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The ecological risk dynamics of pharmaceuticals in micro-estuary environments

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ABSTRACT

Micro-estuarine ecosystems have a surface area < 1 km^2 and are abundant in Mediterranean regions. As a result of their small size, these systems are particularly vulnerable to effects of chemical pollution. Due to fluctuating flow conditions of base-flow dominated by treated wastewater effluents and flood events transporting rural and urban non-point-source pollution, micro-estuaries are under a dynamic risk regime, consequently, struggling to provide ecological services. This two-year study explored the occurrence and risks of pharmaceutical contamination in the Alexander micro-estuary in Israel. Pharmaceuticals were detected in all samples (n=280) at as high as 18 μg L^{-1} in flood events and 14 μg L^{-1} in base-flow. Pharmaceutical mixtures composition was affected by flow conditions with carbamazepine dominating base-flow and caffeine dominating flood events. Median annual risk quotients for fish, crustaceans and algae were 19.6, 5.2, and 4.5, indicating that pharmaceuticals pose high risk to the ecosystem. Ibuprofen, carbamazepine and caffeine were contribute most to the risk quotients. The current work highlights that micro-estuary ecosystems, like the Alexander estuary, are continuously exposed to pharmaceuticals and most likely to other pollutants, placing these ecologically important systems under an elevated risk, in comparison to the more frequently studied large estuarine systems.

INTRODUCTION

Surface waters around the world are contaminated with and influenced by diverse mixtures of organic pollutants, such as solvents, microplastics, flame retardants, pesticides and pharmaceuticals[1,2]. Chronic exposure of stream and estuarine environments to active pharmaceuticals has become a global emerging ecological risk[3]. The main sources of pharmaceuticals in surface water include raw sewage or treated wastewater effluents[4–6], urban runoff[7], and runoff from arable land irrigated with treated wastewater or amended with biosolids or animal manures and slurries[8]. Due to water shortages, irrigation of agricultural systems with treated wastewater is becoming more prevalent, resulting in the ubiquitous presence of pharmaceuticals in runoff water[7] and stream base flows[9].

Flow patterns of semi-arid streams are characterized by low-discharge base flows which are often dominated by, or mixed with effluents and flood events during the rainy season composed of both urban and agricultural runoff[10,11]. Such flow patterns are found in streams in the Mediterranean climate zone, which includes southeastern Spain[12], southern Portugal[13], northwestern China[14], southern California[15] and the Middle East[16]. The eastern zone of the Mediterranean Sea uses large amounts of treated wastewater for agricultural irrigation (87% in Israel[17]), increasing the probability of introducing chemicals, such as pharmaceuticals, into streams from cultivated fields during storm events. Thus, together with urban runoff and sewage overflow, stormwater flood events are a dominant contributor to pharmaceutical loads in streams[7]. While stormwater flow makes up a relatively small proportion, timewise, of the annual flow, it contributes a dominant fraction of the annual water volume[11]. Moreover, flood-event frequency and magnitude are expected to increase in these regions due to climate change[18].

The presence of pharmaceuticals in both base flow and flood events results in chronic exposure of aquatic habitats to pharmaceutical mixtures with changing temporal dynamics.

These flow dynamics change further when entering transitional water bodies, such as lagoons, fjords and estuaries, where geochemical conditions and water residence time changes dramatically[11]. Estuaries, which are a zone of mixed surface and sea water[19], are often characterized by longer water residence times and strong gradients of salinity, temperature and turbidity. These gradients are especially dynamic in micro-estuaries, which are typical to semi-arid zones[11]. These relatively small water bodies of few meters in depth, a few kilometers in length, and a surface area of <1 km^2, are mainly governed by a sandbar at the mouth section. Despite their small size, micro-estuaries are an important ecological and sociological services provider[11]. These environments are generally understudied, and although they are known to frequently suffer from eutrophication[11], little is known on the risk they are subjected to from micropollutants, such as pharmaceuticals, and on their ability to attenuate such toxicants as shown for other similar vegetated water bodies[20].

The occurrence, composition and concentration dynamics of mixtures of pharmaceuticals pose a potential unacceptable risk to aquatic habitats. Although the potential risk of single pharmaceuticals to different aquatic compartments has been previously shown, evaluating risk for mixtures is more challenging[21]. Many tools and models exist for the determination of the risks of pollutant mixtures[22], with the most effective for risk estimation being the concentration addition model[22]. Although this model neglects the possible antagonistic and synergistic interactions of chemicals, it can serve as an important tool for assessing mixture risk and identifying dominant pollutants and threatened taxonomic groups[22].

For a comprehensive evaluation of potential risks of pharmaceuticals in micro-estuarine environments, flux dynamics must be quantified. This calls for high-temporal-resolution sampling combined with comprehensive analytical analysis and the application of appropriate risk models. The current study explored the biannual dynamics of pharmaceutical mixture

fluxes in and out of the micro-estuary and evaluates the risk posed by the pharmaceuticals to aquatic organisms.

MATERIALS AND METHODS

Site and sampling

The Alexander stream main channel flows a distance of 32 km and drains an area of 550 km^2. It starts in the Samaria mountains (Palestinian Territory), crosses the Hefer valley (Israel), and ends at the Mediterranean Sea (Figure S1). The Alexander stream is ephemeral throughout most of its length, receiving some fountain water and mainly treated wastewater from a treatment facility located ~13 km upstream from the estuary head. The Alexander estuary (defined as a micro-estuary[11]) is ~6.5 km long with a maximal depth of ~3 m and average cross-sectional width of 20 m. Detailed characteristics of the Alexander micro-estuary are described elsewhere[11,23,24]. Water upstream from the estuary head (N32.375 E34.912) and adjacent to the estuary mouth (N32.394 E34.869) was sampled at the depth of ~20 cm (Figure S1). Each of the sampling stations was equipped with an automated water sampler (Sigma 900©, Hach Company, Loveland CO; and ISCO 3700 Full-Size Portable Sampler, Teledyne, Lincoln NE) with a carousel containing 24 350mL glass bottles. Samples were taken every 0.25–4 h, with higher sampling frequency during the rising limb of the hydrograph and peak discharge, and lower sampling frequency on the falling limb of the hydrograph. Monthly base-flow water grab samples were collected with a horizontal water sampler (5 L, Model 110B, OceanTest Equipment, Fort Lauderdale FL). All samples were filtered using 90 mm GF/F filters (nominal pore size of 0.7 μm, MGF, Sartorius, Göttingen, Germany) and immediately frozen (-20 °C). A total of 237 flood samples and 44 base-flow samples were analyzed over 2 hydrological years (2016–2018).

