Water Resources Research Center
Annual Technical Report
FY 2003

Introduction

The Maryland Water Resources Research Center (MWRRC) supports Marylands water research and educational needs by funding high priority research projects and sponsoring educational programs and conferences on current water issues. Most of the funded research addresses problems associated with the Chesapeake Bay, a major economic asset in Maryland. The four projects funded in this annual reporting period concern the Bay. Research funding is directed toward supporting graduate students and young faculty members. We annually support at least one summer graduate student, depending on funding. During this reporting period we supported 6 students (two undergraduates and four graduate students) and two USGS interns.

We sponsor/cosponsor seminars and conferences on campus. The 2003 Water Resources conference addressed Maryland Water Policy: What Does the Future Hold? which is described in the Technology Transfer section. A graduate poster session was held in conjunction with the Conference. Seventeen posters were exhibited at this session and the three winning students received a engraved plaque and a cash award.

The Center acts as a focal point at the University on Maryland water issues with both Federal and State agencies. We solicit proposals through our two annual newsletters and an e-mail list of about 60 scientists on the College Park campus. We convene a panel of outside experts to review and rank the submitted proposals. In addition to the USGS requirements, the principle scientists are notified and are requested to submit a half page progress report in 6 months and a full page in one year. Progress on projects are monitored informally on a regular basis. The Director and Associate Director meet with State and Federal personnel on an annual basis, including the Maryland Department of Natural Resources, the Maryland Department of the Environment, and the U.S. Geological Survey, Maryland, Delaware, D.C. District Office.

Research Program

Investigation of isotopic methods for identifying atmospheric deposition of nitrate to the Chesapeake Bay Watershed

Basic Information

Title:	Investigation of isotopic methods for identifying atmospheric deposition of nitrate to the Chesapeake Bay Watershed
Project Number:	2002MD1B
Start Date:	3/1/2002
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	5th congressional district of Maryland
Research Category:	Not Applicable
Focus Category:	Non Point Pollution, Nitrate Contamination, Water Quality
Descriptors:	
Principal Investigators:	James Farquhar

Publication

Our research focuses on developing methods for measuring the isotopic ratios of the three oxygen isotopes and the two nitrogen isotopes of precipitation nitrate and collecting data that will provide a baseline for the coupled oxygen and nitrogen isotopic compositions of precipitation nitrate from the Maryland-DC metropolitan area to the Chesapeake Bay watershed. The work is presently in a no cost extension phase, and the final results of this study are anticipated to be completed by September 2004 when K. Cooney, who is supported by this grant, completes the requirements for an M.S. degree at the University of Maryland.

Problem and Research Objectives

The input of excess nutrients, such as nitrogen, to the Chesapeake Bay can adversely affect the estuarine ecosystem and its living resources. One of the most devastating consequences of eutrophication is the over-production of algae. Algal blooms block sunlight from reaching submerged aquatic vegetation (SAVs), which require solar energy for photosynthesis and growth. In addition to shutting off oxygen production by SAVs, algal blooms lead to further reductions in dissolved oxygen after the algae dies and subsequently decomposes via aerobic processes that can consume most, if not all, of the oxygen available for aquatic wildlife. During the summer, a deep-water "dead zone" extending hundreds of square miles occurs in the Bay's mainstem and spreads into several of the major tributaries. Alarmingly, the volume of low-oxygen and anoxic water in the Bay tripled between 1950 and 1990, and 40% of the Bay's mainstem was deprived of oxygen in July 2003 (CBF Fact Sheet 2003).

Due to complex hydrology and a multitude of natural and anthropogenic sources, quantifying the different sources of nitrogen to the Chesapeake Bay (e.g. atmospheric sources, wastewater treatment plants, fertilizers, manure) has proven to be quite difficult. Estimates of the atmospheric contribution of nitrate to the Bay and its tributaries have varied widely. Fisher and Oppenheimer (1991) estimated that the atmospheric deposition to the watershed was 34% of total nitrogen inputs. Boynton et al. (1995) estimated that atmospheric deposition (wet-fall only) was 12.0% of the inputs for the entire Bay and 12-37% for individual tributaries and concluded that the atmospheric source terms were the greatest sources of uncertainty in their nitrogen budget.

Our research investigates a new isotopic tool that could potentially be used to understand further atmospheric deposition to the Chesapeake Bay. Stable isotopes can be a powerful tool for investigating the sources, transport, and fate of nitrate in various ecosystems (Kendall 1998 and references therein). There are two stable isotopes of nitrogen (^{14}N and ^{15}N) and three stable isotopes of oxygen (^{16}O, ^{17}O, and ^{18}O). The stable isotopic composition of a particular material or compound remains unchanged through time and space unless a chemical, physical, or biological process alters the isotopic makeup of the species involved. Typically, the isotopic composition of a compound is expressed in delta notation, the parts per thousand (or "permil") deviation of a compound's isotopic ratio from the isotopic ratio of a reference material, such as the composition of N_2 in atmospheric air (AIR) or Vienna standard mean ocean water (VSMOW). The results presented for this study will be expressed in delta notation as defined below.

$$\delta^{15}N = \left(\frac{\frac{^{15}N}{^{14}N_{sample}}}{\frac{^{15}N}{^{14}N_{AIR}}} - 1 \right) \times 1000$$

$$\delta^{18}O = \left(\frac{\frac{^{18}O}{^{16}O_{sample}}}{\frac{^{18}O}{^{16}O_{VSMOW}}} - 1 \right) \times 1000$$

$$\delta^{17}O = \left(\frac{\frac{^{17}O}{^{16}O_{sample}}}{\frac{^{17}O}{^{16}O_{VSMOW}}} - 1 \right) \times 1000$$

In the absence of isotopic fractionation processes, the isotopic composition of a substance can be thought of as its fingerprint or as a reflection of the reservoir from which it originated. However, the isotopic character of a substance can change during a process that occurs more readily for a species that is heavier or lighter than another species. For example, H_2O comprised of ^{16}O evaporates more quickly than H_2O comprised of ^{18}O. Therefore, the reservoir from which the water is evaporating will become isotopically heavier over time due to the preferential evaporation of the lighter species. Even though many natural processes affect the isotopic composition of substances, isotopic fractionations can often be understood because the majority of terrestrial reactions are governed by rules determined by the relative mass differences of the isotopic species involved. For example, the magnitude of the fractionation of ^{17}O from ^{16}O is determined by the one atomic mass unit difference between ^{17}O and ^{16}O while the fractionation of ^{18}O from ^{16}O is approximately twice as much because there is a two atomic mass unit difference between ^{18}O and ^{16}O. Therefore, there is a mass-dependent relationship between the ratios of $^{18}O/^{16}O$ versus $^{17}O/^{16}O$ that plots as a line with a slope of approximately 0.5, differing only slightly due to the particular nature of the process.

Exceptions to mass-dependent fractionations exist and can be produced by several processes, including nuclear reactions (decay or production of one or more isotopes), hyperfine interactions that involve spin-spin coupling associated with odd mass nuclei in certain liquid phase photochemical reactions, and a number of gas-phase reactions (e.g. Thiemens 1999). The physical-chemical origin of the mass-independent effect in gas phase reactions is thought in some cases to be related to selection rules that depend on parameters in addition to mass, but in many cases the origin of the effect is still a subject of considerable study. The quantity $\Delta^{17}O$ (= δ^{17} O – 1000 * ((1 + $\delta^{18}O/1000)^{0.52}$ – 1)) is used to describe the deviation of a datum in units of permil from the mass-fractionation line and is a measure of mass-independent processes.

Recent studies have utilized both $\delta^{15}N$ and $\delta^{18}O$ of nitrate to identify major sources of nitrate in catchments characterized by different land uses (e.g. Chang et al. 2002). While there are numerous advantages to using this dual-isotope approach, several different sources of nitrate (e.g. precipitation and soil water) have overlapping isotopic signatures (e.g. Kendall, 1998 and

sources therein). A recent discovery by G. Michalski and coworkers (2002, 2003) could provide a useful tool for differentiating atmospheric sources from all other sources of nitrate. Michalski and colleagues have documented an extremely large mass-independent isotopic fractionation effect for atmospheric nitrate. Whereas $\Delta^{17}O$ is approximately 0 for the majority of nitrate sources (e.g. wastewater and farm runoff), $\Delta^{17}O$ is approximately 20-30.8‰ for nitrate aerosols in La Jolla, California. The effect observed in atmospheric nitrate is believed to be associated with the transference of mass-independent oxygen from ozone to nitrogen oxide species during the oxidation of NO_x (a term that represents the sum of NO and NO_2) to HNO_3 via NOx-cycle chemistry in the atmosphere (Michalski et al. 2003). Since post-depositional isotopic fractionations obey mass dependent fractionation laws, the $\Delta^{17}O$ signature appears to be a conserved tracer of atmospheric nitrate and has been used to investigate atmospheric deposition to a southern California semiarid ecosystem (Michalski et al. 2004).

Our study builds upon the work of G. Michalski and colleagues and addresses the feasibility of using similar techniques to study the atmospheric deposition of NO_x-derived nitrate to the Chesapeake Bay watershed. Our primary objective has been to establish the wet chemistry and mass spectrometric techniques to analyze the $\Delta^{17}O$ of nitrate at the University of Maryland College Park. Our next objective has been to collect a multi-year set of precipitation samples from the Maryland-DC metropolitan area from which we could extract nitrate to be analyzed for $\Delta^{17}O$ in addition to $\delta^{18}O$ and $\delta^{15}N$. Characterizing the magnitude and variability of the $\Delta^{17}O$ of wetfall nitrate to this region is a necessary step in establishing a long-term program to investigate atmospheric deposition to our local watershed.

Methodology

The analytical work for this project involves three separate steps: (1) sample collection, (2) nitrate extraction, and (3) isotopic analysis of the extracted nitrate. Seventy-five Maryland precipitation samples have been collected since November 2002. Sample sites included College Park, Hyattsville Laurel, Kensington, Tacoma Park, and Urbana, where the majority of the samples were collected. Particular storms of interest during the collection period included the event beginning February 15, 2003 during which over two feet of snow accumulated in some areas and Storm Isabel (September 18, 2003), which could represent different sources of atmospheric nitrate and sulfate than the majority of storms in this region. Precipitation samples were collected in HDPE bins that had been washed, rinsed with deionized water and 10% hydrochloric acid, and triply rinsed with Millipore water (the typical cleaning sequence used for the entire procedure described here). The collection bins were placed outside before and collected after rain events. Bins were also used to catch falling snow, and, at the end of storms, more snow was scooped into the bins using clean HDPE lids. At times, in addition to collecting fresh snow samples, additional snow samples were collected from the sample site after the snow had been on the ground for certain periods of time. In order to recover adequate amounts of nitrate for isotopic analysis, the preferred amount of rainwater or melted snow collected for each storm was 12-18 liters. Whenever possible, the precipitation samples were immediately transferred to clean HDPE bottles, frozen, and kept in a frozen state until thawed and filtered prior to ion extraction.

