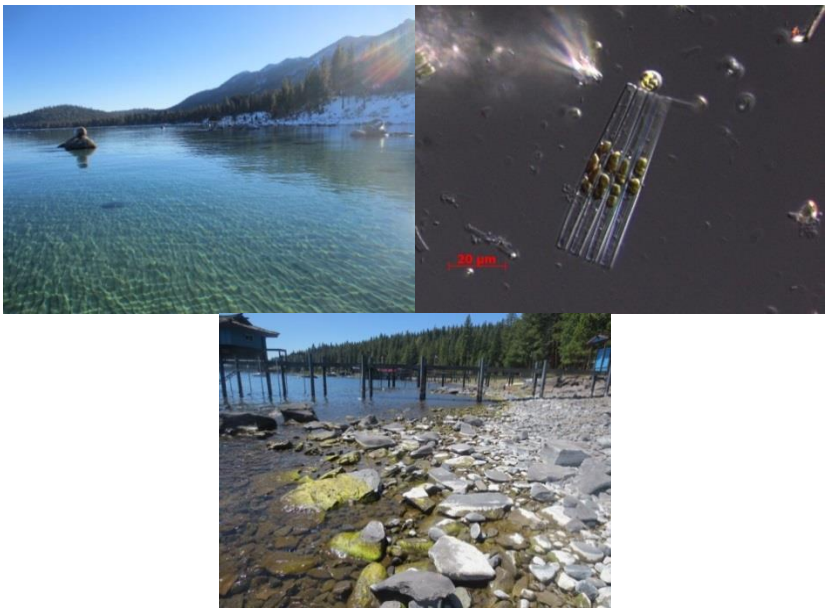
Lake Tahoe Water Quality Investigations

Algal Growth Potential assays • Phytoplankton • Periphyton



Annual Report:
July 1, 2014– June 30, 2015

Submitted to:
State Water Resources Control Board
Lahontan Regional Water Quality Control Board

By:

UC DAVIS
TAHOE ENVIRONMENTAL
RESEARCH CENTER

November 24, 2015

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Agreement No. 13-038-160

Submitted to:
State Water Resources Control Board
Lahontan Regional Water Quality Control Board

Submitted by:
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Executive Summary

This document provides a report of work completed by the U.C. Davis – Tahoe Environmental Research Center (TERC) between July 1, 2014 and June 30, 2015 under Agreement No. 13-038-160: Lake Tahoe Water Quality Investigations. Three primary areas of investigation or tasks were undertaken in this study, which were primarily related to algae growth in the nearshore zone of Lake Tahoe: (1) algal growth potential assays; (2) phytoplankton identification and enumeration; and (3) quantification of periphyton (attached algae) in the littoral zone.

Results from July 1, 2014-June 30, 2015 investigations together with information on project quality assurance and quality control are detailed in the main body of the report. Highlights, including findings from the past year, management implications, and recommendations are summarized in this executive summary.

AGP Assays

The purpose of the Algal Growth Potential (AGP) assay task is to compare levels of algal growth potential in the nearshore to identify emerging problem areas. The Algal Growth Potential (AGP) assay test was conducted as part of the California-Nevada-Federal Joint Water Quality Investigations in the late 1960's and early 1970's (California Department of Water Resources "DWR", 1970-75) to assess the maximum amount of algal growth supported by available nutrients in sampled waters. Lahontan has an existing water quality standard which states that mean annual nearshore AGP at a site will not be greater than two times the mean annual AGP at a mid-lake reference station. Sites with samples having repeatedly high AGP, or which exceed this standard repeatedly would deserve closer scrutiny of algae growth levels, and the environmental factors contributing to that growth.

During 2014 -2015, AGP tests were done approximately every 3 months, on nearshore and mid-lake water samples. While variability was observed in the AGP responses, AGP levels at most sites did not exceed the Lahontan standard. However, one site, Tahoe City, was observed to have relatively high AGP in tests done in late summer 2014 and early summer 2015. The AGP at this site also slightly exceeded the Lahontan standard in 2014[1]. This site is near the Tahoe City Boat ramp and also near Star Harbor which receives inflow from Burton and Polaris creeks**.** This site deserves closer scrutiny in future monitoring.

