Neuroactive pharmaceuticals in estuaries: Occurrence and tissue-specific bioaccumulation in multiple fish species

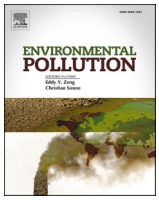
Irina A Duarte, Patrick Reis-Santos, Jerker Fick, Henrique Cabral, Bernardo Duarte, Vanessa F Fonseca

Environmental Pollution 316 (2023) 120531

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Neuroactive pharmaceuticals in estuaries: Occurrence and tissue-specific bioaccumulation in multiple fish species

Irina A. Duarte [a,*], Patrick Reis-Santos [b], Jerker Fick [c], Henrique N. Cabral [d], Bernardo Duarte [a,e], Vanessa F. Fonseca [a,f]

[a] MARE – Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal
[b] Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, South Australia, 5005, Australia
[c] Department of Chemistry, Umeå University, Umeå, Sweden
[d] INRAE, UR EABX, Cestas, France
[e] Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal
[f] Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal

ARTICLE INFO

Keywords:
Psychoactive compounds
Emerging contaminants
Uptake
BAF
Estuarine/marine fish
Estuarine pollution

ABSTRACT

Contamination of surface waters by pharmaceuticals is an emerging problem globally. This is because the increased access and use of pharmaceuticals by a growing world population lead to environmental contamination, threatening non-target species in their natural environment. Of particular concern are neuroactive pharmaceuticals, which are known to bioaccumulate in fish and impact a variety of individual processes such as fish reproduction or behaviour, which can have ecological impacts and compromise fish populations. In this work, we investigate the occurrence and bioaccumulation of 33 neuroactive pharmaceuticals in brain, muscle and liver tissues of multiple fish species collected in four different estuaries (Douro, Tejo, Sado and Mira). In total, 28 neuroactive pharmaceuticals were detected in water and 13 in fish tissues, with individual pharmaceuticals reaching maximum concentrations of 1590 ng/L and 207 ng/g ww, respectively. The neuroactive pharmaceuticals with the highest levels and highest frequency of detection in the water samples were psychostimulants, antidepressants, opioids and anxiolytics, whereas in fish tissues, antiepileptics, psychostimulants, anxiolytics and antidepressants showed highest concentrations. Bioaccumulation was ubiquitous, occurring in all seven estuarine and marine fish species. Notably, neuroactive compounds were detected in every water and fish brain samples, and in 95% of fish liver and muscle tissues. Despite variations in pharmaceutical occurrence among estuaries, bioaccumulation patterns were consistent among estuarine systems, with generally higher bioaccumulation in fish brain followed by liver and muscle. Moreover, no link between bioaccumulation and compounds' lipophilicity, species habitat use patterns or trophic levels was observed. Overall, this work highlights the occurrence of a highly diverse suite of neuroactive pharmaceuticals and their pervasiveness in waters and fish from estuarine systems with contrasting hydromorphology and urban development and emphasizes the urgent need for toxicity assessment of these compounds in natural ecosystems, linked to internalized body concentration in non-target species.

1. Introduction

Pharmaceuticals include a complex variety of compounds with different chemical and therapeutic properties that are used to treat a myriad of health conditions and have greatly improved the global public health. However, the excretion of pharmaceuticals from the human body contributes to their release into wastewaters and subsequently into the aquatic environment, through wastewater effluents, including discharges from wastewater treatment plants (Arnold et al., 2014; Wilkinson et al., 2022). Overall, the main input of pharmaceuticals to aquatic systems includes domestic discharges, yet industrial and hospital effluents as well as other input sources such as pharmaceuticals applied in livestock or aquaculture are also substantial (Arnold et al., 2014) and are occasionally associated with extremely high

* Corresponding author.
E-mail address: iaduarte@fc.ul.pt (I.A. Duarte).

https://doi.org/10.1016/j.envpol.2022.120531
Received 25 August 2022; Received in revised form 6 October 2022; Accepted 24 October 2022
Available online 28 October 2022

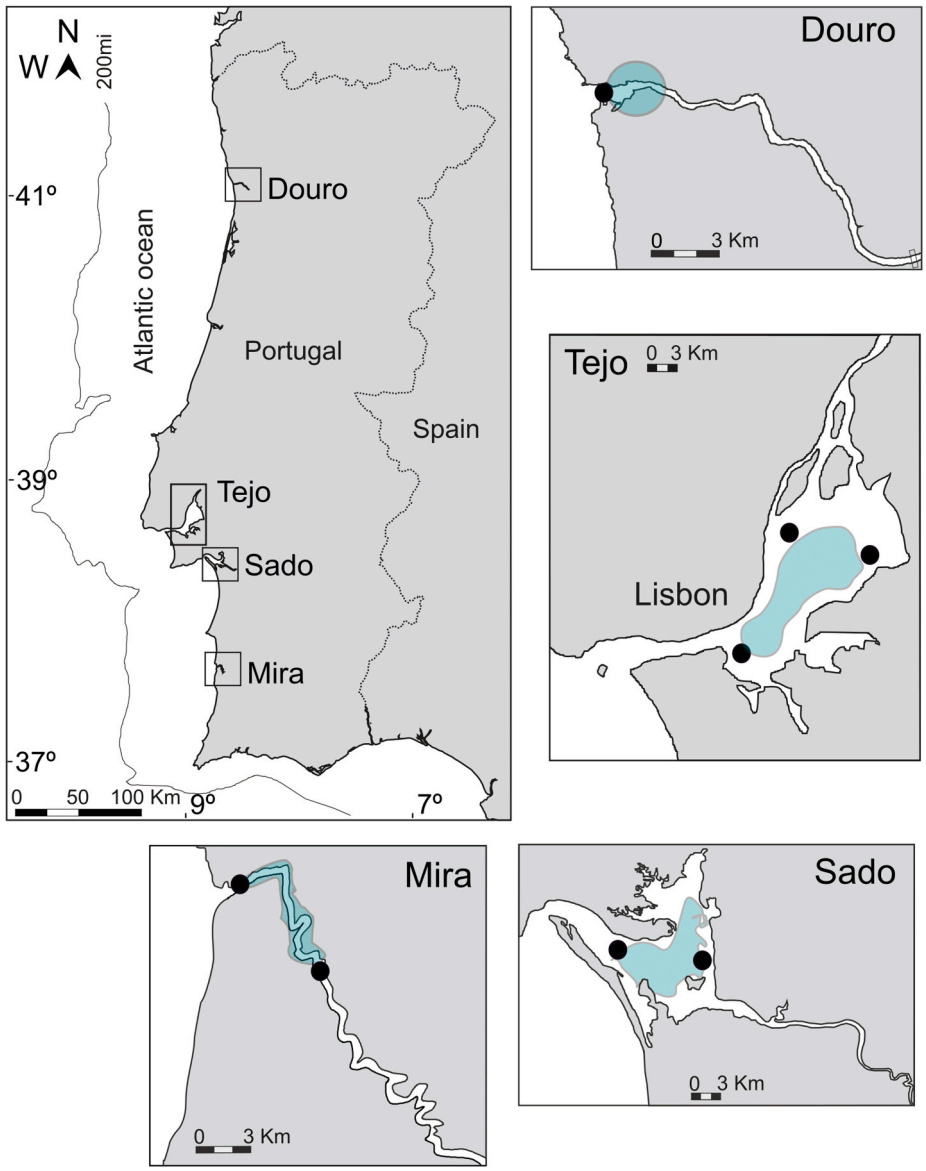


Fig. 1. Sampled estuarine areas (Douro, Tejo, Sado and Mira estuaries), where water (black circles) and fish (coloured areas) samples were collected.

environmental concentrations, including in the mg/L range close to production sites (e.g. Fick et al., 2009; Larsson, 2014). Furthermore, pharmaceutical prescription has increased worldwide in recent years, and this trend is expected to continue, associated with population growth and higher demand and access (Arnold et al., 2014; Bernhardt et al., 2017). Accordingly, many pharmaceutical compounds are frequently detected in wastewaters and end up being detected in various environmental matrices, generally at low μg/L concentrations, such as surface and ground waters or sediments, depending on the pharmaceutical chemical properties and their fate in the environment (aus der Beek et al., 2016; Fatta-Kassinos et al., 2011; Wilkinson et al., 2022; Zhou et al., 2019).

The recognition that pharmaceuticals target evolutionarily conserved pathways among humans and other animals (Gunnarsson et al., 2008) and elicit effects at very low dosages, has spurred interest in the evaluation of environmental concentrations and their potential effects across fish and aquatic invertebrate species (Corcoran et al., 2010; Fabbri and Franzellitti, 2016; Mezzelani et al., 2018). Of the variety of biological effects explored, development, reproductive and behavioural impacts, have been reported in a variety of species and in some cases depicting deleterious effects at environmentally relevant concentrations (Corcoran et al., 2010; Duarte et al., 2022; Fabbri, 2015). Moreover, the potential toxicity and pervasiveness of pharmaceutical compounds in the natural environment worldwide have led to their inclusion in European legislation as emerging contaminants of priority concern, with the potential to threaten aquatic ecosystems and human health (European Parliament and Council, 2013). In this context, selected pharmaceutical compounds, including neuroactive pharmaceuticals such as venlafaxine and its metabolite O-desmethylvenlafaxine, have been recommended for broad-scale assessment in monitoring programmes (European Commission, 2022).

Neuroactive pharmaceuticals target the central nervous system through different modes of action aiming to treat a variety of human conditions, such as depression, anxiety or epilepsy, among many others. Therefore, the continued exposure of fish to neuroactive compounds is expected to elicit responses and effects on sub-individual physiological processes, as well as higher-level individual and ecological impacts such as changes to fish behaviour, growth and reproduction that may threaten fish populations (e.g. Duarte et al., 2022; Hamilton et al., 2016). In toxicity studies, molecular effects are among the most frequently assessed, followed by alterations in fish behaviour, physiology and growth, whereas studies on behavioural changes are pointed

Table 1
Geomorphologic and hydrologic features of all four estuarine systems (Douro, Tejo, Sado and Mira). Resident population and information concerning wastewater treatment in the surrounding municipalities are presented.

	Unit	Treatment level	Douro	Tejo	Sado	Mira
Total area [a]	km^2		10	320	180	5
River flow [a]	m^3s^{-1}		450	300	40	3
Mean depth [a]	m		4	5	6	4
Residence time [a]	Days		2	25	30	15
Volume [a]	10^6 m^3		59	1900	500	27
Resident population [b]	Nº [A]		683,063	1,840,523	257,551	24,717
Percentage of wastewater volume treated in each treatment level [b]	% [A]	Primary	0	27	0	0
		Secondary	50	57	29	32
		Tertiary	50	16	71	68
Percentage of dwellings served by wastewater drainage [b]	% [B]		94	96	88	66

[a] From França et al. (2009)
[b] Statistical data by geographic location (municipalities) in the vicinity of each estuary was obtained through Statistics Portugal (www.ine.pt). The values presented correspond to the sum (A) or average (B) of all municipalities' data from 2019 for each estuarine area.

as of higher sensitivity (Duarte et al., 2022; Melvin and Wilson, 2013). However, reported effects are manifold, and their direction and intensity seem to vary within different species or life-stages (Calisto and Esteves, 2009; Cunha et al., 2019, 2017; Duarte et al., 2022), and generally lack the simultaneous link to internalized tissue concentrations (Duarte et al., 2022; Miller et al., 2018).

Pharmaceutical bioaccumulation has been shown for many therapeutic classes such as antibiotics, antidepressants, among many others, in both invertebrates and vertebrates (Mezzelani et al., 2018; Silva et al., 2015; Świacka et al., 2022). In the particular case of fishes, the bioconcentration of neuroactive pharmaceuticals has been reported for many compounds under controlled laboratory conditions (Duarte et al., 2022) but also in wastewater-impacted aquatic systems (e.g. Arnnok et al., 2017; Grabicova et al., 2017; Lahti et al., 2011). Prediction of neuroactive compounds' bioconcentration, even under controlled conditions, does not seem to be straightforward or directly correlated to the compound's physical and chemical properties, since a multitude of factors such as species, life-stages or tissues, etc, have a large impact (Duarte et al., 2022). Further environmental assessments are needed to fully understand the complexity of neuroactive pharmaceuticals accumulation in fish. In particular, the bioaccumulation patterns in natural aquatic systems are still seldomly explored, and includes mostly freshwater environments (Gaw et al., 2014; Mezzelani et al., 2018; Świacka et al., 2022). Although less studied compared to freshwater systems, estuarine and coastal areas are highly impacted systems, and as a result of the settlement of almost half of the world's population, they are also the major recipients of urban effluents and therefore of many pharmaceutical residues (Fonseca and Reis-Santos, 2019; Martínez et al., 2007). These areas encompass a variety of habitats, supporting the life and development of numerous species, including many fish species that depend on these systems to complete their life cycle. Therefore, a few recent studies have explored pharmaceuticals bioaccumulation in coastal systems, pointing to the importance of assessing their impacts in non-target species (e.g. Ali et al., 2018; Fonseca et al., 2021), yet scarcely any have explored patterns considering a multi-species, multi-tissue and multi-system approach, towards unravelling the fate and potential risk of neuroactive pharmaceutical compounds.

Here, we present a comprehensive assessment of neuroactive pharmaceutical occurrence in surface waters and in wild biota from various estuarine systems along the North Atlantic Portuguese Coast, aiming at further understanding the fate of potentially toxic neuroactive pharmaceuticals, including their bioaccumulation in different organs (muscle, liver and brain) of several fish species, with different life-history strategies and varying habitat use patterns of estuarine systems.

2. Material and methods

2.1. Sampling areas

Four Portuguese estuaries with different morphological features and

Table 2
Summary of fish morphometric and ecological traits from all seven species collected in the four sampled estuaries (Douro, Tejo, Sado and Mira), namely number of individuals (N), mean (and standard deviation) of total length (in mm) and weight (in g). Also shown is the size at maturity (in mm), species ecological guilds based on life cycle and estuarine habitat use (ER – Estuarine Resident, MM – Marine migrant, MS – Marine straggler) as well as the trophic levels.

