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Bioaccumulation potential of the tricyclic antidepressant amitriptyline in a marine Polychaete, *Nereis virens*

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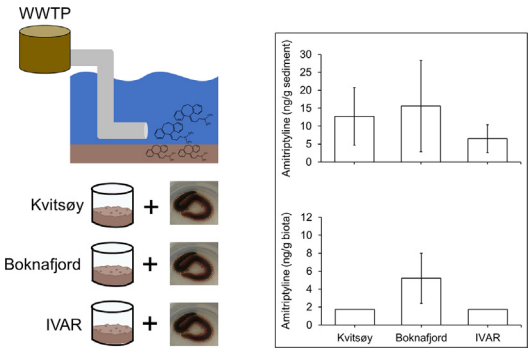
HIGHLIGHTS

- Amitriptyline detected in wastewater treatment plant discharge in Norwegian fjord.
- Sediments surrounding discharge site had measurable levels of amitriptyline.
- Potential for marine polychaetes to uptake amitriptyline after exposure to sediment.

GRAPHICAL ABSTRACT



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ABSTRACT

The continual discharge of pharmaceuticals from wastewater treatment plants (WWTPs) into the marine environment, even at concentrations as low as ng/L, can exceed levels that induce sublethal effects to aquatic organisms. Amitriptyline, a tricyclic antidepressant, is the most prescribed antidepressant in Norway, though the presence, potential for transport, and uptake by aquatic biota have not been assessed. To better understand the release and bioaccumulative capacity of amitriptyline, laboratory exposure studies were carried out with field-collected sediments. Influent and effluent composite samples from the WWTP of Stavanger (the 4th largest city in Norway) were taken, and sediment samples were collected in three sites in the proximity of this WWTP discharge at sea (WWTP discharge (IVAR), Boknafjord, and Kvitsøy (reference)). Polychaetes (*Nereis virens*) were exposed to field-collected sediments, as well as to Kvitsøy sediment spiked with 3 and 30 μg/g amitriptyline for 28 days. The WWTP influent and effluent samples had concentrations of amitriptyline of 4.93 ± 1.40 and 6.24 ± 1.39 ng/L, respectively. Sediment samples collected from IVAR, Boknafjord, and Kvitsøy had concentrations of 6.5 ± 3.9, 15.6 ± 12.7, and 12.7 ± 8.0 ng/g, respectively. Concentrations of amitriptyline were below the limit of detection in polychaetes exposed to sediment collected from Kvitsøy and IVAR, and 5.2 ± 2.8 ng/g in those exposed to Boknafjord sediment. Sediment spiked with 3 and 30 μg/g amitriptyline had measured values of 423.83 ± 33.1 and 763.2 ± 180.5 ng/g, respectively. Concentrations in worms exposed to the amended sediments were 9.5 ± 0.2 and 56.6 ± 2.2 ng/g, respectively. This is the first known study to detect measurable concentrations of amitriptyline in WWTP discharge in Norway and accumulation in polychaetes treated with field-collected sediments, suggesting that amitriptyline has the potential for trophic transfer in marine systems.

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1. Introduction

The global consumption of pharmaceuticals and personal care products (PPCPs) and subsequent release into the environment raises concern to aquatic organisms (Tran et al., 2018). The predominant sources of PPCPs into the environment are from wastewater treatment plants (WWTPs), due to incomplete removal, livestock production, and aquacultural practices (Arnold et al., 2014; Cunha et al., 2017; Tran et al., 2018). PPCPs are considered to be pseudo-persistent in aquatic environments, which can subject aquatic biota to long-term, chronic exposure with a greater potential for those compounds to be bioaccumulated (Cravo et al., 2022; Mezzelani et al., 2020; Ojemaye and Petrik, 2022; Oluwole et al., 2020; Tran et al., 2018). Concentrations of pharmaceuticals, specifically, have been detected in the environment from ng/L concentrations (Aus der Beek et al., 2016) to several mg/L (Fick et al., 2009) in surface water and sediment samples.

Sublethal toxicity, bioaccumulation, and transgenerational effects have been observed following exposures to environmental concentrations of pharmaceutical agents in various aquatic organisms (Arnold et al., 2014; Branchet et al., 2021; De Serrano et al., 2021; Duarte et al., 2022; Qiu et al., 2022; Xuan et al., 2022). However, the potential of pharmaceuticals to be bioavailable to aquatic biota once released into the environment depends on factors such as chemical composition, water currents and temperature, binding affinity to organic carbon, and degradation rates (Chen et al., 2017b; Gaw et al., 2014; Patel et al., 2019). Pharmaceuticals in sediments have been reported to be at higher concentrations relative to surface water, which may be due to sediments acting as a sink for sorbed compounds (Gilroy et al., 2012), making them more bioavailable to benthic-dwelling biota due to bioturbation (Goedkoop and Peterson, 2003; Gunnarsson et al., 1999; Maranho et al., 2014; Reible et al., 1996). The route of uptake, potential for bioaccumulation in benthic species, and capacity for trophic transfer remains less understood for pharmaceuticals, particularly in marine systems.

