



How flood events affect rainbow trout: Evidence of a biomarker cascade in rainbow trout after exposure to PAH contaminated sediment suspensions

Markus Brinkmann^{a,*,1}, Sebastian Hudjetz^{a,1}, Ulrike Kammann^b, Michael Hennig^a, Jochen Kuckelkorn^a, Michelle Chinoraks^a, Catrina Cofalla^c, Steve Wiseman^d, John P. Giesy^{d,e}, Andreas Schäffer^f, Markus Hecker^{e,g}, Jan Wölz^a, Holger Schüttrumpf^c, Henner Hollert^a

^a Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

^b Thünen Institute of Fisheries Ecology, Palmaille 9, 22767 Hamburg, Germany

^c Institute of Hydraulic Engineering and Water Resources Management, RWTH Aachen University, Kreuzherrenstraße 7, 52056 Aachen, Germany

^d Dept. Veterinary Biomedical Sciences, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK, S7N 5B3, Canada

^e Toxicology Centre, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK, S7N 5B3, Canada

^f Department of Environmental Biology and Chemodynamics, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

^g School of the Environment & Sustainability, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK, S7N 5B3, Canada

ARTICLE INFO

Article history:

Received 31 August 2012

Received in revised form 6 November 2012

Accepted 11 November 2012

Keywords:

Sediment re-suspension

Temperature stress

Micronuclei

Lipid peroxidation

ABSTRACT

Increasing frequency and intensity of flood events are major concerns in the context of climate change. In addition to the direct hydrological implications of such events, potential ecotoxicological impacts are of increasing interest. It is vital to understand mechanisms of contaminant uptake from suspended particulate matter (SPM) and related effects in aquatic biota under realistic conditions. However, little is known about these processes. Due to recent changes in climate, during summer temperatures of German rivers frequently exceed 25 °C. Effects of re-suspension of sediments on biota under elevated temperature regimes are likely to differ from those under lower temperature regimes. To elucidate this differential response of aquatic vertebrates, rainbow trout were exposed to suspensions of sediment from the Rhine River that was spiked with a mixture of polycyclic aromatic hydrocarbons (PAH). The experiments were conducted under two different temperature regimes (24 °C or 12 °C). Physicochemical parameters, including concentration of PAHs in SPM, and biomarkers in fish (biliary PAH metabolites, 7-ethoxyresorufin O-deethylase activity, lipid peroxidation (LPO), mRNA expression of some genes and micronuclei) were measured over the course of a 12 d study. Concentrations of pyrene and phenanthrene decreased over time, while no decrease was observed for chrysene and benzo[a]pyrene. The biomarker cascades, more specifically the temporal dynamics of biomarker reactions, did not only show quantitative differences (i.e. different induction intensity or rate of biomarker responses) at the two temperatures but also qualitative differences, i.e. different biomarker responses were observed. A slight significant increase of biliary metabolites in fish was observed in un-spiked sediment at 24 °C. In bile of fish exposed to PAH spiked sediment concentrations of 1-hydroxypyrene and 1-hydroxyphenanthrene increased significantly during the first two days, and then decreased. At 12 °C uptake of PAHs was slower and maximum metabolite concentrations in bile were less than in fish exposed at 24 °C. Following a latency of two days, concentrations of PAH metabolites in bile of fish exposed at 24 °C were followed by a peak in LPO. PAHs spiked into sediments under laboratory conditions were significantly more bioavailable than the PAHs that were already present in un-spiked field-collected sediments.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

As a potential implication of climate change, the number of extreme weather events such as droughts, storms or heavy rainfalls

which have often lead to flood events has been increasing globally (Solomon et al., 2007). As a consequence, it has been predicted that both frequency and intensity of floods will increase in the coming decades (Ikeda et al., 2005; Kay et al., 2006). Not only are there direct hydrological implications of floods that pose a risk to humans and ecosystems, but there will also be the risk of re-suspension of historically polluted sediments in their river basins. Under non-flood conditions sediments are usually a sink for both inorganic and organic contaminants. However, upon re-suspension, these

* Corresponding author. Tel.: +49 0 241 80 26686; fax: +49 0 241 80 22182.

E-mail address: markus.brinkmann@bio5.rwth-aachen.de (M. Brinkmann).

¹ Both authors contributed equally to the paper and share first authorship.

sediments can constitute a secondary source of pollutants, which consequently become available for uptake into aquatic organisms (Ahlf et al., 2002; Hollert et al., 2003; Wölz et al., 2010) and – among other factors – cause that quality goals of the European Water Framework Directive (WFD) might not be met in many river basins (Wilby et al., 2006). To date, the complexity of the various interactions between sediments, pollutants and biota has been little investigated (Ciarelli et al., 1999; Ciutat et al., 2006; Gerbersdorf et al., 2011; Hyötyläinen et al., 2002; Reible et al., 1996; Roberts, 2012).

Besides the occurrence of extreme floods, temperatures in rivers have been constantly increasing during the past few decades. In the case of the Rhine River, Germany, the annual average temperature has risen by 3 °C, of which 2 °C is likely caused by waste heat of power plants and 1 °C to climate change (IKSR, 2004). The number of days per year with temperatures exceeding 23 °C has increased from less than 20 in the 1960s to 40–50 in the 1990s (IKSR, 2004). Days with temperatures exceeding 25 °C are also more frequent.

Metabolic rates of poikilothermic organisms as well as desorption of pollutants from sediments are temperature-dependent (Noyes et al., 2009). It has been shown that the toxicity of sediments and bioaccumulation of pollutants can be temperature-dependent (e.g. Airas et al., 2008; Heinonen et al., 2002; Honkanen and Kukkonen, 2006; Ng and Gray, 2011). Additionally, besides the influence of temperature itself (Hari et al., 2006) effects of environmental stressors, such as ammonia or acidification, were more severe under temperature stress (Morgan et al., 2001). Thus, it is plausible to expect that toxicity and resulting effects of sediment-bound contaminants re-mobilized during flood events could also be affected as a consequence of climate change.

It has been proposed to include hydrodynamics, i.e. transport processes of water in fluvial systems, and sediment mobility as a more realistic approach to assess potential effects of contaminated sediments (Brinkmann et al., 2010b; Chapman and Hollert, 2006; Hollert et al., 2008; Wölz et al., 2009). Until recently, however, toxicologists lacked appropriate laboratory methods to systematically investigate fates and effects of sediment-bound pollutants under flood-like conditions. A first attempt to provide such methodologies was made by the interdisciplinary project “Floodsearch”, which was funded by the German Excellence Initiative at RWTH Aachen University, Germany (Wölz et al., 2009). In this study, rainbow trout (*Oncorhynchus mykiss*) were exposed to artificial sediments that had been spiked with polycyclic aromatic hydrocarbons (PAH). These exposures were made during simulated 5 d flood events in an annular flume, i.e. a circular channel that is typically used for erosion and sedimentation studies. A set of different biomarkers was investigated after exposure and the hypothesis that re-suspension of sediments can result in uptake of particle-bound contaminants and effects in aquatic biota was verified (Brinkmann et al., 2010a; Cofalla et al., 2012; Schuttrumpf et al., 2011). Apart from transient changes in several biomarkers, the frequency of micronuclei (MN) in peripheral erythrocytes – a definitive marker for genetic damage with potential to cause population-level adverse effects (Diekmann et al., 2004a; Diekmann et al., 2004b) – was significantly elevated after exposure to the contaminated sediment. The annular flume provides opportunities for testing of dynamics of contaminants in sediments and their accumulation into and effects on aquatic organisms (Cofalla et al., 2011). However, there is a major drawback of this method, which is that since contact of the instrument lid to the surface of the water is required for generation of the flow in the simulated flood event, animals, and samples of water and sediments cannot be taken during the simulation. Thus, additional experiments had to be conducted to be able to characterize the temporal trends of biomarker induction in exposed animals. It is of vital importance to biomarker experiments to fully understand

the underlying physiological mechanisms and influencing factors (Forbes et al., 2006; Kammann et al., 2012).

The aim of the present study was to investigate the influence changes in temperature regime have on biomarker cascades during exposure to particle-bound PAHs. Here we report the results of experiments conducted to elucidate the time- and temperature-dependency of the toxicological properties of re-suspended sediments on rainbow trout which were exposed to suspensions of sediments. Sediment was used as collected from the environment or spiked with a mixture of PAHs representative of those occurring in sediments of rivers in Germany. These included the following: pyrene (PYR), phenanthrene (PHE), chrysene (CHR) and benzo[*a*]pyrene (BAP). To account for the influence of temperature on uptake and effects of pollutants bound to particles, the experiment was conducted at two average temperatures, 12 °C or 24 °C. The temperature of 12 °C was chosen since optimal growth of rainbow trout was observed at 12–14 °C according to Johnson et al. (1987). The temperature of 24 °C was chosen to represent temperature stress. It is just below the incipient upper lethal temperature (IULT), i.e. the temperature at which animals do not tolerate further temperature increase, according to Bjornn and Reiser (1991). Concentrations of PAHs in suspended particulate matter (SPM), as well as uptake and biotransformation, as determined by concentrations of PAH metabolites in bile, were quantified. Several functional responses of rainbow trout that occurred during re-suspension of pollutants bound to particulates were also measured. Expression of CYP1A (Cytochrome P450 1A) mRNA in liver was used as a potential measure of stimulation of phase I biotransformation of xenobiotics mediated by the aryl hydrocarbon receptor (AhR) at the transcript level. The mixed function mono-oxygenase (MFO) enzyme activity 7-ethoxyresorufin *O*-deethylase (EROD) was used as a measure of CYP1A activity. Glutathione-*S*-transferase (GST) and UDP glucuronyltransferase (UDPGT) mRNA expression were assessed as indicators of potential induction of phase II conjugation of xenobiotics. Expression of Caspase 3 mRNA was used as a measure of potential apoptosis (programmed cell death). Lipid peroxidation (LPO) was used as a measure of oxidative stress. Formation of MN in peripheral erythrocytes was used as a measure of genotoxicity. Biliverdin concentration in bile was measured to estimate gross energy metabolism.

