Nutrient Science Program FY 2015 Progress Update

By:
Emily Novick
Jennifer Sun
David Senn

Acknowledgements

Contents

Table of Figures

1. Introduction

San Francisco Bay has long been recognized as a nutrient-enriched estuary. Nitrogen (N) and phosphorus (P) concentrations in many areas of the Bay substantially exceed those in other estuaries where condition has been impaired by nutrient pollution. However, the Bay has exhibited resistance to some of the classic symptoms of excessive nutrient concentrations that have plagued other nutrient-enriched estuaries, such as high phytoplankton abundance and low dissolved oxygen. High turbidity and strong tidal mixing in the Estuary limit light levels and phytoplankton growth, causing a low proportion of available nutrients to be converted to biomass. Large populations of filter-feeding clams have further limited phytoplankton accumulation. However, observations over the past 10 years are challenging the Bay's resistance to its high nutrient loads. These include:

- a greater than 2 fold increase in summer-fall phytoplankton biomass in South Bay since 1999
- frequent detections of algal species that have been shown in other nutrient-rich estuaries to form harmful algal blooms (HABs);
- frequent detection of the toxins microcystin and domoic acid that are produced by some harmful algae;
- evidence of low dissolved oxygen in some sloughs and tidal creeks; and
- studies suggesting that the chemical forms of nitrogen can decrease phytoplankton productivity or cause shifts toward algal species that poorly sustain the food web.

The combination of high nutrient concentrations and changes in environmental factors that regulate SFB's response to nutrients has generated growing concern about whether the Bay is trending toward, or may already be experiencing, nutrient-related impairment. To address this concern, the San Francisco Bay Regional Water Quality Control Board (SFBRWQCB) worked collaboratively with stakeholders to develop the San Francisco Bay Nutrient Management Strategy[1], which lays out an approach for gathering and applying information to inform key management decisions. The Nutrient Management Strategy calls for several on-going, core programs (assessment framework development, modeling program development, monitoring program development, control/regulatory strategy development) as well as shorter-term special studies to address high priority science questions as they arise.

This document serves as a program update for FY2015, and covers the following topics:

- Ship-based monitoring results for nutrients (Section 2.1) and phytoplankton community composition/toxins (Section 2.2)
- Moored sensor monitoring activities (Section 2.3)
- High-resolution biogeochemical mapping (Section 2.4)
- Nutrient load characterization (Section 2.5)
- Monitoring program development progress (Section 2.6)
- Modeling activities (Section 3),
- Priorities for the upcoming year (Section 4)

---

[1] http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/planningtmdls/amendments/estuarine NNE/Nutrient_Strategy%20November%202012.pdf

2. Monitoring activities

Over the past 40 years, biogeochemical monitoring in SF Bay has occurred via 1-2x monthly ship-based sampling, conducted by researchers at USGS-Menlo Park throughout the entire Bay. Although the USGS' program has yielded one of the premier estuarine water quality datasets worldwide, it was not designed specifically for monitoring nutrient-related issues. The California Department of Water Resources (DWR) manages another ~40 year monthly sampling program in northern SFB and the Delta. Like the USGS program, though, the DWR program is also not specifically designed for nutrients. In addition, the DWR program covers only ~20% of the Bay.

The NMS calls for developing a nutrient-related monitoring program for San Francisco Bay that integrates with and builds upon existing efforts, identifies current gaps in terms of types of measurements or spatial or temporal resolution of sampling and identifies ways to address those gaps. Beginning in 2012, the Nutrient Strategy launched several monitoring activities driven by nutrient-related management questions (See Figure 2.1). While this report will focus mainly on results that have become available in the last year, it is helpful to view them in the context of on-going work by USGS and DWR, and other NMS-related work over the prior 2-3 years. Key observations from these monitoring-related activities are presented in Sections 2.1-2.5, and Section 2.6 provides a brief update on monitoring program development planning.
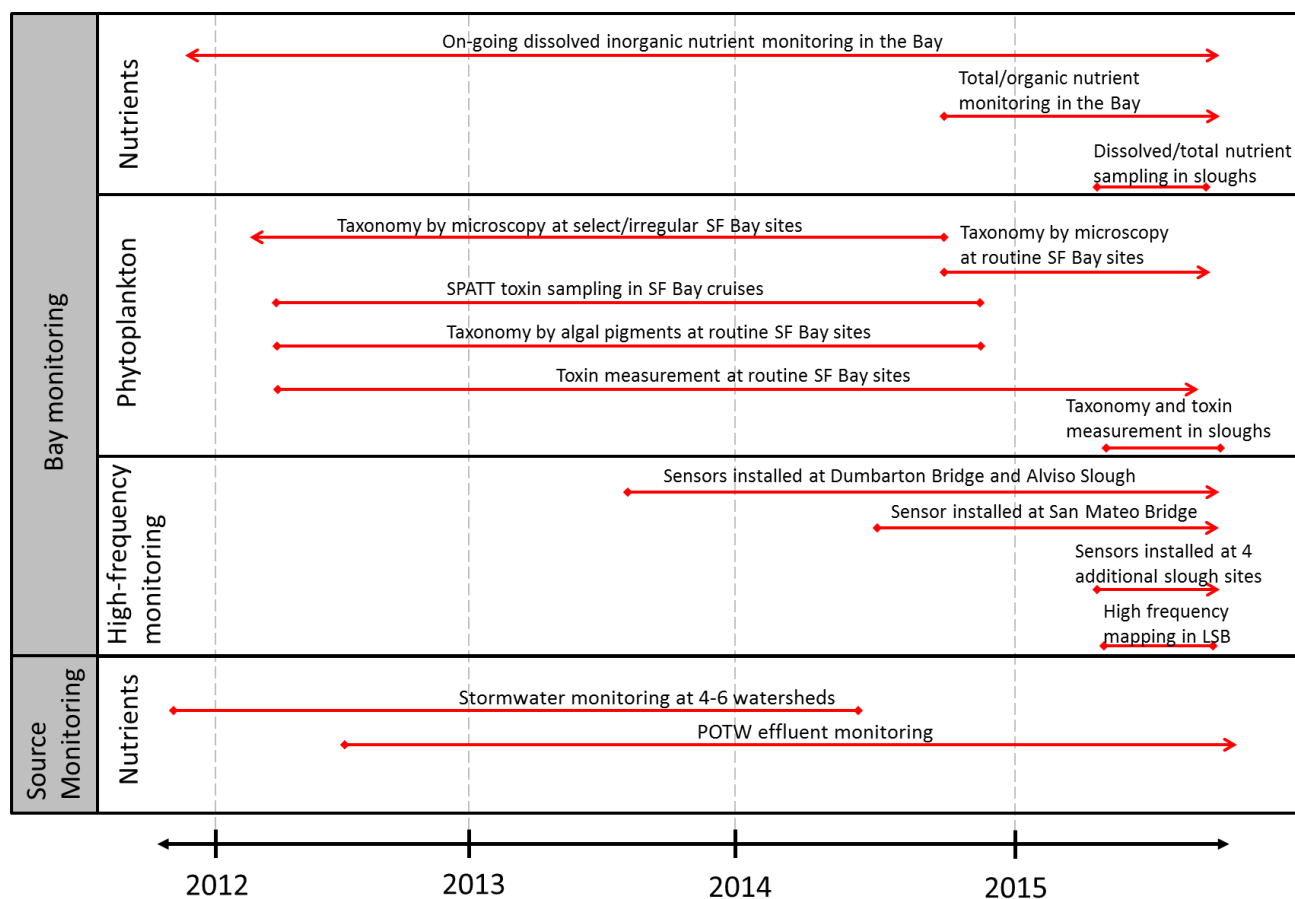
Fig 2.1 Nutrient-related monitoring activities, 2012-present. This report will focus on data from the last year (July 2014-July 2015), but will include older data when relevant

2.1 Ship-based monitoring: nutrients

The USGS research program[2] conducts biweekly (South Bay) and monthly (Bay-wide) surveys aboard the *R/V Polaris*, measuring a range of parameters throughout SFB (Figure 2.2). The Regional Monitoring Program (RMP) achieves some of its Bay monitoring requirements by partnering with and contributing funds (since 1993) to the USGS program. In 2014, the USGS shifted the cost of nutrient analyses to the NMS (14 regular stations, Figure 2.2). The NMS also added total N and total P to the suite of analytes, which were not previously being measured, and are needed for model calibration and nutrient cycling mass balances.

Time series of dissolved inorganic nitrogen (DIN), o-PO$_4$, chl-a concentration and photic depth at a subset of USGS stations for the last decade is shown in Figure 2.3. On average, nutrient concentrations were consistently greatest in South Bay and Lower South Bay. Chl-a concentrations were also greatest in South Bay, despite South Bay having the shallowest photic depth (i.e., depth at which light levels are only 1% of incident light). All subemebayments showed considerable seasonal variability in nutrient concentration and photic depth, and chl-a varied substantially by season and interannually. More detailed discussions of these observations and the underlying drivers of spatial, seasonal, and interannual variability can be found elsewhere (e.g., Cloern et al, 2007; Cloern and Jassby, 2012; SFEI 2014 #731[3]; SFEI 2014 #732[4]).
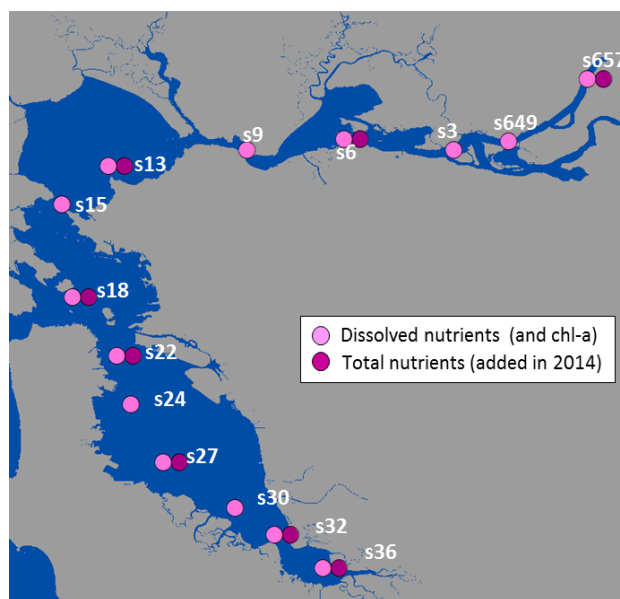
Figure 2.2 Nutrient monitoring locations during 1-2x monthly cruises of the USGS research vessel *R/V Polaris* for 2014-2015. Chl-a sampling occurred at more stations that shown here. When s36 was inaccessible due to tides, sampling occurred at station 34, just north of station 36. Prior to 2014, nutrients sampling occurred at s21, just north of s22, instead of s22.

---

[2] http://sfbay.wr.usgs.gov/access/wqdata/

[3] http://sfbaynutrients.sfei.org/sites/default/files/SFBNutrientConceptualModel_Draft_Final_Oct2014.pdf

[4] http://sfbaynutrients.sfei.org/sites/default/files/LSB_Synthesis_Draft_June%202015.b.pdf
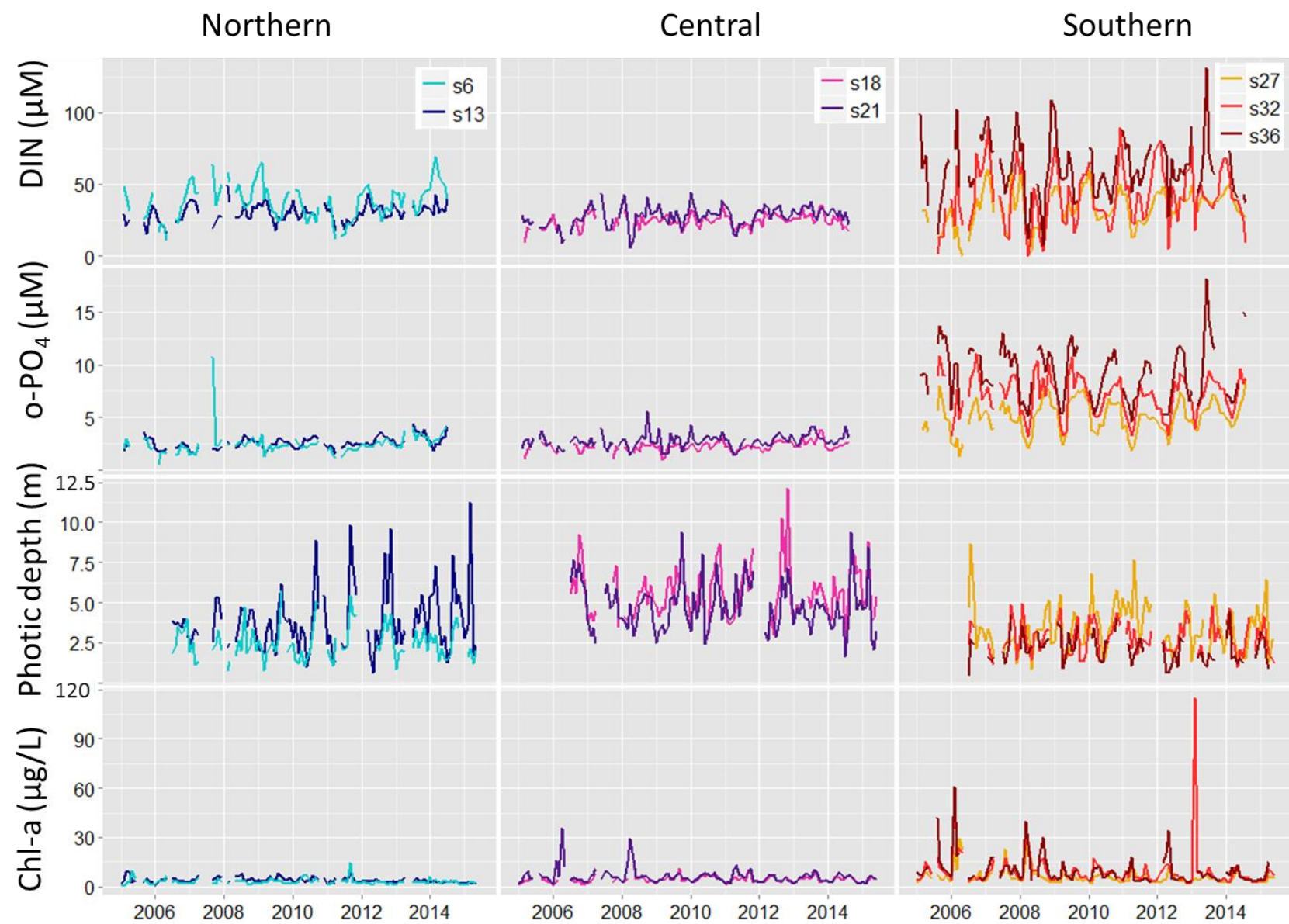
Figure 2.3 Dissolved nutrient concentrations, photic depth and chl-a concentrations at regular *R/V Polaris* monitoring sites, by subembayment, for the last 10 years. Samples were collected in the surface (0-2m)

TN concentrations in SFB (2014-2015) ranged from 17 – 170 µM (Figure 2.3, Table 2.1) and were highest at the northern and southern extents of SFB and lowest in Central Bay, where water exchanges more readily with the coastal ocean. Inorganic N accounted for, on average, 70% of TN Bay-wide and most of the organic nitrogen was present as dissolved organic N (DON). The proportions DIN and DON varied in both space and time, with seasonal component of that variability likely caused by varying freshwater flows (and associated nutrients) and varying intensity of internal nutrient transformation rates. Because of the large DIN contribution to TN, TN and DIN were obviously strongly correlated (Figure 2.4). DON tended to increase proportionally with DIN, with TN:DIN ranging between 1.0-2.0, and most data falling within the range 1.25-1.5, but with a notable subset falling roughly along the 2.0 line. The highest absolute concentrations of organic nitrogen were detected in Lower South Bay (72 µM DON and 55 µM PN), but the TN:DIN was comparable to other subembayments.
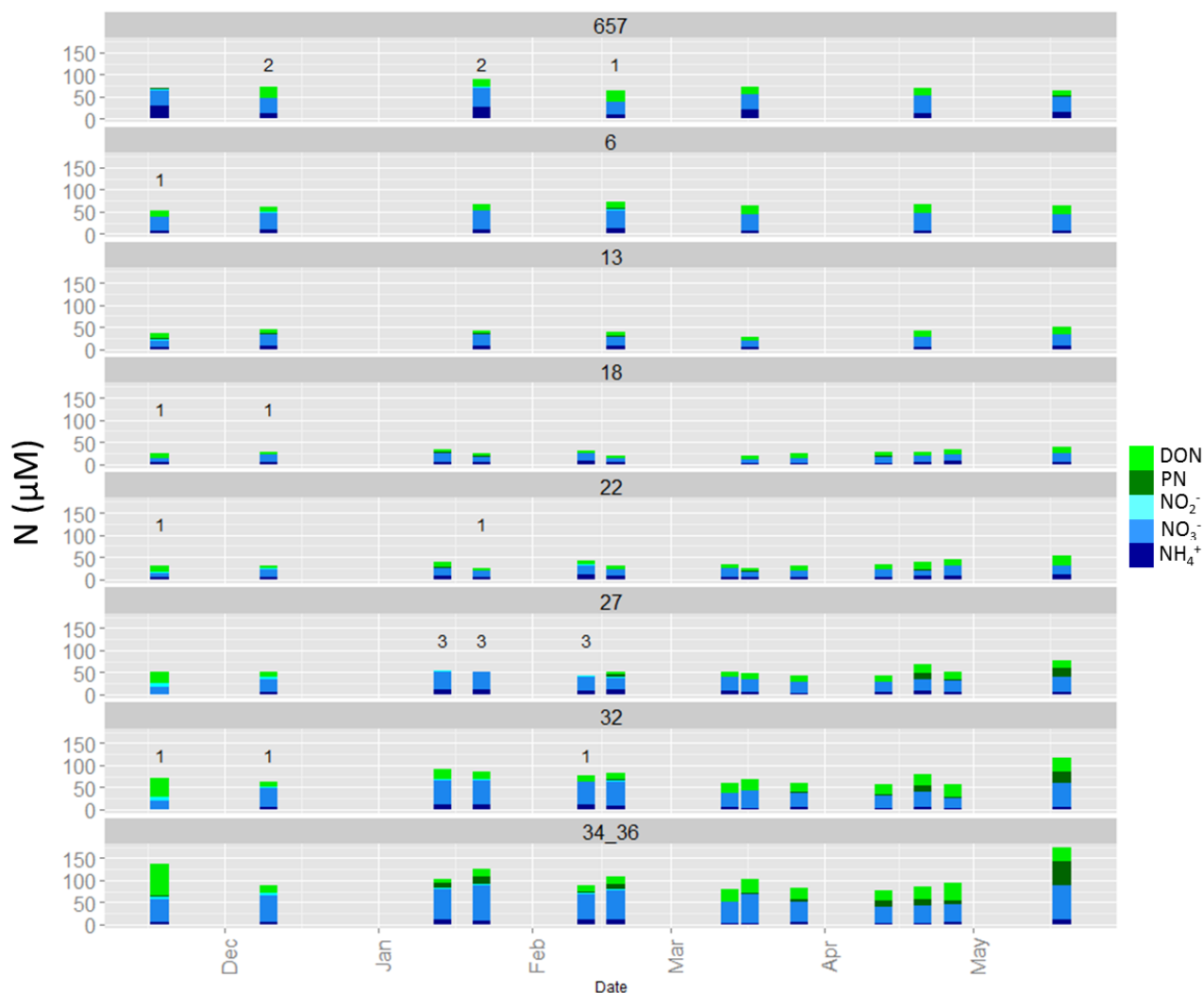
Figure 2.4 Nitrogen speciation and concentrations in open bay samples taken during *R/V Polaris* cruises. Station locations are shown in Figure 2.2. No NO_2^- data is available for samples after Feb 2015, so NO_2^- + NO_3^- is shown (as NO_3^-). Numbers within plot indicate: 1 - calculated PN was negative and assumed small = 0; 2 – TN data unavailable; 3 - TDN data unavailable, therefore PN or PN and DON, respectively not shown

Table 2.1 Average nitrogen speciation in open-Bay samples (PN is when PN >=0) taken during *R/V Polaris* cruises

	%NO2	%NO3	%NH4	%DON	%PN	TN range
Delta	3%	49%	26%	21%	1%	64 − 72 µM
Suisun Bay	3%	58%	13%	25%	1%	52 − 72 µM
San Pablo	4%	52%	14%	27%	3%	28 − 46 µM
Central	4%	47%	16%	32%	1%	17 − 35 µM
South	6%	45%	16%	30%	3%	25 − 78 µM
Lower South	4%	54%	7%	29%	6%	54 − 174 µM
Bay-wide	*4%*	*50%*	*15%*	*27%*	*4%*	*17 − 174 µM*
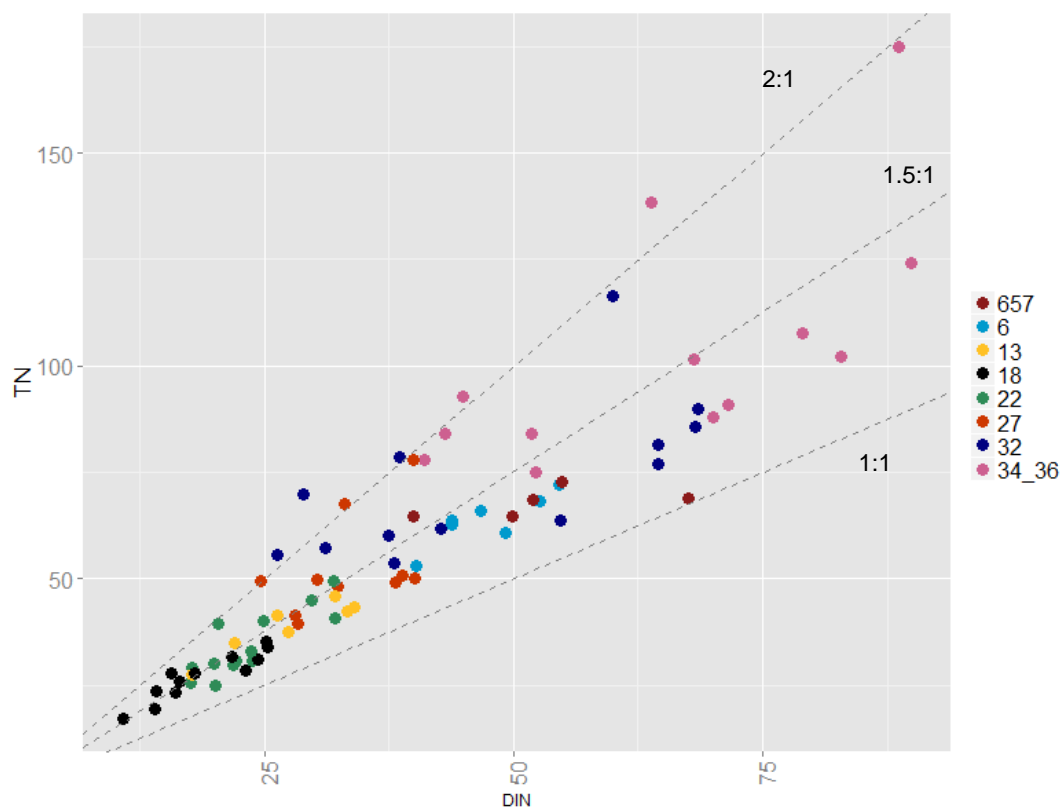
Figure 2.5 TN vs. DIN concentration (in µM), by station, during *R/V Polaris* cruises Nov 2014 − May 2015. Dashed lines show slopes of 1:1, 1.5:1, and 2:1.

Bay-wide, total phosphorous concentrations ranged from 2 − 19 µM and o-PO$_4$ accounted for 76% of TP (Figure 2.6). Most of the non-o-PO4 was present as particle-completed P. The laboratory methods used do not distinguish between P associated with organic vs. inorganic particles; however, because o-PO4 binds readily to the surfaces of common minerals (e.g., iron oxides) it is possible that much of the particulate P is associated with inorganic material. Similar to TN:DIN, values of TP:o-PO$_4$ ranged between 1.0-2.0, but more of the TP:o-PO4 values falling between 1-1.5.
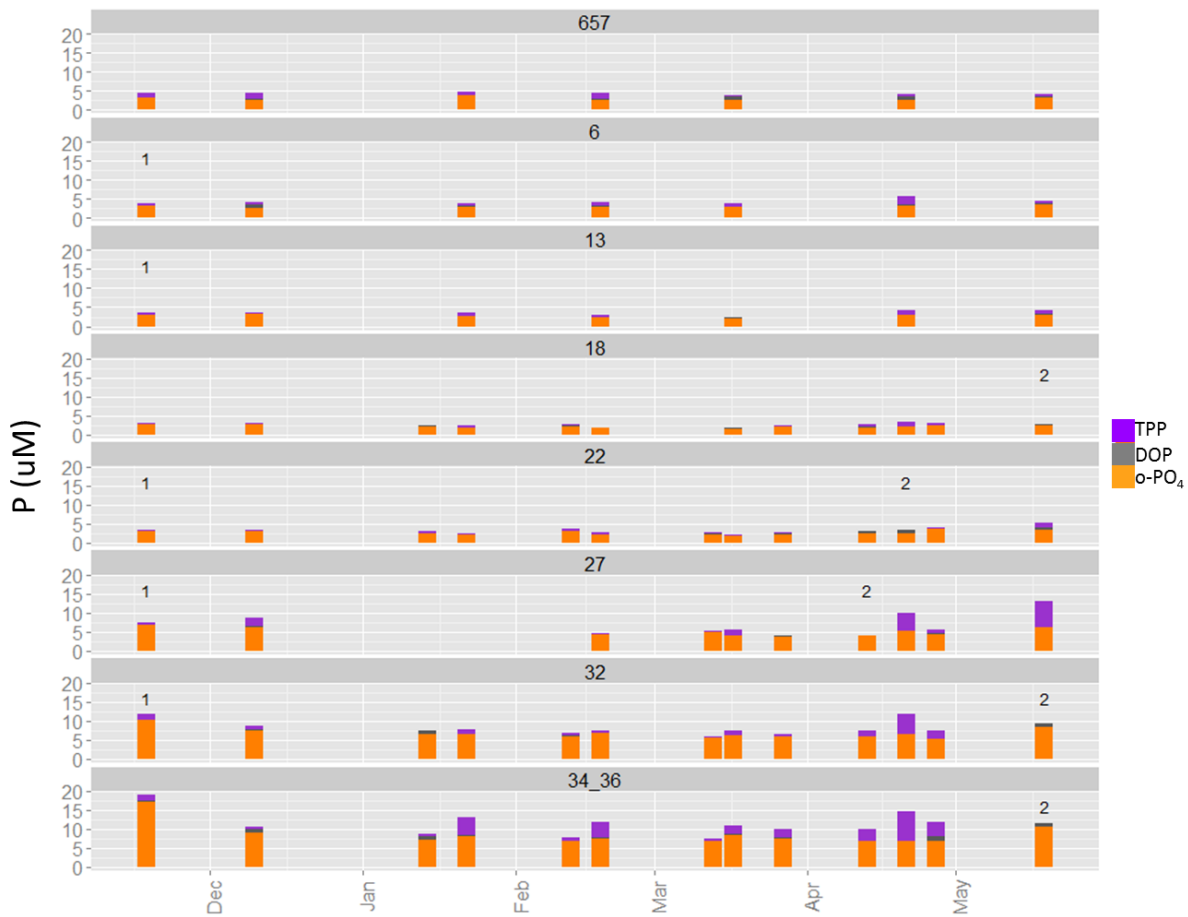
Figure 2.6 Phosphorous speciation and concentrations in open bay samples taken during *R/V Polaris* cruises. Station locations are shown in Figure 2.2. Numbers within plot indicate: 1 – DOP (calculated) was negative (i.e., small) and is not shown; 2 – TPP data not available

Table 2.2 Average phosphorus speciation in open-Bay samples (DOP is when DOP>=0) taken during *R/V Polaris* cruises

	%o-PO4	%DOP	%TPP	TP range
Delta	67%	9%	24%	4 – 5 µM
Suisun Bay	73%	5%	22%	4 – 6 µM
San Pablo	79%	<1%	20%	2 – 4 µM
Central	80%	4%	16%	2 – 3 µM
South	80%	3%	17%	2 – 13 µM
Lower South	79%	2%	19%	6 – 19 µM
Bay-wide	*76%*	*4%*	*20*	*2 – 19 µM*
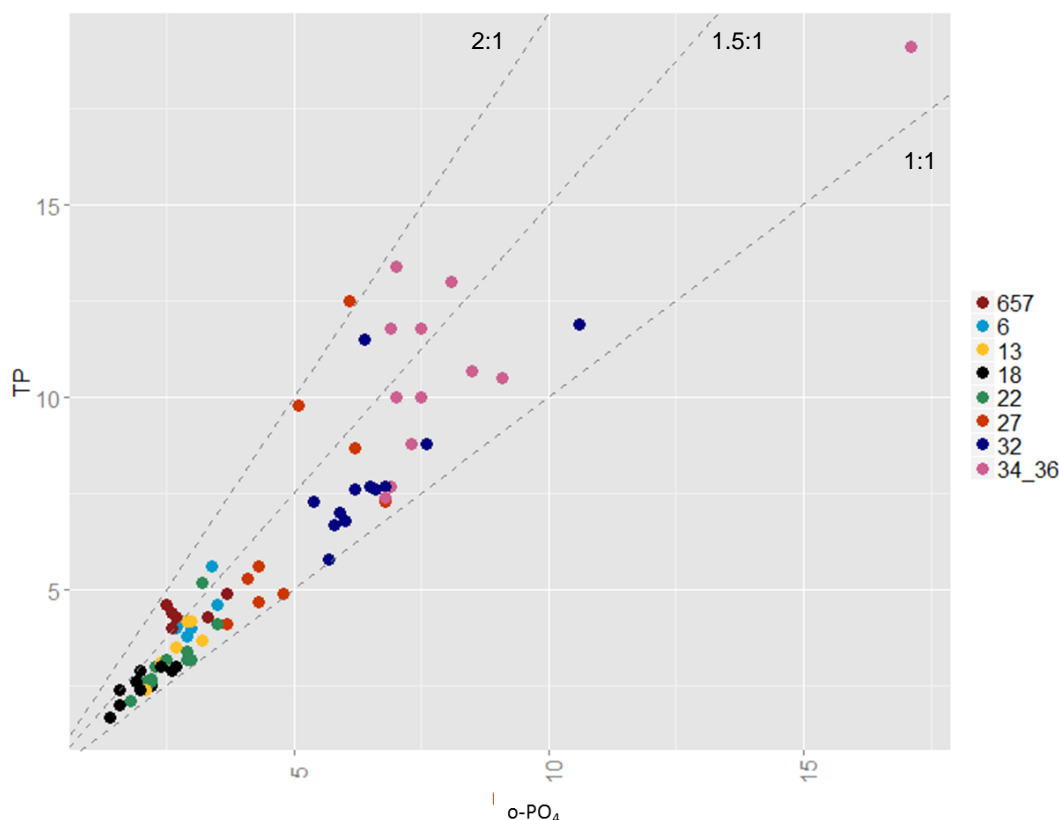
Figure 2.7 o-PO$_4$ vs TP concentration (in μM), by station, during *R/V Polaris* cruises Nov 2014 – May 2015. Dashed lines show slopes of 1:1, 1.5:1, and 2:1.

At the point of this report's writing, only 6 months of TN and TP data were available. Therefore, no inferences can be made at this point about the seasonal or interannual variability of DON and TPP concentrations or the ratios of TN:DIN and TP:o-PO4. However, the fact that TN:DIN and TP:o-PO4 differed substantially from 1:1, and varied in both space in time, underscores the importance of the TN and TP data for mass balances and model calibration and argues for continued measurements.

2.2 Phytoplankton community composition and HAB toxin monitoring

Knowledge about the types and abundances of phytoplankton taxa present in the SFB is important for several reasons:

- Phytoplankton comprise the majority of food for aquatic organisms, and the quality of phytoplankton-derived food can vary depending on the types of organisms present
- Some phytoplankton species produce toxins that adversely impact other organisms or have other harmful effects (rapid biomass accumulation, surface scums). Blooms of these organisms are referred to as harmful algal blooms (HABs) and can have severe impacts on ecosystem health if large HABs occur with sufficient frequency.
- Nutrients, in synergy with many other factors, can influence the types of phytoplankton that grow and the occurrence of HABs.

Phytoplankton community composition data (via microscopy) has been collected in SFB by USGS since approximately 1991, but those measurements have been performed with varying spatial and temporal frequency (typically, only when chl-a > 5 μg/L). Beginning in 2014, NMS funds began supporting analysis

of phytoplankton community composition in samples collected biweekly or monthly at a consistent set of stations throughout SFB and independent of chl-a levels (Figure 2.8). The NMS also funded collaborative phytoplankton and HAB projects between the USGS, UC Santa Cruz, and SFEI, including:

- a pilot project to analyze phytoplankton pigments in archived samples from 2011-2013, to assess the utility of this method for characterizing community composition at the class level.
- Measurement of algal toxins by several techniques to assess condition and inform monitoring program design: spatially-integrated water column toxin measurements (SPATT); toxins accumulated in deployed mussels; and algal toxins in in discrete samples
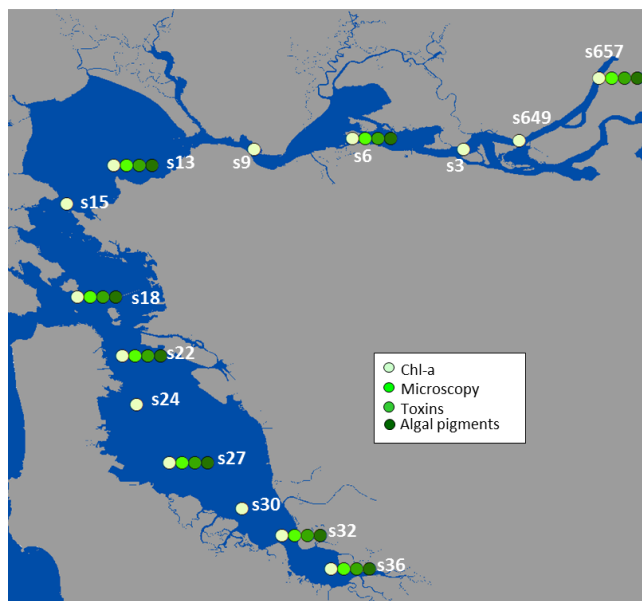
Figure 2.8 Locations of phytoplankton related sampling aboard the *R/V Polaris*. Chl-a sampling has occurred at these (and other) locations since 1975. Microscopy sampling occurred with regularity since Nov 2014, and irregularly before then (back to 1991). Toxins and algal pigment sampling has occurred since 2012.

2.2.1 Phytoplankton community composition by microscopy (Sep 2014-May 2015)

Phytoplankton community composition results, as biovolume (μm^3/mL), indicate that diatoms were the dominant class of organisms throughout the 9 month period Bay-wide (Figure 2.9; for an expanded y-axis scale see Figure A.1.1). Diatoms were especially dominant during bloom conditions, (defined here as chl-a > 10 µg/L, which roughly corresponded to >= ~2e6 μm^3/mL). These observation are generally consistent with past reports on phytoplankton community in San Francisco Bay (Cloern and Dufford, 2005), and consistent with a recent meta-analysis of estuaries worldwide that showed a predominance of diatoms. During low chl-a conditions, other phytoplankton classes accounted for larger proportions of the community biovolume. Cryptophytes accounted for a non-trivial amount (up to 25%) of total biovolume in all subembayments during certain times, and were particularly abundant in South Bay (station 27) and San Pablo Bay (station 13), where they are more than 25% of the biovolume on average and more than half during certain low chl-a samples. Dinoflagellates were almost entirely absent in the Delta, but represented significant portions of the community's biovolume in Central and South Bay, at times nearly half of the total biovolume at stations 22 and 27. Cyanobacteria comprised a larger percentage of biovolume at northern stations (657, 6), but the greater relative abundance of cyanobacteria appears to have been due in part to lower overall biovolume. Non-trivial cyanobacteria biovolume was also observed in Lower South Bay on some dates, although it remained low there as a percentage of total biovolume. This phytoplankton data is the focusing of on-going analysis, with further reporting expected over the next year.
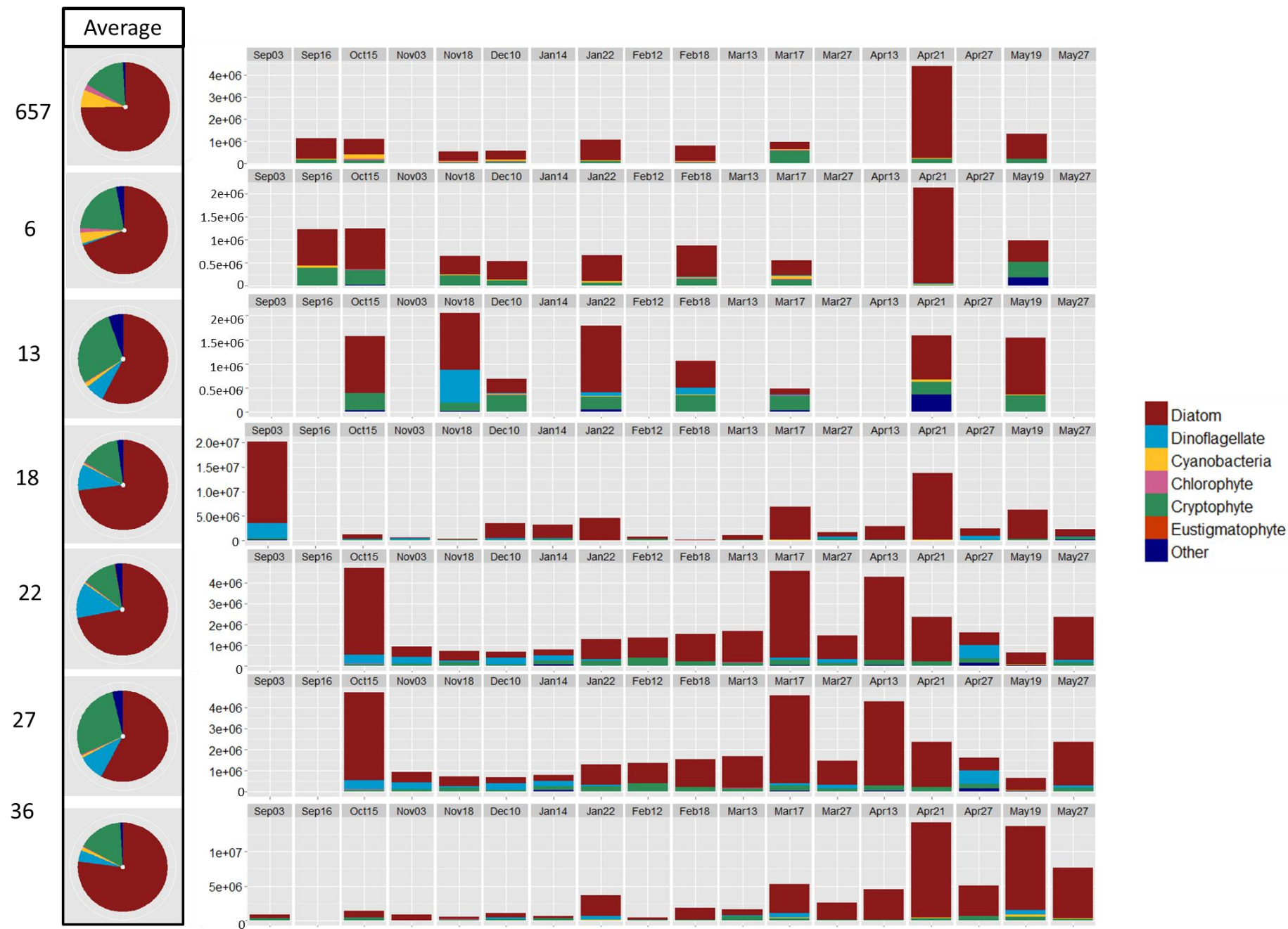
Figure 2.9 Biovolume (µm^3/mL) by phytoplankton class as analyzed by microscopy, Sept 2014 – May 2015. Note: variable y-axis scales between sites.

13

2.2.2 Phytoplankton pigment measurements to assess community composition

Microscopy is an expensive, labor-intensive method for analyzing phytoplankton community composition. Results may also be subject to considerable uncertainty (i.e. different microscopists and methods), and may underrepresent some phytoplankton classes, especially those comprised of small organisms. The NMS funded a collaborative project between USGS, UC Santa Cruz and SFEI to measure algal pigments in a 3-year set of regularly-sampled stations (biweekly or monthly; see locations in Figure 2.8). The methodology and results from this project are presented in more detail in a separate technical report (see Appendix 3 and link[5]) and a forthcoming manuscript, and an overview of the results is presented here. Samples were analyzed for a number of pigments, and a mathematical model (CHEMTAX; NASA SEAHAARE reports) was used to calculate each class' contribution to total biomass (as a proportion of chl-a) based on distinct pigment fingerprints of each class. Paired pigment and microscopy samples were used to tune CHEMTAX for SFB and to evaluate the accuracy of its estimates.

CHEMTAX-derived community composition estimates (as a fraction of total chl-a) agreed well with analysis of paired microscopy samples (biovolume) at the full Bay scale and over the initial 2.5 years of data (See Figure 2 in Appendix 3). Diatoms represented 70% of the biovolume by microscopy and 65% of the biovolume by CHEMTAX. Dinoflagellates were 22% by microscopy and 24% by CHEMTAX, and cryptophytes were 6% in both. CHEMTAX was more sensitive to detecting smaller phytoplankton such as cyanobacteria. While cyanobacteria did not comprise a large portion of the community's biomass Bay-wide and over the entire dataset (<1% on average), they did represent higher proportions of biomass in some subembayments and on some dates (see below). In addition, a disproportionate number of cyanobacteria species are considered harmful, and need to be monitored for that reason. Agreement between the whole-Bay-tuned CHEMTAX model and CHEMTAX tuned for individual subembayments was also evaluated. Differences between microscopy and CHEMTAX were most pronounced in San Pablo (see Figure 3 in Appendix 3). However, adjusting the input parameters for CHEMTAX to be specific to San Pablo Bay, rather than SFB overall, resulted in closer agreement.

The record of phytoplankton community composition, determined by pigments and CHEMTAX, over 2.5 years at a subset of stations reveals interesting seasonal and spatial patterns (Figure 2.10; for an expanded y-axis, see Figure A.1.2). On average Bay-wide, diatoms were the largest contributor to chl-a, particularly during bloom events (chl-a >10 µg/L). Figure 2.10 presents results in terms of proportion of total chl-a contributed by each class, which precludes direct quantitative comparison with the biovolume-related results in Figure 2.9. However, the relative proportions can be compared qualitatively. For example, the pigment-CHEMTAX approach from Nov 2011-Apr 2014 suggests greater relative abundances of cyanobacteria in the Delta/North Bay (stations 657 and 6) and in Lower South Bay (stations 34 & 36), as did microscopy data from 2014-2015 (Figure 2.9). The pigment-CHEMTAX results point to greater proportions of cyanobacteria than microscopy. This may be due to the fact that the samples are from non-overlapping time periods, or it may be due to the pigment-CHEMTAX approach being more readily able to detect small phytoplankton species. The pigment-CHEMTAX time series identified important contributions of dinoflagellates to biomass in Central Bay (station 18), and at other locations on individual dates.

