Environmental Forensics: What is it and what can it do?

John P. Giesy, *Ph.D., FRSC, FSETAC*

Global Water Futures Science Meeting
Hamilton Ontario
June 5, 2018

GLOBAL WATER FUTURES

GWF Project Title:
Next generation solutions to ensure healthy water resources for future generations

John P. Giesy (PI), Canada Research Chair in Environmental Toxicology, Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan
Mark R. Servos (Co-I), Canada Research Chair in Water Quality Protection, Department of Biology, University of Waterloo
Paul D. Jones (Co-I), Northern Ecosystem Toxicology Initiative Chair, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan
Markus Hecker (Co-I), Canada Research Chair in Predictive Aquatic Toxicology, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, **Timothy Jardine** (Co-I), Assistant Professor, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan
Bram Noble (Co-I), Professor, School of Environment and Sustainability, University of Saskatchewan
Paul Craig (Co-I), Assistant Professor, Department of Biology, University of Waterloo,
Barb Katzenback (Co-I), Assistant Professor, Department of Biology, University of Waterloo,
Prof. Xiaowei Zhang, State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing, China
Vince Palace, IISD-Experimental Lakes Area (ELA),

Solutions to Issues for Chemicals

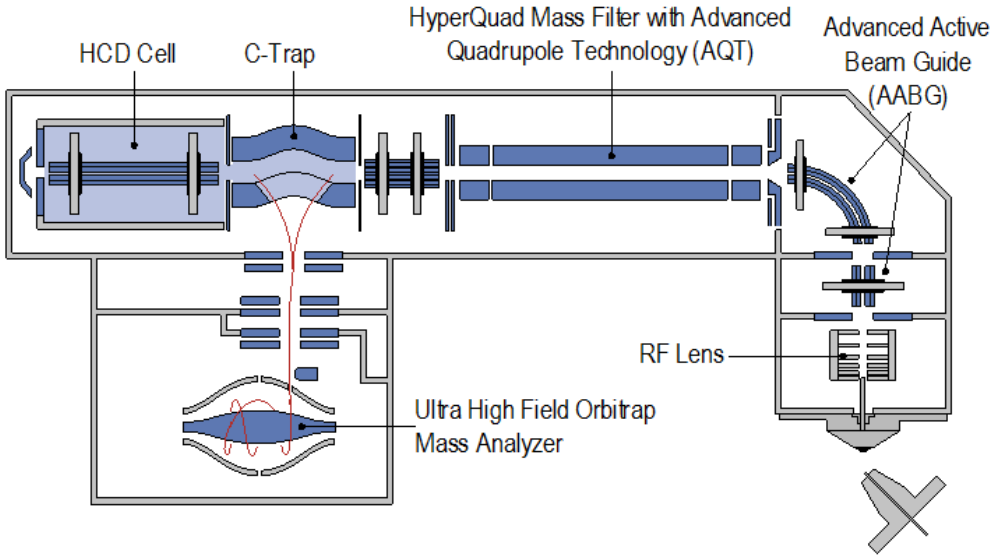
- Use of Untargeted Identification of Chemicals by use of Ultra-high Resolution Mass spectrometry
- Use of Bioassays
- Use of Effect-Directed Fractionation and Identification
- Use of Pull-down Assays
  - Directed for Known Protein Targets
  - Undirected for Unknown Protein Targets
- Use of Mass and Potency Balances
- Use of eDNA to monitor for status and trends in environments

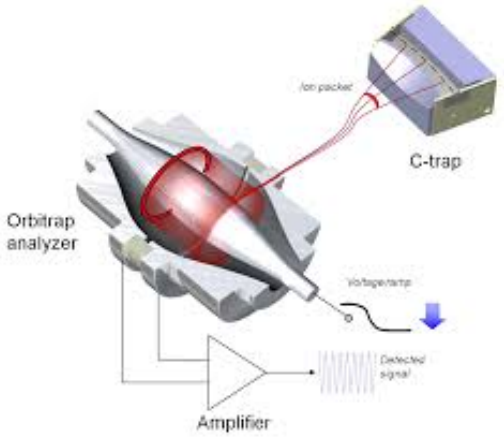
Instruments Available

- Ultra-high Resolution (Orbitrap)Mass Spectrometers
  - 2 interfaced with Liquid Chromatograph (water soluble (Thermo Scientific *Q Exactive* w/ nanoLC)
  - 1 Thermo Scientific Q *Exactive* interfaced with gas chromatograph (neutral volatile and semi volatile)
  - Accurate mass determination to 1 < ppm

- Targeted analyses of small molecules (<400 AMU)
- Identification of novel chemicals (natural and synthetic)
- Untargeted analyses of small molecules
- Metabolomics
- Proteomics
- Lipidomics
- Environmental fingerprinting
- Headspace analyses (volatile compounds)
- Microfibre automated, solid phase analyses

Q-Exactive (Orbitrap) instrument





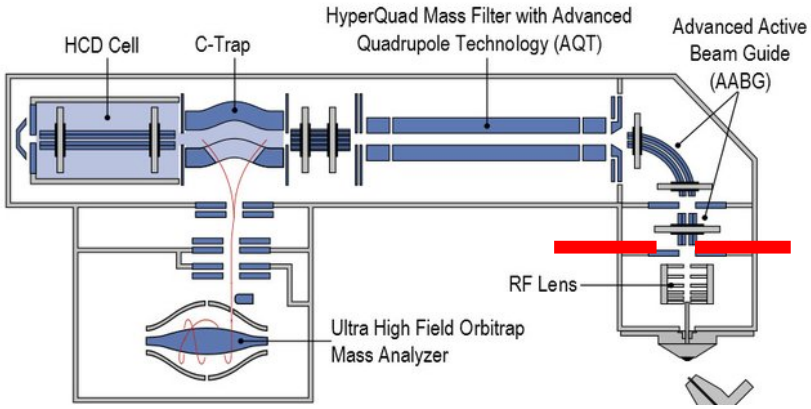
- Ultra-high resolution (<2 ppm)
- Quadrupole can be used to isolate precursor ions
- Collect high resolution MS2 spectra
- Sensitive
- Operated in negative or positive ion modes
- Chemical or electrical ionization

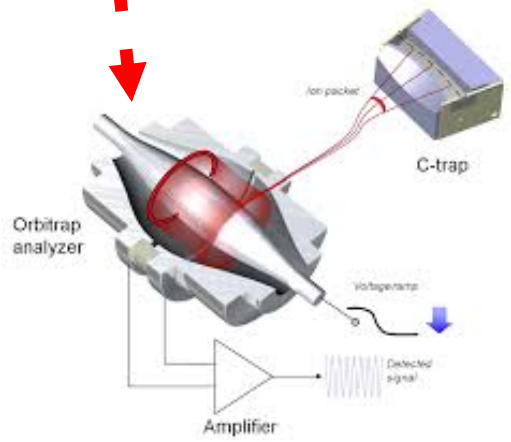
All these characteristics are important for performance

GC Interface

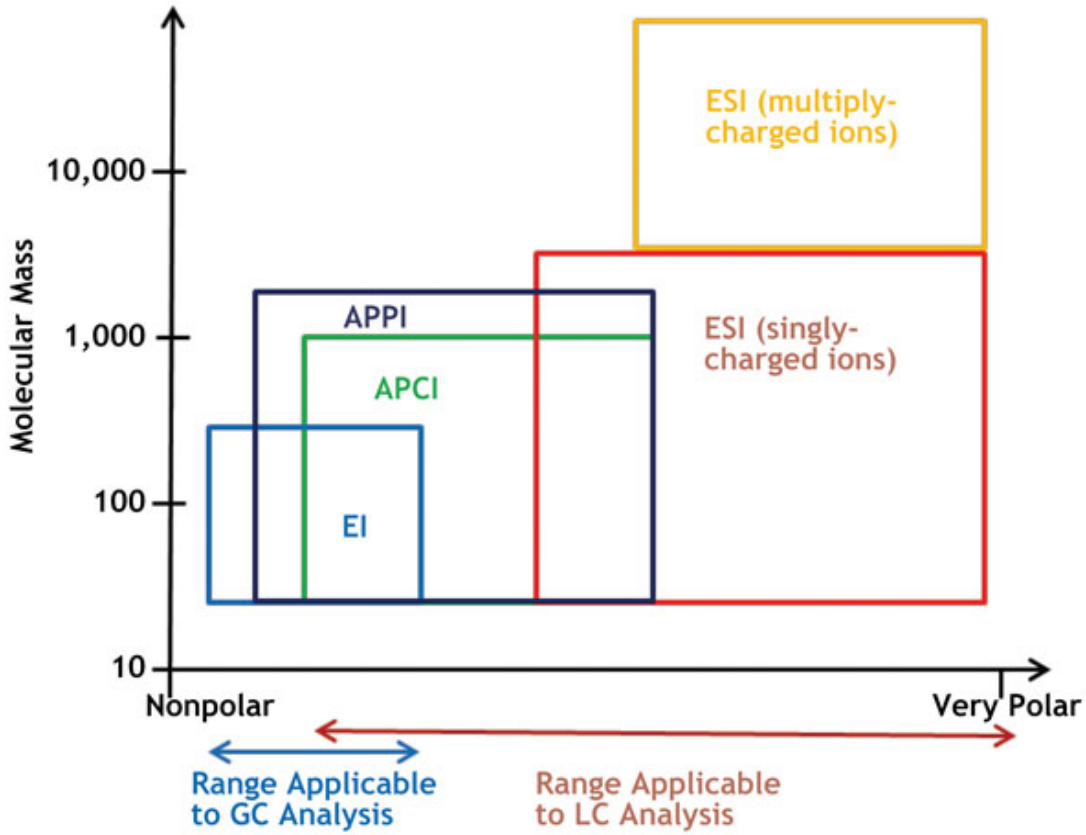
LC Interface

Q Exactive HF Orbitrap)



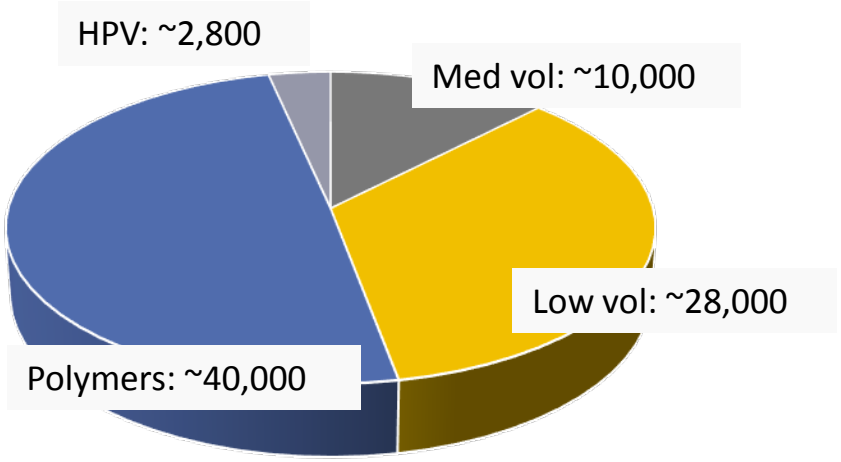


- Resolution 15,000 – 240,000
- Scan rate 1.8 – 25 scans/sec
- High sensitivity (6 fg OFN)
- Quad to isolate precursor ions
- High resolution MS2 spectra (masspec-masspec)
  Fragments for identification
- Positive and negative ionization modes
- Multiple acquisition modes simultaneously
- Common componentry post source
- Common software platform

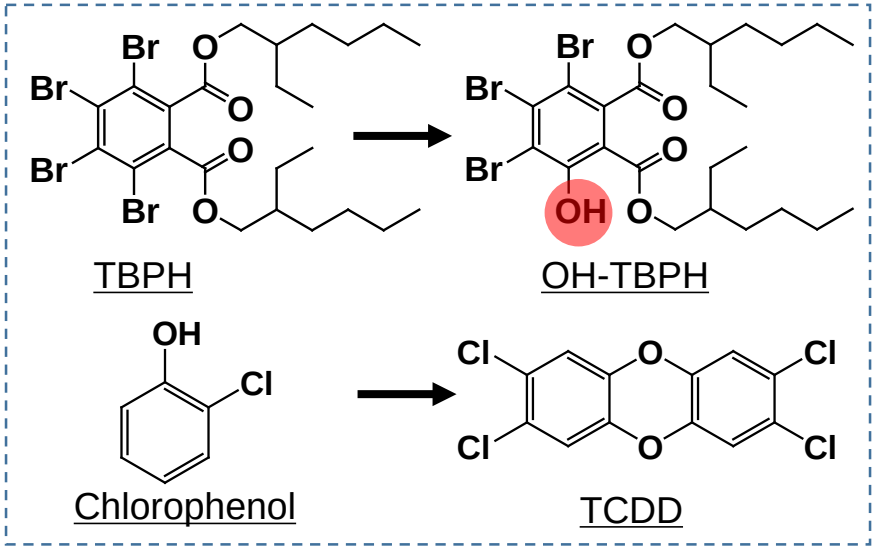

Molecular Mass
10,000
1,000
100
10
ESI (multiply-charged ions)
APPI
ESI (singly-charged ions)
APCI
EI
Nonpolar
Very Polar
Range Applicable to GC Analysis
Range Applicable to LC Analysis

Bottleneck of targeted chemical analysis

most chemicals remain unknown in environmental mixtures



Chemicals in use



Byproducts

Environmental samples are complicated mixtures, the compounds we monitor are less than 0.01% of total number of synthetic compounds

Peng et al., ES&T, 2015, 49, 2999-3006
Muir et al., ES&T, 2006, 40, 7157-7166

DIPIC-Frag* method: untargeted screening of Compounds: Example Brominated Compounds

*Data independent precursor isolation and characteristic fragment

Environmental Science & Technology
2015 50:321-330
Article
pubs.acs.org/est

Untargeted Screening and Distribution of Organo-Bromine Compounds in Sediments of Lake Michigan

Hui Peng,[*,†] Chunli Chen,[†,‡] Jenna Cantin,[†] David M. V. Saunders,[†] Jianxian Sun,[†] Song Tang,[§] Garry Codling,[†] Markus Hecker,[†,§] Steve Wiseman,[†] Paul D. Jones,[†,§] An Li,[○] Karl J. Rockne,[◆] Neil C. Sturchio,[¶] and John. P. Giesy[*,†,∥,⊥,#,∇]

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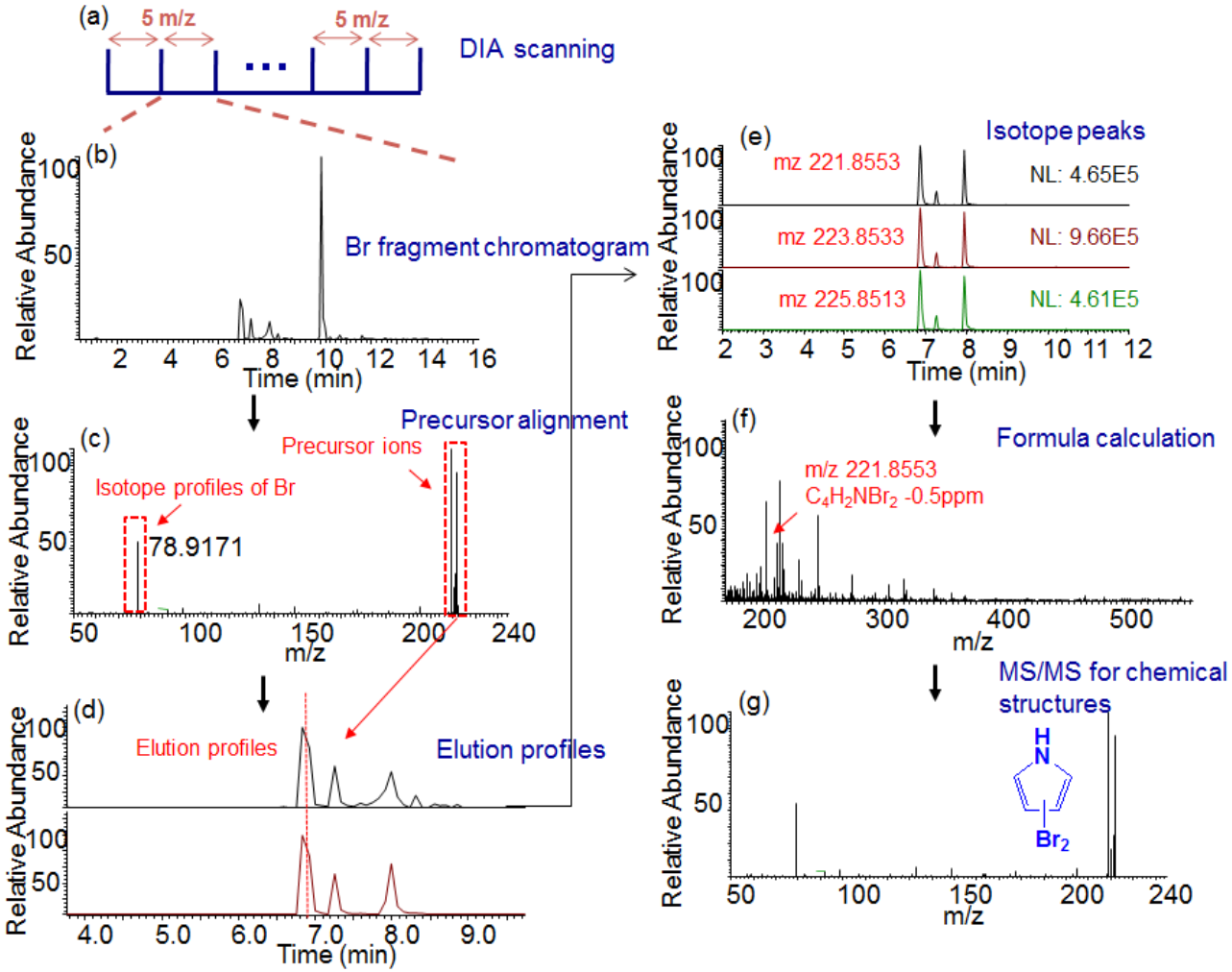
Environmental Science & Technology
2016 50:10097-10105
Article
pubs.acs.org/est

Untargeted Screening and Distribution of Organo-Iodine Compounds in Sediments from Lake Michigan and the Arctic Ocean

