

**Exploring how Clean Water Act enforcement influences
stream macroinvertebrate communities**

A Thesis Presented for the
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Dedication

To the Arkansas River, my hometown river and first aquatic ecology study site.

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Abstract

Enforcement is a key feature of the Clean Water Act. The Clean Water Act's National Pollutant Discharge Elimination System (NPDES) permit program regulates effluent into streams that may impact aquatic life. Yet, authorities do not always enforce permits when violations occur. My research examines macroinvertebrate survey data near NPDES permitted facilities in Kentucky and Tennessee to determine the influence of enforcement actions on aquatic biodiversity. Pre-existing data from the Environmental Protection Agency and state agencies was used to make quantitative and spatial comparisons.

My first study used multiple regression to explore whether enforcement and freshwater community indicators changed together. North Carolina Biotic Index (NCBI) and Percent Clingers both significantly covaried with enforcement in fourth and fifth order streams ($p=0.048^{**}$ and $p=0.056^{*}$ respectively). Enforcement and Ephemeroptera-Plecoptera-Trichoptera (EPT) taxon richness also had a significant relationship in an ecoregion subset encompassing the Mississippi Valley Loess Plains, Interior River Valleys & Hills, and Southeastern Plains ($p=0.079^{*}$). Taxon Richness covaried with enforcement in eastern ecoregions of my study area ($p = 0.095^{*}$).

My second study used linear regression and a differencing design to see whether time and/or distance influenced the observed covariance relationship seen in my first study. EPT composition downstream of NPDES sources improved in time since enforcement ($p= 0.056^{*}$) and distance from where the discharges occurred ($p= 0.051^{*}$). NCBI in communities downstream of an NPDES facility also improved as distance from the facility increased ($p= 0.098^{*}$).

My research suggests that National Pollutant Discharge Elimination System enforcement has a positive influence on aquatic macroinvertebrate communities. Yet, some authorities suggest NPDES permit enforcement is decreasing nationwide. Understanding the relationship between biodiversity and enforcement in aquatic habitats may encourage authorities to reassess enforcement trends regionally and nationally.

Key Terms: Enforcement, Clean Water Act, National Pollutant Discharge Elimination System (NPDES), aquatic macroinvertebrates, biomonitoring, bioassessment

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Chapter 1: Introduction

The effect of environmental law enforcement on biotic communities across spatial scales is understudied. Recent studies have examined the impact of activities regulated by environmental law (Pond *et al.* 2008, Gunn *et al.* 2010, Bernhardt 2012), but the role of enforcement is less understood. Can a biotic improvement in Tennessee and Kentucky streams be detected on a broad spatial scale after enforcement? To address this question, I explored the relationship between National Pollutant Discharge Elimination System (NPDES) enforcement and aquatic macroinvertebrate composition in Tennessee and Kentucky.

My research consists of two studies. Study I determines whether enforcement and freshwater community indicators change together. This study uses a covariance approach and utilizes a multiple regression model to determine whether enforcement and macroinvertebrate biodiversity change together. Study II considers whether freshwater community indicators return to background conditions after enforcement and whether distance from discharge point influences impact of enforcement on freshwater community indicators.

Living waters: Aquatic macro-invertebrates in streams

Freshwater streams and rivers are filled with dynamic living communities. Over 8,600 North American insect species are associated with freshwater environments at some point in their life cycle (Voshell 2009). These aquatic insects, called macroinvertebrates, are large enough to be seen without a microscope (see Figure 1). Aquatic macroinvertebrates are the dominant animals in temperate streams and river systems (Kaplan 2008). Varied in morphology and life history, some live on the rocky substrates of free flowing riffles, while others reside in slow moving pools. Major groups of aquatic insects include Mayflies (Ephemeroptera),



Figure 1. Mayfly (top, left), Stonefly (bottom, left), Caddisfly (bottom, right), and True Fly (top, right).
Photos by Robert G. Henricks (used by permission).

Stoneflies (Plecoptera), Caddisflies (Trichoptera), and True Flies (Diptera) (Hynes 1970). These different groups have varying levels of tolerance to poor water conditions. For example, Diptera groups are usually more tolerant of water pollution, including raw sewage effluent (Voshell 2009). Mayflies, like *Acentrella* and *Stenonema*, need cleaner conditions. Often considered as “canaries in a coal mine” for freshwater systems, researchers use macroinvertebrates for bioassessment to assess water quality (Cairns & Pratt 1993, Gibson *et al.* 1996, KDEP 2002a). Even though these species can tell humans something about water conditions for the sake of human interests (*e.g.* recreation, fishing), they are intrinsically valuable and subject to protection under the Clean Water Act (Karr & Chu 1995, NPS 2008).

Clean Water Act’s purpose of protecting aquatic life

The purpose of the Clean Water Act (CWA) is to restore and maintain the chemical, physical, and biological integrity of waters of the United States (Clean Water Act 2006a). Ecologists have defined “biological integrity” as “[t]he capability of supporting and maintaining a balanced, integrated adaptive community of organisms having a species composition and functional organization comparable to that of natural habitat of the region” (Karr & Dudley 1981). When Congress adopted the CWA in 1972, committees sponsoring the legislation used prevailing ecological concepts of stability and equilibrium when considering “biological integrity” (Adler 2010).

Research demonstrates that some forms of chemical pollution impair biological integrity (Miller *et al.* 1989, Richter *et al.* 1997). Therefore, as the Environmental Protection Agency (EPA) implemented the CWA over several decades, biological integrity was protected by establishing narrative limits highlighting biodiversity and numeric limits on point source

chemical pollution. The EPA regulates this point source pollution through the National Pollutant Discharge Elimination System.

Clean Water Act's National Pollutant Discharge Elimination System

The National Pollutant Discharge Elimination System (NPDES) is one program that helps achieve some Clean Water Act purposes. The NPDES permit program limits pollutant discharges into waters of the United States from point sources to protect biological integrity of surface waters. Subject sources must apply for permits and renew those permits every five years to conduct their activities (CFR 1995). One point source category, which I used in my studies, is major sources. Major sources are facilities with design flows of more than one million gallons/day or facilities that have been designated as major by an EPA Regional Administrator (CFR 1990).

Under the Clean Water Act, major sources with permits must document their compliance with regulatory standards in monthly or quarterly discharge monitoring reports (Clean Water Act 2006c). Because deliberate falsification can be criminally prosecuted, people using monitoring reports for research purposes tend to consider these self-reports truthful (Gray *et. al.* 2011). States store compliance data and report that data to the EPA. Even so, the EPA's database of compliance and enforcement has gaps. If a source violates its NPDES permit, the state authority may initiate enforcement. A state's decision to initiate a Clean Water Act enforcement action is contingent on a broad range of social, economic and political considerations (Stover 2013), and in the past decade, a decrease in enforcement can be seen nationally.

Downward Trends in Environmental Law and NPDES Enforcement Nationally

The Clean Water Act, as well as other pollution control laws, is not self-executing. Enforcement is a key feature to ensure that the aims of the law are actually pursued. However,

there is a trend toward less enforcement of environmental law nationally. In the 1990s, civil enforcements declined substantially and environmental agencies (national and state) were called to justify their compliance schemes (Gray *et. al.* 2011). As seen in Figure 2, the EPA experienced a steady decrease in opening and closing enforcement actions every year since 2009.¹ In 2013, the EPA initiated 2,418 civil judicial and administrative cases to enforce environmental laws (US EPA 2013). The EPA also concluded 2,489 civil judicial and administrative cases in 2013 (US EPA 2013). Federal criminal enforcement cases have decreased since 2009 as well (US EPA 2013).

This trend is also consistent with National Pollutant Discharge Elimination System violation and enforcement. When a facility violates its NPDES permit, a state permitting authority may respond with informal or formal enforcement. Informal enforcement can be a warning letter or notice of violation. Formal enforcement is more intensive. It can be an administrative or judicial proceeding that can result in civil penalties. Because formal enforcement is more intensive, it requires more administrative resources. So, there are usually more informal enforcement actions than formal ones in a given jurisdiction. The EPA and state enforcement agencies summarize enforcement data from NPDES sources in summary administrative reports and the Environmental Compliance and History Online (ECHO) database.

In the past five years, fewer NPDES formal enforcement actions have been concluded.² In 2009, 2,628 formal enforcement actions were closed. The number of cases increased to 2,824 in 2010, but steadily declined to 1,959 in 2014 (see Figure 3). Further, according to 2012 data,

¹ States are normally responsible for environmental law enforcement, so these particular EPA figures pertain to federally enforced cases under special circumstances.

² As of November 2014, ECHO only provided data on enforcement actions concluded, not enforcement actions initiated each year.

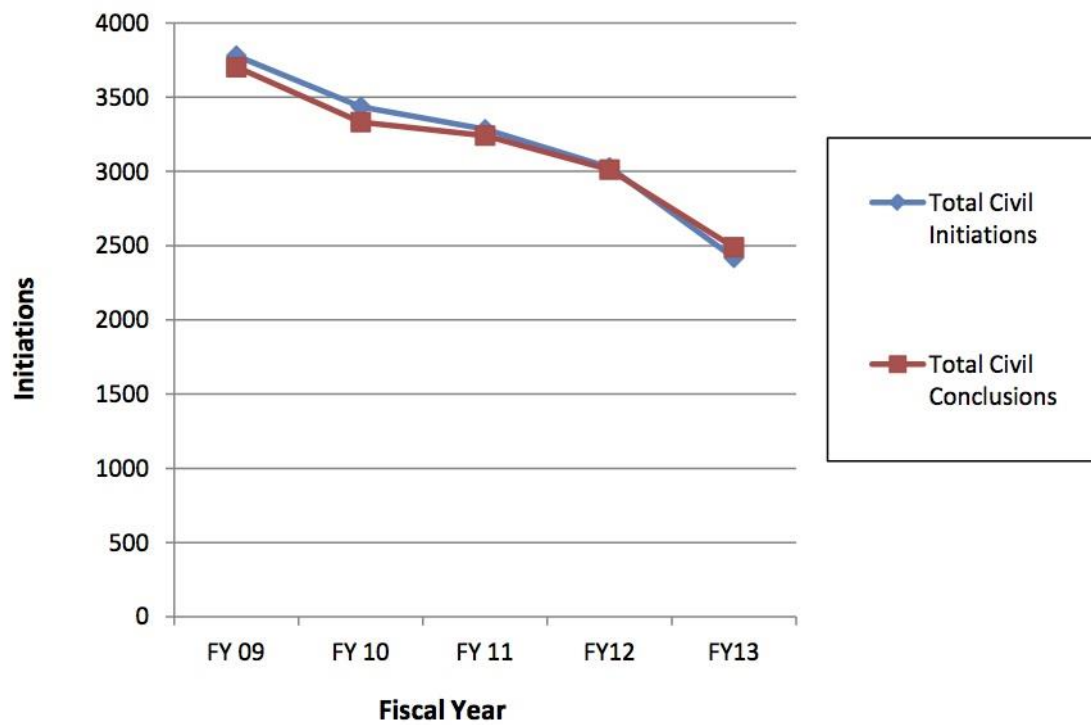


Figure 2. EPA civil enforcement case initiations and conclusions (Fiscal years 2009-2013). From United States EPA. Office of Enforcement and Compliance Assurance. Fiscal Year 2013 EPA Enforcement and Compliance Annual Results. January 13, 2013.

non-major³ NPDES sources had a 24% Category I⁴ violation rate. This means that, of reported sources, nearly a quarter of minor sources were in serious violation of their permits (US EPA 2014a). In the EPA's view, "noncompliance rates are too high and enforcement is too infrequent in the [National Pollutant Discharge Elimination System] program" (US EPA 2014a). Therefore, a decrease in enforcement cannot be explained by an increase in NPDES permit compliance.

Academic research and national data suggest downward enforcement trends, but it is important to consider enforcement at regional and state scales. Macroinvertebrate biodiversity is constrained by local stream habitats, and each state enforces NPDES requirements differently.

Mixed enforcement trends in Tennessee and Kentucky

Recent NPDES enforcement is mixed in Tennessee and Kentucky. In the United States, states are primarily responsible for enforcing NPDES permits (Clean Water Act 2006b). This enforcement model relies on cooperative federalism. The EPA acts on the federal level by providing nationwide standards and support while states issue permits with source-specific limits and are responsible for enforcing these permits (Clean Water Act 2006b). This partnership gives primacy to the states, which must comply with or exceed federal CWA requirements.

Individually, Tennessee and Kentucky have "the primary responsibility to establish... [s]tate NPDES program priorities" that are consistent with national goals and objectives (US

³ Major sources are facilities with design flows capable of more than 1 million gallons/day and facilities that have been designated as major by an EPA Regional Administrator. Minor sources are usually smaller.

⁴ According to the Code of Federal Regulations (CFR), Category I noncompliance includes: "(1) violations of conditions in enforcement orders except compliance schedules and reports; (2) violations of compliance schedule milestones for starting construction, completing construction, and attaining final compliance by 90 days or more from the date of the milestone specified in an enforcement order or a permit; (3) violations of permit effluent limits that exceed the Appendix A 'Criteria for Compliance Reporting in the NPDES Program'; and (4) failure to provide a compliance schedule report for final compliance or a monitoring report."

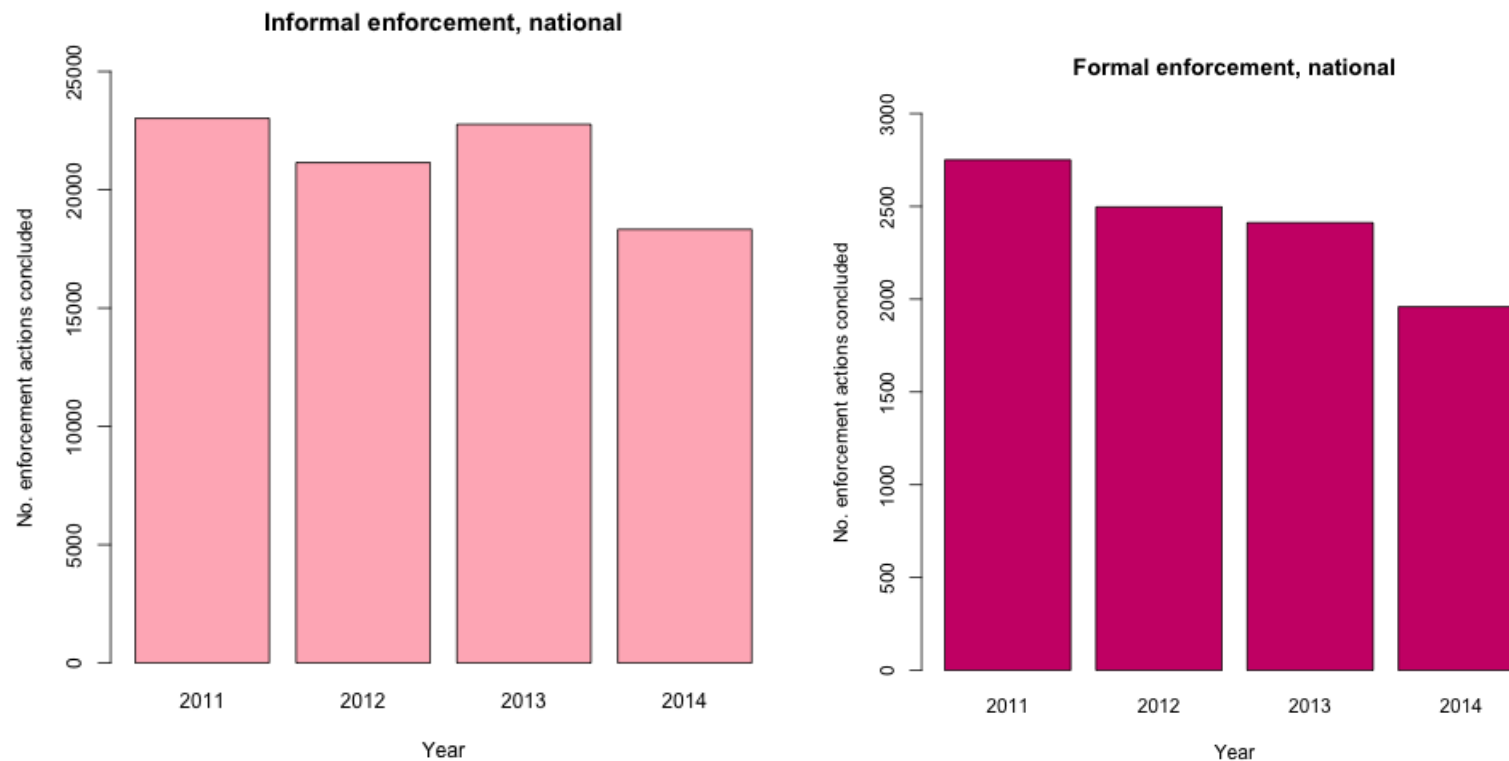


Figure 3 NPDES informal and formal NPDES enforcement nationally for major sources only from 2011 to 2014. State agencies brought these enforcement actions, not the Environmental Protection Agency (ECHO 2014).

