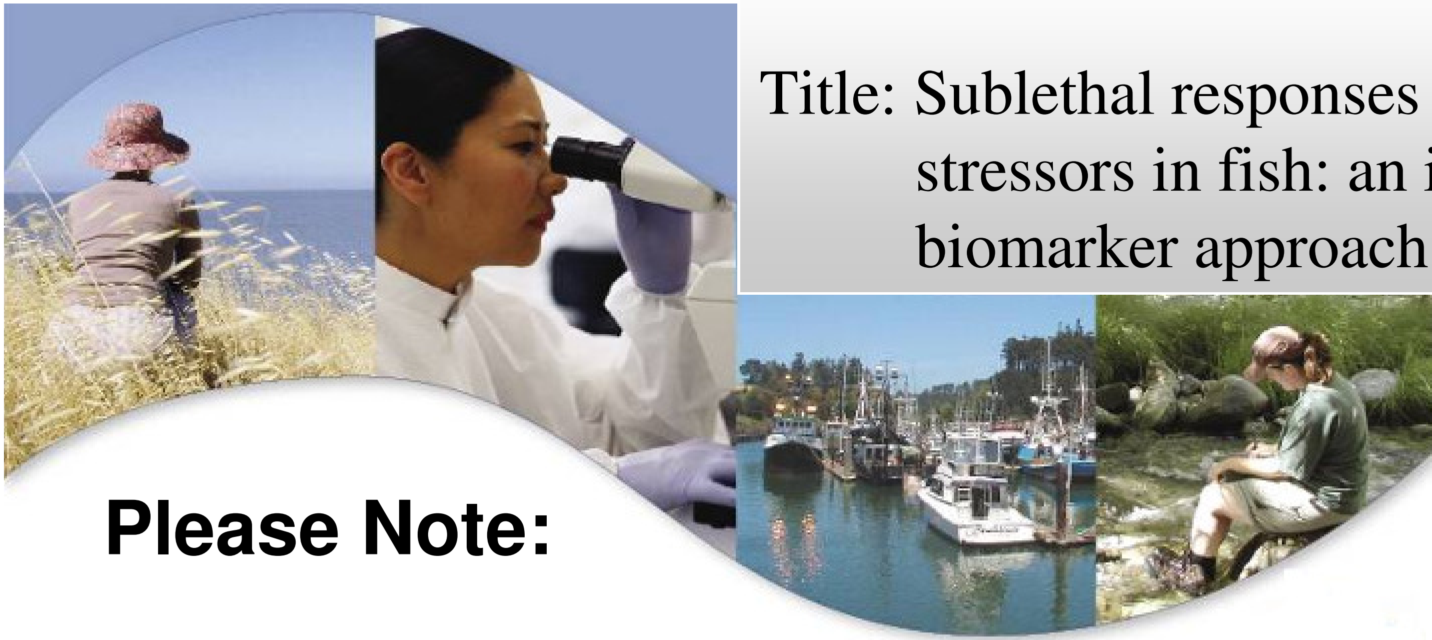
Welcome to SWAMP Webinar

Title: Sublethal responses to environmental stressors in fish: an integrative multi-biomarker approach



Please Note:

- All participants are muted upon log in
- For questions, all participants will be un-muted after the presentation is completed
- Put yourself on mute by pressing *6 so that you do not detract other participants

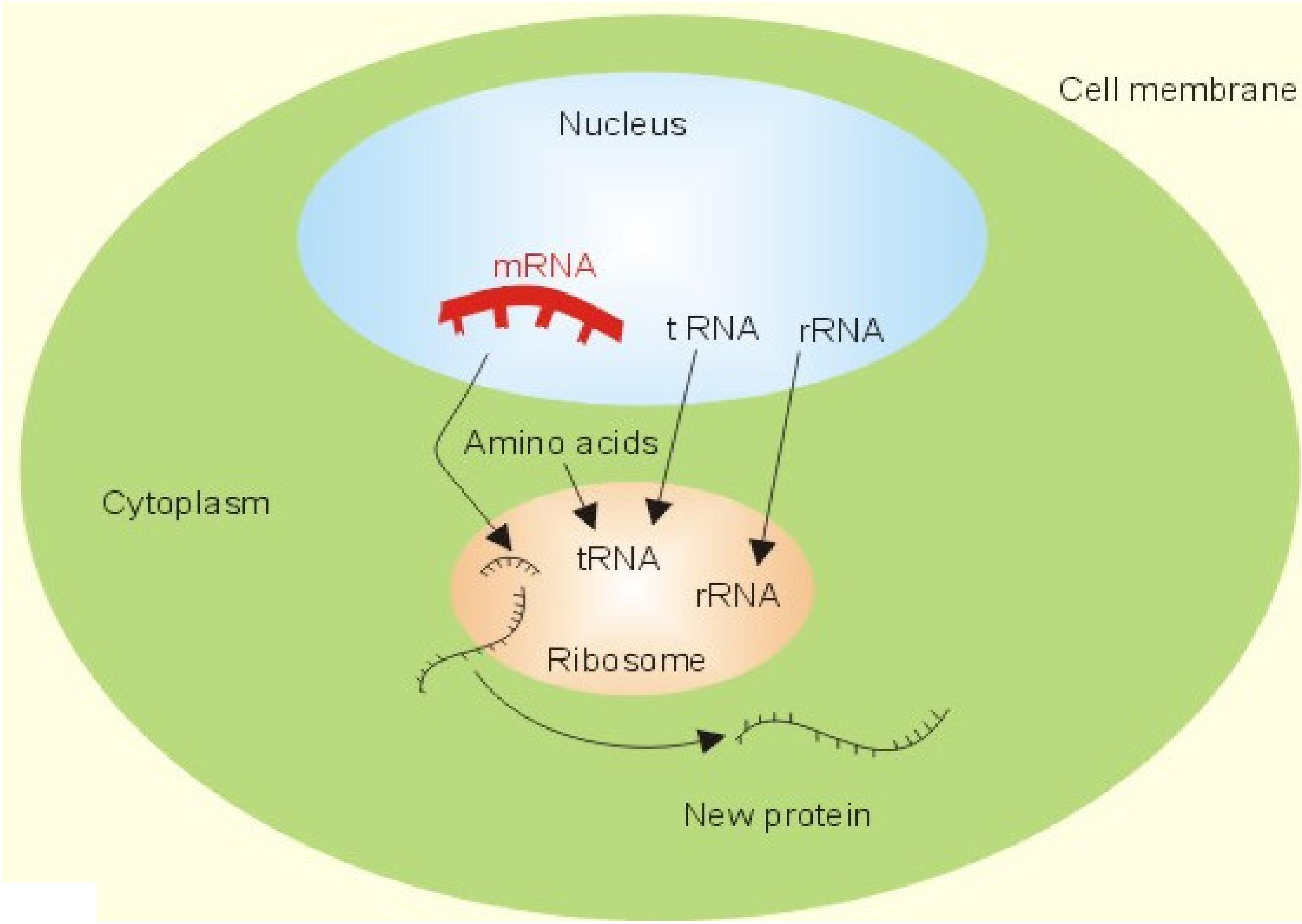
Aquatic Toxicology Laboratory
School of Veterinary Medicine - UC Davis

Sublethal responses to environmental stressors in fish: an integrative multi-biomarker approach

Richard Connon, Sebastian Beggel, Erika Fritsch, Leandro D’Abronzo, Becca Hudson-Davies, Linda Deanovic, and Inge Werner

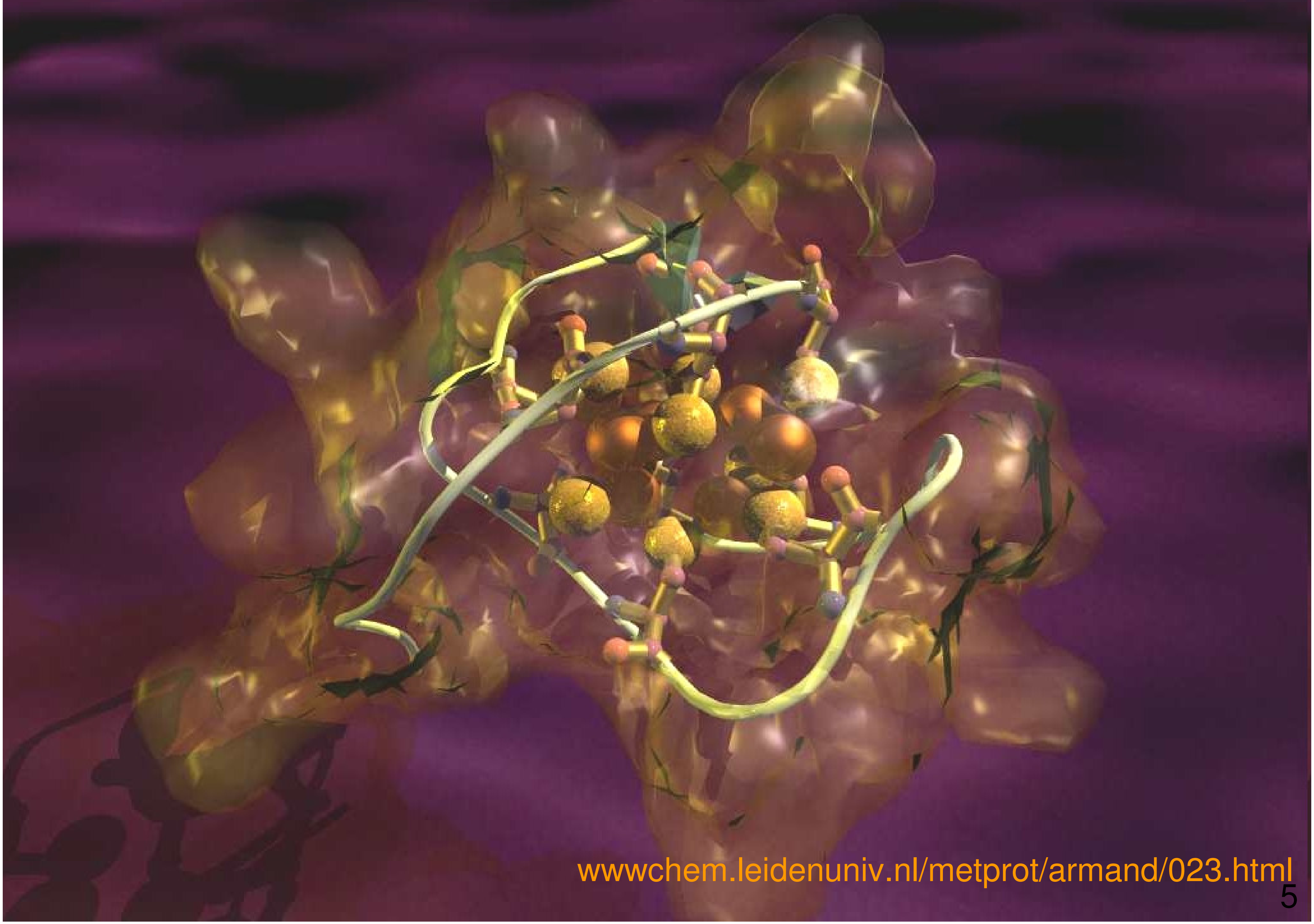
Outline

- Molecular techniques background (5’-10’)
- Links to relevant higher levels of organization
- Application of multiple biomarkers in delta smelt
- Ammonia/um in river water study
- Current, future and proposed work
- Other studies in model species (Inge Werner)

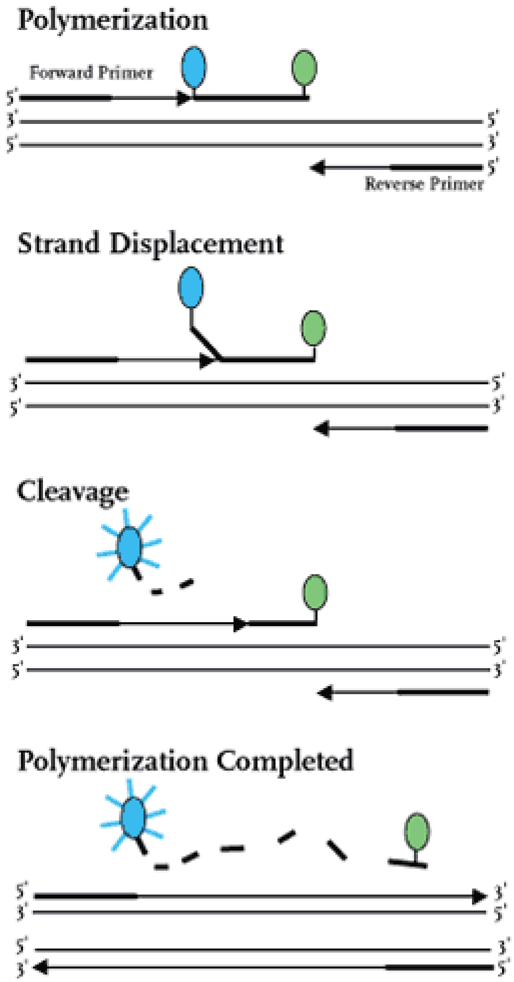


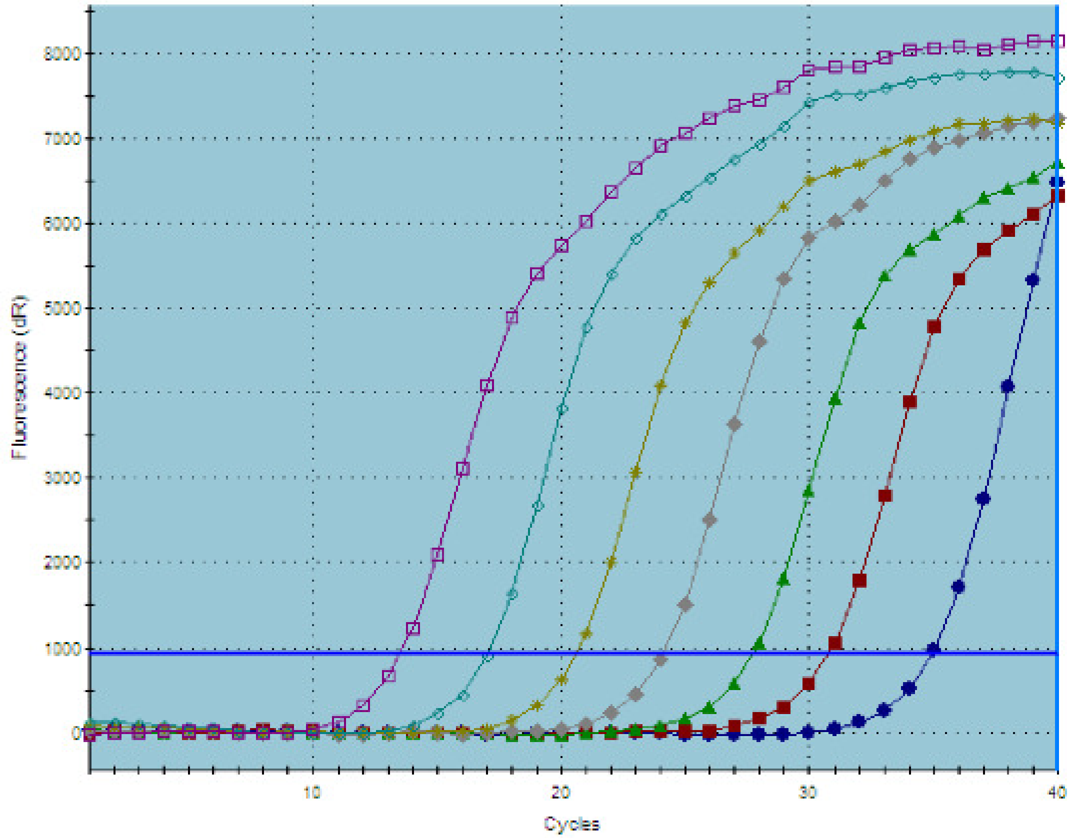
www.mfi.ku.dk/ppaulev/chapter31/kap31.htm