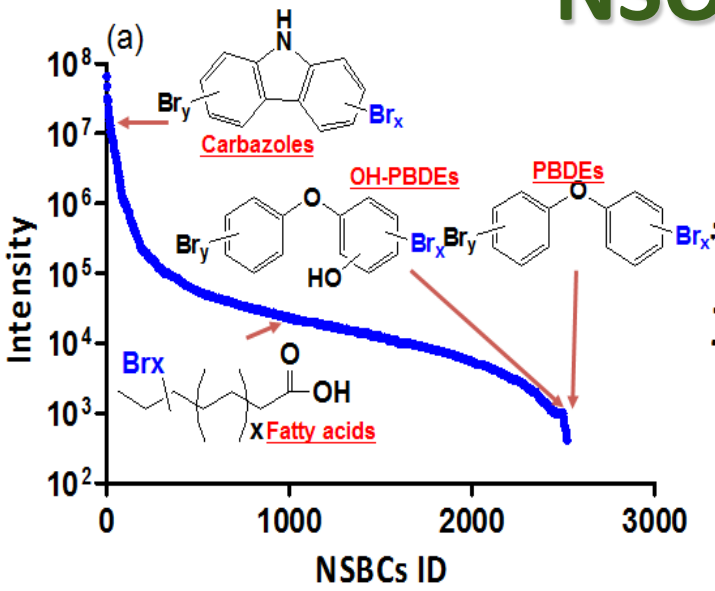
Hui Peng,[*,†] Chunli Chen,[†,‡] Jenna Cantin,[†] David M. V. Saunders,[†] Jianxian Sun,[†] Song Tang,[§] Garry Codling,[†] Markus Hecker,[†,§] Steve Wiseman,[†] Paul D. Jones,[†,§] An Li,[∇] Karl J. Rockne,[○] Neil C. Sturchio,[◆] Minghong Cai,[*,¶] and John P. Giesy[*,†,∥,⊥,#]

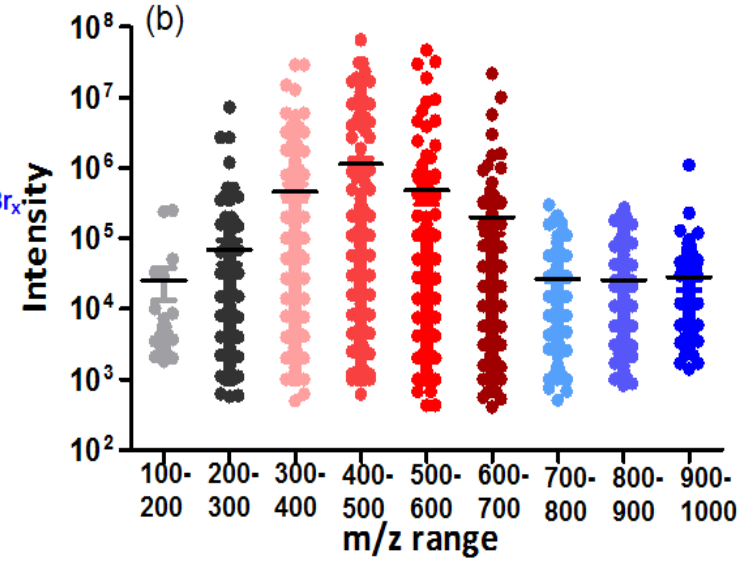
Workflow for DIPIC-Frag

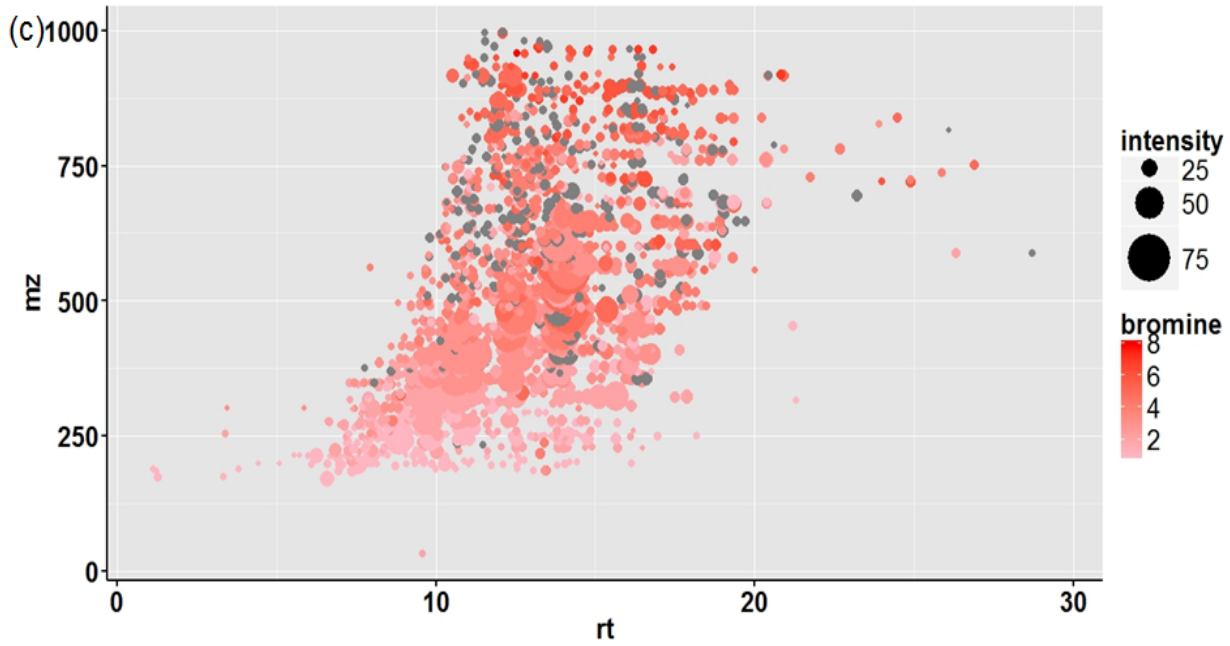


- Data independent precursor isolation and characteristic fragment (DIPIC-Frag) method: APCI to increase compound coverage; Br fragment to increase specificity; DIA windows to expand dynamic range

NSOBC distribution

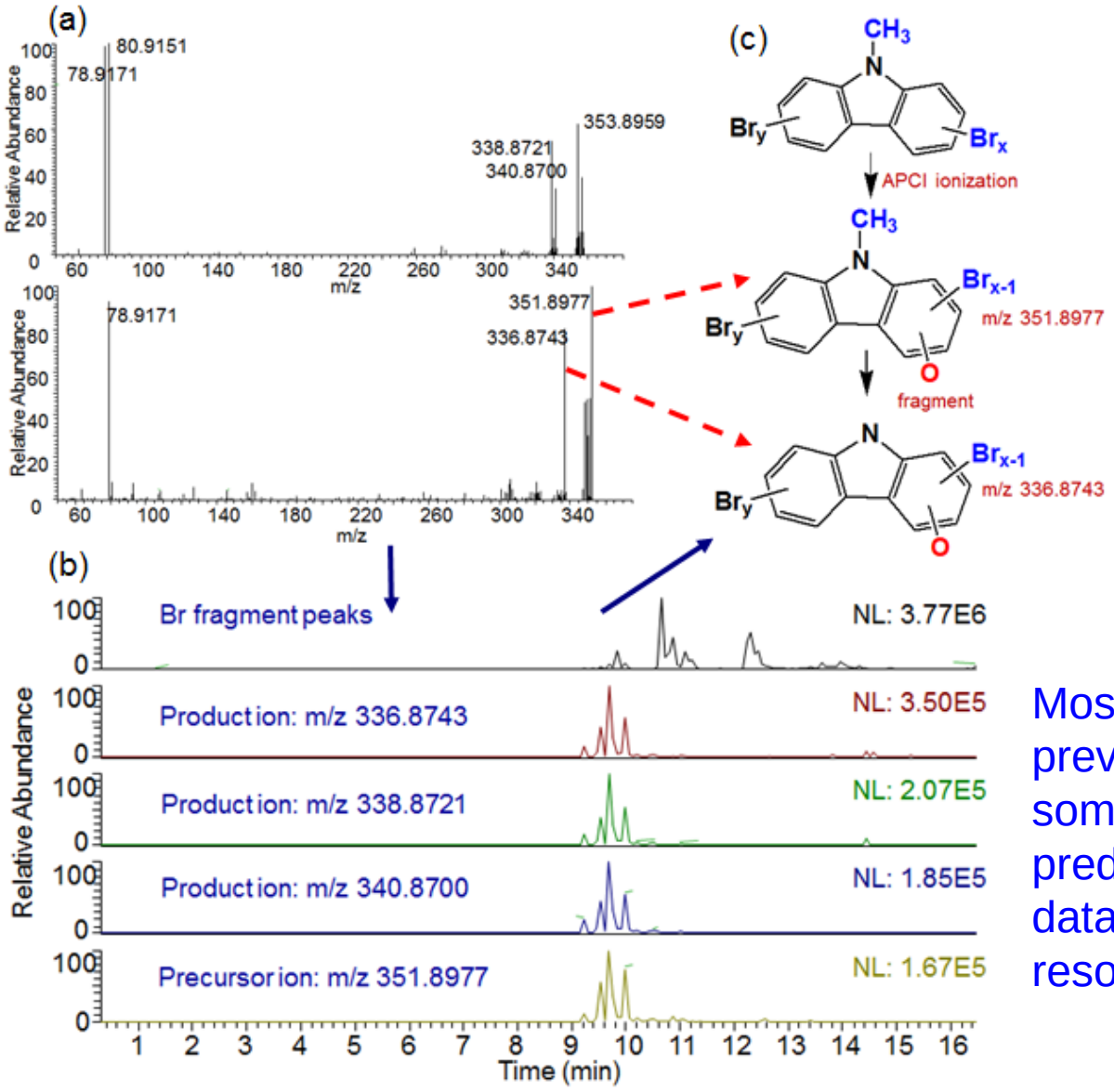






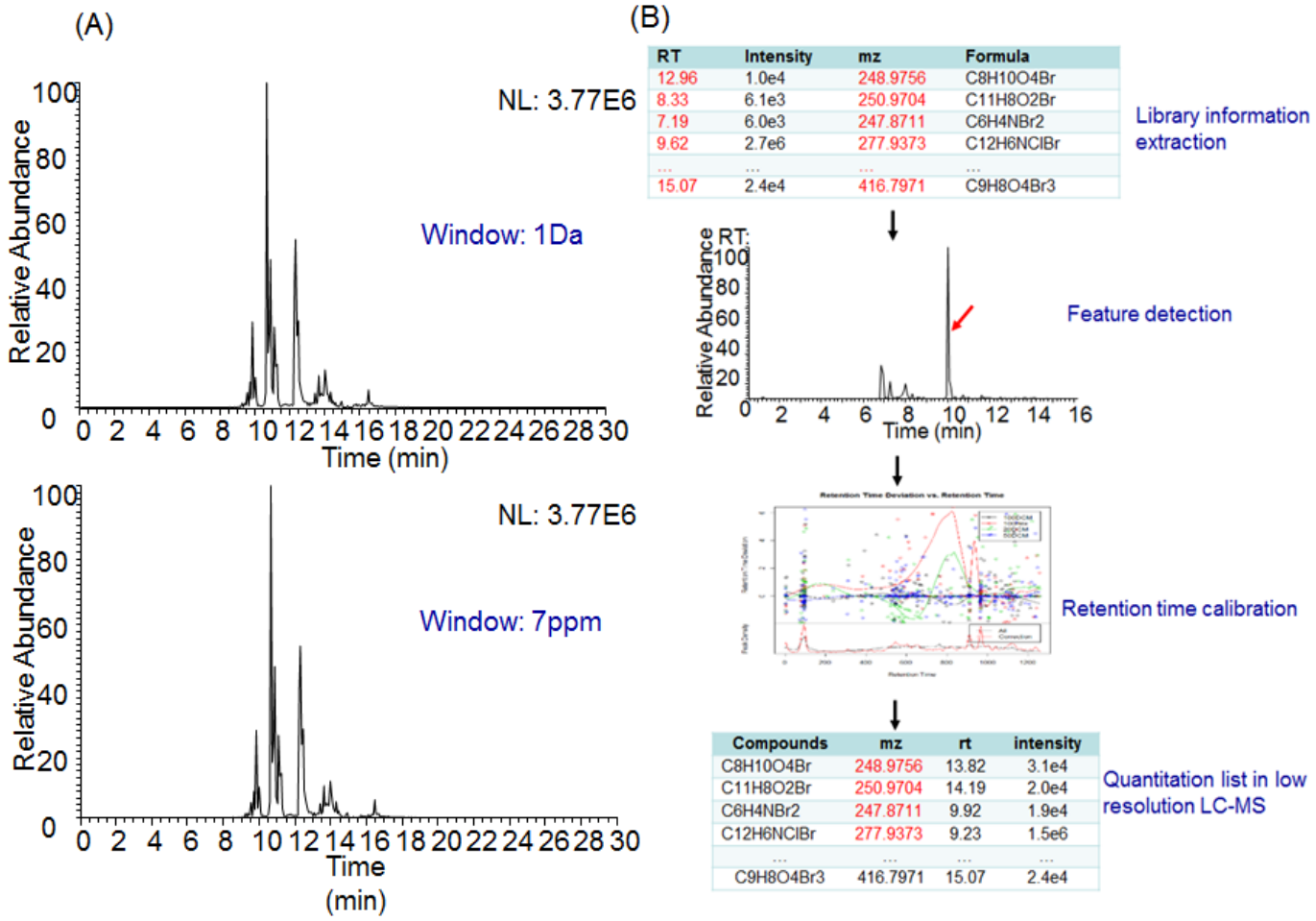
2,520 peaks were detected, precursor ions were identified for 2,163 peaks (86%), formulae were calculated for 2072 peaks (82%), which were corresponding to 1,593 unique NSOBCs compounds

Structure prediction

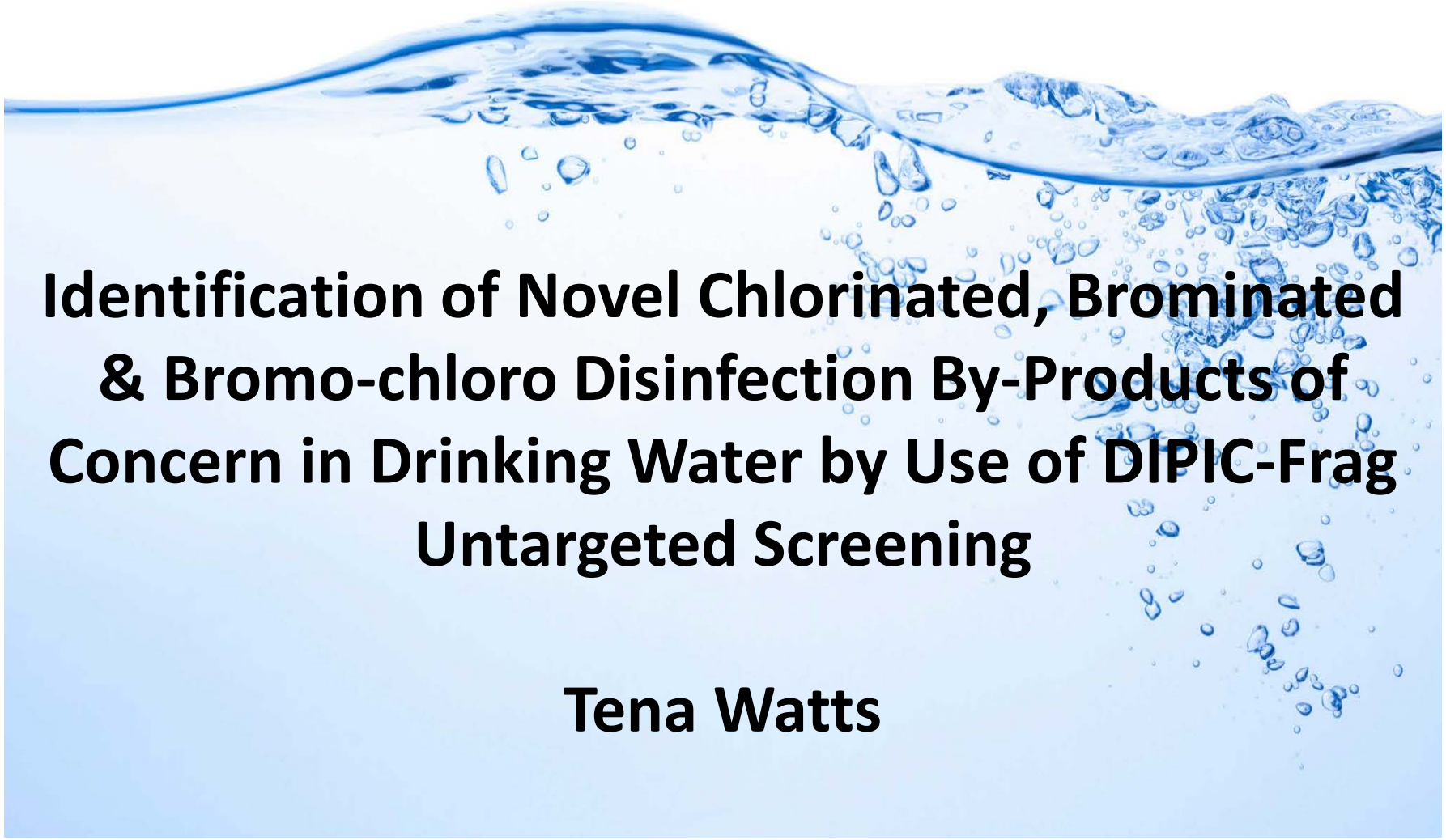


Most compounds have never been previously reported, structures of some novel compounds could be predicted by combining public database search and high-resolution MS^2 spectra