EPA Region 4 2007, US EPA Region 4 2008). Further, both states maintain monitoring and evaluation programs, including enforcement protocols.⁵

In Tennessee, the Tennessee Department of Environment and Conservation (TDEC) issues permits and initiates enforcement for NPDES permittees. The Kentucky Department for Environmental Protection (KDEP) fills this role in its state. As of October 20, 2014, 96 major sources with effective NPDES permits have current violations in Tennessee and Kentucky (see Table 1). One hundred sixty-six major sources with effective NPDES permits violated their permit in the past three years (US EPA ECHO 2014). Sixty-three major sources with effective NPDES permit have had formal enforcement actions against them in the last five years (US EPA ECHO 2014). These findings show how many sources have been subject to enforcement. But since one source can be subject to several enforcement actions, it is important to review the number of recent NPDES enforcement actions in Tennessee and Kentucky overall as well to discern enforcement trends.

Trends in overall NPDES enforcement actions in Tennessee and Kentucky are mixed (see Figure 4)⁶. Formal actions in Tennessee and informal actions in Kentucky peaked around 2011-2012, and then decreased in following years. In Kentucky, closed informal enforcement actions against major dischargers peaked in 2011 at 1,950 cases. In 2012, the number of concluded cases decreased to 1,573 and by 2014 only 1,090 cases closed. In Tennessee, about 20 formal

⁵ EPA may issue an enforcement action against a state-issued permittee when states fail to act. Citizens also may sue NPDES violators in federal court.

⁶ I relied on enforcement data from the EPA Enforcement and Compliance History Online (ECHO) to determine state trends. But, as I mentioned earlier in the introduction, ECHO data does not include all enforcement actions. To verify potential gaps, I followed up with a representative from TDEC about NPDES enforcement data in Tennessee. I also referred to data from the Tennessee Clean Water Network, which researched Clean Water Act enforcement in Tennessee extensively. Even though ECHO data is not exhaustive, it can still reveal important information about enforcement in states.

Table 1. Enforcement and compliance data of major sources with effective NPDES permits in EPA Region 4, Kentucky and Tennessee. (Last updated: October 20, 2014).

Place	Facilities with a Current Violation/s	Facilities with Violations in the Last Three Years	Facilities with Formal Enforcement Actions in the Last Five Years
EPA Region 4 - Alabama, Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, Tennessee and 6 Tribes	580	930	347
Kentucky	34	53	29
Tennessee	62	113	34

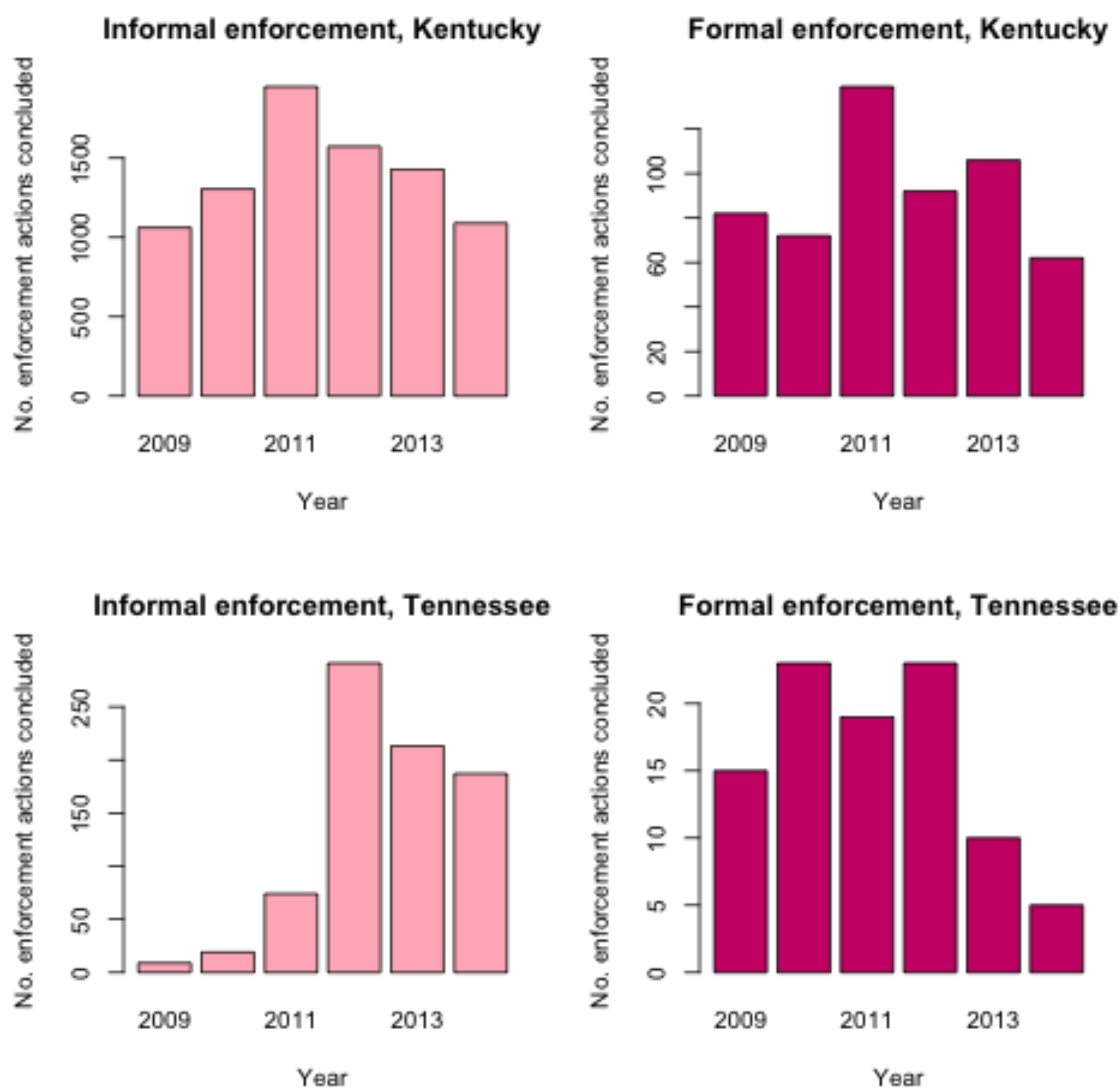


Figure 4. NPDES informal and formal enforcement actions for major sources concluded in Kentucky and Tennessee from 2009 to 2014 (ECHO 2014).

enforcement cases closed each year in 2010, 2011, and 2012⁷. That number halved to 10 in 2013 and halved again in 2014 to 5 formal cases. However, the formal enforcement actions concluded in Kentucky and the informal enforcement actions concluded in Tennessee did not exhibit the same pattern. Thus, enforcement in the two states combined is mixed and I did not find a downward trend in the ECHO data.

Enforcement of the National Pollutant Discharge Elimination System varies in Tennessee and Kentucky and seems to be decreasing nationwide. What could variation in enforcement levels mean for macroinvertebrate communities in streams? Improvements in biotic communities downstream of point sources with improved pollution control technology is well-documented (US EPA 2001, Coombs 2010). Is it possible to detect improvements in aquatic macroinvertebrate composition over a broad spatial scale in response to major point source changes brought about by enforcement?

Why study influence of enforcement on aquatic macroinvertebrates on a regional scale?

Using a small ecological-political region (Tennessee and Kentucky) is an appropriate study area. Because states have autonomy to enforce NPDES, I wanted to include at least two states to account for enforcement variation. Ecologically, the region shares common river basins, but also has spatial heterogeneity (*e.g.* plateau regions, mountainous regions). Having some similarities but enough differences allowed me to test the limits of how much diversity can exist in a given scale and whether relationships between my variables could be detected. Further, researchers are

⁷ According to an enforcement report from a local nonprofit that verified Tennessee enforcement actions with state authorities, there were 231 in 2008 and only 53 in 2004--a 75% reduction (TCWN 2015). The ECHO figures are for major NPDES sources only. The TCWN report includes minor NPDES sources, as well as aquatic resource alteration permits (most of these are minor NPDES sources).

increasingly acknowledging that anthropogenic actions at a landscape scale impair stream ecosystems and the biota through numerous channels (Townsend 2003, Allan 2004).

My research provides a novel approach to evaluating the effect of enforcement on point source impact on biotic integrity, because few studies have used Geographic Information Systems to integrate biotic and legal datasets to understand enforcement impacts over large spatial scales. For example, in conservation planning, researchers have found that a “research implementation gap” exists between well-thought conservation plans and the actual implementation of those plans, particularly for freshwater stream systems (Barmuta 2011, Knight *et al.* 2006, Knight *et al.* 2008).

Prior research suggests that the influence of some land use related factors on stream integrity is scale-dependent (*e.g.* local vegetative cover, regional hydrology) (Allan *et al.* 1997; Martiny 2011). Much research has been done on the role nonpoint pollution has on macroinvertebrate metrics, but the role of point sources should not be neglected. This is important as the Clean Water Act calls for clean water goals to be met “through the control of both point and nonpoint sources of pollution” and our regulatory tools are oriented to address point source pollution (Clean Water Act 2006a).

Chapter 2: Research Questions and Hypotheses

Research Questions

Study I determines whether NPDES enforcement and macroinvertebrate community metrics covary. Study II examines temporal and spatial effects to understand the directionality of the covariance relationship established in Study I. As such, the questions for the two studies are as follows:

- (1) Do spatial patterns in enforcement actions and freshwater community indicators covary when controlling for state, population density and elevation?
- (2) Is there a greater difference between downstream and reference freshwater communities in surveys:
 - (a) taken closer in time to enforcement?
 - (b) taken at closer distances from the discharge point?

Hypotheses

I hypothesized that enforcement and macroinvertebrate composition would covary and that the covariation is driven by enforcement. I thought enforcement should lead to better abiotic conditions for aquatic life. Because all facilities in the dataset violated their NPDES permit in the recent past to some degree (see Figure 5), I assumed enforcement would lead to improved conditions in nearby streams. Conversely, because Study I used a covariance design, I considered the possibility that areas with high biotic integrity were subject to more NPDES enforcement in order to protect that the biodiversity. It was also possible that NPDES enforcement was more likely in areas with low biotic integrity to improve poor conditions.

Further, under Study II, I predicted that downstream surveys taken closer (in time and space) would differ more from their corresponding reference surveys compared to downstream

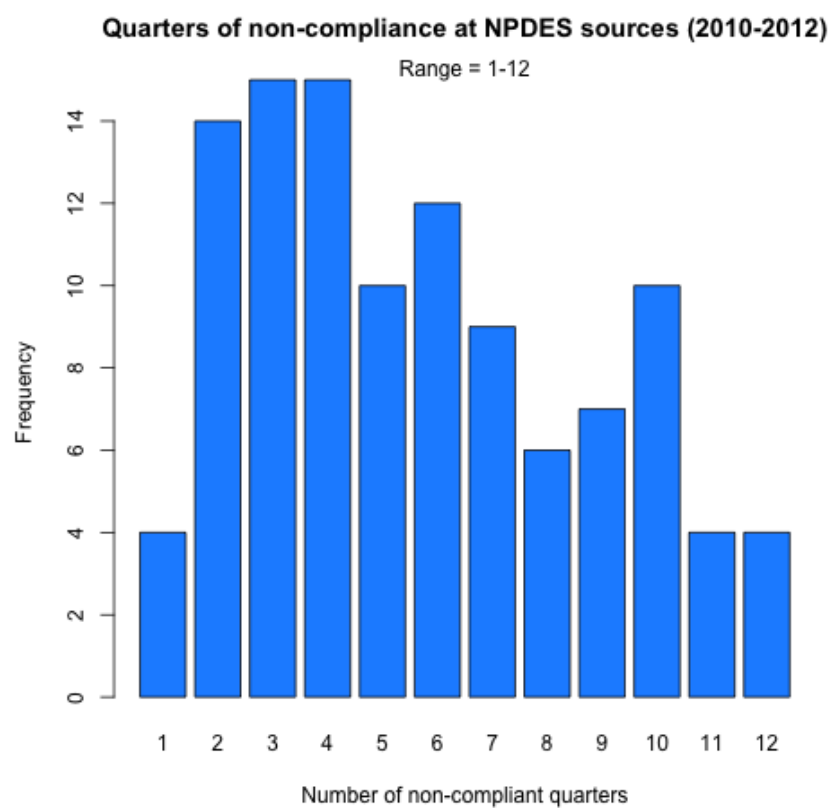


Figure 5. Quarters of noncompliance for NPDES sources in Study I. A small subset of these sources were also used in Study II. Twelve quarters of noncompliance means the facility temporarily violated each quarter over three years.

surveys taken further (in time and space) and their corresponding reference surveys. This hypothesis makes sense because biotic communities need some time to recover after suffering from poor abiotic conditions caused by a nearby violating source. And excess pollution would most likely affect biotic communities closer to the facility than further away. Macroinvertebrates tend to remain in relatively small spatial areas, so they are exposed to more localized pollutants (Merritt & Cummins 1996).

Chapter 3: General Methods

Study Area

Watersheds in Tennessee and Kentucky

My study area covers streams in the Ohio, Tennessee, and Lower Mississippi River basins (see Figure 6). The Ohio River is the largest tributary by volume to the Mississippi River and much of it is impaired due to high bacteria counts that affect the recreational uses of the river. The Ohio River basin covers 528,000 square kilometers and much of its 1578-kilometer length borders the northern edge of Kentucky (US EPA Region 5 2013). Over five million people use the Ohio River for recreation and drinking water (US EPA Region 5 2013). The Ohio River basin experiences water-related problems such as effluent from municipal wastewater treatment plants, combined sewage and storm water overflows, urban storm water, acid mine drainage and toxic pollutants (Ohio University 2014).

Various municipal and production industries exist along the Tennessee River (USGS 1998, 2001). The Upper Tennessee River Basin covers over 50,000 square kilometer (USGS 2001). Elevation in the study area ranges from 189 meters above sea level in Chattanooga to 620 meters at Mt. Mitchell in North Carolina (USGS 2001). The Lower Tennessee watershed covers about 50,500 square kilometers and nearly 2.7 million people lived within the watershed's boundaries in 1995 (USGS 1998). The Lower Tennessee River Basin experiences problems related to nutrient enrichment (USGS 1998).

The segment of the Lower Mississippi River Basin in my study is part of the Yazoo Basin, which covers a 90,000 square kilometer plain from south of Memphis, Tennessee to Vicksburg, Mississippi (US ACE 2014). Parts of the Yazoo River have designated uses to support aquatic life under the Clean Water Act (US EPA 2003).

