Article (refereed) - postprint

Hanamoto, Seiya; Nakada, Norihide; Juergens, Monika D.; Johnson, Andrew C.; Yamashita, Naoyuki; Tanaka, Hiroaki. 2018. **The different fate of antibiotics in the Thames River, UK, and the Katsura River, Japan.** *Environmental Science and Pollution Research*, 25 (2). 1903-1913. https://doi.org/10.1007/s11356-017-0523-z

The different fate of antibiotics in the Thames River, UK and the Katsura River, Japan

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Abstract

Little is known about the mechanisms influencing the differences in attenuation of antibiotics between rivers. In this study, the natural attenuation of four antibiotics (azithromycin, clarithromycin, sulfapyridine, and sulfamethoxazole) during transport along the Thames River, UK, over a distance of 8.3 km, and the Katsura River, Japan, over a distance of 7.6 km were compared. To assist interpretation of the field data, the individual degradation and sorption characteristics of the antibiotics were estimated by laboratory experiments using surface water or sediment taken from the same rivers. Azithromycin, clarithromycin, and sulfapyridine were attenuated by 92%, 48%, and 11% in the Thames River stretch. The first-order decay constants of azithromycin and sulfapyridine were similar to those in the Katsura River, while that of clarithromycin was 4.4 times higher. For sulfamethoxazole the attenuation was limited in both rivers. Loss of sulfapyridine was attributed to both direct and indirect photolysis in the Thames River, but to only direct photolysis in the Katsura River. Loss of azithromycin and clarithromycin was attributed to sorption to sediment in both rivers. The probable explanation behind the difference in loss rates of clarithromycin

between the two rivers was considered to be sediment sorption capacity.

Keywords
antibiotics, natural attenuation, sorption, sediment, direct photolysis, indirect photolysis

Introduction

Antibiotics have been detected worldwide in various environmental media including fresh water (Kolpin et al. 2002; Kasprzyk-Hordern et al. 2008; Shimizu et al. 2013), coastal water (Managaki et al. 2007; Jia et al. 2011; Shimizu et al. 2013), and sediments (Feitosa-Felizzola et al. 2009; Blair et al. 2013; Xu et al. 2014). Due to the potential risk to aquatic organisms (Cooper et al. 2008; Boxall et al. 2012; Cizmas et al. 2015) and possible links to antibiotic resistance (Ågerstrand et al. 2015), antibiotics are considered some of the most important emerging contaminants in aquatic environments. Studies on antibiotic resistant bacteria have shown their wide prevalence in natural environments, including drinking water resources (Sharma et al. 2016), which is one of the most important challenges to the health care sector in the 21st century (Carvalho and Santos 2016). Therefore, in order to assess their risk and to aid in their management, the environmental fate and behaviour of antibiotics should be modeled.

In the aquatic environment, antibiotics may be attenuated by physical, chemical, and/or biological processes. Studies on the natural attenuation of antibiotics during river transport suggest rapid removal is possible for some macrolide, quinolone, and tetracycline antibiotics (Hanamoto et al. 2013; Barber et al. 2013; Luo et al. 2011). However, the reported attenuation of pharmaceuticals, including antibiotics, often differs between rivers (Li et al. 2016; Radke et al. 2010; Kunkel et al. 2011; Dickenson et al. 2011; Acuña et al. 2015; Aymerich et al. 2016).

To understand natural attenuation, we must identify which are the key factors or processes that can explain the different loss rates between rivers. The mechanisms influencing the different attenuation of antibiotics between rivers have been estimated based on general characteristics of rivers such as hydrological, meteorological, and water quality parameters (Li et al. 2016; Dickenson et al. 2011; Acuña et al. 2015). Li et al. (2016) observed the attenuation of pharmaceuticals in four European rivers, and suggested that shallow depth and low turbidity made the photochemical attenuation more efficient in a small river, compared with larger rivers. However, in most cases, no obvious explanation was found for the difference between rivers. It is presumed river characteristics, such as the composition of sediments, dissolved matter, and microbial communities (which are related to sorption, photolysis, and biodegradation processes, respectively), are determining the different fates of antibiotics among rivers. But to date little research has been carried out to resolve the importance of these processes on the different fates.

Thus, the aim of this study was to identify the mechanisms influencing the different fate and behaviour of selected antibiotics between rivers. The natural attenuation of the antibiotics in the Thames River (UK) were compared with the observations for the Katsura River (Japan) examined previously (Hanamoto et al. 2013). To help distinguish the roles of the local degradation potential

and sorption characteristics for the antibiotics in both rivers, laboratory experiments and model estimations were used. The antibiotics studied were two macrolides (azithromycin and clarithromycin) and two sulfonamides (sulfapyridine and sulfamethoxazole). Their physical properties are summarized in the Supporting Information (SI) Table S1. It is desirable to be in a better position to predict the fate of pharmaceuticals and particularly antibiotics in rivers. In this case the significance of the fate and behaviour of the same compounds in very different rivers/climates/topologies was examined essentially to ask how predictable is their loss?

Materials and methods

Site descriptions

Samplings were conducted along an 8.3 km stretch of the Thames River (Fig. 1A), between site 1 (51°42′55″N, 1°14′11″W) and site 3 (51°40′14″N, 1°16′8″W), in Oxfordshire. The stretch receives water from Littlemore Brook (site 2), where treated wastewater is discharged 1.6 km upstream of site 2. The Katsura River stretch (7.6 km) receives water from two wastewater treatment plants (sites a-c) and two tributaries (sites d and e) (Fig. 1B). There is little vegetation and no significant additional inflows along the two river stretches. The Thames River stretch catchment is mostly composed of limestone, clay/mudstone, and sandstone (Smith 2013), while the Katsura River stretch catchment is mostly granite, chalk, clay/mudstone, and sandstone (Ministry of Land, Infrastructure and Transport 2014). Most residents in both catchments are connected to the respective sewer system. These stretches were selected because they are highly impacted by treated wastewater, and antibiotics concentrations were expected to be higher there than elsewhere in each river. The general characteristics of the Thames River stretch and the Katsura River stretch are summarized in Table 1.

87 **Table 1** General characteristics of the Thames and Katsura River stretches.

		Thames River stretch	Katsura River stretch
Water quality parameters[a]	temperature (℃)[b]	18.0 (16.0 - 19.9)	21.6 (9.4 - 27.3)
	pH[b]	8.0 (7.8 - 8.1)	7.4 (7.4 - 7.5)
	suspended solids (mg/L)[b]	8.9 (6.9 - 23.6)	5.8 (3.5 - 19.8)
Hydrological conditions[a]	flow rate (m^3/s)[c]	7.6 (4.0 - 49.5)	22.4 (18.0 - 36.6)
	flow velocity (m/s)[c]	0.35 (0.26 - 0.90)	0.54 (0.50 - 0.67)
	depth (m)[c]	0.53 (0.39 - 1.38)	0.48 (0.44 - 0.56)
	hydraulic radius (m)[c]	0.52 (0.38 - 1.30)	0.47 (0.44 - 0.55)
	friction velocity at sediment-water interface (m/s)[c]	0.031 (0.024 - 0.067)	0.048 (0.045 - 0.058)
	travel time (h)	6.5 (2.6 - 8.9)	8.6 (7.0 - 9.4)
Stretch length (km)		8.3	7.6

[a] Median (minimum - maximum) in the field studies (n = 7, the Thames River; n = 6, the Katsura River), obtained as described in SI "General Characteristics of the Rivers". [b] Data at the most downstream site in each stretch. [c] Average of several sites in each stretch.