2. Materials and methods

2.1. Experimental design

Juvenile rainbow trout were exposed to suspensions of a sediment from the Rhine River, either un-spiked or spiked with a mixture of the following PAHs (nominal concentrations in mg kg⁻¹ dw are given in brackets), purchased from Sigma–Aldrich (Deisenhofen, Germany): PYR (purity ≥99%, 4.1 mg kg⁻¹), PHE (purity 98%, 5.0 mg kg⁻¹), CHR (analytical standard, 3.3 mg kg⁻¹), and BAP (purity ≥96%, 8.3 mg kg⁻¹). Experiments were conducted in 750L glass fiber-reinforced plastic containers (Fig. 1) purchased from AGK Kronawitter (Wallerdsdorf, Germany). Submersible pumps (maximum flow-through 6000 Lh⁻¹) were used to constantly suspend the sediments at a nominal concentration of 10 gL⁻¹. Tanks were aerated at a rate of 25 Lmin⁻¹. In the first experiment the mean temperature was 23.8 ± 0.5 °C, while in the second experiment the mean temperature was 11.9 ± 0.3 °C. Tanks were cooled using submersible coolers (Colora Tauchkühler, Lorch, Germany), which were controlled by analog plug-in thermostats (UT100, Fuva, Erlangen, Germany). In each of the two experiments physicochemical water parameters and concentrations of PAHs (and metabolites, respectively) were measured, after 0 (i.e. untreated control animals), 1, 2, 4, 6, 8, or 12 d, in suspended

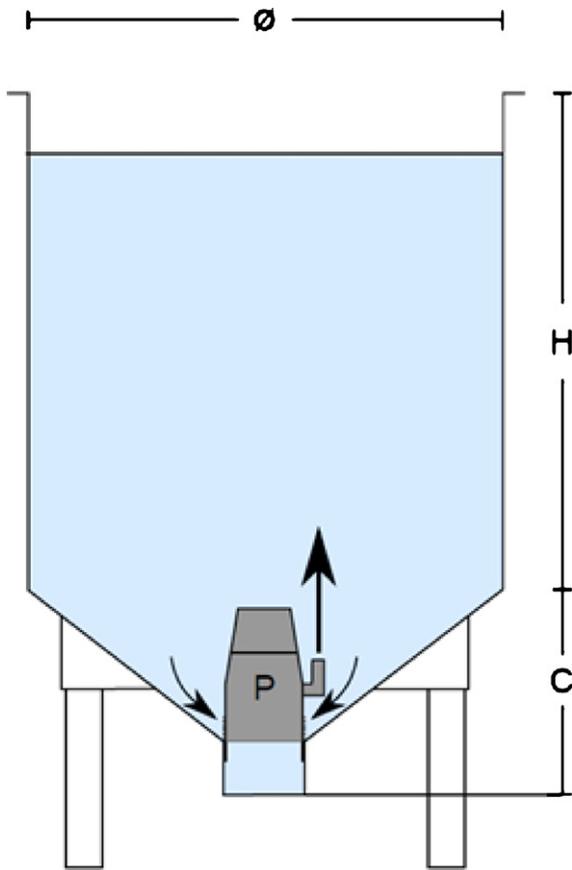


Fig. 1. Re-suspension tanks used for exposure of fish to sediment suspension (P: Pump, Height H: 145 cm, Cone C: 40 cm, Diameter Ø: 80 cm).

sediments and fish ($n = 10$ per sampling point, i.e. 60 individuals per treatment, 10 for each of the two untreated controls, 260 individuals in total). Due to mortality, no animals for biomarker analysis were available for day 12 in the 24 °C spiked sediment treatment and only $n = 4$ animals were assessed in the 24 °C un-spiked treatment. In the same animals, biomarkers of exposure or effect were determined.

The temperature was held constant at 11.9 ± 0.3 °C in the experiment with active cooling, temperature increased during four days from 20 °C to 23.8 ± 0.5 °C in the uncooled experiment. Dissolved oxygen concentrations were 10.5 ± 0.2 mg L⁻¹ in the 12 °C experiments. In contrast, concentrations of dissolved oxygen decreased to concentrations as low as 6.5 mg L⁻¹ in the uncooled experiment during the first four days of the experiment and then averaged at 7.6 ± 0.4 mg L⁻¹. In the 12 °C experiments, total hardness increased from 63 mg L⁻¹ to 84 mg L⁻¹ over the exposure period. In the uncooled experiments hardness almost doubled from 74 to 142 mg L⁻¹. SPM concentrations deviated from the nominal concentration of 10 g L⁻¹ and ranged between 6.0 and 13.6 g L⁻¹.

2.2. Experimental fish

Immature rainbow trout (15–20 g) were purchased from a commercial hatchery (Mohnen Aquaculture, Stolberg, Germany) and

allowed to acclimatize to laboratory conditions for at least 2 months prior to use in experiments. Fish were reared in groups of 100–150 individuals in 1500 L glass fiber-reinforced plastic tanks at RWTH Aachen University, Institute for Environmental Research, Aachen, Germany. In a recirculating system with a 400 L biofilter and UVC-sterilizer, water was continuously exchanged at a rate of 0.1–0.2 full replacements per day with municipal tap water. Light and dark phases were 12 h each. Fish were fed commercial trout pellets (Ecolife 20, 3 mm, Biomar, Brande, Denmark) at a rate of 1–2% bodyweight per day until experimentation. The final weight and length of used fishes was 58 ± 23 g and 161 ± 19 mm, respectively.

All experiments were conducted in accordance with the Animal Welfare Act and with permission of the federal authorities (Landesamt für Natur, Umwelt und Verbraucherschutz NRW, Germany), registration number 8.87-50.10.35.08.225.

2.3. Sediment sampling and spiking

The sediment was collected in April 2010 from the Rhine River (river kilometre 591) close to the fortress Ehrenbreitstein in the vicinity of Koblenz, Germany ($+50^{\circ} 21' 12''$ N, $+7^{\circ} 36' 27''$ E). Samples were collected in cooperation with the German Federal Institute of Hydrology (BFG, Koblenz, Germany). This location was chosen as it is known to be moderately contaminated and to be representative based on particle size and organic carbon content (Heininger et al., 2007). A surface sample (approximately 100 kg ww) was taken by use of a Van Veen grab (Hydrobios, Kiel, Germany) and subsequently stored at 4 °C in darkness prior to experiments. Physicochemical parameters of sediment and water were directly recorded (Table 1).

Aliquots of 7.5 kg dw of the sediment were spiked with PAHs according to OECD guideline 218 (OECD 218, 2004). Briefly, 10% of the sediment (i.e. 750 g dw) that was used in each experiment were dried overnight at 105 °C and thoroughly homogenized. PAHs were dissolved in a mixture of 350 mL *n*-hexane and 150 mL acetone, and added to the dried sediment. After evaporation of the solvent, distilled water was added to reconstitute the original water content of the sediment. The spiked portion was added to the bulk of sediment (90%), thoroughly homogenized using an electric mixer, incubated in darkness at 4 °C for 7 d, and mixed again prior to the experiments. Un-spiked sediments served were investigated for reference and treated identically to the spiked sediments except for the addition of PAHs. After incubation, the sediments were transferred to the exposure tanks to obtain a nominal suspended matter concentration of 10 g L⁻¹.

2.4. Sampling of sediment suspensions and quantification of PAHs

Sediment suspension samples were taken as 1 L duplicates per sampling event and centrifuged for 30 min ($4500 \times g$, 4 °C) in a cooling centrifuge (Rotina 420R, Hettich, Tuttlingen, Germany). The supernatant was filtered through 0.7 µm glass fiber filters (MN-GF 1, Macherey & Nagel, Düren, Germany) under vacuum. Retained SPM and precipitates were pooled and lyophilized (Christ Alpha 1-2, Martin Christ GmbH, Osterode am Harz, Germany). Dried SPM samples were extracted with *n*-hexane (Chromasolv, Sigma-Aldrich) by means of pressurized liquid extraction (PLE) by use of a SpeedExtractor® (SpeedExtractor E-916, BÜCHI Labortechnik GmbH, Essen, Germany). The device was operated under the

Table 1
Basic physicochemical parameters of sediment and water sampled at the harbor of Ehrenbreitstein, Germany.

	Temperature (°C)	pH	Conductivity (µS cm ⁻¹)	Dissolved (O ₂ /mg L ⁻¹)	Redox potential (mV)
Sediment	11.7	7.43	–	–	–275
Water	12.0	7.50	459	11.0	–

Table 2

Limit of detection (LOD), limit of quantification (LOQ) and recoveries for PYR, PHE, CHR and BAP in sediment and suspended matter.

	Pyrene	Phenanthrene	Chrysene	Benzo[a]pyrene
LOD (mg kg ⁻¹)	0.11	0.10	0.17	0.30
LOQ (mg kg ⁻¹)	0.26	0.23	0.39	0.69
Recovery (%)	95	101	103	109

following conditions: two extraction cycles, extraction temperature 100 °C, extraction pressure 120 bar. Extracts were reduced close to dryness using a rotary evaporator between 300 and 500 mbar and 40 °C (WB 2001; Heidolph, Kehlheim, Germany) and a gentle stream of nitrogen. Each extract was re-dissolved in 1 mL of *n*-hexane p.a. (Sigma–Aldrich) which resulted in a concentration equivalent to 5 g dry sediment (SEQ) per 1 mL solvent each. Extracts were stored in the dark at –20 °C until further analysis.

