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Fate and Effects of Enrofloxacin in Aquatic Systems under Different Light Conditions

C. W. KNAPP,[†,#] L. A. CARDOZA,[‡,#] J. N. HAWES,[§,#] E. M. H. WELLINGTON,[§] C. K. LARIVE,[‡,¶] AND D. W. GRAHAM*,[†]

Department of Civil, Environmental and Architectural Engineering and Department of Chemistry, University of Kansas, Lawrence, Kansas 66045, and Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

The fate and effects of fluoroquinolone antibacterials (FQ) in the environment is of significance because of apparent increased FQ resistance in environmental and clinical organisms. Here we simultaneously assessed the fate and effects of enrofloxacin (enro), an FQ often used in agriculture, on the chemistry and in situ microbial communities in receiving waters. We added enro to 25 μg/L in nine outdoor mesocosms maintained under three light conditions (in triplicate): full sunlight typical of the upper epilimnion (100% full-light exposure, FLE), partial shading typical of the lower epilimnion (28% FLE), and near-complete shading typical of the hypolimnion (0.5% FLE). Enro disappearance and ciprofloxacin (cipro) formation were monitored over time using LC/MS, and water chemistry and ambient microbial communities (using denaturing gradient gel electrophoresis; DGGE) were characterized. Enro half-lives were 0.8, 3.7, and 72 days for the 100%, 28%, and 0.5% FLE treatments, respectively, creating three distinct FQ exposure scenarios. Although FQ exposures ranged from ~6 μg/L for 24 h to ~21 μg/L for 30 days, no statistically significant exposure effects were noted in water quality or microbial communities (as indicated by whole-community 16S rDNA DGGE analysis and specific amplification of the QRDR region of gyrase A). Small changes in water chemistry were noted over time; however, changes could not be specifically attributed to FQs. In general, enro addition had minimal effect on water column conditions at the levels and durations used here; however, further investigation is needed to assess effects in aquatic sediments.

Introduction

Fluoroquinolones (FQs) are a class of synthetic antibacterials that have broad-spectrum antibiotic properties. The environmental fate of FQs, like similar agents, is of interest due to their frequent use in human and veterinary medicine and their potential for migration into the environment (*1*−*5*). The fate of FQs is of special concern because they have been shown to be genotoxic in hospital wastewaters (*6*), and their use is apparently leading to increased antibacterial resistance in clinical settings (*7*, *8*). Although ciprofloxacin (cipro) and levofloxacin are important for medical use, enrofloxacin (enro) is of greater interest in environmental systems because it is often used in agriculture and one of its primary degradation products is cipro (*2*).

The environmental fate of FQs is influenced by three primary mechanisms, photodegradation, adsorption, and biodegradation (*9*−*12*), although photodegradation and adsorption appear to be most significant in aquatic systems (*13*, *14*). Enro and cipro are both highly photodegradable with half-lives ranging from about 5 min to 5 h depending upon light intensity, pH, FQ level, phosphorus level, and the presence of organic matter (*10*, *14*, *15*−*17*), and both FQs readily adsorb onto organic particles (*14*, *18*, *19*). Golet et al. (*13*) concluded that adsorption and particle settling was the primary fate-driving mechanism for cipro in Swiss rivers, although Cardoza et al. (*14*) suggested that this may only be true in waters with elevated suspended particle levels. In general, the fate and attenuation of FQs is becoming understood; however, the "effects" of such compounds, especially in aquatic systems, need to be elucidated.

The lack of good effects data is largely due to inherent difficulties of working with FQs in quasi-natural systems. Enro rapidly adsorbs onto organic solids and photodegrades; therefore, creating exposure conditions reflective of different aquatic scenarios is difficult. In fact, we suspect that earlier studies that did not measure FQ levels in real-time (e.g., *20*) may have provided misleading results because nominal FQ levels tend to overestimate biologically active FQ. Further, methods for determining effects on individuals or communities are not trivial because FQs are both bactericidal and can have negative ancillary effects on higher organisms through different mechanisms (*21*, *22*).

The goal of this project, therefore, was to assess quantitatively the fate of enro in aquatic systems while simultaneously evaluating effects of the chemical under field conditions. We provided a single-pulse dose of enro to field mesocosms, each designed to simulate different aquatic systems (*23*), and monitored microbial community changes using denaturing gradient gel electrophoresis (DGGE) and enro disappearance and cipro formation using LC/MS. Enro exposures were "controlled" by varying light supply conditions in different field units to create differential light-related effects. The ultimate goal of using different light supplies was to simulate and compare different portions of the water column where enro might be found, ranging from light-exposed near surface waters to deeper waters where ambient light is very low. In theory, such different light conditions could result in different ambient enro levels and exposure periods that might influence exposure impacts. This is the first study where both the fate and effects of enro were addressed simultaneously in field-scale aquatic systems.