Upcoming work includes a mechanistic exploration of phytoplankton community composition data, including how community composition varies seasonal and spatially, and in response to potential regulating factors (temp, salinity, nutrient concentrations)

---

[5] http://sfbaynutrients.sfei.org/sites/default/files/Algal_Pigment_FinalReport_DRAFT_Oct2014.pdf
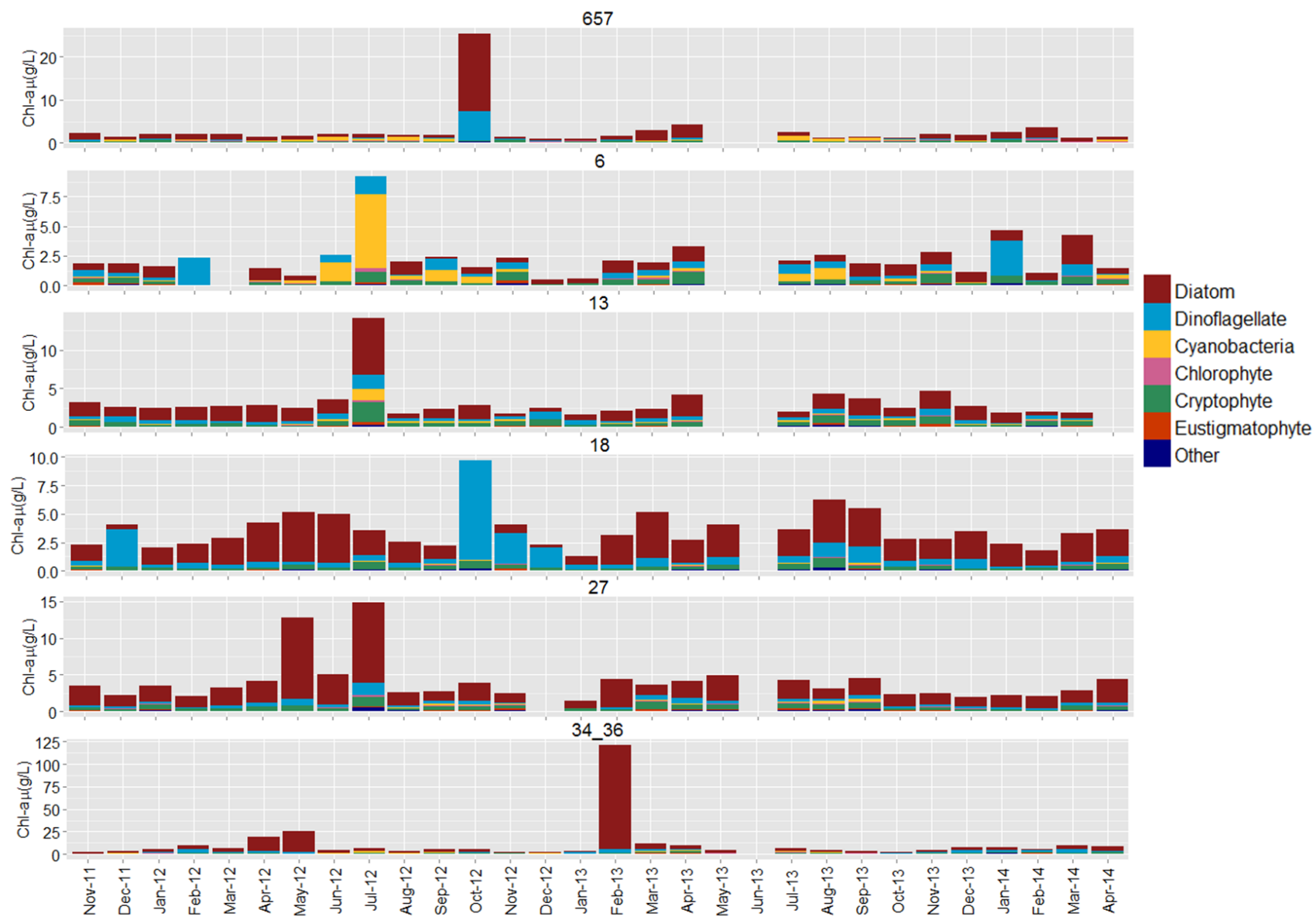
Figure 2.10 Results of algal taxonomy by pigment analysis (CHEMTAX) for November 2011- April 2014, shown as chl-a contribution by class. Note differing y-axis.

2.2.3 Monitoring for algal toxins in San Francisco Bay

Toxins in the Water Column

Some phytoplankton species form harmful algal blooms (HABs) and produce toxins that adversely impact both aquatic life and humans. Links between anthropogenic nutrients and HABs/toxins have been shown in some estuaries. However, the frequency and severity of HABs are influenced by the complex interplay of multiple physical, chemical, and physiological factors, and until recently, there had been no investigations exploring HABs in SFB. Developing an improved understanding of algal toxin levels in SFB and the relationship between nutrients and HABs/toxins are among the highest priority science and monitoring needs of the NMS. Three NMS projects related to algal toxins were launched over the past 3 years as collaborative studies between UC Santa Cruz, USGS, and SFEI. These studies were designed to provide information on current toxin levels in SFB, guide monitoring program development related to toxins, and begin to identify potential toxin sources and factors that may influence HABs and toxin production in the Bay. Initial results from those studies have become available in the past year and are described below.

Beginning in late 2011, USGS began collecting spatially-integrated toxin samples during biweekly (South Bay) and monthly (Full Bay) *R/V Polaris* cruises. The sampling technique involves placing a passive sampler (referred to as Solid Phase Absorption Toxin Tracking, SPATT) in a continuous stream of water that the *Polaris* pumps from the Bay while underway. The SPATT sampler accumulates toxin from the water, and the toxin was later extracted and measured in the laboratory at UCSC. One sampler was used per subembayment, providing spatially-integrated measures of toxin for broad areas of the Bay. The initial focus of the measurements has been on two toxins: domoic acid (DA), produced by the marine diatom, *Pseudo-nitzschia* spp. and microcystin (MC) produced by the cyanobacteria *Microcystis spp.* The full technical report for this project was completed in mid 2015 and is included as an appendix to this report (see Appendix 4 and link[6]).

DA and MC were detected in 97% and 72% of samples, respectively, over three years in all seasons and throughout SFB (Figure 2.11). DA is most commonly associated with marine environments, yet was detected throughout SFB, including its freshest subembayment, Suisun Bay. In an analogous manner, MC is most commonly considered a freshwater algal toxin, yet was observed throughout SFB, including Central Bay and South Bay where salinities commonly exceed 25-30 ppt. In both cases, the toxins' widespread detection raises the question of whether the coastal (DA) and freshwater (MC) inputs were the only sources, or if in some cases the compounds were produced by resident organisms within SFB. While it is possible for both compounds to persist for extended periods after their production, sufficient inputs would be needed for them to be detected after dilution. Observed concentrations in SFB ranged from 0-400 ng /g-resin for domoic acid and 0-25 ng /g-resin for microcystins. On multiple occasions, SPATT samples exceeded 20 ng MC/g-resin and 150 ng DA/g-resin, levels that prior studies using SPATT and water column grab or biota samples found corresponded to OEHHA's 0.8ppb MC alert level for water grab samples and OEHHA's 20 ppm DA threshold for mussel tissue (see Kudela 2015). Since no regulatory guidance is available based on SPATT concentrations, the comparison between SPATT and OEHHA action levels in water or tissue provides a necessary means for assessing the importance of these concentrations. However, the comparisons also need to be considered as approximate because of nontrivial variability in the ratios SPATT:water and SPATT:tissue, potential differences between systems, and some methodological uncertainties (e.g., is it a cumulative signal, or more of an equilibrium signal?; see Kudela 2015).

---

[6] http://sfbaynutrients.sfei.org/sites/default/files/SPATT%20Final%20Report%20May2015.pdf
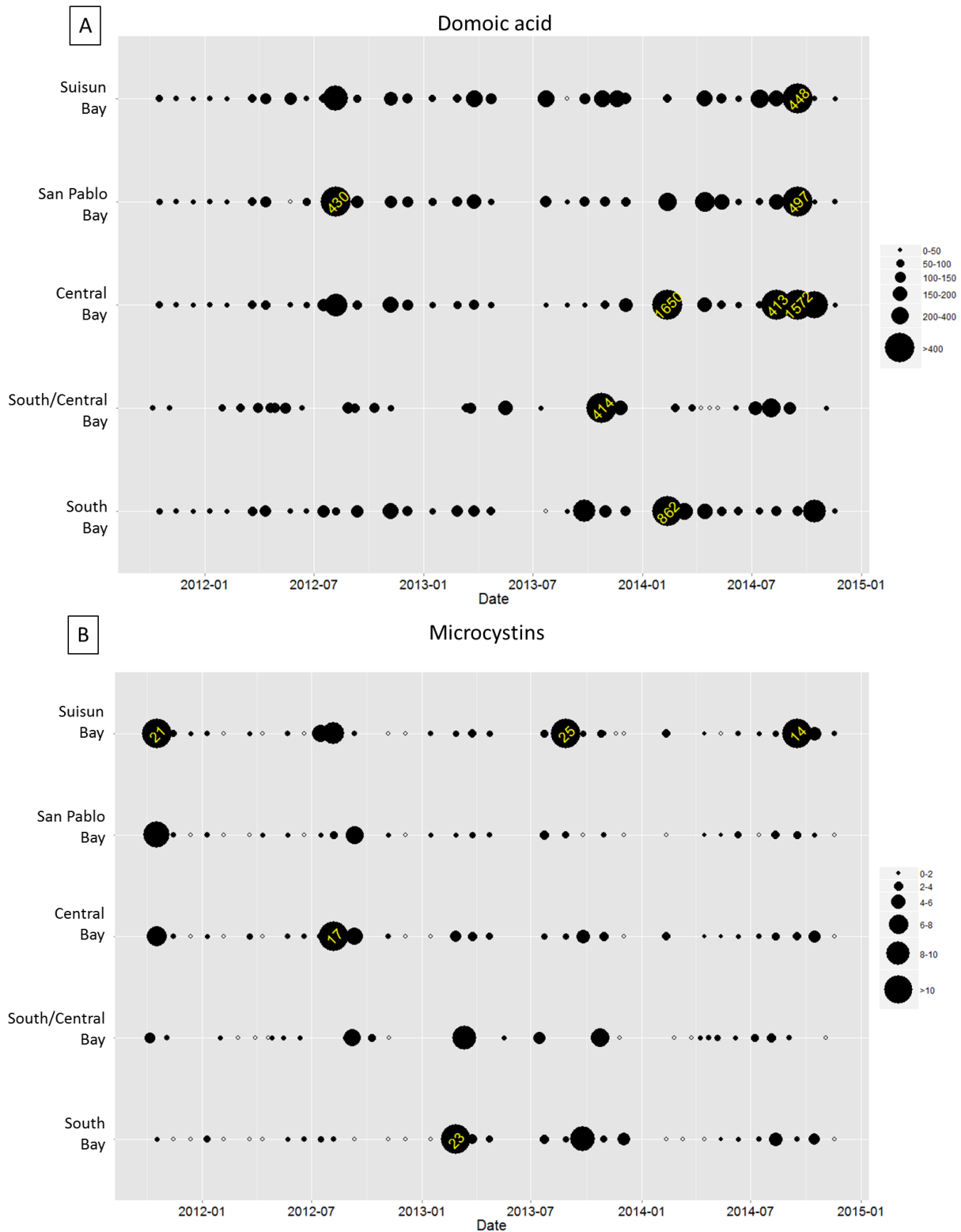
Figure 2.11 SPATT resin concentrations (ng/g) of domoic acid (A) and microcystins (B), by Bay segment. "South/Central Bay" refers to special cruises that stopped at Central Bay (1 SPATT bag for this entire region); other Bay segments were not sampled on these dates. Actual resin concentrations for high values are annotated. Instances were SPATT were deployed but no toxin was detected are noted with an open circle.

The SPATT study was the first SFB-wide investigation of toxins, prior to which toxin levels were essentially unknown. SPATT was an ideal method for this exploratory study. The integrated SPATT signal (combined with the low detection limits of the laboratory techniques) maximized the likelihood that, if DA or MC were present along a transect, they would be detected. It is also a cost-effective monitoring technique because it required only one sample per subembayment. However, the SPATT approach also had its limitations. One limitation was noted above – large uncertainty in translating SPATT concentration to water or biota concentrations. A second limitation is related to the long transects over which SPATT samplers integrated during this initial study, especially the combined transect of South Bay and Central Bay during abbreviated cruises. Long transects are cost-effective (fewer samples to analyze) but make it difficult to glean insights about toxin sources. SPATT measurements are continuing in FY2016, but on some cruises transect lengths will be adjusted to help better discern sources. In addition, toxin concentrations are being measured in a set of from archived filters from grab samples to better resolve spatial variability in toxin levels. Beginning in 2011, USGS began collecting and archiving water column toxin samples at regular stations during *Polaris* cruises (see locations in Figure 2.8). Analysis of those samples is underway and results will be presented in a forthcoming technical report.

Toxins in Biota

Filter feeding bivalves, like mussels and clams, tend to accumulate algal toxins, and can serve as bioindicators of toxin levels. While the SPATT samplers deployed along USGS *Polaris* transects serve as spatially-integrated toxin samplers at a given point in time, bivalves from specific locations can be used as time-integrated bioindicators of algal toxin levels.

During FY2015, we began measuring algal toxins in Bay bivalves. In Fall 2012 and 2014, the Regional Monitoring Program (RMP) deployed mussels (*Mytilus californianus*) at 11 stations throughout San Francisco Bay for 90 days. Researchers at UC-Santa Cruz analyzed tissue aliquots from the mussel samples for MC and DA, and a third compound, saxitoxin, which is produced by marine dinoflagellates from the group *Alexandrium*. DA was the most frequently detected of the three toxins, present in 100% of Bay samples in both 2012 and 2014 (Figure 2.12). MC was also quite commonly detected, present in 80% of 2012 samples and 100% of samples in 2014 (although there were 50% fewer samples collected in 2014). Saxitoxin was the least frequently detected toxin, present in fewer than 50% of Bay samples in 2012 but 80% of Bay samples in 2014. Whereas there was some uncertainty from the SPATT results about the exact locations where toxins were present, mussel observations provide clear location-specific evidence of toxin occurrence. While DA concentrations in these samples were 40-100-fold less than regulatory limits for shellfish consumption (20 ppm), and saxitoxin concentrations were 20-30-fold lower than regulatory limits (800 ppb), several MC concentrations approached or exceeded proposed action levels of 10 ppb.[7] A major limitation of this particular dataset is that mussels were deployed for 90 days, making it difficult to determine what the maximum tissue concentrations were, especially since DA tends to be rapidly excreted by this species of mussels (half-life ~ 1week).

Analyzing toxins in bivalves from around SFB will be a major focus of work in 2015. A large bloom of the harmful diatoms, *Pseudonitzchia* spp., that produce domoic acid occurred off the Pacific Coast during spring 2015. NMS collaborators at UCSC used this event as a natural experiment to examine the degree to which toxins produced along the coast enter SFB, and collected naturally-occurring mussels from around Central Bay. Mussels are also being collected throughout SFB on a biweekly or monthly basis. The biota results, along with water column toxin samples, will shed further light on how toxin levels vary in space and time, and how to best monitor for toxins.

---

[7] http://www.waterboards.ca.gov/water_issues/programs/peer_review/docs/calif_cyanotoxins/cyanotoxins053112.pdf
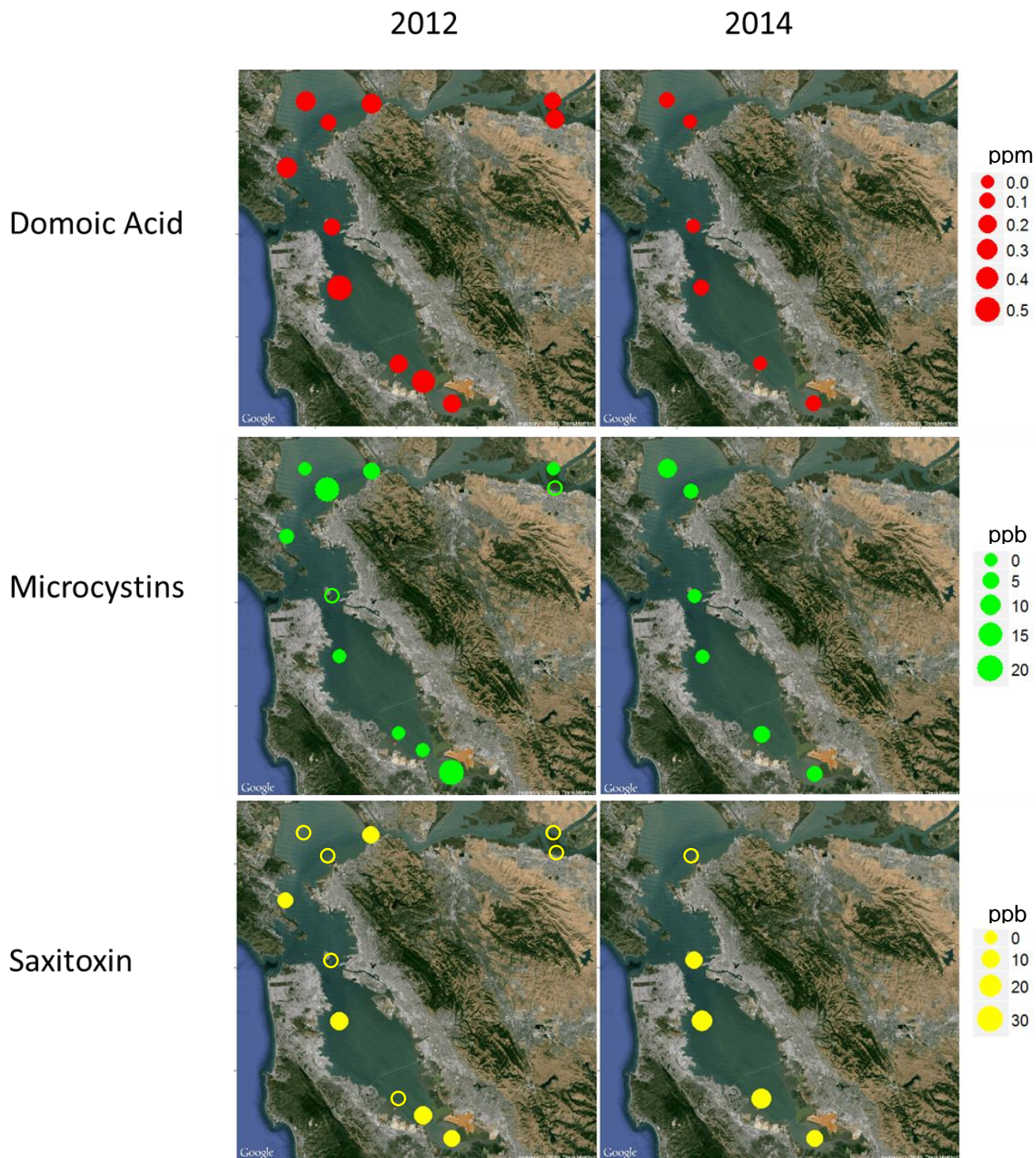
Figure 2.12 Toxin concentrations in mussel samples deployed in collaboration with the RMP in 2012 and 2014. Fewer samples were analyzed in 2014 because of problems retrieving the samples from the Bay. Open circes indicate where samples were collected but toxin was not detected

2.3 High frequency monitoring with moored sensors

Since many nutrient-related processes and parameters vary on tidal or even hourly time scales, water quality data are needed at high temporal resolution, both to assess condition and calibrate biogeochemical models. However, most ambient water quality monitoring in SF Bay over the past 40 years has been carried out in deep subtidal habitats, in particular the Bay's deep main channel, on a biweekly or monthly basis. While some stations in Suisun Bay and the Delta measure chl-a fluorescence and nitrate at high-frequency (operated by USGS and DWR), prior to 2013 no high-frequency chl-a data was being collected in other subembayments. In addition, little systematic high-frequency monitoring was being performed in sloughs and creeks in LSB for chl-a or other parameters, including DO. In 2013, the NMS initiated work to address these data gaps. This section provides an update on Year 2 (FY2015) NMS moored sensor activities and results. Sensor maintenance, data quality, and data management are described to Appendix 2. Moored sensor data from all sites, as well as from select USGS and DWR sites, can also be accessed and manipulated through a beta-version web-based high-frequency data visualization tool that is under development by SFEI for the NMS (www.enviz.org). Recommendations for future years of the NMSP are presented in the Section 4.

NMS moored sensor work began in summer and fall 2013 by installing moorings at the Dumbarton Bridge and in Alviso Slough (Figure 2.13), through a collaborative effort between SFEI and USGS-Sacramento. Sensors measure a variety of parameters at 15-minute intervals (temperature, conductivity, dissolved oxygen, chl-a fluorescence, turbidity, fluorescent dissolved organic matter). In July 2014, a third station was added at San Mateo Bridge. In Spring 2015 additional stations were added in several slough sites and in Coyote Creek (Figure 2.13). The discussion below focuses primarily on DO and chl-a fluorescence. Additional parameters are presented in Figures A.1.3 – A.1.9. Data quality and details related to calibration and maintenance are discussed in Appendix A.2.
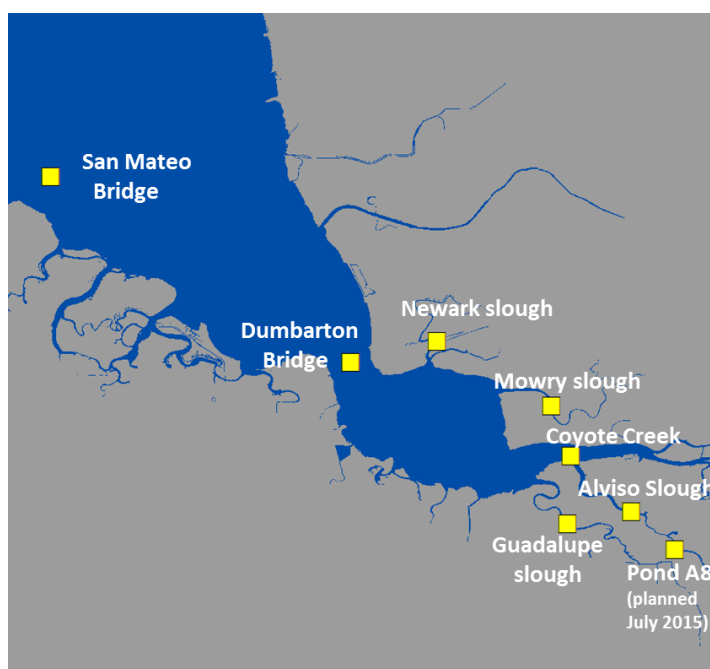
Figure 2.13 Location of moored sensor monitoring locations operated by SFEI in collaboration with researchers at USGS Sacramento. All sites monitor depth, temperature, conductivity, turbidity, chl-a fluorescence and dissolved oxygen. Details about each site can be found in Table 2.3

Table 2.3 Details on moored sensor locations shown in Figure 2.13

	Date installed	Vertical position	Slough or channel?	Site details
Dumbarton Bridge	July 2013	12.5m from bottom 1-4m from water surface, (depending on tide)	Channel	USGS also maintains two sensors here, 4ft and 25ft from bottom. Monitoring T, SpC, DO and turbidity
San Mateo Bridge	July 2014	0.5m from surface at all times (floating)	Channel	USGS also maintains two sensors here, 10ft and 44ft from bottom. Monitoring T, SpC, DO and turbidity
Alviso Slough	Sept 2013	0.5m from bottom 1-4m from water surface	Slough	USGS also maintains a sensor here. Monitoring T, SpC, DO and turbidity. Alviso Slough receives freshwater input (via Guadalupe River) and has connections to former salt ponds upstream and downstream from the sensor site. There is no direct POTW input to Alviso Slough.
Newark Slough	April 2015	1.2m from bottom 0-3m from water surface (depending on tide)	Slough	Newark Slough is a dead-end slough (no freshwater input), has no salt pond connections, and receives no POTW input
Coyote Creek	May 2015	1.2m from bottom 2-5m from water surface (depending on tide)	Slough	Located at the confluence of Coyote Creek and Alviso Slough. Coyote Creek receives freshwater input (via Coyote Creek) and POTW input from the City of San Jose (via Artesian Slough).
Mowry Slough	June 2015	0.5m from bottom 0-3m from water surface (depending on tide)	Slough	Mowry slough is a dead-end slough (no freshwater input), has no salt pond connections, and receives no POTW input
Guadalupe Slough	June 2015	0.5m from bottom 0-3m from water surface (depending on tide)	Slough	Guadalupe slough receives freshwater input (via Saratoga Creek). It has connections to former salt ponds and also receives POTW input from Sunnyvale (via Moffett Channel)
Pond A8 outlet	July 2015	0.5m from bottom	Slough	Located just downstream from the flow gate at former salt pond A8 in a small side channel off Alviso Slough

High-frequency data at the San Mateo Bridge, Dumbarton Bridge and Alviso Slough sites for July 2014 – June 2015 show that important water quality indicators like DO and chl-a fluorescence exhibit complex temporal and spatial variability (Figure 2.14). High quality data were obtained for 80-90% of the year for DO and chl-a (and most other parameters) at these three sites; some data were deemed low quality or lost due to fouling or sensor failure (see Appendix 2 for details). On an annual basis, main channel stations (San Mateo Bridge, Dumbarton Bridge) showed less variability and fewer extreme values than at Alviso Slough. At open channel stations, the relationship between chl-a (µg/L) and RFU is approximately 4:1 (Figure A.2.2), which translates to open Bay chl-a concentrations of 4-24 µg/L over the time period shown in Figure 2.14. The chl-a data suggests that modest blooms lasting 1-2 weeks occurred in Fall 2014 around San Mateo Bridge and Spring 2015 around Dumbarton Bridge and San Mateo Bridge and also reveal substantial high-frequency variability in chl-a levels (3-5 µg/L over hourly time scales), especially at Dumbarton Bridge. This variability would not have been captured by ship-based monitoring, and could be important for carbon and DO budgets and improved mechanistic interpretations of phytoplankton productivity (SFEI 2014 # 732). The chl-a:RFU relationship at Alviso Slough (~2.5:1) differs substantially from the open-Bay relationship and has greater scatter (Figure A.2.2), likely due to a combination of elevated (and variable) suspended sediment and dissolved organic matter levels. Efforts are underway to develop an improved understanding of, and an improved calibration (i.e., decreased uncertainty) for, this relationship through correcting for interferences. Even

with the uncertainty, it is clear that chl-a concentrations in Alviso Slough (2-150 µg/L) substantially exceeded those in the open-Bay, and were highly variable. The large DO supersaturations in early 2015 coincided with periods of elevated chl-a fluorescence, both of which are consistent with high primary production rates. One working hypothesis for the elevated chl-a and supersaturated DO is that high rates of phytoplankton were occurring in an adjacent salt pond that exchanges with Alviso Slough ~2.5 km upstream of the moored station.
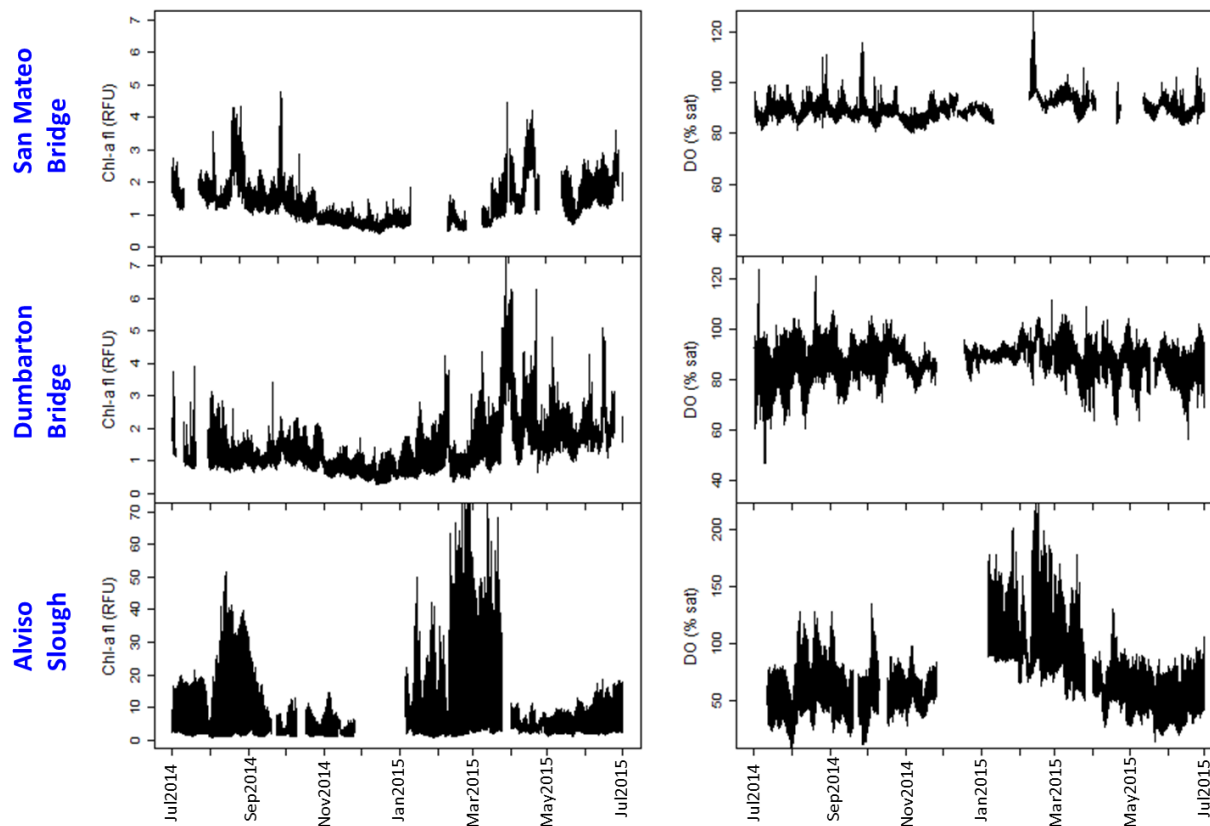
Figure 2.14 Timeseries for high-priority parameters during Year 2 of sensor operation at long-term sites. Data gaps are described in Appendix 2

Focusing on the central tendencies of monthly chl-a and DO data over an annual cycle helps illustrate the seasonal and spatial variability (Figure 2.15). The interquartile range of phytoplankton biomass, inferred from chl-a fluorescence, increased at both open-Bay sites between winter and spring. At the San Mateo Bridge, biomass remained elevated above baseline levels through summer, while biomass levels at the Dumbarton Bridge returned to near-baseline levels by early summer. Although DO concentration (mg/L) dropped to ~6 mg/L during summer months at both San Mateo and Dumbarton, DO% saturation narrowly bracketed ~90% saturation year-round, suggesting that most of the central-tendency DO concentration decrease was due to decreased saturation concentration at higher summertime temperatures and salinities. In terms of both saturation and concentration (mg/L), however, DMB had a large number of lower-DO outliers. We hypothesize that these decreases were due to low-DO water exiting sloughs and creeks during ebb tide and mixing with open-Bay water (SFEI 2014 # 732), and that during flood tide relatively DO-rich water from north of the Dumbarton enters and dominates the DMB DO signal. The chl-a fluorescence signal at Alviso Slough was both much greater and relatively more variable (wider interquartile ranges as a percentage of the median) than at open-Bay sites.
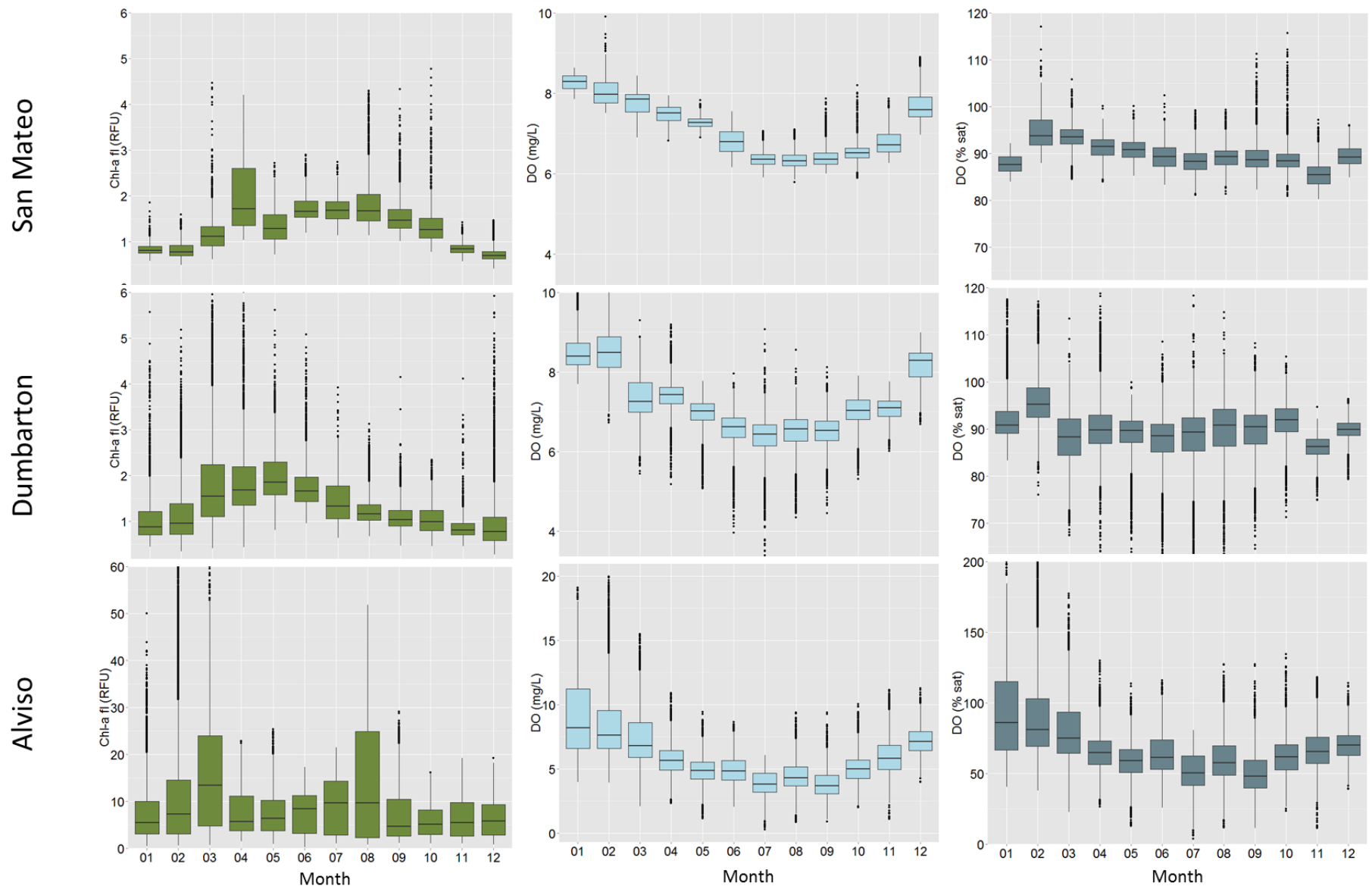
Figure 2.15 Monthly boxplots of high-priority parameters for all available data (2 years at Dumbarton Bridge, 1.5 years at Alviso and 1 year at SMB)

In spring and early-summer 2015, several LSB slough and creek sites were added to the network to better characterize water quality in these habitats, which, until recently, had not been systematically monitored (Figure 2.13, Table 2.3). Recent observations in Alviso Slough, and a review of limited data from several investigations in other sloughs, suggested that low DO occurs commonly along some sloughs (SFEI 2014 # 732, Shellenbarger et. al 2008). The slough and creek sites were selected such that a range of potential physical and biological drivers could be examined (e.g., freshwater input, salt pond connection), both to better characterize the spatial and temporal extent of low DO events and to begin exploring factors that influence DO. Initial observations indicate that these shallow margin habitats are dynamic habitats that exhibit a diversity of responses (Figure 2.16 for chl-a fl and DO% saturation; see Figures A.1.11-A.1.16 for other parameters). In general, DO %saturation was lower and chl-a fluorescence higher in sloughs compared to open-Bay sites, and the signals varied strongly with tidal stage. However, the central tendencies and the amount of variability of chl-a and DO differed substantially among the slough and creek sites. Longer data records and extensive data analysis are needed in order to thoroughly assess DO-related condition and begin developing mechanistic interpretations, and those will be a major focus of NMS activities over the next 1-2 years.
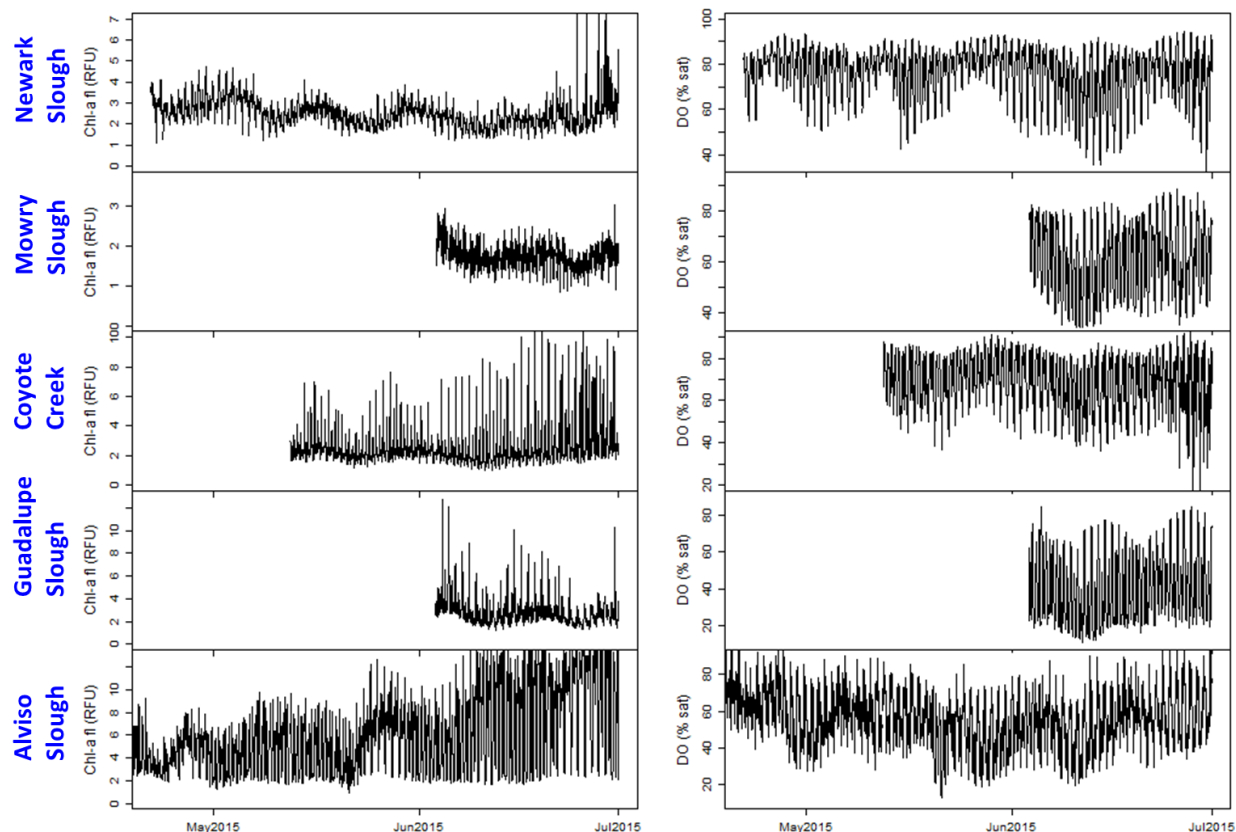
Figure 2.16 Time series of all available data for high-priority analytes at slough sites. Coyote Creek was installed May 2015, and Mowry Slough and Guadalupe Slough were installed June 2015

The DO minima at Dumbarton Bridge during low tide, which reach or dip below the Basin Plan DO standard of 5 mg/L, were a new observation, and one that suggested strong spatial gradients in DO. The pronounced dips at low tide seems to be limited to summer and early fall months, but were evident in 2013-2015. A high priority science goal for the second year of moored sensor monitoring was to identify potential sources of this low DO water, leading us to look further upstream into the LSB's sloughs and Coyote Creek, and the mechanisms controlling DO in these source waters. Figure 2.17 shows DO at

Dumbarton and San Mateo Bridge (channel sites), Coyote Creek (at the southern end of the open Bay channel, near the confluence with Alviso Slough) and Alviso Slough (a true slough end-member). Even at high tide, when open Bay waters have intruded the most in Lower South Bay, a spatial gradient in DO was evident May 2015. DO values at Dumbarton and San Mateo Bridge were comparable (within 0.5mg/L) to those in Coyote Creek but DO values in Alviso Slough were 1-2 mg/L lower than any of these sites. All sites showed a dip in DO on the ebb tide, which was most pronounced at Alviso Slough, with DO values dipping as low as 2 mg/L. The gradient between sites was more pronounced during the neap tide, when we hypothesize that weaker exchange prevented full flushing of low-DO waters from Alviso Slough. Comparison of additional parameters between sites (for the period when all sensors were in place) are shown in Figures A.1.11 – A.1.16
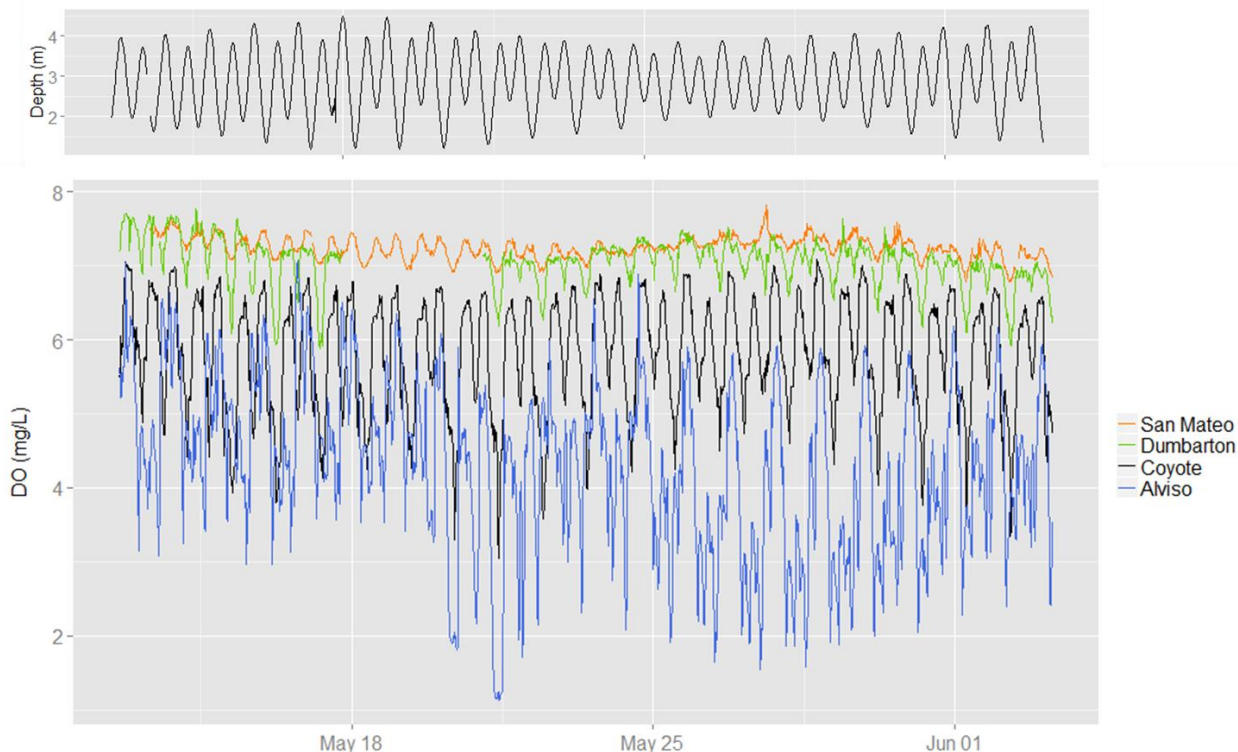
Figure 2.17 Comparison of DO along roughly a longitudinal transect, from a largely open-Bay influenced site (San Mateo) to a tidally open-Bay influenced site (Alviso), for a time period when all sensors were installed

