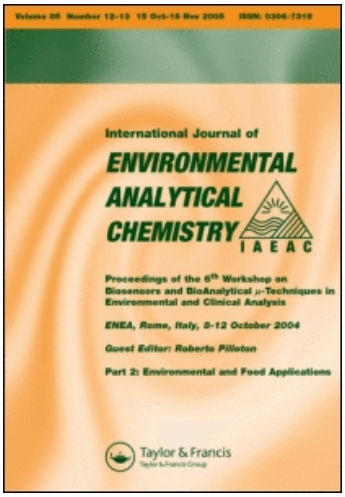

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Occurrence of antibiotics in water from 13 fish hatcheries, 2001–2003

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A 2-year study of extensive and intensive fish hatcheries was conducted to assess the general temporal occurrence of antibiotics in aquaculture. Antibiotics were detected in 15% of the water samples collected during the 2001–2002 collection period and in 31% of the samples during the 2003 collection period. Antibiotics were detected more frequently in samples from the intensive hatcheries (17 and 39%) than in samples from the extensive hatcheries (14 and 4%) during the 2001–2002 and 2003 collection periods, respectively. The maximum ormetoprim, oxytetracycline, and sulphadimethoxine concentrations were higher in samples from the intensive hatcheries (12, 10, and 36 $\mu g\,L^{-1}$), respectively, than in samples from the extensive hatcheries (<0.05, 0.31, and 1.2 $\mu g\,L^{-1}$), respectively. Sulphadimethoxine persisted for a longer period of time (up to 48 days) than ormetoprim (up to 28 days) and oxytetracycline (less than 20 days).

Keywords: Antibiotics; Fish hatchery; Ormetoprim; Oxytetracycline; Sulphadimethoxine

1. Introduction

The recent discovery of antibiotics in streams across the USA has raised awareness and demonstrated the need to monitor and determine sources of antibiotics and their persistence in the environment. It has been estimated that approximately 50% of the annual production of antibiotics in the USA is for human health, and 50% is for agriculture and aquaculture practices [1, 2]. The US Food and Drug Administration (USFDA) has approved antibiotics for use in aquaculture to treat systemic bacterial infections in fish. The approved antibiotics include a combination drug containing ormetoprim and sulphadimethoxine (marketed as Romet® 30), and oxytetracycline HCL (marketed as Terramycin® 10), which are approved for use on catfish and salmonid [3, 4]. These drugs generally are administered directly to the water in medicated feed at fish hatcheries.

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When antibiotics are used in aquaculture, the drugs may be transported out of the hatcheries into open waterways or sewage systems where they may also interact with other environmental contaminants [5]. Antibiotics may enter the environment as a result of leaching from faeces and uneaten antibiotic feed [6]. It has been estimated that 75% of the antibiotics fed to fish enter water through excretion [5]. Previous studies have found antibiotic residues in water, sediment, and fish tissue in aquaculture facilities following treatment with medicated feed [6–9]. Because of public health implications, the apparent increase in antibiotic resistance from areas of agricultural food animal production has led to an increase in studies of bacterial resistance in these settings [1, 10].

To determine the environmental concentrations of antibiotics in water from fish hatcheries across the USA, samples were collected from 13 fish hatcheries during 2001–2002. Three of the hatcheries were selected for a follow-up study in 2003. The objectives of this study were: (1) to assess the occurrence of antibiotics in fish hatcheries in multiple states; (2) to assess the difference in the occurrence of antibiotics in intensive and extensive hatcheries and between the different types of medicated feed treatment; and (3) to assess the difference in the persistence of antibiotics during and after antibiotic treatment. This research was conducted to provide fish-hatchery operators knowledge on antibiotic residues that may be useful in management practices such as recycling water and minimizing the release of water containing trace levels of antibiotics to the aquatic environment. This article presents the results on the occurrence and persistence of three antibiotics, ormetoprim, oxytetracycline, and sulphadimethoxine used in aquaculture.

