Supplementary Information

The ecological risk dynamics of pharmaceuticals in micro-estuary environments

Tom Topaz[1], Alistair Boxall[2], Yair Suari[3], Roey Egozi[4], Tal Sade[3], and Benny Chefetz[1*]

[1]Dept. of Soil and Water Sciences, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 7610001, Israel

[2]Department of Environment and Geography, University of York, Heslington YO10 5NG, United Kingdom

[3]Faculty of Marine Sciences, Ruppin Academic Center, Mikhmoret 402970, Israel

[4]The Soil Erosion Research Station, Soil Conservation and Drainage Division, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel

* benny.chefetz@mail.huji.ac.il

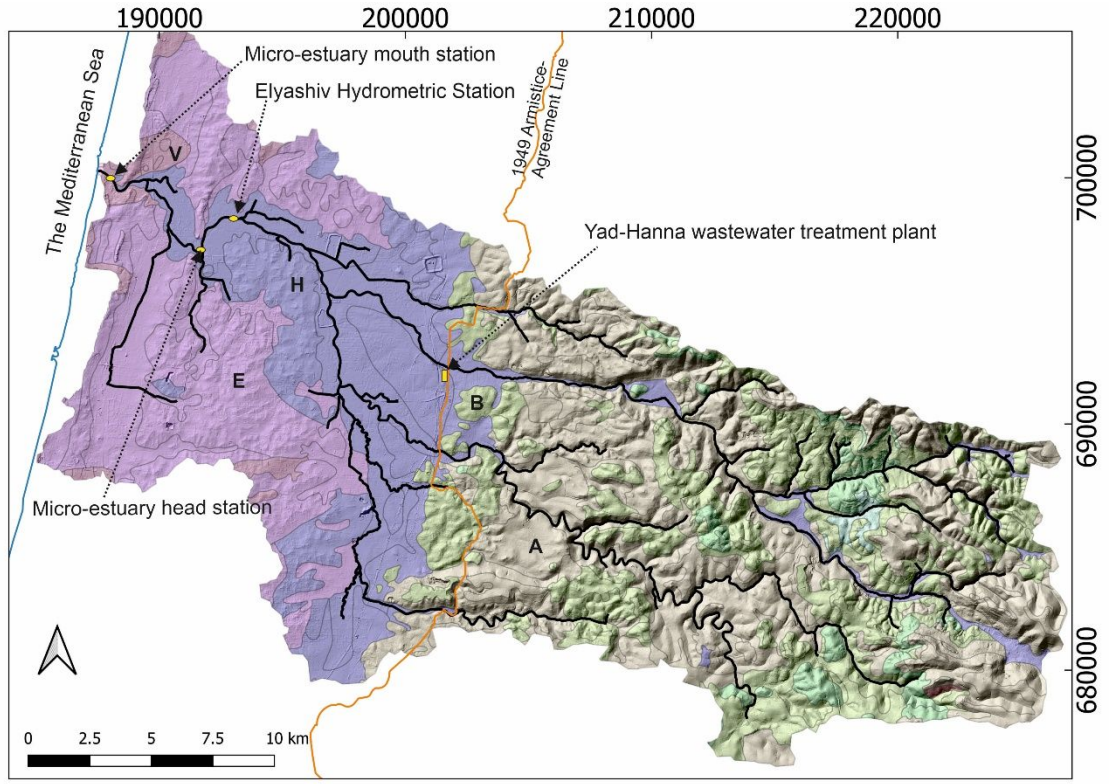

Figure S1. Map of the Alexander watershed with locations of sampling stations. V – Sand dunes, E – Hamra (Sandy-Loam soil), H – Grumusols, B – Light Rendzinas, A – Terra Rossa.

