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Evaluation of two aquatic passive sampling configurations for their suitability in the analysis of estrogens in water

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ABSTRACT

The presence of estrogens in the aquatic environment has been the target of several studies in the last decade. Newly developed passive sampling techniques for polar organic chemicals show great promise for the assessment of long-time exposure of aquatic organisms to emerging contaminants. In the present work, two configurations of the Chemcatcher® passive sampler have been tested for their applicability to the analysis of seven estrogens in water. Accumulation experiments in the laboratory, to calculate the uptake rates, and a field trial show that the polar configuration of this device may be used for the efficient sampling and determination of estrogens in water. Time weighted average concentrations were determined in the field trial and compared with spot sampling concentrations. The detection of estriol using passive sampling, although not found with spot sampling, clearly demonstrates the value of this technique in assessing relevant concentrations of estrogens in the aquatic media.

1. Introduction

Steroid estrogens of human and animal origin enter the environment and reach surface waters mainly due to incomplete removal during waste water treatment and through runoff from sludge amended soil [1,2]. Estrogens have been reported to be present at low concentrations (in the ng L^{-1} range) in the aquatic environment where their strong estrogenic potency has lead to adverse effects in aquatic organism [3–5]. In the last decades environmental chemists have developed several analytical methods to determine their presence in waste water, river, marine and ground water [6]. Commonly, samples are collected strategically in several sampling campaigns and estrogens are analysed using mostly solid phase extraction (SPE) followed by gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) [7]. By working with spot samples only the current concentration of the collected volume can be determined. Thus limited information is obtained from the analysis when the interest of the study is to know the level of exposure that aquatic organisms are experiencing over a longer period of time. Bio-concentration experiments based on the use of living organisms can be employed for this purpose. However, to obtain comparable results the biological variability has to be overcome (e.g. metabolism of the target compounds, mortality of the organism, diversity of species) [8]. Passive sampling technologies have been developed for air, soil and water for a large variety of compound classes [9,10]. Time weighted average (TWA) concentrations over several days (in the case of aquatic membrane samplers up to 56 days [8]) can be determined, less costly than spot sampling and easier to handle and interpret than bio-concentration experiments. The new generation of aquatic passive samplers Chemcatcher® [11–14] and POCIS [15–17] have been used in the monitoring of non-polar and polar organic compounds. They consist of a polymeric receiving phase separated from the sampling media by a diffusion limiting membrane. The choice and combination of different materials of the receiving phase and membrane allow to selectively promote the uptake of the desired compounds. When a smaller deployment time is desired the membrane can be left aside using only the naked receiving phase for a faster uptake onto the passive sampler. This configuration however brings several disadvantages which have to be encountered for, such as faster bio-fouling, unselective sampling, etc. Detailed information about the passive sampling technique, absorption principles and challenges are presented in several reviews [9,10,18].

Most emerging (e.g. pharmaceuticals, estrogens) and several classical contaminants (e.g. modern pesticides, plasticizers) of envi-

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ronmental concern are semi-polar to polar organic compounds with octanol–water partition coefficients ($\log K_{ow}$) < 4. The use of passive samplers for the analysis of pharmaceuticals and pesticides in water has been reported also in combination with toxicological analysis of the samples extracts showing good results [13,19–21].

However, to the authors knowledge there are only two studies which report time weighted average concentrations of estrogens in water [15,22]. In these studies estrogens uptake was performed onto passive samplers using poly(styrenedivinylbenzene)/carbonaceous resin/styrenedivinylbenzene (ENV+/Ambersorb 1500/S-X3 Bio Beads) and poly(divinyl-benzene-co-N-vinylpyrrolidone) (Oasis HLB), both with a polyethersulphone (PES) membrane [15], and a naked sulphonated poly(styrenedivinylbenzene) (SDB-RPS) receiving phase [22]. The latter surveyed a waste water treatment facility at different stages and the passive sampler was deployed for only 4 days.