Timber Cove along the south shore is another site that may deserve close scrutiny. Although AGP did not exceed the Lahontan standard at Timber Cove, that site had the highest AGP among South Shore sites in tests done in late summer 2014 and early summer 2015, as well as in the February, 2015 test. This was particularly notable since initial chlorophyll *a* levels at this site were consistently the lowest of all sites. The algae at this site appear to have significant capacity for growth during those periods as shown by their increase in chlorophyll *a* during the AGP experiment. However, some factor or combination of factors at Timber Cove worked to prevent this growth potential from translating into greater biomass. The Timber Cove site may be influenced by several potential nutrient sources including: (1) nearby stream inflows from the

[1] When only June and Aug. 2014 data used to calculate the annual mean and the mid-lake north site was used as the mid-lake reference site.

U.Truckee/Trout watersheds; (2) nearby urban runoff inputs; (3) localized nutrient inputs from Asian clams, which are abundant in the area; and (4) the existence of an extensive shallow shelf area where bottom sediments may be stirred up by wind/wave activity, boating activity and human activities in the nearshore potentially bringing associated nutrients into the water column. It is unknown what factor or combination of factors resulted in relatively low initial chlorophyll *a* there. Some possible explanations are considered further in the full report.

As part of the AGP tests this year, TERC analyzed the initial lake water samples from all sites for levels of nitrogen and phosphorus. Nutrient levels did not show obvious site to site trends similar to AGP or chlorophyll *a*, although some variation in nutrient levels was observed. The nutrients present in lake water are subject to rapid biological uptake, and may not show large variations from site to site.

This was the first report in which we had AGP from a complete calendar year (2014) to compare with the Lahontan AGP standard. Slightly different conclusions were drawn for the 2014 data based on the whether all data from a year were used in calculation of an annual mean or just summer data, and whether the mid-lake reference data used was a mean of two mid-lake sites, or only consisted of data from the site closest to the nearshore site being evaluated. The Lahontan standard for AGP states that *mean annual AGP at a site should not be greater than two times the mean annual AGP at a mid-lake reference station*. In many of DWR's early studies, evaluations relative to the Lahontan AGP standard for the calendar year were based on AGP tests done **only** in May and August. We tested AGP in Feb., June, Aug., and December. We also collected data from both a north and south mid-lake reference station. So, mean annual AGP was calculated using 4 different combinations of annual mean and mid-lake reference site data. Using 3 of 4 combinations of the 2014 data, there were no violations of the Lahontan Standard indicated. However, when only the summer (June and August) nearshore station data was used and this was compared to the mean of AGP data from only the nearest mid-lake station, one site was found to violate the Lahontan standard in 2014: the Tahoe City site. Additional data may help to elucidate which combination of annual mean and mid-lake reference site data is most appropriate to use in evaluating AGP results relative to the Lahontan standard.

Phytoplankton Enumeration

In the phytoplankton enumeration and identification task, characterization of phytoplankton species and abundance in Lake Tahoe provides important data with regard to the base of the food web and nearshore condition. Change in the number and biodiversity of phytoplankton are indicators of nutrient loading, eutrophication and trophic status. Additionally, data and information generated through this task helps managers to determine if new and undesirable species (e.g. bloom-forming organisms, taste and odor species, or species that indicate a move away from the lake's current ultra-oligotrophic status) are colonizing the lake. Furthermore, these organisms influence lake clarity and there is some evidence that species composition and organism size can be a sentinel for climate change effects in Lake Tahoe (Winder et al., 2009).

