



2011

Investigation of a Pharmaceutical Compound with Artificial Streams: Effects of the Antihistamine Cimetidine on Stream Ecosystem Function.

Paul David Hoppe
Loyola University Chicago

Follow this and additional works at: https://ecommons.luc.edu/luc_theses

 Part of the [Ecology and Evolutionary Biology Commons](#)

Recommended Citation

Hoppe, Paul David, "Investigation of a Pharmaceutical Compound with Artificial Streams: Effects of the Antihistamine Cimetidine on Stream Ecosystem Function." (2011). *Master's Theses*. 489.
https://ecommons.luc.edu/luc_theses/489

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.



This work is licensed under a [Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 License](#).
Copyright © 2011 Paul David Hoppe

LOYOLA UNIVERSITY CHICAGO

INVESTIGATION OF A PHARMACEUTICAL COMPOUND WITH ARTIFICIAL
STREAMS: EFFECTS OF THE ANTIHISTAMINE CIMETIDINE ON STREAM
ECOSYSTEM FUNCTION

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE

PROGRAM IN BIOLOGY

BY

PAUL DAVID HOPPE

CHICAGO, IL

DECEMBER 2011

Copyright by Paul D. Hoppe, 2011
All rights reserved

ACKNOWLEDGEMENTS

I owe gratitude to many people for helping me throughout graduate school. First, I would like to acknowledge my parents for their encouragement and support in my pursuit of working in aquatic science. I would also like to thank my wife Kara for her love and companionship and transferring schools to be with me in Chicago.

Thanks to everyone in the Rosi-Marshall lab. In particular I would like to thank Holly Wellard and Antoine Aubeneau for helping with managing the artificial-stream facility and data analysis. I would also like to thank Sikandar Kahn, Drew Lee, Amatul Salma, Nga Huynh, Jeff Kampman, Jim Nunnally and Yousuf Sayeed for their assistance in collecting and analyzing samples. Thanks to Tim Hoellein for his guidance with nutrient chemistry and data analysis. Dr. Dom Castignetti deserves special thanks for teaching me techniques in High-Performance Liquid Chromatography and biochemistry.

I would like to especially thank my committee members Dr. Martin Berg, Dr. Chris Peterson and Dr. Matt Whiles for their persistence and patience in teaching me about stream ecology, aquatic entomology, statistics and helping me to think critically about science and my research. I give my greatest thanks to my advisor, Dr. Emma Rosi-Marshall for allowing me all the great opportunities I received during graduate school. Thank you for all you have taught me and making my graduate research exciting, challenging, and fun. This work was supported by a grant awarded to Dr. Emma Rosi-Marshall from the Illinois Water Resources Center, Department of Natural Resources.

This work is dedicated to my mother and father Janet and Dave Hoppe, and my wife Kara for their unconditional love and support throughout my life and the course of this thesis.

The river has taught me to listen; you will learn from it, too. The river knows everything; one can learn everything from it. You have already learned from the river that it is good to strive downwards, to sink, to seek the depths.

Herman Hesse, *Siddhartha* (1922)

TABLE OF CONTENTS

AKNOWLEDGEMENTS	iii
LIST OF TABLES	x
LIST OF FIGURES	xi
CHAPTER ONE: AN INTRODUCTION TO PHARMACEUTICAL AND PERSONAL CARE PRODUCTS IN STREAM ECOSYSTEMS.	1
Introduction	1
Background on PPCPs – pharmaceuticals in streams	1
Urban streams	2
Contaminants in streams	4
Background on PPCPs and Aquatic Ecotoxicology	4
Benthic macroinvertebrates as water quality indicators	5
Effects of PPCPs on aquatic ecosystems	6
Antihistamines – review of cimetidine	8
Histamines and invertebrates	9
Research questions and objectives	10
CHAPTER TWO: MEASURING CIMETIDINE IN STREAM WATER.	11
Abstract	11
Introduction	12
Methods	16
Measuring cimetidine	17
Experiment 1	18
Experiment 2	19
Results	20
Experiment 1	22
Experiment 2	24
Discussion	25
CHAPTER THREE: EFFECTS OF THE ANTIHISTAMINE CIEMTIDINE ON STREAM ECOSYSTEM FUNCTION.	28
Abstract	28
Introduction	29
Methods	32
Artificial streams	32
Pharmaceutical compound	34
Experimental design	34

Basal resources	35
Invertebrate populations	36
Data analysis	38
Results	39
Effects of cimetidine on basal resources – streams without invertebrates	39
Effects of invertebrates on basal resources without cimetidine	43
Indirect effects of cimetidine on basal resources – streams with invertebrates	44
Effects of cimetidine on invertebrates	47
Discussion	52
Basal resource response to cimetidine – streams without invertebrates	52
Indirect effects on basal resources – streams with invertebrates	54
Effects on invertebrates	55
Utility of my experimental approach	57
Conclusion	58
APPENDIX A: CIMETIDINE LOSS FROM WATER COLUMN DATA	60
REFERENCES	67
VITA	76

LIST OF TABLES

Table 1. Properties of cimetidine.	15
Table 2. Mean and standard error (SE) of body length distributions and number of <i>G. fasciatus</i> population (per m ²) following 83 day exposure to control water and cimetidine treatments.	47

LIST OF FIGURES

- Figure 1. Molecular structure of cimetidine, N-cyano-N'-methyl'-[2-[[[(5-methyl-1H-imidazol-4-yl) methyl]thio]ethyl]guanidine. 14
- Figure 2. Standard curve using HPLC-UV with six concentrations in: 0, 5.0, 10.0, 30.0, 50.0, 100.0 $\mu\text{g L}^{-1}$. Data are fit using simple linear regression with the natural log of the area under the chromatograph peak on the x-axis with the equation $f = -1332.4642 + 113.2399 * (x)$, $R^2 = 0.993$. 21
- Figure 3. Chromatograph peak of minimal detection limit (MDL) determined by the lowest detectable concentration repeated seven times and multiplying the standard deviation of these measurements by 3.14. The MDL for this HPLC method is 1.1692 $\mu\text{g L}^{-1}$. Cimetidine appears on chromatograph 8 minutes after injection into the HPLC. 21
- Figure 4. Cimetidine (mean and SE) in the water column of the artificial stream experiment over 37 hour time period from artificial streams containing A) no organic matter and no sediment (\bullet), B) sediment with no organic matter (\circ), C) 15.0 grams of organic matter (*Acer rubrum*) (\blacktriangledown). Initial dose was 70 $\mu\text{g L}^{-1}$ of cimetidine. 23
- Figure 5. Cimetidine (mean and SE) loss from water in 500 ml beaker microcosms over 22 hour time period (shown in minutes). A) no organic matter in the dark (\bullet), B) no organic matter exposed to sunlight (\blacksquare), C) organic matter exposed to sunlight (\blacktriangle), and D) organic matter with microbial communities exposed to sunlight (*). 2.5 grams of *Acer rubrum* was used for organic matter treatments and the organic matter inoculated with 30 mL of algal/microbial slurry scraped from rocks. Initial dose was 70 $\mu\text{g L}^{-1}$ of cimetidine. Each treatment had 4 replicates. 24
- Figure 6. Ratios of treatment divided by the mean ash-free dry mass (mg cm^{-2}) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 40

- Figure 7. Ratios of treatment divided by the mean microbial respiration ($\text{mg O m}^{-2} \text{ h}^{-1}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 41
- Figure 8. Ratios of treatment divided by the mean chlorophyll *a* ($\mu\text{g cm}^2$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 42
- Figure 9. Ratios of treatment divided by the mean primary production ($\text{mg O m}^{-2} \text{ h}^{-1}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 43
- Figure 10. Ratios of treatment divided by the mean ash-free dry mass (mg cm^{-2}) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 45
- Figure 11. Ratios of treatment divided by the mean microbial respiration ($\text{mg O m}^{-2} \text{ h}^{-1}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 45
- Figure 12. Ratios of treatment divided by the mean chlorophyll *a* ($\mu\text{g cm}^{-2}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 46

- Figure 13. Ratios of treatment divided by the mean primary production ($\text{mg O m}^{-2} \text{h}^{-1}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment. Data were compared using Tukey's multiple comparison test following repeated measures with non-significant interaction factor. 46
- Figure 14. Mean and standard error (SE) of body length distributions and number of *G. fasciatus* per m^{-2} following 86 day exposure to control water and A) 0.07 $\mu\text{g L}^{-1}$ cimetidine [0.07], B) 0.7 $\mu\text{g L}^{-1}$ cimetidine [x10], C) 7.0 $\mu\text{g L}^{-1}$ cimetidine [x100], D) 70.0 $\mu\text{g L}^{-1}$ cimetidine [x1000]. *P*-values are the result of one-way ANOVA with post hoc Tukey's multiple comparison test to determine differences in size class and number of individuals between control and cimetidine treatments ($n = 3$). * indicates a significant difference from control values. 48
- Figure 15. Mean and standard error (SE) of A) biomass and B) number of individuals for *G. fasciatus* populations following 86 day exposure to control water and cimetidine treatments. *P*-values are the result of one-way ANOVA with post hoc Tukey's multiple comparison test to determine differences in size class and proportion of population between control and cimetidine treatments ($n = 3$). * indicates a significant difference from control values. 49
- Figure 16. Mean and standard error of A) percent survivorship of *P. herricki* following 79 days of exposure to cimetidine treatments, B) instantaneous growth rates of *P. herricki* following a 28 days of exposure to cimetidine treatments and C) instantaneous growth rates of *G. fasciatus* following a 28 days of exposure to cimetidine treatments. *P*-values are the result of one-way ANOVA with post hoc Tukey's multiple comparison test to determine differences between cimetidine treatments [0], [0.07], [x10], [x100], and [x1000] ($n = 3$). Differences in treatments are represented by lower case letters. 51

CHAPTER ONE
AN INTRODUCTION TO PHARMACEUTICALS AND PERSONAL CARE
PRODUCTS IN STREAM ECOSYSTEMS

Introduction

Background on PPCP's - pharmaceuticals in streams

In recent years, the occurrence of pharmaceuticals and personal care products (PPCPs) in rivers has received increased attention; however, the effects of these contaminants on river ecosystems remain unclear (Halling-Sørensen et al. 1998, Daughton and Ternes 1999). PPCPs are a unique suite of contaminants and share these properties: 1) wastewater treatment facilities (WWTFs) do not completely remove these contaminants (Ternes 1998); 2) PPCPs have the potential to degrade, are continually discharged to rivers via wastewater effluent (Daughton and Ternes 1999); and 3) PPCPs are designed to have biological effects, increasing the likelihood they will affect non-target organisms (Henschel et al. 1997). Although enhanced analytical techniques enable us to detect many of these compounds at low concentrations, the methods for understanding ecological effects of PPCPs on stream ecosystem function have not been adequately developed.

Approximately 80,000 chemicals are in use today (Pimentel et al. 1996) and in 2006 14,117 active investigational new drugs were under active investigation for human use (Pisano and Mantus 2008). These compounds have been developed to benefit human

health; however, many of these substances could affect non-target organisms. When pharmaceutical compounds are consumed not all of the active pharmaceutical ingredient (API) is fully metabolized and it is excreted and ends up at WWTFs. Flushing of unused medicines into the waste-stream appears to be less significant than excretion following therapy (Kolpin et al. 2002). Many PPCPs enter and leave WWTFs unaltered or incompletely removed and end up in effluent-receiving rivers (Daughton and Ternes 1999). The average time a compound remains within a typical WWTF ranges between <1 h to a few days, shorter than the degradation half-lives of many PPCPs (Xia et al. 2005, Halling-Sørensen 1998). Combined sewer overflows (CSOs) provide another route that PPCPs enter rivers and streams. During periods of heavy rainfall or snowmelt, storm water and wastewater can exceed the capacity of treatment facilities and combined sewer systems have been designed to handle this overflow. CSOs release excess storm water mixed with wastewater directly into receiving streams and rivers, containing untreated human and industrial waste, likely containing pharmaceutical compounds.

Urban streams

Human population growth results in increased demand on limited supplies of freshwater (Ricciardi and Rasmussen 1999, Jenkins 2003, Kolpin et al. 2002, Revenga et al. 2005, Abel et al. 2007). Protecting the quality of freshwater ecosystems is one of the most crucial environmental issues of our times. Urbanization is a pervasive and rapidly growing form of land use change and results in reduced water quality of aquatic ecosystems (Paul and Meyer 2001). Urban landscapes affect downstream aquatic ecosystems in numerous ways by altering hydrology, increasing nutrient loading and

increased exposure to contaminants, such as pesticides, trace metals and organic contaminants (Paul and Meyer 2001).

The greater metropolis of Chicago, Illinois provides an example illustrating the potential widespread effects of emerging contaminants like PPCPs on aquatic ecosystems. The Chicago area has seven WWTF, including the largest in the world, the Stickney Water Reclamation Plant, and the greater Chicago area has approximately 250 CSO sites. Much of the discharge in urban streams can be dominated by wastewater effluent. For example, in the South Platte River which drains Denver, CO, wastewater effluent constitutes 69% of the annual discharge, at times comprising 100% (Dennehy et al. 1998). Effluent-dominated streams have unique water quality characteristics that differ from stream conditions upstream of effluent point-sources or at regional reference streams (Taylor 2002, Brooks et al. 2002). The potential for PPCPs to affect stream organisms and ecosystem function is an increasing concern for water resource managers because aquatic organisms are continually exposed to these contaminants.

Recently pharmaceutical compounds have been detected in surface waters receiving wastewater effluent in highly urbanized streams (e.g. Kolpin et al. 2002, Gross et al. 2004). During 1999 and 2000, the US Geological Survey conducted a nationwide survey of surface waters and detected numerous PPCPs in surface waters, e.g., hormones, caffeine, painkillers, etc. (Barnes et al. 2002). Newly developed analytical methods may explain the recent detection of these contaminants; presumably the presence of PPCPs in freshwater ecosystems dates back to the time use of chemicals became common (Daughton 2003). Given the large volume of wastewater legally discharged into urban

streams, it is likely that the prevalence and ecological significance of PPCPs in these systems, in particular, may be high.

Contaminants in streams

PPCP contamination may contribute to urban stream degradation (Paul and Meyer 2001, Paul 1999, Rosi-Marshall 2004), similar to contaminants such as trace metals (e.g., Cu, Cd and Pb) that have been shown to adversely affect aquatic communities (Peckarsky and Cook 1981, Norton et al. 1992, Kiffney and Clements 1996, Richardson and Kiffney 2000). For example, increasing Fe^+ may significantly change the structure and function of stream ecosystems (Vuori 1995), and along with other metals, can reduce invertebrate abundance (Richardson and Kiffney 2000). Also, mine drainage has been shown to significantly decrease the survival rates of caddisflies (DeNicola and Stapleton 2002). In addition, the quality of basal food resources of an urban river, as measured by aquatic macroinvertebrate growth rates, declined as the volume of wastewater permitted to discharge into the system increased (Rosi-Marshall 2004). Novel contaminants associated with wastewater treatment effluent present an emerging area of concern because of their ubiquity and potential to antagonistically interact with the other contaminants present in urban waterways.

Background on PPCPs and Aquatic Ecotoxicology

PPCPs are designed for human and veterinary medicine, but these compounds could affect other vertebrates and invertebrates because many target receptors/molecules are evolutionarily conserved (Fent et al. 2006). Concentrations of PPCPs in surface waters range from ng L^{-1} - $\mu\text{g L}^{-1}$, these are below levels needed to induce biological effects with acute exposure. However, chronic exposure to such low concentrations may

result in sublethal effects such as altered feeding behavior, fecundity, and/or growth (De Lange et al. 2006).

Ecotoxicology, as a scientific subdiscipline, emerged from fusion of ecological and toxicological approaches to address applied ecological questions. Traditionally, ecologists have focused on how abiotic and biotic factors influence community and ecosystem dynamics, whereas toxicologists have focused on single-species toxicity tests (Relyea and Hoverman 2006). Ecotoxicology combines these approaches by investigating the effects of compounds on organisms and ecosystems using a more integrated approach. Testing the effects of pharmaceutical compounds on aquatic organisms requires a combination of techniques to adequately address the effects of these compounds in stream ecosystems. Classic toxicology tests link the dose of contaminant and a biological response, typically mortality. Although toxicity tests can control confounding environmental factors, they do not account for ecological variability, especially when stream-dwelling organisms are the subjects of testing (Richardson and Kiffney 2000). Use of artificial streams provide a practical alternative to discrete toxicity tests and can be useful for examining long-term chronic exposure to pollutants (Lamberti and Steinman 1993).

Benthic macroinvertebrates as water quality indicators

Benthic invertebrates are ideal organisms to examine the effects of contaminants on stream communities and have been used since the early 1900's (Carpenter 1924). Because invertebrates are ubiquitous in stream ecosystems, have short life cycles and influence stream ecosystem function, they are ideal indicators of environmental stress, including contaminants, on aquatic ecosystems (Rosenberg and Resh 1993, Richardson

and Kiffney 2000). Effects of contaminants are often documented by changes in macroinvertebrate community structure between upstream reference sites and polluted downstream sites (Rosenberg and Resh 1993, Richardson and Kiffney 2000). This approach has been used effectively to document effects of metal contamination on macroinvertebrate community structure (Chadwick et al. 1986, Roline 1988, Clements 1994, Kiffney and Clements 1996). Such *in situ* studies are not without drawbacks based on measurements, which are not independent from pseudoreplication, and limit the ability to statistically confirm cause and effect inferences. Results can have statistical limitations (e.g., pseudoreplication) that limit cause and effect inferences. Another difficulty is being able to tease out natural differences (e.g. habitat characteristics, physical properties) that may influence changes in invertebrate community structure. Finally, because PPCPs are typically found in urban or suburban streams, elucidating the effects of PPCPs from the effects of land use and other contaminants are impossible within a field setting.

Effects of PPCPs on aquatic ecosystems

The chronic input of pharmaceutical compounds introduces a new research challenge that has received little attention, as most PPCP studies have examined acute doses. Acute toxicity tests are not suitable for understanding the effects of these compounds on ecosystem function (Fent et al. 2006). Toxicology studies typically examine the effects of contaminants on aquatic organisms with exposure assays that do not extend beyond 72 hours, though some recent studies examined chronic dosing exposure of up to 56 days (Watts et al. 2001(a), 2002, Maul et al. 2006, Nentwig 2007). Very few toxicology studies have measured the effects of chronic PPCP exposure on

stream biota (Watts et al. 2001(b), Robinson et al. 2005, Maul et al. 2006, Simon et al. 2006, Isidori et al. 2007) and even fewer have measured the effects on stream ecosystem function (see Halling-Sørensen et al. 1998).

