Water Resources Research Center
Annual Technical Report
FY 2006

Introduction

The New Hampshire Water Resources Research Center, located on the campus of the University of New Hampshire, is an institute which serves as a focal point for research and information on water issues in the state. The NH WRRC actually predates the Federal program. In the late 1950s Professor Gordon Byers (now retired) began a Water Center at UNH. This Center was incorporated into the Federal program in 1965 as one of the original 14 state institutes established under the Water Resource Research Act of 1964. The NH WRRC is currently directed by Dr. William McDowell with administrative and technical assistance from Jeff Merriam and Jody Potter. The NH WRRC is a stand alone organization, in that it is not directly affiliated with any other administrative unit at UNH. The NH WRRC has no dedicated laboratory, administrative or research space on campus and no formal library holdings. To overcome these potential limitations, our website (www.wrrc.unh.edu) is used heavily, and serves as a focal point for information dissemination and includes all NH WRRC publications and results from past research, as well as links to other sites of interest to NH citizens and researchers.

Research Program

Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

Basic Information

Title:	Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds
Project Number:	2003NH21B
Start Date:	3/1/2006
End Date:	2/28/2008
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Congressional District:	NH01
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Surface Water, Nutrients
Descriptors:	
Principal Investigators:	William H. McDowell

Publication

1. Buyofsky, Lauren A., 2006, Relationships between groundwater quality and landscape characteristics in the Lamprey River watershed, "MS Dissertation", Department of Natural Resources, College of Life Science and Agriculture, University of New Hampshire, Durham, NH, 176 pages.

Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

Statement of Critical Regional or State Water Problem
New Hampshire's surface waters are a very valuable resource, contributing to the state's economic base through recreation (fishing, boating, and swimming), tourism and real estate values. Many rivers and lakes also serve as local water supplies. New Hampshire currently leads all New England states in the rate of development and redevelopment (2000 Census). The long-term impacts of population growth and the associated changes in land use to New Hampshire's surface waters are uncertain. Of particular concern are the impacts of non-point source pollution to the state's surface waters (e.g. septic, urban run off, road salt application, deforestation and wetland conversion). Long-term datasets that include year-to-year variability in precipitation, weather patterns and other factors will allow adequate documentation of the cumulative effects of land use change and quantification of the effectiveness of watershed management programs.

Statement of Results or Benefits
> The proposed project will provide detailed, high-quality, long-term datasets which will allow for a better understanding of the impacts of land use change and development on surface water quality. This could occur through the development, testing and refinement of predictive models, accurately assessing the impacts of watershed management practices, and potentially early warning of dramatic changes to surface water quality in the region resulting from rapid development.

Objectives of the Project
> This project allows for the continued collection of long-term water quality data in New Hampshire. It will use UNH staff, students and volunteers from local communities to collect samples from the College Brook watershed (Durham, NH), the Lamprey River Watershed, and the Ossipee River Watershed.

Water samples will be collected from the following sub-projects.

> The **College Brook** watershed, which is dominated by the University of New Hampshire, receives a variety of non-point pollution from several different land uses. Suspended sediments (TSS), pH, conductivity, biological oxygen demand (BOD) and nutrient concentrations (Cl^-, SO_4^{-2}, Na^+, K^+, Mg^{+2}, Ca^{+2}, NO_3, NH_4, PO_4, DOC, TDN, SiO_2) will be measured to assess water quality. Samples from 7 sites will be collected monthly throughout the year. Sampling of College Brook began in 1991. Sample collection will be done by UNH staff and/or students. The Water Quality Analysis Lab at UNH will analyze these samples as part of the non-federal match.

> The **Lamprey River** has been sampled weekly and during rain events since October 1999. Samples are analyzed for total dissolved nitrogen (TDN), nitrate (NO_3-N), ammonium (NH_4-N), DON, DOC and orthophosphate (PO_4-P). Additionally, samples collected since October 2002 are also analyzed for dissolved inorganic carbon (DIC), pH, conductivity, dissolved oxygen (DO), temperature, total suspended sediment, particulate

carbon, particulate nitrogen, silica and major anions and cations. In January of 2004, we began routine sampling of additional Lamprey sites for nitrogen, phosphorus and DOM. Since November of 2003, weekly bulk precipitation samples have been collected at numerous locations throughout the basin for analysis of nitrogen, phosphorus, DOM, major cations and anions and silica. Several homeowners have been monitoring precipitation volume throughout the basin since October 2003.

Precipitation and stream sampling was be scaled back in FY05. Precipitation data from FY04 indicates that chemistry does not vary significantly spatially, therefore we'll only sample from one collector on an event basis. Homeowners will continue to monitor precipitation gages throughout the watershed as precipitation amount is spatially variable. The frequency of stream sampling will be curtailed to monthly (instead of weekly) for 10 of our sampling sites. The remaining 3 streams will continue to be sampled weekly. These water samples will be analyzed by the Water Quality Analysis Lab at UNH.

Volunteers of the Green Mountain Conservation Group will sample streams within the **Ossipee watershed** of New Hampshire. Samples will be collected every 2 weeks from May to November, and monthly during the winter months. There will be approximately 340 samples collected. Water chemistry (Cl^-, SO_4^{-2}, Na^+, K^+, Mg^{+2}, Ca^{+2}, NO_3, NH_4, PO_4, DOC, TDN) will be measured on selected samples by the WQAL as part of the non-federal match. WRRC staff will assist in data interpretation.

Principal Findings and Significance

College Brook
Previous work on College Brook in the early 1990's (McDowell unpublished) shows that the UNH campus had a severe impact on water quality and was negatively affecting stream biota and the integrity of downstream ecosystems. By any yardstick, campus operations could not be considered sustainable. There was clear evidence that the UNH incinerator was causing excessive organic matter loading, resulting in high biochemical oxygen demand (BOD) and low dissolved oxygen (DO) in stream water. Since the incinerator has been closed, BOD and DO are no longer at levels detrimental to instream biota. Our monthly sampling regime was scaled back beginning October 2006 to the 3 stations that have historically shown the greatest changes, and we eliminated the BOD and TSS measurements (both which change little over the reach since the incinerator was closed). The most downstream sampling location is now closer to where the stream empties into the Oyster River in an effort to better quantify inputs to the Great Bay estuary. Analyses of samples collected thru 2006 has been completed and we are in the process of updating our website
http://www.wrrc.unh.edu/current_research/collegebrook/collegebrookhome.htm.

Data from 2000-2006 indicates that the steam is strongly impacted by road salt at its origin, which is essentially a road-side ditch leading to a wetland area. Average Sodium and Chloride concentrations appear to have jumped after year 2000 and remained reasonably constant since then. Concentrations tend to decline downstream. Dissolved Oxygen (DO) is lower at the upstream stations. This difference is presumably due to hydrologic properties of the upstream sampling location which resembles a wetland (i.e.

slow flow, higher organic matter). DO increases downstream as flow becomes faster and re-aeration higher.

Weekly Lamprey Sampling and the Lamprey River Hydrologic Observatory

The Lamprey River watershed is a rural watershed located in southeastern NH and is under large development pressure as the greater area experiences rapid population growth. Our goal for the long-term Lamprey water quality monitoring program is to document changes in water quality as the Lamprey watershed becomes increasingly more developed and to understand the controls on N transformations and losses. We have continued to sample the Lamprey River at the USGS gauging station in Durham, NH (referred to as "LR 73.3"), the North River at the former USGS gauging station in Epping, NH (NR 26.9) and a small tributary to the Lamprey River in Lee, NH (WHB 1.03) for DOC, DON, NO_3-N, NH_4-N, PO_4-P, TDP, TP, Na, Ca, Mg, K, Cl, SO_4, SiO_2, pH, DO, temperature and conductivity on a weekly basis. In addition to these parameters, station LR 73.3 is also sampled for DIC, TSS, Particulate C and Particulate N. The USGS discontinued the operation of the North River gauging station in October 2006 and since then we have been recording weekly stage height and calculating flow based on the USGS rating curve. We are able to record stream flow at WHB 1.03 using an electronic distance meter in combination with a rating curve that we have developed for this site. We have also developed a stream flow model for WHB 1.03 where daily discharge can be estimated from meteorological measurements (such as precipitation and temperature) and this model is useful for estimating historic flows. We continue to collect precipitation at Thompson Farm (UNH property located in Durham, NH) to document nitrogen inputs to the basin and work with NOAA/AIRMAP in an attempt to link to precipitation chemistry to airmass chemistry.

Results of stream chemistry to date show an increase in peak springtime TDN concentration in the Lamprey from 2000 -2004, but lower springtime TDN concentrations since 2004 (Figure 1). Lamprey TDN export is dominated by DON export and the highest TDN flux recorded to date was during 2006 (Figure 2). This high export likely associated with the May 2006 hundred year flood given that the runoff from the Lamprey in 2006 (1056 mm/yr) was twice the long-term average (approximately 500 mm/yr). The variation in DON concentration and export throughout the Lamprey basin is related to % wetland cover and even though NO_3-N does not dominate TDN, variation in NO_3-N concentration and flux is linked to watershed human population density. Results of precipitation monitoring show that wet deposition is the largest input of N to the Lamprey watershed and precipitation chemistry can be linked to airmass chemistry. DOC and TDN in precipitation are related to biogenic airmass sources, NH_4-N, NO_3-N and SO_4-S are related to urban/industrial airmasses and Na and Cl are weakly related to ocean aerosols.
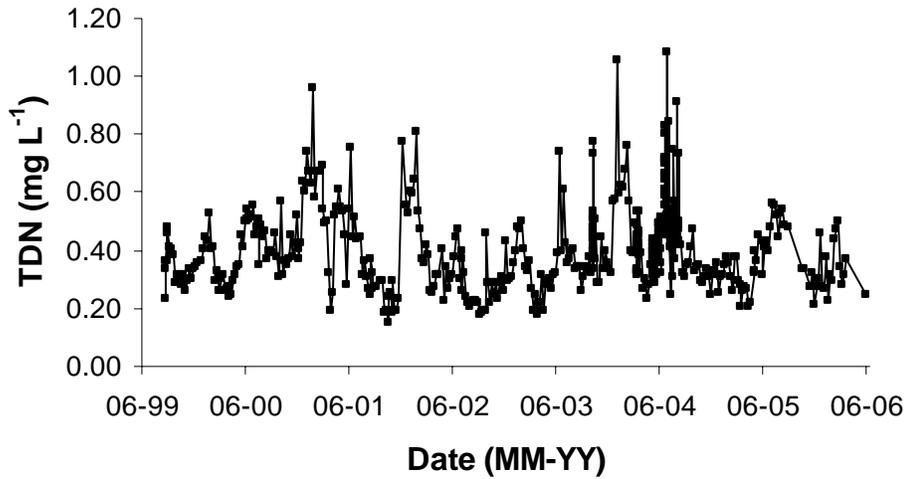
Figure 1. Nitrate concentrations over time in the Lamprey River at the USGS gauging station in Durham, NH.
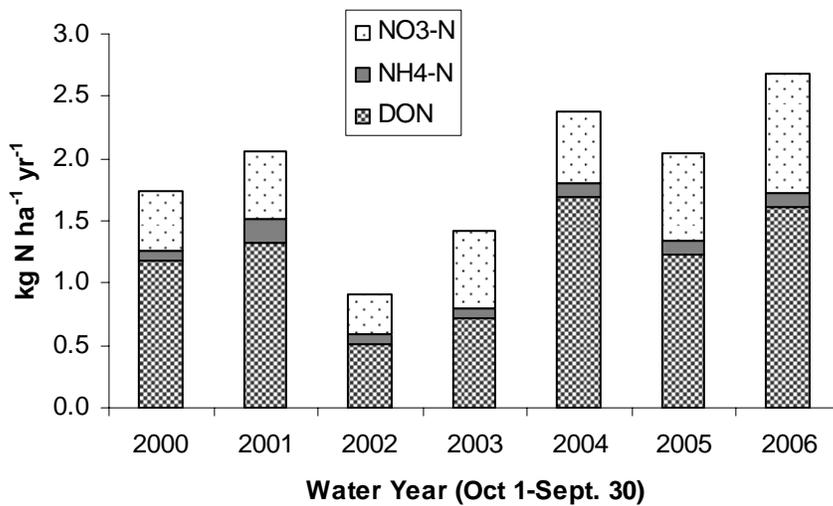

Figure 2. Annual export of dissolved nitrogen species from the Lamprey watershed.