Materials and Methods

Field Mesocosm System. The experiment was performed in the fall of 2003 at the Nelson Environmental Study Area (NESA) located near Lawrence, KS. Nine cylindrical 11.3-m^3 fiberglass tanks, 3.2 m in diameter and 1.4 m deep, were placed into two "host" ponds supplemented with three 39 cm × 53 cm bottom sediment trays to inoculate the units and provide sediment zones. Three of the units were left

* Corresponding author phone: (785)864-2945; fax: (785)864-5379; e-mail: dwgraham@ku.edu.
[†] Department of Civil, Environmental and Architectural Engineering, University of Kansas.
[‡] Department of Chemistry, University of Kansas.
[§] University of Warwick.
[#] Contributed equally to the manuscript.
[¶] Current address: Department of Chemistry, University of California, Riverside, CA 92507.

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open, whereas lids for "partial" shading (black window screen) and "complete" shading (black vinyl plastic) were draped over the other six tanks (each in triplicate) to provide three different light scenarios (*23*). The goal of variable shading was to control sunlight, which in turn would regulate enro degradation rate in the units and simulate different portions of an aquatic water column. This experimental strategy succeeded (see later) with light treatments of (1) "open" or 100% full-light exposure (FLE), (2) "shaded" or 28% FLE, and (3) "dark" or 0.5% FLE, as measured using a LICOR no. LI-185A photon sensor.

Water for the mesocosms was provided from a protected source water pond located at NESA and, after transfer from the storage pond, allowed to "age" in the mesocosms for about 2 months prior to enro addition. All tanks were provided supplemental nitrogen (N, as KNO_3) and phosphorus (P, as H_3PO_4) throughout the experiment to elevate baseline nutrient levels, thus increasing the microbial biomass in the systems (*24*). N and P were added weekly to achieve upper mesotrophic water conditions (~35 μg/L total phosphorus; TP), although actual TP and total nitrogen (TN) levels were allowed to drift as influenced by light conditions in each unit. Enro was added to the units on October 6, 2003 to 25 μg/L; the units were monitored until November 18, 2003.

Water Sampling, Processing, and Analysis. Mesocosms were monitored weekly for physicochemical and molecular microbial water conditions commencing 2 weeks prior to enro addition. Real-time field measurements, including water temperature, pH, conductivity, and dissolved oxygen (DO), were determined at three depths (0.3, 0.7, and 1.2 m) using a Water Checker field monitor (Horiba Instruments) at each sampling event. Water column samples were also collected, using a 1.2-m long, 25-mm diameter PVC tube equipped with a check valve (*25*), for laboratory analysis of TN, TP, total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), chlorophyll *a*, total organic carbon (TOC), dissolved organic carbon (DOC), and enro and cipro. Samples were stored in acid-washed 500-mL and 1000-mL amber glass bottles with Teflon-lined caps for water chemistry and FQ analysis, respectively. Samples were retained on ice in the dark prior to return to the laboratory.

Samples for molecular microbial characterization were collected using the same samplers; however, the samplers were presterilized with ethanol and rinsed three times prior to use. Water samples were collected and rapidly transferred to sterile acid-washed amber bottles, and 200-mL volumes were filtered using 47-mm diameter sterile 0.22-μm Nalgene filters (NNI, Rochester, NY). The filters were frozen immediately on dry ice. Complementary microbial biomass levels were estimated using measured carbon levels and a previously developed correlation between particulate carbon level (POC = TOC − DOC) and biomass from similar mesocosms (*24*); i.e.,

$$\log(\text{biomass}) = 0.64 \log(\text{POC}) + 3.60$$

where biomass is in "μg/L" and POC is in "mg/L" ($n = 47$; $r^2 = 0.68$, $P < 0.01$).

Analytical Procedures. *Enrofloxacin and Ciprofloxacin Analysis*. Samples for enro and cipro analysis were prepared and analyzed as described for cipro (*14*). Typically, 500-mL water samples were filtered using GF/F glass microfiber filters (Whatman Inc., Clifton, NJ), and spiked with 2 mL of formic acid and 500 μL of the surrogate standard flumequine (10 000 μg/L) prior to concentration using solid-phase extraction (SPE). Due to interferences from natural organic matter, a two-step extraction method was used. SPE recoveries were 97 ± 4% for 25 μg/L and 104 ± 11% for 20 μg/L for enro and cipro, respectively.