Thus far, PPCPs examined include analgesics, synthetic hormones, antibiotics, neuroactive compounds, surfactants and antidepressants (for reviews see Halling-Sørensen et al. 1998, Fent et al. 2006). Diclofenac, a common analgesic and anti-inflammatory drug used in humans and livestock affects phytoplankton [lowest EC_{50} (96 h) = 14.5 mg L^{-1}] (Ferrari et al. 2003) and leads to renal lesions and altered gills in rainbow trout *Onchorhynchus mykiss* at $5 \text{ } \mu\text{g L}^{-1}$ (Schwaiger et al. 2004). Consumption of diclofenac-treated livestock has been attributed to population declines of a species of vultures in India due to renal failure induced by exposure to this chemical (Oaks et al. 2004). The synthetic hormone 17α -ethinylestradiol (EE2), an oral contraceptive, induced estrogenic effects in fish at low concentrations (Nash et al. 2004). Male fathead minnows *Pimephalus promelas* failed to develop normal secondary sex characteristics, sex ratios were altered, and no testicular tissue was observed at 4 ng L^{-1} (Länge et al. 2001). Exposure to zebrafish *Danio rerio* at 3 ng L^{-1} caused gonadal feminization and inhibited reproduction (Fenske et al. 2005). Antibiotics are frequently used in production of domestic livestock and bacterial resistance has been documented for six antibiotics and resulted in decreased rates of denitrification (Costanzo et al. 2005). Chronic exposure to $100 \text{ } \mu\text{g L}^{-1}$ ciprofloxacin (Cipro) significantly decreased communities of leaf-associated microbial decomposers (Maul et al. 2006). Neuroactive compounds (e.g. antidepressants, antiepileptics) also affect non-target organisms. The anti-depressant fluoxetine was toxic to phytoplankton (EC_{50} (48 h, alga) = 0.024 mg L^{-1}) (Brooks et al. 2003), and chronic

exposure to $36 \mu\text{g L}^{-1}$ (30 d) stimulated reproduction of *Daphnia magna* and at concentrations of $56 \mu\text{g L}^{-1}$ *Ceriodaphnia dubia* had increased fecundity (Flaherty and Dodson 2005). In another study, fluoxetine reduced reproduction in the midge *Chironomus tentans* (Brooks et al. 2003). Antiepileptic drugs such as diazepam and carbamazepine, inhibited growth of *D. magna* at 12.7 mg L^{-1} and 9.2 mg L^{-1} (Fent et al. 2006).

Antihistamines – review of cimetidine

A group of commonly detected PPCPs in streams are antihistamines (Kolpin et al. 2002, Kosonen and Kronberg 2009). Histamine is a neuroactive amine found in the nervous system of animals from diverse phyla (Hashemzadeh-Gargari and Freschi 1992) and is widely used by vertebrates and invertebrates as neurotransmitters, neuromodulators or neurohormones. Cimetidine HCl (Tagamet[®]) is an H₂ histamine antagonist that has been measured in surface waters at concentrations up to $0.58 \mu\text{g L}^{-1}$ (Kolpin et al. 2002). In humans, cimetidine is commonly used for the treatment of acid related gastrointestinal conditions. Histamine activates the H₂ receptor on the parietal cells of the stomach wall and inhibits the potassium proton pump that releases hydrogen ions in the stomach. Cimetidine inhibits the action of histamine on the acid-producing cells of the stomach and reduces stomach acid. Cimetidine, approved by the Federal Drug Administration in 1977, was the first drug ever to reach more than 1 billion dollars annually in sales. Approximately 60% of cimetidine ingested by humans is excreted unmetabolized (Lorenzo and Drayer 1981). Approximately 163,000 kg of cimetidine are sold each year in the US (Anderson et al. 2004) and ca. 76,610 kg enter WWTFs annually

(Anderson et al. 2004). WWTF remove about 70% of cimetidine; with the remaining 23,626 kg of cimetidine entering US surface waters each year.

The effects of cimetidine on US surface waters are not well understood, but limited research suggests that the bacterium *Vibrio fischeri*, the freshwater invertebrate *Daphnia magna*, and the Japanese medaka fish *Oryzias latipes* all had $LC_{50s} > 100 \text{ mg L}^{-1}$ for 96 h toxicity tests (Kim et al. 2007). Concentrations that cause acute mortality in aquatic organisms are consistently higher than in surface water concentrations (Kolpin et al. 2002), but effects of chronic exposure on stream macroinvertebrates has not been measured.

Histamines and invertebrates

Histamine activates olfactory receptors and stomatogastric neurons in the spiny lobster *Homarus americanus* and is inhibited by cimetidine (Hashemzadeh-Gargari and Freschi 1992; Claiborne and Selverston 1984). Histamine also activates chloride conductance in motor neurons of the lobster cardiac ganglion (Hashemzadeh-Gargari and Freschi 1992). Histamine is the neurotransmitter released by insect photoreceptors and cimetidine reduced the response to light in the common housefly *Musca domestica* (Hardie 1988). In addition to photoreception, histamine is a neuroregulator that has been shown to modulate escape behavior in crayfish (Cattaert et al. 2002). Histamine can independently mediate presynaptic inhibition of olfactory receptor neurons in crustaceans (Wachowiak et al. 2002). Histamine stimulates pyloric rhythm and gastric mill rhythm in the stomatogastric nervous system of the crab *Cancer borealis* and these actions were also blocked by cimetidine (Christie et al. 2004). Although it has not yet been established if there is a class of histamine receptor common to arthropods, research thus

far has shown that histamine is used by many invertebrates (Hardie 1988, Buchner et al. 1993, Witte et al. 2002).

Research Questions and Objectives

Based on this previous research, I hypothesized that chronic exposure to cimetidine would affect aquatic invertebrates, specifically disrupting their growth. My thesis research addressed the following questions: 1) Can cimetidine concentrations be measured in stream water cheaply and efficiently? 2) What is the fate of cimetidine in streams and, more specifically, does it sorb to organic matter or photodegrade? 3) Does cimetidine affect stream-dwelling macroinvertebrates? 4) Does cimetidine affect algae or microbes? 5) Are there indirect effects of cimetidine on basal resources through its effects on macroinvertebrate consumers? In Chapter 2 of my thesis, I describe the method I developed for measuring cimetidine in streams and how I used this method to examine cimetidine fate in streams. In Chapter 3, I describe my experiments that examined the effects of cimetidine on stream-dwelling organisms and ecosystem function.

CHAPTER TWO
MEASURING CIMETIDINE IN STREAM WATER USING A MODIFIED HIGH-
PRESSURE LIQUID CHROMATOGRAPHY METHOD

Abstract

Cimetidine is a common H₂ histamine antagonist that has been measured in stream water, but its fate in stream ecosystems is not currently known. In addition, because cimetidine can inhibit invertebrate physiological functions, it may affect stream-dwelling invertebrates. However, measuring low concentrations of compounds like cimetidine is expensive and previous methods for stream water analysis produced suboptimal results. My goal was to develop a simple and rapid method to measure cimetidine concentrations in stream water that uses high-pressure liquid chromatography containing a reverse-phase column and a variable-wavelength (228 nm) UV detector. I also used this method to examine cimetidine fate in streams and specifically measured its loss in the water through photolysis, in the presence of organic matter (OM), and in the presence of organic matter with microbial communities. In streams without organic matter, cimetidine concentrations remained relatively stable when exposed to sunlight with an estimated half life > 37 hours. In contrast, streams with organic matter had a loss of 2.69 $\mu\text{g L}^{-1} \text{h}^{-1}$ from the water column presumably due to organic matter sorption. In a second experiment, there was little loss of cimetidine from the water column when no organic matter was present. Similar to the first experiment, the loss

rates of cimetidine from the water column in treatments with OM were rapid. When organic matter was inoculated with microbial communities, the loss rate was not significantly different from treatments with organic matter only. The method for measuring cimetidine in stream water developed for this study had a method detection limit (MDL) of $1.168 \mu\text{g L}^{-1}$ and its rapid loss from the water column in the presence of organic matter suggests that cimetidine sorbed to organic matter warrants further study to effectively estimate how much cimetidine is in aquatic ecosystems.

Introduction

Typical wastewater treatment facilities (WWTFs) do not effectively remove pharmaceutical compounds with up to 80% of total pharmaceutical loads discharged into surface waters (Ternes 1998, Cahill et al. 2004). Once in surface waters these chemicals can be transformed by hydrolysis, photolysis, photo-oxidation, or sorb to sediments or organic matter. These processes influence the route of exposure and toxicity of these compounds to organisms (Stern and Walker 1978). Although improved analytical methods, particularly using high performance liquid-chromatography (HPLC) and gas chromatography (GC) have allowed for the detection of PPCPs at sub-microgram concentrations in streams (Ternes 1998, Stackelberg et al. 2004), determining the fate of these chemicals in stream ecosystems remains difficult because reduced concentrations of pharmaceutical compounds may be due to sorption to particles or by biotransformation and degradation (Xia et al. 2005, Sedlak and Pinkston 2001).

Pharmaceutical compounds in surface waters may affect aquatic organisms in different ways, or not at all, depending on their fate and exposure concentration

(Cunningham et al. 2006). These compounds can be degraded through the interaction of hydrogen ions (hydrolysis) or free oxygen radicals (oxidation) and these compounds can also be transformed during wastewater treatment through processes such as chlorination, bromination, sunlight exposure, or fluoride treatment. At times, sister compounds may be as toxic as parent compounds (Buth et al. 2007). Once in surface waters, compounds may remain in the water column, be transported downstream or degraded. In addition, compounds may also bind to sediments or adsorb to organic matter where they can be consumed by organisms. There are various exposure pathways that may result in biological effects on stream-dwelling organisms. Accurately measuring these compounds in surface waters is an essential first step in understanding the fate of PPCPs and potential degradation and sorption rates.

The pharmaceutical compound cimetidine, sold as the nonprescription antacid Tagamet®, is an H₂ histamine antagonist that has an annual usage of 160,000 kg in the US (Anderson et al. 2004, Buth et al. 2007). Human metabolism removes about 50% of the compound and WWTFs remove approximately 70% from the waste stream (Anderson et al. 2004), therefore an estimated 23,000 kg enters US surface waters annually. Cahill et al. (2004) developed an HPLC-electrospray ionization (ESI) mass spectrometry procedure for routine monitoring of a suite of pharmaceutical compounds in surface waters including cimetidine. My goal was to develop a low cost method to measure cimetidine in stream water and to examine the fate of the compound in streams. Initially, I used the Cahill et al. (2004) method for cimetidine extraction and analysis. This method was used in the Kolpin et al. (2002) report, but after testing this method and

through personal correspondence with Edward Furlong (second author on Cahill et al. 2004), it became apparent that this procedure produced sub-optimal results for quantifying cimetidine concentrations. Highly-polar compounds, such as cimetidine, were recovered at less than 50%. Low recovery for polar compounds is believed to be due to poor retention on the polymeric sorbent as a result of not adjusting the pH of the sample for extraction (Cahill et al. 2004).

The function of HPLC is to separate a liquid sample into molecular components. The sample is injected into a stream of high-pressure liquid buffer (mobile phase), which is pushed through a C-18 packed column and in this case the separated sample is read by a ultra-violet (UV) detector. The buffer is typically ethanol or methanol mixed with a salt. Those molecular compounds with the lowest affinity will “wash off” the column first showing up as a peak on the chromatograph. The area under the peak is used as a measure of concentration. An understanding of the molecular compound is necessary when developing analytical techniques. Cimetidine ($C_{10}H_{16}N_6S$) is slightly soluble in water with a solubility of $0.5 \text{ g } 100 \text{ mL}^{-1}$ and has a molecular weight of 252.344, CAS # 051481-61-9 (Figure 1).

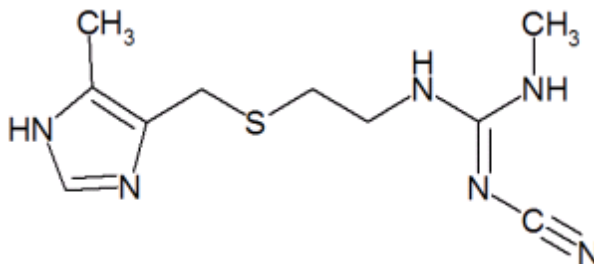


Figure 1. Molecular structure of cimetidine, N-cyano-N'-methyl-N'-[2-[(5-methyl-1H-imidazol-4-yl) methyl]thio]ethyl]guanidine.

Table 1.
Properties of cimetidine that affect concentrations in surface waters

<i>Property</i>	<i>Fate of cimetidine</i>	<i>Source</i>
Solubility	Slightly soluble, $K_{ow} = 1.5$, pH 7	GSK
Sorption	Likely to sorb to soil, sludge, biomass, or sediment if released into the environment. $K_p = 500 \text{ L kg}^{-1}$ with sediment	GSK Anderson et al. 2004
Hydrolysis	Chemically stable in water: half-life > 1 month Chemically stable in ground water: half-life > 37 hours	GSK Hoppe (unpublished)
Photolysis	Half-life in lake water: 2-200 hours Half-life in pristine water: 53-120 minutes Half-life in colored water: 90-900 hours (7 days with 12 hours sunlight per day) Half-life in artificial streams: > 37 hours	GSK Latch et al. 2003 Hoppe (unpublished)
Biodegradation	50%, 3 days, batch activated sludge, not readily biodegradable	GSK

In developing an effective method for measuring cimetidine in stream water I also examined its fate in streams. I used three artificial streams to study the fate of cimetidine. I addressed three questions: 1) Does cimetidine break down by photolysis in artificial streams (or bind to artificial stream construction material)? 2) Does cimetidine bind to sand and gravel? 3) Does cimetidine leave the water column when exposed to organic matter? I performed an additional experiment in chambers to further investigate cimetidine dynamics in the presence of organic matter with and without microbial activity to examine the relative importance of OM or sorption and photo-oxidation from microbial activity.

Methods

I modified a method for measuring cimetidine in human blood serum (Lorenzo and Drayer 1981) to isolate and quantify, N-cyano-N'-methyl'-[2-[[[(5-methyl-1H-imidazol-4-yl) methyl]thio]ethyl]guanidine in stream water samples. Cimetidine was quantified by liquid chromatography with a Rainin Rabbit HPX (Oakland, CA) and a Knauer Variable Wavelength Monitor (Berlin, Germany) using a reverse-phase column μ -Bondpack C-18 column; 4.6 x 250 mm, Waters Associates, (Milford, MA) and ultra-violet (UV) variable-wavelength detector (228 nm). The analog voltage signals from the UV detector were transmitted to a picolog that transformed the light absorbance into a digital signal, which was then exported to a Microsoft Excel file.

I ran the manufacturers standard to establish the quantitative effectiveness of the column, in this case the Rainin C-18 (4.6 x 250 mm) column. I determined the column volume and tubing volume to calculate the amount of time (ml min.^{-1}) needed for the solution to go through the system. In this system 5 ml was added to account for the volume of tubing and the following equation was used to calculate the volume of solution (V) that can be in the system, where r is the radius of the column, and h is the height of column:

$$V = \pi * r^2 * h + 5 \text{ (column volume + tubing)}$$

I set the flow rate at 0.3 ml min^{-1} and solution A was Q-water and the pump was set at 42%. The B solution was 0.3 mL of trifluoroacetic acid added to 300 mL of methanol (1% trifluoroacetic acid) and the pump was set at 58%. The sample was 60 μL acetone, 775 μL toluene and 7 μL uracil in 1 mL of 60% acetonitrile and 40% water.

The range was set at 0.16, wavelength 254 nm and I injected 10 μ L of the sample. Uracil and acetone peaked at 3 minutes 15 seconds and toluene peaked at 13 minutes confirming effectiveness of the column.

Measuring Cimetidine

Pure cimetidine was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). I prepared stock solutions with E-pure water and stored in glass light-resistant bottles in a refrigerator for up to four days. All samples were first filtered through an IC Millex – LG low protein binding hydrophilic 0.2 μ m syringe filter before injection and all solutions and buffers were first filtered with a 0.45 μ m cellulose/acetate filter and degassed.

The isocratic mobile phase consists of HPLC grade methanol in 5mM potassium phosphate K_2HPO_4 acidified to pH 2.8 by the addition of 6N hydrochloric acid as a 10:90 mixture by volume. The attenuation was set at 0.02 to detect lower range of sensitivity and the UV detection light was set at 228 nm. At a flow rate of 2 ml min^{-1} , the retention time for cimetidine is 8.1 minutes. After running the samples, the column was flushed for 20 minutes with 10% methanol in water to prevent build up of salt in the system and then flushed with 100% methanol for 20 minutes to prevent build up of any organic matter.

The y-axis of the chromatograph is absorbency readings from molecules flushed off the column, the x-axis is time. An integration formula (trapezoid function) was used to derive the area under the curve and this was plotted against concentration. A

hyperbolic curve model fit exceptionally well ($R^2 = 1.0$) with the equation for determining concentration (f) from area under the curve (x):

$$f = \frac{1.0 * x}{1 + 2.8275E^{-20} * x}$$

Experiment 1

Artificial streams (4 m x 15.5 cm x 15 cm) were constructed of composite fiberglass with a streambed surface area of 0.62 m². The streams were filled with groundwater that contained low nutrient concentrations: ammonium (NH₄⁺): range = 3.5 – 6.5 µg L⁻¹, soluble reactive phosphorus (SRP): range = 15 – 30 µg L⁻¹. Current velocity was kept constant at 0.26 m s⁻¹ by a Dayton DC gear motor (model 42129b) and a Dayton DC speed control (model 5X412D) (Dayton DC Gear Motor, Niles, Illinois) connected to a rotating stainless steel paddle-wheel. Natural lighting from April sunlight at 41° 59' latitude entered the stream facility through windows that block 50% broad-spectrum light.