2.4 High-resolution biogeochemical mapping

The strong semi-diurnal and spring-neap tidal signature in parameters such as DO and chl-a at moored stations in LSB suggested that there were strong spatial gradients in biogeochemical processes. Characterizing this spatial heterogeneity is important for several reasons: condition assessment; mechanistic understanding of biogeochemical and physical processes; model calibration; and informing future monitoring program design. In April 2015, a collaborative project was launched between USGS, SFEI, and UCSC to perform high-frequency mapping throughout LSB, including sloughs and Coyote Creek at different tidal stages. Multiple nutrient-related parameters (e.g., NO_3^-, NH_4^+, chl-a and DO) were measured at high frequency (1 Hz) using a flow-through system while cruising at speeds of 20-30 km/h. Discrete samples were also collected at multiple sites for sensor calibration (chl-a, NO_3^-, NH_4^+) and for phytoplankton species enumeration, pigment analysis, and toxin measurements. The first 2 mapping trips were April 15-16 and July 15-16 2015, and a third trip is planned for late September. Results for the

full project will be summarized in a forthcoming technical report (Q2 2016), and some initial results are presented below.
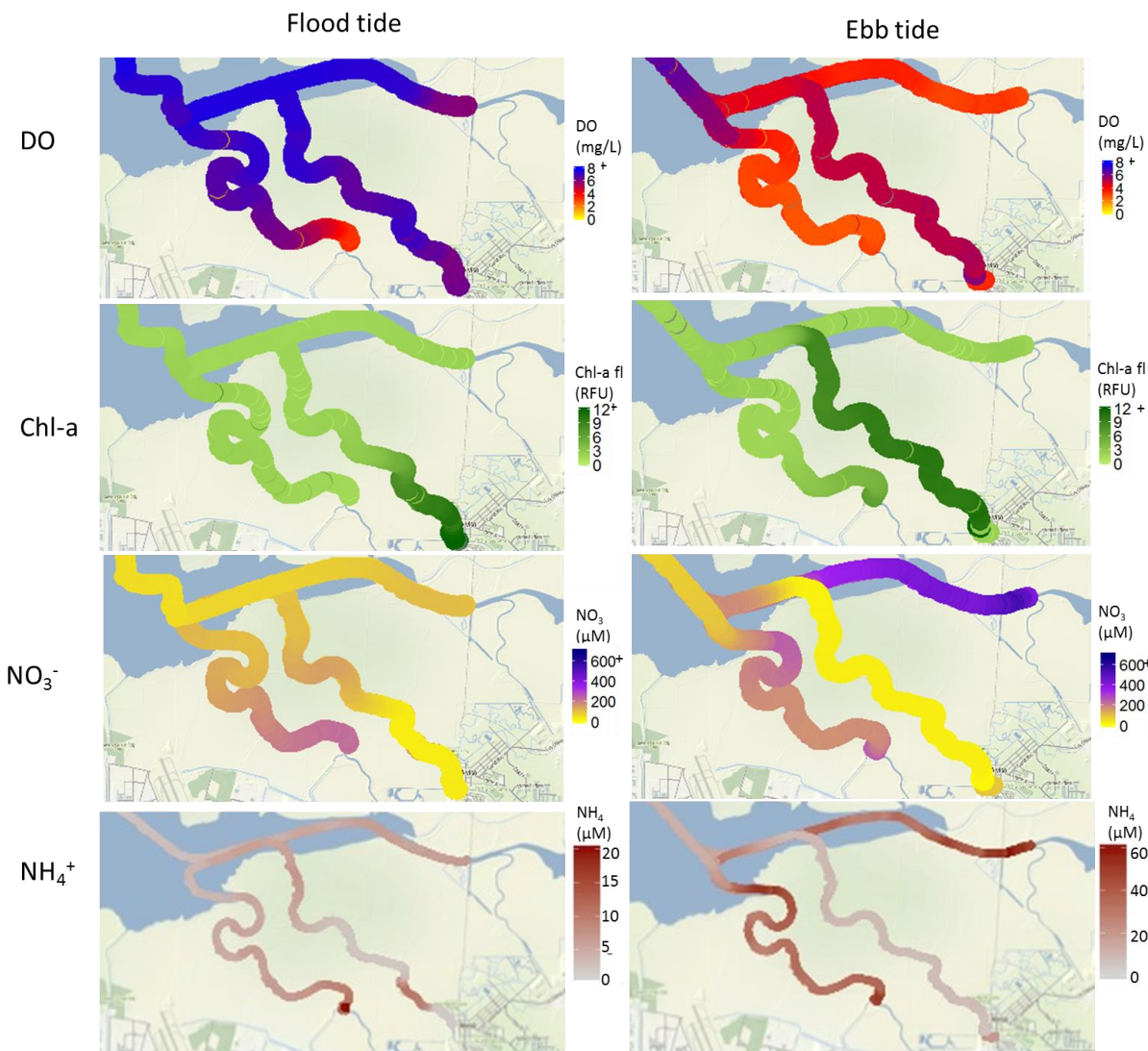
Figure 2.18 High frequency mapping data from July 2015. Data were collected via a flow-through configuration while travelling 20-30 mi/hr. Chl-a is reported in RFU, and scales to ug/L by ~2-3x. NH_4 data courtesy of A. Strong (Stanford). Note different scales on flood and ebb NH_4^+ plots

Chemical signatures in LSB respond to the tides by their aerial coverage expanding and contracting (Figure 2.18). During flood tide, conditions were relatively homogenous in open-Bay areas of LSB and a few kilometers up some sloughs. Further up Guadalupe Slough, however, a region of low-DO/elevated-NO3 water was observed. At the upstream end of Alviso Slough, a ~2 km stretch of water containing high-chl-a and moderately-elevated NH_4^+ water was encountered. On the ebb tide, these water masses with distinct chemical signatures expanded down-slough and into the open Bay. NO_3^- and NH_4^+ concentrations were highest in Guadalupe Slough and Coyote Creek, both of which receive wastewater effluent that would drain toward the open-Bay gradient during ebb tide. Although POTWs in LSB nitrify before discharge, NH_4^+ concentrations approached ~60 µM in Coyote Creek during ebb tide, coincident with peak NO_3^- levels of 600 µM reached. While some of the NH_4^+ may have been due to NH_4^+

regeneration from sediments during organic matter mineralization, much of the NH_4^+ can be explained by residual NH_4^+ present in effluent from the San Jose POTW (NO_3^-:NH_4^+ ~ 15:1), which discharges into Artesian Slough and flows to Coyote Creek. During ebb tide in Alviso Slough, the channel contained high chl-a water (~20-30 ug/L) but among the lowest observed NO_3^- concentrations, perhaps suggesting that much of the NO_3^- had been used during primary production. These observations are consistent with discharge from former salt pond A8, which enters Alviso Slough ~7km upstream from the Alviso-Coyote confluence, exerting strong influence over conditions in Alviso Slough.

2.5 Source monitoring

Accurate quantification of nutrient loads to SFB is a high priority for the NMS. While an initial loads assessment was recently completed (SFEI 2014 #704), additional stormwater and wastewater data have become available since that report was finalized. The purpose of this section is to summarize the recent data, and compare and refine previous load estimates based on this new data.
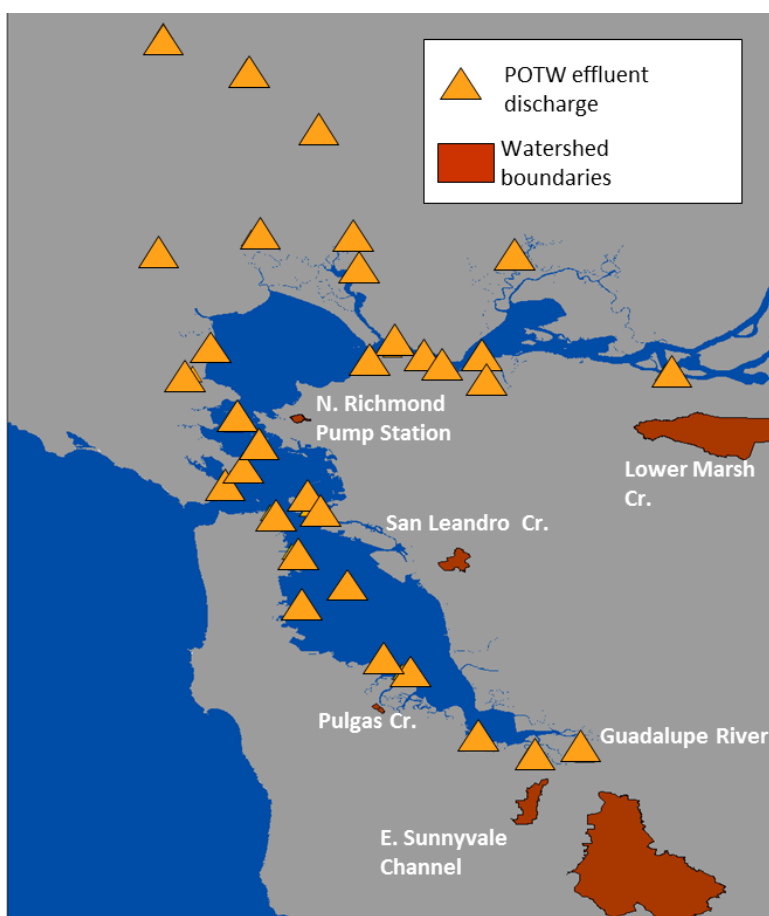
Figure 2.19 Locations of POTW discharges and watershed boundaries monitored for nutrients in 2012-2015

2.5.1 Stormwater monitoring

In October 2012, stormwater nutrient monitoring began in 4-6 watersheds around the Bay Area for NH_4^+, NO_3^-, NO_2^-, TKN (total Kjeldahl N), o-PO_4 and TP (total P) (Figure 2.19). These nutrient analytes were added to a list of analytes already being collected as part of a regional stormwater monitoring effort funded by stormwater agencies and the Regional Monitoring Program. Data from WY 2012 and

WY 2013 were presented in an earlier report (SFEI 2014 #730), and are included again here for comparison with new data from WY2014 data (Figures 2.20 and 2.21).

Results from WY2012 and WY2013 indicated that there was considerable variability in N and P concentrations and speciation within results from individual sites, and also across sites and across storms. Despite the multiple sources of variability, some broad patterns in speciation emerged. NO_3^- and total organic N (TON = TKN − NH_4^+) were the dominant forms of N, with non-trivial amounts of NH4 occasionally observed. The majority of P was present as total particulate phosphorous (TPP = TP − o-PO_4). In WY2014, TON and NO_3^- continued to be the dominant form of across sites, but higher NH_4^+ concentrations were observed in WY 2014 than in previous years at all but one site. o-PO_4 also appeared to represent a slightly larger portion of TP in WY2014. These results are noteworthy considering that previous stormwater loading estimates considered only inorganic forms of nutrients. Pulgas Creek had the maximum N and P values of any site (on 11/19/2013), but in other storms N and P concentrations at this site were comparable to or less than those recorded at other sites.
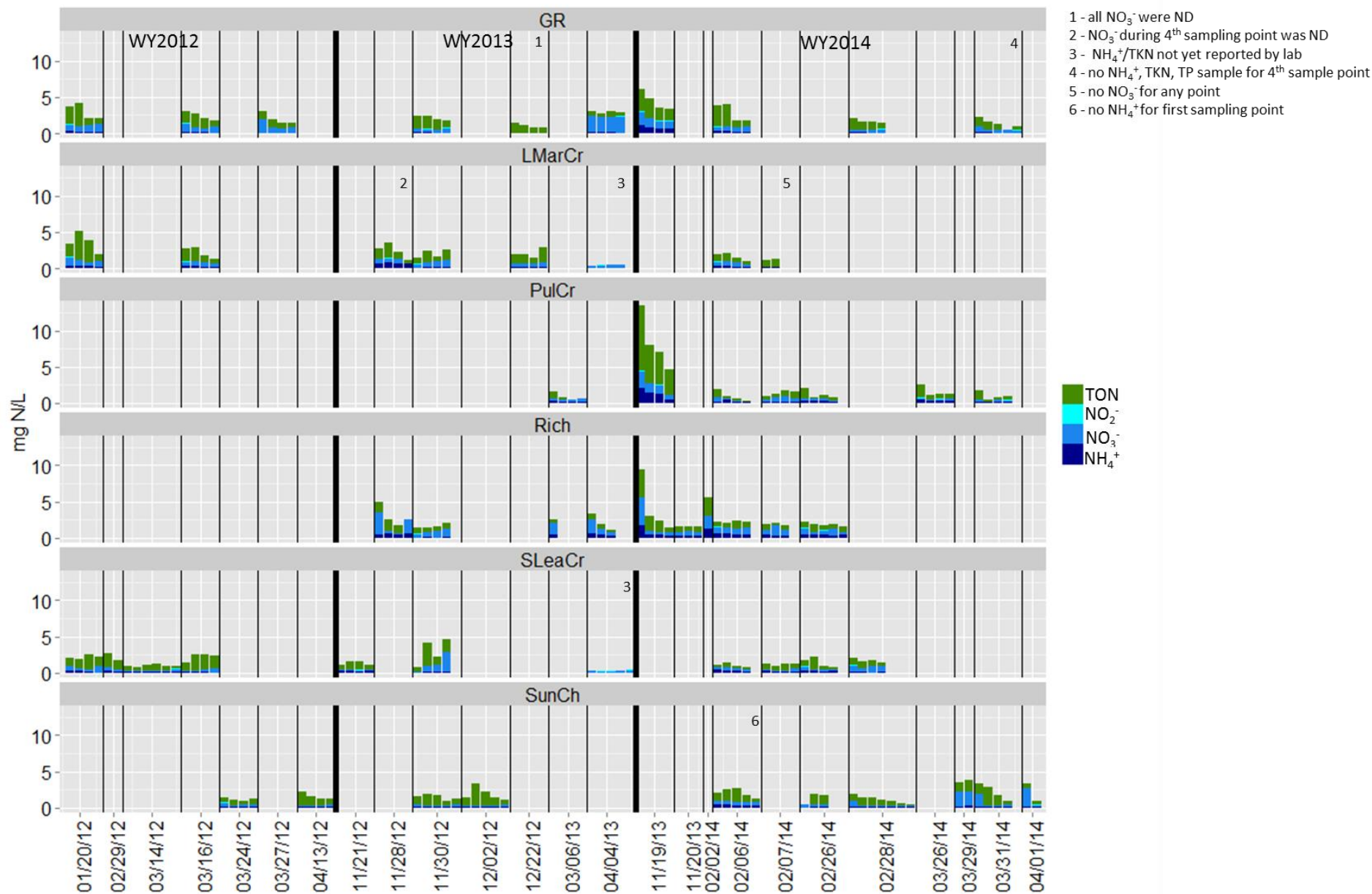
Figure 2.20 Nitrogen monitoring data for WY 2012-2014 by site and storm. Bars represent individual sampling points (typically 4 per storm). Watershed locations are shown in Figure 2.19 and watershed descriptions can be found in Gilbreath (2014). Pulgas Creek and Richmond Pump Station were not monitored in 2014. TON was calculated as the difference of two measured parameters (TKN − NH_4^+).
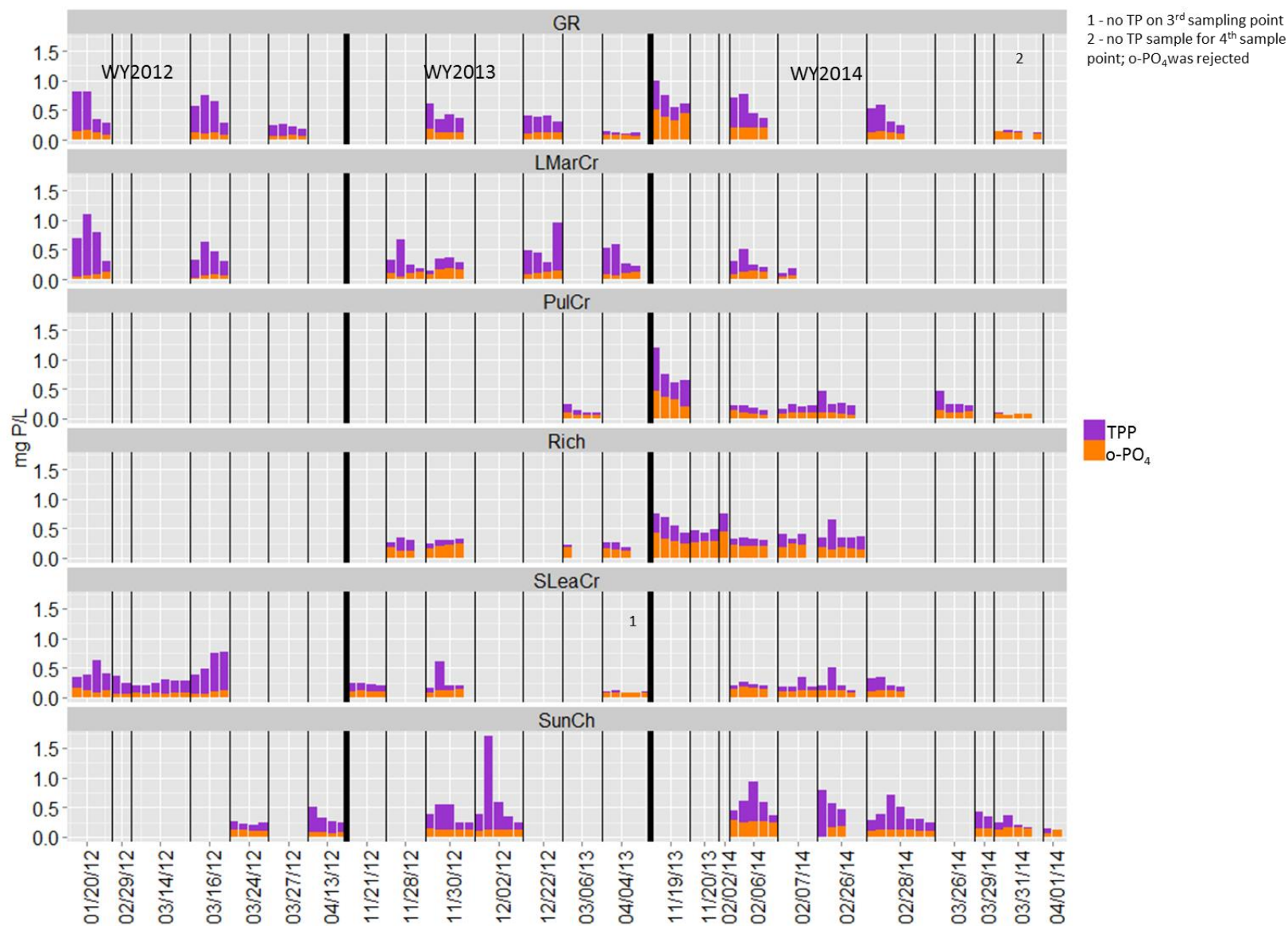
Figure 2.21 Phosphorous monitoring data for WY 2012-2014 by site and storm. Bars represent individual sampling points (typically 4 per storm). Watershed locations are shown in Figure 2.19 and watershed descriptions can be found in Gilbreath (2014). Pulgas Creek and Richmond Pump Station were not monitored in 2014. TPP was calculated as the difference of two measured parameters (TP – o-PO$_4$; all dissolved P was assumed as o-PO$_4$).

2.5.2 POTW loads

San Francisco Bay receives effluent from 42 POTWs. Prior to the 2012 monitoring requirement, NH_4^+ was the only parameter monitored by most POTWs, with the exception of a few POTWs whose permits required additional analytes, and a few POTWs that independently measured those analytes to monitor plant performance. In July 2012 the SFB Regional Water Quality Control Board mandated a 2-year comprehensive effluent monitoring study for all POTWs, requiring a fairly complete set of N and P species to be measured (Table 2.4). After June 2014 the major N and P species and a few minor species continued to be monitored, while several less important species were dropped (see Table 2.4). The dropped analytes introduce very minor inconsistencies in the inter-year comparison presented below, as noted in the Table 2.4 footnote.

The first year of the data, with the expanded analyte list was previously summarized (see Appendix 4, SFEI 2014 #704). In this report 2 years of additional data are added to better characterize temporal variability and variability among plants, and to evaluate the accuracy of previous nutrient loading estimates. While data was available for 42 POTWs across the Bay, the summary below focuses on a subset of POTWs: the 8 largest (by average daily flow) and two additional plants with treatment technologies that differ from most other plants (Napa and Sunnyvale). The discussion below is intended as an overview, and not an in-depth analysis of plant performance or data quality. Several noteworthy data quality issues are summarized in Table 2.5 and in its footnote.

Table 2.4 Measured and calculated analytes monitored in POTW effluent July 2012 – June 2014. Parameters noted with a ** continued to be monitored from July 2014 – present. Data from March – June 2015 was not available at the time of this study..

Parameter	Measured or calculated	Calculation
NO_3^- - nitrate**	measured	
NO_2^- - nitrite	measured	
NH_4^+ - ammonium**	measured	
TKN - Total Kjeldahl Nitrogen**	measured	
SKN - Soluble Kjeldahl Nitrogen	measured	
TN = Total Nitrogen**	calculated	TN = TKN + NO3 +
TDN = Total Dissolved Nitrogen	calculated	TDN = SKN + NO3 +
TON - Total organic N (TON)**, the total amount of	calculated	TON = TKN - NH_4^+
DON - Dissolved organic nitrogen (DON), he amount of	calculated	DON = SKN - NH_4^+
PON - particulate organic nitrogen	calculated	PON = TON - DON
TP - total phosphorous**	measured	
TDP - total dissolved phosphorous	measured	
DIP - dissolved inorganic phosphorous or o-PO₄**	measured	

TPP - total particulate phosphorous, which would include particulate organic phosphorous (POP) + any mineral-complexed P, which would be expected to be small.	Calculated	TPP = TP - TDP
DUR - dissolved non-reactive phosphorous, an approximation for dissolved organic P (DOP)	calculated	DOP = TDP - DIP

In the second 2 years, TON is presented instead of distinguishing DON and PON; and "otherP" is presented, rather than distinguishing DOP, TPP species. NO_2^- was also not monitored after July 2014, so these loads are not considered at all (although, the first two years of data suggests they are trivial, on average less than 5% of TN).

Table 2.5 Percent of calculated values rejected due to data quality concerns. "otherP" refers to any P data from the 3rd year of sampling (July 2014 – present) that is not o-PO4 (only TP and o-PO4 were sampled, so DOP/TPP could not be resolved as they were in the first 2 years)

Plant	DON	PON	TON	DOP	TPP	otherP
Central Contra Costa Sanitary District (CCCSD)	2%	12%	0%	2%	0%	1%
City of Palo Alto	8%	6%	10%	4%	6%	4%
City of Sunnyvale	27%	6%	4%	8%	2%	7%
East Bay Municipal Utility District (EBMUD)	0%	0%	0%	14%	0%	1%
East Bay Dischargers Authority (EBDA)	4%	12%	0%	23%	0%	6%
Fairfield – Suisun Sewer District	0%	4%	0%	23%	0%	13%
Napa Sanitation District	0%	4%	0%	11%	7%	9%
San Jose/Santa Clara (SJSC)	0%	0%	0%	4%	0%	4%
Silicon Valley Clean Water	22%	24%	12%	31%	15%	23%
City and County of SF- Southeast Plant	27%	9%	18%	93%	2%	86%

Several data quality issues were identified, some of which were noted in the previous summary (see Appendix 4, SFEI 2014 #704) and have persisted. Several minor parameters were estimated by difference between two measured values (TON, TDN, TPP, DOP), and calculated values for these parameters were often negative. Some amount of analytical uncertainty is expected, and when possible the results were still retained by assigning a value of zero to the minor parameter. Data was rejected if the calculated difference was more negative than 0.1 mg/L AND if the difference was greater than 5% of the larger value from which it was calculated. Rejected data is not included in Figures 2.23-2.32 or any conclusions below. The frequency with which this occurred varies by plant and analyte but seems most common for DOP and PON and these results should be viewed with this issue in mind. particularly for Silicon Valley SF Southeast. We did not attempt to resolve outliers from the dataset, so no data was rejected on this basis.

Figures 2.22-2.31 present flows, nutrient loads, and nutrient concentrations for each of the 10 plants considered in this report. For the most part, effluent monitoring results from the most recent 2 years are consistent with the results from the first years. In many POTWs, TN and TP loads exhibited substantial temporal variability (i.e, months or seasons, and in some cases between years). TN values at EBMUD were ~50 mg/L-N on average, likely due to its food waste program to support natural gas production / cogeneration. Compared to 'rule-of-thumb' TN concentrations for secondary treatment (25-30 mg/L; Falk et al., 2011), TN was also elevated at EBDA (37 mg/L-N), Silicon Valley (46 mg/L) and SF Southeast (43 mg/L). Average TN values are far below this benchmark at plants with advanced nutrient removal, such as SJSC (15 mg/L-N), Napa (12 mg/L-N) and seasonally at Sunnyvale (10 mg/L-N during summer months). On average, TN was 91% inorganic (ranging from 82% at Napa to 98% at Palo Alto) and either dominantly NH_4^+ or NO_3^- depending on treatment type at a given plant. Sunnyvale and Napa are two interesting exceptions. They are designed to nitrify, but during cooler winter months the nitrification efficiency is reduced and they discharge non-trivial amounts of NH_4^+. On average, organic nitrogen was <10% of TN at all plants, but ranged from very low (~2% at Palo Alto and Fairfield Suisun) to nearly 20% at Napa. TON was on average 75% dissolved. Other than Napa and Sunnyvale, clear seasonal patterns in effluent nitrogen concentrations were not evident.

Rule of thumb estimates for TP in secondary treated wastewater are in the range of 4-6 mg/L-P (Falk et al., 2011). Most POTWs had TP concentrations well below these values. One exception was Sunnyvale, where dredging of treatment ponds led to elevated phosphorous and nitrogen concentrations in 2012. Across all plants phosphorous was mainly in the form of o-PO_4, which represented 75% of TP on average, and ranged from 40% (Napa) to 95% (Palo Alto, Fairfield-Suisun). TPP was non-trivial at CCCSD and Napa, accounting for approximately 30% of the total phosphorous discharged. DUP was on average ~15% of TP across all plants, but was nearly 35% at Napa.
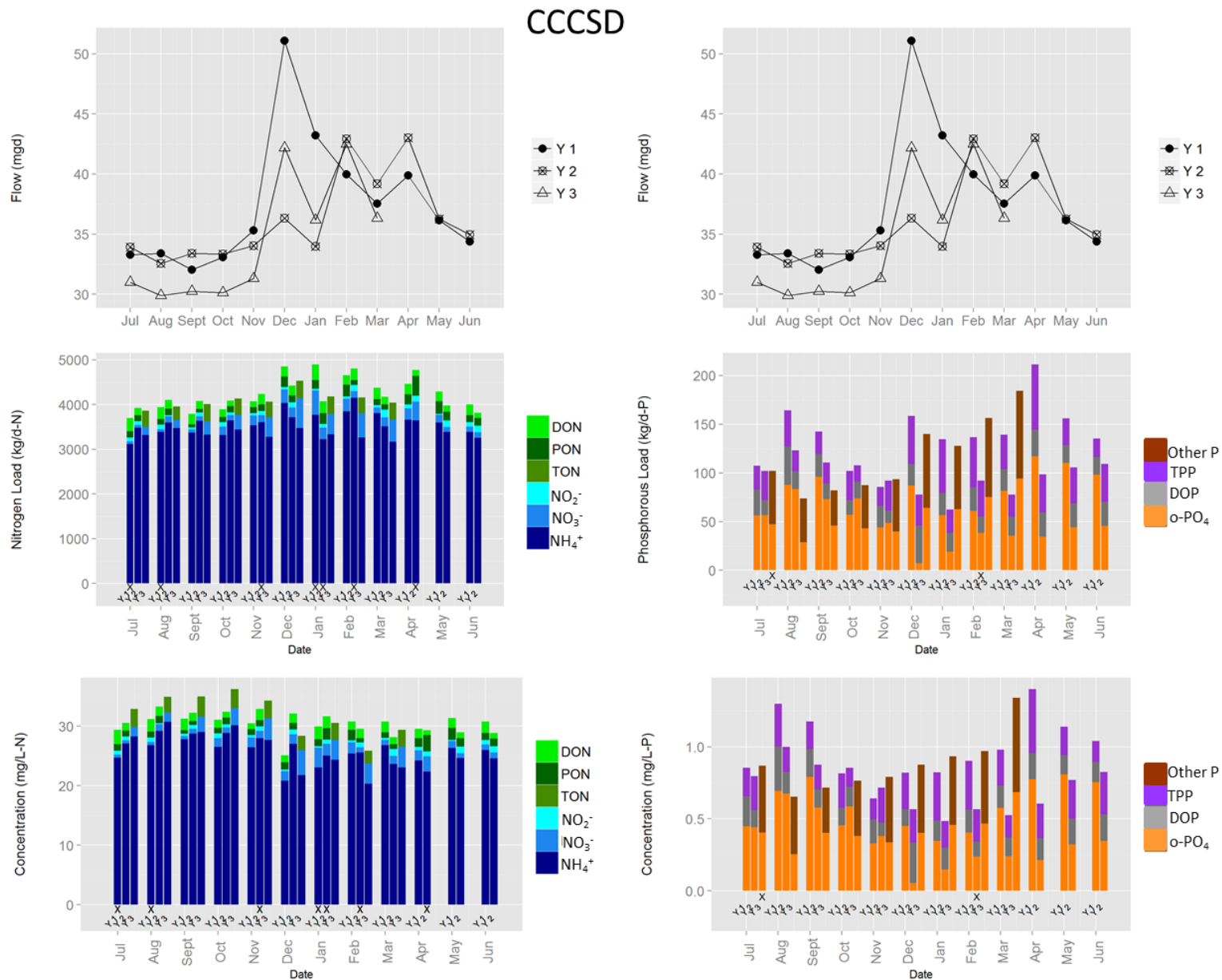
Figure 2.23 Effluent flows, load and concentrations at CCCSD, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO$_4$" and "Other P" forms
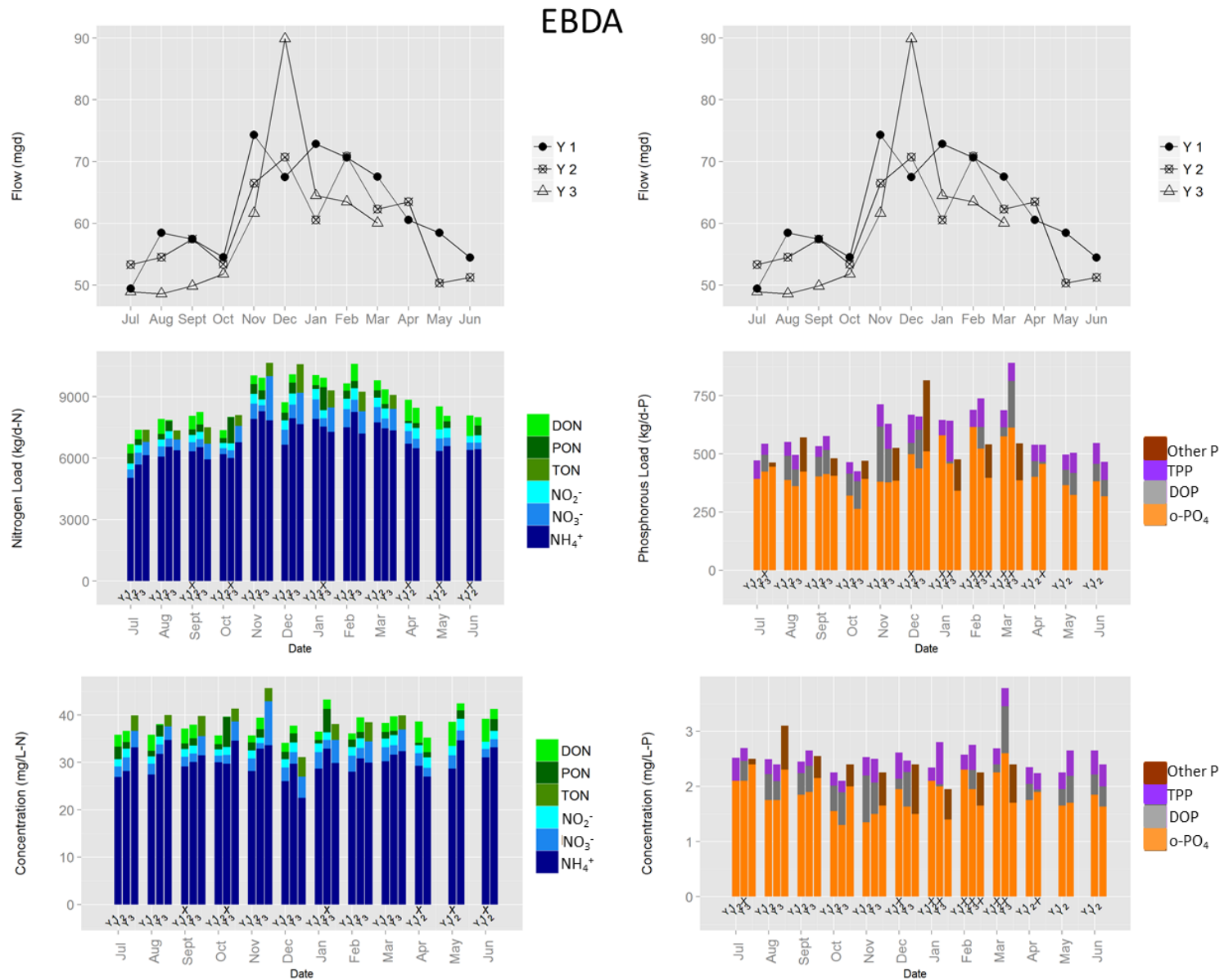
Figure 2.24 Effluent flows, load and concentrations at EBDA, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO_4" and "Other P" forms

Figure 2.25 Effluent flows, load and concentrations at EBMUD, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO₄" and "Other P" forms

