ABSTRACT

Title of Document: RELATING POLLUTANT AND WATER QUALITY PARAMENTERS TO LANDUSE IN A SUBWATERSHED IN THE CHOPTANK RIVER WATERSHED

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Agriculture and animal feeding operations have been implicated as sources of water pollution along the Choptank River, an estuary and tributary of the Chesapeake Bay. This survey examined a subwatershed within the Choptank River watershed for impacts of a poultry facility on its adjacent surface water. Water and sediment samples were collected May – October 2009 under mostly baseflow conditions and analyzed for antibiotics, nutrients, heavy metals, and selected bacteria.

Of the antibiotics recovered, no significant difference was observed spatially, but a significant difference emerged between spring and fall/winter. For nutrients, the greatest phosphorus concentrations were at the subwatershed outlet (4) and at two branches not containing the poultry house (3 and 5); nitrogen concentrations at sites 2 and 5 were as high as site 4. Arsenic concentrations at 2 were lower than both the

low-agriculture (control) site and a site neighboring 3. Bacterial counts in water and sediment remained fairly constant throughout the sampling regime.

RELATING POLLUTANT AND WATER QUALITY PARAMENTERS TO LANDUSE IN A SUBWATERSHED IN THE CHOPTANK RIVER WATERSHED

By

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Dedication

For my parents who taught me to love nature and my mentors who are helping me to understand it.

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Chapter 1: Executive Summary

The Choptank River, a tributary of the Chesapeake Bay, is surrounded by various agricultural practices and has been under scrutiny for impaired water quality. The majority contributor to the poor water quality of this river is speculated to be agricultural facilities and farms, particularly husbandry operations. The intention of this survey was to collect water and sediment samples upstream and downstream of an animal feeding operation to quantify the effects of a single poultry facility on surrounding surface water and micro-ecocosm, measured as the native bacterial species populations of *E.coli* and *Enterococcus*. Pollutants measured include antibiotics, heavy metals, and nutrients. Spikes in representative bacterial populations could be attributed to bolstering from increased substrate availability after runoff events and/or to the influx of these species after runoff events. Results from this survey are intended to assist in gathering data for further investigations, possible understanding of the effectiveness of mitigation efforts mandated by the Federal government, and to assist in decision-making for farmers and policy-makers with regard to nutrient application/management and mitigation implementation.

Chapter 2: Literature Review

2.1 The Chesapeake Bay, the Choptank River, and runoff

The Chesapeake Bay, the largest estuary in the United States (Whitall et al., 2010), is located on the Eastern shore of the United States and is bordered by Maryland, Virginia, Delaware, and the District of Columbia. Its watershed is more extensive, incorporating New York, West Virginia, and Pennsylvania (McConnell et al., 2007). While approximately 58% of the watershed remains undeveloped, 22% is devoted to agriculture (U.S. Environmental Protection Agency, 2010c). According to the Environmental Protection Agency's Guidance for Federal Land Management in the Chesapeake Bay Watershed (2010b), agriculture is responsible for approximately 43% of nitrogen (N), 45% of phosphorus (P), and 60% of the sediment loads released into the Bay. Of this, approximately 17% of N and 19% of P load comes from chemical fertilizers, and 19% of N and 26% of P load comes from manure (U.S. Environmental Protection Agency, 2010b). As such, the Chesapeake Bay has been under scrutiny for water pollution contributing to declining oyster and crab populations, massive algae blooms, loss of submerged aquatic vegetation, and the overall water/organism health among others.

In spite of efforts by the federal government and other non-government organizations to control the amount of pollution entering this important body of water, little dramatic success has been observed. This is partially due to the urban runoff and stormwater contribution to pollution; these sources are the only contributors that are

increasing (U.S. Environmental Protection Agency, 2010b). In May 2009 an executive order was issued by President Obama outlining steps for the restoration and protection of the Chesapeake Bay (Obama, 2009) via (among other points) use of adaptive management for the implementation of current data in decision making, identifying measurable indicators for evaluating environmental conditions, and coordinating programs and strategies among federal agencies for greater effectiveness.

An estuary and tributary of the Chesapeake Bay, the Choptank River is a tidal embayment that spans 2057 km^2 and runs southwest through the eastern shore of Maryland. Since 1998 various segments of the Choptank River have been classified as "impaired waters" under the Federal Clean Water Act due to fecal coliform numbers, nutrients, sediments, and for low scores on the biotic integrity surveys conducted during the 2000-2002 Maryland Biological Stream Survey. Approximately 60% of land use in the Choptank River watershed is devoted to agriculture, producing corn (*Zea mays*), soybean (*Glycine max*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*). Much of this grain supports small- and medium-sized animal feeding operations, mostly poultry with some dairy and horse husbandry. Manure from poultry houses is routinely used as a fertilizer on agricultural fields. Potential pollutants from these activities include sediment, pesticides, nutrients, antibiotics, heavy metals, and non-indigenous microorganisms. Animal feed lots are considered point pollution sources and are subject to the Federal Water Pollution Control Act (or Clean Water Act) of 1972 that created water quality

standards and an accountability system for point sources based on water health limits, industry-specific standards, or technological-based limits (U.S. Environmental Protection Agency, 2010a). However, agricultural fields are non-point sources. Non-point sources are more loosely regulated as they are more of an agglomeration of non-descript origin and liability is harder to assign (U.S. Environmental Protection Agency, 2010e). Their regulation is more in the form of support for State programs that develop methods and practices aimed at non-point source pollution reduction (U.S. Environmental Protection Agency, 2010d).

Runoff as an environmental and public health threat is complex; it can be managed well under ideal conditions, but may be difficult to control under harsher circumstances. Runoff itself is a catchall for soluble or insoluble pollutants that have attached themselves to loose material, susceptible to washing away. The range of substances that fit this description increases classification and management difficulty since the synergy that develops between compounds changes behavior and may be unique to each event. Effective management and prevention requires integration of multiple approaches; management practices should be as diverse as the runoff it is expected to mitigate. Examples of current mitigation strategies for agricultural runoff control/prevention include buffer zones, cover crops, nutrient management plans, and no-till.

In a two year experiment done in Southeastern Spain, Durán-Zuazo et al. (2004) measured the nutrient fluxes of nitrogen and phosphorus in runoff and sediment

coming off of intensely cultivated orchard terraces. Comparing the bare plots to those using sage or thyme as ground cover, runoff and sediment were reduced by as much as 60%. Mitigation strategies should be better tailored to the site itself, flexible, realistic, and economical for greatest effectiveness (Way, 2007). While "blanket" solutions may be easier to understand and follow, they are not as robust as plans that account for the topographical, hydrological, and operational practices of the site.

A healthy freshwater aquatic ecosystem not only supports many trophic levels and organisms in the water, but on land as well. Some important components of a healthy water environment include natural water movement (water body is able to shift flow path season-to-season, includes meanders, flooding, etc.), bank protection (against premature erosiong), appropriate trophic representation (e.g. bacteria, plants, fish, frogs, insects), and good quality water input/head water protection. When natural bed-shifts are obstructed, meanders are straightened, and seasonal flood are prevented, soil fertility decreases, and increases are seen in erosion, sediment movement, and water speed. Banks that are not properly supported after flow path manipulation lead to erosion, possible bank collapse, and an increase in sediment transport downstream, which may lead to the deterioration of downstream surface water and structure. Water quality and pollutant fate are greatly dependent on microorganisms and the relationship they have with their predators (Hahn, 2006). Microbes are the primary degraders of many pollutants, such as oil, gasoline, and antibiotics, and are the backbone of the food web.

How species diversity positively influences ecological processes is not well understood, however, a study done by Cardinale et al. (2002) gives at least one clear example of the ecological differences between systems that have one organism performing a particular function (monoculture) versus one that has many. In streams with a mixed group of suspension feeding hydropsychid caddisflies, six larvae from each of three taxa (*Hydropsyche depravata*, *Ceratopsyche bronta*, and *Cheumatopsyche* sp.) the amount of suspended particle matter consumed was more than 60% greater than that consumed in streams containing only one caddisfly taxa. The scientists' hypothesis that the increase in particle consumption in mixed assemblages was due to facilitative interactions was confirmed when it was found that the mixed assemblage streams had faster near-bed flow and greater catchnet complexity compared to single-taxa streams. In other words, the more complex microcosm outperformed the monoculture (Cardinale et al., 2002). Thus, a decrease in aquatic ecosystem diversity may mean lower water quality and an overall decrease in ecosystem functionality due to the poorer degradation and nutrient cycling capabilities. Headwaters are one of the most difficult resources to protect since many of these streams are very small or hidden and they may not appear on maps used for management (Lowe and Likens, 2005). Headwater contamination may make downstream mitigation strategies less effective since the source has been polluted.

Contamination that would disturb any of these important natural processes might not have a visible or immediate effect on the health of the surrounding ecosystem, but upsetting known and unknown processes may prove ruinous later. For example,

over-harvesting of oysters contributed to the declining health of the Chesapeake Bay and eventually the decline of the population (Rothschild et al., 1994). Stewardship and restraint are important for preventing similar situations so disturbed relationships do not escalate to this magnitude.

2.2 Animal husbandry operations management, runoff, and environmental impact

Animal husbandry is a large contributor to both the economy of the Eastern Shore and water quality issues (Sampson and Morison, 2007; Northcutt and Jones, 2004). Farmers may own their own land and operate a family-farm, possibly leasing additional land for a working farm or operate as contract farmers. Contract farmers typically raise livestock or grow crops for a corporation on a contract (Roth, 1992). Either the farmer or agricultural manager, if the farm is very large, will be in charge of, or manage, soil preparation, tilling, planting, fertilizing, cultivating, spraying, and harvesting the crops. Winter crops, or cover crops, may also be a part of the schedule during the "off-season". For livestock, a contract farmer is responsible for feeding and caring for the animals and must provide land, buildings, equipment, and labor (Roth, 1992). The company for whom the farmer works will provide feed, medicine, and management directions. Contract farmers may or may not own the animals; if they do not, the company will provide them as well (Roth, 1992). Not all livestock farms are birth-to-finish operations; some send their livestock on to finishers where the animals mature and are prepared for market.

To supplement nutrient-poor soil, manure (either produced on-site or imported) or some other nutrient-rich materials are often applied to soils to reestablish fertility for the coming growing season(s) and/or to condition the soil by adding organic matter and structure. Aside from nutrients, biosolids contain (trace) amounts of heavy metals, antibiotics, bacteria, and hormones. Runoff from farms and AFOs may have multiple sources. Field runoff will contain a number of different elements depending on the time of season. If the earth has recently been turned over, runoff will include particulates (loose soil, small gravel, clay), loose seed, pesticides/herbicides, and fertilizer. Any storage facilities will produce small runoff amounts that may contain oil, agrochemical residue, heavy metals from machinery wear, fertilizing material, etc. Animal feeding operations (AFOs) are of particular interest because the high density operations produce high levels of waste concentrating nutrient, metals, and antibiotics, and require a lot of energy input. Previous studies have detected low levels of many classes of antimicrobials in surface and groundwater close to husbandry operations that land-apply their waste, suggesting the waste as a source of antimicrobials (Campagnolo et al., 2002; Meyer et al., 2006). Poultry AFOs, in particular, produce phosphorus in abundance and some level of antibiotic resistant bacteria; antibiotic resistant genes and bacteria like *Salmonella* and *Enterococci* are already known to be transported to surface waters from AFOs (Meyer et al., 2006). The bacteria may leave with runoff or in the gut of free-roaming vectors (e.g. insects) and give rise to more virulent strains of familiar pathogens and other bacteria. In addition, antibiotics uptake by plant and animal tissue has already been proven (Meyer et al., 2006).

Chicken litter removed from poultry houses on the Eastern Shore is commonly piled on a covered clay- or concrete-lined storage pad (Harris, 2010). After every flock of birds, the top few inches of litter from the building is scraped out and moved to the storage pad, a full cleaning (entire removal of bedding) occurring after the 4^{th} to 7^{th} flock (Harris, 2010). Litter is stored until application season, where it may or may not be pelletized before application. Land application of poultry litter is closely supervised, as submission of nutrient management plans is required to ensure that overload or leaching is minimized (Maryland Department of Agriculture, 2010). Weather conditions are also closely observed, as application of these materials will not occur if storms or other harsh weather is imminent. Buffer zones and riparian areas are mandated and encouraged to protect nearby surface and sub-surface water (Maryland Department of the Environment, 2010). Ultimately, the goal is to trap surface material that may wash away and "encourage" the water to percolate down through the soil layers for natural filtration. In areas where the soil is poorly drained (high clay content), mitigation techniques help to slow runoff speed and help disburse the water.

Mitigation is not fool proof. Pollutant infiltration of surface and groundwater from runoff is still a large problem. That is why facility location, storage of manure, and application of manure needs to be done conscientiously, to minimize unintended consequences. And while pollutant degradation is generally beneficial for the environment, the degradation products of some chemicals are more harmful than the

parent compound. This is because the degradation products can be more bioavailable and more toxic. Knowledge and understanding of major transport pathways still need exercise when planning and executing farm operations.

2.3 Type of pollution from poultry husbandry operations and the effects on the immediate environment

Despite the use of mitigation practices, pollutants entering surface water from mixed (poultry) husbandry/agriculture operation include antibiotics, heavy metals, nutrients, and (pathogenic) microorganisms. Some common offenders in these classes are shown in Table 1.

Table 1: Selected pollutants and their land use implications

	Analytes	Indication of…
Antibiotics	Tetracyclines, some ionophores, monensin	Animal husbandry
Nutrients	Phosphorus, nitrogen	Husbandry, field crops, septic systems
Heavy metals	Arsenic	Feed additive, legacy pesticide
Bacteria	*E. coli*, *Enterococcus*	Native species to environment; fecal contamination

The effect most of these constituents (as a pollutant) have on the environment is reasonably understood, but their synergy is not and will be discussed later. The behavior and impacts of antibiotics are still ambiguous.

