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Summary - sammendrag

This report summarises the findings of a screening study into the occurrence of selected pharmaceuticals and hormones in effluents, sludges, surface waters, sediments and biota.

Denne rapporten oppsummerer resultatene av en screeningundersøkelse i forekomsten av utvalgte legemidler og hormoner i avløp, sludges, overflatevann, sedimenter og biota.

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Summary

The screening programme for 2015 focused on the occurrence of selected pharmaceuticals and hormones in wastewater from hospitals, wastewater treatment works (WWTW) influent, effluent and sludges as well as in surface water, sediment and biota in Oslofjord and Lake Mjøsa.

Wastewater effluent from three Norwegian hospitals was shown to contain elevated loads of selected pharmaceuticals (i.e. amiloride, amitriptyline, amlodipine, atorvastatin, azithromycin, ciprofloxacin, clarithromycin, diclofenac, fexofenadine, hydrochlorothiazide, lidocaine and simvastatin). Typically the overall contribution from hospitals, based on the median daily loads, to the receiving wastewater treatment works was < 0.28% for Ullevål, <10% for SI Hamar and <6% or UNN Tromsø.

Treated wastewater from four different WWTWs was shown to contain elevated loads of selected pharmaceuticals. Median daily loads were as high as 2.4 x 10^6 mg day^{-1} for certain pharmaceuticals (i.e. ciprofloxacin and fexofenadine). Effluent median loads of certain pharmaceuticals (i.e. atorvastatin, ciprofloxacin, diclofenac) and hormones were reduced when compared to influent loads, while median daily loads of other pharmaceuticals (i.e. amiloride, amitriptyline, azithromycin, clarithromycin, fexofenadine, lidocaine, simvastatin) were higher or similar in the treated effluent than influent.

Median sludge concentrations ranged from between <2.5 and 415 ng g^{-1} with the following selected pharmaceuticals detected; amitriptyline; azithromycin, clarithromycin, fexofenadine, lidocaine. Hormone sludge concentrations were typically low (<1 ng g^{-1}).

Few residues of the discharged pharmaceuticals were detected in surface water, sediment and biota samples from the recipients of Oslofjord and Lake Mjøsa.

Histopathological analysis of gonads and plasma vittellogenin levels in cod and trout gonads from Oslofjord and Lake Mjøsa respectively showed no effects attributable to the low levels of steroid hormones being discharged from WWTWs.

The overall risk to the Norwegian aquatic environment from inputs of the selected pharamceuticals studied in this report is low with the levels determined below published EQS and PNEC values. For example, the EU Watch List pharmaceutical diclofenac and hormones E2 and EE2 were below the EQS. The risks to agricultural soils from the levels determined in sludges was also deemed to be low, however the absence of $PNEC_{Soil}$ for amitriptyline and fexofenadine suggests that further work should be performed on these two pharmaceuticals with respect to their occurrence.

Sammendrag

Screeningsprogrammet for 2015 fokuserte på forekomsten av utvalgte legemidler og hormoner i avløpsvann fra sykehus, vannrenseanleggs innløp, avløp og slam samt i overflatevann, sediment og biota i Oslofjorden og Mjøsa.

Avløpsvannet fra tre norske sykehus viste seg å inneholde forhøyede mengder av utvalgte legemidler (dvs. amilorid, amitriptylin, amlodipin, atorvastatin, azitromycin, ciprofloksacin, klaritromycin, diklofenak, feksofenadin, hydroklortiazid, lidokain og simvastatin). Det samlede bidraget til de mottagende renseanleggene, basert på median av daglige mengder, var vanligvis <0,28% for Ullevål, <10% for SI Hamar og <6% for UNN Tromsø.

Behandlet avløpsvann fra fire ulike renseanlegg viste seg å inneholde høye mengder av utvalgte legemidler. Median daglige mengder var så høye som 2.4×10^6 mg dag^{-1} for visse legemidler (dvs. ciprofloksacin og feksofenadin). Medianmengden av visse legemidler (dvs. atorvastatin, ciprofloksacin, diklofenak) og hormoner i avløpsvann var redusert sammenlignet med det ubehandlede vannet, mens median daglig mengde av andre legemidler (dvs. amilorid, amitriptylin, azitromycin, klaritromycin, feksofenadin, lidokain, simvastatin) i det behandlede avløpsvannet var høyere eller lik innløpet.

Mediankonsentrasjoner i slam varierte fra mellom <2.5 og 415 ng g^{-1} med de følgende detekterte legemidler: amitriptylin; azitromycin, klaritromycin, feksofenadin, lidokain. Konsentrasjoner av hormoner i slam var vanligvis lave (<1 ng g^{-1}).
Få rester av de utslipte legemidlene ble oppdaget i overflatevann, sediment og biotaprøver fra resipientene i Oslofjorden og Mjøsa.

Histopatologiske analyser av gonadene og plasma vittellogenin-nivåer i torske- og ørretgonader fra Oslofjorden og Mjøsa viste henholdsvis ingen effekter som kan tilskrives de lave nivåene av steroidhormoner som slippes ut fra vannrenseanleggene.
Den samlede risikoen for det norske vannmiljøet fra tilførsel av legemidler er lav, med nivåene fastsatt under de publiserte EQS og PNEC-verdiene. For eksempel var EU Watch list-legemidlet diklofenak og hormonene E2 og EE2 under EQS. Basert på de fastslåtte verdiene for nivåene i slam ble også risikoen for landbruksjord vurdert som lav, men fraværet av $Soil_{PNEC}$ for amitriptylin og feksofenadin viser at videre arbeid bør gjøres på forekomsten av disse to legemidlene.

1. Background and Introduction

1.1 General

The Norwegian Environment Agency in 2015 selected two groups of compounds for target analysis for inclusion in Part 1 of its annual screening programme. These were pharmaceuticals and hormones. The objective of the project was to establish the occurrence of these chemicals in the Norwegian marine and freshwater environments, with particular focus on their sources potential to bioaccumulate. Additional focus was also placed on hospitals as direct sources of pharmaceutical residues to wastewater treatment works (WWTW) and WWTWs as point sources of pharmaceuticals to the Norwegian aquatic environment. The pharmaceuticals and hormones were selected for screening as they have been identified as potentially problematic compounds due to their extensive use and the intrinsically active properties. The European Union has started to look at the regulation of pharmaceuticals in the environment and in order to initiate this work in Norway there is a need for further environmental occurrence data and establish whether pharmaceuticals and hormones are potentially having an adverse environmental impact.

The pharmaceuticals and hormones selected, their uses, and previous occurrence in Noridc samples are summarised in Table 1. Of the pharmaceuticals selected, amiloride, amlodipine, amoxicillin, azithromycin, candesartan, ciprofloxacin, clarithromycin, diclofenac, 17ß-estradiol, 17α-ethinylestradiol, hydrochlorothiazide, ibuprofen, levonorgestrel, sulfamethoxazole had previously been included in screening studies in one or more Nordic country (Table 1). Amitriptyline, atorvastatin, chloramphenicol, fexofenadine, gliclazide, lidocaine, ramipril and simvastatin were included in a Nordic screening study for the first time.

Table 1. Pharmaceuticals selected for screening, their uses and previously reported levels in Nordic screening studies (TemaNord 2012:519)

Pharmaceutical	CAS No.	Use	Concentrations in various matrices							
			WWTW influent (ng L^{-1})	WWTW effluent (ng L^{-1})	Hospital WW (ng L^{-1})	Surface waters (ng L^{-1})	Sludge (ng g^{-1})	Sediment (ng g^{-1})	Biota (ng g^{-1})	Country
Amiloride	2016-88-8	Potassium-sparing diuretic used for treating hypertension	Nd-287	Nd-217	Nd-8	53-552	4-57	Nd-0.9		Faroe Islands, Greenland & Iceland
Amitriptyline	50-48-6	Serotonin-ephinephrine reuptake inhibitor used for treating mental illness								
Amlodipine	88150-42-9	Calcium channel blocker used for treating hypertension and coronary artery disease	Nd-247	Nd-319	Nd-273	Nd-18	Nd-286	Nd-1.5	No data	Denmark, Faroe Islands, Greenland & Iceland
Amoxicillin	26787-78-0	Antibiotic used against a number of bacterial infections	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Norway & Sweden
Atorvastatin	134523-00-5	Lipid-lowering agent used for treating dyslipidemia and to prevent cardiovascular disease								
Azithromycin	83905-01-5	Antibiotic used against a number of bacterial infections	Nd-44	Nd-27		Nd-16	Nd-6		Nd	Sweden
Candesartan	139481-597	Angiotensin II receptor antagonist used for treating hypertension	Nd-57	Nd-111	Nd-251	Nd-5	Nd-50	Nd		Faroe Islands & Iceland
Chloramphenicol	56-75-7	Antibiotic used against a number of bacterial infections when safer antibiotics cannot be used. Rapid inactivation in nature.								

Pharmaceutical	CAS No.	Use	Concentrations in various matrices							
Ciprofloxacin	85721-33-1	Antibiotic used against a number of bacterial infections	Nd-2,920	Nd-1,320	Nd-101,000	Nd.-160	100-7,400	Nd-130	Nd-9	Finland, Norway & Sweden
Clarithromycin	81103-11-9	Antibiotic used against a number of bacterial infections	Nd-620	Nd-780		Nd-11,000		Nd-5	Nd	Sweden
Diclofenac	15307-86-5	NSAID used to treat inflammations and to reduse pain and fever	Nd-9,700	Nd-3,900	Nd-0.6	Nd-880	Nd-560	Nd-3.5	Nd	Faroe Islands, Finland, Greenland, Iceland & Sweden
17ß-estradiol	50-28-2	Sex hormone	Nd-465	Nd-357	Nd-358	Nd-1.2	Nd-310	Nd-130	Nd	Denmark, Faroe Islands, Greenland & Iceland, Norway, Sweden
17α-ethinylestradiol	57-63-6	Contraceptive pill	Nd-54	Nd-40	Nd-20	Nd-3.7	Nd-6800	Nd	Nd-0.9	Denmark, Faroe Islands, Greenland & Iceland, Norway, Sweden
Fexofenadine	83799-24-0	Second-generation antihistamine used to treat allergy symptoms								
Gliclazide	21187-98-4	A sulfonylurea used for treating hyperglcemia								
Hydrochlorothiazide	58-93-5	Diuretic used for treating hypertension and swelling due to fluid build up	Nd-90	22-984	6-345	Nd	Nd-168	Nd		Faroe Islands, Greenland, Iceland
Ibuprofen	15687-27-1	NSAID used to treat inflammations and to reduse pain and fever	Nd-16,000	Nd-7,800	3-12,100	Nd-900	Nd-22,000	Nd-2.8	Nd	Denmark, Faroe Islands, Greenland & Iceland, Norway, Sweden
Levonorgestrel	797-63-7	Synthetic progestogen	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Sweden
Lidocaine	137-58-6	Anaesthetic used to numb tissue in a spesific area and to treat ventricular tachycardia								
Ramipril	8733-19-5	Angiotensin-converting enzyme (ACE) inhibitor used to treat								

Pharmaceutical	CAS No.	Use	Concentrations in various matrices							
		hypertension and congestive heart failure								
Simvastatin	79902-63-9	Statin used to treat dyslipidemia and to prevent atherosclerosis-related complications such as stroke and heart attacks								
Sulfamethoxazole	723-46-6	Antibiotic used against bacterial infections such as urinary tract infections, bronchitis and prostatitis	Nd-1,500	Nd-290	Nd-6,600	Nd-620	Nd-151	Nd-16	Nd-13	Denmark, Norway, Sweden

Nd- Not detected. Blank- no reported data.

2. Materials and Methods

2.1 Sample description and collection

2.1.1 Wastewater treatment plants (WWTWs)

Four wastewater treatment works (WWTW), representative of different sized populations and influent sources, were selected for investigation.

Vestfjorden avløpsselskap (**VEAS**) at Slemmestad is Norway´s largest WWTW receiving municipal wastewater from a population of around 550,000. The plant annually receives between 100-110 x 10^6 m^3 of wastewater that is treated mechanically, chemically and biologically (post-denitrification). The sludge is treated by anaerobic digestion and drying. Treated effluent is discharged into Oslofjord at a depth of approximatley 50 m.

HIAS WWTW receives wastewater from approximatley 52,000 people from the municipalities of Hamar, Løten, Ringsaker, and Stange. The plant is located at Ottestad on Lake Mjøsa with the discharge point at a depth of 15 m around 250 m from the shore. Wastewater is treated mechanically, biologically (not N removal) and chemically. The sludge is treated by thermal hydrolysis (Cambiprocess at 160°C) prior to anaerobic digestion at 38°C.

Rambekk WWTW in the municipality of Gjøvik receives wastewater from approximately 17,900 people plus industry (11,600 PE). The plant is located on the shore of Lake Mjøsa with the discharge point at a depth 7 m. The wastewater is treated mechanically and chemically. The resulting sludge is treated together with external sewage sludge from up to eight municipalities in the region by mesophilic (34-39°C) anaerobic digestion at a pH of approximately 7, followed by drying.

Breivika WWTW in Tromsø municipality receives domestic wastewater from a total of 2,850 households and the University hospital Nord-Norge (UNN Tromsø) (total of ca. 18,000 people equivalents). The wastewater is treated by simple screening (Salsnes Filter SF 6,000 with 0.35 mm mesh size) and the plant has a capacity of 18,700 person equivalents. The sludge that is removed is dewatered in a screw press and sent to Balsfjord municipality (Stormoen) for windrow composting. The treated wastewater is discharged at 30 m depth and ca. 300 m out into the Tromsøy strait.

WWTW sampling

Staff at the respective plants collected all of the WWTW samples. They were kindly asked not to use plastic gloves during samples and to avoid the use of personal care products. Twenty-four hour composite effluent samples were collected by means of an automatic sampling equipment already found at the WWTWs for routine monitoring. The effluent samples were collected in clean glass bottles (1x 2.5 L bottle, 1x 0.5 L bottle and 2x 0.25 L bottles, where the three small bottles were top-filled) and shipped to NIVA. 3 x 0.25 L bottles containing Imsdals water were opened during sampling on one of the sampling days and used as a field blank. Sludge samples were collected on the same days as the effluent samples using a

procedure based on the Mattilsynet guideline for the sampling of sludge, compost and other waste-based fertilizer products. Five core samples of mixed sludge were collected from each facility. Each mixed sample was transferred to 4 glass sample jars using pre-washed stainless steel equipment provided by NIVA.

2.1.2 Hospitals

Three hospitals were selected for this study:

Ullevål University Hospital is one of the largest hospitals in Oslo. It has approximately 45,000 admittances and 400,000 patient consultations annually covering a broad spectrum of somatic illnesses, including oncology and psychiatry patients. The hospital discharges untreated effluent to VEAS WWTW.

SI Hamar Hospital is the main hospital for a population of almost 85,000 people in the Hamar, Stagne, Løten and Ringasker region. Effluent is discharged untreated to HIAS WWTW.