Other ongoing research in the Lamprey watershed involves examining potential nitrogen loss in riparian zones. We have two well fields installed within 10 m of two small streams. In one of the riparian zones, there is a large reduction in NO_3-N (approximately 4.5 mg NO_3-N upslope to 0.2 mg/L NO_3-N near the stream) and an increase in NH_4-N (approximately 0.02 mg NH_4-N upslope to 0.2 mg/L NH_4-N near the stream) over a small distance (approximately 10 m). Nitrate addition experiments have been conducted to evaluate the potential for denitrification loss of N but the results of this study are still preliminary.

4

Ossipee River watershed sampling
Collaboration with the Green Mountain Conservation Group and their sampling of the Ossipee River watershed has continued to be beneficial. Volunteers sampled streams within the watershed every 2 weeks from May to November, with approximately 340 samples collected from 14 sampling locations. Many presentations were made to planning boards, conservation commissions and other local government groups (see Publication, presentations and awards section below). Data have been used to heighten awareness of the impacts of excessive road salting and snow dumping in local streams. Communication with local road agents has led to the remediation in one development where road salting was an issue. Samples collected and data generated from this funding have shown an improvement in water chemistry following reduced salting and snow dumping.

Material in Preparation:

McDowell, W.H., M.L. Daley, B. Sive, R. Talbot. Factors controlling atmospheric deposition at a coastal suburban site. *Journal of Geophysical Research (Atmospheres).*

Conference Proceedings & Abstracts

Daley, M.L., McDowell, W.H., T.E. O'Donnell, and L.A. Buyofsky. 2006. Biogeochemistry of a Suburban Basin. *2006 Watershed Conference.* Concord, NH. November 2006.

Daley, M.L., McDowell, W.H., T.E. O'Donnell, and L.A. Buyofsky. 2006. Biogeochemistry of a Suburban Basin. *State of the Estuaries Conference.* Portsmouth, NH. October 2006.

Gettel, G.M., W.H. McDowell, and O. Pisani. 2006. Using fluorescence to understand the role of carbon quality in nitrogen dynamics in a rapidly suburbanizing watershed in southeastern New Hampshire. *Annual meeting of Northeastern Regional Research Collaborative*, Durham NH October 2006.

Gettel, G.M., W.H. McDowell, and O. Pisani. 2006. Using fluorescence to measure carbon quality at watershed scales and implications for nitrogen retention in a rapidly suburbanizing watershed. *Annual NABS Meeting*, June 2006, Anchorage, Alaska.

McDowell, W.H. 2006. Biogeochemistry of suburban basins. *Boston University.* November, 2006.

McDowell, W.H. 2006. Biogeochemistry of suburban basins. *University of Florida.* November, 2006.

McDowell, W.H. 2006. Biogeochemistry of suburban basins. *China.* April, 2006.

Dissertations and Theses:
Buyofsky, L.A. May 2006. Relationships between groundwater quality and landscape characteristics in the Lamprey River watershed. *Masters Thesis*, University of New Hampshire, Durham, NH.

Information Transfer:

McDowell, W.H. & Associates 2006. Lamprey River Hydrological Observatory: an "invisible" part of Lee. *Lee Town Crier* 26, Fall 2006.

Presentations made by the Green Mountain Conservation Group staff.
3/10/2006 OWC Workshop w/ Board & Town Rep's (GIS, land use planning, watershed planning)
3/24/2006 Natural Resource Based Planning Workshop (regional meeting, Tamworth)
4/8/2006 Water Quality Monitoring Program & Training
4/21/2006 Groundwater Conference
5/3/2006 Tamworth Learning Circle School program (RIVERS)
5/17/2006 Sandwich Elementary School program (RIVERS, macro)
5/18/2006 NRBP Workshop (regional workshop, GIS, watershed planning w/ SPNHF)
5/20/2006 Community School program (WQM)
5/25/2006 Calumet School Program (OLT)
6/1/2006 Calumet Program (OLT)
6/2/2006 Calumet Program (OLT)
6/6/2006 Calumet Program (OLT)
6/8/2006 Summer Camp Program (OLT, Deep Water - Directors)
6/9/2006 Calumet Program (OLT)
6/21/2006 OWC Workshop Madison (GIS, land use planning, watershed planning)
7/4/2006 Calumet Program (OLT)
7/18/2006 Calumet Program (OLT)
7/22/2006 Watershed Weekend (water quality, land use planning, wildlife)
7/25/2006 OWC Workshop Ossipee (GIS, land use planning, watershed planning)
7/27/2006 OWC Workshop Effingham (GIS, land use planning, watershed planning)
8/1/2006 Calumet Program (OLT)
8/2/2006 Valley Vision/TV taping WQM program at Camp Huckins
8/12/2006 SPNHF Workshop – Green Mountain Hike (land use change, land conservation)
8/15/2006 Calumet Program (OLT)
8/23/2006 Macro Training (expansion of WQM program)
8/30/2006 Wetlands Forum (land use planning, protecting water quality in watershed)
9/11/2006 Sandwich Program (RIVERS, macro)

9/21/2006 TNC Moth Program in Pine Barrens
10/5/2006 Land Celebration (land use planning, watershed protection)
10/11/2006 Calumet adult program - Watershed Presentation & WQM
10/18/2006 Ossipee Central School (OLT/Lake/NPS program)
10/19/2006 Ossipee Central School (OLT/Lake/NPS pgrogram)
11/2/2006 Calumet adult program - Watershed Presentation & WQM
11/17/2006 State of the Watershed
12/8/2006 OWC Workshop (regional workshop, natural resource guidebook, watershed protection)
1/18/2007 OWC Workshop Dark Skies/Stars Program (intro. to GMCG & wqm program)
2/10/2007 Tracking (wildlife habitat protection, pine barrens, watershed protection)
2/14/2007 UNHCE Conservation Easement workshop(land conservation, intro. to GMCG as a land trust)
2/21/2007 Green Yards (water quality protection, junkyard monitoring)

FREQUENCY OF REOVIRUS DETECTION IN BIOSOLIDS: COMPARISON OF THE EPA CFR 503 TECHNIQUE TO INTEGRATED CELL CULTURE - REAL TIME PCR

Basic Information

Title:	FREQUENCY OF REOVIRUS DETECTION IN BIOSOLIDS: COMPARISON OF THE EPA CFR 503 TECHNIQUE TO INTEGRATED CELL CULTURE - REAL TIME PCR
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Descriptors:	None
Principal Investigators:	Aaron B. Margolin

Publication

1. Gallagher, Elizabeth M. and Aaron B. Margolin, 2007, Journal of Virological Methods, In Press.

Development of an integrated cell culture—Real-time RT-PCR assay for detection of reovirus in biosolids

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Abstract

The current method for viral detection in biosolids is a plaque assay, as specified by the EPA in the 40 CFR Part 503 rule. Development of an integrated cell culture-polymerase chain reaction (ICC-PCR) assay has allowed detection of viruses that are under-detected and undetected by the plaque assay. This study examined the efficiency of the ICC-PCR method to detect mammalian orthoreovirus, a virus typically under-detected in biosolids. Biosolid samples seeded with mammalian orthoreovirus type 1 (Lang) detected to 3×10^5 plaque forming units (pfu) with a plaque assay, 10^2 pfu equivalents with real-time RT-PCR and no incubation, and 10^8 pfu equivalents with real-time RT-PCR after 7 days incubation. More infectious virus was detected using ICC-real-time RT-PCR than a plaque assay. Twenty-four environmental samples from three locations around the United States did not plaque with the EPA method; however the ICC-PCR detected infectious reovirus in 13 of the samples. Raw biosolids samples accounted for 12 of the positive samples, and 1 positive was from an aerobically digested sample.
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Keywords: ICC-PCR; Real-time PCR; Reovirus; Biosolids

1. Introduction

1.1. Reovirus

Mammalian orthoreovirus is a member of the family Reoviridae and the genus *Orthoreovirus*. The virus has two round icosahedral capsids, and in the intestinal lumen of the mammals, the outer capsid is proteolytically uncoated and made infectious (Golden et al., 2002). The double protein capsids make mammalian orthoreovirus resistant to disinfection. The virus is hardy, remaining infectious in water for long periods of time (Matsuura et al., 1988). There are three serotypes that infect humans which are identical to the serotypes that infect other mammals. Although the pathogenic effects of reovirus are unknown, mammalian orthoreovirus has been isolated from patients with respiratory infections, gastroenteritis, or rashes (Ward and Ashley, 1978). The majority of adults have serum antibodies to all three types of this virus. The virus is shed by infected individuals in feces for several weeks (Fenner and White, 1986). Reovirus is frequently detected in environmental water and is usually the most abundant virus detected (AWWA, 1999).

1.2. Biosolids

Sludge is the solid waste byproduct of the municipal sewage treatment process. One popular method of sludge disposal is land application. The EPA 503 regulations (EPA, 1992) were established in 1993 to define how sewage sludge, known as biosolids, must be processed in order to be land applied. Biosolids must be treated according to approved processes or tested for viable helminth ova and enteric viruses. The enteric virus testing is performed by a plaque assay.

1.3. Detection methods

Cell culture is considered by some experts to be the best way to isolate and determine infectious virus from an environmental sample (Fong and Lipp, 2005). Plaques assays utilizing cell culture are typically used with wastewater and biosolid samples. However, many viruses do not produce plaque and some will only plaque after several passages on a cell line. In addition, a

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cell line with a mixed population of viruses may be infected by only the fastest growing virus (Spinner and DiGiovanni, 2001). Another drawback is the inability to conclusively identify the plaque as originating from viral lysis by visual inspection. Plaque assays have also been shown to be dependant on the amount of cells seeded in the cultureware, the amount of viral inoculum added, and the incubation time (Payment and Trudel, 1985).

PCR detection can be sensitive and specific. The efficiency of viral amplification from environmental samples by PCR is influenced by the ability to recover the virus from the environmental matrix and the purity of the recovered nucleic acid (Metcalf et al., 1995). Traditionally, PCR gives a positive or negative result, however real-time PCR can quantify the amount of virus in the sample. Other advantages include a smaller time frame to obtain results because an agarose gel is not necessary and a closed system which is less likely to be contaminated (Fong and Lipp, 2005). Like traditional PCR, real-time PCR does not indicate infectivity.

Integrated cell culture-PCR (ICC-PCR) overcomes the individual disadvantages of cell culture and PCR. The use of cell culture helps to dilute out any PCR inhibitors that would otherwise have to be removed using techniques, such as columns, which while removing PCR inhibitors, also simultaneously reduce the concentration of virus. In addition to diluting out PCR inhibitors, cell culture provides an in-vitro amplification system which increases the numbers of viruses and enhances the sensitivity of the assay while providing a means of differentiating between infectious and non-infectious virus (Reynolds, 2004). Additionally, ICC-PCR permits evaluation of a much larger percentage of the original sample as compared to traditional PCR. Several studies have considered the sensitivity, efficiency, and ease of ICC-PCR and found it be better than either traditional PCR or cell culture methods alone (Blackmer et al., 2000; Chapron et al., 2000; Greening et al., 2002; Jiang et al., 2004; Ko et al., 2003; Lee and Jeong, 2004; Lee et al., 2005; Reynolds et al., 1997, 1996, 2001).

