



3rd NORMAN workshop

New tools for bio-monitoring of emerging pollutants

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Chronic biotests with *Potamopyrgus antipodarum* – Suitable tools for the detection of endocrine disruption in aquatic ecosystems?

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- Endocrine disruptors (EDs)

- chemicals causing specific effects on endocrine systems at several levels and low concentrations without relevant toxic actions

Background

Test Organism

Spiked water

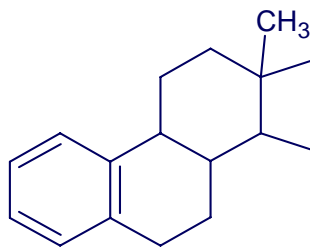
Spiked sediment

Field sediments

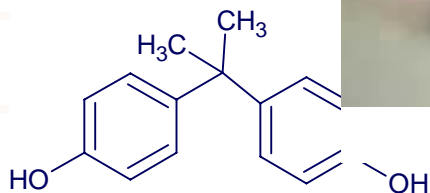
Field sediment extractions

In situ cages

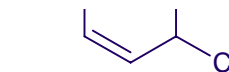
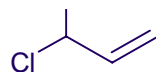
Conclusions



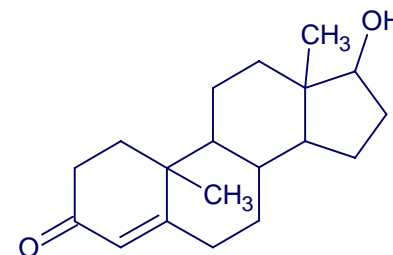
Estradiol



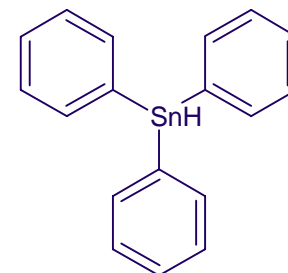
Bisphenol-A



DDT



Testosterone



Tributyltin

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• Effects of endocrine disruptors

Background

Test Organism

Spiked water

Spiked sediment

Field sediments

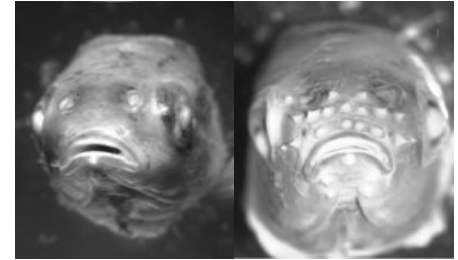
Field sediment extractions

In situ cages

Conclusions

➤ androgenic

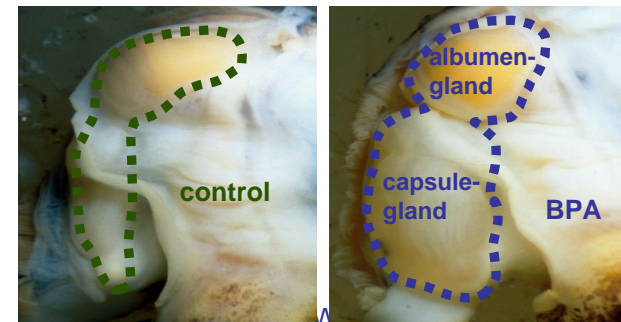
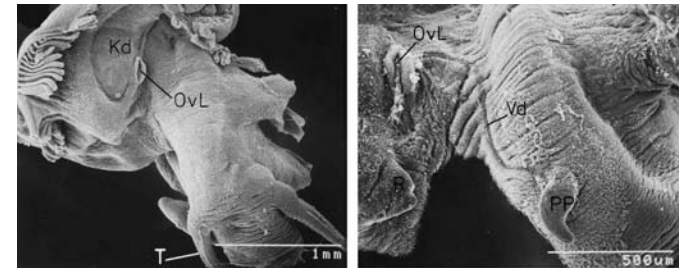
- ✓ masculinisation of females
- ✓ abnormal decrease in reproduction



✓ imposex

➤ estrogenic

- ✓ feminisation of males
- ✓ abnormal increase in reproduction
- ✓ "superfemales"



	<i>Potamopyrgus antipodarum</i>	<i>Marisa cornuarietis</i>	<i>Nassarius reticulatus</i>
Background			
Test Organism	culturing	to be initiated by specimens (e.g. from Belgium)	to be initiated by specimens e.g. from Florida
Spiked water	sexes	females only, parthenogenic	both sexes
Spiked sediment	suitability for habitats	freshwater and estuarine	freshwater only
	exposure	water and sediment	water
Field sediments	test duration	1-2 months	3-6 months
Field sediment extractions	endpoints	mortality, embryo production and development	mortality, VDSI (imposex), egg production
In situ cages	handling of analysis	easy determination	advanced skills required for imposex
Conclusions	sensitivity to EDCs	estrogens (and androgens)	androgens (and estrogens)

Background

**Test
Organism**

Spiked water

Spiked
sediment

Field
sediments

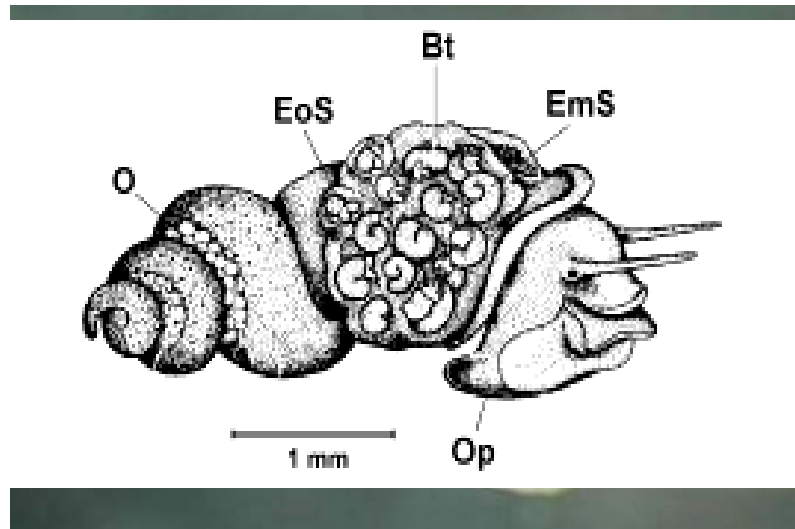
Field sediment
extractions

In situ cages

Conclusions

- *Potamopyrgus antipodarum*

- invasive species, originally from New Zealand
- shell size up to 4.5 mm
- benthic and epibenthic
- tolerant for high salinities and variations in oxygen concentrations and pH



