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deep

ViewPoint
Biosurveillance de l'eau

EEDEMS 2022

Fouilles de données videotracking massives pour l'identification d'empreintes comportementales de l'exposition aux contaminants en écotoxicologie aquatique : application a 3 invertébrés pour la surveillance des rejets

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Encadrants :

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- Jean-Luc Bertand-Krajewski, Jean-Baptiste Aubin – Deep, INSA



La Région
Auvergne-Rhône-Alpes



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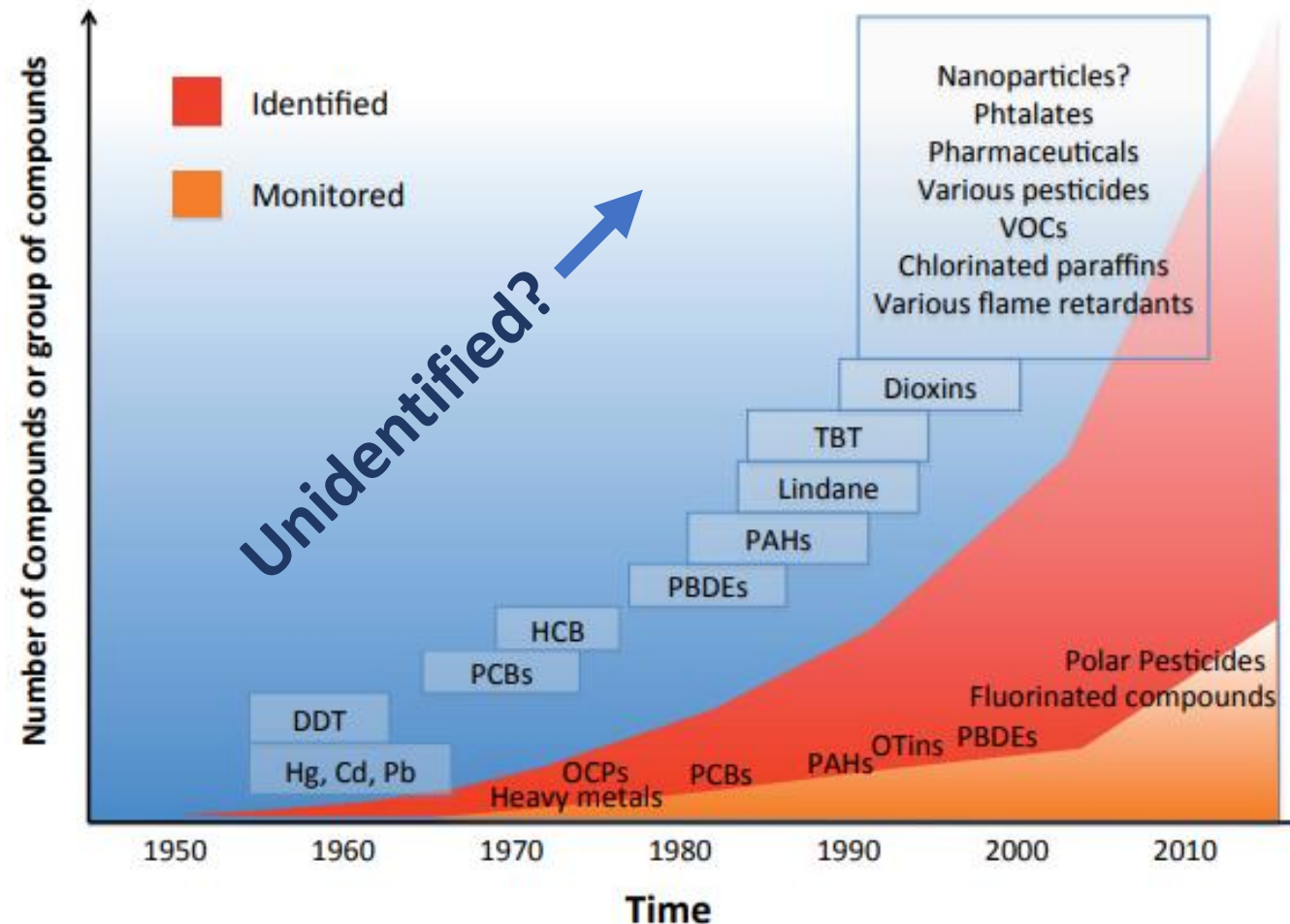
deep

ViewPoint
Biosurveillance de l'eau

- PhD Context and Objectives
- Methodology
- Results
- Outlook

Emerging Aquatic Micropollution

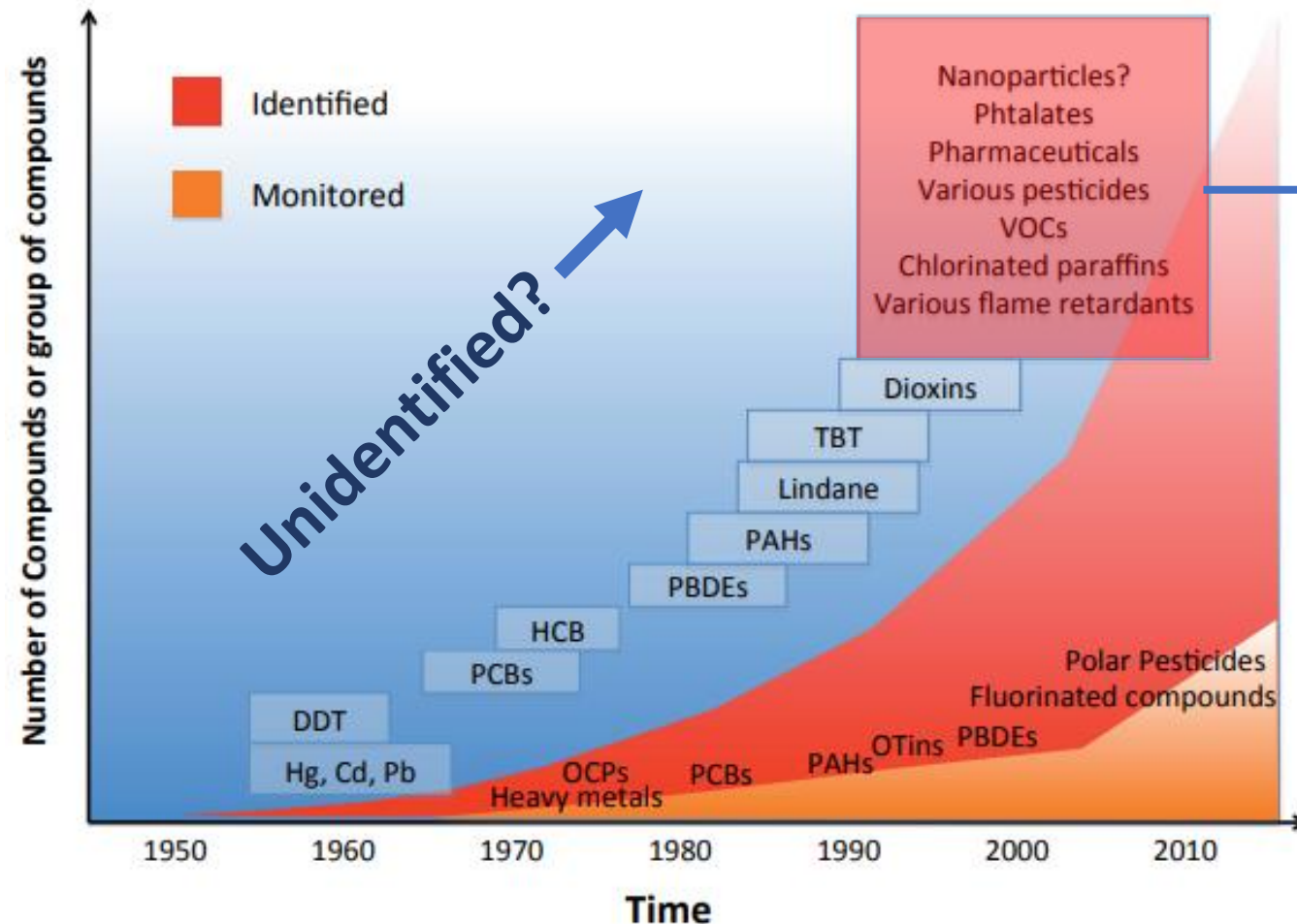
- Negative CEC impact often poorly understood
- Absence of CEC monitoring in wastewater management (WWTPs)
 - One shot campaigns
 - Grab sampling
- Temporal variability in discharge



Source : Chemical Pollution in Europe's Seas : Programmes, Practices and Priorities for Research, Marine Board Position Paper 16. Calewaert J.B. and McDonough N. Marine Board-ESF Ostend, Belgium.

Emerging Aquatic Micropollution

- Negative CEC impact often poorly understood
- Absence of CEC monitoring in wastewater management (WWTPs)
 - One shot campaigns
 - Grab sampling
- Temporal variability in discharge
- Objective : **Real-time CEC identification**

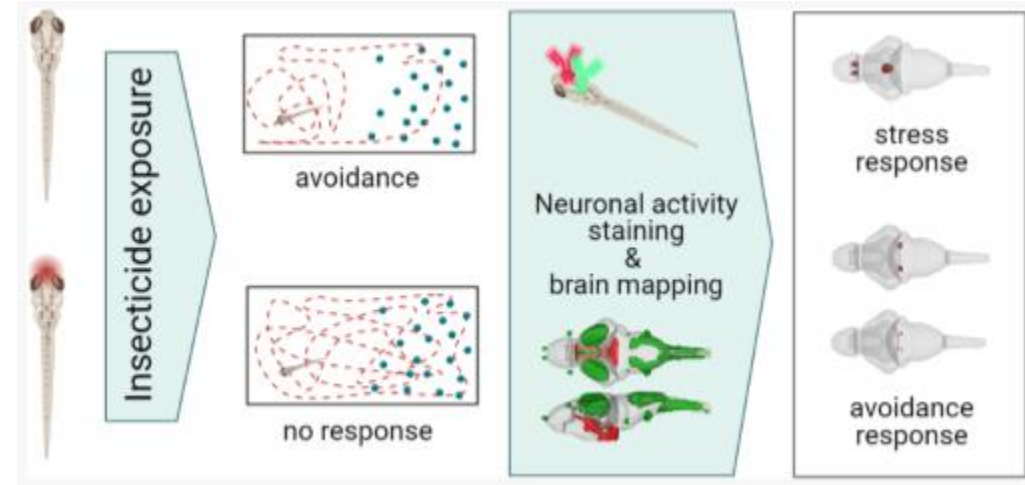


Non-targeted
CEC detection?

