Supporting Information

Temporal Dynamics of Periphyton Exposed to Tetracycline in Stream Mesocosms

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Materials and Methods

Site description. Mesocosms within the U.S. Environmental Protection Agency (U.S. EPA) Experimental Stream Facility (ESF) were used to examine the short and long-term impacts of tetracycline (TC) exposure on the periphyton community in a study conducted from August to October 2007. Periphyton, including grazers and predators, colonized the mesocosms naturally via river water flowing continuously in a semi-controlled environment (i.e., flow velocities, hydraulics, slope, substrate, and irradiance controlled). Foci of simulated conditions in this study were the microhabitat features (i.e., riffle-runs) of natural streams, in which there is a continuous source of upstream organisms for colonization of downstream habitat with reach-scale antibiotic discharge (i.e., point and non-point sources). This flow-through design allowed for potential recolonization of periphyton throughout the experiment. A detailed description of the ESF stream design, lighting, building, and operational logistics is provided elsewhere (*1–2*).

Source water for this study was the Lower East Fork of the Little Miami River (LEFR), Clermont County, OH, USA. The five mesocosms (12-m length) each consisted of five sections: a head tank that delivered water via a broad-crested weir to the system; a tile-lined flume for periphyton measurements; a short transition section; a gravel-substrate flume; and finally, a tail pool. Mesocosm discharge remained constant at 1.6 L s^{-1} in a semi-recirculating mode of operation, with 64% recirculating flow, and 36% LEFR water renewal.

Experiment conditions. Background water quality conditions were continuously measured using Hach Company (Loveland, CO, USA) probes positioned in the tail pool of each mesocosm, and included dissolved oxygen (mg L^{-1}), pH, specific conductance (µS cm^{-1}), water temperature (ºC), oxidation reduction potential (mV), and turbidity (NTU). Parameters were sensed at 5-min intervals and the data acquired along with output from flow controllers, light

meters, and indoor and outdoor climate sensors. Surface grab and intergravel water samples were collected and analyzed for carbon and nutrient species every other week employing Lachat Instruments' (Milwaukee, WI, USA) QuickChem® 8000 Flow Injection Autoanalyzer methods for nitrate (*3*), ammonium nitrogen (*4*), total nitrogen (*5–6*), total phosphorus and total reactive phosphorus (*7*). Total organic carbon was analyzed by wet oxidation with ultraviolet (UV)/persulfate using a Sheena 6000 TOC analyzer (Boulder, CO, USA). Intergravel water was obtained based on diffusion-based solute equilibration by burying a 63-µm covered glass vial filled with water within the gravel substrate.

Tetracycline dosing. After a 21-day colonization period, TC (Sigma-Adrich, St. Louis, MO, USA) was dosed continuously for 28 days so that individual mesocosm concentrations of 0.0, 0.5, 1, 10, and 100 µg L^{-1} were achieved. This range of TC dose was based on environmentally-relevant concentrations reported for streams and rivers throughout the United States (*8*), concentrations found in collection systems and wastewater treatment plant effluents (*9*), and concentrations applied on agricultural lands as liquid manure (*10*). To eliminate the potential for degradation compounds or epimers, stock concentrations were replenished every 3 days in the dosing tanks. The stock concentrations were housed in stock tanks which directly fed the streams TC continuously for 28-d. Treatments were assigned randomly to the mesocosms. After 28 days of dosing, the mesocosms were allowed to recover for an additional 28 days.

Tetracycline analysis. Water samples, collected in duplicate at the head and tail of each mesocosm, and also collected at the source water LEFR inflow, were analyzed for tetracycline, anhydrotetracycline, and epi-tetracycline (Sigma-Aldrich, Saint Louis, MO, USA) throughout the colonization, dosing, and recovery periods using solid phase extraction (SPE) in combination with liquid chromatography and tandem mass spectrometry (LC/MS/MS) every 7 days. Samples

were concentrated using a previously published extraction method (*11*) and extracts were analyzed using an Acquity Ultra Performance Liquid Chromatograph coupled to a Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray ionization source (ESI; Waters Corp, Milford, MA, USA). An average percent recovery from four matrix spike samples was used to report TC concentrations, with the daily average recoveries ranging from 80–108% over the four week sampling period.

Streambed substrate sampling. Two different substrates, surface-sterilized unglazed terra cotta clay tiles (surface area 8870 mm^2) and polypropylene trays filled with gravel (2.0-cm median diameter, 26.0 cm x 15.9 cm x 6.4 cm, surface area 0.043 m^2) were placed in the ESF mesocosms prior to the colonization period. Tiles (n = 3) and trays (n = 2) were randomized by row and column *a priori* and were collected every 7 days during colonization, dosing, and recovery periods.

Periphyton development. Periphyton was collected from two different substrates, tiles (surface area 8870 mm^2) and gravel-filled trays (2.0-cm median particle diameter). Tiles (n = 3 per mesocosm) and trays (n = 2 per mesocosm) were randomly sampled every 7 days during colonization, dosing, and recovery periods, and replaced with clean (HCl-washed) substrate. Periphyton was gently scraped from the tiles, then rinsed into a tared beaker with reagent water, and weighed. The periphyton slurry was hand-mixed and then subsampled for bacteria abundance, algae composition, AFDM, Chl a, antibiotic resistance, and bacteria production. Slurry subsamples for bacteria abundance and algae composition (20 mL) were collected in glass scintillation vials and preserved with a final concentration of 0.5 % gluteraldehyde. Live periphyton slurry was also collected for bacteria resistance plates (23) and cell-specific bacteria productivity analysis (33) and held on ice and transported back to the USEPA National Exposure

Research Laboratory, Cincinnati, OH for further analysis within 6 hours of collection. Periphyton slurry subsamples were filtered on site for AFDM (17) and chlorophyll a (Chl-a) (12)

Periphyton was also gently scraped from the gravel trays, then rinsed into a tared beaker with reagent water, and weighed. Periphyton endpoints collected from the gravel trays include nematodes and macroinvertebrates.

