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1 **EVALUATION OF COMBINED SEWER OVERFLOW IMPACTS**  
2 **ON SHORT-TERM PHARMACEUTICAL AND ILLICIT DRUG**  
3 **OCCURRENCE IN A HEAVILY URBANISED TIDAL RIVER**  
4 **CATCHMENT (LONDON, UK)**

5

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26 **Abstract**

27 The occurrence of pharmaceutical and illicit drug residues potentially arising  
28 from combined sewer overflows (CSOs) in the Central London portion of the  
29 Thames Estuary is presented. Approximately 39 million tonnes of untreated  
30 sewage enter the River Thames at 57 CSO points annually. Differential analysis  
31 of influents and effluents in a major wastewater treatment plant identified seven  
32 potential drug-related CSO markers based on removal rates. Three were  
33 present in influent at concentrations  $>1 \mu\text{g L}^{-1}$  (caffeine, cocaine and  
34 benzoylecgonine). During dry weather, analysis of hourly samples of river water  
35 revealed relatively consistent concentrations for most drugs, including CSO  
36 markers, over a tidal cycle. River water was monitored over a week in January  
37 and July and then daily across six consecutive weeks in November/December  
38 2014. Out of 31 compounds monitored, 27 drug residues were determined in  
39 the River Thames and, combined, ranged between  $\sim 1,000\text{-}3,500 \text{ ng L}^{-1}$ . Total  
40 drug concentration generally declined during extended periods of drier weather.  
41 For CSO markers, short-term increases in caffeine, cocaine and  
42 benzoylecgonine concentration were observed  $\sim 24 \text{ h}$  after CSO events  
43 (especially those occurring at low tide) and generally within one order of  
44 magnitude. Timings of elevated occurrence also correlated well with  
45 ammonium ion and dissolved oxygen data following CSOs. This work also  
46 represents an important study of pharmaceutical occurrence before a major  
47 'Super Sewer' infrastructure upgrade in London aiming to reduce CSOs by  
48 95 %.

49 **Keywords:** river water monitoring, emerging contaminants, high resolution  
50 mass spectrometry, CSOs

## 51 **1. Introduction**

52           Pharmaceuticals as environmental contaminants have been the focus of  
53 much research in the past 20 years. Concentrations, generally in the ng- $\mu$ g L<sup>-1</sup>  
54 range, have now been reported in most environmental compartments including  
55 wastewater [1-3], surface/ground water [4-6], marine water [7-9], solids [10],  
56 biota [11] and even in air [12]. However, the primary source of pharmaceutical  
57 and illicit drug contamination in the receiving environment has been identified  
58 as outputs from wastewater treatment plants (WWTPs), as either treated  
59 effluent or via sludge. In the EU, some pharmaceutical compounds have been  
60 placed on a 'watch-list' until sufficient evidence on the full extent of their impacts  
61 is known [13]. Environmental contamination and effects of illicit drugs have also  
62 been reported, albeit on a smaller scale to pharmaceuticals, and the focus for  
63 these has been largely on their measurement in untreated wastewater to  
64 estimate community consumption patterns [14-16].

65           As part of the wastewater infrastructure of many developed towns and  
66 cities, combined sewers are often used to simultaneously carry storm water and  
67 municipal sewage to urban WWTPs. Such sewers are often designed to carry  
68 several fold the average dry-weather load, but in extreme cases of runoff,  
69 rainfall or snowmelt, capacity can be breached. In these cases, combined  
70 sewer overflow (CSO) events occur to avoid back-flooding of streets and  
71 homes. Storm flow is normally mixed with treated or untreated wastewater and  
72 released directly into a nearby river or water body. Many reports have detailed  
73 the resultant changes in water quality [17] and ecosystem impacts [18] arising  
74 from faecal matter [19], microbial pathogens [20, 21], priority pollutants [22] and  
75 other storm water-related contents [23] .

76           In London, ~39 million tonnes of untreated sewage is discharged into the  
77 river Thames every year on average, but following exceptional wet weather and  
78 flooding in 2014, that total rose to 62 million tonnes [24]. London is mostly  
79 served by a Victorian combined sewer system built by Sir Joseph Bazalgette  
80 following the 'Great Stink' of 1858. From 1831 until its completion in 1865, an  
81 estimated 40,000 Londoners died from cholera. The expansion of London and  
82 an increasing population (>8.3 m) has meant that the system is currently  
83 running at approximately 80 % of its capacity, resulting in more frequent  
84 breaches with CSOs occurring at least once a week, even at times of light  
85 rainfall. London's sewer system contains 57 CSO vents, 36 of which were  
86 assessed as having adverse environmental effects [25, 26]. CSO discharges  
87 were found to reduce the dissolved oxygen (DO) levels in river, introduce  
88 pathogenic organisms and to cause negative aesthetic changes in the river  
89 through the release of sewage, sewage litter, grease and scum directly into the  
90 river. A potential solution has been the Thames Tideway Tunnel, or 'Super  
91 Sewer', currently being built ~66 m under the river over 25 km. This major  
92 upgrade will intercept 34 CSOs and reroute sewage to a relief WWTP at  
93 Beckton in east London. It is due to be completed by 2023 and aims for an  
94 average 95 % reduction in sewage discharged to the river [27].

95           In comparison to prioritised pollutants, the impact of CSOs containing  
96 multiple pharmaceutical residues on receiving waters has received relatively  
97 little attention. A recent study by Kay et al. [28], showed that concentrations of  
98 five compounds monitored over 18 months in non-tidal rivers did not decrease  
99 even 5 km from the nearest WWTP in Northern England, which may potentially  
100 influence risk assessments based on models using first-order decay kinetics in

101 rivers [29]. Repeated sampling was also performed to identify fluctuations  
102 across a day, which showed significant variance in measured concentrations  
103 and, in some cases, across two-three orders of magnitude. A second study by  
104 Benotti and Brownawell near New York City reported concentrations of 12 high-  
105 volume pharmaceutical residues in mixed freshwater-saline regions across  
106 Jamaica Bay during dry and wet weather conditions [30]. Of these, two  
107 compounds had similar or higher concentrations in comparison to dry weather  
108 conditions (acetaminophen and nicotine). Despite being a comprehensive  
109 spatial study, repeated sampling was not performed to monitor temporal  
110 changes at each site. However, this study demonstrated the effect of salinity on  
111 pharmaceutical concentrations. Weyrauch et al. showed that compounds with  
112 removal efficiencies >95 % during wastewater treatment could result in  
113 elevated concentration in river water after CSOs [31]. For example, and though  
114 not a pharmaceutical, concentrations of nitrilotriacetic acid in the River Spree  
115 increased by 10-fold following a CSO and was well removed by a WWTP in  
116 Berlin. Compounds with intermediate removal above ~56 % also showed an  
117 increase in some cases, despite dilution with rainwater. Madoux-Humery et al.,  
118 performed high resolution temporal sampling of sewage outfalls over a year in  
119 Canada [32]. Several CSO markers were monitored and *E. coli* was considered  
120 the best overall. However, of four pharmaceuticals monitored, carbamazepine  
121 was determined to be the best marker of CSOs due to its persistence, specificity  
122 for human use, stability and correlation with *E. coli*. Previous work by the same  
123 group showed that caffeine was correlated with faecal coliforms [33] and its use  
124 as an indicator of wastewater contamination was also shown by other groups  
125 in different parts of the world [34-38]. Acetaminophen was also identified as a

126 suitable CSO marker by other groups [38, 39]. In an alternative approach, Fono  
127 et al. showed that chirality could be exploited to identify raw sewage discharges  
128 and/or CSOs using the ratio of one of the isomers of propranolol to its total  
129 concentration [40]. Aside from CSOs, use of drug markers has also recently  
130 been proposed to differentiate sewage from manure contamination [41]. Save  
131 for a few studies [42-44], the number of pharmaceuticals and especially illicit  
132 drugs included is generally small. More comprehensive analytical methods are  
133 required to fully identify the scale of CSO impacts more broadly regarding such  
134 compounds. Ideally, these should be more tailored to the catchment at the  
135 method development stage. The advent of liquid chromatography-high  
136 resolution mass spectrometry (LC-HRMS) has enabled a more flexible  
137 approach to multi-residue analysis, by allowing targeted, untargeted and  
138 suspect screening to be performed on large numbers of compounds, often  
139 simultaneously [45-48]. However, reports using such approaches for CSO  
140 impact assessment on receiving waters for pharmaceuticals and illicit drugs are  
141 few.

142         The aim of this work was to identify fluctuations in drug concentrations  
143 in the Central London catchment of the River Thames potentially arising from  
144 CSO events. The objectives were (a) to perform a differential quantitative  
145 analysis of influent and effluent wastewater to identify CSO-related drug  
146 markers, and (b) to monitor fluctuations in general drug occurrence, as well as  
147 ammonium and DO in receiving river water during dry and wet weather. In  
148 particular, sampling sites were chosen for their location ~25 km away from any  
149 main WWTP effluent discharge points. This project focused on quantitative  
150 monitoring of a larger number of pharmaceutical and illicit drug compounds than

151 studied previously (n=31), and measured at high frequency, with an analytical  
152 method based on LC-HRMS that was flexibly adapted for the catchment. Also,  
153 this work serves as a potential snapshot of drug contamination before a major  
154 sewer infrastructure upgrade such as the Thames 'Super Sewer' project.