Among pharmaceutical classes detected in the environment, antidepressants are the most commonly administered medications globally (Castillo-Zacarías et al., 2021; Tamblyn et al., 2019). Additionally, prescriptions may underestimate antidepressant use as ~50 % of prescribed antidepressants have been used for combating non-depressive conditions, such as sleep disorders and chronic pain (Mojtabai and Olfson, 2011; Uchida et al., 2007). Amitriptyline, a tricyclic antidepressant, is the most prescribed antidepressant in Norway, with 59,416 individuals prescribed in 2018 (Berg et al., 2019). Concentrations of 243 ng/L have been observed in WWTP discharge effluents in the UK (Baker and Kasprzyk-Hordern, 2013).

Amitriptyline is also considered to be a high risk neuroactive pharmaceutical in fish (Sumpter and Margiotta-Casaluci, 2022) and recent studies have indicated that exposure to environmentally relevant concentrations induced sublethal and lethal effects in aquatic organisms (Castillo-Zacarías et al., 2021; Yang et al., 2014). Early life stage (ELS) (8 hpf) common carp (*Cyprinus carpio*) exposed to 10 μg/L amitriptyline had a significantly reduced total length, increased glutathione reductase activity, and lipid peroxidation by 30 dpf (Sehonova et al., 2017). ELS (4 hpf) zebrafish (*Danio rerio*) exposed to 100 ng/L amitriptyline for 120 h had a significantly induced oxidative stress response, noted by increased superoxide dismutase (SOD) and catalase (CAT) activity and lipid peroxidation (Yang et al., 2014). Additionally, amitriptyline has been shown to accumulate in the livers of brook trout (*Salvelinus fontinalis*) when exposed to 20 % diluted samples of effluent from a Canadian WWTP (Lajeunesse et al., 2011). Concentrations were also measured in liver, gill, muscle, and brain tissue in gilthead bream (*Sparus aurata*) exposed to 200 ng/L (Ziarrusta et al., 2017).

The presence of amitriptyline in WWTP discharges in Norway and capacity for amitriptyline to accumulate in invertebrates within marine systems is largely unknown. To better characterize the potential for this compound to accumulate in biota found within marine waterways in Norway, this study aimed to: i) determine sediment concentrations within sites surrounding a WWTP discharge in a Norwegian fjord; ii) assess the potential for a benthic polychaete (*Nereis virens*) to accumulate amitriptyline following exposure to field-collected sediments; and iii) understand the potential risk for trophic transfer through dietary uptake.

2. Materials and methods

2.1. Chemicals

Amitriptyline hydrochloride (≥98 purity), amitriptyline-d_3 hydrochloride, and sodium azide were obtained from Sigma-Aldrich (Merck Life Science AS, Darmstadt, Germany). Carbamazepine-D_{10} was obtained from Sigma-Aldrich (Merck Life Science AS, Oslo). Formic acid (HCO_2H) (99 %), ammonium hydroxide (NH_4OH), methanol (MeOH), ethyl acetate (EtOAc), sulfuric acid (H_2SO_4), and dichloromethane (DCM) were all HPLC grade and obtained from VWR (VWR International AS, Oslo, Norway).

2.2. WWTP discharge

Untreated (influent) and treated (effluent) wastewater samples from the Stavanger WWTP plant, IVAR, were collected in 1 L glass bottles in October 2020. Influent samples, collected after the initial mechanical screening treatment step, where only larger objects are removed, were taken as multiple grab samples at 11:00 am. Effluent samples were collected from sedimentation tanks after the biological treatment and prior to discharge into the marine environment. Effluent was collected as 24 h composite samples. Samples were transported on ice to the laboratory, where they were stored at −20 °C until further analysis. Since all WWTP samples were collected within 1 h on the same day, differences in concentration levels between influent and effluent samples cannot be used to calculate accurate removal efficiencies of the biological treatment, as the hydraulic retention time of the WWTP from influent to effluent is approximately 6 h.

