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Characterization of the *Microcystis* Bloom and Its Nitrogen Supply in San Francisco Estuary Using Stable Isotopes

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Abstract A suite of particulate and dissolved organic and inorganic stable isotopes were needed to determine the source of the nutrients and cells that initiate and sustain the toxic cyanobacteria bloom of *Microcystis* in San Francisco Estuary. Particulate and dissolved inorganic and organic matter in water and plankton samples were collected biweekly during *Microcystis* blooms in 2007 and 2008. Stable isotopes for particulate and dissolved organic matter, nitrate, and water (POM-δ^{13}C, POM-δ^{15}N, DOC-δ^{13}C, C/N ratio, NO_3-δ^{15}N, NO_3-δ^{18}O, H_2O-δ^{18}O and H_2O-δ^{2}H) were compared with *Microcystis* cell abundance, dissolved organic carbon, chlorophyll *a*, and toxic total microcystins concentration, as well as physical and chemical water quality variables, including streamflow. The isotopic composition of particulate organic matter, nitrate, and water differed for the Sacramento and San Joaquin Rivers and varied along the salinity gradient. The variation of particulate organic matter and water isotopes suggested *Microcystis* primarily entered the estuary from the San Joaquin and Old Rivers, where it was most abundant. Nitrate isotopes along with streamflow variables indicated that the San Joaquin River was a source of nitrate to the estuary. However, stable isotope comparison of the nitrogen in *Microcystis* cells with the dissolved inorganic nitrate in the San Joaquin River indicated that nitrate was not the primary source of nitrogen that supported the bloom. Instead, ammonium from the Sacramento River was the likely sole source of the nitrogen for most of the bloom. Selective uptake of ammonium may have further contributed to the magnitude of the *Microcystis* bloom which increased with the percent of ammonium within the total dissolved inorganic nitrogen pool.

Keywords *Microcystis* · Stable isotopes · Nutrients · Cyanobacteria bloom · Estuary · Streamflow

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Introduction

Summertime blooms of the toxic cyanobacteria *Microcystis aeruginosa* (*Microcystis*) have occurred regularly in San Francisco Estuary (SFE) since 1999 (Lehman et al. 2005). *Microcystis* is the only harmful algal bloom that consistently occurs in the estuary and begins once water temperature reaches above 19°C, total suspended solids drop below 40 mg l^{-1} and net streamflow is low during the summer and fall, usually between June and October (Lehman et al. 2013). These blooms are a threat to SFE because *Microcystis* cells often contain toxic microcystins, which promote tumors and liver cancer in humans and wildlife (Zegura et al. 2003; International Agency for Research on Cancer 2006; Ibelings and Havens 2008) and impact the health and survival of the phytoplankton, zooplankton, and the native fish in the estuary (Lehman et al. 2010; Ger et al. 2010; Acuña et al. 2012a, b). Importantly, the onset of these blooms in 2000 coincided with a decline in fish of interest to SFE, including the endangered delta smelt, longfin smelt, threadfin shad, and striped bass

(Sommer et al. 2007). Although long-term studies have determined the correlation between *Microcystis* cell abundance and physical, chemical and biological variables in SFE, the sources of *Microcystis* cells and nutrients that initiate and sustain the bloom are unknown (Lehman et al. 2005, 2008, 2010, 2013).

Stable isotopes are commonly used to identify the sources and sinks of nutrient and organic matter in aquatic systems because nutrients and particulate (POM) or dissolved organic matter (DOM) derived from different sources often have distinct isotope composition or "fingerprints" (Finlay and Kendall 2007). POM-δ^{13}C and POM-δ^{15}N and the associated C/N ratio were used to separate the sources of organic matter from freshwater and brackish water in the York and St. Lawrence Rivers (Hoffman and Bronk 2006; Martineau et al. 2004). These isotopes can even be used to separate organic matter into phytoplankton and cyanobacteria taxa (Vuorio et al. 2006). Dual nitrate (NO_3-δ^{15}N and NO_3-δ^{18}O) isotopes were successfully used to trace sources of inorganic nitrate and ammonium in large and hydrologically complex aquatic habitats, including the Mississippi, Delaware and Seine Rivers, Lake Lugano, Elkhorn Slough and San Francisco Bay Estuaries, and Monterey Bay (Cifuentes et al. 1989; Chang et al. 2002; Lehmann et al. 2004; Panno et al. 2006; Sebilo et al. 2006; Wankel et al. 2006, 2007, 2009). Dual stable isotopes of water (H_2O-δ^{18}O and H_2O-δ^{2}H) were similarly used to characterize large scale environmental gradients associated with rivers across the United States (Kendall and Coplen 2001). Because overlap is common, the use of multiple isotopes to trace sources and sinks of inorganic and organic matter has often been the most successful approach, particularly if it is accompanied by water quality tracers (Kendall et al. 2007).

Stable isotopes have been used to trace sources and sinks of organic matter and nitrogen with varying success in SFE. Stable nitrogen isotopes were used to demonstrate that groundwater was the primary source of nitrate to the San Joaquin River (Kratzer et al. 2004). Stable carbon isotopes were used to trace the source of particulate and dissolved inorganic carbon along the salinity gradient from the Sacramento River to San Francisco Bay (Spiker and Shemel 1979). Kraus et al. (2008) further demonstrated that different environments in the delta had distinctive δ^{13}C and δ^{15}N values for DOM and POM. Lipid biomarkers, chlorophyll *a* concentration, POM-δ^{13}C, and C/N ratio were also successfully used to determine that phytoplankton was the primary source of POM to SFE (Canuel and Cloern 1995). However, overlapping composition and high seasonal variability made it difficult to trace the origins of organic matter across aquatic and terrestrial habitats with δ^{13}C and δ^{15}N (Cloern et al. 2002).

The purpose of this study was to use multiple stable isotopes to determine if the source of *Microcystis* and its toxin, as well as the nitrogen that supports its growth, were derived from the San Joaquin River, where *Microcystis* cell abundance is elevated. This was achieved by comparing the variation of the stable isotopes of POM (POM-δ^{13}C, POM-δ^{15}N, C/N ratio), nitrate (NO_3-δ^{15}N and NO_3-δ^{18}O) and water (H_2O-δ^{18}O and H_2O-δ^{2}H) with *Microcystis* cell abundance, chlorophyll *a* concentration, toxic microcystins concentration, water quality conditions, and streamflow at sampling stations throughout SFE during the summers of 2007 and 2008. In addition, the stable isotope composition for dissolved organic carbon (DOC-δ^{13}C) was used to address the hypothesis that *Microcystis* blooms affect the quality and/or quantity of the dissolved organic carbon (DOC) in the water column.

Materials and Methods

Site Description

SFE contains an inland delta containing about 200 km^2 of waterways, which receive water from the Sacramento River on the north and the San Joaquin River on the south. Water from these rivers converge near Antioch and flow into a chain of downstream marine bays—Suisun, San Pablo and San Francisco—and creates one of the largest estuaries on the west coast of North America (Fig. 1). The Sacramento River is the largest of the rivers that feed the delta, with an average discharge of 4,795 m^3 s^{-1}, compared with 400 m^3 s^{-1} for the San Joaquin River, over the *Microcystis* bloom period, June through October. The delta contains about 1,300 km of sloughs and 57 levied islands that are used for agriculture and wildlife habitat. Daily streamflow during August and September, when *Microcystis* is most abundant, averages between 466 m^3 s^{-1} and 44 m^3 s^{-1} in the Sacramento and San Joaquin Rivers, respectively. An important feature of the delta is the large quantity of water diverted for agriculture from the southern portion of the delta that causes net negative streamflow in the San Joaquin and Old Rivers (Lehman et al. 2008; www.water.ca.gov/dayflow). This net flow reversal allows water from the Sacramento River to enter the San Joaquin River at the confluence of the two rivers. Water depth in the delta varies from a few meters in the shallow flooded islands in the center of the delta to 13 m in the deep river channels. Tides in the delta reach 2 m in height, with tidal velocities up to 30 cm s^{-1} and tidal excursions up to 10 km. Although the delta has non-limiting nutrient concentrations, the accompanying high total suspended solids concentration prevents development of eutrophic conditions (Jassby 2008).