Figure 2.26 Effluent flows, load and concentrations at Fairfield-Suisun, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO$_4$" and "Other P" forms
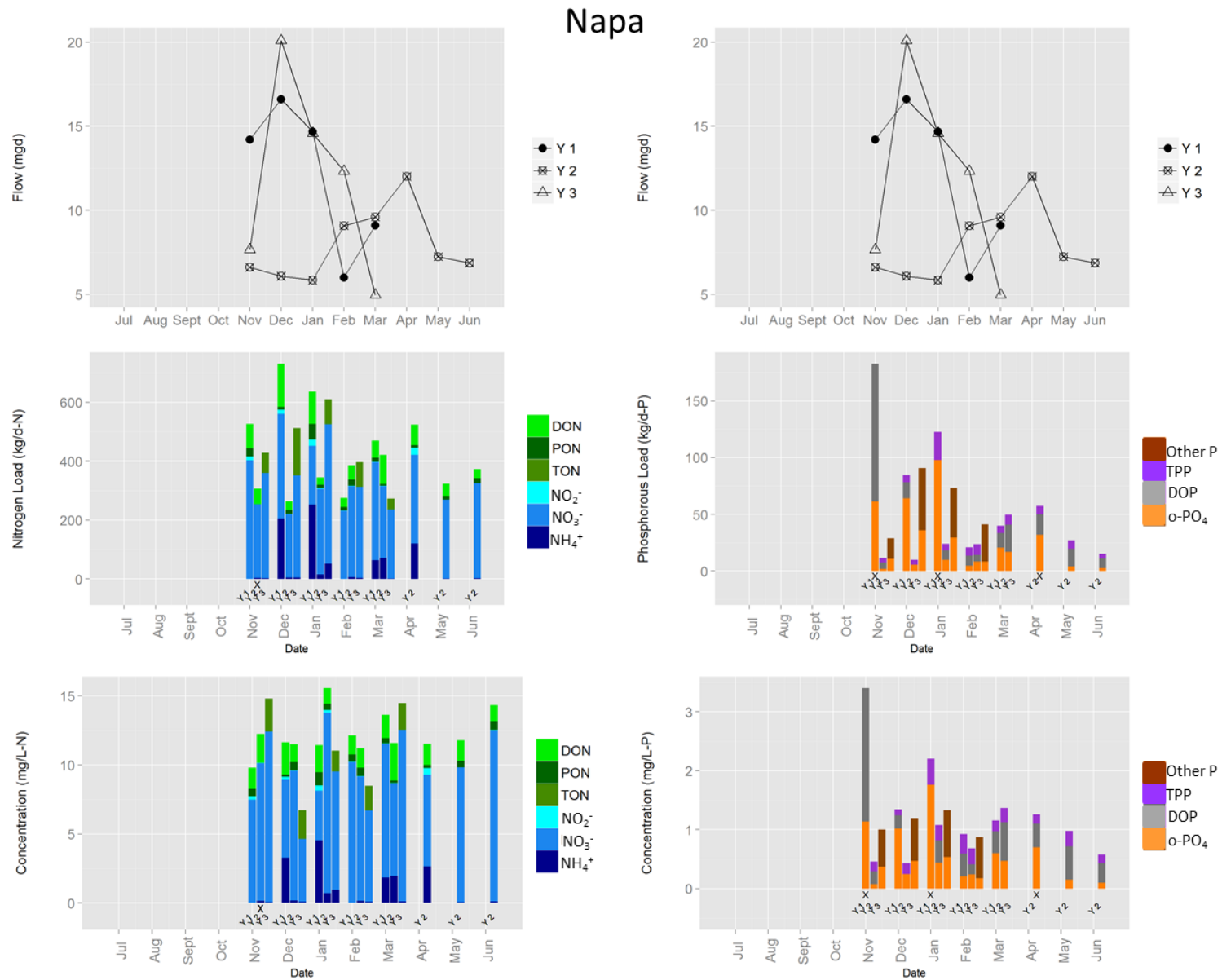
Figure 2.27 Effluent flows, load and concentrations at Napa Sanitation District, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO$_4$" and "Other P" forms
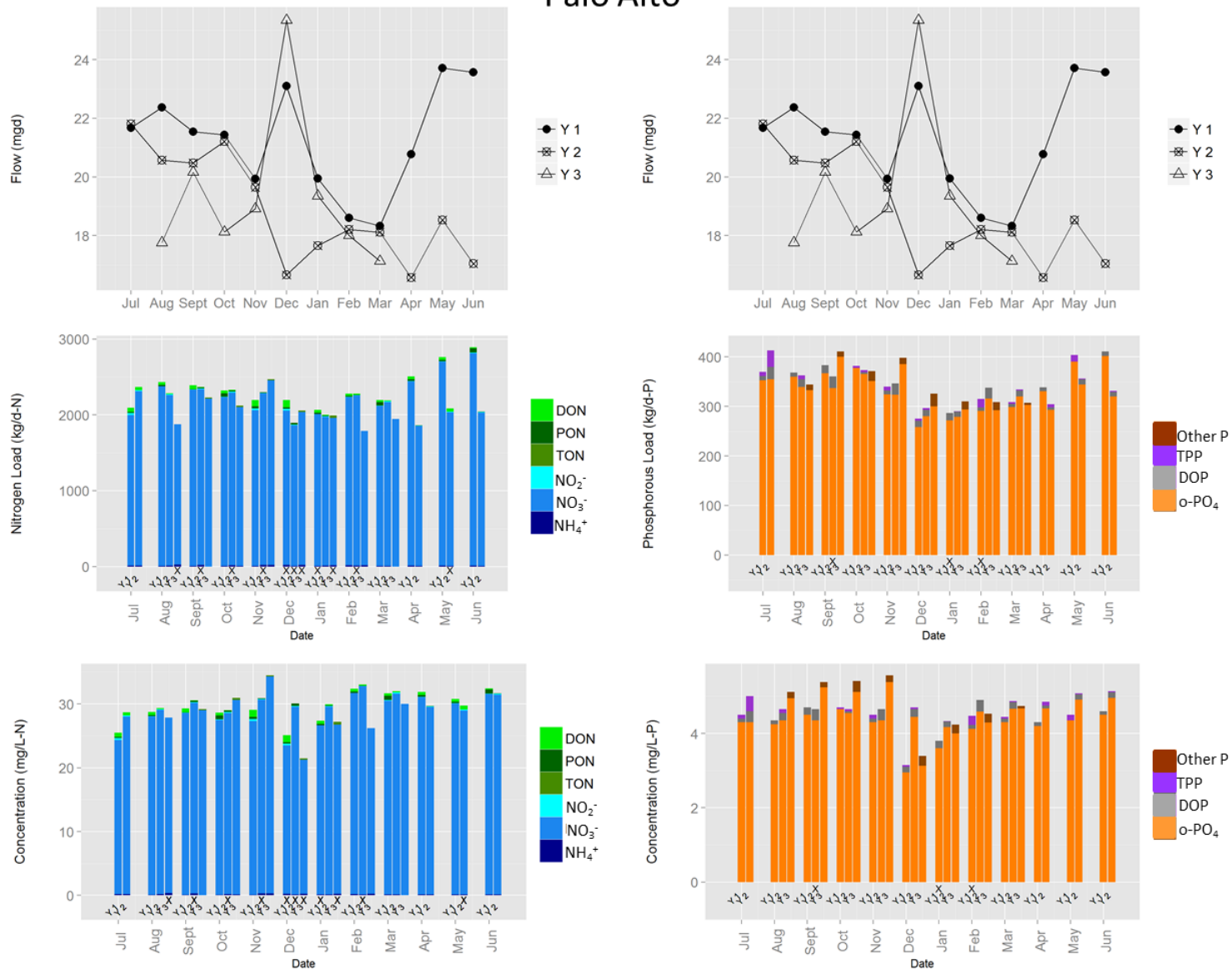
Figure 2.28 Effluent flows, load and concentrations at City of Palo Alto, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO₄" and "Other P" forms
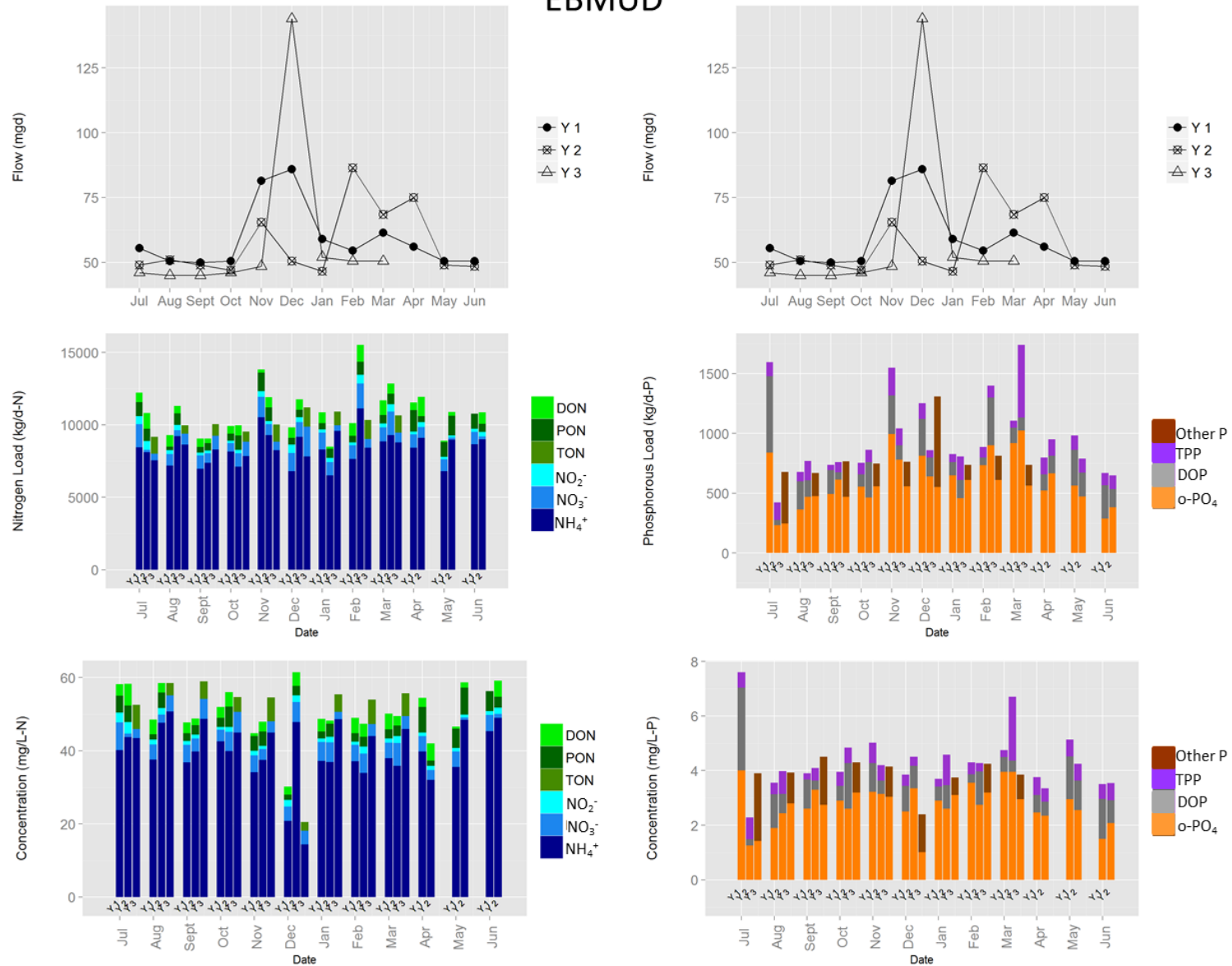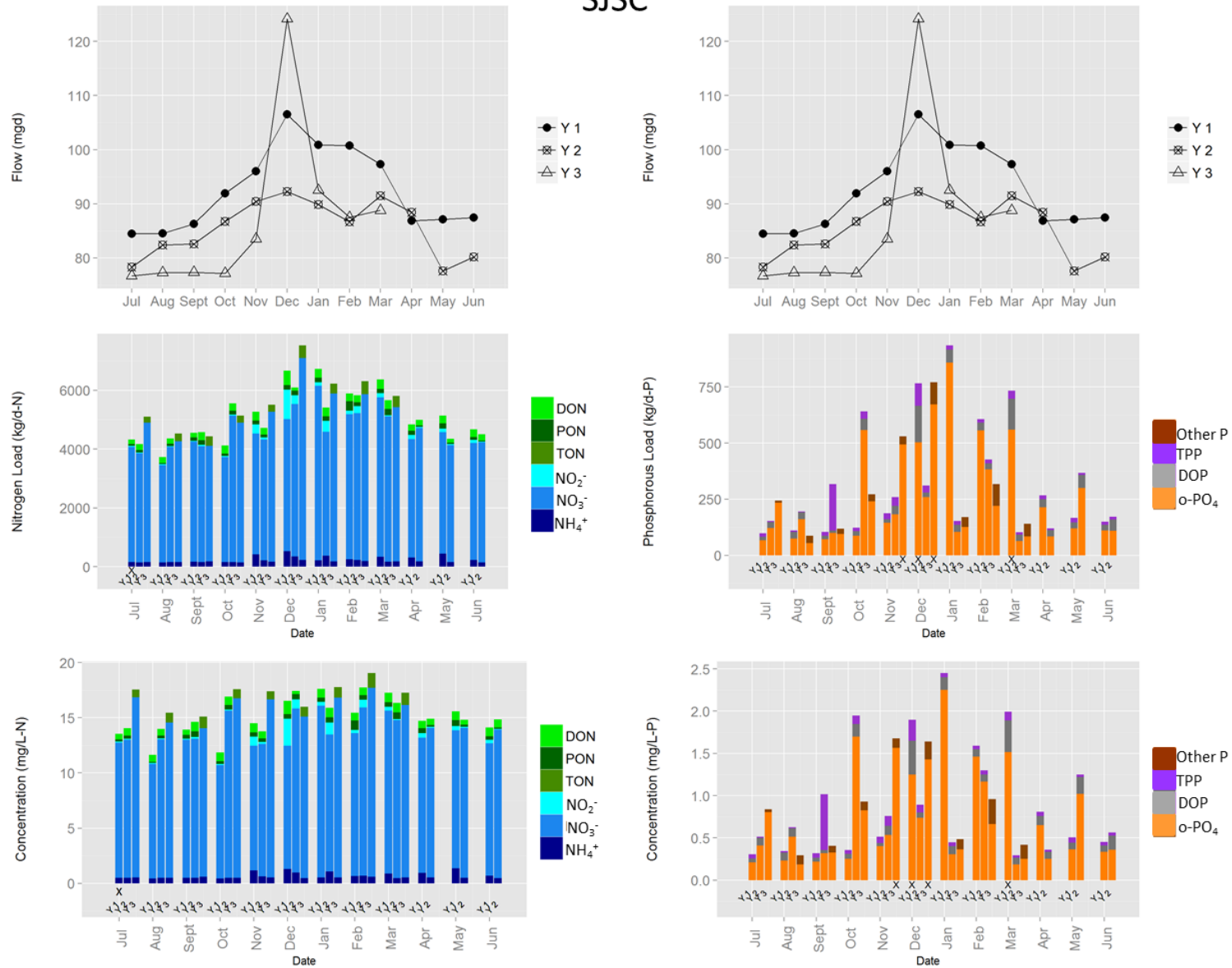
Figure 2.29 Effluent flows, load and concentrations at SJSC, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO$_4$" and "Other P" forms
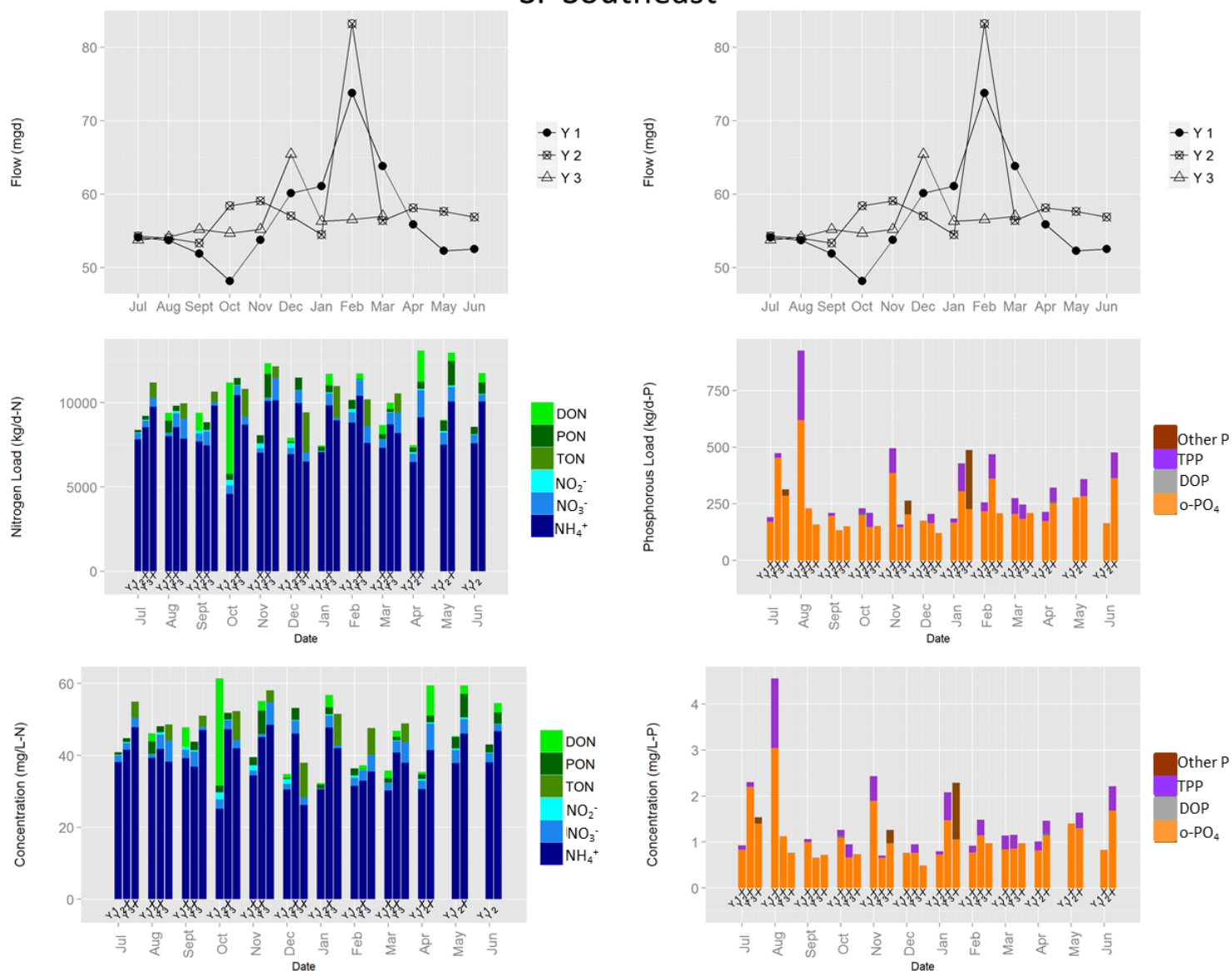
Figure 2.30 Effluent flows, load and concentrations at SF Southeast plant, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO₄" and "Other P" forms
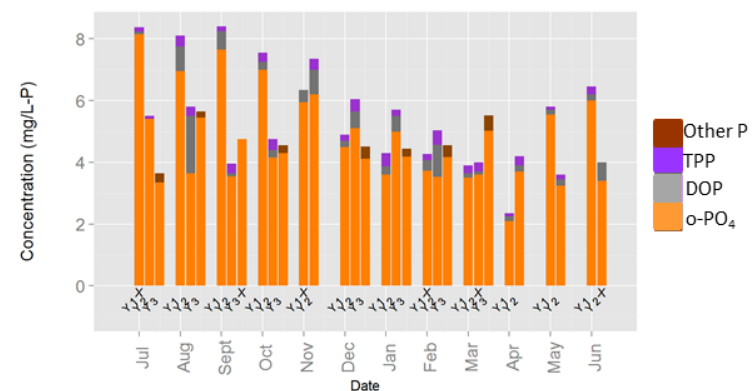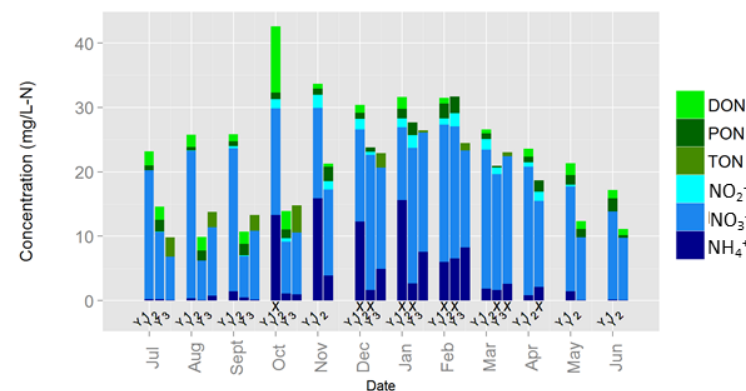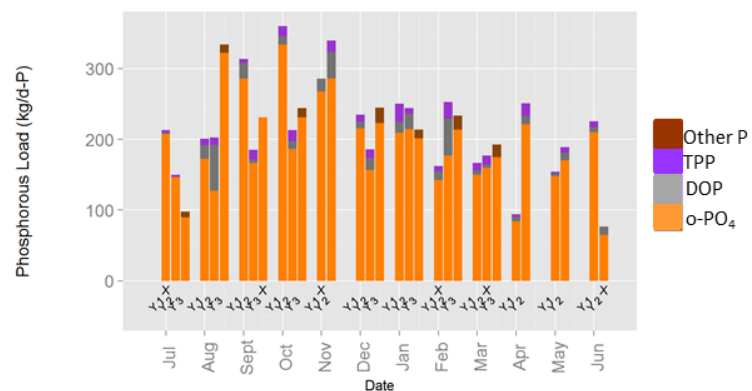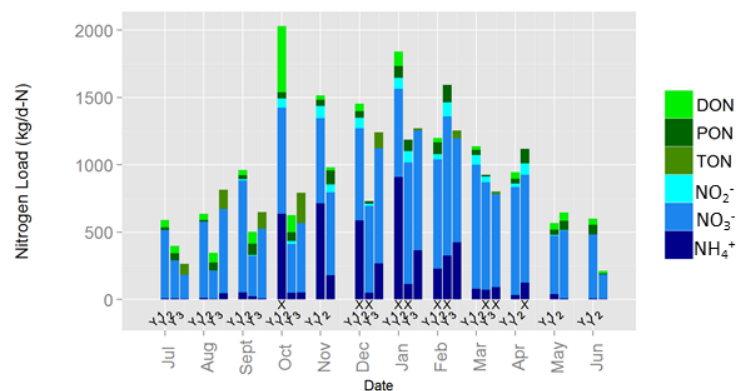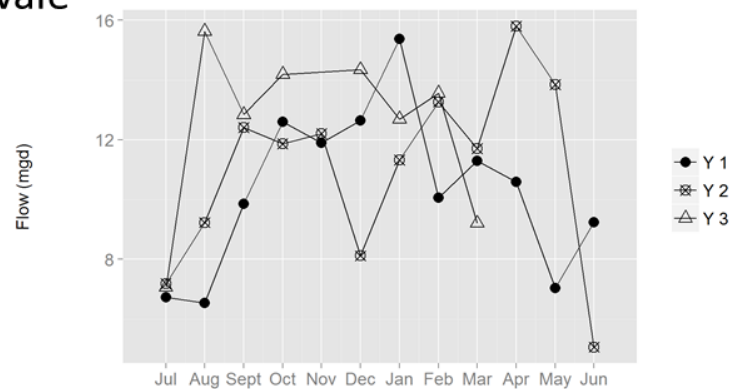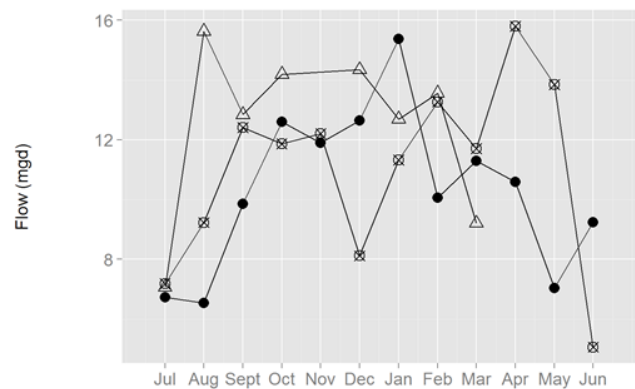
Figure 2.31 Effluent flows, load and concentrations at City of Sunnyvale, by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO₄" and "Other P" forms
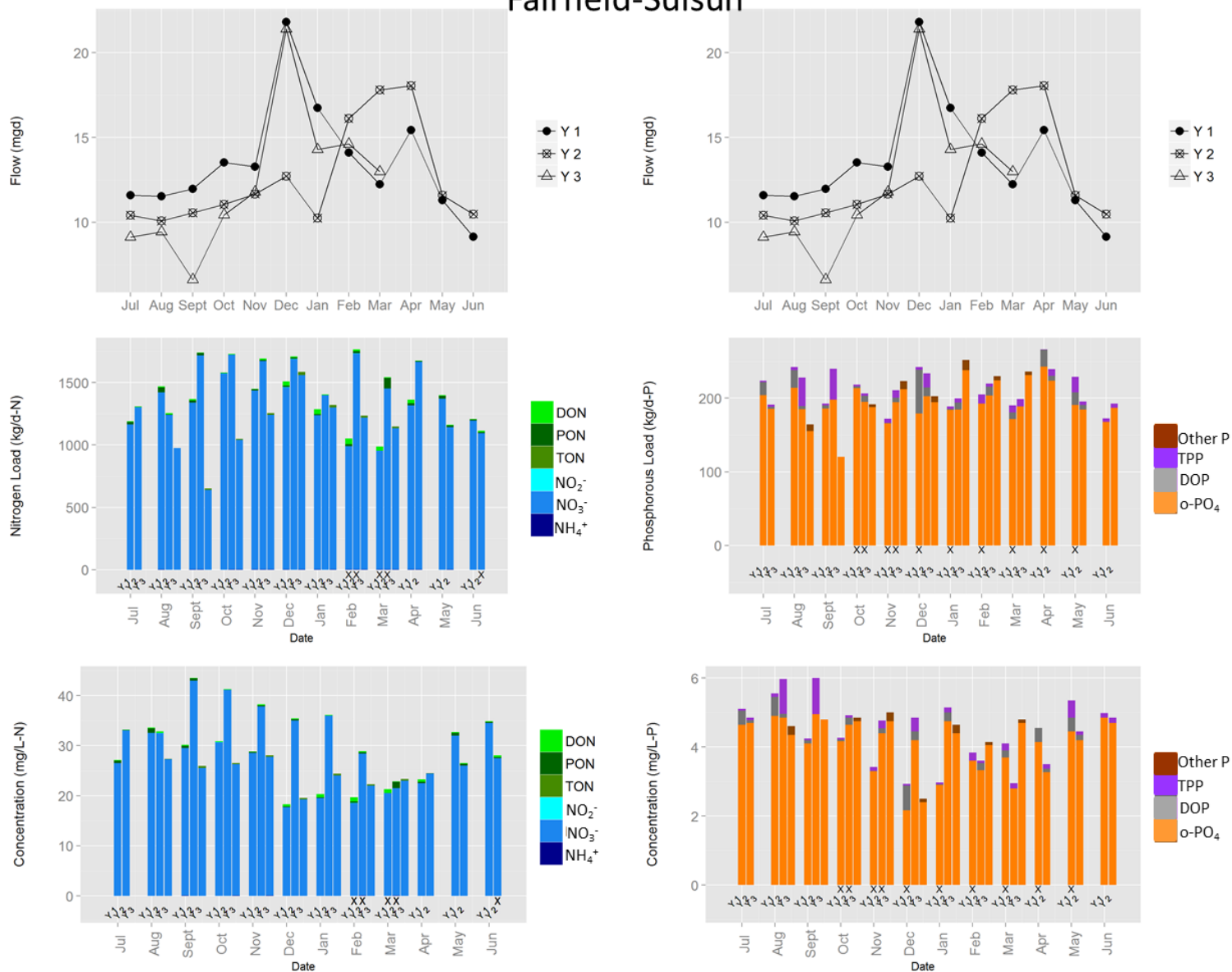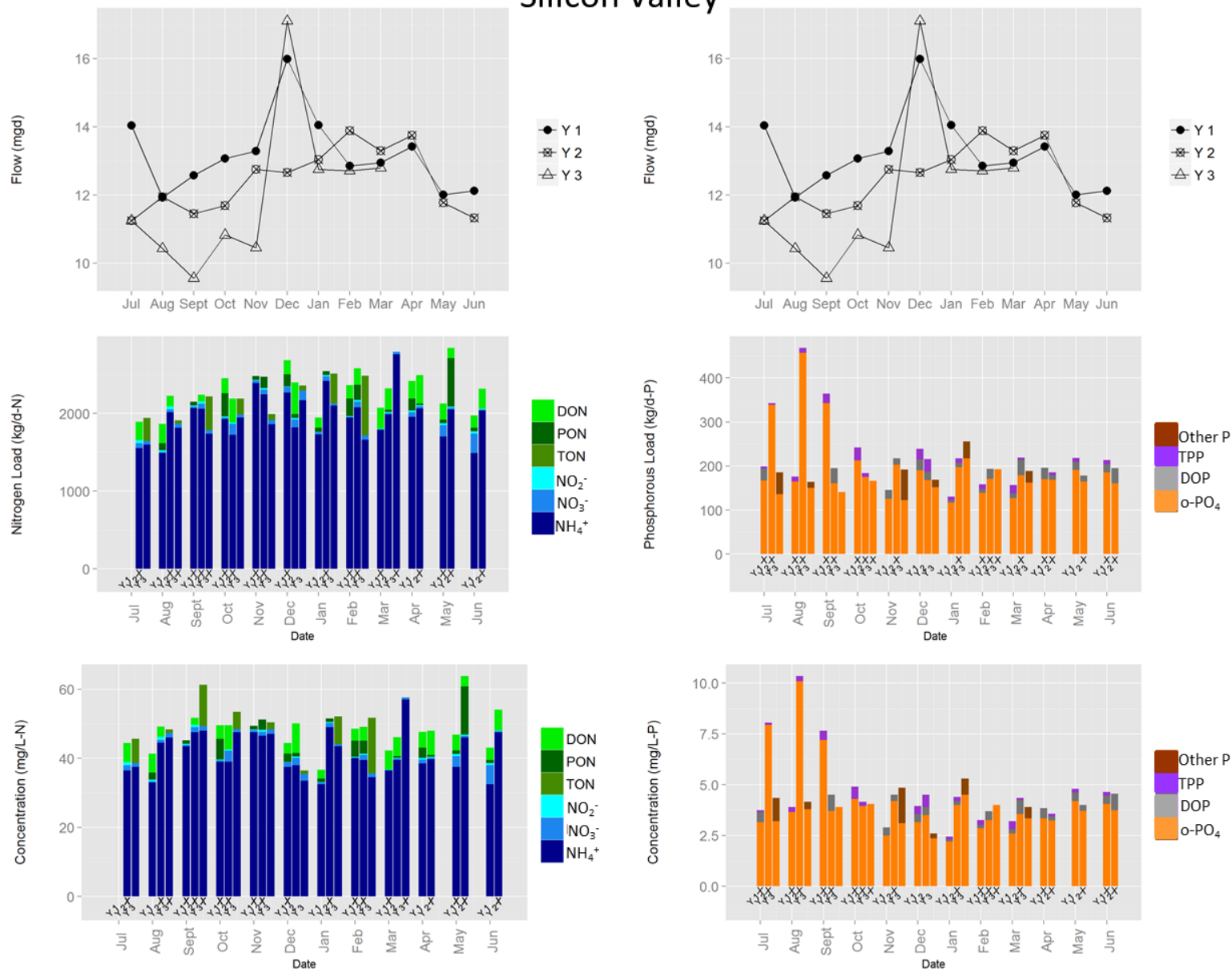
Figure 2.32 Effluent flows, load and concentrations at Silicon Valley Clean Water (formerly South Bay Dischargers Authority), by year and by nutrient type. Instances where calculated concentrations and therefore loads were rejected based on the criteria described above are noted with an "X" and omitted from the plots (most common for DOP and PON). TPP/DOP speciation not available for Yr3, so P data is broken down into "o-PO$_4$" and "Other P" forms

Figure 2.33 shows boxplots of DIN, TN, o-PO$_4$ and TP load by plant. EBMUD had the highest median loads across all species. DIN and TN loads at SF Southeast plant were the second highest, due to relatively high nitrogen concentrations (40-60 mg/L); however, SF Southeast's o-PO4 and TP loads ranked lower. EBDA was the 3rd largest nitrogen discharger and the second largest phosphorous discharger Baywide. Although SJSC had the highest flow rates, its TN and TP loads were on par with smaller POTWs due to advanced nutrient removal at this plant. The largest discharging plants also showed substantial variability in their loads, as much as 20-25%. For EBDA, SJSC and SF Southeast (along with Sunnyvale and CCCSD to a lesser extent) , this variability was more seasonal than interannual, with highest loads in winter (Figures 2.23-2.32), At EBMUD, interannual variability appeared to be greater than seasonal variability..

One purpose of analyzing this dataset was to corroborate previously estimated loads, when considerable less data was available on N and P species. The red dots in Figure 2.33 indicate inorganic N/P loads reported in SFEI 2014 (#704), which was not able to consider organic N or P because of insufficient data. In general, the previously estimated o-PO4 loads agree reasonably well with load estimates based on the longer dataset. However, the DIN loads estimated using the longer dataset are greater in several cases than the previous estimates.
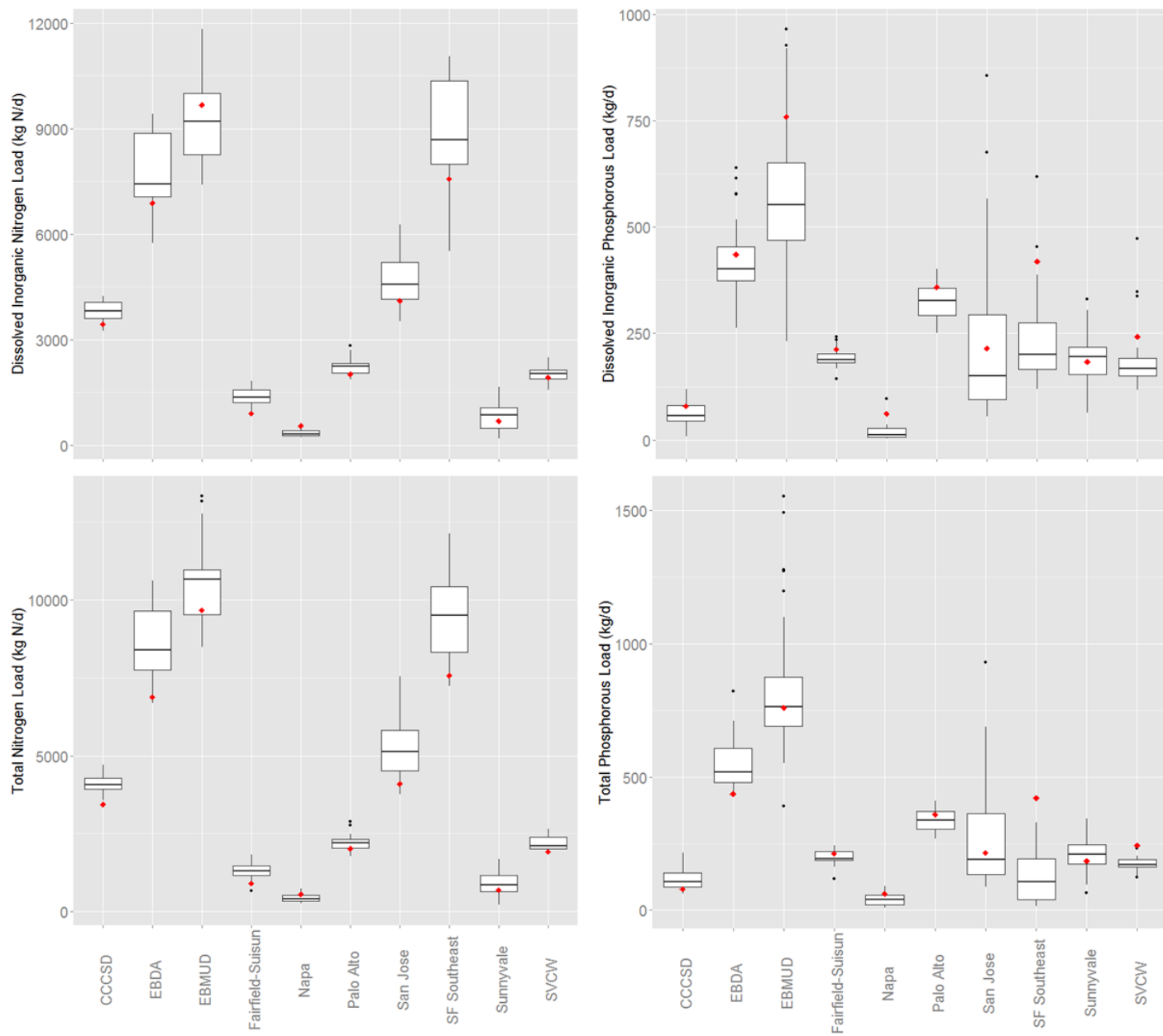
Figure 2.33 TN and TP load boxplots by plant, with red dots showing estimates from SFEI 2014 (#704)

2.6 Monitoring Program Development

Developing and implementing a nutrient-focused monitoring program for San Francisco Bay is one of several major work elements identified in the Nutrient Management Strategy. As an initial step in this process, a nutrient monitoring development plan was crafted that identified "no-regrets" recommendations for program development, and next steps to address remaining data gaps related to future monitoring program structure (SFEI 2014, #724). Table 2.7 summarizes the main recommendations for monitoring program development outlined in monitoring program development plan (#724), as well as a qualitative assessment of progress towards each. Many of the FY2015 and FY2016 projects are well-aligned with these recommendations. In particular progress has been made toward collecting additional ship-board and high-frequency water quality data in the open Bay and in the sloughs, which will also be useful calibration/validation data for model development. Other recommended program areas have received limited attention to date due to funding limitations.

Table 2.7 Progress along monitoring program recommendations

	Monitoring element	Importance to NMS	Status	Key projects underway
Shipboard water quality monitoring	Additional nutrient analytes	• Calibration biogeochemical models • Box models/mass balances of nutrient transformation	● (full)	• DON, PN, TN, DOP, TPP and TP added to monthly Bay sampling in Nov 2014. Data summarized in Section 2.1.1 of this report
	Continue inorganic nutrients	• Continue 40+ year dataset • Calibration biogeochemical models	● (full)	• DIN, o-PO_4 and SiO_2 continued to be monitoring during monthly Bay sampling
	Phytoplankton C, N, chl-a determination	• Assess physiological state of phytoplankton • Improve accuracy of biomass estimates from chl-a	○ (empty)	• No relevant projects underway
	Characterize phytoplankton primary production rates	• Update/calibrate existing Bay primary productivity models (i.e. Cole/Cloern 1987)	○ (empty)	• No relevant projects underway
	Regular monitoring for phytoplankton community composition	• Characterize community composition across a range of conditions • Analyze mechanisms that shape community composition • Optimize monitoring techniques	◔ (quarter)	• Algal taxonomy by microscopy implemented at routine Bay stations during monthly cruises. Preliminary results discussed in Section 2.2.1 of this report • Algal taxonomy by pigments in open Bay pilot project complete. Results discussed in Section 2.2.2 and final project report included in Appendix 3 • Algal taxonomy by microscopy and pigments in sloughs underway. Data not yet analyzed
	Regular monitoring for algal toxins	• Determine frequency, magnitude and duration of algal toxin events • Optimize monitoring techniques	◑ (half)	• Algal toxins measurement and analysis by passive sampler in SF Bay complete. Results discussed in Section 2.2.3 and final project report included in Appendix 4 • Algal toxins measurement by chromatography in open Bay and in sloughs underway. Data not yet analyzed
	Expand ship-board monitoring to the shoals	• Characterize conditions (Historically undersampled) • Evaluate relative importance of shoals in shaping conditions in open Bay	◔ (quarter)	• RMP began margins sampling program, in July 2015, but not nutrient-focused. However, may be an opportunity for piggy-back sampling in the future

47

High-frequency monitoring	Utilize moored sensor for continuous data collection	• Assess condition on high temporal scales • Calibrate biogeochemical models • Optimize monitoring techniques	●	• Continued operation of 2 main channels stations and 1 slough station for 1-2 years • 4 additional slough stations added this year • Results are discussed in Section 2.3
	Coordinated monitoring in shallow margin habitats and sloughs	• Characterize conditions (Historically undersampled) • Evaluate relative importance of marings/sloughs in shaping conditions in open Bay	●	• 4 moored sensors added to soughs in Lower South Bay; additional sensor planned for July 2015. • 2 high-frequency mapping cruises in Lower South Bay sloughs conducted n April and May 2015. Results discussed in Section 2.4
Benthos monitoring	Zoobenthos monitoring	• Grazing rates are needed to estimate biomass accumulation and ultimately calibrate biogeochemical models	◔	• USGS does benthic abundance surveys 3x a year
	Microphytobenthos monitoring	• Determine relative important to overall primary productivity (could be particularly important in shallow habitats)	○	• No relevant projects underway
Priority Data Analyses	Define "priority events" that would need to be captured by monitoring	• Determine problematic chl-a, DO and toxin levels • Characterize frequency, duration, magnitude and extent of these events	◑	• Draft assessment framework report is under development, and expected in Q4 2015.
	Optimize monitoring design based on these needs	• Determine optimal combination of ship-based monitoring and moored sensors • Determine ideal spatial/temporal distribution of ship-based sampling • Optimize optimal monitoring techniques	◔	• Limited progress to date.

3.0 Model results

Biogeochemical modeling is a high-priority element of the Nutrient Management Strategy. Model planning began in earnest in 2013, resulting in a modeling whitepaper completed in 2014 (SFEI 2014 #705) and, more recently, a Detailed Modeling Workplan (SFEI 2014 #733). The workplan covers FY15-FY21 in detail, and beyond this timeframe in less detail. One of the early tasks identified in this workplan is to refine the hydrodynamic model in coordination with collaborators at USGS and Deltares. That work is well underway, with parts of the model recently shared with SFEI modeling staff. A full-time modeler began work at SFEI in August 2015. Major current activities are related to developing a simplified domain model for Lower South Bay and South Bay, and gradually moving toward a more spatially-resolved model. An annual progress report on modeling activities will be distributed in mid-2016. More news on modeling will provided mid fiscal year (Jan/Feb 2016).

4.0 Recommendations/Projects for next year

In June 2015, the Nutrient Steering Committee voted to continue funding nutrient data collection during ship-board monitoring for the same suite of parameters as presented in section 2.1.1. This data is not only important for better characterizing trends in nutrient concentration and speciation, but will be important for biogeochemical model calibration and validation. However, after this additional year of data collection, it may be worthwhile to re-evaluate the list of nutrient parameters being analyzed to see which are most critical to collect (for example, very little particulate N or organic phosphorous was detected during the first year of data collection).

The Nutrient Steering Committee also voted to continue funding for moored sensor monitoring in the open bay and in the sloughs, but at lower levels than the previous years (approximately $100,000 less in FY 2016 than in FY 2015). This difference is mainly due to fewer equipment purchases: in FY 2015, we purchased and installed 5 new moored sensors whereas in FY 2016, we are not requesting any funds for equipment purchase. Instead, we are investing in increased personnel time to improve data quality/management and interpret data from the existing sensors, including a new researcher with experience in sensor design and high-frequency data interpretation who joined SFEI in August 2015. Specific priorities for the next year of the Nutrient Moored Sensor Program, organized by broad goals, include:

- Sensor maintenance/operation
    - Improve real-time data connections at existing moored sensor sites, both to facilitate response to broken sensors or trigger event-based additional sampling
    - Upon successful bench-top and pilot-scale deployments of a high-frequency NO3 sensor (SUNA), deploy at Dumbarton Bridge
- Data collection, QA/QC and management
    - Further automate data collection, processing and storage
    - Improve ability to accurately predict chl-a concentration from chl-a fl signals, which includes continued discrete sample collection during site visits, additional discrete sample collection across a range of environmental conditions and developing/refining single or multi-variate regressions between chl-a concentration and fl, turbidity, etc.
- Establish relationships with existing moored sensor networks
    - Widely distribute Phase 1 of the nutrients visualization tool and prioritize elements for Phase 2. Previous discussions with collaborators at USGS suggest that real-time connections, as well as enhanced, customizable "dashboard" features, are high-priority.
- Use moored sensor data to address priority science and management questions

- o Characterize the frequency, duration and magnitude of low DO in sloughs in LSB
- o Apply time-series analyses to determine the dominant periodicities that control conditions in sloughs and open Bay (seasonal, spring/neap, day/night, semi-diurnal tides)
- o Use simple box models to assess the relative importance of slough/pond drainage in shaping conditions in the open Bay

Phytoplankton analysis is continuing at similar effort for FY 2016 for microscopy, algal pigments and algal toxins. Additional funds are also proposed for FY 2016 for rigorous interpretation of the full set of data, specifically related to exploring how phytoplankton community composition (and related, toxin concentration) is linked to environmental variables such as temperature, salinity, nutrient concentration, etc. After this next year of data, it may be worth considering whether both algal pigment and microscopy data are necessary. Either they are of equal value, and therefore redundant, or one method provides more reliable results and should be preferenced. Additional sampling for algal toxins in Bay biota may be useful for informing what protective levels should be in SFB, and SFEI (in collaboration with UCSC) is currently exploring low-cost options for additional bivalve sampling.

Stormwater monitoring will not continue in FY 2016. Stormwater loads to SFB are of relatively minor importance (SFEI 2014 #704), and although these estimates are considered highly uncertain, the Nutrient Steering Committee has not prioritized additional monitoring or modeling efforts to constrain load estimates. Previous load estimates agree reasonably well with data collected from 2012-2014, so there doesn't seem to be a need to overhaul these estimates on the large scale. To the extent that certain loading data is needed to calibrate/validate a biogeochemical model, POTW monitoring can be intensified. Before requiring large-scale POTW effluent monitoring in the future, aforementioned data quality issues around minor N and P species should be resolved in order to maximize the value of the collection effort.

Many of the above recommended monitoring activities are well aligned with the monitoring program development goals described in Table 2.7. In the next year, we also recommend an increased focus on data analyses to inform program development, an activity that was begun but somewhat interrupted this year.

Nutrient model development is expected to begin in earnest this fiscal year, now that our full-time modeler is on board. The early priorities for model development include spatially-simplified water quality modeling in Lower South Bay, incorporation of existing hydrodynamic model output, and a full-bay conservative tracer study. The simplified LSB model will be used both to test hypotheses on the importance of shoals, sloughs and ponds on nutrient transformations, and as a testbed for sensitivity studies of the biogeochemical model. Collaborators at the USGS have supplied a hydrodynamic model which includes San Francisco Bay and the Delta. Further collaboration on model development will focus on improving forcing and validation in South San Francisco Bay. This model will then be used to drive transport in a full bay tracer study, as well as more complex water quality models.

5.0 References

Cloern, J. E. and R. Dufford (2005). "Phytoplankton community ecology: principles applied in San Francisco Bay." Marine Ecology Progress Series **285**: 11-28.

Cloern, J. E., A. D. Jassby, et al. (2007). "A cold phase of the East Pacific triggers new phytoplankton blooms in San Francisco Bay." Proceedings of the National Academy of Sciences **104**(47): 18561-18565.

Cloern, J. E. and A. D. Jassby (2012). "DRIVERS OF CHANGE IN ESTUARINE-COASTAL ECOSYSTEMS: DISCOVERIES FROM FOUR DECADES OF STUDY IN SAN FRANCISCO BAY." Reviews of Geophysics **50**.

Cloern, J.E., A. Malkassian, R. Kudela, E. Novick, M. Peacock, T. Schraga, and D.B. Senn (2014) The Suisun Bay Problem: Food quality or food quantity? IEP Newsletter; **27 (1)**

Gilbreath, A.N., D.C. Gluchowski, J. Wu, J.A. Hunt, and L.J. McKee (2014). Pollutants of concern (POC) loads monitoring data progress report, water year (WYs) 2012 and 2013. A technical report prepared for the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP), Sources, Pathways and Loadings Workgroup (SPLWG), Small Tributaries Loading Strategy (STLS). Contribution No. 708. San Francisco Estuary Institute, Richmond, California.

Kudela, R., M. Peacock. (2015). Assessing SPATT in San Francisco Bay. A technical report prepared for San Francisco Estuary Institute, Richmond, CA.

SFEI (2014). External Nutrient Loads to San Francisco Bay. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 704

SFEI (2014). Model Development Plan to Support Nutrient Management Decisions in San Francisco Bay. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 705

SFEI (2014). Nutrient Moored Sensor Program Year 1 Progress Update. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 723

SFEI (2014). Development Plan for the San Francisco Bay Nutrient Monitoring Program. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 724

SFEI (2014). Scientific Foundation for San Francisco Bay Nutrient Management Strategy. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 731

SFEI (2014). Nutrient Stormwater Monitoring Results: WY 2012 and WY 2013. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 730

SFEI (2014). Lower South Bay Nutrient Synthesis. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 732

SFEI (2014). San Francisco Bay Nutrient Management Strategy: Detailed Modeling Workplan for FY15-FY21. A technical report prepared by San Francisco Estuary Institute, Richmond, CA. Contribution No. 733

Shellenbarger, G. G., Schoellhamer, D.H., Morgan, T.L., Takekawa, J.Y., Athearn, N.D., and Henderson, K.D. (2008). "Dissolved oxygen in Guadalupe Slough and Pond A3W, South San Francisco Bay, California, August and September 2007." U.S. Geological Survey Open-File Report 2008–1097, 26 p.

