1	The effects of wet wipe pollution on the Asian clam, Corbicula fluminea
2	(Mollusca: Bivalvia) in the River Thames, London
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11	ABSTRACT
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13	The aim of the present study was to evaluate "flushable" and "non-flushable" wet
14	wipes as a source of plastic pollution in the River Thames at Hammersmith, London
15	and the impacts they have on the invasive Asian clam, Corbicula fluminea, in this
16	watercourse. Surveys were conducted to assess whether the density of wet wipes
17	along the foreshore upstream of Hammersmith Bridge affected the distribution of C .
18	fluminea. High densities of wet wipes were associated with low numbers of clams
19	and vice versa. The maximum wet wipe density recorded was 143 wipes m^{-2} and
20	maximum clam density 151 individuals m ⁻² . Clams adjacent to the wet wipe reefs
21	were found to contain synthetic polymers including polypropylene (57%),
22	polyethylene (9%), polyallomer (8%), nylon (8%) and polyester (3%). Some of these
23	polymers may have originated from the wet wipe reefs.
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26	Keywords:
27	Wet wipes
28	Microplastic pollution
29	Corbicula fluminea
30	River Thames
31	FTIR spectroscopy
32	London
33	
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37 **1. Introduction**

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39 The working daytime population of central London is ca. 10 million people 40 (Piggott, 2015) and according to Barnes et al. (2009) areas with a higher population 41 density are often more affected by plastic pollution. In London, the high population 42 density and its associated plastic waste (Morritt et al., 2014; McGoran et al., 2017, 43 2018) have significantly polluted the River Thames. Due to the tidal nature of the 44 Thames, downstream (east) of Teddington Lock, plastic debris is able to accumulate 45 along certain reaches of the river on account of it being deposited on the foreshore 46 with tidal cycles (Thompson et al., 2009). Thames21, a charity working in improve 47 the Thames waterways, conduct a biannual 'Big Count' survey to quantify the 48 amount and types of plastics found along the foreshore of the river. Roughly, one-49 third of the plastic found are toiletry items including wet wipes which comprise 18% 50 of the total litter recorded by Thames21 (2019). Another Thames study (Morritt et al., 2014) found sanitary items ca. 22% of total litter recorded. These toiletry items 51 52 originate from sewage effluent which overflows into the Thames. The overflows also 53 distribute large numbers of microbeads and synthetic fibres (Horton et al., 2017; 54 Mintenig et al., 2017). While sewage treatment works have the potential to remove 55 ~98% of synthetic fibres, many are still released into the watercourse due to such 56 high population densities (Mintenig et al., 2017; Munoz et al., 2018). This is a 57 particular issue after rainfall when the antique treatment works can only deal with 58 small amounts of precipitation by releasing it and sewage directly into the Thames 59 without processing. 60 Synthetic fibres are the most abundant form of plastic pollution found in 61 marine environments and sediments, in particular, are a sink for microplastics 62 (Thompson et al., 2004; Wright et al., 2013). Previous studies have demonstrated the ingestion of synthetic microfibres by some Thames fish species such as the European 63 64 smelt, Osmerus eperlanus, the European flounder, Platichthys flesus and roach

- 65 *Rutilus rutilus* (Horton et al., 2018; McGoran et al., 2017, 2018). The studies by
- 66 McGoran et al. (2017, 2018) found that fibres were the most dominant form of

67 ingested microplastic. Of the fish sampled, benthic species ingested more plastics

than pelagic. This could potentially be due to their close association with sediment