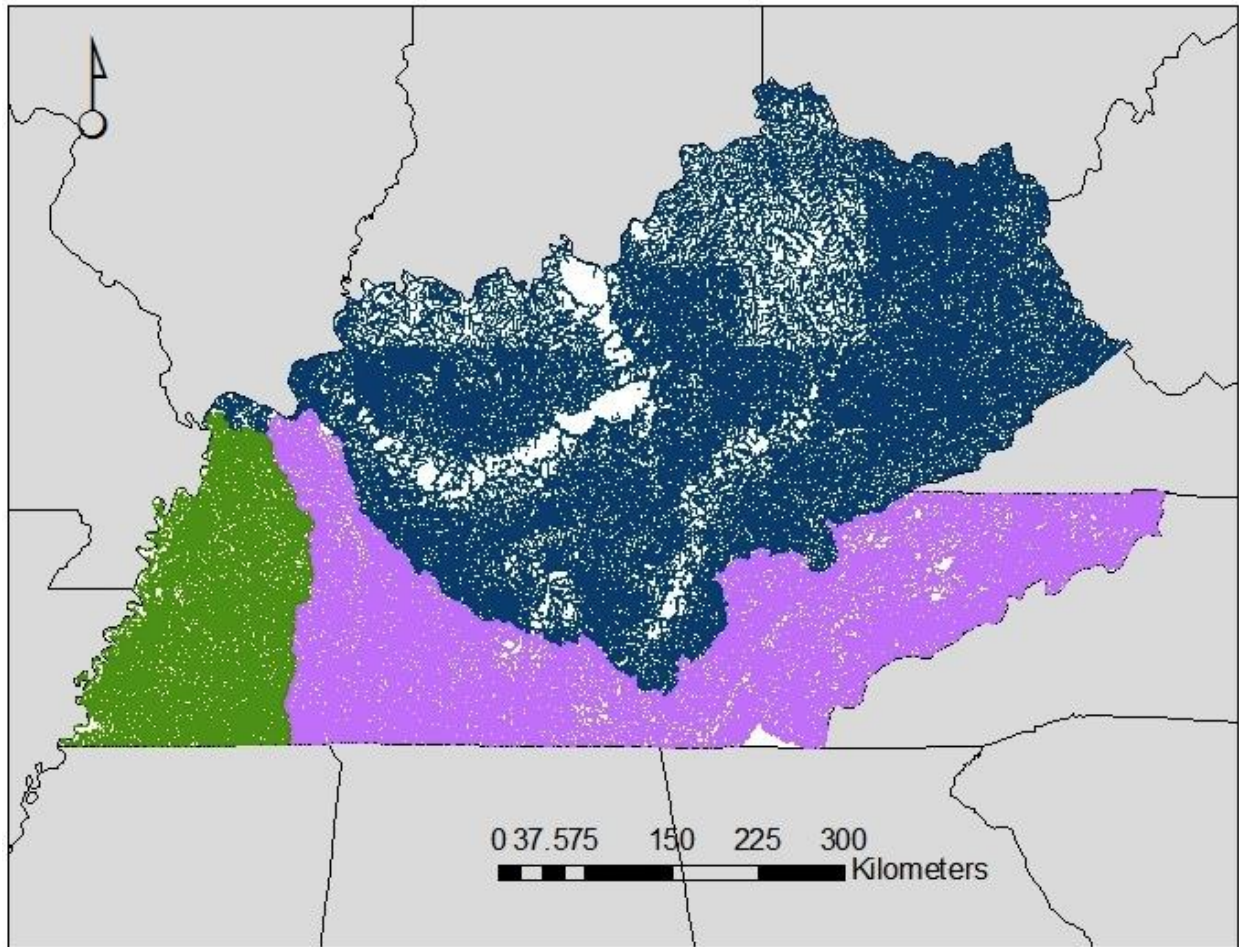


Figure 6. *Map of Tennessee and Kentucky with corresponding river basins.* The dark blue streams are in the Ohio River Basin. The purple streams are in the Tennessee River Basin. The green stream segments are in the Lower Mississippi River Basin.

NPDES facility types

The facilities in my dataset represent various municipal and commercial pollution point sources (see Table 2). However, most facilities are municipal sewage treatment plants (STP). Sewage Treatment Plants are major NPDES sources under the Clean Water Act. Sewage waters contain nitrogen and phosphorus, among other substances (US EPA 2015). After treatment, sewage waters are typically released into local water bodies, which can lead to nutrient pollution (US EPA 2015). If a STP source violates its permit, potentially even more nitrogen and phosphorus enters surface waters.

Survey Station Characteristics

State personnel and contractors conduct macroinvertebrate surveys at designated survey stations along stream reaches of interest following standard protocols. These stations vary in stream characteristics, and sampling is done to meet various regulatory requirements. Based on publicly accessible data, sampling at each station appears infrequent. Stations in my dataset usually did not have sampling done more than once every few years.

Stream Size

Stream order is an indication of stream size. Under the Strahler stream order system, streams that have no tributaries are considered first-order streams and larger streams are higher order (Strahler 1964). When two first order streams join together, the new stream is considered a second-order stream. When two second-order streams meet, a third-order stream forms and so on. A lower order stream does not change the order of a higher stream if they meet. In my research dataset, surveys were taken at stations in first through fifth-order streams (see Figure 7).

Table 2. NPDES Source Types for Covariance Question. Various types of activities are accounted for in the sources, but most sources were municipal sewage treatment plants (N = 86).

Facility Type	Frequency
Ammunition	1
Chemicals	4
Energy	4
Food Processing	1
Landfill	1
Metal Processing	3
Mining	2
Municipal	1
Nuclear	2
Paper Processing	4
Petro-chemical	1
Sewage Treatment Plant (STP)	86
Textile	2
Wood	1

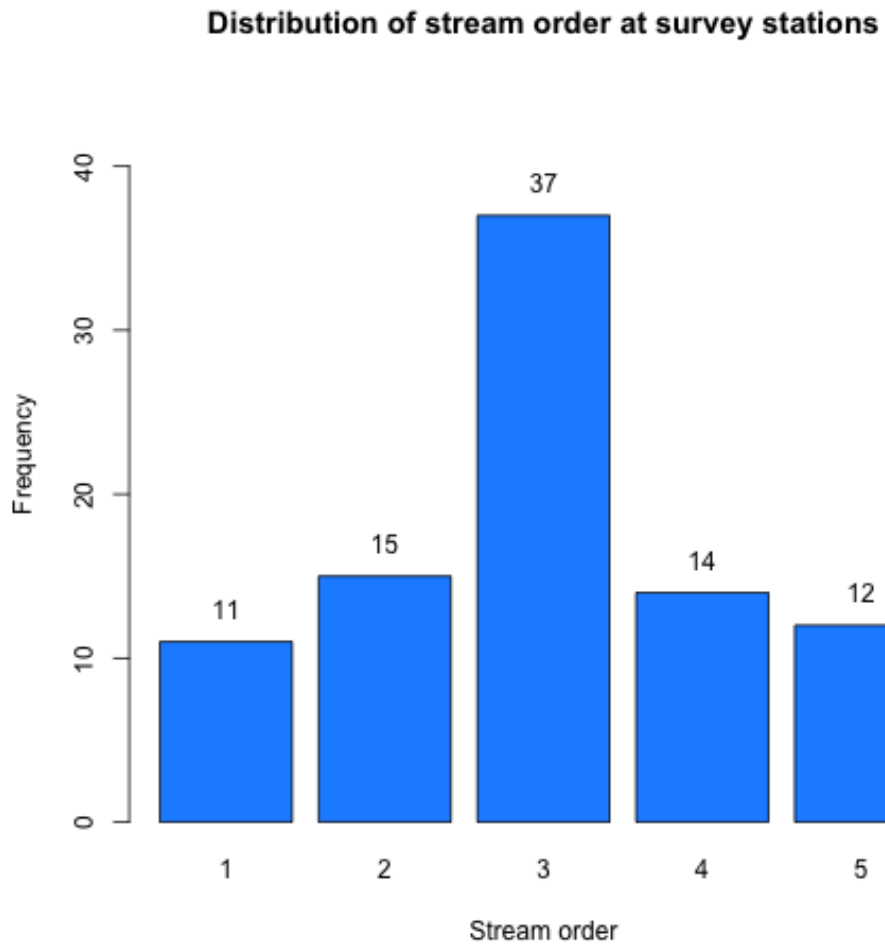


Figure 7. Frequency of Stream order among survey stations. Stream order data was not available at all points.

Ecoregions

My study area covered ten different United States Geological Survey (USGS) Level-III ecoregions of the southeastern United States (see Table 3). An ecoregion is a geographic area with distinct climatic and physiographical features that influence the biotic communities of that region (Vargas *et al.* 1998, Omernik 2004). For example, in the Southeastern Plains, streams tend to have sandy bottoms (US EPA 2014b). This benthic environment is more favorable to some macroinvertebrates than others. Terrestrial, geological, and climatic patterns can also have an impact on aquatic macroinvertebrates (*e.g.* allochthonous leaf litter from oak trees in the Blue Ridge ecoregion).

Most of the survey stations in both Tennessee and Kentucky fell within the Ridge and Valley (N = 41) or the Interior Plateau (N = 41) ecoregions. The Ridge and Valley Ecoregion has a forest cover of about 50% and possesses a rich diversity of aquatic habitats (US EPA 2014b). The Interior Plateau is characterized by relatively low elevations (compared to the Blue Ridge, Ridge and Valley, and Southwestern Appalachians) and oak-hickory forest (US EPA 2014b).

Geographic Information System Designs

My Geographic Information System (GIS) database combines several sources of data from state and federal agencies. This data was available from government websites and through inquiry to open records personnel. After compiling the data, I mapped it in ARCMAP 10.1 to identify candidate sites for both of my studies. I will explain study-specific candidate site parameters and data extraction details in subsequent sections for each study separately. Similar studies examining point feature influence on stream biological communities used comparable designs (Mims and Olden 2013).

Table 3. Survey Station Ecoregions (EPA Level III and IV Ecoregions of the Continental United States 2014)

Ecoregion	Code (Level 3)	Number of survey stations present in ecoregion	Notes particularly related to stream characteristics
Southeastern Plains	65	5	Streams are relatively low-gradient and sandy-bottomed.
Blue Ridge	66	4	Great diversity of aquatic habitats fish species.
Ridge and Valley	67	41	
Southwestern Appalachians	68	2	
Central Appalachians	69	5	Widespread coal mining has caused siltation and stream acidification.
Western Allegheny Plateau	70	1	Diverse fish fauna
Interior Plateau	71	41	
Interior River Valleys and Hills	72	8	
Mississippi Valley Loess Plains	74	8	

My data collection protocol resulted in a Tennessee/Kentucky database of 110 replicates for Study I. For Study II, I had five downstream and reference surveys pairs. My study designs influenced the number of replicates for each study. Study I is a uses a covariance approach, so the GIS design is not as strict. Study II requires a sample site downstream of a NPDES facility, a nearby reference site, and I needed to be able to snap both sites to a stream segment in ARCMAP 10.1. As such, there were significantly more data points in Study I compared to Study II (see Table 4).

Input and response variables

Enforcement (input variable)

The US EPA Enforcement and Compliance History Online (ECHO) database provided enforcement data on major NPDES sources in Kentucky and Tennessee (US EPA ECHO 2014).

I extracted formal and informal enforcement data between 1999 and 2012 for the NPDES sources in my study.⁸ I constructed a loop in the R software environment for statistical computing and graphics (R) to extract enforcement data for each NPDES source and corresponding survey in my study. I classified each NPDES source as being either subject to enforcement or not subject to enforcement based on what enforcement occurred during the three-year timeframe prior to the biotic survey taken near the NPDES source. For my studies, formal and informal actions were grouped together, so a source was considered subject to enforcement (either formal or informal enforcement) or not.

⁸ See the introduction for a description of the difference between formal and informal enforcement actions.

Table 4. Comparison of the number of raw sampling stations in Tennessee and Kentucky and how many met the requirements of each of my study designs.

Group Type	Description	Number of sampling sites
Raw Data	All unique sampling stations in Tennessee and Kentucky	3336
Study I Designs	<u>Candidate Sites</u> : Number of sampling stations within 3 km of a NPDES source	286
	<u>Sites used in the analysis</u> : Number of sampling stations within 3 km of the source when only using one sampling station per source	110
Study II Designs	<u>Candidate Sites</u> : Number of survey stations that could be snapped onto an adjacent stream segment that were downstream of a NPDES source (within 3 km of the NPDES source)	13
	<u>Sites used in the analysis</u> : Number of survey stations that could be snapped onto an adjacent stream segment that were downstream of a NPDES source and could be paired with a reference site within 3 km of the NPDES source	5

Macroinvertebrate metrics (response variables)

I synthesized macroinvertebrate survey data from the Tennessee Department of Environmental Control and Kentucky Department for Environmental Protection databases that are publicly accessible. These surveys followed standard operating procedures determined by each state, but these procedures were modeled after EPA established protocols (Barbour *et al.* 1999, KDEP 2002a, KDEP 2002b, KDEP 2009, KDEP 2011, TDEC 2011).

Under Kentucky and Tennessee Standard Operating Procedures, personnel collected a stream sample using kicknets (Barbour *et al.* 1999). In the lab, a subsample of approximately 200 individuals is taken from the whole field sample. Most of the subsampled specimens were identified to the genus level, or in the case of some Diptera groups, they were identified to the family level (Barbour *et al.* 1999). After processing the samples, researchers calculated several metrics to assess the health of macroinvertebrate biota and overall stream health.

In this study, I used six metrics for further analysis: Taxon Richness, Ephemeroptera-Plecoptera-Trichoptera Taxon Richness, Percent Ephemeroptera-Plecoptera-Trichoptera, Percent Clingers, North Carolina Biotic Index, and percent Oligochaeta/Chironomidae (see Table 5). Some of these measures decrease in response to water pollution while others increase in response to it (TDEC 2011).

Metrics that decrease in response to water pollution

Taxon Richness (TR) is the number of macroinvertebrate groups, typically at the genus-level, in the sample. High taxon levels indicate a stream ecosystem's ability to support varied taxa (Barbour *et al.* 1999). The metric measures the overall variety of the macroinvertebrate assemblage. (Barbour *et al.* 1999). Other metrics consider specific macroinvertebrate groups

Table 5. Macroinvertebrate metrics and value meanings.

Metric	Increases with increased pollution	Decreases with increased pollution
TR		✓
EPT		✓
%EPT		✓
NCBI	✓	
%Clingers		✓
%OC	✓	

and/or take into account differing tolerance levels of macroinvertebrate taxa.

Ephemeroptera-Plecoptera-Trichoptera taxon richness (EPT) is the number of taxa that belong to either the Ephemeroptera, Plecoptera, or Trichoptera insect orders. These orders are for the most part sensitive to water pollution (Merritt & Cummins 1996). So, members of these groups can only flourish in cleaner water. Further, the percent EPT taxon richness (%EPT) out of the entire sample Taxon Richness in a sample is also used to scale the units for more appropriate comparisons between samples. In this study, percent EPT taxon richness excludes *Cheumatopsyche* individuals as this genus is tolerant to water pollution, unlike other Trichoptera genera.

Percent Clingers (%Clingers) shows how many individuals in the sample (as a percentage) are characterized as “clingers.” Because Clingers are macroinvertebrates that “cling” to the stream bottom, %Clingers helps researchers understand how macroinvertebrates are able to use the benthos in which they are situated. Pollution that decreases surface area availability (e.g. nutrient enrichment leading to excess algal growth) may reduce the presence of Clingers (Griffith *et al.* 2009). Aquatic quality impairment usually impedes a clinger’s ability to cling to a rocky benthic environment, sometimes due to nutrient-sensitive algal growth on a rocky substrate (Braccia & Voshell 2006). As such, a low percentage of Clingers may indicate poor water quality whereas a higher incidence of Clingers may indicate better water quality.