2. Experimental

2.1. *Sampling sites*

During 2001–2002, water samples were collected from seven extensive and six intensive fish hatcheries from seven states—Colorado, Iowa, Kansas, Missouri, New York, Oklahoma, and Oregon (figure 1). In 2003, one previously studied extensive fish hatchery and two previously studied intensive fish hatcheries were sampled. These fish hatcheries produce numerous species of fish, some of which include bass (*Micropterus*), catfish (*Ictalurus*), muskellunge (*Esox masquinongy*), salmon (*Oncorhynchus*), trout (*Salmo*), and walleye (*Stizostedion vitreum*). Medicated feed, Romet® 30 and Terramycin® 10, were administered for 5 and 10 days, respectively, to both extensive and intensive hatcheries during the 2001–2002 and 2003 collection periods.

Extensive fish hatcheries typically are earthen ponds in which fish hatchery operators maintain optimum opportunities for fish to spawn and grow [11]. Extensive fish hatcheries can raise both warm- and cool-water fish (15–27°C). In these hatcheries, an undetermined number of fish are placed in the ponds and are weighed at the time of transfer from the fish hatchery to release locations. The ponds typically are drained at the end of the hatchery season and refilled during the next growing season [11].

In contrast, intensive fish hatcheries raise fish in concrete raceways, linear ponds in which the length is approximately 10 times its width, and are operated under a more controlled environment [11]. Hand feeding or mechanical fish feeders assist in meeting the nutritional needs of the fish. Oxygen, ammonia, and nitrate levels are maintained

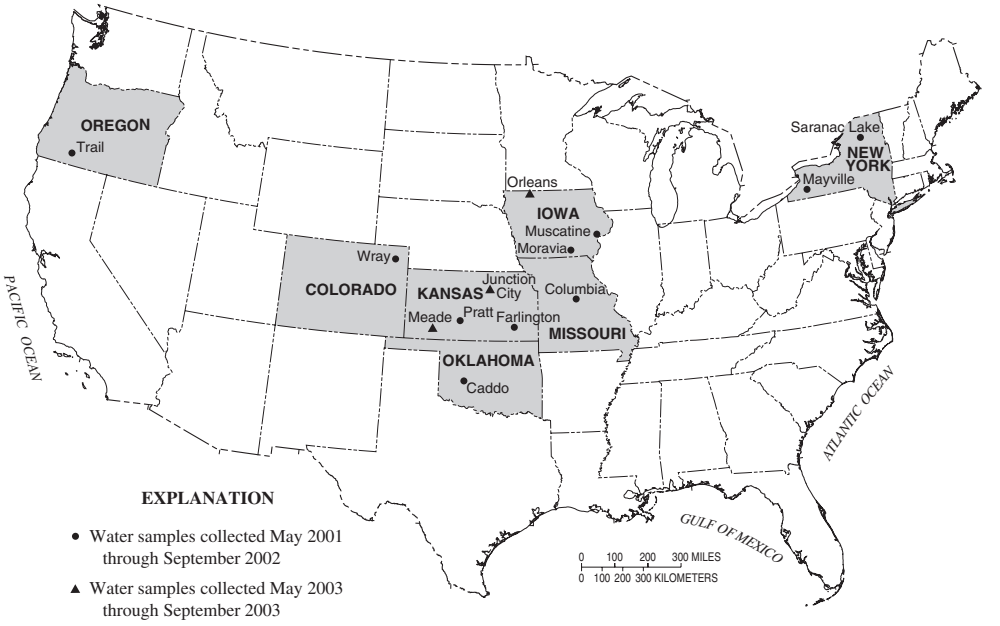

Figure 1. Location of 13 fish hatcheries across the USA sampled during May 2001–September 2002 and three fish hatcheries sampled during May–September 2003.

by the use of filters or rapid exchanges of water in the system to help meet the environmental needs of the fish. In the past, intensive fish hatcheries have been used only to raise cold-water fish (10–16°C); however, with improved technology, it has become practical to raise warm-water fish in intensive fish hatcheries [11].