For this experiment, each stream contained 40 L of groundwater from a 2649.78 L storage tank. Stream 1 was set up to address the question: Does cimetidine bind to the artificial stream fiberglass material or break down by photolysis? Stream 2 was set up to address the question: Does cimetidine bind to sand and gravel? Stream 3 was set up to address the question: Does cimetidine bind to organic matter? I added rinsed coarse playground sand and pea-size gravel to stream 2, whereas streams 1 and 3 had no substrate. After draining and rinsing the streams, I added 3 leaf litter bags of senescent red maple (*Acer rubrum* leaves) (15 grams dry weight total) inoculated with 60 ml of microbial communities to stream 2. Microbial inoculum was scraped from rocks from an

artificial pond in the Loyola aquatic facility to obtain microbial community and combined with 500 ml of ground water. Leaves were collected from Benton CO., MI, USA and transported to the laboratory, air dried and stored in large cardboard flats. Leaves were conditioned in water for five days to leach tannins after drying and storage.

I dosed streams 1, 2 and 3 with $70 \mu\text{g L}^{-1}$ of cimetidine to measure rate of cimetidine loss from the water column when introduced to streams with organic material and measure the rate of loss from photolysis (stream without OM or substrate). I collected four filtered water samples from each treatment after 1, 4, 8, 12, 18, 24, and 37 hours of dosing (Figure 4). Samples were stored in clean light resistant glass scintillated vials in the refrigerator until HPLC analysis.

Experiment 2

This experiment had four treatments consisting of 1) no organic matter in the dark, 2) no organic matter exposed to sunlight, 3) organic matter exposed to sunlight, and 4) microbial inoculated organic matter exposed to sunlight. Each treatment had four replicates consisting of 500 ml glass beakers that were filled with 500 ml of groundwater and placed on a shaker table set at 72 rotations per minute. The treatment without sunlight was placed under a light-proof cardboard box. Collection and pre-experiment preparation of senesced *Acer rubrum* leaves was as described in Experiment 1. I weighed 2.5 grams of leaves using a Mettler Toledo XS105 analytical balance. Microbial inoculum was collected using the same method as experiment 1. I then pipetted 30 ml of microbial slurry into beakers with microbial treatments and pipetted 30 ml of groundwater in treatments without microbial communities to ensure equal amounts of

water in each treatment. Microbial communities were allowed to colonize for two weeks prior to addition of cimetidine.

Each treatment was dosed to a cimetidine concentration of $70 \mu\text{g L}^{-1}$ from prepared stock solution and then sampled at 13 time periods: 5, 10, 20, 40, 60, 120, 180, 240, 300, 360, 440, 480, and 1200 min. Samples were stored in light resistance glass scintillation vials and placed in the refrigerator, stored for less than one week and analyzed on the HPLC. Sample analysis was based on the same HPLC method previously described. Negative controls (blanks) were analyzed during testing and cimetidine concentrations of the blanks were consistently below detectable limits ($1 \mu\text{g L}^{-1}$).

Results

I was able to effectively run water samples and prepare standards using the Rainin C-18 (4.6 x 250 mm) column for extraction and the UV detector to measure cimetidine concentrations. This method is efficient because each sample took 8.1 minutes to be extracted from the column without the cost of using solid-phase extraction cartridges. A five point standard curve was made using concentrations of $0 \mu\text{g L}^{-1}$, $5 \mu\text{g L}^{-1}$, $10 \mu\text{g L}^{-1}$, $30 \mu\text{g L}^{-1}$, $50 \mu\text{g L}^{-1}$ and $100 \mu\text{g L}^{-1}$ of pure cimetidine, $R^2 = 0.993$, $y = -1332.4642 + 113.2399x$ (Figure 2). I determined the method detection limit (MDL) by taking the lowest concentration that I could accurately repeat ($1.0 \mu\text{g L}^{-1}$), measured seven samples from a stock solution, then multiplied by the standard deviation of these measurements by the confidence interval 3.14 (APHA 2005). The MDL for this method was $1.169 \mu\text{g L}^{-1}$ (Figure 3).

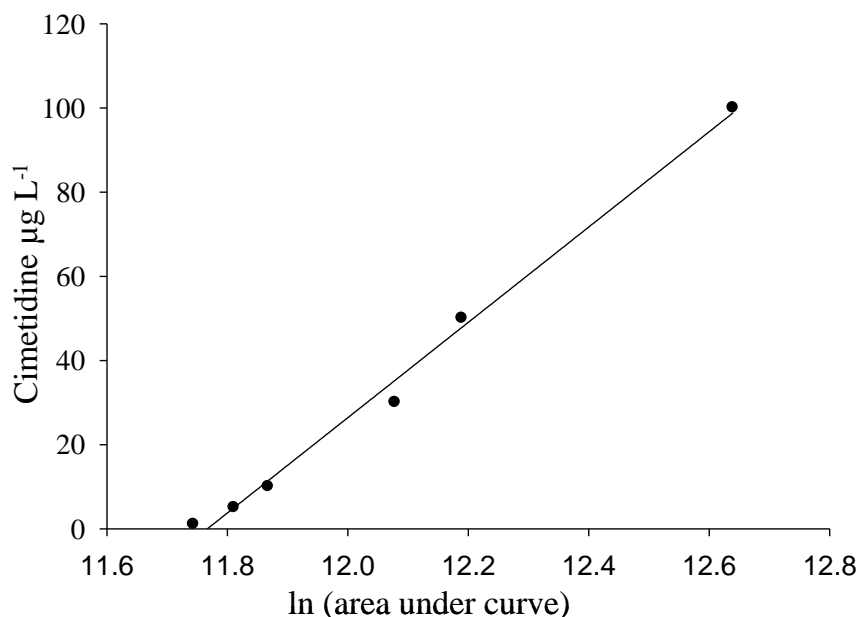


Figure 2. Standard curve using HPLC-UV with six concentrations in: 0, 5.0, 10.0, 30.0, 50.0, 100.0 $\mu\text{g L}^{-1}$. Data are fit using simple linear regression with the natural log of the area under the chromatograph peak on the x-axis with the equation $f = -1332.4642 + 113.2399 * (x)$, $R^2 = 0.993$.

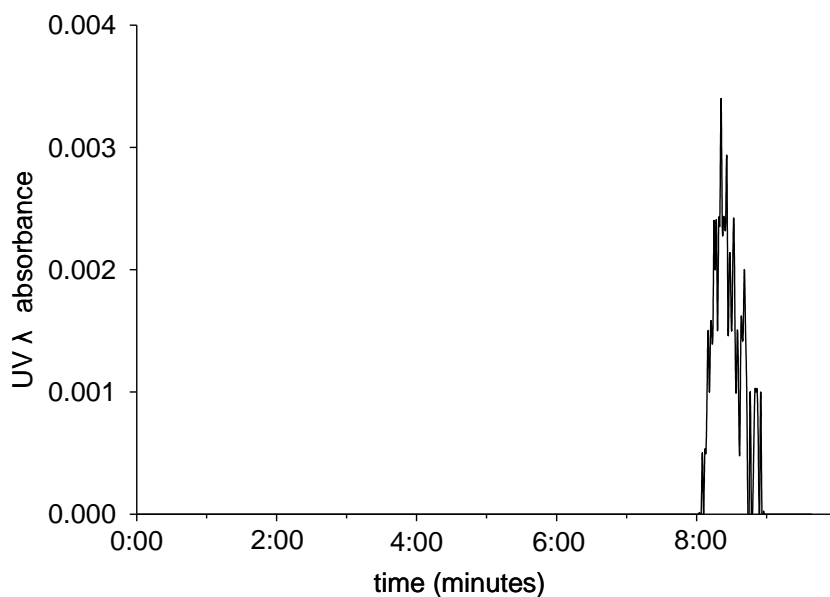


Figure 3. Chromatograph peak of minimal detection limit (MDL) determined by the lowest detectable concentration repeated seven times and multiplying the standard deviation of these measurements by 3.14. The MDL for this HPLC method is $1.1692 \mu\text{g L}^{-1}$. Cimetidine appears on chromatograph 8 minutes after injection into the HPLC.

Experiment 1

Cimetidine concentrations measured in streamwater were similar in sediment and no sediment treatments and remained stable during the 37 h test period (Figure 4). These concentrations remained relatively stable, varying between 48 – 60 $\mu\text{g L}^{-1}$ for about 2.5 days, with a half-life > 37 hours). In contrast, cimetidine rapidly declined in the water column in the presence of leaf packs (Figure 4). These data show that a stream with 40 liters of ground water and 15 grams of inoculated organic matter (0.375 g L^{-1}) has a transfer rate of $2.69 \mu\text{g L}^{-1} \text{ h}^{-1}$ from the water column to organic matter: $\text{rate} = -[(\text{reactant at time}_2) - (\text{reactant time}_1)] / (\text{time}_2 - \text{time}_1)$, where (reactant at time₂) is cimetidine concentration measured at the final time, (reactant time₁) is the concentration at the initial time and (time₂ – time₁) are the corresponding times, $R^2 = 0.998$, $y = -2.189x + 60.577$; $f = (1.9511\text{E-}005 * 51.9458) / (51.9458 + x)$.

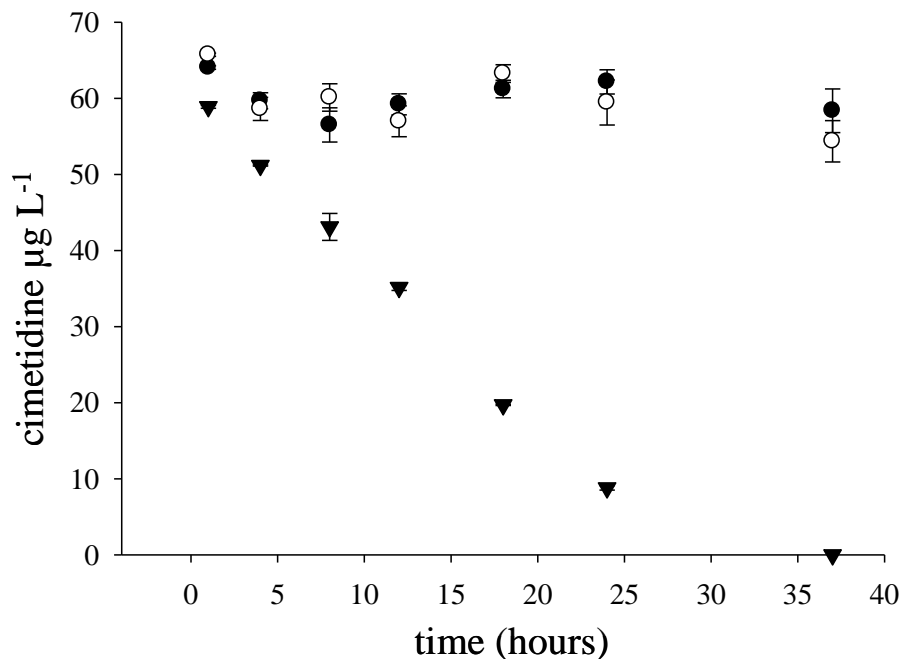


Figure 4. Cimetidine (mean and SE) in the water column of the artificial stream experiment over 37 hour time period from artificial streams containing no organic matter and no sediment (●), sediment with no organic matter (○), 15.0 grams of organic matter (*Acer rubrum*) (▼). Initial dose was 70 µg L⁻¹ of cimetidine.

Experiment 2

In the absence of OM and exposed to sunlight, photodegradation was negligible (Figure 5) and could be fit using a polynomial cubic model ($R^2 = 0.77$). Degradation in the dark with no OM was also negligible ($R^2 = 0.75$). These results are similar to those from the first experiment in the artificial streams. Cimetidine was lost or degraded in beakers kept in the dark with OM absent, with the concentration at 1200 min. being 81% of initial concentration, whereas the light treatment concentration was 76% of the initial concentration at the same sample time (Figure 5).

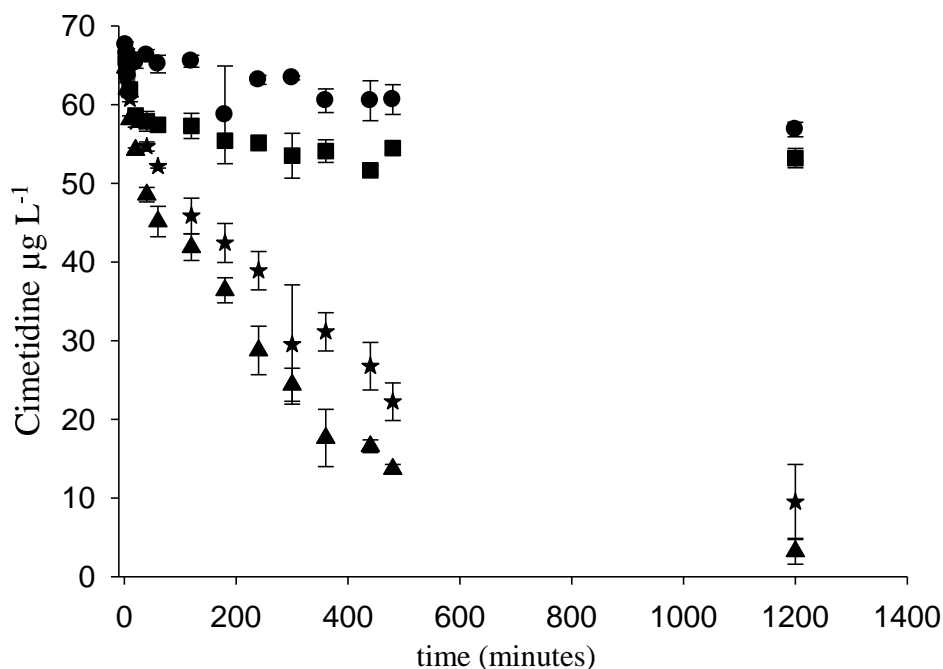


Figure 5. Cimetidine (mean and SE) loss from water in 500 ml beaker microcosms over 22 hour time period (shown in minutes). No organic matter in the dark (●), no organic matter exposed to sunlight (■), organic matter exposed to sunlight (▲), and organic matter with microbial communities exposed to sunlight (*). 2.5 grams of *Acer rubrum* was used for organic matter treatments and the organic matter inoculated with 30 mL of algal/microbial slurry scraped from rocks. Initial dose was $70 \mu\text{g L}^{-1}$ of cimetidine. Each treatment had 4 replicates.

The loss and degradation rates of cimetidine from water were more rapid in the presence of OM when exposed to sunlight compared to no OM exposed to sunlight (ANOVA, $p < 0.001$). The OM with microbial communities followed a similar trend (Figure 5), but the treatment with microbial communities had a lower rate of decrease ($1.36 \mu\text{g L}^{-1} \text{h}^{-1}$, $R^2 = 0.98$). The cimetidine concentration at 1200 minutes with microbial communities was 13% of initial concentration ($9.49 \mu\text{g L}^{-1}$), whereas the concentration with non-inoculated OM was 4% of the initial concentration ($3.23 \mu\text{g L}^{-1}$, $R^2 = 0.97$).

Both OM treatments had less cimetidine than sunlight exposed water without OM (81% of initial concentration).

Discussion

The method modified from Lorenzo and Drayer (1981) was effective for isolating and measuring cimetidine from stream water with a MDL of $1.169 \mu\text{g L}^{-1}$. The method used in the Kolpin et al. (2002) study produced a MDL of $0.0067 \mu\text{g L}^{-1}$, however, their use of HLB (hydrophilic-lipophilic balanced) cartridges produced sub-optimal recovery (less than 50%) for cimetidine Cahill et al. (2004). The method developed in the present study effectively detects concentrations slightly higher than the maximum concentrations measured in US streams by Kolpin et al. (2002), but without the expense of using HLB cartridges. I used a reverse-phase C_{18} column attached to a UV detector on a Rainin HPLC, which is better suited for the more linear molecular shape of the cimetidine molecule (Cahill et al. 2004) and the method presented here is both cost effective and time efficient for isolating and measuring cimetidine.

Photodegradation appears to be an unlikely pathway for degrading cimetidine concentrations in the water column without OM. Cimetidine absorbance maximum is 218 nm and does not appreciably absorb in the wavelength region provided by the solar spectrum (290-3200nm). It is possible that because the windows in the artificial stream facility block 50% of broad-spectrum light and the half-life of cimetidine may be greater if exposed to 100% natural light. My findings are consistent with those of Latch et al. (2003) where cimetidine reacted negligibly under sunlight irradiation (summer sunlight, 45° latitude). They found that the primary mechanism of cimetidine degradation in

Mississippi River water was likely via reaction with O_2 formed from the interaction of sunlight and dissolved organic carbon (DOC) (DOC = 16 mg L⁻¹, pH = 8.0 at their study sites). Their results also did not account for cimetidine adsorbing to organic matter, which can increase the half-life of cimetidine (Latch et al. 2003). Although I was not able to measure sorption rates, I was able to measure removal rates of cimetidine from the water column in the presence of organic matter, which could be indicative of sorption.

Cimetidine readily left the water column in the presence of OM with and without microbial communities. Increased rates of loss from the water column observed in these two studies could be due to several factors. Cimetidine is likely to adsorb to biomass, sludge, soil or sediment (Table 1); however, results from this experiment show that in the absence of OM, cimetidine did not readily bind to inorganic sediments. Extracting cimetidine from OM was beyond the scope of this study, and no other research thus far has described an effective method for such extraction. I attempted several methods for extracting cimetidine from leaf packs with no success. I tried using acetone, hexane/acetone and methanol as extracting solvents with a centrifuge and sonicator to separate cimetidine from the organic matter. None of these methods resulted in cimetidine extraction as measured by HPLC.

Another possible mechanism for cimetidine removal from the water column is photooxidation. This degradation pathway is expected to be a reaction with O_2 formed from the interaction of sunlight with dissolved organic carbon DOC (Latch et al. 2003). OM treatments with and without microbial communities had similar rates of removal from the water column, with the microbial community treatment showing 8.9% less

degradation than the no microbial community treatment after 20 hours. There could be larger amounts of DOC in the treatment without microbes versus that with microbial activity due DOC consumption by microbes, though I have no data to support this. Direct photolysis is not a major breakdown pathway for cimetidine (Latch et al. 2003, Hoppe et al. unpublished); however, the rapid loss of the molecule from the water column in the presence of organic matter suggests cimetidine is either binding to organic matter through sorption mechanisms or degrading through photo-oxidation with DOC.