2.3. Sediment sample collection

Sediment samples were collected from three locations in October 2019 using a stainless steel Van Veen grab (Fig. 1). Site 1 was located about 100 m North of a WWTP discharge (IVAR; 59°02′10.2″N 5°33′08.4″E). Site 2 was located 2 km West of the WWTP discharge (Kvitsøy; 59°03′46.8″N 5°22′33.0″E) that has previously been used as a reference site for studies focusing on legacy contaminants (Nilsen et al., 2012). Site 3 was located 17 km Northeast of the WWTP discharge (Boknafjord; 59°10′37.4″N 5°39′36.2″E). Grab samples were collected in triplicate from the top 2 cm of sediment and mixed as a composite sample from three grabs. Although physical and chemical properties of sediment samples were not measured in this study, a qualitative assessment of the sediment deemed that the dominate characteristic was representative of a fine sand. This is consistent with a previously conducted assessment within the same fjord in Stavanger, which determined that fine sand was the dominant class, with clay and silt comprised of the secondary class (Gomiero et al., 2019).

2.4. Experimental design

Marine polychaetes (*Nereis virens*), with a length of ~13 cm, were obtained from a sea bait aquacultural farm, Topsy Baits (Wilhelminadorp, The Netherlands), and transported to the University of Stavanger within 24 h in cooled seawater after being placed in continuously running seawater for at least 24 h after harvest. Upon arrival, *N. virens* were divided and transferred into 1 L glass beakers that were filled a third of the way with autoclaved sand and 600 mL sterile seawater (collected north of Kvitsøy) in a temperature-controlled incubator (12.6 °C) under a 8:16 light:dark cycle (North European winter light conditions) (7 polychaetes/beaker). *N. virens* were allowed to acclimate for at least 8 days with 100 % seawater renewals conducted and fed live sea angling bait to satiation.

To determine whether uptake of amitriptyline from sediment occurs into biota, polychaetes were placed in one of three sediments collected

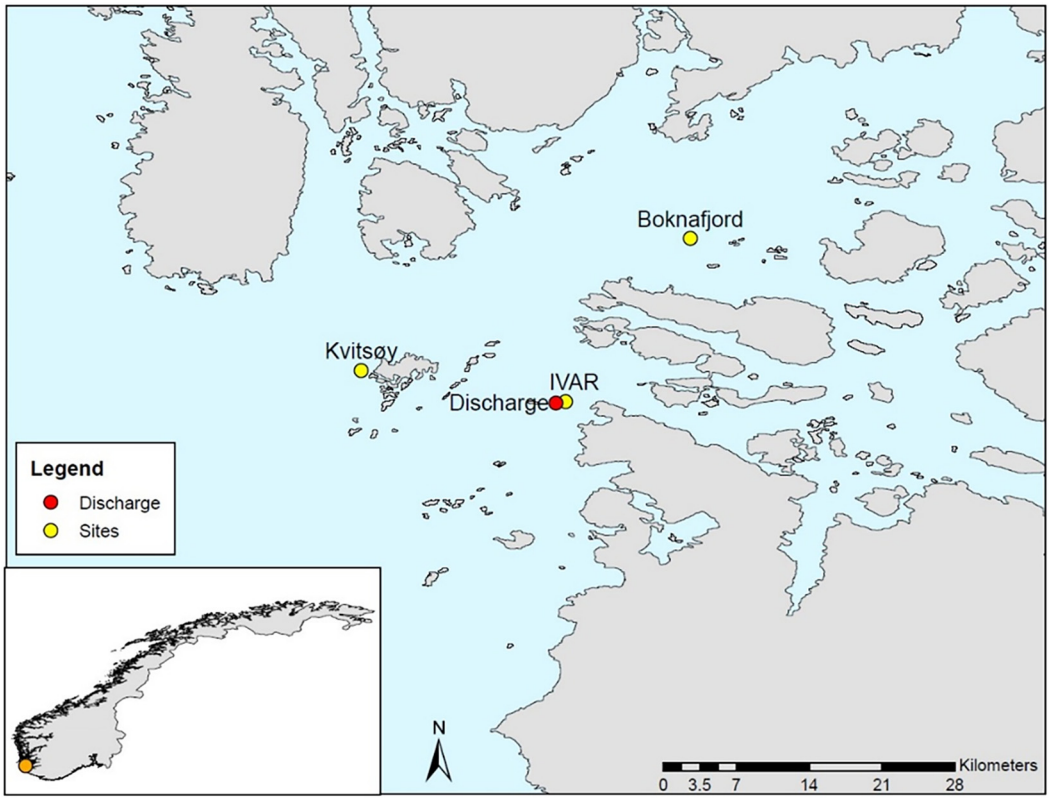

Fig. 1. Sediment sample collection sites within a Stavanger fjord.