2.2. *Sample collection*

During the May 2001–September 2002 collection period, 189 water samples were collected from the inflow, outflow, and ponds or raceways at the 13 fish hatcheries [12] (identified as Extensive Fish Hatcheries A–G and Intensive Fish Hatcheries A–F in table 1). Water samples were collected from one or two ponds or raceways from each fish hatchery except at Extensive Hatchery A and B, where water samples from additional ponds were collected.

During the 2003 collection period, a total of 124 water samples were collected from three of the fish hatcheries sampled in 2001–2002 (table 1). Samples were collected on a monthly basis from the three hatcheries, and additional samples were collected from any pond or raceway receiving antibiotic treatment during the period of the study. At Intensive Hatchery A, once raceways had been treated with antibiotics, samples were collected for the remainder of the season on a biweekly basis to better determine the persistence of antibiotics after medicated treatment.

Grab samples were collected in 250 mL amber glass bottles using methods described by Wilde *et al.* [13]. Water samples were chilled immediately and shipped overnight to the USGS Organic Geochemistry Research Laboratory in Lawrence, KS.

Table 1. Number of water samples analysed and number of antibiotics detected in water samples from fish hatcheries, May 2001–September 2002 and May 2003–September 2003 collection periods[a].

	2001–2002 collection period					2003 collection period			
		Number of detections					Number of detections		
Fish hatcheries[b]	Total samples analysed	Oxytetra cycline	Sulphadi methoxine	Tetra cycline[c]	Ormeto prim[d]	Total samples analysed	Oxytetra cycline	Sulphadi methoxine	Ormeto prim
Extensive fish hatcheries									
Hatchery A	43	1	4	0	0	14	1	0	0
Hatchery A; collection during Romet® 30 treatment	12	0	12	0	–	–	–	–	–
Hatchery A; collection during Terramycin® 10 treatment	–	–	–	–	–	12	0	0	0
Hatchery B	33	2	0	0	–	–	–	–	–
Hatchery C	8	0	0	0	–	–	–	–	–
Hatchery D	9	0	0	0	–	–	–	–	–
Hatchery E	10	0	0	0	0	–	–	–	–
Hatchery F	9	0	0	0	–	–	–	–	–
Hatchery G	6	0	0	0	–	–	–	–	–
Intensive fish hatcheries									
Hatchery A	13	1	7	0	1	31	0	13	7
Hatchery A; biweekly collection	–	–	–	–	–	21	0	6	4
Hatchery A; collection during Romet® 30 treatment	–	–	–	–	–	15	0	10	10
Hatchery A; collection during Terramycin® 10 treatment	–	–	–	–	–	8	7	0	0
Hatchery B	8	1	0	0	–	–	–	–	–
Hatchery C	10	2	0	2	–	–	–	–	–
Hatchery D	10	0	0	0	–	23	2	0	0
Hatchery E	10	0	0	0	–	–	–	–	–
Hatchery F	8	0	0	0	–	–	–	–	–
Totals	189	7	23	2	11 of 3	124	10	29	21

[a]–: no data collected. [b]Samples collected monthly unless otherwise stated. [c]Tetracycline was not detected during the 2003 collection period. [d]Ormetoprim was added to the method in September of 2002; therefore, three of the 189 samples during the 2001–2002 collection period were analysed for ormetoprim.

Water samples were filtered through a 0.7 μm, glass-fibre filter into 125 mL amber glass bottles in the laboratory.

2.3. *Sample analysis*

During the 2001–2002 collection period, water samples from each of the hatcheries were analysed for compounds in the quinoline, sulphonamide, and tetracycline classes of antibiotics. During the 2003 collection period, the beta lactam and macrolide classes of antibiotics were added to the method [14].

The beta lactams and macrolides (BLM), sulphonamides and quinolines (SQ), and tetracyclines (TET) were analysed separately using three online solid-phase extraction (SPE) methods (Triathlon autosampler/Prospekt-2 system, Spark Holland, The Netherlands) and liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) in positive-ion mode (Agilent 1100 LCMSD, Model 1946D, Wilmington, DE). Water samples were extracted in sets of 60, which included a standard curve and also one blank, one duplicate, and one matrix spike after every 10 samples and a continuing calibration check standard every 20 samples. The surrogate standard used for the BLM method was oleandomycin. The SQ method used nalidixic acid and $^{13}C_{16}$ sulphamethazine and the TET method used meclocycline.