Field Sampling

Data were combined from two studies in which water and POM samples were collected similarly at biweekly intervals

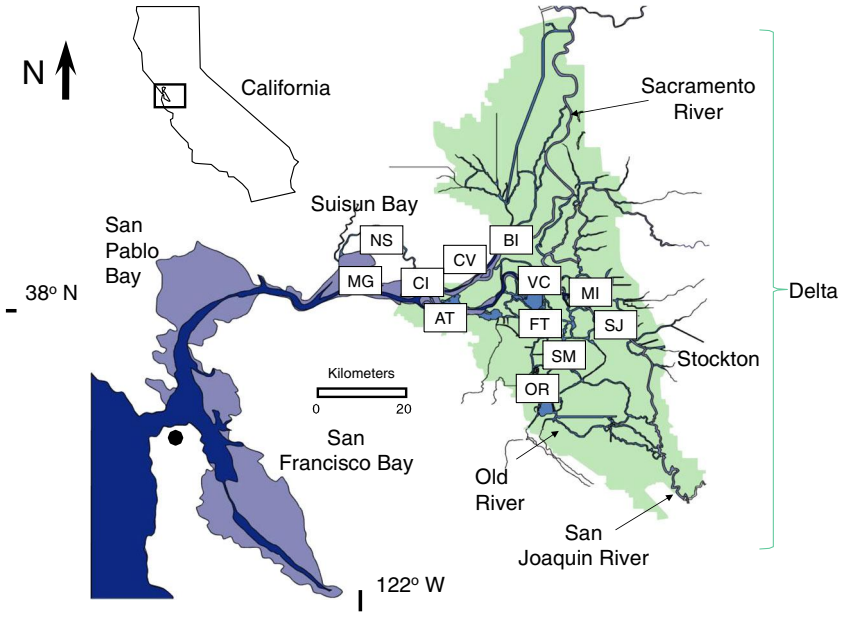

Fig. 1 Map of San Francisco Estuary showing the location of stations in the delta regions of the Sacramento, San Joaquin, and Old Rivers. Stations were located in Suisun Bay: Nurse slough (*NS*) and Middle Ground (*MG*); Sacramento River: Chipps Island (*CI*), Collinsville (*CV*), Brannon Island (*BI*); San Joaquin River: Antioch (*AT*), Venice Cut (*VC*), Mildred Island (*MI*), San Joaquin River (*SJ*); and Old River: Franks Tract (*FT*), Sand Mound (*SM*) and Old River (*OR*)

during *Microcystis* blooms. We defined the bloom season as the period of time when *Microcystis* colonies were visible to the eye on the surface of the water column. Samples were collected at all 12 stations between July and September in 2007 and at stations AT, CI, CV, MI, OR, and SJ between June and September in 2008 (Fig. 1). *Microcystis* and associated phytoplankton, cyanobacteria and organic material were collected from the surface of the water column using a 1- to 3-min horizontal surface tow of a 0.5-m diameter plankton net fitted with a 75-μm mesh. The total volume of the net tow was determined by a General Oceanics 2030R flow meter attached to the net. A net tow was used in order to get a representative sample of the *Microcystis* colonies, which were widely dispersed across the surface of the water column and usually did not form a surface scum. A wide mesh net also facilitated collection of the unusually wide *Microcystis* colonies, which can reach 50,000 μm wide, and reduced clogging from heavy suspended sediment. Cell abundance and both chlorophyll *a* and total microcystins concentration were determined from the concentrated samples in the net tow diluted by the total volume of water sampled by the net. Water for nutrient, DOC, total organic carbon concentration (TOC), and stable isotope analysis was collected by a van Dorn water bottle or diaphragm pump sampler deployed at 0.3 m below the surface.

Replicate *Microcystis* samples for chlorophyll *a* and phaeophytin pigments and toxic total microcystins analysis were filtered through GF/F glass fiber filters. Filters for chlorophyll *a* and phaeophytin concentration were treated with 1 % magnesium carbonate solution to prevent acidity, immediately frozen on dry ice and stored at −4°C until analysis. Pigments were extracted in 90 % acetone and quantified using spectrophotometry (American Public Health Association et al. 1998).

Filters for total microcystins analysis were immediately frozen on dry ice and stored at −80°C until processing for total toxic microcystins concentration using protein phosphate inhibition assay (PPIA), as detailed by Lehman et al. (2005). The PPIA assay is an integrative bioassay, which sums the inhibitory activity of all the different microcystins in the sample into a single value. The results are then expressed in terms of microcystin-LR equivalents. To ensure that the changes in toxicity observed using the PPIA assay were not due to changes in toxin composition, 274 of the more than 600 samples were also analyzed by liquid chromatography coupled with photodiode array and mass selective detection, when the microcystin concentration in the extract solution exceeded 0.5 μg l^{-1} (Boyer 2007).

Microcystis samples for determination of cell abundance were stained and preserved with Lugol's solution. The volume of *Microcystis* colonies within each sample was computed from the area of the colonies and an assumption of a spherical volume using a FlowCAM digital imaging flow cytometer made by Fluid Imaging Technologies (Sieracki et al. 1998). Cell abundance was computed from the average number of individual cells per unit volume. Cell abundance estimates based on FlowCAM measurements were closely correlated with those determined by microscopic analyses (r=0.88, p<0.01). In order to more easily measure the diameter of the colonies, the samples were size fractionated into 12–35, 36–300 and >300 μm diameter sub-samples using sieves, diluted to a maximum of 200 cells ml^{-1} and read at a magnification of either 4× or 2×.

Raw water samples were stored on ice after collection. From these samples, water for chloride, ammonium-N, nitrate-N plus nitrite-N, and soluble reactive phosphorus-P analysis was filtered through a 0.45 μm nucleopore filter

and immediately frozen (American Public Health Association et al. 1998; United States Environmental Protection Agency 1983; United States Geological United States Geological Survey 1985). Water for DOC analysis was filtered through pre-combusted GF/F filters, and the filtrate was kept at 4°C (American Public Health Association et al. 1998). Unfiltered water samples for total and volatile suspended solids and TOC analyses were kept at 4°C (American Public Health Association et al. 1998). All filtration was completed within 4 h of collection. Water temperature, pH, specific conductance, turbidity (NTU), and dissolved oxygen were measured at 0.3 m depth using a Yellow Springs Instrument (YSI) 6600 water quality sonde.

Isotopes

The POM isotopes POM-δ^{13}C, POM-δ^{15}N, and the C/N ratio were used to identify the contribution of *Microcystis* from different rivers, as well as the relative contribution of terrestrial versus phytoplankton organic matter to the POM. Replicate samples for isotopic analysis of POM were filtered through pre-combusted GF/F filters, and the filters were frozen at −4°C until analysis. Filters were ground and vapor acidified to remove any carbonate prior to preparation and analysis for δ^{13}C and δ^{15}N using an Optima mass spectrometer (Kendall et al. 2001). C/N ratio values are reported as atomic ratios.

The nitrate isotopes NO_3-δ^{15}N and NO_3-δ^{18}O were used to determine multiple factors: the use of nitrate as a nitrogen source by *Microcystis*, the contribution of nitrate from different rivers in the estuary, the source of the nitrogen from different habitats, and evidence of nitrification, denitrification or assimilation. Samples for nitrate isotope samples were filtered through 0.45-μm nucleopore filters, and the filtrate was kept frozen until analysis for δ^{15}N and δ^{18}O using a minor modification of the Sigman et al. (2001) and Casciotti et al. (2002) microbial denitrifier method and a custom-designed autosampler connected to an IsoPrime mass spectrometer.

