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})
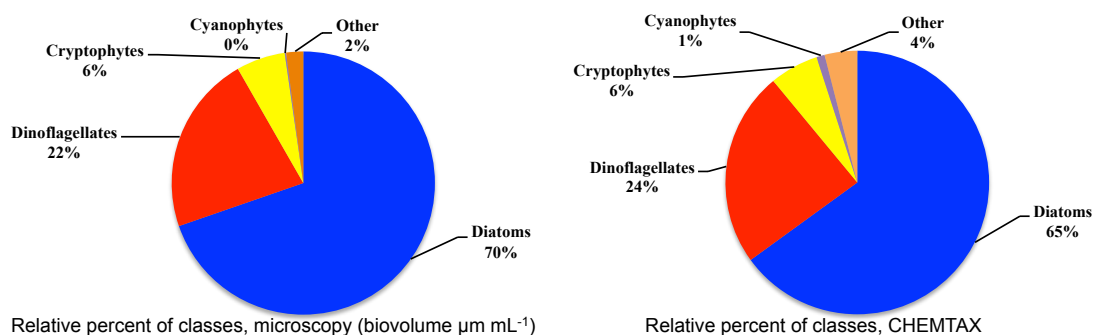
Relative percent of classes, CHEMTAX

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

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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%
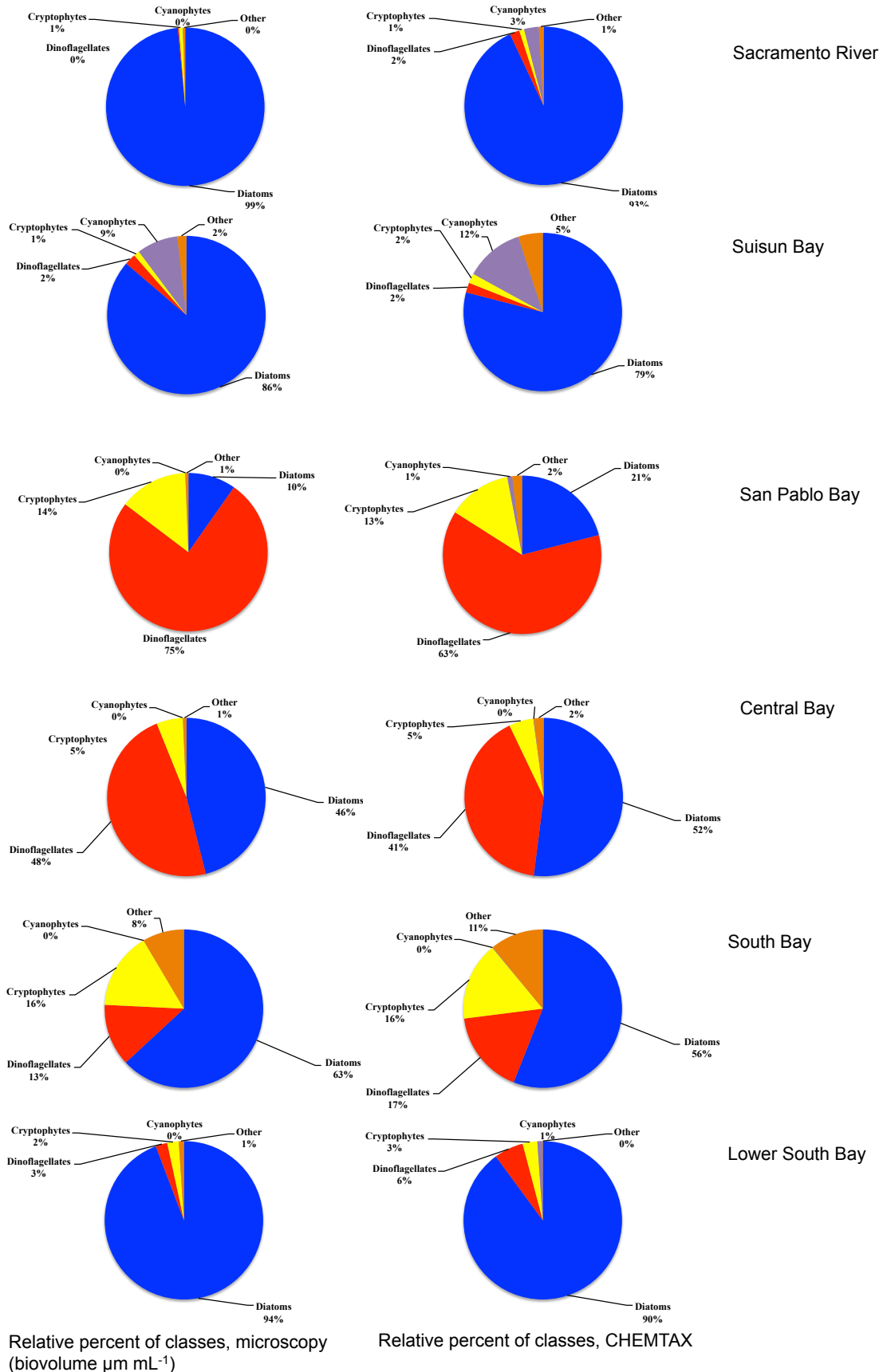
Sacramento River