from Kvitsøy, Boknafjord, or IVAR. Additionally, sediment collected from Kvitsøy were either spiked with a low (3 μg/g) or high (30 μg/g) concentration of amitriptyline for 28 days (Fig. 2), representing a worst-case exposure scenario (Petrie et al., 2015) or 10-fold greater acting as a positive control. The amitriptyline was evenly distributed in the Kvitsøy-spiked sediment by mixing with a sterile metal spatula and placed into an incubator with continual mixing by magnetic stirring bars for 24 h at 13 °C prior to exposure start. Each of the five treatments had a total of three replicates, with 7 polychaetes placed in 1 L glass beakers that were filled a fourth of the way with sediment and brought to a final volume of 900 mL with sterile seawater and kept in a temperature-controlled incubator at 13 °C. Water renewals were conducted biweekly for the course of the 28-day exposure period with 300 mL seawater replaced with new, sterile seawater. The following water parameters were recorded throughout the exposure period: water temperature 12.6 ± 0.1 °C, salinity 36.1 ± 0.4 ppt, pH 8.3 ± 0.1, and dissolved oxygen 9.0 ± 0.3 mg/L.

Following exposures, sediment samples were placed in glass bottles and spiked with 1 g/L sodium azide to prevent microbial degradation (Vanderford et al., 2011) and stored at −20 °C until analysis. Exposure water was transferred into glass bottles and 4 M H_2SO_4 and 100 % MeOH (1:500 v/v) were added and stored at −20 °C until analysis (Chen et al., 2012). Polychaetes were removed from the sediment and rinsed in clean, sterile seawater to eliminate any adhered sediment and stored in glass vials at −20 °C until analysis.

2.5. Sample extractions

2.5.1. Influent and effluent samples

Influent (n = 3) and effluent (n = 3) samples were collected as grab samples in 1 L glass bottles, transported on ice, and stored at −20 °C until analysis. Samples were spiked with the internal standard, Carbamazepine-D_{10}, and filtered through pre-conditioned solid phase extraction (SPE) hydrophilic lipophilic balance cartridges (HLB) 6 cc 200 mg (Waters, Oslo, Norway). Samples were washed with 5 % MeOH, eluted with 100 % MeOH, and evaporated until dry. They were then reconstituted in 20 % MeOH and cleaned on a Spin-x microcentrifuge (nylon mesh; 0.22 μm) at 7000 rpm for 1.5 min. An Agilent 6490 (Agilent Technologies, Santa Clara, CA, USA) triple quadrupole mass spectrometer

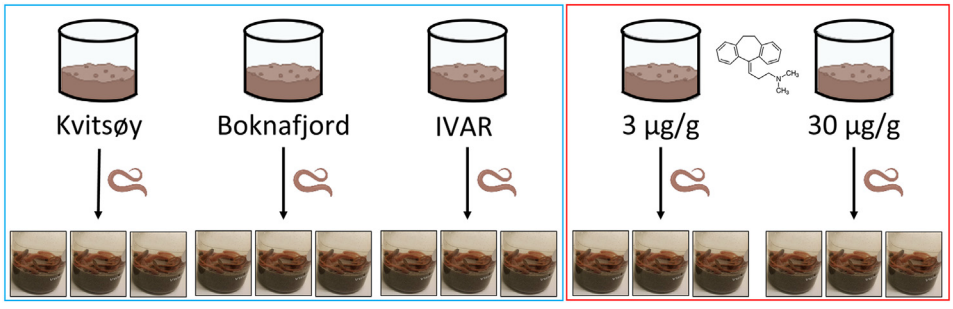

Fig. 2. Experimental setup of *Nereis virens* exposed to field-collected sediment samples, Kvitsøy, Boknafjord, and IVAR, or worst case scenario and positive control samples, which consisted of Kvitsøy sediment spiked with either 3 or 30 μg/g amitriptyline for 28 days.

with an Agilent Jet Stream electrospray ion source was used for the detection and quantitative analysis. The ions were monitored in a positive and negative multiple reaction monitoring (MRM) system. The quantification batch was built using Agilent MassHunter software (Version B.07.00 / Build 7.0.457.0, 2008), which was also used for instrument control, method validation, and quantification. Targeted analytes were quantified on an Agilent 1200 series HPLC (Agilent Technologies, Waldbronn, Germany). 10 μL of the sample was injected on a Zorbax Eclipse plus C18 RRHD (2.1 × 100 mm, 1.8 μm) (Agilent, Palo Alto, USA) with a respective Guard Cartridge (4 μm × 3.0 mm ID) (Zorbax, Agilent, Palo Alto, USA), which were kept at 25 °C. Separations were performed using a binary gradient with mobile phase consisting of water with 0.1 % formic acid (A) pure CH_3CN (B) with a mobile phase flow rate of 0.35 mL/min (v:v). The initial mobile phase proportion was 100 % (A). B was then linearly increased to 100 % over 8 min and held for 7 min. Initial mobile phase conditions were restored over 1 min and the column was allowed to equilibrate for 4 min resulting in a total run time of 20 min.

