BIOAVAILABILITY OF DISSOLVED ORGANIC CARBON AND DYNAMICS OF ACTINOBACTERIA IN THE CALIFORNIA STATE WATER PROJECT

by

ELISABETH RENEE DUNSMUIR WANDZELL

(Under the Direction of JAMES T. HOLLIBAUGH)

ABSTRACT

This thesis examines bioavailability of dissolved organic carbon (DOC) and the dynamics of Actinobacteria in the California State Water Project (SWP). DOC bioavailability was low throughout the SWP. Exposure to the equivalent of a one day of solar irradiation did not change aqueduct DOC bioavailability, but caused a slight increase in reservoir DOC bioavailability. Actinobacteria relative abundance was measured using real-time PCR; changes in assemblage composition were assessed by PCR/DGGE, and phylogenetic affinities of Actinobacteria populations were determined by sequencing cloned 16S rRNA genes. Actinobacteria relative abundance was 22% (range, 9-34%). Actinobacteria in water samples were not closely related to those in soil samples but were closely related to Actinobacteria found in the Sacramento-San Joaquin Delta. Reservoir and aqueduct assemblages differed. Aqueduct Actinobacteria assemblages varied seasonally. Changes in October assemblages correlated with conductivity and optical and chemical properties of DOC, while February assemblages varied with pH, temperature and conductivity.

INDEX WORDS: Dissolved organic carbon, Bioavailability, Photodegradation, Drinking water, Actinobacteria, 16S rRNA, California State Water Project, Sacramento-San Joaquin Delta.

BIOAVAILABILITY OF DISSOLVED ORGANIC CARBON AND DYNAMICS OF ACTINOBACTERIA IN THE CALIFORNIA STATE WATER PROJECT

by

ELISABETH RENEE DUNSMUIR WANDZELL

B.A., California State University Stanislaus, 1989

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2006

BIOAVAILABILITY OF DISSOLVED ORGANIC CARBON AND DYNAMICS OF ACTINOBACTERIA IN THE CALIFORNIA STATE WATER PROJECT

by

ELISABETH RENEE DUNSMUIR WANDZELL

Major Professor: James T. Hollibaugh

Committee: Erin K. Lipp
William L. Miller

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2006

DEDICATION

This thesis is dedicated to my amazing family, friends, everyone in the Marine Science department and my doctors and nurses at Athens Regional Medical Center. Without your incredible support and skill, I would have literally not lived to finish this research. I owe you my life and love and will never forget the impact you have all made on my life.

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my advisor Tim Hollibaugh for not only being a mentor, but also supporting me and my family through the most difficult time of my life. Also, thank you to my committee members Erin Lipp and Bill Miller for all their help and edits. A very special thanks to Nasreen Bano for selflessly passing on her wisdom and experience. I will be eternally grateful to Sylvia Schaefer, Erin Biers, Susan White and many others in the department who not only gave me and my family moral support, but also carried out my experiments until I could return to work. To all my labmates I am grateful to you for providing a great work atmosphere and individual help, including Matt First for answering numerous computer questions, Karen Kalanetra for qPCR protocol and Angel Smith for showing me the basics of molecular lab techniques. Thank you to the Moran Lab and to Wendy Ye for help with titrations, Justine Lyons for help with sequencing and Jennifer Edmunds for statistical help. Thank you to Ramunas Stepanauskas for assisting me with protocol and methods. A big thank you to many of my understanding professors including Aaron Fisk, Rhett Jackson, Judy Meyer and J. Bruce Wallace. Finally, thank you for all the help I received from the USGS in Sacramento, especially Tamara Kraus for her kindness, support and edits, Bryan Downing, Connie Clapton, Travis von Dessonneck and to Rich Losee and others at the Metropolitan Water District of Southern California.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

The California State Water Project (SWP), a more than 400-mile conveyance of aqueducts and reservoirs, was engineered to transport drinking and irrigation water from the Sacramento-San Joaquin Delta in the north to the over 20 million people and over 10 million ha of farmland in the Central and Southern California desert regions. Perhaps the largest project of its time, the SWP is comprised of the California Aqueduct, South Bay Aqueduct, Coastal Branch Aqueduct and several reservoirs and was the culmination of nearly a century of study, planning and politics.

1.1 History of the State Water Project

California's water resources were first officially investigated in 1873 following the influx of settlers during the Gold Rush of 1848, the subsequent population boom in cities such as San Francisco and Los Angeles and the increase of farming in the Central Valley. President Ulysses S. Grant commissioned the Army Corps of Engineers to survey the Central Valley's irrigation needs, proposing development of the watersheds of the Sierras to meet those needs and to mitigate the periodic flooding of Sacramento, San Francisco and the surrounding areas. The state followed with its own comprehensive study from 1873 - 83. This study included drainage and river channel investigations and recommendations for flood control and navigation improvements on the Sacramento River, its tributaries and in the Sacramento-San Joaquin Delta (Figure 1.1). In 1919, based on the recommendations of that extensive study, the concept of a state water

project was first raised by the US Geological Survey. The project involved transporting water from the Sacramento-San Joaquin Delta through the Central San Joaquin Valley and over the Tehachapi mountains into Southern California. Construction of the California State Water Project (SWP) officially began in 1957 after extensive planning, political wrangling and millions of dollars worth of bond acts approved (DWR, 2006).

Currently, the SWP consists of five major reservoirs, five pumping plants and 444 miles of aqueduct (Figure 1.2). This study examines the East Branch (main stem) of the SWP completed in 1973, two of the five reservoirs and a forebay (San Luis Reservoir, Castaic Lake and Elderberry Forebay) and approximately 400 miles of the California Aqueduct from Check 12 in Central California to Devils Canyon Afterbay (DCA) in Southern California.

1.2 SWP Source Water: The Sacramento-San Joaquin Delta

The state of California is host to towering peaks and rushing rivers in the north and arid valleys and deserts in the central and southern regions. The watershed that supplies the Sacramento-San Joaquin Delta, at the juncture of Northern and Central California, encompasses 1.63×10^7 ha, or 40 % of the area of the state (Figure 1.1). The Delta itself is formed by the union of two great rivers; the Sacramento River and the San Joaquin River. The Sacramento River is the longest in the state (377 mi.), originating from the base of Mt. Shasta in the north, and provides approximately 63 - 81 % of the fresh water to the Delta (Stepanauskas et al. 2005). The San Joaquin River drains the Sierra Nevada range in Central California and provides the remaining 19 - 37 %. The total fresh water contribution of the two rivers to the Delta is 35×10^9 m^3 $year^{-1}$ (Jassby and Cloern 2000). Delta water exits naturally at the confluence of these two rivers into Suisun Bay, the

eastern arm of San Francisco Bay and is subject to tidal mixing. Rain and snowfall in the state vary by year, season and area, with floods and droughts periodically occurring in the same year, though yearly precipitation is generally more predictable in the northern California region.

The Delta is an intensely managed 3×10^5 ha system of both natural and engineered channels and lakes, cities and towns (2.6×10^4 ha), diked agricultural fields (2.2×10^5 ha) and relict tidal marshlands, of which approximately 3×10^4 ha is undeveloped (DWR 1993). Jassby and Cloern (2000) also reported 2.6×10^4 ha of this to be open water habitat.

1.3 The California Aqueduct and Delta Mendota Canal

The State Water Project (SWP) and the Delta Mendota Canal (DMC) remove approximately one-third of these freshwater inputs at the southern end of the Delta (3×10^9 and 9×10^9 m^3 $year^{-1}$, respectively; Stepanauskas et al. 2005). Water exits Clifton Court from its southwest corner at H.O. Banks pumping plant, the entrance to the California Aqueduct (mile 0; Figure1.1). Water is pumped out of Clifton Court at low tide to avoid salt-water intrusion into the aqueduct. The aqueduct is an engineered, concrete-lined waterway approximately 12 m (40 ft) wide and 9 m (30 ft) deep. Depth and width of the aqueduct are varied according to projected water capacity required at various locations.

The entrance to O'Neill Forebay is at Check 12 (mile 66) of the California Aqueduct (Figure 1.2) with designated sampling sites along the aqueduct referred to as Checks. The Delta water arriving at Check 12 is mixed with water arriving into the O'Neill Forebay from a diversion along the Delta Mendota Canal (DMC). DMC receives water from two

separate sources. Historically, the water supply originated from the Delta near the San Joaquin River and entered the DMC at Tracy Pumping Plant. However, during yearly periods of low-flow in the summer, the San Joaquin becomes highly polluted and also experiences salt water intrusion from San Francisco Bay. The Delta Cross Channel (DCC) was constructed to alleviate these two problems. Water is diverted 1.5 miles from the Sacramento River through the DCC into the Mokelumne River Systems, following 50 miles of natural channels, to the Intake Channel at the Tracy Pumping Plant. Water is also pumped at low tide to avoid salt water intrusion. The bypass is closed to O'Neill Forebay when the San Joaquin reaches a natural or flood-stage flow.

The East Branch (main stem) of the CA Aqueduct was the section sampled in this study. Aqueduct sampling began in O'Neill Forebay, at Check 12, DMC and at Check 13 (O'Neill Forebay Outlet (OFO)) as the water reenters the aqueduct at Check 13. The aqueduct receives three inputs: Semitropic (groundwater) Inflow (mile 210) and Kern River Inflow (mile 238) between Check 21 and Check 29 and Arvin Edison Inflow (groundwater; mile 277) between Check 29 and Check 41 (Table 2.1).

Water is diverted from the main stem just past Check 41 (mile 303). This water is pumped by Oso Pumping Plant into Quail Lake and then enters a pipeline leading into Warne Powerplant to generate power. Water is then discharged into Pyramid Lake and Elderberry Forebay via the Angeles Tunnel and into Castaic Powerplant. Castaic Lake and Castaic Lagoon is at the end of the West Branch. Water in Castaic Lake then travels directly to drinking water treatment facilities.

South of Check 41, on the main stem, are Check 52 and Check 66. After Check 66, water is used to generate power at Alamo Powerplant. The water is pumped uphill and

then flows downhill through an open aqueduct and four underground pipelines into the Mojave Siphon Powerplant, discharging into Lake Silverwood, with a storage capacity of 73,000 acre-feet. When water is again needed for power, it is discharged into Devil Canyon Powerplant and its two afterbays, one of which is Devils Canyon Afterbay (DCA), the southern-most sampling site (mile 413). Water from DCA is then treated and used for drinking water.

1.4 The Reservoirs

Water is pumped into San Luis Reservoir (Figure 1.3) for storage from O'Neill Forebay primarily during the fall and winter rainy season (September to May). Water is released from San Luis Reservoir dam into O'Neill Forebay beginning in April or May to meet water demands to the south during the dry periods of late spring to early fall. Occasionally, this water also serves a second purpose; that of power generation. Water maybe released from San Luis Dam during the day and then may be pumped back into the reservoir at night from O'Neill Forebay to generate power during peak demand times. This reverse pumping is an infrequent occurrence but is another factor in the complicated SWP system. The reservoir reaches its capacity of 2,027,835 acre-feet and 280 feet in depth in the winter, with levels typically dropping below 22 % of capacity, or 440,000 acre-feet and 120 feet in depth, during the summer months. During extreme drought conditions, levels can drop as low as 17 % of capacity, or 350,000 acre-feet and 105 feet in depth.

Castaic Lake is located at mile 33.2 of the West Branch of the aqueduct near Castaic, California (Figure 1.4). Castaic Lake is termed a 'flow through' reservoir. Unlike San Luis Reservoir, whose water level may drop as low as 17 % of capacity, Castaic Lake's

storage capacity remains above 250,000 acre-feet, or 76 % of capacity. Its maximum storage capacity is 328,702 acre-ft with a maximum depth of 315 feet and normal minimum depth of 235 feet. Water exits Castaic Lake from Castaic dam to the south. This system is similar to San Luis Reservoir and O'Neill Forebay as it contains a pumping plant in the dam (Castaic Powerplant) that also generates power.

Water is frequently pumped back into Elderberry Forebay from Castaic Lake at night to regenerate hydropower energy during the day. This pumping pattern results in a large volume, well-mixed hypolimnion in Castaic Lake and cool water temperatures in Elderberry Forebay (Kraus et al. 2005). During periods of Castaic Lake stratification, the cool water released from Elderberry dam turbines causes air-entrainment, forcing the water to the surface and releasing air bubbles to the atmosphere. Then, the cold water plunges deep into the water column, finding its corresponding density layer. This process causes a significant amount of mixing energy to be imparted to the hypolimnion, as well as to entrained surface water. Unlike San Luis Reservoir, Castaic Lake is 'V'-shaped, with water entering at the northwest branch and exiting to the south. As a result, there may be differences in DOC quality between the east and west branches of the reservoir, though this hypothesis cannot be examined by this study, as discrete samples were composited from the different stations, not individually. Details of this method will be described in the sampling methods section of Chapter 2.

1.5 Jones Tract

Jones Tract in the south Delta, the sight of a levee break on June 3, 2004, was sampled in June, July and November 2004 (Figure 1.5). The flooding of Jones Tract gave rise to concerns that this breach would affect both the quantity and quality of water

entering the SWP. Water from the Upper Jones Tract (UJT) and Lower Jones Tract (LJT) were sampled to follow changes in the water quality as the area was drained and the levee repaired.

1.6 Drinking Water Treatment Concerns

The Sacramento-San Joaquin Delta water, as reported by Jassby and Cloern (2000), contains high concentrations of dissolved organic carbon (DOC) resulting primarily from the inputs from tributaries (69 %) that are primarily biologically refractory DOC from terrestrial plants and soil. Other contributions from the various Delta habitats include both allochthonous (~ 15 %; agricultural drainage, tidal marsh export, wastewater treatment plant discharge and urban runoff) and autochthonous (< 15 %; phytoplankton, higher aquatic plants and benthic microalgae). In addition, Stepanauskas et al. (2005) found the Delta to be a net source of DOC to the water passing through it from the Sacramento and San Joaquin Rivers.

The SWP receives DOC in water arriving primarily from the Delta and also from *in situ* (i.e. algal) origin within the SWP. When this water is disinfected with chlorine or ozone during the drinking water treatment process, a fraction of this DOC reacts to form disinfection byproducts (DBPs; Bergamaschi et al. 1999). DBPs have been linked, in several epidemiological studies, to increased occurrence of bladder cancer (Koivusalo et al. 1997), miscarriages (Waller et al. 1998) and other health concerns. DBPs, however, have been framed the ‘luxury concern of the developed world’ by some scientists and regulators (Driedger and Eyles 2003), since chlorination is the most cost-effective disinfection treatment used to control microbial pathogen contamination. The EPA has mandated regulations that must balance the trade-off of the immediate health risk of

microbial contamination versus the long-term exposure risk to DBPs (Villanueva et al. 2004). With over 240 million Americans exposed to DBPs through ingestion of tap water, dermal exposure (swimming pools, showers) and inhalation (cooking, dishwashers), the U.S. government has taken these health risks seriously.

Treated Delta water frequently exceeds DBP concentrations permitted by the U. S. Environmental Protection Agency (EPA, 2006). Both the amount and source of DOC within the system contributes to the DBP formation potential (DBPFP), both of which can vary significantly throughout the system. This has been reported in water samples collected from the H.O Banks Pumping Plant in Clifton Court Forebay, the entrance to the CA Aqueduct (Amy et al. 1990). Drainage water from Delta islands was estimated to contribute from 20 to 50 percent of the DOC contributing to the formation of DBP trihalomethanes (THM), including chloroform ($CHCl_3$) and bromoform ($CHBr_3$) and their precursors.

When water enters the SWP, it spends anywhere from weeks in transit to years in reservoir storage in the SWP, with a mean residence time of months. This is sufficient time for biological processes, such as microbial degradation and algal blooms and chemical processes such as photooxidation, to alter the amount and quality of both DOC and DBP-forming materials. The bioavailability study detailed in Chapter 2 is just one aspect of a CALFED funded comprehensive study of the SWP currently underway by the US Geological Survey (USGS) and other contributing agencies and organizations. Entitled “Improving Delta Drinking Water Quality: Managing Sources of Disinfection Byproduct-Forming Material in the State Water Project”, this study investigates the DBP formation potential of the SWP water. This information will give water managers an idea

of the potential of SWP water to form DBPs, which will be useful in revising management plans currently governing the SWP.

In addition, the SWP source water, the Sacramento-San Joaquin Delta, has been reported to contain a high abundance of *Actinobacteria* (Stepanauskas et al. 2003). *Actinobacteria* have been reported to cause taste and odor problems in drinking water (Klausen et al. 2004; Zaitlin et al. 2003). The MWD is concerned with the input of *Actinobacteria* into the SWP from the Delta as they distribute SWP water to over 18 million people for use as drinking water.

1.7 SWP Biological and Chemical Processes

DOC is a major pool of energy potentially available for aquatic microorganisms. Bacterioplankton play a central role in the carbon flux in aquatic ecosystems by bringing assimilable dissolved organic carbon (i.e. phytoplankton detritus) back into the foodweb (Pomeroy 1974). DOC is a large fraction (~ 60 %) of the dissolved organic matter (DOM) pool present in freshwaters (Raymond and Bauer 2001).

In my study, DOC was size classified as what passed through a 0.2 um filter and therefore may include a small fraction of colloidal OM (< 0.1 μm), some small bacteria and viruses (0.03 – 0.2μm). Though viral lysis of bacteria is an important component of the food web (Van Hannen et al. 1999), the effect of viruses on the natural occurring bacterial population during incubation was not measured.

The majority of tributary inputs into the Delta, thus potentially entering the SWP are humic, primarily of terrestrial origin and biologically refractory (Jassby and Cloern 2000). Further, these terrestrial inputs mainly originate from vascular, ligninaceous plants and are of high molecular weight (HMW), high in aromatic structures and frequently

colored. Photolysis can break the bonds of a fraction of these high molecular weight, recalcitrant DOC polymers, converting them into labile, low molecular weight compounds (Miller et al. 2002; Moran and Zepp 1997) or into highly photobleached DOC (Moran et al. 2000) more suitable for use as a substrate for bacterial growth. Conversely, carbon can also originate from autochthonous sources (i.e. algal) that can be of low molecular weight (LMW) more biologically labile and low in aromatic UV-absorbing structures. UV radiation exposure may cause condensation reactions within this LMW fraction of the DOC pool, or cause polymerization with terrestrial humic matter, making it increasingly recalcitrant (Tranvik and Kokalj 1998). This may decrease the fraction of DOC substrate available in the short-term for bacterial utilization.

Autochthonous carbon input by net primary production of phytoplankton (NPP) in the Delta was estimated by Jassby and Cloern (2000). NPP is estimated to be ~20 % of the TOC (47.5 ± 5 t day $^{-1}$) and occurs primarily in the spring and summer months (58 and 54 t C day $^{-1}$, respectively) and decreases in autumn (20 t C day $^{-1}$) before becoming an almost negligible addition to the total carbon pool in winter (3.9 t C day $^{-1}$).

While DOC can be made more available through photooxidation due to the relatively shallow depth of the aqueduct and high surface area of the reservoirs, direct UV light exposure has the potential to inhibit bacteria at the surface. However, bacteria may only temporarily experience negative effects of UV exposure, or recover more quickly than other organisms, either at night or by mixing to lower depth (Lindell et al. 1996). More likely, the bacteria benefit from the increased bioavailability of DOM more than they are inhibited by UV exposure (Lindell et al. 1995).

The magnitude and net effect of these processes within the SWP are currently unknown. In addition, a possible restoration of 40,000 ha of Delta agricultural land to wetlands (Fleck et al. 2004) proposed to provide shallow water habitat for spawning and refuge and increase food availability for the Delta fish population in decline since the 1970s (Jassby et al. 2002) may affect both the concentration and composition of the DOC entering the SWP from the Delta. This change in DOC concentration, in turn, may affect not only the microbial food web ecology, but also the drinking water quality in the SWP.

1.8 Study Goals

This study looks at two aspects of microbial processes in the SWP: (1) determining the bioavailability of dissolved organic carbon and (2) characterization of the community composition of *Actinobacteria,* a common fresh water microbe known to cause taste and odor problems. In Chapter 2, an estimate of DOC bioavailability along the SWP is determined both prior to and following photoexposure. Chapter 3 addresses the temporal and spatial composition of the *Actinobacteria* assemblage in the SWP by determining the genetic fingerprint using denaturing gel gradient electrophoresis (DGGE), comparing the population to *Actinobacteria* previously described in the literature through clone library construction and 16S rRNA sequencing and determining the average relative abundance using real-time PCR (qPCR).

1.9 References

Amy, G. L., J. M. Thompson, L. Tan, M. K. Davis, and S. W. Krasner. 1990. Evaluation of THM precursor contributions from agricultural drains. Journal American Water Works Association 82**:** 57-64.

Bergamaschi, B. A., M. S. Fram, C. Kendall, S. R. Silva, G. R. Aiken, and R. Fujii. 1999. Carbon isotopic constraints on the contribution of plant material to the natural precursors of trihalomethanes. Organic Geochemistry **30:** 835-842.

California Department of Water Resources (DWR). 2006. http://www.publicaffairs.water.ca.gov/swp/history_swp.cfm. Accessed 10 April 2006.

California Department of Water Resources (DWR). 1993: Sacramento-San Joaquin Delta atlas. The Resources Agency, Sacramento, 121p.

Driedger, S. M., and J. Eyles. 2003. Different frames, different fears: communicating about chlorinated drinking water and cancer in the Canadian media. Social Science & Medicine 56**:** 1279-1293.

Environmental Protection Agency (EPA). 1993. http://www.epa.gov/OGWDW/mdbp/mdbp.html. Accessed 10 February 2006.

Fleck, J. A., D. A. Bossio, and R. Fujii. 2004. Dissolved organic carbon and disinfection by-product precursor release from managed peat soils. Journal of Environmental Quality 33**:** 465-475.

Jassby, A. D., and J. E. Cloern. 2000. Organic matter sources and rehabilitation of the Sacramento-San Joaquin Delta (California, USA). Aquatic Conservation-Marine and Freshwater Ecosystems 10**:** 323-352.

Jassby, A. D., J. E. Cloern, and B. E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnology and Oceanography 47**:** 698-712.

Klausen, C., N. Jorgensen, M. Burford, and M. O'donohue. 2004. Actinomycetes may also produce taste and odour. Water**:** 45-48.

Koivusalo, M., E. Pukkala, T. Vartiainen, J. J. K. Jaakkola, and T. Hakulinen. 1997. Drinking water chlorination and cancer: a historical cohort study in Finland. Cancer Causes and Control 8**:** 192.

Kraus, T. E. C., B. A. Bergamaschi, B. Downing, and M. S. Fram. 2005. Improving delta drinking water quality: Managing sources of disinfection byproduct-forming material in the State Water Project: Draft Final Data Report. Unpublished.

Lindell, M. J., H. W. Graneli, and L. J. Tranvik. 1996. Effects of sunlight on bacterial growth in lakes of different humic content. Aquatic Microbial Ecology **11:** 135-141

Lindell, M. J., W. Graneli, and L. J. Tranvik. 1995. Enhanced bacterial-growth in response to photochemical transformation of dissolved organic-matter. Limnology and Oceanography 40**:** 195-199.

Miller, W. L., M. A. Moran, W. M. Sheldon, R. G. Zepp, and S. Opsahl. 2002. Determination of apparent quantum yield spectra for the formation of biologically labile photoproducts. Limnology and Oceanography **47:** 343-352.

Moran, M. A., W. M. Sheldon, and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnology and Oceanography **45:** 1254-1264.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnology and Oceanography 42**:** 1307-1316.

Pomeroy, L. R. 1974. Oceans food web, a changing paradigm. Bioscience 24**:** 499-504.

Raymond, P. A., and J. E. Bauer. 2001. Use of C-14 and C-13 natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: a review and synthesis. Organic Geochemistry 32**:** 469-485.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2003. Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquatic Microbial Ecology **31:** 85-98.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2005. Sources, bioavailability and photoreactivity of dissolved organic carbon in the Sacramento-San Joaquin River Delta. Biogeochemistry 74**:** 131-149.

Tranvik, L. J., and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquatic Microbial Ecology 14**:** 301-307.

Van Hannen, E. J., G. Zwart, M. P. Van Agterveld, H. J. Gons, J. Ebert, and H. J. Laanbroek. 1999. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Applied Environmental Microbiology **65:** 795-801.

Villanueva, C. M. and others 2004. Disinfection byproducts and bladder cancer - A pooled analysis. Epidemiology 15**:** 357-367.

Waller, K., S. H. Swan, G. Delorenze, and B. Hopkins. 1998. Trihalomethanes in drinking water and spontaneous abortion. Epidemiology 9**:** 134-140.

Zaitlin, B., S. B. Watson, J. Ridal, T. Satchwill, and D. Parkinson. 2003. Actinomycetes in Lake Ontario: habitats and geosmin and MIB production. Journal American Water Works Association **95:** 113-118.

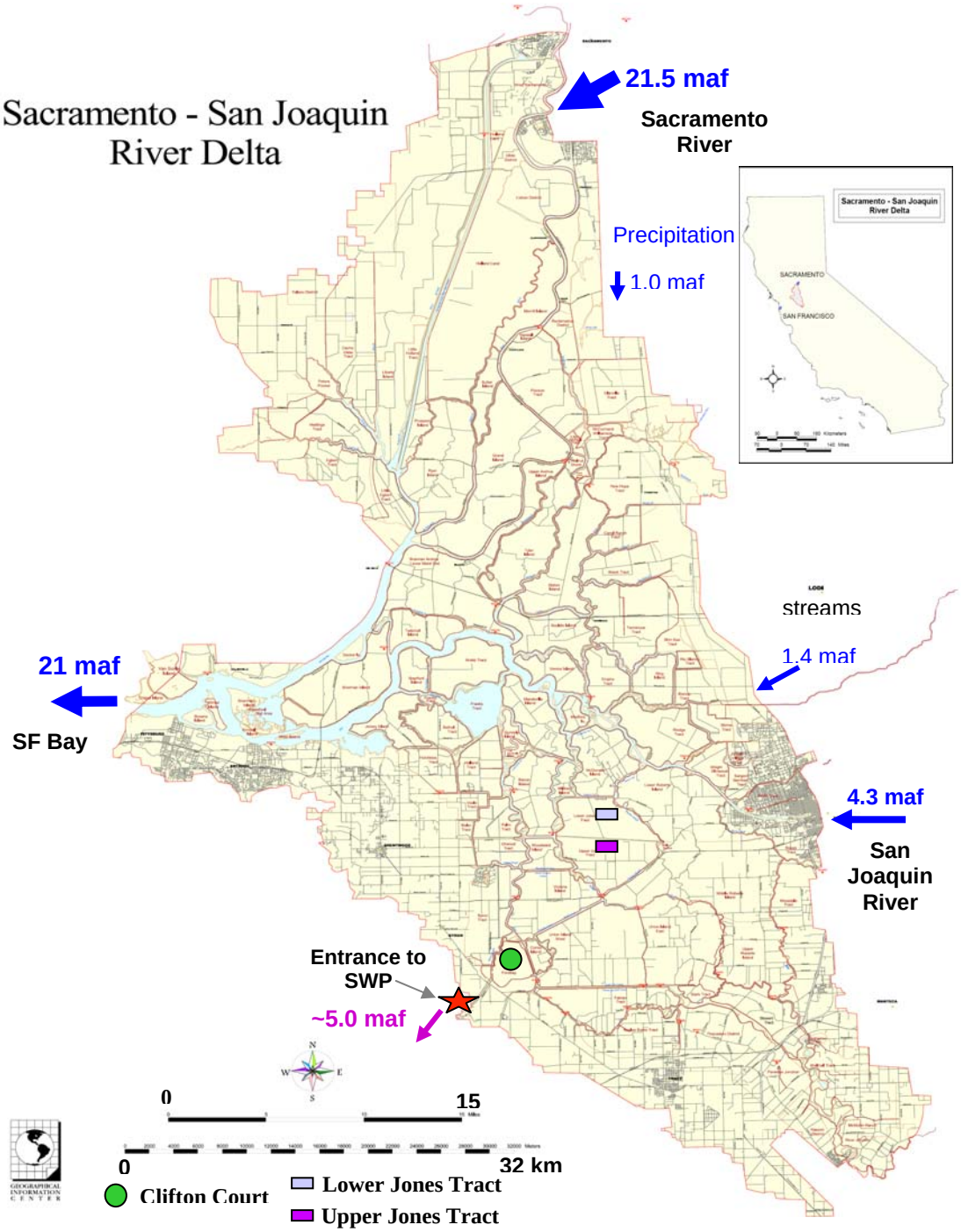

Figure 1.1 Map of the Sacramento-San Joaquin Delta. Adapted from the California State University, Chico website (www.gic.csuchico.edu/projects/watersheds/delta/). Flow data in million acre feet (maf) (DWR (1993) for 1980-1991).

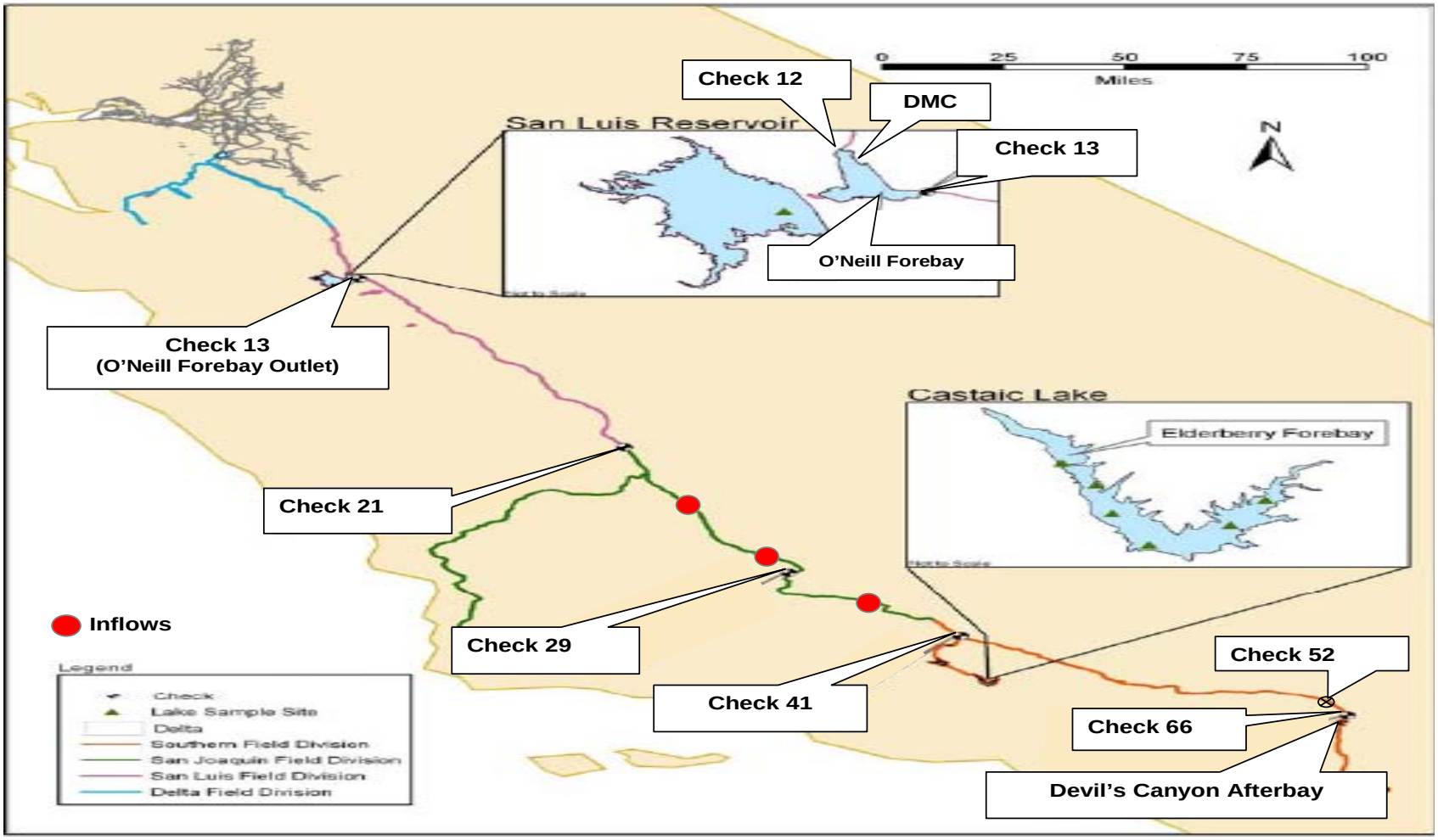

Figure 1.2 Map of the California State Water Project indicating sites sampled along the main stem of the CA Aqueduct, San Luis Reservoir and Castaic Lake. Inputs along the aqueduct, north to south, denoted with a circle: Semitropic Inflow (groundwater), Kern River Inflow, Arvin Edison Inflow (groundwater). Adapted from DWR 2005.

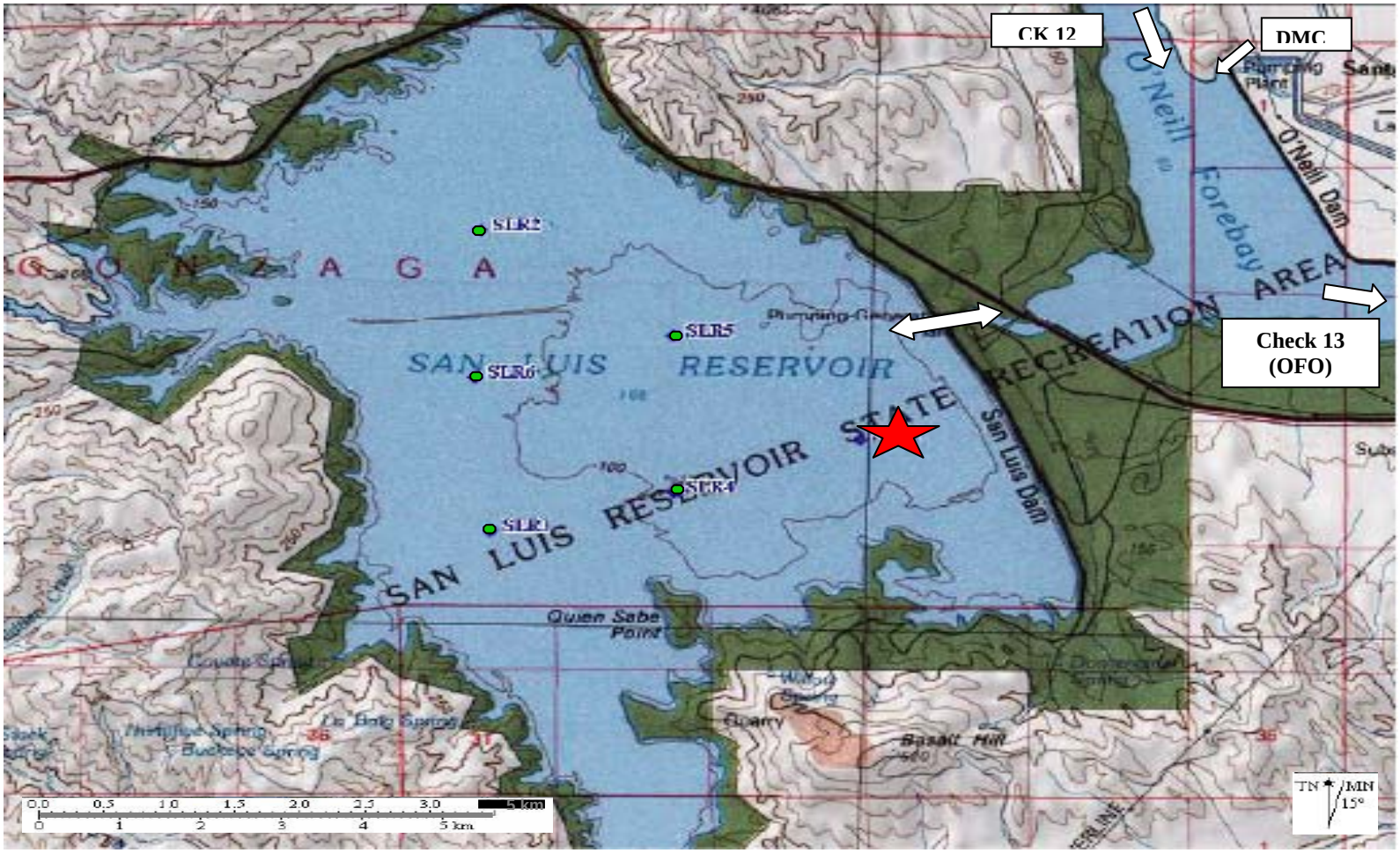

Figure 1.3 Map of the San Luis Reservoir indicating sampling location (star). Arrows indicate water entering O'Neill Forebay from the Delta through the aqueduct at Check 12 (top arrow) and exiting at O'Neill Forebay Outlet (OFO) (right arrow) into the aqueduct south of O'Neill. The double arrow indicates water pumped in and out of San Luis Reservoir. Printed from TOPO!© 2001 National Geographic Holdings (www.topo.com).

