UNIVERSITY OF BIRMINGHAM

Probing the water flea's biochemistry: a truly complex but high information content environmental sample

RSC Recent Advances in the Analysis of Complex Environmental Matrices

28th February 2013

Mark Viant, University of Birmingham, UK

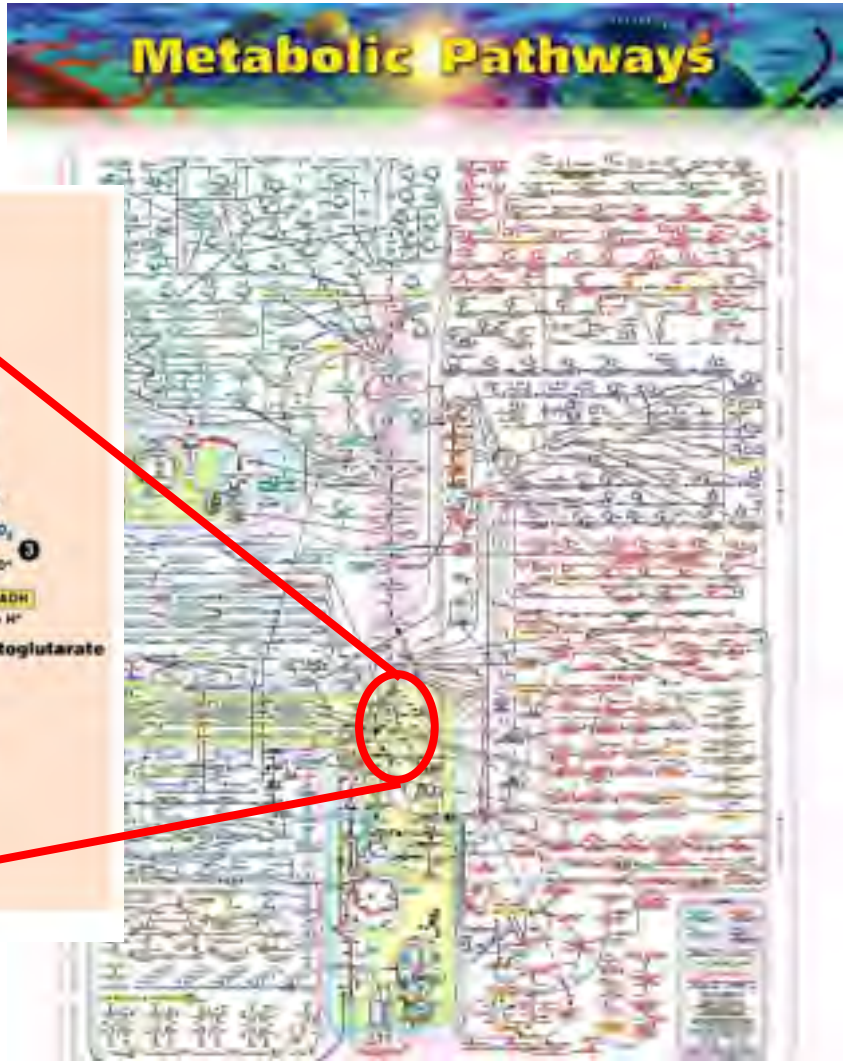
Daphnia – the water flea

- Central component of freshwater ecosystems
- Used extensively worldwide in aquatic toxicity testing
- Easy to culture in lab, rapid life cycle, new generation every few days
- Reproduces asexually producing genetically identical offspring

US National Institutes of Health model organism

To understand genomic responses to environmental stressors that are important factors in human health and well being

What makes up the *Daphnia* "metabolome"?



- Amino acids
- Carbohydrates
- Lipids
- Steroids
- Secondary metabolites…
- Estimated to be 5000-10000 metabolites.

No metabolome of any organism has been thoroughly characterised!!

Environmental stress

What are the effects of these stressors on living organisms?

Can we develop novel information-rich approaches for environmental regulation?

