Water Resources Center
Annual Technical Report
FY 2013

Introduction

Delaware Water Resources Center

June 1, 2014

Understanding the nature of the water quality and water supply problems faced in Delaware, historically and today, requires knowledge of the physiographic nature of the state, its climate, and major land uses. Geologically, Delaware is comprised of the Piedmont and Atlantic Coastal Plain Provinces. Only the northernmost 6% of the state is within the Piedmont, a region created of very old igneous and metamorphic rock. Soils range from well-drained, highly productive silt loams in the Piedmont to well– and excessively well-drained sandy loams and loamy sands in the Coastal Plain. Significant areas of poorly drained soils are also present, particularly in southeastern Delaware. Erosion and surface runoff are the main concerns in the Piedmont, while leaching of contaminants to shallow ground waters is the main water quality problem in the Coastal Plain. Average annual rainfall is plentiful (45 inches/year) and rather constant, averaging 3 to 4 inches/month in winter and spring and 4 to 5 inches/month in summer. Precipitation typically exceeds evapotranspiration by 12 to 18 inches/year, providing 10 to 12 inches/year of ground water infiltration.

Surface water is the main water supply source in the Piedmont, although the Cockeysville Formation is an important local aquifer of fractured marble and dolomite. This province is dominated by the Christina River Basin, fed by rivers that first flow extensively through Pennsylvania and Maryland. Water quality of the White Clay and Red Clay Creeks and Brandywine River is strongly affected by land use and point sources of pollution in neighboring states. Those rivers flow into the Christina River which, in turn, flows into the Delaware River.

Ground water is the major water supply source for the Atlantic Coastal Plain, a province of southeastwardly thickening unconsolidated and semi-consolidated sediments over crystalline basement rock. A primary aquifer in this province for water supply, stream base flow, and confined aquifer recharge is the unconfined Columbia aquifer. In a southwardly expanding wedge, the western portion of this area flows to the Chesapeake Bay through headwaters of the rivers and creeks of the Delmarva Peninsula's eastern shore. The mideast section of the province flows to the Delaware Estuary, fed by the watersheds of 15 creek and river systems. The southwest portion of the state flows into the Inland Bays of Delaware and Maryland and the Atlantic Ocean.

According to the Delaware Office of State Planning Coordination's mosr recent Land Use/Land Cover data set, the major land use in Delaware is agriculture (526,070 acres; 41% of the 1.28 million acres in the state), which is dominated by a large, geographically concentrated poultry industry. Other main land uses are urban (19%), wetlands (19%), forests (15%), open water (4%), and barren land (1%). Delaware has 2509 miles of streams and rivers, 2954 acres of lakes/reservoirs/ponds, 841 square miles of estuarine waters, and 25 miles of ocean coastline. Approximately three-quarters of the state's wetlands are freshwater, and one-fourth is tidal.

Protection of the quality and quantity of the state's surface waters and aquifers is a major concern to all agencies and individuals responsible for water resource management in Delaware. Ground water protection is particularly important given the increasing reliance on this resource for drinking water. In general, the key priority water resource issues today are (not prioritized): (1) enhanced management and control of stormwater runoff, erosion and sediment; (2) improved understanding of sources, transport, fate, and remediation of toxic organics and trace elements; (3) comprehensive management of agricultural nutrients and sediment; (4) identifying sources of pathogenic organisms and preventing human health impacts; (5) increased understanding of the response of aquatic systems to pollutants; (6) identification and protection of wellheads and aquifer recharge areas; (7) better management of water supply and demand and development of a systematic means to deal with droughts and floods; (8) treatment and disposal of on-site sewage; (9)

protection and restoration of wetlands; (10) prevention of saltwater intrusion to potable water supplies; (11) protection of functioning riparian areas; and (12) climate change impacts on water resources, including water quality and water supply.

The Water Resource Issues and Problems of Delaware

Surface Water Quality

Point Sources: Delaware has a number of serious, documented surface water quality problems. Many can be traced back to point source pollution problems in past decades; others reflect ongoing anthropogenic activities that degrade surface water quality. Water quality is a major state environmental priority and mprovements have occurred, particularly since the 1970s, due to the use of state and federal regulatory and funding means to address "end-of-pipe" point sources of surface water pollution. Much of this improvement was due to aggressive use of federal funding, available in the late 1970s and early 1980s under the Clean Water Act, combined with local funding, to expand and improve municipal wastewater treatment systems.

The National Pollution Discharge and Elimination System (NPDES) Program in Delaware has reduced the number of individual "point source" permits to discharge wastewater and stormwater from over 200 in the 1970s to 51 as of 2014. Of those, eight are all or almost all stormwater. NPDES permitting programs have been expanded to address pollution in stormwater runoff from concentrated animal feeding operations (“CAFOs," over 400 potential permittees), construction (2250 permittees as of May 2013), and ongoing industrial activities (363 permittees). Current initiatives include implementation of “Total Maximum Daily Load” (TMDL) requirements, in a long term multi-state effort to reduce PCBs in the Delaware River, and implementation of “Best Available Technology” for cooling water intake structures which draw in tens and hundreds of millions of gallons per day of water from Delaware waters. Major reductions in oxygen demanding materials and toxics in surface waters have been achieved. Future investments in water quality will likely weigh the cost-effectiveness of further reducing point source pollution, versus non-point sources of water quality problems. Currently, the State Clean Water Revolving Fund is providing funds for infrastructure to reduce point source pollution and other pollution sources.

The major surface water quality problems in Delaware include:

Urbanization: A rapidly expanding urban population is increasing pressures on Delaware’s surface waters. Rivers and streams are being affected by elevated temperature and low dissolved oxygen levels that can result from degradation of streambanks and stream channels. In residential and urban areas, increases in impervious surface have resulted in greater and flashier stormwater runoff, leading, in turn, to erosion, sedimentation, shallower water levels and destabilization of stream channels. Biological and habitat quality are also being affected by removal of stream buffers and stream bank "hardening" through use of riprap and concrete.

Drainage: Extensive drainage systems have been installed throughout the state, especially in coastal plain areas. Most were constructed in the 1930s and 1940s by the Civilian Conservation Corps and the Works Progress Administration. At that time, building a drainage ditch system involved channelizing and straightening headwaters of existing natural streams, then constructing ditches out and back from the channelized stream. Upland wetlands were often drained to reduce mosquito populations.

Delaware’s Drainage Program works with tax ditch associations to manage these ditches and restore them when possible. The effects on the biological and habitat quality of the waterway once it is stabilized are just starting to be known. The Drainage Program also manages public ditch projects, which are typically smaller (a few hundred feet) in scope and take place in the upper reaches of streams (typical bottom width is 3 feet) to augment mostly residential and some agricultural drainage. These projects are often carried out by the Conservation Districts. Little is currently known about the impacts to water quality or ecology from such projects. This lack of information may be important since protection of small headwater streams is critical to watershed health. Few streams in Delaware are unaffected by current or historic drainage projects that modify watershed drainage, natural stream channel configuration, buffers, and nutrient transport.

Nutrients: Nutrients are a leading cause of water quality degradation in Delaware. Nutrient effects can be seen especially in lakes, ponds, bays, and estuaries that receive nutrients conveyed by rivers, streams, and ground water. According to the State of Delaware's 2012 "Combined Watershed Assessment Report (305(b)) and Determination for the Clean Water Act Section 303(d) List of Waters Needing TMDLs" (dated April 2013), Delaware waters are generally considered to suffer from eutrophication and low dissolved oxygen related to nutrient enrichment. Primary land-based sources of nutrients in Delaware are agricultural practices, septic systems, and urban runoff. About 41% of Delaware's land area is devoted to agricultural activities and 19% to urbanized uses. Delaware's agricultural industry has a strong broiler industry component that heavily influences the state's overall agricultural nutrient balance and has long created nutrient management problems because of the large amount of manure that must be land applied; commercial inorganic fertilizers used by farmers, other land managers and homeowners also contribute nutrients to ground and surface waters. About 70% of Delaware's cash farm income comes from broilers, with annual production ranging from 260 to 280 million broilers, primarily in Sussex County, the largest broiler chicken producing county in the U.S.

Other Problems: Toxics have affected Delaware waters resulting in fish consumption advisories for the Delaware River and Bay, Atlantic coastal waters including the Inland Bays, and twenty smaller waterbodies in 2009. The primary pollutant is polychlorinated biphenyl (PCB). Chlorinated pesticides, dioxins, and mercury have also been identified. Though PCBs have long been banned, they are persistent in the environment and are transported from land to waters through runoff. Once in runoff, PCBs settle in waterbody sediments where they enter the aquatic food chain. Another problem is pathogenic organisms. New designated uses and surface water quality standards as amended on July 11, 2004 indicate that pathogenic organisms in surface waters have negatively affected shellfish harvesting and caused 86% of Delaware's rivers and streams to not fully support the swimming use; 98% do not fully support the fish and wildlife use. Most waters do not meet standards because of nonpoint source pollution impacts. In 2012 the Department of Natural Resources developed a "Watershed Approach to Toxics Assessment and Restoration" (WATAR), a five year plan to integrate and coordinate assessment and restoration of watersheds impacted by toxics.

Ground Water Quality

The domestic needs of approximately two-thirds of the State's population are met with ground water provided by both public and private wells. Most of the water used for agriculture, Delaware's largest industry, and self-supplied industrial use, is also derived from ground water sources. A shallow water table and highly permeable soils make Delaware's ground water vulnerable to pollution. Shallow unconfined aquifers are especially vulnerable, though deeper confined aquifers are susceptible as well because they subcrop beneath and are recharged by unconfined aquifers.

Major ground water quality problems in Delaware today are:

Nutrients: Nitrates from agriculture and septic systems are, by far, the major contaminant in Delaware's ground water. There are also some concerns about dissolved phosphorus transport to surface waters by shallow ground water flow in parts of the state where shallow water tables are interconnected with surface waters by ditches and/or tiles.

Organics: Hydrocarbons have also been found as have pesticides, though not at levels which cause alarm. A major source of hydrocarbons, such as MBTE, is leaking underground storage tanks (USTs) while agricultural activities are the source of pesticides. There are 12,050 regulated underground storage tanks in the State; 9651 have been properly abandoned and 2399 are still in use. Since the 1980s, 314,040 releases to ground water have been confirmed and 2800 of those (USTs) have been closed. Over the period 2002-2003, 142 sites had confirmed releases with 30 confirmed ground water releases.

Saltwater Intrusion: Problems with private wells occur sporadically from seasonal saltwater intrusion along the Delaware River and the Inland Bays/Atlantic Ocean coastal areas. No major problems have occurred and only one public well in Lewes required abandonment. Saltwater intrusion will become a recurring issue as sea level rises.

Trace Elements: Though not considered a health threat, iron concentrations are a widespread problem in Delaware for cosmetic reasons. Many public water supplies have treatment systems to remove iron. Thirty-four percent of 561 raw ground water samples analyzed by Delaware's Office of Drinking Water in 2002 exceeded the secondary contaminant level standard of 0.3 mg/L. Concerns exist about arsenic in ground waters because of the long-term application of this element in poultry manure to soils overlying shallow drinking water aquifers, the presence of brownfield soils in urban areas that had bee used as tanneries or other industries, and the lowered drinking water standard for arsenic.

Wetlands Quality: The ambient condition of fresh and salt water wetlands was assessed in the Broadkill, Cedar Creek, Mispillion, Little River and Leipsic watersheds. Scientific reports summarizing the condition of existing wetlands, recent changes in wetland acreage and landuse, and management recommendations were created for the Broadkill watershed. Reports and related information can be found on the Delaware Wetlands webpage: http://de.gov/delawarewetlands,

Water Supply: Half of Delaware's population is located in the Piedmont (6% of land area) and uses surface water for drinking water. The other 50% of the population relies on ground water and is spread throughout the remaining 94% of the State. With regard to the amount of water used, ground and surface water are of equal importance; with regard to area served, ground water is overwhelmingly dominant. Capacity concerns are important north of the Christina River due to population concentration and the reliance on surface water. For the rest of the state, the reliance on abundant ground water and a diffuse pattern of development suggest that the supply of potable water is not currently a problem. Recent drought emergencies have brought water supply demand in northern Delaware into conflict with the need to maintain minimum pass-through flows in streams for protection of aquatic resources. Benthic organisms, the foundation of the aquatic food chain, cannot move to avoid dry stream bed conditions. This suggests that not maintaining pass-through flows at all times would be detrimental to stream aquatic life. Required pass-through flows can be high; the need to ensure those flows can result in practices or structures such as reservoirs that are economically inhibitory or may cause as much or greater environmental degradation as occasional dry stream bed periods.

Recent Initiatives Promoting Delaware Water Quality

Non-Tidal Wetlands Conservation and Restoration

Governor Jack Markell signed Senate Bill 78 into law in July 2013. This legislation establishes a Wetlands Advisory Committee to develop comprehensive recommendations for conserving and restoring non-tidal wetlands in Delaware, including evaluating national best practices and standards, evaluating incentive-based programs, and reviewing state and federal wetland permitting processes to identify opportunities to improve efficiency and eliminate redundancy.

Wetlands cover approximately 25 percent of the total land area in the state of Delaware and provide numerous benefits. Wetlands provide flood storage, water purification, and habitat for economically important wildlife. The loss of wetlands in the state has led to flood damage and adverse effects to landowners' safety, welfare, and personal property. Additional information on Senate Bill 78 and the Wetlands Advisory Committee can be found at: http://www.dnrec.delaware.gov/swc/Pages/Wetland-Advisory-Committee.aspx.

Water Quality Standards

Water quality standards for surface waters in Delaware, revised and adopted effective July 11, 2004 by the Delaware Department of Natural Resources and Environmental Control (DNREC), include amendments to protect swimmers by making bacteria standards consistent with U.S. Environmental Protection Agency guidance and 2000 federal Beaches Environmental Assessment and Coastal Health (BEACH) Act requirements.

To ensure that Delaware waters meet state, regional, and national water quality requirements and goals, the State has one of the most extensive water quality monitoring networks in the nation. Our water resources in this State are regularly tested for biological and chemical parameters. The results are reported in even years in the State's Watershed Assessment Report (305(b) report). Waters that do not meet water quality standards are listed in the State's 303(d) list. Both of these reports are available at: http://www.dnrec.delaware.gov/swc/wa/Pages/WatershedAssessment305band303dReports.aspx.

The extensive water quality data have allowed tracking of long term progress made towards improving Delaware's water resources.

Delaware's non-attainment of Clean Water Act standards as described in the 1996 303(d) list was addressed by a federal court order requiring the development of total maximum daily load (TMDL) regulations for nearly the entire state, according to a schedule that concluded in 2010 for nutrients and bacteria. TMDLs establish the maximum amount of pollutants a water body can receive daily without violating water quality standards, allowing the use of these waters for swimming, fishing, and drinking water supplies. TMDLs have been established for nutrients, bacteria, PCBs, and toxics. TMDL analysis documents and regulations can be found at: http://www.dnrec.delaware.gov/swc/wa/Pages/WatershedAssessmentTMDLs.aspx.

Additional programs are in place to ensure continued compliance with the court order and to achieve water quality standards. Now that TMDLs are in place, Pollution Control Strategies (PCSs) and/or Watershed Implementation Plans (WIPs) are developed to address how, where and when pollutant loads will be reduced to achieve TMDL levels. These plans generally offer voluntary and regulatory strategies for urban, suburban and agricultural land uses and are developed through a public process where recommendations are made by Tributary Action Teams (TATs), groups of stakeholders formed with the purpose of addressing water quality concerns.

The PCSs and/or WIPs for the Appoquinimink, Broadkill, Chesapeake Bay, Christina, Inland Bays, Mispillion and Cedar Creek, Murderkill, Nanticoke, St. Jones, and Upper Chesapeake (Chester and Choptank) watersheds are available online at: http://www.dnrec.delaware.gov/swc/wa/Pages/WatershedManagementPlans.aspx.

Other DNREC Water Quality Initiatives Include:

Sediment and Stormwater Management Program: The Delaware Sediment and Stormwater regulations were revised effective January 1, 2014. The revised regulations address management of stormwater volume, provide for a watershed approach to stormwater management, and strengthen construction site stormwater management requirements. More information on the Delaware Sediment and Stormwater program is available at: http://www.dnrec.delaware.gov/swc/Drainage/Pages/RegRevisions.aspx.

Non-point Source (NPS) Pollution: DNREC continues to reduce non-point source pollution through enhanced coordination of the Division of Watershed Stewardship's Cost Share Programs through the USEPA's NPS Management 319 Program and the National Oceanic and Atmospheric Association's (NOAA) Coastal NPS Management 6217 program along with the Delaware Nutrient Management Commission's (DNMC) program through the Delaware Department of Agriculture (DDA) and other programs. The effort allows DNREC to direct millions of dollars every year toward a comprehensive NPS program to reduce pollutant loads, restore streams and buffers, and install best management practices (BMPs) such as cover crops, nutrient management plans, manure storage structures, manure relocation, and urban best management practices within impaired watersheds. More information on the NPS 319 program is available at: http://www.dnrec.delaware.gov/swc/district/Pages/NPS.aspx and information on Delaware's Coastal Management Program is available at: http://www.dnrec.delaware.gov/coastal/Pages/CoastalMgt.aspx.

Stream and Wetland Restoration: Rehabilitating stream corridors by re-establishing natural floodplains and sinuous low-flow channels, stabilizing stream banks, decreasing erosion, improving water quality, increasing wildlife habitat, providing buffers along the streams, establishing wetlands, promoting ground-water recharge and water storage, controlling invasive plant species, reintroducing native plant species, and reducing turbidity and sediment loading into stream channels are examples of the benefits that result from projects DNREC has implemented to improve the ecological quality and biological diversity in the State's watersheds. Over the past few years stream restoration projects have been completed along Mill Creek, Ham Run (tributary to Red Clay Creek), Middle Run (tributary to White Clay Creek) and Silver Lake Park (tributary to Appoquinimink River in Middletown) in New Castle County and along the St. Jones River in Dover (Kent County) at the Silver Lake Park and Mirror Lake projects.

Onsite Wastewater Treatment Systems (Septics): Delaware's "Regulations Governing the Design, Installation and Operation of On-Site Wastewater Treatment and Disposal Systems" were amended in 2002 and 2005 and revised, effective January 11, 2014. The revised regulations include requirements for small residential septic systems of less than 2,500 gallons of wastewater treated per day, as well as large community and commercial systems of more than 2,500 gallons of wastewater treated per day.

Among other changes, the regulations effective Jan. 11, 2014:

a) Require inspection of all septic systems prior to property transfers. Most if not all mortgage lending institutions currently require the inspection of a septic system prior to sale. This requirement informs a buyer of a system's type and condition and protects a homebuyer from acquiring a malfunctioning septic system; b) Clarify the permitting process for siting, installing and maintaining all small systems; c) Create new inspection protocols for system contractors and inspectors; d) Allow homeowners to maintain their own innovative/alternative system, once certified through a homeowner training program; e) Standardize the permitting process for spray irrigation and on-site systems; and f) Include procedures for distributing treated wastewater for agricultural use and other authorized purposes.

Regulations effective Jan. 2015:

a) Require the elimination of cesspools and seepage pits under certain situations; b) Require the upgrade of all new and replacement systems within 1,000 feet of tidal portions of the Nanticoke River and Broad Creek, which will assist Delaware in meeting federal targets to clean up the Chesapeake Bay Watershed; c) Establish statewide performance standards for all innovative/alternative systems; and d) Require all manufacturers of concrete system components (septic tanks, dosing chambers, etc.) to be certified through the On-Site Wastewater Accreditation Program.

Regulations effective Jan. 2016:

a) Require waste haulers to report septic tank pump-out; and b) Create a new licensee category for construction inspectors.

The regulations represent the culmination of more than five years of work by DNREC staff that included 13 workshops and three public hearings, answering questions and gathering input from homeowners, state legislators, realtors, businesses, the wastewater industry, and public utilities. After each workshop and hearing, the draft regulations were amended to reflect public comment.

Delaware's Septic Rehabilitation Loan Program (SRLP) is available to help eligible property owners meet regulatory requirements. The program provides low interest or no interest loans to assist homeowners with the costs of replacing malfunctioning septic systems or cesspools. The program is managed by DNREC's Financial Assistance Branch with technical assistance from the Ground Water Discharges Branch, in partnership with First State Community Action Agency of Georgetown/Dover. To view these regulations, go to: http://www.dnrec.delaware.gov/wr/Information/GWDInfo/Pages/default.aspx.

Source Water Assessment and Protection: The DNREC Source Water Assessment and Protection Program (SWAPP) provides for the assessment and protection of sources of public drinking water, both surface and ground water. The assessment consists of three critical steps: first, delineation of source water areas; second, identification of existing and potential sources of contamination; and finally, assessment of the susceptibility of the source water area to contamination. The Site Index Database identifies the location and status of both existing and potential sources of contamination within the State. Most potential point sources have been mapped and rated. In 2004, the Source Water Protection Program developed a guidance manual for local governments. This document was updated in 2005. For more information on source water protection, go to: http://www.wr.udel.edu/swaphome/index.html. Delaware SWAPP is a cooperative effort between DNREC, Delaware Division of Public Health, and the University of Delaware's Water Resources Agency. A citizen's advisory group (CTAC) was formed to assist DNREC in the development and implementation of the program and to ensure public involvement. SWAPP is a multi-phase program that is expected to be completed in the next few years.

Cooperative Efforts: Cooperation among DNREC, residents, other agencies-state and federal, universities, county and municipal governments, conservation districts, and non-governmental organizations (NGOs) helps bring Delaware water goals to fruition. Pollution Control Strategy development and implementation of TMDL regulations is driven by Tributary Action Teams (TATs). The Center for the Inland Bays, Nanticoke Watershed Alliance, Partnership for the Delaware Estuary, Delaware Nature Society, University of Delaware Cooperative Extension, the Sea Grant Program at the University of Delaware College of Earth, Ocean, and Environment, University of Delaware Water Resources Agency, Delaware State Cooperative Extension, the Camden-Wyoming Rotary Club, the State of Delaware's Nutrient Management Commission, New Castle, Kent and Sussex County governments, Sierra Club, the county conservation districts, USDA, USGS other DNREC divisions and many others have been vital contributors in the development and implementation of PCSs and WIPs.

All of the projects implemented in TMDL watersheds to address water quality concerns require a cooperative effort and partnerships to be formed, not just in government interactions, but between members of TATs and the public as well. Finding a solution for cleaner water will require more innovative solutions, greater regulatory control, additional financial resources, and a willingness to make a change by everyone affecting Delaware's watersheds, as we are all part of the problem and we must work together to find a reasonable solution for everyone.

Delaware Water Resources Center: An Overview

The Delaware Water Resources Center (DWRC) has been a part of the University of Delaware since 1965. From 1965 until 1993 the DWRC was located in the University of Delaware's Research Office. In 1993, the DWRC was formally moved to the College of Agriculture and Natural Resources (CANR) where, since 1997, Dr. Tom Sims, Deputy Dean for Academic Programs and Research, has served as DWRC Director. The

DWRC works with all organizations and agencies in Delaware with an interest or responsibility in water resources. We have a 12- to 15-member Advisory Panel representing a wide variety of water resource backgrounds. We regularly cooperate with the Delaware Water Resources Agency, Delaware Geological Survey, Delaware Department of Natural Resources and Environmental Control, the Center for the Inland Bays, the Delaware Nutrient Management Commission, Delaware State University, USDA Natural Resources Conservation Service, Delaware Nature Society, and The Nature Conservancy, to name but a few. The DWRC has always supported a wide range of water resource related research, education, and information transfer programs. We cooperate with many academic departments and units that conduct water-related research at Delaware State University's College of Agriculture and Related Sciences and the University of Delaware (UD), including the UD Water Resources Agency in the Institute for Public Administration, the Delaware Environmental Institute, the UD Departments of Biological Sciences, Chemistry, Civil and Environmental Engineering, Geography, Geological Sciences, and Plant and Soil Sciences, as well as the UD Colleges of Agriculture and Natural Resources; Arts and Sciences; Engineering; and Earth, Ocean, and Environment. Close communication is maintained between the DWRC and state natural resource agency representatives and water officials to address priority water quality and water quantity concerns in the state. Through efforts such as these, the DWRC has provided key stakeholders a forum for discussion and an opportunity for education regarding water resources.

Section 104 Objectives

The DWRC has defined a three-fold mission to meet the goals of the Water Resources Research Act:

(1) To support research that will provide solutions to Delaware's priority water problems;

(2) To promote the training and education of future water scientists, engineers, and policymakers; and

(3) To disseminate research results to water managers and the public.

To meet these goals we have focused our efforts into three major areas:

(1) Graduate Fellowship Program: A competitive graduate fellowship program supports graduate fellows to conduct research on a three-year cycle.

(2) Undergraduate Internship Program: We initiated a highly successful undergraduate internship program in 2000. DWRC interns work with faculty to conduct research, prepare a written project report, and present their findings at an annual poster session.

(3) Information Transfer: The DWRC website and newsletters are sources of up-to-date information on DWRC activities and water-related issues of importance to Delaware and the region. Our website provides information on water resources problems, links to water-related organizations, internship and job opportunities in water resources, a calendar of upcoming events, and a Kids Zone for teachers and parents. We also co-sponsor state-wide or regional conferences on water resource topics of current interest.

Delaware Water Resources Center Program Goals and Priorities

1. Institute Director: Dr. J. Thomas Sims, T.A. Baker Professor of Soil and Environmental Chemistry, Deputy Dean, College of Agriculture and Natural Resources, Director, Delaware Water Resources Center, 113 Townsend Hall, University of Delaware Newark, DE 19716-2103, Phone: 302-831-2698, FAX: 302-831-6758, email: jtsims@udel.edu

2. Administrative Personnel: Maria Pautler, Program Coordinator, Phone: 302-831-0847, FAX: 302-831-0605, email: mpautler@udel.edu

3. Abstract of Program and Management Overview: The Delaware Water Resources Center (DWRC) research, education and information transfer programs focus on issues of state and regional importance to both water quality and water quantity. Long-term priority areas of the DWRC have included nonpoint source pollution of ground and surface waters, development of ground water supplies, the impact of hydrologic extremes on water supply, and socio-economic factors affecting water supply and water quality. In 2000, the DWRC Advisory Panel identified five specific areas for near-term DWRC research efforts: (1) Agricultural nutrient management and water quality; (2) Basic and applied research on sources, fate, and transport of water pollutants; (3) Quantifying response of aquatic ecosystems to pollutant inputs; (4) Water supply, demand, and conservation, as affected by changing land uses in Delaware and the mid-Atlantic states; and (5) Management and control of stormwater runoff. The FY13 DWRC public water conservation/educational outreach program addressed these issues. DWRC's research program during the same period addressed these concerns by supporting graduate fellowships in water quality, an undergraduate student internship program, and public information forums including an intern research poster session.

2013-2014 DWRC Fellowship and Internship Research Program

Two fellowships were funded for the third and final year in 2013-2014 based on satisfactory progress reporting to the DWRC Advisory Panel:

a) Quantifying the Role of Carbon Amount and Quality for Transport of Contaminants on Our Landscapes: A Watershed Scale Model

Graduate Fellow: Gurbir Dhillon followed by Zhixuan Qin; Advisor: Shreeram Inamdar, Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, University of Delaware.

b) Microbiome of the Eastern Oyster, *Crassostrea virginica*

Graduate Fellow: Eric Sakowski; Advisor: K. Eric Wommack, Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, University of Delaware.

