Overview of Health Assessment Tools in Native Freshwater Mollusks

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USGS science for a changing world

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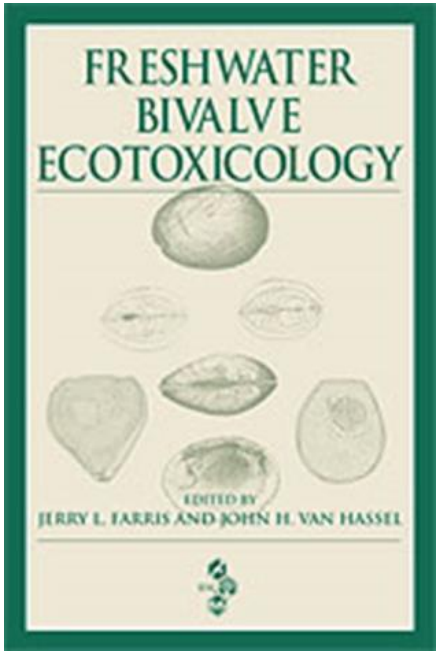
Biomarker Health Focus

Toxicology Driven

- Key Publication:
- Newton, T.J. and W.G. Cope. 2006. Biomarker responses of unionid mussels to environmental contaminants. Chapter 10 (pp. 257-284). In: Farris, J.L. and Van Hassel, J.H. (Eds.), Freshwater Bivalve Ecotoxicology. CRC Press, Boca Raton, FL.



Biomarker Concept

- *Change in a biological response that can be related to exposure to, toxic effects of, or susceptibility to contaminants*
- Biomarkers measured in organisms can provide sensitive indices, or early warning signs, to contaminants or other stressors
- Compared with chemical residue analysis, biomarkers have the advantage of measuring the stress on the organism, thus may be more biologically relevant

Classification of Biomarkers

- *Biomarker of exposure* – relates exposure to a contaminant to the levels of the substance that can be measured within an organism (e.g., Cytochrome P-450 induction from PCB exposure)
- *Biomarker of effect* – biomarker that can be recognized as associated with an established or possible health impairment or disease (e.g., ALAD induction from lead toxicity, growth)
- *Biomarker of susceptibility* – biomarker of an inherent or acquired ability of an organism to respond to exposure to a specific substance (e.g., altered gene or enzyme regulation)