- ovoviviparous
- parthenogenic
- sensitive to endocrine active substances

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Shelled and unshelled embryos of *P. antipodarum* after removal of the shell

Background

**Test
Organism**

Spiked water

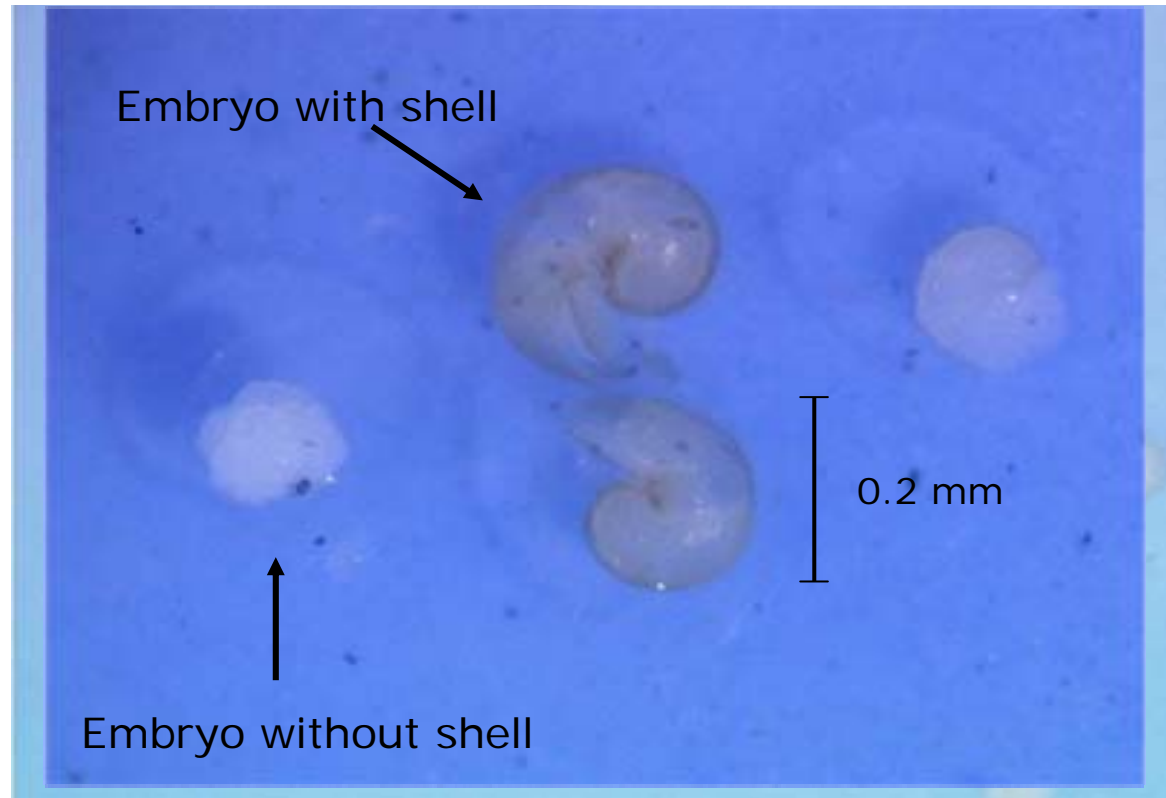
Spiked
sediment

Field
sediments

Field sediment
extractions

In situ cages

Conclusions



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• Test Setup

Background

Test
Organism

Spiked water

Spiked
sediment

Field
sediments

Field sediment
extractions

In situ cages

Conclusions

- $16 \pm 1^\circ\text{C}$
- 16:8 h light/dark
- 10 snails per replicate
- 2-3 replicates
- control / solvent control
- reproduction, growth and embryo development as endpoints
- analysis after 14, 28 (& 56 days)



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Background

Test Organism

Spiked water

Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions

SOP FOR TESTING OF CHEMICALS

Also: **PROPOSAL FOR A NEW GUIDELINE**
Reproduction Test with the Prosobranch Snail *Potamopyrgus antipodarum* for Testing Endocrine Active Chemicals

Content of SOP:

- Part I: Culturing of *Potamopyrgus antipodarum*
- Part II: Reproduction Test using Water Exposure
- Part III: Reproduction Test using spiked Sediment

Version: 2006-02-08



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Background

Test Organism

Spiked water

Spiked sediment

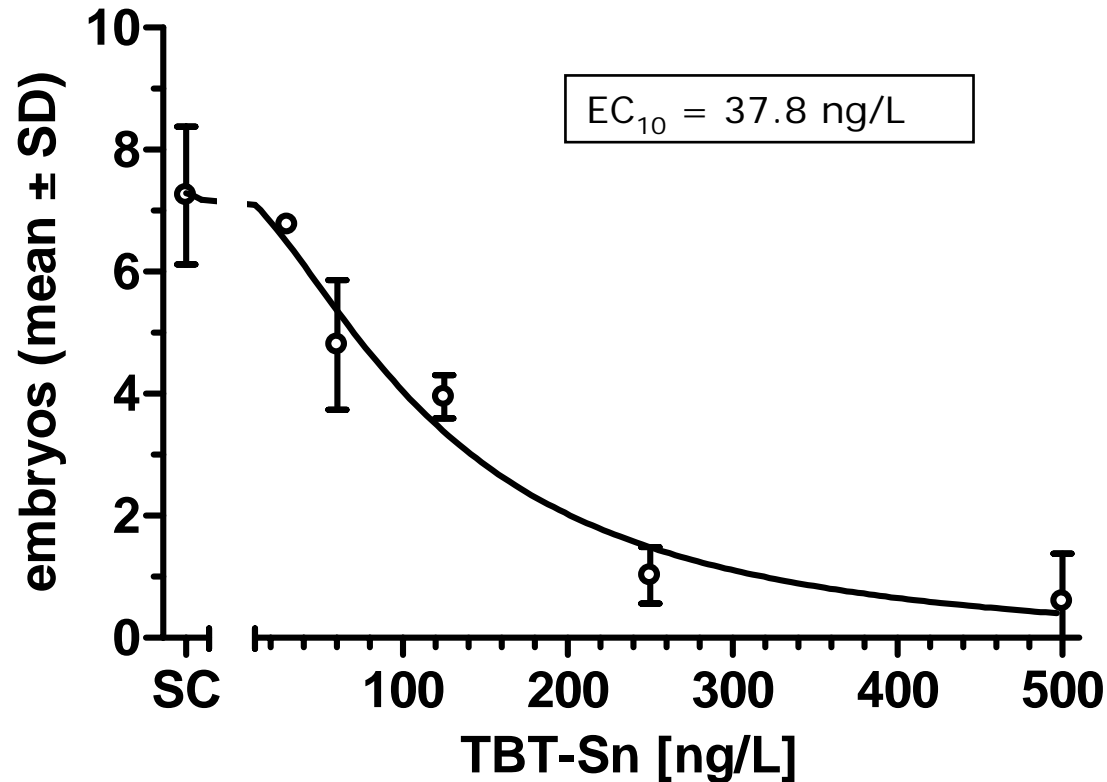
Field sediments

Field sediment extractions

In situ cages

Conclusions

The antifouling compound Tributyltin (TBT) - androgenic -



Effects of Tributyltin on unshelled embryo numbers of *P. antipodarum* after 4 weeks of exposure. n=20;
Regression line is added.