A 6890 N gas chromatograph system coupled with a G2589A-5973 N mass selective detector (MSD) and a 7683 automatic sample injector (all instruments from Agilent technologies, Waldbronn, Germany), which was equipped with an Optima 35 MS capillary column (30.0 m × 0.25 mm i.d. and 0.25 μm film thickness, Macherey and Nagel, Düren, Germany) was used for chromatographic separation of PAHs. Helium was used as carrier gas at a constant flow rate of 1.1 mL min⁻¹. The system operated at the following conditions: injector temperature 250 °C, injection volume 1 μL in splitless mode; GC–MS transfer line temperature 280 °C; ionization by electron impact at 70 eV; oven temperature program: 50 °C for 5 min then ramped up at 10 °C min⁻¹ to 280 °C and held for 15 min. The MSD was operated in SIM mode. Data acquisition and processing was performed with the Agilent Technologies MS ChemStation data analysis software and the NIST MS Search Program. Concentrations of PAHs were calculated after PAHs were quantified in duplicate by means of an external five point calibration curve derived from a standard stock solution (DE-PROM 16, LGC Standards, Wesel, Germany) that contained unlabeled EPA-PAHs, and expressed as mg kg⁻¹ dw. Limits of detection (LOD) and quantification (LOQ) were calculated from chromatograms (ACS Committee on Environmental improvement, 1980) and recovery rates were estimated for unlabeled external standards using pre-cleaned quartz sand (BÜCHI Labortechnik GmbH, Table 2). A one-phase exponential decay model [equation (1.1)] was used for calculation of the half-lives of PYR and PHE in the spiked sediment treatments [equation (1.2)] with GraphPad Prism 5 (GraphPad, San Diego, USA).

$$y = (y_0 - \text{Plateau})e^{-kx} + \text{Plateau} \quad (1.1)$$

$$\text{Half-life} = \frac{\ln(2)}{k} \quad (1.2)$$

Plateau is the PAH concentration at infinite time, y_0 is the PAH concentration at time zero and k the rate constant.

2.5. Determination of physicochemical parameters

Temperature, pH, conductivity and dissolved oxygen concentration were determined by use of calibrated handheld instruments (Hanna, Ann Arbor, USA or WTW, Weilheim, Germany). Total hardness was measured after filtration using the titrimetric Titriplex B method (Merck, Darmstadt, Germany).

2.6. Animal dissection and tissue preparation

After exposure, fish were individually anesthetized in a 10 L container by adding a saturated solution of ethyl4-aminobenzoate (benzocaine, Sigma–Aldrich) and then exsanguinated. Subsequently, length and mass were determined for calculation of

condition index [K, equation (2)], liver somatic index [LSI, equation (3)], and visceral index [VI, equation (4)].

$$K = \frac{W}{L^3} \times 100 \quad (2)$$

$$LSI = \frac{LW}{W} \times 100 \quad (3)$$

$$VI = \frac{(W - CW)}{W} \quad (4)$$

W is the weight of the fish (mg), LW the liver weight (mg), L the standard length (mm) and CW the carcass weight (mg), i.e. the weight of the eviscerated animal.

The gall bladder was evacuated by use of a syringe, and bile transferred to 1.5 mL polypropylene vials (Carl Roth, Karlsruhe, Germany), and stored at –20 °C until determination of PAH metabolite concentrations. The liver was rapidly isolated and mass determined. Explants of liver were cut into four equally sized pieces, transferred into sterile 2 mL cryogenic vials (Greiner Bio-One, Frickenhausen, Germany) and snap-frozen in liquid nitrogen. Samples of liver were stored at –85 °C until preparation.

2.7. Analysis of biomarkers

LPO, MN in peripheral erythrocytes, as well as concentrations of biliary PAH metabolites (selected major phase I metabolites and their phase II conjugates) and biliverdin in bile were determined following the methods of Ohkawa et al. (1979), Rocha et al. (2009), and Kammann (2007), respectively, according to previously published protocols (Brinkmann et al., 2010a). Prior to measurement of EROD activity, pieces of liver explants were thawed carefully and homogenized in 0.1 M phosphate buffer (pH 7.4) at a ratio of 1:10 (w/v) for 20 s using an electric disperser (VWR, Darmstadt, Germany). Subsequently, homogenates were transferred to 1.5 mL micro test tubes (Greiner Bio-One) and centrifuged for 15 min (10,000 × g, 4 °C) in a cooling centrifuge (Rotina 420R, Hettich, Tuttlingen, Germany). Supernatant was carefully transferred into fresh 1.5 mL micro test tubes and stored at –80 °C until measurement of enzymatic activity. EROD activity in liver was measured following the protocol published in Brinkmann et al. (2010a) according to a combination of the methods described by Kennedy and Jones (1994) and Pohl and Fouts (1980). Slight modifications were made to correct for fluorescence quenching of the sample: In addition to the buffer blank, a sample reference was included in which the whole reaction mixture including S9 was mixed. Other than in the reaction wells, acetonitrile was added first to precipitate proteins. Concentrations of protein for the calculation of specific enzyme activities were determined in triplicates by the bicinchoninic acid (BCA) method provided as a kit (Sigma–Aldrich). A minimum coefficient of determination (r^2) of 0.95 was accepted for standard curves in all used assays for protein quantification. Hepatic mRNA expression of some genes was determined via reverse-transcription quantitative real-time PCR according to Zhang et al. (2008) following the protocol published by Brinkmann et al. (2010a). Briefly, total RNA was extracted from preserved liver tissue of individuals according to manufacturer's protocol with a QIAGEN RNeasy Plus Mini Kit (QIAGEN, Mississauga, Ontario, Canada). RNA concentrations were determined by measuring the absorption at 260 nm using a ND-1000 spectrophotometer (Thermo Scientific, Wilmington, USA) and samples were stored at –80 °C until processing. First-strand cDNA was synthesized from 1 μg of total RNA using the Superscript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. mRNA expression was quantified by means of real-time Q-RT-PCR using a 96-well Applied Biosystems 7300 real-time PCR System (Applied Biosystems, Foster City, CA, USA). The

Table 3
Primer pair sequences, amplicon sizes, and accession numbers of the investigated genes used in real-time PCR reactions.

Gene	Primer sequence (5'–3')	Amplicon size (bp)	GeneBank accession no.
AhR β	F: TGGCAAATGGACACACATTC R: AGTCTGTTGGGTTCTGTGG	100	NM.001124252
CYP1A ^a	F: GATGTCAGTGGCAGCTTTGA R: TCCTGGTCATCATGGCTGTA	104	U62796
EL-1 α	F: GAGAACCATTGAAAAGTTGAGAAG R: GCACCCAGGCATACTTGAAG	71	NM.001124339
GST-P	F: TTCAGGGAGGGGAAGGTATC R: GTTGGTGACAAGCCTTCGTT	101	BQ036247
SOD1 ^b	F: TGGTCTGTGAAGCTGATTG R: TTGTCAGCTCTGCAGTCAC	201	NM.001124329
UGT ^c	F: ATAAGGACCGTCCCATCGAG R: ATCCAGTTGAGGTCGTGAGC	112	DY802180
Caspase 3 β ^d	F: GGAGAACAGGAATGAGTCTT R: TCACGTTGTACCCAAACGT	87	FR751081

Source of data: Modified from Brinkmann et al. (2010a).

^a This primer pair was previously published by Wiseman and Vijayan (2007).

^b This primer pair was previously published by Fontagne et al. (2008).

^c This primer pair was previously published by Mortensen and Arukwe (2007).

^d This primer pair was previously published by Bobe et al. (2004).

PCR program included an enzyme activation step at 95 °C (10 min), and 40 cycles of 95 °C (15 s) and 60 °C (60 s). PCR mixtures sufficient for 200 reactions contained 2 mL of SYBR Green master mix (Applied Biosystems), 200 μ L of 10 μ M sense/anti-sense gene-specific primers (Table 3), and 1.6 mL of nuclease-free distilled water (QIAGEN). PCR products of mixed cDNA samples using previously published gene-specific primers (Table 3) were separated on 1% agarose gels with a Gene Ruler DNA size standard (Thermo Scientific) to confirm single PCR amplicons with the desired length. Melting curve analyses were performed during real-time PCR to ensure target specificity and single peak amplification, respectively. For complete method descriptions, please refer to the supplemental material.

2.8. Data analysis

Calculations were performed in spreadsheets by use of Microsoft ExcelTM 2007. Graphs were plotted by use of the software GraphPad Prism 5. Statistical analyses and correlations were conducted by use of the software Sigma Stat 3.11 (Systat Software, Erkrath, Germany). All datasets that did not pass the Kolmogorov–Smirnov test on Gaussian distribution ($p < 0.05$) or Bartlett's test for equal variances ($p < 0.05$) were analyzed by use of nonparametric Kruskal–Wallis ANOVA on ranks ($p \leq 0.001$). The datasets passing both tests were analyzed by use of parametric one-way ANOVA ($p \leq 0.001$). Dunn's method was used as the multiple range test to identify significant differences among treatments. Since most of the data did not fulfill the criteria for two-way or multiple-way ANOVA, this method was not applied in the present study for comparability purposes. For selected comparisons between un-spiked and spiked sediment treatment groups, one-tailed parametric Student's t -tests were used if data were normally distributed and of equal variance. If one of the criteria for the parametric test was not met, non-parametric Mann–Whitney Rank Sum tests were performed. The probability of Type I error (α) was set to $p \leq 0.05$. In the text, values are expressed as mean value \pm standard deviation, unless indicated.

3. Results

3.1. Fish mortality

When exposure was conducted at 12 °C, mortality of rainbow trout was small in both the un-spiked and spiked sediment treatments with 0 and 2%, respectively. However, when conducted at 24 °C, mortalities of 25% and 31% were observed when

fish were exposed to un-spiked and spiked suspended sediment, respectively. Due to the high mortality, no animals for biomarker analysis were available for day 12 in the 24 °C spiked sediment treatment and only $n = 4$ animals were assessed in the 24 °C un-spiked treatment.