Metrics that increase in response to water pollution

North Carolina Biotic Index (NCBI) is a metric that takes into account the composition of a sample and the tolerance values of groups in that sample (TDEC 2011). Each taxon is assigned a tolerance value (t_i) that indicates how well a genus can tolerate water pollution. A higher tolerance value means a group can handle poor water quality, whereas a low tolerance value

means that a genus is not very tolerant of water pollution. To calculate NCBI, the number of individuals of a group (x_i) is multiplied by its tolerance value (t_i), the products of all groups are summed together and then divided by the total number of individuals in a sample (N):

$$NCBI = \frac{\sum x_i t_i}{N}$$

For my research, NCBI had to be calculated for the Kentucky data, because Kentucky authorities used the Holsenhoff Biotic Index instead of NCBI. A higher proportion of individuals from groups sensitive to water quality drives the index down to a lower value. When more groups with higher tolerance values are present in a sample, NCBI goes up. Therefore, a higher NCBI means poorer water quality.

Percent Oligochaeta and percent Chironomidae are the percentage of oligochaeta and Chironomidae individuals in a sample respectively (TDEC 2011). These groups are both relatively tolerate of poor water quality conditions, so a higher percentage of oligochaetes or chironomids indicates lower water quality. It is common practice to combine both measures into one metric, so for this research percent Oligochaeta and Chironomidae are combined into one metric, %OC, (TDEC 2011).

Geographic information system notes and covariate variables

United States Geological Survey (USGS) National Hydrography Dataset (NHD) Plus 2 provided shapefiles of streams and rivers of interest in the Lower Mississippi, Tennessee and Ohio basins. I used the NHD Plus 2 Linear Referencing toolbox in ARCMAP to map stream features and determine stream distance for Study II.

The Study I multiple regression model controlled for state, elevation, and population as covariates to enforcement. I got elevation data from the USGS National Elevation Dataset. I used population density data at the zip code scale from the 2010 United States Census. In Study II, the reference surveys were so close to the downstream surveys that the state, elevation, and population density for both surveys were treated as being equivalent.

Exploratory Data Analysis

I tested the assumptions necessary for regression models, including independence of input variables and normality of errors in response variables. Tables and plots summarizing these findings are in the Appendix. I used boxplots and stacked barplots to determine independence involving categorical variables. I used scatterplots and Pearson's Correlation for collinearity exploratory analysis involving numeric variables. I used Quantile-Quantile (QQ) plots, D'Agostino Tests, and Anderson-Darling Normality Tests to determine the normality of response variables. If response variables were not normal, I log (base 10) transformed them.

Chapter 4: Study I - Enforcement and freshwater community covariance

Summary

Study I examines the relationship between enforcement and freshwater community indicators using a multiple regression model. The regression aimed to determine whether enforcement and freshwater community indicators changed together. Each NPDES source was paired with a nearby survey station for analysis. These surveys were not necessarily downstream of the NPDES source, so the study is a covariance study. I examined directionality of the covariance relationship through Study II.

The multiple regression with all points yielded no relationship between enforcement and aquatic biodiversity. After subsetting by ecoregion and stream order, I found a statistically significant relationship between both NCBI and enforcement as well as percent Clingers and enforcement in larger streams. I also found ecoregion specific relationships. EPT Taxon Richness and enforcement covaried in western ecoregions of my study area. A relationship between enforcement and Taxon Richness was also seen in eastern Tennessee and Kentucky.

Geographic Information System Design

Conceptually, I needed a biotic survey within a short distance of a NPDES permitted facility (see Figure 8). Because the study investigates covariance, it was not essential that the biotic surveys be downstream of the permitted facility. My aim was to determine whether enforcement and freshwater communities change together in the first place. Under my research study design, it was a threshold consideration before I could consider directionality of effect or any other factor.

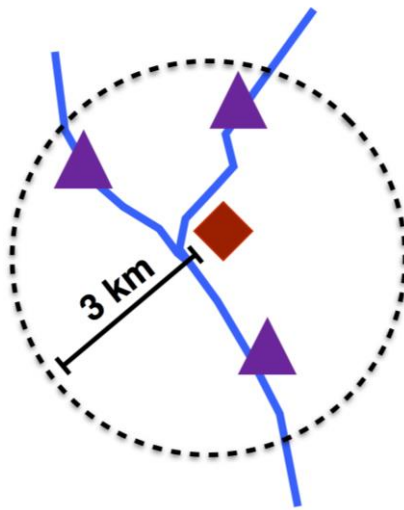


Figure 8. Study I Conceptual Design. Blue lines represent stream segments, purple triangles indicate survey stations, and red square represent NPDES source.

I identified candidate study sites by overlaying NPDES sources and macroinvertebrate surveys onto streams and rivers in Tennessee and Kentucky in the ARCMAP 10.1 Geographic Information System (see Figure 9). I extracted survey stations within a three-kilometer radius of a NPDES source for further analysis.⁹ When I found multiple surveys done at the same survey station, I used only the most recent sample. If I found more than one survey station within the three-kilometer radius of the point source, I used the Near tool in ARCMAP to determine the closest survey station to the NPDES source. After candidate site identification, 110 surveys within a three-kilometer radius of a NPDES sources were selected as data points for the covariance study (see Figure 10).

Data Analysis

After extracting datasets from ARCMAP 10.1, I ran statistical analyses in the R software environment for statistical computing and graphics (R). I used a general linear model for response variables with a normal distribution or could be transformed to conform to a normal distribution. I log transformed (base 10) non-proportion response variables that did not have a normal distribution to improve their fit to normal distribution. I used a generalized linear model with a binomial error structure for two of my proportion metrics (%EPT and %OC), because the range of values was imbalanced.¹⁰

⁹ The coordinates for NPDES sources were facility centroids, facility offices, or actual discharge points. As such, an ample buffer radius was necessary to envelope area outside of the NPDES facility (*i.e.* nearby stream segments with biotic surveys).

¹⁰ %Clingers conformed to a normal distribution.

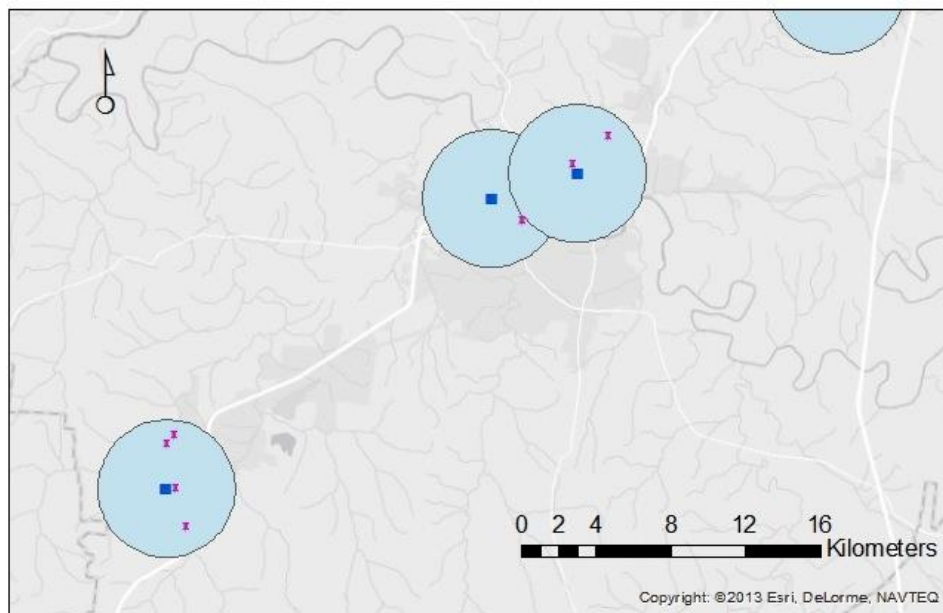


Figure 9. Map of Study I candidate sites with three-kilometer buffer areas (shaded in blue circles), survey stations (pink pin points), and NPDES sources (blue squares).

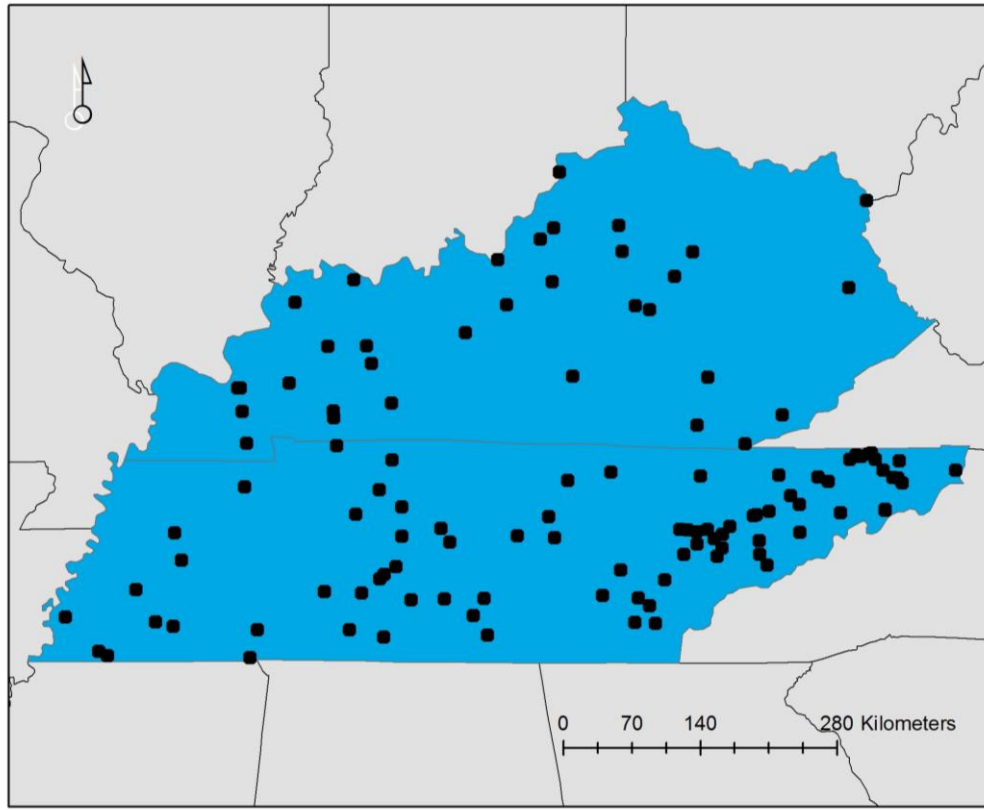


Figure 10. Map of Study I survey points.

Both the general linear model and generalized linear model with a binomial error structure controlled for population density, elevation, and state environmental quality agency:

$$y = \alpha + \beta_1(Enforcement) + \beta_2(State) + \beta_3(PopulationDensity) + \beta_4(Elevation) + \varepsilon$$

These covariates could potentially influence aquatic biodiversity or enforcement.

Enforcement was coded as presence/absence (1 or 0) if a source was subject to either a formal or informal enforcement action. State was coded as 0 and 1 (1 for Kentucky and 0 for Tennessee). Population density and elevation were continuous. Summary statistics for the covariates can be seen in the Appendix.

I used the “summary” and “Anova” functions in R to perform an Analysis of Variance (ANOVA). Alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$. I did a Holm’s, also known as a Sequential Bonferroni, correction on my p values to adjust for making multiple statistical comparisons using the same dataset. Even so, I am using the non-adjusted p-values to interpret my results because Study I is *post hoc* testing of unplanned comparisons which are regarded as hypotheses for further investigation (Armstrong 2014).

After running the multiple regression model with all points together, I subset my dataset by stream order and ecoregion, separately. To enhance statistical power, ecoregions were grouped by similar geographic location (see Table 6 and Figure 11) and streams of similar order were also grouped. I ran the regression models again for subset groupings. Again, I used a Sequential Bonferroni correction to account for the multiple comparisons.

Table 6. Ecoregion Groups

Group 1 (N = 20)	Group 2 (N = 41)	Group 3 (N = 49)
Mississippi Valley Loess Plains	Interior Plateau	Ridge and Valley
Interior River Valleys and Hills	Western Allegheny Plateau	Blue Ridge
Southeastern Plains		Central Appalachians
		Southwestern Appalachians

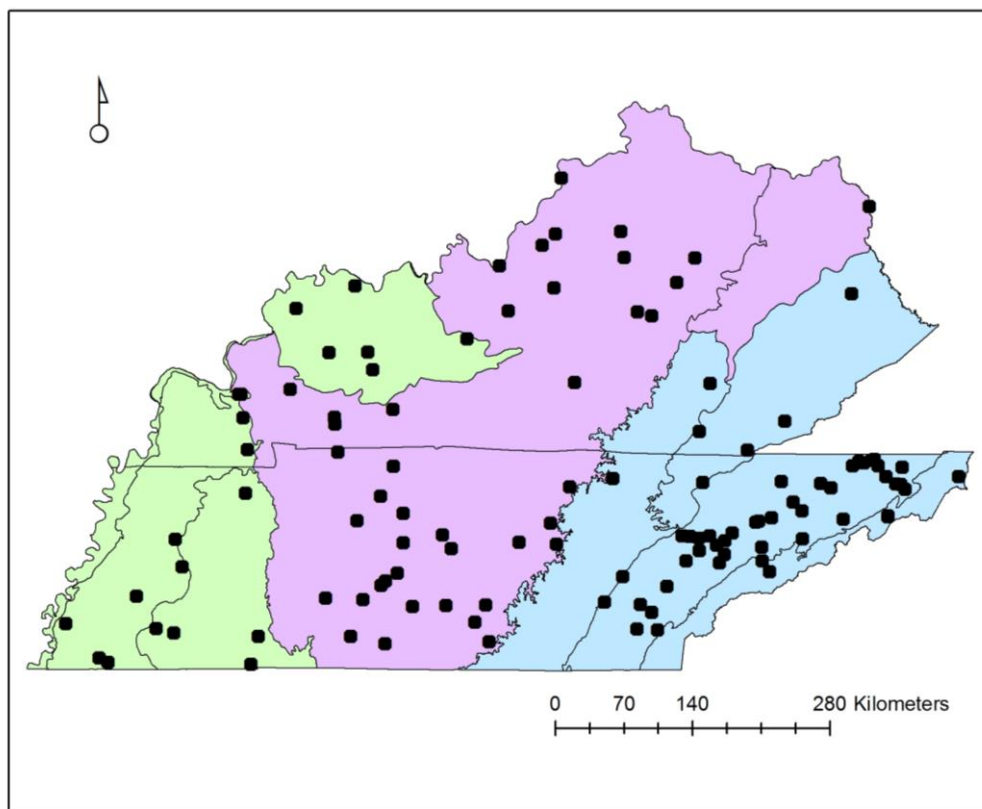


Figure 11. Map of Ecoregion subsets. Group 1 in shaded green, Group 2 is shaded purple and Group 3 is shaded blue.

Results

Summary statistics of all variables and a regression table for model covariates are contained in the Appendix. Most surveys taken were near a NPDES source not subject to enforcement (N = 77). Thirty-three surveys were adjacent to sources that had been subject to enforcement. Enforcement occurred in both states (see Figure 12). Overall, more survey stations and sources are in my dataset are from Tennessee, but similar counts of surveys near sources subject to enforcement are found in Tennessee and Kentucky (N = 18 and N = 21 respectively).

I found no statistically significant relationship between enforcement and any of the freshwater community indicators using the regression model and all 110 study replicates (see Table 7). Models for all metrics had noticeably low r-squared values, meaning the input variables did not do a good job of explaining the distribution of the response variables.

Regression Models for Stream Order Subsets

NCBI and %Clingers covary with enforcement in large streams (see Table 8). Among fourth and fifth order streams, enforcement significantly correlated with NCBI ($p = 0.048^*$) and %Clingers ($p = 0.056^*$). Enforcement coefficients indicate that enforcement had a positive relationship with %Clingers ($\beta = 0.227$) and a negative relationship with NCBI ($\beta = -1.22$). As noted in the general methods section, %Clingers is expected to increase with better water quality. NCBI is expected to decrease with better water quality. Therefore, these observed associations are consistent with my hypotheses that more enforcement leads to better abiotic conditions for macroinvertebrates when considering NCBI and %Clingers.