70 (McGoran et al., 2018). Horton et al. (2018) also found that fibres were the most 71 abundant form of microplastic comprising 75% of all those sampled. All polymers 72 identified were either polyethylene, polypropylene or polyester. These materials are 73 all components of wet wipes; a non-woven cloth that once introduced to waterways 74 can breakdown to release microplastic fibres (Horton et al., 2018; Munoz et al., 75 2018). 76 The Asian clam, Corbicula fluminea has been studied in Chinese rivers to 77 monitor plastic pollution through the ingestion of microplastics, most notably microfibres (Su et al., 2018). These clams are highly efficient filter feeders, filtering 78 79 up to 1L of water per hour (Silverman et al., 1995) and inhabit superficial 80 sedimentary layers by burrowing using their foot and shell (Baudrimont et al., 1997). 81 These clams are an edible species and, as the soft tissue is consumed whole, they 82 provide a direct pathway for the ingestion of microplastics by humans (Su et al., 83 2018). Originating from south-eastern Asia, Corbicula fluminea is an invasive 84 species in the UK that was first recorded in 1998 (Elliott and zu Ermgassen, 2008). It 85 has previously been identified at 4 locations along the River Thames, in West 86 London (Elliott and zu Ermgassen, 2008) and provides an ideal model to assess the 87 potential impacts of plastic pollution. 88 The current study identified C. fluminea at a new location along the tidal Thames on the south bank, just upstream (west) of Hammersmith Bridge, that is 89 90 impacted by a nearby sewage outlet on the north bank and has high densities of 91 plastic pollution in the form of wet wipes and sanitary towels (Fig. 1). The site is on 92 an inside bend of the river, meaning the downstream water velocity is reduced at this 93 point. Consequently, plastic debris suspended in the water column is deposited on 94 the foreshore and subsequently exposed at low tide (Graf and Blanckaert, 2002). A 95 slipway used by St. Paul's School Rowing Club potentially acts further in slowing 96 water flow around the bend of the river, causing wet wipes to be deposited on the 97 downstream side of the foreshore towards Hammersmith Bridge. Here, an 98 investigation was undertaken to determine the density of wet wipes along the foreshore and to assess whether this affects the distribution of the C. fluminea 99 100 population at this site. This study also examined whether microfibres from the wet 101 wipes or other microplastics were being captured by the clams. Based on preliminary

containing microplastics and may be inadvertently consumed when feeding on prey

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102 observations, it was expected that there would be a significant reduction in the

- abundance of *C. fluminea* where densities of wet wipes are higher. From previous
- 104 literature, including the work of Su et al. (2018), it was also expected that the clams
- 105 would filter the surrounding Thames water and contain microfibres, similar in
- 106 polymer composition to that of the wet wipes and sanitary items that are
- 107 accumulating on the south bank foreshore.
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109 **2. Methods & materials**

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113 Fig. 1. (a) The sampling site along the south bank of the River Thames at 114 Hammersmith Bridge. To the far right is the rowing boat slipway of St Paul's School 115 that was used as a distance marker for transects which were set out eastward 116 (downstream) of this point towards the bridge. Scale bar = 60m. (b) Layout of the 12 117 transects along Hammersmith south bank foreshore: yellow arrows indicate where 118 wet wipe surveys were conducted (section 2.2), blue dots indicate where clam 119 samples were collected along the same tidal height (section 2.3) and the red arrow 120 indicates where clam densities were measured along the same tidal height (2.4) 121 (Google Earth Pro). 2.1. Survey site 122 123 Field surveys were conducted along the foreshore of the south bank, just upstream of Hammersmith Bridge (51°29'17.574" N 000°13'55.217" W; Fig. 1a), on 124 125 the River Thames between February and May 2019. This site is located adjacent to 126 the Hammersmith Pumping Station (HPS) which discharges untreated sewage

Field surveys were conducted along the foreshore of the south bank, just upstream of Hammersmith Bridge (51°29'17.574" N 000°13'55.217" W; Fig. 1a), on the River Thames between February and May 2019. This site is located adjacent to the Hammersmith Pumping Station (HPS) which discharges untreated sewage effluent into the river via the combined sewer overflow, due to a limited sewage capacity when precipitation levels are increased (Fig. 3). Thus the survey site is likely to be exposed to HPS effluent. This section of the foreshore was selected based on Thames21 methodology for their Big Count so that the data collected would be comparable with previous studies. During sampling, mounds of wet wipes and other debris were observed and appeared to alter the topography of the foreshore by forming reefs.

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Fig. 2. The amount of sewage discharged (in cubic metres or tonnes) by Hammersmith Pumping Station into the western tideway of the River Thames
 between July 2015 - September 2019 (Thames Water data).