2. Methods and materials

2.1. Propagation of reovirus type 1 (Lang)

Reovirus type 1 (Lang) was chosen for the seeded experiments. The virus was obtained from the American Type Culture Collection (ATTC), Manassas, VA (catalog no. VR-230). BGMK cells (ATTC, Rockville, MD) were grown in 175 cm^2 closed cell culture flasks to confluency using MEM supplemented with 10% fetal bovine serum. Prior to infection, the cells were washed and inoculated with reovirus stock at an MOI of 2 pfu/cell. Flasks were incubated at 37 °C for 90 min with periodic rocking. Post absorption, MEM supplemented with 2% fetal bovine serum was added and the flasks were returned to the incubator. Flasks were checked daily and when approximately 75% of the cell monolayer was exhibiting cytopathic effects (CPE) and the cell layer was sloughing off, the flasks were placed in the freezer until the media was frozen and then removed from the freezer and placed at room temperature to thaw. The process

of freeze-thawing was repeated three times to liberate the virus. Cellular debris were removed by centrifugation two times at $1000 \times g$ (2100 rpm) with a Beckman JA14 rotor for 15 min and the supernatant containing the virus was aliquoted and stored at −80 °C until use.

LLC-MK2 cells were also used to propagate mammalian orthoreovirus type 1 (Lang). The virus was adapted to this cell line with five passages. Similarly to the initial propagation, the cells were grown in 175 cm^2 closed cell culture flasks to confluency using MEM supplemented with 10% fetal bovine serum. After inoculation at an MOI of 4, the propagation proceeded in the exact same manner except on the first four passages instead of storing the supernatant it was used to infect more flasks. On the final passage, the supernatant containing the virus were aliquoted and stored at −80 °C until use.

2.2. Sludge collection

For seeded experiments, 5 l of biosolids were collected from the end of the secondary treatment train at a Concord, MA wastewater treatment plant. This plant serves about 5000 people and treats 1.2 million gallons per day at capacity during the summer months. The treatment process consists of a single-stage trickling filter with intermittent sand beds for winter season polishing. The sludge collected was 3.4% solids. The biosolids were stored at 4 °C until use.

Raw and treated sludge was collected from three different sites: Texas, Pennsylvania, and New Hampshire. The Texas plant uses anaerobic digestion for treatment, the Pennsylvania plant uses liming, and the New Hampshire plant uses composting. One liter of sludge was collected and sent to the laboratory in New Hampshire via overnight mail with the exception of the New Hampshire samples which were collected by the research team and driven to the laboratory. All samples were stored at 4 °C until use.

2.3. Elution of viruses from sludge samples

Viruses were recovered from the sludge samples as designated by the EPA part 503 rules (EPA, 1992). The procedure for recovery of viruses from wastewater solids was an adsorption process reliant upon adsorption of viruses from the liquid phase to the sludge solids, which are concentrated by centrifugation and subsequently eluted. The supernatant was discarded and viruses were desorbed from the solids by physiochemical means and further concentrated by organic flocculation. Decontamination was accomplished by incubation with antibiotics after the viruses were eluted. The concentrated eluent was frozen at −80 °C until evaluation. The losses due by to procedure have been outlined in Katz (2005) and are not significant for reovirus.

2.4. Sample preparations and spiking

Every sludge sample was thawed at 37 °C and vortexed. For each experiment 5 ml of sample was removed from the larger sample and 0.1 µl chloroform per 1 ml sample was added. The sample was then spun at $10,000 \times g$ in a micro-centrifuge

Table 1
Primers used to detect the three types of mammalian reovirus

Serotype	Forward primer	Reverse primer	Position of amplified region
1 (Lang)	gaggagggacacgcgtagtg	ccagatccagaacgaatctcatc	1114–1176
2 (Jones)	cggctacggtgtcaggatct	cgcgcgacgctattttg	1766–1824
3 (Dearing)	ctaccgctgtaccatcgttaagct	tggtacccctccgggatt	112–170

Nucleotides are listed in 5′-3′ direction.

142 for 5 min. After centrifugation the sample was split into three
143 aliquots. These aliquots were then individually diluted three
144 times in PBS to become a dilution series.
145   From the 5 l collected in Concord, MA for the seeded exper-
146 iments, three different aliquots were removed. Spiking of the
147 seeded samples occurred before the first step of the elution pro-
148 cedure. 0.1 ml of reovirus at 10^{-6} was added to the 200 ml in the
149 blender to bring the final concentration of the virus to approx-
150 imately 10^{-5}. Each of these aliquots were seeded and eluted
151 separately.

2.5. Reovirus plaque assay

153   Buffalo Green Monkey Kidney (BGMK) cells were grown
154 to 95–100% confluency in six well plates. The concentrations
155 from the dilution series done under sample preparations were
156 tested. Each concentration was enumerated in triplicate and then
157 averaged to determine the pfu/ml. 0.1 ml of the sample being
158 tested was used as an inoculum and added to each well, and
159 incubated for 90 min with periodic rocking for viral adsorption.
160 After adsorption, 4 ml of agar overlay containing 2% bacto-agar
161 and $2\times$ MEM was added to each well. To enhance plaque for-
162 mation, 100 μl of 1 mg/μl trypsin was added to each well. The
163 agar overlay was then permitted to harden and plates were incu-
164 bated at 37 °C for 7 days. After 7 days, 1 ml formalin was added
165 to each well, and the well plates were placed in the incubator
166 for 24 h. After 24 h, the agar overlay was removed with warm
167 water and gentle tapping, and 1 ml crystal violet was added to
168 visualize the plaque forming units in the cell layer (Brabants,
169 2003).

2.6. Integrated cell culture

171   Each of the samples were done in duplicate; one which was
172 incubated on the cells for 0 days ($T = 0$) and one which was incu-
173 bated for 7 days ($T = 7$). LLC-MK2 cells were grown to 75–90%
174 confluency in six well plates using MEM with 10% FBS. The
175 media was then removed, the cells were washed, and 100 μl of
176 the specified dilution was added. The cells were rocked every
177 15 min for 90 min. After rocking, 4 ml MEM was added. For
178 the $T = 0$ plates, the media was immediately removed and 1 ml
179 trypsin added. The plates were incubated for 20 min at 37 °C to
180 loosen the cells and then placed in the freezer. The $T = 7$ plates
181 were placed in the incubator and after 24 h, 100 μl of 1 mg/ml
182 trypsin was added to each well and the plates were returned
183 to the incubator for an additional 6 days. On the 7th day, the
184 media was removed, trypsin was added, the plates were incu-
185 bated for 20 min and then placed in the freezer. After thawing,

186 RNA extraction was performed on the LLC-MK2 cells in both
187 the $T = 0$ and 7 plates to retrieve any replicated virus.

2.7. RNA extraction procedure

189   Qiagen QIAamp viral RNA mini kit was chosen for RNA
190 extraction from the samples (Qiagen, 1999). The spin-column
191 procedure was performed with the maximum amount of rec-
192 ommended sample, 140 μl. The virus elution was tested with
193 real-time PCR immediately following extraction and then stored
194 at −80 °C for further use.

2.8. Real-time PCR primer design

196   The primers and probe sets were designed using Applied
197 Biosystems Proprietary software, PrimerExpress. They were tar-
198 geted to sections of the genome which diverged in the three
199 types of reovirus. All three sets of primers (Table 1) and probes
200 (Table 2) were based on outer capsid protein (mu-1) in the m2
201 segment of the genome. NCBI blast was used to determine that
202 the primers detected only the intended target organism.

2.9. Quantitative real-time reverse transcription polymerase

205   Primers were received from Applied Biosystems dry and
206 desalted at 80,000 pmol and diluted to 50 μm/l with molecular
207 grade water. The probe was received from Applied Biosystems
208 at 6000 pmol in 60 μl buffer and was diluted to 10 μM/l with
209 molecular grade water. Five μl of the extracted DNA was com-
210 bined with 25 μl of master mix. The master mix contained
211 $1\times$ of TaqMan® One-Step RT-PCR Master Mix supplied by
212 Applied Biosystems (part number: 4309169) containing Ampli-
213 Taq Gold® DNA polymerase, Passive Reference I, and opti-
214 mized buffer components. The master mix also contained $1\times$ of
215 MultiScribe™ Reverse Transcriptase and RNase Inhibitor from
216 Applied Biosystems, 900 nM of the forward primer, 900 nM of
217 the reverse primer, 250 nM of the probe, and sterile molecular
218 grade water. The sample was placed in the ABI Prism 7700 real-
219 time PCR thermocycler. The run consisted of 48 °C for 45 min,

Table 2
Probes used to detect the three types of mammalian reovirus

Serotype	Probe	Position
1 (Lang)	cttggatcagattgctc (tagged with FAM)	1137–1153
2 (Jones)	taatccgaaaggtattttgt (tagged with VIC)	1787–1806
3 (Dearing)	atcacctggaatgct (tagged with FAM)	137–151

They coordinate with the primers listed in Table 1. Nucleotides are listed in 5′-3′
direction.

95 °C for 10 min, and 50 cycles of 94 °C for 20 s, 55 °C for 1 min, and 72 °C for 30 s. The fluorescence was detected at the end of each cycle to determine the cycle threshold value (CT) which is the cycle number at which the fluorescence generated within the reaction crossed the threshold. The CT values for the $T = 7$ samples were compared to the corresponding CT values for the $T = 0$ samples. For the seeded samples, each sample was combined with the reovirus type 1 primers. For the environmental samples, each sample was combined in separate tubes with each of the three primers.

2.10. Correlation

To determine the relationship of plaque forming units to cycle threshold values without cell culture, reovirus type 1 was diluted three times. Aliquots from each concentration were analyzed directly by a plaque assay and by real-time PCR. This entire analysis was repeated four times and plotted to determine the numerical relationship between the variables. To compare the same amount in the plaque assay and the quantitative PCR, the plaque forming units in the 5 µl of sample, which was the amount tested on the PCR, were determined from the plaque forming units per ml found in the plaque assay. This calculation assumed 100% recovery for the extraction method.

2.11. Quality control

The laboratory maintained strict quality assurance methods. The ABI Prism 7700 real-time PCR thermocycler was calibrated monthly using a calibration plate provided by Applied Biosystems. In addition, weekly the background fluorescence was examined and instrument wells emitting light were cleaned with alcohol. In the plaque assay, each run had three wells that served as the negative control and three wells for the positive control. Additionally, each time a sample was diluted into a dilution series; the PBS was tested on a plaque assay to confirm that it did not produce any plaques. For the cell culture portion of the ICC-PCR, a positive and negative well were done each time the assay was run. The positive control was reovirus type 1 and the negative control was a well with no virus at all. For the RNA extraction, the positive and negative controls from the cell culture portion were included. An additional negative control was added, which is referred to as the spin control. This was a tube that had all the buffers added and was placed in the centrifuge each time a spin was required in the protocol. These three controls were included as part of the real-time PCR assay. An additional negative control was added that was just the master mix, and an additional positive control was added that was the master mix with non-manipulated reovirus. These five controls were included with every PCR assay.

