# MODELKEY Newsletter No. 5
# March 2008

**Models for Assessing and Forecasting the Impact of Environmental Key Pollutants on Marine and Freshwater Ecosystems and Biodiversity**

## Introduction

Evidence is increasing that the achievement of a good ecological status according to the EU-WFD till 2015 for many European rivers is a very challenging task. Although it is obvious that in many rivers an improvement of hydromorphological conditions is crucial, renaturation often does not improve the ecological status significantly. Other stressors including toxic contaminants are believed to prevent recovery. MODELKEY is designed to provide tools for identification and assessment of toxic hazards to the quality elements according to WFD and to the aquatic ecosystem as a whole.

With the present newsletter we want to focus on phytobenthos and benthic invertebrates as key elements for ecosystem services including carbon and nutrient cycling, and water purification. You will find a selection of promising investigative monitoring tools that may help you to unravel cause-effect relationships between insufficient ecological quality and contamination at the sites of your concern. This will support you in your attempt to select efficient and cost-effective measures for achieving the goals of the WFD.

## Contents

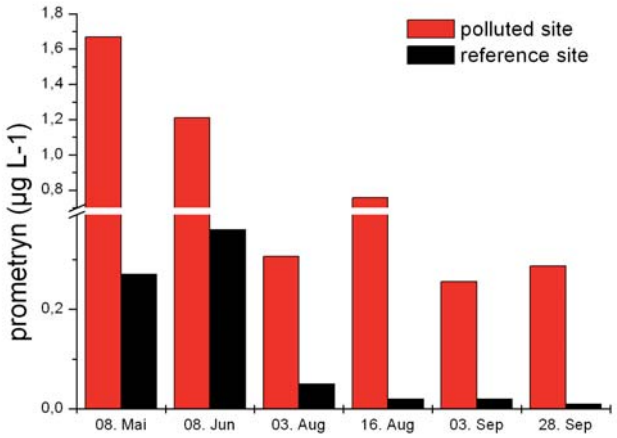


## Biofilm translocation – a tool to assess toxicity at site-scale

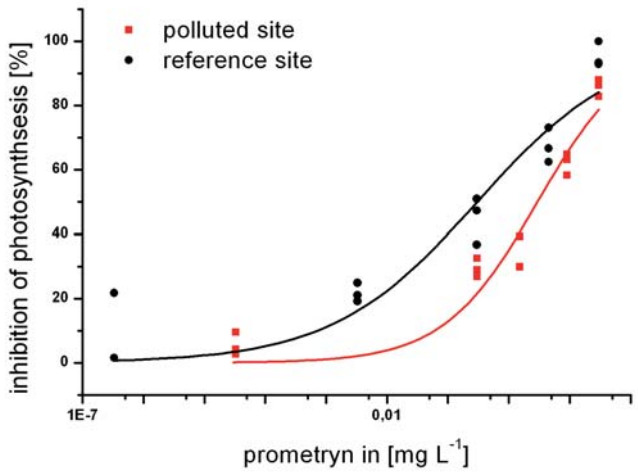
Biofilm translocation is an "*in situ*" essay exploring early changes in biofilms due to the pollutants mixture exposure. Laboratory biofilms - cultivated under controlled and reproducible conditions - are used as a reference and translocated into various sampling sites of the Llobregat. Structural and functional parameters, followed over time, are used as endpoints to describe the magnitude and type of effects of pollutants mixture on biofilms. This tool allows to link toxicity effects observed in the laboratory (using "*in vitro*" and "*in vivo*" essays) with biodiversity changes observed in the field as well as to validate the relationship between biofilm characteristics and the ecological status of the river. The *in-situ* experiments allow an estimation of the impact of emerging toxicants. Moreover, the use of laboratory biofilms as a tool to assess ecological status of rivers is very promising as an early warning system in the aquatic environment.

