1 **PPCPs in Jiulong River estuary (China): Spatiotemporal**
2 **distributions, fate, and their use as chemical markers of**
3 **wastewater**

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Abstract

The occurrence and fate of 50 pharmaceuticals and personal care products (PPCPs) were investigated in the surface water of Jiulong River estuary in the southeast of China in spring, wet season, summer, autumn and winter. Results demonstrated a wide distribution of PPCPs in Jiulong River estuary, where 34 PPCPs were detected at least once and 5 PPCPs were detected in all the samples, including caffeine, diclofenac, metoprolol, methyl paraben, and propyl paraben. Spatial and seasonal variations were observed. Special emphasis was placed on the PPCP fate in the estuary. Most PPCPs showed a non-conservative behavior in the estuary, while the non-steroidal anti-inflammatory drugs and bisphenol A showed a pseudo-conservative behavior. The non-conservative and pseudo-conservative behavior was attributed to the combination of the seawater dilution, the introduction of PPCPs via the sewage water, and the physical, chemical, or biological removal processes. Furthermore, PPCP concentrations showed drastic variations in the turbidity maximum zones. To our best knowledge, this is the first work to indicate the pseudo-conservative behavior of PPCPs in the estuary, and to show the drastic variations of PPCPs in the turbidity maximum zone. In addition, the ratio of labile to conservative PPCPs was calculated to track the source of untreated sewage contamination. Results showed a significantly higher ratio compared to the average value in WWTP effluents, indicating the ubiquitous discharge of untreated domestic wastewater in Jiulong River estuary. In addition, the high ratio of bisphenol A to conservative PPCPs implied the potential input of untreated industrial wastewater in Jiulong River estuary.

45 **Keywords**

46 PPCPs; Estuary; Spatiotemporal distributions; Mixing curves; Sewage markers

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48 **1 Introduction**

49 Pharmaceuticals and personal care products (PPCPs) are a class of emerging
50 contaminants. Great concerns have been raised regarding their possible adverse
51 effects on the ecological safety and human health due to their biological activity and
52 bioaccumulation [1]. Large numbers of studies have investigated PPCP occurrence in
53 the aquatic environment [2-5]. Results showed that PPCPs were widely detected in
54 the wastewater [2-4] and freshwater (mainly river water) [4, 5]. Due to the continuous
55 input of PPCPs into the aquatic systems and constant detection, they are referred to as
56 “pseudo-persistent” contaminants [5]. However, data on the PPCP presence in the
57 estuarine water are few [6]. The investigations of PPCPs in Yangtze estuary (China)
58 [7, 8], Jiulong estuary (China) [9], Douro River estuary (Portugal) [10], Long Island
59 Sound estuary (US) [11], and so on, have provided the basic information of PPCP
60 occurrence and fate in the estuary. Considering the estuarine areas are generally well
61 developed regions with high population density, the further investigation of
62 anthropogenic pollutant (such as PPCPs) is of great need. In addition, the estuarine
63 systems receive considerable contaminated inputs via WWTP effluent and river runoff.
64 The seasonal variations of PPCPs in the WWTP effluent due to the variations of PPCP
65 usage and removal efficiencies [2], and in the river water due to the precipitation
66 dilution [9], might lead to the seasonal variations of PPCPs in the estuaries. Therefore,
67 the investigation of PPCP occurrence in the estuary over an extended period of time is
68 of great interest.

The salinity gradient is one of the main characteristic of the estuarine ecosystem. Generally, the negative relationship between PPCP concentrations and salinities was observed, which were mainly due to the dilution of PPCPs by incoming seawater in the estuary [7, 12]. The investigation of PPCP distribution as a function of salinity in the Jamaica Bay in US showed the conservative behavior of PPCPs in the estuary [12]. However, a non-conservative behavior of PPCPs was observed in the Yangtze Estuary, China [7]. The contradictory results indicated the further investigations are still needed to understand PPCP behavior in the estuary. In addition, the hydrologic condition of the estuarine turbidity maximum (ETM), where the suspended particles were higher than those upstream or seaward [13], is highly dynamic. Studies have shown that there was a significant influence on the distribution of persistent organic pollutants (e.g. polycyclic aromatic hydrocarbons) in ETM [14]. However, to our knowledge, up to now information on the occurrence and fate of PPCPs in the ETM was not available. Therefore, the further understanding of PPCP behavior as mixing with seawater, especially in the ETM, is of great interest.

