



**INRAE**

**deep**

**ViewPoint**  
Biosurveillance de l'eau

*EEDEMS 2022*

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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



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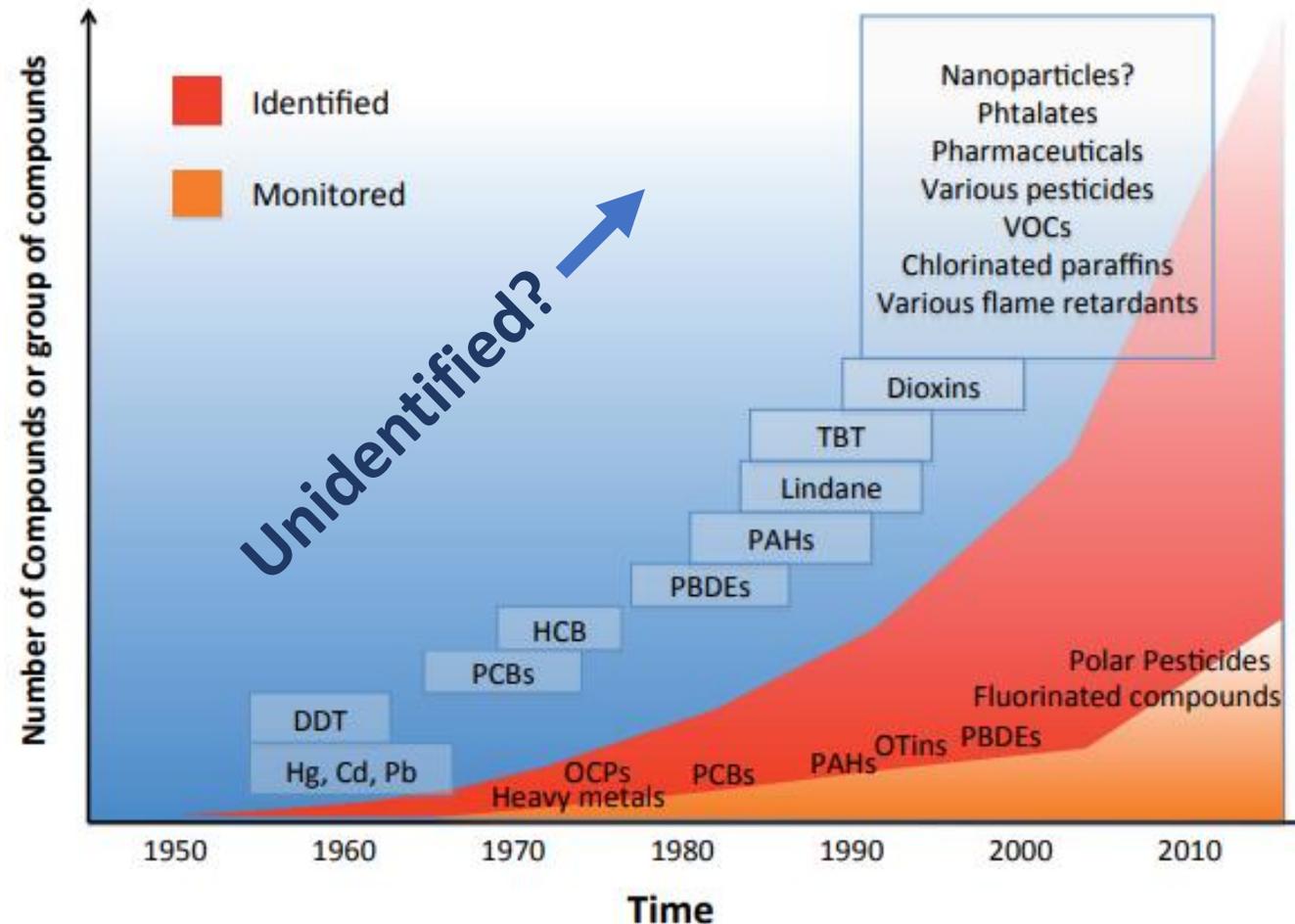
deep

ViewPoint  
Biosurveillance de l'eau

- PhD Context and Objectives
- Methodology
- Results
- Outlook

## Emerging Aquatic Micropollution

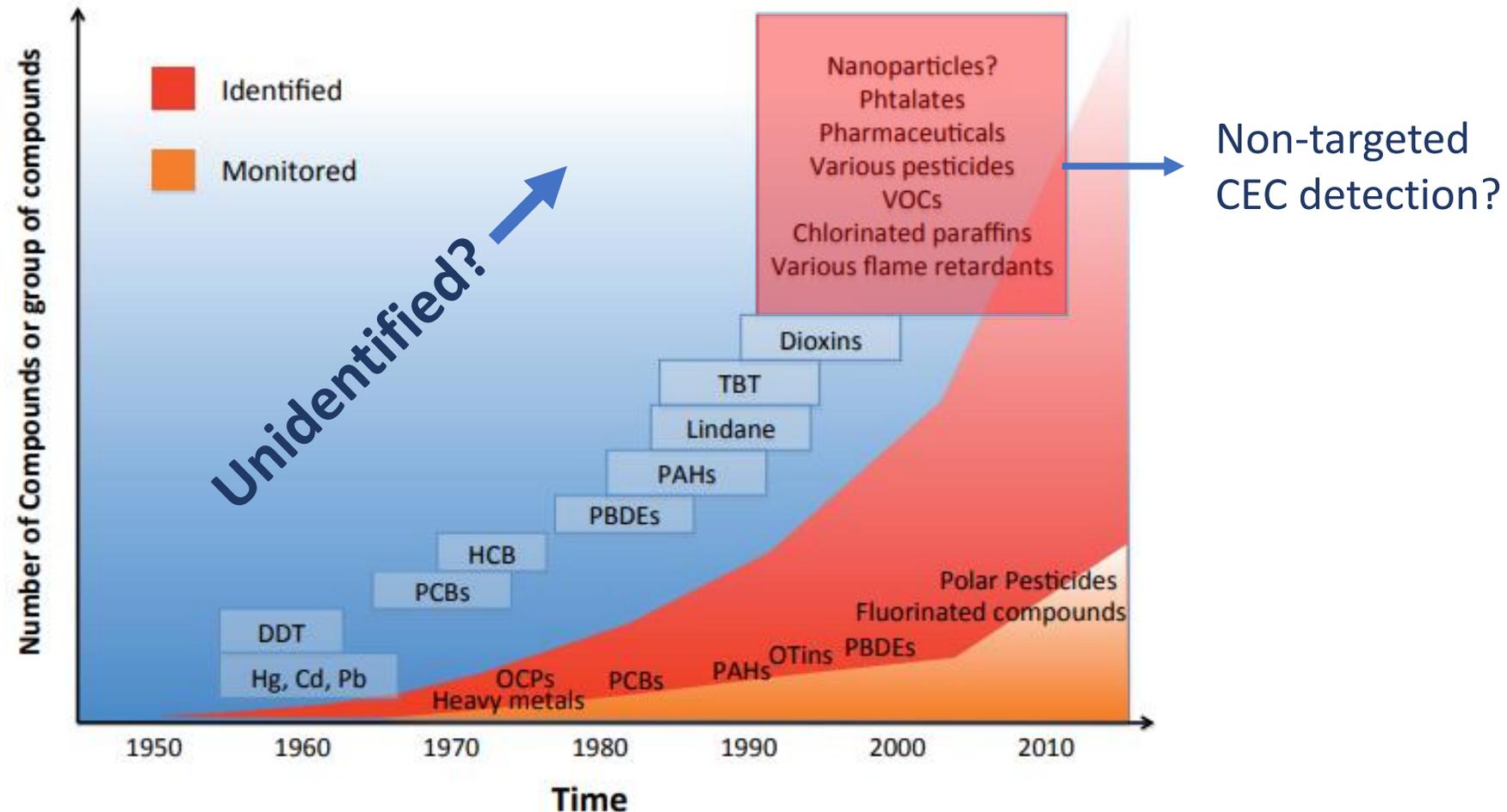
- Negative CEC impact often poorly understood
- Absence of CEC monitoring in wastewater management (WWTPs)
  - One shot campaigns
  - Grab sampling
- Temporal variability in discharge



