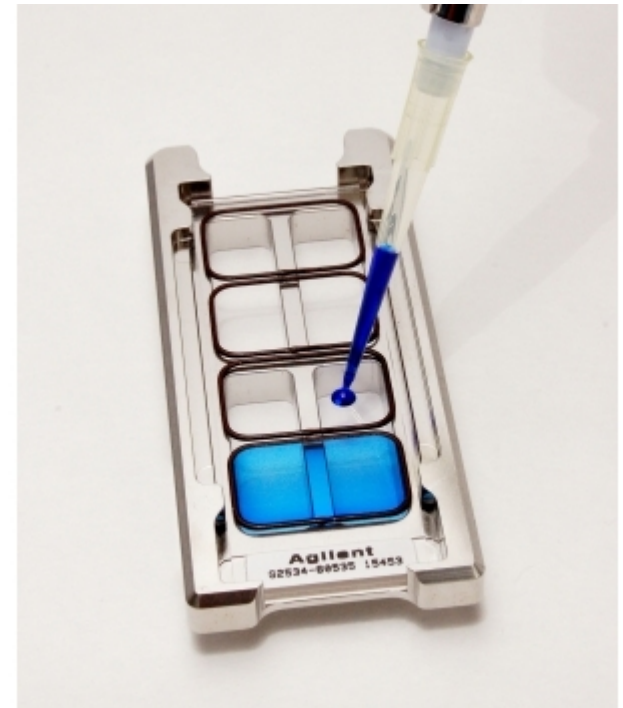


Delta Stewardship Council (DSC) Grant # 201015533, & Bureau of Reclamation (BOR) Gant # R12AP20018

Hypomesus transpacificus: 18,409 features are represented on the microarray, with 2 probe targets per feature, plus Agilent controls on an quadruplex microarray (4x44K).

Menidia beryllina: 14,393 individual genes are represented on the microarray, with 3 probe targets per gene, plus Agilent controls on an Agilent quadruplex microarray (4x44K).

Spirinchus thaleichthys: Agilent quadruplex microarray (4x44K) is currently being developed.



Connon lab and collaborators

Research examples

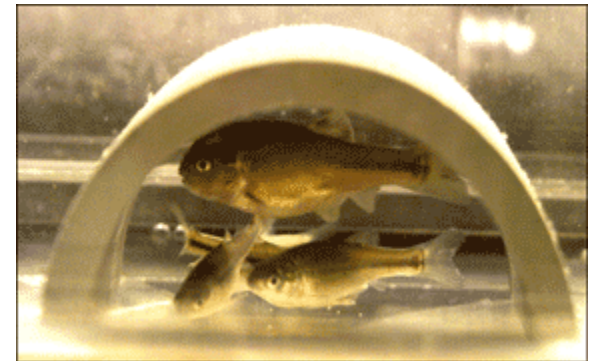
Species

- Fathead minnows
- Delta smelt
- Steelhead
- Silversides*

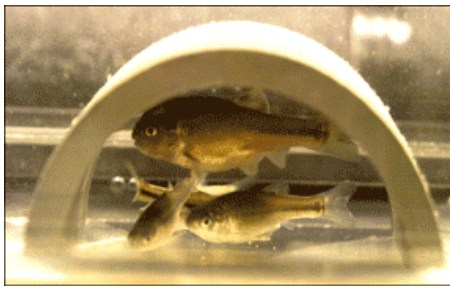


Stressors

- Contaminants
- Salinity
- Turbidity
- Temperature
- Disease
- Multiple



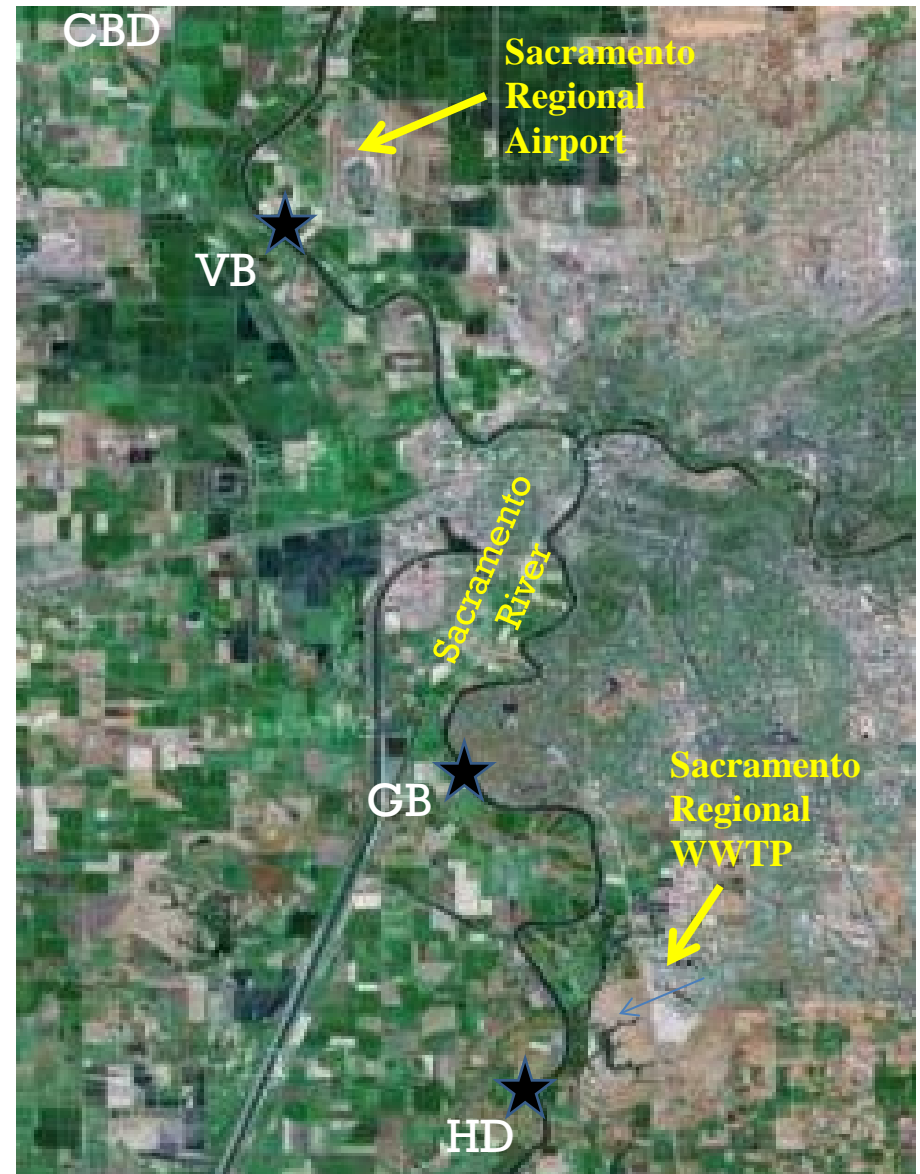
*Susanne Brander (next talk)

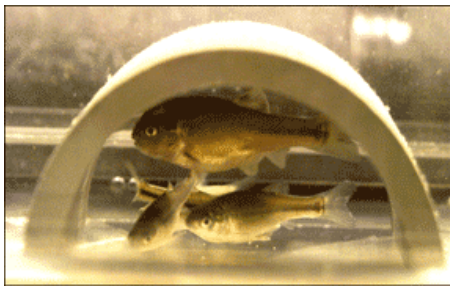


Fathead minnows

- Four study locations sampled for a total of seven weeks
 - Collusa Basin Drain (CBD)
 - Veterans Bridge (VB)
 - Garcia Bend (GB)
 - Hood Field Station (HD)
- Chemical Analysis
 - Organophosphates
 - Pyrethroid Insecticides
 - Pharmaceuticals and Personal Care Products