The nitrate, sulfate, and chloride concentrations of several of the samples were determined using ion chromatography. We are grateful to Dr. Allen Davis for allowing us to use the Dionex DX100 Ion Chromatograph, housed in the Environmental Engineering Lab at the University of Maryland, and to Hunho Kim for training us to properly prepare and store samples for IC analysis and to operate the instrument. At least 20-mL and, in some cases, as much as several liters of each precipitation sample have been archived in a freezer. Future plans include analyzing more of the samples for major ion concentrations for comparison with the nitrogen and oxygen isotope data.

The preparation scheme used to extract nitrate from the water samples and prepare silver nitrate for isotopic analysis incorporated aspects of Silva et al. (2000), Chang et al. (1999), and unpublished methods used by Janet Hannon and others at the USGS in Reston, Virginia. Approximately 15 liters of each natural precipitation sample were filtered using GF/F type and 0.4μm polycarbonate membrane filters and transferred to a clean lowboy with a spigot. Each sample was then gravity dripped through a series of two columns. The first column was loaded with AG50WX-8 cation exchange resin in the H^+ form. This column was intended to minimize dissolved organic carbon contamination of the second column filled with AG2X-8 anion exchange resin, which was used to extract anions (e.g. nitrate and sulfate) from the precipitation samples (Chang et al. 1999). Additionally, since AG50WX-8 resin does not contain nitrogen and retains ammonium, the resin itself can be combusted in order to analyze the nitrogen isotopic composition of the precipitation NH_4^+ (Lehmann et al. 2001). Therefore, for the majority of the samples, this resin has been dried and saved for future analyses.

Nitrate and sulfate ions were then eluted from each anion exchange column using 0.25M potassium chloride solution. Using nitrate test solutions, we monitored the elution of nitrate, and typically the nitrate was recovered using approximately 150-250 ml of the KCl solution. At this stage, each sample (K^+, Cl^-, NO_3^-, and SO_4^{-2} ions in water) was acidified using 2-3 drops of 1M HCl and 0.75 ml 0.5M $BaCl_2$ solution was added to precipitate $BaSO_{4(s)}$. The $BaSO_{4(s)}$ was then removed using a 0.2μm nylon filter and saved for future sulfate sulfur and oxygen isotopic analyses.

Specially made silver resin (AGMP50 cation exchange resin converted from the H^+ form to the Ag^+ form) was then used to remove the Cl^- ions. The cations in solution (e.g. K^+, H^+, and Ba^{+2}) replaced the Ag^+ ions retained on the resin, and the freed Ag^+ ions combined with the Cl^- ions to form $AgCl_{(s)}$. Each solution was then separated from the resin/$AgCl_{(s)}$ mixture and put through a final column filled with the silver resin to ensure that all of the cations had been replaced with Ag^+ ions. Finally, there should only be Ag^+ and NO_3^- ions so that when each sample is freeze-dried, there are pure $AgNO_3$ crystals.

The wet chemistry procedure described above has proven to be very time consuming. In order to expedite the analyses of this sample set, we have processed the precipitation samples in batch mode, working on the same step for numerous samples at a time. Thus far, 61 samples have been processed to the stage in which there are K^+, H^+, Ba^{+2}, Cl^-, and NO_3^- ions in solution. The only steps that need to be completed prior to isotopic analysis include the use of silver resin to remove the chloride ions and replace K^+, H^+, and Ba^{+2} with Ag^+ ions. Making the silver resin is one of the most time consuming and difficult parts of this procedure. A large volume of silver

resin has already been prepared to complete the wet chemistry for this sample set. Eight precipitation samples have already been converted to $AgNO_{3(s)}$ and the remaining samples will likely be completely processed by July 2004.

The final step in our analytical protocol is the conversion of $AgNO_{3(s)}$ to molecular oxygen for isotopic analysis using methods that are modified from techniques used at the University of California, San Diego (Michalski et al. 2002). The manifold that we use for the vacuum pyrolysis of $AgNO_{3(s)}$ is described in Figure 1. This system consists of a platinum resistance furnace in a vacuum chamber that is heated by a 10 volt 15 amp DC current. The $AgNO_{3(s)}$ decomposes to O_2 and NO_2 when heated under vacuum conditions. The O_2 is purified by condensing the NO_2 in a liquid nitrogen-cooled trap. The O_2 is then separated from the frozen NO_2 by freezing it onto a molecular sieve substrate that is kept at the temperature of liquid nitrogen. The purified O_2 is then introduced to a gas-source mass spectrometer for isotopic analysis.

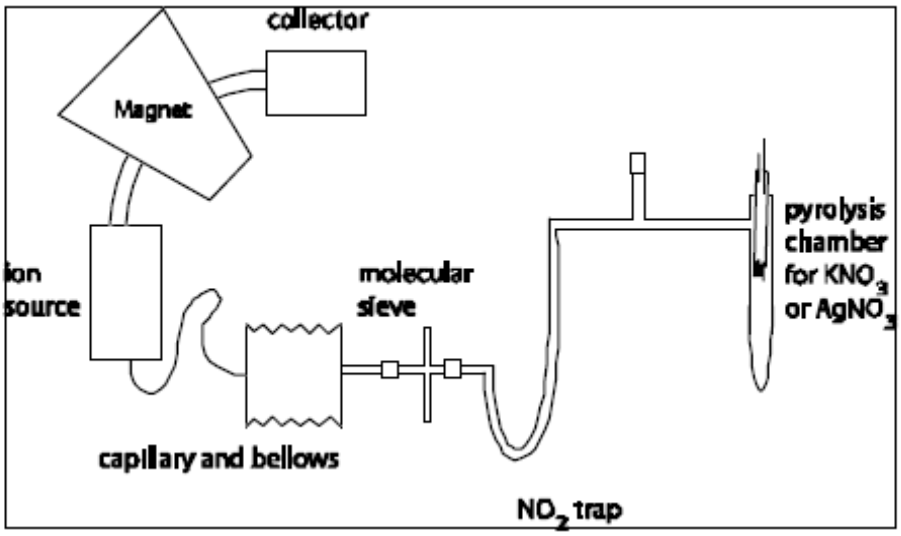


Figure 1. Schematic diagram of nitrate pyrolysis manifold and mass spectrometer. Nitrate samples are wrapped in silver foil and placed in the pyrolysis chamber which is evacuated overnight. The sample is heated using a platinum resistance furnace to generate NO_2 and O_2. The O_2 is purified by condensing NO_2 in a liquid nitrogen-cooled trap and then by transferring the O_2 onto a liquid nitrogen-cooled molecular sieve trap. This purified O_2 is then introduced to the Delta Plus gas-source mass spectrometer for determination of its oxygen isotopic composition. The gas is introduced through the capillary to the ion source where it is ionized and accelerated by a voltage potential. The ion beams are separated according to mass by the magnet and the intensities of the ion beams are measured by an array of Faraday collectors. The isotopic abundances of the gas are measured by isotope ratio measurement relative to a standard.

Principle Findings

We have demonstrated our ability to analyze the $\Delta^{17}O$ of nitrate standard materials. Figure 2 presents the results of our $\Delta^{17}O$ analyses of nitrate standard materials that have been previously analyzed by G. Michalski and coworkers (2002). We have seen good agreement between our methods and theirs and have better reproducibility for $\Delta^{17}O$ than reported by Michalski et al. (2002), which we attribute to the design of our internally heated pyrolysis cell.

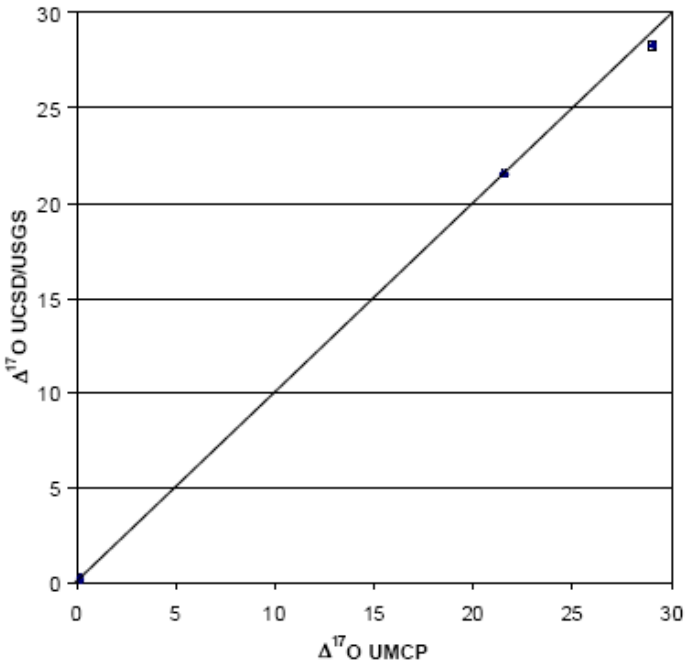


Figure 2 illustrates the $\Delta^{17}O$ values that we have measured for thermal decomposition of two nitrate standards that are reported in Michalski et al (2002) and one nitrate sample that G. Michalski sent to us. The standards fall within error of a slope 1 line indicating a good correspondence between labs. The third sample yielded higher $\Delta^{17}O$ at UMCP that is outside of analytical uncertainty and thought to reflect a blank contribution in the UCSD/USGS data that is not present in the UMCP preparation procedure.

Of the eight samples that have already been converted to silver nitrate, four samples have been analyzed for both $\delta^{18}O$ and $\delta^{15}N$ and two samples have been analyzed for $\Delta^{17}O$. The $\delta^{18}O$ and $\delta^{15}N$ data displayed in Figure 3 were obtained using the TC-EA (thermochemical elemental analyzer) manifold and continuous flow isotope ratio mass-spectrometer (CF-IRMS) managed by Marilyn Fogel and housed at the Geophysical Laboratory of the Carnegie Institution of Washington.

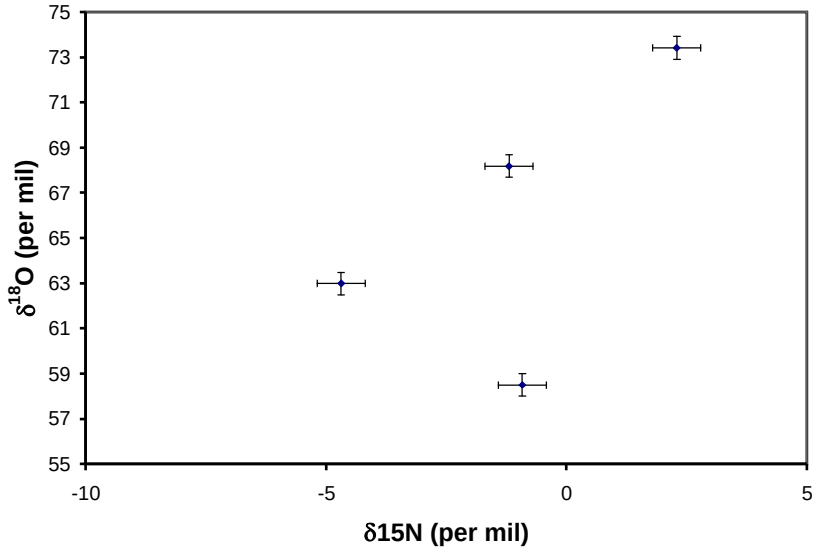


Figure 3: A plot of $\delta^{18}O$ versus $\delta^{15}N$ for four Urbana precipitation samples collected in 2003.

