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APPENDIX

METHODOLOGY

All Stream Team sampling and laboratory analysis is conducted in compliance with a Quality Assurance Project Plan approved by the State Water Resources Control Board. This Quality Assurance Project Plan can be viewed on-line at www.stream-team.org. The following narrative summarizes all Stream Team testing procedures.

Water sampling and chemical analyses

Stream water samples were collected manually at mid-depth near the center of flow. Sample bottles (and caps) of high-density polyethylene (HDPE) were rinsed three times with deionized water before being used, and three times again with sample water immediately prior to being filled. Samples were placed in coolers as soon as possible and transported on ice, and were stored at 4°C once in the laboratory.

Samples for dissolved constituents were generally filtered in the field through Gelman A/E glass fiber filters, pre-flushed with deionized water and then sample water. A syringe was used to force the sample through the filter unit. Stormflow samples with high sediment concentrations could not be field-filtered and were either centrifuged or allowed to settle before filtration in the laboratory. Samples were analyzed for nitrogen (dissolved organic nitrogen, nitrate (NO3 + NO2) and ammonium) and phosphorus (soluble reactive phosphate, SRP). Nitrate, ammonium and phosphate were determined colorimetrically on a Lachat® auto-analyzer. Ammonium was measured by adding base to the sample stream, converting ammonium to ammonia, which diffuses across a Teflon® membrane (Willason and Johnson, 1986) and into phenol red pH indicator. Nitrate was measured using a standard Griess-Ilosvay reaction after Cd reduction (EPA, 1983). Phosphate was measured after reaction with ammonium molybdate and antimony potassium tartrate and reduction by ascorbic acid with heating at 45°C.

Detection limits were 0.3 μmol L-1 for NH4+ and PO43- and 0.5 μmol L-1 for NO3-; accuracy is ±5%. Total dissolved nitrogen (TDN) was determined after persulfate digestion (Valderrama, 1980) followed by measurement of nitrate. The basic persulfate reagent was added to a separate aliquot at the time of initial processing or laboratory filtration, and the digestion completed within one week. The detection limit was 0.5 μmol L-1 and accuracy was + 10%. Dissolved organic nitrogen (DON) was computed as the difference between TDN and dissolved inorganic nitrogen (DIN: nitrate and ammonium).

The goal was to analyze inorganic nutrient samples and begin the digestion of total dissolved nitrogen samples within 48 hours of collection, and we were able to meet this goal for most of the samples collected. However, during winter storm periods, when high sediment concentrations prevented filtration in the field and the laboratory was inundated with hundreds of samples, the 48-hour limit was often exceeded by one to five days. To evaluate the effect of delay, three types of samples were collected from six streams with widely varying nutrient chemistry: (1) samples filtered in the field and analyzed in duplicate within 12 hours; (2) samples filtered in the laboratory on the day of collection, stored at 4°C, and repeatedly re-analyzed after delays of 1-14 days; and (3) an unfiltered sample, stored at 4°C, subsamples of which were repeatedly filtered and analyzed after similar delays. Numerous duplicate and deionized water samples provided quality assessment and control. The average error (the combined error of processing, delay, instrument calibration and analysis) for nitrate was 5-10% (the higher percentage error in the second week of delay), 10% for phosphate, and 20% for ammonium. Samples filtered within two days showed almost no variation in nitrate and phosphate from initial values, while ammonium was usually within 10%. Delays greater than two days did sometimes cause significant increases in ammonium concentrations.

Bacteriological analysis

Water samples for bacteria analysis were collected manually, at mid-depth near the center of flow, in sterile plastic bottles pre-charged with small amounts of sodium thiosulfate to remove residual chlorine (a possible problem below sewage treatment plants and in urban nuisance waters). Samples were placed in coolers, transported on ice, and analyzed within six hours of collection.

Each sample was analyzed for three indicator bacteria: total coliform, E. coli, and enterococci, using IDEXX Colilert® and Enterolert® methodologies (ASTM #D6503-99). Both methods are approved by the US Environmental Protection Agency (EPA, 2003a). The sample, diluted with distilled, bacteria-free water (typically using a dilution of 10:1), was used to fill multiple wells in an analysis tray. Colilert uses two indicators, one that changes color when metabolized by total coliform, and another that fluoresces when metabolized by E. coli; the Enterolert indicator fluoresces when metabolized by enterococci. The number of positive wells after incubation for 18 hours at 35°C (Colilert) or 24 hours at 41°C (Enterolert) provides a statistical determination of concentration. The unit of measure is the "most probable number" of "colony forming units," abbreviated as either "MPN" or "cfu," in 100 ml of sample.