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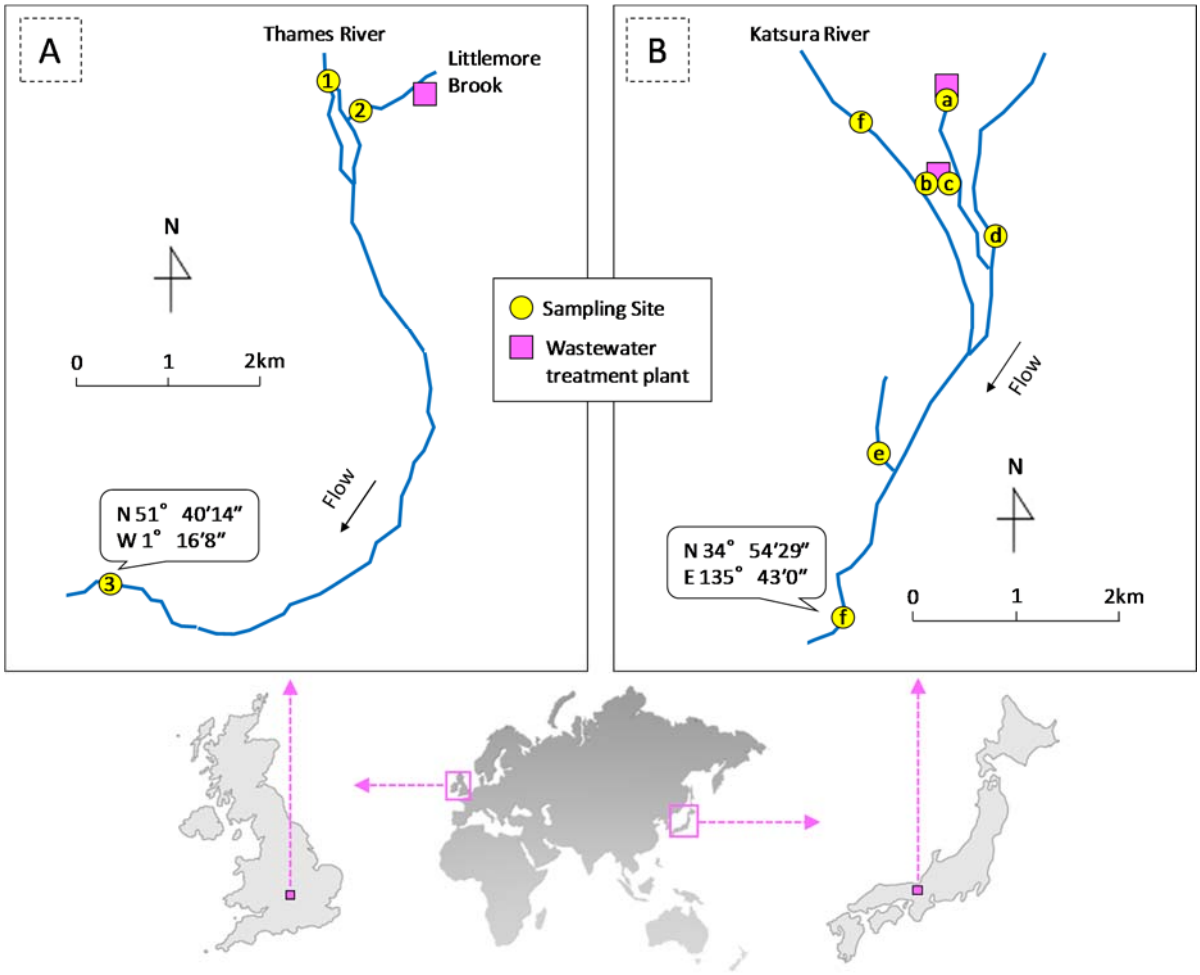


90
91 **Fig. 1** Locations of sampling sites on the (A) Thames River stretch and (B) Katsura River stretch.

92 **Field study**

93 The details of the sampling method in the Thames River stretch are described elsewhere (Nakada
94 et al. 2017). Surface water samples were collected at three sites, once or twice a summer between
95 2012 and 2015, yielding a total of seven samplings. All the samples were collected hourly for 24 h
96 by using automatic water samplers (ISCO Avalanche, ISCO 6712, Hach Sigma SD 900 or Bühler
97 Montec Xian 1000). For the samples collected in 2012 and 2013, the 24 hourly samples were
98 combined to 12 two-hourly samples and subjected to the antibiotics analysis separately. Since the
99 diurnal variations of mass loading of the selected antibiotics were not significant (see "Natural
100 attenuation of antibiotics ~" below), the samples collected in 2014 and 2015 were collected as 24
101 h composite samples by combining the 24 hourly samples. The samples were stored in plastic
102 bottles with ascorbic acid at 1.0 g/L in darkness (to reduce sample deterioration) and taken to the
103 laboratory. The samples were stored in a dark room at 4 □ until treatment. Details of the sample
104 analysis are described in SI "Analysis of Antibiotics". Briefly, samples were filtered and
105 concentrated by solid-phase extraction within two days of collection and the four selected
106 antibiotics and the antiepileptic agent carbamazepine were measured by ultra-performance liquid
107 chromatography coupled to a tandem mass spectrometer and quantified by a surrogate method
108 (Narumiya et al. 2013). Carbamazepine can be considered as a relatively conservative tracer and so
109 was used to estimate the flow rate (see below).

110 The mass balance approach was used to estimate the attenuation of the antibiotics. The amount
111 of an antibiotic attenuated during the transport along the stretch relative to total mass loadings from
112 sites 1 and 2 is defined as the attenuation rate (equation 1). In addition, since most attenuation
113 processes in a river can be regarded as first-order reactions (see "Laboratory experiment and model
114 estimation" described below), a first-order decay constant was determined by equation 2, using the
115 Goal Seek function of Microsoft Excel. The flow rate at site 3 was estimated by subtracting the
116 reported flow rate at the Ock River from that at Sutton Courtenay on the Thames River (Centre for
117 Ecology and Hydrology). The flow rates at sites 1 and 2 were estimated by using the mass balance
118 of carbamazepine, which is persistent in aquatic environments (Nakada et al. 2008; Yamamoto et
119 al. 2009) (equations 3 and 4). The laboratory experiments indicated that carbamazepine was also
120 persistent in the Thames River stretch (see "Biotic and abiotic degradation ~" described below).
121 The travel time was calculated from length and flow velocity, which was estimated from the flow
122 rate using an empirical general relationship for rivers in the UK (Round et al. 1998).

123 $$R_a = \frac{C_1Q_1 + C_2Q_2 - C_3Q_3}{C_1Q_1 + C_2Q_2} \times 100 \quad (1)$$

124 $$C_3Q_3 = C_1Q_1e^{-k_at_1} + C_2Q_2e^{-k_at_2} \quad (2)$$

125 $$Q_1 = \frac{Q_3(C_{CBZ_3} - C_{CBZ_2})}{C_{CBZ_1} - C_{CBZ_2}} \quad (3)$$

126 $$Q_2 = Q_3 - Q_1 \quad (4)$$

127 where R_a is attenuation rate (%); k_a is first-order decay constant (h^{-1}); C is concentration of an

antibiotic in surface water (ng/L); C_{CBZ} is concentration of carbamazepine in surface water (ng/L); Q is flow rate (m^3/s); t is travel time to site 3 (h); and the subscripts are site IDs in Figure 1.

The sampling and calculation methods for the Katsura River stretch were similar to those for the Thames River stretch. The samples were collected from seven sites (Fig. 1B) three times in summer and three times in winter between 2011 and 2012. The details were described in our previous study (Hanamoto et al. 2013).

Laboratory experiment and model estimation

Indirect photolysis, biodegradation, and sorption tests, and model estimations of direct photolysis for the selected antibiotics and carbamazepine (test compounds) were carried out. Carbamazepine, a compound used to estimate the flow rate in the field study, was included here to test its persistence in the river stretches studied.

Direct photolysis estimation

Direct photolysis represents degradation of a compound derived from direct absorption of light by the compound, which follows a first-order reaction (Direct photolysis rate in water by sunlight 1998). Direct photolysis rate constants of the test compounds in the Thames River stretch under average summer conditions were estimated by an equation proposed in our previous study (Hanamoto et al. 2013). The equation considers the attenuation of sunlight in the atmosphere and water, and was derived from equations proposed by Zepp et al. (1977) and Tixier et al. (2002) (see SI equation S2). The parameters used in the estimation were set as follows. Reported values (Hanamoto et al. 2013) were used for the photochemical properties (i.e., quantum yields and molar absorption coefficients). The depth of water was estimated from the reported flow rate (Centre for Ecology and Hydrology), estimated flow velocity (Round et al. 1998), and river width measured in Google Maps©, assuming the river cross-section to be a rectangle. To determine light penetration in water, we collected surface water at site 3 under low flow conditions in 2013 summer and measured the absorptivity between 290 and 490 nm with a UV-Vis spectrophotometer (UV-2500PC, Shimadzu, Kyoto, Japan). The measured value was used for the light absorption coefficient of the water body. Reported values at latitude 50°N in summer under clear sky (Direct photolysis rate in water by sunlight 1998) were used for the spectrum of sunlight at the water surface. Since we could not obtain any monitoring data for UVB or UVA in the UK, those measured in Kyoto city, Japan (Project for monitoring sunlight intensity) were substituted to estimate the average fraction of sunlight blocked by the clouds. Theoretical values for sky radiation were used for the fraction of sunlight reflected at the water surface and the path length of sunlight in the water (Zepp et al. 1977). Since there was little overhanging vegetation along the river stretch, the fraction of sunlight shaded by plants was set to 0. Direct photolysis rate constants in the Katsura River stretch were estimated using the same equation under average summer and winter conditions, because the field studies in the stretch were conducted in both summer and winter (see Table 1). The parameters used in the estimation were obtained in our previous study (Hanamoto et al. 2013) for the Katsura River stretch. The parameters for the Thames and Katsura river stretches are summarized in SI Table S3.

