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Measurement of Oxytetracycline and Emamectin Benzoate in Freshwater Sediments Downstream of Land Based Aquaculture Facilities in the Atlantic Region of Canada

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Abstract This study investigated the occurrence of oxytetracycline (OTC) and emamectin benzoate (EB) in sediments located near the effluent outfall from four freshwater aquaculture facilities in Atlantic Canada. While two facilities had no detectable concentrations of EB or OTC, two facilities had detectable concentrations of one or both of these chemicals. Concentrations ranged from <0.05–18 mg/kg to <0.01–2.5 mg/kg for OTC and EB respectively. Although these values could not be compared with freshwater toxicant values, some of the concentrations of EB and OTC detected were higher than LC_{50} values calculated for marine invertebrates. OTC concentrations measured in this study are also of a magnitude which has been known to produce resistant bacteria.

Keywords Oxytetracycline · Emamectin benzoate · Sediment · Aquaculture

In the Atlantic Region of Canada, there are over 50 land-based aquaculture facilities. The majority of these facilities discharge wastewater into streams or rivers while a smaller number of facilities discharge into lakes or estuaries. Approximately 90 % of the facilities have some form of treatment of the wastewater before discharge into the natural environment. Treatment options can include the following; settling ponds, swirl separators, settling deck, rotary drums and recirculation/reuse technologies. Treatment options are constantly evolving with many facilities moving towards re-use or re-circulation systems. However there are approximately 10 % of the facilities that still discharge the wastewater without any treatment whatsoever. The facilities that discharge without treatment are usually the older facilities or the facilities that contain a very low density of fish on site.

Currently, there are no national use pattern data for individual antibiotics or therapeutants in aquaculture operations. Veterinary drugs are used in aquaculture operations to control and prevent disease outbreaks and infections. There are currently five antibiotics registered for use in Canada by Health Canada by the Bureau of Veterinary Drugs under the authority of the Food and Drug Act: formalin, bronopol, oxytetracycline (OTC) hydrochloride, sulphadiazine plus trimethoprim, sulfadimethoxine plus ormetoprim, and florfenicol (Health Canada 2010). The major theoretical environmental concerns regarding the addition of antibiotics into the environment are that they will: (a) impact and inhibit the growth of natural sediment-dwelling microbes, potentially altering important biochemical processes such as degradation processes and nutrient cycling; and, (b) encourage the development of antibiotic resistance in microbes, which could be transferred to animal and human pathogens (Burridge et al. 2008). A detailed description of the source, dosage and the antimicrobial capabilities of these antibiotics can be found in Scott (2004). Emamectin benzoate (EB) is the active ingredient of the anti sea-lice therapeutant SLICE^{TM} and may be used on salmon smolts in freshwater aquaculture sites just prior to transfer to marine sites to inhibit infestation of sea lice. With the exception of EB, the other ingredients of SLICE^{TM} have shown to have a negligible impact to the environment (Scottish EPA 1999). The main environmental impact of EB is its toxicity to nematodes, arthropods and several other pest taxa (Scott 2004).

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Most of the studies on the potential environmental impact of freshwater fish farms facilities have focussed on nutrients (Viadero et al. 2005), while only a handful have determined the fate of antibiotics or therapeutants used in these facilities (Lalumera et al. 2004; Pouliquen et al. 2009; Thurman et al. 2003). No studies on the presence of antibiotics or therapeutants in the receiving environment related to Canadian freshwater fish facilities were identified in the literature. The purpose of this preliminary study was to assess the sediment concentrations of EB and OTC downstream of freshwater fish farms located in Atlantic Canada.

Materials and Methods

In October 2010, sediment samples were collected within 5 m of the outfall locations of four freshwater fish farms (Facilities A–D, Table 1). An Eckman grab was deployed by hand and the top 2 cm of sediment was obtained from the grab. The sediment was scooped out from the grab with a stainless steel spoon and put into laboratory-certified clean amber jars (250 mL). The jars were kept in a cooler with ice packs and delivered overnight to RPC (analytical laboratory) in Fredericton, New Brunswick. The sediment samples were analysed for EB and OTC.

Emamectin benzoate was determined by solid phase extraction with analysis by high performance liquid chromatography/fluorescence detection (HPLC/FLD) with a reporting limit of 0.01 mg/kg (Riet et al. 2001). OTC was determined by solvent extraction with analysis by liquid chromatography and tandem mass selective detection (LC/MS/MS) with a reporting limit of 0.05 mg/kg (Yuan et al. 2010). Method blank analysis and spike recovery (%) were also determined for all samples by RPC. Two replicates (Facilities A and B) were also sent for analysis to RPC. Percent moisture of each sample was also determined by RPC.

