EREM 79/4
Journal of Environmental Research, Engineering and Management
Vol. 79 / No. 4 / 2023
pp. 86–101
DOI 10.5755/j01.erem.79.4.34064

Pharmaceuticals in Municipal Wastewater – Two Case Studies of Uptake in Fish and Crayfish (*Pacifastacus leniusculus*) in Aquaria Experiment and In-field Sampling

Received 2023/05 | Accepted after revisions 2023/08

https://doi.org/10.5755/j01.erem.79.4.34064

Pharmaceuticals in Municipal Wastewater – Two Case Studies of Uptake in Fish and Crayfish (*Pacifastacus leniusculus*) in Aquaria Experiment and In-field Sampling

Hannes Waldetoft*, Bahare Esfahani, Tomas Viktor, O. Magnus Karlsson

IVL Swedish Environmental Research Institute, Sweden

***Corresponding author:** hannes.waldetoft@ivl.se

In the receiving areas of effluents from municipal wastewater treatment plants (WWTPs), aquatic organisms are threatened by adverse effects due to exposure to pharmaceutical residues. To elucidate the uptake of pharmaceuticals in fish, measurements were made in bile of brown trout (*Salmo trutta*) exposed in aquaria to 100% effluent water and in muscle, liver, kidney, and bile in northern pike (*Esox lucius*), European perch (*Perca fluviatilis*) and common rudd (*Scardinius erythrophthalmus*) from a lake receiving municipal wastewater. Pharmaceuticals were also measured in hepatopancreas of signal crayfish (*Pacifastacus leniusculus*). In addition to the measurements in fish and crayfish, pharmaceuticals were measured in the effluent, upstream and downstream of the WWTPs. In effluent water, pharmaceuticals were detected in the µg/L range, with the highest concentrations being of commonly prescribed NSAIDs and hypertension drugs, such as diclofenac, ibuprofen, naproxen, losartan, and metoprolol. However, the differences in concentrations between different sampling occasions were high, indicating a need for repeated sampling to obtain representative average concentrations. Pharmaceuticals in fish samples showed strong tendencies to species and tissue-specific partitioning. Levels of diclofenac in the brown trout bile were within the range of 4–16 µg/g w.w and naproxen within 37–170 ng/g w.w, while for all other pharmaceuticals, they were below detection limits. Several other pharmaceuticals were present in a similar order of magnitude in the effluent as diclofenac, suggesting diclofenac has a strong partitioning to trout bile. In the wild fish, the highest number of detected pharmaceuticals and the highest levels were observed in kidney of pike. Diclofenac in pike kidney was at most 37 ng/g w.w, followed by propranolol (20 ng/g w.w) and losartan (18 ng/g w.w). In crayfish, no pharmaceuticals were detected. The results suggest that the kidney of pike is a suitable tissue for evaluating pharmaceuticals in fish, while hepatopancreas of signal crayfish is not.

Keywords: pharmaceuticals, fish, crayfish, wastewater.

Introduction

The widespread use of pharmaceuticals and their modest removal efficiencies in wastewater treatment plants (WWTPs) lead to pharmaceuticals being found in surface waters of effluent-receiving streams, lakes, and coastal areas (Fernández-Rubio et al., 2019; Fick et al., 2017; Moreno-González et al., 2015; Tran et al., 2018; Vieno et al., 2007), but also in offshore waters (Björlenius et al., 2018). Concentrations of pharmaceuticals in municipal effluent waters are often in the range of no-detection to a few µg/L, with representation from different therapeutic groups, such as non-steroidal anti-inflammatory drugs (NSAID), antibiotics, selective serotonin reuptake inhibitors (SSRI), beta-blockers, and antifungal pharmaceuticals. Water concentrations in the effluent-receiving areas vary greatly, but concentrations in areas with low dilution are generally higher (Malnes et al., 2022). Concentrations in receiving waters also correlate to the consumption volumes and wastewater treatment technologies (Fatta-Kassinos et al., 2011; Yang et al., 2017), the environmental characteristics of the receiving areas, and the seasonal variations. In addition to pharmaceuticals, other pollutants, such as estrogenic hormones, personal care products, and per- and polyfluoroalkyl substances (PFAS), are not catabolized in WWTPs (Emmanouil et al., 2019; Lenka et al., 2021; Schröder et al., 2016), leading to effluents containing several different pollutants, collectively referred to as micropollutants.

In fish inhabiting WWTP effluent-impacted streams, pharmaceuticals are often found (Brooks et al., 2005; Gelsleichter and Szabo, 2013; Huerta et al., 2012; Schultz et al., 2010), with typical levels ranging between non-detection and low ng/g. Levels at detected quantities have also been found in fish from coastal areas (Álvarez-Muñoz et al., 2015; Liu et al., 2018) and offshore locations (Vieno et al., 2017).

Different types of pharmaceuticals also bioaccumulate differently in different fish tissues, e.g., muscle, liver, blood, brain, and kidney. For example, fish exposed to 100% WWTP effluent water was shown to have quantifiable levels of antidepressants more often in brain and liver compared with muscle and blood plasma (Grabicova et al., 2014). The same pattern with higher concentrations of selective serotonin reuptake inhibitors (SSRI) in liver and brain compared with muscle was also seen in wild fish (Brooks et al., 2005). The opposite pattern was, however, noted for the antihypertension drug diltiazem (Ramirez et al., 2007). In general, how pharmaceuticals bioaccumulate in different species and tissues is poorly understood (Cerveny et al., 2021).

Although concentrations often are low, pharmaceuticals are potent at low concentrations, and fish have, to a great extent, similar receptors and enzymes as humans, which leads to risks of adverse effects for these organisms (Brown et al., 2014; Gunnarsson et al., 2008).

Studies regarding pharmaceuticals in receiving areas often do not use repeated sampling (Hughes et al., 2013), and thus, research assessing temporal variation in pharmaceutical concentrations is scarce. Furthermore, the research has shown contrasting results, with findings of seasonal patterns (Lindholm-Lehto et al., 2016) and no findings of seasonal patterns (Kay et al., 2017).

In this study, measurements in biota are of pike (*Esox Lucius*), perch (*Perca fluviatilis*), common rudd (*Scardinius erythrophthalmus*), and signal crayfish (*Pacifastacus leniusculus*). Concerning fish and crayfish, measurements are biased toward certain species. In pike, measurements have only been made in plasma (Larabie et al., 2017). No measurements of pharmaceuticals have been made in signal crayfish and common rudd. In brown trout (*Salmo trutta*), no exposure studies have been performed to assess pharmaceutical uptake in bile. However, a related species, the rainbow trout, has been studied regarding pharmaceutical uptake in bile and plasma (Lahti et al., 2011). Pharmaceuticals in perch (*Perca fluviatilis*) have been measured previously (Björlenius et al., 2018; Vieno et al., 2017).

Given these research gaps, the aim of this work was to (1) investigate which pharmaceutical substances found in water are also found in different tissues of pike, perch, common rudd, brown trout, and signal crayfish, and (2) assess the temporal variability of pharmaceutical concentrations in both water and biota.

Large investments are being made worldwide to improve wastewater treatment techniques so that the load of micropollutants to aquatic environments can be reduced. Such remediation actions are costly to implement and often imply additional greenhouse gas emissions and a higher resource intensity (Pistocchi et al., 2022). To increase the cost-efficiency and optimize the environmental benefit, it is important to increase the knowledge about pharmaceuticals and other micropollutants associated with emissions from WWTPs.

Methods

Localities and sampling – Fors WWTP

Fors WWTP is situated in the municipality of Haninge, one of the southernmost suburbs of Stockholm (*Fig. 1*). It receives sewage water at approximately 15 000 population equivalents, and the treatment consists of conventional, mechanical, biological (active-sludge and denitrification), and chemical treatment. Processed water is released into a small stream (average flow 4.5 m^3/s) called Vitsån Creek. The proportion of effluent water from Fors WWTP was about 50% of the net flow in Vitsån Creek in late August, 7% in December, and 20% in April. Vitsån holds, at least since the 1990s, a breeding population of brown trout (*Salmo trutta*) despite the prevalence of migration barriers and high ammonia concentrations (Waltersson and Kjellberg, 1997).

Water samples were taken with a clean plastic bucket and transferred to a plastic container. They were delivered to IVL Swedish Environmental Research Institute's laboratory in Stockholm, where they were stored in a freezer (–20°C) before analysis. Sampling points were upstream of the WWTP, in the treated effluent water, and downstream in Vitsån Creek (*Fig. 1*). Water samples were analyzed for 47 pharmaceuticals, of which 17 are antibiotics. All water samples were grab samples.