166 ***Indirect photolysis test***

167 Indirect photolysis represents degradation of a compound driven by reactive species (e.g., singlet
168 oxygen and hydroxyl radical) produced under light irradiance to dissolved matter (e.g. humic
169 substance and nitrate) in surface water. Indirect photolysis rate constants of test compounds in the
170 river stretches were assessed by applying U.S. Environmental Protection Agency's (USEPA)
171 harmonized test guideline 835.5270 (Indirect photolysis screening test 1998) to the surface water
172 with an assumption of extrapolation as described below. Grab samples collected at site 3 in the
173 Thames River stretch were brought to the laboratory, and filtered through a 1 μm pore size glass
174 fiber filter (GF/B, Whatman, UK) to prevent sorption of test compounds to suspended solids. A
175 phosphate buffer solution (10 mM) at pH 7.8 (same as the surface water) was prepared with
176 ultrapure water. The test compounds were added to the filtered surface water and phosphate buffer
177 to give an initial concentration of 5 μg/L each. The 10 mL solutions in 20 mL quartz tubes were
178 exposed to natural sunlight under a clear sky in the daytime for 4 h. The test compounds before and
179 after the exposure were analyzed as described above. The change in concentrations in darkness was
180 negligible (data not shown). The surface water sample was collected in 2015 summer under low
181 flow condition (7^{th} percentile in 2010-2014, obtained in Centre for Ecology and Hydrology), and
182 the experiment was conducted in duplicate in an open space at the Centre for Ecology and
183 Hydrology, UK (51°36′9″N, 1°6′45″W), within two days of the sample collection. The same
184 laboratory conditions and procedures were applied to the surface water sample collected at the
185 Miyamae Bridge, the most downstream site in the Katsura River stretch (site g in Figure 1B), under
186 low flow condition (9^{th} percentile in 2010-2014, obtained in Ministry of Land, Infrastructure and
187 Transport; website). The sunlight exposure to the Katsura River water was conducted in open space
188 at the Research Center for Environmental Quality Management, Japan (35°0′9″N, 135°53′24″E).

189 Since the indirect photolysis is generally a pseudo-first-order reaction (Indirect photolysis
190 screening test 1998), the indirect photolysis rate constant in the test tube was estimated by
191 subtracting the first-order rate constant in the phosphate buffer from that in the surface water
192 (equations 5 and 6). To extrapolate from the rate in the quartz test tube to that in the Thames River
193 stretch, the ratio of sunlight absorbed by influential dissolved matter (i.e., dissolved matter
194 producing reactive species influential on the antibiotics degradation) in the stretch to that in the
195 tube is needed. Calculated sunlight intensities in the tubes with surface water and phosphate buffer
196 were similar (data not shown) due to the short light path length in the tube. Most dissolved matter
197 and also antibiotics absorb sunlight mainly in the 300-400 nm range (Hanamoto et al. 2013).
198 Therefore, the ratio was estimated by dividing the direct photolysis rate constants of antibiotics
199 estimated in the stretch ($k_{d_{env}}$) by those observed in the test tube ($k_{pw_{tub}}$), assuming that the shape
200 of the solar action spectrum of the influential dissolved matter is similar to that of the antibiotics.
201 The indirect photolysis rate constant in the Thames River stretch was then estimated using this ratio
202 (equation 7).

203 $$k_{sw/pw_{tub}} = -\frac{1}{t_i}\ln\frac{C_{sw/pw_{aft}}}{C_{sw/pw_{bef}}} \quad (5)$$

$$k_{i_{tub}} = k_{sw_{tub}} - k_{pw_{tub}} \quad (6)$$

$$k_{i_{env}} = \frac{k_{i_{tub}} k_{d_{env}}}{k_{pw_{tub}}} \quad (7)$$

where k is first-order rate constant (h^{-1}); C is concentration in the water (ng/L); t_i is exposure time (h); k_i is indirect photolysis rate constant (h^{-1}); k_d is direct photolysis rate constant (h^{-1}); and subscripts *tub, env, sw, pw, bef* and *aft* are values in the test tube, aquatic environment, surface water, ultrapure water, before the exposure, and after the exposure.

Biodegradation test

To assess the degradation of test compounds resulting from the activity of microorganisms which live in surface water in the river stretches, a simple simulation test was conducted with reference to USEPA's harmonized test guideline 835.3170 (Shake Flask Die-Away Test 1998). Surface water grab samples collected at site 3 in the Thames River stretch were brought to the laboratory. The test compounds were first dissolved in ultrapure water and then added to the river water samples to give an initial concentration of 0.5 µg/L of each. The solutions were incubated at 20 ± 1 °C in the dark on a rotating shaker at 100 rpm for 24 h. The test compounds in the dissolved phase before and after the incubation were analyzed as described above, and those in the particulate phase were analyzed as described in SI "Analysis of Antibiotics". The amount of a compound in the solution (i.e., total of dissolved and particulate phase) that was lost in the incubation relative to the amount before the incubation was defined as a biodegradation loss (equation 8). Sorption of test compounds to glassware was negligible (Hanamoto et al. 2013). The change in suspended solids concentration during the incubation was negligible (data not shown). The surface water samples were collected twice in 2015 summer under low flow conditions (7^{th} and 12^{th} percentile of the 5 year daily flow data 2010-2014, obtained in Centre for Ecology and Hydrology), and the experiments were conducted in duplicate within a day after the sample collection. The same laboratory conditions and procedures were applied to the surface water samples collected at the Miyamae Bridge in the Katsura River stretch (site g in Figure 1B), although the experiment was conducted only once using sample collected under high flow condition (83^{rd} percentile of the 5 year daily flow data 2010-2014, obtained in Ministry of Land, Infrastructure and Transport; website). Since the biodegradation of a compound of low concentration is a first-order reaction (Shake Flask Die-Away Test 1998), a biodegradation rate constant was determined by equation 9.

$$L_b = \frac{M_{bef} - M_{aft}}{M_{bef}} \times 100 \quad (8)$$

$$k_b = -\frac{1}{t_b} \ln\left(1 - \frac{L_b}{100}\right) \quad (9)$$

where L_b is biodegradation loss (%); k_b is biodegradation rate constant (h^{-1}); M is mass of a compound in the solution (i.e., total of dissolved and particulate phase) (ng); t_b is incubation time (h); and subscripts *bef* and *aft* are values before and after the incubation.

Sorption test

Since the sorption rate of a compound to river sediment is difficult to determine at laboratory scale due to complications by hydrological factors such as hyporheic exchange, the sediment-water partition coefficient was measured to evaluate sediment sorption capacity. The sediment-water partition coefficients of test compounds were estimated in accordance with OECD test guideline No. 106 (Adsorption–desorption using a batch equilibrium method 1995). Sediment grab samples were collected from the top 5 cm in the Thames River stretch - two mixture sites and in the Katsura River stretch - three mixture sites in 2014 summer under low flow conditions and brought to the laboratory, air-dried, and passed through a 2 mm sieve. The solvent used for the sorption experiments with the sediments was surface water from the Katsura River at the Miyamae Bridge (site g in Figure 1B) which was filtered through a 1 μm pore size glass fiber filter, and to which 0.02% sodium azide was added to minimize microbial activity. Sediments (0.15-0.20 g) and river water (50 ml) were put into glass centrifuge tubes and the test compounds were added to give an initial concentration of 200 ng/L each. The tubes were then rotated at 20 ± 1 °C in the dark. After two days, the test compounds in the water and sediment were analyzed as described above and in SI "Analysis of Antibiotics" respectively. We had previously observed that the sediment-water equilibrium for the test compounds was nearly reached within two days and that changes in concentration without sediment were negligible (Hanamoto et al. 2013). The sediment-water partition coefficients were determined by dividing the concentration in the sediment by that in the water (equation 10). A higher coefficient indicates a greater sorption capacity of the sediment. The experiment was conducted in duplicate.

$$K_p = \frac{C_{s_{eq}}}{C_{w_{eq}}} \quad (10)$$

where K_p is sediment-water partitioning coefficient (L/kg); $C_{w_{eq}}$ is concentration in the water at equilibrium (ng/L); and $C_{s_{eq}}$ is concentration in the sediment at equilibrium (ng/kg).