155

## 156 2. **Experimental**

### 157 2.1 *Materials and Reagents*

158 All reagents were of analytical grade or higher. Methanol (MeOH), acetonitrile  
159 (MeCN), dichloromethane (DCM) and dimethyldichlorosilane (DMDCS) were  
160 purchased from Fisher Scientific (Loughborough, UK). Ammonium acetate and  
161 37 % (w/v) hydrochloric acid solution were sourced from Sigma-Aldrich  
162 (Gillingham, Dorset, UK). Ultra-pure water was obtained from a Millipore Milli-  
163 Q water purification system with a specific resistance of 18.2 MΩ.cm (Millipore,  
164 Bedford, USA). All glassware including stock solution vials and evaporation  
165 tubes were silanised to reduce loss of analyte through adsorption to the glass  
166 surfaces. Each component was rinsed with a 50:50 (v/v) MeOH/H<sub>2</sub>O solution  
167 before triplicate rinses with DCM. A 10:90 (v/v) DMDCS/DCM solution was then  
168 used to rinse the container followed by triplicate rinses with each of DCM, 50:50  
169 MeOH:H<sub>2</sub>O solution and water. A total of 51 pharmaceuticals, illicit drugs and  
170 metabolite reference materials were purchased from Sigma Aldrich  
171 (Gillingham, UK) for analytical method development and assessment (See  
172 Table S1. Stock solutions (1,000 mg L<sup>-1</sup>) were prepared in MeOH and working  
173 standard solutions prepared weekly in ultrapure water or LC mobile phase A.  
174 All solutions were stored in silanised amber glass vials at 4 °C in dark  
175 conditions.

176

## 177 2.2 *Sampling sites and procedures*

178 Wastewater influent (immediately after the fine screen) and treated effluent  
179 were taken as seven 24-hour composite samples from a major sewage  
180 treatment works in London (population equivalent = 3.5 million) from 11-17<sup>th</sup>  
181 March 2014 to identify pharmaceuticals and illicit drug residues potentially  
182 indicative of CSO events. A 12-hour diurnal occurrence study was conducted  
183 using 13 hourly grab samples (500 mL) taken on Tuesday 12<sup>th</sup> August 2014, at  
184 Gabriel's Pier, London (51°30'31.0" N; 0°06'35.1" W) covering a period from  
185 07:00 to 19:00 and collected at ~0.5 m depths. A moderate temperature (16-23  
186 °C), mainly dry day (<1 mm rainfall) was chosen to reflect a normal daily river  
187 cycle and free from storm runoff or triggered CSOs. For inter-season  
188 occurrence of pharmaceutical and illicit drug CSO marker candidates, samples  
189 were taken from two sites, again at ~0.5 m depths each time: Site 1 was at  
190 Lambeth Bridge (51°29'42.4"N 0°07'27.8"W) and Site 2 was at Gabriel's Pier  
191 (as above). Of 57 vents in total in London, six CSO vents lay in close proximity  
192 to Site 2 in both directions, spanning from Westminster Bridge to Blackfriars  
193 Bridge. For Site 1, a CSO vent lay within 50 m of the sampling site on the same  
194 bank. Following this, a high frequency sampling campaign was conducted by  
195 taking grab samples over a 6-week period at 09:00 on weekdays from Site 2  
196 from 3<sup>rd</sup> November-13<sup>th</sup> December 2014. All samples of wastewater and river  
197 water were collected in 500 mL Nalgene bottles, transported immediately to the  
198 laboratory (~30-60 min transit time), acidified to < pH 2 with HCl and frozen (-  
199 20 °C) until analysis. Tide heights were also recorded at the river sampling site  
200 at each timepoint using the local tidal gauge pole. Daily rainfall data for the

201 sampling site was gathered from the published CEH-GEAR dataset by Tanguy  
202 et al. [49].

203

### 204 2.3 *Sample pre-treatment and solid phase extraction*

205 Before extraction, samples were thawed and filtered under vacuum using  
206 Whatman GF/F 0.7  $\mu\text{m}$  glass microfiber filters. For matrix-matched standards,  
207 acidified 100 mL sample aliquots were spiked volumetrically before solid phase  
208 extraction (SPE). HyperSep Retain Polar Enhanced Polymer (PEP) cartridges  
209 (200 mg x 6 mL) were selected for SPE of river water and wastewater (Thermo  
210 Fisher Scientific, Runcorn, UK). Cartridges were conditioned with 4 mL MeOH  
211 and 4 mL ultrapure water. Acidified samples (100 mL) were loaded under  
212 vacuum at  $\sim 5 \text{ mL min}^{-1}$  and washed thereafter with 4 mL 5:95 (v/v) MeOH:H<sub>2</sub>O.  
213 The sorbent was dried under vacuum prior to elution for  $\sim 10$  min before elution  
214 with 4 mL MeOH. Eluted extracts were evaporated to dryness under N<sub>2</sub> at 35 °C  
215 and reconstituted in 100  $\mu\text{L}$  of 10 mM ammonium acetate 90:10  
216 water:acetonitrile (mobile phase A) using a positive displacement pipette. The  
217 reconstituted samples were then sonicated for  $\sim 10$  min before being transferred  
218 to an amber HPLC vial fitted with a silanised insert for analysis.

219

### 220 2.4 *Instrumentation*

221 For LC-HRMS analysis, an Accela ultra-high performance LC system, an HTS-  
222 A5 autosampler (at 10 °C) and an Exactive<sup>TM</sup> (Orbitrap) HRMS detector were  
223 used throughout. All separations were performed on a Thermo 150 x 2.1 mm,  
224 2.6  $\mu\text{m}$  Accucore C<sub>18</sub> analytical column fitted with a matching 10 x 2.1 mm, 2.6  
225  $\mu\text{m}$  Accucore C<sub>18</sub> guard column. The LC flow rate was 0.4 mL min<sup>-1</sup>, the

226 temperature was maintained at 24 °C and the injection volume was 20 µL. A  
227 binary gradient elution profile of 90:10 to 20:80 10 mM ammonium acetate in  
228 water:acetonitrile (mobile phase A and B, respectively) was used as follows:  
229 0% B for 2.5 min; 0-30% B from 2.5 to 7.5 min; 30% B from 7.5 to 12.5 min; 30-  
230 40% B from 12.5 to 15 min; 40-100% B from 15.0 to 20.0 min; 100% B from  
231 20.0 to 27.5 min. Re-equilibration time was 7.5 min. The Exactive™ HRMS was  
232 fitted with a heated electrospray ionisation source (HESI-II). All samples and  
233 model solutions were run separately in either positive or negative ionisation  
234 mode at 50,000 FWHM with a scan range of m/z 100–1000. Each acquisition  
235 cycle comprised of a full-scan without higher energy collisional dissociation  
236 (HCD) followed by a full scan with HCD enabled (collision energy: 20 eV; cycle  
237 time: ~2 s). Sheath, auxiliary and sweep gas settings were 50, 10 and 0  
238 arbitrary units, respectively. The capillary temperature was 350 °C; the heater  
239 temperature was 300 °C; and the positive/negative spray voltages were +4.50  
240 kV and –3.00 kV. All acquisition data was processed using Xcalibur v2.0  
241 software. The entire analytical method was validated to ICH guidelines in  
242 wastewater and river water (see Tables S2-S4) [50]. Method development  
243 details are also presented in the Supplementary Information. For wastewater  
244 influent and effluent, the method was found to be quantitative for n=33 and n=38  
245 compounds in untreated influent and treated effluent, respectively. For river  
246 water, the method could reliably quantify n=31 compounds at environmentally  
247 relevant concentrations.

248

249 *2.5 Targeted analysis, quantitation and statistical procedures*

250 Confirmation of target analyte occurrence in all samples was based on the  
251 accurate mass of the protonated/deprotonated precursor ion and its associated  
252 major HCD product ion to within 5 ppm mass accuracy, the ratio between these  
253 two ions (<30 % to a matrix-matched standard) and a matching  
254 chromatographic retention time ( $t_R$ ) to within 15 s. For 24-h composite  
255 influent/effluent wastewater samples, duplicate aliquots were extracted for each  
256 day and determined using matrix-matched calibration using a pooled matrix of  
257 all samples across the week-long sampling period. Background correction was  
258 performed, as needed. Calibration lines were prepared for  $N \geq 5$  points,  
259 alongside triplicate background-corrected quality control samples (50 ng/L) to  
260 allow the accuracy of the method to be monitored. Given that the river was tidal  
261 and brackish, significant variance in analyte matrix effects across days was  
262 observed for a number of compounds (data not shown), so all drugs were  
263 determined in duplicate using 3-point standard addition in each sample  
264 separately for added accuracy. Drug occurrence in all samples is reported as  
265 the average of duplicates with error bars representing the larger of the two  
266 measurements. For temporal occurrence experiments, measured values over  
267 each timeframe were averaged and the associated variance expressed as the  
268 standard deviation, unless otherwise specified.