The University Hospital of Northern Norway (**UNN Tromsø**) is based in Tromsø. The hospital offers treatment for a broad spectrum of somatic illnesses, as well as having specialist departments for oncology and psychiatry patients from the whole of Northern Norway. The hospital effluent constitutes approximately a 10% of the influent to Breivika WWTW.

Hospital effluent sampling

Ullevål University Hospital

An automatic sampler (ISCO 6712; provided by Oslo VAV) was mounted in a manhole (No. 210237) in a part of the sewer that included only, and most probably the main part of, the wastewater coming from the hospital area (See Figure 1). An ISCO 2150 flowmeter (provided by NIVA) was mounted in the same manhole and was coupled to the autosampler to provide 5 x flow-proportional 24-hour composite samples from 50 ml sub-samples collected every 30 m^3 wastewater. 2 L of the sample was transferred to 2x 1 L plastic bottles and brought directly to NIVA for freezing.

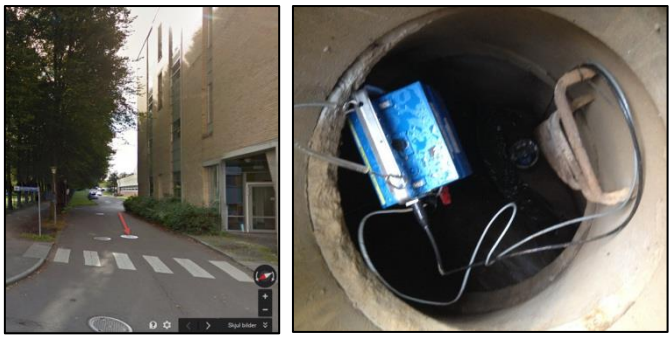


Figure 1. Automatic sampler and flowmeter mounted in manhole at Ullevål hospital.

SI Hamar

An automatic sampler (ISCO 6700; provided by NIVA) was mounted on top of a manhole in a part of the sewer that included only, and the main part of, the wastewater coming from the hospital area. An ISCO 2150 flowmeter (provided by NIVA) was mounted in the same manhole

and was coupled to the autosampler to provide 5x flow-proportional 24 hour composite samples from 50 ml sub-samples ollected every 0.5 m^3 wastewater. 2 L of the sample was transferred to 2x 1 L plastic bottles provided by NIVA and stored frozen until shipment to NIVA.

UNN Tromsø
An automatic sampler (ISCO 6700; provided by NIVA) was mounted in a manhole (S302) upstream a V-shaped overflow (to assure high enough water level in the sewer even during nights) in a part of the sewer that included only, but all, wastewater coming from the hospital area. The sampler collected 5x 24-hour composite samples from 50 ml sub-samples collected every 8 min, preferentially during dry-weather conditions. 2 L of the sample was transferred to 2x 1 L plastic bottles provided by NIVA and stored frozen until shipment to NIVA. A flow meter (ISCO 2150; provided by Tromsø municipality) was mounted through a manhole upstream of the autosampler. This stopped working after the three first samples. The flows during the two last sampling days were estimated based on flow measurements done on days with similar precipitation patterns.

2.1.3 Inner Oslofjord

Surface water
Water was collected at five stations with a Niskin water sampler, at 10 meters depth. The water samples were taken at the same stations as the sediment samples. The water sample was transferred to a 2.5 litre bottle and stored cold until analysis.

Sediment
Sediment samples were collected at five stations along a transect from close to the discharge diffuser from the VEAS WWTW and southward in the deep-water channel of Oslofjord (Figure 2). On the west side of the fjord, the tidal current runs in a southerly direction and is split by a vortex near the middle of the fjord south of Søndre Langåra. There are also currents through Ristsundet on the east side of Håøya, and one current on the west side of Håøya (Gråøyrenna). On rising tide most of the current flows on the east side of Håøya. Sediment stations were placed in the deep channel on both sides of Håøya. The sediment stations were on approximately same depths. Sediment was collected with a stainless steel Van Veen grab. One sample of the top 2 cm of the sediment was collected from each station. Each sample was a mixed composite from three grabs.

Figure 2. Map of the sediment stations in the Oslofjord.

Atlantic cod and herring

Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*) were caught by trawling from the research vessel F/F Trygve Braarud during 19th of August 2015. The cod were caught in the area between Askerlandet and Steilene in the Inner Oslofjord and ranged in size from 1.1 to 5.8 kg. Cod was killed with a blow to the head with a priest (club), and then dissected on-board. Individual samples of liver were removed for chemical analysis and stored in heat-treated (500 °C) glass containers sealed with heat-treated aluminium foil underneath the lids. Samples were stored frozen (-20º C) until analysis.

Blood samples were taken from the cod with heparinized syringes. Plasma was separated with a centrifuge, and then the samples were kept in liquid nitrogen until transfer to -80°C freezer at NIVA. Samples of gonads were dissected out and put in cassettes and then put into two kinds of fixation solutions (Baker's and Davidson's fixation solutions). Gonad samples were then sent to IRIS for pathological analysis.

Individual samples were made of five herring. The samples consisted of whole fish without head and spine.

Shrimp

Northern shrimp (*Pandalus borealis*) were caught by trawling. The shrimps were peeled and split into 5 bulk samples. Each sample comprised of between 50 and 60 individual shrimps.

Blue mussel

Blue mussel (*Mytilus edulis*) was sampled at five stations in the Oslofjord. The blue mussels were sampled from stations nearby the discharge from VEAS, and on stations on increasing distance from the discharge in southward direction (Figure 3; Table 2).

Figure 3. Blue mussels were sampled at five stations (●) in the Oslofjord.

Table 2. Blue mussel stations with distance from the VEAS WWTW	
Blue mussel sampling stations	Distance from VEAS
B1 Sjøstrand	900 meters north
B2 Slemmestad	1.3 km south
B3 Nærsnesbukta	3.2 km south
B4 Håøya, Bjørnhodebukta	9.2 km south
B5 Sætre, Skålevika	12.7 km south

One bulk sample was made from each station. Each sample comprised of soft tissue from 20 blue mussels (length 6-8 cm).

2.1.4 Lake Mjøsa

Surface water
Water was collected at five stations with a Ruttner water sampler, at 15 meters depth (26th May 2015). The water samples were taken at the same stations as the sediment samples. Each water sample was transferred to two l litre PE bottles and stored cold until analysis.

Sediment
Five pooled samples of sediment were taken along a gradient from the discharge point to HIAS and south (26th May 2015). Each pooled sample consisted of three individual subsamples taken from the upper 0-2 cm sediment layer at a water depth of 25–35 m. We used a gravity corer with a core tube and a retractable sediment stopper in stainless steel. The samples were transferred to heat-treated (500 °C) glass containers sealed with heat-treated aluminium foil underneath the lids. The core tube and other sectioning equipment used were thoroughly cleaned with acetone and cyclohexane (HPLC grade) before use, and direct hand contact with the sampling matrix was avoided. They samples were stored frozen (-20 °C) until analysis.

Fish
From Lake Mjøsa, between 11. – 30. August 2015, we collected the following species of pelagic fish: brown trout (*Salmo trutta*), smelt (*Osmerus eperlanus*) and vendace (*Coregonus albula*). Smelt and vendace were caught with gillnets, deployed in the area around the outlet of discharge pipe of the HIAS sewage treatment plant, at a depth of about 20 – 35 m, whereas brown trout were caught north of the town of Gjøvik at a depth of 5 –20 m. The smelt were mainly small-bodied planktivorous individuals, but a few larger cannibalistic individuals were also included.

Smelt and vendace were taken out of the nets as they were hauled, instantly killed with a short blow to the head, wrapped in clean aluminium foil, kept cool and transported to a freezer (-20 °C). Before freezing the aluminium foil wrapped fish were put in polyethylene bags. At no time were the fish allowed to be in contact with plastics or other potentially contaminated surfaces. The time between catch and transfer to the freezer took no longer than 4 hours.

The brown trout were transferred to a water filled container after they were taken out of the nets, transported alive to the shore where a bench with the necessary tools and equipment for sampling of blood and histological samples of gonads and liver were waiting. The biological samples were prepared in accordance with a Standard Operating Procedure. From each fish a blood sample was taken from the caudal vein with a syringe pre-treated with heparin and aprotinin, centrifuged with subsequent removal of supernatant before snap-frozen in liquid nitrogen. A liver tissue sample was dissected and snap-frozen in liquid nitrogen, whereas a gonad sample was transferred to a cassette and formalin fixated. The fish was then wrapped in aluminium foil and transported to a freezer (-20°C) for later dissection and preparation of the muscle samples for chemical analyses.

Before preparing the pelagic fish muscle samples, they were thawed and the total length and weight recorded (Table 3). The fish were then scraped clean of mucus with a solvent washed knife and placed on a cutting board covered with solvent rinsed aluminium foil. A solvent cleaned set of stainless steel dissection tools was used for each fish. The sagittal otoliths were dissected and sex and maturity determined following opening of the abdomen. Samples of lateral skeleton muscles were then dissected and transferred to heat-treated (500 °C) glass containers sealed with heat-treated aluminium foil placed underneath the lids. The samples were then frozen (-20°C) and sent for homogenization before analysis. Five pooled samples were prepared for smelt and vendace, respectively, to obtain sufficient material for chemical analysis, whereas 10 individual samples were prepared for brown trout.

All personnel involved in fieldwork and sample preparation avoided the use of personal care products for at least 24 hours prior to any activity. Dissection and sample preparation was performed outside in a non-urban area. All dissection equipment and aluminium foil that could have direct contact with the samples was cleaned with acetone and cyclohexane (HPLC grade) before use, and direct hand contact with the sampling matrix was avoided.

Table 3. Mean length and weight of the studied fish. Pooled samples were made for smelt and vendace.

			Length (cm)		Weight (cm)	
Species	Sample No.	N	Mean	Std Dev	Mean	Std Dev
Smelt	1	5	21.7	0.6	55.6	9.2
	2	7	17.2	1.3	29.1	7.2
	3	8	15.8	0.2	24.3	1.5
	4	11	14.7	1.0	18.0	4.1
	5	40	11.4	0.6	10.7	1.3
Vendace	1	4	21.4	0.9	75.5	4.7
	2	3	21.7	0.4	74.3	6.5
	3	4	20.8	0.9	73.8	11.3
	4	3	21.4	1.1	75.3	10.5
	5	4	20.7	0.4	69.8	3.6
Brown trout	1:10	10	64.8	13.1	3569	2321

Mysis and zooplankton

Samples of the opossum shrimp *Mysis relicta* and zooplankton were sampled with horizontal net hauls. Epipelagic zooplankton, consisting mainly of the cladocerans *Daphnia galeata* and *Bosmina longispina* together with the copepod *Eudiaptomus gracilis*, were collected at a depth of 3–5 m, whereas Mysis were collected at a depth of 70–110 m (this is a diurnal vertical migrating Mysida, mainly feeding in the epipelagic zone during night-time). The zooplankton net used were made of nylon mesh (single strand thread, mesh size: 500 µm), equipped with a brass cup with a brass mesh, and with an opening diameter of 1 m.

Mysis were separated from copepods in the hypopelagic samples by filtering the samples through a sieve (mesh of stainless steel strands) while flushing gently with water from the lake and handpicking with tweezers. All filtering and separation of samples were done in the boat immediately after net hauling. The samples were kept on the same type of cleaned class jars as the fish, held cool on board until they could be transferred to a freezers (-20°C) no more than 8 hours after sampling. All equipment (glass or metal) and aluminium foil that could be in direct contact with the samples after they were transferred from the net were cleaned with acetone and cyclohexane (HPLC grade) before use, and direct hand contact with the samples was avoided. We prepared 5 samples each of Mysis and epipelagic zooplankton.

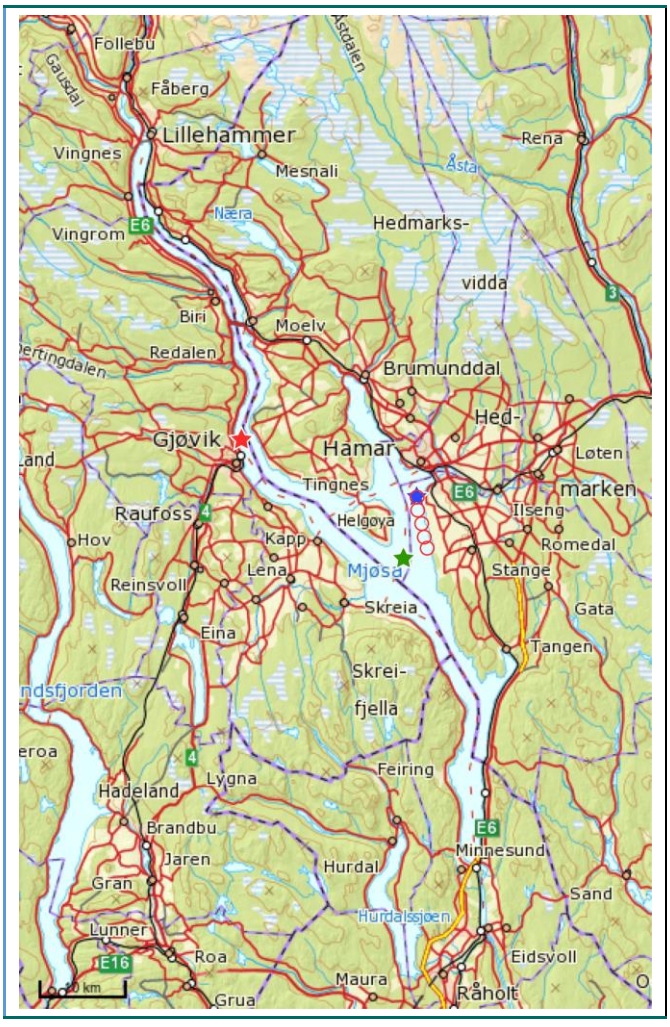


Figure 4. Map showing Lake Mjøsa, the catch sites (blue star: smelt and vendace; red star: brown trout; Mysis and zooplankton: green star) and sediment/water sampling sites (red circles). The location coordinates are given in Table 4

Table 4. Coordinates for the Lake Mjøsa water, sediment and biota sampling stations						
Station	Date	Depth (m)	UTM 33E	UTM 33 N	°E	°N
Sediments/water						
St-1	26.05.2015	sed.: 35, water: 15	286400	6743600	11.059	60.766
St-2	26.05.2015	sed.: 25, water: 15	285941	6742150	11.075	60.759
St-3	26.05.2015	sed.: 25, water: 15	285932	6740684	11.072	60.744
St-4	26.05.2015	sed.: 25, water: 15	286479	6739302	11.084	60.732
St-5	26.05.2015	sed.: 25, water: 15	287021	6737370	11.096	60.715
Fish						
St-1	11.08.15	20–35	286400	6743600	11.059	60.766
St. Gjøvik	13.-30.08.15	10-20	265100	6750000	10.680	60.816
Mysis	10.-13.08.14	70–110	284000	6735000	11.04	60.69
Zooplankton	13.09.15	3-5	284000	6735000	11.04	60.69

2.2 Chemical analysis

2.2.1 Pharmaceuticals

Biota (approx. 1 g wet weight), and sediment and sludge (exactly 0.5 g dry weight) aliquots were taken. Deuterated internal standard was added to each aliquot before the addition of 1 ml 0.1 M zinc sulfate. 1 min of vortex mixing resulting in cell lysis. 4 ml ACN added to further precipitate proteins and extract the pharmaceuticals by further vortexing.