2.5.2. Water samples

Seawater samples (from water within the beaker containing sediment from IVAR, Kvitsøy, Boknafjord, and Kvitsøy sediment spiked with amitriptyline), as well as sterile seawater collected north of Kvitsøy and water obtained at each site sediment was collected from, were extracted using SPE. A subset of water samples were analyzed, i.e. after a 1 week and 3 week exposure timepoint, to evaluate the exposure set up. A total of 900 mL of sampled water was filtered through a series of filters with decreasing pore size (25 μm, 4.7 μm, and 2.5 μm).

SPE cartridges, 6 mL, 500 mg, Oasis HLB (Waters, Milford, MA, USA), were primed with 10 mL MeOH followed by 10 mL Milli-Q water. Water was vacuum filtered at a rate of 10 mL/min and then samples dried under vacuum for 1.5 h, as conducted according to manufacturer's instructions. Samples were eluted as previously reported (Jia et al., 2020). Briefly, 5 mL of MeOH was added to the cartridge followed by 4 mL EtOAc and 3 mL DCM and eluant collected in 20 mL scintillation vials. Samples were dried under a gentle stream of nitrogen, resuspended with 1 mL MeOH, passed through a 0.22 μm polypropylene syringe-tip filter, and stored at −20 °C until ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) analysis.

2.5.3. Sediment and biota measurements of amitriptyline

Sediment samples (between 15 and 22 g) were lyophilized for 6–8 h using a Maxi Dry Lyo Freeze-dryer (MechaTech Systems, Thornbury, UK) and homogenized using a mortar and pestle until a fine powder. Samples were then transferred to 15 mL glass vials and underwent the same extraction as water samples.

For each exposure triplicate ($n = 3$ per sediment treatment), 7 individual *N. virens* from each treatment beaker were pooled and homogenized with an OMNI international tissue homogenizer (OMNI, Kennesaw, GA, USA), placed into 120 mL glass beakers, and lyophilized. Homogenates were transferred to a mortar and pestle and ground into a fine powder and underwent the same extraction as water samples.

A set of 15 sediment (2 g) and biota samples (0.5 g) were each spiked with an internal standard, 100 ng amitriptyline-d_3 hydrochloride. Additionally, a subset of polychaetes from the farm were pooled to determine if background levels of amitriptyline were present. A MeOH and 0.1 % formic acid (HCO_2H) solution in Milli-Q (1:1 v/v) was added to each spiked sample and vortexed for 30 s. Samples were ultrasonicated for 15 min and centrifuged for 10 min at 2800 rpm. The supernatant was transferred to a 1 L glass flask. The extraction process was repeated, for a total of two times with a greater volume, 5:5 (v/v) MeOH and HCO_2H, and supernatant combined and diluted with 300 mL Milli-Q water and 30 μL H_2SO_4 so the total concentration of MeOH was under 10 % prior to undergoing SPE filtration, as previously conducted (Chen et al., 2012). The same process of SPE filtration was conducted as with water, sediment, and biota samples.

2.6. UPLC-MS/MS analysis

Detection of amitriptyline in sediment samples and biota was conducted with a Waters Acquity Ultra-Performance Liquid Chromatography system and Quattro Premier XE tandem Quadrupole Mass spectrometer and was equipped with an Acquity UPLC BEH C18 (100 mm × 2.1 mm, 1.7 μm particle size) column. Chromatographic separation of amitriptyline was conducted using a two-part solvent system: 1) A1 consisted of 0.2 % formic acid and 2) B1 contained MeOH. The injection volume was 10 μL and the flow rate of the mobile phase was 200 μL/min. Amitriptyline was monitored in positive electrospray ionization mode and run through an MRM software system. The process consisted of 95 % A1, 5% B1, followed by B1 at 99 % and then a return to the initial conditions for a reset of the column for the remaining 3 min. Samples were kept at 5 °C in the autosampler before analysis. Each sample was run for 13 min, and amitriptyline had a retention time around 6.33 min. External calibration curves (low end: 10-point from 0.59 to 150 ng/mL ($r^2 = 0.9997$); high end: 13-point from 2.44 to 10,000 ng/mL ($r^2 = 0.9915$)) were used for determining the limit of detection (LOD) and limit of quantification (LOQ), which were 3.5 ng/mL and 10.6 ng/mL, respectively. Spike recoveries were calculated by subtracting the deuterated amitriptyline added from deuterated amitriptyline measured divided by the amount of deuterated amitriptyline added.