Appendix 1 Additional Figures
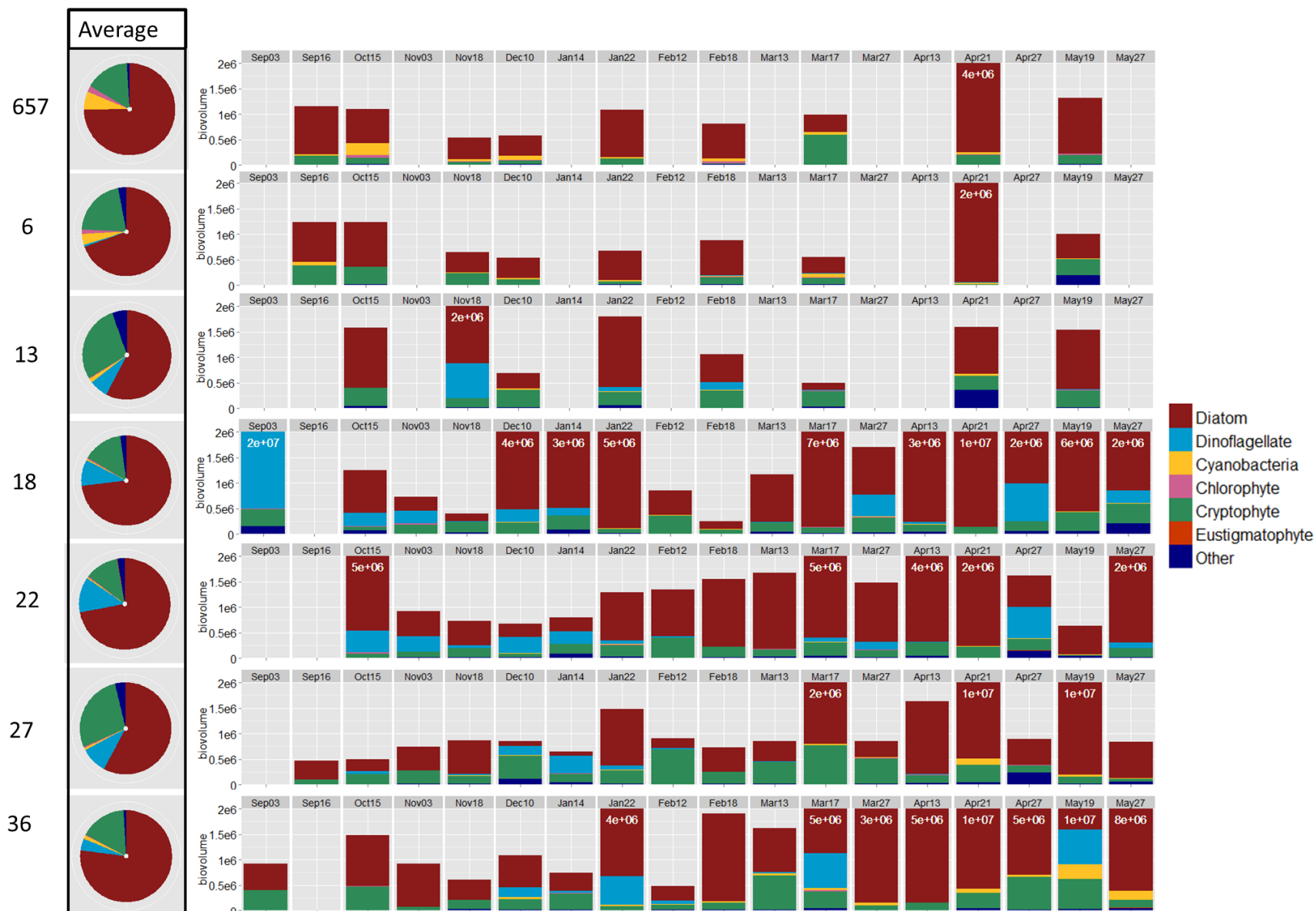
Figure A.1.1 Biovolume (µm³/mL) by phytoplankton class as analyzed by microscopy, Sept 2014 – May 2015. For samples with high diatom biovolume that exceed the y-axis, the upper limit is annotated.

Figure A.1.2 Results of algal taxonomy by pigment analysis (CHEMTAX) for November 2011- April 2014, shown as chl-a contribution by class, with consistent y-axis. Instances of high chl-a, where the yaxis is exceeded, is annotated with the upper bound.
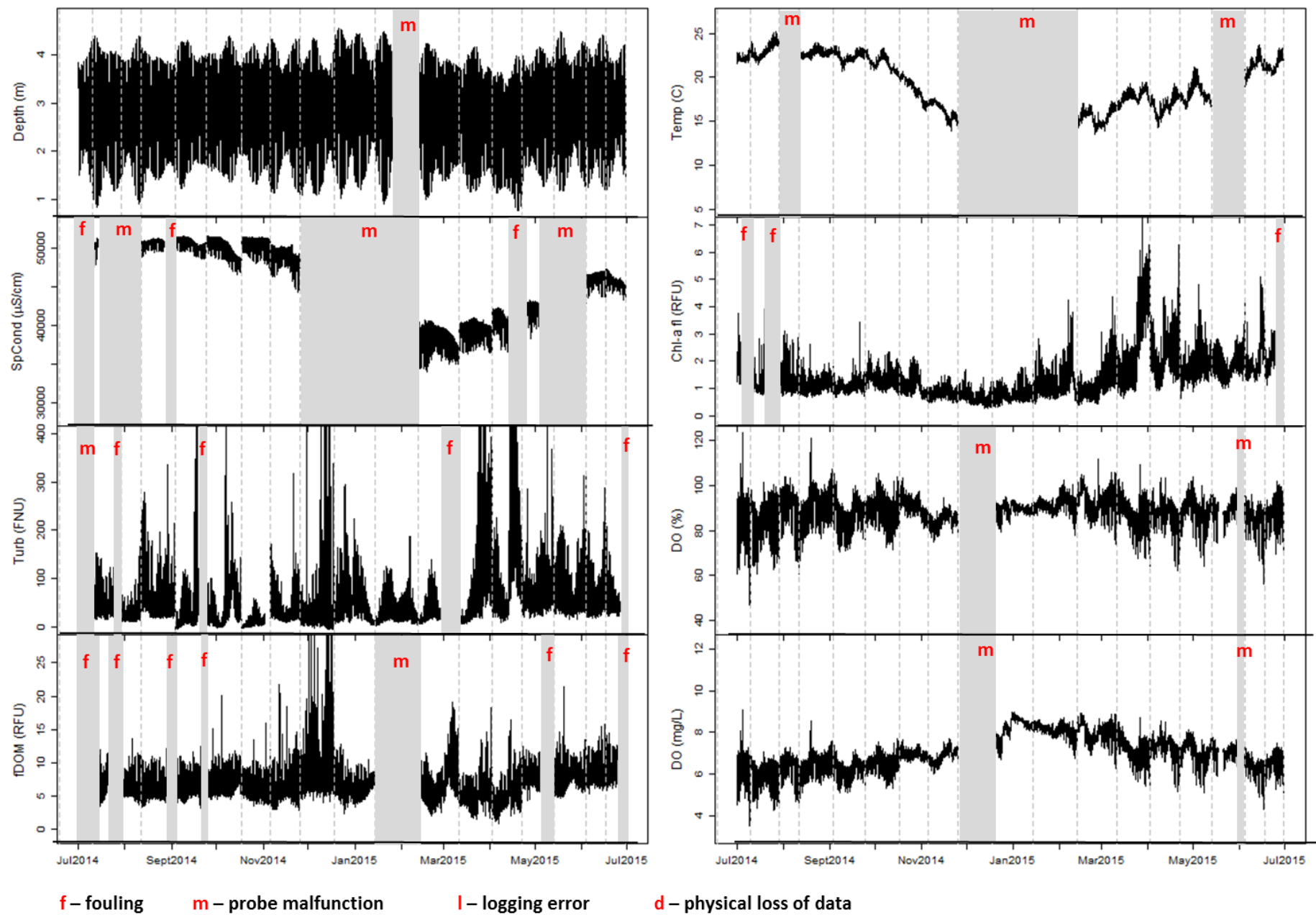
Figure A.1.3 Dumbarton Bridge sensor data. Periods of missing data indicated by grey boxes, with reason noted

f – fouling   m – probe malfunction   l – logging error   d – physical loss of data

f – fouling   **m** – probe malfunction   **l** – logging error   **d** – physical loss of data
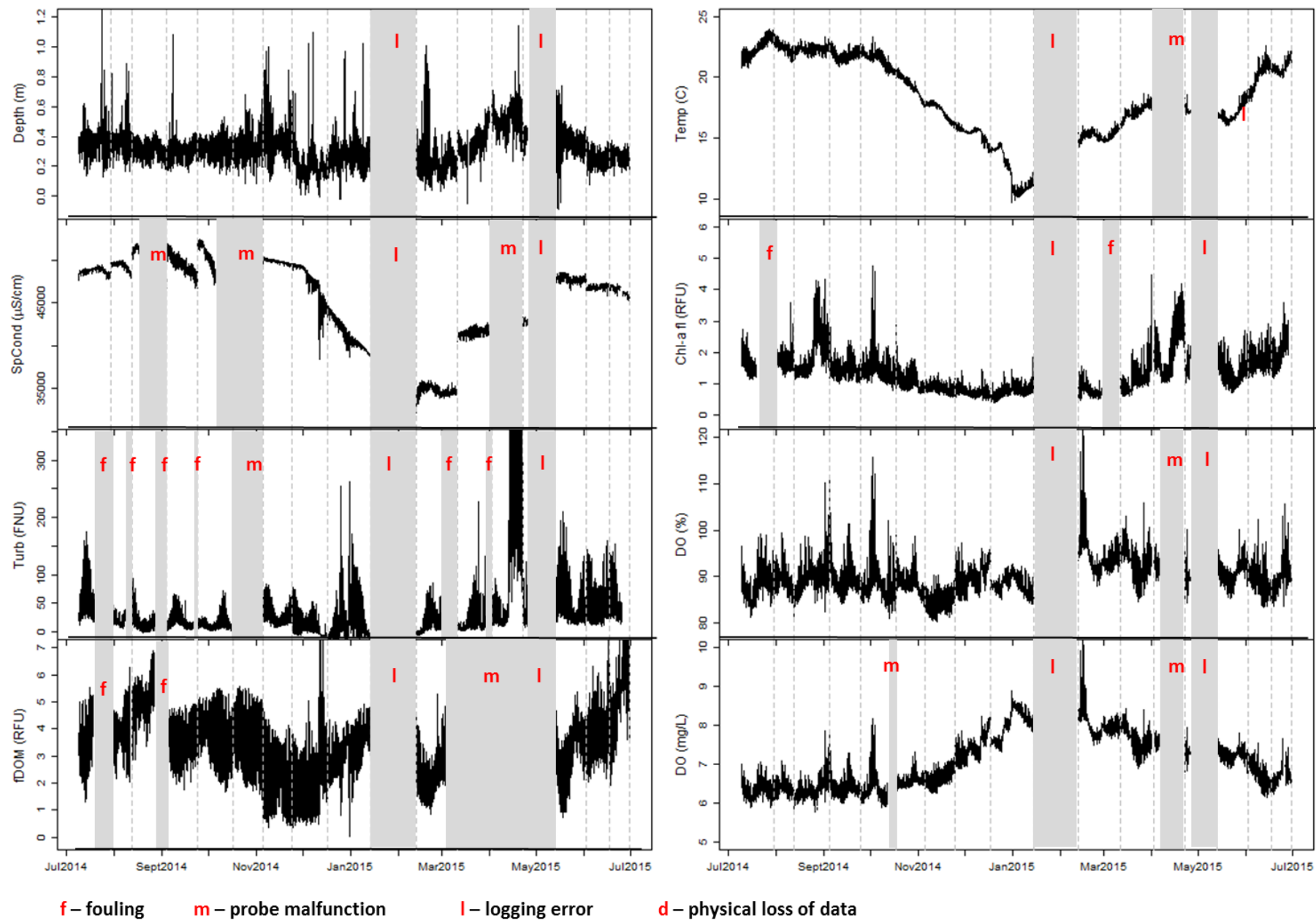
Figure A.1.4 San Mateo Bridge sensor data. Periods of missing data indicated by grey boxes, with reason noted
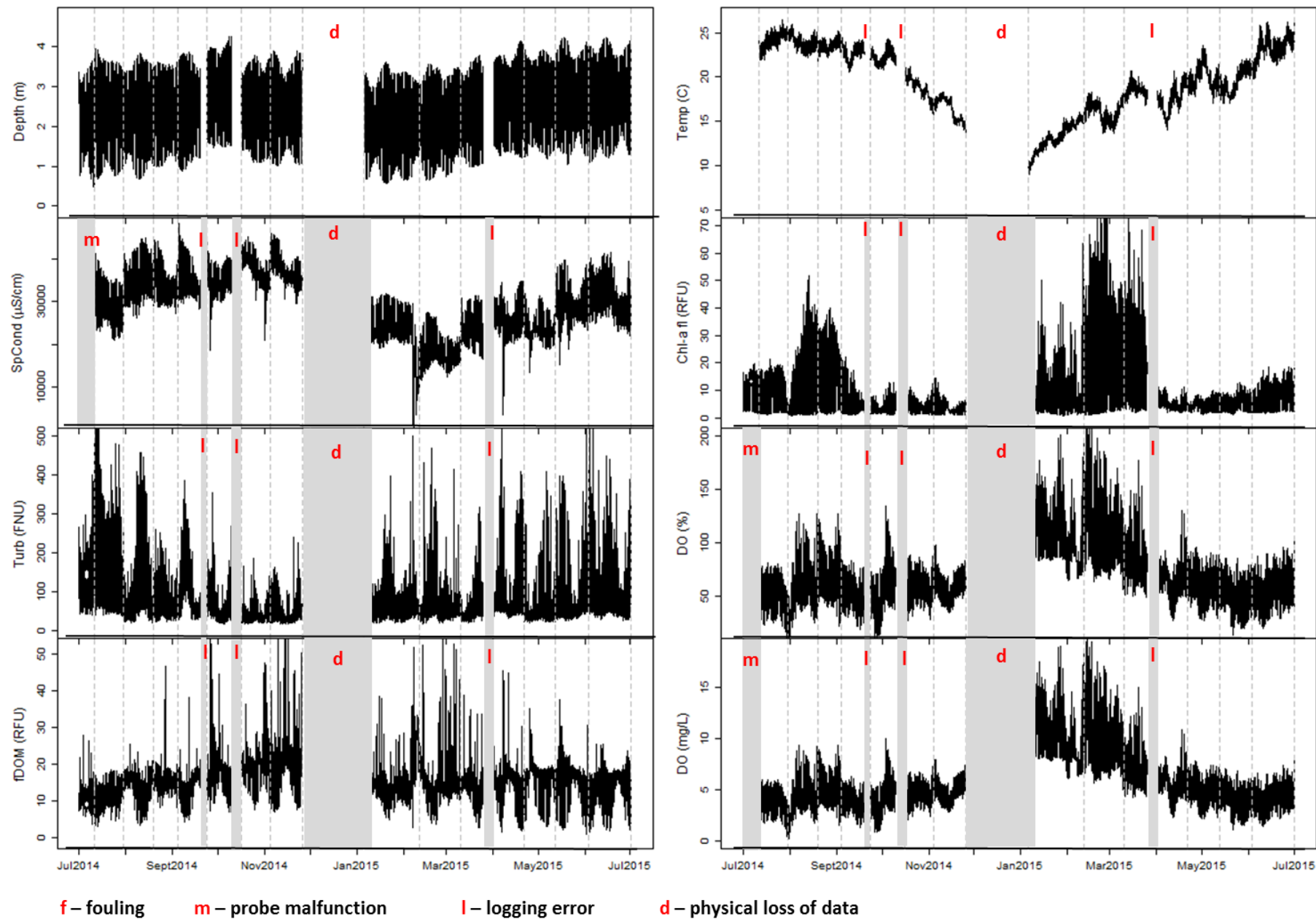
Figure A.1.5 Alviso Slough sensor data. Periods of missing data indicated by grey boxes, with reason noted

f – fouling　　　m – probe malfunction　　　l – logging error　　　d – physical loss of data
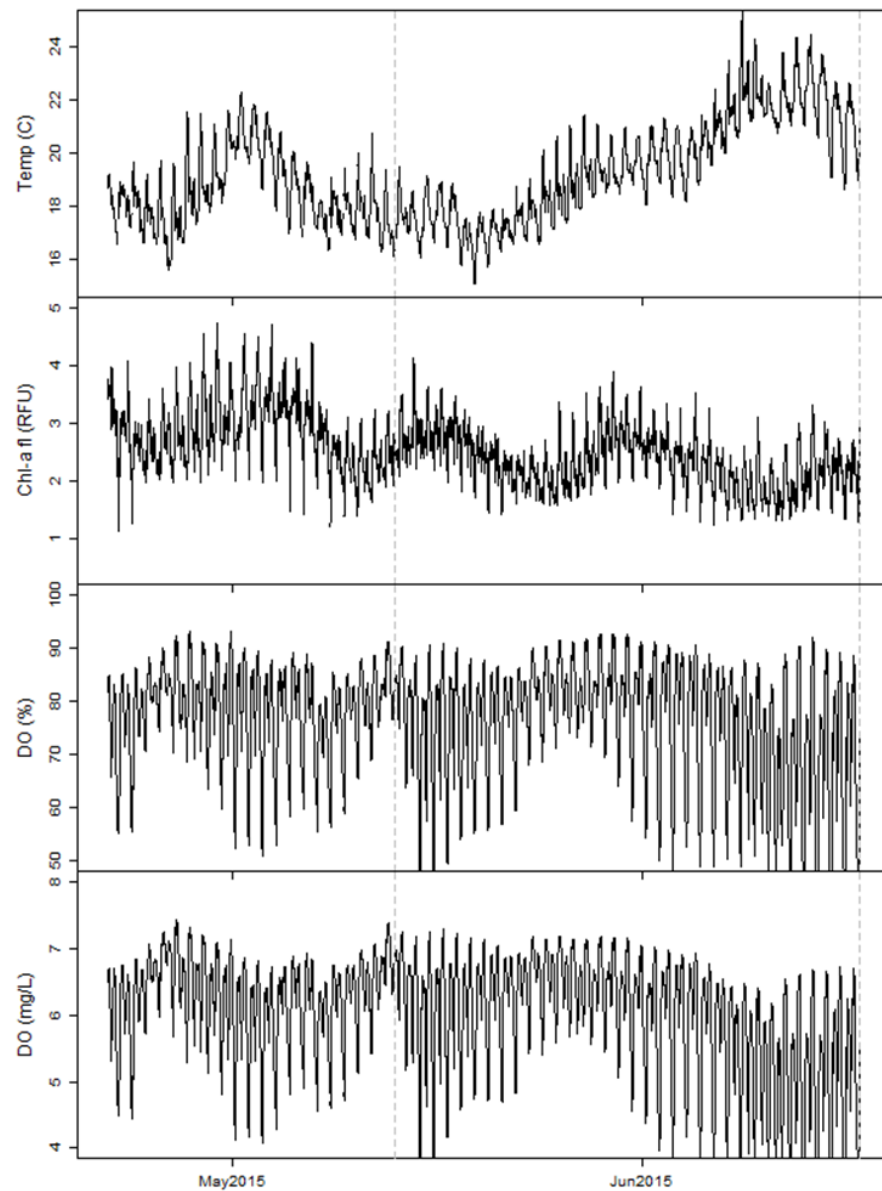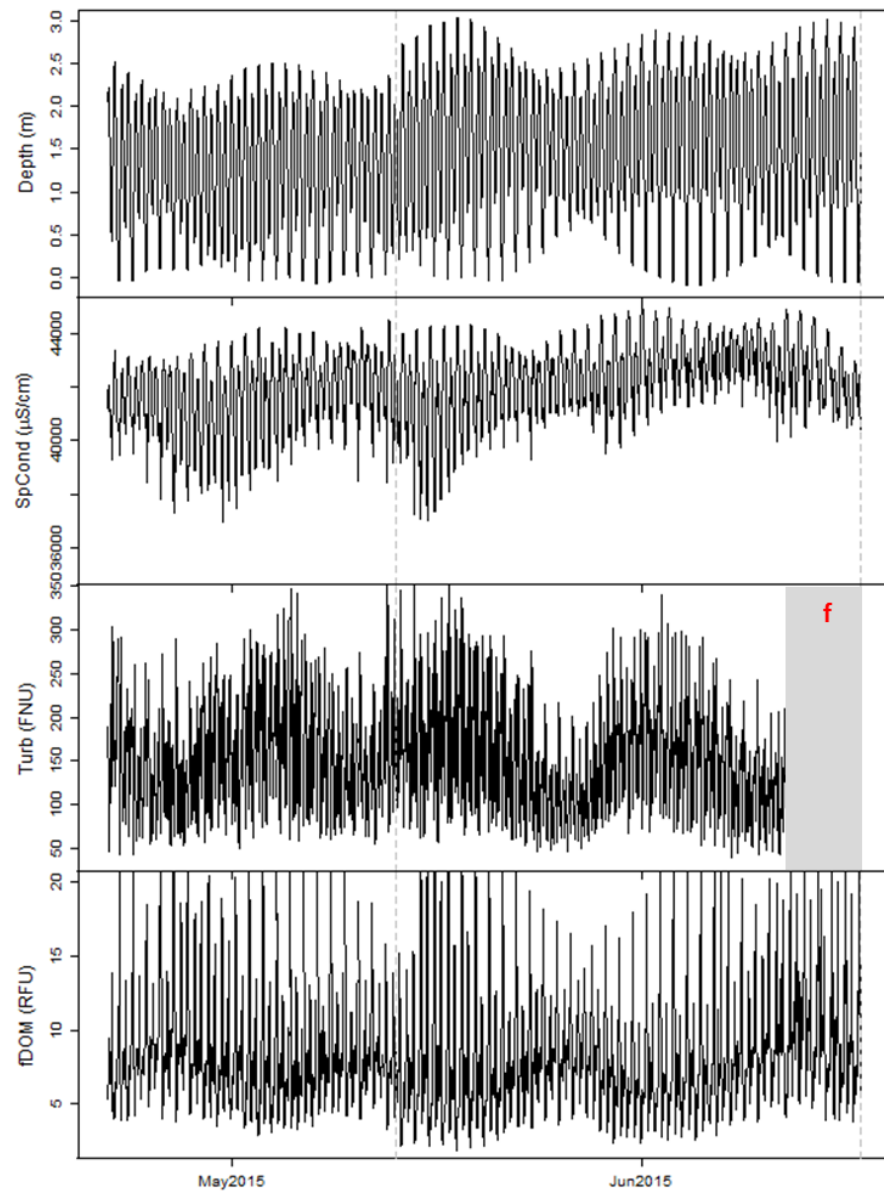
f – fouling   m – probe malfunction   l – logging error   d – physical loss of data

Figure A.1.5 Newark Slough sensor data. Periods of missing data indicated by grey boxes, with reason noted

f – fouling   m – probe malfunction   l – logging error   d – physical loss of data
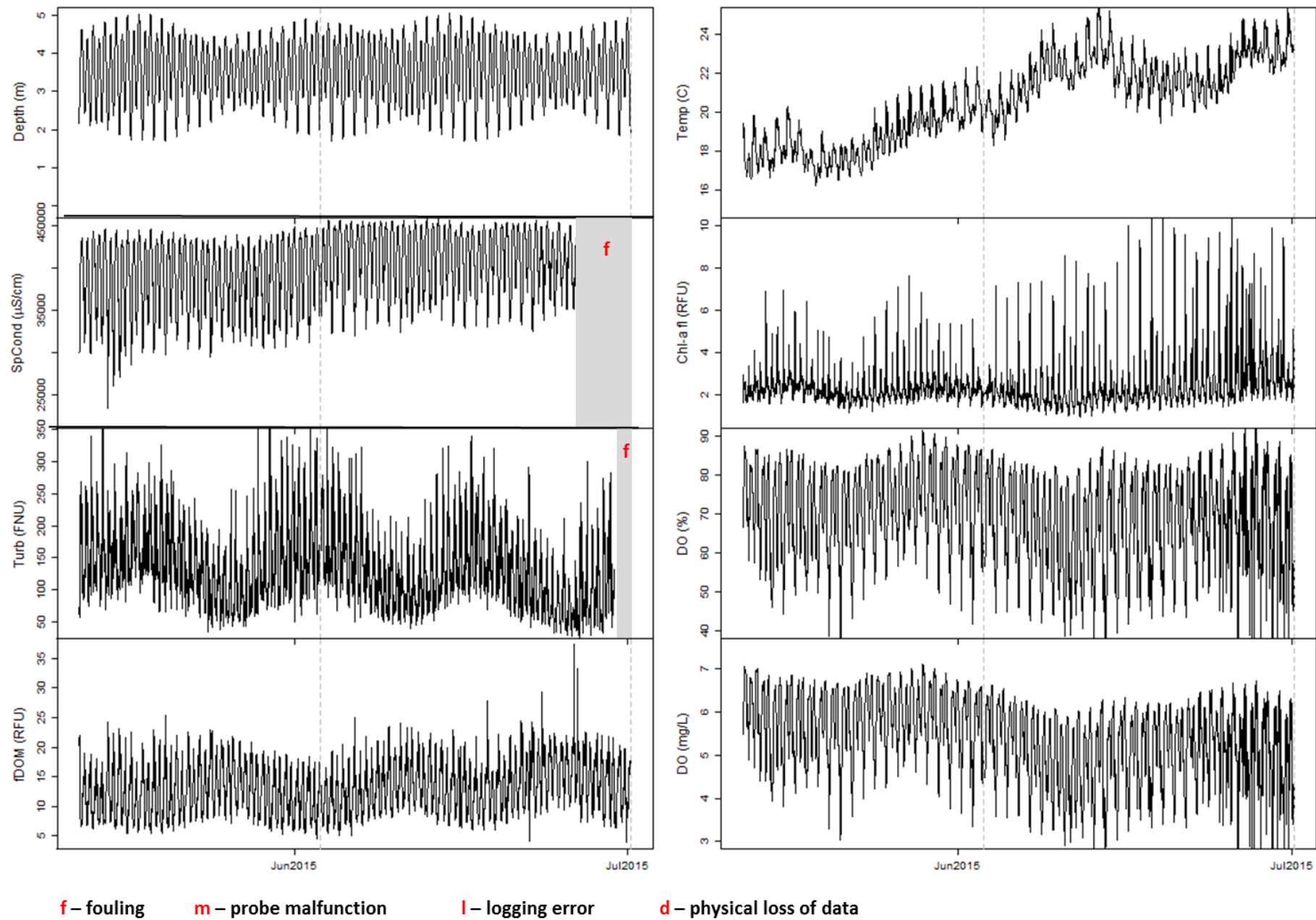
Figure A.1.7 Coyote Creek sensor data. Periods of missing data indicated by grey boxes, with reason noted

f – fouling   **m** – probe malfunction   **l** – logging error   **d** – physical loss of data
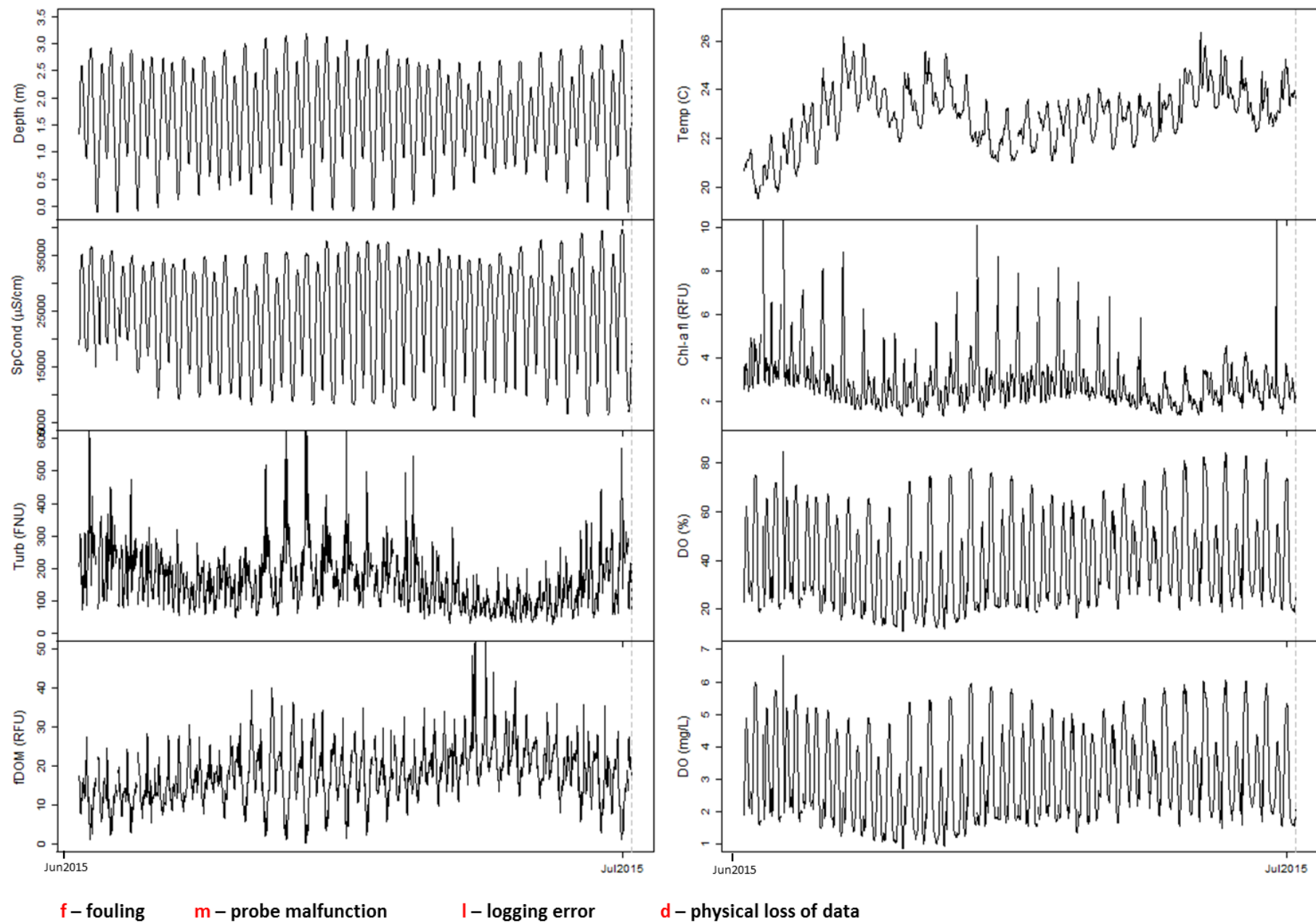
Figure A.1.8 Guadalupe Slough sensor data. Periods of missing data indicated by grey boxes, with reason noted

