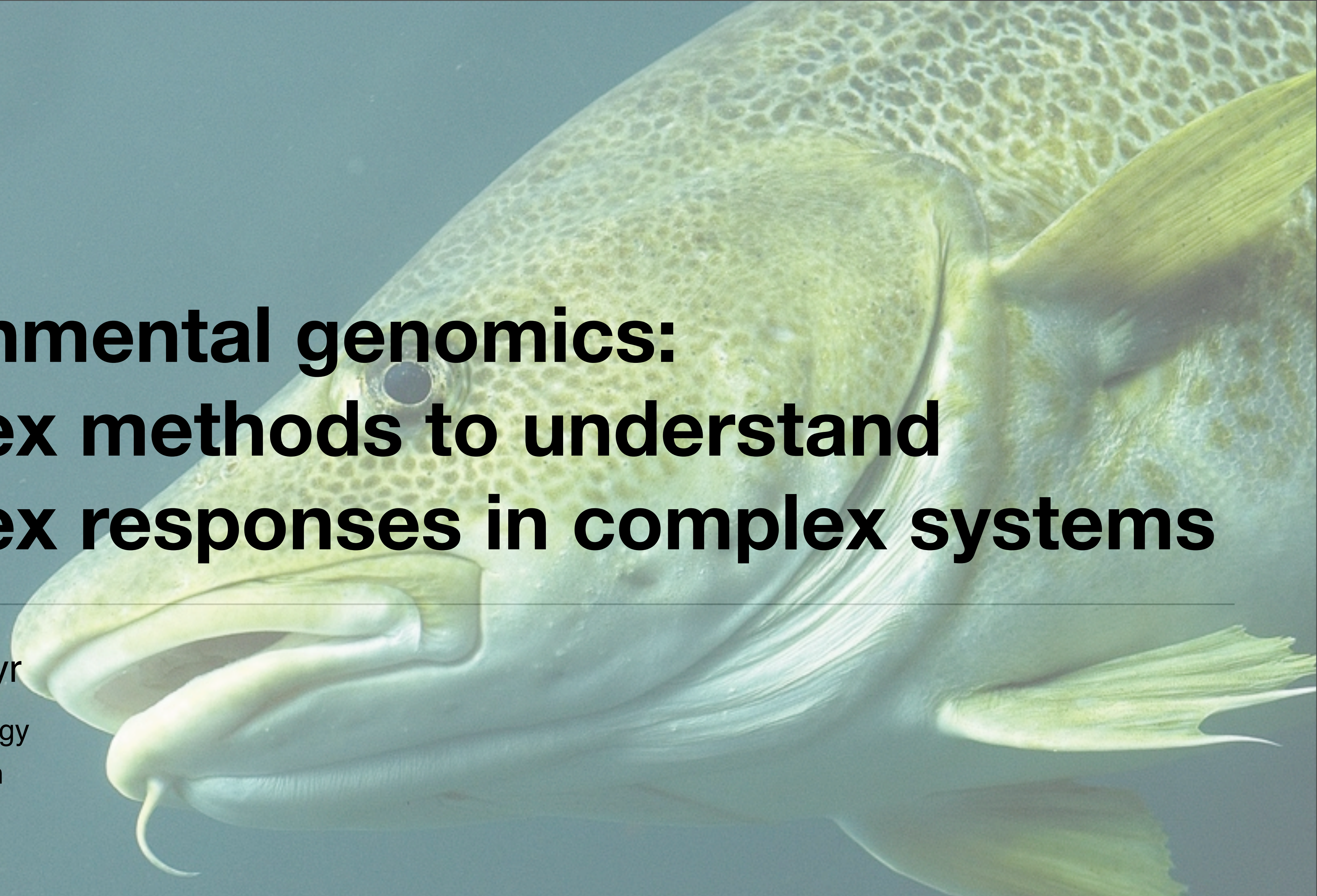
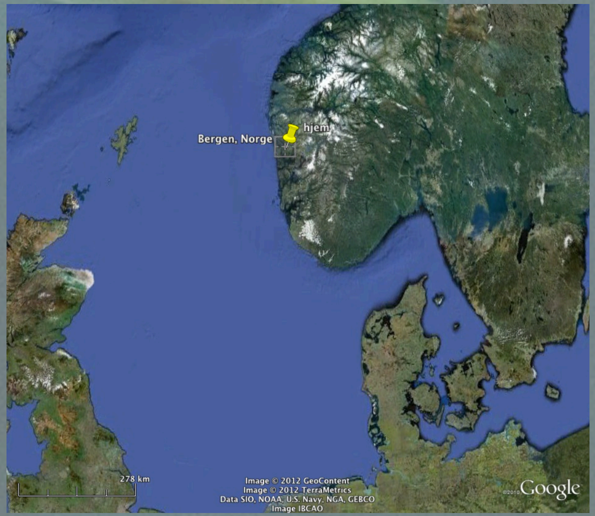


Environmental genomics: complex methods to understand complex responses in complex systems

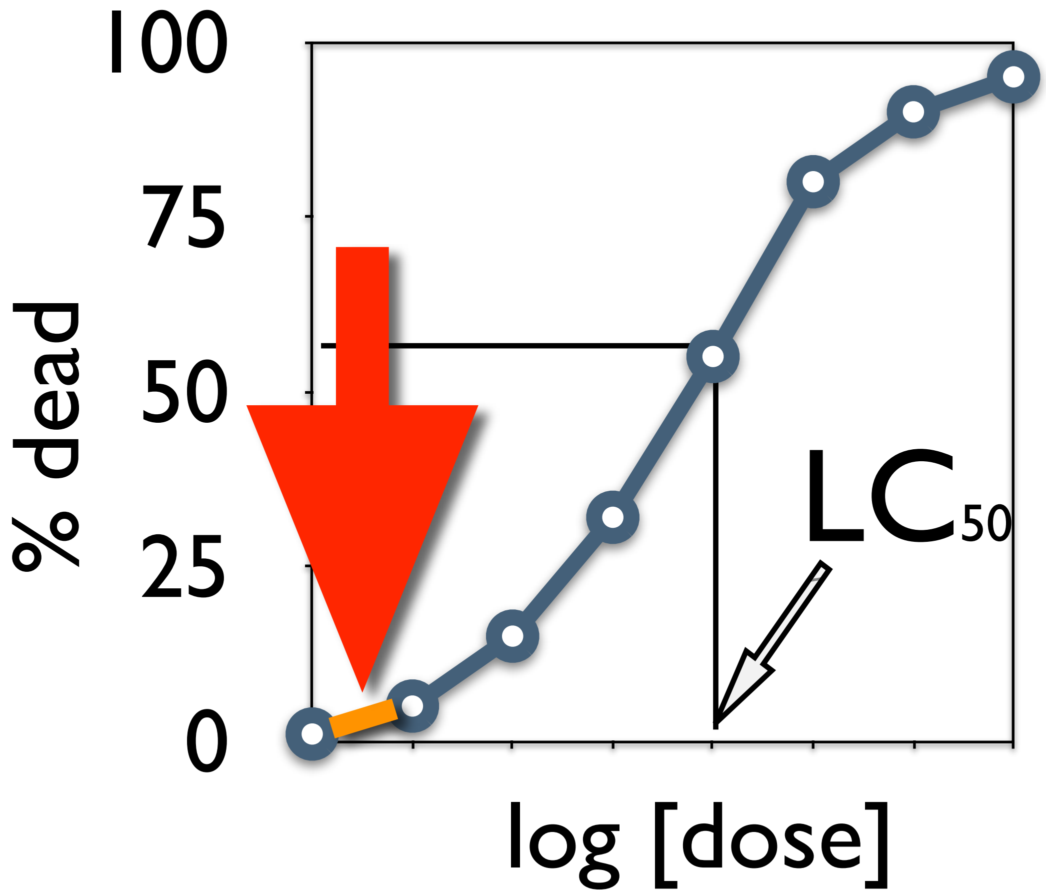
---

Anders Goksøyr

Department of Biology
University of Bergen
Norway



Acute tox goodbye: A paradigm shift in toxicology



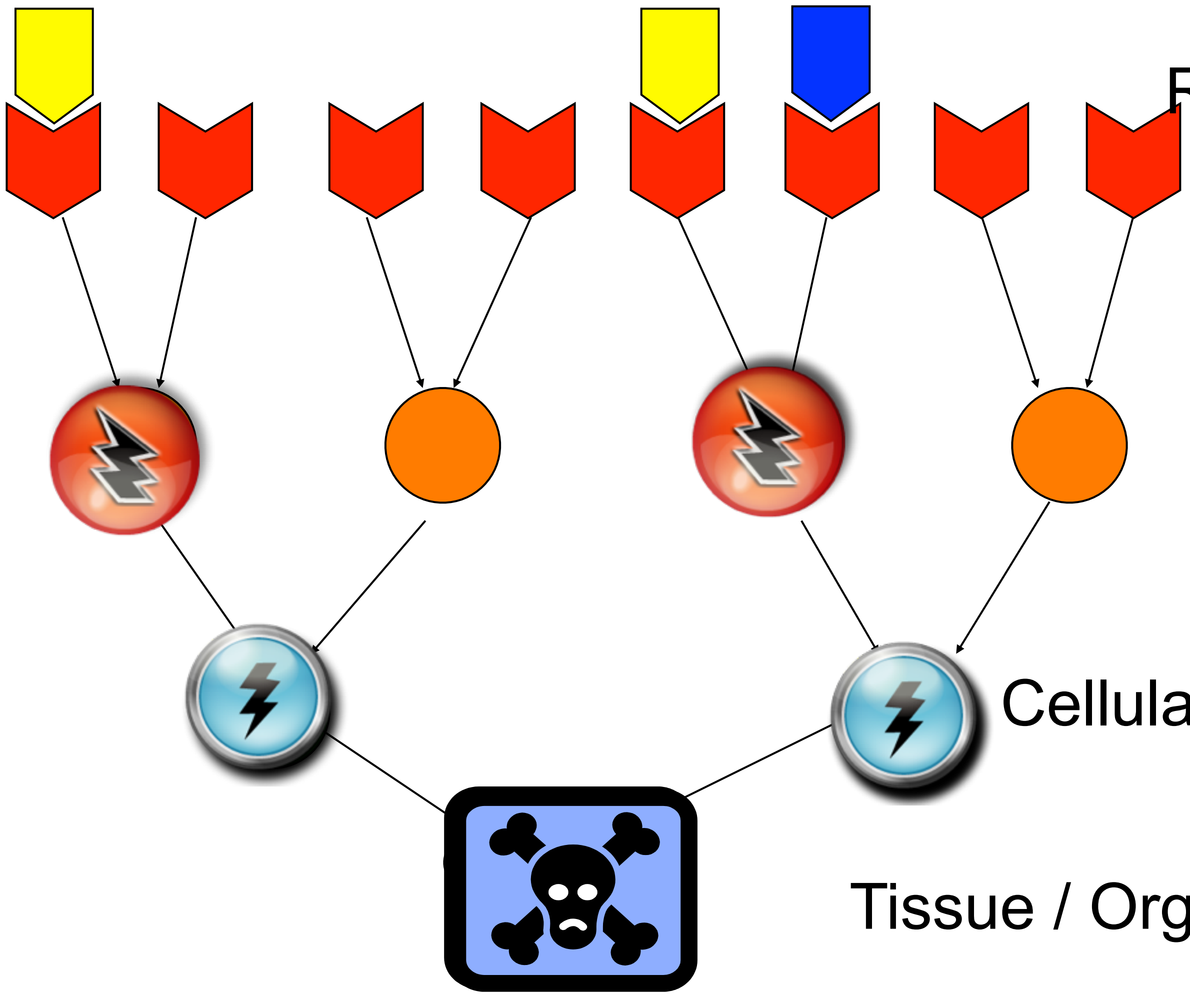
FROM:

- focus on acute effects at high doses

TO:

- focus on the ability of compounds to influence endocrine systems and metabolic homeostasis at low doses

Pathways to toxicity



Compound

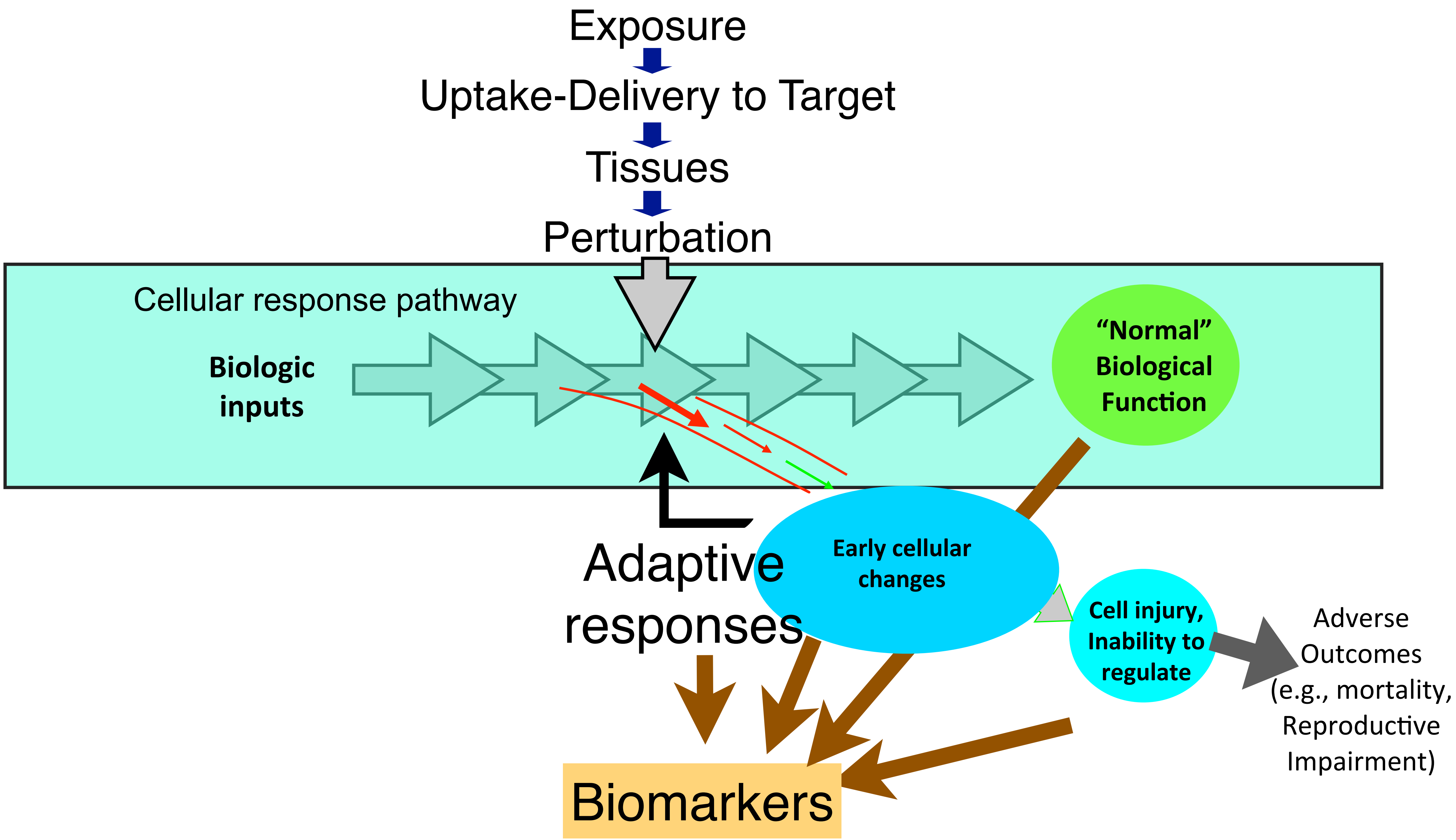
Receptors / enzymes / proteins, etc.
Direct molecular interactions

Signal pathways /
Genomics

Cellular processes

Tissue / Organ / Organism

Adaptation and compensation during a toxic response



Exposure

Uptake-Delivery to Target

Tissues

Perturbation

Cellular response pathway

Biologic inputs

"Normal" Biological Function

Early cellular changes

Adaptive responses

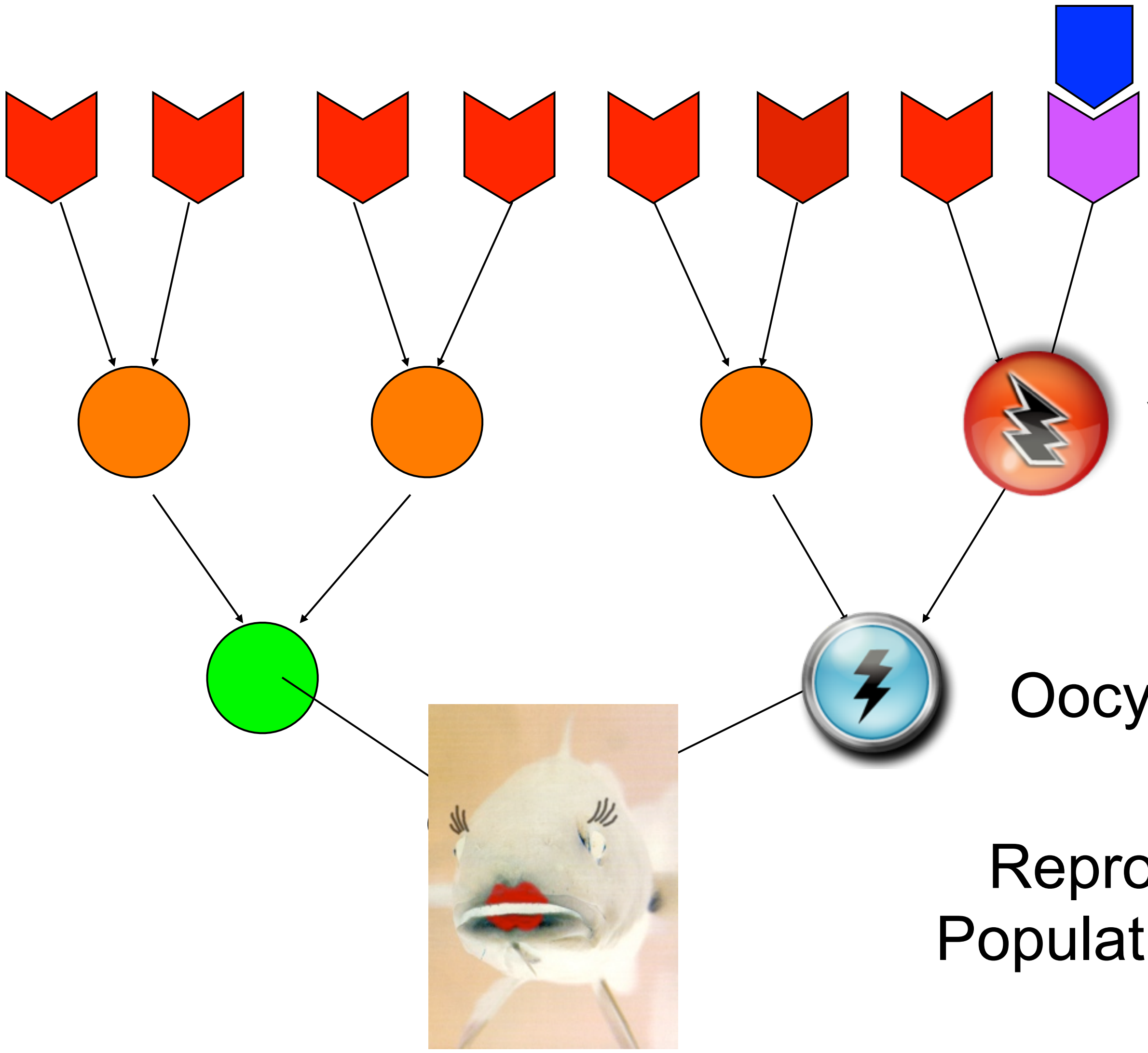
Cell injury, Inability to regulate

Adverse Outcomes (e.g., mortality, Reproductive Impairment)

Biomarkers

Adapted from Toxicity Testing in the 21st Century, NRC

Vitellogenin (VTG) = biomarker for endocrine disrupting compounds (EDC)



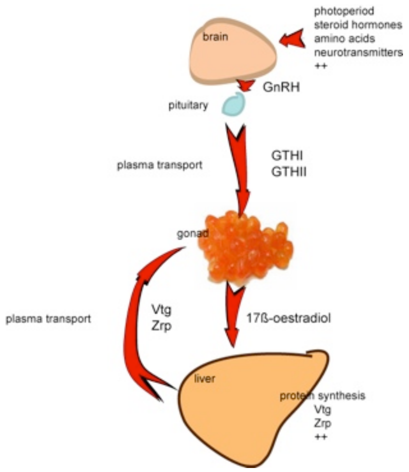
EDC

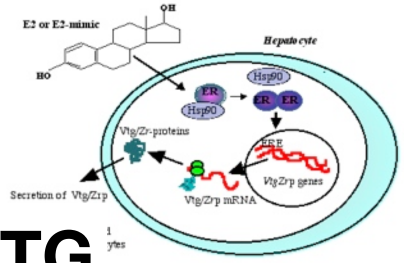
Estrogen-receptor

Vitellogenin

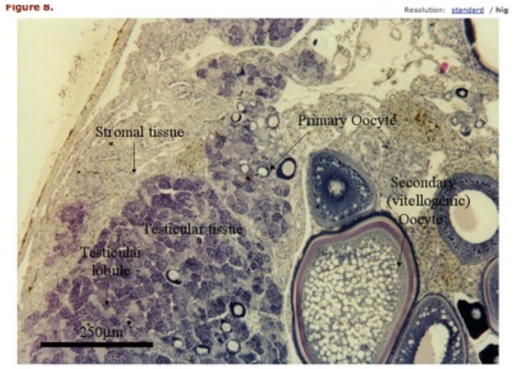
Oocyte development/
ovotestis

Reproduction/
Population effects





VTG



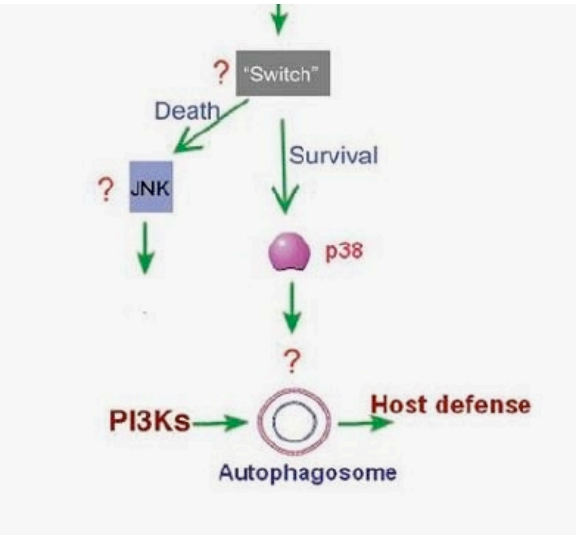
Histological section of a grossly intersex gonad of the gudgeon, *Gobio gobio*, containing testicular tissues and both primary and secondary (vitellogenic) oocytes. Picture is a kind donation from Ronny van Aerle and Charles Tyler.