Seven internships were awarded for 2013-2014 based on a review of proposals submitted by potential undergraduate interns and their advisors to the DWRC Advisory Panel:

a) Use of the White Rot Fungus as a Fungal Bioreactor to Remove *E. coli* from Aqueous Dairy Manure Wastewater

Undergraduate Intern: Anna Brady; Advisor: Anastasia Chirnside, Department of Entomology and Wildlife Ecology, College of Agriculture and Natural Resources, University of Delaware.

b) A Biogeographic Investigation of Viral Diversity within the Eastern Oyster, *Crassostrea virginica*

Undergraduate Intern: Alessandra Ceretto; Advisor: K. Eric Wommack, Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, University of Delaware.

c) Exploring the Viability of Biochar to Treat Stormwater

Undergraduate Intern: Naomi Chang; Advisor: Paul Imhoff, Department of Civil and Environmental Engineering; College of Engineering, University of Delaware.

d) The Varying Impact of Stemflow and Soil Moisture on the Diversity of Soil Bacterial and Fungal Communities in Relation to Soil Respiration

Undergraduate Intern: Katherine Junghenn; Advisor: Delphis Levia, Department of Geography, College of Earth, Ocean, and Environment, University of Delaware.

e) Methane and Carbon Dioxide Fluxes in a Watershed

Undergraduate Intern: Kelsey McWilliams; Advisor: Rodrigo Vargas, Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, University of Delaware.

f) Sustainable Management of Water and Ecosystem Services on a Residential Landscape in Delaware

Undergraduate Intern: Megan Murray; Advisor: Joshua Duke, Department of Applied Economics and Statistics, College of Agriculture and Natural Resources, University of Delaware.

g) Acid Neutralization of Stemflow in a Deciduous Forest: The Role of Edge Effects

Undergraduate Intern: Alexey Shiklomanov; Advisor: Delphis Levia, Department of Geography, College of Earth, Ocean, and Environment, University of Delaware.

Research Program Introduction

None.

Microbiome of the Eastern Oyster, Crassastrea virginica

Basic Information

Title:	Microbiome of the Eastern Oyster, Crassastrea virginica
Project Number:	2010DE171B
Start Date:	3/1/2013
End Date:	8/31/2013
Funding Source:	104B
Congressional District:	At large
Research Category:	Not Applicable
Focus Category:	Ecology, Non Point Pollution, Conservation
Descriptors:	None
Principal Investigators:	Eric Wommack

Publications

1. Sakowski, E. and E. Wommack, 2011, Exploring the Commercial Microbial Communities of the Eastern Oyster, <i>Crassostrea virginica<i> Progress Report, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 16 pages.
2. Pautler, M., ed., 2010, Delaware Water Resources Center WATER NEWS Vol. 10 Issue 2 DWRC Spotlight on Graduate Research, http://ag.udel.edu/dwrc/newsletters/Winter09Spring10/WATERNEWSco-Spring2010.pdf , p. 7.
3. Sakowski, E. and E. Wommack, 2012, Exploring the Commensal Microbial Communities of the Eastern Oyster, Crassostrea virginica. Progress Report, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 16 pages.
4. Sakowski, E. and K.E. Wommack, 2013, Exploring the Commercial Microbial Communities of the Eastern Oyster, Crassostrea virginica. Progress Report, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 16 pages.
5. Pautler, M., ed., 2012, Delaware Water Resources Center WATER NEWS Vol. 12 Issues 1&2 DWRC Spotlight on Graduate Research, http://ag.udel.edu/dwrc/newsletters/Fall11Summer12/WATERNEWSco-Summer2012.pdf , p. 5.
6. Schmidt, H.F., E.G. Sakowski, S.J. Williamson, S.W. Polson, and K.E. Wommack, 2013, Shotgun Metagenomics Indicates Novel Family A DNA Polymerases Predominate within Marine Virioplankton, The International Society for Microbial Ecology Journal, 8, 103-114.

The ISME Journal (2013), 1–12

www.nature.com/ismej

ORIGINAL ARTICLE

Shotgun metagenomics indicates novel family A DNA polymerases predominate within marine virioplankton

Helen F Schmidt[1], Eric G Sakowski[1], Shannon J Williamson[2], Shawn W Polson[1] and K Eric Wommack[1]

[1]*Department of Plant & Soil Science, College of Marine Studies, Delaware Biotechnology Institute, University of Delaware, Newark, DE, USA and* [2]*Lake Pend Oreille Waterkeeper, Sandpoint, ID, USA*

Virioplankton have a significant role in marine ecosystems, yet we know little of the predominant biological characteristics of aquatic viruses that influence the flow of nutrients and energy through microbial communities. Family A DNA polymerases, critical to DNA replication and repair in prokaryotes, are found in many tailed bacteriophages. The essential role of DNA polymerase in viral replication makes it a useful target for connecting viral diversity with an important biological feature of viruses. Capturing the full diversity of this polymorphic gene by targeted approaches has been difficult; thus, full-length DNA polymerase genes were assembled out of virioplankton shotgun metagenomic sequence libraries (viromes). Within the viromes novel DNA polymerases were common and found in both double-stranded (ds) DNA and single-stranded (ss) DNA libraries. Finding DNA polymerase genes in ssDNA viral libraries was unexpected, as no such genes have been previously reported from ssDNA phage. Surprisingly, the most common virioplankton DNA polymerases were related to a siphovirus infecting an α-proteobacterial symbiont of a marine sponge and not the podoviral T7-like polymerases seen in many other studies. Amino acids predictive of catalytic efficiency and fidelity linked perfectly to the environmental clades, indicating that most DNA polymerase-carrying virioplankton utilize a lower efficiency, higher fidelity enzyme. Comparisons with previously reported, PCR-amplified DNA polymerase sequences indicated that the most common virioplankton metagenomic DNA polymerases formed a new group that included siphoviruses. These data indicate that slower-replicating, lytic or lysogenic phage populations rather than fast-replicating, highly lytic phages may predominate within the virioplankton.
The ISME Journal advance online publication, 29 August 2013; doi:10.1038/ismej.2013.124
Subject Category: Integrated genomics and post-genomics approaches in microbial ecology
Keywords: viral ecology; metagenomics; phage diversity

Introduction

Measurements of viral production within marine ecosystems indicate that a significant proportion of the bacterioplankton standing stock is lost to viral lysis (Winget *et al.*, 2011) and that virioplankton populations turn over rapidly, often in less than a day for productive coastal marine ecosystems (Winget *et al.*, 2011). The lytic release of viruses and nutrients is a key mechanism shaping the flow of C and energy through marine ecosystems (Poorvin *et al.*, 2004; Suttle, 2005) and influencing the productivity and composition of marine microbial communities. Although appreciation of the importance of viral processes to ecosystem function has grown, we have only a cursory understanding of the predominant biological characteristics of abundant viral populations that are driving viral effects within the ocean. Such information is crucial to a deeper, mechanistic understanding of the virus–host relationships and how these relationships shape the microbial activities critical to global biogeochemical cycles.

One impediment to these investigations has been the lack of a universal genetic marker that can draw connections between the evolutionary history, diversity and biological characteristics of viruses. By analogy, the use of the 16S ribosomal RNA gene as a universal genetic marker among prokaryotic life has provided a means to investigate links between the phylogeny and population biology of prokaryotic groups. Nevertheless, as many viruses and bacteriophages carry informational protein genes (that is, genes involved in maintenance of genetic information), these genes have been used in studies examining the diversity, biogeography and

Correspondence: KE Wommack, Department of Plant & Soil Science, College of Marine Studies, Delaware Biotechnology Institute, University of Delaware, 15 Innovation Way, Newark, DE 19711, USA.
E-mail: wommack@dbi.udel.edu
Received 1 February 2013; revised 7 June 2013; accepted 18 June 2013

population biology of viruses. In particular, because polymerases are critical to viral replication, these genes can have a disproportionately major role in shaping the evolutionary history and fitness (Gimenes *et al.*, 2011) of the viruses that carry them.

For example, DNA polymerase sequences have been critical to constructing phylogenetic relationships between viruses infecting eukaryotic microalgal host groups (Brussaard *et al.*, 2004), whereas RNA-dependent RNA polymerase gene sequences revealed a remarkable diversity of picornaviruses and other RNA viruses within the virioplankton (Culley *et al.*, 2003, 2006). In the case of bacteriophages, previous studies have utilized DNA polymerase sequences to examine the distribution and diversity of phages related to coliphage T7 belonging to the *Podoviridae* morphological family (Breitbart *et al.*, 2004; Labonté *et al.*, 2009). The ubiquity of T7-like DNA polymerase genes has led some to propose that highly lytic podoviruses are key factors in the virioplankton (Labonté *et al.*, 2009). However, a critical shortcoming of these previous examinations has been the limited ability of PCR approaches to detect novel viral groups on the basis of DNA polymerase gene sequences. A telling example of this shortcoming is the fact that sequences related to *polA* genes from known siphoviruses and myoviruses have not been detected within environmental samples, despite the fact that *polA* is known to be carried by phages within these morphological families (Scarlato and Gargano, 1992; Buechen-Osmond and Dallwitz, 1996; Lohr *et al.*, 2005).

Today, the use of high-throughput DNA sequencing to assess the genetic diversity and composition of natural viral assemblages—shotgun viral metagenomics—enables much broader access to the extant viral genetic diversity and thus deeper investigations of viral population genetics through phylogenetic analysis of viral genes. This study used just such an approach through characterization of virioplankton metagenomic sequences homologous to full-length family A DNA polymerases. The family A DNA polymerases, encoded by the *polA* gene, are a large and varied group of polymerases that includes all bacterial Pol I. In bacteria, Pol I primarily functions as a DNA proofreading enzyme and includes a polymerase domain, a 3′–5′ exonuclease domain, and a 5′–3′ exonuclease domain (Ollis *et al.*, 1985; Beese *et al.*, 1998; Li *et al.*, 1998). However, the *polA* gene is also common in tailed dsDNA phages (Breitbart *et al.*, 2004). Among phages, the protein does not include a 5′-3′ exonuclease and is generally the DNA polymerase primarily responsible for phage genome replication (Tabor and Richardson, 1987; Doublie *et al.*, 1998; Naryshkina *et al.*, 2006). Here we report that well-known structural features of DNA polymerases, which shape its enzymatic activities, provide a framework for understanding the prevalent biological features of phage populations within the virioplankton.

Materials and methods

Viral metagenome sequence libraries (viromes)

Details of library preparation are available in the supplementary material. Sequences from 10 virioplankton metagenomic libraries collected at three sampling sites (Table 1 and Supplementary Figure S1) were analyzed. Libraries from the Dry Tortugas (Andrews-Pfannkoch *et al.*, 2010) and the Chesapeake Bay (Bench *et al.*, 2007; Rusch *et al.*, 2007; Andrews-Pfannkoch *et al.*, 2010) were constructed from environmental viral nucleic acids that had been separated into three fractions: dsDNA, ssDNA and RNA (Andrews-Pfannkoch *et al.*, 2010). Only dsDNA virioplankton were analyzed from the Gulf of Maine sample (Tully *et al.*, 2012). After separation, the ssDNA and RNA fractions were transformed into dsDNA copies and subsequently all dsDNA fragment libraries were constructed using the linker-amplified shotgun library procedure (Andrews-Pfannkoch *et al.*, 2010). Insert DNA from randomly selected clones was sequenced using the Sanger dideoxy-chain terminator method (Sanger *et al.*, 1977) to provide ~64–117 thousand sequence reads (Figure 1), with read lengths of ca. 750 bp. The longer read lengths provided by Sanger sequencing were critical to unambiguous assembly of putative full-length DNA polymerase genes (Wommack *et al.*, 2008). Environmental metadata, sequences and bioinformatic analyses for these libraries are available on the Viral Informatics Resource for Metagenome Exploration (VIROME) website (http://virome.dbi.udel.edu) (Wommack *et al.*, 2012).

Table 1 DNA polymerase A reads and contigs by library

VIROME[a] *Library Name*	*Location*	*Library type*	*polA reads (%*[b]*)*	*polA contigs*[c]
CFA-D[d]	Chesapeake	dsDNA	239 (0.29)	12
CIA-B[d]	Chesapeake	dsDNA	10 (0.18)	0
CBB	Chesapeake	dsDNA	39 (0.69)	1
CBJ	Chesapeake	dsDNA	80 (0.70)	2
CBS	Chesapeake	ssDNA	122 (2.13)	2
CBR	Chesapeake	RNA	12 (0.21)	0
Total	Chesapeake		502 (0.43)	17
DTF	Dry Tortugas	dsDNA	573 (0.88)	37
DTS	Dry Tortugas	ssDNA	47 (0.82)	0
DTR	Dry Tortugas	RNA	33 (0.60)	0
Total	Dry Tortugas		653 (0.86)	37
GMF	Gulf of Maine	dsDNA	680 (1.06)	33

[a]Library identifier in VIROME. Additional library details available at (http://virome.dbi.udel.edu).
[b]*polA* reads per total number of reads in library.
[c]Contigs were assembled with up to 2% gaps and 3% mismatches, and the consensus sequences were translated and used only if they were longer than 300 amino acids and had a conserved domain hit to the DNA polymerase A domain.
[d]Multiple libraries collected at the same site were combined for the purpose of this study.

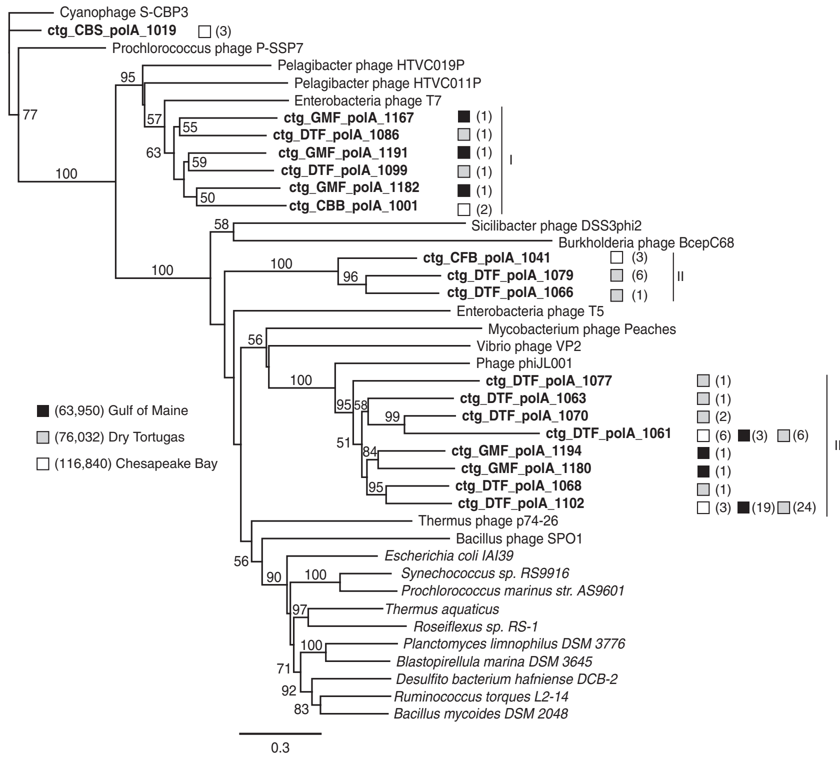


Figure 1 Unrooted maximum likelihood tree of representative peptide sequences from translated metagenomic DNA polymerase A contig clusters with known phage and bacterial Pol I sequences. Known phages are shown in normal text, bacteria in italics and metagenomic sequences in bold. Sequences were trimmed to the polymerase domain. Each metagenomic sequence is the representative sequence of a peptide cluster and numbers in parentheses indicate the number of contigs in the cluster. Shaded boxes indicate the location of virioplankton metagenomic libraries. Roman numerals indicate clades of environmental sequences. Numbers in parentheses in the legend are total sequences from each location. Bootstrap values below 50% are not shown. Scale bar represents 0.3 amino acid substitutions per site.

Metagenomic and phylogenetic analysis

Open reading frames (ORFs) were predicted from all virome reads using MetageneAnnotator (Noguchi *et al.*, 2006, 2008), translated and subsequently clustered (supplementary methods) at a similarity cutoff of 40% (Edgar, 2010). Representative sequences from all of the peptide clusters were searched in the VIROME database to determine which clusters contained a representative peptide sequence with significant homology (BLASTP E-score $\leqslant 10^{-3}$) to a known DNA polymerase. Subsequently, the ORFs within putative DNA polymerase clusters were retrieved from each viral metagenome library. These ORFs were separated by library and assembled with a minimum overlap of 50 bp and maximum of 2% gaps and 3% mismatches (Drummond *et al.*, 2011). Contig consensus sequences were translated into amino acids according to the ORF call, and a conserved domain BLAST search (Marchler-Bauer and Bryant, 2004; Marchler-Bauer *et al.*, 2009, 2011) was run on translated consensus sequences of $\geqslant 300$ amino acids and only those sequences with hits to the polymerase domain were used. Putative DNA polymerase I sequences identified in this study have been deposited in GenBank Acc. KF514434–KF514521.

Each virioplankton DNA polymerase contig translation was clustered using the nearest neighbor algorithm of DOTUR with a minimum similarity of 40%, producing 18 clusters (Schloss and Handelsman, 2005) (Supplementary Table S1). A representative translated contig sequence from each cluster was aligned (MUSCLE, 8 iterations, gap extension penalty: -2) with known phage and bacterial DNA polymerase A sequences (Edgar, 2004), and the alignment was trimmed to exclude all exonuclease domains. A maximum likelihood tree of this alignment (Figure 1) was constructed using the JTT substitution model of PHYML (Guindon *et al.*, 2005) with 100 bootstrap replicates.

The genetic content of dsDNA and ssDNA virioplankton from the Chesapeake Bay were compared using four dsDNA viral metagenome libraries (CFA, CFB, CFC and CFD) and one ssDNA viral metagenome library (CBS) (Figure 2). For the purpose of this comparative analysis the four dsDNA libraries were considered as one library. In actuality, each of these libraries was from a single station in the Chesapeake Bay sampled over a 24 h period.

Phylogenetic relationships between assembled virioplankton DNA polymerases and PCR-amplified virioplankton DNA polymerases were investigated by aligning (MUSCLE, 8 iterations, gap extension

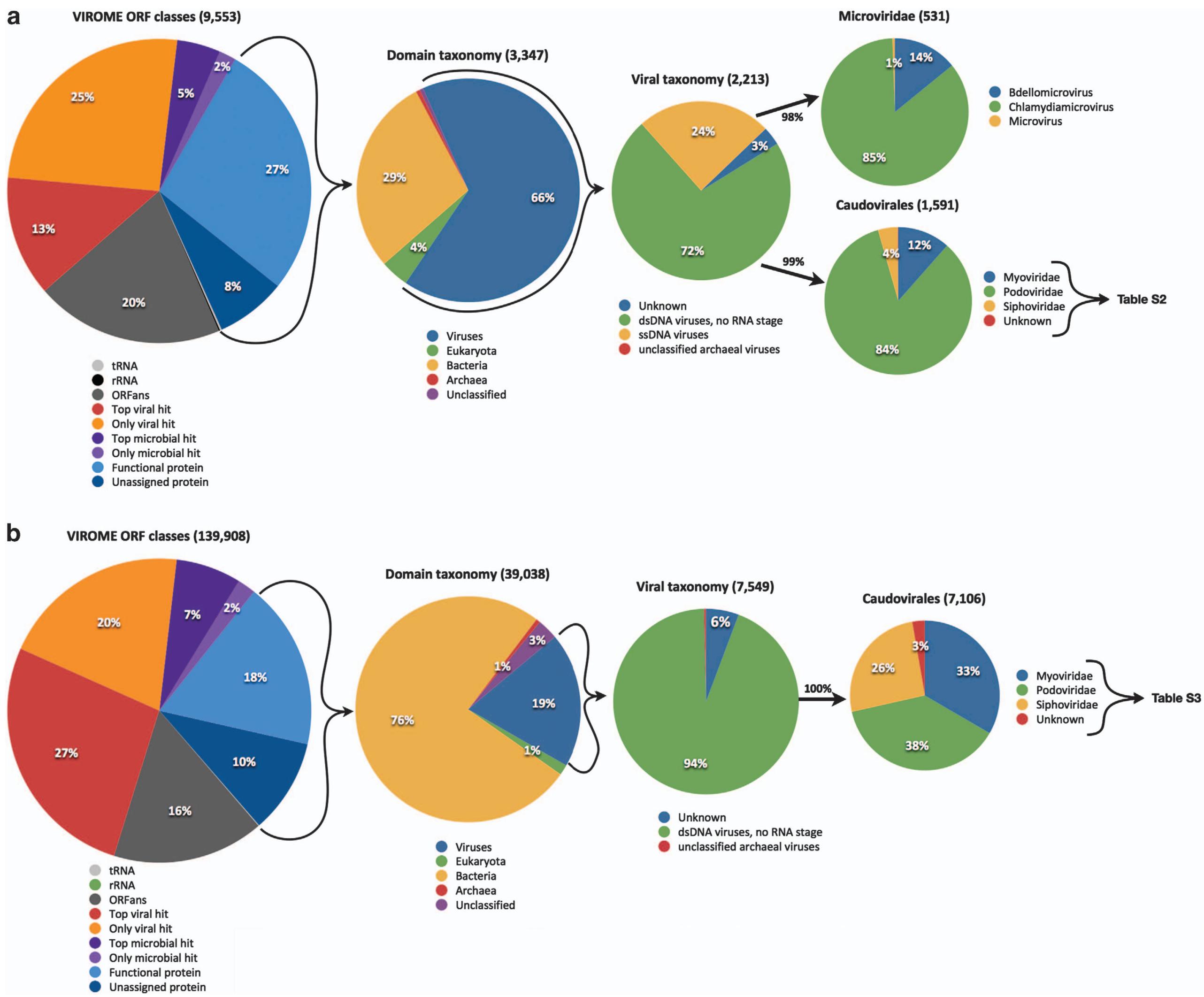


Figure 2 Distribution of BLAST homologs to predicted ORFs from shotgun metagenome sequence libraries of: (**a**) single-stranded DNA; and (**b**) double-stranded DNA virioplankton in the Chesapeake Bay water samples. Virioplankton ORFs within VIROME ORF classes; top viral hit, only viral hit, top microbial hit and only microbial hit showed homology to only environmental peptides. Virioplankton ORFs within the VIROME ORF classes; functional protein or unassigned protein showed homology to a peptide in the UniRef 100 database. ORFans were defined as virioplankton ORFs having no significant homology ($E \leqslant 0.001$) to either an environmental peptide or a UniRef peptide. Subsequent pie charts based on taxonomy of BLAST homologs are based on virioplankton ORFs showing significant homology to a UniRef peptide. Details of virioplankton ORFs with homology to known phages within the Caudovirales are shown in Supplementary Table S2 (ssDNA virioplankton) and Supplementary Table S3 (dsDNA virioplankton).

penalty: -2) (Edgar, 2004) the representative consensus sequences with a large collection of environmental DNA polymerase sequences obtained using degenerate PCR primers (Labonté *et al.*, 2009). All metagenomic sequences were trimmed to match the correct amplicon length, and a maximum likelihood tree (Figure 3) was constructed as described above.

Structural prediction of DNA polymerase from ssDNA virome

Because no known ssDNA viruses have been shown to carry a DNA polymerase gene, the structure of a DNA polymerase A peptide from the CBS ssDNA viral library (ctg_CBS_polA_1019, Supplementary Table S1) was examined using the first approach, automated mode of homology modeling in Swiss-Model Workspace (Peitsch *et al.*, 1995; Guex and Peitsch, 1997; Schwede *et al.*, 2003; Arnold *et al.*, 2006; Kiefer *et al.*, 2009). The amino acid sequence of ctg_CBS_polA_1019 was modeled onto structure 1×9WA of T7 DNA polymerase (Dutta *et al.*, 2004).

Results

Contig assembly and homology to DNA polymerase

Sequence reads showing significant homology (BLASTP E-score $\leqslant 10^{-3}$) to known DNA polymerase I sequences were present in all libraries, although only 6 of the 10 libraries contained contigs that were longer than 300 amino acids and showed homology

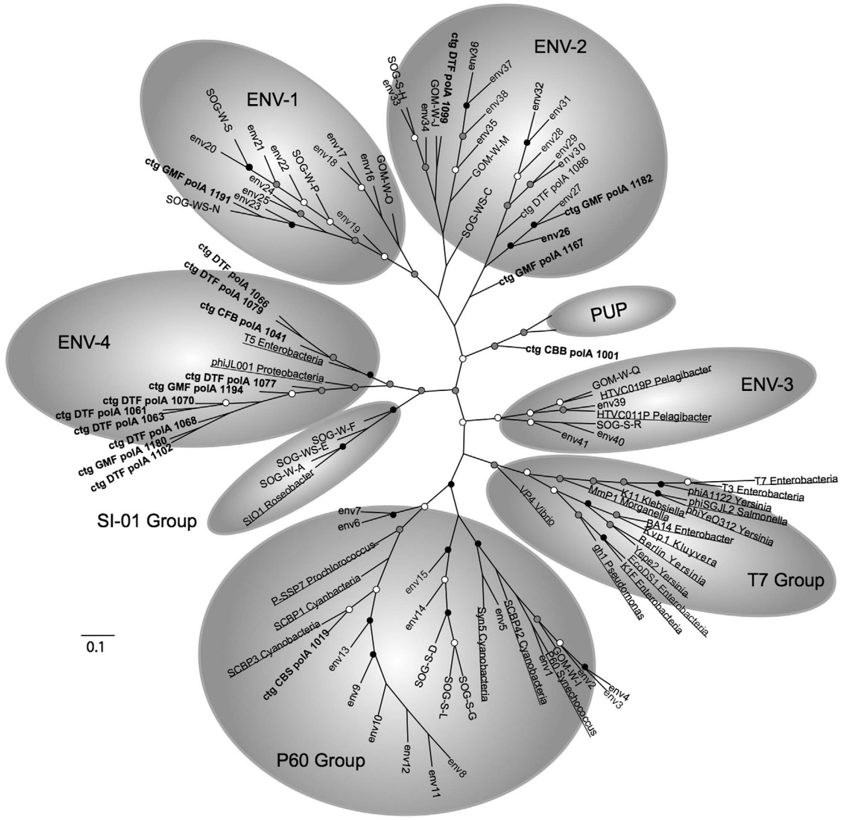


Figure 3 Unrooted maximum likelihood tree based on the alignment of translated metagenomic contigs (bold) with translated DNA Pol A amplicons from degenerate primers from Labonté *et al.* (2009) and Breitbart *et al.* (2004) and known phage (underlined). All metagenomic contigs were trimmed to the amplicon length. Black, gray and white circles indicate nodes with at least 100%, 75% and 50% bootstrap support, respectively. PUP clade as identified in Breitbart *et al.* (2004). ENV clade 1-3 are as designated in Labonté *et al.* (2009) and include all sequences from clade I of Figure 1. ENV-4 is newly identified in this study and contains all sequences from clade II and III of Figure 1. Scale bar represents amino acid substitutions per site.

to the *polA* domain (Table 1). Among dsDNA libraries, sequences from the Dry Tortugas (DTF) yielded the greatest number of usable contigs. With the exception of the CFA-D and CIA-B libraries, all dsDNA libraries showed a frequency of *pol*A-encoding reads between 1.1 and 0.7%, making this one of the most abundant functional proteins detected within the libraries (Supplementary Table S3). The highest observed frequency of *pol* A reads was from the Chesapeake Bay ssDNA library (CBS), but only two viable full-length contigs were assembled from the 122 sequences showing homology to DNA polymerase A (Table 1). The frequency of *polA* reads in the Dry Tortugas RNA library (DTR) was nearly three times that of the Chesapeake RNA library (CBR), but no viable contigs were assembled from either library.

Multiple sequence alignment of translated metagenomic contigs with known DNA polymerase I sequences showed that these putative DNA polymerases contained many conserved residues critical to metal and DNA binding and enzymatic function (Figure 4). Similar to family A DNA polymerases from known phage, contigs from dsDNA libraries had the 3′–5′ exonuclease and DNA polymerase domains but lacked the 5′–3′ exonuclease domain. Contigs from the ssDNA libraries contained the DNA polymerase domain but neither of the exonucleases at the N-terminus (Figure 4).