Water pollution

Air pollution

Water pollution and chemical risk assessment

"Increasing worldwide contamination of freshwater systems with 1000's of industrial chemicals is one of the key environmental problems facing humanity"

Schwarzenbach et al., *Science* 313:1072 (2006)

>70,000 synthetic chemicals used by chemical industry

Full safely assessment completed on <10% of these

EU REACH (Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals, 2006)

- assesses impacts on human health and the environment
- estimated to use 54 million vertebrate animals and cost €9.5 billion

Hartung & Rovida, *Nature* 460:1080 (2009)

Need for high throughput, mechanism-based testing strategies to determine chemical toxicity

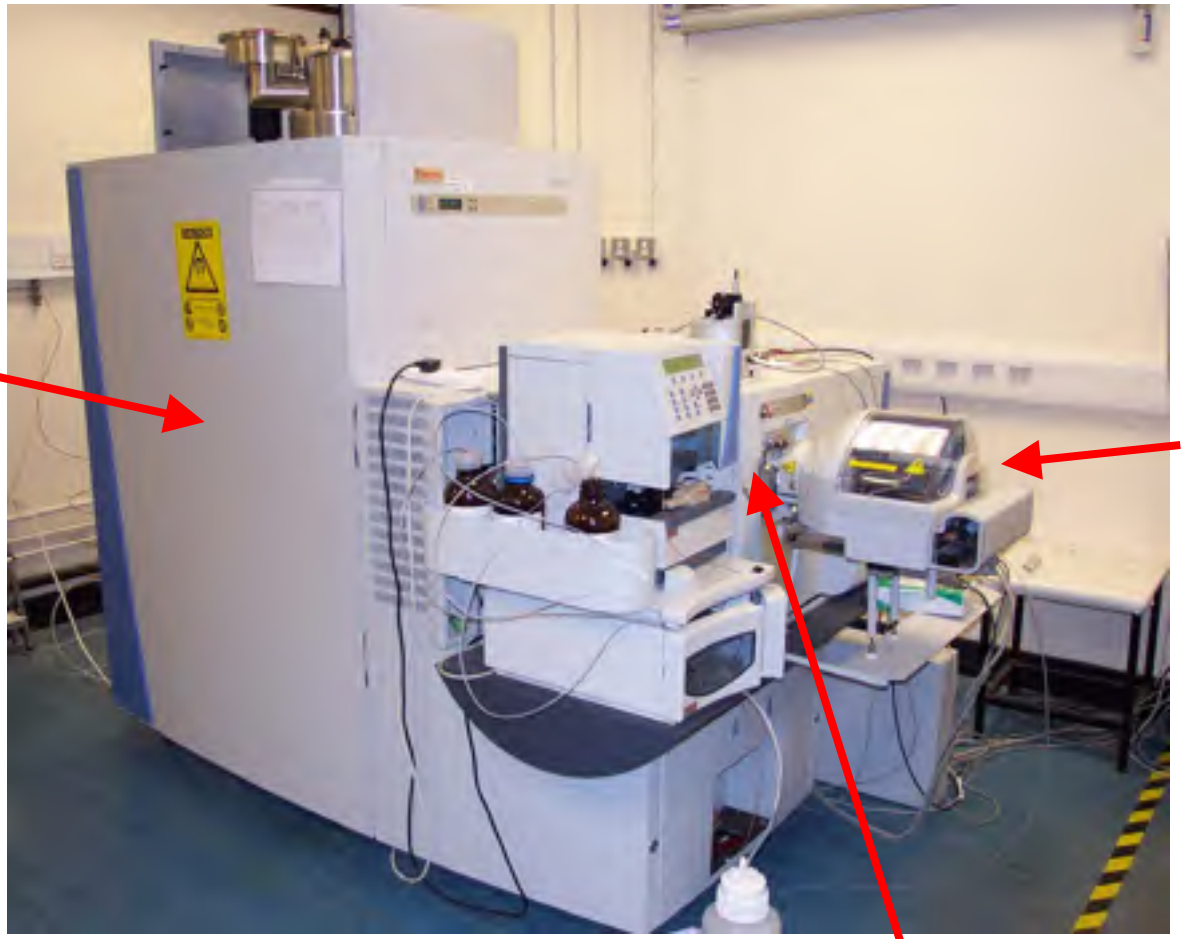
Overview of presentation

1. Direct infusion mass spectrometry (DIMS) based metabolomics – revealing the complexity of the metabolome

2. Metabolic effects of ZnO nanoparticles to *Daphnia magna* – using metabolomics to discover the needle in the haystack! …towards mechanism-based chemical risk assessment

Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry

Thermo Fisher LTQ FT Ultra



Analysis of complex mixtures by FT-ICR mass spectrometry

5710 J. Agric. Food Chem. 2001, 49, 5710−5718

Electrospray Ionization Fourier Transform Mass Spectrometric Analysis of Wine

Helen J. Cooper and Alan G. Marshall*

Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310

Acc. Chem. Res.

Petroleomics: The Next Grand Challenge for Chemical Analysis

ALAN G. MARSHALL*,† AND
RYAN P. RODGERS†

Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306, and Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310-3706

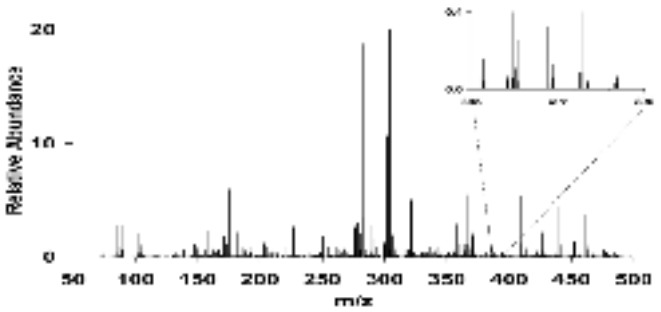
DIMS metabolomics pipeline