DOC-δ^{13}C was used to determine if the bloom released organic carbon and to further identify the *Microcystis* within each river. Water samples for isotopic analysis of DOC were filtered through 0.45-μm nucleopore filters, and the filtrate was analyzed with an automated TOC analyzer connected to an IsoPrime mass spectrometer (St. Jean 2003).

Water isotopes H_2O-δ^{18}O and H_2O-δ^{2}H were used as conservative tracers of the contributions of streamflow from different rivers along the salinity gradient. Water isotope samples were filtered through 0.45 μm nucleopore filters and stored at room temperature in scintillation vials with poly-seal cone caps to prevent evaporation. Both δ^{18}O and δ^{2}H of water were measured with laser spectroscopy using a Los Gatos Research DLT-100 Liquid-Water Isotope Analyzer (Lis et al. 2008).

Isotopic composition was reported in units of parts per thousand (‰):

$$\delta(‰) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1,000,$$

where R represents the ratio of the heavy to light isotope (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, or $^{3}H/^{2}H$) in either the sample or standard. The data are reported relative to the international standards of Pee Dee Belemnite (PDB) for δ^{13}C, Air for δ^{15}N, and Vienna Standard Mean Ocean Water (V-SMOW) for δ^{18}O and δ^{2}H. Analytical precisions for replicate analyses of the same sample were method and isotope dependent, with precisions of: <0.3‰ for δ^{13}C and <0.5‰ for δ^{15}N of POM; <0.3‰ for DOC-δ^{13}C; <0.2‰ for δ^{18}O and <1.0‰ for δ^{2}H of water; and 0.2‰ for δ^{15}N and 1.5‰ for δ^{18}O of nitrate.

Streamflow

Streamflows were estimated from a combination of measured streamflows throughout the delta and computed streamflow from the DSM2 Hydrological Model version 8.0.6, a one-dimensional hydrodynamic and water quality simulation model for the Sacramento–San Joaquin Delta developed by the California Department of Water Resources (http://baydeltaoffice.water.ca.gov/modeling/deltamodeling/models/dsm2/dsm2.cfm). The hydrodynamics module (HYDRO) was calculated from stage, flow, and net flow at 15-min intervals. The water quality and transport module (QUAL) used the hydrodynamics simulated in HYDRO to calculate the average percent volume of water from the Sacramento River or San Joaquin River at each sampling station over 15-min intervals. Measured streamflow data were obtained from the California Department of Water Resources DAYFLOW database (www.water.ca.gov/dayflow).

Data Analysis

Due to the lack of normality associated with small sample size, statistical analyses were computed using nonparametric statistics. Data were reported as the median and standard deviation. The standard deviation of the median was computed from the median absolute deviation, which is a nonparametric measure of the standard deviation of the median that does not require the differences between the median and data values to be normality distributed (SAS Institute 2013). Simple correlation coefficients were computed using Spearman rank correlation (r_s) with SAS Institute software (2013). Similar spatial patterns between isotope variables and *Microcystis* cell abundance were determined using the principal component analysis (PCA) in Primer-e version 6 software (Clarke and R.N. Gorley 2006). Before PCA analysis, biological data

were transformed by the Bray–Curtis dissimilarity index for biological variables, and the chemical isotope data were normalized to the mean (difference from the mean and divided by the standard deviation) by month. Because H_2O-$\delta^{18}O$ and H_2O-$\delta^{2}H$ were inter-correlated at >0.70, only H_2O-$\delta^{2}H$ was used in the PCA analysis.

Results

Particulate and Dissolved Organic Matter

C/N ratios and microscopic analyses indicated that the POM in the seston was primarily derived from phytoplankton and bacteria. Median C/N ratios ranged from 5.9 to 9.7 (median 7.0±1.6) among stations and was ≤7 at most stations (Fig. 3). These median ratios were near the Redfield Ratio value of 6.6, which characterizes phytoplankton, and suggested that the POM was phytoplankton plus bacteria at stations NS, MG, MI, FT, AT, CI, SJ, and VC. Slightly larger median C/N ratios of 7.9 to 8.1 at stations CV and SM, and even larger C/N ratios of 9.2 to 9.7 at stations BI and OR, suggested that there were other sources of POM at these stations.

Median *Microcystis* cell abundance was 39 times greater in the San Joaquin and Old Rivers (5,178±7,509 cells ml^{-1}) than the Sacramento River (132±196 cells ml^{-1}, $p<0.01$; Fig. 2). Because *Microcystis* comprised over 90 % of the plankton by volume, its cell abundance varied closely with chlorophyll *a* concentration ($r=0.77$, $p<0.01$), which was also greater ($p<0.01$) in the San Joaquin and Old Rivers (median 0.13±0.16 ng l^{-1}) than in the Sacramento River (0.01±0.01 ng l^{-1}; Fig. 2). *Microcystis* cell abundance was also correlated ($r=0.77$, $p<0.01$) with total microcystins concentration. Total microcystins concentration in the San Joaquin and Old Rivers reached a median concentration of 0.042±0.059 ng l^{-1} and was orders of magnitude greater ($p<0.01$) than in the Sacramento River of 0.002±0.000 ng l^{-1} (Fig. 2).

Microcystis cell abundance ($r=0.50$) as well as chlorophyll *a* ($r=0.70$) and total microcystins concentration ($r=0.63$) were correlated with more positive POM-$\delta^{13}C$ values throughout SFE ($p<0.01$). Greater POM-$\delta^{13}C$ values occurred in the San Joaquin (−27.95±1.93‰) and Old Rivers (−26.50±1.19‰) than the Sacramento River (−28.20±1.04‰), where *Microcystis* was abundant ($p<0.01$; Fig. 3). POM-$\delta^{13}C$ values also increased with the percentage of San Joaquin River water on ebb ($r=0.40$, $p<0.01$) and slack tide ($r=0.82$, $p<0.05$), when more San Joaquin River water enters SFE from upstream. Differences in POM among the rivers was supported by the negative correlation between *Microcystis* cell abundance, chlorophyll *a* and total microcystins concentration with both oxygen and hydrogen water isotope values ($r=-0.44$, −0.39 and −0.41 for H_2O-$\delta^{18}O$ and $r=-0.42$, −0.37 and −0.40 for H_2O-$\delta^{2}H$, respectively, $p<0.01$; Figs. 2 and 3).

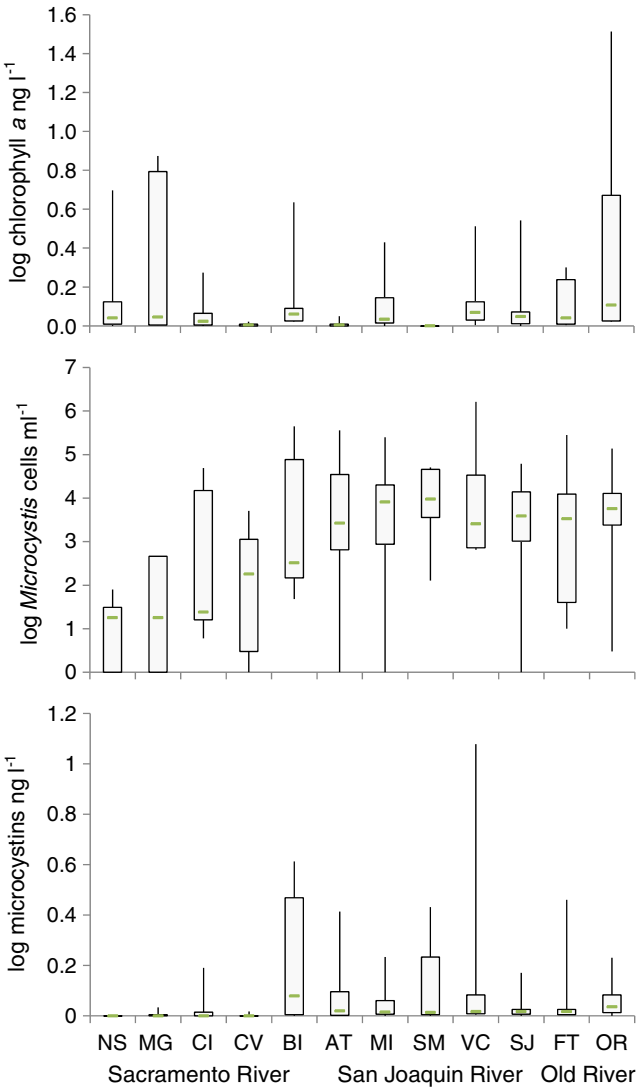

Fig. 2 Median (*bar*), intervals for the 25th and 75th percentiles (*box*), maximum and minimum (*whiskers*) values of log chlorophyll *a* concentration, log *Microcystis* cell abundance, and log total microcystins concentration measured during the summers of 2007 and 2008. Stations names are listed in Fig. 1