f – fouling   **m – probe malfunction**   **l – logging error**   **d – physical loss of data**
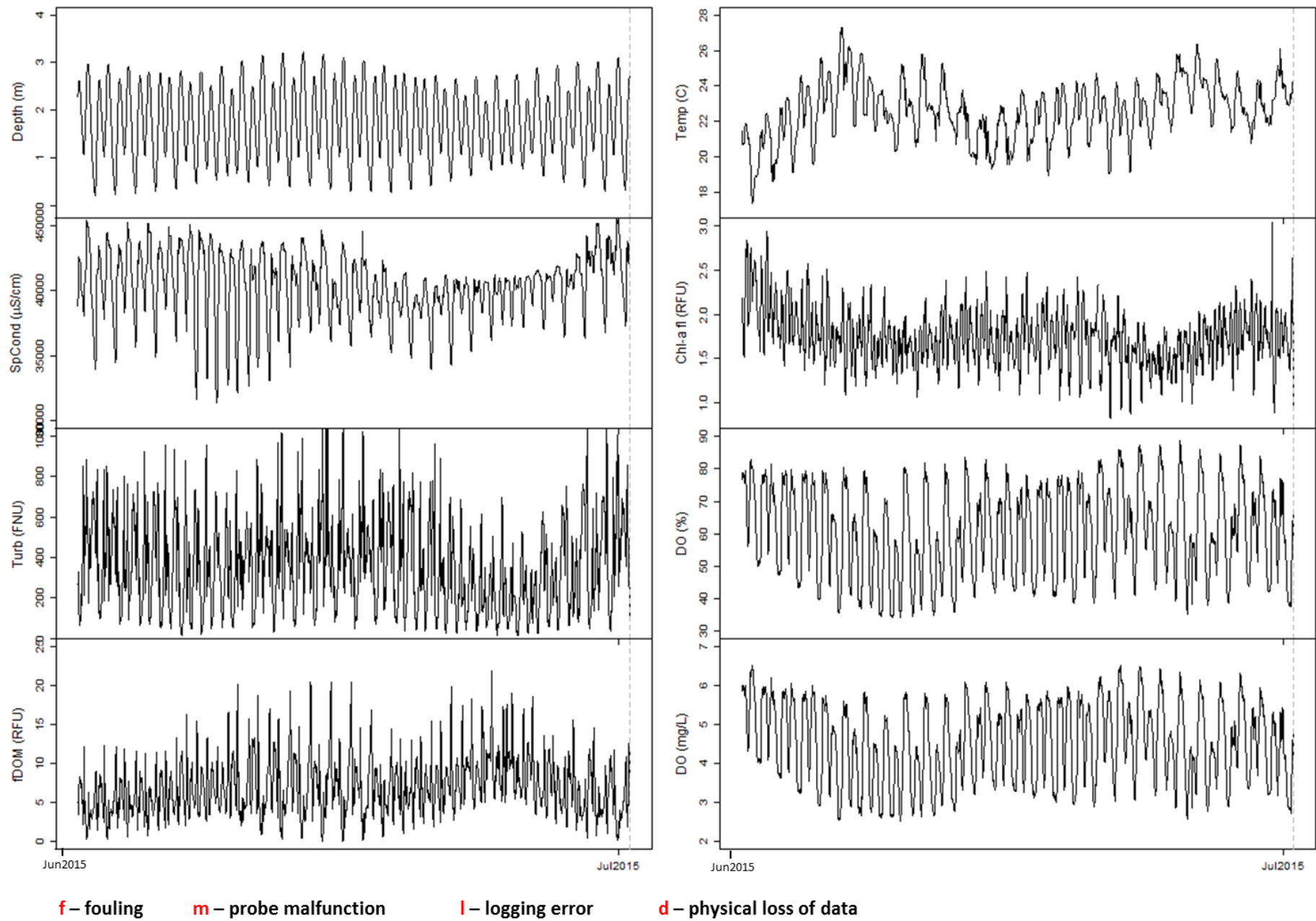
Figure A.1.9 Mowry Slough sensor data. Periods of missing data indicated by grey boxes, with reason noted
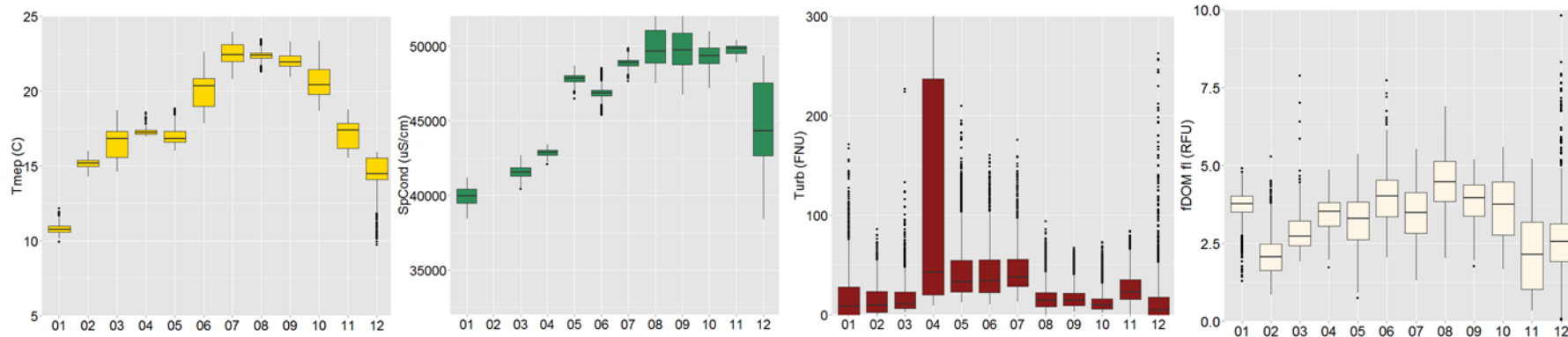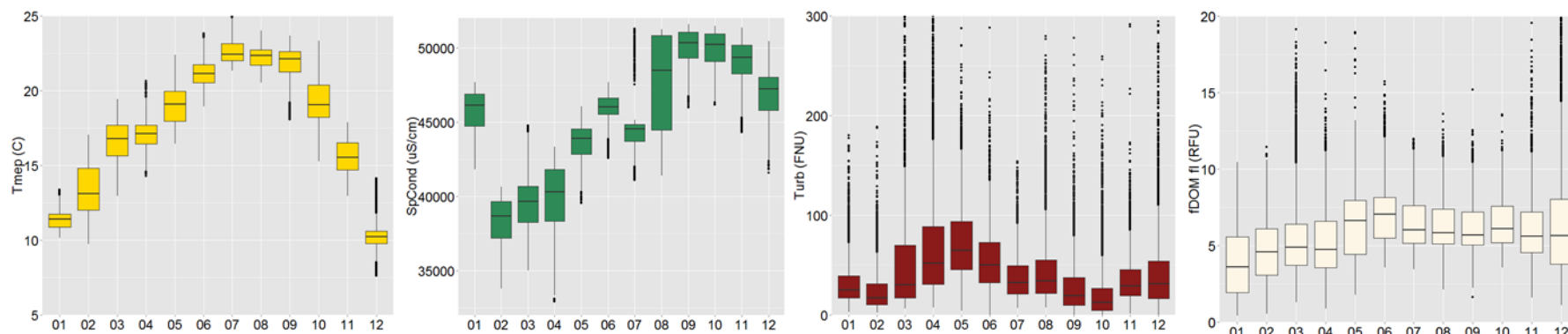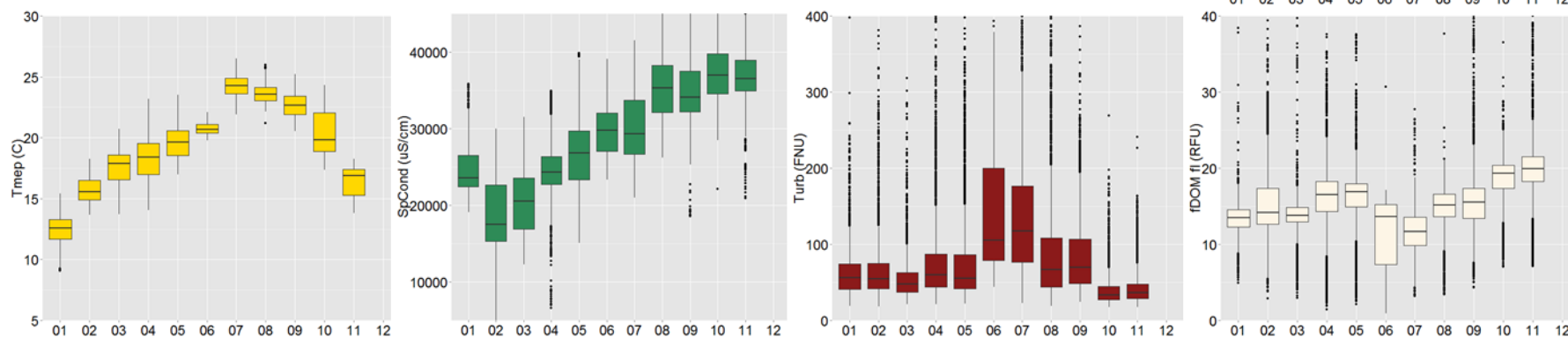
Figure A.1.10 Monthly boxplots of temperature, specific conductivity, turbidity and fluorescent dissolved organic matter at our 3 long-term moored sites for 2013-2015 (Dumbarton and Alviso) and 2014 (for San Mateo)
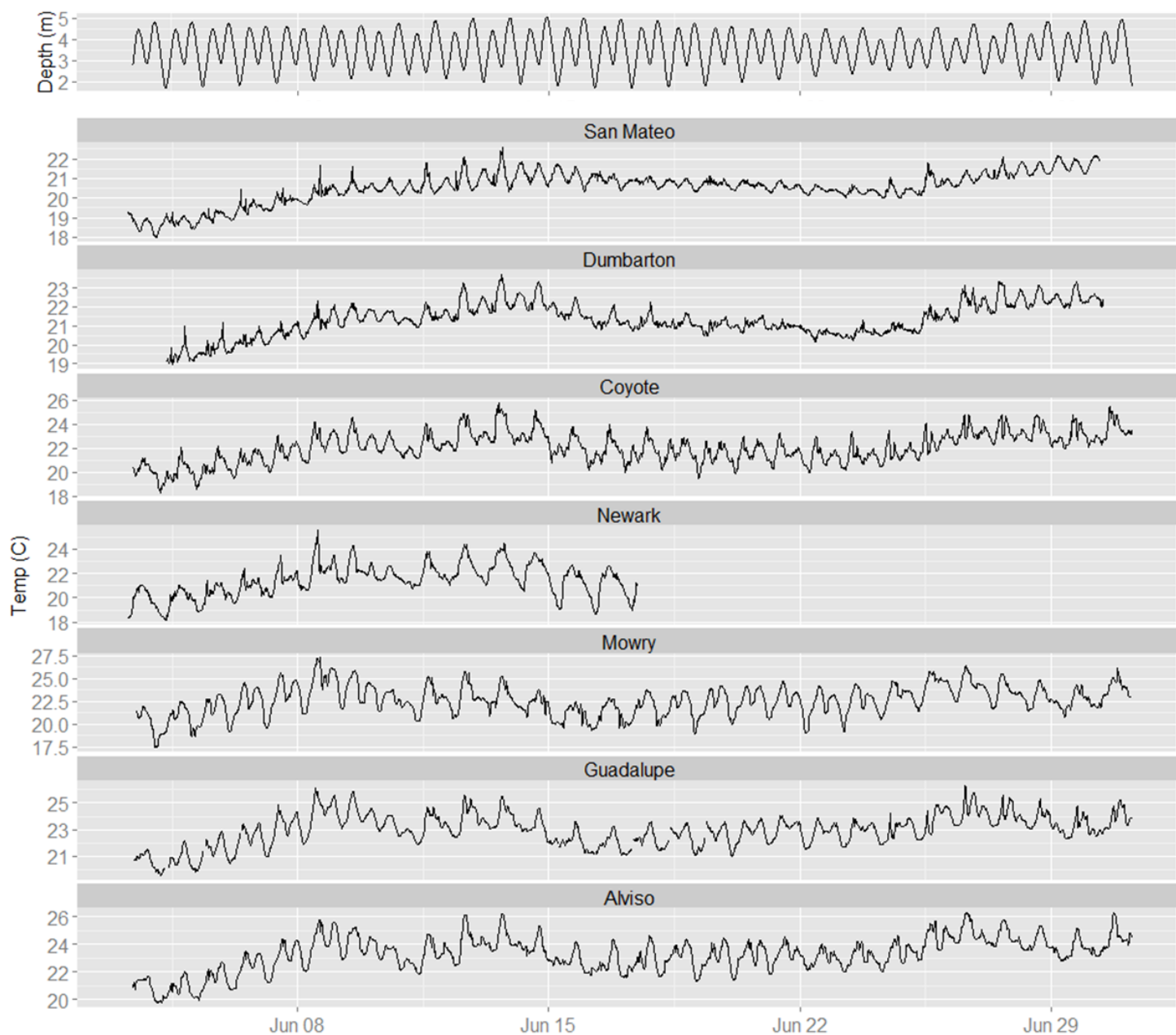
Figure A.1.11 Temp comparison. Depth shown is for Coyote creek
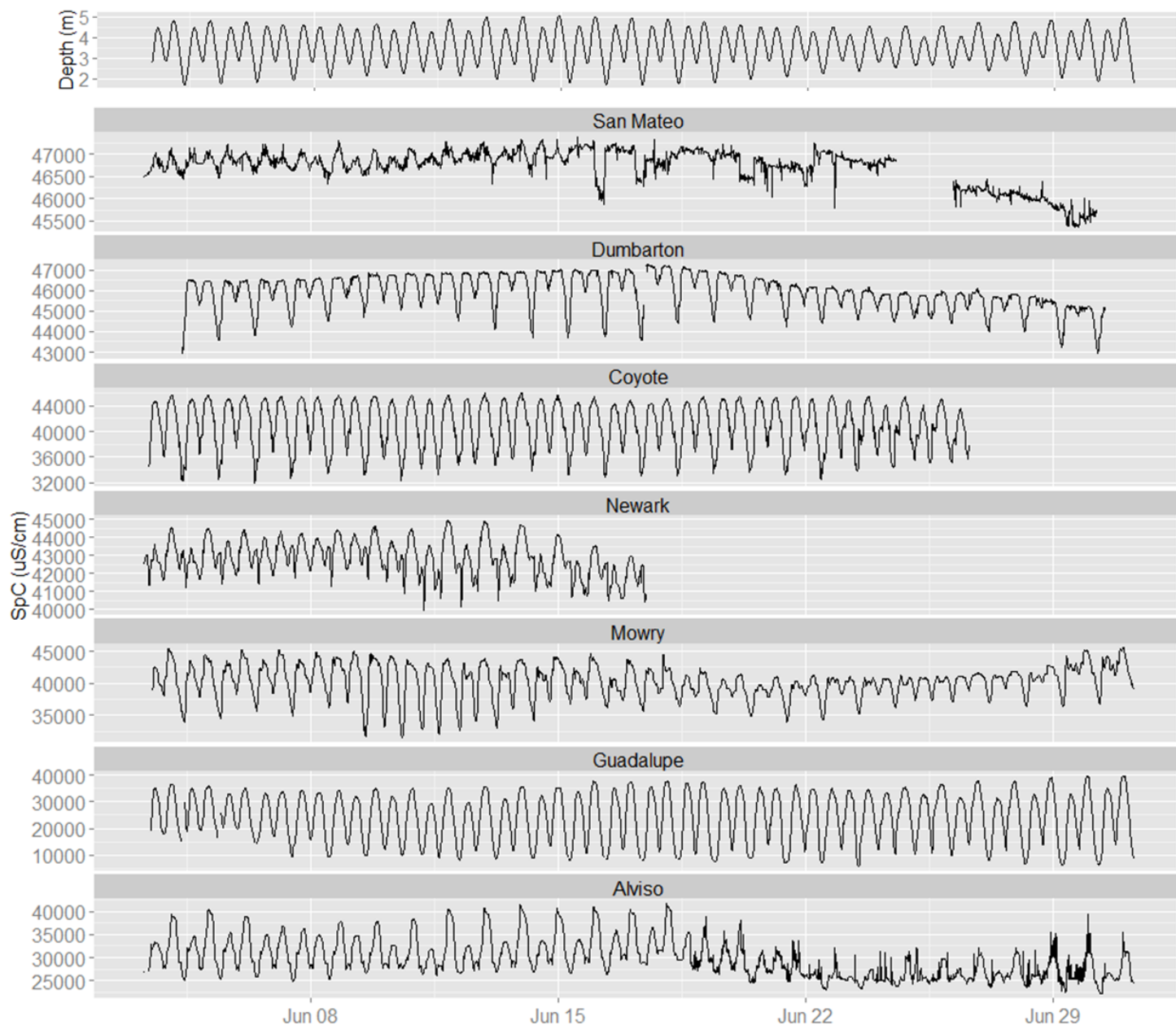
Fig A.1.12 SpC comparison
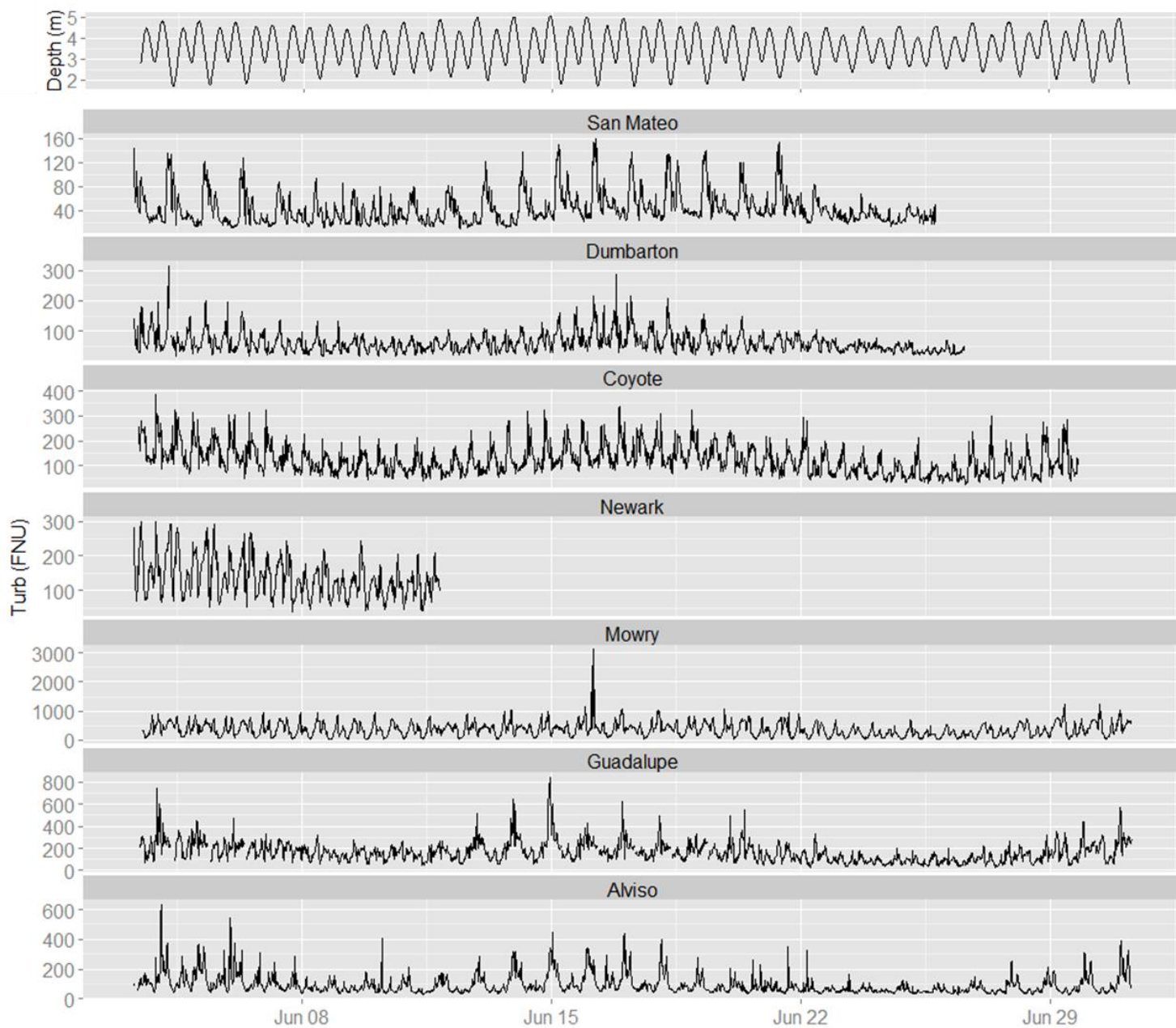
Fig A.1.13 Turbidity comparison across sites
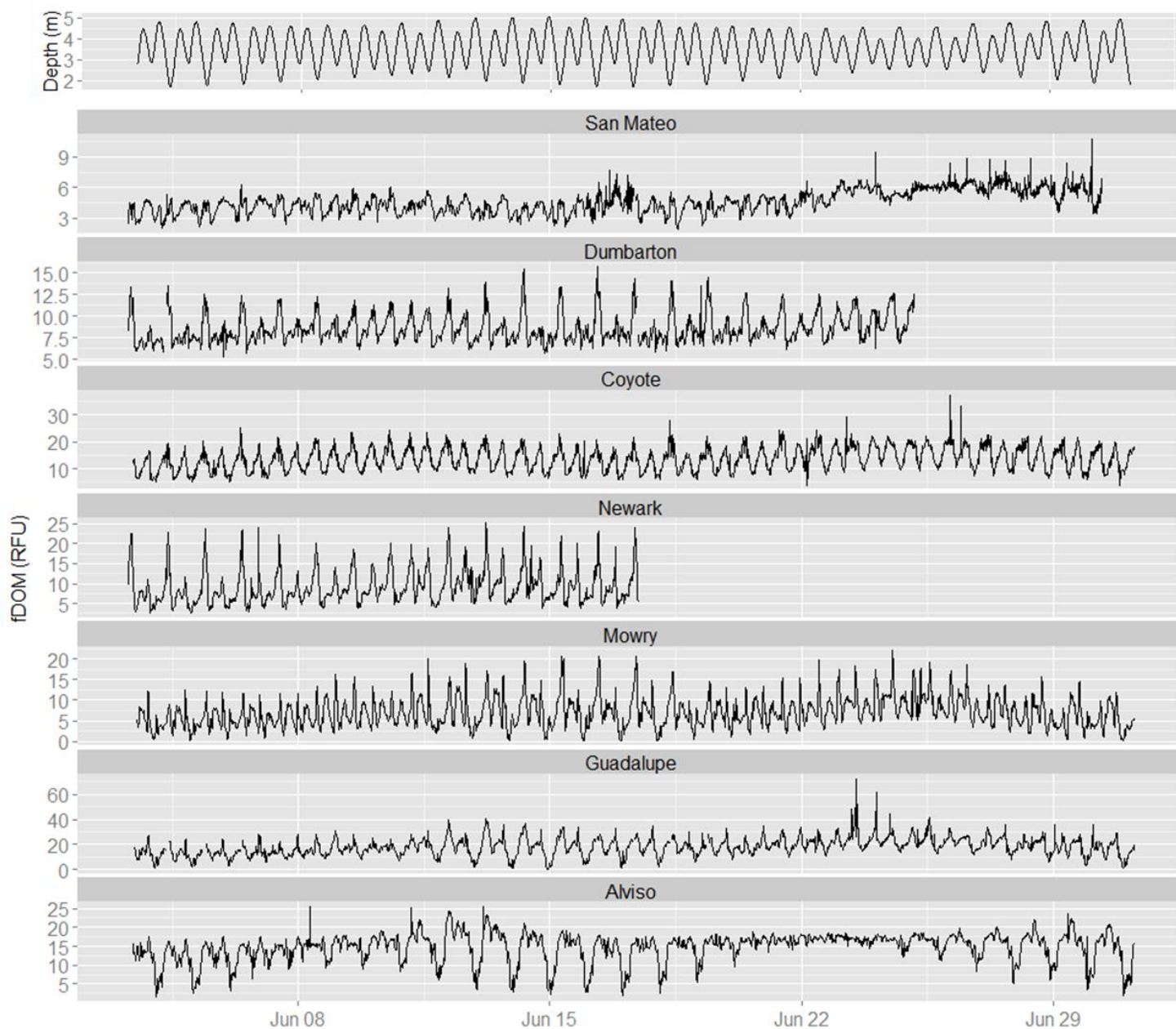
Figure A.1.14 FDOM comparison across sites
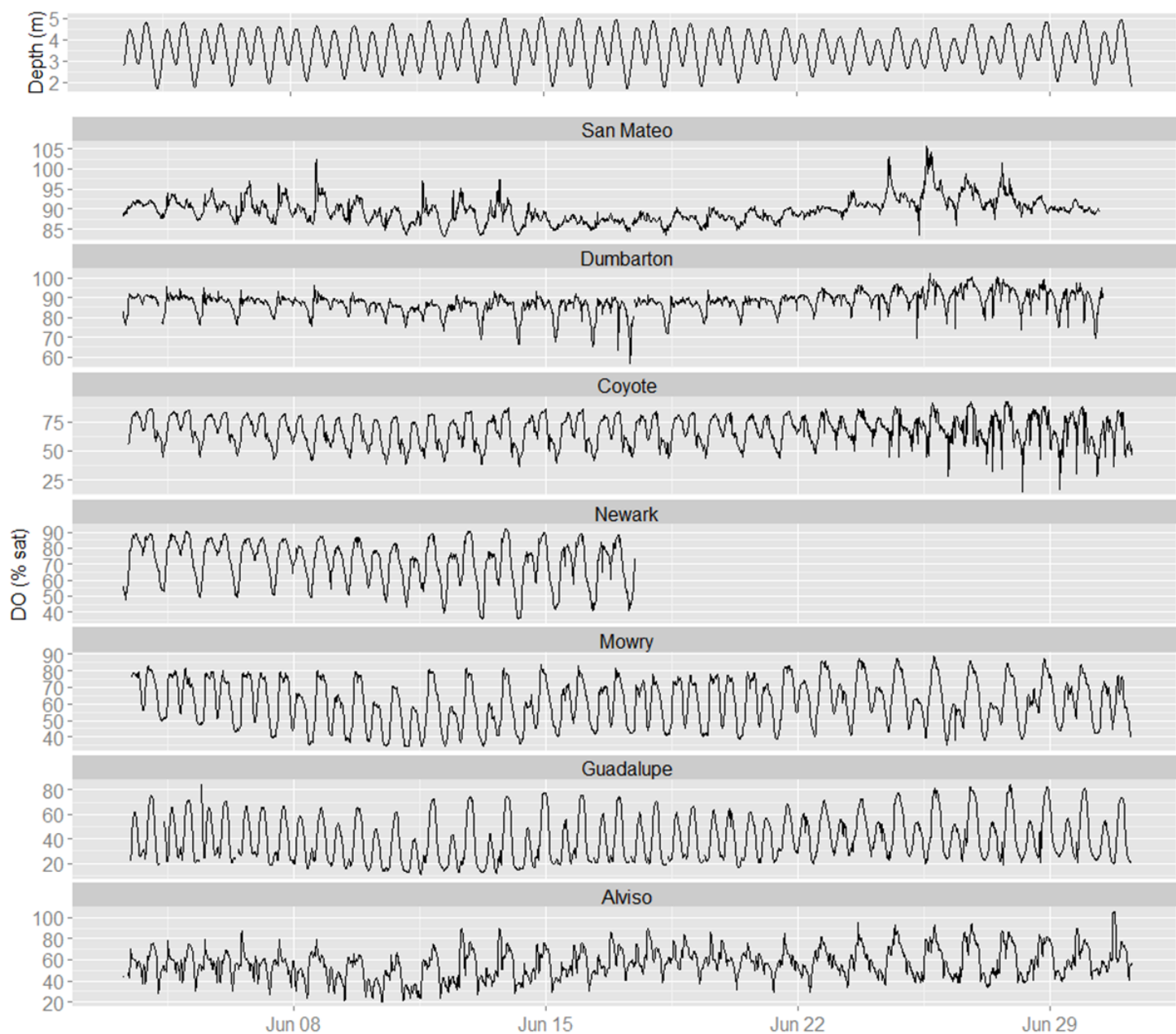
Figure A.1.15 DO % comparison across sites
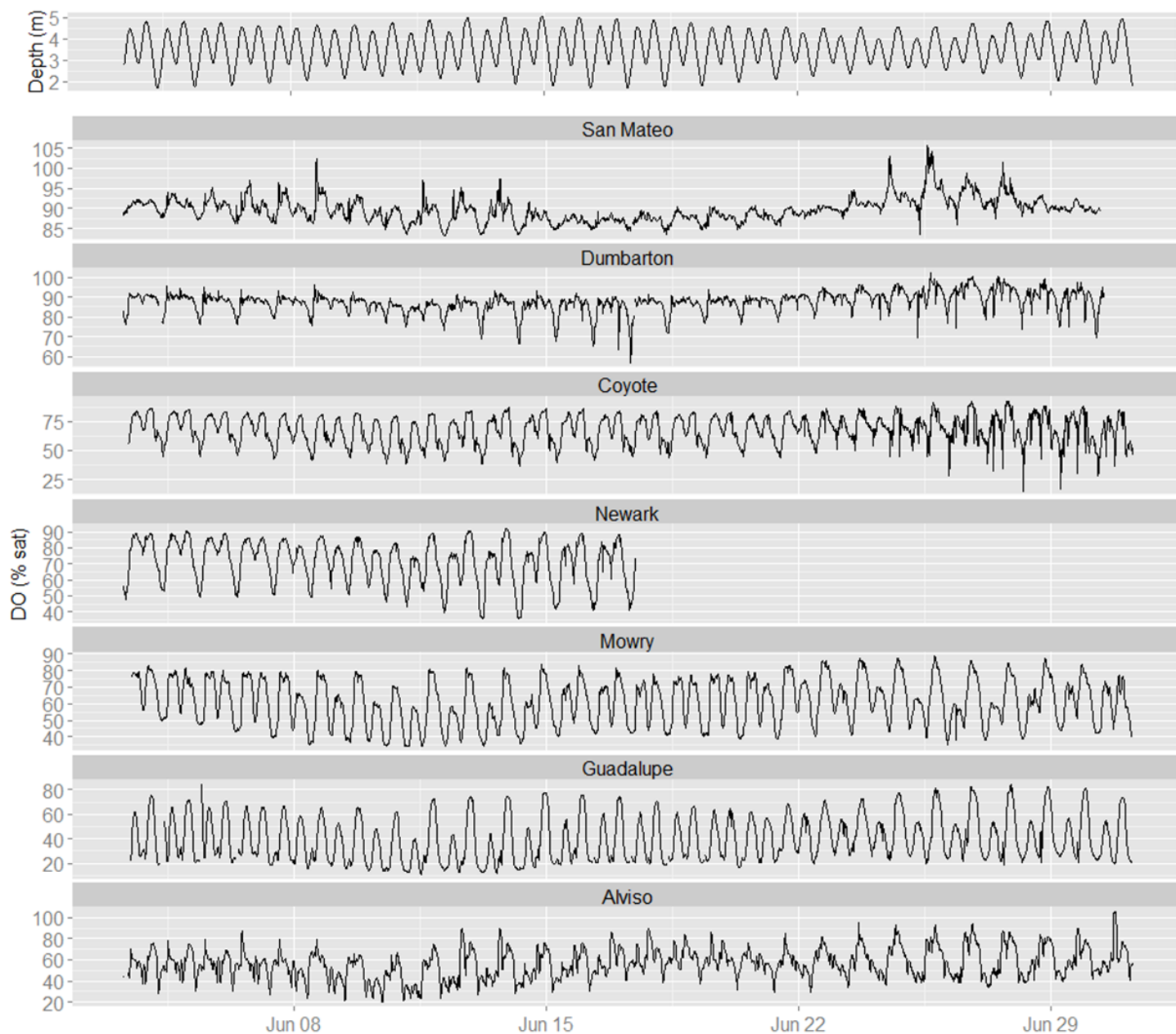
Fig A.1.16 Chl comparison across sites

Appendix 2 Updates on moored sensor maintenance and data management

The goals of the NMSP include: (1) develop capacity to deploy and maintain moored sensors; (2) develop procedures for data management and data quality assurance, including an understanding of sensor accuracy; (3) establish relationships with existing moored sensor programs and determine the role of a moored sensor network in the future nutrient monitoring program; (4) use moored sensor data to address priority science and management questions; (5) use moored sensor data to calibrate and validate biogeochemical models; (6) use moored sensor data to assess condition in SF Bay. The first few years of the NMSP will be disproportionately focused on goals (1)-(3), but we also have some interesting results to present related to (4).

A.2.1 Sensor deployment and maintenance

SFEI and USGS have successfully implemented a variety of different deployment configurations, based on site constraints and scientific priorities. Our sensors deployed during Year 1 of the program were at fixed vertical positions, with variable depths of water above the sensor. This raised some concern that it would be difficult to resolve whether observed tidal variations where due to lateral gradients in a well-mixed water column or vertical gradients, as the sensor moved into a different relative position in the water column depending on tidal stage. To address this, in July 2014, we installed a surface floating sensor at SMB that is in the top ~0.5m of the water column at all times, within the photic depth of SF Bay.

Data management/QA continues to be one of the highest priorities of the NMSP. Common data quality issues we confront are listed below (noted when they occur in Figures A.1.3 – A.1.9), and Table A.2.2 details the frequency and cause of rejected data. One significant data loss (at Alviso Slough in December 2014) does not fall into one of these categories, but instead was due to physical loss of the datafile from that time period.

1. Periodic outliers, particularly in optical instruments (most likely cause by short-lived obstructions of the probes)
2. Sensor calibration drift or calibration inaccuracy
3. Biological fouling and fouling due to fine sediment accumulation
4. Probe malfunction, and ripple effect on calculated parameters (i.e., T/SpC malfunction affecting DO mg/L calculation)
5. Entire sonde shutdown or logging error

Table A.2.2 % data retained, and main reason for omitted data, by site, July 2014-June 2015. Physical loss = backpack stolen. Logging issue is mainly battery failure

		Depth	T	SpC	Chl-a fl	Turb	fDOM	DO %	DO mg/L
Dumbarton Bridge	% retained	95%	70%	55%	85%	85%	80%	90%	90%
	reason rejected	bad probe	bad probe	bad probe	fouling	fouling	fouling	bad probe	bad probe
Alviso Slough	% retained	85%	80%	80%	85%	85%	85%	80%	80%
	reason rejected	physical loss	physical loss	physical loss	physical loss	physical loss	physical loss	physical loss	physical loss
San Mateo Bridge	% retained	90%	85%	75%	85%	75%	70%	85%	85%
	reason rejected	logging issue	logging issue	logging issue	logging issue	logging issue	bad probe	logging issue	logging issue
Newark Slough	% retained	100%	100%	100%	100%	90%	100%	100%	100%
	reason rejected	--	--	--	--	fouling	--	--	--
Coyote Creek	% retained	100%	100%	100%	100%	100%	100%	100%	100%
	reason rejected	--	--	--	--	--	--	--	--
Guadalupe Slough	% retained								
	reason	--	--	--	--	--	--	--	--

	rejected								
Mowry Slough	% retained								
	reason rejected	--	--	--	--	--	--	--	--

During our first year of the NMSP, we developed an automated procedure to remove outliers based on a criteria adapted from that used at the Chesapeake Bay Monitoring Program (cite), where any data point that is more than 3x the hourly rolling average is removed and interpolated around. Occasionally, there is a data point that is not removed automatically but still appears to be an outlier and must be manually removed, but overall this automated procedure has proven efficient and reliable.

We continue to collect field data to assess for the magnitude of sensor drift and biological fouling (procedure described in Appendix A, SFEI 2014 #723), but applying corrections to the datasets is an intensely manual process and has not been successfully implemented to date. One reason for this is that biological fouling is for the most part limited to our two main channel sites, San Mateo Bridge and Dumbarton Bridge. Secondly, calibration drift has not yet been significant enough to justify data rejection. In order to reduce biological fouling at San Mateo and Dumbarton, we now make site visits every 3 weeks year-round (as opposed to 4-6 weeks in the winter during Year 1), which has reduced data loss due to fouling in Year 2. One case study of interest is SpC data loss at Dumbarton Bridge due not to biological fouling but due to fine sediment accumulation in the probe port. The dirtier the sonde carriage became (with build-up of barnacles, mussels and hydroid growth), the worse this problem got because water velocities around the sonde slowed and sediment could more easily fall out of solution. In order to address this problem, we installed a new carriage at DMB in April 2015 and we expect this will reduce SpC data loss.

A significant source of data loss is due to probe malfunction. This affected many types of probes (including fDOM, turbidity and pH), but most significantly impacted the T/SpC probes due to a bad batch of probes distributed by the manufacturer. The last of these probes finally failed at Dumbarton Bridge in May 2015, so from this point forward we should no longer encounter this issue. When these probes failed, it also effected estimates of DO mg/L, since the EXO2 uses T/SpC data from that probe to convert DO % to DO mg/L. To correct this, we used either USGS T/SpC data when available or best estimates based on typical environmental conditions (when USGS data was not available) to perform the conversion. There were a few instances where extreme T/SpC values from the failing probes (1-2 orders of magnitude from typical values) effect DO % accuracy as well (i.e. April 2015 at San Mateo Bridge) and DO mg/L could not be estimated. As detailed previously (cite), substituting either USGS data or best estimates for T/SpC when needed introduces on 0.15 mg/L uncertainty for every 1°C and 0.2 mg/L uncertainty for every 5000 µs/cm, so we believe our DO mg/L estimates are fairly accurate. In order to further assess this, we compared DO mg/L estimates to co-located DO mg/L data from USGS probes, and found reasonable good agreement (see Figure A.2.1, estimated points shown in green). Throughout the last year, we also had a number of our pH probes fail. Due to their high failure rate and the fact that pH is not a high-priority parameter for nutrient-related science and management questions, we chose to phase out pH at all existing sites and not install pH at new sites. This has saved money both on probe repairs and personnel time for data management/processing. If pH proves to be a necessary parameter for model calibration/validation, we can reinstall as needed at select sites.

A.2.2 Data quality
One of the many benefits of collaborating with USGS on moored sensor deployment and maintenance is that co-located sondes at many sites can be an easy way to evaluate the performance of EXO2 probes, as compared to earlier (and perhaps more tried-and-true) models of YSI probes. Figures 2.x compares

data from SFEI and USGS sondes at Alviso Slough for 2014, with 1:1 lines shown in red. USGS also maintains sondes at San Mateo Bridge and Dumbarton Bridge, but they are at different vertical positions on the water column.
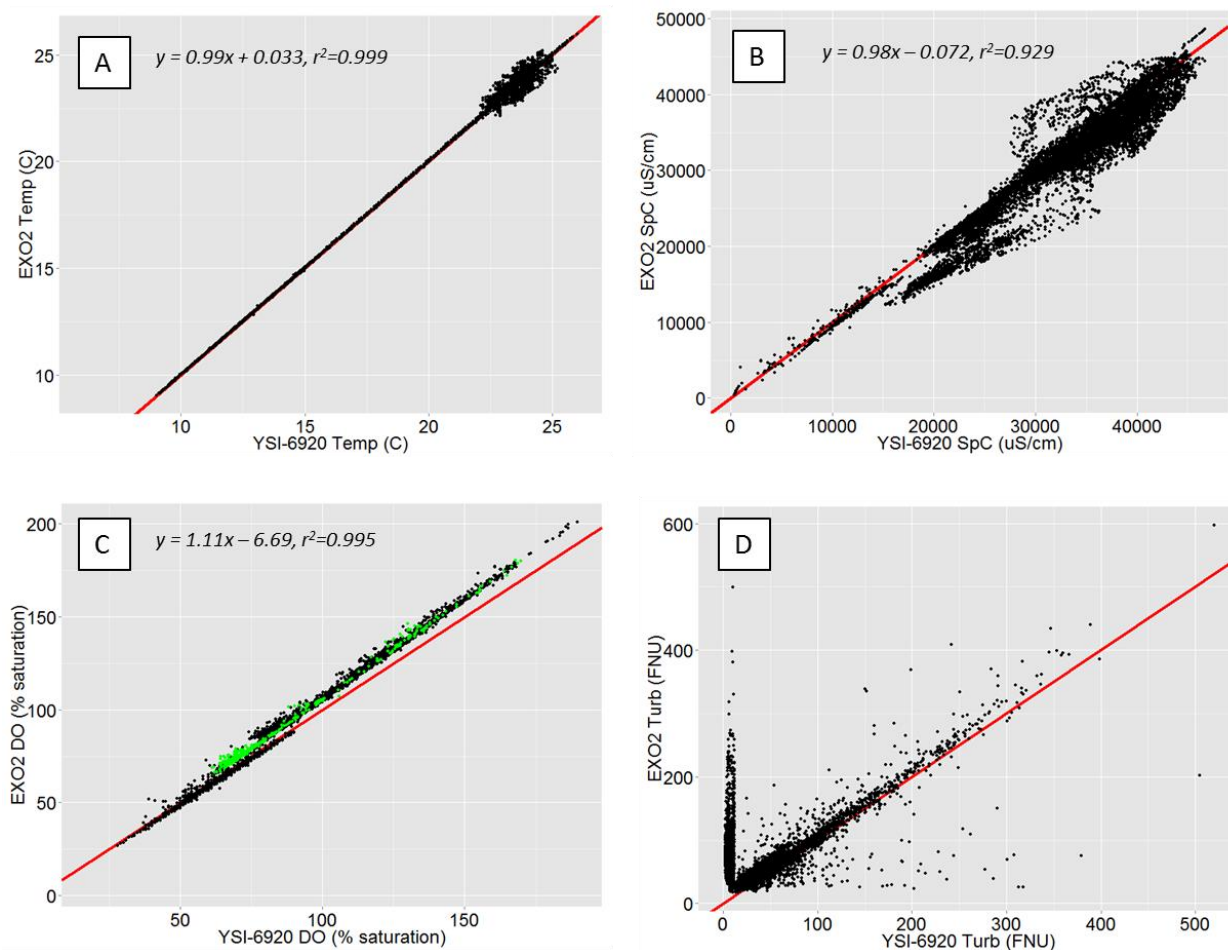
Figure A.2.1 Comparisons of side-by-side deployed YSI 6920 and YSI EXO2 sondes at Alviso Slough for 2014

The results of this comparison are similar to those from previous year (Figure 3.1 in SFEI 2014 #723). SpC and Temp show reasonably good agreement, with regression slopes very close to 1:1 and good r^2 values. EXO2 tends to overestimate DO % compared to the 6920, and turbidity shows the least agreement. There is a cluster of points along the 1:1 line, but also a cluster of points where the EXO2 is responding to something that the 6920 is not, and vice versa. We recently came across a study that suggests that AMCO polymer beads, and not formazin, are better suited to calibrated the EXO2. We will begin using AMCO and investigate whether the instruments show closer agreement.

Chl-a is among one of our highest-priority parameters, but is also subject to the most environmental interference. Our sensor reports in relative fluorescence units (RFU) and accurately inferring chl-a concentration from this is complicated by interferents in the water column (turbidity, decaying phytoplankton) and changes in fl per unit chlorophyll (due to phytoplankton responses to light, varying phytoplankton community composition, etc). A high priority item of the first 2 years of the NMSP was building a library of paired fl/chl samples with which to develop a regression. At the time of last year's report, there were very few samples results, but at this time we now have ~30 samples from our regular site visits, as well as an additional 15 samples from the high-frequency mapping study in April 2015. At

the time of this report, we were still awaiting some results from samples collected during Spring/Summer 2015.

There are two general approaches for developing these regressions: (1) develop multivariate relationships that use ancillary data of potential interferents also collected at moored sensor data (turbidity, temperature, etc) to relate chl-a fl to concentration, or; (2) develop a regression that includes only chl-a fl and concentration, but is season and/or site specific to indirectly account changes in potential interferents. We anticipate using a combination of these two approaches: developing site-specific regressions for each of our instruments, but also including some secondary predictors in our regression (turbidity, fDOM, etc.) where it seem beneficial. Figure A.2.2A shows our chl-a fl:concentration regression for our Dumbarton Bridge sensor. Even without including turbidity, the regression fit is high (r^2=0.94) and the intercept is low (0.30). Adding turbidity does not improve the fit in this case, and turbidity is not considered a significant parameter in the regression (not shown). At Alviso Slough (Figure A.2.2B), our regression results in a poor fit (r^2=0.54), but a reasonable small intercept (1.01). There are two instances of high turbidity (>200 FNU, indicated in Fig 2.XB) that may be affecting the fit, however a multi-variate regression including turbidity improves r^2 only slightly and results in a much larger intercept (6.96). If we force the intercept to 0, which is logical given that zero fl should suggest zero chl-a, then the fit improves (r^2=0.77), however the slope is less than that observed at Dumbarton Bridge. This suggest that there are some site-specific conditions that are attenuating the fl signal more strongly at Dumbarton than at Alviso (or, conversely, amplifying the signal more at Alviso than at Dumbarton). In order to improve the relationship at Alviso Slough, we will need to collect additional samples, particularly at high chl-a values or at high values for potential interferents. One goal of a high-frequency mapping study completed in April 2015 was to collect additional chl-a samples in the channel and slough of LSB, and the results are shown in Figures A.2.2C and A.2.2D (respectively). Like our Alviso Site, the fit resulted in a high intercept and fit improved when the regression was forced through 0. We considered the channel and slough samples separately here in order to highlight the differences in slope, but will likely need to collect additional samples in order to perform any rigorous analysis. In our next mapping cruise scheduled for July 2015, we plan to collect 10-15 samples in each the slough and the channel.
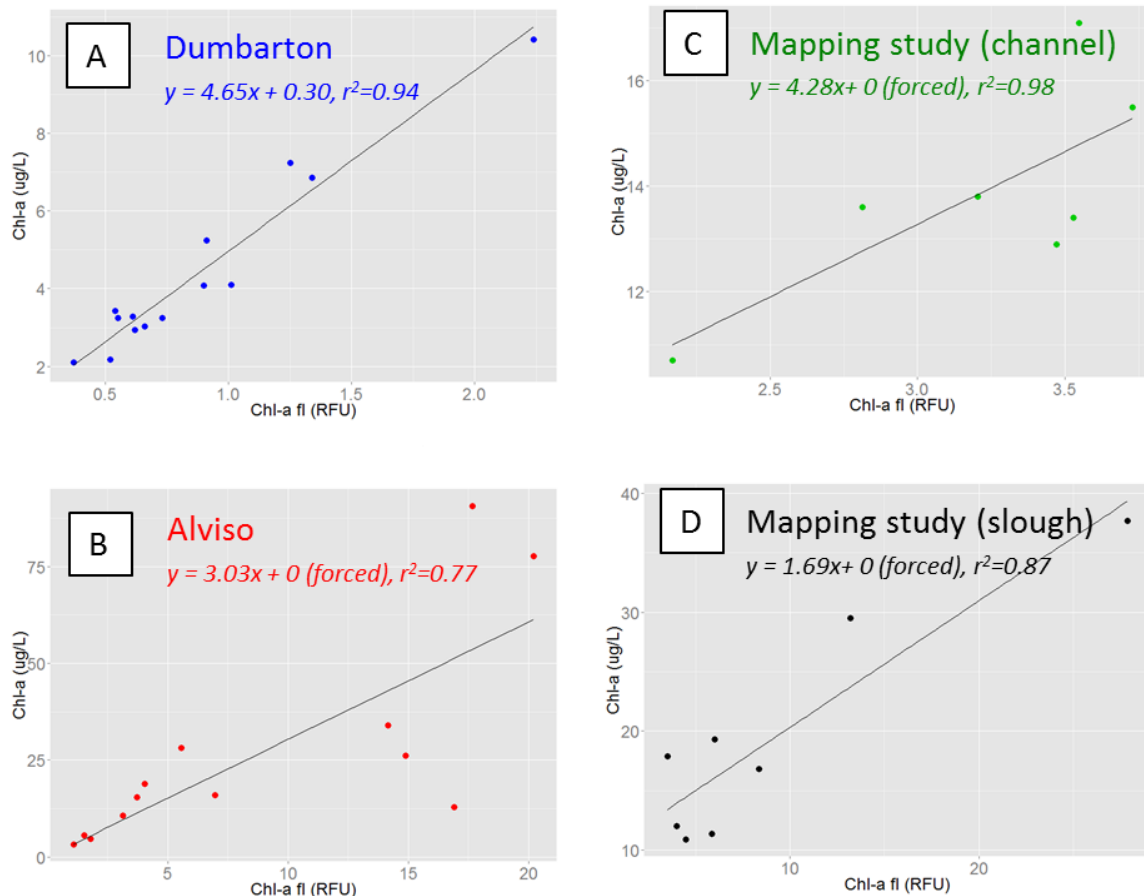
Figure A.2.2 Regression of EXO2 chl-a RFU data with discrete chl-a samples (ug/L) at two of our fixed sites (A,B) and during high frequency mapping work (C,D)

During our mapping study, we were also able to collect calibration samples for our NO3 sensor (A.2.3) We got a very good fit (r2=0.99), but a slope of 0.87. This suggests that the sensor is overestimating the concentration of NO3 in the water column.
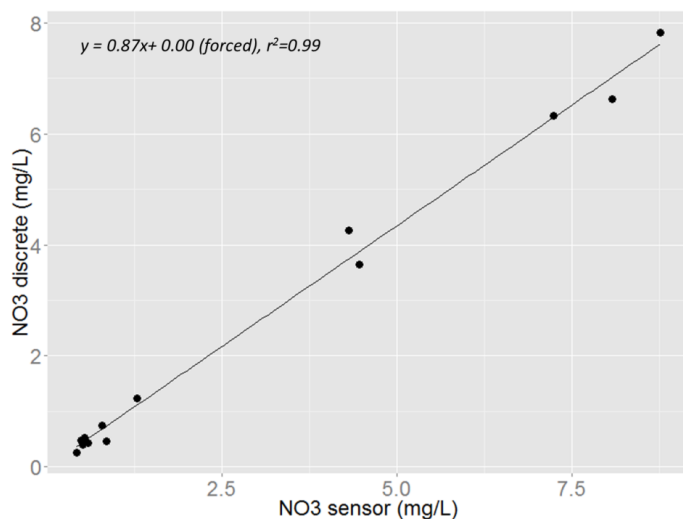
Figure A.2.3 Regression of SUNA NO3 data with discrete NO3 samples during high frequency mapping work in April 2015

Comparison of microscopy and a pigment-based approach to identify phytoplankton classes in San Francisco Bay

San Francisco Bay is a nutrient-enriched estuary, but has not shown the typical symptoms of nutrient over enrichment, such as high phytoplankton biomass and low dissolved oxygen. However, excess nutrients can also adversely affect estuaries by supporting increases in the abundance of harmful algal species, and can lead to other potential shifts in community composition such as changes leading to altered or reduced food quality. Developing an improved understanding of the phytoplankton community-nutrient linkage is among the highest priority nutrient management issues for the Bay, but will require a substantial increase in the spatial and temporal resolution of phytoplankton community data collection. Microscopy is the classic method for phytoplankton taxonomy and biomass quantification, but it is labor-intensive and cost-prohibitive. An alternate method to quantify phytoplankton uses high-performance liquid chromatography (HPLC) pigment analysis, an objective and fast method for identifying the composition of phytoplankton communities, albeit at reduced taxonomic resolution.

In **Task 1.1**, *Calibration and validation of a method for using algal pigments to characterize phytoplankton community composition in San Francisco Bay*, UCSC tested and calibrated/validated an approach in San Francisco Bay that allows phytoplankton community composition to be determined more easily and inexpensively by measuring algal pigment abundance. From this validation approach the total concentration of pigment, as well as an estimate of the classes of phytoplankton present with the sophisticated data analysis program CHEMTAX. Detailed standard operating procedures for both the HPLC methods and the implementation of the factor analysis program CHEMTAX were completed. The validation/testing/calibration was completed using samples from USGS cruises from November 2011 - April 2014, and comparison to duplicate samples analyzed at Horn Point Laboratory analytical HPLC laboratory at the University of Maryland (considered the "gold standard" for pigment analysis).

In **Task 1.2**, *Analyze San Francisco Bay samples, and provide a description of pigments to characterize algal classes in San Francisco Bay*, UCSC using the developed method in **Task 1.1** were contracted to analyze ~100 samples collected on USGS cruise throughout the Bay, with matching microscopy paired samples for comparison to traditional methods. Actual sample count analyzed was 426, including 79 paired microscopy samples. This task also encompasses incorporating long-term monitoring data, which is used to support the findings from this project.

In **Task 1.3**, *Monitoring program development/planning*, participating and planning, contributing to meeting planning, participation in work group meetings, and providing presentations and technical sections for progress reports was completed.

Results - Task 1.1

We tested, calibrated, and validated a method using the Agilent HPLC and standards purchased from DHI Water & Environment. Our final method was adapted from the Horn Point Laboratory method for pigment analysis, adapted to incorporate the unique environment of San Francisco Bay. Estuaries provide a unique challenge for pigment analysis, since samples are originally filtered onto glass fiber filters. For pigment

analysis it is necessary for a comparatively large quantity of pigments to be filtered, difficult in a turbid environment such as an estuary. Our method was adapted to account for this. Provided here completes the **method development from Task 1.1**, and is the finalized standard operating procedure, including a supplies list, reagent preparation, and analysis of samples. The SOP is included as supplemental material 1 at the end of this report.

For **Task 1.1 calibration and validation of the method**, a Carey UV spectrophotometer was used with published extinction coefficients to quantify pigment standards, which were subsequently compared to the HPLC values to provide response factors that were to be used to quantify samples pigment concentrations. This is following standard procedure to quantify pigment concentrations by HPLC and has been described in the literature (NASA SeaHARRE reports). We further validated the method by comparing duplicate samples sent to Horn Point Laboratory. Our samples were within 0.6 - 14.7% CV compared to their chlorophyll-a pigments, and between 0.2 - 18% CV for other ancillary pigments (within the expected % CV between laboratories, < 15% for chlorophyll pigments, and <25% for ancillary pigments, as reported in NASA SeaHARRE-5).

Excitingly, based on this analysis, the UCSC laboratory was asked to participate in the NASA SeaHARRE-8 working group to better calibrate HPLC analysis of pigments between laboratories. This is beyond the scope of this project, but was a brought about because of the methodology completed during this project.

For analysis of samples, and **completion of Task 1.2,** a standard operating procedure was written for use of the factor analysis program CHEMTAX. This program is used to calculate the contribution of the different algal groups to the total chlorophyll a, taking into account the concentration of accessory pigments, which are unique to different classes of phytoplankton and was first described by Mackey et al. 1996. This infers the composition of the phytoplankton community, to the class level, and the output provides the percentage contribution for each chemotaxonomic group to the community. Some knowledge of the original community must be understood, and for this project, that was taken from the USGS multi-decadal time series of phytoplankton microscopic analysis in order to provide a reasonable estimate for the original community, along with published literature values of accessory to chlorophyll a pigments ratios. Included as supplemental material 2 is the initial input matrix ratio, determined from the literature and also with input from the microscopy counts completed in San Francisco Bay.

Results, Task 1.2, all 426 samples from San Francisco Bay and estuary were analyzed. CHEMTAX was used to identify classes of phytoplankton in the Bay, based on their pigments. The classes identified were: diatoms, dinoflagellates, cryptophytes, cyanophytes, chrysophytes, chlorophytes, eustigmatophytes, euglenophytes, haptophytes, and raphidophytes. These classes were identified as likely classes of phytoplankton in San Francisco Bay because of the microscopy counts completed from the USGS samples. The microscopy samples include all of the above classes of phytoplankton, as well as Holotrich ciliates. Ciliates were not included in the CHEMTAX analysis because they are purely heterotrophic or kleptoplastic plankton and therefore only have the marker pigments of the phytoplankton that have been ingested. When using CHEMATX, these pigments would be included with their class that best represents them, and not with ciliates.

For the scope of this project, we have included the results from the comparisons between paired classes from microscopy samples (as relative percentage of biovolume, μm mL^{-1}) and relative percentage from CHEMTAX. Samples are compared as ALL of the paired samples (regardless of year, or location), by subembayment (location), and as single stations (date and location) to investigate the usefulness of CHEMTAX.

There were 79 filters paired with microscopy samples, out of 436, some in each part of the Bay. Figure 1 shows the sampling locations, and subembayment markers.

Fig. 1. Map and subembayments of USGS sampling locations.

When samples are compared across time and space, there is very good matching between the three main classes of phytoplankton (diatoms, dinoflagellates, and cryptophytes; Figure 2). To bring this into perspective, an analysis of USGS microscopy data from 1992 - 2013 indicates that these three classes of phytoplankton have a total biovolume 100 - 1000 times greater than the other classes that were identified. The fourth group identified as being in the same category as diatoms, dinoflagellates, and cryptophytes are the ciliates, but those are unidentifiable with CHEMTAX (or cross identified as the phytoplankton they have ingested).

The diatoms represented 70% of the phytoplankton with microscopy, and 65% with CHEMTAX, the dinoflagellates 22% with microscopy and 24% with CHEMTAX, and the cryptophytes were 6% of the total for both methods (Fig. 2). For the other groups, the chlorophytes, chrysophytes, cyanophytes, eustigmatophytes, euglenophytes, haptophytes, and raphidophytes, the total biovolume made up 2.1% of the relative proportion of phytoplankton from November 2011 - December 2013.