Bacteria abundance. Bacteria abundance in duplicate was determined using a dual stain technique (*14*) and enumerated on an epifluorescence microscope (UV excitation) equipped with a 100x oil immersion objective. On each filter, no fewer than 200 clear-edged cells in 20 microscopic fields were counted. Field blanks were stained and examined using the same technique for every 10 bacteria slides. Bacteria abundance was determined per area of tile.

Cell-specific bacteria productivity assays. Radiolabeled leucine (L-[U-14C] leucine) in sterile water at a specific activity of 261 mCi $mmol^{-1}$ was obtained from Moravek Biochemicals, Inc. (Brea, CA, USA) and unlabelled leucine was purchased from Sigma-Aldrich (Saint Louis, MO, USA). All leucine incorporation analyses were based on a modified combustion method (*14*) on a PerkinElmer Model 307 Sample Oxidizer and analyzed using a PerkinElmer Tricarb 2300 Liquid Scintillation Analyzer (PerkinElmer, Waltham, MA, USA).

Leucine uptake experiments were conducted with periphyton subsamples (n = 6) at 7 and 21 days of dosing and then 7 and 21 days during the recovery period (after dosing was discontinued). Based upon the results of two saturation tests (incubating periphyton slurry from the control mesocosm with increasing leucine [from 1.3 to 51.2 µM and from 10 to 211 µM]), 45 µM was used in the uptake experiments on duplicate vials for each sample. Non-biotic leucine sorption was assessed with formalin "killed" controls. With the exception of the 7-day dosing experiment, where periphyton samples collected from the 0.5 µg L^{-1} TC treatment were lost, all

antibiotic treatments were included in the productivity assays. Leucine incorporation (pmol cm^{-2} h^{-1}) was normalized by bacteria abundance (cells cm^{-2} identified by epifluorescent microscopy) to determine cell-specific uptake per hour (amol h^{-1}).

Tetracycline-resistant bacteria. Antibiotic resistance to TC was determined using dilution plating according to methods described by McArthur and Tuckfield (*15*). Briefly, bacteria from serially-diluted periphyton slurries were plated on control plates with half-strength nutrient agar (n = 2) and TC-enriched plates (100 µg mL^{-1}; n = 2). To control fungal overgrowth, both sets of plates contained 0.36 mM of cycloheximide (*15*). All plates were incubated at room temperature (~23°C) in darkness for 6 days (*15*). Relative frequency of TC-resistant bacteria was calculated as the ratio of TC-resistant colony count to the control count. Mean TC-resistant bacteria from the dosing and recovery periods was reported as a percent abundance of the total TC-resistant bacteria (n = 7). Compositional changes in the bacteria community during dosing and recovery are currently being analyzed independently.

Algae composition. Subsamples for the identification and enumeration of soft algae and diatoms were preserved with a final concentration of 1% gluteraldehyde. Soft algae and diatoms were identified to the lowest possible taxonomic level using a Palmer counting cell at a magnification of 100x and 400x. No fewer than 500 cells or algal units were counted for each sample (*5*) in duplicate. Biovolume measurements were determined for all major algae groups. Algal composition was reported as relative abundance. All subsamples were re-examined by a separate independent taxonomist for verification of identification.

Nematodes and macroinvertebrates. Invertebrates were sampled from the periphyton slurries obtained from gravel trays. Retrieved gravel trays were gently emptied into a 2.0-mm sieve stacked on top of a 250-µm sieve, placed over a tared 20-L bucket. All organic matter was

removed with thorough mixing and rinsing with reagent water. Macroinvertebrates retained on the 250-µm sieve were elutriated and preserved with 75% ethanol. Macroinvertebrates were identified and enumerated per m^{-2} using a dissecting microscope in duplicate. Macroinvertebrates were separated into the following categories: aquatic insects (composed of the orders Coleoptera, Diptera, Ephemeroptera, Lepidoptera, Odonata, Plecoptera, and Tricoptera); mollusks (represented by the families Ancylidae (limpets) and Corbiculidae (Asian clams)); annelids, and crustacea.

Microinvertebrates were subsampled (1 L) from the tared bucket, after the tray contents were passed through the 250-µm sieve, and a final weight was obtained. The microinvertebrate subsample was then filtered through a 63-µm mesh sieve (*16*). Microinvertebrates retained on the sieve were collected into a tared centrifuge tube, preserved with a formalin-phloxine B reagent equal to the sample volume, and then weighed. Nematodes comprised the majority of the microinvertebrate community and were counted using a Sedgewick Rafter Counting Chamber at 100x magnification for each sample (minimum of 100 nematodes per sample). Nematode abundance per tray was corrected by the volume of sample for each consecutive sieving procedure.

Seasonal Trends

The time frame of the experiment spanned late summer and early autumn, resulting in declining light availability and water temperatures, and increases in dissolved oxygen (Table S2). These seasonal trends corresponded significantly with the periphyton mat development (AFDM, Chl *a*, bacteria, and cyanobacteria) (Appendix I)

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TABLE S1. Statistical summary of the separate generalized linear models for each response variable and each time period. Dunnett's test was used post-hoc to test for the lowest observable dose ($\mu g\ L^{-1}$).