Estuary	Species	N	Length (mm)	Weight (g)	Size at maturity (mm)	Ecological guild [a]	Trophic level [b]
Douro	*Dicentrarchus labrax*	5	289.6 ± 15.9	256.2 ± 23.7	361 (230–460) [b]	MM	3.5
	Platichthys flesus	5	283.2 ± 10.4	277.3 ± 34.3	224 (140–300) [b]	MM	3.3
Tejo	*Dicentrarchus labrax*	7	337.6 ± 7.7	368 ± 27	361 (230–460) [b]	MM	3.5
	Halobatrachus didactylus	5	267 ± 20	371.1 ± 97.9	367 (321–438) [c]	ER	4.0
	Solea solea	6	204.8 ± 10.6	86.7 ± 16.4	303 [b]	MM	3.2
Sado	*Dicentrarchus labrax*	1	360	536.8	361 (230–460) [b]	MM	3.5
	Diplodus bellottii	6	172.5 ± 9.4	95 ± 17.5	117 [d]	MM	3.6
	Halobatrachus didactylus	6	202.2 ± 37.7	172.8 ± 86.4	367 (321–438) [c]	ER	4.0
	Solea solea	2	244 ± 36.8	130.5 ± 49.4	303 [b]	MM	3.2
	Sparus aurata	1	272	334	365 (330–400) [b]	MM/MS	3.7
Mira	*Dicentrarchus labrax*	2	309.5 ± 23.3	324.9 ± 72.3	361 (230–460) [b]	MM	3.5
	Diplodus sargus	2	211.5 ± 4.9	185.2 ± 2.7	173 [e]	MM/MS	3.4
	Halobatrachus didactylus	3	147.7 ± 33.4	64.8 ± 52.8	367 (321–438) [b]	ER	4.0
	Sparus aurata	4	227.8 ± 20	174.4 ± 52	365 (330–400) [b]	MM/MS	3.7

[a] Franco et al., (2008);
[b] Fishbase (www.fishbase.org);
[c] Pereira et al., (2011);
[d] Santos et al., (1998), average of sexes;
[e] *Erzini et al. (2001)* in Prista et al., (2003).

Table 3
Neuroactive pharmaceuticals in water samples. Median (Med), minimum (Min), and maximum (Max) concentration values (ng/L) of pharmaceutical analytes detected in surface water samples of Douro, Tejo, Sado and Mira estuaries. The sum of concentrations (∑) and detection frequency (DF, %) per therapeutic group and for all analytes are also shown. < LOQ indicates values below the Limit of Quantification (DF = 0).

Therapeutic Group	Pharmaceuticals	Douro		Tejo		Sado		Mira	
		Med (Min-Max) ng/L	DF (%)N = 3	Med (Min-Max) ng/L	DF (%) N = 9	Med (Min-Max) ng/L	DF (%) N = 7	Med (Min-Max) ng/L	DF (%) N = 6
Opioids	Buprenorphine	< LOQ		< LOQ		< LOQ		< LOQ	
	Codeine	0.5 (0.4–0.7)	100	0.3 (0.1–0.7)	67	0.6 (0.3–22)	100	0.7 (0.3–11)	100
	Tramadol	25 (22–28)	100	21 (18–33)	100	12 (11–1590)	57	17 (6.7–1557)	83
Σ Opioids		26 (22–29)	100	21 (18–33)	100	11 (0.5–1612)	100	15 (0.3–1568)	100
Antiepileptics	Carbamazepine	0.79 (0.77–0.83)	100	0.9 (0.6–1.5)	100	0.5 (0.1–52)	86	0.7 (0.1–61)	100
	Clonazepam	< LOQ		1.1 (0.7–4.9)	44	1.5	14	1.5	17
	Topiramate	< LOQ		< LOQ		< LOQ		15	17
Σ Antiepileptics		0.79 (0.77–0.83)	100	1.5 (0.6–5.9)	100	0.5 (0.1–52)	86	1.1 (0.1–76)	100
Antipsychotics	Chlorpromazine	< LOQ		1.4	11	2.6	14	2.2 (1–3.3)	33
	Clozapine	< LOQ		< LOQ		1.4	14	1.8	17
	Flupentixol	< LOQ		0.9	11	0.6	14	< LOQ	
	Haloperidol	0.6 (0.2–0.9)	100	0.2 (0.1–1.2)	78	0.1 (0.04–1.5)	71	0.2 (0.1–1.7)	100
	Levomepromazine	< LOQ		< LOQ		< LOQ		< LOQ	
	Risperidone	< LOQ		< LOQ		< LOQ		< LOQ	
Σ Antipsychotics		0.6 (0.2–0.9)	100	0.3 (0.1–1.5)	78	0.3 (0.04–5.5)	86	0.3 (0.1–6.8)	100
Anxiolytics	Alprazolam	1.6 (1.3–1.8)	100	1.3 (1.1–1.6)	33	2.3 (1.1–3.5)	29	4.7 (1.6–7.8)	33
	Bromazepam	< LOQ		< LOQ		< LOQ		< LOQ	
	Clobazam	< LOQ		< LOQ		< LOQ		< LOQ	
	Hydroxyzine	0.4 (0.2–0.6)	67	0.4 (0.1–2.1)	89	0.3 (0.1–0.9)	57	0.3 (0.2–0.8)	83
	Lorazepam	5.7 (5.6–6.3)	100	6.7 (5.6–7.7)	22	79	14	9.7 (5.7–73)	67
	Oxazepam	3.9 (3–5.5)	100	5 (1.4–12)	100	2.4 (1.1–171)	86	5.3 (2.8–190)	100
Σ Anxiolytics		11 (11–13)	100	7.1 (2–19)	100	3.1 (1.1–253)	86	12 (2.8–271)	100
Antidepressants	Amitriptyline	0.7 (0.7–0.8)	67	0.7 (0.5–1.6)	56	1.3 (0.6–65)	43	2 (1.2–44)	50
	Bupropion	0.7 (0.7–0.9)	100	0.7 (0.5–1.4)	100	0.4 (0.1–59)	100	0.6 (0.3–48)	100
	Citalopram	0.9 (0.7–2)	100	1.3 (0.8–4.6)	89	0.9 (0.7–54)	43	1.3 (1.1–77)	83
	Duloxetine	0.2 (0.2–0.3)	100	0.4 (0.1–1.1)	67	0.3 (0.1–8.3)	86	1.5 (0.3–7.1)	83
	Fluoxetine	< LOQ		< LOQ		14 (3.5–24)	29	16 (8–133)	50
	Maprotiline	0.5	33	0.7 (0.5–1)	33	0.9 (0.5–1.3)	29	1.5 (0.8–2.9)	67
	Mianserin	0.5 (0.4–0.7)	100	0.6 (0.2–2.9)	89	0.4 (0.2–3.2)	86	0.6 (0.3–3.5)	67
	Mirtazapine	< LOQ		< LOQ		31	14	61	17
	Paroxetine	< LOQ		< LOQ		9.3	14	1.7 (1.1–6.2)	50
	Sertraline	1.3 (1.1–1.5)	67	1.3 (1.1–1.4)	22	113	14	39 (2.4–76)	33
	Venlafaxine	21 (9.4–22)	100	8.5 (1.5–15)	89	6 (0.5–336)	100	12 (0.7–383)	83
Σ Antidepressants		24 (15–26)	100	14 (3.4–17)	100	10 (1.6–705)	100	29 (2–715)	100
Psychostimulants	Caffeine	113 (109–116)	100	62 (28–165)	100	123 (17–344)	100	157 (24–1003)	100
Σ Psychostimulants		113 (109–116)	100	62 (28–165)	100	123 (17–344)	100	157 (24–1003)	100
Anti-dementia drugs	Memantine	0.4 (0.3–0.4)	100	0.8 (0.6–1.6)	100	0.3 (0.1–93)	86	0.8 (0.3–62)	83
Anticholinergic agents	Trihexyphenidyl	0.2 (0.1–0.2)	100	0.1 (0.02–0.3)	78	0.2 (0.1–0.3)	71	0.1 (0.07–0.3)	83
Hypnotics and sedatives	Zolpidem	0.36 (0.36–0.41)	100	0.4 (0.4–0.6)	100	0.5 (0.4–2.8)	100	0.4 (0.3–4.2)	100
Σ Other		0.9 (0.8–0.9)	100	1.3 (1–2.3)	100	0.9 (0.6–96)	100	1.3 (0.5–66)	100
Σ Total without caffeine		63 (58–64)	100	42 (28–68)	100	27 (5.3–2724)	100	76 (6.2–2703)	100
Σ Total		173 (170–179)	100	108 (61–205)	100	150 (26–3068)	100	277 (30–2876)	100
Number of pharmaceuticals		18 (18–19)	100	16 (13–18)	100	14 (11–24)	100	19 (10–26)	100

distinct levels of anthropogenic pressures, including resident population and wastewater-related parameters were sampled (Douro, Tejo, Sado and Mira) (Fig. 1 and Table 1). Douro estuary, located in the north of Portugal, is surrounded by the second most populated metropolitan region in Portugal with a resident population of ca. 0.68 million inhabitants in the vicinity of the watershed. It's an estuary with a relatively low area and volume, with a high river flow (Table 1). The Tejo estuary is surrounded by the most populated metropolitan area in the country (ca. 1.84 million inhabitants in the municipalities surrounding the Tejo watershed), and is the largest estuary in Portugal, characterised by large area and volume (Table 1). The Sado estuary, located south of Tejo, is the second largest estuary in the country, surrounded by less populated areas (ca. 0.26 million inhabitants) than Douro and Tejo, whilst the Mira estuary is the smallest of the estuaries with a smaller area, volume and river flow, and is surrounded by a smaller resident population (ca. 0.02 million inhabitants) (Table 1).

Secondary and tertiary treatments are applied to a considerable portion of all wastewater volume treated across the four estuaries (>73%, Table 1), with the proportion of dwellings served by wastewater drainage on average higher in Douro and Tejo estuaries (above 90%), followed by Sado (88%) and Mira (66%) estuaries.

2.2. Sample pre-treatment and chemical analysis

Fish and water samples were collected in the four estuaries (Fig. 1) during the summer of 2019. Fish species were collected with beam trawls and transported on ice into the laboratory. Individual total length and weight were recorded, and portions of dorsal muscle, liver and brain were collected from a total of 55 fish from seven different species, namely estuarine resident (ER) Lusitanian toadfish *Halobatrachus didactylus*, and marine migrants (MM) and stragglers (MS) namely the European sea bass *Dicentrarchus labrax*, the Senegal sea bream *Diplodus bellottii*, the white sea bream *Diplodus sargus*, the gilthead sea bream *Sparus aurata*, the European flounder *Platichthys flesus* and the common sole *Solea solea* (Table 2). Tissue samples were weighted (0.10 ± 0.01 g) and stored frozen until extraction. Extraction was performed twice with 1.5 mL of acetonitrile, including tissue disruption with zirconium beads for 4 min at 3450 oscillations per minute (Mini Beadbeater, Biospec). After centrifugation at 17,500*g* for 10 min at 4 °C (Beckman Coulter Microfuge 22 R Centrifuge), the supernatant was recovered and the

entire process was repeated, making a final extract volume of 3 mL per sample.

Twenty-five superficial (ca. 0.3 m) water grab samples (1 L) were collected by hand in pre-rinsed bottles, stored frozen and away from light. In the laboratory, samples were acidified with formic acid to pH 3, filtered through GF/C and 0.45 μm polyamide membranes and extracted using OASIS™ HLB cartridges, followed by a washing step with 5 mL of methanol:water (10:90) and final elution with 6 mL of methanol. Both water and fish extracts were dried in a water bath under an N_2 stream at 30 °C.

Before analysis, all extracts were reconstituted in 100 μL of methanol, transferred to glass autosampler vials, and centrifuged for 5 min at 4000 rpm (Mega Star 1.6 R, VWR). An equal amount of deuterated internal standards was added to all samples before extraction. Screening for neuroactive pharmaceuticals included 33 compounds (Table S1), selected based on a combination of commercialization data (INFARMED, 2018), available compound library and previous detection in Portuguese waters (e.g. Reis-Santos et al., 2018). A total of seven therapeutic groups were considered and will be referred to as follows: PS - Psychostimulants, OP - Opioids, AD - Antidepressants, ANX - Anxiolytics, AE - Antiepileptics, AP - Antipsychotics and O - Other (including one anticholinergic agent, one hypnotic and sedative and one anti-dementia drug). Pharmaceutical compound concentrations were calculated by comparison with seven-point standard curves (concentrations ranging between 1 and 250 ng/mL) with internal standards and native compounds (for more details see Supplementary Tables S1 and S2).

All samples were analysed through liquid chromatography–tandem mass spectrometry (LC–MS/MS) following Grabic et al. (2012). Target analytes were separated using Hypersil gold columns and analysed through triple-stage quadrupole mass spectrometer (TSQ Quantiva and Quantum Ultra EMR, Thermo Fisher Scientific) coupled with an Accela LC pump (Thermo Fisher Scientific), an aPAL HTC autosampler (CTC Analytics AG) and equipped with a heated-electrospray ionization (HESI) ion source. Instrument set-up is described in detail in supplement (Tables S1 and S2). Briefly, heated electrospray in positive or negative ion mode was used for ionization and screening of targeted pharmaceuticals and internal standards. Injection of the mobile phase was performed regularly in the analytical runs to detect carry-over effects, and no contamination was observed in either instrumental or procedural blanks. Peak identification was performed with Xcalibur™ 4.3 software (Thermo Fisher Scientific), and results are presented as ng of pharmaceutical compound per L of water or per g of wet weight (ww) of fish tissue.

2.3. Data analyses

Pharmaceutical concentrations in water (ng/L) and biota tissues (ng/g ww) are presented as median, minimum and maximum values for each pharmaceutical, but also as the sum of concentrations (Σ) per therapeutic class and for the total concentration of all pharmaceuticals. A total concentration without caffeine is also given because caffeine consumption in Portugal is mostly unprescribed, and thus differs from all remaining compounds. Detection frequency (%) is presented as the percentage of samples with pharmaceuticals detected above the limit of quantification, out of all samples analysed.