e.g. Metallothionein



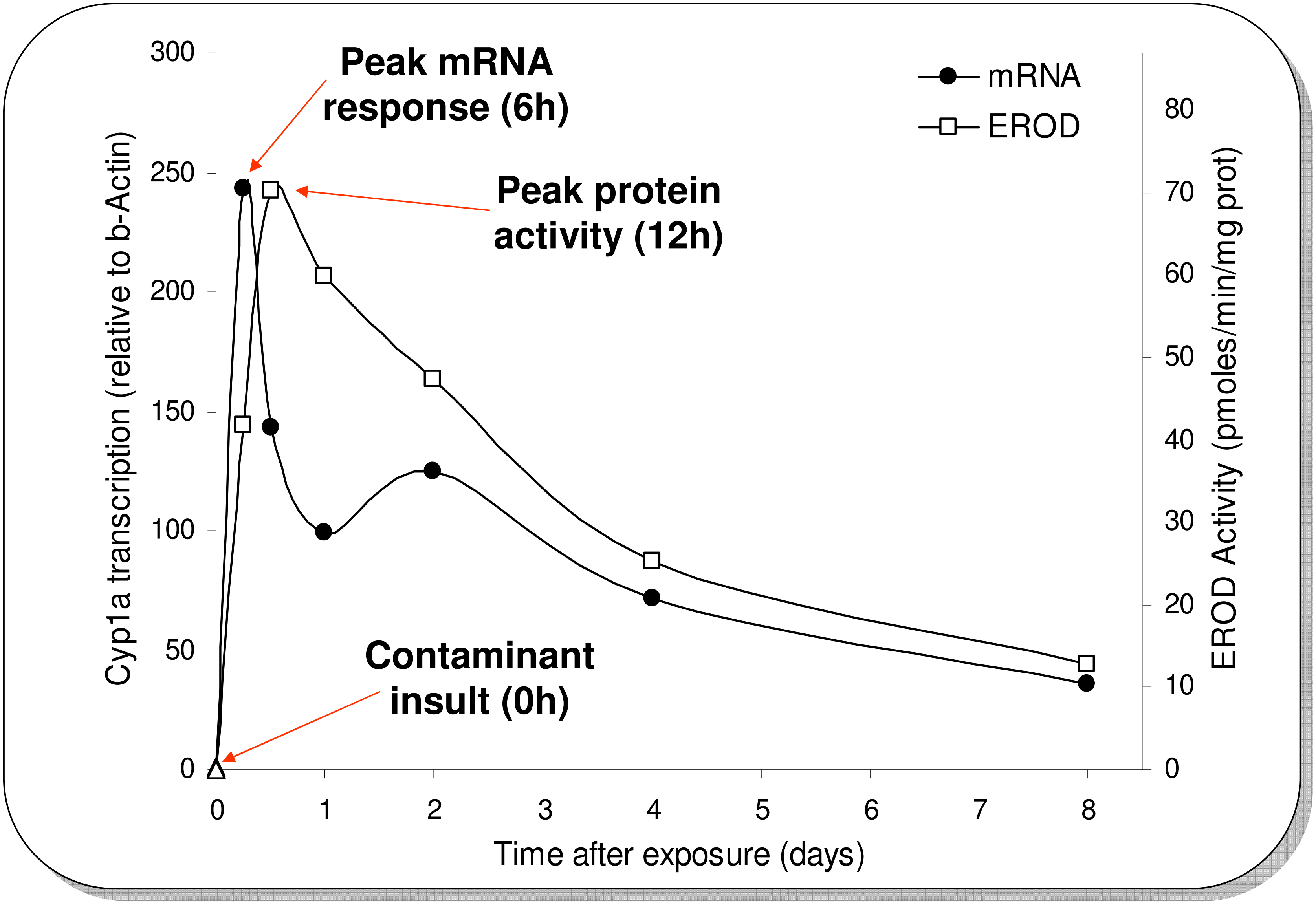
wwwchem.leidenuniv.nl/metprot/armand/023.html





Quantitative PCR

Connon RE, Durieux EDH, D'Abronzo LS, Ostrach DJ, and Werner I. Cytochrome P450 induction in the striped bass (*Morone saxatilis*): signature biomarkers of past and present xenobiotic exposure (in prep).



Time-dependent change in CYP1A **mRNA transcription** and **protein activity** (EROD) expression in striped bass exposed to 10mg.kg^{-1} β-naphthoflavone.

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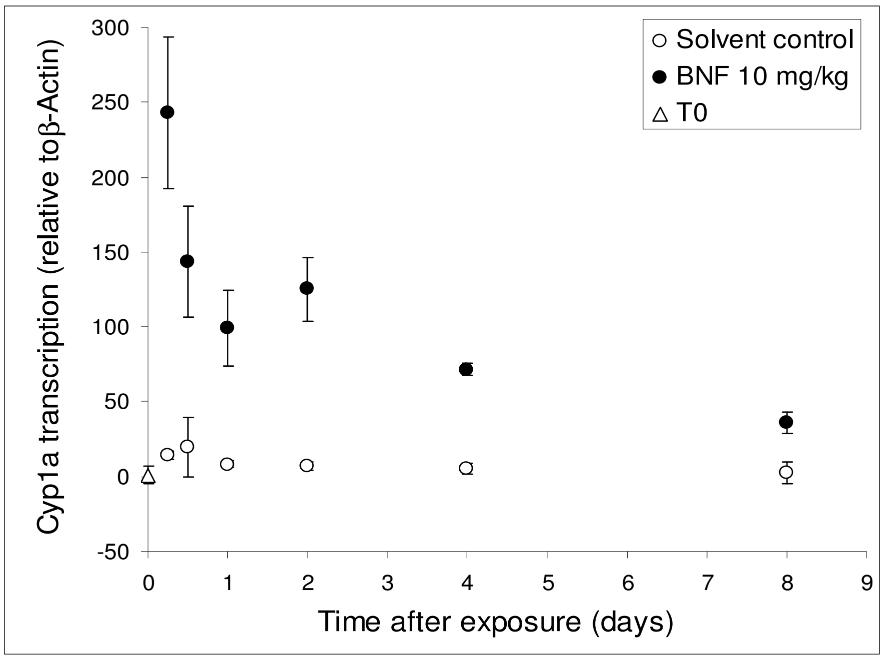

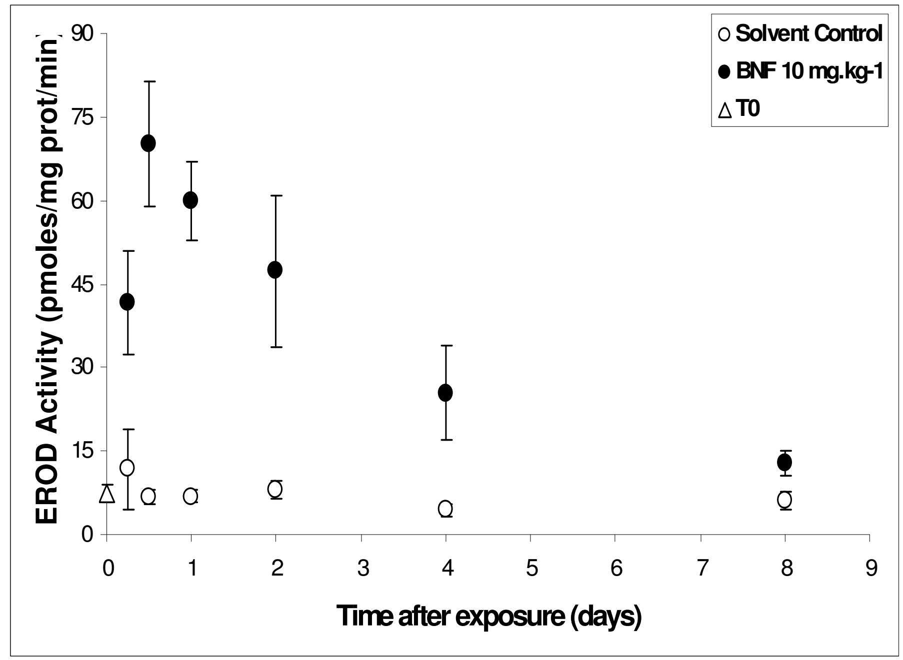

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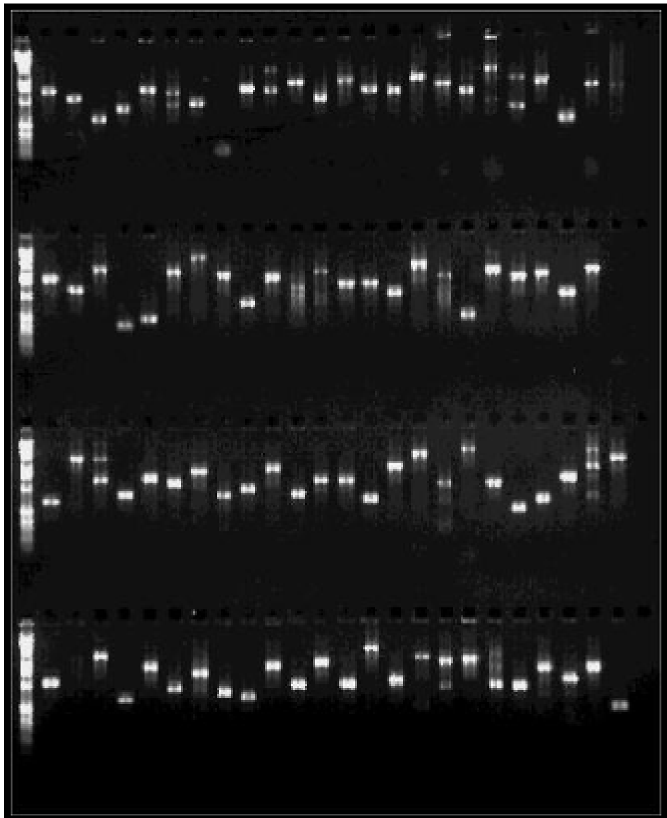
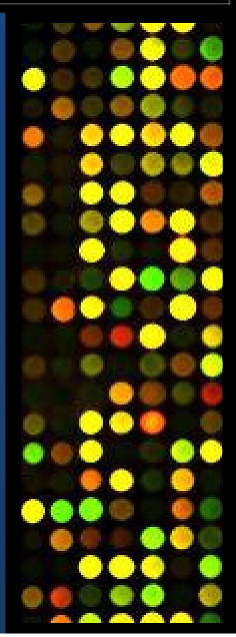
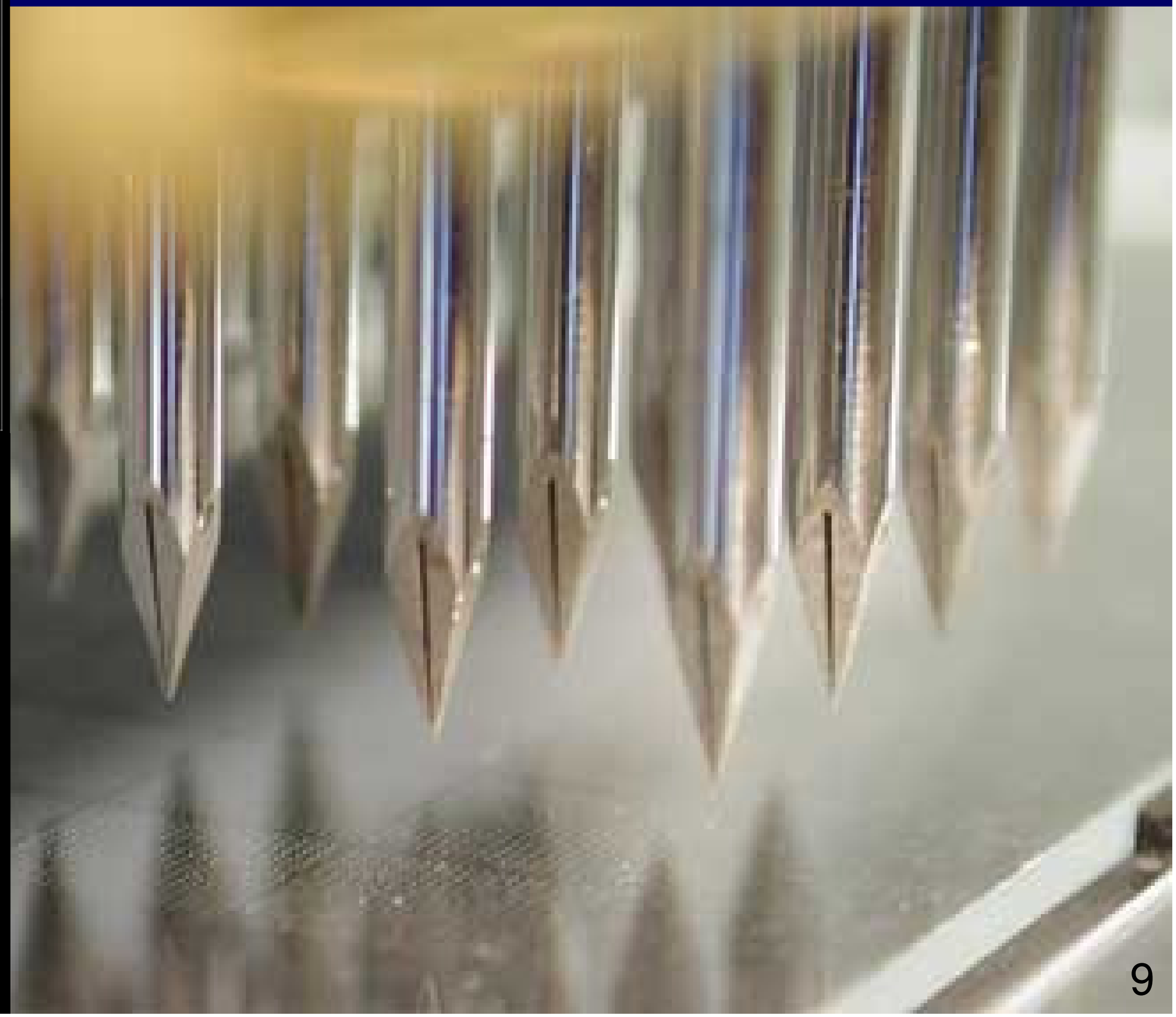


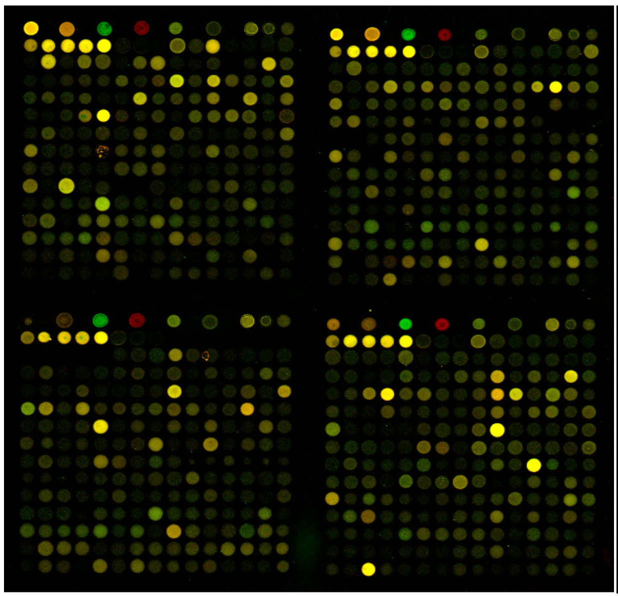


Microarrays

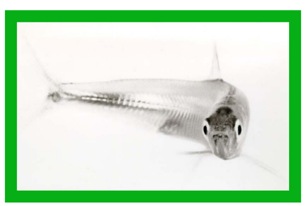
e.g. cDNA library, PCR amplified fragments, purified, and printed onto epoxysilane coated glass slides.