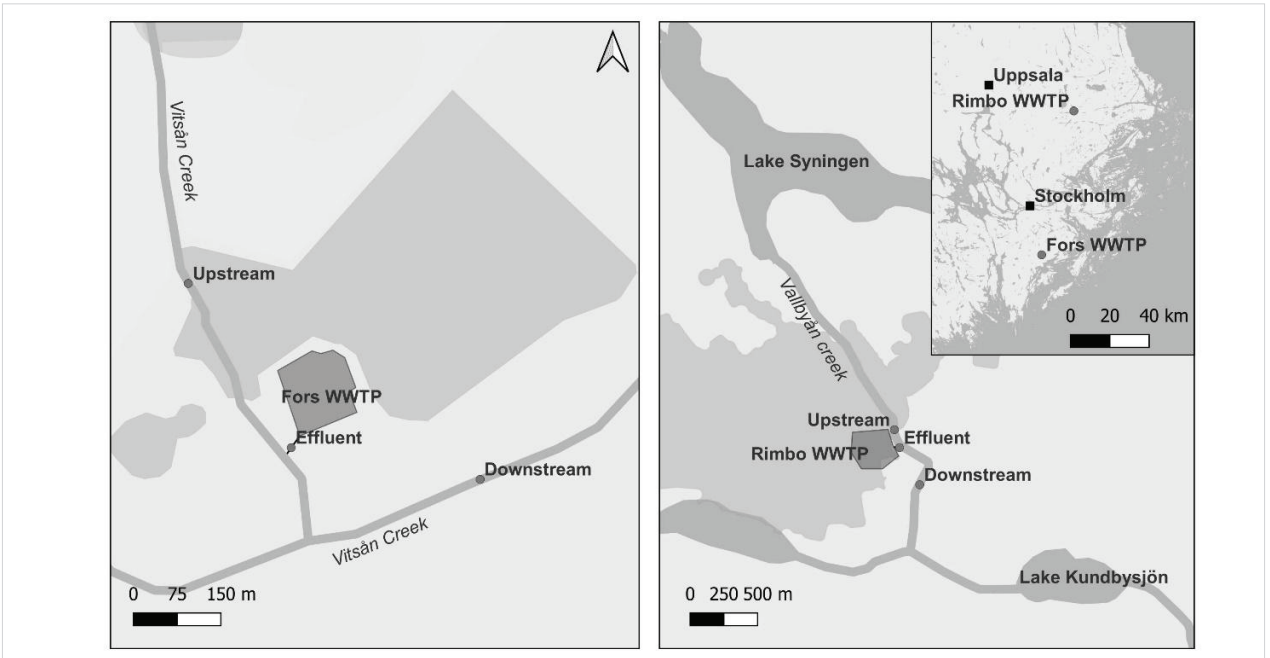
Experimental setup of the fish experiment at Fors WWTP

The experiment was carried out in the fall of 2020 using a mobile experimental facility previously used in Pohl et al. (2018). Two-year-old brown trout from the population inhabiting the stream were purchased from the hatchery used to maintain the trout population in the area. Upon arrival, the trout were transferred to two different 1000 L tanks with a flow of carbon-filtered municipal drinking water for two-week acclimatization. The fish were fed with 3 mm salmon feed pellets.

After the acclimatization, the fish were placed in 3 glass aquaria of 50 L each. Each aquarium contained seven fish. WWTP effluent water was pumped into the experimental facility and, via a 50-L barrel through gravity drainage, to the aquariums. The flow rate was set to 17 L/h per aquarium. The exposure lasted for 28 days, and the fish were inspected and fed daily. In addition, flow rates and oxygen levels were controlled.

After the exposure time, the fish were transferred, without leaving the water, to a container with the same treatment water as in the aquarium. A tranquilizer, MS 222, was added, and after approximately two minutes,

Fig. 1. *Area map with upstream, downstream, and 100% effluent sampling points*



when the tranquilizer had numbed the fish, they were taken out and inspected visually for visible damages. Then, the fish was killed with a blow to the head, and bile samples were taken and frozen (–20°C).

Localities and sampling – Rimbo WWTP

Rimbo WWTP is located in the municipality of Norrtälje. It receives sewage water from about 5000 population equivalents, but also from a large-scale washing facility washing work clothes and other textiles for hospitals in the region, equivalent to approximately 1000 population equivalents. When discharged into Vallbyån Creek, the effluent water accounts for approximately 4% of the creek's net flow at average flow rates, and approximately 10% at minimum flow rates. Vallbyån Creek is regulated, with a minimum allowed flow rate of 0.2 m^3/s during summer and winter. In fall and spring, the maximum net flow is approximately 1 m^3/s. The receiving lake, Lake Kundbysjön, is a small, shallow, and nutrient-rich lake with an average depth of 1 m, a surface area of 0.25 km^2, and a turnover rate of 4 days. It is dense in macrophytes and is surrounded mainly by wetlands and arable lands. Lake Syningen, used as a reference lake, has a surface area of 1.2 km^2, an average depth of 2.5 m, and a turnover rate of 57 days.

Water samples from the upstream, downstream, Lake Syningen, and Lake Kundby sites were taken with 1 L plastic bottles. Samples in effluent water were taken consecutively for five days with a passive sampler before a pooled sample weighted according to the net flow through the WWTP for each sampling day was made. Sampling was made monthly, except for August and January, starting in December 2021 and ending in September 2022. Not all sampling points were analyzed at the same time. The whole sampling scheme is found in the **supplementary information**.

Fish were caught in Lake Syningen and Lake Kundbysjön in February 2022 and May 2022. From the fish caught in February, muscle, kidney, bile, and liver samples were prepared the day after the catch. The fish was stored in a fridge (+8°C), and the resulting samples in a freezer (–20°C) before analysis. From the fish caught in May, kidney samples were prepared, and the whole fish and samples were stored frozen.

Pike from both lakes and perch from Lake Kundbysjön were analyzed for pharmaceuticals in muscle, kidney, liver, and bile. Also, kidney of common rudd from Lake Kundbysjön was analyzed for pharmaceuticals.

Signal crayfish were caught in September 2022 using crayfish cages baited with pieces of common rudd, placed approximately 200 meters upstream of Rimbo WWTPs outlet and 200 meters downstream. The crayfish were kept alive, in water from Vallbyån, until the sampling one day later, when pooled samples of hepatopancreas from 4–5 individuals were prepared. The length and weight of the fish and crayfish are found in the **supplementary information**.

Chemical analysis

Pharmaceuticals were extracted from water samples (200–300 mL) with solid phase extraction (SPE) using a 200 mg Oasis Hydrophilic-Lipophilic-Balanced (HLB) column from Waters (Ireland). Prior to extraction, 50 mg of ethylenediaminetetraacetic acid (EDTA) was added to the sample together with 100 ng of internal standards. Filter-aid (4 g) was added to the column, which was washed with 6 mL of methanol (MeOH) and conditioned with 6 mL of milli-Q water (MQ) before the sample was applied. The column was washed again with 2 mL of MQ water after sample application. Analytes were eluted with 5 mL of MeOH followed by 5 mL of acetone. The extract was then evaporated to dryness under nitrogen stream and heat (40°C) and re-dissolved in 1 mL of MeOH: MQ (1:1) with 0.1% EDTA followed by sonication for 5 min.

Pharmaceuticals were extracted from 0.2 g trout bile with liquid-liquid extraction. The bile was dissolved in 1 mL of dichloromethane (DCM), and 100 ng of internal standards was added to the samples prior to extraction. A saturated sodium chloride salt solution (0.5 mL) was added to the samples, and the analytes were extracted twice with 4 mLof DCM. The samples were shaken for 15 min and centrifuged (HERMLE, Germany) for 5 min at 3000 rpm for each extraction. The DCM phase was transferred to a new test tube and evaporated to dryness under nitrogen stream and heat (40°C). The sample was redissolved in 1 mL of MeOH: MQ (1:1) with 0.1% EDTA and sonicated for 5 min.

Pharmaceuticals were extracted from 0.1–1 g of fish muscle with liquid extraction. Prior to extraction, the sample was homogenized with a mortar, and 100 ng of internal standard, together with 50 mg of EDTA, was added. The samples were extracted with 1.5 ml of MeOH:MQ (7:3) with 0.1% formic acid, followed by

two extractions with acetonitrile (ACN). The extracts of each sample were pooled together and evaporated to dryness under nitrogen stream and heat (40°C) and redissolved in 1 mL of MeOH: MQ (1:1) with 0.1% EDTA followed by sonication for 5 min.

