

# Appendix 2. Methods Used to Analyze Water Quality of Four Watersheds in Eastern Puerto Rico

By Sheila F. Murphy and Robert F. Stallard

## Introduction

The U.S. Geological Survey's Water, Energy, and Biogeochemical Budgets (WEBB) program in Puerto Rico analyzed a core suite of chemical and physical measurements that would indicate the major sources and processes affecting water chemistry of the rivers. More than 6,600 samples from both routine sampling and storm events were collected and analyzed for chemical or physical parameters (or both) from 1991 to 2005 (appendix 1, its table 5). Standard methods of sample collection, processing, field measurements, and laboratory analysis were used throughout the project and are described here.

## Field Sampling

Two principal types of samples, grab and storm event, were collected during the study. Grab samples were initially collected quarterly, but sampling frequency varied depending on staff availability. Onsite measurements included pH, conductivity (microsiemens per centimeter,  $\mu\text{S cm}^{-1}$ ), temperature (degrees Celsius,  $^{\circ}\text{C}$ ), and dissolved oxygen ( $\text{O}_2$ ; milligrams per liter,  $\text{mg L}^{-1}$ ). Water chemistry samples were collected from riverbanks at well-mixed cross sections near each gage site by using dip bottles and gloved hands, following methods described by Edwards and Glysson (1999). Three 1-liter (L) high-density polyethylene (HDPE) bottles were rinsed four times sample water and then filled with sample water and labeled MIN (for sediment mineralogy), IC (for inorganic chemistry), or OC (for organic carbon). One 250-milliliter (mL) clear HDPE bottle was similarly rinsed four times, filled with sample water, and labeled ARC (for archive). Depth-integrated samples for suspended sediment were periodically collected at the same time as a grab sample, following the method of Wilde and others (1999). This sample was collected in a preweighed 125-mL, wide-mouth HDPE bottle labeled TSM (for total suspended mass). Samples were transported on ice to the U.S. Geological Survey Caribbean Water Science Center laboratory as soon as possible, typically within a few hours.

Storm event samples were collected by an ISCO<sup>®</sup> sampler, which was automatically triggered when the river reached a specified stage height. Once triggered, all 24 bottles were filled following a preset program calibrated by using sample storm hydrographs. The sampler was then emptied and restarted manually. Because a small storm could prevent the sampling of a subsequent larger storm, and the number of

samples generated by the initial stage was often too great to process in a reasonable time, threshold stages were gradually raised during the study (appendix 1, its figures 2–6). ISCO<sup>®</sup> bottles were retrieved and transported to the laboratory for processing as soon as the site could be accessed after a storm. Conductivity was measured in the laboratory, but because the samples could not be measured immediately, temperature, pH, and dissolved oxygen were not analyzed.

## Sample Filtration and Preservation

Before filtration, storage bottles and quartz-fiber filters were cleaned. For major ions (MAJ) and nutrients (NUT), 250-mL clear high-density polyethylene (HDPE) and 250-milliliter (mL) amber HDPE bottles, respectively, were leached with hot deionized water overnight, then rinsed three times with deionized water. For trace elements (TE), 250-mL HDPE bottles were soaked overnight with 2 percent hydrochloric acid, followed by three rinses with deionized water and drying at  $60^{\circ}\text{C}$ . For dissolved organic carbon (DOC), 125-mL amber glass bottles were rinsed with deionized water and baked for 1 hour at  $500^{\circ}\text{C}$ . Quartz-fiber filters (used to capture DOC and particulate organic carbon) were soaked overnight in 10 percent hydrochloric acid, rinsed thoroughly in deionized water, and baked for 1 hour at  $500^{\circ}\text{C}$ .