All virioplankton metagenomic DNA polymerases contained three motifs that were conserved throughout DNA polymerases (Figure 4) (Loh and Loeb, 2005). Except where noted, residue number refers to its position in the *E. coli* Pol I. In motif A, Asp705 is immutable because of its binding of catalytic magnesium (Patel and Loeb, 2000). Also highly conserved within motif A are Glu710, which stabilizes the closed form of the enzyme and prevents the incorporation of ribonucleotides (Loh and Loeb, 2005), and Arg712. All of these residues were conserved in the virioplankton DNA polymerases. Motif B, which contacts the nascent base pair, has the key residues Arg754, Lys758, Phe762 and Tyr766 (Loh and Loeb, 2005). These residues were universally conserved in the viroplankton contigs except for Phe762. Of the metagenomic sequences, 12% had phenylalanine, 13% had tyrosine and 75% had leucine in this position. Residues 881-883 of motif C were highly conserved across the reference and metagenomic sequences. His881 coordinates to the sugar of the primer terminus. Asp882 binds to catalytic magnesium and coordinates with Glu883

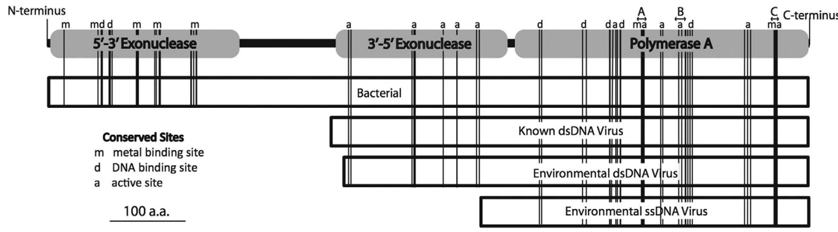


Figure 4 Schematic of typical bacterial, phage and putative environmental viral DNA polymerase I proteins. Lengths in amino acids based on averages from multiple sequence alignments. Capital letters A-C indicate motifs conserved across DNA polymerases (Loh and Loeb, 2005). Vertical lines represent conserved amino acids. All contigs of environmental viral putative DNA polymerase I proteins contained these sites.

via a water molecule (Loh and Loeb 2005). In the crystal structure of phage T7 DNA polymerase, Glu883 is proximal to the C-terminal histidine, which is critical to T7 DNA polymerase function (Doublie *et al*., 1998). However, histidine is not the terminal residue in a number of phage and bacteria including *T. aquaticus*, Enterobacteria phage T5 and 85% of the metagenomic sequences. In motif C, Val880 is evolutionarily conserved in bacteria (Loh and Loeb, 2005) but is substituted with threonine and isoleucine in cultured phage and in the metagenomic DNA polymerases.

Phylogeny of metagenomic DNA polymerases

Phylogenetic analysis showed that bacterial polymerases form a single, well-supported clade that was less diverse than the environmental and known phage sequences (Figure 1). Larger, well-supported clades of virioplankton metagenomic sequences each contained representatives from at least two of the three locations. This analysis confirmed the ubiquity of T7-like podoviruses observed in previous PCR-based studies (Breitbart *et al*., 2004; Labonté *et al*., 2009), as full-length polymerase sequences from all three environments claded with T7 DNA Polymerase I (Clade I, Figure 1). Recently identified T7-like Pelagibacter phages HTVC011P and HTVC019P were also part of this clade. However, this was not the largest clade of environmental sequences and contained only six clusters and a total of seven metagenomic polymerases. In total, the 18 virioplankton DNA polymerase clusters contained 88 contigs assembled from 320 sequence reads (Supplementary Table S1). Thus, T7-like DNA polymerase I sequences accounted for one-third of the virioplankton DNA polymerase clusters, but only 8% of sequenced contigs. Astonishingly, only 5% of the reads contributing to full-length DNA polymerase contigs occurred in the T7-like Clade I (Supplementary Figure S2). These data illustrate that the T7-like DNA polymerases are polymorphic and ubiquitous, yet the phage carrying these genes appeared to be less abundant than other phage groups within the virioplankton.

The second largest group of virioplankton DNA polymerase sequences (Clade II, Figure 1) was distantly related to the DNA polymerase of Enterobacteriophage T5. Although this clade contained only three clusters from the Dry Tortugas and Chesapeake Bay, it comprised a greater number of contigs (10) than the T7-like viruses (Supplementary Table S1), and a larger proportion of the reads (10%) contributing to full-length DNA polymerase contigs (Supplementary Figure S2). Thus polymerases from phages in Clade II were less diverse across the sampling sites than T7-like viruses but more abundant within the virioplankton. The largest group of virioplankton DNA polymerase sequences belonged to Clade III, a clade containing only metagenomic sequences that was distantly related to Proteobacteria phage phiJL001, a siphovirus infecting an α-proteobacterial symbiont of a marine sponge (Lohr *et al*., 2005). This clade contained 44% of all DNA polymerase clusters, 77% of contigs and 83% of reads contributing to full-length DNA polymerase contigs (Supplementary Figure S2). Of the eight clusters within Clade III, six contained one or two contigs and were only found in a single environment. However, the two largest DNA polymerase clusters in this clade (represented by ctg_DTF_polA_1061 & ctg_DTF_polA_1102 (Figure 1)) contained contigs from all three locations and accounted for 76% of reads contributing to full-length DNA polymerase contigs (15% for cluster-ctg_DTF_polA_1061 and 61% for cluster-ctg_DTF_polA_1102). Therefore, a large majority of DNA polymerase-carrying phages within the virioplankton have a DNA polymerase within Clade III.

The final clade of virioplankton DNA polymerase sequences contained a cluster of ssDNA Pol A sequences represented by ctg_CBS_polA_1019 and was distantly related to podoviruses infecting cyanobacteria, Cyanophage S-CBP3 and *Prochlorococcus* phage P-SSP7 (Figure 1). This clade contained 2% of reads contributing to full-length DNA polymerase contigs (Supplementary Figure S2). Although much shorter than the sequence of T7 DNA polymerase, ctg_CBS_polA_1019 aligned well with the polymerase domain of this protein, forming the critical thumb, palm and finger regions of the DNA polymerase structure (Figure 5). As the contig had all the key residues discussed above, this predicted polymerase from the Chesapeake Bay ssDNA virome library would make the necessary

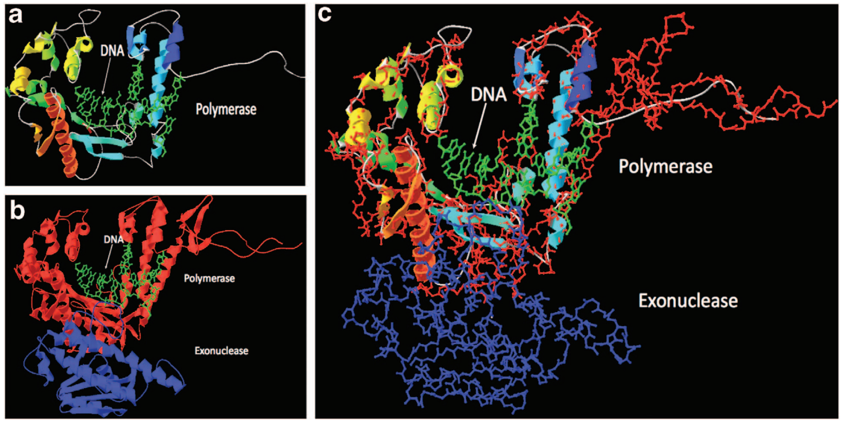


Figure 5 (**a**) Predicted structure of DNA polymerase from a ssDNA virus based on aligning the sequence of ctg_CBS_pola_1019 with the known structure of Enterobacteria Phage T7. DNA Polymerase DNA molecule within the enzyme structure is shown in green; (**b**) Structure of T7 DNA polymerase with the polymerase region in red and the exonuclease region in blue; (**c**) DNA polymerase from ctg_CBS_pola_1019 (ribbon model) overlaid with T7 DNA polymerase (carbon backbone).

contacts with the template DNA molecule. As expected from the conserved domain BLAST and alignment (Figure 4), the ssDNA viral metagenomic sequence did not have either exonuclease structure.

Confirmation of purity of ssDNA library

Comparisons of the Chesapeake ssDNA and dsDNA libraries showed that these viromes had similar proportions – 35% and 28%, respectively – of predicted peptides showing significant BLAST homology ($E \leqslant 10^{-3}$) to a peptide in the UniRef 100 database (Figure 2). These peptides comprised the 'functional protein' and 'unassigned protein' VIROME classes (Wommack *et al.*, 2012) and could be further assessed according to the taxonomic origin and function of the top UniRef BLAST homolog. The taxonomic origin of top BLAST hits against UniRef 100 peptides differed sharply between the viromes. At the domain level, most of the ssDNA virioplankton peptides showed significant homology to other known viral proteins (66%); whereas, only 19% of dsDNA virioplankton peptides hit another known viral peptide. For the ssDNA library, 24% of these viral hits were against known proteins from ssDNA viruses with the majority (85%) belonging to phages within the Chlamydiamicrovirus genus. No predicted peptides from the dsDNA virioplankton library had a top BLAST hit to a ssDNA viral peptide.

Most of the viral hits to dsDNA virioplankton peptides (94%) were from phages within the order Caudovirales (tailed phages). Surprisingly, three quarters of the viral BLAST hits to peptides in the ssDNA virome belonged to tailed phages. The family-level distribution of Caudovirales hits between the ssDNA and dsDNA virioplankton libraries was very different. For the ssDNA library the majority of Caudovirales hits (84%) were to phages within the *Podoviridae*. For the dsDNA virioplankton, hits to phage within the Caudovirales were distributed almost evenly among the three tailed phage families: *Myo*-, *Sipho*- and *Podoviridae*.

The presence of Caudovirales homologs within the ssDNA virioplankton library warranted further investigation to determine the possible functional characteristics of the genes that appear to bridge the evolutionary divide between ssDNA and dsDNA phages. For the ssDNA virome, 85% of peptides with a top UR100 hit in the Caudovirales fell within only seven functional genes, nearly all of which were related to nucleic acid metabolism ((RNA polymerase, endonuclease, uncharacterized protein, DNA polymerase, ssDNA binding protein, DNA primase/helicase and exonuclease) (Supplementary Table S2 and Supplementary Figure S6)). In contrast, the top 85% of Caudovirales hits to the dsDNA viromes included 106 gene descriptions across numerous categories of functional genes (Supplementary Table S3). By and large, only four cyanophages (P-SSP7, P-SSM2, S-CBP3 and Syn5) accounted for the lion's share of top UniRef hits to the ssDNA virome, with podovirus P-SSP7 being the most common (Supplementary Table S2 and Supplementary Figure S6). Recruitment of the Chesapeake Bay and Dry Tortugas ssDNA virome reads to the P-SSP7 genome showed that homology to P-SSP7 genes was largely restricted to genes involved in nucleotide metabolism (Supplementary Figure S4).

Discussion

Key mutations cause biochemical changes relevant to phage lifestyle

The conservation of residues critical to the function of DNA polymerase indicates that the metagenomic contigs likely encoded functional polymerases. However, many of the evolutionarily conserved residues in bacterial polymerases were not conserved in known phage or metagenomic sequences, suggesting that they are less critical to polymerase function or may serve to uniquely alter enzymatic characteristics of this enzyme within bacteria. Of particular interest was residue Phe762, where both known phage and metagenomic sequences frequently encoded tyrosine or leucine instead. At this position, the ssDNA cluster and Clade I (T7-like DNA polymerases) exclusively contained tyrosine, whereas Clades II and III were exclusive for phenylalanine and leucine, respectively (Figure 1).

Mutations in Phe762 are well studied because the site has important roles in discrimination against dideoxynucleotide (ddNTP) incorporation, polymerase activity and fidelity. Site-directed mutagenesis of Phe762 to tyrosine in DNA polymerase I from *E. coli* and *Thermus aquaticus* (*Taq* polymerase) increases the incorporation of ddNTPs 1000-fold or more than the native enzymes (Tabor and Richardson, 1995), a feature essential for DNA sequencing by chain-termination (Tabor and Richardson, 1987). Tyrosine occurs naturally at this position in *Mycobacterium spp.* and some phage polymerases, including that from phage T7 (Tyr526) (Tabor and Richardson, 1987; Doublie *et al*., 1998). Despite a low dNTP:ddNTP incorporation ratio of three (Tabor and Richardson 1995), T7 polymerase contains a strong 3′–5′ exonuclease capable of degrading ddNTPs (Tabor and Richardson, 1987). Thus, the selective pressure of tyrosine at this position in phage T7 is likely not the incorporation of ddNTPs but rather increased efficiency. A Phe762Tyr mutation in the Klenow fragment of *E. coli* decreases the K_m fivefold, resulting in an approximately fourfold increase in catalytic efficiency (k_{cat}/K_m) over wild type (Astatke *et al*., 1998). Similarly, conversion of tyrosine at the corresponding residue in *Mycobacterium tuberculosis* (Tyr737) to phenylalanine results in a sixfold reduction of polymerase activity (Mizrahi and Huberts 1996).

The incorporation of a tyrosine residue at this location might be especially advantageous for highly lytic phage with large burst sizes such as T7-like podoviruses. Indeed, all of the cultured phages with the tyrosine mutation at this position were lytic (Supplementary Figure S3). This included the lytic Pelagibacter phages HTVC011P and HTVC019P, which infect abundant and ubiquitous SAR11 hosts. Together, these data indicate that the members of Clade I are virulent phages.

Like the sequences in Clade III, all cultured lysogenic phages carrying the *pol*A gene contain the leucine substitution in the site corresponding to Phe762 or Phe667 (*T. aquaticus)* (Supplementary Figure S3). This suggests a link between this particular substitution and the biological requirements for a lysogenic life cycle. The induced mutation Phe667Leu in *T. aquaticus* increases the accuracy of *Taq* DNA polymerase threefold compared with the wild type, but simultaneously decreases the specific activity and catalytic efficiency (V_{max}/K_m) (Suzuki *et al*., 2000), whereas the T7 Tyr526Leu mutant polymerase displays a 1000-fold decrease in polymerase activity (Tabor and Richardson, 1987). A more accurate phage polymerase could slow the background mutation rate in these phages and have implications for phage evasion of host resistance through high mutation rates. The decreased efficiency of this polymerase also suggests that the phages that possess it replicate more slowly and may produce fewer progeny per burst. Presumably, such a polymerase would be more suitable to a temperate rather than a virulent phage. Alternatively, this mutation could be an adaptation for replication within hosts with low growth rates, although the presence of leucine in cultivated lysogenic phages with DNA polymerase A spanning a broad diversity of hosts suggests the mutation is directly linked to phage lifestyle. It is important to note that although leucine occurs in the Phe762 position in known phage DNA polymerases and is most frequent among marine phages, the biochemistry of this substitution has only been studied in the context of an induced mutation in *T. aquaticus* Pol I (Suzuki *et al*., 2000) and bacteriophage T7 (Tabor and Richardson, 1987). These cultured and metagenomic phage polymerases offer a new avenue for biochemists to study this mutation in its naturally occurring form.

The unusual case of Pol I in ssDNA virioplankton

Surprisingly, the metagenomic ssDNA libraries showed similar or higher frequencies of *polA* reads than the dsDNA libraries. The two CBS contigs had the essential active sites and necessary predicated shape to be functional polymerases despite their shorter coding length. As ssDNA phages typically have smaller genomes than their dsDNA counterparts, it is logical that the ssDNA phage proteins would minimize gene length while maintaining necessary structural domains for protein function. Also, the fact that ssDNA phages have higher mutation rates than dsDNA phages (Duffy *et al*., 2008) is consistent with the observation that the ssDNA virioplankton DNA polymerase did not include a proofreading exonuclease.

Several lines of evidence indicate that the presence of family A DNA polymerases in the ssDNA virioplankton libraries was not the result of contamination with DNA from dsDNA viruses. First, in the hydroxyapatite chromatography method used to separate the virioplankton nucleic acid fractions, the ssDNA fraction elutes first followed by RNA and finally dsDNA. Thus, the ssDNA and dsDNA fractions are well resolved from one another. Tests on a known mixture of viral nucleic acid types found that each viral nucleic acid eluted in the expected fraction without cross contamination (Andrews-Pfannkoch *et al*., 2010). Second, sequences from the ssDNA Chesapeake Bay library (CBS) were frequently most similar to DNA polymerase genes from podoviruses S-CBP3 and P-SSP7 in BLAST searches. All metagenomic sequence reads within the CBS library were tested for recruitment to the P-SSP7 genome. Contrary to what would be expected in the event of contamination, the ssDNA fragments recruited at high frequency to only a few sites of the P-SSP7 genome, most notably those regions related to genes encoding proteins involved in DNA replication (Supplementary Figure S4 and Supplementary Table S2). Finally, the distribution of BLASTP homologs among taxa and functional gene groups was substantially different for the dsDNA and ssDNA viromes from the Chesapeake Bay water samples (Supplementary Tables S2–S5).

One possible explanation why DNA pol I and other nucleic acid metabolism genes were so readily detected in the Chesapeake Bay and, to a lesser extent, the Dry Tortugas ssDNA viromes may be the procedures used in the preparation of these samples. Other ssDNA virome studies have relied on the propensity of the phi29 DNA polymerase to preferentially amplify small circular DNA molecules to selectively enrich DNA samples with amplified ssDNA viral genomes (Kim *et al.*, 2008). Indeed, because of this preferential bias, all viromes in which multiple displacement amplification has been used to amplify environmental viral genomic DNA are enriched for the presence of ssDNA viral sequences (Angly *et al.*, 2006; Tucker *et al.*, 2011). To our knowledge, this is the first viral metagenomics study to avoid the use of MDA (and the phi29 DNA polymerase) in the process of preparing environmental viral DNA for sequencing. The combination of hydroxyapatite chromatography for selective isolation of ssDNA molecules along with linker amplification may have enabled detection of ssDNA viral groups that have evaded detection in MDA-based library preparation techniques. Recent work has demonstrated that viral metagenomes prepared using linker amplification more accurately preserve the underlying distributions of viruses within a community (Duhaime *et al.*, 2012).

The critical role of virome data

Assessing viral diversity remains a challenge because viruses lack any universal marker genes. To date, studies that have used marker gene polymorphism as a means for investigating viral diversity and population ecology within natural environments have largely used PCR-based approaches that rely on primers designed from known phage genome sequences. For example, bacteriophage structural proteins g20 (portal vertex protein) (Short and Suttle 2005; Sullivan *et al.*, 2008) and gp23 (major capsid protein) (Filee *et al.*, 2005; Jamindar *et al.*, 2012) have been used to identify the diversity and distribution of cyanomyoviruses and T4-like phages. Functional genes like DNA polymerase A (Labonté *et al.*, 2009; Huang *et al.*, 2010) and photosystem genes *psbA* and *psbD* (Bench *et al.*, 2007; Chenard and Suttle 2008) have been used as proxies of T7-like podoviruses and cyanophage diversity, respectively. These marker genes, which have been examined in both cultivated phages and environmental amplicon sequence data, have yielded key insights into the diversity and distribution of their respective phage targets. However, in each case these studies have relied on *a priori* approaches for the design of PCR primers and the degree to which this reliance has limited our view of viral diversity is not well appreciated. The abundant, novel DNA polymerase A sequences identified in this study using a metagenomic approach highlight the limitations of PCR-based approaches for investigations of viral diversity and population ecology.

Adding our representative metagenomic DNA polymerase sequences to a preexisting alignment of T7-like DNA polymerase PCR amplicons (Labonté *et al.*, 2009) did not alter previously defined groups but instead produced an additional clade labeled ENV-4 (Figure 3). Metagenomic contig sequences that were the closest to T7 DNA polymerase on the full-length sequence tree (Figure 1) surprisingly did not fall into the T7 group on the PCR-amplicon tree (Figure 3), but rather claded with the amplicons in the Labonté *et al.* (Labonté *et al.*, 2009) ENV groups and with the amplicons within the PUP clade (Breitbart *et al.*, 2004). The ssDNA virus sequence claded within the cyanophage P60 group, close to cyanophages P-SSP7 and S-CBP3 as on the full-length sequence tree (Figure 1). The remaining 11 representative DNA Pol sequences did not fall into any of the previously described groups, but formed a new clade along with Enterobacteria phage T5 and Proteobacteria phage phiJL001 labeled ENV-4 (Figure 3). This new clade corresponded to Clade II and III in Figure 1 and represented a total of 78 out of 88 contigs. It also included all three sampling locations and 93% of all sequence reads contributing to the assembly of full-length virioplankton DNA polymerases. It is important to note that the degenerate PCR primers used to obtain the T7-like environmental sequences would not have amplified the majority of virioplankton polymerase gene sequences found in this study. As a consequence, an *a priori* approach based on any of the previously reported DNA pol I primer sets would have missed most of the diversity of viruses carrying the DNA polA gene.

Both the full-length (Figure 1) and PCR-amplicon length (Figure 3) analyses of DNA polymerase I from viral metagenomic data indicate that the most abundant DNA polymerase-carrying viruses in coastal and estuarine environments may be similar to siphoviruses, like proteobacteria phage phiJL001. This prediction is supported by observations that myo- and siphoviruses are most frequently isolated from marine environments (Breitbart *et al.*, 2004; Labonté *et al.*, 2009). Moreover, Pol I sequences from cultivated siphoviruses grouped closely with the abundant metagenomic sequences in Clade III

(Supplementary Figure S3). These phages are more likely to be lysogenic or pseudo-lysogenic and have slow replication rates as compared with the highly lytic podoviruses such as coliphage T7, and cyanophages P-SSP7 and P60 (Liu *et al.*, 2004; Lohr *et al.*, 2005; Sabehi and Lindell, 2012).

Relationship with DNA polymerase γ

Recent studies have reported that genes with homology to mitochondrial DNA polymerase γ are abundant within the virioplankton have been found in cyanophage genomes and the Global Ocean Survey data set (Chan *et al.*, 2011; Sabehi *et al.*, 2012). To determine the prevalence of these polymerases in our metagenomic libraries the VIROME databank was queried with the set of S15 DNA pol γ sequences from the mitochondria, cyanophage and the Global Ocean Survey (Rusch *et al.*, 2007; Yooseph *et al.*, 2007) and BroadPhage (John *et al.*, 2011) data sets (BLASTP E-score $\leqslant 10^{-10}$). This gene does not appear to be abundant in our metagenomic libraries, as only seven ORFs with homology to DNA pol γ were found in the VIROME database.

A maximum likelihood tree encompassing all the polymerase groups described in (Filee *et al.*, 2002) and trimmed to their specified conserved regions confirmed that metagenomic Clade I–III are not related to the group of phages encoding DNA pol γ-like polymerases (Supplementary Figure S5). The clades containing metagenomic representative sequences described in Figure 1 remained distinct and did not disrupt previously established relationships, further supporting our finding that these clades are valid groupings linked to polymerase functionality. Because Clade I–III (Figure 1) contained a greater number of sequences than those identified as DNA pol γ across all VIROME libraries, phages carrying these DNA polA genes likely have a larger impact on aquatic ecosystems. These data should ignite subsequent investigations on the biochemistry of unique phage enzymes and how this biochemistry shapes the mechanistic details behind viral impacts on ecosystems.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful for the support of a NSF Microbial Genome Sequencing Program grant (EF 0626826) to SJW and KEW and a Gordon and Betty Moore Foundation grant to KEW and SWP. HFS was supported through summer undergraduate research internships from the Delaware EPSCoR program and the Howard Hughes Medical Institute. EGS was supported through a graduate fellowship from the Delaware Water Resources Center. Usage of the computational infrastructure of the University of Delaware Center for Bioinformatics and Computational Biology Core Facility was made possible by support from Delaware INBRE (NIH NCRR 2 P20 RR016472-09) and Delaware EPSCoR (NSF EPS-0814251). The authors are grateful to Curtis Suttle and Jessica Labonté for providing their alignment of DNA polymerase amplicon sequences.

References

Andrews-Pfannkoch C, Fadrosh DW, Thorpe J, Williamson SJ. (2010). Hydroxyapatite-mediated separation of double-stranded DNA, single-stranded DNA, and RNA genomes from natural viral assemblages. *Appl Environ Microb* **76**: 5039–5045.

Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, Carlson C *et al.* (2006). The marine viromes of four oceanic regions. *PLoS Biol* **4**: 2121–2131.

Arnold K, Bordoli L, Kopp J, Schwede T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **22**: 195–201.

Astatke M, Grindley ND, Joyce CM. (1998). How *E. coli* DNA polymerase I (Klenow fragment) distinguishes between deoxy- and dideoxynucleotides. *J Mol Biol* **278**: 147–165.

Beese LS, Kiefer JR, Mao C, Braman JC. (1998). Visualizing DNA replication in a catalytically active Bacillus DNA polymerase crystal. *Nature* **391**: 304–307.

Bench SR, Hanson TE, Williamson KE, Ghosh D, Radosovich M, Wang K *et al.* (2007). Metagenomic characterization of Chesapeake Bay virioplankton. *Appl Environ Microb* **73**: 7629–7641.

Breitbart M, Miyake JH, Rohwer F. (2004). Global distribution of nearly identical phage-encoded DNA sequences. *Fems Microbiol Lett* **236**: 249–256.

Brussaard CP, Short SM, Frederickson CM, Suttle CA. (2004). Isolation and phylogenetic analysis of novel viruses infecting the phytoplankton Phaeocystis globosa (Prymnesiophyceae). *Appl Environ Microb* **70**: 3700–3705.

Buechen-Osmond C, Dallwitz M. (1996). Towards a universal virus database - progress in the ICTVdB. *Arch Virol* **141**: 392–399.

Chan YW, Mohr R, Millard AD, Holmes AB, Larkum AW, Whitworth AL *et al.* (2011). Discovery of cyanophage genomes which contain mitochondrial DNA polymerase. *Mol Biol Evol* **28**: 2269–2274.

Chenard C, Suttle CA. (2008). Phylogenetic diversity of sequences of cyanophage photosynthetic gene psbA in marine and freshwaters. *Appl Environ Microbiol* **74**: 5317–5324.

Culley AI, Lang AS, Suttle CA. (2003). High diversity of unknown picorna-like viruses in the sea. *Nature* **424**: 1054–1057.

Culley AI, Lang AS, Suttle CA. (2006). Metagenomic analysis of coastal RNA virus communities. *Science* **312**: 1795–1798.

Doublie S, Tabor S, Long AM, Richardson CC, Ellenberger T. (1998). Crystal structure of a bacteriophage T7 DNA replication complex at 2.2A resolution. *Nature* **391**: 251–258.

Drummond A, Ashton B, Buxton S, Cheung M, Cooper A, Duran C *et al.* (2011). Geneious v5.4. http://www.geneious.com/.

Duffy S, Shackelton LA, Holmes EC. (2008). Rates of evolutionary change in viruses: patterns and determinants. *Nat Rev Genet* **9**: 267–276.

Duhaime MB, Deng L, Poulos BT, Sullivan MB. (2012). Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. *Environ Microbiol* **14**: 2526–2537.

Dutta S, Li Y, Johnson D, Dzantiev L, Richardson CC, Romano LJ *et al.* (2004). Crystal structures of 2-acetylaminofluorene and 2-aminofluorene in complex with T7 DNA polymerase reveal mechanisms of mutagenesis. *Proc Natl Acad Sci USA* **101**: 16186–16191.

Edgar RC. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**: 1792–1797.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Filee J, Forterre P, Sen-Lin T, Laurent J. (2002). Evolution of DNA polymerase families: evidences for multiple gene exchange between cellular and viral proteins *J Mol Evol* **54**: 763–773.

Filee J, Tetart F, Suttle CA, Krisch HM. (2005). Marine T4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. *Proc Natl Acad Sci USA* **102**: 12471–12476.

Gimenes MV, Zanotto PMdA, Suttle CA, da Cunha HB, Mehnert DU. (2011). Phylodynamics and movement of Phycodnaviruses among aquatic environments. *ISME J* **6**: 237–247.

Guex N, Peitsch MC. (1997). SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* **18**: 2714–2723.

Guindon S, Lethiec F, Duroux P, Gascuel O. (2005). PHYML Online - a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33**: W557–W559.

Huang S, Wilhelm SW, Jiao N, Chen F. (2010). Ubiquitous cyanobacterial podoviruses in the global oceans unveiled through viral DNA polymerase gene sequences. *ISME J* **4**: 1243–1251.

Jamindar S, Polson SW, Srinivasiah S, Waidner L, Wommack KE. (2012). Evaluation of two approaches for assessing the genetic similarity of virioplankton populations as defined by genome size. *Appl Environ Microbiol* **78**: 8773–8783.

John SG, Mendez CB, Deng L, Poulos B, Kauffman AK, Kern S *et al.* (2011). A simple and efficient method for concentration of ocean viruses by chemical flocculation. *Environ Microbiol Rep* **3**: 195–202.

Kiefer F, Arnold K, Kunzli M, Bordoli L, Schwede T. (2009). The SWISS-MODEL Repository and associated resources. *Nucleic Acids Res* **37**: D387–D392.

