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► To cite this version:

Fanny Desbiolles, Laetitia de Jong, Xavier Moreau, Isabelle Laffont-Schwob, Laure Malleret, et al.. Non-destructive biological and chemical traits to track down long term effects of environmentally relevant low concentrations of pharmaceutical mixtures using two freshwater species. Society of Environmental Toxicology and Chemistry, 26th annual meeting, May 2016, Nantes, France. . hal-01462322

HAL Id: hal-01462322

<https://hal.science/hal-01462322v1>

Submitted on 8 Feb 2017

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Non-destructive biological and chemical traits to track down long term effects of environmentally relevant low concentrations of pharmaceutical mixtures using two freshwater species

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Context & Objectives

Currently, there are a lot of normalised **acute bioassays** that evaluate ecotoxicity:

- Of single pollutants
- Under short time exposures (72h or 96h)
- At high concentrations (mg.L⁻¹)



There is a **need for new models** to evaluate :

- Pollutant mixtures
- Chronic toxicity
- Low environmental concentrations

Pollutants & Freshwater species

Study of 2 pharmaceuticals

not removed in wastewater treatment plants

Carbamazepine (CBZ), OxCarbazepine (OxCBZ)

and one of their metabolite **Acridine-9-carboxylic acid (9-CAA)**



Lemna minor L.

- ❖ Cosmopolitan species and fast growth rate
- ❖ Normalised bioassay for acute & chronic toxicity

Growth conditions:

Light : 9h/day, Moisture : 60%, Temperature : 15°C, Hoagland media (10%)

- ❖ Optical measurement of early changes in primary and secondary metabolites (anthocyanin, chlorophyll, flavonol, phenol and nitrogen balance indexes)

Published ecotoxicity values

Only For CBZ^{1,2}

Lemna minor L., EC₅₀ : 25.5 mg.L⁻¹, growth inhibition, 7d, (chronic toxicity)

Hydra attenuata Pall., EC₅₀ : 15.52 mg.L⁻¹, 96h (chronic toxicity)

LC₅₀ : 29.4 mg.L⁻¹, 96h (acute toxicity)

Hydra attenuata Pall.



- ❖ Minimized genetic variability (polyps propagate asexually by budding)
- ❖ Diploblastic organisation

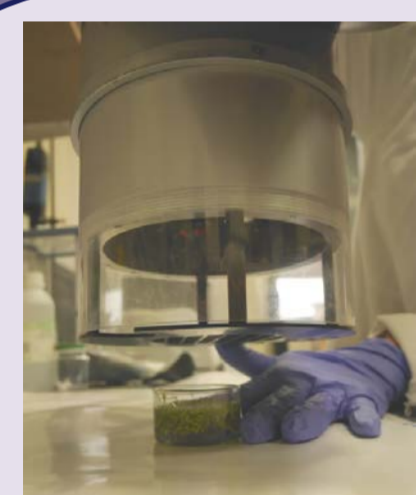
Growth conditions:

Single strain, Light : 12h/day, Temperature : 19.5°C, TES* buffer, EDTA, CaCl₂

- ❖ Monitoring of morphological alterations, reproductive rates, feeding behaviour