2.7. Statistical analysis

Statistical analyses were conducted with IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, New York). A one-way analysis of variance (ANOVA), followed by a Tukey post hoc, was conducted to compare mean differences in amitriptyline concentrations in field-collected sediment samples and uptake in polychaetes. A Student's *t*-test was conducted to compare mean differences in amitriptyline concentrations in sediment from Kvitsøy spiked with 3 or 30 μg/g amitriptyline and uptake in polychaetes. A Levene's test was used to assess homogeneity of variance and Shapiro-Wilk test conducted to assess normality. A p-value <0.05 was used to determine statistical differences.

3. Results and discussion

3.1. Influent and effluent analysis

The Stavanger WWTP receives an average of 68,000 m^3/d of wastewater from approximately 340,000 people from four different municipalities. The plant has a treatment capacity of 80,000 m^3/d, which is the equivalent use of approximately 400,000 people. It treats wastewater from domestic households, a hospital, and industrial sources, using a biological treatment system based on an activated sludge process. The treated effluent is discharged into the marine recipient, 1.6 km Northwest from shore at a depth of 80 m.

Concentrations of amitriptyline in the influent and effluent from this WWTP were detected at 4.93 ± 1.40 ng/L and 6.24 ± 1.39 ng/L, respectively. The percent recovery was 60.58 % in water samples. Those values are within the wide range of previously detected concentrations measured within influent and effluent, globally. The influent concentrations detected from five WWTPs within São Paulo, Brazil ranged from non-detected (nd) to 200 ng/L and effluent from nd to 80 ng/L amitriptyline (Pivetta et al., 2020) and 341 to 5143 ng/L and 53 to 357 ng/L amitriptyline in influent and effluent from the WWTP Cilfynydd in South Wales, respectively (Kasprzyk-Hordern et al., 2009). Amitriptyline measured in wastewater influent in the UK was as high as 2092 ng/L with effluent concentrations measured at 207 ng/L (Petrie et al., 2015). Amitriptyline has a high binding affinity for particulate matter (Petrie et al., 2015), which may explain the slightly higher concentrations detected in influent relative to effluent samples from the WWTP.

Sorption, partitioning, currents, and hydrodynamics play an important role in the movement of PPCPs in the marine environment (Bavumiragira et al., 2022; Bayen et al., 2013; Fabbri and Franzellitti, 2016; Liu et al.,

2013; Tong et al., 2022). Pharmaceuticals absorbed to particulate matter have been reported to be an important route of transport within aquatic systems (Petrie et al., 2014). As such, the concentration of pharmaceuticals in marine environments do not necessarily reflect the proximity from WWTP discharge sites due to hydrodynamic flushing and sorption of compounds to suspended solids (Bayen et al., 2013) and are able to be transported and deposited in sediments, which can act as a sink (Ojemaye and Petrik, 2019). Additionally, it has been previously shown that PPCPs can exhibit negative removal efficiencies (Ashfaq et al., 2017; Zhou et al., 2019), which are likely related to transformations of a conjugated form to a free form, as well as biotransformations of precursors upstream (Chen et al., 2017a, 2017b).