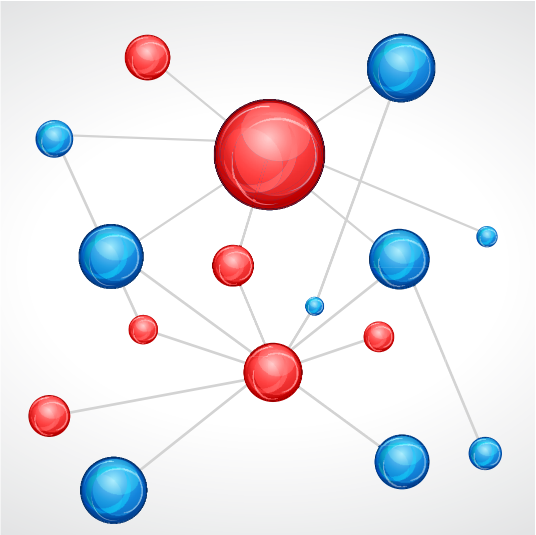
Arukwe and Goksøyr *Comparative Hepatology* 2003 **2**:4 doi:10.1186/1476-5926-2-4

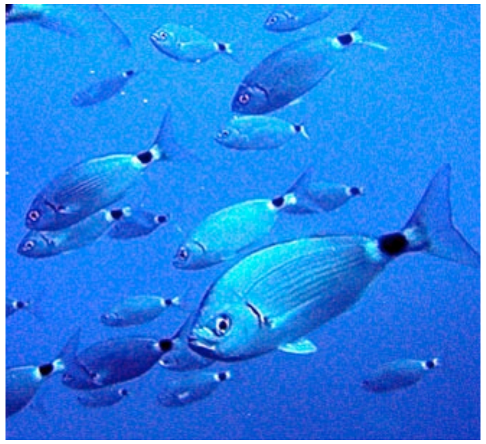
Figures from Arukwe & Goksøyr Comp Hepatol (2003)

Foto: Sonnich Meier

Complexity of systems in ecotoxicology







Single molecule

Cellular Pathways

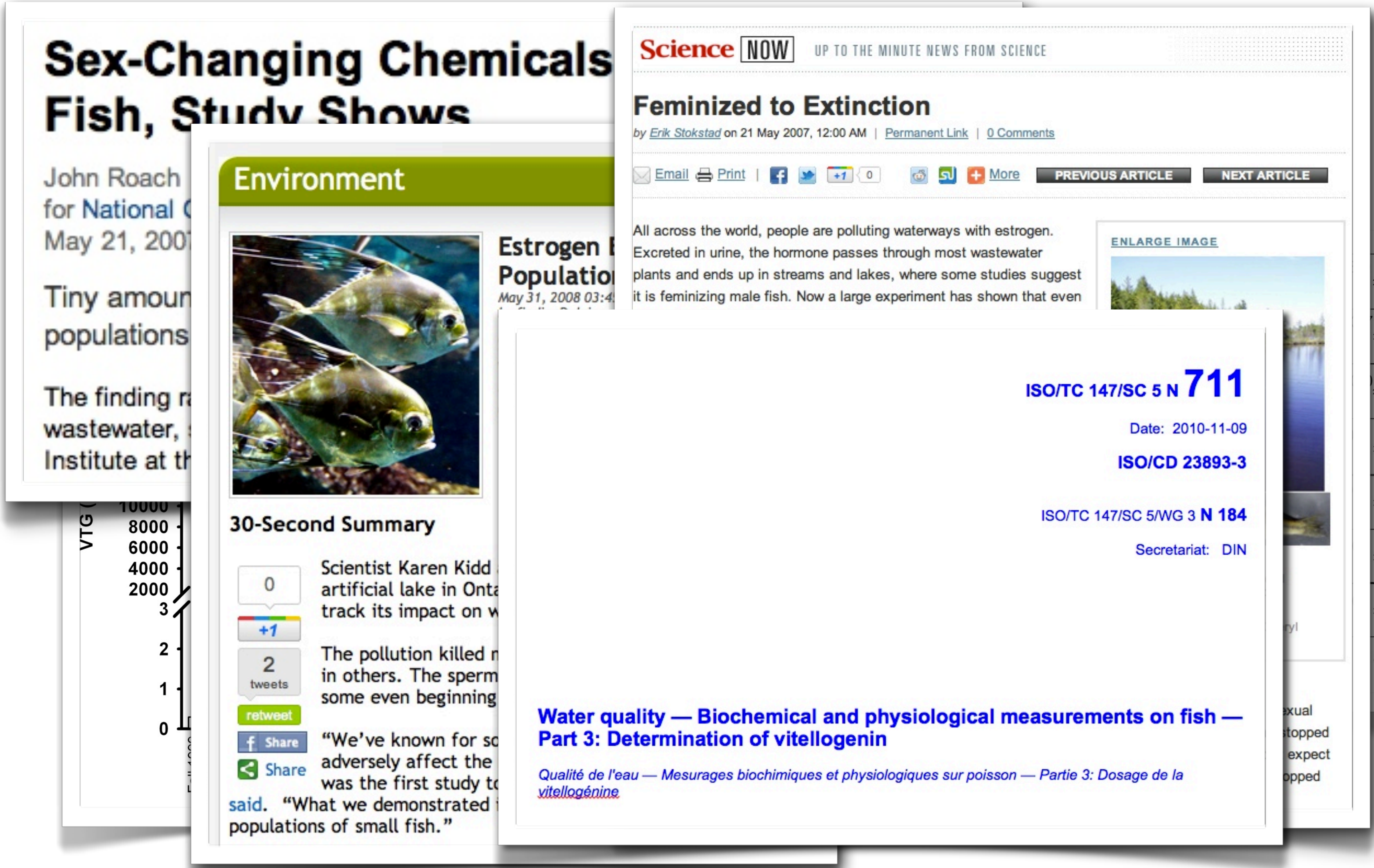
Tissue Networks of interactions

Organism

Populations

Complexity

Mechanistic understanding



Sex-Changing Chemicals Fish, Study Shows

John Roach
for National
May 21, 200

Tiny amoun
populations

The finding r
wastewater,
Institute at th

Environment

Estrogen
Populatio
May 31, 2008 03:4

30-Second Summary

0
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tweets
retweet
Share
Share

Scientist Karen Kidd
artificial lake in Onta
track its impact on w

The pollution killed
in others. The sperm
some even beginning

"We've known for so
adversely affect the
was the first study to
said. "What we demonstrated
populations of small fish."

Science NOW UP TO THE MINUTE NEWS FROM SCIENCE

Feminized to Extinction

by Erik Stokstad on 21 May 2007, 12:00 AM | Permanent Link | 0 Comments

Email Print | +1 0 More PREVIOUS ARTICLE NEXT ARTICLE

All across the world, people are polluting waterways with estrogen. Excreted in urine, the hormone passes through most wastewater plants and ends up in streams and lakes, where some studies suggest it is feminizing male fish. Now a large experiment has shown that even

ENLARGE IMAGE

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ISO/TC 147/SC 5 N 711

Date: 2010-11-09

ISO/CD 23893-3

ISO/TC 147/SC 5/WG 3 **N 184**

Secretariat: DIN

Water quality — Biochemical and physiological measurements on fish — Part 3: Determination of vitellogenin

Qualité de l'eau — Mesurages biochimiques et physiologiques sur poisson — Partie 3: Dosage de la vitellogénine

2011

OPEN ACCESS Freely available online

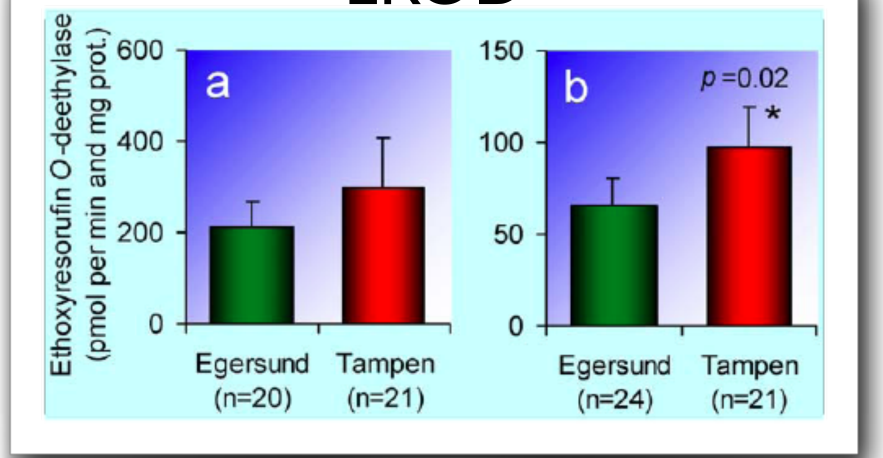
PLoS one

Biomarkers in Natural Fish Populations Indicate Adverse Biological Effects of Offshore Oil Production

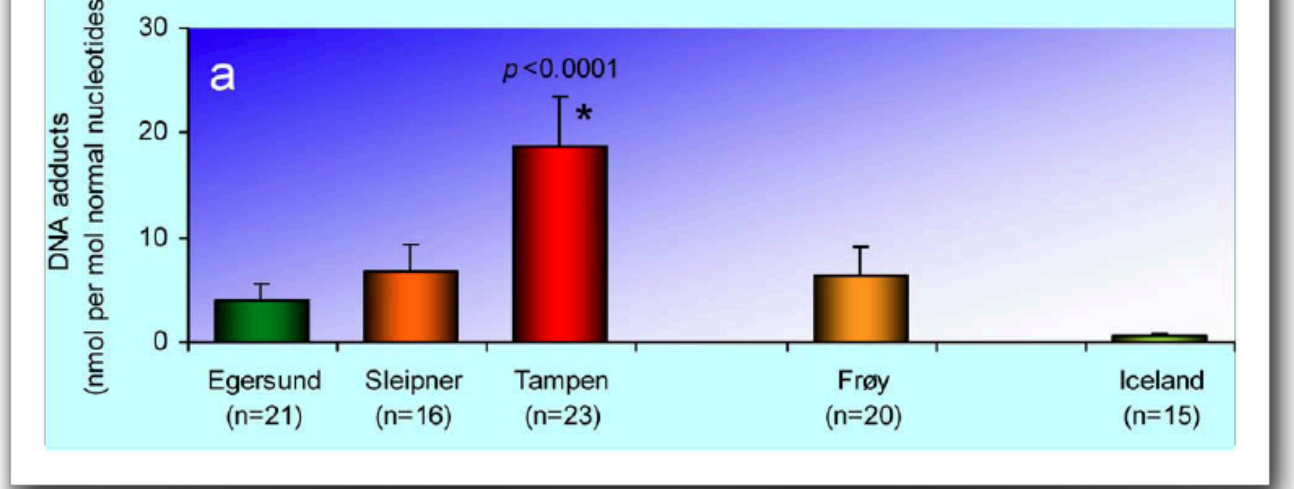
Lennart Balk[1]*, Ketil Hylland[2,3], Tomas Hansson[1]*, Marc H. G. Berntssen[4], Jonny Beyer[5,6], Grete Jonsson[7], Alf Melbye[8], Merete Grung[3], Bente E. Torstensen[4], Jan Fredrik Børseth[5], Halldora Skarphedinsdottir[1], Jarle Klungsøyr[9]

1 Department of Applied Environmental Science (ITM), Stockholm University, Stockholm, Sweden, **2** Department of Biology, University of Oslo, Oslo, Norway, **3** Norwegian Institute for Water Research (NIVA), Oslo, Norway, **4** National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway, **5** International Research Institute of Stavanger (IRIS), Stavanger, Norway, **6** Department of Mathematics and Natural Science, University of Stavanger, Stavanger, Norway, **7** Department of Medical Biochemistry, Stavanger University Hospital, Stavanger, Norway, **8** Marine Environmental Technology, SINTEF Materials and Chemistry, Trondheim, Norway, **9** Institute of Marine Research (IMR), Bergen, Norway

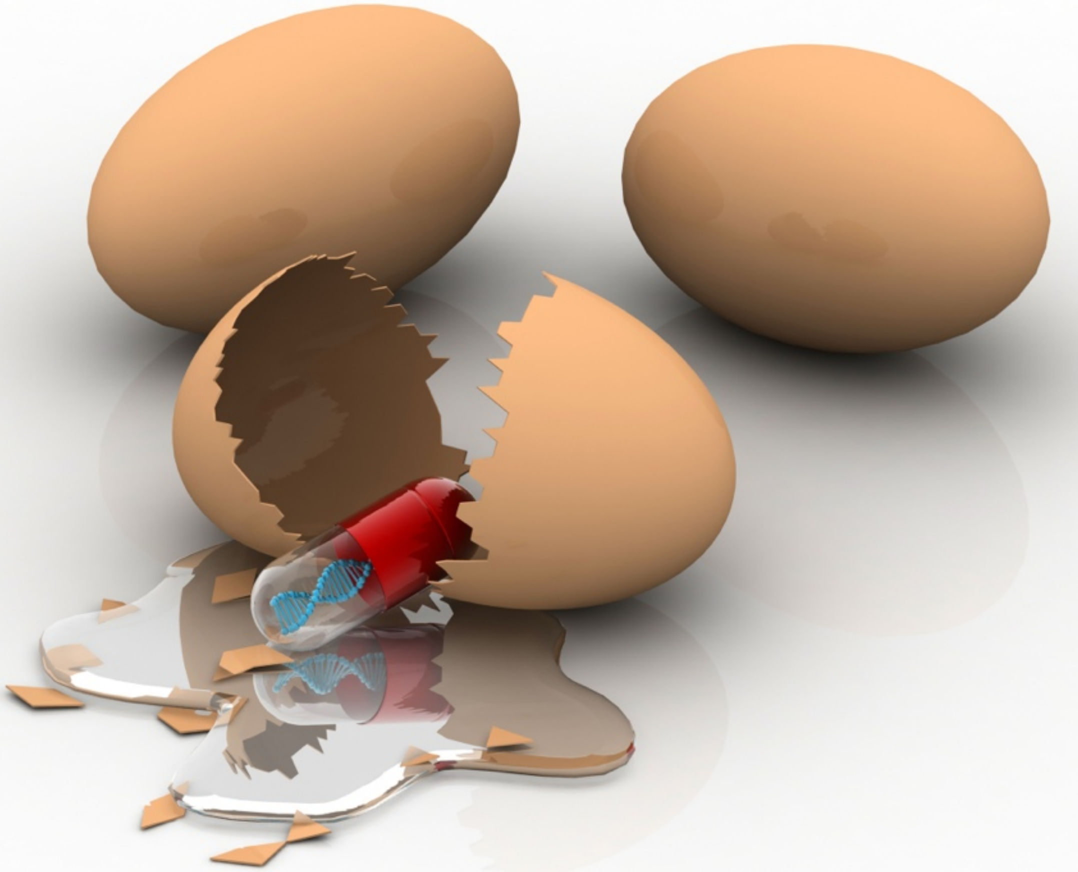
EROD



DNA-skade



The emergence of systems biology



> We're exposed to lots of chemicals but at very low concentrations over time. We need tools to help us understand how complex exposures perturb complex systems.
>
> - *William Suk*

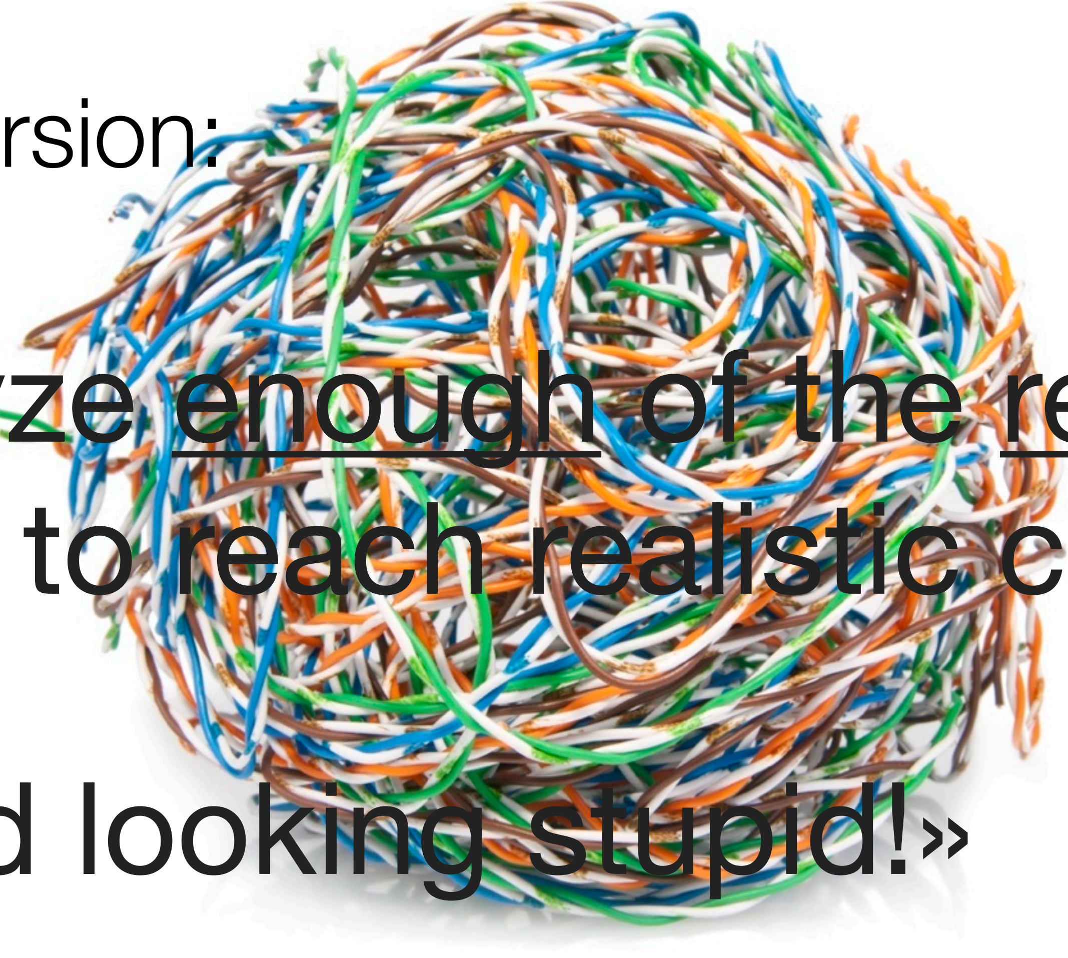
The emerging field of systems biology attempts to harness the power of mathematics, engineering, and computer science to analyze and integrate data from all the "omics" and ultimately create working models of entire biological systems.

Spivey A (2004) Systems biology: the big picture. Environ Health Perspect.

Holistic analysis - the ultimate vs the feasible

Paradoxically, systems biology research suffers from having both too much data and not enough.

the feasible version:



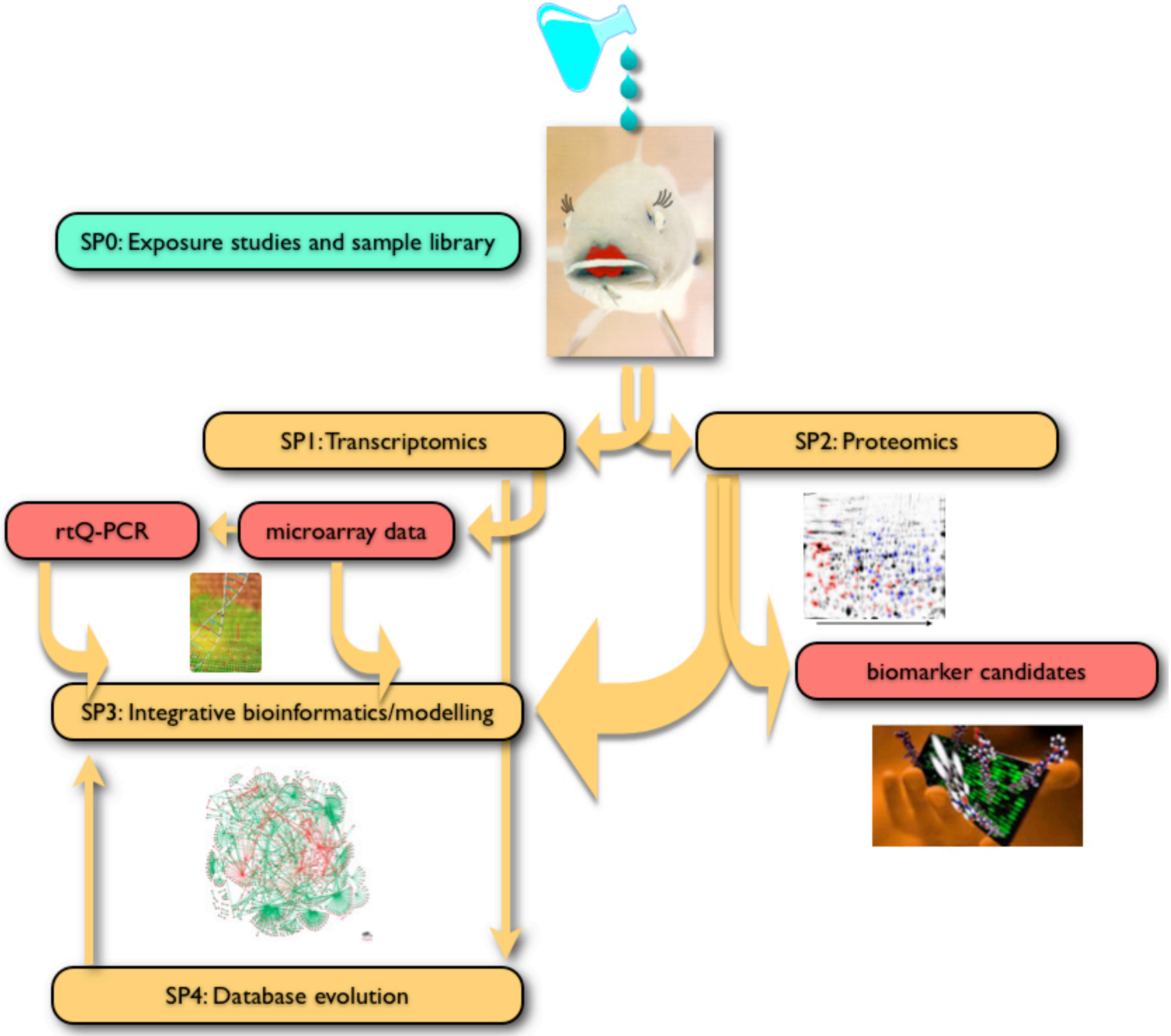
«To analyze <u>enough</u> of the <u>relevant</u> variables to reach realistic conclusions, -

and avoid looking stupid!»