Mass spectrometry library



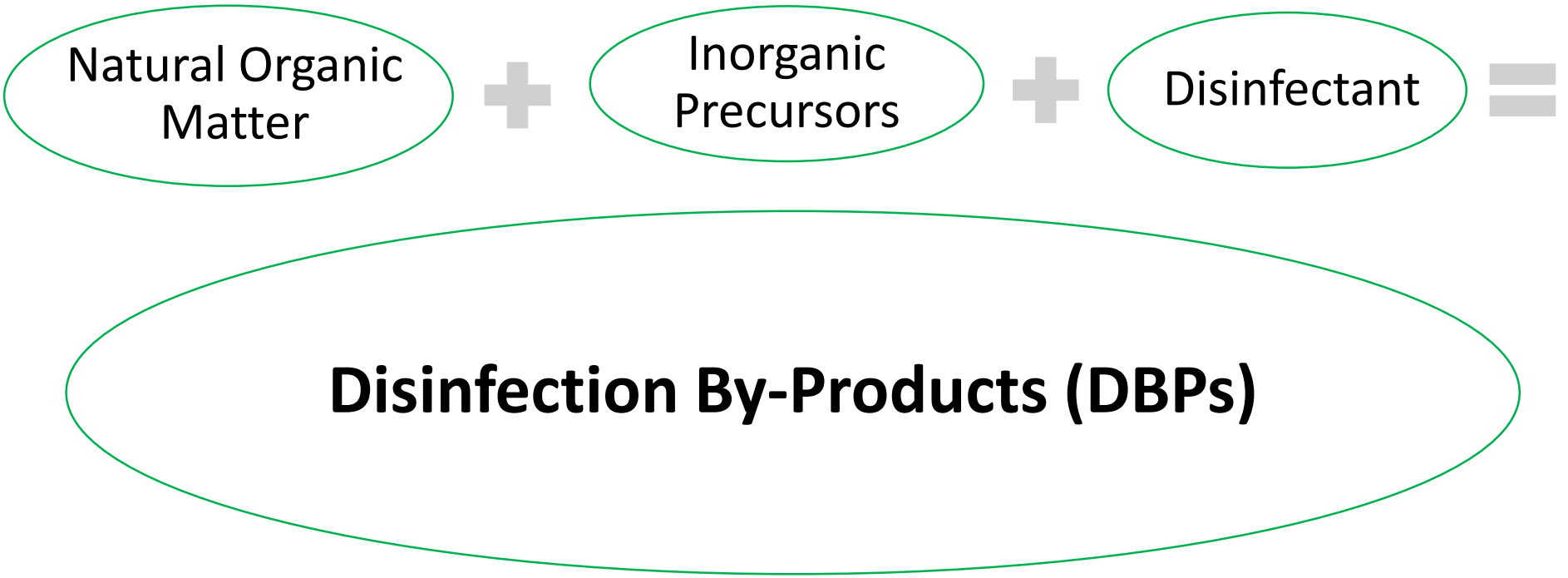
The library established in the present study could be easily adopted by low-resolution mass spectrometry such as LC-triple quadrupole instrument



Identification of Novel Chlorinated, Brominated & Bromo-chloro Disinfection By-Products of Concern in Drinking Water by Use of DIPIC-Frag Untargeted Screening

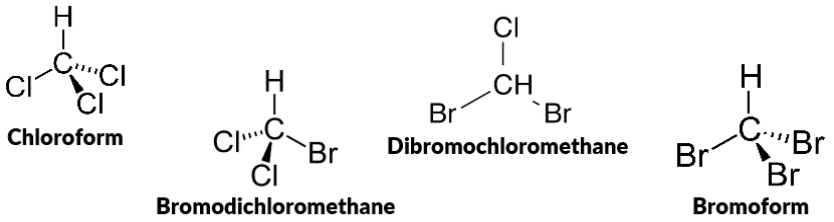
Tena Watts

Water disinfection: process of deactivating or removing pathogens from drinking water by use of physical or chemical technologies

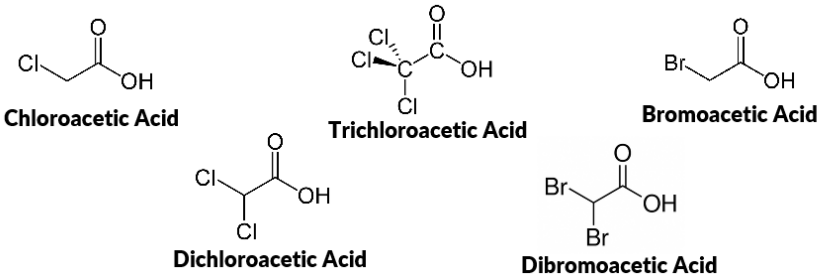


Disinfection By-Products (DBPs)

Trihalomethanes 0.1 mg/L



Haloacetic Acids 0.08 mg/L

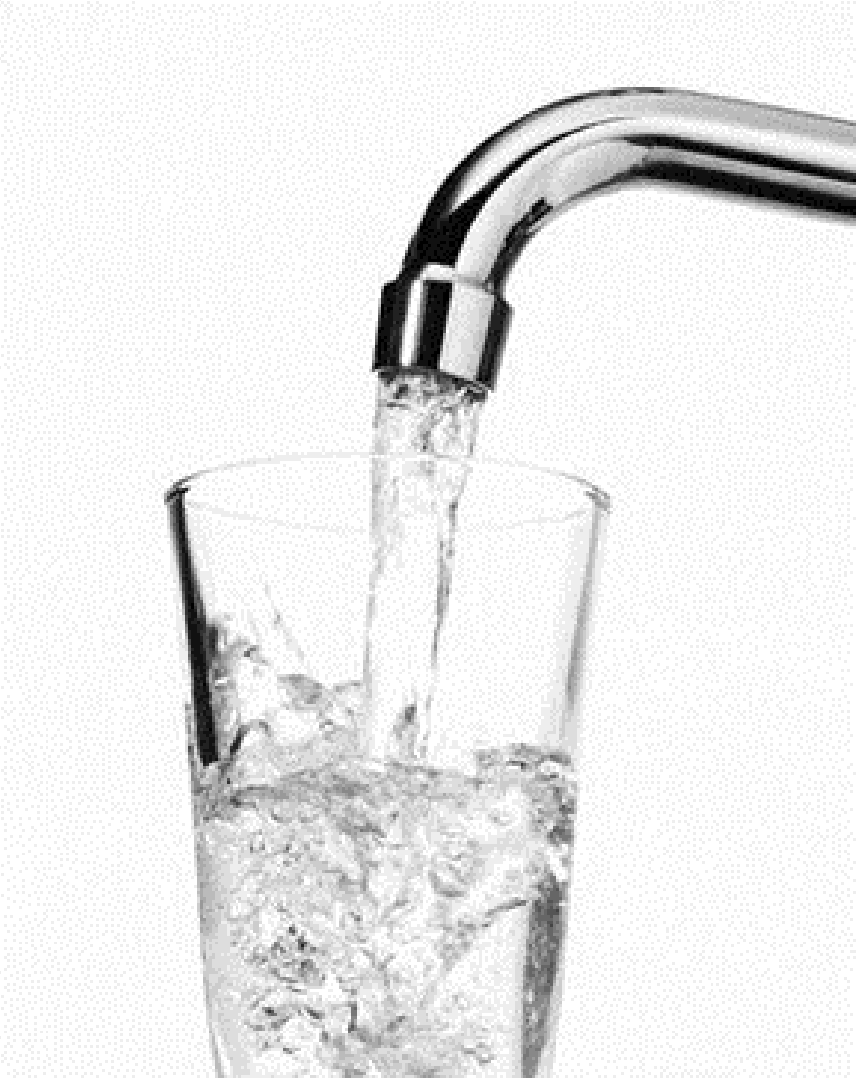


- Genotoxic, bladder cancer and adverse pregnancy outcomes (Jeong *et al*., 2012)
- > 600 compounds have been identified in drinking water
- Only 50% of total organic halide can be accounted for by known DBPs (Richardson *et al*., 2012)
- Many unregulated compounds have enhanced toxicities

Brominated > chlorinated analogues

Research Questions

- What brominated compounds are yet to be identified in drinking water and how can we screen for them?
  - DIPIC-Frag method
    - – Q Exactive UHRMS
    - Optimize conditions
    - Identify novel Br-DBPs
- Can we produce a semi-quantitative method that is reproducible for the analysis of real drinking water extracts?

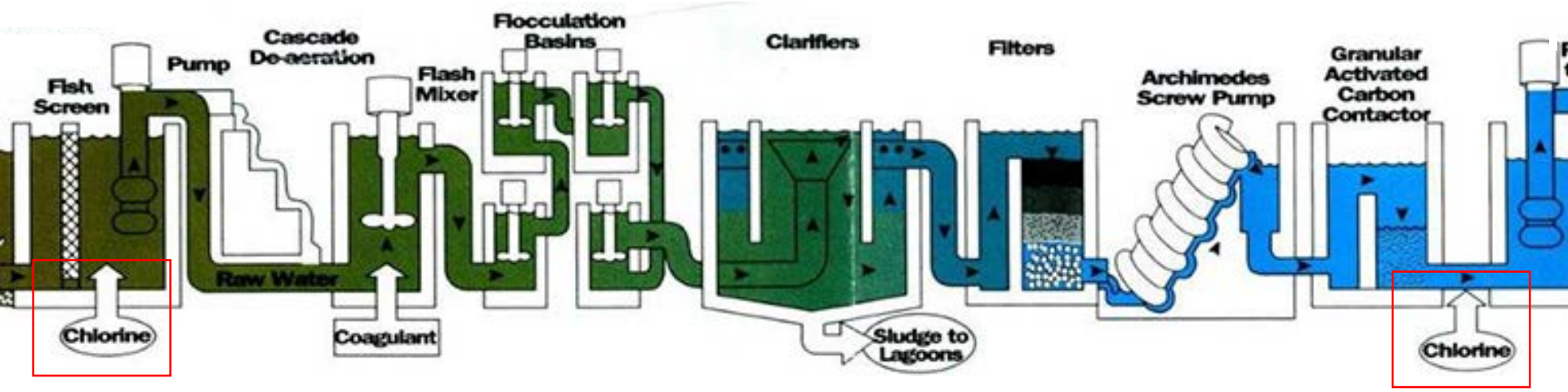


Example: Prairie Water Supplies

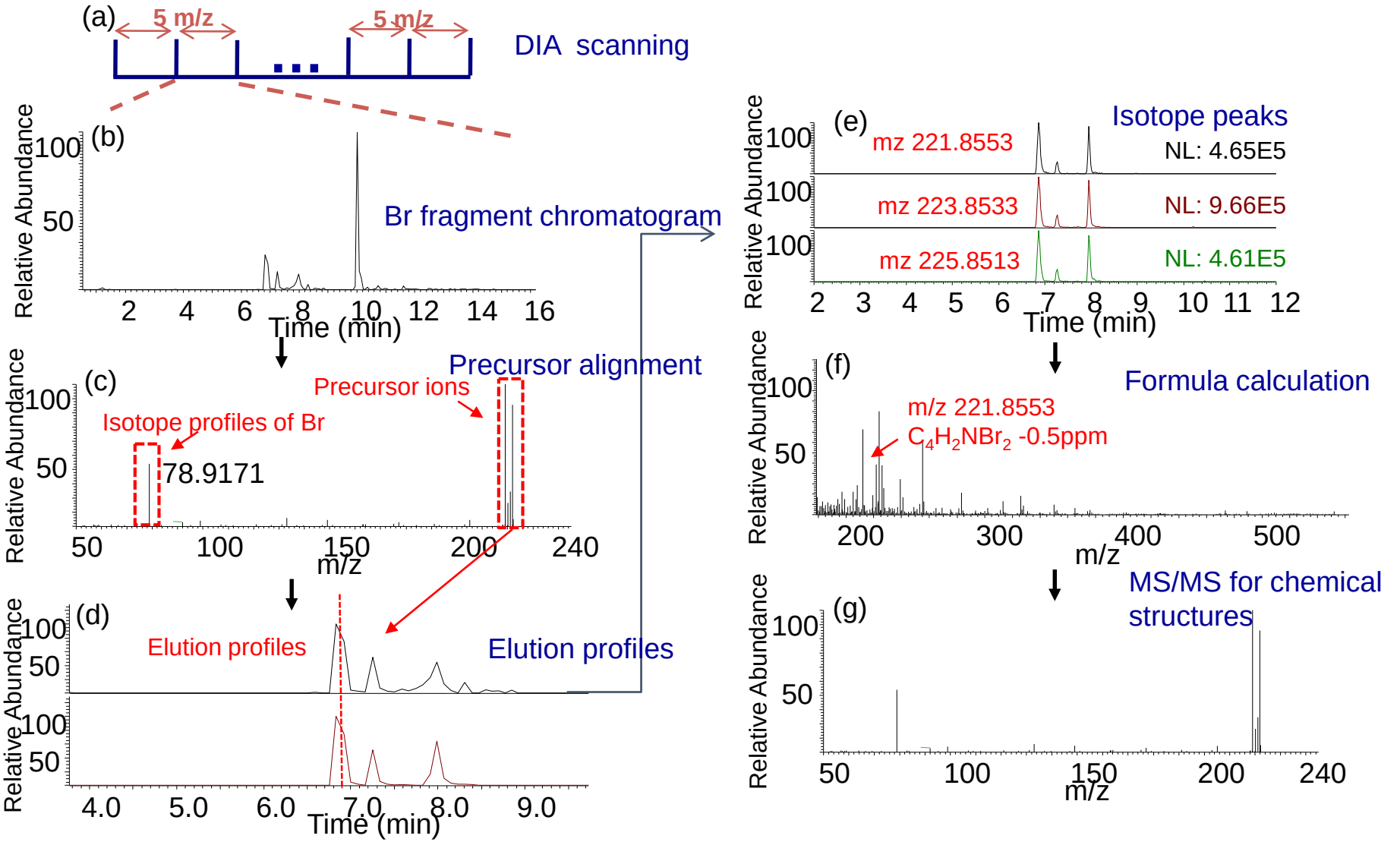
- Little groundwater and much surface water is saline
- South Saskatchewan River
  - Originates in Rocky Mts of Alberta
  - Flows through several metropolitan areas
  - Diefenbaker Reservoir
  - Water supply to 750,000 people
  - Eutrophication resulting in hazardous algal blooms
    - Source water for drinking poor
    - Requires pre-treatment-chlorination
    - Concentrations of chlorine high
    - Contact time is long- 1 km
    - Concentrations of Br- ion high
    - Chlorination forms hypobrous acid
    - Presence of naturally occurring organic acids (humic-fulvic) results in formation of halomethanes (carcinogens)

Buffalo Pound Water Treatment Plant (BPWTP)

- Located northeast of Moose Jaw, SK
- 250,000 customers (Regina and Moose Jaw)
- Water sourced from Buffalo Pound Lake, which is known to contain a high concentration of Br^- and it is quite eutrophic



Data Independent Precursor Isolation and Characteristic Fragment Method (DIPIC-Frag)



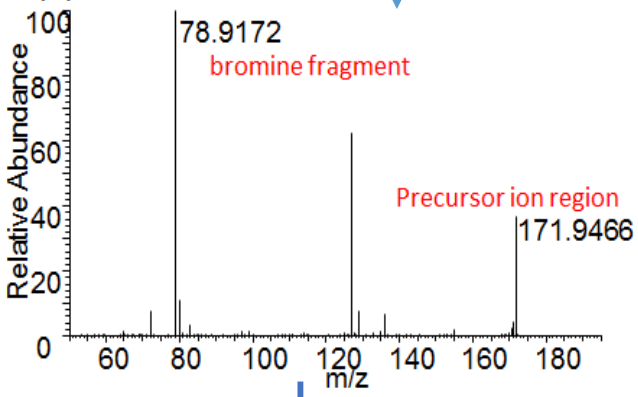
Data Independent Precursor Isolation and Characteristic Fragment Method (DIPIC-Frag)

A)

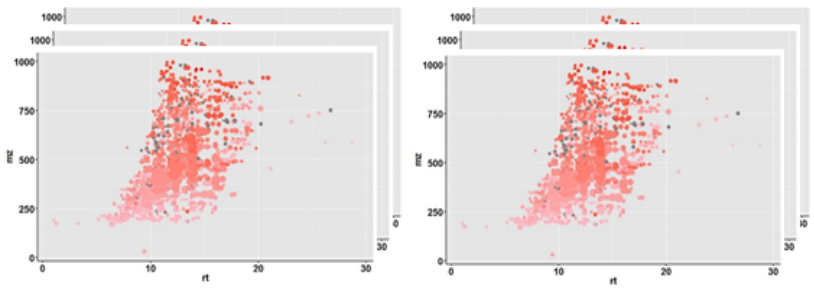
pH 7	HLB	C18	ESI(-)
pH 2	C18	Amide	APCI(-)
	WAX		

UHRMS

(B)



(C)



(D)

$$MSerror = \exp\{-0.5\times(\frac{MZ_{act}-MZ_{pred}}{\delta})^2\}$$

$$Isotope = 1-\sum|r_{act,i}-r_{pred,i}|$$

$$r_i = \frac{I_{M+i}}{I_M}$$

$$\text{Homologue} = \begin{cases} 0, no \\ 1, yes \end{cases}$$

(E)

Compounds	mz	rt	intensity
C2H2O2Br	136.9229	9.91	6.3e5
C4H4OBr	148.9427	6.01	5.6e3
CHO3SClBr	206.8517	3.40	6.1e5
C8H5NBr	193.9603	4.57	3.5e5
...	...	...	...
C5O3ClBr	221.8720	2.43	2.1e6

Predict the formula and compound database

$$MS1 = \exp\{-0.5 \times \left(\frac{mz_{real} - mz_{theoretical}}{\delta}\right)^2\}$$

$$HPLC = \exp\{-0.5 \times \left(\frac{RT_{act.} - RT_{lib.}}{\delta_{RT}}\right)^2\}$$

$$MS2 = \frac{(\sum wA_{act} wA_{lib})^2}{\sum wA_{act^2} \sum wA_{lib^2}}$$

$$w = 1/(1 + \frac{A}{\sum A - 0.5})$$

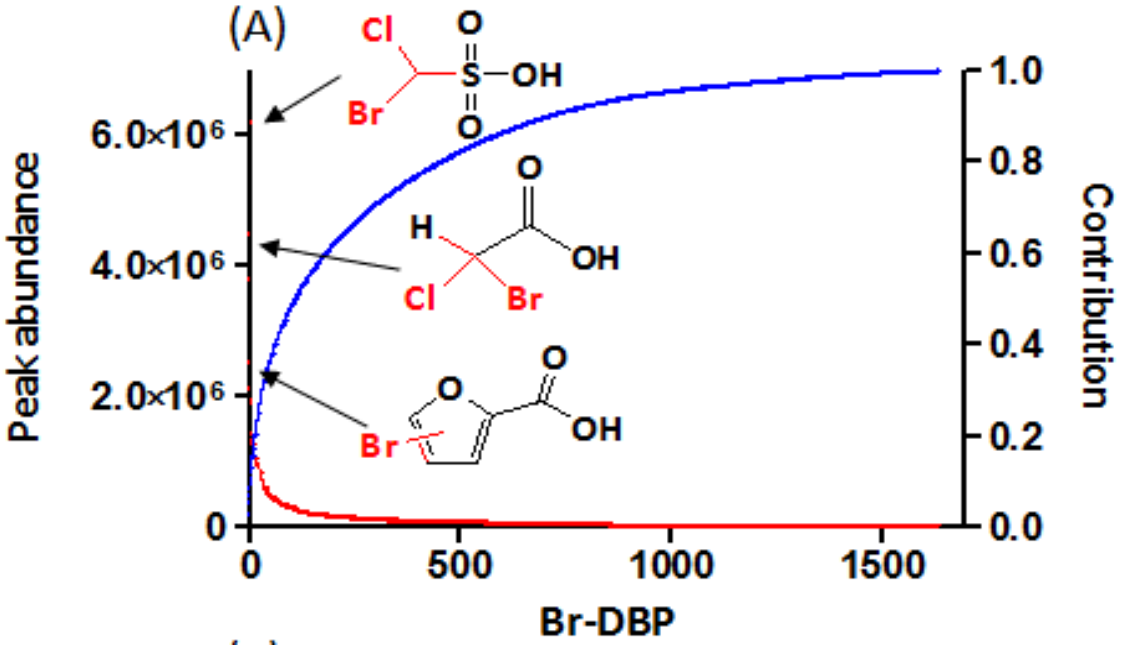
$$\boldsymbol{Score = \frac{Similarity_{HPLC} + Similarity_{MS1} + Similarity_{MS2}}{3}}$$