2.3.1 Antibiotics

Antibiotics are a class of common pollutants in agricultural runoff near AFOs and fields receiving manure. The diet and close quarters of animals in AFOs increases the necessity of antibiotics to prevent flare-ups of bacterial infection propagated by close proximity and to enhance growth of the animal (Schlüsener et al., 2006). Unfortunately, up to 90% of the prophylactic antibiotics taken in are excreted almost immediately afterwards. After the manure is land applied antibiotics are either completely eliminated via mineralization, undergo partial transformation, or are conserved (Schlüsener et al., 2006). One controversy over using drugs prophylactically is antibiotic resistance not only in livestock but in humans as well (Lindsey et al., 2001). Tylosin, for example, is a medication commonly added to livestock feed as a broad range antibiotic. Though tylosin is not given to humans, its structure is similar enough to erythromycin (a common human-administered antibiotic) that the bacteria *Streptococcus* and *Staphylococcus* that have developed resistance to tylosin have also developed a resistance to erythromycin (Lindsey et al., 2001). A classic case in humans is that of penicillin-resistance as a result of overuse and complacency. After its discovery in 1928 by Sir Alexander Fleming, penicillin was a wonder-drug and prescribed for most ailments, whether or not they were related to sickness or infection. This practice led to the downfall of penicillin; by the 1950's and 60's penicillin-resistant *Staphylococcus* became pandemic (DeLeo et al., 2009).

One branch of antibiotics used in animal husbandry operations is ionophores. Ionophores are lipid-soluble molecules produced by many microorganisms that

disrupt ion concentration gradients. Common ionophores used as poultry feed additives include monensin, salinomycin, and narasin, which are all produced by the bacteria *Streptomyces*. These three ionophores are used to treat coccidiosis in broiler chickens, while narasin also prevents necrotic enteritis (Campbell et al., 2006; Kim et al., 2006). Coccidia are spore-forming, single-celled, obligate, intracellular protozoa that infect the intestinal tract of animals. Ionophores are hydrophobic compounds (Kim et al., 2006); their greatest concentration would be expected in the sediment material of water bodies as opposed to the water column. And because ionophores are only used in animal husbandry, their presence may act as a physical marker for the transport of animal pharmaceuticals in the watershed (Kim et al., 2006).

Tetracycline, another antibiotic compound, works by binding to bacterial ribosomes and preventing tRNA access to receptor sites (Lindsey et al., 2001). Tetracyclines, sulfonamides, and macrolides are the most frequently detected pharma groups in the environment (Kim et al., 2006). Tetracycline is not only given to livestock but also humans; it is commonly prescribed for acne. Tetracyclines also sorb very strongly to soil particles, giving them very low mobility (Aga et al., 2005). Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) is commonly added to poultry feed for coccidial control as well as to increase the growth rate of chickens. Most of the roxarsone administered is excreted in manure as the parent material, which when hydrolyzed in the soil, becomes inorganic arsenic. Arsenic is a micronutrient but also a known poison and carcinogen. The levels distinguishing these roles are approximately 12 to 25 µg for nutritional necessity (Hunter, 2008), <5 mg for acute poisoning

(Ratnaike, 2003), and approximately 1 mg/day as a carcinogen (Mass, 1992), though this level may be as low as 0.05 mg/L (or 0.25 mg/day) (Ng et al., 2003). The non-farming community proposed the Poison-Free Poultry Act of 2009 (H.R. 3624) for the amendment of the Federal Food, Drug, and Cosmetic Act to stop the use of this and similar drugs (Chandler, 2009; Botemiller, 2009).

Previous studies for the detection of antibiotics in surface water, specifically in the Chesapeake Bay watershed, have only been detected at very low levels, perhaps due to their hydrophobic nature. Arikan et al. (2008) collected water samples from 15 subwatersheds in Choptank River watershed and from 7 stations on the Choptank River over the course of four different seasons to determine antibiotic presence in water and seasonal variability. One set of sediment samples was also collected. In the 26 river samples, sulfamethoxazole (5 of 26 samples) and sulfadimethoxine (3 of 26 samples) were the only two sulfonamides detected at an average concentration of 0.001 µg/L and 0.002 µg/L, respectively, while chlortetracycline (5 of 26 samples) and oxytetracycline (4 of 26 samples) were the most frequently detected tetracyclines at average concentrations of 0.016 µg/L for both compounds. In the subwatersheds, sulfamethoxazole (3 of 56 samples) and sulfadimethoxine (8 of 56 samples) were detected most frequently at average concentrations of 0.006 µg/L and 0.003 µg/L, respectively in the sulfonamide group, while chlortetracycline (12 of 56 samples) and oxytetracycline (10 of 56 samples) were the most frequently detected tetracyclines at average concentrations of 0.020 µg/L and 0.053 µg/L, respectively. Overall, the concentrations in the subwatersheds were higher than the river; the December

collection yielded the most samples positive for antibiotics. In the sediment samples collected, chlortetracycline (all samples) and sulfamethoxazole (3 of 4 samples) were the two antibiotics seen most often, at average concentrations of 4.6 µg/kg dry weight and 0.10 µg/kg dry weight, respectively. As discussed by Arikan et al. (2008) oxytetracycline, which was the most commonly seen tetracycline in the water column, was not detected in the sediment.

The ability to detect these antibiotics, either as parent or degradation products, makes the most commonly used compounds in husbandry, particularly poultry, facilities good markers for detecting infiltration and runoff into surface waters.

2.3.2 Heavy metals

The largest non-natural sources of heavy metals come from actions and amenities such as water treatment facilities, agriculture, livestock feed additives, machinery, and driving. Heavy metals like arsenic, copper, lead, and zinc are naturally occurring minerals and deposits can be found almost anywhere; their presence in soil and water does not always indicate a dangerous human enterprise. Because heavy metals readily complex with organic matter their greatest concentration would be expected in soil and sediment (Gupta et al., 1996). In previous work, Gupta et al. (1996) determined that heavy metal concentration could be followed by normalizing their presence against various elements. One finding was that heavy metal presence decreased with increasing sampling depth. They also found that heavy metals preferred to complex with sediment material rather than remaining in soil pore water.

In terms of transport, however, surface water and groundwater are the important pathways; hydrology, concomitant redox, sorption, alkylation (Hemond, 1995), and pH determine arsenic movement, form (e.g. As^{3-}, As^{5-}), and whether or not contaminated soil will be a source or sink (Gupta et al., 1996). With the application of manure for the growing season and the heavy rains typical of spring, a large pulse of heavy metals would be expected in surface water. The USGS May 2000 county map shows that approximately 25% of groundwater samples contained arsenic concentrations greater than 1 ppm on the Maryland and Eastern Shore (U.S. Geological Survey, 2009).

Arsenic, as previously mentioned, naturally occurs throughout the environment and in trace concentrations in the human body. Typical anthropogenic sources, both current and historic, include livestock feed additives (e.g., roxarsone, as was mentioned earlier), pesticides (e.g., lead arsenate for the control of the codling moth in apple orchards), smelting (arsenic trioxide is a byproduct), and the burning of fossil fuels. Arsenic is a carcinogen and bioaccumulates, meaning that it is able to enter the food chain from the environment. Children often ingest arsenic when they stick their hands in their mouth after having played in soil contaminated with arsenic. The EPA limit for arsenic is 10 ppb (Table 2) (U.S. Environmental Protection Agency, 2009a), though many natural deposits are much higher than this. Copper, another heavy metal, is used in agriculture as copper sulfate and copper hydroxide to combat vegetable crop fungus and unwanted aquatic vegetation, and in anti-fouling paints to protect boat hulls (Kwok et al., 2008). Though it is an essential micronutrient, copper

is toxic to algae and aquatic invertebrates at elevated levels, 1180 µg/L Cu(I) chloride and 813 µg/L Cu(II) chloride (Kwok et al., 2008).

Table 2: Selected contaminants and their maximum contaminant level values

Contaminant	MCL (mg/L)
Arsenic	0.010
Atrazine	0.003
Copper*	1.3
Fecal coliform, *E. coli*	0
Nitrate-nitrogen	10.0
*	Action Level

To remove arsenic specifically, some wastewater treatment plants oxidize iron to immobilize arsenic by taking it out of the dissolved fraction:

$$Fe^{+2}(soluble) \rightarrow Fe^{+3}(insoluble)$$

$$As^{-3} + Fe^{+3} \rightarrow FeAs$$

$$As^{-5} + Fe^{+3} \rightarrow Fe_5As_2$$

Bicarbonate and phosphate are two other substances that immobilize arsenic; natural water bodies high in alkalinity, iron, and phosphate will bind arsenic so that it is not biologically available. Microbes, however, are able to mobilize arsenic by metabolizing these complexes and releasing arsenic. Unlike antibiotics or other agrochemicals, heavy metals cannot be degraded any further making them dangerous and a nuisance for generations. Like asbestos and lead, arsenic and copper should be phased out of commercial and residential usage in order to protect (general) health and groundwater quality.

2.3.3 Microorganisms

Greater than 90% of the microorganisms in non-extreme aquatic habitats are bacteria (Hahn, 2006), making them one of the most important members of aquatic communities. Disruption of bacterial communities may affect a number of critical processes, such as mineralization and nitrogen cycling. Natural factors affecting overall bacterial community composition in water include water chemistry, water temperature, predation, organic-matter supply, intensity of ultraviolet radiation, habitat size, and retention time (Lindström et al., 2005; Šimek et al., 2001; Crump et al., 2003; Crump et al., 2004; Warnecke et al., 2005; Reche et al., 2005). Viability of the communities is chiefly a function of ultraviolet light exposure and predation/grazing (Brookes et al., 2004; Kashefipour et al., 2006). The effectiveness of solar radiation is depth and turbidity dependent; Brookes et al. (2004) discuss the sharp transition between effective and ineffective UV light. Other characteristics, such as temperature, pressure, and pH do not have large viability effects, as the necessary levels for inactivation are not (normally) reached in natural systems (Brookes et al., 2004). Factors that govern the behavior of individual communities have been more difficult to determine, and little is known about the ecological function of individual species (Hahn, 2006).

Pathogenic bacteria are almost always present in natural surface waters. It is the number in which they exist, antibiotic resistance, and whether or not they are native species that makes their presence a concern. In dealing with animal feeding operations, concerns center not only on release of pathogens from litter/manure into

surface or ground water, but also on zoonoses, the ability of a disease to transfer from animal to human (Gilchrist et al., 2007).

Surface runoff is the major transport process that carries sediment and anything sediment-associated, like chemicals or microbes, off the land and into nearby surface water. Using surface water for irrigation is a practice many farmers resort to when there has been a shortage of rainfall and/or groundwater sources are low. Using surface water to mix with powdered additives for application as a liquid (i.e. powdered pesticides, fertilizers) is another way groundwater resources are conserved during dry times. It has been shown that surface water can be a source of plant pathogen deposition onto the leaves and soil of crops and ornamental plants (Guan et al., 2004; Izumi et al., 2007), where the pathogens may flourish and cause widespread disease, plant death, and soil contamination. Human health is directly affected by the presence of pathogens on food crops—food related illness is a big problem for the food production and handling industry, as well as for medical facilities, who may not be equipped to handle large outbreaks of severe food poisoning.

The direct enumeration of pathogenic species is complex, expensive, and time consuming, making this accurate and direct method little used. Traditional proxy measurements, including particle counting and turbidity, look at related and easy to assess indicator organisms (Brookes et al., 2004). Total and/or fecal coliforms, fecal streptococci, and Enterococci are the indicators commonly used (Brookes et al., 2004;

Kashefipour et al., 2006), though there is argument that these species are not accurate enough in determining real health risk.

Fecal coliforms and *Enterococcus* are the indicator organisms used by wastewater treatment facilities to judge water quality, thus this convention may be hard to change, especially if detection of other (better) indicators cost more time and money. Some other species that may provide a better indication of water quality and to check for AFO contamination are *Cryptosporidium parvum* (for longevity and highly resistant oocysts) (Brookes et al., 2004) and *Clostridium perfringens* spores (resistant to predation, appear in sediment, possibly good conservative indicator) (Brookes et al., 2004). However, these strains are anaerobic and their vegetative forms are hard to cultivate, already detracting from their utility as indicators. Some species related to poultry AFOs are *Bacteroides* group (Meyer et al., 2006), *Bifidobacterium* spp., *Lactobacillus* group, *Veillonella* spp., *Atopobium* spp., and *Campylobacter* spp. (Wise et al., 2007) all of which are anaerobic, opportunists.

Important watershed factors to monitor to understand microbe behavior are soil type, slope, animal density, management practices, and stream flow data (Meyer et al., 2006). The zeta-potential, or aggregation ability, of *Cryptosporidium* oocysts affects settling time. If runoff contains a large amount of suspended material, the oocysts will bind to the inflow particulates, thereby increasing their settling velocity by a factor of two (Brookes et al., 2004). Settling time also has an effect on viability because oocysts are able to travel to colder, darker waters more quickly (Brookes et

al., 2004), improving their chances of survival. Resuspension of *Cryptosporidium* is also likely as they have a strongly negative zeta-potential in neutral pH, making adsorption to clay material in sediment unlikely (Brookes et al., 2004). Though more than 90% of fecal coliform bacteria die within 4 hours of estuary release (Sherwin, 2000; Kashefipour et al., 2006), fecal bacteria typically settle out to sediment and may survive for weeks to months after a waste effluent spill (Burkholder et al., 2007). Resuspension depends on the magnitude of turbulence in the benthic layer, as enough force is needed to break the bed shear (critical turbulent velocity) in order to release bound cells (Brookes et al., 2004).