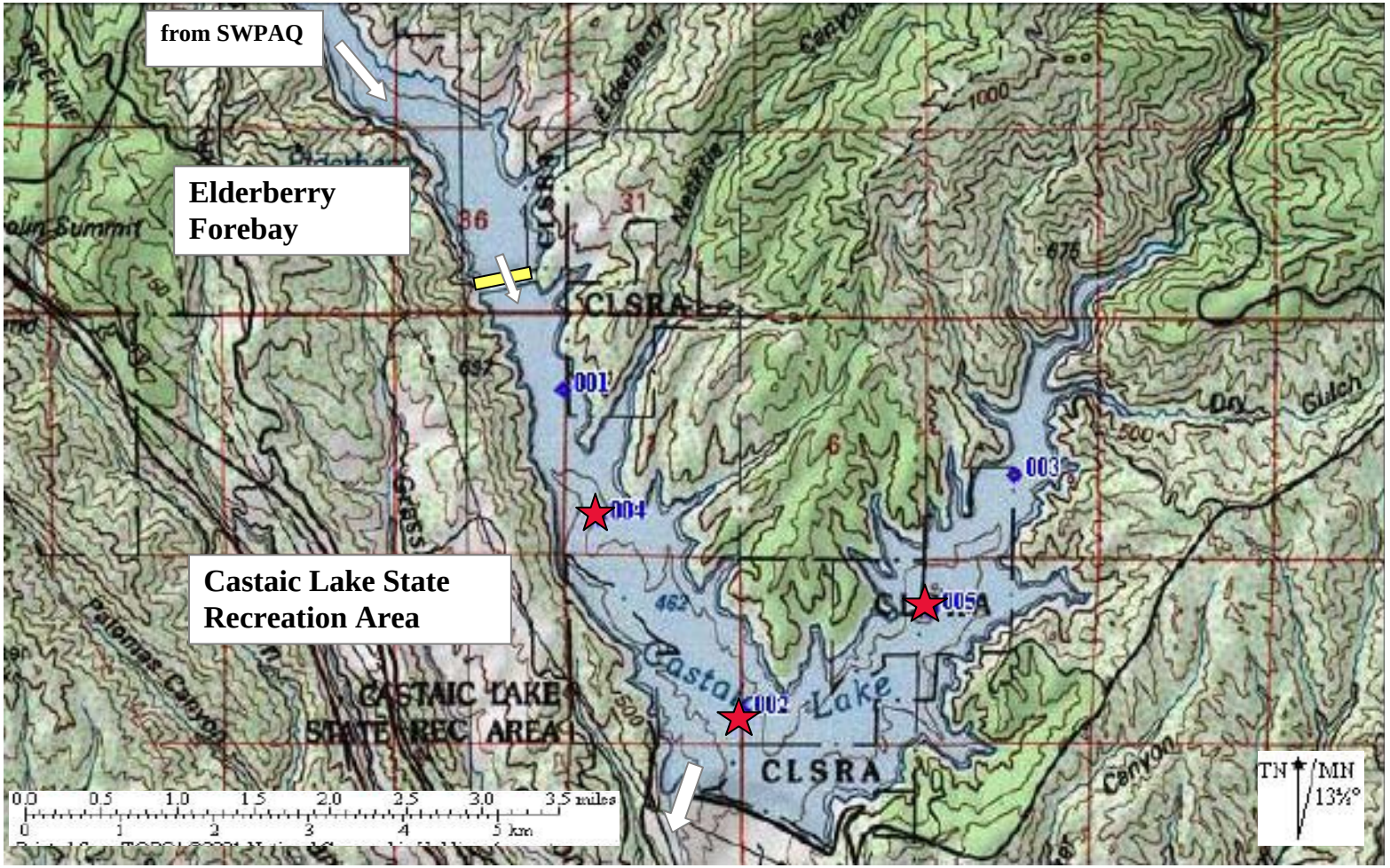

Figure 1.4 Castaic Lake water samples collected at stations denoted with red stars. Top arrow indicates water entering Elderberry Forebay from the aqueduct (SWP AQ), continuing to Castaic Lake through the upper dam (yellow bar). Water exits Castaic Lake through the lower dam (bottom arrow) to water treatment facilities. Adapted from TOPO!© 2001 National Geographic Holdings (www.topo.com).

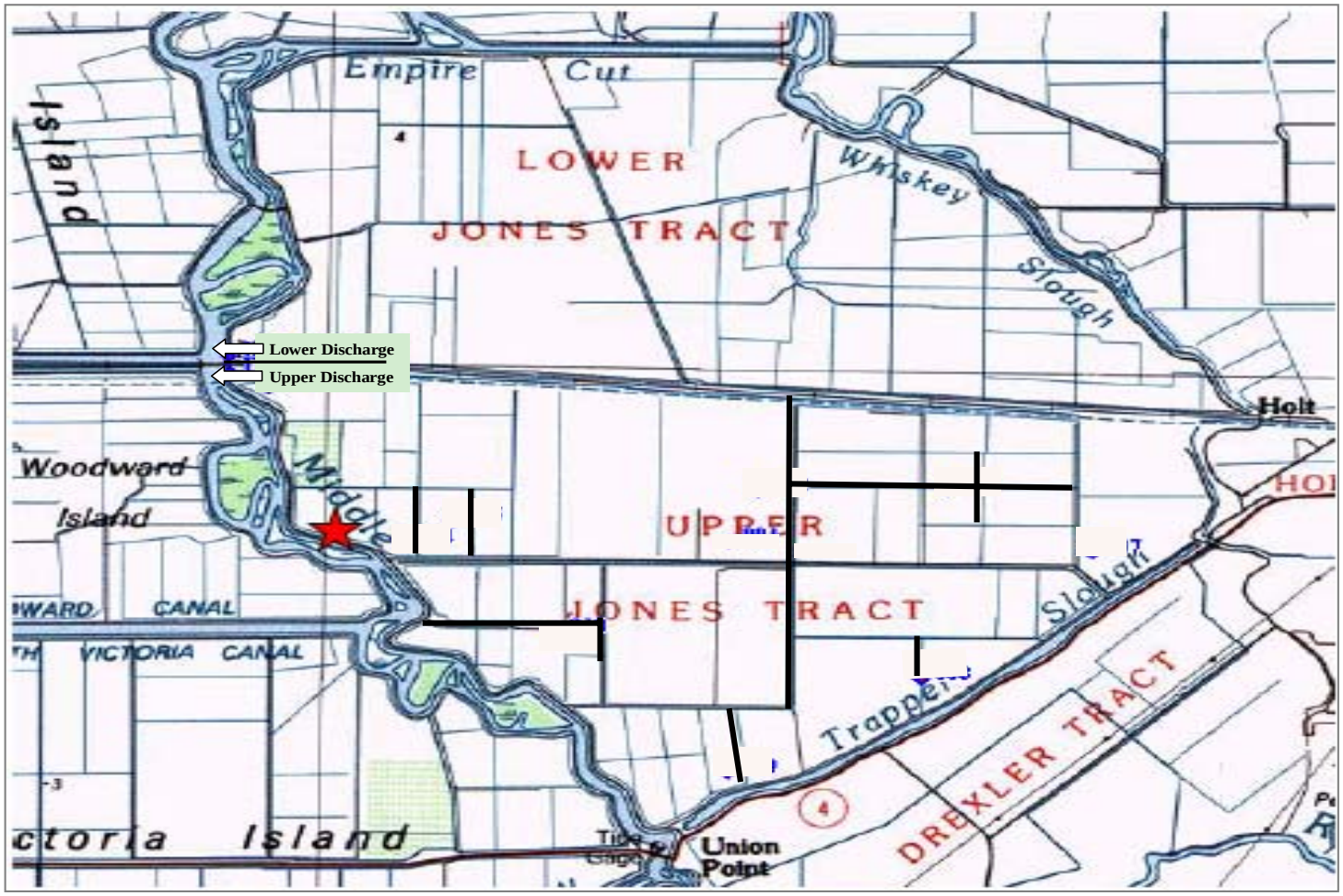

Figure 1.5 Upper and Lower Jones Tract site locations: Arrows indicate sampling sites at the discharge points of Upper and Lower Tracts. Red star indicates approximate location where the levee break occurred. (Adapted from Topo! 2001 National Geographic Holdings, www.topo.com).

CHAPTER 2

DISSOLVED ORGANIC CARBON IN THE STATE WATER PROJECT: SOURCES AND TRANSFORMATIONS

2.1 Introduction

The California State Water Project (SWP) contains seasonally high concentrations of dissolved organic carbon (DOC) arriving from the Sacramento-San Joaquin Delta (Jassby and Cloern 2000) and Delta Mendota Canal (DMC; Figure 1.2). DOC in the SWP is problematic as it can result in the formation of disinfectant byproducts (DBPs) during drinking water treatment (Bergamaschi et al. 1999). Long-term exposure to DBPs has been linked to bladder cancer (Koivusalo et al. 1997) and other health concerns. Degradation of DOC in the SWP during transport to treatment plants may reduce the potential to form DBPs during treatment. Alternatively, DOC produced in the SWP by phytoplankton or periphyton may enhance the concentration of DOC or alter its reactivity. Knowledge of the processes affecting concentrations of DBP-forming materials will help SWP managers implement practices that reduce DBP precursors, thereby ameliorating DBP formation at the treatment plant.

The majority of tributaries to the Delta contain significant concentrations of humic material: colored dissolved organic carbon of high molecular weight (HMW) with a high content of aromatic moieties (Jassby and Cloern 2000). These compounds are also resistant to microbial degradation. A fraction of these HMW, recalcitrant DOC polymers can be converted into low molecular weight (LMW) compounds by photolysis (Miller et

al. 2002; Moran et al. 2000; Moran and Zepp 1997), that are more suitable for use as a substrate for bacterial growth.

Conversely, phytoplankton-derived DOC is primarily LMW, more biologically labile and low in UV-absorbing aromatic structures. However, UV radiation exposure may also cause condensation reactions within this LMW DOC fraction making it increasingly recalcitrant (Tranvik and Kokalj 1998) and decreasing the fraction of DOC substrate immediately available to bacteria.

The concentration of UV absorbing compounds as indicated by absorbance at 250 nm (A_{250}) is useful in distinguishing between terrestrial and phytoplankton-derived carbon (Tranvik and Bertilsson 2001). Terrestrially derived HMW DOC is primarily biologically refractory and optically dense, especially in the UV region of the spectrum, with a high absorbance at 250 nm. Conversely, phytoplankton derived DOC is primarily LMW and more transparent, with a lower absorbance at 250 nm. UV absorbance measured at 250 nm in a 1 cm cuvette, therefore, can be used as an indicator of the relative contribution of the two sources. Furthermore, Tranvik and Bertilsson (2001) have proposed a system for classifying fresh waters based on A_{250}: clearwater ($A_{250} < 0.25$ cm $^{-1}$) vs. humic ($A_{250} > 0.25$ cm $^{-1}$) and oligotrophic (< 4 μg Chl-a/L) vs. eutrophic (> 4 μg Chl-a/L).

Approximately 80 % of the DOC in the 0 – 2 m layer of the epilimnion of a clear-water lake with an average DOC concentration of ~ 4 mg/L and 15 – 20 % of a humic lake can be photochemically consumed annually assuming no new DOC production, a stagnant epilimion and the photosensitivity of DOM is constant (Graneli et al. 1996). In clear lakes with long hydraulic retention times, photooxidation can have a substantial effect on overall DOC transformation, as photooxidation can act to great depths for

longer periods, especially during summer (Lindell et al. 2000). After prolonged exposure, the DOC can become photorefractory and resistant to further photooxidation. This condition can continue until fall/winter mixing changes the nature of DOC in the epilimnion.

Yet, as DOC is being made more available to bacteria through photooxidation, UV radiation also has the potential to inhibit their growth. UV inhibition may only be temporary, as bacteria have the ability to repair damage caused by UV exposure while at depth or at night (Lindell et al 1996). However, Lindell et al. (1995) concluded that bacteria are more likely to benefit from increased DOC bioavailability than to be inhibited by UV.

The magnitude and net effect of these processes within the SWP is currently unknown. In addition, the possible restoration of 40,000 ha of Delta agricultural land to wetlands (Fleck et al. 2004) proposed to enhance Delta fish populations that have been in decline since the 1970s (Jassby et al. 2002) may affect both the concentration and composition of the DOC entering the SWP from the Delta. This change may affect both the microbial processing of DOC and the quality of SWP drinking water.

This study measured DOC bioavailability in the SWP both prior to and following a simulated 24 hr exposure to solar irradiance. These bioavailability data contribute to a larger study of DOC quality and concentration, and of disinfection byproduct formation potential, in the California State Water Project that is a collaboration between scientists from the US Geological Survey Sacramento district office (USGS), the California Department of Water Resources (DWR), the Metropolitan Water District of Southern California (MWD) and the University of Georgia (UGA).

2.2 Sampling Strategy

Locations of the sampling sites along the aqueduct (Figure 1.2) and the reservoirs (Figure 1.3 and 1.4) are detailed in Tables 2.1 and 2.2. Sampling began in May 2004 and was completed in February 2005 (Table 2.3). Jones Tract, a diked, agricultural island in the Delta was flooded by a levee break on June 3, 2004. The flooding and subsequent pumping out of Jones Tract gave rise to concerns that high concentrations of DOC leached from the island's peaty soils would enter the Delta and affect both the quantity and quality of DOC entering the SWP. Accordingly, Jones Tract was sampled July 14, August 24 and November 14, 2004.

Most water samples were obtained using a submersible pump fitted with high purity, plasticizer free Tygon tubing. Water was collected in polyethylene bottles (Nalgene) washed prior to sample collection with 10 % HCl and rinsed repeatedly with DI water. Sample bottles were rinsed three times with sample water prior to filling. Unfiltered samples were collected in 250 mL bottles. For filtered water, 1 L samples were passed sequentially through a 10 μm pore size polypropylene filter and 0.2 μm pore size pleated nylon membrane filter (Osmonics). Samples were immediately placed on ice and transported to the lab where they were refrigerated (4ºC) until analysis. Unfiltered samples collected from Jones Tract by DWR were vacuum filtered in the lab within 24 hours of collection using 0.3 μm nominal pore size GF/F glass fiber filters and refrigerated. Finally samples for Chl-a and Pheop-a collected by the USGS were filtered within 4 h of collection on 0.3 μm nominal pore size GF/F glass fiber filters, wrapped in aluminum foil, placed on dry ice in the field and kept frozen until analysis at the USGS in lab in Sacramento.

Chemical and biological measurements

USGS field measurements of temperature, pH, dissolved oxygen (DO) and conductivity (EC) were measured using a YSI 600 XL sonde. DWR measured EC and DO in the field using a YSI-85 meter and pH and temperature with a Corning 3141 pH meter. Subsamples from all dates were immediately sent on ice to UGA for bioavailability analysis.

Chemical measurements were made by the USGS California Water Science Center Department in Sacramento with the results detailed in a USGS report by Kraus et al. (2005). Measured variables included: DOC; chlorophyll-a and pheophyton-a concentrations; pH; conductivity (EC); specific UVA (SUVA = 100*UVA_{254}/DOC (mg/L)); anions (Cl, Si, SO_4); cations (Na, Ca, K, Mg, Fe, Mn); nitrogen (NH_4, NO_3, NO_2, Total-N) and phosphorus (Ortho-P, Total P). The 0.2 μm filtered and acidified (pH 2) water samples were analyzed with a UV/VIS spectrophotometer (Cary) in a 1 cm path length quartz cuvette recording absorbance from 200 - 750 nm.

Water quality variables were measured by lowering a sonde from a boat at a station in San Luis Reservoir and at three stations in Castaic Lake. The sensor package consisted of a conductivity-temperature-depth meter (Sea Bird SB37), a nine-channel spectral photometer (measuring: 412, 440, 488, 510, 532, 555, 650, 676 and 715 nm; WetLabs AC-9), a fluorometer (WetLabs WetStar) and a data logger (WetLabs DH-4). The pumping and filtering system described above was used to collect water from mid-depth at the checks of the aqueduct. DCA was sampled from a tap coming out of the ground.

Sampling locations – California Aqueduct

In May 2004, almost all water entering O'Neill Forebay originated from San Luis Reservoir (92 %) with the remainder entering from the Delta via Check 12 as reported by SWP Operations for ten days prior to sampling (May 1 - May 11, 2004; Figure 2.1). Water was sampled over three consecutive days, once per check beginning at Check 13, and not composited over time, thus with no attempt to sample the same parcel as it traveled down the aqueduct. As a result, changes in DOC concentration and quality observed during this sampling period could reflect changes in the mixture or quality of source waters. Check 12 and DMC were not sampled.

In October 2004, water entering the aqueduct originated from three sources. SWP Operations reported that water was entering O'Neill Forebay from the CA Aqueduct (Check 12; 33.2 % of daily flow; Figure 2.2) from the Delta Mendota Canal (30.9 %) and from San Luis Reservoir (35.8 %). In response to this complex mixture entering the aqueduct, the "California Aqueduct Water Quality Model" was run as detailed by the USGS (Kraus et al. 2005)**.** The purpose of using this model was to ensure that the same water parcel was optimally sampled as it traveled down the aqueduct. The model, initially developed for the Metropolitan Water District by Harvey Mudd College, simulates the blending, dispersion and transport of a generic tracer added to the aqueduct at a given location and time. A simulated 'tracer' was added to the model at Check 13 at 10:00 am on October 12, 2004 and the model was updated daily as actual pump rates became available. The model provided estimates of tracer concentration, and thus dispersion, for each station downstream of Check 13 over time. Water was collected at each check when

the tracer concentration was predicted to be at its maximum (Figure 2.3) and composted over at least a 3-hour period between 9 am-1 pm PST.

In February, water O'Neill Forebay originated from the Delta via Check 12 (61 %) with the remainder from DMC (37 %; Figure 2.4). The model was run as described above and sampled accordingly.

Sampling locations – reservoirs

Water samples were collected at one station near where the water is pumped into and out of the in San Luis Reservoir from O'Neill Forebay (Figure 1.3). Castaic Lake was sampled from three stations: near the outlet tower (Station 2), on the west branch (Station 4) and on the east branch (Station 5; Figure 1.4).

Reservoir samples were composited from three subsamples of two representative water masses separated by a thermocline, if present, or corresponding to previously established sampling depths during well-mixed conditions (Table 2.4). The location of the thermocline was established by temperature measurements made from a boat.

Elderberry Forebay samples were collected from the Elderberry Outlet Tower, using a Niskin bottle to collect water from 3 - 5 m. Water was filtered on shore as described above.

Jones Tract sampling

Jones Tract (Figure 1.5) was flooded by a levee break on June 3, 2004. This agricultural tract lies below sea level and the average flooded depth was 3.6 m. The levee breach was closed and the flood waters were pumped out beginning on July 12. Jones Tract was sampled by the USGS on July 14, August 24 and October 14 and by DWR on

November 14, 2004. Two sites were sampled: Upper Jones Tract (UJT: 37º 56' 34"N, 121º 31' 89"W); and Lower Jones Tract (LJT: 37º 56' 44"N, 121º 31' 89"W).

July 14 UJT water samples were collected by boat from 2.5 m depth near the drainage pump intake. On August 24, the water level had dropped by 1.2 m and it had dropped by 3.7 m when samples were collected on October 14. Pumping had slowed by November 2 because UJT mostly drained. LJT samples and UJT samples collected in August, October and November samples were taken directly from the pump discharge.

2.3 Analytical Methods

Dissolved organic carbon concentrations

Water quality variables were determined in the lab by the USGS California Water Science Center Department in Sacramento, the Metropolitan Water District (MWD) in Los Angeles and the University of Georgia (UGA). DOC concentrations analyzed at UGA were consistently higher than the DOC samples analyzed by the USGS (Figure 2.5). Therefore, DOC concentrations measured by USGS will be used in referring to environmental data, whereas DOC data measured at UGA will be used in the bioavailability analysis, only. DOC concentrations and temperature measurements for Jones Tract are detailed in Figure 2.6.

The USGS in Sacramento used a Shimadzu TOC-5000A total organic carbon analyzer to determine DOC concentrations in the water samples within 1 day of returning to the lab from the field. Data analyzed by the Metropolitan Water District and Department of Water Resources in California was obtained from their websites. All samples were filtered (0.2 μm) either in the field then acidified to pH 2 (±0.2) in the field or immediately upon returning to the lab. To determine DOC concentration during

processing at UGA, 10 mL subsamples of water were taken at UGA from both the non-irradiated control treatment and immediately following 4 hours of irradiation. The samples were acidified immediately to pH 2 for storage until DOC measurements could be made on a Shimadzu TOC-5000 analyzer.

Irradiations, biological oxygen demand and bioavailability

Water samples were filtered in the field as described previously. Samples were either placed on ice or refrigerated (4 ºC) until arrival at the UGA lab. Refrigeration has been shown to be an adequate means of storage for the 10 days from sample collection to processing (Stepanauskas et al. 2005). Biological oxygen demand (BOD) and determination of bioavailability (BDOC) were carried out essentially as described in Stepanauskas et al. (2005). Briefly, irradiations and bioassays were started within 10 days from the date of sampling and were conducted on an Atlas Sunset CPS solar simulator under a 1 kW Xe lamp producing PAR and UV spectra similar to solar radiation (Miller et al. 2002). Quartz flasks were positioned in a water bath at 4 ºC and the samples were exposed to UV irradiation for 4 h. This simulated UV exposure time was previously calculated to be roughly equivalent to one day of solar radiation at the water's surface in mid-afternoon July in the Sacramento-San Joaquin Delta (Stepanauskas et al. 2005). Control samples were wrapped in foil and also placed in the bath.

A 14-day bioassay was used to determine the concentration of potentially bioavailable DOC using oxygen consumption by the natural microbial assemblage as a measure of DOC metabolism (Covert and Moran 2001). A mixture of inorganic nutrients (final concentration [10 μM $NaNO_3$ and 1 μM NaH_2PO_4]) was added to ensure carbon limitation. Six replicate BOD bottles were prepared and incubated in the dark for 14 days

at 15 ºC. Three of the six bottles were immediately fixed with Winkler reagents prior to incubation and the remaining three bottles were fixed at the end of the incubation. Both initial and final oxygen concentrations were measured using an automated titrator (Mettler Toledo; Pomeroy et al. 1994).

Finally, the BDOC was estimated for both the control and irradiated samples by dividing the oxygen consumption (μM O_2) by the initial concentration of DOC (μM C) measured prior to irradiations. The assumption is that, on average, two atoms of oxygen are needed to oxidize one atom of carbon in the production of CO_2 during microbial respiration.

Because potential residence times in the SWP vary from weeks in the aqueduct to years in the reservoirs, the DOC bioavailability values obtained for the two-week incubation period should be viewed as the lower limit for carbon bioavailability in the reservoirs and as the upper limit in the aqueduct of the SWP.

Field and laboratory measurements

All measurements made in the field including temperature, pH, dissolved oxygen, nutrients and anions are summarized in Table 2.5 and 2.6. $A_{250,}$ a proxy for humic material and colored organic matter (CDOM), was measured in the lab (Table 2.7) and found to be from 0.09 - 0.17 cm^{-1} in the reservoir and aqueduct samples. Tranvik and Bertilsson (2001) described clearwater as having an $A_{250} < 0.25$ cm^{-1}. The SWP water will be classified as clearwater, containing little humic or colored material. Conversely, the absorbance measured in the Delta at Jones Tract ($A_{250} \sim 0.68 - 1.3$ cm^{-1}) classified it as humic and colored.

2.3 Results and Discussion

Bioavailability of dissolved organic carbon

DOC bioavailability was determined prior to and following simulated photoexposure to examine the impact of microbial consumption and transformation of DOC in the SWP. Delta organisms have been reported to consume much of the biologically labile DOC in Delta water (Stepanauskas et al. 2005), therefore it is predicted that the bulk of the DOC pool entering the SWP would be biologically refractory in nature.

Reservoir DOC bioavailability

Mean DOC concentrations were similar at both San Luis Reservoir (3.4 ± 0.2 mg/L) and Castaic Lake (3.2 ± 0.2 mg/L) and Elderberry Forebay (3.3 ± 0.9; Figure 2.7) and were comparable to the average yearly Delta DOC concentration reported at Clifton Court Forebay (3.7 ± 1.6 mg/L; Stepanauskas et al. 2005). Chl-a concentrations were higher in all San Luis Reservoir samples (0.6 µg/L to 39.8 µg/L) than in Castaic Lake samples (1.0 µg/L to 2.9 µg/L). However, this may not reflect the true nature of the reservoirs as samples were collected only one day every two months.

Only San Luis Reservoir was sampled in May 2004. The storage capacity of the reservoir had been reached in March with inflow from the Delta. Water was being released into O'Neill Forebay at the time of sampling (Figure 2.1). San Luis Reservoir upper composite (SLRU) showed greater bioavailability in non-irradiated samples (9.2 %) than the lower composite (SLRL, 0.4 %; Figure 2.7). The decrease in bioavailability in the SLRU sample following irradiation suggests that simulated photochemistry was removing bioavailable DOC at the surface (< 25 m). Conversely, the slight increase in bioavailability following irradiation of the SLRL sample suggests an increase in

bioavailable photoproduct from samples collected at depth (> 40 m). Temperature data revealed a slight stratification between SLRU and SLRL (16 ºC and 13 ºC, respectively) suggesting that a thermocline separated the two carbon pools.

There was a general trend of increased bioavailability following irradiation in all reservoir samples in July (Figure 2.7). Initial (non-irradiated) bioavailability was low (< 4 %) which suggested a biologically refractory DOC pool in all reservoirs. Mid-summer conditions (high temperature, 12 h daylight) suggested that photochemistry may either have contributed to a removal of bioavailable DOC prior to sample collection or resulted in release of DOC photoproducts immediately utilized by microbes prior to sample collection. Simulated solar irradiation exposure contributed to an increase in bioavailable DOC in all samples suggesting release of biologically labile photoproduct subsequently utilized by microbes during the 14-day incubation.

Initial (non-irradiated) bioavailability increased noticeably in September from that measured in July in both San Luis Reservoir and Castaic Lake. The high DOC bioavailability in non-irradiated samples from San Luis Reservoir may have reflected biologically labile carbon input from a large algal bloom occurring at the time of sampling (Chl-a ~ 39 µg/L). Simulated photoexposure also increased bioavailability in all samples indicating a release of labile photoproducts as seen in July. The Castaic Lake samples collected in September denoted with a (*) were not iced in the field for 24 hours, inadvertently and may have been compromised. The possible increase in bacterial and/or algal growth may have lead to the increased bioavailability prior to photoexposure measured in the CASTU sample. Bioavailability measurements from CASTL were omitted from Figure 2.7 as the bioavailability in non-irradiated samples (38 %) was high

and suggested that the sample was indeed compromised. The bioavailability measured in both the non-irradiated and irradiated samples from Elderberry Forebay was similar to that measured from samples collected in July.

Initial (non-irradiated) bioavailability decreased in January, from September in both San Luis Reservoir and Castaic Lake samples. There was no statistically significant trend in bioavailability prior to or following photoexposure. San Luis Reservoir was receiving an inflow of water from O'Neill Forebay and the initial (non-irradiated) bioavailability of 6 % resembled the mean DOC bioavailability in samples collected at O'Neill Forebay in February (5 %). The increase in DOC concentration in Elderberry Forebay reflected the seasonal increase in DOC measured in the aqueduct at the time of sampling. However, the decrease in bioavailability following simulated photoexposure suggested that photochemistry removed bioavailable DOC from the Elderberry sample.

Aqueduct DOC bioavailability

Aqueduct bioavailability calculations showed a great deal of error in May due to instrument error in measuring biological oxygen demand (BOD). As a result, May 2004 bioavailability calculations will not be included in the following results and discussion of aqueduct bioavailability. Environmental data will again be referenced to Table 2.5.

Water arrived into O'Neill Forebay in October 2004 from San Luis Reservoir (36 %), the Delta at Check 12 (33 %) and DMC (31 %; Figure 2.2). There was a slight decrease in the DOC concentration as the water traveled down the aqueduct from Check 13 (3 mg/L) to Check 66 (2.2 mg/L; Figure 2.8).

Bioavailability was similar in all initial (non-irradiated) samples (2 - 4 %) collected in October, suggesting that DOC entering the aqueduct from O'Neill Forebay was relatively

biologically refractory. The slight increase in mean bioavailability following irradiation suggested that very little new substrate was released by photochemistry (mean non-irradiated, 3.2 %; irradiated, 3.5 %). The spike in bioavailability following irradiation at Check 21 could not be explained from either the biological or chemical data gathered at that station. The sample collected in February from Check 21 did not show the same trend, indicating that this station may not be a potential problem area along the aqueduct, though future sampling is suggested.

The O'Neill Forebay samples collected in October from Check 12, 13 and DMC denoted with a (*) were compromised due to the incubator light being mistakenly turned on for 48 hours at the mid-point of the 14-day incubation. This provided light to the system and thus the potential for algal growth. This could have led to the production of DOC and/or oxygen in the closed system thereby causing error in the BOD measurement.

Inflows to the aqueduct were sampled in October and chemical and biological data measured (Table 2.6b). However, neither total inflow volumes nor Chl-a and Pheop-a concentrations were available for these stations. Semitropic (groundwater) and Kern River inflows enter the aqueduct between Check 21 and Check 29 and may account for the increase in the bioavailability measured in the non-irradiated sample from Check 29 from bioavailability measured at Check 21. Arvin Edison Inflow entering prior to Check 41 could also affect bioavailability of the downstream checks. The bioavailability in non-irradiated samples decreased at DCA suggesting DOC degradation as the water resides in Lake Silverwood prior to entering DCA. The slight increase in bioavailability following simulated irradiation indicated that new substrate was released by photochemistry from this primarily biologically refractory DOC pool.

Water entering O'Neill Forebay in February 2005 originated primarily from the Delta via Check 12 (61 %) and DMC (37 %; Figure 2.4). Total DOC concentrations in the aqueduct almost doubled in February (5.8 mg/L) from the median DOC concentration measured in May (3.1 mg/L) and October (2.7 mg/L). The increased in DOC concentration reflected the winter rainfall runoff events and terrestrial DOC inputs to the Delta and the SWP prior to and during sampling. DOC concentrations did not decrease in February as in October as water traveled down the aqueduct from Check 13 to Check 66 (Figure 2.5).

The overall increase in bioavailability prior to irradiation, compared to that measured in October, suggests that a biologically labile DOC component was entering the Forebay from the Delta. Bacteria subsequently utilized this increase in bioavailable DOC as the water traveled south down the aqueduct. The heavy winter rainfall influx into the Delta from the tributaries may have resulted in a shorter Delta residence time. There may have been insufficient time for Delta microbes to consume the increased biologically labile DOC fraction, allowing more biologically labile carbon to enter the SWP in February.

Bioavailability in non-irradiated samples gradually decreased as water traveled from Check 21 to Check 29. Bioavailability in non-irradiated samples then increased slightly at Check 41 and Check 66 as water traveled down the aqueduct. This slight increase may be attributed to a biologically labile fraction entering the aqueduct in the Arvin Edison Inflow prior to, or processes occurring near, Check 41 with an increase in Pheop-a measured there (5 µg/L). Both Chl-a and Pheop-a concentrations increased to 20 µg/L and 17 µg/L at Check 66. A slight increase in initial (non-irradiated) bioavailability at this station suggests *in situ* phytoplankton production at this station. DCA bioavailability

decreased relative to Check 66 as in the two prior samplings, suggesting DOC degradation as the water resides in Lake Silverwood.

Overall, median bioavailability in non-irradiated California Aqueduct samples was low for both October (3.2 ± 0.3 %) and February (3.8 ± 0.6 %). This percentage is lower than the 10.7 ± 6 % median Delta bioavailability calculated by Stepanauskas et al. (2005) in Clifton Court near the entrance to the SWP. The SWP bioavailability is also low compared to averages calculated from literature reviews of the total fraction of biologically labile DOC in lakes (14 %) and rivers (19 %; Søndergaard and Middelboe 1995).

Jones Tract DOC bioavailability

Initial bioavailability (12 %; Figure 2.9) in Upper Jones Tract was higher in July than at all other sampling dates and substantially higher than the median DOC concentration measured in San Luis Reservoir in July (3.1 mg/L). The DOC concentration in the monthly samples from the pump discharge increased from July to November in both Upper and Lower Jones Tract samples. However, while the water was being pumped out of the tracts, there was a decrease in initial (non-irradiated) DOC bioavailability measured in the pump discharge. The decrease in non-irradiated bioavailability suggests that the Delta water that initially flooded Jones Tract contained a biologically labile fraction. However, though Chl-a and Pheop-a concentrations were high throughout the samplings (14 - 55 µg/L and 14 - 39 µg/L, respectively; Table 2.6), this did not translate to increased bioavailability in the water that was pumped out of the tracts to the Delta (Figure 2.9).

The initial 12 % bioavailability in Upper Jones Tract in July was comparable to the mean bioavailability reported for Delta stations located near Jones Tract including Frank's Tract (13.3 ± 6 %), Mildred Island (11.8 ± 5 %), Mandeville Tip (10.2 ± 3.6 %), and Prisoner's Point (10.4 ± 4 %; Stepanauskas et al. 2005) and the 12 % median Delta DOC bioavailability reported by Sobczak and Findlay (2002). However, this bioavailability percentage is low compared to global averages for rivers (19 %) and lakes (14 %; Søndergaard and Middelboe, 1995). DOC in Jones Tract appeared to be of low nutritional value due to large terrestrial vs. algal inputs and nutrient replete conditions. Such conditions contribute to high bacterioplankton utilization of the biologically labile fraction of DOC (Jassby and Cloern 2000).

Effect of simulated solar irradiation on DOC

Reservoirs

The effect of simulated solar irradiation was compared to initial bioavailability of DOC in reservoir samples (Figure 2.10). Irradiation effect was calculated by subtracting DOC bioavailability before irradiation from DOC bioavailability after irradiation. There was a general trend in decreasing effect of irradiation with increasing initial bioavailability for all reservoir samples. The mean effect of irradiation was low in San Luis Reservoir (2.6 ± 7.8 %), Castaic Lake (2.6 ± 2.0 %) and Elderberry Forebay (3.13 ± 4.5 %). Though simulated photoexposure did transform a small fraction of the DOC into a more biologically labile form, the increase in mean bioavailable DOC following photoexposure for all samples was low over the course of the 14-day incubation (2.7 ± 5.7 %). However, given the mean residence time in the reservoirs of month to years,

photochemical transformation may be a substantial contributor to the biologically labile fraction of the DOC pool in the reservoirs.

California Aqueduct

The general trend of the decreasing effect of irradiation with increasing initial bioavailability was also apparent in California Aqueduct samples (Figure 2.11). Mean irradiation effects were low for both October (0.7 ± 1.8 %) and February (0.6 ± 0.6 %). The DOC pool transiting the aqueduct appears to be primarily biologically refractory in nature. With a residence time in the aqueduct generally equivalent to the 14-day incubation time, photoexposure appears to have only a minimal effect (0.7 ± 1.7 %) on the biological lability of the DOC pool in the California Aqueduct.

Jones Tract

Irradiation did not have an effect on mean bioavailability (0.01 ± 2.1 %) in either Upper (-1.1 ± 1.7 %) or Lower Jones Tract (1.7 ± 1.7 %; Figure 2.12). In addition, as increased residence time increased DOC concentration, DOC bioavailability did not increase accordingly in samples collected from pump discharge from both tracts into the Delta during the 4 month pump out.

2.5 Conclusion

In conclusion, initial DOC bioavailability was low for the reservoirs, the aqueduct and Jones Tract. The effect of simulated UV irradiation was minimal in both the aqueduct and Jones Tract samples. However, UV exposure had a greater effect on the samples from the reservoirs, likely due to increased residence time in the reservoir catchments (Figure 2.13). The general trend of decreasing effect of irradiation with increasing initial bioavailability was apparent across all locations.