1 g QuECHERS (80-85% Magnesium Sulfate and 15-20 % Sodium Acetate; Waters, Sweden) extraction powder was added to each extract and mixed by vortex for 1 min. The samples were centrifuged at 2500 rpm for 6 minutes and the supernatent (300 µL) transferred to deep-well 96-well plate (2 ml). 600 µL of ultrapure water was added to each well. The well plates were centrifuged at 3500 g for 10 minutes. The calibration standard were also transferred to 96-well plate and diluted as per samples. The samples were then analysed on a ultra performance liquid chromatograph coupled to a high resolution mass spectrometer. The uncertainty (relative standard deviation; RSD) of the concentrations determined using this method is estimated to be around 25%.

2.2.2 Hormones

Aliquots of freshwater (approx. 450 ml) and effluent (approx. 200 ml) were liquid/liquid extracted with dichloromethane. The dichloromethane was evaporated, the residue redissolved in small amount of acetonitrile and split into two aliqots. One was derivatized with dansylchloride and used for the determination of estradiol and ethynylestradiol and the other was derivatized with perfluoroglutaric anhydride and used for determination of levonorgestrel.

Sediment and sludge samples (1-2 g) were extracted with acetonitrile/hexane (50:50). The acetonitrile was removed and reduced in volume, divided in 2 and derivatised as with the aqueous phase samples. Deuterium labeled hormones were used as internal standards for

quantification. The instrument used was LC/MS-MS (Accela liquid chromatograph with TSQ Vantage mass-spectrometer).

2.2.3 Biomarkers and pathology

Vitellogenin analysis

Vitellogenin (VTG) production was measured through a capture ELISA as previously described by Sundt and Björkholm (2011) and Tollefsen et al. (2003), with minor modifications. In brief, frozen plasma samples of brown trout (*Salmo trutta*) and cod (*Gadus morhua*) were thawed on ice and diluted 1:50 - 1:5000 in a coating buffer (50 mM bicarbonate-carbonate buffer, pH 9.6), followed by dilution of trout VTG (with 3–3000 ng/ ml) and Cod Vtg (0.24-250 ng/ ml) (Biosense Laboratories, Bergen, Norway). The diluted samples, buffer blanks and respective VTG standard were transferred to a 96-well Maxisorp Nunc-immunoplate (Nunc, Roskilde, Denmark) in triplicate, sealed and incubated in the dark at 4°C for <16 h. The plates were rinsed three times with washing buffer (0.05% Tween-PBS, pH 7.2 (T-PBS)), followed by the addition of the blocking solution (2% bovine serum albumin (BSA)-PBS) and incubated for 1 h in the dark at room temperature. Following incubation, the plates were rinsed three times in T-PBS and a poly-clonal rabbit anti-cod VTG antibody (CS-1, 1:1000x diluted in PBS containing 1% BSA, Biosense Laboratories, Bergen, Norway) and a mono-clonal mouse anti-salmon Vtg antibody (BN-5, 1:6000× diluted in PBS containing 1% BSA, Biosense Laboratories, Bergen, Norway) was added in respective plates, followed by incubation at 37°C for 2 h. The plates were rinsed three times with T-PBS and the secondary antibody goat anti-rabbit IgG (1:3000x diluted in PBS containing 1% BSA, Bio-Rad, Herculeas, CA, USA) and goat anti-mouse IgG (1:6000x diluted in PBS with 1% BSA, Bio-Rad, Herculeas, CA, USA) were added respectively, and incubated at 37°C for 1 h. Following incubation, the plates were rinsed five times with T-PBS and the HRP enzyme substrate (TMB plus2, KEMENTEC diagnostics, Taastrup, Denmark) was added to all wells to start the colour development. The plates were incubated in the dark at room temperature for 15 min before colour development was stopped with 1 M H_2SO_4. Absorbance was measured at 450 nm using a Versamax microplate reader (Molecular Devices, USA). The total amount of VTG protein in the plasma samples was calculated using the known amount (ng/ ml) of VTG in respective standards.

Histology

Histological parameters are commonly used as markers of health status in various fish species. The identification of pathologies and diseases are increasingly being used as indicators of environmental stress since they provide a definite and ecologically relevant end-point for chronic/ sub chronic contaminant exposure. The application of histological markers in fish can include measures of reproductive and metabolic condition, and allows for the detection of various pathogens that may affect population mortality. The data generated from this type of analysis in various organs (i.e. gills, gonads, digestive gland) is helpful in providing information for biomonitoring programme (Corbett et al., 2011).
Histopathological alterations illustrate a definitive endpoint of historical exposure, intermediate between initial biochemical changes and reproductive capability and growth (Stentiford et al., 2003, Salamat et al., 2013).

Histopathology in digestive gland and gill
10 samples from cod collected from the Oslofjord, and 10 samples from trout collected from Lake Mjøsa were analysed. Gonad were dissected by IRIS and placed in histo-cassettes and into histological fixative (3.7% formaldehyde) for wax sections. Tissue samples were no thicker than 1 cm to ensure proper fixation. Samples were then stored at 4˚C until embedding.

Histological sections (3 µm) were prepared at Stavanger University Hospital (SUS). The tissues were examined for health parameters related to physiological conditions, inflammatory and non-specific pathologies and those associated with pathogen and parasite infections. Gonad abnormalities were scored using the criteria suggested by Benly et al. (2008) and Sensini et al. (2008). Each alteration was scored according to its severity and frequency (0 = absence of alteration, 1 = ≤ 10 % of the histological section showed the alteration, 2 = between 10% and 50% of the histological section showed the alteration, 3 = between 50% and 100% of the histological section showed the alteration).

The presence of parasites and non-specific inflammation were scored as absent (0) or present (1). All micrographs were captured using an AxioCam MRc5 (Zeiss) digital camera mounted on a *Zeiss Axioplan 2* light microscope (Göttingen, Germany). The slides were analysed blind. The stage of the gonads was also evaluated.

2.2.4 Support Parameter analysis

Particle Size Analysis
Wet sediment was shaken using a mechanical fractionator fitted with < 63 µm sieves. Dry weight measurements were used for the particle size calculations.

Sediment TOC
Freeze dried sediment sample aliquots (0.5-10 mg) were heated in a furnace at 1,800 ºC in the presence of oxygen free helium. The carbon dioxide gas produced was passed through a chromatography column and the total organic carbon was measured.

Water DOC
Samples (4 ml) were injected into an inorganic carbon chamber and 0.5 ml 21% phosphoric acid was added. The inorganic bound carbon from carbonates, bicarbonates and dissolved CO_2 is released to an NDIR detector for CO_2 quantification.

Lipid content
Lipids were calculated gravimetrically following solvent extraction and performed in duplicate for each sample. A column packed with an aliquot of the sample (approximately 0.5-1 g liver or 5 g filet) and sodium sulphate was extracted with 100 ml dichloromethane. The solvent extract was then evaporated to dryness and the remaining lipids dried at 110 ºC to constant weight.

δ13C/δ13N ratio analysis

Samples were dried at 60 oC for 24 hours before grinding to fine powder. Approx 1 mg of sample was combusted in the presence of O2 and Cr2O3 at 1700 oC in a Eurovector element analyser. Reduction of NOx to N2 was done in a Cu oven at 650 oC. H2O was removed in a chemical trap of Mg(ClO4)2 before separation of N2 and CO2 on a 2 m Porapolt Q GC column. The C/N ratio was quantified on the basis of the m/z 44/28 ratio. N2 and CO2 were directly injected online to an isotope ratio mass spectrometer (Nu Instruments Horizon) for the determination of δ13C and δ13N. The mean stable N-isotope ratios, δ15N, reflects the relative trophic position of the organisms. Likewise, the stable C-isotope ratio, δ13C, reflects the carbon sources of the organism. A low δ13C/δ13N ratio indicates influence from a pelagic food chain whereas a higher ratio indicates a more littoral food chain. We have lipid-adjusted all the δ13C-ratios in order to remove the effect of 13C-depleted lipids in the fatty burbot samples.

3. Results and Discussion

3.1 Hospital effluent

3.1.1 Pharmaceuticals

Twelve of the 19 pharmaceuticals were detected in the hospital effluents from Ullevål, SI Hamar and UNN Tromsø (Figure 5). These were amiloride, amitriptyline, amlodipine, atorvastatin, azithromycin, ciprofloxacin, clarithromycin, diclofenac, fexofenadine, hydrochlorothiazide, lidocaine and simvastatin. The data were transformed to daily loads (mg day^{-1}) in order to aid comparison and assess the contribution of hospital effluent to the connected WWTW influent load. Multiplying the measured concentrations by the flow of effluent achieved this. Loads were calculated for the pharmaceuticals not detected using a value of 0.5 x LOD. The median effluent pharmaceutical loads from Ullevål hospital were up to 54 mg/day for ciprofloxacin with loads of lidocaine (24 mg day^{-1}) and fexofenadine (21 mg day^{-1}) being higher than the other pharmaceuticals, which were typically present at loads of <5 mg day^{-1}. Effluent from both SI Hamar and UNN Tromsø also contained elevated loads of ciprofloxacin (582 and 41 mg day^{-1} respectively) and lidocaine (1,093 and 26 mg day^{-1} respectively). In general the pharmaceutical loads from all three hospitals was < 5 mg day^{-1} with a few exceptions (e.g. atorvastatin and amiloride). This is in the same range as previously reported for Norwegian hospitals (Thomas et al., 2007; Langford & Thomas, 2009).

3.1.2 Hormones

E2 and EE2 were detected in all three of the hospital effluents (Figure 6; Figure 7; Figure 8). Levonorgestrel was not detected in any of the hospital effluents. Median daily E2 loads were typically between 8 and 42 mg day^{-1} and EE2 between 0.08 and 0.18 mg day^{-1}.

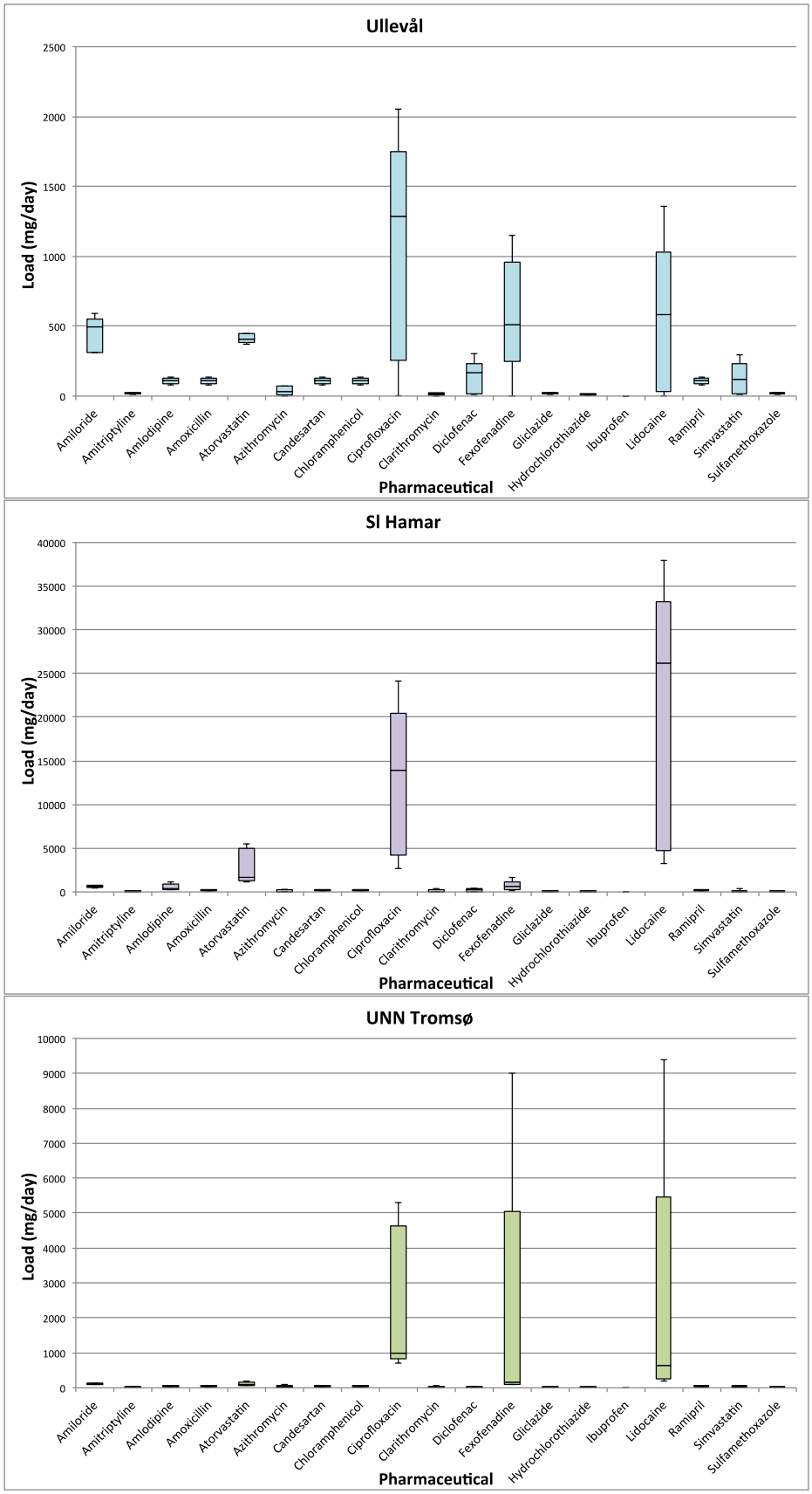


Figure 5. Box and whisker plots showing median (n=5), first quartile, third quartile, maximums and minimum daily loads from hospital effluents from Ullevål, Sl Hamar and UNN Tromsø