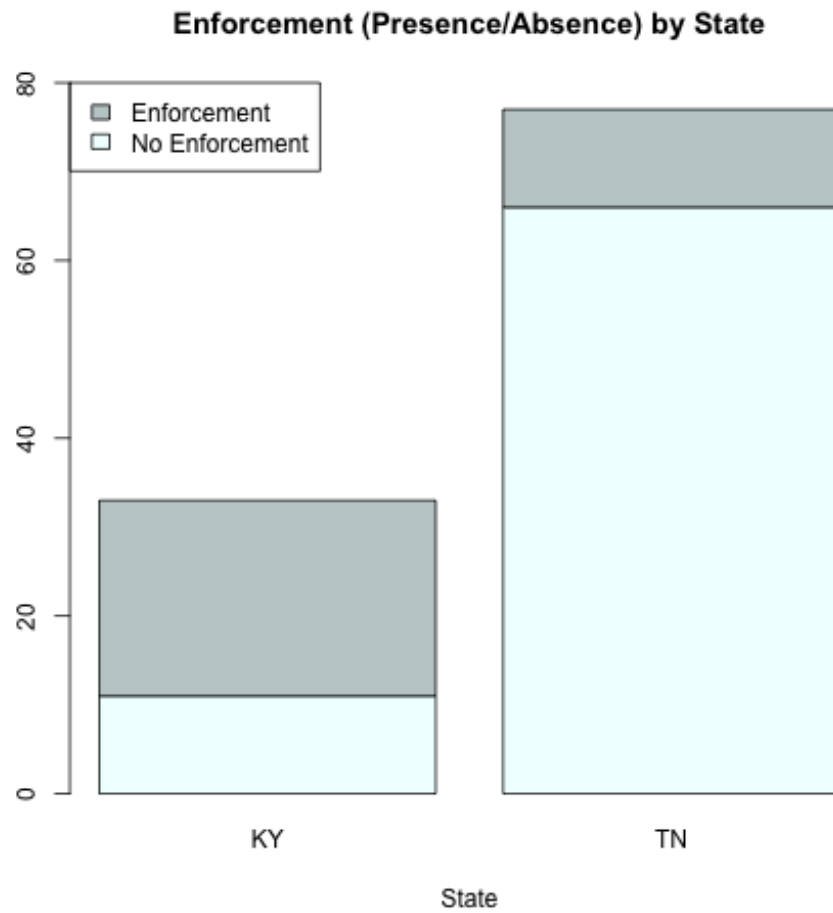


Figure 12. Collinearity of state and enforcement for Study I.

Table 7. ANOVA Table for Covariance Models. Regression table for model using for all points (N = 110). My alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$. No significant relationship between any of the metrics and enforcement were found.

Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
TR	-5.721e-02	3.748e-02	0.1299	0.7794	0.05798
EPT	-6.770e-02	7.478e-02	0.367	1.0000	0.03409
%EPT	-2.031e-01	6.323e-01	0.7480	1.0000	-
NCBI	-4.538e-02	3.054e-01	0.8821	1.0000	0.07106
%Clingers	8.321e-03	5.627e-02	0.8827	1.0000	0.04557
%OC	-2.460e-01	5.514e-01	0.655	1.0000	-

Table 8. ANOVA Table for Stream Order Subsets. My alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$. NCBI and % Clingers covary with enforcement in larger streams. The directionality of the relationship is consistent with my hypothesis.

First & Second Order Streams	Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
	TR	-5.271e-02	1.075e-01	0.629	1	0.07909
	EPT	-7.936e-02	1.976e-01	0.6923	1	0.01515
	%EPT	1.028e-01	1.339e+00	0.939	1	-
	NCBI	-0.3096137	0.8484120	0.719	1	0.145
	%Clingers	-1.125e-01	1.301e-01	0.397	1	0.2042
	%OC	-0.7467415	1.1252778	0.507	1	-
Third Order Streams	Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
	TR	-7.941e-02	5.724e-02	0.1756	1	0.1583
	EPT	-9.617e-02	1.300e-01	0.465	1	0.1072
	%EPT	-0.5229921	1.1616631	0.653	1	-
	NCBI	2.973e-01	5.458e-01	0.590	1	0.1173
	%Clingers	6.992e-02	1.108e-01	0.53298	1	0.07584
	%OC	1.574e-01	1.053e+00	0.881	1	-
Fourth & Fifth Order Streams	Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
	TR	7.428e-03	7.215e-02	0.9190	1	0.2483
	EPT	1.779e-01	1.215e-01	0.1580	0.6320	0.2036
	%EPT	2.679e-01	1.179e+00	0.820	1	-
	NCBI	-1.2242989	0.5820328	0.0477**	0.2862	0.0477
	%Clingers	2.216e-01	1.097e-01	0.0563*	0.2862	0.2952
	%OC	-6.183e-01	1.268e+00	0.626	1	-

Regression Models for Ecoregion Subsets

EPT Taxon Richness and Taxon Richness covaried with enforcement in certain ecoregion groupings. Ecoregion subsetting model ANOVA results are summarized in Table 9. In Ecoregion Group 1, EPT Taxon Richness and enforcement covary ($p = 0.079^*$). Taxon Richness has a significant relationship with enforcement in Ecoregion Group 3 ($p = 0.095^*$). But the directionality of both the TR and EPT Taxon Richness relationship is contrary to my hypotheses. I see a negative association between EPT Taxon Richness and enforcement in Ecoregion Group 1 ($\beta = -0.271$). Taxon Richness also has a negative relationship with enforcement in Ecoregion Group 3 ($\beta = -0.870$).

Because Taxon Richness and EPT Taxon Richness are expected to increase with less pollution, these results are inconsistent with my hypothesis. Further, because Study I used a less rigorous GIS Design, I needed to conduct a study with an enhanced GIS design to determine if enforcement was driving how macroinvertebrate communities changed.

Table 9. ANOVA Table for Ecoregion Subsets. My alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$. EPT Taxon Richness has a significant relationship with enforcement in Group One. Taxon richness and enforcement have a significant relationship in Group Three. Yet, the directionality of the relationships go against my hypothesis.

Ecoregion Group 1	Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
	TR	-1.597e-01	1.181e-01	0.196071	0.980355	0.192
	EPT	-0.2709528	0.1437934	0.0791*	0.4746	0.2629
	%EPT	-0.745881	1.614543	0.644	1	-
	NCBI	0.5500379	0.5704577	0.350232	1	0.249
	%Clingers	-0.1212523	0.0948639	0.221	0.980355	0.2683
	%OC	0.6903686	1.1900209	0.562	1	-
Ecoregion Group 2	Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
	TR	4.219e-02	4.866e-02	0.3917	1	0.2272
	EPT	1.680e-02	1.234e-01	0.892	1	0.07905
	%EPT	-0.2419480	0.9456696	0.798	1	-
	NCBI	-2.264e-01	4.779e-01	0.639	1	0.02556
	%Clingers	1.033e-01	9.451e-02	0.2816	1	0.08473
	%OC	-0.6480278	0.9191516	0.481	1	-
Ecoregion Group 3	Response Variable	β (enforcement)	Standard Error	p-value	Bonferroni adjusted p-value	R ²
	TR	-8.700e-02	5.096e-02	0.094833*	0.568998	0.2381
	EPT	5.178e-03	1.059e-01	0.961	1	0.05053
	%EPT	8.166e-02	1.013e+00	0.936	1	-
	NCBI	-1.629e-01	3.863e-01	0.675	1	0.03236
	%Clingers	-2.901e-02	7.464e-02	0.6994	1	0.06134
	%OC	-4.075e-01	1.016e+00	0.688	1	-

Chapter 5: Study II – Spatio-temporal elements of enforcement influencing macroinvertebrate communities

Summary

Study II takes a closer look at spatio-temporal elements of NPDES enforcement and how they influence macroinvertebrate biodiversity. Examining the time since enforcement and how far biotic communities are from NPDES sources helped me better understand the observed covariance relationship seen in Study I. Study I established a covariance relationship between enforcement and macroinvertebrate communities exists in some cases. This present study considers how the time since enforcement and how far biotic communities are from NPDES sources. Understanding these spatio-temporal influences supported my overall hypothesis that enforcement influences biodiversity to some extent. As such, my study questions are:

- (i) Time: Do freshwater community indicators return to background levels after enforcement?
- (ii) Distance: Does the impact of enforcement on freshwater community indicators vary based on distance from discharge point?

Study II has a more robust Geographic Information System Design. In the first study, not all biotic surveys were downstream of the discharge point or conducted after an enforcement action. Further, potentially impacted survey locations were not compared to unimpaired reference sites with similar background conditions. This second study addresses these outstanding issues with a more experimentally robust geographic information design and data time series. As noted in the general methods section, the vigorous design meant that I had few data points : five paired sites altogether (see Table 4).

I expect enforcement actions to improve abiotic conditions, so these downstream macroinvertebrate communities will be more like the reference macroinvertebrate communities after enforcement. I assumed that these reference conditions are ecologically similar to downstream sites (*i.e.* same elevation, surrounding population density), because they are close in distance.

My hypothesis for Question 1 above is that a larger difference between downstream and reference communities negatively correlates with time since enforcement (*i.e.* difference decreases as time since enforcement increases). As time goes on, the negative influence of excess NPDES pollution on biotic communities will wane.

For Question 2, my hypothesis is that a greater difference between the downstream and reference communities will exist closer to NPDES sources. Excess pollution will have a greater influence closer to the source and that its impact farther along the stream reach will dissipate due to other factors (*e.g.* pollutants settling in the benthos near discharge points).

Geographic Information Design

In ARCMAP 10.1, I identified candidate pairings by overlaying several datasets as layers and extracting surveys that met design conditions. The paired survey design that I constructed required (Figure 13):

- (A) a NPDES source that had been subject to enforcement,
- (B) a downstream station with a biotic survey taken after the enforcement action, and
- (C) a biotic survey taken near the downstream survey that could act as an unimpacted reference survey.

Biotic surveys and NPDES sources from Tennessee and Kentucky were mapped as points.

Streams were mapped as lines. The survey and source features were then snapped onto the

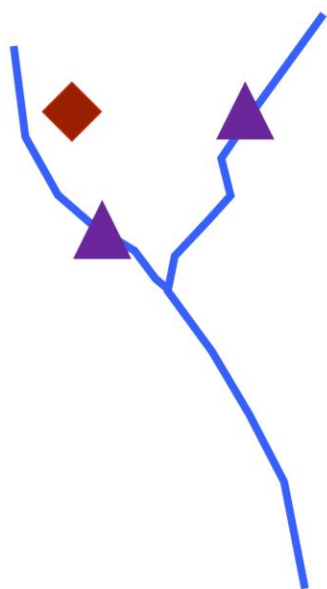


Figure 13. Study II Conceptual Design. The red square is the NPDES source subject to enforcement. The left purple triangle is an impacted downstream survey. The right triangle is an unimpaired reference site. In the study, the difference between the downstream and reference surveys represent one paired data point.

stream segments. I used the Linear Referencing Toolbox to determine stream flow direction and stream distance. I set a three-kilometer buffer around each NPDES source. If there were two surveys within a three-kilometer radius of a NPDES source and one survey could be classified as a biotic survey downstream of a NPDES source done after enforcement and the other could be classified as an unimpaired reference survey, I selected that pair as a Study II site (see Figure 14). I selected reference sites as close to the downstream site as possible, because as geographic distance increases, community similarity between two points generally decreases (Nekola & White 1999).

Data Analysis

I used the R software environment for statistical computing and graphics (R) to perform exploratory data analysis and conduct statistical analyses.¹¹ After extracting points from ARCMAP 10.1, I calculated the difference between reference and downstream indicators for each of my response variables. To calculate the difference (Δy), I subtracted downstream community metrics from reference community metrics:

$$\Delta y = \text{Reference Indicator} - \text{Downstream Indicator}$$

As discussed in the general methods section, some macroinvertebrate community metrics decrease with better abiotic conditions while others increase with better abiotic conditions (See Table 5). Therefore, when considering the difference, the direction and quadrant location of my hypothesized relationship between the input and response variables vary based on whether the metric is known to increase or decrease with more pollution (see Figure 15). To test whether

¹¹ I could not use the D'Agostino or Anderson-Darling Normality Test, because my sample size was too small. Instead, I used QQ plots to test the normality of response variables for Study II. The QQ plots determined that all response variables are consistent with a normal distribution.

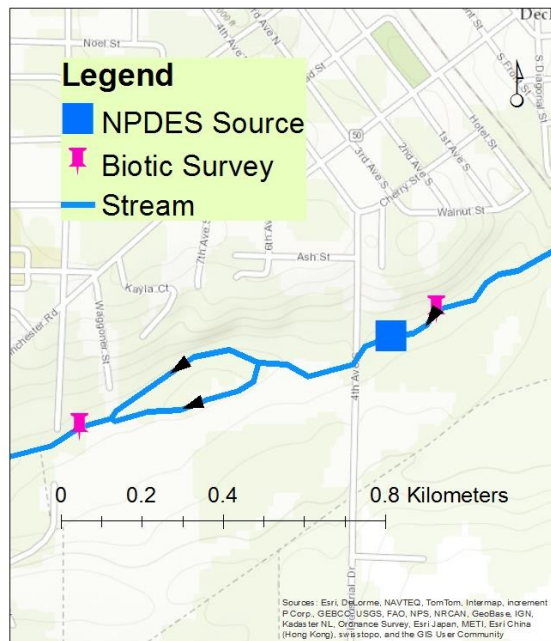


Figure 14. Study II Study Site. See supplemental material for a terrestrial base view of another Study II site.

Δy actually indicated an improvement from reference condition or not, I performed a preliminary paired, one-tail t-test ($\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$) with the following hypotheses, where, μ_1 = mean reference indicator and μ_2 = mean downstream indicator:

For metrics that increase with good water quality

Null hypothesis: $\mu_1 - \mu_2 \leq 0$

Alternative hypothesis: $\mu_1 - \mu_2 > 0$

For metrics that decrease with good water quality

Null hypothesis: $\mu_1 - \mu_2 \geq 0$

Alternative hypothesis: $\mu_1 - \mu_2 < 0$

For my scatterplots, the difference between downstream survey and reference survey was plotted against time. That net change was also plotted against distance from NPDES source. The net change or difference was used to cancel out the influence of ambient factors (*e.g.* population density, ecoregion) as these background factors for the downstream survey and the reference survey were assumed to be similar due to their proximity.

I constructed a linear regression model for each freshwater community indicator metric for time and space. Again, alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$. I did not perform a Holm's correction, because Study II explores the hypotheses stemming from Study I results and Study II has a small number of comparisons (Armstrong 2014).