146 *2.2. Wet wipe surveys*

147 A total of 12 transects were arranged along the foreshore at the 148 Hammersmith sampling site during low tide. The transects were laid from the bottom 149 of the bricked bank where it meets the shoreline to the low water line at 5m intervals 150 between 45–100m from the rowing slipway (Fig. 1b). Quadrats (1m²) were then placed along each transect at 5m intervals. At each quadrat, the number of wet wipes 151 152 present was recorded along with the number of sanitary items and other 153 miscellaneous plastics. Wet wipes were found by counting those present on the 154 surface and by using a hand trowel to make shallow excavations at each quadrat down-shore from the distance mark. All wipes down to a depth of ~4cm inside the 155 156 1m² quadrat were pulled out of the sediment and counted as well as any that protruded into the quadrat. Fragmented wet wipes were counted as individuals. Only 157 158 a limited number of transects were completed within one tidal cycle, so data were collected over 5 days (11th, 18th, 26th February; 5th, 8th March 2019). Differences in 159 160 tidal heights restricted the number of quadrats that could be completed on each visit, 161 as some days only allowed for measures to be made down to 20m from the 162 riverbank.

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164 2.3. Corbicula fluminea surveys

The density of wet wipes and live clams (no empty shells) were counted 165 within 0.5m² quadrats. Twenty-four quadrats were placed at ca. 2m intervals 166 between 45m and 85m from the slipway along the same tidal height of the foreshore, 167 168 and the total number of wet wipes and the total number of clams counted per $0.5m^2$ 169 quadrat. Quadrats were placed at a distance of 25.4–30.3m from the bank along the 170 same tidal height. The range of 45-85m was selected as previous surveys showed 171 that this area had the greatest variation in the density of wet wipes. Wet wipes and 172 clams were counted as per the methods outlined in section 2.2.

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174 2.4. Corbicula fluminea sampling

Asian clams were collected from the survey site in March 2019 and used for laboratory analysis. These were gathered at horizontal distance points 45m, 70m, 85m and 95m downstream from the slipway. For each of these 4 stations, a total of 3 quadrats were placed at low tide at times of 1000hrs, 1100hrs and 1145hrs (2 hours 13 minutes, 1 hour 13 minutes and 28 minutes respectively) before the predicted 180 time of low water at 1213hrs based on Port of London Authority 2019 tide tables. A total of 8 clam samples were collected from the south bank foreshore at 181 182 Hammersmith Bridge; 1 sample at 45m from the slipway, 2 at 70m, 3 at 85m and 2 183 at 90m. Due to the shape of the river and the foreshore, collecting clams at the same 184 distance measured from the riverbank would have resulted in environmental 185 heterogeneity across samples. Therefore the clams were gathered from the same tidal 186 height, as determined by marking the height of the receding tide with stakes, and 187 consequently, each collection point was comparable.

- The presence or absence of C. *fluminea* was recorded for each of the 12 188 quadrats in total and if present, samples of clams were collected. Only live clams 189 190 were counted and collected with empty shells being disregarded. This meant that 191 different numbers of clams were collected from each of the 4 stations due to 192 presence/absence. Where possible, a minimum of 30 individuals were collected per 193 quadrat, washed in situ with Thames water to remove sediment using a sieve, 194 transported back to the laboratory in clean plastic bags and stored in a laboratory 195 freezer prior to further analyses. A control sample of 12 clams was collected from 196 Chelsea Embankment (North Bank of Thames, just upstream (west) of Albert Bridge) on the 1st May 2019. This was used a site for comparison, as wet wipes were 197 198 absent from the foreshore. Due to their smaller population size, it was not possible to 199 collect large quantities of clams at this location.
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201 2.5. Sample processing

202 In the laboratory, all clams were again washed with filtered deionized water 203 (10µm) to remove residual sediment and any microplastics adhered on the surface of 204 the shell. The shell width of each clam was recorded to the nearest 0.01mm using 205 digital Vernier callipers. The soft tissue of each bivalve was dissected out of the shell 206 with a new scalpel blade, cleaned once more with filtered water $(10\mu m)$ to remove 207 microplastics potentially present in the shell. The clam tissue was then dried by 208 blotting with tissue paper, transferred to 50mL Falcon tubes and frozen at -20°C to 209 await further analysis. The soft tissue wet weight of each individual was weighed to 210 the nearest 0.0001g and digested in 50mL of 10% potassium hydroxide. The 211 solutions were then mixed and kept at 60°C in an oven for approximately 12hrs. 212 After this time, the samples were filtered through individual 10 µm CycloporeTM

Polycarbonate Membrane Filters using a Millipore vacuum filtration system. The
filters containing organic/synthetic debris were placed in Petri dishes and sealed with
Parafilm, prior to further analysis.