For grab samples, weights of all sample bottles were recorded upon return to the Caribbean Water Science Center laboratory, and the archive (ARC) bottle was frozen. Water in the remaining sample bottles was filtered as soon as practicable. The 1-liter (L) sediment mineralogy (MIN) sample was filtered through a 0.2-micrometer ( $\mu\text{m}$ ) cellulose acetate (Millipore<sup>®</sup>) filter in a polycarbonate filter apparatus. The filter and funnel were rinsed with sample water four times, and the filtrate was used to rinse the MAJ and NUT storage bottles four times (the final rinse was retained in the bottles for later use, as described later in this paragraph). After the entire MIN bottle was filtered, the weight of the empty bottle was recorded, and the filter was air dried and saved for later mineralogical analysis. A 0.2- $\mu\text{m}$  polycarbonate (Nucleopore<sup>®</sup>) filter was then placed in the same polycarbonate filtration apparatus and rinsed three times using the rinse water stored in the MAJ and NUT bottles. Approximately 50 mL of sample water from the inorganic chemistry (IC) bottle was then filtered and discarded; this step serves to “clog” the filter, which ensures that the filtrate is subjected to a consistent filter pore size. Sample from the IC bottle was then filtered into the MAJ bottle and refrigerated. If the sample

filtered rapidly, the filter was left in the holder, but if the MAJ sample was excessively slow to filter, the filter was replaced with a new 0.2- $\mu\text{m}$  polycarbonate filter, and the filter and filter apparatus were rinsed three times with rinse water and “clogged” as described above. The IC sample was then filtered into the emptied NUT bottle, preserved with 0.25 mL chloroform, and refrigerated.

Simultaneously, the 125-mL total suspended mass (TSM) sample bottle was agitated and filtered through a preweighed 0.2- $\mu\text{m}$  polycarbonate filter in a Teflon filtration funnel. The TSM bottle and filtration apparatus were then rinsed three times with rinse water from the MAJ or NUT bottles to wash any remaining particulate matter onto the filter. Most of the filters were dried at 105°C and then weighed. The dried weight minus the prefilter weight represented total suspended sediment. Some filters were placed in a preweighed crucible and ashed at 550°C. The final weight minus the prefilter weight represented total suspended bedrock.

Sample from the IC bottle was then filtered through a new 0.2- $\mu\text{m}$  Nucleopore® filter and the Teflon® filtration apparatus. Rinse water from the MAJ and NUT bottles was passed through the filter and filter funnel three times into a waste container. Approximately 50 mL of sample water from the IC bottle was filtered to “clog” the filter, and all filtrate was discarded. Sample water from the IC bottle was then filtered into the TE bottle and was treated to 1 percent hydrochloric acid with ultrahigh purity hydrochloric acid.

Sample water from the OC (organic carbon) bottle was then filtered. In the first 6 years of the study, OC samples were pressure filtered through a silver-membrane filter. After 1996 (because the manufacturer discontinued these filters), organic carbon (OC) samples were passed through an acid-washed, prebaked, quartz-fiber filter in the Teflon® filtration apparatus. The bottle was agitated during filtration. At least half of the bottle’s contents was filtered through the quartz-fiber filter into a waste container and discarded. The next volume was filtered into the DOC bottle and preserved with 0.25 mL concentrated phosphoric acid (44 Normal). The remaining OC sample was filtered and discarded. The quartz-fiber filter was air dried and stored for later particulate organic carbon analysis.

For event samples, the 1-L ISCO® bottles were refrigerated upon return to the laboratory. The sample bottles were weighed and processed as soon as possible. Polycarbonate filters and prebaked, rinsed, quartz-fiber filters were weighed and stacked in the polycarbonate filtration apparatus, with the quartz-fiber filter on top. The filters and filtration apparatus were rinsed twice with sample water, and filtrate was fed into a waste container. Sample water was then filtered into a NUT bottle, followed by a MAJ bottle (both rinsed twice with filtrate), followed by a TE bottle (which was not rinsed). The remainder of the 1-L bottle was agitated and filtered into a clean glass beaker. A DOC bottle was filled from the beaker, and the remaining filtrate was poured back into the 1-L ISCO® bottle to rinse remaining sediment onto the filter. The storage bottles were preserved in the same manner as grab samples. The empty 1-L sample bottle was weighed, and this weight was subtracted from the initial bottle weight to

obtain water-sample weight. Filters were placed in a petri dish and dried as pairs; when dry, the filters were weighed together, then separately. The weight of sediment on the filters was obtained by subtracting filter dry weights from final weights; it was then divided by the sample weight to obtain suspended-sediment concentrations.