Eganhouse (2004) has defined molecular markers as “organic substances that carry information about sources of organic matter or contamination” [15]. PPCPs have anthropogenic origin and WWTP effluent is the major source of their occurrence in the environment [16]; therefore, PPCPs are being applied as molecular markers to indicate the sewage discharge to the surface waters [17]. Carbamazepine [18] and acesulfame [19] have been used frequently as sewage marker because of their conservative behavior that they are not eliminated in the WWTPs and are quite

91 persistent in the surface waters. In addition, the utilization of both conservative
92 markers and labile markers has been suggested [20] as it could indicate the type of
93 sewage (raw or treated) discharged to the surface waters [9]. As some rural areas in
94 China do not have the proper wastewater treatment processes and the untreated
95 wastewater might be directly discharged to the surface water, the application of
96 PPCPs as sewage markers will identify the potential hotspots of raw wastewater or
97 treated wastewater effluent discharge.

98 The objectives of the present study were to (1) determine the distribution and
99 abundance of PPCPs in order to understand their spatial and seasonal variations in
100 waters of Jiulong River estuary, (2) understand the PPCPs behavior as a function of
101 salinity in the estuary, (3) evaluate the pollution source using PPCPs as sewage
102 marker.

104 **2 Materials and methods**

105 **2.1 Study area**

106 The Jiulong River estuary is a shallow estuary in the southeast coast of China. It
107 is characterized as a semi-diurnal tide with a typical subtropical monsoon climate.
108 The climate in the watershed of Jiulong River and its estuary is characterized with
109 five seasons according to the temperature and precipitation, including spring (March
110 and April), wet season (May and June), summer (July to September), autumn
111 (October and November), and winter (December to February) [21]. The estimated
112 precipitation and temperature in each season in 2014 is shown in Table S1 in the

Supplemental Information (SI). Jiulong River estuary receives freshwater from Jiulong River, which flowed through the area of animal husbandry, industry, and large cities. Under the rapid economic growth and urbanization in the watershed of Jiulong River and its estuary, the water pollution has become a serious problem.

2.2 Sampling

The sampling sites in Jiulong River estuary are shown in Fig. 1. To get a better understanding, three samples (S1-S3) were collected from the upper estuary, which includes narrow channels and freshwater outflow, four samples (S4-S7) were collected from the middle estuary, and four samples (S8-S11) were collected from the lower estuary, which is mainly mixed with seawater. The ETM is between Site S3 and S4.

Water samples were collected on April 17th, June 4th, August 7th, October 10th, and December 12th in 2014 to represent spring, wet season, summer, autumn, and winter, respectively. For each sampling, samples were collected along a salinity gradient from the upper estuary to the lower estuary. Sampling location, salinity, and other hydrographic data are listed in SI Table S2. Generally, the salinity ranged from 0.0 at the upper estuary to 31.1 at the lower estuary.

Fig.1 Sampling sites in Jiulong River Estuary

2.3 Chemicals

50 PPCPs were selected and investigated mainly based on their high detection frequencies and high concentrations reported in the previous studies [2]. Detail information of PPCPs is provided in SI Table S3. The stock solutions of individual PPCPs were prepared in methanol and stored at -20 °C in the dark.