Variable	Period	Source of variation	df	F	P-value	Lowest Observable Dose ($\mu g\ L^{-1}$)	Box-Cox Transformation
Antibiotic resistance	Dosing	Time	4	36.3	<0.0001	0.05	1/(x+1)
		TC Treatment	4	2.4	NS		
		Time * TC	16	2.6	0.017		
	Recovery	Time	3	46.9	<0.0001	100	sqrt(x+1)
		TC Treatment	4	3.0	0.044		
		Time * TC	12	2.3	0.047		
Bacteria abundance	Dosing	Time	4	26.3	<0.0001	0.05	log10(x)
		TC Treatment	4	37.5	<0.0001		
		Time * TC	16	4.8	0.0003		
	Recovery	Time	4	25.2	<0.0001	1	square root
		TC Treatment	4	57.4	<0.0001		
		Time * TC	16	4.9	0.0002		
Leucine uptake	Dosing	Time	1	5.94	0.019	10	square root
		TC Treatment	4	3.17	0.022		
		Time * TC	3	1.01	NS		
	Recovery	Time	1	7.51	0.009		no transformation
		TC Treatment	4	1.34	NS		
		Time * TC	3	0.68	NS		
Ash-Free Dry Mass	Dosing	Time	4	20.9	<0.0001	10	1/(sqrt(x+1))
		TC Treatment	4	48.5	<0.0001		
		Time * TC	16	5.4	<0.0001		
	Recovery	Time	4	3.0	0.042	100	log10(x+1)
		TC Treatment	4	20.6	<0.0001		
		Time * TC	16	1.2	NS		
Chlorophyll *a*	Dosing	Time	4	13.4	<0.0001	10	1/(sqrt(x+1))
		TC Treatment	4	28.7	<0.0001		
		Time * TC	16	4.3	<0.0001		
	Recovery	Time	4	3.5	0.015	100	1/(sqrt(x+1))
		TC Treatment	4	34.9	<0.0001		
		Time * TC	16	2.3	0.013		

Table S1 continued.

Variable	Period	Source of variation	df	F	P-value	Lowest Observable Dose (μg L^{-1})	Box-Cox Transformation
Cyanobacteria	Dosing	Time	4	806.9	<0.0001	0.5	no transformation
		TC Treatment	4	717.6	<0.0001		
		Time * TC	16	157.3	<0.0001		
	Recovery	Time	4	6.9	0.001	100	no transformation
		TC Treatment	4	79.7	<0.0001		
		Time * TC	16	3.4	0.003		
Nematode abundance	Dosing	Time	4	3.6	0.020	100	no transformation
		TC Treatment	4	3.2	0.031		
		Time * TC	16	1.9	NS		
	Recovery	Time	4	13.4	<.0001	100	no transformation
		TC Treatment	4	7.8	0.000		
		Time*TC	16	2.2	0.034		

TABLE S2. Mean background conditions by colonization, dosing, and recovery periods.[1]

	Experiment Period					
	colonization		dosing		recovery	
Parameter	**mean**	**±*SEM***	**mean**	**±*SEM***	**mean**	**±*SEM***
Climate						
Irradiance (µmol m^{-2} s^{-1})	52.1	*0.5*	47.6	*0.5*	42.9	*0.4*
% Outside, Open Canopy Irradiance	15.2		14.8		20.4	
Outside Air Temperature (ºC)	25.7	*0.06*	22.6	*0.08*	18.9	*0.07*
Continuous Water Quality						
Water Temperature (ºC)	26.7	*0.02*	24.3	*0.03*	20.3	*0.03*
Specific Conductance (µS cm^{-1})	396	*0.2*	377	*0.2*	358	*0.3*
pH (-log [H])	7.7	*0.001*	7.68	*0.001*	7.69	*0.001*
ORP (mv)	382	*0.6*	451	*0.2*	437	*0.2*
Turbidty (NTU)	15.8	*0.05*	16.6	*0.09*	15.2	*0.3*
Dissolved Oxygen (mg L^{-1})	7.5	*0.006*	7.86	*0.01*	8.71	*0.01*
Biweekly Surface Water Grabs						
Total Phosphorus (µg L^{-1})	194	*5*	280	*10*	222	*10*
Total Reactive Phosphorus (µg L^{-1})	163	*2*	232	*8*	233	*1.00*
Total Nitrogen (µg L^{-1})	1890	*20*	2110	*40*	2230	*20*
Ammonium (µg L^{-1})	11.5	*1*	8.31	*0.70*	12.1	*2*
Nitrite-Nitrate (µg L^{-1})	1410	*8*	1690	*50*	1720	*30*
Total Organic Carbon (mg L^{-1})	4.59	*0.05*	4.12	*0.03*	4.08	*0.03*
Biweekly Intergravel Water Samples						
Total Phosphorus (µg L^{-1})	203	*4*	474	*20*	415	*30*
Total Reactive Phosphorus (µg L^{-1})	177	*2*	258	*10*	279	*10*
Total Nitrogen (µg L^{-1})	1960	*20*	1770	*70*	1830	*100*

Ammonium (µg L^{-1})	5.65	*2*	73.1	*20*	22.5	*10*
Nitrite-Nitrate (µg L^{-1})	1480	*17*	1140	*90*	1090	*90*
Total Organic Carbon (mg L^{-1})	4.70	*0.1*	4.32	*0.1*	4.51	*0.04*
Target Tetracycline Concentrations						
Head Tank						
0.0 µg L^{-1}	0.00	*0.0*	0.00	*0.0*	0.00	*0.0*
0.5 µg L^{-1}	0.00	*0.0*	0.415	*0.08*	0.00	*0.0*
1 µg L^{-1}	0.00	*0.0*	0.990	*0.2*	0.00	*0.0*
10 µg L^{-1}	0.00	*0.0*	12.8	*2*	0.00	*0.0*
100 µg L^{-1}	0.00	*0.0*	115	*30*	0.00	*0.0*
Tail Tank						
0.0 µg L^{-1}	0.00	*0.0*	0.00	*0*	0.00	*0.0*
0.5 µg L^{-1}	0.00	*0.0*	0.465	*0.1*	0.00	*0.0*
1 µg L^{-1}	0.00	*0.0*	0.958	*0.2*	0.00	*0.0*
10 µg L^{-1}	0.00	*0.0*	13.2	*2*	0.00	*0.0*
100 µg L^{-1}	0.00	*0.0*	110	*30*	0.00	*0.0*

[1]No significant difference for mesocosm-specific parameters was observed among treatments, means represent pooled data across the five mesocosms. Intergravel nutrient species differed significantly among periods.