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

## Emerging Aquatic Micropollution

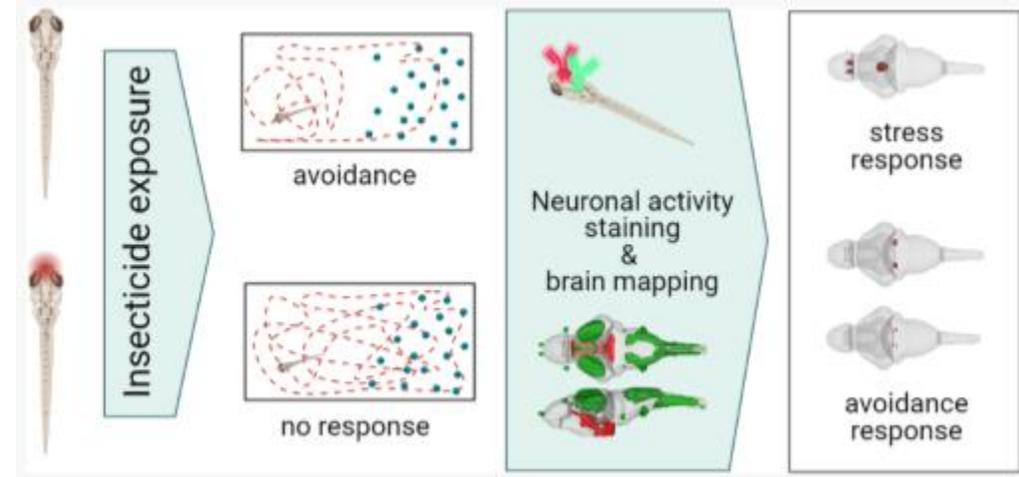
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- Temporal variability in discharge
- Objective : **Real-time CEC identification**



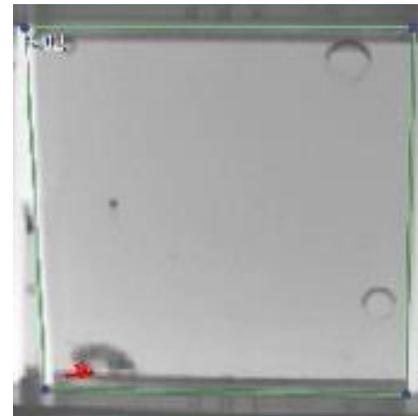
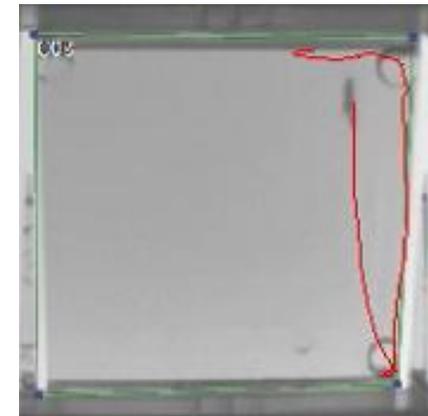
Source : Chemical Pollution in Europe's Seas : Programmes, Practices and Priorities for Research, Marine Board Poosition Paper 16. Calewaert J.B. and McDonough N. Marine Board-ESF Ostend, Belgium.

## 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

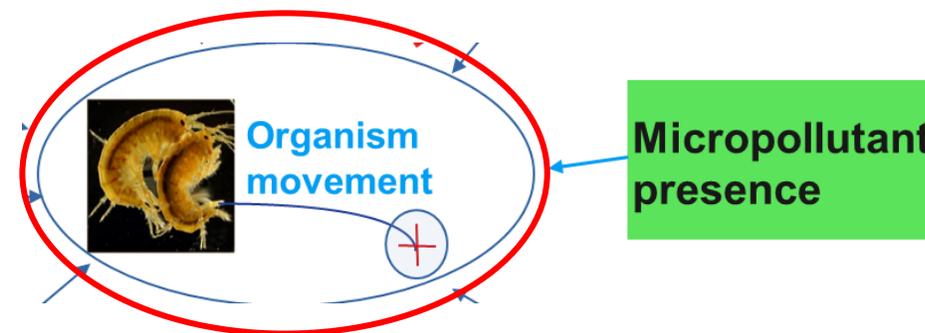