George Somero, 28th ESCPB, Bilbao, Spain, Sept. 2, 2012

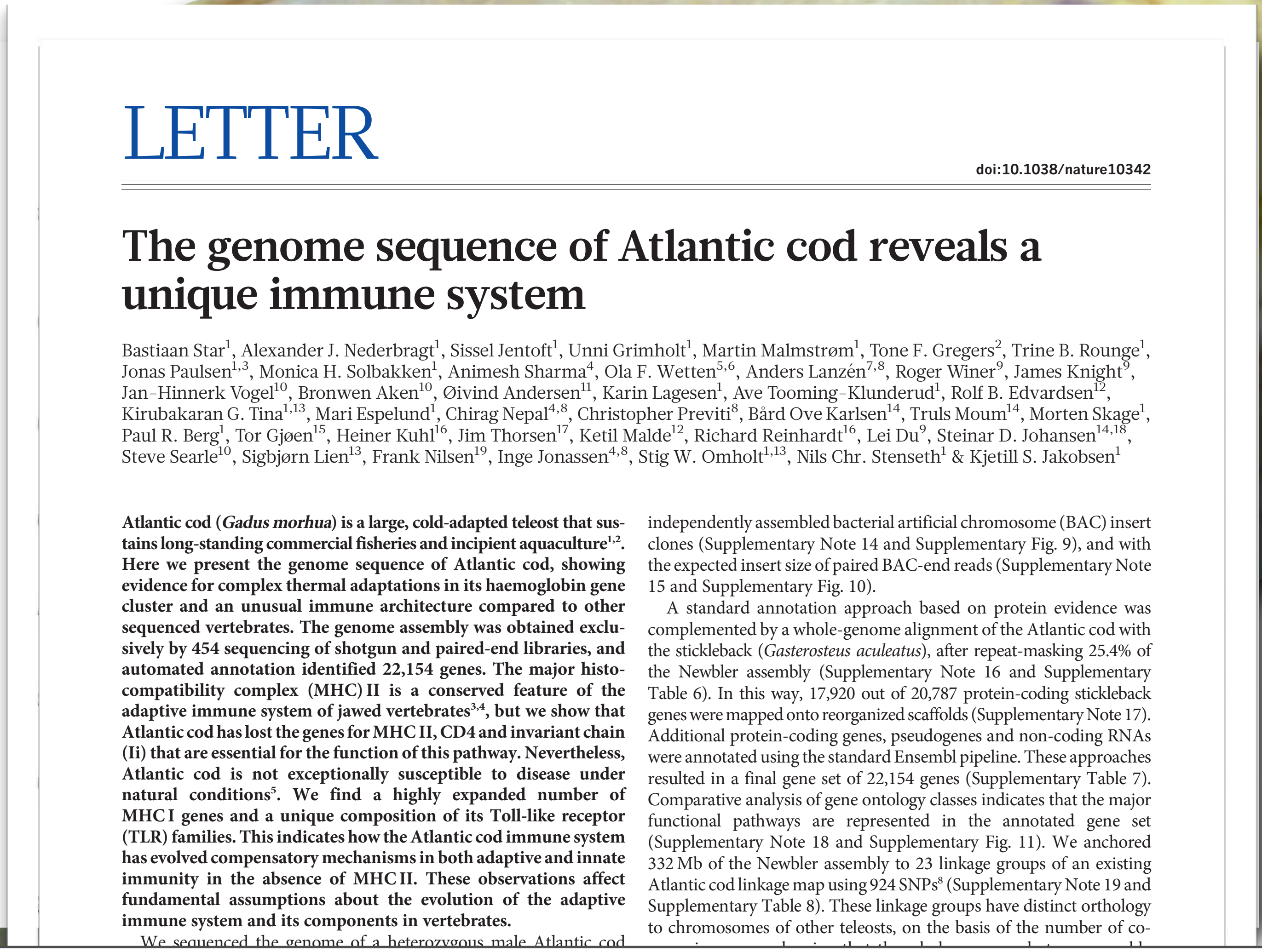
Integrative environmental genomics of cod: iCod Project (NFR, 2009-2012)

- Observe transcriptional and translational responses of the cod genome
- Integrate into mechanistic insights using a systems biology approach
- Based on established competence in cod physiology, transcriptomics, proteomics, bioinformatics



Atlantic cod (Gadus morhua)

- Important species in North-Atlantic fisheries
- Increasing use in aquaculture
- Spawning grounds in important oil production areas
- Genome information available through 454 and EST-sequencing (www.codgenome.no/Star et al., Nature 2011)



LETTER

doi:10.1038/nature10342

The genome sequence of Atlantic cod reveals a unique immune system

Bastiaan Star[1], Alexander J. Nederbragt[1], Sissel Jentoft[1], Unni Grimholt[1], Martin Malmstrøm[1], Tone F. Gregers[2], Trine B. Rounge[1], Jonas Paulsen[1,3], Monica H. Solbakken[1], Animesh Sharma[4], Ola F. Wetten[5,6], Anders Lanzén[7,8], Roger Winer[9], James Knight[9], Jan-Hinnerk Vogel[10], Bronwen Aken[10], Øivind Andersen[11], Karin Lagesen[1], Ave Tooming-Klunderud[1], Rolf B. Edvardsen[12], Kirubakaran G. Tina[1,13], Mari Espelund[1], Chirag Nepal[4,8], Christopher Previti[8], Bård Ove Karlsen[14], Truls Moum[14], Morten Skage[1], Paul R. Berg[1], Tor Gjøen[15], Heiner Kuhl[16], Jim Thorsen[17], Ketil Malde[12], Richard Reinhardt[16], Lei Du[9], Steinar D. Johansen[14,18], Steve Searle[10], Sigbjørn Lien[13], Frank Nilsen[19], Inge Jonassen[4,8], Stig W. Omholt[1,13], Nils Chr. Stenseth[1] & Kjetill S. Jakobsen[1]

Atlantic cod (*Gadus morhua*) is a large, cold-adapted teleost that sustains long-standing commercial fisheries and incipient aquaculture[1,2]. Here we present the genome sequence of Atlantic cod, showing evidence for complex thermal adaptations in its haemoglobin gene cluster and an unusual immune architecture compared to other sequenced vertebrates. The genome assembly was obtained exclusively by 454 sequencing of shotgun and paired-end libraries, and automated annotation identified 22,154 genes. The major histocompatibility complex (MHC) II is a conserved feature of the adaptive immune system of jawed vertebrates[3,4], but we show that Atlantic cod has lost the genes for MHC II, CD4 and invariant chain (Ii) that are essential for the function of this pathway. Nevertheless, Atlantic cod is not exceptionally susceptible to disease under natural conditions[5]. We find a highly expanded number of MHC I genes and a unique composition of its Toll-like receptor (TLR) families. This indicates how the Atlantic cod immune system has evolved compensatory mechanisms in both adaptive and innate immunity in the absence of MHC II. These observations affect fundamental assumptions about the evolution of the adaptive immune system and its components in vertebrates.

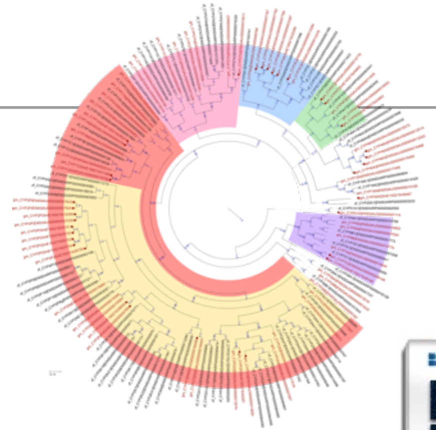
We sequenced the genome of a heterozygous male Atlantic cod

independently assembled bacterial artificial chromosome (BAC) insert clones (Supplementary Note 14 and Supplementary Fig. 9), and with the expected insert size of paired BAC-end reads (Supplementary Note 15 and Supplementary Fig. 10).

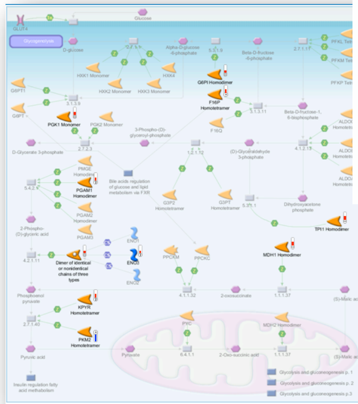
A standard annotation approach based on protein evidence was complemented by a whole-genome alignment of the Atlantic cod with the stickleback (*Gasterosteus aculeatus*), after repeat-masking 25.4% of the Newbler assembly (Supplementary Note 16 and Supplementary Table 6). In this way, 17,920 out of 20,787 protein-coding stickleback genes were mapped onto reorganized scaffolds (Supplementary Note 17). Additional protein-coding genes, pseudogenes and non-coding RNAs were annotated using the standard Ensembl pipeline. These approaches resulted in a final gene set of 22,154 genes (Supplementary Table 7). Comparative analysis of gene ontology classes indicates that the major functional pathways are represented in the annotated gene set (Supplementary Note 18 and Supplementary Fig. 11). We anchored 332 Mb of the Newbler assembly to 23 linkage groups of an existing Atlantic cod linkage map using 924 SNPs[8] (Supplementary Note 19 and Supplementary Table 8). These linkage groups have distinct orthology to chromosomes of other teleosts, on the basis of the number of co-

Environmental genomics approach

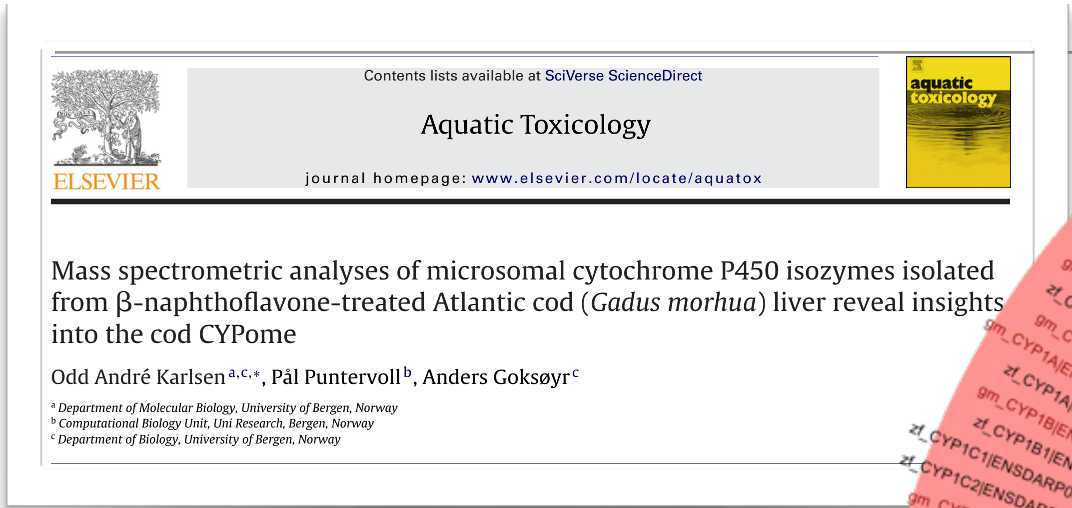
- Genomics
  - Mining the genome for defensome genes, incl. the CYPome
    - e.g. Karlsen et al., 2012
- Transcriptomics
  - Custom made 135k cod oligonucleotide arrays
  - 44k EST ”contigs” (Cod Genome Sequencing Consortium)
  - 60 nucleotide probes
  - Coverage of nearly all genes
  - Q-PCR validation
- Proteomics
  - 2D gel electrophoresis and Delta2D image analysis
    - e.g. Bohne-Kjersem et al., 2009; 2010; Berg et al. 2010; 2011
  - MALDI-TOF and Q-TOF LC-MS/MS
  - ORBITRAP
  - Label-free and gel-free approaches
  - iTRAQ
  - *SID-SRM and MRM*
- Bioinformatics tools
  - KEGG and DAVID: gene ontology, enrichment analysis
  - Metacore GeneGo: pathway, interactome analysis etc.



12x135k arrays
(Roche-NimbleGen)



Cod vs zebrafish CYPome



Contents lists available at SciVerse ScienceDirect

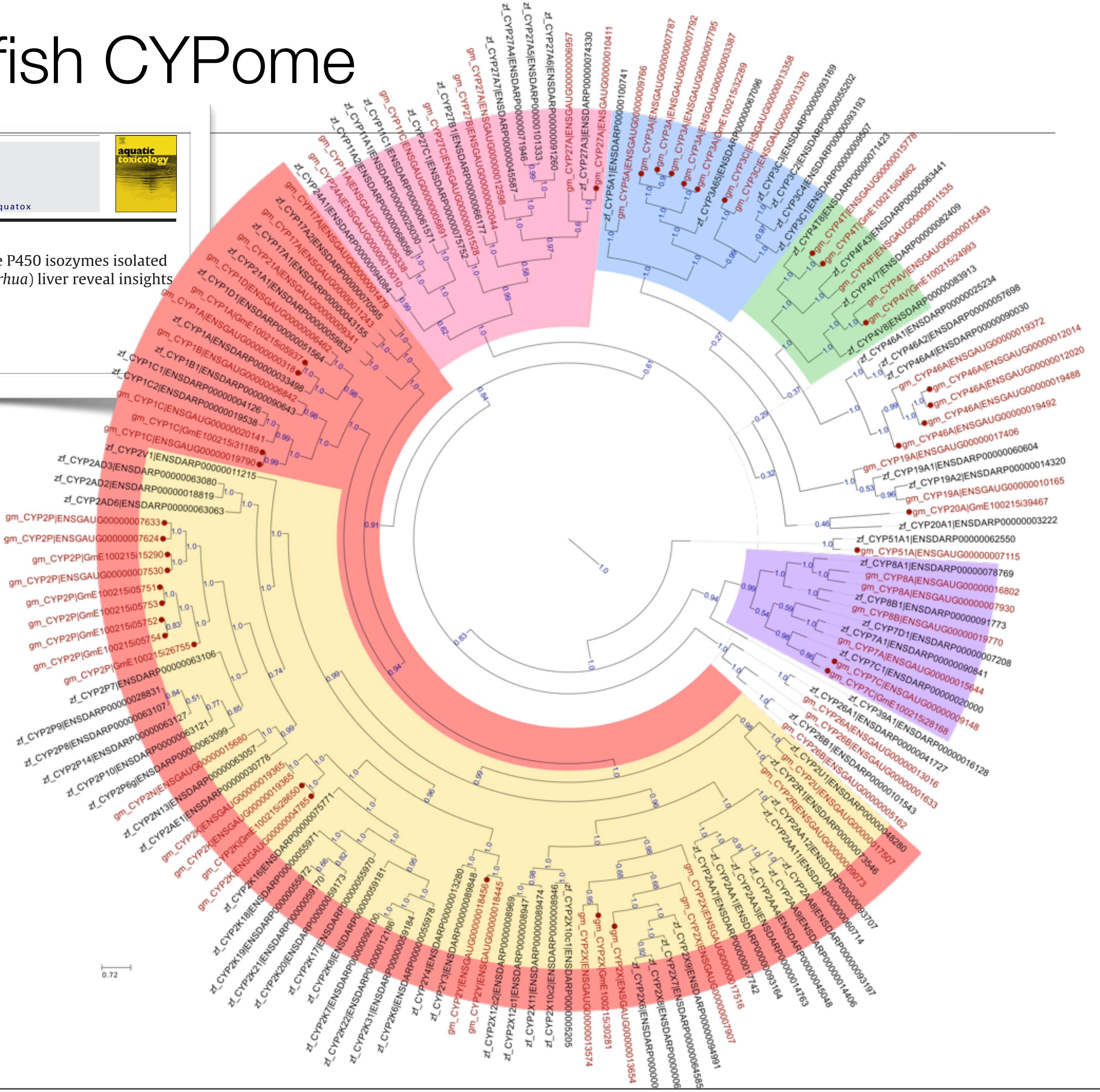
Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

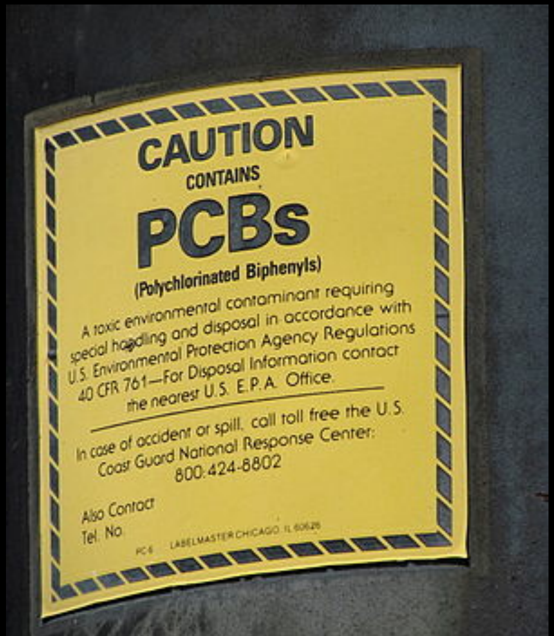
Mass spectrometric analyses of microsomal cytochrome P450 isozymes isolated from β-naphthoflavone-treated Atlantic cod (*Gadus morhua*) liver reveal insights into the cod CYPome

Odd André Karlsen[a,c,*], Pål Puntervoll[b], Anders Goksøyr[c]

[a] Department of Molecular Biology, University of Bergen, Norway
[b] Computational Biology Unit, Uni Research, Bergen, Norway
[c] Department of Biology, University of Bergen, Norway



Chemical contaminants of most concern for ocean and human health



CAUTION

CONTAINS

PCBs

(Polychlorinated Biphenyls)

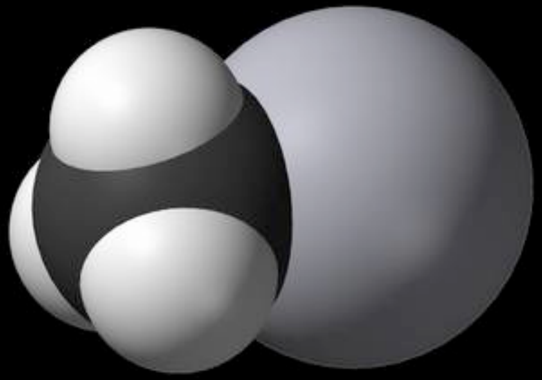
A toxic environmental contaminant requiring special handling and disposal in accordance with U.S. Environmental Protection Agency Regulations 40 CFR 761—For Disposal Information contact the nearest U.S. E.P.A. Office.

In case of accident or spill, call toll free the U.S. Coast Guard National Response Center: 800-424-8802

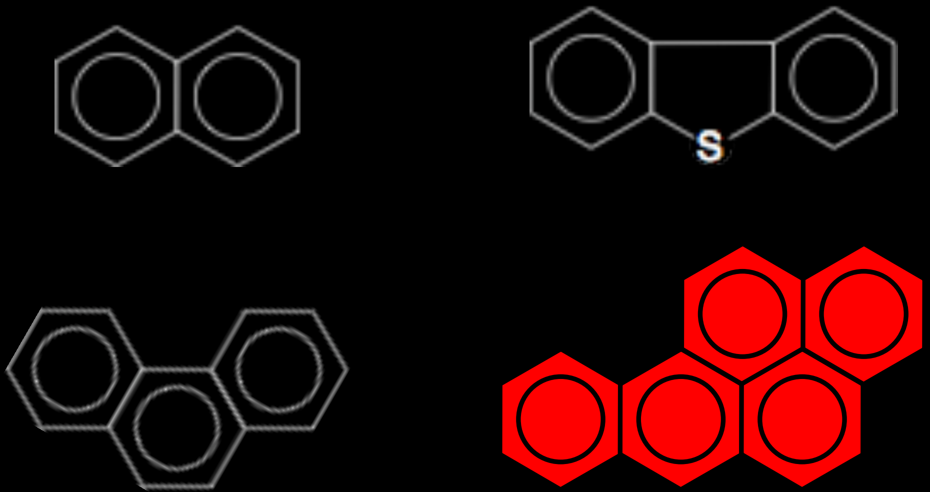
Also Contact
Tel. No.

PC 6 LABELMASTER CHICAGO, IL 60626

Polychlorinated biphenyls (PCBs)



Methylmercury (MeHg)



Polycyclic aromatic hydrocarbons (PAHs)

New flame retardants, PPCPs, phenolics, perfluorinated compounds, nanomaterials, pesticides, many more

Chemicals of emerging concern (CECs)

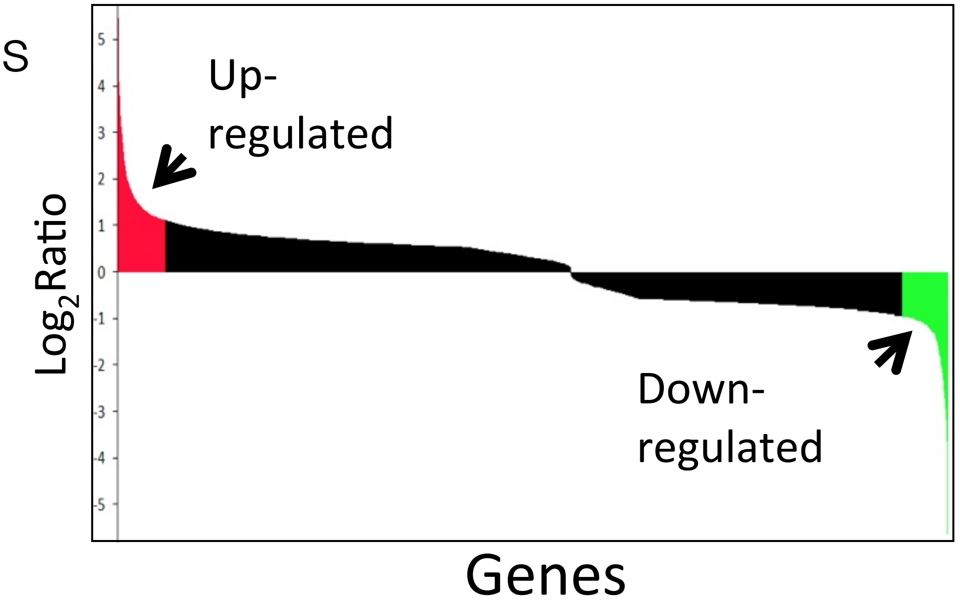
MeHg exposure of cod: Transcriptomics

MeHg treatment:

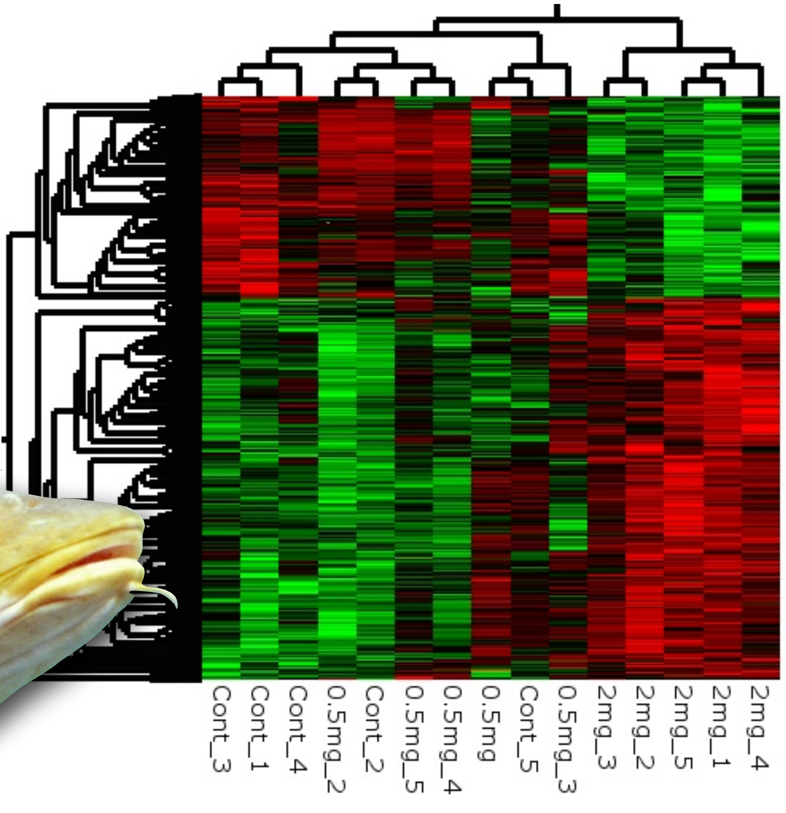
Control	(n=5)
0.5 mg/Kg BW	(n=5)
2 mg/Kg BW	(n=5)

Differentially expressed genes

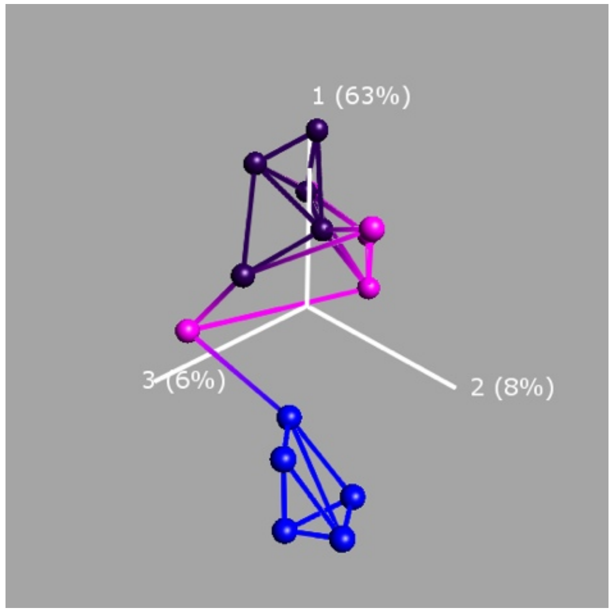
Up-regulated: 429
Down regulated: 226
Total: 655