The mean DOC bioavailability along the CA Aqueduct of 3.4 ± 0.4 % was lower than the reservoir mean of 4.4 ± 0.9 %. Compared to 10.7 ± 6 % reported at Clifton Court in the Delta in 2000 - 2001 (Stepanauskas et al. 2005) and the 12 % median Delta DOC bioavailability reported by Sobczak and Findlay (2002), the DOC quality of the water in the SWP appears to be much less available for the microbial population than that in the Delta.

While the geomorphology of the Delta ranges from tidal marshes and wetlands to agricultural land, the engineered conveyance systems of the SWP consist primarily of cement lined aqueducts and treeless reservoirs. As geomorphology has been reported to determine the carbon resources available to the foodweb (Burt and Pinay 2005; Maloney et al. 2005; Wallace et al 1997), it may be assumed that the majority of the available DOC transiting throughout the SWP aqueduct may be substantially different, and potentially of lower quality, than DOC in the Delta.

Further, a majority of the biologically refractory aromatic carbon compounds that have a higher propensity to form DBPs (Reckhow et al. 1990) may not photooxidized by UV and consequently not consumed by bacteria in the SWP. The possibility of transformations to occur during photoexposure is more likely in the reservoirs, due to longer residence times (Miller and Moran 1997). However, San Luis Reservoir primary production did not appear to contribute to an increase in biologically labile DOC entering O'Neill Forebay and the aqueduct during the spring and summer water release in contrast to the amount of DOC entering the SWP from the Delta in February.

The trend of decreasing effect of irradiation with increasing initial bioavailability agreed with the trend reported in the Delta by Stepanauskas et al. (2005). The effects of

UV exposure varied among previous studies, from an increase in bioavailable photoproducts (Miller et al. 2002; Lindell et al. 1995) to a decrease in bioavailable DOC (Obernosterer et al. 1999; Tranvik and Bertilsson 2001).

State Water Project source water will continue to originate from the Delta despite various processes occurring *in situ.* Future restoration projects undertaken in the Delta that may alter DOC concentrations could also have an impact on the quality of water entering in the SWP. However, most of the water that enters the SWP has a much lower DOC concentration than that measured in the Delta. The engineering of Clifton Court, O'Neill and Elderberry forebays appears to have had a definite effect on reducing the concentration of DOC both entering and transiting throughout the SWP. Consequently, the majority of the management of the source water entering the SWP may not lie fully in the hands of the SWP managers, but may instead be a primary concern of the Delta land and watershed managers.

2.6 References

Bergamaschi, B. A., M. S. Fram, C. Kendall, S. R. Silva, G. R. Aiken, and R. Fujii. 1999. Carbon isotopic constraints on the contribution of plant material to the natural precursors of trihalomethanes. Organic Geochemistry **30:** 835-842.

Burt, T. P., and G. Pinay. 2005. Linking hydrology and biogeochemistry in complex landscapes. Progress in Physical Geography **29:** 297-316.

Covert, J. S., and M. Moran. 2001. Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. Aquatic Microbial Ecology **25:** 127-139.

Fleck, J. A., D. A. Bossio, and R. Fujii. 2004. Dissolved Organic Carbon and Disinfection By-Product Precursor Release from Managed Peat Soils. Journal of Environmental Quality **33:** 465-475.

Graneli, W., M. Lindell, and L. Tranvik. 1996. Photo-oxidative production of dissolved inorganic carbon in lakes of different humic content. Limnology and Oceanography **41:** 698-706

Jassby, A. D., and J. E. Cloern. 2000. Organic matter sources and rehabilitation of the Sacramento-San Joaquin Delta (California, USA). Aquatic Conservation-Marine and Freshwater Ecosystems **10:** 323-352.

Jassby, A. D., J. E. Cloern, and B. E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnology and Oceanography **47:** 698-712.

Koivusalo, M., E. Pukkala, T. Vartiainen, J. J. K. Jaakkola, and T. Hakulinen. 1997. Drinking water chlorination and cancer: a historical cohort study in Finland. Cancer Causes and Control **8:** 192.

Kraus, T. E. C., B. A. Bergamaschi, B. Downing, and M. S. Fram. 2005. Improving delta drinking water quality: Managing sources of disinfection byproduct-forming material in the State Water Project: Draft Final Data Report. Unpublished.

Lindell, M. J., H. Graneli, and S. Bertilsson. 2000. Seasonal photoreactivity of dissolved organic matter from lakes with contrasting humic content. Canadian Journal of Fisheries and Aquatic Sciences **57:** 875-885.

Lindell, M. J., H. W. Graneli, and L. J. Tranvik. 1996. Effects of sunlight on bacterial growth in lakes of different humic content. Aquatic Microbial Ecology **11:** 135-141.

Lindell, M. J., W. Graneli, and L. J. Tranvik. 1995. Enhanced bacterial-growth in response to photochemical transformation of dissolved organic-matter. Limnology and Oceanography **40:** 195-199.

Maloney, K. O., P. J. Mulholland, and J. W. Feminella. 2005. Influence of catchment-scale military land use on stream physical and organic matter variables in small southeastern plains catchments (USA). Environmental Management **35:** 677-691.

Miller, W. L., and M. A. Moran. 1997. Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. Limnology and Oceanography **42:** 1317-1324.

Miller, W. L., M. A. Moran, W. M. Sheldon, R. G. Zepp, and S. Opsahl. 2002. Determination of apparent quantum yield spectra for the formation of biologically labile photoproducts. Limnology and Oceanography **47:** 343-352.

Moran, M. A., W. M. Sheldon, and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnology and Oceanography **45:** 1254-1264.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnology and Oceanography **42:** 1307-1316.

Obernosterer, I., B. Reitner, and G. J. Herndl. 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. Limnology and Oceanography **44:** 1645-1654.

Pomeroy, L., J. Sheldon, and W. Sheldon. 1994. Changes in bacterial numbers and leucine assimilation during estimation of microbial respiration rates in seawater by the precision Winkler method. Applied and Environmental Microbiology **60:** 328-332.

Reckhow, D. A., P. C. Singer, and R. L. Malcolm. 1990. Chlorination of humic materials - by-product formation and chemical interpretations. Environmental Science & Technology **24:** 1655-1664.

Sobczak, W. V., and S. Findlay. 2002. Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpaths. Ecology **83:** 3194-3209.

Søndergaard, M., and M. Middelboe. 1995. A cross-system analysis of labile dissolved organic carbon. Marine Ecology-Progress Series **118:** 283-294.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2005. Sources, bioavailability and photoreactivity of dissolved organic carbon in the Sacramento-San Joaquin River Delta. Biogeochemistry **74:** 131-149.

Tranvik, L. J., and S. Bertilsson. 2001. Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. Ecology Letters **4:** 458-463.

Tranvik, L. J., and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquatic Microbial Ecology **14:** 301-307.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science **277:** 102-104.

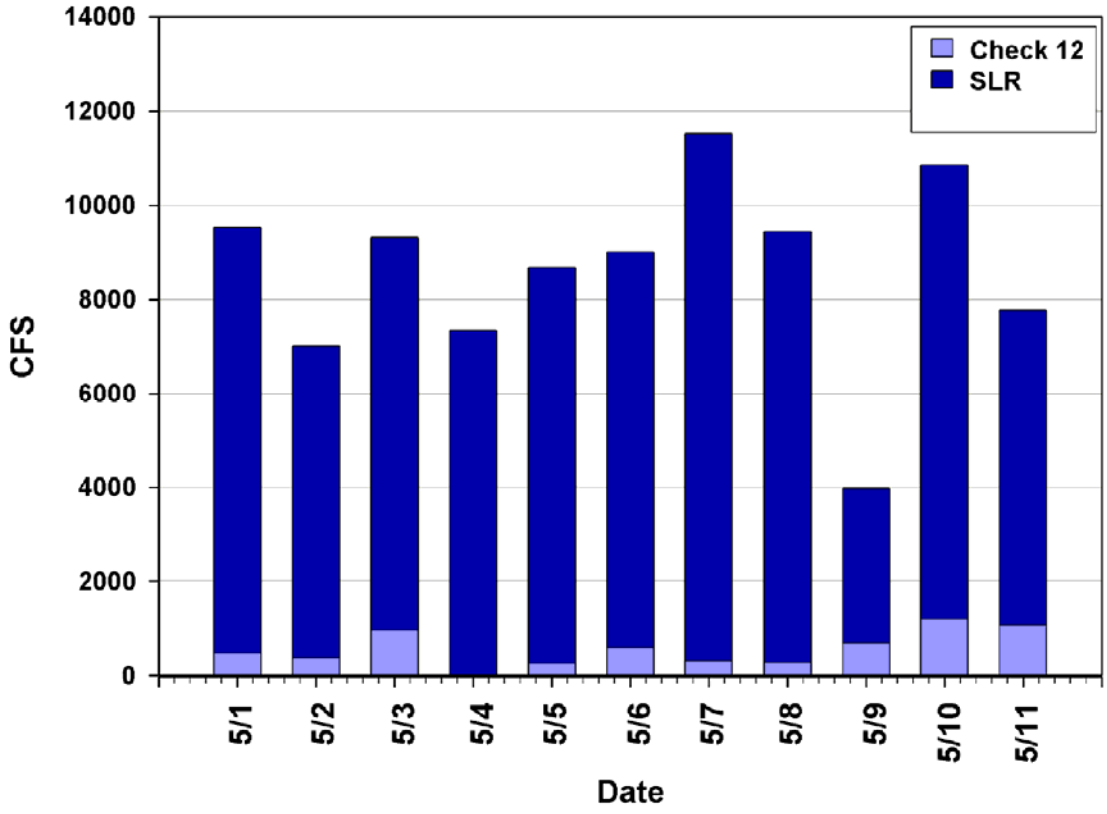

Figure 2.1 Inflow (CFS) to O'Neill Forebay May 2004 from Check 12, San Luis Reservoir (SLR) and Delta Mendota Canal (DMC). Adapted from Department of Water Resources State Water Project Monthly Operation Data. Accessed at the website (http://wwwoco.water.ca.gov/monthly/monthly.menu.html).

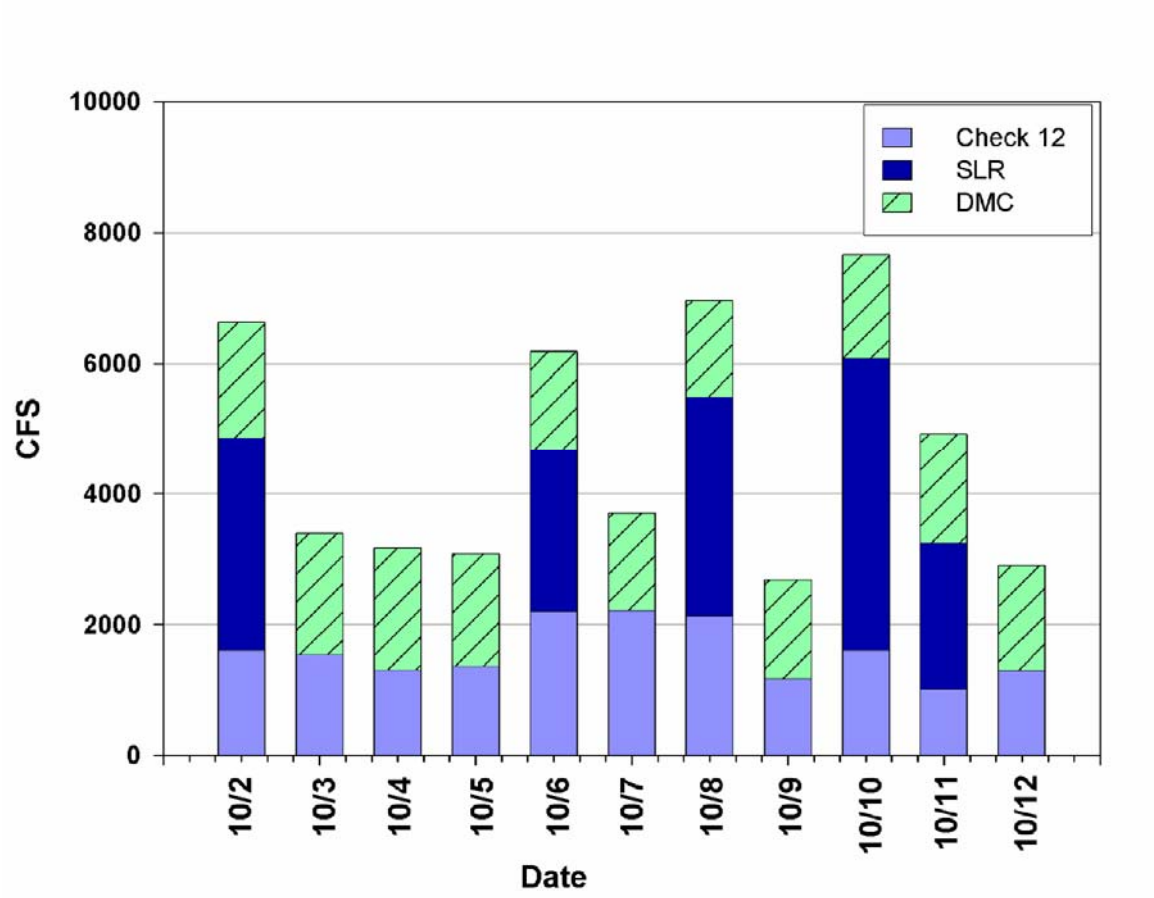

Figure 2.2 Inflow (CFS) to O'Neill Forebay October 2004 from Check 12, San Luis Reservoir (SLR) and Delta Mendota Canal (DMC). Adapted from Department of Water Resources State Water Project Monthly Operation Data. Accessed at the website (http://wwwoco.water.ca.gov/monthly/monthly.menu.html).

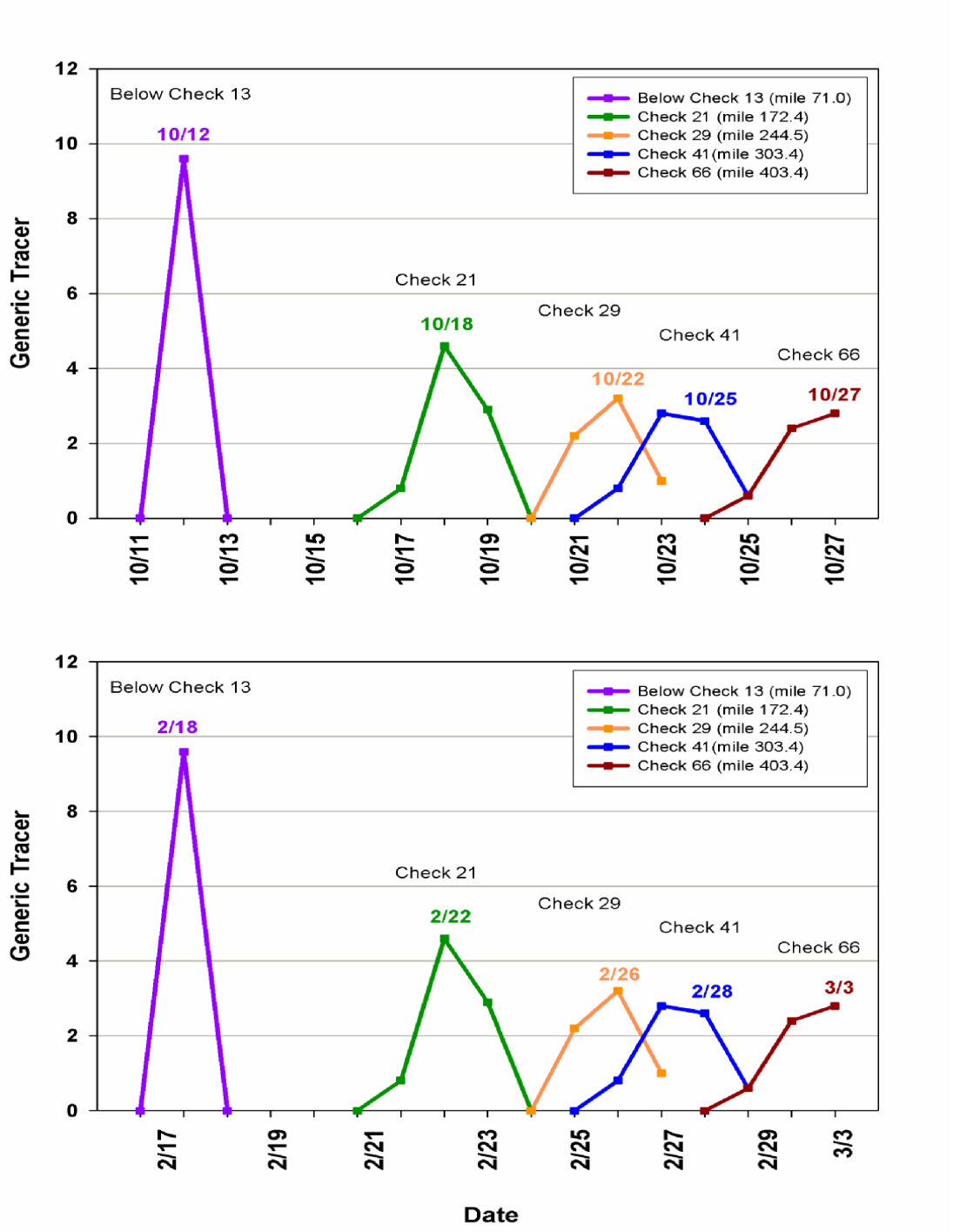

Figure 2.3 Results from the 'California Aqueduct Water Quality Model' run following a generic tracer to predict optimum sampling dates along the CA Aqueduct in (Top) October 2004 and (Bottom) February 2005. Model developed for the Metropolitan Water District by Harvey Mudd College.

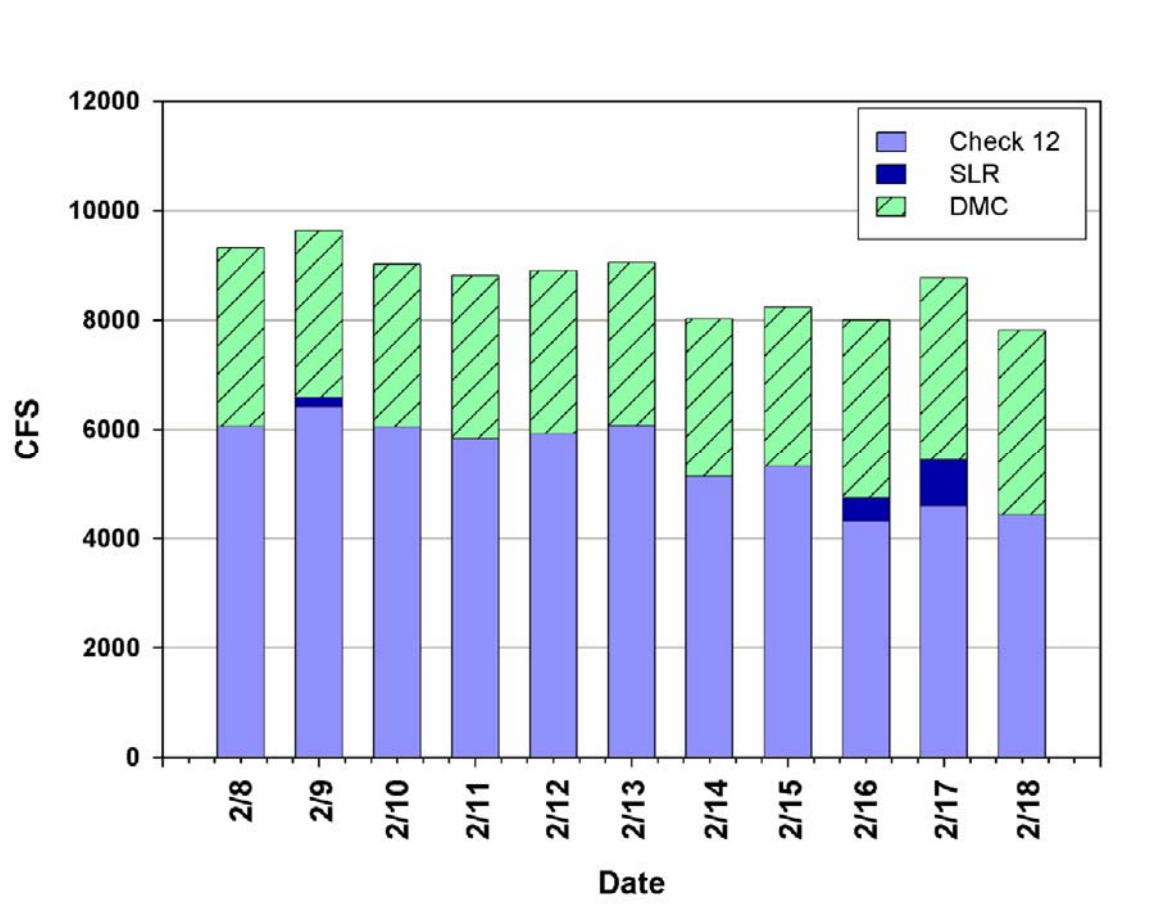

Figure 2.4 Inflow (CFS) to O'Neill Forebay February 2005 from Check 12, San Luis Reservoir (SLR) and Delta Mendota Canal (DMC). Adapted from Department of Water Resources State Water Project Monthly Operation Data. Accessed at the website (http://wwwoco.water.ca.gov/monthly/monthly.menu.html).

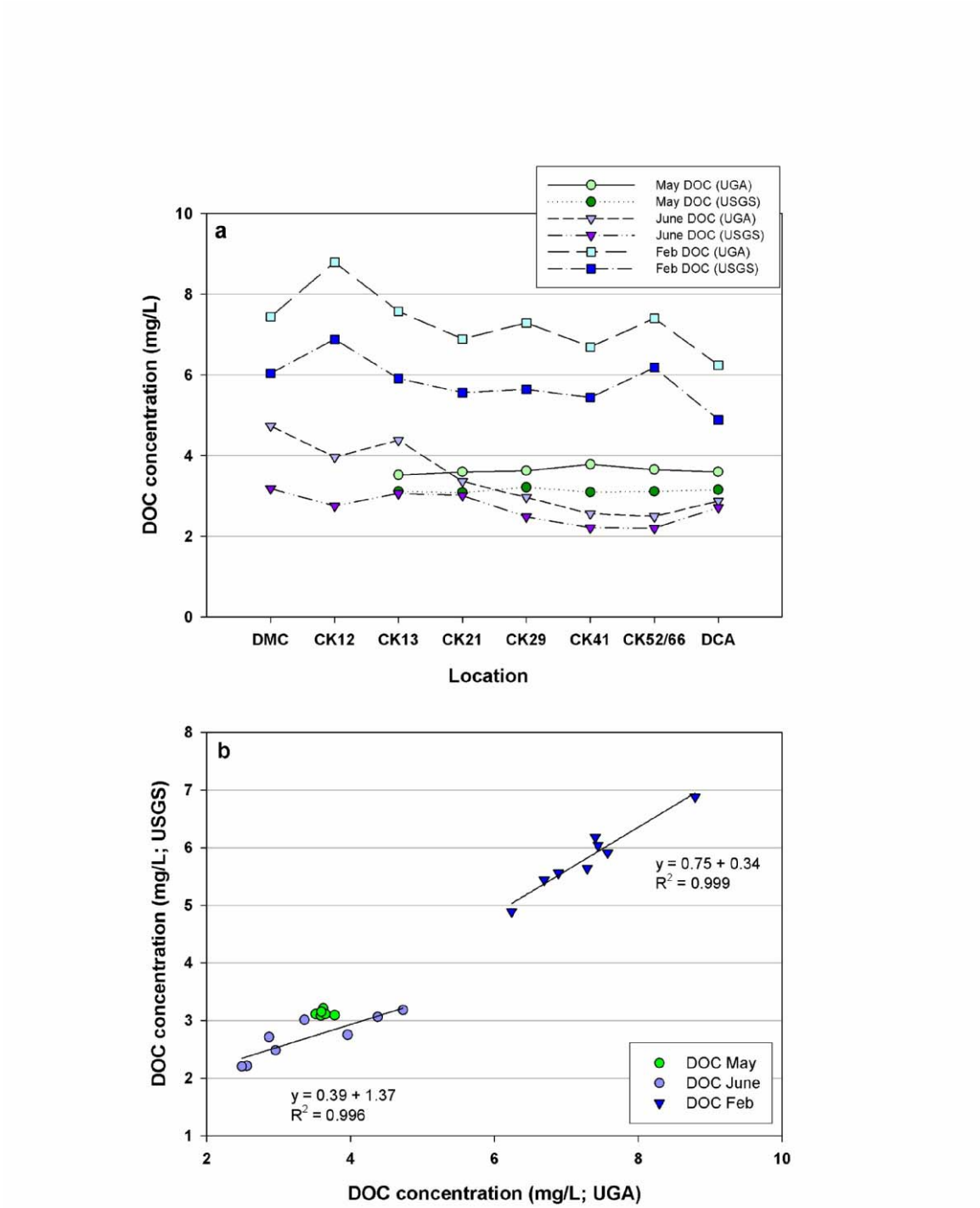

Figure 2.5 DOC concentrations in water samples taken along the California Aqueduct. (a) UGA vs. USGS measured dissolved organic carbon (DOC) concentrations, May and October 2004 and February 2005 and (b) Linear relationship between concentrations measured by UGA and USGS. Samples collected at Checks (CK) followed by station number, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA).

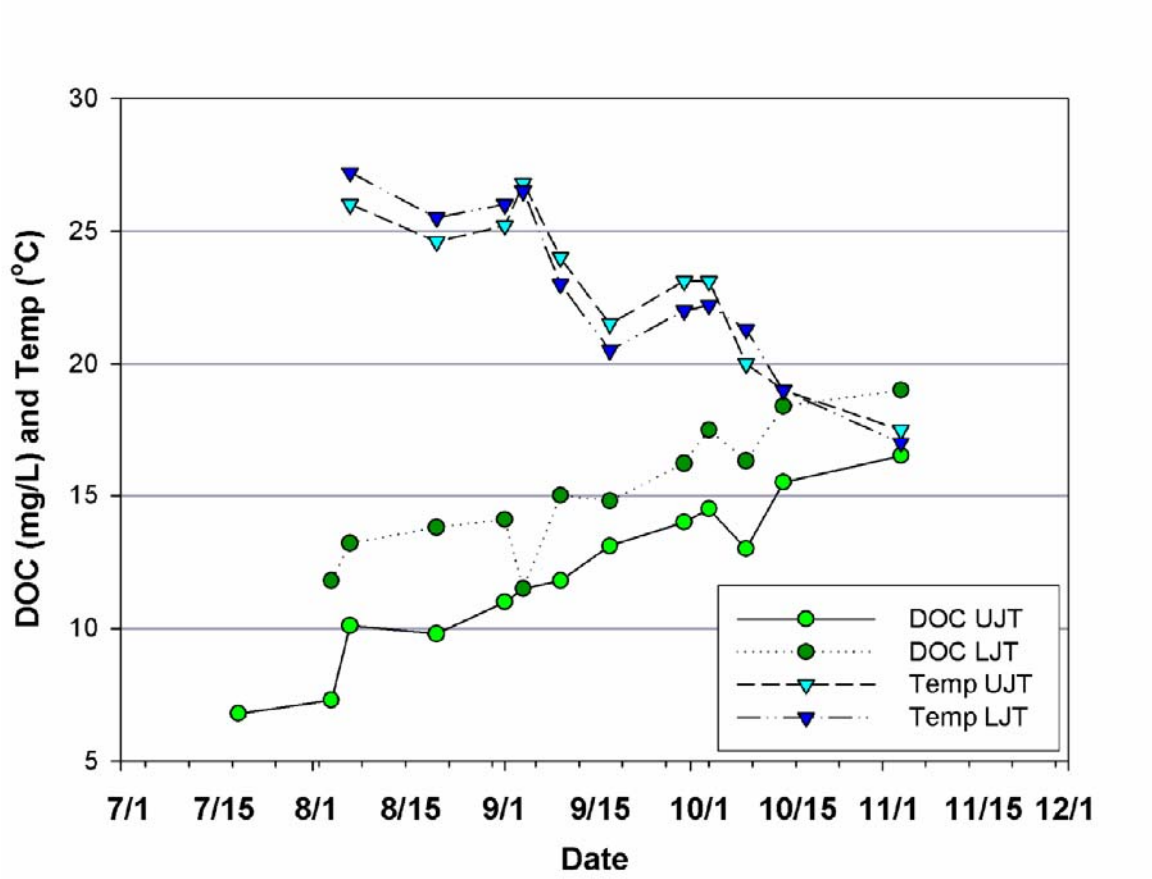

Figure 2.6 Upper and Lower Jones Tract DOC concentrations (mg/L) and temperature (°C), July - October 2004.

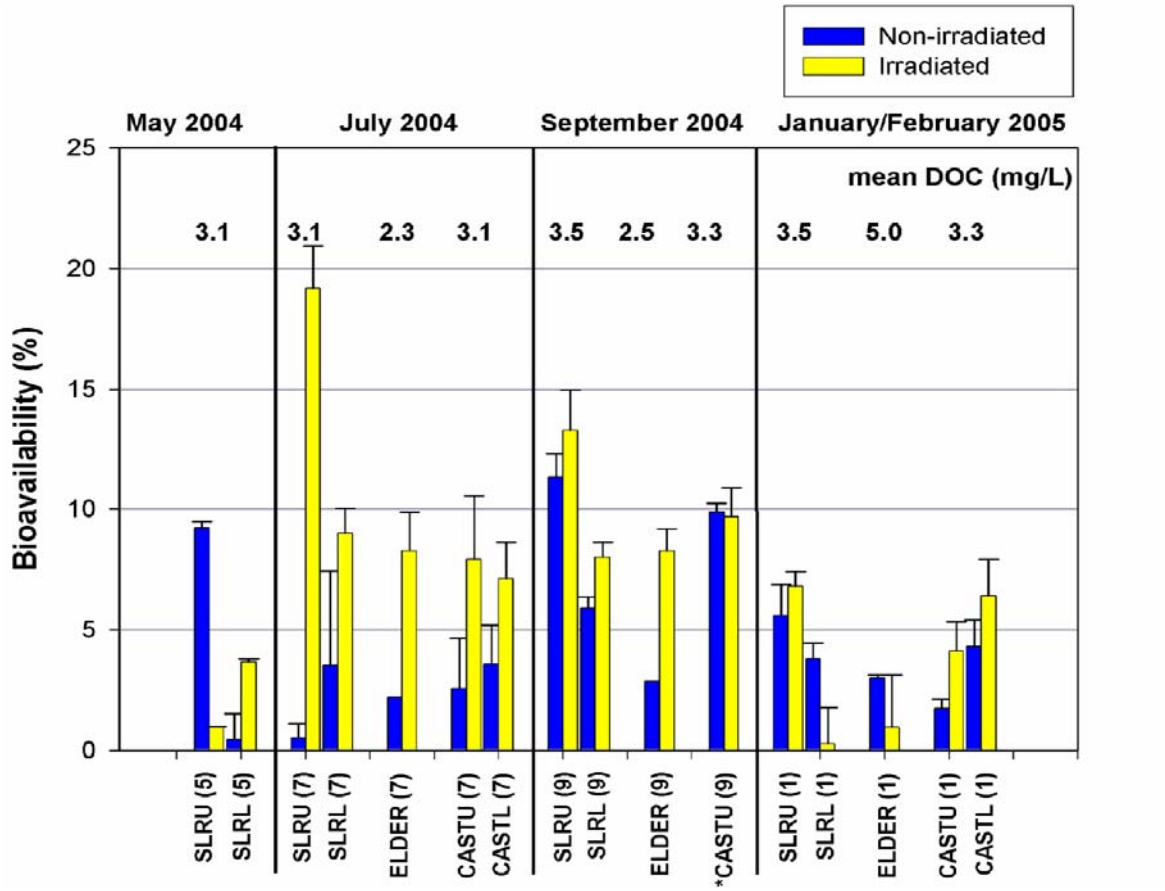

Figure 2.7 DOC bioavailability in irradiated and non-irradiated samples from water samples collected at San Luis Reservoir, Castaic Lake and Elderberry Forebay. DOC concentration Upper San Luis Reservoir (SLRU); Lower San Luis Reservoir (SLRL); Upper Castaic Lake (CASTU); Lower Castaic Lake (CASTL); Elderberry Forebay (ELDER). Sample months are denoted as follows: May (5), July (7) and September (9) 2004 and January (-1) and February (-2) 2005. Percent bioavailability = Mean BOD(uM)/DOC(uM) and standard deviation. (*) denotes compromised sample.

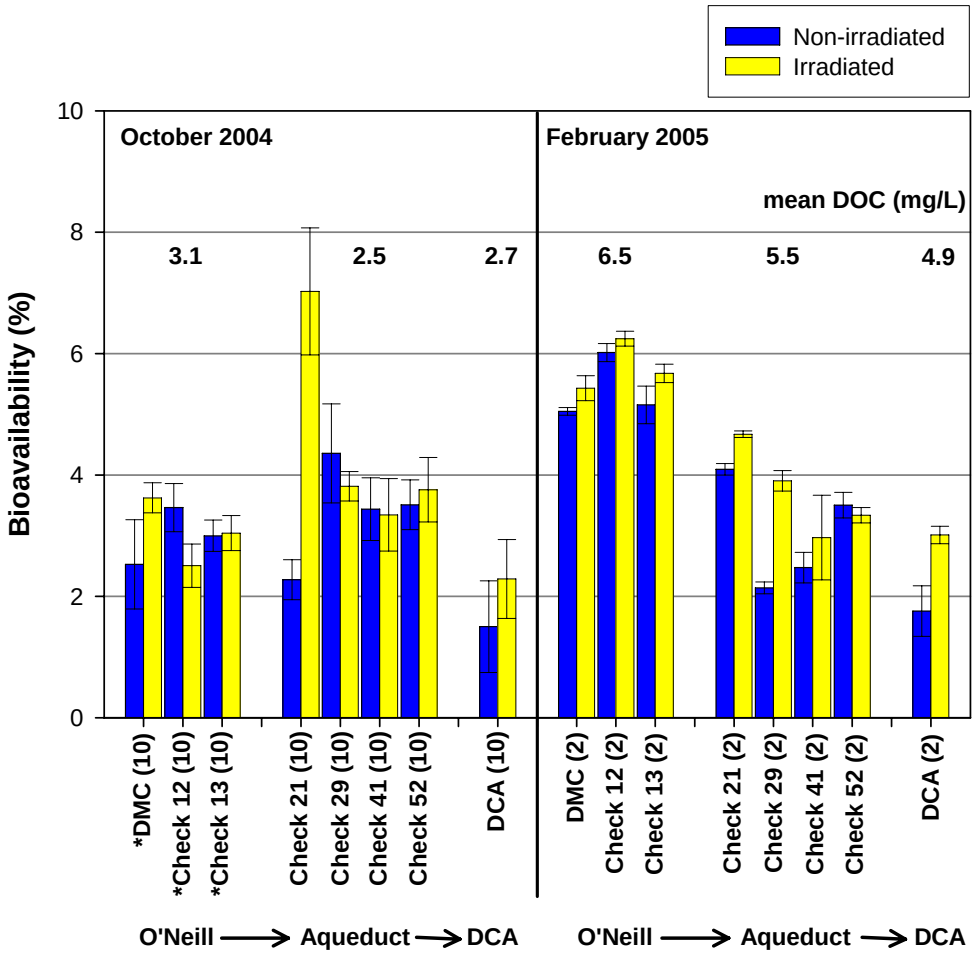

Figure 2.8 DOC bioavailability in irradiated and non-irradiated samples and DOC concentration from water samples collected along the California Aqueduct. Samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA). Sample months are denoted as follows: October 2004 (10) and February 2005 (2). Percent bioavailability = Mean BOD(uM)/DOC(uM) and standard deviation. Asterisks (*) denotes compromised samples.