## Community changes can be linked to toxicant exposure in the field

Algal communities are one of the first to interact with dissolved phytotoxicants in streams and rivers. Therefore they may show effects of toxicants earlier than higher organisms like insects, fishes and mammals. PICT (Pollution-Induced Community Tolerance) is assumed to establish causal relationships between toxicant exposure and effects in ecosystems. Furthermore the PICT-methodology is able to detect effects in a community, consisting of different species.
A longterm exposure (several weeks) to a toxicant can induce a replacement of sensitive species with more tolerant organisms. For this reason, the whole community shows a higher tolerance to the toxicant than a community growing without toxic exposure. In this study, the impact of the herbicide prometryn on algal communities was investigated in a heavily polluted stream in the Elbe River - catchment (Germany) and was compared to an unpolluted reference river. Therefore, algae were colonised at both sites and water samples for chemical analysis were collected to assess the prometryn exposure at the same time. The algal community of the polluted river showed a 4.5 times higher tolerance to prometryn in comparison to the reference site. These results were verified under controlled laboratory conditions, where algal communities exposed to prometryn (20 µg $L^{-1}$) showed a 2.6 - 8 times higher tolerance than the unexposed controls.
Consequently, PICT can be used to detect effects on communities in a complex field situation and can be linked to toxicant exposure. For this reason, PICT may be considered as a higher-tier ecotoxicological tool in the risk-assessment of chemicals.



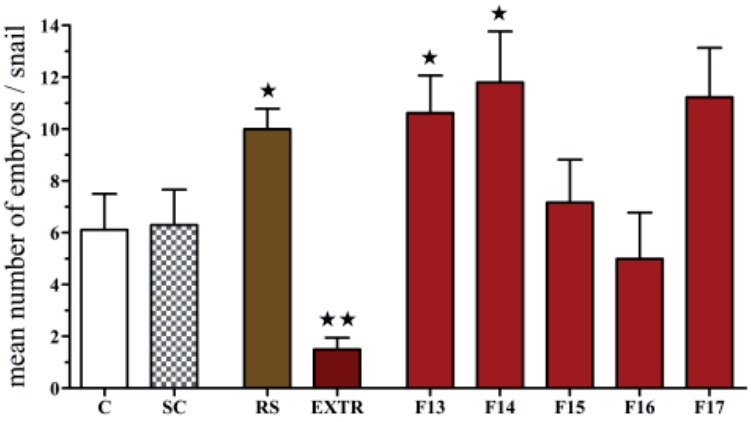

*Prometryn concentrations in µg $L^{-1}$, sampling at the stated dates from the water phase of the polluted (■) and reference (■) site.*




*Concentration-response curve of the inhibition of photosynthesis of algal communities, after three weeks growing at the polluted (■) and reference (■) site.*

## Effect directed analysis of estrogenic compounds in sediments using *in vivo* biotests with the mud snail *Potamopyrgus antipodarum*

The New Zealand mud snail *Potamopyrgus antipodarum*, an invader which occurs almost everywhere in Europe, has already been reported to be a very sensitive organism to endocrine disrupting compounds. Mono substance tests revealed an increasing reproduction of this snail after exposure to estrogens and a decreasing reproduction after exposure to androgens. This outstanding sensitivity is used within the MODELKEY project to identify “estrogenic hotspots” and their responsible key pollutants in the observed river basins. First, sediment contact tests were carried out with the raw sediments collected at the sites of interest, where 50% of the investigated hotspots led to an increasing embryo production of *P. antipodarum*. Afterwards, sediment extractions followed by high resolution fractionation techniques were performed. Extracts and fractions were subsequently spiked to clean, artificial sediments, and the results gave useful information about which group of compounds could explain the observed effects in the raw sediments. The figure below shows the results of the hotspot “Most” at the river Bilina, a tributary to the Elbe, as an example. For the crude extract of