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Background

Test Organism

Spiked water

Spiked sediment

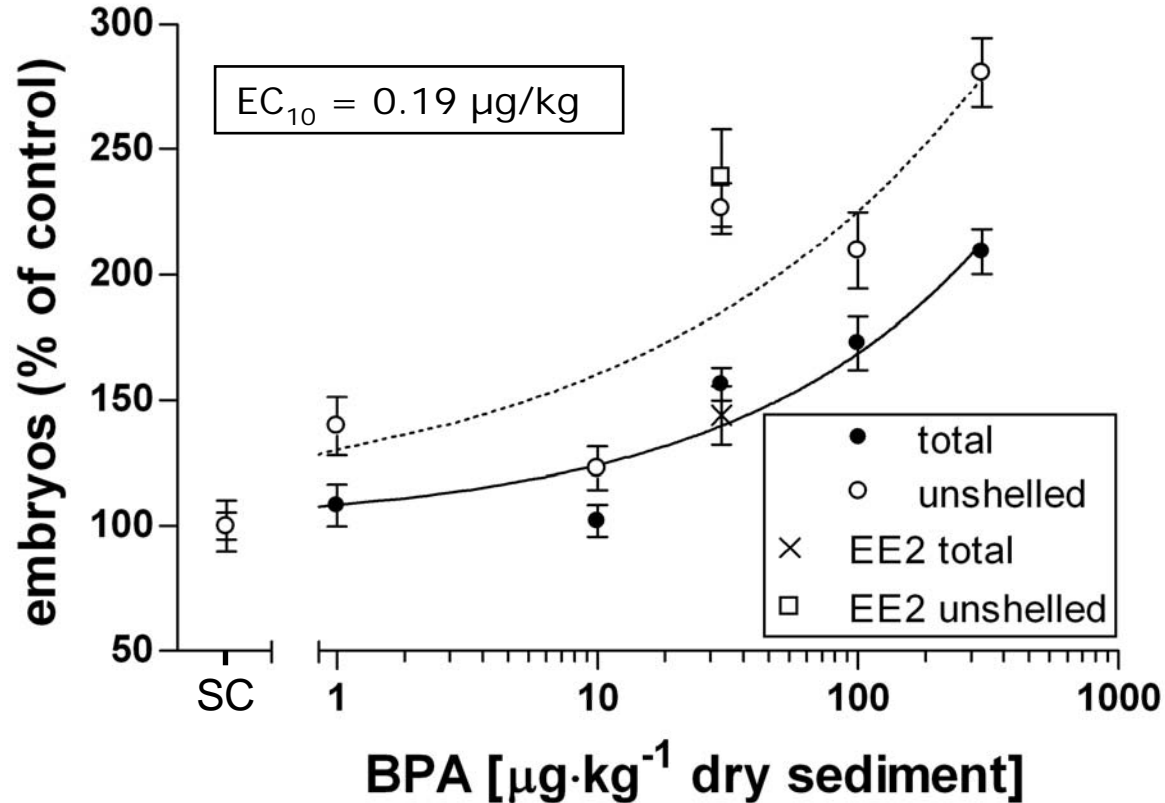
Field sediments

Field sediment extractions

In situ cages

Conclusions

The plasticizer Bisphenol A (BPA) - estrogenic -



Effects of Bisphenol A and EE₂ on embryo numbers (± SEM) of *P. antipodarum* (without shell and total) after 4 weeks of exposure. n=20; Regression lines are added. (Data from Duft et al., 2003)