Results and discussion

Natural attenuation of antibiotics in the rivers Thames and Katsura

Concentrations of the selected antibiotics and carbamazepine in the Littlemore Brook (site 2), a wastewater-impacted tributary, were much higher than those in the Thames River (sites 1 and 3, Fig. 2A). The mass loadings from site 2 were higher than those from site 1 for azithromycin and clarithromycin, and vice versa for the others (Fig. 2B). This difference between compounds would be mainly attributable to the attenuation of the two macrolides during the transport in the stretch upstream of site 1. Coefficients of variations (CVs) of concentrations within a day at the major sources (i.e., site 2 for the two macrolides and site 1 for the others) were low (below 20%, see SI Figure S1), indicating diurnal variation of concentration would not produce substantial errors in estimating attenuations and flow rates by equations 1-4. Higher CVs of the two macrolides in sites

1 and 3 might be attributed to temporal variability of attenuation in the Thames River.

The attenuation rates for the Thames River stretch were low for the two sulfonamides, sulfamethoxazole (-2% on average) and sulfapyridine (11%), and higher for the two macrolides, clarithromycin (48%) and azithromycin (92%), indicating substantial losses for the macrolides. The estimated average half-lives in the Thames River stretch were 29.2, 4.5, and 1.9 h for sulfapyridine, clarithromycin, and azithromycin, respectively. The decay constants in the Thames River were 4.4, 1.2, and 1.2 times higher than those in the Katsura River for clarithromycin, azithromycin, and sulfapyridine, respectively (Fig. 3). Thus, the fate of clarithromycin, in particular, was very different between the rivers. Though there was no statistically significant difference in the decay constants of antibiotics between the rivers even for clarithromycin (p = 0.087), this was attributable to their high fluctuation between sampling days and the limited number of samples. The fluctuation of the decay constants would be mainly driven by the daily variation in environmental factors such as sunlight intensity and flow velocity (see "Mechanistic considerations for ~" described below). Since surface water samples were collected as composite samples, the sampling error is minimal.

The limited attenuation of the sulfonamides in both rivers is similar to several observations in Swedish rivers (Bendz et al. 2005; Li et al. 2016), American rivers (Barber et al. 2013), and a Spanish river (Aymerich et al. 2016). No report on the decay constant was found for the investigated macrolides, but that of erythromycin, a similar macrolide antibiotic, was reported in Iberian rivers (0.17 h^{-1}) (Acuña et al. 2015). This is comparable to that of clarithromycin observed in the Thames River stretch.

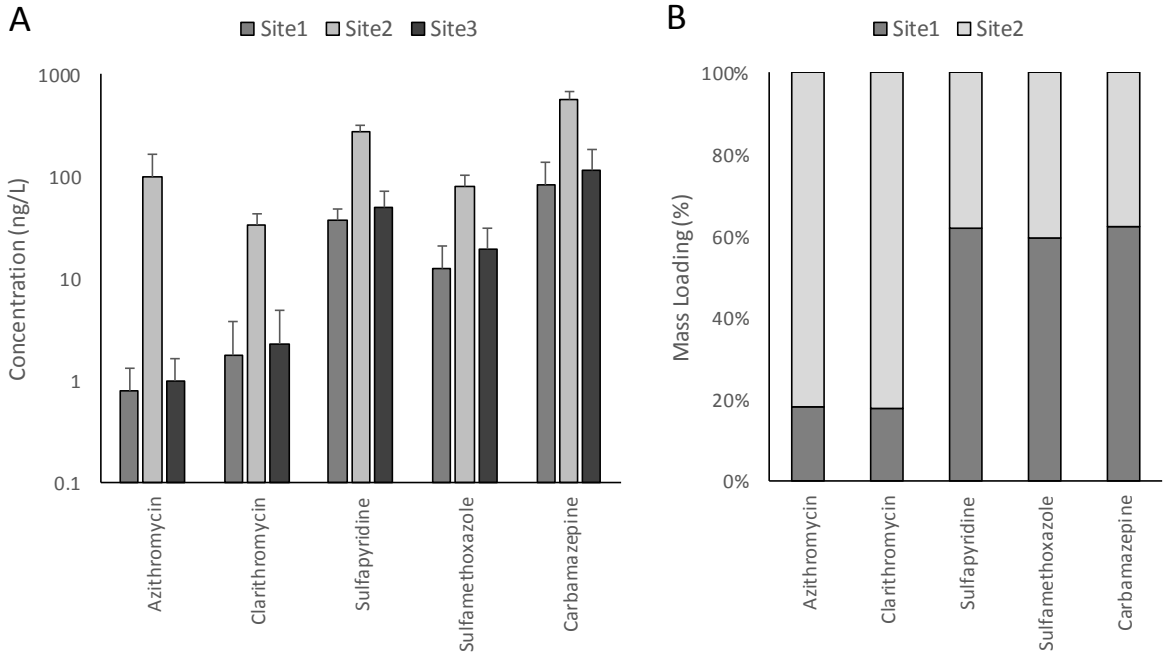
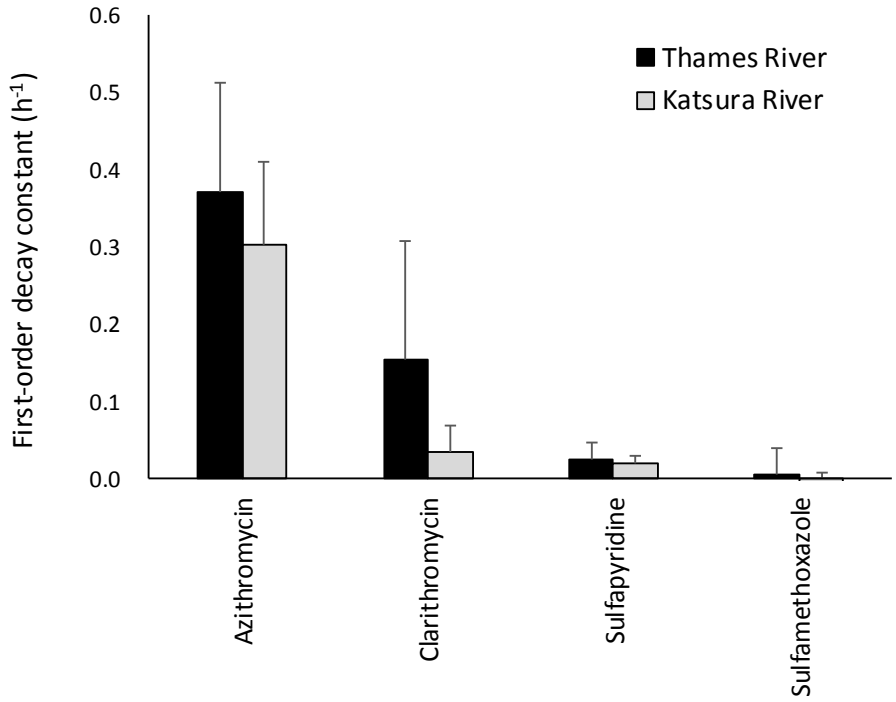


Fig. 2 (A) Measured concentrations and (B) source distribution of antibiotics and carbamazepine in the Thames River stretch. For A, the vertical bars denote means, and the error bars denote standard deviations (n = 7). For B, the vertical bars denote medians (n = 7).



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299 **Fig. 3** Comparison of the first-order decay constants of the antibiotics between the Thames River
300 stretch and the Katsura River stretch (means and standard deviations). The decay constant in the
301 Katsura River was measured in a previous study (Hanamoto et al. 2013). For sulfapyridine, there
302 was a significant difference between the summer and winter sampling campaigns in the Katsura
303 River stretch (see SI Figure S2), so only the three decay constants obtained in summer were
304 included, while all six decay constants were included for the other antibiotics. The number of
305 samples was n = 7 for all antibiotics in the Thames River; n = 3 for sulfapyridine in the Katsura
306 River; n = 6 for the other antibiotics in the Katsura River.

307

308 **Biotic and abiotic degradation and sorption characteristics of antibiotics in the Thames and**
309 **Katsura Rivers**

310 The direct photolysis rate constant of sulfapyridine was 0.011 h^{-1} in the Thames River stretch
311 and 0.022 h^{-1} in the Katsura River stretch under average summer conditions and below 0.010 h^{-1} in
312 the Katsura River stretch in winter. The lower rate constant in the Thames River compared to the
313 Katsura in summer was mainly due to a higher light absorption coefficient of the water body (see
314 SI Figure S3). The direct photolysis of sulfapyridine was attributed to desulfonation and/or
315 denitrification, as well as hydroxylation of photo-oxidized heterocyclic rings (Baena-Nogueras et
316 al. 2017). The rate constants of the other antibiotics were below 0.010 h^{-1} in both rivers.