Samples were extracted for the BLM and SQ methods using HLB Prospekt cartridges (Waters, Milford, MA) and for the TET method using a proprietary Glyphosate Prospekt cartridge (Spark-Holland, The Netherlands). Simetone was used as an internal standard for all three methods. All of the prepared samples were loaded onto the online SPE Triathalon autosampler. During analysis, the online SPE lines were rinsed with solvents and solutions configured with the Triathalon autosampler and the high-pressure dispenser. The cartridges were rinsed just prior to extraction. Ten millilitres of sample was pulled up through the sample vial into a Teflon sample loop and dispensed through the Prospekt SPE cartridge.

The antibiotics for each method were eluted and separated using an LC gradient with mobile phases A and B. Mobile phase A was 5 mM ammonium acetate for the BLM class, and 0.3% formic acid was used for the SQ and TET classes of antibiotics. Acetonitrile was used as mobile phase B for the BLM and SQ classes, and methanol was used for the TET class of antibiotics. The initial flow rates of mobile phases A and B were decreased and contained a higher proportion of mobile phase B to elute the Prospekt SPE cartridge.

During elution, the LC autosampler injected 20 μL of the internal standard. The isocratic mobile phase (mobile phase C) was used to increase the aqueous phase in the mobile-phase stream to focus the compounds eluted from the SPE cartridge onto the head of the LC column. Mobile phase C was 5 mM of ammonium-acetate for the BLM class, and 0.3% formic acid was used for the SQ and TET classes of antibiotics. After the mobile-phase flow was passed through the SPE cartridge, the flow rate was increased to 0.36 mL min^{-1}, and the isocratic pump flow was turned off. A 3.0-X 150-mm Luna C18(2) (Phenonomenex, Torrance, CA) with 3 μm packing was used to separate the antibiotics for each of the three methods. The LC column was rinsed for 5 min with 100% mobile phase B at the end of the gradient and then equilibrated at initial conditions for 5 min before the next sample analysis.

Individual antibiotic compounds were analysed using selected-ion monitoring and were quantitated using the ratio of the area of the base-peak ion of the analyte to the area of the base-peak ion of the internal standard. Compound confirmation was based on the presence of the molecular ion and one to two confirming ions. The method reporting limit (MRL) ranged from 0.05 to 0.10 $\mu g\,L^{-1}$ for all analysed compounds.

3. Results and discussion

3.1. *Occurrence of antibiotics in fish hatchery water*

During the 2001–2002 collection period, there were 33 antibiotic detections in 28 (15%) of the 189 samples collected (table 1). Sulphadimethoxine was the most frequently detected antibiotic, present in 23 of the 189 (12%) water samples collected. Twelve of the 23 samples, which contained sulphadimethoxine, were collected during treatments of Romet® 30 at Extensive Hatchery A. Oxytetracycline was detected in seven (4%) and tetracycline was detected in two (1%) of the 189 samples collected. The presence of tetracycline (an antibiotic not registered for use in aquaculture) may represent trace impurities present in the oxytetracycline or may be a possible transformation product of oxytetracycline [12]. Ormetoprim, which was added to the analytical method late in the first study (September 2002), was detected in one of the three samples analysed for this compound.

During the 2003 collection period, there were 60 antibiotic detections in 38 (31%) of the 124 samples collected (table 1). The most frequently detected antibiotic was sulphadimethoxine present in 29 (23%) of the 124 samples collected. Ormetoprim was detected in 21 (17%) and oxytetracycline in 10 (8%) of the 124 samples collected. Tetracycline was not detected during the 2003 collection period. All of the antibiotic detections occurred in samples from Intensive Fish Hatchery A except for one oxytetracycline detection in a sample from Extensive Fish Hatchery A and two oxytetracycline detections in samples from Intensive Fish Hatchery B.