Owing to the enormous volume of event samples (even after threshold stages were increased), analyses performed on storm event samples were eventually reduced to conductivity, silica, chloride, and sometimes potassium and suspended sediment. For these analyses, sample water was filtered through a 0.2- $\mu\text{m}$  polycarbonate (Nucleopore®) filter into a 30- or 60-mL “Si/Cl” clear HDPE bottle (which had been leached by hot deionized water). The bottle was rinsed three times with sample water, filled, and refrigerated.

Samples were periodically shipped, on ice, to the U.S. Geological Survey National Research Program laboratory in Boulder, Colo., for laboratory analyses.

## Chemical Analyses

Most analyses were performed at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. A subset of samples was analyzed at the Kiowa Environmental Chemistry Laboratory of the University of Colorado, Boulder; at the Department of Geosciences of the University of Montana, Missoula; or at the U.S. Geological Survey Panola Laboratory in Atlanta, Ga., as described below.

### Alkalinity

Alkalinity was determined at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. From 1991 to 1998, alkalinity was determined on an autotitrator that used Gran titration. After 1998, a new autotitrator was obtained, and an incremental inflection point titration was used. For both methods, a blank, a sample replicate, three standards (50, 500, and 1,000 milliequivalents per liter ( $\mu\text{eq L}^{-1}$ )), and a U.S. Geological Survey standard reference water sample (SRWS) (U.S. Geological Survey, 2012) were analyzed every 14 samples. Samples were reanalyzed if the regression coefficient ( $r^2$ ) of the slope of the standards was less than 0.99, or if replicate samples deviated by more than 10 percent. If alkalinity was greater than the highest standard, the sample was diluted and reanalyzed. When the incremental inflection point titration was used, if alkalinity was below the detection limit, then the sample was spiked with 1 mL of 1,000  $\mu\text{eq L}^{-1}$   $\text{KHCO}_3$ , reanalyzed, and the spike was then subtracted.

### Major Cations

Major cations (sodium, potassium, magnesium, and calcium) were analyzed with inductively coupled plasma (ICP) spectroscopy. From 1991 to 1995, samples were analyzed at

the Department of Geosciences of the University of Montana. U.S. Geological Survey SRWSs were run approximately every seven samples. If measured concentrations did not agree within specified limits of certified values (limits varied from element to element, but were always within 10 percent), samples were reanalyzed. Spike recoveries and replicates were also analyzed on 10 percent of the samples, randomly selected.

From 1995 to 2005, major cations were analyzed at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo., with a Perkin Elmer Optima 3300 DV<sup>®</sup> inductively coupled plasma–atomic emission spectroscopy (ICP–AES) by using techniques described by Garbarino and Taylor (1979). Five or six calibration standards were analyzed for each analysis run; each had an  $r^2$  value greater than 0.999. Samples with concentrations above the highest standard were diluted and reanalyzed. Twelve to 17 laboratory blanks, interspersed throughout each analysis run, were used to calculate detection limits for each analyte for each analysis run. SRWSs, analyzed at a frequency exceeding 30 percent of samples, were interspersed throughout each analysis run. Each sample was analyzed in triplicate, and the mean of the three concentration values was reported. Precision was calculated as the standard deviation of the three values; if one of the replicates substantially disagreed with the other two, it was discarded and the average and standard deviation of the remaining two were used to calculate concentration and precision. When all three values substantially disagreed, the sample was reanalyzed.