3.2. Chemical analyses of PAH concentrations

After the sediment-conditioning period of 7 d, directly prior to addition of sediments to exposure tanks, concentrations of PAHs in un-spiked sediments from the harbor Ehrenbreitstein (EBR) were significantly lower than nominal concentrations (C_0 , Table 4). PAH concentrations in SPM during the experiments were expressed relative to C_0 as the C/C_0 ratio (Fig. 2). In contrast to CHR and BAP, where measured concentrations did not show clear trends as a function of exposure time, a constantly decreasing C/C_0 ratio was observed in the spiked sediments for PHE and PYR. In the un-spiked treatments, concentrations of all PAHs remained constant (Fig. 2). The data were fitted using a one-phase exponential decay model. The calculated half-lives of PYR and PHE in the SPM resulting from spiked sediment were significantly shorter in exposure chambers where the temperature was at 24 °C compared to those where the temperature was 12 °C (Table 5). The three-ring substance PHE generally had shorter half-lives than the four-ring substance PYR. Half-lives of PHE and PYR were 1.8- and 5.2-fold greater, respectively, in chambers held at the greater temperature.

3.3. Morphometric indices

Morphometric indices of exposed fish determined to assess changes in health and condition during the experiments, such as condition index (K) were negatively proportional to exposure time. However, the relationship for K was statistically significant in only the 12 °C un-spiked treatment on day 12 compared to un-exposed animals (One-way ANOVA with Dunnett's post hoc test, $p \leq 0.05$). The VI was significantly lower than the initial values after 4 and 6 days in the un-spiked and spiked experiments conducted at 12 °C, respectively, and on the days 2 and 4 in the spiked experiment conducted at 24 °C (One-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$). The LSI was not significantly different from the initial control values in fish exposed to either concentration of PAHs at either temperature during the course of the exposure. There were no significant differences in K , VI or LSI between spiked and un-spiked sediments.

Table 4
Nominal and measured concentrations (C_0) of the PAHs used for spiking prior to the addition of sediments to exposure tanks.

Experiment		Phenanthrene (mg kg ⁻¹ dw)	Pyrene (mg kg ⁻¹ dw)	Chrysene (mg kg ⁻¹ dw)	Benzo[a]pyrene (mg kg ⁻¹ dw)
Un-spiked sediment	Measured	0.27	0.51	0.29	0.16
Spiked sediment	Nominal	5.00	4.10	3.30	8.30
	Measured	1.88	2.29	1.63	2.32

Values in italic typesetting are below the LOQ.

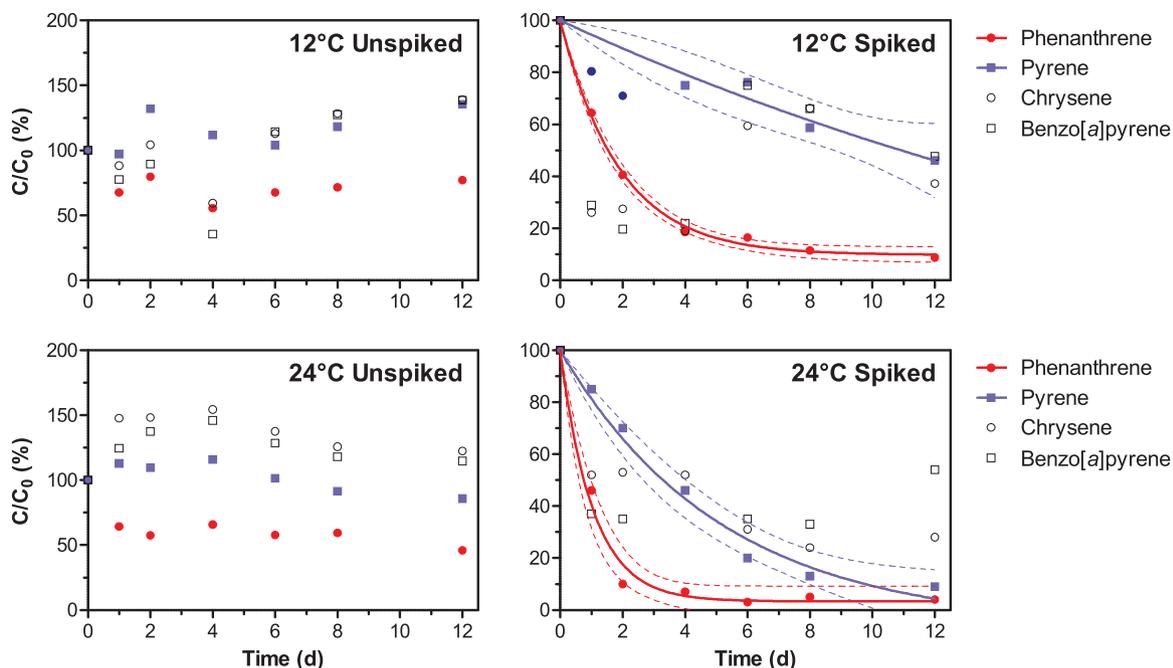


Fig. 2. PAH concentration changes in SPM relative to the initial concentration in the sediment (as given in Table 3) during the experiments at 12 and 24 °C in spiked and un-spiked treatments were measured by means of GC–MS. Solid lines represent regression with a one-phase exponential decay model, dashed lines the 95% confidence intervals.

3.4. Biological analysis

Significant concentrations of PAH metabolites, i.e. the sum of original hydroxylated PAHs and those after deconjugation of phase II products with β -glucuronidase/arylsulfatase, were observed in bile of fish from all treatments, including the un-spiked sediment from the harbor Ehrenbreitstein (Fig. 3). Concentrations of PAH metabolites in bile of fishes in the spiked treatments described a hyperbolic uptake phase followed by a depuration phase that was more pronounced at 24 °C. No such depuration phase was observed in the un-spiked treatments. In the PAH spiked treatment group concentrations of metabolites were significantly greater than the control (confidence interval method, Newman, 2008) at 12 °C and 24 °C. When fish were exposed to spiked sediment, concentrations of PAH metabolites increased over the first days, and then decreased later in the experiment. Dynamics of concentrations of PAH metabolites in bile were markedly different between the two temperatures. The maximum concentration of 1-hydroxy-PYR was detected on day 8 at 12 °C and on day 2 at 24 °C. A similar pattern of accumulation was observed for 1-hydroxy-PHE, where the

maximum concentration was detected on day 4 at 12 °C and on day 2 at 24 °C. Concentration of 3-hydroxy-BAP constantly increased in bile until day 8 at 12 °C. At 24 °C, the concentration of this metabolite was significantly elevated already at day 2, with no further increase.

In the un-spiked sediment experiments, uptake and biotransformation at 12 and 24 °C resulted in significantly increased 1-hydroxy-PHE concentrations compared to initial values after 2 and 1 d (2.5 and 3.5-fold), respectively, and significantly greater 1-hydroxy-PYR concentrations (18.7 and 15.3-fold) compared to initial values were measured after 4 and 2 d, respectively (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$). Concentrations of 3-hydroxy-BAP were lower than the LOQ in bile of fish exposed to un-spiked experiment. To estimate effects of metabolic rates of fish, concentrations of biliverdin were measured. When fish were exposed at 12 °C there was no statistically significant difference in concentrations of biliverdin between those exposed to spiked or un-spiked sediments (Fig. 4). In fish exposed at 24 °C, concentrations of biliverdin increased from 160 ng mL⁻¹ in the untreated fish to 1209 ng mL⁻¹ in the un-spiked

Table 5
Half-lives of PYR and PHE during the experiments with spiked sediments at 12 and 24 °C, respectively.

Experimental temperature (°C)	Pyrene		Phenanthrene	
	Mean $t_{1/2}$ (d)	Coefficient of determination, r^2	Mean $t_{1/2}$ (d)	Coefficient of determination, r^2
12	18.58	0.96	1.32	0.99
24	3.58	0.99	0.73	0.99

Data were computed with a one-phase exponential decay model.