The results from 2001–2002 show that antibiotics are present in water from both extensive and intensive hatcheries (table 1). The greater frequency of antibiotic detections during the follow-up study in 2003 was probably a function of the sampling design, in which more samples were collected during and shortly after antibiotic treatments. There were 23 antibiotic detections during the monthly collection periods in 2003 (table 1). However, there were 37 additional detections of antibiotics with the biweekly and antibiotic treatment samples.

In addition to the more frequent detection of antibiotics during treatment, concentrations also were greater than during non-treatment time periods. In 2003, the maximum concentrations of ormetoprim (12 $\mu g\,L^{-1}$) and sulphadimethoxine (36 $\mu g\,L^{-1}$) were greater during Romet® 30 treatments than during the monthly collections (0.12 and 0.69 $\mu g\,L^{-1}$), respectively (table 2). The maximum concentration of oxytetracycline during Terramycin® 10 treatments was 9 $\mu g\,L^{-1}$, and it was not detected during the monthly collection periods (table 2). These results show the importance of sample collection during treatment periods to better determine the occurrence, persistence, and concentrations of antibiotics within aquaculture environments.

Table 2. Percent detections and median and maximum concentrations ($\mu g\,L^{-1}$) for samples collected during May 2001–September 2003[a].

	Ormetoprim			Oxytetracycline			Sulphadimethoxine			Tetracycline		
		Concentration			Concentration			Concentration			Concentration	
Sample collection	Percentage detections	Median[b]	Max.	Percentage detections	Median[b]	Max.	Percentage detections	Median[b]	Max.	Percentage detections	Median[c]	Max.
2001–2002 collection period												
Monthly collection; $n = 177$	nc[c]	nc[c]	0.08	4	0.65	10	6	0.28	15	1	0.36	0.61
Collection during Romet® 30 treatment; $n = 12$	nd	nd	nd	nd	nd	nd	100	0.30	1.2	nd	nd	nd
2003 collection period												
Biweekly collection; $n = 21$	14	0.05	0.07	nd	nd	nd	29	0.37	0.48	nd	nd	nd
Monthly collection; $n = 68$	10	0.09	0.12	3	0.35	1.2	19	0.42	0.69	nd	nd	nd
Collection during Romet® 30 treatment; $n = 15$	67	7.9	12	nd	nd	nd	67	23	36	nd	nd	nd
Collection during Terramycin® 10 treatment; $n = 20$	nd	nd	nd	35	1.4	9	nd	nd	nd	nd	nd	nd

[a]*n*: number of samples collected; nc: not calculated; nd: antibiotic compound not detected. [b]Ormetoprim was added to the method in September 2002; therefore, three samples were analysed during the 2001–2002 collection period. [c]Medians computed as medians of those samples with detections.

3.2. *Comparison of results from extensive and intensive fish hatcheries*

Antibiotics were detected more frequently in intensive hatcheries (17 and 39%) than extensive hatcheries (14 and 4%) during the 2001–2002 and 2003 collection periods, respectively (table 3). Sulphadimethoxine was the most frequently detected antibiotic, occurring in 10% of the samples from extensive hatcheries and in 23% of the samples from intensive hatcheries. For the extensive hatcheries, oxytetracycline was detected in 3% of the samples, and ormetoprim and tetracycline were not detected in either collection period. For the intensive hatcheries, oxytetracycline was detected in 8% of the samples, ormetoprim in 14%, and tetracycline in 1% of the samples. Maximum concentrations were greater in samples from the intensive hatcheries compared with the extensive hatcheries as well. The maximum concentrations of oxytetracycline and sulphadimethoxine were 10 and 36 $\mu g\,L^{-1}$, respectively, in samples from the intensive hatcheries and were 0.31 and 1.2 $\mu g\,L^{-1}$, respectively, in samples from the extensive hatcheries.