Results and Discussion

Oxytetracycline (OTC) concentrations ranged from below the detection limit of the laboratory (<0.05 mg/kg) to 18 mg/kg (Table 1). Two out of the four sampling sites had no detectable concentrations of OTC in sediment located at the outfall. EB concentrations ranged from below the detection limit of the laboratory to 2.5 mg/kg. Three out of the four sites had no detectable concentrations of EB (Table 1). Usage patterns (quantity or frequency) of these chemicals in these facilities remain unknown. Spike recoveries for OTC averaged 95 % while average recovery for EB was 60.3 %. Method blank for OTC was equal to <0.05 mg/kg while method blank for EB was equal to <0.01 mg/kg.

Table 1 Concentrations of OTC and EB at the effluent outfall of Canadian freshwater fish facilities

Sample locations	OTC (mg/kg)	EB (mg/kg)
Facility A effluent	18	2.5
Facility A effluent-duplicate	18	1.8
Facility B effluent	<0.05	<0.01
Facility B effluent-duplicate	<0.05	<0.01
Facility C effluent	<0.05	<0.01
Facility D effluent	0.14	<0.01

Antibiotics and chemotherapeutants have been known to enter the environment from uneaten foods, urinary discharges and from feces (Lalumera et al. 2004; UMA 2005). Given feed waste and poor digestive adsorption, it is probable that greater than 95 % of the OTC provided is not assimilated and leaves the farm via the effluent (Samuelsen 1989; Pouliquen et al. 2009). OTC which ends up in sediment is then degraded or slowly diffused back to the overlying water (Samuelsen 1989; Hirsh et al. 1999). Samuelsen (1989) measured detectable concentration of OTC found in sediment 3–6 months following feeding of the antibiotic while Lalumera et al. (2004) detected OTC 2 years after last treatment. Using models, the environmental fate of OTC from hatchery operations was determined by Rose and Pedersen (2005) to have a disappearance time half life of 118 days in sediment which is within the calculations from Samuelsen et al. (1992) who measured OTC half life of 87, 124 and 144 days. The calculated and measured half-lives of OTC suggest that the OTC measured in this study were probably from the current year's usage as well as past year's usage. Rose and Pedersen (2005) predicted that OTC concentrations would reach a steady state of approximately 4.0 mg/kg directly downstream of the outfalls and concentrations were expected to be 0.2 mg/kg at 4.4 km from the outfall. In comparison, one of our study sites had a concentration of 18 mg/kg at the outfall which is 4 times greater than the predicted concentration calculated by Rose and Pedersen (2005). Emamectin benzoate is lipophilic which suggests that this chemical will partition or remain in highly organic particles (UMA 2005). McHenery and MacKie (1999) calculated a half life of 164–175 days for emamectin benzoate since they were also able to measure concentrations of EB 4 and 12 months post treatment under marine fish farm sites. Based on the half-life of EB, it is likely that the EB detections measured as part of this study may be from application of EB within this and last year's usage of the chemotherapeutant.

Oxytetracycline (OTC) concentrations are seldom determined in filtered water samples. For example, OTC was detected in only 4 % of the 189 freshwater effluent samples (filtered with a 0.7 μm filter) from hatcheries located in the

United States with a range of 0.23–10 μg/L (Thurman et al. 2003). Results of OTC concentration in river sediments receiving municipal wastewater treatment plant (MWWTP) effluents ranged from 139–262 mg/kg to 235–712 μg/L in water (Li et al. 2008) with highest concentration at the stations located closest to the MWWTP discharge. Pouliquen et al. (2009) reported detections of OTC in freshwater sediments downstream of fish farms with a maximum concentration of less than 1 mg/kg while Lalumera et al. (2004) detected OTC in sediments downstream of freshwater fish farms at concentrations raging from 0.0004 to 0.246 mg/kg. In comparison, our study has detected the highest OTC concentration in freshwater sediments from published literature. The highest detected concentration of OTC (18 mg/kg) in this study was between 18 and 73 times higher than the highest detection in freshwater sediment at fish farm facilities but also 27 times less than the highest concentration of OTC detected in marine sediments (Samuelsen 1989) and 14 times less than the OTC concentration measured downstream of a MWWTP (Li et al. 2008). Emamectin benzoate concentrations in freshwater sediment could not be found in the published literature. However, a few studies have measured EB in marine sediments usually under or close to aquaculture sites. McHenery and MacKie (1999) measured concentrations of EB under cage sites from below the detection limit to a maximum of 0.0027 mg/kg. SEPA (2004) was able to quantify EB concentrations in 3 out of 66 sediment samples with a range for those from 0.0061 to 0.0213 mg/kg. In comparison, our highest detection of EB (2.5 mg/kg) was two and almost three orders of magnitude higher than the detections published in McHenery and MacKie (1999) and SEPA (2004).