2.3.4 Nutrients

Nitrogen and phosphorus are essential life nutrients that regulate many cell functions. In abundance, however, they can cause population booms of aquatic plants and other autotrophs (eutrophication), which can lead to other problems such as hypoxia (reduced dissolved oxygen), leaching (loss of nutrients/minerals due to excess rain or irrigation), algae blooms, and fish kills (the result of low dissolved oxygen in the water). The current maximum contaminant level for nitrate-nitrogen is 10 ppm (U.S. Environmental Protection Agency, 2009a). Nutrient pollution comes from many different sources. According to the U.S. EPA (2009b), the most common contributors of excess nitrogen and phosphorus are fertilizers (commercial/agricultural and residential), runoff from croplands, AFO facilities, and urban/suburban areas, wastewater treatment plant discharge, and overflow/leaks from septic systems. According to Gupta et al. (1996), high levels of nitrogen in the water column usually

indicate the presence of a sewage treatment plant, whereas high organic matter content refers to agricultural runoff.

Phosphorus and nitrogen follow different transport pathways; phosphorus tends to move with surface runoff, whereas nitrogen travels downward through soil and then moves out with groundwater. A study done by Chaubey et al. (2007) examined the interaction of phosphorus and nitrogen with benthic sediments and how these nutrients were transported in-stream. They found that there was a seasonal shift in sediment behavior in their study site, a pasture dominated watershed in Arkansas. During the winter and spring months, sediment appeared to be releasing dissolved inorganic P, whereas during the summer and fall, sediment acted as a sink; the amount of P exchange ranged from 0.4 to 1.0 mg/kg in dry sediment. Because no significant retention of nitrate-nitrogen was seen, Chaubey et al. (2007) deduced that the headwaters in the agricultural lands might be a source of downstream nitrate-nitrogen transport. Finally, they concluded that the stream's ability to assimilate nutrients was the deciding factor in the magnitude and behavior of transported nutrients (Chaubey et al., 2007).

In a related experiment, Edwards et al. (2000) determined that nutrient runoff was greatest when precipitation occurred after a long dry spell. They also found that N and P reacted differently to forage height; N runoff was greatest when forage was at its tallest, whereas P runoff was greatest when forage was shortest. As in the previous study, the ability of the surrounding environment to assimilate nutrients is

vital to pollution control. By managing the surrounding vegetation, targeted nutrients can be minimized in runoff.

Controlling nutrient pollution is a key factor in managing overall water health. Low oxygen presence affects all levels of aquatic life and can be directly attributed to the abundance of certain aquatic autotrophs. High levels of submerged aquatic vegetation indicate good water clarity and light penetration, and healthy levels of algae, which in turn is indicative of health nutrient levels.

2.3.5 Pollutant synergy and current limitations

Studies of pollutant synergy show a positive correlation between the presence of xenobiotics and organism/environmental health. Though the chemical mechanisms of compound interaction are not well understood, certain combinations of different classes are known to either amplify or have no effect on environmental impact. However, species differ in sensitivity and reaction to these combinations.

A recent experiment by White et al. (2010) focused on four peanut fungicides and how each would affect the dissipation kinetics of metolachlor (a pesticide), the soil microbial community, and the efficacy and environmental fate of metolachlor. Metolachlor is typically applied at planting time and is followed by fungicide application once the crop emerges. White et al. (2010) determined that the fungicide chlorothalonil dramatically increased the half-life of metolachlor from 56 to 99 days, compared to the other fungicides, which seemed to increase metolachlor's dissipation.

Significant reductions in the metolachlor-related metabolites, metolachlor ethane sulfonic acid (MESA) and metolachlor oxanilic acid (MOA) were also observed in the chlorothalonil treated soils indicating a modification in the glutathione degradation pathway. Minimal impacts on soil microbial activity were observed based on lipid biomarker analysis. Overall, it was strongly suggested that chlorothalonil could increase the soil persistence and alter detoxification processes related to the metabolism of metolachlor and possibly other agrochemicals with similar degradation pathways (White et al., 2010).

Investigations done in 1981 by Anderson et al. exposed clams to sublethal doses of benzo[a]pyrene, hexachlorobenzene, and pentachlorophenol for 18 weeks to examine the physiological effects (especially the ability to resist bacterial infection) of chronic exposure to pollutant levels that do not necessarily increase mortality or morbidity. They found that clams exposed to these compounds were unable to clear infections completely, and that clearance was not dependent on duration of exposure, but on the amount of burden the clam tissue carried. In other words, the clams' ability to resist bacterial infection was impaired by the presence of pollutants (Anderson et al., 1981).

A similar study by Cedergreen et al. (2006) found that the herbicide prochloraz increased the inhibition of ergostrol (a precursor of vitamin D_2) when combined with certain pesticides, though the inhibitory effect was not consistent across the range of species studied. In some cases, prochloraz increased the potency of some insecticides (by lowering the concentration needed for 50% immobilization). This is cause for

concern, given that many of the pesticides studied are often applied together and the amount put on is not adjusted for this increase.

In a wider investigation done by Bocquené et al. (1995), the effects of several classes of pesticides on the biomarker acetylcholinesterase (AChE) were examined in four different marine species: dragonets (*Callionymus lyra*), soles (*Solea solea*), prawns (*Palaemon serratus*), and oysters (*Crassostrea gigas*). The pesticides tested consisted of three organophosphorus (OP) compounds, two carbamates (C), two organochlorines, atrazine (a triazine), and isoproturon (a urea substitute), along with the following metal-chloride compounds: zinc, cadmium, mercuric, methylmercury, and tributyltin chloride, and arsenite (As_2O_3). Toxicant interaction was based on how much of each toxicant was needed to achieve at least 50% inhibition; thus combinations were either additive, synergistic, antagonistic, or ineffective (no interaction) (Figure 1).

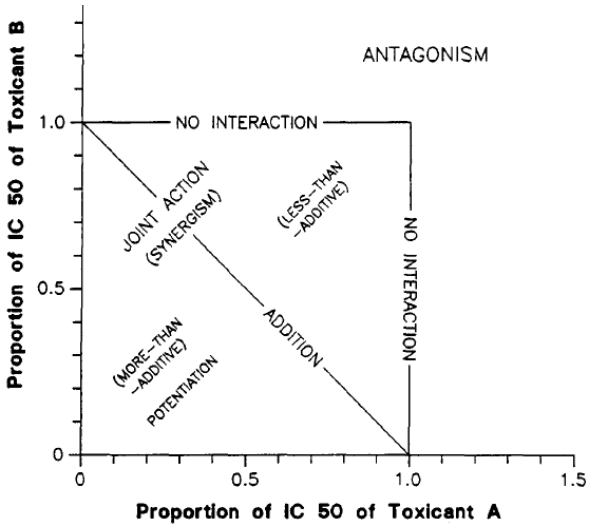


Figure 1: Joint action of two toxicants. Diagram from Anderson et al. 1981, originally modified from Gaddum et al. 1948

Of the various compound combinations, all of the OP-C pairings were synergistic and very strongly so, meaning that the combined effect of the compounds was much greater than the effect of each compound alone. Of the various metals, arsenic from arsenite was the only one that enhanced the synergy of the OP-C pairings. In the end, duration of exposure greatly influenced the detrimental effects of the mixtures and it was the dragonet that showed the most sensitivity to exposure (Bocquené et al., 1995). This experiment, unlike the one conducted by Anderson et al. (1981), not only investigated the interaction amongst different pesticides, but also their interaction with other classes of pollutants, like heavy metals. Cross-effects of different classes of pollutants (nutrients, pesticides, heavy metals, pathogens, antibiotics) have not been widely studied, though they are the most environmentally relevant. Natural relationships, like pollutant synergy, are very complex and involve a lot cross-interaction and feedback loops. Though the specific mechanisms that govern the synergistic relationship itself may not be important, a general understanding of broad associations is much needed.

Chapter 3: Site selection and study design

A sub-watershed within the Choptank River watershed was examined more closely to study the impact of a poultry facility on the adjacent surface water. The surveying sites were selected because of the relatively simple layout of the surface water network (Y-shaped, few bends and branches), the close proximity of the (single) poultry operation to the surface water, and the presence of a ditch running from the poultry facility into the nearby surface water. The land adjacent to the poultry operation is used for agriculture (corn and soybeans), the sampling sites are accessible via public land, and there is historic data for the subwatershed outlet since 2004. Land nearby the poultry facility is small suburban, allowing for the comparison of poultry/no-poultry effects. Also, the sampling sites are located on well-drained soil.

The simplicity of the stream layout is important for flow and pollutant distribution modeling, as well as for determining general sources for the pollutants measured. Because there are only two large branches, there is little interference carried into the water body from other parts of the subwatershed. The single poultry operation allows for a better understanding of the impact an "individual" has on the surrounding environment and if this can be extrapolated to understand areas that have multiple operations on similar land. The sites on neighboring branches help describe the cumulative effects seen at subwatershed outlet, as the activity seen there is not only from agriculture and husbandry, but from a small suburban population as well.

Finally, the biosecurity of each facility is extremely important as cross-contamination may cause outbreaks of disease, and therefore, flock loss. In this sense, to gain (safe) access to samples, it was deemed best that sites should be accessible from public areas, to avoid biosecurity concerns.

Water grab samples and sediment samples were collected immediately upstream and downstream of the poultry operation (sites 1 and 2, respectively), at the outlet of the sub-watershed (site 4), as well as on pertinent branches of the stream system (sites 3, 5, and 6), under approximately baseflow conditions (Figure 2). A seventh site located near the sub-watershed was used as a control, where very little agricultural or other human activities have occurred over the past 30 years. This control site is the same used by McCarty et al. (2008) and Whitall et al. (2010). Water quality parameters were measured at all sites. Samples were collected during the field production season (May – October) for one year. GIS data were obtained for all sites and were used to determine their proximity to water ways, hydrology, and surrounding land use. This project was designed to be used in conjunction with data collected for the Conservation Effects Assessment Project (CEAP).

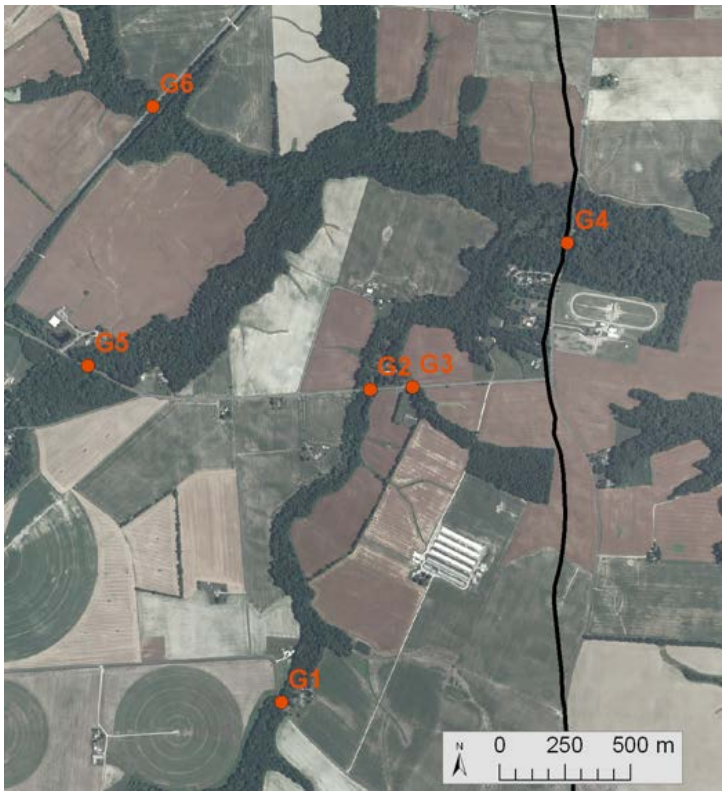


Figure 2: Aerial view of sampling sites; control site (G7) not shown

3.1 *Survey focus*

The focus of this environmental survey was to determine if a single poultry operation had a measurable effect on the surrounding environment, particularly water quality; to test for the appearance of agriculturally-related chemicals downstream of a poultry operation, to assess the potential relative contributions of poultry-related chemicals to a single operation, to observe the mitigation practices used to prevent surface water contamination, and to compare findings to other agriculturally-related contributions. The survey also addresses the issue of whether suspected contaminants are actually present and whether seasonality and on-the-ground practices affect measured concentrations.

The potential customers of the results from the survey are land use managers attempting to implement mitigative measures in a complex setting and scientists/policy makers who seek to create sound policy protecting land and water resources. Findings from this research should also help prioritize where maximum benefits can be achieved, especially in terms of cover cropping, managing runoff, and manure application, and how to treat other areas with mixed-use land.

3.2 *Sampling protocol*

3.2.1 Water sampling

Water samples for pesticides and antibiotics analysis were collected in soap-washed/methanol-rinsed 19 liter stainless steel cans using a stainless steel bucket. Before filling each can, the bucket was rinsed with the water to be sampled and emptied aside to prevent cross contamination between sites. Samples were collected from the center of the stream, closest to the culvert where YSI readings and depth measurements were taken. Collected samples were kept on ice to prevent degradation while in the field and during transport. In the lab, samples were stored for no more that 24 hours at 4°C until processed. Water samples for nutrients were collected using a stainless steel bucket and stored in clear, acid-washed 250 mL screw-top bottles, in duplicate. Bottles were kept on ice, transported to the lab, and then stored at 4°C. Processing took place no more than 24 hours after collection.

Water samples for bacteria analysis were collected in 500 mL screw-top amber bottles that had been previously soap washed, methanol rinsed, and baked for 4 hours.