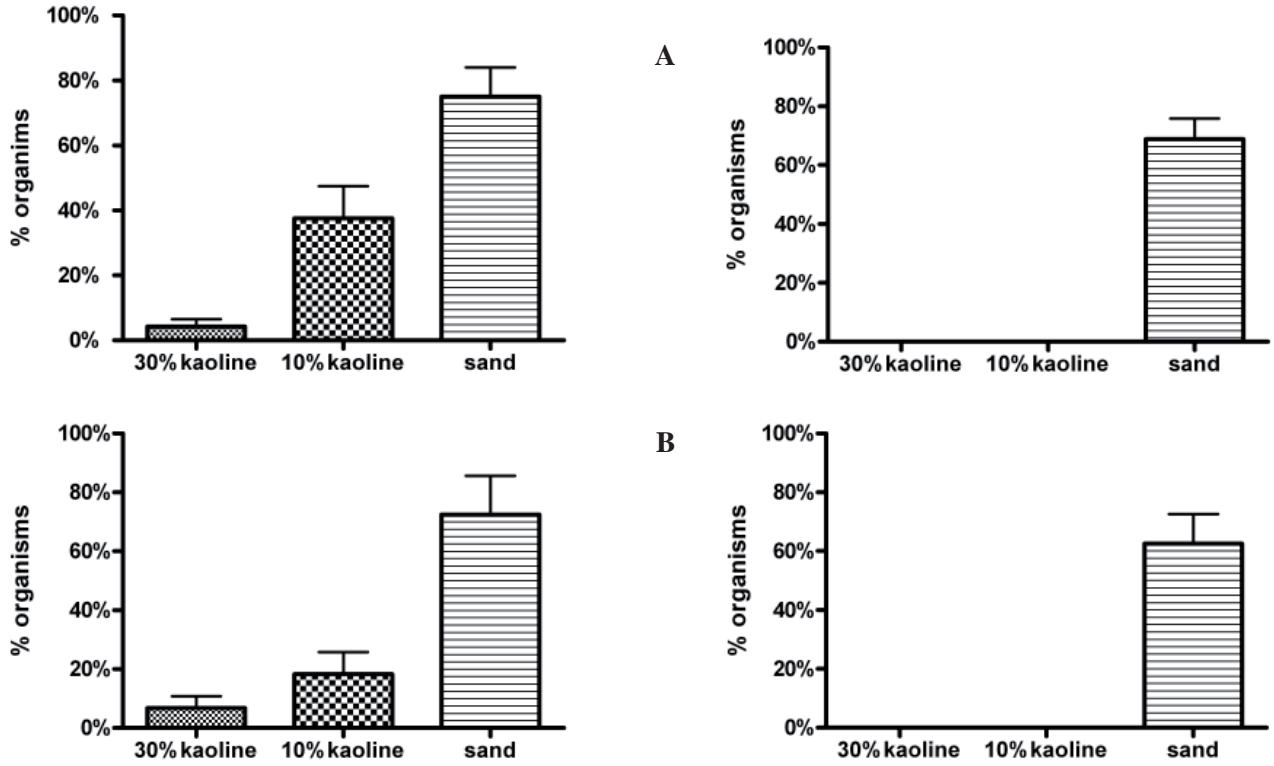



*Sediment contact tests with sediments, extracts and fractions of the river Bilina at Most. Mean number (± SEM) of embryos per Potamopyrgus antipodarum; n=20; C=control, SC=solvent control, RS=raw sediment, EXTR=extract, F=fraction; ★ significant differences to solvent control; p < 0.01 - 0.05, Mann-Whitney U-test.*

the sediment a significant decrease in reproduction was observed, which may be due to a higher toxicity deriving from a higher bioavailability in the artificial sediments. However, in the raw sediment and also after fractionation, a significant increase in reproduction was observed for the fractions 13 and 14 of the same sediment. Therefore we can assume that the most hazardous natural and/or xeno estrogens are located in the fractions 13, 14 and probably also 17. Further steps will include a detailed screening of the respective fractions, followed by an effect confirmation where the most problematic compounds found in those fractions will be tested again in mono substance tests with *P. antipodarum*.

## Sediment preference of the bivalve *Sphaerium corneum*

Large grain sizes are linked to streams with a high stream velocity and consequently high oxygen levels. Because toxicants tend to bind to small grain size fractions, the pollution will consequently be higher on locations with a lot of fine sediments and a lower stream velocity. In general, more macroinvertebrate species are found on locations with sand sediments compared to clay sediments. Consequently, the question can be asked whether some of the species which are mainly found on sites with sand sediments really prefer sediments with larger grain sizes or if they are simply avoiding the pollution present in smaller grain sizes.
To test this hypothesis, we conducted flume experiments with the common freshwater bivalve *Sphaerium corneum*, collected at two non-polluted sites




*% Sphaerium corneum organisms of the Schijn (A) and Breiloop (B) burrowing in the different sediment types.*

*% Sphaerium corneum organisms of the Schijn (A) and Breiloop (B) totally buried in the different sediment types.*

(Schijn Rundvoortbrug and Breiloop) in the Scheldt basin. To investigate the sediment preference of *Sphaerium corneum* 3 artificial sediments were used (sand, sand+10% kaoline and sand+30% kaoline). Endpoints that were looked at were e.g. burrowing activity on the sediment surface and total burying into the sediment.
The *Sphaerium* organisms of both sites showed a significant preference for sand sediments, 70-80% of the organisms showed burrowing activity in the sand sediment compared to 20-40% with 10% kaoline mixture and not even 10% with the 30% kaoline mixture. Total burying was only observed in the sand sediments at the end of the tests.

