A year-long study oF the spatial occurrence and relative distribution Of pharmaceutical residues in sewage effluent, receiving MARINE waters and marine bivalves

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**Abstract**

Reports concerning the quantitative analysis of pharmaceuticals in marine ecosystems are somewhat limited. It is necessary to determine pharmaceutical fate and assess any potential **risk of exposure to aquatic species and ultimately, seafood consumers. In the work presented herein, analytical methods were optimised and validated for the quantification of pharmaceutical residues in wastewater effluent, receiving marine waters and marine mussels (*Mytilus* spp.). Selected pharmaceuticals included two non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac and mefenamic acid), an antibiotic (trimethoprim), an antiepileptic (carbamazepine) and a lipid regulator (gemfibrozil). This paper also presents the results of an *in situ* study in which caged *Mytilus* spp. were deployed at three sites on the Irish coastline over a 1-year period. In water samples, pharmaceutical residues were determined using solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The extraction of pharmaceuticals from mussel tissues used an additional pressurised liquid extraction (PLE) step prior to SPE and LC-MS/MS.** Limits of quantification between 15 and 225 ng.L-1 were achieved in wastewater effluent, between 3 and 38 ng.L-1 in marine surface water and between 4 and 29 ng.g-1 dry weight in marine mussels. Method linearity was achieved for pharmaceuticals in each matrix with correlation coefficients of R2>0.976. All five selected pharmaceuticals were quantified in wastewater effluent and marine surface waters. This work has demonstrated the susceptibility of the *Mytilus* spp. to pharmaceutical exposure following the detection of pharmaceutical residues in the tissues of this mussel species at measurable concentrations.

**Keywords:** Pharmaceuticals · Marine water · Marine bivalves · Effluent · Liquid chromatography · Mass spectrometry

****1. Introduction****

**Current knowledge on the distribution pathways and fate of pharmaceuticals in the aquatic environment is somewhat limited and has emerged as an environmental issue. Unlike other environmental contaminants, pharmaceuticals have many physicochemical and biological properties which must be taken into account when predicting or assessing their fate in the environment. Human pharmaceuticals are excreted into the sewage system as a mixture of the parent compound and metabolites, comprising mostly of either transformation products or conjugated glucuronides (**[**Heberer, 2002**](#_ENREF_19)**). These conjugates are easily cleaved during wastewater treatment, releasing the parent compound into the treated wastewater, and subsequently into the environment (**[**Jelic et al., 2011**](#_ENREF_26)**;** [**Ternes, 1998**](#_ENREF_40)**). Veterinary medicines also enter the environment, mainly via medicated fish feed and agricultural soil leaching (**[**Boxall, 2004**](#_ENREF_9)**;** [**Heberer, 2002**](#_ENREF_19)**). Although susceptible to degradation or transformation, their continuous introduction into the aquatic environment in reality confers some degree of pseudo-persistence (**[**Hernando et al., 2006**](#_ENREF_22)**). The seemingly ubiquitous presence of pharmaceuticals in the aquatic environment has been reported over the past decade or so, with over eighty pharmaceuticals and their metabolites detected at low µg.L-1 concentrations in municipal sewage effluent, surface and groundwater worldwide (**[**Fatta-Kassinos et al., 2011**](#_ENREF_16)**;** [**Heberer et al., 2000**](#_ENREF_20)**;** [**Lapworth et al., 2012**](#_ENREF_29)**;** [**Roberts and Thomas, 2006**](#_ENREF_35)**;** [**Stan and Heberer, 1997**](#_ENREF_39)**;** [**Ternes, 1998**](#_ENREF_40)**).** In order to investigate their fate, the quantitative determination of pharmaceuticals in aquatic ecosystems is necessary.

**One of the main concerns surrounding pharmaceutical release into surface waters is their potential for bioaccumulation in biota. The polar nature of most pharmaceuticals make them directly bioavailable to filter feeding organisms such as bivalves. Bivalves, such as mussels, are natural filter feeders which have been previously utilised in POP monitoring programmes because of their high bioaccumulation capacities, fixed location and high populations in marine waters (**[**Hunt and Slone, 2010**](#_ENREF_28)**;** [**Monirithet al*.*, 2003**](#_ENREF_38)**).** The uptake of pharmaceuticals has been previously observed in wild mussel species collected from the Mediterranean Sea, San Francisco Bay and the Bohai Sea in China (Bueno et al*.*, 2013; Klosterhaus et al*.*, 2013; Liet al*.*, 2012). **The use of caged sample studies allows for the measurement of exposure levels as a function of time, making it easier to assess and compare the extent of pollution between contaminated sites. With regard to pharmaceutical exposure studies using caged mussels,** recent studies carried out by Bringolf et al. (2010) and Wille et al*.* (2011) have involved the exposure of caged *Mytilus* spp. to effluent contaminated marine and freshwater surface waters. While salicylic acid measured in the high ng.g-1 range, mostly low and fluctuating concentrations were measured for other detected pharmaceuticals, including carbamazepine and fluoxetine, in exposed mussel species.

**There is comparatively larger knowledge of the fate of pharmaceuticals in the human body and during wastewater treatment processes, (**[**Debska et al., 2004**](#_ENREF_15)**;** [**Fent et al., 2006**](#_ENREF_17)**) but little research has been performed regarding pharmaceutical fate studies after effluent release into surface waters, particularly in the marine environment. Pharmaceuticals in the environment need to be quantified by means of *in situ* studies in order to assess the pharmaceutical residues present in ‘real’ environmental matrices. Pharmaceuticals are mostly polar compounds and are designed to be biologically active at low concentrations. Numerous effects on the reproduction and growth of non-target aquatic species have been observed following toxicity studies of pharmaceuticals at environmentally relevant concentrations (**[**Boxall, 2004**](#_ENREF_9)**;** [**Huerta et al., 2012**](#_ENREF_24)**;** [**Quinn et al., 2011**](#_ENREF_33)**;** [**Schmidt et al., 2011**](#_ENREF_37)**). Besides toxicity to aquatic species, trace pharmaceutical concentrations have been previously detected in drinking water in Greece and the US (**[**Benotti et al., 2009**](#_ENREF_7)**;** [**Heberer et al., 2002**](#_ENREF_21)**) and in cooked seafood (**[**McEneff et al., 2013**](#_ENREF_30)**;** [**Uno, 2002**](#_ENREF_42)**;** [**Uno et al., 2006a**](#_ENREF_43)**;** [**Uno et al., 2006b**](#_ENREF_44)**;** [**Uno et al., 2010**](#_ENREF_45)**).** **The presence of pharmaceuticals in water and seafood may potentially act as risk to the consumer either through direct effect or indirectly through potential antimicrobial resistance (**[**Cabello, 2006**](#_ENREF_13)**).** In order to study the possible environmental and human health risks posed by these contaminants at environmentally relevant concentrations, information regarding their occurrence in the aquatic environment, particularly in aquatic species, is urgently required.