2008 & 2009 Study Sites

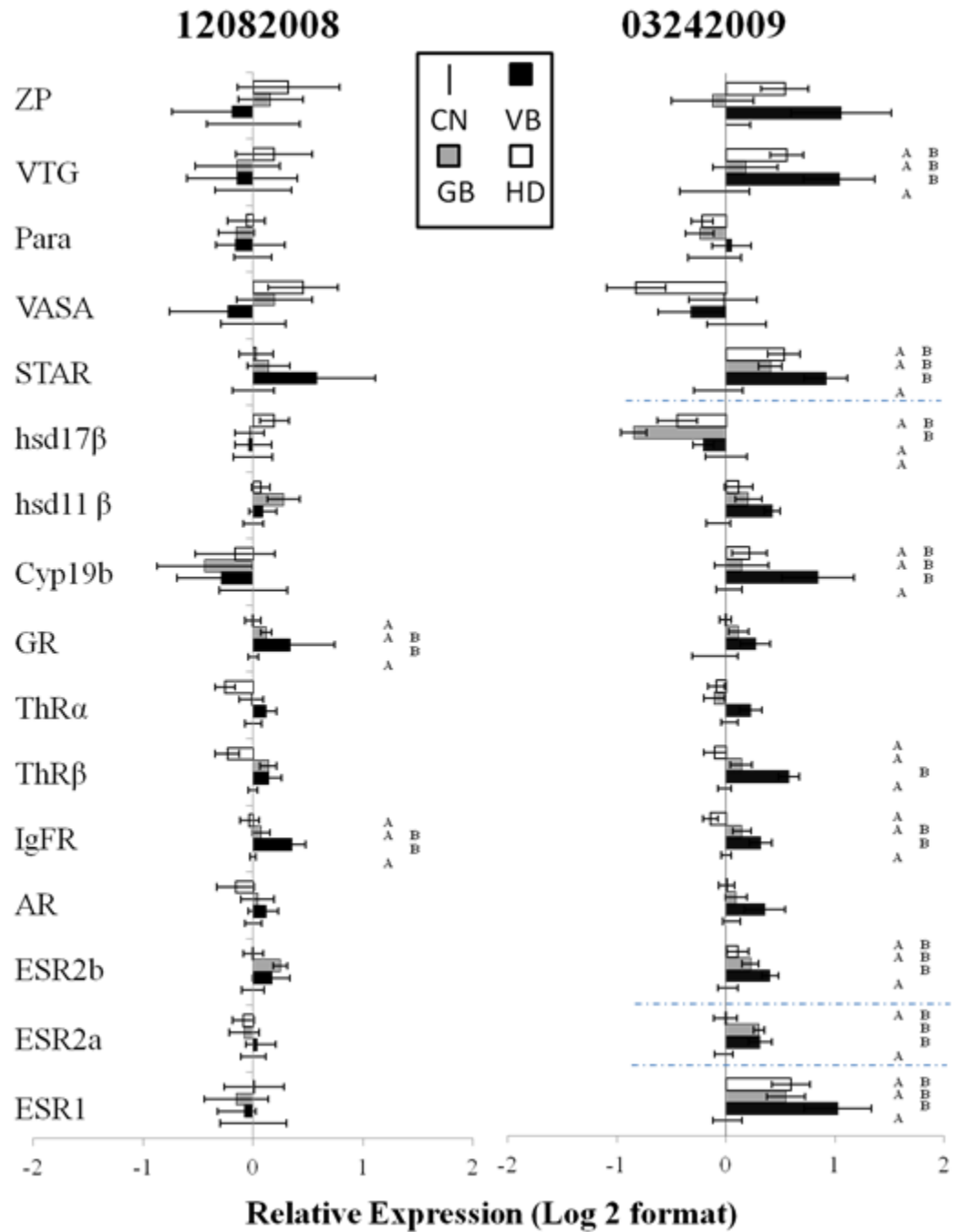


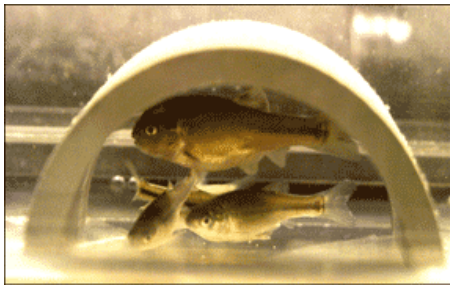


Fathead
minnows

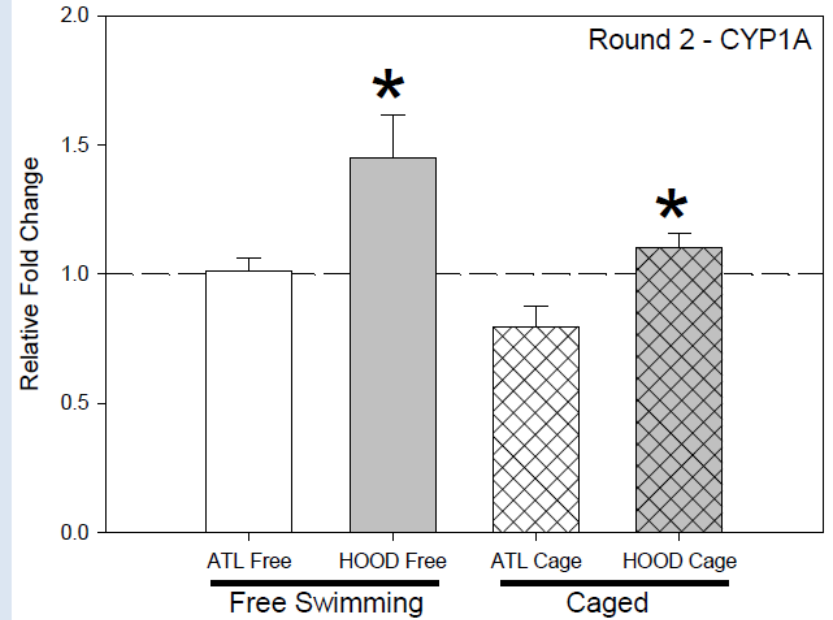
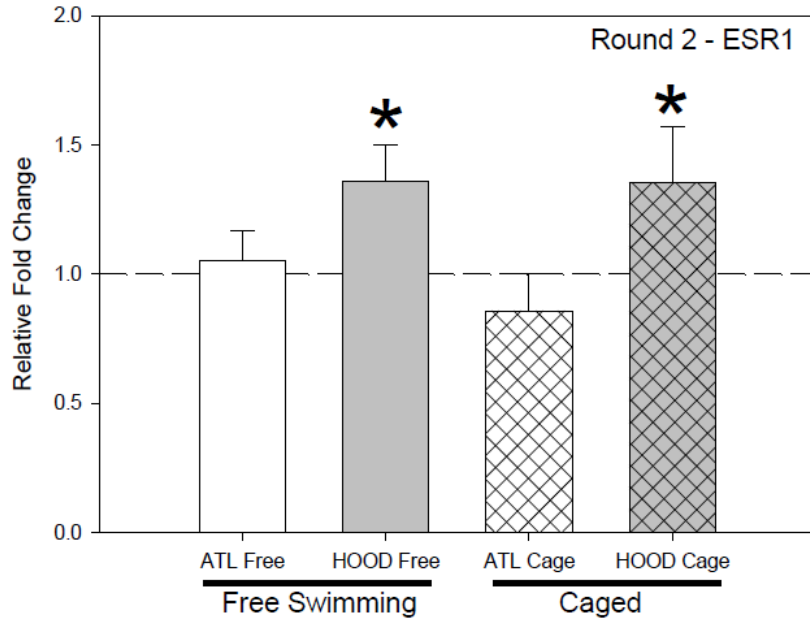
Endocrine Responses