In the present study the passive sampler device Chemcatcher® has been tested for its applicability to the determination of estrogens in environmental waters. The compounds investigated, selected based on their abundance in the human body, their frequency of detection in environmental waters, and their estrogenic potency, comprise five natural, free (estradiol, estrone, estriol) and conjugated (estradiol glucuronide, estrone sulphate) estrogens, and two synthetic chemicals (ethinylestradiol, diethylstilbestrol). These compounds have $\log K_{ow}$ < 4, with the exception of diethylstilbestrol that has $\log K_{ow}$ 5.07 (see Table 1). The Chemcatcher® sampler has two main configurations, both using C18 Empore® disks as receiving phase: a non-polar configuration for analytes with $\log K_{ow}$ 3–6, equipped with a low density polyethylene (LDPE) diffusion membrane, and a polar configuration for analytes with $\log K_{ow}$ < 4, equipped with a polyethersulphone (PES) membrane. Determination of the concentration of the analytes in the samplers and in the water was performed using ultrasonication or SPE followed by detection with HPLC–MS/MS. Both sampler configurations have therefore been evaluated for its performance in the uptake and analysis of estrogens through the comparison of the results obtained in laboratory experiments with those obtained in a field trial and in the analysis of spot samples.

2. Materials and methods

2.1. Standards

Chemcatcher® PTFE devices were supplied by Portsmouth University. C18 and SDB Empore® disks were obtained from 3 M (St. Paul, MN, USA). Polyethersulphone (PES) membranes were supplied by Pall Corporation (East Hills, NY, USA) and low density polyethylene (LDPE) membranes, 40 μm thick, were a gift from Portsmouth University. Sodium sulphate cartridges were purchased from Varian, Inc. (Palo Alto, USA). LiChrolut RP-C18 cartridges were purchased from Merck (Darmstadt, Germany).

Octanol was supplied by Riedel de Haën (Seelze, Germany), and HPLC-grade water and all other solvents (gas chromatography quality) were supplied by Merck (Darmstadt, Germany).

High purity (98–99.9%) standards of the investigated estrogens estradiol, estrone, estriol, estradiol-17-glucuronide, estrone-3-sulphate, ethinylestradiol and diethylstilbestrol, and of their deuterated analogues estradiol-d_5, estrone-d_4, estriol-d_3, estrone-3-sulphate-d_4 and ethinylestradiol-d_4 were purchased as powders from Sigma (St Louis, MO, USA) and C/D/N Isotopes (Sainte-Foy-La-Grande, France), respectively. Stock standard solutions for each of the analytes were prepared in methanol at 1 mg mL^{-1} and stored in the dark at 4 °C. Working standard mixtures at concentrations ranging between 0.1 and 1000 ng mL^{-1} were prepared by appropriate dilution of the stock solutions in methanol (concentration of internal standards when used 50 ng mL^{-1}).

2.2. Passive sampler

The Chemcatcher® sampling device, consisting of a PTFE body, was used with C18 Empore® disks as receiving phase, and with or without a PES or a LDPE membrane. Before use the diffusion limiting membranes were washed for several hours by soaking them in methanol (PES) or hexane (LDPE) and let dry at room temperature. Preparation of the receiving phase was performed by conditioning the disks in methanol until they were translucent, following permeation by passing 10 mL of methanol and 20 mL of HPLC water through them. The disks, saturated with water, were placed into the sampler PTFE body and carefully covered with the PES membrane avoiding the formation of air bubbles in-between. The sampler configuration with the LDPE membrane had its receiving phase prepared in the same way and further dried under vacuum during 30 min, then impregnated until saturation with n-octanol by adding 1 mL of a solution of octanol in acetone (45%, v/v) [12]. After evaporation of the acetone the disk was placed into the sampler body and covered with the membrane with the same care as described above. After exposure, the samplers were disassembled, the diffusion membrane removed, and the Empore® disks analysed.

2.3. Experimental set-up

A flow-through system with monitored constant water temperature, turbulence and concentration of the studied compounds

Table 1
SRM transitions, $\log K_{ow}$, and limits of detection (LOD) and quantification (LOQ) in ng L^{-1} for time weighed average concentrations of selected estrogens after 4, 16 and 28 days of exposure using the Chemcatcher® configuration C18-PES.