**The aim of this study was to measure the occurrence and relative distribution of five pharmaceuticals in samples of wastewater effluent, marine surface water and marine mussels collected from three sites around the Irish coastline. Based on sales data for Ireland (**[**Irish Health Service Executive, 2010**](#_ENREF_2)**), the UK (**[**The National Health Scheme Information Centre of England, 2010**](#_ENREF_1)**) and previous reports of pharmaceuticals detected in Irish effluent (**[**Lacey et al., 2008**](#_ENREF_27)**), five pharmaceuticals were chosen from four different therapeutic classes: an antiepileptic (carbamazepine); two NSAIDs (diclofenac and mefenamic acid); a lipid regulator (gemfibrozil); and an antibiotic (trimethoprim). The chemical structures of these compounds and their physicochemical properties are given in Table 1. To the author’s knowledge, this study was the first to quantify a range of pharmaceuticals in marine waters and marine mussels across a 1-year period.**

**2. Materials and Methods**

2.1Reagents, chemicals and consumables

Spectranal grade acetonitrile and water and analytical grade acetone, acetonitrile, ethyl acetate and methanol were purchased from Fisher Scientific (Cheshire, UK). Dichloromethane, dichlorodimethylsilane, ammonium hydroxide solution, acetic acid and sulphuric acid were purchased from Aldrich (Gillingham, UK). Analytical grade carbamazepine (≥98 %), diclofenac sodium salt (≥98 %), gemfibrozil (≥99 %) and mefenamic acid (≥99 %) were obtained from Sigma-Aldrich (Steinheim, Germany) and trimethoprim (≥98 %) was ordered from Fluka (Buch, Switzerland). Ultra-pure water was obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA). Ottawa sand (20-30 mesh) was ordered from Fisher Scientific (Cheshire, UK) and activated, neutral aluminium oxide was ordered from Sigma-Aldrich (Steinheim, Germany).

Stock solutions (1000 mg.L-1) of individual analytes were prepared in methanol and stored in a freezer at –20 °C and in the dark, for optimum stability. Working mixed standards were prepared weekly in either methanol or, where required, in 80:20 13 mM ammonium acetate in water:acetonitrile (v:v).

2.2Sampling and experimental design

Blue mussels (*Mytilus* spp. which includes both *Mytilus edulis* and *Mytilus galloprovinicialis*) were sourced in the west of Ireland (Lettermullen, Co. Galway), from a Class A bivalve mollusc production area, designated by the Sea-Fisheries Protection Authority of Ireland under EC Regulation 854/2004. Animals chosen for this study were of the same size class (4-6 cm) and were collected in March pre-spawning. Mussels were placed on wet seaweed and transported to the laboratory in a cooler box. Shells were wiped free of debris and seaweed and the animals were depurated over 7 days in a large tank of artificial seawater (ASW) consisting of Peacock Seamix ASW (NaCl 65.5 %, MgSO4 8.25 %, MgCl2 6 %, CaCl2 3 %, KCl 1.6 %, insolubles 0.05 %, H20 15.6 %) dissolved in dechlorinated tap water to a salinity of 33 parts per thousand (ppt) at 13 °C (±1 °C). Mesh cages (1 m x 0.5 m) were constructed and divided into two sections to reduce the loss of mussels in the event of the cage tearing (images shown in Figure S1 (a) & (b) of the supplemental data). Each cage was filled with 2 x 300 mussels, which, over time, were found to attach themselves to the inside wall of the cage. Cages were deployed at a control site (CON) and two effluent exposure sites off the east (EXP1) and west (EXP2) coasts of Ireland. CON was located in a Class A defined enclosed bay, approximately 0.3 km off the west coast of Ireland. EXP1 was set up on the east coast of Ireland, approximately 100 m downstream from a wastewater effluent outfall pipe in a lower river estuary. WWTP1 is a secondary treatment facility close to EXP1 that carries out tertiary treatment (UV) during the summer months. It has a population equivalent (PE) of approximately 1.7 million and an outflow of approximately 550,000 m3.day-1. EXP2 was located in a shallow bay on the west coast of Ireland, above a wastewater effluent diffuser pipe, approximately 0.4 km south of WWTP2. WWTP2 is a secondary treatment facility with a PE of approximately 110,000 and an outflow of 49,000 m3.day-1. A year-long cage experiment was carried out from March 2011 to March 2012. Mussels (n=45) were sampled monthly alongside grab samples of surrounding marine surface waters. Samples of 24-h composite effluent samples were collected from each WWTP. Silanised amber Winchester glass bottles (2.5 L) were used to collect marine surface waters and effluent samples (glassware preparation and silanisation procedure described in S1.0 of the supplemental data). When it was not possible to use this approach such as in the case of marine surface water from EXP2, samples were first collected in a pre-washed stainless steel bucket and then transferred into a silanised, amber Winchester glass bottle. All samples were transported to the laboratory in a cooler box. Mussels were de-shelled, pooled and frozen at −80 °C. Aqueous samples were stored at 4 °C and extracted by SPE within 72-h of collection.