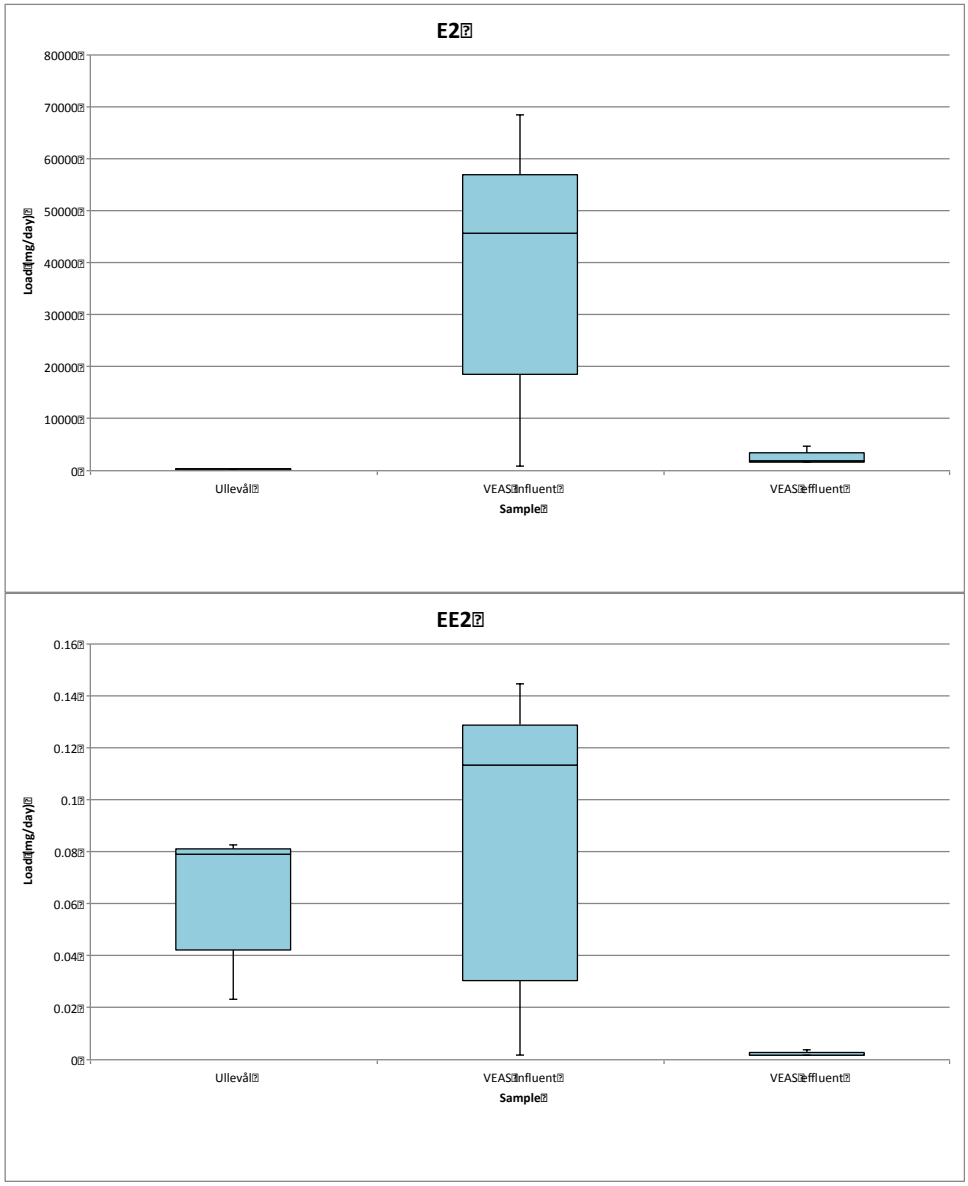


Figure 6. Box and whisker plots showing median (n=5), first quartile, third quartile, maximums and minimum daily loads of E2 and EE2 for Ullevål hospital effluent and VEAS WWTW influent and effluent

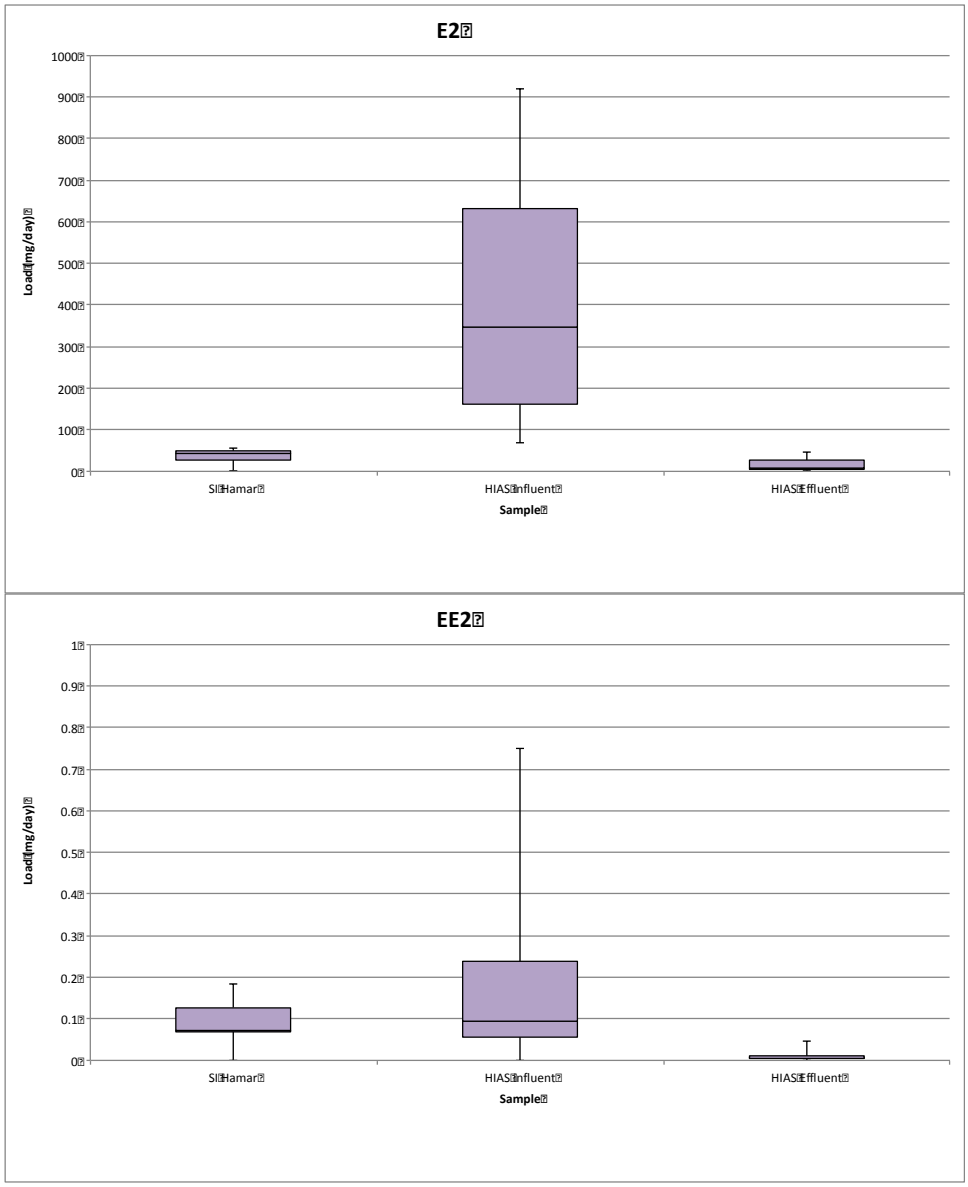


Figure 7. Box and whisker plots showing median (n=5), first quartile, third quartile, maximum and minimum daily loads of E2 and EE2 for SI Hamar effluent and HIAS WWTW influent and effluent

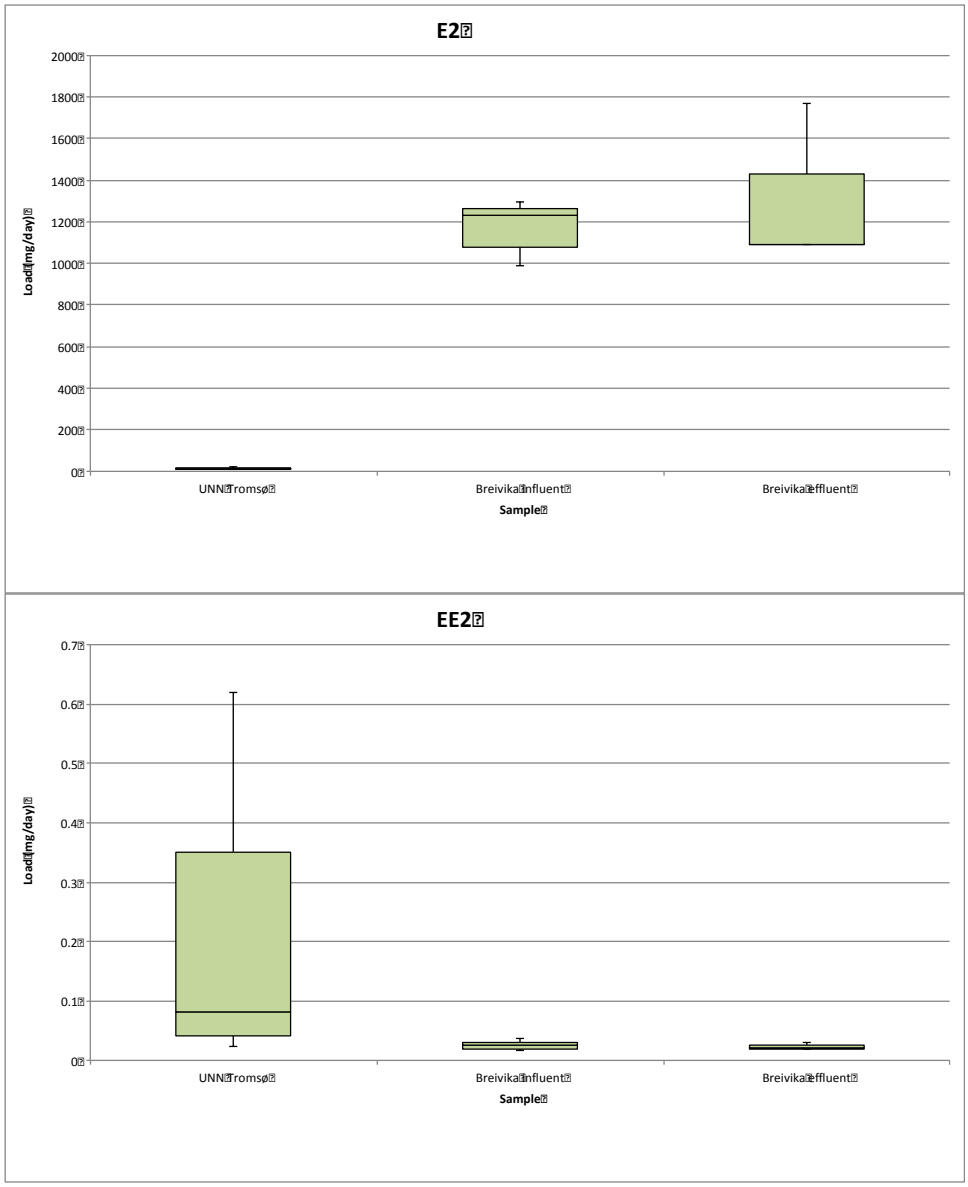


Figure 8. Box and whisker plots showing median (n=5), first quartile, third quartile, maximums and minimum daily loads of E2 and EE2 for UNN Tromsø effluent and Breivika WWTW influent and effluent

3.2 Wastewater treatment plants

3.2.1 Pharmaceuticals

Nine of the 19 pharmaceuticals were detected in either wastewater treatment works influent and/or effluent (Table 5). In the figures presented (Figure 5;

Figure 9; Figure 10; Figure 11) the concentrations determined have been transformed to loads and for compounds detected <LOQ, a value of LOQ x 0.5 has been used.

VEAS WWTW

Comparison of the median (n=5) pharmaceutical loads in the VEAS influent and effluent samples showed loads of up to 2.4 x 10^6 mg day^{-1} (ciprofloxacin and fexofenadine;

Figure 9). For the other pharmaceuticals analysed the loads were typically <50,000 mg/day. Comparison of influent with effluent loads for the detected pharmaceuticals changes in concentrations of between 23% (atorvastatin) and 475 % (simvastatin; Table 5).

The contribution in terms of daily loads from Ullevål Hospital to VEAS, based on a comparison of the median values was typically < 0.5% for all analysed pharmaceuticals. This compares with similar values for most pharmaceuticals in previous studies investigating the inputs of pharmaceuticals from Ullevål hospital to VEAS WWTW (Thomas et al., 2007; Langford & Thomas, 2009).

Four of the selected pharmaceuticals were detected in sludges from VEAS (Figure 10). Amitryptyline was detected at a median concentration of 77 mg kg^{-1}, azithromycin at 60 mg kg^{-1}, clarithromycin at a median concentration of 7.7 mg kg^{-1}, fexofenadine at a median concentration of 658 mg $kg^{-1}$and lidocaine <5 mg kg^{-1}.

Table 5. Comparison of the pharmaceutical loads in effluent and influent based on median concentrations

Pharmaceutical	VEAS	HIAS	Breivika	Rambekk
Amiloride	109	109	91	95
Amitriptyline	218	227	101	91
Amlodipine	100	86	101	100
Atorvastatin	23	67	104	56
Azithromycin	177	297	103	100
Ciprofloxacin	94	88	118	92
Clarithromycin	188	155	198	100
Diclofenac	86	194	146	49
Fexofenadine	157	106	172	114
Hydrochlorothiazide	100	65	100	800
Lidocaine	119	37	281	220
Simvastatin	475	3440	172	575

Table 6. Percentage contribution of each pharmaceutical from the three selected hospitals to the respective WWTW influent based on median loads

Pharmaceutical	Ullevål	SI Hamar	UNN Tromsø
Amiloride	0.05	2.5	0.2
Amitriptyline	0.04	2.5	0.2
Amlodipine	0.04	10	0.3
Amoxicillin	0.04	1.6	0.2
Atorvastatin	0.03	2.1	0.3
Azithromycin	0.01	1.6	0.3
Candesartan	0.04	1.6	0.2
Chloramphenicol	0.04	1.6	0.2
Ciprofloxacin	0.05	83	0.5
Clarithromycin	0.01	6.2	0.1
Diclofenac	0.05	9.3	0.03
Fexofenadine	0.03	3.5	0.4
Gliclazide	0.04	1.6	0.2
Hydrochlorothiazide	0.04	1.9	0.2
Lidocaine	0.25	786	5.5
Ramipril	0.04	1.6	0.2
Simvastatin	0.28	3.7	0.1
Sulfamethoxazole	0.04	1.6	0.2