Field-derived bioaccumulation factors (BAF, L/kg) were calculated as the ratio between pharmaceutical concentrations detected in fish tissues and the median concentrations detected in the corresponding estuarine waters. Pharmaceuticals' estimated log octanol-water partition coefficient values ($\log K_{ow}$) for uncharged molecules, were obtained via KOWWIN™ program by EPI Suite™ (Estimation Programs Interface). Correlations between neuroactive pharmaceuticals' lipophilicity ($\log K_{ow}$) and field bioaccumulation factors (BAF), as well as between trophic levels and summed pharmaceutical concentrations, were tested through Spearman rank correlation (r) analysis, where a significant level of 0.05 was considered.

Principal Component Analysis (PCA) was performed in water and fish data (sums per therapeutic class, where values below quantification limits were replaced by LOQ/2, a common procedure applied in previous studies (e.g. Osorio et al., 2016), after normalization (water) and scaling (both water and fish data), to explore potential patterns in pharmaceutical occurrence and concentration related to estuaries and species. R software (R Core Team, 2019) was used to create all figures and to perform PCA and correlation analysis.

3. Results and discussion

3.1. Neuroactive pharmaceuticals in surface waters

A total of 28 out of the thirty-three neuroactive pharmaceuticals analysed were detected in the 25 water samples (Table 3), with individual water samples showing a minimum of 10 and a maximum of 26 different compounds. Neuroactive compounds from all the seven therapeutic groups considered were detected in at least one of four estuaries: two opioids, three antiepileptics, four antipsychotics, four anxiolytics, eleven antidepressants, one psychostimulant and three other pharmaceuticals used in different therapeutic treatments (Table 3). A high detection frequency was observed for the majority of screened compounds, with several being detected in over 70% of water samples in all four estuaries, such as carbamazepine, haloperidol, oxazepam, venlafaxine, memantine and trihexyphenidyl, whilst a few others were present in every sample, namely caffeine, bupropion and zolpidem. These findings demonstrate the pervasiveness and diversity of neuroactive pharmaceuticals in the analysed estuarine waters, across four different estuaries with large differences in population density and footprint, and reflect the general trend found in previous studies across aquatic systems and other therapeutic groups (e.g. aus der Beek et al., 2016; Gaw et al., 2014; Mezzelani et al., 2018; Ojemaye and Petrik, 2019; Wilkinson et al., 2022).

Pharmaceutical concentrations ranged between 0.02 and 1590 ng/L for individual analytes, whereas the concentration of the pharmaceutical mixture (∑ Total) ranged between 26 and 3068 ng/L per sample, and between 5.3 and 2724 ng/L excluding caffeine, which is mostly a non-prescribed drug. The sum of pharmaceutical concentrations reached higher median values in the Mira estuary (277 ng/L), followed by the Douro (173 ng/L), Sado (150 ng/L) and Tejo (108 ng/L) estuaries, whereas the maximum concentrations were observed in Sado and Mira estuaries (above 3060 and 2870 ng/L, respectively). The range of concentrations for individual neuroactive pharmaceuticals detected in this study is within the range (up to thousands of ng/L) of previously reported surface water concentrations in other European estuaries (e.g. Aminot et al., 2016; Fernández-Rubio et al., 2019; Zhou et al., 2019), albeit our study stands out by considering a broader suite of neuroactive compounds.

Despite differences in hydromorphology and population density in the vicinity of these estuarine systems, there were no clear contamination patterns regarding the presence of different therapeutic groups across the estuaries, with all therapeutic groups occurring in all estuaries (Table 3 and Fig. S1). Conspicuously, the highest concentrations, for all therapeutic groups, were detected in the two less populated estuaries, Sado and Mira, in some cases exceeding thousands of ng/L (Table 3). There is an inherent variability associated with pharmaceutical occurrence in single event water grab samples and previous studies have shown daily, weekly and seasonal variations associated with pharmaceutical consumption and occurrence, highlighting the complexity of pharmaceutical presence in wastewaters and surface receiving waters (e.g. Aminot et al., 2016; Letsinger et al., 2019; Paíga et al., 2019; Pereira et al., 2016). Still, these values could be related to the higher mass loads detected in the southern regions of Portugal likely associated with an older population and increased seasonal population linked to tourism in the summer months (Pereira et al., 2016), on top of the lower percentage

of dwellings served by wastewater treatment in the vicinity areas of these estuaries, down to 83 and 66% in Sado and Mira estuaries, respectively, compared to 94 and 96% in Douro and Tejo (Table 1). As shown in a previous study, reduced treatment is correlated to higher and more unpredictable releases of pharmaceuticals (Fork et al., 2021), and thus direct contributions of untreated wastewater cannot be fully discarded.

Overall, the occurrence of pharmaceutical compounds differed among and within therapeutic groups, with psychostimulants reaching higher median concentrations, followed by antidepressants, opioids and anxiolytics (Table 3). The psychostimulant caffeine was found in every water sample analysed, and it was the compound with higher median concentrations in all estuaries (between 62 up to 157 ng/L), being higher in the Mira and Sado estuaries, followed by Douro and Tejo, with concentrations ranging between 16 up to 1003 ng/L (Table 3). Caffeine is highly consumed in Portugal as generally worldwide (e.g. Quadra et al., 2020), and although secondary wastewater treatment has been shown to reach exceptional removal efficiencies in some cases (Adeleye et al., 2022), high consumption and permanent release of caffeine seem to be contributing to its ubiquity and high concentrations in aquatic systems, reaching thousands of ng/L in marine and estuarine waters (Vieira et al., 2022), in line with those found in this study.

Within opioids, buprenorphine was not detected in any water sample, whereas codeine was detected in all water samples from Douro, Sado and Mira estuaries, at concentrations ranging from 0.1 up to 22 ng/L. Tramadol was also ubiquitous in the Douro and Tejo water samples, but not in Sado (57%) or Mira (83%) yet reaching much higher concentrations up to 1590 ng/L, in the latter. Although opioid consumption in Portugal is relatively low in comparison with other European countries (OECD, 2019), it has been increasing in recent years, with tramadol, codeine and buprenorphine being among the most prescribed opioids, for instance, in the Lisbon metropolitan area (Caldeira et al., 2021). Moreover, recent studies have shown that many opioids are not efficiently removed by wastewater treatment, some even following tertiary treatment (Asimakopoulos et al., 2016; Campos-Mañas et al., 2018), resulting in its frequent detection in environmental water samples, as in our study, in particular tramadol and codeine, two of the most frequently detected opioids (Campos-Mañas et al., 2018). Likewise, buprenorphine, which was not detected in our water samples, is reported as less frequently detected in both wastewater influents and effluents, and occurring at much lower concentrations compared to codeine or tramadol (Asimakopoulos et al., 2016; Campos-Mañas et al., 2018). Tramadol reached by far the highest concentrations of all three opioids considered, yet similar concentrations (reaching thousands of ng/L) have been previously reported for coastal waters (e.g. Sousa et al., 2020).

The eleven antidepressants screened for in this study were all detected in the estuarine waters. Eight of the antidepressants were found in all four estuaries, whereas the other three, namely fluoxetine, mirtazapine and paroxetine, were found exclusively in Sado and Mira waters (Table 3). Bupropion, venlafaxine, duloxetine and mianserin were frequently detected in all four estuaries (DF $>$ 67%), yet reaching different maximum concentrations (Table 3). Higher median and maximum concentrations were observed for venlafaxine (maximum surpassing 300 ng/L in Sado and Mira), whereas sertraline and fluoxetine also reached more than 100 ng/L, despite being less frequently detected. Antidepressants are of the most widely screened and detected pharmaceutical classes, being found worldwide in a vast range of concentrations and in different environmental matrices (aus der Beek et al., 2016; Calisto and Esteves, 2009; Sehonova et al., 2018; Wilkinson et al., 2022). The concentrations found in our samples are in agreement (ca. from below tens up to hundreds of ng/L) with those previously detected in estuarine (e.g. Fernández-Rubio et al., 2019; Reis-Santos et al., 2018) and marine waters (e.g. Björlenius et al., 2018; Nödler et al., 2014; Togola and Budzinski, 2008).

Four out of six anxiolytic pharmaceuticals were detected in estuarine waters. Oxazepam was frequently found in all four estuaries (DF $>$ 86%) at concentrations ranging from 1.1 up to 190 ng/L. This is in agreement with the concentrations reported in previous studies (ca. tens of ng/L) in riverine (e.g. Fick et al., 2017; Wang et al., 2017), estuarine and sea waters (Björlenius et al., 2018; Fernández-Rubio et al., 2019), as well as being the most prevalent benzodiazepine in wastewaters (Asimakopoulos et al., 2016) due to generally low removal percentage following wastewater treatment (e.g. de Boer et al., 2022; de Jesus Gaffney et al., 2017; Kosjek et al., 2012). Alprazolam, hydroxyzine and lorazepam were also present in all four estuaries, yet detection frequencies varied from 14 up to 100%, with concentrations up to 7.8, 2.1 and 79 ng/L, respectively, which have also been found in previous studies in surface waters from the Atlantic coast and other locations worldwide (aus der Beek et al., 2016; Fernández-Rubio et al., 2019; Fick et al., 2011).

Carbamazepine was the most frequently detected antiepileptic pharmaceutical in estuarine water. It was found in every sample from the Douro, Tejo and Mira estuaries, and 86% of samples in the Sado estuary, at concentrations ranging from 0.1 to 61 ng/L. Carbamazepine is a commonly prescribed antiepileptic worldwide, known to be able to resist wastewater treatment at low concentrations and is the most frequently detected antiepileptic in wastewaters and in the environment worldwide (Adeleye et al., 2022; aus der Beek et al., 2016; Cardoso-Vera et al., 2021; Zhang et al., 2008). Hence, in estuarine and coastal waters carbamazepine has been found to reach maximum concentrations of thousands of ng/L (e.g. McEneff et al., 2014), and many studies frequently report 100% detection in surface waters (Cardoso-Vera et al., 2021). Other antiepileptics analysed included clonazepam and topiramate, which were less frequently detected (up to 44 and 17%, respectively) and at concentrations up to 4.9 and 15 ng/L, respectively. Very few studies have screened for these pharmaceuticals in surface waters, so there is still limited information, though there are reports of no detection or detection at the same range of concentrations (below 20 ng/L) as found here (e.g. Pivetta et al., 2020; Renganathan et al., 2021).

Of all six antipsychotic pharmaceuticals analysed in the water, haloperidol was the most common, being present in all samples from Douro and Mira estuaries and on more than 70% of samples from Tejo and Sado systems, at concentrations ranging from 0.04 up to 1.7 ng/L. On the other hand, chlorpromazine, clozapine and flupentixol were seldom detected in the water, with frequencies between 11 and 33% in Tejo, Sado and Mira estuaries, whereas levomepromazine and risperidone were not detected (Table 3). Despite the presence of some of these antipsychotics in wastewater effluents (e.g. Loos et al., 2013), few studies have assessed their occurrence in surface waters, yet they are generally not detected or detected at low ng/L concentrations (e.g. aus der Beek et al., 2016; Dehm et al., 2021; Escudero et al., 2021; Kondor et al., 2020), although some exceptionally high concentrations of clozapine (up to 78 μg/L) have been observed in South Africa's Umgeni and Msunduzi rivers (Matongo et al., 2015a, 2015b). To the best of our knowledge, we present the first record of clozapine in estuarine waters.

Within the Other pharmaceutical compounds group, which includes anti-dementia drug memantine, anticholinergic agent trihexyphenidyl and hypnotic sedative zolpidem, high detection frequencies were observed, $>$70% for all compounds, with concentrations ranging from 0.02 up to 93 ng/L. Detected concentrations of zolpidem are in the same range of concentrations previously found in surface waters, whereas memantine reached higher concentrations than in previous studies, and trihexyphenidyl concentrations were lower than previously reported (e.g. aus der Beek et al., 2016; Brieudes et al., 2017; Dehm et al., 2021). Notwithstanding, for all three compounds, the concentrations found in this study are lower than the maximum reported in wastewaters (Fick et al., 2011; Loos et al., 2013).

Overall, a highly diverse suite of neuroactive compounds was detected in surface waters from the four estuaries. Almost half (15) of the neuroactive compounds screened were found at concentrations above the threshold defined for studies on environmental fate and effects (10 ng/L) according to the European Medicines Agency (EMA) and

Table 4
Neuroactive pharmaceuticals in fish samples. Median (Med), Minimum (Min), and Maximum (Max) concentration values (ng/g ww) of pharmaceutical analytes detected in different fish tissues (brain, liver and muscle) collected from Douro, Tejo, Sado and Mira estuaries. The sum of concentrations (∑) and detection frequency (DF, %) per therapeutic group and for all analytes (∑ Total) are also shown. < LOQ indicates values below the Limit of Quantification (DF = 0).