Pharmaceuticals were extracted from 0.1g to 5 g of kidney or liver from fish and hepatopancreas from crayfish with liquid extraction. Prior to extraction, the sample was homogenized with a mortar, and 100 ng of internal standard, together with 50 mg of EDTA, was added. The sample was extracted twice with 5 mL of ACN and shaken for 1 h on a shake table (Edmund Bühler, Germany), followed by centrifugation at 3500 rpm for 10 min. The extracts of each sample were pooled together, and the fat was removed by extraction with hexane. The remaining ACN extract was evaporated to dryness under nitrogen stream and heat (40°C) and redissolved in 1 mL of MeOH: MQ (1:1) with 0.1% EDTA followed by sonication (Bandelin, Germany) for 5 min. Finally, all samples of water and fish were centrifuged for 5 min at 14 000 rpm, and the supernatant was transferred to vials for analysis on high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

Pharmaceuticals were analyzed on a binary liquid chromatography system (UFLC-HPLC) from Shimadzu with an autoinjector coupled to an API-4000 triple quadrupole mass spectrometer (MS/MS) from Sciex. Electrospray ionization (ESI) was utilized in both positive and negative modes. The analysis was performed in the multiple ion monitoring mode (MRM). Internal standards used for quantification included Carbamazepine-$^{13}C^{15}N$, Diclofenac-$^{13}C_6$, Atenolol-d_7, and Metoprolol-d_7. The chromatographic separation of pharmaceuticals was performed with a gradient elution program of 25 min on a C18 X-Bridge column (3.0 x 50 mm, 2.5 µm) from Waters (Ireland) at 35°C with a flow rate of 0.3 mL/min. The mobile phases A and B consisted of 0.1% acetic acid in Milli-Q (A) and methanol (B). The chromatographic program started with a linear gradient from 0–90% B for 10 min, followed by a 10 min plateau at 90% B before quickly returning to initial conditions with 100% A, which was held for 2 min.

Different chromatographic conditions were used for the analysis of tramadol on the same instrument. A biphenyl Core-shell column (3.0 x 100 mm, 2.6 µm, 100Å) from Phenomenex (California, USA) was used at 40°C with a flow rate of 0.5 mL/min. The mobile phases A and B consisted of Milli-Q water with 0.1% formic acid (A) and Methanol with 0.1% formic acid (B). The chromatographic program started with a linear gradient from 5–70% B under 5 min followed by a quick increase to 95%, B which was held for 4 min before returning to initial conditions of 5%, B which was held for 1 min.

Analyte concentrations in samples were quantified from an 8-point calibration curve (500, 200, 100, 50, 20, 10, 5, and 0 ng/mL) which was analyzed together with the samples. All sample values were blank subtracted and corrected for recovery, which was estimated by spiking a duplicate of a sample from each matrix.

Chemicals and reagents

Methanol, acetone, acetonitrile, dichloromethane, and hexane (all HPLC grade) were purchased from Rathburn Chemicals Ltd (Walkerburn, Scotland). Acetic acid (> 99.8%) was purchased from VWR (United Kingdom), formic acid (98%) was purchased from Merck (St Louise, USA), and ethylenediaminetetraacetic acid was purchased from Merck (Darmstadt, Germany). Filter aid was purchased from CDS (USA), and sodium chloride (high purity grade) from VWR (Ohio, USA). Water was purified with reverse osmosis, followed by additional filtration on a Millipack system from Merck (France). The standards and internal standards used (Carbamazepine-$^{13}C^{15}N$, Atenolol-d_7, Metoprolol-d_7, Diclofenac-$^{13}C_6$) were all analytical grade (> 98% purity) and were purchased from Sigma Aldrich and Merck.

Statistics

The association between pharmaceutical concentrations in water samples from the same time but different sampling points was evaluated via the coefficient of determination (R^2). The R^2 value is the proportion of the variation in one of the variables that can be explained by the other.

The purpose of providing R^2 values is to quantify to what degree the receiving lake or creek concentrations can be determined from the effluent concentrations. Note that the Pearson correlation coefficient is the square root of R^2.

The variability in pharmaceutical concentrations is also expressed as the coefficient of variation (CV), which is the standard deviation divided by the mean, expressed as a percentage.

Results and Discussion

Pharmaceuticals in water

Pharmaceuticals at Fors were present between < LOD and 12 µg/L in the effluent. All substances, except risperidone and simvastatin, were detected in the effluent in at least one of the three sampling occasions. The five pharmaceuticals with the highest average concentrations in the effluent were, in descending order: losartan, metoprolol, furosemide, paracetamol, and hydrochlorothiazide (*Table 1*). All of these were present at concentrations above 1000 ng/L. These five pharmaceuticals are among the most prevalent in Swedish WWTP effluents, usually detected in the 100 ng/L to above 1000 ng/L range (Falås et al., 2012). Concentrations upstream of Fors WWTP were either < LOD or at low ng/L. The exception is losartan, detected at 240 ng/L in one of the samples. In Vitsån Creek, all substances except for risperidone, simvastatin, and ramipril were detected in at least one of the three sampling occasions.

Table 1. *Concentrations, mean (min–max) ng/L, of pharmaceuticals in water samples from Fors WWTP and Rimbo WWTP. Sites at Rimbo: Lake Syningen, upstream and downstream of discharge point in Vallbyån Creek, effluent, and Lake Kundbysjön. Sites at Fors: Upstream, effluent, and downstream of discharge point in Vitsån Creek. Concentrations below LOD were set to zero. Concentrations between LOD and LOQ were set to the average of LOD and LOQ*

	Fors WWTP			Rimbo WWTP				
Substance	Upstream	Effluent	Downstream	Syningen	Upstream	Effluent	Downstream	Kundby
Amlodipine	< LOD	110 (56–190)	10 (< LOD–30)	-	-	-	-	-
Atenolol	< LOD	300 (200–440)	130 (56–250)	< LOD	< LOD	59 (13–96)	3.1 (< LOD–10)	< LOD
Bisoprolol	< LOD	150 (100–200)	64 (21–100)	-	-	-	-	-
Caffeine	100 (100–100)	8300 (8300–8300)	2400 (2400–2400)	-	-	-	-	-
Carbamazepine	< LOD	850 (440–1400)	410 (98–830)	< LOD	0.25 (< LOD–2)	140 (38–210)	3.2 (< LOD–26)	< LOD
Citalopram	< LOD	620 (380–1100)	220 (55–470)	< LOD	< LOD	130 (29–270)	8.5 (< LOD–21)	2.2 (< LOD–8.7)
Diclofenac	5.8 (< LOD–18)	1700 (920–2800)	740 (230–1500)	< LOD	2.4 (< LOD–9.2)	760 (120–1700)	66 (12–210)	23 (< LOD–83)
Fluconazole	< LOD	150 (130–180)	74 (20–140)	< LOD	< LOD	100 (18–200)	4.2 (< LOD–21)	< LOD
Fluoxetine	< LOD	53 (26–91)	15 (6.6–31)	-	-	-	-	-
Furosemide	< LOD	2800 (1600–3700)	1200 (350–2300)	< LOD	< LOD	1000 (150–2000)	60 (< LOD–260)	< LOD
Hydrochlorothiazide	4.4 (< LOD–8.5)	1900 (1000–3200)	890 (240–1800)	-	-	-	-	-
Ibuprofen	6.3 (< LOD–19)	430 (58–1100)	130 (58–240)	< LOD	0.38 (< LOD–3)	1200 (< LOD–4500)	160 (< LOD–610)	34 (< LOD–100)
Ketoconazole	< LOD	35 (< LOD–70)	35 (< LOD–70)	< LOD	< LOD	75 (75–75)	< LOD	< LOD
Ketoprofen	< LOD	130 (93–150)	48 (36–63)	-	-	-	-	-