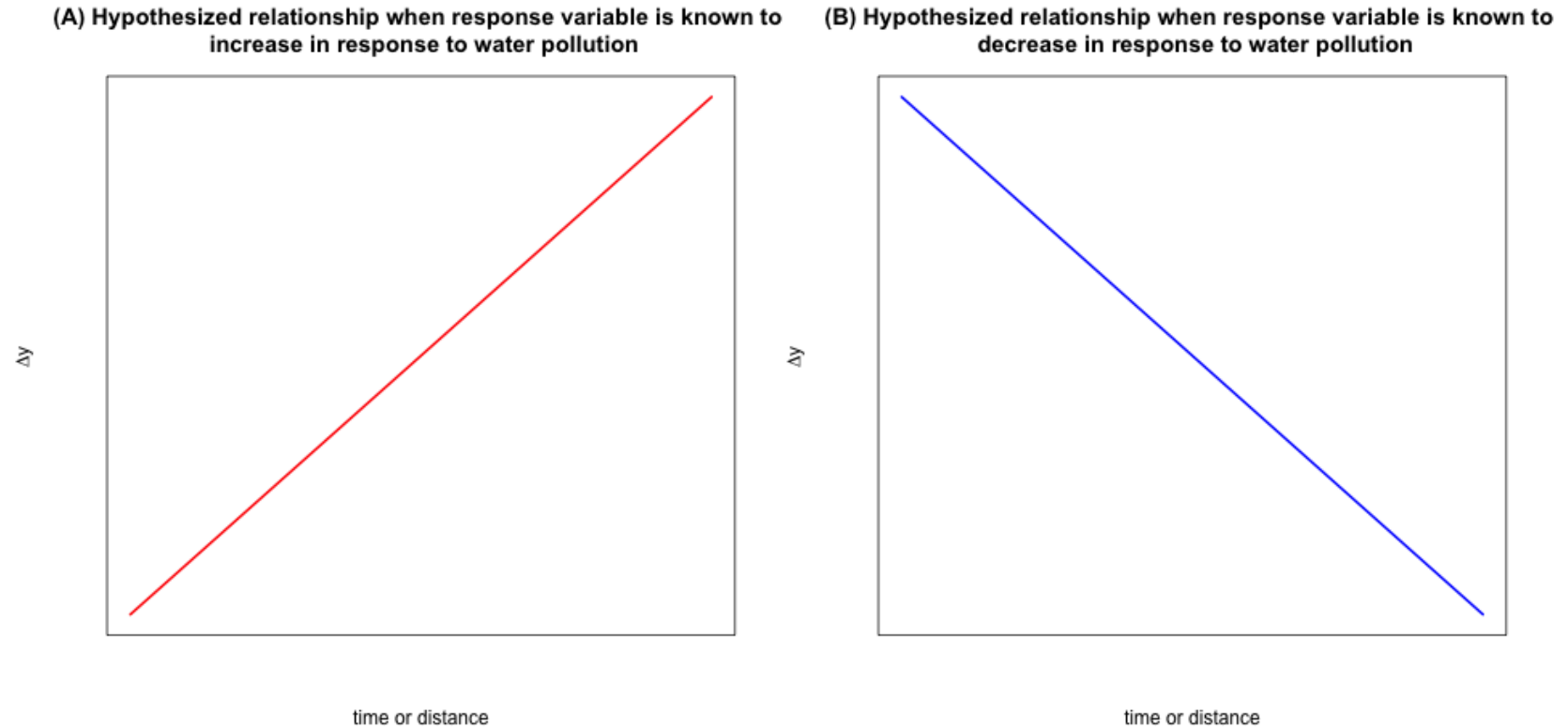


Figure 15 (A) Hypothesized relationship between time or distance and difference between downstream and reference communities for response variables that are known to increase with more pollution and (B) Hypothesized relationship between time or distance and difference between downstream and reference communities for response variables that are known to decrease with more pollution.

I used Pearson's Correlation to examine the correlation between the input and response variables. A correlation coefficient near positive one suggests the two variables have a strong positive relationship, whereas a coefficient near negative one suggests a strong negative relationship. A coefficient of zero indicates no relationship between the two variables.

Results

I found time and distance relationships for some community indicators that suggest NPDES enforcement influences aquatic macroinvertebrate community structure for the better. Summary statistics for my response variables are in the Appendix.

The first sub-section of my results section discusses whether Δy indicates an improvement from reference conditions for both community indicators that increase in response to water pollution and community indicators that decrease in response to water pollution. In light of directional differences, I will discuss Study II results between metrics and space or time separately according to whether the metric increases or decreased in response to pollution.

Does the difference between reference and downstream indicators indicate improvement?

Only in the case of NCBI was there evidence that the reference sites were in significantly better condition than the impacted downstream sites. My testing indicated that reference sites were not always of better biotic quality, but I can still use them as experimental controls.

Community indicators that increase in response to water pollution

North Carolina Biotic Index (NCBI) and Percent Oligochaeta and Chironomidae (%OC) are expected to increase with water pollution. As seen in Figure 16, change in downstream NCBI and distance from pollution source have a strong, positive relationship ($p = 0.098^*$). The relationship between the two variables was highly correlative ($\rho = 0.81$). This change in NCBI is

a biotic improvement, because higher NCBI values indicate low water quality. As seen in Figure 17, the relationship between change in NCBI and time since enforcement was not statistically significant under my analysis of variance. However, the variables correlate moderately ($\rho = 0.68$). Overall, the relationship direction between NCBI and distance/time is consistent with my hypothesis.

No significant relationship between %OC and time since enforcement or distance from pollution source was determined. The scatterplots for %OC are in the Appendix.

Community indicators that decrease in response to water pollution

Taxon Richness (TR), Ephemeroptera-Plecoptera-Trichoptera (EPT) related metrics, and Percent Clingers (% Clingers) are expected to decrease in response to water pollution. I detected a statistical relationship ($p = 0.056^*$) between EPT Taxon Richness and time since enforcement (see Figure 18). Further, time since enforcement and change in EPT Taxon Richness had a correlation coefficient of -0.87, which indicates a strong negative relationship. As seen in Figure 19, I also found a relationship between EPT Taxon Richness and distance from pollution source ($p = 0.051^*$). This relationship was also strongly negative ($\rho = -0.88$).

Results for %EPT were consistent with the EPT Taxon Richness distance and time relationship regarding enforcement (*e.g.* both metrics had negative relationships with input variables), but not statistically significant. Scatterplots in the appendix display %EPT results. The metric correlated with time since enforcement and distance from NPDES discharge ($\rho = -0.75$ and $\rho = -0.83$ respectively). I did not find any meaningful time or distance relationship between Taxon Richness or %Clingers. The scatterplots for both metrics are in the Appendix.

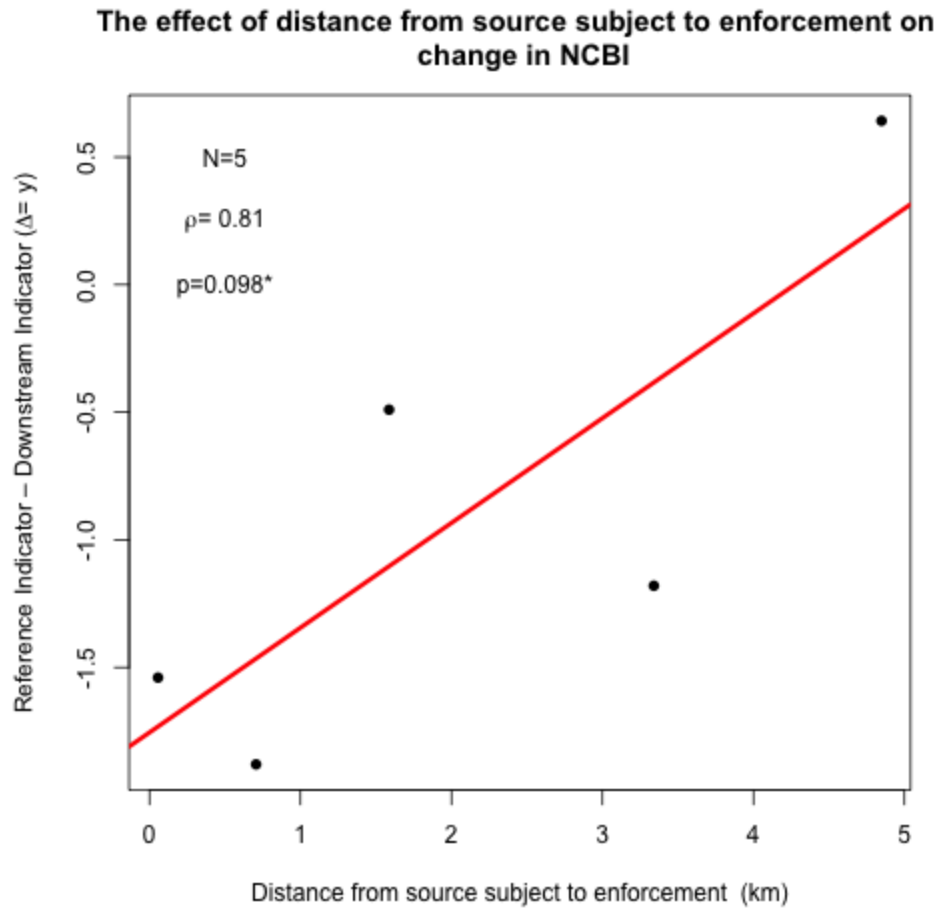


Figure 16. Scatter plot of NCBI difference and distance from NPDES Source. Change in NCBI positively correlates with distance from pollution source ($\rho = 0.81$). The relationship between the two variables is statistically significant ($p=0.098^*$).

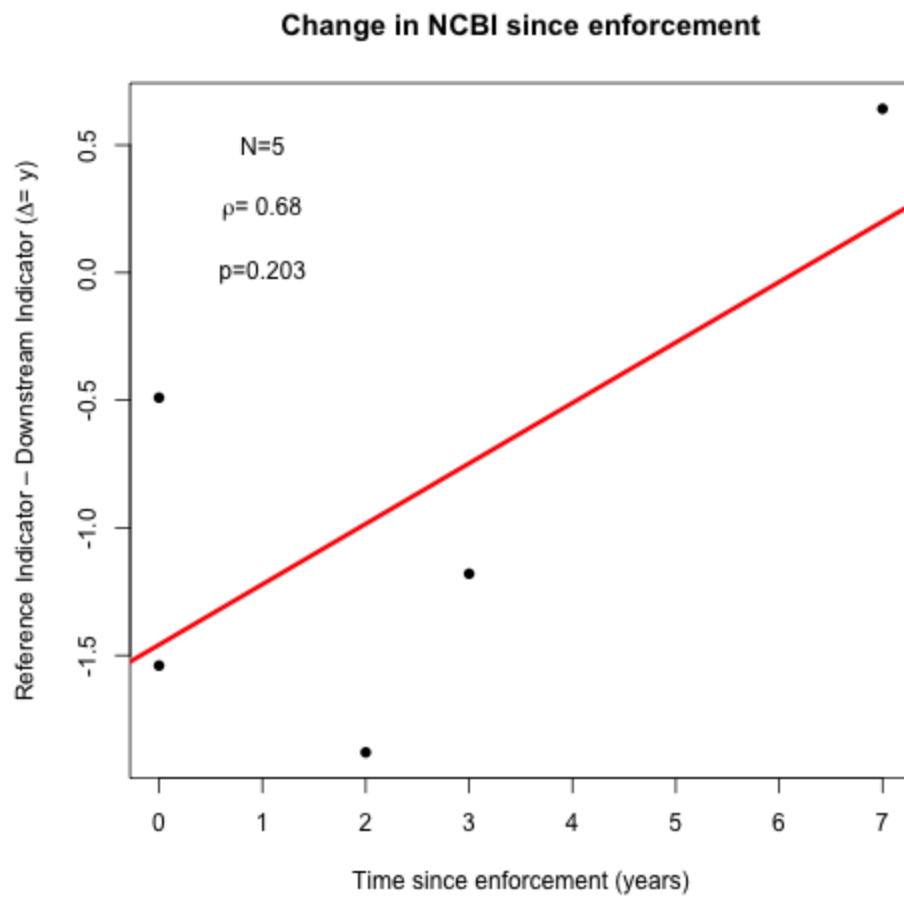


Figure 17. Scatter plot of NCBI difference and time since enforcement. Change in NCBI moderately correlates to time since enforcement ($\rho = 0.68$). Yet, the relationship between the two variables is not statistically significant ($p = 0.203$).

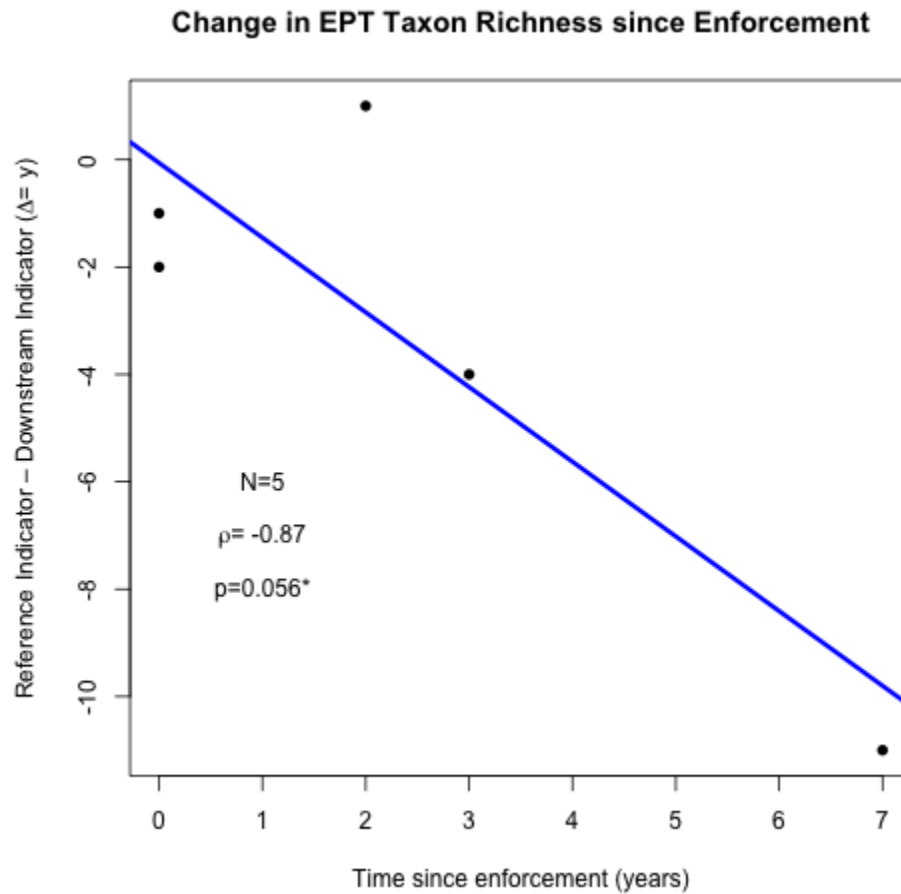


Figure 18. Scatter plot of EPT difference and time since enforcement. Change in EPT Taxon Richness negatively correlates with time since enforcement ($\rho = -0.87$). The relationship between the two variables is statistically significant ($p=0.056^*$).

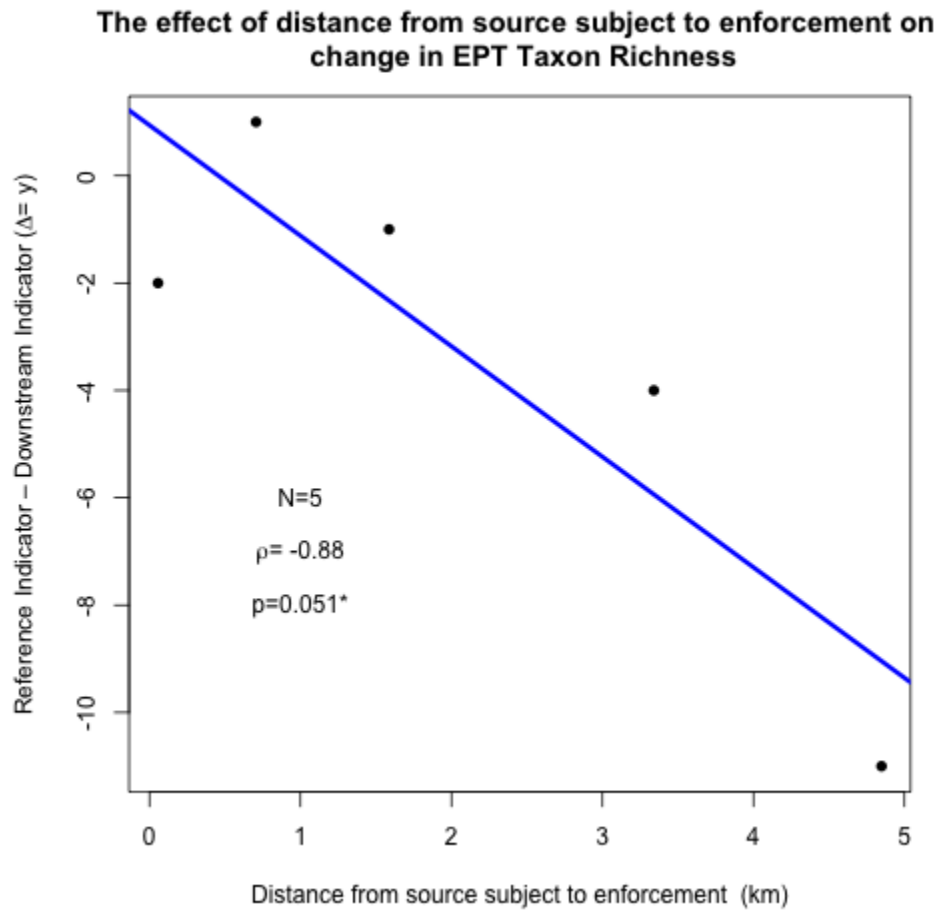


Figure 19. Scatter plot of EPT difference and distance from NPDES Source. Change in EPT Taxon Richness negatively correlates with distance from NPDES source ($\rho = -0.88$). The relationship between the two variables is statistically significant ($p=0.051^*$).