Therapeutic Group	Analyte	Brain		Liver		Muscle	
		Med (Min-Max) ng/g	DF (%) N = 50	Med (Min-Max) ng/g	DF (%) N = 55[a]	Med (Min-Max) ng/g	DF (%) N = 55
Opioids	Buprenorphine	< LOQ		< LOQ		< LOQ	
	Codeine	0.8 (0.5–1.7)	28	0.9 (0.6–1.6)	21	0.7 (0.7–1.1)	13
	Tramadol	< LOQ		< LOQ		< LOQ	
Σ Opioids		0.8 (0.5–1.7)	28	0.9 (0.6–1.6)	21	0.7 (0.7–1.1)	13
Antiepileptics	Carbamazepine	1.4	2	< LOQ		< LOQ	
	Clonazepam	< LOQ		< LOQ		< LOQ	
	Topiramate	12 (1.3–207)	72	8 (1.1–86)	62	2.3 (1.2–20)	47
Σ Antiepileptics		12 (1.3–207)	72	8 (1.1–86)	62	2.3 (1.2–20)	47
Antipsychotics	Chlorpromazine	< LOQ		< LOQ		< LOQ	
	Clozapine	< LOQ		< LOQ		< LOQ	
	Flupentixol	< LOQ		< LOQ		< LOQ	
	Haloperidol	< LOQ		0.2 (0.1–0.5)	23	< LOQ	
	Levomepromazine	< LOQ		< LOQ		< LOQ	
	Risperidone	0.2 (0.1–0.3)	72	0.2 (0.1–0.6)	69	0.2 (0.1–0.4)	65
Σ Antipsychotics		0.2 (0.1–0.3)	72	0.2 (0.1–0.6)	73	0.2 (0.1–0.4)	65
Anxiolytics	Alprazolam	< LOQ		< LOQ		< LOQ	
	Bromazepam	< LOQ		< LOQ		< LOQ	
	Clobazam	< LOQ		< LOQ		< LOQ	
	Hydroxyzine	< LOQ		1.4	2	< LOQ	
	Lorazepam	< LOQ		5.3	2	< LOQ	
	Oxazepam	< LOQ		< LOQ		< LOQ	
Σ Anxiolytics		< LOQ		6.7	2	< LOQ	
Antidepressants	Amitriptyline	< LOQ		< LOQ		< LOQ	
	Bupropion	< LOQ		0.2 (0.1–0.4)	10	0.1	2
	Citalopram	< LOQ		< LOQ		< LOQ	
	Duloxetine	1.6	2	1.7 (1.7–3)	6	< LOQ	
	Fluoxetine	< LOQ		< LOQ		< LOQ	
	Maprotiline	< LOQ		< LOQ		< LOQ	
	Mianserin	< LOQ		2	2	1.1	2
	Mirtazapine	< LOQ		< LOQ		< LOQ	
	Paroxetine	< LOQ		< LOQ		< LOQ	
	Sertraline	< LOQ		< LOQ		< LOQ	
	Venlafaxine	1.1 (0.5–13)	30	1.8 (0.6–4.3)	19	0.9 (0.5–2.5)	22
Σ Antidepressants		1.4 (0.5–13)	30	1.7 (0.1–6.1)	27	0.8 (0.1–2.5)	24
Psychostimulants	Caffeine	7.5 (5.5–9.7)	6	12	2	5.3	2
Σ Psychostimulants		7.5 (5.5–9.7)	6	12	2	5.3	2
Anti-dementia drugs	Memantine	< LOQ		< LOQ		< LOQ	
Anticholinergic agents	Trihexyphenidyl	0.13 (0.11–0.14)	4	0.12 (0.11–0.12)	4	0.2	2
Hypnotics and sedatives	Zolpidem	< LOQ		< LOQ		< LOQ	
Σ Other		0.13 (0.11–0.14)	4	0.12 (0.11–0.12)	4	0.2	2
Σ Total		9 (0.1–207)	100	5.8 (0.1–86)	95	1.5 (0.1–21)	95
Number of pharmaceuticals		2 (1–5)		2 (0–6)		1 (0–3)	

[a] The number of samples (N) varies for some of the analytes screened. For more details see Supplementary Table S3.

reaching maximum concentrations over 150 times higher. Moreover, the ubiquity and diversity of these compounds are outstanding, with more than 10 and up to 26 compounds being detected in every sample collected in distinct estuaries.

3.2. Neuroactive pharmaceuticals in fish

Thirteen out of the 33 neuroactive pharmaceuticals screened were detected in at least one of the fish tissues (i.e., brain, liver and muscle tissues), and included one opioid, two antiepileptics, two antipsychotics, two anxiolytics, four antidepressants, one psychostimulant and one compound from the Other pharmaceutical compounds group (Table 4). Notably, all brain samples (50) and 95% of liver and muscle samples (55 each) contained at least one neuroactive pharmaceutical. Still, in fish brain and liver tissues, a median of 2 neuroactive pharmaceuticals were detected, with individual samples showing up to 6 different compounds, whereas in the muscle samples a maximum of 3 different compounds per sample were detected (Table 4). Pharmaceutical concentrations ranged between 0.1 and 207 ng/g for individual analytes, with antiepileptic topiramate, antidepressant venlafaxine and the psychostimulant caffeine exhibiting the highest concentrations (Table 4). The sum of all neuroactive pharmaceutical concentrations (∑ Total) per sample reached higher median concentrations in the brain (9 ng/g), followed by the liver (5.8 ng/g) and muscle (1.5 ng/g) tissues (Table 4 and Fig. 2). The same pattern was also observed for the maximum tissue concentrations, with brain reaching 207 ng/g followed by liver 86 ng/g and muscle 21 ng/g (Table 4). Laboratory (e.g. Huerta et al., 2016; McCallum et al., 2017; Valdés et al., 2016) and field studies (e.g. Brooks et al., 2005; Liu et al., 2018) have shown similar accumulation patterns among tissues, with brain and liver tissues showing higher concentrations than muscles. These patterns and the presence of different pharmaceuticals among tissues have implications towards the choice of tissues for pharmaceutical quantification and environmental risk assessment (e.g. Duarte et al., 2022; Miller et al., 2018).

Overall, the bioaccumulation of neuroactive pharmaceuticals in fish species differed among and within therapeutic groups, with antiepileptics reaching higher summed concentrations, followed by psychostimulants, anxiolytics and antidepressants (Table 4 and Fig. 2). Antiepileptics and antipsychotics were among the most frequently detected therapeutic groups, with frequencies of detection higher than 47% in all three tissues.

Although frequently detected in water, the antiepileptic carbamazepine was only detected in one brain sample of *P. flesus* from the Douro estuary, at 1.4 ng/g, a slightly higher value than those reported by Liu

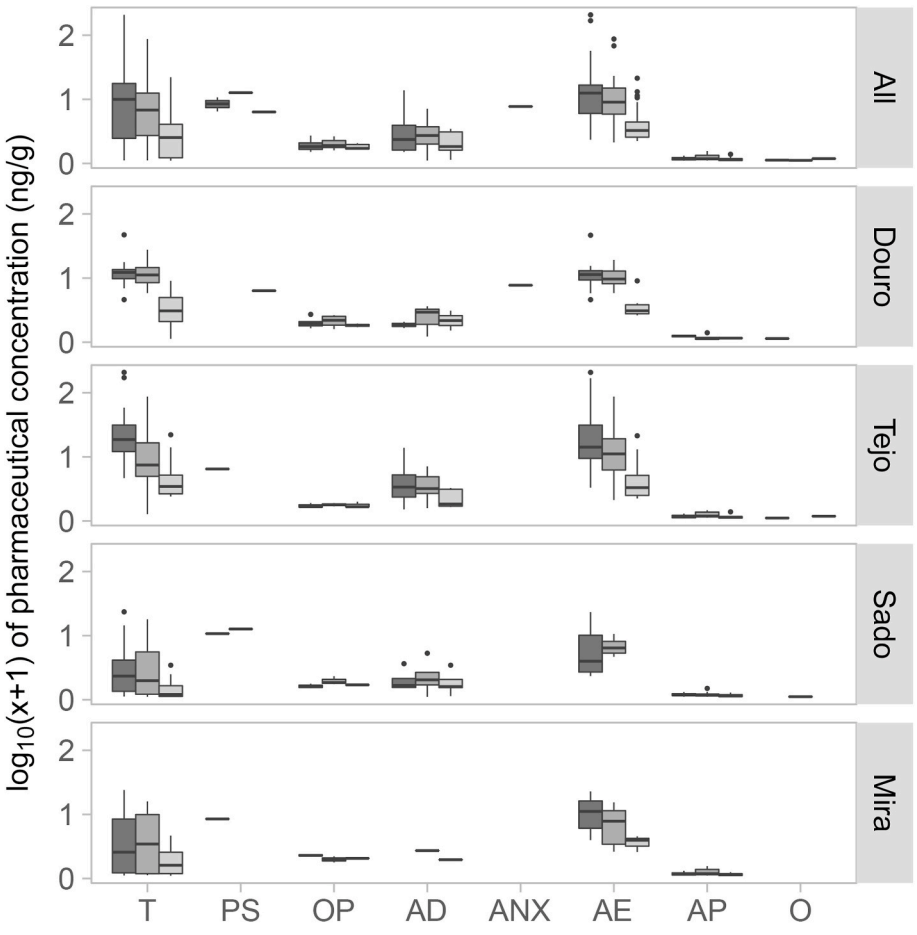


Fig. 2. Sums (∑) of pharmaceutical concentrations per therapeutic group, in different fish tissues (brain, liver and muscle), in all and each estuary (Douro, Tejo, Sado and Mira). Values are presented as $\log_{10}(x+1)$ of pharmaceutical concentrations (ng/g ww). Left (dark grey), centre (grey) and right (light grey) boxplots correspond to brain, liver and muscle, respectively. Boxplots show median, 25th and 75th percentiles, upper and lower whiskers extending at most 1.5 times the interquartile range (IQR) to maximum and minimum values, respectively. Therapeutic groups are the following: T - Total, PS - Psychostimulants, OP - Opioids, AD - Antidepressants, ANX - Anxiolytics, AE - Antiepileptics, AP - Antipsychotics and O - Other.

et al. (2015) and Tanoue et al. (2015) (up to 1 ng/g) in the brains of freshwater species collected in riverine systems in China and Japan, respectively. Moreover, no carbamazepine residues were previously detected in liver and muscle tissues from wild fish collected in the Tejo estuary (Fonseca et al., 2021) but have been found in wild fish species from other locations worldwide (Świacka et al., 2022). Topiramate was frequently detected in all tissues (DF > 47; brain > liver > muscle) from 5 out of 7 species, with higher median and maximum concentrations in the brain (12 and 207 ng/g, respectively), followed by liver and muscle samples (Table 4). In line with our results, a similar range of concentrations has been reported in the liver of wild *D. labrax* juveniles and one adult collected in the Tejo estuary, up to 244.4 ng/g (Fonseca et al., 2021), yet whilst our results show its repeated occurrence at similar elevated levels, there is a general lack of field studies targeting this pharmaceutical in fish, and this should be prioritised, considering the high concentrations observed. Also, no bioaccumulation of the antiepileptic clonazepam was observed, and this is, to our knowledge, the first study to target this compound in wild fish, whilst studies concerning clonazepam occurrence in aqueous matrices and exposure effects are still scarce, as mentioned in recent review studies (Cunha et al., 2019, 2017).

Contrary to the high occurrence in water samples (and reaching up to 1003 ng/L), the psychostimulant caffeine was present in only 6% of fish brain samples, and 2% of both liver and muscle tissues, with concentrations between 5.3 and 12 ng/g. Other field studies also reported caffeine bioaccumulation in muscle, liver and gills of different fish species at the same magnitude (up to 74 ng/g), in wet and dry weights (Li et al., 2020; Ondarza et al., 2019; Vieira et al., 2022), yet no behavioural effects were observed at higher internal concentrations (from 29 up to 68 ng/g) in *Perca fluviatilis* juveniles (Cerveny et al., 2022) whereas changes in biochemical and behavioural endpoints were reported only at substantially higher external concentrations, above several thousands of ng/L and up to mg/L range (e.g. Ladu et al., 2015; Li et al., 2012; Santos-Silva et al., 2018).

Of the six anxiolytics analysed, only hydroxyzine and lorazepam were found, and in one liver sample of *P. flesus* from the Douro estuary, at 1.4 and 5.3 ng/g, respectively. Fonseca et al. (2021) and Huerta et al. (2018) screened for anxiolytic lorazepam in wild fish, with no detection in liver or muscle tissues in different estuarine and freshwater fish species, while Rojo et al. (2019) detected a maximum of 0.23 ng/g in the muscle of 1 out of 3 freshwater fish species. A previous study also documented low hydroxyzine uptake in liver of fish caged in a wastewater-influenced stream (0.3 up to 1.2 ng/g) and also no detection in brain and muscle tissues (Grabicova et al., 2017). Though not detected, this is, to the best of our knowledge, the first screening for clobazam in water and fish in estuarine areas. Whilst anxiolytics such as alprazolam, bromazepam were also not detected in wild fish (e.g. Fonseca et al., 2021; Martínez-Morcillo et al., 2020; Peña-Herrera et al., 2020), oxazepam bioaccumulation was reported in the plasma of wild riverine species *Squalius cephalus* at 25 ng/mL (Cerveny et al., 2021) as well as in *Perca fluviatilis'* bile below 3 ng/g (UNESCO and HELCOM, 2017).

Four out of eleven antidepressants screened were detected in fish tissues: bupropion, duloxetine, mianserin and venlafaxine. Of all four, venlafaxine was the most pervasive, and detected in 5 out of the 7 species, and in 30, 19 and 22% of brain, liver and muscle tissues (concentrations ranging from 0.5 up to 13 ng/g). Bupropion, duloxetine and mianserin were less frequently detected, at maximum concentrations of 0.4, 3 and 2 ng/g. Antidepressants amitriptyline, citalopram, fluoxetine, maprotiline, mirtazapine, paroxetine and sertraline were not found in

any fish tissues. Multiple studies have reported antidepressants' bioaccumulation in fish collected in the wild, including those screened in this study, yet with considerable variability (Miller et al., 2018; Silva et al., 2015; Świacka et al., 2022). Venlafaxine is commonly detected in wild fish, found in various tissues including brain, liver and muscles, and within the low ng/g (ww and dw) range (e.g. Arnnok et al., 2017; Huerta et al., 2018; Schultz et al., 2010). Bioaccumulation of bupropion, mianserin and duloxetine in wild fish has been previously assessed (Arnnok et al., 2017; Cerveny et al., 2021; Grabicova et al., 2017; Schultz et al., 2010), despite being seldom considered compared to other antidepressants (Silva et al., 2015; Świacka et al., 2022). On the other hand, sertraline and fluoxetine are frequently detected in wild fish tissues at concentrations below or close to our LOQ of 10 and 5 ng/g, respectively (Brooks et al., 2005; Meador et al., 2016; Schultz et al., 2010), though there are studies showing bioaccumulation up to hundreds of ng/g (e.g. Du et al., 2014; Fonseca et al., 2021; Ramirez et al., 2009). Its fast metabolism evidenced by higher concentrations of metabolites compared to the parent compounds (Arnnok et al., 2017; Miller et al., 2018; Schultz et al., 2010), in combination with the lower occurrence in our water samples might justify its absence in our fish samples, although metabolites were not screened to confirm this hypothesis. While the remaining antidepressants are comparatively less studied in wild fish, their monitoring should not be disregarded as they are frequently detected in surface waters and in wild biota (Calisto and Esteves, 2009; Silva et al., 2015; Świacka et al., 2022).

