Spatial and temporal occurrence of pharmaceuticals in UK estuaries

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Abstract

There is a lack of data on the occurrence of pharmaceuticals in estuaries worldwide, with little understanding of their temporal and spatial variations globally. Ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were measured in twelve estuaries in the UK. Initially, these compounds were monitored in the Humber Estuary, where samples were taken every two months over a twelve month period in order to assess their spatial and temporal variations. Ibuprofen was found at some of the highest concentrations ever measured in an estuary globally (18 – 6297ng l^{-1}), with paracetamol also measured at relatively high concentrations (4 – 917 ng l^{-1}) in comparison to the other compounds. In terms of spatial distribution, a pattern was observed, where highest concentrations were found at a site where wastewater is discharged, whilst compound concentrations were often lower upstream and downstream of this site. The downstream profile of pharmaceuticals differed temporally with concentrations highest downstream when input from wastewater effluent was highest. Eleven further estuaries were sampled around the UK in order to put the occurrence of pharmaceuticals seen in the Humber Estuary into a wider context. Pharmaceutical concentrations in the other estuaries sampled were less than 210 ng l^{-1}, but, again, ibuprofen and paracetamol were found at concentrations higher than other compounds, whereas diclofenac and citalopram were absent from many estuaries. The Humber, which is the receiving environment for the

sewage effluent for approximately 20% (13.6 million people) of the population of England, was observed to have the highest overall concentration of pharmaceuticals in contrast to the other estuaries sampled, thereby representing a worst case scenario for pharmaceutical pollution.

Keywords: Pharmaceuticals; Emerging Contaminants; Estuary; Occurrence; Temporal Distribution; Spatial Distribution

1. Introduction

Despite the extensive and long-term use of pharmaceuticals, it has only been in the past few decades that interest in pharmaceutical pollution has gained popularity and now hundreds of pharmaceuticals have been detected in the aquatic environment (Hughes et al. 2013; Gaw et al. 2014). Their presence in the aquatic environment is sustained through continuous input from wastewater treatment plants (WWTPs), as well as from improper disposal, agriculture and aquaculture (Godoy et al. 2015). Pharmaceuticals are designed to be biologically active, often at low levels, and their presence in surface water has led to concern over their potential biological effect (Santos et al. 2010). Many pharmaceuticals (e.g. diclofenac and fluoxetine) have been found to illicit a negative response on biota in laboratory exposures at concentrations similar to those found in the aquatic environment (Eades and Waring 2010; Franzellitti et al. 2013; Minguez et al. 2016).

The fate of pharmaceuticals is best understood in the freshwater environment, with input, environmental conditions, biological degradation and sediment-related processes playing a prominent role in their spatial and temporal distribution (Li 2014). Pharmaceuticals often show a decline in concentration downstream from input sources as the result of dilution, degradation and partitioning to sediment (Kunkel and Radkle 2012). However, due to the prevalence of WWTPs, this leads to the continuous input of pharmaceuticals into the environment. As a result, these processes are not enough to sufficiently remove compounds leading to their high detection in the aquatic environment and potentially, transportation into estuaries and coastal waters (Ebele et al. 2017).

Estuaries are receiving waters, often for many rivers, acting as a confluence for contaminants, therefore increasing the potential risk of pharmaceutical pollution in these environments (Ridgway and Shimmield 2002). Estuaries are ecologically important to ecosystem services, providing habitat for many species and acting as an area for recreation and transport (Ridgway and Shimmield 2002). Despite this, few studies have measured the occurrence of pharmaceuticals in estuaries, and those that do, exist typically lack the resolution to determine spatial and temporal patterns (Table 1). Studies which have investigated the spatial and temporal patterns of pharmaceuticals are often locally focused, monitoring only one estuary (for example Tamtam et al., 2012; Hedgespeth et al. 2012; Cantwell et al. 2017) and it is important to determine if any patterns seen are relevant at a wider scale. It is important to examine the fate of these compounds across a wider spatial scale in order to determine whether they pose a risk to the environment.

This study aimed to further contribute to the overall picture of pharmaceutical contamination in estuaries. Five target compounds — ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were chosen for the present study, based on their prevalent usage and predicted risk to the aquatic environment (National Health Service 2017; Roos et al. 2012). To the author's knowledge, citalopram has not previously been monitored in the estuarine environment (Table 1). Moreover, monitoring of the aforementioned compounds is limited, with some of these measurements dating back almost 15 years. The target compounds were measured every other month over a twelve month period at various sites in the Humber Estuary to determine their spatial and temporal occurrence. In addition, eleven further estuaries, located in other parts of the UK, were selected in order to determine whether concentrations observed in the Humber were representative of other estuaries.

79 **Table 1:** Maximum concentrations of ibuprofen, paracetamol, diclofenac and trimethoprim detected in

80 estuaries globally (ng l^{-1}). Citalopram has not previously been monitored in any estuaries.

Region	Estuary	Ibuprofen	Paracetamol	Diclofenac	Trimethoprim	Reference
Asia	Jiulong, China	21	13	11		Sun et al. (2016)
	Hailing Bay, China				37	Chen et al. (2015)
	Qinzhou Bay, China			7		Cui et al. (2019)
	Yangtze, China			<MDL		Yang et al. (2011)
	Yangtze, China				330	Zhang et al. (2012)
	Yangtze, China		<MDL			Zhao et al. (2015)
Europe	Seine, France				45	Tamtam et al. (2008)
	Elbe, Germany	1		1		Weigel et al. (2002)
	Arade, Portugal	28	88	31		Gonzalez-Rey et al. (2015)
	Douro, Portugal				16	Madureira et al. (2010)
	Tejo, Portugal	<MDL	11	52	8	Reis-Santos et al. (2016)
	Bilbao, Spain		440	650	2046	Mijangos et al. (2018)
	Plentzia, Spain		49	22	6	Mijangos et al. (2018)
	Urdaibai, Spain		321	35	3	Mijangos et al. (2018)
	Belfast Lough, UK	376	<MDL	<MDL	32	Thomas and Hilton (2004)
	Mersey, UK	386	<MDL	195	569	Thomas and Hilton (2004)
	Tees, UK	88	<MDL	191	17	Thomas and Hilton (2004)
	Thames, UK	928	<MDL	125	<MDL	Thomas and Hilton (2004)
	Thames, UK				19	Munro et al. (2019)
	Tyne, UK	755		90	46	Thomas and Hilton (2004)
North America	Charleston Harbour, USA	8	28			Hedgespeth et al. (2012)
	Jamaica Bay, USA	38	156		125	Benotti and Brownawell (2007)
	Narragansett Bay, USA		60		18	Cantwell et al. (2017)