Background

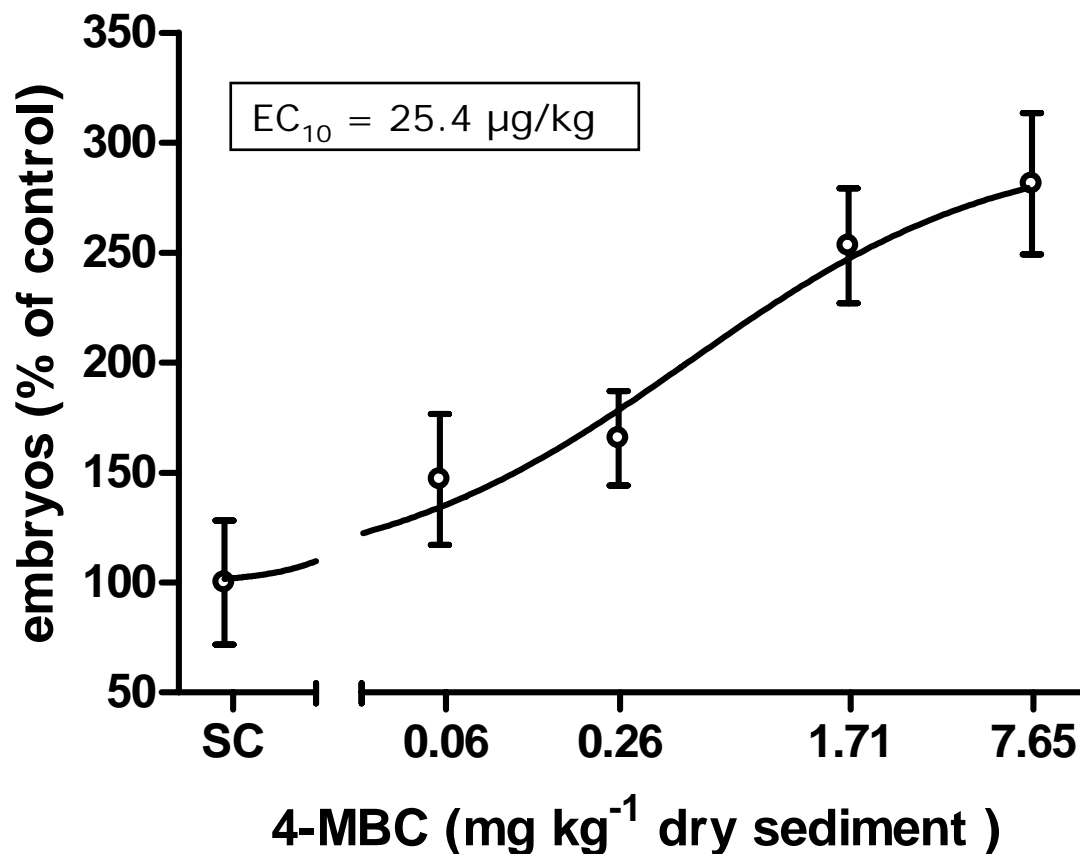
Test Organism

Spiked water

**Spiked
sediment**Field
sedimentsField sediment
extractions

In situ cages

Conclusions

The UV filter 4-methylbenzylidencamphor (4-MBC)
- estrogenic -

Effects of 4-Methylbenzylidencamphor (4-MBC) on mean numbers (\pm SD) of unshelled *P. antipodarum* embryos after 8 weeks of exposure. n=20; Regression line is added. (Data from Schmitt et al., 2007)

Background

Test Organism

Spiked water

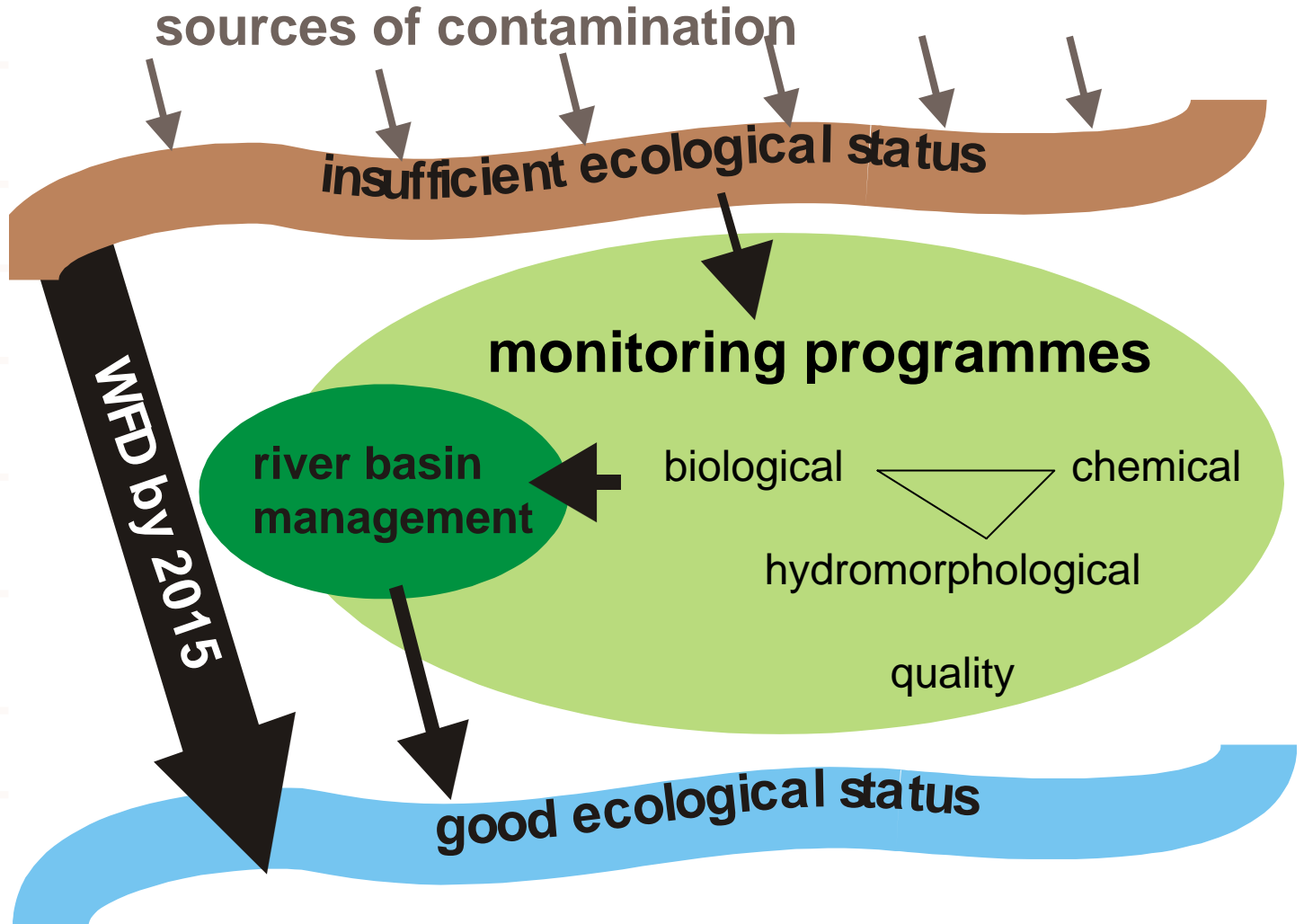
Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions



Background

Test Organism

Spiked water

Spiked
sediment

**Field
sediments**

Field sediment
extractions

In situ cages

Conclusions

- **sampling sites**

- were identified based on monitoring data
- sediments from three different river basins
 - ✓ Elbe/Bilina (Czech Republic)
 - ✓ Scheldt/Schijn (Belgium, The Netherlands)
 - ✓ Llobregat/Anoia (Spain)
- both reference and polluted site for each river
- sampled at the same time (summer 2006)
- stored dark and cool

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Macro invertebrate community at the Schijn (Belgium)

Background

Test Organism

Spiked water

Spiked sediment

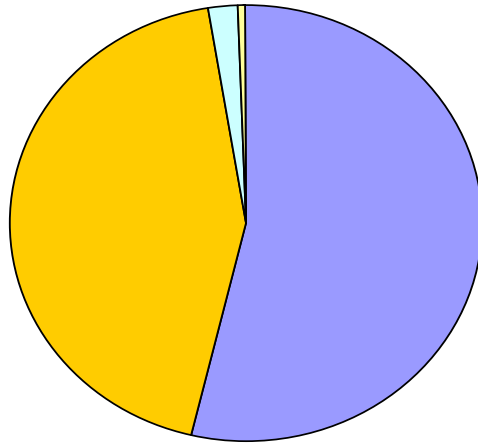
Field sediments

Field sediment extractions

In situ cages

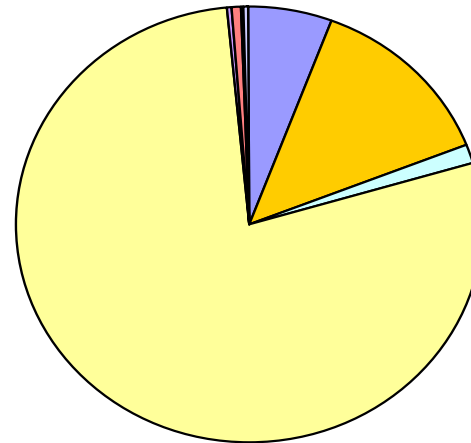
Conclusions

Polluted Site



205 organisms in total

Reference Site



1030 organisms in total

- Gastropoda
- Bivalvia
- Hirudinea
- Crustacea
- Ephemeroptera
- Diptera
- Megaloptera
- Trichoptera