2.4 Sample preparation and analysis

One liter of surface water was collected from each sampling site in amber glass bottle and stored in a cooler with ice. Sample preparation and analysis were performed according to our previous study [2, 9]. Briefly, samples were filtered (GF/F glass microfiber filters, Whatman), acidified to pH 2 using hydrochloric acid, added with 250 mg $\text{Na}_4\text{EDTA} \cdot 2\text{H}_2\text{O}$, and spiked with surrogate standards. Oasis HLB cartridge (500 mg 6 mL, Waters) were preconditioned with 20 mL methanol, 6 mL

water, and 6 mL water with pH 2.0, and then loaded by 1000 mL water samples. After the cartridges drying, analytes were eluted with 12 mL of methanol and 6 mL of acetone: methanol (1:1). The eluates were collected, evaporated to dryness with nitrogen stream and reconstituted to 1.0 mL with methanol. The whole sample preparation was performed within 48 h.

Instrumental analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Chromatographic separation of analytes was performed using a Kinetex C18 column (100 mm x 4.6 mm, 2.6 μ m, Phenomenex, USA) by a Shimadzu LC system (Shimadzu, Japan). A binary gradient with a flow rate of 0.5 mL/min was used. For the negative electrospray ionization mode (ESI), the mobile phase A contained 5 mmol/L ammonium acetate in water, while mobile phase B was methanol. For the positive ESI, the mobile phase A contained 0.1% formic acid in water, while mobile phase B was methanol. The sample injection volume was 10 μ L. The mass spectrometric measurements were carried out on an ABI triple quadrupole (QQQ) MS. The monitored target and reference ions are shown in SI Table S3.

2.5 Quality control

The identification of PPCPs was performed by LC-MS/MS with multiple reaction monitoring (MRM) mode, using the two highest precursor and product ion transition pairs. The ratios of product transitions were calculated to ensure the PPCP identification. An instrumental blank, procedural blank, blank spike, sample duplicate, and sample spike in duplicate were applied for each sampling. All instrumental and

procedural blanks were below the method detection limits (MDLs). As shown in Table S3, more than 80% of the recoveries were in the range of 60-120% in blank spiked samples and 50-120% in the matrix spiked samples (50 ng/L), which met the requirements for simultaneous analysis of diverse targets in the challenging matrices [22]. The MDLs were estimated by combining the PPCP recovery and instrumental quantification limits, which were calculated at S/N ratios ≥ 10 . The MDLs of the target PPCPs were in the range of 0.01-2 ng/L (SI Table S3).

3 Results and Discussions

3.1 PPCP occurrence in estuary

The ranges of PPCP concentration and detection frequencies in each sampling season are presented in Table 1 that clearly demonstrated wide distribution of PPCPs in Jiulong River estuary. Of the 50 monitored PPCPs, 5 PPCPs, including caffeine, diclofenac, metoprolol, methyl paraben, and propyl paraben were detected in all the samples. Ten PPCPs, including acetaminophen, ibuprofen, fenoprofen, indomethacin, ofloxacin, bisphenol A, triclocarban, triclosan, benzophenone-3 and octocrylene were detected in more than 50% samples. In addition, 16 PPCPs (ethenzamide, sulfamerazine, sarafloxacin, tetracycline, benzyl paraben, propranolol, sotalol, acetophenone, losartan, loratadine, fluoxetine, miconazole, glibenclamide, thiabendazole, clofibric acid, and clenbuterol) were below the MDLs in every sample. All the other PPCPs were detected at least once. PPCP detection frequencies in this study were in accordance with the 2012-2013 cruises in Jiulong River Estuary [9].

192 Table 1 The concentration range (ng/L) and the detection frequencies of each PPCP in Jiulong River estuary in the five sampling seasons