	New York Bay, USA	162	14	Cantwell et al. (2018)
	San Francisco, USA		4	Klosterhaus et al. (2013)
Oceania	Sydney, Australia	31		Birch et al. (2015)

2. Methods

2.1 Study Area

The Humber Estuary is a macrotidal estuary located in Yorkshire, on the East Coast of England, UK (Figure 1). It is 303 km^2, has an average depth of 6.5 m and is the confluence for the Rivers Ouse, Trent and Hull which pass through some of the largest urban areas in the UK, thus it is the receiving water for approximately 20% of UK effluent (European Environment Agency, 2019; Table 2). Samples were collected from nine sites along a 65 km stretch on the North side of the estuary (Figure 1). Two of these were located in the River Ouse: A1 (20 km from Humber) was the furthest upstream and A2 was located less that 1km upstream from the confluence with the Humber Estuary. The furthest site upstream in the Humber Estuary (R1) was the receiving site for effluent from Melton WWTP, which serves a population equivalent (PE) of 12,255 (European Environment Agency, 2017). Three sites (R2-R4) were positioned every 2 km downstream from R1. Three final sites (A3-A5) were located 20km from R1 in the lower estuary and 15 km from the mouth. Further information on site location can be found in Supplementary material S1. The Humber Estuary is an important site for conservation and has been designated as a Special Protection Area (SPA), also containing a Special Area of Conservation (SAC). It is also a vital habitat for many species of international importance, providing habitat for 4.1% of the red knot (*Calidris canutus*) and 5.7% of the common redshank *(Tringa tetanus*) international populations, and as a result has also been designated as a RAMSAR site (Buck et al. 1997)

Samples were also collected from eleven further estuaries which encompassed a range of estuary types, tidal ranges and sizes (Table 2). The total PE was calculated for the WWTPs

in the catchment area of each estuary (Table 2); further information on the proximity of WWTPs to the sampling sites in each estuary can be found in supplementary material S2. Many of these estuaries have been designated as SACs, SPAs and RAMSAR sites as the result of the sensitive and important species resident to them.

Table 2: Information on the type and size of estuaries sampled (Davidson et al.1991). Information on the number of WWTPs and the population equivalent served in 2014 was calculated from an interactive wastewater treatment map (European Environment Agency 2019).

Estuary	Type	Estuary Area (km^2)	Tidal Type	Number of WWTPs in Catchment	Total PE (000s)
Cromarty	Complex	92.3	Mesotidal	3	15.6
Forth	Complex	84.0	Macrotidal	33	1 613.3
Humber	Coastal Plain	303.6	Macrotidal	304	13 674.7
Mersey	Coastal Plain	89.1	Macrotidal	30	3 689.7
Portsmouth	Ria	15.9	Macrotidal	2	383
Severn	Coastal Plain	556.8	Macrotidal	171	6 724.4
Solway	Complex	420.6	Macrotidal	20	314.9
Tay	Complex	121.3	Mesotidal	12	167.6
Tees	Coastal Plain	13.5	Macrotidal	9	844.9
Thames	Coastal Plain	46.5	Macrotidal	198	16 510.5
Tyne	Complex	7.9	Macrotidal	6	1 092.8
Ythan	Barbuilt	2.8	Mesotidal	1	11.2

2.2 Sampling

2.2.1 Seasonal Monitoring

Sampling was carried out in the Humber Estuary, UK, every two months from October 2016 to August 2017 at sites R1-R4 (Figure 1). Samples were also collected from four additional sites (A1-A2 and A4-A5) in October, February and June, and a further site (A3) in February and June (Figure 1). Sampling was carried out during a high neap tide (± 3 hours) to minimise differences in diurnal concentrations as the result of tides (Lara-Martin et al. 2014). At each site, 3 x 1 L of surface seawater were collected in amber glass bottles and temperature, pH and dissolved oxygen were determined using a HACH meter and salinity (0 – 27 ppt) measured with a refractometer (Supplementary material S1). Water samples were kept on ice or in the fridge at 4 °C and extracted within 48 hours for analysis of pharmaceuticals.

2.2.2 UK Wide Monitoring

Sampling was carried out in eleven additional UK estuaries in order to provide a wider context for the concentrations of pharmaceuticals seen in the Humber Estuary (Figure 1). Sampling was carried out in August and September 2017 and samples were also collected during high tides (± 3 hours). Within each estuary, sites were chosen in the upper, middle and lower parts of the estuary and 1 L of water was collected at each of these in amber glass bottles. (Supplementary material S2). Temperature, pH, dissolved oxygen and salinity (0-34 ppt) were determined as above and samples stored and extracted in the same manner (Supplementary material S2).