- **Accurately predict compound formula by combining MS1, isotopic peaks, MS2 fragment, and homologue information.**

Results

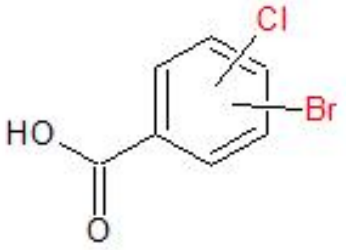
- Halo-acetic acids (HAAs) found to be among the most abundant Br-DBPs, but some novel Br-DBPs were also detected with similar or even greater abundances

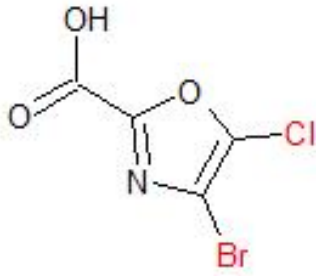
- The top 50 Br-DBPs contributed to 35.6% of total abundance (mass of OBrs

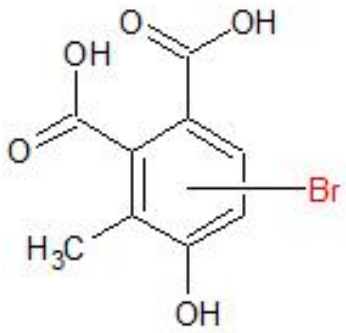


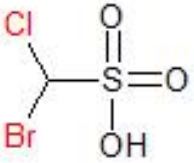
Results

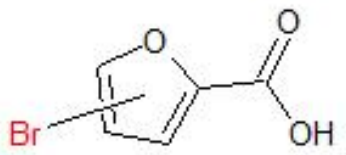
- Predicted structures for 41/50 most abundant Br-DBPs
- Of these 41 Br-DBPs, 18 were found to be aromatic acids or phenols
- 7 high-abundance heteroatomic Br-DBPs containing nitrogen or sulfur were detected



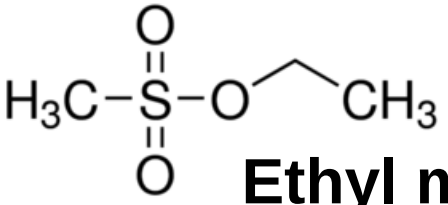




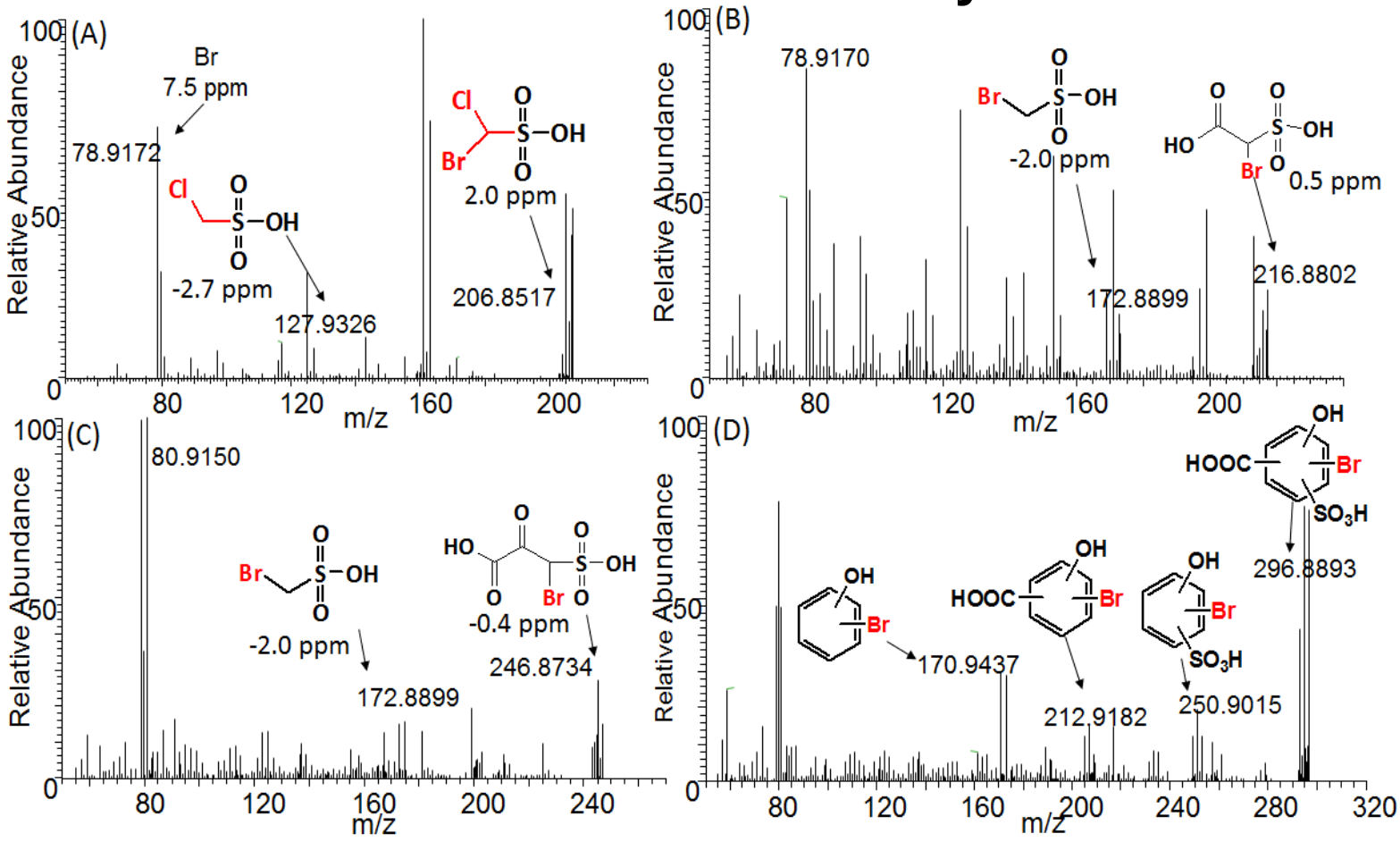




Sulfonic Acids



Ethyl methanesulfonate



Conclusions

1) Established a library of ~700 Br-DBPs; most of these Br-DBPs have not been previously reported in drinking water

2) The method showed good precision on actual drinking water samples, by use of HLB-pH 2

3) Novel heteroatomic DBPs showed unexpectedly high abundance

Environmental Science & Technology
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Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process-Affected Water

Garrett D. Morandi,[†] Steve B. Wiseman,[†] Alberto Pereira,[‡] Rishikesh Mankidy,[†] Ian G. M. Gault,[‡] Jonathan W. Martin,[*,‡] and John P. Giesy[*,†,§,∥,⊥,#,∇,○]

[†]Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

2015 49:12395−12404.

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Effect of Lipid Partitioning on Predictions of Acute Toxicity of Oil Sands Process Affected Water to Embryos of Fathead Minnow (*Pimephales promelas*)

Garrett D. Morandi,[†] Kun Zhang,[‡] Steve B. Wiseman,[†] Alberto dos Santos Pereira,[‡] Jonathan W. Martin,[‡] and John P. Giesy[*,†,§,∥,⊥,#]

Envir. Sci. Technol. 50:8858-8866.

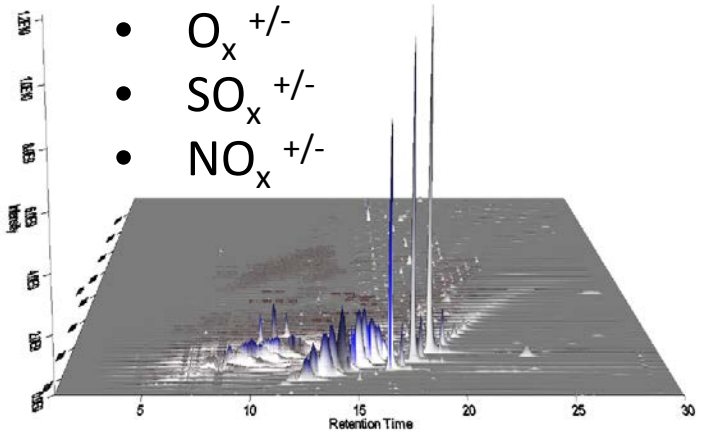
Real environmental samples: A Complex Mixture

Three basic questions:

- What are chemical components?
- What is potential toxicity?
- **What are causative chemicals?**

Chemicals

- O_x $^{+/-}$
- SO_x $^{+/-}$
- NO_x $^{+/-}$

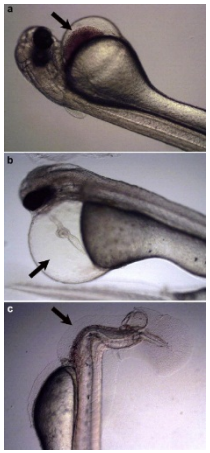


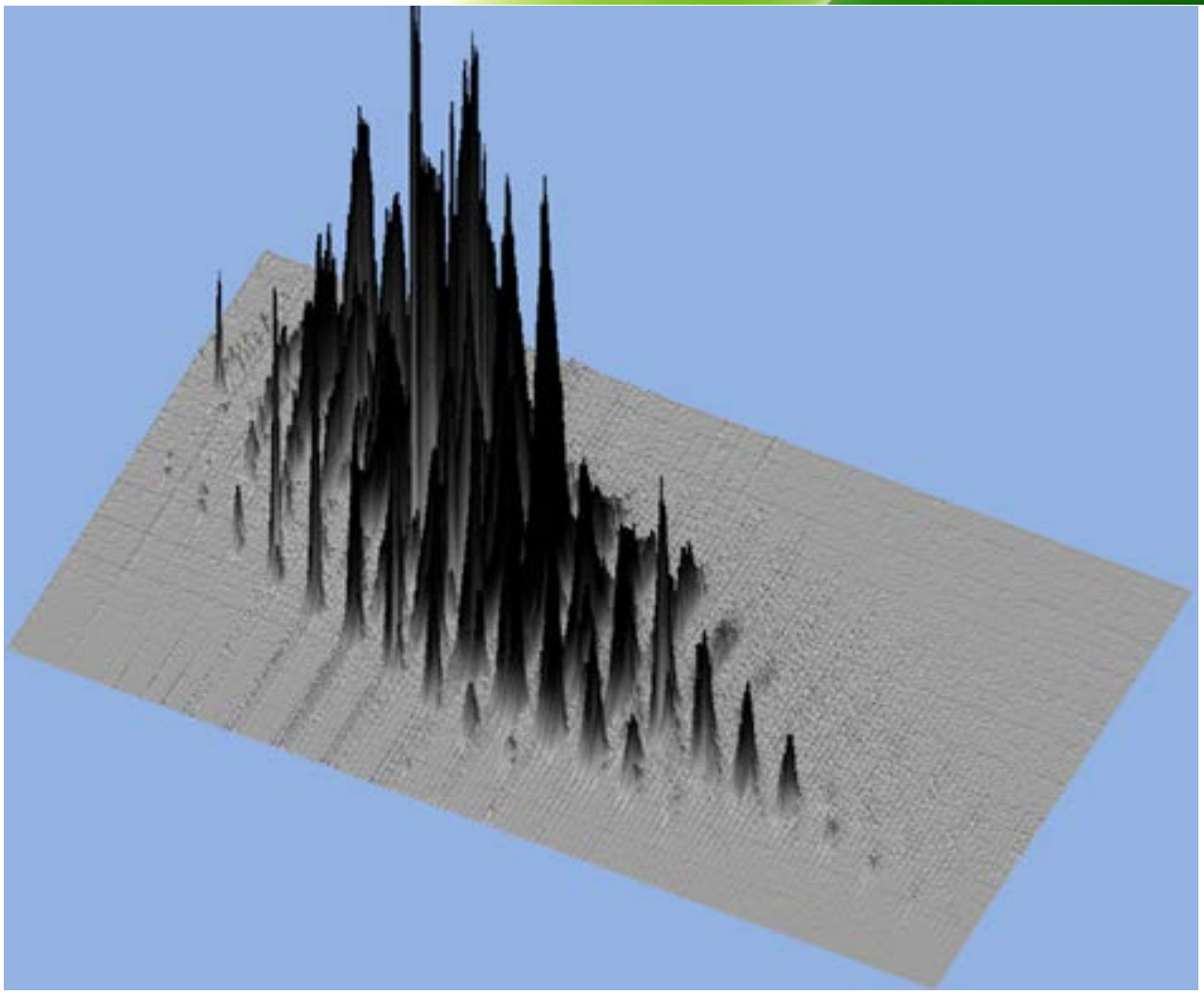
Thousands of dissolved organic compounds

?

Toxicity

- Acute toxicity;
- Deformities;
- Nuclear receptors;
- Endocrine disruption;
- Immune toxicity;
- Oxidative stress





Estimated to be more than 250,000 individual chemicals in OSPW

Our approach

1. **Identify chemical species in sample using LC-Orbitrap mass spectrometry**

(*i*) Name by accurate mass

Orbitrap Mass Spectrometer

2. **Calculate aqueous concentrations**
   - Assume response factor of one for all chemical species
   - Concentration ~ Relative intensity

$$[M]_i = \frac{(RI_i * Mo)}{\text{Molecular mass } i}$$

3. **Assessment of chemical species potency**
   - Use Target lipid model of Di Toro *et al.,* 2000

Environmental Science & Technology
Article
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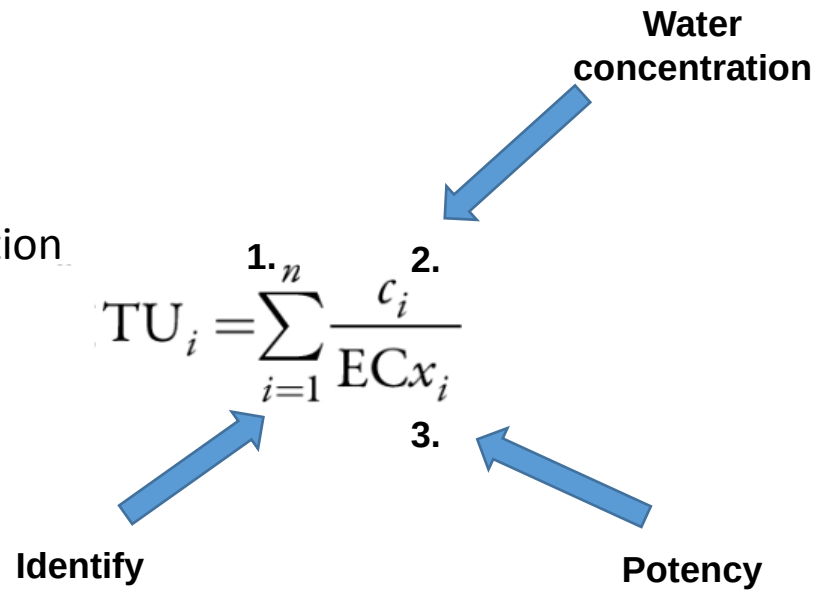
Bioconcentration of Dissolved Organic Compounds from Oil Sands Process-Affected Water by Medaka (*Oryzias latipes*): Importance of Partitioning to Phospholipids

Kun Zhang,[†] Steve Wiseman,[‡] John P Giesy,[‡,#,§,∥,⊥] and Jonathan W. Martin*[,†]

Envir. Sci. Technol. 50:6574−6582.

Some assumptions in model development

- All chemicals contributing to hazard of the sample can be detected (ESI^+ and ESI^-).
- Mode of acute toxic action – Narcosis
- Hazard of mixture follows concentration addition
  - Toxic units



Predicting potency of chemical species

- Target Lipid Model (TLM) has been developed to estimate the 96-hr LC50 of narcotic chemicals by use of K_{OW}

TLM:

$$Log\ (LC50)_i = -0.945 \cdot log\ (K_{OW})_i + Log\ Cbb$$

Can predict K_{ow} from mass or measure empirically
Can also use K_{mw}

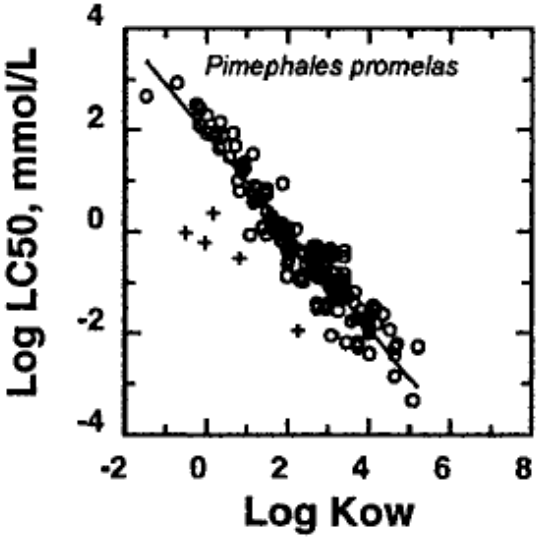


Figure. Log(LC50) versus log(kow) for *Pimephales promelas* for chemicals acting by a narcosis mode of action (Di Toro *et al*., 2000).

Spreadsheet Model

Identify

Concentration:

$$[M]_i = \frac{(RI_i * Mo)}{\text{Molecular mass } i}$$

Toxicity:

$$Log\ (LC50)_i = -0.945 log\ (D_{OW}/D_{MW})_i + Log\ Cbb$$

TU Calculated: (Concentration / Tox.)