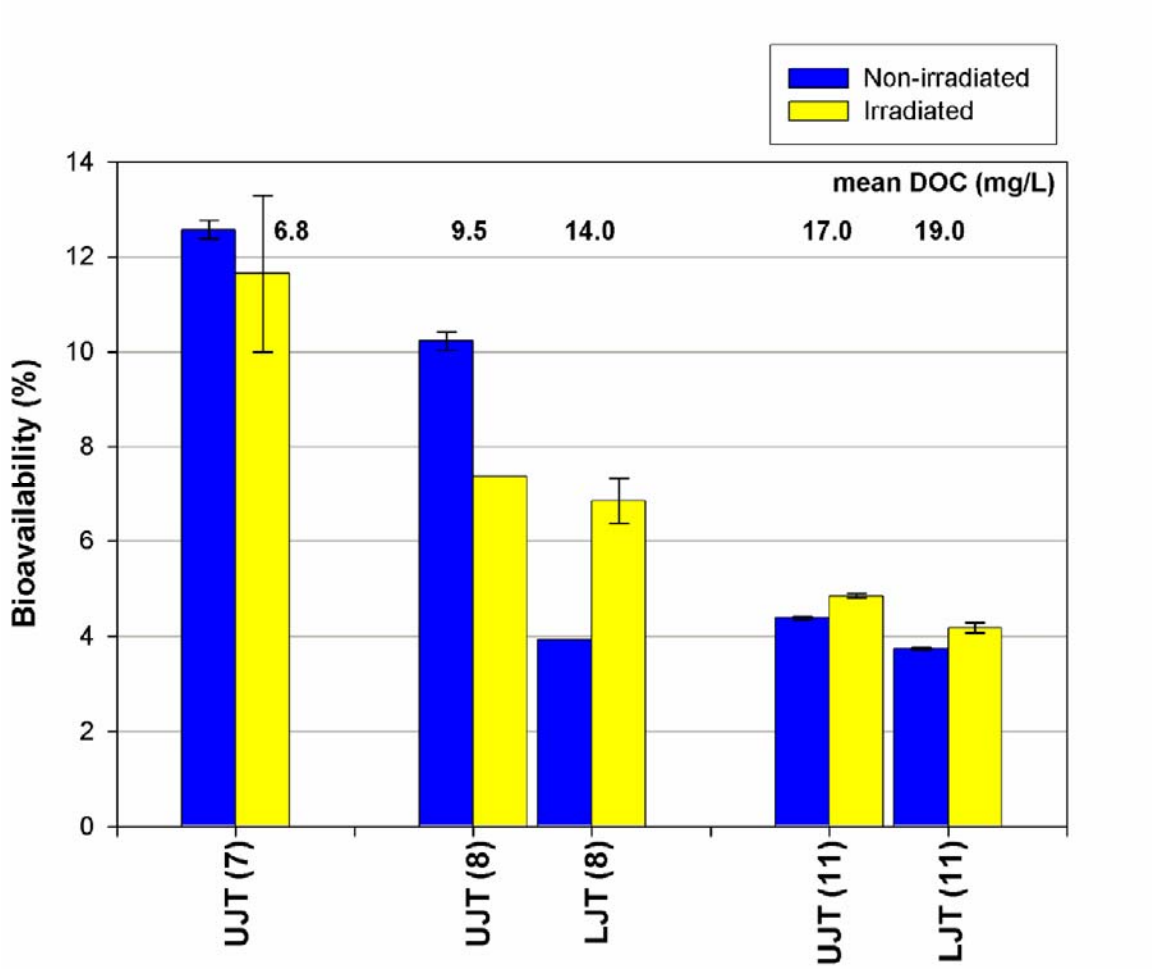

Figure 2.9 DOC bioavailability and concentration in water samples from Upper (UJT) and Lower (LJT) Jones Tracts. Sample months are denoted in parentheses: July 2004 (7), August 2004 (8) and November 2004 (11). Percent bioavailability = Mean BOD(uM)/DOC(uM) and standard deviation.

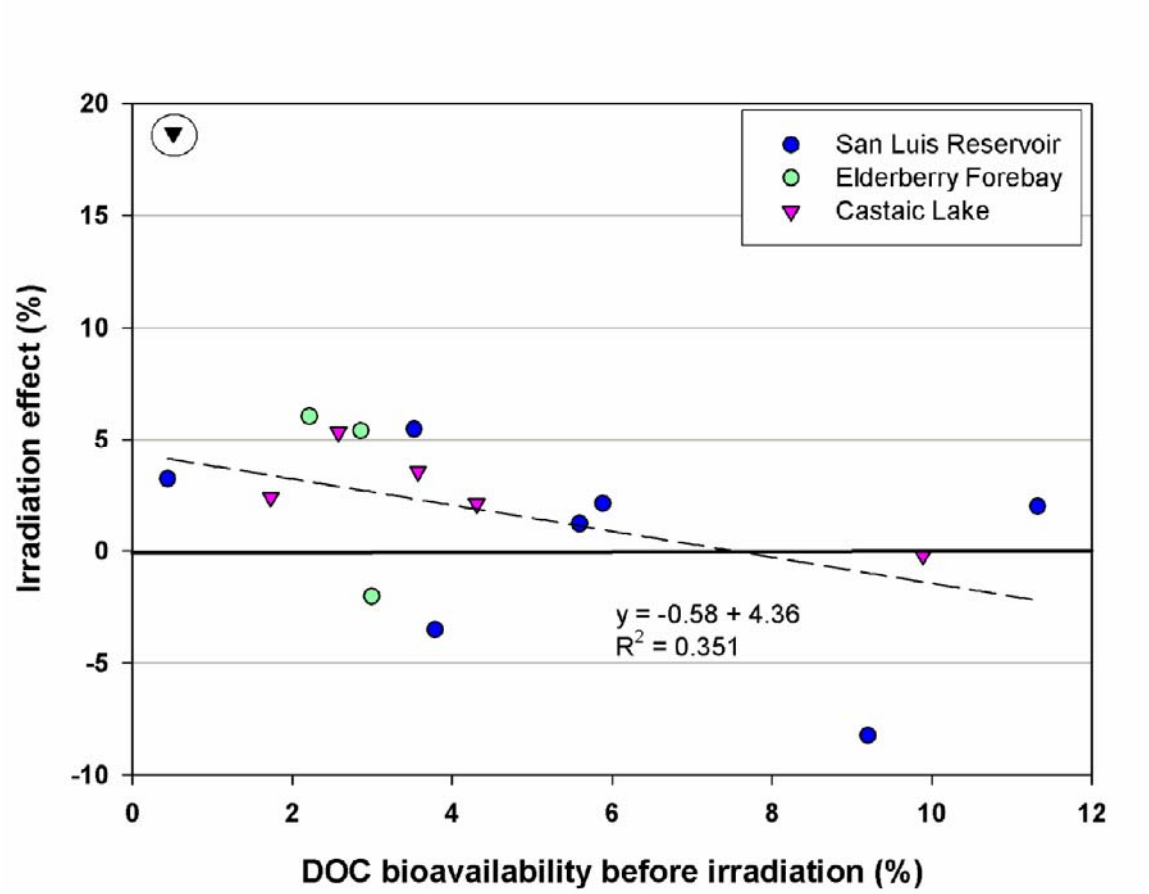

Figure 2.10 Irradiation effect vs. bioavailability before irradiation in samples from San Luis Reservoir, Castaic Lake and Elderberry Forebay. Irradiation effect was calculated by subtracting DOC bioavailability before irradiation from DOC bioavailability after irradiation. San Luis Reservoir samples were collected in May, July and September 2004 and January 2000. Castaic Lake and Elderberry Forebay samples collected July and September 2004 and February 2005 from. San Luis Reservoir outlier (SLRU(7)) circled.

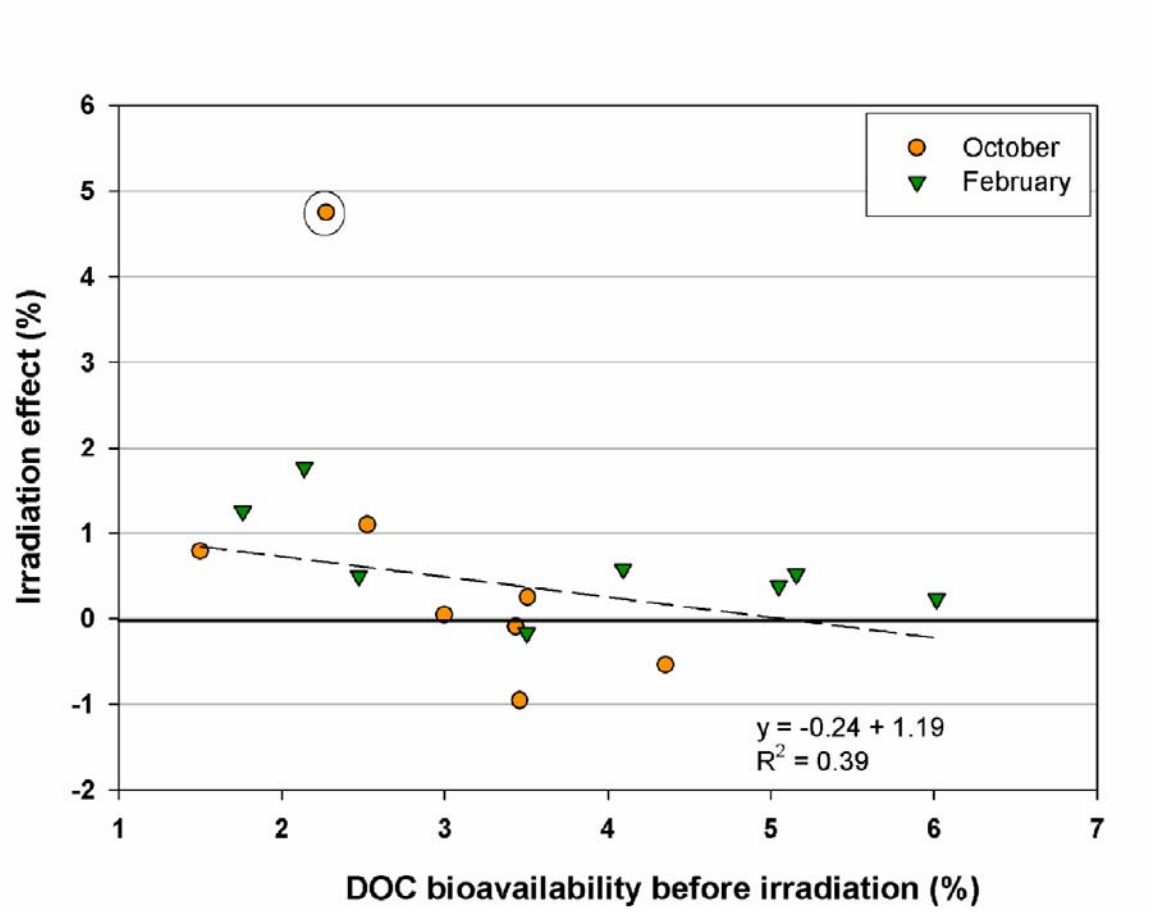

Figure 2.11 Irradiation effect vs. bioavailability before irradiation in samples from the California Aqueduct. Irradiation effect was calculated by subtracting DOC bioavailability before irradiation from DOC bioavailability after irradiation. Samples collected in October 2004 and February 2005. October outlier (Check 21) circled.

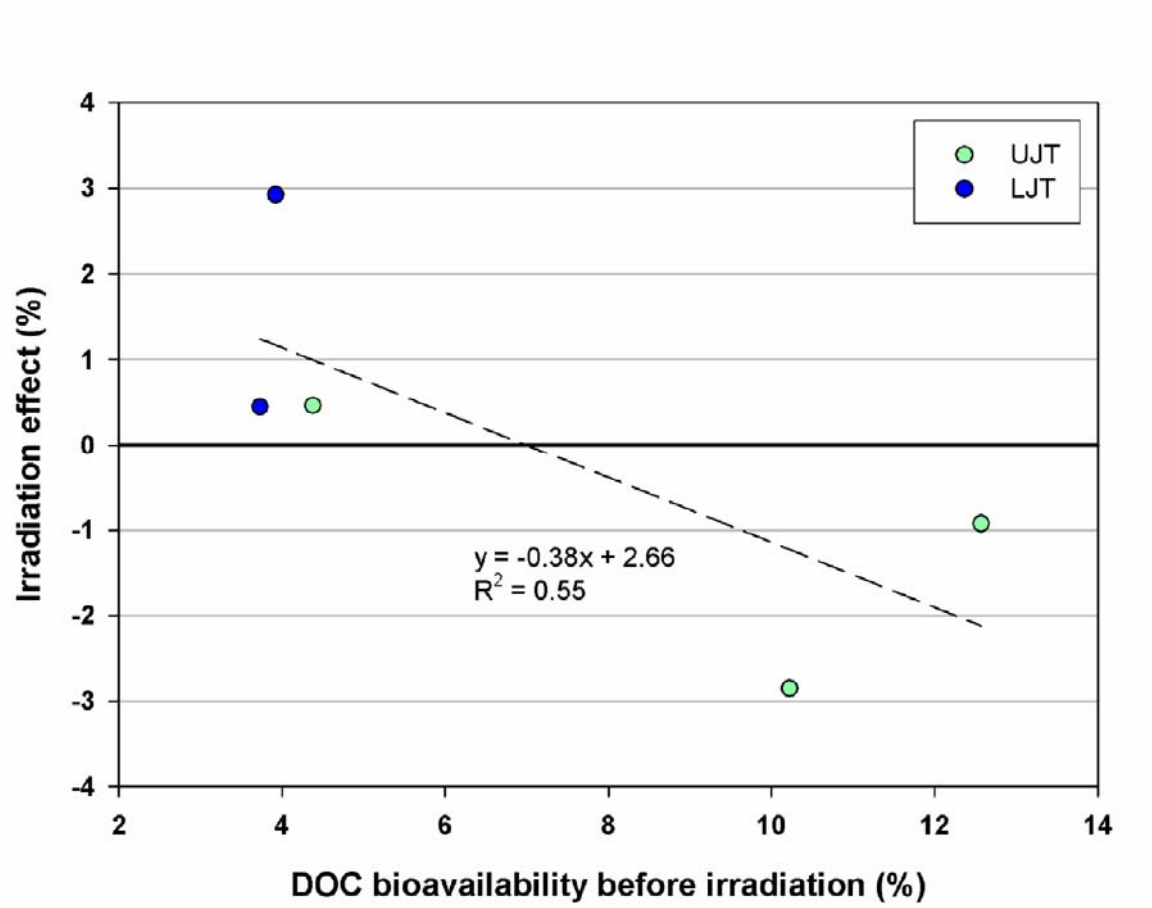

Figure 2.12 Irradiation effect vs. bioavailability before irradiation at Jones Tract. Irradiation effect was calculated by subtracting DOC bioavailability before irradiation from DOC bioavailability after irradiation. Samples collected in July, August and November 2004.

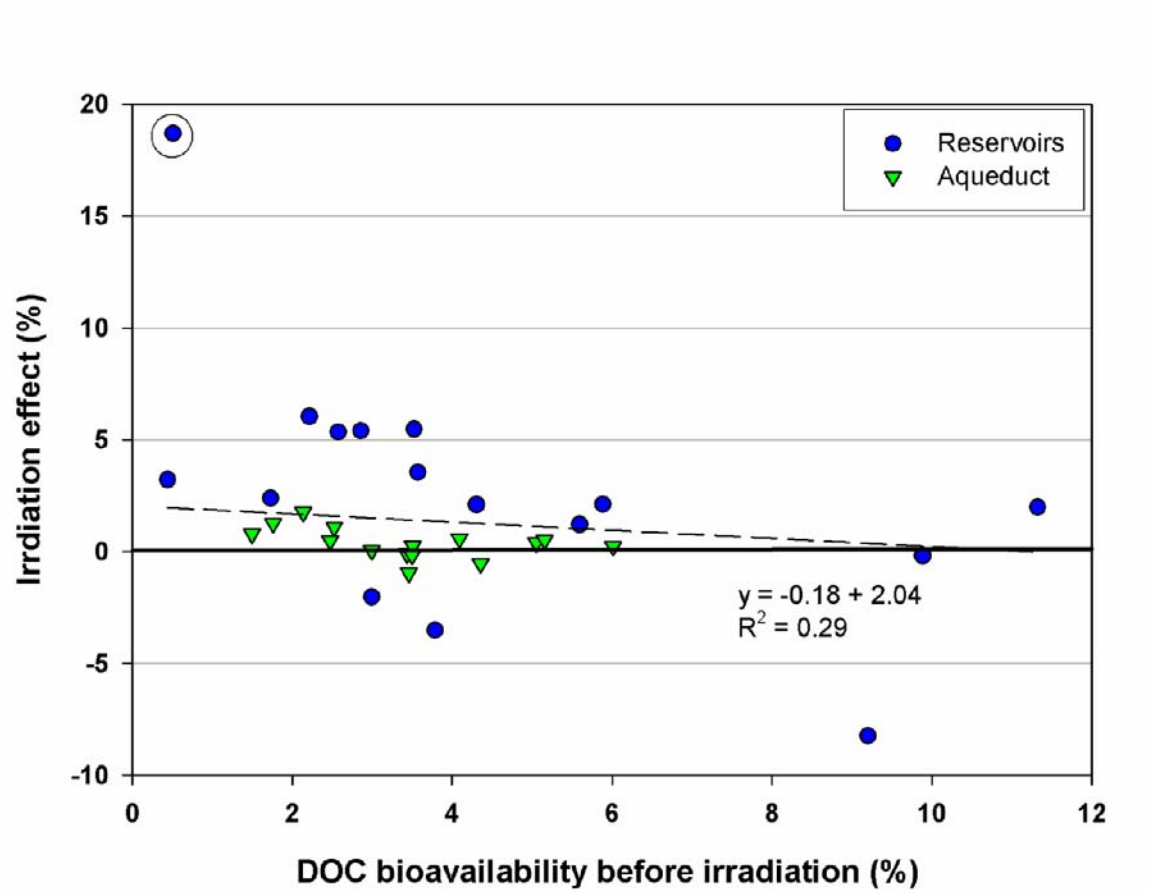

Figure 2.13 Irradiation effect vs. bioavailability before irradiation for all reservoir (circles) and aqueduct (triangles) water samples. Irradiation effect was calculated by subtracting DOC bioavailability before irradiation from DOC bioavailability after irradiation. San Luis Reservoir outlier (SLRU (7)) circled.

Table 2.1 Sampling station location along the California Aqueduct. California Aqueduct entrance (mile 0) is located at H.O. Banks Pumping Plant at Clifton Court. California Aqueduct samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA). Groundwater inflows along the California Aqueduct: Semitropic, Kern River and Arvin Edison

Site	Mile	Latitude	Longitude
Check 12	**66.33**	**37° 11' 80" N**	**121° 05' 80" W**
Delta Mendota Canal (DMC)	**67.00**	**37° 06' 55" N**	**121° 02' 02" W**
Check 13 (O'Neill Forebay Outlet)	**70.89**	**37° 07'40" N**	**121° 01' 50" W**
Check 21	**172.26**	**36° 01' 50" N**	**119° 97' 70" W**
Semitropic Inflow	**209.80**	**35° 33' 07" N**	**119° 39' 15" W**
Kern River Inflow	**238.19**	**35° 17' 13" N**	**119° 20' 09" W**
Check 29	**244.54**	**35° 23' 10" N**	**119° 33' 70" W**
Arvin Edison Inflow	**277.30**	**35° 03' 13" N**	**119° 02' 02" W**
Check 41	**303.41**	**34° 83' 10" N**	**118° 71' 00" W**
Check 52	**343.74**	**34° 32' 60" N**	**117° 30' 30" W**
Check 66	**403.41**	**34° 32' 60" N**	**117 19' 43" W°**
Devils Canyon Afterbay (DCA)	**412.88**	**34° 10' 47" N**	**117° 19' 43" W**

Table 2.2 Reservoir sampling station locations at San Luis Reservoir, Elderberry Forebay and Castaic Lake.

Site	Latitude	Longitude
San Luis Reservoir		
Station 1	**37° 03' 15" N**	**121° 05' 24" W**
Elderberry Forebay	**34° 33' 46" N**	**118° 38' 02" W**
Castaic Lake		
Station 2	**34° 12' 91" N**	**118° 36' 28" W**
Station 4	**34° 32' 34" N**	**118° 37' 21" W**
Station 5	**34° 32' 08" N**	**118° 35' 20" W**

Table 2.3 Sampling dates along the California Aqueduct, May and October 2004 and February 2005.

Site	Mile	May 2004	October 2004	February 2005
Check 12	**66.33**		**10/12**	**2/18**
Delta Mendota Canal (DMC)	**67.00**		**10/12**	**2/18**
Check 13 (O'Neill Forebay Outlet)	**70.89**	**5/11**	**10/12**	**2/18**
Check 21	**172.26**	**5/11**	**10/19**	**2/22**
Semitropic Inflow	**209.80**		**10/21**	
Kern River Inflow	**238.19**		**10/21**	
Check 29	**244.54**	**5/13**	**10/22**	**2/26**
Arvin Edison Inflow	**277.30**		**10/22**	
Check 41	**303.41**	**5/13**	**10/25**	**2/28**
Check 52	**343.74**		**10/27**	
Check 66	**403.41**	**5/12**		**3/4**
Devil's Canyon Afterbay (DCA)	**412.88**	**5/12**	**10/26**	**3/4**

Table 2.4 Sampling dates, station locations and depths sampled to create Upper and Lower composites at San Luis Reservoir and Castaic Lake. Elderberry Forebay sampled at 3-5 m on the same days as Castaic Lake.

	Date	Composite	Station	Depths (m)
San Luis Reservoir				
	5/20/2004	**Upper**	**Station 1**	**5 + 10+ 25**
		Lower	**Station 1**	**40 + 60**
	7/19/2005	**Upper**	**Station 1**	**1.5 + 7.5 + 15**
		Lower	**Station 1**	**23 + 33.5**
	9/22/2005	**Upper**	**Station 1**	**1 + 10+ 15**
		Lower	**Station 1**	**25 + 35**
	1/11/2005	**Upper**	**Station 1**	**1 + 10 + 15**
		Lower	**Station 1**	**30 + 40**
Castaic Lake				
	7/20/2004	**Upper**	**Station 2+4+5**	**1 + 5 + 8**
		Lower	**Station 2+4+5**	**20 + 40 + 60**
	9/23/2004	**Upper**	**Station 2+4**	**1 + 5 + 10**
		Lower	**Station 2+4**	**20 + 40 + 60**
	2/27/2005	**Upper**	**Station 2+4+5**	**1 + 5 + 10**
		Lower	**Station 2+4+5**	**20 + 40 + 60**

Table 2.5 (following page) Chemical and biological data for all State Water Project sample dates and locations. Data are mean values. Electric conductivity (EC); dissolved oxygen (DO); chlorophyll-a concentration (Chl-a); pheophyton-a concentration (Pheop-a); dissolved organic carbon concentration (DOC); biological oxygen demand (BOD); bioavailability of DOC in non-irradiated samples (initial BDOC); specific UV absorbance (SUVA). Aqueduct samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA). Reservoir samples composited into upper and lower depths: Upper San Luis Reservoir (SLRU); Lower San Luis Reservoir (SLRL); Upper Castaic Lake (CASTU); Lower Castaic Lake (CASTL); Elderberry Forebay samples collected at 3-5m (ELDER). Sample months denoted in parentheses: May (5), July (7) and September (9) 2004 and January (1) and February (2) 2005.

		Temp °C	pH	EC uS/cm	DO mg/L	Chl-a ug/L	Pheop-a Ug/L	DOC mg/L	Mean BOD µM	Initial BDOC %	SUVA L/mg-m	NH_4-N mg/L	$N0_3$-N+ N_2-N mg/L	NO_2-N mg/L	Total N Unfil mg/L	Ortho P mg/L	Total P mg/L	Cl mg/L	Si mg/L	SO_4 mg/L	Na mg/L	Ca mg/L	K mg/L	Mg mg/L	Fe mg/L	Mn mg/L
May	**Check 13**	14.1	7.8	466	11.6	0.8	0.7	3.1	17.7	6.0	3.37	<0.04	0.77	<0.008	0.98	0.085	0.101	69.9	16.0	36.0	51.6	21.3	3.0	13.5	9.2	7.9
'04	**Check 21**	16.4	7.9	478	10.9	0.9	1.5	3.1	-2.0	-0.7	3.33	<0.04	0.78	<0.008	1.06	0.082	0.108	72.6	15.4	37.0	52.2	21.4	3.0	13.6	7.3	2.7
	Check 29	18.4	8.1	504	11.6	1.9	2.3	3.2	1.8	0.6	3.07	<0.04	0.68	<0.008	0.97	0.078	0.106	73.5	15.0	37.9	50.0	22.2	2.9	13.8	6.1	1.3
	Check 41	19.9	8.1	497	9.9	4.9	6.3	3.1	15.1	4.8	3.22	<0.04	0.61	<0.008	0.95	0.071	0.122	72.1	14.2	37.4	49.0	21.8	2.9	13.8	6.4	<0.6
	Check 66	18.6	8.3	475	9.6	1.0	0.8	3.1	13.4	4.4	3.17	<0.04	0.58	<0.008	0.89	0.068	0.104	71.7	12.5	39.0	51.7	21.1	3.0	13.7	<6.0	1.1
	DCA	19.4	8.0	426	10.8	--	--	3.2	5.0	1.7	3.33	<0.04	0.70	0.015	0.97	0.078	0.099	58.2	12.9	39.3	44.9	21.1	2.8	12.8	8.9	0.6
Oct	**DMC**	19.3	7.5	536	8.6	3.9	5.1	3.2	10.0	2.5	3.18	<0.04	0.43	<0.008	0.72	0.044	0.078	103.3	13.7	31.1	67.7	20.0	3.8	15.0	13.8	0.8
'04	**Check 12**	19.4	7.7	574	9.8	2.1	2.3	2.8	11.4	3.5	2.75	<0.04	0.19	<0.008	0.47	0.062	0.078	101.8	12.4	25.3	65.0	18.0	3.6	14.8	<6.0	<0.6
	Check 13	19.7	7.8	504	7.8	3.1	5.2	3.1	10.9	3.0	3.06	<0.04	0.27	<0.008	0.58	0.070	0.094	87.8	15.5	30.6	60.1	20.3	3.3	14.7	6.9	2.0
	Check 21	19.4	7.7	561	8.1	1.5	2.0	3.0	6.4	2.3	3.01	<0.04	0.28	<0.008	0.58	0.070	0.092	87.9	13.8	30.6	59.4	19.2	3.4	14.0	<6.0	<0.6
	Check 29	19.1	8.2	531	10.1	0.5	0.4	2.5	10.7	4.4	2.48	<0.04	0.56	<0.008	0.83	0.061	0.079	79.8	14.6	36.6	60.2	21.2	2.9	11.8	<6.0	<0.6
	Check 41	19.3	8.2	523	9.1	5.1	5.7	2.2	7.3	3.4	2.21	<0.04	0.75	<0.008	0.99	0.058	0.087	78.0	14.2	39.3	59.5	22.9	2.8	11.3	<6.0	<0.6
	Check 52	16.9	8.2	480	9.1	1.9	2.3	2.2	7.3	3.5	2.20	<0.04	0.77	<0.008	1.05	0.056	0.080	73.8	13.8	38.6	55.8	22.6	2.7	10.7	<6.0	<0.6
	DCA	17.3	7.9	468	9.2	--	--	2.7	3.6	1.5	2.71	<0.04	0.58	0.017	0.81	0.066	0.092	73.4	11.8	29.6	53.8	20.7	2.9	11.4	7.2	0.8
Feb	**DMC**	12.9	7.6	575	9.6	1.9	4.1	6.0	31.3	5.1	3.43	0.07	1.50	0.020	2.23	0.147	0.220	--	17.9	--	53.2	27.7	3.3	15.7	88.0	14.0
'05	**Check 12**	12.6	7.7	439	9.7	0.8	1.8	6.9	44.1	6.0	3.32	0.06	1.19	0.010	1.80	0.118	0.167	--	18.8	--	34.5	22.7	3.2	12.9	117.0	15.8
	Check 13	12.6	7.4	507	9.7	1.0	2.0	5.9	32.5	5.2	3.56	0.06	1.34	0.016	1.84	0.129	0.177	53.4	18.4	48.7	45.3	25.7	3.3	14.5	76.0	14.9
	Check 21	13.0	7.6	536	10.9	2.5	3.3	5.6	23.5	4.1	3.50	<0.04	1.46	0.010	2.03	0.136	0.200	--	17.7	--	48.9	27.0	3.4	14.8	33.0	3.1
	Check 29	13.7	7.5	514	9.1	0.9	1.8	5.6	13.0	2.1	3.37	<0.04	1.45	<0.008	1.98	0.149	0.260	57.2	18.0	53.4	48.0	27.1	3.4	15.2	33.0	1.6
	Check 41	14.3	8.4	449	9.6	1.6	5.2	5.4	13.8	2.5	3.53	<0.04	1.44	<0.008	1.87	0.146	0.220	56.5	17.0	53.8	45.9	26.5	3.4	15.1	19.0	1.5
	Check 66	13.4	8.8	449	11.0	20.3	17.3	6.2	21.6	3.5	3.05	<0.04	1.36	<0.008	1.81	0.129	0.189	55.8	16.6	53.0	48.7	26.6	3.4	14.9	44.0	0.7
	DCA	11.2	8.4	407	11.2	--	--	4.9	9.1	1.8	3.50	<0.04	1.43	0.009	1.79	0.109	0.136	54.9	17.3	40.3	44.1	23.3	3.4	12.6	58.0	3.5
May	**SLRU**	16.0	7.9	470	--	2.2	0.5	3.2	30.0	9.2	2.80	0.07	0.63	0.018	1.03	0.071	0.092	72.6	17.3	37.9	50.7	21.8	3.0	13.0	<6.0	7.2
'04	**SLRL**	13.0	--	467	--	--	--	3.1	1.4	0.4	3.05	<0.04	0.86	<0.008	1.07	0.101	0.113	72.8	16.1	37.2	50.4	21.8	3.0	13.1	<6.0	1.0
Jul	**SLRU**	20.0	8.2	466	--	9.3	5.0	3.2	1.4	0.5	2.95	<0.04	0.47	<0.008	0.92	0.058	0.092	73.2	16.4	40.3	51.1	23.0	3.0	13.7	<6.0	0.9
'04	**SLRL**	19.0	--	--	--	--	--	3.1	9.3	3.5	2.91	<0.04	0.59	0.011	0.87	0.078	0.089	70.5	--	39.4	--	--	2.8	--	--	--
Sep	**SLRU**	21.2	8.2	444	8.6	39.8	5.0	3.6	35.3	11.3	2.62	0.05	0.21	0.009	0.52	0.086	0.102	70.9	16.7	34.8	46.3	20.6	2.8	13.4	<6.4	<0.8
'04	**SLRL**	21.9	8.4	442	4.0	--	--	3.4	19.1	5.9	2.60	<0.04	0.11	<0.008	0.55	0.069	0.113	71.1	16.9	34.8	47.0	20.8	--	13.7	<6.4	2.8
Jan	**SLRU**	12.0	7.4	495	8.7	0.6	1.0	3.5	20.2	5.6	2.93	<0.04	0.73	<0.008	1.09	0.094	0.107	82.9	17.0	37.5	59.6	22.5	3.4	14.7	12.6	1.9
'05	**SLRL**	11.9	7.7	495	8.7	--	--	3.5	14.1	3.8	2.84	<0.04	0.74	<0.008	1.07	0.095	0.108	83.4	17.0	37.7	60.2	22.5	3.5	14.8	13.0	1.9
Jul	**ELDER**	--	--	--	--	--	--	2.3	5.9	2.2	3.31	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
'04	**CASTU**	23.0	8.1	435	--	1.1	0.7	3.1	7.1	2.6	3.20	<0.04	0.50	<0.008	0.81	0.034	0.053	63.6	14.9	44.6	24.3	13.3	2.8	13.3	<6.4	0.9
	CASTL	18.0	7.7	444	--	--	--	3.0	9.0	3.6	3.13	<0.04	0.66	<0.008	0.94	0.060	0.073	63.4	14.7	44.5	24.4	13.2	2.8	13.2	<6.4	0.9
Sep	**ELDER**	--	--	--	--	--	--	2.5	6.1	2.9	2.45	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
'04	**CASTU**	23.3	7.9	464	5.8	1.0	0.3	3.3	24.0	9.9	2.51	<0.04	0.52	<0.008	0.70	0.056	0.074	62.9	15.9	40.3	22.1	13.2	2.6	13.2	<6.4	1.6
Feb	**ELDER**	12.1	7.2	545	10.6	--	--	4.9	12.2	3.0	2.99	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
'05	**CASTU**	12.2	7.9	481	12.3	2.9	1.7	3.4	6.6	1.7	2.92	<0.04	0.59	<0.008	0.83	0.049	0.079	51.7	15.6	60.0	42.3	31.3	2.9	13.8	<6.0	1.7
	CASTL	12.9	7.9	488	12.5	--	--	3.2	14.8	4.3	2.92	<0.04	0.60	<0.008	0.84	0.053	0.074	54.9	15.6	53.8	43.2	29.0	2.8	13.6	<6.0	3.0

Table 2.6 Chemical and biological data for (a) Upper (UJT) and Lower (LJT) Tract, July – November 2004 and (b) inflows into the California Aqueduct: Semitropic (groundwater), Kern River and Arvin Edison (groundwater) along the CA Aqueduct October 2004. Data are mean values. Electric conductivity (EC); dissolved oxygen (DO); chlorophyll-a concentration (Chl-a); pheophyton-a concentration (Pheop-a); dissolved organic carbon concentration (DOC); biological oxygen demand (BOD); bioavailability of DOC in non-irradiated samples (initial BDOC); specific UV absorbance (SUVA).

a

		Temp °C	pH	EC uS/cm	DO mg/L	Chla ug/L	Ph-a ug/L	DOC mg/L	Mean BOD µM	Initial BDOC %	SUVA L/mg-m	NH_4-N mg/L	$N0_3$-N+ N_2-N mg/L	NO_2-N mg/L	Total N Unfil mg/L	Ortho P mg/L	Total P mg/L	Cl mg/L	Si mg/L	SO_4 mg/L	Na mg/L	Ca mg/L	K mg/L	Mg mg/L	Fe mg/L	Mn mg/L
Jul '04	UJT	--	7.3	300	--	--	--	6.8	61.7	12.6	3.70	0.26	<0.060	<0.008	1.05	0.22	0.13	36.8	13.55	23.5	28.7	19.3	3.00	9.0	355	106
Aug '04	UJT	24.5	7.1	360	7.5	41.3	14.0	9.5	101.7	10.2	4.60	0.16	<0.060	0.011	1.52	0.05	0.21	41.0	13.90	23.7	31.5	22.5	3.93	10.3	30	75
	LJT	25.5	7.0	350	5.8	13.8	21.9	14.0	50.8	3.9	5.21	0.11	0.34	0.049	1.96		0.15	40.6	8.35	28.0	33.1	20.9	4.99	9.0	168	47
Nov '04	UJT	20.5	7.2	375	7.5	55.0	33.0	17.0	67.3	4.4	4.02	0.05	<0.060	<0.008	1.88	0.02	0.13	52.3	0.68	26.5	39.1	22.7	4.12	12.2	168	6
	LJT	21.5	7.2	355	9.0	43.1	39.0	19.0	70.2	3.7	4.50	<0.04	<0.060	<0.008	2.15	0.02	0.15	46.3	0.53	28.9	37.4	20.2	5.37	9.1	415	24

b

October 2004	Temp °C	pH	EC uS/cm	DO mg/L	Chla ug/L	Ph-a ug/L	DOC mg/L	BDOC %	SUVA L/mg-m	NH_4-N mg/L	$N0_3$-N+ N_2-N mg/L	NO_2-N mg/L	Total N Unfil mg/L	Ortho P mg/L	Total P mg/L	Cl mg/L	Si mg/L	SO_4 mg/L	Na mg/L	Ca mg/L	K mg/L	Mg mg/L	Fe mg/L	Mn mg/L
Semitropic	22	9.0	484	7.0	--	--	0.5	--	1.7	0.04	1.72	0.013	1.90	0.027	0.110	65.5	19.2	80.5	76.7	26.7	0.504	0.3	3.0	1.3
Kern River	20	8.2	375	7.0	--	--	0.6	--	2.7	<0.04	2.08	0.005	2.07	<0.006	0.007	39.2	19.9	44.3	45.2	34.5	0.902	1.0	12.5	1.5
ArvinEdison	--	8.0	318	--	--	--	0.8	--	1.9	<0.04	2.63	0.008	2.82	0.026	0.038	20.8	18.8	24.3	34.0	67.7	3.665	7.8	3.63	1.1

Table 2.7 Spectral data, absorbance at 250 nm (A_{250}), for all location in the SWP and Jones Tract in the Delta, May - October 2004. Aqueduct samples collected at Checks (CK), Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA). Reservoir samples composited into upper and lower depths: Upper San Luis Reservoir (SLRU); Lower San Luis Reservoir (SLRL); Upper Castaic Lake (CASTU); Lower Castaic Lake (CASTL); Elderberry Forebay samples collected at 3-5m (ELDER) and Upper (UJT) and Lower (LJT) Tract collected from pump discharge. Sample months denoted in parentheses: May (5), July (7) and September (9) 2004 and January (1) and February (2) 2005. Asterisk (*) denotes compromised samples.