2.3Analytical method

The analytical method previously established for WWTP influent and effluent ([Lacey et al., 2008](#_ENREF_27)) was re-optimised for the determination of pharmaceutical residues in ASW ([McEneff et al., 2013](#_ENREF_30)) and has also been applied to effluent and marine surface water in this experiment. Briefly, samples (500 mL) were filtered, adjusted to pH 4 with dilute sulphuric acid and extracted onto Strata-X solid phase extraction (SPE) cartridges (6 mL, 200 mg, Phenomenex, Cheshire, UK). Samples were eluted with 50:50 ethyl acetate:acetone (v:v), evaporated and reconstituted in 250 µL starting mobile phase before LC-MS/MS analysis. For marine mussels, the same method was carried out with the addition of a pressurised liquid extraction (PLE) step prior to SPE. This method was previously carried out on marine mussels exposed to pharmaceuticals under *in vivo* laboratory conditions and is described in more detail ([McEneff et al., 2013](#_ENREF_30)). Prior to sample extraction, mussels were frozen at –80 °C, freeze-dried, homogenised and sieved to 125 µm. Pressurised liquid extraction (PLE) was performed on a Dionex ASE® 200 Accelerated Solvent Extractor (Dionex Corp., Sunnyvale, CA, USA). A mixture of 1 g of freeze-dried biotic sample with 10 g of Ottawa sand (20–30 mesh, Fisher Scientific, Cheshire, UK) was placed in the extraction cell on top of 20 g of aluminium oxide, with the remaining dead volume filled with sand. A combination of acetonitrile:water (3:1) was used as the extraction solvent. Extraction was carried out at 60 °C for three cycles of each 5 min. The solvent extracts (≈55 mL) were dried under nitrogen while heated to 40 °C with a Turbovap LV to a final volume less than 10 mL. Samples were further diluted to 200 mL with ultra-pure water, before undergoing SPE and reconstitution, as described above, before LC-MS/MS analysis.

The Agilent 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) consisted of a binary pump, autosampler, UV detector and Waters Sunfire C18 column (3.5 µm, 2.1 x 150 mm, Waters Corp., MA, USA) and guard column (3.5 µm, 2.1 mm x 10 mm, Waters Corp., MA, USA). Operating conditions carried out for liquid chromatography are shown in Table 2. A Bruker Daltonics HCT ion trap mass spectrometer equipped with an atmospheric pressure interface-electrospray ionisation (API-ESI) source was used for pharmaceutical identification and quantification. Mass spectrometric analysis was carried out in selected reaction monitoring (SRM) mode, measuring the fragmentation of the ions produced from each analyte. Fragmentation amplitudes were set at 0.5 and 0.6 in negative and positive ESI modes, respectively. At least two transitions were selected for the confirmation of each analyte with the most intense fragment ion selected for analyte quantification (Table 3). Ionisation and mass spectrometric conditions were optimised by direct infusion using a syringe pump (Cole-Parmer 74900 Series) to deliver 300 μL.h-1 of analyte solution and parameter settings are summarised in Table S 1 of the supplemental data.

2.4 Quality control

Calibration curves (n=12) were constructed in each matrix in triplicate to assess method performance for both effluent and marine surface water. Samples (500 mL, n=12 in triplicate) were spiked with the selected pharmaceuticals at concentrations from 5 to 5000 ng.L-1. Signal to noise (S/N) ratios are a measure of the signal strength relative to the background noise. Limits of detection (LODs) (S/N ratio=3:1) and limits of quantification (LOQs) (S/N ratio=10:1) were calculated for each pharmaceutical using samples spiked at three low-level concentrations and prepared in triplicate (n=9). Precision, reproducibility and recovery studies were carried out by spiking sample matrices (n=6) pre-extraction to concentrations of 0.5 µg.L-1 for effluent and marine surface water. Recovery was calculated by comparing spiked extracts to final extracts of the unspiked matrix (n=3) reconstituted in 250 µL of mobile phase A containing the expected 100 % recovery concentration. Sample extracts spiked post extraction were compared to a 1 mg.L-1 analyte mixture prepared in mobile phase A to measure the extent of ion suppression or enhancement in ESI-MS. Extracted ion chromatograms of pharmaceuticals spiked at 1 µg.L-1 in effluent and marine surface waters are shown in Figures S2 and S3, respectively, of the supplemental data. Method performance for marine mussels was previously discussed ([McEneff et al., 2013](#_ENREF_30)) and extracted ion chromatograms for pharmaceuticals spiked at 100 ng.g-1 in marine mussels are available in Figure S4 of the supplemental data.

Blank effluent and marine surface water samples contained low levels of some of the target analytes (pharmaceutical concentrations shown in Table S2 of the supplemental data). Calibration curves were corrected for these concentrations. None of the target analytes were detected in solvents, reagents, ultra-pure water or marine mussel tissue (sampled from Lettermullan, Co. Galway) used for these validation studies. All sample and standard measurements were carried out in triplicate unless otherwise stated.

**3. Results and Discussion**

3.1Analytical method

LC-MS/MS was the method of analysis chosen for this study due to the limited volatility and polar nature of pharmaceuticals. In negative mode ESI-MS, [M–H]− precursor ions were produced for diclofenac, gemfibrozil and mefenamic acid and [M+H]+ precursor ions were produced for carbamazepine and trimethoprim in positive mode ESI-MS. SRM was carried out under both positive and negative ESI modes, yielding reliable MS/MS data for the confirmation and quantification of all observed analytes (Table 3). A selection of EICs of each pharmaceutical, occurring at their highest concentrations in either effluent, marine surface waters or marine mussels, are shown in Figure 1.

3.2 Quality control

Method performance for effluent and marine surface water was improved with minor adjustments of the SPE eluting solvent, LC run time, mobile phase composition and gradient conditions. Instrumental precision measured ≤1.3 % for retention time of all analytes in each matrix. Correlation coefficients >0.99 (n≥8), with the exception of mefenamic acid (R2≈0.98), were obtained in both matrices over the ranges of 0.025 to 5 µg.L-1 using negative ESI-MS/MS mode and 0.005 to 2.5 µg.L-1 using positive ESI-MS/MS mode on the LC-MS/MS instrument. LOQs were as low as 15 to 225 ng.L-1 and 3 to 38 ng.L-1 in effluent and marine surface water, respectively. Due to the complexity and high salt content of marine surface water, pharmaceutical detection in this matrix is difficult and has not been reported to a wide extent. Larger SPE washing volumes were previously used to achieve higher rates of recovery in marine surface waters but, after testing greater than 6 mL volumes, no increase in recovery was observed. Method performance results correlated with previous data reported for the analysis of 13 pharmaceuticals in natural sea water ([Wille et al., 2010](#_ENREF_47)). Recoveries of analytes ranged from 62 to 99 % in effluent and 56 to 110 % in marine surface water for n=6 replicates of a 1 µg.L-1 standard mix (RSD <11 %) (Table 3). Signal suppression was observed for all analytes in marine surface water and for all analytes except gemfibrozil in effluent. The signal enhancement of gemfibrozil in effluent is in stark contrast to the signal suppression of gemfibrozil in marine surface water. At 56 %, gemfibrozil underwent the highest matrix induced suppression out of the five pharmaceuticals in marine surface water but, signal was not greatly reduced as recoveries >100 % were achieved. Most noticeable in the effluent matrix was the signal suppression observed for the pharmaceuticals in positive ESI-MS mode, i.e. trimethoprim and carbamazepine, at 63-65 %. In contrast, these compounds were the least suppressed analytes in the marine surface water matrix. The high salt content of marine surface waters suggests that salt residues may still be present in the sample extract and co-eluting with the selected analytes. Method performance results for marine mussels have been previously discussed ([McEneff et al., 2013](#_ENREF_30)). All validation results for individual pharmaceuticals in effluent, marine surface water and marine mussel tissue are shown in Table 3.