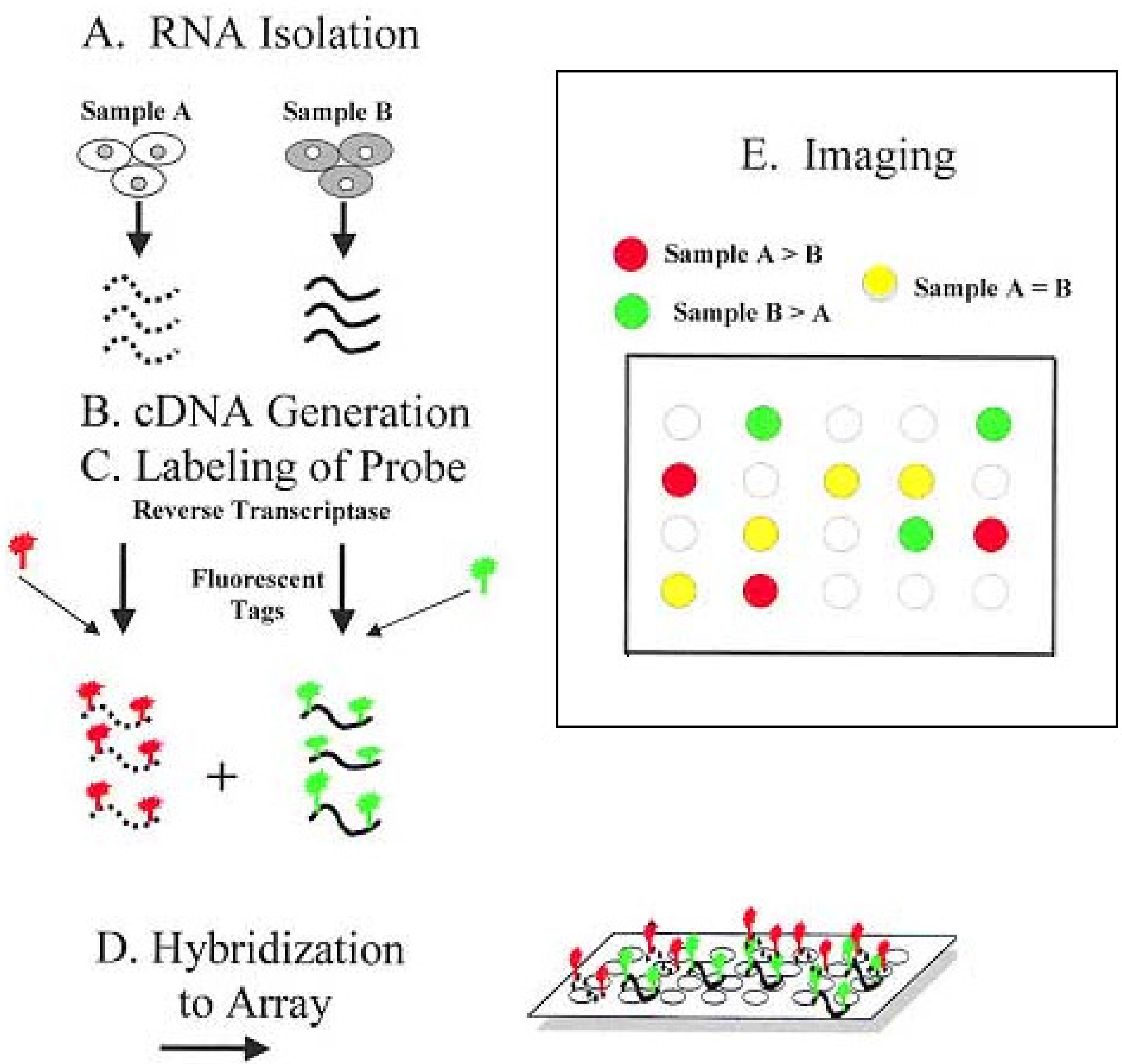


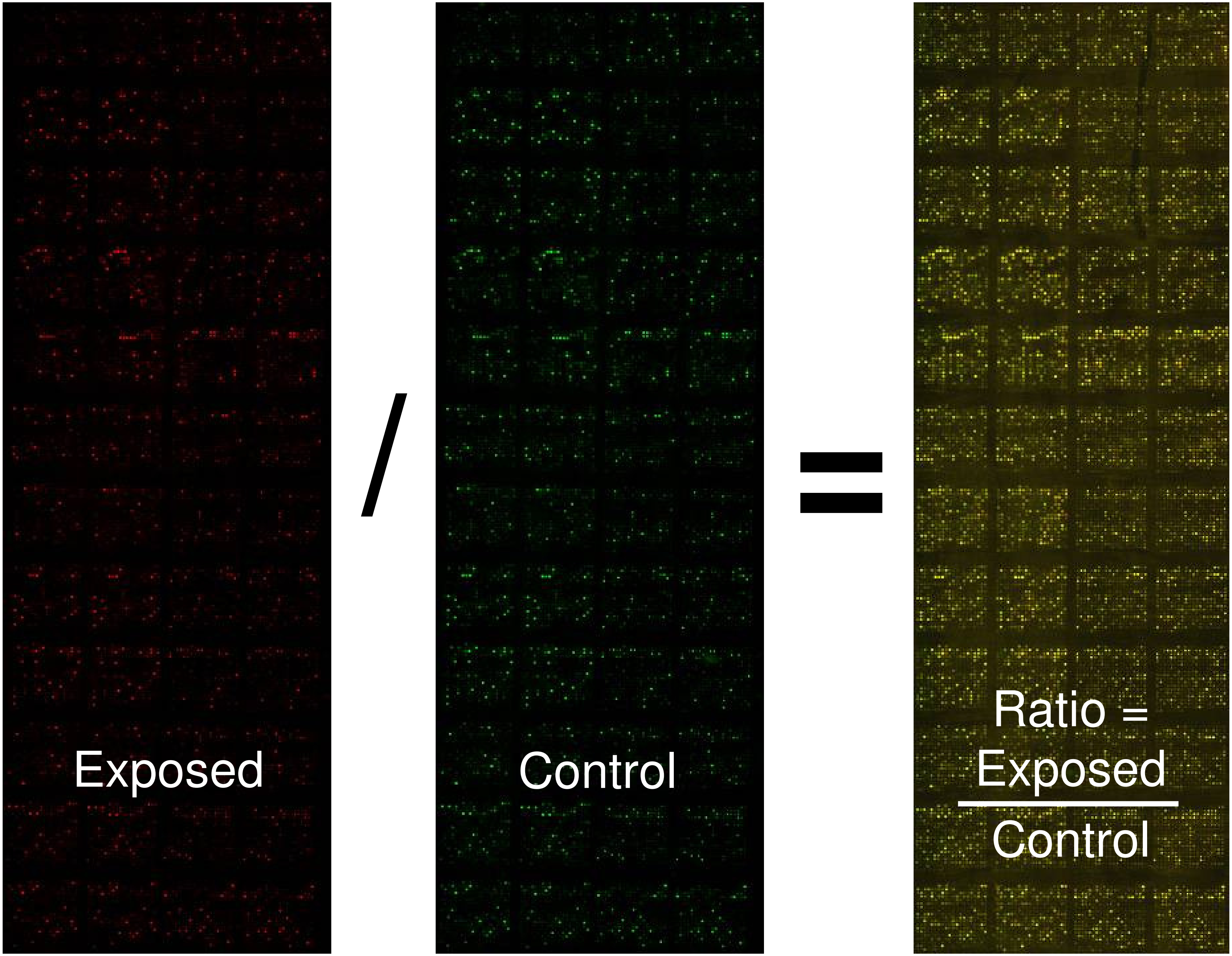
What is a microarray and how does it work?



Exposed Control






Exposed
/
Control
=
Ratio = $\frac{\text{Exposed}}{\text{Control}}$

Microarray differentially expressed gene list

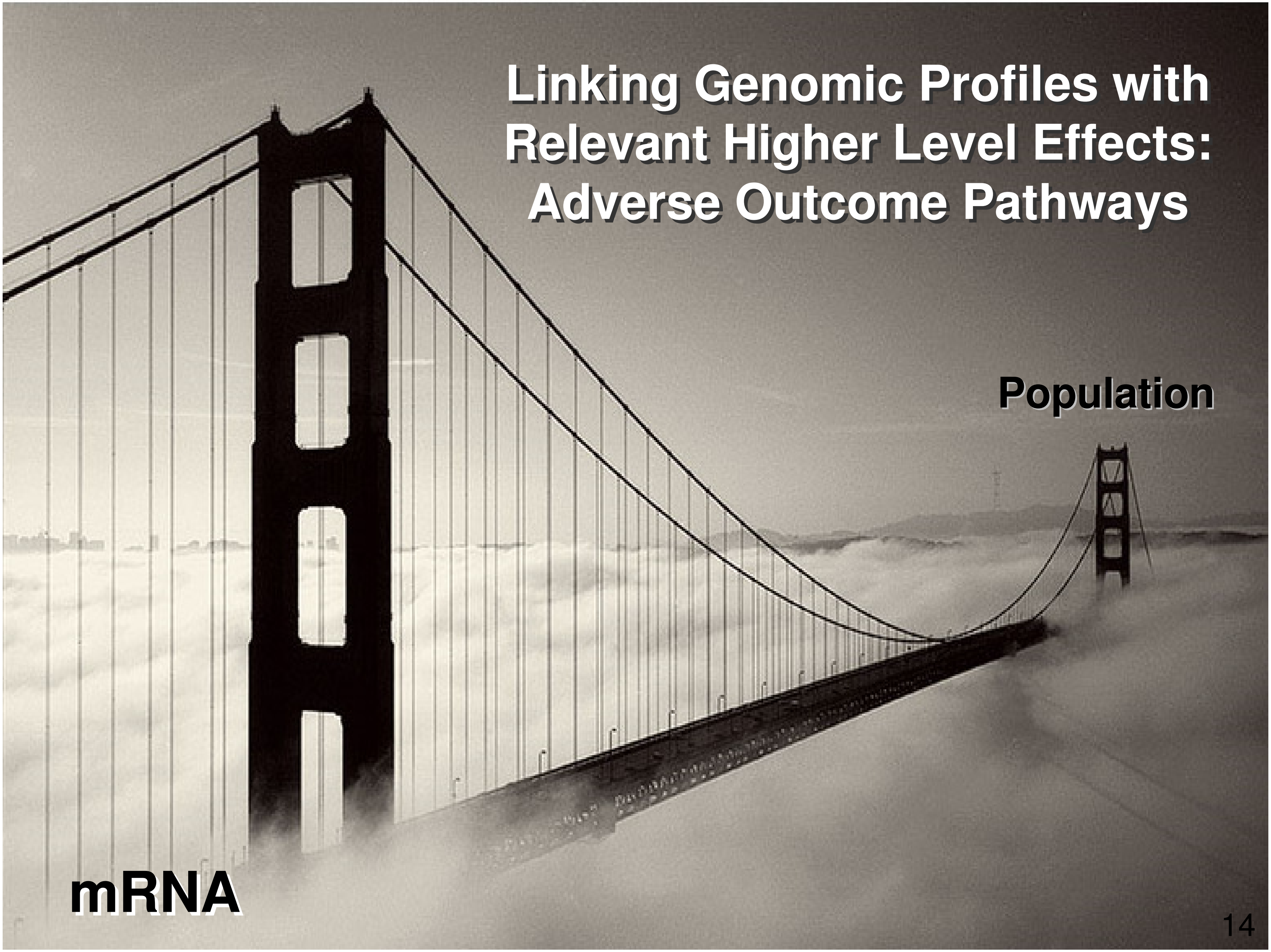
Smelt Clone ID	Response	Fold	Gene most similar to	Species Match	Accession No	E-Value
DS[96]_39_B_06	Up	2.36	1-acylglycerol-3-phosphate O-acyltransferase 3	*Danio rerio*	NP_998590	4.00E-68
DS[96]_77_C_03	Up	4.88	actin alpha 2, skeletal muscle	*Pagrus major*	BAF80060	1.00E-94
DS[96]_03_E_12	Up	3.75	actin, alpha 2, smooth muscle, aorta	*Danio rerio*	AAH75896	e-107
DS[96]_19_B_07	Up	2.92	actin, alpha, cardiac muscle 1 like	*Danio rerio*	NP_001001409	e-141
DS[96]_34_A_06	Up	3.11	actin, alpha, cardiac muscle 1 like	*Danio rerio*	NP_001001409	e-148
DS[96]_29_G_10	Up	3.28	actin, alpha, cardiac muscle 1 like	*Danio rerio*	NP_001001409	e-145
DS[96]_12_G_03	Up	4.58	actin, alpha, cardiac muscle 1 like	*Danio rerio*	NP_001001409	e-125
DS[96]_21_H_11	Up	6.10	actin, alpha, cardiac muscle 1 like	*Danio rerio*	NP_001001409	e-127
DS[96]_38_C_06	Up	2.51	actin, beta	*Acanthopagrus schlegelii*	AAR84618	e-122
DS[96]_05_A_08	Up	1.83	acyl-CoA synthetase long-chain family member 5	*Tetraodon nigroviridis*	CAG06540	e-102
DS[96]_47_G_04	Up	3.47	aldolase a, fructose-bisphosphate	*Danio rerio*	NP_919358	e-124
DS[96]_63_E_08	Up	1.86	alpha tubulin, (protein LOC573122)	*Danio rerio*	NP_001098596	e-120
DS[96]_05_B_06	Up	3.06	amylase-3 protein	*Pseudopleuronectes americanus*	AAF65827	e-144
DS[96]_70_B_05	Up	3.36	amylase-3 protein	*Tetraodon nigroviridis*	CAC87127	3.00E-54
DS[96]_34_D_11	Down	4.54	APEX nuclease (apurinic/apyrimidinic endonuclease) 2	*Xenopus tropicalis*	NP_001006804	6.00E-25
DS[96]_01_C_12	Up	1.80	apolipoprotein	*Tetraodon nigroviridis*	CAG03661	1.00E-38
DS[96]_88_F_11	Up	2.28	apolipoprotein A-I	*Danio rerio*	NP_571203	1.00E-81
DS[96]_74_G_02	Up	3.34	apolipoprotein A-I	*Danio rerio*	NP_571203	1.00E-81
DS[96]_72_D_09	Up	7.67	apolipoprotein A-I	*Danio rerio*	NP_571203	6.00E-73
DS[96]_11_H_09	Up	3.99	apolipoprotein A-I-1 prec[illegible]	*Oncorhynchus mykiss*	O57523	8.00E-76
DS[96]_88_D_02	Up	4.81	apolipoprotein A-I-2 precursor	*Oncorhynchus mykiss*	O57524	4.00E-71
DS[96]_02_A_11	Up	2.72	apolipoprotein A-IV	*Danio rerio*	AAH93239	1.00E-73
DS[96]_41_F_02	Up	2.23	apolipoprotein B	*Salmo salar*	CAA57449	3.00E-24
DS[96]_62_B_05	Up	2.02	apoli[illegible] CII	*Oncorhynchus mykiss*	AAG11410	1.00E-19
DS[96]_65_E_08	Up	2.17	apol[illegible]	*Oncorhynchus mykiss*	AAG11410	3.00E-19
DS[96]_45_B_03	Up	4.16	apolipoprotein Eb	*Danio rerio*	NP_571173	2.00E-38
DS[96]_79_H_01	Up	4.42	arachidonate 12-lipoxygenase	*Danio rerio*	NP_955912	4.00E-23
DS[96]_77_B_08	Up	2.56	arachidonate 12-lipoxygenase	*Danio rerio*	NP_955912	5.00E-35
DS[96]_86_F_12	Up	3.87	astacin like metallo-protease	*Oryzias latipes*	NP_001098207	2.00E-83
DS[96]_17_B_07	Up	4.34	astacin like metallo-proteas[illegible]	*Oryzias latipes*	NP_001098207	7.00E-50
DS[96]_07_A_09	Up	2.17	c1q-like protein	*Dissostichus mawsoni*	ABN45966	3.00E-38
DS[96]_54_A_03	Down	1.69	calcitonin receptor-like receptor	*Oncorhynchus gorbuscha*	CAD48406	5.00E-56
DS[96]_53_D_10	Down	1.58	calcium binding protein 39	*Danio rerio*	NP_998666	1.00E-76
DS[96]_71_F_06	Up	2.27	calpain 1 protein	*Danio rerio*	AAH91999	2.00E-68
DS[96]_37_C_02	Up	1.82	carboxypeptidase H	*Paralichthys olivaceus*	AAO92752	1.00E-82
DS[96]_27_A_09	Up	2.91	cardiac muscle ATP synthase,alpha 1,	*Danio rerio*	NP_001070823	7.00E-62
DS[96]_05_C_08	Up	5.76	cell division cycle 14 homolog A	*Danio rerio*	CAP09233	3.00E-19
DS[96]_30_G_11	Up	2.34	chitin binding Peritrophin-A domain	*Danio rerio*	AAH45331	4.00E-69
DS[96]_33_H_06	Up	3.75	chitinase	*Oncorhynchus mykiss*	CAD59687	9.00E-68
DS[96]_69_C_08	Up	4.25	chitinase1	*Paralichthys olivaceus*	BAD15059	e-127
DS[96]_71_C_06	Up	3.93	chymotrypsinogen 2-like protein	*Sparus aurata*	AAT45254	1.00E-20
DS[96]_56_B_04	Down	2.01	cofilin 2 (muscle)	*Danio rerio*	NP_991263	5.00E-84
DS[96]_59_A_01	Down	2.35	cofilin 2 (muscle)	*Danio rerio*	NP_991263	3.00E-83
DS[96]_67_A_02	Up	5.20	corticotropin-lipotropin A precursor	*Oncorhynchus mykiss*	Q04617	7.00E-63
DS[96]_26_G_05	Down	1.59	cytochrome P450, family 46, subfamily A, polypeptide 1	*Danio rerio*	NP_001018358	2.00E-65
DS[96]_11_H_05	Up	1.86	DAZAP2-like protein (deleted in azoospermia-associated)	*Takifugu rubripes*	NP_001072102	5.00E-59
DS[96]_18_C_06	Down	1.78	dopachrome tautomerase	*Salmo salar*	ABD73808	1.00E-85
DS[96]_20_B_11	Down	1.66	E3 ubiquitin-protein ligase MARCH2	*Danio rerio*	Q1LVZ2	2.00E-87
DS[96]_68_G_10	Up	5.08	elastase 2-like protein	*Sparus aurata*	AAT45251	2.00E-89

Microarrays, biomarkers in monitoring

- Provides information on the mode of action
- Allows test organisms to tell us what we should be looking at
- Permits the development and selection of biomarkers without preconceived ideas
- No prior knowledge of the system required
- Identifies specific effects on organism/condition in question

mindpowersystem.com/15/interrogation-methods/

Linking Genomic Profiles with Relevant Higher Level Effects: Adverse Outcome Pathways