- Reproducible testing protocol
- External bias testing
- Testing of robustness

### 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

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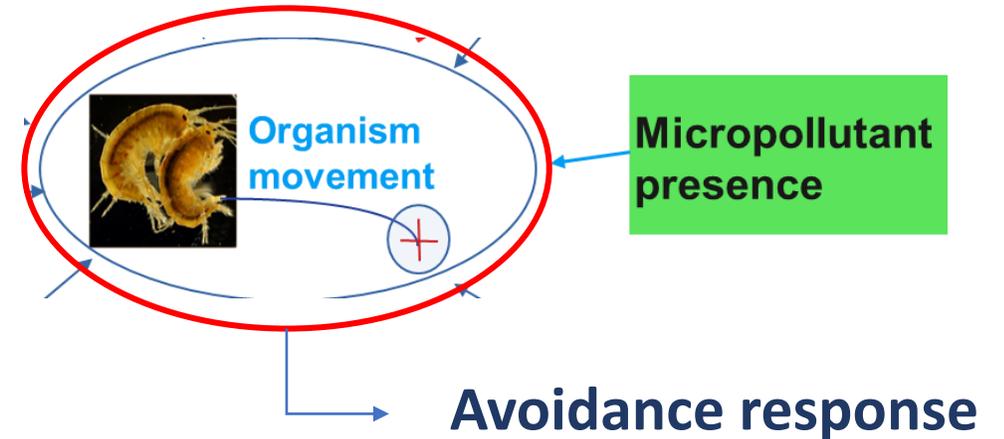
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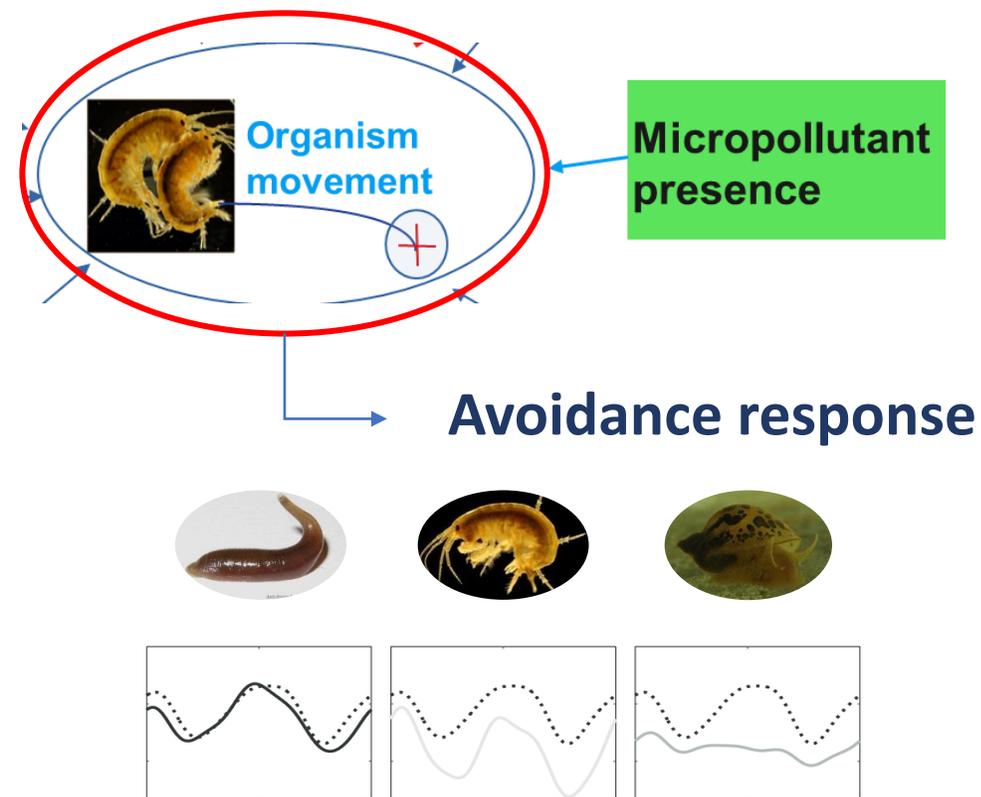
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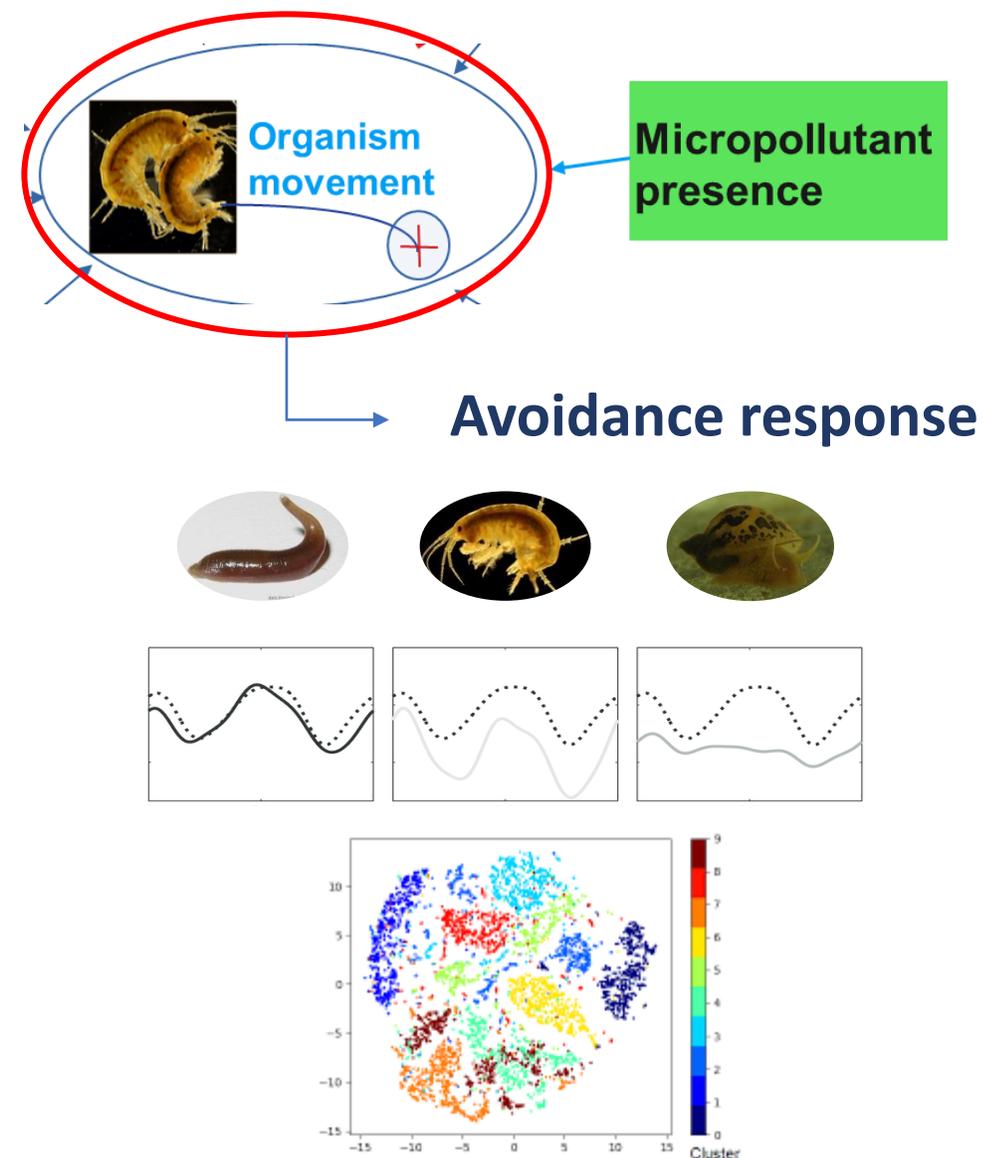
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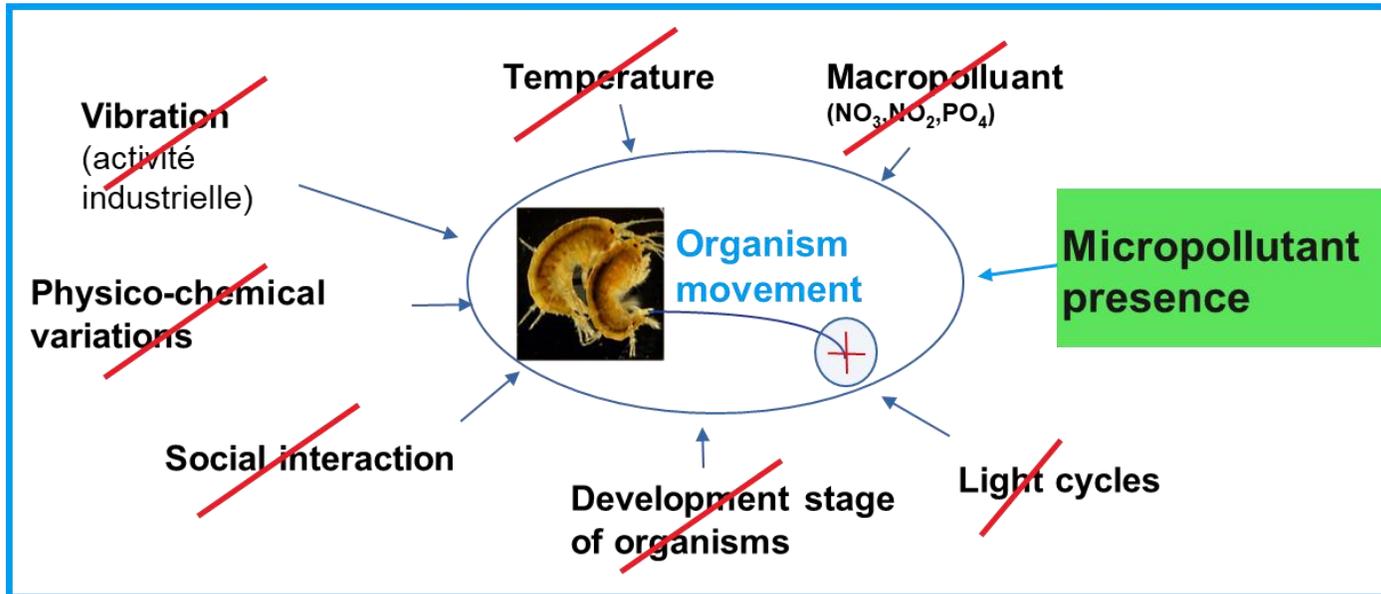
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- Massive testing
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## Lab Testing