	A	B	C	D	E	F
1	Composition [M-H]-	Relative Intensity	Concentration (mmol/L)	log Kow (estimated)	Potency compound (LC50)	TU
2	C11 H19 O	7.91E-06	8.71E-06	-1.40	3.35	3.93E-09
3	C12 H21 O	2.16E-06	2.19E-06	-0.73	2.71	4.30E-09
4	C11 H17 O	5.26E-05	5.85E-05	-1.49	3.43	2.20E-08
5	C12 H19 O	3.00E-05	3.08E-05	-1.97	3.88	4.08E-09
6	C13 H21 O	9.63E-06	9.18E-06	-1.25	3.20	5.76E-09
7	C14 H23 O	2.41E-06	2.14E-06	-0.55	2.54	6.23E-09
8	C11 H15 O	3.91E-05	4.41E-05	-1.89	3.80	6.94E-09
9	C12 H17 O	3.58E-05	3.72E-05	-2.09	4.00	3.76E-09
10	C13 H19 O	1.72E-05	1.66E-05	-1.30	3.25	9.32E-09
11	C12 H15 O	2.24E-05	2.35E-05	-1.68	3.61	5.79E-09
12	C13 H17 O	1.88E-05	1.83E-05	-1.14	3.10	1.46E-08
13	C14 H19 O	1.00E-05	9.08E-06	-1.24	3.19	5.86E-09
14	C15 H21 O	5.39E-06	4.57E-06	-1.21	3.16	3.15E-09
15	C12 H13 O	1.75E-05	1.86E-05	-1.98	3.89	2.41E-09
16	C13 H15 O	2.17E-05	2.13E-05	-1.70	3.62	5.09E-09
17	C14 H17 O	1.48E-05	1.36E-05	-1.39	3.33	6.31E-09
18	C15 H19 O	1.65E-05	1.41E-05	-1.46	3.40	5.63E-09
19	C16 H21 O	1.72E-05	1.38E-05	-1.24	3.19	8.87E-09
20	C17 H23 O	1.41E-05	1.07E-05	-1.08	3.04	9.79E-09
21	C18 H25 O	9.87E-06	7.07E-06	-1.61	3.54	2.04E-09
22	C19 H27 O	2.08E-06	1.41E-06	-0.41	2.41	5.53E-09
23	C13 H13 O	9.14E-06	9.09E-06	-1.65	3.58	2.40E-09

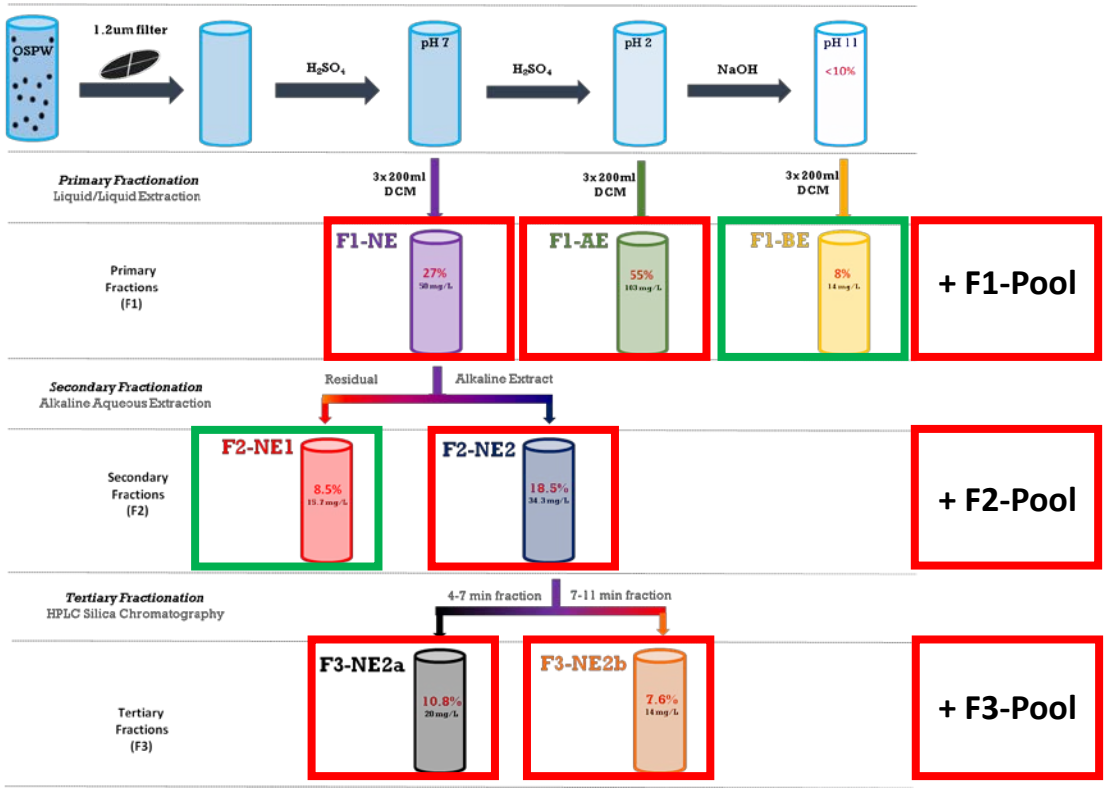
If TU ≥ 1 Expect LC50 or greater

Sum TU and predict toxicity

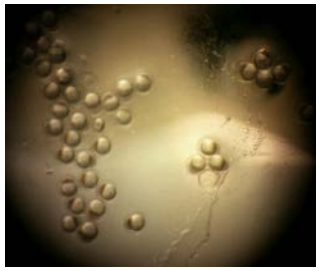
Test of model

With observed LC50

No observed LC50



	No. samples
Acute toxicity (LC50)	8
Total	10



96-hr embryo-lethality assay
Pimephales promelas

Model results

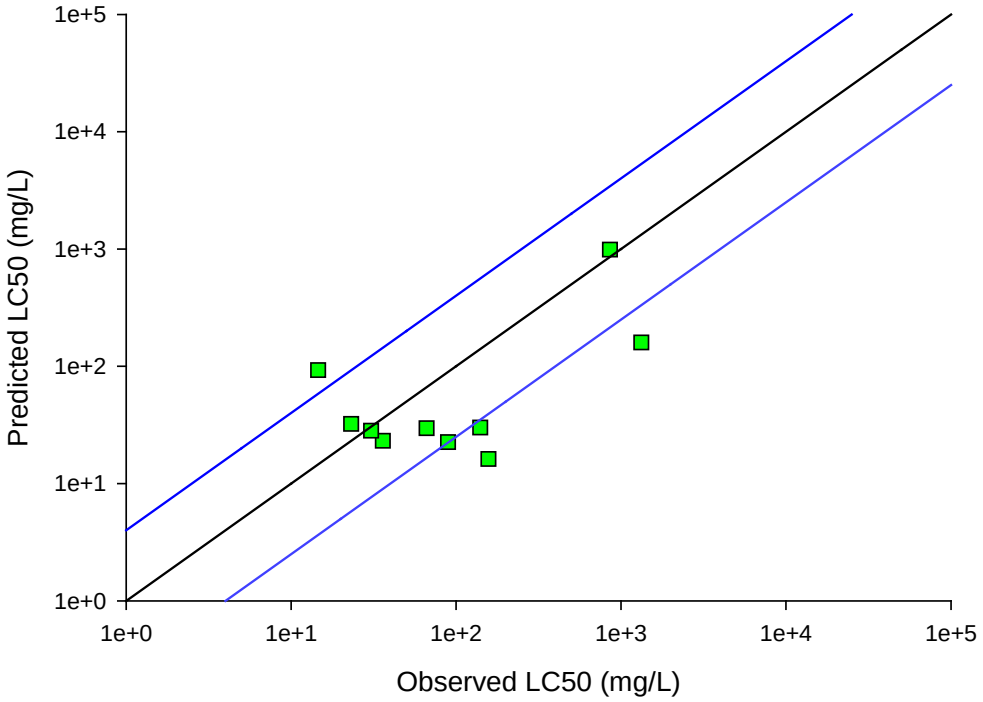


Figure 2. Model predicted LC50 v. observed LC50, blue-line is a 5-fold difference from observed.

Table 2. Percent of samples greater than X-fold different from observed.

Fold difference from Observed LC50	Model (n = 8)
2 – fold	50%
4 – fold	75%
> 10 - fold	0%

- **All LC50s predicted within 10-fold of observed.**

Contribution of chemical class, carbon number ranges to toxicity of the F1-Pool sample.

Chemical Class	Percent TU (%) of dissolved organic fraction of OSPW			
	C5-15	**C16-20**	**C21-25**	**C26-30**
SO^+	5.79	20.3	2.59	3.25
SO_2^-	0.85	7.75	0.15	<0.01
NO^+	8.33	7.40	1.24	<0.01
$O2^-$	4.42	11.9	0.91	<0.01
$O2^+$	7.05	7.93	6.12	0.03
O^+	2.41	1.43	0.20	<0.01
O^-	<4.60E-4	<4.60E-4	<4.60E-4	<4.60E-4
Total TU	**29%**	**57%**	**11.2%**	**2.8%**

- $O_2^{+/-}$ and SO^+ chemical classes contribute most of predicted toxicity (~70%)
- Carbon number range C5-20 contribute > 85% of predicted toxicity
  - C16-20 predominate (>57%)

Conclusions

- Developed a model to predict the acute lethality of dissolved organic chemicals in OSPW to embryos of Fathead minnow (100% of predictions were within biological variability associated with test)

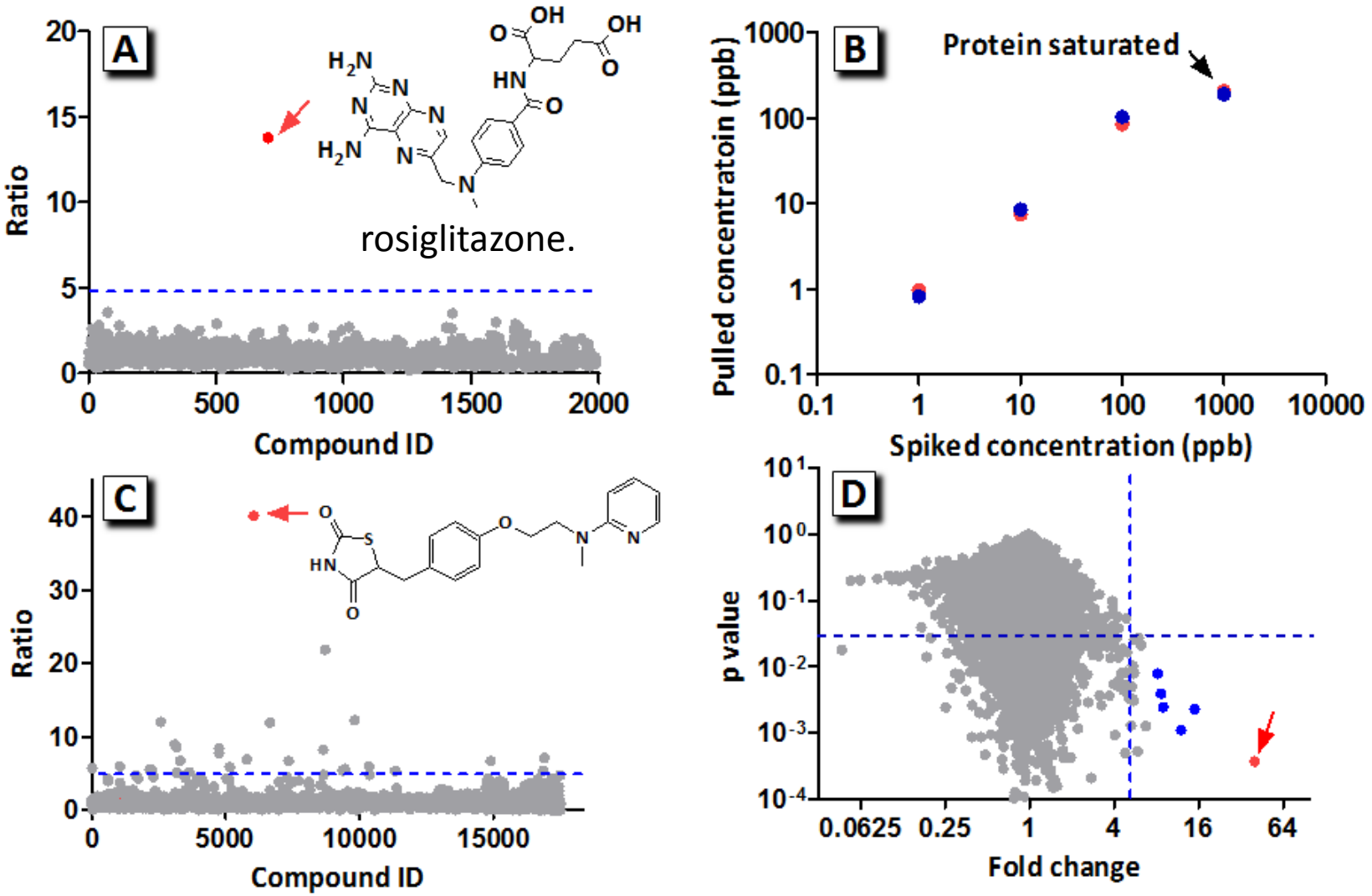
- Chemical class contributions confirms results of EDA
    - i.e. $O^{+/-}$, $O_2^{+/-}$, SO^+, NO^+ and SO_2^- are responsible for most toxicity

- SO^+ were identified as the most potent chemical class
    - SO^+ are among persistent chemicals in OSPW

Pull-down combined with untargeted analysis (PCUA) strategy to robustly identify causative chemicals in mixtures of residues in samples of food, human tissues or environmental matrices

Example: PPARγ agonistic activity

Case studies using pull-down system



Untargeted Strategy to Identify Causative Chemicals

Phenotype-based toxicity (acute toxicity, ROS)

Unknown protein target

Effect-Directed Analysis (EDA)

Fractionation of Active Fractions

Chemical Analysis

Compare

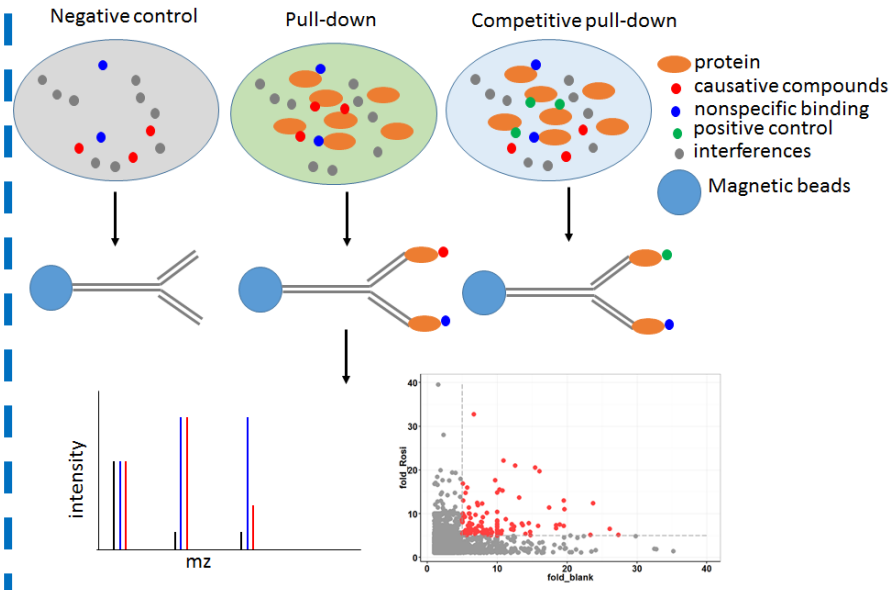
Effects in Bioassay

Identification of Active Chemicals

Target-based toxicity (Nuclear receptors…)

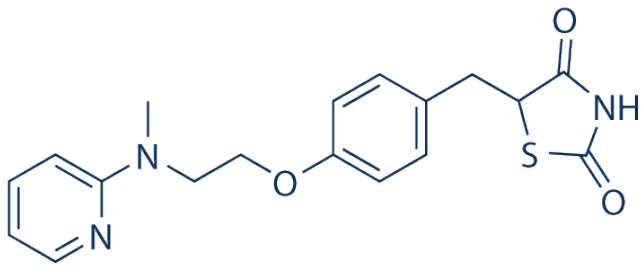
Known protein Target

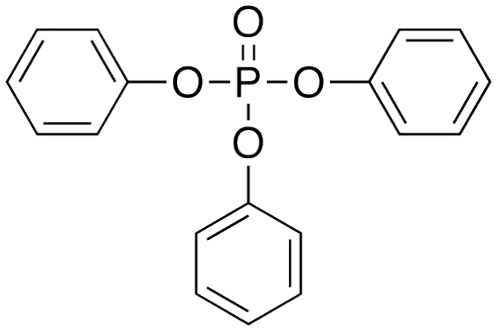
Pull down system

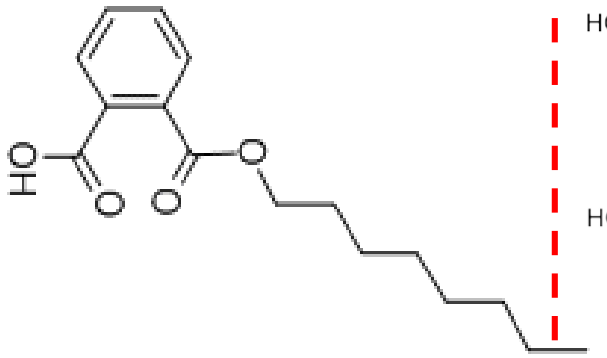


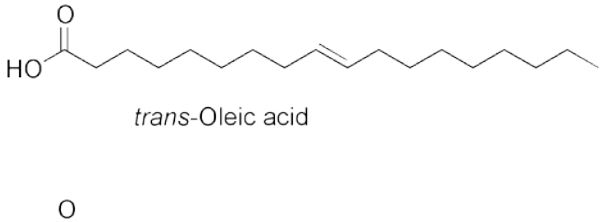
Case 1: PPARγ Activation (known protein target)

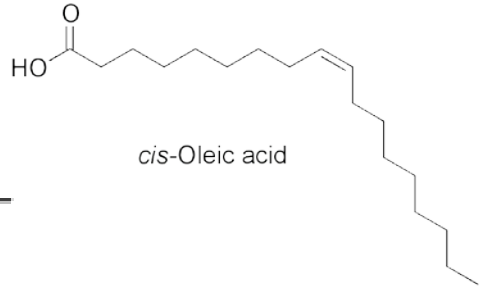
- PPARs regulate intracellular lipid flux and adipocyte proliferation and differentiation.
- PPARγ ligands might promote development of obesity.
- Activated by structurally diverse ligands