3.2. Suspended amitriptyline in overlaying water and seawater

Although amitriptyline has a high binding affinity to particulate matter, bioturbation can enhance the desorption of pharmaceuticals bound in sediments (Gilroy et al., 2012), and previously reported that polychaetes can enhance the desorption of contaminants bound in marine sediments (Gunnarsson et al., 1999). Water collected from underlaying sediment samples in exposure beakers after a 1- and 3-week duration had the following concentrations: Kvitsøy (0.0062 and 0.03 ng/mL), Boknafjord (0.0037 and 0.002 ng/mL), IVAR (0.03 and 0.009 ng/mL), Kvitsøy 3 μg/g spike (0.44 and 0.72 ng/mL), and Kvitsøy 30 μg/g spike (3.52 and 2.83 ng/mL), respectively. Seawater collected from a site located north of Kvitsøy, as well as from each site sediment was collected from, did not have any detected amitriptyline present (below LOQ). The pore water was not separated from overlaying water in the current study. Future studies should consider differences in concentrations of amitriptyline in overlaying versus pore water, which could provide a continual input into the environment (Xu et al., 2014), in addition to bioturbation influences. The bioturbation of *N. virens* during this exposure period may have increased the rate of desorption of amitriptyline from the sediment, being resuspended into the overlaying water, as previously reported to occur in other benthic invertebrates exposed to sediment spiked with pharmaceuticals with log K_{ow}'s < 4 (Gilroy et al., 2012). Amitriptyline has a log K_{ow} = 4.92 and suggested that it is generally sorbed to sediment's organic material as a sink, being less available to biota (Al-Khazrajy and Boxall, 2016; Katayama et al., 2010). Following resuspension of compounds due to bioturbation (Gilroy et al., 2012), benthic-dwelling species have been reported to accumulate both parent compounds and metabolites (Andrzejczyk et al., 2020; Arnnok et al., 2017; Cerveny et al., 2021; Valdés et al., 2016).

3.3. Sediment and biota analysis

Sediment samples collected from Kvitsøy, Boknafjord, and IVAR had 12.7 ± 8.0 ng/g, 15.6 ± 12.7 ng/g, and 6.5 ± 3.9 ng/g amitriptyline detected, respectively (Fig. 3A). The concentrations of amitriptyline between the sites did not significantly differ from each other ($p > 0.05$). The Kvitsøy sediments amended with 3 and 30 μg/g amitriptyline had final concentrations of 423.8 ± 33.1 ng/g and 763.2 ± 180.5 ng/g, respectively (Fig. 3B).

The concentrations of amitriptyline detected were within the range of those previously reported in sediment collected from estuarine, marine, and freshwater systems. Sediment samples from a Baltic Sea estuary ranged from 0.7 to 25.1 ng/g dry wt (Kucharski et al., 2022), between <0.10 to 0.40 ng/g amitriptyline dry wt in marine sediment samples collected in the Bay of Cádiz (Maranho et al., 2015), and up to 80 ng/g dry wt in river sediment samples receiving WWTP discharge in the Itaipu reservoir region, Brazil (Costa Junior et al., 2020). In polychaetes, the body burden concentration of amitriptyline was below the LOD in those exposed to sediment collected from Kvitsøy and IVAR, and present at measurable concentrations, 5.2 ± 2.8 ng/g, in sediment collected from Boknafjord (Fig. 4A). The deposition of amitriptyline to areas of the fjord that are distant from the WWTP discharge location, such as Boknafjord, are likely due to hydrodynamic flushing and sorption capacity to suspended solids (Bayen et al., 2013). Subsequent modelling studies assessing water movement, discharge rate, and physical and chemical properties of amitriptyline are warranted to understand the dispersion within the fjord to locations distant from the WWTP discharge source.

However, the concentration of amitriptyline was not significantly different between the sites ($p > 0.05$). The concentration of amitriptyline in polychaetes from Kvitsøy-spiked sediment was 9.5 ± 0.2 ng/g and 56.6 ± 2.2 ng/g in the 3 μg/g and 30 μg/g spiked samples, respectively (Fig. 4B). The concentrations of amitriptyline in Kvitsøy sediment spiked with 30 μg/g amitriptyline was significantly greater than Kvitsøy sediment spiked with 3 μg/g ($p < 0.001$). The bioaccumulation of compounds into invertebrates can cause concern for trophic transfer (Xie et al., 2017), particularly for commonly consumed organisms that comprise the diet of ecologically and economically important fish, such as the Atlantic cod (Grabicová et al., 2020). Amitriptyline was reported to bioaccumulate in the livers of brook trout (*Salvelinus fontinalis*) at an average concentration of 0.29 ng/g when exposed to 20 % diluted samples of effluent from a WWTP, with an average concentration detected at 3.7 ng/L in WWTP effluent, which is a 78-fold bioaccumulative potential (Lajeunesse et al., 2011). These findings suggest that amitriptyline is capable of being taken up and accumulated in marine polychaetes, which poses a potential route of exposure not currently assessed when considering risk to higher trophic levels.