The $\delta^{15}N$ values of atmospheric nitrate generally range from -15 to +15‰ (Kendall 1998 and references therein). Our $\delta^{15}N$ results range from approximately -5 to +5‰, which relates closely to the results reported by Russell and coworkers (1999) who analyzed precipitation samples collected near the Chesapeake Bay from 1993-1994. The $\delta^{15}N$ of atmospheric nitrate is believed to be representative of the nitrogen isotopic composition of the NO_x source. The interpretation of the $\delta^{15}N$ values obtained by Russell et al. (1999) was that NO_x derived by the combustion of fossil-fuels was the dominant source of the atmospheric nitrate to the Chesapeake Bay watershed. In contrast to $\delta^{15}N$, the $\delta^{18}O$ signature of atmospheric nitrate is not believed to reflect the NO_x source because of the addition of oxygen atoms (e.g. from ozone) during the oxidation of NO_x to HNO_3. A large range of $\delta^{18}O$ values for atmospheric nitrate have been reported (+18 to 70‰), but most precipitation samples that have been analyzed are in the range of +55 to +75‰ (Kendall 1998 and references therein). The four samples that we have analyzed for $\delta^{18}O$ are consistent with this range. The $\delta^{18}O$ data is not likely to be relevant in determining the NO_x source, but this information might add to our understanding of the atmospheric chemistry that results in the mass-independent atmospheric nitrate signature.

In order to measure $\Delta^{17}O$, $AgNO_{3(s)}$ is pyrolyzed according to the technique described earlier, and the oxygen gas produced is analyzed via a Delta Plus dual-inlet mass spectrometer (MS) managed by J. Farquhar and housed in the Stable Isotope Laboratory of the University of Maryland. The results of the first two completed analyses are depicted in Table 1.

Sample Date	**δ18O (‰)**	**Δ17O (‰)**
5-16-03	**63.0±0.5**	**23.0±0.1**
5-26-03	**64.9±0.5**	**21.9±0.1**

Table 1 displays the data obtained for the first two Urbana rainwater samples analyzed for $\Delta^{17}O$ via dual inlet MS. The $\delta^{18}O$ values shown here were obtained using the TC-EA CF-IRMS techniques described in the text.

Nitrate in rainwater samples collected in Urbana on May 16th and 26th 2003 revealed mass-independent oxygen isotopic signatures. Presumably, our entire set of precipitation samples will demonstrate that atmospheric deposition from the Maryland-DC metropolitan area to the Chesapeake Bay watershed is characterized by nonzero $\Delta^{17}O$ values. Our preliminary results are consistent with the range of $\Delta^{17}O$ values of atmospheric nitrate in La Jolla, California (20-30.8‰) reported by G. Michalski and collaborators (2003). Future results will allow us to characterize the magnitude and seasonal variability of our region's wetfall $\Delta^{17}O$ values so that we can compare our results to the La Jolla aerosol $\Delta^{17}O$ values and potentially use our findings in a wide-scale investigation of atmospheric deposition to the Chesapeake Bay watershed.

Significance

The work supported during this reporting period has established the techniques needed to analyze the $\Delta^{17}O$ of precipitation nitrate at the University of Maryland. In addition, we have collected 75 precipitation samples from November 2002 to May 2004, made significant progress on preparing the samples for mass spectrometric analysis, and characterized the isotopic signature for a subset of samples. Our work will establish the baseline for the magnitude and seasonal variability of the isotopic composition of regional atmospheric nitrate, which is necessary information for using the $\Delta^{17}O$ tool in a wide-scale study of atmospheric deposition to the Chesapeake Bay watershed. Our long term goals include continuing the collection of precipitation nitrate data in addition to analyzing other sources of nitrate to the Chesapeake Bay for $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$. Ultimately, we will want to use isotopic data to place constraints on the atmospheric contributions of nitrate to the Bay.

References cited:

Boynton W.R., J.H. Garber, R. Summers W.M. Kemp. 1995. Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. Estuaries. 18(1B): 285-314.

Chang, C.C.Y., J. Langston, M. Riggs, D.H. Campbell, S. Silva, and C. Kendall. 1999. A method for nitrate collection for $\delta_{15}N$ and $\delta_{18}O$ analysis from waters with low nitrate concentrations. Can. J. Fish. Aquat. Sci. 56: 1856-1864.

Chang, C.C.Y., C. Kendall, S.R. Silva, W.A. Battaglin, and D.H. Campbell. 2002. Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River basin. Can. J. Fish. Aquat. Sci. 59: 1874-1885.

Chesapeake Bay Foundation. 2003. Bay Resources Fact Sheet: The Chesapeake Bay's Dead Zone.

Kendall, C. 1998. Tracing nitrogen sources and Cycling in Catchments. In: Isotope Tracers in Catchment Hydrology. Eds. C. Kendall and J.J. McDonnell. Amsterdam: Elsevier Science B.V., 519-576.

Lehmann, M.F., S.M. Bernasconi, and J.A. McKenzie. 2001. A Method for the extraction of ammonium from freshwaters for nitrogen isotope analysis. Anal. Chem. 73:4717-4721.

Michalski, G., J. Savarino., J.K. Böhlke, and M. Thiemens. 2002. Determination of total oxygen isotopic composition of nitrate and the calibration of a $\Delta^{17}O$ nitrate reference material. Anal. Chem. 74: 4989-4993.

Michalski, G., Z. Scott, M. Kabiling, and M.H. Thiemens. 2003. First measurements and modeling of $\Delta^{17}O$ in atmospheric nitrate. Geophys. Res. Lett. 30(16) ASC 14-1-.

Michalski, G., T. Meixner, M. Fenn, L. Hernandez, A. Sirulnik, E. Allen, and M.H. Thiemens. 2004. Tracing atmospheric nitrate deposition in a complex semiarid ecosystem using $\Delta^{17}O$. Envion. Sci. Technol. 38: 2175-2181.

Fisher, D.C. and M. Oppenheimer. 1991. Atmospheric nitrogen deposition and the Chesapeake Bay estuary. Ambio. 20(3-4): 102-108.

Russell, K.M., J.N. Galloway, S.A. Macko, J.L. Moody, and J.R. Scudlark. 1998. Sources of nitrogen in wet deposition to the Chesapeake Bay region. Atmospheric Environment. 32 (14-15): 2453-2465.

Silva, S. R., C. Kendall, D.H. Wilkison, A.C. Ziegler, C.C.Y. Chang, and R.J. Avanzino. 2000. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. J. Hydrology. 228: 22-36.

Thiemens M. H. 1999. Mass-independent isotope effects in planetary atmospheres and the early solar system. Science. 283(5400): 341-345.

A study of Chesapeake Bay oysters: genes, markers and conservation -Summer Research Assistantship

Basic Information

Title:	A study of Chesapeake Bay oysters: genes, markers and conservation -Summer Research Assistantship
Project Number:	2002MD10B
Start Date:	6/1/2003
End Date:	10/1/2003
Funding Source:	104B
Congressional District:	5th Congressional District Maryland
Research Category:	Not Applicable
Focus Category:	Ecology, Conservation, Methods
Descriptors:	
Principal Investigators:	Allen Davis, Colin G Rose

Publication

1. in prep

A study of Chesapeake Bay oysters: genes, markers and conservation

Colin Rose (recipient of Water Resources Research Center grant, summer 2003)

Problem and Research Objectives

Wright (1931) described the opposing influences of genetic drift and migration in the evolution of a population. As a consequence of their finite size, populations are subject to random fluctuations in allele frequencies from generation to generation. Given enough time, every allele will reach fixation or go extinct. Isolated populations inevitably diverge from one another, since each population experiences drift randomly across its genome. The speed with which fixation or loss of alleles occurs, and consequently the rate of divergence among populations, is affected by subpopulation size and rate of migration into the population. Even low levels of migration can reduce the amount of drift that populations experience, slowing the rate of differentiation. Given sufficiently high levels of migration, populations stop undergoing genetic drift separately from one another and evolve as one large demographic unit.

Dispersal facilitates the movement of genetic information between populations, homogenizing them when there is a high level of gene flow. Since dispersal is often necessary for gene flow, species with limited dispersal capabilities are expected to show more population differentiation than species with the potential for long-distance dispersal. Nevertheless, long distance dispersal does not rule out differentiation, even at small scales (Hilbish 1996). Examples of mechanisms for local differentiation in high dispersal species are isolation by distance, sweepstakes events, and secondary contact.

Isolation by distance (IBD), described by Wright (1943), is the genetic differentiation that increases with geographic distance when recruitment tends to be local. Given a stepping-stone or island model of dispersal, genetic differentiation accumulates even when long distance migration occurs if most recruitment is local or with neighboring populations (Palumbi 2003). If gene flow is independent of physical proximity, an IBD pattern will not form.

A second explanation for local differentiation in spite of long distance dispersal was suggested by Hedgecock (1994), who noted that high fecundity and stochasticity of larval success could lead to extreme variance in reproductive success. He equated reproduction in some species to a "sweepstakes" in which most individuals are unsuccessful at reproduction, while a few individuals add a disproportionately large number of offspring to the next generation. A potential result of the sweepstakes hypothesis is a dramatic shift in allele frequencies from generation to generation depending on the number of individuals reproducing and their genetic makeup. Another prediction is that individuals of the same age class will show reduced levels of genetic diversity compared with the rest of the population, since they come from just a few breeding adults.

Studies of *Crassostrea virginica*, the American oyster, along the Atlantic coast of Florida have demonstrated high levels of differentiation across small geographic distances. *C. virginica* is sessile in its adult stage, though young oysters spend their first three weeks as planktonic larvae before settling and metamorphosing into adults. Like many marine species with a long larval stage, the American oyster is considered to have the potential for long distance dispersal (Avise 1998). After macrogeographic studies indicated that the east coast of Florida is a genetic break for oyster populations (Karl and Avise 1992; Reeb and Avise 1990), Hare and Avise (1996) examined the region in greater detail using both mitochondrial and nuclear loci. They

found that allele frequencies shift 50-75% in a sharp genetic cline approximately 20 km wide near Cape Canaveral. Within this cline there exists random mating, but outside the contact zone there is limited introgression of haplotypes. The authors suggest that poor habitat and estuarine retention prevent migration between the populations in northern and southern Florida. Genetic differences between Atlantic and Gulf of Mexico populations have been maintained in secondary contact, despite potential for long-distance dispersal.

In this study we examine Chesapeake Bay for population structure in the American oyster. Chesapeake Bay is an estuary nearly 300 km long and up to 65 km across that has historically supported large populations of *C. virginica*. It is unclear whether sweepstakes events influence population structure in the bay. It is also unclear whether there are barriers to gene flow, or the degree to which recruitment takes place locally. Chesapeake bay is an outlet for several large rivers and many smaller ones, creating complex hydrographic patterns that in some regions may be retentive (Southworth and Mann 1998). Thus, the possibility exists that oyster larvae do not move freely throughout the bay, but are retained within subestuaries by currents. On the other hand, there is evidence that oyster larvae actively swim (Baker and Mann 2003); larvae might escape retentive subestuaries and disperse far from their parents.