Compound	SRM transitions		$\log K_{ow}$[a]	LOD			LOQ		
	Quantifier	Qualifier		4 d	16 d	28 d	4 d	16 d	28 d
E2-G	447 → 113	447 → 271	–	1.40	0.35	0.20	4.68	1.17	0.67
E1-S	349 → 269	349 → 145	–	0.04	0.01	0.01	0.14	0.03	0.02
E1-S-d_4	353 → 273								
E3	287 → 171	287 → 145	2.45	0.44	0.11	0.06	1.46	0.37	0.21
E3-d_3	290 → 173								
E1	269 → 145	269 → 143	3.13	0.22	0.05	0.03	0.73	0.18	0.10
E1-d_4	273 → 147								
E2	271 → 145	271 → 183	4.01	0.60	0.15	0.09	2.01	0.50	0.29
E2-d_5	276 → 147								
EE2	295 → 145	295 → 159	3.67	2.73	0.68	0.39	9.09	2.27	1.30
EE2-d_4	299 → 147								
DES	267 → 222	267 → 237	5.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

E2-G, estradiol-17-glucuronide; E1-S, estrone-3-sulphate; E3, estriol; E1, estrone; E2, 17-β-estradiol; EE2, ethinyl estradiol; DES, diethystilbestrol; n.d., not detected.
[a] http://www.syrres.com/what-we-do/databaseforms.aspx?id=386.

was employed for laboratory exposure experiments. The sampler devices were placed in a stirring turntable in a 25 L stainless steel tank with an overflow to waste while a cooling unit together with a thermostat regulated the temperature and two peristaltic pumps continuously introduced distilled water (33 mL min^{-1}) and a solution of the target chemicals in methanol (30 ng mL^{-1} at 0.1 mL min^{-1}) (for a detailed description see De la Cal et. al. [14]).

2.4. Calibration experiment

The suitability of both, polar and non-polar, configurations was evaluated by comparing the accumulation of each compound in the disks in exposure experiments performed during 48 h at constant temperature (21 °C), turbulence (45 rpm) and concentration of estrogens in water (1 μg L^{-1}). The concentration was confirmed by analysing water samples from the outlet of the tank. The theoretical behaviour of this accumulation has been described as an exponential approach to the steady state [23,24]. However, during the initial stage of exposure, the uptake and accumulation kinetics of the analytes in the receiving phase should be linear, according to the equation:

$$m(t) = m(0) + C_w R_s t$$

where m represents the mass of analyte in the receiving phase at exposure time t (day) or initial time (0), C_w is the concentration in water during that period (ng L^{-1}) and R_s is the sampling rate of the device, that is, the equivalent extracted water volume per unit of time (L day^{-1}). The R_s value for each of the estrogens investigated was worked out from this equation at the conditions tested.

2.5. Sampling sites

The Llobregat River basin, situated in the NE part of Spain, is the main water source for the Barcelona metropolitan area and its surroundings. The high population density, and the intensive industrial and agricultural activities performed in this area impact on the ecological status of the Llobregat river and its tributaries (e.g. Anoia, Cardener, Rubí creek). The Llobregat basin receives the input of various sewage treatment plant effluents and by surface runoff also residues from agricultural employment. Furthermore, the occurrence of natural salt formations and the corresponding mining exploitations in the basin (Cardona, Suria and Sallent mining sites) have caused an increase in the salinity of the water. Several studies have reported the presence of organic pollutants (e.g. hydrocarbons, flame retardants, pesticides, surfactants, plasticizers, pharmaceuticals) in this region [25–30].

Fig. 1 shows the geographical location selected for the field trial, a place situated in El Papiol, near Barcelona, which is under the control of the Catalan Water Agency (ACA) where the passive samplers were protected from vandalism and consequent loss, and where the general water parameters, such as temperature, pH, conductivity and dissolved oxygen, are continuously monitored by an Automatic System of Hydrological Information (SAIH) or the Automated Net of Water Quality Control (XACQA) run by the ACA. The passive samplers were placed in this site in two irrigation channels that run side by side (see Fig. 1), simply divided by a concrete wall, conducting water one from the Anoia River and the other one from the Rubí Creek.