3. Results

3.1. Seeded experiment

In the seeded experiments, plaque forming units, the CT value at $T = 0$, and the CT value at $T = 7$ were compared. The most dilute sample in which reovirus was found in each assay was 10^{-2} for $T = 0$, 10^{-8} for $T = 7$, and 10^{-5} for the plaque assay (Fig. 1). The comparison between the $T = 0$ days and the $T = 7$ days shows that more dilute portions of the sample contained virus after incubation in cell culture (Fig. 2). The $T = 7$ days compared to the plaque forming units shows that the ICC-PCR method detected the most dilute amount of virus (Fig. 3).

3.2. Environmental samples

When environmental samples were tested, 54% of the samples were positive by ICC-PCR and none were positive by the plaque assay method (Table 3). Each location had positive samples but of the treatments only aerobic digestion had a positive sample (Table 4). The positive samples summarized by treat-
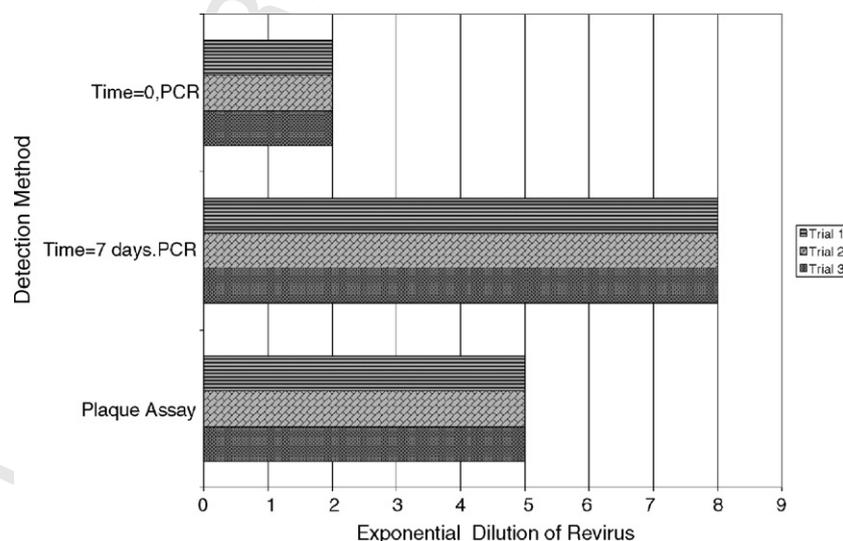

Fig. 1. The sensitivity of the ICC-PCR vs. the plaque assay. In the seeded experiments, the more dilute sample in which reovirus was detected was 10^{-2} for the $T = 0$ ICC-PCR, 10^{-8} for the $T = 7$ ICC-PCR and 10^{-5} for the plaque assay. This shows the sensitivity of the ICC-PCR vs. the plaque assay.
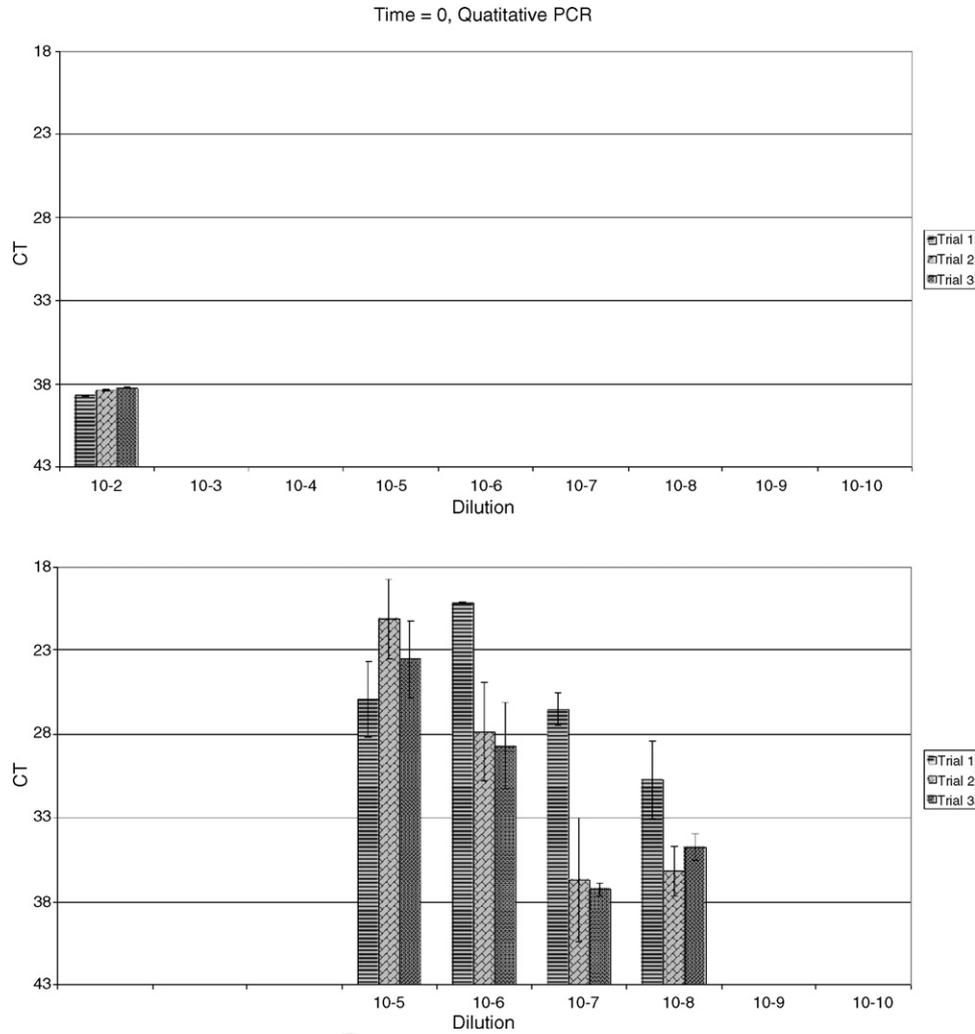
Fig. 2. ICC-PCR detected infectious virus. The $T = 7$ ICC-PCR detected reovirus in each of the dilution 10^{-3} to 10^{-8} where reovirus was not detected in the $T = 0$ ICC-PCR. This indicates that each of these 10-fold dilutions contained infectious virus.

ment show that 80% of the untreated samples were positive and 11% of the treated samples were positive (Table 5). The types of reovirus varied among the different locations, reovirus type 3 was detected at the Texas location and reovirus type 1 detected at the New Hampshire and Pennsylvania locations (Table 6). The PFU from the plaque assay was plotted as a scatter plot along with the CT values from the real-time PCR to show the rela-

Table 3
Out of the 24 environmental biosolids samples that were tested for reovirus, 13 tested positive by ICC-PCR, and none tested positive by the plaque assay technique

States tested	Number of samples tested	Positive for infectious reovirus by	
		ICC-PCR	Plaque assay
NH	9	5	0
TX	9	6	0
PA	6	2	0
Total	24	13	0
Percent positive (%)		54	0

Table 4
The environmental samples are divided into the treatment types which shows how many untreated are positive and how many treated are positive broken out by location

Location	Treatment	Undiluted (positive/total)	10^{-1} (positive/total)
Texas	Untreated	3/3	3/3
	Limed	0/3	
New Hampshire	Untreated	3/3	2/3
	Composted	0/3	
Pennsylvania	Untreated	1/3	
	Digested	1/3	

Table 5
When the samples are grouped by the treatment type (disregarding the locations), 80% of the untreated samples are positive and 11% of the untreated samples are positive

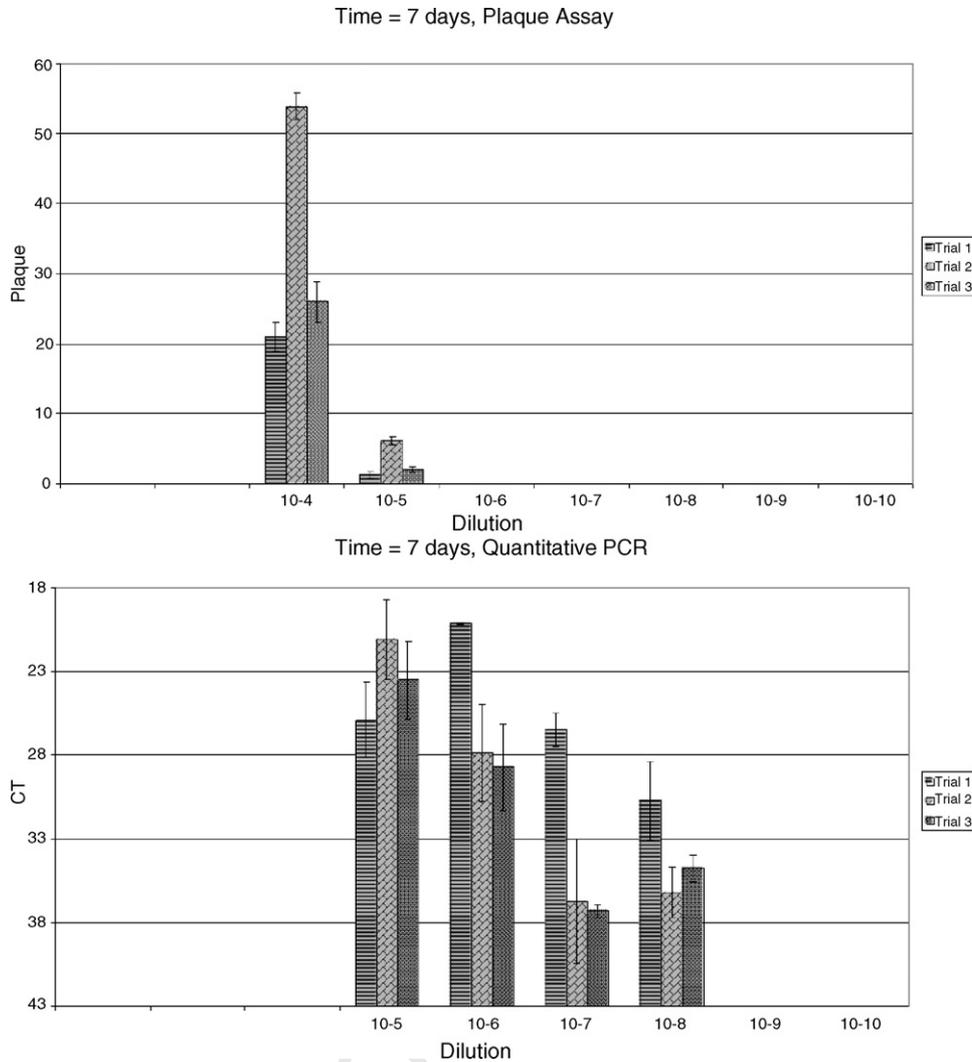| Treatment	Positive by ICC-PCR/ total samples	Percent positive by ICC-PCR (%)
Untreated	12/15	80
Treated	1/9	11

Fig. 3. ICC-PCR detected the most viruses. The top graph shows the plaque assay detected reovirus to a dilution of 10^{-5} and the bottom graph shows the ICC-PCR detected the virus to 10^{-8}.

Table 6
The type of reovirus detected based on sample location

State	Reovirus type(s) found
Texas	Type 3 (Dearing)
New Hampshire	Type 1 (Lang)
Pennsylvania	Type 1 (Lang)

tionship between the two variables. The environmental samples can then be plotted on the line to estimate the final concentration of virus (Fig. 4). The $T = 0$ samples are not listed for the environmental samples because they were all negative.

4. Discussion

It is clear that integrated cell culture with real-time RT-PCR detects more mammalian orthoreovirus than the plaque assay method. To determine infectivity using ICC-PCR, the CT value for a $T = 7$ sample was compared to the corresponding CT value for the $T = 0$ sample. If the CT value for $T = 7$ was lower, it was concluded that the lower CT value was from an increased con-

centration of target viral nucleic acid, indicating viral replication and infectious virus. Evaluation of the results shows that incubation on cells yielded infectious virus and upon comparison to the plaque assay, demonstrates the limitations of using the plaque assay as the method of choice when evaluating biosolids for infectious virus.