106 **Analysis of pharmaceuticals**

107 Water samples were defrosted overnight and 200-mL aliquots were spiked with 10 µL of a
108 mixture of isotopically labeled internal standards (see detailed information in SI) and
109 concentrated using SPE cartridges (Strata-X, 200 mg, Phenomenex, Torrance, CA).
110 Pharmaceuticals were quantified by LC–HRMS analysis using a Q Exactive Plus hybrid FT
111 mass spectrometer coupled with a Dionex Ultimate 3000 RS UPLC (Thermo Fisher Scientific,
112 Waltham, MA). Instrumental parameters, limits of quantification are recoveries are shown in
113 the SI and in Table S1.

114

115 **Ecotoxicological risks: Single-compounds**

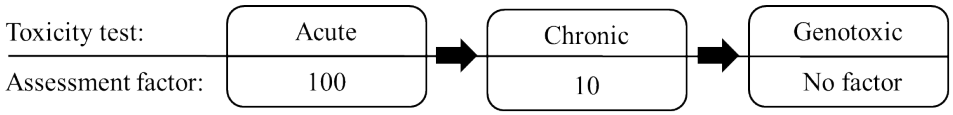
116 Experimental data on the apical effects (72 h algal growth; 21 d daphnid reproduction, 28
117 d fish growth and reproduction etc.) of the studied compounds were obtained from the
118 published literature. The values were then used alongside assessment factors proposed by the
119 European Chemicals Agency[25] (Table S2) to derive predicted no-effect concentrations for the
120 aquatic habitat ($PNEC_{aquatic}$) (Table 1 and Table S3)[25]. In the case where only genotoxicity tests
121 results were available, no assessment factors were applied due to the sensitivity of such
122 experiments. Risk characterization ratios for each study compound for each sample monitored
123 were then calculated using Equation 1. A risk quotient (RQ) ≥1 was considered an unacceptable
124 risk for the aquatic habitat. RQs were calculated based on a single-compound approach:

125 $$RQ_p = \frac{MC}{PNEC_{aquatic}} \quad (1)$$

126 where RQ_p is the pharmaceutical RQ, and MC is the measured concentration of the
127 pharmaceutical.

128 **Ecotoxicological risks: Pharmaceutical mixtures**

129 RQs were also calculated for the mixture of the studied compounds in each sample. It would
130 be inappropriate to use a mixture of endpoints for different taxonomic groups to assess the risk
131 of the mixtures. Therefore, we initially estimated PNECs for each pharmaceutical for each
132 taxonomic group ($PNEC_{fish}$, $PNEC_{crustaceans}$ and $PNEC_{algae}$) using the following prioritization
133 and assessment factors diagram.



134

135 The assessment factor for chronic tests represents the transition from laboratory conditions
136 to the field, with an additional factor applied for the conversion of the acute test to chronic
137 levels. When no acute or chronic tests were available, the more sensitive test of genotoxicity
138 was used with no assessment factor implementation. In the rare case where no experimental
139 value was available, the toxicity calculation used by the Ecological Structure–Activity
140 Relationship model (ECOSAR) was used and regarded as an acute toxicity value. PNEC values
141 determined for 16 out the 19 analyzed pharmaceuticals, with indications of data origin and the
142 applied factors, are presented in Table 1.

143 The PNECs were then used along with the measured concentration data to estimate RQs
144 for the mixture for the separate taxonomic groups using Equation 2:

145 $$RQ_{tg} = [\sum_{i=1}^{16}(\frac{MC_i}{PNEC_{i,tg}}) \quad (2)$$

146 where RQ_{tg} is the pharmaceutical risk quotient for a specific taxonomic group *tg*, MC_i is the
147 measured concentration of pharmaceutical *i* in the sample, and $PNEC_{i,tg}$ is the PNEC of
148 pharmaceutical *i* for taxonomic group *tg*.

149

150

151 **Table 1.** Predicted no effect concentrations (PNEC; μg L^{-1}) of the studied pharmaceuticals for
152 the aquatic environment and for fish, crustaceans and algae taxonomic groups. Assessment
153 factors are presented in Table S3.

Name	Therapeutic activity	Aquatic	Fish	Crustacean	Algae
Carbamazepine	Anticonvulsants	0.05[26]	2500[27]	0.05[26]	0.2[28]
Lamotrigine		150[29]	600[30]	1000[29]	750[29]
Lorazepam		0.005[31]	0.05[31]	37[31]	20[31]
Acetaminophen	Anti-inflammatories	0.5[32]	0.5[32]	95[33]	0.5[34]
Diclofenac		0.1[35]	0.1[35]	46[36]	100[27]
Ibuprofen		0.01[37]	0.01[37]	3.2[38]	1[34]
Ketoprofen		0.03[39]	0.3[39]	436.5[40]	162.1[40]
Naproxen		15[41]	10[42]	15[41]	620[41]
Metoprolol	β-Blocker	7.3[43]	1000[44]	88[44]	73[43]
Sildenafil	Erectile dysfunction	0.026[45]	0.026[45]	N/A	N/A
Clofibric acid*	Lipid regulators	0.49[46]	0.49[46]	1[47]	10[48]
Bezafibrate		0.034[45]	0.034[45]	2.3[49]	6000[49]
Gemfibrozil		0.38[45]	0.38[45]	7.8[49]	312[49]
Caffeine	Stimulant	1[34]	30[50]	12[51]	1[34]
Sulfamethoxazole	Sulfonamides	0.6[27]	800[27]	1[29]	0.6[27]
Sulfapyridine		0.012[31]	0.35[31]	0.012[31]	19.3[52]

154

155 *Metabolite of Clofibrate.