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217 2.6. Polymer identification

218 The filtered clam digestions were observed under a Nikon stereomicroscope 219 (model C-LEDS) to identify any microparticles including potential microplastics. All 220 particles that appeared to be sediment or chitin films were not counted. 221 Microparticles were counted on the Petri dish and categorised by colour and shape 222 (i.e., fibre/film). Due to their minute size, the length of each microparticle could not 223 be measured. An AutoIMAGE Perkin-Elmer Fourier Transform Infrared 224 Spectroscopy (FTIR) at the Natural History Museum (NHM) was used to analyse a 225 sub-sample of microparticles, including microplastic fibres to identify polymer 226 composition. Approximately 20% of the total counted particles from digestions were 227 separated and analysed as a sub-sample due to handling limitations. These particles 228 were placed under an FTIR microscope and visualised using AutoImage Microscope, 229 the aperture recorded, and the material identified using Spectrum Spectrometer 230 containing a spectra library compiled by NHM staff. The spectrum produced by each 231 particle was compared with that of the library by examining the peaks produced to 232 find the most appropriate matches. The percentage match was recorded along with 233 whether the particle was organic, synthetic or semi-synthetic. Those that did not 234 produce a comparable spectrum were disregarded and recorded as 'n/a'. Particles 235 recorded as semi-synthetic were labelled as such if FTIR identified them as either as 236 viscose, chipboard or cellophane.

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238 2.7. Wet wipe sampling

Samples of wet wipes and sanitary items were collected from the study site. These items were placed in plastic bags and transported to the laboratory where they were washed with filtered water (10μ m) and stored in bags for later analysis. Clear fibres were extracted from each of these items using tweezers to pick out single fibres in order to analyse the types of polymers present using FTIR. All 10 of the extracted fibres were analysed as per the method outlined in section 2.6.

246 2.8. Experimental quality control

247 In order to remove contamination from all water used, reverse osmosis water 248 was filtered through 10µm Cyclopore[™] Polycarbonate Membrane Filters using a 249 Millipore vacuum filtration system. Laboratory coats made of 100% cotton were 250 worn to eliminate polyester fibres as a source of contamination. Eco nitrile gloves 251 were worn at all times in order to prevent any further outside microplastic 252 contamination. All dissections, digestions and filtrations were carried out under a 253 laminar flow hood, Airstream® ESCO Class II Biohazard Safety cabinet, model 254 number AC2-4E1. The 10% KOH solution was filtered through a 10µm 255 polycarbonate membrane in a vacuum filtration system prior to use. An open Petri 256 dish containing a damp polycarbonate filter was placed under the laminar flow hood 257 during filtration as an atmospheric control. These could then later be compared with 258 isolated microplastics from digestions and filtrations. Any control dishes that 259 contained synthetic/semi-synthetic particles were accounted for by subtracting the 260 fraction (if significant i.e., > 0.5) from the total count of that material from the 261 samples.

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- 263 2.9. Statistical Analysis

264 All statistical analyses were performed using the software R, version 3.4.1. Using data collected from wet wipe surveys (Table 1), a series of correlation tests 265 266 were used to assess whether there was a relationship between the distance from the slipway or distance from the riverbank and the average number of wet wipes per m². 267 268 A Shapiro-Wilk test was used on the data for each of the different variables to test 269 whether the data were normally distributed. If data were normally distributed, then a 270 Pearson's Correlation was performed, however, if not then a Spearman's Rank 271 Correlation was used as a non-parametric alternative. This was repeated for 2018 and 272 2017 data provided by Thames21.

The same process was used to assess whether there was a relationship between the distance from the slipway and the number of Asian clams sampled per $0.5m^2$ and again to test for a relationship between the number of wet wipes and the number of clams per $0.5m^2$. After finding that there was a linear relationship between the number of wet wipes and the density of clams, a linear regression was used to determine the relationship between these variables.