While opioids buprenorphine and tramadol were not detected in fish, codeine concentrations were generally the same across tissues, though more frequently found in fish brain followed by liver and muscle, reaching maximum concentrations of 1.7 ng/g in the brain (Table 4). Codeine bioaccumulation in fish has been described in various freshwater fish species collected in the field (Rojo et al., 2019; Valdés et al., 2016) and at the same range of concentrations as in this study (up to 1.1 ng/g in Rojo et al. (2019)). Tramadol has also been reported to accumulate in different fish tissues (Grabicova et al., 2017; Hubená et al., 2020; Tanoue et al., 2017), yet despite the high concentrations found in our water samples (up to 1590 ng/L), it was not detected in fish, which might be associated with the relatively high LOQ in our samples (50 ng/g) and with the different exposure conditions from our wild samples, when compared to a likely continuous exposure concentration in these studies from the experimental design in the laboratory and in caged in the field trial. In fact, Tanoue et al. (2017) and Hubená et al. (2020) reported mean brain concentrations of 4.6 ng/g in *Pimephales promelas* adults and 1.8 ng/g in *Squalius cephalus* after long-term (23 and 42 days, respectively) exposures to 1 μg/L (the same range as some of the highest concentrations found in our water samples), whereas Grabicova et al. (2017) detected tramadol in liver and kidney of *Salmo trutta* caged for 3 months in a stream influenced by wastewater effluents, on average from 1.7 up to 6 ng/g, with all these studies pertaining to continuous exposure conditions yet all reporting concentrations below our quantification limit.

Only two antipsychotics were detected in fish: risperidone was found in all three tissues, although more frequently detected in brain (72%), followed by the liver (69%) and muscle (65%) of all species sampled, with concentrations ranging from 0.1 up to 0.6 ng/g; whereas haloperidol was found only in the liver (23%) of 5 species, at concentrations up to 0.5 ng/g. Risperidone has been frequently found in the tissues of wild fish (Cerveny et al., 2021; Grabicova et al., 2017) and fish exposed to treated effluents (e.g. Fick et al., 2010), even when, as in our study, it is not detected in the medium (Fick et al., 2010; Grabicova et al., 2017). Likewise, several studies reported haloperidol concentrations in wild fish at very low ng/g (usually below 1 ng/g or 1.2 ng/mL in plasma), such as in brain (Tanoue et al., 2015), blood plasma (Cerveny et al., 2021; Fick et al., 2010), liver or muscle (Tanoue et al., 2015). Accordingly, Tanoue et al. (2015) have shown a higher partition of haloperidol in the liver than any other fish tissue analysed, including brain and muscle, which is in line with the detection of this pharmaceutical only in the liver. Both Cerveny et al. (2021) and Fick et al. (2010) studies pointed risperidone and haloperidol as of high risk for fish, as concentrations in fish plasma were either above or close to human therapeutic plasma concentrations, implying potential exposure effects. Our results corroborate these studies, as these were the only two out of six antipsychotics to bioaccumulate in fish, even when it was not detected in the medium as observed for risperidone.

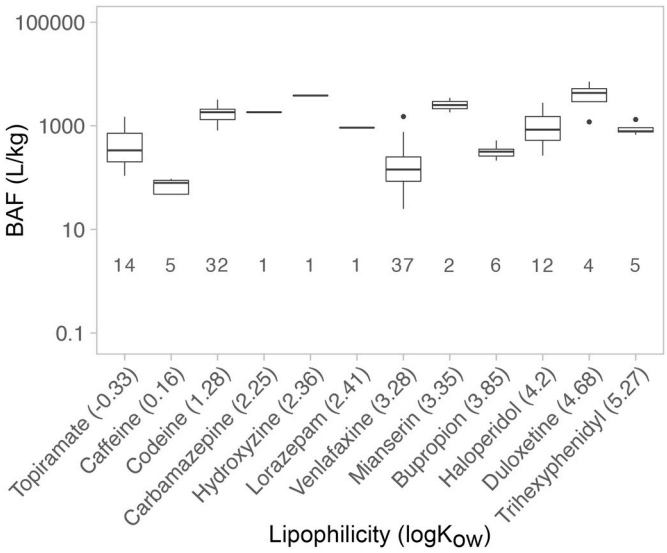


Fig. 3. Field-derived bioaccumulation factors (BAF, L/kg) of neuroactive pharmaceuticals with increasing lipophilicity (logK$_{ow}$). BAF values (N shown under each boxplot) were calculated as the ratio between pharmaceutical concentrations detected in fish tissues and the median concentrations detected in the corresponding estuarine waters. Boxplots show median, 25th and 75th percentiles, upper and lower whiskers extending at most 1.5 times the interquartile range (IQR) to maximum and minimum values, respectively.

Bioaccumulation in fish of the pharmaceutical compounds considered within the Other compounds group was only observed for trihexyphenidyl (4% of samples), with concentrations ranging from 0.11 to 0.2 ng/g, lower than those previously reported in *P. flesus* from the baltic sea (UNESCO and HELCOM, 2017).

Overall, bioaccumulation patterns of the different therapeutic groups in fish were consistent among all four estuaries (Fig. 2). Generally larger contributions for summed concentrations were from antiepileptics, psychostimulants, anxiolytics and antidepressants groups (Table 4 and Fig. 2), which follows patterns in previous studies. For example, Muir et al. (2017) screened for 127 pharmaceuticals and personal care products in the plasma of both caged *Carassius auratus* and wild *Cyprinus carpio*, with more than half of the compounds detected in fish tissues being antidepressants and their metabolites. Following the screening of 20 pharmaceuticals in 8 different fish species, Huerta et al. (2018) also found antiepileptics and antidepressants to be the most prevalent therapeutic groups among the seven groups considered, including for example β-blockers or anti-inflammatory drugs. In their work, Arnnok et al. (2017) screened for 24 pharmaceutical compounds in 10 different fish species and also found higher concentrations of antidepressants compared to other classes of pharmaceuticals such as antibiotics or anti-inflammatory drugs.

Although variations in occurrence and concentrations were observed in the waters from the four estuaries, bioaccumulation patterns were similar in fish from all the systems (Fig. 2). This has also been observed in other field studies where sampling occurred in various locations, suggesting that bioaccumulation patterns are mostly determined by the chemical properties of pharmaceuticals rather than the range of concentrations found in the medium (e.g. Muir et al., 2017; Yang et al., 2020). Despite maximum water concentrations found in Sado and Mira estuaries (the two less populated estuaries, despite seasonal variability),

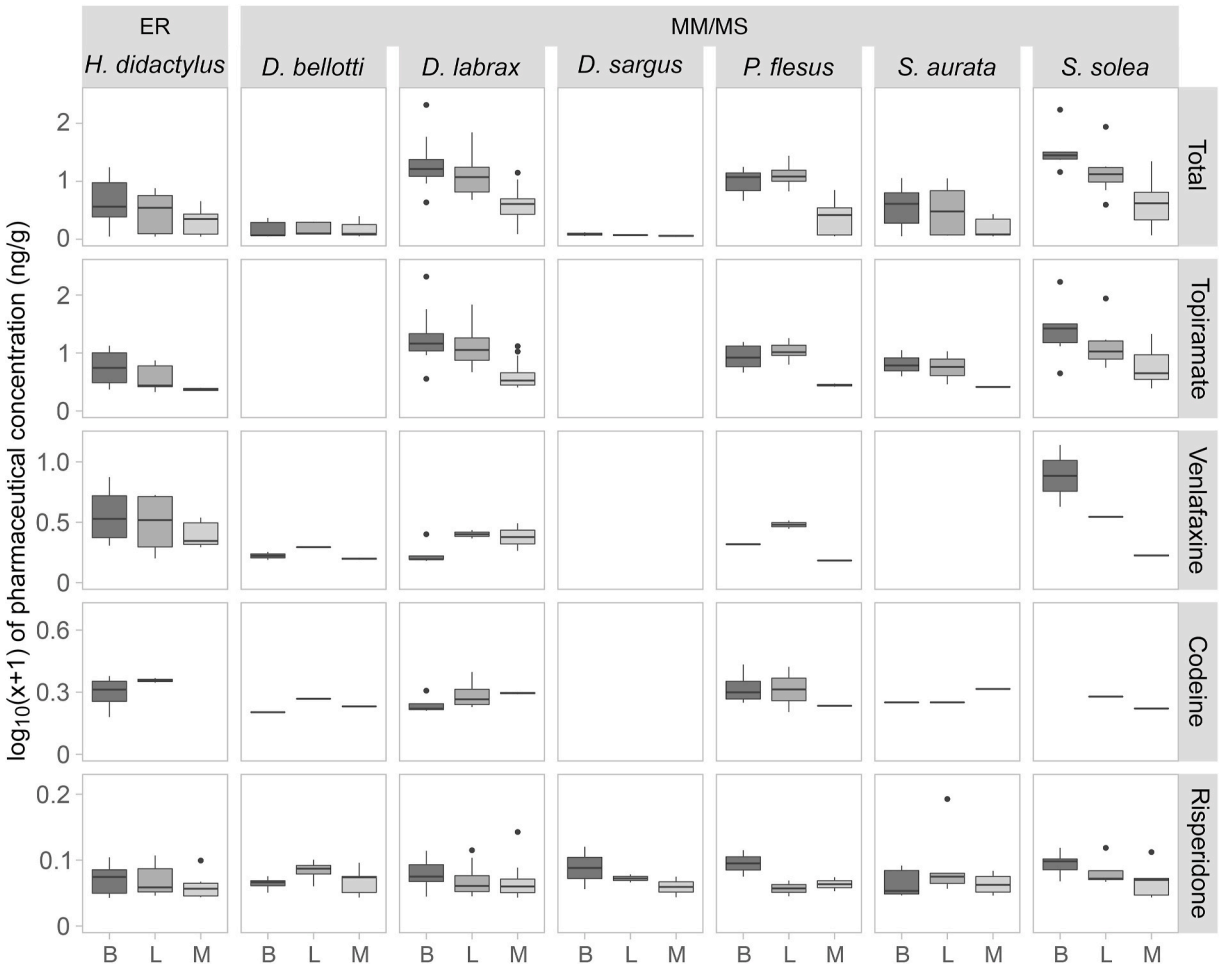


Fig. 4. Concentrations of all neuroactive pharmaceuticals (Total) and of the most frequently detected pharmaceuticals (Topiramate, Venlafaxine, Codeine and Risperidone) in fish brain (B), liver (L) and muscle (M) in each fish species, namely estuarine resident (ER) *Halobatrachus didactylus,* and marine migrants and stragglers *Diplodus bellottii*, *Dicentrarchus labrax*, *Diplodus sargus*, *Platichthys flesus*, *Sparus aurata* and *Solea solea*. Concentrations (ng/g ww) are presented as $\log_{10}(x+1)$, and scales differ between plots. Boxplots show median, 25th and 75th percentiles, upper and lower whiskers extending at most 1.5 times the interquartile range (IQR) to maximum and minimum values, respectively.

fish accumulated higher levels of pharmaceuticals in Douro and Tejo estuaries, evidencing the impacts of the constant pharmaceutical inputs from highly populated areas. Usually, bioconcentration of lipophilic compounds is estimated through the octanol/water partition coefficient (K_{ow}) and has been shown to increase with increasing lipophilicity for different chemicals (Arnot and Gobas, 2006; Bintein et al., 1993; Mackay, 1982). Accordingly, lipophilicity thresholds have been set to estimate chemical bioaccumulation and to determine the need for environmental risk assessment of chemical substances in European guidelines. However, in the particular case of neuroactive pharmaceuticals, it seems that this factor alone may not be the best predictor for bioaccumulation in fish tissues, as their uptake and bioconcentration is influenced by parameters such as salinity, pH or exposure time, but also by species-specific traits, life-stage or tissues (Duarte et al., 2022). Accordingly, we tested if a correlation between field-derived bioaccumulation factors (BAF) and compounds' lipophilicity existed, and no significant correlation was found when considering all BAF values ($r = 0.2$, p-value $= 0.53$, Fig. 3), nor when considering each tissue independently ($r > 0.2$, p-value > 0.3), confirming that the prediction of bioaccumulation of neuroactive compounds through compounds' lipophilicity may not be straightforward.