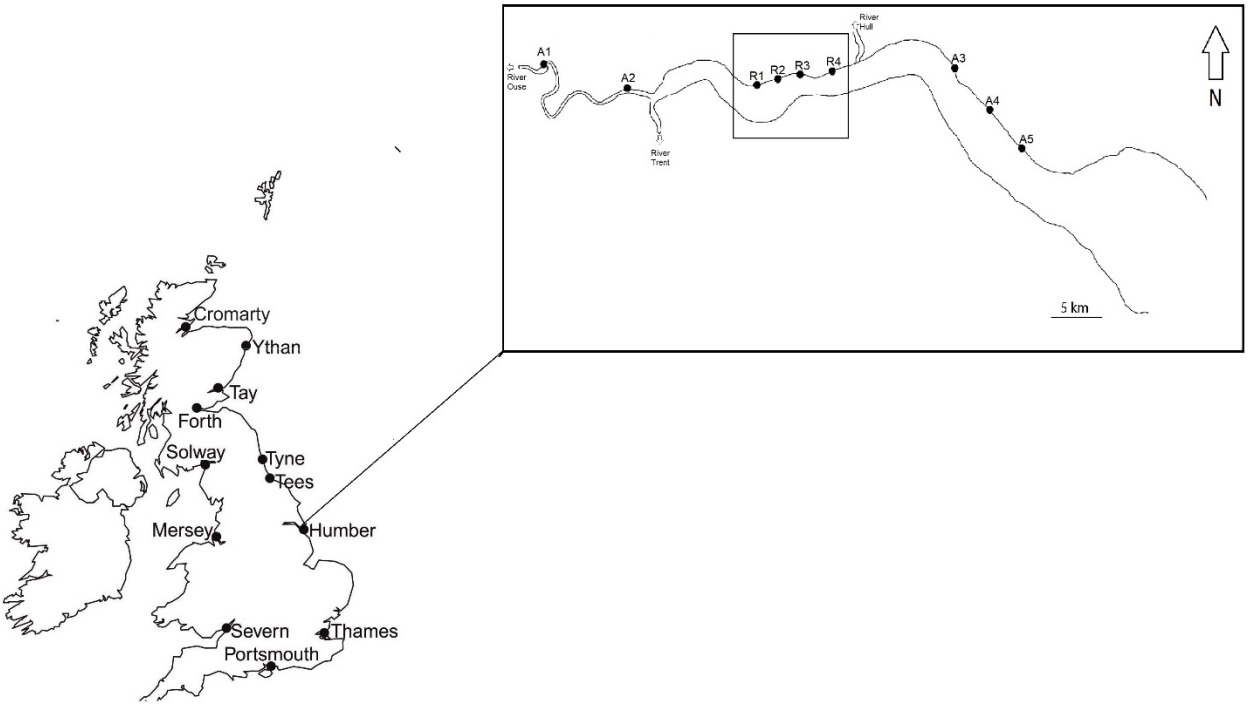

Figure 1 Map of field sites for seasonal and UK wide monitoring of selected pharmaceuticals. The sites in the box (R1-R4) indicate those which were sampled every two months. A1-A2 and A3-A5 were sampled every four months.

2.3 Chemical Analysis

2.3.1 Study Compounds

Five study compounds — ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram, were chosen for monitoring (Table 3). Standards of diclofenac sodium (≥98.5), acetaminophen (≥99%), citalopram (≥98), ibuprofen (≥98%), and trimethoprim (≥98%) were supplied by Sigma-Aldrich Ltd. (Dorset, UK).

Table 3: Physico-chemical characteristics of the study compounds. Physico-chemical data obtained from Alygizakis et al. (2016), Bayen et al. (2013) and Kasprzyk-Hordern et al. (2007). Prescription data obtained from (National Health Service 2019; Supplementary material S3).

Compound	Therapeutic Use	Prescriptions (kg year^{-1})	Water Solubility (mg l^{-1})	Log$_{KOW}$	Molecular Weight	pKa
Ibuprofen	Nonsteroidal anti-inflammatory drug (NSAID)	82,756	41.05	3.97	206.29	9
Paracetamol	Painkiller	2,169,244	22.7	0.9	151.16	9.9
Diclofenac	NSAID	5459	4.52	4.51	296.15	4.2
Trimethoprim	Antibiotic	8444	171.1	1.4	290.32	7.1
Citalopram	Antidepressant	9204	4.02	3.74	324.39	9.4

2.3.2 Solid Phase Extraction

A composite sample was made, by combining the 3 x 1L surface water samples collected from each site during seasonal monitoring, or from each of the estuaries during the UK-wide survey; they were added together in a 5 L beaker and stirred vigorously for two minutes. A 500 mL subsample was taken and filtered through a 0.45 µm cellulose filter (Scientific Laboratory Supplies, Hessle, UK) under vacuum. Solid phase extraction was performed on the filtered water samples using Oasis HLB cartridges (Waters Corporation, Massachusetts, USA), which were conditioned with 5 mL 100% methanol followed by 5 mL deionised water at a rate of 1 mL min^{-1}. The sample was loaded on to the cartridge at a rate of 10 mL min^{-1}, during which care was taken not to let the sorbent material dry out. The cartridges were then rinsed with 5

mL deionised water. The sorbent was dried under vacuum for 15 minutes to remove excess water prior to elution. Elution was performed with 5 mL 0.1% trifluroacetic acid in methanol, followed by a further 5 mL. The eluent was evaporated to dryness using a rotary evaporator (40ºC, speed 7) and reconstituted with methanol: water (10:90).

SPE recovery was evaluated by spiking known concentrations (100, 200, and 1000 ng l^{-1}) of all study compounds into three replicates each of artificial seawater made up to 20 ppt in deionised water (Supplementary material S4). The mean recovery across all concentrations was used to correct the measured environmental concentration (Table 4).

Table 4: Mean method detection limits (± standard deviation), mean method quantification levels (± standard deviation) and mean recovery (± standard deviation) of target compounds.

Compound	MDL (ng l^{-1})	MQL (ng l^{-1})	Recovery (%)
Citalopram	0.34 (0.25)	1.18 (0.85)	43 (5.5)
Diclofenac	1.77 (1.35)	5.91 (4.49)	20 (11.0)
Ibuprofen	1.45 (0.41)	4.83 (1.38)	73 (34.0)
Paracetamol	3.28 (1.82)	10.93 (6.07)	86 (34.1)
Trimethoprim	0.07 (0.04)	0.24 (0.12)	63 (10.6)

2.3.3 UltraperformanceTM-ESI-(QqLIT) MS/MS analysis

Analysis was carried out according to Gros et al. (2012). Briefly, chromatographic separations were performed with a Waters Acquity Ultra-Performance liquid chromatograph system equipped with two binary pumps systems (Milford, Massachusetts, USA), and coupled to a 5500 QTRAP hybrid quadrupole-linear ion trap mass spectrometer with a turbo ion spray source (Applied Biosystems, Foster Systems, Foster City, CA, USA). Citalopram and trimethoprim were analysed under positive electrospray ionisation (PI) using an Acquity HSS T_3 column (50 mm x 2.1 mm, 1.8µm particle size) and ibuprofen, paracetamol and diclofenac were analysed under negative ion (NI) electrospray using an Acquity BEH C_{18} column (5 mm x 2.1 mm, 1.7 µm particle size), both from Waters Corporation.