In order to minimise the matrix effect on the quantification of ions using external calibration, standard addition (n=4, unless otherwise stated) was performed in effluent and marine surface water samples. However, for the quantification of pharmaceuticals in marine mussels, external calibration curves were prepared in matrix-matched samples to avoid the high cost of stable isotope-labelled internal standards and the lengthy processing times for larger amounts of samples. For quality control of the method, an injection of starting mobile phase was run between each sample with no carry over observed.

3.2 Application to sewage effluent, marine surface waters and caged *Mytilus* spp. exposed along the Irish coast

The developed methods were applied to 24-h composite effluent samples (WWTP1 and WWTP2), marine surface water samples and marine mussel samples (CON, EXP1 and EXP2). Samples from CON, EXP1 and WWTP1 were collected for twelve months. Due to rough sea weather and the consequential loss of a cage, a shorter exposure was carried out at EXP2, where marine mussels were sampled for three months and effluent and marine surface water samples were collected from WWTP2 and EXP2 for four months.

*3.2.1 Sewage effluent*

As can be deduced from Tables 4 and 5, pharmaceutical residues were quantified in sewage effluent entering Irish surface waters. All five of the selected pharmaceuticals were detected in >85 % of effluent samples collected from each exposure site. Effluent discharged from WWTP2 was found to contain the highest concentrations of pharmaceutical residues, except for carbamazepine, with >1 µg.L-1 measured for at least one sampling point over the four month period. Diclofenac and mefenamic acid were measured at environmentally relevant concentrations of 2.63 µg.L-1 and 2.80 µg.L-1, respectively, from WWTP2 and carbamazepine measured at concentrations up to 3.16 µg.L-1 from WWTP1. In comparison, WWTP1 caters for fifteen-fold more people than WWTP2 and pharmaceutical concentrations detected over the same four months (May to August 2011) were found to be slightly lower. This may be as a result of the tertiary treatment carried out on the wastewater at WWTP1 from May to September. UV treatment has been shown to partially remove some antibiotics in drinking water but this is at a UV dose approximately 100 times greater than that typically supplied for effluent disinfection ([Adams et al., 2002](#_ENREF_3)) therefore, pharmaceutical residues in UV treated effluent may be slightly reduced but not completely eliminated. When compared to the concentrations of pharmaceutical residues present in the effluent throughout the year, no obvious reduction in pharmaceutical concentrations was observed for UV treated effluent collected from WWTP1.

 In comparison to pharmaceutical concentrations detected in effluent from WWTPs worldwide, slightly higher levels were reported in the effluent from the selected Irish WWTPs in this study. In a previous study carried out by Lacey et al. (2012), the occurrence of pharmaceuticals in influent and effluent from three WWTPs in Ireland was reported at overall higher levels than those reported in this study. From August 2007 to July 2008, the presence of diclofenac, mefenamic acid, trimethoprim and carbamazepine was reported in the effluent from WWTP1 at concentrations up to 0.5 µg.L-1, 9.1 µg.L-1, 0.6 µg.L-1 and 6.5 µg.L-1, respectively. Gemfibrozil was detected at 0.1 µg.L-1 in the influent but was not present in the treated effluent. In comparison, this study reports concentrations of the same compounds at much lower concentrations particularly in the cases of mefenamic acid and carbamazepine, and maximum concentrations measured for diclofenac and trimethoprim measuring slightly higher with a difference of 1.2 µg.L-1 between maximum values from both studies. Gemfibrozil measured <LOD-0.65 µg.L-1 in effluent from WWTP1 throughout the study. Residues of diclofenac and mefenamic acid measured higher in the effluent from WWTP2 at concentrations up to 2.6 µg.L-1 and 2.8 µg.L-1, respectively. WWTP2 is an activated sludge treatment facility with no tertiary treatment similar to the other WWTPs selected in the study carried out by Lacey et al. (2012). Although population equivalents of the other Irish WWTPs were approximately half that of WWTP2, diclofenac residues were recorded in their effluent at concentrations up to 3 µg.L-1.

Removal efficiencies of WWTPs are dependent on the treatment technology in place, wastewater retention time in each phase of treatment, solid retention time (SRT) and weather conditions such as rainfall (Vienoet al*.*, 2007). Pharmaceutical concentrations in the influent and effluent from WWTP1 were previously compared by Lacey et al. (2008). All of the selected pharmaceuticals in this study, except for trimethoprim, were previously measured at higher concentrations in the effluent from WWTP1 than concentrations measured in the influent. This suggests cleavage of conjugated metabolites to the parent compound, as previously reported by Ternes et al*.* (1999). Although no SRT data was available, it may be recommended for the selected WWTPs to extend the length of the SRT during activated sludge treatment as it has been proved that longer SRTs can greatly improve the removal efficiencies of pharmaceuticals (Claraet al., 2005; Lishmanet al., 2006).