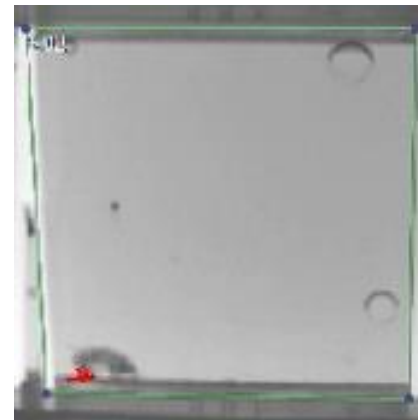
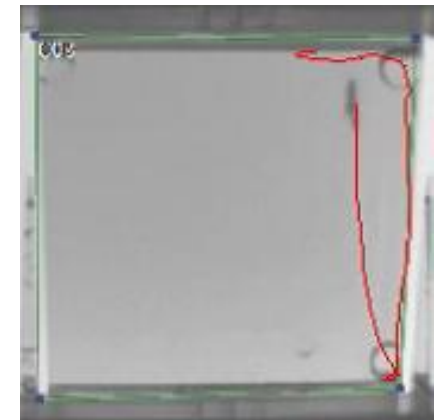
Source : Chemical Pollution in Europe's Seas : Programmes, Practices and Priorities for Research, Marine Board Poosition Paper 16. Calewaert J.B. and McDonough N. Marine Board-ESF Ostend, Belgium.

Effect Based Wastewater Surveillance

- Known ecotoxicology biomarker :
Avoidance Behaviour
 - Sensitive bio-activity measure
 - Quick response time
- Challenge : Quantify avoidance behaviour to indicate **micro-pollution presence** :
 - Centre of gravity tracking
 - Parallel organism tracking (ToxMate)



Source : Sub-Lethal Peak Exposure to Insecticides Triggers Olfaction-Mediated Avoidance in Zebrafish Larvae/ Konemann et. Al.

Low activity**Avoidance**

2 Gammarus fossarum tracked in the ToxMate over 2 seconds

Hypotheses

1. Avoidance behaviour response to micropollutants

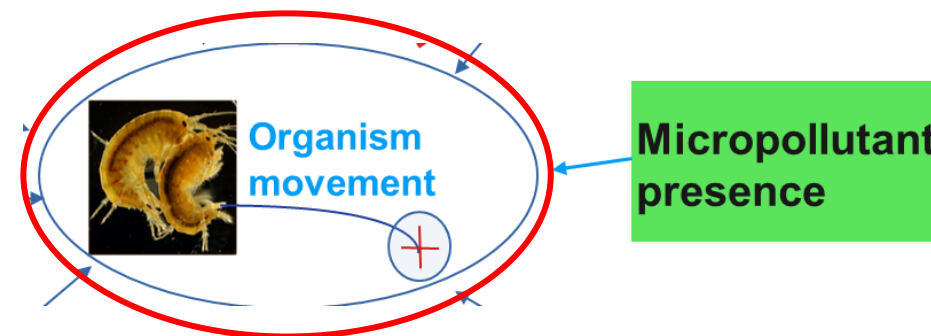
- Reproducible testing protocol
- External bias testing
- Testing of robustness

2. Multi-dimensional testing for pattern identification

- Difference in responses to chemicals
- Machine learning testing

3. Data-Mining and AI improvements

- Massive testing
- Targeted industrial testing
- Links to chemical structure



Hypotheses

1. Avoidance behaviour response to micropollutants

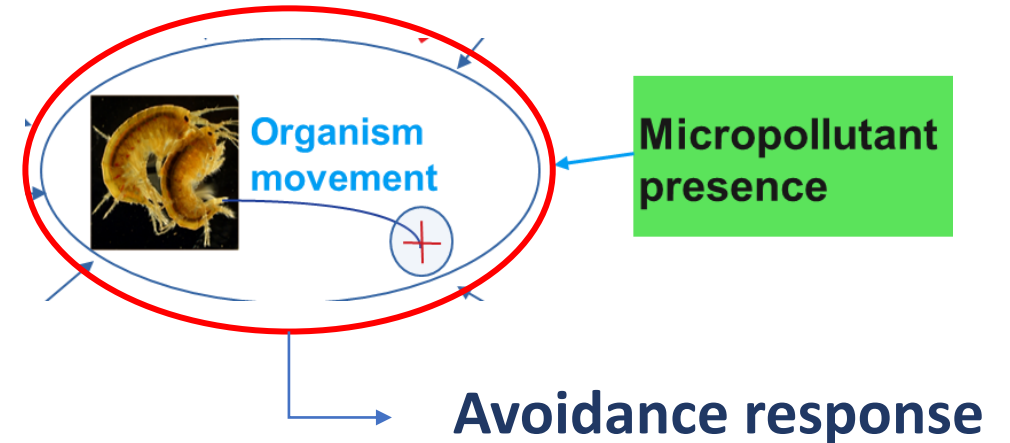
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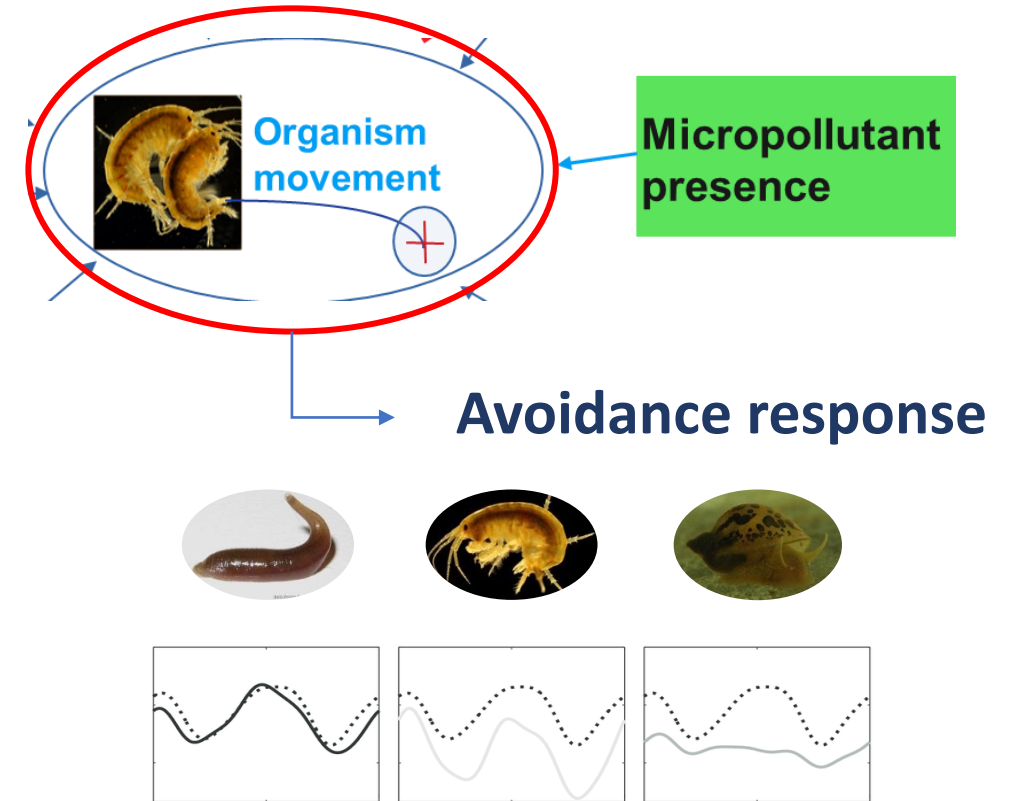
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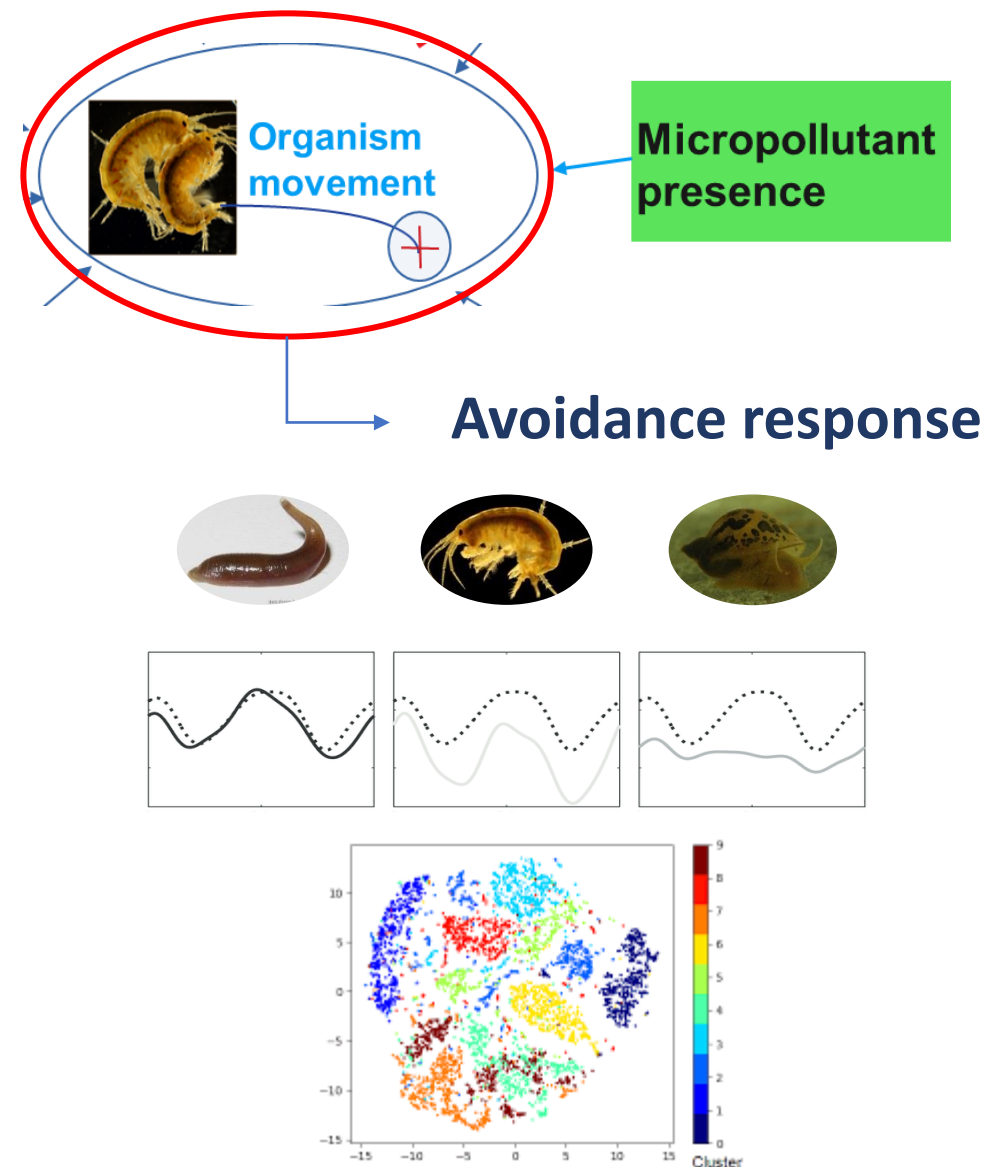
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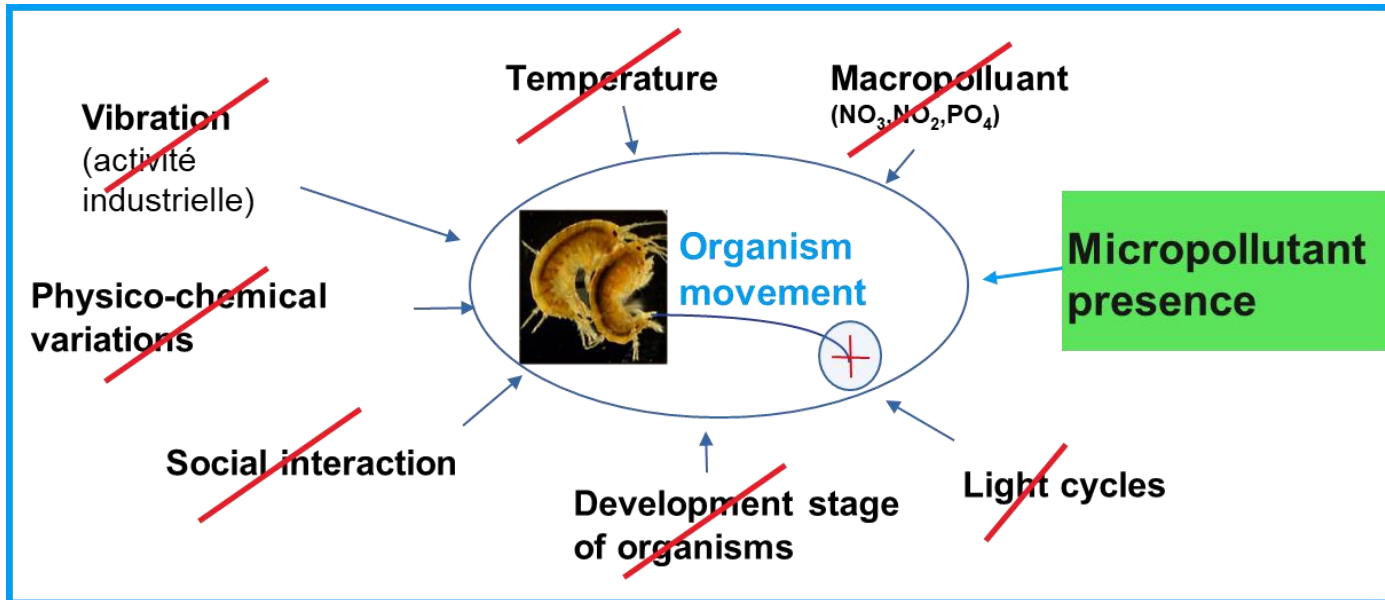
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- Targeted industrial testing
- Links to chemical structure