Fritsch et al, in preparation





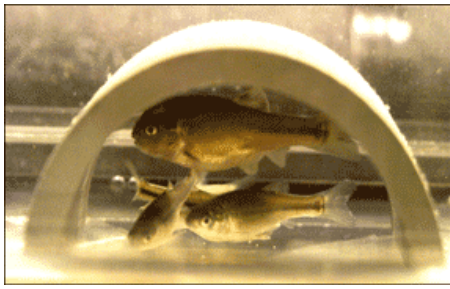
Fathead minnows



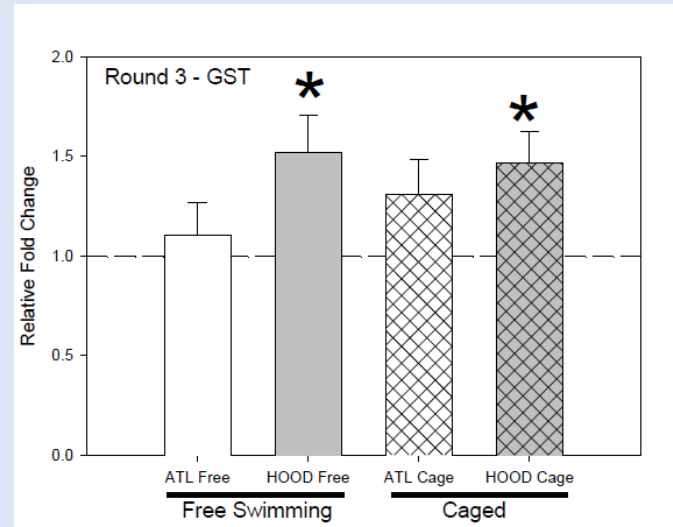
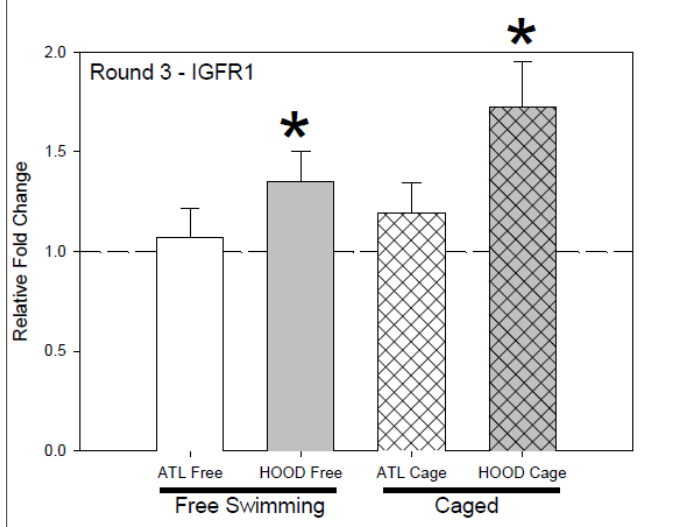
Response to estrogens and mimics



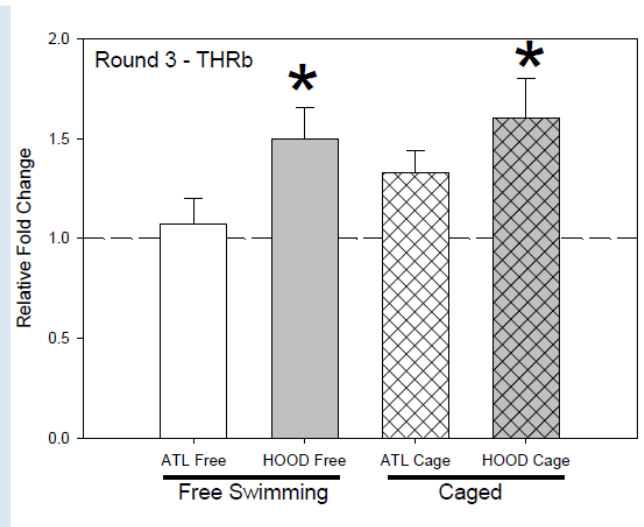
Response to exogenous compounds



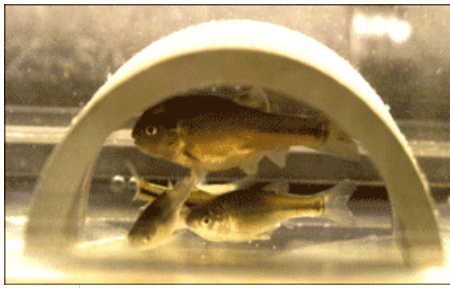
Fathead minnows



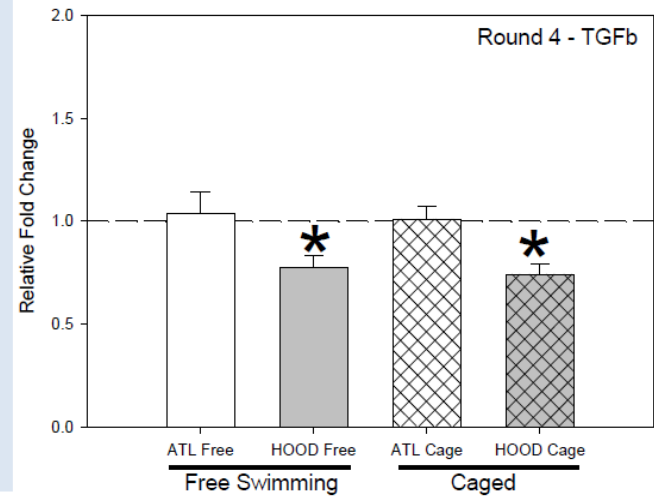
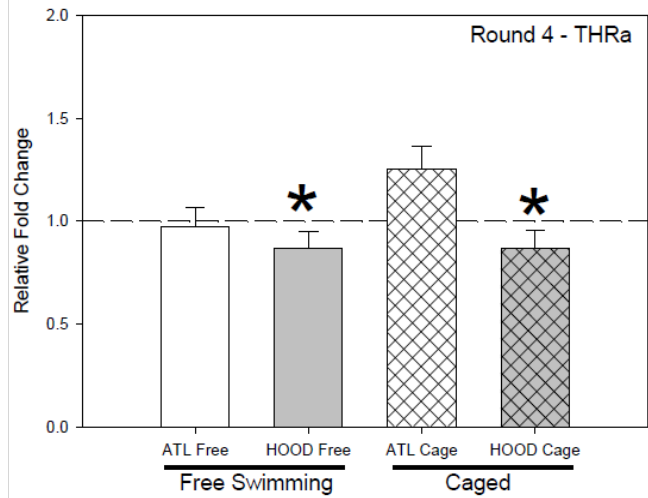
↑
Growth and Development →



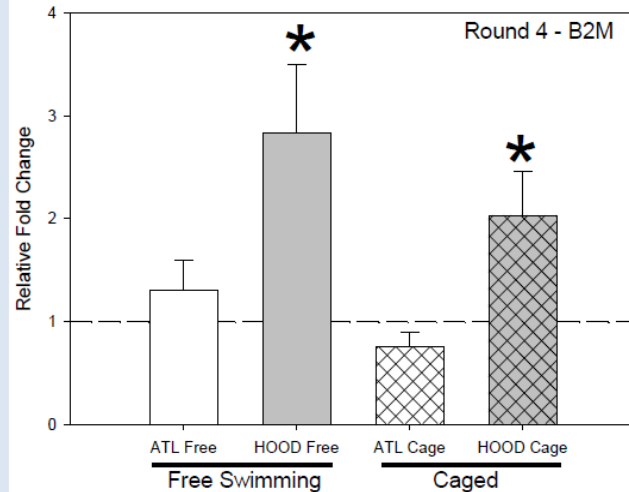
↑
Oxidative Stress



Fathead minnows



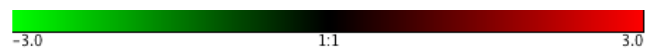
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Growth and Development