*3.2.2 Marine surface water*

The pseudo-persistence of pharmaceuticals in the aquatic environment was observed with the continuous detection of pharmaceutical residues in marine surface waters, at slightly lower concentrations than those detected in effluent. The antiepileptic, carbamazepine, had the highest concentration in marine surface waters, detected at 1.41 µg.L-1 at EXP1. Trimethoprim was repeatedly detected at both exposures with much higher concentrations of up to 0.87 µg.L-1 detected at EXP2. Residues of diclofenac and mefenamic acid varied in their concentrations each month but measured highest at EXP2 with concentrations of 0.55 µg.L-1 and 0.61 µg.L-1, respectively. The lipid regulator, gemfibrozil, was the most infrequently detected pharmaceutical in marine surface water but was measured at concentrations of up to 0.64 µg.L-1 at EXP2. The highest pharmaceutical concentrations in marine surface waters were detected at EXP2, with the exception of carbamazepine. None of the selected analytes were detected in marine surface water samples from the CON site. A selection of monitoring studies have been carried out previously in European estuarine and marine surface waters. In a study carried out by Thomas and Hilton (2004), 14 pharmaceuticals were monitored in British estuaries of the Thames, the Tyne, the Mersey, the Tees and Belfast Lough. From the targeted list, 9 pharmaceuticals were detected in the estuarine water samples collected. Ibuprofen and trimethoprim were detected at the highest concentrations, measuring at 928 ng.L-1 and 569 ng.L-1, respectively, and clotrimazole was the most frequently detected pharmaceutical with a median concentration of 7 ng.L-1. A number of other studies have analysed marine water samples collected from the North Sea, its estuaries and harbours (Buseret al*.*, 1998; Langford and Thomas, 2011; Weigelet al*.*, 2002; Weigelet al., 2004). Eight pharmaceuticals were previously detected in Belgian coastal waters, with concentrations of salicylic acid and carbamazepine measured most frequently at concentrations up to 860 ng.L-1 (Wille et al*.*, 2010). Monitoring studies in the Mediterranean Sea revealed the presence of eight pharmaceuticals, including verapamil, atenolol and metolol in the low ng.L-1 range. In comparison to previous mornitoring studies, pharmaceutical residues measured in marine surface waters from EXP1 and EXP2 are similar to those previously detected in British and Belgian estuaries but, overall, rank higher than the average pharmaceutical concentrations detected in other European marine surface waters.

 The presence of high pharmaceutical concentrations in Irish WWTP effluent is the reason for the higher than average pharmaceutical concentrations measured in Irish marine surface waters. The cumulative pharmaceutical concentrations in effluent and marine surface water from both EXP1 and EXP2 exposure sites were determined and shown in Figures 2 and 3, respectively. The flow rate of the effluent through the WWTP reveals a direct impact on the cumulative pharmaceutical concentrations in marine surface waters with higher concentrations measured with higher flow rates. Insufficient data does not allow for the determination of the dilution factor at each sampling site however, as observed from the results in Tables 4 and 5, the short distances of the sites (outlined in Section 2.2) from the effluent outfall pipe did not allow for dilution of the pharmaceutical concentrations to negligible levels. Besides short sampling distances from the outfall pipe, other factors which may also explain the high pharmaceutical levels in marine surface waters, include the restriction of access to sampling sites at high tide, requiring sample collection to be carried out at low tide, and the continuous flow of effluent from the outfall pipe, regardless of tidal changes.

In some cases, pharmaceutical levels in marine surface waters measured greater than those in the WWTP effluent. Pharmaceutical measurements are based on one sample of effluent and marine surface water collected from each site over a period of 1-2 days each month, depending on weather conditions. The pharmaceutical concentrations in the effluent should not be taken as the exact same concentrations exposed to the marine surface water sampled during the same month. Higher loads of certain pharmaceuticals may occur over time depending on human consumption.

*3.2.3 Marine mussels*

The accumulation of a large number of pharmaceuticals in solid media such as sludge was the focus of recent work in our laboratory. These studies support some of the findings shown here for pharmaceutical occurrence in wastewaters and their potential for uptake in mussels ([Barron et al., 2009](#_ENREF_4); [Barron et al., 2010](#_ENREF_5); [Barron et al., 2008](#_ENREF_6)). Besides the physicochemical properties of pharmaceutical compounds, the potential for pharmaceuticals to bioconcentrate in aquatic biota also depends on several biological factors such as age, species, diet, habitat and reproductive cycle ([Meredith-Williams et al., 2012](#_ENREF_31)). Bioconcentration of pharmaceuticals in biological tissue may be possible due to a small portion of unionized species of the compounds, remaining in the aqueous phase, which has a greater affinity for lipophilic matter. With a pKa of 6.6, trimethoprim is the only pharmaceutical of those selected not to be completely ionised in marine surface water at pH 8.0. This may be the reason why a very low but constant concentration of trimethoprim was detected in exposed mussel tissue. Residues of trimethoprim in the *Mytilus* spp. from EXP1 were quantified at concentrations of 9.22 ng.g-1 and 7.28 ng.g-1 for the respective months of October and November, during which time tertiary treatment was not carried out on the wastewater. Carbamazepine and mefenamic acid were also detected in mussel tissues from both exposure sites but at <LOQ levels. Although mefenamic acid and carbamazepine have slightly higher LOQ values, their concentrations in surrounding marine waters were on average slightly higher than those detected for trimethoprim and thus, their presence in mussel tissues may be as a result of the uptake of residual levels, although this may also be the case for trimethoprim. It is important to note that the mussels were not depurated before analysis, therefore, remains in the mussel digestive tract were included in the measurements. Selected pharmaceuticals had a broad range of temporal variability and particularly in the cases of gemfibrozil and mefenamic acid, residues dropped below detectable levels in marine surface waters possibly due to the higher salt content causing these pharmaceuticals to become less soluble, and ultimately, ‘salt out’ of solution. None of the selected analytes were detected in marine mussel samples collected from the CON site.