(Oligocheata and Chironomidae are not yet included)

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Background

Test Organism

Spiked water

Spiked sediment

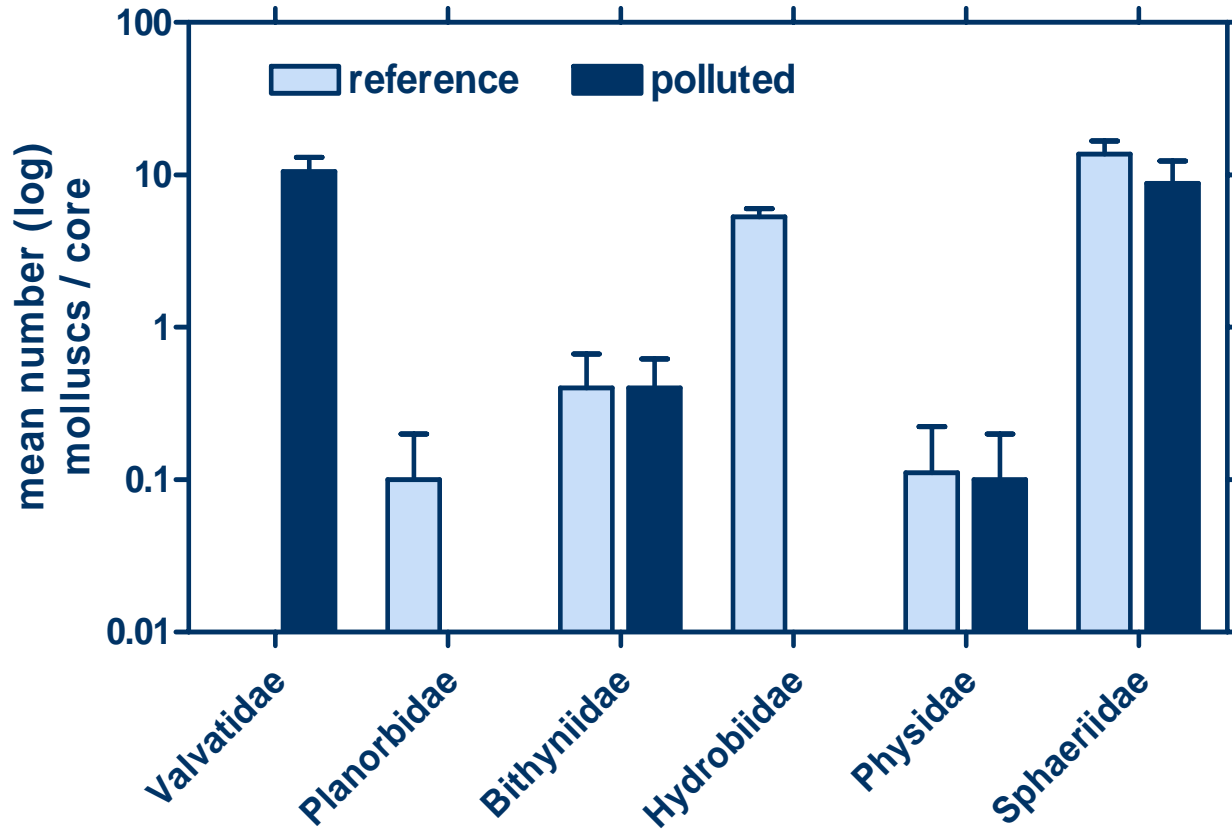
Field sediments

Field sediment extractions

In situ cages

Conclusions

Composition of molluscs at the Schijn (Belgium)



Mean numbers (log) of molluscs found per sediment core in the river Schijn at reference site (Run) and polluted site (Een) in summer 2006

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Chronic biotests with *Potamopyrgus antipodarum* – Suitable tools for the detection of endocrine disruption in aquatic ecosystems?

Background

Test Organism

Spiked water

Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions



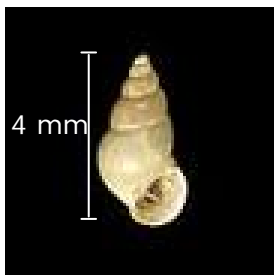
Field sediments from three different river basins



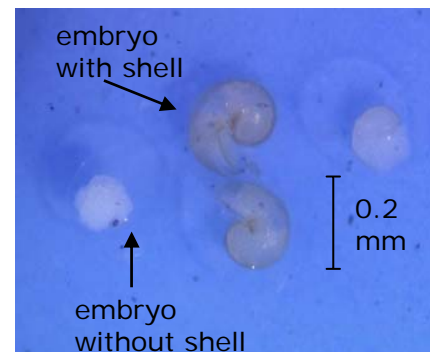
Laboratory culture of *Potamopyrgus antipodarum*