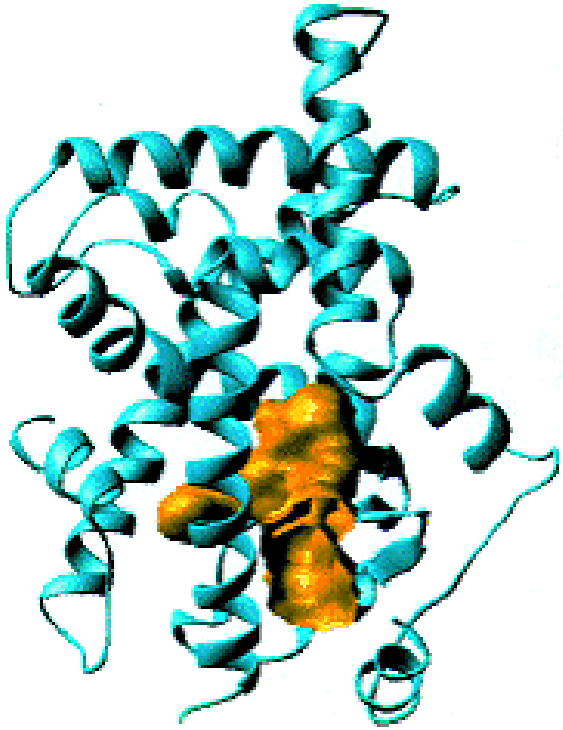




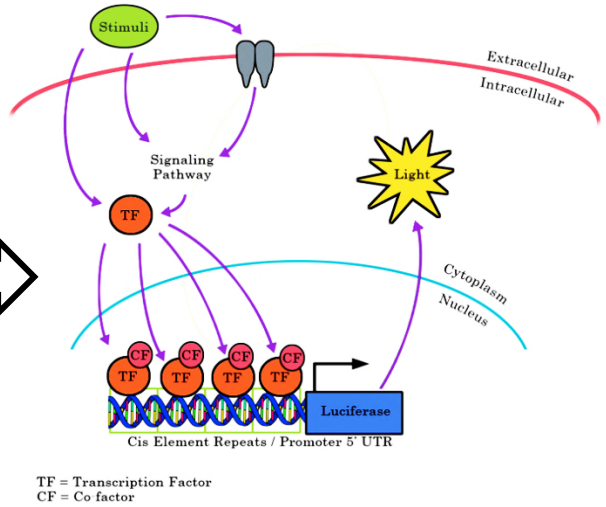


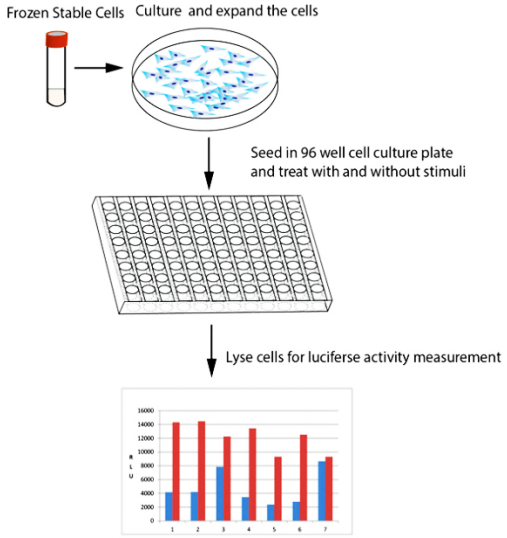






EDA: Chemical Analysis and Bioassay

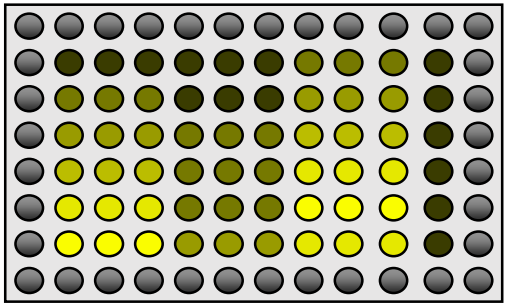




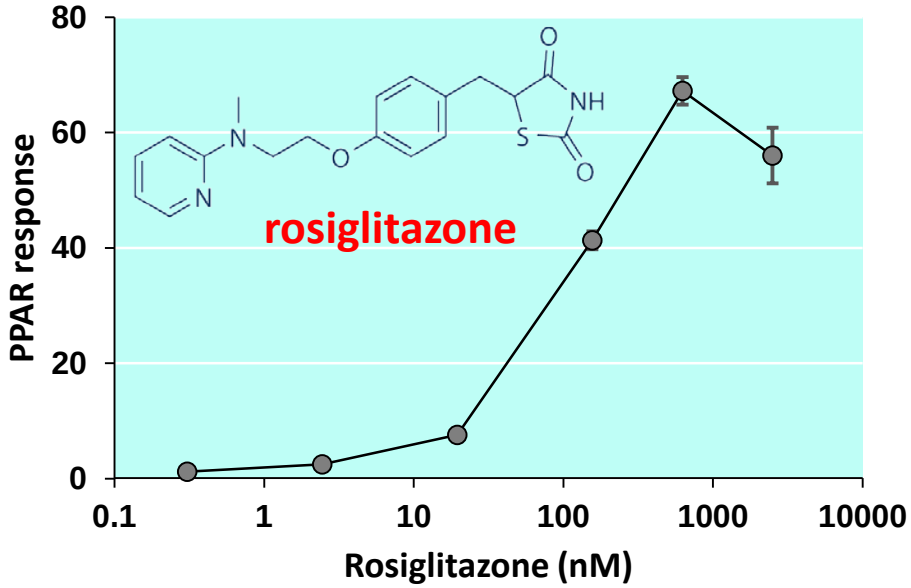
Chemical Analysis:
Ultrahigh-Resolution Mass Spectrometry

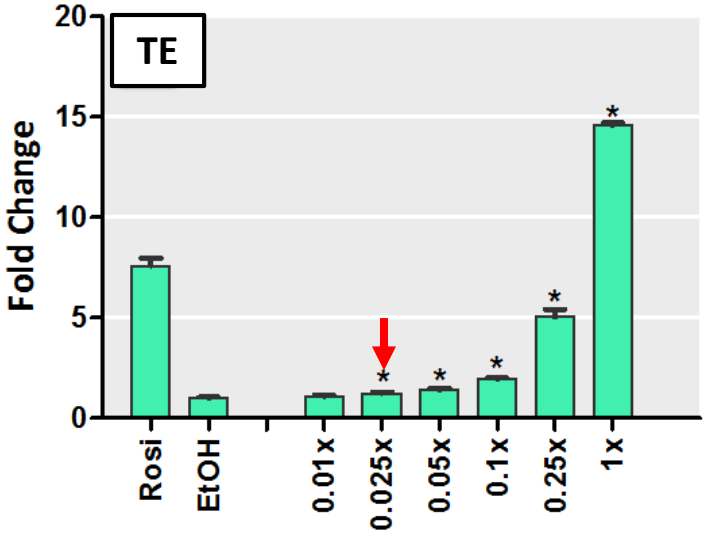
Bioassay:
NRF2 Luciferase Reporter System (High Throughput)

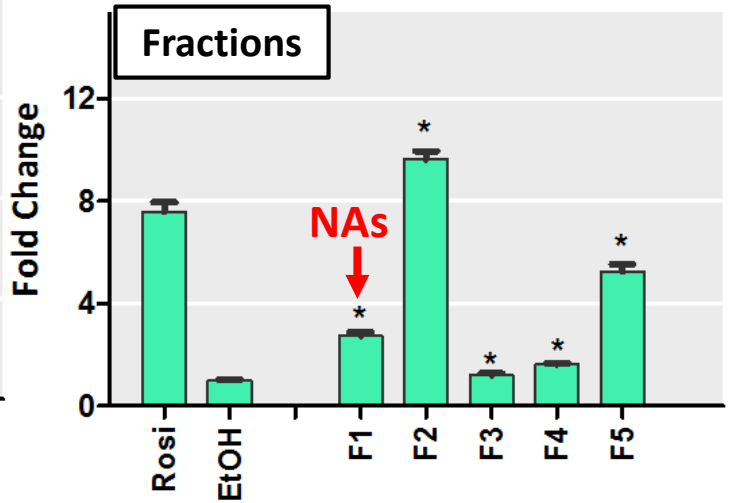
PPARγ Activation - Luciferase Reporter Gene Assay



Human PPAR-G
cell reporter system







- Strong activity was detected
- But NAs are not the causative chemicals

Conclusions

	Pull-down strategy (known protein target)	Effect-Directed Analysis (unknown protein target)
ADV:	Useful to identify unknown ligands	Quantitative mass balance analysis
	Compatible to multiple ligands	Easy for operation
	Expand dynamic range	
	Time-effective	
DISADV:	Need tagged-protein	Difficulty to identify unknown ligands
	No mass balance information	Complicated by multiple ligands
		High noise
		Time-consuming

The emergence of DNA as a detectable and quantifiable unit of observation in biodiversity science is arguably the **SINGLE MOST IMPORTANT** technical advance in ecology in our life-times.

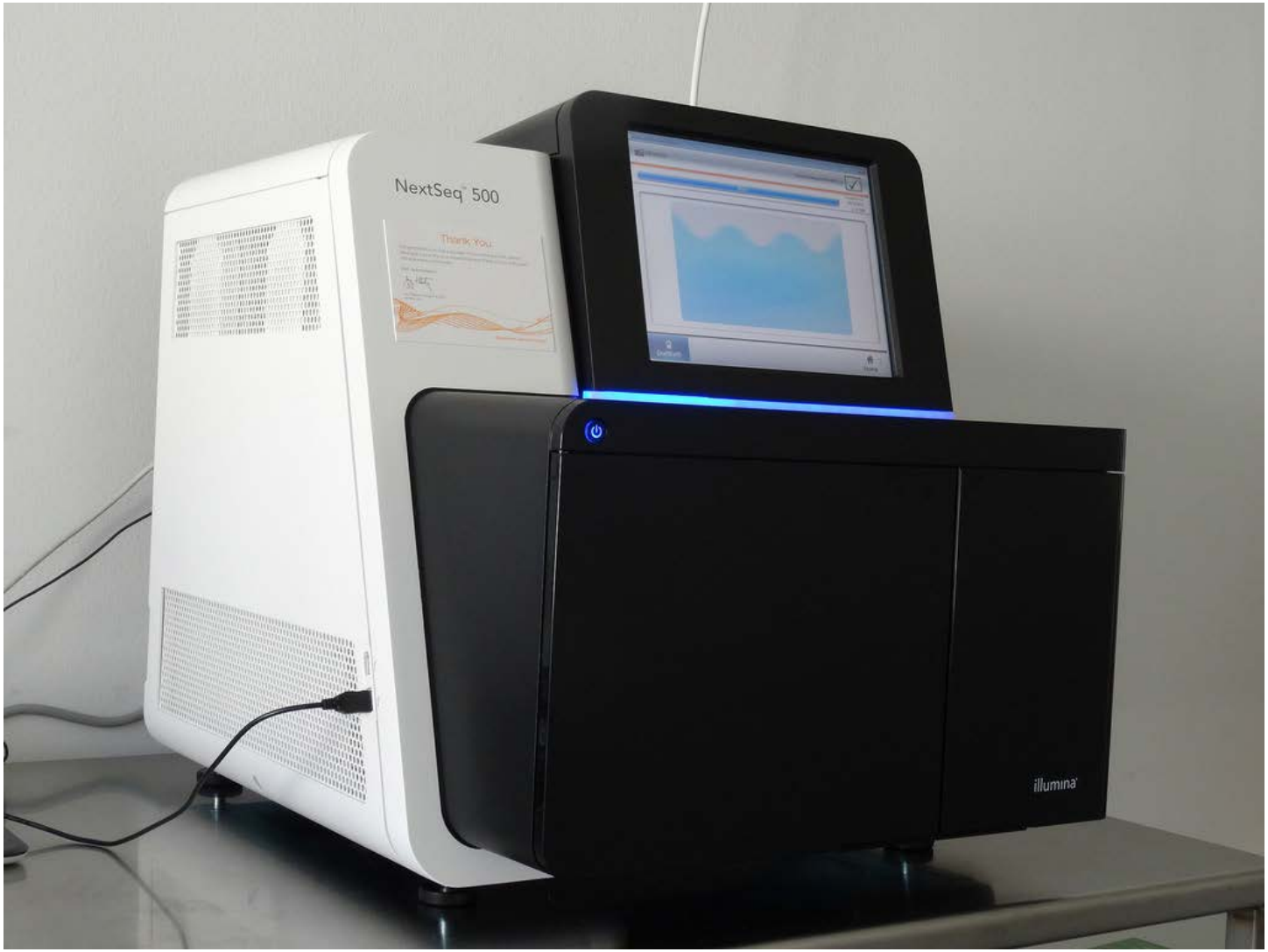
Environmental Science & Technology

Article

pubs.acs.org/est

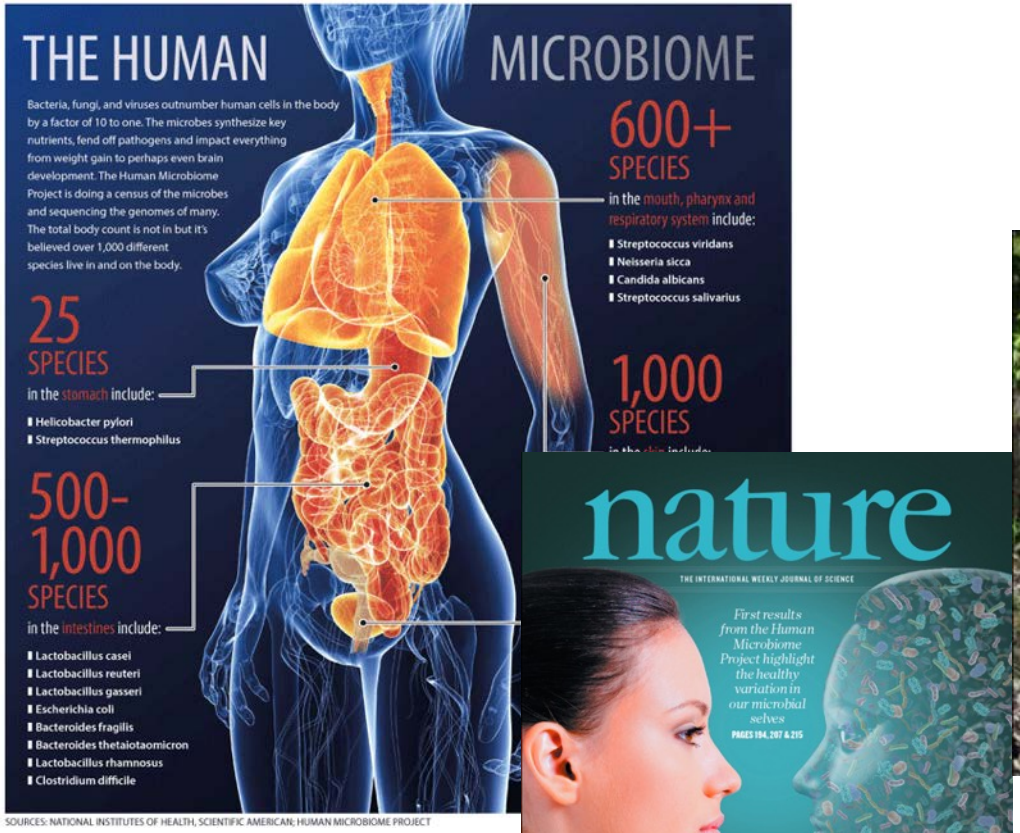
Ecogenomics of Zooplankton Community Reveals Ecological Threshold of Ammonia Nitrogen

Jianghua Yang,[†] Xiaowei Zhang,[*,†] Yuwei Xie,[†] Chao Song,[†] Jingying Sun,[†] Yong Zhang,[‡] John P. Giesy,[†,§,∥] and Hongxia Yu[†]



NextSeq 5000 DNA Sequencer

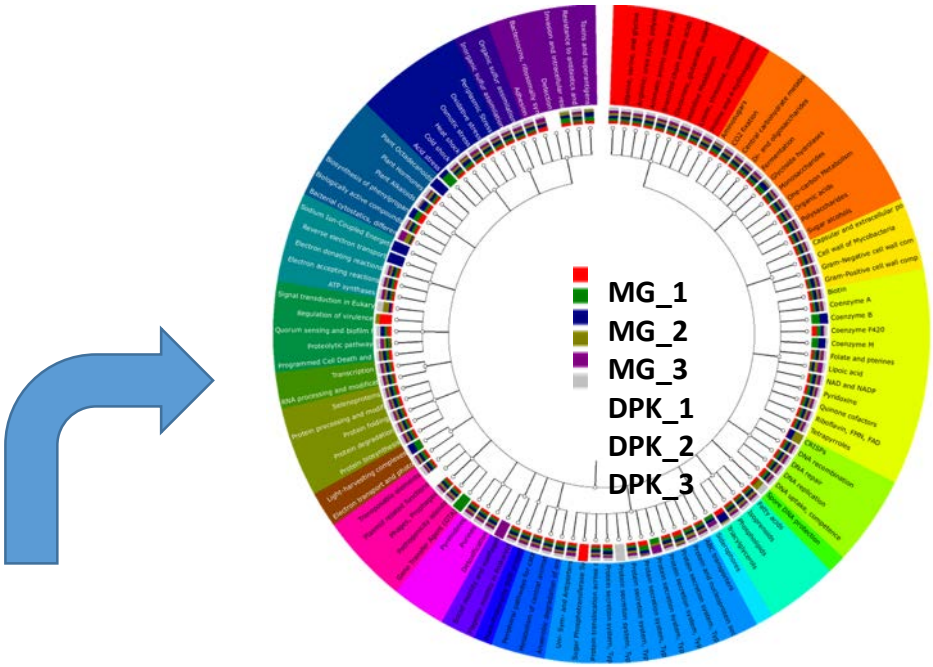
Microbiome



Nitrogen cycling is controlled by sediment microbiome

Environmental Microbiome

Nitrogen cycling is controlled by sediment microbiome

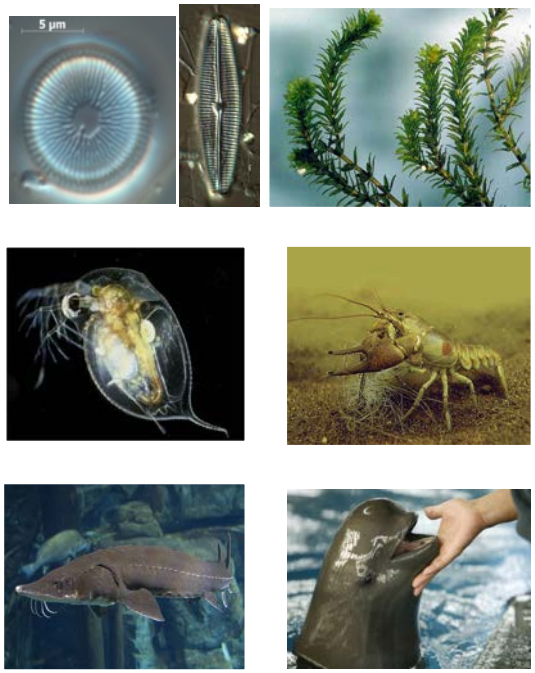


- Amino Acids and Derivatives
- Carbohydrates
- Cell Division and Cell Cycle
- Cell Wall and Capsule
- Cofactors, Vitamins, Prosthetic Groups, Pigments
- DNA Metabolism
- Dormancy and Sporulation
- Fatty Acids, Lipids, and Isoprenoids
- Iron acquisition and metabolism
- Membrane Transport
- Metabolism of Aromatic Compounds
- Miscellaneous
- Motility and Chemotaxis
- Nitrogen Metabolism
- Nucleosides and Nucleotides
- Phages, Prophages, Transposable elements, Plasmids
- Phosphorus Metabolism
- Photosynthesis
- Potassium metabolism
- Protein Metabolism
- RNA Metabolism
- Regulation and Cell signaling
- Respiration
- Secondary Metabolism
- Stress Response
- Sulfur Metabolism
- Virulence, Disease and Defense