	Fors WWTP			Rimbo WWTP				
Substance	Upstream	Effluent	Downstream	Syningen	Upstream	Effluent	Downstream	Kundby
Losartan	78 (< LOD–240)	7800 (3500–12000)	3300 (2700–3900)	< LOD	2.9 (< LOD–12)	1300 (250–2500)	96 (22–330)	36 (< LOD–73)
Metoprolol	< LOD	3400 (1700–5800)	1600 (410–3200)	< LOD	2 (< LOD–7.8)	1000 (270–1600)	76 (< LOD–170)	28 (< LOD–84)
Naproxen	13 (< LOD–38)	460 (110–1100)	140 (61–260)	< LOD	1.6 (< LOD–4.6)	680 (170–1600)	44 (11–88)	18 (< LOD–54)
Oxazepam	< LOD	1500 (290–3600)	400 (120–730)	< LOD	< LOD	330 (70–580)	20 (6.9–47)	10 (< LOD–20)
Paracetamol	32 (28–36)	2800 (52–7700)	980 (30–2700)	< LOD	< LOD	45 (< LOD–210)	11 (< LOD–59)	7 (< LOD–28)
Propranolol	< LOD	250 (140–400)	90 (24–180)	< LOD	< LOD	54 (12–120)	2.7 (< LOD–9.5)	1 (< LOD–4)
Ramipril	< LOD	10 (< LOD–30)	< LOD	-	-	-	-	-
Ranitidine	< LOD	40 (26–47)	7.2 (< LOD–22)	-	-	-	-	-
Risperidone	< LOD	< LOD	< LOD	-	-	-	-	-
Sertraline	< LOD	230 (99–400)	60 (20–140)	< LOD	< LOD	28 (6–50)	1.4 (< LOD–4.1)	< LOD
Terbutaline	< LOD	15 (7–23)	6.8 (< LOD–14)	-	-	-	-	-
Tramadol	< LOD	900 (730–1000)	500 (170–880)	< LOD	< LOD	240 (81–350)	9.1 (< LOD–32)	6.5 (< LOD–26)
Venlafaxine	< LOD	590 (500–730)	340 (130–660)	< LOD	1.1 (< LOD–7.2)	380 (77–980)	27 (< LOD–110)	4.6 (< LOD–12)
Warfarin	< LOD	16 (4–32)	6.3 (< LOD–15)	-	-	-	-	-
Zolpidem	< LOD	3.9 (2.5–5.5)	1.4 (< LOD–4.1)	< LOD	< LOD	1.3 (< LOD–3.3)	< LOD	< LOD
Sulfamethoxazole	< LOD	170 (92–250)	97 (11–220)	< LOD	1.9 (< LOD–15)	370 (69–710)	31 (< LOD–130)	< LOD
Simvastatin	< LOD	< LOD	< LOD	-	-	-	-	-
Clarithromycin	-	-	-	< LOD	< LOD	53 (< LOD–110)	5.1 (< LOD–15)	0.49 (< LOD–2)
Erythromycin	-	-	-	< LOD	< LOD	12 (< LOD-68)	0.25 (< LOD–2)	< LOD
Trimethoprim	-	-	-	0.65 (< LOD–2)	0.24 (< LOD–2)	57 (23–97)	3 (< LOD–10)	< LOD
Ciprofloxacin	-	-	-	< LOD	< LOD	130 (43–220)	< LOD	< LOD
Methotrexate	-	-	-	< LOD	< LOD	0.4 (< LOD–2)	< LOD	< LOD

The correlation between concentrations in the effluent and in Vitsån Creek was strong, with an R^2 ranging between 0.92 and 0.99 (*Fig. 2*). The difference in the slope in each panel of the Figure is due to the seasonal variation in net flow in Vitsån Creek, affecting the dilution of the effluent. This high correspondence in concentrations indicates that the spatial distribution of pharmaceuticals in Vitsån Creek is homogenous and stable over time, at least on a daily basis.

Concentrations of diclofenac in the downstream samples ranged within 230–1500 ng/L (T*able 1*) and are thus considered elevated since they exceed the European environmental quality standard (EQS) at 100 ng/L (HVMFS, 2019) in all measurements. This is a range in which salmonoid fish in laboratory studies have shown histological and cytological changes (Hoeger et al., 2005; Mehinto et al., 2010; Schwaiger et al., 2004; Triebskorn et al., 2004), meaning that there is a risk of the brown trout population in Vitsån Creek suffering negative effects from the diclofenac exposure. However, the concentrations of diclofenac in the effluent are not abnormally high in the treated effluent compared with other WWTPs. Diclofenac concentrations in treated effluent water of around 1000 ng/L are common (Brown et al., 2007; Fick et al., 2010; Meyer et al., 2016). The key factor to the elevated concentrations in Vitsån Creek is likely the low dilution of the effluent when it enters Vitsån Creek.

In the effluent from Rimbo WWTP, pharmaceuticals were present between < LOD and 4.5 µg/L. The five pharmaceuticals in the highest effluent concentration were, in descending order, losartan, ibuprofen, metoprolol, furosemide, and diclofenac. Hydrochlorothiazide, which was detected at elevated concentrations in the effluent from Fors, was not measured at Rimbo (*Table 1*). Notable is that losartan, metoprolol, and furosemide belonged to the pharmaceuticals with the highest concentrations in both cases. There is, however, a clear difference in the magnitude of the concentrations between Fors and Rimbo, with generally higher concentrations in the effluent from Fors. At Rimbo, the highest average concentration was 1300 ng/L, while at Fors, seven substances exceeded this level. That the magnitude of the concentrations differs between different WWTPs is expected. Large variations in effluent concentrations have been shown in a screening of Swedish WWTPs (Andersson et al., 2006).

The variability in the effluent concentrations was large at Rimbo WWTP (*Fig. 3*). For example, in the sampling in April, none of the substances were detected above 500 ng/L, and the R^2 values ranged between 0.3 and 0.98, see e.g. the sampling in September. At the sampling in December, two substances detected in the effluent above 2000 ng/L (ibuprofen and furosemide) were not detected in Vallbyån Creek. The varying net flow in Vallbyån Creek, within the approximate range of 0.2–1.0 m^3/s, in

Fig. 2. *Concentrations (ng/L) of pharmaceuticals in effluent water and the receiving stream, Vitsån Creek, for each of the three sampling occasions. Each dot represents one pharmaceutical. Substances with concentrations larger than 2500 ng/L in the effluent are labelled. Lines are ordinary least square (OLS) fits*

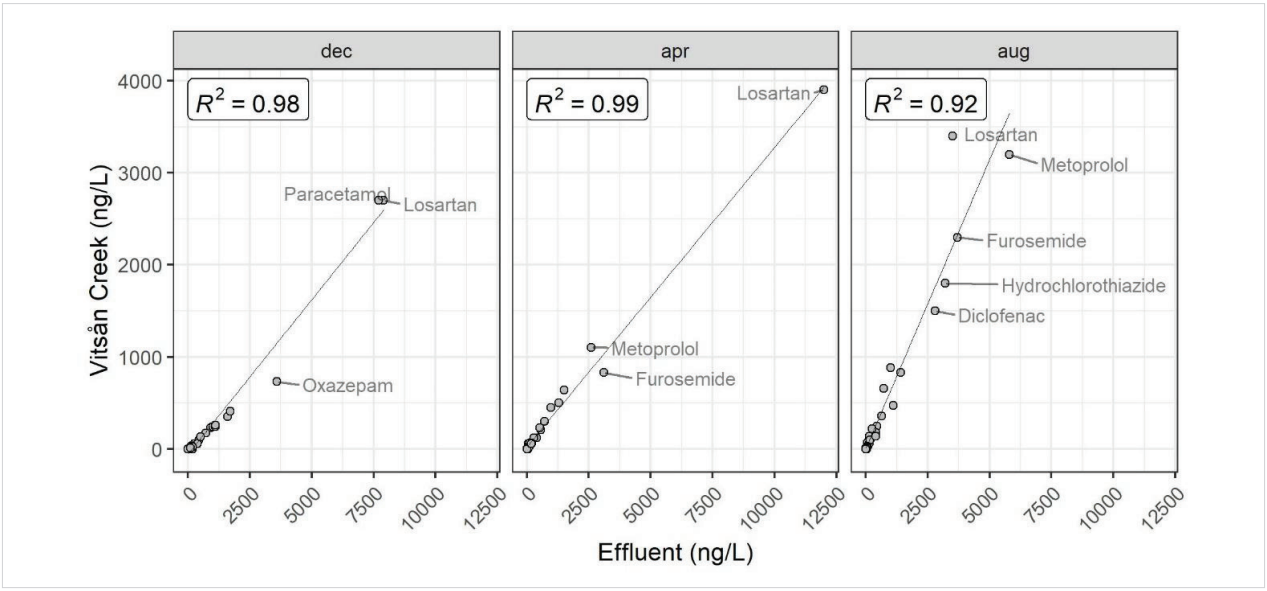
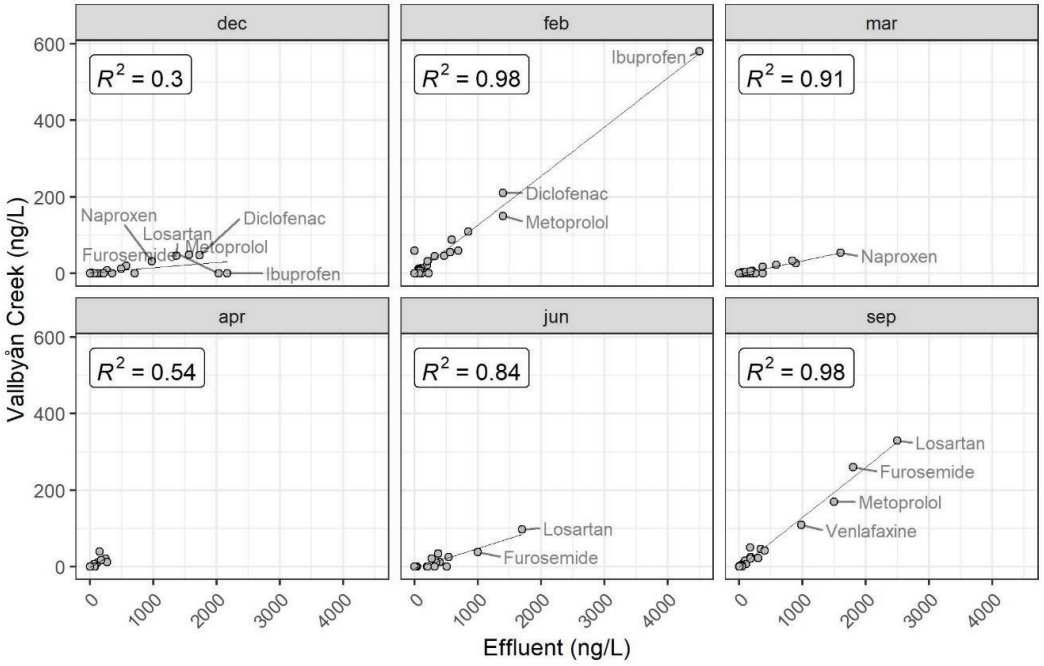