All of the seeded dilutions from 10^{-3} to 10^{-8} contained no detectable virus at $T = 0$ in the ICC-PCR and all contained detectable virus at $T = 7$, which means that each of the dilutions contained at least one virus particle that was able to replicate itself. While it is possible that there was only one infectious virus particle in each of the sample tubes, it is unlikely given that each sample tube was a 10-fold dilution of the previous. Of the dilutions that contained virus at $T = 7$, dilutions 10^{-6}, 10^{-7}, and 10^{-8} did not form plaques in the plaque assay. The samples were taken from the exact same tube to minimize dilution inaccuracies. However, there is a larger sample amount tested in a plaque assay then in a PCR tube, and therefore this comparison is somewhat biased to the plaque assay and underestimates the sensitivity of the ICC-PCR. If the three logs detected at $T = 0$ are subtracted from the eight logs detected at
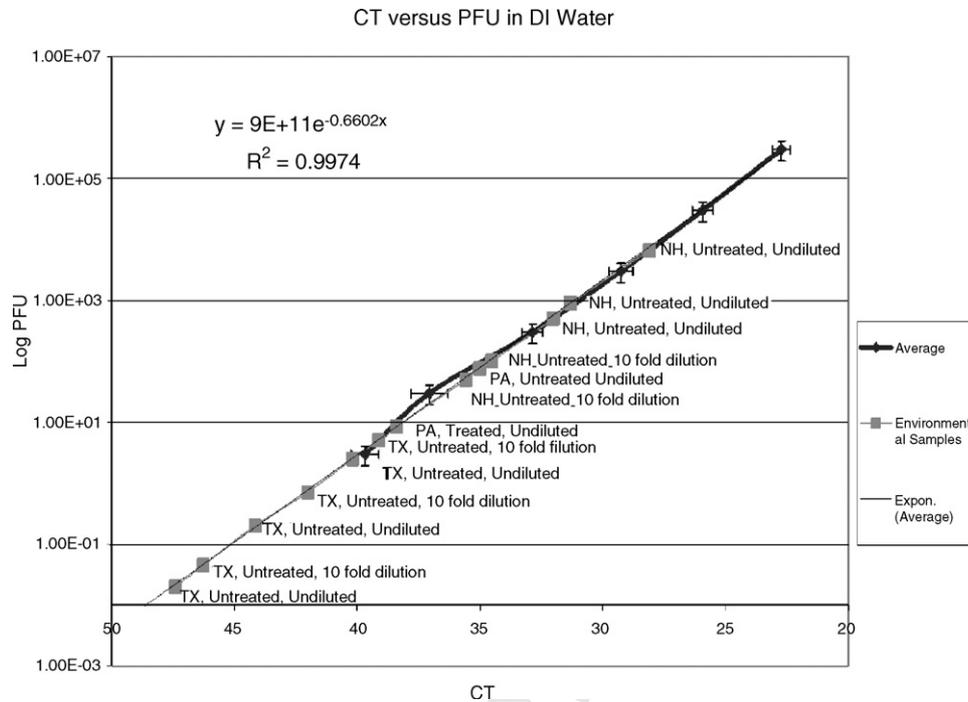
Fig. 4. Relationship between PFU and CT. The scatter plot of the average PFU vs. the CT demonstrates a relationship. The environmental samples are placed on the line created by this relationship and their final concentrations can be estimated.

$T = 7$, this is an increase of six logs. This data could be interpreted as only a one log difference between the ICC-PCR and the plaque assay. However, because each tube was a 10-fold dilution and contained decreasingly smaller amounts with more than one infectious virus present in each dilution, it is better to compare the $T = 0$, $T = 7$, and plaque assay within each dilution. Even if only one log more was detected, the seeded samples demonstrated that the ICC-PCR was a more sensitive method.

One of the problems with plaque assays is cellular damage due to toxicity. This is a problem with the plaque assay because it does not directly detect any part of the virus, but rather points to viral presence by cell lysis. This makes interpretation of the plaque assay more questionable than the ICC-PCR method as described in the study. The ICC-PCR directly detects the viral nucleic acid and for the final analysis the sample can be cleaned to a greater degree because removing the viral capsid proteins does not affect PCR. If there were some toxic effects of the sample, they would have occurred in the cell culture portion of the experiment, and the amount of cells available for infection overcomes this issue.

Similar to the seeded samples, the ICC-PCR method was more sensitive than the plaque assay with the environmental samples. The samples did not plaque, but 54% tested positive for reovirus. For locations where the first three samples of a treatment type yielded a positive result when undiluted samples were used, the sample was diluted 10-fold. A large portion of the samples were positive at the more dilute concentrations indicating the potential for a very small amount of sample to multiply to detectable levels of virus. Based on a positive result, it is not possible to know the exact amount of virus in the biosolids and the small sample size should not be misleading as it relates to

risk when consideration is given to the total amount of material applied to a field.

The three sets of primers and probes allowed for differentiation between the types of reovirus detected. In addition to regional variation, the differential detection of the virus types may also be a reflection of different original starting concentrations or differential growth rates among the types after 7 days of incubation.

The correlation between the CT from the real-time PCR and the PFU from the plaque assay can be seen on the scatter plot. The line was extended and the environmental samples were plotted on the line to estimate the amount of virus detected in the ICC-PCR. This is the amount of virus present in the sample after 7 days incubating in cell culture. There should exist a relationship between the amount of virus in the sample before incubation and the amount of virus in the sample after incubation, but that relationship was not determined in this study and needs further exploration. In this study, samples were evaluated on a semi-quantitative basis using a series of dilutions. Ultimately it may be possible to correlate the amount of virus at some incubation time to an original starting concentration to make ICC-PCR truly quantitative.

Very few studies have used ICC-PCR to detect wastewater viruses. Only one other study known to the authors has used a quantitative ICC-PCR method. Astrovirus was quantified using a dilution technique and detected by PCR (Grimm et al., 2004). This study did not compare the results to a cell culture method.

Previously published work clearly demonstrates that ICC-PCR is more effective than both the plaque assay method and the total culturable virus assay-most probable number assay (TCVA-MPN) for the detection of virus.

Several other studies have been done in a water matrix as compared to a sludge or bisosolids matrix. In one such study, poliovirus and hepatitis A virus was detected by ICC-PCR quicker than cell culture alone (Reynolds et al., 1997). Another study found ICC-PCR to be more rapid and more sensitive for virus detection than looking for cellular cytopathic effects (CPE) (Reynolds et al., 1996). In another study, ICC-PCR detected enterovirus and adenovirus in 13 samples while the TCVA-MPN assay did not detect any virus (Lee et al., 2005). When water samples from across the United States were tested for enteroviruses, adenoviruses and astroviruses comparing the TCVA-MPN method to ICC-PCR, ICC-PCR detected viruses in 48% more samples than TCVA-MPN (Chapron et al., 2000).

Previous work using seeded samples has also been done. In water seeded with adenovirus, ICC-PCR occurred more rapidly than virus detection by the TCVA-MPN assay (Ko et al., 2003). In a study where samples seeded with poliovirus were UV disinfected and then tested for virus using ICC-PCR and TCVA-MPN, ICC-PCR detected poliovirus at the later time points, where TCVA-MPN did not (Blackmer et al., 2000). In another seeded study, researchers compared two different ICC-PCR methods of virus detection to a $TCID_{50}$ assay for the detection of hepatitis A virus. They demonstrated that hepatitis A virus was still infectious by ICC-PCR but not by the more traditional $TCID_{50}$ method 60 h after treatment (Jiang et al., 2004).

In samples of sewage, marine water, and surface water Hepatitis A virus and enteroviruses were detected by ICC-PCR more rapidly than TCVA-MPN (Reynolds et al., 2001). In detecting enteroviruses and adenoviruses in sewage, sludge, river water, and shellfish in New Zealand, ICC-PCR detected more adenoviruses than the plaque assay, although more enteroviruses were detected by the plaque assay than ICC-PCR. However, only the media was removed for testing and therefore the cell layer was not tested, and this may have affected the ICC-PCR results as viruses are intracellular (Greening et al., 2002).

Reovirus has been studied extensively because of its presumed prevalence in water and biosolids (AWWA, 1999). Prior to this study, detection by plaque assay has underestimated the amount of reovirus present. This study has shown that the ICC-real-time RT-PCR method can be used to determine reovirus presence and concentration. With this new method for detection and quantification of infectious reovirus, further work needs to be done to estimate the risk of land applied biosolids.

Uncited reference

Flint et al. (2000).

References

AWWA (American Water Works Association), 1999. Waterborne pathogens. AWWA Manual of Water Practices M48. American Water Works Association, Denver, CO.

Blackmer, F., Reynolds, K.A., Gerba, C.P., Pepper, I.L., 2000. Use of integrated cell culture-PCR to evaluate the effectiveness of inactivation by chlorine. Appl. Environ. Microbiol. 66, 2267–2268.

Brabants, J.J., 2003. Evaluation of bacteriophage and viral persistence during alkaline stabilization in sludge and biosolids intended for land application. Ph.D. Thesis. University of New Hampshire, Durham.

Chapron, C.D., Ballester, N.A., Fontaine, J.H., Frades, C.N., Margolin, A.B., 2000. Detection of astroviruses, enteroviruses, and adenovirus types 40 and 41 in surface waters collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. Appl. Environ. Microbiol. 66, 2520–2525.

EPA, 1992. Control of Pathogens and Vector Attraction in Sewage Sludge (Including Domestic Septage) Under 40 CFR Part 503. US Environmental Protection Agency, No. EPA/525/R-92/013. Government Printing Office, Cincinnati, OH.

Fenner, F., White, D.O., 1986. Medical Virology, third ed. Academic Press Inc., New York.

Flint, S.J., Enquist, L.W., Krug, R.M., Racaniello, V.R., Skala, A.M., 2000. Principles of Virology, Molecular Biology, Pathogenesis, and Control. ASM Press, Washington, DC.

Fong, T.T., Lipp, E.K., 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiol. Mol. Biol. Rev. 69, 357–371.

Golden, J.W., Linke, J., Schmechel, S., Thoemke, K., Schiff, L.A., 2002. Addition of exogenous protease facilitates reovirus infection in many restrictive cells. J. Virol. 76, 7430–7443.

Greening, G.E., Hewitt, J., Lewis, G.D., 2002. Evaluation of integrated cell culture-PCR (C-PCR) for virological analysis of environmental samples. J. Appl. Microbiol. 93, 745–750.

Grimm, A.C., Cashdollar, J.L., Willams, F.P., Fout, G.S., 2004. Development of an astrovirus RT-PCR detection assay for use with conventional, real-time, and integrated cell culture/RT-PCR. Can. J. Microbiol. 50, 269–278.

Jiang, Y.-J., Liao, G.Y., Zhao, W., Sun, M.-B., Qian, Y., Bian, C.-X., Jiang, S.D., 2004. Detection of infectious Hepatitis A virus by integrated cell culture/strand-specific reverse transcriptase-polymerase chain reaction. J. Appl. Microbiol. 97, 1105–1112.

Katz, B.D., 2005. Evaluation of bacteriophage and enteric virus inactivation in alkaline stabilized biosolids intended for land application. M.S. Thesis. University of New Hampshire, Durham.

Ko, G., Cromeans, T.L., Sobsey, M.D., 2003. Detection of infectious adenovirus in cell culture by mRNA reverse transcriptase-PCR. Appl. Environ. Microbiol. 69, 7377–7384.