156

157 **RESULTS AND DISCUSSION**

158 Our study focused on a typical micro-estuary in the Alexander Stream, which is in a semi-
159 arid Mediterranean climate zone and which drains a watershed of mixed land use (agricultural
160 and municipal) that is highly influenced by treated wastewater[11,23]. Here we report data for
161 base-flow and flood-event durations over 2 hydrological years (2016–17 and 2017–18) (Table

S4) which were within the normal annual flow-regime cycle based on measurements from 50 years[23]. In addition to the temporal and spatial dynamics data for pharmaceuticals, our analysis evaluated ecosystem health by determining risk and its drivers.

Pharmaceutical occurrence and concentration: Estuary head and mouth

All water samples collected at the head or mouth of the Alexander estuary during flood events or under base-flow conditions were contaminated with pharmaceuticals or their metabolites (Table 2). The five most frequently detected pharmaceuticals in the flood-event samples were carbamazepine, diclofenac, bezafibrate, caffeine, and sulfamethoxazole. Under base-flow conditions, the ubiquitous pharmaceuticals were carbamazepine, lamotrigine, bezafibrate, gemfibrozil, caffeine, and sulfamethoxazole. These occurrence patterns are similar to those in European rivers, where carbamazepine, diclofenac, caffeine, and sulfamethoxazole are highly ubiquitous[53].

Noticeable differences in substances in the base-flow and flood conditions were found. For example, ibuprofen was detected in ~90% of the flood-event samples but in only 27% of the base-flow sample. In contract, lorazepam was detected in only ~20% of flood-event samples compared to ~80% in the base-flow samples. Changes between flow conditions were also pronounced in terms of concentrations of pharmaceutical groups (Table 2). Anticonvulsants, sulfonamides, and the erectile dysfunction drug sildenafil exhibited higher average concentrations in base-flow samples than in the flood-event samples. Anti-inflammatories, lipid regulators, the stimulant caffeine, and the β-blocker metoprolol showed the opposite trend with higher concentrations in the flood-event vs. base-flow samples. These clear differences suggest a dominant influence of pharmaceuticals' environmental stability on their transport dynamics in the stream. The less environmentally persistence anti-inflammatories and caffeine (as calculated by Aminot et al. based on DT_{50} values[54]) are rapidly degraded in wastewater-treatment plants and are therefore present at low concentrations under base-flow conditions.

187 The more environmentally persistence pharmaceuticals, such as anticonvulsants[54], are found
188 in relatively high concentrations under both base-flow and flood conditions.

189 **Table 2.** Occurrence (%) and concentration (ng L^{-1}) of pharmaceuticals and their metabolites in water samples collected from the head and mouth

190 of the Alexander micro-estuary during flood Events (2016-17 and 2017-18; n = 236) and base flow (2017 and 2018; n = 44).

	Floods events					Base-flow				
	Occurrence	**Average**	**Median**	**Max**	**Min**	**Occurrence**	**Average**	**Median**	**Max**	**Min**
Carbamazepine	100	414	180	4,953	25	100	643	644	1,947	52
Epoxy carbamazepine	100	28	15	294	1	98	57	60	179	0
trans-Dihydroxy carbamazepine	100	425	162	3,857	19	100	1,233	1,125	3,458	68
Lamotrigine	97	30	14	235	0	100	111	113	235	9
Lamotrigine-N-oxide	17	0.3	0	7	0	18	1	0	9	0
Acetaminophen	39	152	0	2,127	0	18	12	0	184	0
Diclofenac	100	126	77	730	0	80	99	46	692	0
Ibuprofen	89	297	172	3,291	0	27	71	0	592	0
Ketoprofen	60	20	17	158	0	41	9	0	34	0
Naproxen	17	40	0	1,146	0	34	25	0	174	0
Metoprolol	82	7	2	1,038	0	66	5	2	77	0
Sildenafil	51	3	1	179	0	82	10	6	139	0
Clofibric acid	60	148	17	11,765	0	41	5	0	36	0
Bezafibrate	100	94	66	1,041	5	91	39	18	248	0
Gemfibrozil	83	263	22	4,428	0	91	140	27	2,704	0
Lorazepam	19	0.4	0	4	0	77	2	2	4	0
Caffeine	100	2,859	2,746	12,969	19	100	828	60	11,559	11
Sulfamethoxazole	100	51	14	656	0	100	115	82	656	1
Sulfapyridine	98	10	4	133	0	89	11	7	87	0

191

Overall, pharmaceutical concentrations found in the Alexander estuary were comparable to those found in other surface-water bodies, with a slight tendency toward the upper end of the concentration ranges[2,53]. Further comparison to other estuarine environments was performed using the German Environmental Agency pharmaceuticals in the environment database[55]. A total of 336 entries of mean and maximum values for 12 pharmaceuticals derived from 16 countries were considered. Average concentration of pharmaceuticals in the Alexander micro-estuary was on average ~7 folds higher than compiled entries from the database with metoprolol being the only compound showing a lower average concentration in the Alexander estuary compared to other estuarine systems (Table S5).

