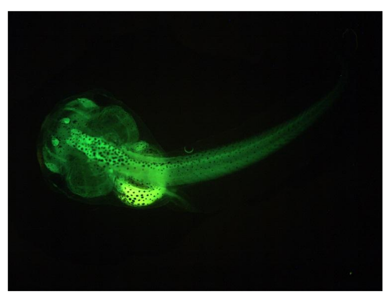

Testing of endocrine disruptors in wastewater

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INTRODUCTION

Measuring effects of mixtures of contaminants in water require the use of biological methods. Chemical analysis is not sufficient to predict consequence of micropollutants, whereas a comprehensive diagnosis of effects is needed to protect water ecosystem. Previous studies have shown the use of fluorescent aquatic organism such as *Xenopus laevis* tadpoles and *Oryzias latipes* fish fry to quantify hormonal disruption of thyroid and estrogenic functions in treated waste water (Castillo et al. 2013, Fini et al 2007 & 2009). Measuring hormonal balance of these organisms reveals their capacity to adapt to the aquatic environment, therefore providing a global quantification of water quality. The higher the pollution, the more the larvae light up. BIOTTOPE project (BIological tools to Optimize Treatment Technology to remOve microPollutants and Endocrine disruptors) is dedicated to adapt waste water treatment solutions for reducing micropollutants and endocrine disruptors impact on aquatic ecosystem. To this aim we have developed the FrogBox®, an automated tool integrating small aquatic organisms to assess quality of waste water treatment plants effluents on site by continuous biological monitoring.

METHODS

Endocrine disruption assessment

Small aquatic biological models (tadpoles of the amphibian xenopus or fry of the medaka fish) are used for the detection of endocrine active molecules. These small model organisms harbor genetic markers which fluoresce in the presence of endocrine disrupters. The fluorescence could be quantified in 96 well plate using a fluorescent plate reader

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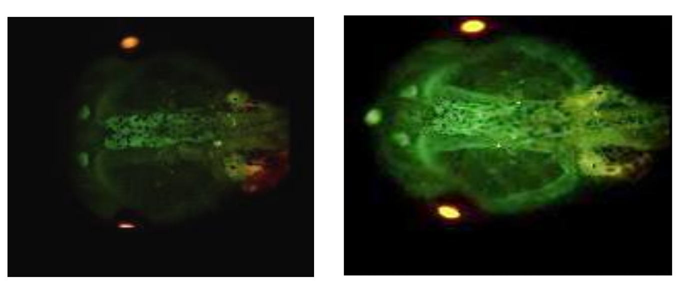
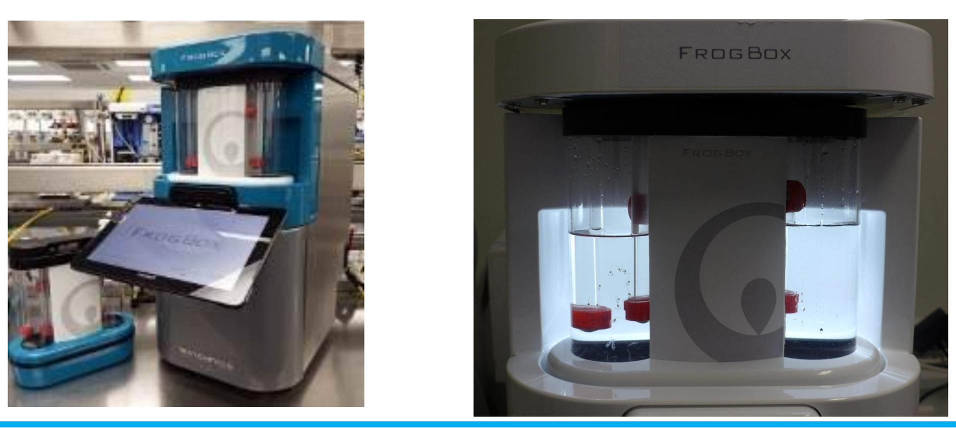
Tadpoles in reference sample not spiked (left) and spiked (right) with T3 hormone