Besides these results, *Sphaerium* can endure moderate pollution levels and they occur in Flanders mainly on sand sediments. This indicates that they clearly have a sediment preference and are not just avoiding pollution or low oxygen levels. Literature showed that also the marine bivalves have preferences for certain grain sizes.
It can be concluded that other factors than pollution, e.g. grain size, should be taken into account as well when modelling aquatic ecosystems because the grain size influences the distribution of macroinvertebrates in freshwater ecosystems and also the bioavailability of contaminants in sediments.

## Patterns in the relationships between emergent toxicants and biological structure: Llobregat case study

The assessment and conservation of biodiversity have been the focus of much attention in recent years. Trends and causes of species loss provide information for ecological risk assessment in areas strongly affected by human activity.
It is often difficult to establish relationships between the chemical characteristics of river water and biological community status. In the Llobregat basin, and within the framework of the MODELKEY project, the assessment of the effects of toxicants on the biological communities has been approached as a multidisciplinary study at site scale.

The Llobregat River is characterized by high discharge fluctuations, which reflect the Mediterranean climate. The headwaters of this river are characterized by abundant agricultural activities while middle and lower reaches have dense industrialised areas. More than 5 million people live in this basin and eutrophication is important. In several sites of the lower part of the river the chemical analyses revealed the presence of emerging contaminants, some of them endocrine disrupter compounds (EDC's): estrogens and progestogens and pharmaceuticals and alkylphenolic compounds.

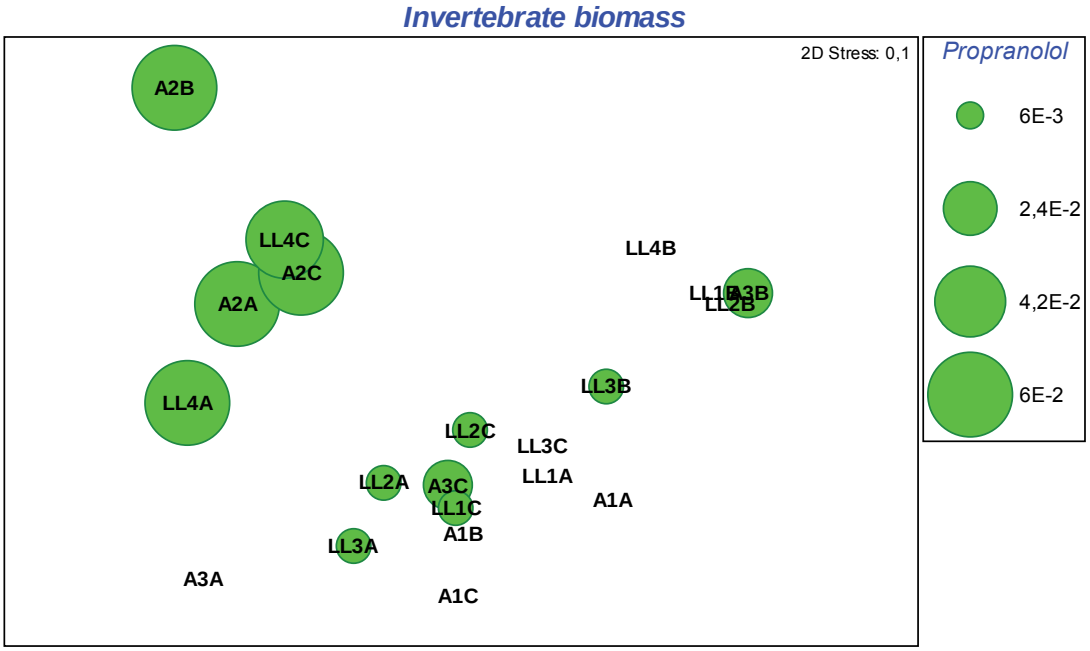
*List of biological, chemical and toxicant parameters measured in the Llobregat River.*