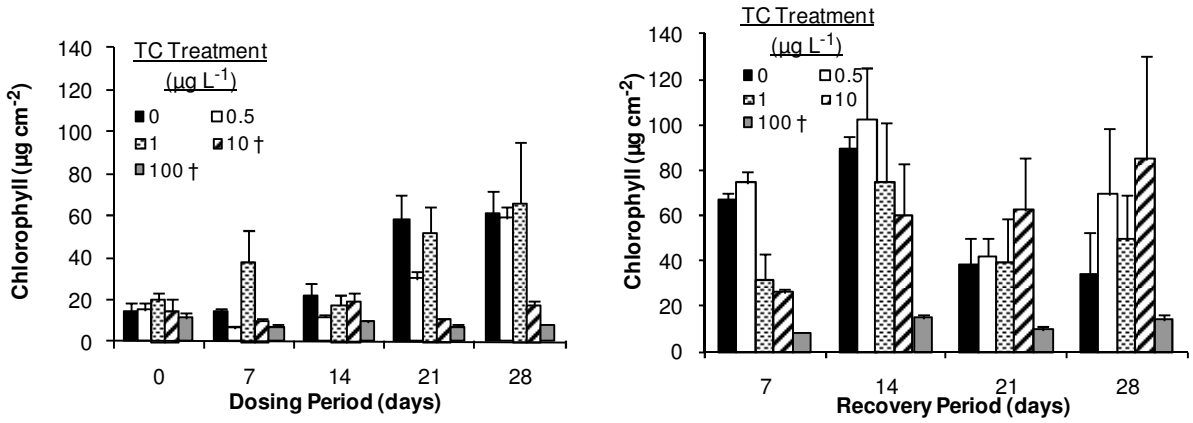

Figure S1. Mean chlorophyll *a* (µg cm^{-2}) during tetracycline (TC) exposure (Dosing) and after TC dosing was discontinued (Recovery). Significant difference relative to control, Dunnett's (†).

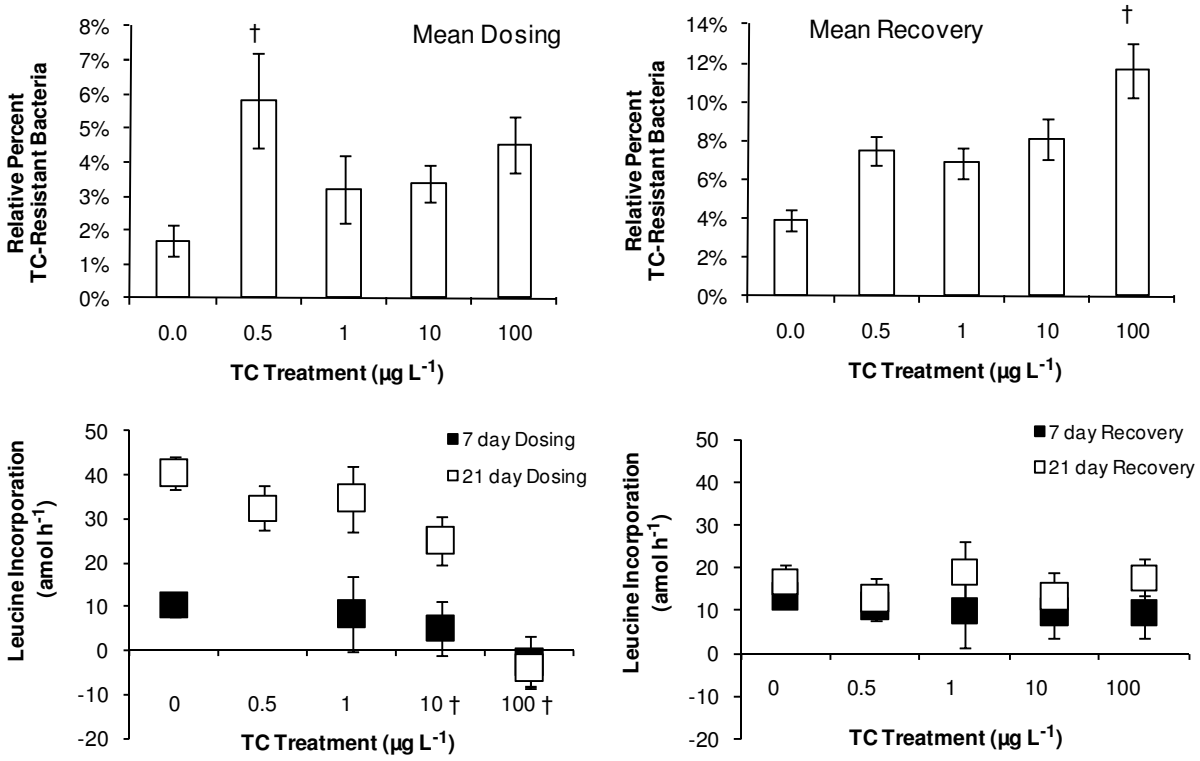

Figure S2. Pooled mean relative percentage of tetracycline (TC)-resistant bacteria relative to control plates (± SEM) and cell-specific leucine incorporation (amol h^{-1}) (± SEM) (Black 7 day, White 21 day) during tetracycline (TC) exposure (Dosing) and after TC dosing was discontinued (Recovery). Significant difference relative to control, Dunnett's (†).