Neuroactive pharmaceutical bioaccumulation showed a prevalent pattern among species, with higher summed concentrations in the brain followed by the liver and muscle tissues (Fig. 4). This pattern was evident for all species, including resident species *H. didactylus*, as well as for marine migrants and stragglers such as *D. labrax*, *S. aurata* or *S. solea*. This pattern could also be generally observed among the most frequently detected neuroactive pharmaceuticals, such as topiramate, venlafaxine and risperidone (Fig. 4). Notwithstanding, not all species seem to accumulate neuroactive pharmaceuticals in the same range of concentrations, i.e., some were found to accumulate higher summed concentrations, such as *D.labrax* and *S.solea* (up to hundreds of ng/g), and to a less extent *P. flesus, H. didactylus* and *S. aurata* (up to tens of ng/g), whereas both *Diplodus* species showed reduced concentrations (up to 1.5 ng/g) (Fig. 4). This may be the result of bioconcentration rates being influenced by different metabolic rates, linked to health status, feeding regimes, life-stage or size (Arnot and Gobas, 2006). Differences in bioaccumulation among wild fish species are known, and its association with species' ecological traits, including different habitat use, feeding strategies or trophic levels has been studied (e.g. Arnnok et al., 2017; Du et al., 2014; Fonseca et al., 2021; Huerta et al., 2018; Rojo et al., 2019). We hypothesised that estuarine resident species, that spend their whole life cycle inside the estuary would have increased pharmaceutical accumulation compared to marine migrant or straggler species, which use the estuaries as nurseries or occasionally for feeding purposes, and thus spend comparatively less time inside the estuarine environment. Yet, our results show an unclear pattern in the bioaccumulation of different therapeutic groups across species with different habitat use

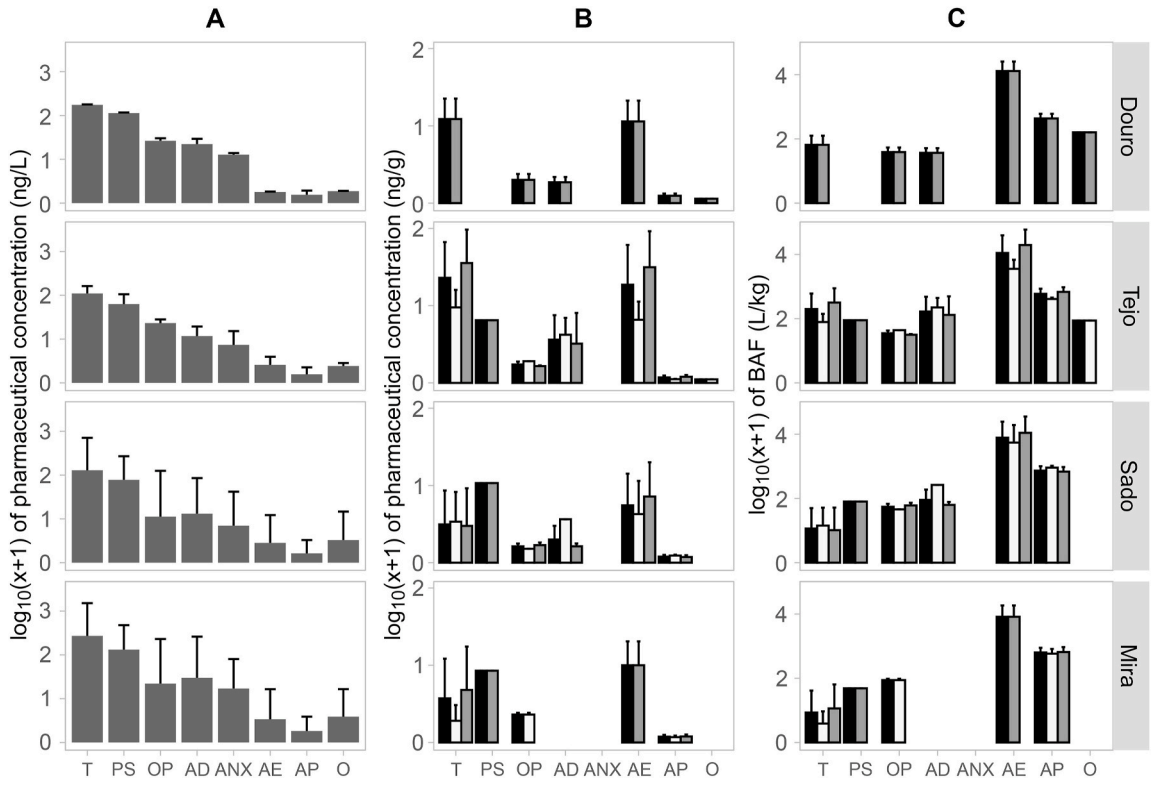


Fig. 5. Mean (and standard deviation) of the summed concentrations per therapeutic class in the water, in ng/L (A) and fish brain (tissue concentrations in ng/g ww (B) and bioaccumulation factors in L/kg (C) are presented). Tissue concentrations and BAF from fish brain are given for all species (All, black bars), for estuarine resident species (ER, white bars) and marine migrant or straggler species (MM/MS, grey bars). Values are presented as $\log_{10}(x+1)$ and scales differ between plots. Therapeutic groups are the following: T - Total, PS - Psychostimulants, OP - Opioids, AD - Antidepressants, ANX - Anxiolytics, AE - Antiepileptics, AP - Antipsychotics and O - Other (including anticholinergic agents, hypnotics and sedatives, anti-dementia drugs).

classifications (Fig. 5). This reveals how exposure to neuroactive compounds and consequent bioaccumulation in fish tissues does not imply exposure to contamination sources throughout their entire life or even large extended periods. Accordingly, it is known that pharmaceutical uptake and bioconcentration can occur in short timeframes (e.g. Liu et al., 2021; Wang and Gardinali, 2013), supporting the idea that all fish species tend to bioaccumulate neuroactive compounds, regardless of the time they spend in more prone areas inside the estuary. Notwithstanding, the specimens sampled here are late juveniles/young adults, which have most likely spent their first year(s) inside the estuary, which may contribute to the higher and comparable concentrations in marine migrants such as *D. labrax*, *S. solea and P. flesus* and those found in estuarine resident species *H. didactylus*.

Moreover, we addressed the potential link between bioaccumulation and fish trophic levels and found no significant correlations between total pharmaceutical concentrations (median values) and species trophic levels (TL, Table 2), for each of the three tissues, brain, liver and muscle ($r > -0.54$, p-value > 0.24). This points to a general bioaccumulation among all fish species, independently of trophic level, which is also highlighted by the overlap of data points obtained through the principal component analysis (Fig. S2), showing that no specific pattern of bioaccumulation can be highlighted among species or estuaries.

Overall, the bioaccumulation of neuroactive compounds was observed for all seven fish species, in all four estuaries, with higher contributions from antiepileptics, psychostimulants, anxiolytics and antidepressants. A similar bioaccumulation pattern was generally evident among all species, revealing overall higher bioaccumulation in brain tissue, followed by liver and muscle, highlighting the importance of tissue selection in future bioaccumulation studies. No clear patterns were evident considering species' different habitat uses, including resident species, marine migrants or straggler species, and there was no obvious bioaccumulation pattern in relation to the different trophic levels, indicating a general uptake of neuroactive pharmaceuticals among the seven fish species, despite higher summed concentrations could be found in some species.

4. Conclusion

This study analyses the occurrence of a broad suite of neuroactive pharmaceuticals of various therapeutic groups in estuarine surface waters and its bioaccumulation in three different tissues of seven species of fish with different life-history strategies and habitat use patterns. In the water, all seven therapeutic groups were frequently detected in all four estuaries (>78%) and almost half (15) of all neuroactive compounds exceeded concentrations of 10 ng/L, defined as the threshold level for studies on environmental fate and effects. With 10 and up to 26 neuroactive compounds detected in individual water samples, our results reveal a complex mixture of a suite of compounds in all four estuaries, despite differences in hydromorphology and urban development in the vicinity of the estuarine systems.

The bioaccumulation of neuroactive compounds was observed in all seven fish species collected in the different estuaries, with neuroactive compounds being detected in every fish brain and in 95% of fish liver and muscle tissues. A bioaccumulation pattern was evident among species, and in all estuaries, revealing overall higher bioaccumulation in the brain followed by liver and muscle tissues. Moreover, no clear uptake patterns linked to different habitat use or trophic levels were found, pointing to a conspicuous uptake of neuroactive pharmaceuticals among the different fish species.

Here, we reveal the ubiquity of neuroactive compounds in estuarine waters and the bioaccumulation of these compounds across multiple

estuarine and marine fish species, independently of their estuary of capture, habitat use or trophic level. These results are key for improved risk assessment, yet information linking internalized concentrations to toxic effects is still scarce, though crucial for defining threshold safety levels to manage the risk of these compounds in the environment. Moreover, despite recent efforts concerning the impacts of pharmaceuticals in estuarine and marine environments, there is still a considerable knowledge gap regarding these key ecosystems when compared to freshwater systems, that needs to be addressed.

Credit author statement

Irina A. Duarte: Conceptualization, Methodology, Writing – original draft. Patrick Reis-Santos: Writing – review & editing. Jerker Fick: Conceptualization, Methodology, Writing – review & editing. Henrique N. Cabral: Conceptualization, Formal analysis, Writing – review & editing. Bernardo Duarte: Writing – review & editing. Vanessa F. Fonseca: Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank all involved in fish and water sampling and processing. This study had the support of Fundacao para a Ciencia e a Tecnologia (FCT) via strategic projects UIDB/04292/2020 and UIDP/04292/2020 awarded to MARE, through project LA/P/0069/2020 granted to the Associate Laboratory ARNET, and project grant PTDC/MAR-EST/3048/2014. IAD was funded with an FCT PhD grant (SFRH/BD/138376/2018), co-funded by the European Union through the European Social Fund (ESF). VFF and BD were funded with FCT researcher contracts (DL57/2016/CP1479/CT0024 & 2021.00244.CEECIND and CEECIND/00511/2017, respectively).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120531.

References

Adeleye, A.S., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O. A., Zhu, Y., 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. J. Hazard Mater. 424, 127284 https://doi.org/10.1016/j.jhazmat.2021.127284.

Ali, A.M., Rønning, H.T., Sydnes, L.K., Alarif, W.M., Kallenborn, R., Al-Lihaibi, S.S., 2018. Detection of PPCPs in marine organisms from contaminated coastal waters of the Saudi Red Sea. Sci. Total Environ. 621, 654–662. https://doi.org/10.1016/j.scitotenv.2017.11.298.

Aminot, Y., Le Menach, K., Pardon, P., Etcheber, H., Budzinski, H., 2016. Inputs and seasonal removal of pharmaceuticals in the estuarine Garonne River. Mar. Chem. 185, 3–11. https://doi.org/10.1016/j.marchem.2016.05.010.

Arnnok, P., Singh, R.R., Burakham, R., Pérez-Fuentetaja, A., Aga, D.S., 2017. Selective uptake and bioaccumulation of antidepressants in fish from effluent-impacted niagara river. Environ. Sci. Technol. 51, 10652–10662. https://doi.org/10.1021/acs.est.7b02912.

Arnold, K.E., Brown, A.R., Ankley, G.T., Sumpter, J.P., 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos. Trans. R. Soc. B Biol. Sci. 369 https://doi.org/10.1098/rstb.2013.0569, 20130569.

Arnot, J.A., Gobas, F.A., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14, 257–297. https://doi.org/10.1139/a06-005.

Asimakopoulos, A.G., Kannan, K., 2016. Neuropsychiatric pharmaceuticals and illicit drugs in wastewater treatment plants: a review. Environ. Chem. 13, 541 https://doi.org/10.1071/EN15202.

aus der Beek, T., Weber, F.-A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A., 2016. Pharmaceuticals in the environment-Global occurrences and perspectives. Environ. Toxicol. Chem. 35, 823–835. https://doi.org/10.1002/etc.3339.

Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. Front. Ecol. Environ. 15, 84–90. https://doi.org/10.1002/fee.1450.

Bintein, S., Devillers, J., Karcher, W., 1993. Nonlinear dependence of fish bioconcentration on n -Octanol/Water partition coefficient. SAR QSAR Environ. Res. 1, 29–39. https://doi.org/10.1080/10629369308028814.

Björlenius, B., Ripszám, M., Haglund, P., Lindberg, R.H., Tysklind, M., Fick, J., 2018. Pharmaceutical residues are widespread in Baltic Sea coastal and offshore waters – screening for pharmaceuticals and modelling of environmental concentrations of carbamazepine. Sci. Total Environ. 633, 1496–1509. https://doi.org/10.1016/j.scitotenv.2018.03.276.

Brieudes, V., Lardy-Fontan, S., Vaslin-Reimann, S., Budzinski, H., Lalere, B., 2017. Development of a multi-residue method for scrutinizing psychotropic compounds in natural waters. J. Chromatogr. B 1047, 160–172. https://doi.org/10.1016/j.jchromb.2016.07.016.

Brooks, B.W., Kevin Chambliss, C., Stanley, J.K., Ramirez, A., Banks, K.E., Johnson, R.D., Lewis, R.J., 2005. Determination of selected antidepressants in fish from an effluent-dominated stream. Environ. Toxicol. Chem. 24, 464–469. https://doi.org/10.1897/04-081R.1.

Caldeira, D., Broeiro, P., Cimadeira, F., Costa, J., Lourenço, A., Meireles, C., 2021. Opioids prescribing trend between 2013 and 2017 in the Lisbon and tagus valley region, Portugal. Int. J. Clin. Pharm. 43, 323–327. https://doi.org/10.1007/s11096-020-01199-7.

Calisto, V., Esteves, V.I., 2009. Psychiatric pharmaceuticals in the environment. Chemosphere 77, 1257–1274. https://doi.org/10.1016/j.chemosphere.2009.09.021.

Campos-Mañas, M.C., Ferrer, I., Thurman, E.M., Agüera, A., 2018. Opioid occurrence in environmental water samples - a review. Trends Environ. Anal. Chem. 20, e00059 https://doi.org/10.1016/j.teac.2018.e00059.

Cardoso-Vera, J.D., Elizalde-Velázquez, G.A., Islas-Flores, H., Mejía-García, A., Ortega-Olvera, J.M., Gómez-Oliván, L.M., 2021. A review of antiepileptic drugs: Part 1 occurrence , fate in aquatic environments and removal during different treatment technologies. Sci. Total Environ. 768.

Cerveny, D., Grabic, R., Grabicová, K., Randák, T., Larsson, D.G.J., Johnson, A.C., Jürgens, M.D., Tysklind, M., Lindberg, R.H., Fick, J., 2021. Neuroactive drugs and other pharmaceuticals found in blood plasma of wild European fish. Environ. Int. 146, 106188 https://doi.org/10.1016/j.envint.2020.106188.

Cerveny, D., Cisar, P., Brodin, T., McCallum, E.S., Fick, J., 2022. Environmentally relevant concentration of caffeine - effect on activity and circadian rhythm in wild perch. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-022-19583-3.