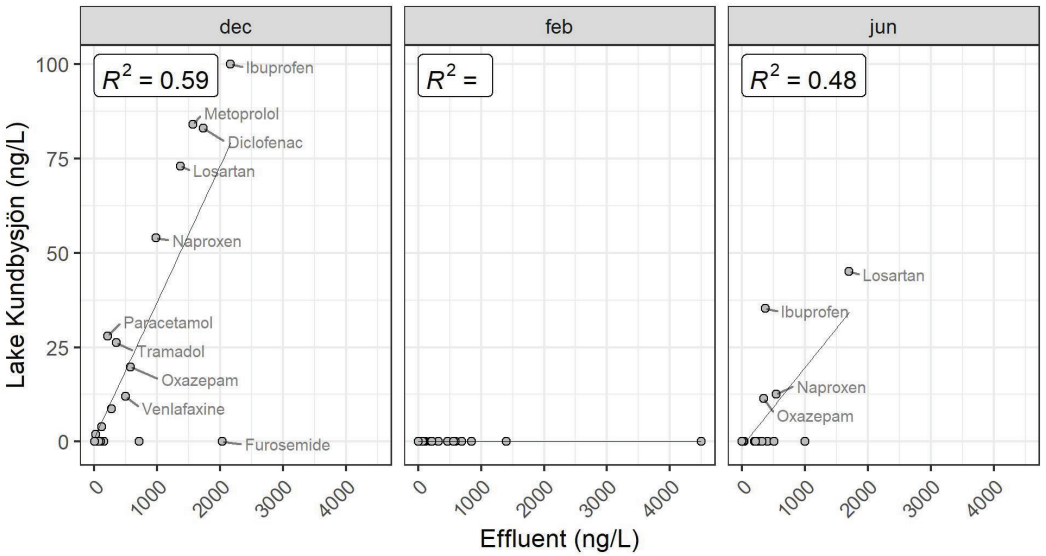
Fig. 3. *Concentrations (ng/L) of pharmaceuticals in effluent water and the receiving stream, Vallbyån Creek, for each of the sampling occasions. Each dot represents one pharmaceutical. Substances with concentrations larger than 1000 ng/L in the effluent are labelled. Lines are ordinary least square (OLS) fits. December samples are from 2021, other months are in 2022*



combination with the variability in the pharmaceutical concentration in the effluent, leads to large variability in pharmaceutical concentration in Vallbyån Creek. For example, diclofenac had an average concentration in Vallbyån Creek at 66 ng/L, but the standard deviation was 76 ng/L, which means that the coefficient of variation (CV) is larger than 100%. This level of variation is similar for most substances. The CV values for ibuprofen, losartan, and metoprolol were 169%, 105%, and 96%, respectively. In an eighteen-month repeated measures study of diclofenac, erythromycin, ibuprofen, mefenamic acid, and propranolol in effluent and receiving waters at seven WWTPs in the UK, similar variability in the receiving water concentrations was reported (Kay et al., 2017). In terms of exposure to aquatic organisms, the results from Rimbo show that the exposure is far from constant, but instead rather variable. The difference between Fors and Rimbo, which suggests a stronger relationship between effluent concentrations and receiving creek concentrations in Fors compared with Rimbo, could, to some extent, be a consequence of higher dilution of the effluent and lower effluent concentrations at Rimbo. At Rimbo, the dilution is up to 90%, whilst at most 50% in Fors. Low concentrations that are substantially diluted are likely to appear more variable than higher concentrations that are less diluted, partly due to the higher chance of the concentration being below detection limits. Another relevant note, likely to confound the comparison between the two sites, is that the sampling in the effluent at Fors was grab samples taken the same day as the receiving water samples, while the effluent samples at Rimbo were pooled weekly samples, thus representing a weekly average effluent concentration.

Concentrations in Lake Kundbysjön showed highly varying correlations to effluent concentrations, depending on the sampling occasion (*Fig. 4*). In the December sampling, the correlation was high, although with the clear exception of furosemide, with high concentrations in the effluent but surprisingly low in Lake Kundbysjön. In February, none of the pharmaceuticals were detected in Lake Kundbysjön. This was somewhat surprising, and the reason for this discrepancy is not easily explained. However, results in Randefelt (2019) support that the December concentrations are most representative of the true winter conditions since it was shown that pharmaceutical concentration reduction in constructed wastewater receiving wetlands was lower in winter conditions (–3–30% reduction) as compared with summer conditions (10–97%). In contrast to this, Kay et al.

Fig. 4. *Concentrations (ng/L) of pharmaceuticals in effluent water and the receiving stream, Vitsån Creek, for each of the sampling occasions. Each dot represents one pharmaceutical. Substances with concentrations larger than 1000 ng/L in the effluent or larger than 10 in Lake Kundbysjön are labelled. December samples are from 2021; other months are in 2022. The R^2 value was undefined in February since there was no variance in Lake Kundbysjön*



(2017) found no temporal pattern during a monthly 18 month sampling campaign. However, winter peaks can occur due to an increase in pharmaceutical consumption and reduced degradation in surface waters (Lindholm-Lehto et al., 2016; MacLeod and Wong, 2010; Sui et al., 2011; Valcárcel et al., 2013) but conversely, some pharmaceuticals have been detected in higher concentrations during the summer months (Lindholm-Lehto et al., 2016; Papageorgiou et al., 2016). In June, only ibuprofen, losartan, naproxen and losartan were detected in Lake Kundbysjön.

Pharmaceuticals in fish – Fors

Levels of diclofenac were within the range of 4.6–16 µg/g w.w in bile of trout exposed to the conventional effluent. Naproxen was detected within the range of 37–170 ng/g vv (*Table 2*), although one of the replicates could not be evaluated for naproxen due to poor recovery in the chemical analysis. No other pharmaceutical could be found in the fish bile, even though the concentrations of many substances in the effluent are approximately the same magnitude as diclofenac (*Table 1*). This suggests a strong partitioning of diclofenac to trout bile and, at the same time, a weak partitioning of the other substances, considering that the trout were exposed to 100% effluent. Taking the dilution of the effluent in Vitsån Creek (7–50%) into account, diclofenac levels in the natural population of brown trout in Vitsån Creek could be in the 1000 ng/L range.

In comparison, diclofenac levels in bile were 5.2 ng/g in perch from a coastal area in Stockholm receiving effluent water from a large WWTP (Vieno et al., 2017). In an effluent receiving lake in Finland, diclofenac levels in bile of bream (*Abramis brama*) and roach (*Rutilus rutilus*) were between not detected and 148 ng/g (assuming the bile density to be 1 g/mL) (Brozinski et al., 2013). That the partitioning to the bile is strong for diclofenac is supported by Lahti et al. (2011), in which rainbow trout were exposed to diclofenac, naproxen, ibuprofen, bisoprolol, and carbamazepine. These pharmaceuticals were then measured in bile and plasma, and the average bioconcentration factor was highest for diclofenac and also two to four orders of magnitude higher in bile compared with plasma.