Relative percent of classes, microscopy (biovolume μm mL^{-1})

Relative percent of classes, CHEMTAX
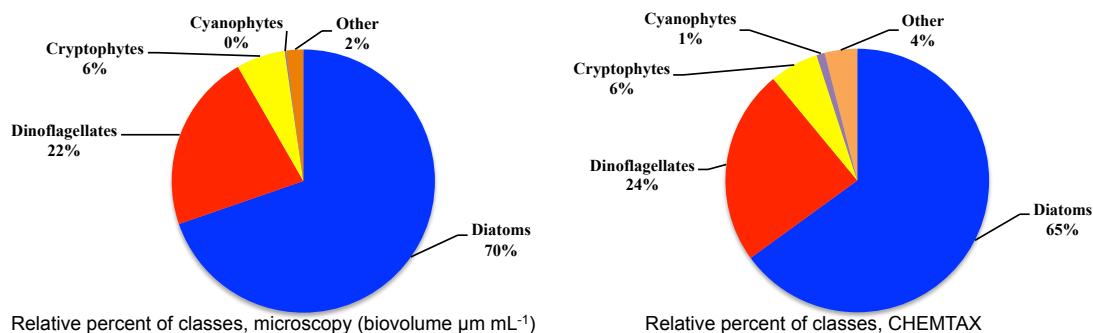
Fig. 2. Comparison of phytoplankton classes by microscopy and CHEMTAX program for San Francisco Bay for 79 paired samples spanning 3 years.

In terms of "matching" biovolume and CHEMTAX, the quality of the matching is very good, compared to the minimal literature that has compared similar samples. While there is some discrepancy between the total percentage of diatoms (5% difference between microscopy and CHEMTAX) and dinoflagellates (2%) this is likely because some of the marker pigments for dinoflagellates and diatoms are the same, making it difficult to split the two groups sometimes. To overcome this, it is essential to fine-tune the input matrix for CHEMTAX to accurately reflect when the pigment ratio should be for a dinoflagellate or a diatom. An example of this will be in Figure 4 and supplemental material 3.

For figure 2, we included the phytoplankton class "cyanophytes" because, while not one of the major classes of phytoplankton we see in the Bay, they are often considered a group of problem phytoplankton - responsible for many of the harmful algal blooms that can occur in San Francisco Bay. CHEMTAX identifies that 1% of the chlorophyll is composed of cyanophytes (compared to <0.3% for microscopy). The discrepancy here, is likely due to the microscopy counts and not the CHEMTAX program, as cyanophytes are picoplankton, and often too small to identify during microscopy counts. The pigments are present in the samples though, and easily identified by CHEMTAX based on the representative marker pigments that are only present in cyanophytes. Furthermore, as evident from the "other" pie section in figure 1, CHEMTAX is identifying a greater proportion of the classes attributed to the other groups of phytoplankton besides diatoms, dinoflagellates, cryptophytes or cyanophytes. This is to be expected, as CHEMTAX can be used to easily identified rare and very small cells, which are often missed during microscopy. It is not a false positive put out by the CHEMTAX program (as can happen, if the input ratio is incorrect), as each of these smaller groups was present and identified by microscopy, but, due to the nature of microscopy, could be easily overlooked if not part of the dominant community.

Figure 3 breaks the dataset into locations, splitting San Francisco Bay into 5 subembayments. Based on the physical and chemical properties in the Bay, we would expect there to be differences in the classes of phytoplankton that are present. This is apparent in a N - S transect, from the Sacramento River to the Lower South Bay (Fig. 1). For both microscopy and CHEMTAX, the northern portion of the Bay (Sacramento River, and Suisun Bay) is > 79% diatoms, the central portion of the Bay (San Pablo, Central Bay) has >41% dinoflagellates, and the southern portion of the Bay (South Bay, Lower South Bay) has >56% diatoms. Another difference is the larger portion of cryptophytes in the north, and cyanophytes in the south. The central Bay had the most variance - sometimes the classes usually incorporated into "other" would make up >5% of the chlorophyll present (i.e., eustigmatophytes). Every subembayment except San Pablo Bay had diatoms as the largest percentage. San Pablo Bay was dominated by dinoflagellates. The difference between microscopy and CHEMTAX was greatest for San Pablo Bay (11% for diatoms, 12% for dinoflagellates, and 1% for cyanophytes). We used this discrepancy as a test example to investigate whether the variance can be improved.

To mitigate the differences between microscopy and CHEMTAX, the input ratio for the program can be fine tuned to the location. In Figure 4, the ratio matrix has been fine tuned for San Pablo Bay (supplemental material 3). With the matrix set for the smaller area of San Pablo, the comparison of the two main groups is more similar than using the matrix for the entire Bay (i.e., 70% dinoflagellates for the tuned matrix, 63%
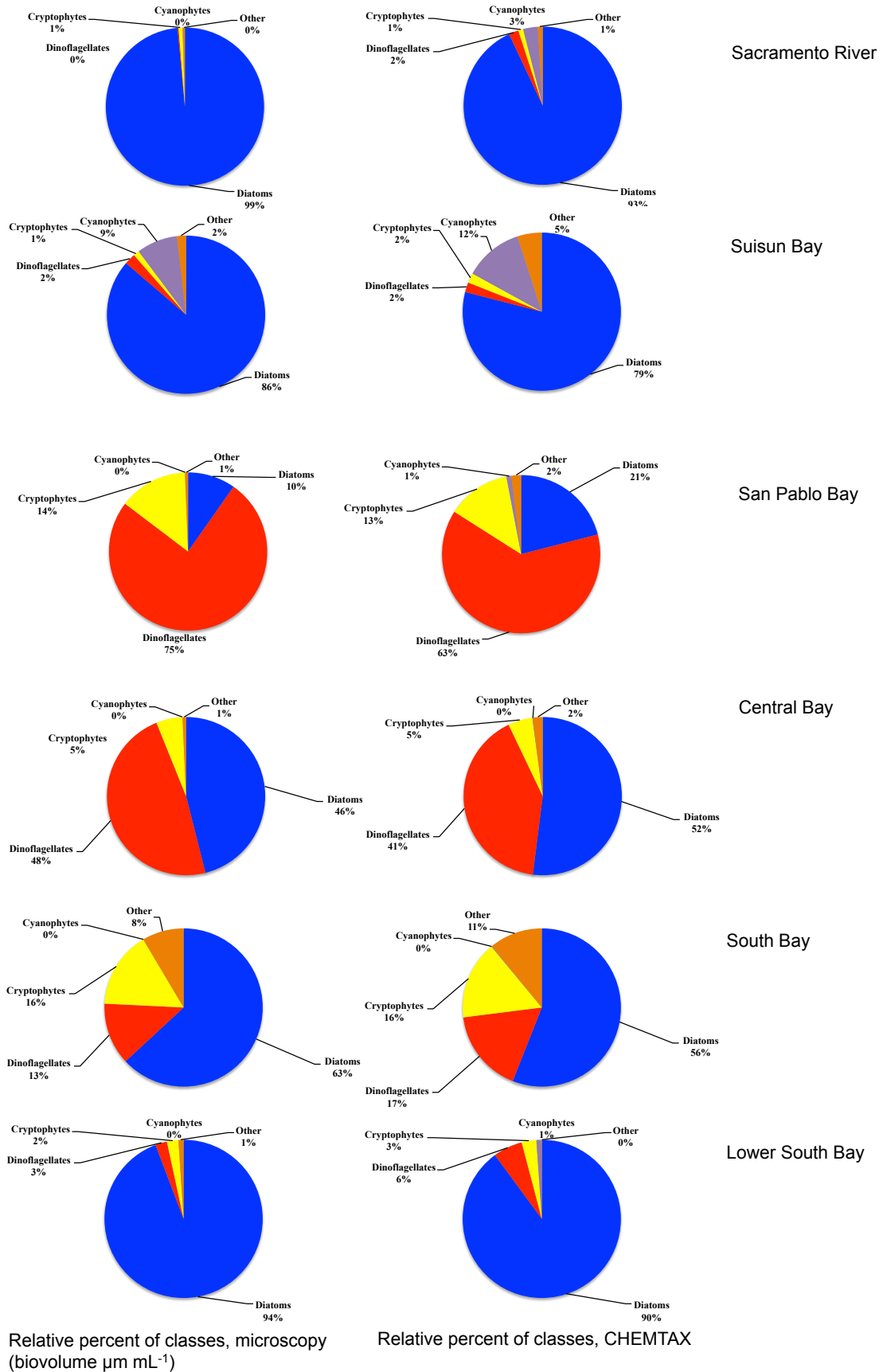
Fig. 3. Comparison of phytoplankton classes by microscopy and CHEMTAX program for the 5 subembayments in San Francisco Bay.

Relative percent of classes, CHEMTAX,
input matrix tuned for San Francisco Bay

Cyanophytes
1%

Other
2%

Diatoms
21%

Cryptophytes
13%

Dinoflagellates
63%

Cyanophytes
0%

Other
1%

Diatoms
10%

Cryptophytes
14%

Dinoflagellates
75%

Relative percent of classes, microscopy
(biovolume µm mL^{-1})

Cyanophytes
1%

Other
2%

Diatoms
12%

Cryptophytes
15%

Dinoflagellates
70%

Relative percent of classes, CHEMTAX,
input matrix tuned for San Pablo Bay

Fig. 4. San Pablo Bay used as a test example of fine-tuning the CHEMTAX input
matrix for a closer match between microscopy and pigment analysis.

dinoflagellates for the San Francisco Bay matrix, and 75% for the microscopy
biovolume). Regardless of the "tuning" of the matrix, there is still a greater proportion
identified as cyanophytes than when using microscopy - again, because CHEMTAX is
able to identify rare and small picoplankton that microscopy cannot.

On a pair-by-pair match (microscopy and CHEMTAX) there was a variety of
some matching well, and others being quite different. There was no discernable pattern,
but it may have to do with the way the input matrix is generated. As the Bay is a large
geographic area, to have an appropriate matrix for the entire Bay, it may not be the best
match for every station. In Figure 5, taken from Central Bay, station 18, December 18th
2012, there is <1% differences for all classes. But, in a snapshot of November 4th, 2011,
from the same station, there are large differences between the classes (1% - 14%; Fig. 6).

Central Bay, Station 18, December 18th 2012
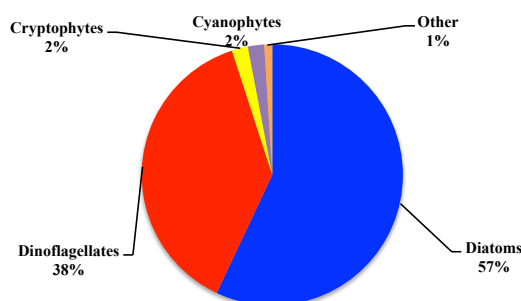
Cryptophytes 2%   Cyanophytes 2%   Other 1%

Dinoflagellates 38%

Diatoms 57%

Relative percent of classes, microscopy (biovolume μm mL^{-1})

Cryptophytes 3%   Cyanophytes 3%   Other 1%

Dinoflagellates 37%

Diatoms 56%
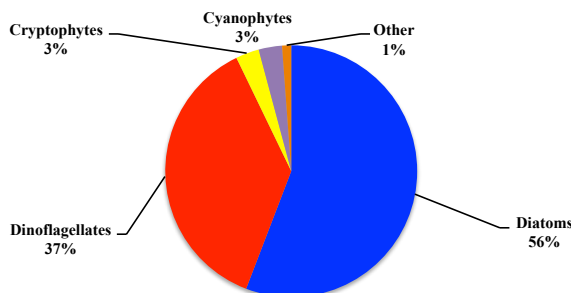
Relative percent of classes, CHEMTAX, input matrix tuned for San Pablo Bay

Fig. 5. Comparison of a Central Bay Station that matches closely between microscopy and CHEMTAX.

Central Bay, Station 18, November 4th 2011
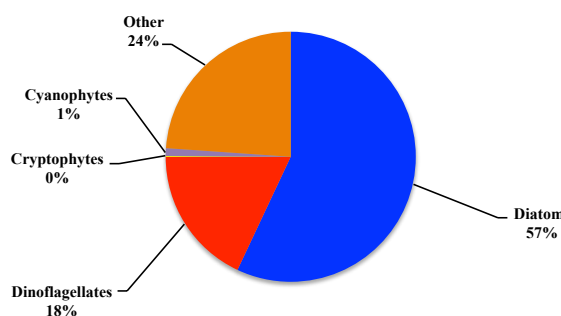
Other 24%

Cyanophytes 1%

Cryptophytes 0%

Dinoflagellates 18%

Diatoms 57%

Relative percent of classes, microscopy (biovolume μm mL^{-1})

Cyanophytes 0%   Other 13%

Cryptophytes 0%

Dinoflagellates 13%

Diatoms 74%
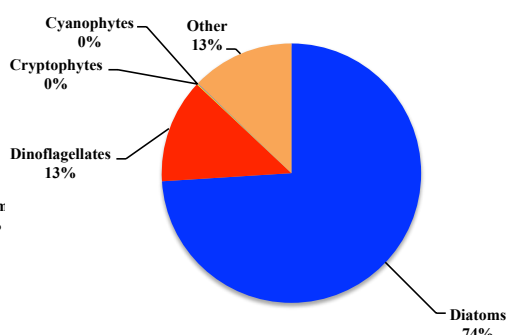
Relative percent of classes, CHEMTAX, input matrix tuned for San Pablo Bay

Fig. 6. Comparison of a Central Bay station that does not match closely between microscopy and CHEMTAX.

CHEMTAX does remarkably well at identifying small and rare phytoplankton classes that are likely being missed by microscopy, and a input matrix for the entire Bay is reasonable at determining the classes of phytoplankton that are present. Microscopy can provide a more detailed approach to identifying phytoplankton in San Francisco Bay, as it can be used to identify phytoplankton species. For a more in-depth look at individual locations or time-series, a fine-tuned matrix can be used. We suggest that due to the time- and labor-intensiveness of microscopy, that HPLC samples for pigment analysis be

collected to augment the gaps in data, and provide a high resolution for areas of interest within the Bay.

Results for Task 1.3, include the presentation of work at three quarterly meetings at USGS, technical work for the Nutrient Technical Working Group Meeting in their written report, delivered at the preliminary meeting, and presented at a secondary meeting, the South Bay Summit, the national meeting for the Association of Limnology and Oceanography in Honolulu, Hawaii, as an article in Estuary News (interviewed on August 20th, 2014), accepted as an abstract for the Bay-Delta Conference in October 2014, and in preparation for a peer reviewed journal article. Both Melissa Peacock and Raphael Kudela participated in planned meetings and working groups for the purpose of developing a long-term monitoring plan for San Francisco Bay. Included are the titles of presentations:

Peacock, M.B., R.M. Kudela, and D.B. Senn. Pigment Analysis of San Francisco Bay by HPLC. Quarterly meeting at USGS. December 2013.

Peacock, M.B., R.M. Kudela, D.B. Senn, T.S. Schraga, and J.E. Cloern. Using Algal pigments to characterize phytoplankton community composition in San Francisco Bay. Honolulu, HI, February 22-27th 2014

Peacock, M.B., R.M. Kudela, and D.B. Senn. Results of Pigment Analysis of San Francisco Bay by HPLC. Quarterly meeting at USGS. March 2014.

Peacock, M.B., R.M. Kudela, and D.B. Senn. Using Algal pigments to characterize phytoplankton community composition in the South Bay. Nutrient Technical Working Group Meeting at SFEI. May 2014

Peacock, M.B., R.M. Kudela, and D.B. Senn. Using Algal pigments to characterize phytoplankton community composition in the Lower South Bay. South Bay Summit, USGS. June 2014

Peacock, M.B., R.M. Kudela, and D.B. Senn. Results of Pigment Analysis of San Francisco Bay by HPLC. Quarterly meeting at USGS. July 2014.

Peacock, M.B., R.M. Kudela, D.B. Senn, T.S. Schraga, and J.E. Cloern. What does a pigment-based analysis tell us about the phytoplankton community composition in San Francisco Bay? Bay-Delta Conference, Sacramento, October 28-30th 2014.

Wong, Kathleen. Revealing Plankton Pigments. Estuary News, September 2014 issue.

The examples provided in this report are intended to show that the method works well and that it provides valuable information to understand the ecology of the Bay. We will include the presentation of this work at the Bay-Delta Conference in October 2014, and will and submit a manuscript with the full dataset on this work for a peer-reviewed article in a relevant journal.

Supplemental Material 1

UCSC San Francisco Bay PIGMENT SOP

<u>Supplies:</u>
1 mL exact measurement pipette
200 uL pipette and tips
5 mL cryovials (1 per sample)
sonicator
3 mL syringe filters (1 per
sample)
0.22 um Teflon filters (1 per
sample)
4 mL scintillation vials (1 per
sample)
parafilm
spatula
pH probe (dead)
stir bar
spatula

1 2L corning glass bottle amber
or covered in tinfoil
1 1000 mL volumetric flask
1 1000 mL graduated cylinder
1 200 mL graduated cylinder
1 stir plate
1 vacuum filter cup with 0.22 um
filter
HPLC sample vials and lids (1
per sample + 32)
crimper
ice
cooler
1 transfer pipette

Chemicals:
0.4 M Tetrabutylammonium hydrate titrant (TbA)
Acetic acid (OPTIMA grade)
Acetone (OPTIMA grade)
Methanol (OPTIMA grade)
Vitamin E Acetate
Acetone (HPLC grade)
Methanol (HPLC grade)

Reagents:
Solvent A - 30:70: 0.028 M TbAA, pH = 6.5: Methanol (OPTIMA grade)
Solvent B - 100% Methanol (OPTIMA grade)
Solvent C - 100% Acetone (OPTIMA grade)
Solvent D - 50% Methanol (HPLC grade)

Injection buffer - 90:10 TbAA:Methanol (OPTIMA grade)
90% Acetone (OPTIMA grade) with Vitamin E acetate

If glassware is acid washed, should be rinsed with acetone (HPLC grade) before use

REAGENT PREPERATION
90 % acetone w/ vitamin E acetate
1. Dilute 100% acetone (OPTIMA) with MQ until 90%. Add vitamin E Acetate (stored at 4C) for the optimal response of ~600 mAU on the HPLC. Store in a glass corning bottle, @-20C.

0.028 M Tetrabutylammonium acid (TbAA)
1. 0.4 M concentrated TbA to 0.0028 M TbA with MQ (70 mL TbA into 1000 mL MQ). Should be stored in a 2L glass corning bottle, in the dark (or wrapped in tinfoil). BE CAREFUL. TbA is VERY BASIC (pH = 12). Usually make up 2 L.
2. Add stir bar, and place on stir plate
3. Calibrate pH probe (use pH of 4 and 7).
4. Add Acetic Acid (OPTIMA) drop wise with transfer pipette, watching pH closely. As it nears ~pH of 10, dilute the acetic acid ~ 1000-fold. This uses very little acetic acid (less than 5 mL). Once the pH is ~10, if will drop VERY QUICKLY (1-2 drops can bring the pH down more than 1 unit). Be very slow and careful, do not overshoot to 6.5 pH. Continue to dilute the acetic acid if the pH is dropping too quickly.
5. Reagent is stable ~1 month at room temperature in the dark.

Injection Buffer
1. Add TbAA reagent and 100% Methanol (OPTIMA) in a 90:10 ratio for injection buffer (usually ~100 mL covers ~200 samples).

2. Filter with the vacuum filter. Store in a dark (or amber) glass corning bottle at room temperature for 1 month.
3. Add 1.8 mL to HPLC vials and cap/crimp. Each one of these injection vials should cover 4 samples.

Solvent A
1. Add TbAA reagent and 100% Methanol (OPTIMA) in a 30:70 ratio for solvent A. ~1 L will last approx. 18 hours of runtime.
2. Filter using a vacuum filter. Must be filtered weekly or anytime a noticeable precipitate is present. Batches can be mixed, as long as they are filtered.
3. Store in an amber or dark (tinfoil) glass corning bottle at room temperature. Stable for ~1 month.

Solvent B
1. Add 100% Methanol (OPTIMA) to Solvent B bottle. 1 L lasts ~ 30 hours.

Solvent C
1. Add 100% Acetone (OPTIMA) to Solvent C bottle. Need very little (a few 100 mL for 100 hours of runtime).

Solvent D
1. Dilute 100% Methanol (HPLC) to 50% with MQ. Add to Solvent D bottle.

SAMPLE PREPERATION
1. Prepare a (dark) cooler with ice and cyrovial rack.
2. Remove 90% acetone with Vit E from -20C freezer - should be room temperature for use.
3. Label and weigh cryovial tubes (with tops on). Record weight.
4. Add 1-3 mL of 90% acetone with vit E (depends on sample) using calibrated 1 mL pipette. Cap immediately. Acetone should be ~ room temperature.
5. Weigh cryovial with acetone (and tops).
6. Keep cryovials on ice until chilled. (20 minutes - 60 minutes, can put cooler in -20C freezer to cool quicker).
7. Label scintillation vials. Lids should be foil lined. If using used scintillation vials, should be rinsed with acetone beforehand and allowed to dry.

THIS SHOULD BE DONE IN THE DARK FROM HERE ON
8. Add filters to cryovials, make sure that entire filter is covered by acetone. Quickly cap (after each sample, don't leave caps off, acetone evaporates quickly and changes the H_2O percentage).
9. Place cryovials in -20C freezer for 1 hour.
10. Sonicate the filters, breaking up completely. Usually takes 10-30 seconds at ~sonication level 6-8. Keep cryovials on ice while sonicating. DO NOT LET

THE SAMPLES BECOME HEATED. Make sure the filter pieces are pushed entirely under the acetone.

11. Place samples in -80C freezer for AT LEAST 4 hours. Can be kept for up to 24 hours. This is a good stopping point if not completing the entire process.
12. Filter the samples into the scintillation vials, using 3 mL syringe and 0.22 um Teflon filters. Make sure all the filter paste and liquid has been transferred to the syringe from the cryovial. (It's easier to do this when filter is frozen, use the spatula to remove pieces of filters. Takes practice to not drop filter milkshake, so do it over a counter).
13. Parafilm the lids. Keep samples in the dark until transfer (ASAP) to -80C. Samples can be kept until analyzed, but should be run ASAP, at least within a month (pigments degrade).
14. Transfer (at least) 200 uL to HPLC vials and cap/crimp them (ASAP, acetone evaporating again) either the day of the run (not more than 28 samples) or the day before (keep at -80C).

STANDARD PREPARATION
1. Each 'run' (or within a 24 hour period) should have a chl a std, a mixed pigment standard, a vit E acetone blank, and a MQ blank. You will also need an injection buffer vial for each of those.
2. The chl a, vit e, and MQ vials will be run multiple times (add sample accordingly). Mixed pig will only be run once.

Supplemental Material 2

Class / Pigment	chl c3	chl c2	chl c1	MG-DVP	PER	but	fuco	hex	Neo	pras	Viola	Diadino	Diato	Allo	zea	lut	chl b	echin	GYRO	BE car	Bbcar	chl_a
CYANO-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.215	0	0	0.191	0	0	0	1
CYANO-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.656	0	0	0	0	0	0.118	1
CHLORO	0	0	0	0	0	0	0	0	0.041	0	0.067	0	0	0	0.039	0.172	0.334	0	0	0.007	0.092	1
PRAS-3	0	0	0	0.028	0	0	0	0	0.093	0.222	0.099	0	0	0	0.057	0.011	0.911	0	0	0.031	0.004	1
EUGLENO	0	0	0	0	0	0	0	0	0.021	0.009	0	0.213	0.019	0	0.072	0	0.33	0	0	0.023	0	1
CRYPTO	0	0.104	0	0	0	0	0	0	0	0	0	0	0	0.379	0	0	0	0	0	0.023	0	1
DIATOM-1	0	0.179	0.087	0	0	0	0.775	0	0	0	0.001	0.163	0.028	0	0.003	0	0	0	0	0	0.019	1
DIATOM-2	0.083	0.284	0	0	0	0	0.998	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RAPHIDO	0	0.064	0.013	0.002	0	0	0.492	0	0	0	0.156	0.046	0	0	0.062	0	0	0	0	0	0	1
EUSTIG	0	0	0	0	0	0	0	0	0	0	0.155	0	0	0	0	0	0	0.003	0	0.024	0	1
HAPTO-1	0	0.042	0.061	0.006	0	0	0.306	0	0	0	0	0.337	0.017	0	0	0	0	0	0	0	0.064	1
DINO-1	0	0.245	0	0	0.804	0	0	0	0	0	0	0.177	0.076	0	0	0	0	0	0	0	0.022	1
DINO-2	0.205	0.125	0	0	0	0.079	0.219	0.135	0	0	0	0.079	0	0	0	0	0	0	0.043	0	0	1

Matrix for San Francisco Bay

Supplemental Material 3

Pigment Selection	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Class / Pigment	chl c3	chl c2	chl c1	MG-DVP	PER	but	fuco	hex	Neo	pras	Viola	Diadino	Diato	Allo	zea	lut	chl b	echin	GYRO	BE car	Bbcar	chl_a
CYANO-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.215	0	0	0.191	0	0	0	1
CYANO-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.656	0	0	0	0	0	0.118	1
CHLORO	0	0	0	0	0	0	0	0	0.041	0	0.067	0	0	0	0.039	0.172	0.334	0	0	0.007	0.092	1
PRAS-3	0	0	0	0.028	0	0	0	0	0.094	0.222	0.099	0	0	0	0.057	0.011	0.911	0	0	0.031	0.004	1
EUGLENO	0	0	0	0	0	0	0	0	0.021	0.009	0	0.213	0.019	0	0.072	0	0.33	0	0	0.023	0	1
CRYPTO	0	0.204	0	0	0	0	0	0	0	0	0	0	0	0.379	0	0	0	0	0	0.023	0	1
DIATOM-1	0	0.179	0.087	0	0	0	0.775	0	0	0	0.001	0.163	0.028	0	0.003	0	0	0	0	0	0.019	1
DIATOM-2	0.083	0.284	0	0	0	0	1.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RAPHIDO	0	0.064	0.013	0.003	0	0	0.492	0	0	0	0.156	0.046	0	0	0.062	0	0	0	0	0	0	1
EUSTIG	0	0	0	0	0	0	0	0	0	0	0.155	0	0	0	0	0	0	0.003	0	0.024	0	1
HAPTO-1	0	0.042	0.061	0.006	0	0	0.306	0	0	0	0	0.337	0.017	0	0	0	0	0	0	0	0.064	1
DINO-1	0	0.245	0	0	0.804	0	0	0	0	0	0	0.177	0.076	0	0	0	0	0	0	0	0.022	1
DINO-2	0.205	0.125	0	0	0	0.079	0.219	0.135	0	0	0	0.079	0	0	0	0	0	0	0.043	0	0	1

Matrix fine-tuned for San Pablo Bay

University of California Santa Cruz
Assessing SPATT in San Francisco Bay
SFEI Contract 1051

Final Report

This project involves using Solid Phase Adsorption Toxin Tracking (SPATT) to detect and quantify microcystin and other phytotoxins in San Francisco Bay, and to undertake controlled experiments using SPATT whose goal is to improve the ability to translate SPATT-derived measurements into average ambient concentrations of phytotoxins.

In **Task 1**, SPATT was deployed on San Francisco Bay cruises in a flow-through configuration and at fixed sites as part of on-going monitoring work for phycotoxins in San Francisco Bay. As discussed with SFEI, one SPATT was deployed per basin in the surface-sampling flow-through system during the monthly *Polaris* cruises. Based on adjustments to cruise schedules and cruise types, SPATT were routinely deployed on all available cruises.

In **Task 2** controlled experiments were conducted in the laboratory to better characterize partitioning of phytotoxins out of solution and into the SPATT. Experiments were designed to evaluate measurement reproducibility, and whether reproducibility can be optimized by adjusting SPATT configuration. Options include:

> a. Controlled experiments carried out in simulated flow-through systems in which SPATT will be exposed to brackish water and seawater containing concentrations of a surrogate compound for toxins, e.g.. microcystin-RR or similar. Toxin will be quantified as a function of both dissolved concentration and exposure time. This "calibration" information will allow for more accurate back-calculations of average ambient concentrations in natural systems.

> b. Time-series "bottle" experiments in which SPATT will be exposed in containers holding brackish water with known concentrations of a surrogate compound for toxins (e.g., microcystin-RR). SPATT will be removed at multiple time points and toxin uptake will be measured. This information will aid in characterizing the uptake kinetics of microcystin under conditions simulating deployments at a single site.

Research priories for Task 2 were identified collaboratively by Kudela and SFEI, and a project plan was developed that is feasible within the available budget.

Results—Task 1
We have processed 155 SPATT samples from USGS cruises, between October

2011 and November 2014. Additional samples (through April 2015) have also been obtained and processed, but were not included in a recent analysis as part of a separate SFEI effort. For convenience, data presented here are limited to the 155 SPATT, but we continue to process the samplers.

While we anticipated 60 SPATT per year, several of the USGS cruises were canceled or reduced in geographic range in 2013 due to ship issues. For each SPATT we have analyzed for domoic acid (DA) and microcystins LR, RR, YR, and LA. These four congeners are identified by OEHHA as the primary microcystin toxins in California, and are considered to be of equivalent toxicity. We therefore sum the congeners to report "total microcystin".

Preliminary data analysis was conducted on the SPATT and USGS underway data for presentation at several meetings:

Kudela, RM, C Mioni, M Peacock, T Schraga. San Francisco Bay acts as a reservoir and mixing bowl for both marine and freshwater toxins. Coastal and Estuarine Research Federation, 3-7 November 2013, San Diego, CA.

Kudela, RM, C Mioni, M Peacock, T Schraga. San Francisco Bay acts as a reservoir and mixing bowl for both marine and freshwater toxins. Eastern Pacific Oceans Conference, Fallen Leaf Lake, California, September 17-19, 2013.

Kudela, R, Peacock, M, Schraga, T, Senn D. 2014. Does San Francisco Bay have a harmful algal bloom problem? 2014 Bay-Delta Science Conference, 28-30 October 2014, Sacramento, CA.

Those presentations are used as the basis for this interim report.

Between 2011-2014, 25 Full Bay and 28 South Bay cruises were analyzed. From those samples, 71.5% were positive for microcystins and 96.5% were positive for domoic acid (Figure 1). Concentrations ranged from 0-400 ng/g domoic acid, and 0-25 ng/g microcystins (Figure 2). Peaks in both toxins were coincident in time, and appear to be related to river flow. Moderate river flow is associated with the highest toxin concentrations. Spatially, toxins were fairly uniformly distributed throughout the four basins (Figure 3). During some periods there was clear separation based on temperature-salinity (T-S) properties, with domoic acid associated with "marine" waters and microcystins associated with "fresh" waters. However at other times toxins were distributed without a clear pattern through the Bay (Figures 4, 5).

The range of toxin concentrations, range of environmental parameters, and length of the time-series (3+ full years of data) make this dataset conducive to statistical modeling to identify relationships between toxins and environmental drivers or correlates (see Recommendations below).

Results—Task 2
We have conducted several "bottle" experiments to evaluate SPATT adsorption under representative conditions. In particular, we recently expanded the SPATT methodology to include anatoxin-a. This is a potent neurotoxin also known as "sudden death factor". While there are no reports of anatoxin-a for San Francisco

Bay, we have routinely seen elevated levels in the Eel River, and occasionally get positive hits in nearby Pinto Lake. We recently concluded a laboratory calibration for anatoxin-a, looking at adsorption and recovery efficiency, effect of different source waters, and effect of temperature on adsorption.

We can now quantify SPATT (using HP20 resin) characteristics for domoic acid, microcystins, and anatoxin-a. Excitingly, we can use a single extraction method to analyze all three toxins from the same SPATT. We can also analyze for okadaic acid (Diarrhetic Shellfish Poisoning), extending our capability to 4 toxins that cover the majority of compounds expected in San Francisco Bay.

Because SPATT and grab samples (or indicator organisms) are fundamentally different measurement methods, we do not recommend a direct calibration factor between the various toxin detection methods. Rather, we provide ranges of SPATT concentrations that correlate to management action levels. For example, OEHHA recommends an alert or action level of 0.8 ppb total microcystins. Based on the large comparative dataset, this would be equivalent to a threshold concentraton of ~1-4 ng/g for SPATT (see below). Based on that criteria, San Francisco Bay appears to approach this alert level seasonally (Figure 2).

Additional Analyses
We requested an extension to the contract to more fully characterize SPATT. We proposed to complete the following:

• SPATT deployment/analysis through 2014, providing a full 3-year record
• Finish characterization of toxin uptake in a simulated flow-through system
• Prepare a peer-reviewed publication describing the presence of toxins in San Francisco Bay

We further recommended the following. These five recommendations are beyond the scope of the current contract, but could be implemented within a 12-18 month contract at a similar cost to this contract.

1) Continue SPATT deployment beyond the scope of this contract;
2) Analyze matched filter samples from the USGS cruises for particulate toxins, to further calibrate the SPATT data; this could also be compared to an existing dataset of HPLC pigments and microscopy samples;
3) Analyze archived mussel tissue provided by the RMP as a pilot dataset, to determine whether additional sample analysis is warranted. This would directly link toxins to trophic accumulation.
4) Develop a method for saxitoxins. This is the only toxin group that we know is in SFB that is not currently included in our analysis. It requires some personnel time to set up the method, and supplies costs.
5) Analyze archived SPATT for anatoxin-a and okadaic acid.

Results from the Contract Extension

Following this document, we provide a separate write-up for the laboratory characterization (the second bullet from the proposed contract extension). Analysis of SPATT through 2014 was also completed, and an initial write-up with peer-review is ongoing as part of the following report and manuscript:

Sutula, Martha , Raphael Kudela, James Hagy, Gry Mine Berg, Suzanne Bricker, James E. Cloern, Richard Dugdale, Lawrence W. Harding, Jr., and David Senn. 2015 (in prep.). Scientific Basis for Assessment of Nutrient Impacts on San Francisco Bay.
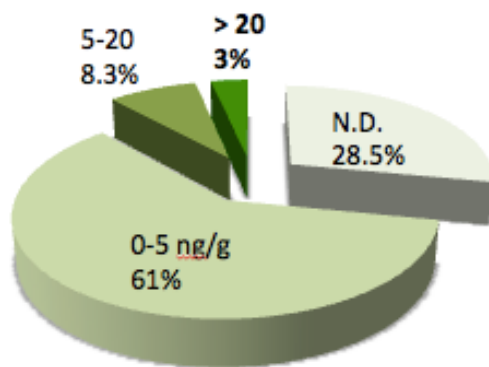
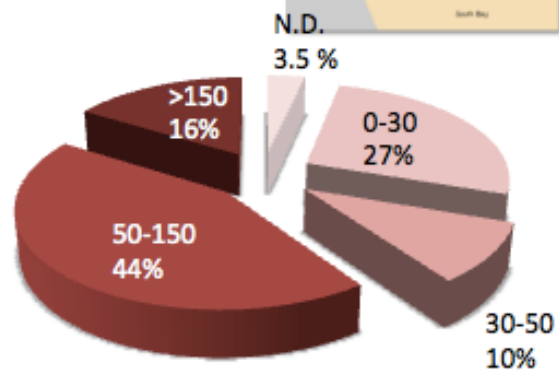Figure 1. Summary results from the USGS cruises.
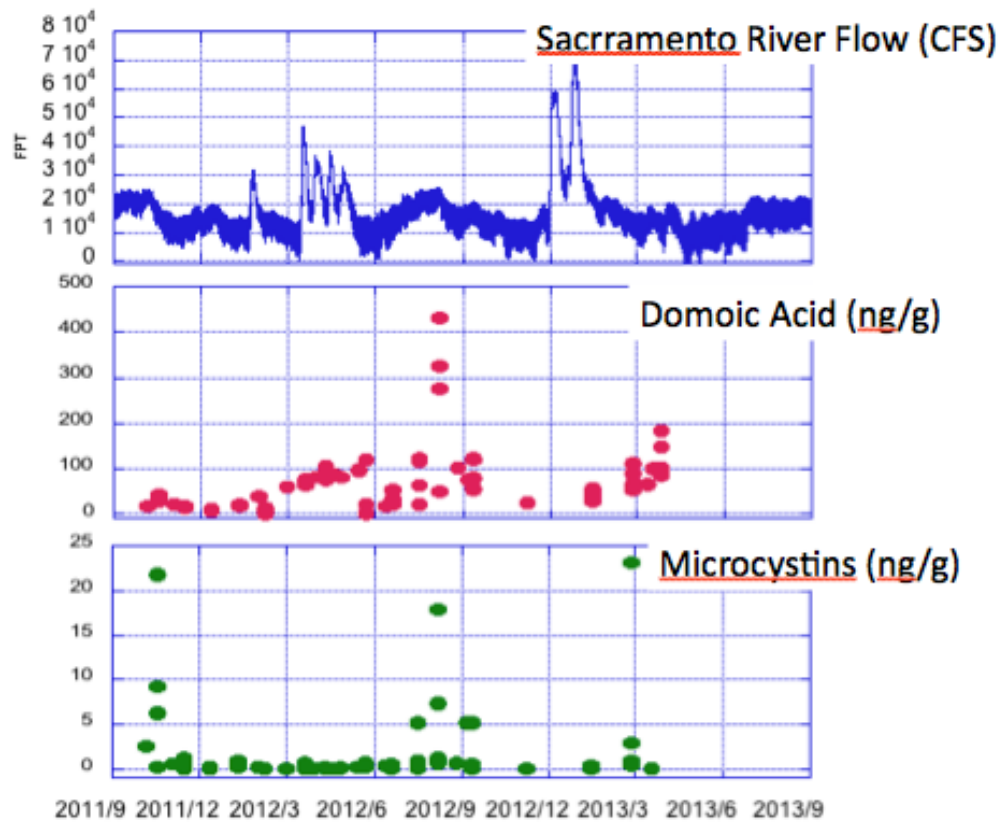
Figure 2. Toxin data shown as a time-series, with river flow (top). Toxins are generally associated with moderate flow in the autumn. The two peaks in autumn 2011 and summer 2012 are shown in more detail in Figures 4-5.
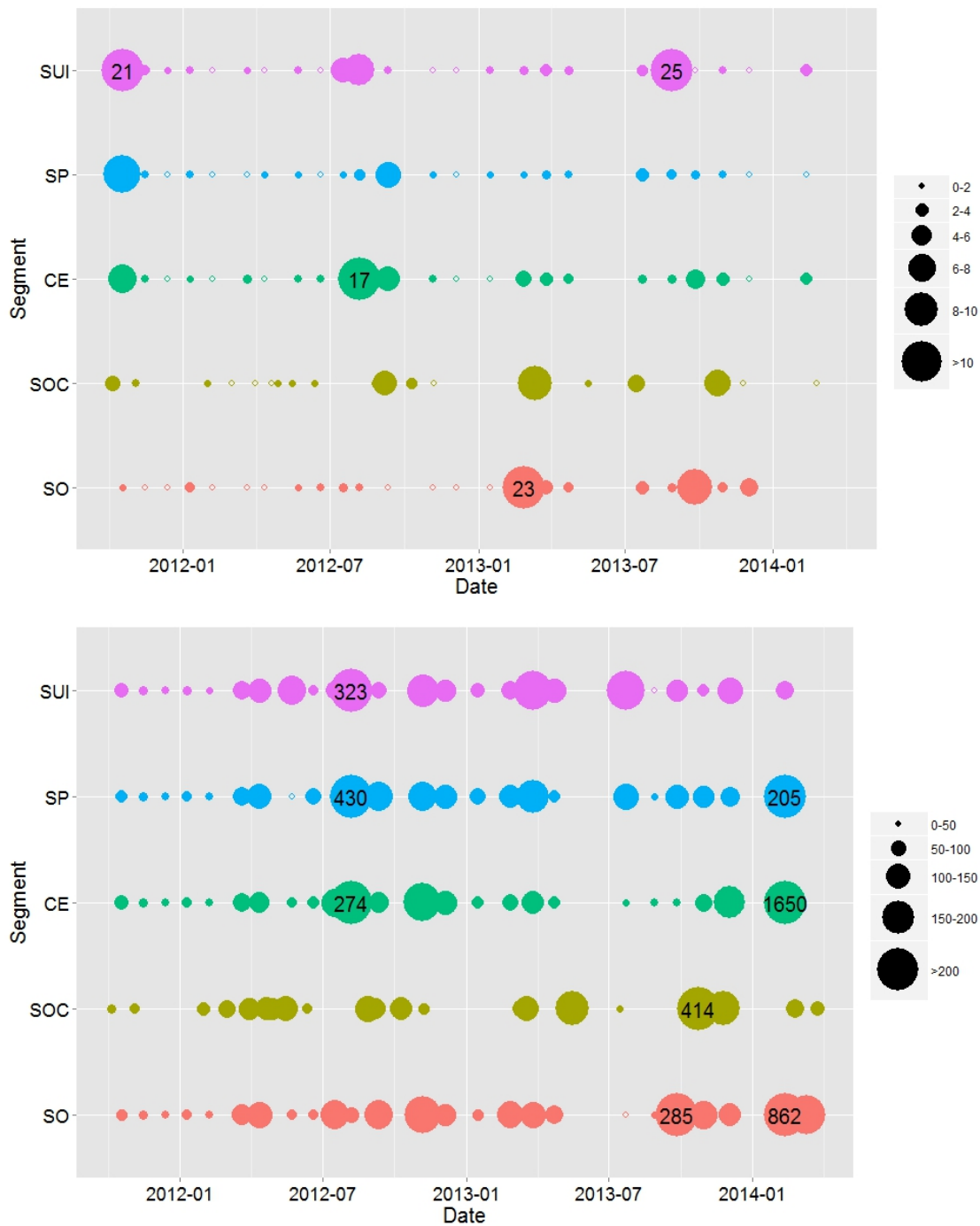
Figure 3. All of the SPATT data shown as concentration (larger circle equals more toxin). Note that microcystins are easily detectable, but fairly low. DA values are fairly high. Letter codes refer to subembayment: SO=South Bay, SOC=South Central, CE=Central, SP=San Pablo, SUI=Suisun.