Linking Molecules with Higher Levels of Biological Organization

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Bioenergetics

Histopathology

Immunology

Physiology

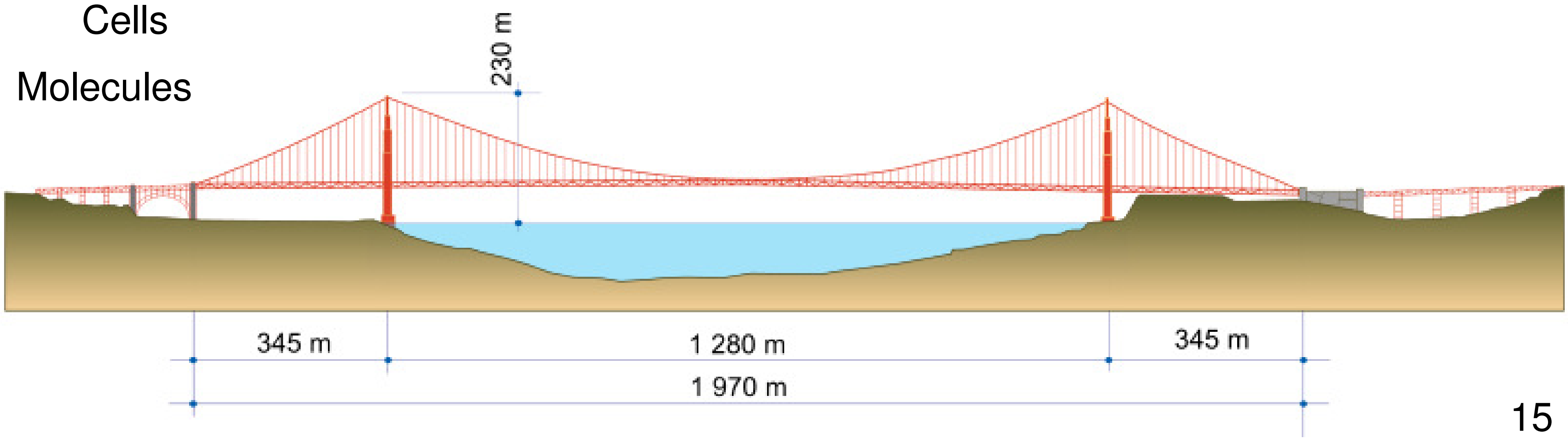
Detoxification

Cells

Molecules

Development and Reproduction

Population and Community



Linking Molecules with Higher Levels of Biological Organization

…

Bioenergetics

Histopathology

Immunology

Physiology

Detoxification

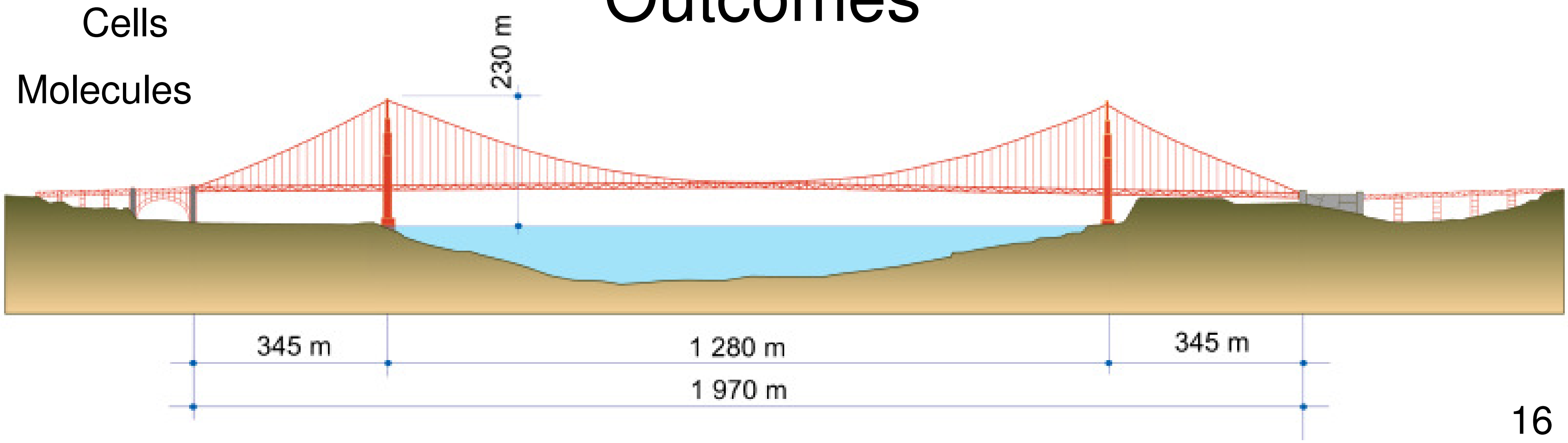
Cells

Molecules

Adverse Outcomes

Development and Reproduction

Population and Community



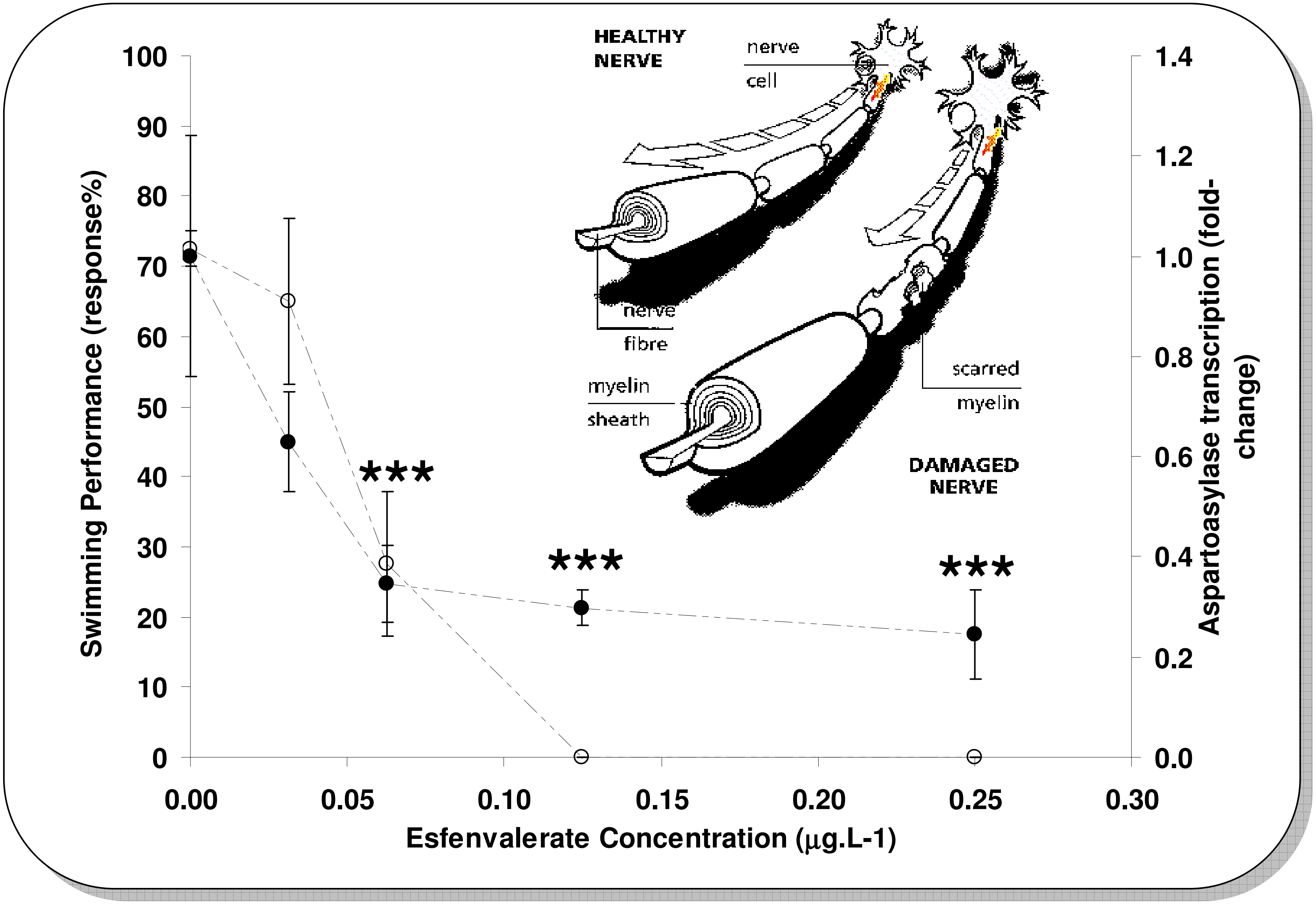
Measure it

Swim it

…

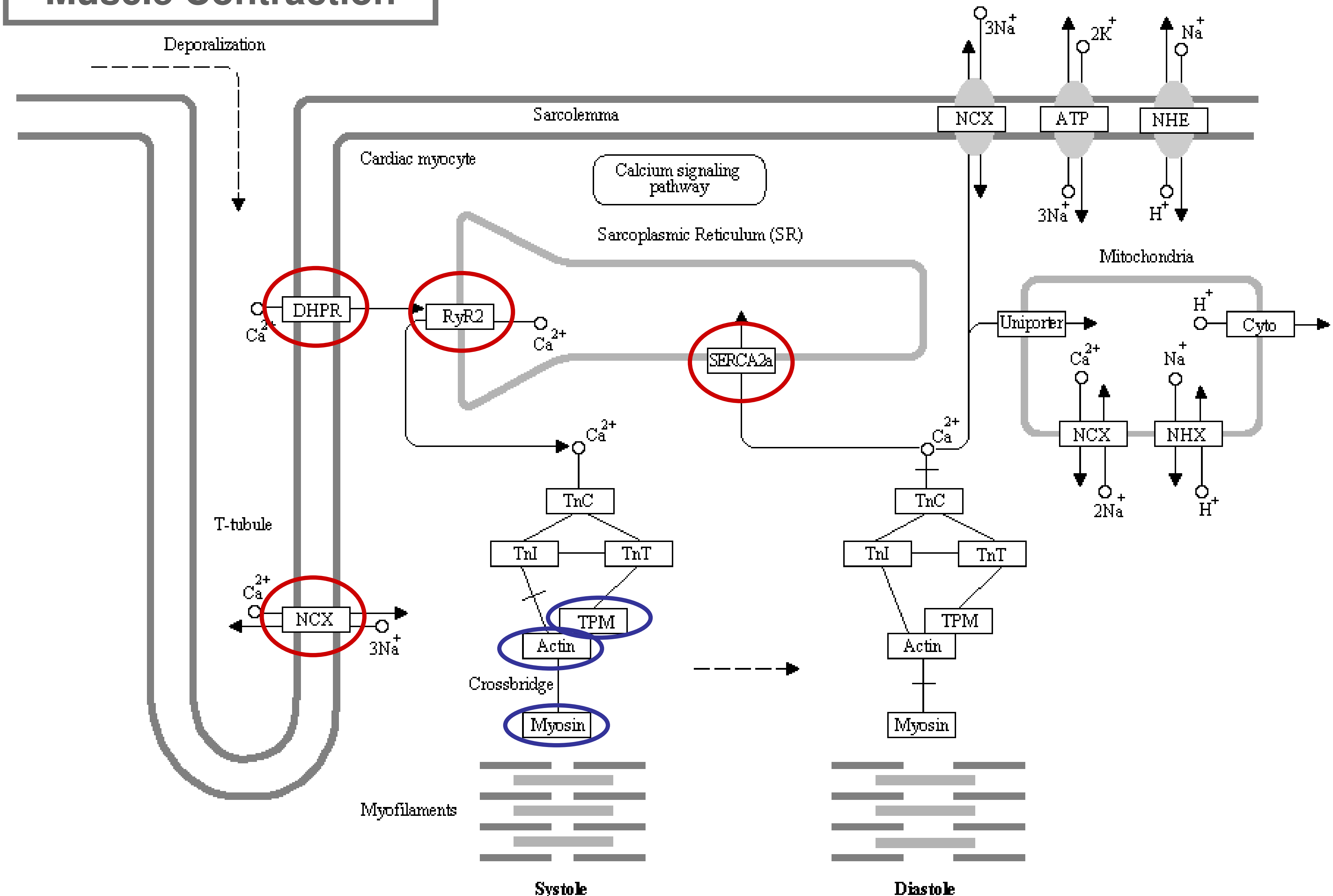
Bridge it

Connon RE, et al (2009). Linking mechanistic and behavioral responses to sublethal esfenvalerate exposure in the endangered delta smelt; Hypomesus transpacificus (Fam. Osmeridae). BMC Genomics.



Swimming performance and aspartoacylase transcription in larval delta smelt exposed to esfenvalerate

Muscle Contraction



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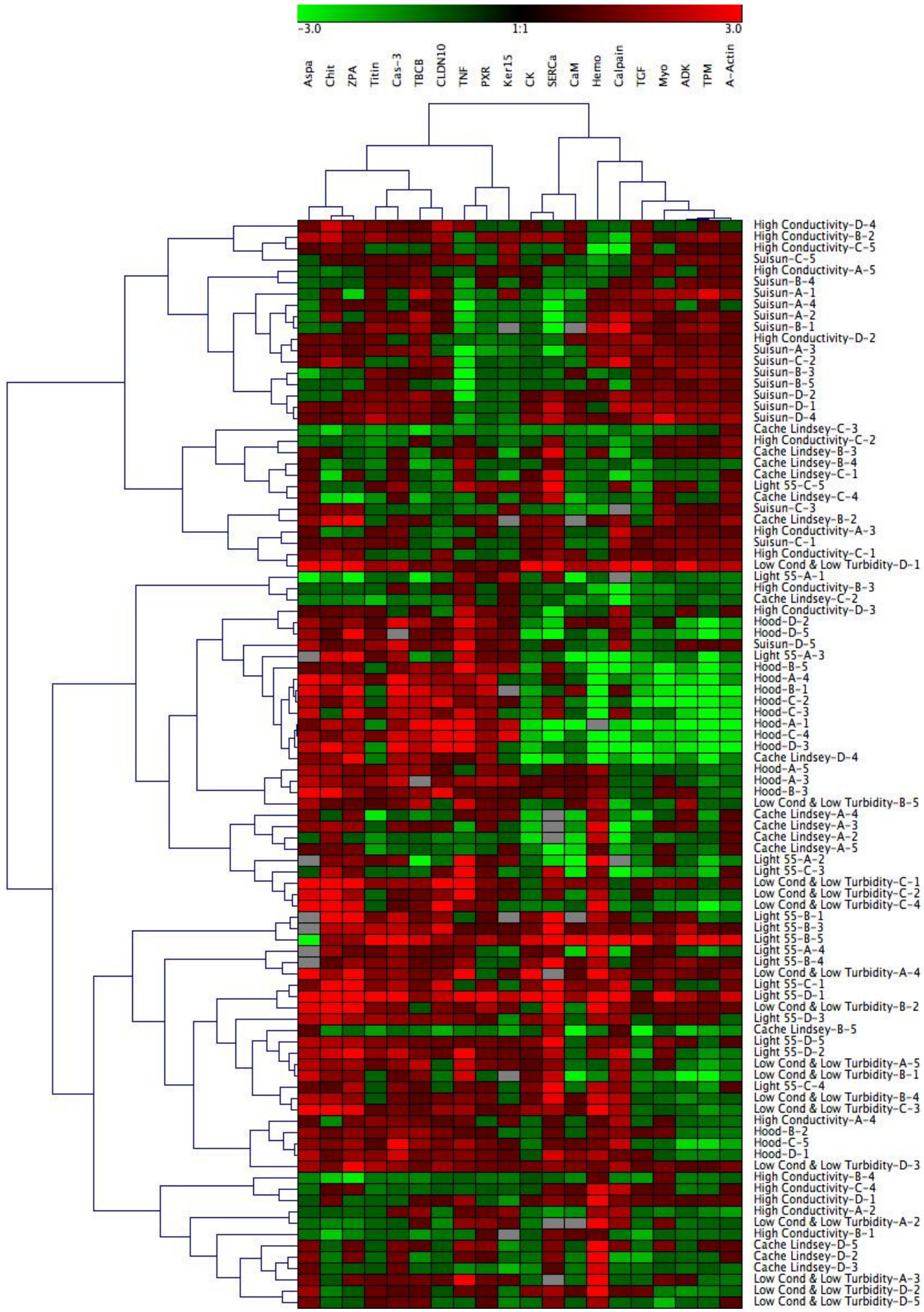
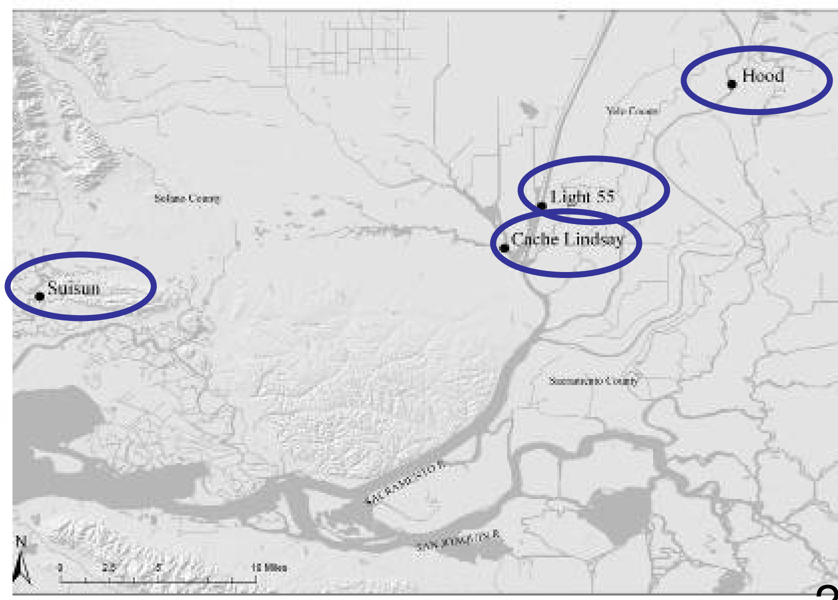