All data acquisition was performed in Analyst 2.1 software. Quantification of analytes was performed by selective reaction monitoring (SRM), monitoring two transitions for each compound as described in Gros et al. (2012). Method detection limits (MDL) and Quantification levels (MQL) were determined for each of the compounds based on a signal-to-noise ratio of 3 and 10, respectively (Table 4).

2.4 Statistical Analysis

Statistical analysis was performed in R 3.3.1. In order to determine if there was a difference in the occurrence of pharmaceutical between sampling months, concentrations from Melton, North Ferriby, Hessle East and Hessle West were grouped together, as these sites were sampled during all of the sampling periods. A Friedman's Test followed by a Nemenyi post-hoc test were conducted using the PMCMR package (Pohlert 2014). All data is presented in graphs created by the ggplot2 package (Wickham 2016).

3. Results

3.1 Humber Estuary

Pharmaceuticals were frequently detected (58 - 97% of samples for individual study compounds) in the Humber Estuary (Table 5) and concentrations followed the order of ibuprofen>paracetamol>diclofenac>trimethoprim>citalopram. Whilst mean concentrations were in the order of 100 ng l^{-1} or below, maximum concentrations were approximately 5 to 10 times higher (Table 5; supplementary material S5). Maximum levels of ibuprofen and paracetamol detected in the Humber are the highest concentrations reported in estuaries to date (Table 1). Furthermore, this is the first study to detect in the estuarine environment (Table 1).

Table 5: Pharmaceutical concentrations (ng l^{-1}) in surface water in the Humber Estuary (n=38) during a 12 month sampling campaign. Values were corrected based on mean recovery values (Table 3). Max = maximum concentration, SD = standard deviation. Detection rate is the amount of samples above the method quantification limit (MQL).

Compound	Detection Rate (%)	Max (ng l^{-1})	Mean (ng l^{-1})	SD
Ibuprofen	97.37	6297.14	665.58	1481.49
Paracetamol	73.68	916.88	88.65	163.66
Diclofenac	57.89	250.8	51.44	68.29
Trimethoprim	92.11	247.02	27.43	54.56
Citalopram	89.47	42.93	6.39	7.66

A general pattern was observed in the occurrence of pharmaceuticals in the Humber surface water, with pharmaceutical concentrations peaking at sampling site R1 (Figures 2) and concentrations upstream (samplings sites A1-A2) and downstream (sampling sites R2-A5) of this site similar to each other. Conversely, this pattern was not consistent in that the chemical concentrations at some of the sampling periods (for instance: paracetamol and diclofenac in June), displayed a reduction in levels downstream (A3-A5). Maximum concentrations were generally seen at sampling site R1 although during some of the sampling periods, they also occurred at sites R2-R4.

Of the three months where all sites were sampled, February had the highest detection rates and concentrations of pharmaceuticals at downstream sites (A3-A5), whilst many of the compounds were absent at these sites in October and June (Figure 2). In contrast, ibuprofen was an exception to this with compounds found at these sites during all of the sampling periods. Citalopram also showed little decline in downstream concentrations in June, and was present at A3-A5, at concentrations similar to or higher than many of the sites further upstream (Figure 2). There appeared to be a relationship between the concentration of pharmaceuticals

at R1 and those seen at the other sites; typically, a higher concentration at R1 resulted in a higher presence at sites further downstream (Figure 2).

Sites R1-R4 were sampled more frequently than the other sites, and Trimethoprim was the only compound to show a statistically significant difference between sampling months (Friedman's Test, chi-squared = 14.71, $p < 0.05$) with concentrations, significantly higher in winter (December and February; 3.29 – 166.54 ng l^{-1}), compared to October and the summer months (June and August; 0 – 142 ng l^{-1}; Figure 3). Nevertheless, the difference was almost significant for ibuprofen ($p = 0.054$) and citalopram ($p = 0.051$). For citalopram, February had the highest concentrations (3.74 – 42.93 ng l^{-1}), whereas ibuprofen concentrations were higher in April and June (186.37 – 6297.14 ng l^{-1}; Figures 3) in comparison to the other sampling periods. All compounds had lowest mean concentrations in August (Figures 3), with no peaks seen at sampling site R1 (Figure 2).