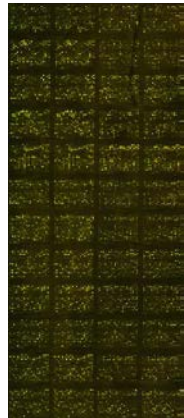
↑
← Immune Function



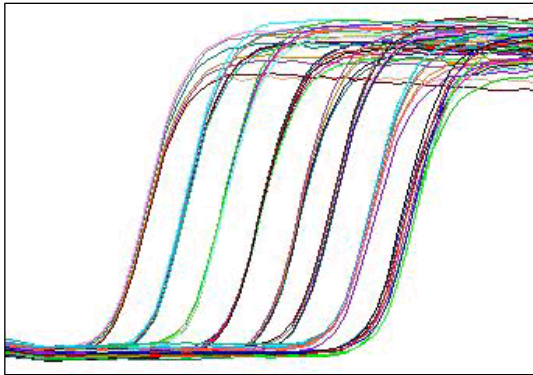
Delta smelt



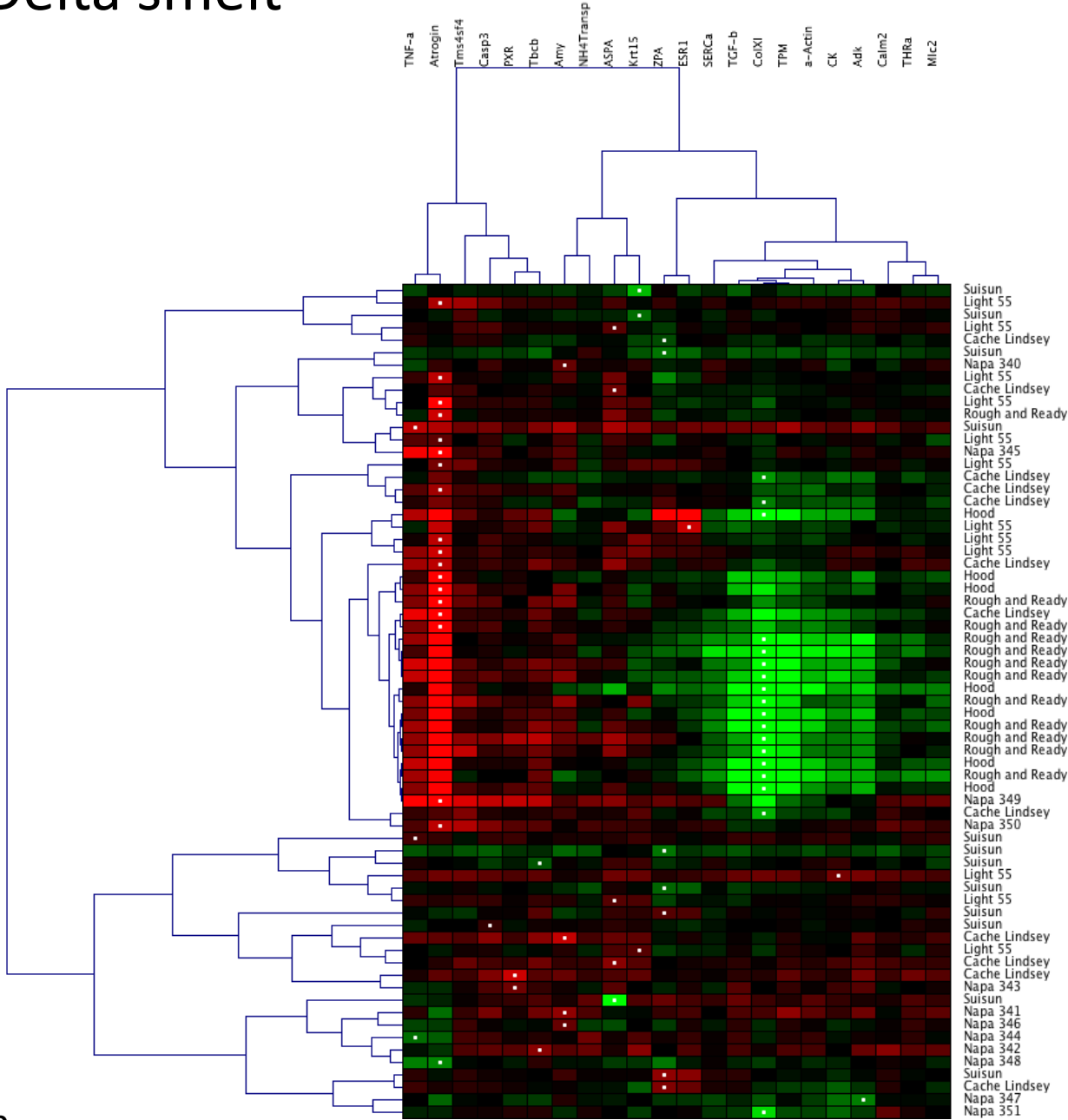
Microarrays



Quantitative PCR

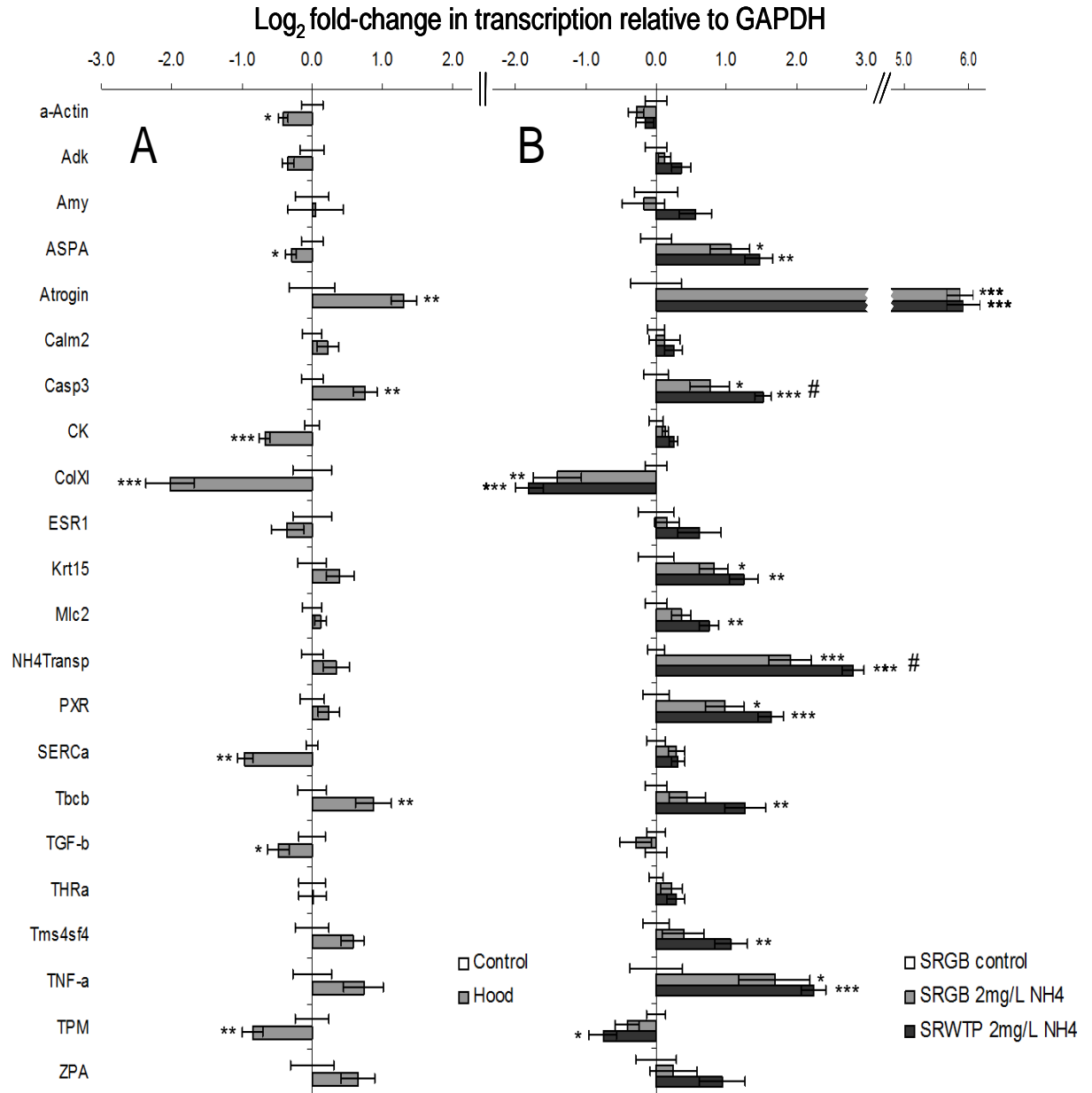
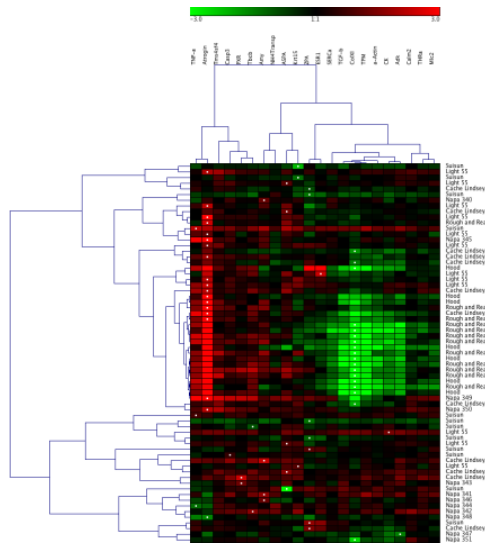