Both FQs were analyzed using an HP1100 series HPLC/MSD (Agilent, Palo Alto, CA) equipped with a diode array detector and a Supelco Discovery HS C-18 column (Bellefonte, PA); operating details are provided in Cardoza et al. (*14*). Cipro, enro, nalidixic acid (the internal standard), and flumequine eluted at retention times of 11.2, 12.1, 21.6, and 22.3 min, respectively. Quantification was performed using the PDA peak area measured at 260 or 270 nm for enro and cipro with peak areas for flumequine and nalidixic acid measured at 285 nm. Cipro (100−100 000 μg/L) standards were prepared in RO-deionized water, where each solution contained flumequine (50 000 μg/L) and nalidixic acid (50 000 μg/L). Enro standards (100−100 000 μg/L) contained 25 000 μg/L of flumequine and 50 000 μg/L of nalidixic acid. All solutions were stored at 4 °C in the dark to reduce photodegradation.

Other Water Chemistry Analyses. DOC and TOC were determined using a Dohrmann organic carbon analyzer. Chlorophyll *a* was extracted directly from filters and analyzed spectrophotometrically (*27*). SRP and TP were analyzed spectrophotometrically (Shimadzu UV-160) with and without filtration, respectively, followed by digestion with potassium persulfate for TP (*26*, *27*). TDN and TN also were determined spectrophotometrically with and without filtration, respectively, followed by alkaline persulfate digestion (*28*). For TP and TN, digests were always stored at 4 °C for less than 2 days prior to analysis, whereas SRP and TDN were performed immediately upon return to the laboratory.

Molecular Microbial Analyses. Sample processing and preparation for DGGE was performed using methods similar to Muyzer et al. (*29*). Typically, three frozen filters harvested from each tank (per treatment) were combined to provide a single composite sample for analysis. The filters were placed in a 2-mL Fast Prep tube containing 0.5 g of 0.1 mm zirconia beads and combined with 500 μL of DNA extraction buffer (50 mM sodium acetate, 10 mM EDTA; adjusted to pH 8.5), phenol, and 35 μL of 20% (w/v) SDS. Samples were then subjected to two bead-beating steps in a Ribolyser (Hybaid) at speed 6.5 for 45 s. The initial supernatant was removed after centrifugation at 10 000*g* for 10 min and retained on ice. A fresh 250 μL aliquot of DNA extraction buffer was readded to the Fast Prep tubes that underwent further bead-beating at speed 6.5 for 30 s. The second supernatant was combined with the first supernatant for further processing.

The combined sample was mixed with an equal volume of phenol, and the aqueous phase was extracted and separated by centrifugation at 10 000*g* for 10 min. This step was repeated with equal volumes of phenol/chloroform (1:1 v/v) and chloroform. The product DNA was precipitated by the addition of a 0.5 volume of 7.5 M ammonium acetate and a 0.6 volume of 2-propanol and stored at −20 °C overnight. The pellet was washed with 80% (v/v) ethanol and then aspirated and resuspended in 50 μL of sterile deionized water prior to amplification.

Two amplification reactions were performed on the total community DNA extracted from the samples. The variable V3 region of the 16S rRNA gene (rDNA) was amplified using eubacterial primers F341 and R534 (*Escherichia coli* numbering) according to Muyzer et al. (*29*). Additionally, primers GYRAF/GYRAR and QRDRF/QRDRR were used to amplify a 469 bp fragment of the DNA gyrase subunit A gene from the total community DNA in a nested PCR (Table 1). DNA gyrase is the enzymatic target of enro, and amino acid polymorphisms in the C-terminus region (known as the quinolone resistance determining region or QRDR) are believed responsible for the majority of enro resistance (*30*). PCR products for all samples (which were processed in duplicate) were pooled prior to DGGE analysis.

Amplicons were analyzed on a 10% acrylamide denaturing gradient gel at a temperature of 60 °C for 5.5 h at 200 V. The

TABLE 1. PCR Primers Used in This Study

primer pair	sequence (5′−3′)[a]	target gene	amplicon size
GYRAF	GCGCGATGGCCTGAARCCNGTNCA	*gyrA*	429
GYRAR	CGTCGCCATGCCCACNGCDATNCC		
QRDRF	GCGCGATGGCCTGAA	*gyrA*	469
QRDRR	CGTCGCCATGCCCAC[b]		
F341[c]	CCTACGGGAGGCAGCAG[b]	bacteria 16S rDNA	233
R534[c]	ATTACCGCGGCTGCTGG		

[a] Degenerate bases: R (A or G), D (A or G or T), and N (A or C or G or T). [b] GC clamp attached at the 5′ end for DGGE. [c] Source: Muyzer et al. (*29*).