Kim KH, Chang HW, Nam YD, Roh SW, Kim MS, Sung Y *et al.* (2008). Amplification of uncultured single-stranded DNA viruses from rice paddy soil. *Appl Environ Microbiol* **74**: 5975–5985.

Labonté JM, Reid KE, Suttle CA. (2009). Phylogenetic analysis indicates evolutionary diversity and environmental segregation of marine Podovirus DNA polymerase gene sequences. *Appl Environ Microb* **75**: 3634–3640.

Li Y, Korolev S, Waksman G. (1998). Crystal structures of open and closed forms of binary and ternary complexes of the large fragment of Thermus aquaticus DNA polymerase I: structural basis for nucleotide incorporation. *Embo J* **17**: 7514–7525.

Liu M, Gingery M, Doulatov SR, Liu Y, Hodes A, Baker S *et al.* (2004). Genomic and genetic analysis of Bordetella bacteriophages encoding reverse transcriptase-mediated tropism-switching cassettes. *J Bacteriol* **186**: 1503–1517.

Loh E, Loeb LA. (2005). Mutability of DNA polymerase I: implications for the creation of mutant DNA polymerases. *DNA Repair* **4**: 1390–1398.

Lohr JE, Chen F, Hill RT. (2005). Genomic analysis of bacteriophage Phi JL001: Insights into its interaction with a sponge-associated alpha-proteobacterium. *Appl Environ Microb* **71**: 1598–1609.

Marchler-Bauer A, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH *et al.* (2009). CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res* **37**: D205–D210.

Marchler-Bauer A, Bryant SH. (2004). CD-Search: protein domain annotations on the fly. *Nucleic Acids Res* **32**: W327–W331.

Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C *et al.* (2011). CDD: a Conserved Domain Database for the functional annotation of proteins. *Nucleic Acids Res* **39**: D225–D229.

Mizrahi V, Huberts P. (1996). Deoxy- and dideoxynucleotide discrimination and identification of critical 5’ nuclease domain residues of the DNA polymerase I from Mycobacterium tuberculosis. *Nucleic Acids Res* **24**: 4845–4852.

Naryshkina T, Liu J, Florens L, Swanson SK, Pavlov AR, Pavlova NV *et al.* (2006). Thermus thermophilus bacteriophage phi YS40 genome and proteomic characterization of virions. *J Mol Biol* **364**: 667–677.

Noguchi H, Park J, Takagi T. (2006). MetaGene: prokaryotic gene finding from environmental genome shotgun sequences. *Nucleic Acids Res* **34**: 5623–5630.

Noguchi H, Taniguchi T, Itoh T. (2008). Metageneannotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. *DNA Res* **15**: 387–396.

Ollis DL, Brick P, Hamlin R, Xuong NG, Steitz TA. (1985). Structure of large fragment of *Escherichia coli* DNA-polymerase-I complexed with Dtmp. *Nature* **313**: 762–766.

Patel PH, Loeb LA. (2000). DNA polymerase active site is highly mutable: evolutionary consequences. *Proc Natl Acad Sci USA* **97**: 5095–5100.

Peitsch MC, Wells TNC, Stampf DR, Sussman JL. (1995). The Swiss-3dimage Collection and Pdb-Browser on the Worldwide Web. *Trends Biochem Sci* **20**: 82–84.

Poorvin L, Rinta-Kanto JM, Hutchins DA, Wilhelm SW. (2004). Viral release of iron and its bioavailability to marine plankton. *Limnol Oceanogr* **49**: 1734–1741.

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S *et al.* (2007). The Sorcerer II global ocean sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biol* **5**: 398–431.

Sabehi G, Lindell D. (2012). The P-SSP7 cyanophage has a linear genome with direct terminal repeats. *PLoS One* **7**: e36710.

Sabehi G, Shaulov L, Silver DH, Yanai I, Harel A, Lindell D. (2012). A novel lineage of myoviruses infecting cyanobacteria is widespread in the oceans. *Proc Natl Acad Sci USA* **109**: 2037–2042.

Sanger F, Nicklen S, Coulson AR. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**: 5463–5467.

Scarlato V, Gargano S. (1992). The DNA polymerase-encoding gene of Bacillus subtilis bacteriophage SPO1. *Gene* **118**: 109–113.

Schloss PD, Handelsman J. (2005). Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microb* **71**: 1501–1506.

Schwede T, Kopp J, Guex N, Peitsch MC. (2003). SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res* **31**: 3381–3385.

Short CM, Suttle CA. (2005). Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. *Appl Environ Microbiol* **71**: 480–486.

Sullivan MB, Coleman ML, Quinlivan V, Rosenkrantz JE, Defrancesco AS, Tan G *et al.* (2008). Portal protein diversity and phage ecology. *Environ Microbiol* **10**: 2810–2823.

Suttle CA. (2005). Viruses in the sea. *Nature* **437**: 356–361.

Suzuki M, Yoshida S, Adman ET, Blank A, Loeb LA. (2000). Thermus aquaticus DNA polymerase I mutants with altered fidelity. Interacting mutations in the O-helix. *J Biol Chem* **275**: 32728–32735.

Tabor S, Richardson CC. (1987). DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc Natl Acad Sci USA* **84**: 4767–4771.

Tabor S, Richardson CC. (1995). A single residue in DNA-polymerases of the *Escherichia Coli* DNA-polymerase-I family is critical for distinguishing between deoxyribonucleotides and dideoxyribonucleotides. *Proc Natl Acad Sci USA* **92**: 6339–6343.

Tucker KP, Parsons R, Symonds EM, Breitbart M. (2011). Diversity and distribution of single-stranded DNA phages in the North Atlantic Ocean. *ISME J* **5**: 822–830.

Tully BJ, Nelson WC, Heidelberg JF. (2012). Metagenomic analysis of a complex marine planktonic thaumarchaeal community from the Gulf of Maine. *Environ Microbiol* **14**: 254–267.

Winget DM, Helton RR, Williamson KE, Bench SR, Williamson SJ, Wommack KE. (2011). Repeating patterns of virioplankton production within an estuarine ecosystem. *Proc Natl Acad Sci USA* **108**: 11506–11511.

Wommack KE, Bhavsar J, Polson SW, Chen J, Dumas M, Srinivasiah S *et al.* (2012). VIROME: a standard operating procedure for analysis of viral metagenome sequences. *Stand Genomic Sci* **6**: 427–439.

Wommack KE, Bhavsar J, Ravel J. (2008). Metagenomics: read length matters. *Appl Environ Microbiol* **74**: 1453–1463.

Yooseph S, Sutton G, Rusch DB, Halpern AL, Williamson SJ, Remington K *et al.* (2007). The Sorcerer II Global Ocean Sampling expedition: expanding the universe of protein families. *PLoS Biol* **5**: e16.

Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)

Quantifying the Role of Carbon Amount and Quality for Transport of Contaminants on Our Landscapes: A Watershed-Scale Model

Basic Information

Title:	Quantifying the Role of Carbon Amount and Quality for Transport of Contaminants on Our Landscapes: A Watershed-Scale Model
Project Number:	2010DE173B
Start Date:	3/1/2013
End Date:	8/31/2013
Funding Source:	104B
Congressional District:	At large
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Hydrogeochemistry, Nutrients
Descriptors:	None
Principal Investigators:	Shreeram P. Inamdar

Publications

1. Dhillon, G. and S. Inamdar, 2011, Quantifying the Relative Contributions of Particulate and Dissolved C to the Total Carbon Export from the Catchment: A Watershed Scale Model Progress Report, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 6 pages.
2. Pautler, M., ed., 2010, Delaware Water Resources Center WATER NEWS Vol. 10 Issue 2 DWRC Spotlight on Graduate Research, http://ag.udel.edu/dwrc/newsletters/Winter09Spring10/WATERNEWSco-Spring2010.pdf , p. 7.
3. Dhillon, G. and S. Inamdar, 2012, Quantifying the Relative Contributions of Particulate and Dissolved C to the Total Carbon Export from the Catchment: A Watershed Scale Model Progress Report, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 21 pages.
4. Dhillon, G., 2012, Comparison of Particulate and Dissolved Organic Carbon Exports from Forested Piedmont Catchments, M.S. Dissertation, Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, University of Delaware, Newark, Delaware, 122 pages.
5. Dhillon, G., and S. Inamdar, 2013, Extreme Storms and Changes in Particulate and Dissolved Organic Carbon in Runoff: Entering Uncharted Waters?, Geophysical Research Letters, 40, 1-6.
6. Inamdar, S., G. Dhillon, S. Singh, S. Dutta, D. Levia, D. Scott, M. Mitchell, J. Van Stan, and P. McHale, 2013, Temporal Variation in End-Member Chemistry and Its Influence on Runoff Mixing Patterns in a Forested, Piedmont Catchment, Water Resources Research, 49, 1828-1844.
7. Dhillon, G.S., and S. Inamdar, 2013, Storm Event Patterns of Particulate Organic Carbon (POC) for Large Storms and Differences with Dissolved Organic Carbon (DOC), Biogeochemistry, 118, 61-81.
8. Pautler, M., ed., 2014, Delaware Water Resources Center WATER E-NEWS Vol. 13 Issue 2, Spotlight on DWRC – UD WATER Research and Investigations, http://ag.udel.edu/dwrc/newsletters/WATERENEWS-Mar2014.pdf , p. 1.

Biogeochemistry (2014) 118:61–81
DOI 10.1007/s10533-013-9905-6

Storm event patterns of particulate organic carbon (POC) for large storms and differences with dissolved organic carbon (DOC)

Gurbir Singh Dhillon · Shreeram Inamdar

Received: 5 November 2012 / Accepted: 19 August 2013 / Published online: 3 September 2013

Abstract This study compared the storm event patterns, sources, and flow paths for particulate (POC) and dissolved organic carbon (DOC <0.45 μm) with a special focus on responses during large storm events. The study was conducted in a 12 ha forested catchment in the mid-Atlantic, Piedmont region of USA. A total of 14 storm events were sampled over a 16-month period (September 2010 to December 2011) including large, intense storms (precipitation >150 mm) associated with two hurricanes—Nicole (2010) and Irene (2011). Storm-event concentrations for suspended sediment (SS), POC and DOC varied between 10–7589, 0.05–252, and 0.7–18.3 mg L^{-1}, respectively. Within-event POC concentrations continued to increase for the large hurricane storms whereas DOC displayed a dilution at peak streamflow discharge. Flow-weighted mean POC concentrations decreased for closely spaced, successive storm events whereas no such decrease was observed for DOC. These results suggest that there are important differences in the supply and transport (leaching rates and kinetics) for POC and DOC which occur at different temporal scales. The % POC content of SS was highest for the summer events. Summer events also registered a sharper increase in DOC with stream discharge and then a decline for peak flow, suggesting critical seasonal controls on storm-event POC and DOC responses. End-member mixing analysis revealed POC is transported with surface runoff while DOC is transported by saturation overland flow and rising groundwater into the soil horizons. A mixing model for sediment sources failed to identify key end-members but event mixing patterns revealed near-stream sources for small events and more distal, upland sediment sources for large and intense storms. This study highlights the need to better understand POC and DOC responses in headwater catchments especially for the large, intense, storm events that are predicted to increase in intensity with climate change.

Keywords Climate change · Organic carbon · Watersheds · Storm events · Runoff · Water quality

Responsible Editor: Eran Hood

G. S. Dhillon · S. Inamdar (✉)
Plant and Soil Sciences Department, University of Delaware, 152 Townsend Hall, 531 S. College Avenue, Newark, DE 19716, USA
e-mail: Inamdar@udel.edu

G. S. Dhillon
Soil Sciences Department, University of Saskatoon, Saskatoon, SK, Canada

Introduction

Organic carbon, which can be transported in both dissolved (DOC) and particulate forms (POC) plays a key role in many ecological processes and has major implications for environmental and human health.

DOC influences the acid–base chemistry of acid sensitive freshwater systems (Herczeg et al. 1985), affects the complexation, solubility and mobility of trace metals such as iron, aluminum, zinc and mercury (Buffle 1984; Driscoll et al. 1988; Hope et al. 1994), and attenuates light penetration into aquatic ecosystems (Cory et al. 2007; Brooks et al. 2005). POC acts as a carrier for the transport of organic chemicals (Ni et al. 2008) and is believed to be responsible for the export of hydrophobic contaminants (Luo et al. 2009). Both POC and DOC regulate aquatic metabolism (Cole 2013) and can influence drinking water quality when C reacts with chlorine to form carcinogenic disinfection byproducts (DBPs, Chow et al. 2008; Hrudey 2009). At regional to global scales, organic carbon transported through streams and rivers is a globally significant carbon flux that has recently received considerable attention (Battin et al. 2008; Butman and Raymond 2011; Cole et al. 2007). Thus, understanding how POC and DOC amounts vary in runoff and the mechanisms and flow paths responsible is important.

Exports of organic carbon from catchments are strongly influenced by storm events, especially the large ones (Fellman et al. 2009; Hood et al. 2006; Inamdar et al. 2006, 2011; Raymond and Saiers 2010; Townsend-Small et al. 2008). Previous studies (Bass et al. 2011; Crisp and Robson 1979; Oeurng et al. 2011; Wiegner et al. 2009) have reported that storm events that constituted only 10–20 % of the total study period contributed to >80 % of POC and >70 % of DOC exports. While the concentrations and exports of both POC and DOC have typically been known to increase with storm events, the relative response of these constituents is not always similar (Dhillon and Inamdar 2013; Pawson et al. 2008; Wiegner et al. 2009). Wiegner et al. (2009) reported a twofold increase in concentration of DOC, but an 11-fold increase in the concentration POC during storms. This difference can be even greater for storms of exceptional intensity and magnitude. Jeong et al. (2012) working in a 38 ha headwater catchment in South Korea, showed that one extreme event that contained 20 % of the annual precipitation was responsible for 62 and 23 % of the annual POC and DOC load, respectively. Our recent work in the catchment used for this study showed that extremely large storms associated with Atlantic hurricanes (precipitation in excess of 100 mm) resulted in POC exports that were 6–8 times the DOC values (Dhillon and Inamdar 2013). Future climate-change predictions suggest that the intensity of storm events is likely to increase for the northeast USA with a greater potential for stronger hurricanes and tropical storms (Bender et al. 2010; Karl et al. 2009). Understanding how POC and DOC responses would respond and differ for such climate-driven extreme events is important for assessing impacts on ecosystem processes and services.

Studies have also revealed that there are subtle, but important distinctions between storm-event responses of POC and DOC (Alvarez-Cobelas et al. 2012; Kim et al. 2010). While there is variation among events, POC concentrations typically tend to peak earlier than DOC on the discharge hydrograph (Coynel et al. 2005; Jeong et al. 2012). In addition, rate of change (increase and decrease) for POC is much quicker than that for DOC (Oeurng et al. 2011; Johnson et al. 2006; Pawson et al. 2012). Alexandrov et al. (2003), Coynel et al. (2005), Pawson et al. (2008), and Rovira and Batalla (2006) have also reported a gradual reduction in POC concentrations for close, successive storm events while the same was not known for DOC. These discrepancies suggest important differences in POC and DOC with respect to sources, hydrologic flowpaths, and the leaching kinetics of these constituents. Investigating these differences and understanding the mechanisms responsible is important for developing realistic and reliable mechanistic models for C transport and fate in catchments.

The primary goal of this study was to investigate the runoff patterns and sources of POC during storm events, and simultaneously, investigate how and why POC responses differed from DOC for the largest storm events. This study builds on our recent work in a 12 ha forested catchment in the mid-Atlantic Piedmont where we have already characterized the hydrologic flow paths and developed a conceptual runoff model (Inamdar et al. 2013); identified the sources of DOC in the catchment (Inamdar et al. 2012); and characterized the temporal patterns and flow paths for DOC during storm events (2011). Thus, this background on runoff flow paths and DOC provided us a strong footing for comparing against POC results. We have also (Dhillon and Inamdar 2013) compared POC and DOC mass exports for a wide range in storm magnitude—from small to large events associated with remnants of hurricanes. This comparison indicated that while POC exports were comparable to

DOC for small to moderate events, the POC exports outpaced DOC and increased exponentially for the largest storm events (Dhillon and Inamdar 2013). Here, we further examine these storm events to investigate the reason for these disparate responses in POC and DOC. In addition, we assess the relationship between POC and suspended sediment (SS). The data set includes SS, POC and DOC concentrations in stream runoff (12 ha outlet) for 14 storm events sampled from September 2010 through December 2011. Precipitation during this study period was greater than previous years (2008–2010) and contained three large storms (precipitation in excess of 100 mm), two of which were associated with remnants of Hurricanes Nicole (2010) and Irene (2011). To identify the sources of sediment and POC for storms, soil and sediment sources were sampled spatially at multiple catchment locations and a mixing model framework was used to determine potential sediment end-members. Specific questions that we address are:

- How do the responses of POC and DOC compare for storm events of varying magnitude, intensity and timing (seasonal occurrence as well as sequential storm events)?
- How do the POC concentrations relate to SS concentrations?
- How do the within-event temporal patterns of POC and DOC compare?
- What are the sources of SS and POC in the catchment?

While previous studies have investigated POC patterns and sources, the work presented here is novel in that we focus on differences in POC and DOC for storms of varying magnitude, intensity and seasonal occurrence and explain these patterns using mixing models for runoff flow paths and sediment sources.

Site description and methods

Site description

The monitored catchment (12 ha) (Fig. 1) is part of an ongoing study on C (Inamdar et al. 2011, 2012, 2013) and is located within the Fair Hill Natural Resources Management Area (39°42′N, 75°50′W) in Cecil County, Maryland. It is part of the Big Elk Creek drainage basin and lies within the Piedmont physiographic region and drains into the Chesapeake Bay. Cecil County has a humid, continental climate with well-defined seasons. The 30 year climate norm (1981–2010) for northeastern Maryland reveals a mean annual precipitation of 1,205 mm. Snowfall is ~450 mm and is concentrated in a few events with a quick melt of the snowpack. The highest mean monthly air temperature is 25.7 °C, occurring in July. The lowest mean monthly air temperature of −0.1 °C occurs in January (Maryland State Climatologist Office Data Page, http://metosrv2.umd.edu/~climate/, accessed June 21, 2013).

The study catchment is underlain by the Mt. Cuba Wissahickon formation and includes pelitic gneiss and schist with subordinate amphibolite and pegmatite. The soils belong to the Glenelg series which consists of deep, well-drained, soils on nearly level to moderately steep slopes. On hillslopes, soils are coarse loamy, mixed, mesic Lithic Dystrudepts while seasonal water saturation in the valley bottoms leads to the formation of Oxyaquic Dystrudepts. The catchment is covered with deciduous forest (61 % areal cover) with pasture along the edges. Dominant tree species are *Fagus grandifolia* (American beech), *Liriodendron tulipifera* (yellow poplar), and *Acer rubrum* (red maple) (Levia et al. 2010).

Hydrologic monitoring

Stream flow discharge was monitored at the outlet of the 12 ha catchment (ST3 in Fig. 1) using a 6-in. Parshall flume and the water flow depths were recorded every 15–20 min using a Global Water Instrumentation, Inc. logger and pressure transducer. Discharge was computed using measured water levels and standard flume equations. Depth to groundwater (from the soil surface) was available from two locations within the catchment—LW2 and LW5 (Fig. 1). Groundwater wells consisted of PVC pipes (5 cm diameter) extending ~2 m below the ground surface that were continuously slotted from a depth of 0.3 m below the soil surface. Water levels in these wells were recorded every 30 min using Global Water loggers. Precipitation and air temperature data were available at 5-min frequency from a Delaware Earth Observation System (DEOS) weather station located in the Fairhill NRMA, about 1,000 m from the outlet of the catchment (DEOS 2012).

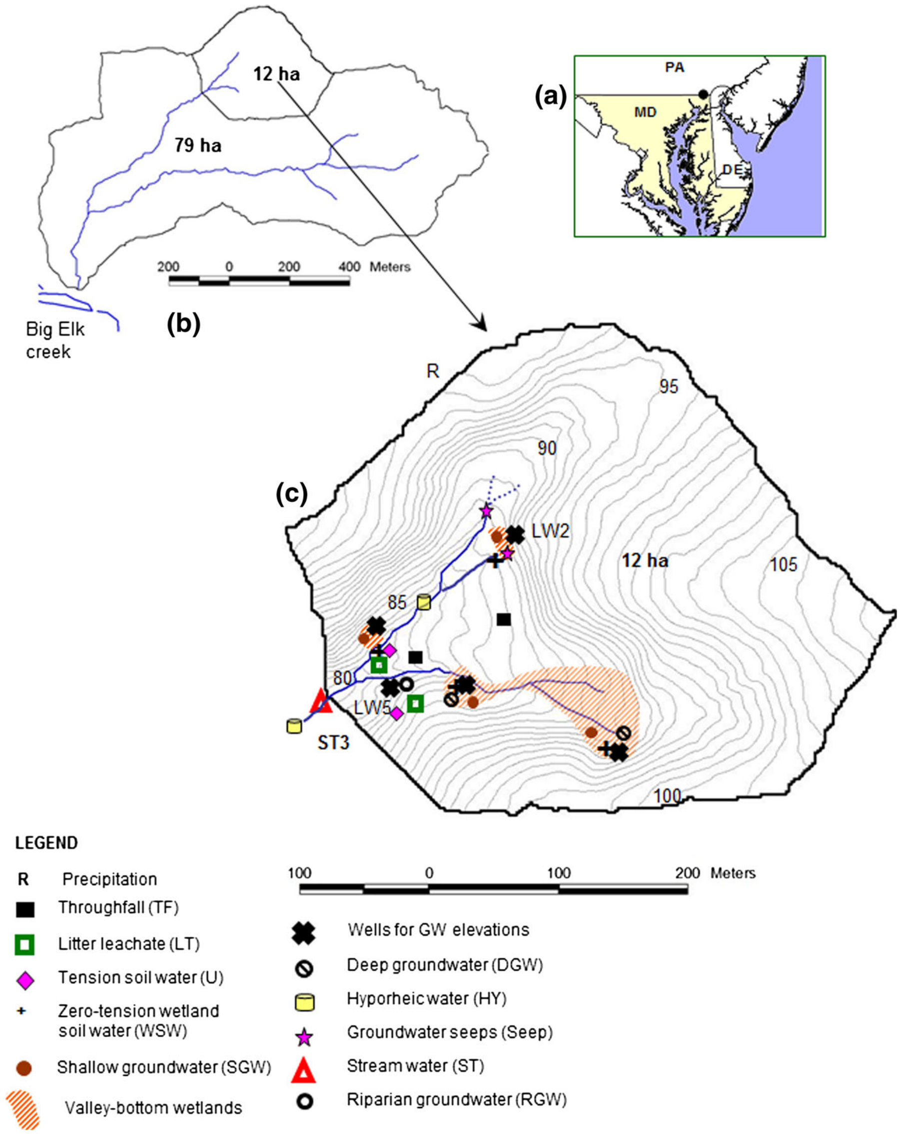


Fig. 1 **a** Study location in Piedmont region of Maryland; **b** the 12 ha study catchment; and **c** instrumentation and sampling within the 12 ha study site

Runoff sampling and pre-processing

SS, POC and DOC data were available for 14 storm events collected over a 16 month period from September 2010 to December 2011 (Fig. 2). Baseflow sampling was performed once a month for stream water, however, since SS and POC concentrations were below detection for the initial set of samples, baseflow sampling was limited to DOC only. Storm event sampling for stream water was performed using an automated ISCO sampler installed at the outlet of the 12 ha catchment and triggered to sample when the rainfall amount exceeded 2.54 mm in a 1 h period (or in some event manually triggered on the expected arrival of the storm). ISCO samples were collected in the "non-uniform time" program mode with a sampling frequency that ranged from as low as 15 min on the hydrograph rising limb to 3 h on the recession limb.

All stream water samples were collected in HDPE bottles and filtered through a 0.45 µm filter paper (Millipore, Inc.; Catalog #HAWP 04700) within 24 h of collection and stored at 4 °C. The weight of the particulate material was determined after drying the filters at 103–105 °C for 1 h and the weight of the sediment was divided by the sample volume to obtain the concentration of SS in mg L^{-1}. The SS represented a size range of 0.45 μm to about 1 mm.

Chemical analyses of water and sediment samples

The dissolved (<0.45 μm) constituents were determined at the Biogeochemistry Laboratory at SUNY-ESF, NY, which is a participant in the USGS QA/QC program (Inamdar and Mitchell 2007). The water samples were analyzed for: major cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and silica (Si) using a Perkin-Elmer ICP-AEC Div 3300 instrument; and dissolved organic carbon (DOC) using the Tekmar–Dohrmann Phoenix 8000 TOC analyzer. The sediment collected on the

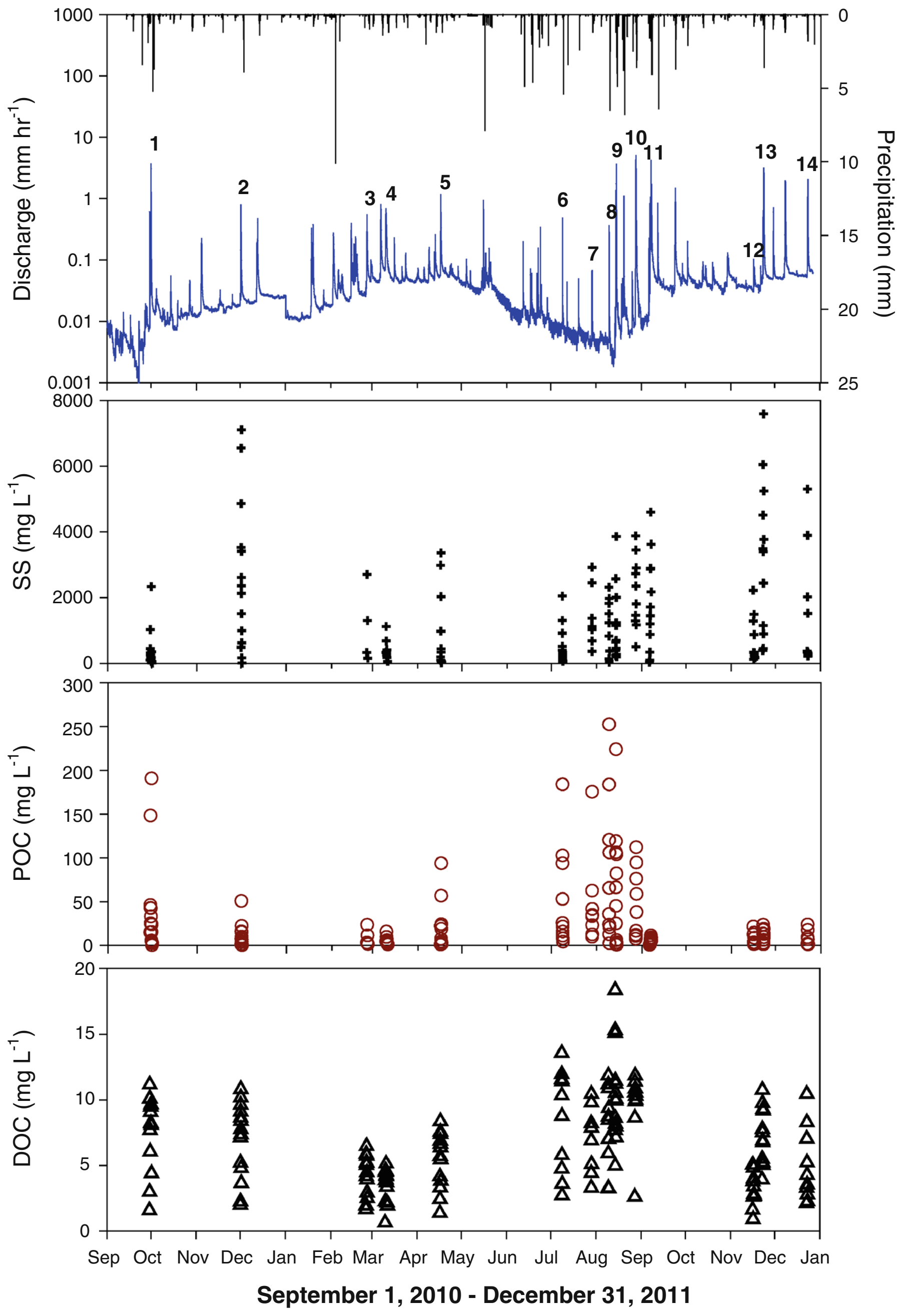


Fig. 2 Time-series plot for the study period (September 2010–December 2011) for precipitation (mm) and discharge (mm h^{-1}); SS (SS, mg L^{-1}); POC and DOC concentrations (mg L^{-1}) for the sampled events. The numbers for the 14 sampled storm events are indicated in the figure

filters was analyzed at the University of Delaware (UD) soil testing laboratory. Total organic carbon (TOC) in the samples was determined using the Elemental TC and TN analyzer—Elementar VarioMax following Dumas method and reported as % C content of the sediments. The % C content (hereafter % POC) was multiplied with the concentration of SS (mg L^{-1}) to determine the concentration of particulate organic carbon (POC) in runoff (mg L^{-1}). Where sufficient sediment was available (e.g., the large events) the sediment was scraped off the filter and combusted. For samples where the sediment amount was small and could not be scraped, the filter along with the sediment was combusted. The % C content for these samples was corrected by subtracting the mean of % C content of blank filters. This procedure was replicated to check for consistent results. Major cations and trace elements (Mn, Al, Cu, Zn) in the sediment were determined by microwave digestion of sediment samples followed by analysis through inductively coupled plasma mass spectrometry (ICP-MS).