Sample preparation and LC–HRMS analysis

Water samples (200 mL) were spiked with 10 µL mixture of isotopically labelled internal standards (2 µg mL^{-1}) of 10,11-dihydro-10-hydroxy-carbamazepine-D3, bezafibrate-D4, caffeine-D9, carbamazepine-13CD3, carbendazim-D4, diclofenac-D4, gemfibrozil-D6, ibuprofen-D3, ketoprofen-D3, lamotrigine-13C3, metoprolol-D7, naproxen-D3, sildenafil-D3, sulfamethoxazole-D4, and sulfapyridine-13C6. Then the sample was loaded on SPE cartridge (Strata-X, 200 mg, Phenomenex) at about 3-5 mL min^{-1}, thereafter the cartridge was vacuum-dried and eluted with 2 mL of methanol. Methanol was then evaporated under a stream of nitrogen to 250-350 µL. The final volume was filtered through 0.2 µm regenerated cellulose membrane filters (Teknokroma) and transferred to glass vials with inserts.

Samples were analyzed by LC-HRMS system using Q Exactive Plus hybrid FT mass spectrometer coupled with Dionex Ultimate 3000 RS UPLC (Thermo Fisher Scientific, Waltham, MA) equipped with a heated electrospray ion (ESI) source. Chromatographic separation was carried out using Acclaim C18 column (2.1×150 mm, particle size 2.2 µm, Dionex) at a flow rate of 0.6 mL min^{-1} at 40 °C. Injection volume was 5 µL. Elution was performed using the following program: 1 min at 95% of solvent A (water with 1.5% acetic acid) and 5% of solvent B (acetonitrile); linear gradient for 14 min where solvent B was increased to 95%; and then 5 min at isocratic condition (95% solvent B). Higher concentration of the acetic acid in water (1.5%) was chosen to obtain narrow and symmetrical peak shapes of polar compounds and for increasing sensitivity upon positive ESI. For analysis of compounds upon negative ESI, similar chromatographic parameters were used besides of using water with 0.1% acetic acid as solvent A to improve ionization (sensitivity).

Analysis was carried in two separate runs, one for positive ESI mode and another for negative ESI mode. Mass spectral data was acquired in full scan (m/z 130-600) and validated using all-ion fragmentation at resolving power set to 70,000. The scan rate was 6 Hz for the full scan mode and 6 Hz for the all-ion fragmentation mode. Analysis parameters of ESI were as follows: spray voltage 3 kV, capillary temperature 300 °C, sheath gas rate (arb) 40, auxiliary gas rate (arb) 10, and ESI capillary temp. 350 °C.

Quantification was done based on external standards using calibration curves composed of 13 points between concentration of 0.25 to 2500 ng L^{-1}. Limit of detection, determined as the instrumental limit of detection (ILOD), was set as the minimal detectable amount of an analyte determined by on-column injection of a particular calibration sample. Limit of quantification was determined as the concentration measured at accuracy ≥70%. Recovery for each analyte was determined at 5, 25, 100 and 200 ng L^{-1}. Recoveries are presented in Table S1.

Table S1. Selected physico-chemical properties of the studied analytes, ionization mode (pESI, positive electro spray ionization; nESI, negative electro spray ionization), instrumental limit of quantification (ILOQ; determined for injection volume of 5 µL), limit of quantification for 200 mL samples (LOQ), and recoveries (% ± standard deviation).