1.  *Daphnia magna* exposures

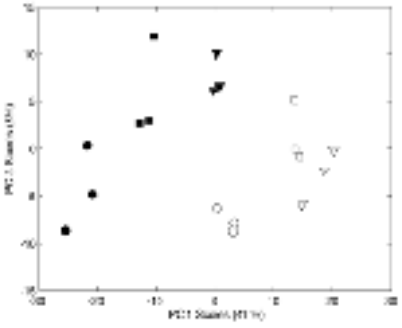
2.  Extract metabolites

3.  FT-ICR mass spectrometry

4. Signal processing

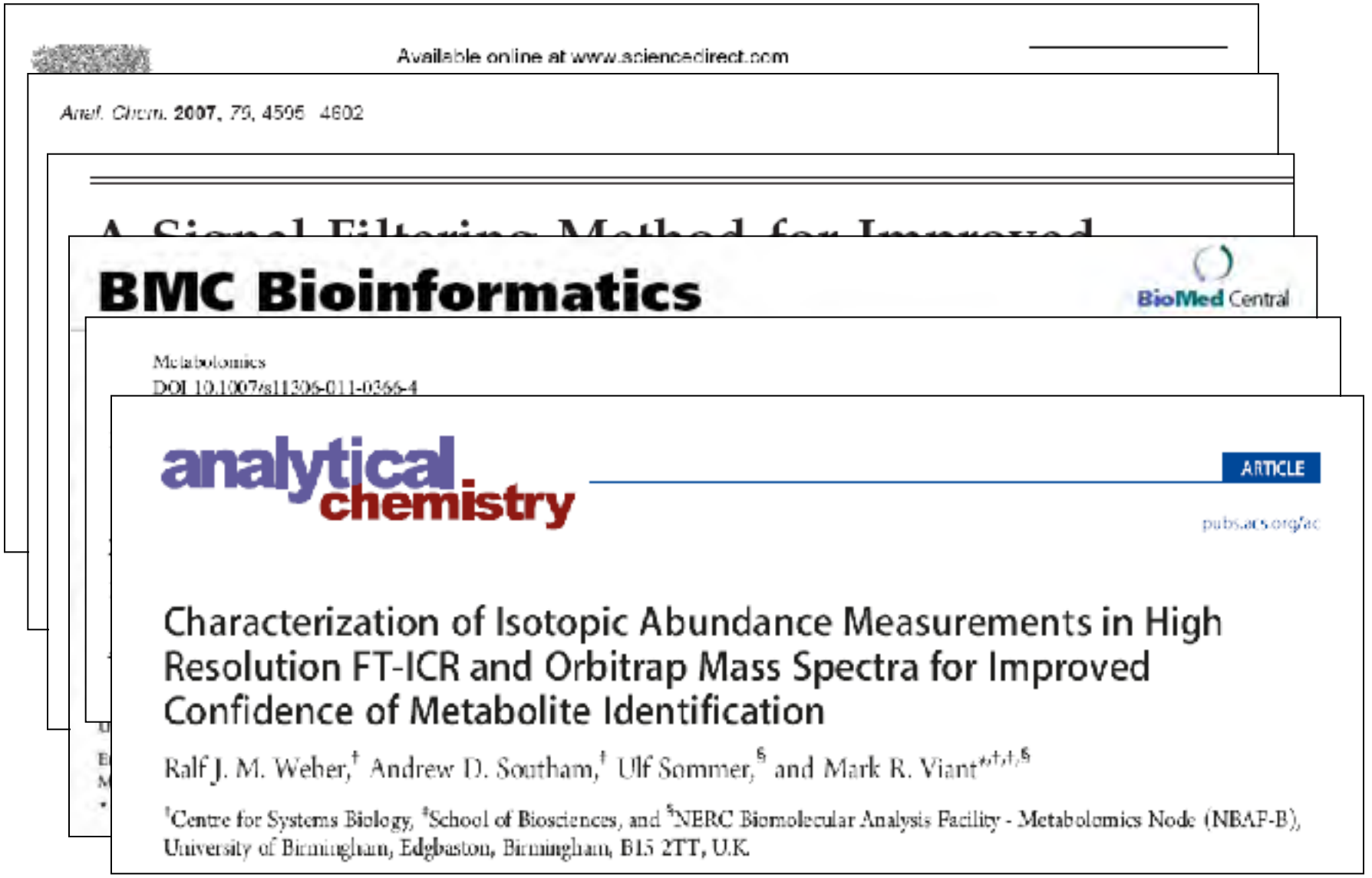


› 4000 signals in each spectrum

5.  Multi- and univariate statistical analyses

6. Metabolite identification (accurate mass, MS/MS)

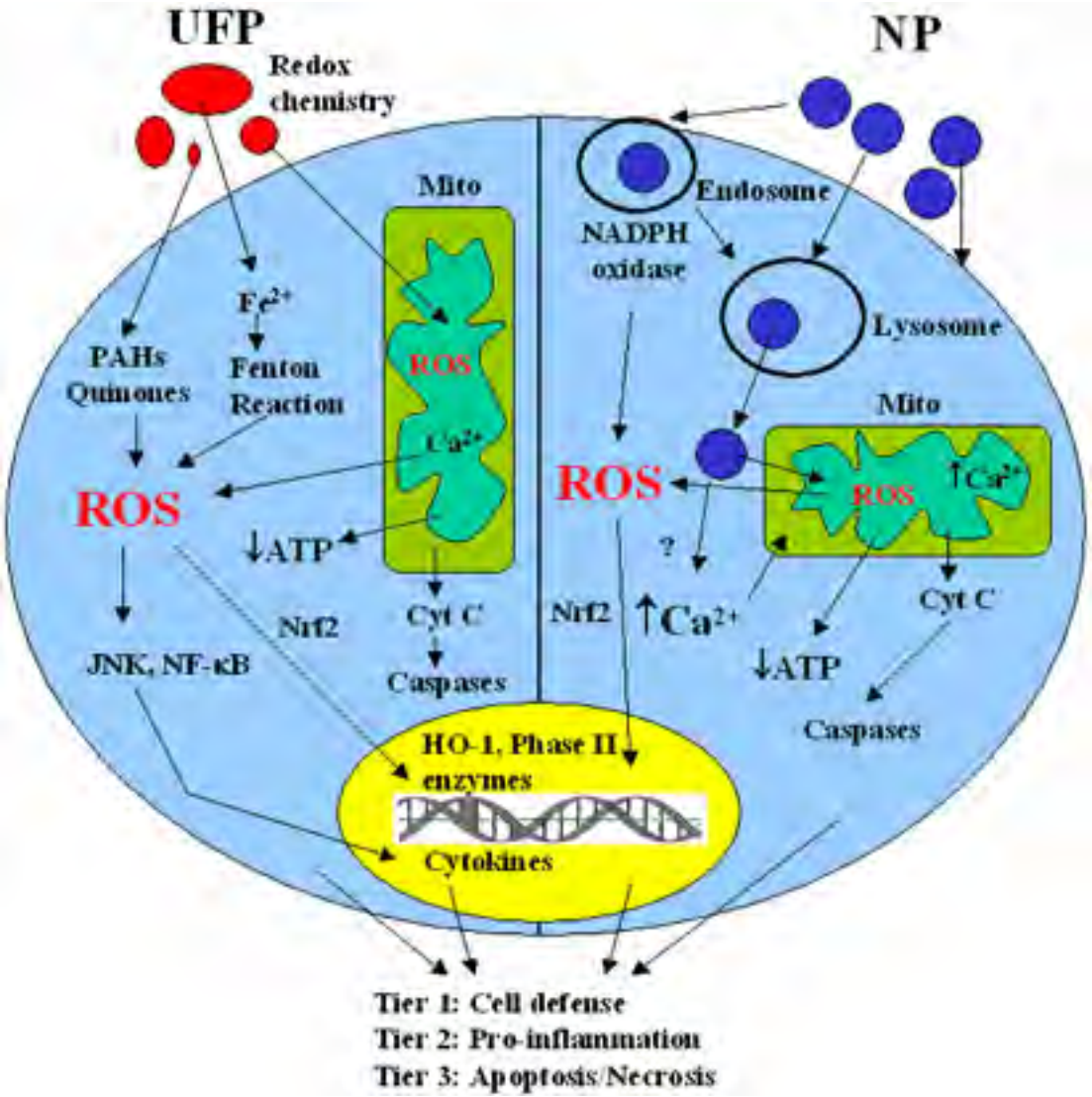
Direct infusion FT-ICR mass spectrometry



Case Study: Engineered nanomaterials

- Potentially transformative in medicine, electronics, computing, etc
- Multi-billion $ industry
- Production has increased dramatically over the past 5 yrs
- Concerns over environmental and human health risks associated with nanoparticle exposure → need for responsible development.

Toxicity of metal oxide nanomaterials believed to result from oxidative stress



Li et al 2008

Metabolic effects of ZnO nanoparticles in *Daphnia*

Why use metabolomics?

1. Investigate suspected mechanism of toxicity (oxidative stress)
2. Attempt to discover genuinely novel mechanisms of toxicity to feed into mechanism-based chemical risk assessment.

"There are known knowns; there are things we know we know.

We also know there are known unknowns; that is to say we know there are some things we do not know.

But there are also unknown unknowns – there are things we do not know we don't know."