Subsystems annotation and abundance

Environmental Macrobiome

Biodiversity of Aquatic Ecosystems



Biodiversity in Terrestrial Ecosystem

Measurement of Macrobiome

DNA Meta-barcoding

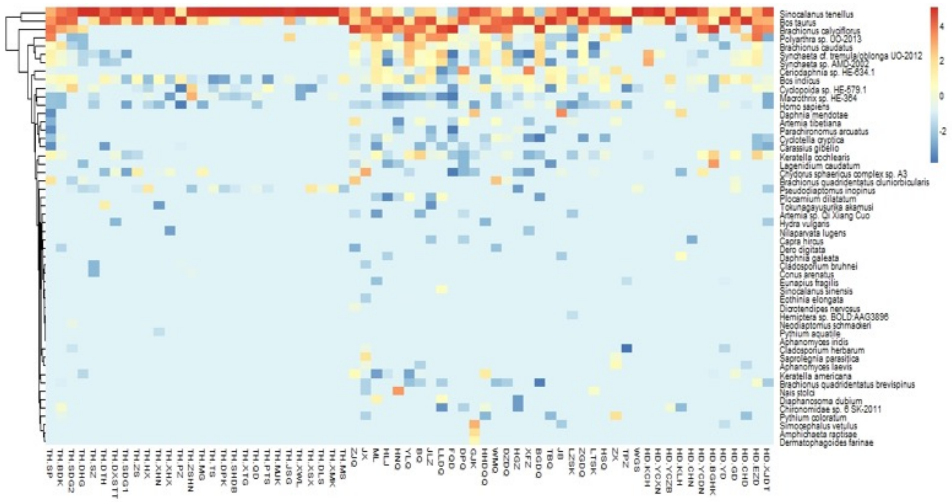
Operational Taxonomic Units (OTUs)

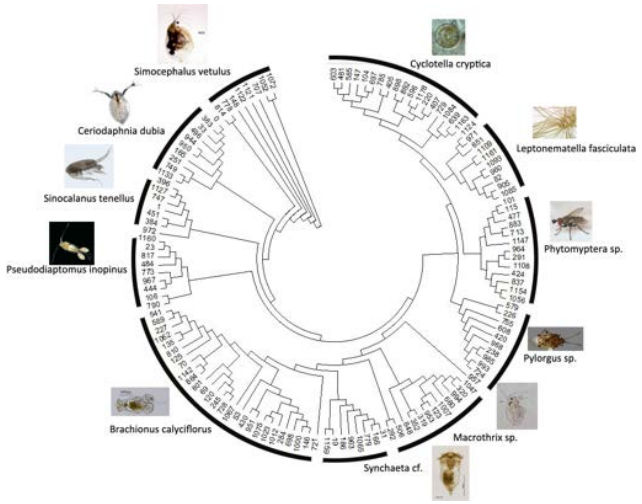
Advantages of Metabarcoding

- More rapid and cost effective
- More comprehensive than traditional visual taxonomy
- Can use to monitor biodiversity
- Can use to monitor for invasive species

HTP Biodiversity assessment by Environmental DNA

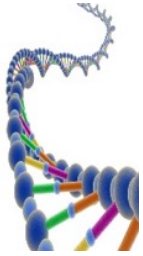
Lakes Rivers





Library

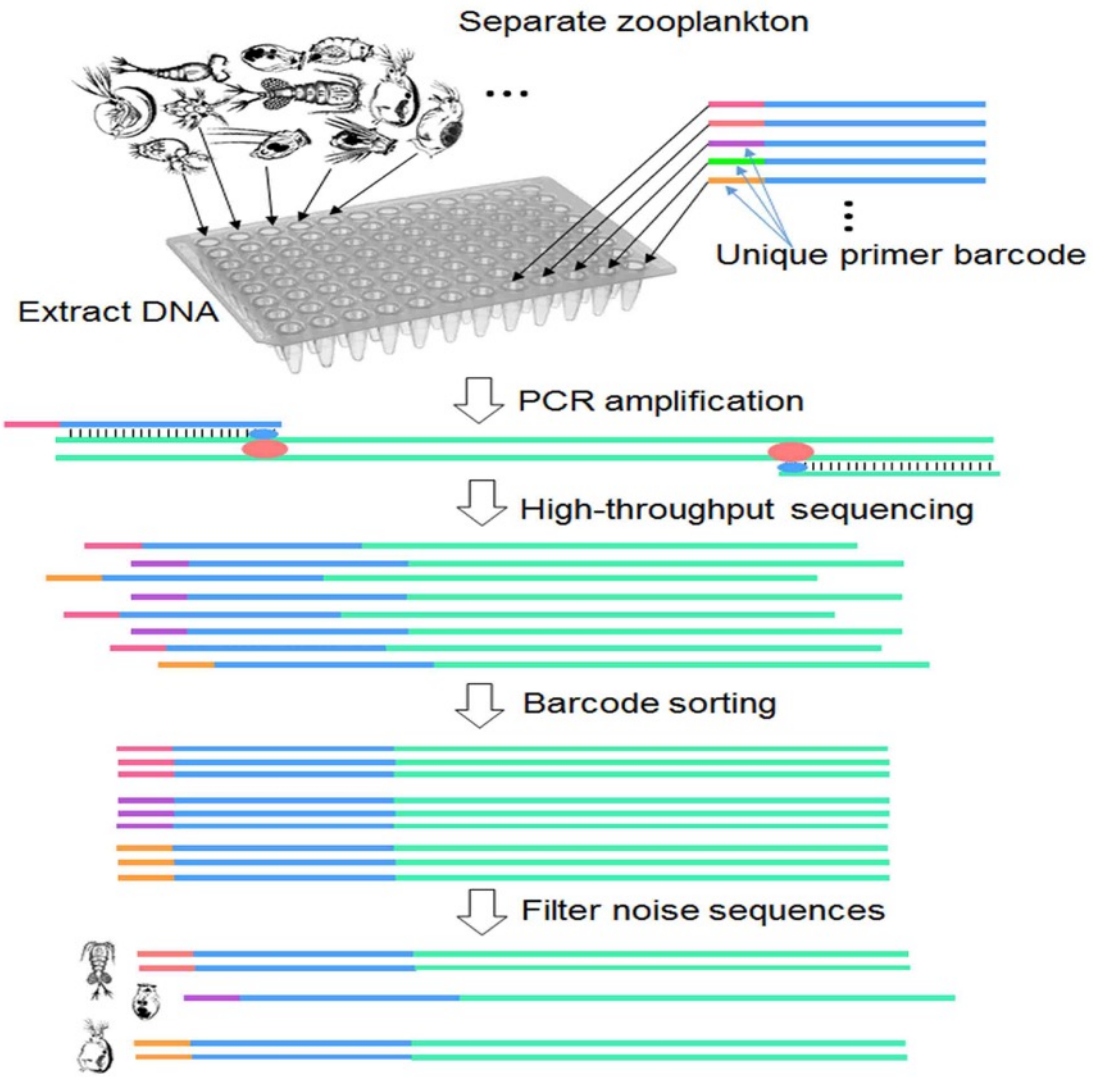
Lake



DNA

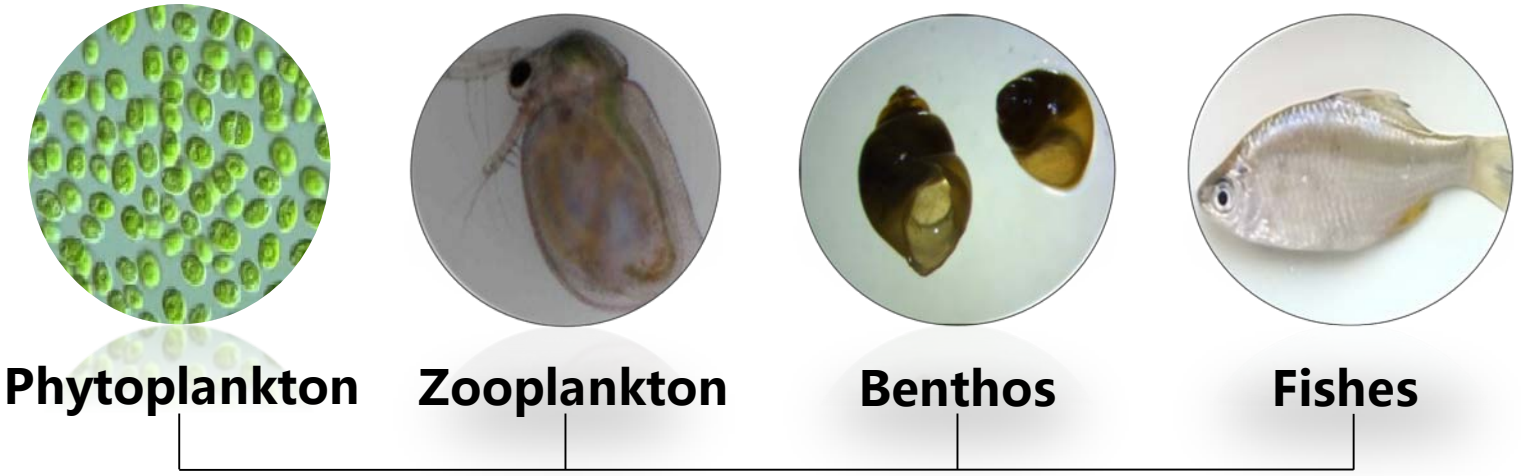
DNA Sequence

1) High Throughput Barcoding Protocol

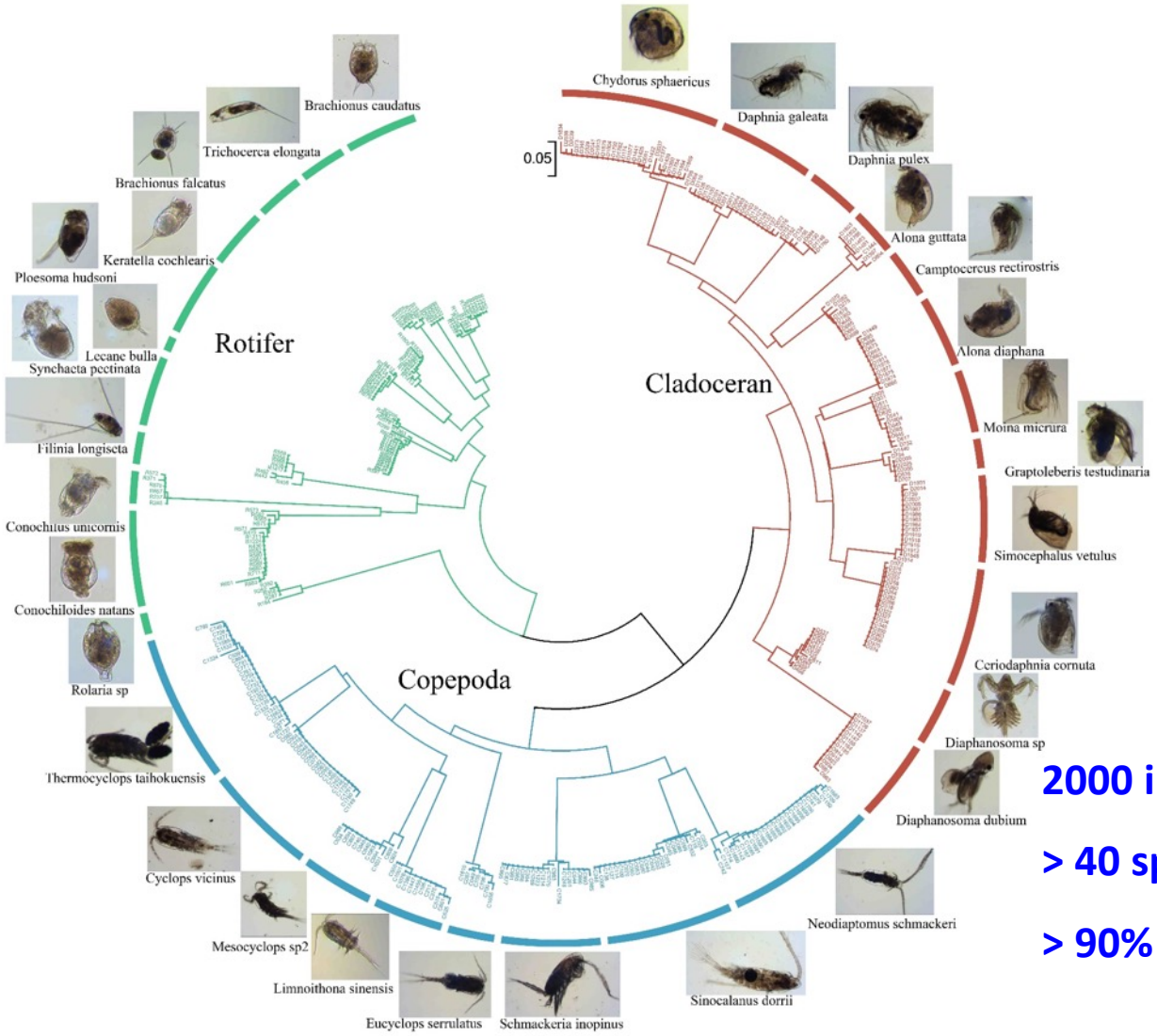


Schematic diagram of parallel barcode recovery using multiple identifier (MID) tagging and next-generation sequencing (NGS) protocol.