Genomic profiling:

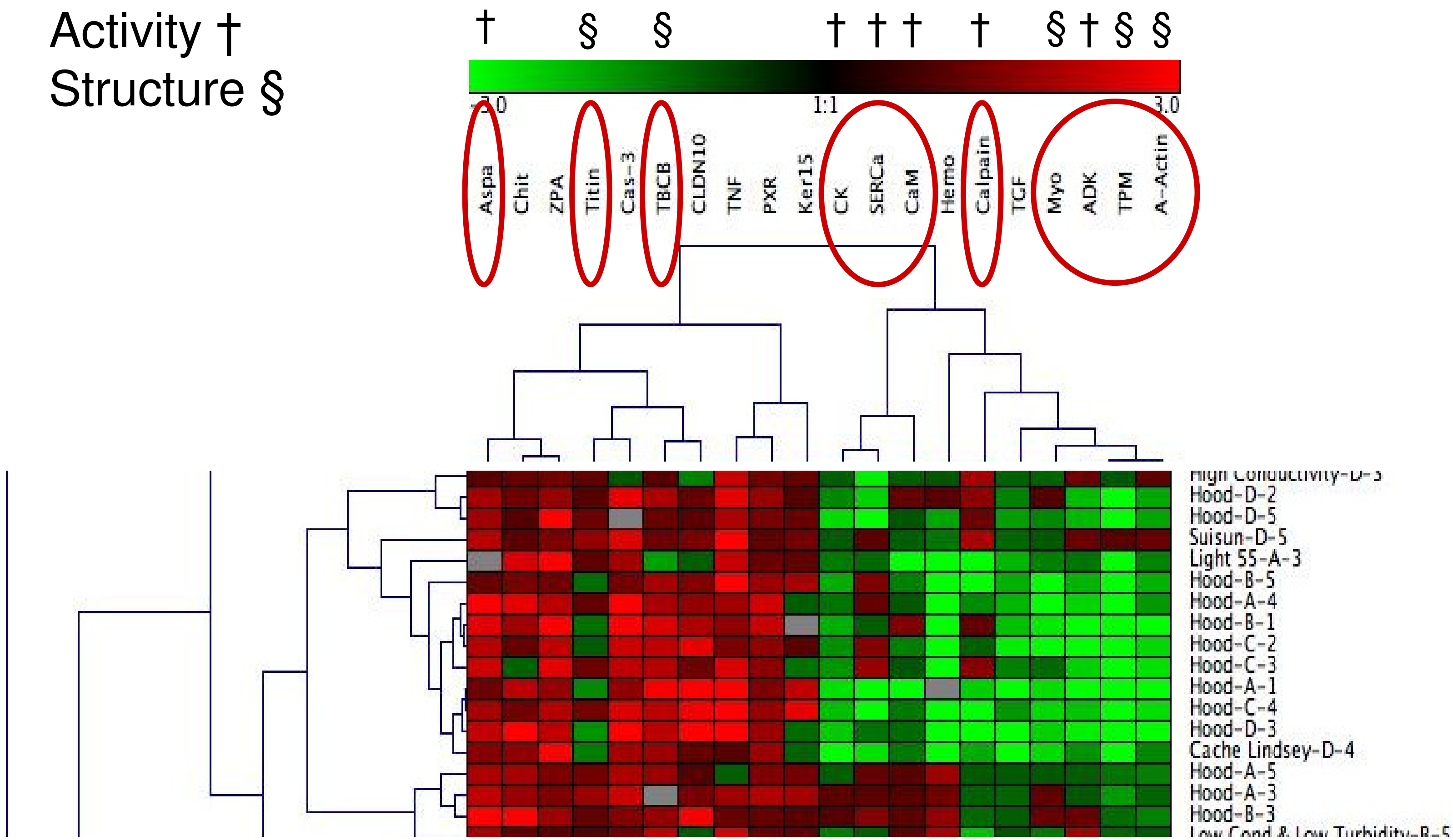
Responses in juvenile delta smelt exposed for 7 days to ambient samples from four sites in the Bay-Delta System, along with respective high and low EC controls



Genomic profiling: Hood

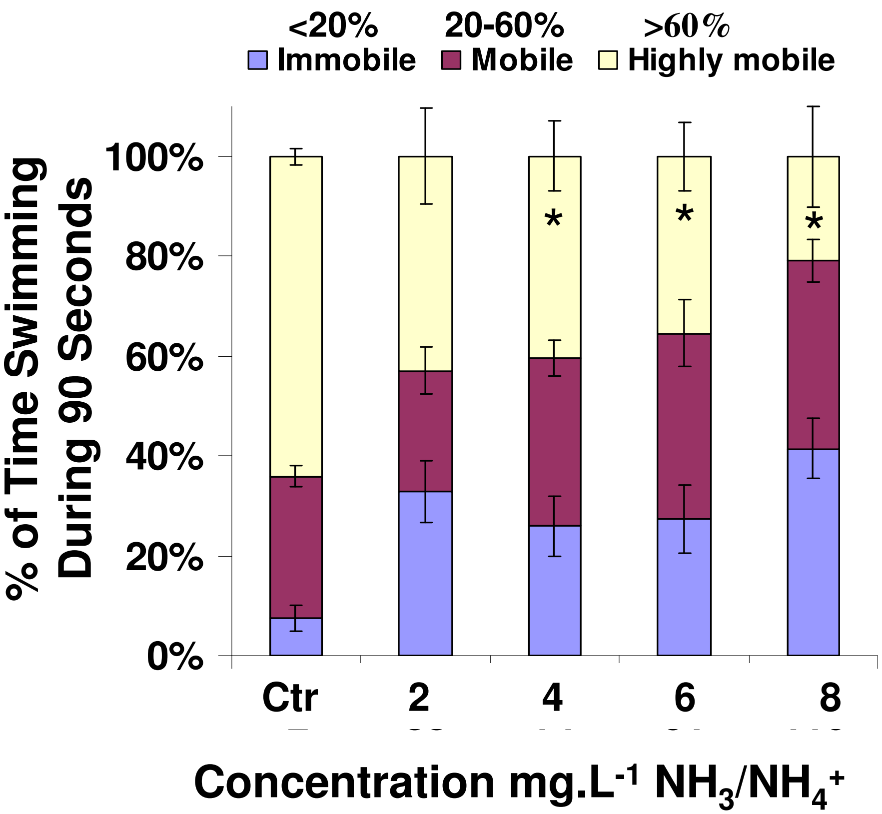
Key:

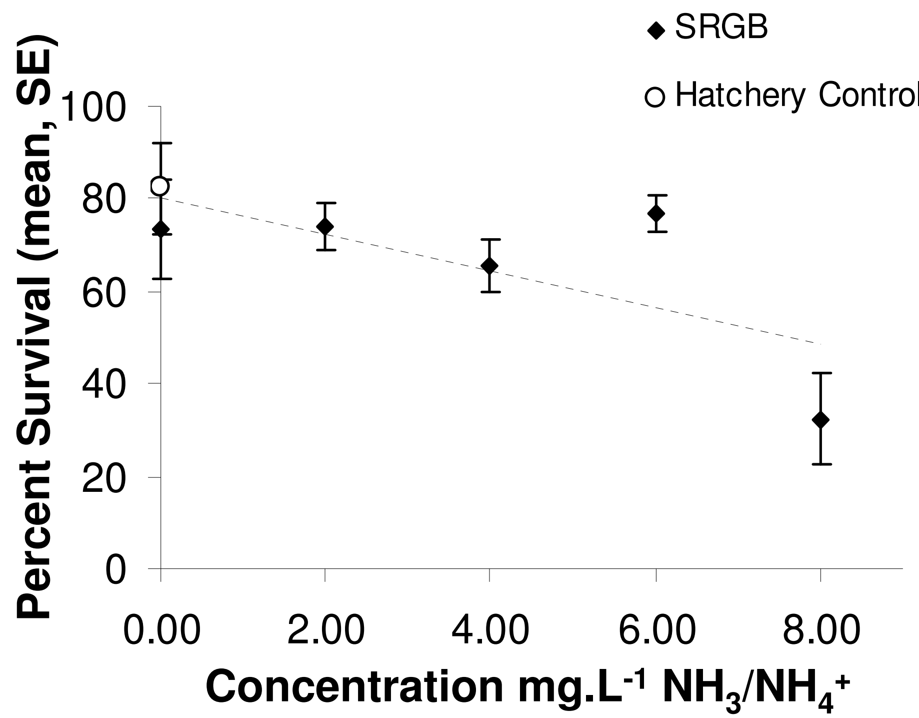
Activity †
Structure §



Survival and swimming of Larval Delta Smelt after 7-d Exposure to **Ammonium Chloride**

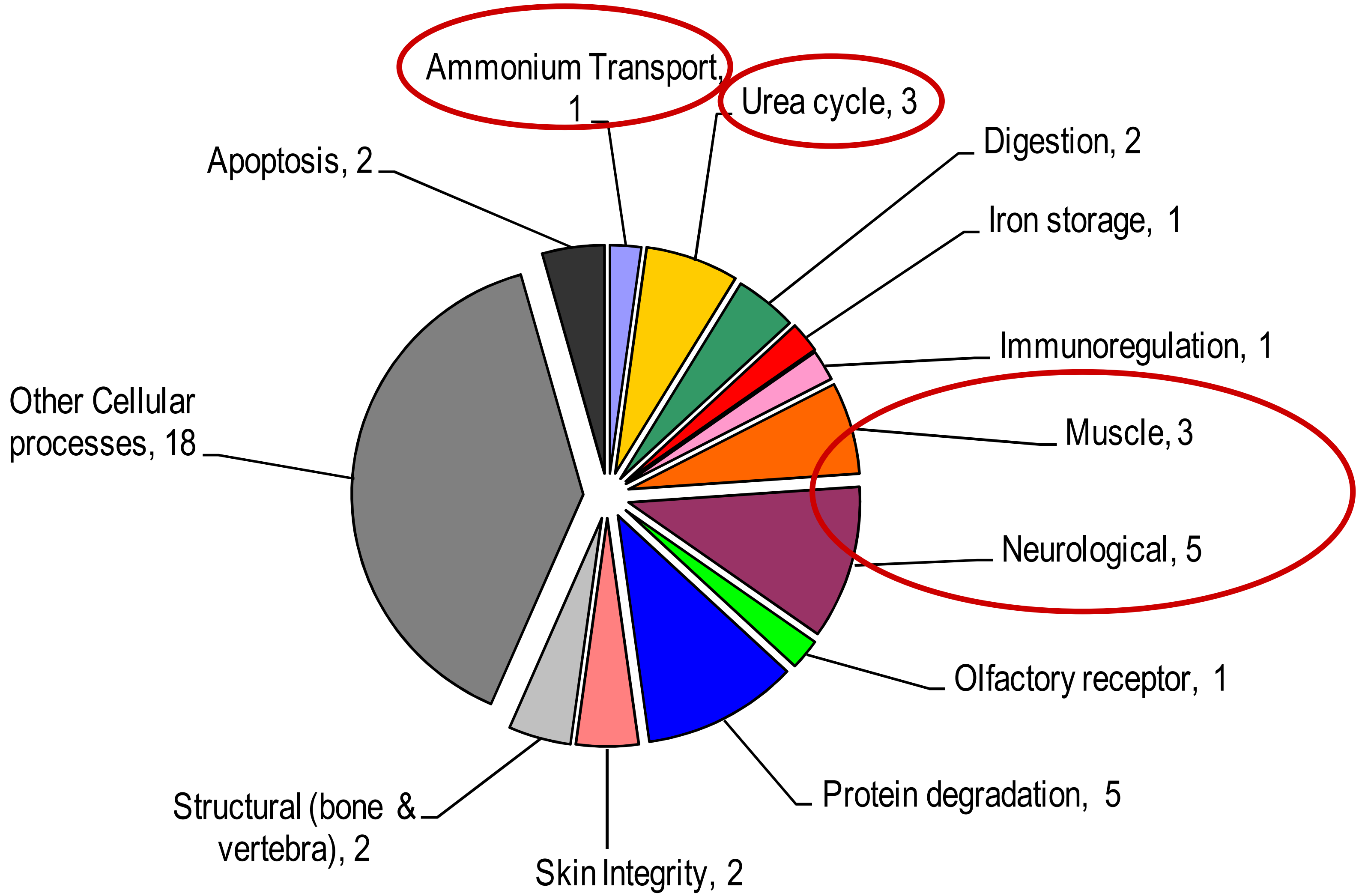
(in Sacramento River water from Garcia Bend)



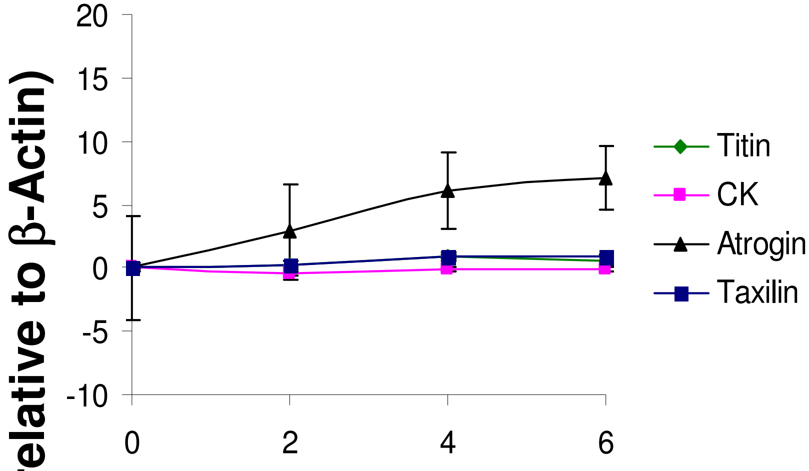


	NH4+Cl (mg.L-1 NH3/NH4+)
7-d LC50	7.5

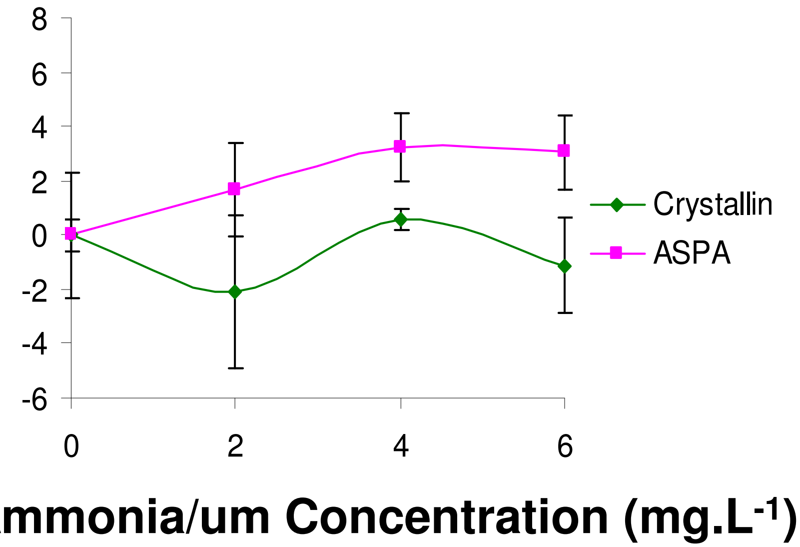
Functional classification of genes responding to 4 mg.L^{-1} NH_3/NH_4^+ from NH_4Cl