	Spring		Wet season		Summer		Autumn		Winter	
	Range	Freq (%)	Range	Freq (%)	Range	Freq (%)	Range	Freq (%)	Range	Freq (%)
Acetaminophen	BLD-7.56	60	BLD-12.5	91	BLD-0.841	91	BLD-1.86	70	3.55-9.85	100
Ibuprofen	BLD-18.3	60	2.73-20.7	100	BLD-20.3	91	BLD-13.0	50	3.28-19.3	100
Ketoprofen	BLD-36.6	60	BLD	0	BLD	0	BLD	0	5.6-12.8	100
Fenoprofen	BLD	0	BLD-241	91	1.97-29.5	100	1.90-33.5	100	6.67-37.7	100
Diclofenac	1.69-11.0	100	1.27-8.88	100	1.44-7.21	100	0.819-7.37	100	1.94-9.23	100
Antipyrine	BLD-8.5	60	BLD-8.32	55	BLD-6.70	55	BLD-4.49	10	BLD	0
Propyphenazone	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Indomethacine	BLD-2.67	90	BLD-0.837	18	BLD-1.28	64	BLD-1.04	30	BLD-1.06	64
Mefenamic acid	BLD	0	1.69-3.14	100	BLD	0	BLD-0.893	50	BLD	0
Codeine	BLD	0	BLD	0	BLD-0.356	82	BLD	0	BLD	0
Ethenzamide	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Naprofen	0.525-1.78	100	BLD-0.592	82	BLD	0	BLD	0	BLD	0
Crotamiton	BLD-0.234	20	BLD	0	0.211-1.08	100	BLD	0	BLD	0
Sulfamethoxazole	BLD-1.07	20	BLD	0	BLD	0	BLD	0	BLD	0
Sulfamethazine	BLD-1.86	20	BLD	0	BLD-2.15	9.1	BLD	0	BLD	0
Sulfamerazine	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Enrofloxacin	BLD-1.45	10	BLD	0	BLD-4.52	9.1	BLD	0	BLD	0
Sarafloxacin	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Oxytetracycline	BLD-15.0	30	BLD	0	BLD-16.9	27	BLD	0	BLD	0

Tetracycline	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Ofloxacin	BLD-20.2	80	BLD-32.8	64	1.53-11.7	100	BLD-6.04	60	BLD-11.4	18
Caffeine	9.47-120	100	129-1920	100	51.2-3060	100	8.07-65.3	100	17.2-813	100
Bisphenol A	20.8-364	100	21.5-925	100	10.6-95.0	100	BLD-39.3	30	11.1-100.5	100
Propyl paraben	1.91-68.3	100	3.4-69.9	100	1.06-10.1	100	0.440-1.59	100	1.11-5.22	100
Methyl paraben	2.23-53.4	100	1.68-39.4	100	2.98-68.8	100	1.41-7.27	100	2.65-29.1	100
Benzyl paraben	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Triclosan	2.64-96.5	100	0.298-1.08	100	BLD-18.1	9.1	0.574-1.79	100	6.31-72.0	100
Triclocarban	BLD-1.95	60	1.77-12.6	100	0.765-46.6	100	0.334-2.02	100	1.07-3.33	100
Metoprolol	8.13-36.0	100	0.940-5.41	100	8.66-30.2	100	0.788-21.5	100	3.99-26.0	100
Propranolol	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Sotalol	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Aspartame	BLD-2.9	10	BLD-4.31	45	BLD-4.13	82	BLD-16.6	20	BLD-1.45	9.1
Benzophenone-3	6.01-80.9	100	BLD-532	84	3.83-11.0	100	BLD-2.55	18	1.18-4.39	100
Acetophenone	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Octocrylene	BLD-31.6	90	BLD-2.37	63	0.378-1.77	100	0.182-2.55	100	BLD-0.782	73
3-4'-methy-ben-2-one	BLD	0	BLD-14.9	91	BLD	0	BLD-2.74	18	BLD-10.4	9.1
Losartan	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Loratadine	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Fluoxetine	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Carbamazepine	1.01-2.04	100	BLD	0	BLD-3.84	18	BLD	0	BLD	0
Diazepam	BLD	0	BLD-2.61	27	BLD	0	BLD	0	BLD	0
Miconazole	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Glibenclamide	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Cyclophosphamide	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0

Thiabendazole	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Clofibric acid	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Gemfibrozil	BLD-0.274	60	BLD	0	BLD-0.193	0	BLD	0	BLD	0
Clenbuterol	BLD	0	BLD	0	BLD	0	BLD	0	BLD	0
Pirenzepine	BLD	0	BLD-0.432	9.1	BLD	0	BLD	0	BLD	0
Sildenafil	BLD	0	0.448	9.1	BLD	0	BLD	0	BLD	0