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



## 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)

## Industrial Application

- Validation for operational use in industry



# ToxMate™

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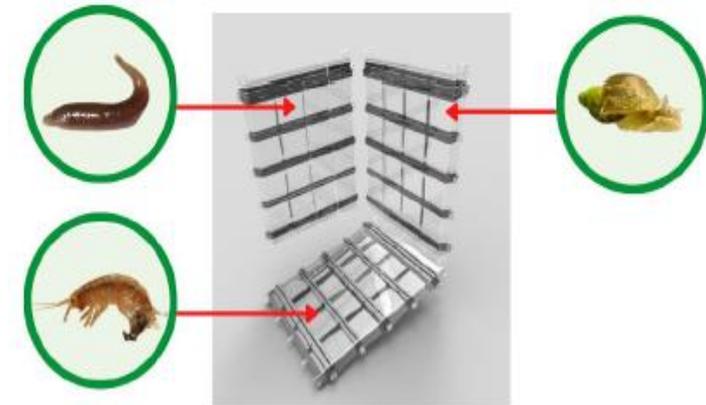
## Methodology

## 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*

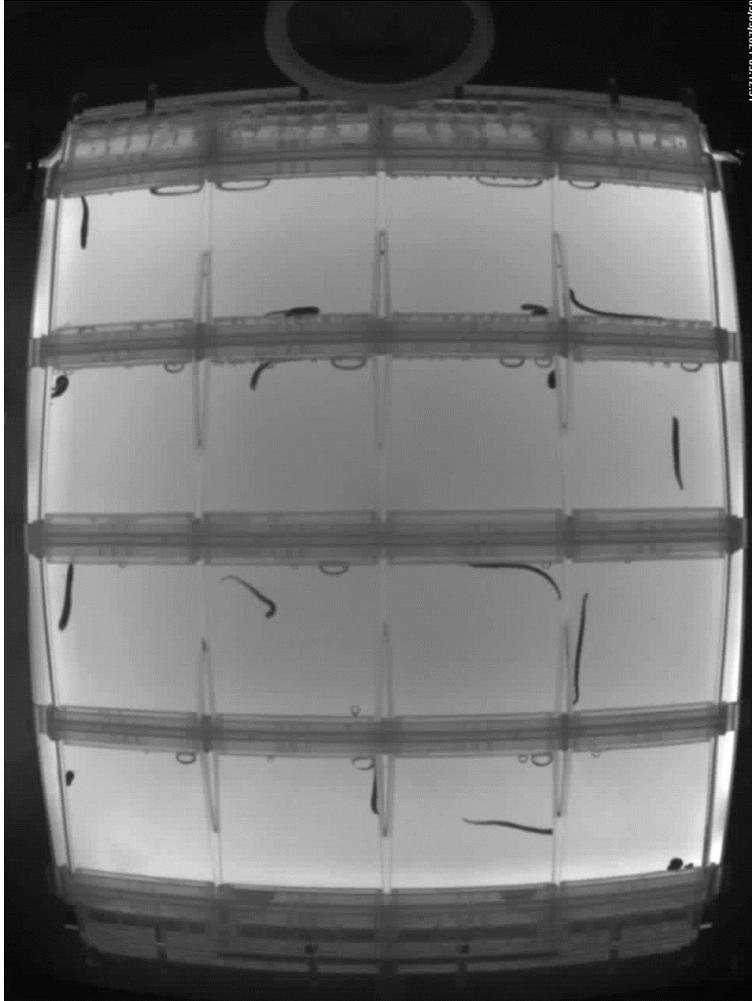


*3 observation panels for 3 bio-model species with constant water current.*

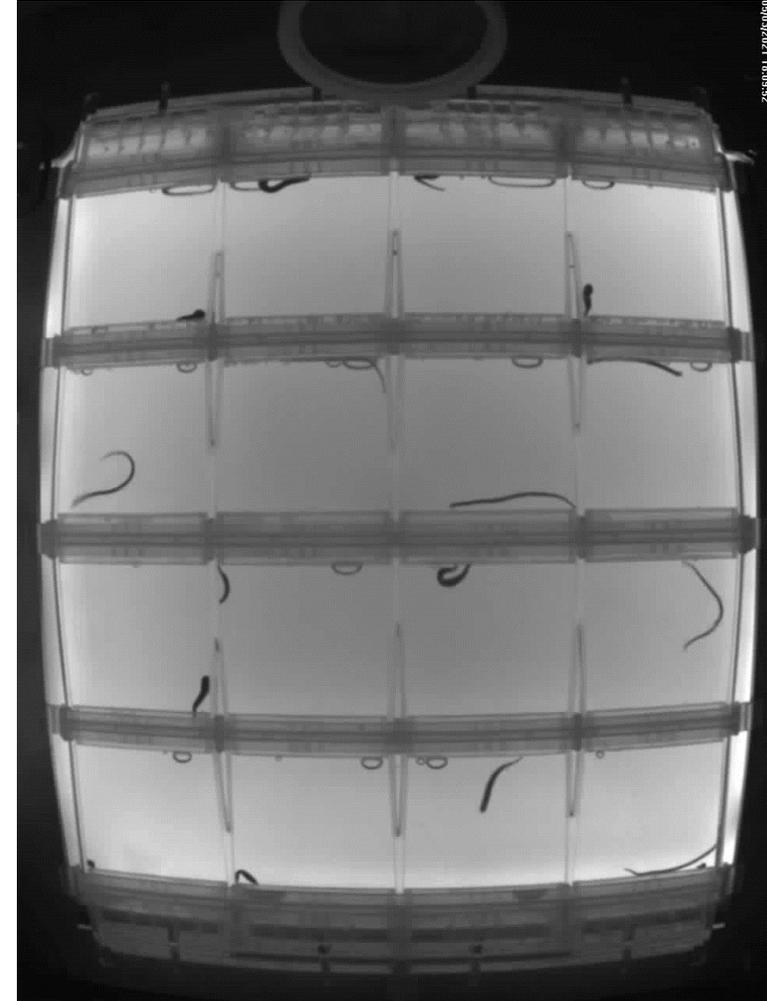
## Videotracking software

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

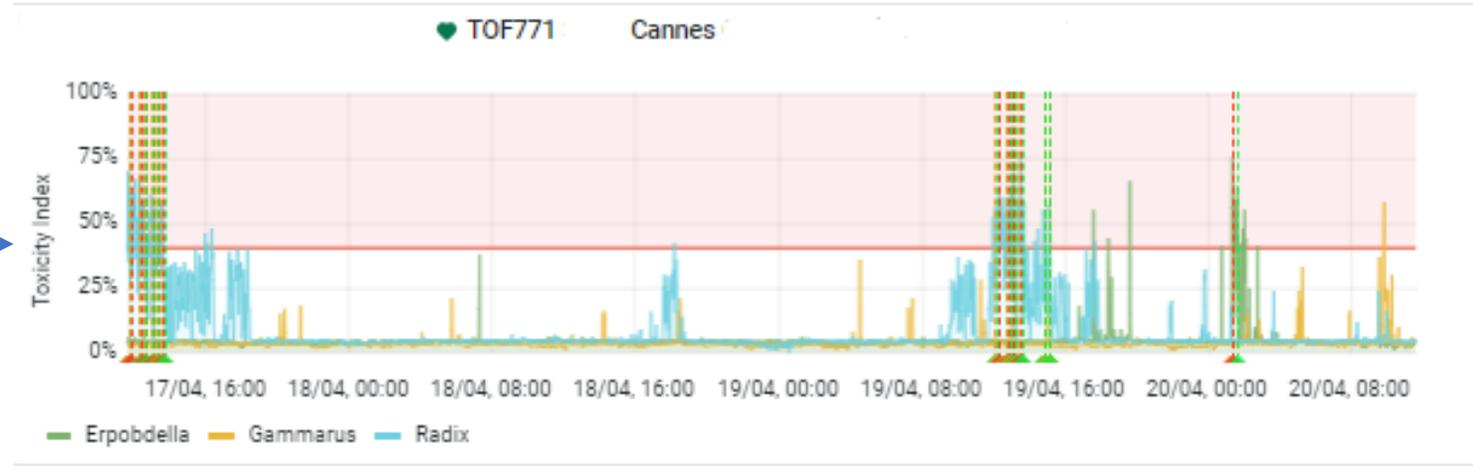


## Avoidance

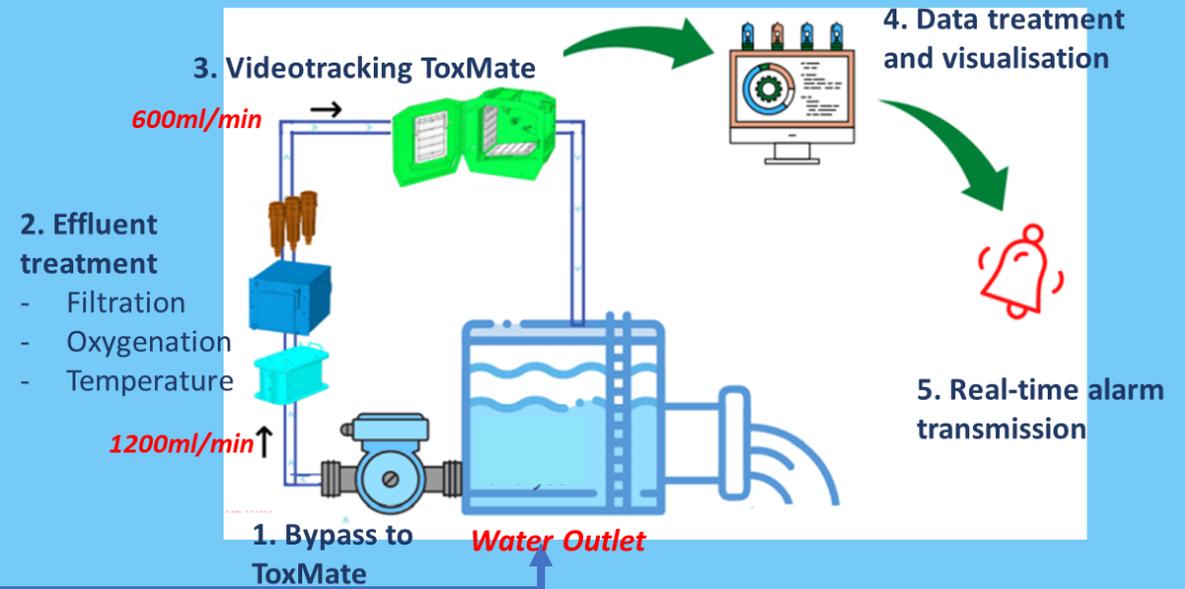


## WWTP operational surveillance

- 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 Cannes France.