269 All statistical treatment of data was performed in Microsoft Excel. For  
270 quantitation/calibration, lines-of-best-fit were applied and coefficients of  
271 determination ( $R^2$ ) calculated. For correlations between tide height/rainfall and  
272 drug concentration (Figure S4), the Pearson correlation ( $R$ ) was calculated and  
273 significance tested by considering a  $p$ -value threshold of 0.05 to reject the null  
274 hypothesis. For statistical comparisons of drug removal efficiency from

275 wastewater, data was first checked for normality and the  $p$ -value quoted  
276 following application of the specified test.

277

## 278 *2.6 Suspect screening of wastewater and river water*

279 Suspect screening was performed on wastewater samples only to differentially  
280 identify unique drugs/metabolites or those with potentially higher  
281 concentrations in influent. Post-acquisition automated peak selection was  
282 performed using Thermo TraceFinder™ version 3.1 software which contained  
283 a library of HRMS spectra for  $n=1,492$  pesticides, herbicides, fungicides,  
284 pharmaceuticals, metabolites and illicit drugs. Following this, predicted  $t_R$  for  
285 potentially new compounds was performed using a previously developed neural  
286 network algorithm (Trajan v6.0, Trajan Software Ltd., Lincolnshire, UK) using  
287 reference  $t_R$  data for 166 pharmaceuticals, illicit drugs and metabolites  
288 measured in influent and effluent wastewater extracts [51]. Compounds were  
289 tentatively identified using a  $t_R$  window of  $\pm 1.3$  min and an accurate  $m/z$  within  
290 5 ppm of its calculated  $m/z$ . Lastly, an 80% fit threshold to theoretical isotope  
291 profile was set, with an acceptable intensity threshold deviation for each isotope  
292 ion set at 25% of the theoretical value.

293

## 294 *2.7 DO, ammonium and conductivity monitoring*

295 Percentage DO, pH, conductivity (as a measure of salinity), and ammonium  
296 concentration were taken at 15-minute intervals by the Environment Agency  
297 (EA), UK and analysed at three sites (Putney, Brentford and Hammersmith)  
298 using YSI6600 systems (Environmental Monitoring Systems, Herts, UK). DO  
299 was measured as % saturation using the YSI optical DO Sensor. The Sonde

300 software automatically compensated for the effect of temperature. River pH was  
301 measured using a combination electrode with an Ag/AgCl reference electrode.  
302 Ammonium was measured using an YSI ion selective electrode and the  
303 reference being provided by the pH combination electrode. Conductivity ( $\mu\text{S}$   
304  $\text{cm}^{-1}$ ) was reported as specific to 25 °C and was calibrated using a solution of  
305 KCl. The YSI6600 sensors were calibrated every 4 weeks following standard  
306 EA operating procedures.

307

### 308 **3. Results and Discussion**

#### 309 *3.1 Differential analysis of influent and effluent wastewaters and* 310 *identification of candidate CSO markers*

311 To shortlist a selection of CSO-related pharmaceutical and illicit drug markers,  
312 differential analysis of influent and effluent wastewaters was performed. Direct  
313 analysis of in-sewer CSO samples was not performed due to limited access.  
314 Two important criteria were considered. Candidate CSO drug markers were  
315 shortlisted where they were: (a) ideally only present in untreated influent  
316 wastewater (i.e. high removal efficiency in the WWTP); and (b) remained at  
317 measurable and relatively consistent concentrations every day (i.e., minimal  
318 seasonal variation or recreational usage patterns should be evident).

319 All determined drug concentrations are presented in Tables S5 and S6  
320 and summarised in Figure 1. A total of 14 compounds were quantifiable almost  
321 every day in untreated influent wastewaters and two of these were unique to it,  
322 i.e. diazepam and sulfapyridine, present at  $76 \pm 14$  and  $184 \pm 96$   $\text{ng L}^{-1}$ ,  
323 respectively, which were both selected as candidates. Prescription drug  
324 concentrations were generally consistent across the week in both influent and

325 effluent (except for sulfapyridine, which was not detected on one day). Both  
326 bezafibrate and furosemide were quantifiable in influent at similar  
327 concentrations ( $\sim 400 \text{ ng L}^{-1}$ ), but less than the lower limit of quantification  
328 (LLOQ) in effluent. This corresponded to an  $>10$ -fold lower concentration, so  
329 both were considered as potential CSO markers. Tramadol exhibited the  
330 opposite trend, with significantly higher levels detected in effluent at  $1,138 \pm 106$   
331  $\text{ng L}^{-1}$  ( $p = 3 \times 10^{-7}$ , Student's two-tailed  $t$ -test), with over a two-fold concentration  
332 increase observed between both matrices. Nine other compounds were present  
333 at quantifiable levels on a regular basis in effluent. Extensive wastewater  
334 monitoring over the past five years as part of the £130 m UK Water Industry  
335 Research (UKWIR) Chemical Investigation Programme (CIP) Phase 2 (CIP2)  
336 has played a key role in the selection of substances and sites for future controls  
337 and remedial measures [52, 53]. It included up to 73 individual determinands  
338 across 44 WWTPs from 2015-2017 including data for six pharmaceutically-  
339 related compounds for which removal rates could be calculated: diclofenac ( $42$   
340  $\pm 29$  %), ibuprofen ( $98 \pm 4$  %), propranolol ( $28 \pm 24$  %), carbamazepine ( $-8$   
341  $\pm 35$  %), carbamazepine epoxide ( $30 \pm 28$  %) and fluoxetine ( $43 \pm 22$  %) [54].  
342 The London-based WWTP studied here was not included within the 44 CIP2  
343 sites. Comparative removal rates for this WWTP could be calculated reliably  
344 here for carbamazepine ( $-61$  %, i.e., more concentrated in the effluent) and  
345 propranolol ( $34$  %), and an estimation made for fluoxetine ( $65$  %; occurrence  
346 was  $< \text{LLOQ}$ , but  $> \text{LOD}$  in influent).

347 For the selected illicit drugs, most were quantifiable during the week  
348 except for methylenedioxymethamphetamine (MDMA) and generally increased  
349 over the weekend. This was consistent with recreational consumption trends

350 seen previously [15]. Ketamine was eliminated as a candidate CSO marker, as  
351 it was present at slightly higher concentrations in effluents than influents ( $58 \pm 5$   
352 and  $42 \pm 9$  ng L<sup>-1</sup>, respectively) and measurements also lay close to the LLOQ.  
353 Ketamine has been shown to display partial transformation in sewer transit  
354 (<25 %) [55], as well as variable and even negative removal rates following  
355 wastewater treatment [56, 57]. Possible reasons for higher concentrations in  
356 effluent include residence times below 24 h, as well cleavage of conjugated  
357 metabolites and desorption from particulate matter during treatment [58-60].  
358 Mephedrone was detected at low levels in all samples and quantifiable at  $83$   
359  $\pm 45$  ng L<sup>-1</sup> in six out of seven influent samples (<LLOQ in effluent). Interestingly,  
360 concentrations of cocaine and its metabolite benzoylecgonine remained high in  
361 influent wastewater across the week with only a relatively minor increase in  
362 occurrence over the weekend (%RSD <10 % for benzoylecgonine and <25 %  
363 for cocaine), which is not consistent with many other cities. London is known as  
364 one of the highest consumers of cocaine and this result suggested everyday  
365 usage [16]. Cocaine was detected at significantly higher levels in influent ( $p$   
366  $= 3 \times 10^{-5}$ ; Student's two-tailed  $t$ -test) as well as analyte concentrations in effluent  
367 at ~30-fold lower levels, which represented >99 % removal efficiency at this  
368 WWTP. While WWTP removal performances can differ between sites, similar  
369 removal of cocaine and benzoylecgonine from influent has been reported in  
370 other parts of UK and globally, even up to 100 % [57, 61]. Given their metabolic  
371 linkage, both were given further consideration as CSO markers. In addition to  
372 these compounds, caffeine was also detected only in influent. However, its  
373 concentration was so high that it lay outside of the quantifiable range when  
374 using background corrected matrix-matched standard addition. However,

375 previous work using stable isotope internal standards showed that caffeine  
376 concentration in untreated wastewater from London was quite stable at  $23 \pm 2$   
377  $\mu\text{g L}^{-1}$  across a full week [15]. Caffeine has also been shown to be removed  
378 almost completely by wastewater treatment processes by both aerobic and  
379 anaerobic degradation [57, 62]. Caffeine was therefore retained as a candidate  
380 CSO marker and more reliable measurements in river water matrix were  
381 possible when present at a diluted concentration. Another compound, salicylic  
382 acid, was present at excessively high concentrations to quantify it in influent  
383 and was not detected in effluent. However, the poor method performance for  
384 this compound, observed in all three matrices assessed, meant it was not  
385 suitable for quantitative monitoring and was eliminated for use.