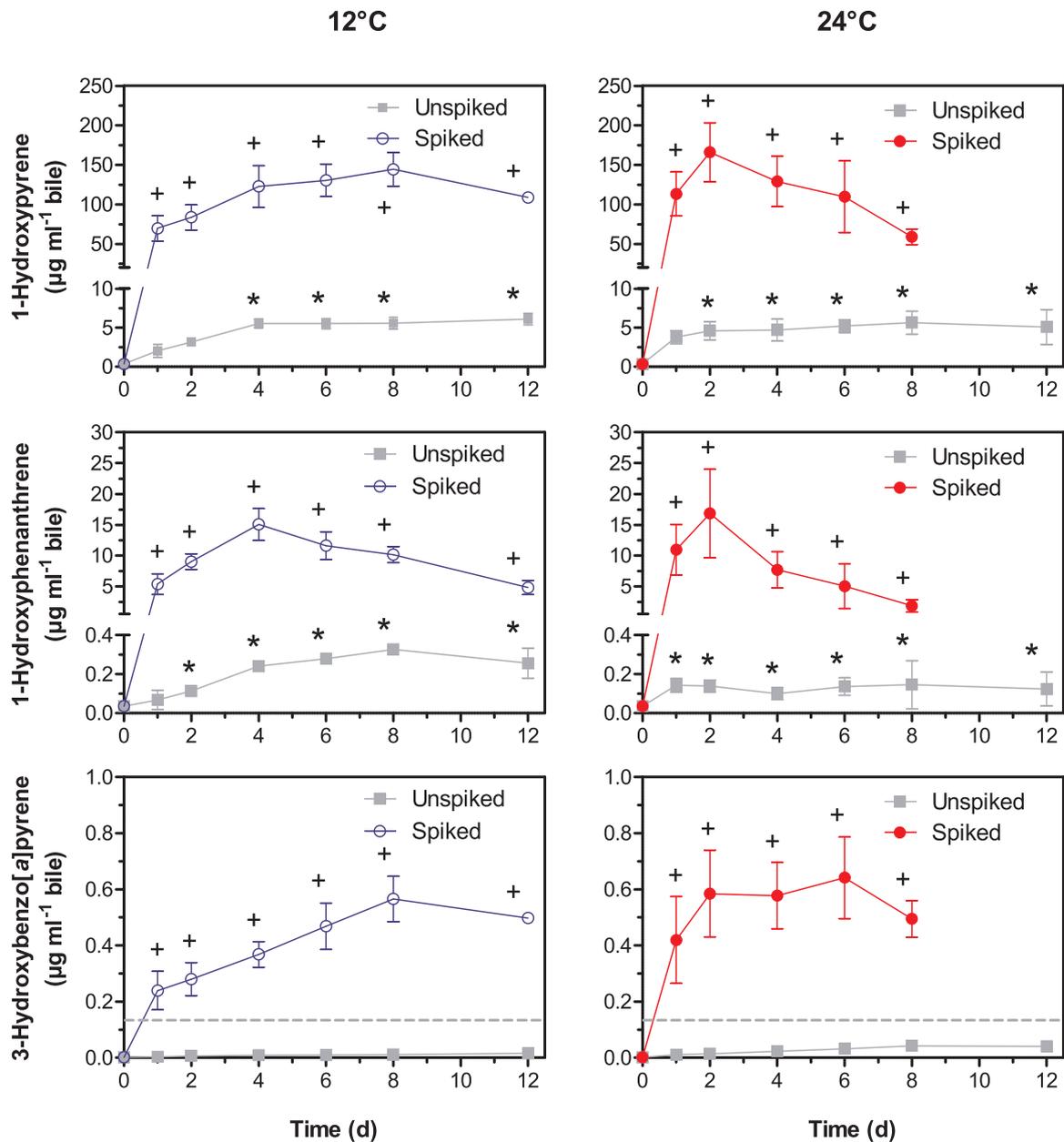


Fig. 3. Absolute biliary metabolite concentrations (following treatment with β -glucuronidase/arylsulfatase) during the experiments (left: 12 °C, right: 24 °C) in the un-spiked (■) and the spiked treatments (○/●). Symbols give the mean of $n=10$ animals, error bars the 95% confidence intervals. The dashed line marks the LOQ for 3-hydroxybenzo[a]pyrene. Asterisks denote significant differences between control and un-spiked treatments (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$), plus symbols between control and spiked treatments.

treatment on day 12. During the first 4 d of exposure, concentrations of biliverdin in bile of fish exposed to spiked sediment at 24 °C were significantly greater than those in bile of fish exposed to un-spiked sediment (significant on day 4, Mann–Whitney Rank Sum test, $p \leq 0.05$).

With the exception of the experiment conducted at 12 °C with spiked sediment, significant differences in EROD activities in trout livers were observed as a function of exposure time (Fig. 5). In the 12 °C experiment with un-spiked sediment, EROD activity was significantly higher than that of untreated animals after 8 and 12 days exposure (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$). When exposed at 24 °C, EROD activity was significantly lower after 4 days exposure to both the spiked and un-spiked sediment compared to untreated animals (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$). When exposed to the suspension of spiked sediment at 24 °C, EROD

activity in livers of fish was significantly 1.9-fold greater than that in livers of fish exposed to un-spiked sediment on day one (t -test, $p \leq 0.05$). Expression of mRNA of *CYP1A*, *GST*, *UDPGT*, and *Caspase 3* in liver was not significantly up-regulated relative to that of untreated controls after 1, 4 or 8 days, respectively, in any treatment (data not shown).

There were no statistically significant differences in LPO measured as equivalent concentrations of malondialdehyde (MDA) as a function of exposure time with the exception of the 24 °C PAH-spiked experiment (Fig. 6). In fish from this treatment group a constant increase of LPO was observed during the first 4 d of exposure (significant, Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$), and which then subsequently decreased to concentrations measured in fish from the other treatment groups. The average LPO concentration in this treatment was $414 \pm 271 \text{ nmol g}^{-1}$ liver on day four, which was

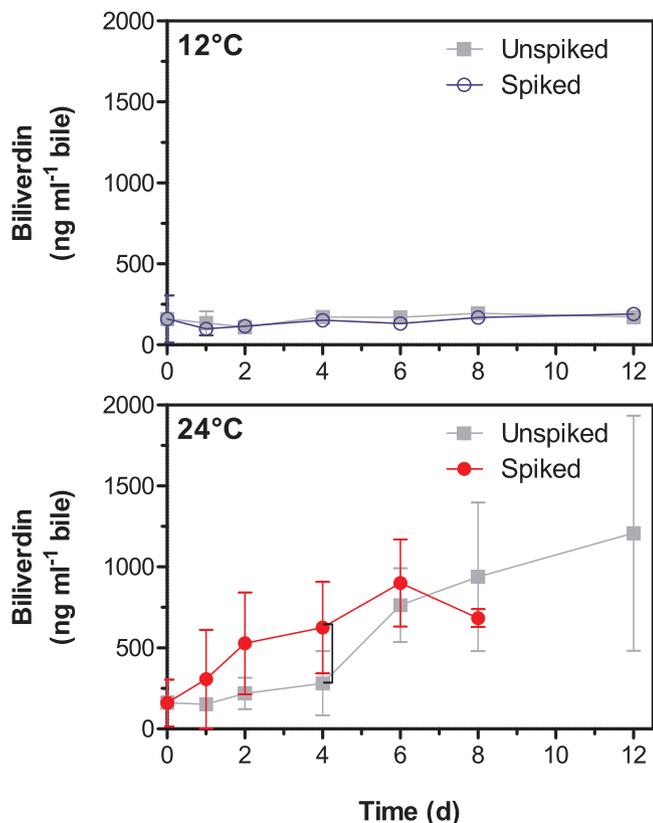


Fig. 4. Dynamics of biliary biliverdin concentrations during the experiments (top: average temperature 12 °C, bottom: average temperature 24 °C) in the un-spiked (■) and the spiked treatments (○/●). Symbols represent mean values of $n=10$ animals, error bars the 95% confidence intervals. Brackets denote significant differences between the experiments (Mann–Whitney Rank Sum test, $p \leq 0.05$).

approximately 2.7-fold greater than that of unexposed control fish at this temperature.

MN in peripheral erythrocytes showed a generally increasing trend during exposure of fish in all treatments and at both temperatures. However, significant differences between the spiked and un-spiked treatments were only observed in the 12 °C group on days 6 and 12 (2.2 and 2.1-fold, respectively; Fig. 7).

4. Discussion

4.1. Dissipation of the PAHs from sediment–water systems

Since rainbow trout were exposed to suspensions of sediments at constant SPM concentrations, changes in PAH levels during the experiments could have affected the results. The rate of biodegradation of PAHs in fluvial systems containing greater concentrations of SPM (i.e., 4–10 g L⁻¹) was found to be greater than that in systems with lower concentrations of SPM by Xia et al. (2006). Most likely, this effect is driven by the greater surface area at the water–sediment interface at which microbial degradation takes place. Microbial mineralization of phenanthrene was 5–10-fold greater if the sediment was frequently re-suspended compared to a un-disturbed sediment bed (LeBlanc et al., 2006). The shortest half-life for microbial degradation in this particular study was 100 d, while other researchers found half-lives in the range of 40 d to several months for phenanthrene (Apitz et al., 1999; Heitkamp and Cerniglia, 1987). In the present study, significantly shorter half-lives were observed (Table 5) that were comparable to those determined in sediment slurries (e.g. Shiaris, 1989). Volatilization of sediment-bound PAHs due to the intense aeration of the

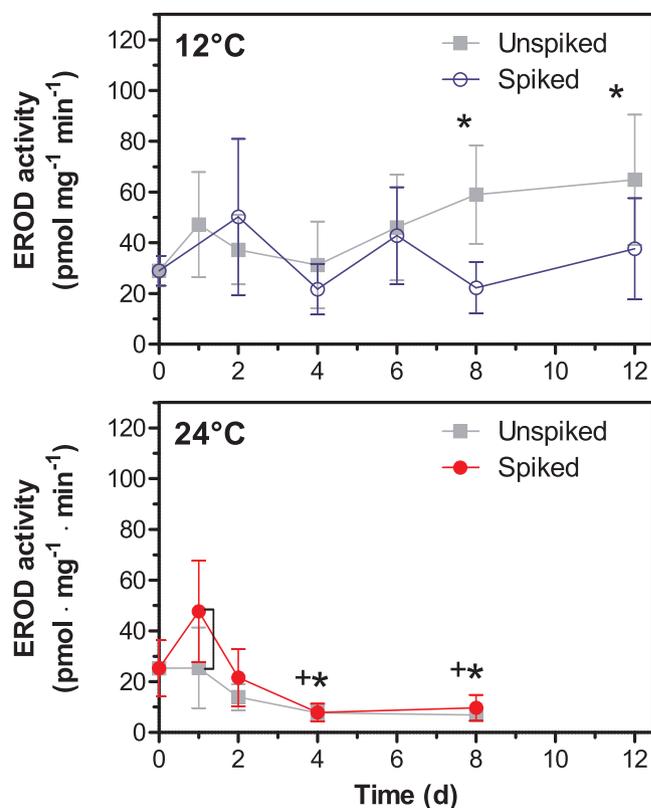


Fig. 5. Dynamics of hepatic EROD activity during the experiments (top: average temperature 12 °C, bottom: average temperature 24 °C) in the un-spiked (■) and the spiked treatments (○/●). Symbols represent mean values of $n=10$ animals, error bars the 95% confidence intervals. Brackets denote significant differences between the experiments (t -test, $p \leq 0.05$), asterisks differences between control and un-spiked treatments, and plus symbols between control and spiked treatments (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$).

experimental containers, as well as uptake and biotransformation by exposed fish might also represent an important dissipation pathway for the lower weight PAHs, such as PHE and PYR (e.g. Ravikrishna et al., 1998; Valsaraj et al., 1997). These potential routes of dissipation were likely to have led to constantly decreasing concentrations of PAHs, especially of those with lower molecular weight. Since only the concentration of parent PAHs was

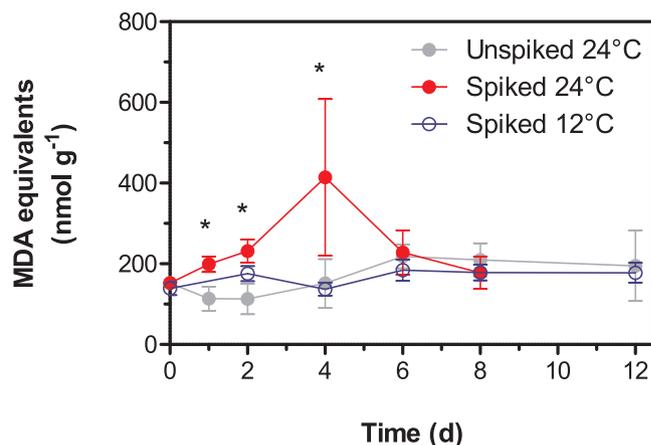


Fig. 6. Dynamics of hepatic lipid peroxidation during the experiments in the un-spiked (■) and the spiked treatments (○/●). Symbols represent mean values of $n=10$ animals, error bars the 95% confidence intervals. Plus symbols denote significant differences between control and spiked treatments (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$).