Sample date	Sample #	Station	A_{250}	Sample date	Sample #	Station	A_{250}
5/20/2004	9	SLRU(5)	0.13	5/11/2004	1	CK13(5)	0.14
5/20/2004	10	SLRL(5)	0.12	5/11/2004	2	CK21(5)	0.14
7/19/2004	58	SLRU(7)	0.13	5/13/2004	6	CK29(5)	0.14
7/19/2004	59	SLRL(7)	0.13	5/13/2004	5	CK41(5)	0.14
9/22/2004	123	SLRU(9)	0.14	5/13/2004	4	CK66(5)	0.12
9/22/2004	124	SLRL(9)	0.14	5/12/2004	3	DCA(5)	0.15
1/11/2004	179	SLRU(1)	--				
1/11/2004	178	SLRL(1)	--				
7/21/2004	92	Elder(7)	0.13	10/12/2004	164	*DMC(10)	0.17
9/22/2004	136	Elder(9)	0.12	10/12/2004	165	*CK12(10)	0.14
2/27/2005	177	Elder(2)	--	10/12/2004	166	*CK13(10)	0.13
7/20/2004	74	CASTU(7)	0.13	10/19/2004	167	CK21(10)	0.14
7/20/2004	75	CASTL(7)	0.13	10/22/2004	168	CK29(10)	0.11
9/23/2004	140	*CASTU(9)	0.12	10/25/2004	169	CK41(10)	0.09
2/27/2005	211	CASTU(2)	--	10/27/2004	170	CK52(10)	0.11
2/27/2005	212	CASTL(2)	--	10/26/2004	171	DCA(10)	0.12
7/19/2004	52	UJT(7)	0.70	10/14/2004	161	*UJT(10)	0.90
8/24/2004	113	UJT(8)	0.70	10/14/2004	160	*LJT(10)	1.31
8/24/2004	53	LJT(8)	1.00	11/2/2004	176	UJT(11)	0.95
				11/2/2004	175	LJT(11)	1.18

CHAPTER 3

DYNAMICS OF ACTINOBACTERIA IN THE CALIFORNIA STATE WATER PROJECT

3.1 Introduction

Actinobacteria are Gram positive, primarily aerobic bacteria with a high G+C content generally above 50 mol % (Stackebrandt et al. 1997). Recent studies have shown aquatic *Actinobacteria* to be one of the dominant bacterial groups in surface water (Cottrell et al. 2005), accounting for up to 60 - 70 % of the bacterioplankton in freshwater systems (Glockner et al. 2000; Warnecke et al. 2005). Others studies have concluded that aquatic *Actinobacteria* are part of a globally distributed, numerically limited set of freshwater bacterioplankton types (Glockner et al. 2000; Zwart et al. 2002).

Actinobacteria in lakes and reservoirs used for drinking water can be a concern to water managers because they can produce organic compounds that give the water an earthy taste and odor (Klausen et al. 2004; Zaitlin et al. 2003b). For example, the genus *Streptomyces* (Order: *Actinomycetales*, Family: *Streptomycetaceae*), has been reported to produce the odorous compounds geosmin and/or 2-methylisoborneol (MIB; Jensen et al. 1994; Klausen et al. 2004; Klausen et al. 2005; Sugiura and Nakano 2000; Zaitlin et al. 2003a). Humans have very low thresholds for perception of both geosmin and MIB. Geosmin produces an earthy-smelling odor detectable at 0.004 - 0.2 µg/l, whereas the woody/camphor odor of MIB is detectable from 0.018 - 0.1 µg/l (Jensen et al. 1994). These compounds are responsible for what is commonly perceived as a “soil” smell in drinking water and are most often encountered when raw water is taken from surface

water supplies (Klausen et al. 2005). In addition, some *Actinobacteria* form branching filaments and have spores that are resistant to treatment, which can lead to colonization of drinking water treatment facilities and distribution systems by *Actinobacteria*. Earlier studies (Niemi et al. 1982) found that eutrophic conditions and overland runoff can contribute to a higher abundance of *Actinobacteria* in water.

Particle-attached strains require a substrate such as sediment (Sugiura and Nakano 2000) or aggregate on suspended particles (Crump et al. 1999; Zaitlin et al. 2003a). Crump et al. (1999) found that particle-attached and free-living organisms fall into separate phylogenetic clusters. Most of the free-living strains in freshwater (Glockner et al. 2000; Sekar et al. 2003) and marine (Bull et al. 2005) systems are very small and slow growing, making isolation difficult. As a result, successful isolation of a representative free-living, freshwater *Actinobacteria* has only recently been reported (Hahn et al. 2003). Their relatively small size and Gram positive cell wall structure may contribute to their abundance by providing protection against protistan grazing (Hahn et al. 2003; Pernthaler et al. 2001).

The State Water Project (SWP) was engineered to transport drinking and irrigation water to the Central and Southern California desert regions from the Sacramento-San Joaquin Delta. The effects of the natural microbial population on drinking water quality in the SWP are thus of concern to many agencies, most notably the Metropolitan Water District of Southern California (MWD), which distributes SWP water to over 18 million people. Previous studies of SWP water quality ascribed taste and odor problems to algae and cyanobacteria in southern California reservoirs (Izaguirre 1992; Izaguirre and Taylor 1998). However, the MWD is also concerned about the potential contribution of

Actinobacteria to taste and odor problems, as they were found to represent up to 32 % of the natural microbial population in waters of the Sacramento-San Joaquin Delta, the source waters for the SWP (Stepanauskas et al. 2003).

My study examined the distribution of *Actinobacteria* in the California Aqueduct and in three reservoirs of the California State Water Project (Figure 1.2) during two different seasons: late summer (October) 2004 and late winter (February) 2005. Since residence times vary greatly between the aqueduct and reservoirs, this study was designed to determine (1) the percentage of *Actinobacteria* in the total bacterial community in the SWP both temporally and spatially, (2) whether the phylogenetic diversity of *Actinobacteria* varies seasonally in the reservoirs and in the aqueduct, (3) if changes in the diversity of these phylotypes are related to environmental variables and (4) how *Actinobacteria* phylotypes in the SWP are related to previously classified, globally distributed clusters of freshwater *Actinobacteria*, including strains known to cause taste and odor problems. Understanding the relationship between *Actinobacteria* distributions and water quality will help water managers reduce or eliminate taste and odor problems at the source, thereby potentially reducing the need to for additional chemical treatment at water treatment facilities.

3.2 Materials and Methods

Sampling strategy

Water enters O'Neill Forebay at three points: Check 12, the Delta-Mendota Canal (DMC) and San Luis Reservoir (Figure 1.2). In late summer, water enters from the California Aqueduct (Check 12; 33.2 % of daily flow) and from the Delta Mendota Canal (DMC; 30.9 % of daily flow) during the day and was being released from San Luis

Reservoir into O'Neill at night to generate power (35.8 % of daily flow; Figure 2.2; DWR, 2005). In late winter, water arriving at O'Neill Forebay originated from the Delta via Check 12 (61 %) and from DMC (37 %) with only a minor input (2 %) from San Luis Reservoir (Figure 2.4). Reservoir and aqueduct sampling thus captured both inflow scenarios: (1) late summer water entering from all three sources and (2) late winter water entering only from the Delta (Check 12 and DMC).

Sample collection

Water samples were collected from San Luis Reservoir in late winter (January) 2005, from the aqueduct in late summer 2004 (October) and late winter (February) 2005 and from Castaic Lake and Elderberry Forebay in late winter (February) 2005 (Table 2.3). Samples were collected in 1L Nalgene bottles, either using a submersible pump fitted with Tygon tubing or a Niskin bottle as described in Chapter 2. At San Luis Reservoir, water samples were collected at one station located near the dam, which is also the site where water is pumped into and out of the reservoir from O'Neill Forebay (Figure 1.3). At Castaic Lake, water was collected from three stations: near the outlet tower (Station 2), on the west branch (Station 4) and on the east branch (Station 5; Figure 1.4).

At each station, reservoir water samples were composited from three samples of two water masses separated by a thermocline, if present, or corresponding to previous sampling depths during well-mixed conditions. Elderberry Forebay samples were collected from the Elderberry Outlet Tower using a Niskin bottle sampler to collect water from approximately 3 - 5 m and were not composited.

Soil samples were collected to determine if the *Actinobacteria* population in the reservoirs was derived from terrestrial populations introduced into the catchment

primarily from soil runoff (allochthonous) or if they were free-living (autochthonous). A soil sample was collected from a typical grassy area above San Luis Reservoir on January 11 and from a similar area above Castaic Lake on February 27, 2005. Grass was removed from the topsoil using a trowel and the top 2 cm of soil in a one-third meter square was placed into a Ziploc bag. All clods were broken up and a sample was taken from the bag with a 50 mL centrifuge tube. The sample was immediately placed on ice and shipped back to the lab.

Water samples are identified according to location and season with aqueduct samples collected at stations named 'Check' (Figures1.2 and 1.3). Those samples collected in October are designated (10), in January (1) and in February (2). Samples collected from the aqueduct are named Delta-Mendota Canal (DMC), Check 12, 13, 21, 29, 41, 52/66 and Devil's Canyon Afterbay (DCA). San Luis Reservoir samples were collected in January 2005, designated with a (1) and named upper composite (SLRU) and lower (SLRL) composite. Castaic Lake samples were collected in February (2) and named upper (CASTU) and lower composite (CASTL). Elderberry Forebay samples were collected in February (2) from one depth (3 - 5 m) and named ELDER.

Chemical and biological measurements in water samples

Chemical and biological measurements for the aqueduct and reservoir water samples are detailed in Tables 2.5 and for the inflows to the aqueduct in Table 2.6. Temperature, pH, dissolved oxygen (DO) and conductivity were measured in the field using a YSI model 600XL sonde. The following determinations were made at the USGS California Water Science Center Department in Sacramento and are compiled in a USGS report (Kraus et al. 2005): DOC; chlorophyll-a and pheophyton-a concentrations; pH;

conductivity (EC); specific UVA absorbance (SUVA = 100*UVA_{254}/DOC (mg/L)); anions (Cl, SiO_4, SO_4); cations (Na, Ca, K, Mg, Fe, Mn); nitrogen (NH_4, NO_3, NO_2, Total-N); and phosphorus (Ortho-P, Total P). Biological oxygen demand and bioavailability of DOC were determined at UGA as detailed in Chapter 2.

DNA extraction

Particulate material was collected from water samples for subsequent extraction of microbial DNA by pressure filtration through Millipore Sterivex filter cartridges (0.22 μm). Cartridges were filled with 1.8 mL lysis buffer [50 mM Tris (pH 8.3), 40 mM EDTA and 0.75 M sucrose], capped, frozen and shipped on dry ice and stored frozen at -70 ºC until processing. Blanks were prepared by filling an unused Sterivex cartridge with only lysis buffer. Total community DNA was extracted from the Sterivex filters as described by Ferrari and Hollibaugh (1999). Briefly, each Sterivex cartridge received 40 μL of lysozyme (50 mg mL^{-1}) and incubated at 37 ºC for 60 min. Then, 50 μL of proteinase K (20 mg mL^{-1}) and 100 μL of a 20 % (wt/vol) solution of sodium dodecyl sulfate (SDS) were added and each cartridge was incubated at 55ºC for 2 h. DNA was purified from an 800 μL subsample of this lysate by sequential extraction with 800 μL of phenol-chloroform-isoamyl-alcohol (25:24:1), then chloroform-isoamyl alcohol (24:1) and finally *n*-butanol. The final aqueous layer was removed and placed in a Centricon-100 concentrator (Amicon), mixed with 500 μL TE buffer [10 mM Tris and 1 mM EDTA (pH 8.0)] and centrifuged at 1000 x *g* for 10 min, with this last step repeated.

DNA was extracted from soil samples (0.75 g) in the lab using the UltraClean Soil DNA Isolation Kit (MoBio), according the manufacturer's instructions**.** The molecular

weight and concentration of DNA in both the water and soil extracts were determined by electrophoresis on 1.5 % agarose gels.

PCR amplification and denaturing gradient gel electrophoresis

The v.3 region of the 16S rRNA gene was amplified from extracted DNA using *Actinobacteria* specific primers targeting positions 235-252 (primer 235F; 5'-GCGGCCTATCAGCTTGTT-3') and 517-533 (primer 517R; 5'-ATTACCGCGGCTGCTGG-3') of the *Escherichia coli* gene. A 40 bp GC clamp (Myers et al. 1985) was added to the 5'end of the 235F primer and fluorescein attached to the 5' end of primer 517R.

PCR reaction mixtures of 25 µL were made using PuReTaq^{TM} Ready-To-Go PCR beads (Amersham Biosciences) according to manufacturer's instruction. The final concentration of primer in each reaction was 1 µM and each reaction used 1.0 µL of template DNA. The reaction was run on a thermal cycler with an initial denaturation at 95 ºC for 10 min followed by 30 cycles consisting of denaturation (30 s at 95 ºC), annealing (30 s at 52 ºC) and extension (1 min at 72 ºC). A final extension step consisting of 10 min at 72 ºC was included. Negative controls were obtained from filters through which no water had passed. The success of the PCR reactions was determined on 1.5 % agarose gel with a 100-bp DNA ladder examined using a UVP transilluminator. The products of this mixed template amplicons were then resolved by denaturing gradient gel electrophoresis (DGGE, below).

Clone libraries

Clone libraries were constructed from mixed template PCR amplifications using the same forward primer as that used for DGGE, except it contained one degenerated base

pair (primer 235F; 5'-CGCGGCCTATCAGTWGTTG-3') in combination with an *Actinobacteria* specific, degenerate reverse primer targeting positions 664-681 of the *Escherichia coli* gene (primer 664R; 5'-GGGGAGANKGGAATTCCT-3'). All primers were synthesized by Operon Technologies, (Oakland, CA). PCR conditions used were as described above for PCR for DGGE. Products of the appropriate size (~ 400 bp) were recovered from a 1.5 % agarose gel and DNA was extracted using the QiaQuik gel extraction kit (QIAGEN, Valencia, CA). The 16S genes were cloned into the pCR 2.1 vector (Invitrogen Corp, CA) according to the manufacturer's protocol. The transformed cells containing the insert were plated on Luria-Bertani (LB) media containing 100 μg of ampicillin ml^{-1}, 80 μg of 5-bromo-4-chloro-2-indolyl-ß-D-galactopryanoside (X-Gal) ml^{-1} and incubated overnight at 37 ºC.

A total of 550 containing inserts (white colonies) from the 13 clone libraries were then selected randomly, placed in 96 well plates in LB media freezing media and incubated overnight at 37 ºC. Then, 100 μL was transferred to a new 96-well plate and sent to the UGA core facility for sequencing using M13 forward primer (primer M13F; 5' -TCCCAGTCACGACGTCGT-3').

Phylogenetic analysis

Primer sequences were not included in the in phylogenetic analysis. Sequences (~425 bp) were aligned using the PILEUP tool of the GCG Wisconsin Suite, version 10.0 (Genetics Computer Group, Inc). Phylogenetic trees were constructed using Jukes-Cantor distances and the neighbor-joining method (PHYLIP package). All sequences ≥ 98 % similar were considered to be the same phylotype. Sequences were compared to database sequences by using the Basic Local Alignment Search Tool (BLAST; Altschul et al.

1997). The closest sequences from GenBank and representative sequences from each phylotype were used in the final phylogenetic tree using positions 265-663 of *E. coli* for water samples and positions 259-623 of *E. coli* for soil samples. The PHYLIP package (Felsenstein 1993) was used to infer phylogenic trees and bootstrap analysis (100 replicates) using the neighbor joining method and Jukes-Cantor evolutionary distances.

Statistical analysis of phylotypes and sequence population diversity

Rarefaction curves were produced using software available online (http://www.uga.edu/strata/software/Software.html). Rarefaction is used to estimate the likelihood that all phylotypes were sampled had the sample sizes been lower. As the rarefaction curve reaches saturation, the more likely it is that most of the phylotypes in the original population have been detected in the sample.

Coverage (C) was determined using the formula: $C = 1- (n_1/N$; Mullins et al. 1995), with n_1 the number of phylotypes that occurred only once in the library and N the total number of clones in the library. This formula determines the homologous coverage within the library at a predetermined ≥ 98 % relatedness. The calculated coverage describes how well the sample represents the individual library.

The LIBSHUFF program (Singleton et al. 2001) was also used to test significant differences in the composition of pairs of libraries. Sequences were aligned, as described previously for phylogenetic analysis, using the PILEUP tool with evolutionary distance (≥ 98 %) calculated using the Jukes-Cantor algorithm. The LIBSHUFF program also uses coverage to determine relatedness of the clone libraries comparing homologous coverage and heterologous coverage curves at a ≥ 98 % relatedness. Heterologous coverage ($C = 1-(N_{xy}/n_x)$ is determined by the number of sequences found in library X that are not found in

library *Y,* with n_x the number of sequences in *X*. The distance between the two coverage curves is then calculated using the Cramer von Mises test statistic with a random shuffling technique after which the data is ranked. The two libraries are considered significantly different with a calculated value of $P < 0.05$.

Quantitative PCR

Quantitative PCR (qPCR) assays were conducted in polypropylene 96-well plates using a BioRad iCycler iQ Real-time PCR Detection System. Each 25 µL reaction contained: 12.5 µL SYBR Green Supermix [100 mM KCl, 40 mM Tris HCl, (pH 8.4), 0.4 mM of each deoxyribonucleoside triphosphate (dATP, DCTP, dGTP and dTTP), iTaq DNA polymerase, 50 units/mL, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein and stabilizers]; 1 µM of each primer and 0.5 µL DNA template. Primers used were (1) specific for the *Actinobacteria* 16S rRNA gene (235F and 664R) and (2) universal *Eubacterial* primers 356F and 517R. Forward primer 235F and 664R detailed above for clone libraries. *Eubacterial* DNA was amplified with forward primer at positions 340-356 (primer 356F; 5' -CCTACGGGAGGCAGCAG-3') of the *Escherichia coli* gene and reverse primer 517R. PCR conditions were as follows: initial denaturation at 95 °C for 5 min followed by 40 cycles consisting of denaturation (15 sec at 95 ºC), annealing (30 sec at 52 ºC) and extension (30 sec at 72 ºC). A dissociation curve was run to ensure that the desired product was quantified.

Plasmid standards for *Actinobacteria* were prepared as described in Suzuki et al. (2000) from a clone sequence used in construction of clone libraries, described previously. To make the general *Eubacteria* standards, genomic DNA from a culture of *E. coli* K12 was extracted with the PowerSoil DNA kit (MoBio) according to

manufacturer's protocol. *Eubacterial* primers targeting the base pair positions 1352-1369 (primer 1369F; 5' -CGGTGAATACGTTCYCGG-3') of the 16S rRNA gene and positions 1492-1510 (primer 1492R; 5' -GGTTACCTTGTTACGACTT-3') of the 16S rRNA gene were used to amplify the region of interest. The amplified gene was cloned using the TOPO TA cloning kit (Invitrogen). Several clones were selected and plasmids were isolated using the Qiaprep Plasmid Miniprep Kit (Qiagen) and linearized by restriction digest with *Not*I (Promega). Linearized plasmids were run on a 1.5 % agarose gel, bands excised and DNA extracted with QIAquick gel extraction kit (Qiagen). DNA concentrations of linearized plasmids for standards for both *Actinobacteria* and *Eubacteria* were determined fluorometrically using a PicoGreen Quant-it dsDNA Quantitation kit (Invitrogen). Gene copy numbers (μL^{-1}) were calculated and diluted for a standard concentration range of 10^1 to 10^8 copies (μL^{-1}). Each DNA sample was run in triplicate on each plate along with at least four standard concentrations per assay. A negative control (*E. coli)* standard was also included in the *Actinobacteria* amplification. Standard curves ranging from 10^1 to 10^8 gene copies (μg^{-1}) were run with both the *Actinobacteria* and *Eubacteria* specific qPCR assays. As detailed in Suzuki et al. (2000), from the standard curves, threshold cycle (C_T) numbers were calculated for each reaction. The number of cycles necessary for the fluorescence emission of reporter dye to exceed a set threshold value (threshold cycle number [C_T]) relative to standards was used to calculate target gene copy numbers in the unknown samples. PCR amplification efficiency in the assays was estimated by the slopes of standard curves (regression line of C_T verses log N, the log of the initial gene copy numbers in standard templates).

DGGE analysis of PCR product

DGGE banding patterns were used to assess richness of the *Actinobacteria* assemblage in the SWP using the methods described in Bano and Hollibaugh (2002). Briefly, PCR products were separated by electrophoresis (CBS Scientific) for 13 h at constant voltage of 4.2 volts cm^{-1} and constant temperature of 60 °C on 6.5 % polyacrylamide gels containing a 40 to 70 % gradient of denaturant (urea and formamide) in 1 X TAE buffer [40 mM Tris-20 mM sodium acetate and 1 mM ethylenediamiamine-tetraacetic acid (EDTA) with pH adjusted to 7.4 with 20 mM acetic acid]. The gels were scanned with an FMBIO II MultiView (Hitachi) gel scanner to measure fluorescein fluorescence. The resulting electropherogram image of each sample is referred to as a fingerprint.

Phylogenetic information was obtained for bands of interest as follows. Bands were excised from gels, immersed in 30-50 µL of nuclease free water and incubated at 55 ºC for 1 h. The DNA eluted from these bands was amplified with primers 235F and 517R using conditions described above for PCR/DGGE. The PCR product was then purified with QIAquick PCR Purification Kit (Qiagen) according to manufacturer's instructions then sequenced using the ABI Prism Big Dye Terminators V 3.0 sequencing kit (Applied Biosystems). Each 10 µL sequencing reaction consisted of 4 µL Big Dye, 1 µL of either 235F or 517R primer and 50-100 ng of PCR product. PCR conditions were as follows: initial denaturation at 98 ºC for 2 min 30 sec followed by 25 cycles consisting of denaturation (25 sec at 98 ºC), ramping down (1 ºC/sec to 50 ºC), annealing (15 sec at 50 ºC), ramping up (1 ºC/sec to 60 ºC) and extension at 60 ºC for 4 min. The PCR product was purified using Sephadex and dried in a SpeedVac. The dry pellets were sequenced at the University of Georgia core facility.

To associate sequences from clone libraries with DGGE bands unambiguously, cloned nearly full length amplicons were used as template for PCR/DGGE as described above and run on a DGGE gel along with environmental DNA. The band in the fingerprint from a sample with the same electrophoretic mobility as the band generated from a cloned amplicon was assumed to represent the same sequence (data not shown). This was verified where possible by comparing sequences obtained from the DGGE band with the sequences from cloned amplicons.

DGGE gel image analysis

DGGE gel images were analyzed with Molecular Analyst – Fingerprint Plus software (version 1.12, BioRad) as in Ferrari and Hollibaugh (1999). Gel banding patterns were normalized to compensate for differences between gels by aligning bands from universal standards and two common bands (internal markers) that were present in all samples. Individual bands (operational taxonomic units, OTUs) were then identified automatically by the software using a tolerance of 1 % of the migration distance to discriminate between two bands. A similarity matrix for all samples was then constructed using the Jaccard Coefficient Index based on the presence or absence of bands in each fingerprint. A dendogram was generated based on the similarity matrix using the unweighted pair group method with arithmetic mean (UPGMA). DGGE bands of the soil samples resolved poorly and were eliminated from further analysis.

Comparison with environmental data

Statistical analyses of the relationship between DGGE band distributions and environmental variables were performed with PRIMER v5 software, version 5.2.9 (PRIMER E Ltd.). Environmental data were not collected for all samples (Table 2.5 and

2.6). Consequently, ELDER-2, SLRL-2 and CASTL-2 were included in the initial analysis of DGGE band distributions by non-metric multidimensional scaling (NMS), but excluded from the subsequent comparison of the environmental variables with the DGGE bands by Spearman rank correlation. In addition, NO_x was excluded from all analyses as it was below detection levels for almost all samples (Table 2.5). SO_4^- and Cl^- were excluded as they were not measured at all stations and, when included in preliminary analyses, did not increase the amount of variance explained by the Spearman rank correlation (data not shown). Data from the inputs along the aqueduct (Figure 2.6) were only included in the NMS analysis of the environmental samples.

Non-metric multidimensional scaling (NMS)

The NMS analysis (Kruskal's non-metric procedure) constructs a configuration of the samples based on similarity matrix conditions. Thirty-seven DGGE bands in the 20 samples were ordinated using a presence-absence transformation and Bray-Curtis similarity to rank the band data. To rank environmental data, normalized-Euclidian distance was used with no transformation. To avoid local minima, the NMS was run with 500 randomized starts.

One-way analysis of similarities (ANOSIM)

One-way analysis of similarities (ANOSIM; Clarke and Warwick 2001) was used independently to test the significant similarities between (1) the DGGE banding patterns seasonally and by location and (2) the environmental data. The ANOSIM procedure is a rough analogue of the standard univariate 1-way ANOVA. The similarity matrices described previously for NMS were used in the ANOSIM analysis. Then, a test statistic (R) is generated that indicates the degree of separation between the different groups.

Complete dissimilarity (separation) is given a score of 1 and complete similarity (no separation) is given a score of 0.

Spearman rank correlation

The Spearman rank correlation coefficient is used for evaluating the degree of linear association or correlation between two independent variables (Gauthier 2001). It is similar to the Pearson's (parametric) product moment correlation, but it is a non-parametric technique. Unlike Pearson's correlation, which operates on the raw data, the Spearman correlation uses ranked data. The advantages of using the Spearman rank correlation for our data set is that it is unaffected by the distribution of the population. Also, it can be used with very small data sets and there are no requirements that the data be collected over regularly spaced intervals. The disadvantages lie in the loss of information that occurs when data are converted to ranks.

The routine used by the PRIMER v5 programs (BIO-ENV) rank correlates the matching elements in the two matrices using the coefficient ρ_s for the standard Spearman rank correlation (Clarke and Warwick 2001). The measure of agreement is calculated between the two similarity matrices; the fixed triangular matrix of DGGE bands and each of the possible triangular matrix combinations for environmental data. The value of ρ_s is computed for all possible combinations of environmental variables; combinations of which are considered at steadily increasing levels of complexity. The ρ_s value is not a demonstration that certain environmental variables are the cause of the biological pattern, since the real causal variable may not have been measured, but may instead be strongly correlated with one or more variables that did cause the DGGE band pattern.

The DGGE band matrix was compared to the environmental data matrix in three ways: (1) all bands included; (2) with Elderberry Forebay excluded (anions and nutrients were not measure at Elderberry Forebay); and (3) with only aqueduct samples included, excluding DCA-2, as it was only sampled once. Checks 52 and 66 are located near enough to each other to be considered together (Figure 1.2). The statistical significance of Spearman correlations was tested at a 95 % confidence level using the critical values listed in Gauthier (2001).

3.3 Results

Analysis of DGGE banding patterns

The band-calling program recognized a total of 36 different DGGE bands (OTUs) in water samples (Figure 3.1). The *Actinobacteria* primer set (235F, 664R) has been reported to amplify 16S rRNA genes from *Verrucomicrobia* (Fierer et al. 2005). One DGGE band in our samples was identified as *Verrucomicrobia* and was removed from the data set prior to statistical analysis. Only 21 (5 %) of the total of 426 sequences obtained from the clone libraries were characterized as *Verrucomicrobia*.

DGGE fingerprints of individual samples contained 13 to 22 OTUs (Table 3.1a). The average number of OTUs was similar in both October (18 ± 1.3) and February (17 ± 0.5) samples, though not statistically significant (Table 3.1b). However, there were differences in the distribution of some of the OTUs, both seasonally and spatially (Figure 3.2). *Actinobacteria* richness in O'Neill Forebay was statistically significantly lower in February than in October. The richness of the *Actinobacteria* assemblage in O'Neill Forebay samples (20 ± 1.2) was greater than in aqueduct samples (16.5 ± 1.4) in October,

while in February richness was lower (16.3 ± 0.3) in O'Neill Forebay samples than in aqueduct samples (17.5 ± 0.7), though neither was statistically significant.

Actinobacteria phylotypes in DGGE bands

The DGGE fingerprints of all samples (Figure 3.2) contained eight major OTUs at positions 177, 254, 280, 332, 345, 358, 391 and 464. DNA sequences obtained from six bands representing four OTUs (280, 332, 358 and 391; Figure 3.3) were determined to match sequences found in clone libraries (Figure 3.4). Two other OTUs were identified by sequencing DNA from DGGE bands (OTUs 186 and 212; Figure 3.2), but they were not present at all sites. DGGE fingerprints showed recurring patterns (Figure 3.2), including a change in OTU composition between Check 29 and Check 41 of the aqueduct. Five OTUs (166, 194, 294, 323 and 371) appeared in the aqueduct from Check 12 through either Check 21 or Check 29 in both seasons. Of these, OTUs 166 and 194 were present in San Luis Reservoir and also in the aqueduct until Check 29 and Check 21, respectively, but only OTU 166 was found at Elderberry Forebay. OTU 294 was found in San Luis Reservoir, at Checks 12-29; and in both Elderberry Forebay and Castaic Lake in late summer, but it was only found at Check 41 and southward in late winter. OTUs 323 and 371 were present at Checks 12-29, and OTU 323 was also present at Castaic Lake in February.

Four OTUs (171, 186, 199 and 220) were present at Check 41 and southward in February with only OTU 186 present in October. Three of these (186, 199, 220) were also present in Elderberry Forebay and Castaic Lake. OTU 266 was present in the aqueduct only in late winter, though it was also present in both San Luis Reservoir and Castaic Lake samples in later winter. OTU 315 appeared in the Elderberry Forebay

sample, yet it was only present in the epilimnion of San Luis Reservoir and Castaic Lake. Three OTUs were only found in reservoir samples: San Luis Reservoir (391), Castaic Lake (209) and Elderberry Forebay (224).

Similarity of Fingerprints

An analysis of the relationships between samples based on the similarity of their fingerprints (Figure 3.4) illustrated the influence of mixing in O'Neill Forebay on assemblage composition from Check 13 until Check 29 in both late summer and late winter. However, there may have been a change in assemblage composition either at, or just before, Check 41. The analysis also indicated that the *Actinobacteria* assemblages in San Luis Reservoir were distinct from those in Castaic Lake. Elderberry Forebay most closely resembled the late winter samples collected from the aqueduct.

NMS analysis of DGGE bands

DGGE bands clustered by season and location in the NMS analysis (Figure 3.5). DGGE banding patterns from O'Neill Forebay and aqueduct through Check 29 in both seasons appeared to be more similar than DGGE bands from Check 41 and southward. All differences between samples were statistically significant as determined by ANOSIM except for ON10 vs. AQ2 and AQ10 vs. AQ2 and (Table 3.2). Banding patterns from the three reservoirs were distinct. The small number of samples ($n = 20$) prohibited direct comparison between locations.

NMS of environmental variables

NMS analysis of environmental data was first run with all samples included. Due to a limited number of measurements from Elderberry Forebay (Table 2.5), only the variables of temperature, pH, EC, DO, DOC, BDOC and SUVA were used for the first NMS

analysis (Figure 3.6). The two seasons clustered separately, reflecting late summer versus late winter differences in water composition. The late summer samples clustered together, indicating that water composition remained relatively homogenous, as it traveled south to Check 52. In late winter, the environmental data measured for each station did not cluster together. The environmental data collected at the reservoirs showed the water composition to be distinct from the aqueduct and also among the three reservoirs.

An NMS analysis of the complete environmental data set was then performed for aqueduct samples (Figure 3.7) to allow for more relevant seasonal comparisons. A change in composition as water traveled down the aqueduct is apparent in both seasons. Environmental data collected from the late summer sample sites grouped much more tightly than late winter data. The groundwater inflow variables measured from Semitropic, Kern River and Arvin Edison (Table 2.6b) did not show a relationship to aqueduct water samples (Figure 3.8).

Spearman rank correlation analysis

I used the Spearman rank correlation coefficient to compare matrices of DGGE fingerprints with environmental data. The data were analyzed three ways, with (1) all locations included, (2) only reservoir samples and (3) only aqueduct samples. Aqueduct samples were subsampled with (a) a combination of both seasons (b) only October samples and (c) only February samples (Table 3.3). The rank correlation (ρ_s) was compared to critical values for significance at the 95 % confidence interval (Gauthier 2001).

The correlation between matrices of the DGGE fingerprints and the environmental variables were only statistically significant using just data from aqueduct samples. When

aqueduct samples from October and February were combined, DGGE fingerprints were statistically significantly correlated with the environmental variables pH and SUVA (ρ_s = 0.682; Table 3.3). DGGE fingerprints from late summer aqueduct samples were significantly correlated with environmental variables EC, DOC, BDOC, Chl-a, SUVA and calcium ion (ρ_s = 0.804), while DGGE fingerprints were the most significantly correlated with temperature, pH and EC in late winter (ρ_s = 0.757).

qPCR

The relative abundance of aquatic *Actinobacteria* in the SWP ranged from 12 - 34 % in late summer versus 9 - 40 % in late winter in the California Aqueduct and 11 - 33 % in the reservoirs (Table 3.4). There was not a statistically significant pattern in overall relative abundance; however, there was a decrease in relative abundance at Checks 21 and 29 in late summer and at Check 29 in late winter. Also, there was an increase in relative abundance at Check 41 in both seasons. Yet, DGGE fingerprints did not correlate with relative abundance when included in the Spearman rank correlation analysis (data not shown). The relative abundance of *Actinobacteria* in the soil samples as determined by qPCR was 4.9 % at San Luis Reservoir and 14.7 % at Castaic Lake.

Phylogenic diversity of *Actinobacteria*

Sequences of *Actinobacteria* 16S rRNA genes retrieved from water and soil samples did not cluster together (Figure 3.9). For ease in further description of results, these clone libraries will be discussed separately.

Sequences from water samples

A total of 404 sequence retrieved from aqueduct and reservoir water samples resulted in 40 different (similarity < 98 %) *Actinobacteria* phylotypes (Table 3.5). All but three

clones (97 %) were phylogenetically associated with the freshwater *Actinobacteria* clusters *acI, acII* and *acIV*, as described by Warnecke et al. (2004) and expanded by Allgaier and Grossart (2006; Figure 3.10). Ten of the remaining 13 clones clustered in the newly proposed freshwater *Actinobacteria acSLT* cluster (Allgaier and Grossart 2006). Of the three remaining clones, two were 99 % similar to isolates of *Corynebacterium* and *Mycobacterium,* respectively and one clone was distantly related (92 %) to a *Streptomyces* isolate.

Sequences of 261 cloned inserts fell into the *acI* cluster. There were 27 in the *acI-A* subcluster and 234 clones in the *acI-B* cluster. Allgaier and Grossart (2006) further subdivided the *acI-B* group into *scb1, 2, 3* and *4*. Of the *acI-B* clones, 115 clustered as *scb-1* and 34 as *scb-2*. There were 10 clones in the *acII* cluster and 120 clones in the *acIV* cluster.

The sequences of DGGE bands (Figure 3.11) were identical to sequences from clones representing the following phylotypes (Figure 3.10): Band 391 (SWP-W39), Band 358 (W26), Band 332 (W22) and Band 280 (W16). These four OTUs accounted for 151 clones (37 %) of the 404 clones sequenced from water samples. Sequences from these DGGE bands were 99 - 100 % similar to the sequences of clones representing phylotypes Band 186 (SWP-W25), Band 212 (W10) and Band 410 (W38; Figure 3.11). Two of these OTUs (W10 and W38) were found in San Luis Reservoir and Castaic Lake, but only appeared in the aqueduct in late summer when water was being released from San Luis Reservoir (Figure 3.3). Clone W25 (Band 186) was also found in the reservoirs, yet was only apparent from Check 41 southward in both seasons.