386 Application of HRMS database searching (TraceFinder) and reference  
387 to matching predicted chromatographic retention times resulted in tentative  
388 identification of  $n=32$  more drug residues in influent and  $n=28$  more in effluent  
389 across the week (Tables S7 and S8). For influent only, two detectable  
390 chromatographic peaks were present for four compounds in extracted ion  
391 chromatograms within their 1.3 min retention window even at 5 ppm mass  
392 accuracy/isotope profile matching (i.e., matching hydrocortisone, salbutamol,  
393 testolactone and acetylsalicylic acid, but not confirmed with reference  
394 standards). A total of 14 compounds were detected in influent at higher signal  
395 intensities than effluent at least once across the week (Figure 2 and Table S9).  
396 Eleven compounds were tentatively identified in effluent every day, including  
397 nine also present in influent every day. However, two unresolved isomers  
398 (quinine and quinidine) were present at markedly higher signal intensities in  
399 influent and were used together as a combined signal as potential CSO

400 markers. It was expected that of the two, quinine was likely to be the dominant  
401 compound given its widespread use in tonic waters.

402 A total of seven target analytes (bezafibrate, benzoylecgonine, caffeine,  
403 diazepam, sulfapyridine, cocaine and furosemide) were shortlisted as  
404 candidate CSO markers quantitatively. Quinine and quinidine were used  
405 together as qualitative CSO markers. For the six-week monitoring study, all  
406 other compounds were still included for river water monitoring, even if not  
407 considered as potential CSO markers to assess the potential contribution of  
408 CSOs in general.

409

### 410 3.2 *Diurnal variation in drug concentrations in the River Thames*

411 The river sampling sites in Central London lay within the Thames Estuary,  
412 where river levels often change by up to seven metres, twice a day. River flow  
413 is relatively small compared with the volume of the tide and therefore, is well  
414 mixed. Generally, the entire water mass travels in and out of the estuary with  
415 tidal cycles. When CSOs discharge to the river, it takes approximately one  
416 month for litter and sewage to exit the estuary to the sea in Winter and up to  
417 three months in Summer [24]. River water is also brackish to the top of the  
418 estuary at Teddington Lock, which lies west of the city. Previous research has  
419 shown that varying salinity, dissolved organic carbon (DOC) and/or suspended  
420 particulate matter (SPM) can influence drug concentrations in tidal waters [42,  
421 43]. Therefore, fluctuations in drug concentration were monitored over a tidal  
422 cycle on a day free from storm water runoff or CSOs to understand the impact  
423 of fresh/saline water changes. From a qualitative perspective, n=24/31  
424 compounds included in the validated method were detected at least once

425 across the day at Site 2 (Table S10) showing that the selection of compounds  
426 was highly relevant to this catchment and benefited greatly from the use of  
427 flexible full-scan LC-HRMS-based methods. Of these, n=18 drug residues were  
428 quantifiable and n=13 of those determined at all sampled time points. Figure  
429 3(a) shows that four potential CSO marker drugs were quantifiable and  
430 remained relatively low in concentration. As perhaps expected, caffeine was  
431 present at the highest concentration across the day at  $112 \pm 48 \text{ ng L}^{-1}$ , and it  
432 presented a minor correlation with tide. No obviously apparent correlation with  
433 tide was observed for the other three CSO markers and all remained below  $\sim 20$   
434  $\text{ng L}^{-1}$ . Figure 3 (b)-(d) show the other determined pharmaceutical residues,  
435 again most of which showed low and relatively consistent concentration  
436 profiles. Tramadol and carbamazepine concentrations were the highest  
437 between  $\sim 100\text{-}300 \text{ ng L}^{-1}$  over the 12-hour period. Tramadol occurrence has  
438 been linked to hospital effluent contribution to CSOs, but was present at lower  
439 concentrations in untreated wastewaters here [63]. Trimethoprim,  
440 sulfamethazine, carbamazepine and ketamine were the only obvious cases  
441 showing any correlation with tide or water conductivity. These almost doubled  
442 in concentration at high tide which was in contrast to observations for  
443 pharmaceuticals by some other researchers [42, 43]. Three of London's five  
444 WWTPs (Beckton, Riverside and Crossness) discharge treated wastewater into  
445 the Thames  $\sim 25\text{-}30 \text{ km}$  to the east of the Central London location (Site 2) and  
446 serve a combined population equivalent of  $\sim 5.9$  million ( $\sim 71 \%$  of Greater  
447 London). The remainder of the population is served mainly by Mogden WWTP,  
448 which discharges effluent  $\sim 25 \text{ km}$  west of Site 2 ( $\sim 2$  million population  
449 equivalent). Therefore, concentration rises with high tide are likely due to drug

450 residues from more treated effluent entering downstream being swept inland  
451 towards Site 2. Therefore, and in general, drug residues were not removed from  
452 the sampling site by a tidal cycle and concentrations largely remained relatively  
453 consistent. This was particularly useful for CSO markers considering that river  
454 water conductivity changed from  $\sim 650\text{-}1,000 \mu\text{S cm}^{-1}$  across the tidal cycle on  
455 this date showing the salt water influx/efflux.

456

### 457 3.3 *Inter-season occurrence of pharmaceutical and illicit drug CSO marker* 458 *candidates*

459 CSOs were categorised into two main types. CSO Type 1 comprised of storm  
460 water combined with untreated sewage, which was discharged directly into the  
461 river. CSO Type 2 represented heavily diluted storm water that was screened,  
462 settled in tanks and mixed with fully treated wastewater at a major WWTP  
463 before release to the river. Public notifications of either CSO type corresponded  
464 to two monitored sites in London: (a) Hammersmith pumping station (CSO Type  
465 1) and (b) Mogden WWTP (CSO Type 2). Weather in January 2014 was one of  
466 the wettest on record since 1910 with  $\sim 135$  mm rainfall and available data from  
467 Hammersmith Pumping Station alone revealed  $\sim 1,637,456 \text{ m}^3$  of CSO Type 1  
468 discharge and  $2,505,000 \text{ m}^3$  of Type 2 from Mogden WWTP [64]. However, the  
469 total volume of either CSO type was likely much higher given that several more  
470 pumping stations and CSO vents exist across the Central London catchment.  
471 Across 2014, 16 million tonnes of untreated sewage were discharged into the  
472 River Thames from just the central London CSO vents covering the two  
473 sampling points selected. Three of these (the Hammersmith, Lots Road, and  
474 Western Pumping Stations) contributed 11 million tonnes to that total. One Type

475 1 CSO event occurred during the week sampled in winter on 16<sup>th</sup> January, 2014  
476 at 21:50 hours, but after a grab sample was taken. However, concentrations of  
477 caffeine and benzoylecgonine increased at both Sites 1 and 2 on the following  
478 day (Figure 4). Furthermore, at Site 1 increases in concentration were also  
479 observed for bezafibrate and cocaine, most likely as it lay so close to a CSO  
480 vent, but this trend was not observed at Site 2. Caffeine had the highest  
481 concentration overall and reached a maximum of 1,520 ng L<sup>-1</sup> at Site 1 and  
482 ~13 h after this Type 1 CSO. Its high concentration was prolonged in this  
483 instance and took roughly two days to return to baseline concentrations. No  
484 CSOs occurred during the week of sampling in July, 2014. Only ~44 mm rainfall  
485 was recorded for the month with 24,000 m<sup>3</sup> of Type 1 CSO discharge from  
486 Hammersmith Pumping Station and no Type 2 CSO discharge from Mogden  
487 WWTP. By comparison, caffeine concentrations were much lower in Summer  
488 and rarely reached >200 ng L<sup>-1</sup>. Detection of all other substances was  
489 intermittent. Interestingly, baseline concentrations of bezafibrate and  
490 benzoylecgonine remained relatively consistent with the January samples,  
491 despite recorded rainfall and tidal height differences of >3.5 m across all  
492 sampling timepoints. At this time of year, salinity of the river was also much  
493 higher and more affected by tide as its freshwater composition was much lower  
494 (conductivity of ~600-700  $\mu$ S in the Winter dates studied versus 900-3,000  $\mu$ S  
495 in Summer)

496

497 *3.4 Longitudinal daily monitoring of pharmaceutical and illicit drug*  
498 *occurrence in the River Thames over six weeks*

499 Site 2 was selected for a longitudinal occurrence study of all 31  
500 pharmaceuticals given its convenience, reliability and safety of access during  
501 bad weather across six weeks in Autumn and Winter, 2014. Furthermore, it  
502 represented an equidistant point in the river between the major west and east  
503 WWTP discharge points (~25 km in either direction). A total of 27 drug residues  
504 were determined in the River Thames (Figure 5). The total (summed)  
505 concentration of all compounds monitored varied from ~1-3.5  $\mu\text{g L}^{-1}$ .