The average pharmaceuticals cumulative concentration at the estuary head was slightly higher during flood events, with 5 μg L^{-1} compared to 4 μg L^{-1} under base-flow conditions (Figure 1). These findings contradict the assumption that dilution of the base-flow water, dominated by treated wastewater, by stormwater runoff significantly reduces the overall pharmaceutical concentration in flood events. Our findings suggest that pharmaceuticals in runoff events originate from another source, perhaps the urbanized part of the watershed. The increased concentrations of rapidly degradable compounds such as ibuprofen and caffeine during flood events support the assumption of an urban influence, suggesting sewage overflow mixed with urban runoff as a source for pharmaceuticals in the stream and estuary.

Within-season dynamics were also observed during the rainy season, where the highest concentration peaks (18.6–17 μg L^{-1}) were recorded at the estuary head during the first flood event for each year (F2016-17 #1, F2017-18 #1; Figure 1). The peak concentration for F2016-17 #1 was dominated by caffeine (41%) and anticonvulsants (29%), whereas the two consecutive peaks of F2017-18 #1 were composed mainly of caffeine (76% and 67%). We assume that cumulative concentration peaks at the beginning of a rainy season are attributed to the flushing of pharmaceuticals accumulated in bed sediments and pore water in the upper

tributaries during the wastewater base flow of the dry season. Although dilution had little influence on overall dynamics, high-discharge flood events (e.g., F2018 #4) exhibited a significant dilution effect resulting in low accumulation of pharmaceutical concentrations.

Under base-flow conditions, pharmaceutical concentrations were dominated by the anticonvulsant carbamazepine and its metabolites. In general, the cumulative base-flow concentrations were lower than those of the flood events with one exception, the sample collected on 7 March 2018, which contained elevated concentrations of caffeine and anti-inflammatories (Figure 1). This rise in concentration was composed mainly of caffeine (~12 μg L^{-1}) and ibuprofen (~1 μg L^{-1}), probably from a recorded malfunction in the sewage-treatment facility upstream. The malfunction resulted in approximately 2 weeks of raw sewage being spilled into an upper tributary, carrying the more degradable compounds into the estuary and elevating the cumulative pharmaceuticals concentration. On the other hand, a sharp drop in cumulative concentrations in the estuary head was observed during the dry season of 2018. At that time, the base flow was redirected from the stream for irrigation, lowering the annual median to 2.3 μg L^{-1}, in comparison to 3.7 μg L^{-1} in 2017. The vast changes in pharmaceutical concentrations and mixture composition from sewage spills during base flow and sewage overflow during the rainy season, and on the other hand, from redirection of reclaimed wastewater for irrigation, emphasize the lack of a proper watershed-management effect on estuarine and coastal environmental pollution.

While flow types dictate the composition and concentration of pharmaceutical mixtures entering the estuary, the conditions in the estuary may influence the overall pharmaceutical concentration, and more importantly, cause shifts in pharmaceutical mixture composition. During flood events, cumulative pharmaceuticals concentration was generally lower in the estuary mouth vs. head. As an example, the peak concentration of the F2016-17 #1 event decreased by >20% along its flow in the estuary to 14.3 μg L^{-1} at the mouth (Figures 1 and 2).

Although the total contribution of anticonvulsants and caffeine to the cumulative concentration was maintained, the proportion of caffeine decreased from 41% to 28%, while that of the anticonvulsants increased from 29% to 44%. In the case of the F2017-18 #1 event, mouth samples were not collected since discharge was very low (Table S4), and the flood water was contained in the estuary and strongly diluted by the base flow and seawater intrusion.

The estuary water body holds ~300,000 m^3 over a length of 6.5 km, resulting in long-duration replacement of base-flow water with storm water. This enables the estuary to hold on to low water volumes, associated with small flood events, prolonging their residence time. The effect is on the scale of complete retention in the estuary, such as for F2017-18 #1, to hours, e.g., F2016-17 #1 where the first flood wave entering the estuary arrived at its mouth after 10 h. Therefore, the presence of a base-flow signature of pharmaceutical mixture composition is highly pronounced in the first flood-event outflow samples. In general, an increased contribution of anticonvulsants and lipid regulators to the cumulative pharmaceuticals concentration was recorded in outflux samples of all flood events (Figures 1 and 2).

A reduction in pharmaceutical concentration was observed under base-flow conditions along the flow in the estuary, where anticonvulsant concentrations dropped by ~25% from head to mouth during the 2017 base flow. Reduction was also pronounced in a spring 2018 water sample where the elevated caffeine concentrations dropped by almost 50%. Pharmaceutical addition and removal along the estuary under the different flow conditions are complex and derive from the dynamics between the multiple sources, the compound characteristics and persistence, and sorption–desorption processes. These changes may influence the risk potential and differentiate the waters entering the estuary from those exiting to the near-shore environment.

Ecotoxicological risks: Single compound approach

Due to dilution cycles, continuous changes in geochemical conditions, and the diverse exposed taxonomic groups, there is a need for a dynamic ecological risk evaluation. We focused our efforts on the changes in risk between the head and mouth of the estuary under different flow conditions (Figure 3). Under base flow conditions, at the head of the estuary, carbamazepine and diclofenac were found to pose an unacceptable risk, with median RQ of ~15 and ~ 1 respectively. Sildenafil and lorazepam had median RQ values of 0.3 and 0.5, respectively, posing minor risk. All other compounds exhibited median RQ values < 0.1 indicating an acceptable risk. Carbamazepine risk was also found to pose an unacceptable risk at the head of the estuary during flood-events, with a median RQ of 2.5, under these conditions ibuprofen and caffeine were also found to pose an unacceptable risk with median RQs of ~18 and ~3, respectively. Diclofenac and acetaminophen also showed RQ values higher than 1 on several occasions. Our data show that flood risk is driven by more readily degradable, but still toxic, compounds into the estuary that are otherwise absent from the system. Moreover, flood water eventually replaces the estuarine volume to become background water, dominating the habitat for weeks, and imposing elevated risk during the rainy season.