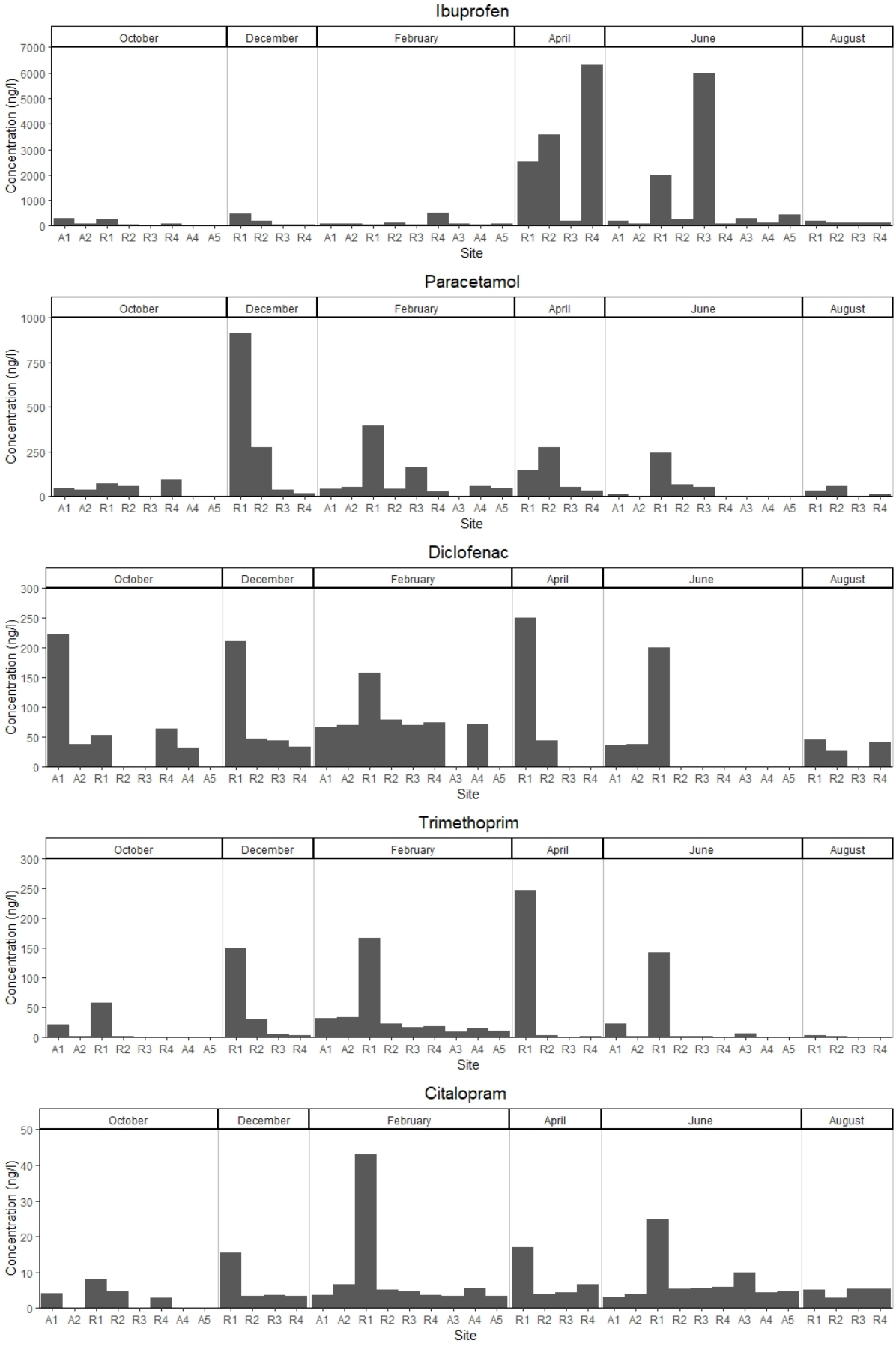

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Figure 2 Concentrations of target analytes at nine sites in the Humber Estuary. Values were corrected based on mean recovery values (Table 3). Sites are listed from furthest upstream (A1) to furthest downstream (A5). R1-R4 were sampled every sampling event, whilst the other sites were only sampled in October, February and June, except for A1 which was not sampled in October.

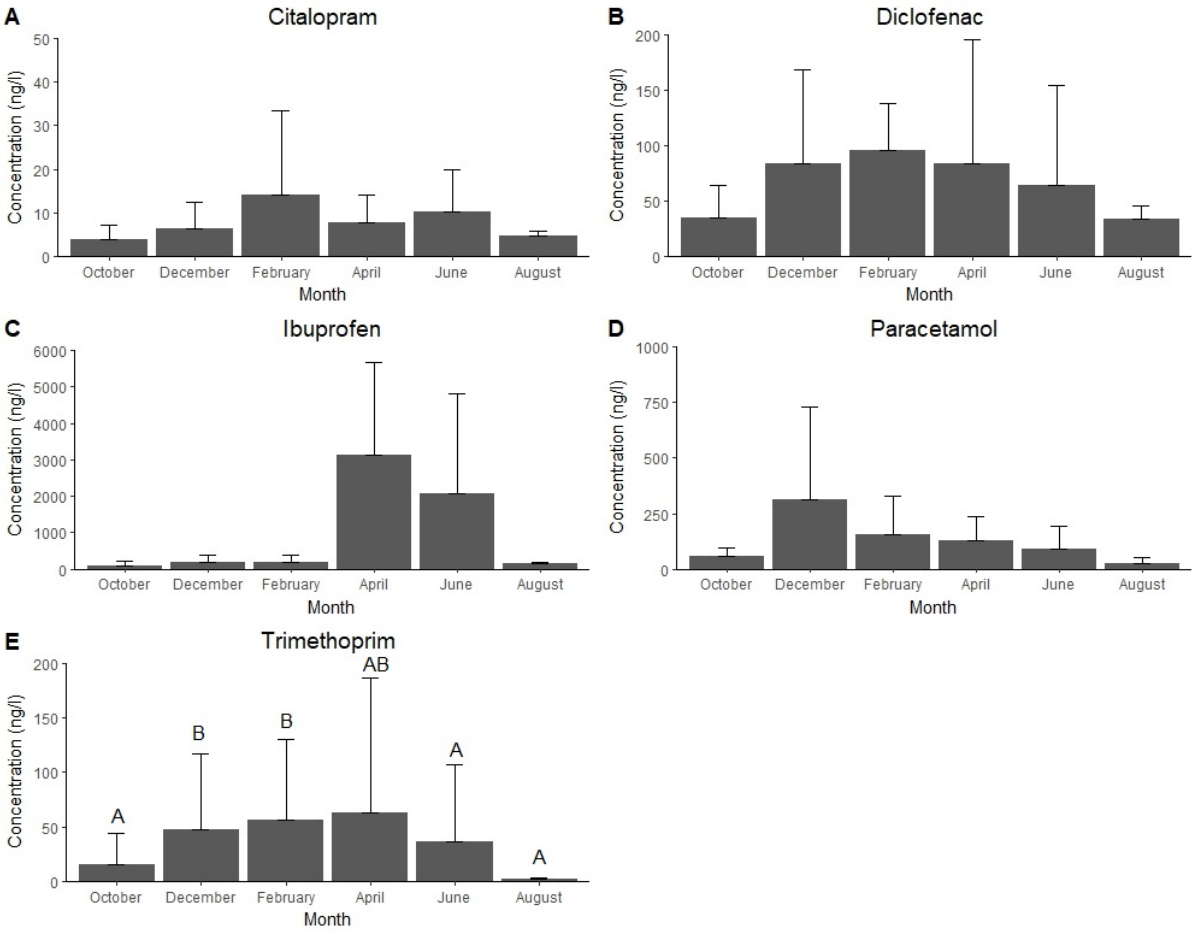

Figure 3 Mean bi-monthly concentrations (± one standard deviation) of (A) Ibuprofen (B) Paracetamol (C) Diclofenac (D) Trimethoprim and (E) Citalopram at the four sites monitored most frequently (R1-R4). Values were corrected based on mean recovery values (Table 3). Letters denote statistically significant difference (Friedman's Test).