## Silica

Silica was analyzed in more than 4,600 grab and event samples, all at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. Silica was measured either by an ultraviolet/visible (UV/Vis) spectrophotometer or by ICP–AES, and sometimes by both. For spectrophotometer analyses, a blank, four standards, a SRWS, and a laboratory replicate were analyzed on a Perkin Elmer<sup>®</sup> UV/Vis spectrophotometer for every nine samples. A regression of the standards calculated sample concentration. Samples were reanalyzed if blanks were greater than the instrument detection limit (1 micromole per liter ( $\mu\text{mol L}^{-1}$ )) of silica, if absorbance values of replicates differed by more than 0.025 in the 0.100 to 0.500 absorbance range or by more than 0.050 in the 0.500 to 1.000 absorbance range, or if the observed value of the SRWS was greater than 5 percent of its most probable value.

For ICP–AES analyses, silica was analyzed on a Leeman Labs Direct Reading Echelle<sup>®</sup> dual view, sequential multielement ICP spectrometer. The detection limit was 0.05 milligrams per liter ( $\text{mg L}^{-1}$ )  $\text{SiO}_2$ . Blanks, SRWSs, and laboratory replicates were analyzed several times during each analytical run. Replicate determinations were performed and were within 2 percent.

## Potassium

Potassium was determined either on an atomic adsorption spectrophotometer or by ICP–AES at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. For atomic absorption, samples were analyzed on a Perkin Elmer 5000<sup>®</sup> atomic adsorption spectrophotometer using a three-point standard curve. Replicates and several SRWSs were analyzed every 10 samples. If SRWS concentrations deviated more than 5 percent from certified values, the instrument was restandardized and analyses were repeated.

For ICP–AES analyses, potassium was analyzed on a Leeman Labs Direct Reading Echelle<sup>®</sup> dual view, sequential multielement, ICP spectrometer. The detection limit was 0.04  $\text{mg L}^{-1}$  potassium. Blanks, SRWSs, and laboratory replicates were analyzed several times during each analytical run. Replicate determinations were within 3 percent of the original value.

## Major Anions

When complete chemistry was analyzed, nitrate, sulfate, and chloride were determined by ion chromatography. Samples were usually analyzed at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo., on a Dionex<sup>®</sup> ion chromatograph. Three blanks, six standards, a SRWS, and a laboratory replicate were analyzed every ninth sample. In 1996, owing to a high volume of samples and to problems with instruments, samples were analyzed at the U.S. Geological Survey's Panola Laboratory in Atlanta, Ga., by ion chromatography. Six standards and two SRWSs were analyzed at the beginning of each run. After every 10 samples, a replicate sample and a random selection of 2 reference samples, including SRWSs and blanks, were analyzed.

In many samples chloride was analyzed, but nitrate and sulfate were not. Like silica, chloride was analyzed in more than 4,600 grab and event samples. It was analyzed either by ion chromatography, as described above, or with a Buchler Chloridometer<sup>®</sup>, which operates by coulometric titration with silver ions, at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. Four calibration standards were analyzed at the beginning and end of each run and were used to calculate sample concentrations. Blanks and SRWSs were analyzed every 10 samples. All samples were run in duplicate, and if the relative standard deviation was greater than 5 percent, they were reanalyzed. A 70  $\mu\text{mol L}^{-1}$  chloride spike was added to every sample to ensure a clean titration end point for samples with low chloride concentrations.

## Nitrite

Most samples were analyzed for nitrite at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo., on a Perkin-Elmer<sup>®</sup> spectrophotometer by using a four-point calibration curve. Blanks and intermediate standards were analyzed throughout each run, and

approximately 10 percent of samples were run in duplicate. Samples were reanalyzed if the blank concentration was greater than the instrument detection limit ( $0.2 \mu\text{mol L}^{-1}$  of nitrogen), if the observed value of the SRWS was greater than 5 percent of its most probable value, or if absorbance values of replicates differed by more than 0.005 in the 0.030 to 0.100 absorbance range, by more than 0.020 in the 0.100 to 0.500 absorbance range, or by more than 0.030 in the 0.500 to 1.000 absorbance range. Some samples were analyzed at the U.S. Geological Survey Panola Laboratory in Atlanta, Ga., on an ALPKEM® nutrient analyzer; samples were analyzed in triplicate, and a seven-point calibration curve was run before and after each analytical run.