Samples were collected in the middle of the water column either at stream center and/or above the depositional zone. Samples were kept on ice during transport to the lab, where stored at 4°C. Sample processing was conducted no more than 48 hours later. Water samples for metals analysis were collected in acid washed, 150 mL HDPE Nalgene plastic bottles, each containing 1 mL 1N nitric acid to prevent sample degradation. These samples were not temperature or time sensitive and therefore kept at ambient temperature until analyzed, usually within 1 week of collection.

3.2.2 Sediment sampling

Sediment samples collected for antibiotics and bacterial analysis were gathered using wide-mouthed, 150 mL amber jars which had been soap-washed, methanol rinsed, and baked for 4 hours. The top 4-6 cm of stream bed was collected, using the jar itself to scoop up the sediment. Care was taken to collect the easily-disturbed top layer, along with the water directly above the sediment. Samples were taken from the middle of the stream and from the depositional zone. Samples were kept on ice in the field and then stored at 4°C until processing. Bacterial analysis was conducted within 48 hours of collection, while antibiotics analysis was conducted within a week of collection. Sediment samples could have been frozen for longer storage, but a week limit was imposed to prevent sample accumulation.

3.3 *Materials and methods*

3.3.1 Bacterial methods

Water samples were shaken to re-suspend all bacteria and particles that may have settled during storage. Appropriate sample volumes were added to IDEXX 100 mL sample bottles, according to detection limits of Colilert-18 and Enterolert test kits (IDEXX Laboratories, Inc.). Two dilutions were made using sterile deionized (DI) water. Each dilution was treated as a replicate and separate sets were prepared for each group of interest (*Enterococcus* or *E. coli*). The appropriate nutrient indicator was added to each sample; bottles were shaken by hand to completely dissolve indicator and then poured into labeled IDEXX trays (IDEXX Quanti-Tray/2000, IDEXX Laboratories, Inc.). Trays were sealed using an IDEXX Quanti-Tray sealer and incubated at either 35°C for 18 hrs (*E. coli*) or at 41°C for 24 hrs (*Enterococcus*). Quantification was conducted by counting positive (fluorescent under long UV radiation) wells and interpreted using the MPN table provided with the trays.

A water layer was part of some sediment samples; this was gently poured off and saved for later analysis (same methodology as water samples). Sediment samples were prepared according to modified standard methods (Camper et al., 1985; U.S. Food and Drug Administration, 2002; Garzio-Hadzick et al., 2010). For each sediment sample, 10 g was weighed and added to a blender with 100 mL sterile DI water. This mixture was blended on high speed for 2 min, poured into a 250 mL beaker, and set aside to settle for approximately 1 hr. After settling, the appropriate volume of supernatant was added to a 100 mL IDEXX bottle, according to detection

limits of Colilert-18 and Enterolert test kits (IDEXX Laboratories, Inc.) and diluted with sterile DI water. Each of the two dilutions was treated as a replicate and separate sets were made for each group of interest (*Enterococcus* or *E. coli*). The appropriate nutrient indicator was added to each sample and bottles were shaken by hand to dissolve the indicator completely before being poured into labeled IDEXX trays (IDEXX Quanti-Tray/2000, IDEXX Laboratories, Inc.). Trays were sealed using an IDEXX Quanti-Tray sealer and incubated at either 35°C for 18 hrs (*E. coli*) or at 41°C for 24 hrs (*Enterococcus*). Quantification was done by counting positive wells and interpreted using the MPN table provided with the trays.

3.3.2 Heavy metals methods

Samples were prepared and analyzed according to previously modified methods (Anderson and Isaacs, 1995; Arikan et al., 2008). For each water sample, 100 g was weighed out into acid-washed 200 mL beakers. Beakers were evenly spaced on a hot plate and heated until water was gently evaporating, but not boiling. Samples were reduced to 2 mL and then removed from the hot plate. After cooling to room temperature, the beaker walls were gently rinsed with a small amount of 1N HNO_3, swirled, and then emptied into a labeled 10 mL volumetric flask. This was repeated until flask volume was reached. Two sets of arsenic standards and blanks were included among each sample batch as method standards. Each volumetric flask was inverted twice to mix the sample fully before taking a 4 mL aliquot and transferring it to a labeled falcon tube. To this 4 mL volume, 1.5 mL hydrochloric acid, 2.0 mL of a 5.0% potassium iodide in ascorbic acid solution, and 2.5 mL of a 1.73M sulfamic acid

solution was added. Sample tubes were tightly covered with parafilm, vortexed, and set aside for 15-20 minutes before running. Samples were run on an Optima 4300 DV ICP-OES (Inductive Coupled Plasma- Optical Emission Spectroscopy) with four instrument standards (5, 10, 25, 50 ppb), two instrument "check" standards (1.0, 2.5 ppb), two quality checks (5.0, 50.0 ppb), and a blank. A wavelength of 188.79 nm was used for detection on the ICP-OES with a detection limit of 0.14 µg/L as described by Anderson et al. (1995).

3.3.3 Nutrients methods

Nutrient analysis was done using methods previously established for CEAP samples (McConnell et al., 2007). Total suspended solids (TSS) were measured concurrently. Samples were first left to warm up to room temperature after having been stored in refrigerator. Using forceps for handling, Pall GN-6 Grid filter membranes (d. 47 mm, 0.45 µm posre size) were weighed, recorded, and placed grid-side up on a filter head. Samples were shaken, 100 mL measured, and passed through the membrane. Filters were then removed and placed in aluminum pans for drying. When completely dry, each filter was re-weighed for TSS measurement. The filtrate was divided between a 5 mL borosilicate test tube for phosphate measurement and a 10 mL scintillation bottle for ammonia/nitrate/nitrite measurement on a Lachet QuikChem FIA+ 8000. All glassware and graduated cylinders were washed with DI water three times between replicates. A persulfate digestion (Pote and Daniel, 2000) was carried out using 50 mL of unfiltered sample for total phosphorus measurement: 50 mL of a well shaken sample was added to a 250 mL Erlenmeyer flask, along with 0.5 g potassium

persulfate and 1 mL 0.3% (v/v) sulfuric acid/water solution. Flasks were boiled until liquid was reduced to approximately 10 mL. After cooling, sample was transferred to a 50 mL volumetric flask and brought up to volume with DI water, after which 20 mL of this volume was transferred to another vial. Concentrations were measured colormetrically on a Lachet QuikChem FIA +8000.

3.3.4 Antibiotics analysis

Antibiotic extractions and analysis from water

Water samples for antibiotics analysis were collected concurrently with pesticide samples; duplicate 1 L samples were collected in baked 1 L amber bottles. Analysis proceeded according to methods previously used by Arikan et al. (2008), Campagnolo et al. (2002), Hirsch et al. (1998), and Lindsey et al. (2001). In brief, samples were augmented with 1 g (1 mg/mL) tetra-sodium EDTA and pH was adjusted to approximately 3.0 with concentrated sulfuric acid. Blank-spikes and samples-spikes were supplemented with a solution of monensin, tetracycline, oxytetracycline, chlortetracycline, doxycycline, narasin, lasalocid, salinomycin, and monensin in neutralized methanol for a final concentration of 2 ppm prior to EDTA addition. Samples were rapidly stirred until EDTA completely dissolved. Extraction was done by drawing the samples by vacuum (20 mL/min) through a 500 mg HLB Oasis cartridges from Waters, pre-conditioned using 6 mL methanol, 6 mL 0.5 N hydrochloric acid, and 6 mL water. After extraction, cartridges were rinsed with 12 mL water to remove excess EDTA. Samples were eluted with 15 mL methanol then concentrated under nitrogen gas to 0.5 mL. Volume was brought back to 1 mL

using DI water and transferred to a 2 mL amber autosampler vial. Simetone was added as an internal standard (0.1 ppm) to each vial before storage at -20°C until analysis. Samples were run on a Waters 2695 LC and Micromass Quattro Ultima MS with an electrospray source using an XBridge C18 5 µm 2.1 x 150 mm column from Waters in conjunction with an XBridge C18 5 µm 2.1 x 10 mm guard cartridge. Both positive and negative ionization modes were used for detection. Solvent A was 70:30 1% formic acid: methanol, solvent B was 100% water, solvent C was 50:50 water: methanol, and solvent D was 100% methanol. LC conditions were set to flow: 0.250 mL/min, flow ramp: 2.0, column temperature: 45 °C, and an injection total volume of 20 µL. The solution profile was 0.0 - 1.0 min 50% A and 50% B, from 1.0 - 13.0 min 50% A and 50% B, from 13.0 - 15.0 min 70% A and 30% D, from 15.0 - 20.0 min 7% A and 93% D, from 20.0 - 21.0 min 7% A and 93% D, from 21.0 - 30.0 min 100% D, from 30.0 - 31.0 min 100% D, and from 31.0 - 40.0 min 50% A and 50% B. Samples were diluted 10-fold with 50:50 methanol: water for peak clarity and noise reduction. Ionization gases used were argon and nitrogen.

Antibiotic extractions and analysis from sediment

Excess water was removed from sediment samples to reduce moisture to less than 12% (Schlüsener and Bester, 2006). Whatman Glass Microfibre filters (d. 70 mm, 0.7 µm pore size) were weighed dry and recorded. 10 mL DI water was added to filters under vacuum until dripping almost completely stopped, then weighed wet and recorded. After pouring excess water onto filter under vacuum, sediment was mixed and added to filter except for 5 g which was set aside for moisture content analysis.

Dried sample and filter were reweighed and weight was reduced to 20-50 g, depending on amount of original sample. Accelerated solvent extraction (ASE) cells (33 mL) were loaded with two glass fiber filters, followed by 10 g baked sand (Fisher), the sediment sample plus filter, and 20 µL of 5 ppm simatone spike before being packed tightly with more sand. Samples were extracted with a Dionex ASE 300 using methods described by Schlüsener and Bester (2006). ASE methods used 1% (v/v) aqueous ammonia in methanol under the following condtions: preheat: 0 min, static: 10 min, flush: 70%, purge: 180 sec, cycles: 2, pressure: 140 bar, temperature: 80°C. Glacial acetic acid (150 µL) was added to extracts and mixed on a vortex for 15 sec. Volume was reduced to 5 mL under nitrogen gas in a 60 °C water bath, brought up to 20 mL with DI water then concentrated again to 10 mL. Extracts were further cleaned before analysis using methods from Schlüsener and Bester (2006). Diol SPE cartridges from UCT (2000 mg/15 mL) were conditioned using 10 mL methanol and by 10 mL water before use. Extracts were passed through at about 5 mL/min under vacuum then rinsed with 10 mL water. Cartridges were then eluted twice with 4 mL of acetonitrile: 0.1 M aqueous ammonium acetate (3:2 v/v) each time. From this eluate, 0.8 mL was transferred to a 2 mL autosampler vial for LCMS analysis using previously described conditions.

3.3.5 Statistical analysis

A one-way analysis of variance (ANOVA), nonparametric Kruskal-Wallis test was run on all samples, as more than three unmatched groups were compared. Gaussian distribution is not assumed. The Kruskal-Wallis test ranks all values, lowest to

highest, and assigns each value a rank from 1 (smallest) to *N* (the total number of values). A single value (Kruskal-Wallis statistic) is then calculated from the discrepancies among the rank sums, *K* (Equation 1) (Devore, 2004). Here, *i* is a random sample (for example, you will have *I* number of groups), *J* is the size of the groups (number of values), and $R_{i\circ}$ represents the total of the ranks in the *i*th sample.

$$K = \frac{12}{N(N+1)} \sum_{i=1}^{I} \frac{R_{i\circ}^2}{J_i} - 3(N+1) \qquad \textbf{Equation 1}$$

Finally, *K* is used to calculate significance in equation 2 (Devore, 2004),

$$k \geq x_{\alpha,I-1}^2 \qquad \textbf{Equation 2}$$

where α is significance level and $x_{\alpha,I-1}^2$ can be found in a critical values table for chi-squared distributions.

3.4 *Sources and propagation of error*

Sources and propagation of error in this study include warped glassware due to its repeated baking for decontamination, improperly cleaned glassware, inaccurate pipets and contaminated tips, indirect measurement of volume (e.g., 4 L volume for pesticide analysis is measured by filling bottle to volume height of a factory-filled solvent bottle), balance/scale error (some weights were very small and had to be scaled up to obtain a more accurate weight reading), rounding, reagent differences due to brand, reagent/solution and dilution preparation (if either weight of dry reagent

added to solution or volume were inaccurate), reagent age, cartridge imperfections (used for analyte extractions and extract clean-up), inaccurate prediction of baseflow conditions, possible cross-contamination of samples when stored in the ice chest for transport to the laboratory, lack of completely mixing or shaking samples before analysis, and improper refrigeration of samples (at one point samples had to be relocated to several refrigerators in other buildings since the lab refrigerator was not holding the proper temperature).