Analytical chemists and aquatic ecologists have historically used disparate methods for measuring the fate and effects of pharmaceutical compounds in stream ecosystems. My goal was to integrate both approaches into an efficient and complete diagnosis and understanding of the effects of cimetidine in streams using HPLC-UV and artificial streams. The method I developed is effective for measuring cimetidine in streams and allowed me to readily examine the fate of cimetidine dissolved in stream water, and increases our understanding of a previously unexamined compound. The rapid sorption of cimetidine to organic matter may indicate that stream-dwelling organisms may be exposed to this compound via feeding. In addition, previous estimates of actual exposure of aquatic organisms to cimetidine concentrations in surface waters (e.g. Kolpin et al. 2002) may be conservative because this compound readily binds to organic matter.

CHAPTER THREE
EFFECTS OF THE ANTIHISTAMINE CIMETIDINE ON STREAM ECOSYSTEM
FUNCTION

Abstract

Pharmaceutical compounds have been widely detected in surface waters but their effects on stream ecosystems are unknown. Cimetidine (Tagamet®), a widely used H₂ histamine antagonist used to treat heartburn, is a commonly detected pharmaceutical and personal care product (PPCP) in surface waters. Because histamine regulates invertebrate olfactory and stomatogastric function, I predicted that cimetidine may affect stream-dwelling invertebrates. To measure the chronic effects of cimetidine on stream invertebrates, I conducted a long-term (83d) artificial-stream experiment. A range of cimetidine concentrations (0.07 µg L⁻¹ to 70.0 µg L⁻¹) were added to streams supporting populations of the amphipod *Gammarus fasciatus* and the beetle *Psephenus herricki*. Because cimetidine might also influence food resources of invertebrates, I dosed a second set of streams that lacked invertebrates to measure the direct effects of cimetidine on algae. Growth rates of *P. herricki* and growth and biomass accrual of reproducing *G. fasciatus* populations were measured as response variables for invertebrates. In all streams, I measured chlorophyll *a*, ash-free dry mass, primary production, and microbial respiration to examine the effects of cimetidine on algal biofilms. The paired streams with and without invertebrates allowed me to examine direct effects of cimetidine on

invertebrates and effects of the compound on basal resources as mediated through invertebrates. *P. herricki* individual growth rates were reduced in the presence of cimetidine, but *G. fasciatus* individual growth rates were not different among treatments. However, *G. fasciatus* size distribution was significantly different in treatments with the lowest concentration of cimetidine $0.07 \mu\text{g L}^{-1}$ (ANOVA, $p = 0.002$) with no individuals in the three smallest size classes. Biomass and density of *G. fasciatus* were lower across all cimetidine treatments compared to the control and density was significantly lower than control when cimetidine concentrations were $0.7 \mu\text{g L}^{-1}$ (ANOVA, $p = 0.035$). I found no consistent effect of cimetidine on biomass and production within algal biofilms (chl *a*, AFDM, primary production and microbial respiration) in streams with or without invertebrates. Understanding the effects of novel compounds currently detected in surface waters will require a substantial effort, the artificial stream approach I employed prove useful in quantity effects of such compounds. Pharmaceutical compounds, such as cimetidine, detected in surface waters may have effects on lotic ecosystem function; however, effects of these compounds are complex and merit further study.

Introduction

In recent years, the occurrence of pharmaceuticals and personal care products (PPCPs) in lotic systems has received increased attention; however, effects of these contaminants on stream ecosystem structure and function are unclear (Halling-Sørensen et al. 1998, Daughton and Ternes 1999, Cunningham et al. 2006). These novel contaminants enter surface waters following incomplete breakdown in both human digestion and wastewater treatment processes. PPCPs typically spend from less than 1 h to a few days in wastewater treatment facilities (WWTFs); less than the half-lives of

many PPCPs (Halling-Sørensen 1998, Xia et al. 2005). As a result of incomplete removal by WWTFs and combined sewer overflows (CSOs), which combine excess stormwater and untreated sewage, PPCPs enter and potentially affect receiving waters. PPCPs are also typically detected in urban waterways and may represent an additional stressor in these already degraded ecosystems (Grimm et al. 2000, Paul and Meyer 2001).

Previous research has demonstrated that there are acute effects on aquatic organisms from PPCPs such as analgesics, synthetic hormones, antibiotics, neuroactive compounds, surfactants and antidepressants (see reviews by Halling-Sørensen et al. 1998, Cunningham et al. 2006, Fent et al. 2006). PPCPs typically occur in surface waters at low concentrations (ranging from ng L^{-1} to $\mu\text{g L}^{-1}$), below levels that cause acute toxicity. However, chronic exposure may result in sublethal effects, e.g. changes in feeding behavior, fecundity and/or growth (De Lange et al. 2006). The widespread use of these compounds results in their repeated addition to surface waters and as such they have been classified as pseudopersistent compounds (Nilsen et al. 2007) to which stream organisms are exposed (see Watts et al. 2001(a), 2002, Maul et al. 2006, Nentwig 2007). A fundamental goal of aquatic ecotoxicology is to understand how contaminants affect organisms at the population level (Truhaut 1975), but there has been very little experimental work addressing the chronic inputs of PPCPs on population-level responses in streams (Widdows and Donkin 1991, Xia et al. 2005, Cunningham et al. 2006).

In an extensive study of PPCP occurrence in US surface waters Kolpin et al. 2002 documented detectable levels of 82 of the 95 compounds they measured and these compounds may affect stream ecosystems. Although PPCPs are specifically designed for human and veterinary medicine, they may affect other vertebrates and even invertebrates

because many target receptors/molecules are evolutionarily conserved (Fent et al. 2006). Antihistamines, which include H₁ and H₂ receptor antagonists used to treat symptoms of allergies and heartburn, are frequently detected in US surface waters. Histamines, neuroactive amines in the nervous systems of animals from diverse phyla (Hashemzadeh-Gargari and Freschi 1992), are widely used by vertebrates and invertebrates as neurotransmitters, neuromodulators or neurohormones. Several neurological studies have demonstrated that histamine activates photoreception, olfactory receptors and stomatogastric neurons in invertebrates; antihistamines block these actions (Claiborne and Selverston 1984, Hardie 1988, Hashemzadeh-Gargari and Freschi 1992, Wachowiak and Cohen 1999, Cattaert et al. 2002, Wachowiak et al. 2002, Christie et al. 2004). Because invertebrates use histamines for these important physiological functions, I predicted that the presence of these compounds in surface waters might have adverse affects on stream-dwelling invertebrates.

A widely used antihistamine is cimetidine (Fig. 1), an H₂ histamine antagonist sold worldwide as the gastrointestinal drug Tagamet[®]. Cimetidine has been measured in surface waters at concentrations up to 0.58 µg L⁻¹ (Kolpin et al. 2002). Because about 60% of the original dose is excreted by humans following ingestion (Lorenzo and Drayer 1981), of the 163,000 kg of cimetidine sold in the US each year, approximately 76, 610 kg enter WWTFs (Anderson et al. 2004). Approximately 70% of cimetidine is degraded within WWTFs (Anderson et al. 2004) and it has been estimated that approximately 23,626 kg of cimetidine enter US surface waters annually (Buth et al. 2003). Based on the known neurological effects of cimetidine on invertebrates, I hypothesized that exposure to cimetidine may affect normal histamine function in aquatic stream-dwelling

invertebrates, possibly disrupting their growth rates with potential consequences for stream ecosystem function.

Chronic exposure to low doses of cimetidine could affect stream biota, including microbial and algal communities, as well as invertebrates and fishes. Consequently, stream ecosystem functions such as nutrient cycling, primary and secondary productivity and organic matter dynamics could be altered. My objective was to measure the effects of chronic exposure to the antihistamine cimetidine on invertebrate growth and production, as well as measure its direct effects on stream algae. In addition to possible direct effects on invertebrates and algae, cimetidine may also induce cascading effects on algae from altered invertebrate feeding. I used a long-term (83 day) artificial stream experiment to test the effects of four cimetidine concentrations on algal biofilms and macroinvertebrates. My approach examined the effect of cimetidine on individual growth rates and population-level effects on two invertebrates, *Gammarus fasciatus* and *Psephenus herricki* as well as direct and indirect effects on algal biofilms. An additional objective of this work was to develop a method using artificial streams to measure the effects of chronic exposure of a PPCP on stream ecosystem function.

Methods

Artificial streams

I conducted this experiment from July through September 2006 in 30 recirculating artificial streams located in an indoor greenhouse (windows block 50% incoming solar radiation) facility at Loyola University Chicago. Water temperature was measured continuously in four randomly chosen streams using Hobo[®] data loggers (Onset Computer Corp.) and was consistently $19 \pm 3^{\circ}\text{C}$. Artificial streams (4 m x 15.5 cm x 15

cm) were constructed of composite fiberglass with a streambed surface area of 0.62 m². Streams were filled to 9.5 cm depth (76 L) with groundwater and 50% of the volume was replaced weekly to mimic exposure in rivers and streams. Nutrient concentrations in the groundwater were low: ammonium (NH₄⁺): range = 3.5 – 6.5 µg L⁻¹, soluble reactive phosphorus (SRP): range = 15 – 30 µg L⁻¹. Cimetidine was not detected in any groundwater samples throughout the study. Current velocity in each stream was kept constant at 0.26 m s⁻¹ by a Dayton DC gear motor (model 42129b) and a Dayton DC speed control (model 5X412D, Dayton DC Gear Motor, Niles, Illinois) connected to a rotating stainless steel paddle-wheel. I placed several substrate types into each stream: unglazed clay tiles (ten 12 cm x 12 cm and six 4 cm x 4 cm), pre-rinsed pea-size gravel (2.2 kg), and coarse sand (2.8 L). I inoculated each stream with 60 ml of algal slurry comprised of epipellic algae from Nippersink Creek (McHenry County, Illinois). All visible macroinvertebrates were removed from the algal inoculum prior to addition. I allowed 6 weeks for periphyton communities to develop (visibly abundant growth on all substrates) before beginning the cimetidine additions. I also added 20 leaf packs (5g) to each stream using senesced red maple (*Acer rubrum*) collected from the Ottawa National Forest in the Upper Peninsula of Michigan. Leaves were dried and stored in large cardboard flats and covered with cardboard sheets out of direct sunlight. Leaf packs were pre-conditioned in a separate set of streams to allow tannins and dissolved organic carbon to leach by submerging them in groundwater for three consecutive 3 day periods, replacing stream water between each iteration. The leaching process was repeated 3 times.

Pharmaceutical compound

Cimetidine (Sigma-Aldrich Chemical Co., St. Louis, Missouri) stock solutions were prepared with E-pure[®] water and stored in a refrigerator in the dark for up to four days. Cimetidine concentrations of 0.07 $\mu\text{g L}^{-1}$, 0.7 $\mu\text{g L}^{-1}$, 7.0 $\mu\text{g L}^{-1}$, or 70 $\mu\text{g L}^{-1}$ were administered every 2-d for 86 days to all treatments except the control, which received no cimetidine. The lowest concentration of 0.07 $\mu\text{g L}^{-1}$ was the median concentration measured from 84 samples taken from streams throughout the US (Kolpin et al. 2002).

I modified the procedure of Lorenzo and Drayer (1981), designed to measure cimetidine in human blood serum, to measure cimetidine in stream water using HPLC-UV. This method resulted in a minimum detection limit of 1.68 $\mu\text{g L}^{-1}$. The concentrations of cimetidine in stock solutions and stream water were analyzed by liquid chromatography with a Rainin Rabbit HPX (Oakland, CA) and a Knauer Variable Wavelength Monitor (Berlin, Germany) using a μ -Bondpack C-18 column; 4.6 x 250 mm, Waters Associates (Milford, MA). I also measured photolysis-induced degradation of cimetidine, and rates of sorption of this compound to organic matter (Hoppe et al. unpublished). Preliminary studies demonstrated that cimetidine readily binds to organic matter but does not readily degrade by photolysis (Chapter 2 and Latch et al. 2003). I dosed the streams every other day for the duration of the experiment to mimic exposure in streams receiving wastewater effluent.

Experimental design

The effects of cimetidine on invertebrates and basal food resources were examined using 30 artificial streams, 15 of which streams contained invertebrates and 15 streams without invertebrates. Cimetidine concentrations were administered to streams

with and without invertebrates. There were two types of control to which cimetidine was not added: streams with and without invertebrates. Each treatment was replicated 3 times. This design allowed me to measure direct effects of cimetidine on basal resources (streams without invertebrates) and its effects on invertebrates (streams with invertebrates). In addition, an indirect effect of cimetidine on resources mediated by invertebrates could be assessed by comparing streams with invertebrates to those without. For example, a difference in algal production at a given cimetidine concentration in streams with invertebrates compared to streams without invertebrates, would suggest an effect of cimetidine on invertebrates that cascaded down to algal productivity. If there was a cimetidine effect on algae, a cascading up effect could be assessed through measuring differences in invertebrate biomass between treatments.

Basal resources

Every other week, I measured algal biomass as chlorophyll *a* concentration (chl *a*) and ash-free dry mass (AFDM), as well as primary production and respiration. To measure chl *a*, 4 cm x 4 cm clay tiles were scraped, made into slurry and subsamples were filtered onto 0.7 μm glass fiber filters (GF/F Pall Corporation, East Hills, New York). I extracted chl *a* using the hot ethanol/warm water bath method and analyzed them on a Shimadzu PharmaSpec UV-1700 UV-visible spectrophotometer (APHA 2005). To measure AFDM, a second algal slurry subsample was filtered onto pre-ashed, pre-weighed glass fiber filters. The filters were oven dried (50°C for 24 h), weighed on an analytical balance and then ashed in a muffle furnace at 550°C for 1 h and reweighed to obtain AFDM (Benfield 2006).

Respiration and primary production were measured using light and dark chambers (Hill et al. 2002). I placed tiles and a streamwater blank (to account for water column changes in oxygen concentration) into 130 ml specimen containers (Fisher Scientific, Pittsburgh, Pennsylvania) filled with stream water collected from the treatment and recorded initial dissolved oxygen (DO) with a YSI DO meter (model 550A, YSI Inc. Yellow Springs, OH) as (mg L^{-1}) and water temperature. Specimen cups were capped, using care to eliminate air bubbles and placed inverted in streams. After approximately two hours I measured final DO and temperature. Respiration was measured with the same procedure but samples were covered with shade cloth and cardboard to block sunlight. Respiration and primary production rates were calculated as the change in $\text{mg O}_2 \text{ cm}^{-2} \text{ time}^{-1}$. Gross primary production (GPP) was then calculated as the sum of net production and respiration (Wetzel and Likens 2000).

Invertebrate populations

Two common riverine invertebrates representing different functional feeding groups were used: *Gammarus fasciatus* (Class Crustacea) a common riverine amphipod and functionally classified as a shredder (DeLong et al. 1993) and *Psephenus herricki*, or water penny beetles, (Coleoptera: Psephenidae) a common invertebrate and classified as a scraper (Merritt et al. 2008). I used these two invertebrate taxa to represent possible differences in exposure to cimetidine from representatives of two functional feeding groups. *G. fasciatus* (P_1 generation) were collected from cobble substrata at several shoreline locations of the Calumet River south of Chicago, IL in September of 2005. Amphipods were transported to the aquatic facility, placed in artificial ponds containing nutrient-enriched well water, and maintained at approximately 20°C under natural light

conditions. Oviparous females were transferred to a second pond for the production of the F₁ generation. I collected *Psephenus herricki* from Hickory Creek, Will County, near Joliet, IL and used these individuals in the experiment.

I selected individuals of each species from approximately the same size class for use in stream experiments. Prior to adding invertebrates to the artificial streams, I measured wet weight (WWT; mg) of all individuals by carefully drying individuals between two sheets of filter paper for approximately 30 seconds, so that adhering water was removed, and weighing to the nearest 0.1 mg using a Mettler analytical balance (type XS105). I also took photographs (7.1 megapixel Pentax Optio) of each treatment population on 1 mm graph paper and measured invertebrate lengths using Image-Pro Plus software (Media Cybernetics, Inc.). Body lengths of *G. fasciatus* individuals in each subsample were measured from behind the eye to the tip of the third uropod along the curve of the dorsal surface (Blockwell et al. 1999) and *P. herricki* individuals were measured between the dorsal posterior and anterior tips. I placed 50 *G. fasciatus* of similar size and 50 *P. herricki* of similar size in each stream on July 6, 2006. Invertebrates were allowed to acclimate 1-wk before initiating cimetidine additions.

At the end of the experiment (83 d after cimetidine treatments began), I sampled the macroinvertebrate populations by collecting all organic matter from each artificial stream on a 0.01 mm sieve. All *P. herricki* were collected and because *G. fasciatus* populations had increased substantially, I reconstituted all the organic matter from each stream with 4 L of water, suspended the material, and collected three 125 ml subsamples. Each subsample was sorted to collect all *G. fasciatus*. Organisms were preserved in formalin (37% formaldehyde) along with a small amount of Rose Bengal stain. Final

counts and lengths were measured using the imaging technique previously described.

Individual length measurements were used to determine size structure of populations and also used for length to mass regressions. Biomass accrual was measured as change in biomass using wet-weights:

$$\text{Change in biomass} = \frac{\ln \text{ final WWT} - \ln \text{ initial WWT}}{\text{Stream bed surface area}}$$

In addition to free-living population level measurements, individual instantaneous growth rates (IGRs) of *G. fasciatus* and *P. herricki* were measured using flow-through growth chambers (Toby tea boys, Plymouth Tea Co., Chatham, Massachusetts) placed in the artificial streams. Individuals from the same population source were added to streams. In each growth chamber, I placed one *G. fasciatus* and one *P. herricki* and 5 g of gravel with periphyton that had been exposed to 5 weeks of chronic cimetidine dosing from the artificial streams. I added both organisms to one chamber because I was measuring growth and not their effect on algal resources and chambers and space were limited. Initial photographs were taken of each individual and 6 growth chambers were placed in each stream. After 28 days the final length of each individual was measured. I converted lengths to mass using length mass regression (Benke et al. 1999) and instantaneous growth rate (Huryn and Wallace 1986) was calculated as:

$$\text{IGR} = \frac{\ln \text{ final WWT} - \ln \text{ initial WWT}}{\text{days}}$$

Data analysis

I used repeated measures analysis of variance (rmANOVA) to examine the effect of cimetidine and time on all response variables including chl *a*, AFDM, primary

production and microbial respiration in replicate streams ($n = 3$) with and without invertebrates. If a significant interaction between cimetidine treatment and date resulted, I used one-way ANOVA across treatments for each sample date using a Bonferroni adjusted p-value of $0.05/8$ sample dates = 0.00626 to determine significant differences (Zar 1999). In addition, I used rmANOVA followed by Tukey's multiple comparison test (MCT) for each treatment to compare changes among basal resources among dates. For all rmANOVAs, I used a first-order autoregressive covariance structure (after Simon et al. 2005). This process allowed covariance among effects while comparing only two parameters (day and treatment).