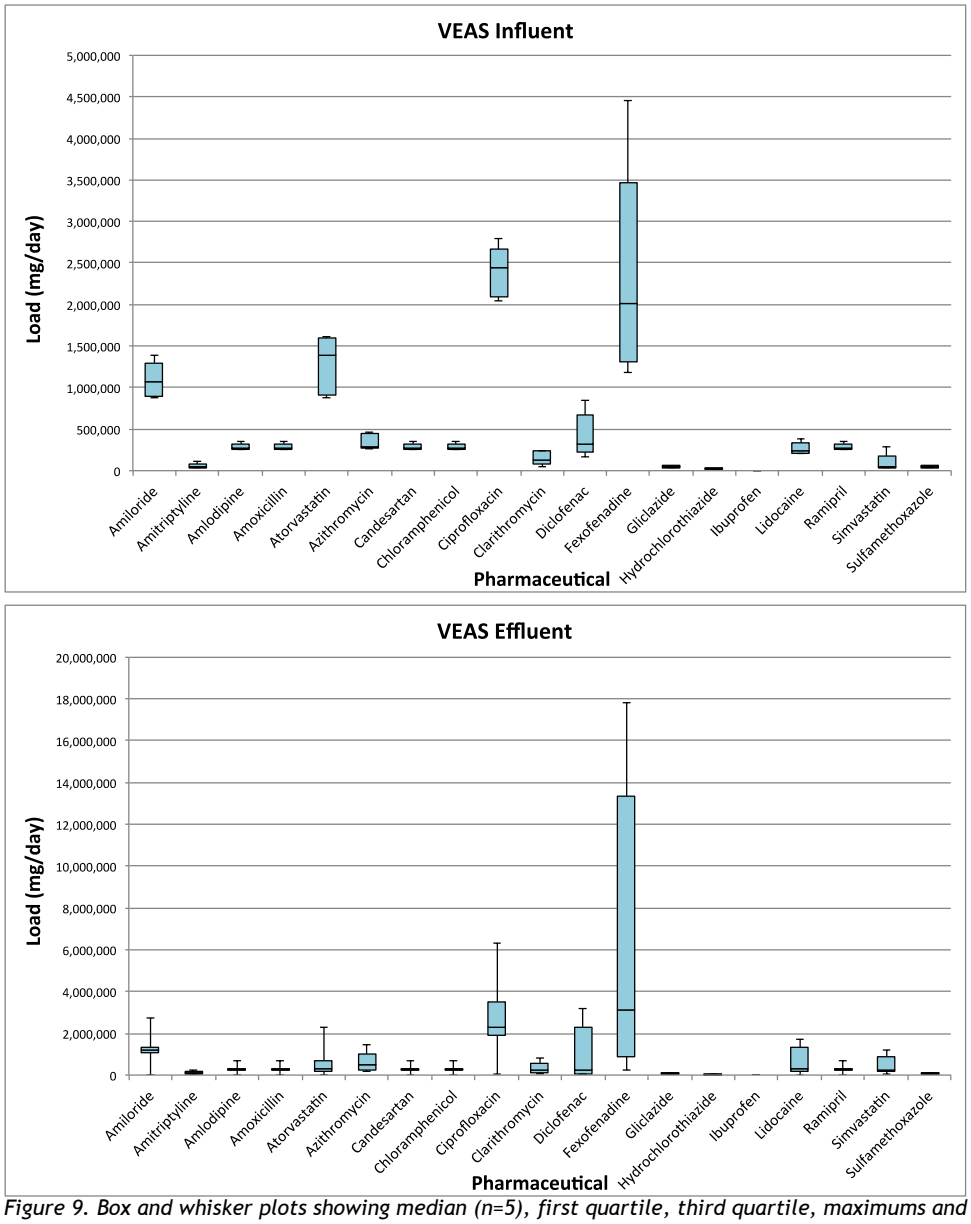


Figure 9. Box and whisker plots showing median (n=5), first quartile, third quartile, maximums and minimum daily loads for VEAS WWTW influent and effluents

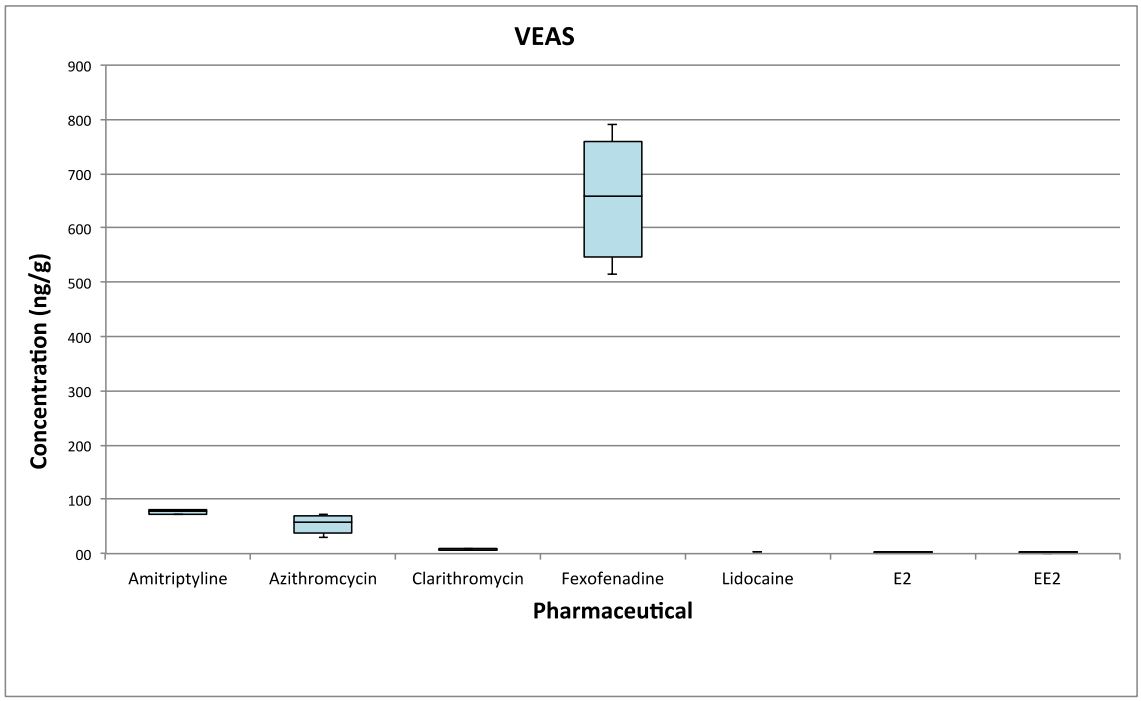


Figure 10. Box and whisker plots showing median (n=5), first quartile, third quartile, maximums and minimum concentration of pharmaceuticals and hormones in sludges from VEAS WWTW

HIAS WWTW

Influent and effluent from HIAS WWTW contained median pharmaceutical loads of up to 21,600 mg day^{-1} (atorvastatin). Elevated median loads of ciprofloxacin (11,600 mg day^{-1}) and fexofenadine (10,400 mg day^{-1}) were detected in HIAS influent (Figure 11). The difference in influent and effluent median daily loads for the detected pharmaceuticals showed a reduction in effluent concentration for amlodipine (14%), atorvastatin (33%), ciprofloxacin (12%), hydrochlorothiazide (35%) and lidocaine (63%) indicating some degree of removal at the WWTW before discharge into Lake Mjøsa (Table 5). For a number of the other compounds the median daily loads were greater in the effluent than the influent. For example for amitriptyline, azithromycin, clarithromycin, diclofenac and simvastatin there was an increase in the median effluent loads when compared with the influent. A possible explanation for this may be a high variability in the emission of these particular drugs in the HIAS catchment, resulting in a high level of variability in the concentrations measured, or also due to deconjugation of pharmaceuticals excreted as their sulphate or glucuronide congregates.
The contribution in terms of median daily pharmaceutical loads from SI Hamar to HIAS shows that the hospital typically contributes around 2% for each pharmaceutical analysed, apart from amlodipine (10%), ciprofloxacin (83%), clarithromycin (6%), diclofenac (9%) and lidocaine. The contribution is generally higher than Ullevål, however this proportion is clearly due to the larger dilution at VEAS from other sources than domestic wastewater.
Four of the selected pharmaceuticals were detected in sludge from HIAS (Figure 12). Amitryptyline was detected at a median concentration of 80 mg kg^{-1}, azithromycin at <30 mg kg^{-1}, clarithromycin at a median concentration of 5,6 mg kg^{-1}, fexofenadine at a median concentration of 447 mg $kg^{-1}$and lidocaine 10.9 mg kg^{-1}.

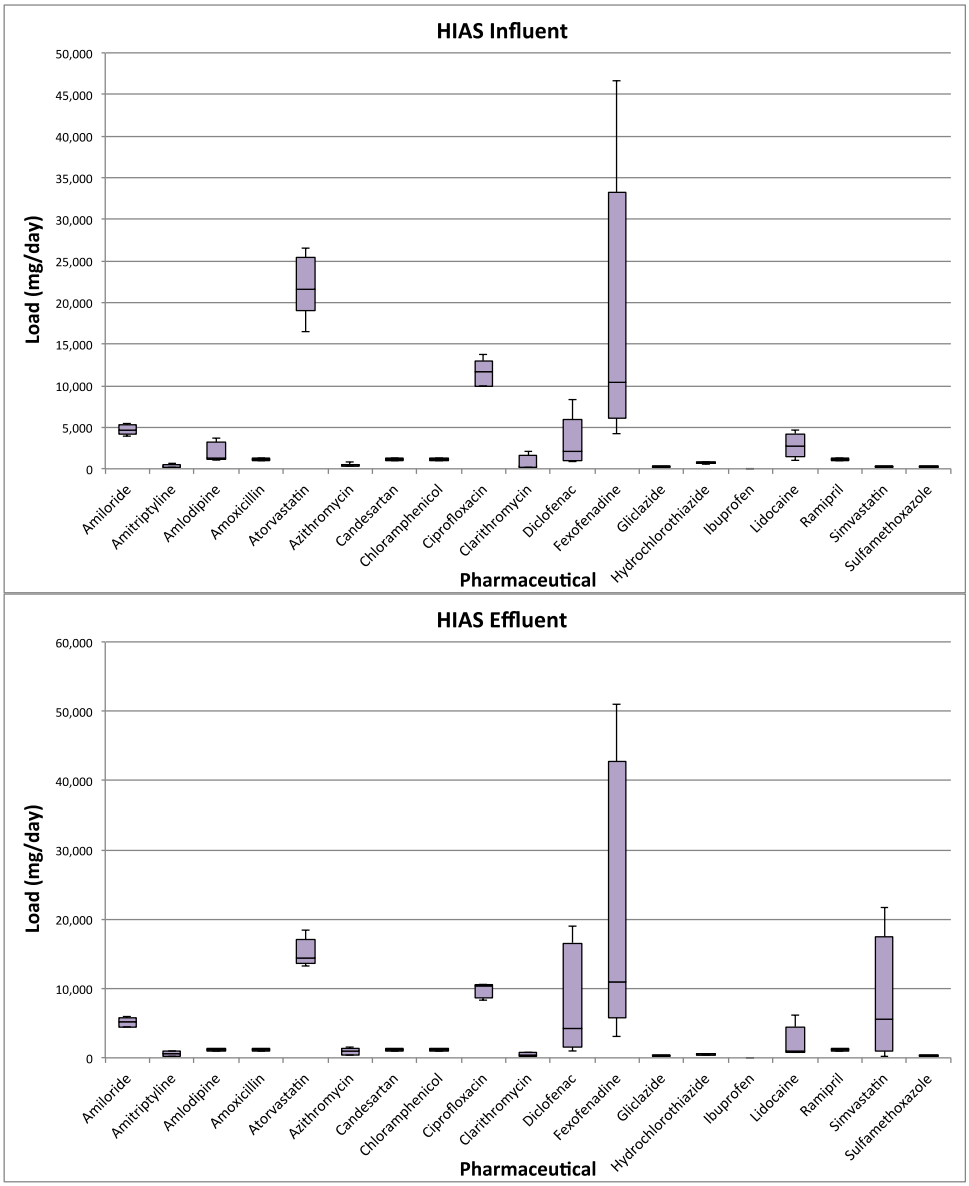


Figure 11. Box and whisker plots showing median (n=5), first quartile, third quartile, maximums and minimum daily loads for HIAS WWTW influent and effluents

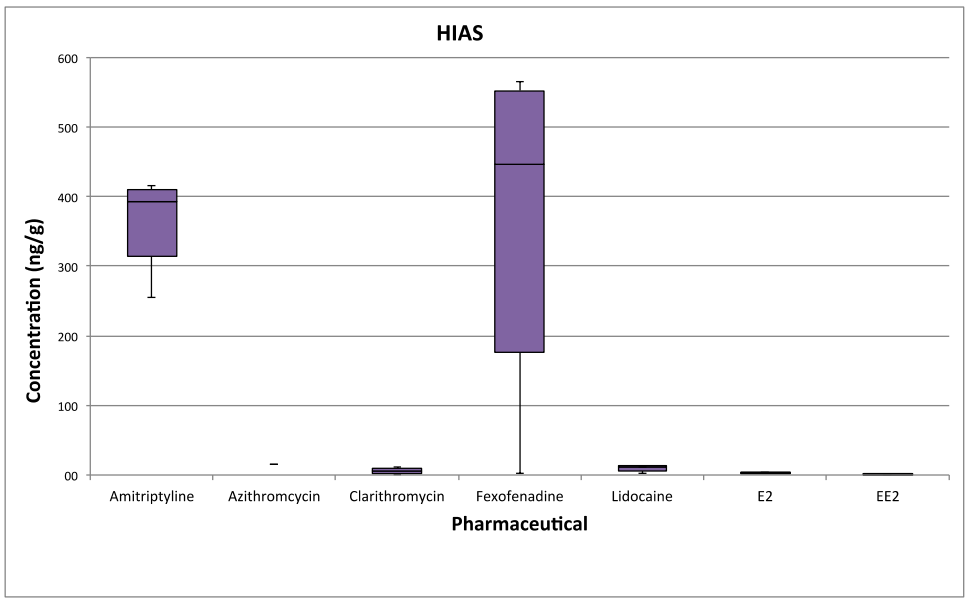


Figure 12. Box and whisker plots showing median (n=5), first quartile, third quartile, maximum and minimum concentration of pharmaceuticals and hormones in sludges from HIAS WWTW

Rambekk WWTW

Daily median influent and effluent loads determined at Rambek WWTW were generally below 4,225 mg day^{-1} (ciprofloxacin). Elevated median daily loads were also determined for amiloride (2,680 mg day^{-1}), atorvastatin (2,952 mg day^{-1}) and fexofenadine (2,306 mg day^{-1}). Of those pharmaceuticals, the median effluent daily loads of amiloride were only slight reduced (5%), while atorvastatin loads were reduced by around 45%, ciprofloxacin by 8% and diclofenac by 50%.

Five of the selected pharmaceuticals were detected in sludge from Rambekk (Figure 14). Amitryptyline was detected at a median concentration of 252 mg kg^{-1}, azithromycin at 34 mg kg^{-1}, clarithromycin at a median concentration of 8.4 mg kg^{-1}, fexofenadine at a median concentration of 348 mg $kg^{-1}$and lidocaine at 14 mg kg^{-1} in a single sample and <5 mg kg^{-1} in the other 4.