Chapter 4: Results and discussion

Environmental analysis involves looking for subtle relationships among many different constituents. Ten pollutants were measured as indicators of water/ecological health and impact, and synergy; 9 antibiotics, 2 classes of nutrients (phosphorus and nitrogen), 1 heavy metal, and 2 bacterial species. Samples were taken from seven different sites over the course of three seasons (spring, summer, and fall); Figure 3, below, illustrates weather conditions. Appendices A-D shows all data for measured constituents, broken down by pollutant class (antibiotics, biological, heavy metals, and nutrients). In total, over 680 environmental samples were analyzed and were compared both spatially and temporally. Each pollutant was first assessed separately,

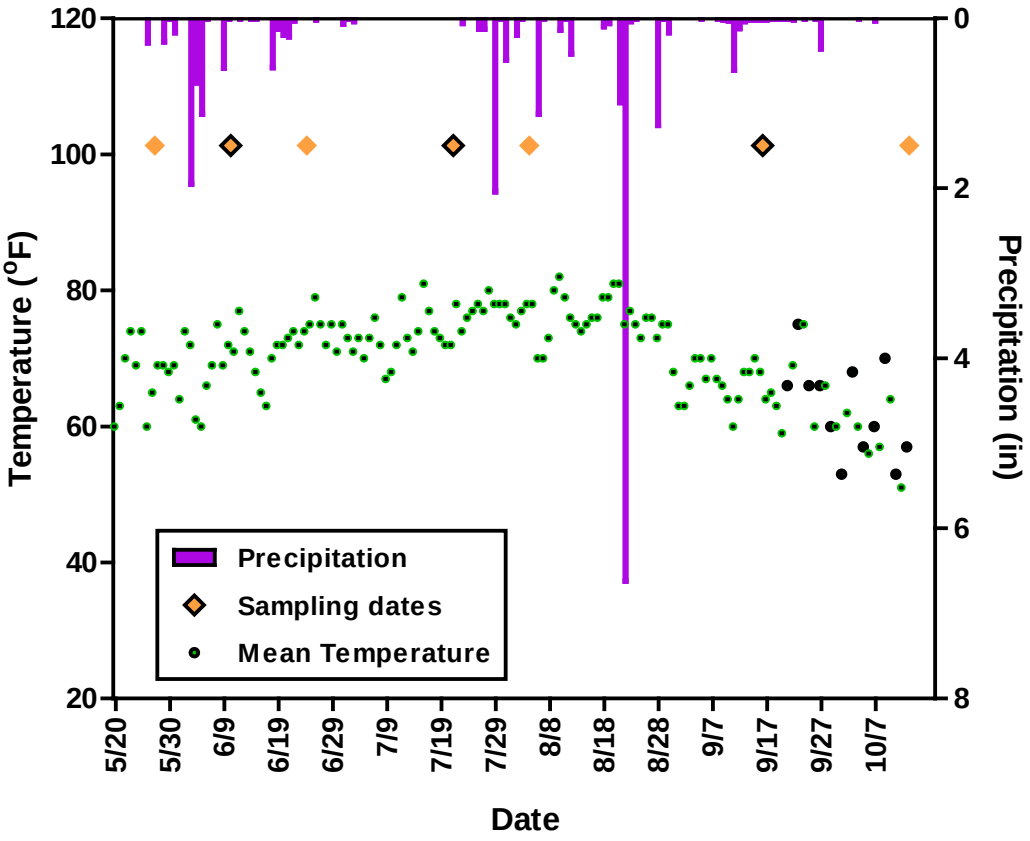


Figure 3: Temperature and precipitation for 2009 sampling season

to determine any "internal" connections then comparisons were made across pollutant class to look for larger associations. One-way, non-parametric ANOVA were run for each data set and resultant pair/p-values are shown on each figure for that particular constituent. Virtually no significant temporal changes were observed in phosphorus or nitrogen concentrations (Appendix E), indicating that land application of manure and other soil amendments in the spring did not cause a spike in the presence of these constituents in the water column. However, as baseflow conditions were mainly observed, runoff of these constituents may have been missed.

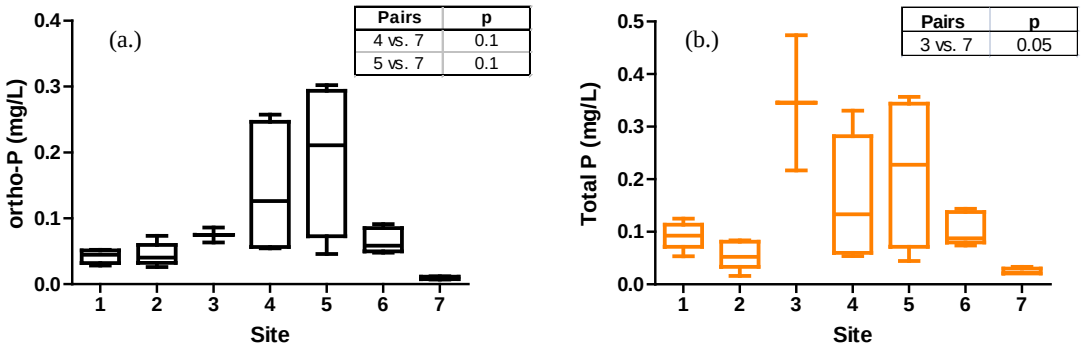


Figure 4: (a.) ortho-P and (b.) total phosphorus present in water column at sampling sites

A significant difference was found in the median orthophosphate (ortho-P) concentrations of the samples collected between the low-agriculture control (7) and sites 4 and 5 (figure 4a, above), though total phosphorus (total P) (Figure 4b, above) did not show this same relationship. Based on figure 4a, site 5 appears to be the major inorganic phosphorus (represented by ortho-P) contributor to site 4, while site 3 is the major overall phosphorus donor, figure 4b. Ortho-P also appears to be the major fraction in the total phosphorus measured, with the exception of sites 1 and 3. Site 3 in particular has a much higher organic phosphorus composition though it does

not seem to have elevated the amount of organic phosphorus seen at site 4; total phosphorus at site 4 remains chiefly influenced by ortho-P. This lack of effect may come from the difference in stream size and volume; site 3 is much smaller than the other sites and is also prone to drying out during the driest months of the year, hence the low number of water samples. Also, sites 4 and 5 have wider buffer zones than sites 2 and 3 and run through a greater tract of land before the sampling locations signifying that the higher ortho-P levels may be coming from multiple upstream sources. This may also be seen in the significant differences between sites 4 and 5 to 7 with respect to ortho-P; some human related activity is influencing the amount of inorganic phosphorus. In regards to the significant difference in total P between site 3 and 7, the high organic portion at site 3 may be due to the close proximity of the poultry house. Phytic acid is the organic fraction excreted in poultry litter. The vegetation and land characteristics observed at site 3 compared to 2 may also explain the large difference in TP measured. Site 3 is mostly grass and few trees with a sharp drop to the stream bed whereas site 2 has abundant low brush, more trees, a wider buffer zone between human activity and the stream, and a gradual decline to the bed. Site 2 discourages overland flow better than site 3, leading to lower TP levels in adjacent water.

Another contributor to the increasing ortho-P levels in figure 4a may be explained by the total dissolved solids (TDS) also measured when samples were taken (figure 5a, below). The linear relationship and an $R^2 = 0.73$ seen in figure 5b (below) between ortho-P and TDS provides a fair predictor as to the amount of inorganic phosphorus

that may be present in water. A similarly linear relationship between total P and TDS is not seen.

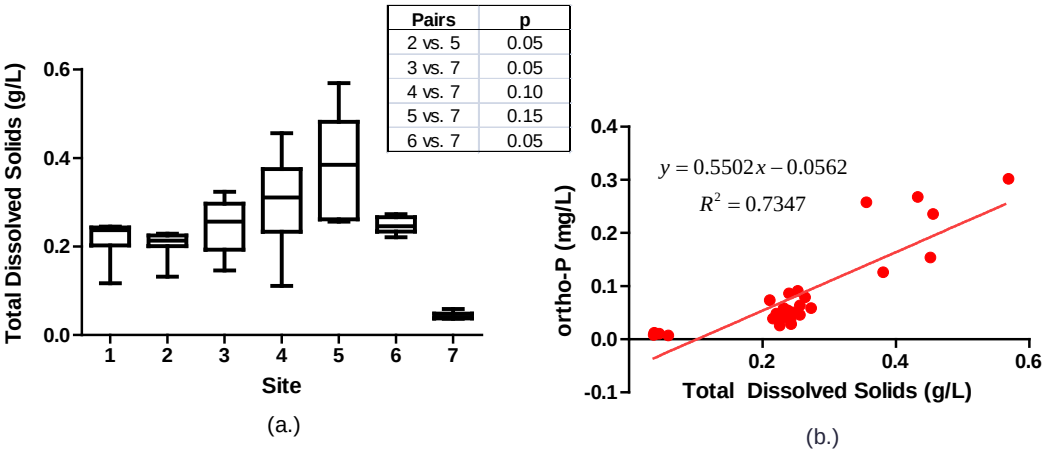


Figure 5: (a.) Total dissolved solids measured in water column at sampling sites and (b.) ortho-P with respect to total dissolved solids at all sites

Nitrogen levels observed at the sampling sites show a different spatial relationship from phosphorus. Ammonia levels are fairly consistent across the sites (not shown), nitrate/nitrite and inorganic-N show a signified difference between directly downstream of the poultry house (2), the control (7), and a well-buffered stream branch that runs through a more urban area located upstream (6) (Figure 6, below). Inorganic-N (sum of ammonia and nitrate/nitrite) at these sites appears to be composed mainly of nitrate/nitrite. The dissimilarity in nitrogen release between sites 2 and 3 highlights the importance of site specificity. They are only approximately 200 m apart yet behave quite differently. It is likely that site 2 has greater groundwater contribution than site 3, explaining both the high level of N observed and the frequent dryness of site 3. Similarly, the low N levels seen at sites 6 and 7 may be due to low groundwater contributions, although the low phosphorus level also

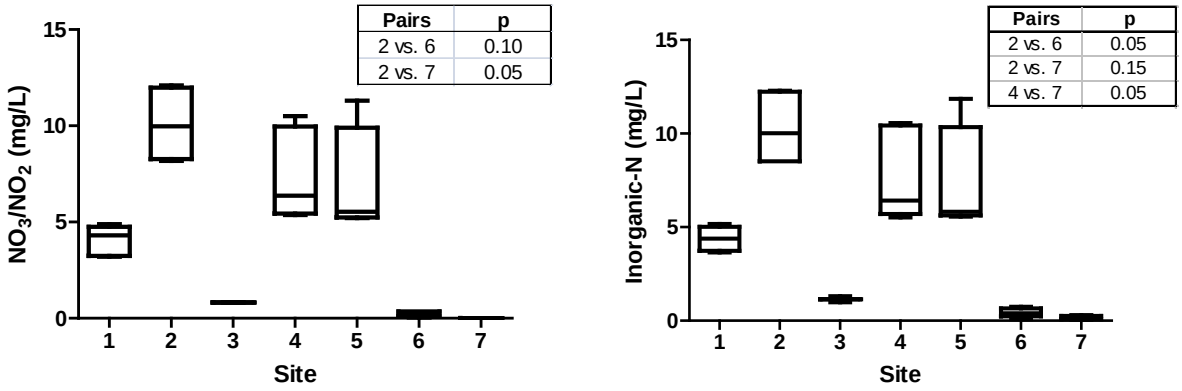


Figure 6: Nitrate/nitrite and (total) inorganic nitrogen measured in water column at sampling sites

seen at site 7 probably indicates that low levels of human activity are the cause for low overall nutrient levels.

Arsenic levels only showed significant differences in a spatial comparison (figure 7, below). The median total arsenic concentration at site 3 was significantly different than the median concentrations observed at sites 2 and 5; median pH at these sites showed no real differences. It is possible that sites 3 and 7 may be natural sources of

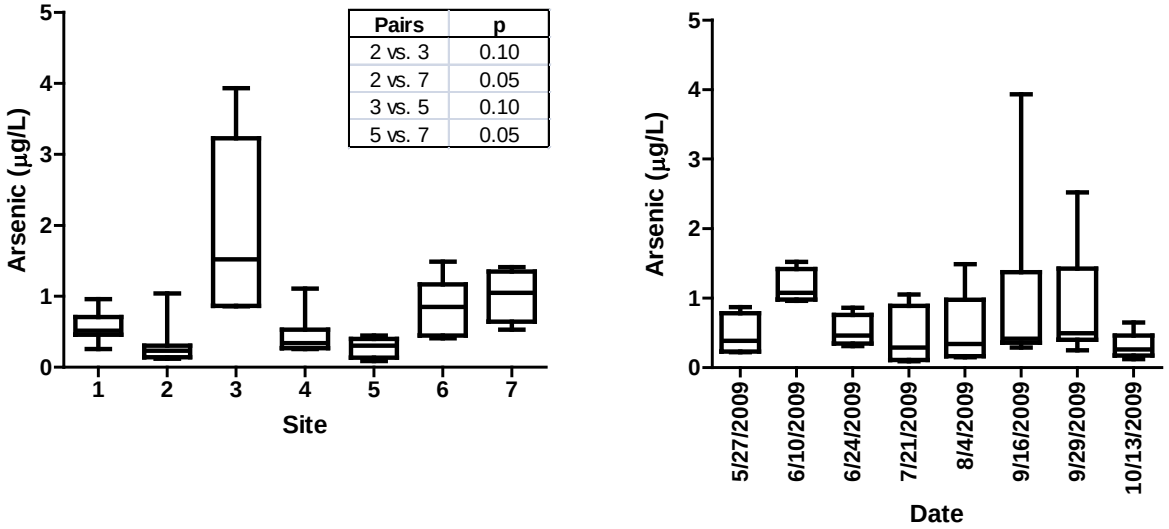


Figure 7: Arsenic measured in the water column

arsenic. Concentrations of arsenic and phosphorus were compared at each site;

higher values of arsenic appear to correspond to higher values of phosphorus suggesting that arsenic transport occurs via overland flow like phosphorus. However, no apparent relationship exists between nitrogen and arsenic when comparing the concentrations at each site (figure 8b).