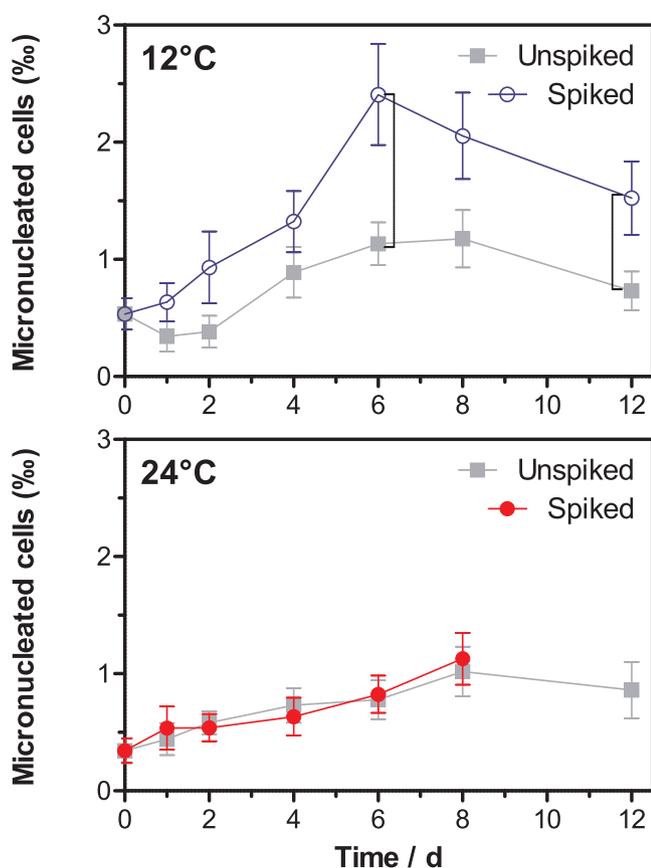


Fig. 7. Dynamics of micronuclei in peripheral erythrocytes during the experiments (top: average temperature 12 °C, bottom: average temperature 24 °C) in the un-spiked (■) and the spiked treatments (○/●). Symbols represent mean values of $n = 10$ animals, error bars the SEM. Brackets denote significant differences between the experiments (t -test, $p \leq 0.05$).

determined, it cannot be excluded that transformation products with different toxicity and behavior were formed.

In contrast to the experiments performed in this study, new suspended particles are permanently re-suspended from the sediment bed in the annular flume that was used in the predecessor project. This results in quasi-flow-through conditions, which minimizes dissipation during the simulated floods. Aging of spiked PAHs can lead to lower rates of desorption and less biodegradation (Fu et al., 1994; Hatzinger and Alexander, 1995; Kan et al., 1994; White et al., 1997). This effect was also observed in the current study, where no significant reduction of sediment-bound PAH concentration over time was found in the experiments with field-aged sediments from the harbor Ehrenbreitstein. Since the same processes affect bioavailability, it seems likely that the differences observed for uptake of the PAHs in fish comparing spiked and un-spiked sediment were due to differing desorption rates (Reid et al., 2000).

4.2. Differences between spiked and naturally aged sediment-bound PAHs

Substantial differences in desorption and subsequent bioavailability of organic contaminants can occur between spiked and naturally aged sediments (Reid et al., 2000). Such differences in desorption of PAHs were apparent in the fraction available for uptake by rainbow trout during this study. While the concentration of sediment-bound PHE was 7.0-fold greater in spiked than in un-spiked sediments (i.e. with naturally aged PAH contamination), the maximum biliary concentration of 1-hydroxy-PHE was 120 and

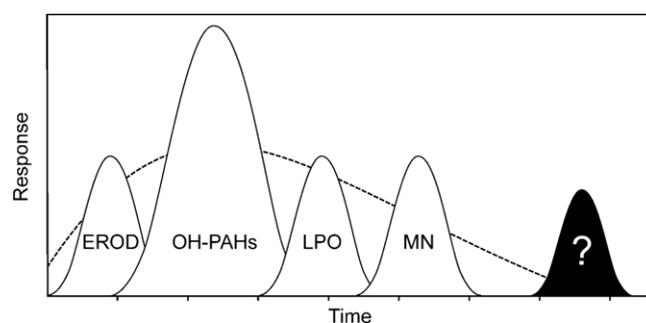


Fig. 8. Conceptual model of the observed biomarker dynamics, in which distinct peak responses follow each other in a cascade-like pattern (combination of experiments at 12 and 24 °C): 7-ethoxyresorufin-*O*-deethylase (EROD, 24 °C), hydroxylated PAH metabolites (OH-PAHs, 12 and 24 °C), lipid peroxides (LPO, 24 °C) and micronuclei (MN, 12 °C). Biomarkers with small peaks were significantly altered only at one temperature following exposure to PAH spiked sediment suspensions, the biomarker with a high peak, i.e. biliary metabolites were altered at both temperatures. The black peak represents a possible adverse effect that might follow the observed biomarker cascade; the dashed line represents a hypothesized integrated stress level.

45-fold greater in fish exposed to spiked relative to un-spiked sediment at 12 °C or 24 °C, respectively. The same effect was observed for PYR. The use of spiked sediment increased the bioavailable fraction significantly.

4.3. Dynamics of biomarker responses during exposure: biomarker cascades

Since no sampling was possible during simulated floods in the project Floodsearch, only qualitative information on biomarkers could be derived (Brinkmann et al., 2010a; Wölz et al., 2009). In the exposure experiments of the present study, biomarkers were monitored kinetically to provide detailed insights in their dynamics as a function of extended exposure to suspended matter. Uptake and effects of PAHs followed a cascade-like pattern in the spiked treatment groups, as indicated by a series of peak biomarker responses (Fig. 8). Due to dissipation of PYR and PHE from the system, the experiment can be subdivided in an uptake and a quasi-depuration phase, in which the concentrations of biliary PAH metabolites increased and decreased, respectively. The peak concentrations of metabolites observed on day two were followed by a peak of LPO on day four at 24 °C. Production of MN, a marker for genetic damage that can be predictive of potential population level effects (Diekmann et al., 2004b), was observed on day 6 in the 12 °C treatment (Fig. 7). EROD activity was significantly induced in the un-spiked treatment group compared to untreated control animals after 8 and 12 d at 12 °C (Kruskal–Wallis one-way ANOVA on ranks with Dunn's post hoc test, $p \leq 0.05$), but not compared to the spiked sediment treatment (Mann–Whitney Rank Sum test, $p \leq 0.05$). Exposure time or concentration at 12 °C might not have been sufficient to induce EROD since the PAHs used for spiking are considered weak to moderate AhR agonists (Barron et al., 2004). At 24 °C, however, a significant induction of EROD activity comparing animals from the spiked and un-spiked treatments on day one (t -test, $p \leq 0.05$) was followed by a decrease, which was most probably non-specific due to temperature stress (cf. Whyte et al., 2000). Although induction of biomarkers was transient, potential long-term adverse effects of exposure to particle-bound PAHs cannot be excluded. Thus, an experiment to monitor health and performance of fish over a longer period of time following short exposures to contaminated SPM should be conducted to answer the question if the observed biomarker cascade is followed by a potentially adverse effect (question mark, Fig. 8).

4.4. Influence of temperature (stress) on the biomarker cascade

Since degradation, bioavailability and effects of particle-bound pollutants can vary depending on temperature, exposure experiments were conducted at an average temperature representative for rivers in Central Europe (12 °C) and under temperature stress (24 °C) to investigate a range of possible consequences of sediment re-suspension.

In some studies, critical thermal maxima for salmonid fish species were investigated by constantly increasing water temperature. The reported temperatures with significant effects on growth and survival of rainbow trout ranged from 24 to 27 °C, depending on other environmental parameters (Myrick and Cech, 2004). The greater mortality of rainbow trout in the 24 °C experiment of the present study (25 and 31% for the un-spiked and spiked treatment, respectively) could thus be explained by direct effects of temperature.

Gross energy metabolism of fish was significantly greater at 24 °C as represented by the elevated excretion rate of biliverdin (cf. Avery et al., 1992), which was similar to rates of uptake and biotransformation of PAHs. Although maximum concentrations of PAH metabolites in bile did not differ much between the two temperatures, PAH uptake was approximately 2-fold faster at 24 °C compared to that at 12 °C. Significant uptake of PAHs from the spiked sediment was observed at 12 °C, but elevated hepatic LPO was only found at 24 °C after 4 d (Fig. 6). BAP has been previously shown to be converted to a 1,6-quinone metabolite in fish via the precursor 1-hydroxy-BAP (Di Giulio and Hinton, 2008), which was reported to cause oxidative stress (Lemaire et al., 1994). Please note that only the metabolite 3-hydroxy-BAP was measured in the present study. Seubert and Kennedy (2000), however, have shown that 1-hydroxy- and 3-hydroxy-BAP co-occur among other metabolites in the investigated species. Rainbow trout appeared to compensate for oxidative stress at lower temperature so that an increased level of LPO was only observed in the spiked 24 °C treatment. Similar effects have been observed by other researchers who concluded that the rate of turnover of lipids was more rapid at lower temperatures, and which has a protective function (Grim et al., 2010; Lushchak, 2011; Lushchak and Bagnyukova, 2006; Parihar and Dubey, 1995). Significant differences between the spiked and un-spiked treatments concerning MN in peripheral erythrocytes have only been observed at 12 °C.