2.6. Analytical methods

Extraction of the estrogens from the C18 disks used in combination with LDPE membranes was based on the method described

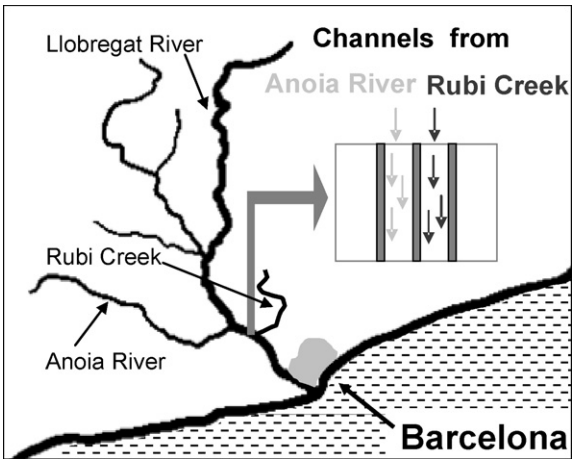

Fig. 1. Location of the field trial sites.

in Kingston et al. [11]. The disk is placed in a vial and extracted twice with 15 mL acetone and 10 mL acetone:hexane (1:1, v/v) using ultrasonication. The combined extracts are dried over a sodium sulphate cartridge and concentrated using a stream of nitrogen [14]. The octanolic residue is complemented with methanol to a final volume of 1 mL. The disks employed with PES membranes were processed in the same way but using a mixture of acetone:methanol 1:1 (2 × 10 mL) for ultrasonic extraction.

Extraction of the water samples was carried out with the automated sample processor ASPEC XL. SPE was performed with LiChrolut RP-18 (500 mg) cartridges from Merck (Darmstadt, Germany) following a methodology published earlier [31,32]. Briefly, river water samples (250 mL) and spiked water samples from the exposure experiments (50 mL) are passed through the cartridges previously conditioned with methanol and water, and after washing of the cartridges with HPLC water, the compounds are eluted with 2 × 4 mL methanol. The extracts are then concentrated under a N_2-stream and reconstituted with 250 μL methanol for further analysis.

Disks and water extracts from the field trial were reconstituted with a methanolic solution of the deuterated internal standards at a concentration of 50 ng mL^{-1}.

Analysis of the estrogens in the extracts was performed by LC–MS/MS with a system consisting of a Waters Alliance 2690 LC pump equipped with an autosampler and connected to a Quattro LC triple-quadrupole mass spectrometer from Micromass (Manchester, UK).

Separation was achieved on a Purospher STAR-RP-18ec column (250 × 4 mm, 5 μm) preceded by a guard column (4 × 4 mm, 5 μm) of the same packing material (Merck, Darmstadt). Chromatography at 0.2 mL min^{-1} was performed with a 45 min gradient starting from 10% acetonitrile in water, increasing to 50% acetonitrile in 5 min and continuing to 80% in 20 min. During the following 5 min the column was cleaned with 100% acetonitrile, readjusted to the initial conditions in 2 min, and equilibrated for further 13 min.

MS/MS detection was performed with an electrospray interface (ESI) operating in the negative ion mode acquiring two selected reaction monitoring (SRM) transitions per compound (see Table 1). Other optimized MS conditions were as follows: capillary voltage, 3.5 kV; source temperature, 150 °C; desolvation temperature, 450 °C; extractor voltage, 2 V; and RF lens, 0.4 V. Nitrogen was used as nebulising and drying gas. The flow-rate of the nebulising gas was set at 60 L h^{-1}, and that of the desolvation gas at 550 L h^{-1}. Argon was used as collision gas with a pressure of 2.58×10^{-3} mbar [33].

Quantification, based on peak areas, was performed by the external standard method during method optimization and throughout the laboratory exposure experiments. Field trial samples were quantified by the internal standard method.