						Recovery			
	Molecular weight g/mol	**log *D* pH 7.8**	**p*K*a**	**Ionization Mode**	**ILOQ (ng L^{-1})**	**5 ng L^{-1}**	**25 ng L^{-1}**	**100 ng L^{-1}**	**200 ng L^{-1}**
Acetaminophen	151.165	0.45	9.38	pESI	2.5	35.5 ±5.8	31.2 ±2.6	37.9±11.3	38.4±3
Bezafibrate	361.822	-0.22	3.61	pESI	5	97.9 ±0	99.5 ±5.6	98.5±1.2	98±1.3
Caffeine	194.194	-0.07	14	pESI	10	-	-	118.6±23.5	99.9±6.8
Carbamazepine	236.274	2.45	13.9	pESI	0.25	97.7 ±0	99.8 ±3.5	98.7±2.4	98.9±3
Clofibric acid	214.65	-2.05	3.18	nESI	2.5	122.8 ±9.7	123.9 ±3.3	111.5±14.8	109.2±6
Diclofenac	296.147	0.86	4.15	pESI	10	-	96 ±2.9	88.8±3	91.5±4
Epoxy carbamazepine	252.273	1.58	15.96	pESI	1.25	95.5 ±6.5	93.3 ±1.3	99.7±6.7	93±5
Gemfibrozil	250.338	0.1	4.5	nESI	5	97.2 ±7.2	102.2 ±1.7	101.5±1.9	102.2±3
Ibuprofen	206.285	0.88	4.91	nESI	2.5	104.2 ±0	100.5 ±1.5	101±2.5	101.8±2.3
Ketoprofen	254.285	-0.23	4.45	pESI	10	-	109.8 ±3	99.8±1.1	102.9±3
Lamotrigine	256.09	0.4	5.7	pESI	1	89.6 ±0	97.8 ±1.5	101.1±1.7	102.1±2.1

						Recovery			
	Molecular weight g/mol	**log *D* pH 7.8**	**p*K*a**	**Ionization Mode**	**ILOQ (ng L^{-1})**	**5 ng L^{-1}**	**25 ng L^{-1}**	**100 ng L^{-1}**	**200 ng L^{-1}**
Lorazepam	321.157	2.32	8.53, 9.21	pESI	2.5	85.2 ±11.3	85.2 ±4.3	88.2±7.8	95.5±4.6
Metoprolol	267.369	1.87	9.6	pESI	0.5	102.3 ±0	98.9 ±2.8	103.1±2.9	100±1.7
Naproxen	230.263	-0.47	4.15	nESI	12.5	-	100.4 ±1.7	97.8±1.5	99.7±1.9
Sildenafil	474.58	0.08	5.99	pESI	2.5	114.4 ±7.4	99.6 ±2.8	97±6.9	93.7±7.6
Sulfamethoxazole	253.276	0.89	1.6, 5.7	pESI	2.5	105.9 ±6.4	104 ±3.3	109.7±1.8	104±3.3
Sulfapyridine	249.288	0.21	8.43	pESI	2.5	98 ±0	98.7 ±2.7	102.2±1.4	102±1.7
trans-dihydroxy carbamazepine	270.29	0.81	-1.5, 11.7	pESI	2.5	90.6 ±11.6	99.7 ±6.9	104.6±6.5	105.5±4

Table S2. Assessment factors to derive $PNEC_{aquatic}$ as proposed by the European Chemicals Agency.

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the baseset (fish, Daphnia and algae)	1,000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10

Table S3. Predicted no effect concentrations (PNEC; μg L^{-1}) of selected pharmaceuticals and their affiliation factors.

	Therapeutic activity	PNEC							
		Assessment factor	Aquatic	Assessment factor	Fish	Assessment factor	Crustacean	Assessment factor	Algae
Carbamazepine	Anticonvulsant	10	0.05[1]	10	2500[2]	10	0.05[1]	10	0.2[3]
Lamotrigine		50	150[4]	100	600[5]	10	1000[4]	10	750[4]
Lorazepam		1,000	0.005[6]	100	0.05[6]	100	37[6]	100	20[6]
Acetaminophen	Anti-inflammatory	10	0.5[7]	10	0.5[7]	10	95[8]	10	0.5[9]
Diclofenac		10	0.1[10]	10	0.1[10]	10	46[11]	10	100[2]
Ibuprofen		10	0.01[12]	10	0.01[12]	10	3.2[13]	10	1[9]
Ketoprofen		100	0.03[14]	10	0.3[14]	100	436.5[15]	100	162.1[15]
Naproxen		10	15[16]	10	10[17]	10	15[16]	10	620[16]
Metoprolol	β-Blocker	1,000	7.3[18]	100	1000[19]	100	88[19]	100	73[18]
Sildenafil	Erectile dysfunction	N/A	0.026[20]	N/A	0.026[20]	N/A	N/A	N/A	N/A
Clofibric acid*	Lipid regulator	10	0.49[21]	10	0.49[21]	10	1[22]	10	10[23]
Bezafibrate		N/A	0.034[20]	N/A	0.034[20]	10	2.3[24]	10	6000[24]
Gemfibrozil		N/A	0.38[20]	N/A	0.38[20]	10	7.8[24]	10	312[24]
Caffeine	Stimulant	10	1[9]	10	30[25]	10	12[26]	10	1[9]
Sulfamethoxazole	Sulfonamide	10	0.6[2]	10	800[2]	10	1[4]	10	0.6[2]
Sulfapyridine		1,000	0.012[6]	100	0.35[6]	100	0.012[6]	100	19.3[27]