4. Conclusions

Amitriptyline was detected at measurable concentrations in the effluent of the Stavanger WWTP. Marine polychaetes exposed to field-collected sediments from locations surrounding the WWTP discharge site, along with those exposed to spiked sediments, for 28 days were capable of accumulating amitriptyline. The ability of lower trophic level species to accumulate amitriptyline raises concern for higher trophic level species. Future studies

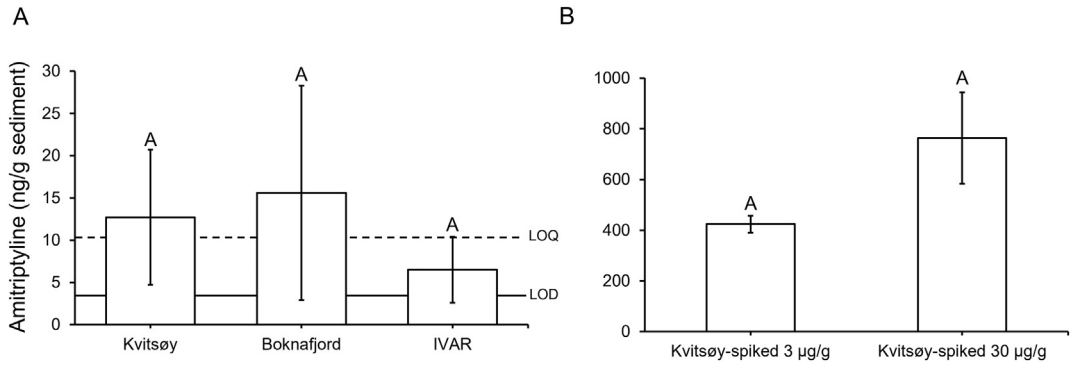

Fig. 3. Average (±SD) amitriptyline concentrations from A) field-collected sediments (Kvitsøy, Boknafjord, and IVAR) and B) Kvitsøy sediment spiked with 3 or 30 μg/g amitriptyline following a 28-d exposure with *N. virens*. (A) One-way ANOVA, Tukey post hoc, $p < 0.05$, $n = 3$ per treatment; B) Student's *t*-test, $p < 0.05$, $n = 3$ per treatment). Uppercase letters used to denote significant differences between exposures.

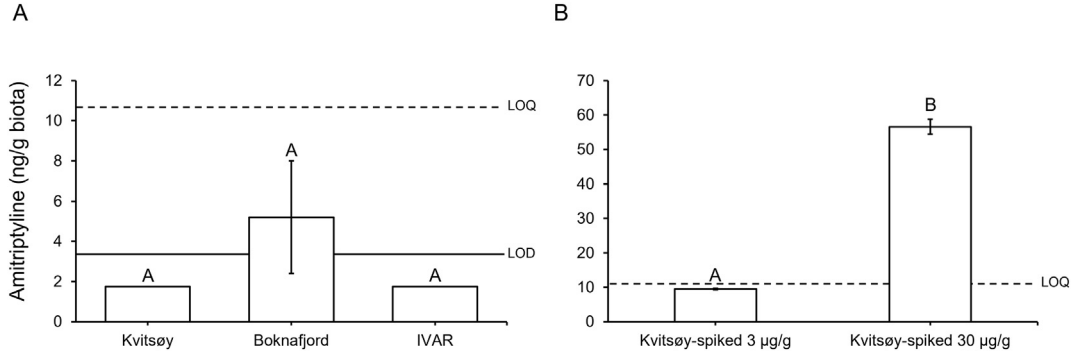

Fig. 4. Average (±SD) amitriptyline concentrations in polychaetes exposed to A) field-collected sediments (Kvitsøy, Boknafjord, and IVAR) and B) Kvitsøy sediment spiked with 3 or 30 µg/g amitriptyline following a 28-d exposure. (A) One-way ANOVA, Tukey post hoc, $p < 0.05$, $n = 3$ per treatment; B) Student's *t*-test, $p < 0.05$, $n = 3$ per treatment). Uppercase letters used to denote significant differences between exposures.

assessing the capacity of amitriptyline to accumulate in biota are warranted to determine rates of biotransformation to metabolites, such as nortriptyline, following consumption, as assessing concentrations of amitriptyline alone may be underestimating the bioaccumulation potential following uptake (Ziarrusta et al., 2017).

CRediT authorship contribution statement

Jason T. Magnuson: Conceptualization, Formal analysis, Visualization, Writing – original draft. **Zoe Longenecker-Wright:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Ivo Havranek:** Formal analysis, Investigation, Writing – review & editing. **Giovanna Monticelli:** Methodology. **Hans Kristian Brekken:** Methodology, Data curation. **Roland Kallenborn:** Resources. **Daniel Schlenk:** Conceptualization, Writing – review & editing. **Magne O. Sydnes:** Conceptualization, Writing – review & editing, Funding acquisition. **Daniela M. Pampanin:** Conceptualization, Writing – review & editing, Project administration.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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