3.2 UK-wide sampling

Pharmaceuticals were detected in all of the estuaries sampled around the UK but only at concentrations in the low ng l^{-1} range and were generally present at concentrations lower than those detected in the Humber Estuary (Figure 4). The order of pharmaceuticals were similar

to that found in the Humber (ibuprofen>paracetamol>diclofenac>citalopram>trimethoprim), except trimethoprim was found at lowest concentrations (supplementary material S6). Ibuprofen and trimethoprim were present in all of the estuaries sampled, whereas diclofenac was only detected in two of the other estuaries, the Cromarty and Thames (Figure 4). The Thames and Humber were the only estuaries to contain all of the compounds. The Humber had the overall highest concentration of pharmaceuticals, and only the Cromarty and Tay had a total concentration of pharmaceuticals over 200 ng l^{-1} (Figure 4).

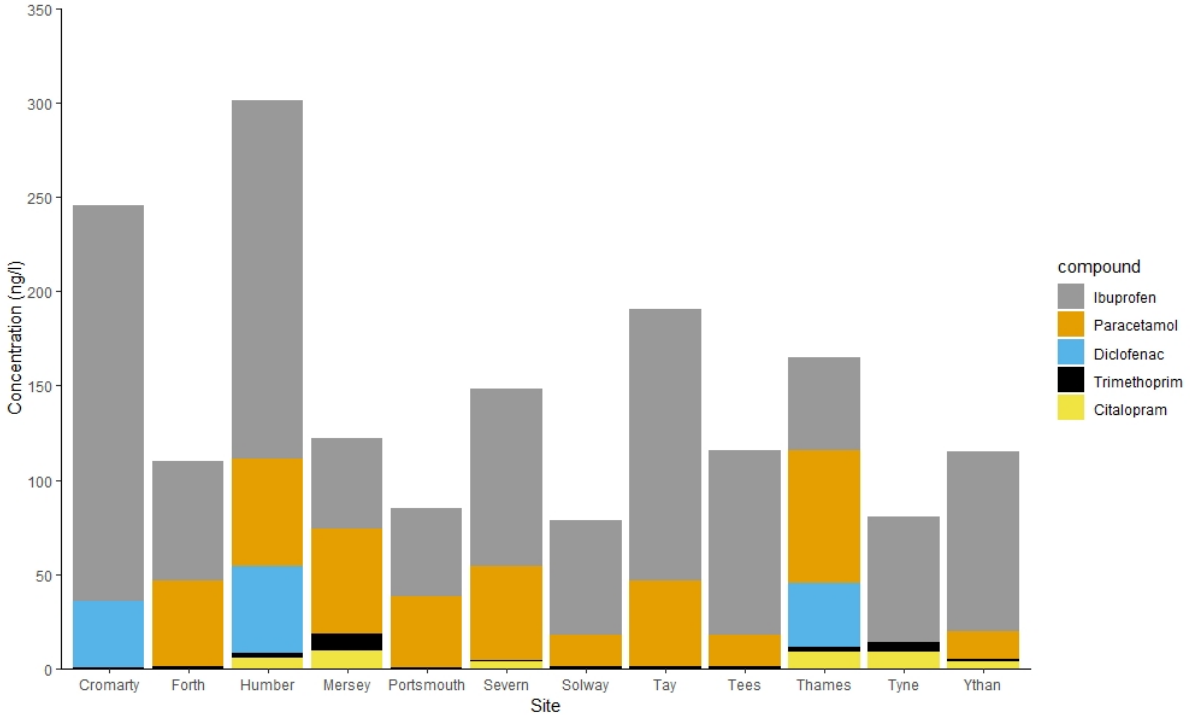

Figure 4 Concentrations of citalopram, diclofenac, ibuprofen, paracetamol and trimethoprim across eleven estuaries in the UK. Concentrations have been corrected for recovery (Table 3). Concentrations reported for the Humber are maximum concentrations measured in August, when the wider UK survey was undertaken.

4. Discussion

Most monitoring studies to date have been carried out in freshwater systems as it was originally thought that estuaries and coastal waters would dilute compounds so that they would

be undetectable (Fabbri and Franzellitti 2016). Despite this hypothesis, pharmaceutical contamination was found to be widespread as all of the estuaries monitored contained at least three of the target analytes at levels of a similar magnitude to those found in the freshwater environment, and higher than those measured in many other estuaries (Hughes et al. 2013; Table 1). The levels of pharmaceuticals detected in this study, contribute to the overall picture on pharmaceutical pollution and add to the growing evidence that it is a global issue (aus der Beek et al. 2016). Our work indicates that the limited monitoring carried out to date may not have captured peak concentrations that occur in these environment and clearly highlights that further work is needed.

Ibuprofen was detected at the highest concentrations and in all of the estuaries sampled, with its occurrence not only exceeding levels detected in other estuaries (Table 1), but also those seen in river water both in the UK (Barbara Kasprzyk-Hordern et al. 2008; Kay et al. 2017; Burns et al. 2017, 2018), as well as globally (Hughes et al. 2013). Ibuprofen has only been measured in 7 estuaries previously, with maximum concentrations all under 100 ng l^{-1} (Table 1). Further monitoring studies should include ibuprofen as a priority to determine if high concentrations seen in the UK are similar to those elsewhere.