TABLE 2. Water Column Conditions before and after Enrofloxacin Addition under the Three Light Conditions

	0.5% FLE		28% FLE		100% FLE	
parameter	before[a]	after	before	after	before	after
pH	8.00 (0.02)[b]	7.93 (0.10)	**9.01 (0.09)**	**8.75 (0.26)**	**9.59 (0.07)**	**9.13 (0.37)**
conductivity[c]	0.303 (0.009)	0.307 (0.009)	0.215 (0.004)	0.215 (0.007)	0.208 (0.002)	0.209 (0.003)
DO[d]	**5.37 (0.87)**	**8.21 (1.84)**	13.1 (1.01)	13.8 (1.28)	15.6 (0.90)	14.9 (1.54)
temperature (°C)	**16.2 (0.90)**	**13.3 (3.1)**	**15.8 (1.1)**	**13.2 (3.1)**	**16.0 (1.2)**	**13.4 (3.7)**
TP[e]	52.2 (10.9)	61.2 (11.6)	**31.3 (4.2)**	**21.3 (7.0)**	**29.7 (7.32)**	**19.9 (5.71)**
SRP[e]	44.0 (9.78)	41.5 (19.8)	**10.1 (7.03)**	**3.00 (2.57)**	2.71 (2.41)	2.49 (2.08)
TN[e]	989 (132)	1085 (193)	697 (91)	607 (87)	834 (97)	865 (150)
TDN[e]	1100 (141)	1217 (152)	687 (56)	645 (134)	768 (150)	718 (119)
TOC[d]	4.82 (0.31)	5.07 (0.29)	**7.65 (0.72)**	**9.03 (0.73)**	**10.6 (1.39)**	**13.4 (0.82)**
DOC[d]	4.18 (0.40)	4.50 (0.40)	7.10 (0.97)	7.95 (0.70)	**9.19 (1.66)**	**11.5 (0.97)**
Chlorophyll *a*[e]	0.71 (0.58)	2.8 (1.31)	5.38 (1.98)	5.06 (2.61)	6.80 (1.98)	4.26 (2.74)
Biomass[f]	2820 (1700)	2630 (2190)	2690 (2820)	4370 (3630)	5130 (5010)	6030 (3800)

[a] Before and after the addition of enrofloxacin. [b] 95% confidence interval. [c] mS/cm. [d] mg/L. [e] μg/L. [f] μg/L as estimated from calculated particulate organic carbon (POC) levels. Bold values denote significant differences (*t*-test, $P < 0.05$) among values before and after enro addition within individual light treatments.

gradients used were 30−70% and 40−70% urea denaturant for the 16S rDNA and DNA gyrase A primer sets, respectively. Gels were prepared and run in 1× Tris−acetate−EDTA (TAE) buffer and were stained using SYBR green I (Molecular Probes). Gel visualization was performed using a Gene Genius bio imaging system (SynGene).

Data Analysis. *Water Chemistry and Enrofloxacin Decay Rate.* Mean physiochemical conditions in each tank were calculated based on a minimum of 8 samples per tank after FQ exposure ("after" samples) and 4 samples per tank prior to FQ exposure ("before" samples). The means for each parameter were then combined for all tanks under each treatment to produce overall means per treatment. The 95% confidence intervals (C. I.) were calculated using standard errors associated with each mean; a Student's *t*-test assuming unequal variances was used to compare means. Enro levels over time were pooled for the three tanks under each treatment, and overall decay coefficients were estimated using a first-order model. Estimated half-lives were then calculated using the decay coefficients. All statistical analyses employed SPSS (*31*).

Comparison of DGGE Banding Patterns. DGGE banding patterns for each amplification reaction were assessed with the GeneTools analysis program (version 3.03.03, SynGene). This software matches bands across lanes on a single gel image (or across multiple gels if reliable markers are included) and then creates a profile for each chosen lane (minus background) using band intensity versus R_f distance. Each track/lane profile was compared with related profiles, including the "active matching standard" (a user defined track/lane), and quantitative comparisons between lanes were developed based on the number of matching bands per profile. Final dendrograms were produced that displayed lane relatedness to assess whether there were measurable differences among tracks pre- and postantibiotic addition. Selected bands were excised and sequenced for band identification as described in the Supporting Information.

Results

Mean Water Conditions in the Mesocosms. Table 2 summarizes mean water column conditions under the three treatments before and after enro addition. In general, the 100% FLE units had the highest pH, DO, DOC, TOC, chlorophyll *a*, and biomass levels, and the lowest SRP and conductivity levels of the three treatments, suggesting they were dominated by photosynthesis relative to decomposition and were P-limited for microbial growth (*23*). The 28% FLE units had intermediate levels of all measured parameters and were also dominated by photosynthesis, although there appeared to be a shift from N to P limitation around the time of enro addition as suggested by changing SRP and TDN levels. In contrast, the 0.5% FLE units were decomposition dominated as indicated by low pH, DO, DOC, TOC, chlorophyll *a*, and biomass levels, and high conductivities. Elevated SRP and TDN levels in the 0.5% FLE tanks suggested they were either light- and/or C-limited for microbial growth, which is consistent with earlier data for "dark" units (*23*).