317 The first-order rate constant of sulfapyridine observed in the indirect photolysis experiment in
318 the Thames River water was 2.4 times higher than that in the ultrapure water, while the constant of
319 sulfapyridine in the Katsura River water was similar to that in the ultrapure water (see SI Figure
320 S4). The estimated indirect photolysis rate constant of sulfapyridine in the Thames River stretch
321 was 0.015 h^{-1}, while that in the Katsura River stretch was below 0.010 h^{-1}. Previously, significant

indirect photolysis of sulfapyridine was reported in water from a constructed wetland, and this was attributed not to nitrate but to a portion of dissolved organic matter (Challis et al. 2013). Since there is no other study on indirect photolysis of sulfapyridine under sunlight, further mechanistic studies should be conducted to elucidate constituents of dissolved organic matter determining the indirect photolysis of sulfapyridine. The decrease in concentrations of the other antibiotics during the sunlit experiment were below 20%, yielding indirect photolysis rate constants of below 0.010 h^{-1} in both rivers.

The biodegradation losses derived from the laboratory experiments over 24 h were below 20% and the biodegradation rate constants were below 0.010 h^{-1} for all antibiotics in both rivers. The reported biodegradation rate constants of the antibiotics, which were observed in Katsura River water under the five-day incubation test, were also below 0.010 h^{-1} (Hanamoto et al. 2013).

The relative sediment-water partitioning coefficients were azithromycin > clarithromycin ≫ sulfapyridine > sulfamethoxazole in both rivers. The partitioning coefficients for the Thames River sediments were 1.4 and 5.5 times (on average) higher than those for the Katsura River sediment for azithromycin and clarithromycin, respectively. Since the two macrolides mostly exist in cationic forms in surface water (Sibley et al. 2008), their sorption is likely to be due to coulombic attraction to negatively charged surface sites on sediments (e.g. permanent negative charge on aluminosilicate clays, deprotonated surface hydroxyl groups on sediment metal oxides, and deprotonated surface hydroxyl or carboxylic acid groups on sediment organic matter, Vasudevan et al. 2009). Because of the multiple sorption sites, sorption capacities of macrolides did not correlate with general properties of soils (e.g. organic carbon content, cation exchange capacity) (Kodešová et al. 2015; Srinivasan et al. 2014), and there is no related mechanistic study with sediments. Given the geologic differences between the two catchments (see "Site descriptions" above), mineralogical compositions of sediments seem to be different between the rivers, and this might have caused the much higher partitioning coefficient of clarithromycin in the Thames River than in the Katsura River. Therefore, further mechanistic studies should be conducted to elucidate sediment constituents determining the sorption capacity of clarithromycin. As for the difference in partitioning coefficients between the compounds, previous studies on sediments indicated that the octanol-water partitioning coefficient (K_{ow}), the indicator of hydrophobicity of a compound, could not explain the different sorptivity between pharmaceuticals (Yamamoto et al. 2009; Schaffer et al. 2012). Given the hydrophilic and ionizable properties of pharmaceuticals and negatively charged surface sites on sediments, the fraction of pharmaceuticals existing in cationic form would be the most influential factor differentiating antibiotics sorptivities. Therefore, the observed low sorptivities of the two sulfonamides could be because they mostly exist in anionic or neutral forms within the common environmental pH range (Gao and Pedersen, 2005).

For carbamazepine, all the degradation rate constants were below 0.010 h^{-1}, the partitioning coefficient was not measurable due to its low sorptivity, and its predicted volatilization is expected to be negligible (Hanamoto et al. 2013) in both rivers, indicating the validity of using it as a conservative tracer to estimate the flow rate in the river stretches.

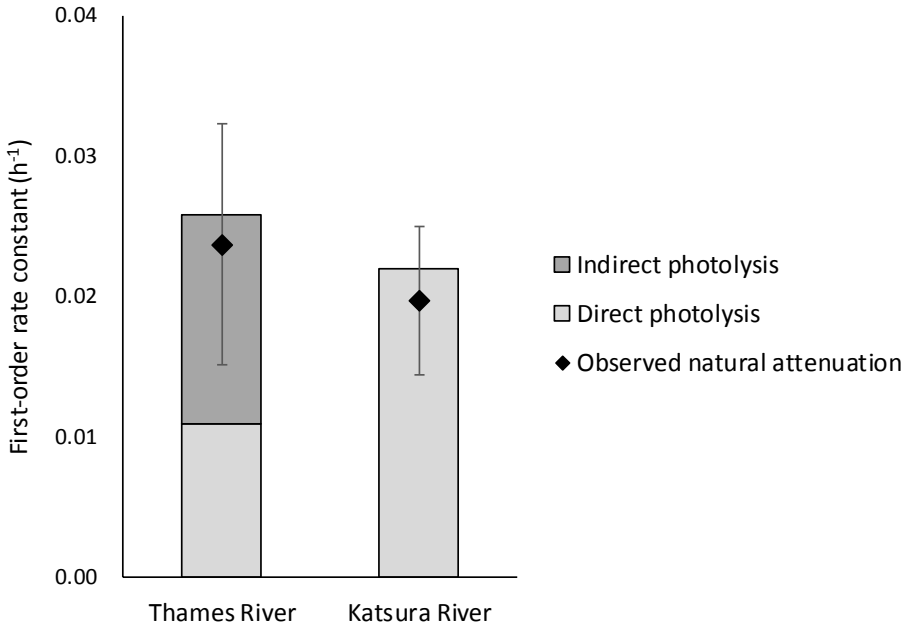
Mechanistic considerations for the difference in attenuation between the rivers

The degradation and volatilization (Hanamoto et al. 2013) rates were negligible and sediment sorption capacity was low in both rivers for sulfamethoxazole, which is consistent with its limited natural attenuation (see Figure 3). Since biodegradation and volatilization (Hanamoto et al. 2013) were negligible and sediment sorption capacity was low in both rivers for sulfapyridine, its major loss mechanism during transport along the river stretches was direct and/or indirect photolysis. The decay constants of sulfapyridine observed in the river stretches were comparable to the sum of its direct and indirect photolysis rate constants under average summer conditions (Fig. 4), indicating the assumption made in extrapolating the indirect photolysis rate constants observed in the test tubes into those in the river stretches (see "Indirect photolysis test" described above) did not produce a substantial error in the estimate. Therefore, the attenuation of sulfapyridine can be considered to be mainly due to both direct and indirect photolysis in the Thames River, but to only direct photolysis in the Katsura River. The difference in indirect photolysis between the rivers is attributable to constituents of dissolved organic matter (see "Biotic and abiotic degradation ~" above).

The degradation and volatilization (Hanamoto et al. 2013) rates were also negligible but the sediment sorption capacity was high in both rivers for azithromycin and clarithromycin. Therefore, the major loss mechanism during transport along the river stretches for the two macrolides would be sorption to sediment, which is consistent with their reported high concentrations in sediments (Blair et al. 2013; Feitosa-Felizzola et al. 2009; Xu et al. 2014; Luo et al. 2011). The much higher decay constant of clarithromycin in the Thames River than in the Katsura River was attributable to the higher sediment sorption capacity in the former, given their positive relationship shown in Figure 5. Sorption rate constant of a compound (i.e., mass transferred from overlaying water into sediment per unit time) is determined by a driving force, which is defined as difference in sediment pore water and overlaying water concentrations, and a mass transfer coefficient (Thibodeaux 1996). The sediment sorption capacity is an influential factor determining the driving force of sorption to sediment.