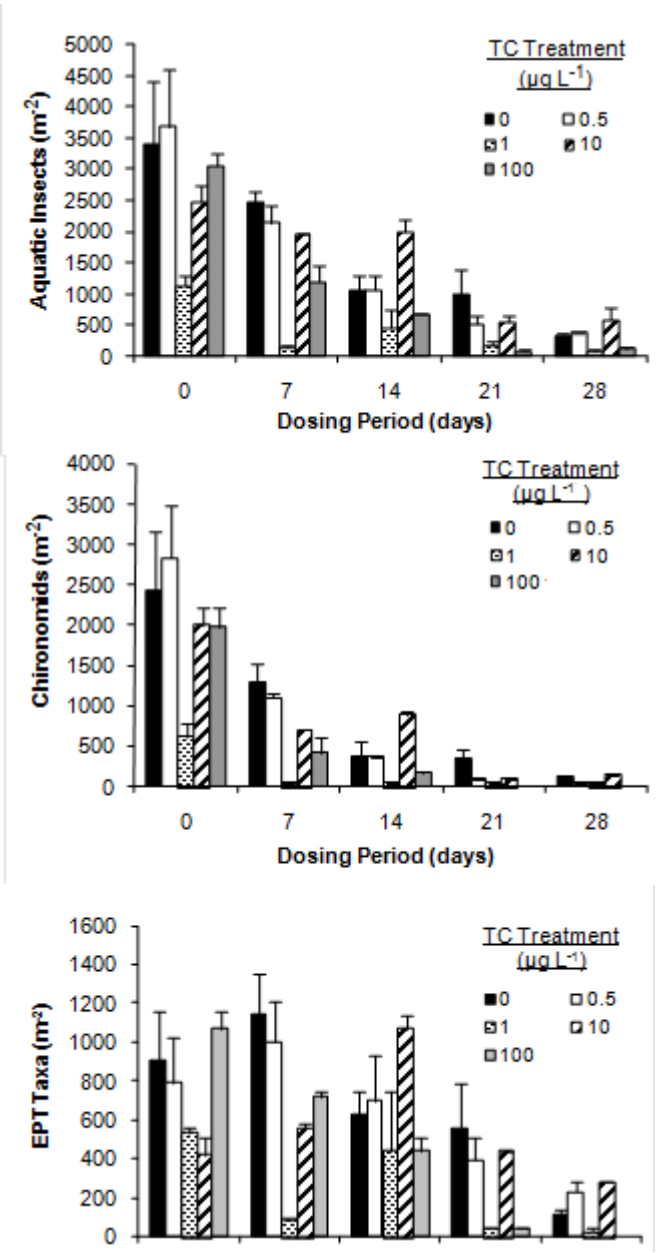

Figure S3. Mean aquatic insects (top), chironomids m^{-2} (center), and EPT taxa (bottom) (± SEM) during the tetracycline (TC) exposure.

APPENDIX I

			Tetracycline Exposure			
Endpoint	**Sampling Date**	**Control**	**0.5 µg L^{-1}**	**1.0 µg L^{-1}**	**10 µg L^{-1}**	**100 µg L^{-1}**
AFDM (mg/cm^2)	**Dosing 0 d**	3.50	3.81	3.65	1.52	3.24
AFDM (mg/cm^2)	**Dosing 0 d**	1.83	5.03	7.39	2.04	2.61
AFDM (mg/cm^2)	**Dosing 0 d**	3.46	4.89	5.69	3.70	3.78
AFDM (mg/cm^2)	**Dosing 7 d**	3.41	2.39	1.61	1.99	1.08
AFDM (mg/cm^2)	**Dosing 7 d**	4.29	1.53	14.12	2.48	1.36
AFDM (mg/cm^2)	**Dosing 7 d**	2.64	1.40	6.77	2.29	1.25
AFDM (mg/cm^2)	**Dosing 14 d**	2.46	1.51	4.75	2.82	0.57
AFDM (mg/cm^2)	**Dosing 14 d**	1.93	2.31	3.41	1.52	0.81
AFDM (mg/cm^2)	**Dosing 14 d**	7.15	2.61	4.06	2.89	0.77
AFDM (mg/cm^2)	**Dosing 21 d**	10.39	7.84	24.63	1.93	1.19
AFDM (mg/cm^2)	**Dosing 21 d**	17.67	7.89	15.65	0.80	1.02
AFDM (mg/cm^2)	**Dosing 21 d**	20.80	8.94	4.38	1.48	0.69
AFDM (mg/cm^2)	**Dosing 28 d**	15.52	15.20	14.15	4.17	1.72
AFDM (mg/cm^2)	**Dosing 28 d**	26.94	25.06	10.97	2.50	1.48
AFDM (mg/cm^2)	**Dosing 28 d**	16.06	13.97	38.13	3.76	1.41
AFDM (mg/cm^2)	**Recovery 7 d**	22.46	20.93	17.40	9.19	1.53
AFDM (mg/cm^2)	**Recovery 7 d**	25.40	22.29	8.37	6.42	1.33
AFDM (mg/cm^2)	**Recovery 7 d**	23.53	19.01	9.94	6.79	1.46
AFDM (mg/cm^2)	**Recovery 14 d**	27.67	30.19	22.50	7.17	1.42
AFDM (mg/cm^2)	**Recovery 14 d**	28.97	35.98	61.44	28.85	0.98
AFDM (mg/cm^2)	**Recovery 14 d**	27.28	14.62	5.31	5.16	1.28
AFDM (mg/cm^2)	**Recovery 21 d**	17.17	22.05	8.41	22.39	2.21
AFDM (mg/cm^2)	**Recovery 21 d**	21.76	45.96	6.21	19.77	3.48
AFDM (mg/cm^2)	**Recovery 21 d**	35.20	27.76	38.57	36.73	7.41
AFDM (mg/cm^2)	**Recovery 28 d**	16.54	13.18	4.17	9.29	6.06
AFDM (mg/cm^2)	**Recovery 28 d**	16.32	48.25	19.06	15.77	9.41
AFDM (mg/cm^2)	**Recovery 28 d**	32.12	34.70	60.87	49.04	13.20