Antibiotics were not detected in samples from the effluent, influent, or ponds during either collection period at the extensive hatcheries. During the 2001–2002 study, Extensive Fish Hatchery A used Romet® 30 in four ponds in which there were no detections of antibiotics in samples from the influent, ponds, or effluent of the hatchery. During the 2003 collection period, Extensive Fish Hatchery A used Terramycin® 10 in three ponds, and there were no detections of antibiotics in the samples of untreated water.

Antibiotics, however, were detected in samples from the effluent, raceways, and influents of the intensive hatcheries. During the 2001–2002 collection period, antibiotics occurred once in a sample from the effluent of Intensive Fish Hatchery A and once in a sample from the influent of Intensive Fish Hatchery B. During the 2003 collection period, antibiotic results showed, after a 5-day treatment of Romet® 30 to five raceways at Intensive Hatchery A, that there were three detections of antibiotics in samples of the effluent, three detections in samples from the influent, and seven detections in samples from two raceways not treated with medicated feed, which included four detections of ormetoprim and nine detections of sulphadimethoxine (table 3).

Conversely, after a Terramycin® 10 treatment was completed in two raceways at Intensive Fish Hatchery A, oxytetracycline was not present in samples from the influent, untreated raceways, or effluent of the hatchery. Concentrations in treated raceways ranged from 0.35 to 9.0 $\mu g\,L^{-1}$ during the 10-day treatment (not shown). A study conducted by Bebak-Williams *et al.* [7] on oxytetracycline in freshwater recirculating systems showed that oxytetracycline chelates divalent cations and binds readily to sediments, so it is more likely to accumulate in system water, biofilter sand, and sediment (fish faeces and uneaten feed), which may explain the absence of oxytetracycline in untreated water.

The absence of antibiotics in untreated water in the ponds of extensive hatcheries may be a result of uncirculated water within the hatchery. Because of the unintentional exposure of fish to antibiotics in untreated raceways of intensive hatcheries, further research should be conducted to assist in determining the advantages of recycling water within a hatchery and to better determine the occurrence of sulphadimethoxine and the absence of oxytetracycline in untreated water.

Table 3. Percent detections and median and maximum concentrations ($\mu g\,L^{-1}$) for samples collected from extensive and intensive hatcheries, 2001–2002 and 2003[a].

	Ormetoprim			Oxytetracycline			Sulphadimethoxine			Tetracycline			
		Concentration			Concentration			Concentration			Concentration		
	Percentage detections	Median[b]	Max.	Percentage detections	Median[b]	Max.	Percentage detections	Median[b]	Max.	Percentage detections	Median[b]	Max.	Total percentage detections
Extensive hatcheries													
2001–2002 collection period; $n=130$	nd	nd	nd	2	0.23	0.31	12	0.24	1.2	nd	nd	nd	14
2003 collection period; $n=26$	nd	nd	nd	4	0.30	0.30	nd	nd	nd	nd	nd	nd	4
2001–2002 and 2003 collection periods; $n=156$	nd	nd	nd	3	0.27	0.31	10	0.24	1.2	nd	nd	nd	12
Intensive hatcheries													
2001–2002 collection period; $n=59$	nc[c]	nc[c]	0.08	7	2.0	10	12	0.53	15	3	0.36	0.61	17
2003 collection period; $n=98$	21	0.12	12	9	1.2	9	30	0.45	36	nd	nd	nd	39
2001–2002 and 2003 collection periods; $n=157$	14	0.12	12	8	1.4	10	23	0.45	36	1	0.36	0.61	27

[a]*n*: number of samples collected; nc: not calculated; nd: antibiotic compound not detected. [b]Ormetoprim was added to the method in September 2002; therefore, only three samples were analysed during the 2001–2002 collection period. [c]Medians computed as medians of those samples with detections.