Hierarchical cluster analysis based on genes most significantly regulated by MeHg

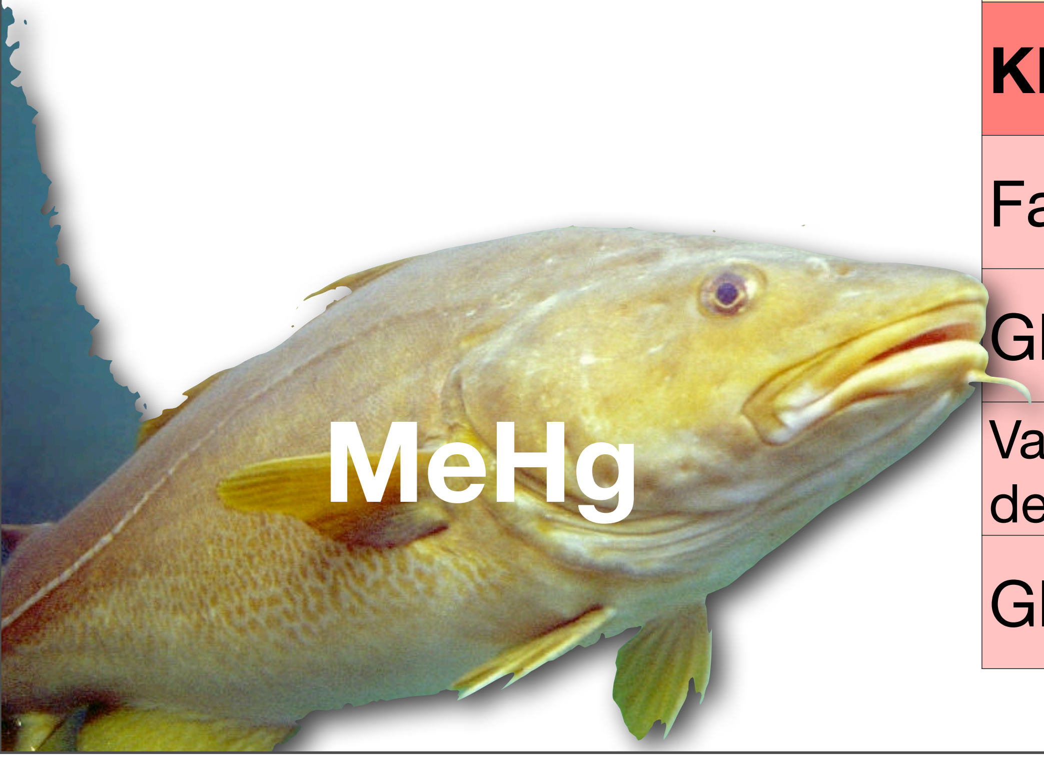


Principal component analysis



Yadetie et al., Aquat. Toxicol. (in press)

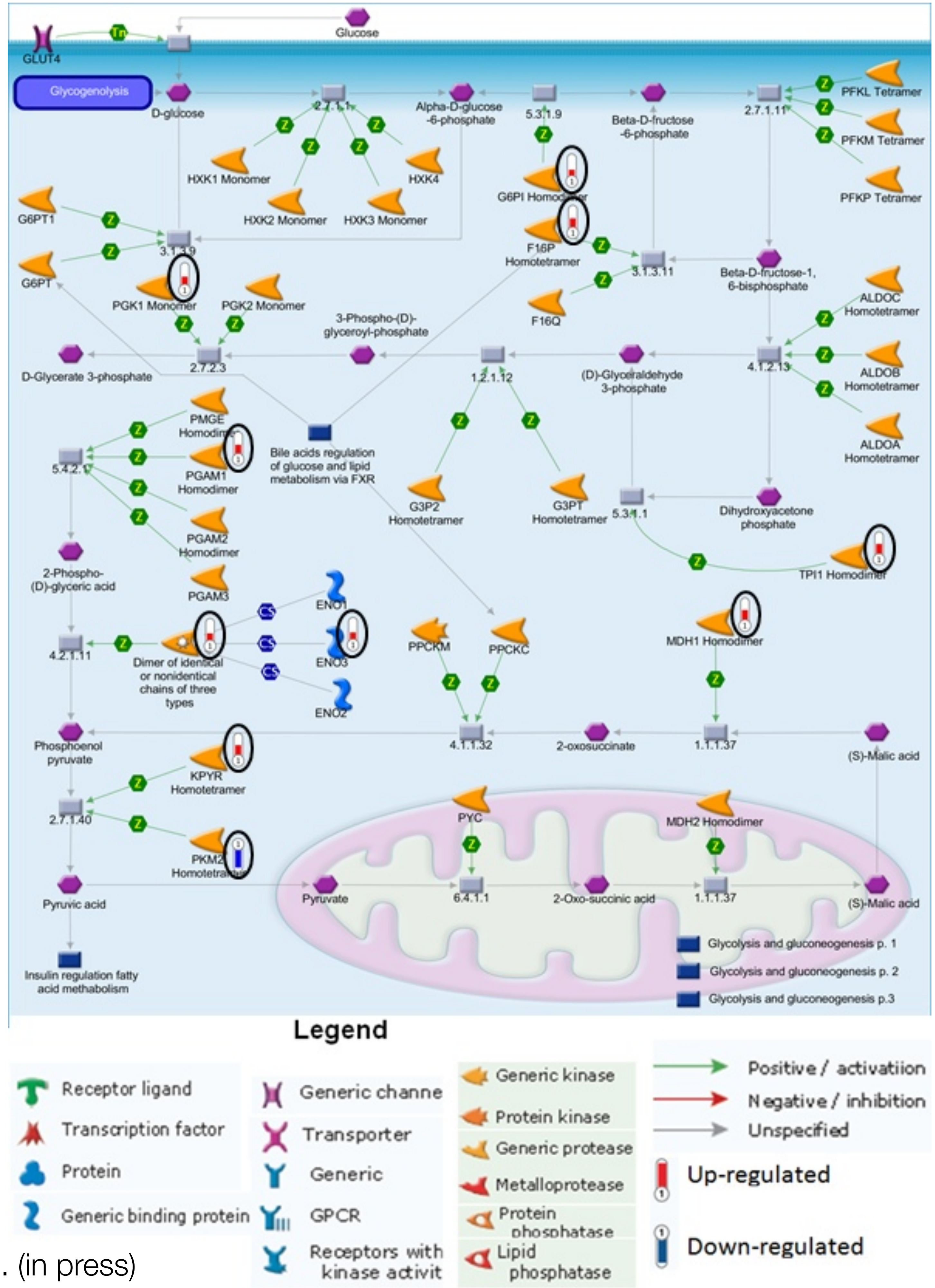
MeHg exposure of cod:
Enriched GO terms and KEGG pathways



Gene ontology term	**Count**	**PValue**	**Benjamini**	**FDR**
Amino acid catabolism	9	1.7E-04	2.9E-02	0.2
Amino acid metabolism	20	2.0E-04	1.7E-02	0.2
Glycolysis	8	5.6E-04	3.2E-02	0.7
Acyl-CoA metabolism	6	8.1E-04	3.5E-02	1.0
Fatty acid metabolism	16	8.9E-04	3.1E-02	1.1
KEGG pathway				
Fatty acid metabolism	10	3.4E-05	5.5E-03	0.0
Glycolysis / Gluconeogenesis	12	3.6E-05	2.9E-03	0.0
Valine, leucine and isoleucine degradation	10	7.6E-05	4.1E-03	0.1
Glutathione metabolism	10	2.2E-04	8.7E-03	0.3

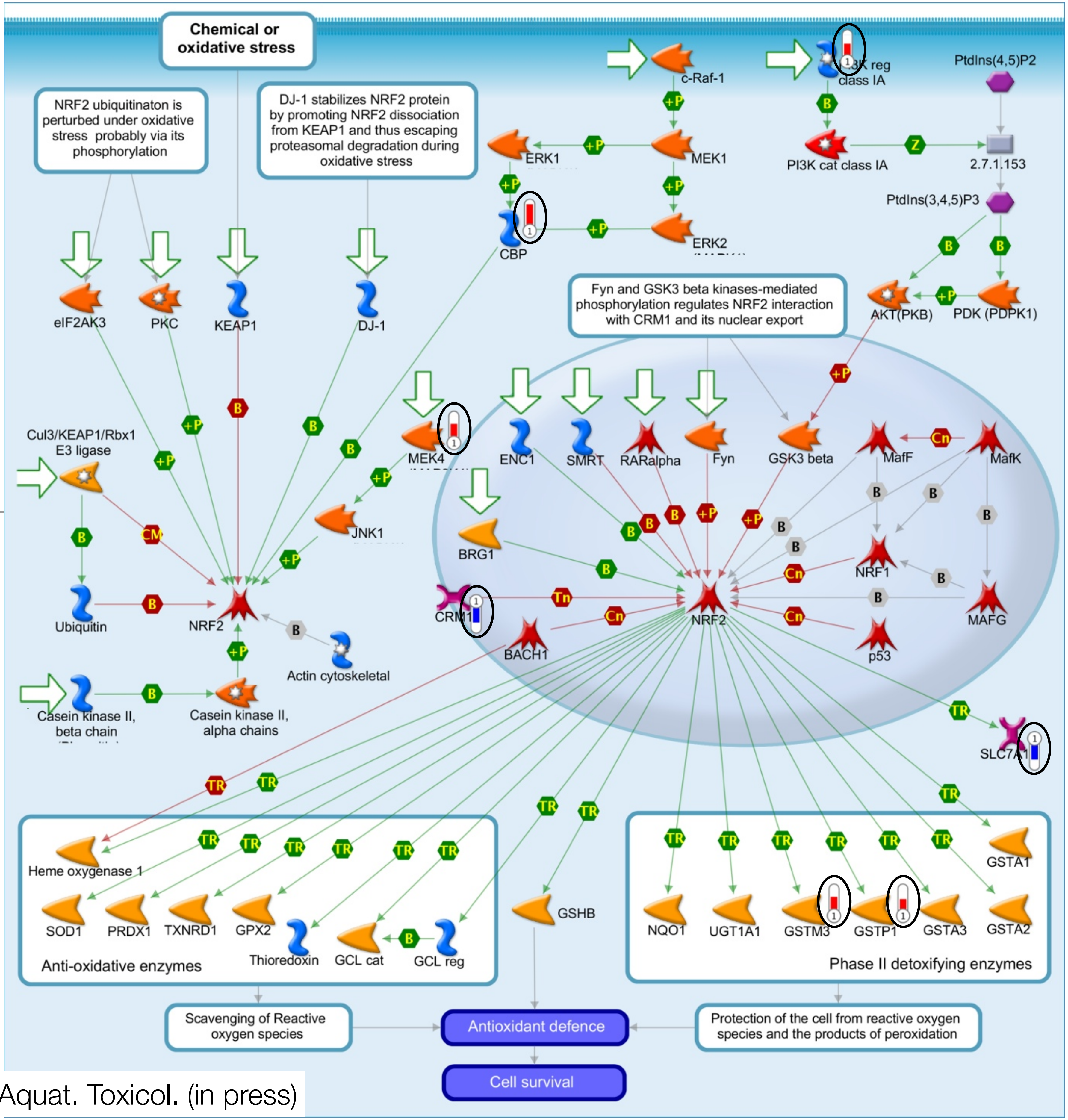
Yadetie et al., Aquat. Toxicol. (in press)

Metacore top scored map “Glycolysis and gluconeogenesis”



Yadetie et al., Aquat. Toxicol. (in press)

Map of NRF2 mediated regulation of oxidative stress response pathway affected by MeHg



Chemical or oxidative stress

NRF2 ubiquitinaton is perturbed under oxidative stress probably via its phosphorylation

DJ-1 stabilizes NRF2 protein by promoting NRF2 dissociation from KEAP1 and thus escaping proteasomal degradation during oxidative stress

Fyn and GSK3 beta kinases-mediated phosphorylation regulates NRF2 interaction with CRM1 and its nuclear export

Anti-oxidative enzymes

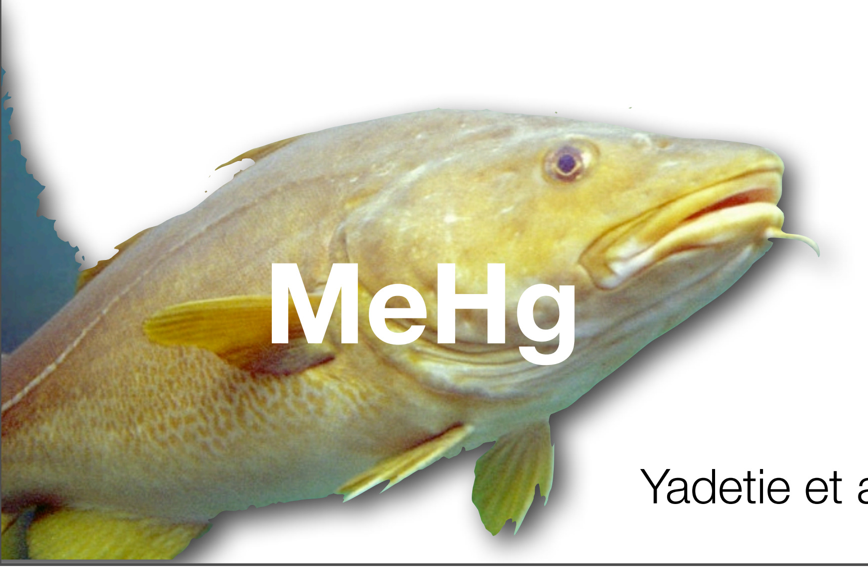
Phase II detoxifying enzymes

Scavenging of Reactive oxygen species

Antioxidant defence

Protection of the cell from reactive oxygen species and the products of peroxidation

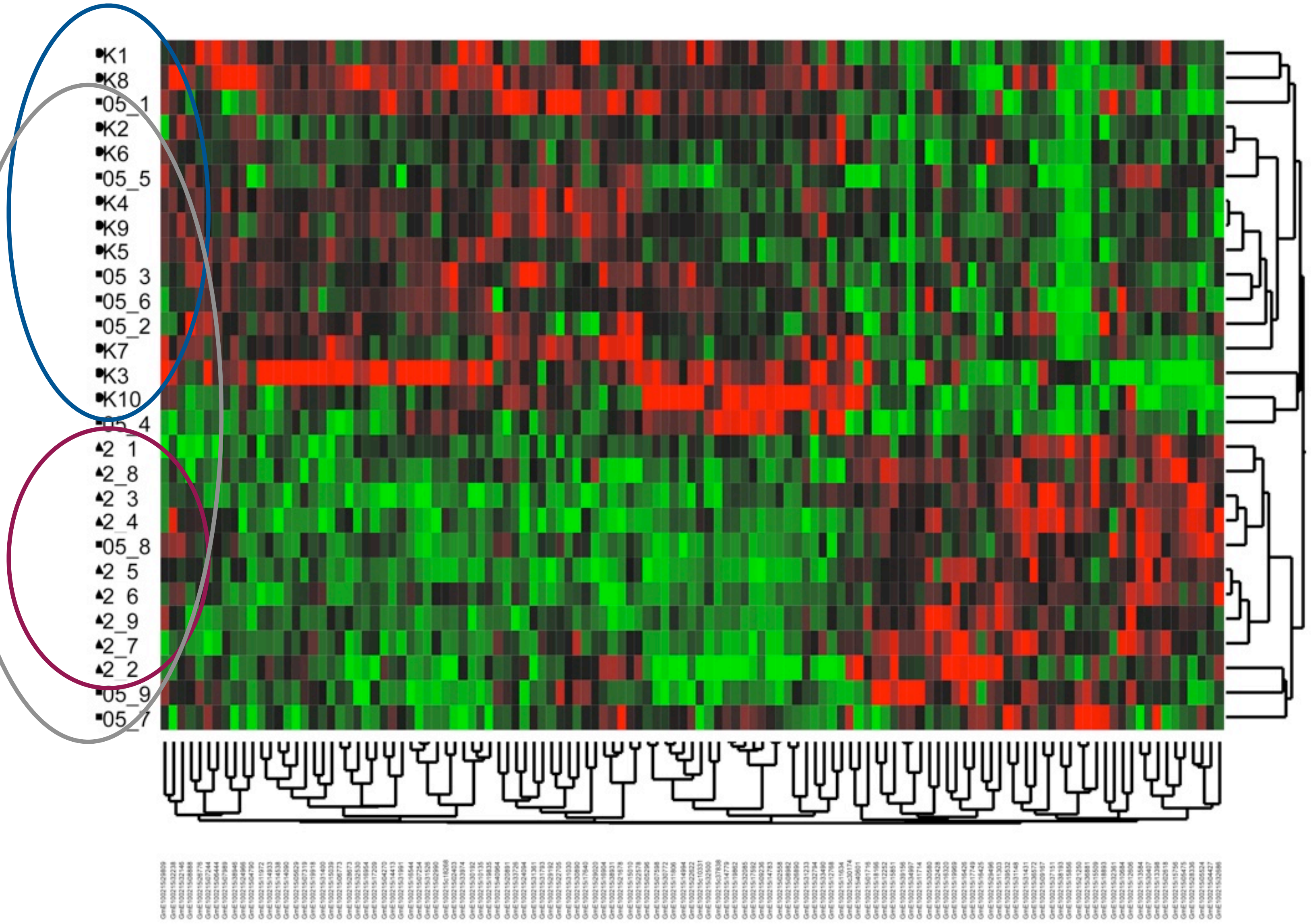
Cell survival



Yadetie et al., Aquat. Toxicol. (in press)

MeHg exposure: Label-free proteomics analysis

39 up-regulated proteins
78 down-regulated proteins



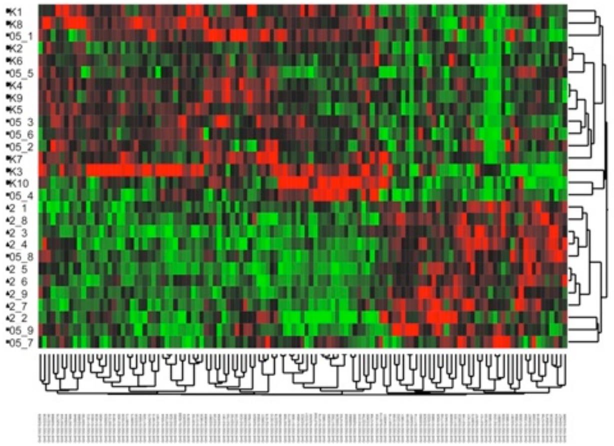
Two-way clustering of all normalised data from all groups

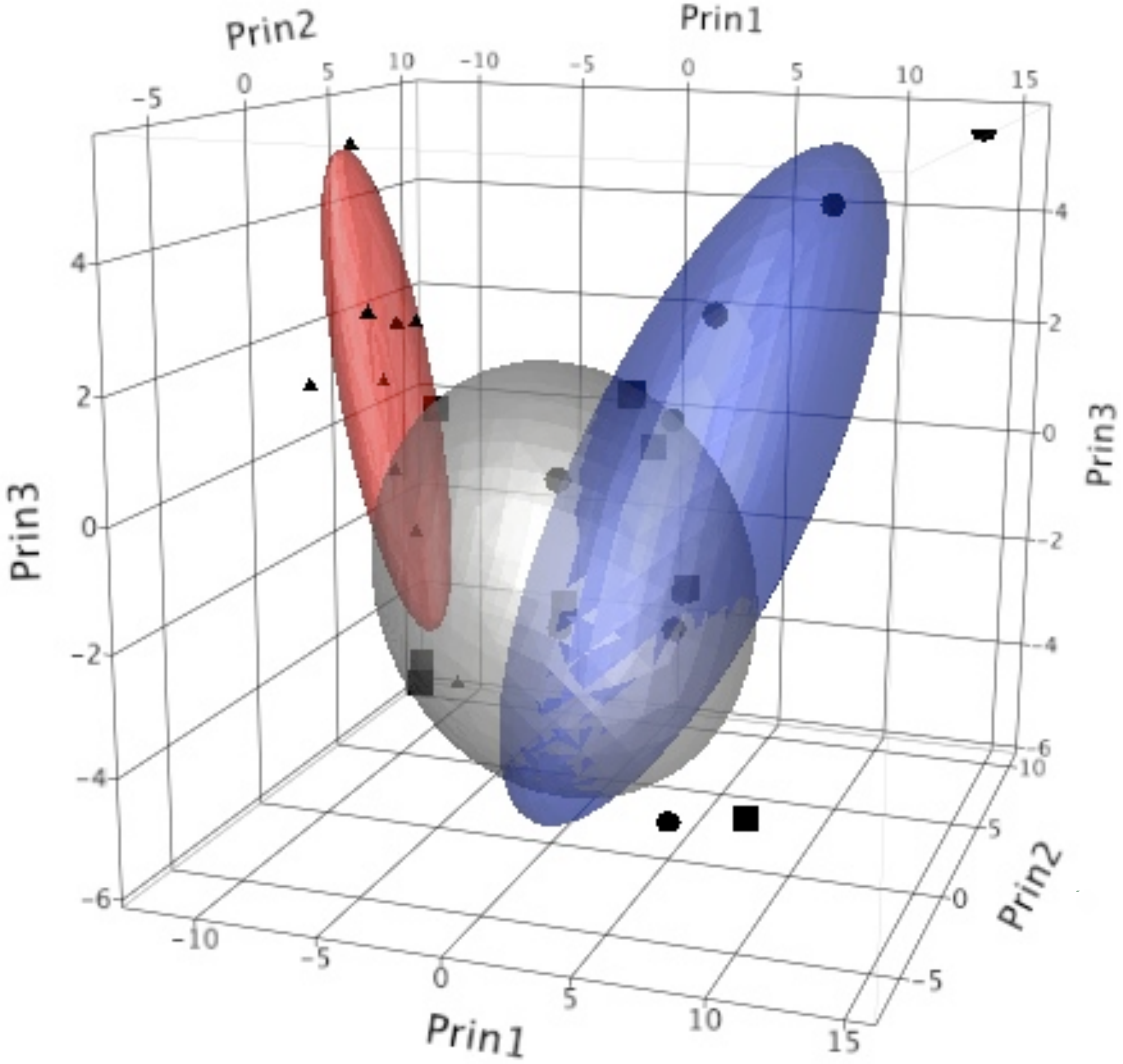
0 mg/kg

0,5 mg/kg

2 mg/kg

MeHg/Proteomics: Principal component analysis (PCA)