Connon et al, in preparation





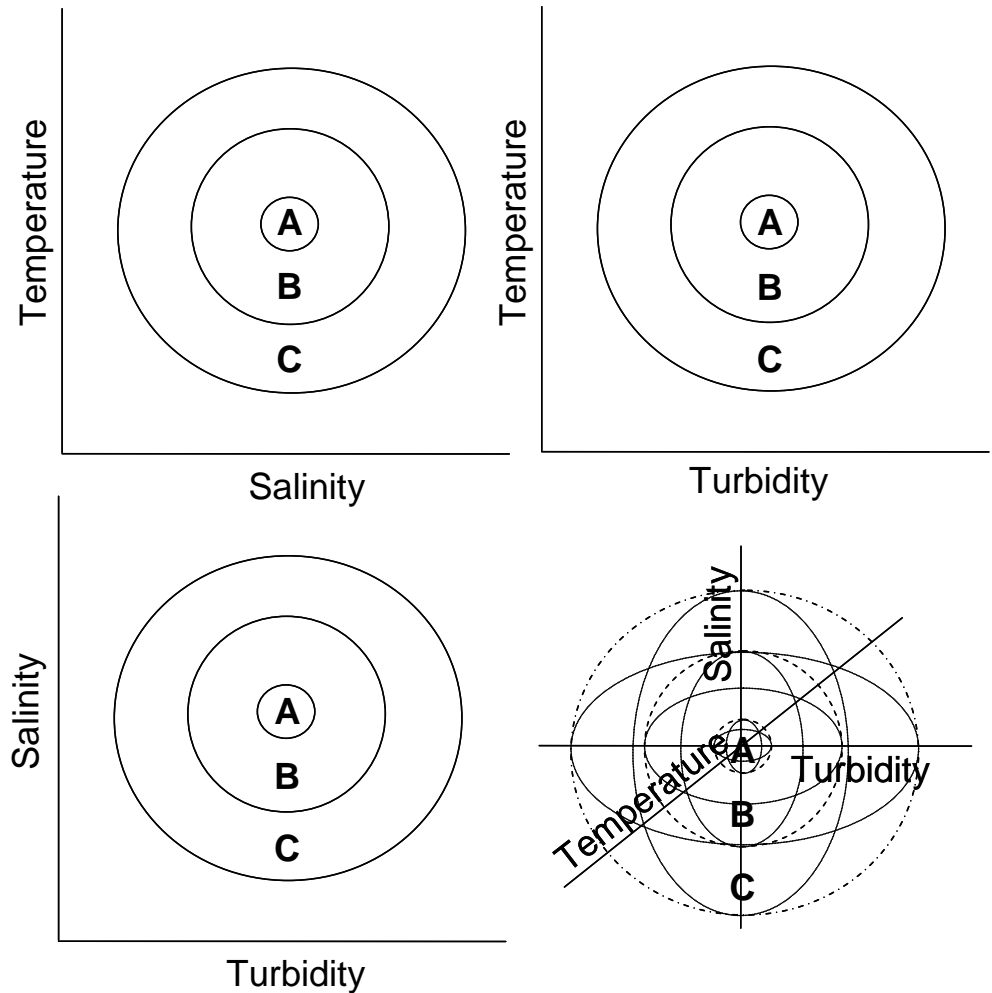
Delta smelt





Delta smelt – Fundamental Niche

A hypothetical characterization of a fundamental niche through stress and performance assessments, as determined by turbidity, salinity and temperature.

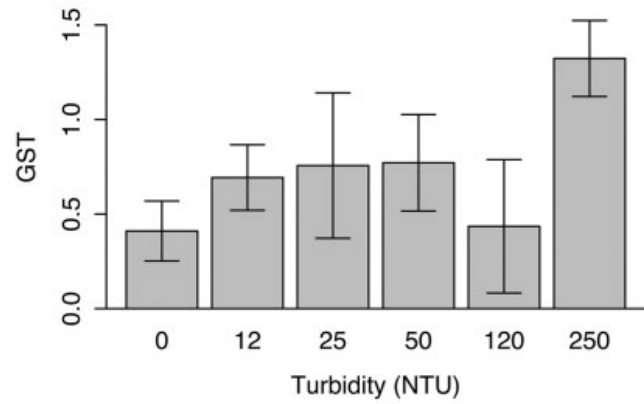
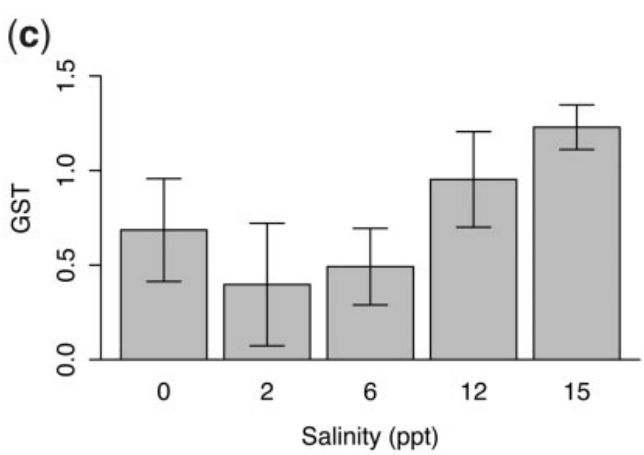
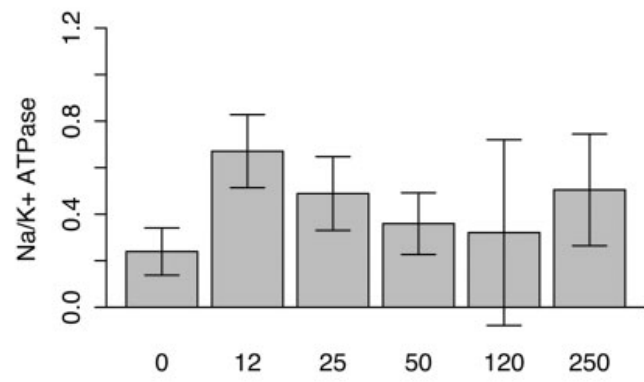
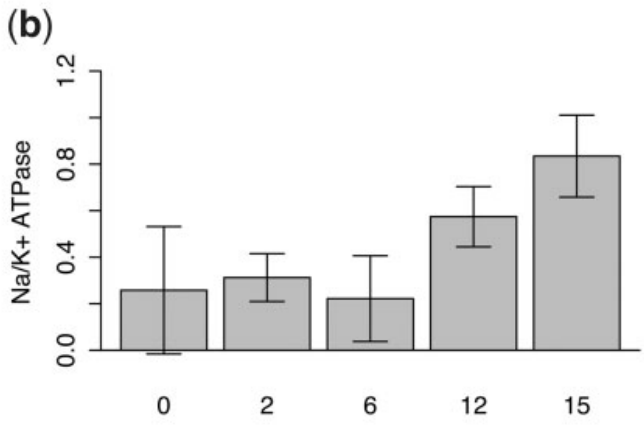
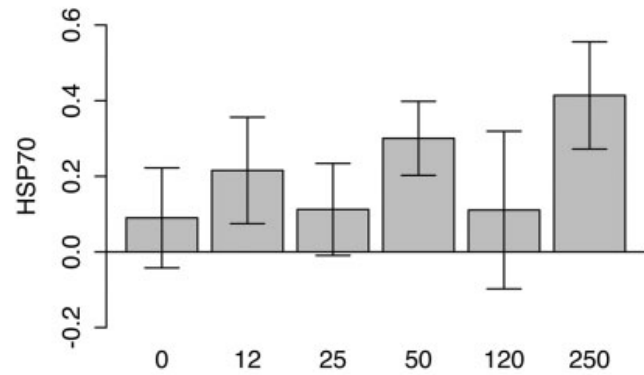
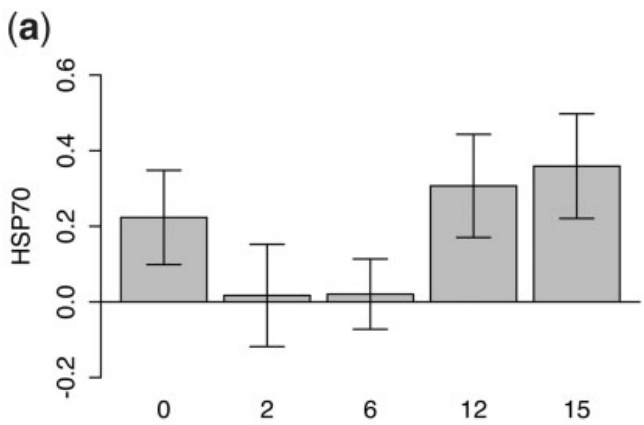