Concentrations of paracetamol, diclofenac and trimethoprim were similar to those seen in other global estuaries, with mean concentrations less than 100 ng l^{-1} (Table 1). Whilst maximum concentrations of paracetamol were similar to those detected in rivers (Barbara Kasprzyk-Hordern et al. 2008; Burns et al. 2017), concentrations of diclofenac and trimethoprim were considerably lower (Hughes et al. 2013; Nakada et al. 2017). In the present study, water samples were collected at high tide, when concentrations would be expected to be lowest, so it is possible that these levels could be higher at other points in the tidal cycle (Yang et al. 2016). This is the first study to measure the occurrence of citalopram, however concentrations were low and did not exceed 50 ng l^{-1}. These low concentrations are in agreement with previous studies which have monitored citalopram in rivers (Hughes et al.

2013). Despite these low concentrations, PNECs for citalopram are below this (Minguez et al. 2016).

Whilst an overall widespread occurrence of pharmaceuticals was seen in the UK, patterns in their spatial and temporal distributions within and between estuaries were observed.

4.1 Humber Estuary

4.1.1 Spatial Variation

It is generally expected that pharmaceutical concentrations will decrease downstream due to physical processes in an estuary leading to their breakdown and removal (Daughton 2016). The spatial pattern of pharmaceutical occurrence in the Humber Estuary followed this pattern to a degree; peak concentrations were found in the middle of the estuary, particularly at R1, where samples were collected next to an outlet from a wastewater treatment plant (WWTP), indicating that WWTPs could be a significant source of pharmaceuticals in the Humber Estuary. Input from WWTPs has been attributed as the largest source of pharmaceutical pollution in the aquatic environment (Caldwell 2016). In some cases maximum concentrations were detected outside of this site; in April and June, maximum concentrations for paracetamol and ibuprofen occurred at sites R2-4. It is difficult to determine what caused these peaks as composite sampling can lead to uncertainty in the representativeness of samples in cases such as this, however these sites are within 6km from R1, so it is possible that the large increases seen at these sites are still due to input at R1, and fluctuations of concentrations between these sites are the result of sampling timing or within sample variation (Ort et al. 2010). The site (R4) which showed the highest levels (6.2 µg l^{-1}) of ibuprofen was also 7km upstream from the confluence of the River Hull. Transport of pharmaceuticals from this tributary upstream during high tide could also account for the increases seen. The River Trent, located near the confluence with the Ouse (Figure 1), will also account for the addition of further pharmaceuticals. Inputs of pharmaceuticals in other studies have also been attributed

to other sources such as improper disposal, leaching from landfills or through veterinary usage and subsequent runoff of these compounds into the aquatic environment, which could account for these differences. (Bound and Voulvoulis 2005; Ebele et al. 2017).

Dilution plays a key role in the fate of pharmaceuticals in the aquatic environment and the decrease in concentrations after R1 is presumably caused by dilution away from the input source (Baker and Kasprzyk-Hordern 2013). Decline of pharmaceutical concentrations downstream the estuary was observed more in some compounds than others, and as a result, is unlikely to be fully explained by dilution. Degradation of pharmaceuticals has been found to be a significant factor affecting the fate of pharmaceuticals and could account for these differences (Caracciolo et al. 2015). Citalopram experienced the lowest decrease in concentration downstream, and was typically the same concentration, or higher at A5 than A1, which could be explained by the low degradation which has been observed in other studies (Metcalfe et al. 2010; Styrishave et al. 2011). Ibuprofen, paracetamol and trimethoprim also showed little decline in concentration beyond initial dilution after R1, which is consistent with what has been seen at other sites. These compounds have been found up to 10 km downstream from a WWTP (Bendz et al. 2005, Kay et al. 2016, Burns et al. 2018), and trimethoprim has even been found 200 km downstream from an WWTP (Tamtam et al. 2008). Further WWTPs are located within the estuary (European Environment Agency, 2019) which could also account for this lack in decline. Diclofenac on the other hand, was not detected at A3 or A5 during any of the sampling periods, but was found at A4. The downstream decline of diclofenac has been found to be variable, with some studies finding it to be more persistent than others (Bendz et al. 2005; Wilkinson et al. 2017). Removal of compounds through degradation and sorption to sediment has been found to be highly dependent on environmental conditions and sediment type.

4.2.1 Temporal Variation

Seasonal differences of pharmaceuticals have been observed in a number of studies and these are often attributed to changes in usage and local environmental conditions (Golovko et

al. 2014b; Moreno-González et al. 2014). Trimethoprim was the only compound to show significant temporal differences in concentrations (at sites R1-R4), with average winter concentrations over double that of those during the summer months. Previous studies have explained the seasonal occurrence of antibiotics in winter due to their higher usage in those months to treat seasonal infections (Verlicchi and Zambello 2016). The temporal differences seen in the occurrence of trimethoprim in the Humber Estuary appeared to follow this pattern, as prescriptions were highest in October 2016 to March 2017 and lowest in August 2017 (Supplementary material S4). Trimethoprim has been observed to have higher winter concentrations in some studies (Golovko et al. 2014b) but not in others (Burns et al. 2018). Burns et al. (2018) found higher levels of trimethoprim during spring in the Ouse (upstream from A1), which was attributed to hydrological differences seen between the seasons sampled. As a result, it is likely that the temporal differences in trimethoprim are the result of different site specific conditions or daily variations. Temporal variations in other studies have also been explained by lower temperatures, leading to lower degradation (Golovko et al. 2014a), however, input at R1 was highest in April. The other target compounds have exhibited in other locations, but did not in the Humber. Paracetamol, for instance, has been detected at high concentrations in spring in some rivers but winter in others, whilst other studies found no temporal variations (Paíga et al. 2016; Ma et al. 2017; Burns et al. 2018).