0 mg/kg

0,5 mg/kg

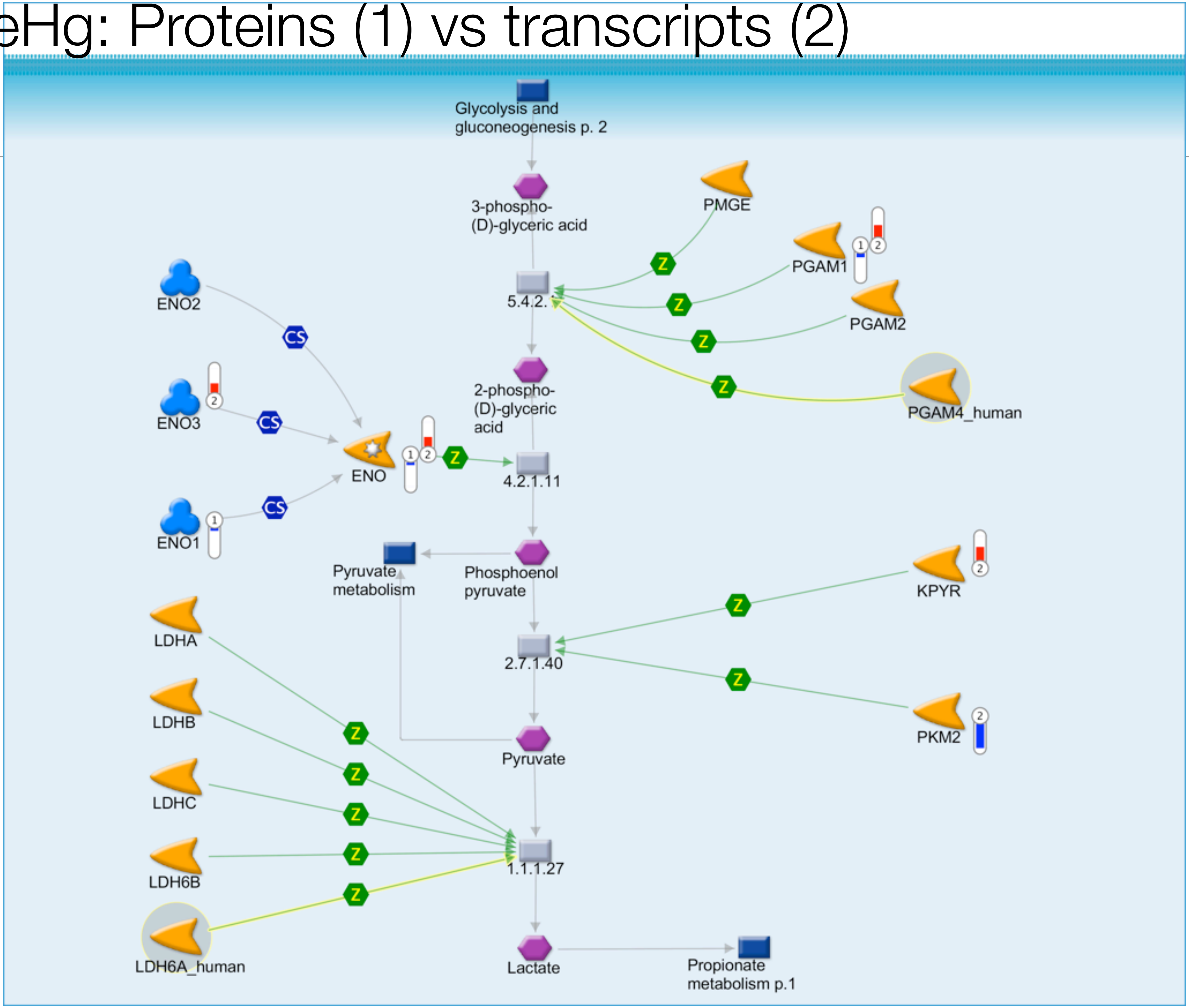
2 mg/kg

3D PCA-plot of all normalised data

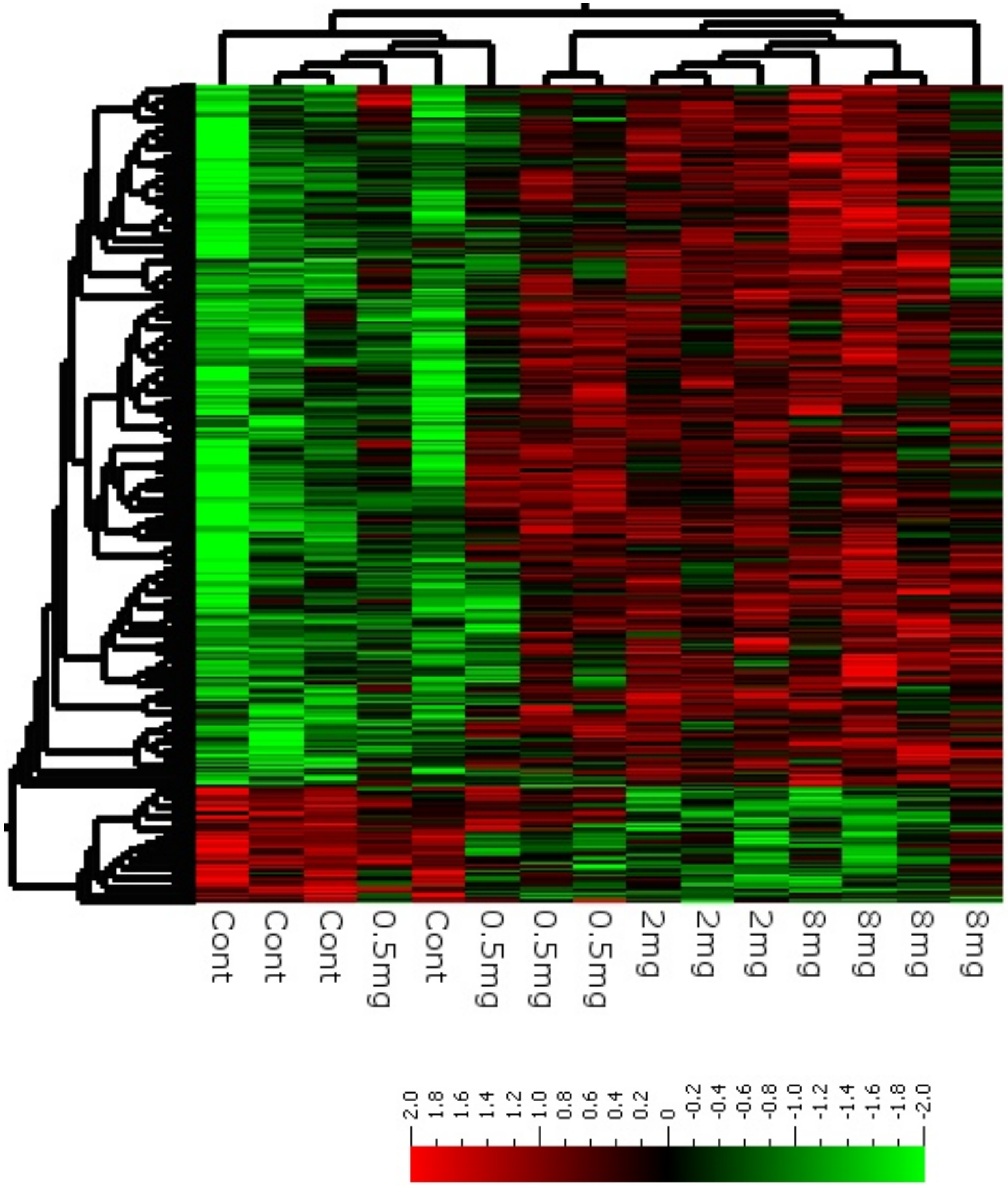
Pathways (Metacore): proteome (🟧) vs transcriptome (🟦) data

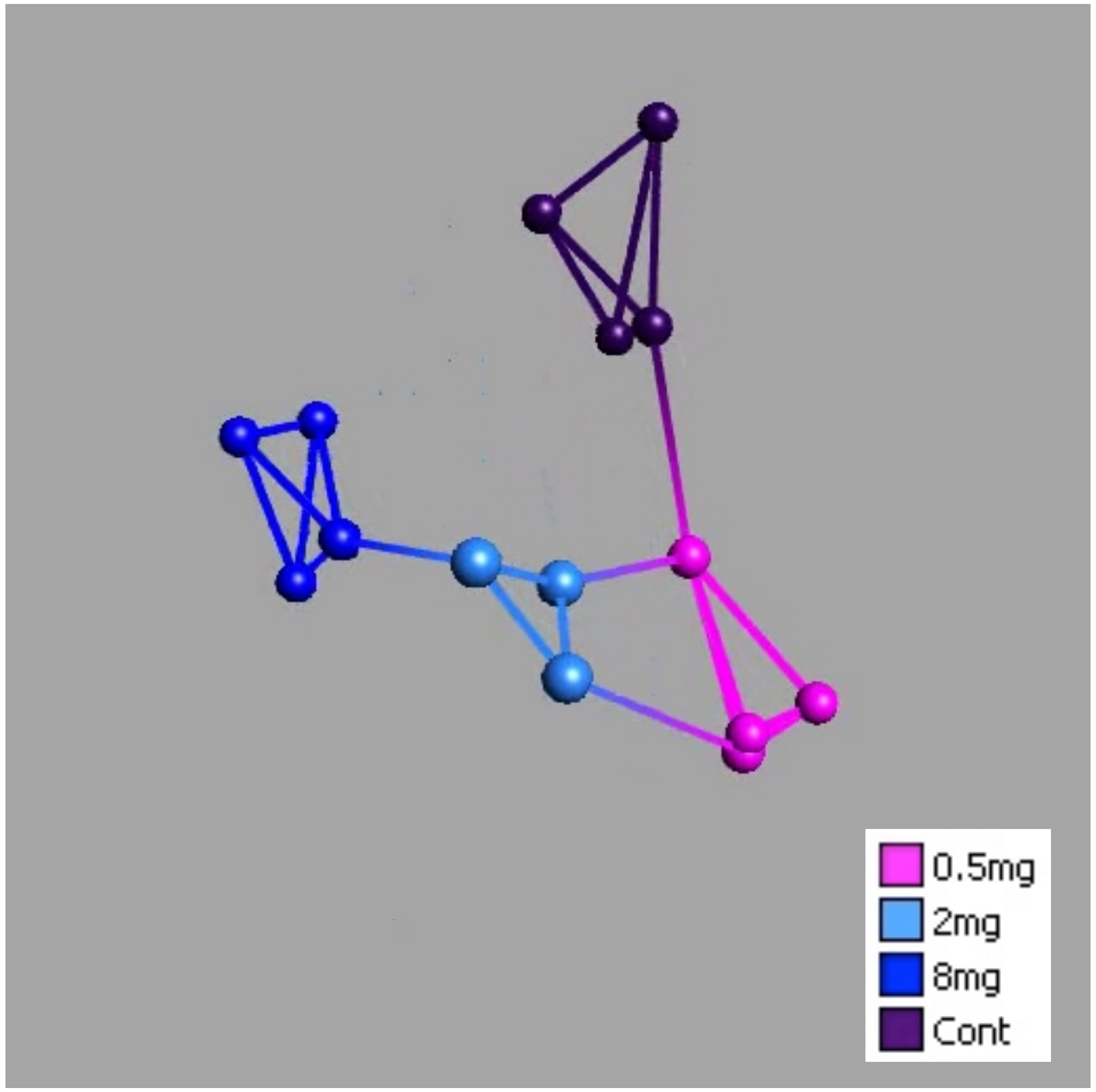
#	Maps	pValue	min(pValue) ↑
1	Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling	4.856e-3 1.310e-6	1.310e-6
2	Immune response_Alternative complement pathway	2.159e-1 6.809e-6	6.809e-6
3	Glycolysis and gluconeogenesis (short map)	7.844e-3 8.505e-6	8.505e-6
4	Cell adhesion_Chemokines and adhesion	3.272e-5 1.719e-2	3.272e-5
5	Cell adhesion_Integrin-mediated cell adhesion and migration	3.207e-3 3.396e-5	3.396e-5
6	Cytoskeleton remodeling_Cytoskeleton remodeling	3.590e-3 8.024e-5	8.024e-5
7	Phenylalanine metabolism/ Rodent version	3.379e-1 3.422e-4	3.422e-4
8	Glycolysis and gluconeogenesis p.3 / Human version	4.168e-4 3.620e-4	3.620e-4
9	Glycolysis and gluconeogenesis p.3	4.168e-4 3.620e-4	3.620e-4
10	Cytoskeleton remodeling_Regulation of actin cytoskeleton by Rho GTPases	3.663e-4 2.924e-3	3.663e-4
11	Phenylalanine metabolism	3.420e-1 3.798e-4	3.798e-4
12	Cell adhesion_Endothelial cell contacts by non-junctional mechanisms	4.168e-4 1.333e-1	4.168e-4
13	Cytoskeleton remodeling_Neurofilaments	4.716e-4 2.791e-2	4.716e-4
14	NRF2 regulation of oxidative stress response	4.404e-2 5.322e-4	5.322e-4
15	Glycine, serine, cysteine and threonine metabolism	9.450e-4 1.103e-1	9.450e-4
16	Glycine, serine, cysteine and threonine metabolism/ Rodent version	1.016e-3 1.168e-1	1.016e-3
17	Development_Role of CDK5 in neuronal development	1.179e-3 2.293e-1	1.179e-3

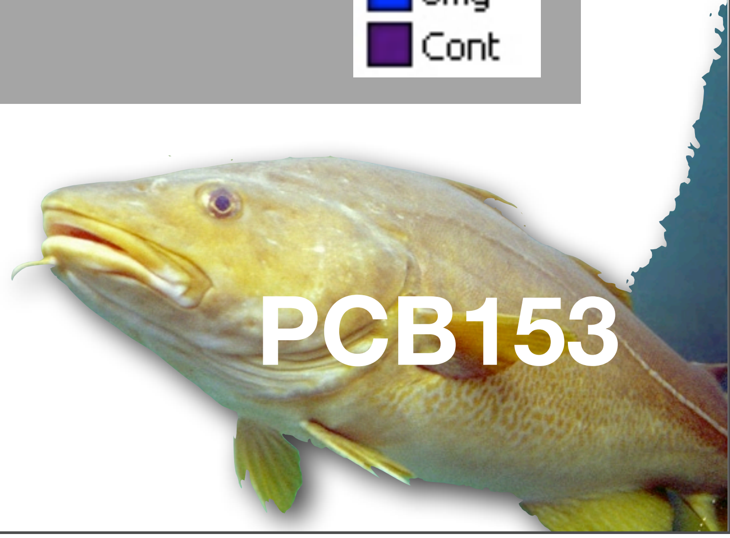
MeHg: Proteins (1) vs transcripts (2)



PCB153 exposure of cod: Transcriptomics





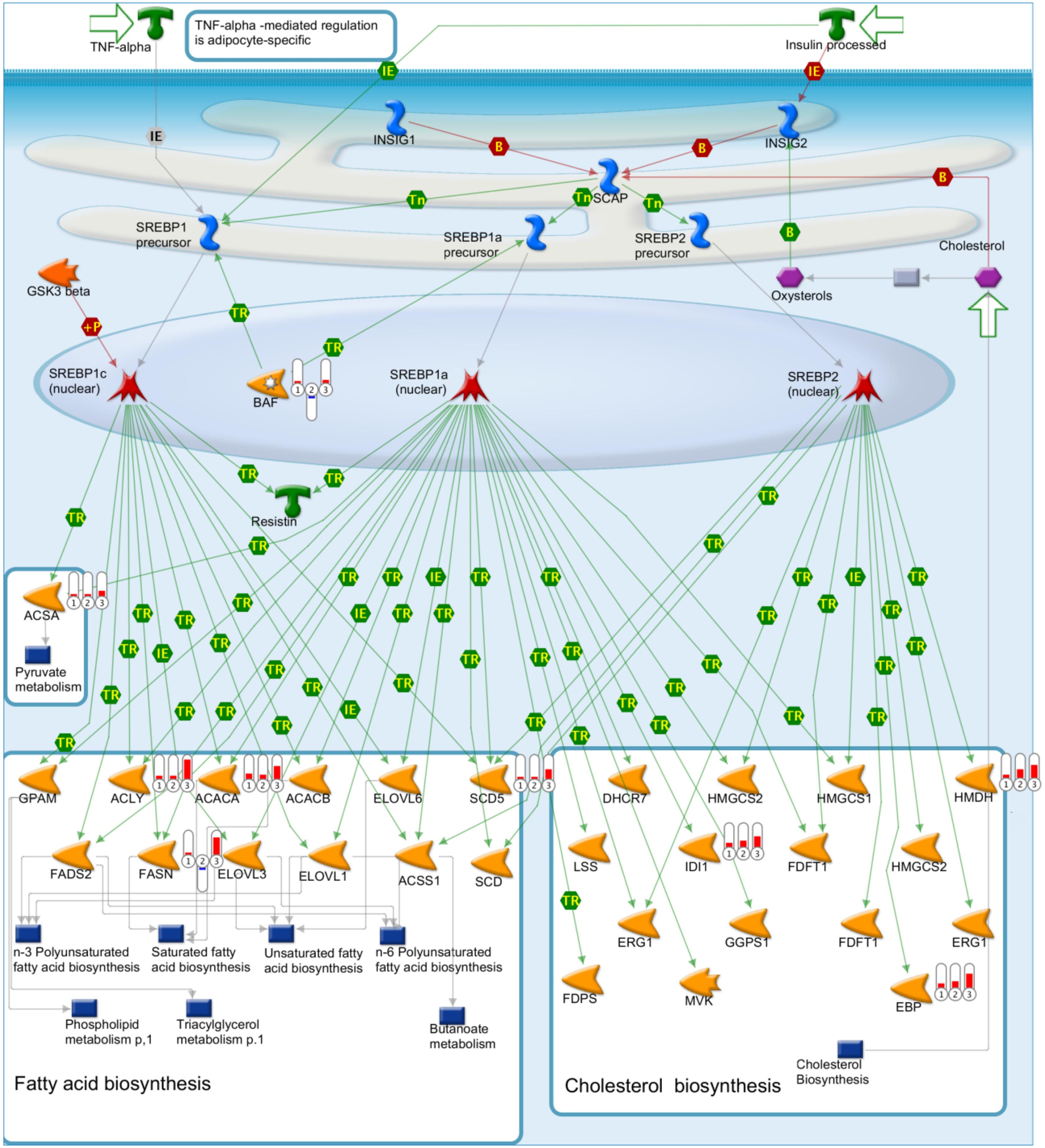


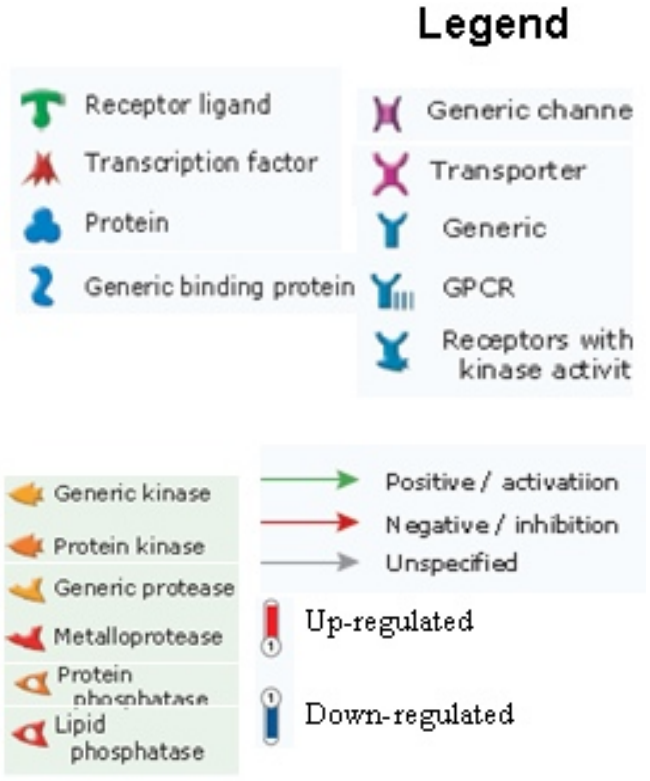
Significantly enriched GeneGo Pathway maps

#	Maps
1	SCAP/SREBP Transcriptional Control of Cholesterol and FA Biosynthesis
2	Development_PACAP signaling in neural cells
3	Immune response_CCR5 signaling in macrophages and T lymphocytes
4	Signal transduction_Calcium signaling
5	Immune response_MIF - the neuroendocrine-macrophage connector
6	Neurophysiological process_Corticoliberin signaling via CRHR1
7	Reproduction_GnRH signaling
8	Neurophysiological process_Thyroliberin in cell hyperpolarization and excitability
9	Cell adhesion_Role of tetraspanins in the integrin-mediated cell adhesion
10	Development_Gastrin in differentiation of the gastric mucosa
11	HIV-1 signaling via CCR5 in macrophages and T lymphocytes
12	Development_VEGF signaling via VEGFR2 - generic cascades
13	Development_GH-RH signaling
14	Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling
15	Renin-Angiotensin-Aldosterone System
16	Cell adhesion_Histamine H1 receptor signaling in the interruption of cell barrier integrity
17	Possible pathway of TGF-beta 1-dependent inhibition of CFTR expression
18	Immune response_CD16 signaling in NK cells
19	Immune response_Function of MEF2 in T lymphocytes
20	Cell adhesion_Gap junctions
21	Cell cycle_Start of DNA replication in early S phase

Only significant pathways are shown (FDR<5)

SCAP/SREBP Transcriptional Control of Cholesterol and FA Biosynthesis (GeneGo map, p = 2.4e-7)

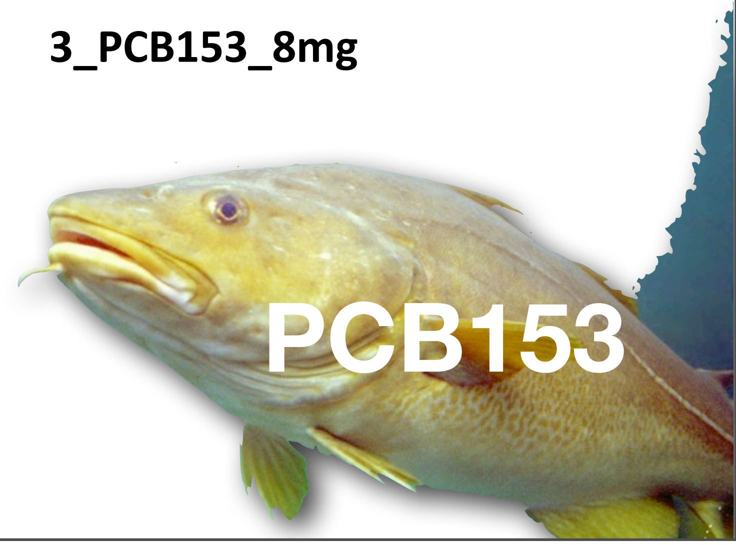




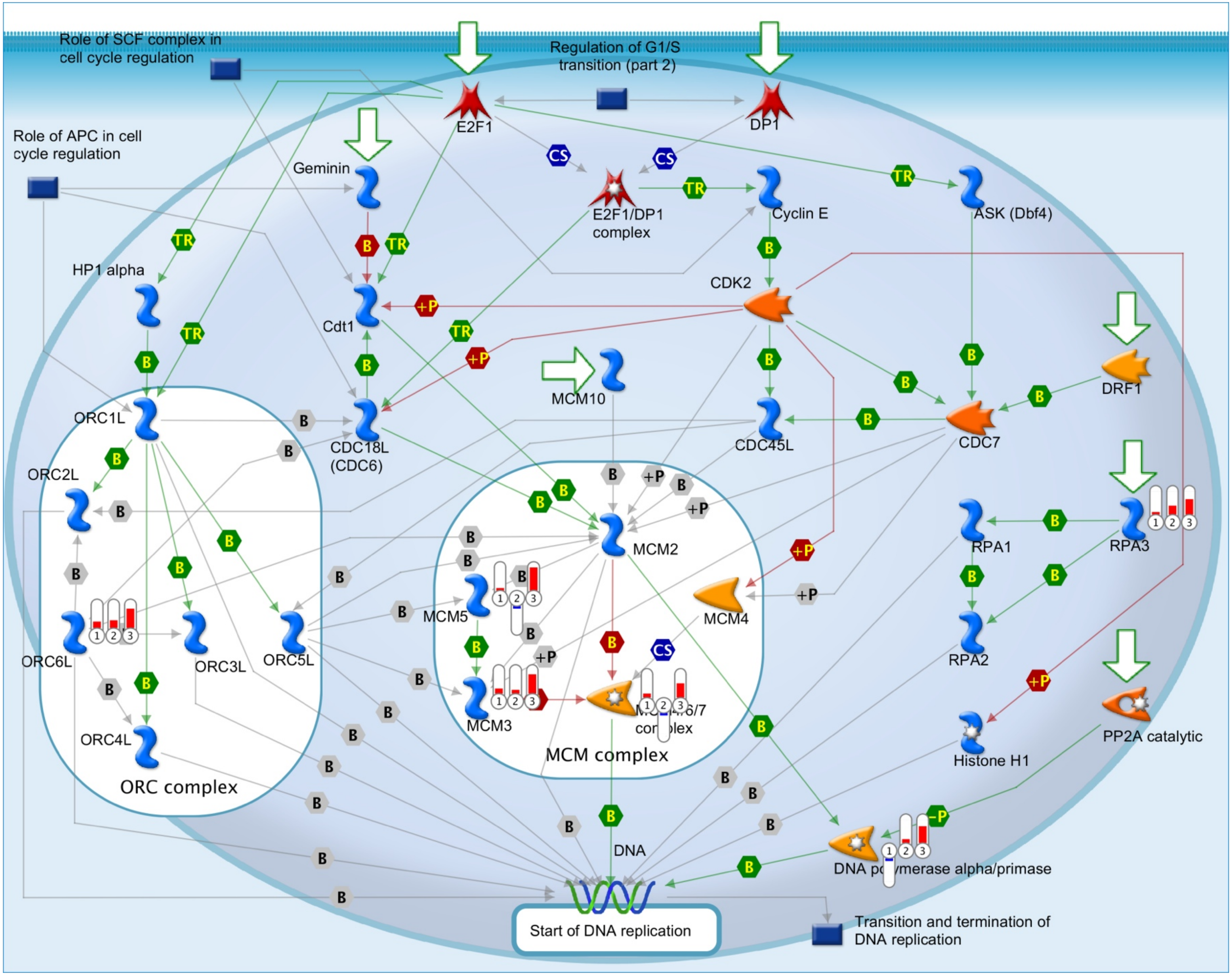
1_PCB153_0.5mg

2_PCB153_2mg

3_PCB153_8mg



Cell cycle_Start of DNA replication in early S phase (GeneGo map, p = 2.2e-3)