Another possible factor differentiating the driving force of sorption between the rivers could be biodegradation of clarithromycin within the sediment. Though there is no related study on sediments, degradation rates of the macrolides were quite low within soils (Kodešová et al. 2016) and biosolids-amended soils (Walters et al. 2010) (half lives were mostly over 100d), which indicates biodegradation within sediment is likely to be low for clarithromycin. The sorption rate constant is also affected by hydrological parameters such as flow velocity, hydraulic radius, and hyporheic exchange, which are determinants of the mass transfer coefficient, and replacement of surface sediment during high flow events, which affects the driving force. However, these parameters could not explain the fact that the fate of only clarithromycin was very different between the two rivers, since such parameters do not have substance-specific effects (Thibodeaux 1996). The sorption to suspended solids was also not considered to be playing an important role given that the concentration was low in both rivers (see Table 1). Thus, though there are some unquantified

400 factors, the sediment sorption capacity would be a key factor explaining the different fate of
401 clarithromycin between the rivers. Further studies to estimate the sorption rate constant should be
402 conducted and compared with the observed attenuation to help confirm this.
403



404
405 **Fig. 4** Comparison of the observed first-order decay constant and the estimated photolysis rate
406 constants for sulfapyridine under average summer conditions. Since there was a significant
407 difference in the decay constants between the summer and winter sampling campaigns in the
408 Katsura River stretch, the comparison was conducted under summer conditions. Means and standard
409 errors of measurements in summer sampling campaigns were substituted for the decay constants (n
410 = 7 for the Thames River; n = 3 for the Katsura River). The decay constant in the Katsura River
411 was measured in a previous study (Hanamoto et al. 2013).
412

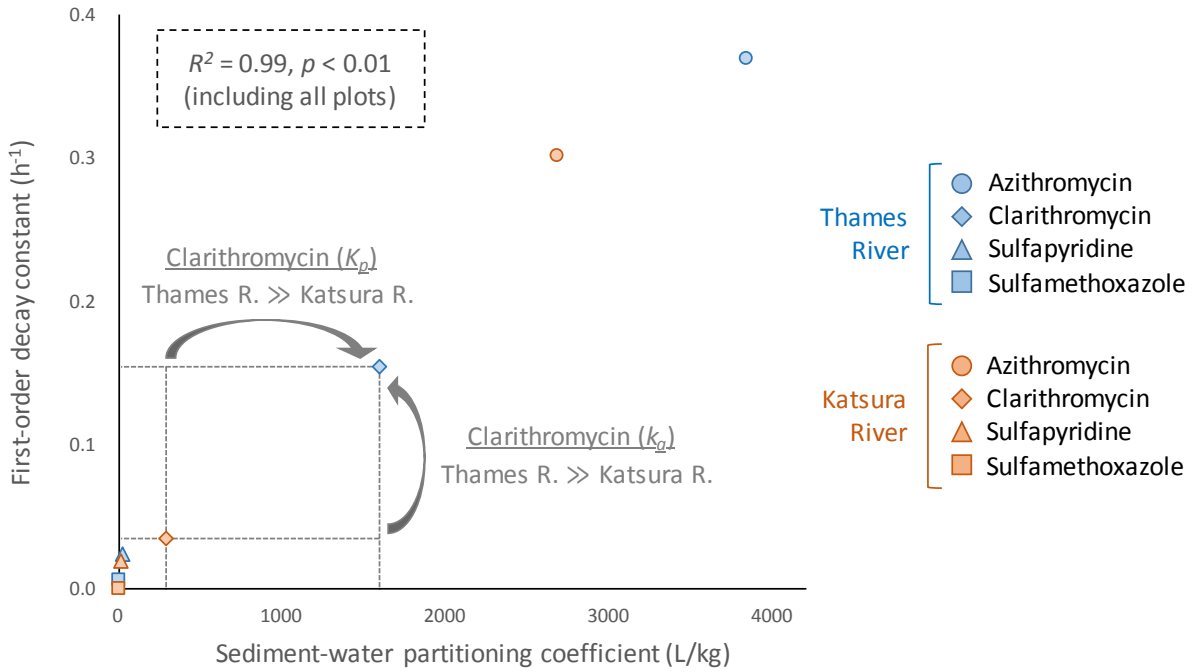


Fig. 5 Comparison of the first-order decay constants (k_a) and the sediment-water partitioning coefficients (K_p) for antibiotics. Means were substituted for the decay constant (n = 7 for all antibiotics in the Thames River; n = 3 for sulfapyridine in the Katsura River; n = 6 for the other antibiotics in the Katsura River). The decay constants in the Katsura River were measured in a previous study (Hanamoto et al. 2013). A higher partitioning coefficient indicates a greater sorption capacity of the sediment. Since the partitioning coefficient of sulfamethoxazole was not available due to its low sorptivity, it was plotted as 0.

Conclusions

The field study revealed that the decay constants in the Thames River were 4.4, 1.2, and 1.2 times higher than those in the Katsura River for clarithromycin, azithromycin, and sulfapyridine respectively, while the attenuation was limited in both rivers for sulfamethoxazole. River characterization highlighted sediment sorption capacity played an important role in the different loss rates of clarithromycin between the two rivers. Attenuation of azithromycin was also attributed to sorption to sediment in both rivers. Both direct and indirect photolysis affected attenuation of sulfapyridine in the Thames River, while indirect photolysis was negligible in the Katsura River. These findings provide a better understanding of the key factors differentiating natural attenuation of antibiotics between rivers. Future work should focus on the sediment properties which determine the sorption capacity of the macrolides. In addition, more information is needed on the types of dissolved organic matter determining indirect photolysis of sulfapyridine, as well as on sorption rate constants of the macrolides in the rivers.

Acknowledgments

We thank the Japanese Ministry of the Environment and DEFRA-supported UK/Japan cooperation. We thank Dr. SangJung Lee, Dr. Vimal Kumar, Dr. Masaru Ihara, and several students of the Research Center for Environmental Quality Management, Kyoto University, for their assistance in the laboratory and in the fieldwork. We thank Dr. Michael J. Bowes of the Centre for Ecology and Hydrology for providing water quality data. This work was supported by the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (A) (25257304, 23254003, and 26257302) and funding from the UK Natural Environment Research Council.

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SUPPORTING INFORMATION

The different fate of antibiotics in the Thames River, UK and the Katsura River, Japan

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Selected Antibiotics

Table S1. Physical properties of the selected antibiotics.

	therapeutic class	formula	molecular weight	pK_a	$\log K_{ow}$	Henry's Law constant (atm-m^3/mole)
azithromycin	macrolide antibiotics	$C_{38}H_{72}N_2O_{12}$	749.0	8.7	4.0	5.3×10^{-29}
clarithromycin	macrolide antibiotics	$C_{38}H_{69}NO_{13}$	748.0	9.0	3.2	1.7×10^{-29}
sulfapyridine	sulfonamides	$C_{11}H_{11}N_3O_2S$	249.3	2.7, 8.4	0.35	1.1×10^{-13}
sulfamethoxazole	sulfonamides	$C_{10}H_{11}N_3O_3S$	253.3	1.6, 5.7	0.89	6.4×10^{-13}

There data were obtained from Syracuse Research Corporation, PhysProp Database (http://www.srcinc.com/what-we-do/databaseforms.aspx?id=386) or EPI Suite (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm)

Analysis of Antibiotics

The four selected antibiotics and carbamazepine (test compounds) in the dissolved phase were analyzed as follows. A 500 mL surface water sample was filtered through a glass fiber filter (GF/B, 1.0 μm, Whatman, UK), and spiked with a surrogate standard mixture (containing 50 μg each of five isotopically labelled pharmaceuticals) (Narumiya et al., 2013). The test compounds in the dissolved phase were concentrated by solid-phase extraction through Oasis HLB cartridges (500 mg, 6cc, Waters, Japan). The cartridges containing the sample concentrate and surrogate standards were stored in a refrigerator for up to a few weeks, then extracted with 6 mL of methanol before being measured by ACQUITY ultra-performance liquid chromatography system (Waters, USA) coupled to a Micromass Quattro micro API Tandem Quadrupole system (Waters, USA). An ACQUITY BEH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters, USA) was used for separation. Measured concentrations were quantified by the surrogate method (Narumiya et al., 2013). Details of the analytical method and quantification limit of test compounds can be found in previous studies (Okuda et al. 2009; Narumiya et al. 2013).

The test compounds in the particulate phase of the water and sediments were analyzed as follows. Suspended solids trapped on a 1-μm pore size glass fiber filter or 0.20-g subsamples of the sediment were spiked with a surrogate mixture, and accelerated solvent extraction (ASE) was performed with an ASE 200 system (Thermo Fisher Scientific Inc., Waltham, MA, USA) in 11-mL ASE cells under the following conditions: 100 °C, 10 min static, 3 cycles, 2000 psi, and 60% flush. Methanol/water (1:1, v/v) with 0.5% (v/v) aqueous ammonia (pH = 11) was used as a solvent. The extracts were filtered through a cellulose filter (ASE extraction filters, Thermo Fisher Scientific) installed at the bottom of the cell, diluted with ultrapure water to reduce the concentration of methanol to <5% (v/v), and had ascorbic acid (2 g/L) and ethylenediaminetetraacetic acid disodium (1 g/L) added to them. Solid-phase extraction was used to clean-up the extracts by using Oasis HLB cartridge (200 mg, 6cc, Waters, USA). Test compounds were treated, measured, and quantified as described above.