Sediment contact test



Endpoint growth



Endpoint reproduction

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Background

Test Organism

Spiked water

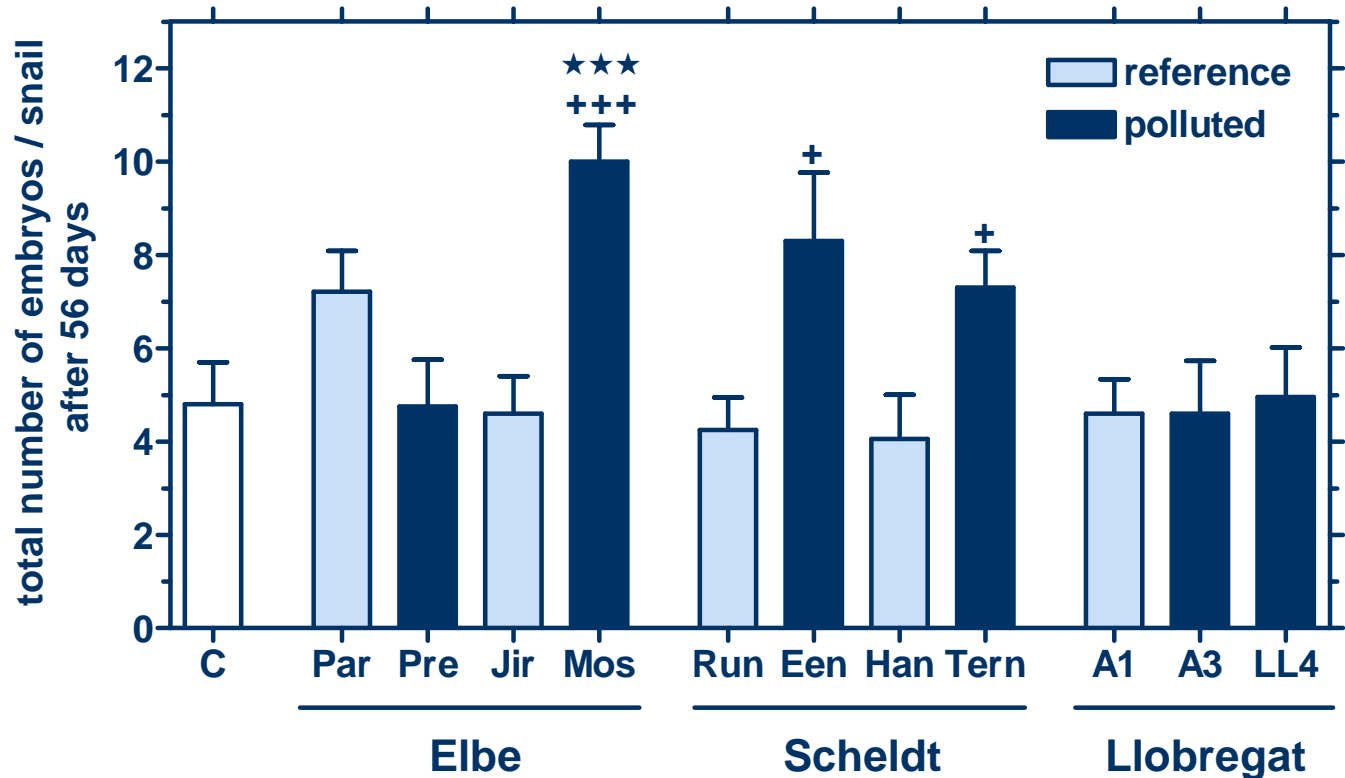
Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions



Mean (± SEM); C=artificial sediment control; n=20; ★ significant differences to control; + to corresponding reference site; + p < 0.05, ★★/+++ p < 0.001; Mann-Whitney U-test)

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- possible explanations

Background

Test Organism

Spiked water

Spiked
sediment

**Field
sediments**

Field sediment
extractions

In situ cages

Conclusions

- effects of estrogenic substances at sites where embryo number increased
 - ✓ 50 ng/l BPA* in water samples of the river Bilina
 - ✓ 27 – 428 ng/g Nonylphenol* in sediment samples of the river Elbe and tributaries
- effects of different sediment characteristics
 - ✓ variances in TOC and grain size distribution are expected
 - ✓ reproduction of *P. antipodarum* is not influenced by variances in TOC content between 1.2 and 9%#

*Stachel et al., 2003

#Duft et al., 2003

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Background

Test Organism

Spiked water

Spiked sediment

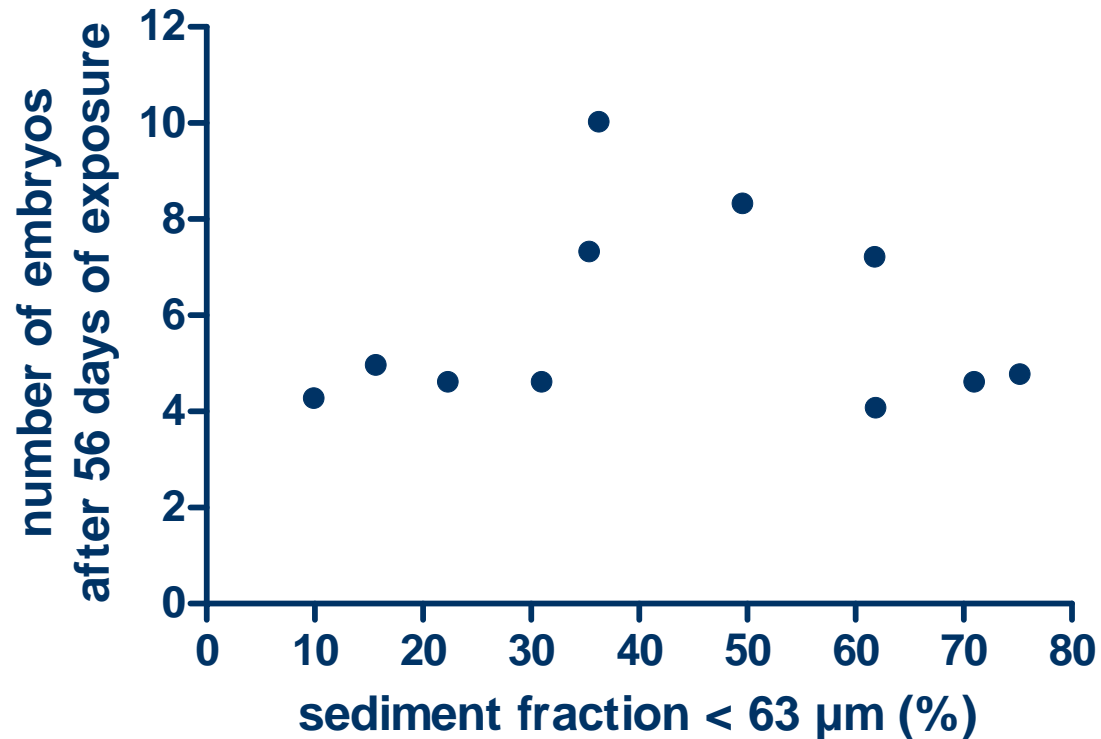
Field sediments

Field sediment extractions

In situ cages

Conclusions

reproduction vs grain size



Correlation of the mean number of *P. antipodarum* embryos and the sediment fraction < 63 μm.
Pearson correlation, $r^2 = 0.57$, $p = 0.007$