193 BLD: below the method detection level.

194

195 Caffeine was the most abundant PPCP with the concentrations up to 3060 ng/L
196 (summer, S1). Similar level of caffeine was observed in the seafront of Thessaloniki,
197 Greece [23]. The high caffeine level was probably due to high consumption of tea in
198 the surrounding area and the direct discharge of raw wastewater. Bisphenol A was also
199 of high abundance and detected in almost all the samples. The median concentration
200 of bisphenol A was 58.0 ng/L, which was slightly higher compared to the median
201 concentration (with the value of 42.6 ng/L) in the same sampling area collected in
202 2012-2013 [9], indicated the increasing trend of bisphenol A in this estuary. Due to the
203 estrogenic activity of bisphenol A, a potential risk might be escalated for the aquatic
204 life, which indicated the necessity of long-term monitoring [24]. For the non-steroidal
205 anti-inflammatory drugs (NSAIDs) group, acetaminophen, ibuprofen, diclofenac, and
206 fenopfen, were of high detection frequencies and concentrations, with the highest
207 concentrations of 12.5, 20.7, 11.0, and 240 ng/L, respectively. For the antibiotics,
208 ofloxacin was frequently detected, while sulfonamides were of low detection
209 frequencies and concentrations. Generally, the NSAIDs were within the comparable
210 levels with the other estuary investigations [25, 26], while antibiotics in Jiulong River
211 estuary were lower than previous studies [8, 27]. For the other pharmaceuticals,
212 metoprolol was detected in all the samples with a concentration range of 0.788-36.0
213 ng/L. The widely detection was probably due to the low removal efficiencies during
214 the wastewater treatment processes [28], and the relative low attenuation in the
215 surface water [29]. Carbamazepine was widely detected in the surface water and was
216 applied as sewage marker [17] due to its persistence during the wastewater treatment
217 processes [28] and its recalcitrance in the surface water [30]. However, in the present
218 study, carbamazepine was only detected in spring with a concentration range of

1.01-2.04 ng/L. The detection frequency and concentrations of carbamazepine in this study were significantly lower (Mann-Whitney test, $p < 0.01$) compared to the 2012-2013 cruises in Jiulong River Estuary [9]. In addition, for the personal care products, the preservatives (methyl paraben, propyl paraben), the antimicrobial agent (triclocarban), the UV-filters (octocrylene and benzophenone-3) were of high detection frequencies ($>81\%$). The statistical analysis conducted in the next section was mainly based on those frequently detected PPCPs which were of relative high concentrations.

3.2 Seasonal variations

PCA analysis was conducted to investigate the detection frequencies of PPCPs among different sampling seasons. If a PPCP was detected, the value was marked as 1, while if it was below the MDLs, it was marked as 0. As shown in Fig. 2, the PPCP detection frequencies of most samples could be clustered into separate groups among different sampling seasons. For example, winter samples were clustered separately, which was probably due to the higher detection frequencies of NSAIDs. The higher detection of NSAIDs in winter was in accordance with previous study [31]. Summer samples were grouped separately, which might be because of lower detection frequencies of NSAIDs and antimicrobial agent (e.g. triclosan), and higher detection frequencies of antipruritic drug (e.g. crotamiton). Spring samples were separated, which might be due to the fluctuation of NSAIDs and higher detection of anticonvulsant drug (e.g. carbamazepine). However, autumn and wet season samples overlapped and separated from the other seasons. The average precipitation in Jiulong River area was around 210 mm and 750 mm during October and June, respectively [32]. Previous studies have shown that PPCP detection frequencies were lower in the

wet season due to the precipitation dilution [6, 9]. In the present study, the similar PPCP detection frequencies and pattern in the wet season and autumn (dry season) indicated that besides the dilution of river flow due to the precipitation, other parameters would also affect the PPCP detection frequencies. Tides in estuaries play a key role in the movement of water. The currents and mixing processes dilute the fresh water in the estuary which contains anthropogenic contaminants, including PPCPs. As shown in SI Table S2, the salinities of the wet season samples were lower or marginally lower compared to the other samples ($p<0.05$ or $p<0.1$) that indicated the less dilution of PPCP concentrations by the seawater in the wet season.