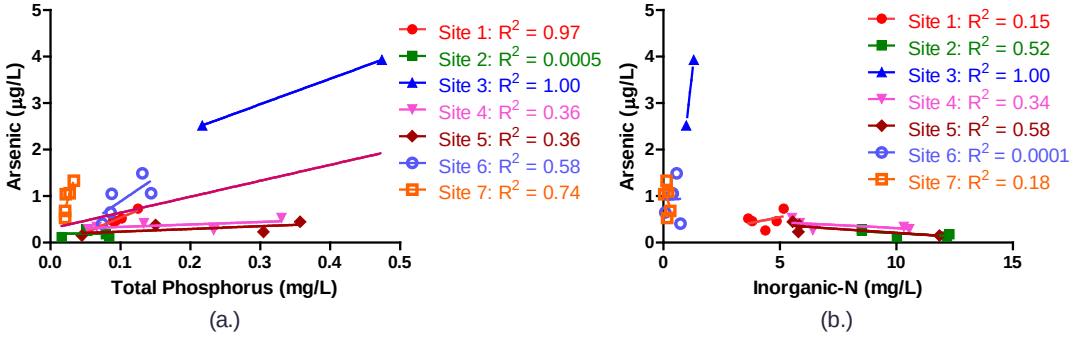


Figure 8: Arsenic profile at each site compared to (a.) total phosphorus and (b.) inorganic nitrogen

Examining the arsenic concentrations as a function of time at sites 2, 3, and 7 (figure 9, below), site 3 had 3-4 times greater arsenic values in September than the downstream-poultry site and control, while the control had a moderately consistent level of arsenic during the collection period. It should be noted that during these

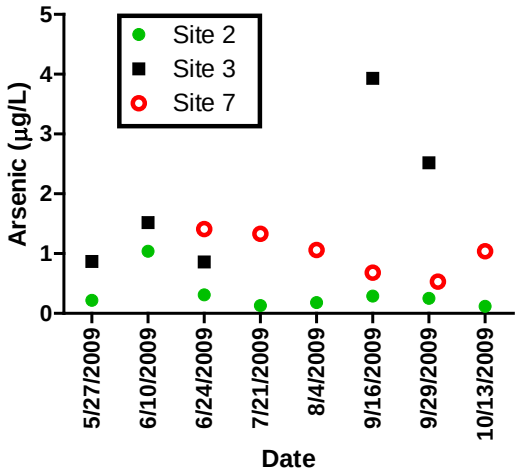


Figure 9: Arsenic concentrations in water column at selected sites

summer months, site 3 was dry and no water could be collected for arsenic analysis. In all cases, sites 3 and 7 had greater arsenic values than site 2.

No significant differences were observed in the median total arsenic concentrations of each sampling event (figure 7), though the median value on 6/10/2009 is slightly higher than those of the rest of the sampling season. This corresponds to the precipitation occurring prior to collection (figure 3). Also, median pH values for the sampling event that occurred after the spring rains (June 10) are significantly lower than the median pH values of events during the drier summer months (July 21 and August 4).

A negative correlation was observed when comparing arsenic to inorganic-N, examining all sites for a particular date (figure 10). Linear regression analysis gives R^2 values between 0.55 and 0.97 for individual dates. As a whole (all dates together), $R^2 = 0.60$. This relationship is not seen between arsenic and ammonia. This high nitrogen/low arsenic relationship suggests that groundwater contributions of arsenic are minimal, and that arsenic movement mostly occurs via particle-phase/overland flow like phosphorus.

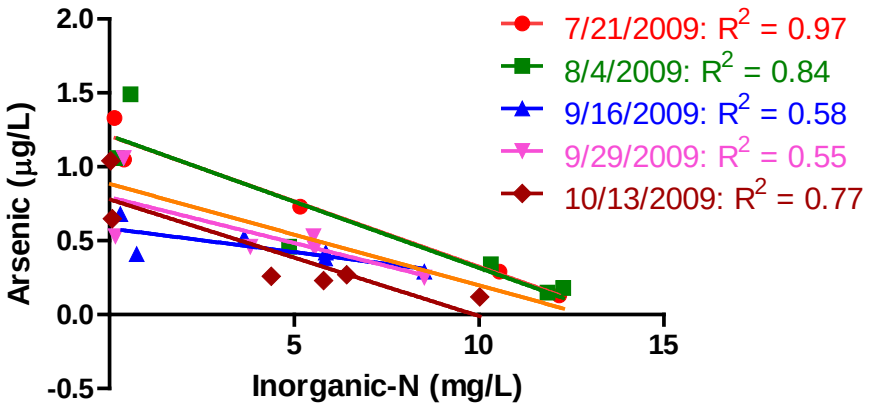


Figure 10: Comparison of arsenic concentrations to inorganic nitrogen, by date

The average recovery from the water column was 79% for the tetracycline compounds, and 52% for ionophores. Simatone was used as the internal standard. A summary of the compounds recovered from the water samples is shown below (Table 3). No significant difference was observed spatially for the four most frequently observed antibiotics (chlortetracycline, oxytetracycline, tetracycline, and monensin).

Table 2: Target antibiotics analyzed in water column samples, their method detections limit, and limit of quantification

Compound	Avg. lab spike recovery (%) (*n*=7)	MDL (ng/mL)	LOQ (ng/mL)
Tetracycline	119 ± 48	2.8	8.4
Oxytetracycline	104 ± 40	1.9	5.7
Chlortetracycline	71 ± 43	2.3	7.0
Doxycycline	48 ± 45	2.8	8.5
Naracin	38 ± 15	0.70	2.1
Monensin	62 ± 6	0.37	1.1
Salinomycin	52 ± 38	1.7	5.2
Lasalocid	35 ± 33	1.8	5.3
*Simatone**	*110 ± 34*	--	--

* Simatone was used as the internal standard and no MDL was determined

In terms of temporal differences, there was a clear and significant difference observed between the spring and fall/winter dates (Figure 11, below). June sampling dates were consistently different from the sampling dates in early September and mid-October. Samples from late September (9/29/2009) were discarded due to poor recovery of the internal standard. A significant temporal difference was also observed with the internal standard between spring/summer and fall/winter, possibly

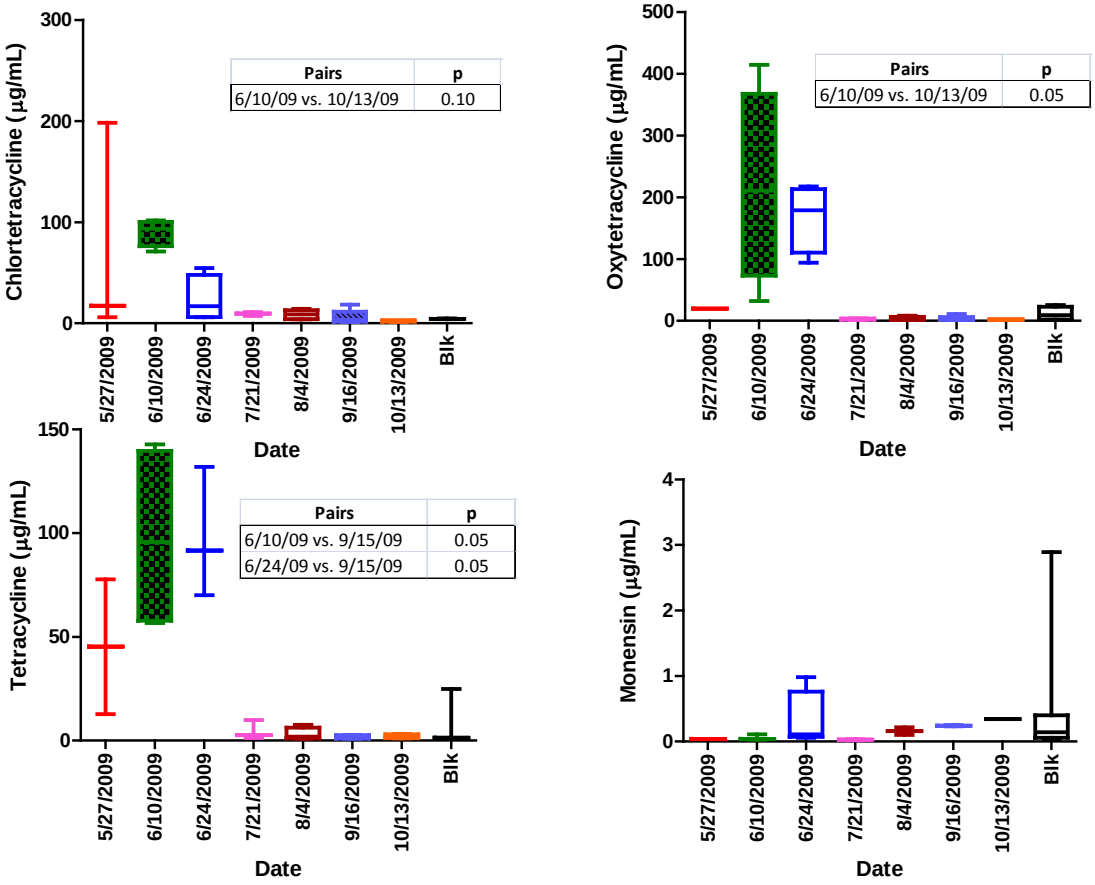


Figure 11: Most frequently recovered antibiotics in water column at sampling sites

indicating a change in matrix conditions as reflected by instrument sensitivity. Standard curves were compared between batches to determine if there were instrument conditional differences between sample runs but no significant differences

were observed between standard curves; each was inside the other's 95% confidence interval. Unlike nutrient and arsenic samples, antibiotics show significant variation between dates (figure 11) as opposed to location (Appendix F). Due to their hydrophobic nature, antibiotics are prone to sediment sequestration; however, the small rain just before the 6/10/2009 collection suggests that three of four antibiotics in figure 11 moved via water transport. In order to see if this transport was driven by groundwater or overland flow, comparison to corresponding nitrogen and phosphorus concentrations was done since each is known to correspond mainly to one transport method or the other. However, nutrient data was not collected prior to July sampling dates and antibiotics levels do not show much movement after the June 10th sampling. Linear regression analysis of antibiotics to nutrients from July onward does not yield consistent or robust trends either spatially or temporally, although (mostly) positive slopes of phosphorus comparison (figure 12, below) may indicate that antibiotics move via overland flow.

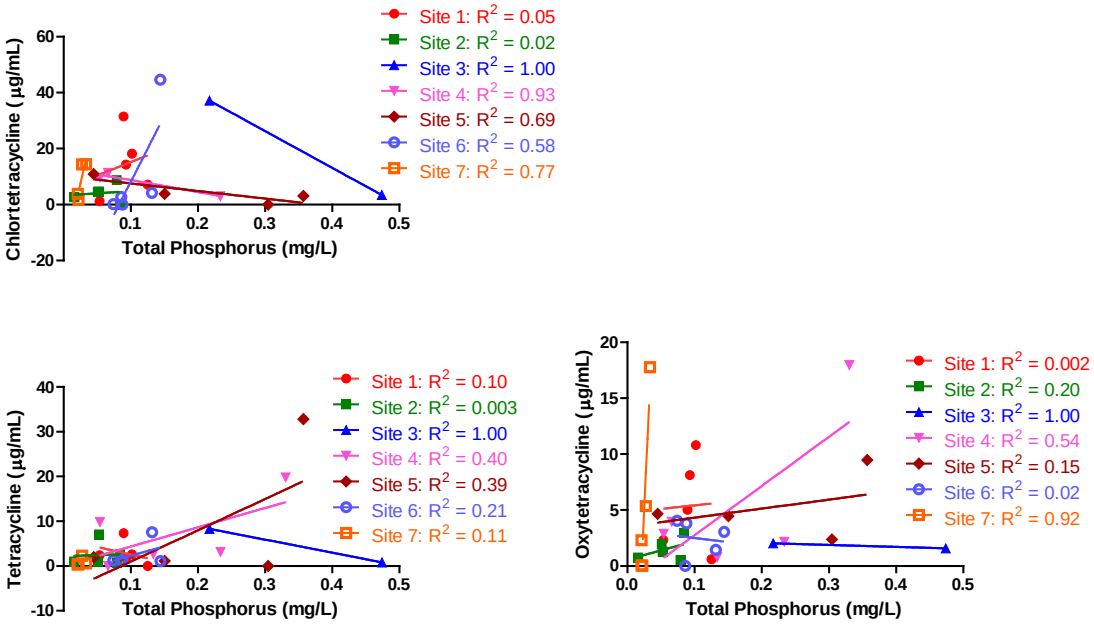


Figure 12: Select antibiotics concentration with respect to total-P, by site

The control site showed a higher concentration of each antibiotic found in the water column, with respect to site 2, during most dates in the spring time (Figure 13, below). During the summer and fall dates, both showed almost no presence of

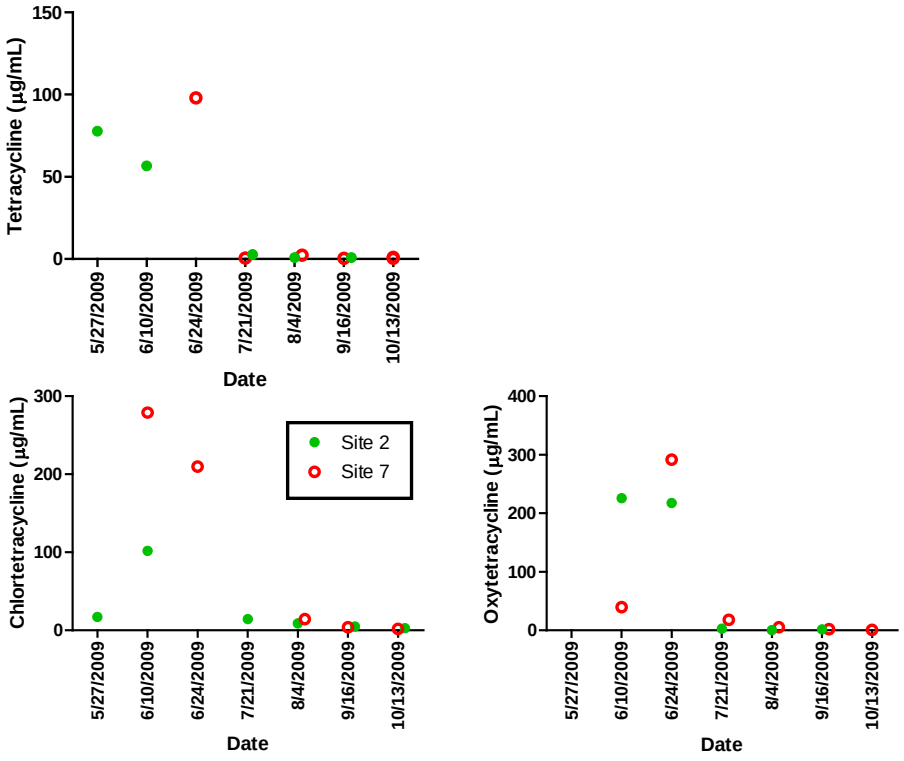


Figure 13: Select antibiotics as measured at sites 2 and 7

antibiotics. This suggests that site 7 may not be a good control for antibiotics despite its heavy forestation and low agriculture activity.