			Tetracycline Exposure			
Endpoint	**Sampling Date**	**Control**	**0.5 µg L^{-1}**	**1.0 µg L^{-1}**	**10 µg L^{-1}**	**100 µg L^{-1}**
Chl a (ug/cm2)	**Dosing 0 d**	12.34	9.00	13.53	8.51	9.36
Chl a (ug/cm2)	**Dosing 0 d**	8.64	12.97	25.16	9.68	8.33
Chl a (ug/cm2)	**Dosing 0 d**	22.76	22.86	21.26	25.94	16.11
Chl a (ug/cm2)	**Dosing 7 d**	10.39	6.76	36.77	9.02	5.95
Chl a (ug/cm2)	**Dosing 7 d**	14.34	6.37	65.14	8.79	7.62
Chl a (ug/cm2)	**Dosing 7 d**	17.67	7.42	12.41	12.98	8.72
Chl a (ug/cm2)	**Dosing 14 d**	14.29	9.57	23.81	26.59	9.84
Chl a (ug/cm2)	**Dosing 14 d**	16.51	13.54	20.18	13.04	10.75
Chl a (ug/cm2)	**Dosing 14 d**	34.47	13.00	8.49	18.83	8.03
Chl a (ug/cm2)	**Dosing 21 d**	62.28	25.06	68.57	8.96	8.48
Chl a (ug/cm2)	**Dosing 21 d**	37.86	36.17	58.79	11.52	7.79
Chl a (ug/cm2)	**Dosing 21 d**	75.69	29.49	29.35	11.39	4.87
Chl a (ug/cm2)	**Dosing 28 d**	55.69	56.03	124.30	14.44	9.08
Chl a (ug/cm2)	**Dosing 28 d**	46.81	52.30	36.03	21.62	7.52
Chl a (ug/cm2)	**Dosing 28 d**	82.22	68.95	37.99	15.43	7.79
Chl a (ug/cm2)	**Recovery 7 d**	68.26	75.48	20.57	27.64	8.19
Chl a (ug/cm2)	**Recovery 7 d**	61.18	83.29	54.04	27.63	8.51
Chl a (ug/cm2)	**Recovery 7 d**	70.99	65.44	21.19	23.66	9.21
Chl a (ug/cm2)	**Recovery 14 d**	92.34	61.42	29.21	35.85	16.34
Chl a (ug/cm2)	**Recovery 14 d**	78.29	140.67	120.36	105.29	13.81
Chl a (ug/cm2)	**Recovery 14 d**	96.93	106.09	75.77	39.47	13.81
Chl a (ug/cm2)	**Recovery 21 d**	16.86	56.57	13.98	22.42	7.90
Chl a (ug/cm2)	**Recovery 21 d**	56.57	41.26	76.07	101.24	10.00
Chl a (ug/cm2)	**Recovery 21 d**	42.42	28.26	29.13	63.46	11.61
Chl a (ug/cm2)	**Recovery 28 d**	72.09	16.10	90.24	176.34	9.76
Chl a (ug/cm2)	**Recovery 28 d**	16.66	106.58	47.36	30.64	12.10
Chl a (ug/cm2)	**Recovery 28 d**	12.37	10.69	64.86	29.65	18.54

Tetracycline Exposure

Endpoint	Sampling Date	Control	0.5 µg L^{-1}	1.0 µg L^{-1}	10 µg L^{-1}	100 µg L^{-1}
Bacteria (cells per cm^2)	**Dosing 0 d**	117392486	97865615	121154161	92608813	79361185
Bacteria (cells per cm^2)	**Dosing 0 d**	86589743	129674967	129453596	85917669	70705637
Bacteria (cells per cm^2)	**Dosing 7 d**	92902009	50750192	62594544	71146572	49238451
Bacteria (cells per cm^2)	**Dosing 7 d**	176140064	84229171	67124112	79439226	47795198
Bacteria (cells per cm^2)	**Dosing 14 d**	263175724	102885106	142352125	113129836	33794960
Bacteria (cells per cm^2)	**Dosing 14 d**	272825034	145002329	166997851	98755108	42671780
Bacteria (cells per cm^2)	**Dosing 21 d**	269228579	199877304	259244783	53539950	93523000
Bacteria (cells per cm^2)	**Dosing 21 d**	394556940	350701908	230930802	23422390	27586051
Bacteria (cells per cm^2)	**Dosing 28 d**	851380336	467737098	295546832	141408890	150272089
Bacteria (cells per cm^2)	**Dosing 28 d**	646497416	445004597	297544701	84690252	88821223
Bacteria (cells per cm^2)	**Recovery 7 d**	1247667254	1130411798	396055122	223319785	77816895
Bacteria (cells per cm^2)	**Recovery 7 d**	1536023269	1054783656	424466005	241673175	111966140
Bacteria (cells per cm^2)	**Recovery 14 d**	1434036692	1492657123	1563956502	475232874	162457772
Bacteria (cells per cm^2)	**Recovery 14 d**	1324056016	1995833592	2562117887	1037557771	212292253
Bacteria (cells per cm^2)	**Recovery 21 d**	913641376	993853201	938742609	1284072504	202855066
Bacteria (cells per cm^2)	**Recovery 21 d**	944797317	1707825204	363894782	1196192166	153074129
Bacteria (cells per cm^2)	**Recovery 28 d**	1223574676	1249242268	434939944	604574024	208285372
Bacteria (cells per cm^2)	**Recovery 28 d**	1110199092	1813943988	750610914	829268024	268613490
Cyanobacteria (% of Total Alage Count)	**Dosing 0 d**	0.65	0.97	0.80	0.63	0.90
Cyanobacteria (% of Total Alage Count)	**Dosing 0 d**	0.89	0.78	0.67	0.97	0.75
Cyanobacteria (% of Total Alage Count)	**Dosing 7 d**	3.19	1.24	0.46	0.61	0.12
Cyanobacteria (% of Total Alage Count)	**Dosing 7 d**	4.21	1.92	0.35	0.43	0.20
Cyanobacteria (% of Total Alage Count)	**Dosing 14 d**	9.43	8.77	1.27	2.23	0.35
Cyanobacteria (% of Total Alage Count)	**Dosing 14 d**	11.40	10.20	3.20	2.56	0.20
Cyanobacteria (% of Total Alage Count)	**Dosing 21 d**	28.22	11.96	9.91	2.43	0.37
Cyanobacteria (% of Total Alage Count)	**Dosing 21 d**	26.70	12.30	8.74	3.45	0.43
Cyanobacteria (% of Total Alage Count)	**Dosing 28 d**	26.83	23.79	18.07	2.50	0.00
Cyanobacteria (% of Total Alage Count)	**Dosing 28 d**	27.80	22.80	19.84	2.10	0.00