POM-$\delta^{15}N$ values were greater in the Sacramento (10.0±2.4‰) and San Joaquin (10.4±1.7‰) Rivers than the Old River (8.2±0.89‰, $p<0.05$; Fig. 3). POM-$\delta^{15}N$ was also correlated with ammonium concentration ($r=0.44$, $p<0.01$) in the San Joaquin River, and the correlation was higher during flood tide, when the percentage of Sacramento River water was greater at each station ($r=0.60$, $p<0.05$). In contrast, POM-$\delta^{15}N$ was not correlated with the NO_3-$\delta^{15}N$ values in the San Joaquin River. The relatively lower NO_3-$\delta^{15}N$ compared with the POM-$\delta^{15}N$ (ratio <1) for most samples indicated that nitrate was not the primary source of nitrogen for the bloom (Fig. 4). In fact, only 11 % of the samples had NO_3-$\delta^{15}N$ values greater than the POM-$\delta^{15}N$. These samples

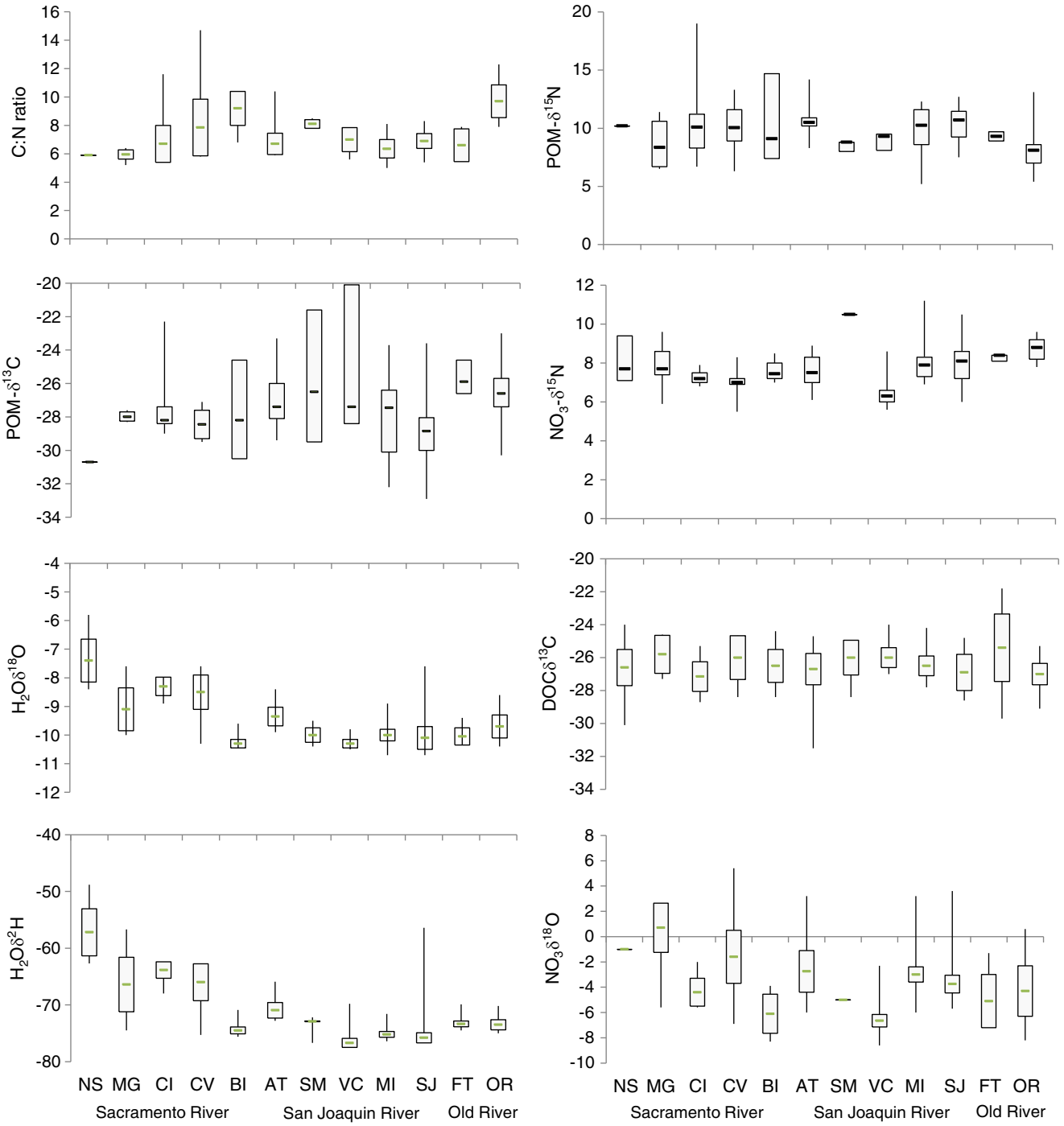

Fig. 3 Median (*bar*), intervals for the 25th and 75th percentiles (*box*), maximum and minimum (*whiskers*) values of the carbon to nitrogen ratio (C:N), and POM-δ^{13}C, H_2O-δ^{18}O, H_2O-δ^{2}H, POM-δ^{15}N, NO_3-δ^{15}N, DOC-δ^{13}C, and NO_3-δ^{18}O isotopes measured at stations in the Sacramento, San Joaquin, and Old Rivers during the summers of 2007 and 2008. Stations names are listed in Fig. 1

came from stations SJ, OR, and MI in the San Joaquin and Old Rivers and station MG in the Sacramento River. The relatively greater NO_3-δ^{15}N value compared with the POM-δ^{15}N value ($p<0.05$; ratio >1) of samples in Old River indicated that *Microcystis* and other primary producers in Old River were more likely to use nitrate as a nitrogen source than the other rivers.

Environmental Factors

Water quality variables differed among the rivers in the estuary. More brackish water in the lower Sacramento River was associated with an order of magnitude greater specific conductance and chloride than the freshwater in the San Joaquin and Old Rivers (Table 1). The Sacramento River was also

Table 1 Median and standard deviation of environmental variables measured in the Sacramento (1), San Joaquin (2) and Old (3) Rivers during the summer of 2007 and 2008