506 Over the course of the study, 13 CSOs were triggered due to heavy  
507 rainfall (Table S11). In all, six Type 1 CSOs were recorded over the six-week  
508 period, which were most relevant to this study. Of these, four samples were  
509 taken within 24 hours following a CSO event. Available Type 1 CSO-related  
510 records from the Hammersmith, Lotts Road and Western pumping stations  
511 showed that a combined total of 1,883,485 and 204,150  $\text{m}^3$  of untreated  
512 sewage mixed with storm water was discharged into the Central London region  
513 of the River Thames in November and December months, respectively [64].  
514 Measured total concentrations of illicit drugs and pharmaceuticals decreased in  
515 general throughout November and December (Figure 5 and Table S12).  
516 Approximately 75 % (~80-90 mm) of the total rainfall fell in the first three weeks.  
517 Dilution with freshwater arising from the upper Thames may have been a  
518 contributor to this decline, amongst other factors such as changing temporal  
519 consumption patterns, varying WWTP performance, changing river water  
520 chemistry (e.g., salinity, etc.), molecular stability and biological activity. On the  
521 other hand, prolonged elevated concentrations following CSOs could have  
522 arisen here where several events occurred in rapid succession, especially in  
523 the first three weeks, and which were slowly removed by the tide. The top five

524 most concentrated compounds on average across the six weeks were caffeine  
525 (477 ±313 ng L<sup>-1</sup>), diazepam (305 ±558 ng L<sup>-1</sup>), tramadol (220 ±75 ng L<sup>-1</sup>),  
526 carbamazepine (154 ±99 ngL<sup>-1</sup>) and amitriptyline (102 ±57 ngL<sup>-1</sup>). Temporal  
527 variance in measured concentrations across the 30 sampled days was, as  
528 perhaps expected, high and not likely to only include any impact of CSOs, but  
529 also changes in community consumption behaviour, illness/disease treatments  
530 or seasonal consumption patterns influencing the concentrations in treated  
531 wastewater effluents [65]. Where Type 1 CSOs occurred, no readily identifiable  
532 spikes in total concentration of all drugs determined were observed within a 24  
533 to 48hour period, nor any correlations with tide height, daily rainfall, or a ratio  
534 of both ( $R^2 < 0.1$  in all cases). Principal component analysis did not yield any  
535 further classification between daily concentrations determined for all 27  
536 compounds (Figure S2). In addition, five out of six Type 1 CSOs were also  
537 accompanied by Type 2 CSOs, which may have served to dilute untreated  
538 wastewater entering the Thames Tideway further. Some additional interesting  
539 observations were made. The illicit drugs ketamine and mephedrone were  
540 detected almost every day at 12 ±4 ngL<sup>-1</sup> and 9 ±2 ng L<sup>-1</sup>, respectively. The  
541 latter was banned in the UK in 2010, but was still determined in wastewater  
542 influent, effluent and river water here in 2014. However, despite being present  
543 at higher concentrations in influent, its concentration flux did not align with  
544 CSOs, likely in part due to recreational use increasing over the weekend.

545         When focussing on the seven shortlisted candidate CSO markers, some  
546 trends became more evident, but were very complex to interpret. Firstly,  
547 concentrations of caffeine, cocaine and its metabolite benzoylecgonine in river  
548 water showed a correlation with some CSOs. As their concentrations in

549 untreated wastewater was regularly  $>1 \mu\text{g L}^{-1}$ , this was perhaps expected over  
550 the other four compounds. Elevated concentrations were mainly detected in  
551 samples taken on the following day (Figure 6) especially following the two  
552 heaviest rainfall events and CSOs on 23<sup>rd</sup> November and 11<sup>th</sup> December, 2014,  
553 both during the lower portion of incoming flood tidal phases. For the latter date,  
554 two CSOs were triggered on the following day at 06:25 (Type 1) and 08:58  
555 (Type 2) just before the sample was taken and which enabled subsequent  
556 determination of all compounds at higher concentrations, even within 3 hours  
557 following a Type 1 discharge. However, neither cocaine nor benzoylecgonine  
558 were detected at obviously elevated levels following Type 1 CSOs on the 4<sup>th</sup> or  
559 14<sup>th</sup> November. On both occasions, the river was at the top of its tidal phase  
560 and dilution may have occurred. As before, elevated caffeine concentration  
561 following CSOs seemed prolonged over several days in comparison to cocaine,  
562 especially after the heaviest rain event on the 22<sup>nd</sup>/23<sup>rd</sup> November.  
563 Concentrations of diazepam were high across the first two weeks of the  
564 campaign and then decreased markedly thereafter and did not correlate with  
565 any one CSO event directly. Short-term elevated concentrations may be more  
566 prolonged for this compound given its potential for sorption to sediment [66].  
567 Following the CSO event on the 4<sup>th</sup> November, elevated concentrations of  
568 sulfamethazine and sulfamethoxazole occurred, and a mild rise in  
569 concentration of sulfapyridine over the following 48 h. However, sulfapyridine  
570 was not useful to indicate other Type 1 CSO events across the remainder of  
571 the campaign. Lastly, furosemide and bezafibrate yielded no apparent trends  
572 and were removed from further interpretations.

573 The majority of compounds tentatively identified during suspect  
574 screening as being indicative of influent wastewater were not present in river  
575 water. However, the combined signal for the stereoisomers quinine/quinidine  
576 was detected every day ( $[M+H]^+$  m/z 325.1910), but revealed no obvious co-  
577 incidence with CSO events (Figure S3). However, achieving chromatographic  
578 resolution of both compounds and quantification is still required to fully evaluate  
579 their individual value as CSO markers. Furthermore, the use of signal intensities  
580 from LC-HRMS analysis was likely subject to variable matrix interference due  
581 to the influence of seawater with tide, especially over the first week of the  
582 sampling campaign (Figure 7(a)). However, for the majority of the six weeks,  
583 conductivity measurements indicated that the river was predominantly  
584 composed of freshwater (600-800  $\mu$ S), mainly arising from influx of upstream  
585 sources to Teddington Lock experiencing heavy rainfall and run-off.

586

### 587 *3.5 Ammonium, pH and %DO*

588 Comparison of drug concentrations with ammonium and %DO data  
589 gathered simultaneously from Putney, Hammersmith and Brentford (each  
590 ~5-7 km apart) in the west of the city revealed correlations with most Type 1  
591 CSOs (Figure 7 (b)-(d)). Interestingly, and despite their distances apart, the  
592 changes in ammonium/%DO concentrations at each site aligned well with each  
593 other, indicating that CSOs may be triggered across the length of the network  
594 simultaneously. However, and in agreement with some of the drug  
595 measurements here, poorly discernable changes in ammonium concentration  
596 or %DO were observed for Type 1 CSOs on the 4<sup>th</sup>, 8<sup>th</sup> or 9<sup>th</sup> November (only  
597 observed clearly at the Brentford site). The pH of the river remained relatively

598 constant over the six weeks ( $\text{pH} = 7.77 \pm 0.09$ ), and very minor reductions of  
599  $<0.25$  pH units were observed during periods of elevated ammonium  
600 concentration.

601 The duration of CSO impacts could be interpreted from ammonium  
602 and %DO data (unfortunately, data for CSO duration and discharge volumes  
603 were not available for specific dates). Generally, and like CSO drug markers,  
604 changes occurred within 24 h after a CSO and returned to normal levels  $\sim 24$  h  
605 later. A mild positive, but statistically significant correlation ( $R = 0.6023$ ;  
606  $p = 0.0049$ ) existed between total concentrations of the three main CSO drug  
607 markers determined on the following day with tide height:daily rainfall ratio at  
608 the time of sampling (Figure S4). Therefore, it was concluded that there exists  
609 a fine balance between tide height/direction, rainfall and time ( $<24$  h here)  
610 before an influent wastewater-specific drug can be measured in the river to  
611 potentially indicate CSO influx. The Type 1 CSO event on the 23<sup>rd</sup> of November  
612 2014 was the most prominent and prolonged from these data which explains  
613 why concentrations of some CSO drug markers increased so markedly. The  
614 Putney site is closest by distance to Site 2 chosen for drug monitoring ( $\sim 11$  km).  
615 Despite being more central, smaller changes in ammonium and %DO were  
616 observed across the six-week period. Therefore, proximity to a local CSO vent  
617 will likely affect measurements overall. Ideally, more sites should be monitored  
618 across this catchment to more fully understand spatial impacts of  
619 pharmaceuticals and illicit drugs from CSOs on receiving waters. However,  
620 despite short-lived peaks in concentration, longer term concentrations of  
621 pharmaceuticals and illicit drugs in CSO material may decline overall upon

622 completion of the Thames Tunnel, which aims to reduce annual sewage  
623 discharge via CSOs by 95 % [27].

624

## 625 **Conclusions**

626 Of 31 compounds monitored quantitatively, 27 pharmaceuticals and illicit drug  
627 residues were determined in river water in the Thames Tideway in daily  
628 measurements over six weeks. However, occurrence and total concentrations  
629 of pharmaceuticals and illicit drugs as a whole showed no short-term correlation  
630 with specific CSO events (total concentration lay between  $\sim 1.0\text{-}3.5 \mu\text{g L}^{-1}$ ).