Corcoran, J., Winter, M.J., Tyler, C.R., 2010. Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish. Crit. Rev. Toxicol. 40, 287–304. https://doi.org/10.3109/10408440903373590.

Cunha, D.L., de Araujo, F.G., Marques, M., 2017. Psychoactive drugs: occurrence in aquatic environment, analytical methods, and ecotoxicity - a review. Environ. Sci. Pollut. Res. 24, 24076–24091. https://doi.org/10.1007/s11356-017-0170-4.

Cunha, D.L., Mendes, M.P., Marques, M., 2019. Environmental risk assessment of psychoactive drugs in the aquatic environment. Environ. Sci. Pollut. Res. 26, 78–90. https://doi.org/10.1007/s11356-018-3556-z.

de Boer, S., González-Rodríguez, J., Conde, J.J., Moreira, M.T., 2022. Benchmarking tertiary water treatments for the removal of micropollutants and pathogens based on operational and sustainability criteria. J. Water Proc. Eng. 46, 102587 https://doi.org/10.1016/j.jwpe.2022.102587.

de Jesus Gaffney, V., Cardoso, V.V., Cardoso, E., Teixeira, A.P., Martins, J., Benoliel, M. J., Almeida, C.M.M., 2017. Occurrence and behaviour of pharmaceutical compounds in a Portuguese wastewater treatment plant: removal efficiency through conventional treatment processes. Environ. Sci. Pollut. Res. 24, 14717–14734. https://doi.org/10.1007/s11356-017-9012-7.

Dehm, J., Singh, S., Ferreira, M., Piovano, S., Fick, J., 2021. Screening of pharmaceuticals in coastal waters of the southern coast of Viti Levu in Fiji, South Pacific. Chemosphere 276, 130161. https://doi.org/10.1016/j.chemosphere.2021.130161.

Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A., Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., Brooks, B.W., 2014. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. Philos. Trans. R. Soc. B Biol. Sci. 369, 20140058 https://doi.org/10.1098/rstb.2014.0058.

Duarte, I.A., Fick, J., Cabral, H.N., Fonseca, V.F., 2022. Bioconcentration of neuroactive pharmaceuticals in fish: relation to lipophilicity, experimental design and toxicity in the aquatic environment. Sci. Total Environ. 812 https://doi.org/10.1016/j.scitotenv.2021.152543.

Escudero, J., Muñoz, J.L., Morera-Herreras, T., Hernandez, R., Medrano, J., Domingo-Echaburu, S., Barceló, D., Orive, G., Lertxundi, U., 2021. Antipsychotics as environmental pollutants: an underrated threat? Sci. Total Environ. 769, 144634 https://doi.org/10.1016/j.scitotenv.2020.144634.

European Commission, 2022. Decision (EU) 2022/1307 of 22 July 2022 Establishing a Watch List of Substances for Union-wide Monitoring in the Field of Water Policy Pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union.

European Parliament and Council, 2013. Directives of 12 August 2013 Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy. Official Journal of the European Union.
Fabbri, E., 2015. Pharmaceuticals in the environment: expected and unexpected effects on aquatic fauna. Ann. N. Y. Acad. Sci. 1340, 20–28. https://doi.org/10.1111/nyas.12605.
Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: focus on exposure and biological effects in animal species. Environ. Toxicol. Chem. 35, 799–812. https://doi.org/10.1002/etc.3131.
Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal. Bioanal. Chem. 399, 251–275. https://doi.org/10.1007/s00216-010-4300-9.
Fernández-Rubio, J., Rodríguez-Gil, J.L., Postigo, C., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2019. Psychoactive pharmaceuticals and illicit drugs in coastal waters of North-Western Spain: environmental exposure and risk assessment. Chemosphere 224, 379–389. https://doi.org/10.1016/j.chemosphere.2019.02.041.
Fick, J., Söderström, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D.G.J., 2009. Contamination of surface, ground and drinking water from pharmaceutical production. Environ. Toxicol. Chem. 28, 2522. https://doi.org/10.1897/09-073.1.
Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Larsson, D.G.J., 2010. Therapeutic levels of levonorgestrel detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents. Environ. Sci. Technol. 44, 2661–2666. https://doi.org/10.1021/es903440m.
Fick, J., Lindberg, R.H., Kaj, L., Brorström-Lundén, E., 2011. Results from the Swedish National Screening Programme 2010: Subreport 3. Pharmaceuticals.Pubmed Partial Author stitle stitle Volume Page.
Fick, J., Brodin, T., Heynen, M., Klaminder, J., Jonsson, M., Grabicova, K., Randak, T., Grabic, R., Kodes, V., Slobodnik, J., Sweetman, A., Earnshaw, M., Barra Caracciolo, A., Lettieri, T., Loos, R., 2017. Screening of benzodiazepines in thirty European rivers. Chemosphere 176, 324–332. https://doi.org/10.1016/j.chemosphere.2017.02.126.
Fonseca, V.F., Reis-Santos, P., 2019. Ecotoxicology of pharmaceuticals in coastal and marine organisms. In: Ecotoxicology of Marine Organisms. CRC Press, Taylor & Francis Group, Boca Raton, pp. 158–184. https://doi.org/10.1201/b22000-7. A science publishers book.».
Fonseca, V.F., Duarte, I.A., Duarte, B., Freitas, A., Pouca, A.S.V., Barbosa, J., Gillanders, B.M., Reis-Santos, P., 2021. Environmental risk assessment and bioaccumulation of pharmaceuticals in a large urbanized estuary. Sci. Total Environ. 783 https://doi.org/10.1016/j.scitotenv.2021.147021, 147021.
Fork, M.L., Fick, J.B., Reisinger, A.J., Rosi, E.J., 2021. Dosing the coast: leaking sewage infrastructure delivers large annual doses and dynamic mixtures of pharmaceuticals to urban rivers. Environ. Sci. Technol. 55, 11637–11645. https://doi.org/10.1021/acs.est.1c00379.
França, S., Costa, M.J., Cabral, H.N., 2009. Assessing habitat specific fish assemblages in estuaries along the Portuguese coast. Estuar. Coast Shelf Sci. 83, 1–12. https://doi.org/10.1016/j.ecss.2009.03.013.
Franco, A., Elliott, M., Franzoi, P., Torricelli, P., 2008. Life strategies of fishes in European estuaries: the functional guild approach. Mar. Ecol. Prog. Ser. 354, 219–228. https://doi.org/10.3354/meps07203.
Gaw, S., Thomas, K.V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. Trans. R. Soc. B Biol. Sci. 369, 20130572 https://doi.org/10.1098/rstb.2013.0572.
Grabic, R., Fick, J., Lindberg, R.H., Fedorova, G., Tysklind, M., 2012. Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry. Talanta 100, 183–195. https://doi.org/10.1016/j.talanta.2012.08.032.
Grabicova, K., Grabic, R., Fedorova, G., Fick, J., Cerveny, D., Kolarova, J., Turek, J., Zlabek, V., Randak, T., 2017. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Res. 124, 654–662. https://doi.org/10.1016/j.watres.2017.08.018.
Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Larsson, D.G.J., 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ. Sci. Technol. 42, 5807–5813. https://doi.org/10.1021/es8005173.
Hamilton, P.B., Cowx, I.G., Oleksiak, M.F., Griffiths, A.M., Grahn, M., Stevens, J.R., Carvalho, G.R., Nicol, E., Tyler, C.R., 2016. Population-level consequences for wild fish exposed to sublethal concentrations of chemicals - a critical review. Fish Fish. 17, 545–566. https://doi.org/10.1111/faf.12125.
Hubená, P., Horký, P., Grabic, R., Grabicová, K., Slavík, O., Randák, T., 2020. Environmentally relevant levels of four psychoactive compounds vary in their effects on freshwater fish condition: a brain concentration evidence approach. PeerJ 8, e9356. https://doi.org/10.7717/peerj.9356.
Huerta, B., Margiotta-Casaluci, L., Rodríguez-Mozaz, S., Scholze, M., Winter, M.J., Barceló, D., Sumpter, J.P., 2016. Anti-anxiety drugs and fish behavior: establishing the link between internal concentrations of oxazepam and behavioral effects. Environ. Toxicol. Chem. 35, 2782–2790. https://doi.org/10.1002/etc.3448.
Huerta, B., Rodriguez-Mozaz, S., Lazorchak, J., Barcelo, D., Batt, A., Wathen, J., Stahl, L., 2018. Presence of pharmaceuticals in fish collected from urban rivers in the U.S. EPA 2008–2009 National Rivers and Streams Assessment. Sci. Total Environ. 634, 542–549. https://doi.org/10.1016/j.scitotenv.2018.03.387.
INFARMED, 2018. Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.
Kondor, A.C., Jakab, G., Vancsik, A., Filep, T., Szeberényi, J., Szabó, L., Maász, G., Ferincz, Á., Dobosy, P., Szalai, Z., 2020. Occurrence of pharmaceuticals in the Danube and drinking water wells: efficiency of riverbank filtration. Environ. Pollut. (Amsterdam, Neth.) 265, 114893. https://doi.org/10.1016/j.envpol.2020.114893.
Kosjek, T., Perko, S., Zupanc, M., Zanoški Hren, M., Landeka Dragičević, T., Žigon, D., Kompare, B., Heath, E., 2012. Environmental occurrence, fate and transformation of benzodiazepines in water treatment. Water Res. 46, 355–368. https://doi.org/10.1016/j.watres.2011.10.056.
Ladu, F., Mwaffo, V., Li, J., Macrì, S., Porfiri, M., 2015. Acute caffeine administration affects zebrafish response to a robotic stimulus. Behav. Brain Res. 289, 48–54. https://doi.org/10.1016/j.bbr.2015.04.020.
Lahti, M., Brozinski, J.-M., Jylhä, A., Kronberg, L., Oikari, A., 2011. Uptake from water, biotransformation, and biliary excretion of pharmaceuticals by rainbow trout. Environ. Toxicol. Chem. 30, 1403–1411. https://doi.org/10.1002/etc.501.
Larsson, D.G.J., 2014. Pollution from drug manufacturing: review and perspectives. Philos. Trans. R. Soc. B Biol. Sci. 369, 20130571 https://doi.org/10.1098/rstb.2013.0571.
Letsinger, S., Kay, P., Rodríguez-Mozaz, S., Villagrassa, M., Barceló, D., Rotchell, J.M., 2019. Spatial and temporal occurrence of pharmaceuticals in UK estuaries. Sci. Total Environ. 678, 74–84. https://doi.org/10.1016/j.scitotenv.2019.04.182.
Li, Z., Lu, G., Yang, X., Wang, C., 2012. Single and combined effects of selected pharmaceuticals at sublethal concentrations on multiple biomarkers in Carassius auratus. Ecotoxicology 21, 353–361. https://doi.org/10.1007/s10646-011-0796-9.
Li, S., He, B., Wang, J., Liu, J., Hu, X., 2020. Risks of caffeine residues in the environment: necessity for a targeted ecopharmacovigilance program. Chemosphere 243, 125343. https://doi.org/10.1016/j.chemosphere.2019.125343.
Liu, J., Lu, G., Xie, Z., Zhang, Z., Li, S., Yan, Z., 2015. Occurrence, bioaccumulation and risk assessment of lipophilic pharmaceutically active compounds in the downstream rivers of sewage treatment plants. Sci. Total Environ. 511, 54–62. https://doi.org/10.1016/j.scitotenv.2014.12.033.
Liu, J., Dan, X., Lu, G., Shen, J., Wu, D., Yan, Z., 2018. Investigation of pharmaceutically active compounds in an urban receiving water: occurrence, fate and environmental risk assessment. Ecotoxicol. Environ. Saf. 154, 214–220. https://doi.org/10.1016/j.ecoenv.2018.02.052.
Liu, Y.-H., Lv, Y.-Z., Huang, Z., Guan, Y.-F., Huang, J.-W., Zhao, J.-L., Ying, G.-G., 2021. Uptake, elimination, and toxicokinetics of selected pharmaceuticals in multiple tissues of Nile tilapia (Oreochromis niloticus) exposed to environmentally relevant concentrations. Ecotoxicol. Environ. Saf. 226, 112874 https://doi.org/10.1016/j.ecoenv.2021.112874.
Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47, 6475–6487. https://doi.org/10.1016/j.watres.2013.08.024.
Mackay, D., 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. 16, 274–278. https://doi.org/10.1021/es00099a008.
Martínez, M.L., Intralawan, A., Vázquez, G., Pérez-Maqueo, O., Sutton, P., Landgrave, R., 2007. The coasts of our world: ecological, economic and social importance. Ecol. Econ. 63, 254–272. https://doi.org/10.1016/j.ecolecon.2006.10.022.
Martínez-Morcillo, S., Rodríguez-Gil, J.L., Fernández-Rubio, J., Rodríguez-Mozaz, S., Míguez-Santiyán, M.P., Valdes, M.E., Barceló, D., Valcárcel, Y., 2020. Presence of pharmaceutical compounds, levels of biochemical biomarkers in seafood tissues and risk assessment for human health: results from a case study in North-Western Spain. Int. J. Hyg Environ. Health 223, 10–21. https://doi.org/10.1016/j.ijheh.2019.10.011.
Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015a. Pharmaceutical residues in water and sediment of Msunduzi river, KwaZulu-natal, South Africa. Chemosphere 134, 133–140. https://doi.org/10.1016/j.chemosphere.2015.03.093.
Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015b. Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. Environ. Sci. Pollut. Res. 22, 10298–10308. https://doi.org/10.1007/s11356-015-4217-0.
McCallum, E.S., Krutzelmann, E., Brodin, T., Fick, J., Sundelin, A., Balshine, S., 2017. Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals. Sci. Total Environ. 605 (606), 578–588. https://doi.org/10.1016/j.scitotenv.2017.06.073.
McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. Sci. Total Environ. 476– 477, 317–326. https://doi.org/10.1016/j.scitotenv.2013.12.123.
Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of emerging concern in a large temperate estuary. Environ. Pollut. (Amsterdam, Neth.) 213, 254–267. https://doi.org/10.1016/j.envpol.2016.01.088.
Melvin, S.D., Wilson, S.P., 2013. The utility of behavioral studies for aquatic toxicology testing: a meta-analysis. Chemosphere 93, 2217–2223. https://doi.org/10.1016/j.chemosphere.2013.07.036.
Mezzelani, M., Gorbi, S., Regoli, F., 2018. Pharmaceuticals in the aquatic environments: evidence of emerged threat and future challenges for marine organisms. Mar. Environ. Res. 140, 41–60. https://doi.org/10.1016/j.marenvres.2018.05.001.
Miller, T.H., Bury, N.R., Owen, S.F., MacRae, J.I., Barron, L.P., 2018. A review of the pharmaceutical exposome in aquatic fauna. Environ. Pollut. (Amsterdam, Neth.) 239, 129–146. https://doi.org/10.1016/j.envpol.2018.04.012.
Muir, D., Simmons, D., Wang, X., Peart, T., Villella, M., Miller, J., Sherry, J., 2017. Bioaccumulation of pharmaceuticals and personal care product chemicals in fish exposed to wastewater effluent in an urban wetland. Sci. Rep. 7, 16999 https://doi.org/10.1038/s41598-017-15462-x.
Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85, 50–59. https://doi.org/10.1016/j.marpolbul.2014.06.024.