To test the analytical reproducibility of the test compounds in the dissolved phase, the following

experiment was conducted. Three ultrapure water samples (200 mL) were spiked with a surrogate standard mixture containing 50 ng of each and the test compounds were analyzed as described above. The relative standard deviations of absolute recoveries of the test compounds in the water samples ranged from 1.1% (carbamazepine) to 12.8% (sulfapyridine), indicating that the analytical procedure was accurate. In addition, to validate the method for sediment samples, the following experiment was conducted. The sediment sample collected in the Katsura River was passed through a 2-mm sieve, and ten 0.20 g subsamples were obtained. Five were spiked with a test compounds mixture containing 500 ng of each compound, and all ten were additionally spiked with a surrogate mixture containing 50 ng of each. Then, the test compounds were analyzed as described above. The accuracy of the analytical method was evaluated using the absolute and relative recoveries (over extraction, solid-phase extraction, and measurement) of the spiked test compounds. The absolute recoveries of the test compounds in the sediment ranged from 70.2% (carbamazepine) to 88.6% (sulfamethoxazole); their relative recoveries ranged from 83.4% (carbamazepine) to 101.4% (azithromycin); and the relative standard deviations of the relative recoveries ranged from 2.5% (carbamazepine) to 8.3% (sulfapyridine). Thus, the analytical procedure of the sediment samples was also deemed accurate for the test compounds.

General Characteristics of the Rivers

We measured water quality parameters at site 3 in the Thames River during the sampling in 2015, while for the years 2012-2014 those measured within ± 2 days of our sampling 22.4 km downstream of site 3 in Wallingford (Personal communication from Dr. Michael J. Bowes) were used. Since the inflows between site 3 and Wallingford are small compared with the flow at site 3, the water quality was similar (Table S2). Reported data at the Miyamae Bridge (Ministry of land, infrastructure and transport) were used for the water quality parameters in the Katsura River, though concentration of suspended solids was estimated from turbidity (estimation accuracy, R^2=0.91).

The flow rates at site 3 in the Thames River were estimated by subtracting the reported flow rate at the Ock River from those at Sutton Courtenay on the Thames River (Centre for Ecology and Hydrology). For the Katsura River, the reported flow rates at the Miyamae Bridge were used (Ministry of land, infrastructure and transport). The flow velocities in the Thames River were estimated using a general flow rate-flow velocity relationship for rivers in the UK (Round et al. 1998), while those in the Katsura River were estimated using the relationship developed in our previous study (Hanamoto et al. 2013). The depth of water in the Thames River was estimated from the flow rate, the flow velocity, and river width measured in Google Maps©, assuming the river cross-section to be a rectangle, while that in the Katsura River was estimated using the flow rate-water depth relationship developed in our previous study (Hanamoto et al. 2013). The hydraulic radius was estimated from the river depth and width assuming the river cross-section to be rectangle for the Thames River, while that in the Katsura River was estimated using the river cross- section diagram obtained in our previous study (Hanamoto et al. 2013). Friction velocity at the sediment-water interface was estimated by the equation S1, assigning a value of 0.025 to Manning's roughness coefficient. The travel time was calculated from length and the flow velocity.

$$v_* = \frac{vn\sqrt{g}}{R^{1/6}} \quad \text{(S1)}$$

where v_* is friction velocity at the sediment-water interface (m/s); v is flow velocity (m/s); n is Manning's roughness coefficient (s/m$^{1/3}$); g is gravity acceleration (m/s^2); and R is hydraulic radius (m).

Table S2. Comparison of water quality between Wallingford and site 3.

	date	time	temperature (℃)	pH	suspended solids (mg/L)
Wallingford[a]	August 24, 2015[b]	14:40[b]	18.4[b]	7.9[b]	10.8[b]
Site 3	August 24, 2015	11:00	19.4	8.0	8.12

[a] 22.4 km downstream of site 3. [b] Reference 2.

Field Study

The diurnal variation in concentrations of the test compounds in the Thames River stretch are shown in Figures S1. Comparison of the first-order decay constants of the antibiotics in the Katsura River stretch obtained in summer and winter in a previous study (Hanamoto et al. 2013) are shown in Figure S2.

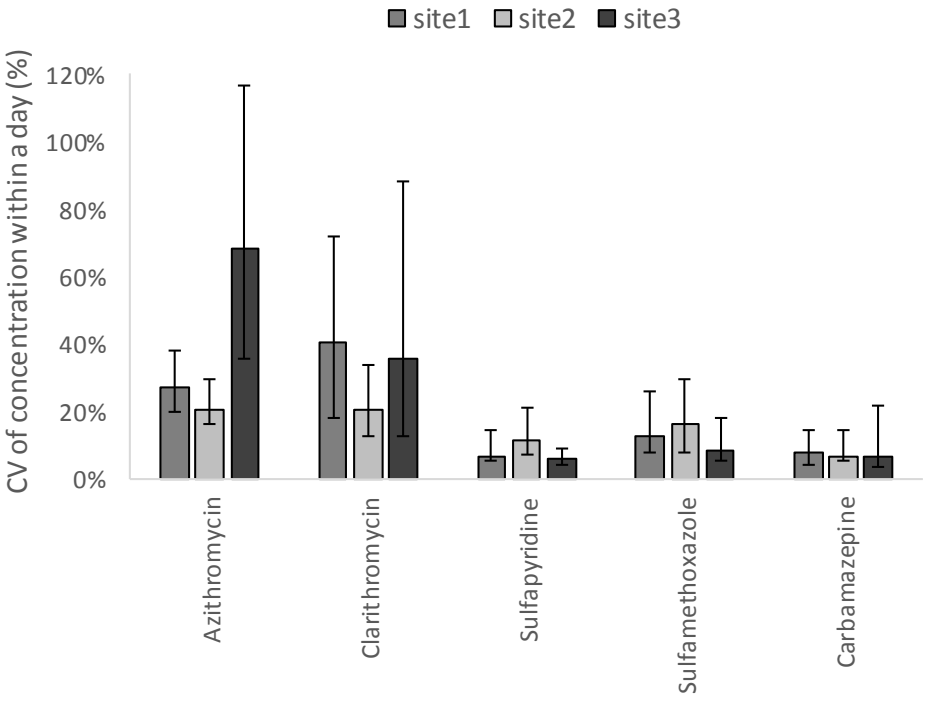

Figure S1. Coefficients of variation (CV) of concentration of antibiotics and carbamazepine within 24 hours (12 samples). The vertical bars denote median and the error bars denote maximum and minimum (n = 4).

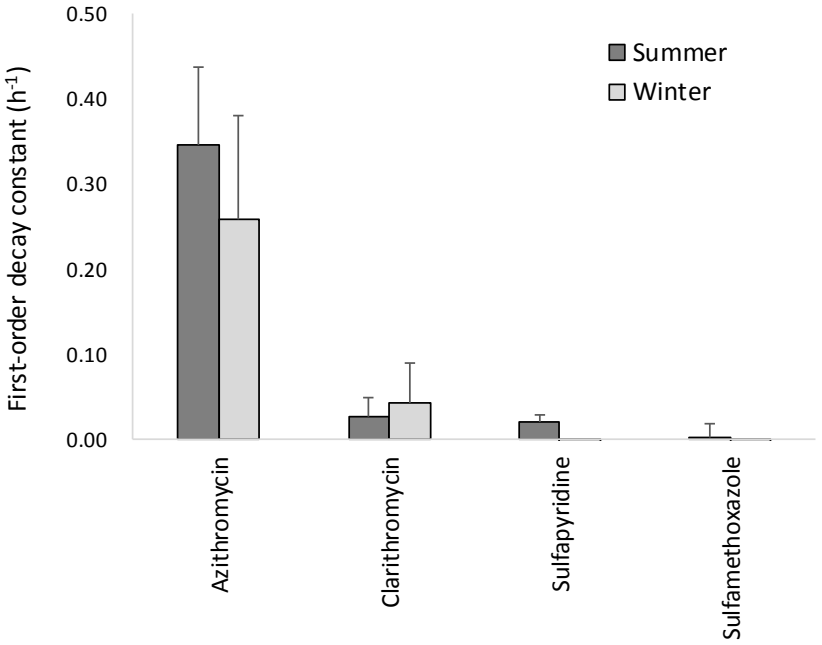

Figure S2. Comparison of the first-order decay constants of the antibiotics in the Katsura River stretch obtained in summer and winter sampling campaigns in a previous study (Hanamoto et al. 2013). The vertical bars denote means and the error bars denote the standard deviation (n = 3 for both summer and winter).