I used a one-way ANOVA with Tukey's MCT to determine significant differences in size classes, change in biomass, number of individuals, and IGR's for *G. fasciatus* and percent survivorship and IGR's for *P. herricki*. Instantaneous growth rates of invertebrates were natural log (ln) transformed and percent survivorship were arcsine-square root transformed to meet assumptions of ANOVA. Statistical analyses were done using SAS (version 9.1; SAS Institute, Cary, North Carolina) or SYSTAT (version 10.2; SYSTAT software, San Jose, California).

Results

Effects of cimetidine on basal resources –streams without invertebrates

Basal resource response to cimetidine was examined by comparing each cimetidine treatment relative to the control (treatment/control). A ratio greater than 1 indicates a greater response in the treatment relative to the control. In general algal biofilm AFDM and chlorophyll *a* did not change in response to cimetidine when macroinvertebrates were absent. However, biofilm functions of microbial respiration and

primary production displayed low dose responses. For example, biofilm AFDM was not different among treatments (rmANOVA, Figure 6, $F_{4,7} = 0.81$, $p = 0.52$) or through time (rmANOVA, $F_{4,7} = 1.15$, $p = 0.34$); however, microbial respiration differed significantly among treatments (rmANOVA, Figure 7, $F_{4,7} = 2.80$, $p = 0.03$) with the lowest ($0.07 \mu\text{g L}^{-1}$) and low (x10) concentrations having higher respiration rates relative to the control, and over time (rmANOVA, $F_{4,7} = 75.05$, $p < 0.001$).

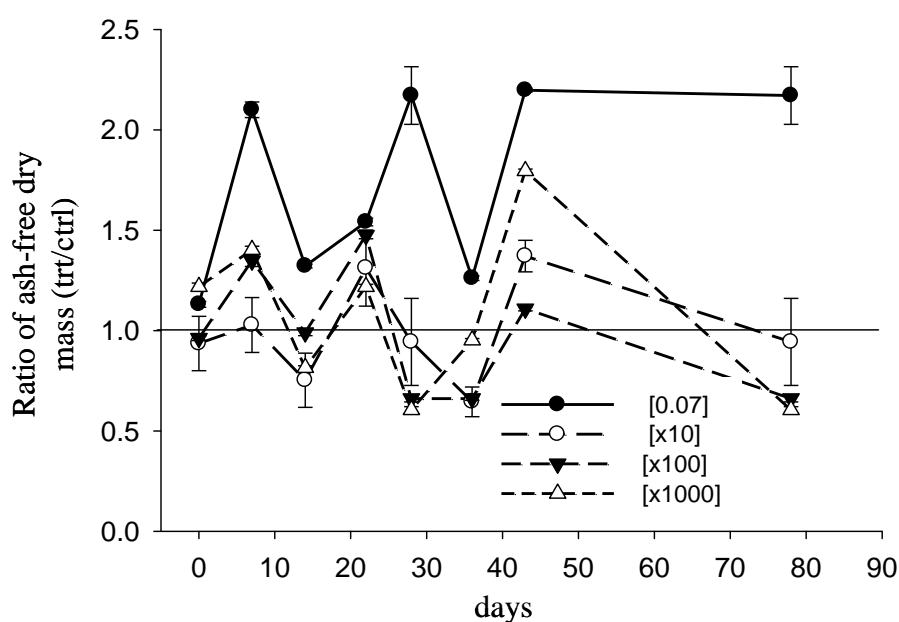


Figure 6. Ratios of treatment divided by control [0] of mean ash-free dry mass (mg cm^{-2}) with standard error bars from clay tiles for each cimetidine treatment [0], $[0.07 \mu\text{g L}^{-1}]$, [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment.

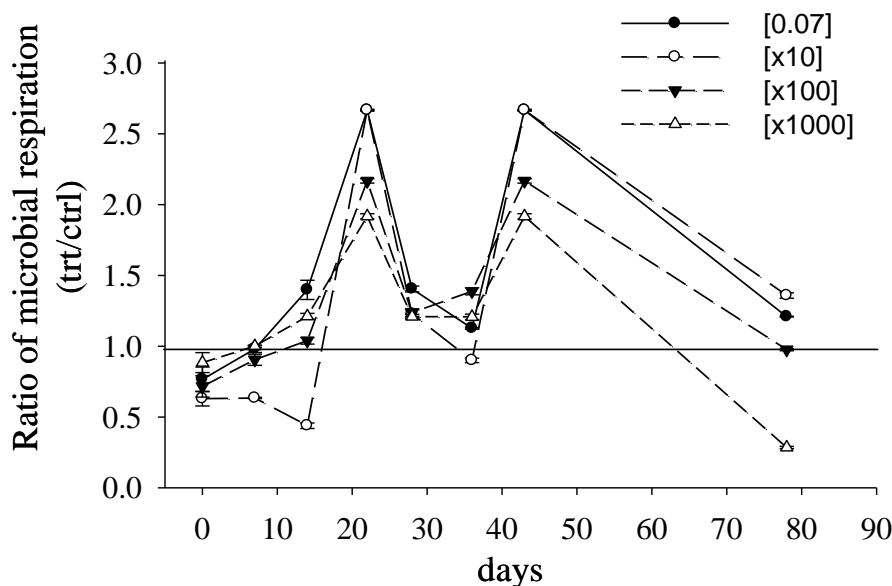


Figure 7. Ratios of treatment divided by control [0] of mean microbial respiration ($\text{mg O m}^{-2} \text{h}^{-1}$) with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment.

Chlorophyll *a* concentrations were the same among cimetidine treatments relative to the control (rmANOVA, Figure 8, $F_{4,7} = 0.73$, $p = 0.57$) but different over time (rmANOVA, $F_{4,7} = 25.02$, $p < 0.0001$). In contrast, primary production was significantly different among treatments relative to the control (Figure 9, rmANOVA, $F_{4,7} = 4.82$, $p = 0.001$) with the low concentration (x10) having lower rates of primary production relative to the control (rmANOVA, $F_{4,7} = 4.82$, $p = 0.0016$) and over time (rmANOVA, $F_{4,7} = 3.66$, $p = 0.0018$). In streams without invertebrates, the greatest differences between the cimetidine treatments relative to the control were during the first three weeks of the experiment, with the greatest differences found in microbial respiration and primary production (Figs. 7 and 9).

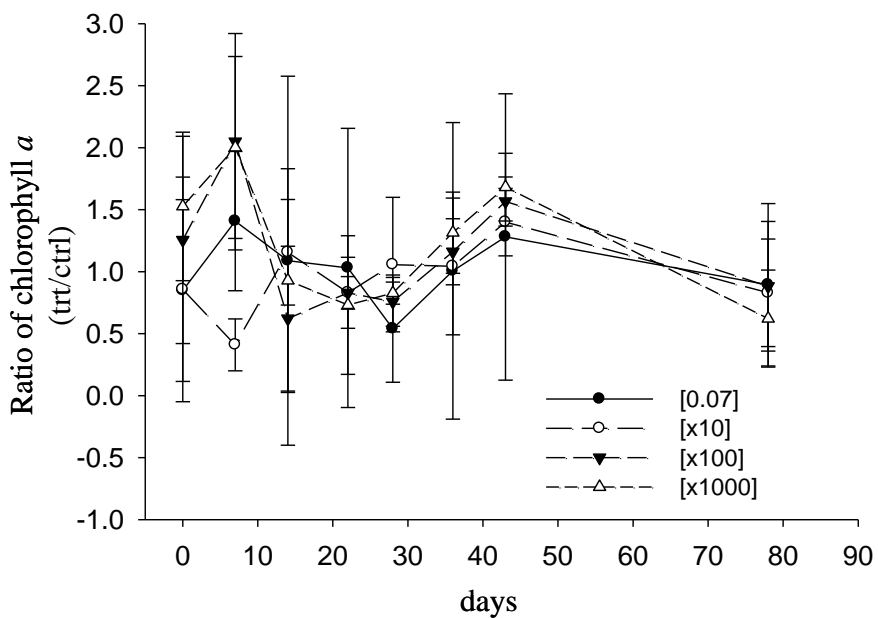


Figure 8. Ratios of treatment divided by the mean of chlorophyll *a* ($\mu\text{g cm}^{-2}$) of the control [0] with standard error bars from clay tiles for each cimetine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment.

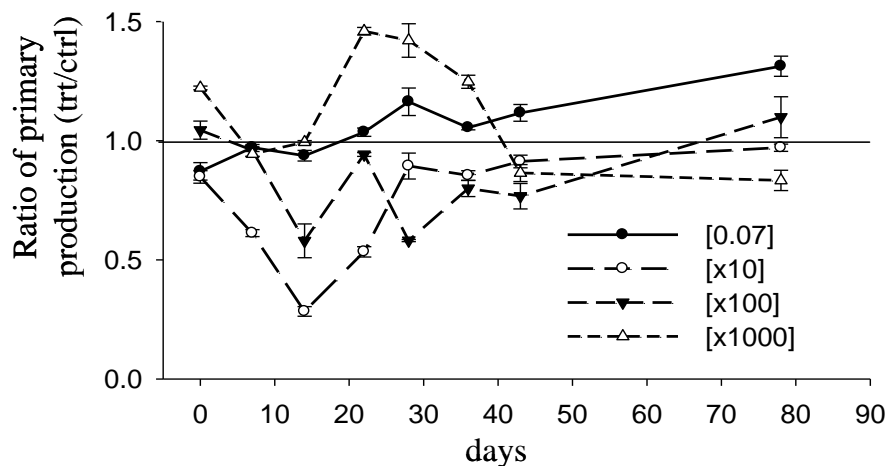


Figure 9. Ratios of treatment divided by the mean of primary production ($\text{mg O m}^{-2} \text{h}^{-1}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams without macroinvertebrates during 83 day artificial stream experiment.

Effects of invertebrates on basal resources without cimetidine

The 1-way ANOVA of control (no cimetidine) in streams with and without invertebrates suggests no differences in basal resources when consumers were present or absent. In the control streams, macroinvertebrates did not have an observable effect on basal resources implying very little grazer effects in these systems, possibly due to prolific algal growth. In treatments dosed with cimetidine, there were no cascading effects of cimetidine on basal resources via changes in invertebrate activity. AFDM, chlorophyll *a*, and primary production were not significantly different between treatments with and without invertebrates. For example, there were no significant differences in chlorophyll *a* between streams with invertebrates and those without invertebrates ($F_{1,28} = 17.3, p = 0.303$). Within the first 36 days rates in microbial respiration differed between streams with and without invertebrates, with higher rates occurring in the absence of invertebrates.

Indirect effects of cimetidine on basal resources - streams with invertebrates

In streams containing invertebrates, biofilm AFDM and chlorophyll *a* concentrations did not differ among cimetidine treatments relative to the control (AFDM, rmANOVA, Figure 10, $F_{4,7} = 1.35$, $p = 0.26$; chl *a*, rmANOVA, Figure 12, $F_{4,7} = 1.22$, $p = 0.307$) but did differ significantly over time (chl *a* $F_{4,7} = 23.21$, $p < 0.0001$; AFDM $F_{4,7} = 7.05$, $p < 0.001$). A similar trend was observed with microbial respiration with no differences among treatments relative to the control (rmANOVA, Figure 11, $F_{7,80} = 7.05$, $p = 0.19$) but significant variation over time ($F_{7,80} = 51.08$, $p < 0.001$). The lowest cimetidine concentration exhibit higher rates for the final two sampling dates (days 43 and 78). Similarly, rates of primary production did not differ among treatments relative to the control (rmANOVA, Figure 13, $F_{4,80} = 3.17$, $p = 0.053$) but differed over time ($F_{4,7} = 8.01$, $p < 0.0001$) with the low concentration ($0.07 \mu\text{g L}^{-1}$) having lower rates relative to the control ($F_{4,80} = 3.30$, $p = 0.012$).

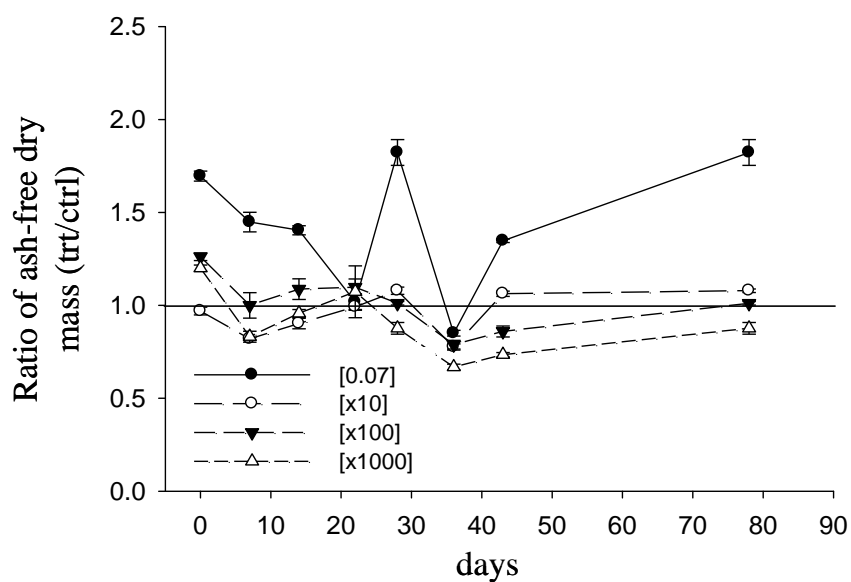


Figure 10. Ratios of treatment divided by the mean of ash-free dry mass (mg cm^{-2}) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment.

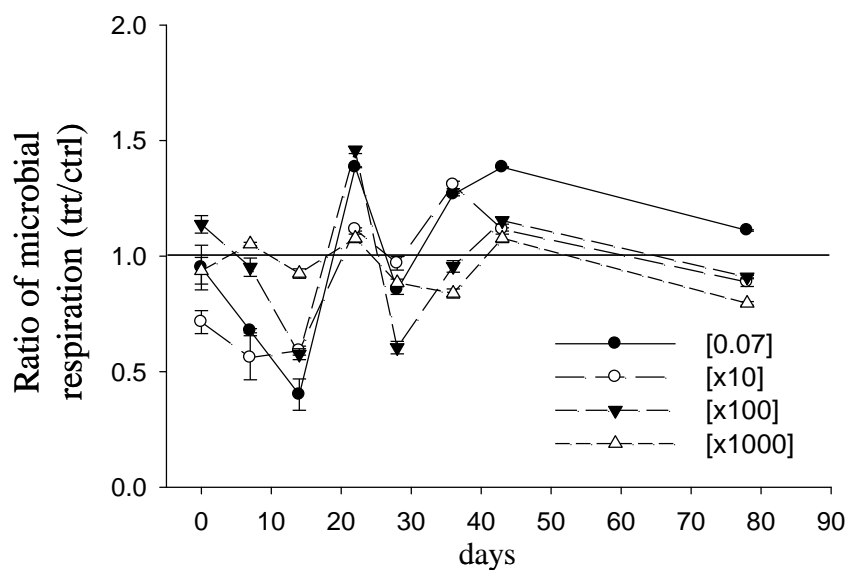


Figure 11. Ratios of treatment divided by the mean microbial respiration ($\text{mg O m}^{-2} \text{h}^{-1}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment.

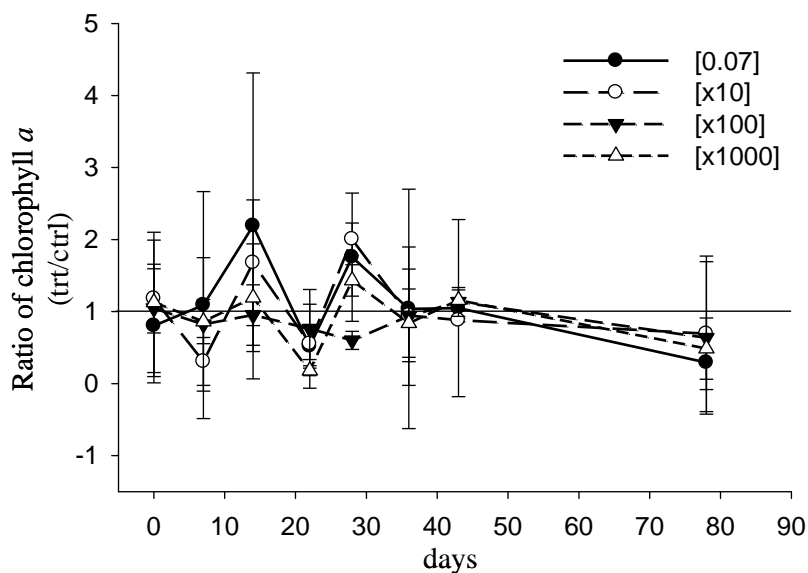


Figure 12. Ratios of treatment divided by the mean chlorophyll a ($\mu\text{g cm}^{-2}$) of the control [0] with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment.