*Metabolite of the lipid regulator clofibrate.

Aquatic affiliation factors were determined using ECHA technical guide[28]. Fish, crustaceans, and algae affiliation factors were determined according to experimental setup where chronic toxicity experiment factor was 10, acute toxicity experiment factor was 100, ECOSAR calculation was considered as acute and received a factor of 100, and genotoxic experiments were used without applying an affiliation factor.

Table S4. Characteristics of flow events at the Head of the Alexander Estuary during 2 hydrological years (F, flood event; BF, base-flow).

	Date (dd/mm/yyyy)	Duration (days)	Peak discharge ($m^3 s^{-1}$)	Volume (m^3)
F2016-17 #1	02/12/2016	1.3	7.2	370,000
F2016-17 #2	13/12/2016	1.6	16.5	640,000
F2016-17 #3	25/12/2016	3.2	51.6	3,340,000
F2016-17 #4	16/02/2017	0.6	7.3	270,000
BF2017	01/03/2017 – 31/12/2017	330	0.2	2,900,000
F2017-18 #1	25/12/2017	0.2	1.9	75,000
F2017-18 #2	01/01/2018	0.4	1.1	43,000
F2017-18 #3	05/01/2018	1.9	31.5	1,380,000
F2017-18 #4	25/01/2018	2.6	104	8,620,000
BF2018	07/02/2018 – 27/11/2018	330	0.3	3,270,000

Table S5. Pharmaceuticals (average concentrations in µg L^{-1}) in the Alexander micro-estuary and in other estuaries as available in the German Environmental Agency (UBA) database.

Compound	Alexander	UBA
Carbamazepine	0.450	0.102
Acetaminophen	0.130	0.070
Diclofenac	0.121	0.069
Ibuprofen	0.261	0.050
Ketoprofen	0.018	0.002
Naproxen	0.038	0.020
Metoprolol	0.007	0.026
Clofibric acid*	0.125	0.009
Bezafibrate	0.086	0.004
Gemfibrozil	0.244	0.009
Sulfamethoxazole	0.061	0.045
Sulfapyridine	0.010	0.009