In similar exposure **studies using caged mussels,** a recent study deployed five cages of blue mussels off the Belgian coast. Over a six-month period, five pharmaceuticals were detected in mussel tissues including salicylic acid residues, measuring up to 490 ng.g-1 dry weight([Wille et al*.*, 2011](#_ENREF_46)). While salicylic acid measured in the mid ng.g-1 range, mostly low and fluctuating concentrations were measured for all other pharmaceuticals including carbamazepine, ofloxacin, propranolol and paracetemol. In a separate study in North Carolina, the antidepressant, fluoxetine, was detected in caged mussel tissues up to 79 ng.g-1 wet weight after 14 days exposure in a wastewater effluent channel. Fluoxetine was also detected in mussels caged approximately 100 m downstream from the effluent outfall pipe at concentrations of 9.8 ng.g-1 wet weight where residues in surrounding waters measured up to 7.3 ng.L-1 ([Bringolf et al., 2010](#_ENREF_10)). **The presence of estrogens in Irish seawater and their potential for uptake in marine mussels was only recently investigated. Although low ng.L-1 levels of estrone were detected in marine surface waters, no uptake was determined in exposed mussel tissues due to high levels of interference occurring from the co-extraction of unwanted compounds (**[**Ronan and McHugh, 2013**](#_ENREF_36)**). Most recently, wild marine mussels were collected from several diversely impacted areas in the Mediterranean Sea with traces of carbamazepine measuring up to 3.5 ng.g-1 dry weight in mussels residing from wastewater exposed waters (Bueno et al*.*, 2013). Similarly, wild ribbed horse mussels were collected from five near shore sites in San Francisco Bay and analysed for up to 104 PPCPs.** Carbamazepine and gemfibrozil were detected in the surrounding marine surface waters at concentrations measuring between 38-92 ng.L-1 but, mussels collected from the same sites were found to contain only very low residues of carbamazepine and sertraline at concentrations up to 2.4 ng.g-1 and 0.3 ng.g-1 wet weight, respectively (Klosterhauset al., 2013). Uptake of pharmaceuticals has been previously confirmed in other wild aquatic species such as fish ([Brooks et al., 2005](#_ENREF_11); [Fick et al., 2010](#_ENREF_18); [Huerta et al., 2013](#_ENREF_24); [Togunde et al., 2012](#_ENREF_41)) with glucuronised parent compounds measuring at higher concentrations to the unmetabolised compounds in one particular study ([Lahti et al., 2011](#_ENREF_28)). The cost and limited availability of glucuronised pharmaceutical compounds did not allow for further investigation into the bioaccumulation of metabolites within the mussel.

*3.2.4 Risk assessment of pharmaceutical compounds*

The requirement for a detailed risk assessment of pharmaceuticals is a priority to ensure there are no major risks to the environment and human health. The risks for the aquatic environment, associated with the measured water concentrations of the targeted pharmaceuticals within this study, cannot be fully assessed as there are currently no regulatory guidelines established for widely used and widespread occurring pharmaceuticals. Only recently, diclofenac has been added to a new ‘watch list’ included in the EU Water Framework Directive 2013/39/EU and may be added to the priority list at a later date following further monitoring and toxicity studies. Due to insufficient data, no maximum allowable concentration for diclofenac in surface waters or biota has been set. In this case, annual average values for surface waters are used as the maximum limits, hence, a value of 0.01 µg.L-1 was set for diclofenac in marine surface waters. This set limit is approximately 30 times lower than the average concentrations of diclofenac detected in Irish marine surface waters over a 1-year period, indicating pollution and high risk to the Irish aquatic environment. Diclofenac is the only pharmaceutical, excluding hormones, to be listed under EU regulations. However, there are numerous other pharmaceuticals present at high concentrations in marine surface waters which are also known to cause chronic effects in aquatic organisms at low doses such as carbamazepine and gemfibrozil ([Quinnet al*.*, 2004](#_ENREF_14); Quinn et al., [2011](#_ENREF_15); [Schmidtet al., 2011](#_ENREF_17); Schmidt et al., [2013](#_ENREF_18)).

The European Medicines Evaluation Agency (EMEA) is the regulatory body responsible for carrying out environmental risk assessments on pharmaceuticals prior to their licensing in European countries. If predicted environmental concentrations (PECs) are >0.01 µg.L-1, risk is then determined by the ratio of PEC/predicted no effect concentration (PNEC), where PNEC values are based on results from acute toxicity studies. In order to perform a more comprehensive risk assessment, available information on the chronic toxic effects of the selected pharmaceuticals i.e. lowest observed effect concentration (LOEC), were used instead. The pharmaceutical monitoring data produced for carbamazepine from this study highlights a risk to carp in the selected Irish coastal zones. The ratio determined for diclofenac measured just less than 1 for rainbow trout highlighting potential risk for chronic effects (Triebskorn et al., 2007).

 It may be suggested that the low levels of pharmaceutical residues detected in exposed marine mussels to date are so low that they pose no environmental risk or toxicological threat. However, in this study, selected pharmaceuticals were continuously detected in surrounding marine surface waters at average concentrations in the mid to high ng.g-1 range, except for gemfibrozil which was detected sporadically. Previous chronic exposure studies have tested environmentally relevant concentrations of pharmaceuticals on the *Mytilus* spp. and other aquatic organisms with potentially lethal effects observed, such as increased oxidative stress, lesions on vital organs and reduced enzyme function in cells ([Contardo-Jaraet al*.*, 2011](#_ENREF_6); [Cuklev et al., 2011](#_ENREF_8); [Lajeunesseet al., 2011](#_ENREF_11); [Mehinto et al., 2010](#_ENREF_13); [Quinnet al*.*, 2004](#_ENREF_14); Quinn et al., [2011](#_ENREF_15); [Schmidt et al., 2011](#_ENREF_17); Schmidt et al*.*, [2013](#_ENREF_18); [Schwaigeret al., 2004](#_ENREF_19)). Due to the ability of these compounds to readily metabolise, there is a need to combine both chemical and biological analysis particularly for the assessment of pharmaceutical uptake in naturally exposed aquatic organisms, as seen in this case where low uptake does not necessarily indicate low exposure or risk.

Wild mussels were observed at EXP1 and a considerable number of crab and oyster pots were observed in close proximity to the effluent outfall pipe at EXP2. The presence of pharmaceuticals in the caged mussels suggests a possibility for pharmaceutical uptake in wild and farmed species with potential exposure to humans via ingestion. The measurement of pharmaceuticals in aquatic species that are important in terms of human consumption, such as mussels, is very useful in estimating the human exposure and dietary intake of these pharmaceuticals. It was observed that the European maximum residue limit (MRL) for trimethoprim in all food producing species (50 ng.g-1) was not exceeded in marine mussels collected from one of the most highly contaminated sites in Ireland, deeming it safe for human consumption (European Commission Regulation No. 37/2010, 2009). A recent study carried out by McEneff et al*.* (2013) investigated the effect of cooking on pharmaceutical residues in exposed marine mussels. Trimethoprim was the only pharmaceutical to be reduced after cooking, hence, marine mussels with MRL values acceptable before cooking should still be acceptable for human consumption after cooking. It is suggested there is a very low risk of pharmaceutical exposure to humans via dietary ingestion of marine mussels.