279	Pivot tables were used in Microsoft Excel to compare all data recorded for
280	FTIR analysis of microparticles. This was followed by correlation tests which were
281	conducted to assess the potential relationships between different variables and the
282	distribution of microparticles and microplastics contained in C. fluminea. These
283	variables included the dry soft tissue weight (g) of each individual, the distance from
284	the slipway at which the sample was collected and the number of wet wipes present
285	at each distance. A Pearson's correlation was selected for data that were normally
286	distributed and a Spearman's rank used as a non-parametric alternative for non-
287	normal data.
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289	3. Results
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Distance from slipway/m	45	50	55	60	65	70	75	80	85	90	95	100
Distance from bank/m												
0	0	0	0	0	0	0	0	0	0	0	0	0
5	5	0	0	0	0	1	0	1	0	0	0	1
10	31	63	23	15	27	14	3	14	6	6	20	12
15	85	118	143	52	90	8	5	9	4	0	31	34
20	143	130	102	62	102	34	0	1	0	0	5	1
25	102	3	44	84	114	1	0	0		0		
30				0	1	0						

Table 1.

313 The number of wet wipes counted per m^2 along 12 transects of the Hammersmith foreshore, measured from the riverbank down to low water at

314 5m intervals.

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317 *3.1. Wet wipe surveys*

318 The counts of wet wipes along 12 transects (Fig. 1b) on the Hammersmith 319 south bank foreshore can be seen in Table 1. These results provide an overview of 320 wet wipe distribution along the foreshore by showing densities indicated as a heat 321 map. A colour scale of red through to green indicates areas of high to low densities 322 of wet wipes accordingly. Table 1 is similar to those produced in both 2017 and 323 2018 from Thames21 data, showing that the highest densities of wet wipes appear to 324 be closer to the slipway and further down the shore between 15–25m. Further data 325 collected by Thames21 shows that one of the largest wet wipe mounds has in fact 326 increased in height by 0.7m between 2014–2018 and a further 0.7m between 327 September 2018 and May 2019. This increased in spite of efforts by Thames21 to 328 remove thousands of wet wipes during their annual Big Count which saw a total of 23,000 wet wipes collected on 23rd March 2019. 329 330 The data from each year (2017–2019) were used to assess whether there was a relationship between the average number of wet wipes counted per m² and the 331 332 distance from the rowing slipway. There was a weak negative correlation of in 2017 (Spearman's rank correlation = -0.31; S = 110, p = 0.46). In 2018 there was a 333 334 stronger negative correlation (95% CI: -0.98 and -0.72, Pearson's correlation = -0.92; t = -7.16, d.f. = 9, p < 0.01). There was also a strong negative correlation in 335 2019 (Spearman's rank correlation = -0.73; S = 494, p = 0.01). These results indicate 336 that the average density of wet wipes decreases with increasing distance from the 337 338 slipway (Fig. 3a). Gaps in the data for 2017 may account for the insignificant 339 correlation for this year. There was an outlier at 45m where there was a markedly 340 lower average number of wet wipes than other years which may have skewed the 341 data. The average number of wet wipes per m² was also compared with the 342

measured distance from the riverbank to assess whether there was a correlation between the two variables (Fig. 3b). In 2017 there appeared to be a strong positive correlation (Spearman's rank correlation = 0.82; S = 512.87, p < 0.01). The data for 2018 showed a strong positive correlation (Pearson's correlation = 0.81; t = 4.80, d.f. = 12, p < 0.01). The weak positive correlation found in 2019 was non-significant (Pearson's correlation = 0.35; t = 0.83, d.f. = 5, p = 0.44).



Fig. 3. The average number of wet wipes per m² along 12 transects of the Hammersmith south bank foreshore. (a) 5m intervals from the rowing slipway. (b) 5m intervals from the bottom of the riverbank down to low water.