Variable	Sacramento River	San Joaquin River	Old River	Significance
Ammonium (mg l^{-1})	0.05±0.01	0.03±0.02	0.03±0.02	1>2, 3 and 2>3
Nitrate (mg l^{-1})	0.35±0.07	0.30±0.15	0.20±0.15	1>3
Soluble reactive P (mg l^{-1})	0.06±0.02	0.06±0.02	0.06±0.02	ns
Silica (mg l^{-1})	14.00±1.19	14.50±1.33	12.70±2.82	ns
Chloride (mg l^{-1})	1570±1533	44±28	122±67	1>2, 3 and 3>2
Specific conductance (μS cm^{-1})	4778±5502	305±116	483±314	1>2, 3 and 3>2
Dissolved oxygen (mg l^{-1})	8.80±0.44	8.50±0.59	8.80±1.19	ns
pH	7.90±0.15	8.10±0.30	8.10±0.59	ns
Secchi disk depth (cm)	60±30	108±50	104±53	2, 3>1
Water temperature (°C)	21.30±1.04	23.10±1.93	23.50±1.33	2, 3>1
Dissolved organic carbon (mg l^{-1})	2.00±0.44	2.20±0.44	2.30±0.30	2, 3>1
Total organic carbon (mg l^{-1})	2.10±0.44	2.30±0.30	2.40±0.30	2, 3>1
Total suspended solids (mg l^{-1})	16.50±9.63	4.00±4.45	6.00±2.96	1>2, 3 and 2>3
Volatile suspended solids (mg l^{-1})	3.00±1.48	1.00±1.48	1.00±1.48	1>2, 3

Significant differences among the rivers at the 0.05 level or higher are indicated (e.g., 1>2, 3 means that river #1 (the Sacramento River) has a significantly higher value for the variable than rivers #2 and #3)

cooler than the San Joaquin and Old Rivers by about 2°C. Water transparency (Secchi disk depth) was a factor of 2 lower in the Sacramento River and accompanied by a factor of 4 greater suspended and volatile solids than the San Joaquin or Old Rivers. In contrast, the San Joaquin and Old Rivers had greater TOC and DOC than the Sacramento River. All rivers had relatively high and similar soluble reactive phosphorus and silica concentrations; only nitrate and ammonium concentrations were greater in the Sacramento River.

Chlorophyll *a* concentration and *Microcystis* cell abundance were negatively correlated with chloride ($r=-0.35$ and -0.30, respectively) and total suspended solids concentration ($r=-0.32$ and -0.35, respectively) and positively correlated with water temperature ($r=0.34$ and 0.35, respectively, $p<0.01$). Elevated specific conductance and chloride concentration characterized the Sacramento River and was accompanied by greater suspended and volatile solids than in the San Joaquin or Old Rivers, where *Microcystis* was abundant (Table 1). Both chlorophyll *a* concentration and *Microcystis* cell abundance were greater in the San Joaquin and Old Rivers, where water temperature was relatively high (Table 1).

Microcystis cell abundance ($r=-0.30$, $p<0.05$), chlorophyll *a* concentration ($r=-0.42$, $p<0.01$), and total microcystins concentration ($r=-0.38$, $p<0.01$) increased in the San Joaquin River when the net streamflow was low. High POM-δ^{13}C values, which characterized the *Microcystis* bloom in the San Joaquin and Old Rivers, were also unexpectedly correlated with a coincident decrease in the percentage of San Joaquin River water at each station ($r=-0.41$, $p<0.01$). The association between low San Joaquin River flow and high POM-δ^{13}C values was supported by the correlation between POM-δ^{13}C and the percentage of Sacramento River water at each station ($r=0.32$, $p<0.05$; Fig. 5).

DOC concentration did not increase with *Microcystis* cell abundance ($r=-0.47$), chlorophyll *a* concentration ($r=-0.51$), or microcystin concentration ($r=-0.52$) in the San Joaquin River ($p<0.01$; Table 1). Instead, DOC concentration increased with the percentage of San Joaquin River water at stations in the Sacramento, San Joaquin, and Old Rivers

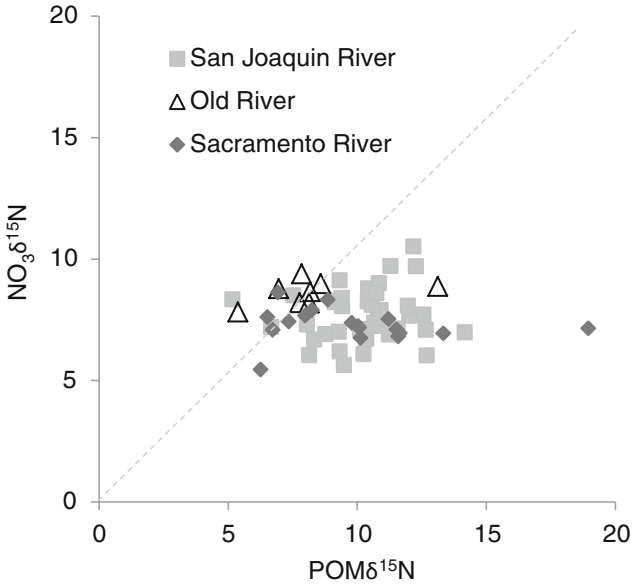

Fig. 4 Plot of NO_3-δ^{15}N versus POM-δ^{15}N at stations within the Sacramento (*diamond*), San Joaquin (*square*), and Old (*triangle*) Rivers during the *Microcystis* blooms of 2007 and 2008. The *dotted line* indicates the 1:1 ratio

(r=0.59, 0.53, 0.68, respectively, p<0.01). DOC concentration was also greater when the net flow in the San Joaquin River was elevated (r=0.33; p<0.01). The importance of high streamflow to DOC concentration was supported by the increase in DOC concentration when nitrate concentration was high (r=0.53 and 0.55, respectively, p<0.01) and Secchi disk depth was low (r=−0.36 and −0.46, respectively, p<0.01) in the San Joaquin and Old Rivers. Nitrate increased (r=0.52 and 0.80, respectively, p<0.01) while Secchi disk depth decreased (r=−0.40 and −0.75, respectively, p<0.01) with streamflow in both the San Joaquin and Old Rivers.

DOC-δ^{13}C increased with *Microcystis* cell abundance in the San Joaquin River (r=0.32, p<0.05), even though the median DOC-δ^{13}C values (−26.3±1‰) were not significantly different among rivers. DOC-δ^{13}C also varied inversely with DOC concentration in both the San Joaquin and Old Rivers (r=−0.31 and −0.53, respectively, p<0.05). The uncoupling of DOC-δ^{13}C and DOC concentration was supported by correlations with environmental variables. Opposite to DOC, DOC-δ^{13}C was greater at low streamflow (r=−0.48, p<0.01), high Secchi disk depth (r=0.45, p<0.01), and low nitrate concentration (r=−0.48, p<0.01) in the San Joaquin River.

Nitrogen

Median NO_3-δ^{15}N values were greater in the San Joaquin and Old Rivers (8.1±1.2‰) than the Sacramento River (7.2±0.4 ‰, p<0.05; Fig. 3). The NO_3-δ^{15}N values increased with the percentage of San Joaquin River water at stations in both the San Joaquin and Old Rivers (r=0.72 and 0.71, respectively, p<0.01). NO_3-δ^{15}N values were also greater on ebb tide in the San Joaquin River, when there was even more San Joaquin River water at each station (r=0.56, p<0.01). Elevated NO_3-δ^{15}N values were associated with greater nitrate concentration in the San Joaquin River (r=0.40, p<0.01) and the percentage of San Joaquin River water (r=0.57, p<0.01). High NO_3-δ^{15}N values in the San Joaquin River were also positively correlated with elevated TOC and water temperature (r=0.31 and 0.24, respectively, p<0.05), which is characteristic of the San Joaquin River (Table 1). Conversely, NO_3-δ^{15}N values were negatively correlated with the high chloride, total suspended solids, and ammonium concentration characteristic of the Sacramento River (r=−0.26, −0.21 and −0.28, respectively, p<0.05; Table 1). Median NO_3-δ^{18}O values were more variable than those for NO_3-δ^{15}N and not significantly different among rivers (−2.8±3.3‰, −3.2±2.2 ‰ and −4.4±2.7‰ for the Sacramento, San Joaquin and Old Rivers, respectively; Fig. 3). However, NO_3-δ^{18}O values were correlated with the oxygen water isotopes (r=0.35, p<0.01) and nitrate concentration (r=0.55, p<0.05) in Old River.