3. Results and discussion

3.1. Analysis of sampler disks and water samples

The C18 Empore® disks were extracted by ultrasonication with different solvents. We investigated a total of four solvent and solvent mixtures for this procedure: acetone, acetone:methanol (1:1, v/v), acetone:hexane (1:1, v/v) and acetone followed by acetone:hexane (1:1, v/v). The choice of including hexane in combination with acetone as a potential extraction mixture responds to the fact that for the non-polar sampler other compounds, namely, polybrominated flame retardants (PBDEs) and organochlorine pesticides were simultaneously investigated. The results of this study are published elsewhere by De la Cal et al. [14]. The prepared disks were spiked by passing 100 mL of water with 500 ng of each estrogen (100 μL of 5 μg mL^{-1} standard solution) and extracted with the different solvents ($n = 4$). In general recoveries were always higher than 68% (with relative standard deviations (RSD) always <20%), with the only exception of estradiol glucuronide which was recovered only about 50% with all solvents tested. However, as expected the highest recoveries were achieved with acetone:methanol (1:1, v/v) (>95%, RSD < 8%). This solvent was therefore chosen for the extraction of the disks used in combination with the PES membranes. For the extraction of the octanol soaked disks used in combination with the LDPE membranes, the use of acetone followed by acetone:hexane (1:1, v/v), was selected as optimum taking into consideration the extraction recoveries achieved for both the estrogens and the other compounds investigated (PBDEs and pesticides). This extraction protocol yielded 68–94% recoveries for estrogens (except for estradiol glucuronide), around 10% lower, on average, than those achieved with acetone:methanol (1:1, v/v). Estradiol glucuronide could not be recovered at all with acetone followed by acetone hexane (1:1, v/v) when octanol was present. The presence of 25% octanol in the extracts coming from the non-polar configuration was also found to affect the LC–MS/MS analysis of estrogens, increasing the quantitation limits by a factor of 4–6 to 0.34–32 ng $disk^{-1}$.

The extraction of the water samples yielded recoveries ($n = 3$, spiked at 100 ng L^{-1}) higher than 90% with RSD < 4% for all compounds except for diethylstilbestrol (66%, RSD 23%).

3.2. Passive sampler evaluation

The suitability of the different sampler configurations was evaluated by comparing the accumulation efficiency of each compound. The accumulation factor in litres (L) is defined as C_s/C_w where C_s is the amount of compound accumulated in the receiving disk (ng) and C_w its measured concentration in the water (ng L^{-1}). Several studies have shown that the uptake rate R_s (L day^{-1}) of these types of passive samplers is independent from the concentration of the analyte [35] and is linear over several days of exposure.

The accumulation experiments were performed in a flow-through system for 48 h, as described earlier, at 21 °C, 45 rpm and with a theoretical estrogen concentration in the water of ~1 μg L^{-1}. Four replicates of each configuration were prepared together with two blanks. In addition four samplers with a naked C18 Empore® disk were evaluated in order to compare the diffusion limiting capacities of the membranes.

The uptake of the non-polar (LDPE membrane) and polar (PES membrane) sampler configurations was as expected strongly reduced in comparison to the sampler with the naked C18 disk. Diethylstilbestrol accumulated 2–20 times more than the other estrogens in the naked C18 sampler, and at the same time was the only compound not detected in the polar and non-polar samplers. By analysing the membranes it was found that DES was sampled with an accumulation factor of 10 L although no diffusion to the receiving disk took place. As for the other estrogens they were sampled 10–80-fold less with the use of the LDPE membrane (estrone sulphate 1400-fold less) as compared to the naked disk. The polar sampler equipped with a PES membrane limited their uptake onto the disk by a factor of 6–14 indicating that satisfactory uptake rates are obtained with this configuration.

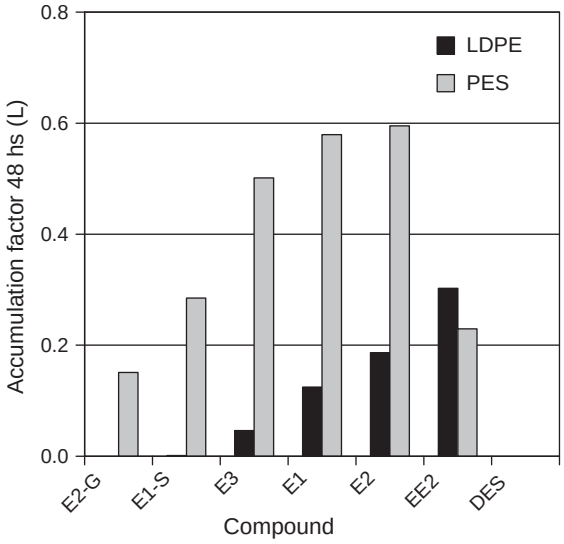

Fig. 2. Accumulation factor (C_s/C_w) in L after 48 h exposure of the sampler using a LDPE and a PES membrane. E2-G, estradiol-17-glucuronide; E1-S, estrone-3-sulphate; E3, estriol; E1, estrone; E2, 17-β-estradiol; EE2, ethinyl estradiol; DES, diethylstilbestrol.