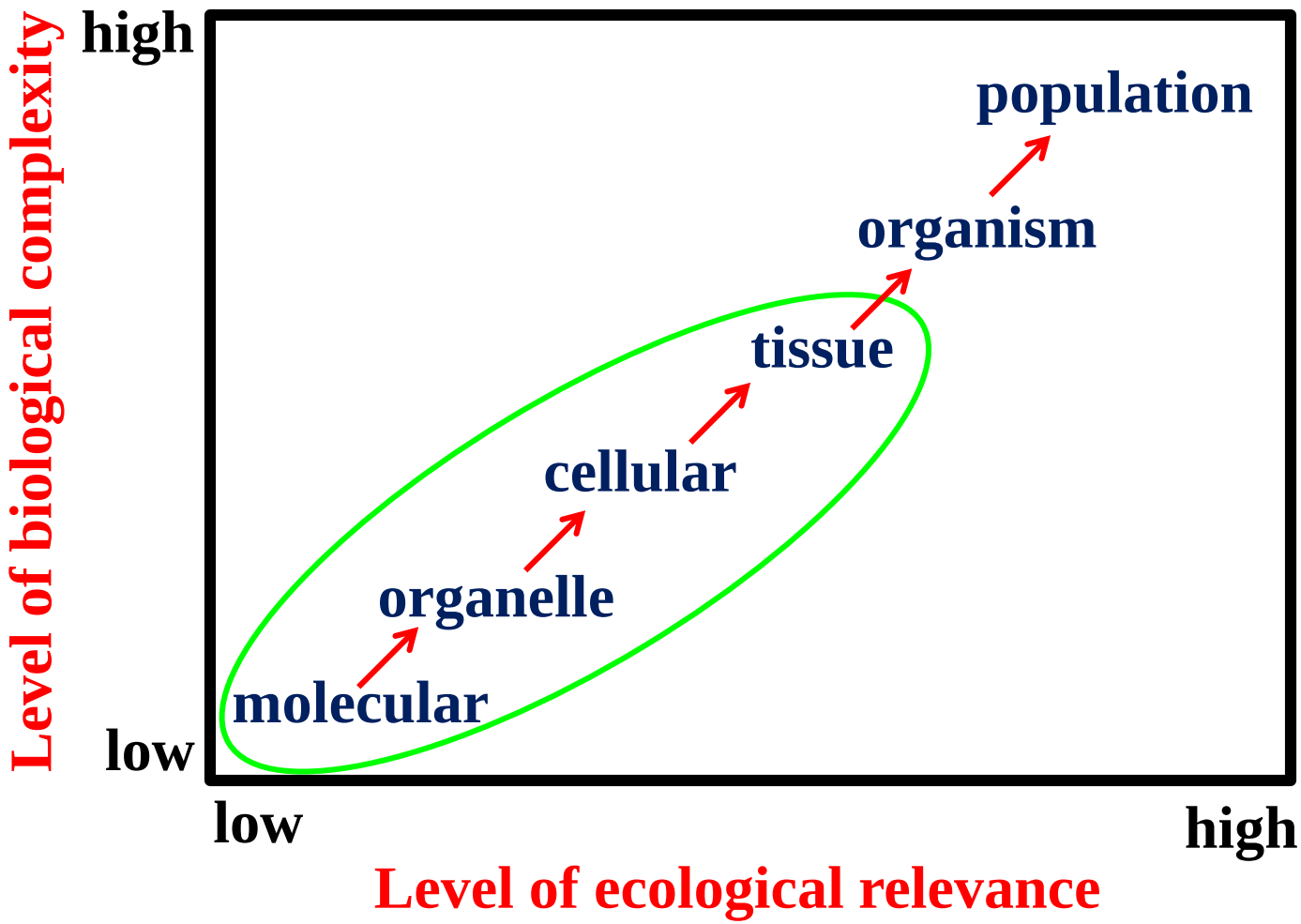
World Health Organization 1993

Biomarker Criteria

- Reliable, inexpensive, simple assay
- Sensitive response, early warning
- Well defined baseline data (natural variation vs. contaminant induced)
- Confounding factors are well understood
- Mechanisms of action for response to exposure understood or established
- Impact of biomarker response to organism health established

Van der Oost et al. 2003

Levels of Biological Organization



Biomarkers in Aquatic Biota (non-unionid)

Category	Example
Biotransformation enzymes	Cytochrome P450, EROD, Glutathione-S-transferase
Oxidative stress	Lipid peroxidation, GPOX, GRED, SOD
Biotransformation products	PAH metabolites in bile
Amino acids & proteins	Amino acids, stress proteins, metallothioneins
Hematological	Serum transaminases
Immunological	Cell- & humoral-mediated immunity, phagocytosis
Reproductive & endocrine	Imposex, vitellogenin
Neuromuscular	Cholinesterases
Genotoxic	DNA damage
Physiological & morphological	Histopathology, ion regulation, condition indices, energetics, valve activity, growth

Van der Oost et al. 2003

Biomarkers in Unionid Mussels

Category	Example
Biotransformation enzymes	Several Phase I & Phase II enzymes
Oxidative stress	Lipid peroxidation, GPOX, GRED, SOD
Biotransformation products	None
Amino acids & proteins	Metallothioneins
Hematological	Porphyrin profiles
Immunological	Phagocytosis, cell viability
Reproductive & endocrine	Vitellogenin-like proteins, ALP
Neuromuscular	Cholinesterases
Genotoxic	DNA strand breakage
Physiological & morphological	Histopathology, ion regulation, digestive processes, condition indices, energetics, valve activity, growth