Sequences from soil samples

The 91 *Actinobacteria* 16S rRNA genes sequenced from soil sample libraries resulted in 38 phylotypes (Table 3.6). All of the clones sequenced were in the class *Actinobacteria*, with the majority of the clones (90 %) closely related to previously isolated members of subclass *Actinobacteridae* (Figure 3.12). Sequences of all clones (except one) from soil samples were > 96 % similar to previously isolated *Actinobacteria* of the suborders *Frankineae, Corynebacterineae, Micromonosporineae, Micrococcineae, Propionbacterineae, Pseudonorcardineae and Streptomycineae.*

Assemblage richness

Coverage values for my libraries ranged from 44 - 98 % for water samples and 0 - 77 % for soil samples (Table 3.7). Castaic Lake samples had the highest coverage (93 %) for an individual library and reflected the large number of clones in this library. When all libraries were combined, coverage of water samples was 98 %. Check 41 in October appeared to be the most diverse (44 %) with Check 29 in October the least diverse (82 %). Combined October (67 %) and February (69 %) library coverage was similar. Rarefaction curves for water samples (Figures 3.13 and 3.14 a, b) and for soil samples (Figure 3.14 c) indicate different patterns of diversity in the clone libraries, however, the number of clones in each library was not equal and there was also a low number of total clones in each library.

LIBSHUFF

LIBSHUFF analysis showed that few libraries were closely related (Table 3.8). Libraries constructed from O'Neill Forebay samples were not statistically significantly different from aqueduct libraries in both October and February. SLRU libraries were not

statistically significantly different from SLRL libraries, nor were CASTU and CASTL libraries. Also, San Luis Reservoir and Castaic Lake libraries were not significantly different from each other. The influence on aqueduct populations of the input from San Luis Reservoir during late summer was apparent. When water was being released from San Luis Reservoir in October, the San Luis Reservoir clone library was not significantly different from the O'Neill Forebay library or from the aqueduct library. However, when no water was being released from San Luis Reservoir in February, there was no similarity between the San Luis Reservoir libraries and the libraries from either O'Neill Forebay or the aqueduct.

3.4 Discussion

Phylogenetic analyses of freshwater microbial communities worldwide have shown *Actinobacteria* to be abundant in systems of varying trophic status. These studies used 16S rRNA sequencing preceded by various qualitative methods to assess composition, such as denaturing gel gradient electrophoresis (DGGE; Crump and Hobbie 2005; Selje et al. 2005), terminal restriction fragment length polymorphism (T-RFLP; Allgaier and Grossart 2006; Boucher et al. 2006; Eiler and Bertilsson 2004; Stepanauskas et al. 2003) and fluorescent *in situ* hybridization probes (FISH; Allgaier and Grossart 2006; Burkert et al. 2003; Glockner et al. 2000; Warnecke et al. 2005) to assess differences between *Actinobacteria* assemblages. The *Actinobacteria* sequences obtained in these studies clustered similarly within phylogenetic trees, though the habitats varied from river systems to oligotrophic, mesotrophic and eutrophic lakes and reservoirs. In addition, the freshwater *Actinobacteria* of these systems were not introduced into their aquatic systems from the catchment, but were of autochthonous origin. However, very little is known

about their functional role in the ecosystem due to the low number of organisms cultured. I also found *Actinobacteria* in the California State Water Project to be autochthonous and not related to the catchment soil bacteria (Figure 3.9). The diversity and abundance of these phylotypes closely resembled that of *Actinobacteria* sequenced from its source water, the Sacramento-San Joaquin Delta (Stepanauskas et al. 2003). I found 22 % average relative abundance of aquatic *Actinobacteria* across all sites in the SWP (Table 3.4), which agreed with the 20 – 30 % frequency of *Actinobacteria* reported in the Sacramento-San Joaquin Delta (SF21 clone library; Stepanauskas et al. 2003). DGGE fingerprints and cloned sequences from all SWP samples contained the same major *Actinobacteria* phylotypes in both seasons (Figure 3.3). This suggests that these major phylotypes dominated both the aqueduct and reservoir populations throughout both seasons and possibly throughout the year.

All but three of the sequences cloned from water samples were > 96 % similar to previously sequenced, globally distributed freshwater *Actinobacteria*. In addition, the majority of these phylotypes clustered primarily with cloned sequences into the previously described *Actinobacteria* clusters *acI* and *acIV* (Figure 3.10). These two clusters dominate many freshwater systems (Allgaier and Grossart 2006; Boucher et al. 2006; Warnecke et al. 2004). Though closely related, they differ in their phylogenetic origins (Warnecke et al. 2004). Cluster *acI* has shown an adaptive radiation within the freshwater pelagic zone, whereas cluster *acIV*, though also aquatic, has diverse lineages and has apparently radiated across several different habitats, from marine to soil to freshwater. Globally distributed *Actinobacteria* phylotypes do not necessarily coexist

within all habitats and numerous phylotypes are present in varying seasonal abundances (Crump and Hobbie 2005; Eiler and Bertilsson 2004; Stepanauskas et al. 2003).

Influence of seasonal and environmental conditions on diversity

The DGGE fingerprints from water samples revealed that many phylotypes are present across all sites. NMS analyses were conducted to distinguish subtle differences in the distributions of *Actinobacteria* assemblages. Assemblage compositions were unique to different sites throughout the SWP, most notably the reservoirs and the aqueduct (Figure 3.5). Though *Actinobacteria* richness did not vary greatly seasonally (Table 3.1), some phylotypes were more abundant in either late summer or late winter.

Two sets of environmental data were also analyzed by NMS. The analysis of environmental data measured for all stations (Figure 3.6) was limited to just seven measurements (Table 2.5). However, results from this analysis were very similar to the NMS analysis of the additional environmental data collected at aqueduct stations (Figure 3.7) which included nutrient and ion concentrations. This suggests that the additional chemical variables did not contribute significantly to explaining the distribution of *Actinobacteria*. This agreed with a previous study reporting that diverse *Actinobacteria* assemblages displayed similar seasonal dynamics that were independent of basic limnological features (i.e. trophic status, pH, alkalinity, PO_4 and DOC; Allgaier and Grossart 2006). Spearman rank correlation analysis (Table 3.3) supported the NMS analysis, with few correlations found between environmental variables that may have influenced distribution of *Actinobacteria* and the DGGE fingerprints.

Late summer *Actinobacteria* distributions did correlate with a number of optical and chemical characteristics of DOC, including DOC concentration, BDOC, SUVA and

phytoplankton-derived DOC inferred from Chl-a concentrations. Stepanauskas et al. (2003) also reported a correlation between DOC characteristics and the relative abundance of *Actinobacteria* in the Delta during the summer and fall. This suggests that the amount of available carbon may contribute to changes in community composition correlating with seasonal variance in organic carbon concentration in the SWP, mainly due to terrestrial inputs to the Delta associated with heavy winter rainfall (Jassby and Cloern 2000; Stepanauskas et al. 2005).

Another measure of carbon quality is specific UVA absorbance (SUVA = $100*UVA_{254}$/DOC (mg/L)), a measure to estimate the amount of aromaticity within the DOC pool. The average SUVA values were lower in late summer than in late winter (Figure 2.5). However, community composition did not correlate with SUVA in late winter, perhaps because DOC concentration and bioavailability were so much greater then than in late summer. Average Chl-a concentrations were higher in late summer than in late winter, indicating an input of phytoplankton-derived DOC into the system. However, with labile carbon reported to be a low percentage of the carbon pool in the SWP (Figures 2.7 and 2.8), it may have only localized effects on bacterial communities.

Seasonal fluctuations of electric conductivity (EC) in the SWP were apparent (Table 2.5) and corresponded to seasonal fluctuations of EC reported in the Delta by Stepanauskas et al. (2003). *Actinobacteria* community composition correlated with EC in the SWP in both the late summer and late winter and was also reported to correlate with EC in the summer and fall in the Delta (Stepanauskas et al. 2003) and in other studies (Olapade et al. 2005; Schauer et al. 2005).

I found a correlation between community composition and pH as reflected in DGGE fingerprints in late winter samples due to an increase in pH along the aqueduct from O'Neill Forebay (pH ~7.5) to Check 66 (pH ~8.8) that was not seen in late summer (pH ~7.8 to ~8.2; Table 2.5). The overall trend of rising pH in the aqueduct in the late winter samples is consistent with either the dissolution of the calcium carbonate from the aqueduct's cement liner ($CaC0_3 + H_20 \rightarrow Ca^{+2} + C0_3^{-2} \rightarrow Ca^{+2} + HCO_3^- + OH^-$) and/or from the loss of organic acids (i.e. phenolic or carboxylic) due to biological consumption or photodegradation of carbon along the aqueduct. pH was also reported to be a factor in bacterial composition in the Delta (Stepanauskas et al. 2003) and other recent studies (Lindstrom et al. 2005; Lindstrom and Leskinen 2002; Yannarell and Triplett 2005).

Community composition correlated with temperature in February. Late summer temperature was higher (19 ºC) than in late winter (13 ºC). These factors were reported to influence bacterial community distribution in the Delta (Stepanauskas et al. 2003) and in other freshwater systems (Lindstrom et al. 2005).

Finally, a correlation between community composition and calcium in late summer may have been spurious. Though calcium ions are involved in many physiological processes, both intra- and extracellularly, I was not able to explore the physiological aspects of *Actinobacteria* that may explain this correlation. There was no correlation between community composition and calcium ion concentration in late winter, perhaps due to the increased calcium concentration measured in the aqueduct, most likely attributed to runoff.

Phylogenetic characteristics of sequenced DGGE bands

Four of the DGGE bands that were sequenced represented four of the eight major bands (Figure 3.11) apparent throughout all seasons and locations (Figure 3.3) in the SWP. The *acI-B* cluster (Figure 3.10) included the most abundant phylotype W22 (Band 332), which was 98 % similar to a clone sequenced from an oligotrophic lake in Germany (STH11-4). Also included in *acI-B* was phylotype W16 (Band 280) which was a 100 % match to several globally distributed strains, including a sequence retrieved from 500 m in oligotrophic Crater Lake (CL500-67) and from 3 m in an Arctic lake (TLM07/TLMdgge34). The cluster *acI-A* included phylotype W26 (Band 358), which was 99 % similar to clone S7 found in an oligo/mesotrophic, shallow German lake. Both the *acI-A* and *acI-B* clusters of SWP clones were closely associated with one another as in Allgaier and Grossart (2006), unlike the more distant clustering reported by Warnecke et al. (2004). Phylotype W39 (Band 391) was in cluster *acIV* and was related to two clones from the Sacramento-San Joaquin Delta (SFD1-11 and SFD1-5).

Phylogenic composition of *Actinobacteria* assemblages from reservoirs

DGGE fingerprints contained three prominent bands that appeared in San Luis Reservoir samples and were subsequently found in the aqueduct in late summer. Sequences obtained from two of the DGGE bands (Band 212 and 410) were related to phylotypes SWP-W10 and W38, respectively (Figure 3.11). Both phylotypes were similar to sequences retrieved from samples collected in a flooded island in the Sacramento-San Joaquin Delta. W10 was 100 % identical to SFD1-39 (*acI-B*) and W38 was 99 % similar to SFD1-11 (*acIV-A*).

Common characteristics of reservoirs include long residence time (Crump et al. 2004; Lindstrom et al. 2005), high chlorophyll concentrations (Stepanauskas et al. 2003) and the taxonomic composition of the predator community (Pernthaler et al. 2001). These factors are reported to contribute to dominance of particular *Actinobacteria* phylotypes. In addition, the presence of these phylotypes in Castaic Lake and Elderberry Forebay (Figure 3.3) further suggests that reservoir conditions in the SWP provide an optimum habitat for these *Actinobacteria*.

Phylogenic composition of *Actinobacteria* assemblages from the aqueduct

The composition of the *Actinobacteria* assemblage entering the aqueduct apparently changed as the water traveled south. The NMS analysis of DGGE bands (Figure 3.5) showed that the *Actinobacteria* assemblage remained relatively stable through Check 29 in both seasons. The high winds present year-round and shallow depth contributes to mixing in O'Neill Forebay which may have translated to the similarity in *Actinobacteria* community composition seen from O'Neill Forebay to Check 29. However, an apparent change in composition, prominent in both seasons, occurred between Checks 29 and 41. This change may be attributed to factors described previously, including residence time, input of new phylotypes from groundwater along the aqueduct, or changes in predation. It is also possible that this apparent shift in community composition is an artifact as the upstream and downstream samples were analyzed on two different DGGE gels.

With residence time reported to be one factor in determining bacterial abundance, a generic tracer model (Figure 2.3) was used to determine aqueduct residence time in both seasons. It was estimated to take 7 days for a tracer to reach Check 21 with an additional

3 days travel time in between each check (Check 29, 41 and 52/66). The total residence time from Check 13 to 66 was estimated to be 15 days.

Actinobacteria are characteristically slow growing and have been out-competed by faster growing bacterial strains in culture (Burkert et al. 2003). As a result of the short aqueduct residence time, *Actinobacteria* entering the aqueduct from O'Neill Forebay may not have sufficient time to become established and therefore may be grazed in the first week. Mean relative abundance of Actinobacteria decreased at Check 21 and 29 in late summer and at Check 29 in late winter (Table 3.4). In addition, there was an increase in relative abundance at Check 41 in both seasons. Also, different phylotypes emerged at Check 41 (Figure 3.3) where there was also an increase in Chl-a and Pheop-a concentrations in both seasons (Table 2.5). It has been reported that *Actinobacteria* phylotypes may have an advantage in the summer and fall when DOC is enriched in the system (Stepanauskas et al. 2003).

Emergence of new phylotypes at Check 41 may also be attributed to bacteria entering the aqueduct from groundwater (Hancock et al. 2005). Two inflows enter the aqueduct prior to Check 29, Semitropic (groundwater) and Kern River, and one groundwater input (Arvin Edison) prior to Check 41. Environmental variables measured in these waters (Table 2.6b) were very different and their composition was not similar to of the aqueduct samples (Figure 3.8). However, the volumes and timing of the pump-ins that would be necessary to calculate whether these inputs could have a significant effect on water composition were not available. Other phenomena that may change along the aqueduct, such as "hot-spots" of microbial activity occurring on microaggregates of organic matter

(Simon et al. 2002) or biogeochemical hotspots due to converging hydrological flowpaths (McClain et al. 2003) are not apparent from sampling methods employed for this study.

Clone libraries

The phylogenetic affinities of 404 cloned *Actinobacteria* 16S rRNA gene sequences from 16 libraries are shown in Table 3.5. Rarefaction curves (Figures 3.13 and 3.14) show that coverage was becoming saturated when libraries were combined however, coverage was low for individual libraries. DGGE analysis of OTU distributions (above) provided a robust comparison of samples.

Soil samples

Sequences retrieved from reservoir catchment soil were not closely related to water samples from either the aqueduct or reservoirs (Figure 3.10). Soil sample libraries (a total of 91 sequences) were richer (Figure 3.12) than those from water samples as illustrated by rarefaction curves (Figure 3.14). Also, DGGE did not prove to be a reliable method for screening soil *Actinobacteria* assemblages in this study. In general, when the bacterial diversity is high, bands may not resolve (Calvo et al. 2004) as they are too light or too near each other to be reliably detected. Also high humic concentrations in the sample can inhibit the PCR reaction (Watson and Blackwell 2000). The *Actinobacteria* sequences retrieved from soil samples were typical of globally distributed environmental assemblages previously isolated or cloned (Chanal et al. 2006; Mummey and Stahl 2004).

The most abundant soil phylotype, S35 (23 clones), clustered with the suborder *Frankineae* and was 99 % related to isolates from Australian (Ellin6023) and Spanish (GEO1A-10) soils. Other phylotypes in this cluster were most closely related to isolates of the genus *Modestobacter*, found in a soil core from a dairy research center in Australia

(Ellin165; 99 % similarity) and also in samples from the Transantarctic mountains (AA826; 98 % similarity). The remaining phylotypes were similar to isolates and clones recovered from arid soil in Australia (clone 0319-6E4; 97 % similarity) and from a stone monument in Italy (BC503; 100 % similarity).

Five of the sequences in the soil library were most closely related (98 – 100 % similarity) to sequences from isolates belonging to the suborder *Propionbacterineae*, that were isolated from two aquatic habitats and one soil habitat. Phylotypes in this cluster (S9 through S13) were most closely related to isolates of the species *Norcardiodes* (99 % similarity); to freshwater isolates (MWH-CaK6; 99 % similarity), to an isolate from an aquatic biofilm in the UK (NRRL B-3381; 99 % similarity).The second largest suborder, *Pseudonorcardineae,* contained 14 clones. The most abundant phylotype in this cluster, S17 (6 clones), was related (97 % similarity) to an isolate from Minnesota farm soil (AKYG500). Other related phylotypes were isolated or cloned from agricultural soil in Austria (ACF42; 99 % similarity) and plant rhizospheres in China (ga49; 99 % similarity).

The suborder *Micrococcineae* contained 9 clones. Phylotypes S5 and S6 were identical to sequences from isolates of the genus *Arthrobacter* recovered from a glacial ice core (Muzt-E04) and from terrestrial subsurface sediment in Washington state (SMCC ZAT200), respectively. Other phylotypes were related to a *Plantibacter* isolate from the oxic sediment layer in the Wadden Sea in Germany (GWS-SE-H149; 98 % similarity) and to a *Micrococcineae* clone from subsurface water of the Kalahari Shield, South Africa (EV818CFSSAHH7; 99 % similarity).

The suborder *Micromonosporineae* contained five clones in two phylotypes related to the species *Micromonospora*. An isolate from Malaysian soil (410F05) was 98 % similar to phylotype S23, and phylotype S24 was related (98 % similarity) to an isolate from the marine environment in the UK (i19).

The remaining phylotypes were classified in the suborder *Actinobacteridae* were related, respectively, to clones from agricultural soil in Austria (ACF44; 99 % similarity), marine sediments in the UK (ASb01; 96 % similarity) and arid soil in New Mexico (C0224; 96 % similarity).

qPCR of soil samples

The qPCR estimate of the relative abundance of *Actinobacteria* in the soil samples was 4.9 % at San Luis Reservoir and 14.7 % at Castaic Lake. These abundances agreed with published (Fierer et al. 2005) estimates of the relative abundances of soil *Actinobacteria*: 5 % in forest soils to 15 % in desert soils.

3.5 Conclusion

Actinobacteria are abundant in the SWP; are related to *Actinobacteria* previously reported in the Sacramento-San Joaquin Delta; and are also related to previously classified, globally distributed freshwater *Actinobacteria*. *Actinobacteria* in SWP water samples are not related to *Actinobacteria* from catchment soils, indicating that they are not simply "washed in" to the reservoirs of the SWP by overland runoff. Factors that appear to contribute to changes in the SWP *Actinobacteria* assemblage include temperature, DOC characteristics, pH, electric conductivity and residence time in the reservoirs. Undetermined processes occurring along the aqueduct may contribute to a shift in the *Actinobacteria* assemblage between Check 29 and Check 41.

3.6 Management Implications

This study has provided the groundwork for understanding the dynamics of aquatic *Actinobacteria* in the California State Water Project. This characterization will inform future research focusing on determining the function of these uncultivated strains in this highly managed ecosystem. Whether strains of aquatic *Actinobacteria* found in the State Water Project have the ability to produce musty taste and odor compounds has not been confirmed; however, several lines of evidence suggest that they might play an important role in this process.

Previous culture-based studies demonstrate taste and odor production by strains of *Actinobacteria* (Jensen et al. 1994; Klausen et al. 2005; Lanciotti et al. 2003) and mass spectroscopy (Bruce et al. 2002) demonstrates the presence of these compounds in the environment. However, less than 1 % of microorganisms known to exist in the environment can be cultured using standard culturing techniques (Amann et al. 1995), with the ability to produce geosmin/MIB potentially lost through repetitive subculturing (Zaitlin et al. 2006). New techniques, however, may provide access to the remaining 99 % (Giovannoni et al. 2005). In the mean time, studies targeting the pathways that encode enzymes of the secondary pathways leading to production of geosmin and other problematic metabolites (Omura et al. 2001; Wanke et al 2001; Spiteller et al. 2002) would provide useful and practical information needed to understand the roles of these globally distributed phylotypes in water quality.

3.7 References

Allgaier, M., and H.-P. Grossart. 2006. Diversity and seasonal dynamics of Actinobacteria populations in four lakes in northeastern Germany. Applied Environmental Microbiology **72:** 3489-3497.

Altschul, S. F. and others 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Research **25:** 3389-3402.

Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. Microbiological Reviews **59:** 143-169.

Bano, N., and J. T. Hollibaugh. 2002. Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. Applied Environmental Microbiology **68:** 505-518.

Boucher, D., L. Jardillier, and D. Debroas. 2006. Succession of bacterial community composition over two consecutive years in two aquatic systems: A natural lake and a lake-reservoir. FEMS Microbiology Ecology **55:** 79-97.

Bruce, D., P. Westerhoff, and A. Brawley-Chesworth. 2002. Removal of 2-methylisborneol and geosmin in surface water treatment plant in Arizona. Journal of Water Supply: Research and Technology **51:** 183-197.

Bull, A. T., J. E. M. Stach, A. C. Ward, and M. Goodfellow. 2005. Marine Actinobacteria: perspectives, challenges, future directions. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **87:** 65-79.

Burkert, U., F. Warnecke, D. Babenzien, E. Zwirnmann, and J. Pernthaler. 2003. Members of a readily enriched β-proteobacterial clade are common in surface waters of a humic lake. Applied Environmental Microbiology **69:** 6550-6559.

California Department of Water Resources (DWR). 2005. Division of Operations and Maintenance: State Water Project Operations Control Office. State Water Project Monthly Operations Data. http://wwwoco.water.ca.gov/monthly/monthly.menu.html. Accessed 20 May 2005.

Calvo, L., X. Vila, C. A. Abella, and L. J. Garcia-Gil. 2004. Use of the ammonia-oxidizing bacterial-specific phylogenetic probe Nso1225 as a primer for fingerprint analysis of ammonia-oxidizer communities. Applied Microbiology and Biotechnology **63:** 715-721.

Chanal, A., V. Chapon, K. Benzerara, M. Barakat, R. Christen, W. Achouak, F. Barras, T. Heulin. 2006. The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. Environmental Microbiology **8:** 514-525.

Clarke, K., and R. Warwick. 2001. Change in marine communities: An approach to statistical analysis and interpretation. 2nd Edition. PRIMER-E: Plymouth

Cottrell, M. T., L. A. Waidner, L. Y. Yu, and D. L. Kirchman. 2005. Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environmental Microbiology **7:** 1883-1895.

Covert, J. S., and M. Moran. 2001. Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. Aquatic Microbial Ecology **25:** 127-139.

Crump, B., E. Armbrust, and J. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Applied Environmental Microbiology **65:** 3192-3204.

Crump, B. C., and J. E. Hobbie. 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnology and Oceanography **50:** 1718-1729.

Crump, B. C., C. S. Hopkinson, M. L. Sogin, and J. E. Hobbie. 2004. Microbial biogeography along an estuarine salinity gradient: Combined influences of bacterial growth and residence time. Applied Environmental Microbiology **70:** 1494-1505.

Eiler, A., and S. Bertilsson. 2004. Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environmental Microbiology **6:** 1228-1243.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package), v.3.5 ed. University of Washington, Seattle.

Ferrari, V. C., and J. T. Hollibaugh. 1999. Distribution of microbial assemblages in the Central Arctic Ocean Basin studied by PCR/DGGE: analysis of a large data set. Hydrobiologia **401:** 55-68.

Fierer, N., J. A. Jackson, R. Vilgalys, and R. B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied Environmental Microbiology **71:** 4117-4120.

Gauthier, T. D. 2001. Detecting trends using Spearman's rank correlation coefficient. Environmental Forensics **2:** 359-362.

Giovannoni, S. J., L. Bibbs, J. C. Cho, M. D. Stapels, R. Desiderio, K. L. Vergin, M. S. Rappe, S. Laney, L. J. Wilhelm, H. J. Tripp, E. J. Mathur, D. F. Barofsky. 2005. Proteorhodopsin in the ubiquitous marine bacterium SAR11. Nature **438:** 82-85.

Glockner, F. and others 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. Applied Environmental Microbiology **66:** 5053-5065.

Hahn, M. W., H. Lunsdorf, Q.L Wu, M. Schauer, M.G. Hofle, J. Boenigk, P. Stadler. 2003. Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia. Applied Environmental Microbiology **69:** 1442-1451.

Hancock, P. J., A. J. Boulton, and W. F. Humphreys. 2005. Aquifers and hyporheic zones: Towards an ecological understanding of groundwater. Hydrogeology Journal **13:** 98-111.

Izaguirre, G. 1992. A copper-tolerant phormidium species from Lake Mathews, California, that produces 2-methylisoborneol and geosmin. Water Science and Technology **25:** 217-223.

Izaguirre, G., and W. D. Taylor. 1998. A pseudanabaena species from Castaic Lake, California, that produces 2-methylisoborneol. Water Research **32:** 1673 - 1677.

Jassby, A. D., and J. E. Cloern. 2000. Organic matter sources and rehabilitation of the Sacramento-San Joaquin Delta (California, USA). Aquatic Conservation-Marine and Freshwater Ecosystems **10:** 323-352.

Jassby, A. D., J. E. Cloern, and B. E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnology and Oceanography **47:** 698-712.

Jensen, S. E., C. L. Anders, L. J. Goatcher, T. Perley, S. Kenefick, and S. E. Hrudey. 1994. Actinomycetes as a factor in odor problems affecting drinking-water from the North Saskatchewan River. Water Research **28:** 1393-1401.

Klausen, C., N. Jorgensen, M. Burford, and M. O'donohue. 2004. Actinomycetes may also produce taste and odour. Water**:** 45-48.

Klausen, C., M. H. Nicolaisen, B. W. Strobel, F. Warnecke, J. L. Nielsen, and N. O. G. Jorgensen. 2005. Abundance of Actinobacteria and production of geosmin and 2-methylisoborneol in Danish streams and fish ponds. FEMS Microbiology Ecology **52:** 265 - 278.

Kraus, T. E. C., B. A. Bergamaschi, B. Downing, and M. S. Fram. 2005. Improving delta drinking water quality: managing sources of disinfection byproduct-forming material in the State Water Project: Draft Final Data Report. Unpublished.

Lanciotti, E., C. Santini, E. Lupi, and D. Burrini. 2003. Actinomycetes, cyanobacteria and algae causing taste and odours in water of the River Arno used for the water supply of Florence. Journal of Water Supply: Research and Technology-Aqua **52:** 489-500.

Lindstrom, E. S., M. P. Kamst-Van Agterveld, and G. Zwart. 2005. Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Applied Environmental Microbiology **71:** 8201-8206.

Lindstrom, E. S., and E. Leskinen. 2002. Do neighboring lakes share common taxa of bacterioplankton? Comparison of 16S rDNA fingerprints and sequences from three geographic regions. Microbial Ecology **44:** 1-9.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Goffman, S. C. Hart, J. W. Harvey, C. A. Johnson, E. Mayorga, W. H. McDowell, G. Pinay. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems **6:** 301-312.

Mullins, T.D., R.L. Krest, S.J. Giovanni. 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnology and Oceanography. **40**: 148-158.

Mummey, D. L., and P. D. Stahl. 2004. Analysis of soil whole- and inner-microaggregate bacterial communities. Microbial Ecology **48:** 41-50.

Myers, R. M., S. G. Fischer, L. S. Lerman, and T. Maniatis. 1985. Nearly all single base substitutions in DNA fragments joined to a GC-clamp can be detected by denaturing gradient gel-rlectrophoresis. Nucleic Acids Research **13:** 3131-3145.

Niemi, R. M., S. Knuth, and K. Lundström. 1982. Actinomycetes and fungi in surface waters and in potable water. Applied Environmental Microbiology **43:** 378-388.

Olapade, O. A., X. Gao, and L. G. Leff. 2005. Abundance of three bacterial populations in selected streams. Microbial Ecology **49:** 461-467.

Omura, S. et al. 2001. Genome sequence of an industrial microorganism Streptomyces avermitilis: Deducing the ability of producing secondary metabolites. Proceedings of the National Academy of Sciences of the United States of America **98:** 12215-12220.

Pernthaler, J. et al. 2001. Predator-specific enrichment of Actinobacteria from a cosmopolitan freshwater clade in mixed continuous culture. Applied and Environmental Microbiology **67:** 2145-2155.

Schauer, M., C. Kamenik, and M. W. Hahn. 2005. Ecological differentiation within a cosmopolitan group of planktonic freshwater bacteria (SOL Cluster, Saprospiraceae, Bacteroidetes). Applied Environmental Microbiology **71:** 5900-5907.

Sekar, R., A. Pernthaler, J. Pernthaler, F. Warnecke, T. Posch, and R. Amann. 2003. An improved protocol for quantification of freshwater Actinobacteria by fluorescence in situ hybridization. Applied and Environmental Microbiology **69:** 2928-2935.

Selje, N., T. Brinkhoff, and M. Simon. 2005. Detection of abundant bacteria in the Weser estuary using culture-dependent and culture-independent approaches. Aquatic Microbial Ecology **39:** 17-34.

Simon, M., H. P. Grossart, B. Schweitzer, and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquatic Microbial Ecology **28:** 175-211.

Singleton, D. R., M. A. Furlong, S. L. Rathbun, and W. B. Whitman. 2001. Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Applied and Environmental Microbiology **67:** 4374-4376.

Spiteller, D., A. Jux, J. Piel, and W. Boland. 2002. Feeding of [5,5-H-2(2)]-1-desoxy-D-xylulose and [4,4,6,6,6-H-2(5)]-mevalolactone to a geosmin-producing Streptomyces sp and Fossombronia pusilla. Phytochemistry **61:** 827-834.

Stackebrandt, E., F. A. Rainey, and N. L. Ward-Rainey. 1997. Proposal for a new hierarchic classification system, Actinobacteria classi s nov. International Journal Systematic Bacteriology **47:** 479-491.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2003. Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquatic Microbial Ecology **31:** 85-98.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, J. T. Hollibaugh. 2005. Sources, bioavailability and photoreactivity of dissolved organic carbon in the Sacramento-San Joaquin River Delta. Biogeochemistry **74:** 131-149.

Sugiura, N., and K. Nakano. 2000. Causative microorganisms for musty odor occurrence in the eutrophic Lake Kasumigaura. Hydrobiologia **434:** 145–150.

Suzuki, M. T., L. T. Taylor, and E. F. Delong. 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. Applied Environmental Microbiology **66:** 4605-4614.

Wanke, M., K. Skorupinska-Tudek, and E. Swiezewska. 2001. Isoprenoid biosynthesis via 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway. Acta Biochimica Polonica **48:** 663-672.

Warnecke, F., R. Amann, and J. Pernthaler. 2004. Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environ Microbiol **6:** 242-253.

Warnecke, F., R. Sommaruga, R. Sekar, J. S. Hofer, and J. Pernthaler. 2005. Abundances, identity, and growth state of Actinobacteria in mountain lakes of different UV transparency. Applied and Environmental Microbiology **71:** 5551-5559.

Watson, R. J., and B. Blackwell. 2000. Purification and characterization of a common soil component which inhibits the polymerase chain reaction. Canadian Journal of Microbiology **46:** 633-642.

Yannarell, A. C., and E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology **71:** 227-239.

Zaitlin, B., and S. B. Watson. 2006. Actinomycetes in relation to taste and odour in drinking water: Myths, tenets and truths. Water Research **40:** 1741-1753.

Zaitlin, B., S. Watson, J. Dixon, and D. Steel. 2003a. Actinomycetes in the Elbow River Basin, Alberta, Canada. Water Quality Research Journal of Canada **38:** 115-125.

Zaitlin, B., S. B. Watson, J. Ridal, T. Satchwill, and D. Parkinson. 2003b. Actinomycetes in Lake Ontario: habitats and geosmin and MIB production. Journal American Water Works Association **95:** 113-118.

Zwart, G., B. C. Crump, M. Agterveld, F. Hagen, and S. K. Han. 2002. Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquatic Microbial Ecology **28:** 141-155.

.

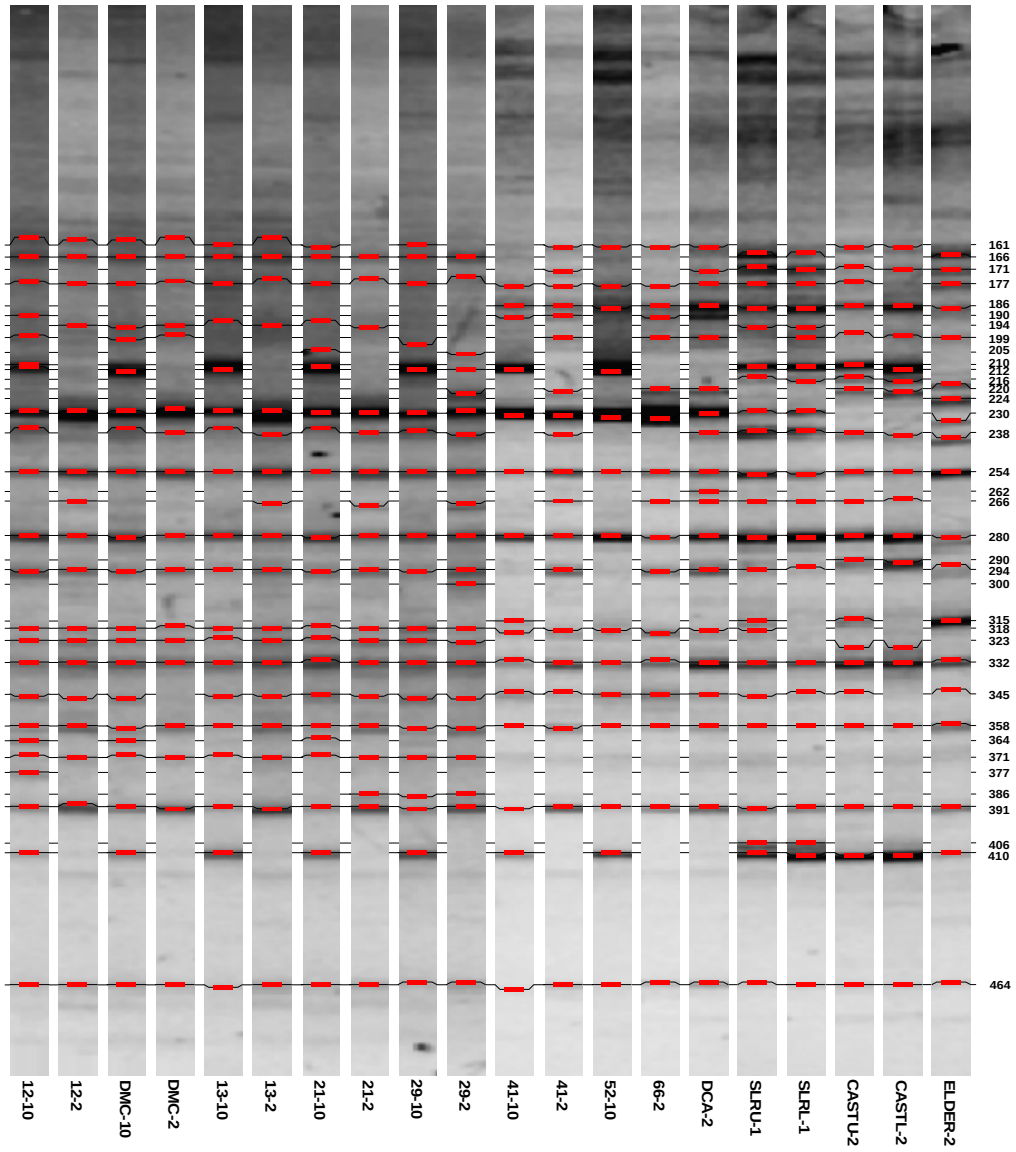

Figure 3.1 Aligned DGGE gel band positions of water samples from the State Water Project. Sample identified as follows (bottom of gel image): Aqueduct samples denoted by station number, including Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA) denoted by location number followed by month sampled: October (10) 2004, January 2005 (-1) and February 2005 (2). Upper San Luis Reservoir (SLRU-1); Lower San Luis Reservoir (SLRL-1); Upper Castaic Lake (CASTU-2); Lower Castaic Lake (CASTL-2); Elderberry Forebay (ELDER-2). Band position numbered along the side of the gel image.