| | |
|---|---|
| Biological parameters | diatom composition and density in the substrate<br>macroinvertebrate density and biomass in the soft sediment<br>bacterial density<br>exoenzymatic activities<br>chlorophyll a concentration |
| Physico-chemical parameters | temperature<br>oxygen concentration<br>pH<br>conductivity<br>nutrient concentration (phosphate, total P, nitrate, nitrite, ammonium)<br>anions and cations<br>TOC<br>suspended solids |
| Toxicant parameters | metal concentration (Ba, Pb, Al, Mn, Zn)<br>pesticides (bentazone, 2.4-D, MCPA, mecoprop, propanyl, fenitrothion, isoproturone, atrazine, diurone, deisopropylatrazine)<br>estrogenic compounds (Estrone, Estradiol, Estriol, EE, DES, E1, E2, E3)<br>alkylphenolic compounds (LAS, CDEA, NP1, NP2, NP9, OP1EC)<br>pharmaceuticals drugs (analgesics and anti-inflammatories, lipid regulators and cholesterol lowering statin drugs, psychiatric medication, anti-ulcer agent, histamine H1 and H2 receptor antagonists, antibiotics, ß-blockers) |

Simultaneous samples were taken along the lower reach of the Llobregat River during spring and autumn of 2005 and 2006. This sampling strategy is crucial for the multivariate treatment of the data in order to find relationships between the water quality status and the biological community structure. The biological, chemical and toxicant parameters analysed are listed in the table to the left.

Chemical and biological results were contrasted with the routine BEST in the PRIMER v. 6, statistical package (Plymouth Marine Laboratory, UK, 2006) and using a Redundance Analysis (CANOCO program). These procedures were applied to find the best match between the among-sample patterns of biological assemblages and the environmental variables associated with those samples.

Preliminary results show that the abundance and biomass of macroinvertebrate community are mainly related with some pharmaceutical agents (some analgesics and β-blockers). Nevertheless further analysis is required to confirm these relationships.




*MDS (multidimensional scaling) of macroinvertebrate biomass at the sampling sites (LL: Llobregat river, A: Anoia tributary; number: site number; A, B, C: sampling date, A: spring 2005, B: autumn 2005, C: spring 2006). Superimposed circles represent propranolol concentration [ng/ml]. Sites A2 and LL4 are the most polluted with a particular benthic community.*

## MODELKEY events and announcements

| MODELKEY Agenda | Topic | Contact |
|---|---|---|
| **General MODELKEY Events** | | |
| **15-17 April 2008, Toulouse, France** | **General MODELKEY Meeting 2008**<br>**Target group: MODELKEY partners** | **Michaela Hein (michaela.hein@ufz.de)** |

***For more events see next page please***

## MODELKEY events and announcements

| MODELKEY Agenda | Topic | Contact |
|---|---|---|
| **Training courses** | | |
| August 2008, UJOE, University of Joensuu, Finnland | Old sins and new threats in aquatic environments: Utilisation of advanced on-site methods in monitoring and risk assessment of organic contaminants<br>Target group: MODELKEY and KEYBIOEFFECTS partners and young researchers | Jussi Kukkonen<br>jussi.kukkonen@joensuu.fi |
| 29 September - 3 October 2008, CNRS, Toulouse, France | Patterning and clustering of the effects of toxic substances on biota in freshwater systems<br>Target group: MODELKEY and KEYBIOEFFECTS partners and young researchers | Sovan Lek<br>lek@cict.fr |
| **Workshops** | | |
| 22 - 24 September 2008, Federal Instiute of Hydrology (BfG), Koblenz, Germany | Water Framework Directive Implementation in relation to Priority and Emerging Pollutants<br>The workshop will focus on<br>• WFD implementation<br>• Case studies<br>• Application of innovative scientific tools for WFD implementation<br>• Current status of river basin management plans<br>Target group: all scientists and stakeholders interested in pollutants in relation to the WFD | Thomas Ternes<br>ternes@bafg.de<br><br>more information soon available at www.modelkey.org |

## Related events

| RISKBASE Agenda | Topic | More information |
|---|---|---|
| 15-17 May 2008, Budapest, Hungary | 2nd RISKBASE General Assembly and 2nd Thematic Workshop WP 1b | www.riskbase.info |