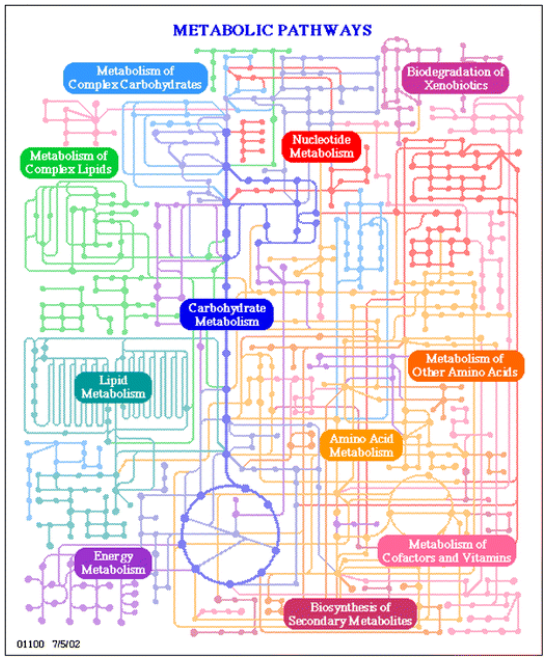
Newton and Cope 2006

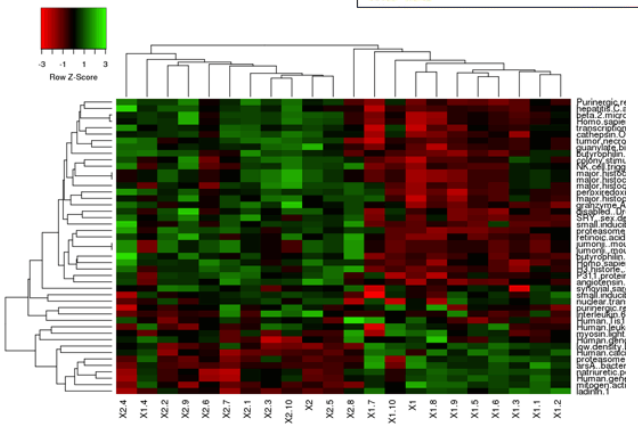
Promising Endpoints

- Those involving analysis of effects on sensitive life stages and reproductive activity
- Includes behavioral and physiological categories (e.g., abortive glochidia release, mantle flap, and foot movement)
- Bringolf et al. 2010 Fluoxetine
- Leonard et al. 2014 EE2
- Newton et al. 2017 Bayluscide®

Promising Endpoints

- Those involving analysis of effects on superpathways, protein, and gene expression
- Includes molecular and physiological categories (e.g., metabolomics, proteomics, transcriptomics)
- Roznere et al. 2014 Captivity
- Leonard et al. 2014 EE2
- Bertucci et al. 2017 Metals



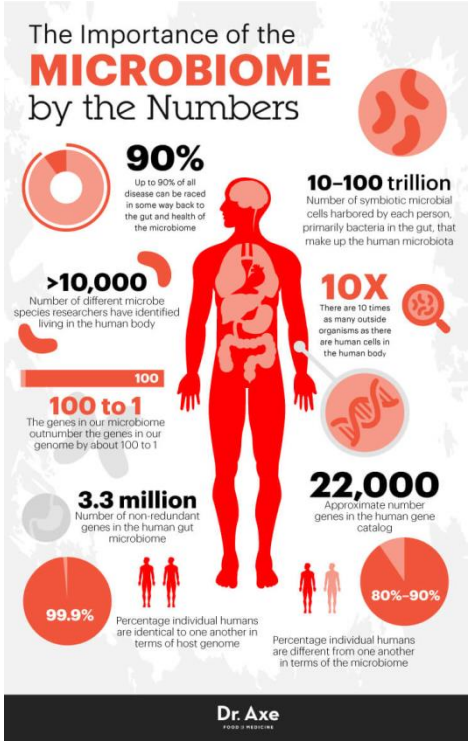


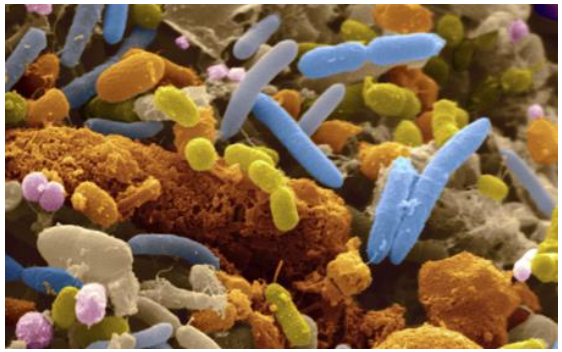
Promising Endpoints

- Those involving non-lethal sampling (i.e., biopsy, hemolymph) to assess health and condition
- Includes hematological, immunological, physiological, reproductive, and endocrine categories (e.g., hemolymph chemistry profiles, fatty acids, cell count)
- Issues: Hemolymph still not well-characterized; Fe vs. Cu, cell types, activity; normal ranges
- Fritts et al. 2015 Methods, parameters
- Gustafson et al. 2005 Technique, reference range