It can be concluded that some effects of PAHs on fish observed in the present study were only apparent in combination with temperature stress during exposure, while others were only apparent at 12 °C. The biomarker cascades did not only show quantitative differences (i.e. different induction intensity or rate of biomarker responses) at the two temperatures but also qualitative differences, i.e. different biomarker responses were observed. The correlation between gross energy metabolism and some of the biomarkers (i.e., biliary metabolites and LPO) supports the hypothesis that a combination of chemical exposure and other environmental stressors can lead to enhanced effects in aquatic biota (Holmstrup et al., 2010). Since temperatures of German rivers frequently exceed 25 °C during summer as a result of dissipated heat from power plants and due to climate change (IKSR, 2004), it can be assumed that re-suspension of sediments under these conditions could potentially have higher impact on aquatic biota compared to lower temperatures, as in the case of dissolved pollutants (e.g. Airas et al., 2008; Heinonen et al., 2002; Honkanen and Kukkonen, 2006).

Acknowledgments

This study has been generously supported by a Boost-Funds project of the Exploratory Research Space (ERS) at RWTH Aachen

University, as part of the German Excellence Initiative. The RWTH Aachen University Undergraduate Research Opportunities Program (UROP) provided funding for a graduate student from the United States of America who performed the micronucleus assay in our laboratory. Furthermore, we thank the German Academic Exchange Service (DAAD PPP, project number 50154858) for providing travel funds for Jochen Kuckelkorn, who performed mRNA expression studies at the University of Saskatchewan. We would like to thank the German Federal Institute of Hydrology (Bundesanstalt für Gewässerkunde, BfG), especially Denise Spira and Dr. Georg Reifferscheid, for assistance and support during sampling of the sediments. Drs. John Giesy and Markus Hecker were supported through the Canada Research Chair program. The authors want to express their gratitude for the great and helpful effort that was put into the manuscript by the two anonymous reviewers.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquatox.2012.11.010>.

References

- ACS Committee on Environmental improvement, 1980. Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Analytical Chemistry* 52, 2242–2249.
- Ahlf, W., Hollert, H., Neumann-Hensel, H., Ricking, M., 2002. A guidance for the assessment and evaluation of sediment quality—A German approach based on ecotoxicological and chemical measurements. *Journal of Soils and Sediments*, 37–42.
- Airas, S., Leppänen, M., Kukkonen, J.V.K., 2008. Effects of temperature and oxygen concentration in sediment toxicity testing. *Ecotoxicology and Environmental Safety* 70, 475–482.
- Apitz, S.E., Arias, E., Clawson, S.A., Lin, E.W., Melcher, R.J., Hemmingsen, B.B., 1999. The development of a sterile, PAH-spiked, aged marine sediment for biodegradation experiments: chemical results. *Organic Geochemistry* 30, 891–900.
- Avery, E.H., Lee, B.L., Freedland, R.A., Cornelius, C.E., 1992. Bile pigments in gall-bladder and freshly-secreted hepatic duct bile from fed and fasted rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Part A: Physiology* 101, 857–861.
- Barron, M.G., Heintz, R., Rice, S.D., 2004. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. *Marine Environmental Research* 58, 95–100.
- Bjornn, T.C., Reiser, D.W., 1991. Habitat requirements of salmonids in streams. In: Meehan, W.R. (Ed.), *Influences of Forest and Rangeland Management on Salmonid Fishes and their Habitat*. American Fisheries Society, Bethesda, Maryland, pp. 83–138.
- Bobe, J., Nguyen, T., Jalabert, B., 2004. Targeted gene expression profiling in the rainbow trout (*Oncorhynchus mykiss*) ovary during maturational competence acquisition and oocyte maturation. *Biology of Reproduction* 71, 73–82.
- Brinkmann, M., Hudjetz, S., Cofalla, C., Roger, S., Kammann, U., Giesy, J.P., Hecker, M., Wiseman, S., Zhang, X., Wölz, J., Schüttrumpf, H., Hollert, H., 2010a. A combined hydraulic and toxicological approach to assess re-suspended sediments during simulated flood events. Part I—multiple biomarkers in rainbow trout. *Journal of Soils and Sediments* 10, 1347–1361.
- Brinkmann, M., Hudjetz, S., Keiter, S., Seiler, T.B., Wölz, J., Hallare, A.V., Hollert, H., Cofalla, C., Roger, S., Schüttrumpf, H., Gerbersdorf, S.U., 2010b. Toxizität und Risk Assessment fluvialer Sedimente und Schwebstoffe: Eine kurze Übersicht bisheriger und neuerer Entwicklungen. *Umweltwiss Schadst-Forsch* 22, 651–655.
- Chapman, P.M., Hollert, H., 2006. Should the sediment triad become a tetrad, pentad or possibly even a hexad? *Journal of Soils and Sediments* 6, 4–8.
- Ciarelli, S., van Straalen, N.M., Klap, V.A., van Wezel, A.P., 1999. Effects of sediment bioturbation by the estuarine amphipod *Corophium volutator* on fluoranthene resuspension and transfer into the mussel (*Mytilus edulis*). *Environmental Toxicology and Chemistry* 18, 318–328.
- Ciutat, A., Widdows, J., Readman, J.W., 2006. Influence of cockle *Cerastoderma edule* bioturbation and tidal-current cycles on resuspension of sediment and polycyclic aromatic hydrocarbons. *Marine Ecology Progress Series* 328, 51–64.
- Cofalla, C., Roger, S., Frings, R.M., Schüttrumpf, H., Brinkmann, M., Hudjetz, S., Wölz, J., Hollert, H., Gerbersdorf, S.U., Hecker, M., Kammann, U., Lennartz, G., Schäffer, A., 2011. Eine Methode zur Bestimmung der Wechselwirkungen zwischen Sedimentdynamik und Ökotoxikologie. *Hydrologie und Wasserbewirtschaftung* 11, 227–230.
- Cofalla, C., Hudjetz, S., Roger, S., Brinkmann, M., Frings, R., Wölz, J., Schmidt, B., Schäffer, A., Kammann, U., Hecker, M., Hollert, H., Schüttrumpf, H., 2012. A combined