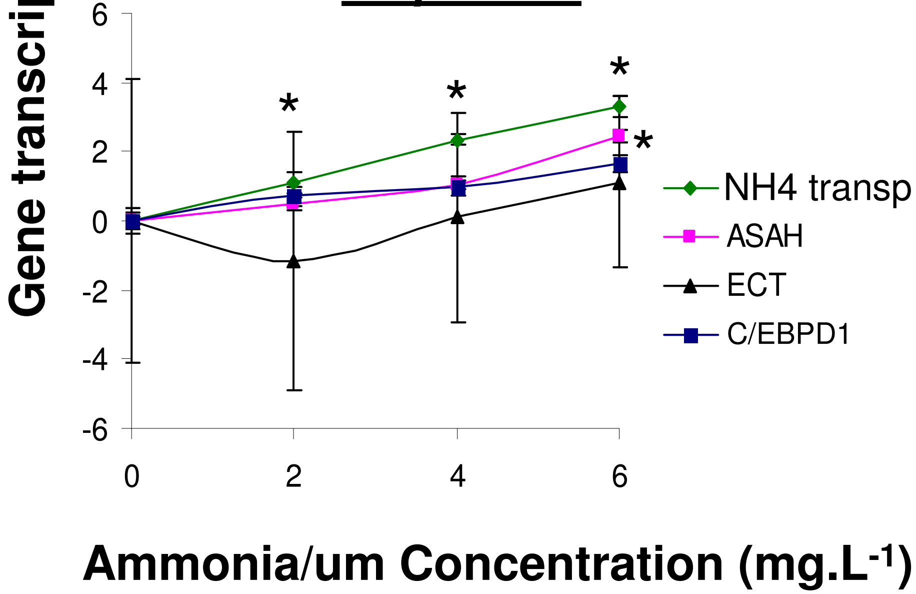
Muscular responses



Neurological responses



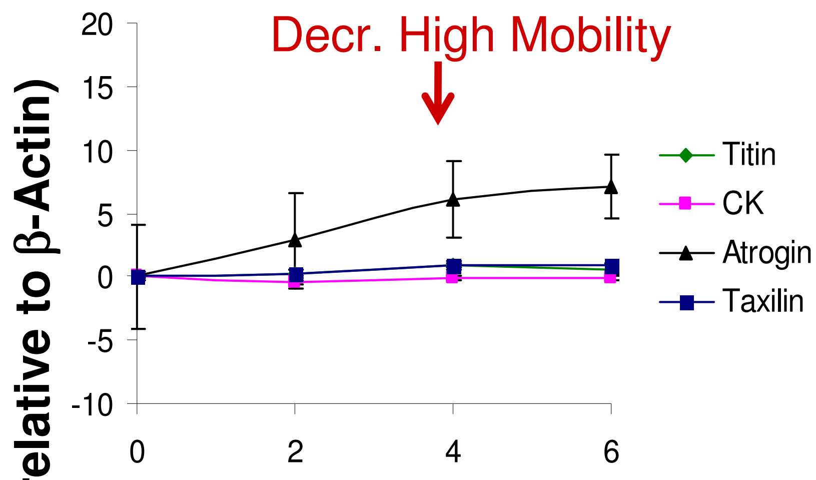
Direct link to ammonia responses



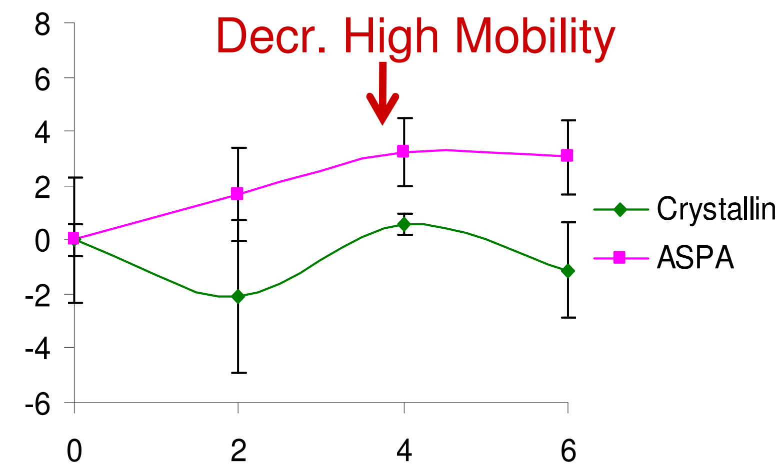
Molecular biomarker responses (n=10)

Genes were selected from top 10 statistically significant responses identified with microarray – and others from previous tests (e.g. Creatine Kinase, Aspartoacylase)

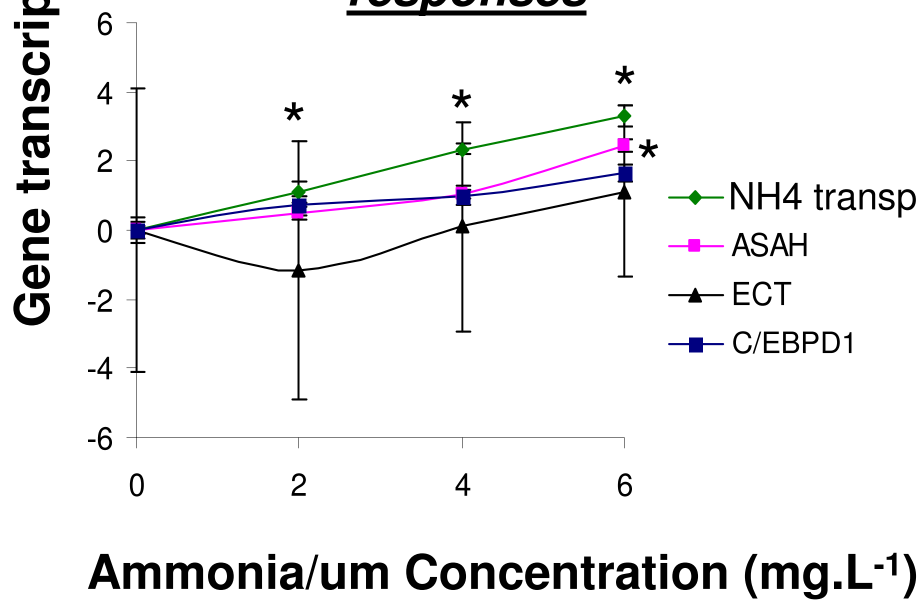
Muscular responses

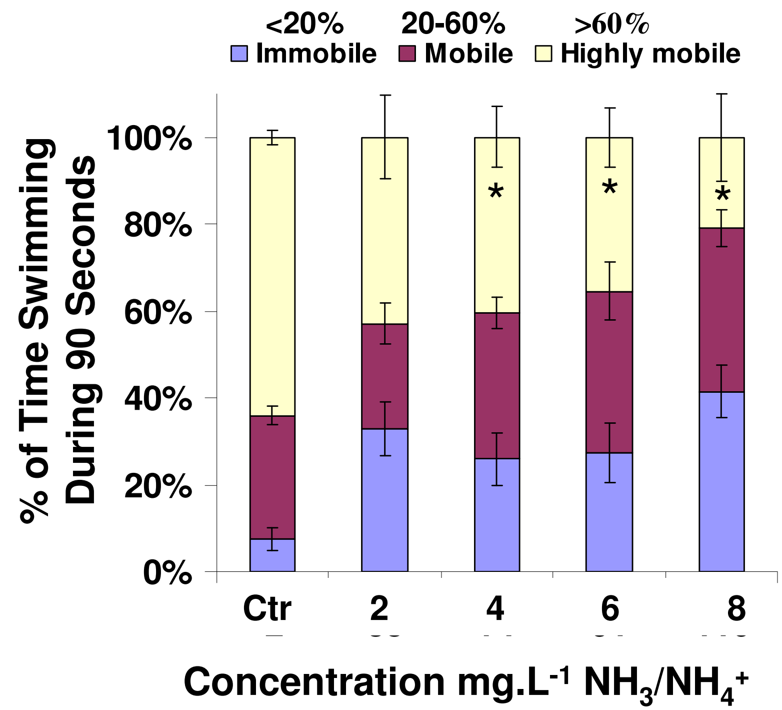


Neurological responses

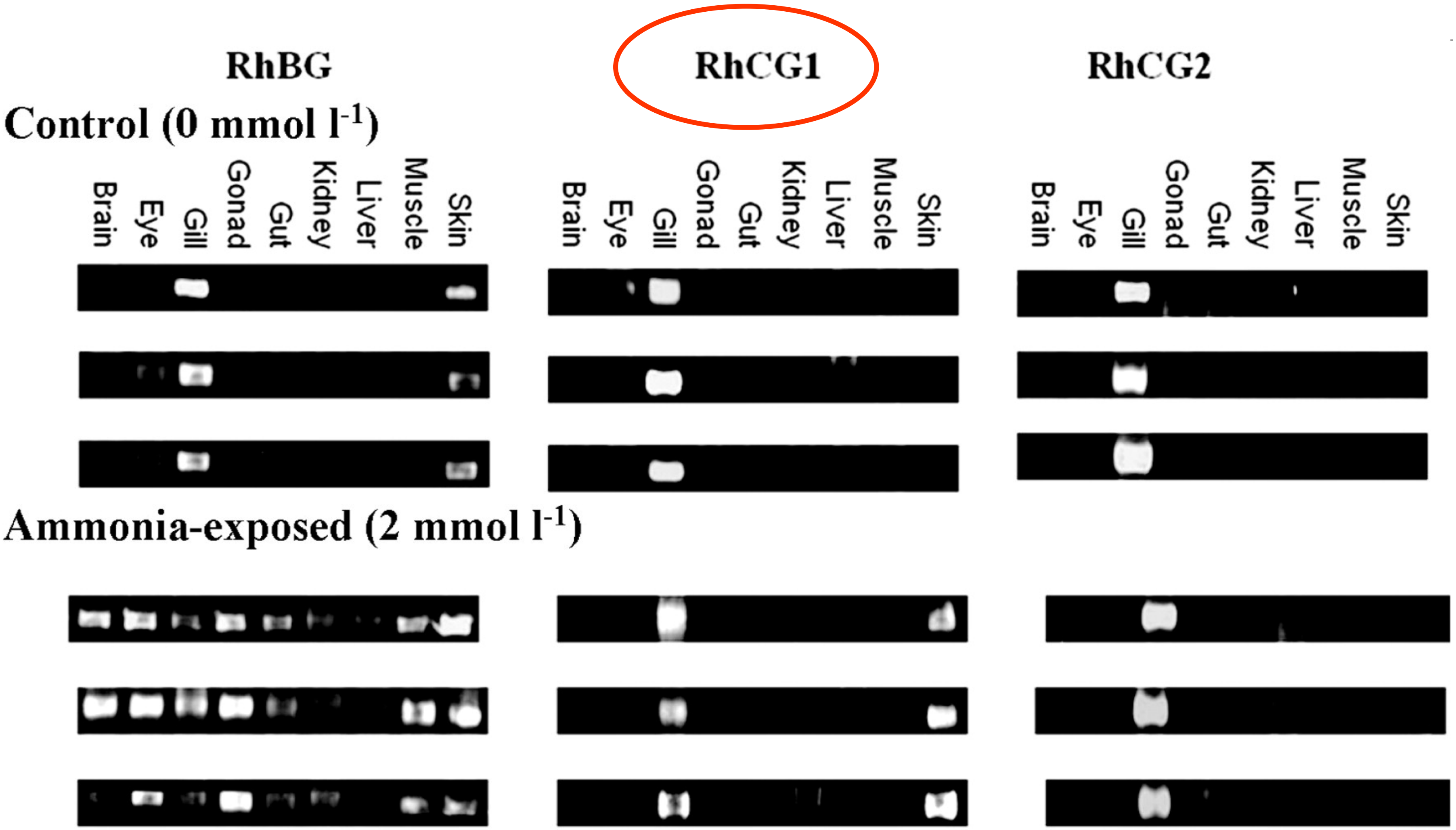


Direct link to ammonia responses





Hung CYC, et al (2007) Rhesus glycoprotein gene expression in the mangrove killifish *Kryptolebias marmoraturs* exposed to elevated environmental ammonia levels and air. J. Experimental Biology.



Quantitative PCR mRNA expression of *RHbG, RhCG1 and RhCG2* in control and ammonia-exposed *K. marmoratus.*

Current, future and proposed work

- Generating contaminant-specific genomic profile database, using microarray and qPCR technology
- Assaying field based exposures
- Developing biomarkers linked to reproduction

Questions we're seeking answers to:

- Can we use profiling system to aid TIE investigations based on chemical modes of action?
- Do all fish species respond comparatively to equitoxic contaminant concentrations?

Molecular Biomarkers as Tools in Toxicity Testing and Field Monitoring

?

Major Challenges:

- Laboratory toxicity testing with resident species;
  - Often difficult to culture and handle.
  - Require extensive method development.
  - Can generally not be used for in-depth studies evaluating ecologically important chronic endpoints, such as reproductive success, behavior and/or growth.
- Interpretation of biomarker data collected in field monitoring studies.

Model Fish Species in Toxicity Testing

- **Rainbow Trout (*survival, embryo test, growth*)**
- **Fathead Minnow (*growth, survival, reproduction*)**
- **Zebrafish (*everything?)***

Important Questions:

- **Are expression patterns of selected biomarker genes reflective of species sensitivity and stressor type across species?**

- **Are molecular biomarkers mechanistically linked to contaminant type and ecologically relevant effects?**

….. Connecting the Dots…..

Delta Smelt Biomarkers

Other Available Biomarker Information

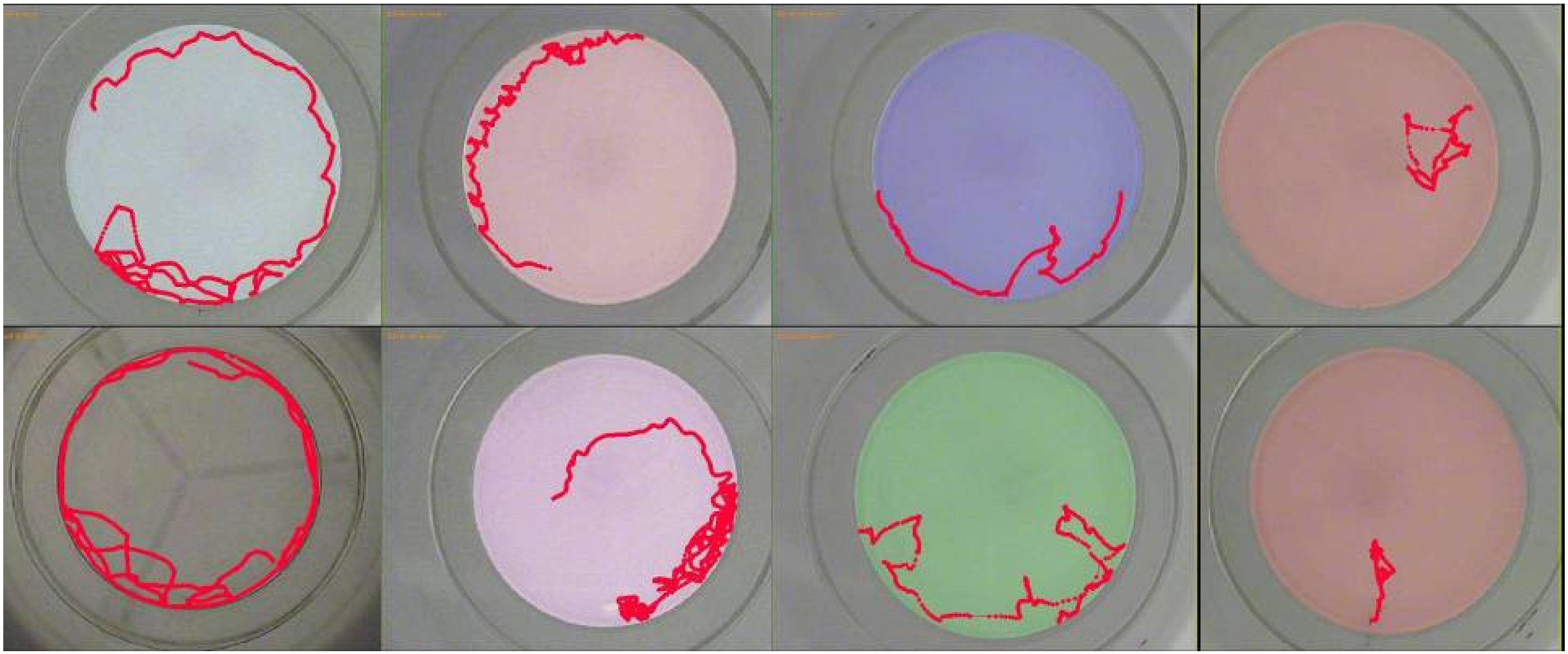
Fathead Minnow

Establish **Linkages** (the “Bridge”) to **Ecologically Relevant Adverse Effects**: growth, reproduction, behavior, survival