Table 2. *Levels of detected pharmaceuticals in brown trout bile (ng/g) in three replicates (A–C) per treatment. Other substances could not be detected in any sample."-" could not be evaluated due to poor recovery in the chemical analysis*

Substance	Rep A	Rep B	Rep C
Diclofenac (ng/g vv)	16 000	8 900	4 600
Naproxen (ng/g vv)	170	37	-

Pharmaceuticals in fish and crayfish – Rimbo

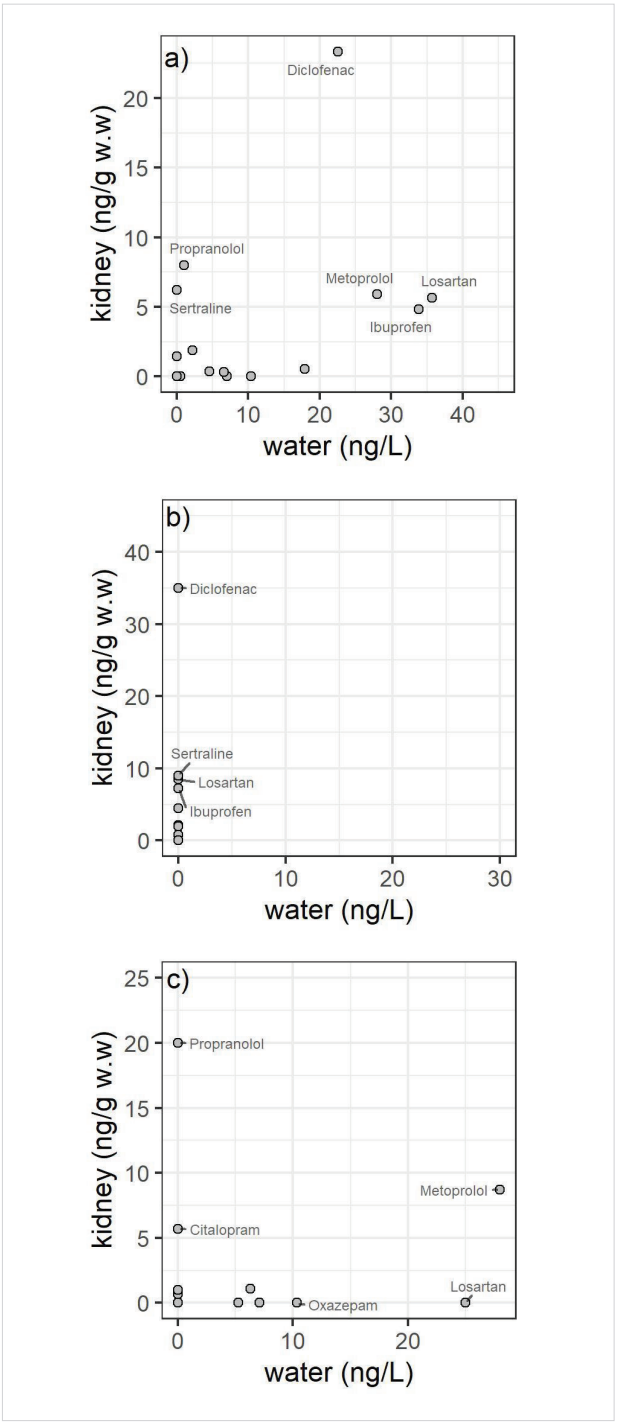
The fish tissue with the highest number of detected pharmaceuticals in fish was kidney of pike, in which 11 of the 22 analyzed pharmaceuticals were detected (*Table 3*). The highest concentration in kidney was diclofenac (37 ng/g w.w), followed by losartan (17 ng/g w.w) and ibuprofen (12 ng/g w.w). None of the pharmaceuticals were detected in kidney of perch. In kidney of rudd, tramadol and venlafaxine were detected at low concentrations. Despite the small sample size of perch and rudd, this indicates a difference between the species regarding the uptake of pharmaceuticals. Rudd, however, was a pooled sample of kidney from five individuals, thus representing an average of these five. In muscle, naproxen (< LOD–0.5 ng/g w.w) and atenolol (< LOD–2 ng/g w.w) were detected in pike from Lake Syningen, atenolol in perch from Lake Kundbysjön, and atenolol and paracetamol (12–26 ng/g vv) in pike from Lake Kundbysjön. In liver and bile, only atenolol was detected. A note is that all concentrations of sertraline were at trace levels (between LOD and LOQ). The table reports the average of LOD and LOQ.

That pharmaceutical residues accumulate differently in different tissues is expected. The results found here, that pharmaceuticals are more often found in kidney than liver, is however not supported by results from European

Table 3. *Concentrations, mean (min–max) ng/L, of pharmaceuticals in fish samples from Lake Syningen, upstream and downstream of discharge point in Vallbyån Creek, and Lake Kundbysjön. Concentrations below LOD set to zero. Only pharmaceuticals that were detected in at least one sample are shown. Concentrations between LOD and LOQ set to the average of LOD and LOQ.*all three samples of signal crayfish, one sample upstream and two samples downstream of the discharge point*

Site	Kundby									Syningen				Vallbyån
Matrix	Bile		Liver		Muscle		Kidney			Bile	Liver	Muscle	Kidney	Hepato.
Species	Perch	Pike	Perch	Pike	Perch	Pike	Perch	Pike	Rudd	Pike	Pike	Pike	Pike	Crayfish*
Atenolol	< LOD	2 (2–2)	2	2 (2–2)	2 (2–2)	9.2 (9.2–9.2)	< LOD	< LOD	< LOD	1 (0–2)	2 (2–2)	0.67 (0–2)	< LOD	< LOD
Citalopram	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.9 (0–5.7)	< LOD	< LOD	< LOD	< LOD	0.21 (0–0.64)	< LOD
Diclofenac	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	23 (0–37)	< LOD	< LOD	< LOD	< LOD	11 (0–18)	< LOD
Furosemide	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.4 (0–4.3)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ibuprofen	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	4.8 (0–12)	< LOD	< LOD	< LOD	< LOD	2.3 (0–4.5)	< LOD
Losartan	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	5.7 (0–17)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Metoprolol	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	5.9 (4.5–8.7)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Naproxen	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.53 (0–1.1)	< LOD	< LOD	< LOD	0.17 (0–0.5)	0.17 (0–0.5)	< LOD
Paracetamol	< LOD	< LOD	< LOD	< LOD	< LOD	19 (12–26)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Propranolol	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	8 (2–20)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sertralin	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	6.2 (0.65–9)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Tramadol	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.33 (0–0.99)	0.98	< LOD	< LOD	< LOD	< LOD	< LOD
Venlafaxine	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.37 (0–1.1)	0.18	< LOD	< LOD	< LOD	0.06 (0–0.18)	< LOD

Fig. 5. *a) Average concentrations in Lake Kundbysjön vs. average concentrations in pike kidney from Lake Kundbysjön. Substances with water concentration > 20 ng/L or kidney concentration > 4 ng/L are labeled. b) Concentrations in Lake Kundbysjön, February 2022 vs. average concentrations in pike kidney from Lake Kundbysjön, caught in February 2022. c) Concentrations in Lake Kundbysjön, May 2022 vs. concentrations in pike kidney from Lake Kundbysjön, caught in May 2022*



chub (*Squalius cephalus*) from 10 streams in the Czech Republic (Grabicová et al., 2020), in which no apparent difference in detection frequency between liver and kidney could be noted. Considering only the samples of perch and rudd in this study, it seems to be coherent. In perch kidney, no pharmaceuticals were detected, and in liver, only atenolol at low concentrations. The sample size is, however, very small, with only one sample of perch and rudd, respectively. The tissue that stands out here is kidney of pike, with generally high detection frequencies.

The relation between water concentrations in Lake Kundbysjön and levels in pike kidney is weak for matching sampling occasions or averages (*Fig. 5*). However, the pharmaceuticals in the highest concentrations in water were losartan, ibuprofen, metoprolol, and diclofenac, and the highest average concentrations in pike kidney were diclofenac, sertraline, metoprolol, losartan, ibuprofen, and propranolol, indicating that at least some of the substances found in highest concentrations in water can be found in fish. Propranolol and sertraline were not detected, or in low concentrations, in water but were among the substances detected in fish. Likely, the bioconcentration factors in pike kidney differ significantly for each substance, and such estimates are not available in the literature and are not calculated here due to the considerable temporal variability in pharmaceutical concentrations in fish and lake water.

Sertraline was found at trace levels (between 0.3 and 14 ng/ w.w) in pike kidney, which means that the concentration is uncertain. Elevated concentrations of sertraline in fish compared with the surrounding water can be expected since sertraline is a highly fat-soluble substance, reflected in a high log-Kow value. However, findings in Lagesson et al. (2016) suggest that Kow is of limited use for predicting the uptake of pharmaceuticals in natural systems. It is also expected that several confounding variables contribute to the weak relation between water and fish concentrations. To begin with, water was sampled on four occasions in Lake Kundbysjön, while fish were sampled only twice. Also, pharmaceuticals fluctuate significantly in fish between different sampling occasions and do not correspond directly with water concentrations. Diclofenac, for example, was detected between 33–37 ng/g w.w in pike kidney from the sampling in Lake Kundbysjön in February, and it was below the detection limit of 0.1 ng/g w.w in pike kidney in May. In February, diclofenac was not detected in the lake water (see supplementary information). Pharmaceutical concentrations are also dependent on trophic transfers, possibly leading to increased concentrations in

fish even if the water concentrations are decreasing for a period of time (Lagesson et al., 2016). Possible confounding factors when comparing fish from the different sampling occasions could be differences in age and size of the fish, as well as differences in feeding habits, the reproductive cycle, and movement patterns. The effect of size differences, however, does not appear to be the case here since the pikes caught in May were not larger compared with the pikes caught in February.