* 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid

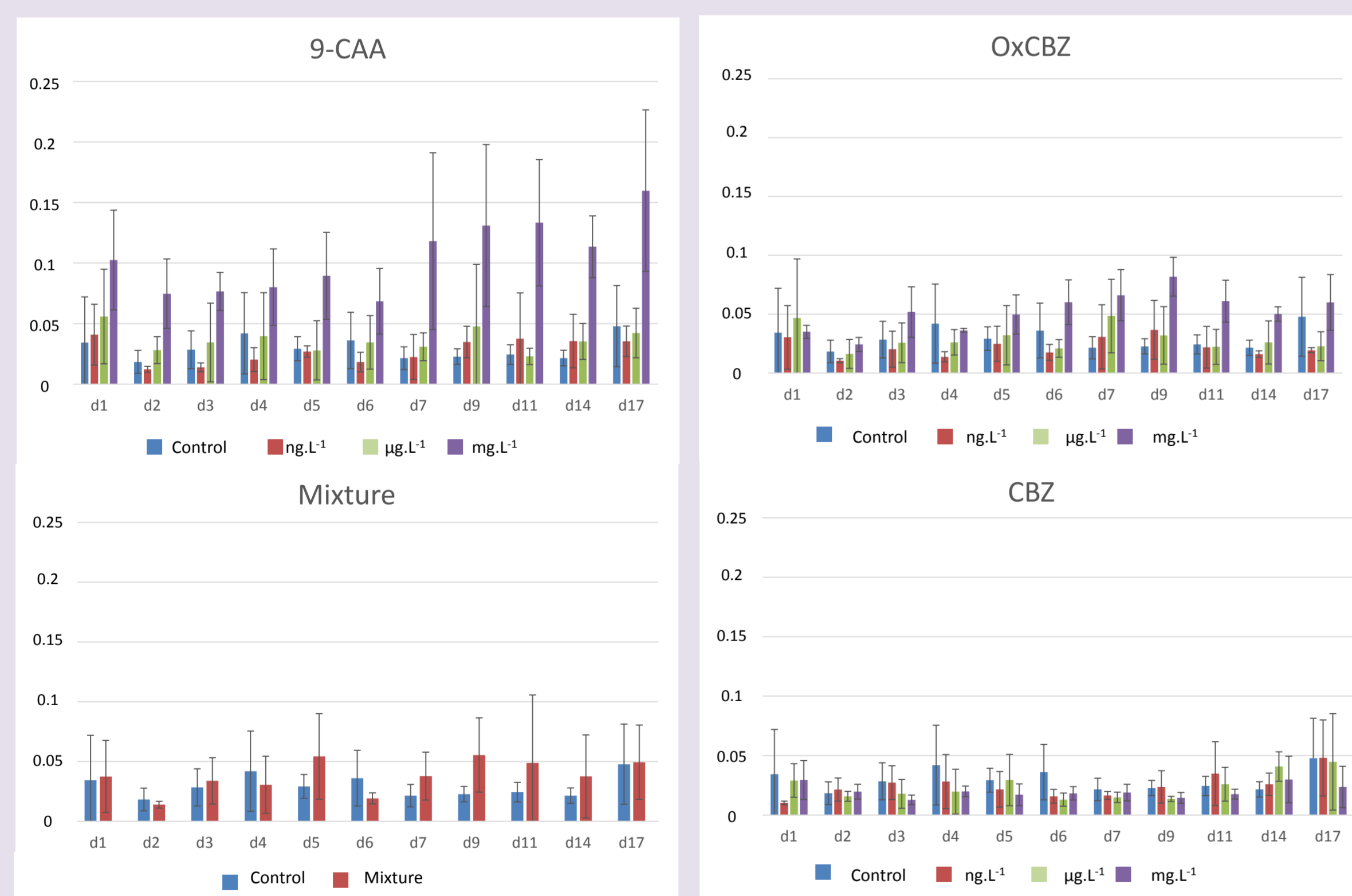
Results



Compounds	Concentration range		
	600 ng.L ⁻¹	600 µg.L ⁻¹	60 mg.L ⁻¹
CBZ	27 ng.L ⁻¹	270 µg.L ⁻¹	24 mg.L ⁻¹
OxCBZ	900 ng.L ⁻¹	900 µg.L ⁻¹	15 mg.L ⁻¹
Mixture	Σ _{CBZ+OxCBZ+9CAA}		



Compounds	Effluent realistic concentrations
CBZ	605 ng.L ⁻¹
OxCBZ	27 ng.L ⁻¹
9-CAA	900 ng.L ⁻¹
Mixture	Σ _{CBZ+OxCBZ+9CAA}



Phenolic index of compounds and control during a 17 day-long exposure

➤ Phenolic index:

The highest concentration of 9-CAA induced an early metabolic **stress** in *Lemna minor* L. as shown by the increase of the phenolic index probably due to a **defence mechanism**.

For OxCBZ, a slight metabolic stress was observed latter whereas neither CBZ nor the mixture at low concentrations impacted this index.

➤ Nitrogen balance index:

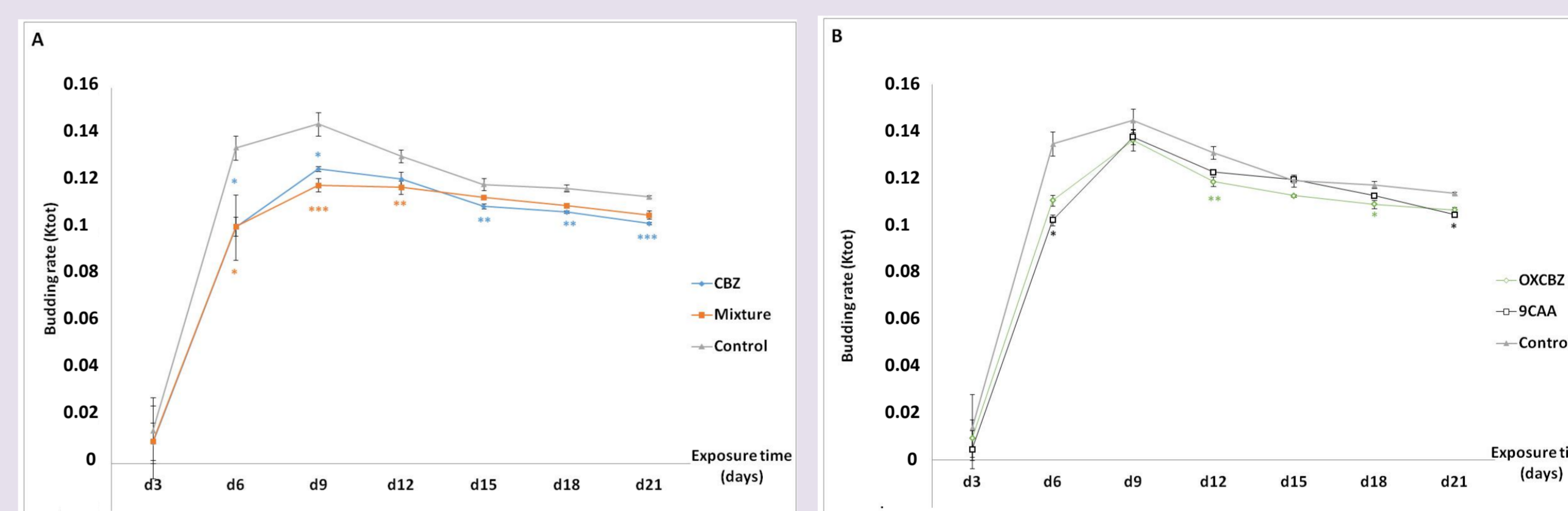
OxCBZ and CBZ at the highest concentrations increased the nitrogen balance index after 11 days. It shows a modification of **allocation of nitrogen and carbon** that may **impact photosynthesis** in of duckweed.

➤ Chlorophyll index:

This primary metabolite was **stable** for all the compounds and their mixture.

➤ Anthocyanin and flavonol indexes were below detection limits.

➤ Morphological changes, reflecting **progressive intoxication**, were observed after only one day of exposure : 21% of polyps exposed to CBZ, OxCBZ or 9-CAA showed first signs of intoxication and 16,6% of polyps exposed to the mixture showed more pronounced signs of intoxication. Interestingly, after 6 days of exposure morphological signs of intoxication tended to disappear and polyps exhibited normal morphological stage until the end of exposure (21 days). These observations suggest that *H. attenuata* possesses **defence mechanisms** to face intoxication.



A-B : Budding rate (Ktot) of exposed polyps and controls followed during 21 days.

K_{tot} values were calculated³ with the following formula $K_{tot} = (\ln(n_{tot}) - \ln(n)) / d$
n: number of polyps at the beginning of the experiment (d0); n_{tot}: the sum of attached buds plus the number of polyps (i.e. detached buds and initial polyps) after x days of exposure (d3 to d21).
*:P< 0,05, **:P< 0,01, ***:P< 0,001, significantly different from control (n= 24 in triplicate; Kruskal-Wallis followed by Dunn test).

➤ In exposed polyps, **slowing down and decrease of budding rate were observed** (Figs A-B) that could be related to morphological changes. Indeed, as the decrease of budding rate occurred from the d6 after exposure, i.e. the time required to the *Hydra* polyps to restore their normal morphology, trade-offs between the survival of polyps and the reproductive success of the population could be hypothesized. **As polyps exposed to CBZ and mixture never reached the maximal budding rate observed in control**, these compounds have negative effect on the reproductive success.

➤ Whatever the exposure conditions, all *Hydra* polyps were still able to catch and ingest prey : no alteration of feeding behaviour was observed.

Conclusion

❑ Both models enable to study **non-destructive biological pertinent endpoints**.

- ❑ The macrophyta, *Lemna minor*, showed **deleterious effects only at high concentrations**. This specie does not seem highly sensitive to these compounds under the tested conditions.
- ❑ Bioassays using *Hydra attenuata* showed that the selected **compounds at environmental concentrations induced deleterious effects** at early (morphological changes) and later stages (budding rates). Such negative effects raise the question of the impact on natural populations that are chronically exposed to pollutants.
- ❑ These preliminary results could help to develop **new tools** for water quality monitoring and **for chronic toxicity assessment at realistic environmental concentrations**.

References

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³ De Jong L., Pech N., de Aragão Umbuzeiro G., Moreau X. 2016. Multi-scale biomarker evaluation of the toxicity of a commercial azo dye (Disperse Red 1) in an animal model, the freshwater cnidarian *Hydra attenuata*. *Water Research* 96 : 62-73.

Acknowledgement

The authors thank the Provence-Alpes-Côtes d'Azur region and the society Seakalia (Marseille, France) for funding this research through a PhD grant (DEB n°141198/2014)