378 *3.2.* Corbicula fluminea density and distribution

- The density of *C. fluminea* appeared to be much higher further down the foreshore towards low water when assessing the presence or absence of them at each quadrat leading to low tide. In addition, there were greater abundances of *C. fluminea* further downstream and away from the rowing slipway, where there were fewer wet wipes in general along this part of the foreshore. This was also supported by additional sampling to assess clam densities in the area.
- 385 There was a significant positive correlation between the approximate distance from the rowing slipway and the number of clams per $0.5m^2$ with the greatest density 386 of clams found at 80m from the slipway with 151 per 0.5m² (Fig. 4; Spearman's rank 387 388 correlation = 0.56; S = 1020.6, p < 0.01). Therefore, the null hypothesis can be rejected. These results parallel those shown in Fig. 3a, which are supported by the 389 390 negative correlation of -0.73 (section 3.1) between the distance from the rowing slipway and the average number of wet wipes per $0.5m^2$. These data suggest an 391 392 inverse relationship between the number of wet wipes and the density of clams per
- 393 quadrat.
- Where there are higher counts of wet wipes there a few or no clams. In comparison, clams occurred in numbers at locations with few or no wet wipes. There was negative correlation between the number of wet wipes and the number of clams
- 397 present per $0.5m^2$ quadrat (Fig. 4b; Spearman's rank correlation = -0.76; S =
- 4052.50, p < 0.01). The retention time of wet wipes on the foreshore may also
- influence the apparent correlation however, this could not be determined.
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413 Fig. 4. The number of *C. fluminea* and wet wipes per $0.5m^2$ surveyed in 2019 (a) 414 with increasing distance from the rowing slipway at the same tidal height (between 415 25.4m at 85m from the slipway and 30.3m at 45m from the slipway) along the 416 Hammersmith foreshore. (b) with linear regression fitted trendline (adjusted R² = 417 0.2).

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421 3.3. Polymer identification





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426 Fig. 5. The types of polymers found from a sample of N=35 synthetic

427 fibres/fragments identified using FTIR spectroscopy of 281 microparticles extracted

428 from the digested soft tissue of C. fluminea.

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430 A total of 1404 microparticles were identified from the 9 samples of digested 431 clams (N = 227) with a range of 0-24 particles counted per individual clam (an average of 6.40 particles per individual discounting control samples). All particles 432 433 were classified as fibres, apart from two that were considered to be film. Of these, 281 were analysed using FTIR spectroscopy. All particles were categorised as either 434 synthetic, semi-synthetic, organic or n/a (see above) with 12% identified as 435 436 synthetic, 38% as semi-synthetic, 40% as organic and 10% as undescribed due to 437 inability to identify them. Nine different types of synthetic polymer (N = 35) were 438 identified from the samples analysed as shown in Fig. 5. There was an average of 439 0.77 synthetic particles per individual. 440

441 3.4 Polymer content 442 The soft tissue weight of each clam was compared with the total number of 443 microparticles that were extracted after filtration. The test indicated a weak, but 444 significant correlation of (Spearman's rank correlation = 0.27; S = 12083, p < 0.01). 445 The weak correlation may be explained by the characteristically small size range of 446 C. fluminea resulting in a range in soft tissue weight of 0.1126g to 2.6748g. 447 There were no significant correlations between the distance from the slipway with 448 the total number of particles for each of the 4 stations; with the proportion of 449 synthetic particles for each of the 4 stations; and with the average number of 450 particles per individual clam per station (Pearson's correlation, p > 0.05 in all cases). 451 All 5 of the clear fibres from the wet wipes were identified as polyester and 452 the 5 clear from the sanitary items were identified as polypropylene or 453 polyallomer/polypropylene. Polyethylene/propylene is found in the top sheets of 454 sanitary pads, and polyethylene is also found in the back sheets (Woeller and 455 Hochwalt, 2015). These results, along with those shown in Fig. 5, would suggest that 456 the clams at this site predominantly contain fibres which potentially originate from 457 sanitary items as opposed to wet wipes, among other possible sources. 458 A total of 7 microparticles were analysed from the Chelsea Embankment 459 control sample using FTIR. None were identified as synthetic. This is in keeping 460 with the lack of wet wipes at this site, so it was not expected that any fibres 461 identified were of potential wet wipe origin. Four of these fibres, however, were 462 identified as viscose (a cellulose-based material), which is commonly found in 463 sanitary items, another major contributor to pollution on the Hammersmith foreshore 464 (Always, 2019; Woeller and Hochwalt, 2015). Out of 8 control Petri dishes used 465 during sampling, only two were found to contain microparticles and each one only 466 contained 1 particle per Petri dish. One of these particles was too small for FTIR 467 analysis, the other was identified as viscose. The control dish containing the viscose 468 fibre was used during digestions of two samples. Of those samples, 24 other viscose 469 fibres were identified, leaving a ratio of 1 in 24 (0.04) as a potential source of 470 contamination. This ratio was considered insignificant as a source of contamination 471 and therefore was not deducted from the final count. 472 473 4. Discussion

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475 *4.1. Wet wipe surveys*

The distance of the transect downstream from the rowing slipway appeared to negatively affect the number of wet wipes counted, with higher numbers being found nearer the rowing slipway. The reason for higher levels of deposition at this section of the foreshore is most likely due to the reduced velocity on the inside bend causing deposition at the apex of the bend on this inner side (Graf and Blanckaert, 2002).

