



SIXTH FRAMEWORK PROGRAMME

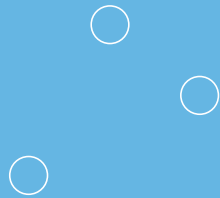


# Ecotoxicological impact assessment of upgrading technologies

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- Introducing the problem: Impacts
- Effects of selected emerging contaminants
  - Endocrine disruptors (EDs)
  - Pharmaceuticals
- Ecotoxicological impact assessment
  - Case study 1: Ozonation (WWTP Regensdorf, CH)
  - Case study 2: Ozonation and activated carbon treatment (WWTP Neuss, D)
- Conclusions

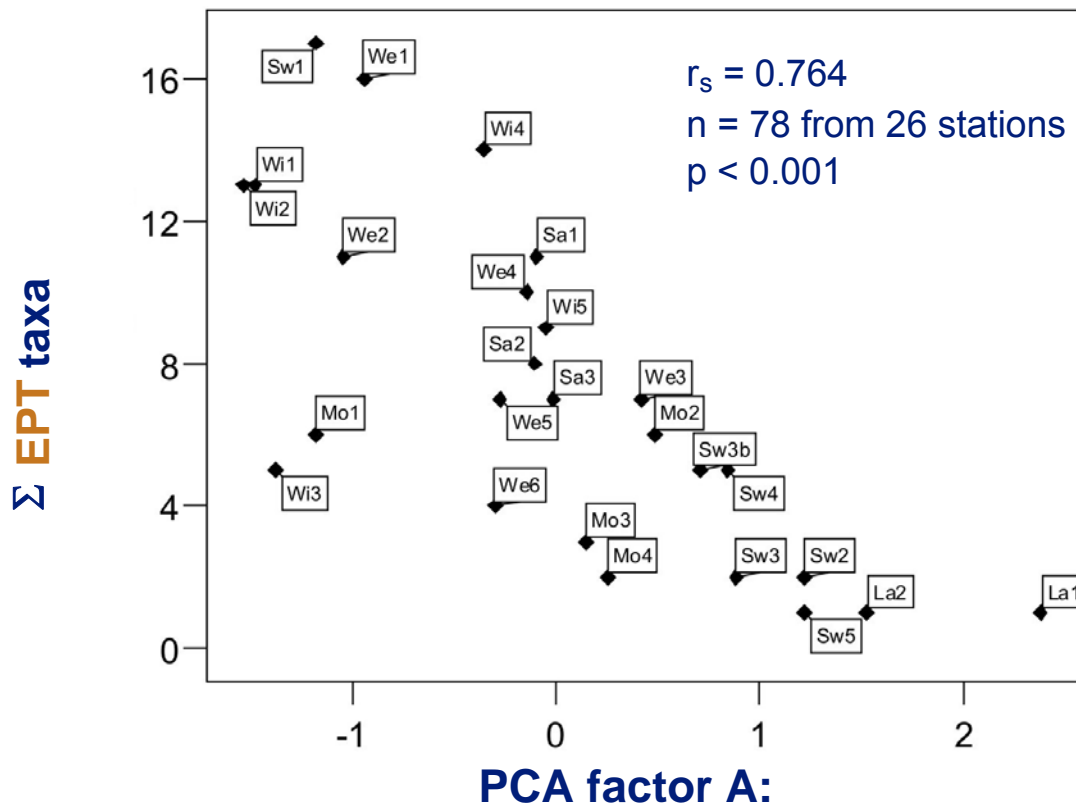


Surface waters receive  
10.5 billion m<sup>3</sup> wastewater  
per year in Germany

## Wastewater and emerging contaminants

- Several **10,000 chemicals** enter the sewer system
- Polar to medium polar contaminants are often **not removed** in WWTP
- Emerging contaminants with **particular relevance**:
  - high biological activity,
  - thus effects in the  $\mu\text{g/L}$  range and below,
  - present as complex mixtures
- Examples: **Endocrine disruptors (EDs)** and **pharmaceuticals**

- Loss of sensitive wildlife groups:



- concentration of 11 emerging contaminants
- wastewater content

**E** = Ephemeroptera,  
mayflies



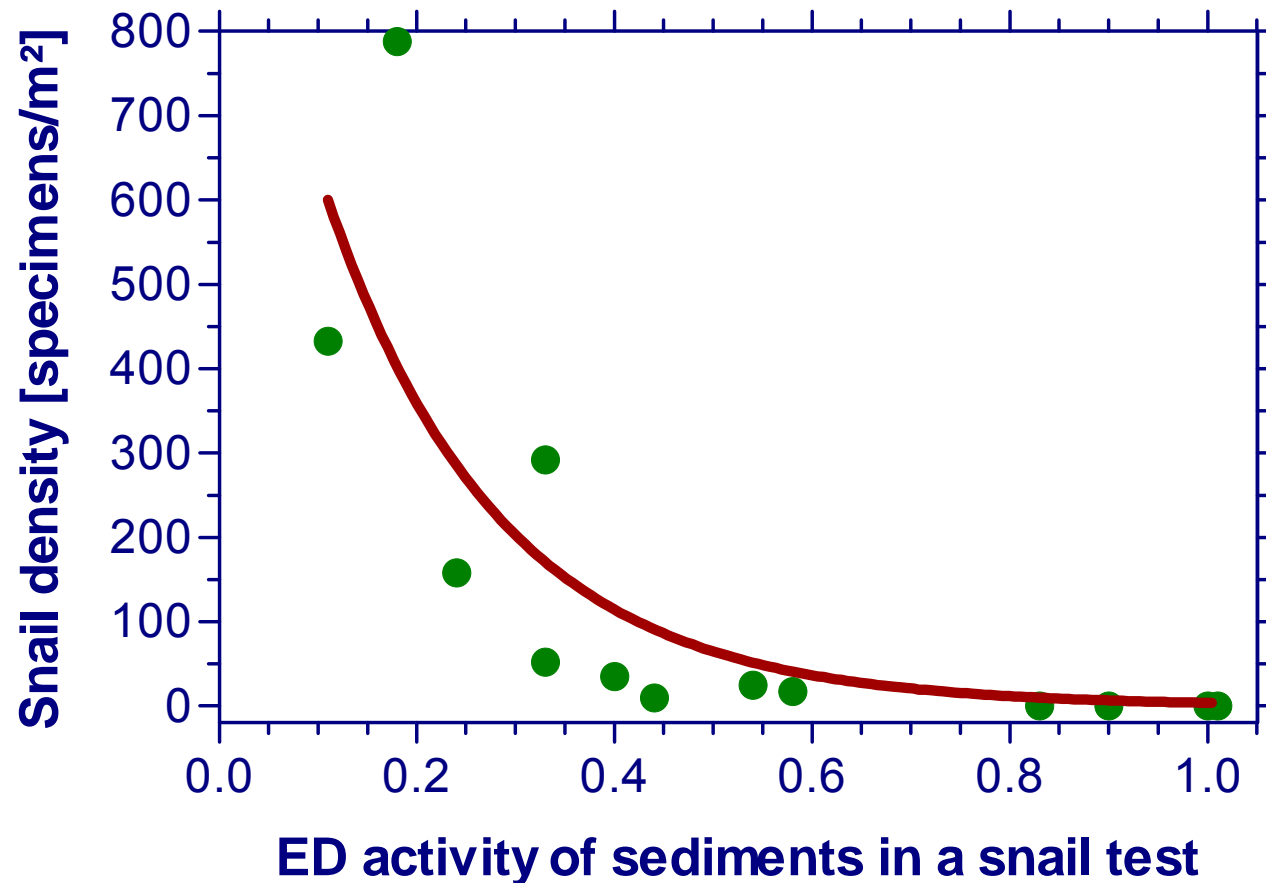
**P** = Plecoptera,  
stoneflies



**T** = Trichoptera,  
caddis flies



... correlated with the exposure to emerging contaminants such as EDs in the river Elbe:



## What is an endocrine disrupter (ED)?

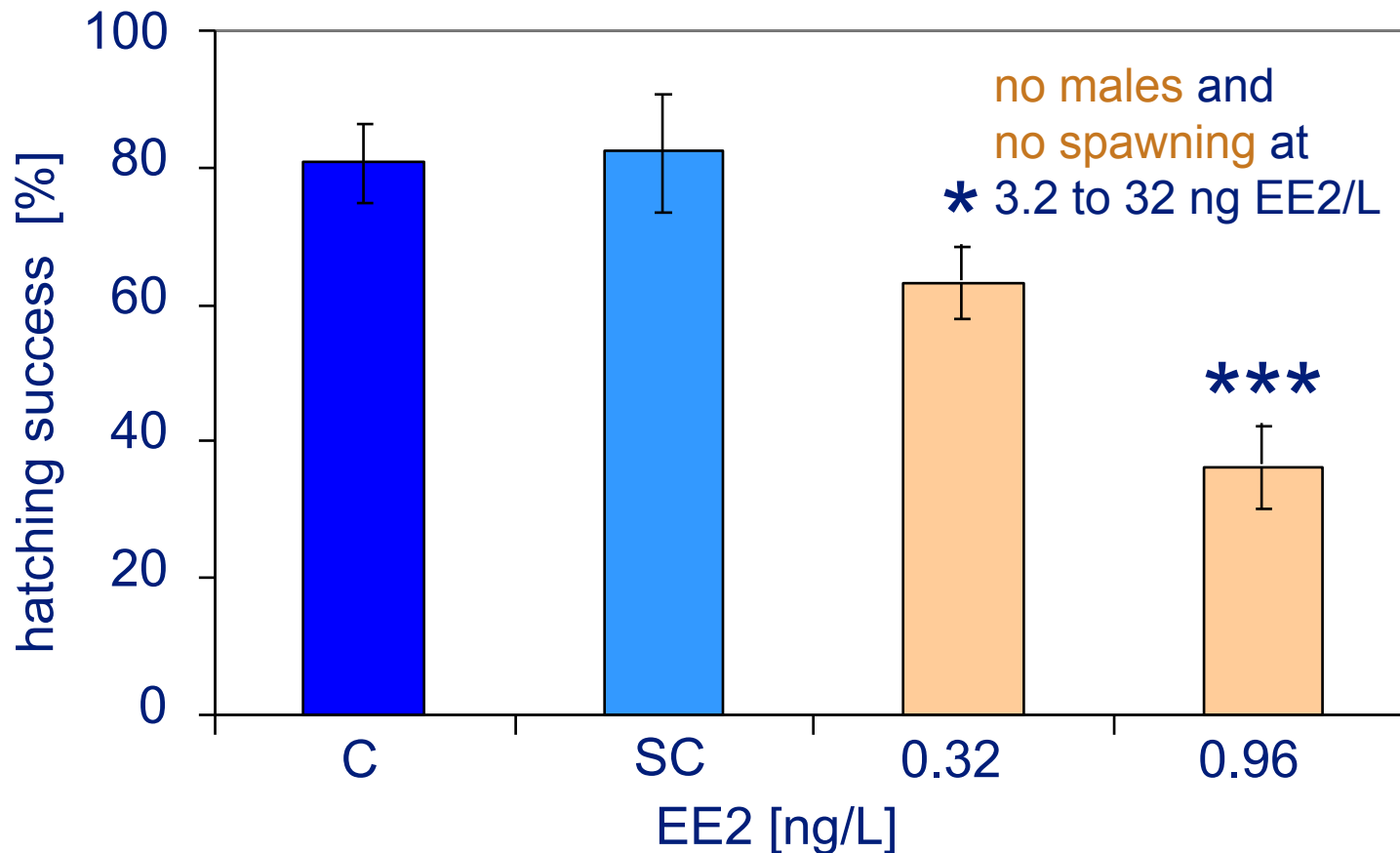
- EU definition (COM(1999) 706):

Exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations

- EDs may **affect growth, development and reproduction** by modulating the hormone system, often at trace concentrations



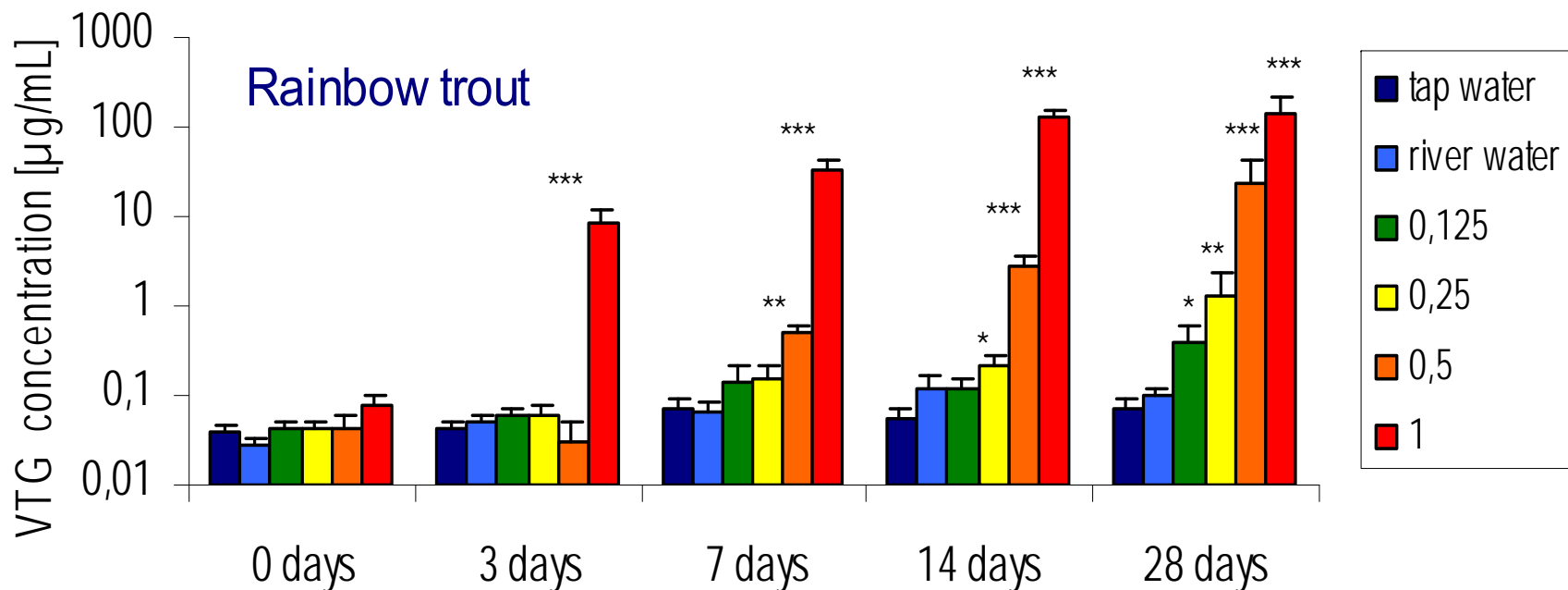
- Lack of males and **no reproduction** in fathead minnows beyond 1 ng EE2/L:





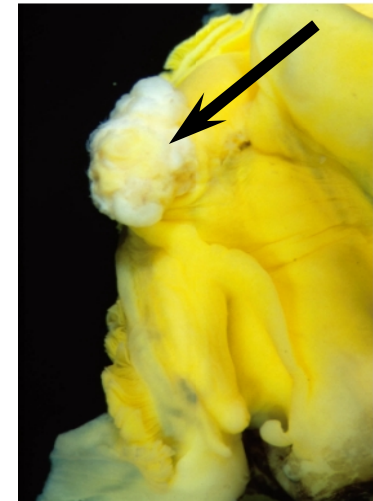
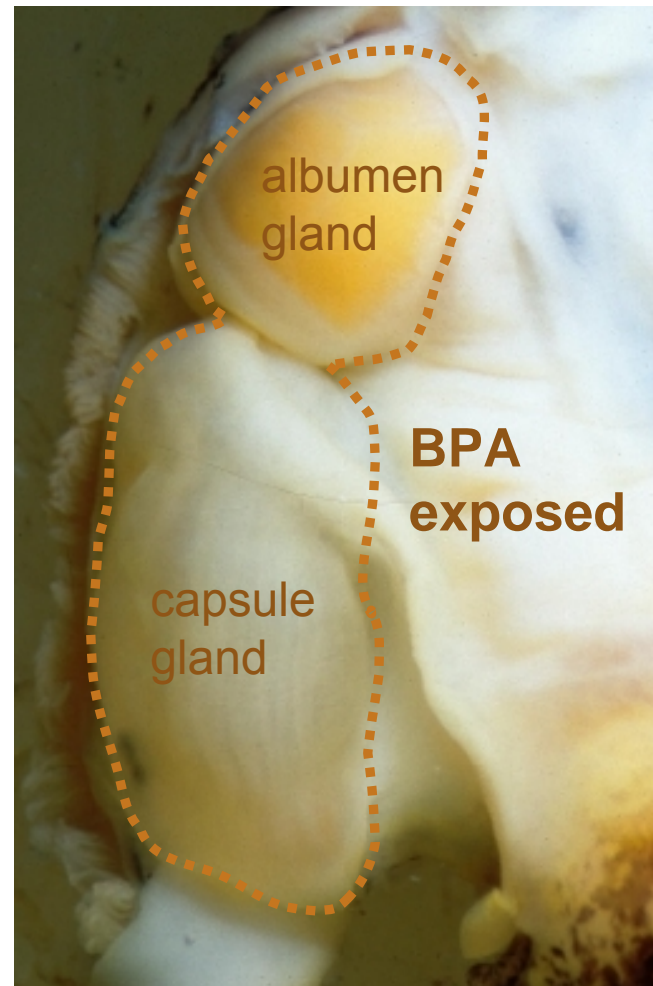
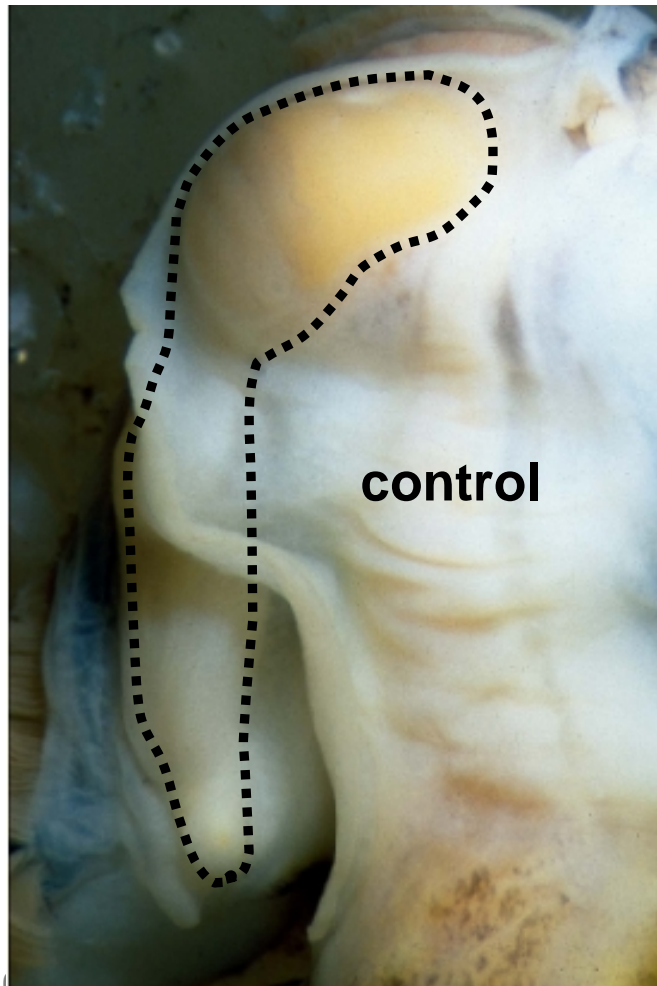


- Vitellogenin (VTG) induction in male rainbow trout exposed to WWTP effluents:





- **'Superfemales'**: Enlarged sex glands ... & ruptured oviducts:



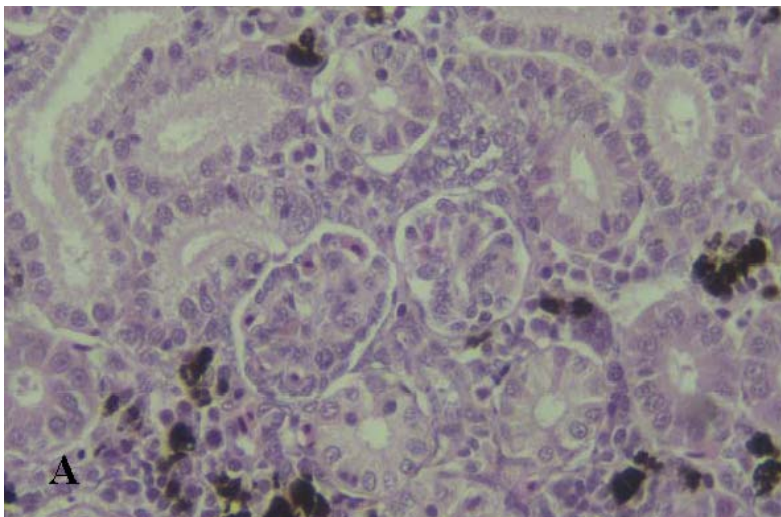
Oehlmann et al. (2006):  
EHP 114, 127-133

## Sensitivity differences between species

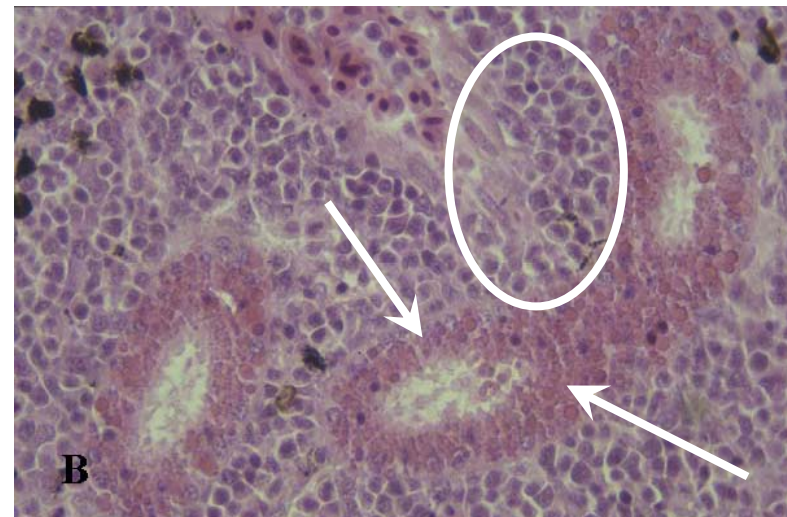
- Huge differences in sensitivity for many EDs:
  - snails more sensitive to BPA than fish (by factor 1,000)
- Resulting Environmental Quality Standard (EQS) for BPA:
  - 1.6  $\mu\text{g/L}$  to protect fish populations,
  - 0.0014  $\mu\text{g/L}$  based on effects in snails
- Predicted Environmental Concentration for European surface water ( $\text{PEC}_{\text{water}}$ ): 0.12  $\mu\text{g/L}$