Lee, H.K., Jeong, Y.S., 2004. Comparison of total culturable virus assay and multiplex integrated cell culture-PCR for reliability of waterborne virus detection. Appl. Environ. Microbiol. 70, 3632–3636.

Lee, S.-H., Lee, C., Lee, K.W., Cho, H.B., Kim, S.-J., 2005. The simultaneous detection of both enteroviruses and adenoviruses in environmental water samples including tap water with an integrated cell culture-multiplex-nested PCR procedure. J. Appl. Microbiol. 98, 1020–1029.

Matsuura, K., Ishikura, M., Nakayama, T., Hasegawa, S., Morita, O., Uetake, H., 1988. Ecological studies on reovirus pollution of rivers in Toyama Prefecture. Microbiol. Immunol. 32, 1221–1234.

Metcalf, T.G., Melnick, J.L., Estes, M.K., 1995. Environmental virology: from detection of virus in sewage and water by isolation to identification by molecular biology—a trip of over 50 years. Annu. Rev. Microbiol. 49, 461–487.

Payment, P., Trudel, M., 1985. Influence of inoculum size, incubation temperature, and cell culture density on virus detection in environmental samples. Can. J. Microbiol. 31, 977–980.

Qiagen, 1999. QIAamp Viral RNA Mini Kit Handbook 01/99. QIAGEN Worldwide, Valencia, CA.

Reynolds, K.A., 2004. Integrated cell culture/PCR for detection of enteric viruses in environmental samples. Methods Mol. Biol. 268, 69–78.

Reynolds, K.A., Gerba, C.P., Pepper, I.L., 1996. Detection of infectious enteroviruses by an integrated cell culture-PCR procedure. Appl. Environ. Microbiol. 62, 1424–1427.

Reynolds, K.A., Gerba, C.P., Abbaszadegan, M., Pepper, I.L., 2001. ICC/PCR detection of enterovirus and hepatitis A virus in environmental samples. Can. J. Microbiol. 47, 153–157.

Reynolds, K.S., Gerba, C.P., Pepper, I.L., 1997. Rapid PCR-based monitoring of infectious enteroviruses in drinking water. Water Sci. Technol. 35, 423–427.

Spinner, M.L., DiGiovanni, G.D., 2001. Detection and identification of mammalian reoviruses in surface water by combined cell culture and reverse transcription-PCR. 67, 3016–3020.

Ward, R.L., Ashley, C.S., 1978. Heat inactivation of enteric viruses in dewatered wastewater sludge. Appl. Environ. Microbiol. 36, 898–905.

Protecting water supply quality through improved watershed planning and management

Basic Information

Title:	Protecting water supply quality through improved watershed planning and management
Project Number:	2006NH52B
Start Date:	3/1/2006
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	NH01
Research Category:	Social Sciences
Focus Category:	Management and Planning, Economics, Law, Institutions, and Policy
Descriptors:	None
Principal Investigators:	John M Halstead, Thomas P. Ballestero

Publication

Title: "Protecting Water Supply Quality through Watershed Planning and Management"
Principal Investigator: John M. Halstead, Department of Resource Economics and Development, University of New Hampshire

1. Problem

Management plans for natural resources can be seen as multi-attribute goods that involve many different interest groups (including specific stakeholders, experts, and public citizens) and numerous decision criteria. In recent years it has become more accepted that the management of scarce or sensitive natural resources requires both expert and public input. The former provides a basis for understanding natural systems and forecasting the likely outcomes of different policy or management options, while the latter is necessary to ensure that public values and concerns are incorporated in management alternatives and that local knowledge is considered. This research is part of a larger natural resource management project in which there are very structured procedures for the involvement of experts. However, the public and stakeholder involvement methods were much less developed. Much of the research on public participation in environmental decision-making states that traditional methods of public hearings and public comment periods are not effective on their own as a means for involving these groups in the decision process.

With increasing population and development comes increased demand for water by all members of society including businesses, landowners, and local citizens. As with most environmental policy issues, balancing competing demands of all river water users can often be contentious and confrontational. The state of New Hampshire has recognized the need to address the challenge of river management and in 2002, a broad coalition of New Hampshire business and conservation interests joined together to enact compromise legislation which

became Chapter 278, Laws of 2002 (from House Bill 1449-A). The legislation calls for a pilot program for instream flow protection on two of the fourteen designated rivers - the Lamprey River in the coastal watershed and the Souhegan River in the Merrimack watershed. In order to manage important water resources. The Instream Flow Study is a highly technical process that involves engineers, hydrologists, and biologists. However, there is limited involvement of public and stakeholder values in the study.

2. Objectives

This research developed a management model for assessing the needs and values of water users and watershed residents. The objective was to create and implement a decision analytic approach to river management that involves stakeholders and citizens with experts. To maintain the health and maximal functionality of a scare resource, states across America are developing water management plans.. This research set out to create a framework for structuring a water management plan that includes expert, stakeholder, and public opinions while providing information to and offering recommendations for the Lamprey Instream Flow Study and Water Management Plan development. In the pages to come, recommendations for a more balanced, structured, and inclusive water management process will be made based upon research findings.

3. Methods

A series of social science research methods, including interviews and two survey tools, were used to involve the public and stakeholders in the process while eliciting their values about the river. Interviews focused on gaining local knowledge about how stakeholders (those that were designated by the New Hampshire Department of Environmental Services as affected water users, affected dam owners, and others including watershed association members, local and university officials). A stakeholder survey followed the interviews and was meant to verify

interpretations from the interviews as well as prioritize potential conflicts in the management of the Lamprey River. Next, a larger, survey was sent to 1,000 randomly selected public residents of the Lamprey River watershed. The survey utilized a modified Dilman approach and included many qualitative and quantitative questions that helped to prioritize aspects of river management. Because natural resource management requires tradeoffs, conjoint analysis was utilized to force respondents to make tradeoffs among hypothetical water management plan alternatives. Respondents were asked to rank hypothetical water management plans from one to 10 with , one being the most preferred and 10 being the least preferred. From this ranking, the application of conjoint analysis allows the determination of part worths for each of the four attributes of a water management, which were ecological health, recreational use, community business use, and withdrawal amounts.

4. Major Findings and Significance

Results from the stakeholder interviews and surveys indicated that the stakeholders have a complex relationship with the river. For example, they may use the river as a crucial part of their business production while at the same time enjoy fishing in the Lamprey River. The respondents to the public watershed survey (we obtained a 25% response rate) indicated that they were much more focused on the ecological and public water supply components of the river. This process allowed the identification of potential conflicts in the watershed when it comes for the actual implementation of the water management plan. Additionally, it identified and characterized the values and perspectives of stakeholders and the public. In general, recommendations for a more balanced, structured, and inclusive water management process are being made to the state based on the findings of the interviews and surveys. Because the Lamprey River study is part of a pilot program for the State, we recommend that future Instream

3

Flow Studies incorporate stakeholder and public values in these studies. We also make recommendations that are applicable to all natural resource managers and these include encouraging managers and decision makers to involve stakeholders and the public in the decision process early and throughout, utilizing a structured decision analysis process that focuses on values, assessing areas of possible conflict and evaluating management alternatives in a manner that is comprehensive to the public, experts, and other decision makers.

5. Publications, presentations, awards

Research findings presented at the following conferences:

-2nd Potawatomi Student Industrial Ecology Conference, Angola, Indiana. March 23-25, 2007

-NH Water Conference, Concord, NH, April 9, 2007

-Northeast Agricultural and Resource Economics Association Meeting, Rehoboth Beach, DE
June 10-13, 2007

-United States Society for Ecological Economics Conference, New York, NY. June 24-26, 2007

1. Number of students supported: Ms. Shannon Rogers, MS Degree in Resource

Administration and Management granted May, 2007. Currently pursuing PhD in Natural

Resources at University of New Hampshire.

Water Quality Change-Effects of Development in Selected Watersheds

Basic Information

Title:	Water Quality Change-Effects of Development in Selected Watersheds
Project Number:	2006NH60B
Start Date:	3/1/2006
End Date:	2/28/2008
Funding Source:	104B
Congressional District:	NH01
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Nutrients, Surface Water
Descriptors:	
Principal Investigators:	Jeffrey Schloss

Publication

2006 WRRC Report for project dates March 1, 2006 to February 28, 2007:

Title- **Water Quality Change:-Effects of Development on Nutrient Loading in Selected Watersheds**

PI: Jeffrey Schloss, Extension Professor in Zoology (UNH Center for Freshwater Biology) and Water Resources Specialist, UNH Cooperative Extension.

Statement of regional or State water problem-

The waters of New Hampshire represent a valuable water resource contributing to the state's economic base through recreation, tourism, and real estate revenues. Some lakes and rivers serve as current or potential water supplies. For most residents (as indicated by boating and fishing registrations and shoreline re-development) our waters help to insure a high quality of life. As documented in the 2000 Census, New Hampshire currently leads all of the New England states in the rate of new development and redevelopment. The long-term consequences of the resulting pressure and demands on the state's precious water resources remain unknown. Of particular concern is the response of our waters to increasing non-point source pollutant loadings due to watershed development and land use activities.

Of all the in-depth watershed nutrient budget measurements and modeling efforts that have been attempted in NH none have primarily focused on change detection due to development as they were either base-line studies on relatively pristine lakes or focused on specific problems such as internal nutrient loading from past sewage outfalls, or septic systems in the water table of a seepage lake. In addition nitrogen species were only monitored for less than a handful of studies and the measurement technologies at the time were not sensitive enough to provide much usable data. The opportunity to add nitrogen monitoring and support GIS land change analysis to co-occurring externally funded phosphorus watershed nutrient budget studies on two lakes that had previous budgets done in the past provides a true cost-effective project that directly addresses Statewide concerns.

Alone, these watershed nutrient budgets represent only short-term examinations of non-point source pollution nutrient loadings to the lake. A longer-term monitoring program conducted through differing weather years at both shallow and deep sites is required to best estimate the lake response to the loadings due to development over time.

Nature, scope and objectives of the project and task status .-

This project has allowed for the continued collection of long-term water quality data over a substantial spatial and temporal scale. Both project components employed utilize a combination of students and volunteer citizen water quality monitors to collect samples (and preserve for analysis) from a wide range of lake and stream watersheds throughout the state which are part of the NH Lakes Lay Monitoring Program (LLMP), a 28 year long-term sampling effort.

Emphasis on LLMP efforts has been to maintain and expand tributary monitoring for participating lakes to allow for long-term change detection through a number of years with differing weather regimes.

As other funding sources are already available for water quality analysis costs and related expenses, funding from this project will provide support for student lab and field technicians and for supervision, data management and data analysis by the project director.

NOTE: We have envisioned this effort as providing the foundation to further assess the impacts of land use and the effectiveness of watershed management strategies using long-term data sets in future years.

To summarize, objectives of this study include:

1. To add nitrogen analysis to an already funded project developing a water and total phosphorus budget for a lake watershed that has experienced land cover change since a previous study was undertaken over a decade ago.

Status: Project has been initiated, QAPP was approved by EPA New England in the Fall of 2006 and sampling was started in November of 2006.

2- The continued collection and analysis of long-term water quality data in selected watersheds.
Status: 12 new monitors trained directly, 20 indirectly; 50+ monitors re-trained and tested, thirty eight additional tributary sites added on Newfound Lake and Mendums Pond as part of a Water/Nutrient Budget for the respective lakes (to add to Winnipesaukee and Bow Lake efforts). Over 950 deep lake site trips by volunteers; 82 deep lake site trips by CFB field team (students and faculty),596 shallow lake and tributary site trips made by volunteers; 104 tributary sample trips made by CFB team.