- hydraulic and toxicological approach to assess re-suspended sediments during simulated flood events—part II: an interdisciplinary experimental methodology. *Journal of Soils and Sediments* 12, 429–442.
- Diekmann, M., Hultsch, V., Nagel, R., 2004a. On the relevance of genotoxicity for fish populations I: effects of a model genotoxicant on zebrafish (*Danio rerio*) in a complete life-cycle test. *Aquatic Toxicology* 68, 13–26.
- Diekmann, M., Waldmann, P., Schnurstein, A., Grummt, T., Braunbeck, T., Nagel, R., 2004b. On the relevance of genotoxicity for fish populations II: genotoxic effects in zebrafish (*Danio rerio*) exposed to 4-nitroquinoline-1-oxide in a complete life-cycle test. *Aquatic Toxicology* 68, 27–37.
- Di Giulio, R.T., Hinton, D.E., 2008. *The Toxicology of Fishes*. CRC Taylor & Francis, Boca Raton.
- Fontagne, S., Lataillade, E., Breque, J., Kaushik, S., 2008. Lipid peroxidative stress and antioxidant defence status during ontogeny of rainbow trout (*Oncorhynchus mykiss*). *Br J Nutr* 100, 102–111.
- Forbes, V.E., Palmqvist, A., Bach, L., 2006. The use and misuse of biomarkers in ecotoxicology. *Environmental Toxicology and Chemistry* 25, 272–280.
- Fu, G., Kan, A.T., Tomson, M., 1994. Adsorption and desorption hysteresis of PAHs in surface sediment. *Environmental Toxicology and Chemistry* 13, 1559–1567.
- Gerbersdorf, S., Hollert, H., Brinkmann, M., Wieprecht, S., Schüttrumpf, H., Manz, W., 2011. Anthropogenic pollutants affect ecosystem services of freshwater sediments: the need for a triad plus x approach. *Journal of Soils and Sediments* 11, 1099–1114.
- Grim, J.M., Miles, D.R.B., Crockett, E.L., 2010. Temperature acclimation alters oxidative capacities and composition of membrane lipids without influencing activities of enzymatic antioxidants or susceptibility to lipid peroxidation in fish muscle. *Journal of Experimental Biology* 213, 445–452.
- Hari, R.E., Livingstone, D.M., Siber, R., Burkhardt-Holm, P., GÜTtinger, H., 2006. Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. *Global Change Biology* 12, 10–26.
- Hatzinger, P.B., Alexander, M., 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environmental Science and Technology* 29, 537–545.
- Heininger, P., Höss, S., Claus, E., Pelzer, J., Traunspurger, W., 2007. Nematode communities in contaminated river sediments. *Environmental Pollution* 146, 64–76.
- Heinonen, J., Honkanen, J., Kukkonen, J.V.K., Holopainen, I.J., 2002. Bisphenol A accumulation in the Freshwater Clam *Pisidium amnicum* at Low Temperatures. *Archives of Environmental Contamination and Toxicology* 43, 50–55.
- Heitkamp, M.A., Cerniglia, C.E., 1987. Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. *Environmental Toxicology and Chemistry* 6, 535–546.
- Hollert, H., Haag, I., Dürr, M., Wetterauer, B., Holtey-Weber, R., Kern, U., Westrich, B., Färber, H., Erdinger, L., Braunbeck, T., 2003. Investigations of the ecotoxicological hazard potential and risk of erosion of contaminated sediments in lock-regulated rivers. *UWSF-Z Umweltchem Ökotoxikol* 15, 5–12.
- Hollert, H., Dürr, M., Haag, I., Wölz, J., Hilscherova, K., Blaha, L., Gerbersdorf, S.U., 2008. Influence of hydrodynamics on sediment ecotoxicity. In: Westrich, B., Förster, U. (Eds.), *Sediment Dynamics and Pollutant Mobility in Rivers*. Springer, Heidelberg, pp. 401–416.
- Holmstrup, M., Bindsbøl, A.-M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.-R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C., Spurgeon, D.J., 2010. Interactions between effects of environmental chemicals and natural stressors: a review. *Science Total Environment* 408, 3746–3762.
- Honkanen, J.O., Kukkonen, J.V.K., 2006. Environmental temperature changes uptake rate and bioconcentration factors of bisphenol A in tadpoles of *Rana Temporaria*. *Environmental Toxicology and Chemistry* 25, 2804–2808.
- Hyytiäläinen, T., Karels, A., Oikari, A., 2002. Assessment of bioavailability and effects of chemicals due to remediation actions with caging mussels (*Anodonta anatina*) at a creosote-contaminated lake sediment site. *Water Research* 36, 4497–4504.
- Ikeda, T., Yoshitani, J., Terakawa, A., 2005. Flood management under climatic variability and its future perspective in Japan. *Water Science and Technology* 51, 133–140.
- IKSR, 2004. *Wärmebelastung der Gewässer im Sommer 2003 – Zusammenfassung der nationalen Situationsberichte*. Internationale Kommission zum Schutz des Rheins. IKSR-Bericht Nr. 142d.
- Johnson, J.E., Kramer, R.P., Larson, E., Bonebrake, B.L., 1987. Trout growth, harvest, survival and microhabitat selection in the Green River, Utah, 1978–1982. Final Report, Flaming Gorge Tailwater Fisheries Investigations. Utah Division of Wildlife Resources, Salt Lake City.
- Kammann, U., 2007. PAH metabolites in bile fluids of dab (*Limanda limanda*) and flounder (*Platichthys flesus*) – spatial distribution and seasonal changes. *Environmental Science and Pollution Research* 14, 102–108.
- Kammann, U., Lang, T., Wosniok, W., 2012. Biological effects monitoring in marine research. *Environmental Sciences Europe* 24, 1.
- Kan, A.T., Fu, G., Tomson, M.B., 1994. Adsorption/desorption hysteresis in organic pollutant and soil/sediment interaction. *Environmental Science and Technology* 28, 859–867.
- Kay, A.L., Jones, R.G., Reynard, N.S., 2006. RCM rainfall for UK flood frequency estimation. II. Climate change results. *Journal of Hydrology* 318, 163–172.
- Kennedy, S.W., Jones, S.P., 1994. Simultaneous measurement of cytochrome p450a1 catalytic activity and total protein-concentration with a fluorescence plate reader. *Analytical Biochemistry* 222, 217–223.
- LeBlanc, L.A., Gulnick, J.D., Brownawell, B.J., Taylor, G.T., 2006. The influence of sediment resuspension on the degradation of phenanthrene in flow-through microcosms. *Marine Environmental Research* 61, 202–223.
- Lemaire, P., Matthews, A., Forlin, L., Livingstone, D.R., 1994. Stimulation of oxyradical production of hepatic microsomes of flounder (*Platichthys flesus*) and perch (*Perca fluviatilis*) by model and pollutant xenobiotics. *Archives of Environmental Contamination and Toxicology* 26, 191–200.
- Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology* 101, 13–30.
- Lushchak, V.I., Bagnyukova, T.V., 2006. Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress. *Comparative Biochemistry and Physiology – Part C: Pharmacology, Toxicology and Endocrinology* 143, 30–35.
- Morgan, I.J., McDonald, D.G., Wood, C.M., 2001. The cost of living for freshwater fish in a warmer, more polluted world. *Global Change Biology* 7, 345–355.
- Mortensen, A.S., Arukwe, A., 2007. Effects of 17 alpha-ethynylestradiol on hormonal responses and xenobiotic biotransformation system of Atlantic salmon (*Salmo salar*). *Aquat Toxicol* 85, 113–123.
- Myrick, C.A., Cech, J.J., 2004. Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know? *Reviews in Fish Biology and Fisheries* 14, 113–123.
- Newman, M.C., 2008. What exactly are you inferring? A closer look at hypothesis testing. *Environmental Toxicology and Chemistry* 27, 1013–1019.
- Ng, C.A., Gray, K.A., 2011. Forecasting the effects of global change scenarios on bioaccumulation patterns in great lakes species. *Global Change Biology* 17, 720–733.
- Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., Levin, E.D., 2009. The toxicology of climate change: Environmental contaminants in a warming world. *Environment International* 35, 971–986.
- OECD 218, 2004. OECD guideline 218: Sediment–water chironomid toxicity using spiked sediment. Organisation for Economic Co-operation and Development, Berlin.
- Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95, 351–358.
- Parihar, M.S., Dubey, A.K., 1995. Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase. *Comparative Biochemistry and Physiology – Part C: Pharmacology, Toxicology and Endocrinology* 112, 309–313.
- Pohl, R.J., Fouts, J.R., 1980. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Analytical Biochemistry* 107, 150–155.
- Ravikrishna, R., Valsaraj, K.T., Yost, S., Price, C.B., Brannon, J.M., 1998. Air emissions from exposed, contaminated sediment and dredged materials: 2. Diffusion from laboratory-spiked and aged field sediments. *Journal of Hazardous Materials* 60, 89–104.
- Reible, D.D., Popov, V., Valsaraj, K.T., Thibodeaux, L.J., Lin, F., Dikshit, M., Todaro, M.A., Fleeger, J.W., 1996. Contaminant fluxes from sediment due to tubificid oligochaete bioturbation. *Water Research* 30, 704–714.
- Reid, B.J., Jones, K.C., Semple, K.T., 2000. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. *Environmental Pollution* 108, 103–112.
- Roberts, D.A., 2012. Causes and ecological effects of resuspended contaminated sediments (RCS) in marine environments. *Environment International* 40, 230–243.
- Rocha, P.S., Luvizotto, G.L., Kosmehl, T., Böttcher, M., Storch, V., Braunbeck, T., Hollert, H., 2009. Sediment genotoxicity in the Tietê River (São Paulo, Brazil): in vitro comet assay versus in situ micronucleus assay studies. *Ecotoxicology and Environmental Safety* 72, 1842–1848.
- Schüttrumpf, H., Brinkmann, M., Cofalla, C., Frings, R., Gerbersdorf, S., Hecker, M., Hudjetz, S., Kammann, U., Lennartz, G., Roger, S., Schaffer, A., Hollert, H., 2011. A new approach to investigate the interactions between sediment transport and ecotoxicological processes during flood events. *Environmental Sciences Europe* 23, 39.
- Seubert, J.M., Kennedy, C.J., 2000. Benzo[a]pyrene toxicokinetics in rainbow trout (*Oncorhynchus mykiss*) acclimated to different salinities. *Archives of Environmental Contamination and Toxicology* 38, 342–349.
- Shiaris, M.P., 1989. Phenanthrene mineralization along a natural salinity gradient in an Urban Estuary, Boston Harbor, Massachusetts. *Microbial Ecology* 18, 135–146.
- Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 996.
- Valsaraj, K.T., Choy, B., Ravikrishna, R., Reible, D.D., Thibodeaux, L.J., Price, C.B., Brannon, J.M., Myers, T.E., 1997. Air emissions from exposed, contaminated sediments and dredged materials. 1. Experimental data in laboratory microcosms and mathematical modelling. *Journal of Hazardous Materials* 54, 65–87.
- White, J.C., Kelsey, J.W., Hatzinger, P.B., Alexander, M., 1997. Factors affecting sequestration and bioavailability of phenanthrene in soils. *Environmental Toxicology and Chemistry* 16, 2040–2045.
- Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *CRC Critical Reviews in Toxicology* 30, 347–570.
- Wilby, R.L., Orr, H.G., Hedger, M., Forrow, D., Blackmore, M., 2006. Risks posed by climate change to the delivery of Water Framework Directive objectives in the UK. *Environment International* 32, 1043–1055.

- Wiseman, S.B., Vijayan, M.M., 2007. Aryl hydrocarbon receptor signaling in rainbow trout hepatocytes: Role of hsp90 and the proteasome. *Comp Biochem Physiol C Toxicol Pharmacol* 146, 484–491.
- Wölz, J., Cofalla, C., Hudjetz, S., Roger, S., Brinkmann, M., Schmidt, B., Schäffer, A., Kammann, U., Lennartz, G., Hecker, M., Schüttrumpf, H., Hollert, H., 2009. In search for the ecological and toxicological relevance of sediment re-mobilisation and transport during flood events. *Journal of Soils and Sediments* 9, 1–5.
- Wölz, J., Fleig, M., Schulze, T., Maletz, S., Lübcke-von Varel, U., Reifferscheid, G., Kühlers, D., Braunbeck, T., Brack, W., Hollert, H., 2010. Impact of contaminants bound to suspended particulate matter in the context of flood events. *Journal of Soils and Sediments* 10, 1174–1185.
- Xia, X.H., Yu, H., Yang, Z.F., Huang, G.H., 2006. Biodegradation of polycyclic aromatic hydrocarbons in the natural waters of the Yellow River: Effects of high sediment content on biodegradation. *Chemosphere* 65, 457–466.
- Zhang, X., Hecker, M., Tompsett, A.R., Park, J.W., Jones, P.D., Newsted, J., Au, D., Kong, R., Wu, R.S.S., Giesy, J.P., 2008. Responses of the Medaka HPG axis PCR array and reproduction to Prochloraz and Ketoconazole. *Environmental Science and Technology* 42, pp. 8614–8614.