Lab Testing

- Conditioning of organisms in lab conditions to reach a **minimal activity reference**
- Avoidance behaviour to detect CEC presence



Industrial Application

- Validation for operational use in industry

Model macro-invertebrates

Gammarus fossarum



Erpobdella testacea



Radix auricularia



- Pharmaceuticals
 - PAHs
 - Solvents / Flame retardants
- (Dangerous substances for the aquatic environment found in WWTP outlets. Technial report 2021 - INERIS)



- >NOEC Gammarus
(No observed effect concentration)

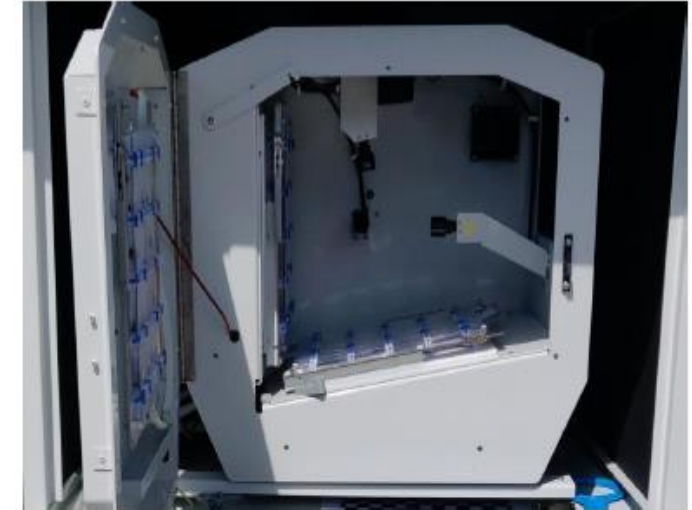


ToxMate™

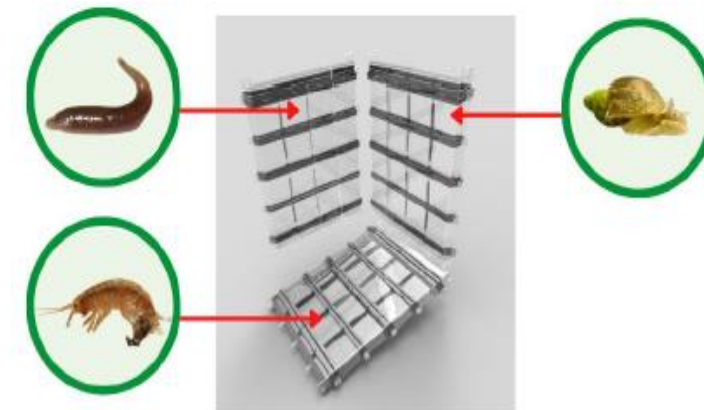
Methodology

ToxMate Surveillance

- 2014 — Development of ToxMate surveillance station
- 2016 — **Lab Testing** first data collection
- 2018 — Industrial adaptation for long-term operational use



Interior of ToxMate surveillance station with observation cameras for videotracking

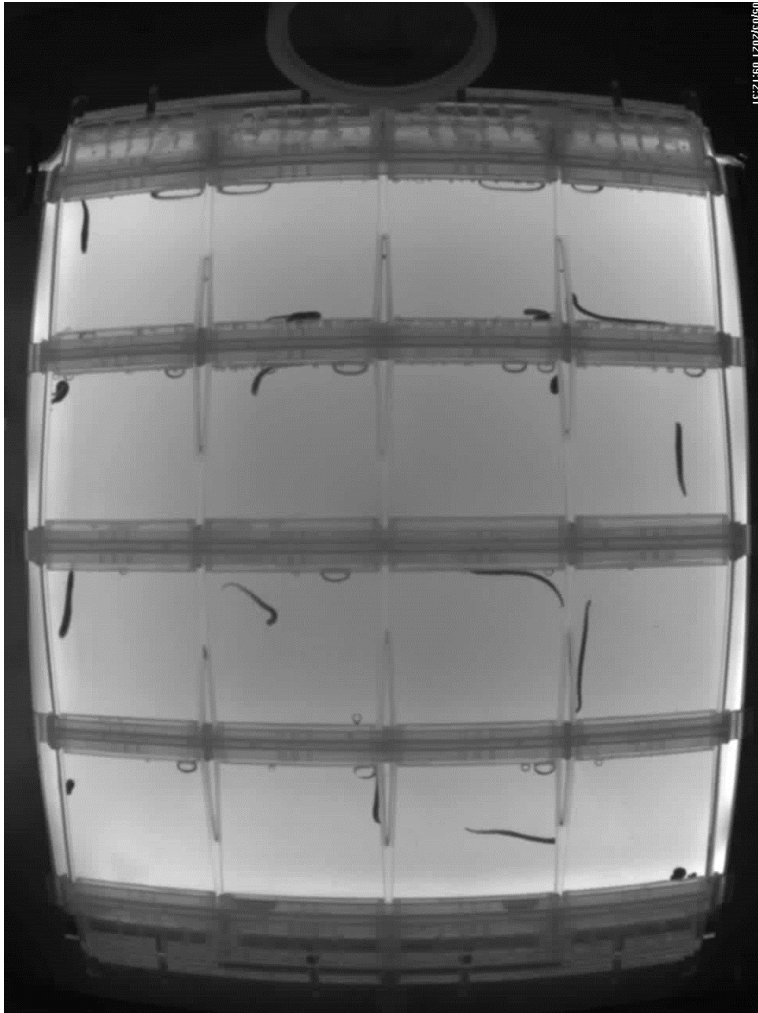


3 observation panels for 3 bio-model species with constant water current.