Toxicant Identification Tools for Model Species

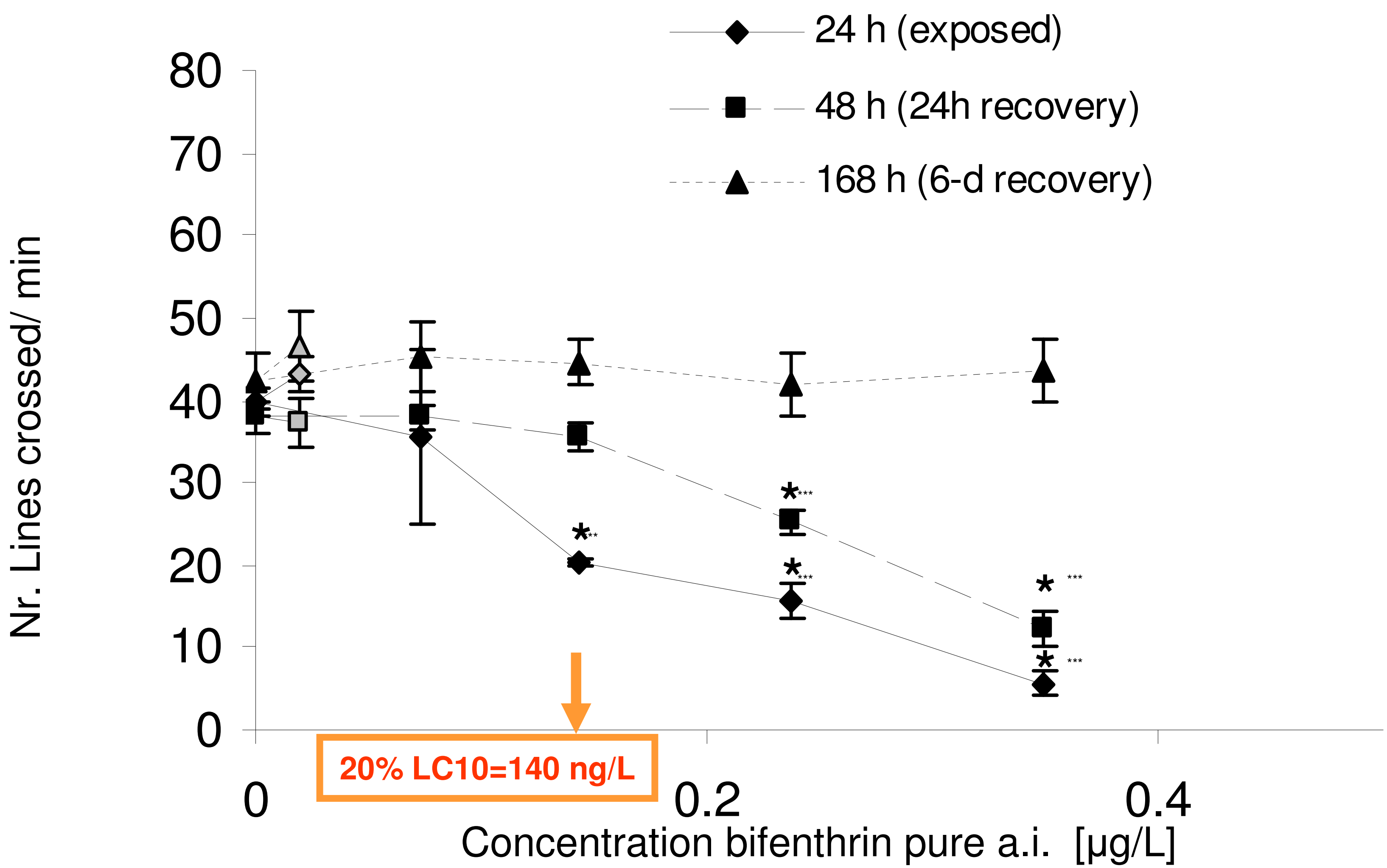
Mechanism-Based, Interpretable Biomarkers for Field Studies with Resident Species

Neuromuscular Effects of Pesticides: Swimming Behavior of Larval Fathead Minnow after 24 h Exposure to Talstar® (Bifenthrin)



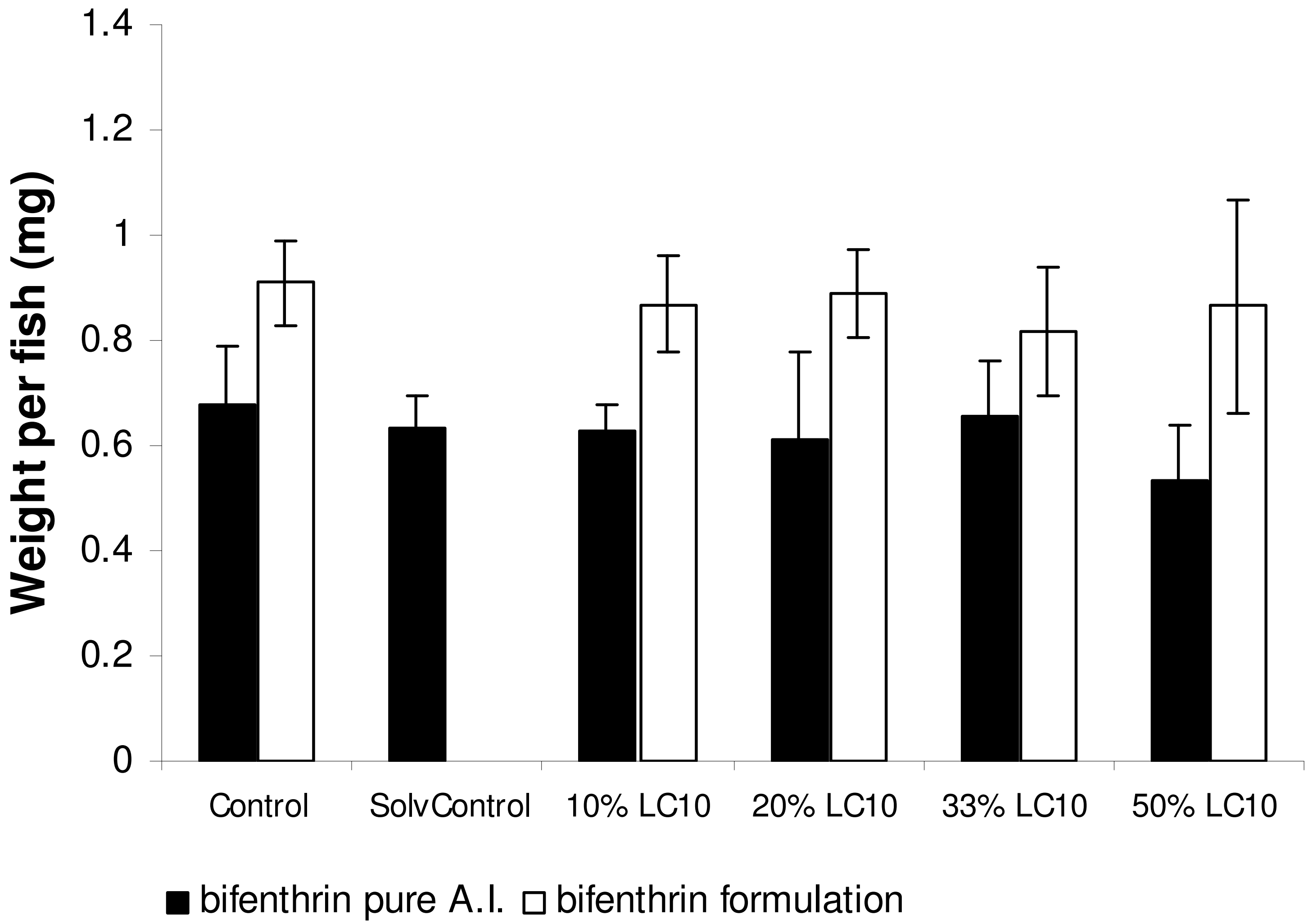
Control 20% LC10 33% LC10 50% LC10

Swimming Activity in Fathead Minnows Exposed to Bifenthrin for 24 h



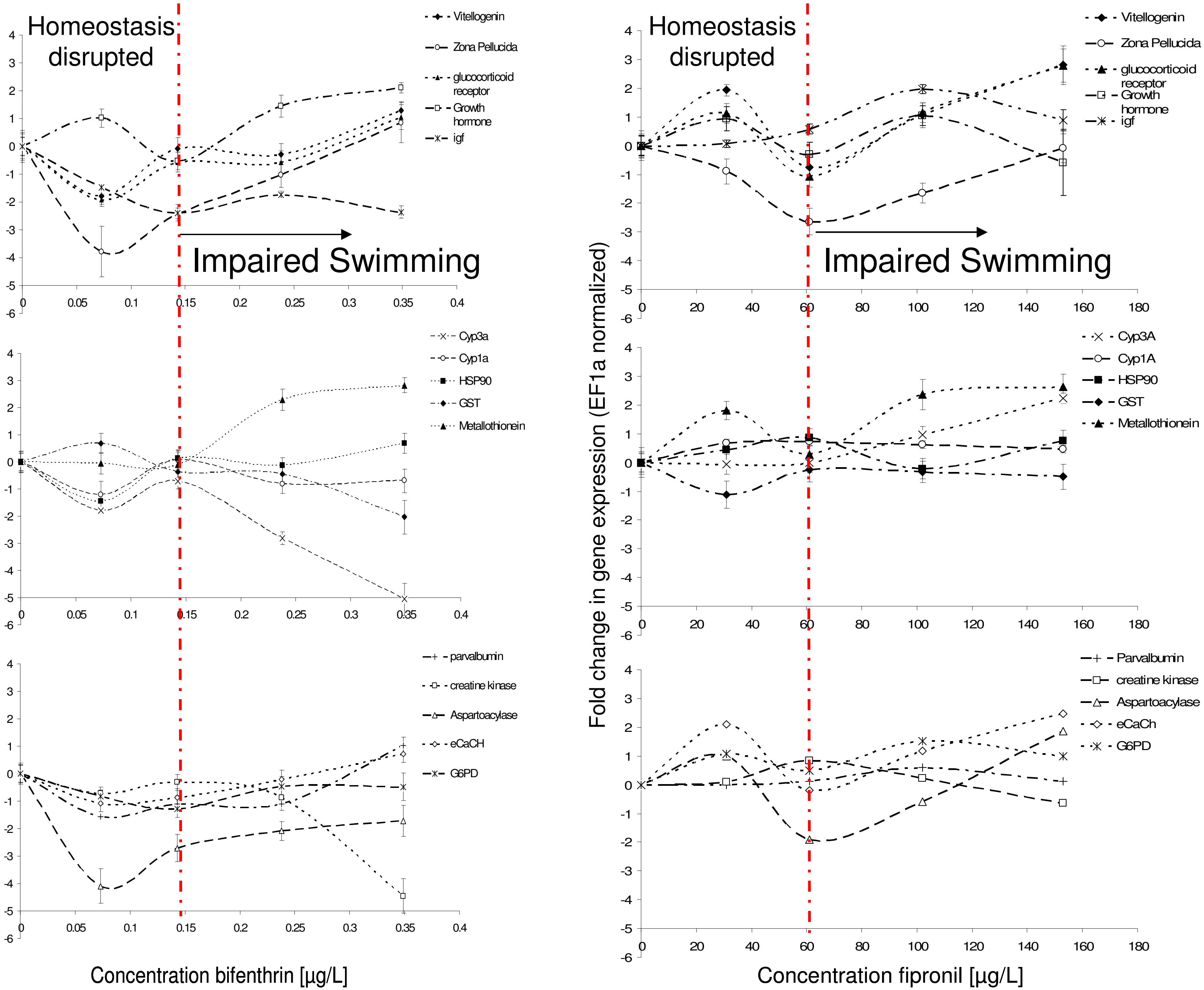
Beggel S, Werner I, Connon RE, Geist JP. (2010) *Science of The Total Environment*. 408: 3169-3175.

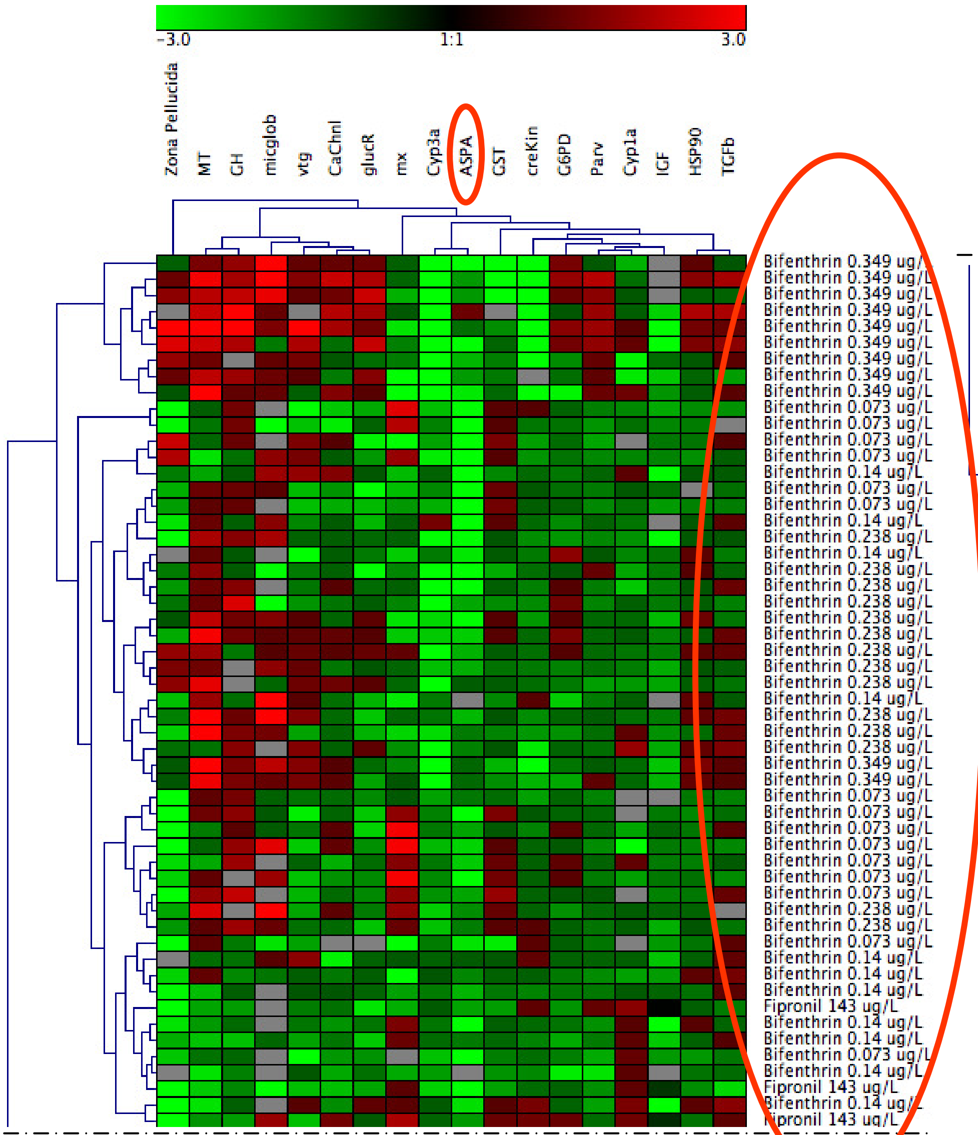
Growth in Fathead Minnows

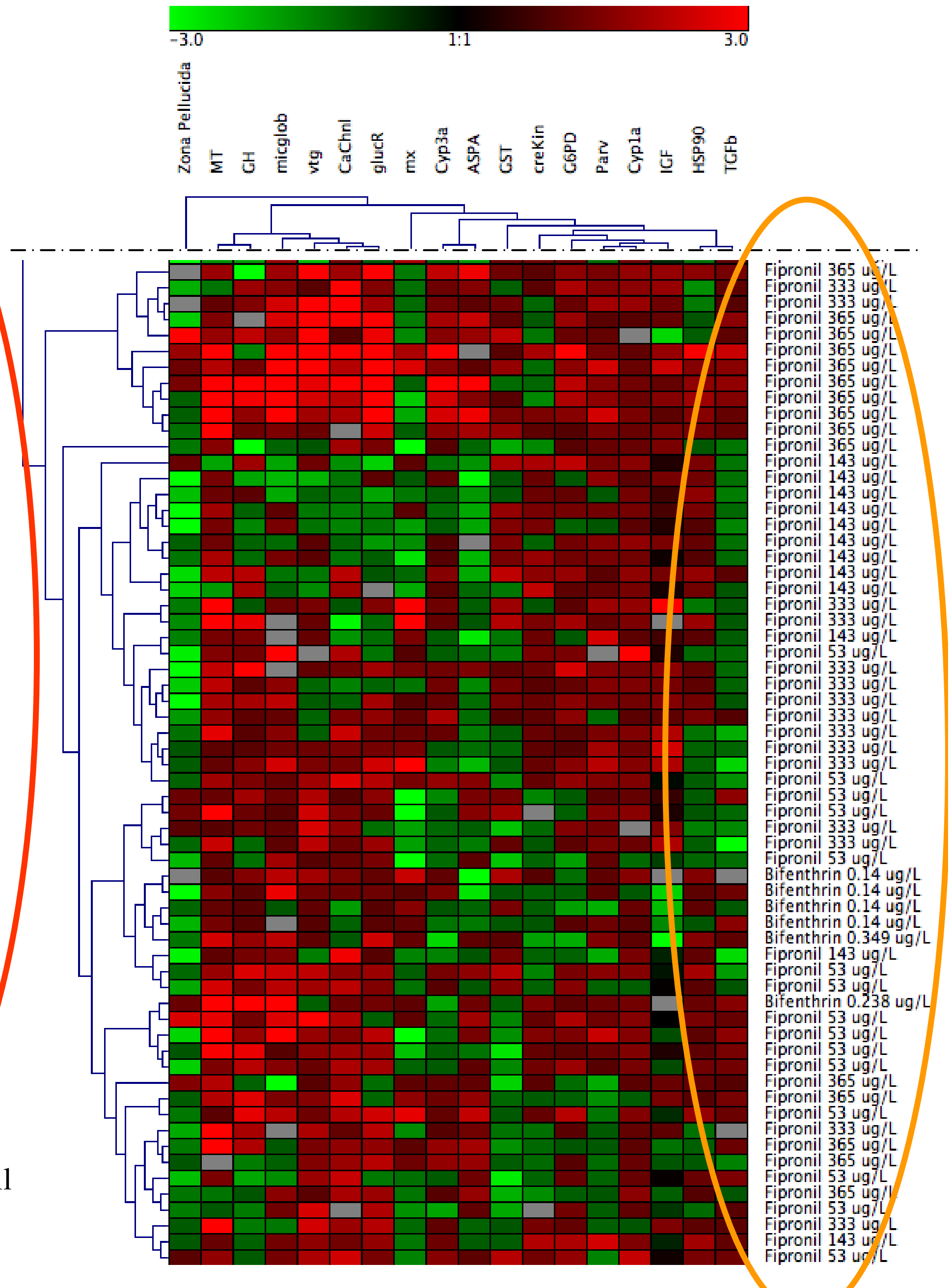


Selected Molecular Biomarkers in Fathead Minnow

Gene Function	Gene
General Stress Response	HSP70, HSP90
Detoxification	Metallothionein, Glutathione-S Transferase, CYP1A
Endocrine Disruption/ Development	Vitellogenin, CYP3A, Zona Pellucida Protein, Glucocorticoid Receptor
Iron Storage	Ferritin
Neuromuscular Function/Energy	Parvalbumin, Creatine Kinase, Epithelial Ca+ Channel, Aspartoacylase, Titin, Myozenin, G6PD
Growth	Insuline-Like Growth Factor, Growth Hormone
Apoptosis	Caspase 1
Immune System	Microglobulin, IL8, Mx Protein, Nramp