Chapter 6: Discussion & Conclusions

Summary of key findings

My thesis has four key findings that suggest how Clean Water Act (CWA) National Pollutant Discharge Elimination System (NPDES) enforcement influences stream macroinvertebrate communities. First, North Carolina Biotic Index (NCBI) and percent Clingers (%Clingers) covary with NPDES enforcement of major facilities in large streams ($p = 0.048^*$ and respectively $p = 0.056^*$). The directionality of both NCBI and %Clingers were consistent with my hypothesis that more enforcement leads to improved biotic conditions. Second, NPDES enforcement at major facilities and macroinvertebrate community structure change together in some ecoregions. EPT Taxon Richness and enforcement covary in western Tennessee and Kentucky ecoregions ($p = 0.079^*$, $\beta = -0.271$). Taxon Richness and enforcement covary in eastern ecoregions of both states as well ($p = 0.095^*$, $\beta = -0.870$). Yet, the directionality of the relationships was contrary to my hypothesis.

The key findings of Study II address space and time to help interpret the mixed signals of the observed covariance relationships in my first study. The first finding of Study II is that downstream macroinvertebrate communities closer to NPDES facilities subject to enforcement are more impacted for NCBI and EPT metrics than those further away. NCBI strongly correlates with distance from pollution source in a manner consistent with my hypothesis ($\rho = 0.81$). EPT Taxon Richness and distance also correlate in a direction that suggests pollution from point sources is influencing downstream communities ($\rho = -0.88$). The second key finding is that EPT Taxon Richness downstream of NPDES facilities subject to enforcement improved in time since enforcement. By two to three years after enforcement, my linear regression suggests a noticeable improvement in EPT Taxon Richness that continues into future years (see Figure 17).

Suggested sampling regime

My findings can inform a potential sampling regime that is designed to track how enforcement influences biotic communities. Such a regime would need to consider spatial extent, stream size, sampling before and after a possible three-year benchmark, and knowing the appropriate metrics to use.

Spatial extent and stream size matter. As noted in the introduction, research has demonstrated that some land activity impacts on stream health are scale dependent (Allan *et al.* 1997, Martiny 2011). My research suggests NPDES enforcement influence is also scale dependent. For Study I, I first ran my regression model across a small region (Tennessee and Kentucky) ignoring ecoregions and stream size. That scale was too large to detect any relationship between enforcement and macroinvertebrate communities. But at the ecoregion level, I found relationships between enforcement for Taxon Richness and EPT Taxon Richness. Further, stream size was an important consideration. Enforcement changed with NCBI and %Clingers when considering stream size. Any successful sampling and regulatory regime examining NPDES enforcement would need to consider the scale of analysis and stream size.

Three years after enforcement, noticeable improvements in biotic indicators can be seen. I noticed marked improvements in EPT Taxon Richness and NCBI around the three-year mark. As such, it would be essential to measure freshwater community indicators several times before and after the three-year threshold.

More research should be done to explore which metrics are best suited to study enforcement and biodiversity relationships. It is hard for me to deduce with confidence why the particular metrics in my study were most sensitive to NPDES enforcement. NCBI and %Clingers covary with NPDES enforcement only in larger streams. I detected Taxon Richness and EPT Taxon

Richness change with enforcement change in some ecoregions. Why did these metrics have a marked relationship with enforcement in Study I? What are the best metrics to consider when exploring NPDES enforcement on aquatic macroinvertebrate biodiversity? My dataset spanned diverse ecoregions, stream orders, and facility types. However, my dataset of NPDES sources only included major facilities under the CWA. Further, most of these facilities were sewage treatment plants. Appropriate metrics for exploring enforcement's impact on aquatic macroinvertebrate communities could vary based on scale, pollution source type, or other factors.

Limitations due to small sample size

One important limitation of my research is the small data sample size. As seen in Table 4, as my Geographic Information Design became more robust to address more causal connections, my dataset got smaller and smaller. Additional research using more data points in more states, ecoregions, etc. would test the validity of my findings in other landscapes. Further, incorporating NPDES minor sources (*e.g.* small scale processing, construction sites of one acre or more, small sewage treatment plants) would provide a more complete picture of NPDES enforcement's influence.

Understanding enforcement's power in the face of enforcement decline

NPDES enforcement influences macroinvertebrate stream communities in Tennessee and Kentucky in a positive manner for a number of freshwater community indicators. Future analysis including more states and samples can determine if this pattern holds true across the region and beyond. With this potential, consistent permit enforcement could help achieve the national-scale aspirations of the Clean Water Act to enhance and maintain biological integrity.

These results come at a key moment. National enforcement of the CWA is waning and states must consider enforcement regimes. It is important for federal agencies, states, and citizens alike to understand the potential of responsible environmental law enforcement's ability to improve the living waters of streams in our own communities.

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Appendix

Table 10. Study I mean, median, and range of input variables.

Input variable	N	Mean	Median	Range
Enforcement				
- No enforcement	77	-	-	-
- Some enforcement	33	-	-	-
State				
- Kentucky	33	-	-	-
- Tennessee	77	-	-	-
Population Density (persons/sq km)	-	910	420	37.609 - 25264.306
Elevation (m)	-	260	250	82 - 777

Table 11 State regression table for model using for all points (N = 110). My alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$.

Response Variable	β (state)	Standard Error	p-value	Adjusted p-value	R ²
TR	9.328e-02	3.765e-02	0.0148**	0.0888*	0.05798
EPT	6.091e-02	7.513e-02	0.419	1	0.03409
%EPT	-5.591e-02	6.287e-01	0.9291	1	-
NCBI	3.847e-01	3.068e-01	0.2126	1	0.07106
%Clingers	-2.001e-02	5.653e-02	0.7241	1	0.04557
%OC	-2.205e-01	5.525e-01	0.690	1	-

Table 12 Population density regression table for model using for all points (N = 110). My alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$.

Response Variable	β (population density)	Standard Error	p-value	Adjusted p-value	R ²
TR	1.209e-06	5.917e-06	0.8385	1	0.05798
EPT	-4.476e-06	1.180e-05	0.705	1	0.03409
%EPT	-2.723e-05	1.217e-04	0.8229	1	-
NCBI	1.534e-05	4.820e-05	0.7509	1	0.07106
%Clingers	6.634e-06	8.883e-06	0.4568	1	0.04557
%OC	2.247e-05	8.171e-05	0.783	1	-

Table 13 Elevation regression table for model using for all points (N = 110). My alpha thresholds to determine statistical significance were $\alpha = 0.05^{**}$ and $\alpha = 0.10^{*}$.

Response Variable	β (elevation)	Standard Error	p-value	Adjusted p-value	R ²
TR	2.610e-05	1.280e-04	0.8388	1	0.05798
EPT	3.618e-04	2.553e-04	0.159	0.6360	0.03409
%EPT	3.793e-04	2.052e-03	0.185	0.6360	-
NCBI	-1.943e-03	1.042e-03	0.0652*	0.3912	0.07106
%Clingers	3.392e-04	1.921e-04	0.0803*	0.4015	0.04557
%OC	5.203e-04	1.808e-03	0.774	1	-

Table 14 Study II mean, median, and range of response variables.

Response variable	Mean	Median	Range
<i>Decreases with more pollution</i>			
- Δ TR	-4	-3	-11 – 3
- Δ EPT	-3	-2	-11 – 1
- $\Delta\%$ EPT	- 6%	-6%	-35.69% - 23.30%
- Δ %Clingers	4%	-2%	-26.7% - 27.5%
<i>Increases with more pollution</i>			
- Δ NCBI	-0.89	-1.18	-1.88 – 0.64
- Δ %OC	-14.75%	-22%	-65.5% - 34.17%



Figure 20. Satellite view of Study II site pair.

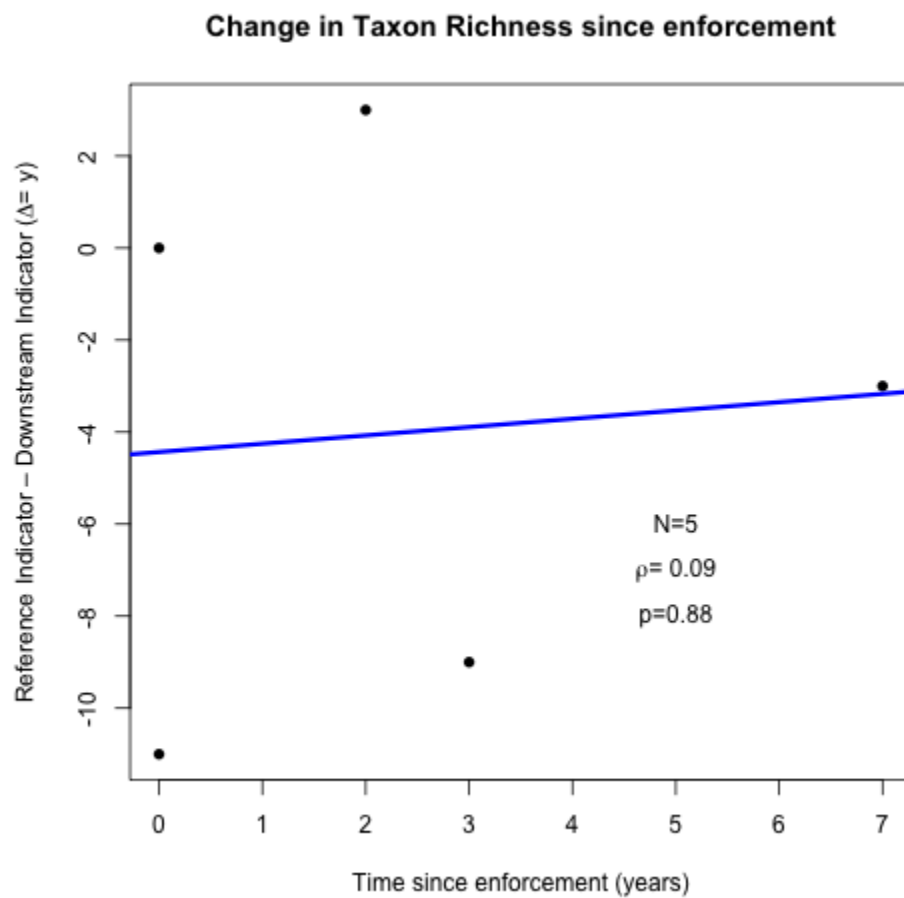


Figure 21. Scatter plot of TR difference and time since enforcement.

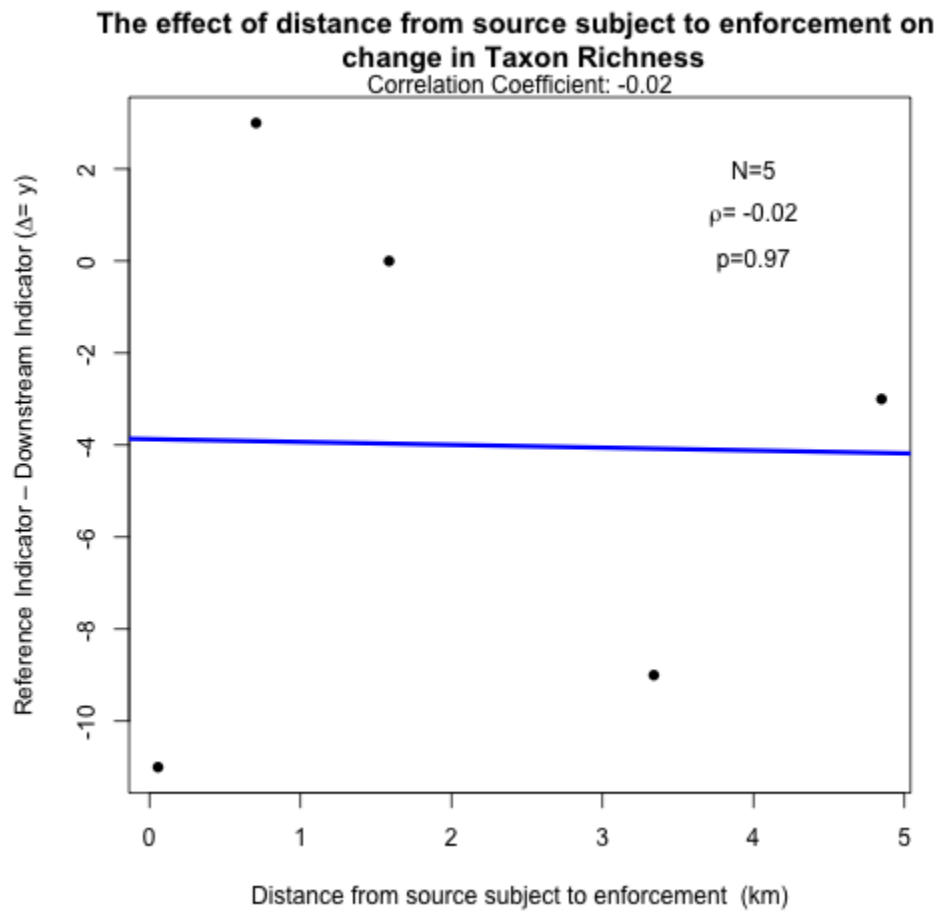


Figure 22. Scatter plot of TR difference and distance from NPDES Source.

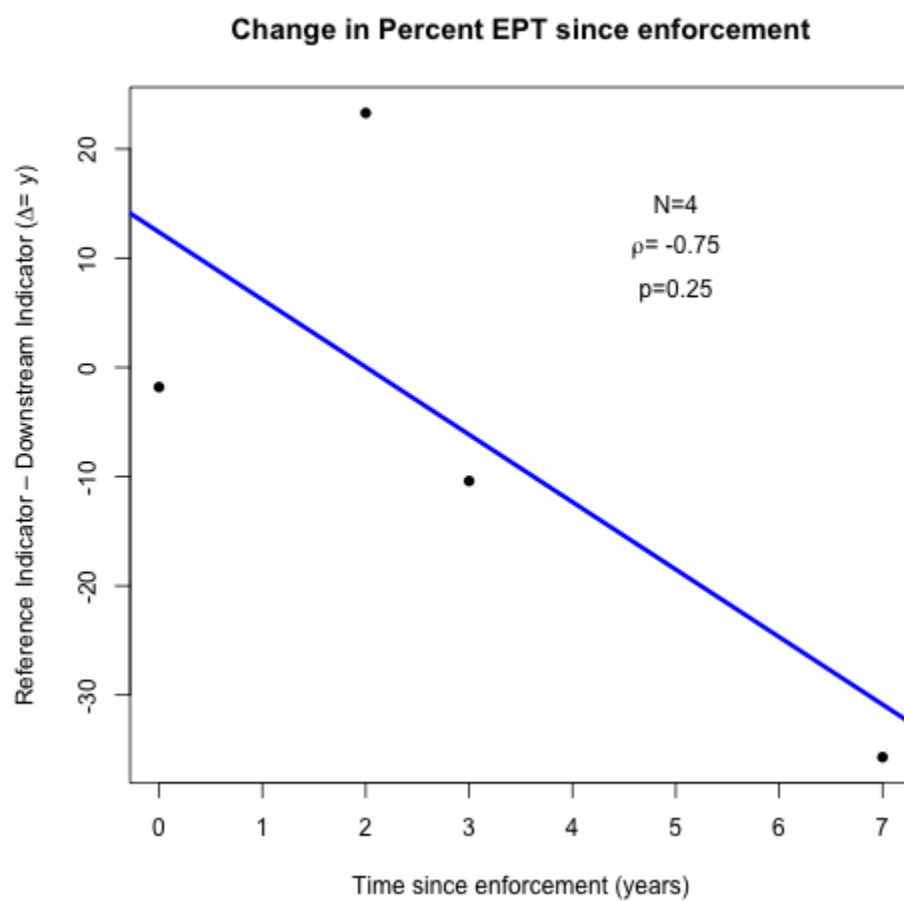


Figure 23. Scatter plot of %EPT difference and time since enforcement.