Fig. 2 Seasonal variations in the detection frequencies of PPCPs in the Jiulong River Estuary in different sample seasons

The seasonal variations in the concentrations of PPCPs, which were of high detection frequencies and concentrations, are shown in Fig. 3. The NSAIDs, including ibuprofen, diclofenac, fenoprofen, and acetaminophen, were generally higher in winter and the wet season, particularly acetaminophen, with $p<0.01$. The higher NSAIDs in the estuary were also observed in the previous study [31], which might be due to the higher usage in the winter seasons [33]. Our previous study showed that lower PPCP concentrations were observed in the wet season in Jiulong River compared to the dry season and normal season due to the rainfall dilution [9].

265 However, in the estuarine system, both rainfall and seawater might dilute the PPCP
266 concentrations. As shown in Table S1, the precipitations in the wet season and
267 summer were close with the value of 656 and 531 mm, respectively, while the
268 salinities were marginally lower ($p<0.1$) in the wet season compared to summer. The
269 less dilution by the seawater during the wet season might lead to high PPCP
270 concentrations. For the personal care products, including two preservatives, one
271 plasticizer, one antimicrobial agent, and two UV filters, higher concentrations were
272 observed in wet seasons, which was probably due to the less dilution of seawater,
273 since the salinity was lower ($p<0.05$) or marginally lower ($p<0.1$) compared with the
274 other sampling seasons. However, metoprolol showed an opposite trend, for which
275 lower concentrations were observed in wet season. Lara-Martin et al. found a relative
276 high concentration of metoprolol in the suspended solids and sediments due to the
277 positively charged of the amine group ($pka>8$) [34], which indicated that the
278 metoprolol concentration in the aquatic phase could be also affected by the
279 participation on solid phase.

280

281 Fig. 3 Seasonal variations in the PPCP concentrations (Letter A, B, and C, represent differences by one-way ANOVA at $p < 0.05$).

3.3 Spatial variations

In general, PPCP concentrations in the estuary decreased from the confluence of river to the sea (Fig. 4). For example, the total PPCPs concentrations in summer sampling was 3250 ng/L and 106 ng/L in S1 and S11, while in autumn it was 188 ng/L and 34.7 ng/L, respectively. Similar decrease trend from the river to the seawater was observed in the previous studies [8, 12]. The high PPCP levels near the river was probably due to the high burden of anthropogenic activities in the river water, including the effluent from WWTPs in the lower Western Prong of Jiulong River, and the non-point source pollution from the discharge of untreated wastewater [9]. The PPCP levels decreased towards sea as the fresh water was diluted by the PPCP-free seawater. However, the decreased trend was discrete. As shown in Fig. 4, a less decrease or an increase of the total PPCP levels was observed in sites S2, S4-S6 in spring and wet season, in S3 and S10 during winter, which might be due to the direct discharge of wastewater.

Fig. 4 Spatial variations of PPCP concentrations

3.4 PPCP concentrations as a function of salinity

The concentration-salinity mixing curves were applied to interpret the behavior of dissolved constituents based on the traditional approaches developed to identify the conservative and non-conservative behavior in the estuary [12, 35]. If the dissolved constituent was conservative in the estuary, the decrease in concentration was mainly due to the dilution of seawater and consequently, the mixing curve was linear. Otherwise, the nonlinearity indicated the non-conservative behavior, which may be caused by the removal processes such as adsorption onto the particles, photolytic, hydrolytic, or microbial degradation and transformation, or the introduction of PPCP contaminated sewage water [12].