Results of the phytoplankton enumeration indicated that bio-volume (an estimate of the amount of algae present) was quite high by February 2015 and reached a maximum at most sites in May 2015. Typically spring and summer are the height of phytoplankton growth activity. Blue-green algae, in February and May 2015 greatly influenced the total bio-volume at many stations. There

was only one species of blue-green, *Aphanothece sp*., a very small (3µm) solitary cell which has the capacity to fix nitrogen from the atmosphere. Due to the predominance of blue-green algae at many sites, any differences directly attributable to unique station conditions were overshadowed. An odd occurrence seen in February2015 was the dominance of a small centric diatom, *Cyclotella gordonensis*, which typically is seen only during summer stratified months of July and August. This alga is an excellent competitor under conditions of low nutrient, high light and warmer temperatures, which suggests that in February, most stations at shallow depths were stable and nutrient deficient. Overall, seasonal fluctuations as well as whole lake phytoplankton blooms tended to overwhelm differences among near shore stations.

Periphyton Quantification

The purpose of the periphyton quantification task is to assess biomass levels of nearshore attached algae (periphyton) around the lake. Excessive attached algae biomass coats the rocks in the spring in many areas around the lake and bright green filamentous algae occur along portions of the shoreline in the summer. Nearshore periphyton can adversely impact the aesthetic, beneficial use of the shore zone in areas where thick growth develops. The amount of periphyton biomass can reflect local nutrient loading and also be affected by long-term environmental changes. Monitoring trends in periphyton biomass is important in assessing local and lake-wide nutrient loading trends.

Results from the periphyton monitoring indicated that WY 2015 was similar to WY 2014 in that generally low periphyton biomass was measured at the 9 routinely monitored sites. Annual maximum chlorophyll *a* levels in 2015 were relatively low among all sites, ranging from 16.82 mg/m^2 at Sugar Pine Pt. to 47.49 mg/m^2 at Pineland. Sites along the northwest shore (Pineland, Tahoe City and Dollar Pt.) did not show substantially greater peak annual biomass than other routine sites as they have in many previous years.

In the 2015 spring synoptic in which approximately 50 sites were assessed using a rapid assessment method called the Periphyton Biomass Index (PBI), generally light biomass was also observed with some areas of slightly heavier growth interspersed along the shoreline. There were a few areas of moderate biomass observed during the spring synoptic, including Dollar Pt., South Fleur du lac and Timber Cove. Reduced inputs of nutrients during this dry year likely contributed to the generally low periphyton biomass around the lake.

Two areas of particularly heavy biomass were observed in 2015. One site, the Tahoe City Tributary site is a spring synoptic monitoring site that has been monitored every spring since the mid-2000's. Spring biomass there has remained relatively high the past three years in spite of the ongoing drought. It is probable that nutrient inputs from the tributary there are contributing to the elevated periphyton growth.

The other site, though not a routinely monitored spring synoptic site, is located just to the west of the Garwoods spring synoptic site and was observed to have unusually heavy periphyton growth along shore. Heavy algal growth has also been observed there in some previous years. Steady inflow of water seeping from the ground in the backshore was observed there and the seepage water was found to have slightly elevated levels of both nitrogen and phosphorus (NO_3-N=

86µg/l; NH_4-N=2µg/l) and phosphorus (SRP=29 µg/l). This inflow supported persistent growth of stalked diatoms (*Gomphoneis sp.*) throughout the summer of 2015 along with *Cladophora sp.*, which is not found at many sites in Lake Tahoe. Although it appears this seepage water is subsurface or groundwater, the extent to which there is an anthropogenic contribution of nutrients to this water is unknown. With the unusually heavy growth of periphyton observed at this site, it would be desirable to learn more about the factors contributing to the heavy growth there.

In contrast to the two heavy growth sites noted above, a site near the Ward Creek mouth (Ward Cr. synoptic site) has shown a substantial decline in biomass the past two water years, with chlorophyll *a* decreasing from a very high level of 379 mg/m^2 in 2013 to 7 mg/m^2 in 2015. Nearby Pineland also showed a substantial decline from very high total biomass in 2013 (242 mg/m^2) but chlorophyll biomass was similar the past two years (32mg/m^2).

It is interesting to note that a "drier than normal year" doesn't necessarily equate to a low periphyton year. While periphyton biomass was low to moderate this year (2015), in contrast, annual maximum biomass was quite high at Pineland and Tahoe City in 2012 and 2013 and at Rubicon Pt. in 2012 and Dollar Pt. in 2013 - yet these were years of lower precipitation and part of the current 4-year drought. Many factors likely interact to contribute to the level of periphyton growth in a year (e.g., nutrient inputs with surface runoff, enhanced inputs from urban/disturbed areas, groundwater, lake mixing, and atmospheric deposition, lake level, substrate availability, and wind/ wave events). The timing of events and previous year's conditions may also play a role.