A: optimal response,
B: lowest observable effect level,
C: maximum observable effect level threshold



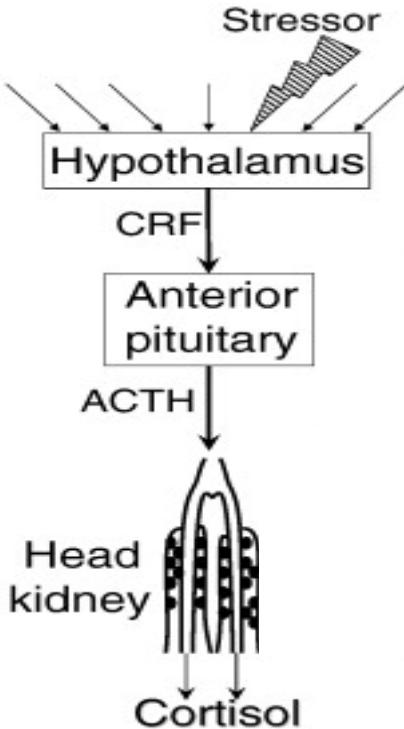
Juvenile Delta smelt

Log₂ Fold-change in transcription



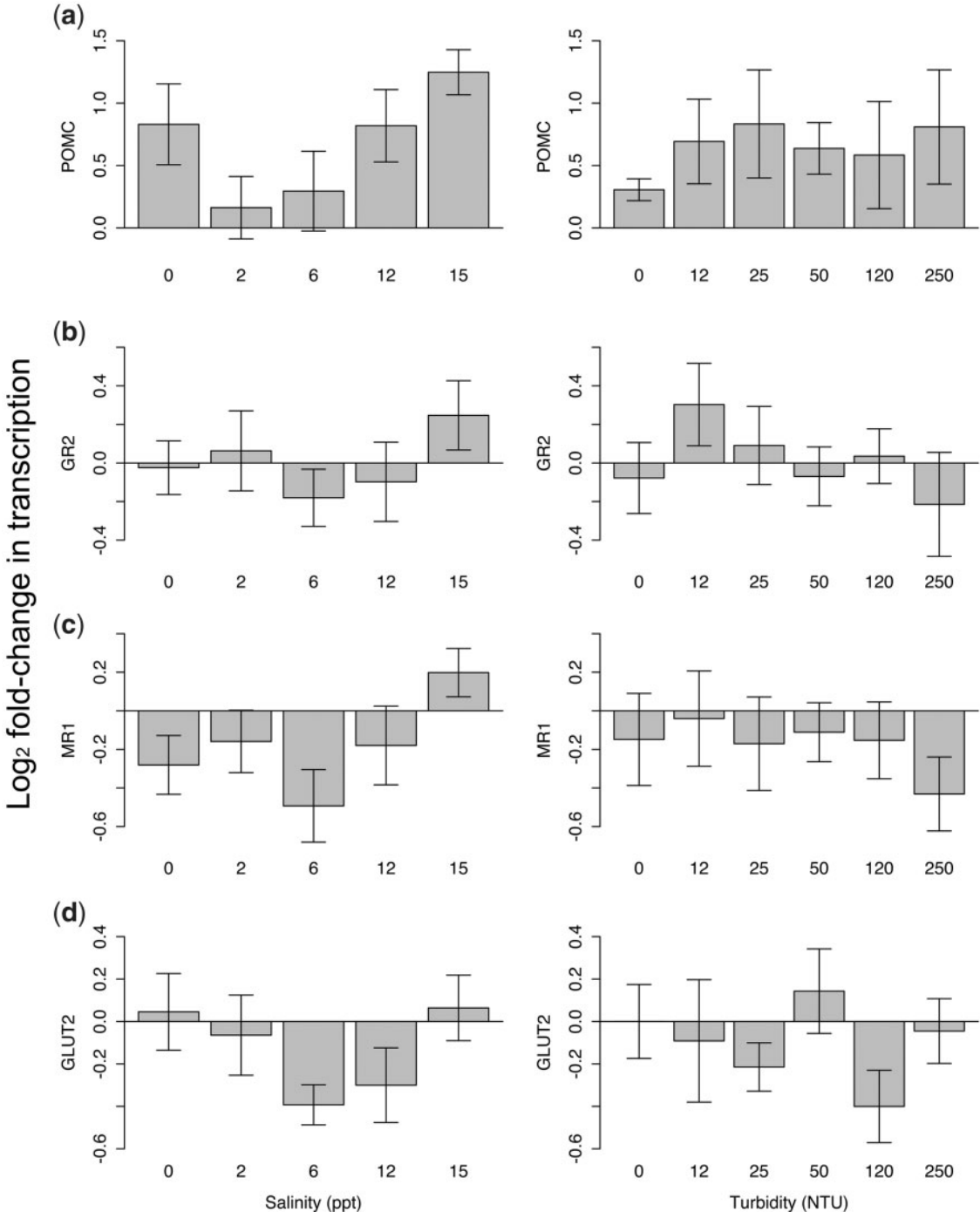


Juvenile Delta smelt



Simplified overview the hypothalamus–pituitary–interrenal (HPI) axis anatomy and signaling cascade.