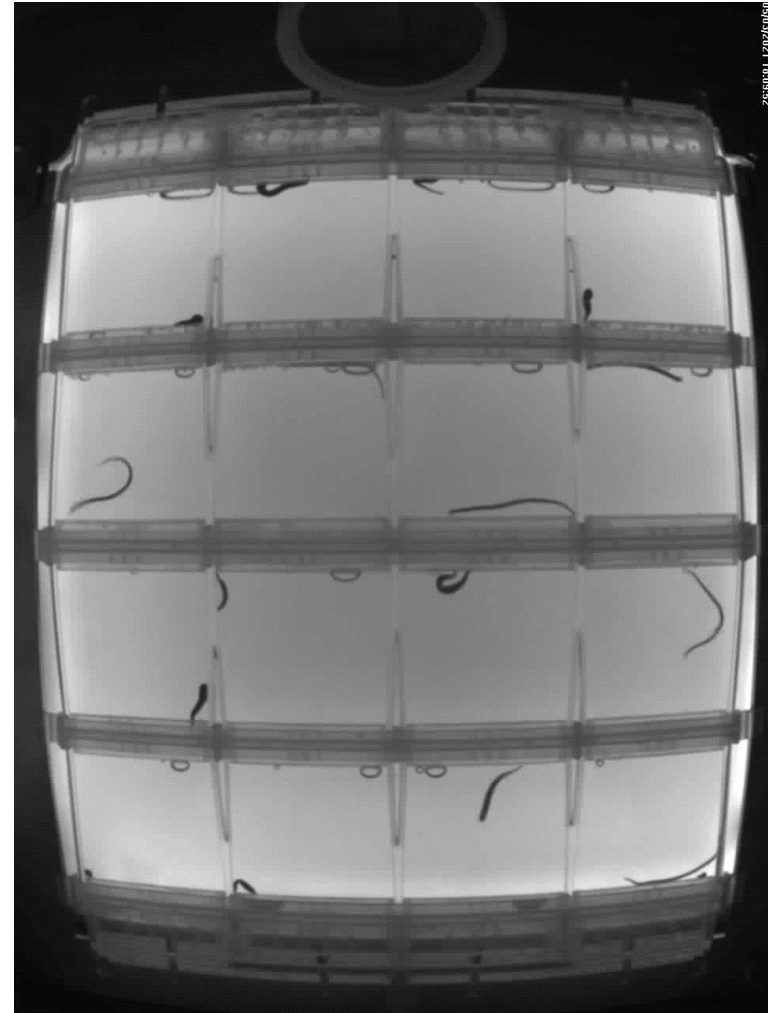
Videotracking software

Sampling	< 40 ms
Repetability	16 organisms, 3 species
Data Output	~ 300 000 datapoints / minute
	RAW – C.O.G. : (x, y) + Area
	XLS – Automatic agregation
	AVI– images XVID

Minimal activity state

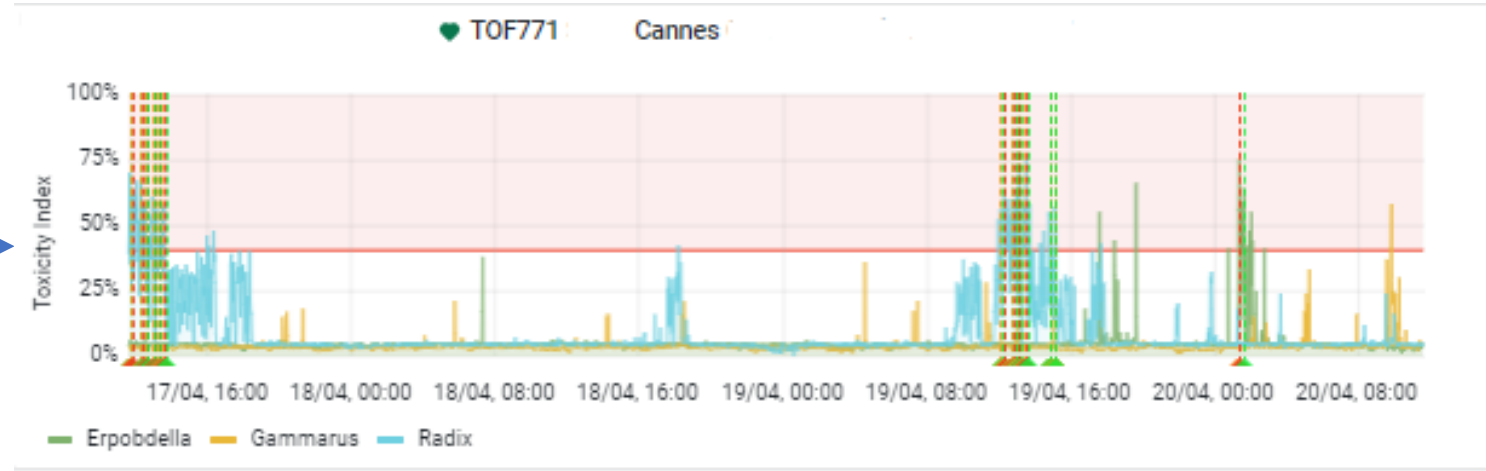


Avoidance

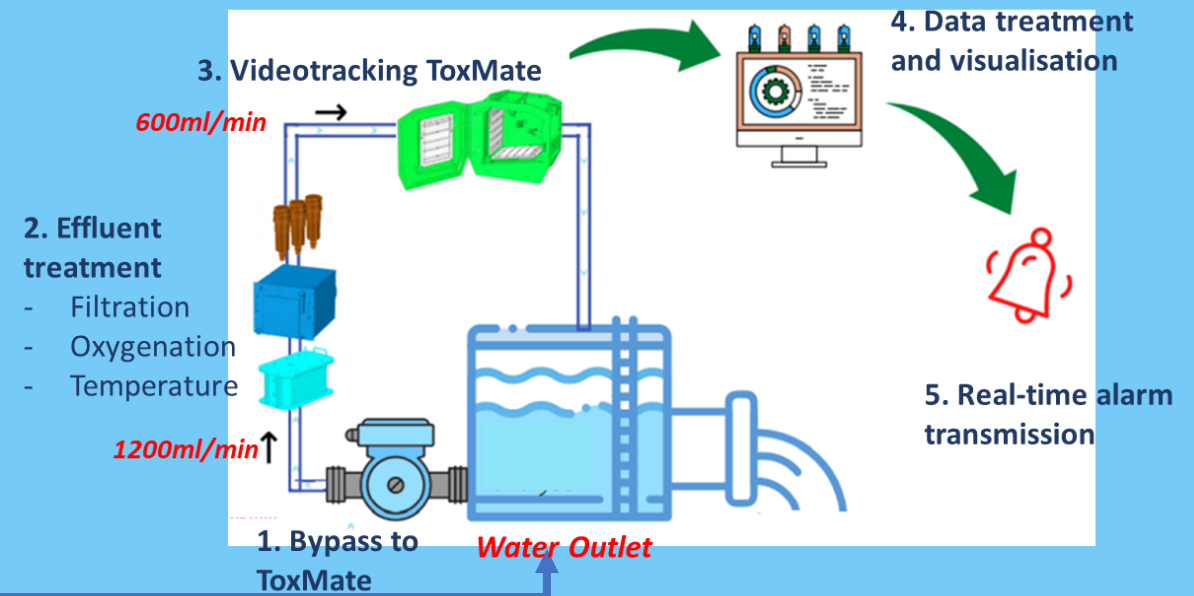


WWTP operational surveillance

- WWTP outlet bypassed to ToxMate
- Continuous surveillance (>2years)
 - **Critical moment detection**
- **2019-present** : >20 ToxMates deployed for continuous surveillance across Europe



Alert Panel for industrials at WWTP site in Canne France.