Some differences in water conditions were noted before and after enro addition, although it was difficult to define whether these changes resulted from enro addition or were related to seasonal changes in water conditions in autumn. For example, mean temperatures declined and DOC and TOC levels significantly increased in all treatments after enro addition (*t*-test, all $P < 0.05$). TP also significantly declined under the two higher light treatments; however, none of these changes in physiochemical conditions can be unequivocally attributed to enro because such changes might also result from carbon release due to bacterial die-off or due to seasonal senescence.

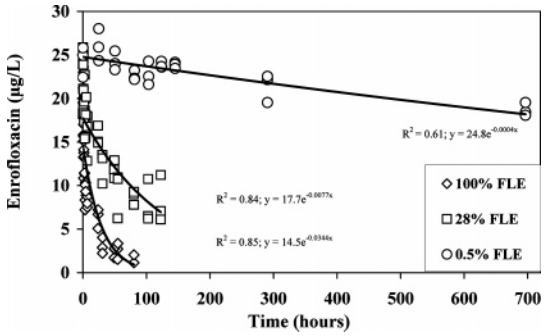

FIGURE 1. Enrofloxacin disappearance vs light supply (expressed as full-light equivalent, FLE).

Enrofloxacin Transformation Patterns. Figure 1 presents enro disappearance patterns for the three light conditions. First-order decay coefficients for the 100%, 28%, and 0.5% FLE treatments were 0.034, 0.0077, and 0.0004 hr^{-1}, respectively, which correspond to half-lives of 0.8, 3.7, and 72 days for the three treatments. Mean midday light intensity was 2880 $\mu mol/m^2/s$ ($\pm$ 710, 95% confidence intervals) during the 5 days immediately after enro addition. As noted, there were significant temperature differences among treatments (normalized data, *ANOVA*; $F_{2,110} = 11.09$, $P < 0.01$). However, mean differences were less than 0.25 °C among treatments and major temperature effects were considered unlikely. In summary, light exposure clearly influenced enro fate in the systems and created three distinct enro exposure scenarios. Enro was present in the 100% FLE units for only ~1 day, whereas enro was present for >5 days in 28% FLE units and for >30 days in 0.5% FLE units.

Ciprofloxacin Formation from Enrofloxacin. Cipro is a major degradation product of enro, therefore, it was also monitored over time to estimate the "combined" antibacterial level in the systems. Figure 2 summarizes cipro formation patterns under each treatment. Figure 2A shows that cipro was formed in the 100% FLE units within minutes of enro addition, cresting about 4 h after exposure. Cipro level was approximately 2 μg/L for about 5 days after enro addition and then dropped below detection limits (<0.5 μg/L). As such, the combined antibacterial level (the sum of enro and cipro masses) was ~6 μg/L for about 24 h in the 100% FLE units.

In contrast, Figure 2B shows that cipro levels were >4.5 μg/L for ~5 days after enro addition in the 28% FLE units, suggesting a combined antibacterial level of ~10 μg/L for about 5 days in this treatment. Finally, Figure 2C shows that cipro was first detected 12 days after enro addition in the 0.5% FLE units but remained at very low levels (<2 μg/L) for 30 days after exposure. Therefore, the 0.5% FLE units retained ~21 μg/L combined antibacterial level, mostly enro, for ~30 days in these systems.

DGGE Analysis. DGGE was used to compare microbial community similarities and differences before and after enro addition under the three exposures. In general, minimal differences were noted among microbial communities in samples collected before and after enro addition as determined by the PCR analyses. Figure 3 summarizes total 16S-rDNA DGGE banding patterns for the three treatments and Table S1 summarizes identified bands (see the Supporting Information). Specific bands appear and disappear over time under each treatment (e.g., bands C (uncultured bacterium) and D (*Acidovorax delafieldii*) appear at day 12 in the 100% FLE treatment), but no broad changes in the number of bands, or extensive losses or gains in individual bands, were noted around enro addition. Band A in the 100% FLE treatment (*Pseudomonas putida*) and Band E in the 28% FLE treatment