No significant temporal differences were observed among the median concentrations of *E. coli* or *Enterococcus* in sediment samples but both showed changes in the water column. Water column concentration for *Enterococcus* on June 10 was consistently greater than samples collected on other dates (Figure 14a, below), a trend reflected by the higher aquatic concentrations of antibiotics and slight elevation of arsenic

observed on June 10th as well (Figures 11 and 9, respectively); this was not observed in *E. coli*. June 10th had very high water levels and approximately a half inch of rain had come down the previous day. Other sampling dates had some rainfall just prior to collection (8/4 and 9/16), but no more than 0.02 inches. Precipitation of the same magnitude came within 4-7 days of the other sampling events. Significant differences were observed in the median concentrations of *Enterococcus* in the water column between sites 1-3 and the control (Figure 14b, below), a trend also seen in *E.coli*. This consistent significant difference between each organism at site 2 and 7 is also seen in the significantly higher levels of inorganic nitrogen at site 2 over 7 (Figure 6).

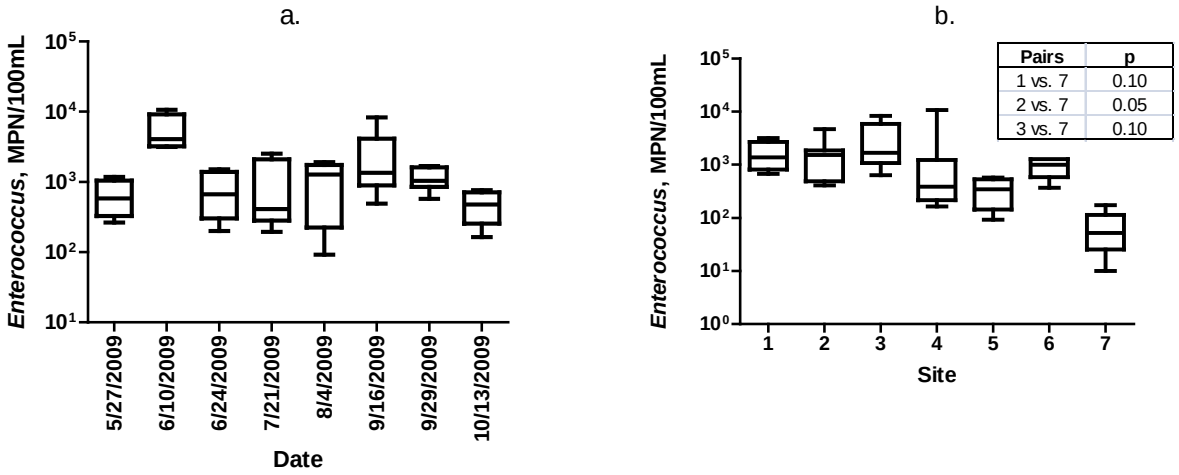


Figure 14: (a) Enterococcus in water column by date, (b) Enterococcus in water column at each site

Whether this is helping bolster the native population is uncertain as this relationship is not observed at other sites. Overall, presence of *Enterococcus and E. coli* at each site were similar in both the water column and sediment, though temporally the median shifted more in the sediment, but not significantly. This slight shift was not observed in the water column by either organism. The lack of dramatic variation in

the representative bacterial population may mean that the low level of change seen by other constituents was not enough to cause an alteration, that there are other parameters that need to be taken into account in order to see greater changes, or that because the early spring flush was not captured in these samplings, any changes that may have occurred were missed. Also, that sampling sites and dates are too broad (far apart) to see movement. Other confounding factors include bacterial reservoirs, undocumented runoff (additional nourishment from runoff substrates), and the infiltration/competition of bacteria from other runoff sources. Also, elevated bacterial populations at non-control sites versus the control may represent a natural difference in native species presence.

Chapter 5: Conclusions and future work

5.1 *Conclusions*

Subtle relationships and changes were noted between measured constituents. More concrete findings would be possible with greater sampling frequency, closer spacing of sampling sites, and sampling for more than a single season. Interconnectivity between analyte concentrations and biological activity (bacterial population measurements) can be suspected but not proven due to the low number of samples, the high number of confounding factors, and the relatively low environmental concentrations measured.

Median analyte concentrations fell within maximum contaminant level standards. Nitrate-N median concentrations did not exceed 10 mg/L and median arsenic values remained below 1.5 µg/L. Overall median concentrations for the four most frequently detected antibiotics (chlortetracycline, oxytetracycline, tetracycline, and monensin) in the subwatershed water samples did not exceeded previous study findings by Arikan et al. (2008), although several dates during the spring did reach medians of 0.2 µg/µL for oxytetracycline and 0.1 µg/µL for chlortetracycline and tetracycline. Monensin median concentration did not exceed 0.001 µg/µL.

A robust linear relationship was observed between ortho-P and TDS which supports the concept that phosphorus is tied to particulate matter and overland flow. Whether this particular linear relationship is unique to the sites chosen will need further examination; it is likely that the regression variables will change with the addition of

sites. A greater amount of nutrient runoff to the water column was expected on the 8/4/2009 based on the dry conditions throughout July. However, the majority of runoff was likely missed as approximately 2 in of rain fell the week before. Higher phosphorus concentrations at site 3 in particular may also be tied to the short forage of the location, discussed by Edwards et al. (2000) to promote phosphorus runoff.

Arsenic levels at site 3 were greater than levels at sites 2 and 7. In terms of transport, arsenic was found to have a positive correlation to total P and strong negative correlation to inorganic N. This implies arsenic mobility as related more closely with runoff than groundwater.

A seasonal variation in antibiotic concentration is suggested based on water column recoveries; the summer and fall months were very low compared to the spring when greater values were recovered. A (mostly) positive correlation between antibiotics and phosphorus was found implying that antibiotics may be moving to surface water via overland flow like phosphorus. Antibiotics' hydrophobic nature promotes association with organic material and sediments which are typically washed into surface water during runoff events. In addition, previous work by Cheubey et al. (2007) suggests that sediments release inorganic-P during winter and spring and act as a sink during the fall and summer. This behavior coupled with greater rain events during spring months may explain the high antibiotic and phosphorus concentrations in the spring and low concentrations in the fall and summer.

Median levels of the bacteria remained fairly steady between sampling sites and dates although some significant variation was seen between sites. It is possible that this stems from the relatively low concentrations of nutrients, arsenic, and antibiotics observed in the water column; thus the concentrations were not high enough to cause a dramatic alteration in population numbers. It is unknown whether community composition was affected since only two indicators were measured. Other confounding factors regarding the interpretation of bacterial data include not capturing the early spring flush, broad sampling sites and dates, presence of bacterial reservoirs, undocumented runoff (additional nourishment from runoff substrates), natural site (microbial) differences (more samples needed), and the infiltration/competition of bacteria from other runoff sources.

Overall, the poultry facility had an effect on water quality, though hydrologic and geomorphic characteristics of each site also played a large role in this observed impact. Relatively high constituent concentrations at the larger stream locations, like sites 4 and 5, suggest that pollution may also be more of a cumulative effect.

5.2 *Future work*

Bacteria are good indicators of ecological structure and are under-utilized as early warning systems. Results from this project will further promote using "sentinel" bacteria along with analytical chemistry for assessing ecological impact of possibly disruptive human activities, as well as encourage the need for more integrated studies. Cumulative and synergistic effects of pollution should also be investigated (multiple

poultry facilities on a given segment of stream/river), given that there is evidence of several analytes traveling to surface water using similar means. In-depth soil and hydrological investigations at these sites are needed, as well as similar multi-variable data on other soil types and hydrologic areas. These types of studies will provide the needed data for land managers and producers for placement of facility and mitigation practices to support strong natural processes and bolster weaker ones.

Appendices

A. Antibiotics: Water column concentrations

	Chlortetracycline (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	198.1	17.2	5.9	*ND*	*ND*	*ND*	*ND*	0.0	2.3
6/10/2009	95.8	101.6	91.2	71.1	*ND*	*ND*	278.8	0.0	1.1
6/24/2009	6.6	*ND*	54.7	26.8	*ND*	5.9	209.7	0.0	1.4
7/21/2009	7.1	0.0	*ND*	9.6	10.9	0.0	14.4	4.7	1.6
8/4/2009	14.3	8.7	*ND*	11.3	3.8	4.2	14.4	3.9	3.3
9/16/2009	18.2	4.6	3.4	*ND*	3.1	0.2	3.8	4.2	1.2
10/13/2009	1.3	2.7	*ND*	3.0	*ND*	2.7	1.7	*ND*	2.3

	Doxycycline (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	0.0	0.0	0.0	*ND*	*ND*	*ND*	*ND*	0.0	1.1
6/10/2009	0.0	97.5	40.7	0.0	*ND*	*ND*	0.0	0.0	0.4
6/24/2009	22.8	*ND*	75.6	61.5	*ND*	0.0	0.0	0.0	0.8
7/21/2009	0.0	0.0	*ND*	0.0	*ND*	0.0	0.0	0.0	0.5
8/4/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.0	2.7
9/16/2009	0.0	0.0	0.3	1.8	0.0	*ND*	0.8	0.0	1.0
10/13/2009	*ND*	1.0	*ND*	*ND*	*ND*	1.2	2.4	0.0	2.6

	Lasalocid (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	0.1	0.0	0.0	*ND*	*ND*	*ND*	*ND*	0.0	1.7
6/10/2009	0.0	0.0	0.0	0.0	*ND*	*ND*	0.0	0.0	1.6
6/24/2009	0.0	*ND*	0.0	0.0	*ND*	0.0	0.0	0.0	1.6
7/21/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.4	1.4
8/4/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.1	0.0	1.1
9/16/2009	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10/13/2009	0.1	0.1	*ND*	0.6	0.1	0.0	0.0	0.0	0.8

	Monensin (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	0.0	0.0	*ND*	*ND*	*ND*	*ND*	*ND*	0.0	1.5
6/10/2009	0.1	0.0	0.0	0.0	*ND*	*ND*	0.0	0.0	1.7
6/24/2009	0.1	*ND*	1.0	0.1	*ND*	0.1	0.1	0.4	1.4
7/21/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.1	1.5
8/4/2009	0.0	0.0	*ND*	0.2	0.1	0.0	0.0	0.1	1.5
9/16/2009	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.2	1.4
10/13/2009	0.0	0.0	*ND*	0.3	0.0	0.0	0.0	2.9	1.7

	Narasin (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	0.0	0.0	0.0	*ND*	*ND*	*ND*	*ND*	0.0	1.1
6/10/2009	0.0	0.0	0.0	0.0	*ND*	*ND*	0.0	0.0	1.3
6/24/2009	0.0	*ND*	0.0	0.0	*ND*	0.0	0.0	0.0	1.0
7/21/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.0	1.1
8/4/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.0	1.1
9/16/2009	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2
10/13/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.0	0.6

	Oxytetracycline (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	*ND*	*ND*	19.3	*ND*	*ND*	*ND*	*ND*	*ND*	3.4
6/10/2009	195.4	225.9	31.8	414.9	*ND*	*ND*	39.8	14.5	2.7
6/24/2009	93.9	*ND*	217.6	199.2	*ND*	158.9	291.6	25.3	1.7
7/21/2009	0.6	3.0	*ND*	2.9	2.5	3.8	17.8	*ND*	2.6
8/4/2009	8.1	0.6	*ND*	4.0	4.7	1.4	5.4	*ND*	3.7
9/16/2009	10.8	2.0	1.6	0.8	4.5	4.0	2.3	3.4	2.6
10/13/2009	2.3	0.8	*ND*	2.2	2.4	*ND*	*ND*	*ND*	2.8

	Salinomycin (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	0.0	0.0	0.0	*ND*	*ND*	*ND*	*ND*	0.0	1.8
6/10/2009	0.0	0.0	0.0	0.0	*ND*	*ND*	0.0	0.0	2.3
6/24/2009	0.0	*ND*	0.0	0.0	*ND*	0.0	0.1	0.0	1.7
7/21/2009	0.0	0.0	*ND*	0.0	0.0	0.1	0.0	0.0	1.5
8/4/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	0.0	1.3
9/16/2009	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
10/13/2009	0.0	0.0	*ND*	0.0	0.0	0.0	0.0	18.6	0.4

	Tetracycline (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	0.0	77.7	12.7	*ND*	*ND*	*ND*	*ND*	*ND*	2.9
6/10/2009	142.8	56.6	61.0	130.0	*ND*	*ND*	*ND*	*ND*	2.2
6/24/2009	91.6	*ND*	131.8	*ND*	*ND*	70.0	97.9	24.8	2.1
7/21/2009	0.0	2.6	*ND*	9.8	*ND*	1.2	0.6	1.2	1.8
8/4/2009	1.7	0.8	*ND*	*ND*	2.0	7.6	2.3	1.3	4.2
9/16/2009	2.6	1.0	0.8	2.7	1.1	1.3	0.4	*ND*	3.2
10/13/2009	2.4	1.0	*ND*	3.2	*ND*	1.4	0.4	*ND*	3.7