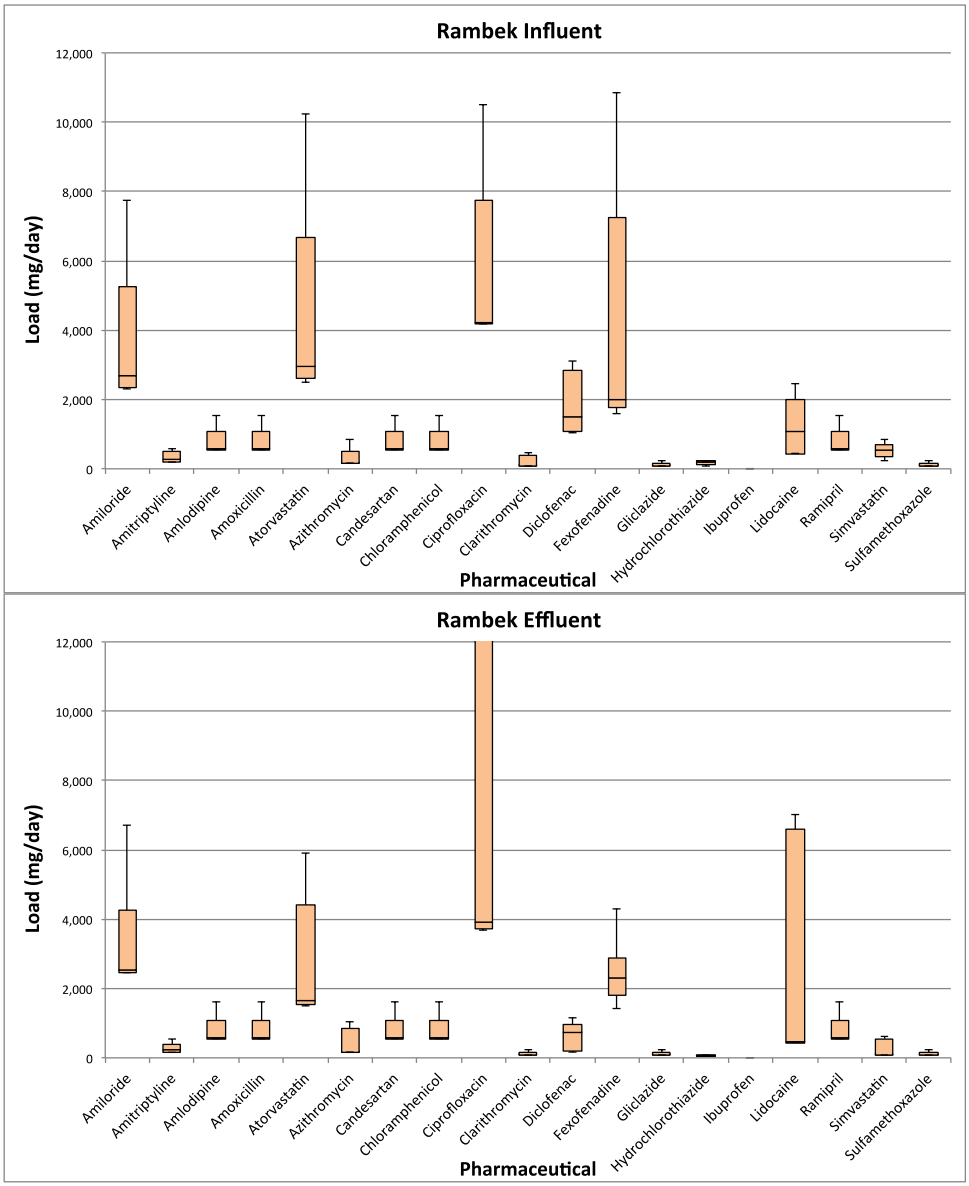


Figure 13. Box and whisker plots showing median (n=5), first quartile, third quartile, maximum and minimum daily loads for Rambekk WWTW influent and effluents

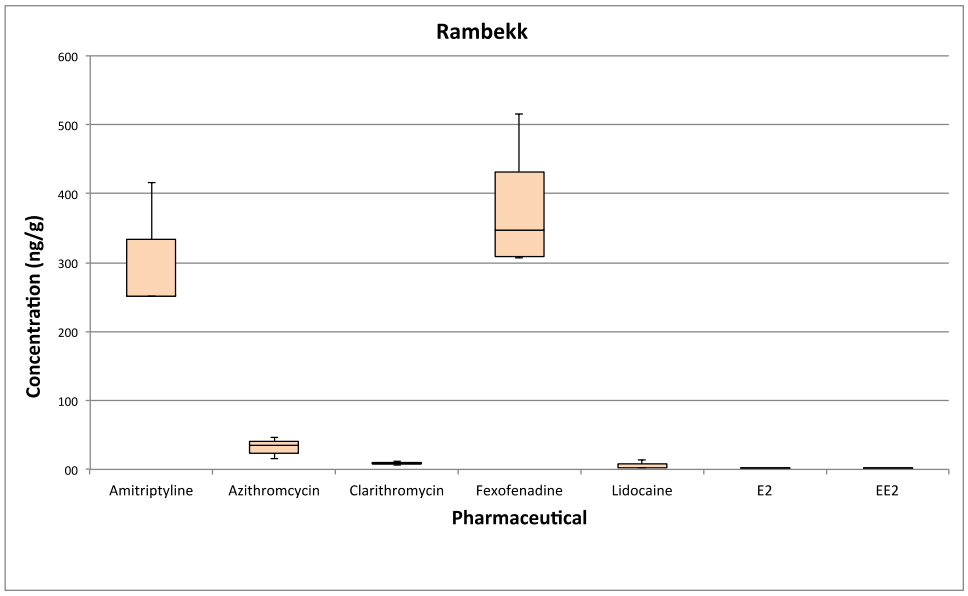


Figure 14. Box and whisker plots showing median (n=5), first quartile, third quartile, maximum and minimum concentration of pharmaceuticals in sludges from Rambekk WWTW

Breivika WWTW

Median influent and effluent loads of pharmaceuticals from Breivika were as high as 224,700 mg day^{-1} (Ciprofloxacin; Figure 15). Elevated median loads were also observed for amiloride (66,284 mg day^{-1}), atorvastatin (36,070 mg day^{-1}), diclofenac (13,492 mg day^{-1}), fexofenadine (36,482 mg day^{-1}), lidocaine (11,589 mg day^{-1}) and simvastatin (11,740 mg day^{-1}). In general there was little difference between the median influent and effluent loads determined at Breivika WWTW. Since Breivika influent only receives basic treatment it is not surprising that there is little difference in loads.

The contribution of UNN Tromsø to the overall loading is in general low, with the greatest contribution from ciprofloxacin at 0.5% of the total influent daily load.

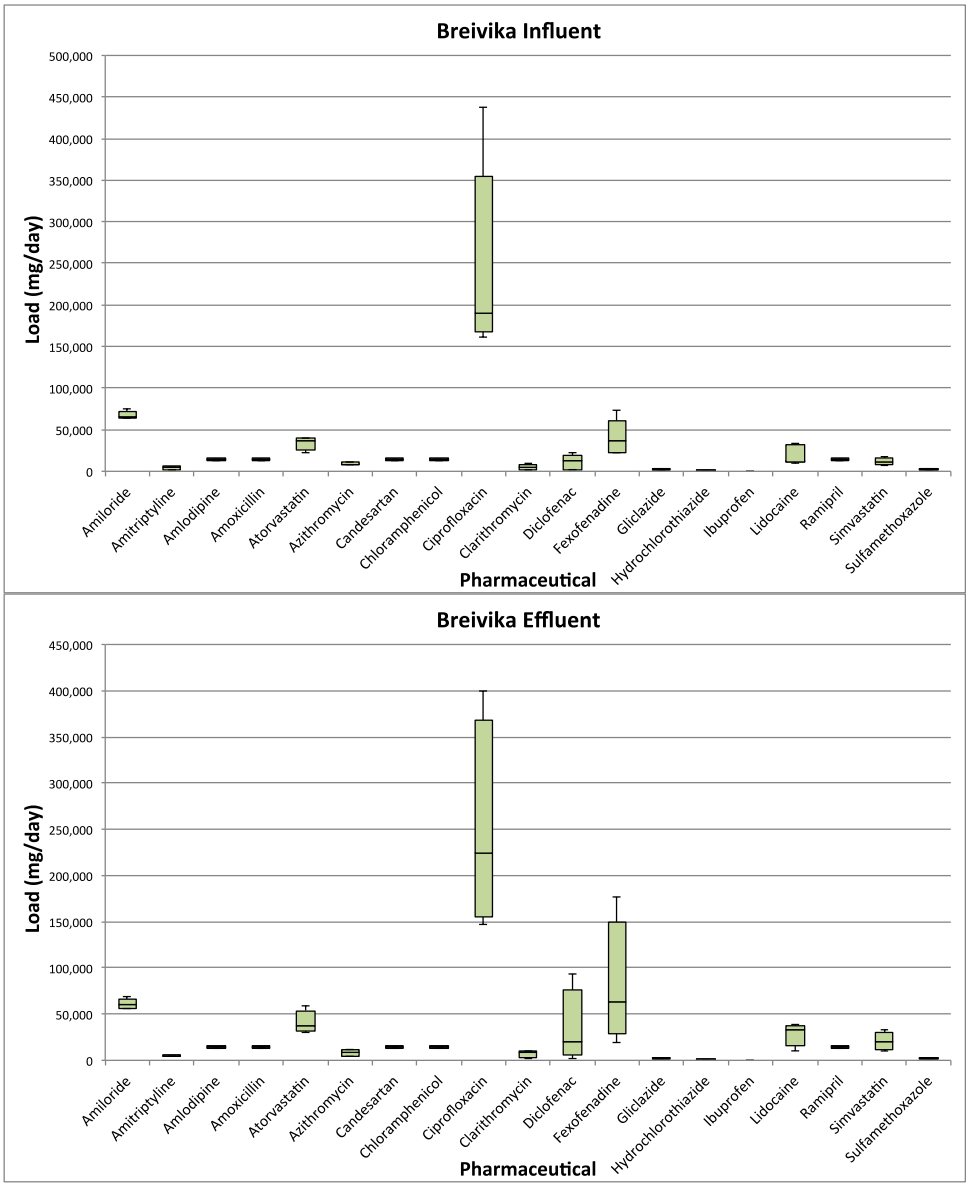


Figure 15. Box and whisker plots showing median (n=5), first quartile, third quartile, maximum and minimum daily loads for Breivika WWTW influent and effluents

3.2.2 Hormones

VEAS WWTW

The median influent daily load of E2 in VEAS WWTW influent was 45,600 mg day^{-1}, which was reduced to 1,972 mg day^{-1} following treatment (Figure 6). The daily loads of EE2 were much lower at 0.11 and 0.01 mg day^{-1} for influent and effluent respectively. As is shown in Figure 6 there is a significant reduction in the E2 and EE2 loads following treatment. The median sludge concentration of E2 was 1 ng g^{-1} and EE2 was 0.33 ng g^{-1} (

Figure 9).

HIAS WWTW

The median influent daily load of E2 in HIAS WWTW influent was 345 mg day^{-1}, which was reduced to 7 mg day^{-1} following treatment (Figure 7). The daily loads of EE2 were much lower at 0.23 and 0.01 mg day^{-1} for influent and effluent respectively. As with VEAS WWTW there is a significant reduction in the E2 and EE2 loads following treatment (Figure 7). The median sludge concentration of E2 was 2.9 ng g^{-1} and EE2 was 0.8 ng g^{-1} (Figure 11).

Rambekk WWTW

Unfortunatley no data are available on the levels of E2 and EE2 in Rambekk influent. Median daily effluent loads were 66 and 0.07 mg day^{-1} respectivley for E2 and EE2. These are in a similar range to the other WWTW effluents analysed in this study. The median sludge concentration of E2 was 1.1 ng g^{-1} and EE2 was 0.9 ng g^{-1} (

Figure 13).

Breivika WWTW

The median influent daily load of E2 in Breivika WWTW influent was 1230 mg day^{-1}, which was reduced to 1088 mg day^{-1} following treatment (Figure 8). The daily loads of EE2 were much lower at 0.025 and 0.021 mg day^{-1} for influent and effluent respectively. Unlike with the other WWTWs that are fitted with much more advanced treatment than Breivika there is little reduction in the hormone loads (Figure 8).

3.3 Inner Oslofjord

3.3.1 Pharmaceuticals

None of the 19 pharmaceuticals was detected in the Oslofjord surface water and sediment samples collected. Very few of the biota samples collected from Oslofjord contained detectable concentrations of the selected pharmaceuticals. Two samples of cod liver contained levels of amiloride (30 and 41 $\mu g\ g^{-1}$), while all samples of shrimp from Oslofjord contained amiloride (18-57 (median=29) $\mu g\ g^{-1}$). A previous screening study in 2008 showed the occurrence of certain pharmaceuticals (naproxen, morphine and carbamazepine) in surface waters from Oslofjord up to 400 m from the point of discharge. Many of the pharmaceuticals detected in the discharged effluent were not detected in the receiving environment and this is also the case in the present study. This suggests that the selected compounds are sufficiently labile to be transformed following release into the fjord and/or there is sufficient dispersion to reduce their concentrations to below that, which is detectable.

3.3.2 Hormones

E2 was detected in all 5 surface water samples from Oslofjord at concentrations of between 0.2 and 0.25 ng L^{-1}. EE2 was detected in a single surface water sample at 0.007 ng L^{-1}. E2 was detetcted in 2 of the 5 sediment samples at concentrations of 0.09 and 0.23 ng g^{-1}. EE2 was not detected in Oslofjord sediments. E2 was detected in a single cod liver sample at 0.55 ng g^{-1}, in a single herring sample at 0.21 ng g^{-1} and in all blue mussel samples at concentration of between 0.15 and 0.29 ng g^{-1}. None of the shrimp samples contained E2. EE2 was detected in a single herring sample at a concentration of 0.04 ng g^{-1} and in 3 of the 5 blue mussel samples at concentrations of between 0.007 and 0.02 ng g^{-1}. No other biota samples contained detectable concentrations of EE2.

3.3.3 Vitellogenin levels in cod plasma

VTGg was detected at low concentrations in the blood plasma of male cod (median 8.95 ng ml^{-1}; Figure 16). The ICES BAC for male cod has been established at 2,300 ng ml^{-1} (ICES SGIMC, 2012). This is almost 3 fold higher than the median value for male cod caught in Oslofjord and indicates no response to estrogens. The median VTG concentration for female cod was also low (15.87 ng ml^{-1}) and most likely reflects the natural reproductive stage of the female cod sampled.