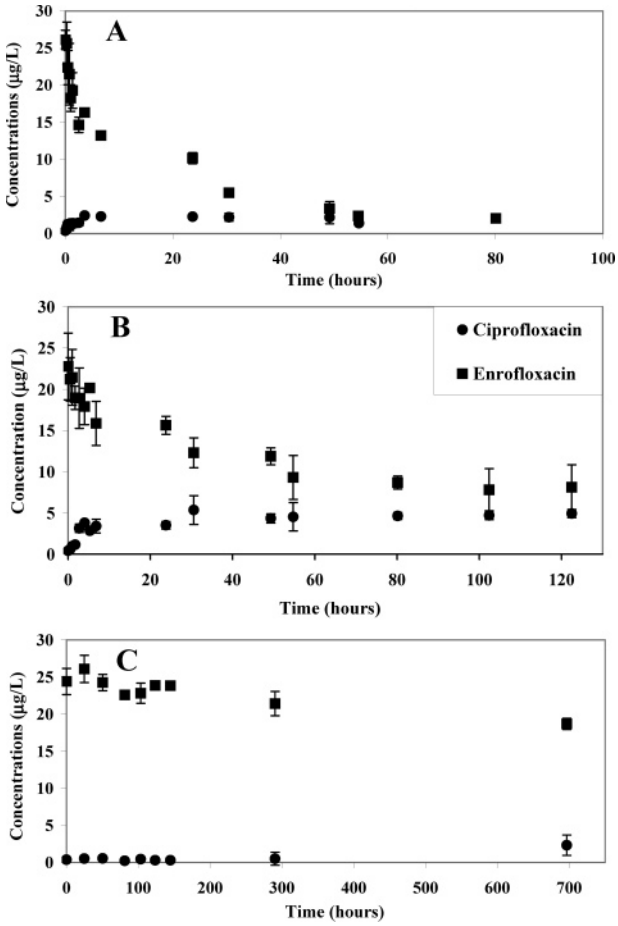

FIGURE 2. Ciprofloxacin formation curves from enrofloxacin: (A) in tanks receiving full sunlight, 100% FLE (full-light equivalent); (B) partially shaded tanks, 28% FLE; (C) completely shaded tanks, 0.5% FLE.

(*Cyanothece* sp.) both appeared after enro addition; however, these were isolated responses.

This broad "lack of pattern" is confirmed by Figure 4, which presents a general relatedness dendrogram for the 0.5% FLE treatment for the 16S rDNA. Although mild clustering of bands is noted before and after enro addition (e.g., the minus versus plus days), such clustering was not dramatic or consistent with time and cannot be unequivocally attributed to FQ exposure. Similar dendrograms were seen for the 28% and 100% FLE treatments (data not shown).

Figure 5 and Table S2 (in the Supporting Information) show DGGE analyses and band identifications for total community *gyrA* from the 100% and 0.5% FLE treatments, providing a general picture of presumptive FQ antibacterial resistance sequences under these exposures. Although some band shifts were noted, few new bands appeared post-enro addition. Band B, which was identified as *Novosphingobium aromaticivorans* (Table S2), did appear after enro addition and contained one conserved mutation at position 83 (Ala-83-Ser) and three nonconserved mutations at positions 73, 93, and 116, suggesting that this organism may have been selected by enro addition. Additionally, Band D (*Cytophaga hutchinsonii*) also had a mutation at key position 83 (often associated with quinolone resistance; *32*). However, few other changes in community *gyrA* were noted under either short-term (100% FLE) or long-term (0.5% FLE) enro exposures (e.g., Band C (*C. hutchinsonii*) disappeared after enro addition, even though it contained one nonconserved mutation at position 62). Similar observations were made