United States Secretary of Defense Donald Rumsfeld

Experimental design

Daphnids

ZnO NPs

0.03, 0.1, 0.3, 1.0 ppm

Zn^{2+} control

bulk ZnO control

untreated control

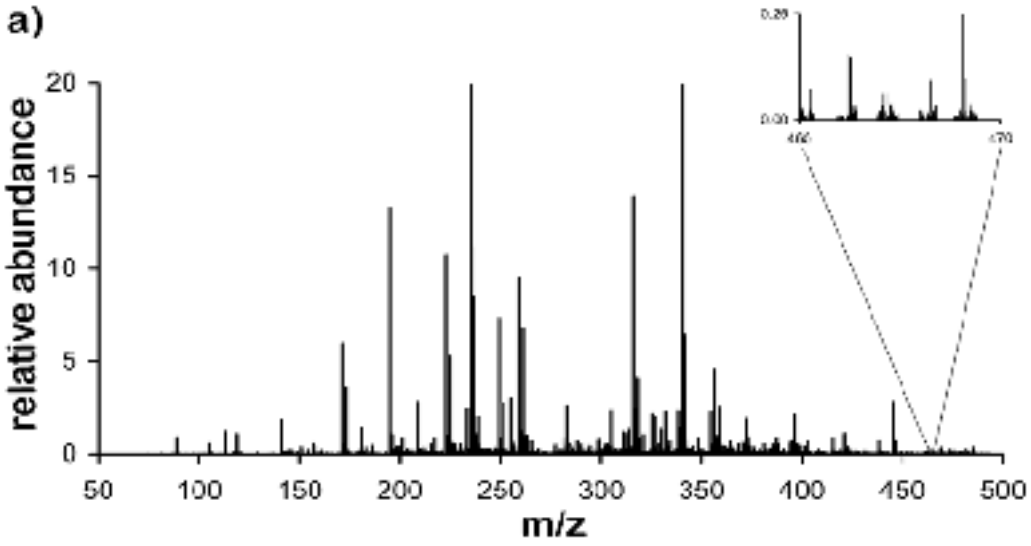
Whole organism extracts

FT-ICR mass spectrometry

Data mining

Nanoparticle characterisation & uptake

FT-ICR mass spectral data



Each spectrum: > 4000 signals from low molecular weight metabolites

Entire dataset: 80 spectra (n=10 replicates from each of 8 groups)

Aim: data mine these > 320,000 signals to investigate metabolic responses to ZnO nanoparticles, dissolved Zn^{2+}, bulk ZnO, untreated controls

Some data removed until after publication

What are function(s) of these sulfated lipids?

Function 1: potentially anionic gut surfactants to induce micelle formation and enhance the solubilisation of food

1. Amphiphilic structures are consistent with anionic surfactants

lipophilic tail

polar head group

2. Structural similarity to known alkyl sulfates discovered in the digestive juices of the snail *H. pomatia*

Collatz et al., J. Comp. Physiol. 1975, 96:123-9.

What are function(s) of these sulfated lipids?

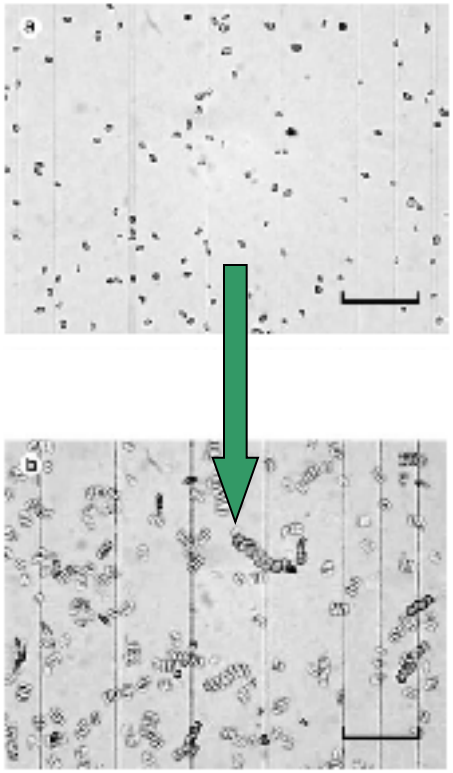
Function 2: known kairomone chemical messenger in *Daphnia*

Yasumoto et al., Tetrahedron Letters 2005, 46:4765-7.

Kairomone: a chemical emitted by an organism that benefits an individual of another species, without benefiting the emitter

No benefit, likely a loss

Chemical signal (kairomone)



Unicellular algae senses kairomone…

…changes morphology to protect itself

"There are known knowns; there are things we know we know.