631 Following differential analysis of influent and effluent wastewater, seven  
632 compounds were shortlisted as potentially being influent wastewater specific  
633 and three of these were present at concentrations  $>1,000 \text{ ng L}^{-1}$  in influent (i.e.  
634 caffeine, cocaine and benzoylecgonine). In river water, these three compounds  
635 showed noticeably elevated concentrations  $\sim 24\text{-}48 \text{ h}$  after CSO events  
636 following major rainfall events and aligned with ammonium and %DO data. It  
637 was found that there existed a fine balance between tide height, direction and  
638 rainfall, before any elevated concentrations of these CSO markers were  
639 recorded. Therefore, CSO releases should be ideally aligned with the onset of  
640 the ebb tidal phase to enable sufficient dilution to occur. However, even with  
641 dilution, more research is required to understand the longer-term impacts of  
642 CSOs on drug occurrence in receiving waters and particularly any potential  
643 improvements following a major infrastructure upgrade such as that planned in  
644 London to mitigate them.

645

646

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659

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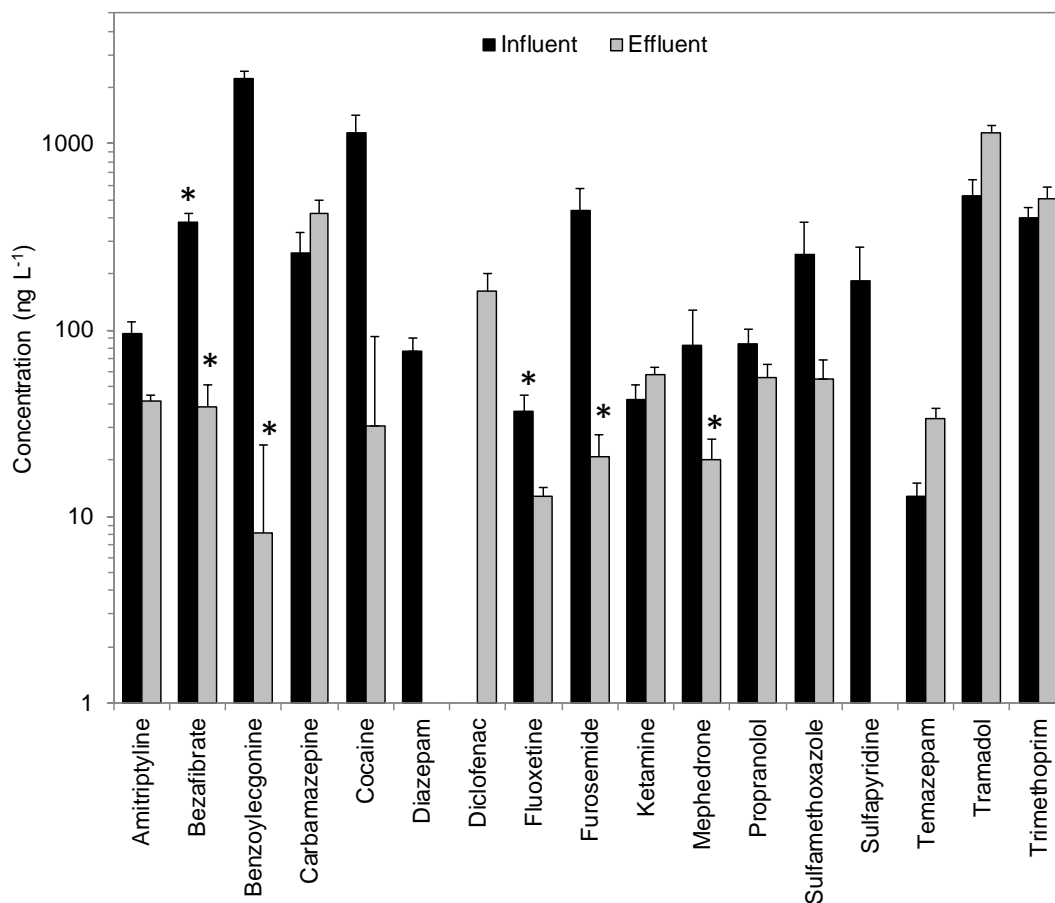
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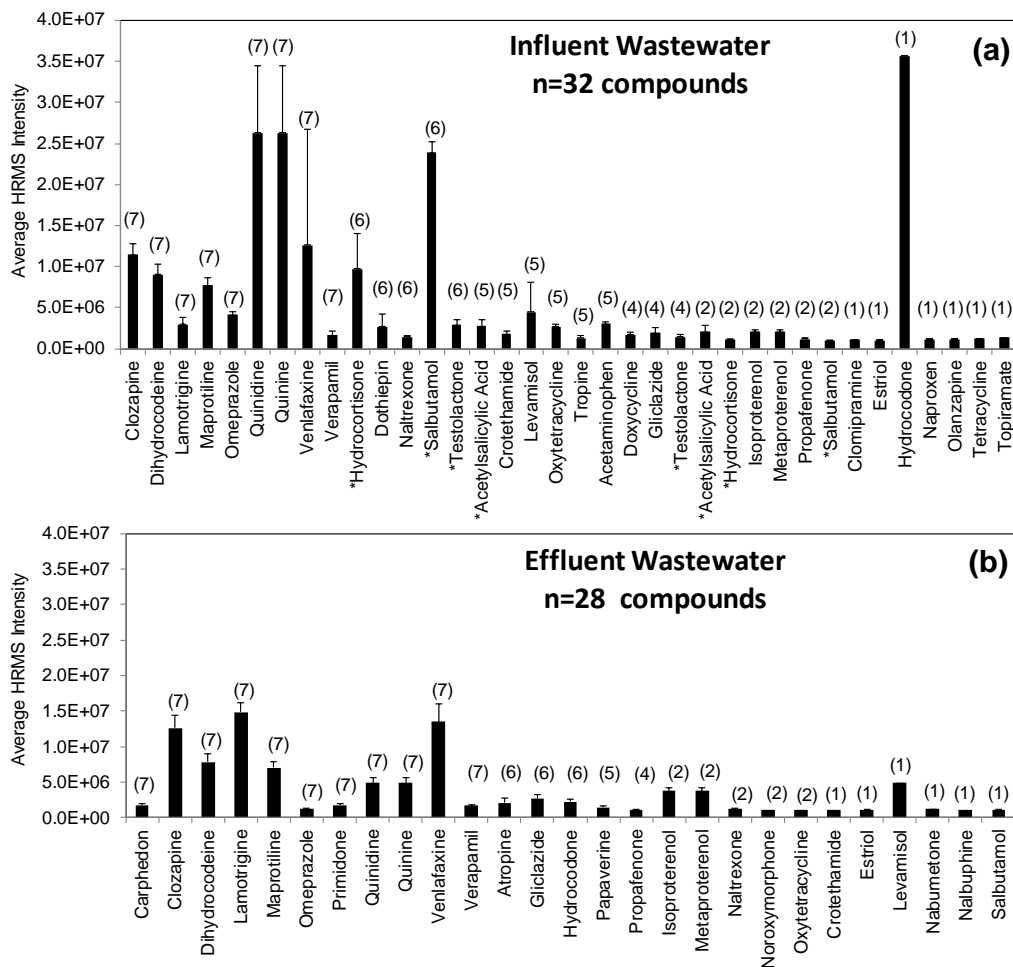
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**Figure 1.** Differential analysis of drug occurrence in untreated influent and treated effluent wastewaters from a major treatment works in London in n=7 consecutive 24-h composite samples in March, 2014. Bars marked with \* represent semi-quantitative measurements as values were <LLOQ, but >LOD. Error bars represent the standard deviation of the means of all measurements for each compound across the 7-day period.