Under the base-flow regime, risk quotients for the estuary outflow were lower than for the head of the estuary. Median RQ values for carbamazepine and diclofenac were reduced from 15 to 10 and from 1 to 0.1, respectively (Figure 3). These drops in RQ may be the consequence of dissipation processes in the estuary due to prolonged water residence time under base-flow conditions. For some pharmaceuticals, RQ values in the outflow during flood events were also reduced compared to the head with the estuary with drops of 10% and 30% was observed for ibuprofen and caffeine in outflow samples. On the other hand, an increase in median RQs was observed for carbamazepine and gemfibrozil, from 2.5 and 0.01 to 7 and 0.6, respectively. These increases in relatively environmentally stable pharmaceuticals suggest additional

sources of treated wastewater along the flow in the estuary, and perhaps, resuspension and desorption processes in the estuary. These changes show that the risk regime expands beyond temporal variations to spatial location along the estuarine flow, and reflects the complex dynamics of pharmaceutical mixtures transported through the estuary.

Ecotoxicological risks: Analysis per taxonomic groups

While single-compound risk is crucial to identifying key stressors, the estuarine environment is exposed to multiple pharmaceuticals which may affect each taxonomic group differently. Furthermore, use of the concentration addition model to estimate cumulative risk enables targeting risk assessments to specific taxonomic groups, such as fish, crustaceans and algae (Figure 4). The overall annual RQ medians at the head of the estuary for fish, crustaceans and algae were 20, 3.5 and 4.5, respectively. For fish, median RQ values for flood events was ~22 vs. only 4.5 under base-flow conditions (Figure 4). Risk for crustaceans was elevated in the base flow with median RQ values of ~11 compared to 3.5 in the flood events. For algae, the RQ values for the flood and base flow conditions were relatively similar (4.5 and 3.5, respectively). Only 3 inflow water samples (out of 151), collected from the base flow during the peak dry season of 2018, exhibited RQ values lower than 1 (i.e., no risk) for fish (Figure S2). For crustaceans, 11 samples (collected at the end of F2017 #3 and beginning of F2017 #4 events) showed RQ values lower than 1; and 10 samples exhibited no risk for algae, mainly samples from F2017 #4 at peak discharge and the base flow in the 2018 dry season.

The highest RQ values of 338 and 227 were calculated for fish during the second and third flood events of 2016 (F2016-17 #2 and #3), in agreement with its elevated median RQ in flood events (Figure 4 and S2). Although the median RQ for crustaceans was higher during base flow, peak RQ values of 32 and 27 were measured at the beginning of the first flood event of each of the rainy seasons, suggesting first season wash of the upper watershed. Similar to

317 crustaceans, the RQ for algae peaked at 16 and 19 at the beginning of the first two flood events
318 of each of the rainy seasons.

319 The anti-inflammatory ibuprofen, which was less dominant in terms of concentration
320 (Figures 1 and 2), was the compound found to contribute most to the RQ for fish, (Figure S2).
321 Yet even in the absence of ibuprofen, 95% of the water samples showed unacceptable risk to
322 fish with RQ values higher than 1. In these samples, risk was mainly derived from the lipid
323 regulator bezafibrate and the anti-inflammatory diclofenac. Risk for crustaceans was derived
324 almost entirely from the anticonvulsant drug carbamazepine, in both flood events and base
325 flow, with a small contribution from the stimulant caffeine. An additional contribution to risk
326 for crustaceans was the lipid regulator metabolite clofibric acid. Unlike risk to fish, removal of
327 the dominant contributor (i.e., carbamazepine) from the cumulative risk calculation for
328 crustaceans resulted in only ~20% of the samples exceeding an RQ value of 1. Risk to algae
329 was mostly driven by the stimulant caffeine, contributing 56% of the risk on average, followed
330 by carbamazepine, responsible for 30% of the RQ, mainly during base flow (Figure S2). Minor
331 contributions to risk for algae were from ibuprofen and sulfamethoxazole. Omitting both
332 caffeine and carbamazepine from the cumulative risk calculations for algae still resulted in
333 ~25% of the samples exceeding an RQ of 1, all from flood events.

334 The overall median RQ values for estuary mouth samples were relatively similar to those
335 for the head samples (Figure 4). The most pronounced difference in risk to crustaceans was
336 found during flood events, being over twofold higher in mouth vs. head samples, with a median
337 RQ of ~8. Median RQ for algae during flood events was slightly higher in the outflow (5.5),
338 even though the dominant toxicant, caffeine, had decreased in concentration (Figures 1 and 2).
339 This additive effect was due to the increase in carbamazepine concentration, which was also
340 found to be a main contributor of risk to algae (Figures S2 and S3). The same trend was found
341 for cumulative risk to fish during flood events, being slightly elevated in outflow samples with

a median RQ of 23 (Figure 4). The reduction in ibuprofen concentration was substituted by the elevation in gemfibrozil concentration and risk to fish. Base-flow conditions at the mouth of the estuary were similar to those at the estuary head, where the biggest recorded difference was a decrease of 25% median RQ for fish. It is important to note that the elevated risk to algae from caffeine, due to the sewage spill in March 2018, dropped by almost 50% from head to mouth (Figures S2 and S3). The observed removal emphasizes the strong dissipation processes that might occur along the estuary under long water residence times.