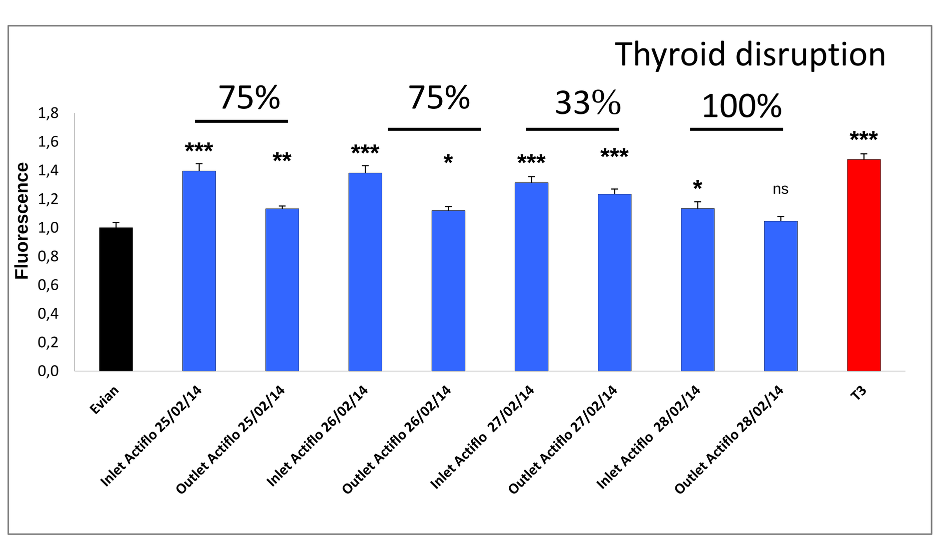
Flow-through Fluorescence Monitoring using the FrogBox

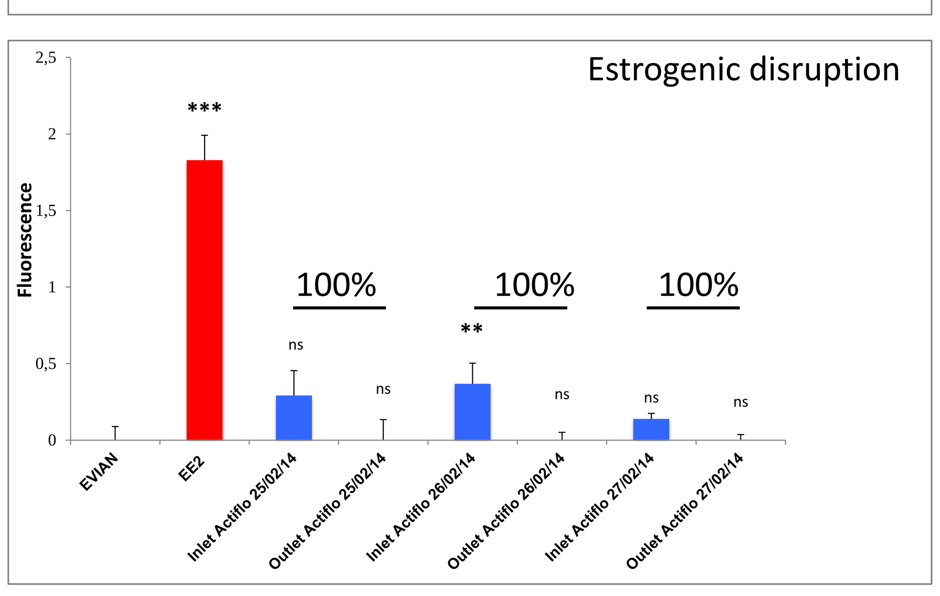
The FrogBox is basically compose of two reservoirs containing the larvae, a transparent measurement flow cell and a pumps to move the frog or the fish larvae from one reservoir to another. Whilst in the first reservoir, the embryos are exposed to the water to analyse. During the transfer from one reservoir to the other, the embryos pass through a measurement cell, whereby any changes in the fluorescence generated due to the presence of endocrine disruptors be detected via an optic array and a camera. The data from the camera is then interpreted using a image analysis software to quantify the change in fluorescence intensity.