- Damage in **kidney**, gill and liver at  $\geq 1 \mu\text{g/L}$  by the anti-inflammatory drug
- Example kidney: **Protein accumulation**, epithelial **degeneration** and interstitial **proliferation**

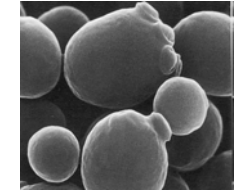


*Oncorhynchus mykiss*, control

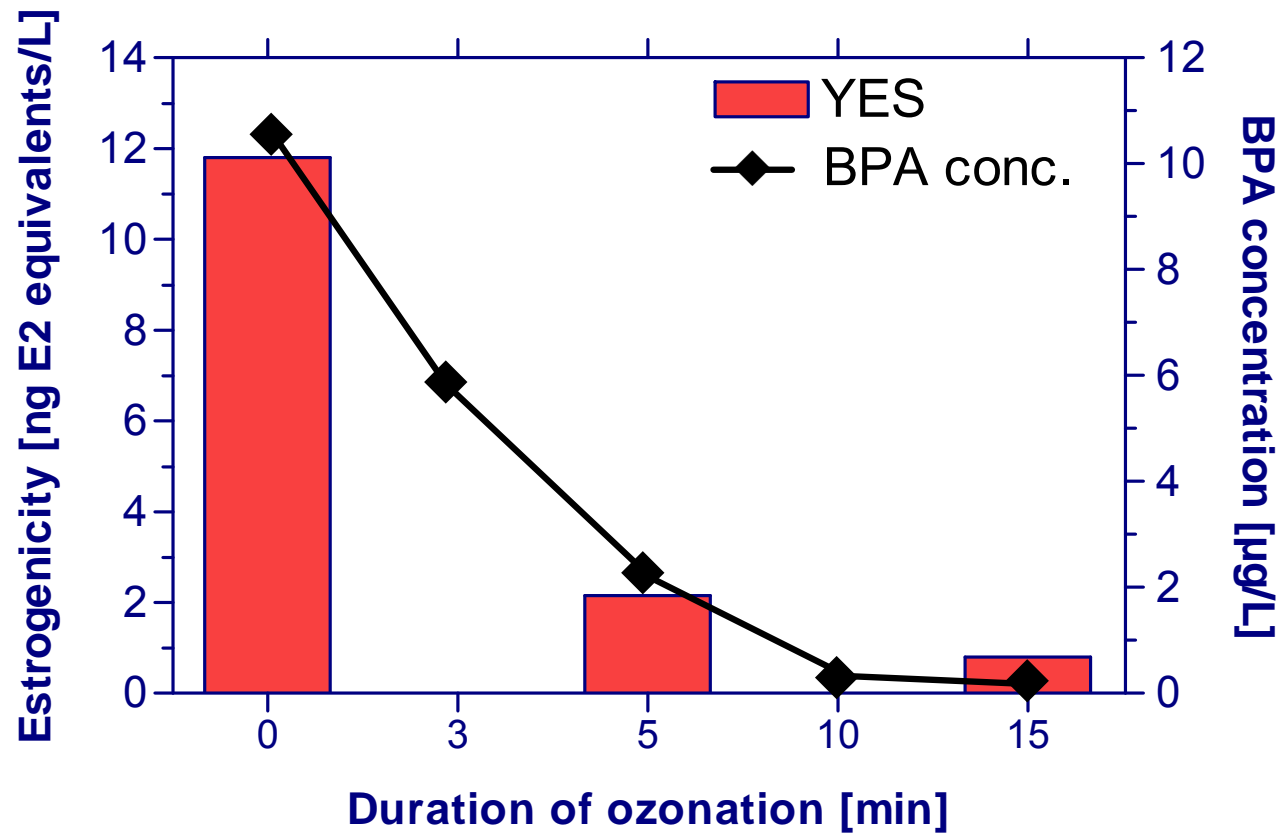


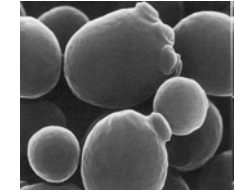
*O. mykiss*, 100  $\mu\text{g}$  diclofenac/L

- **Advantages** compared to *in vivo* tests:  
sensitive, specific, easy to use and inexpensive
- **Disadvantages**: no assessment of transformation products,  
test batteries necessary and not available for many MoA
- **Examples**:
  - ED activity: E-screen, **YES**, **YAS**
  - Mutagenicity: umu test, Ames test
  - Various cytotoxicity tests

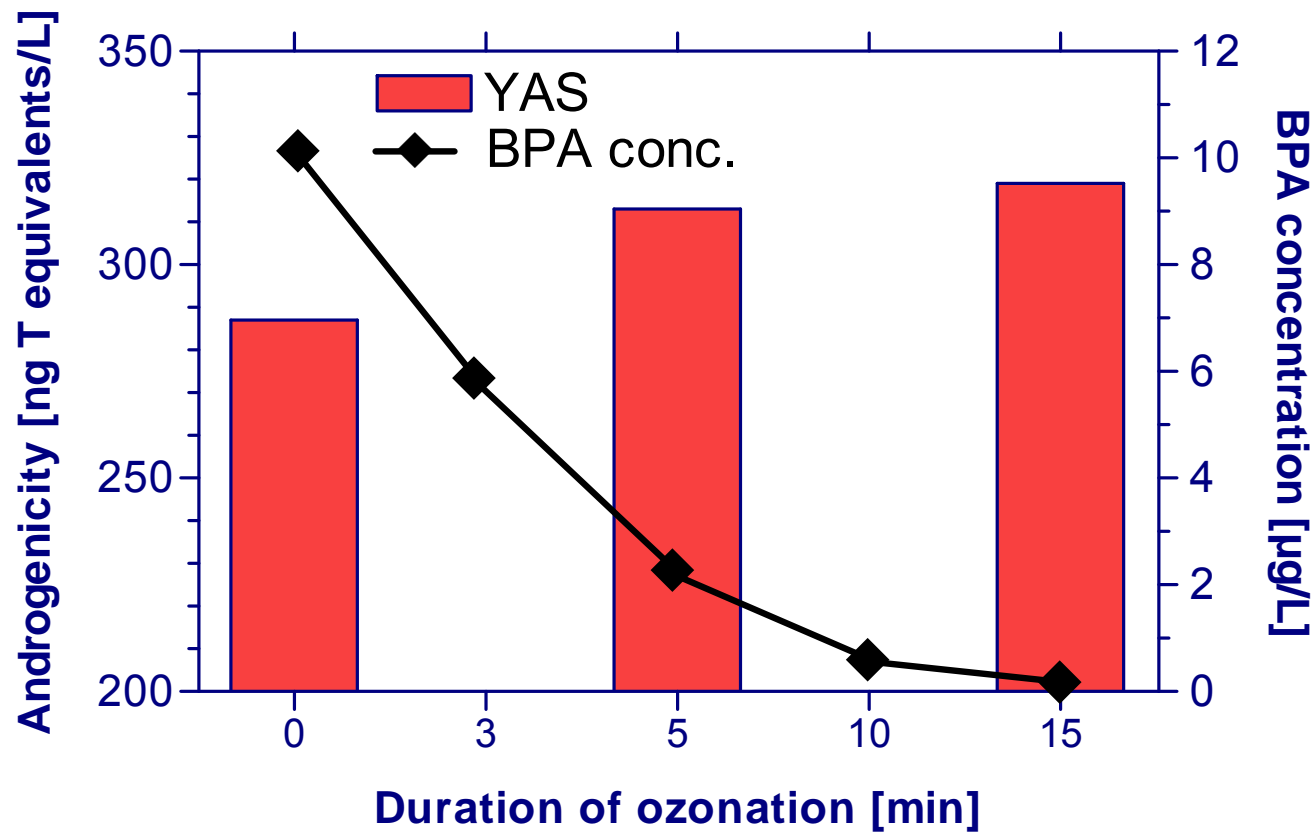


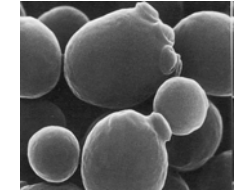
- **Estrogenic activity** in the **YES** and BPA concentration during ozonation (WWTP Düsseldorf):