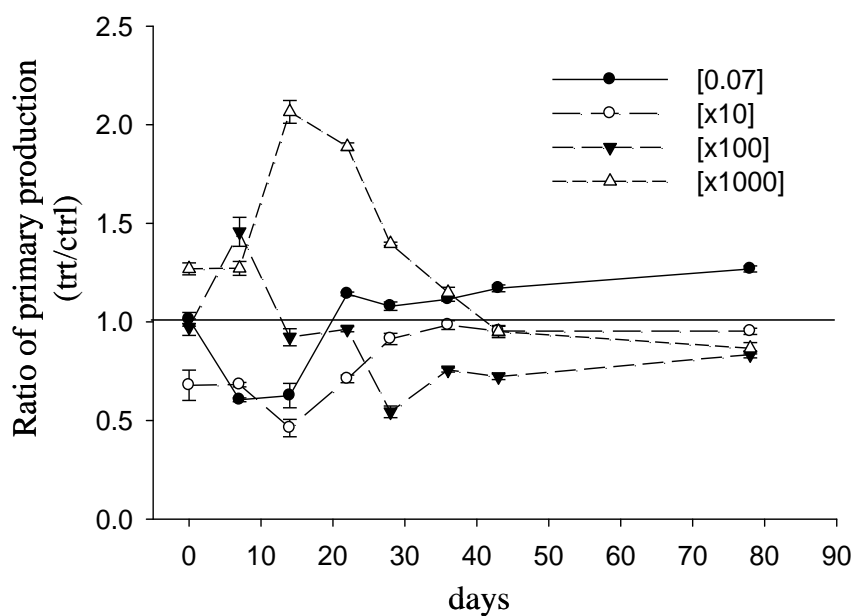


Figure 13. Ratios of treatment divided by the mean primary production ($\text{mg O m}^{-2} \text{h}^{-1}$) of the control [0] of with standard error bars from clay tiles for each cimetidine treatment [0], [0.07 $\mu\text{g L}^{-1}$], [x10], [x100], and [x1000] for streams with macroinvertebrates during 83 day artificial stream experiment.

Effects of cimetidine on invertebrates

After three months of chronic exposure to cimetidine, size distribution within *G. fasciatus* were significantly different among all cimetidine treatments relative to the control (Table 2). All concentrations of cimetidine yielded significantly reduced numbers in the smallest size class, including individuals less than 4 mm (Table 2, $F_{4, 10} = 8.99$, $p = 0.002$) ($0.07 \mu\text{g L}^{-1}$ $p = 0.003$; $\times 10$ $p = 0.022$; $\times 100$ $p = 0.006$; and $\times 1000$ $p = 0.005$). The size classes 9-10 mm and 10-11 mm also were significantly lower in the lowest ($0.07 \mu\text{g L}^{-1}$) and highest ($\times 1000$) cimetidine concentrations compared to the control (Figure 14A and 14D, $p = 0.025$ and $p = 0.017$, respectively).

Table 2. Mean and standard error (SE) of body length distributions and number of *G. fasciatus* population (per m^2) following 83 day exposure to control water and cimetidine treatments.

	< 4mm	4 - 5mm	5 - 6mm	6 - 7mm	7 - 8mm	8 - 9mm	9 - 10	10 - 11	> 11mm
Treatment ($\mu\text{g L}^{-1}$)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Control [0]	700 (104.08)	216.6 (120.18)	200 (150.0)	883 (268.22)	1316.6 (376.75)	2050 (301.38)	2250 (637.05)	1183.3 (164.1)	516.6 (145.29)
[0.07]	0 (0.0)	0 (0.0)	0 (0.0)	66.6 (16.6)	366.6 (158.98)	416.66 (158.98)	366.6 (187.82)	333.3 (169.14)	116.6 (60.09)
[x10]	83.3 (44.09)	100 (76.37)	166.6 (60.09)	550 (208.16)	900 (284.31)	1033.3 (540.31)	600 (160.72)	483.3 (164.14)	166.6 (16.6)
[x100]	16.6 (16.66)	16.6 (16.66)	266.6 (92.72)	883.3 (358.62)	1266.6 (294.86)	1566.6 (519.88)	1083.3 (404.48)	516.6 (268.22)	400 (275.37)
[x1000]	16.6 (16.66)	83.3 (33.33)	250 (115.47)	383.3 (158.98)	666.6 (441.90)	583.3 (433.33)	350 (275.37)	100 (76.37)	66.6 (66.6)
ANOVA	$p < 0.001$, <0.05	$p = 0.226$, >0.05	$p = 0.377$, >0.05	$p = 0.137$, >0.05	$p = 0.268$, >0.05	$p = 0.094$, >0.05	$p = 0.025$, <0.05	$p = 0.017$, <0.05	$p = 0.205$, >0.05

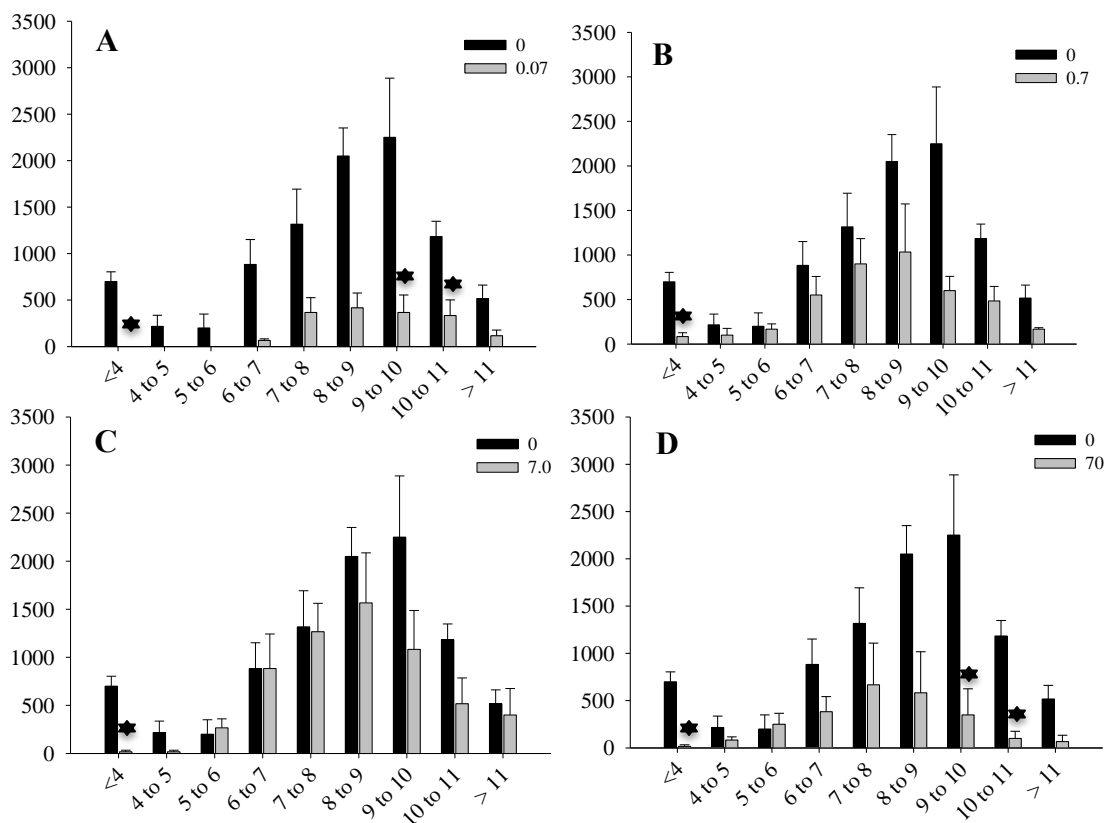


Figure 14. Mean and standard error (SE) of body length distributions and number of *G. fasciatus* per m² following 86 day exposure to control water and A) 0.07 µg L⁻¹ cimetidine [0.07], B) 0.7 µg L⁻¹ cimetidine [x10], C) 7.0 µg L⁻¹ cimetidine [x100], D) 70.0 µg L⁻¹ cimetidine [x1000]. * indicates a significant difference from control values (p ≤ 0.05).

Change in *G. fasciatus* biomass did not significantly differ among treatments

(Figure 15A, $F_{4, 10} = 1.285$, $p = 0.339$). However, there was a trend of lower biomass (grams m⁻²) across all treatments relative to the control. The low dose (0.07 µg L⁻¹) had 26.7% less biomass m⁻² than the control, (0.7 µg L⁻¹) had 28.6% less biomass compared to the control, (x100) had 19.3% less biomass compared to the control, and (70.0 µg L⁻¹) had 28.4% less biomass compared to the control. I found the same trend for density (individuals m⁻²) among the treatments (Figure 15B). The (x10) treatment had

significantly (53.8%) fewer individuals than the control (Fig. 15B, $F_{4,10} = 3.77$, $p = 0.035$), and both $0.07 \mu\text{g L}^{-1}$ and (x1000) supported 44 % fewer individuals than the control.

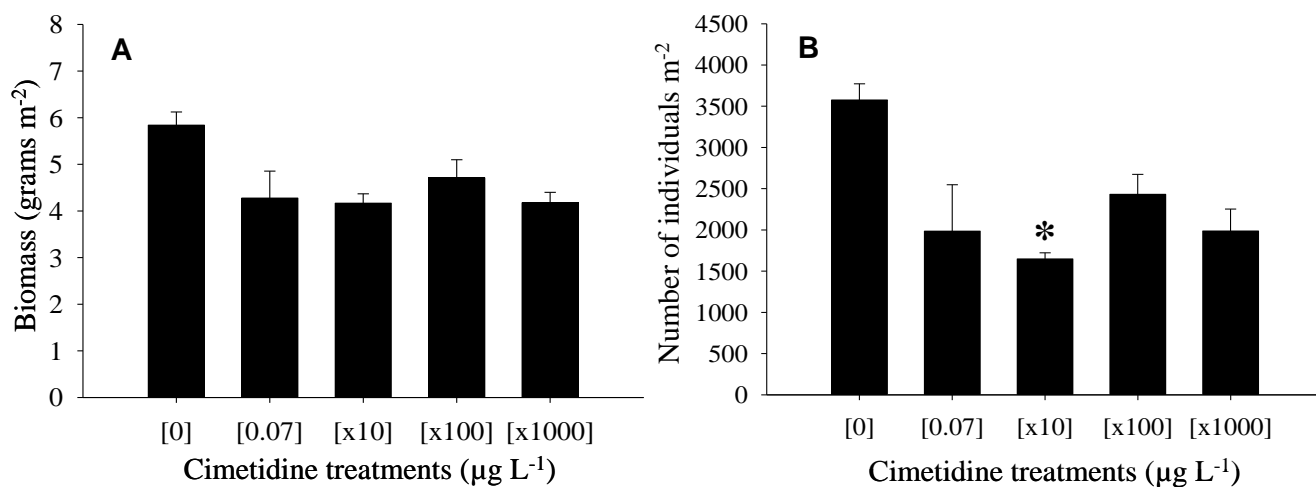


Figure 15. Mean and standard error (SE) of A) biomass and B) number of individuals for *G. fasciatus* populations following 86 day exposure to control water and cimetidine treatments. * indicates a significant difference from control values.

G. fasciatus had varied IGR's when exposed to cimetidine in individual growth chambers (Figure 16C, $F_{4,25} = 1.949$, $p = 0.133$). No differences in IGR's were observed between the low dose ($0.07 \mu\text{g L}^{-1}$), (x100) and control, but the (x10) treatment had 16.2% faster growth and (x1000) had 31.0% slower growth than the control, but these were not significant differences.

IGR's of *P. herricki* were not significantly different among treatments during the 28 day incubation in individual growth chambers (Figure 16B, $F_{4,25} = 1.411$, $p = 0.259$). However, there was a trend showing lower IGR's among all treatments compared to the control. The lowest dose ($0.07 \mu\text{g L}^{-1}$) had growth rates 67.5% less than the control, (x10) had 45% less growth compared to the control, (x100) had 84.5% less growth

compared to the control, and (x1000) had 58% less growth compared to the control (Figure 16B). In addition, percent survivorship was significantly reduced in (x100) and (x1000) treatments when compared to the control (Figure 16A, $F_{4,1} = 5.05$, $p = 0.017$).

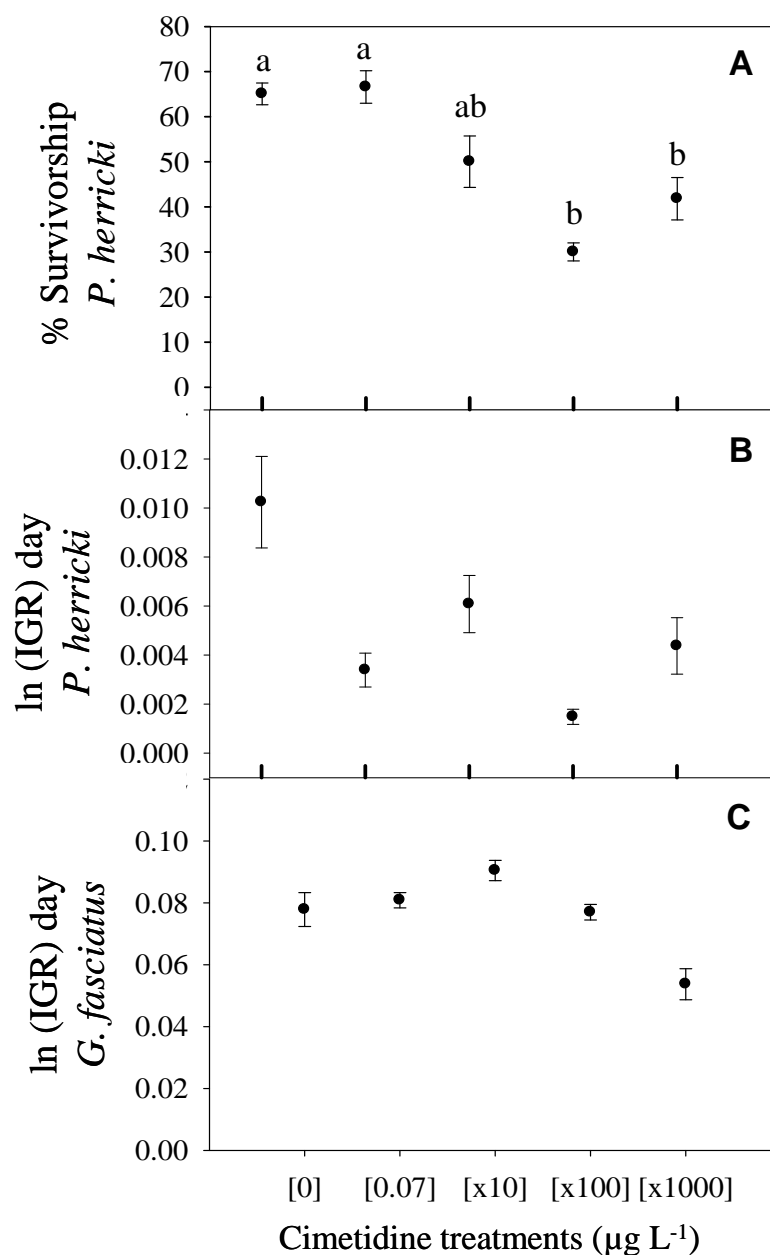


Figure 16. Mean and standard error of A) percent survivorship of *P. herricki* following 79 days of exposure to cimetidine treatments, B) instantaneous growth rates of *P. herricki* following a 28 days of exposure to cimetidine treatments and C) instantaneous growth rates of *G. fasciatus* following a 28 days of exposure to cimetidine treatments. Significant differences in panel A) are represented by lower case letters ($p \leq 0.05$).

Discussion

To document the long-term effects of a novel compound, the antihistamine cimetidine, on stream ecosystem structure and function I evaluated a range of integrated response variables, including the effects on algae, invertebrates and indirect effects of invertebrates on basal resources. My experimental approach employed measurable endpoints at both the individual and population level in aquatic invertebrates. Cimetidine sorbs to organic matter (Anderson et al. 2004, Hoppe et al. unpublished) such as leaves and algae that are consumed by invertebrates. My results support the hypothesis that invertebrates consuming food resources exposed to cimetidine have decreased growth.

Basal resources response to cimetidine - streams without invertebrates

Little is known about how multiple contaminants; both known and novel, associated with urban and suburban areas affect stream ecosystems (see Cleuvers 2003, Meyer et al. 2005). PPCPs represent a class of contaminants entering urban and suburban streams that may have effects across many trophic levels. Nilsen et al. (2007) found that the antihistamine diphenhydramine (Benadryl[®]) was the most frequently detected PPCP in sediment samples from the Lower Columbia River. In addition, a pilot study by the EPA found that diphenhydramine was one of the most frequently detected PPCP in fish liver and fillet tissue samples from streams throughout the US (www.epa.gov/waterscience/ppcp/studies/fish-tissue.html, Nov. 1, 2008). By measuring a suite of ecological endpoints, my study is the first to demonstrate that antihistamines can affect invertebrate population, growth and production. The combined effects of the numerous biologically active compounds detected in surface waters could be have long-

term effects that may be best addressed through chronic dosing experiments measuring ecosystem functions.

My experimental design allowed me to measure the effects of a novel compound on both consumers and their food resources. Cimetidine treatments had little effect on AFDM and chlorophyll *a*, but cimetidine did affect both primary production and microbial respiration on clay tiles initially at low doses. I found no evidence that cimetidine was utilized as a source of C or N; as increased concentrations did not yield increased rates of algae production, which would be expected if cimetidine was being used as a nutrient and C and N are limited.

There was an increase in biofilm biomass (AFDM) for the lowest dose ($0.07 \mu\text{g L}^{-1}$) compared to the control on days 7, 28, 43, and 78, whereas the other doses remained similar to each other and the control. These increased amounts of OM in the low dose treatments are difficult to explain because the fluctuating trend is similar to the other treatments, except for day 78. If cimetidine can be used as a source of carbon or nitrogen, then these data show that this occurs only at low concentrations. This seems unlikely because all other concentrations showed little response to increased concentrations of cimetidine and there were no statistical differences among treatments.

Compared to other biofilm measurements, chl *a* was most variable among replicate streams, and least variable among treatments. Primary production was a more sensitive indicator of cimetidine effects. Primary production was significantly lower in response to cimetidine from days 7 – 28, but by days 32 to 78 these streams were similar to the control. However, across cimetidine concentrations the results varied: the highest concentration ($\times 1000$) had greater rates of primary production on days 22, 36 and 36, and

the medium concentration (x10) and high concentration (x100) were similar to the control. A similar trend was observed with microbial respiration, which could have increased if cimetidine was used as a source of carbon or nitrogen. Other pharmaceutical compounds may also affect stream biofilms by enhancing eutrophication, rather than toxicity. Further exploration into the biotic and abiotic degradation pathways are required to differentiate the potential for toxic or fertilizing effects of PPCPs on stream biofilms.

To my knowledge, no other studies have examined the chronic effects of a histamine antagonist on stream basal resources. My study indicates the importance of measuring both structural biofilm attributes, AFDM and chl *a*, and response variables related to ecosystem function, including primary production and microbial respiration. Using a chronic dosing regimen coupled with long-term measurements of microbial respiration and primary production could help researchers better understand the magnitude of responses to a novel compound. If measurements were based only on day 22, results would indicate a strong dose response; however, by continuing the experiment beyond this point no significant differences among cimetidine concentrations and the control were observed. These findings suggest that cimetidine may have effects on algae in streams, but they may not have long-term detectable consequences in the field.