3.3. *Persistence of antibiotics in fish hatchery water*

Romet® 30 was administered to channel catfish for five consecutive days at a rate of 50 mg of active ingredients per kilogram of body weight per day. Sulphadimethoxine concentrations in water from Intensive Fish Hatchery A were higher (12–36 $\mu g\,L^{-1}$) than concentrations in samples from Extensive Fish Hatchery A (0.10–1.2 $\mu g\,L^{-1}$) during and following a 5 day treatment period of Romet® 30. Concentrations of sulphadimethoxine peaked by the third day of treatment at Intensive Fish Hatchery A, and after 17 days, concentrations decreased to trace levels (figure 2). However, trace concentrations of sulphadimethoxine were present after 48 days in one sample from Intensive Fish Hatchery A and after 41 days in one sample from Extensive Fish Hatchery A. Samples were collected only once per month during the first study (2001–2002) at Extensive Fish Hatchery A; therefore, the time of the peak sulphadimethoxine concentrations is unknown.

Ormetoprim, the other active ingredient in Romet® 30, was not analysed in samples collected during 2001–2002 from Extensive Fish Hatchery A. Concentrations of ormetoprim in Intensive Hatchery A were lower (3.1–12 $\mu g\,L^{-1}$) than sulphadimethoxine concentrations (12–36 $\mu g\,L^{-1}$) during Romet® 30 treatments. Ormetoprim concentrations, like sulphadimethoxine, peaked on the third day of treatment, and after 17 days, concentrations decreased to trace levels (figure 3).

Terramycin® 10 was administered to channel catfish for 10 days at the rate of 2.5–3.75 g of medicated feed per 45 kg (100 lb) of fish. When Terramycin® 10 treatments were used at Intensive Fish Hatchery A, oxytetracycline was detected in seven of eight samples collected from two raceways with concentrations ranging from 0.35–9.0 $\mu g\,L^{-1}$ (figure 4). However, when Terramycin® 10 was administered in three of the ponds at Extensive Fish Hatchery A, there were no detections of oxytetracycline during the treatment period and only one detection of 0.30 $\mu g\,L^{-1}$ on day 22 (not shown). Oxytetracycline levels began to peak between day 5 and day 8.

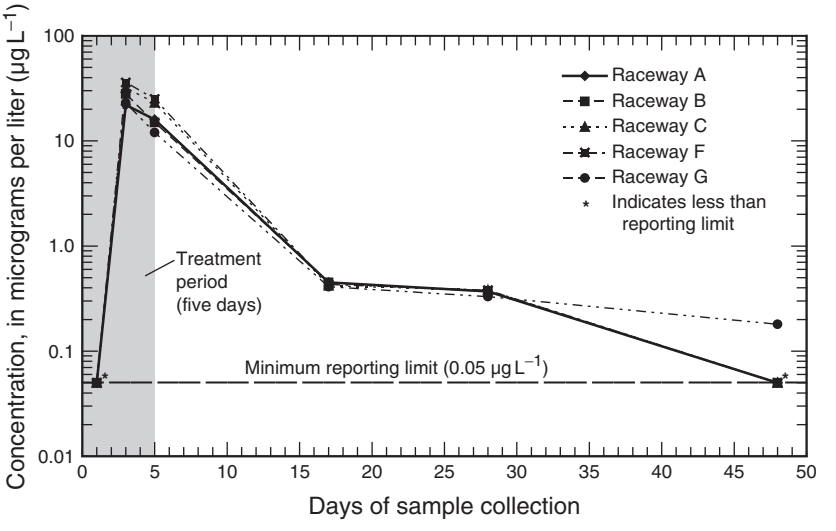

Figure 2. Changes in concentrations of sulphadimethoxine (Romet® 30) in water samples from treated raceways at Intensive Fish Hatchery A.