The calculated accumulation factors of each estrogen are displayed in Fig. 2. As can be seen the non-polar sampler shows a profile of increasing accumulation with increasing $\log K_{ow}$ of the compound. This profile, typical of non-polar samplers, is well known [35] and is characterized also for a dramatic decrease of the uptake of compounds with $\log K_{ow}$ higher than 6 [14]. The uptake rates (R_s) of the estrogens by the non-polar sampler, when determined, were very low, between 0.00005 and 0.013 L day^{-1}, and would not be relevant for their analysis in environmental waters.

The polar sampler using a PES membrane, on the other hand, showed a good uptake performance of the estrogens (see Fig. 2). Accumulation factors higher than 0.4 L were obtained for the main estrogens estradiol, estrone and estriol, while ethinyl estradiol and also the two conjugated metabolites were sampled to a lesser extent.

The uptake rates R_s calculated for the estrogens were in the range of 0.077–0.304 L day^{-1}. These values are of the same order of magnitude as other R_s reported in the literature for organic contaminants using the POCIS or Chemcatcher® passive sampler device with PES membranes [13,15,19,21,36]. Fig. 3 illustrates the reported R_s and those measured in this study as a function of the $\log K_{ow}$ of the studied analytes. From this figure, a clear trend with respect to the influence of the $\log K_{ow}$ of the compound on its uptake in a polar sampler configuration cannot be derived; however, this might be due to the fact that the accumulation and calibration tests performed differ in several parameters.

The sampling rate of estrogens was shown to be in a satisfactory range, however, in order to find a relevant application for their

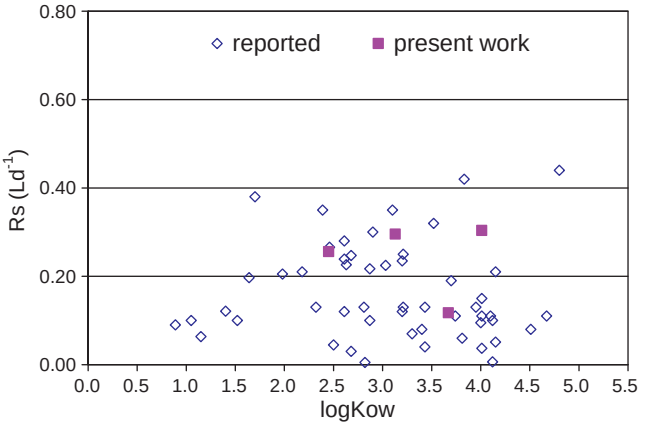

Fig. 3. Correlation of log K_{ow} with reported uptake rates R_s, using PES as diffusion membrane, from the present study (estriol, estrone, ethinyl estradiol, 17-β-estradiol) and reported in the literature [13,15,19,21,36].

Table 2
Water quality parameters of the River Anoia and Creek Rubi measured during the field trial period. Values are expressed as minimum–maximum (average).

Parameters	Anoia	Rubi
Temperature (°C)	8.8–12 (10)	12–16 (13)
pH	8.2–8.4 (8.3)	7.9–8.1 (8.0)
Dissolved oxygen (mg L^{-1})	6.6–8.7 (7.7)	3.3–6.7 (4.8)
Conductivity (μS cm^{-1})	1680–1880 (1810)	2140–2350 (2260)

analysis in environmental waters the limits of quantitation have to be also in a relevant range. Table 1 shows the limits of quantitation of the resulting time weighted average concentrations obtained for each estrogen taking into account the whole analytical procedure and a sampling time of 4, 16 and 28 days. As it can be seen from this experiment a minimum sampling time of 16 days is recommended in order to detect estrogens in the lower ng L^{-1} levels.

A field trial to assess the performance of the non-polar and polar passive samplers was conducted from the 6th to the 22nd of February 2006. The sampling sites were two irrigation channels flowing side by side in El Papiol near Barcelona (see Fig. 1). Five replicates of each sampler configuration were prepared in the laboratory together with two blanks. In addition three replicates of another polar sampler with styrenedivinylbenzene (SDB) as receiving phase and PES as diffusion limiting membrane were also exposed on the two sites.