Homeostasis disrupted
Impaired Swimming
Vitellogenin
Zona Pellucida
glucocorticoid receptor
Growth hormone
igf
Cyp3a
Cyp1a
HSP90
GST
Metallothionein
parvalbumin
creatine kinase
Aspartoacylase
eCaCH
G6PD
Cyp3A
Cyp1A
Parvalbumin
Fold change in gene expression (EF1a normalized)
Concentration bifenthrin [µg/L]
Concentration fipronil [µg/L]



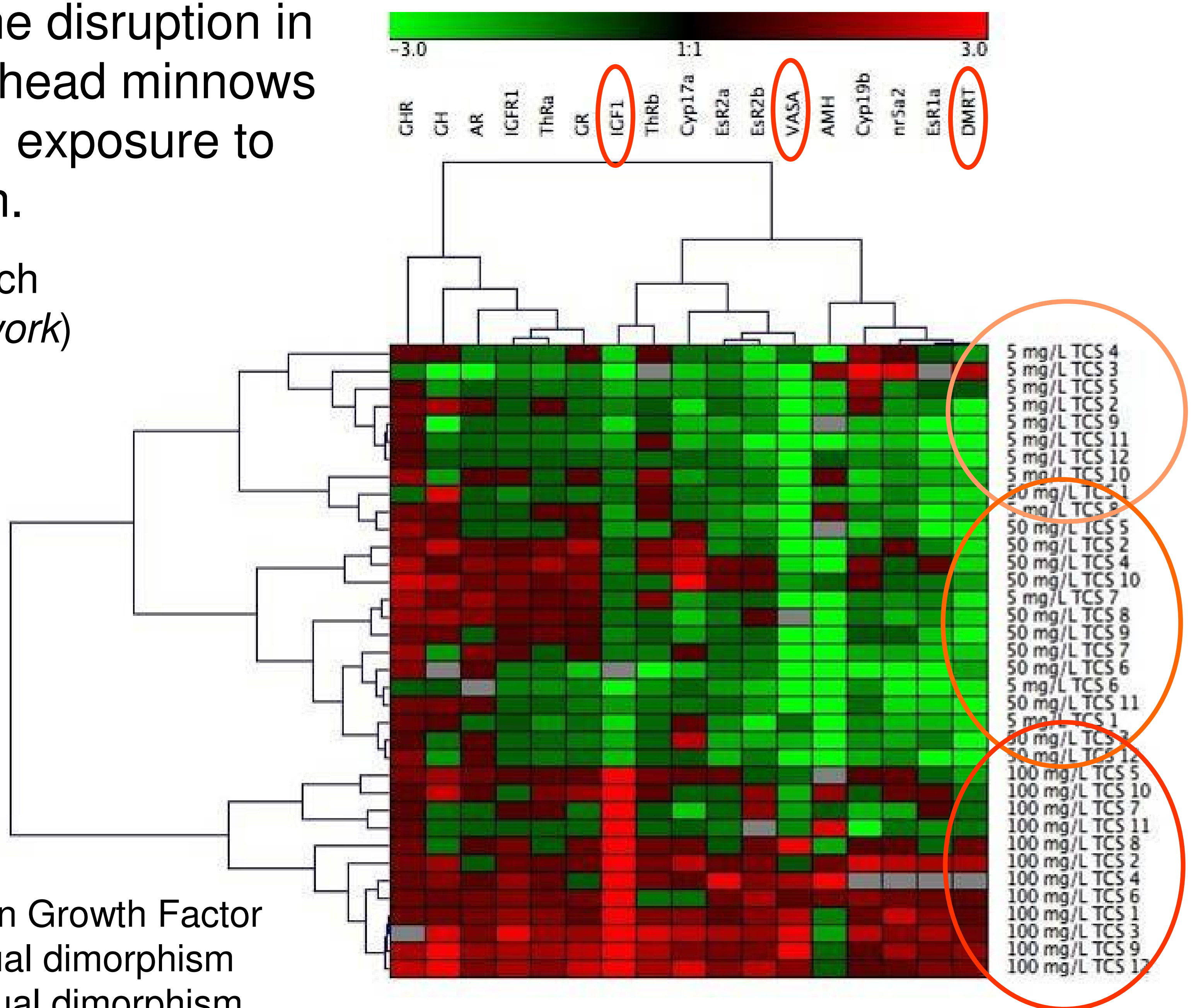


Genomic fingerprinting (Heatmaps).
Molecular biomarkers distinguish between bifenthrin and fipronil exposed fathead minnow.

Beggel S, Werner I, Connon RE, Geist JP. (in preparation). Changes in gene expression in larval fathead minnow (*Pimephales promelas*) following short-term exposure to bifenthrin and fipronil.

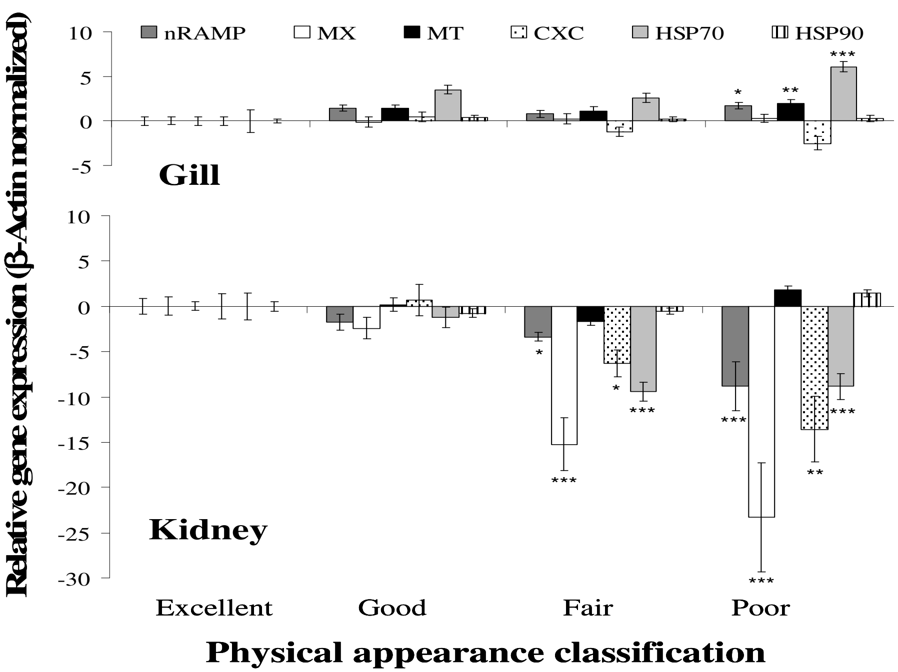
Endocrine disruption in larval fathead minnows following exposure to Triclosan.

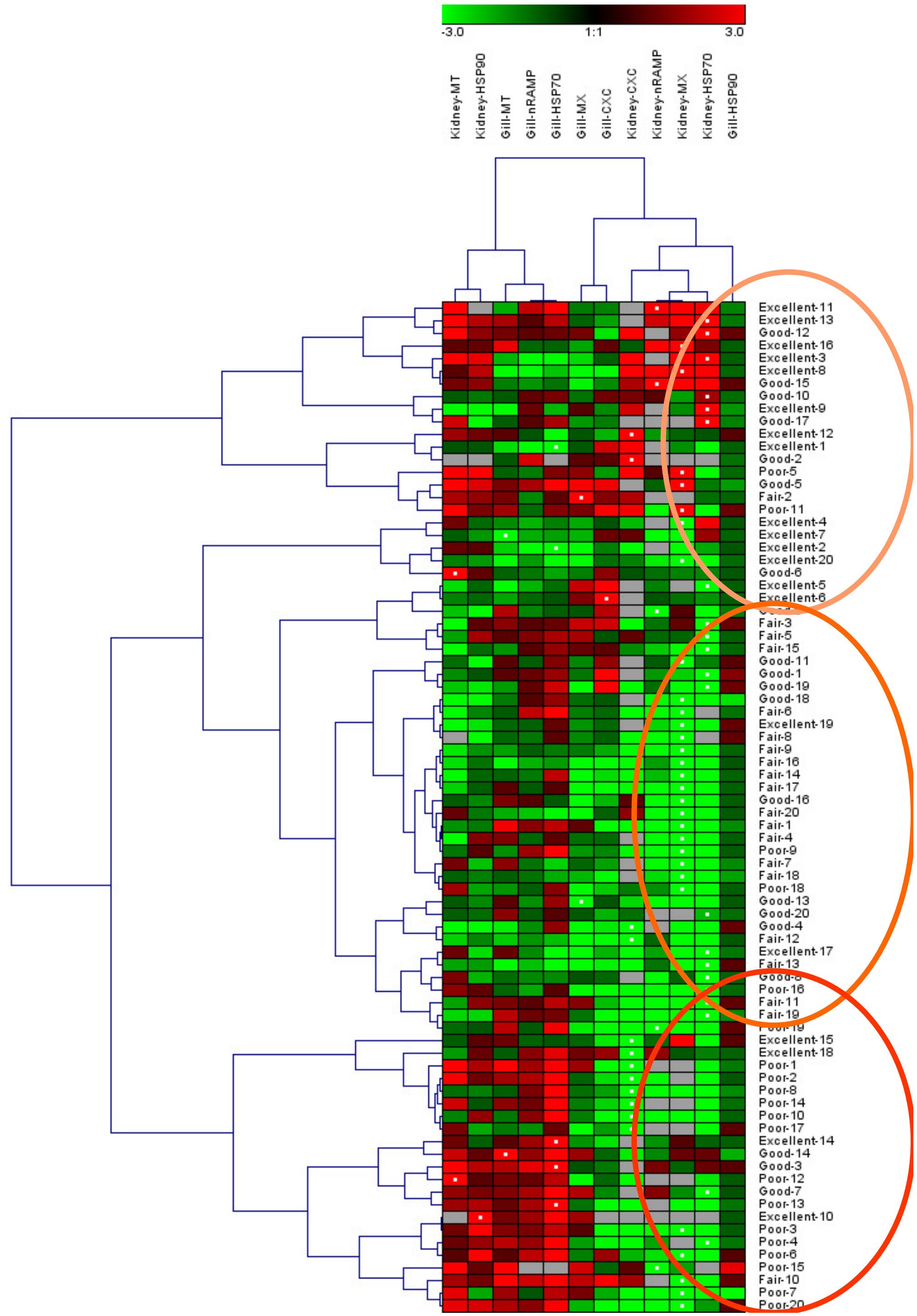
Erika Fritsch
(*Current work*)



IGF1=Insulin Growth Factor
VASA: sexual dimorphism
DMRT: sexual dimorphism

Expression of selected biomarker genes in Columbia River Steelhead Trout

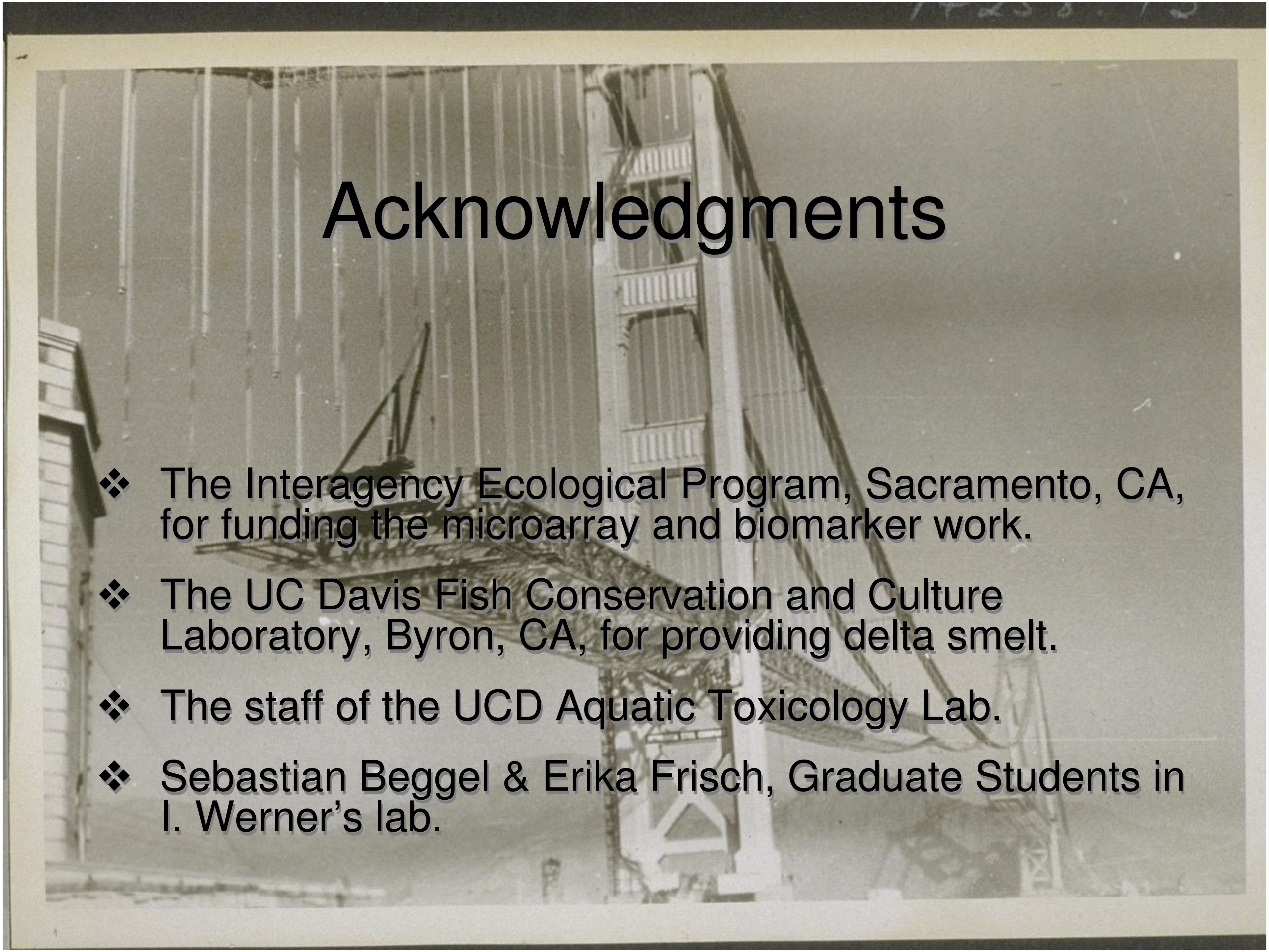




D'Abronzo et al., in prep.

Conclusions

- Microarray technology allows us to identify new mechanism-based molecular biomarkers.
- The multi-biomarker approach (heatmaps) integrates old and new information.
- Shows great promise in distinguishing the impact of different stressors (TIE).
- Allows mechanism based interpretation of biomarker signals.
- Leads to a better understanding of linkages (“adverse outcome pathways”).



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