Microscopy:
- Cryptophytes 1%
- Cyanophytes 0%
- Other 0%
- Dinoflagellates 0%
- Diatoms 99%

CHEMTAX:
- Cryptophytes 1%
- Cyanophytes 3%
- Other 1%
- Dinoflagellates 2%
- Diatoms 93%

Suisun Bay

Microscopy:
- Cryptophytes 1%
- Cyanophytes 9%
- Other 2%
- Dinoflagellates 2%
- Diatoms 86%

CHEMTAX:
- Cryptophytes 2%
- Cyanophytes 12%
- Other 5%
- Dinoflagellates 2%
- Diatoms 79%

San Pablo Bay

Microscopy:
- Cyanophytes 0%
- Other 1%
- Diatoms 10%
- Cryptophytes 14%
- Dinoflagellates 75%

CHEMTAX:
- Cyanophytes 1%
- Other 2%
- Diatoms 21%
- Cryptophytes 13%
- Dinoflagellates 63%

Central Bay

Microscopy:
- Cyanophytes 0%
- Other 1%
- Cryptophytes 5%
- Diatoms 46%
- Dinoflagellates 48%

CHEMTAX:
- Cyanophytes 0%
- Other 2%
- Cryptophytes 5%
- Diatoms 52%
- Dinoflagellates 41%

South Bay

Microscopy:
- Cyanophytes 0%
- Other 8%
- Cryptophytes 16%
- Dinoflagellates 13%
- Diatoms 63%

CHEMTAX:
- Other 11%
- Cyanophytes 0%
- Cryptophytes 16%
- Dinoflagellates 17%
- Diatoms 56%

Lower South Bay

Microscopy:
- Cryptophytes 2%
- Cyanophytes 0%
- Other 1%
- Dinoflagellates 3%
- Diatoms 94%

CHEMTAX:
- Cryptophytes 3%
- Cyanophytes 1%
- Other 0%
- Dinoflagellates 6%
- Diatoms 90%

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

Relative percent of classes, CHEMTAX

Fig. 3. Comparison of phytoplankton classes by microscopy and CHEMTAX program for the 5 subembayments in San Francisco Bay.