The mixing curves of PPCP concentration over salinity are shown in Fig. 5. Generally, the nonlinearity was observed for most PPCPs except for the NSAIDs. For example, in spring, summer, and autumn, the levels of the two preservative, methyl paraben and propyl paraben, were low in the upper reaches of estuary (S1 and S2), sharply increased in S3 and S4, then linearly decreased in the lower estuary. However, in wet season and winter, the high preservative levels were observed in the lower estuary. For caffeine, an increase concentration was observed in S4-S5 and S2-S6 towards the lower estuary in spring and wet season, respectively. For benzophenone-3, octocrylene, triclocarban, and metoprolol, the correlation between concentrations and salinity was discrete. The non-conservative behavior and the higher levels in the lower estuary indicated the introduction of PPCPs from the sewage water in the Jiulong River estuary. In addition, a rapid decrease of caffeine concentration was observed from S1 to S5 in summer, which was faster than that expected from the mixing of fresh and saline end-member concentrations. The rapid decrease indicated the natural

attenuation of caffeine, which was mainly via the photodegradation and biodegradation rather than adsorption [12, 19, 36].

As shown in Fig. 5, the near linear decrease curves were observed for the NSAID levels over salinity, including ibuprofen, fenoprofen, diclofenac, and acetaminophen ($R^2 > 0.75$). The mixing curves of bisphenol A ($R^2 > 0.71$) in all samples except in wet season also showed near linearity. The nearly linear decreased concentrations of NSAIDs and bisphenol A with increase of salinity seemed to indicate the conservative behavior during their transport in the estuary, for which the decrease was mainly due to the dilution of PPCP-free seawater. However, since PPCPs might be introduced via the sewage water as shown above, the linear concentration-salinity relationships of NSAIDs and bisphenol A was probably due to the combination of seawater dilution, the introduction of PPCPs via the sewage water, and the physical, chemical, or biological removal processes. Therefore, the behavior of NSAIDs and bisphenol A in the Jiulong River estuary was pseudo-conservative.

339 Fig. 5 Mixing curves of PPCP concentration over salinity

It's worthy to note that in the ETM, PPCP concentrations showed drastic variations. For example, the concentrations of methyl paraben, propyl paraben, metoprolol, benzophnone-3, and octocrylene were drastically increased in S4 right after the ETM (S3) in the spring sampling. The concentration of bisphenol A drastically decreased in S3-5, which was the ETM in the wet season sampling. The ETM is the mixing zone of salt and fresh water, which is influenced by the interaction between runoff and tide. Within the ETM, there are considerable increases in the suspended particle concentrations [37], which are from both the upstream and the re-suspension of the sediment. The drastic decrease of PPCP concentration in ETM was probably because of the adsorption of PPCP onto the high concentration of suspended particles due to their floc property [37] in ETM. In addition, previous study showed that ETM may trap the river-borne particles which contained the organic pollutants (e.g. polycyclic aromatic hydrocarbons) [38]. The release of PPCP from the suspended particles or sediment due to the variations of the ionic strength in the estuarine system might increase PPCP levels in the aquatic phase [39]. The discharge of sewage water which contained high concentrations of PPCPs might also lead to the increase of PPCPs in the ETM [9]. So far, the investigation of PPCP fate in ETM is few [7]. The drastic PPCP variations in the ETM suggested that it's important to consider the suspended particles for the PPCP determination in the estuary, particularly for those samples with high levels of suspended particles or for the hydrophobic PPCP compounds.