			Tetracycline Exposure			
Endpoint	**Sampling Date**	**Control**	**0.5 µg L^{-1}**	**1.0 µg L^{-1}**	**10 µg L^{-1}**	**100 µg L^{-1}**
Cyanobacteria (% of Total Alage Count)	**Recovery 7 d**	24.48	32.95	24.73	15.64	0.20
Cyanobacteria (% of Total Alage Count)	**Recovery 7 d**	25.40	29.80	32.00	18.50	0.37
Cyanobacteria (% of Total Alage Count)	**Recovery 14 d**	27.40	39.56	23.60	29.34	0.00
Cyanobacteria (% of Total Alage Count)	**Recovery 14 d**	26.90	27.80	30.10	25.10	0.21
Cyanobacteria (% of Total Alage Count)	**Recovery 21 d**	27.54	15.60	19.68	33.90	0.60
Cyanobacteria (% of Total Alage Count)	**Recovery 21 d**	28.40	29.40	32.10	28.10	0.21
Cyanobacteria (% of Total Alage Count)	**Recovery 28 d**	22.71	32.00	30.31	19.89	1.81
Cyanobacteria (% of Total Alage Count)	**Recovery 28 d**	29.00	27.10	22.90	26.40	1.00
Nematode (nematode per m^2)	**Dosing 0 d**	131337	130941	134968	119366	142859
Nematode (nematode per m^2)	**Dosing 0 d**	138457	109647	81570	159795	88936
Nematode (nematode per m^2)	**Dosing 7 d**	142924	156689	93285	132387	106585
Nematode (nematode per m^2)	**Dosing 7 d**	158121	189980	163332	106978	217070
Nematode (nematode per m^2)	**Dosing 14 d**	159231	151004	137327	183372	137116
Nematode (nematode per m^2)	**Dosing 14 d**	177119	123316	133532	177774	107749
Nematode (nematode per m^2)	**Dosing 21 d**	163182	140080	147301	172037	105807
Nematode (nematode per m^2)	**Dosing 21 d**	218768	167044	227358	93059	99168
Nematode (nematode per m^2)	**Dosing 28 d**	253474	328108	185241	73035	51216
Nematode (nematode per m^2)	**Dosing 28 d**	166528	273423	237116	200050	143190
Nematode (nematode per m^2)	**Recovery 7 d**	198086	232952	241265	73123	181651
Nematode (nematode per m^2)	**Recovery 7 d**	271778	208900	270445	184128	117119
Nematode (nematode per m^2)	**Recovery 14 d**	225055	133125	282031	147585	159228
Nematode (nematode per m^2)	**Recovery 14 d**	300785	187071	226488	248187	128538
Nematode (nematode per m^2)	**Recovery 21 d**	322859	323291	675543	304715	217531
Nematode (nematode per m^2)	**Recovery 21 d**	222916	235136	415399	574480	97015
Nematode (nematode per m^2)	**Recovery 28 d**	647947	458901	616289	169619	377439
Nematode (nematode per m^2)	**Recovery 28 d**	492610	203355	564729	278260	274667

		Tetracycline Exposure				
Endpoint	**Sampling Date**	**Control**	**0.5 µg L^{-1}**	**1.0 µg L^{-1}**	**10 µg L^{-1}**	**100 µg L^{-1}**
Leucine uptake (amol h^-1)	**Dosing 7 d**	17.18	.	-52.14	1.03	-9.60
Leucine uptake (amol h^-1)	**Dosing 7 d**	16.96	.	-15.77	16.51	-11.72
Leucine uptake (amol h^-1)	**Dosing 7 d**	11.62	.	43.37	7.71	25.21
Leucine uptake (amol h^-1)	**Dosing 7 d**	3.99	.	-20.46	-22.75	13.16
Leucine uptake (amol h^-1)	**Dosing 7 d**	19.98	.	87.52	19.86	9.04
Leucine uptake (amol h^-1)	**Dosing 7 d**	7.21	.	68.99	7.46	-21.31
Leucine uptake (amol h^-1)	**Dosing 21 d**	48.66	41.19	52.70	19.02	4.41
Leucine uptake (amol h^-1)	**Dosing 21 d**	55.32	48.21	51.17	11.12	5.67
Leucine uptake (amol h^-1)	**Dosing 21 d**	26.91	4.02	43.40	41.87	-5.07
Leucine uptake (amol h^-1)	**Dosing 21 d**	34.73	17.68	57.72	36.83	-18.91
Leucine uptake (amol h^-1)	**Dosing 21 d**	43.79	28.12	11.41	-0.18	13.83
Leucine uptake (amol h^-1)	**Dosing 21 d**	52.63	27.37	16.31	16.55	-4.74
Leucine uptake (amol h^-1)	**Recovery 7 d**	8.97	12.84	21.63	8.66	10.45
Leucine uptake (amol h^-1)	**Recovery 7 d**	10.18	13.10	18.41	0.25	16.19
Leucine uptake (amol h^-1)	**Recovery 7 d**	9.06	-20.45	7.93	5.41	-2.60
Leucine uptake (amol h^-1)	**Recovery 7 d**	25.05	-17.20	3.37	16.76	11.84
Leucine uptake (amol h^-1)	**Recovery 7 d**	.	9.27	9.41	-14.61	16.08
Leucine uptake (amol h^-1)	**Recovery 7 d**	.	9.18	5.16	8.05	4.63
Leucine uptake (amol h^-1)	**Recovery 21 d**	12.01	.	11.76	14.64	11.38
Leucine uptake (amol h^-1)	**Recovery 21 d**	9.85	.	8.61	12.78	8.32
Leucine uptake (amol h^-1)	**Recovery 21 d**	9.40	9.64	31.26	9.88	17.00
Leucine uptake (amol h^-1)	**Recovery 21 d**	8.68	14.48	33.29	3.44	4.86
Leucine uptake (amol h^-1)	**Recovery 21 d**	15.90	16.35	12.32	20.73	41.56
Leucine uptake (amol h^-1)	**Recovery 21 d**	14.77	10.73	15.89	18.70	23.38
Percent Tetracycline Resistant Colonies (%)	**Dosing 0 d**	0.00	0.00	0.00	0.00	0.00
Percent Tetracycline Resistant Colonies (%)	**Dosing 0 d**	0.00	0.00	0.00	0.00	0.00
Percent Tetracycline Resistant Colonies (%)	**Dosing 7 d**	0.00	0.00	0.00	2.63	0.00
Percent Tetracycline Resistant Colonies (%)	**Dosing 7 d**	0.00	0.00	0.00	1.54	0.00
Percent Tetracycline Resistant Colonies (%)	**Dosing 14 d**	1.67	16.44	2.33	10.00	9.76