Storm event attributes and catchment hydrologic conditions

The start of a storm event was defined with the rise in streamflow discharge following precipitation. The end of the event was defined when the streamflow discharge returned to within 10 % of the pre-event values. Catchment hydrologic conditions during and prior to the storm events were characterized using a number of metrics (Table 1): total amount of precipitation for the storm event (mm), maximum 5-min rainfall intensity (mm), total specific discharge for the event (mm per unit catchment area), peak specific discharge (mm h^{-1}), and ratio of total specific discharge to total precipitation for the event (runoff ratio). Antecedent soil moisture conditions prior to the storm events were characterized with: (a) 7-day sum of precipitation (API7, mm); (b) 24-h average of stream runoff (AR24, mm); and (c) 7-day average of groundwater depths (m, groundwater index) for wells LW2 and LW5.

Flow-weighted means and correlation analyses

The flow-weighted mean concentration (Cm) for SS, POC and DOC for individual storm events was calculated using the formula :

$$Cm = \sum_{i=1}^{i=n} Ci \cdot Qi \Big/ \sum_{i=1}^{i=n} Qi$$

where Ci was the measured concentration of SS, POC or DOC and Qi was the corresponding discharge value at time i during the storm event. To investigate how SS, POC and DOC concentrations varied with storm event attributes and catchment antecedent moisture conditions (Table 1), a Pearson correlation analysis was performed between flow-weighted mean event concentrations of SS, POC and DOC and the hydrologic metrics.

End-member mixing model analysis (EMMA) and storm-event DOC patterns

EMMA for hydrologic flow paths and runoff sources has already been implemented for the study catchment (Inamdar et al. 2011, 2013). The potential runoff sources were—rainfall, throughfall, litter leachate, tension soil water, wetland soil water, shallow groundwater, riparian groundwater and deep groundwater (sampling locations in Fig. 1 here and Fig. 5 in Inamdar et al. 2013). EMMA was performed following the procedures of Hooper (2003) and Inamdar (2011). Selected tracers included Na^{+}, Ca^{2+}, Al, Si, and DOC. EMMA space was defined by streamflow chemistry and individual watershed runoff sources (mean concentrations) were projected in this space to determine which runoff end-member influenced runoff chemistry.

As described previously in Inamdar et al. (2013), surficial and groundwater end-members were clearly differentiated in EMMA space with the surficial sources (rainfall, throughfall, and litter leachate) on the right-hand side of the mixing space and the soil water and groundwater sources being clustered together on the left-hand side (Fig. 5 in Inamdar et al. 2013). While there were differences among individual events, overall, all storm events displayed a consistent temporal pattern in runoff source or end-member sequencing (Fig. 8 in Inamdar et al. 2013). Stream water was composed of groundwater sources (e.g., seeps) at the start of event; contributions from throughfall and litter leachate composed the rising limb of the discharge hydrograph, and soil water and shallow groundwater sources contributed to the recession limb of the hydrograph. Using the procedures of Inamdar et al. (2013) temporal patterns for

Table 1 Hydrologic attributes of the 14 sampled storm events during for the study period September 2010 through December 2011

Event no.	Date	Season	Duration (h)	Precipitation			Streamflow discharge				7-day GW depth[a]	
				Amount (mm)	Intensity (mm)	API7 (mm)	Amount (mm)	Peak (mm h^{-1})	RR	AR24 (mm)	LW2 (m)	LW5 (m)
High POC concentration events[a]												
1	9/30/2010[b]	Su	61:15	151	5.2	20.3	13.5	3.7	0.09	0.2	0.3	0.8
8	8/9/2011	Su	14:30	21	6.5	7.2	0.5	0.4	0.02	0.6	0.2	0.7
9	8/14/2011	Su	30:45	104	4.9	31.3	9.3	3.6	0.09	0.1	0.2	0.7
10	8/27/2011[c]	Su	59:00	155	3.6	20.5	32.7	5.0	0.21	22.3	0.2	0.6
Medium POC concentration events[a]												
5	4/16/2011	Sp	30:00	37.7	1.2	23.7	7.1	1.2	0.19	1.3	0.1	0.4
6	7/8/2011	Su	19:45	23.4	5.4	3.7	0.8	0.5	0.03	0.2	0.1	0.7
7	7/28/2011	Su	7:00	11.0	1.2	0.7	0.1	0.1	0.01	0.1	0.2	0.7
Low POC concentration events[a]												
2	12/1/2010	W	56:30	34.7	3.9	4.8	3.8	0.8	0.11	0.4	0.3	0.5
3	2/25/2011	W	62:16	21.2	1.0	10.1	6.3	0.5	0.30	0.7	0.1	N/A
4	3/10/2011	Sp	48:30	45.9	1.2	45.4	11.1	0.7	0.24	1.7	0.1	N/A
11	9/6/2011[d]	Su	44:00	102	4.1	0.9	16.5	4.2	0.16	0.3	0.2	0.4
12	11/16/2011	F	40:15	17.1	0.8	2.6	2.0	0.1	0.12	1.6	0.0	0.5
13	11/22/2011	F	84:15	52.8	3.6	26.1	16.1	3.2	0.31	1.1	0.0	0.5
14	12/23/2011	W	48:00	35.0	1.8	1.6	9.5	2.0	0.27	1.3	0.0	0.4

Seasons: *Su* summer (June–September), *F* autumn (October–November), *W* winter (December–February), *Sp* spring (March–May)

[a] The storm events have been grouped based on their flow-weighted mean POC concentration (POCm) (High—POCm >60 mg L^{-1}; Medium—POCm 10–60 mg L^{-1}; Low—POCm <10 mg L^{-1}).; API7 is the sum of precipitation for 7 days preceding the event; AR24 is average antecedent stream discharge for 24 h; RR is the runoff ratio (ratio of discharge amount and total precipitation for event); 7-day GW depth is the average of groundwater depth for 7 days preceding the event at wells LW2 and LW5

[b] Storm associated with remnants of hurricane Nicole

[c] Storm associated with remnants of hurricane Irene

[d] Storm associated with remnants of Tropical depression Lee

selected storms used in this study along with the runoff end-members are reported in EMMA space in Fig. 3.

The within-event temporal patterns for DOC were described in Inamdar et al. (2011). In general, storm-event DOC concentrations increased with the increase in discharge, peaked at or after the discharge peak, and receded slowly on the hydrograph recession limb. Primary sources of DOC in the catchment were identified as throughfall, litter leachate, and surficial soil water and were assumed to occur in that order.

Sediment sources and mixing model

Soil and sediment samples to characterize the potential sources of runoff sediment were sampled from multiple locations spatially distributed within the 12 ha watershed during July 2010 (Dhillon 2012). Eleven different locations were sampled which accounted for four potential sediment sources—riparian wetlands (eight samples from two sites), upland soils (sixteen samples from four upland sites at varying elevations and distance from stream), stream bed (six samples from three sites in the stream bed) and stream bank (eight samples from two sites). At each sampling location, samples were collected from the A and B soil horizons and each sample had one replicate. Prior to the analysis, the samples were homogenized using pestle and mortar and sieved through a 2 mm sieve. Similar to SS, these soil samples were analyzed for the full suite of major cations and trace elements.

Using procedures similar to those for EMMA for hydrologic flow paths (Inamdar 2011), an EMMA analyses was also performed to identify the sources or end-members for runoff sediment (or POC). Runoff sediment chemistry replaced stream water chemistry and watershed runoff sources were replaced with sediment and soil chemistry sampled at various locations in the catchment. Sediment tracers selected included—Al, Cu, Zn, Mn, Ca^{2+}, Mg^{2+}, Fe, and K^{+} based on linear mixing trends in bivariate plot analysis (Inamdar 2011). The sediment sources were then projected into the EMMA mixing space defined by the runoff sediment chemistry. Sources that enclose the runoff sediment indicate potential end members. The intent here was primarily to identify the potential sediment sources and not to determine the specific amounts or proportions of sediment contributed by these individual sources.

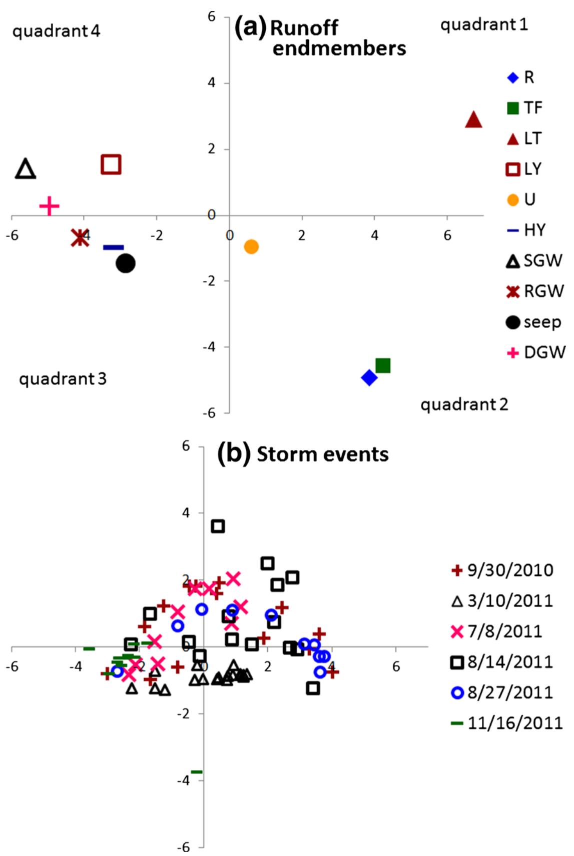


Fig. 3 Runoff end-members (**a**) and selected storm events (**b**) in EMMA space to determine the influence of runoff end-members on stream water chemistry. Runoff end-members include: *R* rainfall, *TF* throughfall, *LT* litter leachate, *LY* zero-tension soil water, *U* tension soil water, *HY* hyporheic water, *SGW* shallow groundwater, *RGW* riparian groundwater, *seep* hillslope seeps, and *DGW* deep groundwater

Results

Hydrologic attributes of sampled storm events

A total of 14 storm events were sampled over the study period of 16 months from September, 2010 to December, 2011 (Fig. 2; Table 1). The total precipitation for the 16-month study period was 1,842 mm. Of this, 1,462 mm of precipitation was observed in year 2011 (January through December). The annual precipitation for 2011 was higher than the previous years—2008 (1,052 mm), 2009 (1,238 mm) and 2010 (972 mm). Total stream discharge measured at the 12 ha outlet (ST3) for the 16 month study period was 497 mm, resulting in an annual runoff ratio of 0.27.

Of the 14 storm events, seven events were sampled in summer (June–September), two in autumn (October–November), three in winter (December–February) and two in spring (March–May). The maximum 5-min rainfall intensity for the events varied from 0.8 to 6.5 mm (Table 1). Peak streamflow discharge during storm events varied from 0.07 to 5.0 mm h^{-1} (Fig. 2). The largest amount of precipitation (155 mm) was recorded for the event of August 27, 2011 (event 10, Table 1), which was associated with remnants of hurricane Irene (NOAA, 2012) and had a precipitation return period of 25 years (Appendix C, Ward and Trimble 2004). This event also produced the highest peak discharge (5.0 mm h^{-1}) and highest total amount of streamflow discharge (32.7 mm) for the study period. Similarly, the event of September 30, 2010 (event 1) was associated with remnants of Hurricane Nicole (NOAA, 2012) and yielded a rainfall amount of 151 mm and had a precipitation return period of 25 years (Appendix C, Ward and Trimble 2004). In contrast, storm events of July 28, 2011 (event 7) and November 16, 2011 (event 12) produced the lowest discharge peaks. Event 7 (July 28, 2011) produced the lowest amount of streamflow runoff during the study period. Among all events, the summer events of September 30, 2010, August 9, 2011, August 14, 2011 and August 27, 2011 had the highest rainfall intensity (Table 1) while the events of February 25, 2011 and November 16, 2011 had the lowest rainfall intensity. The events of February 25, 2011, November 22, 2011 and December 23, 2011 had the highest runoff ratios while the summer events of July 8, 2011, July 28, 2011 and August 9, 2011 had the lowest runoff ratios.

Low values of API7 and AR24 and high values of 7-day GW depth (Table 1) indicated drier catchment conditions. Based on a combination of these indices, the catchment was at its driest preceding the event of September 30, 2010. Catchment conditions were also very dry during the summer events of July 28, 2011, August 9, 2011 and August 14, 2011. The catchment was at its wettest prior to the event of December 23, 2011 when water levels for well LW2 were at the soil surface (zero values in Table 1). Similarly, the catchment was also very wet during the events of November 16, 2011 and November 22, 2011. In general, soil moisture conditions were driest during later summer (August–September).

The events with the largest precipitation inputs (e.g., September 30, 2010 and August 14 and 27, 2011; Table 1) produced the strongest shifts of stream water composition towards surficial end-members (Fig. 3). In contrast, the smallest event in terms of precipitation amount and intensity (e.g., November 16, 2011, Table 1), barely produced any shift away from the region of groundwater end-members, suggesting minimal contributions from surficial runoff sources. Other events with intermediate precipitation amounts (March 10 and July 8, 2011) produced mixing loops and shifts that were between the two extremes described above.

Storm event concentrations of SS, POC and DOC

SS, POC and DOC concentrations increased rapidly with the increase in stream flow (Fig. 2), however, the increase in POC concentrations outpaced the increase in DOC concentration such that the peak concentration of POC was 2–20 times higher than that of DOC for individual storm events. DOC concentrations ranged from 0.7 to 18.3 mg L^{-1} while POC and SS concentrations ranged from 0.05–252 to 10–7,589 mg L^{-1}, respectively. SS concentrations were especially influenced by streamflow discharge. The top three events with the highest streamflow discharges (August 27, September 6 and November 22, 2011; Table 1) also had the highest flow-weighted mean SS concentrations (Table 2), but not in the order of the discharge amounts. In addition, the late fall and winter events of November 22, 2011 and December 1, 2010 which occurred under wet antecedent conditions and had high stream discharge produced the highest peak SS concentrations of 7,589 and 7,102 mg L^{-1}, respectively. The influence of runoff amount on SS is further supported by the Pearson correlation values (Table 3). Correlation was highest for total and peak streamflow discharge with the relationship being significant ($p < 0.05$) for peak discharge.

In contrast to SS, storm event POC concentrations revealed a slightly different trend. Flow-weighted mean POC concentrations were highest for the high-flow summer events (e.g., September 30, and August 9, 14, and 27, Fig. 2 and Table 2) but fairly low for the other high-flow events that occurred in fall and winter (e.g., November 22 and December 23, 2011). POC concentrations were also very low for the high-flow summer event of September 9, 2011. Mean POC concentration was highest for the summer event of August 9, 2011 despite having relatively low precipitation and discharge amounts. However, this event did have the highest precipitation intensity of 6.5 mm h^{-1} among all the recorded events (Table 1). The influence of precipitation intensity is supported by the significant correlation between POC concentrations and precipitation intensity ($p < 0.01$; Table 3).

It should be noted that POC concentrations were determined by multiplying the % POC content of sediment with the SS amounts; and % POC contents for the summer events were highest whereas the winter and late fall events had the lowest % POC (Table 2). This seasonal trend is obvious when SS and POC are plotted against each other (Fig. 4). This plot displays a triangular region extending from the origin with summer events falling along the lower edge with high POC concentration per unit SS (mild slope of the SS–POC relationship) and the winter and late fall events forming the upper edge with low POC content per unit SS mass (steep slope). Events from spring (e.g., April 16) occupied a region between these two extremes (Fig. 4). Very large summer events with some depletion of POC (e.g., August 27, 2011) were shifted to the left and in a region in the middle whereas summer events where considerable depletion of POC occurred (e.g., September 9, 2011, further details below) were completed shifted towards the edge containing the winter events.

A comparison of POC versus DOC for the storm events also revealed important similarities as well as differences. The top five events with flow-weighted mean POC concentrations (September 30, August 9, 14, 27 and July 8) also had the highest DOC values. However, DOC concentrations were also elevated for

Table 2 Flow-weighted mean and peak concentrations (mg L^{-1}) of SS, POC and DOC for sampled storm events in runoff at the outlet of the 12 ha catchment

Events		SSC (mg L^{-1})		POC (mg L^{-1})		DOC (mg L^{-1})		% POC
No.	Date	FW mean	Maximum	FW mean	Maximum	FW mean	Maximum	FW mean
High POC concentration events[a]								
1	9/30/2010[b] (Su)	801	2,330	67.8	190	8.2	11.2	7.8
8	8/9/2011 (Su)	1,366	2,310	112	252	9.0	11.9	8.9
9	8/14/2011 (Su)	1,905	3,854	70.3	223	10.0	18.3	5.4
10	8/27/2011[c] (Su)	2,169	3,874	66.4	112	10.0	11.9	3.1
Medium POC concentration events								
5	4/16/2011 (Sp)	560	3,356	14.5	93.7	5.4	8.4	4.6
6	7/8/2011 (Su)	653	2,042	58.4	183	9.5	13.6	8.7
7	7/28/2011 (Su)	1,458	2,917	33.3	175	7.3	10.4	2.9
Low POC concentration events								
2	12/1/2010 (W)	1,824	7,102	8.3	50.5	6.1	10.8	0.5
3	2/25/2011 (W)	1,038	2,702	9.0	23.4	3.9	6.5	0.9
4	3/10/2011 (Sp)	421	1,113	5.9	15.5	3.3	5.1	1.4
11	9/6/2011[d] (Su)	2,883	4,599	7.1	11.3	N/A[e]	N/A	0.3
12	11/16/2011 (F)	546	2,266	5.3	21.8	3.3	5.1	1.0
13	11/22/2011 (F)	3,032	7,589	8.2	23.4	7.5	10.8	0.3
14	12/23/2011 (W)	2,007	5,298	9.0	23.7	5.3	10.5	0.5

[a] The storm events have been grouped based on their flow-weighted mean POC concentration (POCm) (High—POCm is more than 60 mg L^{-1}; Medium—POCm is between 10 and 60 mg L^{-1}; Low—POCm is less than 10 mg L^{-1}). The storm events within the groups have been listed in the chronological order

[b] Storm associated with remnants of hurricane Nicole

[c] Storm associated with remnants of hurricane Irene

[d] Storm associated with remnants of Tropical depression Lee

[e] DOC was not measured

the high-flow winter and late fall events of December 1, 2010, and November 22 and December 23, 2011, whereas POC values for these events were among the lowest (Table 2). Not surprisingly then, correlation analyses (Table 3) indicated that while both POC and DOC were significantly correlated with precipitation intensity ($p < 0.01$), only DOC was strongly correlated with peak streamflow discharge ($p < 0.05$). When pooled by seasons, *t*-tests indicated that POC and DOC concentrations for summer were significantly greater ($p < 0.05$) than the other seasons but there was no significant difference among the spring, fall, and winter events.

An important distinction between POC and DOC responses occurred for a sequence of successive storm events that occurred within a period of 1 month (August 9, 2011–September 8, 2011; Events 8–10 in Fig. 5). Event 8 (August 9, 2011) had a high flow-weighted mean POC concentration of 112 mg L^{-1} and peak POC concentration of 252 mg L^{-1} (Table 2) despite a low discharge value (Table 1). Following this high, flow-weighted POC concentrations displayed a gradual decline for events 9–11 (Fig. 5) despite elevated streamflow discharge for events 10 and 11 (Table 2). These storm events also revealed a gradual impoverishment in the % POC. Event 8 had a % POC value of 8.9 % which decreased to 5.4 % in event 9, 3.1 % in event 10 and 0.26 % in event 11. Although DOC was not measured for Event 11, DOC concentrations clearly did not follow the decline indicated by POC values. It should be noted though that despite the declining trend, the magnitude of flow-weighted POC concentrations was still much greater than DOC.

Table 3 Pearson correlation matrix among the hydrologic variables and flow-weighted mean POC (POC_m) and DOC (DOC_m) concentrations

Parameter	SS_m	POC_m	DOC_m
Q_t	0.50	0.01	0.28
Q_{max}	0.63*	0.18	0.56*
AR24	0.21	0.22	0.32
P_t	0.32	0.33	0.52
PI_{max}	0.28	0.76**	0.82**
AP7d	−0.14	0.04	−0.01
AP24	0.30	0.27	0.47
RR	0.28	−0.61*	−0.48
LW2	0.02	0.59*	0.55*
LW5	−0.07	0.82**	0.67*

Correlation is significant at $p < 0.05$ level for * and $p < 0.01$ for **; Q_t—total discharge; Q_{max}—peak discharge; AR24—average antecedent stream discharge for 24 h; P_t—total precipitation; PI_{max}—peak 5-min precipitation intensity; AP7d—7-day antecedent precipitation preceding the event; AP24—24-h antecedent precipitation preceding the event; RR—runoff ratio—ratio of discharge amount and total precipitation for event; LW2 and LW5—average of groundwater depth for 7 days preceding the event at wells LW2 and LW5 respectively

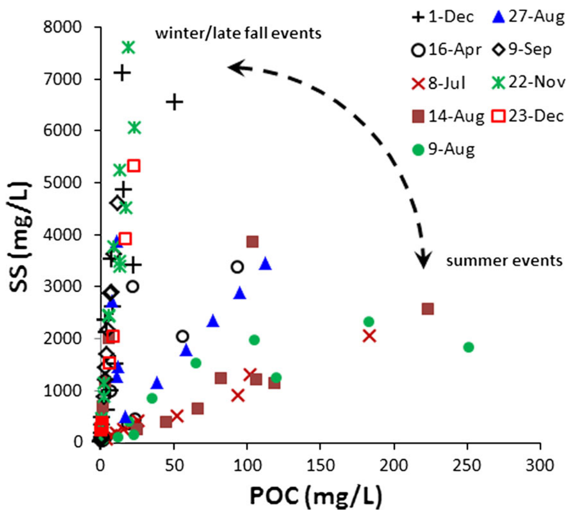


Fig. 4 Relationship between SS and particulate organic carbon (POC) concentration for selected events highlighting the change in slopes of the relationship between summer and winter events

Within-event temporal patterns of SS, POC and DOC

Within-event temporal patterns of SS, POC, and DOC for four events of different magnitude—September 30, 2010, July 8, 2011, August 14, 2011, and August 27, 2011 are presented in Figs. 6, 7, 8 and 9. Except for the Hurricane Irene event of August 27, SS and POC followed similar temporal patterns with a peak in concentrations on the rising limb of the streamflow hydrograph and decline in concentrations thereafter. SS concentrations for the event of August 27 displayed two separate peaks while the POC concentrations occurred as a single delayed peak. In terms of magnitude, however, there were notable differences in the responses of SS and POC, especially for large storms consisting of two sub events (Figs. 6, 8). Concentrations of SS increased with streamflow discharge for the event of September 30 and the POC peak was higher for the second subevent which also had higher discharge. This pattern was also repeated for the event of April 16, 2011 (figure not included). Conversely, for the event of August 14, 2011 (and all other events with two sub-events), peak POC concentrations for the second subevent were lower than the first subevent even though the discharge values were higher for the second subevent (Fig. 8). The low POC concentrations for the second sub-events were due to the lower % POC content of the soil sediments. For e.g., for the event of August 14, 2011, the % POC dropped from 8.7 % during the first discharge peak to 2.7 % during the second discharge peak. This indicates that POC values may not always closely follow the trend in SS concentrations.

DOC concentrations also generally increased with streamflow discharge, but there was greater temporal variability in DOC patterns than those observed for POC (Figs. 6, 7, 8, 9). Typically, the peak in DOC concentrations followed the POC peaks. Unlike POC, DOC concentrations however, decreased much more gradually through hydrograph recession and did not drop back to the pre-event values for most of the events. While most storms revealed an increase in DOC with discharge and a peak in concentration in the vicinity of the discharge peak (e.g., Fig. 7 and Inamdar et al. 2011), the largest storm events—September 30, 2010 (event 1), August 14, 2011 (event 9), and August 27, 2011 (event 10)—revealed a slight decrease in DOC concentrations at or near the peak discharge (Figs. 6, 8, 9). POC did not follow this pattern. All three of these events had rainfall amounts in excess of 100 mm, with events 1 and 10 exceeding 150 mm of rainfall. For events 1 and 9 (Figs. 6, 8), DOC concentrations did indeed peak with discharge for

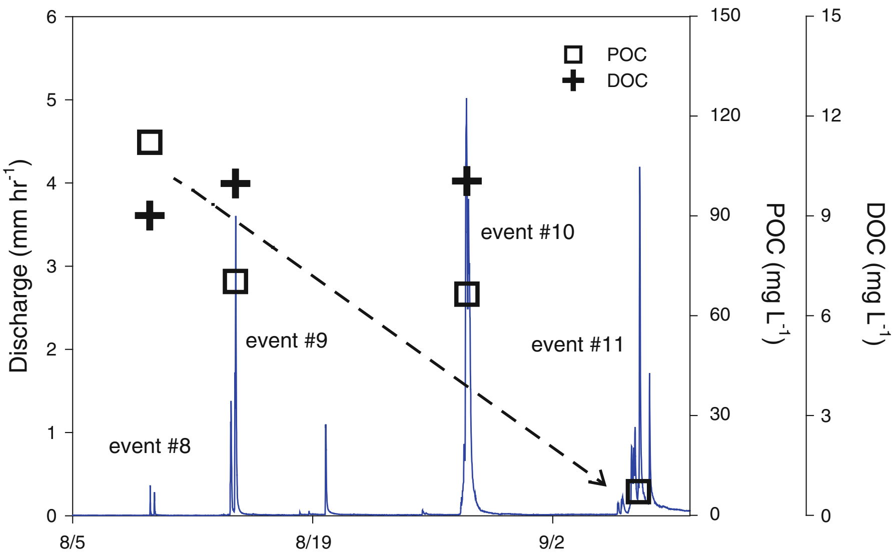


Fig. 5 Streamflow discharge and flow-weighted mean POC and DOC concentrations for four successive storm events in summer, 2011 at the outlet of the 12 ha catchment. Storm events included—August 9 (event 8), August 14 (event 9), August 27 (event 10), and September 6 (event 11). DOC concentrations for event 11 were not available. Flow-weighted POC concentrations decreased systematically for the four sequential events but the same response was not reproduced for DOC

the first subevent, but for the second subevent which had a larger discharge peak, a slight dilution of DOC is observed. Similarly, for the event of August 27, 2011, DOC concentrations increased quickly on the rising limb of the hydrograph (Fig. 9) but then dipped slightly when discharge was at its maximum. The same pattern however did not extend to within-event export of POC.

The difference in responses for POC and DOC with streamflow discharge is further illustrated by the concentration-discharge plots of POC and DOC for all the 14 events (Fig. 10). These CQ plots for DOC also provide additional insights into the influence of event magnitude and seasonality on DOC. The increase in DOC concentrations with discharge for the summer events (e.g., July 8 and 28, August 14 and 27 and September 30) was much more rapid (steeper slope) than the non-summer events (e.g., April 16, March 10, December 23) which displayed a more gradual increase (Fig. 10 inset). Furthermore, the CQ pattern for the summer events also displayed a leveling-off in DOC concentrations with increasing discharge and a subsequent decline for very high discharge values (Fig. 10). This response was not apparent for the non-summer events. From Fig. 10 (inset), it appears that, in general, for our study site, DOC concentrations increased sharply until about a discharge of 0.2 mm h^{-1}; then leveled off until a discharge rate of 1.0 mm h^{-1}, followed by a decline in concentrations thereafter. However, no such behavior is replicated by POC.