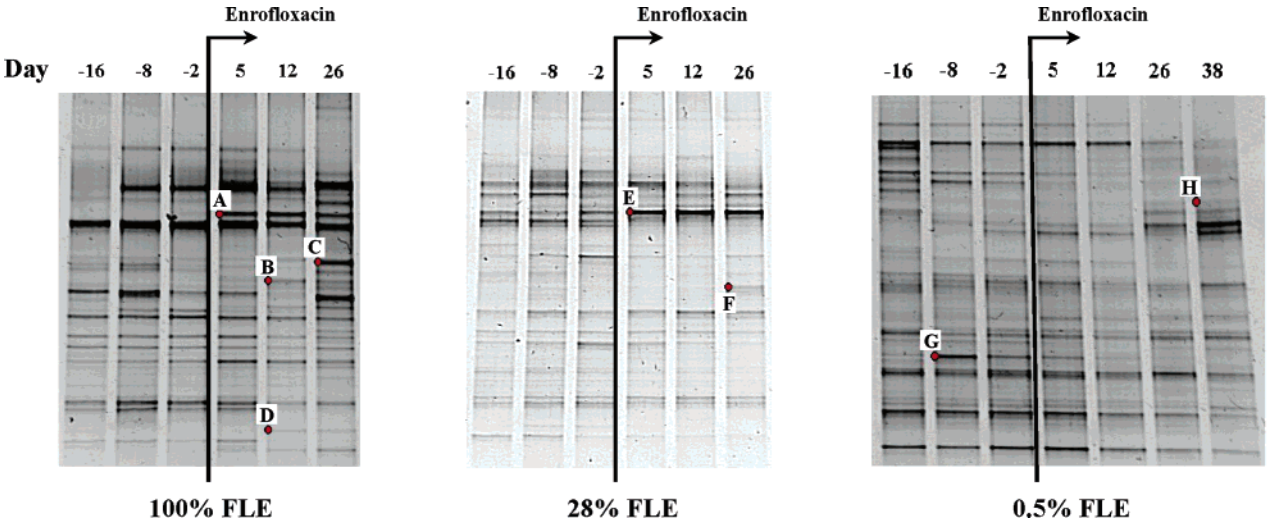

FIGURE 3. DGGE analysis of 16S-rDNA PCR products from different light treatments before and after enrofloxacin addition. Letters indicate excised bands; see Table S1 in the Supporting Information for identification.

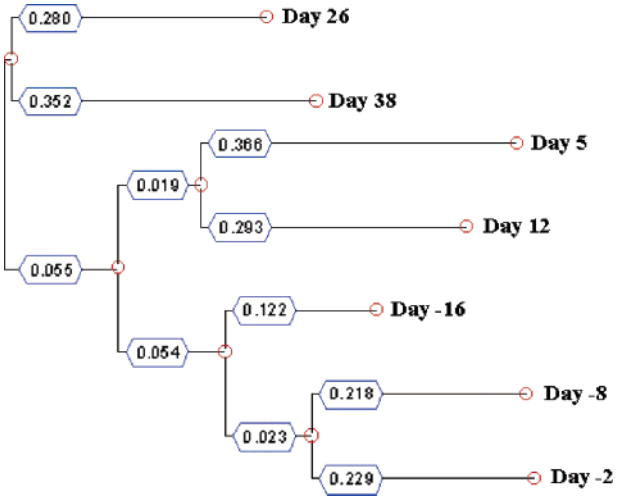

FIGURE 4. Dendrogram of sample relatedness based upon GeneTools analysis of DGGE tracks—16S rDNA analysis of samples collected from completely shaded tanks, 0.5% FLE.

for *Psuedomonas aeruginosa gyrA* genotypes as reported elsewhere (*33*).

Discussion

Fluoroquinolone antibiotics have been used successfully in both human and veterinary medicine for about 25 years. However, concern has developed over possible negative impacts of these compounds on the environment and in the development of antibacterial resistance via environmental exposures or exposure through the food supply (*8*). Although there is strong evidence that FQ use in agriculture, especially for nontherapeutic applications (*34*), has had a major impact on antibacterial resistance in pathogens via food systems, there is still minimal evidence of similar impacts through the water supply.

The purpose of this study was to assess the fate and effects of enrofloxacin in aquatic mesocosms to assess both ecological impacts and possible in situ antibacterial resistance development due to environmental exposure. Enro was selected for this study because it is the most commonly used FQ in agriculture (*35*) and one of its main breakdown products is cipro, a critical antibacterial agent used in human medicine. A unique component of the study was the use of different light supply conditions to produce three different enro and cipro exposures due to differential light exposure. This strategy permits a comparison of enro fate and effects in different portions of the water column that have different levels of light exposure.

Figures 1 and 2 confirm that light supply regulates the decay rate of enro and the formation of cipro in the different systems. If light is present, enro rapidly photodegraded to cipro, and cipro is subsequently further degraded. As such, FQ exposure times and levels were inversely related to light supply, providing three antibacterial exposures: ~6 μg/L for ~24 h at 100% FLE (short duration—lower concentration), ~10 μg/L for 5 days at 28% FLE (moderate duration—higher concentration), and ~21 μg/L for ~30 days at 0.5% FLE (longer duration—high concentration). These data indicate that enro released into lower light environments will last much longer than in near-surface light-exposed locations. It should be noted that we had considered providing enro at a constant supply rate to simulate continuous inputs (as opposed to a single pulse); however, at the mesocosm scale, this approach required prohibitively large quantities of enro, and the single-dose approach was used in this preliminary study.

Effects of FQ exposure were assessed using two general measures: changes in water chemistry and changes in the molecular microbial community pre- and post-enro addition. In fact, changes in water chemistry were noted around enro addition; however, most changes were not statistically significant nor could be clearly attributed to enro. This experiment was performed in midautumn: a time for seasonal change in water conditions. Given this, changes in water conditions would have to have been dramatic after enro addition to differentiate between "seasonal" versus "enro" effects. No such major changes were seen implying that enro had minimal impact on water chemistry.