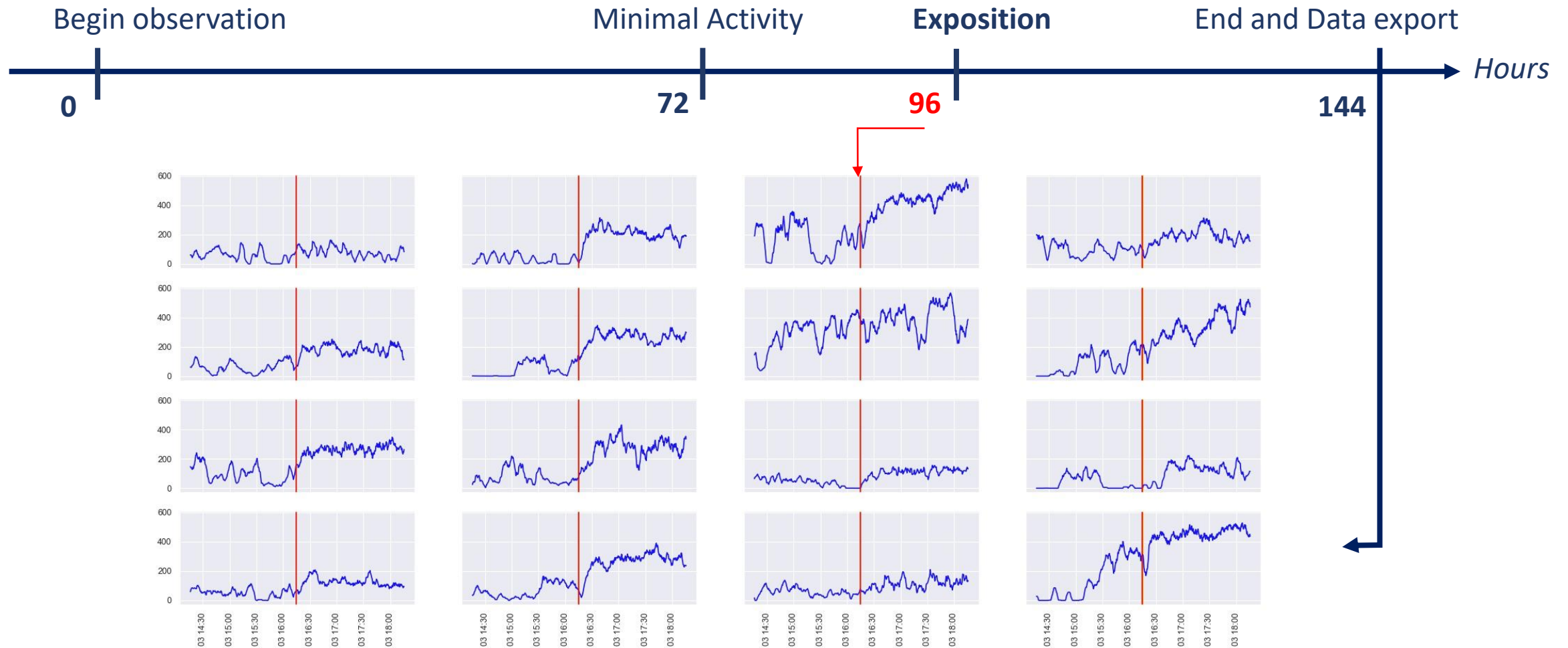




ToxMate™

Results

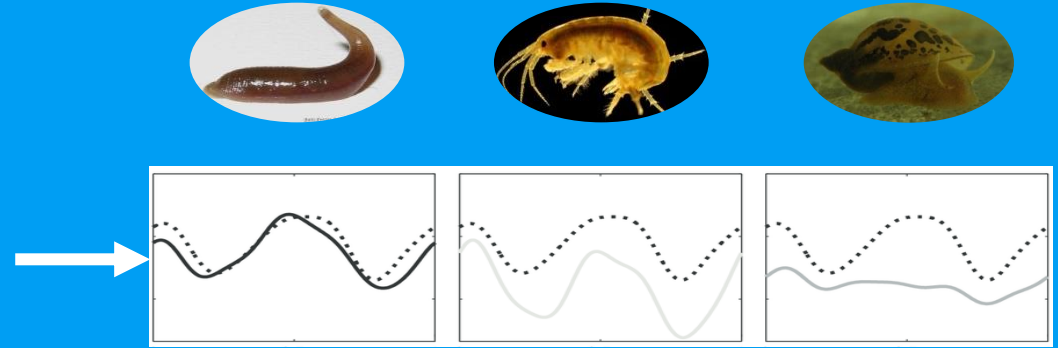
One experimental result set



Signal processing

- Accumulation of 16 individual trajectories

*Movement
curves*



Experimental Protocol Definition

Acclimation

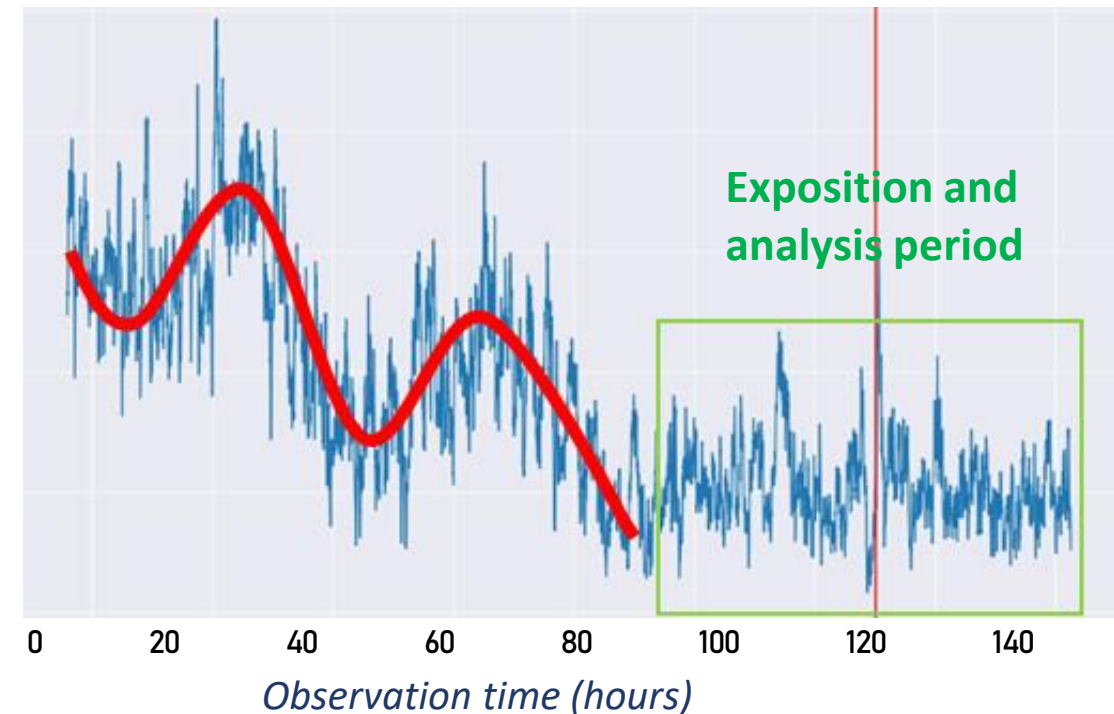
- ToxMate conditioning reduces external confusion
- CEC exposition after 72 hours

Minimal activity

- Reference behaviour (close to zero)

Gammarus mean activity measure (n=16)

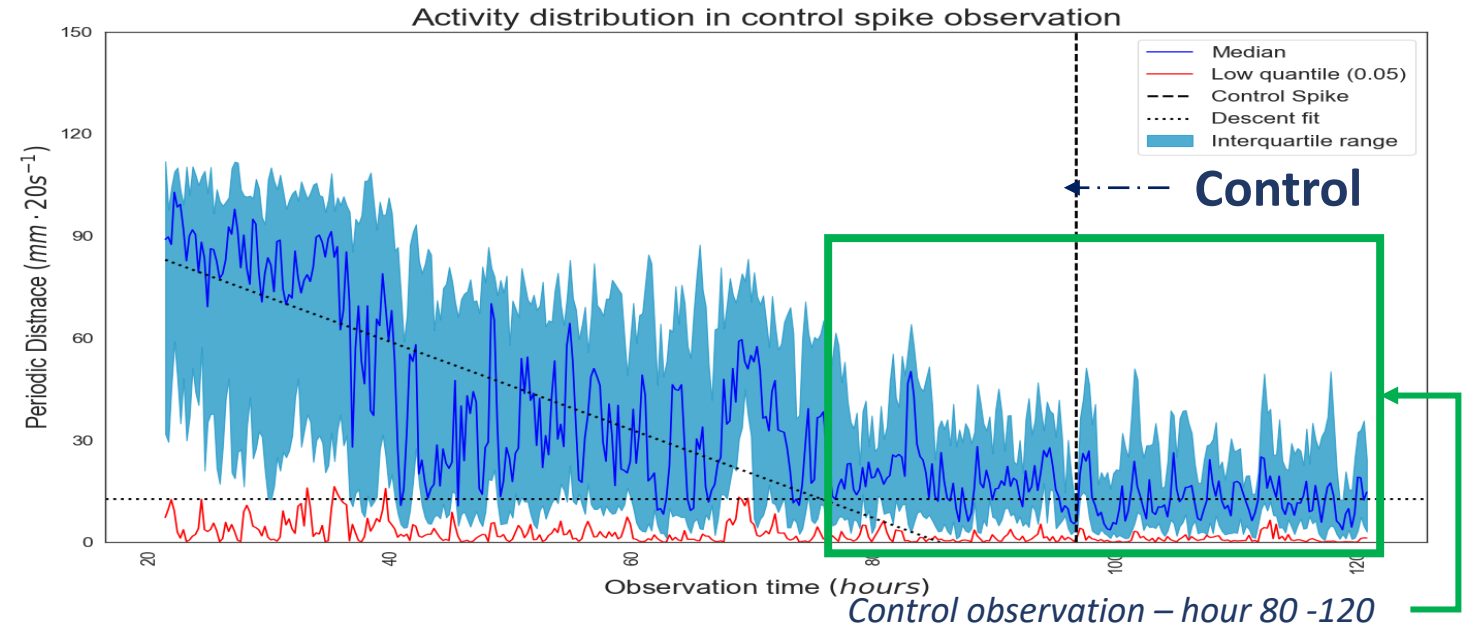
Day cycle presence reduced



Signal processing

Reproducibility

- Distribution shows **minimal activity** in lower quantile movements
- Observation period after 3 days