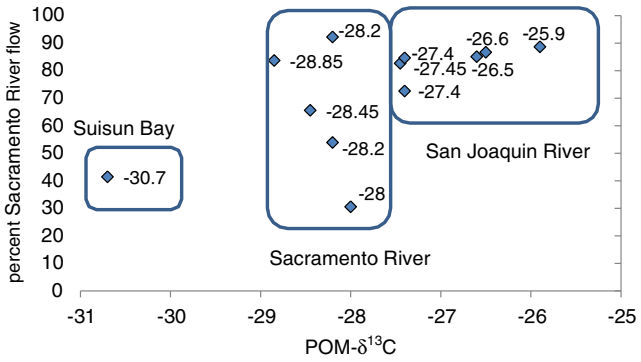

Fig. 5 POM-δ^{13}C values associated with the percentage of Sacramento River flow for stations sampled in the summers of 2007 and 2008

Microcystis abundance was closely associated with the percentage of ammonium within the total dissolved inorganic nitrogen pool. Maximum cell abundance occurred when the ammonium concentration comprised 20 % to 25 % of the total dissolved inorganic nitrogen within the Sacramento (r=0.69, p<0.01), San Joaquin (r=0.38, p<0.01), and Old Rivers (r=0.44, p<0.05; Fig. 6). The percentage of ammonium increased when the net flow was low in the San Joaquin (r=−0.28, p<0.05) and Old Rivers (r=−0.45, p<0.05).

Low H_2O-δ^{18}O and H_2O-δ^{2}H water isotope values were correlated with *Microcystis* cell abundance in the San Joaquin and Old Rivers (r=−0.44 and −0.42, respectively, p<0.01; both H_2O-δ^{18}O and H_2O-δ^{2}H had the same correlation coefficient). Median water isotope values ranged from −10.3‰ to −7.4‰ for H_2O-δ^{18}O and −76.7‰ to −57.2‰ for H_2O-δ^{2}H and were significantly greater (p<0.05) in the Sacramento River than the San Joaquin and Old Rivers (−8.9±1.6‰, −10.0±0.7‰ and −10.0±0.6‰, respectively, p<0.05 for H_2O-δ^{18}O and −64.6±9.8‰, −73.4±4.0‰ and −73.3±1.5 ‰, respectively, p<0.05 for H_2O-δ^{2}H; Fig. 3). The association between more positive water isotope values and saltier water was supported by the greater water isotope values at the brackish water station AT and the most seaward station NS than at stations SJ, VC, and MI in the freshwaters of the upper San Joaquin River (Fig. 3). Both water isotopes were also positively correlated with high chloride, nitrate, and total suspended solids (r=0.63, 0.67 and 0.44, respectively, p<0.01 for H_2O-δ^{18}O and r=0.37, 0.53 and 0.68, respectively, p<0.01 for H_2O-δ^{2}H), which characterized the more brackish water stations in the lower Sacramento River (Table 1). H_2O-δ^{18}O (r=−0.57, p<0.01) and H_2O-δ^{2}H (r=−0.53, p<0.01) water isotope values also decreased with the percentage of freshwater from the upper Sacramento River.

PCA Analysis

Three PCA axes described 75 % of the variation in the isotope signatures (Table 2). The first axis primarily described NO_3-δ^{15}N followed by H_2O-δ^{2}H and POM-δ^{13}C, while the second axis primarily described POM-δ^{13}C followed by NO_3-δ^{15}N. The third axis varied most closely with the water isotope H_2O-δ^{2}H followed by the C/N ratio.

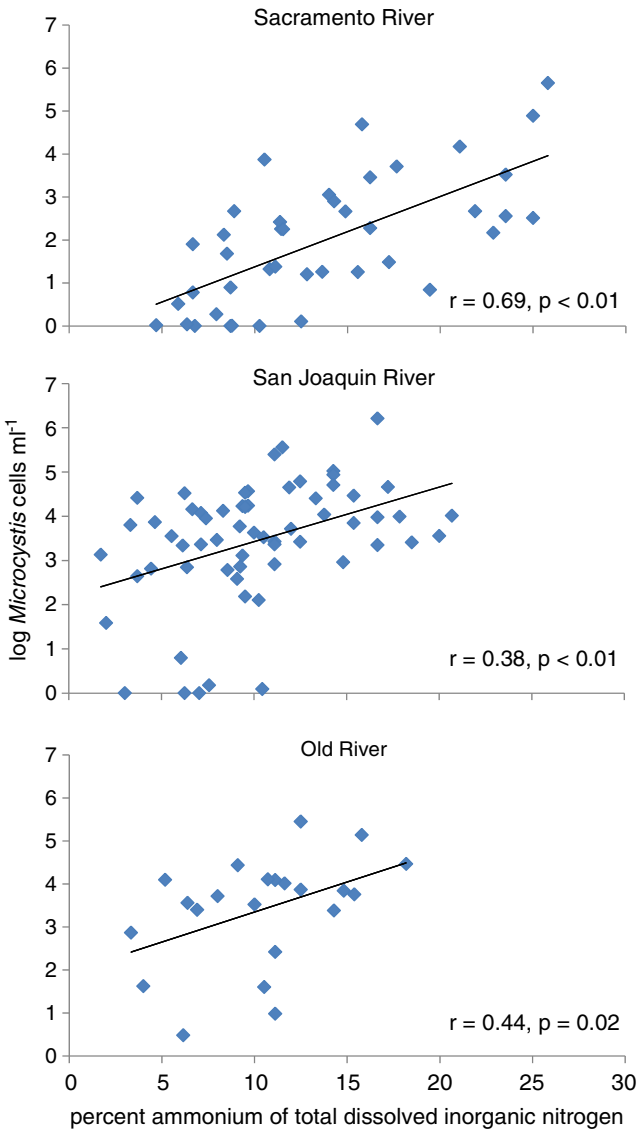

Fig. 6 Variation of *Microcystis* cell abundance with the percent ammonium of the total dissolved nitrogen pool at stations within the Sacramento, San Joaquin and Old Rivers

Average PCA scores computed for each station indicated that PCA axis 1 separated San Joaquin River stations with more positive average scores (0.24±1.34) from Sacramento River stations with more negative scores (−0.32±1.30), while PCA axis 2 separated the more positive scores of the Sacramento River stations (0.19±1.20) from more negative scores of the Old River stations (−0.42±2.10). The third PCA axis separated positive scores for Old River stations (0.50±1.70) from negative scores for San Joaquin River stations (−1.80±1.30).

Within the ordination plot for PCA axes 1 and 2, as well as the ordination plot for PCA axes 2 and 3 (not shown), *Microcystis* abundance plotted near the center of the PCA ordination; slightly negative on PCA axis 1 and slightly positive on PCA axis 2 (Fig. 7). *Microcystis* cell abundance varied most closely with the NO_3-$\delta^{15}N$ and POM-$\delta^{13}C$ isotopes, but the associations were opposite. The negative correlation between *Microcystis* cell abundance and PCA axis 1 (r=−0.44, p<0.01) indicated that *Microcystis* cell abundance increased with POM-$\delta^{13}C$ values (negative score on PCA axis 1) and decreased with NO_3-$\delta^{15}N$ nitrogen and the water isotope H_2O-δ^2H and H_2O-$\delta^{18}O$ values (positive scores on PCA axis1) in the San Joaquin River. The contradictory association between POM-$\delta^{13}C$ and NO_3-$\delta^{15}N$ on PCA axis 1 and PCA axis 2 reflected the differing conditions in the San Joaquin River, where POM-$\delta^{13}C$ and NO_3-$\delta^{15}N$ varied in opposition, and the Sacramento River, where POM-$\delta^{13}C$ and NO_3-$\delta^{15}N$ varied together (Fig. 3). The positive association between *Microcystis* abundance and POM-$\delta^{13}C$ for PCA axis 1 was supported by the positive correlation between *Microcystis* abundance in the San Joaquin River and PCA axis 2 (r=0.26, p<0.05). *Microcystis* abundance in the San Joaquin River was not correlated with PCA axis 3.