Sources for runoff sediment from the mixing model

EMMA plot including potential sediment sources and runoff sediment for storm events are included in Fig. 11. Nearly all of the sediment sources were located in quadrant 3 of the EMMA plot (Fig. 11a). The wetland and stream bed sources were closer to the origins of the axis while the upland and stream bank sources were located further away from the origin and in the lower portion of the quadrant 3. When compared against runoff sediment chemistry (Fig. 11b) it is clear that the sampled sources did not enclose the runoff sediment chemistry and that there were likely additional sources of sediments which were not sampled. The large shifts of runoff events towards quadrant 4 suggest that there was another unknown source in this quadrant which was not identified in this study. Despite our inability to capture all sediment sources the storm sediment patterns reveal some interesting trends. High rainfall intensity (>3.5 mm h^{-1}, red symbols in Fig. 11b) summer events such as September 30, 2010 (event 1), July 28, 2011 (event 7), August 9, 2011 (event 8), August 14, 2011 (event 9) and August 27, 2011 (event 10) were spread out in the third and fourth quadrants of the EMMA space. On the other hand, most of the low intensity (<3.5 mm h^{-1}; green symbols in Fig. 11b) winter and spring storm events such as events of December 1, 2010 (event 2), February 25, 2011 (event 3) and March 10, 2011 (event 4) were clustered on the right-hand side of the EMMA space in the vicinity of the stream bed end-member.

Fig. 6 Precipitation (mm), streamflow discharge (mm h^{-1}), and SS, POC and DOC concentrations (mg L^{-1}) for the event of September 30, 2010 (remnants of hurricane Nicole) at the outlet of the 12 ha catchment. A slight dilution in DOC concentrations can be seen with the second discharge peak but no such response was seen for POC

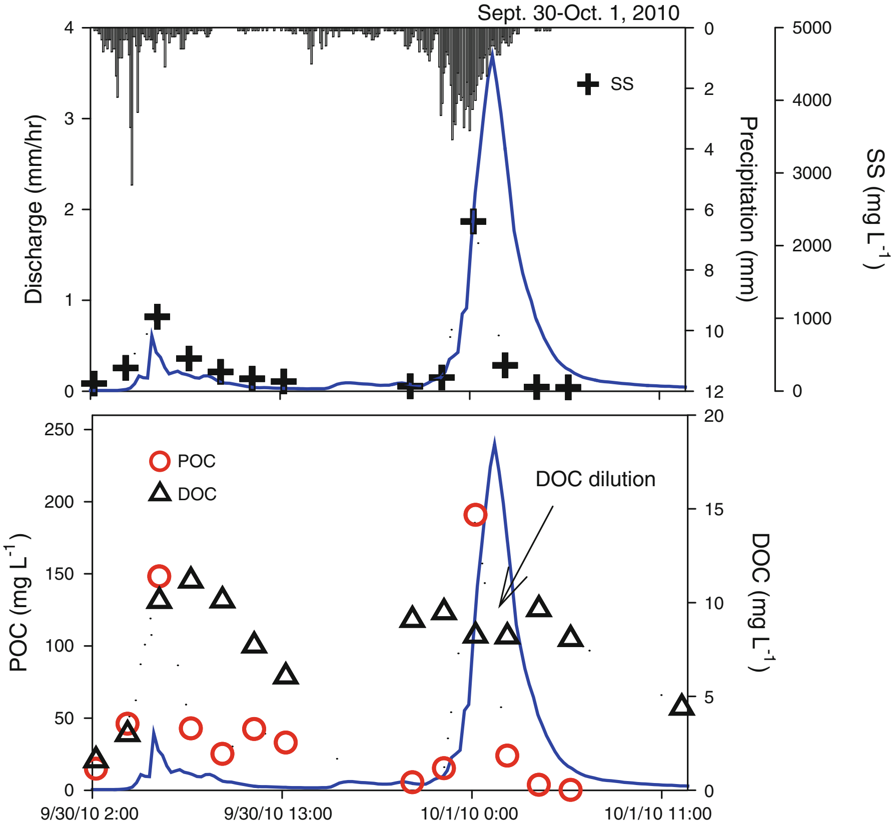
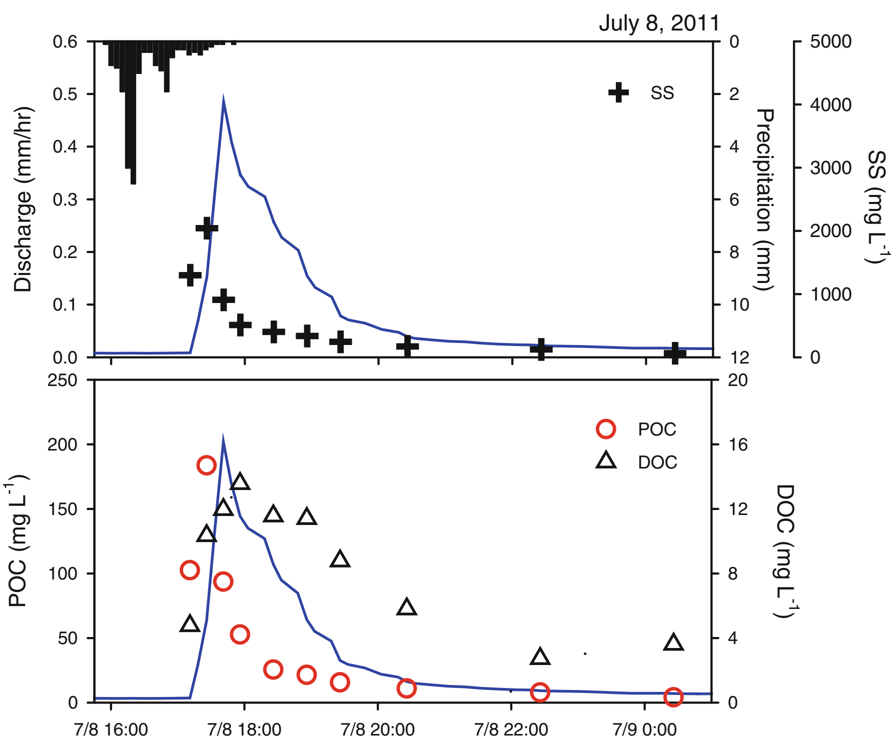


Fig. 7 Precipitation (mm), streamflow discharge (mm h^{-1}), and SS, POC and DOC concentrations (mg L^{-1}) for the event of July 8, 2011 at the outlet of the 12 ha catchment



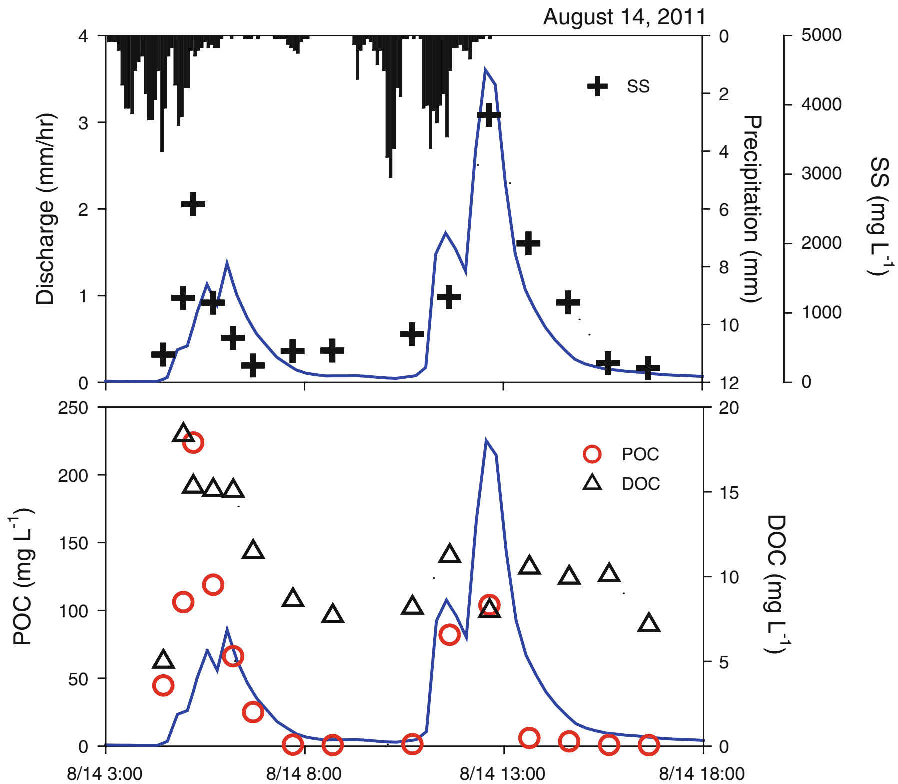


Fig. 8 Precipitation (mm), streamflow discharge (mm h^{-1}), and SS, POC and DOC concentrations (mg L^{-1}) for the event of August 14, 2011 at the outlet of the 12 ha catchment

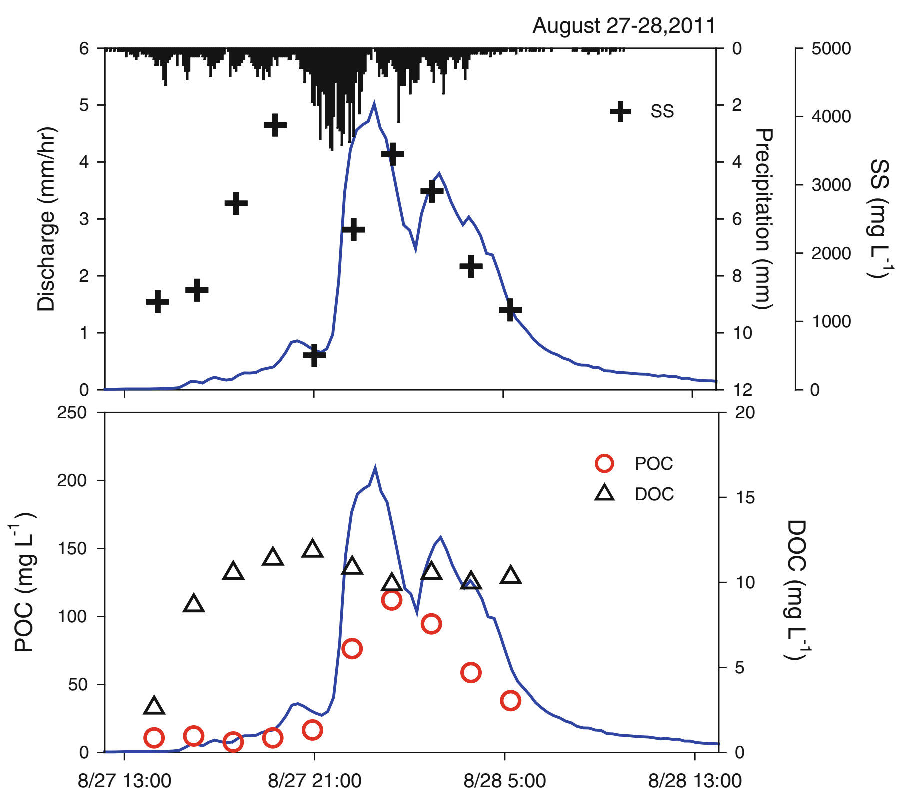


Fig. 9 Precipitation (mm), streamflow discharge (mm h^{-1}), and SS, POC and DOC concentrations (mg L^{-1}) for the event of August 27, 2011 (remnants of hurricane Irene) at the outlet of the 12 ha catchment

Discussion

Temporal patterns, flowpaths, and sources of POC and DOC

We performed EMMA analyses for both—the dissolved as well as the sediment phases of storm runoff. The dissolved phase EMMA characterized the hydrologic flow paths whereas the sediment EMMA enabled us to identify the potential sources of sediment (e.g., stream bed, stream bank erosion, and uplands). Our previous work on DOC for this study site (Inamdar et al. 2011) has already shown that storm event DOC peaks, with the exception of few events, typically occurred at or after the discharge peaks producing an anticlockwise concentration-discharge (CQ; Evans and Davies 1998) hysteresis loop. Using EMMA analyses, we attributed this DOC leaching pattern

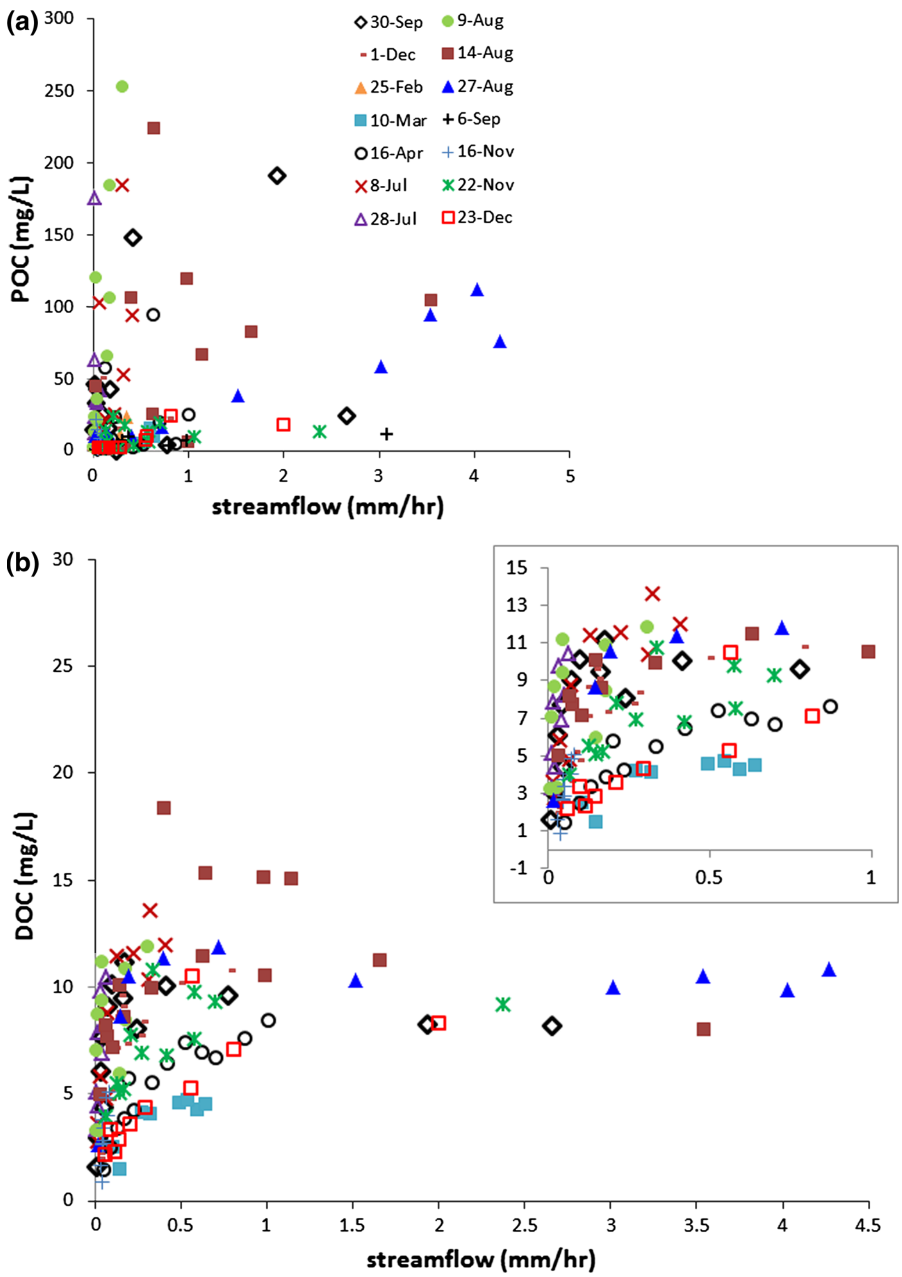


Fig. 10 POC and DOC concentrations versus streamflow discharge (CQ plots) for the 14 storm events. Summer storms compared to winter and spring events (note *inset*) displayed a sharper increase in DOC with discharge and then a decline for the largest flows. No such response was seen for POC

(e.g., Inamdar et al. 2011) to throughfall and litter leachate contributions followed by soil water contributions from surficial soils. Using the same model and rationale, the earlier expression of POC on the rising limb of the discharge hydrograph (and therefore clockwise CQ hysteresis) suggests that POC moved with saturation overland flow associated with event water (precipitation, throughfall and litter leachate end-members). The match in timing and magnitude of elevated event POC concentrations and the large shifts in EMMA space towards surficial runoff sources (Fig. 3) further supports this argument.

Unlike our EMMA for hydrologic flow paths, the EMMA for sediment sources did not yield definitive results since some of the key end-members could not be identified (Fig. 11). We did, however, find that the high-intensity summer events displayed greater diversity of sediment sources compared to the low-intensity storm events (Fig. 11). The low-intensity events were clustered between the near-stream sources such as wetlands, stream beds and another unidentified source. While the high-intensity summer events also showed a major contribution from the near-stream sources, they were more spread out on the EMMA plots and indicated an increased contribution of distal upland sources as well as the stream bank. This is possibly due to the increased erosive ability of runoff associated with high precipitation-intensity events. This is in agreement with previous studies such as Walling and He (1999) who have observed that the contribution of sediment from stream banks is substantially greater during high versus low flows. Using $\delta^{13}C$ and $\delta^{15}N$ isotopes of SS, Jung et al. (2012) found that POC derived from eroded mineral soil fractions increased with increasing rainfall intensity and peak discharges. In our case, however, a more comprehensive sampling may be required to capture the complete spectrum of sediment sources as well as their evolution during the storm events.

Most previous studies have typically employed CQ hysteresis loop analyses to identify the origins of runoff sediment. Jeong et al. (2012) reported clockwise hysteresis patterns for both POC and DOC and attributed shallow hydrologic flowpaths and steep slope gradients for the quick mobilization of C in stream runoff. Coynel et al. (2005) and Oeurng et al. (2011) found a combination of both clockwise as well as anticlockwise loops for POC; with anticlockwise loops being associated with larger storm events or

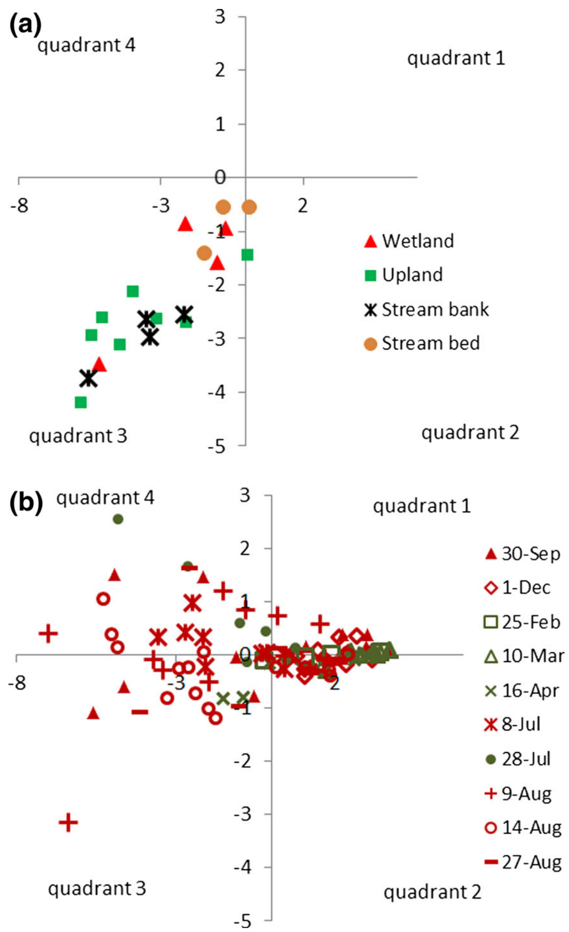


Fig. 11 **a** Distribution of sediment end-members in the mixing model space; **b** comparison of selected storm events in mixing space to evaluate the relative changes in sediment chemistry. In **b** events with precipitation intensity >3.5 mm h^{-1} are indicated in *red* symbols whereas events with low intensity (<3.5 mm h^{-1}) are indicated in *green* symbols. Events with precipitation intensity >3.5 mm h^{-1} display a larger spread in mixing space indicating greater changes in sediment chemistry and more diverse sediment end-members. (Color figure online)

events occurring under wet catchment conditions. Oeurng et al. (2011) attributed the clockwise loops to POC originating from near-stream sources whereas the anticlockwise loops were associated with POC from more distal sources as erosive forces associated with larger storm events mobilized POC from hillslopes and upper reaches of the catchments. Similarly, Coynel et al. (2005) attributed the clockwise loops to POC-rich riparian sources while the anticlockwise patterns were credited to distal, less-erodible, and POC-poor sources. Extending the rationale of these studies, we hypothesize that the clockwise POC loops for most storm events in our study indicate the dominance of near and within-stream POC sources in the catchment (as alluded by the sediment EMMA). Conversely, the anticlockwise POC loops for the large runoff events of August 27, 2011 (event 10) and September 6, 2011 (event 11) were likely due to the exhaustion of easily-erodible near and within stream sediment sources and the enhanced contribution of more compact, erosion-resistant soils and/or distal upland sources of sediment (as also suggested by EMMA). Both of these events had high precipitation amounts (Table 1) and were the last of four successive storm events within a month (events 8–11).

Storm event patterns of SS and POC

SS concentrations are often used as a surrogate for POC (Alvarez-Cobelas et al. 2012; Hope et al. 1994), and while our data reveal strong correlations between SS and POC there were key changes in the slopes of these relationships with events (Fig. 4). The four back-to-back events of August (Fig. 5) clearly showed that the % POC content of SS could change with sequential events and that this could be because of POC depletion of sediment sources due to hydrologic flushing; and/or mobilization of distal or upland sources of sediment with increasing hydrologic intensity which may be lower in their % POC contents. This phenomenon of exhaustion of POC for successive closely-spaced events has also been reported in previous studies (Alexandrov et al. 2003; Coynel et al. 2005; Oeurng et al. 2011; Rovira and Batalla 2006; Veyssy et al. 1999). Veyssy et al. (1999) explained this phenomenon through a conceptual model which proposes that the carbon-rich litter fraction is depleted in the earlier storm events resulting in the export of mineral-rich SS in the later events.

The slopes of the SS–POC relationships in Fig. 4 and the mean % POC content for storm events in Table 2, however, also suggest a strong seasonal pattern driven by hydrology and biotic processes. The summer events in general were much higher in % POC content than the winter and late fall events. Two potential explanations (among others) could be the hydrologic nature of the summer events and/or biogeochemical conditions that may enhance the production/supply of POC. Summer storms are generally associated with convective weather systems that generate high-intensity, short-duration precipitation with a greater potential for forest floor disturbance and soil/sediment erosion as opposed to frontal systems in autumn and spring that generate long-duration, low-intensity precipitation. Our POC data already indicates a strong correlation with precipitation intensity and these events likely facilitated greater surface runoff and erosion and thus delivered larger amounts of carbon-rich sediment to the stream. Jeong et al. (2012) have also reported that rainfall intensity was a critical factor in influencing the storm event concentrations of POC. The summer events were also likely influenced by the repeated cycles of drying and rewetting of the soil surface and forest floor. Repeated cycles of drying and wetting of soils have been shown to enhance mineralization rates and therefore the production and release of carbon from the soil (Borken and Matzner 2009; Lundquist et al. 1999). It is likely that elevated summer temperatures along with drying and wetting cycles enhanced the breakdown of organic matter on the forest floor which was then flushed out with runoff associated with high-intensity summer events. These observations clearly suggest that we need to be extremely careful while using SS–POC relationships for predictions since the form (slope) of such relationships could change with season as well as for closely spaced back-to-back storm events.

POC and DOC exports patterns for storm events of varying magnitude and timing

The sharper rise in POC versus DOC has been reported for other studies (Johnson et al. 2006; Pawson et al. 2012). Johnson et al. (2006) found that the storm-event POC concentrations were 150 times their baseflow values while the DOC concentrations increased four times. The range in POC and DOC concentrations from this study is similar to the values reported for other studies (Oeurng et al. 2011; Johnson et al. 2006; Pawson et al. 2012). Concentrations in an 1,110 km^2 agricultural catchment in France (Oeurng et al. 2011) ranged between 0.1–173.2 mg L^{-1} for POC and 1.5–7.9 mg L^{-1} for DOC, which is slightly less than our values. In contrast, our POC concentrations were considerably less than those reported for an eroding peatland catchment in UK that yielded an average of 1.5–1,220 mg L^{-1} for POC and 13.58–27.52 mg L^{-1} for DOC (Pawson et al. 2012). It should be noted that in our study, the peak and flow-weighted mean concentrations for POC (Table 2) were always greater than the corresponding DOC values across all events. This is unlike observations by Jeong et al. (2012) where POC concentrations were less than DOC for small discharge events, but exceeded the DOC values for events with higher discharge values.

Our previous observations on C mass exports (Fig. 3 in Dhillon and Inamdar 2013) indicated that the differences in POC and DOC were largest for the hurricane-associated storms (September 30, 2010 and August 27, 2011) and while POC mass export increased exponentially DOC was constrained to a linear increase. A thorough examination of within-event patterns and among-event differences in POC and DOC presented here help explain these differences. Within-event patterns of POC and DOC for the large hurricane events (Figs. 6, 9) showed that not only was the increase in DOC concentrations less than POC, but that DOC also displayed a dilution trend at peak stream flow discharges. This response for our study catchment suggests that while new POC stocks continued to be mobilized by erosive forces associated with increasing storm runoff the DOC supply could not keep up with the rate of runoff increase. Thus, for extremely large events (>100 mm precipitation) the DOC response in our catchment was supply-limited while the same phenomenon did not extend to POC. We believe this is one reason that explains the large disparity in POC and DOC mass exports observed in Fig. 3 in Dhillon and Inamdar (2013). In contrast to the within-event patterns, POC depletion was observed for consecutive storm events (Fig. 5) but the same pattern was not reproduced for DOC. The depletion of POC for the event of September 6, 2011 (last of the four sequential events), is also reflected in the mass exports in Fig. 3 of Dhillon and Inamdar (2013) with POC amounts for this event being much lower and even comparable to the DOC values. These observations suggest that POC and DOC could both become supply-limited for storm events but the time scale and thresholds at which this occurs differs for the two constituents. It appears that catchment supplies of DOC cannot keep up with very high runoff rates which occur at the time scale of hours/minutes but these supplies recover quickly between storms (days). On the other hand, POC supplies tend to run out with continuous hydrologic flushing by closely-spaced, (days) multiple, events. Future climate-change predictions (Karl et al. 2009) indicate increasing storm intensity/magnitude with longer intervening dry periods. Based on our observations, such future events will increase the disparity in DOC and POC exports from catchments with DOC dilution occurring at peak flows.

The CQ plots in Fig. 10 also provided key insights into the differential nature of POC and DOC flushing with stream discharge. DOC displayed a more consistent pattern while the same was not seen for POC. DOC was rapidly mobilized with runoff during high-intensity summer events (indicated by the steep increase), but at the same time, this intensity resulted in DOC supply being outpaced by event water input. This is seen in the leveling off and decline of DOC concentrations. A similar response was however not observed for the long duration and low intensity spring and winter storms which revealed a more gradual increase in DOC with discharge (Fig. 10). We hypothesize that such DOC responses are likely occurring for other catchments similar to ours but with a different set of thresholds and slopes for the DOC response curves. The shapes of these curves would be dictated by the DOC pools in the catchment (supply) and the runoff sources and rate of runoff increase (transport). Understanding and quantifying the slopes of the DOC curves and the discharge thresholds would significantly advance our understanding of C response to storm events.