Quality control was evaluated by analyzing laboratory "blanks" (zero bacteria samples), duplicate field samples, and by performing multiple tests on single samples. The reproducibility of the bacteria results can be evaluated by examining the differences between duplicate field samples. Two duplicates (consecutive samples taken at the same location) were collected on each sampling day. A measure of reproducibility is the difference proportion, the absolute value of the difference between two samples divided by the average value, or

$$\text{difference proportion} = (2 \mid N1 - N2 \mid) / (N1 + N2)$$

where N1 and N2 are the concentrations of the first and second samples (Kayhanian et al., 2005). The mean and median difference proportions for the bacteria analyses are shown in Table A1.

Table A1. Average and median difference proportions (expressed as a percentage ± the standard deviation) of duplicated samples collected in Channelkeeper sampling programs, 2001 - 2005.

		MPN/100 ml	%	%
	no. of duplicates	average concentration	average difference proportion	median difference proportion
E.coli	124	460	43.3 ± 38.9	34.9 ± 48.6
enterococci	126	485	55.7 ± 50.9	42.3 ± 63.6
total coliform	116	4670	37.2 ± 34.7	27.0 ± 43.4

In-field measurements

Portable, hand-held meters were used to take field measurements for dissolved oxygen, pH, conductivity, water temperature and turbidity. Measurements were typically taken near the center of flow, below the surface in the upper half of the water column. The objective was to obtain measurements characteristic of the bulk of stream flow and not a spectrum of variation at the testing location. All instruments were calibrated according to manual instructions us-

ing certified laboratory standards on the day prior to sampling. Table A2 shows the type and accuracy of each meter used.

Table A2. Meters and accuracy.

Meter	Accuracy
YSI Model 55 Dissolved Oxygen/Temperature Meter	± 0.3 mg/L or 2%, ± 0.2°C
Oakton CON 410 Conductivity/TDS/Temperature Meter	± 1%, ± 0.5°C
LaMotte 2020 Turbidimeter	± 2% or 0.05 NTU
Oakton Waterproof pH Tester 2 (prior to April 2005)	± 0.1 pH
Oakton pH/mV/Temperature Meter (April 2005 and later)	± 0.01 pH

At each site, three readings were taken in three different areas of the creek with each meter (six for stream temperature using temperature scales on both the conductivity and dissolved oxygen meters). For the turbidimeter, two separate sample vials are tested three times each. All readings are later averaged to produce the final result that is entered into the database.

After sampling, all results are checked for quality control purposes. Any suspicious results are re-tested within six hours at the lab using a 500 ml sample collected at each location and transported on ice. Suspicious results are those that (1) are unusual in light of past measurements at the location, (2) have widely varying multiple measurements, or (3) are expressed in doubtful units (e.g., milli vs. micro, or ppt vs. ppm). The "back-up" samples were also used in cases of on-site equipment failure or suspected meter malfunctions.

The difference proportion used to evaluate duplicate bacteria samples can also be used to examine the repeatability of multiple measurements. In this case, the difference between maximum and minimum measurements is expressed as a percentage of the average of all measurements (typically either three, in the case of dissolved oxygen, conductivity and pH, or six for turbidity and water temperature). The median difference proportions for each parameter for all measurements made by both the Ventura and Goleta Stream Teams from June 2004 through July 2005 are shown in Table A3.

The repeatability of measurements is usually very good. With the exception of turbidity, a majority of the multiple measurements are within a few percentage points of each other. Turbidity measurements are afflicted by problems similar to those that effect bacteria concentrations: a spatially and temporally varying dispersion in stream flow. In addition, turbidity can vary with stream velocity, and its measurement is particularly susceptible to errors in collection and measurement, e.g., disturbing bottom sediment while collecting samples and/or failure to properly clean sample vials. This occasionally accounts for proportional errors greater than 100%.

Table A3. Median difference proportions (expressed as a percentage) and standard deviations of multiple parameter measurements collected in Channelkeeper sampling programs, June 2004-July 2005.

parameter	n	unit	median value	maximum value	minimum value	median standard deviation	median difference proportion
VENTURA STREAM TEAM							
dissolved oxygen	142	mg/L	8.86	17.43	4.05	0.09	2.1%
% saturation	142	%	94.1	196.5	53.8	1.09	2.1%
pH	142	units	8.15	9.03	6.95	0.04	1.0%
conductivity	142	µS/cm	1091	2747	335	3.8	0.8%
temperature	126	° C	16.9	24.6	6.2	0.15	2.1%
GOLETA STREAM TEAM							
dissolved oxygen	129	mg/L	9.33	19.76	3.41	0.15	3.4%
% saturation	125	%	94.4	32.8	98.2	1.65	3.3%
pH	130	units	8.17	8.90	7.10	0.03	0.7%
conductivity	142	µS/cm	1923	47600	164	23.1	1.8%
temperature	117	° C	16.9	27.1	7.2	0.23	3.1%
turbidity	118	NTU	3.96	309.5	0.13	0.30	16.4%

Santa Barbara Channelkeeper

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The volunteers listed above are those who participated in three or more Ventura Stream Team sampling events. However, Channelkeeper also sincerely thanks the hundreds more who donated their time to Ventura Stream Team over the past five years.