481 The positive correlation between the distance from the riverbank and the 482 abundance of wet wipes is most likely explained by variations in the sediment down 483 the foreshore. At the top of the foreshore near the bank, there was much more 484 shingle and drier sediment, due to reduced tidal immersion at this height. This means that wet wipes are unlikely to be deposited in this area. Wipes are therefore 485 486 deposited further down the foreshore at \sim 15–25m, where they combine with other 487 objects such as leaf litter and anthropogenic litter in general to form these reefs. 488 There were fewer wet wipes after 25m down the foreshore, potentially due to a much 489 higher immersion time. The weak correlation found for the two variables in 2019 490 may be explained by a reduced number of replicate quadrats down the foreshore as 491 they were only placed every 5m.

492 The methods used in 2019 were an adaptation of Thames21 methodology 493 from previous years. Previous years data employed a less systematic approach, with 494 different numbers of transects and quadrats laid out at uneven intervals. This was so 495 surveys could target areas with the highest densities in order to provide a better 496 estimate of the total number of wet wipes. The sampling method in 2019 used 497 transects set out at 5m intervals along the foreshore and sampling quadrats at 5m 498 intervals from the bank down to the low water line, effectively a grid. Seasonality 499 may also account for variations in the data, as levels of rainfall may determine the 500 number of wet wipes that are deposited along the foreshore due to flooding or 501 changes in the river's current speed.

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503 *4.2. Bivalve density and distribution*

The presence and absence of *C. fluminea* in relation to the distance from the riverbank indicated a wider distribution of clams at heights further down the foreshore at ca. 30-35m. This was the lowest height for low water sampled, with greater tidal exposure, and most likely had a higher abundance of clams due to a greater immersion time needed for their characteristic filter feeding. The results show that there were greater densities of *C. fluminea* the further away from the

510 slipway which is paralleled by low densities of wet wipes further from the slipway, 511 thus supporting the hypothesis that the density of clams is higher where wet wipe 512 densities are lower. In addition, there were almost no C. fluminea present in areas 513 with a large abundance of wet wipes. This suggests that the wet wipes are causing a 514 physical disturbance to the distribution of C. fluminea, as has been demonstrated in 515 other studies (Aloy et al., 2011; Green et al., 2015; Richards and Beger, 2011). This 516 interaction is not surprising because wet wipes cause a smothering effect as outlined 517 by Goldberg (1997). The accumulation of plastics covering sediments creates a 518 blanketing effect which can result in anoxia by inhibiting gas exchange/redox 519 potential between river water and sediment pore water (Goldberg, 1997; Green et al., 520 2015). This has been observed when studying the surface sediments beneath wet 521 wipe accumulations where a black layer of anoxic sediment can be observed. Clams 522 inhabit areas near the surface of sediment in order to filter feed from the water 523 column (Sousa et al., 2008). Therefore, the anoxic layer produced by wet wipe 524 smothering makes this area uninhabitable for the clams.

- 525
- 526 4.3. Polymer content

527 The results from FTIR analysis indicate that *C. fluminea* along the foreshore 528 at Hammersmith contain small quantities of synthetic fibres with an apparent lack of 529 wet wipe-related polymers. No significant relationship was found between the 530 distance from the slipway and the proportion of synthetic particles in each of the 4 531 stations although this may be, in part, due to the limited number of samples used in 532 these analyses.

533 There were few C. *fluminea* individuals present where wet wipe abundance 534 was high, almost a presence or absence distribution. Interestingly there was a higher 535 number of particles where there were fewer wet wipes. The absence of wet wipes 536 and subsequently the lack of synthetic fibres in clams from the Chelsea Embankment 537 control site, support the hypothesis that the wet wipes and other plastic debris found 538 at Hammersmith are the possible sources of synthetic polymers found in C. fluminea. 539 It was expected that the soft tissue weight of the individual clams would be 540 positively correlated with the number of particles (Welden et al., 2018), but this was 541 not the case in the present study. Both 'flushable' and 'non-flushable' wet wipes have been found to contain 542

543 polyester (PET). In addition, 'flushable' wipes are known to contain many more