	Simatone (µg/mL)								
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**blk**	**spk**
5/27/2009	12.1	10.0	4.9	*ND*	*ND*	*ND*	*ND*	1.3	86.5
6/10/2009	41.1	26.1	13.5	13.7	*ND*	*ND*	14.3	2.3	129.9
6/24/2009	22.8	*ND*	25.9	31.8	*ND*	15.4	17.9	42.8	143.5
7/21/2009	36.7	52.2	*ND*	42.0	46.9	47.0	30.4	37.4	108.4
8/4/2009	25.3	26.5	*ND*	28.5	32.6	35.0	40.6	28.0	113.0
9/16/2009	41.6	39.8	52.0	57.9	58.1	54.3	55.4	62.5	85.8
10/13/2009	131.0	128.1	*ND*	126.0	74.5	84.9	80.7	133.6	75.7

ND: Not Detected
Samples from 9/29/2009 not included in tables due to poor internal standard recovery

B. Biological: *Enterococcus* and *E.coli* present in water column and sediment

Water Column	*Enterococcus* (MPN/100mL)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5/27/2009	1181.1	522.9	637.7	263.3	*NC*	*NC*	*NC*
6/10/2009	3150.4	4690.0	3441.0	10683.0	*NC*	*NC*	*NC*
6/24/2009	670.0	405.0	1506.5	200.0	*BDL*	1265.3	173.0
7/21/2009	2529.0	1669.5	*NC*	411.0	194.0	367.5	52.0
8/4/2009	1572.5	1917.0	*NC*	356.0	92.0	1272.0	*BDL*
9/16/2009	2729.0	1447.5	8287.0	1263.5	492.0	1029.5	41.0
9/29/2009	935.0	1600.0	1673.0	1114.0	574.0	956.0	10.0
10/13/2009	764.0	476.0	*NC*	164.0	343.5	659.0	52.0

Sediment	*Enterococcus* (MPN/100mL)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
6/10/2009	21715.4	1850.7	206.5	*NC*	*NC*	*NC*	*NC*
6/24/2009	475.3	200.2	231.5	223.1	85.5	206.5	20.9
7/21/2009	5003.0	483.9	*NC*	291.1	648.5	595.0	*BDL*
8/4/2009	2212.4	483.8	9241.8	185.8	791.6	569.5	41.1
9/16/2009	564.3	392.2	8522.4	611.0	*ADL*	228.0	122.6
9/29/2009	411.1	*NC*	304.4	534.7	262.3	386.8	43.4
10/13/2009	12620.8	309.5	1805.4	386.6	1609.0	1090.3	84.3

Water Column	*E. coli*(MPN/100mL)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5/27/2009	1335.2	494.3	611.7	307.2	*NC*	*NC*	*NC*
6/10/2009	2446.9	2105.6	686.1	7301.0	*NC*	*NC*	*NC*
6/24/2009	947.3	1510.0	444.5	221.5	237.5	679.5	110.0
7/21/2009	3218.0	2785.0	*NC*	325.5	802.5	7270.0	10.0
8/4/2009	1238.0	2563.5	*NC*	311.5	104.5	1041.5	87.5
9/16/2009	4611.0	1565.5	263.0	470.0	422.5	407.0	313.0
9/29/2009	2034.5	896.5	449.5	383.5	1031.0	173.0	554.5
10/13/2009	1308.5	723.5	*NC*	151.5	76.0	60.0	110.0

Sediment	*E. coli*(MPN/100mL)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
6/10/2009	22284.4	4387.7	376.7	*NC*	*NC*	*NC*	*NC*
6/24/2009	84.5	758.0	267.1	84.2	137.9	527.4	*BDL*
7/21/2009	3426.2	710.2	*NC*	648.4	24312.4	4571.5	*BDL*
8/4/2009	1459.4	843.9	645.2	1168.5	2811.6	1439.1	385.1
9/16/2009	1078.2	943.6	732.6	903.9	*ADL*	*BDL*	341.7
9/29/2009	172.5	*NC*	2372.3	1784.1	800.9	7219.9	1396.5
10/13/2009	8875.0	1311.6	723.7	1448.4	2165.0	1969.3	94.9

NC: Not Collected
BDL: Below Detection Limit
ADL: Above Detection Limit

C. Heavy metals: Arsenic concentration in water column

	Arsenic (µg/L)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5/27/2009	0.51	0.22	0.87	0.26	*NC*	*NC*	*NC*
6/10/2009	0.96	1.04	1.52	1.11	*NC*	*NC*	*NC*
6/24/2009	0.65	0.31	0.86	*NC*	0.38	0.46	1.41
7/21/2009	0.73	0.13	*NC*	0.29	0.09	1.05	1.33
8/4/2009	0.46	0.18	*NC*	0.34	0.15	1.49	1.06
9/16/2009	0.52	0.29	3.93	0.42	0.38	0.41	0.68
9/29/2009	0.46	0.25	2.52	0.53	0.45	1.06	0.53
10/13/2009	0.26	0.12	*NC*	0.27	0.23	0.65	1.04

NC: Not Collected

D. Nutrients: Phosphorus and nitrogen measured in the water column

	ortho-P (mg/L)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
7/21/2009	0.04	0.07	*NC*	0.05	*ND*	0.08	0.01
8/4/2009	0.04	0.04	NC	0.06	0.05	0.09	0.01
9/16/2009	0.05	0.04	0.06	0.13	0.15	0.05	0.01
9/29/2009	0.05	0.05	0.09	0.26	0.27	0.05	0.01
10/13/2009	0.03	0.03	*NC*	0.24	0.30	0.06	0.01

	Total Phosphorus (mg/L)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
7/21/2009	0.13	0.08	*NC*	0.05	*ND*	0.09	0.03
8/4/2009	0.09	0.08	*NC*	0.07	0.04	0.13	0.03
9/16/2009	0.10	0.05	0.47	0.13	0.15	0.07	0.02
9/29/2009	0.09	0.05	0.22	0.33	0.36	0.14	0.02
10/13/2009	*ND*	*ND*	*NC*	*ND*	*ND*	*ND*	*ND*

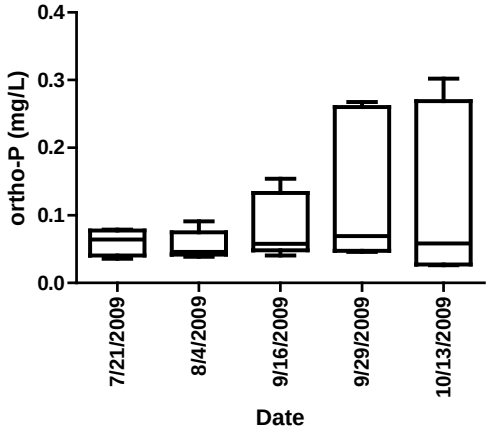
	NH_4 (mg/L)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
7/21/2009	0.27	0.07	*NC*	0.05	*ND*	0.03	0.13
8/4/2009	0.26	0.43	*NC*	0.88	0.55	0.36	0.21
9/16/2009	0.45	0.34	0.47	0.32	0.64	0.38	0.30
9/29/2009	0.50	0.15	0.18	0.16	0.20	0.13	0.15
10/13/2009	0.07	0.05	*NC*	0.06	0.09	0.04	0.04

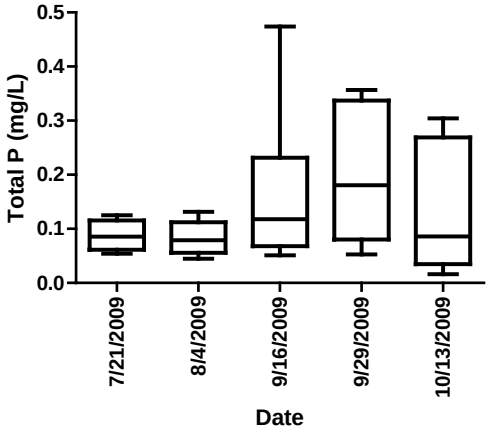
	NO_3/NO_2 (mg/L)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
7/21/2009	4.90	12.10	*NC*	10.50	*ND*	0.36	0.00
8/4/2009	4.61	11.85	*NC*	9.44	11.30	0.21	0.00
9/16/2009	3.19	8.18	0.83	5.54	5.21	0.36	0.00
9/29/2009	3.31	8.37	0.80	5.36	5.36	0.26	0.01
10/13/2009	4.30	9.98	*NC*	6.36	5.71	0.04	0.00

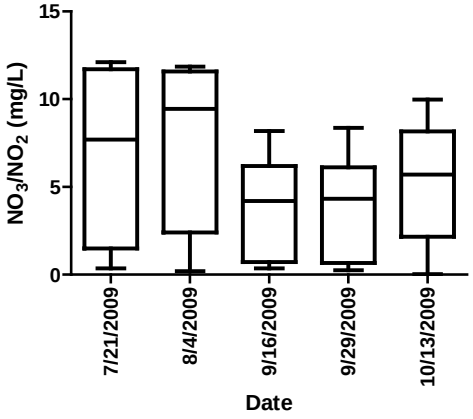
	Inorganic Nitrogen (mg/L)						
Sampling Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**
7/21/2009	5.17	12.17	*NC*	10.55	*ND*	0.39	0.13
8/4/2009	4.86	12.28	*NC*	10.32	11.85	0.57	0.21
9/16/2009	3.65	8.52	1.30	5.86	5.84	0.74	0.30
9/29/2009	3.81	8.52	0.98	5.52	5.56	0.39	0.16
10/13/2009	4.38	10.02	*NC*	6.42	5.80	0.08	0.04

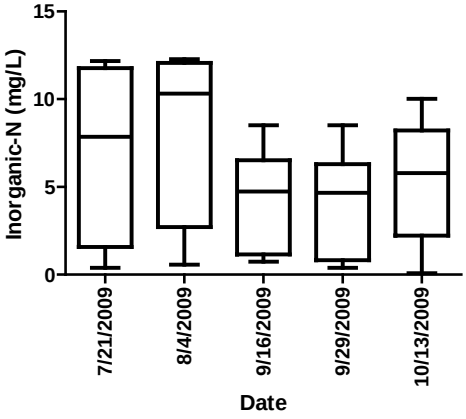
NC: Not Collected
ND: Not Detected

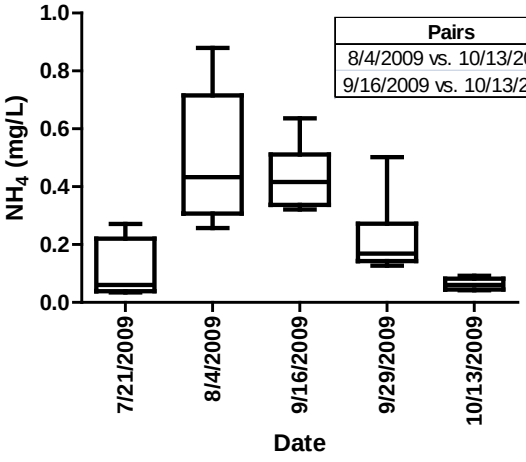
E. Changes in phosphorus and nitrogen concentrations separated by sampling event





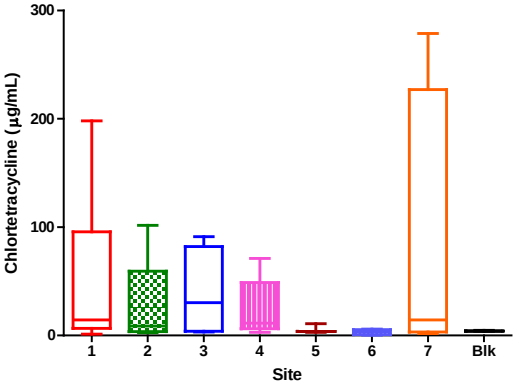


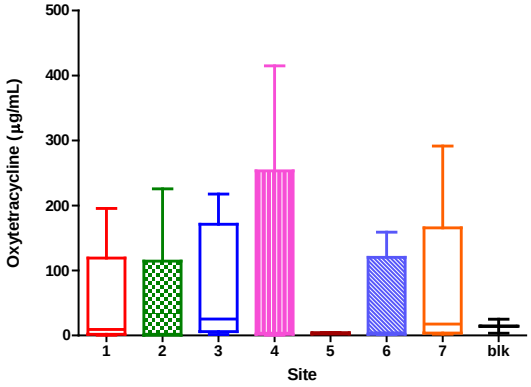


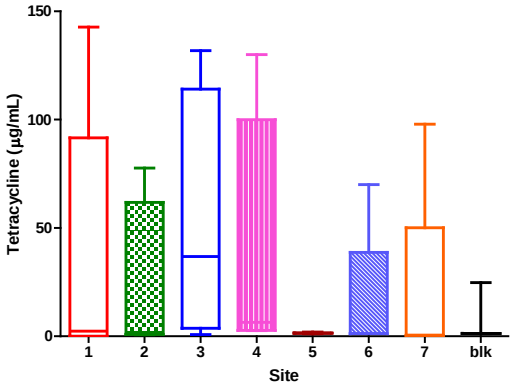


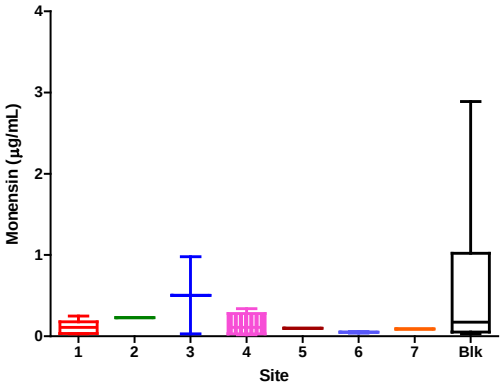
Pairs	p
8/4/2009 vs. 10/13/2009	0.05
9/16/2009 vs. 10/13/2009	0.05

F. Variation of antibiotics in water column, separated by site









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