Temporal variations in the downstream pattern of pharmaceuticals were also observed, with the greatest variation seen at the sites furthest downstream (A3-A5). Pharmaceuticals were mostly absent from these sites in October, with the exception of ibuprofen, where concentrations were reduced. Sampling at high tide could account for the absence of these pharmaceuticals downstream as the result of increased dilution or transport of contaminants upstream (Munro et al. 2019). Pharmaceutical concentrations often fluctuate diurnally as the result of timing of effluent discharges from WWTPs and combined sewer overflows (CSOs), as well as variations in wastewater as the result of consumption patterns (Xu et al. 2007). To an extent, there was a pattern in the presence of compounds at R1 consistent with those seen

downstream the estuary, so it is possible that the temporal variations could be the result of these daily variations, instead of conditions seen seasonally. The concentration of pharmaceuticals at R1 were lowest in October and the low input could, in part, account for the absence of compounds seen at sites furthest downstream (A3-A5). Likewise, concentrations for the majority of compounds were highest at R1 during February where concentrations were highest at sites furthest downstream (A3-A5). This is further evidence that there is a difference in input from WWTPs. R1 is not the only site at which wastewater is discharged, but if these other sites exhibit the same temporal variations, then it could explain the differences observed in concentrations at A3-A5. WWTP removal has been found to be less efficient during the winter time due to lower temperatures and decreased biodegradation, leading to higher concentrations in effluent (Vieno et al. 2005). At R1, concentrations for all compounds were lowest in August when temperatures were warmest (Supplementary material S1).

4.3 UK Estuaries

The Humber Estuary was shown to represent a worst case scenario in terms of pharmaceutical pollution, with all five pharmaceuticals present at relatively high concentrations. Of the estuaries sampled, it was the second highest impacted by WWTPs, with a PE of approximately 13.7 million people. The Thames, which was the most impacted, was the only other estuary to contain all five compounds. A higher presence of pharmaceuticals is frequently seen in large urban areas due to their increased usage (Hong et al. 2018). With the exception of both the Humber and the Thames estuaries, there was no apparent relationship between the number of WWTP and concentrations (Table 2). The Cromarty Firth, which was the receiving water of only 3 WWTPs (15,600 PE), exhibited similar levels of pharmaceuticals to the Humber. This could be explained by differences WWTP efficiency, as technology used in WWTPs can greatly affect the removal of pharmaceuticals. For example, ibuprofen removal has been reported to be between 7% and 99% at different WWTPs (Radjenovic et al. 2007; Jelic et al. 2015). It is possible that the removal efficiency of

WWTPs could differ between areas, with rural areas being less efficient as they are serving smaller populations. Rural areas are more likely to have a higher occurrence of septic tanks, which could contribute to the elevated levels seen in the Cromarty (Hanamoto et al. 2018). Whilst the Humber experienced the lowest concentration in August, it is possible that seasonal variations in population in areas like the Scottish Highlands (a tourist destination), where the Cromarty is located, could be responsible for these higher concentrations, increasing pressure on WWTPs. Pharmaceuticals in a Portuguese river have previously shown higher concentrations which was thought to be the result of increased summer populations (Rocha et al. 2014).

The presence of pharmaceuticals is greatly influenced by environmental conditions and proximity of the sampling site to input sources, possibly accounting for some of the apparent differences in concentrations observed between estuaries. Water samples from different locations in the estuary were mixed together and a subsample was taken to obtain a snapshot of the presence of pharmaceuticals, and it is likely that these concentrations will vary depending on these factors. This could possibly explain the absence of diclofenac, which in the Humber study was frequently undetected in sites downstream the estuary. Citalopram also had a low detection (50%) in estuaries, however, it was detected in estuaries which have the highest PE.

There are also likely to be more complex interactions in play which further affect the occurrence of pharmaceuticals in estuaries and can help to explain the spatial differences seen. Differences in site specific conditions such as salinity profiles and hydrology can affect sorption processes, degradation and dilution. Undoubtedly, these processes, in conjunction with daily variations in rainfall and temperature, are likely to be responsible for differences in concentrations in estuaries between sampling periods, yet it is still clear that pharmaceutical pollution is a ubiquitous problem in estuaries (Tamtam et al. 2008).

Ibuprofen, paracetamol, diclofenac and trimethoprim were previously monitored in the Mersey, Thames, Tees and Tyne estuaries (as well as Belfast Lough) in 2002 (Thomas and Hilton,

2004). It was also found that ibuprofen was present at highest concentrations. Paracetamol, however, was not detected in any of the estuaries sampled in 2002, which indicates that the occurrence of this compound could be rising. A rise in pharmaceuticals would be consistent with what has been found in other areas. For example, analysis of sediment cores in Jamaica showed an overall rise in pharmaceutical concentrations over time, with these concentrations doubling over the last decade (Lara-Martin et al. 2015). This highlights the importance of establishing baseline measurements of pharmaceuticals, in order to determine areas most at risk and therefore require continued monitoring. The Humber Estuary likely poses the greatest risk, particularly due to the high level concentrations of ibuprofen. Other large urban estuaries (such as the Thames and Severn) may also warrant a further detailed study. However, as seen with the Cromarty, focus on monitoring should be extended to rural areas as well.

5. Conclusion

All five target analytes — ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were detected in twelve estuaries in the UK. Diclofenac is a compound that has been highlighted as a potential concern, yet paracetamol and ibuprofen were consistently detected at higher concentrations and at levels which could be toxic to aquatic organisms (Vestel et al. 2016). In particular, the concentrations of ibuprofen measured indicate that the limited monitoring of pharmaceuticals in estuaries around the globe to date has not accurately quantified peak concentrations. Whilst trimethoprim was detected in every sample it was only present at concentrations in the low ng l^{-1} range. Citalopram was present at lowest concentrations, but also showed the least change in concentration downstream the estuary. A more intensive monitoring regime of the Humber Estuary showed that pharmaceutical input from WWTPs is a significant source and could explain the overall higher concentrations of pharmaceuticals in large urban estuaries. Despite this, a rural estuary had the highest concentration of ibuprofen which may be due to lower removal at smaller rural sewage works.

More detailed studies need to be undertaken in order to understand the complex interactions taking place in estuaries which could affect the fate of pharmaceuticals.

Whilst there was little significant variation of pharmaceutical concentrations between sampling periods in the Humber Estuary, August typically had the lowest input from WWTPs and overall lowest concentrations, which is when samples were taken from estuaries throughout the UK. Consequently, it could be expected that pharmaceutical concentrations may exceed those measured. Additionally, samples were taken on a high tide when it would be expected that concentrations are lowest due to dilution. This study provides an important baseline of pharmaceutical measurements in the UK, and highlights ibuprofen as a compound which may warrant further assessment. This work provides further evidence to the growing problem of pharmaceutical pollution, highlighting that it is not only an urban and localised issue.

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