Overall, the seasonal occurrence of storms and associated attributes (e.g., precipitation intensity) was a critical factor that influenced the disparity between POC and DOC responses. We speculate that the occurrence of high-intensity hurricane events towards the tail end of the summer further enhanced the disparity between POC and DOC responses and exports. Events of similar magnitude in winter or spring (e.g., with lower intensity) may not have produced the same level of differences between POC and DOC. We also recognize that the differences in POC and DOC responses were especially apparent at the small 12 ha scale of our headwater catchment. It is well recognized that POC concentrations are elevated for small, headwater catchments (Alvarez-Cobelas et al. 2012; Pawson et al. 2012; Townsend-Small et al. 2008) and that POC concentrations decrease with increasing catchment scale as POC and sediment settle out as they are transported through the fluvial network (Battin et al. 2008; Hope et al. 1994).

Conclusions

High-frequency sampling of particulate and dissolved organic C in stream runoff from a headwater catchment during a large, intense, storm events, a few associated with remnants of hurricanes, provided rare and critical insights into storm-event C patterns. Key conclusions that that can be derived from this study are:

- The responses of POC and DOC differed substantially within large storm events as well as for closely spaced, sequential storm events. During large storms, DOC yielded a dilution trajectory at peak discharge, while POC concentration continued to rise. Flow-weighted mean POC concentrations decreased for closely-spaced sequential storms, suggesting an exhaustion of the POC pool, whereas the same pattern was not replicated by DOC. These observations suggest that there are important distinctions in the supply and transport of these two C constituents which may be apparent at different time scales and hydrologic thresholds.
- Seasonal hydrologic and biochemical conditions of storms had important implications for both POC and DOC. The % POC content of SS for summer storm was much greater than corresponding values for winter events. This suggests caution should be used if SS is used as a surrogate for POC. Similarly, DOC concentrations increased much sharply with discharge for summer versus non-summer events, but then also leveled off and displayed a dilution pattern at high flows. Thus, seasonal hydrologic and biogeochemical conditions may have an important role in shaping POC and DOC responses.
- The differences in the temporal patterns of POC and DOC during storm events as well as the nature and direction of C–Q hysteresis loops of POC and DOC alluded to different sources and flow paths of POC and DOC within the catchments. EMMA analysis revealed that while surficial flowpaths are important for both POC and DOC, POC is quickly mobilized with surface runoff while DOC expression in runoff is delayed and occurs in concert with surface runoff and leaching of surficial soil horizons. Near-stream, C-rich sediment sources contribute to POC during small to moderate storms but distal, mineral-rich sediment sources could become more important for the larger events.
- The sharp increases and changes in POC and DOC concentrations during storm events underscore the need to perform high-frequency sampling during storm events for accurate and thorough understanding of C response patterns and thresholds. Daily, weekly, or monthly sampling regimes will be unable to identify key responses that are critical to advancing our mechanistic understanding of C.

This work underscores the importance of POC in headwater catchments and for large events and the need to develop more realistic and robust models of POC and DOC supply and transport from headwater catchments. Considering future climate-change predictions of increasing intensity of the largest storms for the northeastern USA (Bender et al. 2010) such information is particularly urgent.

Acknowledgments The authors would like to acknowledge the support of Delaware Water Resources Center (DWRC) for providing a research assistantship to Gurbir Singh Dhillon for his master's thesis research. Instrumentation and sampling in the study catchments was funded through an existing grant from the National Science Foundation (NSF, Hydrologic Sciences Program, EAR-0809205). We also thank Dr. Myron Mitchell's Biogeochemistry Laboratory at Syracuse for the analyses on water samples and Karen Gartley and the UD Soils Laboratory for the POC analyses. We would like to thank Captain Wayne Suydam and the Fair Hill NRMA staff for providing access to the study site. We also thanks Drs. Tom Sims and Yan Jin for their suggestions and insights during Gurbir's MS thesis research. We would also like to recognize the support of graduate student colleagues including Shatrughan Singh, Sudarshan Dutta, Rachael Vaicunas and Zhixuan Qin for sample collection and analyses.

References

Alexandrov Y, Laronne JB, Reid I (2003) Suspended sediment transport in flash floods of the semiarid northern Negev. IAHS Publications, Israel, pp 346–352

Alvarez-Cobelas M, Angeler D, Sánchez-Carrillo S, Almendros G (2012) A worldwide view of organic carbon export from catchments. Biogeochemistry 107:275–293

Bass AM, Bird MI, Liddell MJ, Nelson PN (2011) Fluvial dynamics of dissolved and particulate organic carbon during periodic discharge events in a steep tropical rainforest catchment. Limnol Oceanogr 56:2282–2292

Battin TJ, Kaplan LA, Findlay S, Hopkinson CS, Marti E, Packman AI, Newbold JD, Sabater F (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 1:95–100

Bender MA, Knutson TR, Tuleya RE, Sirutis JJ, Vecchi GA, Garner ST, Held IM (2010) Modeled impact of

anthropogenic warming on the frequency of intense Atlantic hurricanes. Science 327:454–458
Borken W, Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob Change Biol 15:808–824
Brooks PD, O'Reilly CA, Diamond SA, Campbell DH, Knapp R, Bradford D, Corn PS, Hossack B, Tonnessen K (2005) Spatial and temporal variability in the amount and source of dissolved organic carbon: implications for UV exposure in amphibian habitats. Ecosystems 8:478–487
Buffle J (1984) Natural organic matter and metal-organic interactions in aquatic systems. Met Ions Biol Syst 18:165–221
Butman D, Raymond PA (2011) Significant efflux of carbon dioxide from streams and rivers in the United States. Nat Geosci 4:839–842. doi:10.1038/ngeo1294
Chow AT, Dahlgren RA, Zhang Q, Wong PK (2008) Relationships between specific ultraviolet absorbance and trihalomethane precursors of different carbon sources. J Water Supply Res Technol 57(7):471–480
Cole J (2013) Freshwater in flux. Nat Geosci 6(1):13–14. doi:10.1038/ngeo1696
Cole J, Prairie Y, Caraco N, McDowell W, Tranvik L, Striegl R, Duarte C, Kortelainen P, Downing J, Middelburg J (2007) Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:172–185
Cory RM, McKnight DM, Chin Y, Miller P, Jaros C (2007) Chemical characteristics of fulvic acids from Arctic surface waters: microbial contributions and photochemical transformations. J Geophys Res 112:G04S51. doi:10.1029/2006JG000343
Coynel A, Etcheber H, Abril G, Maneux E, Dumas J, Hurtrez JE (2005) Contribution of small mountainous rivers to particulate organic carbon input in the Bay of Biscay. Biogeochemistry 74:151–171
Crisp DT, Robson S (1979) Some effects of discharge upon the transport of animals and peat in a north Pennine headstream. J Appl Ecol 16:721–736
Delaware Environmental Observing Station (DEOS) (2012). http://www.deos.udel.edu/ Accessed 30 Oct 2012
Dhillon G (2012) Comparison of particulate and dissolved organic carbon exports from forested piedmont catchments. Master's Thesis, University of Delaware, Newark, DE
Dhillon G, Inamdar S (2013) Extreme storms and changes in particulate and dissolved organic carbon in runoff: entering uncharted waters? Geophys Res Lett 40:1322–1327. doi:10.1002/grl.50306
Driscoll CT, Johnson NM, Likens GE, Feller MC (1988) Effects of acidic deposition on the chemistry of headwater streams: a comparison between Hubbard Brook, New Hampshire, and Jamieson Creek, British Columbia. Water Resour Res 24:195–200
Evans C, Davies TD (1998) Causes of concentration/discharge hysteresis and its potential as a tool for analysis of episode hydrochemistry. Water Resour Res 34:129–137
Fellman JB, Hood E, Edwards RT, D'Amore DV (2009) Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. J Geophys Res 114:G01021
Herczeg AL, Broecker WS, Anderson RF, Schiff SL, Schindler DW (1985) A new method for monitoring temporal trends in the acidity of fresh waters. Nature 315:133–135
Hood E, Gooseff MN, Johnson SL (2006) Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon. J Geophys Res 111:G01007
Hooper RP (2003) Diagnostic tools for mixing models of stream water chemistry. Water Resour Res 39:1055
Hope D, Billett M, Cresser M (1994) A review of the export of carbon in river water: fluxes and processes. Environ Pollut 84:301–324
Hrudey SE (2009) Chlorination disinfection by-products, public health risk tradeoffs and me. Water Res 43:2057–2092
Inamdar SP (2011) The use of geochemical mixing models to derive runoff sources and hydrologic flow paths in watershed studies. In: Levia D, Carlyle-Moses D, Tanaka T (eds) Forest hydrology and biogeochemistry: synthesis of research and future directions. Springer, New York
Inamdar SP, Mitchell MJ (2007) Storm event exports of dissolved organic nitrogen (DON) across multiple catchments in a glaciated forested watershed. J Geophys Res 112:G02014
Inamdar SP, O'Leary N, Mitchell MJ, Riley JT (2006) The impact of storm events on solute exports from a glaciated forested watershed in western New York, USA. Hydrol Process 20:3423–3439
Inamdar SP, Singh S, Dutta S, Levia D, Mitchell M, Scott D, Bais H, McHale P (2011) Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. J Geophys Res 116:G03043
Inamdar SP, Finger N, Singh S, Mitchell M, Levia D, Bais H, Scott D, McHale P (2012) Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed, USA. Biogeochemistry 108:1–22
Inamdar SP, Dhillon G, Singh S, Dutta S, Levia DF, Mitchell MJ, Van Stan J, Scott D, McHale P (2013) The controls of end-member chemistry and hydrologic conditions on the temporal patterns of runoff in a forested, Piedmont catchment. Water Resour Res 49:1828–1844. doi:10.1002/wrcr.20158
Jeong JJ, Bartsch S, Fleckenstein JH, Matzner E, Tenhunen JD, Lee SD, Park SK, Park JH (2012) Differential storm responses of dissolved and particulate organic carbon in a mountainous headwater stream, investigated by high-frequency, in situ optical measurements. J Geophys Res 117:G03013
Johnson MS, Lehmann J, Selva EC, Abdo M, Riha S, Couto EG (2006) Organic carbon fluxes within and streamwater exports from headwater catchments in the southern Amazon. Hydrol Process 20:2599–2614
Jung BJ, Lee HJ, Jeong JJ, Owen J, Kim B, Meusburger K, Alewell C (2012) Storm pulses and varying sources of hydrologic carbon export from a mountainous watershed. J Hydrol 440–441:90–101
Karl TR, Melillo JM, Peterson TC, Hassol SJ (2009) Global climate change impacts in the United States. Cambridge University Press, Cambridge
Kim S, Kim J, Kim K (2010) Organic carbon efflux from a deciduous forest catchment in Korea. Biogeosciences 7:1323–1334

Levia D, Van Stan J, Mage S, Kelley-Hauske P (2010) Temporal variability of stemflow volume in a beech-yellow poplar forest in relation to tree species and size. J Hydrol 380:112–120
Lundquist E, Jackson L, Scow K (1999) Wet–dry cycles affect dissolved organic carbon in two California agricultural soils. Soil Biol Biochem 31:1031–1038
Luo J, Ma M, Liu C, Zha J, Wang Z (2009) Impacts of particulate organic carbon and dissolved organic carbon on removal of polycyclic aromatic hydrocarbons, organochlorine pesticides, and nonylphenols in a wetland. J Soils Sediments 9:180–187
Maryland State Climatologist Office Data Page. http://metosrv2.umd.edu/~climate/cono/norm.html. Accessed 21 June 2013
Ni HG, Lu FH, Luo XL, Tian HY, Zeng EY (2008) Riverine inputs of total organic carbon and suspended particulate matter from the Pearl River Delta to the coastal ocean off South China. Mar Pollut Bull 56:1150–1157
Oeurng C, Sauvage S, Coynel A, Maneux E, Etcheber H, Sánchez-Pérez JM (2011) Fluvial transport of suspended sediment and organic carbon during flood events in a large agricultural catchment in southwest France. Hydrol Process 25:2365–2378
Pawson R, Lord D, Evans M, Allott T (2008) Fluvial organic carbon flux from an eroding peatland catchment, southern Pennines, UK. Hydrol Earth Syst Sci Discuss 12:625–634
Pawson R, Evans M, Allott T (2012) Fluvial carbon flux from headwater peatland streams: significance of particulate carbon flux. Earth Surf Process Landf 37:1203–1212
Raymond PA, Saiers JE (2010) Event controlled DOC export from forested watersheds. Biogeochemistry 100:197–209
Rovira A, Batalla RJ (2006) Temporal distribution of suspended sediment transport in a Mediterranean basin: the Lower Tordera (NE Spain). Geomorphology 79:58–71
Townsend-Small AT, McClain ME, Hall B, Noguera JL, Llerena CA, Brandes JA (2008) Suspended sediments and organic matter in mountain headwaters of the Amazon River: results from a 1-year time series study in the central Peruvian Andes. Geochim Cosmochim Acta 72:732–740
Veyssy E, Etcheber H, Lin R, Buat-Menard P, Maneux E (1999) Seasonal variation and origin of particulate organic carbon in the lower Garonne River at La Reole (southwestern France). Hydrobiologia 391:113–126
Walling DE, He Q (1999) Using fallout lead 201 measurements to estimate soil erosion on cultivated land. Soil Sci Soc Am J 63:1404–1412
Ward AD, Trimble SW (2004) Environmental hydrology. Lewis Publishers, New York
Wiegner TN, Tubal RL, MacKenzie RA (2009) Bioavailability and export of dissolved organic matter from a tropical river during base-and stormflow conditions. Limnol Oceanogr 54:1233

Use of the White Rot Fungus as a Fungal Bioreactor to Remove E. coli from Aqueous Dairy Manure Wastewater

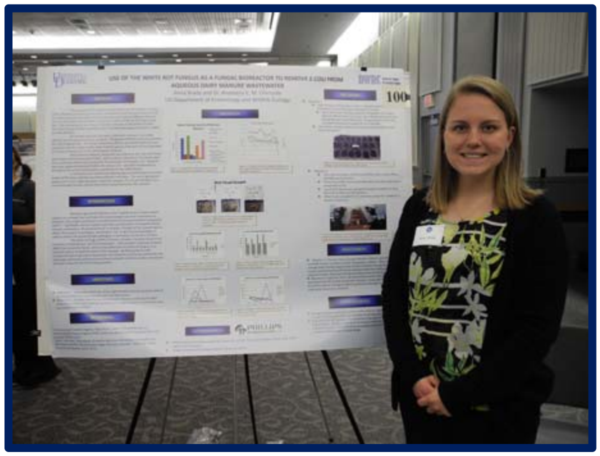
Basic Information

Title:	Use of the White Rot Fungus as a Fungal Bioreactor to Remove E. coli from Aqueous Dairy Manure Wastewater
Project Number:	2013DE249B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Water Quality, Non Point Pollution, None
Descriptors:	None
Principal Investigators:	Anastasia Chirnside, Anna Brady

Publications

1. Brady, A., and A.E.M. Chirnside, 2014, Use of the White Rot Fungus as a Fungal Bioreactor to Remove *E. coli* from Aqueous Dairy Manure Wastewater, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 7 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 7.

Undergraduate Internship Project #1 of 7 for FY13



Intern ***Anna Brady's*** project, co-sponsored by the ***DWRC*** and the ***UD's College of Agriculture and Natural Resources***, was titled "Use of the White Rot Fungus as a Fungal Bioreactor to Remove *E. coli* from Aqueous Dairy Manure Wastewater," She was advised by Dr. Anastasia Chirnside of the ***UD's*** Department of Entomology and Wildlife Ecology.

"The DWRC undergraduate research experience opened doors to explore new topics and help me discover what areas I want to focus on after graduation - through this internship, I was able to work with a professor who has similar interests, as well as address relevant water quality issues facing the state of Delaware today… Doing undergraduate research gave me a new perspective on career possibilities, and I am grateful that I was able to pursue my interest in wastewater management in such a tangible way." – Anna Brady

Abstract

The purpose of this research project was to see if the white rot fungus, *Pleurotus ostreatus*, could be used as a filter to remove *Escherichia coli* from liquid dairy manure wastewater. This was carried out through two objectives. The first objective was to grow up 3 replications of 8 different biomass mixtures inoculated with the WRF to see in which 2 biomass mixtures the fungus grew best. The second was to see which of those 2 biomass mixtures, or the control of spent mushroom compost (SMC), eliminated *E. coli* the best.

For the first objective, the spent mushroom compost + corn stalks (SMCCS) and the spent mushroom compost + *Phragmites* (SMCPH) mixtures promoted the most fungal growth. These biomass mixtures, along with a live control (non-autoclaved sample of SMC) and a killed control (autoclaved sample of SMC), were studied further in their ability to remove *E. coli* from liquid dairy manure. This was done at two different dilutions: 100% and 50% manure.

The results showed that SMCCS and SMCPH were the most successful, possibly linked to their water holding capacity: they were not too saturated or too dry in comparison to the other biomass mixtures. For the second objective, the results were not as conclusive about how successfully the biomass mixtures eliminated *E. coli*. However, there was a general downward shift in the amount of *E. coli* colonies counted throughout the month of observation in objective 2. Better drainage in the bioreactors would allow for better growth conditions for the fungus.

In conclusion, this research provided background information for further studies of *Pleurotus ostreatus* as a bioremediation technique. The use of agricultural waste, such as corn stalks and spent mushroom compost, for manure filtration is an environmentally friendly method that will be researched further this summer.

A Biogeographic Investigation of Viral Diversity within the Eastern Oyster, Crassostrea virginica

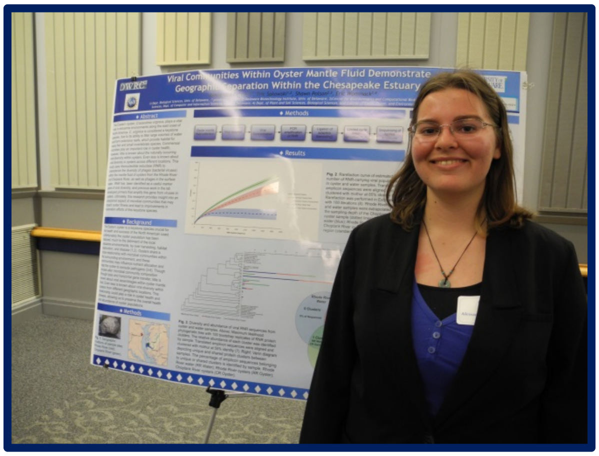
Basic Information

Title:	A Biogeographic Investigation of Viral Diversity within the Eastern Oyster, Crassostrea virginica
Project Number:	2013DE250B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Water Quality, Non Point Pollution, Sediments
Descriptors:	None
Principal Investigators:	Eric Wommack, Alessandra Ceretto

Publications

1. Ceretto, A., E. Sakowski, S. Polson, and E. Wommack, 2014, Viral Communities within Oyster Mantle Fluid Demonstrate Geographic Separation within the Chesapeake Estuary, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 12 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 7.

Undergraduate Internship Project #2 of 7 for FY13



Intern ***Alessandra Ceretto's*** project, co-sponsored by the ***DWRC*** and the ***UD's*** Department of Plant and Soil Sciences was titled "A Biogeographic Investigation of Viral Diversity within the Eastern Oyster, *Crassostrea virginica*." She was advised by Dr. K. Eric Wommack the ***UD's*** Department of Plant and Soil Sciences.

Abstract

The Eastern oyster, *Crassostrea virginica*, plays a vital role in estuarine environments along the east coast of North America. *C. virginica* is considered a keystone species, due to its ability to filter large volumes of water and form extensive reefs, which provide habitat for many fish and small invertebrate species. Commensal microbes play an important role in oyster health; however, little is known about the naturally occurring viral diversity within oysters. Even less is known about viral diversity in oysters across different locations. This study uses ribonucleotide reductase (RNR) to characterize the diversity of phages (bacterial viruses) within the mantle fluid of oysters from the Rhode River and Choptank River, as well as phages in the surface water. RNR has been identified as a useful marker gene of viral diversity, and previous work in the lab developed primers that amplify this gene from viruses in oysters. Ultimately, this research provides insight into an unexplored aspect of microbial communities that may impact oyster fitness and lead to improvements in restoration efforts of this keystone species.

Exploring the Viability of Biochar to Treat Stormwater

Basic Information

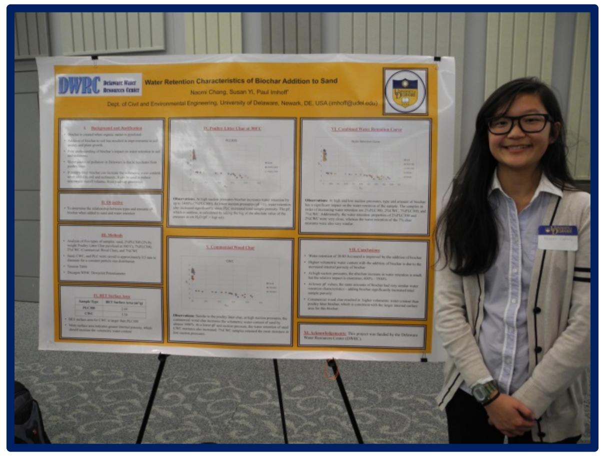
Title:	Exploring the Viability of Biochar to Treat Stormwater
Project Number:	2013DE251B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At large
Research Category:	Engineering
Focus Category:	Surface Water, Water Quality, Treatment
Descriptors:	None
Principal Investigators:	Paul Imhoff, Naomi Chang

Publications

1. Chang, N., S. Yi, and P, Imhoff, 2014, Water Retention Properties of Biochar and Sand Mixtures, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 6 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 4.

Undergraduate Internship Project #3 of 7 for FY13

Intern ***Naomi Chang's*** project, co-sponsored by the ***DWRC*** and the ***UD's*** Department of Plant and Soil Sciences was titled "Exploring the Viability of Biochar to Treat Stormwater." She was advised by Dr. Paul Imhoff of the ***UD's*** Department of Civil and Environmental Engineering.



Abstract

Biochar is created when organic materials are pyrolized at various temperatures. It is being considered as an additive to poor soils to improve crop yield and soil fertilization. Despite significant prior research, the effects of biochar-addition on many soil processes are not well understood. The water retention properties of biochar-amended sediments using two types of biochar were examined in this study using a tension table and a Decagon WP4C Dewpoint Potentiometer. Five types of samples were analyzed: 30/40 Accusand with no biochar, 30/40 Accusand containing 2%PLC300, 7%PLC300, 2%CWC, and 7%CWC, where PLC300 is poultry litter char (PLC) pyrolized at 300˚C and CWC is commercial wood char (CWC) that was pyrolized at 600˚C. All biochar was rinsed until the electrical conductivity was reduced to below 50 µS, and then along with the sand sieved to be around 0.5 mm in diameter. By assuring the diameter of the biochar is similar to sand particle diameters, any change in the water retention will not be a result of a variation in the particle size distribution. At higher pressures, the matric potential was measured using a Decagon WP4C Dewpoint Potentiameter. At lower suction pressures, a tension table was used.

Addition of biochar significantly increased the water retention properties of the sand at both high and low suction pressures. In particular, the 7%CWC sample was able to retain the most amount of moisture of all samples tested for all pressures. The addition of different amounts of a particular type of biochar also affected water retention in a systematic fashion. At high suction pressures, there is little difference in absolute volume of water retained between the three samples - sand, 2%PLC300, and 7%PLC300. However, percentage-wise, the 2%PLC300 sample exhibited over a 600% increase in volumetric water content over the pure sand sample, while the 7%PLC300 exhibited almost a 1500% increase. Similar to the poultry litter char, at high suction pressures the addition of CWC results in a significant increase in volumetric water content on a percentage basis. Compared to pure sand, the volumetric water content is over 400% larger for the 2%CWC sample and over 1500% larger for the 7%CWC sample. Overall, the 7%CWC sample had the highest water retention followed by 7%PLC300, 2%CWC, 2%PLC300, and sand. This increased ability to retain water at all pressures is due to the high internal porosity of the biochar. BET surface area analyses determined that PLC300 had a surface area of 2.68 m^2/g and the CWC had a surface area of 3.38 m^2/g. An increase in surface area correlates with an increase in water retention.

Based on these findings, biochar addition significantly affects the water retention properties of the sand/biochar mixture. To further determine what potential impact the addition of biochar could have on the soils in Delaware, research is currently being conducted on the addition of different amounts and types of biochar to sandy loam, a common Delaware soil. The effect of biochar addition on hydraulic conductivity and infiltration rates is also being tested.

The Varying Impact of Stemflow and Soil Moisture on the Diversity of Soil Bacterial and Fungal Communities in Relation to Soil Respiration

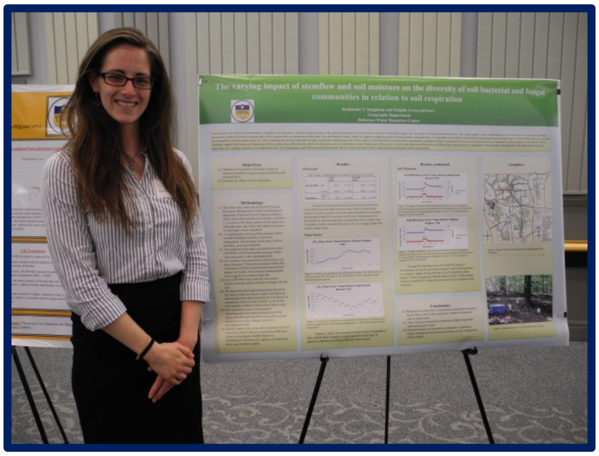
Basic Information

Title:	The Varying Impact of Stemflow and Soil Moisture on the Diversity of Soil Bacterial and Fungal Communities in Relation to Soil Respiration
Project Number:	2013DE252B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Hydrogeochemistry, Geochemical Processes, Solute Transport
Descriptors:	None
Principal Investigators:	Delphis Levia, Katherine Junghenn

Publications

1. Junghenn, K., and D. Levia, 2014, The Varying Impact of Stemflow and Soil Moisture on the Diversity of Soil Bacterial and Fungal Communities in Relation to Soil Respiration, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 7 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 7.

Undergraduate Internship Project #4 of 7 for FY13



Intern ***Katherine Junghenn's*** project, co-sponsored by the ***DWRC*** and ***UD's College of Earth, Ocean, and Environment***, was titled "The Varying Impact of Stemflow and Soil Moisture on the Diversity of Soil Bacterial and Fungal Communities in Relation to Soil Respiration." She was advised by Dr. Delphis Levia of the ***UD's*** Department of Geography.

Abstract

Soil moisture in forested environments is spatially heterogeneous. A better understanding of the spatial patterning of soil moisture in forests could yield insights into "hot spots" of biogeochemical activity. This study sought to better understand soil moisture and soil respiration dynamics around the trunks of American beech and yellow poplar trees in northeastern Maryland. Our preliminary results, based on targeted sampling periods during summer, indicated that: (1) respiration in microbial communities surrounding American beech trees is both higher and more variable as compared to yellow poplar trees; (2) only American beech trees experience higher respiration rates during rain events; and (3) American beech soil microbial communities experience higher respiration but lower soil moisture levels as compared to yellow poplar on the whole. These findings suggest that American beech and yellow poplar trees differentially affect soil moisture and soil respiration in near-trunk areas. However, it is likely that observed differences are affected and confounded by a multitude of other factors, including the individual life history of a particular tree, canopy structure of an individual tree, soil temperature, and differential soil properties. More work is needed to better understand the intricacies of these relationships.

Methane and Carbon Dioxide Fluxes in a Watershed

Basic Information

Title:	Methane and Carbon Dioxide Fluxes in a Watershed
Project Number:	2013DE253B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At large
Research Category:	Climate and Hydrologic Processes
Focus Category:	Climatological Processes, Geochemical Processes, Hydrogeochemistry
Descriptors:	None
Principal Investigators:	Rodrigo Vargas, Kelsey McWilliams

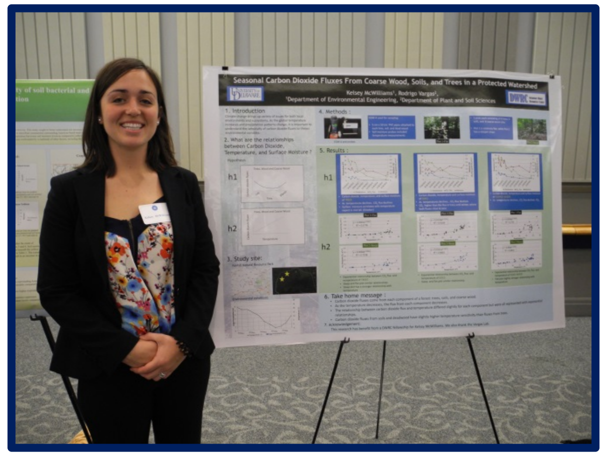
Publications

1. McWilliams, K., and R. Vargas, 2014, Seasonal Carbon Dioxide Fluxes from Coarse Wood, Soil and Trees in a Protected Watershed, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 8 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 7.