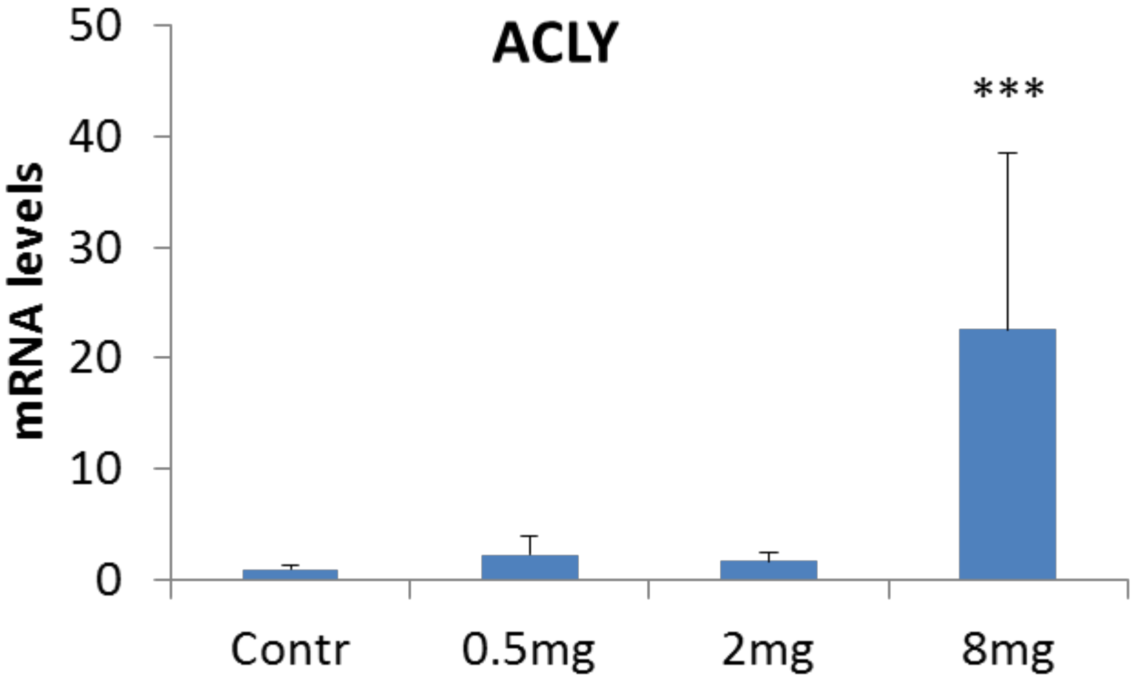


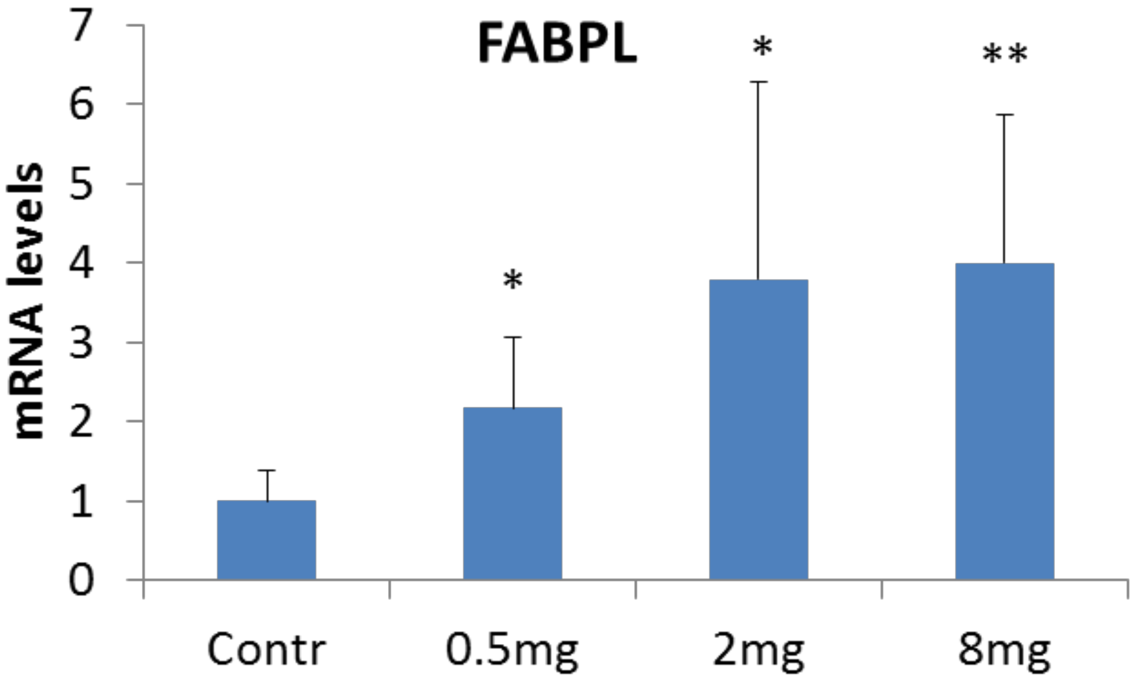
1_PCB153_0.5mg

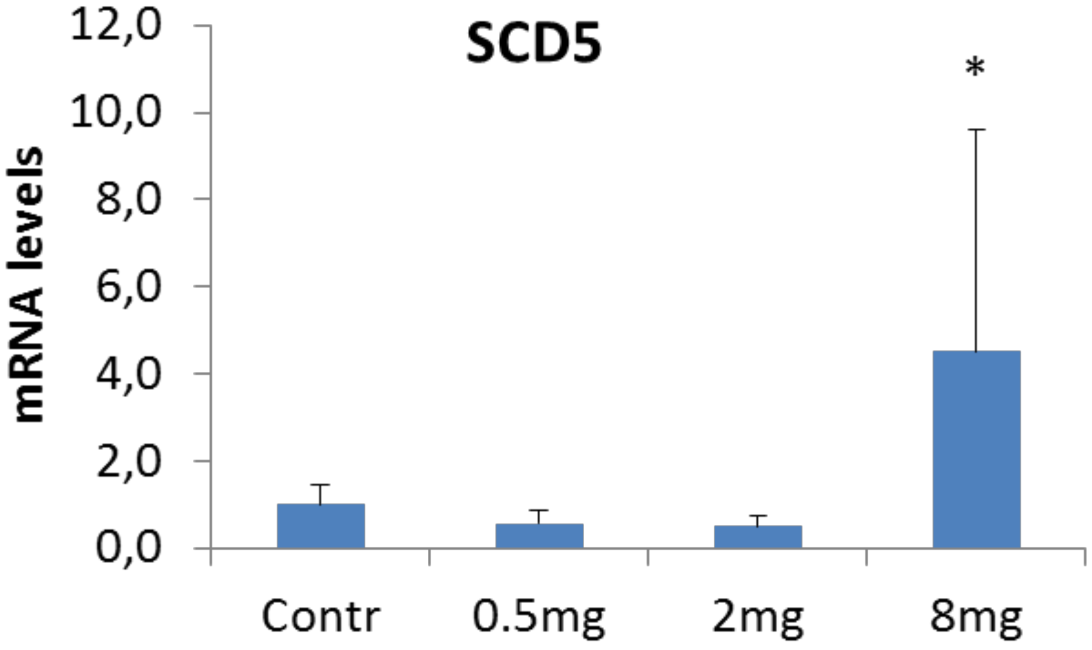
2_PCB153_2mg

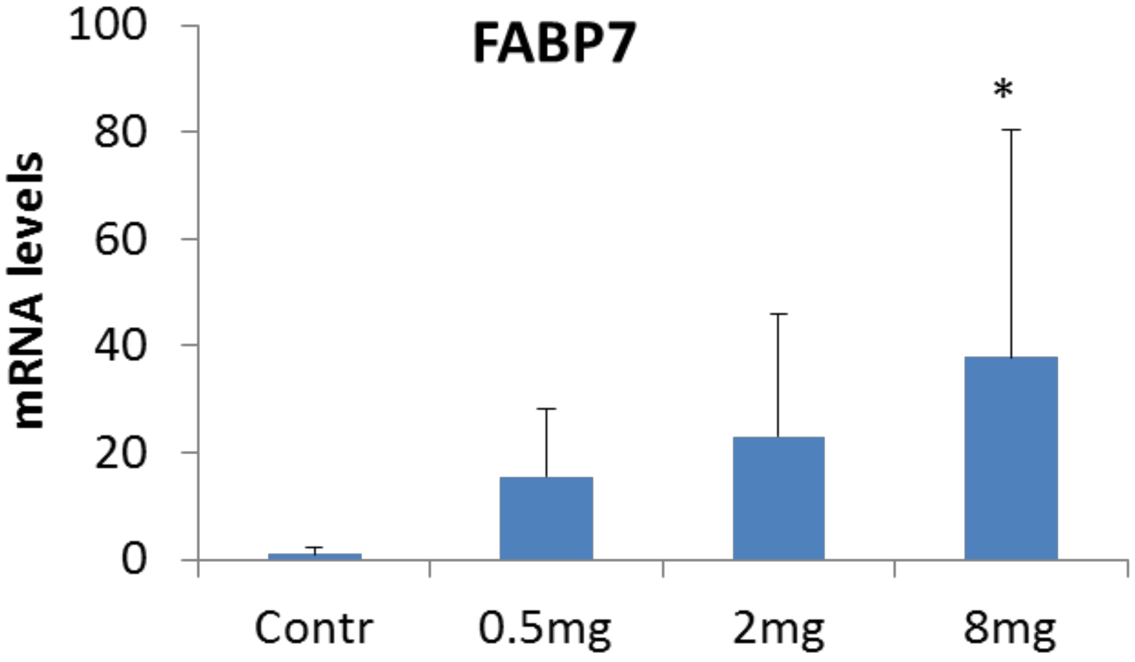
3_PCB153_8mg

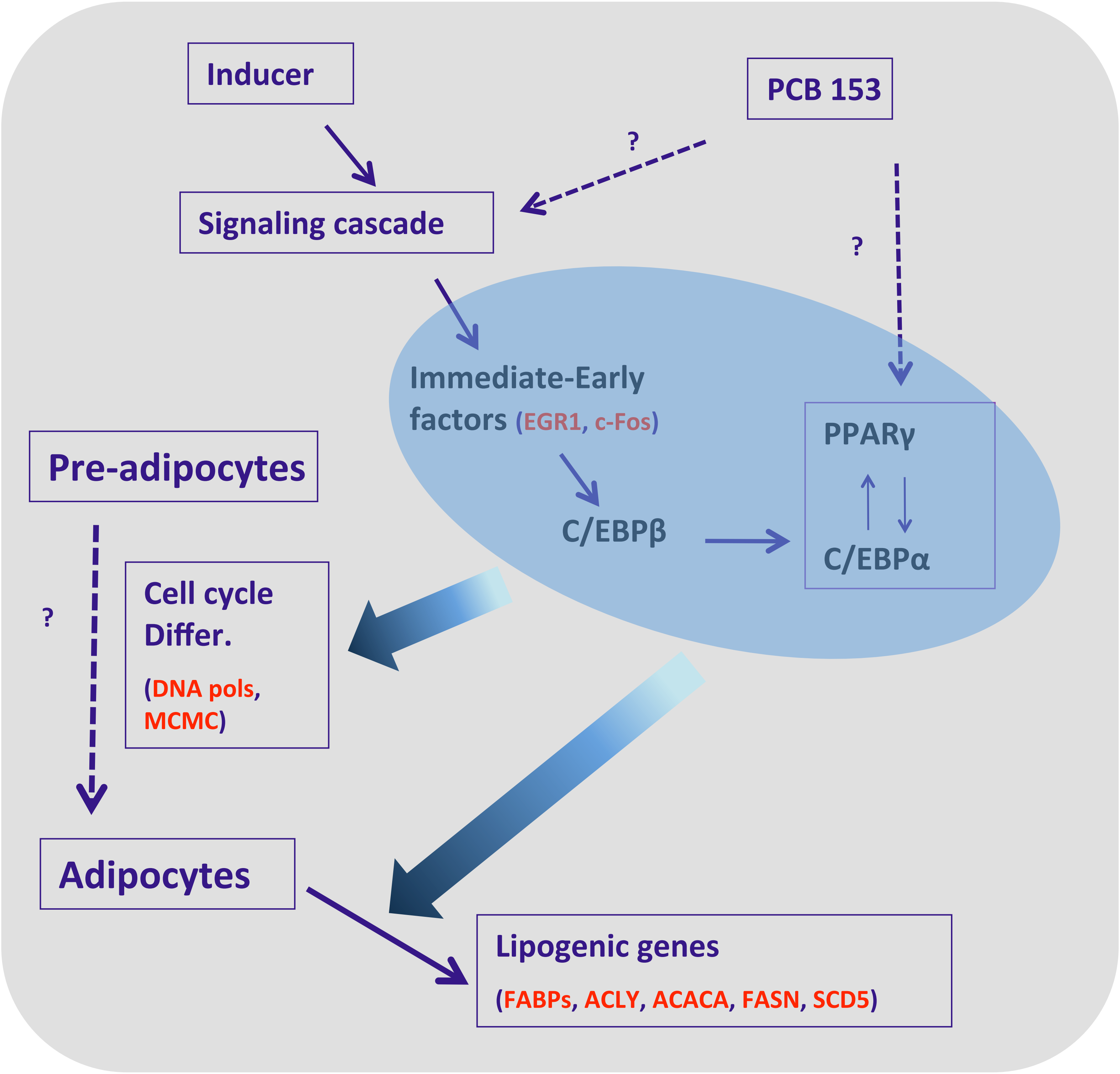
qPCR: Lipogenic genes











Inducer

PCB 153

?

Signaling cascade

?

Immediate-Early factors (EGR1, c-Fos)

PPARγ

C/EBPα

Pre-adipocytes

C/EBPβ

?

Cell cycle Differ.

(DNA pols, MCMC)

Adipocytes

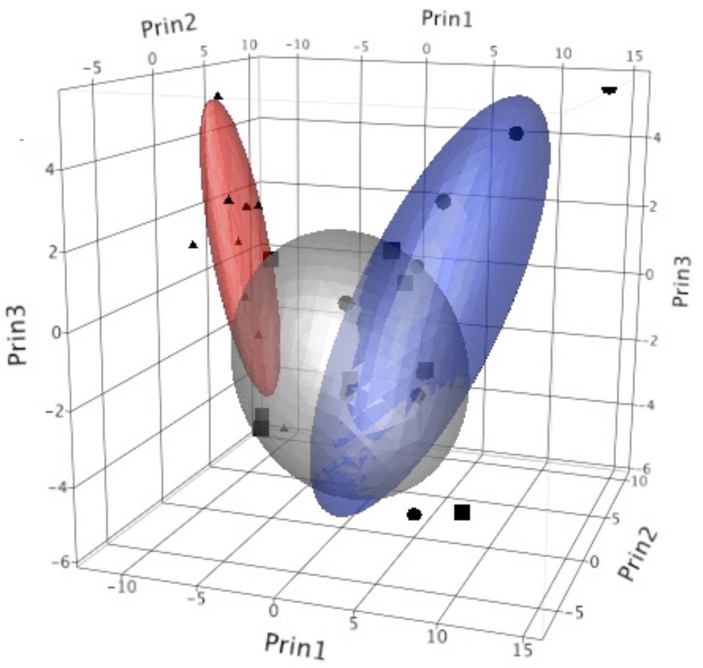
Lipogenic genes

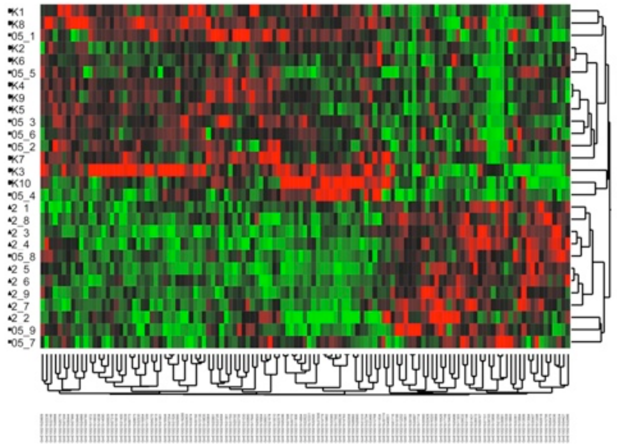
(FABPs, ACLY, ACACA, FASN, SCD5)

Summary of major pathways affected by PCB153

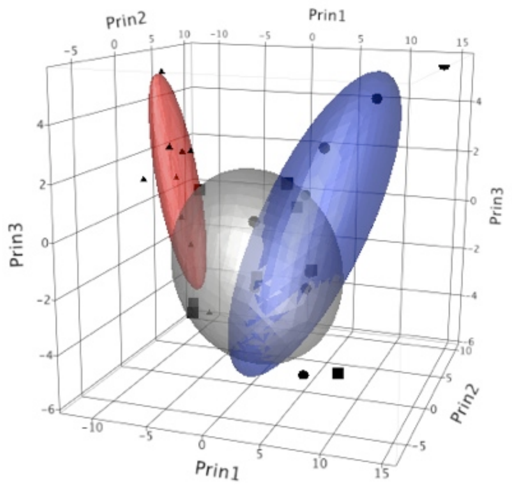
- Lipid metabolism
- DNA metabolism and Cell cycle
- Tissue remodeling and wound repair
- Immune response
- Xenobiotic metabolizing enzymes

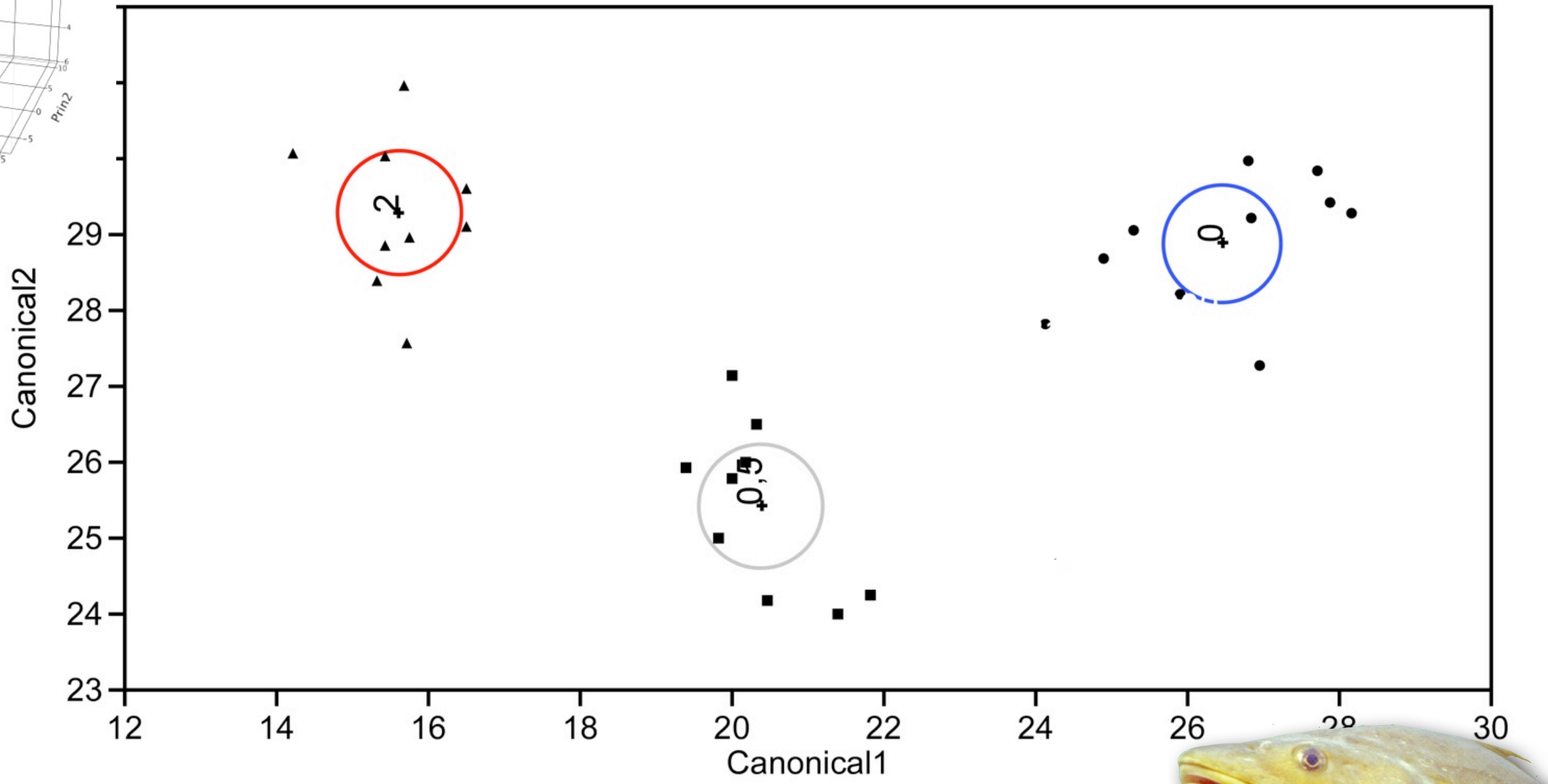
How can omics results be used for biomarker development?