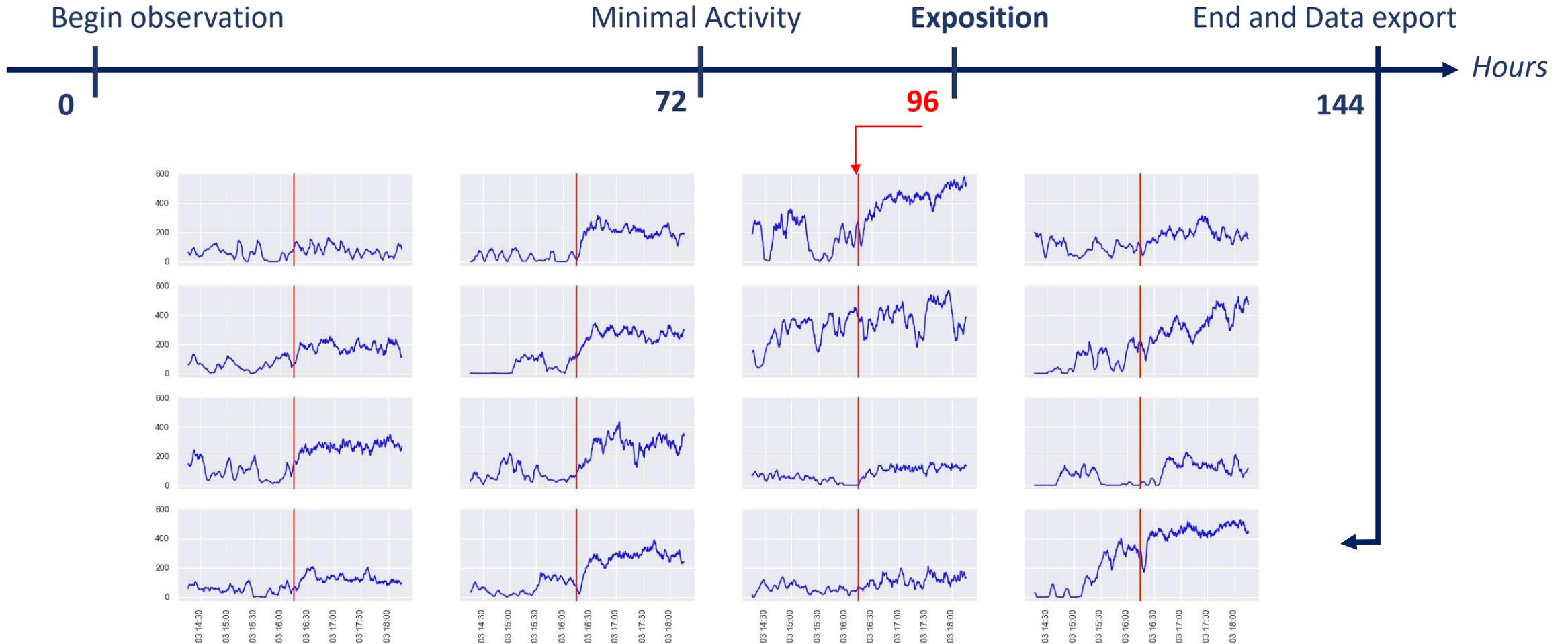


# ToxMate™

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## Results

## One experimental result set

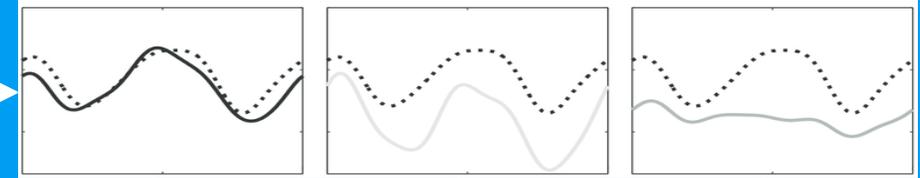


## Signal processing

- Accumulation of 16 individual trajectories



*Movement curves*



## Experimental Protocol Definition

### Acclimation

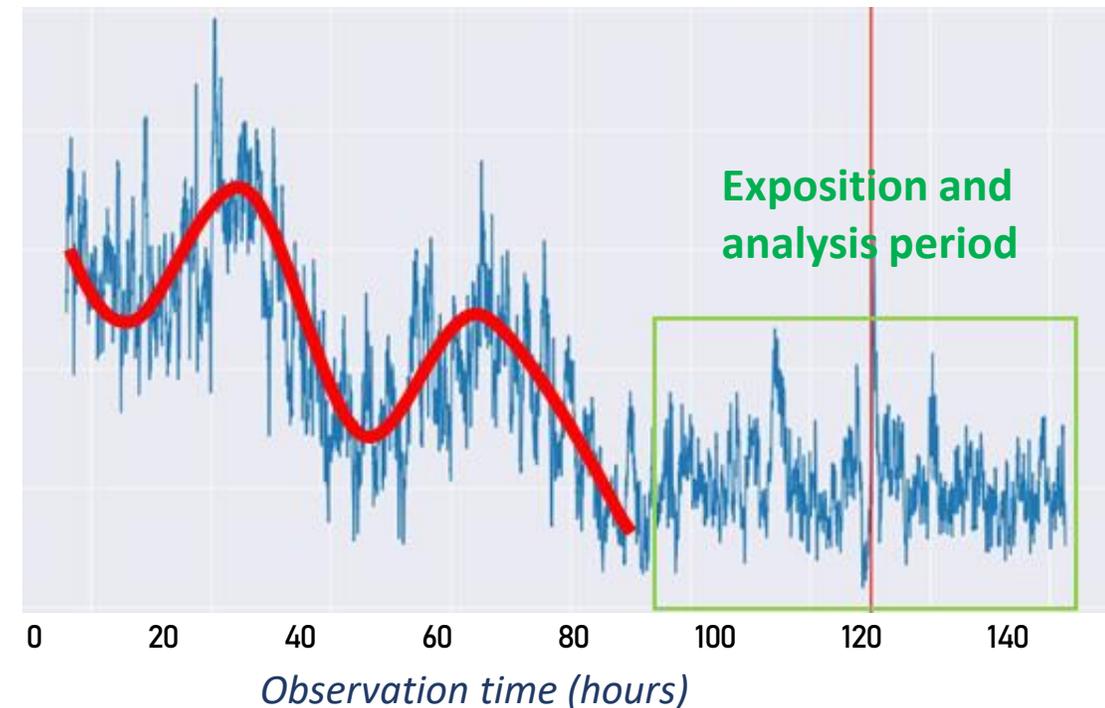
- ToxMate conditioning reduces external confusion
- CEC exposition after 72 hours

### Minimal activity

- Reference behaviour (close to zero)