Ecotoxicological risks: Effects of geochemical conditions

Although geochemical factors were not included in our risk calculation, they can affect directly or indirectly the ecological risk in micro-estuaries. Temperature in the micro-estuary ranges between 15-30 °C, peaks in the dry season when treated wastewater flows dominates the system (Figure S4). Temperature rise in dry season (i.e., base flow condition) is expected to pose abiotic stress via reducing the level of dissolved oxygen. In practice, the highly nutrient loaded wastewater induces eutrophication processes resulting in sever oxygen deficiency throughout the micro-estuary water column (Figure S4). The continuous anoxic-hypoxic conditions, which may be harmful for aquatic organisms on its own, along with low rates of water replacement[11], and possibly reduced microbial degradation of pharmaceuticals under these conditions[56], may result with elevated stress to the aquatic habitat during the dry season.

The reactivity of pharmaceuticals might be also affected by geochemical conditions, and in turn, influence the ecological risk. In micro-estuaries, pharmaceuticals are exposed to rising levels of salinity along the flow and with depth of the water column (Figure S4). This may affect the potential calculated toxicity of compounds to some extent[52,57,58]. Yet salinity may more profoundly affect pharmaceuticals risk indirectly, due to alteration of sediment-water partitioning. Elevated salinity is expected to increase adsorption of organic pollutants, reducing

the concentration in water column (and lowering the risk to organisms within that media), but increasing the concentration in bed sediments and the resulted risk to benthic dwellers. However, these effects are more pronounced for hydrophobic compounds rather than to the studied pharmaceuticals exhibiting low log *D* (Table S1).

Environmental implications

Estuarine environments, such as the Alexander stream, are continuously exposed to organic pollutants[59], among them biologically active pharmaceuticals[3,23,53]. Pharmaceuticals are introduced to estuarine habitats via runoff from rural and municipal zones during the rainy season. During the long and dry summer periods, pharmaceuticals are introduced via wastewater which made up the sole water source for these ecosystems. The composition of the pharmaceutical mixtures is highly coupled to the flow patterns, thus exposing the estuary to a changing risk regime.

The use of general $PNEC_{aquatic}$ and RQ as risk parameters is of great value in locating dominant drivers, but the use of taxonomic group PNECs allowed a more practical risk assessment, especially in systems with fluctuating flow conditions. While general aquatic risk showed elevated risk from caffeine during flood events, in practice, caffeine risk peaked at the beginning of the flood events and mainly affected algae, which are most likely washed from the system during this flood stage. On the other hand, ibuprofen is mostly toxic to fish, which stays within the estuary. Nevertheless, risk to algae extends beyond the flood's first wave and remains a concern as these primary producers are extremely important to micro-estuaries, dictating the oxygen balance in these highly eutrophicated ecosystems.

Ibuprofen, carbamazepine and caffeine were the main compounds contributing to risk for different taxonomic groups. However, their removal from the system is not expected to fully mitigate the ecosystem, as the level of the mixture's risk remains high. Caffeine and ibuprofen

originate mainly from raw or semi-treated wastewater and can be controlled by preventing sewage spills and overflows. Carbamazepine, on the other hand, is diffusive and more persistent, and its removal from the system is more challenging. Along with lipid regulators and sulfonamides, removing carbamazepine's adverse effect requires the complete removal of treated wastewater from the stream flow, and therefore must include control of the arable land runoff from fields irrigated with treated wastewater. The latter is of high importance since flood tails water, posing potential risk to algae and elevated risk to fish, enters the estuary in flow with decreasing velocities, eventually acquiring base-flow residence times of ~20 days.

The Alexander micro-estuary is exposed annually to chronic risk throughout its entire length and under the different flow conditions. This reflects the high potential risk in hypereutrophic micro-estuaries to several taxonomic groups from a mixture of pharmaceuticals originating from both point and diffuse pollution sources. The current work findings highlight the need to update water regulations, which do not currently take pharmaceutical mixtures into account. Furthermore, watershed-management interfaces are required in semi-arid region streams and estuaries to reduce pharmaceutical influx and prolong water residence time, thereby enhancing the natural removal processes.

Supporting Information

Information is provided about internal standards used for LC-HRMS; selected pharmaceuticals properties and analytical method parameters (Table S1). Assessment factors determination for $PNEC_{aquatic}$ are provided in Table S2 and pharmaceuticals assessment factors and PNECs are presented in Table S3. Sampled flow events characteristics are listed in Table S4. A map of the study site is shown in Figure S1. Risk quotient distribution between pharmaceuticals during 2 hydrological years is presented in Figures S2 and S3. Geochemical