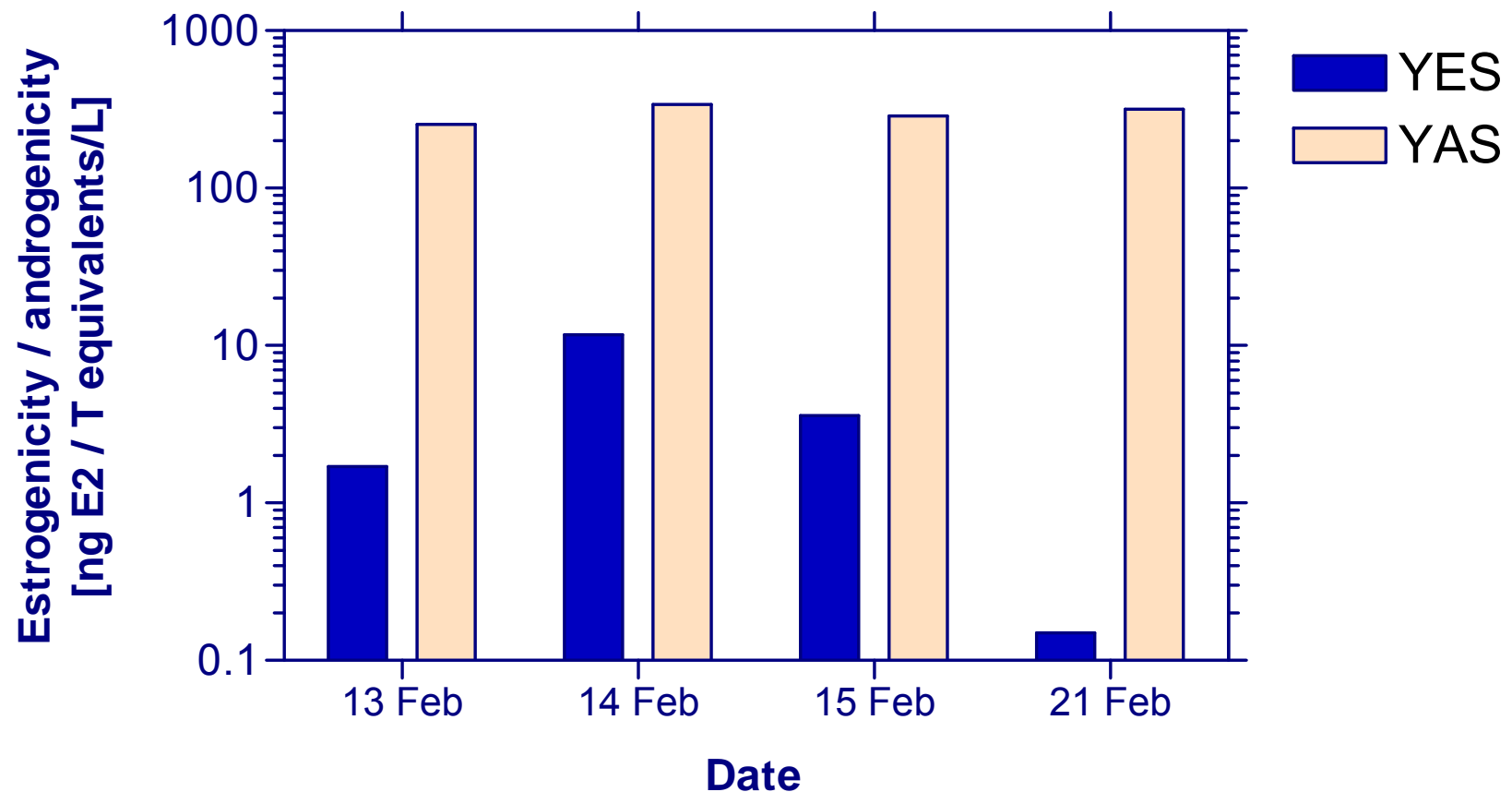


- **Androgenic activity** in the **YAS** and BPA concentration during ozonation (WWTP Düsseldorf):





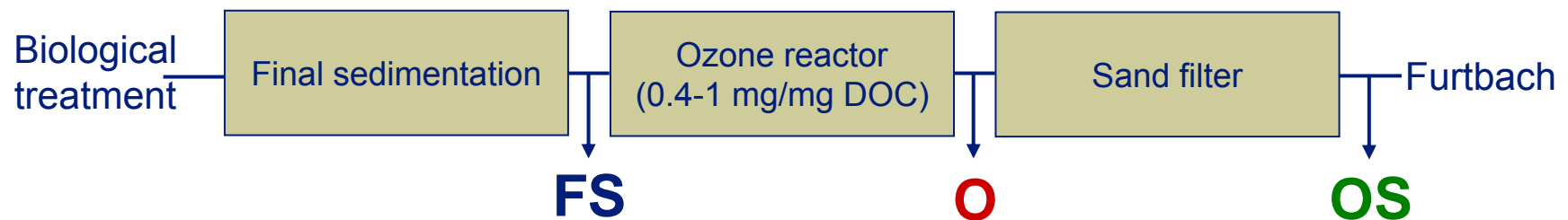
- Androgenic vs. estrogenic activity in WWTP Düsseldorf effluents:





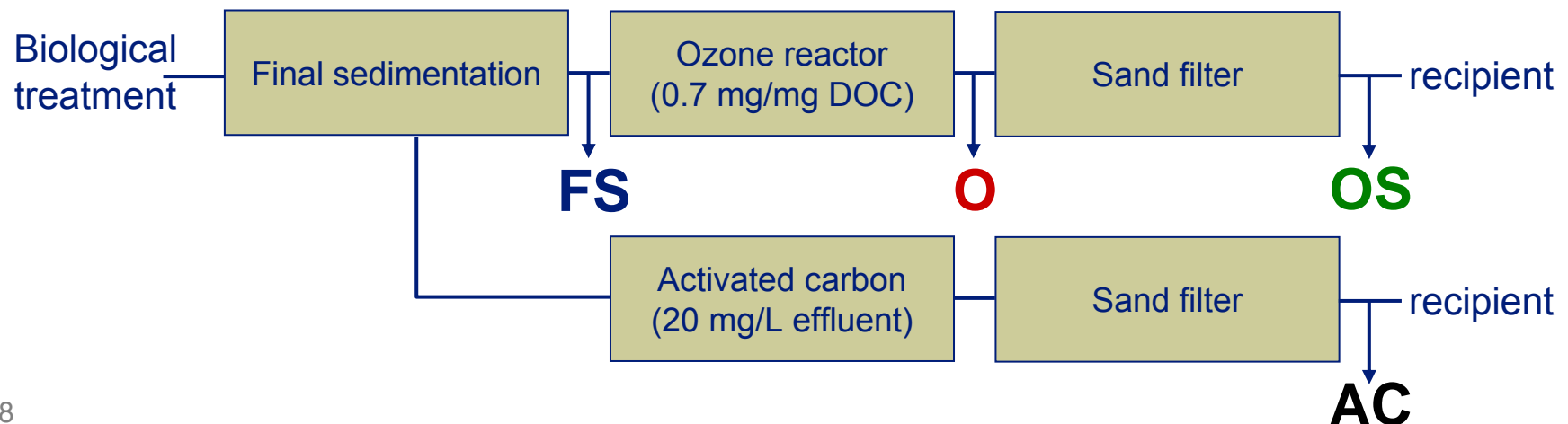


- **Case study 1: WWTP Regensdorf (25,000 PE)**
- Comparative onsite testing of three treatment steps, including full-scale ozonation:
  - After final sedimentation (FS)
  - After ozone reactor (O)
  - After sand filter (OS)
  - Control (C)










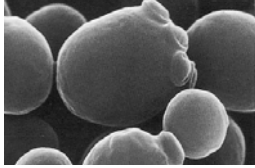
- **Case study 2: WWTP Neuss (120,000 PE)**
- Pilot treatment plant with ozonation and powdered activated carbon treatment in parallel lines; samples tested :
  - After final sedimentation (FS)
  - After ozone reactor (O)
  - After sand filter (OS)
  - After activated carbon treatment (AC)
  - Control (C)



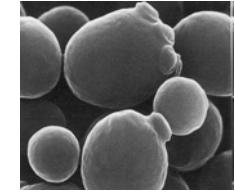
- Continuous exposure under flow-through conditions:



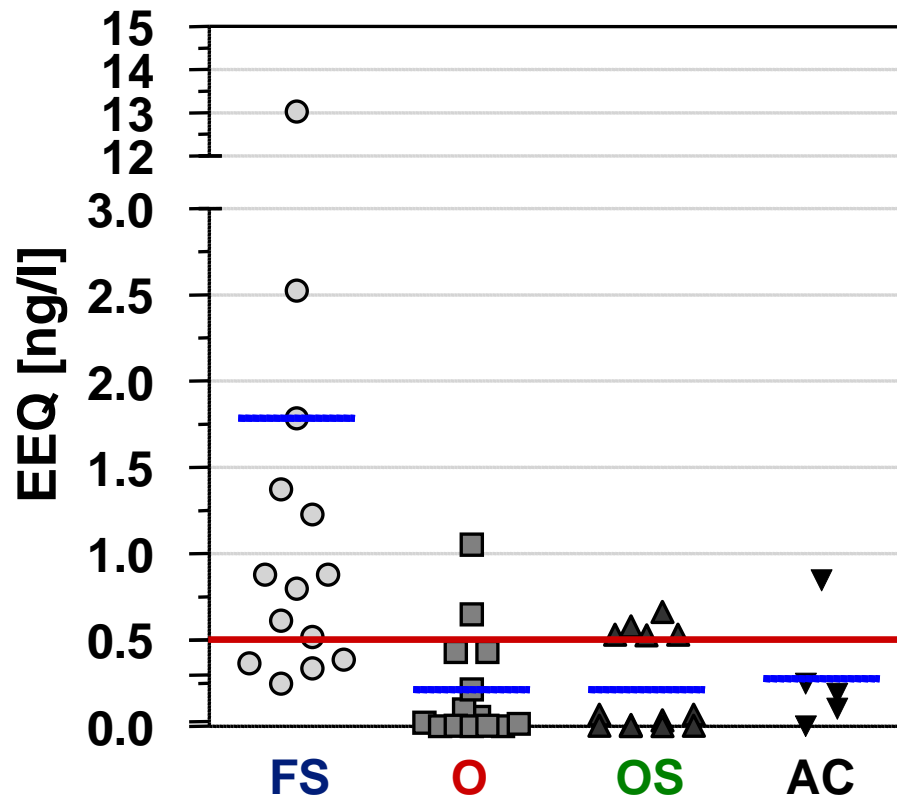
## In vivo and in vitro test systems

Species	Duration (d)	Endpoints	Media exchange/d
 <i>Lemna minor</i>	7	frond area biomass	6 fold
 <i>Chironomus riparius</i>	28	development reproduction	4 fold
 <i>Lumbriculus variegatus</i>	28	reproduction biomass	4 fold
 <i>Potamopyrgus antipodarum</i>	28	reproduction	4 fold
 <i>Oncorhynchus mykiss</i> ( <b>FELST</b> )	65	development biomass vitellogenin	2-6 fold
 YES/YAS + anti-screens		(anti-) estrogenicity (anti-) androgenicity	

**NO EFFECTS**



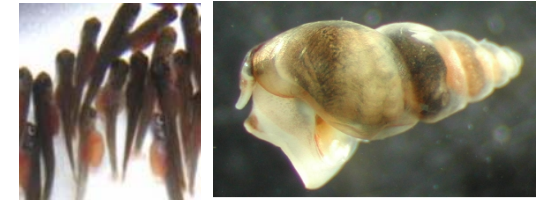
- **YES (Yeast Estrogen Screen):** Reduction of estrogenicity below EQS values after advanced treatment :



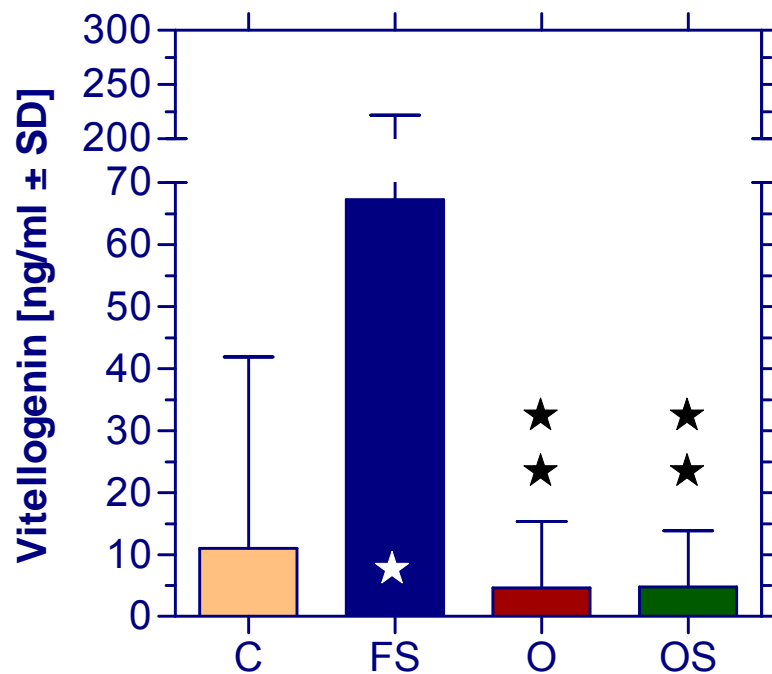
Pooled data from WWTPs  
Regensdorf and Neuss