Indirect effects on basal resources –streams with invertebrates

Invertebrates did not have an observable effect on stream biofilm structure and function in control streams (i.e. no cimetidine). In addition, cimetidine had no affect on the degree to which invertebrates influenced AFDM, chlorophyll *a*, primary production and microbial respiration, but there were differences over time. However, the responses

were once again moderated after day 36, with greatest increases in basal resource response observed on day 22 with the lowest dose ($0.07 \mu\text{g L}^{-1}$) and the high dose (x100). These changes in responses over time highlight the importance of examining the effects of PPCPs with chronic dosing experiments to understand changes in long-term responses. The trends for indirect basal resource responses to cimetidine were restricted to microbial respiration, showing the low and high doses having the greatest effects. No other studies have examined the effects of an antihistamine on microbial respiration and my results suggest that these effects may merit further investigation.

Effects on invertebrates

My results demonstrate that low concentrations of a pharmaceutical compound can have effects on non-target organisms such as invertebrates in streams. My results support the hypothesis that invertebrate growth is negatively affected by chronic exposure to cimetidine either by direct exposure in the water column or indirectly, by way of consuming food resources exposed to cimetidine. Specifically, low chronic exposure to cimetidine ($0.07 \mu\text{g L}^{-1}$) reduced the population growth of *G. fasciatus* and the survivorship and growth of *P. herricki*. Cimetidine negatively affected both *G. fasciatus* and *P. herricki*, but the responses differed between these taxa. All cimetidine treatments significantly reduced the numbers of *G. fasciatus* individuals in the smallest (neonates and juveniles) individuals over the 3 month experiment. The lowest dose $0.07 \mu\text{g L}^{-1}$ and highest dose 70.0 (x1000) also significantly reduced the number of individuals in the 9 – 11mm size classes, demonstrating that cimetidine can affect both the early and adult stages of *G. fasciatus*. This further indicates that cimetidine may influence recruitment success, growth and development of these populations. In addition,

cimetidine also reduced *G. fasciatus* population biomass and density across all cimetidine treatments, but not significantly. Therefore, it is likely that *G. fasciatus* exposed to the lowest and highest cimetidine treatment in this study might have been unable to achieve the same level of fitness or energetic balance as control individuals. In contrast there was no measurable difference in individual growth rates of *G. fasciatus* during the 28 day chamber experiment. This could be explained by less exposure time (compared to 3 months) in the streams.

P. herricki had decreased survivorship in the (x100) and (x1000) treatments and the IGR's were decreased in all cimetidine treatments compared to the control. These declines in growth, survivorship and reproduction suggest that cimetidine may be affecting stomatogastric function as has been observed in other studies (Claiborne and Selverston 1984, Hardie 1988, Hashemzadeh-Gargari and Freschi 1992, Wachowiak and Cohen 1999, Cattaert et al. 2002, Wachowiak et al. 2002, Christie et al. 2004). As a regulator of the stomatogastric nervous system, histamine has been shown to control intestinal motility and feeding in insects (Hartenstein 1997). Measuring growth has commonly been used to provide an indication of the fitness of individuals and populations in response to biotic stressors as it represents a composite of physiological and biological processes (Kiffney and Clements 1996). Together, my results suggests that invertebrate growth rates may be compromised when exposed to cimetidine, although the strength of effect may differ depending on the tolerance of the species. My data suggest that populations of *G. fasciatus* and *P. herricki* exposed to cimetidine were unable to sustain the same level of fitness as control populations.

Overall, my data suggest cimetidine could influence stream ecosystem function in a top-down manner by negatively influencing populations of invertebrate shredders and grazers. Aquatic invertebrates are important to many different processes in riverine ecosystems (e.g. Wallace and Webster, 1996). For example, they have a significant influence on nutrient cycling and the processing of organic material produced in the stream as well as inputs from the riparian zone. Predators, such as fish, depend on macroinvertebrates as a substantial food resource. Antihistamine inputs into rivers could negatively affect invertebrate production and ultimately affect those species that consume invertebrates, as well as alter organic matter processing and nutrient cycling.

Utility of my experimental approach

My experimental design involved an ecotoxicological approach to measure effects of a novel contaminant on the structure and function of both resources (algal and microbial communities) and consumers (invertebrates). Maul et al. (2006) used a similar approach to assess effects of ciprofloxacin on leaf-associated microbial communities and leaf-processing invertebrates (*Gammarus* spp. and *Lepidostoma liba*). They found that Ciprofloxacin (a commonly used antibiotic) influenced microbial communities, but not invertebrates. Studies that examine effects of individual compounds on stream function and structure are important, as they provide data necessary to evaluate chronic exposure of the combination of PPCPs detected in surface waters. Models that incorporate ecosystem structure and function in relation to the transport and fate of PPCPs will aid in the management of pharmaceutical inputs into streams and rivers.

This research has shown that an artificial stream approach is an effective tool for quantifying chronic effects of low concentrations of novel contaminants found in urban

ecosystems. The paired design of streams with and without invertebrates allowed me to conclude that cimetidine did not significantly influence algae and microbial respiration, but it did affect invertebrates. One can easily imagine an alternative finding if the compound of study affected algae, but not invertebrates. Urban areas can have many compounds present (Kolpin et al. 2002) and necessitate developing laboratory techniques that examine the effects of these compounds on stream ecosystem function (Relyea and Hoverman 2006). Also, reproducing populations of *G. fasciatus* allowed me to examine the effects of long-term exposure on population dynamics mimicking natural systems.

Conclusion

Concentrations of cimetidine that have been detected in streams throughout the US ($0.07 \mu\text{g L}^{-1}$, Kolpin et al. 2002) can reduce population growth of *G. fasciatus* and reduce survivorship and growth of *P. herricki*; however, cimetidine had no detectable long-term effects on basal resources. Approximately 20,000 kg of cimetidine enters US streams per year (Anderson et al. 2004) and my research demonstrates that cimetidine may negatively affect invertebrates. Cimetidine is just one PPCP out of the 95 PPCPs detected by Kolpin et al. (2002): other PPCPs detected include antibiotics, steroids and hormones, analgesics (such as ibuprofen), stimulants, and anti-depressants. The potential effects of these compounds, both individually and in combination, on aquatic ecosystems is staggering. The diverse modes of action of this variety of compounds in combination with the diversity of organisms present in streams suggest the effects of PPCPs on streams could be widespread and research will require a substantial effort to understand the ecological implications. The detection of PPCP's in urban streams, as well as in drinking water has recently received widespread media attention. Because these

compounds are designed to have biological effects, it is critically important for aquatic scientists to understand how these drugs affect aquatic organisms. To effectively measure the effects of chronic exposure, mechanistic experiments and models are needed to address the transport, fate, and ecotoxicological effects of these compounds on our nation's rivers and streams.

APPENDIX A

CIMETIDINE LOSS FROM WATER COLUMN DATA

Values for cimetidine standard curve. Data points are a linear fit with the polynomial equation: $y = -1332.4642 + 113.2399 * x$.

Values for cimetidine standard curve					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.9966	0.9931	0.9914	3.4945		
	Coefficient	Std. Error	t	P	VIF
y0	-1332.4642	56.7346	-23.4859	<0.0001	1581.5376<
a	113.2399	4.7047	24.0693	<0.0001	1581.5376<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	2	13477.154	6738.5771		
Residual	4	48.8458	12.2114		
Total	6	13526	2254.3333		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	1	7074.4876	7074.4876	579.3325	<0.0001
Residual	4	48.8458	12.2114		
Total	5	7123.3333	1424.6667		

Concentrations of cimetidine ($\mu\text{g L}^{-1}$) in treatments: 1) no sediment, no organic matter (OM), 2) sediment and no organic matter, 3) organic matter with microbial communities in the light, over a 37 hour time period in artificial streams.

TREATMENTS	1 hour	4 hours	8 hours	12 hours	18 hours	24 hours	37 hours
no sediment, no OM	64.040	59.678	56.500	59.210	61.218	62.165	58.370
standard dev.	0.919	4.217	8.975	5.509	4.626	6.345	5.740
standard error	0.460	2.109	4.487	2.755	2.313	3.173	2.870
sediment with no OM (mean)	65.723	58.610	60.105	56.980	63.248	59.443	54.350
standard dev.	0.840	6.078	7.207	8.146	4.650	0.897	5.460
standard error	0.420	3.039	3.603	4.073	2.325	0.449	2.730
OM (mean)	58.883	51.168	43.098	35.138	19.718	8.798	0.000
standard dev.	0.734	0.349	0.337	1.554	0.322	1.162	0.000
standard error	0.367	0.175	0.168	0.777	0.161	0.581	0.000

Values for cimetidine rates of loss in artificial streams. All data are cubic fit with the polynomial equation: $f = y^0 + a * x + b * x^2 + c * x^3$.

No sediment, no OM					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.9209	0.8480	0.6960	1.3862		
	Coefficient	Std. Error	t	P	VIF
y0	64.9841	1.5556	41.7747	<0.0001	8.8152<
a	-1.6505	0.4268	-3.8667	0.0306	236.4699<
b	0.1130	0.0284	3.9732	0.0285	983.1355<
c	-0.0020	0.0005	-3.9838	0.0283	359.6047<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	4.0000	25373.9615	6343.4904		
Residual	3.0000	5.7647	1.9216		
Total	7.0000	25379.7261	3625.6752		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	3.0000	32.1626	10.7209	5.5793	0.0959
Residual	3.0000	5.7647	1.9216		
Total	6.0000	37.9272	6.3212		
Sediment, no OM					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.8279	0.6854	0.3708	3.0074		
	Coefficient	Std. Error	t	P	VIF
y0	65.7266	3.3749	19.4753	0.0003	8.8152<
a	-1.5356	0.9260	-1.6583	0.1958	236.4698<
b	0.1002	0.0617	1.6243	0.2028	983.1353<
c	-0.0018	0.0011	-1.6830	0.1910	359.6046<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	4.0000	25074.3569	6268.5892		
Residual	3.0000	27.1332	9.0444		
Total	7.0000	25101.4901	3585.9272		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	3.0000	59.1170	19.7057	2.1788	0.2695
Residual	3.0000	27.1332	9.0444		
Total	6.0000	86.2502	14.3750		

Concentrations of cimetidine ($\mu\text{g L}^{-1}$) in treatments: 1) no organic matter in the dark, 2) no organic matter in the light, 3) organic matter in the light, 4) organic matter with microbial communities in the light.

TREATMENTS (n=3)	Concentration ($\mu\text{g L}^{-1}$) at 2.5 minutes	5 min.	10 min.	20 min.	40 min.	60 min.	120 min.	180 min.	240 min.	300 min.	360 min.	440 min.	480 min.	1200 min.
no organic matter in the dark (mean)	67.59	66.58	65.93	65.30	66.27	65.15	65.51	58.70	63.14	63.39	60.49	60.49	60.64	56.83
standard dev.	0.67	0.87	1.25	1.22	1.24	1.95	1.32	10.76	0.98	0.43	2.60	4.40	3.27	1.60
standard error	0.39	0.50	0.72	0.70	0.72	1.12	0.76	6.21	0.57	0.25	1.50	2.54	1.89	0.92
no organic matter in light (mean)	65.96	64.35	61.91	58.62	57.86	57.43	57.29	55.42	55.13	53.50	54.08	51.66	54.48	53.20
standard dev.	1.17	2.12	1.03	1.06	2.18	1.35	2.77	0.66	1.66	4.93	2.49	1.11	1.14	2.11
standard error	0.68	1.22	0.60	0.61	1.26	0.78	1.60	0.38	0.96	2.85	1.44	0.64	0.66	1.22
organic matter in light (mean)	64.71	62.01	58.13	54.23	48.55	45.14	41.88	36.40	28.75	24.39	17.63	16.58	13.69	3.23
standard dev.	0.77	1.35	0.76	0.45	1.59	3.34	2.93	2.74	5.32	3.64	6.30	1.40	0.99	2.85
standard error	0.44	0.78	0.44	0.26	0.92	1.93	1.69	1.58	3.07	2.10	3.64	0.81	0.57	1.65
organic matter with microbial community in light (mean)	63.72	63.41	60.71	57.80	54.67	52.16	45.85	42.40	38.89	29.51	31.12	26.75	22.24	9.49
standard dev.	0.72	0.92	0.59	1.17	1.00	0.40	3.93	4.29	4.22	13.13	4.21	5.24	4.13	8.26
standard error	0.41	0.53	0.34	0.68	0.58	0.23	2.27	2.48	2.44	7.58	2.43	3.03	2.38	4.77

Values for cimetidine rates of loss from the water column with no organic matter in microcosms. All data are cubic fit with the polynomial equation: $f = y^0 + a * x + b * x^2$.

No organic matter (dark)					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.8993	0.8088	0.7514	1.6548		
	Coefficient	Std. Error	t	P	VIF
y0	6.6785E+01	8.1580E-01	8.1865E+01	<0.0001	3.4025E+00
a	-3.0800E-02	1.3100E-02	-2.3570E+00	0.0402	136.5385<
b	5.0690E-05	3.9601E-05	1.2800E+00	0.2294	1256.2815<
c	-2.6638E-08	2.4591E-08	-1.0833E+00	0.3041	664.3765<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	4.0000E+00	5.6189E+04	1.4047E+04		
Residual	1.0000E+01	2.7384E+01	2.7383E+00		
Total	1.4000E+01	5.6216E+04	4.0155E+03		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	3.0000E+00	1.1584E+02	3.8613E+01	14.1009	0.0006
Residual	1.0000E+01	2.7384E+01	2.7383E+00		
Total	1.3000E+01	1.4322E+02	1.1017E+01		
No organic matter (light)					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.9105	0.8291	0.7778	2.0156		
	Coefficient	Std. Error	t	P	VIF
y0	6.2634E+01	9.9370E-01	6.3033E+01	<0.0001	3.4025E+00
a	-6.2000E-02	1.5900E-02	-3.9001E+00	0.0030	136.5386<
b	1.0000E-04	4.8235E-05	2.5368E+00	0.0295	1256.2815<
c	-6.4374E-08	2.9952E-08	-2.1492E+00	0.0571	664.3765<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	4.0000E+00	4.6015E+04	1.1504E+04		
Residual	1.0000E+01	4.0626E+01	4.0626E+00		
Total	1.4000E+01	4.6056E+04	3.2897E+03		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	3.0000E+00	1.9703E+02	6.5676E+01	16.1660	0.0004
Residual	1.0000E+01	4.0626E+01	4.0626E+00		
Total	1.3000E+01	2.3766E+02	1.8281E+01		

Values for cimetidine rates of loss from the water column with organic matter in microcosms. All data are cubic fit with the polynomial equation: $f = y^0 + a * x + b * x^2$.

Organic matter (light)					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.9906	0.9812	0.9756	3.0821		
	Coefficient	Std. Error	t	P	VIF
y0	6.0110E+01	1.5194E+00	3.9560E+01	<0.0001	3.4025E+00
a	-1.8550E-01	2.4300E-02	-7.6303E+00	<0.0001	136.5386<
b	2.0000E-04	7.3758E-05	3.2261E+00	0.0091	1256.2816<
c	-1.0238E-07	4.5801E-08	-2.2353E+00	0.0494	664.3766<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	4.0000E+00	2.3937E+04	5.9843E+03		
Residual	1.0000E+01	9.4995E+01	9.4994E+00		
Total	1.4000E+01	2.4032E+04	1.7166E+03		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	3.0000E+00	4.9681E+03	1.6560E+03	174.3297	<0.0001
Residual	1.0000E+01	9.4995E+01	9.4994E+00		
Total	1.3000E+01	5.0631E+03	3.8947E+02		
Organic matter with microbial communities (light)					
R	Rsqr	Adj Rsqr	Standard Error of Estimate		
0.9941	0.9881	0.9846	2.1126		
	Coefficient	Std. Error	t	P	VIF
y0	6.2025E+01	1.0415E+00	5.9554E+01	<0.0001	3.4025E+00
a	-1.4320E-01	1.6700E-02	-8.5917E+00	<0.0001	136.5386<
b	2.0000E-04	5.0557E-05	3.3323E+00	0.0076	1256.2817<
c	-7.1368E-08	3.1394E-08	-2.2733E+00	0.0463	664.3766<
Analysis of Variance:					
Uncorrected for the mean of the observations:					
	DF	SS	MS		
Regression	4.0000E+00	2.9325E+04	7.3312E+03		
Residual	1.0000E+01	4.4632E+01	4.4631E+00		
Total	1.4000E+01	2.9369E+04	2.0978E+03		
Corrected for the mean of the observations:					
	DF	SS	MS	F	P
Regression	3.0000E+00	3.7191E+03	1.2397E+03	277.7653	<0.0001
Residual	1.0000E+01	4.4632E+01	4.4631E+00		
Total	1.3000E+01	3.7638E+03	2.8952E+02		

REFERENCES

- Abel, R., D. Allan, and B. Lehner. 2007. Unlocking the potential of protected areas for freshwaters. *Biological Conservation* 134:48-63.
- APHA, AWWA, and WEF. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, D.C.
- Anderson, P. D., V. J. D'Aco, P. Shanahan, S. C. Chapra, M. E. Buzby, V. L. Cunningham, B. M. Duplessie, E. P. Hayes, F. J. Matrocco, N. J. Parke, J. C. Rader, J. H. Samuelian, and B. W. Schwab. 2004. Screening analysis of human pharmaceutical compounds in U.S. surface waters. *Environmental Science and Technology* 38:838-849.
- Barnes, K.K., D.W. Kolpin, M.T. Meyer, E.M. Thurman, E.T. Furlong, S.D. Zaugg, L.B. Barber. 2002. Water-quality data for pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000 Open-File Report 02-94.
- Benfield, E.F. 2006. Decomposition of leaf material. *Methods in Stream Ecology*, 2nd edn. (eds F.R. Hauer and G.A. Lamberti) pp. 711-720. Elsevier, Amsterdam.
- Benke, A. C., and A. D. Huryn, L. A. Smock and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to southeastern United States. *Journal of the North American Benthological Society* 18:308-343.
- Blockwell, S. J., S. J. Maund, and D. Pascoe. 1999. Effects of the organochlorine insecticide lindane (γ -C₆H₆Cl₆) on the population responses of the freshwater amphipod *Hyalella azteca*. *Environmental Toxicology and Chemistry* 18:1264-1269.
- Brooks, B.W., C.M. Foran, S.M. Richards, J. Weston, P.K. Turner, J.K. Stanley, K.R. Solomon, M. Slattery and T.W. La Point. 2003. Aquatic toxicology of Fluoxetine. *Toxicology Letters* 142:169-183

- Brooks, S.S., M.A. Palmer, B.J. Cardinale, C.M. Swan, and S. Ribblett. 2002. Assessing stream ecosystem rehabilitation: limitations of community structure data. *Restoration Ecology* 10:156–168.
- Buchner, E., S. Buchner, M. Burg, A. Hofbauer, W. Pak and I. Pollack. 1993. Histamine is a major mechanosensory neurotransmitter candidate in *Drosophila melanogaster*. *Cell and Tissue Research* 273:119-125.
- Buth, J.M., W. Arnold and K. McNeill. 2003. Unexpected products and reaction mechanisms of the aqueous chlorination of cimetidine. *Environmental Science and Technology* 41:6228-6232.
- Cahill, J. D., E. T. Furlong, M. R. Burkhardt, D. W. Kolpin, and L. G. Anderson. 2004. Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography A*:171-180.
- Calabrese, E. J. and L. A. Baldwin. 2003. Toxicology rethinks its central belief. *Nature* 421:691-692.
- Carpenter K.E. 1924. A study of the fauna of rivers polluted by lead mining in the Aberystwyth District of Cardiganshire. *Annals of Applied Biology* 11:1–23.
- Cattaert, D., M. Le Bon, and D. Le Ray. 2002. Efferent controls in crustacean mechanoreceptors. *Microscopy Research and Technique* 58(4):312-324.
- Chadwick J.W., S.P. Canton, R.L. Dent. 1986. Recovery of benthic invertebrate communities in Silver Bow Creek, Montana, following improved metal mine wastewater treatment. *Water Air Soil Pollution* 28:427–438.
- Christie, A. E., Stein, W., Quinlan, J. E., Beenhakker, M. P., Marder, E. and M. Nusbaum. 2004. Actions of a histamine/peptidergic projection neuron on rhythmic motor patterns in the stomatogastric nervous system of the crab *Cancer borealis*. *Journal of Comparative Neurology* 469:153-169.
- Claiborne, B. J. and A. I. Selverston. 1984. Histamine as a neurotransmitter in the stomatogastric nervous system of the spiny lobster. *Journal of Neuroscience* 4:708-721.
- Clements W.H. 1994. Benthic community responses to heavy metals in the Upper Arkansas River Basin, Colorado. *Journal of the North American Benthological Society* 13:30–44.