3- The dissemination of the results of the analysis to cooperating agencies, water managers, educators and the public on a local, statewide and regional basis.
Status:
        Reports:
30 Individual Reports produced for each lake and or watershed association participating;
"Winnipesaukee Tributary Study Analysis Report" *provided to the Lake Winnipesaukee Association*
        Peer reviewed reports:
"Quality Assurance Project Plan for the Mendum's Pond Watershed Study" *(November 2006)*
        Public Presentations:
"The State of the Watershed" *to the Saco River monitoring program of the Green Mountain Conservation Group (Ossippee Watershe,) November 2006.*
"A Summary of the Winnipesaukee Tributary Sampling Effort Results" *to a joint public meeting of the Meredith, Laconia and Guilford Town Boards (October 2006).*
Over a Quarter Century of Lake Monitoring: What have we learned? Invited presentation NH Lakes Congress (June 2006)
"Meredith Bay, Winnipesaukee Monitoring Overview"-*special televised Selectman's meeting, Meredith NH (March 2006)*
        Class Presentations:
Introduction to Water Resources Management (Natural Resources)- "NH Lakes: Issues and Concerns"
Lake Biology (Zoology/Plant Biology)- "The NH Lakes Lay Monitoring Program"
Watershed Ecology (Natural Resources)- "Lake Ecology", "Lake Water Quality Sampling" and "Following the Flow: Nonpoint Source Pollution in NH Watersheds"
Multidisciplinary Lakes Management (Zoology/Plant Biology)- "Analysis a long term datasets"
        Academia /Professional Societies:
Plymouth State University (invited seminar) "The Squam Lakes Watershed: Analysis of 25 Years of Monitoring"
North American Lakes Management Society/EPA (invited) – Choosing a Sampling Scheme for the National Lakes Assessment: Stressors and Indicators" (April 2006)

4- To offer undergraduate and graduate students the opportunity to gain hands-on experience in water quality sampling, laboratory analysis, data management and interpretation.
Status: See table below- 9 undergraduate and 4 graduate students were directly involved

5- To further document the changing water quality in a variety of watersheds throughout the state in the face of land use changes and best management efforts.
Status: See above – in process

6- To determine next steps for further analysis of long-term data sets and GIS spatial data on land cover.
Status: in process to be done at end of next study year.

In addition we have secured additional funding to support a masters student (assistantship for two year) who will focus on the groundwater component of the Mendum's Pond Study and for extended sampling of total phosphorous in the Newfound Lake Watershed.

Expected Activity Timeline for 2007 effort:

March - April 2007	Continue to modify and update water quality and GIS databases.
	Initiate run-off sampling and seepage (Mendums Pond).
April - June 2007	Continue to manage volunteer, student and staff sampling efforts.
	Analyze samples collected
	Continue to modify and update water quality and GIS databases.
	Work with NH DES to facilitate data sharing for:
	Statewide database
	Shared web site
	STORET uploads
Sept 2007- Feb 2008	Analyze results in lab, prepare summaries and reports

Methods, procedures and facilities:-

An EPA approved QAPP (Schloss 2006) for the watershed water/nutrient budget is being followed that includes volunteer sample collection and gage readings and student technicians sampling and conducting stream flow measurements using a Doppler water velocity meter (SonTek/YSI).

Lake and stream monitoring through the LLMP generally involves a minimum of monthly sampling starting at spring runoff through to lake stratification and weekly to bi-weekly sampling through to fall mixis. Water clarity, chlorophyll a, acid neutralizing capacity, dissolved organic color, dissolved oxygen and nutrients (total N, total P and nitrate) will be the default suite of parameters measured for lakes while nutrients, turbidity, dissolved organic color and flow will be the parameters of choice for the lake tributary work. On occasion, student field teams will travel to join the volunteer monitors to perform quality assurance checks and do more in-depth analysis and lake profiling.

As stated above the primary scope of this project is to maintain the long-term data collection effort but in addition, land cover changes to study subwatersheds will be documented on our established GIS data base and any new management practices or conservation efforts will also be documented

This project will be coordinated from the University of New Hampshire, which will supply the office and laboratory space (analytical and computer). The Center for Freshwater Biology Analytical Water Quality Laboratory has a Quality Assurance Project Plan for surface water analysis on file with the US Environmental Protection Agency Region 1 Office (EPA New England). Besides nutrient analysis (Total Phosphorus, Total Nitrogen, Nitrate), other water quality capabilities include chlorophyll a, dissolved oxygen, dissolved CO_2, acid neutralizing capacity, specific conductivity, pH, ORP, turbidity, water clarity, iron and E.coli. The lab can also provide field sampling, field water quality instruments, automated data loggers and water velocity measurement equipment. The lab can also provide the use of Real-Time Differential GPS units for watershed surveying and ground truth sampling.

The Water Resource Center Laboratory, which follows standard methods, has the capability to perform ion chromatography for a variety of anions and cations, organic carbon analysis and total nitrogen analysis. It also can provide field and automated sampling equipment.

UNH Cooperative Extension will provide vehicles for travel for PI's, students and interns at a cost (mileage) basis. A dedicated GIS PC Windows XP workstation will be provided for use including ArcGIS and ArcView Software, ArcView Extensions: Spatial Analyst, 3-D Analyst, Image Analysis and ArcPress. This will be used in addition to other data input PC stations, laser printers and a large format (36" wide) ink jet plotter that will be made available for the project.

The project will utilize an extensive GIS database for the study subwatersheds created through previous WRRC funding to the PI. Updated and additional GIS data can be made available through the UNH Complex Systems Research Center, which manages the NH GRANIT statewide GIS data depository. The extensive data directory contains statewide GIS data layers (usually at 1:24,000 scale) including hydrology, geology, soils, National Wetlands Inventory, land-use, land cover, and digital elevation models. Also available are Landsat Thematic Mapper, SPOT Panchromatic and digital orthophoto imagery.

Students impacted for 2006-2007 (supported by and/or worked on aspects of project)

Field of Study	Undergraduate	Masters	Ph.D.	**Total**
Marine and Freshwater Biology	2	1		3
(Plant Biology or Zoology)				
Microbiology	1			1
Environmental Conservation	1			1
Water Resources	2	1	1	4
(Natural Resources or Hydrology Dept.s)				
Extension Education (Colorado State)		1		1
English	1			1
Psychology	1			1
Undecided	1			1
TOTAL:	**9**	**3**	**1**	**13**

Major Findings:
As the study is currently in the data collection phase we do not have any major findings to report to date.

Grant No. 06HQGR0143 Determining the Effectiveness of the Clean Air Act and Amendments for the Recovery of Surface Waters in the Northeastern U.S.

Basic Information

Title:	Grant No. 06HQGR0143 Determining the Effectiveness of the Clean Air Act and Amendments for the Recovery of Surface Waters in the Northeastern U.S.
Project Number:	2006NH86S
Start Date:	3/6/2006
End Date:	3/5/2011
Funding Source:	Supplemental
Congressional District:	1
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Steve Kahl, William H. McDowell

Publication

1. Hunt, K., J.S. Kahl, J. Rubin, and D. Mageean, 2007. Assessing the science-based needs of stakeholders; a case study on acid rain research and policy. Journal of Contemporary Water Research and Education, in press.
2. Rosfjord, C., K. Webster, J.S. Kahl, S.A. Norton, I. Fernandez, and A. Herlihy, 2007. Anthropogenically-driven changes in chloride complicate interpretation of base cation trends in lakes recovering from acidic deposition. Environmental Science and Technology, in press.

Annual Report to

USGS WRD WRRI, Reston, VA and US EPA, Corvallis OR

March, 2007

Determining the effectiveness of the Clean Air Act and Amendments on the recovery of surface waters in the northeastern US

IAG 06HQGR0143

Principal Investigators: *Steve Kahl[1], Katherine Webster[2], and Bill McDowell[3]*
[1]Plymouth State University
[2]University of Maine
[3]University of New Hampshire

Overview of activities this period

A summary of progress on the project plan are provided below and discussed on the following page:

Project Activity	2006 Q2	2006 Q3	2006 Q4	2007 Q1	2007 Q2	2007 Q3	2007 Q4	2008 Q1	2008 Q2	2008 Q3	2008 Q4	2009 Q1	2009 Q2	2009 Q3	2009 Q4	2010 Q1	2010 Q2	2010 Q3	2010 Q4	2011 Q1
project period	■	■	■	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
funding received	■	■	■	■																
RLTM outlets				░				▒				▒				▒				▒
RLTM drainage lakes		■	■		▒	▒	▒		▒		▒		▒	▒			▒	▒	▒	
RLTM seepage lakes		■	■		▒	▒			▒		▒		▒	▒			▒	▒	▒	
original LTM lakes			■					▒				▒				▒				
HELM subset			■					▒				▒				▒				
BBWM - EB	■	■	■	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
TIME lakes		■				▒			▒			▒			▒			▒		
sample analyses		■	■	■		▒		▒		▒		▒		▒		▒		▒		▒
zooplankton analyses			▒	▦	▒	▒														
progress report				■				▒				▒				▒				▒
annual data report	■				▦															

▒ = project plan   ▦ = in progress   ■ = completed   ░ = activity cancelled

Field sampling. Field sampling for all main project components recommenced in the summer of 2006 upon receipt of funding in late spring. All project field objectives in the summer and fall of 2006 were accomplished as planned. Data collection had continued despite the project being in non-cost extension during 2004 and 2005. Spring sampling for all lakes in Maine will commence in April.

Analytical. Analyses are complete for all samples collected through the end of 2006, except for aluminum which has an extended holding time. PSU has ordered an AAS HGA instrument for analysis of Al, due to the last minute closure of the laboratory at UMaine that previously served this project. Inter-laboratory comparisons were completed between PSU, UNH, and UMaine (prior to closure of the Mitchell Center laboratory).

Samples from East Bear Brook at BBWM, which are collected on a regular basis year around, are being analyzed in a contract laboratory at UMaine. It is not yet known if this laboratory will be able meet EPA DQOs or if they will provide an annual QA report.

Zooplankton analyses. Samples from 1986 have been archived and checked. A small number of sample bottles had broken or leaked. We have gained access to a FlowCam (Fluid Imaging, Inc.) for sizing the zooplankton and received training from staff at the EPA laboratory in Chelmsford who are using a similar instrument to analyze their zooplankton samples. This would save significant time in processing samples. We plan to run the 1986 and 2004 size spectra and cladoceran species analyses over the spring and summer. The EPA lab in Chelmsford has also made arrangements for a permanent archiving of the zooplankton samples at Yale University.

Data reporting. All data collected through 2004 have been delivered to EPA. The next delivery of data to EPA is expected to be done in late spring, after evaluation of inter-laboratory comparisons.

Presentation of findings. Several publications have resulted from this project since the final report for the previous LTM/TIME grant.   Several presentations have also been made to a variety of audiences, including a talk invited by EPA OAR at the 2005 ESA annual meeting, and a talk invited by EPA OAR at the 2006 international mercury meeting that developed recommendations for national Hg monitoring based on the success of TIME/LTM.   Publications and talks are listed at the end of this report.

Project status: We have requested funding for field season 2007, and are aware that there is uncertainty for funding in FY08. Therefore, we are planning to not sample the outlets of drainage lakes in the spring of 2007 to facilitate the option of stretching the funding out into 2008 for baseflow sampling for RLTM lakes and summer sampling for TIME lakes. The outlet work is expensive and difficult, and we believe that this option provides for more cost-effective use of funds under the present uncertain scenario.