This observation is confirmed by DGGE analysis of total community 16S rDNA and *gyrA* before and after enro addition shown in Figures 3 and 5. Although individual bands appear and disappear around enro addition, no major pattern of change is seen in either community composition, community diversity, or in the selection of apparent resistant organisms as suggested by *gyrA* data. Furthermore, dendrograms, such Figure 4, describing relatedness among DGGE gel patterns showed no consistent changes under any exposure scenario. In broad terms, the microbial community did not dramatically change after enro was added either in terms of diversity or apparent resistance.

There are several explanations for the observed "lack of effect". First, FQ levels used in this experiment were low

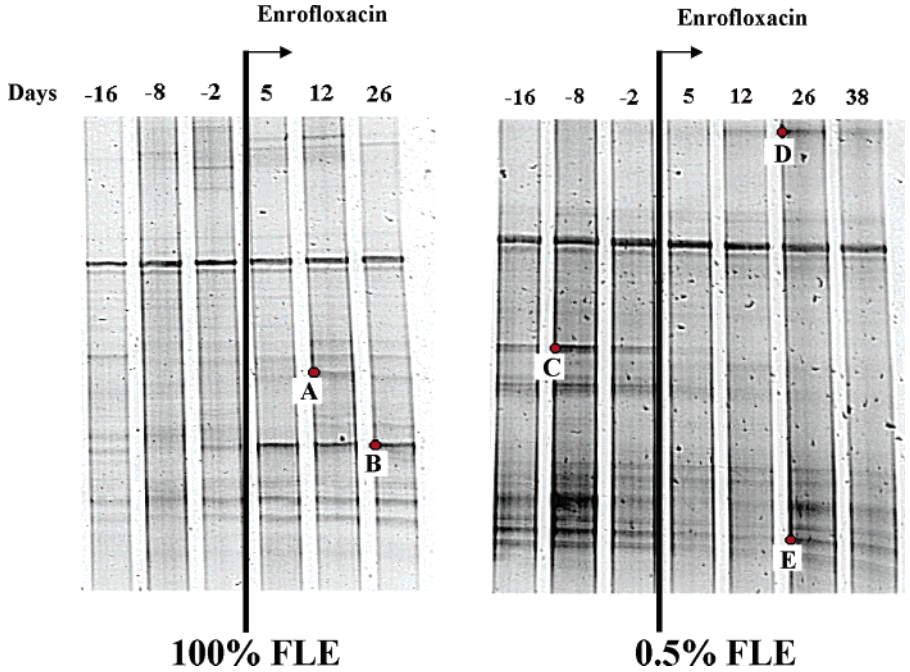

FIGURE 5. DGGE analysis of *gyrA* PCR products from the shaded tanks (0.5% FLE) and tanks receiving full sunlight (100% FLE) units. Letters indicate excised bands; see Table S2 in the Supporting Information for identification.

compared to medical or veterinary applications. Our 25 ug/L enro addition was near the lowest MIC (minimal inhibitory concentrations) observed for some enteric *E. coli* (~16 to 31 μg/L enro), but was well below the MIC for most clinical isolates (average ~750 μg/L; *36*, *37*). Therefore, the lack of effect was not surprising given the relatively short half-life of the FQs in the water columns.

A second possible explanation for the minimal effects is related to the method employed. DGGE is a well-established technique for assessing microbial community characteristics, however, it does not reflect all organisms; it only detects numerically dominant species in a system. Therefore, impacts on less abundant groups would not likely be detected. Regardless, the method provided insights into genetic diversity within and around the QRDA region and provided preliminary evidence for possible resistance genotypes within the system. Further, the method should be very useful for future screening environments of FQ resistance. Finally, it is possible that major "effects" due to FQ release to the environment imply do not occur in the water column. For example, it is possible that more significant impacts might have been seen in the sediments where prolonged exposures are more likely, although preliminary data suggest that such impacts on sediment communities might also be minimal (data not shown).

In summary, results show that enro is readily converted to cipro in aquatic systems, although the half-life of both compounds is short if light is present. Three different FQ exposures were generated, but none of the FQ exposures had major impacts on water quality or the microbial community. This does not mean that FQ use and possible release may not have negative effects (because evidence from medicine indicates significant resistant development in the food supply). However, this does indicate that FQs are short-lived in aquatic systems, and exposed microbial communities are not likely to have negative impacts at the concentrations and exposure times used here. Specifically, data suggest that small amounts of FQs to surface waters do not necessarily have negative effects via water, although additional work must be performed to verify the effects of FQs in sediments where higher exposures might prevail.

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Supporting Information Available

Materials and methods related to the excision and sequencing of bands from the DGGE; identification of organisms from 16S rDNA and gyrase A DGGE analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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