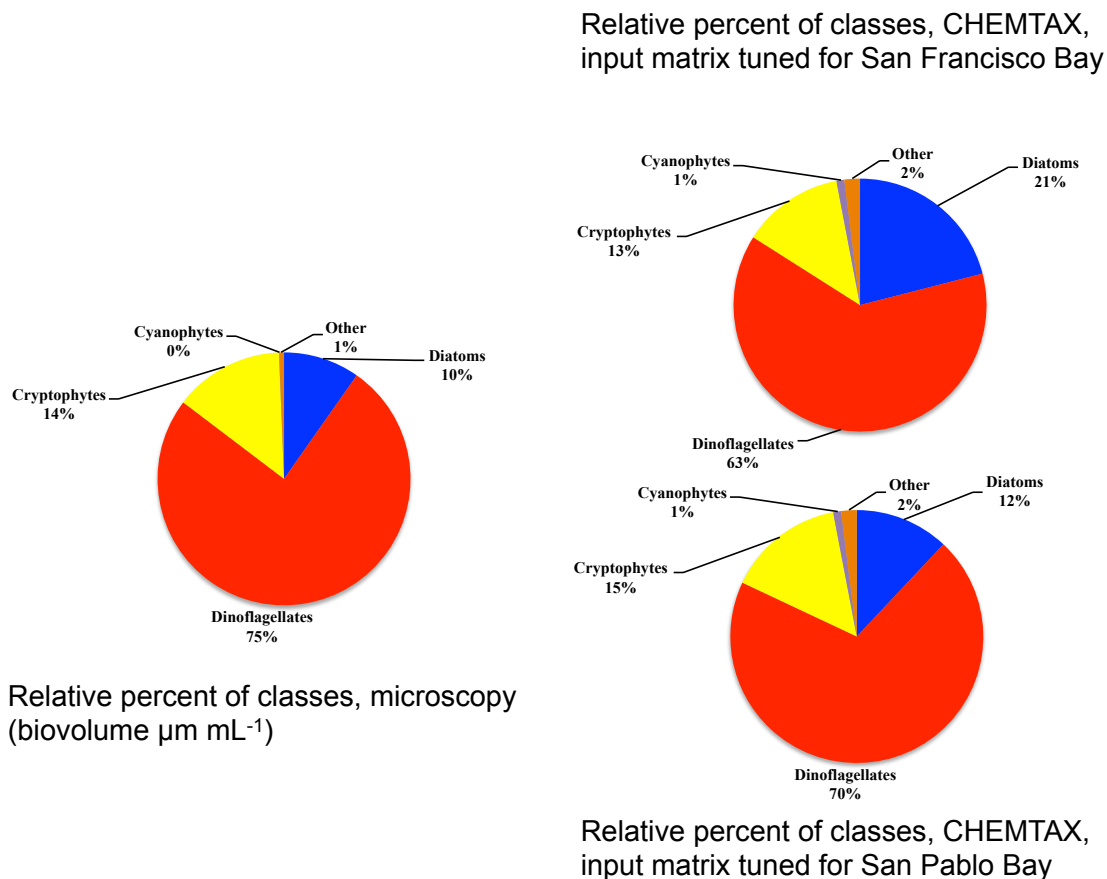Relative percent of classes, CHEMTAX,
input matrix tuned for San Francisco Bay

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,
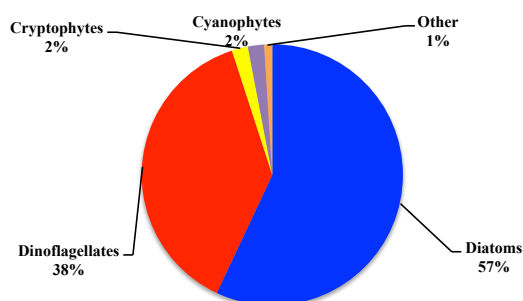input matrix tuned for San Pablo Bay

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).
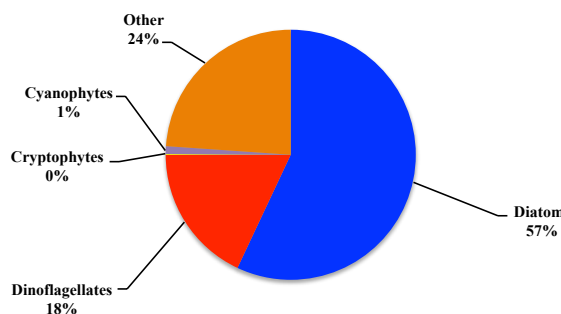
Central Bay, Station 18, December 18th 2012

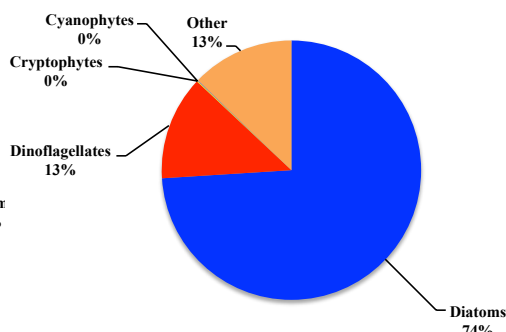Relative percent of classes, microscopy
(biovolume µm mL⁻¹)
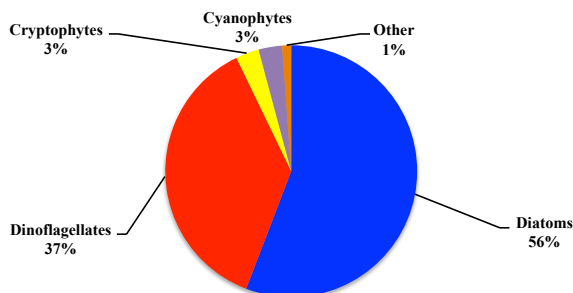
Relative percent of classes, CHEMTAX,
input matrix tuned for San Pablo Bay

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

Central Bay, Station 18, November 4th 2011

Relative percent of classes, microscopy
(biovolume µm mL⁻¹)

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	0.064	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	0.043	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	0.064	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	0.043	0	1

Matrix fine-tuned for San Pablo Bay