In hepatopancreas of signal crayfish, all samples had levels of pharmaceuticals below the detection limit (*Table 3*). When considering the bioconcentration factor in hepatopancreas for ibuprofen in a similar type of crayfish (*Procambarus clarkii*), which was estimated to be within the range of 1.47–1.78 L/Kg (Trombini et al., 2021), these results appear reasonable. A BCF of 1.78 and a water concentration of 177 ng/L ibuprofen, which is the average downstream concentration in Vallbyån Creek, corresponds to an estimated hepatopancreas concentration of 0.32 ng/g – a concentration that is below the detection limit of 1.1 ng/g.

Considering both the results in signal crayfish, pike, perch, and rudd, although the sample size is relatively small, thus not allowing for any statistical hypothesis testing, there is an indication that the highest concentrations of pharmaceuticals are found in piscivorous fish (pike) higher in the food chain compared with detritivorous (common rudd). Pike had both the highest detection frequency and concentrations. In perch, which, however, from 2–3 years old, is piscivorous, and in crayfish, no substances were detected. In common rudd, only tramadol and venlafaxine were detected at a low concentration.

The indications found here regarding differences between species with different feeding strategies are not supported by Rojo et al. (2019), which found the lowest uptake of pharmaceuticals in piscivorous fish. Three species – *M. obtusidens* (omnivorous), *P. lineatus* (detritivorous), and *S. brasiliensis* (piscivorous) – in the Uruguay River were studied for which both the highest concentrations and detection frequencies were found in the omnivorous species, followed by the detritivorous species and lastly the piscivorous species.

Conclusions

The conducted study shows that pharmaceutical concentrations in receiving waters and effluent waters can be largely variable throughout the year, thus shedding light on the necessity of repeated sampling to obtain representative average concentrations. However, there is an indication that the variability in concentrations and the correlation between effluent concentrations and receiving water concentrations are site dependent.

This study also further strengthens the notion that concentrations of pharmaceuticals in fish vary substantially between and within species and between different tissues. Here, pharmaceuticals were detected mainly in kidney of pike, in contrast to muscle, liver, and bile samples and samples of perch and common rudd. This, although limited by the low sample size, suggests that kidney of pike is a good indicator of pharmaceutical exposure to fish since 11 of the 22 pharmaceuticals were detected in pike kidney at ecologically relevant water concentrations. The correlation between pharmaceuticals in pike kidney and water concentrations was weak. Likely explanations for this are large spatial and temporal variations both in water and fish, in combination with the fact that the uptake in fish is substance dependent.

In signal crayfish, the pharmaceutical concentrations in its hepatopancreas were below the detection limit for all substances, even though the exposure is higher than for the fish since it lives in the effluent receiving stream. This indicates generally low bioaccumulation of pharmaceuticals in hepatopancreas of signal crayfish.

The results showed clear variation in the uptake of different pharmaceuticals in bile of brown trout exposed to 100% effluent. Despite several pharmaceuticals with different therapeutic effects being present in the effluent in the µg/L range, only the NSAIDs diclofenac and naproxen could be detected in bile, suggesting that analysis of pharmaceuticals in only fish bile will not give a comprehensive picture of the exposure. This notion is strengthened by the previously mentioned analyses of wild fish, with generally low detection frequencies in bile of pike, perch, and common rudd.

Acknowledgments

We want to thank Musbau Adeoye Bello and Emma Gunnerblad for their assistance with water sampling, Joakim Hållén and Jonas Röttorp for help with fishing, and Inger Abrahamsson for fish sample preparation. Funding was supplied by the SIVL foundation, Norrtälje Vatten and Avfall AB, and Haninge municipality.

References

Álvarez-Muñoz, D., Rodríguez-Mozaz, S., Maulvault, A. L., Tediosi, A., Fernández-Tejedor, M., Van den Heuvel, F., Kotterman, M., Marques, A., and Barceló, D. (2015). Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe. Environmental research, 143, 56-64. https://doi.org/10.1016/j.envres.2015.09.018

Andersson, J., Woldegiorgis, A., Remberger, M., Kaj, L., Ekheden, Y., Dusan, B., Svenson, A., Brorström-Lundén, E., Dye, C., and Schlabach, M. (2006). Results from the Swedish National Screening Programme 2005 - Subreport 1: Antibiotics, anti-inflammatory substances and hormones. IVL Swedish Environmental Reseach Institute Report B1689.

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

Brooks, B. W., Chambliss, C. K., Stanley, J. K., Ramirez, A., Banks, K. E., Johnson, R. D., and Lewis, R. J. (2005). Determination of select antidepressants in fish from an effluent-dominated stream. Environmental Toxicology and Chemistry: An International Journal, 24(2), 464-469. https://doi.org/10.1897/04-081R.1

Brown, A. R., Gunnarsson, L., Kristiansson, E., and Tyler, C. R. (2014). Assessing variation in the potential susceptibility of fish to pharmaceuticals, considering evolutionary differences in their physiology and ecology. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1656), 20130576. https://doi.org/10.1098/rstb.2013.0576

Brown, J. N., Paxéus, N., Förlin, L., and Larsson, D. J. (2007). Variations in bioconcentration of human pharmaceuticals from sewage effluents into fish blood plasma. Environmental Toxicology and Pharmacology, 24(3), 267-274. https://doi.org/10.1016/j.etap.2007.06.005

Brozinski, J.-M., Lahti, M., Meierjohann, A., Oikari, A., and Kronberg, L. (2013). The anti-inflammatory drugs diclofenac, naproxen and ibuprofen are found in the bile of wild fish caught downstream of a wastewater treatment plant. Environmental science and technology, 47(1), 342-348. https://doi.org/10.1021/es303013j

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

Emmanouil, C., Bekyrou, M., Psomopoulos, C., and Kungolos, A. (2019). An insight into ingredients of toxicological interest in personal care products and a small-scale sampling survey of the Greek market: delineating a potential contamination source for water resources. Water, 11(12), 2501. https://doi.org/10.3390/w11122501

Falås, P., Andersen, H. R., Ledin, A., and Jansen, J. L. C. (2012). Occurrence and reduction of pharmaceuticals in the water phase at Swedish wastewater treatment plants. Water Science and Technology, 66(4), 783-791. https://doi.org/10.2166/wst.2012.243

Fatta-Kassinos, D., Meric, S., and Nikolaou, A. (2011). Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Analytical and bioanalytical chemistry, 399, 251-275. https://doi.org/10.1007/s00216-010-4300-9

Fernández-Rubio, J., Rodríguez-Gil, J. L., Postigo, C., Mastroianni, N., de Alda, M. L., Barceló, D., and 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., Brodin, T., Heynen, M., Klaminder, J., Jonsson, M., Grabicova, K., Randak, T., Grabic, R., Kodes, V., and Slobodnik, J. (2017). Screening of benzodiazepines in thirty European rivers. Chemosphere, 176, 324-332. https://doi.org/10.1016/j.chemosphere.2017.02.126

Fick, J., Lindberg, R. H., Parkkonen, J., Arvidsson, B. r., Tysklind, M., and Larsson, D. J. (2010). Therapeutic levels of levonorgestrel detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents. Environmental science and technology, 44(7), 2661-2666. https://doi.org/10.1021/es903440m

Gelsleichter, J., and Szabo, N. J. (2013). Uptake of human pharmaceuticals in bull sharks (Carcharhinus leucas) inhabiting a wastewater-impacted river. Science of The Total Environment, 456, 196-201. https://doi.org/10.1016/j.scitotenv.2013.03.078

Grabicová, K., Grabic, R., Fedorova, G., Kolářová, J., Turek, J., Brooks, B. W., and Randák, T. (2020). Psychoactive pharmaceuticals in aquatic systems: A comparative assessment of environmental monitoring approaches for water and fish. Environmental Pollution, 261, 114150. https://doi.org/10.1016/j.envpol.2020.114150

Grabicova, K., Lindberg, R. H., Östman, M., Grabic, R., Randak, T., Larsson, D. J., and Fick, J. (2014). Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Science of The Total Environment, 488, 46-50. https://doi.org/10.1016/j.scitotenv.2014.04.052

Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., and Larsson, D. J. (2008). Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environmental science and technology, 42(15), 5807-5813. https://doi.org/10.1021/es8005173

Hoeger, B., Köllner, B., Dietrich, D. R., and Hitzfeld, B. (2005). Water-borne diclofenac affects kidney and gill integrity and selected immune parameters in brown trout (Salmo trutta f. fario). Aquatic Toxicology, 75(1), 53-64. https://doi.org/10.1016/j.aquatox.2005.07.006

Huerta, B., Rodríguez-Mozaz, S., and Barceló, D. (2012). Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. Analytical and bioanalytical chemistry, 404(9), 2611-2624. https://doi.org/10.1007/s00216-012-6144-y

Hughes, S. R., Kay, P., and Brown, L. E. (2013). Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environmental science and technology, 47(2), 661-677. https://doi.org/10.1021/es3030148

HVMFS. (2019). HVMFS 2019:25. Havs- och vattenmyndighetens föreskrifter om klassificering och miljökvalitetsnormer avseende ytvatten.