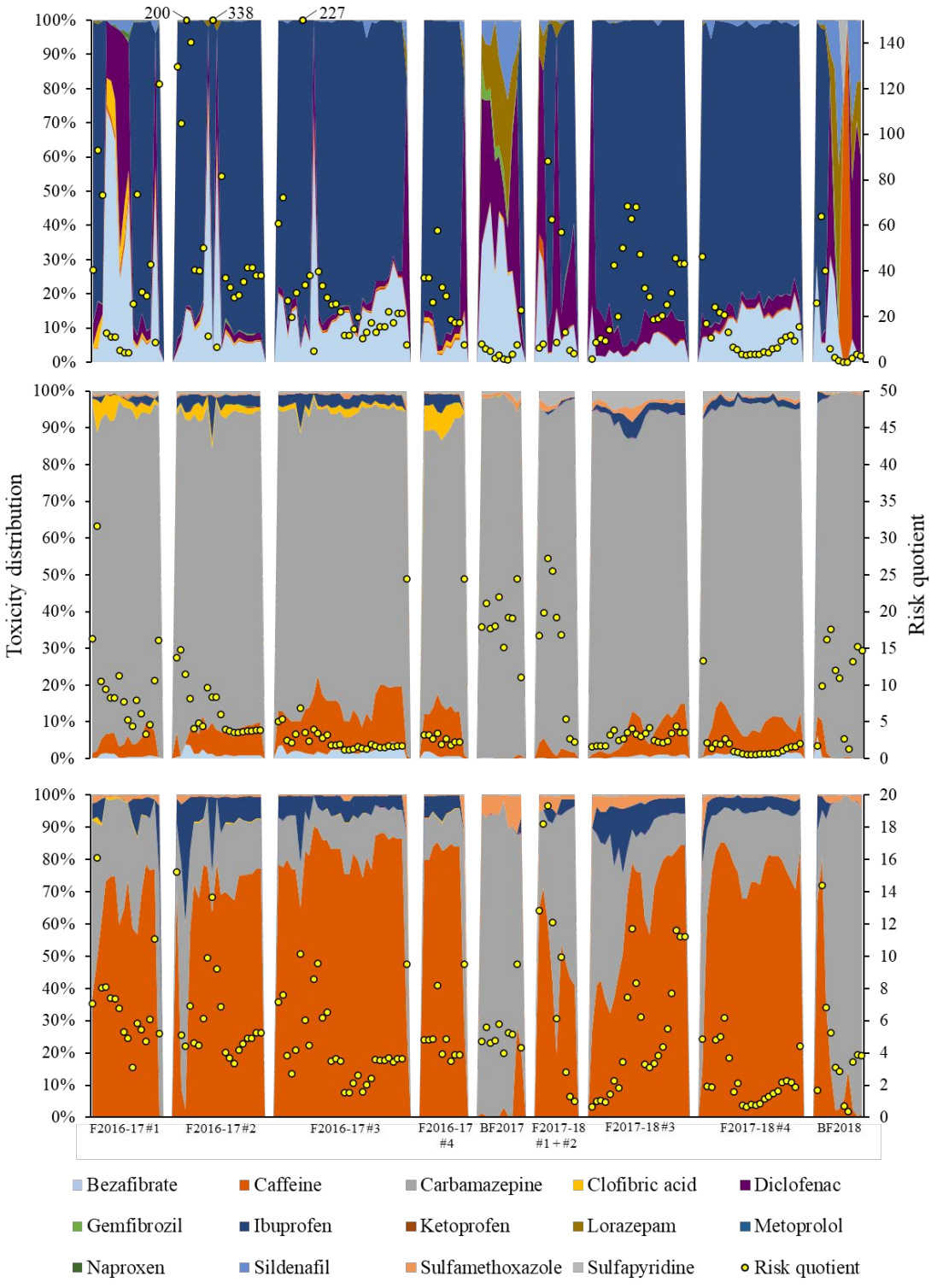

Figure S2. Different pharmaceuticals' proportional fraction (left Y axis, %) of the calculated risk quotients (yellow dots, right Y axis) during 2 hydrological years at the Alexander micro-estuary. Data for fish (top), crustaceans (middle) and algae (bottom) calculated for water samples collected from the head of the estuary.