Percent Tetracycline Resistant Colonies (%)	**Dosing 14 d**	9.09	23.21	18.75	7.25	14.81
			Tetracycline Exposure			
Endpoint	**Sampling Date**	**Control**	**0.5 µg L^{-1}**	**1.0 µg L^{-1}**	**10 µg L^{-1}**	**100 µg L^{-1}**
Percent Tetracycline Resistant Colonies (%)	**Dosing 21 d**	0.29	0.00	0.00	0.00	4.08
Percent Tetracycline Resistant Colonies (%)	**Dosing 21 d**	0.86	0.00	1.15	2.17	2.17
Percent Tetracycline Resistant Colonies (%)	**Dosing 28 d**	0.00	1.08	0.33	0.00	0.67
Percent Tetracycline Resistant Colonies (%)	**Dosing 28 d**	0.00	0.67	0.67	1.47	0.00
Percent Tetracycline Resistant Colonies (%)	**Recovery 7 d**	2.80	7.25	12.05	12.03	23.40
Percent Tetracycline Resistant Colonies (%)	**Recovery 7 d**	2.99	11.83	14.95	22.47	12.62
Percent Tetracycline Resistant Colonies (%)	**Recovery 14 d**	3.00	0.67	1.15	1.96	6.45
Percent Tetracycline Resistant Colonies (%)	**Recovery 14 d**	1.23	9.30	3.37	2.38	25.00
Percent Tetracycline Resistant Colonies (%)	**Recovery 21 d**	9.60	14.63	6.99	6.71	5.26
Percent Tetracycline Resistant Colonies (%)	**Recovery 21 d**	7.81	8.28	8.88	9.57	8.59
Percent Tetracycline Resistant Colonies (%)	**Recovery 28 d**	.	.	.	.	.
Percent Tetracycline Resistant Colonies (%)	**Recovery 28 d**	.	.	.	.	.

LC/MS/MS	Target TC Concentration	0	0.5 µg L^{-1}	1.0 µg L^{-1}	10 µg L^{-1}	100 µg L^{-1}
LEFLMR Influent	**Dosing 7 d**	nd	.	.	.	.
LEFLMR Influent	**Dosing 7 d**	nd	.	.	.	.
Mesocosm Head Tank	**Dosing 7 d**	nd	0.54	1.1	11.1	97.9
Mesocosm Head Tank	**Dosing 7 d**	nd	0.48	1.0	10.4	94.4
Mesocosm Tail Tank	**Dosing 7 d**	nd	0.71	1.0	12.5	88.7
Mesocosm Tail Tank	**Dosing 7 d**	nd	0.57	1.1	9.5	84.0
LEFLMR Influent	**Dosing 14 d**	nd	.	.	.	.
LEFLMR Influent	**Dosing 14 d**	nd	.	.	.	.
Mesocosm Head Tank	**Dosing 14 d**	nd	0.33	0.8	12.0	135.5
Mesocosm Head Tank	**Dosing 14 d**	nd	0.33	0.7	17.0	128.0
Mesocosm Tail Tank	**Dosing 14 d**	nd	0.35	0.6	12.0	126.9
Mesocosm Tail Tank	**Dosing 14 d**	nd	0.46	0.9	19.0	129.9
LEFLMR Influent	**Dosing 21 d**	nd	.	.	.	.
LC/MS/MS	**Target TC Concentration**	**0**	**0.5 µg L^{-1}**	**1.0 µg L^{-1}**	**10 µg L^{-1}**	**100 µg L^{-1}**
LEFLMR Influent	**Dosing 21 d**	nd	.	.	.	.
Mesocosm Head Tank	**Dosing 21 d**	nd	0.43	1.02	12.75	93.00
Mesocosm Head Tank	**Dosing 21 d**	nd	0.38	0.97	12.70	92.80
Mesocosm Tail Tank	**Dosing 21 d**	nd	0.43	0.98	12.22	84.89
Mesocosm Tail Tank	**Dosing 21 d**	nd	0.39	1.05	11.40	80.33
LEFLMR Influent	**Dosing 28 d**	nd	.	.	.	.
LEFLMR Influent	**Dosing 28 d**	nd	.	.	.	.
Mesocosm Head Tank	**Dosing 28 d**	nd	0.44	0.98	12.69	134.00
Mesocosm Head Tank	**Dosing 28 d**	nd	0.38	1.31	14.09	146.33
Mesocosm Tail Tank	**Dosing 28 d**	nd	0.37	1.11	13.11	140.88
Mesocosm Tail Tank	**Dosing 28 d**	nd	0.43	1.01	15.89	146.11