Kay, P., Hughes, S. R., Ault, J. R., Ashcroft, A. E., and Brown, L. E. (2017). Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. Environmental Pollution, 220, 1447-1455. https://doi.org/10.1016/j.envpol.2016.10.087

Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., and Klaminder, J. (2016). Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web-Insights from a field experiment. Science of The Total Environment, 568, 208-215. https://doi.org/10.1016/j.scitotenv.2016.05.206

Lahti, M., Brozinski, J. M., Jylhä, A., Kronberg, L., and Oikari, A. (2011). Uptake from water, biotransformation, and biliary excretion of pharmaceuticals by rainbow trout. Environmental Toxicology and Chemistry, 30(6), 1403-1411. https://doi.org/10.1002/etc.501

Larabie, S. P., Houde, M., and Gagnon, C. (2017). Determination of the bioavailability of selected pharmaceutical residues in fish plasma using liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography A, 1522, 48-55. https://doi.org/10.1016/j.chroma.2017.09.055

Lenka, S. P., Kah, M., and Padhye, L. P. (2021). A review of the occurrence, transformation, and removal of poly-and perfluoroalkyl substances (PFAS) in wastewater treatment plants. Water research, 199, 117187. https://doi.org/10.1016/j.watres.2021.117187

Lindholm-Lehto, P. C., Ahkola, H. S., Knuutinen, J. S., and Herve, S. H. (2016). Widespread occurrence and seasonal variation of pharmaceuticals in surface waters and municipal wastewater treatment plants in central Finland. Environmental Science and Pollution Research, 23, 7985-7997. https://doi.org/10.1007/s11356-015-5997-y

Liu, S., Bekele, T.-G., Zhao, H., Cai, X., and Chen, J. (2018). Bioaccumulation and tissue distribution of antibiotics in wild marine fish from Laizhou Bay, North China. Science of The Total Environment, 631, 1398-1405. https://doi.org/10.1016/j.scitotenv.2018.03.139

MacLeod, S. L., and Wong, C. S. (2010). Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. Water research, 44(2), 533-544. https://doi.org/10.1016/j.watres.2009.09.056

Malnes, D., Ahrens, L., Köhler, S., Forsberg, M., and Golovko, O. (2022). Occurrence and mass flows of contaminants of emerging concern (CECs) in Sweden's three largest lakes and associated rivers. Chemosphere, 294, 133825. https://doi.org/10.1016/j.chemosphere.2022.133825

Mehinto, A. C., Hill, E. M., and Tyler, C. R. (2010). Uptake and biological effects of environmentally relevant concentrations of the nonsteroidal anti-inflammatory pharmaceutical diclofenac in rainbow trout (Oncorhynchus mykiss). Environmental science and technology, 44(6), 2176-2182. https://doi.org/10.1021/es903702m

Meyer, W., Reich, M., Beier, S., Behrendt, J., Gulyas, H., and Otterpohl, R. (2016). Measured and predicted environmental concentrations of carbamazepine, diclofenac, and metoprolol in small and medium rivers in northern Germany. Environmental monitoring and assessment, 188(8), 1-16. https://doi.org/10.1007/s10661-016-5481-2

Moreno-González, R., Rodriguez-Mozaz, S., Gros, M., Barceló, D., and León, V. (2015). Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). Environmental research, 138, 326-344. https://doi.org/10.1016/j.envres.2015.02.016

Papageorgiou, M., Kosma, C., and Lambropoulou, D. (2016). Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Science of The Total Environment, 543, 547-569. https://doi.org/10.1016/j.scitotenv.2015.11.047

Pistocchi, A., Andersen, H., Bertanza, G., Brander, A., Choubert, J.-M., Cimbritz, M., Drewes, J. E., Koehler, C., Krampe, J., and Launay, M. (2022). Treatment of micropollutants in wastewater: Balancing effectiveness, costs and implications. Science of The Total Environment, 850, 157593. https://doi.org/10.1016/j.scitotenv.2022.157593

Pohl, J., Björlenius, B., Brodin, T., Carlsson, G., Fick, J., Larsson, D. J., Norrgren, L., and Örn, S. (2018). Effects of ozonated sewage effluent on reproduction and behavioral endpoints in ze-

brafish (Danio rerio). Aquatic Toxicology, 200, 93-101. https://doi.org/10.1016/j.aquatox.2018.04.014

Ramirez, A. J., Mottaleb, M. A., Brooks, B. W., and Chambliss, C. K. (2007). Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. Analytical chemistry, 79(8), 3155-3163. https://doi.org/10.1021/ac062215i

Randefelt, J. (2019). Spillvattenvåtmarkers reningseffekt på aktiva läkemedelssubstanser (Publication Number UPTEC W 19044,) Stockholm University].

Rojo, M., Álvarez-Muñoz, D., Domanico, A., Foti, R., Rodriguez-Mozaz, S., Barceló, D., and Carriquiriborde, P. (2019). Human pharmaceuticals in three major fish species from the Uruguay River (South America) with different feeding habits. Environmental Pollution, 252, 146-154. https://doi.org/10.1016/j.envpol.2019.05.099

Schröder, P., Helmreich, B., Škrbić, B., Carballa, M., Papa, M., Pastore, C., Emre, Z., Oehmen, A., Langenhoff, A., and Molinos, M. (2016). Status of hormones and painkillers in wastewater effluents across several European states-considerations for the EU watch list concerning estradiols and diclofenac. Environmental Science and Pollution Research, 23, 12835-12866. https://doi.org/10.1007/s11356-016-6503-x

Schultz, M. M., Furlong, E. T., Kolpin, D. W., Werner, S. L., Schoenfuss, H. L., Barber, L. B., Blazer, V. S., Norris, D. O., and Vajda, A. M. (2010). Antidepressant pharmaceuticals in two US effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. Environmental science and technology, 44(6), 1918-1925. https://doi.org/10.1021/es9022706

Schwaiger, J., Ferling, H., Mallow, U., Wintermayr, H., and Negele, R. (2004). Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout. Aquatic Toxicology, 68(2), 141-150. https://doi.org/10.1016/j.aquatox.2004.03.014

Sui, Q., Huang, J., Deng, S., Chen, W., and Yu, G. (2011). Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environmental science and technology, 45(8), 3341-3348. https://doi.org/10.1021/es200248d

Tran, N. H., Reinhard, M., and Gin, K. Y.-H. (2018). Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water research, 133, 182-207. https://doi.org/10.1016/j.watres.2017.12.029

Triebskorn, R., Casper, H., Heyd, A., Eikemper, R., Köhler, H.-R., and Schwaiger, J. (2004). Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss). Aquatic Toxicology, 68(2), 151-166. https://doi.org/10.1016/j.aquatox.2004.03.015

Trombini, C., Kazakova, J., Montilla-López, A., Fernández-Cisnal, R., Hampel, M., Fernández-Torres, R., Bello-López, M. Á., Abril, N., and Blasco, J. (2021). Assessment of pharmaceutical mixture (ibuprofen, ciprofloxacin and flumequine) effects to the crayfish Procambarus clarkii: A multilevel analysis (biochemical, transcriptional and proteomic approaches). Environmental research, 200, 111396. https://doi.org/10.1016/j.envres.2021.111396

Valcárcel, Y., Alonso, S. G., Rodríguez-Gil, J., Castaño, A., Montero, J., Criado-Alvarez, J., Mirón, I., and Catalá, M. (2013). Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). Environmental Science and Pollution Research, 20, 1396-1412. https://doi.org/10.1007/s11356-012-1099-2

Vieno, N., Hallgren, P., Wallberg, P., Pyhälä, M., Zandaryaa, S., and Commission, B. M. E. P. (2017). Pharmaceuticals in the aquatic environment of the Baltic Sea region: a status report (Vol. 1). UNESCO Publishing.

Vieno, N. M., Härkki, H., Tuhkanen, T., and Kronberg, L. (2007). Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. Environmental science and technology, 41(14), 5077-5084. https://doi.org/10.1021/es062720x

Waltersson, U., and Kjellberg, M. (1997). Havsöringens reproduktion i Stockholms län 1995. Länsstyrelsen i Stockholms län report 1997:06.

Yang, Y., Ok, Y. S., Kim, K.-H., Kwon, E. E., and Tsang, Y. F. (2017). Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. Science of The Total Environment, 596, 303-320. https://doi.org/10.1016/j.scitotenv.2017.04.102