- Cleuvers, M. 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicology Letters* 141:185-194.
- Costanzo, S. D., J. Murby, and J. Bates. 2005. Ecosystem response to antibiotics entering the aquatic environment. *Marine Pollution Bulletin* 51:218-223.
- Cunningham, V. L., M. E. Buzby, T. Hutchinson, F. Mastrocco, N. J. Parke, and N. Roden. 2006. Effects of human pharmaceuticals on aquatic life: Next steps. *Environmental Science and Technology* 40:3456-3462.
- Daughton, C. G. 2003. Pollution and the Combined Activities, Actions, and Behaviors of the Public: Pharmaceuticals and Personal Care Products. *NorCal Society of Environmental Toxicology and Chemistry News* 14:5-15.
- Daughton, C.G. and T.A. Ternes. 1999. Pharmaceutical and personal care products in the environment: Agents of subtle change? *Environmental Health Perspectives* 107 (Supplement 6):907-938.
- De Lange, H. J., W. Noordoven, A. J. Murk,, M. Lurling and E. T. H. M. Peeters. 2006. Behavioral responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. *Aquatic Toxicology* 78:209-216.
- Delong, M. D., R. B. Summers, and J. H. Thorp. 1993. Influence of food type on the growth of a riverine amphipod, *Gammarus fasciatus*. *Canadian Journal of Fisheries and Aquatic Sciences* 50(9):1891-1896.
- DeNicola, D.M. and M.G. Stapleton. 2002. Impact of acid mine drainage on benthic communities in streams: the relative role of substratum vs. aqueous effects. *Environmental Pollution* 119:303-315.
- Dennehy, K.F., D.W. Litke, C.M. Tate, S.L. Qi, P.B. McMahon, B.W. Bruce, R.A. Kimbrough and J.S. Heiny. 1998. Water Quality in the South Platte River Basin, Colorado, Nebraska, and Wyoming, 1992-95: U.S. Geological Survey Circular 1167, on line at: <http://water.usgs.gov/pubs/circ1167>.
- Fenske M, G. Maack, C. Schafers and H. Segner. 2005. An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, *Danio rerio*. *Environmental Toxicology and Chemistry* 24:1088–1098.
- Fent, K., A. Weston, and D. Caminada. 2006. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* 76:122-159.

- Ferrari, B., N. Paxeus, R. Lo Giudice, A. Pollio, and J. Garric. 2003. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibrac acid, and diclofenac. *Ecotoxicology and Environmental Safety*. 55:359-370.
- Flaherty, C.M. and S.I. Dodson. 2005. Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction. *Chemosphere* 61:200-207.
- Grimm, N. B., M. J. Grove, S. T. A. Pickett and C. L. Redman. 2000. Integrated approaches to long-term studies of urban ecological systems. *BioScience* 50:571-584.
- Gross, B., J. Montgomery-Brown, A. Naumann and M. Reinhard. 2004. Occurrence and fate of pharmaceuticals and alkylphenol ethoxylate metabolites in an effluent dominated river and wetland. *Environmental Toxicology and Chemistry* 23:2074-2083.
- Halling-Sørensen B, S. N. Nielsen, P. F. Lanzky, F. Ingerslev, H. C. H. Holten-Lutzhof and S. E. Jorgensen. 1998. Occurrence, fate, and effects of pharmaceutical substances in the environment - a review. *Chemosphere* 36:357-393.
- Hardie, R. C. 1988. Effects of antagonist on putative histamine receptors in the first visual neurophile of the housefly (*Musca domestica*). *Journal of Experimental Biology* 138:221-241.
- Hartenstein, V. 1997. Development of the insect stomatogastric nervous system. *Trends in Neuroscience* 20:421-427.
- Hashemzadeh-Gargari, H. and J. E. Freschi. 1992. Histamine activates chloride conductance in motor neurons of the lobster cardiac ganglion. *Journal of Neurophysiology* 68:9-15.
- Henschel, K.-P., A. Wenzel, M. Diedrich, and A. Fliedner. 1997. Environmental hazard assessment of pharmaceuticals. *Regulatory Toxicology and Pharmacology* 25:220-225.
- Hill, B. H., A. T. Herlihy and P. R. Kaufmann. 2002. Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern U.S.A. *Freshwater Biology* 47:185-194.
- Huryn, A. D., and J. Bruce Wallace. 1986. A method for obtaining in situ growth rates of larval chironomidae (Diptera) and its application to studies of secondary production. *Limnology and Oceanography* 31:216-222.

- Isidori M, A. Nardelli, L. Pascarella, M. Rubino and A. Parrella. 2007. Toxic and genotoxic impact of fibrates and their photoproducts on non-target organisms. *Environmental International* 33:635–641.
- Jenkins, M. 2003. Prospects for Biodiversity. *Science* 302:1175-1177.
- Kiffney, P.M. and W.H. Clements. 1996. Effects of metals on stream macroinvertebrate assemblages from different altitudes. *Ecological Applications* 6:472–481.
- Kim, Y., K. Choi, J. Jung, S. Park, P.-G. Kim and J. Park. 2007. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environmental International* 33:370-375.
- Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A National Reconnaissance. *Environmental Science and Technology* 36:1202-1211.
- Kosonen J. and L. Kronberg. 2009. The occurrence of antihistamines in sewage and in recipient rivers. *Environmental Science and Pollution Research International* 16:555-564.
- Lamberti, G.A. and A.D. Steinman, Research on disturbance in artificial streams. 1993. Pages 347-350 in G.A. Lamberti and A.D. Steinman (editors). *Research in artificial streams: applications, uses, and abuses*. *Journal of the North American Benthological Society* 12: 313-384.
- Länge, R., T.H. Hutchinson, C.P. Croudace, F. Siegmund, H. Schweinfurth, P. Hampe, G.H. Panter, J.P. Sumpter. 2001. Effects of the synthetic estrogen 17 alpha ethinylestradiol on the lifecycle of the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* 20:1216–1227.
- Latch, D. E., B. L. Stender, J. L. Packer, W. A. Arnold, and K. Mcneil. 2003. Photochemical Fate of Pharmaceuticals in the Environment: Cimetidine and Ranitidine. *Environmental Science and Technology* 37:3342-3350.
- Lorenzo, B, and D.E. Drayer. 1981. Improved method for the measurement of cimetidine in human serum by reverse-phase high-pressure liquid chromatography. *Journal of Laboratory and Clinical Medicine*. 97:545-550.
- Maul, J. D., L. J. Schuler, J. B. Belden, M. R. Whiles, and M. J. Lydy. 2006. Effects of the antibiotic ciprofloxacin on stream microbial communities and detritivorous macroinvertebrates. *Environmental Toxicology and Chemistry* 25:216-224.

- Merritt, R. W., K. W. Cummins and M.B. Berg (Editors). 2008. An introduction to the aquatic insects of North America. 4th ed. Kendall/Hunt, Dubuque, Iowa.
- Meyer, J. L., M. J. Paul and W. K. Taulbee. 2005. Stream ecosystem function in urbanizing landscapes. *Journal of the North American Benthological Society* 24:602-612.
- Nash J.P., D.E. Kime, L.T. Van der Ven, P.W. Wester, F. Brion, G. Maack, P. Stahischmidt-Allner and C.R. Tyler. 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethinylestradiol causes reproductive failure in fish. *Environmental Health Perspectives* 112:1725–1733.
- Nentwig, G. 2007. Effects of pharmaceuticals on aquatic invertebrates. Part II: The antidepressant drug fluoxetine. *Archives of Environmental Contamination and Toxicology* 52:163-170.
- Nilsen, E. B., R. R. Rosenbauer, E. T. Furlong, M. R. Burkhardt, S. L. Werner, L. Greaser, M. Noriega. 2007. Pharmaceuticals, Personal Care Products and Anthropogenic Waste Indicators Detected in Streambed Sediments of the Lower Columbia River and Selected Tributaries. In 6th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, National Ground Water Association, Costa Mesa, CA; Paper 4483, p 15.
- Norton, S.B., D.J. Rodier, J.H. Gentile, W.H. Van der Shalie, W.P. Wood and M.W. Slimak. 1992. A framework for ecological risk assessment at the EPA. *Environmental Toxicology and Chemistry* 11:1663–1672.
- Oaks J.L, M. Gilbert, M.Z. Virani, R.T. Watson, C.U. Meteyer, B. Rideout, H.L. Shivaprasad, S. Ahmed, M.J.I. Chaudhry, M. Arhad, S. Mahmood, A. Ali and A.A. Kahn. 2004. Diclofenac residues as a cause of population decline of white-backed vultures in Pakistan. *Nature* 427:630–633.
- Paul, M.J. 1999. Stream ecosystem function along a land use gradient. PhD Thesis, University of Georgia, Athens, GA.
- Paul, M. J. and J. L. Meyer. 2001. Streams in the urban landscape. *Annual Review of Ecology and Systematics*. 32:333-365.
- Peckarsky B.L. and K.Z. Cook. 1981. Effect of Keystone mine effluent on colonization of stream benthos. *Environmental Entomology* 10:864–871.
- Pimentel, D., J. Houser, E. Preiss, O. White, H. Fang, L. Mesnick, T. Barsky, S. Tariche, J. Schreck, and S. Alpert. 1996. Water resources: agriculture, the environment, and society. *BioScience* 47(2): 97-106.

- Relyea, R. and J. Hoverman. 2006. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. *Ecology Letters* 9:1157-1171.
- Revenge, C., R. Campbell, R. Abell, P. de Villiers, and M. Bryer. 2005. Prospects for monitoring freshwater ecosystems towards the 2010 targets. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360(1454): 397–413.
- Ricciardi, A. and J.B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. *Conservation Biology* 13: 1220-1222.
- Richardson, J.S and P.M. Kiffney. 2000. Responses of a macroinvertebrate community from a pristine, southern British Columbia, Canada, stream to metals in experimental mesocosms. *Environmental Toxicology and Chemistry* 19:736-743.
- Robinson, A.A., J.B. Belden and M.J. Lydy. 2005. Toxicity of fluoroquinolone antibiotics to aquatic organisms. *Environmental Toxicology and Chemistry* 24:423-430.
- Roline R.A. 1988. The effects of heavy metal pollution of the upper Arkansas River on the distribution of aquatic macroinvertebrates. *Hydrobiologia* 160:3-8.
- Rosenberg D.M. and V.H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In Rosenberg DM, Resh VH, eds, *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman & Hall, London, UK, pp 1-9.
- Rosi-Marshall, E.J. 2004. Quality of suspended fine particulate matter along an urban river. *Freshwater Biology* 49:515-525.
- Schwaiger, J., H. Ferling, U. Mallow, H. Wintermayr, and R.D. Negele. 2004. Toxic effects of the non-steroidal ant-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquatic Toxicology* 68:141-150.
- Sedlak, D.L., and K.E. Pinkston. 2001. Factors Affecting the Concentrations of Pharmaceuticals Released to the Aquatic Environment. Proceedings of the National Groundwater Association, 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, October 9-11, 2001, National Groundwater Association. Minneapolis, Minnesota and Westerville, Ohio.
- Simon, K. S., C. R. Townsend, B. J. F. Biggs and B. W. Bowden. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. *Journal of the North American Benthological Society* 25(1):1-18.

- Stackelberg, P. E., E. T. Furlong, M. T. Meyer, S. D. Zaugg, A. K. Henderson, and D. B. Reissman. 2004. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of the Total Environment* 329:99-113.
- Stern, A.M. and C.R. Walker. 1978. "Hazard Assessment of Toxic Substances: Environmental Fate Testing of Organic Chemicals and Ecological Effects Testing." Estimating the Hazard of Chemical Substances to Aquatic Life, ASTM STP 657, John Cairns, Jr., K.L. Dickenson, and A.W. Maki, Eds. American Society for Testing and Materials, pp. 81-131.
- Taylor, D.I. 2002. Eutrophication of the lower Charles, Mystic and Neponset rivers, and of Boston Harbor: a statistical comparison. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2002-20. p. 58.
- Ternes, T.A. 1998. Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32:3245-3260.
- Truhaut, R. 1975. Ecotoxicology - A new branch of toxicology: A general survey of its aims, methods and prospects. Pages 3-23 in McIntyre, A. D. and C. F. Mills (editors). *Ecological toxicology research. Effects of heavy metals and organohalogen compounds*. Plenum, New York, NY, USA.
- Vuori, K. 1995. Direct and indirect effects of iron on river ecosystems. *Annales Zoologici Fennici* 32:317-329.
- Wachowiak, M. and L. Cohen. 1999. Presynaptic inhibition of primary olfactory afferents mediated by different mechanisms in lobster and turtle. *Journal of Neuroscience* 19(20):8808-8817.
- Wachowiak, M., L. B.Cohen, and B. W. Ache. 2002. Presynaptic inhibition of olfactory receptor neurons in crustaceans. *Microscopy Research and Technique* 58:365-375.
- Wallace, J.B, Grubaugh, J.W., and M.R. Whiles. 1996. Biotic indices and stream ecosystem processes: results from an experimental study. *Ecological Applications* 6:140-151.
- Watts, M., M., D. Pascoe, K. Carroll. 2001(a). Chronic exposure to 17 α -ethinylestradiol and bisphenol A –effects on development and reproduction in the freshwater invertebrate *Chironomus riparius* (Diptera: Chironomidae). *Aquatic Toxicology* 55:113-1

- Watts, M. M., D. Pascoe, and K. Carroll. 2001(b). Survival and precopulatory behavior of *Gammarus pulex* (L.) exposed to two xenoestrogens. *Water Research* 35:2347-2352.
- Watts, M. M., D. Pascoe, and K. Carroll. 2002. Population responses of the freshwater amphipod *Gammarus pulex* (L.) to an environmental estrogen, 17 α -ethinylestradiol. *Environmental Toxicology and Chemistry* 21:445-450.
- Wetzel, R. G. and G. E. Likens. 2000. *Limnological Analysis*. 3rd ed. Springer-Verlag, New York, New York.
- Widdows, J. and P. Donkin. 1991. Role of physiological energetics in ecotoxicology. *Comparative Biochemical Physiology* 100:69-75.
- Witte, I., H-J. Kreienkamp, M. Gewecke and T. Roeder. 2002. Putative histamine-gated chloride channel subunits of the insect visual system and thoracic ganglion. *Journal of Neurochemistry* 83:504-514.
- Xia, K., A. Bhandari, K. Das, and G. Pillar. 2005. Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids. *Journal of Environmental Quality* 34:91-104.
- Zar, J. H. 1999. *Biostatistical analysis*. 4th ed. Prentice-Hall, Englewood Cliffs, New Jersey.

VITA

Paul David Hoppe graduated from Winona State University with a Bachelor of Science in 2002 under the advisement of Dr. Michael Delong. His undergraduate work included two years as a student researcher at the Large River Studies Center where he investigated spatial and temporal variability in food quality of transport organic matter in the Upper Mississippi River. He continued at the University of Minnesota in Chemistry and afterwards worked as a Marine Fisheries Technician on the Ocean Salmon Project collecting data for the California Department of Fish and Game and the Pacific States Marine Fisheries Commission. In the fall of 2005 Paul was awarded a Research Assistantship with Dr. Emma Rosi-Marshall and began his work investigating the effects of pharmaceuticals on stream ecosystems. While at Loyola University he was the first to develop and use the artificial stream facility to conduct experiments. During this time he also was the instructor for two Biology classes, served on the M.S. Graduate Committee and mentored a high school student who placed fourth at the International Science Fair. He also conducted research in Ghana for one month collecting data related to the distribution of the mycobacterium *M. ulcerans* in streams and ponds. Currently Paul is living in Seattle with his wife Kara and enjoys spending time in the Cascade and Olympic Mountains.