### Day cycle presence reduced

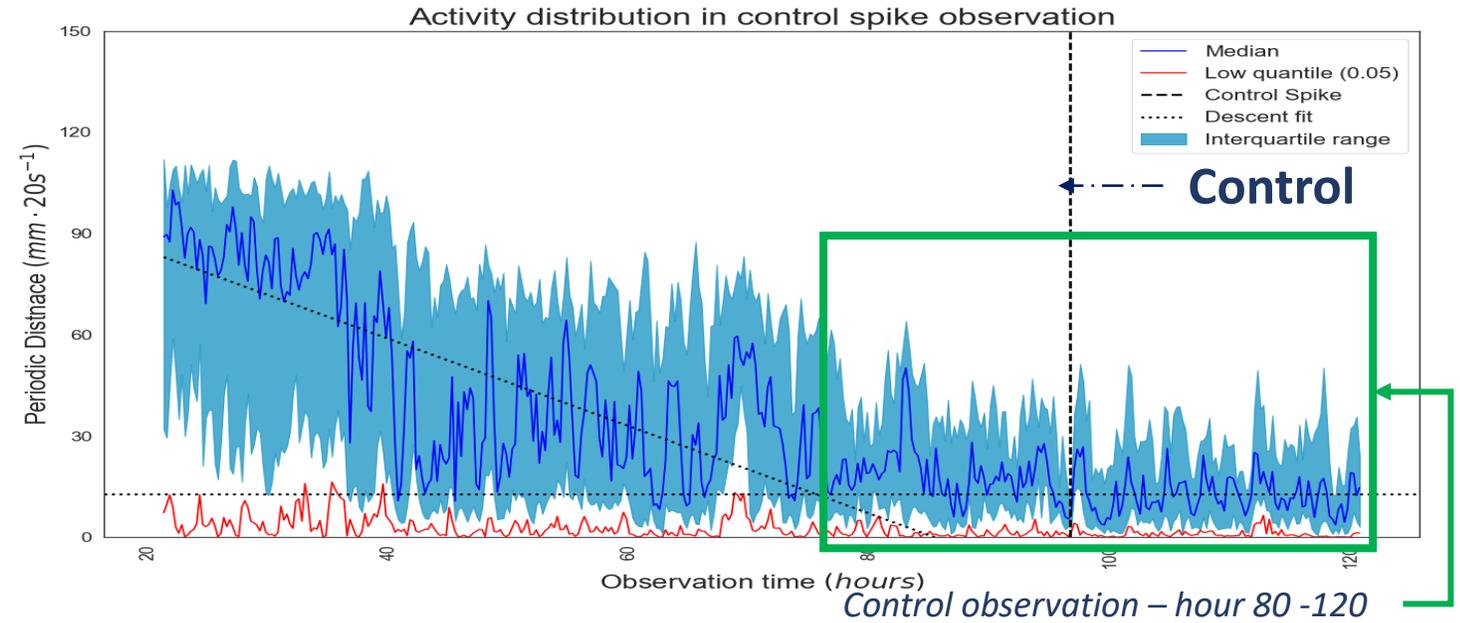
*Gammarus mean activity measure (n=16)*



## Signal processing

## Reproducibility

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

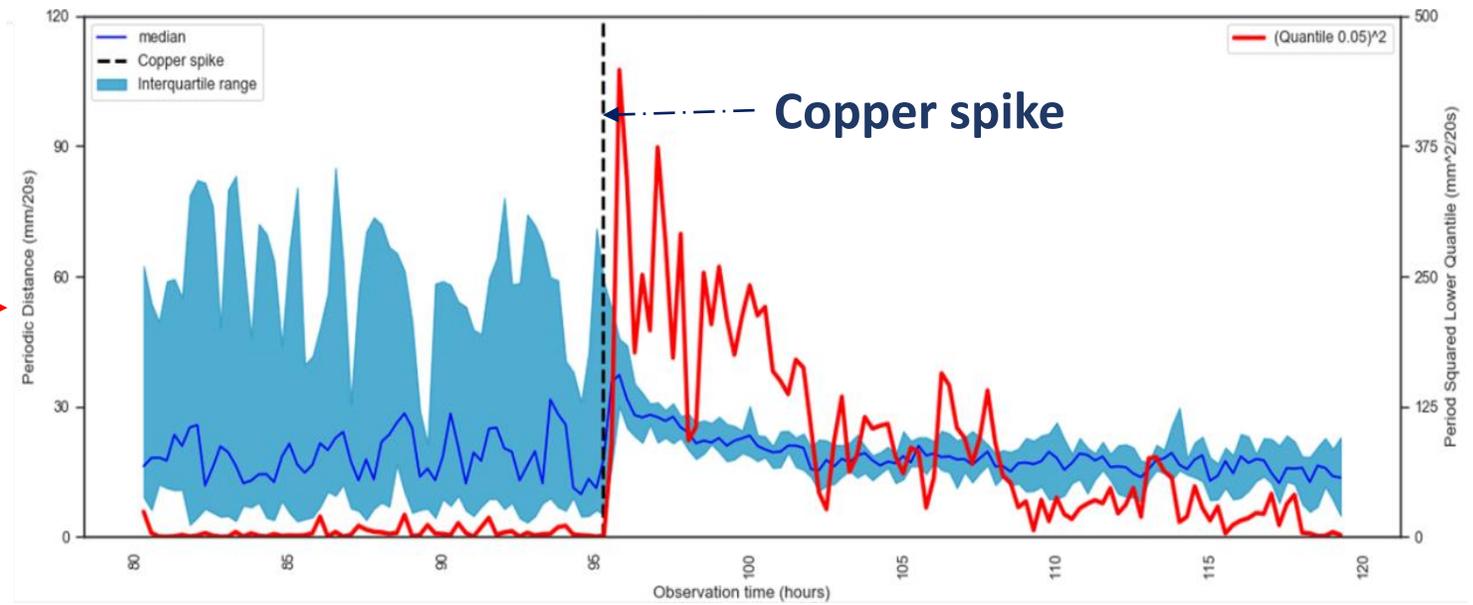
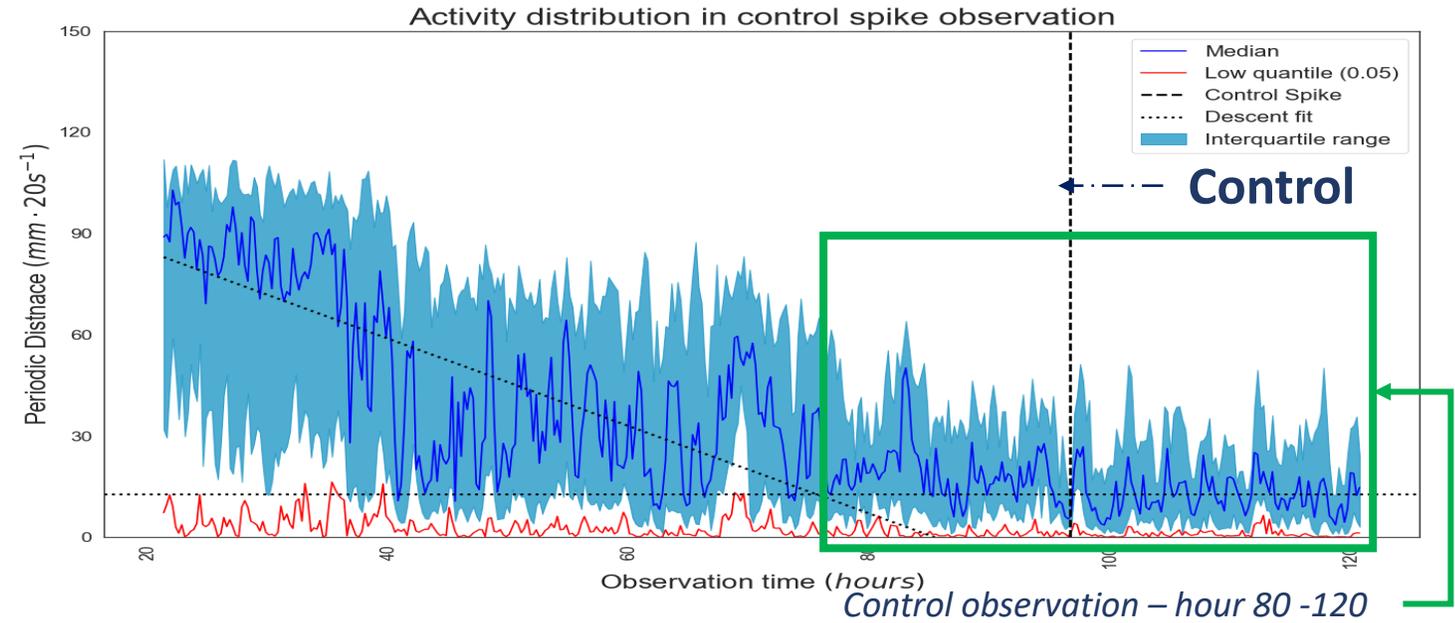


## Signal processing

### Reproducibility

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

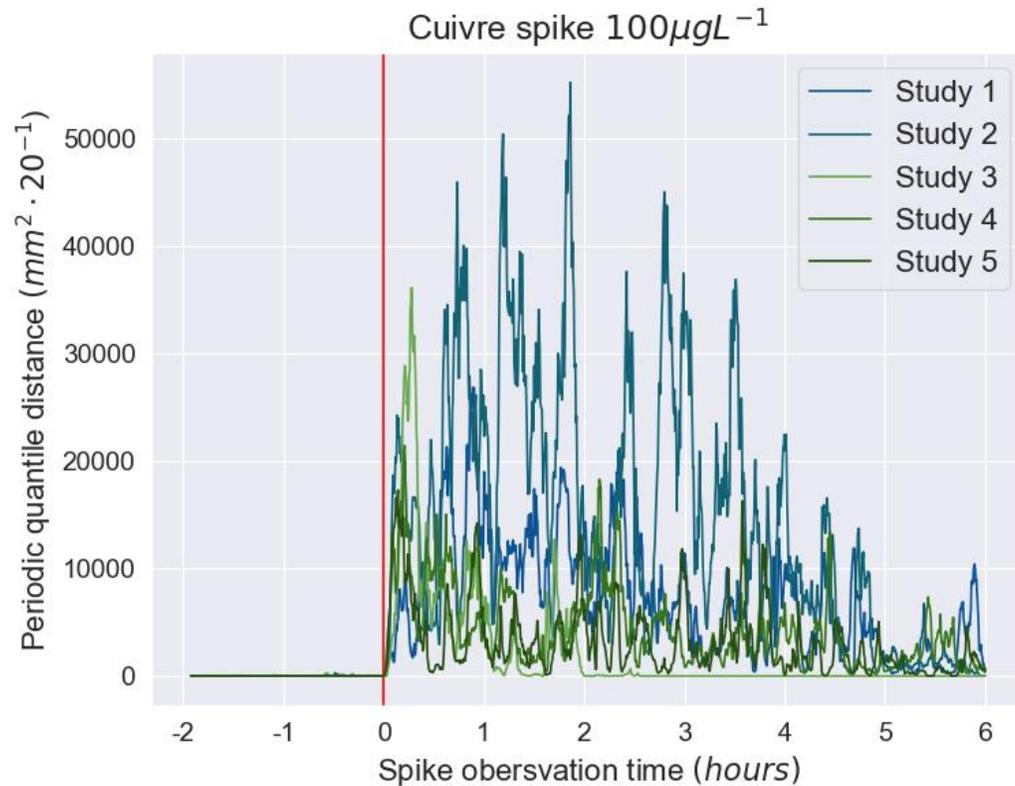
Micropollutant observation – hour 80 -120



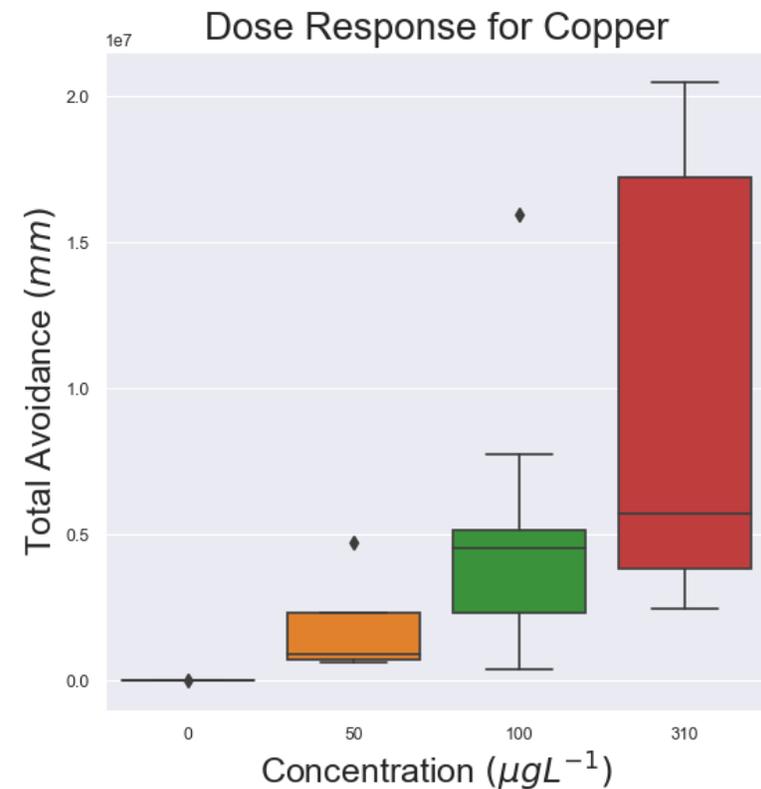
## CEC exposition spikes

## Repetitions for reference chemicals

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



- Dose response in avoidance behaviour?