Figure 4. Toxin data plotted in T-S space. For this period, microcystins are clearly coming from the Delta, and spreading into the rest of the Bay, while DA is coming from Central Bay and spreading into the rest of the Bay, suggesting that sometimes, it's simply conservative mixing that is moving the toxins around.
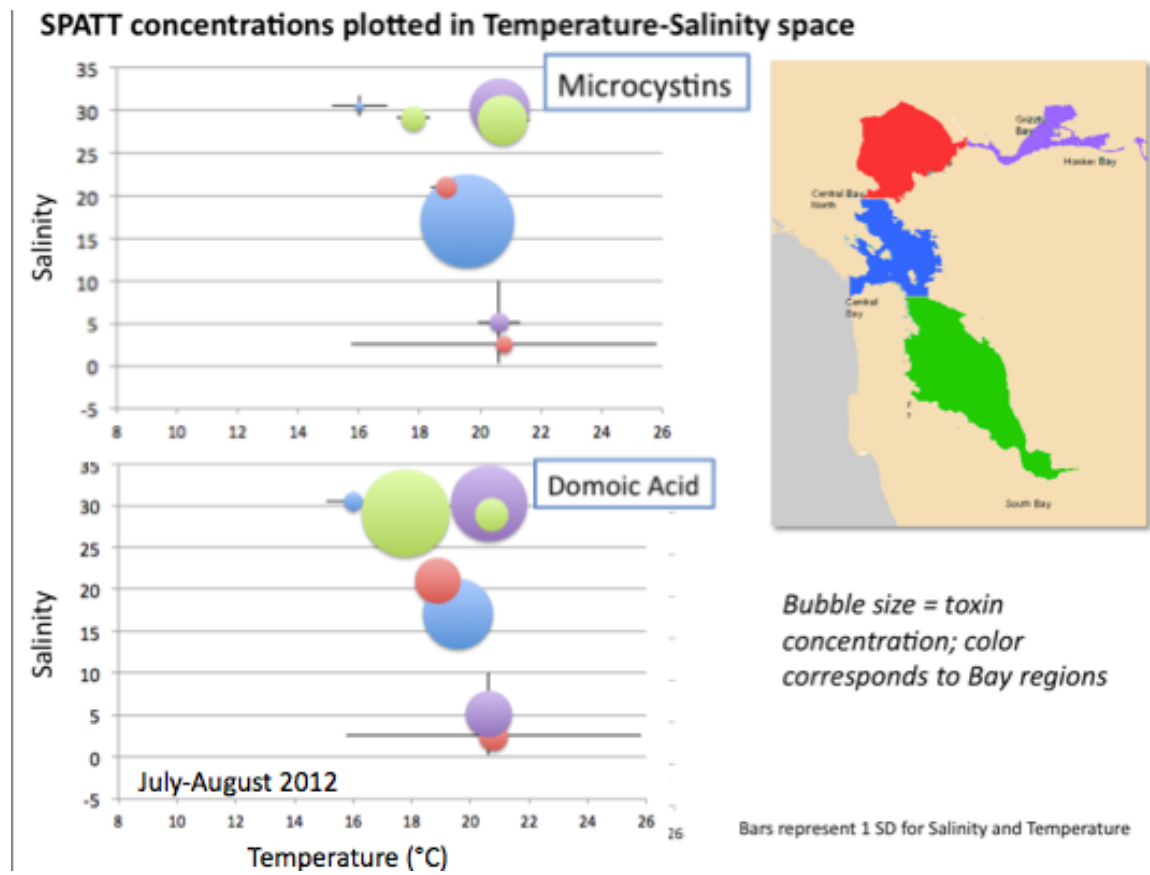
Figure 5. Another example, from July-August 2012. It is not as clear how the geographic patterns relate to environmental forcing. Highest microcystins are in Central Bay, with moderate levels in South Bay and the Delta. There is evidence (not shown) that microcystins are coming in from a separate South Bay source, possibly the sloughs and salt ponds. The DA is highest in South Bay, and pretty high in the Delta, suggesting transport of cells that eventually release toxin.

Results from Contract Extension
Calibration of SPATT—Background

A primary objective of this project was to intercalibrate SPATT toxin data for microcystins and domoic acid such that data from the USGS underway mapping aboard the R/V *Polaris* can be qualitatively related to regulatory limits. OEHHA recommended 0.8 ppb for the sum of total (particulate and dissolved) microcystin LR, RR, YR, and LA. There are no formal guidelines for domoic acid, but regulatory limits for fish and shellfish is 20 ppm in tissue.

It is not possible to *directly* compare SPATT values to the regulatory guidance because (a) SPATT measures dissolved, and not total toxin; (b) SPATT toxins and grab samples for domoic acid are not equivalent to toxin levels in tissue; (c) SPATT integrates spatially and temporally. Additionally, SPATT is generally considered to be more sensitive than grab samples (Lane et al. 2010, 2012; Kudela 2011; Gibble and Kudela, 2014). Given these caveats, it is still desirable to relate SPATT concentrations to regulatory limits/guidelines.
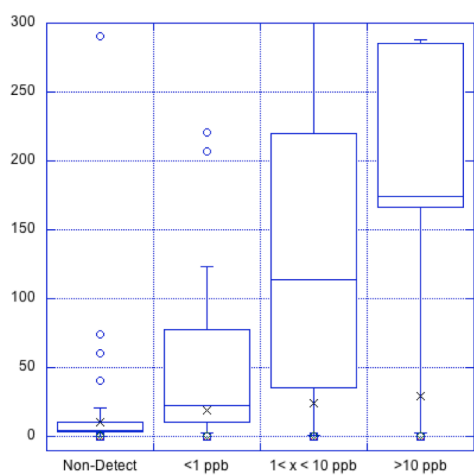

Figure 1. SPATT data from Pinto Lake, showing the correspondence between grab sample bins and SPATT values.

An initial attempt to provide an intercomparison used environmental data from long time-series at the Santa Cruz Municipal Wharf (Lane et al. 2010) and from Pinto Lake, California (Kudela 2011). For both of those programs SPATT, using the HP20 resin, are deployed weekly, with matching samples for dissolved and particulate domoic acid, and mussel tissue (SCMW), and dissolved and total microcystins (Pinto Lake). Using those data, SPATT values were binned into ranges corresponding to grab samples or mussel samples: non-detect, < 1 ppb, 1-10 ppb, and > 10 ppb for microcystins, and 0-5, 5-10, 10-20, and >20 ppm domoic acid in mussel tissue. Ranges were determined by binning the corresponding SPATT data an calculating the median, mean, and standard deviation. These data are depicted graphically for microcystins in Figure 1 and the ranges are provided in Tables 1-2.

As part of laboratory characterization, resin capacity and equilibration times were evaluated when SPATT were developed (Lane et al. 2010; Kudela 2011). Since then, adsorption/desorption of microcystin LR was more rigorously evaluated (Zhao et al. 2013) and HP20 was again identified as the optimal resin for environmental use, with linear absorption characteristics over several days. HP20 was also identified as the best resin for use with lipophilic toxins in seawater for prolonged (days) deployment, with reasonably linear uptake and a combination of good adsorption

and desorption capabilities; other resins performed better under some circumstance, but were found not to be as universally applicable to a broad range of toxins, deployment times, and recovery methods (Zendong et al. 2014). Thus there is growing acceptance of HP20 resin as a "universal" SPATT resin, with the best overall combination of characteristics.

Table 1. SPATT concentrations corresponding to total microcystins from matching grab samples.

Microcystin Grab Sample (ppb)	SPATT (ng/g)
Non-Detect	5-13
< 1 ppb	20-50
1< x < 10 ppb	50-200
> 10 ppb	175-275

Table 2. SPATT concentrations corresponding to mussel tissue domoic acid concentrations from matching mussel samples (SPATT were deployed weekly; mussels samples were collected weekly).

Domoic Acid Mussel (ppm)	SPATT (ng/g)
0-5 ppm	0-30
5-10 ppm	30-50
10-20 ppm	50-75
>20 ppm	>150

Calibration of R/V *Polaris* Underway Measurements

In order to translate the general characteristics of HP20 SPATT, a simulation was set up in the laboratory to mimic conditions on the R/V *Polaris* cruises. The following assumptions were made:

1) Transects include fresh, brackish, and marine waters;
2) Individual SPATT deployments are for no longer than 12 hours;
3) SPATT adsorption may differ when using a flow-through system compared to passive (static) water bodies such as Pinto Lake and Santa Cruz Wharf;

4) Temperature and salinity vary over the transects, potentially influencing toxin adsorption;
5) SPATT samplers are stored frozen prior to analysis.

Given these assumptions, the laboratory experiment was designed to mimic typical field conditions. A large volume (~16 L) of low-salinity water (Sacramento River water with Monterey Bay water mixed in, final salinity ~10). A recent study (Fan et al. 2014) showed HP20 adsorption varies with salinity, but not significantly so compared to other sources of variability, so it was assumed that salinity did not need to be directly tested again. The water was spiked with an initial concentration of ~34 ppb MC-LR, and 82 ppb domoic acid (a trace amount, ~3 ppb, of MC-YR was also present). The water was subsequently diluted to create a series of toxin concentrations for testing SPATT adsorption.

Adsorption kinetics should also be sensitive to temperature, since adsorption is a physical-chemical interaction between the resin and the sorbents (toxins). This was tested as part of the laboratory trial by testing adsorption at 3 temperatures (22°C, 15°C, 4°C) and three time periods (20 minutes; 1 hour; 2 hours). For each time point 2-3 SPATT were soaked in a large (~2 L) volume, with the ambient toxin concentration tested before and after each SPATT exposure to account for uptake.

For the SPATT adsorption tests (other than temperature), two methods were employed. First, SPATT were exposed for 15 minutes in a glass, 2L container with spiked water at 5 concentrations. This was designed primarily to calibrate SPATT uptake using the method employed by Peggy Lehman (DWR) in a previous field experiment. For that study, Bay and Delta water were collected into a container and SPATT were added for 15 m. Second, the large (~16L) carboy was connected to a peristaltic pump and water was recirculated through a 2L glass container (about 1.5L was in the container), using a flow rate of 2.5 L/min, which is a typical flow rate for underway mapping systems. The SPATT were prepared/deployed following the same methods as for the USGS cruises. For each time point, the SPATT were removed, allowed to drain, placed in 50 mL plastic centrifuge tubes, and frozen. The SPATT Were subsequently thawed and toxin was extracted using the standard UCSC protocol (10 mL 50% MeOH, 20 mL 50% MeOH, 20 mL 50% MeOH with 1M ammonium acetate). An additional step, collection of the Milli-Q rinse water, was added to test for loss of toxin during processing. As per UCSC protocol, each eluate fraction was run separately on an Agilent 6130 LC/MS, and the total toxin per SPATT sampler was calculated based on volumes and concentrations of extract (see also Lane et al. 2010; Kudela 2011; Gibble and Kudela 2014).Pictures of the flow-through setup are provided in Figure 2.

For the flow-through experiment, replicate (2-3) SPATT were placed in the flow-through container and allowed to absorb for 20 minutes to 24 hours. The spiked water was then diluted to adjust the toxin concentration, and additional SPATT were tested. This was repeated for 4 concentrations. Additional SPATT were tested

during the transitions, to determine how quickly SPATT exposed to high toxin concentrations would equilibrate to a lower concentration.

Figure 2. Laboratory setup for the flow-through testing of SPATT. Upper photo shows the carboy, receiving container, and pump. Lower photo shows SPATT (in embroidery hoops) within the receiving container.

Calibration of SPATT--Results

Temperature: There was no significant difference (ANOVA, p>0.05) for SPATT toxin concentrations of both microcystin and domoic acid as a function of temperature. This is consistent with previous laboratory experiments conducted when SPATT methodology was first developed.

Milli-Q Rinse: For standard processing of SPATT, the Milli-Q (deionized water) rinse is not tested for toxin. As part of these experiments Milli-Q volumes and toxin concentrations were measured. While toxins were detected in the rinse water, it was a few percent of the total extracted toxin (for both microcystin and domoic acid), as previously reported (Lane et al. 2010, Kudela 2011). While this lost toxin could be important for cases where very low toxin levels are of interest, SPATT is already more sensitive than grab samples so this is considered to be an acceptable loss.

Microcystins, 15 minute exposure: Adsorption of MCY-LR and MCY-YR was linear as a function of concentration. Toxins were easily detected after 15 minutes of exposure. Previous comparison of SPATT to grab samples exhibited a calibration factor of about 10-50x (SPATT is 10-50x more sensitive than grab samples), as exhibited in Table 1. Shorter exposure resulted in a calibration factor of about 5x, as seen in Figure 3. Given typical underway mapping speeds, 15 minutes would roughly correspond to spatial scales of about a kilometer, and assuming exposure to toxin occurred for 0-15 minutes at a given concentration, the SPATT factor would be 1-5x.
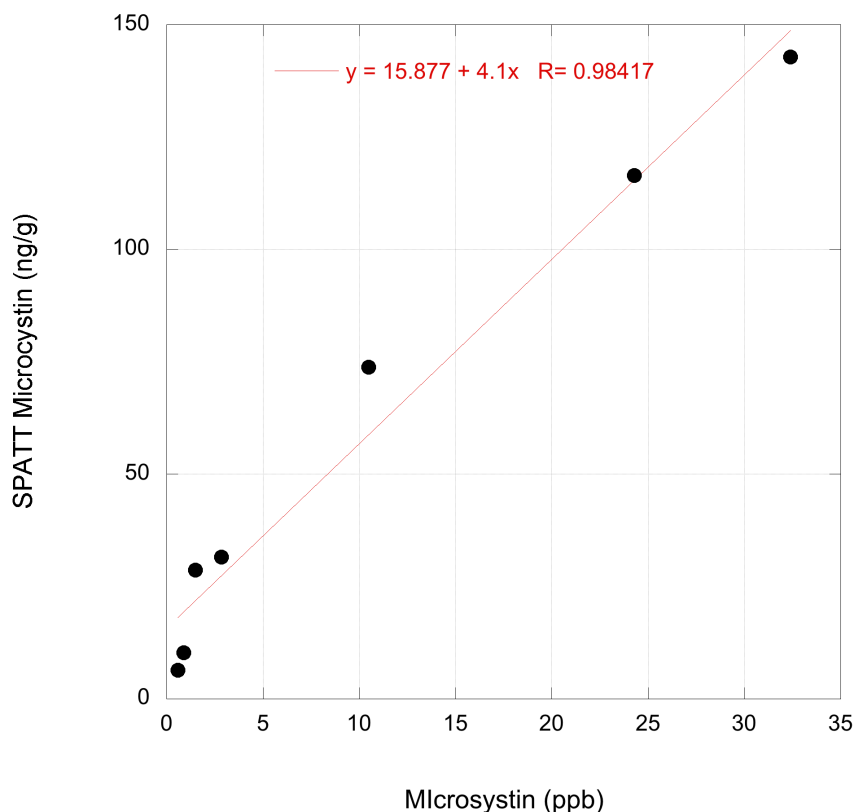

Figure 3. SPATT versus ambient water concentration for microcystins for SPATT exposed to constant concentration of toxin for 15 minutes. When forced to a zero-intercept, the calibration factor is 4.97x.

Microcystins, > 1 hour exposure: Testing of SPATT showed that microcystins equilibrate in approximately 1 hour. The calibration of toxin versus SPATT was therefore recalculated using SPATT exposed for 1-24 hours to estimate the upper-limit calibration factor. Results are presented in Figure 4. Linearity is excellent, and the calibration factor increases considerably compared to the 15 minute exposure, with a calibration factor of 271x.
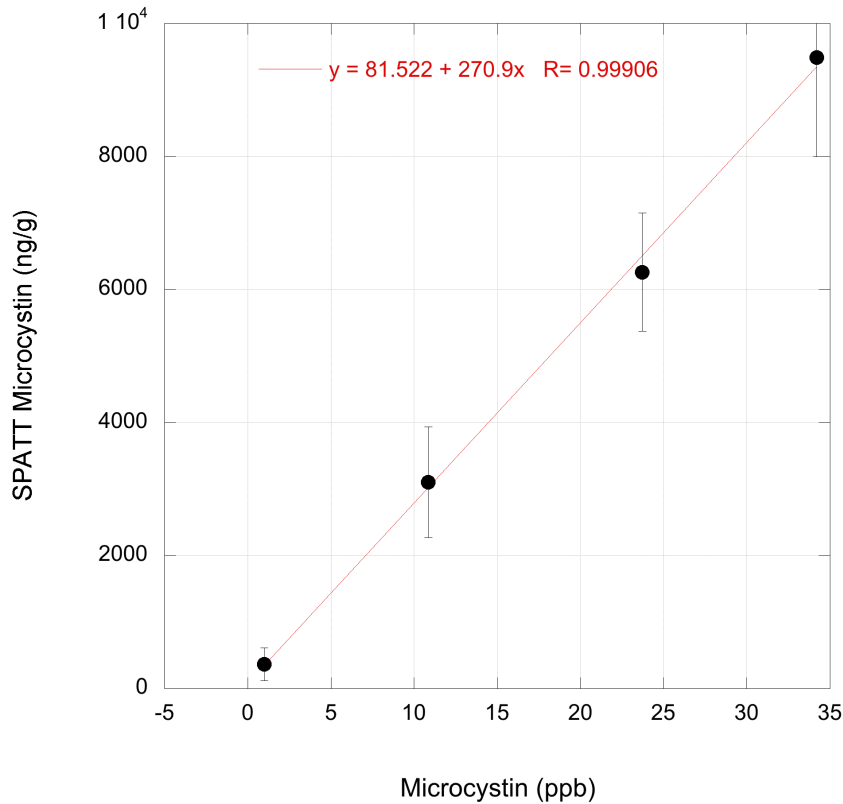

Figure 4. SPATT versus ambient water concentration for microcystins with SPATT exposed to a constant concentration for >1 hour.

Microcystins, transferred to lower concentration: When SPATT were allowed to equilibrate at a higher toxin concentration and were then exposed to water of lower concentration, similar kinetics were observed (not shown) with equilibrium occurring in ~1 hour, and a linear decrease over the first 60 minutes observed.

Field Calibration of SPATT Microcystins: the laboratory data for adsorption kinetics (time) and toxin levels (concentration) were used to develop a matrix showing the relationship between field SPATT observations and potential ambient toxin concentrations. The matrix is shown in Figure 5, together with statistics showing the total microcystin concentrations observed from October 2011- November 2014 for San Francisco Bay. The suggested "alert level" of 1 ng/.g microcystins is indicated, along with the estimated non-detect limit.
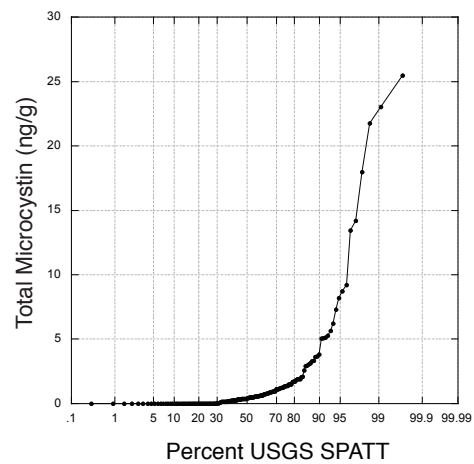
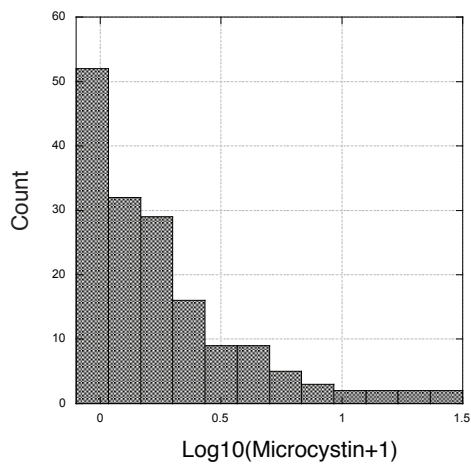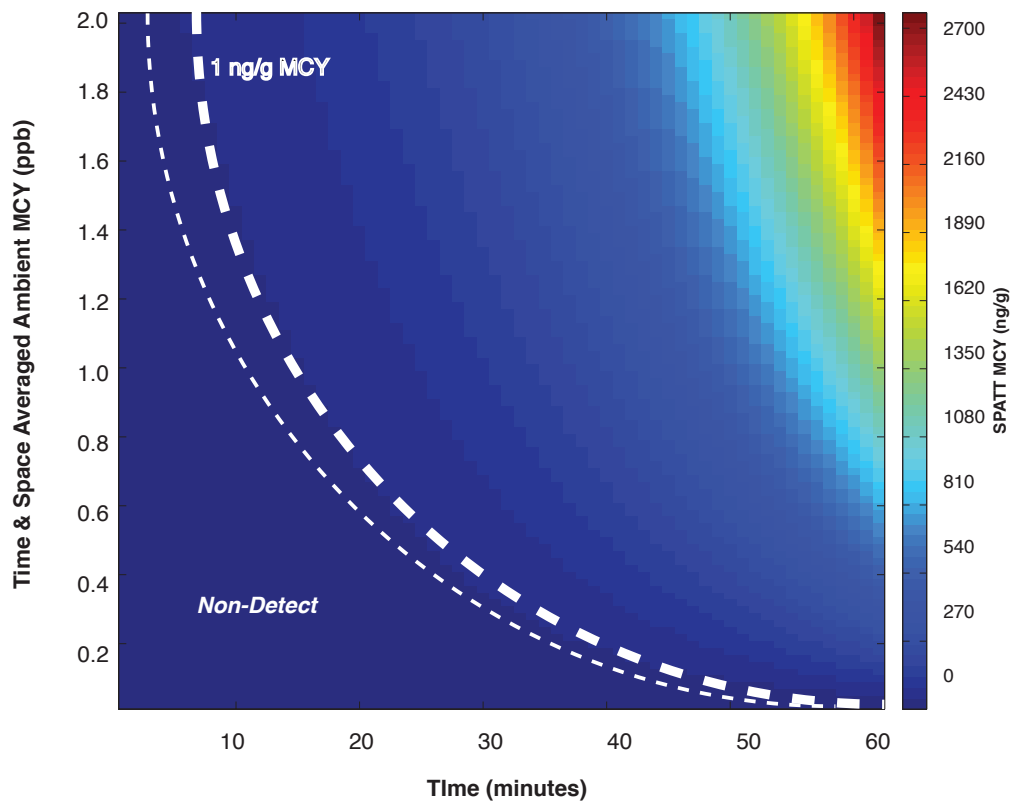Figure 5. The top graph shows the SPATT concentrations that would be measured as a function of exposure time versus concentration. Note that toxin levels would increase/decrease in response to exposure (x-axis) to water with higher/lower concentration, with an equilibrium time of ~1 hour. The lower panels show the histogram of toxin concentrations observed in SFB (left) and cumulative percent (lower right).

Domoic Acid, 15 minute exposure: Adsorption of DA was exponential rather than linear (as seen for microcystins). Toxins were easily detected after 15 minutes of exposure. This makes calibration of SPATT more difficult, since it strongly depends on how long the SPATT are exposed. Data are presented in Figure 6.
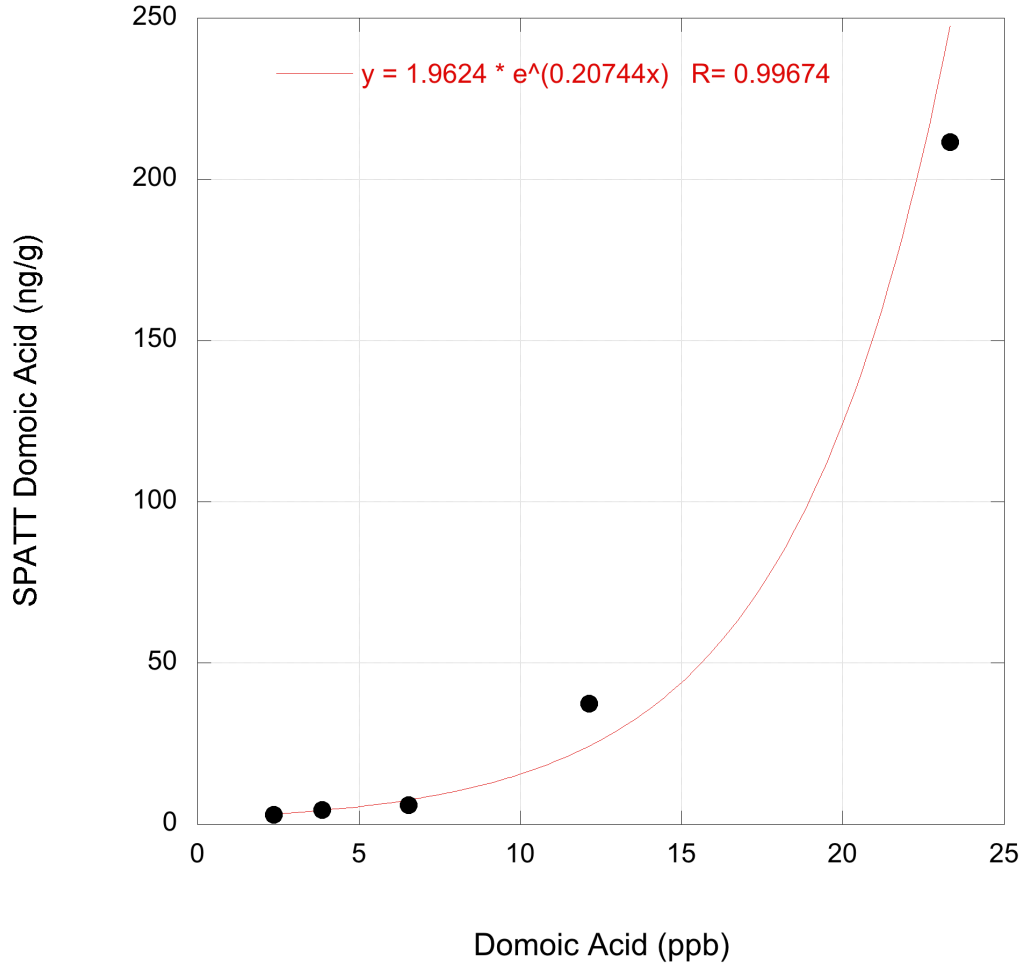

Figure 6. SPATT versus ambient water concentration for domoic acid for SPATT exposed to constant concentration of toxin for 15 minutes.

Domoic Acid, > 20 hour exposure: Testing of SPATT showed that domoic acid continues to be adsorbed for up to 24 hours, while other studies (Lane et al. 2010, Zendong et al. 2014) shows that SPATT continues to adsorb toxins for multiple days, but is quasi-linear when multiple days are included. Results for up to 24 hour exposure for varying concentrations of domoic acid are presented in Figure 7. As with 15 minute exposure the data fit an exponential curve, suggesting that SPATT concentrations of domoic acid may underestimate low values and overestimate high values, compared to what would be assume using a linear relationship.

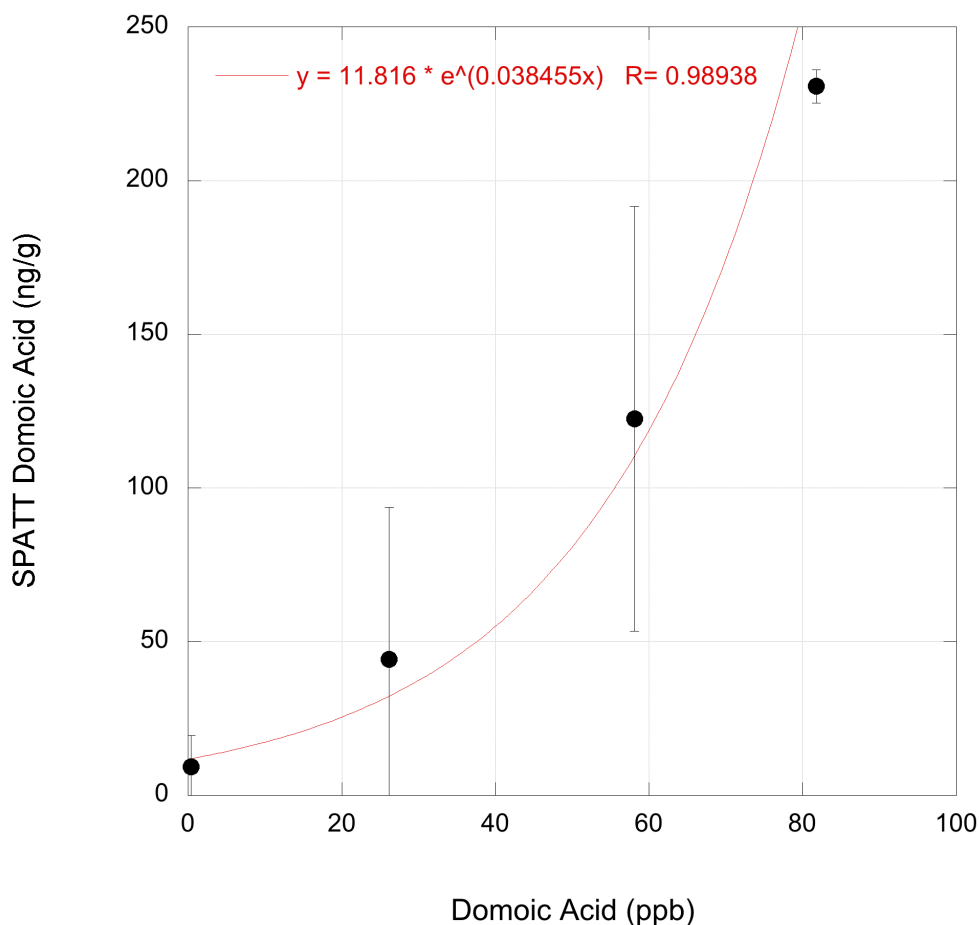$$y = 11.816 * e^{(0.038455x)} \quad R= 0.98938$$

Figure 7. SPATT versus ambient water concentration for domoic acid for SPATT exposed to constant concentration of toxin for >20 hours.

Domoic Acid, transferred to lower concentration: When SPATT were allowed to equilibrate at a higher toxin concentration and were then exposed to water of lower concentration, similar kinetics were observed (not shown) with equilibrium initially fast, and then slowing down. The net result would be to (again) overestimate concentrations when exposed to high levels of domoic acid, compared to a linear response for time-averaged concentrations.

Field Calibration of SPATT Domoic Acid: the laboratory data for adsorption kinetics (time) and toxin levels (concentration) were used to develop a matrix showing the relationship between field SPATT observations and potential ambient toxin concentrations. The matrix is shown in Figure 8, together with statistics showing the total domoic acid concentrations observed from October 2011-November 2014 for San Francisco Bay. The suggested "alert level" of 75 ng/.g domoic acid is indicated, along with the estimated non-detect limit.

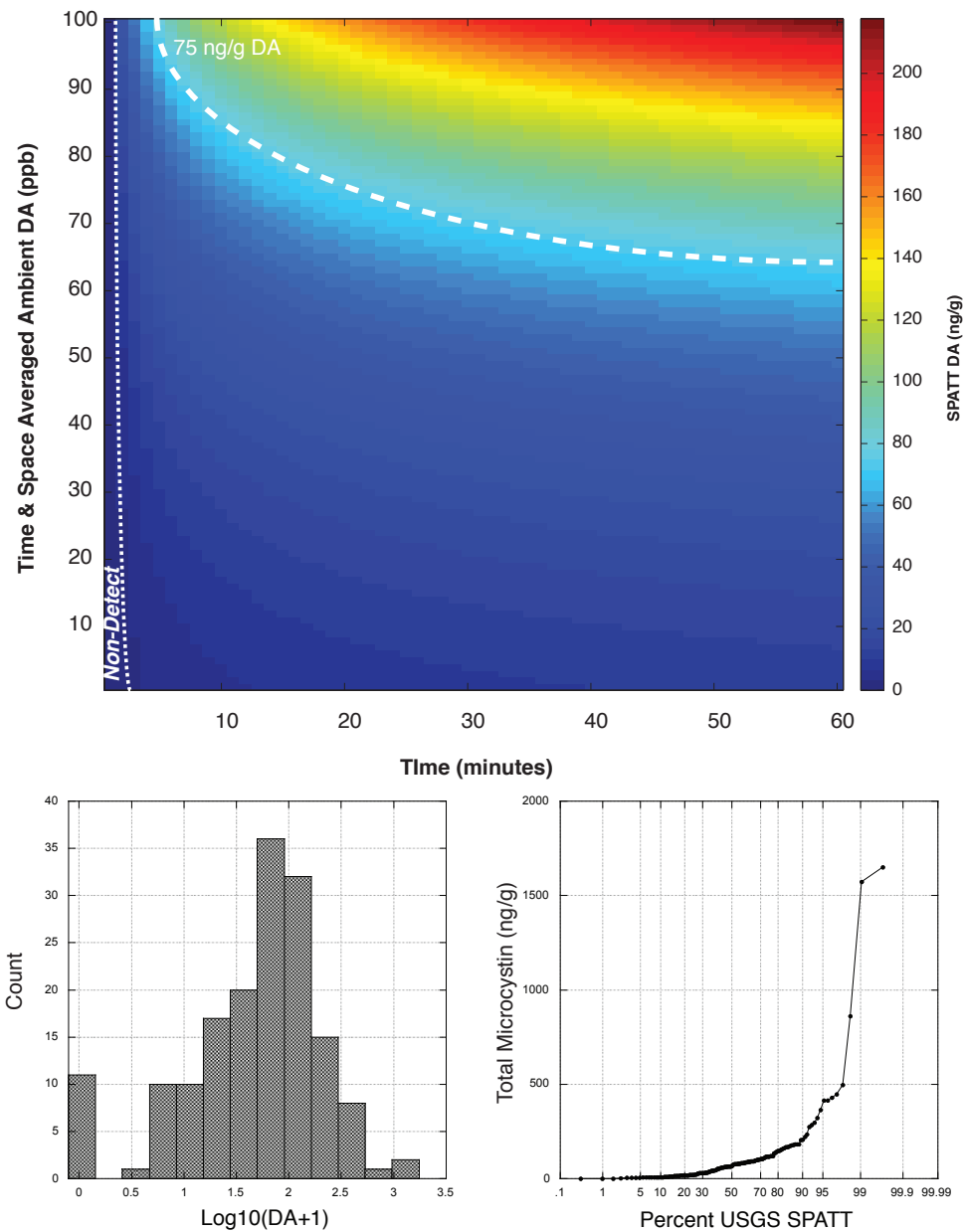Figure 8. The top graph shows the SPATT concentrations that would be measured as a function of exposure time versus concentration. Note that toxin levels would increase/decrease in response to exposure (x-axis) to water with higher/lower concentration, with an equilibrium time of ~several days. The lower panels show the histogram of toxin concentrations observed in SFB (left) and cumulative percent (lower right).

Calibration of SPATT—Comparison to Mussels

Mussel samples were obtained from the RMP monitoring program for 2012 and 2014. This provides a direct comparison between a regulatory measurement (tissue samples) and SPATT from approximately the same time period and location, keeping in mind that the SPATT are deployed in surface water on a subembayment scale for a few hours, while mussels are deployed at depth for ~6 months.
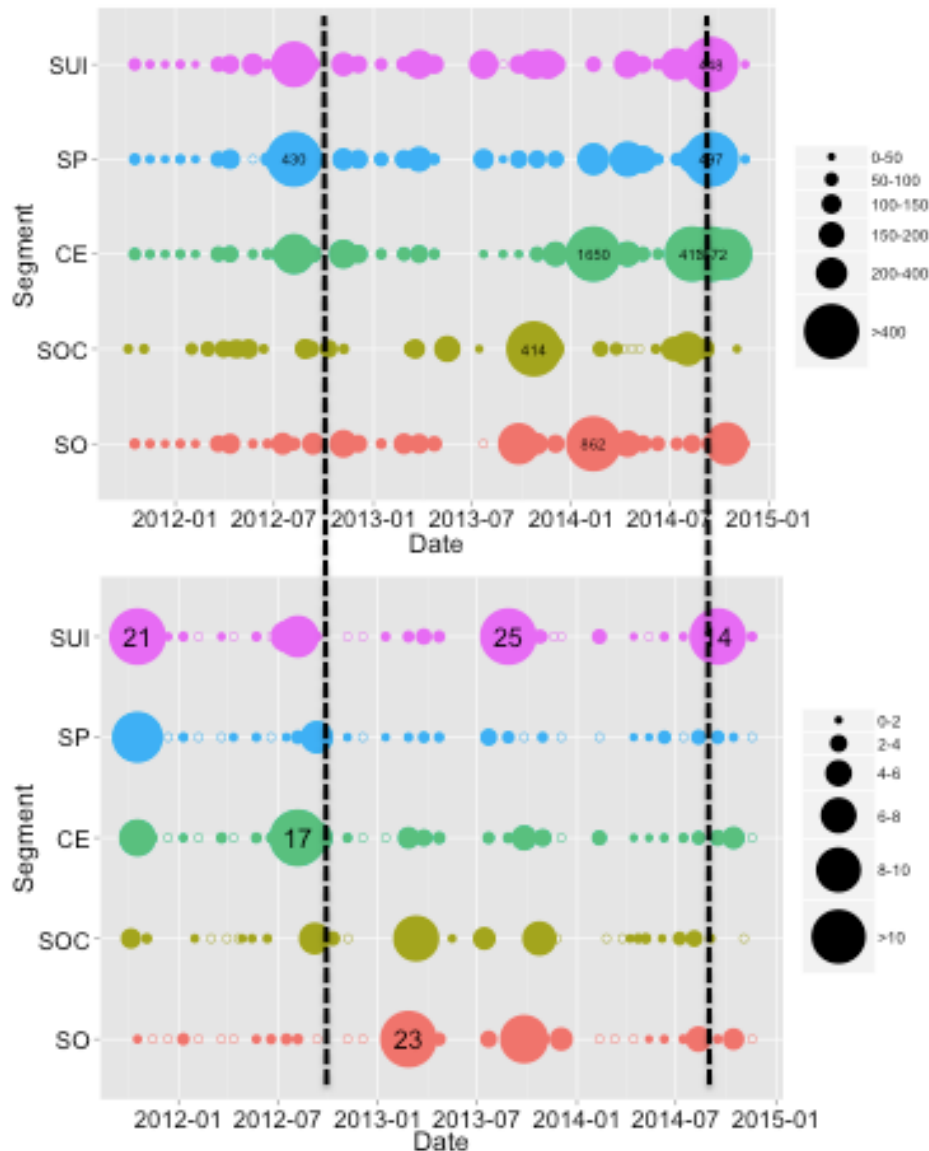

Figure 9. SPATT time-series, with bivalve retrieval dates overlayed as dashed lines. Note that bivalves were retrieved shortly after widespread toxin throughout the Bay for both DA and microcystins.

Figure 9 shows the SPATT time-series for microcystins and DA, with the mussel collection. Note that toxin was detected in mussels immediately following periods when SPATT indicated widespread presence within the Bay. For the mussel samples, 100% of sites had detectable domoic acid, while 82% (2012) and 100% (2014) of mussels had detectable microcystins. Of the two, the microcystins were closer to regulatory closure, with a maximum value of ~22 µg/kg (WHO guidelines recommend closure at 24 µg/kg). Comparison of SPATT with the mussel data suggest that a microcystin level of 10-20 ng/g SPATT would be too conservative, so more recent recommmendations have lowered this to 1 ng/g. Similarly, presumably because of the non-linearity in uptake, DA values of 30-50 ng/g are probably too conservative, and the new recommended value is 75 ng/g (these values are reported in Sutula et al., in prep; "Scientific Basis for Assessment of Nutrient Impacts on San Francisco Bay").

Calibration of SPATT--Recommendations

Based on this initial pilot study of field-deployed SPATT and laboratory calibration, it seems clear that the SPATT time-series should be continued as part of the USGS cruises. Discussions with USGS and SFEI have explored the possibility of further dividing the Bay into subembayments consistent with the analysis performed by Sutula et al. (in prep.). This would primarily mean adding a Lower South Bay SPATT sampler, and separating Central Bay and North Central Bay. It is also recommended that, if possible, additional mussel samples be collected since this is the most unambiguous comparison between SPATT and ecosystem impairment. As part of separate SFEI funding, analysis is also underway to compare discrete filter samples with SPATT, but this will be subject to sampling variability (in previous comparisons, >50% of grab samples were negative while SPATT was positive) and to issue with limits of detection using filters due (primarily) to the heavy sediment load encountered when filtering whole water. It would also be useful to conduct a statistical analysis of SPATT relative to environmental conditions, to identify likely drivers of variability. Finally, additional laboratory testing of SPATT adsorption/desorption (for example, in response to salinity) could be carried out.

Ranking these recommendations by feasibility, cost, and impact, the following is proposed (from highest to lowest), with the recommendation followed by comments [in brackets]:

1) Continue SPATT time-series.

[SPATT is ongoing, primary limitation is availability of funds for both deployment and analysis of the data].

2) Collect additional mussel (or other invertebrate) samples for toxin analysis compared to SPATT. Ideally, deploy SPATT co-located with mussels.

[Feasible, but RMP currently conducts experiments every 2 years. So costs increase considerably if more frequent sampling is desired].

3) Add Lower South Bay and North Central Bay to the existing SPATT time-series.

[Minimal additional effort; would require permission from USGS, and would increase current costs by about 25%].

4) Collect/analyze discrete plankton samples for toxins to compare with SPATT.

[This is underway as part of separate funding; it's not clear that it will provide a direct intercalibration, given the past issues with comparing grab samples and SPATT].

5) Conduct retrospective analysis of SPATT versus environmental conditions to identify drivers of variability.

[This is probably a high priority, but the longer the time-series, the more valuable the analysis; analyzing now would primarily capture the drought period. Consider waiting until the drought ends, or anticipate analyzing again in the future. This could be a task for SFEI via the funded project for Blakely in 2015-16].

6) Conduct additional laboratory intercalibration.

[This could be done, but given the data already presented and the recent publications on SPATT, the chemistry is reasonably well-constrained. It would be a low priority compared to intercalibration with field samples].

Final Recommendation for interpreting SPATT: as documented in the Sutula et al. (in prep.) document, current recommendations based on statistical analysis, comparison with other field sites, and comparison with limited mussel samples is to consider "elevated" toxin concentrations equivalent to 1 ng/g total microcystins or 75 ng/g domoic acid for SPATT deployed by subembayment in San Francisco Bay. Values should be considered as "ranges" rather than absolute concentrations. For example, reasonable ranges, based on these updated thresholds, would be <1, 1-10, >10 for microcystins (no threat, moderate threat, high threat), and <50, 50-150, >150 for domoic acid. These ranges could be improved with additional mussel sampling, using logistic regression to define probability ranges (e.g. Lane et al. 2009; Anderson et al. 2011).

References

Anderson, CR, RM Kudela, C Benitez-Nelson, E Sekula-Wood, CT Burrell, Y Chao, G Langlois, J Goodman, and DA Siegel. 2011. Detecting toxic diatom blooms from ocean color and a regional ocean model, *Geophysical Research Letters* 38, L04603, doi:10.1029/2010GL045858.

Gibble, CM, RM Kudela. 2014. Detection of persistent microcystin toxins at the land-sea interface in Monterey Bay, California. Harmful Algae, 39: 146-153. doi:10.1016/j.hal.2014.07.004.

Kudela, RM. 2011. Characterization and Deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and salt water. *Harmful Algae*, 11: 117-125, doi: 10.1016/j.hal.2011.08.006.

Lane, JQ, GW Langlois, and RM Kudela. 2012. Update on the application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of domoic acid. Proceedings of the 14th International Conference on Harmful Algae, Intergovernmental Oceanographic Commission of UNESCO.

Lane, JQ, P Raimondi, and RM Kudela. 2009. The development of a logistic regression model for the prediction of toxigenic *Pseudo-nitzschia* blooms in Monterey Bay, California. *Marine Ecology Progress Series*, 383: 37-51.

Lane, JQ, CM Roddam, GW Langlois, and RM Kudela 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of domoic acid and saxitoxin in coastal California, *Limnology and Oceanography Methods,* 8:645-660.

Lin, F., G. Sun, J. Qiu, Q. Ma, P. Hess, A. Li. 2014. Effect of seawater salinity on pore-size distribution on a poly(styrene)-based resin and its adsorption of diarrhetic shellfish toxins. J. Chromatography A, 1373: 1-8.

Zendong, Z., C. Herrenknecht, E. Abadie, C. Brissard, C. Tixier, F. Mondeguer, V. Séchet, Z. Amzil, P. Hess. 2014. Extended evaluation of polymeric and lipophilic sorbents for passive sampling of marine toxins. Toxicon, 91:57-68.

Zhao, H., et al. 2013. Mechanism and application of solid phase adsorption toxin tracking for monitoring microcystins. *Journal of Chromatography A* 1300: 159-164.