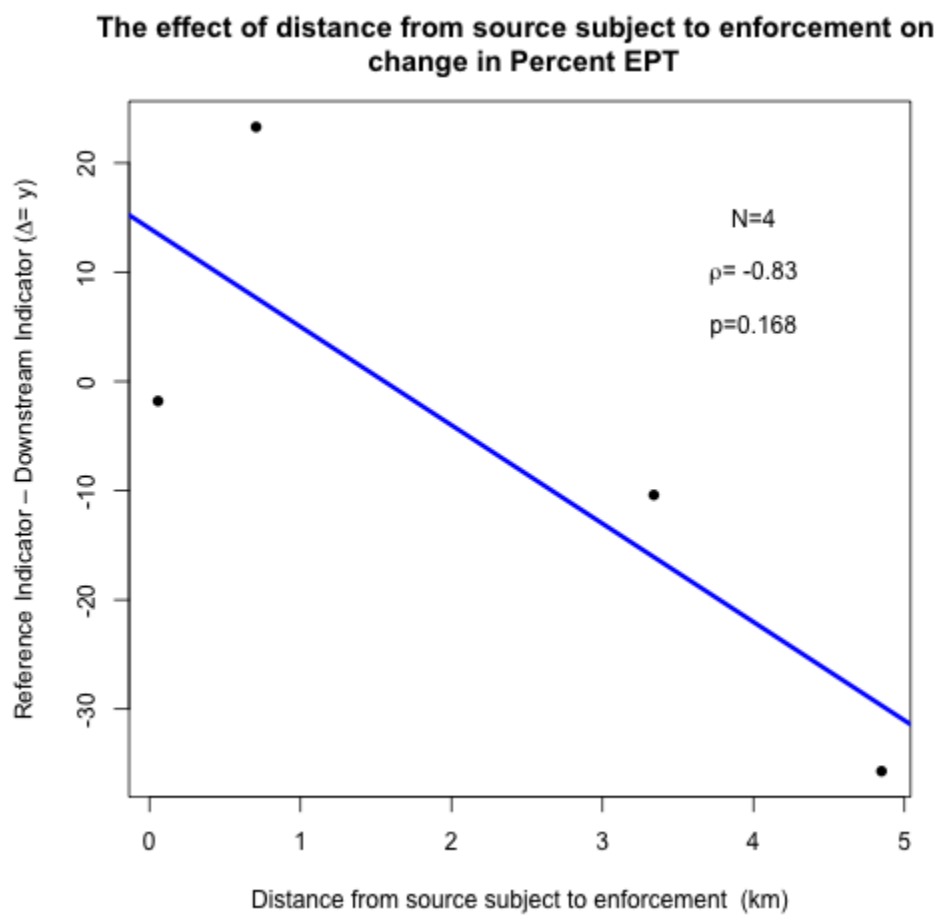


Figure 24. Scatter plot of %EPT difference and distance from NPDES Source.

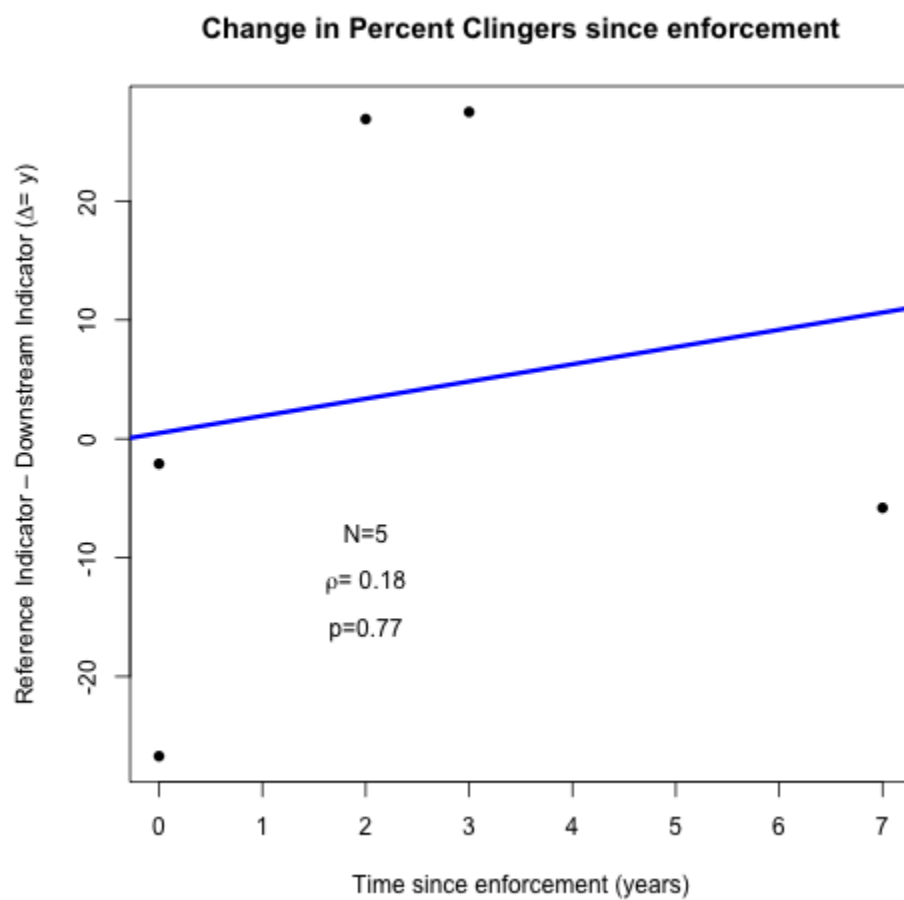


Figure 25. Scatter plot of %Clingers difference and time since enforcement.

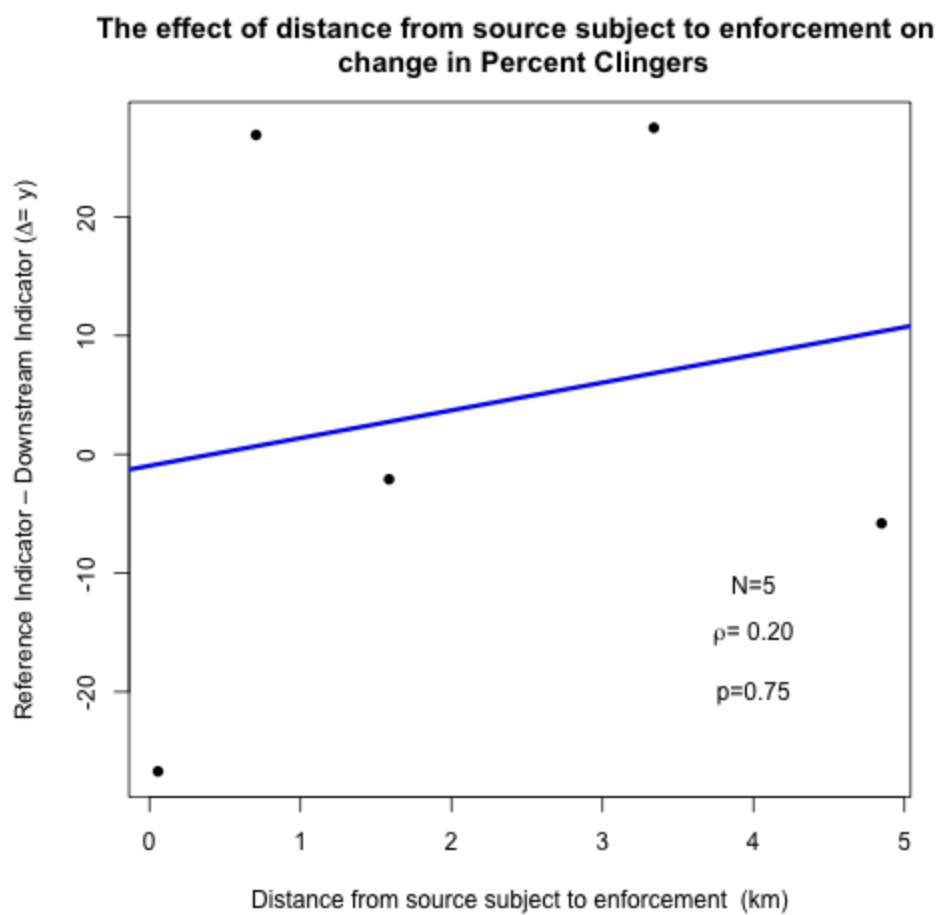


Figure 26. Scatter plot of %Clingers difference and distance from NPDES Source.

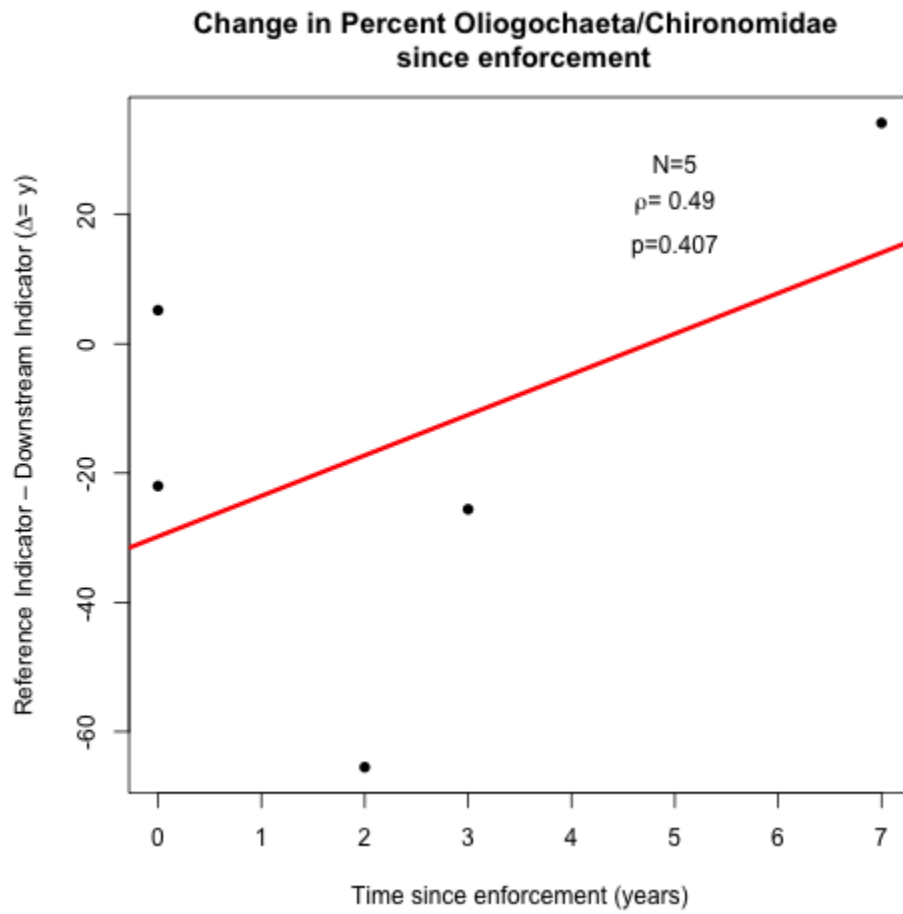


Figure 27. Scatter plot of %OC difference and time since enforcement.

**The effect of distance from source subject to enforcement on
change in Percent Oligochaeta/Chironomidae**

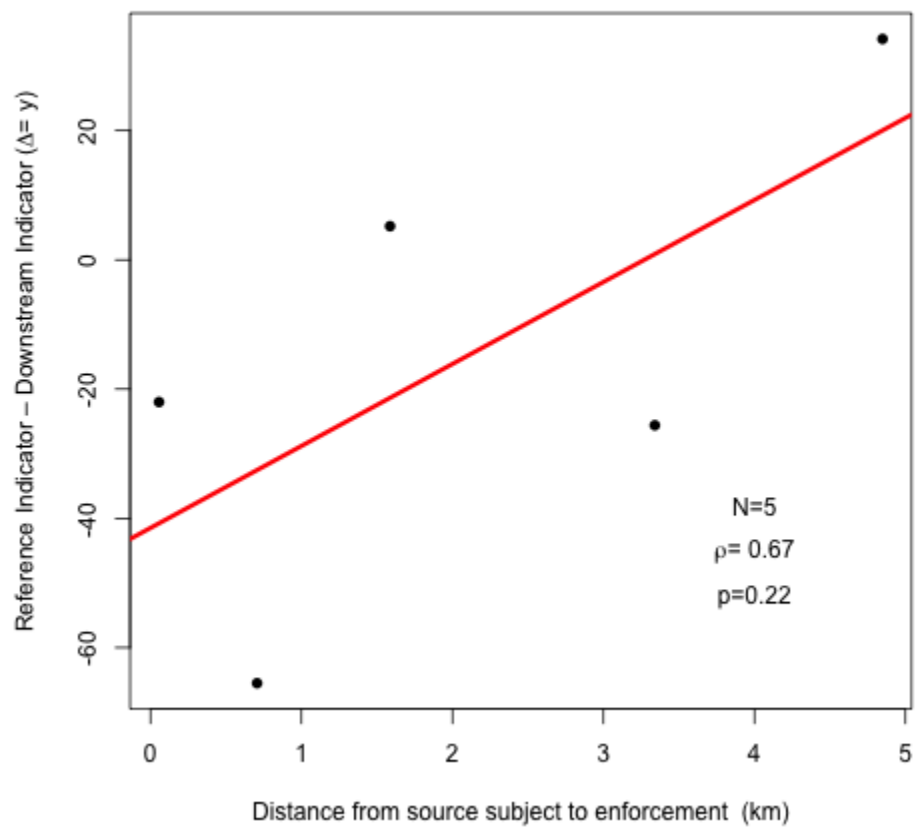


Figure 28. Scatter plot of %OC difference and distance from NPDES Source.

Vita

Shelby R. B. Ward, *Esq.*, is an attorney and aquatic ecologist. Her work focuses on environmental law and freshwater ecosystems. In her solo law practice, Shelby has represented low-income clients in family law disputes and worked with a local environmental non-profits. As a scientist, she explores the impact of Clean Water Act enforcement on macroinvertebrate communities in streams and identifies macroinvertebrates to genus and species.

In her recent past, Shelby acted as an Independent Consultant for the National Parks Conservation Association (NPCA)'s southeast regional office by advising on Clean Air Act related issues. She is also a co-founder of the annual Appalachian Public Interest Environmental Law Conference ("APIEL"). She is a member of the NPCA Next Generation Advisory Council.

Shelby holds a *Juris Doctorate* from the University of Tennessee College of Law and a Bachelor of Science in Biology and a Bachelor of Arts in History from Howard University.

Outside of work, Shelby tweets about law, conservation, and ecology on Twitter (@sw285). She is a native of Wichita, Kansas.