Undergraduate Internship Project #5 of 7 for FY13

Intern ***Kelsey McWilliam's*** project, co-sponsored by the ***DWRC*** and the ***Delaware Environmental Institute*** was titled, "Methane and Carbon Dioxide Fluxes in a Watershed." She was advised by Dr. Rodrigo Vargas of the ***UD's*** Department of Plant and Soil Sciences.

"The DWRC internship gave me a unique opportunity to work with an amazing student at UD. I am impressed about the quality and motivation of the students participating in this internship and I hope this support can continue to enrich undergraduate and faculty experiences at UD." – Dr. Rodrigo Vargas



Abstract

Climate change brings up a variety of issues for both local environments and ecosystems. As the global temperature increases and precipitation patterns change, it is important to understand the sensitivity of carbon dioxide fluxes to various environmental variables. The carbon dioxide flux for trees, soil and coarse wood was analyzed weekly from July 2013 to March 2014. An EGM-4 was used with a chamber that was inserted into a 10-cm diameter PVC pipe for each tree, soil and coarse wood sample. Two sites consisted each of 8 trees, 8 soil sites and 8 coarse woods. Plot 1 was relatively flatter than plot 2.The soil moisture, temperature and wood surface moisture were also taken weekly. The two hypotheses were (1) carbon dioxide flux would follow a temporal pattern with higher flux in the summer and lower flux in the winter and (2) temperature and carbon dioxide would be linearly correlated. The highest carbon dioxide fluxes were produced by the soil in August at a concentration of 0.2 µmol CO_2 m^2s^{-1}. For all components the carbon dioxide fluxes were found to correlate to either surface moisture or temperature. From July to early September, surface moisture was the limiting factor. The carbon dioxide flux correlated to the pattern of temporal surface moisture. As the temperature declined and the surface moisture increased around November, the carbon dioxide flux depended on the temperature. As the temperature declined, carbon dioxide fluxes in each component declined reaching close to zero µmol CO_2 m^2s^{-1} in the dead of winter. The carbon dioxide flux was also analyzed with its relationship to temperature. For each component there was an exponential relationship with temperature. Trees and soil sites in both plot 1 and plot 2 held similar relationships. The coarse wood on plot 1 has a stronger relationship with temperature than the steeper plot (plot 2). Carbon dioxide fluxes come from each component of a forest: trees, soils and coarse wood. As the temperature decreases, the flux from each component decreases. The relationship between carbon dioxide flux and temperature differed slightly for each component but were all represented with exponential relationships. Carbon dioxide fluxes from soils and deadwood have slightly higher temperature sensitivity than fluxes from trees. With the impacts of climate change in Delaware's near future, it is now more than ever before important to understand the carbon cycle of a forest ecosystem.

Sustainable Management of Water and Ecosystem Services on a Residential Landscape in Delaware

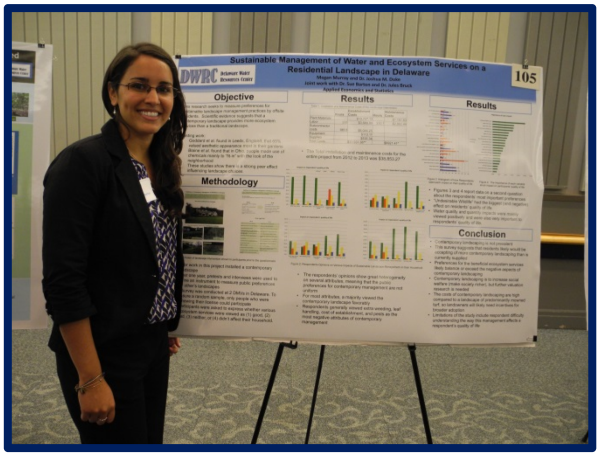
Basic Information

Title:	Sustainable Management of Water and Ecosystem Services on a Residential Landscape in Delaware
Project Number:	2013DE254B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	a
Research Category:	Water Quality
Focus Category:	Water Quality, Water Quantity, Non Point Pollution
Descriptors:	None
Principal Investigators:	Joshua Duke, Megan Murray

Publications

1. Duke, J., J. Bruck, S. Barton, and M. Murray, 2014, The Ecosystem Services of Residential Landscapes: A Delaware Study Site, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 49 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 7.
3. Pautler, M., ed., 2014, Delaware Water Resources Center WATER E-NEWS Vol. 13 Issue 1, DWRC News, http://ag.udel.edu/dwrc/newsletters/WATERENEWS-Feb2014.pdf , p. 2.

Undergraduate Internship Project #6 of 7 for FY13



Intern ***Megan Murray's*** project, co-sponsored by the ***DWRC*** and the ***UD College of Agriculture and Natural Resources***, was titled "Sustainable Management of Water and Ecosystem Services on a Residential Landscape in Delaware." She was advised by Dr. Joshua Duke of the ***UD's*** Department of Applied Economics and Statistics.

"Megan did a fantastic job for me this year, and we are grateful for DWRC support of this research. Your support enabled Megan to get fully integrated with a research team, though she is only in her third year of undergraduate studies. With Drs. Barton and Bruck, Megan and I are embarking on a journal article based on this research. We will acknowledge DWRC support in this article and let you know if it gets accepted." – Dr. Joshua Duke

Abstract

This report describes research on a sustainable landscape intervention in Delaware that altered a residential landscape in order to enhance ecosystem services. This intervention was termed "contemporary" landscaping. Data were collected on installation and management costs and a survey of perceived impacts to off-site residents was conducted. The landscape intervention occurred in the suburban "Applecross" development in northern New Castle County, Delaware. The affluent neighborhood has houses with large yards on lots of about 1.2 acres. The intervention sought to apply recent scientific advances to enhance ecosystem services, especially water quality protection. The intervention consisted of reducing the lawn space from 98% of the yard to less than 50%. Native plants and various types of land cover were introduced, including a constructed forested area and separate meadow. With this landscape intervention came many ecosystem services including:

• Water quantity and quality improvements;
• Aesthetic changes; and
• Expanded habitats.

The intervention cost approximately $32,000 to establish. Though high, this cost aligns with landscaping costs in similar affluent neighborhoods. An intercept survey of non-neighboring Delaware residents was conducted to understand public preferences for this type of intervention, particularly the off-site received costs and benefits of the altered ecosystem services. An additional, small survey was conducted with neighbors. The survey data show a majority of the ecosystem service changes were perceived to have a positive impact on people's quality of life, though some had a negative or no effect. The most important impacts were found to be:

• Undesirable wildlife might be present (negative);
• Better flood control (positive); and
• Better water quality (positive).

The neighbors' survey had an inadequately small sample, but generally matched the results of the other Delaware residents. In sum, the research shows that contemporary landscapes may possibly increase social welfare, but high establishment costs will preclude many landowners from adoption. Further valuation research is needed to determine benefits and cost estimates.

The research suggests that even though contemporary landscapes are not prevalent, it may not be due to preference for traditional yards with extensive lawn space. Rather, there may be a mismatch between public benefits and landowner costs.

Acid Neutralization of Stemflow in a Deciduous Forest: The Role of Edge Effects

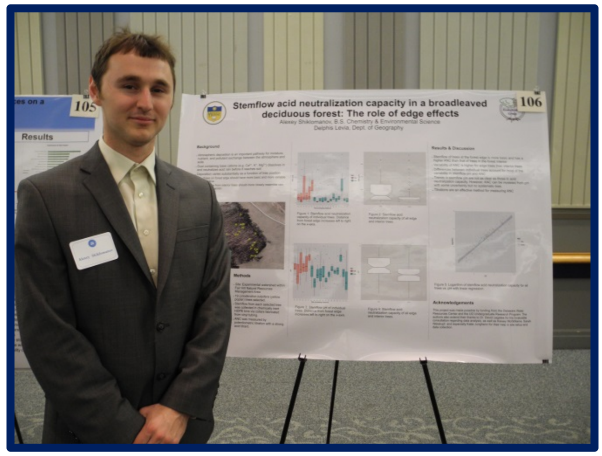
Basic Information

Title:	Acid Neutralization of Stemflow in a Deciduous Forest: The Role of Edge Effects
Project Number:	2013DE255B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At large
Research Category:	Biological Sciences
Focus Category:	Acid Deposition, Geochemical Processes, Water Quality
Descriptors:	None
Principal Investigators:	Delphis Levia, Alexey N Shiklomanov

Publications

1. Shiklomanov, A., and D. Levia, 2014, Stemflow Acid Neutralization Capacity in a Broadleaved Deciduous Forest: The Role of Edge Effects, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 5 pages.
2. Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, Introducing Our 2013-14 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 7.

Undergraduate Internship Project #7 of 7 for FY13



Intern ***Alexey Shiklomanov's*** project, co-sponsored by the ***DWRC*** and the ***UD's Water Resources Agency*** was titled "Acid Neutralization of Stemflow in a Deciduous Forest: The Role of Edge Effects." He was advised by Dr. Delphis Levia of the ***UD's*** Department of Geography.

Abstract

Atmospheric deposition is an important pathway for moisture, nutrient, and pollutant exchange between the atmosphere and soils. However, atmospheric deposition can also alleviate acid inputs to the soil. Depositional fluxes have been found to vary substantially as a function of tree position relative to the forest edge. This study sought to: (1) develop an effective field observation strategy capable of detecting differences in ANC (acid neutralization capacity) in relation to forest edge and interior locations; and (2) measure and analyze the differential ANC of stemflow from trees located at a range of distances from the edge of the forested watershed. Such work would increase our knowledge of forest biogeochemical cycling, and specifically forests' vulnerability to soil acidification. Stemflow was collected from nineteen *Liriodendron tulipifera* L. (yellow poplar) trees from representative locations within an experimental watershed in northeastern Maryland. ANC was measured by potentiometric titration. Results indicate that edge trees had higher and more variable median stemflow ANC and pH than interior trees. On a tree-by-tree basis, trends were confounded by inter-individual variability, which merits further investigation.

UD Watershed Team Ecological Restoration

Basic Information

Title:	UD Watershed Team Ecological Restoration
Project Number:	2013DE256B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	a
Research Category:	Water Quality
Focus Category:	Water Quality, Non Point Pollution, Management and Planning
Descriptors:	None
Principal Investigators:	James Thomas Sims, Gerald Kauffman

Publication

1. Pautler, M., ed., 2014, Delaware Water Resources Center WATER E-NEWS Vol. 13 Issue 2, Spotlight on DWRC – UD WATER Research and Investigations, http://ag.udel.edu/dwrc/newsletters/WATERENEWS-Mar2014.pdf , p. 1.

Stormwater Solutions for the University of Delaware's North Campus: Hydrologic Modeling in the Piedmont Watershed

Jillian Allen, Tim D'Agostino, Hannah Diehl
UD Watershed Action Team for Ecological Restoration (UD WATER)

1. Introduction

Stormwater runoff is an increasing problem for the health of water quality and water supply in the White Clay Creek National Wild and Scenic River. The Laird campus, in the Piedmont, at the University of Delaware contains a large amount of impervious cover which increases runoff and pollutant loads detrimental to the White Clay Creek (Fig. 1). Reduction of impervious cover through best management practices such as rain gardens and reforestation will reduce the detrimental impact of stormwater on the quality of White Clay Creek.

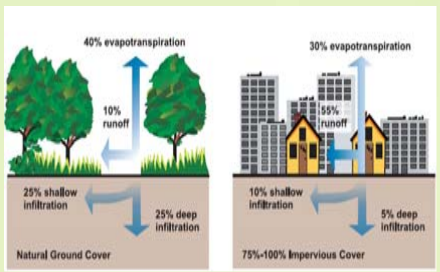


Fig. 1: Effect of impervious cover on runoff and infiltration rates in urban areas.

2. Research Objectives

To reduce runoff and pollutant loads, develop a watershed model to determine how changes in the stormwater system will improve water quality and reduce flooding in: (1) Fairfield Run, (2) Blue Hen Creek, (3) a gully system tributary to White Clay Creek

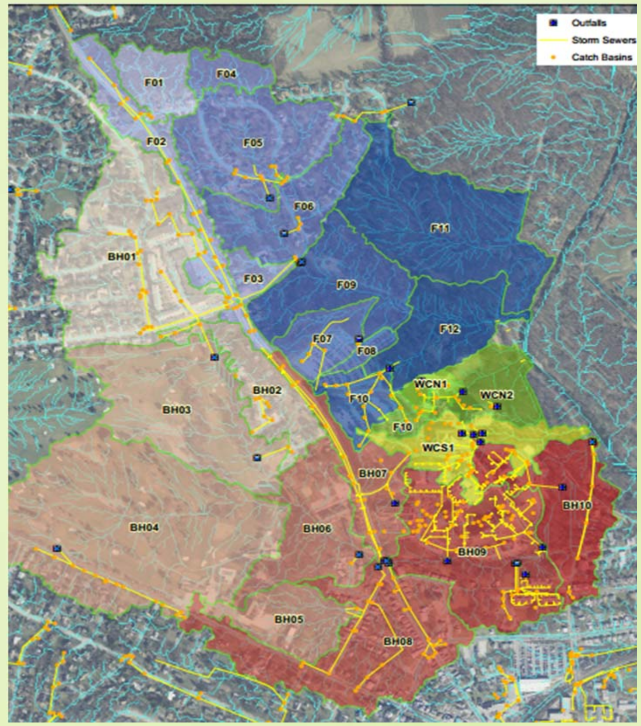


Fig. 2: Piedmont subcatchments along Fairfield Run (blue), Blue Hen Creek (red), gully system (green)

3. Methods

Each watershed was modeled using an EPA hydrology/hydraulics program called SWMM (Storm Water Management Model). The SWMM model was developed according to the following methods:

Task 1 - Delineate the watersheds (Fig. 2) into appropriate sections according to topography (contour lines) and the stormwater network using GIS data This was done using GIS data and for time and convenience sake was prepared for us.

Task 2 - Create a backdrop map for the SWMM model that includes the subcatchments, pipes, and outfalls that convey the stormwater runoff.

Task 3 – Input nodes (manholes) and outfalls to create the framework of the SWMM model (Fig. 3).

Task 4 – Connect the nodes and outfalls using links (stormwater pipes) as depicted in Fig. 4.

Task 5 – Define and draw the catchments on the map to complete the basic watershed model (Figs. 5, 6, 7).

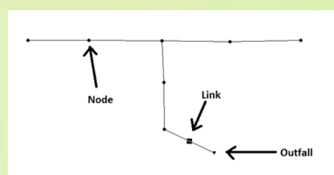


Fig. 3: Nodes, links, and Outfalls in the SWMM model

4. Results

Fig. 4: Gully system SWMM model

The gully system has the following parameters:
1. Area: 14 acres
2. Links: 37
3. Nodes: 39
4. Outfalls: 7

4. Results

Fig. 5: Fairfield Run SWMM model

Fairfield Run has the following parameters:
1. Area: 139.4 acres
2. Links: 61
3. Nodes: 66
4. Outfalls: 5

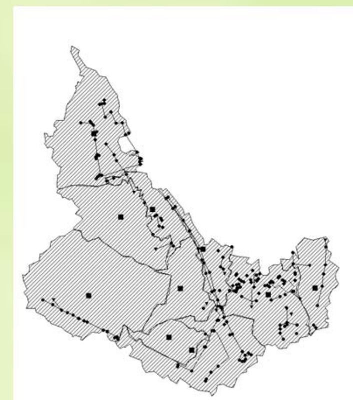


Fig. 6: Blue Hen Creek SWMM model

Blue Hen Creek has the following parameters:
1. Area: 246.3 acres
2. Links: 133
3. Nodes: 174
4. Outfalls: 18

5. Future Work

1. Input rainfall data collected from the field to run the model.
2. Watershed data such as area, pipe diameter and length, and invert elevation must be put into the model.
3. Run the model with alternate inputs (such as BMPs installed).
4. Expansion of the SWMM model to include the entire University of Delaware campus and Newark.

6. Acknowledgements

We would like to acknowledge our mentors Gerald Kauffman and Andrew Homsey for all of their help throughout the course of this project . We would also like to thank the WRA for the funding for this project.

Information Transfer Program Introduction

None.

DWRC Information Transfer

Basic Information

Title:	DWRC Information Transfer
Project Number:	2010DE197B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	At Large
Research Category:	Not Applicable
Focus Category:	Water Quality, Water Supply, Education
Descriptors:	None
Principal Investigators:	James Thomas Sims, Maria Pautler

Publications

There are no publications.

Information Transfer Program

The following section describes all Delaware Water Resources Center information transfer activities during FY13, consolidating reporting into a single (extended) project **#2010DE197B**. Most activities from the DWRC's FY12 Information Transfer project (**#2010DE197B)** continued into this year.

The FY13 DWRC Information Transfer Activities include:

- Delaware Water Resources Center Electronic Publication WATER NEWS (2000 – 2006 = print; 2007 – present = electronic)
- Delaware Water Resources Center Electronic Newsletter WATER E-NEWS (2002 – present)
- Delaware Water Resources Center Website (3rd edition launched in 2009)
- Delaware Water Resources Center E-group / Courses Link (2002 – present)
- Delaware Water Resources Center Intern Project Poster Session / Advisory Panel Annual Meeting (2001 – present)
- Delaware Statewide Conference Co-sponsor and Participant (2001 – present, when held)

Basic Information:

Delaware Water Resources Center Electronic Publication WATER NEWS

Title:	"WATER NEWS"
Issues during FY12:	Volume 13 Issues 1&2 (Fall-Winter 2012 and Spring-Summer 2013)
Description:	Online 8-page newsletter published biannually by the Delaware Water Resources Center (In FY13 there was a one-time 8-page newsletter)
Lead Institute:	Delaware Water Resources Center
Principal Investigators:	Dr. J. Thomas Sims, Director; Maria Pautler, Editor

WATER NEWS is received electronically by over 300 recipients in water-related academic, government, public and private agency, agriculture and industry positions in Delaware and the surrounding area as well as 100 nationwide contacts for water resource issues. It may be accessed via the Delaware Water Resources Center website at: http://ag.udel.edu/dwrc/newsletters.html.

FY13 topics included:

- DWRC Annual Luncheon and Poster Session – April 19, 2013
- Introducing Our 2013-14 Interns
- Spotlight on Graduate Research
- The UD WATER Project
- Spotlight on Undergraduate Internships
- DWRC History, Goals, Advisory Panel, Contacts

Basic Information:

Delaware Water Resources Center Electronic Newsletter WATER E-NEWS

Title:	"WATER E-NEWS"
Issues during FY12:	Feb. 2014, Mar. 2014
Description:	Brief online "highlights" newsletter published periodically by the Delaware Water Resources Center
Lead Institute:	Delaware Water Resources Center
Principal Investigators:	J. Thomas Sims, Director; Maria Pautler, Editor

WATER E-NEWS is received electronically by over 300 recipients in water-related academic, government, public and private agency, agriculture and industry positions in Delaware and the surrounding area. The current issue and back issues dating to its August 2002 inception may be accessed via the DWRC website at: http://ag.udel.edu/dwrc/newsletters.html.

Featured in each issue of WATER E-NEWS are:

I. News items about the DWRC, including undergraduate internships and graduate fellowships

II. Jobs in Water Resources

III. Upcoming Water Conferences / Events

IV. Water Resources Information / Training

Basic Information: Delaware Water Resources Center Website

Title:	Website: http://ag.udel.edu/dwrc
Start Date:	Third edition; since February 2009
End Date:	Ongoing
Description:	Comprehensive site serving Delaware water resources community
Lead Institute:	Delaware Water Resources Center
Principal Investigators:	Dr. J. Thomas Sims, Director; Maria Pautler, Administrator

The website contains:

- **Delaware Water Resources Center (DWRC) and Director's News:** Latest updates on DWRC activities and information on the DWRC's mission, history, and role in the National Institute of Water Resources (NIWR).
- **Delaware Water Concerns:** Summary of the major areas of concern related to Delaware's ground and surface waters, with links to key organizations and agencies responsible for water quality and quantity.
- **Projects and Publications:** Descriptions of DWRC's undergraduate internship and graduate fellows programs, annual conference proceedings, and project publications dating back to 1993. Abstracts from the undergraduate internship projects are prevalent to educate current undergraduates and faculty about the types of research that can be done under this program.
- **Advisory Panel:** Purpose, contact information and e-mail links for the DWRC's Advisory Panel.
- **Request for Proposals and Application Forms:** For undergraduate interns, graduate fellowships and other funding opportunities available through the DWRC.
- **Internships and Job Opportunities:** Information on undergraduate and graduate internships from a wide variety of local, regional, and national sources along with current job opportunities in water resource areas.
- **Water Courses and Faculty:** Link to search engine for current list of University of Delaware water resource courses. List of researchers at Delaware universities with an interest in water resources research; also, science and natural resource curricula links.
- **Water Resources Contacts:** Links to local, regional, and national water resource agencies and organizations categorized as government, academia, non-profit, and US Water Resource Centers.
- **Calendar:** Upcoming local, regional, and national water resources events sponsored by the DWRC and other agencies, such as conferences, seminars, meetings, and training opportunities.
- **Newsletters:** Access to DWRC newsletters dating back to 1993.
- **Annual and 5-year Reports:** DWRC annual and 5-year reports, dating to 1993.
- **KIDS' Zone:** Water resources activities and information for kids and teachers.

Basic Information: Delaware Water Resources Center E-group / Courses Link

Title:	Delaware Water Resources Center / Water Resources Agency E-group, originating from the online listing of Delaware water teachers and researchers found on the DWRC website: http://ag.udel.edu/dwrc/faculty_researchers.html
Start Date:	Since December 2001
End Date:	Ongoing
Description:	E-group and link to university water resources courses taught, serving Delaware water resources community
Lead Institute:	Delaware Water Resources Center
Principal Investigators:	J. Thomas Sims, Director; Maria Pautler, Administrator

The online listing of approximately 70 researchers at the University of Delaware, Delaware State University, and Wesley College found on the Delaware Water Resources Center website at http://ag.udel.edu/dwrc/faculty_researchers.html forms the foundation for a broader e-group list maintained by the DWRC reaching additional academic, public, private, and government water community contacts, who are notified via an e-mail newsletter of events and job postings of interest in water resources.

The website also links to a search engine and site for water-related courses currently offered by the researchers.

Basic Information:
Delaware Water Resources Center Intern Project Poster Session /
Annual Advisory Panel Meeting

Title:	University of Delaware 2014 Undergraduate Research Scholars Poster Session with DWRC Advisory Panel Meeting
Date:	April 24, 2014
Description:	Undergraduate interns presented their 2013-2014 DWRC-funded projects following the annual meeting of the DWRC Advisory Panel
Lead Institute:	University of Delaware Undergraduate Research Program Co-sponsors: Delaware Water Resources Center, Charles Peter White Fellowship in Biological Sciences, Chemistry and Biochemistry Alumni Fellowship, College of Agriculture and Natural Resources, Howard Hughes Medical Institute, McNair Scholars Program, National Science Foundation, Northeastern Chemical Association, NUCLEUS, State of Delaware.
Principal Investigators:	Iain Crawford, Director, UD Undergraduate Research Program (icrawf@udel.edu); J. Thomas Sims, Director, DWRC (jtsims@udel.edu)

On April 24, 2014, the undergraduate student interns who had been funded in 2013-2014 by the DWRC, accompanied by their advisors, presented the results of their research at an informal poster session sponsored by the University of Delaware Undergraduate Research Program. Over 95 UD Science and Engineering Scholars joined the DWRC interns to present to a crowd of over 500 visitors. The DWRC Advisory Panel also convened for lunch with the interns and their advisors and then held their annual meeting prior to the poster session. DWRC Director Tom Sims described the Center's plans for 2014-2015 with regard to research funding and public education outreach efforts.

Poster Presentations by 2013-2014 DWRC Undergraduate Interns – April 24, 2014

1) Brady, Anna. Poster Presentation April 24, 2014. Use of the White Rot Fungus as a Fungal Bioreactor to Remove *E. coli* from Aqueous Dairy Manure Wastewater. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

2) Ceretto, Alessandra. Poster Presentation April 24, 2014. A Biogeographic Investigation of Viral Diversity within the Eastern Oyster, *Crassostrea virginica*. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

3) Chang, Naomi. Poster Presentation April 24, 2014. Exploring the Viability of Biochar to Treat Stormwater. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

4) Junghenn, Katherine. Poster Presentation April 24, 2014. The Varying Impact of Stemflow and Soil Moisture on the Diversity of Soil Bacterial and Fungal Communities in Relation to Soil

Respiration. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

5) McWilliams, Kelsey. Poster Presentation April 24, 2014. Methane and Carbon Dioxide Fluxes in a Watershed. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

6) Murray, Megan. Poster Presentation April 24, 2014. Sustainable Management of Water and Ecosystem Services on a Residential Landscape in Delaware. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

7) Shiklomanov, Alexey. Poster Presentation April 24, 2014. Acid Neutralization of Stemflow in a Deciduous Forest: The Role of Edge Effects. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

8) Allen, Jillian, Timothy D’Agostino, and Hannah Diehl. Poster Presentation April 24, 2014. Stormwater Solutions for the University of Delaware’s North Campus: Hydrologic Modeling in the Piedmont Watershed. 2014. University of Delaware Undergraduate Research Scholars Poster Session, University of Delaware, Newark, Delaware.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	7	0	0	0	7
Masters	1	0	0	0	1
Ph.D.	2	0	0	0	2
Post-Doc.	0	0	0	0	0
Total	10	0	0	0	10

Notable Awards and Achievements

Research Program: The Delaware Water Resources Center (DWRC) has funded ten research grant projects during March 2013 through February 2014 that address state water resources priorities identified by the DWRC's Advisory Panel. Two of these projects are graduate fellowships with research focuses on 1) quantifying carbon amount and quality for transport of contaminants in landscapes and 2) microbiome of the eastern oyster. The remaining projects were undergraduate internships researching 1) removal of *E. coli* from wastewater; 2) viral diversity in oysters; 3) use of biochar to treat stormwater; 4) stemflow, soil moisture, and soil respiration; 5) methane and carbon dioxide fluxes in a watershed; 6) water and ecosystem services on a residential landscape; 7) acid neutralization of stemflow in a forest; and 8) the UD WATER Project – stormwater solutions on the UD campus (three undergraduate interns reporting).

Former DWRC Graduate Fellow Gurbir Dhillon's work was highlighted in the National Institutes of Water Resources 2014 NIWR-USGS partnership brochure in a summary titled, "Entering uncharted waters with carbon runoff."

Publications from Prior Years

1. 2012DE235B ("Developing Scientifically-Based Food Safety Metrics for Water Management and Irrigation Methods") - Other Publications - Cook, L., and K. Kniel, 2013, Developing Scientifically-Based Food Safety Metrics for Water Management and Irrigation Methods, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 6 pages.
2. 2012DE234B ("The Returns to Best Management Practices: Evidence from Early Proposals for Nutrient Trading in the Chesapeake Bay Watershed") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 3.
3. 2012DE235B ("Developing Scientifically-Based Food Safety Metrics for Water Management and Irrigation Methods") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 2.
4. 2012DE236B ("Water Quality Impacts of Landscape Best Management Practices that Enhance Vegetation") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 3.
5. 2012DE237B ("Improving Irrigation Management through Soil Moisture Monitoring and Automated Control of Sprinkler and Sub-Surface Drip Irrigation") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 3.
6. 2012DE238B ("Characterization of Viral Diversity within the Mantel Fluid of the Eastern Oyster, Crassostrea virginica") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 2.
7. 2012DE239B ("Water Quality Management in Urban Ecosystems") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 3.
8. 2012DE240B ("Preventing Formation of Toxic Chlorination Byproducts in Water Using Zerovalent Iron") - Other Publications - Pautler, M., ed., 2013, Delaware Water Resources Center WATER NEWS Vol. 13 Issues 1&2, DWRC Annual Poster Session – April 19, 2013. http://ag.udel.edu/dwrc/newsletters/Fall12Summer13/WATERNEWSco-Summer2013.pdf , p. 3.