Previous work has examined allozymes from *C. virginica* in Chesapeake Bay (Buroker 1983). Sampling from ten oyster bars in the bay, significant genetic differentiation was found for 23 of 41 alleles, with mean F_{ST}=0.016 across all loci. Principle component analysis grouped the oysters into four groups, roughly segregated by latitude, which do not correlate with any obvious environmental variables. One subdivision includes just one population, another contains three neighboring populations, and two are groupings of populations scattered through Chesapeake Bay. Because the neutrality of allozymes has been called into question (Karl and Avise 1992), these data would be strengthened by confirmation using independent nuclear markers. The non-IBD pattern to come out of the principle component analysis raises the question of whether the signal has come from processes acting in space, time, or a combination of both.

Brown and Paynter (1991) performed an analysis of mtDNA haplotypes from three native Chesapeake Bay populations and found no evidence of genetic structure. The study, though, may suffer from a lack of statistical power. First, sample sizes were quite low (n=138) compared with Buroker's study (n=1640). Second, the Chesapeake Bay populations are characterized by a single, common mitochondrial haplotype, with many low frequency or unique haplotypes; there are fewer intermediate frequency haplotypes than are predicted under neutral expectations (Ewens 1972). It is precisely those intermediate frequency haplotypes that are informative in tests of differentiation, particularly when signal is subtle, as Buroker suggested it is.

Here we estimate population structure in Chesapeake Bay *C. virginica* with eight microsatellite loci. In order to distinguish the potential effects of IBD, specimens were collected from across the bay (spatial sampling). To detect sweepstakes events, specimens were collected from different cohorts (temporal sampling); short-term temporal variation might occur at a single site, only detectable by sampling genetic variation in different age cohorts. Sources of genetic variation could change with scale, so the spatial and temporal frameworks were investigated both at large and small scales.

C. virginica was once distributed continuously throughout Chesapeake Bay, but fishing, habitat degradation, and disease have reduced the oysters to a fraction of their former numbers and fragmented the remaining populations (Jackson 2001; Kennedy 1996). Because of their importance to the economy and ecology of Chesapeake Bay, oysters have become the focus of

intensive restoration plans (Breitburg et al. 2000; Mann and Evans 2004). As these efforts take shape, it will be important to determine the extent to which population differentiation exists and what is responsible for levels of gene flow (Botsford et al. 2003).

Methodology

Sampling design

Sampling was performed in order to discern both spatial and temporal processes of population structure in Chesapeake Bay. To this end, spatial sampling was performed at sites separated from one another by distances that range from kilometers to hundreds of kilometers. Additionally, in two rivers adults and juveniles (spat) were both collected in order to test for differentiation over a single generation. To test for finer-scale temporal heterogeneity, spat in Little Choptank River were sampled at two-week intervals.

With the goal of testing for both spatial and temporal heterogeneity, a total of 954 specimens from 15 locations were collected in or near Chesapeake Bay (**figures 1, 2**). Abbreviations and descriptions of sampled sites are in **Table 1**. Adult and juvenile specimens were collected together except in the Piankatank and Little Choptank Rivers, where they were collected separately.

Adult oysters were collected by dredge or by hand from subtidal reefs. Spat in Little Choptank River were sampled from oyster shell deployed as spat collectors. Spat collectors hung approximately one meter below low tide and were replaced every two weeks in the breeding season (approximately June through September). After removing the collectors from the water, spat were removed. Spat in Piankatank River were collected after settlement by dredge. After collection, oysters were stored on ice until they were processed in laboratory.

DNA extraction, amplification, and genotyping

Oysters were destructively sampled for DNA extraction. Gill and/or mantle tissue was removed from specimens, and 20 mg were used for DNA extraction. In the case of very small spat, DNA was extracted from the entire oyster (including shell), or after removal of the "gut". DNA extraction was performed using the DNeasy 96 Tissue kit (Qiagen Inc, Valencia, CA) following the standard protocol provided.

Eight microsatellite loci ("2i23", "2g14", "i24b", "2j24", "2i4", "1g3", "Cvi9", "Cvi12") were used in this study. Following amplification, PCR products were electrophoresed with an ABI-Prism 3100 automated sequencer (Applied Biosystems). Allele sizes were estimated using Genescan and Genotyper version 2.5 (Applied Biosystems). Specimens with more than two non-amplifying loci were removed from the data set before analysis.

Analysis

Wright's *F*-statistics (Wright 1951) are used here to describe levels of genetic subdivision and to measure deviations from Hardy-Weinberg expectations. *F*-statistics are hierarchical parameters that quantify the variance of genetic variability that exists between and among populations. If significant levels of variability occur between populations, the result is a positive F_{ST} value, indicating population structure. F_{ST} with a value of zero indicates that populations are not differentiated, either because of recent divergence or because of high levels of gene flow. In addition, the statistics measure differences in observed and expected levels of heterozygosity within populations; differences give a nonzero value of F_{IS}, which demonstrates that the data are not consistent with Hardy-Weinberg expectations. The F_{ST} estimator θ (Weir and Cockerham

1984) was calculated using F-STAT version 2.9.3.2 (Goudet 1995; Goudet 2001). Estimates of F_{ST} were calculated for overall genetic subdivision and for every pairwise comparison of populations. Bootstrap analysis provided 95% confidence intervals for *F*-statistics. F-STAT was also used to calculate number of alleles, gene diversity, and allelic richness.

Isolation by distance was tested with a Mantel test (10,000 permutations) using the ISOLDE ancillary module in GenePop version 3.4 (Raymond and Rousset 1995). In order to test for a linear relationship between genetic and geographical distances, pairwise values of $F_{ST}/(1-F_{ST})$ were correlated with the logarithm of the shortest aquatic distance between the populations (Rousset 1997).

Principal Findings

The eight microsatellite loci were highly polymorphic at the sampled localities (**Table 2**). Overall the level of population structure as measured by θ was not significant; the estimate of F_{ST} is estimated to be 0.001, with a 95% confidence interval between 0 and 0.002. Pairwise comparisons of θ (**Table 3**) demonstrated low levels of population differentiation, but none of the values were statistically different from zero after Bonferroni correction for multiple comparisons ($p<0.0004$).

No statistical values of θ were detected between adult oysters and spat in either the Piankatank or Little Choptank Rivers. Additionally, allele frequencies remained nearly constant across generations (**Table 4**)

A Mantel test demonstrates that correlation of geographic distance and pairwise estimates of F_{ST} is highly significant ($p=0.006$). Regression of the logarithm of distance and $\theta/(1-\theta)$ is shown in **Figure 3**. Fitting $\theta/(1-\theta)$ to $a + b\,Ln$ (distance), a = -0.0025 and b = 0.0007.

Significance

Two principal conclusions come from the analyses in this report. The first finding here is that there is an absence of temporal heterogeneity in the Piankatank and Little Choptank Rivers. The sweepstakes hypothesis has received a great deal of attention in highly fecund marine species like the oyster, and is generally believed to be an important determinant in the structure of populations (Moberg and Burton 2000). The data in this study, however, demonstrate patterns in Chesapeake Bay that are contrary to the predictions of the sweepstakes hypothesis. This investigation does not totally discount the possibility that sweepstakes events can happen, but rather shows that they do not play an important role in Chesapeake Bay. Since sweepstakes events reduce the effective size of a population (Hedgecock 1994), the effective population size of oysters in Chesapeake Bay may be quite large compared with previous expectations.

The second important finding from this study is that a significant, though very subtle isolation by distance pattern has been demonstrated in Chesapeake Bay oysters. Given the long-distance dispersal capability of oyster larvae, and the fact that Chesapeake Bay is small in comparison with the potential dispersal distance, isolation by distance within Chesapeake Bay is surprising and remarkable. Until recently, highly polymorphic markers like microsatellites were not available for population genetic analysis. The inclusion of these markers in tests of population structure permits the detection of extremely subtle genetic differentiation (Waples 1998). Two previous studies of oysters have failed to find either a consistent—or any—pattern in of differentiation in Chesapeake Bay. The data in this study not only demonstrate that differentiation exists, but also that a biologically meaningful explanation for the pattern.

Isolation by distance can only exist when recruitment is local (Slatkin 1993), so oyster larvae must not disperse as freely in Chesapeake Bay as has been previously assumed.

This information has important consequences for future restoration efforts. In particular, the design of reserves will have to take into consideration that oyster larvae, though capable of long-distance dispersal, may be more likely to settle near their source. This may mean that the use of many small, neighboring reserves, and not a limited number of large ones, will be the most effective design in ensuring gene flow among populations (Palumbi 2003). Since sustainable reserves require interbreeding, it will be necessary to test whether restoration sites are capable of exchanging migrants in large enough numbers to counteract the effects of inbreeding and increased chance of extinction. These results are tentative, and difficult because of their subtle nature; we suggest further sampling and analysis before making suggestions for restoration policy in Chesapeake Bay. Nonetheless, these data present a new picture that contradicts previous expectations of oyster populations in Chesapeake Bay.

Works Cited

Avise, J. C. 1998. Conservation genetics in the marine realm. Journal of Heredity 89:377-382.

Baker, P., and R. Mann. 2003. Late stage bivalve larvae in a well-mixed estuary are not inert particles. Estuaries 26:837-845.

Botsford, L. W., F. Micheli, and A. Hastings. 2003. Principles for the design of marine reserves. Ecological Applications 13:S25-S31.

Breitburg, D. L., L. D. Coen, M. W. Luckenbach, R. Mann, M. Posey, and J. A. Wesson. 2000. Oyster reef restoration: convergence of harvest and conservation strategies. Journal of Shellfish Research 19:371-377.

Brown, B. L., and K. T. Paynter. 1991. Mitochondrial DNA analysis of native and selectively inbred Chesapeake Bay oysters, *Crassostrea virginica*. Marine Biology 110:343-352.

Buroker, N. E. 1983. Genetic differentiation and population structure of the American oyster *Crassostrea virginica* (Gmelin) in Chesapeake Bay. Journal of Shellfish Research 3:153-167.

Ewens, W. J. 1972. Sampling theory of selectively neutral alleles. Theoretical Population Biology 3:87-112.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86:485-486.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). Institut de Zoologie et d'Ecologie Animale, Universite de Lausane, Lucerne

Hare, M. P., and J. C. Avise. 1996. Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). Evolution 50:2305-2315.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population size of marine organisms? Pp. 122-134 *in* A. R. Beaumont, ed. Genetics and evolution of aquatic organisms. Chapman & Hall, London.