OECD, 2019. Addressing Problematic Opioid Use in OECD Countries. OECD Publishing, Paris. https://doi.org/10.1787/a18286f0-en.
Ojemaye, C.Y., Petrik, L., 2019. Pharmaceuticals in the marine environment: a review. Environ. Rev. 27, 151–165. https://doi.org/10.1139/er-2018-0054.
Ondarza, P.M., Haddad, S.P., Avigliano, E., Miglioranza, K.S.B., Brooks, B.W., 2019. Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: implications for protected areas influenced by urbanization. Sci. Total Environ. 649, 1029–1037. https://doi.org/10.1016/j.scitotenv.2018.08.383.
Osorio, V., Larrañaga, A., Aceña, J., Pérez, S., Barceló, D., 2016. Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers. Sci. Total Environ. 540, 267–277. https://doi.org/10.1016/j.scitotenv.2015.06.143.
Paíga, P., Correia, M., Fernandes, M.J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J.G., Freire, C., Delerue-Matos, C., 2019. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: hourly variation. Sci. Total Environ. 648, 582–600. https://doi.org/10.1016/j.scitotenv.2018.08.129.
Peña-Herrera, J.M., Montemurro, N., Barceló, D., Pérez, S., 2020. Analysis of pharmaceuticals in fish using ultrasound extraction and dispersive spe clean-up on que Z-Sep/C18 followed by LC-QToF-MS detection. MethodsX 7, 101010. https://doi.org/10.1016/j.mex.2020.101010.
Pereira, T.J., Silva, G., Costa, M.J., Costa, J.L., 2011. Life strategies of Halobatrachus didactylus (Bloch and Schneider, 1801) in the Tagus estuary: comparison among different morphotypes. Estuar. Coast Shelf Sci. 93, 328–335. https://doi.org/10.1016/j.ecss.2011.04.013.
Pereira, A.M.P.T., Silva, L.J.G., Lino, C.M., Meisel, L.M., Pena, A., 2016. Assessing environmental risk of pharmaceuticals in Portugal: an approach for the selection of the Portuguese monitoring stations in line with Directive 2013/39/EU. Chemosphere 144, 2507–2515. https://doi.org/10.1016/j.chemosphere.2015.10.100.
Pivetta, R.C., Rodrigues-Silva, C., Ribeiro, A.R., Rath, S., 2020. Tracking the occurrence of psychotropic pharmaceuticals in Brazilian wastewater treatment plants and surface water, with assessment of environmental risks. Sci. Total Environ. 727, 138661 https://doi.org/10.1016/j.scitotenv.2020.138661.
Prista, N., Vasconcelos, R.P., Costa, M.J., Cabral, H., 2003. The demersal fish assemblage of the coastal area adjacent to the Tagus estuary (Portugal): relationships with environmental conditions. Oceanol. Acta 26, 525–536. https://doi.org/10.1016/S0399-1784(03)00047-1.
Quadra, G.R., Paranaíba, J.R., Vilas-Boas, J., Roland, F., Amado, A.M., Barros, N., Dias, R.J.P., Cardoso, S.J., 2020. A global trend of caffeine consumption over time and related-environmental impacts. Environ. Pollut. (Amsterdam, Neth.) 256, 2016–2021. https://doi.org/10.1016/j.envpol.2019.113343.
Ramirez, A.J., Brain, R.A., Usenko, S., Mottaleb, M.A., O'Donnell, J.G., Stahl, L.L., Wathen, J.B., Snyder, B.D., Pitt, J.L., Perez-Hurtado, P., Dobbins, L.L., Brooks, B.W., Chambliss, C.K., 2009. Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. Environ. Toxicol. Chem. 28, 2587. https://doi.org/10.1897/08-561.1.
Reis-Santos, P., Pais, M., Duarte, B., Caçador, I., Freitas, A., Vila Pouca, A.S., Barbosa, J., Leston, S., Rosa, J., Ramos, F., Cabral, H.N., Gillanders, B.M., Fonseca, V.F., 2018. Screening of human and veterinary pharmaceuticals in estuarine waters: a baseline assessment for the Tejo estuary. Mar. Pollut. Bull. 135, 1079–1084. https://doi.org/10.1016/j.marpolbul.2018.08.036.
Renganathan, J., S, I.U.H., Ramakrishnan, K., Ravichandran, M.K., Philip, L., 2021. Spatio-temporal distribution of pharmaceutically active compounds in the River Cauvery and its tributaries, South India. Sci. Total Environ. 800, 149340 https://doi.org/10.1016/j.scitotenv.2021.149340.
Rojo, M., Álvarez-Muñoz, D., Dománico, A., Foti, R., Rodriguez-Mozaz, S., Barceló, D., Carriquiriborde, P., 2019. Human pharmaceuticals in three major fish species from the Uruguay River (South America) with different feeding habits. Environ. Pollut. (Amsterdam, Neth.) 252, 146–154. https://doi.org/10.1016/j.envpol.2019.05.099.
Santos, M.N., Monteiro, C.C., Erzini, K., Lasserre, Gerard, 1998. Maturation and gill-net selectivity of two small sea breams (genus Diplodus) from the Algarve coast (south Portugal). Fish. Res. 36, 185–194. https://doi.org/10.1016/S0165-7836(98)00100-3.
Santos-Silva, T.G., Montagner, C.C., Martinez, C.B.R., 2018. Evaluation of caffeine effects on biochemical and genotoxic biomarkers in the neotropical freshwater teleost Prochilodus lineatus. Environ. Toxicol. Pharmacol. 58, 237–242. https://doi.org/10.1016/j.etap.2018.02.002.
Schultz, M.M., Furlong, E.T., Kolpin, D.W., Werner, S.L., Schoenfuss, H.L., Barber, L.B., Blazer, V.S., Norris, D.O., Vajda, A.M., 2010. Antidepressant pharmaceuticals in two U.S. Effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. Environ. Sci. Technol. 44, 1918–1925. https://doi.org/10.1021/es9022706.
Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., Faggio, C., 2018. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: a review. Sci. Total Environ. 631–632, 789–794. https://doi.org/10.1016/j.scitotenv.2018.03.076.
Silva, L.J.G., Pereira, A.M.P.T., Meisel, L.M., Lino, C.M., Pena, A., 2015. Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: uptake, bioaccumulation and ecotoxicology. Environ. Pollut. (Amsterdam, Neth.) 197, 127–143. https://doi.org/10.1016/j.envpol.2014.12.002.
Sousa, J.C.G., Barbosa, M.O., Ribeiro, A.R.L., Ratola, N., Pereira, M.F.R., Silva, A.M.T., 2020. Distribution of micropollutants in estuarine and sea water along the Portuguese coast. Mar. Pollut. Bull. 154, 111120 https://doi.org/10.1016/j.marpolbul.2020.111120.
Świacka, K., Maculewicz, J., Kowalska, D., Caban, M., Smolarz, K., Świeżak, J., 2022. Presence of pharmaceuticals and their metabolites in wild-living aquatic organisms – current state of knowledge. J. Hazard Mater. 424, 127350 https://doi.org/10.1016/j.jhazmat.2021.127350.
Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J.-W., Isobe, T., Shinohara, R., Kunisue, T., Tanabe, S., 2015. Uptake and tissue distribution of pharmaceuticals and personal care products in wild fish from treated-wastewater-impacted streams. Environ. Sci. Technol. 49, 11649–11658. https://doi.org/10.1021/acs.est.5b02478.
Tanoue, R., Margiotta-casaluci, L., Huerta, B., Runnalls, T.J., Nomiyama, K., Kunisue, T., Tanabe, S., Sumpter, J.P., 2017. Uptake and Metabolism of Human Pharmaceuticals by Fish: A Case Study with the Opioid Analgesic Tramadol 12825–12835. https://doi.org/10.1021/acs.est.7b03441.
Togola, A., Budzinski, H., 2008. Multi-residue analysis of pharmaceutical compounds in aqueous samples. J. Chromatogr. A 1177, 150–158. https://doi.org/10.1016/j.chroma.2007.10.105.
UNESCO and HELCOM, 2017. Pharmaceuticals in the aquatic environment of the Baltic Sea region - A status report. In: UNESCO Emerging Pollutants in Water Series - No1. UNESCO Publishing, Paris.
Valdés, M.E., Huerta, B., Wunderlin, D.A., Bistoni, M.A., Barceló, D., Rodriguez-Mozaz, S., 2016. Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals in fish under field and controlled laboratory experiments. Evidences of carbamazepine metabolization by fish. Sci. Total Environ. 557 (558), 58–67. https://doi.org/10.1016/j.scitotenv.2016.03.045.
Vieira, L.R., Soares, A.M.V.M., Freitas, R., 2022. Caffeine as a contaminant of concern: a review on concentrations and impacts in marine coastal systems. Chemosphere 286, 131675. https://doi.org/10.1016/j.chemosphere.2021.131675.
Wang, J., Gardinali, P.R., 2013. Uptake and depuration of pharmaceuticals in reclaimed water by mosquito fish (Gambusia holbrooki): a worst-case, multiple-exposure scenario. Environ. Toxicol. Chem. 32, 1752–1758. https://doi.org/10.1002/etc.2238.
Wang, C., Hou, L., Li, J., Xu, Z., Gao, T., Yang, J., Zhang, H., Li, X., Du, P., 2017. Occurrence of diazepam and its metabolites in wastewater and surface waters in Beijing. Environ. Sci. Pollut. Res. 24, 15379–15389. https://doi.org/10.1007/s11356-017-8922-8.
Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M., Luarte, T., Petrosyan, V., Perikhanyan, Y., Mahon, C.S., McGurk, C.J., Hofmann, T., Kormoker, T., Iniguez, V., Guzman-Otazo, J., Tavares, J. L., Gildasio De Figueiredo, F., Razzolini, M.T.P., Dougnon, V., Gbaguidi, G., Traoré, O., Blais, J.M., Kimpe, L.E., Wong, M., Wong, D., Ntchantcho, R., Pizarro, J., Ying, G.-G., Chen, C.-E., Páez, M., Martínez-Lara, J., Otamonga, J.-P., Poté, J., Ifo, S. A., Wilson, P., Echeverría-Sáenz, S., Udikovic-Kolic, N., Milakovic, M., Fatta-Kassinos, D., Ioannou-Ttofa, L., Belušová, V., Vymazal, J., Cárdenas-Bustamante, M., Kassa, B.A., Garric, J., Chaumot, A., Gibba, P., Kunchulia, I., Seidensticker, S., Lyberatos, G., Halldórsson, H.P., Melling, M., Shashidhar, T., Lamba, M., Nastiti, A., Supriatin, A., Pourang, N., Abedini, A., Abdullah, O., Gharbia, S.S., Pilla, F., Chefetz, B., Topaz, T., Yao, K.M., Aubakirova, B., Beisenova, R., Olaka, L., Mulu, J. K., Chatanga, P., Ntuli, V., Blama, N.T., Sherif, S., Aris, A.Z., Looi, L.J., Niang, M., Traore, S.T., Oldenkamp, R., Ogunbanwo, O., Ashfaq, M., Iqbal, M., Abdeen, Z., O'Dea, A., Morales-Saldaña, J.M., Custodio, M., de la Cruz, H., Navarrete, I., Carvalho, F., Gogra, A.B., Koroma, B.M., Cerkvenik-Flajs, V., Gombač, M., Thwala, M., Choi, K., Kang, H., Ladu, J.L.C., Rico, A., Amerasinghe, P., Sobek, A., Horlitz, G., Zenker, A.K., King, A.C., Jiang, J.-J., Kariuki, R., Tumbo, M., Tezel, U., Onay, T.T., Lejju, J.B., Vystavna, Y., Vergeles, Y., Heinzen, H., Pérez-Parada, A., Sims, D.B., Figy, M., Good, D., Teta, C., 2022. Pharmaceutical pollution of the world's rivers. Proc. Natl. Acad. Sci. USA 119, 1–10. https://doi.org/10.1073/pnas.2113947119.
Yang, H., Lu, G., Yan, Z., Liu, J., Dong, H., Bao, X., Zhang, X., Sun, Y., 2020. Residues, bioaccumulation, and trophic transfer of pharmaceuticals and personal care products in highly urbanized rivers affected by water diversion. J. Hazard Mater. 391, 122245 https://doi.org/10.1016/j.jhazmat.2020.122245.
Zhang, Y., Geißen, S.-U., Gal, C., 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere 73, 1151–1161. https://doi.org/10.1016/j.chemosphere.2008.07.086.
Zhou, S., Di Paolo, C., Wu, X., Shao, Y., Seiler, T.-B., Hollert, H., 2019. Optimization of screening-level risk assessment and priority selection of emerging pollutants – the case of pharmaceuticals in European surface waters. Environ. Int. 128, 1–10. https://doi.org/10.1016/j.envint.2019.04.034.