**EQS**

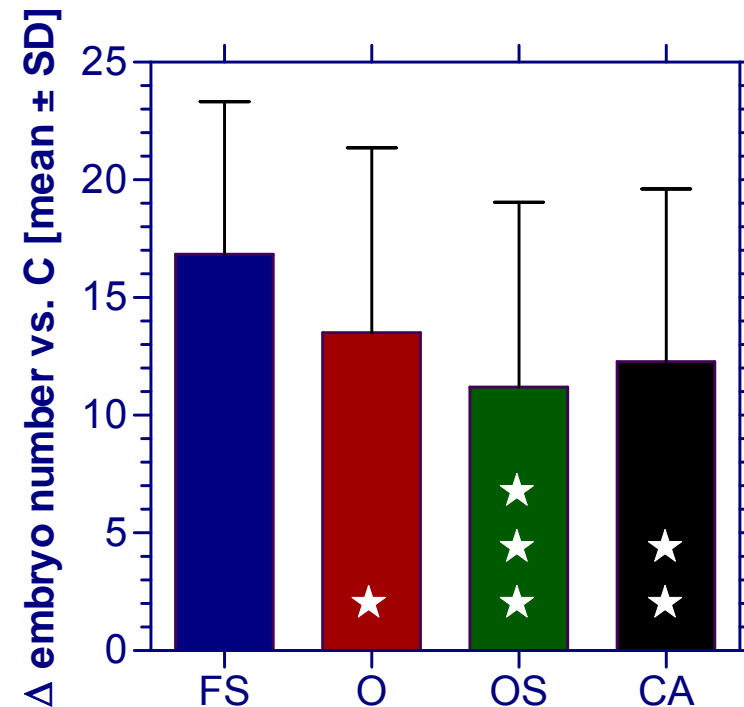
according to Moltmann et al. (2007):  
UBA Final Report FKZ 20524205.



- In vivo* confirmation of **reduced estrogenicity** following ozonation and activated carbon treatment: Vitellogenin in fish in Regensdorf (left) and embryos in snails in Neuss (right)

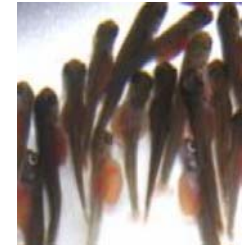


Significant vs. FS (dark asterisks) or C (white asterisk):  
★ =  $p < 0.05-0.01$ ; Kruskal-Wallis with Dunn's