Hilbish, T. J. 1996. Population genetics of marine species: the interaction of natural selection and historically differentiated populations. Journal of Experimental Marine Biology and Ecology 200:67-83.
Jackson, J. B. C. 2001. What was natural in the coastal oceans? Proceedings of the National Academy of Sciences of the United States of America 98:5411-5418.
Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters - implications from nuclear RFLPs. Science 256:100-102.
Kennedy, V. S. 1996. The ecological role of the eastern oyster, *Crassostrea virginica*, with remarks on disease. Journal of Shellfish Research 15:177-183.
Mann, R., and D. A. Evans. 2004. Site selection for oyster habitat rehabilitation in the Virginia portion of the Chesapeake Bay: a commentary. Journal of Shellfish Research 23:41-49.
Moberg, P. E., and R. S. Burton. 2000. Genetic heterogeneity among adult and recruit red sea urchins, Strongylocentrotus franciscanus. Marine Biology 136:773-784.
Palumbi, S. R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications 13:S146-S158.
Raymond, M., and F. Rousset. 1995. Genepop (Version-1.2) - Population-Genetics Software for Exact Tests and Ecumenicism. Journal of Heredity 86:248-249.
Reeb, C. A., and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics 124:397-406.
Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219-1228.
Slatkin, M. 1993. Isolation by Distance in Equilibrium and Nonequilibrium Populations. Evolution 47:264-279.
Southworth, M., and R. Mann. 1998. Oyster reef broodstock enhancement in the Great Wicomico River, Virginia. Journal of Shellfish Research 17:1101-1114.
Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity 89:438-450.
Weir, B. S., and C. C. Cockerham. 1984. Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38:1358-1370.
Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.
Wright, S. 1943. Isolation by distance. Genetics 28:114-138.
Wright, S. 1951. The Genetical Structure of Populations. Annals of Eugenics 15:323-354.

Figure 1 – Collection sites in Chesapeake Bay

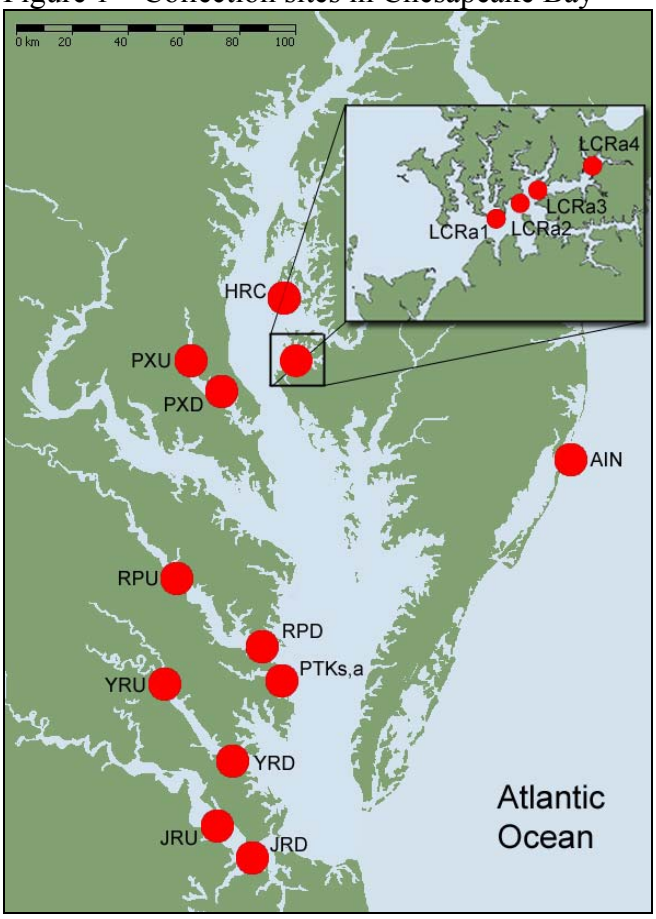


Figure 2 – Temporal sampling sites in Little Choptank River

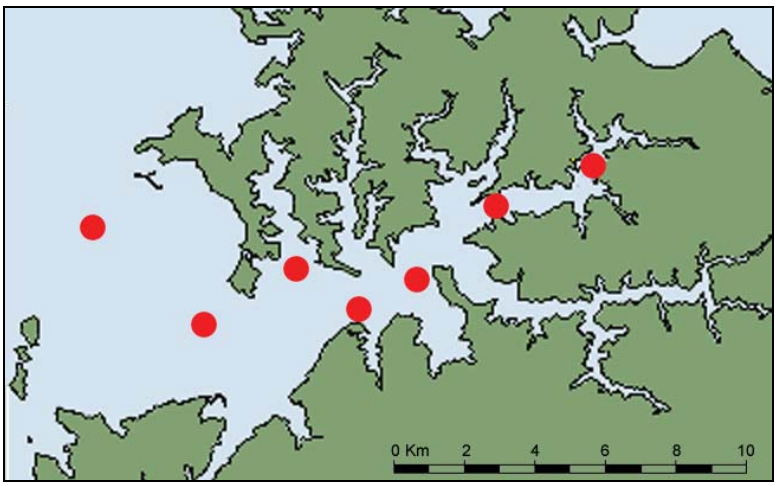


Table 1 – Sampling sites

Site	Description	Sample Size	Latitude, Longitude
PTKs	Piankatank spat	48	37°31.35′ N, 76°21.2′ W
PTKa	Piankatank adults	47	37°30.58′ N, 76°20.53′ W
HRC	Harris Creek	50	38°45.05′ N, 76°17.75′ W
AIN	Assateague Island	50	38°14.39′ N, 75°08.74′ W
LCRs	Little Choptank River spat	163	38°34′ N, 76°10′ W
LCRa1	Little Choptank River adults – site 1	59	38°32.02′ N, 76°14.64′ W
LCRa2	Little Choptank River adults – site 2	50	38°32.61′ N, 76°13.62′ W
LCRa3	Little Choptank River adults – site 3	46	38°32.91′ N, 76°13.07′ W
LCRa4	Little Choptank River adults – site 4	57	38°34.08′ N, 76°10.57′ W
PXD	Patuxent River downriver	50	38°23.51′ N, 76°33.53′ W
PXU	Patuxent River upriver	50	38°30.44′ N, 76°40.19′ W
JRD	James River downriver	50	36°55.62′ N, 76°27.37′ W
JRU	James River upriver	38	37°04.17′ N, 76°35.12′ W
RPD	Rappahannock River downriver	50	37°36.32′ N, 76°24.75′ W
RPU	Rappahannock River upriver	50	37°50.67′ N, 76°45.67′ W
YRD	York River downriver	50	37°15.25′ N, 76°31.43′ W
YRU	York River upriver	50	37°30.25′ N, 76°47.85′ W

Table 2 – Gene diversity per locus and per population

	2g14	2i23	2i4	2j24	Cvi12	Cvi9	i24b	1g3
PTKs	0.942	0.892	0.922	0.865	0.824	0.911	0.905	0.631
PTKa	0.947	0.912	0.900	0.876	0.794	0.900	0.885	0.534
HRC	0.948	0.895	0.915	0.868	0.833	0.906	0.901	0.577
AIN	0.944	0.923	0.928	0.867	0.762	0.900	0.885	0.572
LCRs	0.950	0.897	0.918	0.879	0.867	0.907	0.866	0.606
LCRa1	0.951	0.872	0.924	0.866	0.873	0.904	0.909	0.599
LCRa4	0.951	0.893	0.931	0.866	0.857	0.910	0.887	0.703
LCRa2	0.938	0.892	0.936	0.833	0.861	0.913	0.909	0.596
LCRa3	0.947	0.916	0.928	0.842	0.864	0.893	0.877	0.588
PXD	0.947	0.858	0.919	0.889	0.874	0.915	0.906	0.555
PXU	0.948	0.878	0.931	0.869	0.876	0.909	0.850	0.536
JRD	0.960	0.908	0.915	0.876	0.889	0.898	0.877	0.652
JRU	0.946	0.870	0.938	0.887	0.862	0.924	0.905	0.625
RPD	0.945	0.878	0.914	0.867	0.868	0.909	0.888	0.703
RPU	0.945	0.926	0.939	0.871	0.824	0.884	0.906	0.632
YRD	0.950	0.880	0.936	0.835	0.863	0.898	0.897	0.656
YRU	0.936	0.896	0.900	0.870	0.855	0.921	0.871	0.718

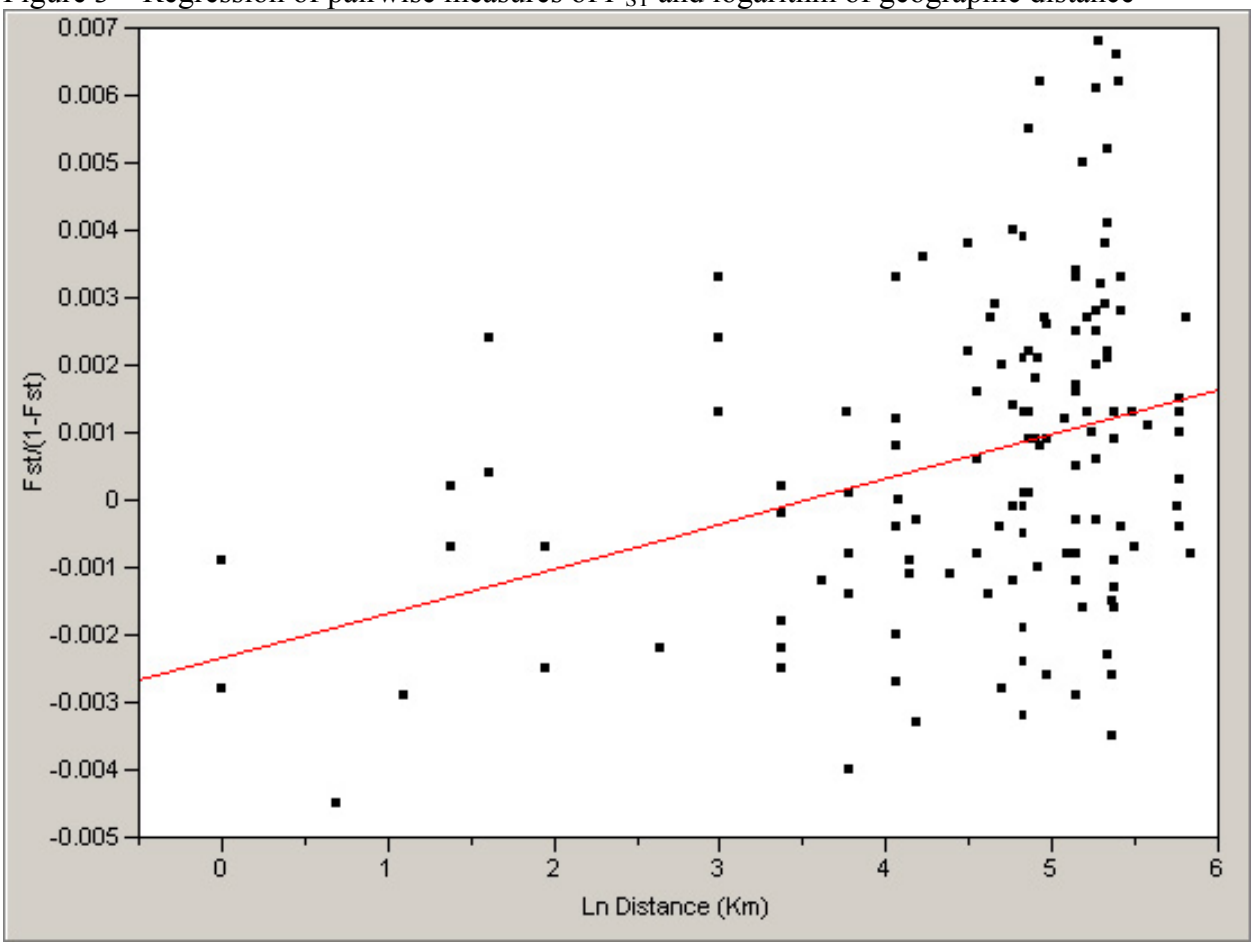
Table 3 – Pairwise values of θ, an unbiased estimator of F_{ST} (below the diagonal) and associated p-values (above the diagonal). Significant values ($p<0.05$) are in bold