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917 **Figure 2.** Average signal intensity for each compound tentatively identified by918 retrospective *in silico* suspect screening in (a) untreated influent and (b)

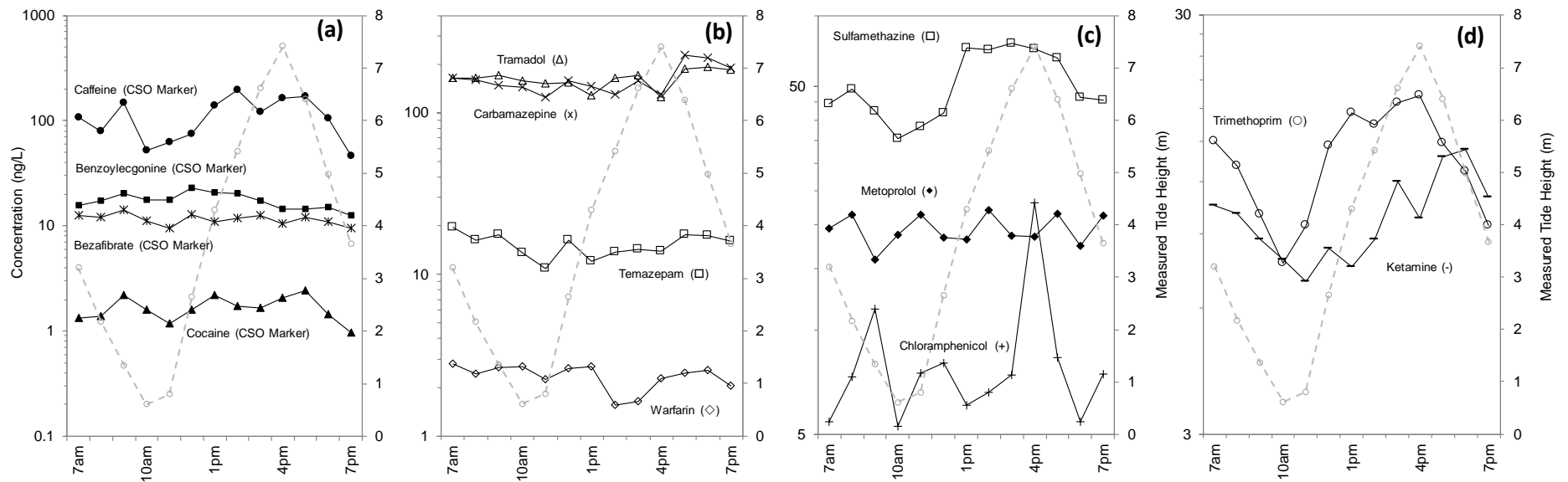
919 treated effluent wastewaters. Their corresponding occurrence frequency out of

920 7 days is shown in parenthesis. Bars represent the mean and whiskers

921 represent the standard deviation of that number of daily measurements in (c)

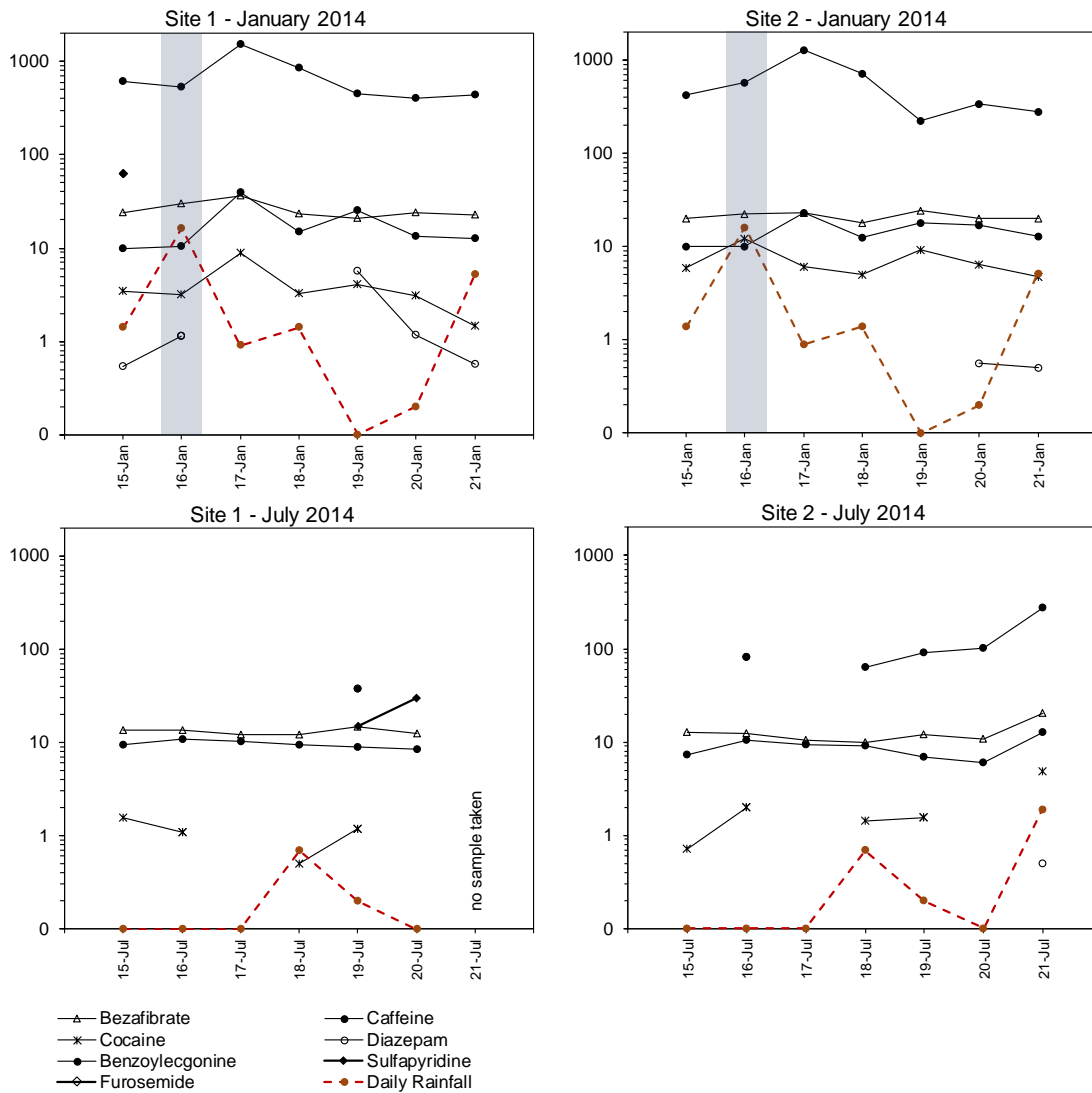
922 and (d). Compounds marked with \* represent those where two matching

923 predicted  $t_R$  values ( $\pm 1.30$  min threshold) and HRMS signals ( $\delta < 5$ ppm for924  $[M+H]^+$  or  $[M-H]^-$ ) were obtained.



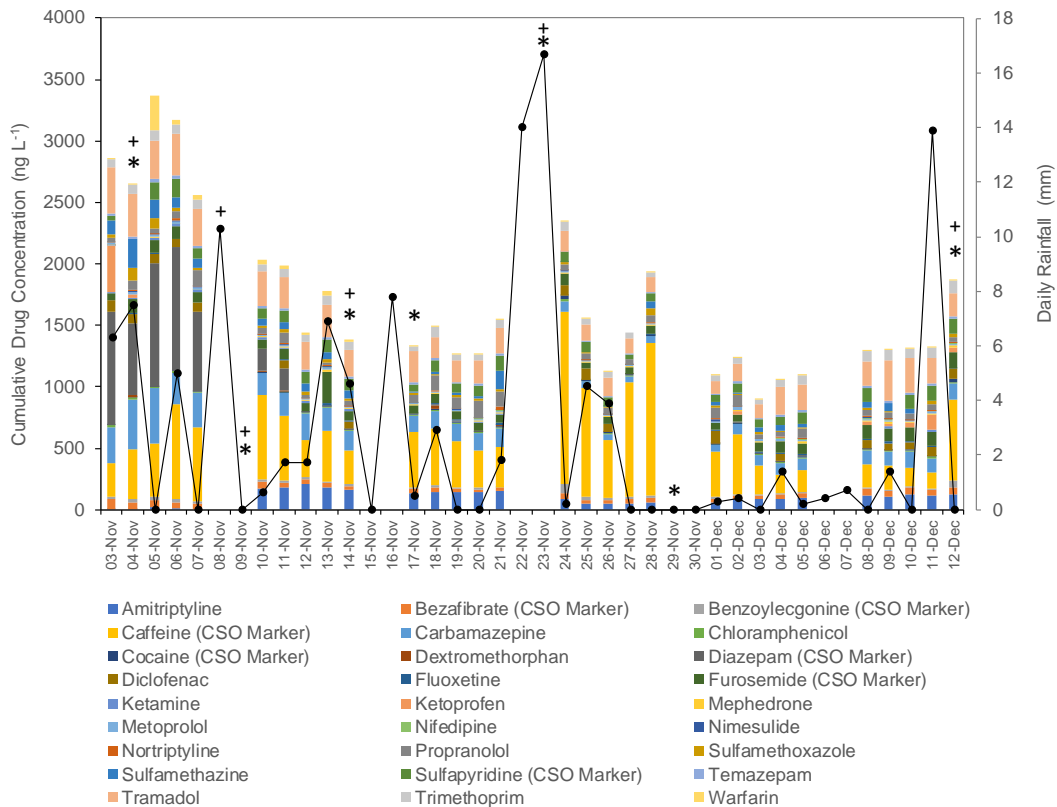
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**Figure 3.** Diurnal variation in (a) CSO marker drug compounds and (b)-(d) all other drug compounds determined above the LLOQ in the River Thames on the 14<sup>th</sup> August, 2014. Black datapoints represent the mean of n=2 replicate grab sample analyses. Grey dashed lines represent the measured tide height at the time of sampling. No CSOs occurred on this day (<1 mm rainfall).