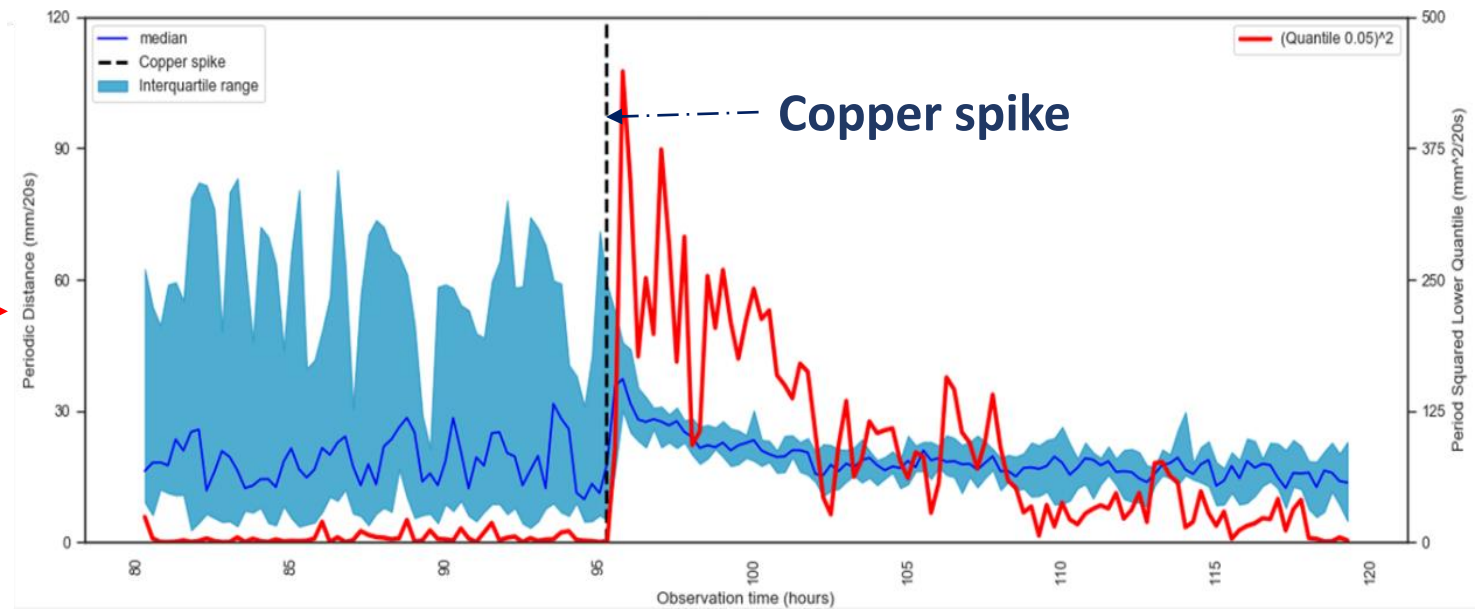
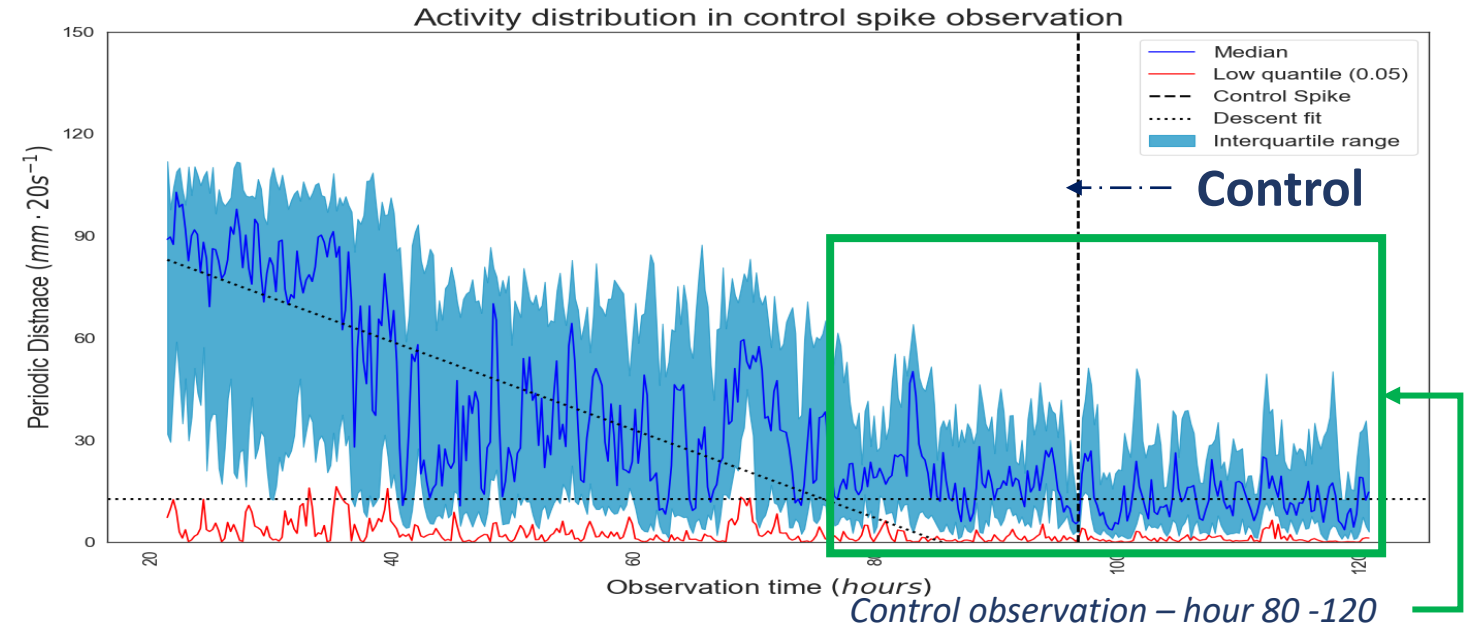


Signal processing

Reproducibility

- Distribution shows **minimal activity** in lower quantile movements
- Observation period after 3 days
- Instantaneous avoidance reaction in activity signal upon introduction of chemical

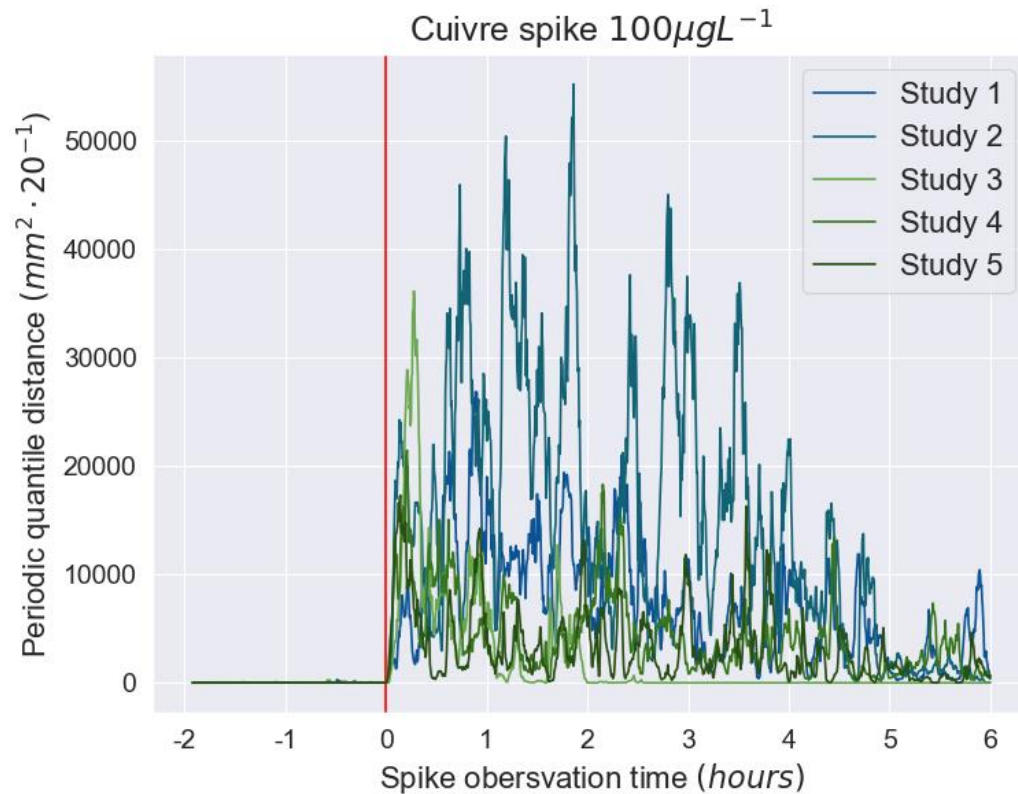
Micropollutant observation – hour 80 -120



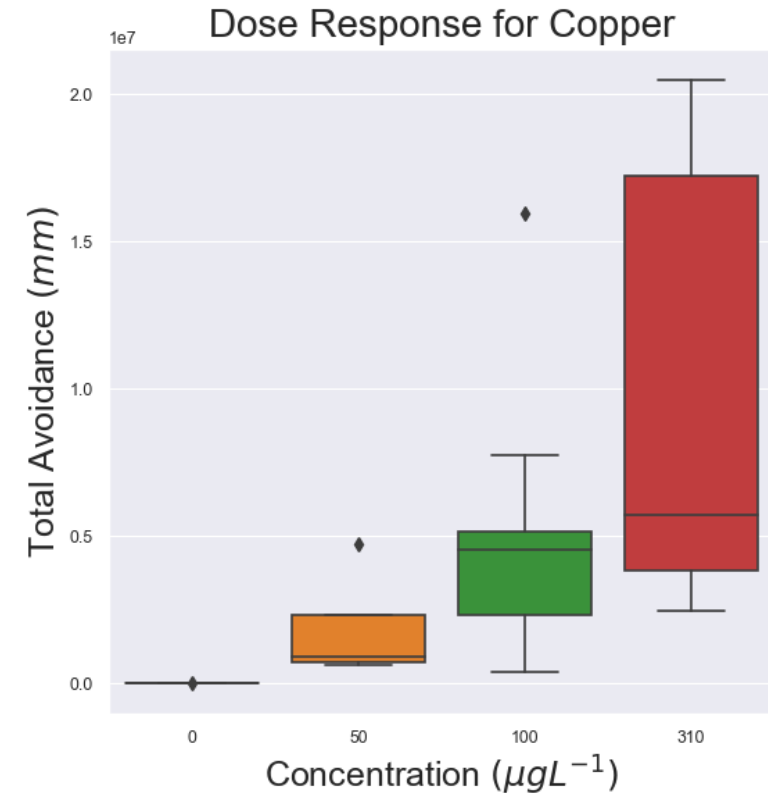
CEC exposition spikes

Repetitions for reference chemicals

- 5 repetitions for copper ($100\mu\text{g/L}$)



- Dose response in avoidance behaviour?