	PTKs	**PTKa**	**HRC**	**AIN**	**LCRs**	**LCRa1**	**LCRa4**	**LCRa2**	**LCRa3**	**PXD**	**PXU**	**JRD**	**JRU**	**RPD**	**RPU**	**YRD**	**YRU**
PTKs	-	0.837	0.683	0.978	0.314	0.776	0.872	0.704	0.863	0.899	0.344	0.211	0.874	0.093	0.699	0.678	0.484
PTKa	-0.0018	-	0.302	0.886	0.139	0.570	0.838	0.197	0.394	0.585	0.101	0.263	0.619	0.176	0.539	0.388	0.448
HRC	-0.0026	0.0009	-	0.709	0.261	0.911	0.445	0.547	0.490	0.943	0.706	0.049	0.292	**0.042**	0.051	0.351	0.072
AIN	-0.0035	-0.0026	-0.0008	-	0.739	0.846	0.538	0.740	0.299	0.914	0.565	**0.037**	0.141	**0.019**	0.543	0.385	0.602
LCRs	-0.0001	0.0013	-0.0002	0.001	-	0.546	0.530	0.111	0.690	0.603	0.082	0.160	0.620	**0.026**	**0.010**	0.061	0.057
LCRa1	-0.0019	0.0021	-0.0025	0.0003	-0.0007	-	0.954	0.983	0.901	0.874	0.664	0.128	0.176	0.338	0.253	0.972	0.420
LCRa4	-0.0032	0.0001	0.0002	-0.0004	-0.0009	-0.0025	-	0.255	0.367	0.538	0.120	0.397	0.222	0.131	**0.039**	0.679	0.706
LCRa2	-0.0005	0.0039	-0.0018	0.0015	0.0024	-0.0045	0.0004	-	0.934	0.734	0.392	0.118	0.109	0.009	0.417	0.665	**0.047**
LCRa3	-0.0024	0.0013	-0.0022	0.0013	-0.0007	-0.0029	0.0002	-0.0028	-	0.408	0.476	0.078	0.424	0.108	0.283	0.514	0.135
PXD	-0.0012	0.0014	-0.0033	-0.0001	-0.0008	-0.004	0.0001	-0.0014	-0.0014	-	0.685	0.501	0.664	0.271	0.726	0.631	0.076
PXU	0.0009	0.0018	-0.0011	0.0027	0	-0.002	0.0008	0.0012	-0.0027	-0.0022	-	0.047	0.244	0.038	**0.036**	0.167	**0.006**
JRD	0.0022	0.0038	0.0033	**0.0068**	0.0006	0.0025	-0.0003	0.0061	0.0028	0.002	0.0038	-	**0.047**	0.249	0.058	0.381	0.179
JRU	-0.0028	0.002	-0.0007	0.004	-0.0016	-0.0013	0.0009	0.0013	-0.0009	-0.0015	-0.0004	**0.0013**	-	0.332	0.767	0.922	0.530
RPD	0.0024	0.0033	**0.0027**	**0.0062**	**0.0022**	0.0013	0.0001	0.0055	0.0009	-0.0001	0.0021	0.0006	-0.001	-	0.393	0.623	0.115
RPU	-0.0011	-0.0009	0.0027	0.0011	**0.0033**	0.0017	**0.0034**	0.0016	0.0005	0.0012	**0.005**	0.0062	-0.0016	0.0013	-	0.949	0.283
YRD	-0.0004	0.0033	0.001	0.0029	0.0025	-0.0029	-0.0008	-0.0012	-0.0003	-0.0008	0.0013	0.0036	-0.0014	-0.0003	-0.0004	-	0.926
YRU	-0.0008	0.0016	0.0028	0.0013	0.0022	0.0021	-0.0023	**0.0052**	0.0041	0.0032	**0.0066**	0.0029	0.0008	0.0027	0.0026	-0.0012	-

Table 4 – Comparison of gene diversity in spat and adults (LCRa is a combination of LCRa1-4)

	2g14	2i23	2i4	2j24	Cvi12	Cvi9	i24b	1g3
LCRs	0.950	0.897	0.918	0.879	0.867	0.907	0.866	0.606
LCRa	0.946	0.891	0.927	0.849	0.862	0.905	0.895	0.625
PTKs	0.942	0.892	0.922	0.865	0.824	0.911	0.905	0.631
PTKa	0.947	0.912	0.900	0.876	0.794	0.900	0.855	0.534

Figure 3 – Regression of pairwise measures of F_{ST} and logarithm of geographic distance



Fate of Alkylphenolic Compounds in Wastewater Treatment

Basic Information

Title:	Fate of Alkylphenolic Compounds in Wastewater Treatment
Project Number:	2003MD28B
Start Date:	3/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	5th District of Maryland
Research Category:	None
Focus Category:	Toxic Substances, Waste Water, Water Quality
Descriptors:	None
Principal Investigators:	Alba Torrents, Clifford Paul Rice

Publication

Yearly project report March 2003 - February 2004

Fate of alkylphenolic compounds in wastewater treatment plants and a sub-estuary of the Chesapeake Bay

Alba Torrents
Department of Civil and Environmental Engineering
University of Maryland
College Park, MD 20742

Clifford Rice
Environmental Chemistry Laboratory
USDA/ARS
Beltsville, MD 20705

Ph.D. Student: Jorge Loyo Rosales

Statement of Problem and Research Objectives

Recently, a series of studies have raised concerns about the presence of persistent organic pollutants in natural waters and the capability of wastewater treatment plants (WWTPs) to remove such chemicals from their effluents. The last decade has also seen an increased interest in pollutants suspected to interfere with the endocrine system, commonly referred to as endocrine disrupters. One such compound is 4-nonylphenol (NP), which was found to produce the same effects as estradiol in a line of cancer cells (Soto et al. 1991). NP is a precursor in the synthesis of the nonylphenol ethoxylates (NPnEOs), one class of alkylphenol ethoxylates (APnEOs). APnEOs are nonionic surfactants that have been widely used in industrial processes and as detergents in both industrial and household applications for more than thirty years. After being used, the APnEOs are discharged into wastewater and are treated in WWTPs. During wastewater treatment, APnEOs are subject to microbial degradation processes that produce different metabolites (NP among them), which are ultimately released into natural waters. The APnEOs have been signaled as a current pollution issue in the Chesapeake Bay and were highlighted in a recent workshop on emerging contaminants organized by the Scientific and Technical Advisory Committee of the Chesapeake Bay Program on October 18, 2002 at Solomons Island. Currently, little information exists on the presence of these compounds in the State of Maryland or the Chesapeake Bay itself. The main objective f the proposed work is to model the distribution and fate of APnEOs and their degradates (APs) in a sub-estuary of the Chesapeake Bay and a WWTP and, at the extent possible, determine how operating parameters at the plants control the concentrations of these endocrine-active substances in the estuary.

Methodology

A main limitation for their study is the limitation of analytical techniques for their analysis in natural waters. As part of this project we proposed to develop a protocol for the sampling and analysis of NPnEOs and OPnEOs and their derivatives in effluent and natural samples. We also proposed the development of a "Mass Balance and Distribution" model.

Principal Findings

During the first year of the project, we have focused our work in the development of the required analytical methods, and we have initiated the development of the theoretical model.

Analytical method development

As stated in the proposal, additional method development was necessary to address the analytical needs of the project. When the proposal was submitted, we had developed a method for the extraction and quantification of nonylphenol (NP), octylphenol (OP) and their ethoxylated derivatives with up to 5 ethoxylate units (APnEO, n = 1 – 5), which has been published since then (Loyo-Rosales et al 2003). In the last year, we have expanded that method to include NPnEO with n = 6 to 16, which are extracted simultaneously to the APnEO with n = 0 to 5. The main limitation was the lack of adequate analytical standards for these compounds because they are not available individually, only as technical mixtures. Besides, most of these mixtures are not characterized, and the relative content of each ethoxymer is unknown. We attempted to use Marlophen 810 (Chemische Werke, Hüls, Germany), characterized by Ahel et al (2000), but we discovered that this mixture not only contains the NPnEO, but also the OPnEO, rendering it useless for quantitative purposes. Therefore, we used Surfonic N-95 (Schenectady International, Schenectady, NY), which was characterized by Huntsman Corporation (Austin, TX), and we were able to successfully quantify NPnEO (n = 6 – 16). Unfortunately, there are no

characterized mixtures of the OPnEOs available; therefore, we are monitoring these analytes only qualitatively.

As part of the proposed model, it is necessary to quantify the analytes of interest in the particulate matter in the water. We developed an analytical method based on Soxhlet extraction with methanol and LC/MS/MS analysis. In order to obtain the particulate, one liter of water is vacuum-filtered with two pre-weighed glass microfibre filters (GF/A and GF/F, particle retention 1.6 and 0.7 μm respectively, Whatman Inc., Clifton, NJ; previously baked at 400°C for 4 h to eliminate any possible NP contamination) in a glass filter holder (Millipore Corporation, Billerica, MA). The filtrate is used for water analysis of the APnEO and the filters are allowed to dry overnight in a desiccator under vacuum. Once dry, the filters are weighed again to calculate particulate concentration and then spiked with a ^{13}C-labeled internal standard and Soxhlet-extracted with methanol for 8 hrs. The extracts are then evaporated to approximately 5 mL in a rotary evaporator, transferred to 15-mL glass centrifuge tubes and further reduced to 0.5 mL under a gentle nitrogen stream. After adding 0.5 mL of carbon-free deionized water, the extracts are filtered using an Acrodisc LC 13-mm syringe filter with a 0.2- μm PVDF membrane (Pall Gelman Laboratory, Ann Arbor, MI) into a 2-mL LC vial; the syringe and filter are rinsed with 0.5 mL of a 50:50 methanol/water mixture that is added to the extract. Finally, volume is adjusted to 1.5 mL and the extracts analyzed by LC/MS/MS. Recoveries for this extraction method vary for the different compounds and range from 73 to 100%.

Besides the APnEO, we are also interested in modeling other metabolites, such as their carboxylated derivatives. Due to their ionic nature, these cannot be extracted along with the APnEO, and a separate extraction method was developed for them. In this method, water samples are filtered as described above and part of the filtrate is acidified to pH 2 with HCl, and extracted with dichloromethane (DCM) in a separation funnel. DCM is evaporated and exchanged to approximately 5 mL methanol in a rotary evaporator and treated as above. Recovery was calculated for the three carboxylated metabolites (NP0EC, 93%; NP1EC, 93%; and OP0EC, 94%) for which standards are available. Additionally, we have been able to identify

carboxylated derivatives with higher molecular weight, and we plan to monitor them qualitatively.