## Phosphate

Phosphate was determined on a Perkin Elmer® UV/Vis spectrophotometer at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo., by using a four-point calibration curve. A blank, four standards, a SRWS, and a replicate were analyzed every nine samples. Samples were reanalyzed if blanks were greater than the instrument detection limit ( $0.03 \mu\text{mol L}^{-1}$  phosphate), if absorbance values of replicates differed by more than 0.010 in the 0.030 to 0.100 absorbance range or by more than 0.020 in the 0.100 to 0.500 absorbance range, or if the observed value of the SRWS was greater than 5 percent of its most probable value.

## Ammonium Ion

Ammonium ion was determined on a Perkin Elmer® UV/Vis spectrophotometer at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo., by using a four-point calibration curve. A blank, four standards, a U.S. Geological Survey SRWS, and a laboratory replicate were analyzed for every nine samples. Samples were reanalyzed if the absorbance of the blank was greater than 0.030, if the observed value of the SRWS was greater than 5 percent of its most probable value, or if absorbance values of replicates differed by more than 0.010 in the 0.030 to 0.100 absorbance range, by more than 0.020 in the 0.100 to 0.500 absorbance range, or by more than 0.030 in the 0.500 to 1.000 absorbance range.

## Dissolved Organic Carbon

Dissolved organic carbon was determined on an OI Analytical TOC analyzer model 700® at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. The instrument utilizes persulfate oxidation to produce  $\text{CO}_2$  gas, which is then trapped and detected with infrared light. Samples were run in replicate, with a four- or five-point calibration curve. Calibration standards were run every 10 to 12 samples. If replicate sample analysis did not agree within 5 percent of the original, samples were reanalyzed.

## Particulate Organic Carbon

Particulate organic carbon analyses were made on overbank sediments and on sediment collected on quartz fiber filters. Samples were analyzed for total carbon on a Coulometrics Total Carbon Combustion Apparatus Model 5120® at the U.S. Geological Survey National Research Program laboratory in Boulder, Colo. This method measures both  $\text{CaCO}_3$  and total organic carbon. The assumption was made that all carbon in samples was organic. One or two  $\text{CaCO}_3$  standards, instrument blanks, and quartz-fiber filter blanks were run each day of analysis. Owing to the small amount of sample, replicates were not analyzed.

## Additional Analyses

In 2003 and 2006, a review of analyses found some gaps in analytes, and a subset of 170 samples was analyzed at the Kiowa Environmental Chemistry Laboratory of the University of Colorado. Analyses included alkalinity (by titration), major cations (by atomic absorption), major anions (by ion chromatography), and ammonia, phosphate, silica, and nitrogen species (by colorimetric methods). Methods and detection limits are described at the Kiowa Environmental Chemistry Laboratory Web site (Kiowa Environmental Chemistry Laboratory, 2011). Replicates and SRWSs were submitted with water samples.

## References

- Edwards, K.E., and Glysson, G.D., 1999, Field methods for measurement of fluvial sediment, *in* National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, book 3, ch. C2, 89 p.
- Garbarino, J.R., and Taylor, H.E., 1979, An inductively coupled plasma atomic-emission spectrometric method for routine water quality testing: *Applied Spectroscopy*, v. 33, p. 220–226.
- Kiowa Environmental Chemistry Laboratory, 2011, Kiowa Environmental Chemistry Laboratory procedure manual, accessed March 29, 2011, at <http://snobear.colorado.edu/Kiowa/Kiowaref/procedure.html>.
- U.S. Geological Survey, 2012, Standard Reference Sample Project: U.S. Geological Survey, accessed January 24, 2012 at <http://bqs.usgs.gov/srs/>.
- Wilde, F.D., and Radtke, D.B., 1998, Field measurements, *in* National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, ch. A6, p. 3–20.