MeHg/Proteomics: Discriminant analysis for biomarker discovery





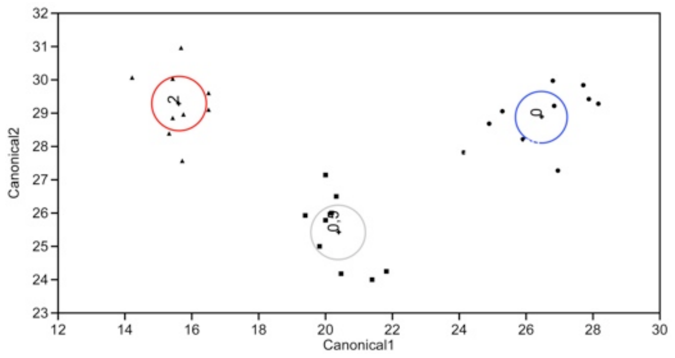
0 mg/kg

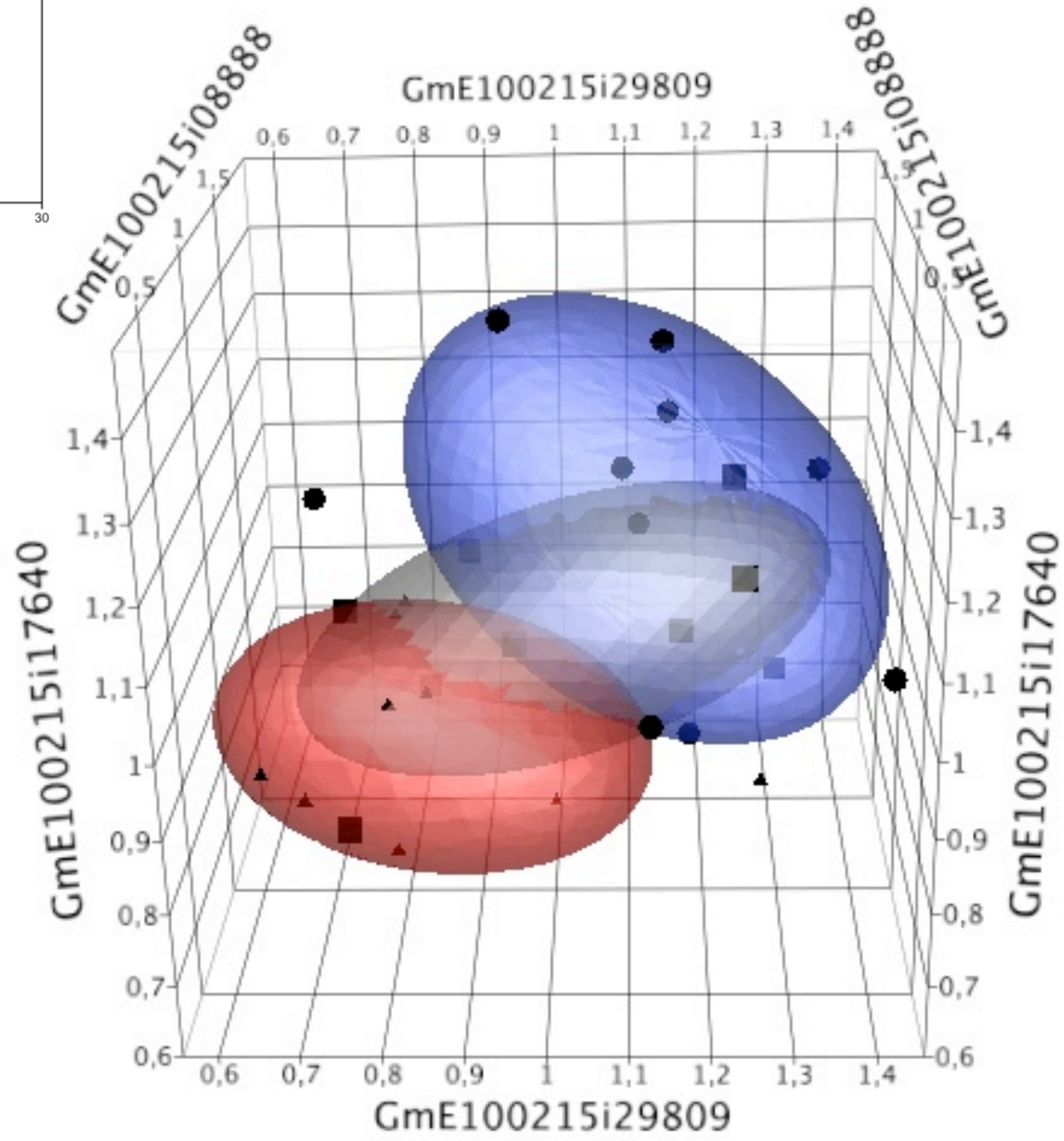
0,5 mg/kg

2 mg/kg

Discriminant Analysis seeks to find a way to predict a classification (*X*) variable based on known continuous responses (*Y*). It can be regarded as inverse prediction from a multivariate analysis of variance (MANOVA). (JMP v. 7.0.2, SAS Institute)

Discriminant-based biomarkers: does it work?



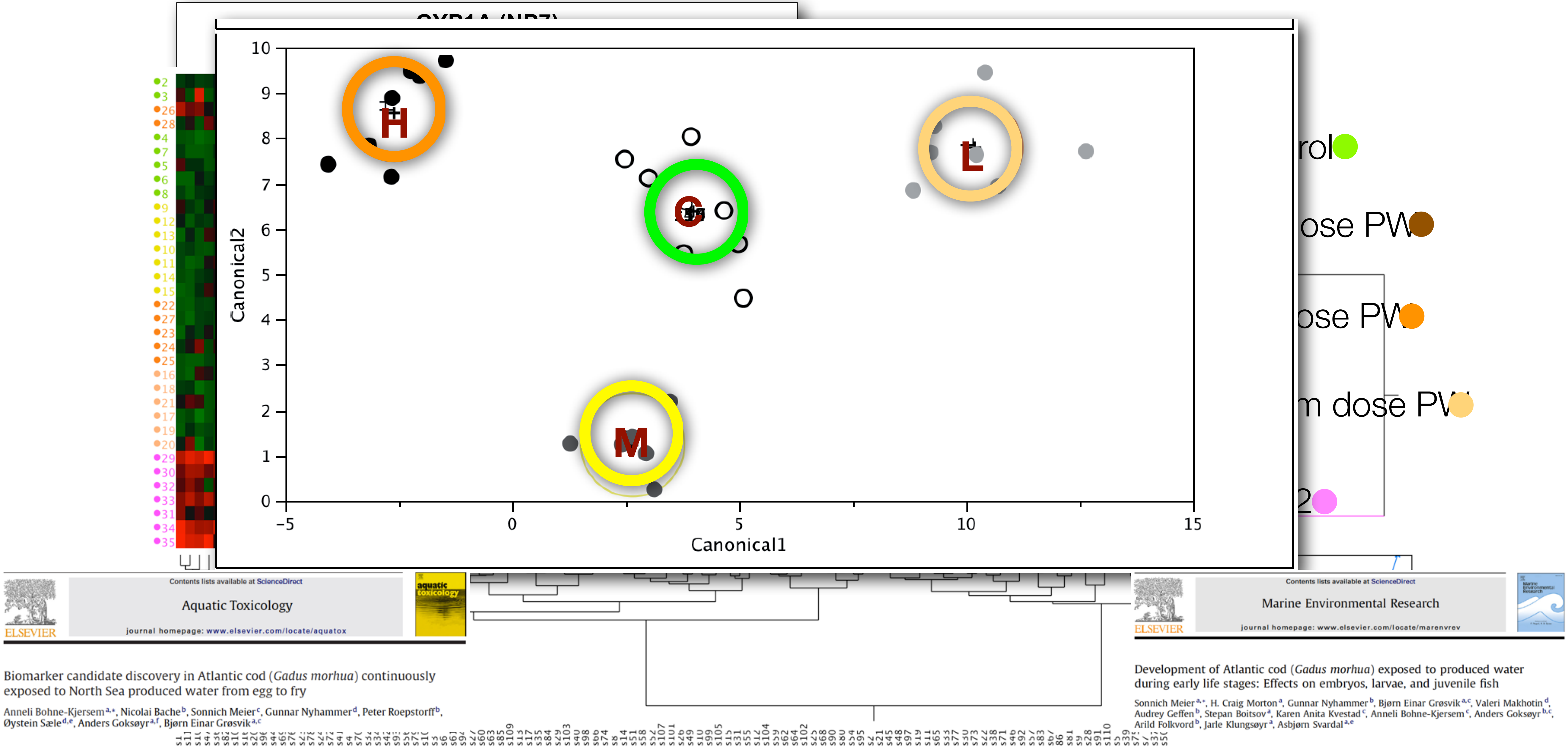


0 mg/kg

0,5 mg/kg

2 mg/kg

Biomarkers identified by proteomics
- reflects effects of produced water with high sensitivity



Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Biomarker candidate discovery in Atlantic cod (*Gadus morhua*) continuously exposed to North Sea produced water from egg to fry

Anneli Bohne-Kjersem[a,*], Nicolai Bache[b], Sonnich Meier[c], Gunnar Nyhammer[d], Peter Roepstorff[b], Øystein Sæle[d,e], Anders Goksøyr[a,f], Bjørn Einar Grøsvik[a,c]

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Development of Atlantic cod (*Gadus morhua*) exposed to produced water during early life stages: Effects on embryos, larvae, and juvenile fish

Sonnich Meier[a,*], H. Craig Morton[a], Gunnar Nyhammer[b], Bjørn Einar Grøsvik[a,c], Valeri Makhotin[d], Audrey Geffen[b], Stepan Boitsov[a], Karen Anita Kvestad[c], Anneli Bohne-Kjersem[c], Anders Goksøyr[b,c], Arild Folkvord[b], Jarle Klungsøyr[a], Asbjørn Svardal[a,e]


Photo: J. Skadal and I. Rønnestad

Bohne-Kjersem et al., Aq Tox (2010); Meier et al., MER (2010)

Biomarker verification:
Stable isotope dilution multiple reaction monitoring (SID-MRM)

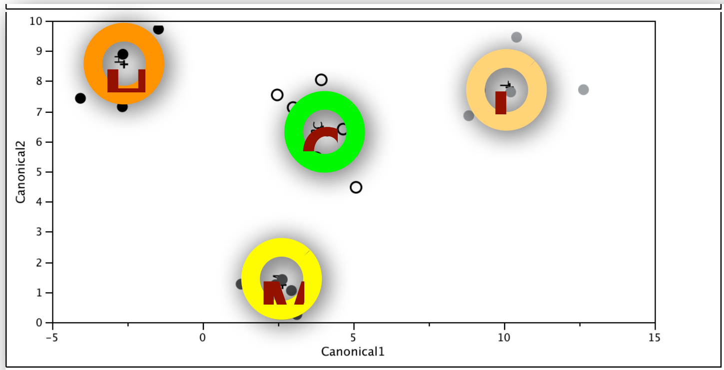
Candidate Proteins

↓

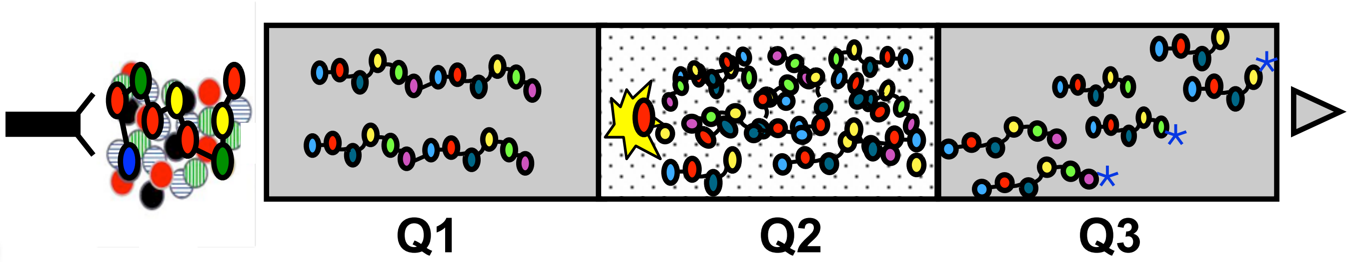
Define “Signature peptides” for protein candidates

↓

Synthesize “heavy" isotope labeled internal standards

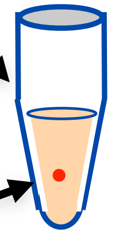


MRM-MS



Spike “heavy” Internal stnd. peptides

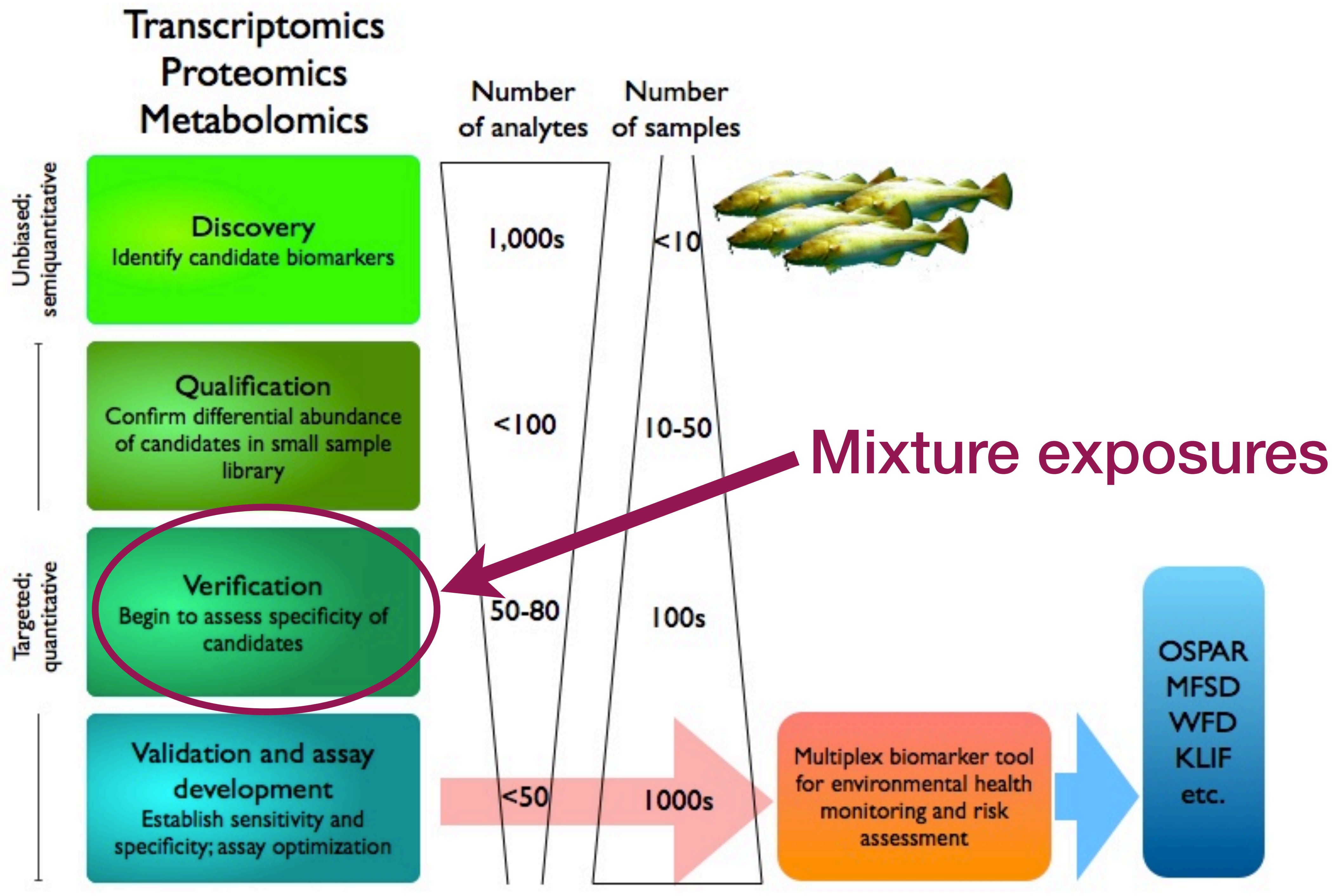
Endogenous “light” peptides



↓

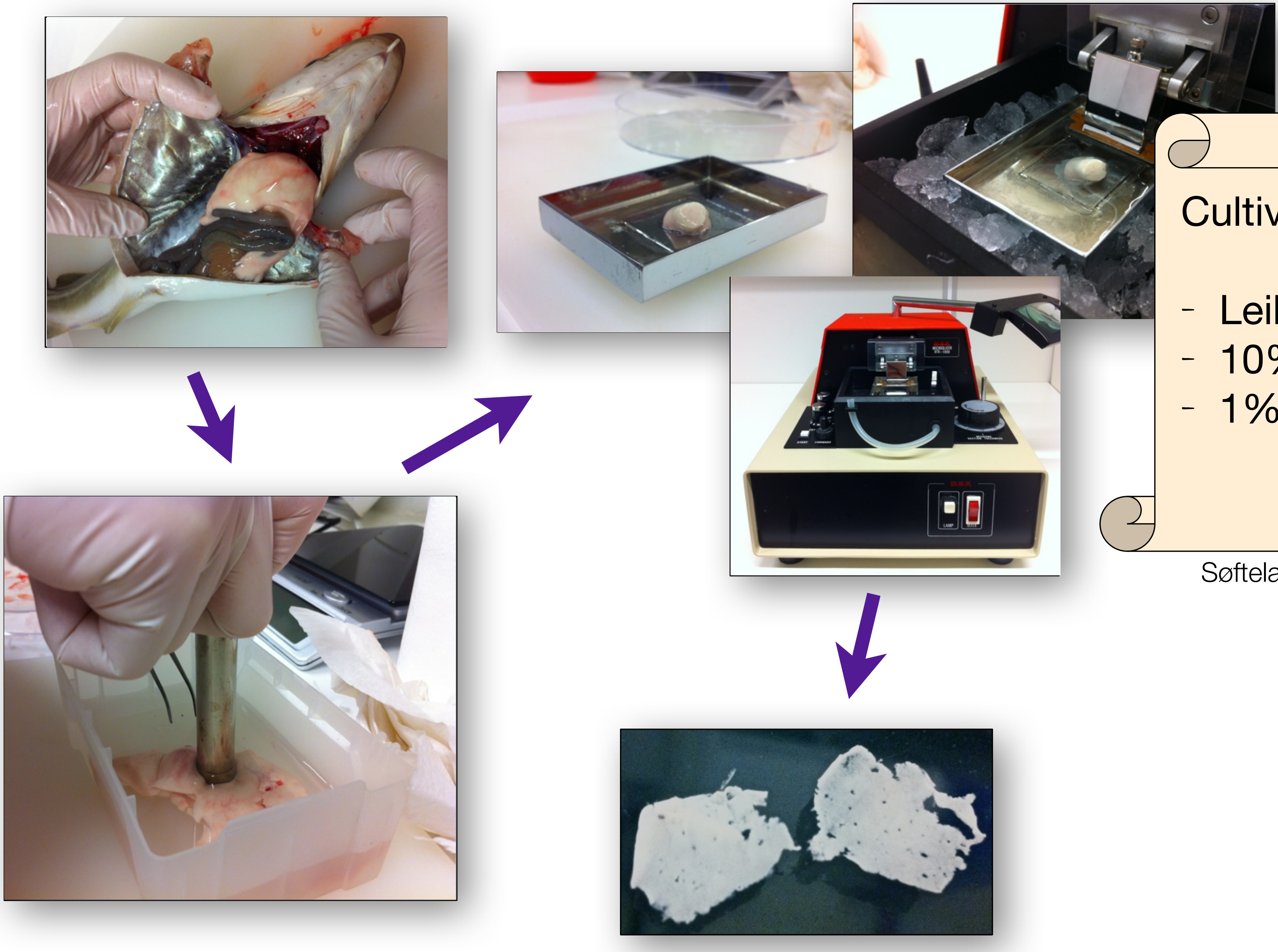
Ratio “light” peptide to “heavy” peptide

Where next? iCod 2.0



based on Rifai et al. Nature Biotech (2006)

Procedure for cod liver slice preparation



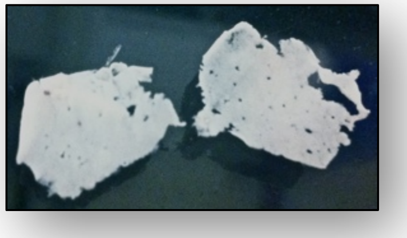
Cultivation media:

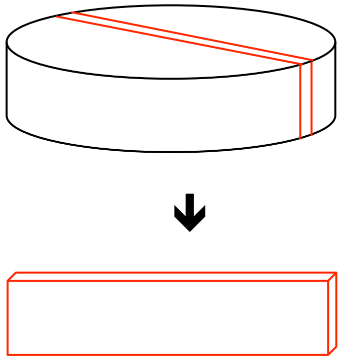
- Leibovitz 15
- 10% FBS
- 1% antibiotica