2) DNA Barcode Database of an Aquatic Ecosystem



DNA Barcode Library of Zooplankton from Lake Tai



2000 individuals

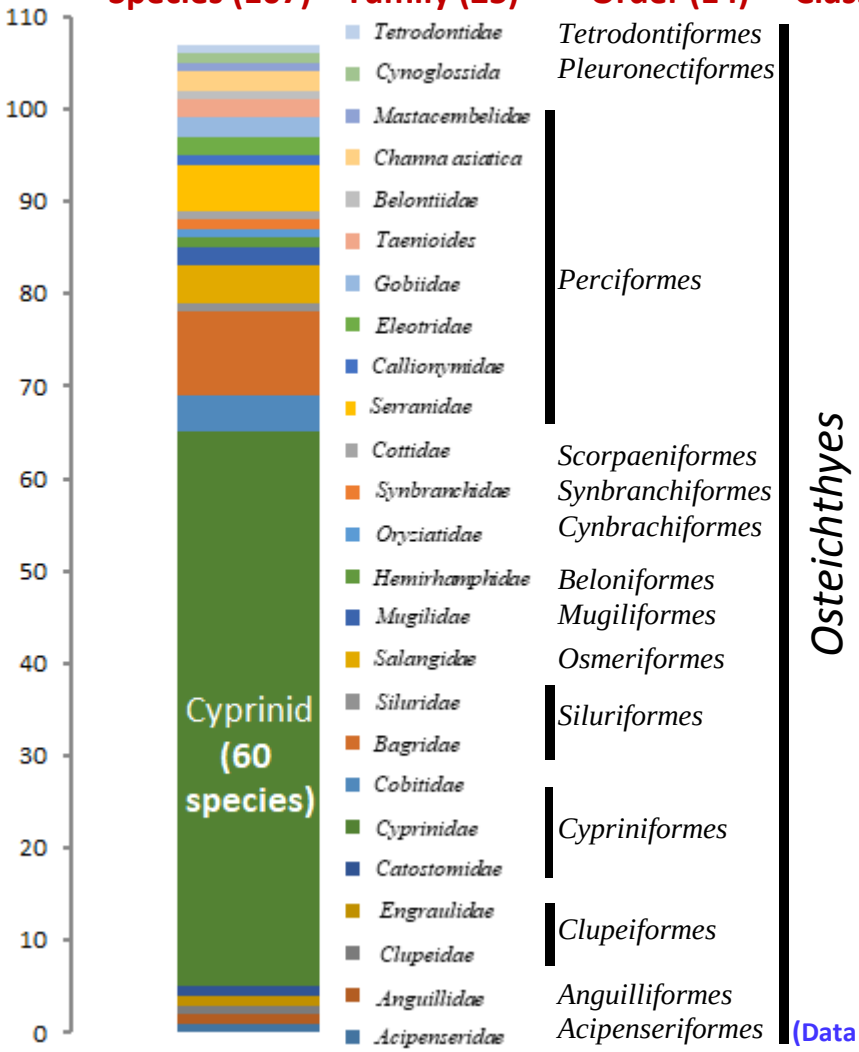
> 40 species

> 90% Environmental samples

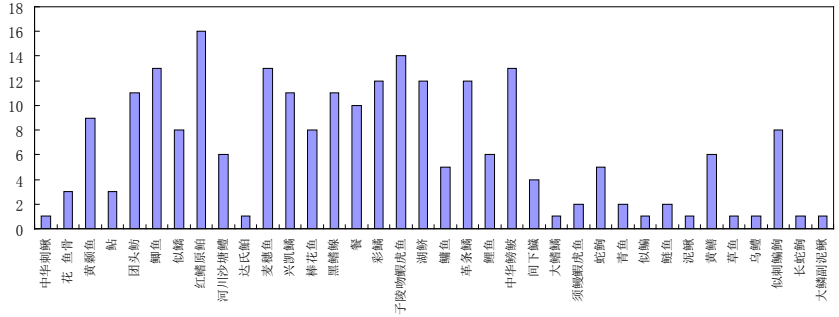
Database of Benthic Macro-invertebrates

DNA barcode Database of Chinese freshwater fishes

Abundance and richness of fishes in Lake Tai



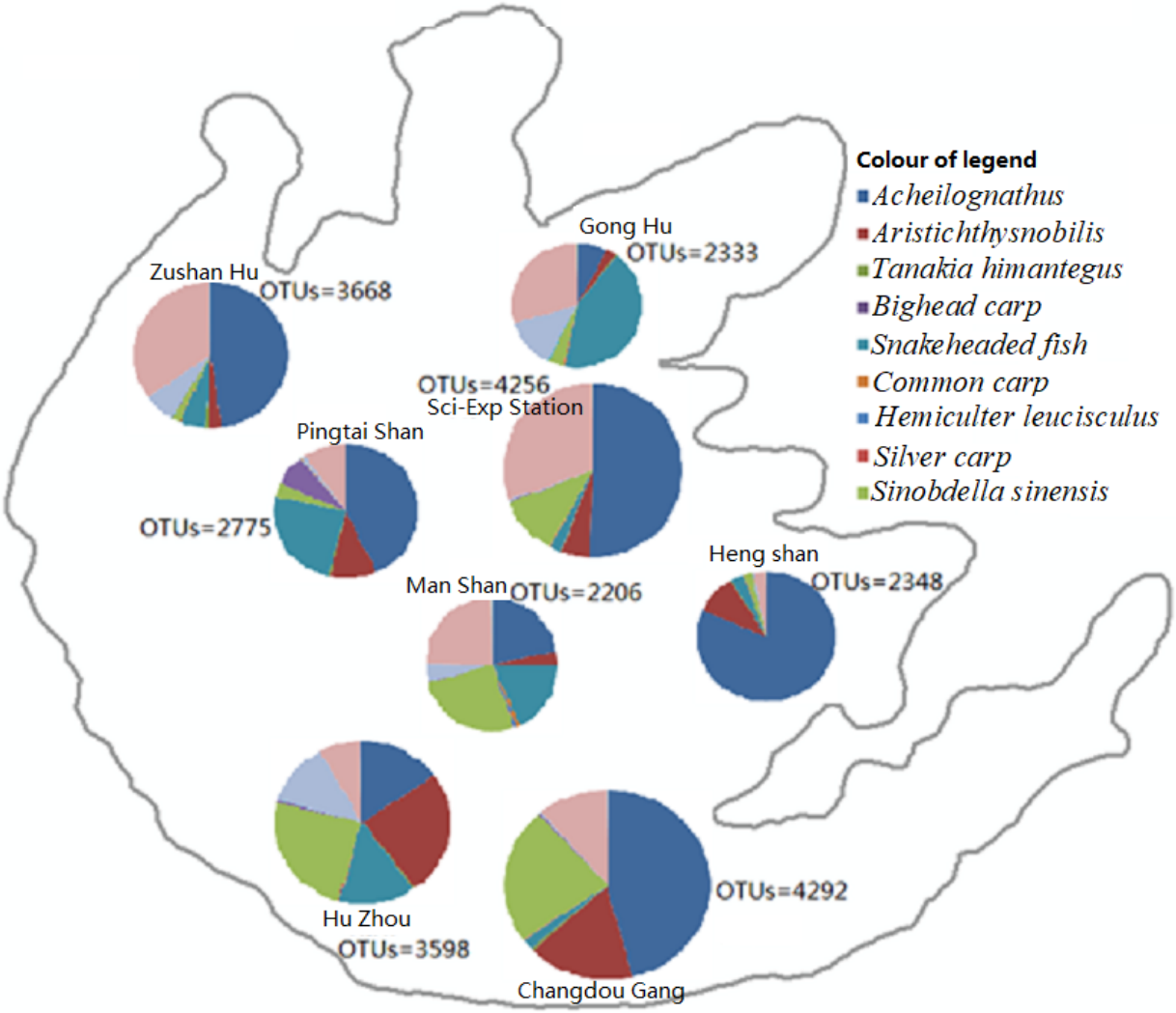
DNA barcoding database of fishes in Lake Tai



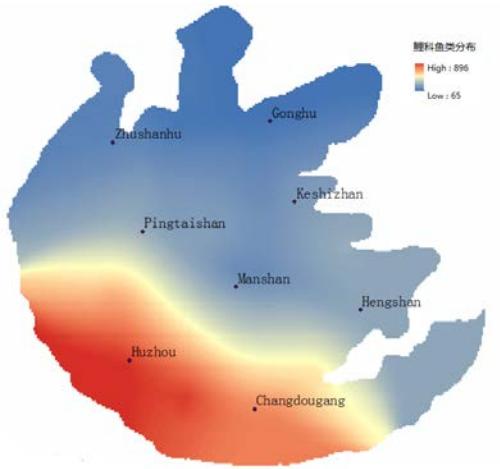
Totally, 36 spices, 234 individuals

(Data from <<Fishes of Lake Tai>>)

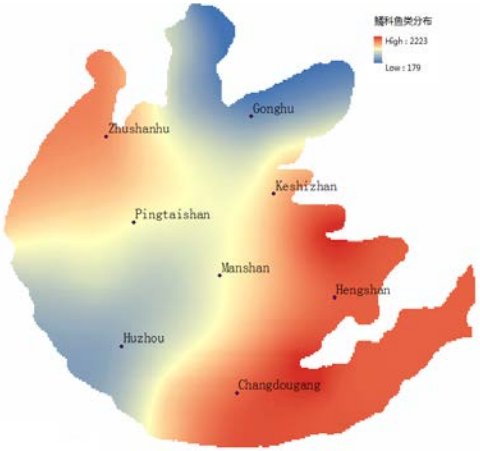
Map of biodiversity of Fish community in Lake Tai (2014)



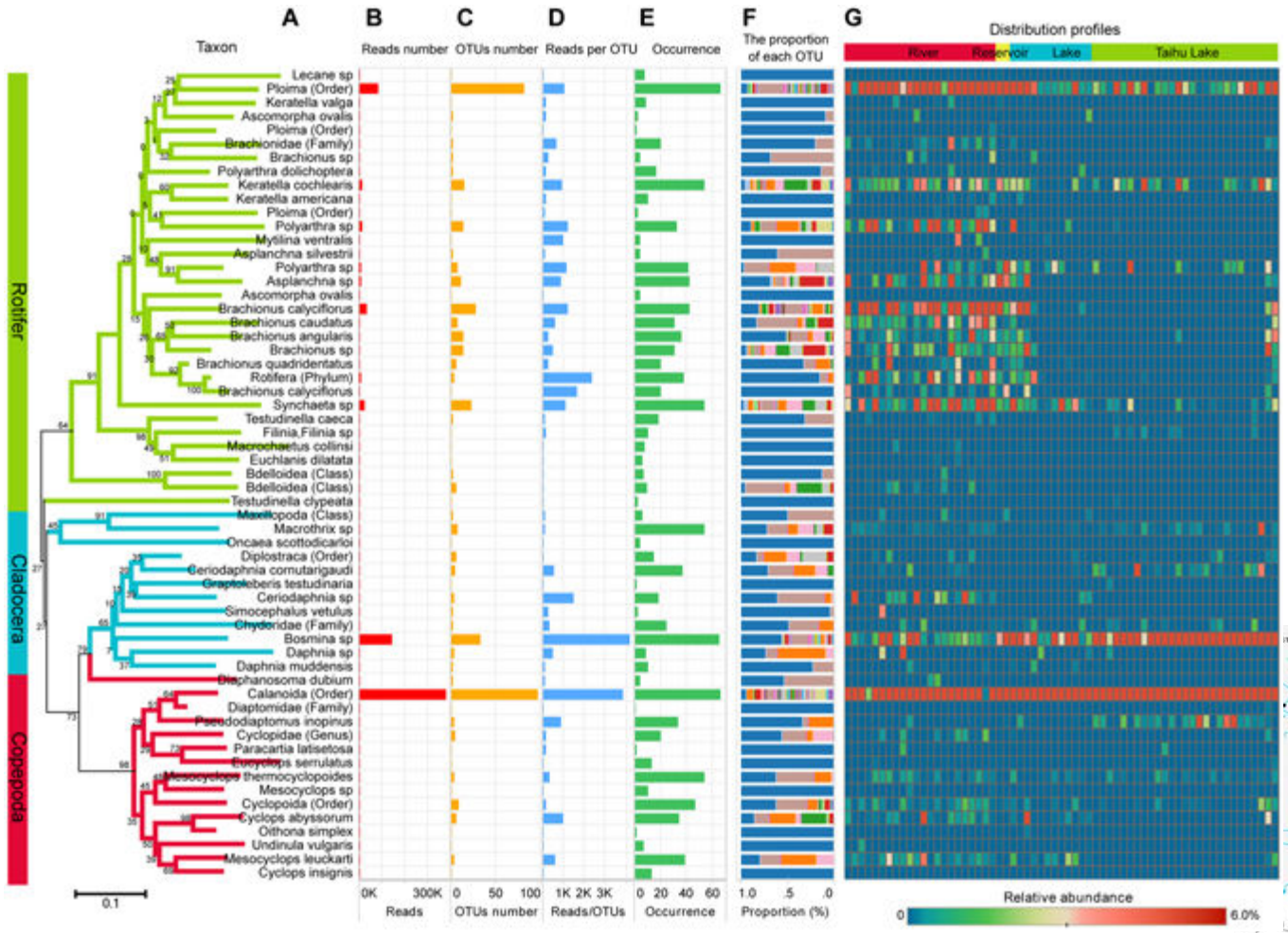
Distribution of Cyprinidae



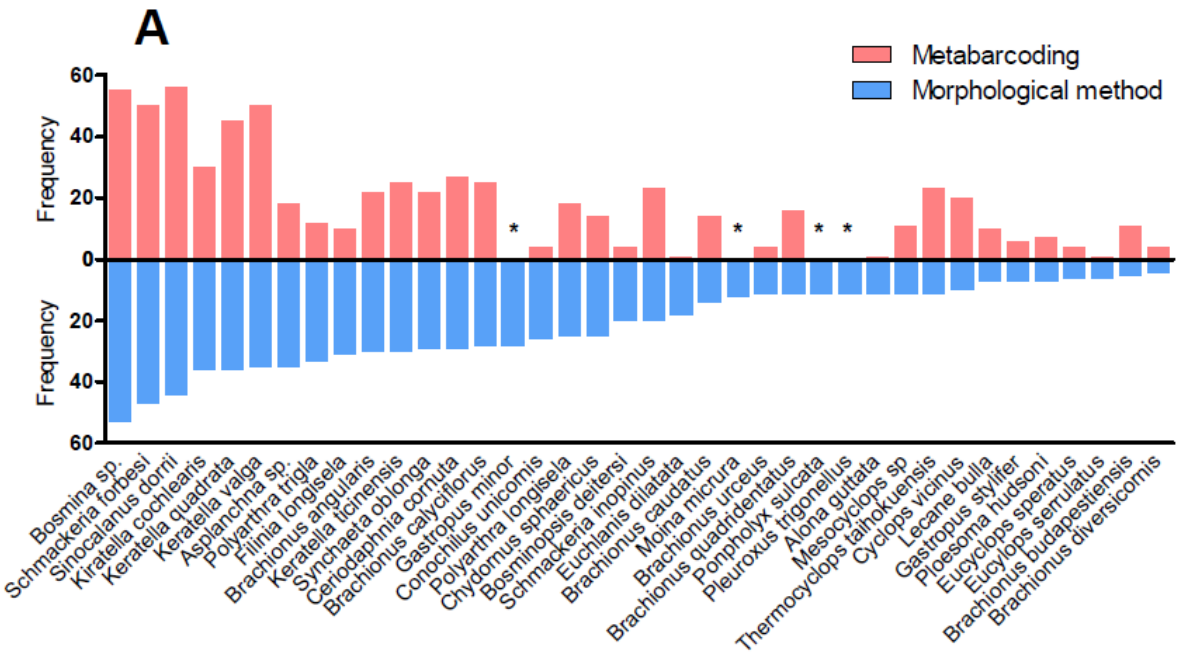
Distribution of Acheilognathus



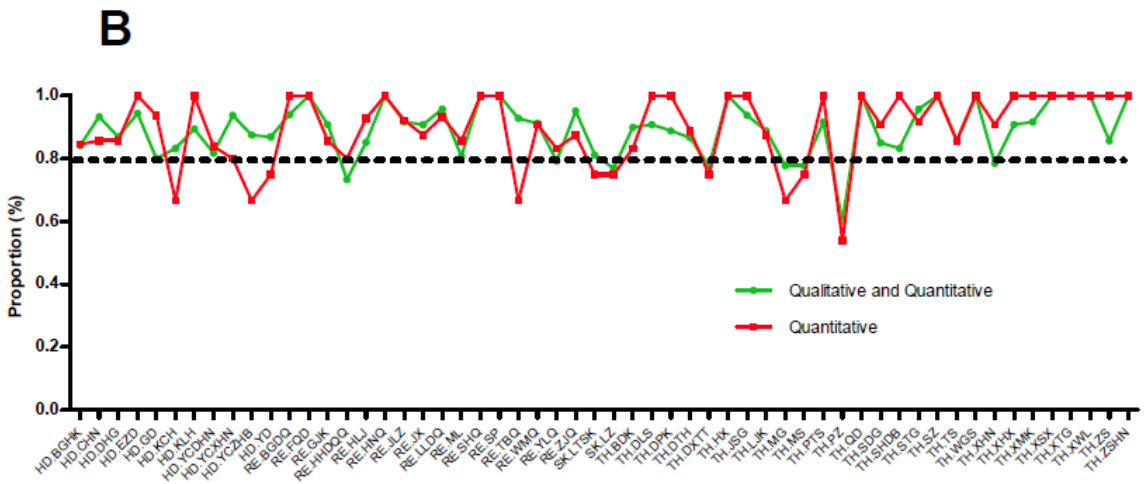
Map of biodiversity of freshwater zooplankton community in China



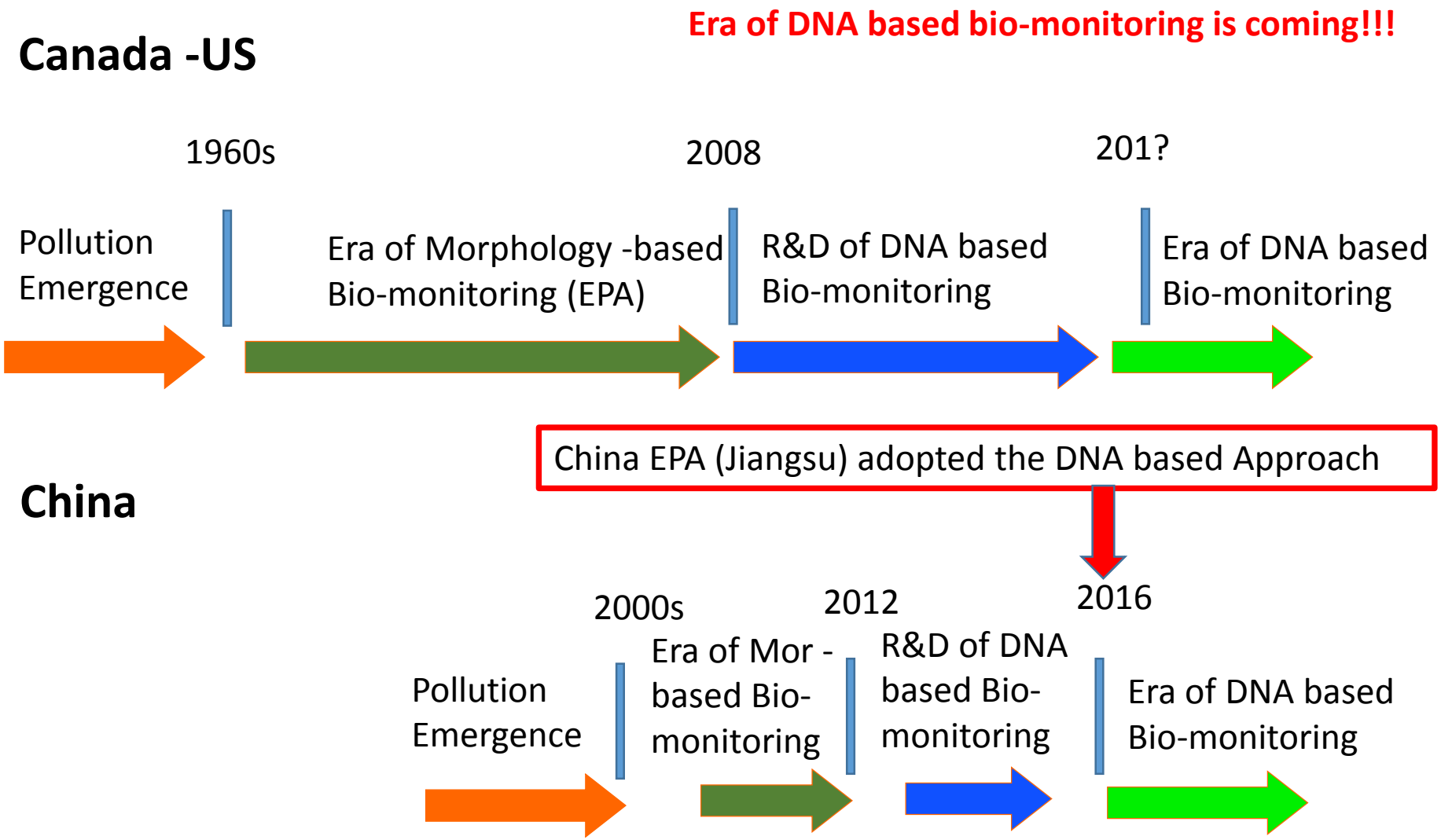
Comparison of metabarcoding and traditional visual taxonomy



Avg 80% identification



Science Translation: toward to Environ Management



Thank you!!!!!!

Global Water Futures Program

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