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Background

Test Organism

Spiked water

Spiked sediment

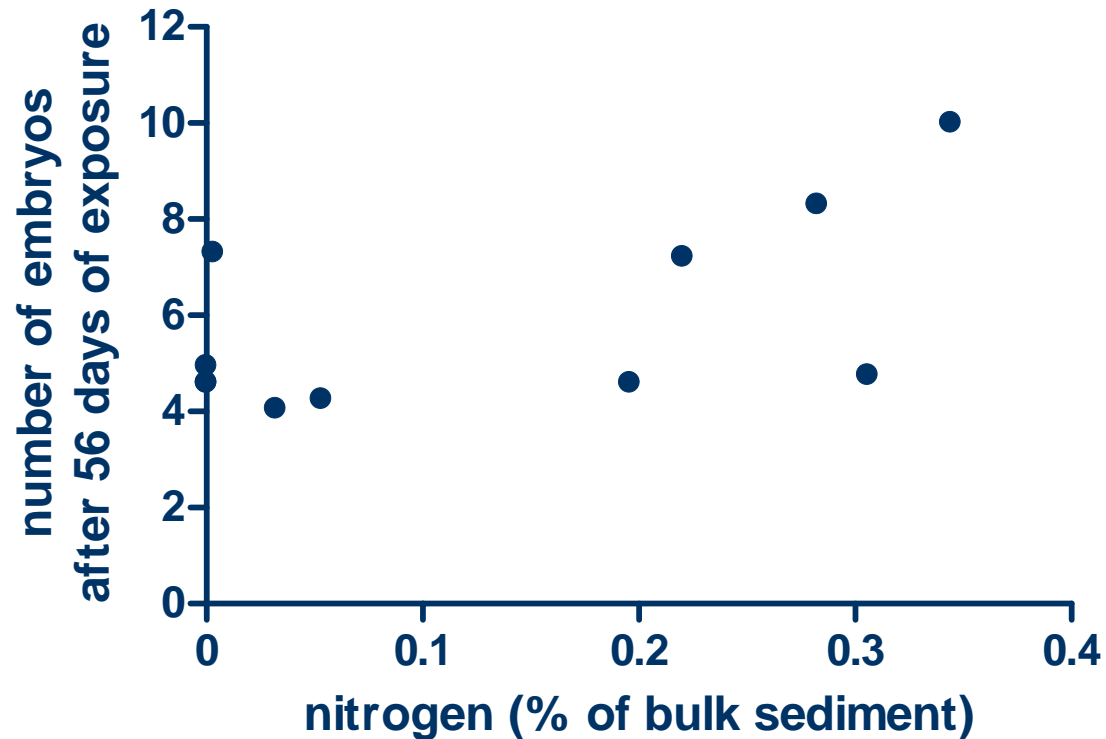
Field sediments

Field sediment extractions

In situ cages

Conclusions

reproduction vs nitrogen content



Correlation of the mean number of *P. antipodarum* embryos and the nitrogen content in bulk sediment. Pearson correlation, $r^2 = 0.36$, $p = 0.05$

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Chronic biotests with *Potamopyrgus antipodarum* – Suitable tools for the detection of endocrine disruption in aquatic ecosystems?

Background

Test Organism

Spiked water

Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions



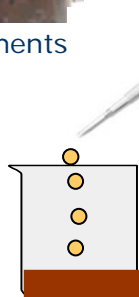
Field sediments



Extraction/Fractionation



Laboratory culture of *Potamopyrgus antipodarum*



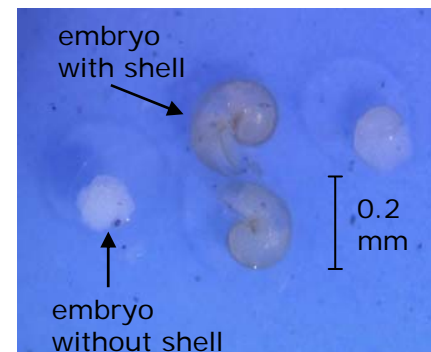
Spiked artificial sediment



Endpoint growth



Sediment contact test



Endpoint reproduction

Background

Test Organism

Spiked water

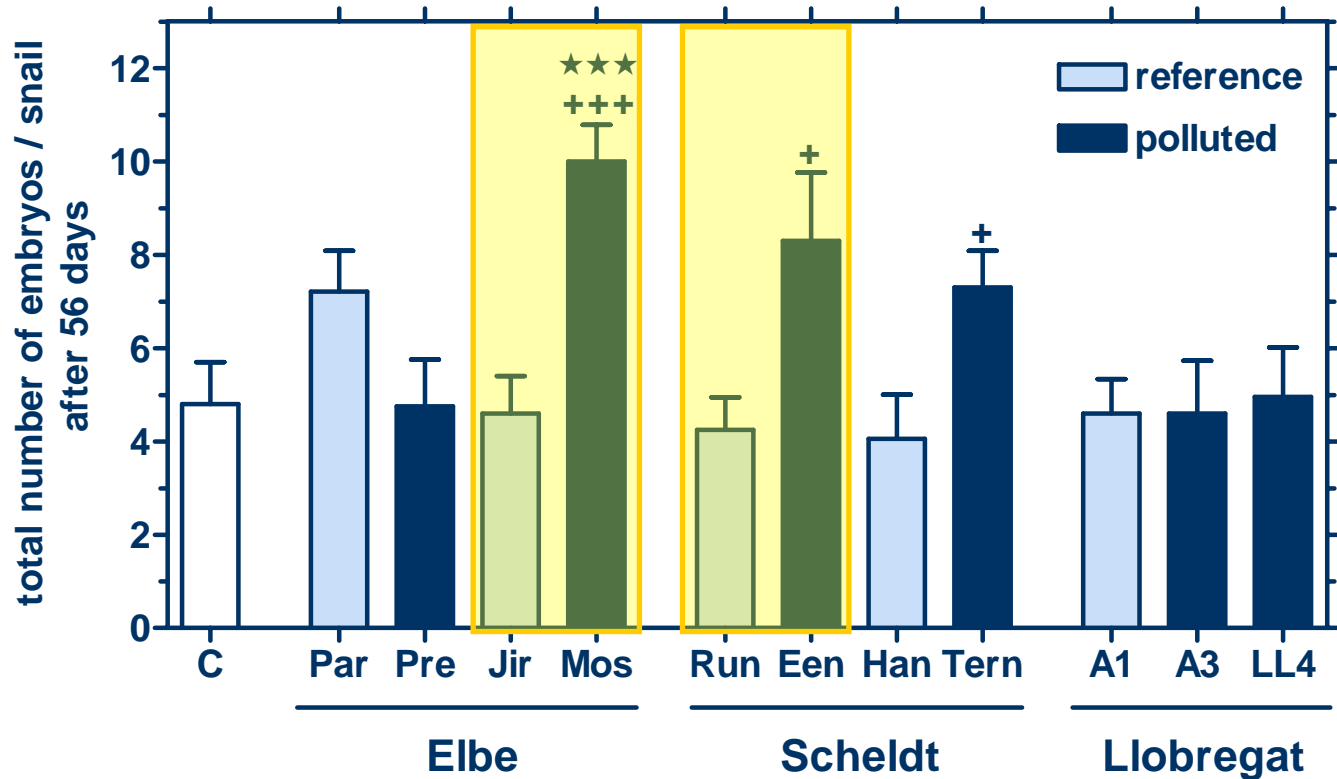
Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions



Mean (± SEM); C=artificial sediment control; n=20; ★ significant differences to control; + to corresponding reference site; + p < 0.05, ★★★/+++ p < 0.001; Mann-Whitney U-test)

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Background

Test Organism

Spiked water

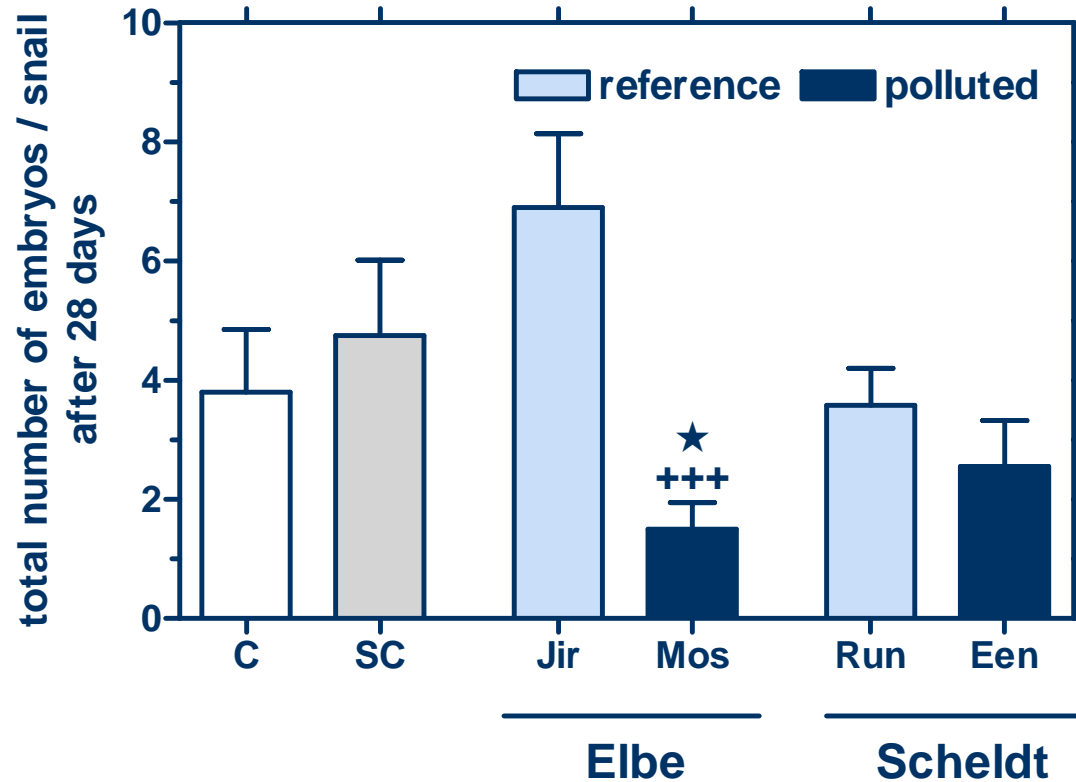
Spiked sediment

Field sediments

Field sediment extractions

In situ cages

Conclusions



Mean number (\pm SEM); n=20; C=control, SC=solvent control with dichlormethane; ★ significant differences to solvent control; + significant differences to corresponding reference site; ★ p < 0.05, +++ p < 0.001; Mann-Whitney U-test)

Effect Directed Analysis

Background

Test Organism

Spiked water

Spiked
sediment

Field
sediments

**Field
sediment
extractions**

In situ cages

Conclusions

- identifying certain groups of compounds which could be responsible for the observed effects
 - ✓ fractionation of the field sediment extracts into 4 different fractions according to their chemical characteristics
 - ✓ spiking the artificial sediments with solutions of each field sediment fraction



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In situ biotests

Background

Test Organism

Spiked water

Spiked
sediment

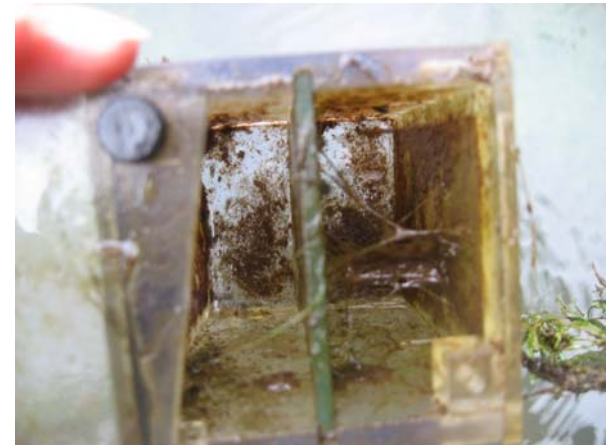
Field
sediments

Field sediment
extractions

In situ cages

Conclusions

- exposure via the water phase and biofilm
- pre-exposure of the cages for 14 days to enable sufficient growth of biofilm
- exposure of 20 snails per cage for 28 days
- mortality, growth and reproduction as endpoints



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Background

Test Organism

Spiked water

Spiked
sediment

Field
sediments

Field sediment
extractions

In situ cages

Conclusions

- **Conclusions**

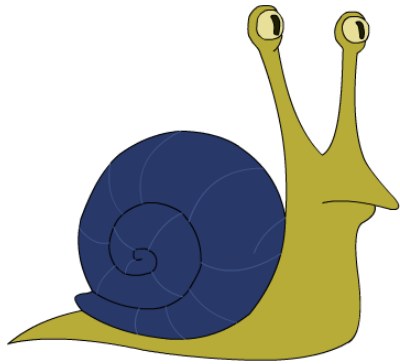
- the reproduction of *Potamopyrgus antipodarum* is an appropriate endpoint for measuring effects of endocrine active chemicals in the laboratory
- the biotests with *P. antipodarum* can be used in various different ways regarding different ways of exposure
- *P. antipodarum* is a promising test organism for the assessment of endocrine disruption in field sediments

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THANK YOU