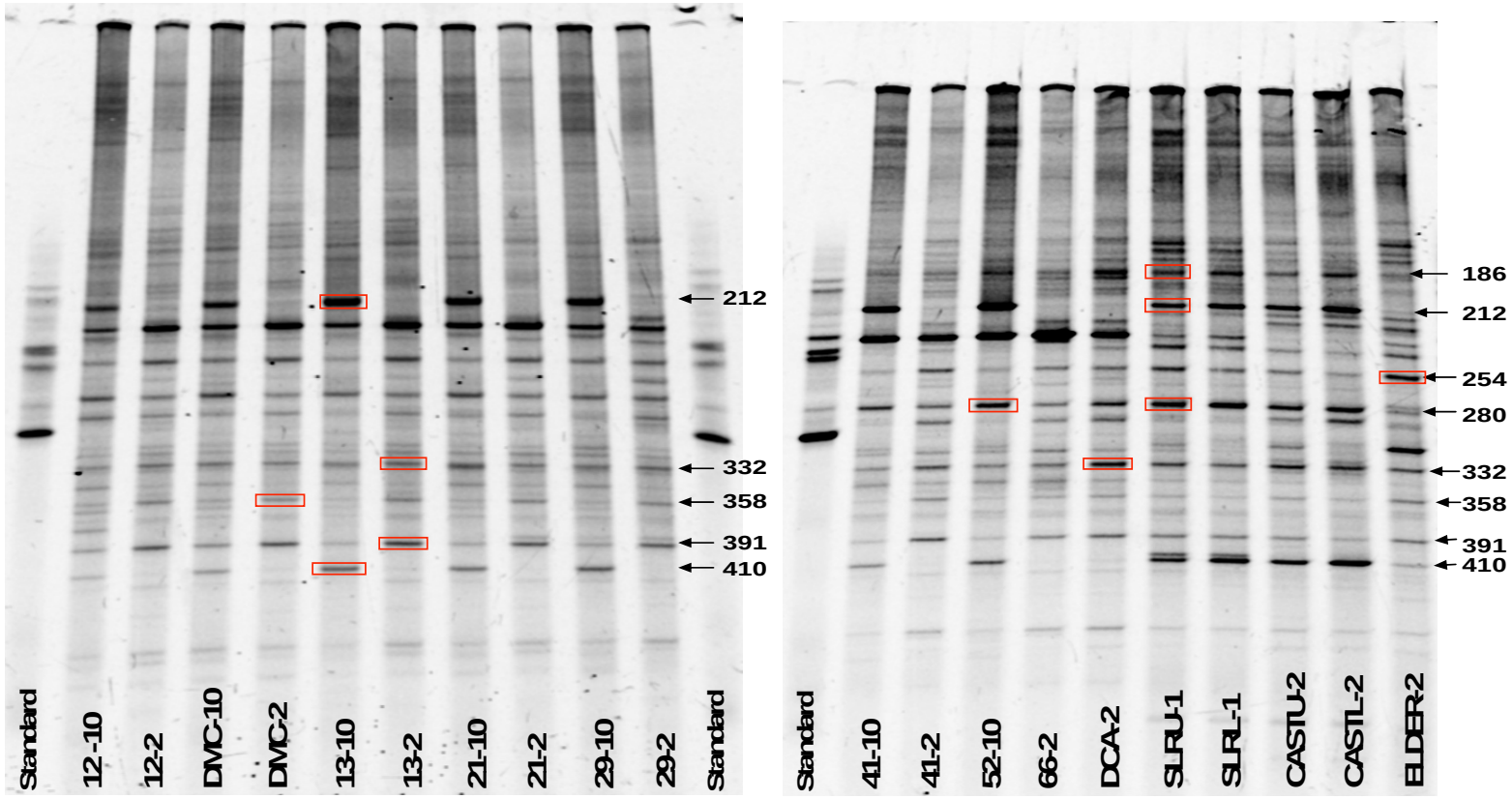

Figure 3.2 DGGE fingerprints of water samples from the State Water Project. Stations named as follows: aqueduct samples denoted by station number, including the Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA) denoted by location number followed by month sampled October (10) 2004, January 2005 (1) and February 2005 (2). Upper San Luis Reservoir (SLRU1); Lower San Luis Reservoir (SLRL-1); Upper Castaic Lake (CASTU-2); Lower Castaic Lake (CASTL-2); Elderberry Forebay (ELDER-2). Sequenced bands surrounded by boxes. Bands numbers (center and right-hand column) correspond to Molecular Analyst band position number (Figure 3.1).

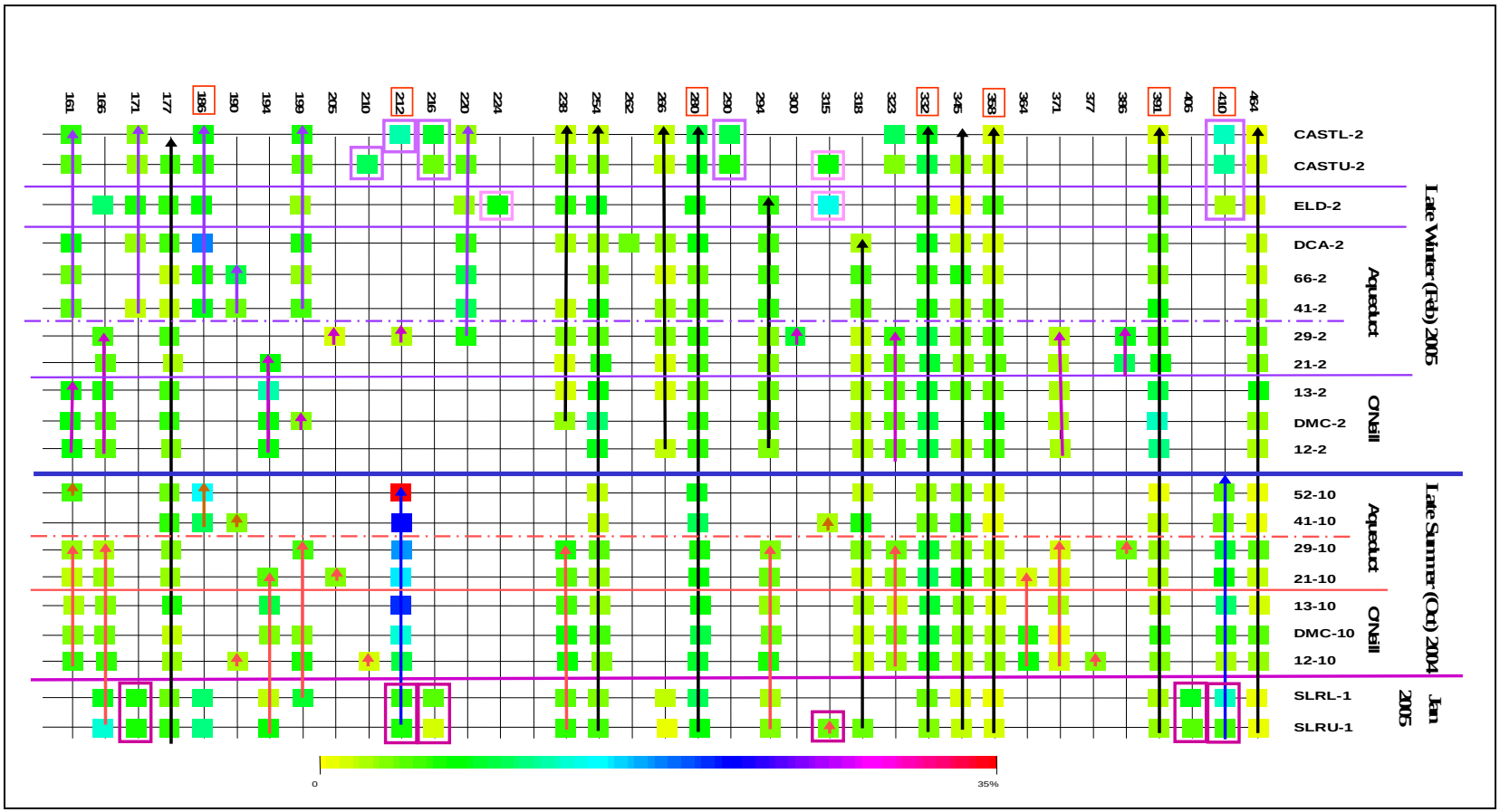

Figure 3.3 DGGE gel band intensity of water samples from the State Water Project. Samples identified as follows: Aqueduct samples denoted by station number, including Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA) denoted by location number followed by month sampled: October (10) 2004, January 2005 (1) and February 2005 (2)Upper San Luis Reservoir (SLRU-1); Lower San Luis Reservoir (SLRL-1); Upper Castaic Lake (CASTU-2); Lower Castaic Lake (CASTL-2); Elderberry Forebay (ELDER-2). DGGE band position (Figure 3.1) numbered along the side of the gel. Band position surrounded by boxes denote sequenced DGGE band. Phylotypes within the band matrix surrounded by boxes are unique to the reservoirs. Dotted lines indicate a distinct change in phylotype assemblage between Check 29 and 41.

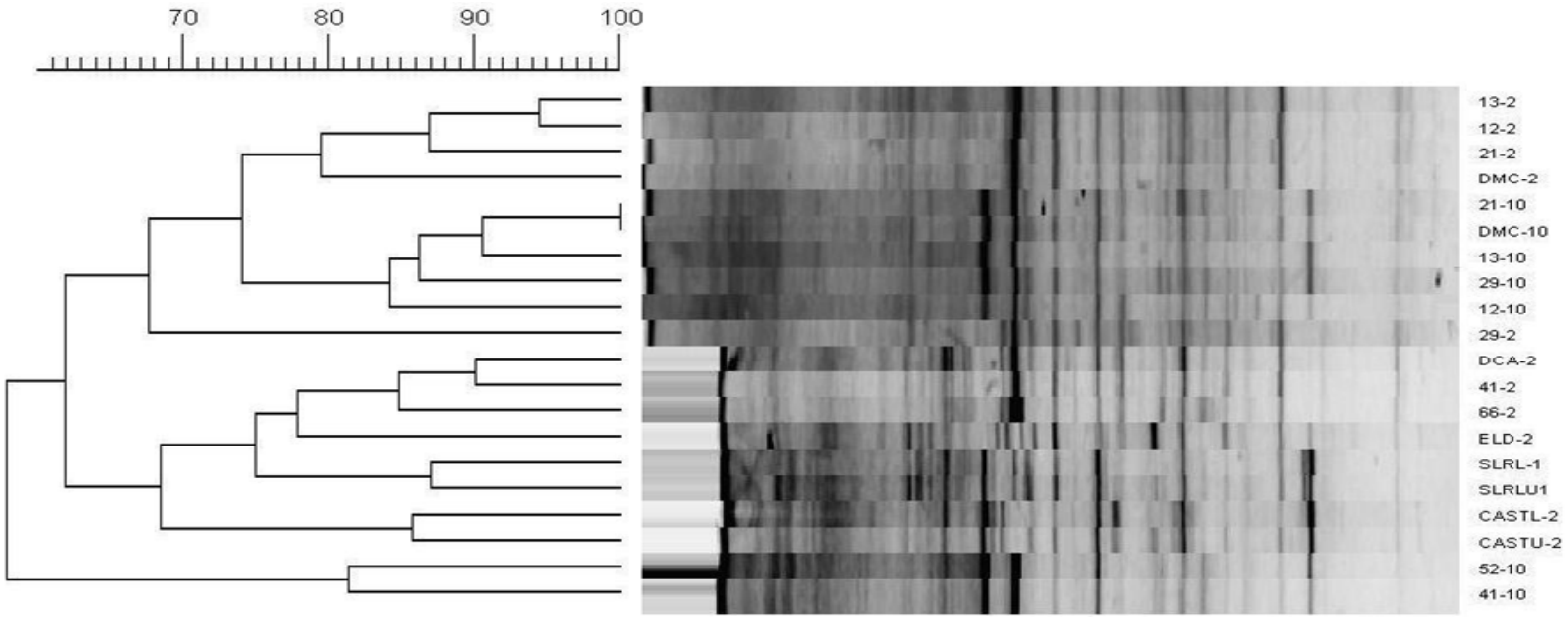

Figure 3.4 Similarity dendogram created by standardizing two DGGE gels containing samples from the State Water Project. Scale: percent similarity of banding patterns derived from cluster analysis. Band positions are shown in Figure 3.1. Samples identified as follows: October (10) 2004, January 2005 (1) and February 2005 (2); Aqueduct samples denoted by station number, including Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA) denoted by location number followed by month sampled; Upper San Luis Reservoir (SLRU-1); Lower San Luis Reservoir (SLRL-1); Upper Castaic Lake (CASTU-2); Lower Castaic Lake (CASTL-2); Elderberry Forebay (ELDER-2).

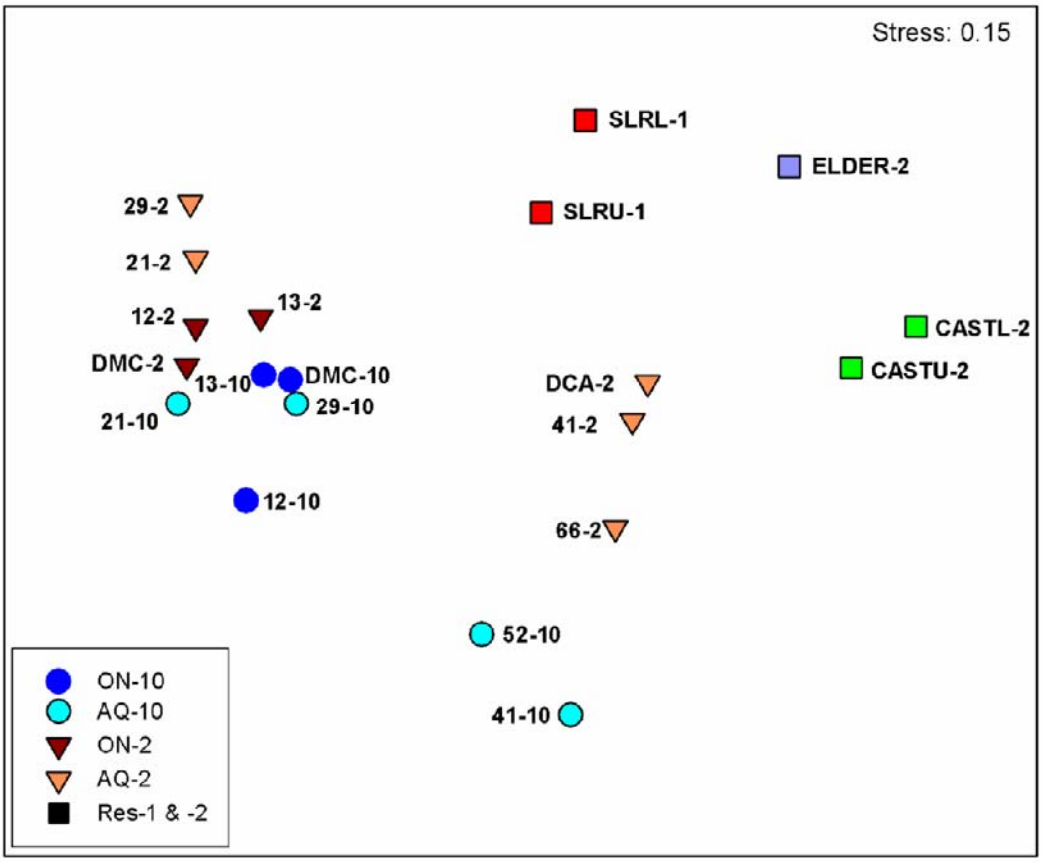

Figure 3.5 Non-metric multi-dimensional scaling (NMS) analysis of DGGE banding patterns, all stations included. Late summer (October) denoted with diamonds; late winter (February) denoted with squares; triangles indicate reservoir samples. O'Neill Forebay (DMC, Check 12 and Check 13); the aqueduct (Check 21, 29, 41, 52/66, DCA); San Luis Reservoir (upper (SLRU-1) and lower (SLRL-1) composites) taken in January; and Castaic Lake (upper (CASTU-2) and lower (CASTL-2) composites) taken in February.

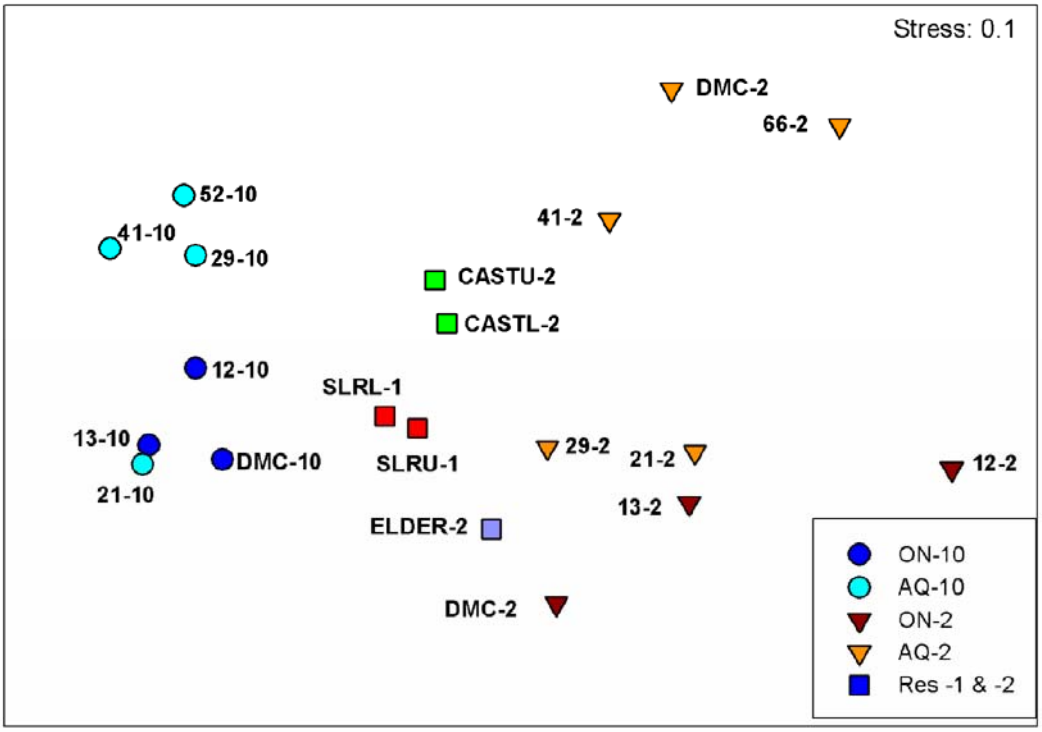

Figure 3.6 Non-metric multidimensional scaling (NMS) analysis of SWP water samples using environmental data available for all samples (T, pH, EC, DO, DOC, BDOC, SUVA). Late summer (October) denoted with diamonds; late winter (February) denoted with squares. O'Neill Forebay (DMC, Check 12 and Check 13); the Aqueduct (Check 21, 29, 41, 52/66, DCA); San Luis Reservoir (upper (SLRU-1) and lower (SLRL-1) composites) taken in January; and Castaic Lake (upper (CASTU-2) and lower (CASTL-2) composites) taken in February.

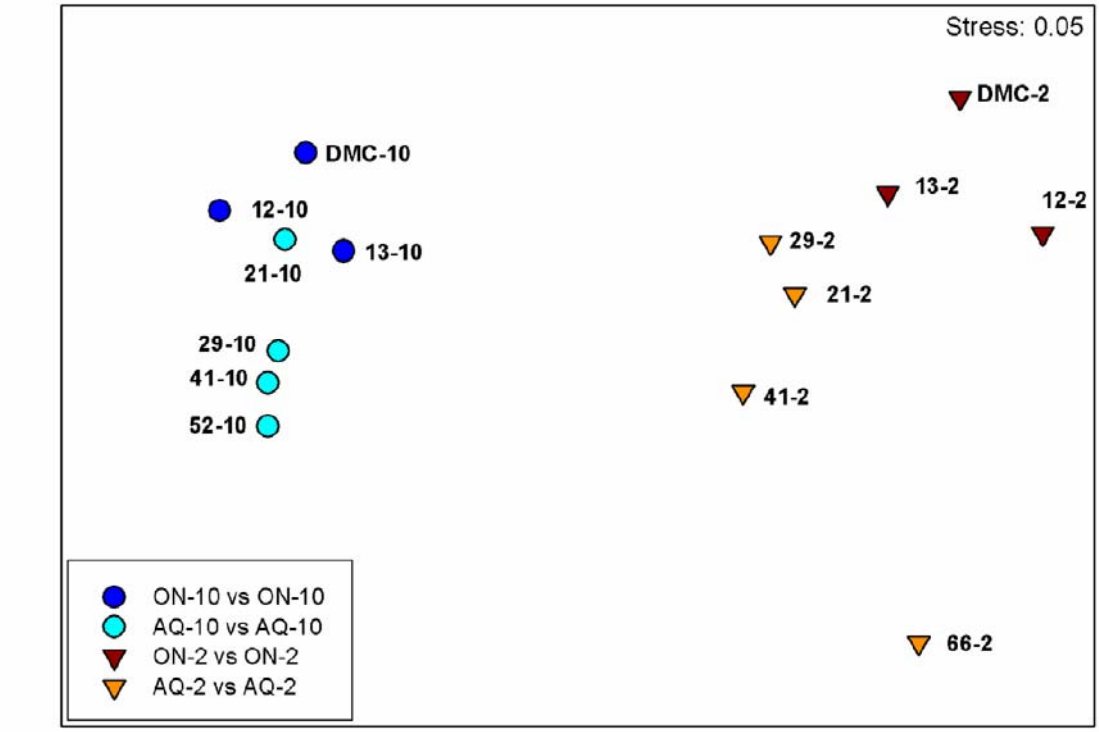

Figure 3.7 Non-metric multidimensional scaling (NMS) analysis of California Aqueduct water samples (DCA excluded) using environmental data. All environmental data included. Late summer (October) denoted with diamonds; late winter (February) denoted with squares. O'Neill Forebay (DMC, Check 12 and Check 13); the Aqueduct (Check 21, 29, 41, 52/66, DCA); San Luis Reservoir (upper (SLRU-1) and lower (SLRL-1) composites) taken in January; and Castaic Lake (upper (CASTU-2) and lower (CASTL-2) composites) taken in February.

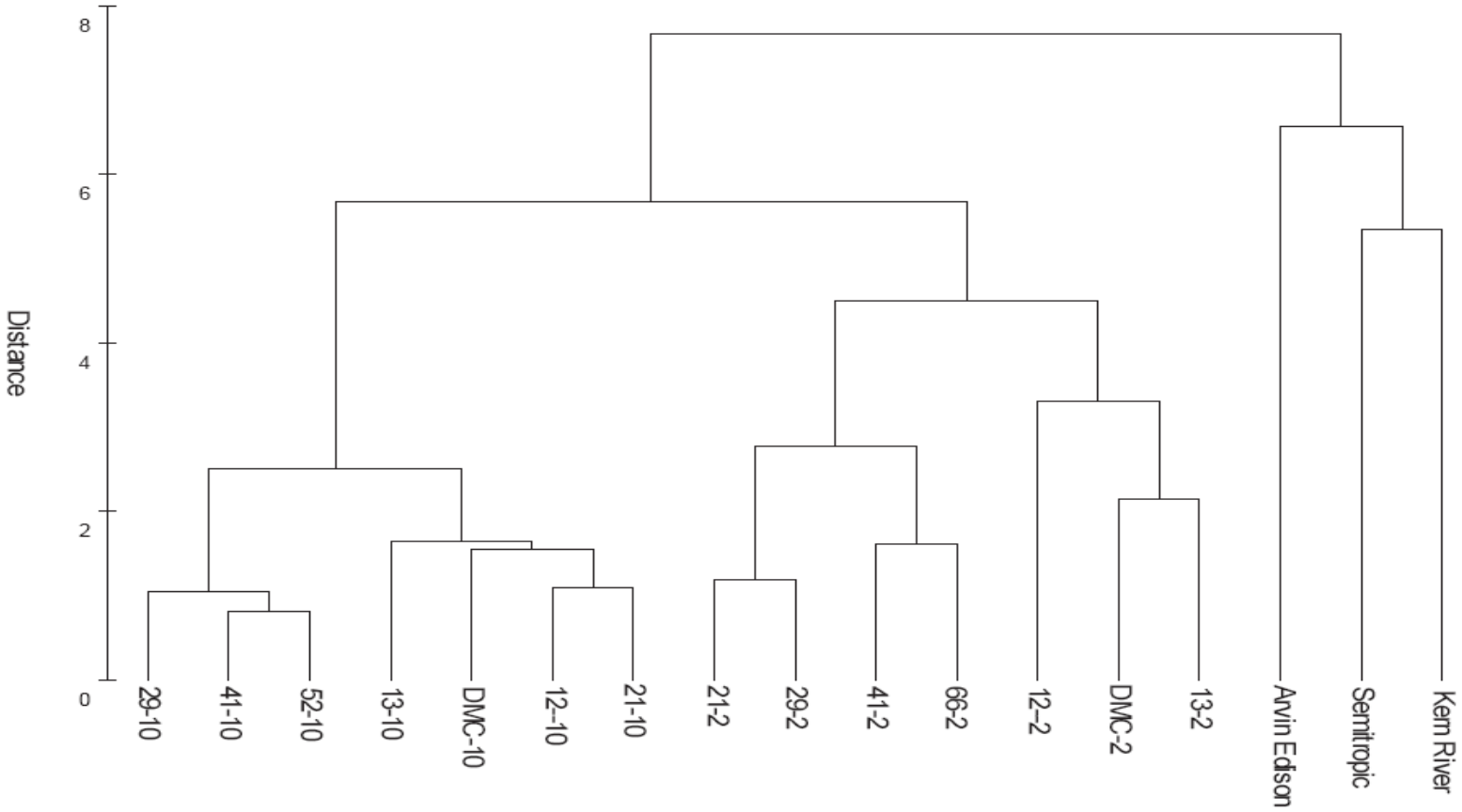

Figure 3.8 Similarity dendogram based on environmental data for California Aqueduct water samples (DCA excluded) and for inflows along the aqueduct (Table 2.6) in October (10) 2004 and February (2) 2005. Dendogram constructed with a normalized-Euclidian matrix with no transformation. Aqueduct sample denoted by station number, including Delta Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA).

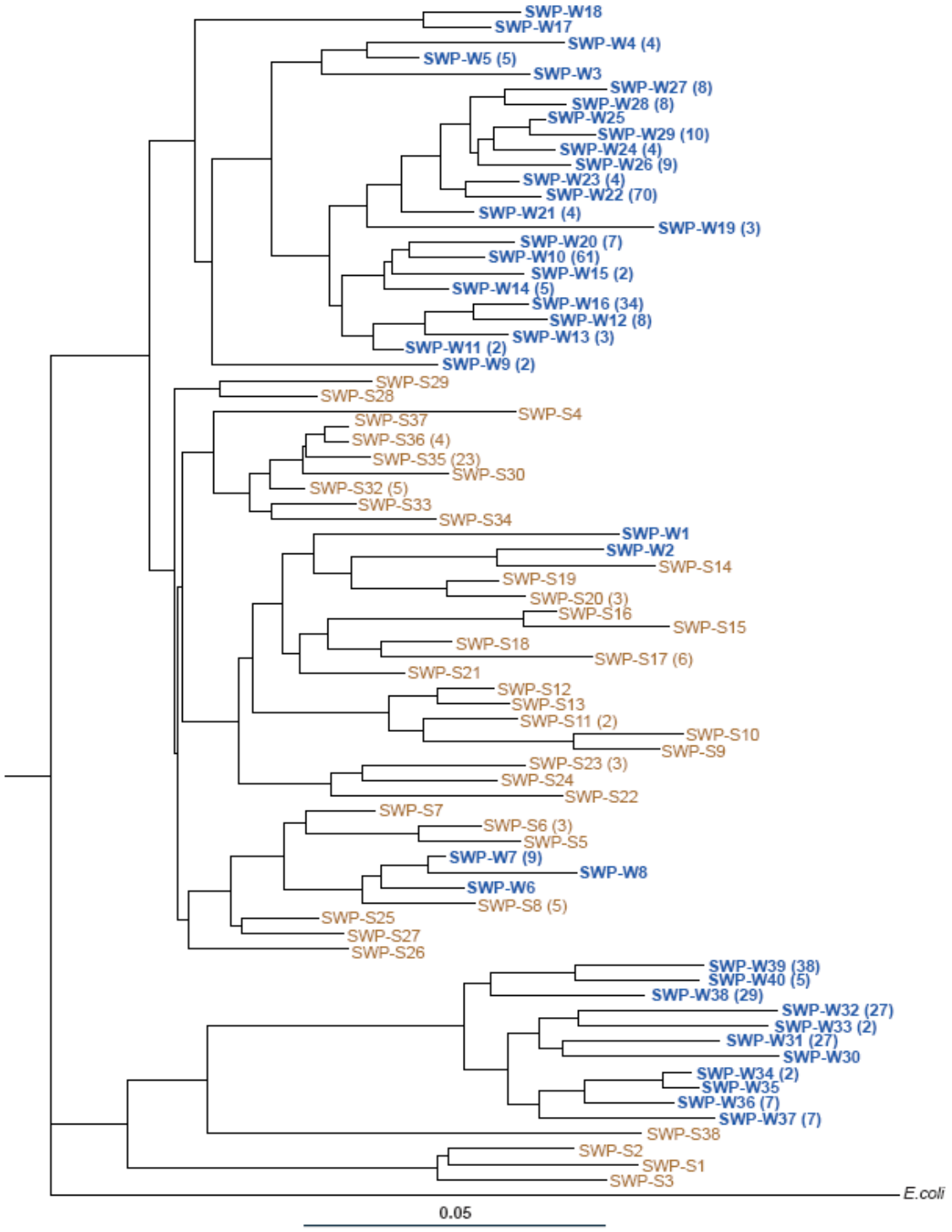

Figure 3.9 Neighbor-joining trees showing phylogenic relationships of representative 16S rRNA sequences (350bp) cloned from State Water Project water and soil samples. The trees are unrooted, with E. coli as the outgroup. Clones from water samples denoted with a 'W' and clones from soil samples denoted with an "S". Number of clones (> 1) in each phylotype in parentheses following clone name.

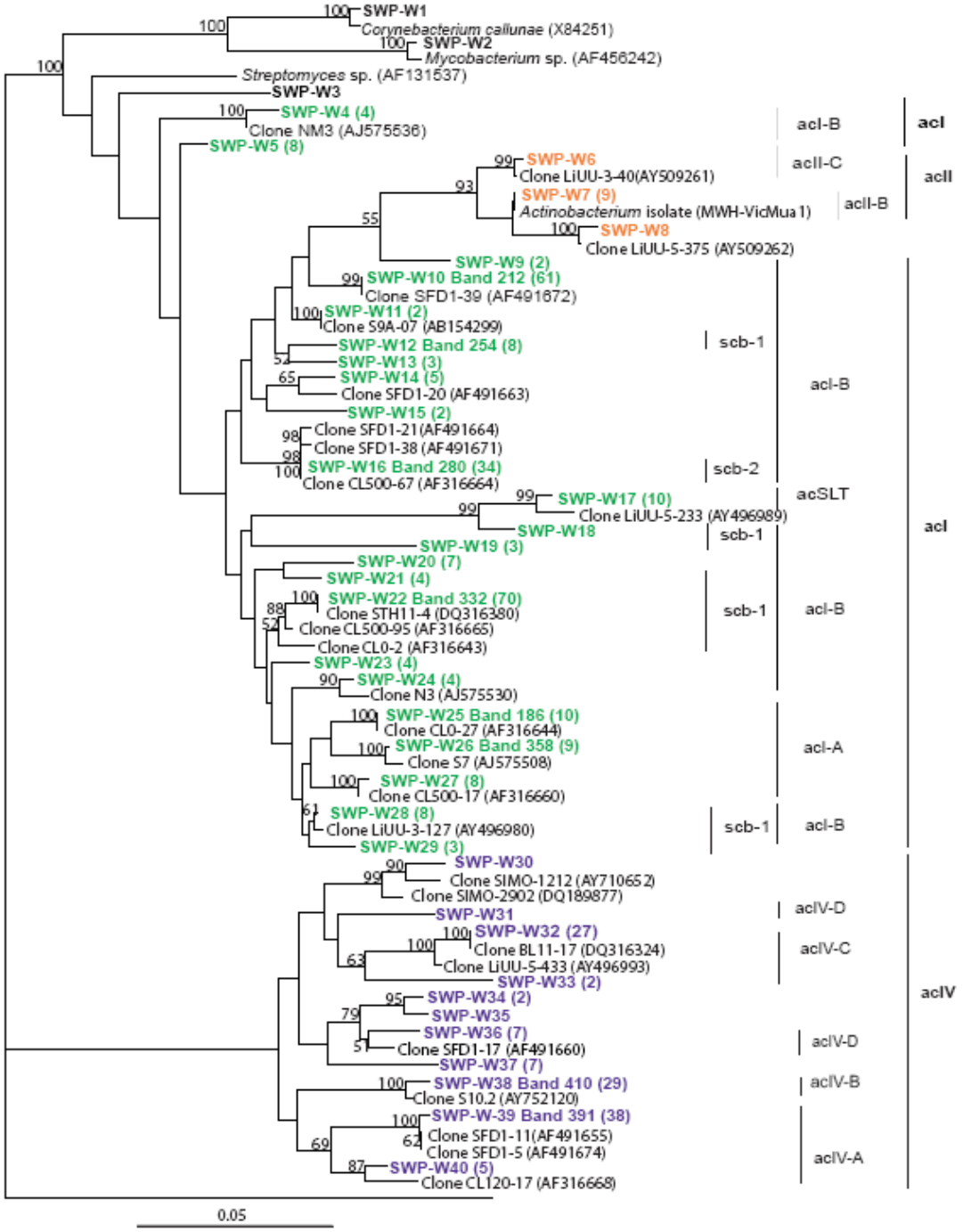

Figure 3.10 Neighbor-joining trees showing phylogenic relationships of representative 16S rRNA sequences cloned from State Water Project water samples (BOLD) to closely related reference sequences from GenBank. The trees are unrooted, with *E. coli* as the outgroup. Number of clones in each phylotype (> 1) in parentheses following clone name.

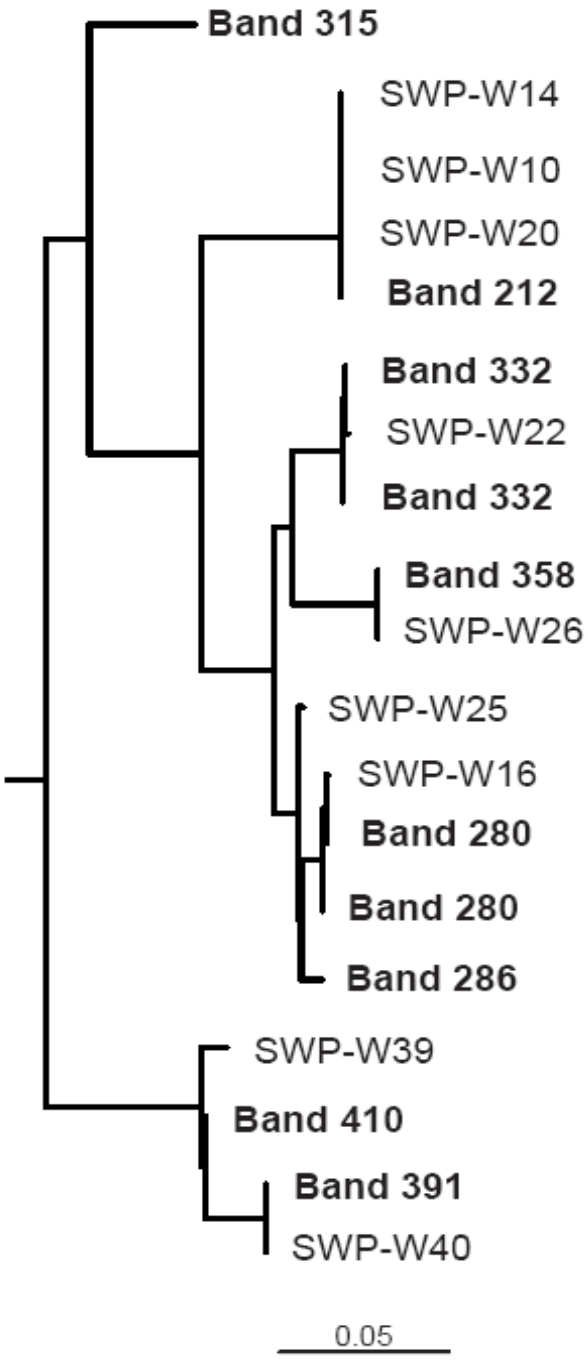

Figure 3.11 Phylogenetic tree showing relationships between sequences obtained from DGGE bands and clone amplicons in water samples from the State Water Project. Band numbers correspond to band position number (Figure 3.1). Phylotype names begin with "W" and correspond to those listed in the phylogenetic tree of SWP water samples (Figure 3.10).