There have been a few studies to determine toxicity of OTC from water samples to a variety of organisms (algae, bacteria, invertebrates and fish). However since OTC is predicted to precipitate to sediment (Samuelsen 1989; Hirsh et al. 1999) it is of importance to determine toxicity to sediments concentrations of OTC. To that effect, Mayor et al. (2008) presented a 10 day LC_{50} value for a mud shrimp (*Corophium volutator*) of 0.414 mg OTC/kg. Based on the LC_{50} value calculated by Mayor et al. (2008), and although the mud shrimp is a marine organism, it is possible that the maximum concentration of OTC detected in our study (18 mg/kg) has a toxic effect to freshwater invertebrates living in the sediment at the outfall from Facility A (Table 1). The only other value for OTC detected in this study (0.14 mg/kg at Facility D) is lower than the LC_{50} value calculated by Mayor et al. (2008). However the relevance of marine to freshwater comparison cannot be established. The greater risk from OTC usage has often been described as the increase of bacterial resistance due to low level exposure of OTC (Samuelsen 1989). Although few toxicant data are available, Mayor et al. (2008) calculated a 10 day LC_{50} value for *Corophium volutator* and *Hediste diversicolor* of 0.153 and 0.138 mg EB/kg respectively. SEPA (1999) calculated a 10 day LC_{50} for a sand flea of 0.19 mg/kg. The Scottish EPA (2004) also published a predicted no effect concentration (PNEC) of 0.00076 mg/kg based on a maximum acceptable toxicant concentration (MATC) of 0.0763 mg/kg for a marine polychaete which included a 100 fold uncertainty factor. Only one facility in our study had detectable concentrations of EB (1.8–2.5 mg/kg, Table 1). The concentration detected at facility A is above the toxicity threshold for all three marine invertebrates described previously. However since our facilities discharged into freshwater environment, the effect of EB on freshwater invertebrates remains unclear.

Samuelsen (1989) described a situation where low OTC does not kill bacteria but offers favorable conditions for resistant strains to evolve. For example, Stachowiak et al. (2010) pointed to hatcheries as a source of tetracycline resistant microorganisms even in the absence of recent use of antibiotics. Stachowiak et al. (2010) also described the possibility of cross resistance to tetracyclines that was induced by the use of biocides (quaternary ammonium, hydrogen peroxide and hypochlorous acid) in fish production. While Herwig et al. (1997) found that a concentration of 1–4 mg/kg of OTC in sediment led to 3–9 % of bacteria being OTC resistant, Kerry et al. (1996) measured 15–25 % of bacteria as OTC resistant with a nominal concentration of 4.6 mg/kg in sediment. By comparison, the levels of OTC-resistant bacteria at background locations averaged 0.6 % (Herwig et al 1997). The minimum effect concentration (MEC) that retarded the growth rate of sensitive strains for OTC was calculated as 20 mg/kg (O'Reilly and Smith 2001). The MEC has the potential of providing an estimate of a matrix-specific environmental breakpoint below which it is reasonable to predict that no impact on resistance frequencies will occur (O'Reilly and Smith 2001). Our results suggest that the maximum concentration of OTC measured in this study has the potential to produce some resistance to the bacteria located in the sediment downstream of the effluent outfall at Facility A. Timing of the OTC treatment may also have an effect on bacterial resistance. Samuelsen et al. (1992) measured a 100 % resistance to OTC immediately after treatment while the resistance dropped to 20 % after 72 days post-treatment. Scott (2004) mentioned that the knowledge was incomplete in regards to the role of organic load, water chemistry and sediment adsorption properties in affecting bacterial resistance.

Unfortunately, microbial resistance measurements were not part of this study design.

While the facilities sampled during this study had different mechanisms of wastewater treatment (swirl

separator, settling deck, rotary drum filter, re-use technologies), it is interesting to note that some proportion of OTC and EB used in some facilities were able to bypass these mechanisms and enter the environment. Phong et al. (2009) calculated that a 50 μm filter was capable of a 500- to 650-fold concentration of OTC into the filter which is a way to prevent environmental release. Meanwhile, Rose and Pedersen (2005) predicted that 76 % of OTC could be removed through settling ponds and that those ponds would have a 95 % upper confidence limit of 21 mg/kg in settling ponds. The level predicted by Rose and Pedersen (2005) is of the same order of magnitude to that of Facility A in our study (18 mg/kg).

Overall, 2 out of 4 facilities had no detectable concentrations of OTC and EB in sediments near their outfalls. One facility had the highest concentrations of OTC and EB detected in freshwater sediments found in the published literature. There were no published reports found on the toxicant of either of these chemicals to freshwater invertebrates. The concentrations of OTC and EB detected at this facility were above the toxicity threshold for marine invertebrates. Lastly, the concentration of OTC in sediments detected as part of this study is comparable with levels which may offers favorable conditions for microbial resistance to evolve.

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