Table 2 Principal component analysis scores for three axes that describe variation in stable isotope measurements and the percentage of the variation that each axis describes during *Microcystis* blooms in 2007 and 2008

Variable	Axis 1	Axis 2	Axis 3
NO_3-$\delta^{15}N$	0.746	0.539	−0.130
H_2O-δ^2H	0.411	−0.085	0.786
C/N molar ratio	−0.228	−0.032	0.567
POM-$\delta^{13}C$	−0.402	0.816	0.160
POM-$\delta^{15}N$	0.246	−0.188	−0.131
Percent variation	34	21	20

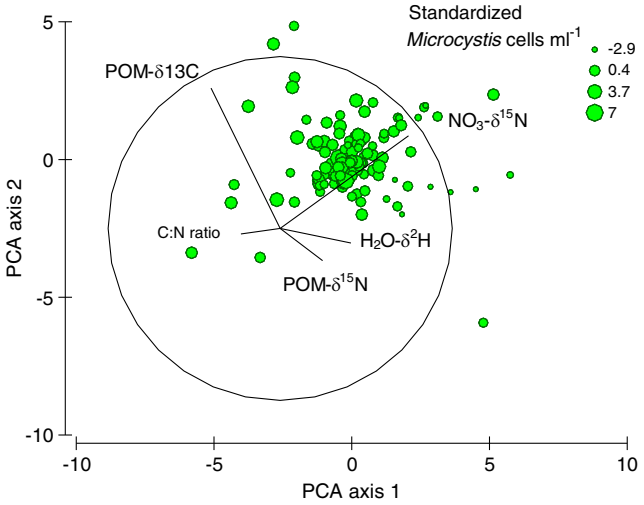

Fig. 7 Ordination plot of the first and second principal component axes computed with principal component analysis of water, nitrate, and POM isotopes collected during *Microcystis* blooms in 2007 and 2008. The principal component axes were overlaid with standardized *Microcystis* cell abundance collected during the same period

Discussion

Microcystis Abundance and Biomass

The surface seston during the *Microcystis* bloom was primarily composed of cyanobacteria and phytoplankton. Among stations, the range of median C/N ratios between 5.9 and 9.7 and POM-δ^{13}C values between −30.7‰ and −25.9‰ confirmed that most of the organic matter in the Sacramento, San Joaquin, and Old Rivers was derived from phytoplankton and cyanobacteria (Finlay and Kendall 2007; Hoffman and Bronk 2006). Previous measurements of POM-δ^{13}C and the C/N ratio before the establishment of the *Microcystis* bloom in 1999 also indicated that phytoplankton was the primary source of POM to SFE (Canuel and Cloern 1995). However, at some stations, there was probably a small amount of terrestrial organic matter in the seston, because the median C/N ratio was slightly larger than the Redfield ratio of 6.6, commonly measured for phytoplankton and bacteria. The C/N ratio of terrestrial leaf litter can reach 20, and even higher for soil organic matter, which ranged from 8 to 25 (Kendall et al. 2007). C/N ratios of emerging and floating terrestrial and salt marsh plants similarly ranged from 11.6 to 51.0 in the San Joaquin–Sacramento River Delta and San Francisco Bay (Cloern et al. 2002). The presence of terrestrial organic matter could have also decreased the POM-δ^{13}C values in the samples somewhat (Kendall et al. 2001). However, the organic matter from aquatic macrophytes would have had little effect on POM-δ^{13}C, because macrophytes generally have POM-δ^{13}C values similar to co-existing phytoplankton (Finlay and Kendall 2007).

The San Joaquin River was the primary source of *Microcystis* cells to the delta. *Microcystis* cell abundance was correlated with POM-δ^{13}C in the San Joaquin and Old Rivers, where POM-δ^{13}C was more positive than at stations seaward in the Sacramento River. The variation of *Microcystis* cell abundance with water isotopes also suggested a difference in the source of cells among the rivers. Although elevated POM-δ^{13}C may be produced by many factors, including allochthonous loading, species composition, primary productivity, concentration of the DIC pool, and isotopic composition (Gu et al. 2006), the strong correlation between POM-δ^{13}C and *Microcystis* cell abundance suggested that the *Microcystis* cell carbon directly influenced POM-δ^{13}C values. As a freshwater cyanobacterium, *Microcystis* would have a slightly higher POM-δ^{13}C value compared with the marine phytoplankton seaward (Hoffman and Bronk 2006). This increase in POM-δ^{13}C with *Microcystis* cell abundance was supported by the 0.3‰ to 1.6‰ decrease in δ^{13}C values between stations landward in the San Joaquin and Old Rivers and stations seaward in the Sacramento River. Relatively high POM-δ^{13}C values were also measured for *Microcystis* blooms in Lake Kinneret (−23.4‰) and in the surface scum of shallow lakes in Florida and China, where *Microcystis* is common (−28.6‰ to −17.6‰; Gu and Schelske 1996; Xu et al. 2007). Cyanobacteria generally have greater POM-δ^{13}C values than those for diatoms (−30.6‰ to −26.6‰) or chrysophytes in co-occurring samples (−34‰ to −31‰; Vuorio et al. 2006). Low POM-δ^{13}C values similarly characterized the Sacramento River, where brackish water diatoms and chrysophytes were abundant (Lehman et al. 2010). It is also possible that the differing POM-δ^{13}C values in fresh and brackish water partly reflect multiple *Microcystis* populations. Previous DNA research suggested that SFE may have both freshwater and brackish water populations (Moisander et al. 2009).

It is also likely that the higher POM-δ^{13}C of the *Microcystis* in the San Joaquin and Old Rivers reflects the long residence time and accumulation of biomass associated with the bloom. Long residence time allows the processes of uptake and respiration to decrease the δ^{13}C of the dissolved inorganic carbon pool and subsequently increase the POM-δ^{13}C of phytoplankton and bacteria (Gu et al. 2006; Finlay and Kendall 2007). However, the slow growth of *Microcystis* may limit the loss of carbon in the dissolved inorganic carbon pool (Lehman et al. 2008). The very large *Microcystis* colonies in SFE may also use δ^{13}C less rapidly due to the lag time required for carbon to diffuse across wide diameter colonies (Xu et al. 2007). It was further suggested that the mechanisms affecting carbon assimilation and the resulting POM-δ^{13}C values in the *Microcystis* surface scum can differ from those of other seston deeper in the water column (Xu et al. 2007). Some of these processes may have contributed to the relatively lower POM-δ^{13}C values for *Microcystis* measured in this study compared to phytoplankton blooms previously measured in SFE, where POM-δ^{13}C values were −19‰ to −17‰ and carbon was limited (Canuel and Cloern 1995).

The increase in DOC-δ^{13}C values with *Microcystis* cell abundance in the San Joaquin River indicated that the bloom may release extracellular products. *Microcystis* can produce extracellular polysaccharides or other substances that enhance colony formation, inhibit feeding in zooplankton, and disrupt the aquatic food web (Lurling 2003; Huang et al. 2007; Yang et al. 2008). The release of extracellular products by *Microcystis* has also been attributed to changes in photosynthesis, growth rate, and production of photosynthetic pigments (Sedmak and Kosi 1998; Singh et al. 2001; Sedmak and Eleršek 2006; Jia et al. 2008; Vassilakaki and Pflugmacher 2008). Such dissolved extracellular substances were hypothesized to have contributed to the absence of the usually common green alga *Scenedesmus* spp. and the abundance of cryptophytes during *Microcystis* blooms in SFE (Lehman et al. 2010). Certainly, the presence of dissolved microcystins during the *Microcystis* bloom in SFE indicated that the bloom was periodically accompanied by dissolved extracellular substances (Lehman et al. 2005, 2008, 2010).