The passive sampler devices were attached on both sides of a rectangular metal grid (30 × 50 cm, 1 cm meshes) with their uptake surface facing outwards. The metal grid was immersed vertically in line with the water flow down to 30–40 cm below water surface. The uptake surface of the samplers was therefore not perpendicular to the water flow. Moreover, spot water samples were collected at days 0, 4, 7, 11 and 16.

Table 2 presents the water parameters measured mostly daily from the SAIH and XACQA automated systems. The temperature in the Rubí Creek was on average 3 °C higher than in the Anoia River, and the dissolved oxygen concentration was lower. The conductivity was in both cases fairly high, probably due to the elevated salt content in the region as mentioned before.

As it is shown in Table 3, none of the estrogens was found in the non-polar samplers. One non-polar sampler was lost during sampling and another had lost its octanolic content. Three of the non-polar samplers had their membranes loosen during sampling and a thick biofilm was formed inside the sampler covering the receiving disk and interfering with the uptake of the compounds.

The polar samplers, however, showed almost no formation of biofilm and their membranes were good in place when sampling was finished. The analysis of the samplers revealed the accumulation of estrone, estrone sulphate and estriol in the polar configurations. The accumulated amounts are summarized in Table 3. Relative standard deviations were all below 20%, except for the estrone sulphate measured in the SDB samplers deployed in the Rubí Creek (36%) and the estriol found in the C18 samplers placed in the Anoia River (25%). The levels found in both polar samplers were fairly similar, except in the case of the estrone measured in the Rubí Creek, which was found at 8 ng $disk^{-1}$ (RSD 8%) in the C18 disks but not in the SDB disks, with no plausible explanation for this difference since the samplers were all intact and none showed a particularly high formation of biofilm.

Meanwhile, the analysis of spot water samples revealed the presence of estrone and estrone sulphate but not of estriol. Fig. 4 illustrates the results of the spot water sampling carried out in five different days over the passive sampler deployment period of 16 days together with the time weighted average concentrations (TWA) calculated from the analysis of the C18-PES polar samplers using the sampling rates derived from the laboratory accumulation experiment. In all cases, the TWA concentration is lower than the average of the concentrations measured in the spot water samples, which could be due to the fact that temperatures during the field trial were in average 8–10 °C lower than in the laboratory experiments, since according to the literature [35] the temperature has a great influence in the sampling rate. Using the average measured water concentrations and the accumulated amounts of estrone and estrone sulphate in the disks we can calculate R_s values obtained under real sampling conditions assuming that the spot sample values are representative over the whole period.

The field R_s calculated in this way for estrone sulphate in the Anoia River and in the Rubí Creek were 1.6 and 2.7 times lower, respectively, than its experimental R_s, and similarly lower values were obtained for estrone (factor 3.3 and 1.8, respectively). These differences may have their origin in the different environmental conditions existing in the studied sites as compared to the laboratory conditions, which point out the need for calibrating the passive sampling devices under different laboratory experimental conditions (temperature, turbulence, etc.), and/or may result from the scattered, not representative, concentrations measured in the spot water samples. The detection of estriol in the passive samplers but not in the spot water samples supports this latter assump-

Table 3
Estrogens (ng $sampler^{-1}$) detected in 3 passive sampler configurations after 16 days exposure in the Anoia River and Rubi Creek. Standard deviations were calculated from 5 respectively 3 replicates of the samplers with C18 or SDB receiving phase and LDPE and/or PES diffusion membrane.

	Anoia			Rubi		
	SDB–PES	C18–PES	C18–LDPE	SDB–PES	C18–PES	C18–LDPE
E1-S	1.33 ± 0.06	1.54 ± 0.09	<0.34	0.80 ± 0.29	0.65 ± 0.11	<0.34
E1	3.89 ± 0.75	4.11 ± 0.38	<4.6	<0.80	8.43 ± 0.71	<4.6
E3	3.90 ± 0.27	3.52 ± 0.89	<9.4	<3.0	<3.0	<9.4

E1-S, estrone-3-sulphate; E1, estrone; E3, estriol.