Søfteland et al. 2010

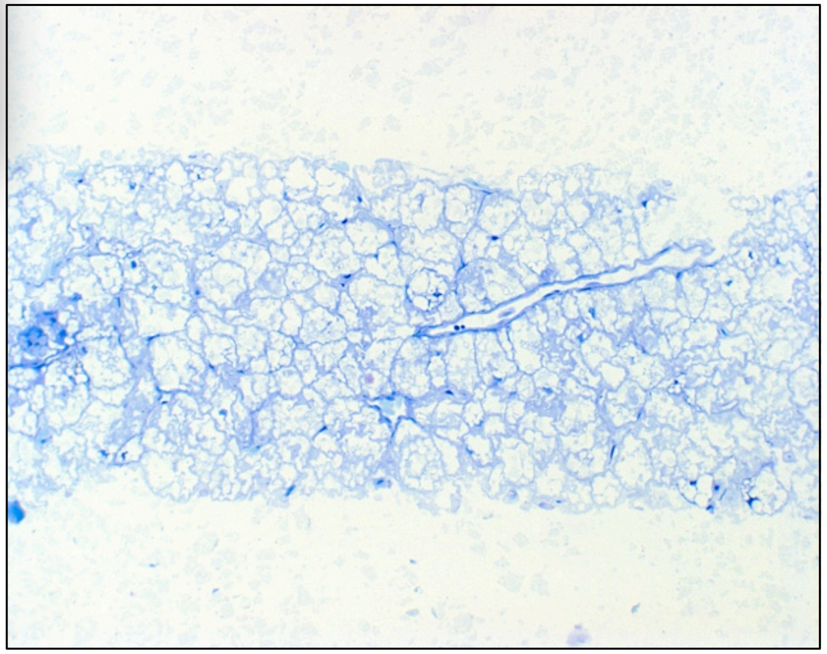
Marta Eide, PhD project

Histology of cod liver slices

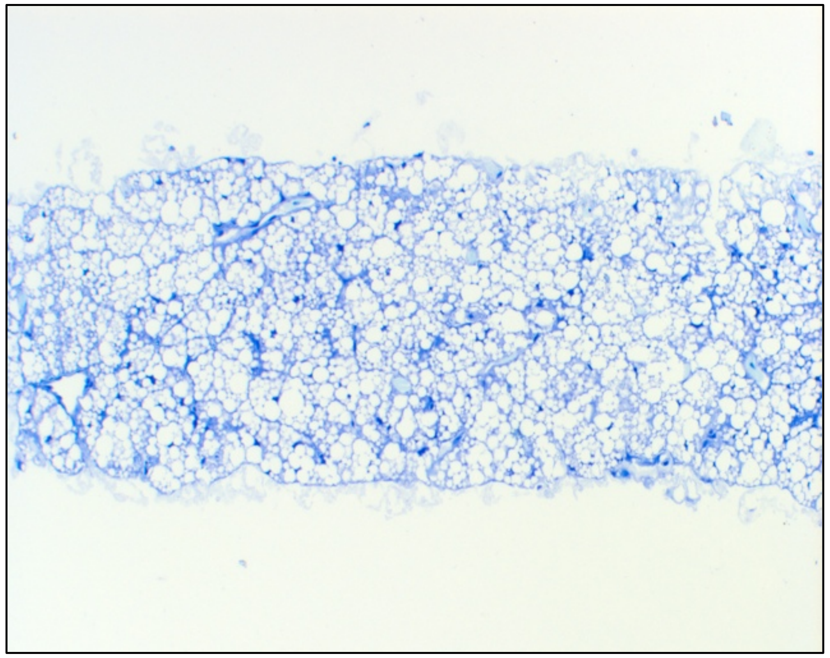




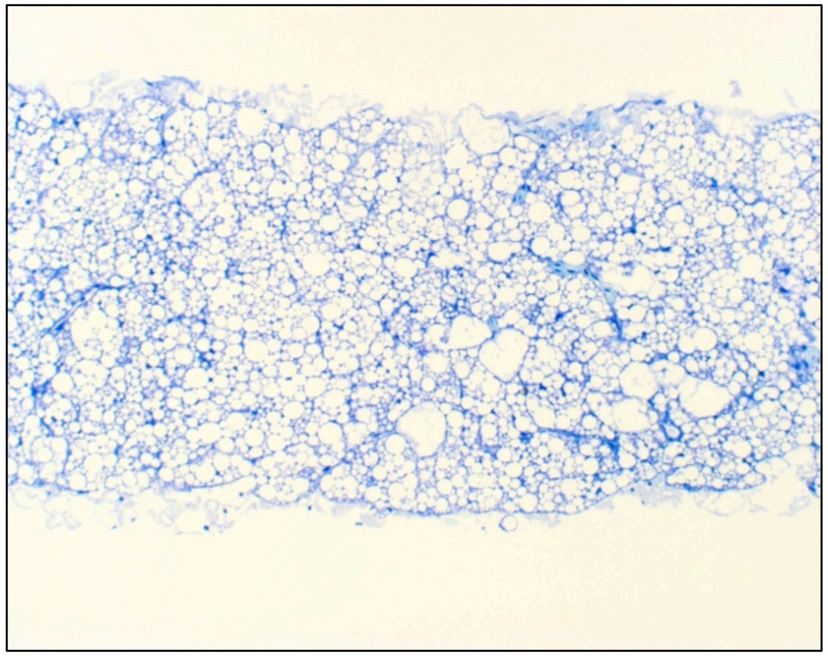
0 h



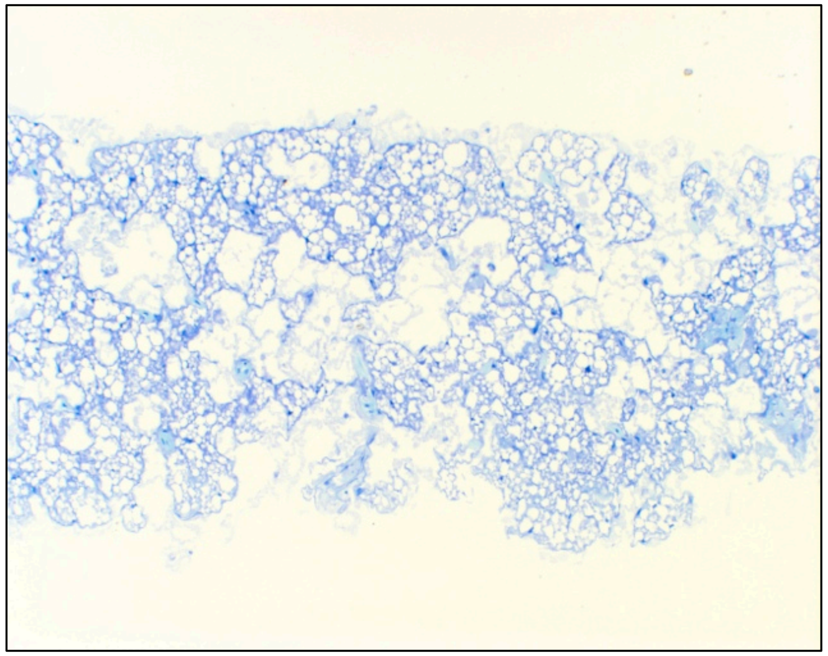
24 h



48 h



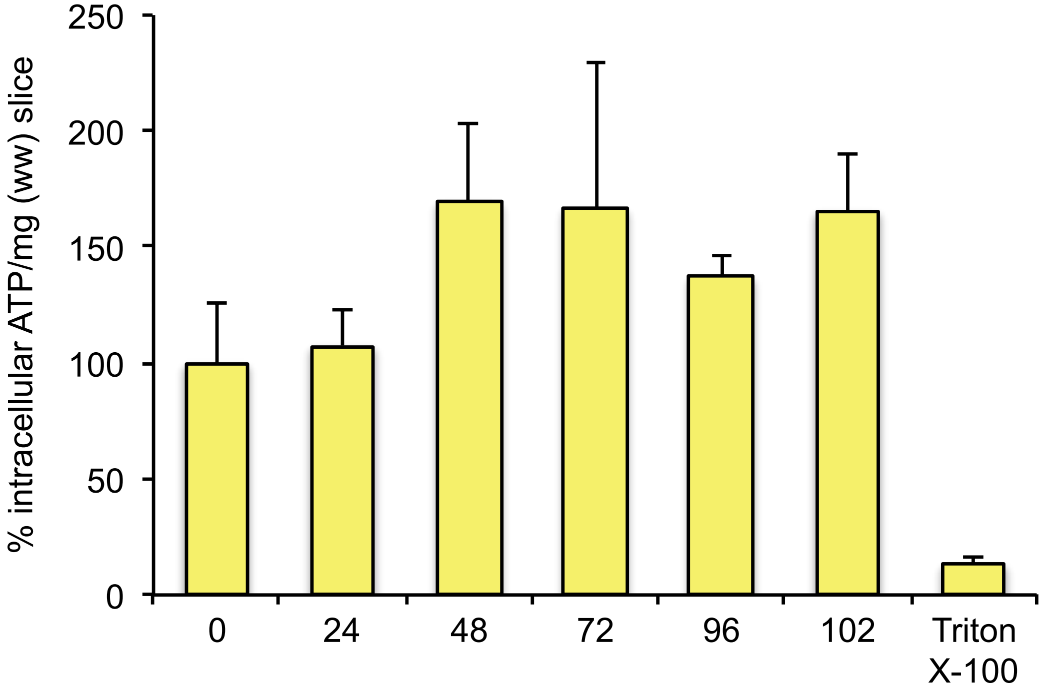
72 h



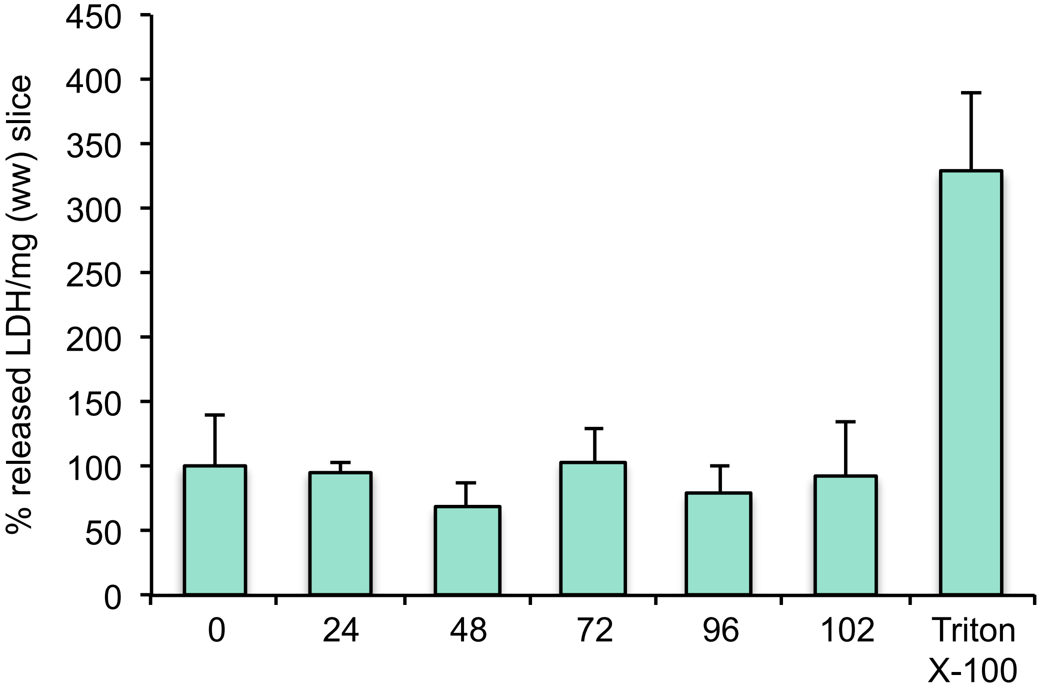
Marta Eide,
PhD project

Viability of cod liver slices

ATP



LDH

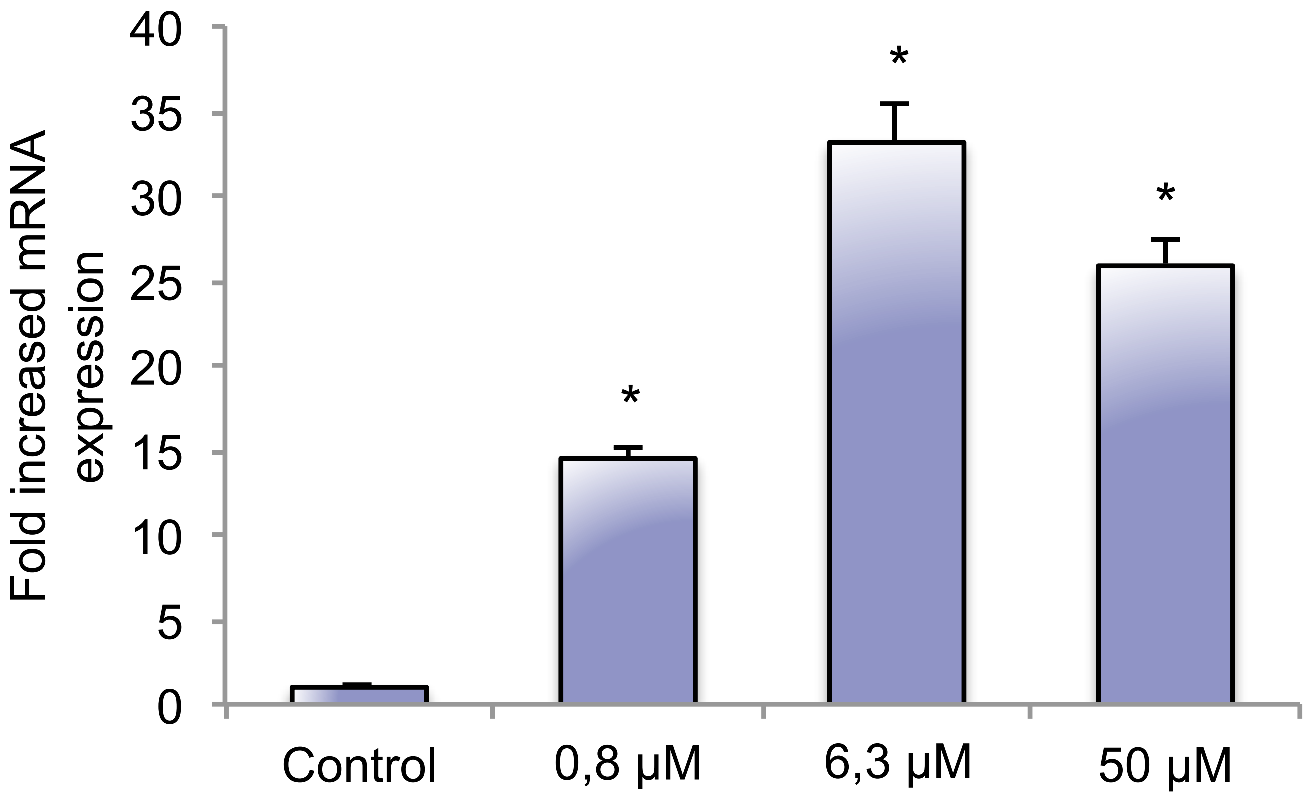


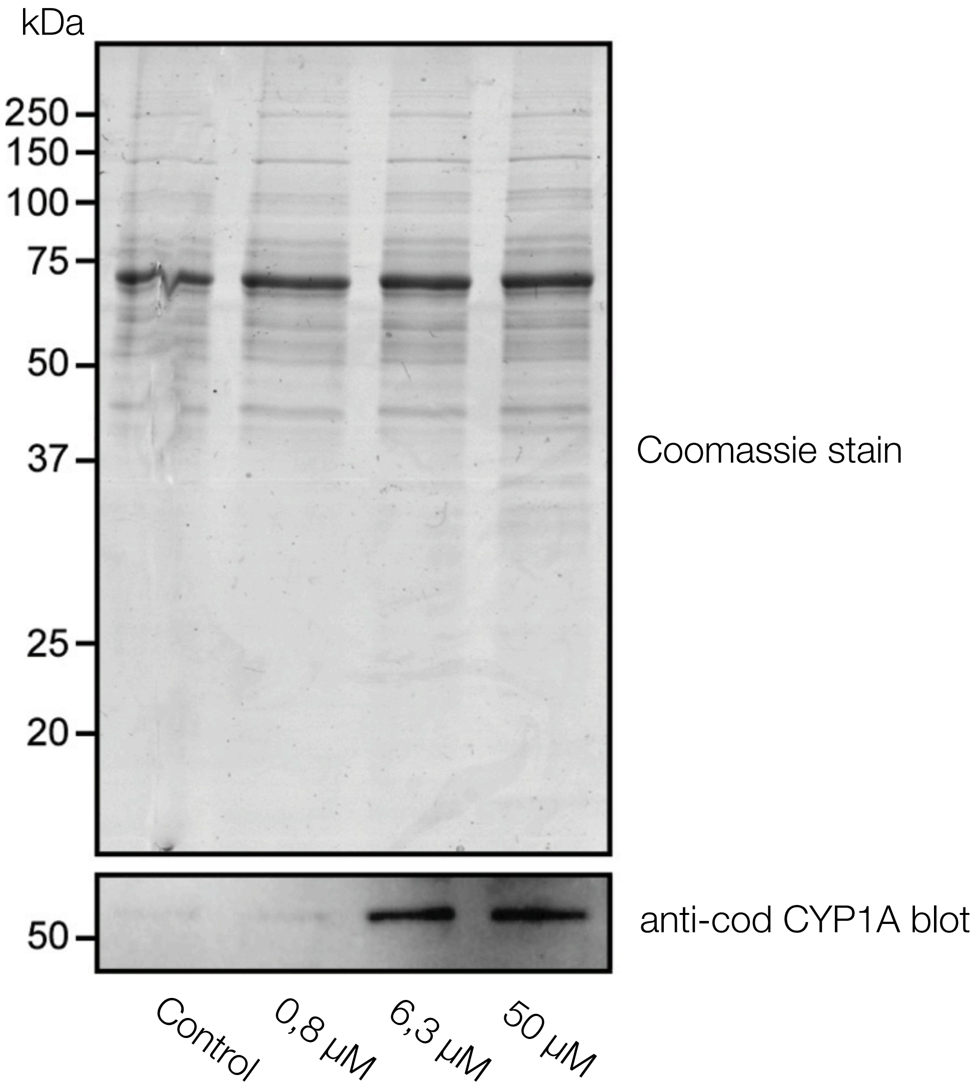
Levels of lactate dehydrogenase (LDH) released from obtained slices (A) and adenosine triphosphate (ATP) within the slices (B), measured with the commercial kits Cytotoxicity Detection LDH Plus Kit (Roche) and ATP Lite 1Step (Perkin Elmer), respectivly. Values compared to fresh (1 h) slices. Exposure to 0.5% Triton X-100 at 72-120 h serves as control for cell death. Statistical analyses performed with Student's t-test, $n = 3$, ** indicating $p < 0.01$, * $p < 0.05$.

Marta Eide, PhD project

Cod liver slice culture: Initial exposure studies

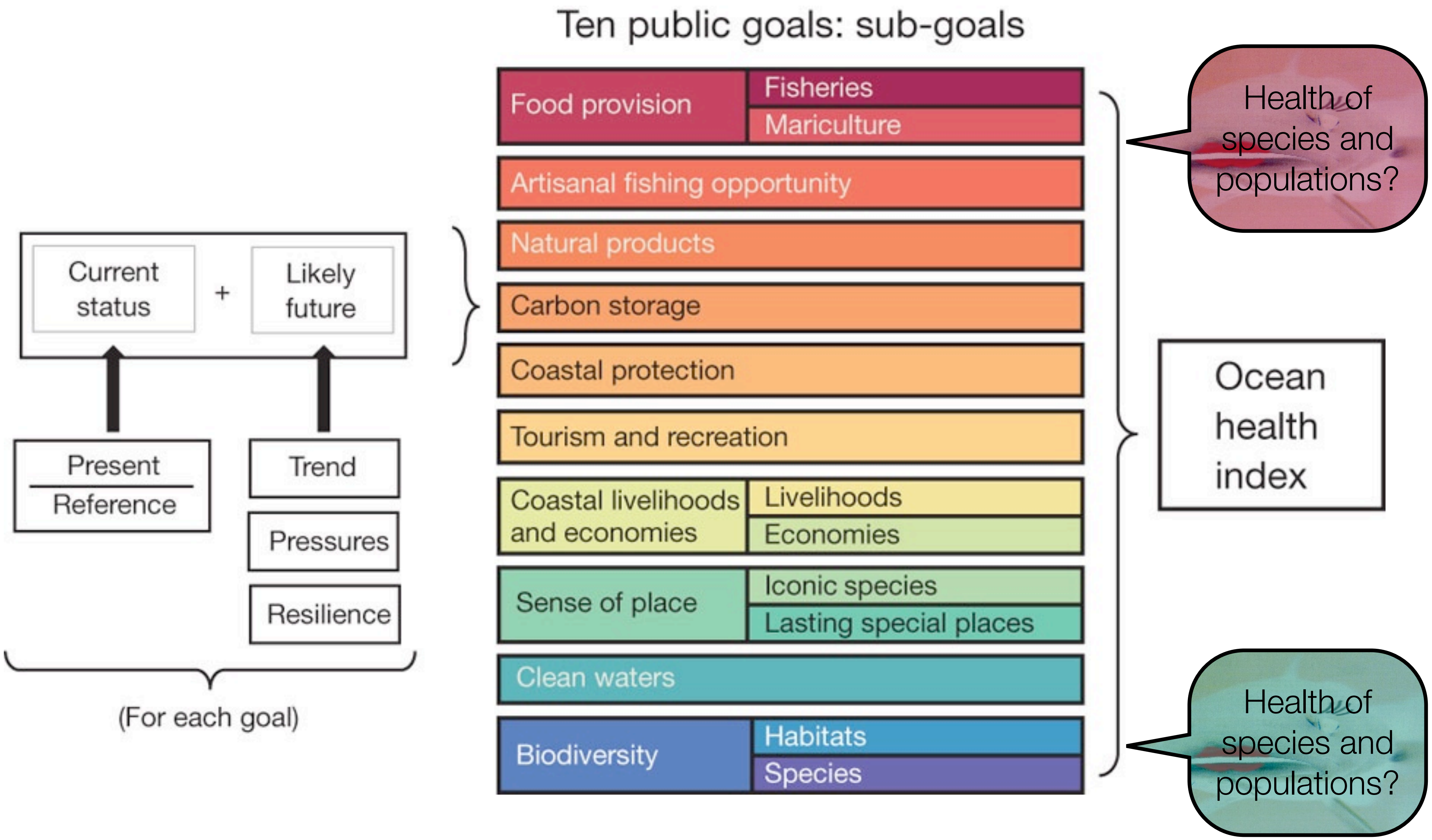
Increased CYP1A1 transcription and translation following 24 h exposure to β-naftophlavone





Marta Eide, PhD project

An index to assess the health and benefits of the global ocean?



Health of species and populations?

Health of species and populations?

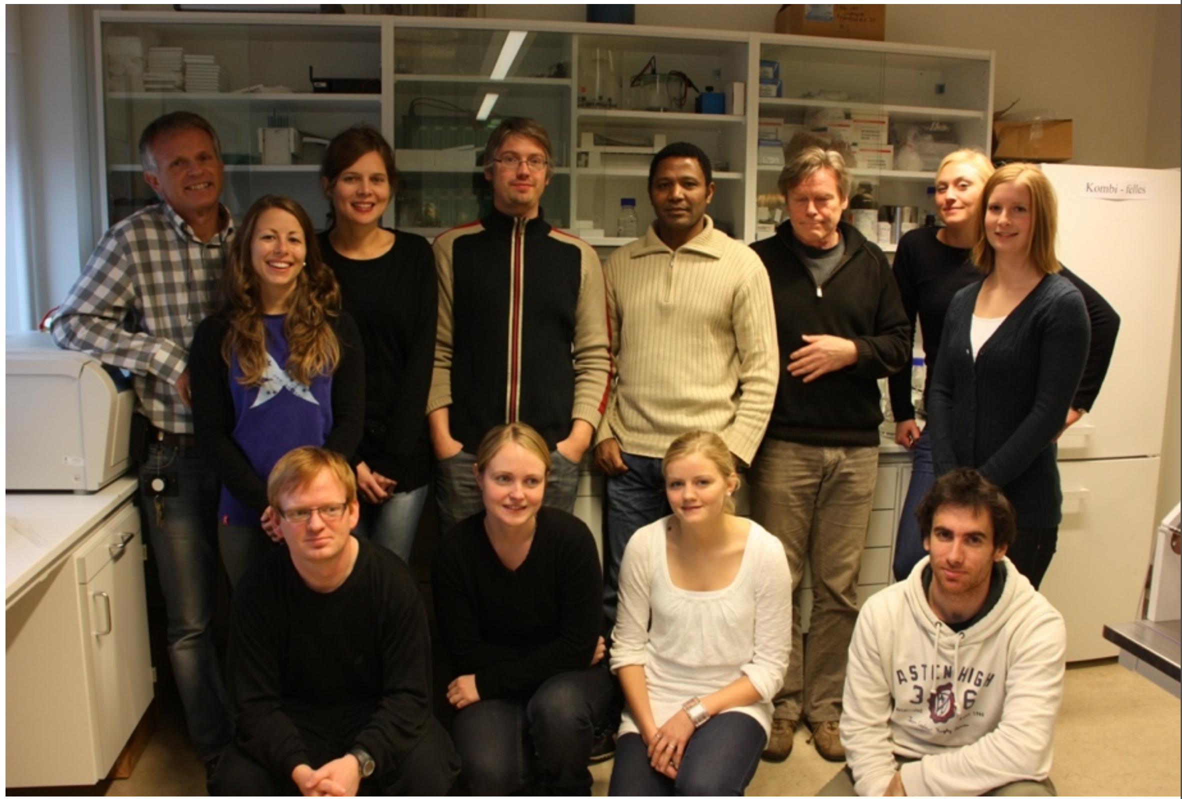
BS Halpern *et al.* *Nature* **488**, 615-620 (2012) doi:10.1038/nature11397

Acknowledgements

- **Odd André Karlsen**, **Fekadu Yadetie**, Roger Lille-Langøy, Marta Eide, Marianne Brattås, Silje Bjørneklett, Karin Berg, Anneli Bohne-Kjersem, Rune Male

- iCod partners:
- Pål Olsvik, NIFES, Bergen, Norway
- Bjørn Einar Grøsvik, Inst. Marine Research, Bergen
- Pål Puntervoll, CBU, UiB/UNI, Bergen
- Ketil Hylland, Dept. of Biology, UiO, Oslo
- Stig W. Omholt, CIGENE/UMB, Ås
- Peter Roepstorff, University of Southern Denmark
- Christer Hogstrand, King´s College, London, UK

The Research Council of Norway




Välkommen till