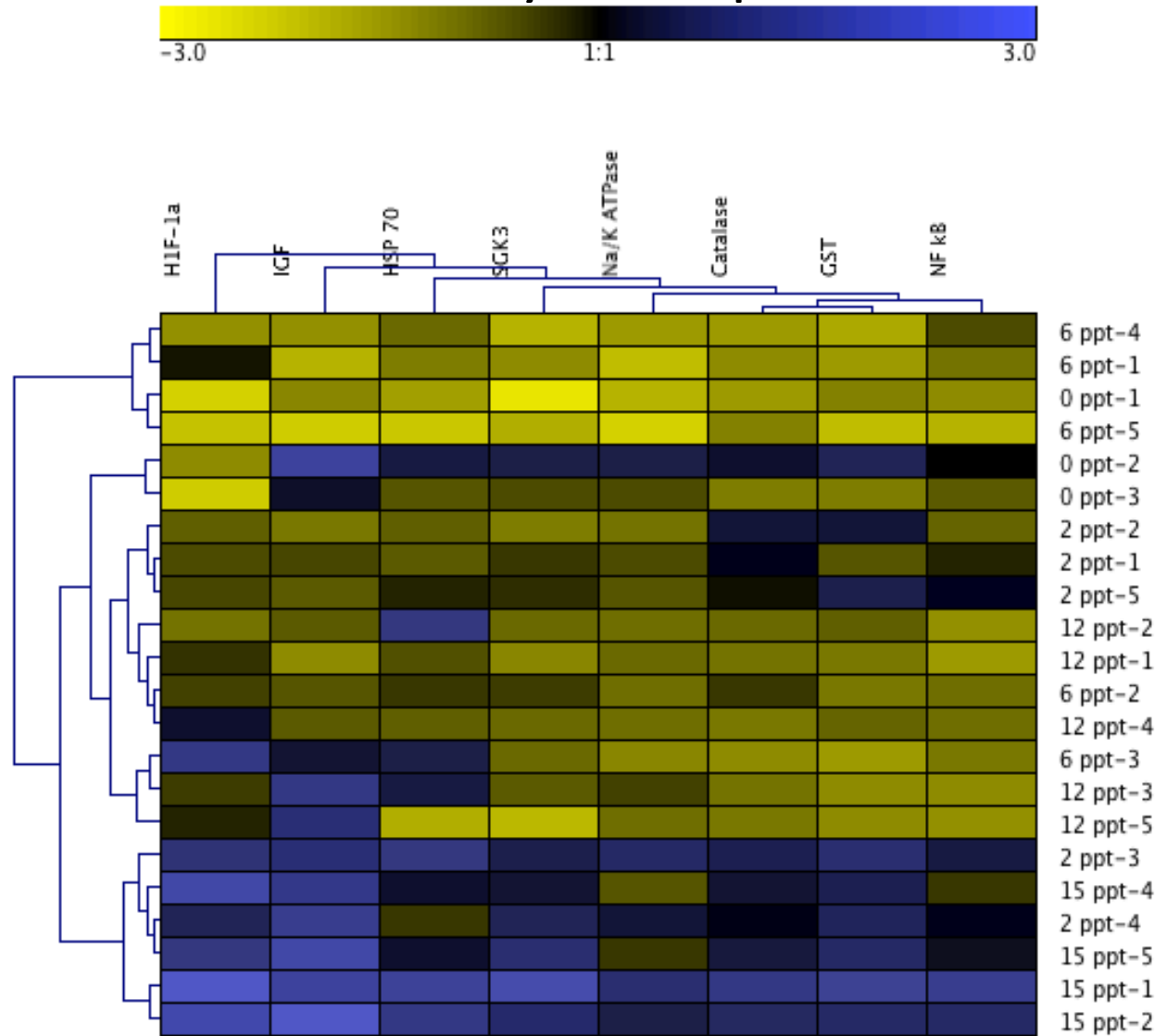
Hasenbein et al, 2013





Adult Delta smelt - Gills

Fast Responses: Salinity tolerance after only 2 h exposure

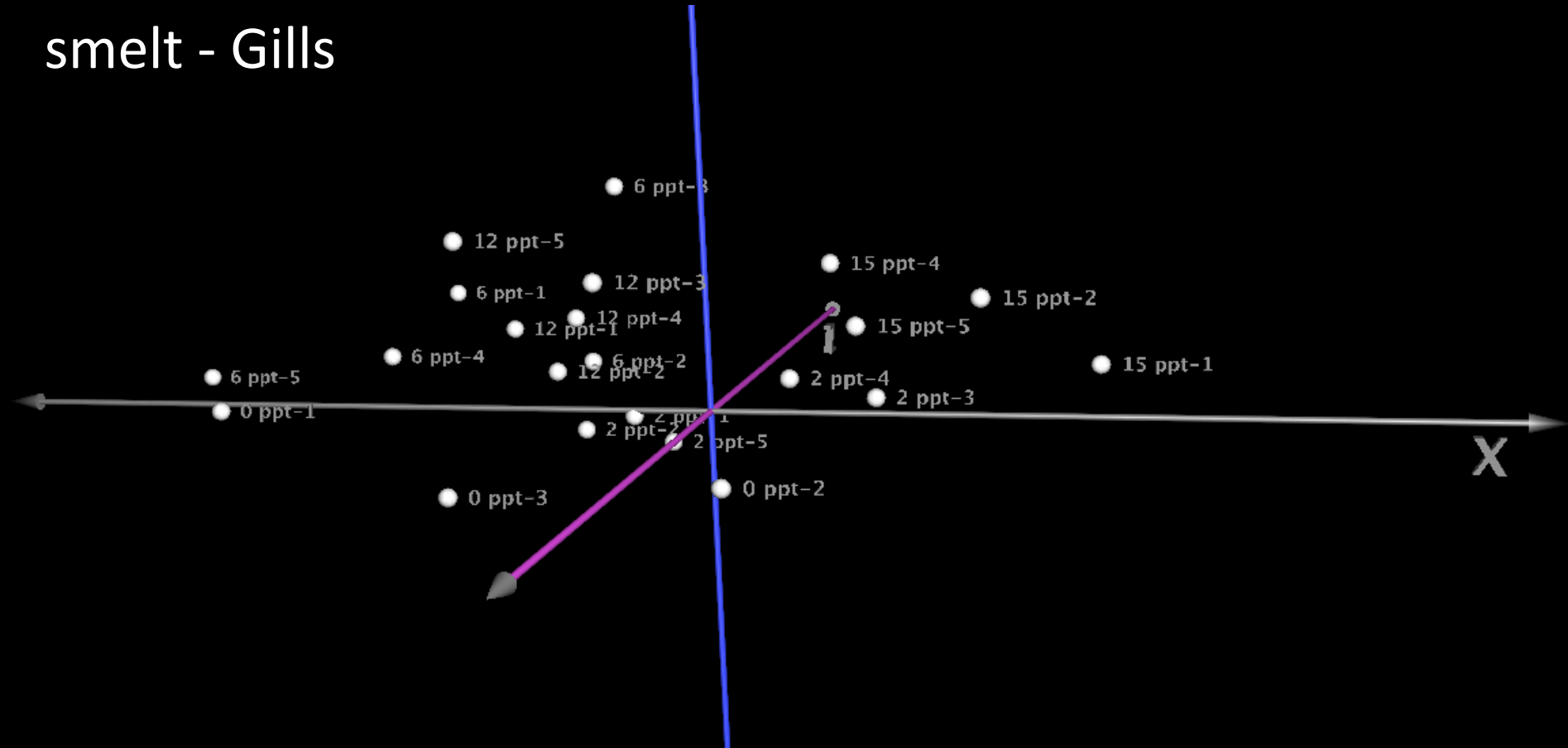


Hasenbein et al, preliminary results (unpublished)



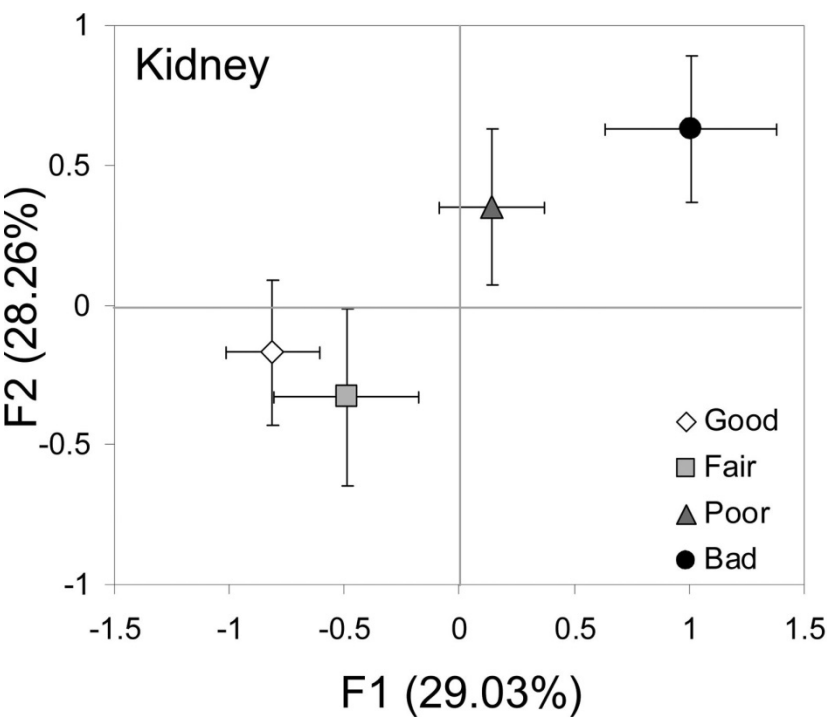
Fast Responses: Salinity tolerance after only 2 h exposure