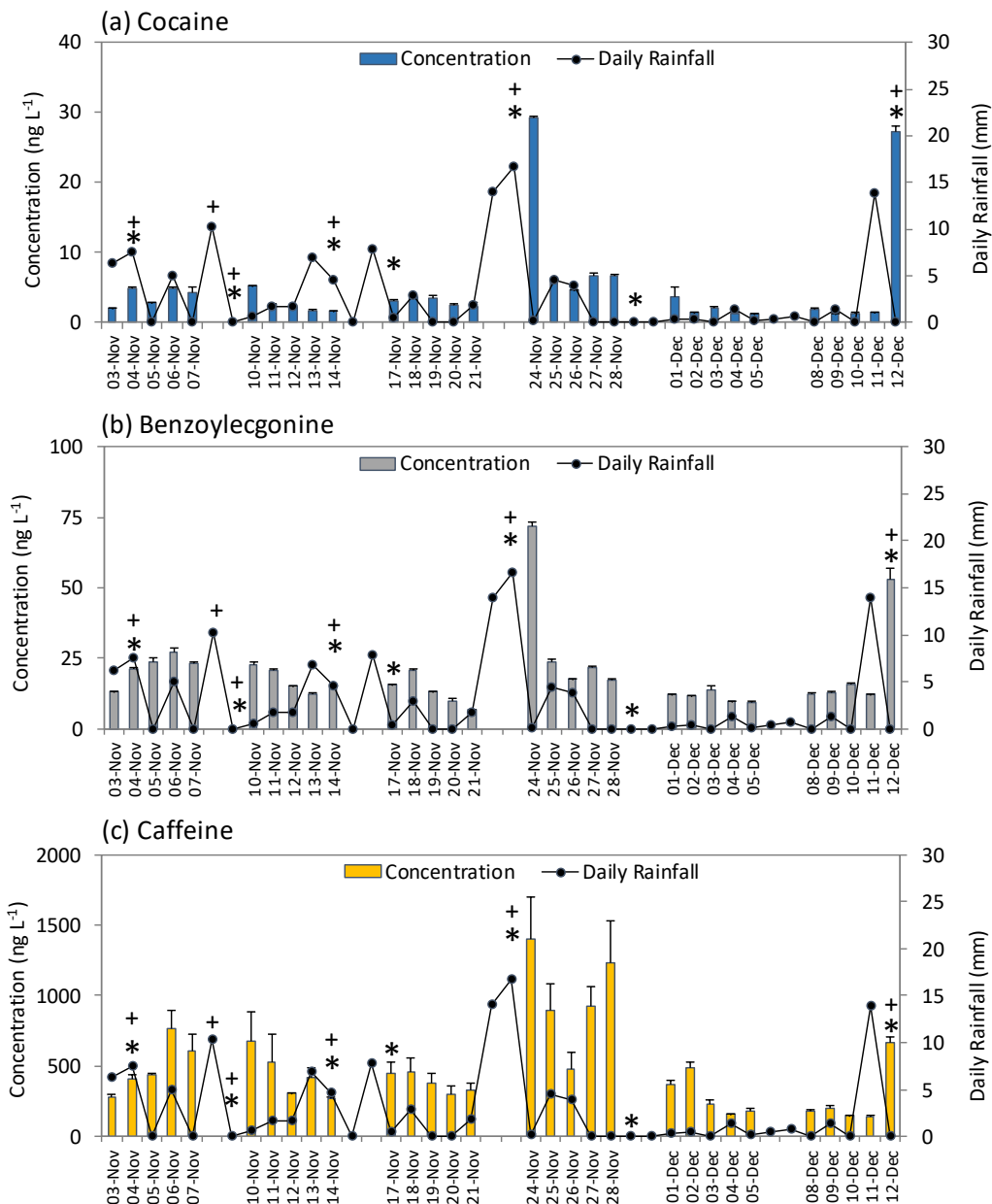
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**Figure 4.** Measured concentrations of seven shortlisted candidate drug CSO markers in samples of Thames River water from two sites in January and July 2014 and overlaid with daily rainfall. A Type 1 CSO occurred on on 17<sup>th</sup> January, 2014 at 21:50 hours (shaded in grey). Note: No sample was taken from Site 1 on 21<sup>st</sup> July, 2014. All measurements represent the mean of n=2 replicates.



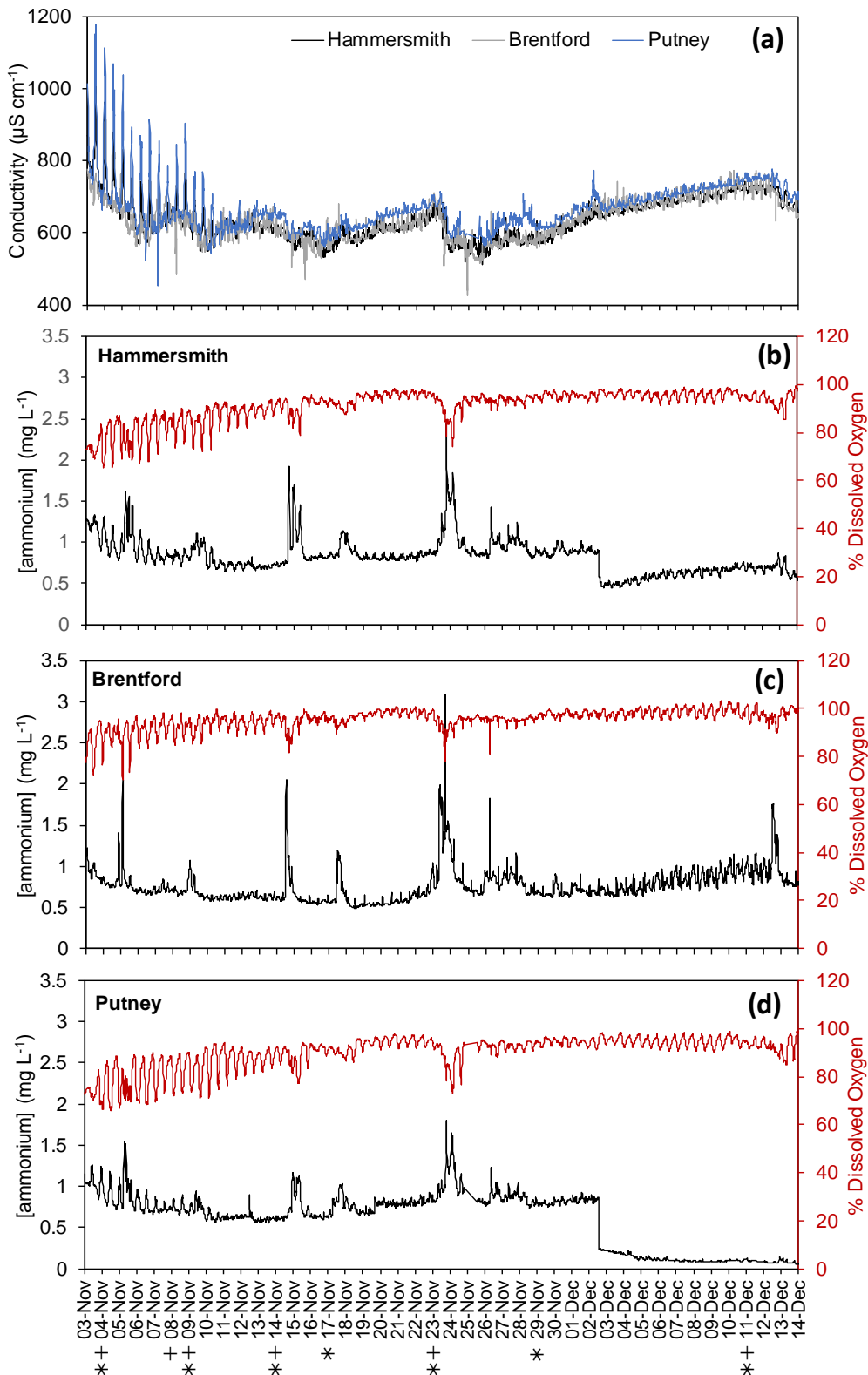
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**Figure 5.** Cumulative concentration of all drug residues determined on weekdays in the River Thames across Nov-Dec, 2014. Dates marked with + are Type 1 CSOs where storm water and untreated sewage were combined and released directly into the river. Dates marked with \* represent Type 2 CSO events where storm water was mixed with treated wastewater effluent at a WWTP and then released into the river (where both + and \* exist, two such CSOs occurred on the same date, also see Table S11).



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**Figure 6.** Occurrence of three drug CSO markers in river water from the Thames over six weeks in Nov-Dec, 2014 (overlaid with daily rainfall). Dates marked with + or \* are as in Figure 5. Bars represent the mean of two replicates and whiskers represent the maximum value measured.



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954 **Figure 7.** Continuous monitoring data at three sites on the River Thames in  
 955 Nov-Dec, 2014 for (a) conductivity and (b)-(d) % DO (red)/ammonium  
 956 concentration (black) at Hammersmith, Brentford and Putney sites,  
 957 respectively. Data-acquisition frequency =15 min. Dates marked with +/\*  
 958 represent CSO Types 1 and/or 2, respectively.