Project overview

Objectives. This proposed research is part of the EPA program to collect long-term data on the trends and patterns of response in surface waters sensitive to acidic deposition.   The goals and methods are hierarchical from intensive site-specific to statistical regional statistical populations. The objectives are to:

1) document the changes and patterns in aquatic chemistry for defined sub-populations and sites that are known to be susceptible to acidification or recovery,
2) evaluate linkages in changes in surface waters, if any, to changes in deposition that are related to regulatory goals;
3) characterize the effectiveness of the Clean Air Act Amendments in meeting goals of reducing acidification of surface waters and improving biologically-relevant chemistry in the northeastern US, and
4) provide information for assessment of the need for future reductions in atmospheric deposition based on the rate of recovery (or not) of the systems under study.

In 2007, we will also evaluate changes in biological condition using zooplankton collected in 2004 from 145 ELS-II lakes in the northeast, as part of our 20th anniversary re-analysis of the Eastern Lake Survey.

Approach. The schedule of tasks ranges from weekly to annual, continuing data records that range from 12 to 22 years. We will evaluate chemistry on a weekly basis year-round at the small watershed-scale at BBWM, weekly during the spring melt period at LTM lakes outlets, quarterly in LTM, and during an annual index period for the HELM and TIME lakes.   These project components provide a *statistical framework* for inferring regional chemical patterns using TIME and LTM (and ELS-II under separate funding). The *long-term records* of LTM, HELM and BBWM provide seasonal and annual variability information, and provide seasonal context for the annual surveys.

Expected Results. This information is fundamental for EPA to meet the Congressional mandate for reporting on the effectiveness of the Clean Air Act Amendments (CAAA). The highly effective combination of site-specific data within the regional context will provide for the recognition and understanding of declining SO_4, base cation depletion, and changes in N-saturation or DOC contributions to acid-base status.   The results are also central to the decisions on additional emission reductions that may be needed to produce recovery.

Recent publications using project information

Campbell, J, J. Hornbeck, M. Mitchell, M. Adams, M. Castro, C. Driscoll, J.S. Kahl, and others, 2004. Input-output budgets for inorganic nitrogen for 24 watersheds in the northeastern United States. Water Air Soil Pollut., 151:373-396.

Dupont, J., T. Clair, C. Gagnon, D. Jeffries, J.S. Kahl, S. Nelson, and J Peckenham, 2005. Estimation of critical loads of acidity in the northeastern US and eastern Canada. Environ. Monit. Assess. 109:275-291.

Hunt, K., J.S. Kahl, J. Rubin, and D. Mageean, 2007. Assessing the science-based needs of stakeholders; a case study on acid rain research and policy. Journal of Contemporary Water Research and Education, in press.

Kahl, J.S., J. Stoddard, R. Haeuber, S. Paulsen, R. Birnbaum, F. Deviney, D. DeWalle, C. Driscoll, A. Herlihy, J. Kellogg, P. Murdoch, K. Roy, W. Sharpe, S. Urquhart, R. Webb, and K. Webster, 2004. Response of surface water chemistry to changes in acidic deposition: implications for future amendments to Clean Air Act. Environmental Science and Technology, Feature Article 38:484A-490A.

Lawler, J., J. Rubin, B.J. Cosby, I. Fernandez, J.S. Kahl, S. Norton, 2005. Predicting recovery from acidic deposition: Applying a modified TAF (Tracking Analysis Framework) Model to Maine' High Elevation Lakes, Water Air Soil Pollut. 164:383-389.

Norton, S., I. Fernandez, J.S. Kahl, and R. Reinhardt, 2004. Acidification trends and the evolution of neutralization mechanisms through time at the Bear Brook Watershed, Maine, USA. Water, Air, Soil, Pollution Focus 4:289-310.

Rosfjord, C., K. Webster, J.S. Kahl, S.A. Norton, I. Fernandez, and A. Herlihy, 2007. Anthropogenically-driven changes in chloride complicate interpretation of base cation trends in lakes recovering from acidic deposition. Environmental Science and Technology, in press.

Recent presentations using project information

Kahl, J.S. 2006 (invited). Acid rain in New England: using high elevation lakes as sentinels of change. Maine Mountain Conference, October 21, 2006. Rangeley, Maine

Kahl, J.S., *et al.*, 2006 (invited). The design of a national mercury monitoring network: Learning from the EPA acid rain experience. The Eighth International Mercury Conference, Madison WI, August 8, 2006.

Kahl, J.S. *et al.*, 2006. Obfuscation of trends in base cations by regional salt contamination. Hubbard Brook Committee of Scientists annual meeting, July 12, 2006.

Kahl, J.S., 2006 (invited). 'Natural and human-derived sources of acidity in Maine Atlantic Salmon Rivers'. Atlantic Salmon Commission workshop on acidity, Bangor ME. April 10, 2006.

Kahl, J.S., 2005 (invited). The intersection of environmental science and environmental policy. NH Charitable Foundation Lakes Region annual meeting, Meredith, NH, September, 2005.

Kahl, J.S., 2005 (invited). Tracking response and recovery in surface waters in the northeastern US. Annual meeting of the Ecological Society of America, Montreal, August, 2005.

Kahl, J.S., and Catherine Rosfjord, 2005 (invited). Acid rain and the Clean Air Act in the northeastern US. Annual meeting of the NH-ME Androscoggin River Watershed Council, Bethel, June, 2005

Kahl, J.S., 2005 (invited). Developing a lake research agenda for NH. NSF workshop on lake research infrastructure in the northeast, Colby Sawyer College, April 2005.

Kahl, J.S., S. Nelson, and A. Grygo, 2004. Surface water chemistry data for the northeastern US for interpreting climate and acid rain trends. Northeast Ecosystems Research Consortium meeting, Durham, NH, October, 2004.

Kahl, J.S., K. Webster, M. Diehl, and C. Rosfjord, 2004. Successes of the Clean Air Act Amendments of 1990. Maine Water Conference invited plenary talk, Augusta, ME, 2004.

Kahl, J.S. and K. Johnson, 2004. Acid-Base Chemistry and Historical Trends in Downeast Salmon Rivers.   Maine Water Conference, Augusta ME, April 2004.

Kahl, J.S., 2004 (invited).   The Clean Air Act Amendments of 1990; testing a program designed to evaluate environmental policy. Lecture, Colby College. April, 2004

Information Transfer Program

Seed Funding for the First Annual NH Water Resources Conference

Basic Information

Title:	Seed Funding for the First Annual NH Water Resources Conference
Project Number:	2005NH54B
Start Date:	3/1/2006
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	NH02
Research Category:	Not Applicable
Focus Category:	Education, Management and Planning, None
Descriptors:	None
Principal Investigators:	Jeffrey Kahl, Paul Currier, William H. McDowell, Kevin McGuire, Keith Robinson, Jeffrey Schloss

Publication

FINAL REPORT

Seed funding for a new New Hampshire Water Resources Conference

submitted by:
The Center for the Environment, Plymouth State University

June 27, 2007

<u>Principal Investigators</u>:
    Steve Kahl, *Plymouth State University*
    Keith Robinson, *USGS, Pembroke.*
    Paul Currier, *NHDES, Concord*
    Kevin McGuire, *Plymouth State University/US Forest Service*
    Jeff Schloss, *NH Cooperative Extension*
    Bill McDowell, *University of NH*

<u>Problem</u>:
New Hampshire faces a host of water resource-related issues, including flooding, drought, non-point source pollution, lake eutrophication, erosion and sedimentation, acid rain, mercury bioaccumulation, and invasive aquatic plants. Management, regulation, research, and education on these issues is handled by a myriad of organizations ranging from federal, state, and local governments, to NGOs, planning agencies, academia, and volunteers. Forums that foster communication among the disparate groups in NH with interests in water resources were lacking.

<u>Objectives</u>:
The primary objective of this project was to develop a **New Hampshire Water Conference** to provide an annual forum for water resource professionals, consultants, citizens, researchers, students, regulators, and planners to exchange information and present new findings on water resources issues in the state. An important role of the conference was to provide an opportunity for attendees to interact and establish an active network of water resource professionals.

<u>Methods</u>:
The first annual New Hampshire Water Conference was held on April 9, 2007 at the Grappone Conference Center in Concord. The conference drew over 200 people, including researchers, legislators, water system operators, land use planners, and government officials. Governor John Lynch spoke to the attendees about the importance of New Hampshire's water resources. Seven state legislators were in attendance.

The conference was organized by the Plymouth State University's Center for the Environment, NH Geological Survey, NH Department of Environmental Services, NH Water Resources Research Center at UNH, NH Water Works Association, US

Environmental Protection Agency, and US Geological Survey. The organizing team represented the diversity we hoped to attract to the conference:

Keith Robinson, Chair	US Geological Survey
Kevin McGuire, Co-Chair	Center for the Environment, Plymouth State University
Celia Chen	Department of Biological Sciences, Dartmouth College
Paul Currier	NH Department of Environmental Services
Trish Garrigan	US Environmental Protection Agency - New England
Brian F. Goetz	Weston and Sampson Engineers, Inc.
June Hammond Rowan	Center for the Environment, Plymouth State University
Steve Kahl	Center for the Environment, Plymouth State University
Bill McDowell	University of New Hampshire
Jeffrey Schloss	University of New Hampshire
David R. Wunsch	New Hampshire Geological Survey, NH DES

The conference theme was "*Sustainability of New Hampshire's Water Resources in a Developing Landscape.*" The current knowledge of the quality, quantity and use of water was examined through talks and sessions on the current conditions of New Hampshire's water resources, water demand trends, projected household costs for water, effects of climate change, and the sustainability and management of surface and ground water. The day closed with a panel discussion on the future outlook on the sustainability of our water resources.

Findings and Significance:
An evaluation form was distributed at the conference. On a scale of 1 to 5, 80% of the people completing the evaluation rated the conference as 4 or 5. Participants at the conference commented that the day provided needed information about the state's water. A copy of the evaluation results is attached.

Publications, presentations, awards:
Information about the conference was posted on a new conference website, www.nhwaterconference.org that includes speaker presentations. This site is maintained by the Center for the Environment.

Number of students supported (and degree level, undergrad, Master, PhD):
The Center for the Environment used other funding to support attendance at the conference for 8 master's students and one undergraduate student. In addition, there were 8 graduate students from UNH and other universities at the conference.

Summary:
Based on the success of the first New Hampshire Water Conference, we aim to make the conference an annual event. Planning is underway for the 2nd New Hampshire Water Conference on **April 16, 2008**. For more information, visit nhwaterconference.org.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	13	0	0	0	13
Masters	26	0	0	0	26
Ph.D.	2	0	0	0	2
Post-Doc.	1	0	0	0	1
Total	42	0	0	0	42

Notable Awards and Achievements

Data collected related to the "Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds" project have been used to heighten awareness of the impacts of excessive road salting and snow dumping in local streams. Communication with local road agents has led to the remediation in one development where road salting was an issue. Samples collected and data generated from this funding have shown an improvement in water chemistry following reduced salting and snow dumping.

The first annual New Hampshire Water Conference was held on April 9, 2007 at the Grappone Conference Center in Concord. The conference drew over 200 people, including researchers, legislators, water system operators, land use planners, and government officials. Governor John Lynch spoke to the attendees about the importance of New Hampshire's water resources. Seven state legislators were in attendance. The conference theme was Sustainability of New Hampshire's Water Resources in a Developing Landscape. The current knowledge of the quality, quantity and use of water was examined through talks and sessions on the current conditions of New Hampshire's water resources, water demand trends, projected household costs for water, effects of climate change, and the sustainability and management of surface and ground water. The day closed with a panel discussion on the future outlook on the sustainability of our water resources.

Publications from Prior Projects

None