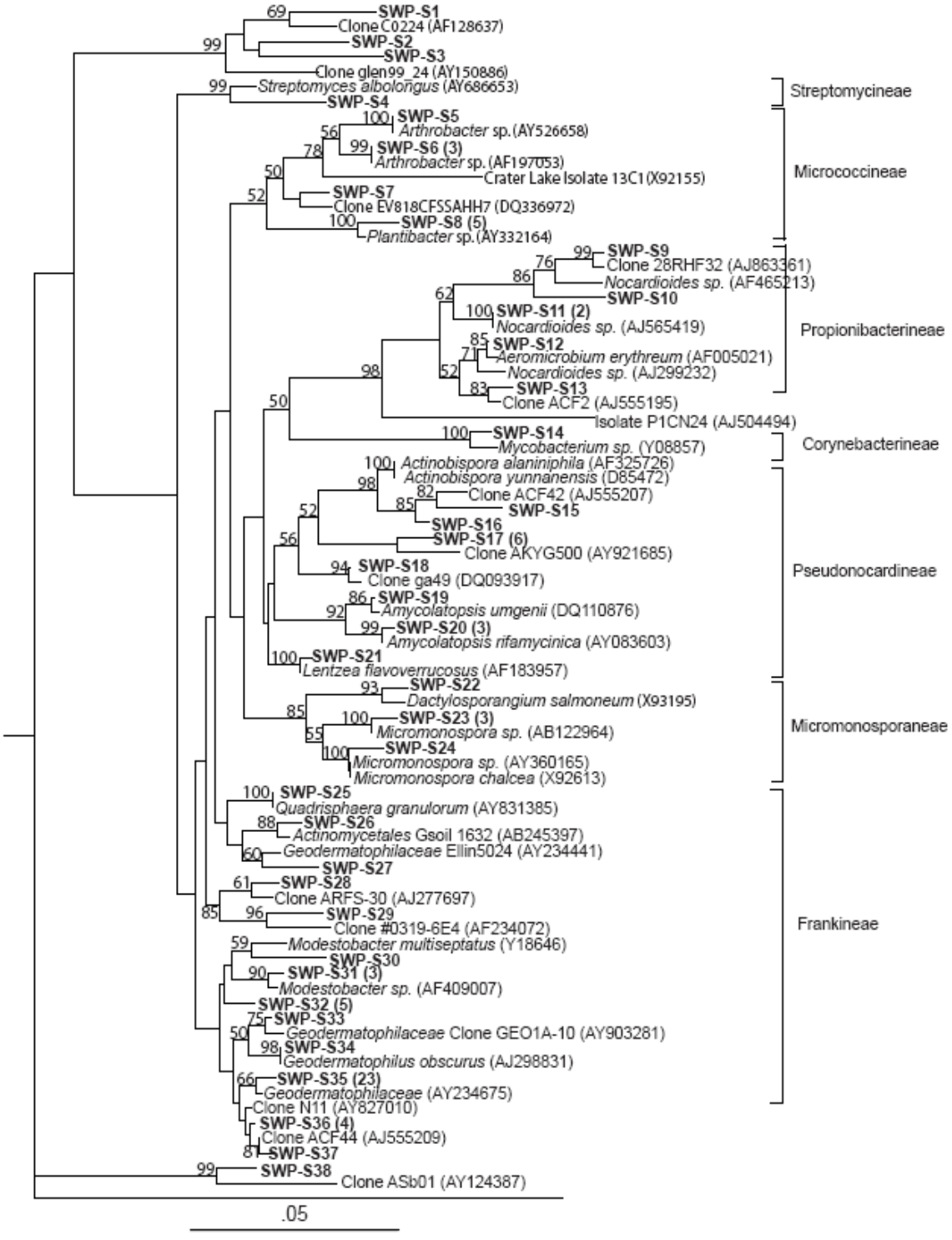

Figure 3.12 Neighbor-joining trees showing phylogenic relationships of representative 16S rRNA sequences cloned from State Water Project soil samples (BOLD) to closely related reference sequences from GenBank. The trees are unrooted, with *E. coli* as the outgroup. Number of clones (> 1) in each phylotype in parentheses following clone name.

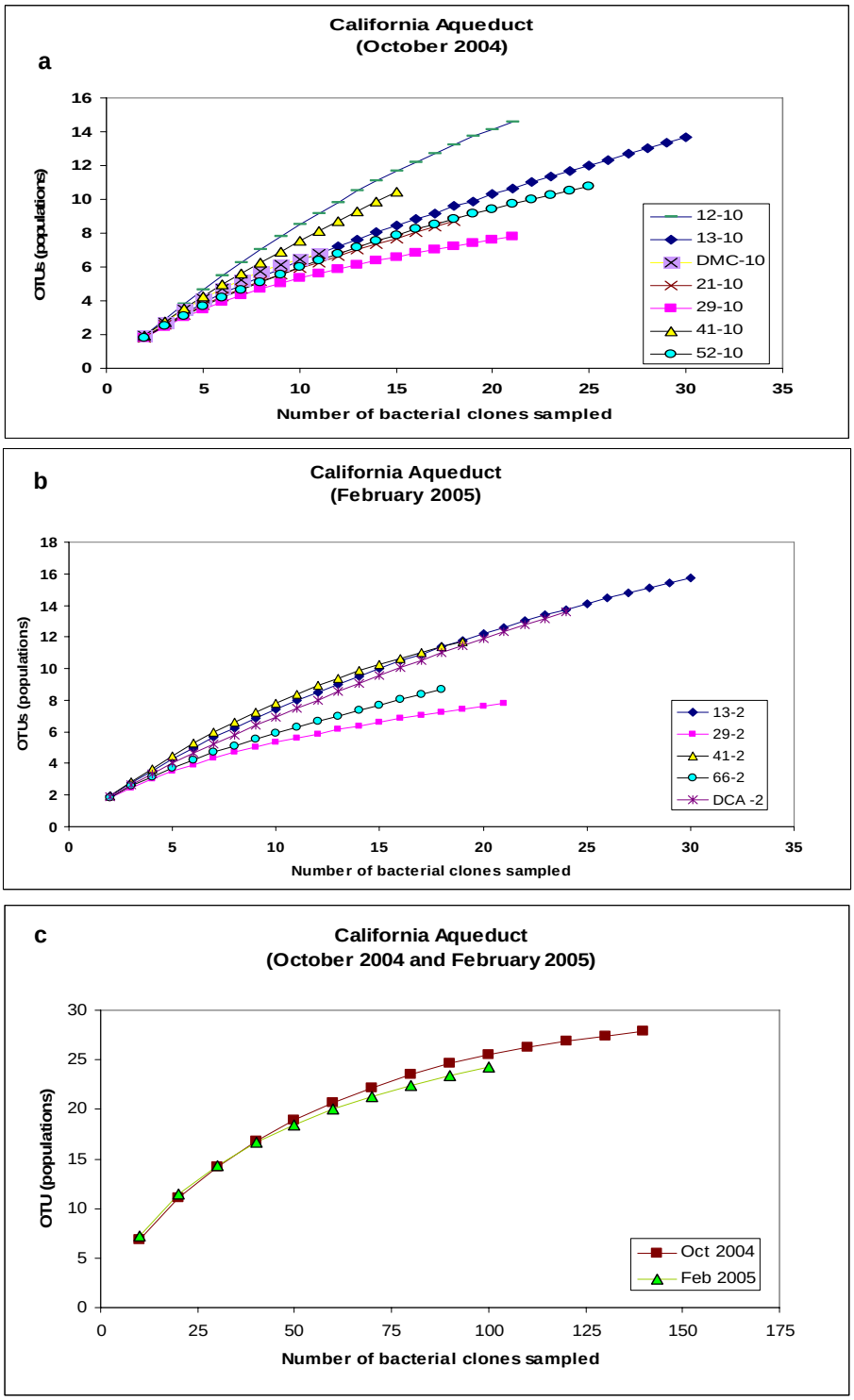

Figure 3.13 Rarefaction curves generated for 16S rRNA genes in clone libraries from water samples collected from the aqueduct of the State Water Project, October 2004 and February 2005: (a) October clone libraries, (b) February clone libraries, (c) combined libraries for October and February. Stations named as follows: Aqueduct samples denoted by station number, including Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA).

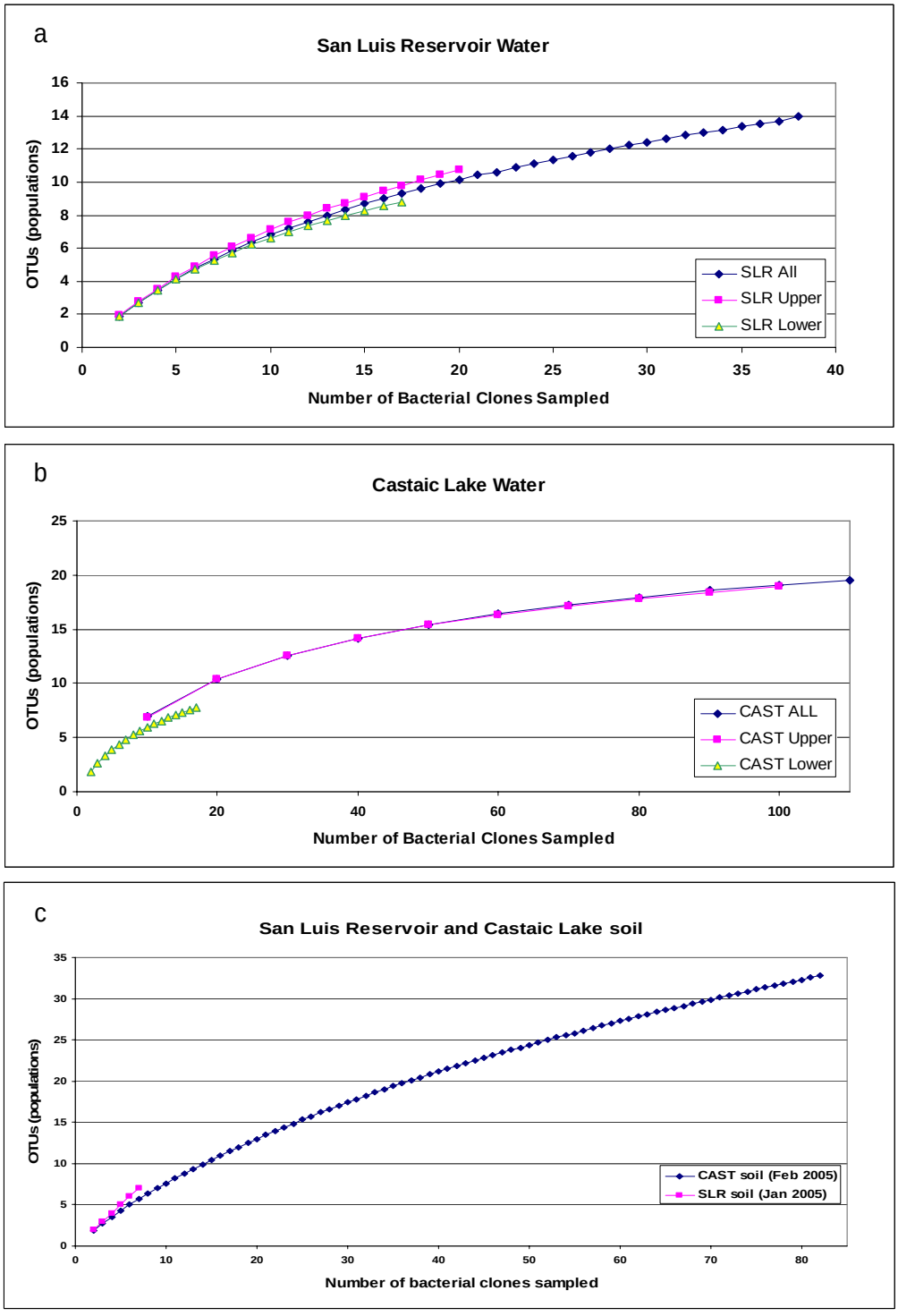

Figure 3.14 Rarefaction curves generated for 16S rRNA genes in clone libraries from water samples collected from upper and lower composites at (a) San Luis Reservoir (SLR; January 2005) and (b) Castaic Lake (CAST; February 2005) and (c) clones from soil samples collected at both reservoirs.

Table 3.1 (a) Total number of distinct DGGE bands (number of phylotypes) per samples (lane) for each water sample as tabulated from band positions from Figure 3.1 and (b) ANOVA significance levels. Total number of *Actinobacteria* phylotypes found in DGGE gels was 36. Dashed line indicates no sample taken. Stations named as follows: Aqueduct samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA); Upper San Luis Reservoir composite (SLRU); Lower San Luis Reservoir (SLRL); Upper Castaic Lake composite (CASTU); Lower Castaic Lake composite (CASTL); Elderberry Forebay (ELDER). O'Neill Forebay cluster (DMC, Check 12 and Check 13); Aqueduct Cluster (Check 21, 29, 41, 52/66, DMC). (b) ANOVA significance levels (*p*) of the absolute numbers of DGGE bands. Significance values ($p \leq 0.05$) are highlighted in bold.

a

	October	**February**
DMC	20	16
Check 12	22	16
Check 13	18	17
Check 21	20	17
Check 29	19	20
Check 41	14	18
Check52/66	13	15
DCA	--	17
Seasonal Mean	**18 ± 1.3**	**17 ± 0.5**
SLRU-1	--	21
SLRL-1	--	19
ELD-2	--	17
CASTU-2	--	20
CASTL-2	--	17
O'Neill Forebay Mean	**20 ± 1.2**	**16.3 ± 0.3**
CA Aqueduct Mean	**16.5 ± 1.4**	**17.5 ± 0.7**

b

Aqueduct (all sites) October *vs*. February	0.488	df = 13	F = 0.51
O'Neill Forebay *vs*. Aqueduct (October)	0.187	df = 6	F = 2.33
O'Neill Forebay *vs*. Aqueduct (February)	0.374	df = 7	F = 5.99
O'Neill Forebay October *vs*. February	**0.038**	df = 5	F = 7.71
Aqueduct October *vs*. February	0.642	df = 7	F = 5.99

Table 3.2 Analysis of similarities (ANOSIM) test using the DGGE band matrix (Bray-Curtis similarity with presence-absence transformation). Locations grouped as follows: O'Neill Forebay (Check 12, 13 and DMC) in October 2004 (ON10) and February 2005 (ON2); aqueduct (Check 21, 29, 41, 52/66, DCA) in October (AQ10) and February (AQ2); San Luis Reservoir (SLR); Castaic Lake (CAST). Significantly similar groups in bold ($R < 0.500$; $p < 0.05$).

	R	p
ON10 vs ON2	0.667	0.100
ON10 vs AQ10	-0.065	0.629
ON10 vs AQ2	**0.456**	**0.036**
ON10 vs SLR	1.000	0.100
ON10 vs CAST	1.000	0.100
ON2 vs AQ10	0.491	0.086
ON2 vs AQ2	0.062	0.357
ON2 vs SLR	1.000	0.100
ON2 vs CAST	1.000	0.100
AQ10 vs AQ2	**0.388**	**0.048**
AQ10 vs SLR	0.518	0.067
AQ10 vs CAST	0.911	0.067
AQ2 vs SLR	0.464	0.095
AQ2 vs CAST	0.509	0.905

Table 3.3 Spearman rank correlation (ρ_s) of the DGGE bands matrix and environmental data matrices of water samples from the aqueduct and reservoirs of the State Water Project. Samples are grouped by location. Number of samples per group (*n*). Critical values of 95 % from Gauthier (2001).Groups are named as follows: (a) Aqueduct All (DCA excluded) includes combined samples from October and February; (b) October aqueduct water samples only; (c) February aqueduct sample only (DCA excluded).

	a Aqueduct All (DCA excl) *n* = 14	**b** Aqueduct (Oct) *n* = 7	**c** Aqueduct (Feb) (DCA excl) *n* = 7
Temp			**0.757**
pH	**0.682**		**0.757**
EC		**0.804**	**0.757**
DOC		**0.804**	
BDOC		**0.804**	
SUVA	**0.682**	**0.804**	
Chl-a		**0.804**	
Ca		**0.804**	
Critical value 95 %	**0.464**	**0.714**	**0.714**

Table 3.4 Quantitative PCR (qPCR) determinations of *Actinobacteria* versus *Bacteria* relative abundance of DNA in water and soil samples taken from the State Water Project in October 2004 and January and February 2005. Relative abundance percentages are mean values with standard deviations. Stations named as follows: Aqueduct samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA); Upper San Luis Reservoir composite (SLRU); Lower San Luis Reservoir (SLRL); Upper Castaic Lake composite (CASTU); Lower Castaic Lake composite (CASTL); Elderberry Forebay (ELDER). For each station: (-) denotes sample not collected; (nv) denotes sample unable to be quantified using qPCR.

	Oct '04	Jan '05	Feb '05
Check 12	**nv**	**-**	**24.6 ± 4.9 %**
DMC	**nv**	**-**	**24.9 ± 17.4 %**
Check13	**29.6 ± 14 %**	**-**	**11.4 ± 15 %**
Check21	**12.7 ± 2.5 %**	**-**	**39.8 ± 13.2 %**
Check29	**12.7 ± 0.9%**	**-**	**11.1 ± 4 %**
Check 41	**33.9 ± 19.2%**	**-**	**27.5 ± 15 %**
Check 52	**12.2 ± 2 %**	**-**	**-**
Check 66	**-**	**-**	**8.9 ± 2.1 %**
DCA	**-**	**-**	**23.0 ± 9.1 %**
SLRU	**-**	**11.2 ± 2.6 %**	**-**
SLRL	**-**	**33.1 ± 3.1 %**	**-**
ELDER	**-**	**-**	**nv**
CASTU	**-**	**-**	**nv**
CASTL	**-**	**-**	**31.8 ± 11.5 %**
SLR soil	**-**	**4.9 ± 0.3 %**	**-**
Cast soil	**-**	**-**	**14.7 ± 6.8 %**

Table 3.5 Number of clones per phylotypes for water samples taken from the aqueduct and reservoirs of the State Water Project in October 2004, and January and February 2005. Phylotype name corresponds to the neighbor joining tree of water samples (Figure 3.10). Stations named as follows: Aqueduct samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon afterbay (DCA); Upper San Luis Reservoir composite (SU); Lower San Luis Reservoir (SL); Upper Castaic Lake composite (CU); Lower Castaic Lake composite (CL).

	SWP-W1	SWP-W2	SWP-W3	SWP-W4	SWP-W5	SWP-W6	SWP-W7	SWP-W8	SWP-W9	SWP-W10	SWP-W11	SWP-W12	SWP-W13	SWP-W14	SWP-W15	SWP-W16	SWP-W17	SWP-W18	SWP-W19	SWP-W20	SWP-W21	SWP-W22	SWP-W23	SWP-W24	SWP-W25	SWP-W26	SWP-W27	SWP-W28	SWP-W29	SWP-W30	SWP-W31	SWP-W32	SWP-W33	SWP-W34	SWP-W35	SWP-W36	SWP-W37	SWP-W38	SWP-W39	SWP-W40	Water Total
12--10				1					1					2	1	2	2	1				2			1		1					3				1		2	1	1	22
DMC-10					1					3												2	2								1				2		1			12
13-10					1		1			11	1		2	1		3	1					5						1			1				1		1	1		31
21-10						1				7						1			1			2									1						1	4		18
29-10							1			9						3						4	1		1						1						2			22
41-10				1						2	1	1		1		1										1	1								1		1	5		16
52-10									1	11		2	1		1	1			1	2		3			1	2														26
SU-1		1					2			3				1		2			1			4				1		1								1	4		21
SL-1										4		1				4						2				1						1					1	2	2	18
13-2	1		1				1	1								4	1			2		8		2	2		2	2	1				1	1	1				31
29-2																	1					2																		3
41-2				1												3	2					2			2	1		2			3		1			1	1		20
66-2					1		1										2					4		1	1	1			1							7			19
DCA-2				1	1					1						2	1					9		1	1	1	1	2	1		1					2			25
CU-2					3		3			7		4				6				3	4	21			1	1	2			1	10	2			1	7	9	16	1	102
CL-2					1					3						2							1				1				5						4	1	18
Total	1	1	1	4	8	1	9	1	2	61	2	8	3	5	2	34	10	1	3	7	4	70	4	4	10	9	8	8	3	1	1	27	2	2	1	7	7	29	38	5	404

Table 3.6 Number of clones per phylotypes from soil samples taken from San Luis Reservoir (SLR; January 2005) and Castaic Lake (CAST; February 2005).Phylotype names correspond to the neighbor joining tree of soil samples (Figure 3.11).

	SWP-S1	SWP-S2	SWP-S3	SWP-S4	SWP-S5	SWP-S6	SWP-S7	SWP-S8	SWP-S9	SWP-S10	SWP-S11	SWP-S12	SWP-S13	SWP-S14	SWP-S15	SWP-S16	SWP-S17	SWP-S18	SWP-S19	SWP-S20	SWP-S21	SWP-S22	SWP-S23	SWP-S24	SWP-S25	SWP-S26	SWP-S27	SWP-S28	SWP-S29	SWP-S30	SWP-S31	SWP-S32	SWP-S33	SWP-S34	SWP-S35	SWP-S36	SWP-S37	SWP-S38	Total
CAST	1		1	1	1	2	1	5	1		2	1	1	1	1	2	6	1		3	3	1	1	3	1	2	1	1		1	3	5	1	2	22	4	1		83
SLR		1				1	1			1									1										1							1			1	8
Total	1	1	1	1	1	3	2	5	1	1	2	1	1	1	1	2	6	1	1	3	3	1	1	3	1	2	1	1	1	1	3	5	1	2	23	4	1	1	91

Table 3.7 Coverage values ($C = 1-(n_1/N)$) calculated for SWP clone libraries of SWP water and soil samples (Table 3.5 and 3.6) with n_1 the number of phylotypes that occurred only once in the clone library and N the number of total clones in the library. Stations named as follows: Aqueduct samples collected at Checks, Delta-Mendota Canal (DMC) and Devil's Canyon Afterbay (DCA); Upper San Luis Reservoir composite (SLRU); Lower San Luis Reservoir (SLRL); Upper Castaic Lake composite (CASTU); Lower Castaic Lake composite (CASTL); Elderberry Forebay (ELDER).

Site	Clones in library	Coverage
Check 12--10	22	59 %
DMC-10	12	75 %
Check 13-10	31	68 %
Check 21-10	18	72 %
Check 29-10	22	82 %
Check 41-10	16	44 %
Check 52-10	26	77 %
Check 13-2	31	71 %
Check 29-2	3	67 %
Check 41-2	20	70 %
Check 66-2	19	68 %
DCA-2	25	60 %
SLRU-1	21	71 %
SLRL-1	18	78 %
CASTU-2	102	95 %
CASTL-2	18	78 %
Total	404	98 %
October (all)	147	69 %
February (all)	98	67 %
SLR (all)	39	74 %
CAST (all)	120	93 %
SLR soil	8	0 %
CAST soil	91	77 %

Table 3.8 LIBSHUFF analysis of clone libraries constructed from water and soil samples from the aqueduct and reservoirs of the State Water Project. Samples are grouped by location. Groups are named as follows: O'Neill Forebay (DMC, Check 12 and 13) in October 2004 (ON10) and February 2005 (ON2); California Aqueduct (Check 21, 29, 41, 52/66) in October (AQ10) and February (AQ2); San Luis Reservoir (SLR); Castaic Lake (CAST); Composites of upper and lower depths from San Luis Reservoir and Castaic Lake, respectively (SLRU, SLRL; CASTU, CASTL), San Luis Reservoir soil (SLR soil): Castaic Lake soil (CAST soil) Significantly similar groups (R > 0.005) denoted in bold text.

	AQ2	**ON2**	**AQ10**	**ON10**	**SLR**	**CAST**
ON10	0.001	0.001	**0.475**	--	**0.059**	0.001
AQ10	0.001	0.002	--	--	**0.509**	0.001
ON2	**0.029**	--	--	0.001	0.001	0.001
AQ2	--	--	--	--	0.001	0.001
SLR soil	--	--	--	--	0.001	--
CAST soil	--	--	--	--	--	0.001
SLRU vs SLRL		**0.390**	--	--	--	--
CASTU vs CASTL		**0.079**	--	--	--	--
SLR vs CAST		**0.618**	--	--	--	--

CHAPTER 4

SUMMARY

4.1 Summary

The California State Water Project (SWP) is a highly managed system of reservoirs and aqueducts designed to transport water to Central and Southern California from the Sacramento-San Joaquin Delta. Much of the water is used for irrigation; however, SWP managers distribute SWP water to over 20 million people for use as drinking water. Water from the Delta contains high concentrations of dissolved organic carbon (DOC; Jassby and Cloern 2000) and a high abundance of *Actinobacteria* (Stepanauskas et al. 2003). This combination may lead to problems at the treatment plant: formation of disinfectant by-products caused by the reaction of DOC with disinfectants (Bergamaschi et al. 1999) and undesirable tastes and odors resulting from *Actinobacteria* secondary metabolites (Jensen et al. 1994; Klausen et al. 2004; Sugiura and Nakano 2000; Zaitlin et al. 2003b). Long-term exposure to disinfection byproducts have been linked to bladder cancer (Koivusalo et al. 1997) and other health concerns.

Bacterial degradation and photooxidation of DOC may reduce the potential for the formation of disinfectant by-products. Photolysis is an important process in this regard because it can break bonds in high molecular weight (HMW) DOC polymers, converting them into labile, low molecular weight (LMW) compounds (Miller et al. 2002; Moran and Zepp 1997). These photolysis products may be more suitable substrates for bacterial growth. Conversely, exposure to UV radiation may cause condensation or polymerization reactions within the LMW fraction of the DOC pool making it increasingly recalcitrant

(Tranvik and Kokalj 1998) and potentially decreasing the fraction of DOC substrate available for immediate bacterial utilization. The net effect of this process on disinfectant by-product formation potential is not known and is one of the goals of the broader study to which this thesis contributes.

Actinobacteria have been reported to represent 5 - 32 % of the bacterial community in Delta waters (Stepanauskas et al. 2003). Some strains of *Actinobacteria* cause taste and odor problems in drinking water (Klausen et al. 2004; Zaitlin et al. 2003b). The effects of *Actinobacteria* on drinking water quality in the SWP are of prime concern to agencies that distribute SWP water for use as drinking water.

Bioavailability of dissolved organic carbon

This study examined DOC bioavailability in the SWP, prior to and following photoexposure. Water samples were collected from the main branch of the California Aqueduct and in three reservoirs; San Luis Reservoir, Castaic Lake and Elderberry Forebay. In addition, DOC bioavailability was determined in samples of water being pumped from submerged, peaty farm land in Jones Tract. The flooding of Jones Tract following a levee breach gave rise to concerns that this and anticipated future breaches, both planned as restoration measures and accidental as in the case of Jones Tract, would adversely affect the quality of water entering the SWP.

Mean DOC bioavailability was found to be low in both the California Aqueduct (3.4 ± 0.4 %), the reservoirs (4.4 ± 0.9 %). The effect of simulated solar irradiation on DOC bioavailability was minimal in California Aqueduct (0.7 ± 1.7 %) and Jones Tract samples (0.1 ± 2.1 %). The effect of exposure to simulated solar irradiance was greater in the reservoirs (2.7 ± 5.7 %). However, higher bioavailability in the reservoirs did not

appear to contribute to an increase in bioavailability in the aqueduct in the summer months when water was being released from San Luis Reservoir. Furthermore, photooxidation did not appear to have an affect on the lability of DOC in Jones Tract samples, as the long residence time of water in Jones Tract appeared to result in decreased bioavailability during the 4 month pump out, from a mean of 12 % in July to 4 % in November.

There was a general trend of decreasing effect of irradiation with increasing initial bioavailability across all SWP locations throughout the year and also in Jones Tract samples. This agreed with a similar trend reported in a study of the Sacramento-San Joaquin Delta by Stepanauskas et al. (2005).

Low DOC bioavailability and minimal increase in bioavailability following photoexposure of SWP and Jones Tract water suggests that the majority of the biologically refractory DOC in the SWP that may have a higher propensity to form DBPs may not photooxidized by UV. Consequently, this DOC may not be removed by natural processes occurring in the distribution system prior to delivery to drinking water treatment plants. Changes in management practices or habitat-restoration projects undertaken in the Delta thus may have a direct effect on the quality and concentration of carbon delivered to consumers by the SWP.

Dynamics of *Actinobacteria*

This study also examined dynamics of *Actinobacteria* in the SWP. *Actinobacteria* have been shown to cause taste and odor problems in drinking water through the formation of secondary odorous secondary metabolites, notably the compounds geosmin and/or 2-methylisoborneol (MIB; Jensen et al. 1994; Klausen et al. 2004; Sugiura and

Nakano 2000; Zaitlin et al. 2003b). Others have reported that problematic *Actinobacteria* strains enter aquatic systems from terrestrial runoff (Niemi et al. 1982) in addition to being abundant in sediment (Sugiura and Nakano 2000) and aggregating on suspended particles (Crump et al. 1999; Zaitlin et al. 2003a). We sought to characterize aquatic *Actinobacteria* assemblages, to determine their origin and fate, and to identify species that might cause taste and odor problems.

We found *Actinobacteria* to be abundant in the SWP, with the average relative abundance ranging from 9 – 40 % (mean, 24 %). SWP phylotypes are related to *Actinobacteria* previously reported in the Sacramento-San Joaquin Delta and to previously classified, globally distributed, freshwater *Actinobacteria*. Factors potentially contributing to changes in the *Actinobacteria* assemblage include temperature, DOC characteristics (DOC concentration and bioavailability, chlorophyll-a, SUVA), pH, conductivity and residence time in the reservoirs. Undetermined processes occurring along the California Aqueduct between Check 29 and Check 41 may be contributing to a shift in the *Actinobacteria* assemblage as SWP water flows southward. In addition, *Actinobacteria* in SWP water samples are not related to *Actinobacteria* residing in the soil of the catchments.

Whether these aquatic strains of *Actinobacteria* in the State Water Project or elsewhere have the ability to produce musty taste and odor compounds is unknown; however, several lines of evidence suggest an important role for them. Previous culture-based studies (Jensen et al. 1994; Klausen et al. 2005; Laniciotti et al. 2003) and mass spectroscopic analyses (Bruce et al. 2002) demonstrated the production of these compounds by *Actinobacteria* in cultures and their presence in the aquatic environments.

The phylotypes we encountered in the SWP were related to strains that produce geosmin and MIB, but we did not recover phylotypes matching these isolates. *Actinobacteria* are difficult to culture and it was not within the scope of this project to test SWP isolates for geosmin and MIB production.

Future Directions

Isolation of freshwater *Actinobacteria* should be encouraged to provide representative strains that can be used to determine their ecological role in the environment. Also, research that focuses on the development of molecular techniques targeting the pathways responsible for production of undesirable secondary metabolites (Omura et al. 2001; Wanke et al 2001; Spiteller et al. 2002) in these free-living, uncultivated *Actinobacteria* strains should also be encouraged, with the ability to produce geosmin/MIB potentially lost through repetitive subculturing (Zaitlin et al. 2006). Within the SWP, the apparent shift in the structure of the *Actinobacteria* assemblage occurring near Check 41 and in the reservoirs should be investigated. These studies would include further DNA analysis, additional sampling to characterize inflows to the California Aqueduct, measurement of additional microbiologically relevant variables (for example, specific fractions of DOC) and determining the changes in biogeochemistry within the system. Also research to identify the source of DOC used by *Actinobacteria* in the SWP (Schrader and Blevins 2001), whether it is derived from Delta DOC or from *in situ* production by phytoplankton and/or periphyton, would provide knowledge useful to managers seeking to optimize SWP water quality.

4.2 References

Bergamaschi, B. A., M. S. Fram, C. Kendall, S. R. Silva, G. R. Aiken, and R. Fujii. 1999. Carbon isotopic constraints on the contribution of plant material to the natural precursors of trihalomethanes. Organic Geochemistry **30:** 835-842.

Bruce, D., P. Westerhoff, and A. Brawley-Chesworth. 2002. Removal of 2-methylisborneol and geosmin in surface water treatment plant in Arizona. Journal of Water Supply: Research and Technology **51:** 183-197.

Crump, B., E. Armbrust, and J. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River,its estuary, and the adjacent coastal ocean. Applied Environmental Microbiology. **65:** 3192-3204.

Jassby, A. D., and J. E. Cloern. 2000. Organic matter sources and rehabilitation of the Sacramento-San Joaquin Delta (California, USA). Aquatic Conservation-Marine and Freshwater Ecosystems **10:** 323-352.

Jensen, S. E., C. L. Anders, L. J. Goatcher, T. Perley, S. Kenefick, and S. E. Hrudey. 1994. Actinomycetes as a factor in odor problems affecting drinking-water from the North Saskatchewan River. Water Research **28:** 1393-1401.

Klausen, C., N. Jorgensen, M. Burford, and M. O'donohue. 2004. Actinomycetes may also produce taste and odour. Water**:** 45-48.

Klausen, C., M. H. Nicolaisen, B. W. Strobel, F. Warnecke, J. L. Nielsen, and N. O. G. Jorgensen. 2005. Abundance of actinobacteria and production of geosmin and 2-methylisoborneol in Danish streams and fish ponds. FEMS Microbiology Ecology **52:** 265.

Koivusalo, M., E. Pukkala, T. Vartiainen, J. J. K. Jaakkola, and T. Hakulinen. 1997. Drinking water chlorination and cancer: a historical cohort study in Finland. Cancer Causes and Control **8:** 192 - 200.

Laniciotti, E., C. Santini, E. Lupi, and D. Burrini. 2003. Actinomycetes, cyanobacteria and algae causing taste and odours in water of the River Arno used for the water supply of Florence. Journal of Water Supply: Research and Technology-Aqua **52.7:** 489-500.

Miller, W. L., M. A. Moran, W. M. Sheldon, R. G. Zepp, and S. Opsahl. 2002. Determination of apparent quantum yield spectra for the formation of biologically labile photoproducts. Limnology and Oceanography **47:** 343-352.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnology and Oceanography **42:** 1307-1316.

Niemi, R. M., S. Knuth, and K. Lundström. 1982. Actinomycetes and fungi in surface waters and in potable water. Applied Environmental Microbiology **43:** 378-388.

Omura, S. and others 2001. Genome sequence of an industrial microorganism Streptomyces avermitilis: Deducing the ability of producing secondary metabolites. Proceedings of the National Academy of Sciences of the United States of America **98:** 12215-12220.

Schrader, K. K., and W. T. Blevins. 1999. Effects of selected environmental conditions on biomass and geosmin production by Streptomyces halstedii. Journal of Microbiology **37:** 159-167

Spiteller, D., A. Jux, J. Piel, and W. Boland. 2002. Feeding of [5,5-H-2(2)]-1-desoxy-D-xylulose and [4,4,6,6,6-H-2(5)]-mevalolactone to a geosmin-producing Streptomyces sp and Fossombronia pusilla. Phytochemistry **61:** 827-834.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2003. Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquatic Microbial Ecology **31:** 85-98.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, J. T. Hollibaugh. 2005. Sources, bioavailability and photoreactivity of dissolved organic carbon in the Sacramento-San Joaquin River Delta. Biogeochemistry **74:** 131-149.

Sugiura, N., and K. Nakano. 2000. Causative microorganisms for musty odor occurrence in the eutrophic Lake Kasumigaura. Hydrobiologia **434:** 145–150.

Tranvik, L. J., and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquatic Microbial Ecology **14:** 301-307.

Wanke, M., K. Skorupinska-Tudek, and E. Swiezewska. 2001. Isoprenoid biosynthesis via 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway. Acta Biochimica Polonica **48:** 663-672.

Zaitlin, B., S. Watson, J. Dixon, and D. Steel. 2003a. Actinomycetes in the Elbow River Basin, Alberta, Canada. Water Quality Research Journal of Canada **38:** 115-125.

Zaitlin, B., S. B. Watson, J. Ridal, T. Satchwill, and D. Parkinson. 2003b. Actinomycetes in Lake Ontario: habitats and geosmin and MIB production. Journal American Water Works Association **95:** 113-118.

Zaitlin, B., and S. B. Watson. 2006. Actinomycetes in relation to taste and odour in drinking water: Myths, tenets and truths. Water Research **40:** 1741-1753.