Nitrogen Source

Ammonium was the primary nitrogen source that supported *Microcystis* growth throughout the bloom season in SFE. If nitrate had been a significant source of nitrogen to the *Microcystis* bloom, then POM-δ^{15}N of the primary producers would have been about 4‰ lower than the δ^{15}N of the co-occurring nitrate, as long as the nitrate concentrations are not limiting (Finlay and Kendall 2007). POM-δ^{15}N of the primary producers was lower than the δ^{15}N of the co-occurring nitrate for only 11 % of the samples in this study. A similar difference was measured for previous studies in the upper San Joaquin River between Lander and Mossdale and the upper Sacramento River between Freeport and Isleton (Kratzer et al. 2004; Dugdale et al. 2007).

However, for 89 % of the samples in this study, the NO_3-δ^{15}N in the water column was lower than the POM-δ^{15}N of the primary producers, which indicated that the primary source of nitrogen for the bloom was not the co-occurring nitrate, but another source of nitrogen, most likely ammonium. That nitrate concentration did not influence the *Microcystis* bloom was also supported by previous research indicating that the N/P molar ratio, which is strongly influenced by relatively high nitrate concentration, was not correlated with *Microcystis* abundance or biomass (Lehman et al. 2013). This poor correlation between *Microcystis* cell abundance or biomass and the N/P molar ratio contradicted the general hypothesis that the total dissolved nitrate plus ammonium and the subsequent N/P molar ratio should control phytoplankton and cyanobacteria blooms in SFE (Glibert 2012).

Elevated ammonium concentration characterized the summer in the Sacramento, San Joaquin, and Old Rivers, where median ammonium concentration ranged from 0.03±0.01 to 0.05±0.01 mg l^{-1} and contributed between 5 % and 25 % of the total dissolved inorganic nitrogen in the water column. Elevated ammonium concentrations were probably maintained solely by the daily release of ammonium from the Sacramento wastewater treatment facility, which has increased over time and averages 391 metric tons of ammonium-N per month (Jassby 2008). Ammonium produced from organic matter decomposition may have contributed to the ammonium concentration in the water column, but the contribution was probably small because organic matter is not released by the wastewater treatment plant, organic matter concentration from phytoplankton and cyanobacteria production is relatively low, and the ammonification rate is relatively slow. Similarly, the daily production of ammonium from ammonification of organic matter and phytoplankton was small compared to the daily load of ammonium from the Stockton wastewater treatment facility in the San Joaquin River (Lehman et al. 2004). Ammonium concentrations also remained high in the delta despite uptake, because the phytoplankton and cyanobacteria biomass during *Microcystis* blooms, is comparatively small and occurs only on the surface of the 12-m-deep water column (Parker et al. 2012; Lehman et al. 2013).

Other sources of nitrogen, such as urea, cannot be ruled out as potential sources of nitrogen for the *Microcystis* bloom, because we did not have the NH_4-δ^{15}N isotopic values needed to verify that the nitrogen source for the POM was ammonium from the treatment plant. However, ammonium concentrations measured during this study were well above the 0.007 to 0.025 mg l^{-1} concentration range needed to support *Microcystis* blooms in Lake Erie and Steilacoom Lake, WA (Jacoby et al. 2000; Chaffin et al. 2011). *Microcystis* can effectively use small amounts of ammonium in the water column because it can outcompete most other primary producers for ammonium due to its low half saturation constant and elevated uptake rate (Takamura et al. 1987; Yoshida et al. 2007). In addition, nitrogen uptake studies confirmed that ammonium concentration alone was sufficient to support growth of the phytoplankton community in the upper Sacramento River during the spring (Parker et al. 2012).

The San Joaquin River was the primary source of the elevated NO_3-δ^{15}N in the delta. NO_3-δ^{15}N values at each station increased with the percentage of San Joaquin River water and were greater during ebb tide, when the percentage of San Joaquin River water increased in SFE. The elevated NO_3-δ^{15}N values of the San Joaquin River water may be influenced by factors such as inputs of nitrate in animal waste from dairy farms in the San Joaquin River watershed, in situ processes such as phytoplankton uptake, and denitrification plus the influence of a waste water treatment plant at Stockton (Kratzer et al. 2004).

Conversely, the low NO_3-δ^{15}N in the Sacramento River was probably the result of extensive nitrification of ammonium waste from the Sacramento waste water treatment plant upstream of the delta. This pattern was supported by the seaward decrease in median NO_3-δ^{18}O values from −2‰ to −4‰ in the Sacramento River between stations CV and CI, which suggested that new nitrate with low NO_3-δ^{18}O values was added from upstream of station CI. The inverse correlation between NO_3-δ^{18}O and the percentage of Sacramento River water also suggested that nitrate in the Sacramento River was derived from nitrification of ammonium downstream of the treatment plant. NO_3-δ^{15}N and NO_3-δ^{18}O were similarly used to identify nitrification and denitrification from waste water treatment plant discharge as the source of nitrate in the Seine River, France, and rivers in the northwest and mid-Atlantic, USA (Mayer et al. 2002; Sebilo et al. 2006).

Streamflow

The percent of water from each river was the most important environmental factor affecting the *Microcystis* bloom and the associated variation in stable isotope signals of dissolved and particulate matter. POM, C/N, nitrate, and water isotopes were

correlated with the percentage of Sacramento or San Joaquin River water at each station and varied along the salinity gradient. In previous research, the spatial distribution of POM and nitrate isotopes were similarly controlled by hydrodynamics and linearly correlated with the salinity gradient between San Francisco Bay seaward and the rivers landward (Canuel and Cloern 1995; Wankel et al. 2006). C/N and POM isotopic composition, and nitrate isotopes commonly vary with the hydrodynamics in large river systems, such as the Mississippi and Colorado Rivers and large estuaries, such as the St. Lawrence River and Chesapeake Bay (Martineau et al. 2004; Hoffman and Bronk 2006).

However, the streamflow relationships that facilitated the development of the *Microcystis* bloom and the isotopic signals associated with it were more complex than merely a gradient from fresh to salt water in SFE. *Microcystis* was more abundant in the San Joaquin and Old Rivers when a majority of the water in the lower San Joaquin River was from the Sacramento River, not the San Joaquin River as expected. In fact, *Microcystis* abundance was positively correlated with the percentage of Sacramento River water and negatively correlated with the percentage of San Joaquin River water. This seeming contradiction is partially due to the reduced flushing time associated with reverse net streamflow in the San Joaquin River, which is caused by agricultural diversion during the summer. Reversed net streamflow draws Sacramento River water across the estuary into the lower San Joaquin River upstream of the confluence at Antioch. The resulting high residence time at the interface between the seaward flowing San Joaquin River water and landward flowing Sacramento River could facilitate accumulation of *Microcystis* colonies in the San Joaquin River for sufficient time to allow assimilation of ammonium from the Sacramento River into the *Microcystis* colonies and affect the isotopic signals of DOM and POM (Finlay and Kendall 2007; Lehman et al. 2008).

Increasing percentages of Sacramento River water in the lower San Joaquin River channel would also influence the bloom by affecting water quality conditions. Sacramento River water would contribute proportionately larger quantities of ammonium into the San Joaquin River each day with daily tidal mixing, because the Sacramento River water contains elevated ammonium concentration from waste water treatment discharge (Jassby 2008). *Microcystis* cell abundance was strongly correlated with the percentage of ammonium within the total dissolved inorganic nitrogen pool for each river. Other water quality conditions, including salinity, water temperature, water transparency, and total suspended solids, would also be affected by the percentage of Sacramento River water in the lower San Joaquin River. The presence of Sacramento River water in the lower San Joaquin River partly explains why positive POM-δ^{13}C values, indicators of *Microcystis* in the San Joaquin River, were negatively correlated with high NO_3-δ^{15}N, an indicator of nitrate from the San Joaquin River, within PCA analysis. Clearly, a combined knowledge of multiple isotopic signals, water quality, and hydrology is needed to gain a complete understanding of the processes that affect *Microcystis* blooms in SFE.

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