Adult Delta smelt - Gills

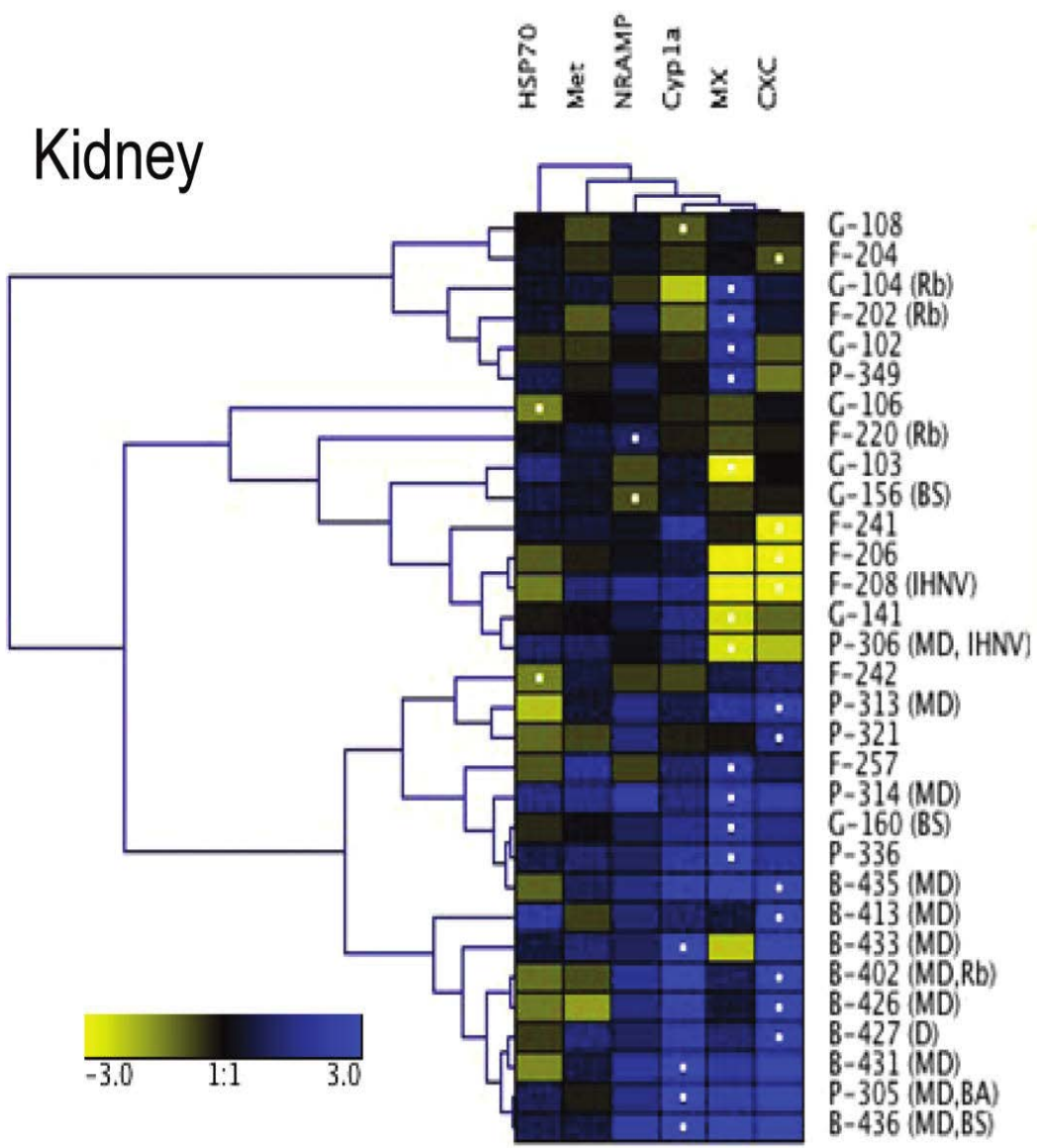




Steelhead

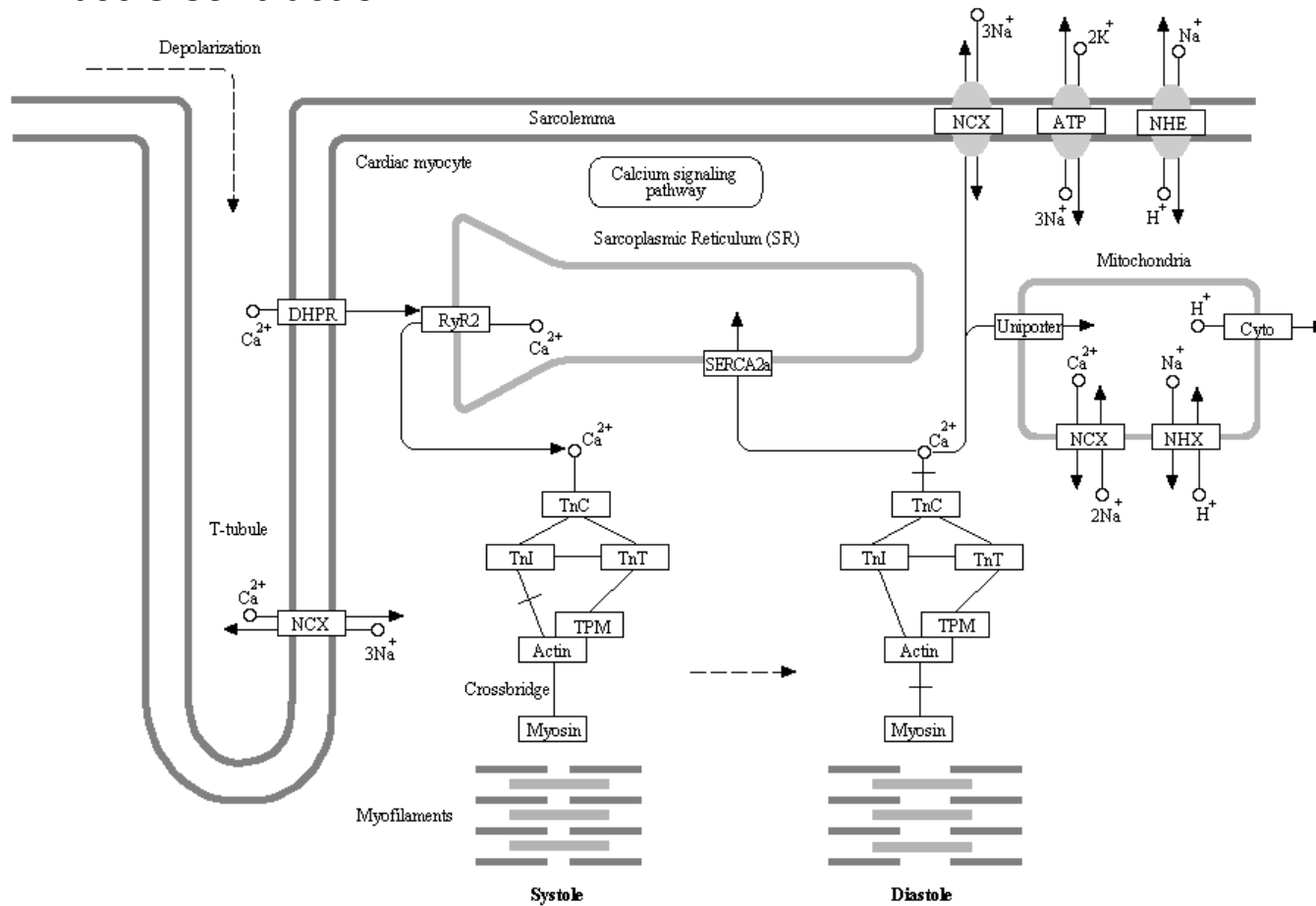


Kidney



Pathway analyses

Muscle Contraction



Summary

- Transcriptomics: multi-biomarker tool
- Applied as a diagnostics tool:
 - Contaminants
 - Disease
 - Environmental stressors
 - Multiple stressors
 - Toxicity Identification Evaluation (TIE)
 - Predictive of long term effects

Note: Studies are conducted in association with tests at higher level of biological organization, some of which are presented in subsequent talks.



74233.75



Acknowledgments:



DELTA
STEWARDSHIP
COUNCIL