416 conditions in the Alexander micro-estuary in the two-years study duration are shown in Figure
417 S4. These materials are available free of charge via the Internet at http://pubs.acs.org/.
418
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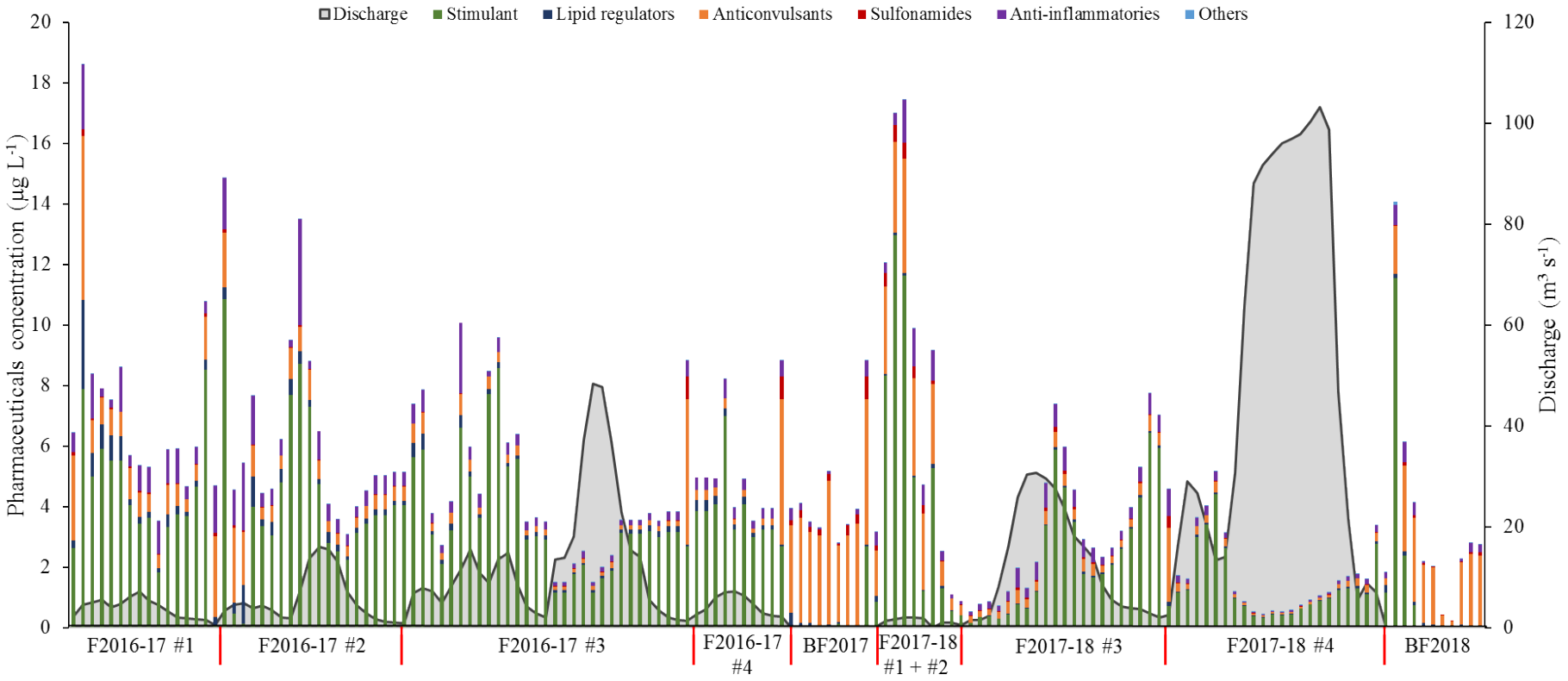
625



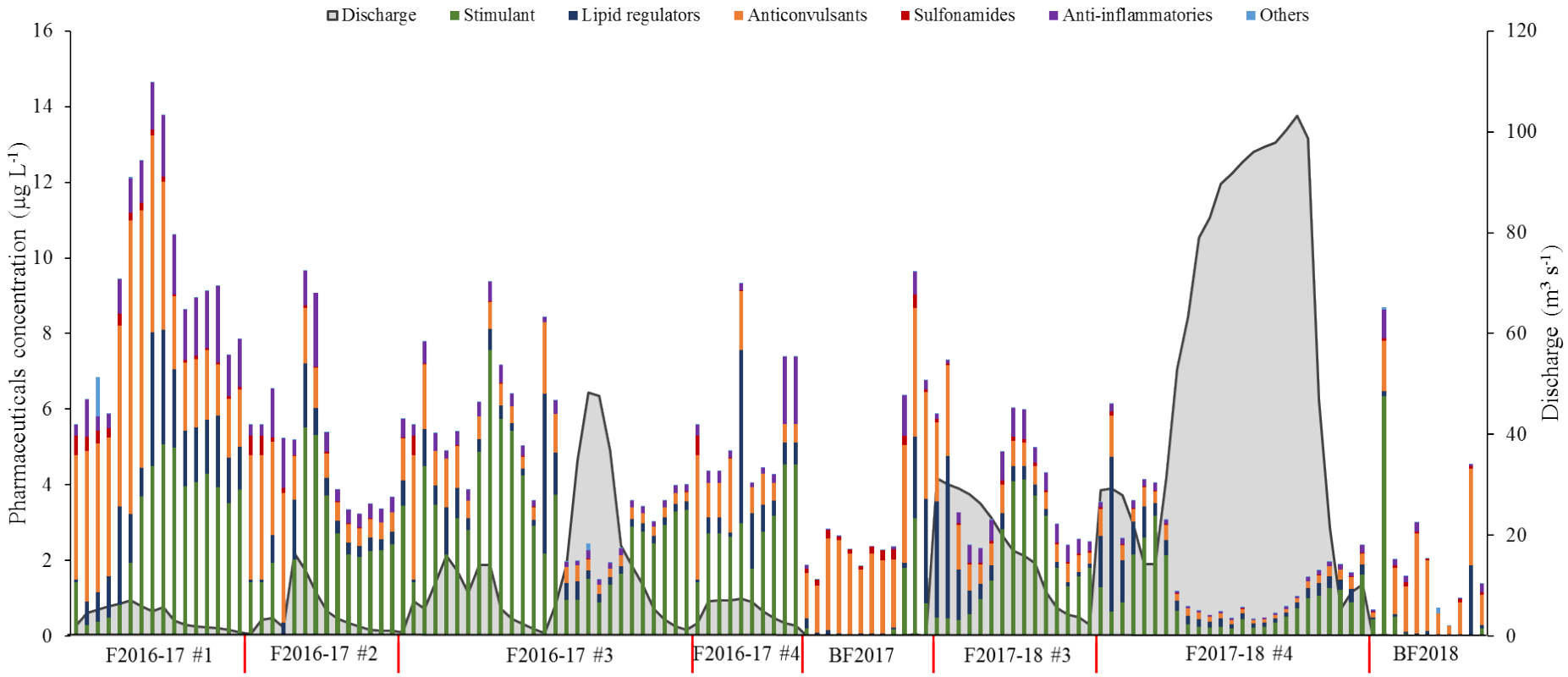
626

627 **Figure 1.** Concentrations of groups of pharmaceuticals (left axis) and flow discharges (right axis) during 2 hydrological years (Table 2) measured