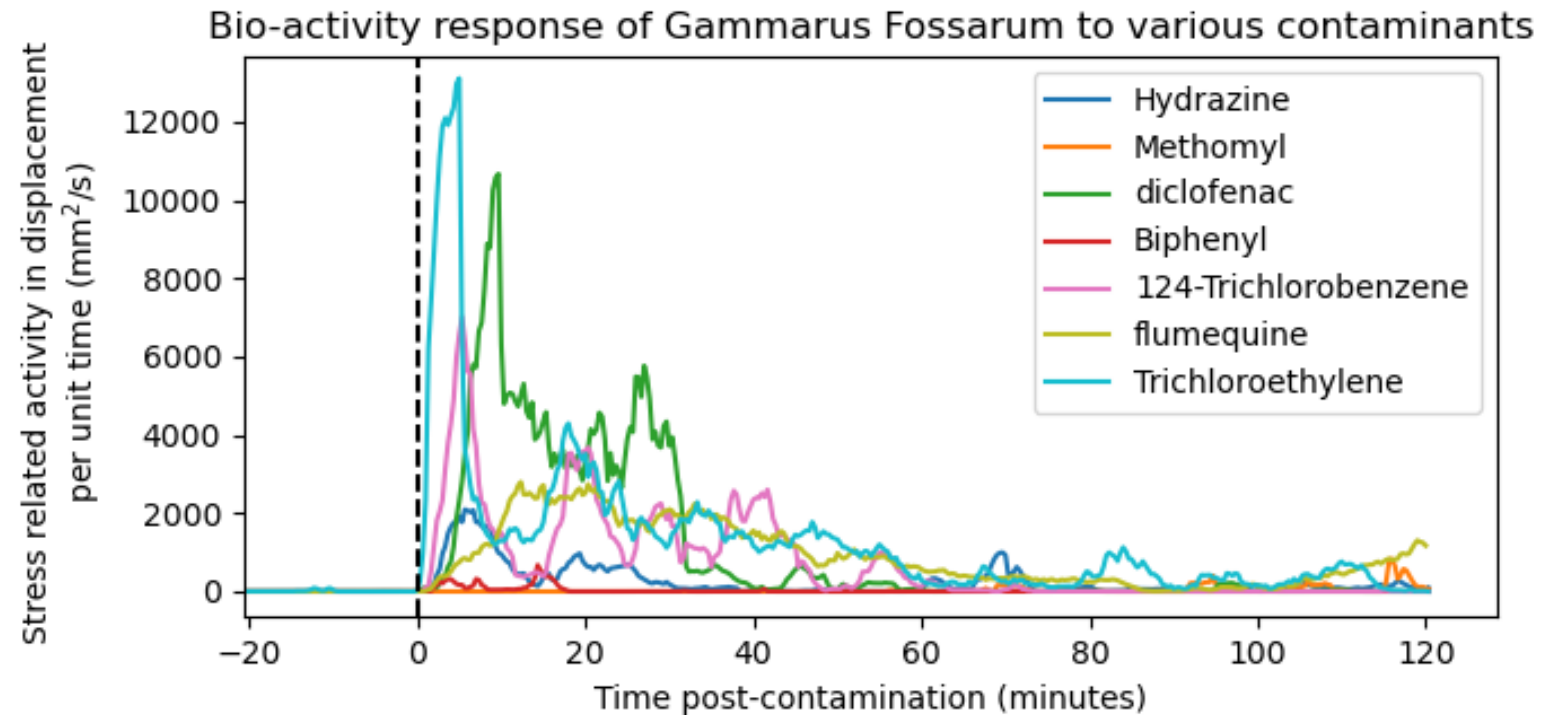
CEC exposition spikes

Diverse molecule selection

- Molecules of concern in WWTP across France selected

Varied response patterns
(*Gammarus*)

- Avoidance observed for numerous micropollutants (>50)
- Differences in *Gammarus* response

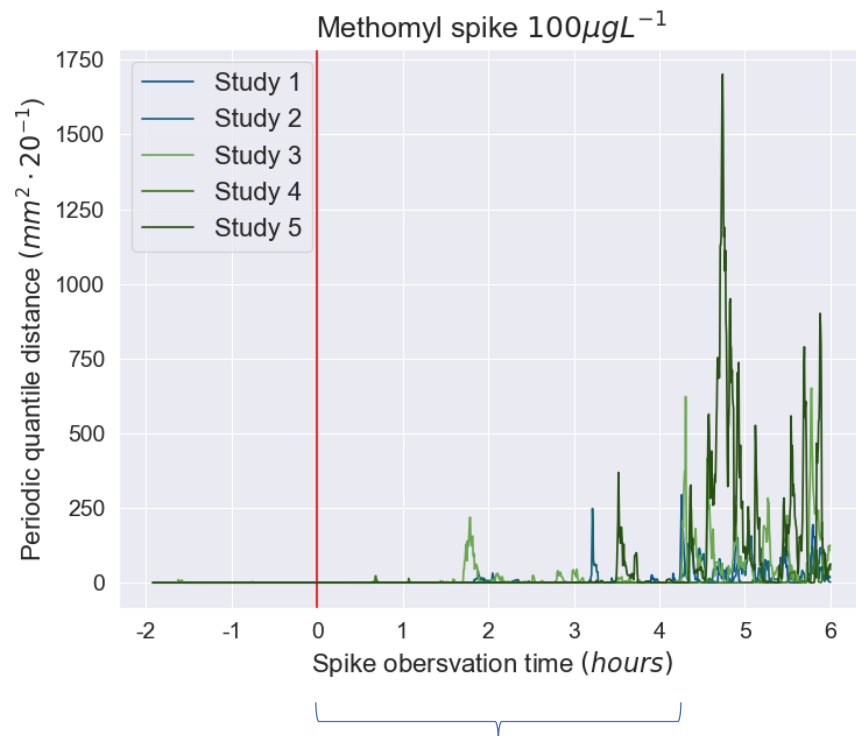


Gammarus avoidance bio-activity profiles for 7 CEC spikes

CEC exposition spikes

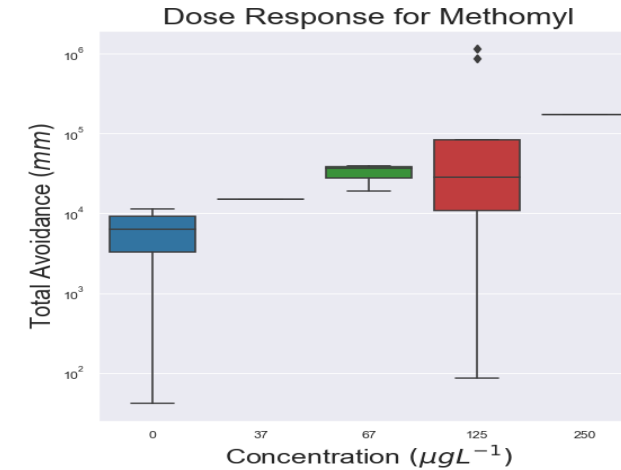
Repetitions for reference chemicals

- 5 repetitions for methomyl (125 $\mu\text{g/L}$)



Delayed response? – 4 hour avoidance?