The FrogBox (left). Tadpole swimming into the larvae cartridge in "day" mode (right)

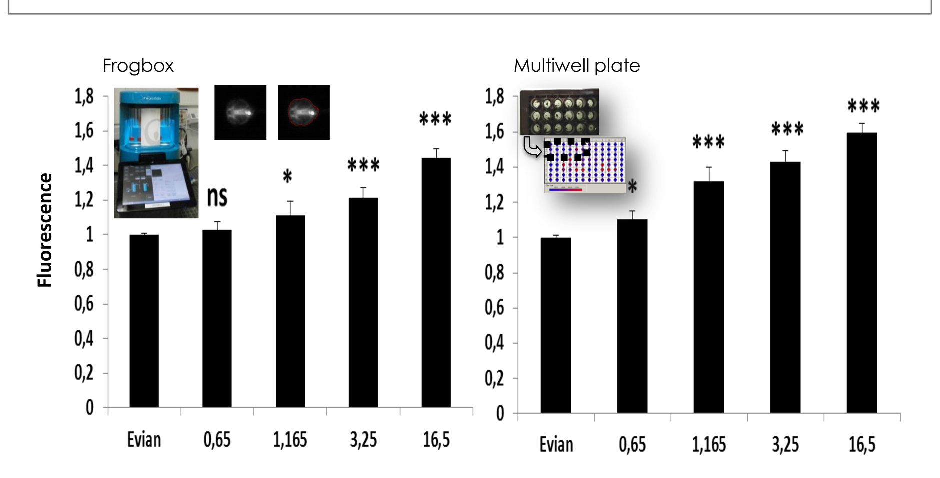


RESULTS



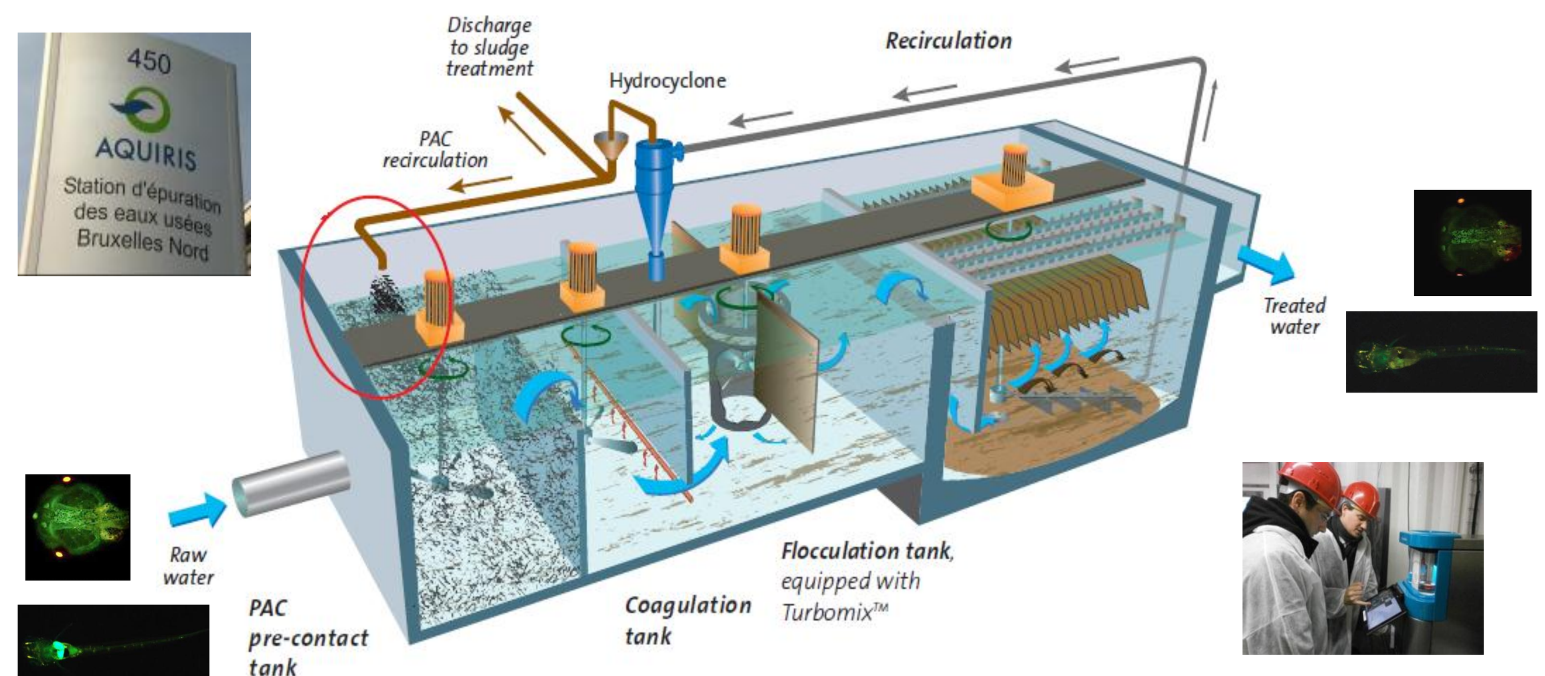


Endocrine disruption assessment using WatchFrog small model organisms shows the efficiency of the Actiflo ® Carb technology for the removal of micropollutants. The percentage indicated correspond to the reduction of the endocrine effect between the inlet and the outlet of the Actiflo®Carb



Dose responses of thyroid hormone T3 on fluorescent tadpoles show the comparable sensitivity of the FrogBox and a mutiwellplate fluorescence reader. FrogBox quantification of fluorescence (left), multiwell plate fluorescence quantification (right).

DISCUSSION



Using of FrogBox prototypes to follow upstream and downstream water quality of the Actiflo ® Carb technology. The Actiflo ® Carb treatment is based on activated carbon adsorption with an accelerated settling step. This tertiary treatment is placed just after the traditional water sewage plant treatment

The small size and the transparency of the aquatic larvae (fish and amphibians) bearing fluorescent labels make them ideally suited to this type of approach. Other advantages of the fish and amphibian models are:

- Fish and amphibian larvae are easy to breed and for a relatively low cost.
- Fish and amphibian larvae exclusively develop in water making them ideal organisms to detect fresh water/liquid pollutions.