4. Conclusion

For the first time, the spatial occurrence of five targeted pharmaceuticals in the aquatic environment was monitored over a 12 month period. Analytical techniques such as PLE, SPE and LC-MS/MS were combined, optimised and applied to wastewater effluent, marine surface water and *Mytilus* spp. samples collected from two impacted sites and a control site on the Irish coastline. The presence of all five targeted pharmaceuticals was confirmed in the low ug.L-1 in effluent and in the high ng.L-1 in exposed marine surface water with residues of carbamazepine and trimethoprim measuring highest in marine surface water collected at the exposure sites. Three of the five detected pharmaceuticals in marine surface waters were also found to occur in exposed *Mytilus* spp., with residues of trimethoprim measuring at concentrations up to 9.22 ng.g-1 dry weight. This study has confirmed the uptake of pharmaceuticals in marine bivalves at measurable quantities and also highlights the inability of mussels to act as reliable bioindicators of pharmaceutical pollution due to temporal variations observed in the data. These findings will contribute to pharmaceutical fate studies and aid in the assessment of ecological and health risks posed by these contaminants. This research has highlighted the need for further research into the transformation and fate of pharmaceuticals in the aquatic environment.

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**Figures and Tables**

**Table 1** Chemical structure, class and physicochemical properties ([Bones et al., 2006](#_ENREF_8); [Brown et al., 2007](#_ENREF_12)) of selected pharmaceutical compounds

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Trimethoprim | Diclofenac | Carbamazepine | Mefenamic acid | Gemfibrozil |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
| *Antibiotic* | *Anti-inflammatory* | *Antiepileptic* | *Anti-inflammatory* | *Lipid regulator* |
| CAS No. 738-70-5 | CAS No. 15307-79-6 | CAS No. 298-46-4 | CAS No. 61-68-7 | CAS No. 25812-30-0 |
| Mr 290.32 | Mr 296.15 | Mr 236.27 | Mr 250.34 | Mr 241.28 |
| p*K*a 6.60 | p*K*a 4.15 | p*K*a 13.90 | p*K*a 4.20 | p*K*a 4.80 |
| Log*K*ow 0.65 | Log*K*ow 3.91 | Log*K*ow 2.30 | Log*K*ow 4.16 | Log*K*ow 3.56 |

**Table 2** Liquid chromatography operating conditions

|  |
| --- |
| Liquid chromatography conditions |
| Column | Waters Sunfire 150 x 2.1 mm,3.5 µm particle size |  |
| Flow rate | 300 µl min-1 |  |
| Injection volume | 10 uL |  |
| Mobile phase | A | 80 % 13 mM ammonium acetate (aq.),20 % acetonitrile |
|  | B | 100 % acetonitrile |
| Gradient | 0-2 min | 0 % B |
|  | 2-3 min | 50 % B |
|  | 3-9 min | 65 % B |
|  | 9-10 min | 100 % B |
|  | 10-14 min | 100 % B |
|  | 14-15 min | 0 % B |
| Re-equilibration time | 15 min |  |



**Fig. 1** Extracted ion chromatograms of the most concentrated occurrence of pharmaceuticals in samples of effluent, marine surface water and marine mussels collected from exposure sites over one year

Diclofenac

Marine surface water

EXP2, MO2

*m/z transition: 294→250*

Trimethoprim

Mussel tissue

EXP1, MO6

*m/z transition: 291→123*

Carbamazepine

Effluent

WWTP1, MO7

*m/z transition: 237→194*

Gemfibrozil

Effluent

WWTP2, MO3

*m/z transition: 249→121*

Mefenamic acid

Marine surface water

EXP2, MO2

*m/z transition: 240→196*

**Table 3** SRM transitions of quantification ions (underlined) and confirmation ions, limits of quantification (LOQs), regression coefficients and recoveries of selected pharmaceuticals in effluent, marine surface water and marine mussel samples

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compound |  |  | Effluent |  | Marine surface water |  | Marine mussels |
|  | tr \* (min) | SRMtransitions | LOQ (ng.L-1) n=9a | *R2*n≥24b | Recovery(%)n=6 |  | LOQ (ng.L-1)n=9a | *R2*n≥24b | Recovery(%)n=6 |  | LOQ (ng.g-1)n=9a | *R2*n≥24b | Recovery(%)n=6 |
| Trimethoprim | 5.9 | 291→123 (+)291→230 (+) | 49 | 0.993 | 88±6 |  | 3 | 0.987 | 56±4 |  | 4 | 0.985 | 91±9 |
| Diclofenac | 8.1 | 294→250 (−)294→236 (−) | 225 | 0.991 | 70±9 |  | 22 | 0.995 | 110±11 |  | 29 | 0.990 | 83±8 |
| Carbamazepine | 8.2 | 237→194 (+)237→192 (+) | 15 | 0.995 | 99±7 |  | 4 | 0.995 | 86±5 |  | 6 | 0.987 | 100±5 |
| Mefenamic acid | 9.0 | 240→196 (−)240→223 (−) | 219 | 0.976 | 62±8 |  | 29 | 0.977 | 91±7 |  | 23 | 0.990 | 104±12 |
| Gemfibrozil | 11.5 | 249→121 (−)249→127 (−) | 35 | 0.995 | 66±11 |  | 38 | 0.995 | 108±5 |  | 18 | 0.993 | 100±20 |

\*Average time recorded for each pharmaceutical in marine mussel tissues

a Three datapoints carried out in triplicate

b Eight datapoints carried out in triplicate

**Table 4** Detected concentrations of pharmaceuticals (CBZ=carbamazepine, DCF=diclofenac, GEM=gemfibrozil, MFA=mefenamic acid, TRM=trimethoprim) present in effluent (µg.L-1), marine surface water (MSW) (µg.L-1) and mussels (ng.g-1 dry weight) sampled from WWTP1 and EXP1 from May 2011 to April 2012