544 synthetic fibres such as high-density polyethylene (HDPE), polyethylene/vinyl 545 acetate (PEVA/EVA), polypropylene (PP), low-density polyethylene (LDPE), 546 expanded polystyrene (EPS) and polyurethane (PU; Munoz et al., 2018). Therefore, 547 it was expected that some, if not all, of these polymers, would be identified from the 548 wet wipes forming the reefs at Hammersmith. The samples of wet wipes and sanitary 549 towels collected from the Hammersmith foreshore only appeared to contain PET and 550 PP fibres respectively, although other polymers being present cannot be discounted. 551 Flushable and non-flushable wet wipes both contain PET and flushable wipes also 552 contain PP, HDPE and PEVA/EVA all of which were captured by the clams (Munoz 553 et al., 2018). The term captured is used, as this study did not isolate the gut of the 554 organisms sampled, and therefore it is uncertain whether the route of microplastic 555 entry was ingestion. This would suggest that the fibres being captured by the clams 556 have potentially been released by wet wipes on the foreshore and in the water 557 column. PP and viscose fibres/particles were two of the most abundant fibres 558 identified. PP, which accounted for 57% of all synthetic fibres/particles were also 559 identified in every fibre analysed from the sample of sanitary items. Another 560 potential source of fibres is from clothing in domestic washing machines which feed 561 directly into waterways, and in a single wash, a garment can shed over 1900 fibres 562 (Browne et al., 2011).

563 Variations in the amounts of particles contained by individual C. fluminea may be explained by a number of factors. Some of the clams may have fed more 564 565 recently in relation to when they were sampled meaning their gut contents were 566 likely to include more microplastics than those that have had time for gut depuration. 567 Su et al., (2018) found C. fluminea showed relatively high retention of microplastic 568 fibres. Future research would benefit from depuration studies to assess gut retention 569 times of microplastics in C. fluminea. Seasonality may affect the number of 570 microplastics captured by the clams. Studies have demonstrated that marine species 571 such as Lepidorhumbos boscii and Nephrops norvegicus were found to ingest fewer 572 plastic items in winter (Welden and Cowie, 2016; Vassilopoulou and Haralabous, 573 2008). Bivalves are potentially one of the groups most impacted by microplastic 574 pollution and have therefore been used widely as bioindicators (Li et al., 2019; Ward 575 et al., 2019). Clams have a wide distribution, are easily accessible, in abundance and 576 are sessile (Li et al., 2019). Therefore, C. fluminea is a valuable indicator of 577 freshwater plastic pollution due to their inability to discriminate between

578 microplastics and other particles during feeding activities and within the digestive 579 system (Su et al., 2018). The effects of capturing and potentially ingesting synthetic 580 polymers have not been demonstrated in the present study, as was the case for Su et 581 al. (2018), but this species is relatively understudied. More research would be needed 582 to assess the potential physiological effects of microplastic ingestion by *C. fluminea*. 583

584 4.4. Wider implications

While there were only small amounts of captured microplastics observed in *C. fluminea* examined for the present study there is evidence for a clear physical effect of wet wipe accumulation on the distribution of the clams. Both fibre capture and physical impact on the habitat have potential implications for other species, including native species, that are of a greater conservation concern such as the depressed river mussel *Pseudanodonta complanata* and the pearl mussel

591 *Margaritifera margaritifera* (ZSL, 2018).

592

593 **5.** Conclusions

594

595 This study is the first to demonstrate how wet wipes, as a form of plastic 596 pollution, can affect organisms in the River Thames. Accumulations of wet wipes 597 have the potential to affect the distribution of aquatic biota most likely due to the 598 physical disturbance to the environment. This disturbance also conceivably reduces 599 the feeding activity of C. fluminea. Furthermore, this invasive species may act as a 600 valuable indicator of plastic pollution in the Thames and by implication the potential 601 impacts on other Thames biota. As such the study provides valuable information for 602 the conservation of biodiversity in the river. Research conducted by Thames21 and 603 Thames Tideway suggests that the problem is increasing in severity, with one of the 604 largest wet wipe mounds showing a height increase of 1.4m since 2014. Our results 605 provide further evidence for the environmental impacts caused by the inappropriate 606 disposal of wet wipes and similar products and the need for greater public awareness 607 of these impacts, improved labelling on packages and appropriate legislation. 608

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