- Their development is fast and can be totally completed in a laboratory facility. The female of both species can lay a large number of eggs. A medaka fish (*Oryzias latipes*) female can lay 200-300 eggs every two to three weeks. An Amphibian (*Xenopus laevis*) female can lay several thousand eggs in one clutch.
- The read-out of the system (fluorescence emission) makes it easy to read and interpret by a non-specialized technician.

The FrogBox on-line monitoring tool that we developed offers unique advantages :

- On site direct monitoring of the endocrine disruptive effect
- Automatized reading
- Easy to use for end-users
- Biological results converted into a comprehensive scale of endocrine disruption
- Sensitivity of detection comparable to the one obtained with laboratory instruments

CONCLUSIONS

WatchFrog small model organisms have been used to demonstrate the efficiency of conventional biological activated sludge treatment plants and Actiflo®Carb tertiary treatment to eliminate endocrine disruptive chemicals.

The FrogBox will be soon associated with a new software for end-users that is under development. It will integrate the following features:

- A simplified interface for end-users.
- Automatized reading.
- An optimized image analysis.
- Live results analysis and presentation into a graphic representation.
- An alarm when the fluorescence of the larvae reaches a threshold indicating an endocrine disruption.

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BIOTTOPE
LIFE11 ENV/FR/742

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