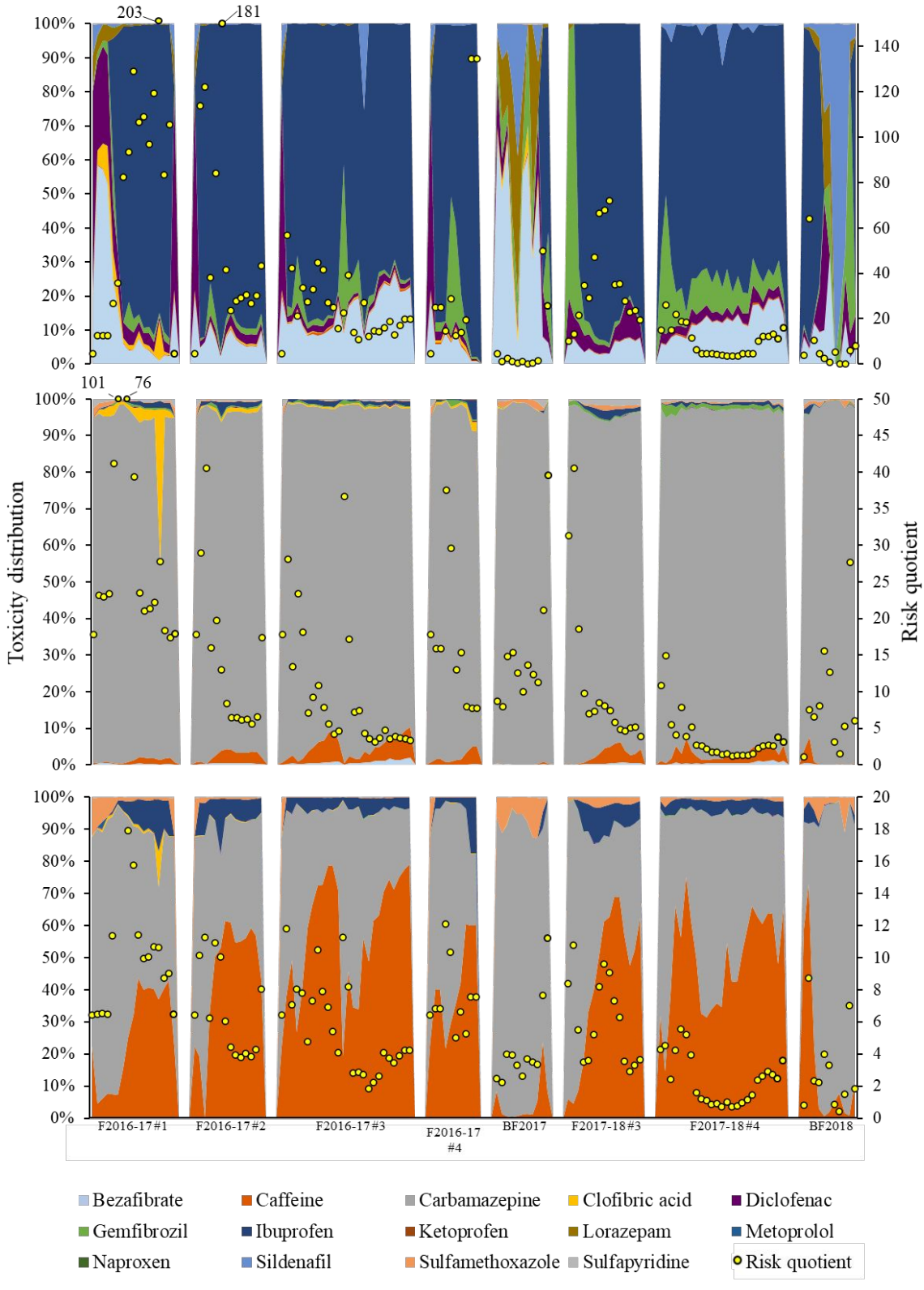

Figure S3. Different pharmaceuticals' proportional fraction (left Y axis, %) of the calculated risk quotients (yellow dots, right Y axis) during 2 hydrological years at the Alexander micro-estuary. Data for fish (top), crustaceans (middle) and algae (bottom) calculated for water samples collected from the mouth of the estuary.