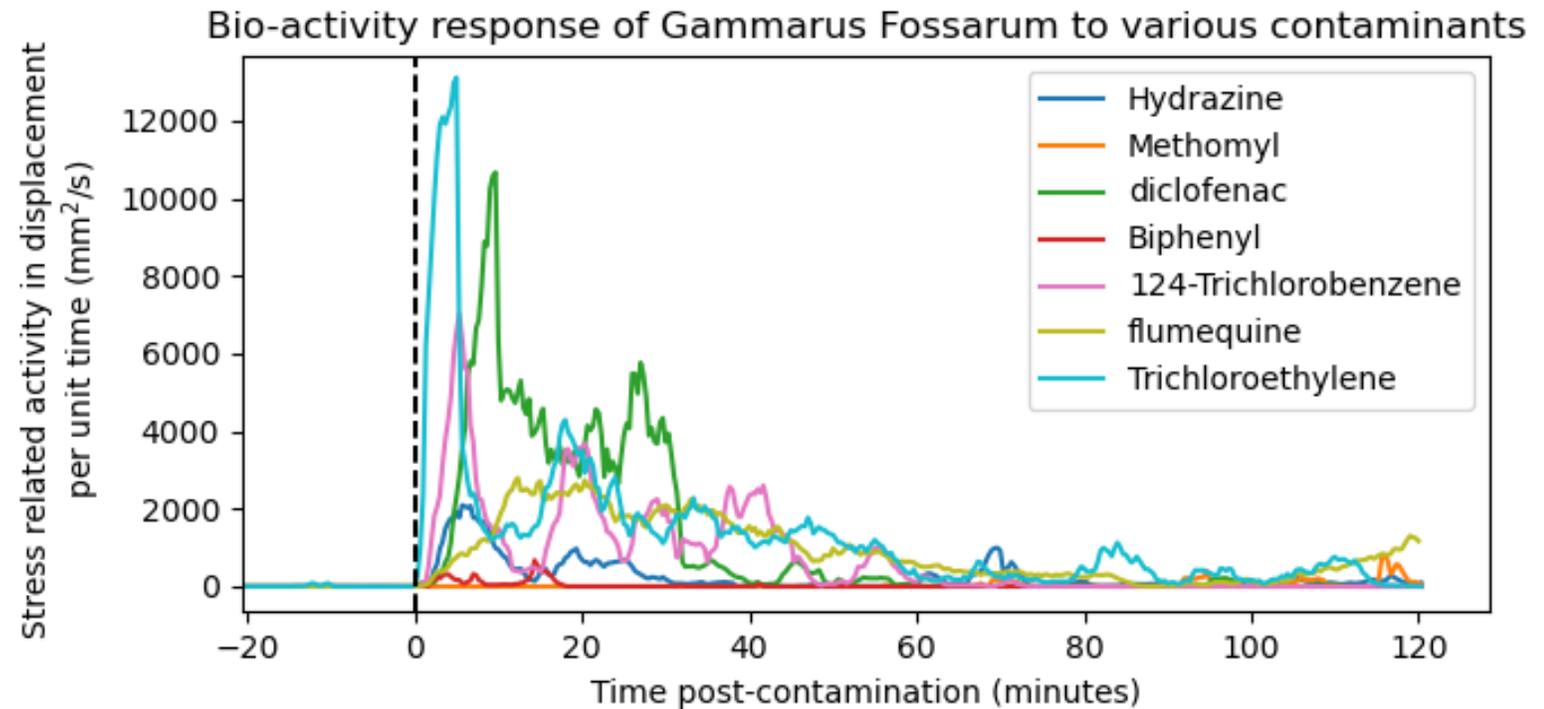
## CEC exposition spikes

## 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

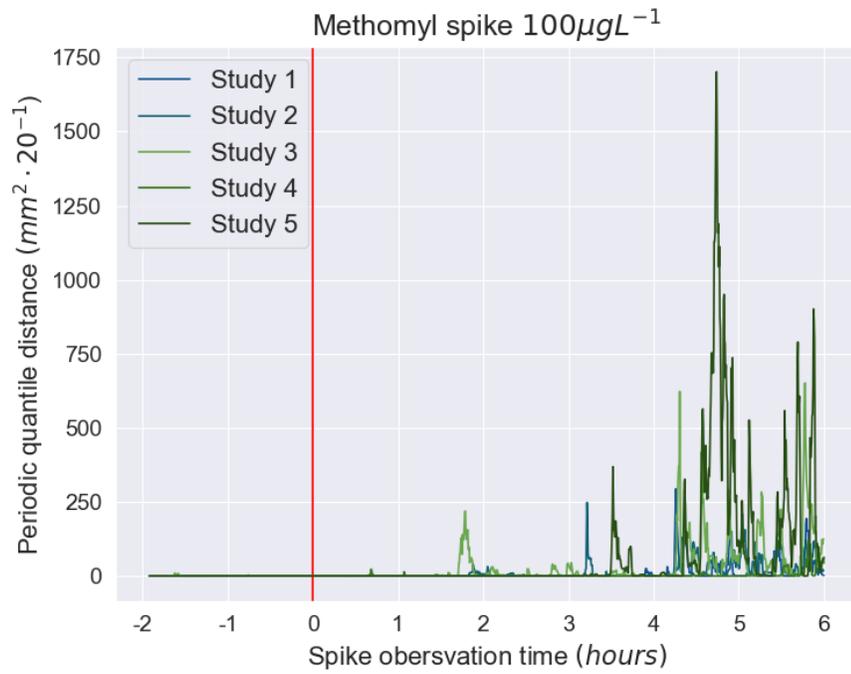


*Gammarus* avoidance bio-activity profiles for 7 CEC spikes

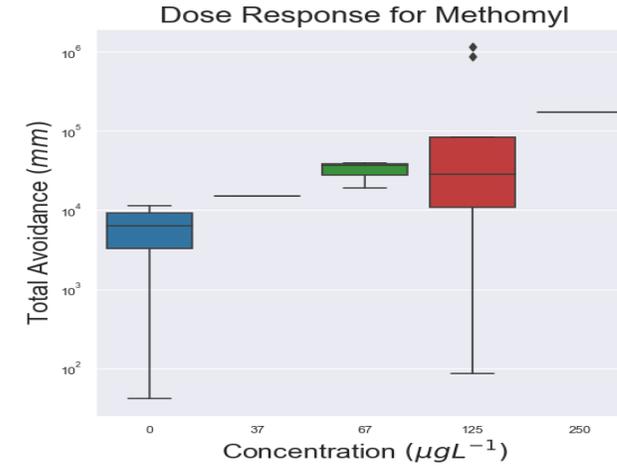
## CEC exposition spikes

### Repetitions for reference chemicals

- 5 repetitions for methomyl (125ug/L)



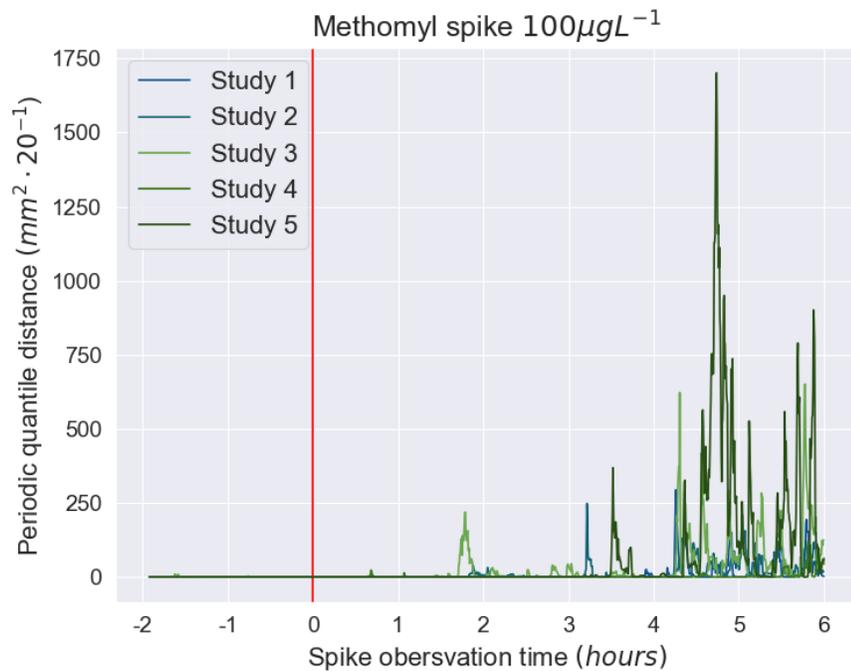
- Dose response in avoidance behaviour



## CEC exposition spikes

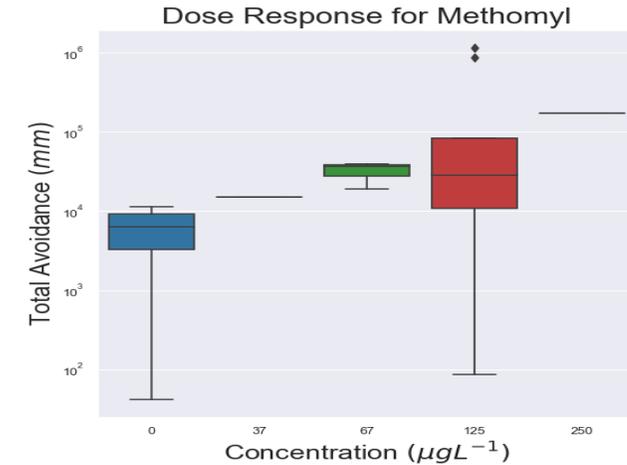
### Repetitions for reference chemicals

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Delayed response? – 4 hour avoidance?