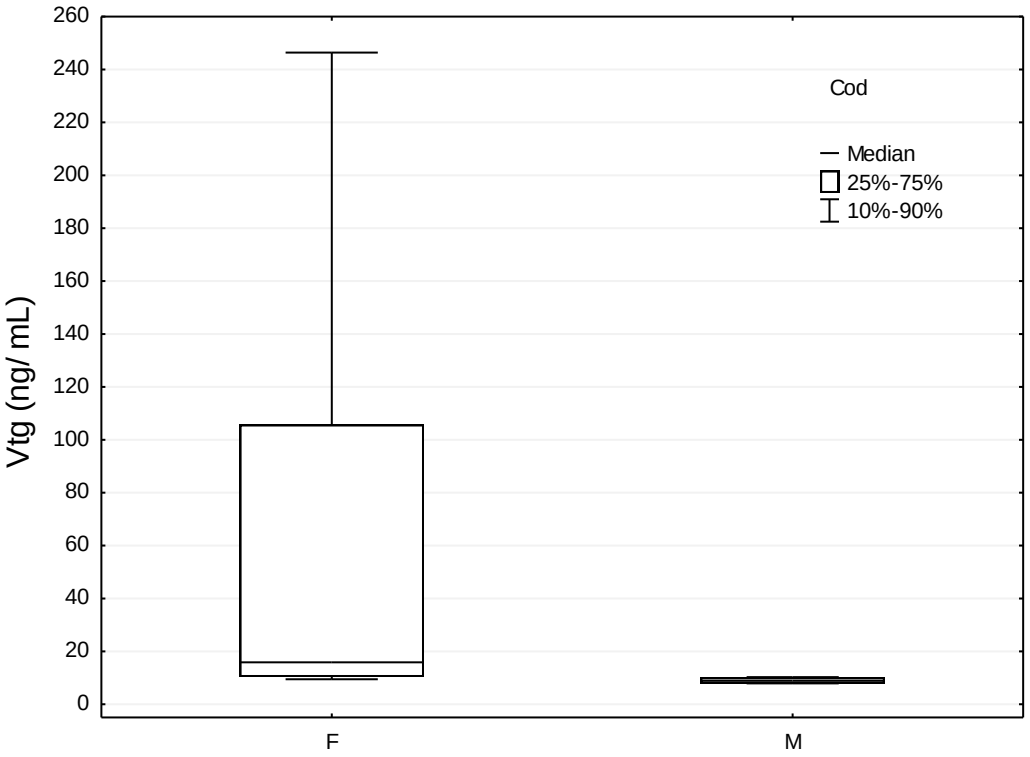


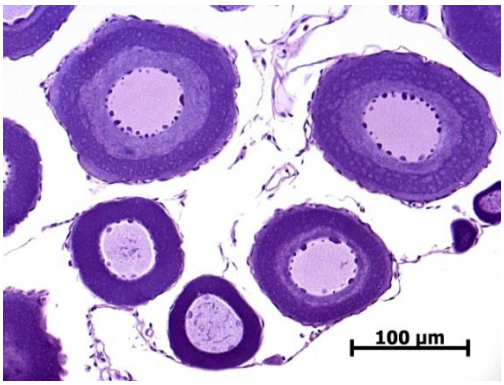
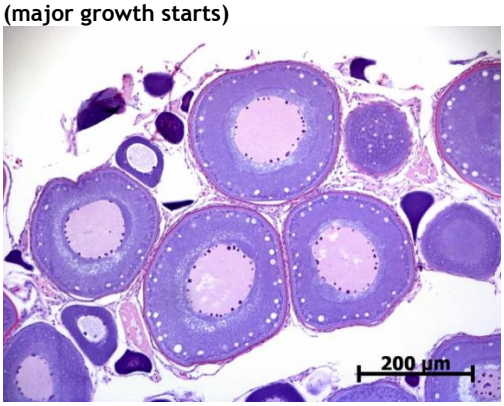
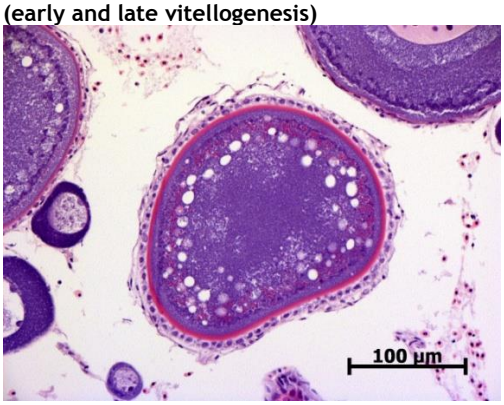
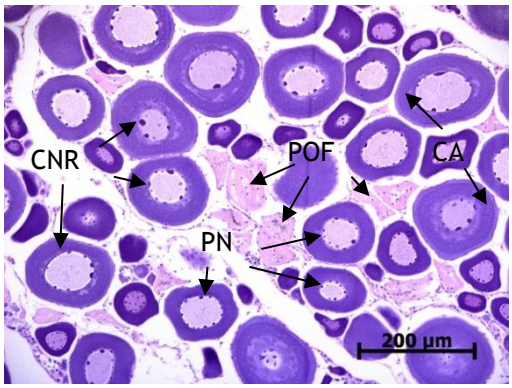
Figure 16. Vitellogenin concentrations in blood plasma samples taken from Atlantic cod from the Oslo fjord (median, quartiles, 10/ 90 percentiles, ng/ ml, F - female n=7, M - male n = 3).

3.3.4 Histological evaluation of cod gonads

A table was developed to score the gonadal development of female (Table 7) and male (Table 8), Atlantic cod. Results of the histological evaluation of the gonads are reported in Table 9 for the female fish and Table 10 for the male fish. In general, all cod were caught after they had spawned. Gonad stages were either spent (stage 5) or spent – ripening (stage 6). No particular histopathological conditions were found in general in this group of fish and a few females (n=3) showed a low degree of granulomatous inflammation.

Table 7. Summary of reproductive stages of female Atlantic cod

Reproductive stage	Description
1. Immature (small growth)	Ovary wall thin. Oocytes vary from small (20 µm) with pale uniform cytoplasm to larger with basophilic cytoplasm apart from ølight ring of mitochondria around nucleus. Oocytes up to about 130 µm in diameter are irregular in outline, then round. Several nucleoli at periphery of nucleus - **perinuclear stage PN**. Ring of mitochondria moves away - **circumnuclear ring stage CNR**.

	
2. Mature Ripening 1 **(major growth starts)** 	Zona radiata (**ZR**) appears as eosinophilic ring. Cortical alveoli appear in peripheral cytoplasm – **CA** ** Image is from stage 5, to show oocytes in stage of major growth but not the characteristics of the ovary*
3. Ripening 2 **(early and late vitellogenesis)** 	Yolk droplets **YD** first appear as small inclusions between vesicles, then enlarge and fill whole cytoplasm, restricting CA to periphery. Zona radiate widens and forms two layers in which radial striations can be seen. Irregular outlines of nucleus, nucleoli detached from periphery ** Image is from stage 6 (spent ripening), to show oocytes in stage of vitellogenesis but not the characteristics of the ovary*
4. Ripe and Spawning	Oocytes hydrates in batches. Yolk as homogenous mass.
5. Spent 	Empty follicles (**post ovulatrory follicles POF**) and a few atretic hydrated oocytes. Small oocytes: formation of zona radiata, CA

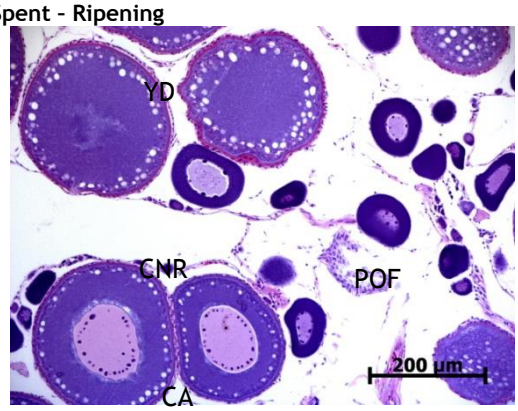
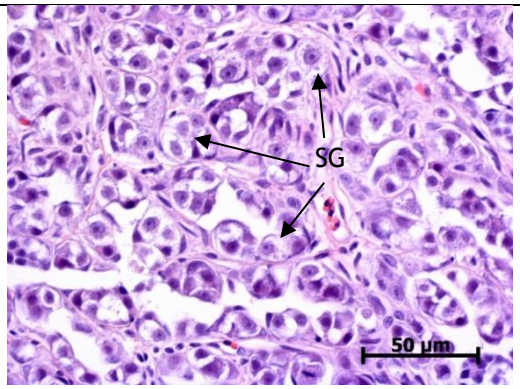
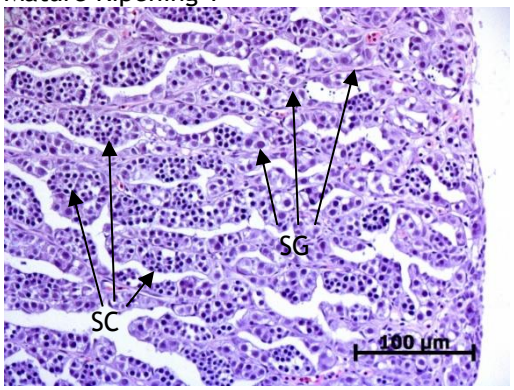
6. Spent - Ripening  **6*. Resting** **(see stage 5)**	Oocytes start vitellogenesis, empty follicles but no atretic hydrated oocytes Larger oocytes in CNR stage

Table 8. Summary of reproductive stages of male Atlantic cod

Reproductive stage	Description
1. Immature	Wall thin. Spermatogonia present single or in small groups

	
2. Mature Ripening 1 	Cysts of spermatocytes form
3. Ripening 2	All stages of development. Earlier stages along distal edge. In later stages cysts containing mature spermatozoa break down and coalesce to form tubules filled with spermatozoa, but lined with developing cysts. Few spermatozoa in large efferent ducts near mesochrium
4. Ripe and Spawning	All tubules and efferent ducts packed with spermatozoa. Few developing cysts left in early part of stage. Single or small groups spermatogonia in lining of tubules will give rise to SZ in next spawning season.
5. Spent	Tubules contain SZ. But few in large efferent ducts near mesochrium. Spermatogonia in distal part of testis

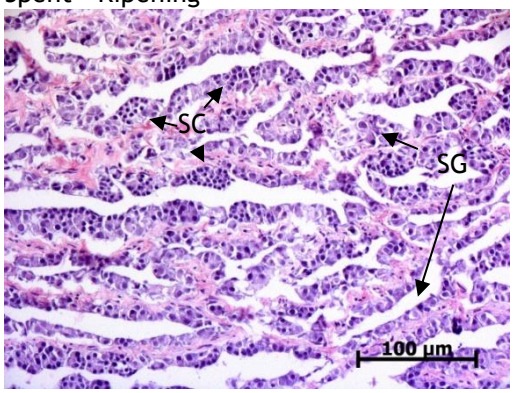
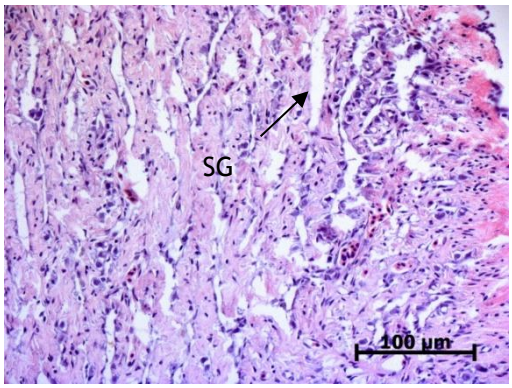
6. Spent – Ripening 	Cysts of spermatogonia SG and spermatocytes SC form in distal part of testis, SZ visible in proximal part
6*. Resting  7.	Distally only spermatogonia SG present. SZ may still be present in efferent ducts

Table 9. Results for female cod

Fish code n	1	3	5	6	7	9	10
Stage	5	6	6	5	6	5	6
Increased oocyte atresia							
Perifollicular cell hyperplasia/hypertrophy							
Decreased vitellogenesis							
Changes in gonadal staging							
Interstitial fibrosis							
Egg debris in the oviduct							
Increased vascular or interstitial proteinaceous fluid							
Granulomatous inflammation		1				3	1
Parasite							
Postovulatory follicles	1	1	1	3	2	3	1
Atretic follicles							
mmc		1				1	2

Blank: No observed occurrence.

Table 10. Results for male cod		
Fish code n	2	4
Stage	5/6	2/6*
Increased proportion of spermatogonia		
Presence of testis-ova		
Increased testicular degeneration (apoptotic)		
Interstitial (Leydig) cell hyperplasia/hypertrophy		
Decreased proportion of spermatogonia		
Interstitial fibrosis		
Increased vascular or interstitial proteinaceous fluid	2	
synchronous gonad development		
Altered proportions of spermatozoa or spermatocytes		
Gonadal staging		
Granulomatous inflammation		1
Parasite		

Blank: No observed occurrence.

3.4 Lake Mjøsa

3.4.1 Pharmaceuticals

None of the 19 pharmaceuticals was detected in the Lake Mjøsa surface water, sediment or biota samples collected.

3.4.2 Hormones

E2 was not detected in any of the surface water samples from Lake Mjøsa, while EE2 was detected in a single surface water sample at 0.013 ng L^{-1}. Neither E2 nor EE2 were detected in Lake Mjøsa sediment. E2 was detected in 7 of the trout livers at concentrations of between 0.01 and 0.32 ng g^{-1}, whilst EE2 was below the LOD. E2 was detected at concentrations of between 0.02 and 0.03 in 3 of the 5 European smelt samples, in 4 out of 5 vendance at concentrations of between 0.05 and 0.12 ng g^{-1}, and in 4 out of 5 of the Mysis samples at between 0.01 and 0.04 ng g^{-1}. EE2 was not detected in any of these samples.

3.4.3 Vitellogenin in trout plasma

Vitellogenin (VTG) was detected at low concentrations (median 8.75 ng ml^{-1}; Figure 17) in blood plasma samples of the two male brown trout and was indicative of typical low background concentrations in male fish. Although species-specific ICES background assessment criteria (BAC) are not currently available for brown trout with respect to VTG, BAC values are available for cod and flounder. In these cases a BAC of 2,300 ng ml^{-1} VTG and 1,300 ng ml^{-1} VTG for male cod and flounder respectively are reported (ICES SGIMC, 2011). Therefore, VTG levels in male fish >1,300 ng ml^{-1} would be needed to indicate an estrogenic response, which did not occur in the present screening. However, only 2 males were sampled of the 10 fish collected. As expected, female VTG concentrations were markedly higher

(median 1,522 ng ml^{-1}) than the males. These VTG concentrations were within the typical range reported in reference brown trout populations (Bjerregaard et al., 2006).