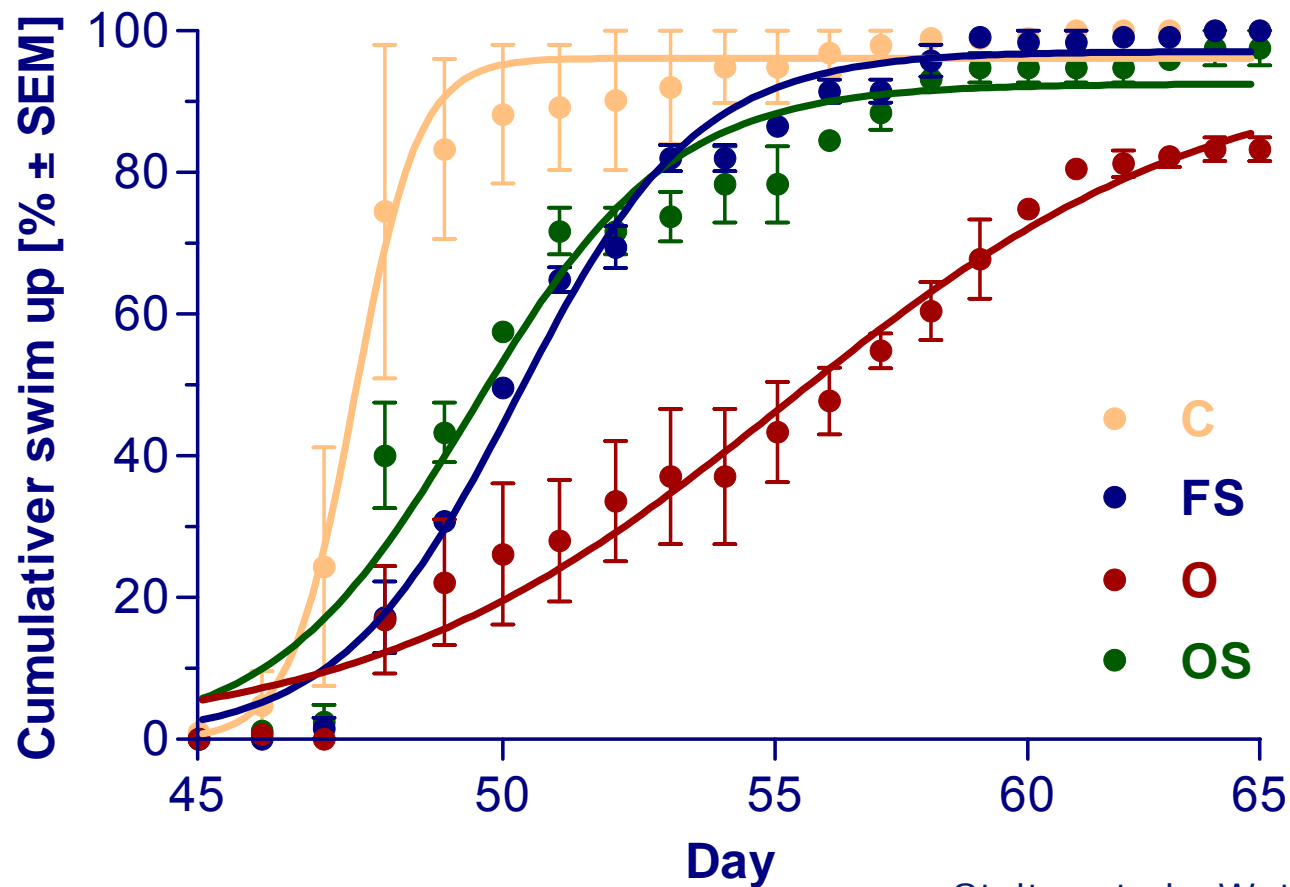


Significant vs. FS:  
★ =  $p < 0.05-0.001$ ; Kruskal-Wallis with Dunn's

Magdeburg & Stalter: unpublished

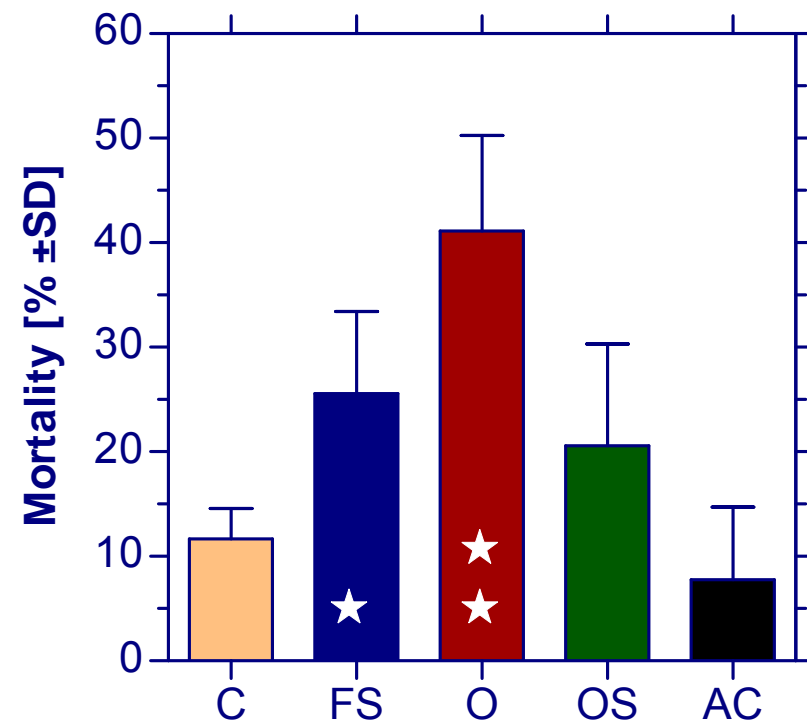
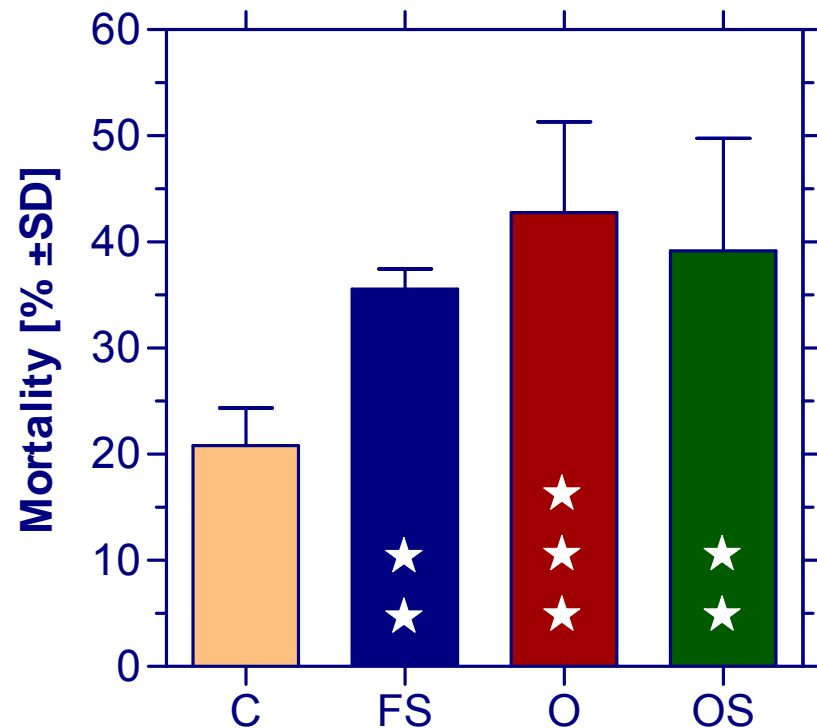


- Delayed development in the fish early life stage toxicity test (FELST) at WWTP Regensdorf, significant for O:





- Significantly **enhanced mortality** in all treatments at Regensdorf (left) and in **FS** and **O** at Neuss (right):

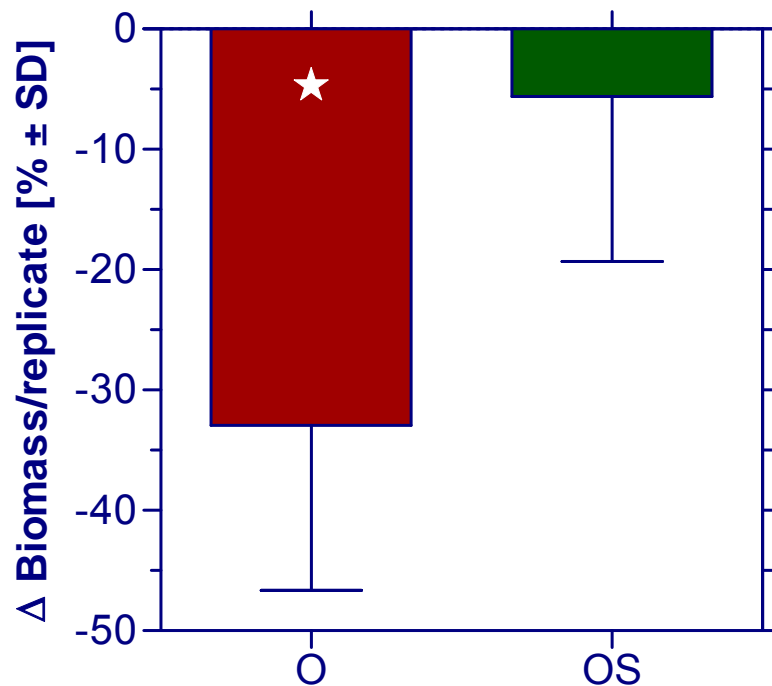


Significant vs. C: ★ =  $p < 0.05-0.001$ ; Fisher's exact test



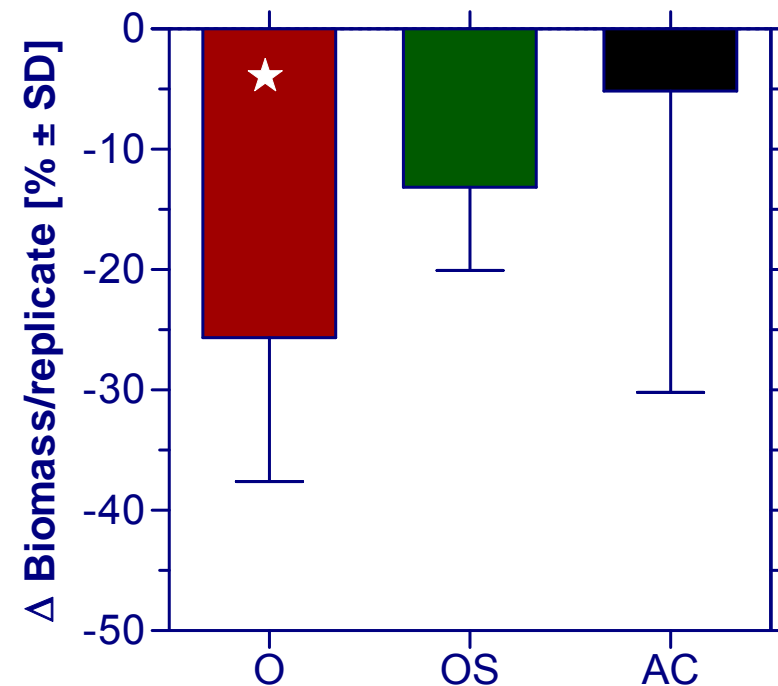


- Significantly **reduced biomass** and reproduction for **O** but not for **OS** and **AC** at Regensdorf (left) and Neuss (right):



Significant vs. FS:

★ =  $p < 0.05$ ; Kruskal-Wallis with Dunn's



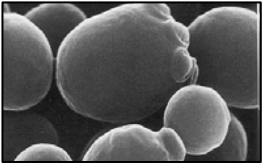


Significant vs. FS:

★ =  $p < 0.05$ ; Kruskal-Wallis with Dunn's



Species	Endpoints	O	OS	AC
<i>Lumbriculus variegatus</i>	reproduction	■	■	■
	biomass	■	■	■
<i>Oncorhynchus mykiss</i> (FELST)	development	■	■	■
	biomass	■	■	■
	mortality	■	■	■

- Significant negative effects compared to FS
- No difference compared to FS
- Significant positive effects compared to FS
- Different results for both WWTPs

	Species	Endpoint	FS	O	OS	AC
	<i>Saccharomyes cerevisiae</i> (YES)	estrogenicity	■	■	■	■
	<i>Potamopyrgus antipodarum</i>	reproduction	■	■	■	■
	<i>Oncorhynchus mykiss</i> (FELST)	vitellogenin	■	■	■	

- Significant negative effects compared to other wastewater samples
- Significant positive effects compared to FS

- Concentrations of 'emerging contaminants' **may exceed EQS** values in surface waters, requiring advanced treatment processes in WWTP for micropollutant removal
- During ozonation **toxic metabolites** may arise temporarily, however these effects are **annihilated during sand filtration**
- *In vivo* tests represent the '**gold standard**' for effluent testing but are costly and time consuming
- *In vitro* assays are a **promising alternative** in the future but exhibit still some disadvantages currently

- The support of the staff at WWTPs Regensdorf and Neuss is greatly acknowledged
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