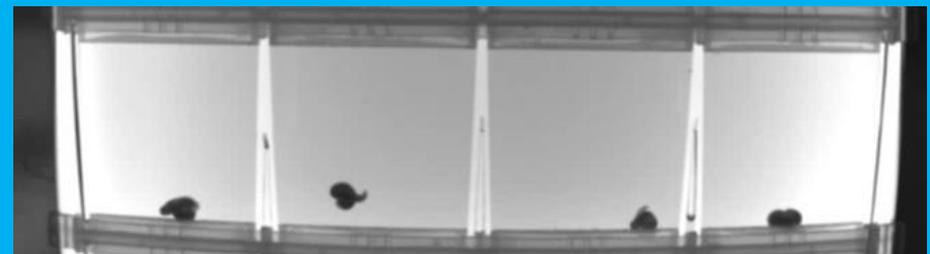
- Dose response in avoidance behaviour



- Multi-species advantage, erpobdella response :



Immediate drop in activity to zero movement



## CEC exposition multi-spikes

## Fingerprint detection

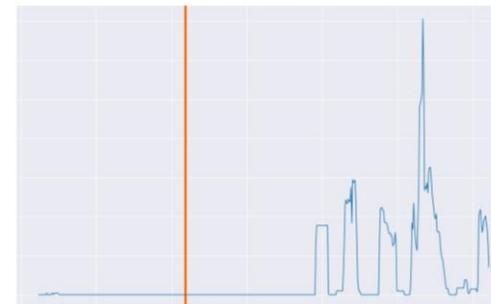
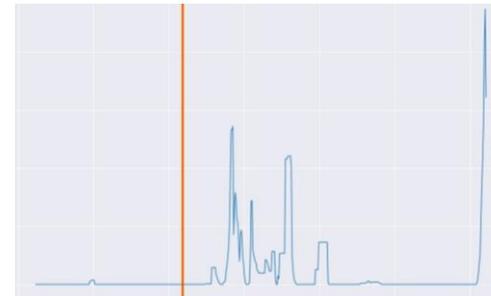
- Better response definition through multi-dimensional signal

## Response patterns

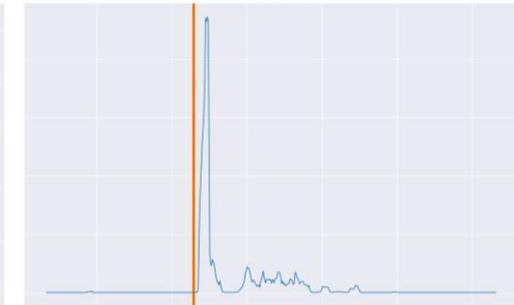
- Initial classification method developed for a group of substances :
  - ACP fonctionnelles
  - Classification de courbes
- Further clustering may reveal links between contaminants (>100 reponses)



## Hydrazine spike reaction



## Trichloroethylene spike reaction



Varying multispecies response for two solvent micropollutants

## Current classification models

### Curve description

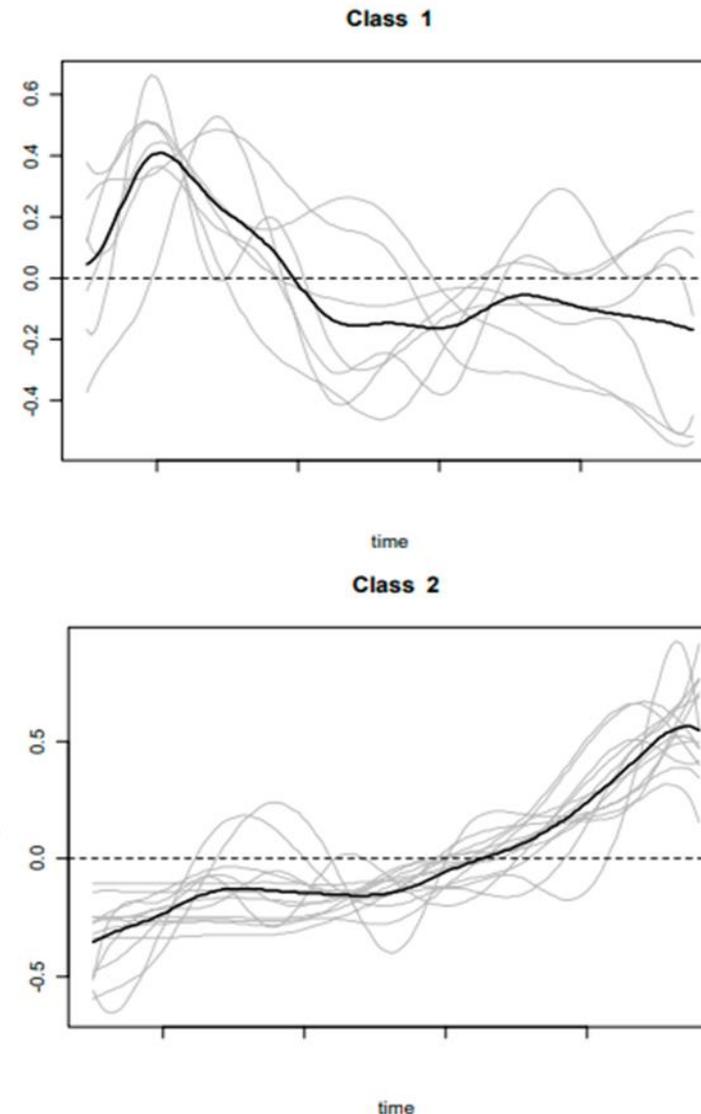
- Functional Principal Component Analysis (FPCA)
- Curve Classification methods

### Machine Learning

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

### Future work.

- Neural network techniques

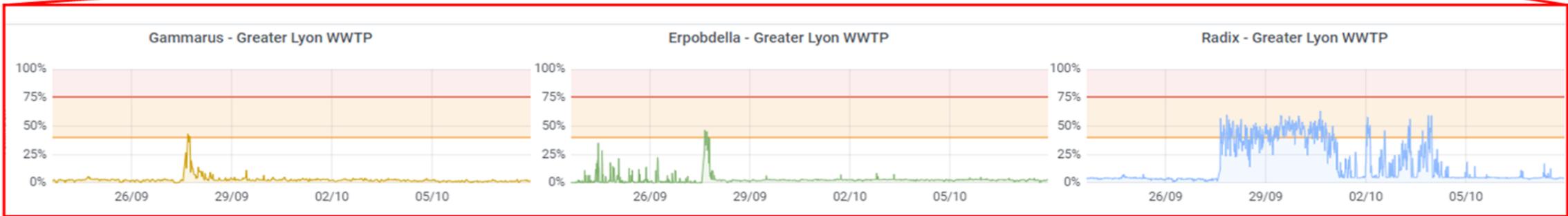
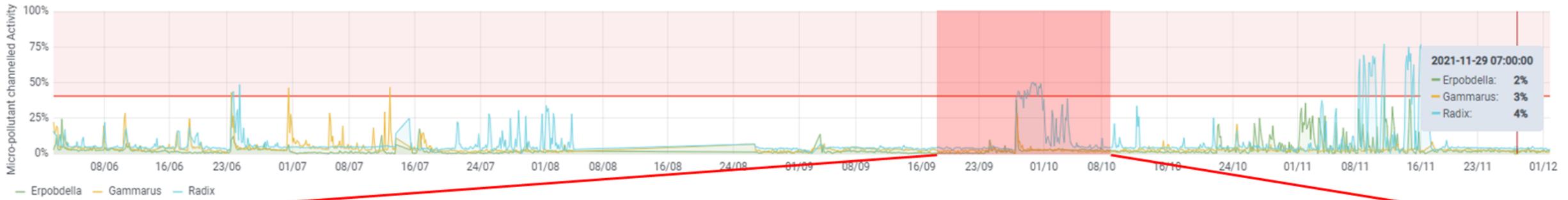


*Classes of mean activity response for the model substances*

## 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
- Equipe expérimentale** - L. Garnero, H. Queau, K. Montalbano, T. Cavanna, C. Grant
- Equipe technique** - M. Dauphin, A Deletang,

*Questions?*