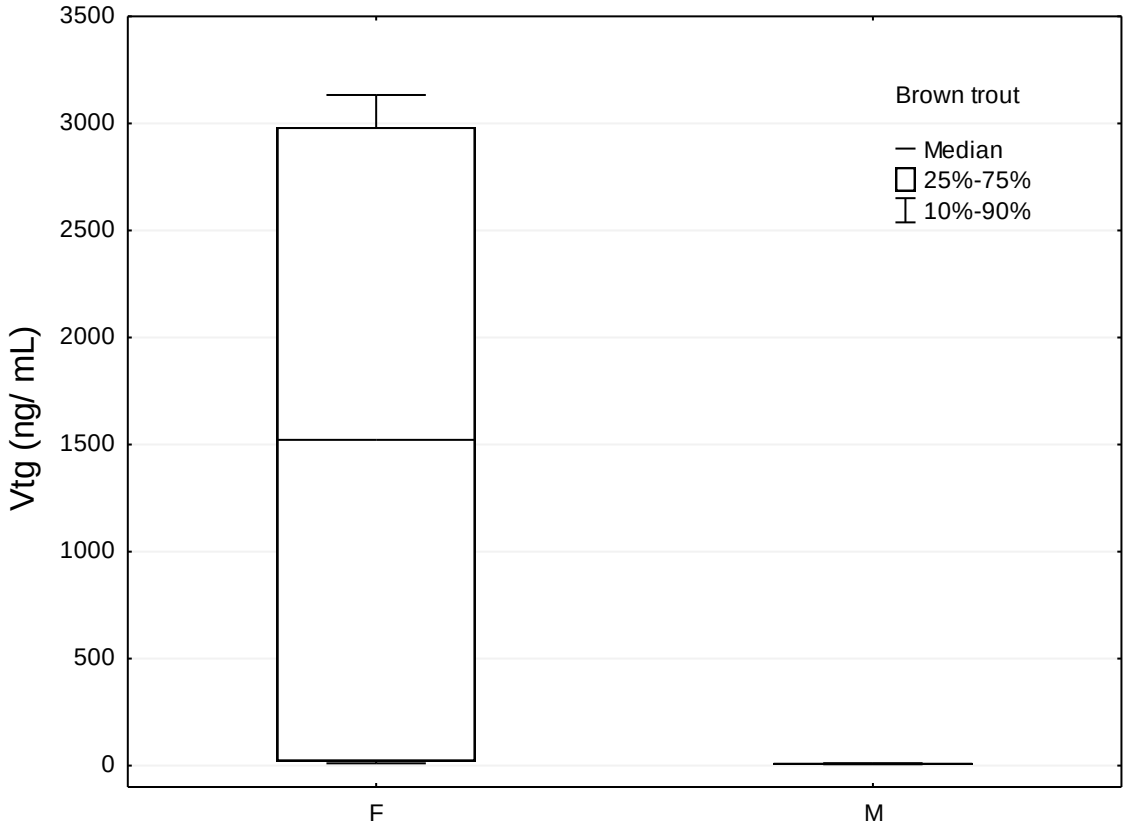


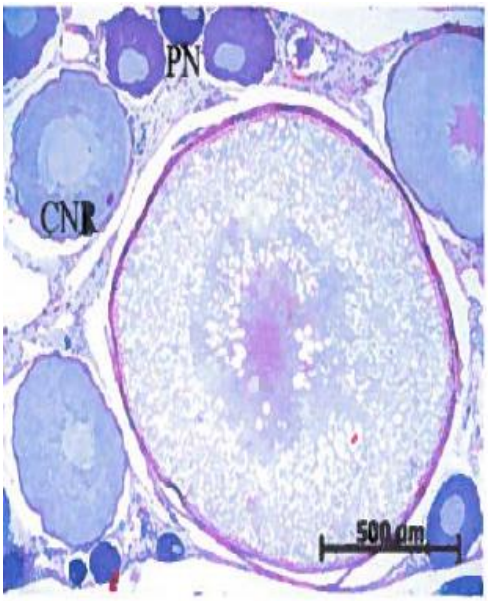
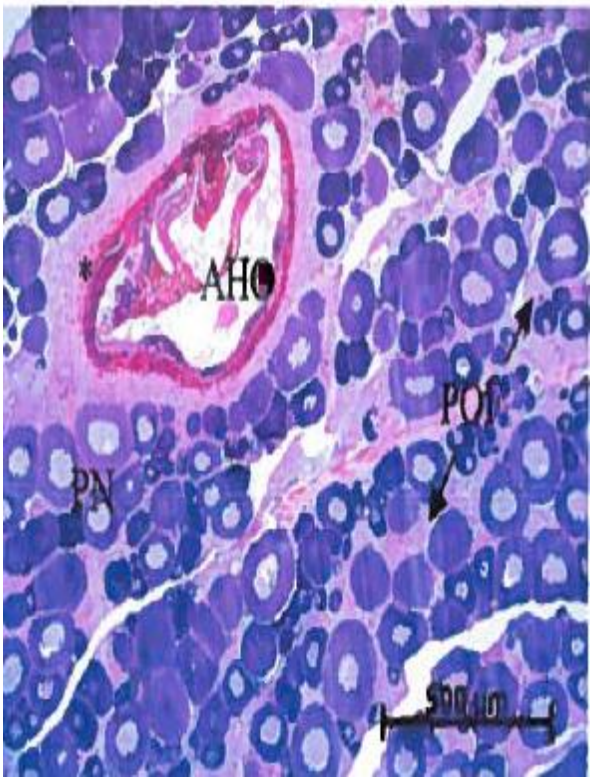
Figure 17. Vitellogenin concentrations in blood plasma samples taken from brown trout from lake Mjøsa (median, quartiles, 10/ 90 percentiles, ng/ ml, F - female n= 8, M - male n=2).

3.4.4 Histological evaluation of trout gonads

A table is also presented to report the scoring of gonad development and abnormalities from trout (Table 11). The results of the histological evaluation of trout gonads are reported for the female fish as no male fish were caught (Table 12). As regards trout, half of the individuals were on stage 2 (ripening) and the remaining were on stage 4 (spent). Also in this case, almost no abnormalities were observed in the fish gonads.

Table 11. Reproductive stages of follicles in female trout

Reproductive stage of female trout	Description
1. Immature or Previtellogenic follicles (small growth)	Ovary wall thin. Oocytes vary from small (10-20 µm) with pale uniform cytoplasm to larger with basophilic cytoplasm apart from light ring of mitochondria around nucleus. Oocytes up to about 130 µm in diameter are irregular in outline, then round. Several nucleoli at periphery of nucleus - **perinuclear stage PN.** Ring of mitochondria moves away - **circumnuclear ring stage CNR.**

2. Ripening: Vitellogenic follicles  	Exogenous yolk formation **YF** – eosinophilic yolk granules, migrate from periphery to the centrum. Zona radiata and granulosa increase
3. Ripe and Spawning – mature follicles	Oocytes hydrates in batches. Yolk as homogenous mass.
 **4. Spent**	Empty follicles (post ovulatrory follicles POF) and a few atretic hydrated oocytes AHO. * microphages and fibroblasts (fibrous capsule)
5. Resting	

Blank: No observed occurrence.

Table 12. Results from female trout											
Fish code n	test	*	**	***	****	*****	MØ6/5	bad	bad	bad	bad
Stage	4	2	2	2	2	2	2	4	4	4	4
Increased oocyte atresia	3										
Perifollicular cell hyperplasia/hypertrophy											
Decreased vitellogenesis											
Changes in gonadal staging											
Interstitial fibrosis	3										
Egg debris in the oviduct											
Increased vascular or interstitial proteinaceous fluid											
Granulomatous inflammation					1						
Parasite											
Postovulatory follicles											
Atretic follicles											
mmc			3								

Blank: No observed occurrence.

4. Environmental Risk

EU generated environmental quality standards (EQS) are available for three of the selected substances in surface waters; diclofenac (100 ng L^{-1}), E2 (0.4 ng L^{-1}) and EE2 (0.035 ng L^{-1}). None of the surface water samples collected contained levels of these compounds above the EQS. For the remaining compounds all were below LoDs that were below available predicted no-effect concentrations. This suggests that the risks to the two selected recipients from the current loads of pharmaceuticals being released are low.

A crude assessment of the levels detected in sludges was performed based on a previous assessment of contaminants in sludges applied on Norwegian soils (VKM, 2009). For two of the pharmaceuticals screened there are available PNECs for agricultural soils (atorvastatin 11 ng g^{-1}; ciprofloxacin 26 ng g^{-1}). Since the levels in sludges does not exceed these levels for agricultural soils then the risk is considered low. In the absence of published PNEC for a number of the selected pharmaceuticals a cut off of 100 ng g^{-1} was used as previously reported by Eriksen et al. 2009. Of the screened pharmaceuticals only amitriptyline and fexofenadine exceed this cut-off and it is recommended that further work be performed to evaluate the potential risks posed to agricultural soils from the occurrence of these two pharmaceuticals.

The low levels of the hormones detected in sludges, combined with rapid biodegradation rates suggest that their occurrence is unlikely to pose a risk to the environment when land applying sludges (Clarke & Smith, 2011).

5. Recommendations

One major challenge in screening for the occurrence of pharmaceuticals in the environment is the large number of pharmaceuticals used in society and selecting those to study. Here and in previous screening reports we have evaluated the occurrence of a few pharmaceutical compounds, typically prioritised based on their volume of use, previous occurrence in the environment and potential to cause harm. Such an approach is useful but may not necessarily identify the few pharmaceuticals that may occur in receiving environments. Since Norway has a very good prescription register (Norwegian Prescription Database; www.norpd.no) and there is a clear link between WWTW influent concentrations and the amounts excreted it is possible to estimate, with relatively good confidence, the levels expected to be present in influent samples. In addition the advent of high-resolution mass spectrometric (HRMS) analysis has facilitated the potential to qualitatively screen for a large number of compounds in a single analysis, so-called suspect screening. We therefore propose that future screening of pharmaceuticals should use an informed suspect screening approach where firstly an evaluation as to the levels expected to be present in WWTW influent, based upon prescription data, to ensure detectable levels are likely to be present, followed by HRMS suspect screening of sources (influents and effluents) and immediate receiving waters. We recommend that biota samples should only be analysed for those pharmaceuticals known to occur in the receiving environment. This would form part of a tiered approach where firstly

sources and receiving waters would be suspect screened by HRMS, those compounds shown to be present quantified and finally determined in biota samples. Such an approach would allow a much broader coverage of different pharmaceuticals whilst focusing efforts on first establishing sources before looking for bioaccumulation and occurrence in biota. This we believe would be an improved use of resources whilst providing a more robust assessment of the potential hazard posed by the occurrence of pharmaceuticals in the environment.

6. Conclusions

Wastewater effluent from three Norwegian hospitals was shown to contain elevated loads of selected pharmaceuticals (i.e. amiloride, amitriptyline, amlodipine, atorvastatin, azithromycin, ciprofloxacin, clarithromycin, diclofenac, fexofenadine, hydrochlorothiazide, lidocaine and simvastatin). Typically the overall contribution, based on median daily loads, to the receiving wastewater treatment works was < 0.28% for Ullevål, <10% for SI Hamar and <6% or UNN Tromsø.

Treated wastewater from four different WWTWs was shown to contain elevated loads of selected pharmaceuticals. Median daily loads were as high as 2.4 x 10^6 mg day^{-1} for certain pharmaceuticals (i.e. ciprofloxacin and fexofenadine). Effluent median loads of certain pharmaceuticals (i.e. atorvastatin, ciprofloxacin, diclofenac) and hormones were reduced when compared to influent loads, while median daily loads of other pharmaceuticals (i.e. amiloride, amitriptyline, azithromycin, clarithromycin, fexofenadine, lidocaine, simvastatin) were higher or similar in the treated effluent than influent.

Median sludge concentrations ranged from between <2.5 and 415 ng g^{-1} with the following selected pharmaceuticals detected; amitriptyline; azithromycin, clarithromycin, fexofenadine, lidocaine. Hormone sludge concentrations were typically low (<1 ng g^{-1}).

Few residues of the discharged pharmaceuticals were detected in surface water, sediment and biota samples from the recipients of Oslofjord and Lake Mjøsa.

Histopathological analysis of gonads and plasma vittellogenin levels in cod and trout gonads from Oslofjord and Lake Mjøsa respectively showed no effects attributable to the low levels of steroid hormones being discharged from WWTWs.

The overall risk to the Norwegian aquatic environment from inputs of pharamceuticals is low with the levels determined below published EQS and PNEC values. For example, the EU Watch List pharmaceutical diclofenac and hormones E2 and EE2 were below the EQS. The risks to agricultural soils from the levels determined in sludges was also deemed to be low, however the absence of $Soil_{PNEC}$ for amitriptyline and fexofenadine suggests that further work should be performed on these two pharmaceuticals with respect to their occurrence.

7. References

Benly ACK, Koksal G, Ozkul A. 2008. Sublethal ammonia exposure to Nile tilapia (*Oreochromis niloticus L.)*: effects on gill , liver and kidney histology. Chemosphere 72:1355-1358.

Bjerregaard LB, Madsen AH, Korsgaard B, Bjerregaard P. 2006. Gonad histology and vitellogenin concentrations in brown trout (*Salmo trutta*) from Danish streams impacted by sewage effluent. Ecotoxicology, 15:315–327.

Corbett PA, King CK, Mondon JA. 2015. Application of a quantitative histological health index for Antarctic rock cod (*Trematomus bernacchii*) from Davis Station, East Antarctica, Marine Environmental Research 109:28-40.

Clarke BO, Smith SR. 2011. Review of 'emerging' organic contaminants in sludges and assessment of international research priorities for the agricultural use of sludges, Environment International 37(1): 226-247.

ICES SGIMC, 2011. Report of the study group on integrated monitoring of contaminants and biological effects (SGIMC) 14-18 March 2011, Copenhagen, Denmark. ICES CM 2011/ACOM:30. 265pp.

Langford K, Thomas KV. 2009. Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works, Environment International 35(5): 766-77.

Sensini C, Torre CD, Corsi I, Focardi S. 2013. First observations of histopathological effects of 2,4,6-trinitrotoluene (TNT) in gills of European eel *Anguilla anguilla* (Linnaeus, 1758). Cell biology and toxicology 24:621-628.

Stentiford GD, Longshaw M, Lyons BP, Jones G, Green M, Feist SW. 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants, Marine Environmental Research 55(2):137-15.

Sundt RC and Björkblom C. 2011. Effects of produced water on reproductive parameters in prespawning Atlantic cod (*Gadus morhua*). Journal of Toxicology and Environmental Health, Part A, 74(7-9):543-554.

TemaNord 2012:519. PPCP monitoring in the Nordic Countries –Status Report. http://www.miljodirektoratet.no/old/klif/publikasjoner/2954/ta2954.pdf

Thomas KV, Dye C, Schlabach M, Langford KH. 2007. Source to sink tracking of selected human pharmaceuticals from two Oslo city hospitals and a wastewater treatment works. Journal of Environmental Monitoring 9:1410-1418.

Tollefsen K-E, Mathisen R, Stenersen J. 2003. Induction of vitellogenin synthesis in an Atlantic salmon (*Salmo salar)* hepatocyte culture: a sensitive in vitro bioassay for the oestrogenic and anti-oestrogenic activity of chemicals. Biomarkers 8(5):394-407.

Vitenskapskomiteen for mattrygghet (VKM). 2009. Risk assessment of contaminants in sewage sludge applied on Norwegian soils. http://www.vkm.no/dav/2ae7f1b4e3.pdf

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