628 at the head of the Alexander micro-estuary. Horizontal axis presents water samples in chronological order, where F represents flood events and

629 BF represents base flow.

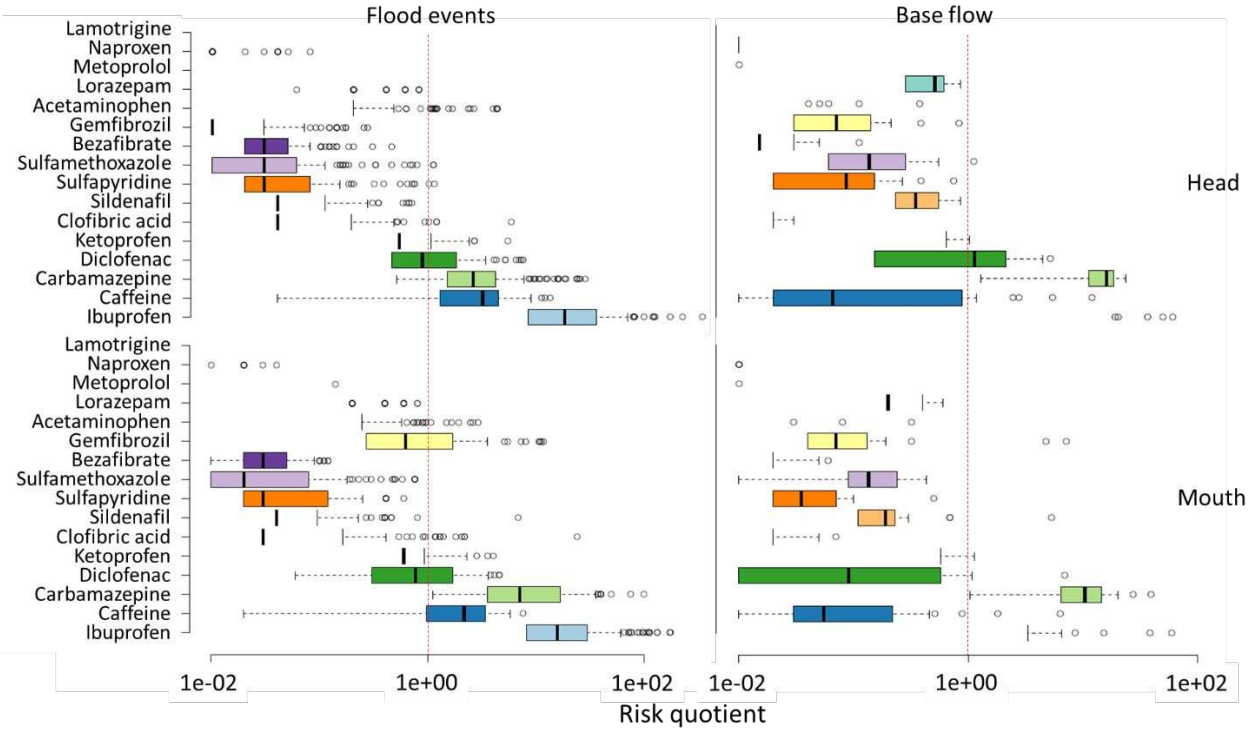
630



631

632 **Figure 2.** Concentrations of groups of pharmaceuticals (left axis) and flow discharges (right axis) during 2 hydrological years (Table 2) measured
633 at the mouth of the Alexander micro-estuary. Horizontal axis presents water samples in chronological order, where F represents flood events and
634 BF represents base flow.

635



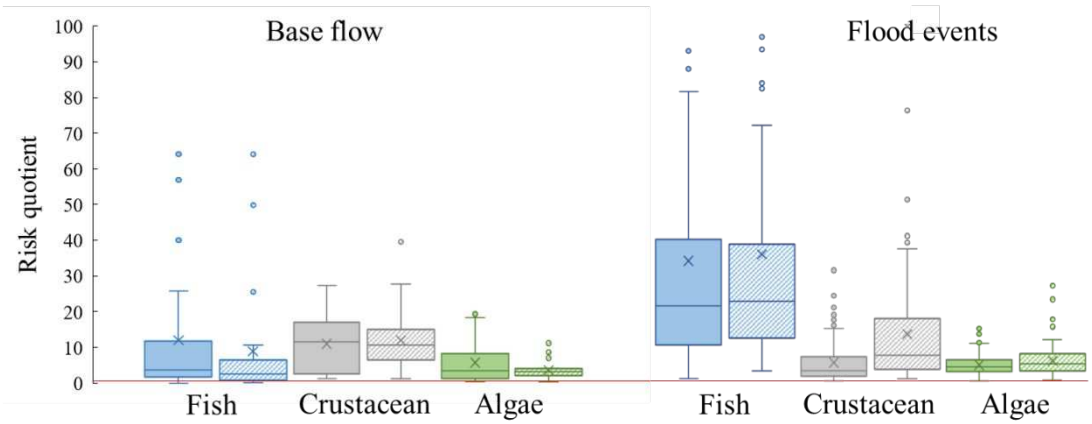
636

637 **Figure 3**. Pharmaceutical risks in flood and base-flow water samples collected at the head and

638 mouth of the Alexander micro-estuary. Risk quotients were calculated using point of no effect

639 concentrations for the aquatic habitat ($PNEC_{aquatic}$).

640



641

642 **Figure 4.** Cumulative pharmaceutical risk in the Alexander micro-estuary. A risk quotient of
643 1 (red line) marks toxicity benchmarks (Table 1). Cumulative pharmaceutical risk to fish,
644 crustaceans and algae at the head of the estuary (solid, n = 151) and mouth of the estuary
645 (diagonal stripes, n = 130). Exceptional samples from head (7) and mouth (9) samples in flood
646 events, with risk quotients ranging from 100 to 338 for fish, were omitted.