Theoretical model

For our initial approach, we constructed a model of Back River in a commercial modeling environment (Stella, isee systems, Lebanon, NH). Only NP was considered and the river was divided into four cells (see figure 1). The first and northernmost cell includes the influent from the two major tributaries, Herring Run and North East Creek and ends before Back River WWTP. The second cell includes the effluent from the WWTP and ends at Muddy Gut. The third cell starts at Muddy Gut and ends at Greenhill Cove. The fourth and last cell runs from Greenhill Cove to the mouth of the river into the Chesapeake Bay. The last two cells receive water only from the preceding cell; no other inputs were considered. This division was based on the location of the influents to the river and specific geographic features; i.e. points where the river turns. Each section of the river was modeled as a well-mixed reactor. The following processes were included in each cell: advection, dispersion, volatilization, photolysis, partition into suspended solids and net deposition into sediments. Advection was modeled as a function of flow rate (Q) and concentration (total concentration of the chemical, including both dissolved and bound species). The flow rate was assumed to be constant and values used were ten times smaller than the actual flow rates to account for tidal flow. Dispersion was modeled as a function of concentration gradients between sections, dispersion coefficient – constant for all sections in the river –, cross sectional area and volume of each section. Volatilization was modeled as a flux out of the water, assuming the concentration of these chemicals is equal to zero in the atmosphere. Photolysis of NP and deposition into sediments were modeled as a first order reaction. Flow diagrams of the model are depicted in figure 2.

The preliminary results of our model suggest that, after reaching steady state, NP will be present in the water at a concentration of 0.5, 0.7, 0.4 and 0.08 μg/L for cells 1 to 4 respectively. This

values are very close to actual concentrations measured in the river in January 2001 (0.3, 0.4, 02 and 0.05 μg/L respectively). The steady-state amount of NP in the water represents approximately 10 % of the total amount entering the Back River over a period of 10 days. Photodegradation appears to be the most important removal process for NP in the water (49% of the NP entering the Back River), whereas deposition into sediments is the second most relevant process (35%), and volatilization losses are minimal (3%). Even with these losses, 13% of the NP would be transported into the Chesapeake Bay. These results are summarized in figure 3.

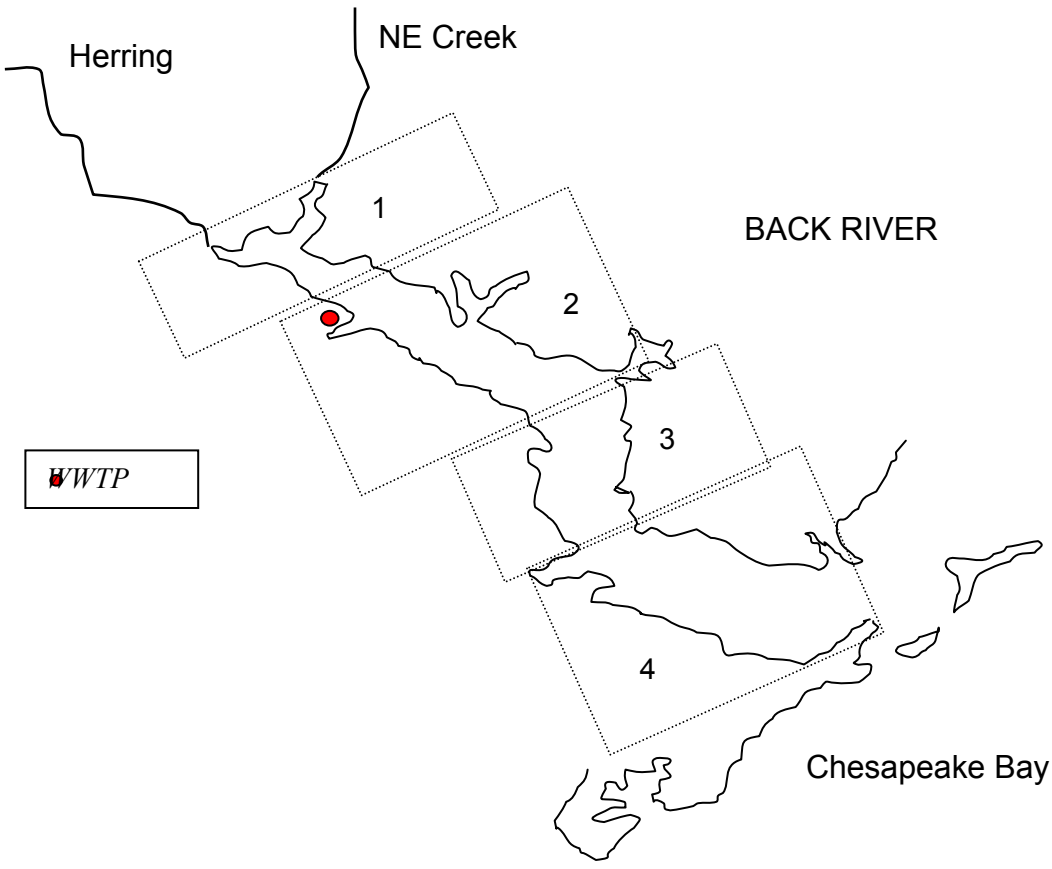
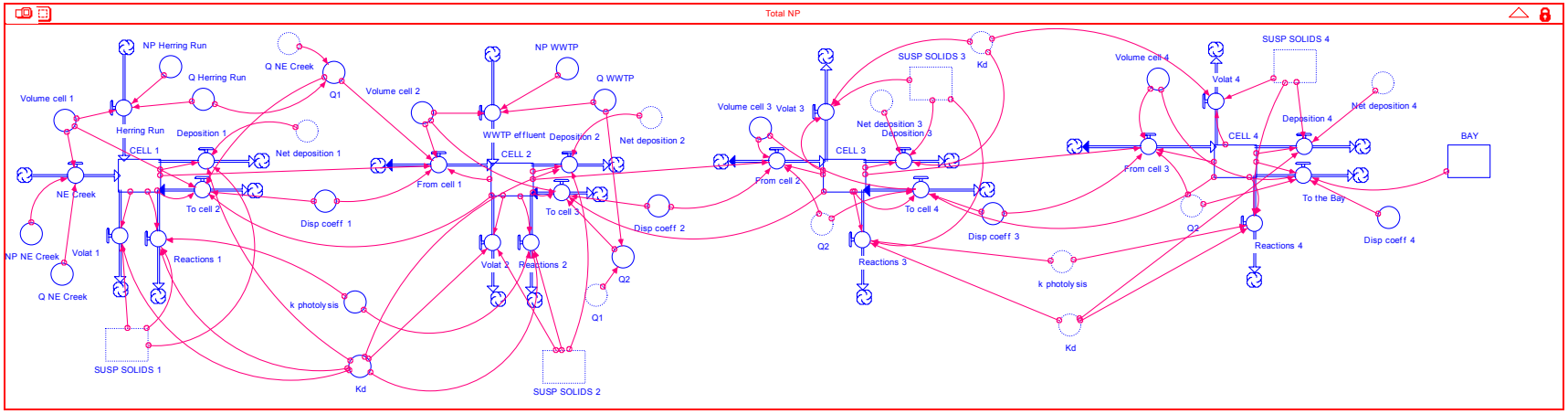


Figure 1. Scheme of the Back River showing the four sections of the model.

Figure 2. Flow diagram for the Stella model of NP distribution in the Back River.



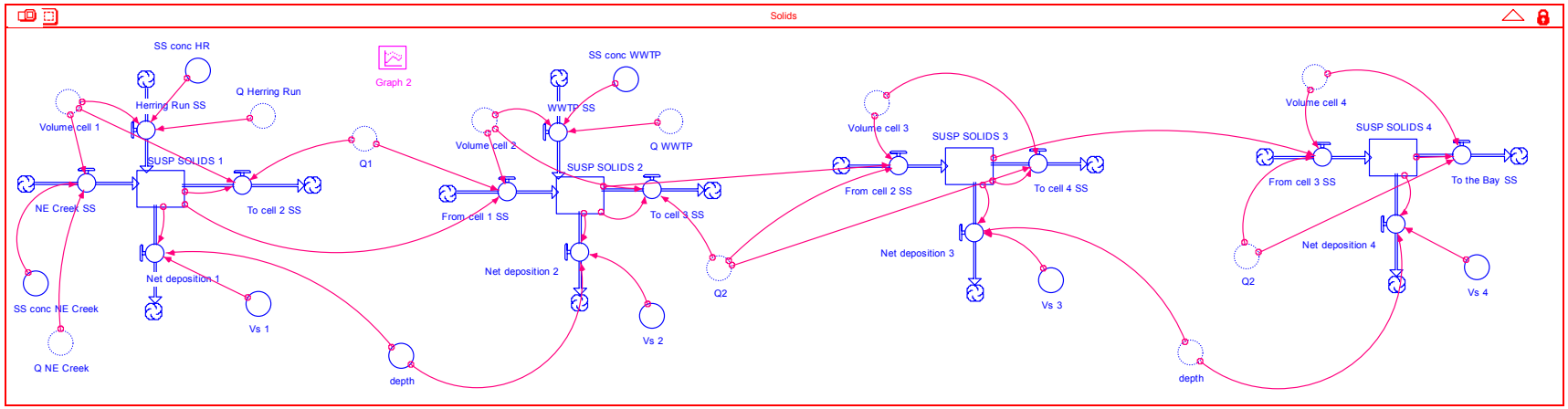
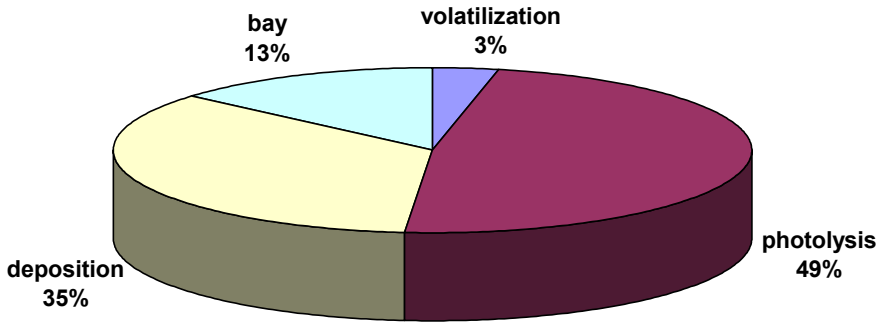


Figure 3. NP distribution in the Back River



Current and future activities

For the second year of the project, we are working on adding more compounds to the model (APnEO) and improving its accuracy by focusing on several items, mainly:

- Adequate quantification of the NP and APnEO inputs into the system. Estimates from previous samplings were used until now; improving the accuracy of these figures will result in better estimates of the equilibrium concentrations in the river. We are currently planning two sampling trips to the Back River that will include collection of both sediment and water along the river. One sampling event will be conducted during the summer and the second one in the winter to account for temperature variations. At the same time, sampling will be conducted at the wastewater treatment plant to better estimate the compounds input to the river.
- A better description of the river's hydrology, including the tidal nature of its flow. We attempt to include variations in the flow and improve our understanding of dispersion in the system.
- The model in its present form does not consider biodegradation. Although it is still debated whether NP is subjected to biodegradation, some studies show evidence that it is. However,

it is widely accepted that APnEO are degraded by microorganisms in the environment and that this is a relevant removal process.

- A better understanding of the suspended solids behavior, including transport along the Back River, deposition and resuspension. These are important parameters, because NP and the lower molecular weight APnEO tend to partition to solids; therefore, the rate at which they are deposited into the sediments has a strong influence on their removal from the water.