Laboratory Experiments

The direct photolysis rate constant (k_p) of a compound in river *i* was estimated as

$$k_{p_i} = \varphi \times \left\{ \frac{UVB_i \times \left(1 - R_{UVB_i}\right) \times \left(1 - B_{UVB_i}\right)}{UVB_t} \times \sum_{\lambda = 297.5}^{315} \frac{L_\lambda \times \left(1 - 10^{-\alpha_{\lambda_i} \times l_i}\right) \times \varepsilon_\lambda}{\alpha_{\lambda_i} \times D_i} + \frac{UVA_i \times \left(1 - R_{UVA_i}\right) \times \left(1 - B_{UVA_i}\right)}{UVA_t} \times \sum_{\lambda = 315}^{490} \frac{L_\lambda \times \left(1 - 10^{-\alpha_{\lambda_i} \times l_i}\right) \times \varepsilon_\lambda}{\alpha_{\lambda_i} \times D_i} \right\} \quad \text{(S2)}$$

where φ is quantum yield of the compound (–); UVB and UVA are sunlight intensity at Earth's surface in those wavelengths (W/m^2); R_{UVB} and R_{UVA} are fraction of sunlight reflected at the surface of the water body in those wavelengths (–); B_{UVB} and B_{UVA} are fraction of sunlight shaded by aquatic and overhanging vegetation in those wavelengths (–); UVB_t and UVA_t are theoretical sunlight intensity at Earth's surface in those wavelengths (W/m^2); L_λ is theoretical sunlight intensity at Earth's surface at wavelength λ (10^{-3} Einstein cm^{-2} h^{-1}); α_λ is light absorption coefficient of the water body at wavelength λ (m^{-1}); l is path length of sunlight in the water body (m); ε_λ is molar absorption coefficient of the compound at wavelength λ (M^{-1} cm^{-1}); and D is depth of the water (m). This equation was proposed in a previous study (Hanamoto et al. 2013).

The parameters used for the estimation of the direct photolysis constant in the Thames and Katsura river stretches are summarized in Table S3.

Table S3. Parameters used for the estimation of the direct photolysis constants of test compounds in the Thames and Katsura river stretches.

		Outline of used data	
		Thames River stretch[a]	Katsura River stretch[b]
UVB, UVA	Sunlight intensity at Earth's surface in UVB or UVA	The ratios UVB/UVB_t and UVA/UVA_t were estimate from observed and teoritical sunlight intensity in Kyoto city	Reported value in Kyoto city
UVB_t, UVA_t	Theoretical sunlight intensity at Earth's surface in UVB or UVA		Theoretical value at latitude 40°N
L_λ	Theoretical sunlight intensity at Earth's surface at wavelength λ	Theoretical value at latitude 50°N	Theoretical value at latitude 40°N
B_{UVB}, B_{UVA}	Fraction of sunlight shaded by plants in UVB or UVA	Since there is little overhanging vegetation along the river stretches, these were set to 0	
R_{UVB}, R_{UVA}	Fraction of sunlight reflected at water surface in UVB or UVA	Theoretical value for sky radiation	Theoretical value for sky radiation
l	Path length of sunlight in water body	Theoretical value for sky radiation	Theoretical value for sky radiation
α_λ	Light absorption coefficient of water body at wavelength λ	Measurements at site 3	Measurements at site *f*
D	Depth of water	Estimate from reported flow rate	Estimate from reported flow rate
φ	Quantum yield of the compound	Reported value	Reported value
ε_λ	Molar absorption coefficient of the compound at wavelength λ	Reported value	Reported value

[a] Details and references for the Thames River stretch were shown in the main text "Direct photolysis estimation". [b] Details and references for the Katsura River stretch were shown in a previous study (Hanamoto et al. 2013).

The light absorption coefficient of the surface water at site 3 in the Thames River was compared with those reported at the Miyamae Bridge in the Katsura River (Hanamoto et al. 2013) (Figure S3). The light absorption coefficient was much higher in the Thames River, which is attributable to higher suspended solids and dissolved organic matter in the Thames River.

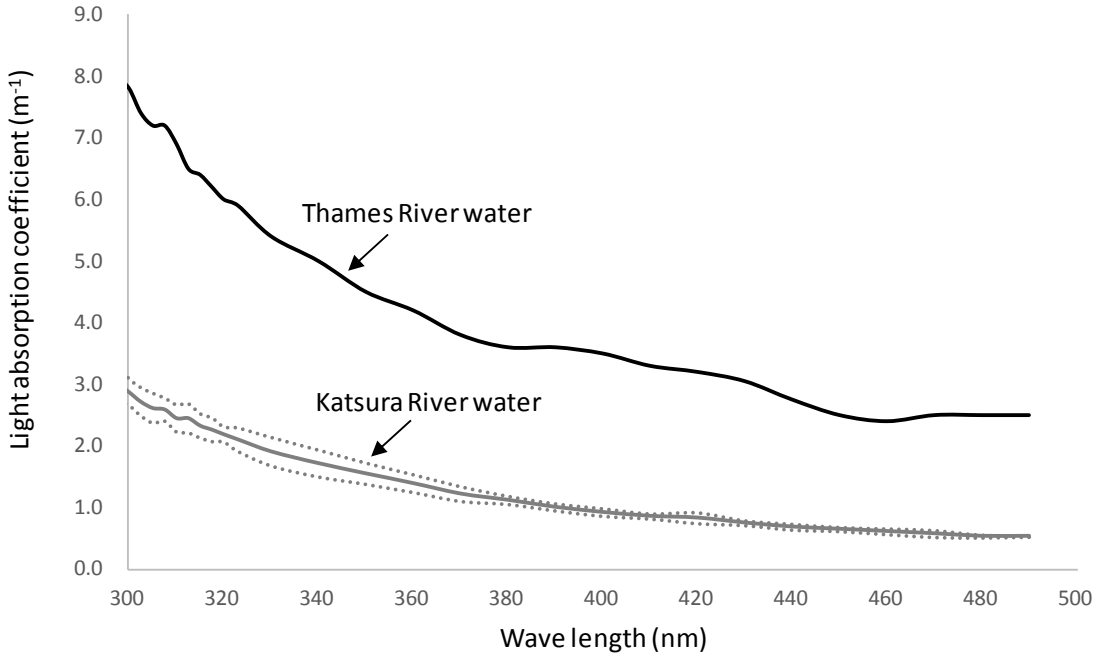

Figure S3. The light absorption coefficient of surface water at site 3 in the Thames River compared with those reported at the Miyamae Bridge in the Katsura River (Hanamoto et al. 2013). The solid line denote means and dashed line denote standard deviations in year-round sampling for the Katsura River (n = 6), whereas the absorption of the Thames River surface water was only measured once during low flow conditions in 2013 summer.

The first-order rate constants of sulfapyridine observed in the indirect photolysis experiments are shown in Figure S4.

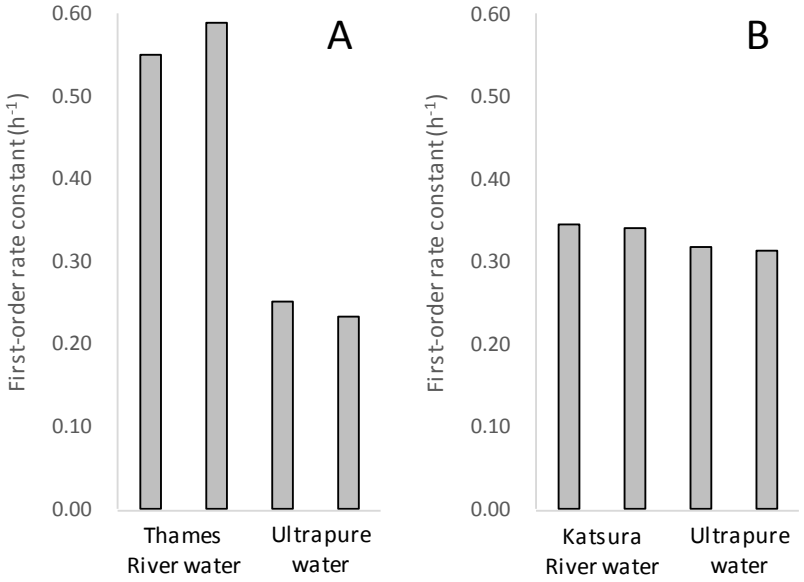

Figure S4. The first-order rate constants of sulfapyridine observed in the indirect photolysis experiments conducted in (A) the UK and (B) Japan. The experiment was conducted in duplicate. Irradiation times in the UK and Japan were 4.0 h and 2.2 h, respectively. The concentrations decreases were 49% (ultrapure water in Japan) to 90% (Thames River water). The concentration decreases of the other antibiotics and carbamazepine during the experiment were not appreciable (< 20%) in all samples.

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