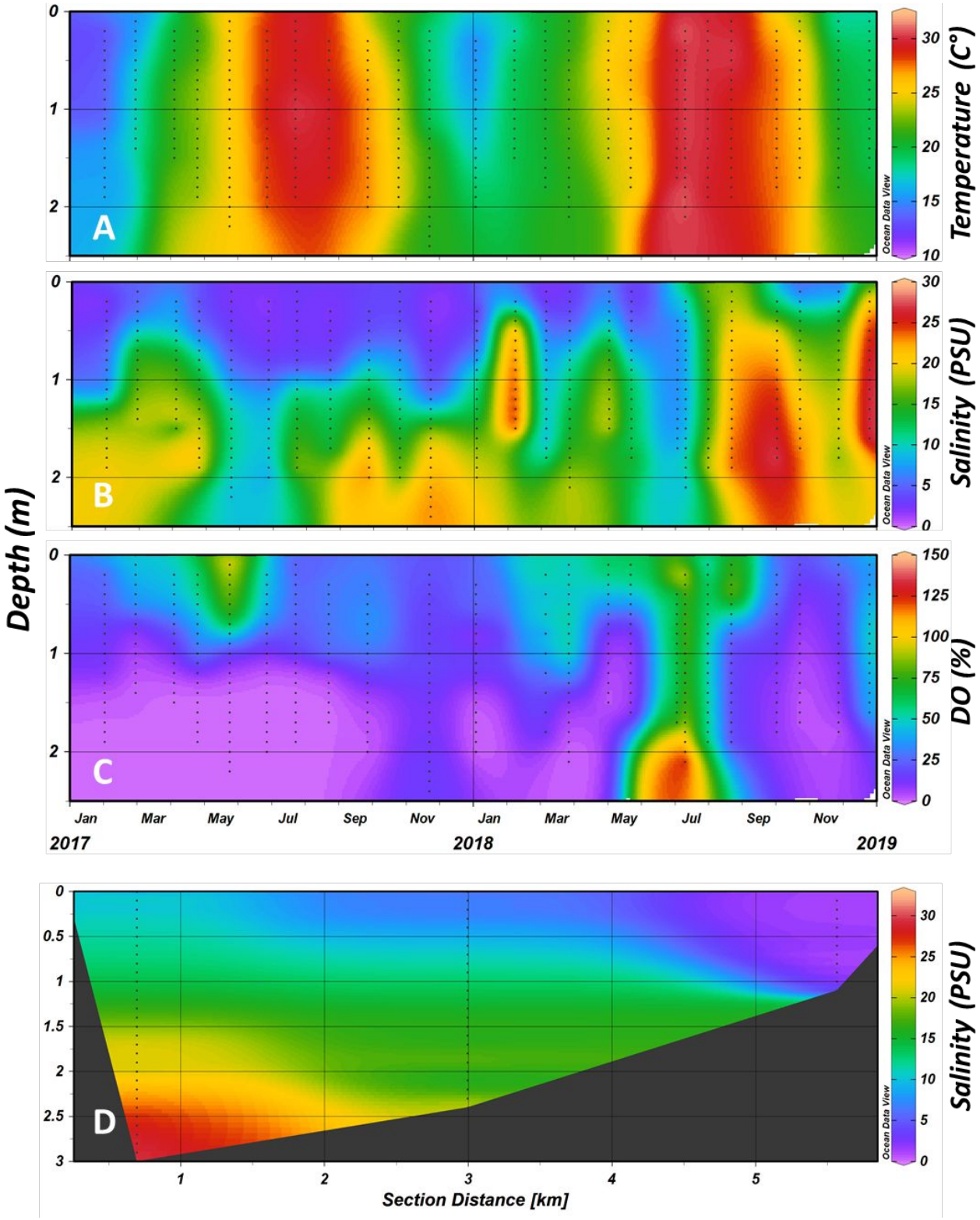

Figure S4. Temperature (A), Salinity (B and D), and dissolved oxygen (% of saturation; C) at the Alexander micro-estuary (January 2017 to December 2018). Plot D shows the average salinity distribution along the micro-estuary throughout the study period.