Significance

We currently have a sound analytical methodology for the study of NP and degradates. We have also developed a Mass balance and distribution model for Back River. During the second year we will concentrate in obtaining WWTP discharge values and water and sediment values to refine the model and assess the relative importance of all the modeled processes. Once such a model is available, it would be extremely useful for policy makers as they could assess the distribution of NPs in natural systems. Furthermore, the framework of this model could be use to develop models for other emerging organic pollutants.

References

Ahel, M., Giger, W., Molnar, E., Ibric, S. (2000), "Determination of nonylphenol polyethoxylates and their lipophilic metabolites in sewage effluents by normal-phase high-performance liquid chromatography and fluorescence detection". *CCACAA* 73(1), 209-227.

Loyo-Rosales, J. E., Schmitz-Afonso, I., Rice, C. P., Torrents, A. (2003), "Analysis of octyl- and nonylphenol and their ethoxylates in water and sediments by liquid chromatography/tandem mass spectrometry". *Anal. Chem.* 75(18), 4811-4817.

Response of Macroinvertebrates to Road Salt Runoff in Headwater Streams

Basic Information

Title:	Response of Macroinvertebrates to Road Salt Runoff in Headwater Streams
Project Number:	2003MD30B
Start Date:	3/1/2003
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	5th District of Maryland
Research Category:	None
Focus Category:	Non Point Pollution, Water Quality, Conservation
Descriptors:	None
Principal Investigators:	William Lamp

Publication

1. No publications have been submitted for this project.

Title	Response of Macroinvertebrates to Road Salt Runoff in Headwater Streams
Project No.	2003MD30B
Start Date	3/1/2003
End Date	2/28/2004
Funding Source	104B
Cong. Dist.	5th District of Maryland
Research Category	None
Focus Category	NonPoint Pollution, Water Quality, Conservation
Descriptors	None
Principal Investigator	William Lamp

2003-2004 USGS Annual Report for Project Number 2003MD30B
Response of Macroinvertebrates to Road Salt Runoff in Headwater Streams

Principal Investigators: William Lamp and Saundra Crane

PROBLEM AND RESEARCH OBJECTIVES:

As stated in the project proposal, little research has been conducted on the effects of salt toxicity and salinity to aquatic biota in running water systems in the mid-Atlantic region of the United States. Increasing suburbanization in the Piedmont physiogeographic region of Virginia and Maryland heightens the risk of loss and adverse impacts to headwater streams. These streams provide habitat for benthic invertebrate organisms which are key components of trophic food webs in aquatic systems. Both acute and chronic effects on aquatic biota may occur from road salt inputs into small streams.

Research Objectives:

1. To quantify the stormflow loading of road salt in a gradient of rural – urban streams subsequent to a snowstorm event.
2. Measure the macroinvertebrate response (mortality and drift) to salt loading of an unimpacted headwater stream.
3. Measure mortality response of macroinvertebrate species to acute levels of high slat concentrations

The final objective, to compare mortality of macroinvertebrate species to different cation sources of salt, was dropped from the study because of cost constraints and lack of access to appropriate equipment.

METHODOLOGY

Objective 1: Fifteen streams representing unimpacted to highly urbanized conditions were selected. Four are located in Loudoun County, VA, 1 in Great Falls National Park, VA (Fairfax County), 4 in Montgomery County, 1 in Prince Georges County (on the University of Maryland College Park campus), and 5 in Howard County, MD. Baseline conditions were measured by taking monthly samples from each stream beginning

August 2003. pH, temp, DO (through December 2003), Conductivity and Specific Conductivity measurements were taken using an YSI probe. Chloride levels (mg/l) were measured using Hach silver nitrate titration kit. Because chloride is inert and does not dissipate over time, 2/3 of the samples still require processing.

Access to streams during snow events proved more difficult than expected so select sites were monitored daily (or multiple times/day) during and following snow events. The Fairfax County and 2 Loudoun County sites were monitored in January 2004 during a snow event that for 10 days until the snow melted. Surge Creek on the UMD campus was also monitored for a week following the snow event. For other short term snow events (November 2003, February 2004), measurements and water samples were taken at a subset of sites. In addition, measurements were taken during two rain storm events in 2003 to compare the dilution effect of snow versus rain discharge.

To obtain a baseline inventory of the invertebrate population in the stream sites, three moss packs were placed at each site for approximately 4 weeks in February 2004 and then collected. At seven of the sites, 1 -3 packs were either lost or desiccated at the time of retrieval, making an assessment of invertebrate community. Sampling is planned for late Fall 2005 to reassess the invertebrate community.

Objective 2: In March 2004, an experiment was conducted at the Central Maryland Research and Education Center (CMREC), Clarksville, Howard County, MD. Drift nets were stationed above the salt input site and 125 feet below the input site in a stream designated Field Stream. A 1280 mg/l chloride solution was pumped into the stream at a constant rate for 5.5 hours. Conductivity readings just below the pumping station monitored the amount of solution entering the stream. Drift net samples were collected prior to the initiation of pumping, beginning at 3:30pm and then every two hours for a total of six samples and then again at sunrise the next morning. A control stream also had two drift net collection sites; samples were collected from 3:30pm until 6pm and at 6:30m the following morning. Samples were noted for mortality or unresponsiveness prior to preserving the invertebrates in alcohol. The invertebrate samples are still being identified and analyses comparing within and across stream sites is not yet complete.

Objective 3: Two laboratory experiments were run to determine acute effects to high NaCl concentrations. The initial experiment was performed primarily to test the technique and used *Gammerus spp.* NaCl concentrations of 0mg/l, 50mg/l, 500mg/l and 5000mg/l were used with 3 organisms per flask and 6 replicates per concentration. Mortality was low and could not be attributed to NaCl concentrations. A second experiment was run in January 2004 using *Tuplidae spp.* Using NaCl concentrations of 0, 50, 500, and 5000mg/l, 3 individuals were placed in a tray in their own mesh containers and provided with leached maple leaves for refugia (to reduce stress). The trays were aerated and stream water was used from the site where the *Tuplidae spp*. were collected. The stream, Fishing Creek in Frederick County, MD, has a low conductivity reading for ions and meets the criteria for this study designation of “unimpacted.”

There were four replicates of each NaCl concentration. Every 24 hours the invertebrates were assessed for mortality and degree of responsiveness (e.g. response to gentle prodding). The experiment was run for 96 hours. While the data has not been statistically analyzed as of June 2004, mortality was extremely low at all NaCl levels.

FINDINGS

There are no findings to date for this study. Completion of water sample analyses is expected by August 2004 and statistical analysis of the experimental data by November 2004. Additional data will be collected from a subset of streams during Fall 2004 and if possible, another snowstorm event will be monitored. Data from additional snow events will be added to the findings in Spring 2005.

Information Transfer Program

What Does the Future Hold? On October 24, 2003, the Maryland Water Resources Research Center sponsored a Conference on Water Policy in Maryland-What Does The Future Hold?, at the Stamp Student Union. Opening remarks were given by Nariman Farvadin, Dean, Clark School of Engineering. Allen P. Davis, Director Maryland Water Resources Research Center chaired the session. The Conference attracted an outstanding group of speakers, highlighted by the keynote speaker, Dr. M. Gordon Wolman of The Johns Hopkins University. University of Maryland faculty speakers included Dr. Matthias Ruth, Public Affairs; Dr. Doug Parker, Agriculture & Resource Economics; and Dr. James Cohen, Urban Studies and Planning Program. Water supply speakers included Dr. Roland Steiner, Washington Suburban Sanitary Commission, and Mr. Paul Swartz, Susquehanna River Basin Commission. Dr. Robert Summers, Head of Water Management, Maryland Department of the Environment also spoke. One major issue discussed was that future urban, commercial and agricultural consumers will all be competing for the same limited water supply in the State. A summary of several of the talks can be downloaded at the center website www.waterresources.umd.edu.

Some selected observations from the Conference include: . Users must be prepared to pay more for water in the future. . It will take decades to address some of the major water problems in the State . Many water problems exist in isolated areas without options or flexibility to solve these problems . Unlike Florida and New Jersey, Maryland does not have a comprehensive water supply plan prepared by any state agency . Based on a four states survey, including Maryland, there is currently a poor level of coordination between water supply planning and growth management planning . Public water supply, power generation and agriculture are the major users of water from the Susquehanna River. . Conservation plans developed by the Washington Suburban Sanitary Commission shows that 4.2 million gallons per day (mgd) could be saved by the year 2009 (only 2.5 percent of current supply). The District of Columbia Water and Sewer Authority estimates that its water conservation plan will save 6 mgd by the year 2012. . The average flows from the Susquehanna River comprise over 50% of the freshwater inputs to the Bay, at an average rate of 18 million gallons per minute near Havre de Grace, Maryland.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	2	0	0	0	2
Masters	1	0	1	0	2
Ph.D.	3	0	1	0	4
Post-Doc.	0	0	0	0	0
Total	6	0	2	0	8

Notable Awards and Achievements

Anubha Goel, a Ph.D. student in the Department of Civil and Environmental Engineering, University of Maryland, won a Student Travel Award from the Agrochemical Division of the American Chemists Society to present a Poster Presentation at the ACS National Meeting in New York City, 7-11 Sept, 2003. Her Poster title was Temporal Trends in Wet-Deposition of Pesticides to the Choptank River Watershed, Chesapeake Bay. Goel, A., Kuang, Z., McConnell, L.L., Torrents, A., Merrit, D. and Tobash, S. Author affiliations are the Dept. of Civil and Environmental Engg, University of Maryland; Environmental Quality Laboratory, Beltsville Agricultural Research Center, USDA, Beltsville, MD 20705; and UMCES, Horn Point Laboratory, Cambridge, MD, 21613. Additionally, Anubha Goel won a Student Travel Award from the 2003 Student Travel Award Program (SETAC) and a Jacob K. Goldhaber Travel Grant (Univ. Maryland), for a Poster presentation at the SETAC 24th Annual Meeting at Austin, Texas, 9-13 November, 2003. Her Poster title was: Pesticides in the Atmosphere over the Choptank River Watershed, Chesapeake Bay. Goel, A., McConnell, L.L., Torrents, A.,Kuang, Z., Meritt, D. and Tobash, S.. Affiliations are University of Maryland, MD 20742; U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705; and the UMCES, Horn Point Laboratory, Cambridge, MD, 21613. As part of the Water Policy Conference, the Center sponsored a Poster Contest open to all graduate students. One of the objectives of the Contest was to provide conference attendees with a broad overview of the extensive water research programs underway at the University of Maryland. Seventeen poster were exhibited from an array of Colleges, Departments and Schools across the University. An outside panel of scientists judged the posters. The first prize winner was Holly Menninger, Department of Biology, Terrestrial-Aquatic Linkages: Herbaceous Vegetation and Headwater Streams, second prize was awarded to Eunyoung Hong, Department of Civil and Environmental Engineering, Sustainable Oil And Grease Removal From Storm Water Runoff Hotspots Using Bioretention, and third prize to Rachel Gilker, Natural Resources Sciences and Landscape Architecture, Nitrogen And Phosphorus Concentrations In Surface And Groundwater Under Management Intensive Grazing . Each of the winning students were awarded an engraved plague and a cash award.

Publications from Prior Projects