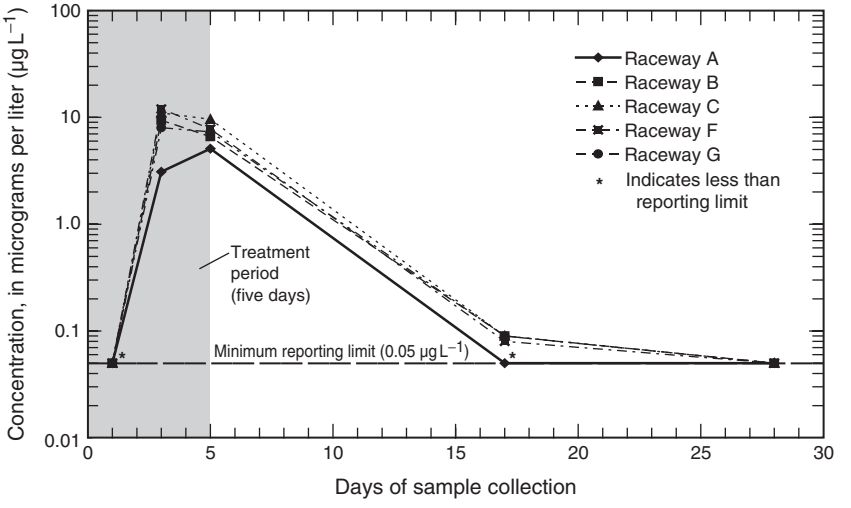

Figure 3. Changes in concentration of ormetoprim (Romet® 30) in water samples from treated raceways at Intensive Fish Hatchery A.

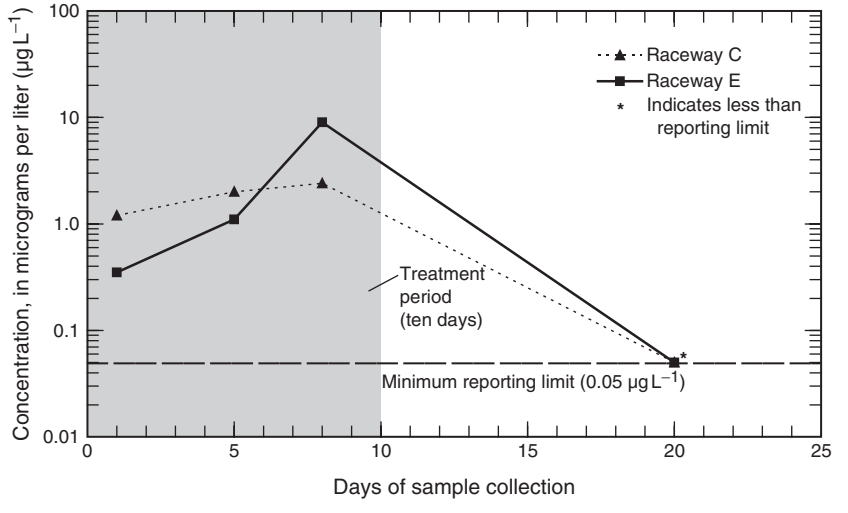

Figure 4. Changes in concentrations of oxytetracycline (Terramycin® 10) in water samples from treated raceways at Intensive Fish Hatchery A.

Samples were collected on day 20 and did not contain antibiotics. These data indicate that the persistence of sulphadimethoxine was greater than ormetoprim and oxytetracycline in the water. However, previous studies have shown that oxytetracycline readily binds to sediment [7], which may be the reason that it was not detected in water samples analysed after 20 days. The data also suggest that antibiotics may persist at detectable levels longer in intensive hatchery water than in extensive hatchery water. This may be due to less sorption occurring in concrete raceways than in earthen ponds.

4. Conclusion

Water from fish hatcheries contained measurable concentrations of antibiotics derived from medicated fish feed in 15 and 31% of samples taken in 2001–2002 and 2003, respectively, indicating a potential low-level source of antibiotics to the environment. The sample analysis indicates that sulphadimethoxine persisted for longer periods of time than ormetoprim and oxytetracycline.

Although antibiotic detections in samples from hatchery effluents were relatively uncommon, results do show that there is the potential for antibiotics to be transported outside the hatchery into the aquatic environment. Results of the study show evidence of unintentional exposure of low levels of antibiotics to healthy fish in raceways that were not treated with antibiotics.

The results of this study will help inform fish-hatchery operators of the importance of recycling water and minimizing the release of water containing trace levels of antibiotics to the aquatic environment. More research is needed to better understand the processes and pathways of antibiotics and their degradation products in the sediment and residue accumulated in the bottom of the extensive ponds and intensive concrete raceways.

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