Promising Endpoints

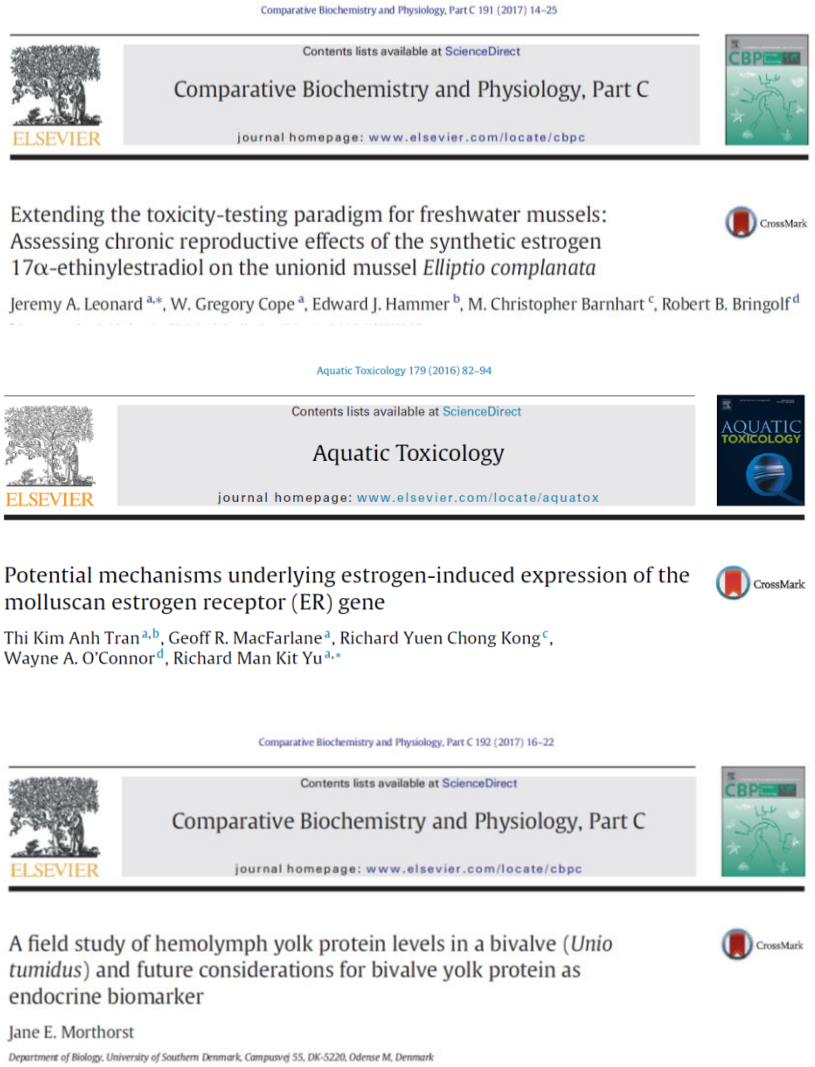
- Those involving analysis of the microbiome of the gut and external environment (i.e., shell, sediment) of all life stages
- Microbiome – the collective genome of the indigenous microbes (microflora), both internal and external
- Will be critically important in diet, propagation, immunology, toxicant sensitivity
- Cova Arias, Auburn University
- Rob Knight, UC San Diego, (https://knightlab.ucsd.edu/)
- Black et al. 2017 Mussel Sediment





Non-Valid Endpoints – Vitellogenin (Vtg) and Alkaline-Labile Phosphatase (ALP)

- Vtg's are the major precursor of egg-yolk proteins in fish and vertebrates
- Early studies evaluated Vtg-like proteins as indicators of estrogenic exposure with an ALP assay
- Data now suggests that mussels are not estrogen receptor responsive
- No Vtg production in mussels; non-genomic estrogen signaling
- Vg-induction, and specifically ALP, is not a useful biomarker to assess estrogenic contamination in mussels



Comparative Biochemistry and Physiology, Part C 191 (2017) 14–25

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Comparative Biochemistry and Physiology, Part C

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

Extending the toxicity-testing paradigm for freshwater mussels: Assessing chronic reproductive effects of the synthetic estrogen 17α-ethinylestradiol on the unionid mussel *Elliptio complanata*

Jeremy A. Leonard [a,*], W. Gregory Cope [a], Edward J. Hammer [b], M. Christopher Barnhart [c], Robert B. Bringolf [d]

Aquatic Toxicology 179 (2016) 82–94

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Aquatic Toxicology

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Potential mechanisms underlying estrogen-induced expression of the molluscan estrogen receptor (ER) gene

Thi Kim Anh Tran [a,b], Geoff R. MacFarlane [a], Richard Yuen Chong Kong [c], Wayne A. O'Connor [d], Richard Man Kit Yu [a,*]

Comparative Biochemistry and Physiology, Part C 192 (2017) 16–22

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A field study of hemolymph yolk protein levels in a bivalve (*Unio tumidus*) and future considerations for bivalve yolk protein as endocrine biomarker

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Holistic Health Focus

- Much progress, but still uncertain what constitutes a ‘healthy’ mussel?
- Variation over time, space; among individuals, species
- Applied biomarkers will help, but need basic science
- Advocate for funding of a mussel health initiative that will model a human health clinical approach
- Need well-defined baseline data (natural variation vs. contaminant induced)—relation to health!!!