We also know there are known unknowns; that is to say we know there are some things we do not know.

But there are also unknown unknowns – there are things we do not know we don't know."

United States Secretary of Defense Donald Rumsfeld

Take home message
--- the "omics" technology ---

Metabolomics & Biomarker Discovery

- Non targeted DIMS to discover the needle in the haystack

Specialised analytical & computational lab

Translate biomarkers

Environmental Chemistry

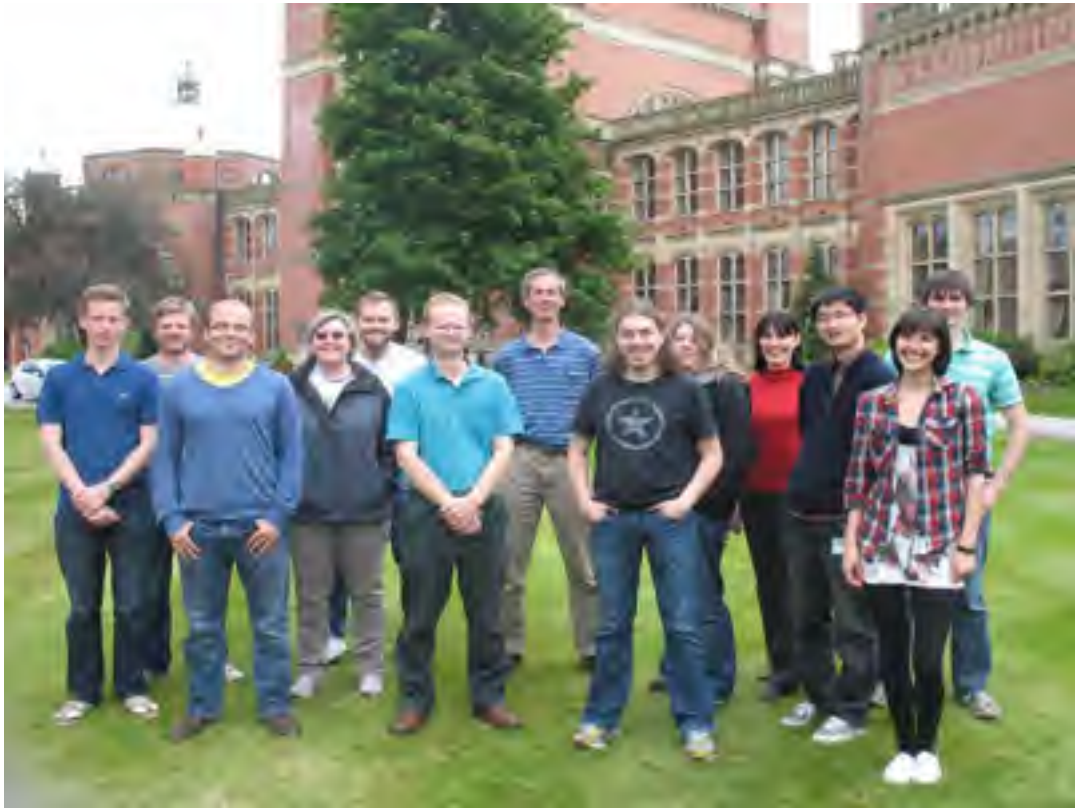
- High throughput, targeted LC-MS/MS analysis of sulfated lipids in water column

Could be conducted in regulatory lab

Environment Agency

defra

Environmental Metabolomics Lab at Birmingham



Daphnia NP study
Dr Nadine Taylor
Dr Ulf Sommer
Dr Ruth Merrifield
Dr Kay van Damme
Prof Charles Tyler (Exeter Univ.)
Dr Julia Fabrega (Exeter Univ.)
Prof Tamara Galloway (Exeter Univ.)
Dr Ratna Tantra (NPL)

http://www.biosciences-labs.bham.ac.uk/viant

NATURAL ENVIRONMENT RESEARCH COUNCIL

BBSRC bioscience for the future

Cefas

Environment Agency