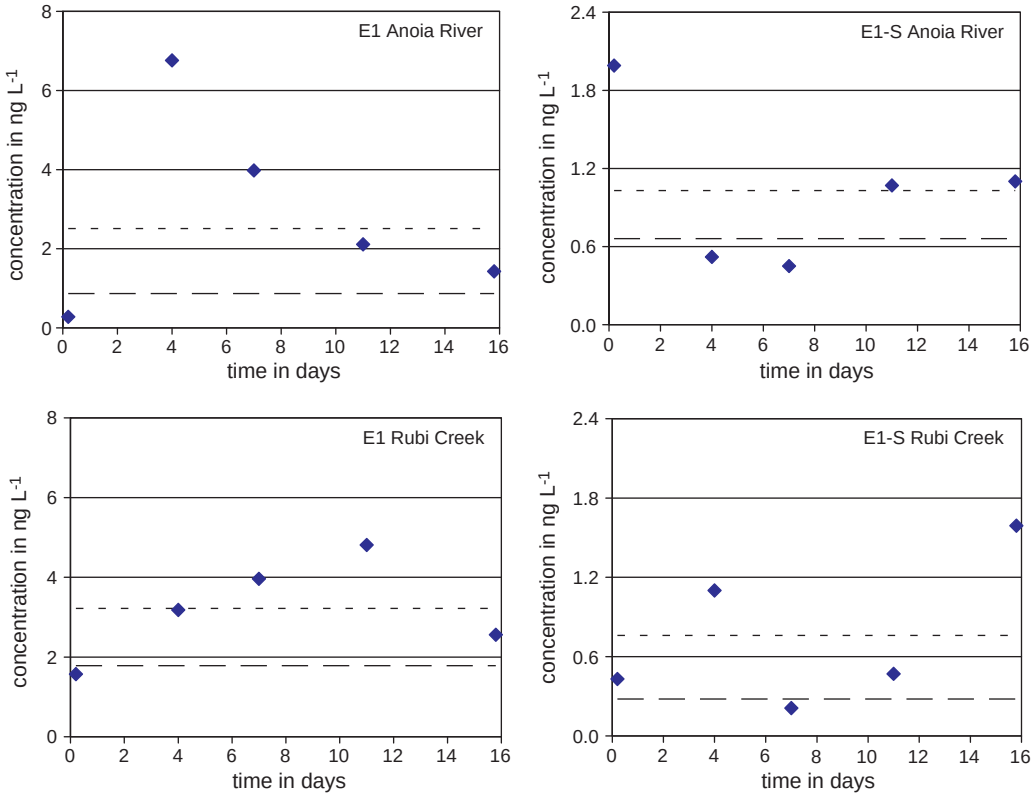

Fig. 4. Concentrations in water of estrone (E1) and estrone sulphate (E1-S) in the Anoia River and the Rubí Creek during the field trial. Values in "♦" represent levels measured in spot samples collected at days 0, 4, 7 and 16; "– – –" average of measured values; "— — —" time weighed averaged concentration calculated using the R_s determined in the 48 h accumulation experiment.

tion and demonstrates the value and interest of performing passive sampling.

4. Conclusion

The use of passive sampler devices for the analysis of polar organic contaminants is currently under investigation. Little information is available so far about their suitability for the determination of estrogens in environmental waters. The present study demonstrates that the passive sampler Chemcatcher® with a polar configuration based on the use of a C18 disk as receiving phase and PES as diffusion limiting membrane is capable of accumulating the most environmentally relevant estrogens (estradiol, estrone, estriol, ethynyl estradiol, estrone sulphate and estradiol glucuronide), and can therefore be used for the integrated sampling and analysis of these compounds in environmental waters at the low ng L^{-1} level. Uptake rates calculated for estrone and estrone sulphate in the course of a field trial where these two compounds were detected in both the passive sampling disks and the spot water samples showed slightly lower accumulation rates under field conditions than under laboratory conditions. Time weighted average concentrations in water calculated from the analysis of passive samplers were slightly lower than those calculated from the analysis of spot water samples, which can be attributed to different environmental conditions in terms of e.g. temperature, and/or turbulence, and/or to the limited information provided by spot sampling. The detection of estriol in the passive samplers but not in the spot water samples during the field trial reinforces the value of passive sampling. Further experiments to evaluate the uptake rates in different conditions (e.g. temperature, turbulence) will provide a solid fundament for achieving more reliable TWA concentrations of estrogens in water.

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