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| EXP1 | May 2011 | Jun 2011 | Jul 2011 | Aug 2011 |
|  | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* |
| TRM | 0.07±0.11 | 0.20±0.06 | < 4\* | 1.19±0.16 | 0.56±0.06 | < 4\* | 0.49±0.13 | 0.24±0.08 | < 4\* | 0.65±0.07 | 0.57±0.02 | < 4\* |
| DCF | 0.75±0.06 | 0.11±0.11 | n.d. | 1.22±0.30 | 0.46±0.05 | n.d. | 0.84±0.20 | n.d. | n.d. | 1.52±0.15 | 0.06±0.05*a* | n.d. |
| CBZ | 0.94±0.13 | 0.18±0.18 | n.d. | 0.86±0.07 | 0.31±0.06 | n.d. | 1.91±0.08 | 0.51±0.03 | < 6\* | 2.37±0.14 | 0.64±0.09 | < 6\* |
| MFA | 1.25±0.11 | n.d. | n.d. | 0.54±0.10 | 0.08±0.08 | n.d. | 0.73±0.12 | n.d. | n.d. | 0.42±0.18 | 0.14±0.01*a* | < 23\* |
| GEM | 0.57±0.15 | n.d. | n.d. | 0.45±0.14 | 0.29±0.04 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
|  | Sep 2011 | Oct 2011 | Nov 2011 | Dec 2011 |
|  | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* |
| TRM | 0.59±0.19 | 0.35±0.08 | < 4\* | 0.85±0.05 | 0.16±0.07 | 9.22±1.54 | 0.34±0.05 | 0.29±0.10 | 7.28±1.70 | 1.08±0.21 | 0.35±0.04 | < 4\* |
| DCF | 0.51±0.04 | 0.30±0.01 | n.d. | 0.88±0.13 | 0.39±0.03 | n.d. | 1.22±0.16 | 0.46±0.12 | n.d. | 0.31±0.07 | 0.26±0.02 | n.d. |
| CBZ | 2.33±0.19 | 1.30±0.20 | < 6\* | 2.49±0.13 | 1.41±0.15 | n.d. | 3.16±0.32 | 0.97±0.19 | n.d. | 1.86±0.16 | 0.88±0.13 | < 6\* |
| MFA | 0.49±0.04 | 0.13±0.11*a* | < 23\* | 0.46±0.04 | 0.31±0.11 | < 23\* | 1.07±0.22 | 0.11±0.11 | n.d. | 0.42±0.11 | 0.27±0.05 | n.d. |
| GEM | 0.10±0.09 | n.d. | n.d. | 0.16±0.07 | n.d. | n.d. | 0.31±0.05 | n.d. | n.d. | 0.09±0.13 | 0.11±0.09 | n.d. |
|  | Jan 2012 | Feb 2012 | Mar 2012 | Apr 2012 |
|  | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* |
| TRM | 1.20±0.10 | 0.21±0.02 | < 4\* | 0.65±0.07 | 0.07±0.03 | < 4\* | 0.60±0.16 | 0.52±0.06 | < 4\* | 0.06±0.11 | 0.21±0.05 | < 4\* |
| DCF | 0.60±0.17 | 0.25±0.08 | n.d. | 0.99±0.15 | 0.37±0.12 | n.d. | 1.40±0.17 | 0.38±0.03 | n.d. | 1.69±0.20 | 0.42±0.13 | n.d. |
| CBZ | 0.62±0.09 | 0.52±0.11 | < 6\* | 0.51±0.10 | 0.58±0.12 | < 6\* | 0.76±0.16 | 0.47±0.05 | < 6\* | 0.99±0.05 | 0.36±0.09 | < 6\* |
| MFA | 1.50±0.19 | 0.37±0.05 | < 23\* | 0.80±0.05 | 0.32±0.09 | < 23\* | 1.52±0.26 | 0.48±0.09 | n.d. | 1.55±0.31 | 0.41±0.07 | < 23\* |
| GEM | 0.36±0.04 | 0.24±0.10 | n.d. | 0.65±0.15 | 0.37±0.10 | n.d. | 0.43±0.11 | 0.14±0.15 | n.d. | 0.53±0.07 | 0.40±0.07 | n.d. |

n.d. not detected

*a* Quantification carried out using a three point standard addition plot

\*Pharmaceutical residues detected at concentrations lower than LOQ value

**Table 5** Detected concentrations of pharmaceuticals (CBZ=carbamazepine, DCF=diclofenac, GEM=gemfibrozil, MFA=mefenamic acid, TRM=trimethoprim) present in effluent (µg.L-1), marine surface water (MSW) (µg.L-1) and mussels (ng.g-1 dry weight) sampled from WWTP2 and EXP2 from May 2011 to August 2011

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| EXP2 | May 2011 | Jun 2011 | Jul 2011 | Aug 2011 |
|  | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* | *Mussels* | *Effluent* | *MSW* |
| TRM | 0.95±0.25 | 0.76±0.10 | < 4\* | 1.17±0.26 | 0.80±0.14 | < 4\* | 0.50±0.08 | 0.74±0.11 | < 4\* | 1.03±0.14 | 0.87±0.14 |
| DCF | 2.63±0.19 | 0.22±0.19 | n.d. | 1.61±0.04 | 0.55±0.05 | n.d. | 0.45±0.16 | n.d. | n.d. | 1.01±0.14 | 0.24±0.01 |
| CBZ | 0.57±0.16 | 0.08±0.04 | n.d. | 0.40±0.19 | 0.05±0.12 | n.d. | 0.27±0.06 | 0.16±0.10 | n.d. | 1.71±0.21 | 0.40±0.10 |
| MFA | 2.42±0.11 | n.d. | < 23\* | 2.80±0.15 | 0.61±0.14 | < 23\* | 0.52±0.07 | 0.27±0.09*a* | < 23\* | 0.53±0.11 | 0.21±0.15*a* |
| GEM | 0.84±0.20 | n.d. | n.d. | 1.48±0.25 | 0.49±0.16 | n.d. | 1.57±0.24 | 0.64±0.14*a* | n.d. | 0.57±0.21 | n.d. |

n.d. not detected

*a* Quantification carried out using a three point standard addition calibration

\*Pharmaceutical residues detected at concentrations lower than LOQ value

Fig.2 Cumulative pharmaceutical concentrations detected in effluent from WWTP1 and marine surface water from EXP1 and WWTP flow rates (May 2011–April 2012)

Fig.3 Cumulative pharmaceutical concentrations detected in effluent from WWTP2 and marine surface water from EXP2 and WWTP flow rates (May 2011–August 2011)

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