3.5 Application of PPCPs as sewage markers

In this study, the ratio of labile to conservative PPCPs was calculated to indicate the sewage type. The labile markers were selected based on their high removal efficiencies during the wastewater treatment processes, and high detection frequencies in the estuary samples, while the conservative markers were selected based on their low removal efficiencies during the wastewater treatment processes, the high detection frequencies both in the sewage effluent and in the estuary samples. Our previous study investigated PPCP removal in 3 WWTPs nearby the Jiulong River estuary in the four seasons in 2014. Results suggested that acetaminophen, caffeine, propyl paraben, and bisphenol A could be used as the labile markers since they were readily removed in the WWTPs with average removal efficiencies higher than 98%, while ketoprofen, diclofenac, crotamiton, metoprolol, carbamazepine, triclocarban, and triclosan could be used as the conservative markers, since they were persistent in the WWTPs [28]. Considering the detection frequencies which are shown in Table 1, propyl paraben, acetaminophen, bisphenol A and caffeine can be used as the labile markers, and triclocarban, diclofenac, and metoprolol can be used as the conservative markers in this study. In addition, significantly higher concentration of bisphenol A was observed in the industrial wastewater, indicating the industrial source of bisphenol A [28]. Therefore, the ratio of bisphenol A to the conservative PPCPs could indicate the discharge of industrial raw water to the estuary, while the ratio of caffeine, acetaminophen, and methyl paraben to the conservative PPCPs could indicate the discharge of domestic raw water.

As shown in Fig. 6, the ratio of labile to conservative PPCPs in the Jiulong River estuary was significantly higher compared to the average value (0.48) in three WWTP effluents from four season samplings [28], which indicate the ubiquitous direct discharge of domestic wastewater. As Heisler's investigation (2008) demonstrated a

389 link of raw sewage and potentially harmful algal blooms in the aqueous environment
390 due to the elevated nutrient input [40], the present study indicate a risk of algal
391 blooms in the Jiulong River estuary. In addition, the ratios were higher in wet seasons
392 and summer, which was in accordance with our previous study in Jiulong River [9].
393 As show in SI Table S1, the precipitation in the wet season and summer in 2014 was
394 656.0 and 530.9 mm, respectively and therefore, the combined sewage overflow
395 might occur in Jiulong River estuary. Moreover, the ratio of bisphenol A to the
396 conservative PPCPs in most of the samples was higher than the average ratio (0.25) in
397 WWTP effluents. Especially at S1, S2, and S11, significantly high ratios of bisphenol
398 A to the conservative PPCPs were observed, which indicated the possible direct
399 discharge of industrial wastewater.

400

401

402 Fig. 6 Ratios of labile and conservative PPCPs in the Jiulong River estuary (the red dotted line indicated the average ratio in three WWTPs)

4 Conclusions

In this study, the occurrence and distribution of 50 PPCPs in the surface water of Jiulong River estuary was investigated over one year. Results demonstrated a wide distribution of PPCPs in the estuary at concentrations in the low ng/L to low $\mu\text{g/L}$ range. Both spatial and seasonal variations were observed.

Special emphasis was placed on the PPCP fate in the estuary. Most PPCPs showed a non-conservative behavior in the estuary, as evidenced by the non-linear relationships between PPCP concentrations with salinities. The NSAIDs and bisphenol A showed a pseudo-conservative behavior with a nearly linear decrease of concentration with increase of salinity. The non-conservative fate of PPCPs was probably due to the combination of the introduction of PPCPs via the sewage water, seawater dilution, and the physical, chemical, or biological removal processes. In addition, in the ETM zones, PPCP concentrations showed drastic variations.

The ratio of labile to conservative PPCPs was calculated to indicate the sewage discharge. The significantly higher ratios compared to the average value in WWTP effluents indicated the ubiquitous direct discharge of untreated domestic and industrial wastewater in Jiulong River estuary. The higher ratios in wet season and summer demonstrated the combined sewage overflow. Results suggest the need to improve the wastewater treatment in the Jiulong River watershed area.

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539

540 Figure captions:
541 Fig.1 Sampling sites in Jiulong River Estuary
542 Fig. 2 Seasonal variations in the detection frequencies of PPCPs in the Jiulong River
543 Estuary in different sample seasons
544 Fig. 3 Seasonal variations in the PPCP concentrations (Letter A, B, and C, represent
545 differences by one-way ANOVA at $p < 0.05$).
546 Fig. 4 Spatial variations of PPCP concentrations
547 Fig. 5 Mixing curves of PPCP concentration over salinity
548 Fig. 6 Ratios of labile and conservative PPCPs in the Jiulong River estuary (the red
549 dotted line indicated the average ratio in three WWTPs)
550