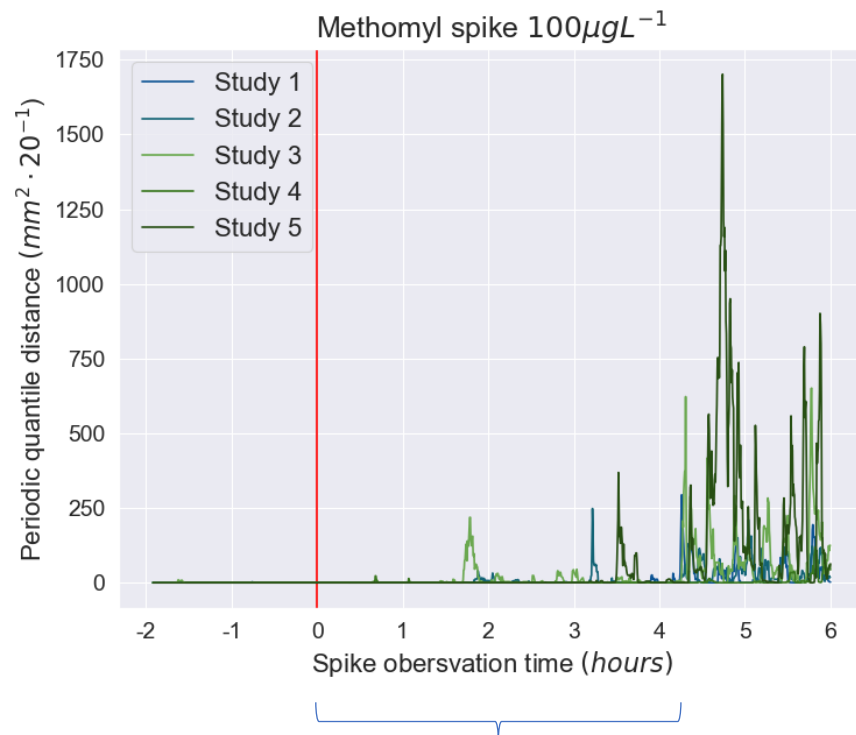
- Dose response in avoidance behaviour



CEC exposition spikes

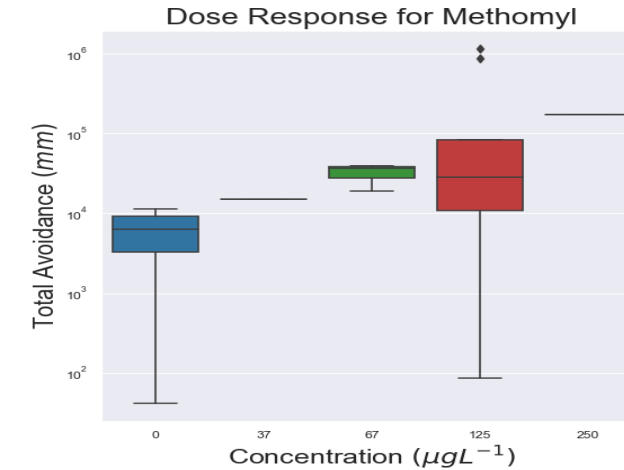
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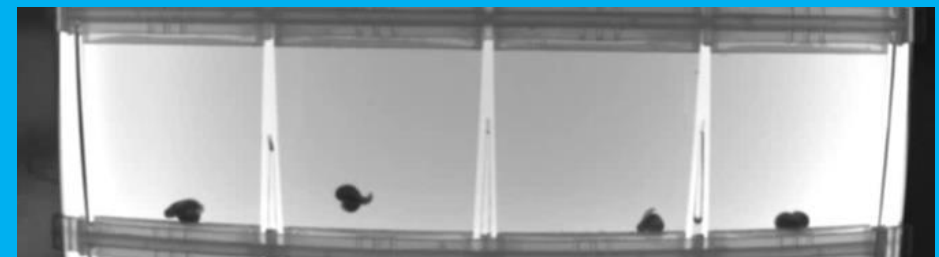
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- Multi-species advantage, erpobdella response :



Immediate drop in activity
to zero movement



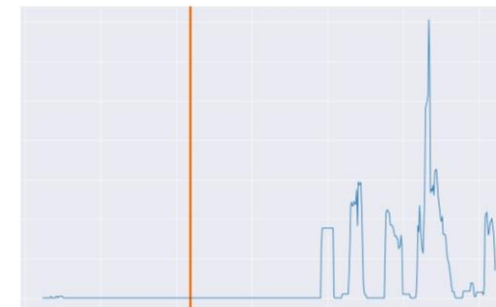
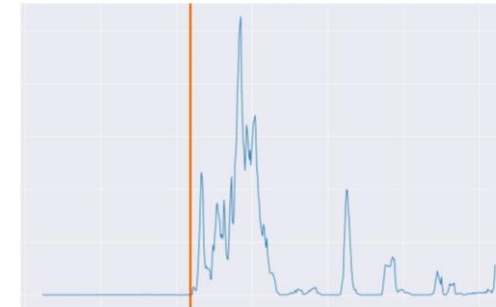
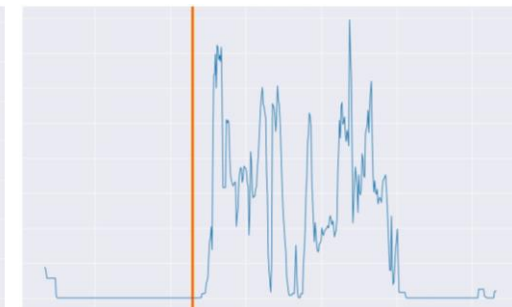
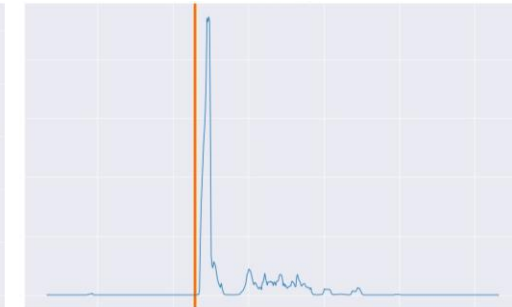
CEC exposition multi-spikes

Fingerprint detection

- Better response definition through multi-dimensional signal

Response patterns

- Initial classification method developed for a group of substances :
 - ACP fonctionnelles
 - Classification de courbes
- Further clustering may reveal links between contaminants (>100 reponses)

Hydrazine
spike reactionTrichloroethylene
spike reaction

Varying multispecies response for two solvent micropollutants

Current classification models

Curve description

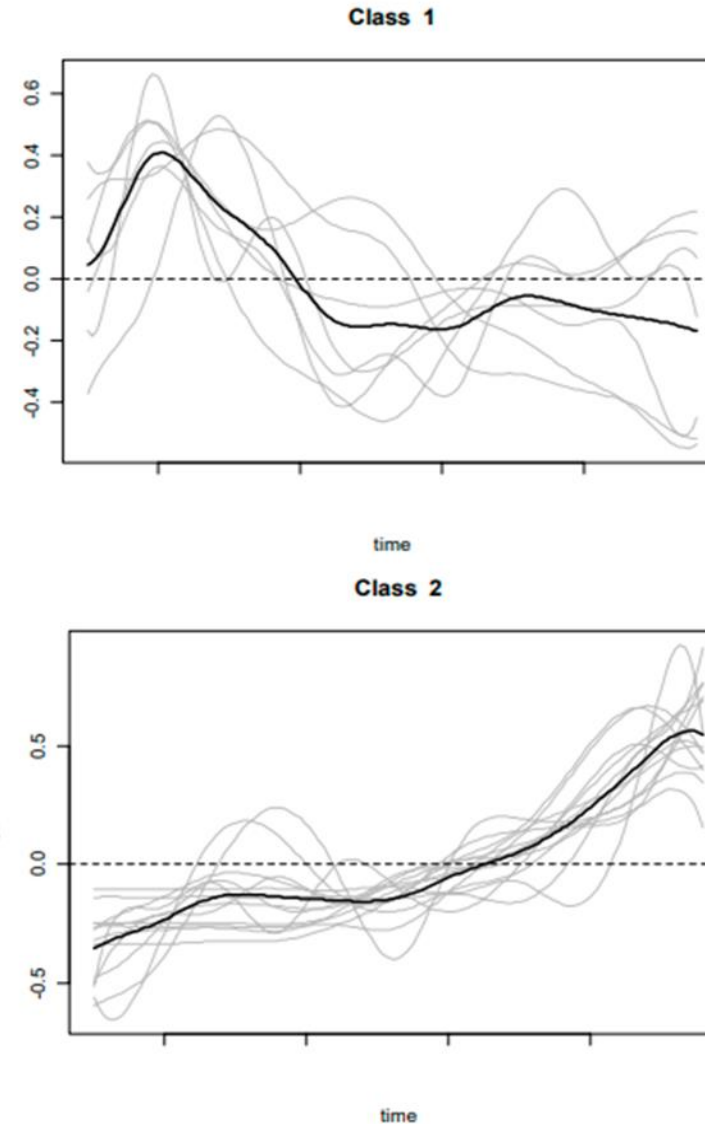
- Functional Principal Component Analysis (FPCA)
- Curve Classification methods

Machine Learning

- Supervised classification
 - Type random Forest
- Unsupervised classification
 - Type hierarchical clustering

Future work.

- Neural network techniques

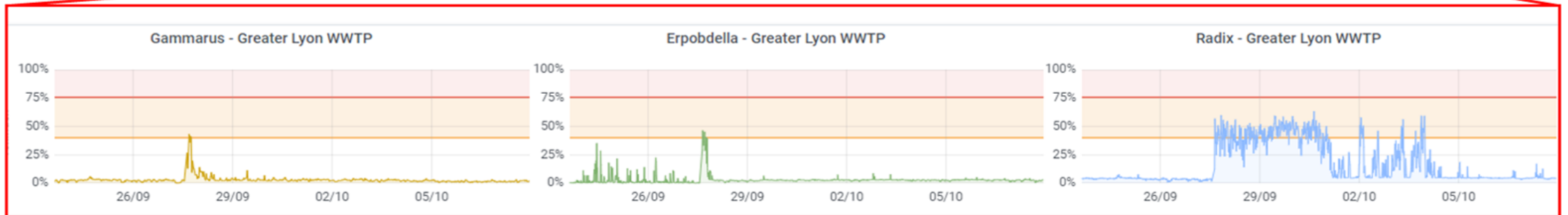
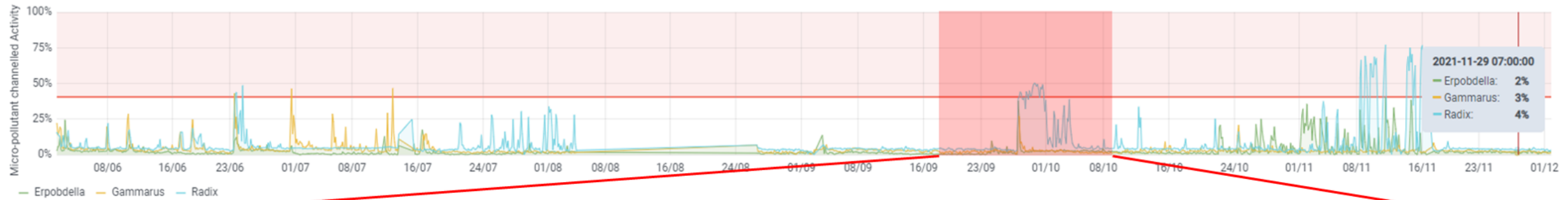


Classes of mean activity response for the model substances

Critical moment detection in industry

Observation over long periods in WWTPS

6 Month monitoring at Greater Lyon Conglomeration Wastewater Plant



- Difference in activity profile for the 3 species
- **Behavioural fingerprinting?**

Merci



- | | |
|-----------------------------|--|
| Directeurs | - A. Chaumot, JB Aubin, JL Bertrand-Krajewski, O. Geffard, A. Decamps, D. Neuzeret |
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| Equipe technique | - M. Dauphin, A Deletang, |

Questions?