The lake level was extremely low during WY 2015 which had an impact on the predominant algae observed during this period. Lake surface elevation was below the natural rim (6223.00 ft.) for the majority of WY 2015 and the 0.5m sampling depth was 1.64 ft. (or 0.5m) below this. Sampling at 0.5m resulted in collection of algae from the cyanobacteria (blue-green algae) zone of periphyton growth at most sites. This type of algae was very apparent as a dark-colored layer of material on rocks above a receding waterline, along portions of the east shore later in the summer 2015.

Finally, this year there was a somewhat weaker relationship between PBI and measured chlorophyll *a* concentration. The variation in the PBI to Chlorophyll *a* relationship this year may have related to variation in the levels of live algae in the periphyton. The PBI measurement utilizes filament length (or thickness of the algae) and percent coverage to estimate the level of biomass at a site. The periphyton coating a rock may contain variable amounts of both live and dead algae, as well as non-living organic mat and stalk material. Chlorophyll *a* is primarily indicative of living algal material. Areas of periphyton with similar thickness and percent coverage, yet different amounts of live algae could have different levels of chlorophyll for the same measured PBI. For the 2015 data, the association between PBI and an estimate of total organic matter (AFDW) was slightly better than that between PBI and chlorophyll *a*. The weak association between the 2015 PBI and chlorophyll *a* data serves as a reminder that PBI is not a perfect surrogate for chlorophyll *a* (i.e., living biomass). We would recommend chlorophyll *a* be used in preference to PBI when available.

Introduction

This report presents the results of work completed by the U.C. Davis – Tahoe Environmental Research Center (TERC) between July 1, 2014 and June 30, 2015 under Agreement No. 13-038-160: Lake Tahoe Water Quality Investigations. Three primary areas of investigation or tasks were undertaken in this study, which were primarily related to algae growth in the nearshore zone of Lake Tahoe: (1) algal growth potential assays; (2) phytoplankton identification and enumeration; and (3) quantification of periphyton (attached algae) in the littoral zone. The results from these investigations are detailed in the Sections I-III in the report. Quality assurance and quality control details for the investigations are presented in Section IV of the report. A detailed summary of Algal Growth Potential Assay data is presented in Appendix 1 and the phytoplankton enumeration standard operating procedure is presented in Appendix 2.

Section I. Algal Growth Potential Assays

With increasing focus on the environmental health of the nearshore the AGP test was reinstituted in August 2013 to evaluate algal growth potential at different nearshore and offshore stations around Lake Tahoe. The purpose of these experiments is to compare levels of algal growth in the nearshore and offshore to identify potential problem areas, and to evaluate conditions relative to an established water quality standard. Availability of the nutrients, nitrogen (N) and phosphorus (P) in the water, and levels of nutrients previously taken up by phytoplankton (known as luxury uptake) are important factors that contribute to growth.

Methods

AGP assay tests are performed on samples collected from 13 stations (Figure 1, Table 1) four times per year (in spring, summer, fall and winter). Samples of lake water (usually from a depth between 0.5-1.5m) are collected from a boat, using a Van Dorn water sampler. Many of the current sites are in proximity to sites sampled by DWR in their study of Lake Tahoe in the 1970's (DWR, 1970-1975). Two open-water reference sites are also sampled, one near mid-lake north (U.C. Davis's MLTP station), and the other a mid-lake south site (similar to that used by DWR). A sample for phytoplankton identification and enumeration is also collected directly from the Van Dorn sampler and treated with Lugol's reagent at the time water is collected for the AGP assay. Lake water from each site for the AGP assay is filtered through an 80 µm size mesh netting to remove large zooplankton, and collected in 4 liter HDPE bottles. The samples are kept near lake temperature in the dark in a cooler and returned to the lab at TERC where the experiment is usually started the same day.

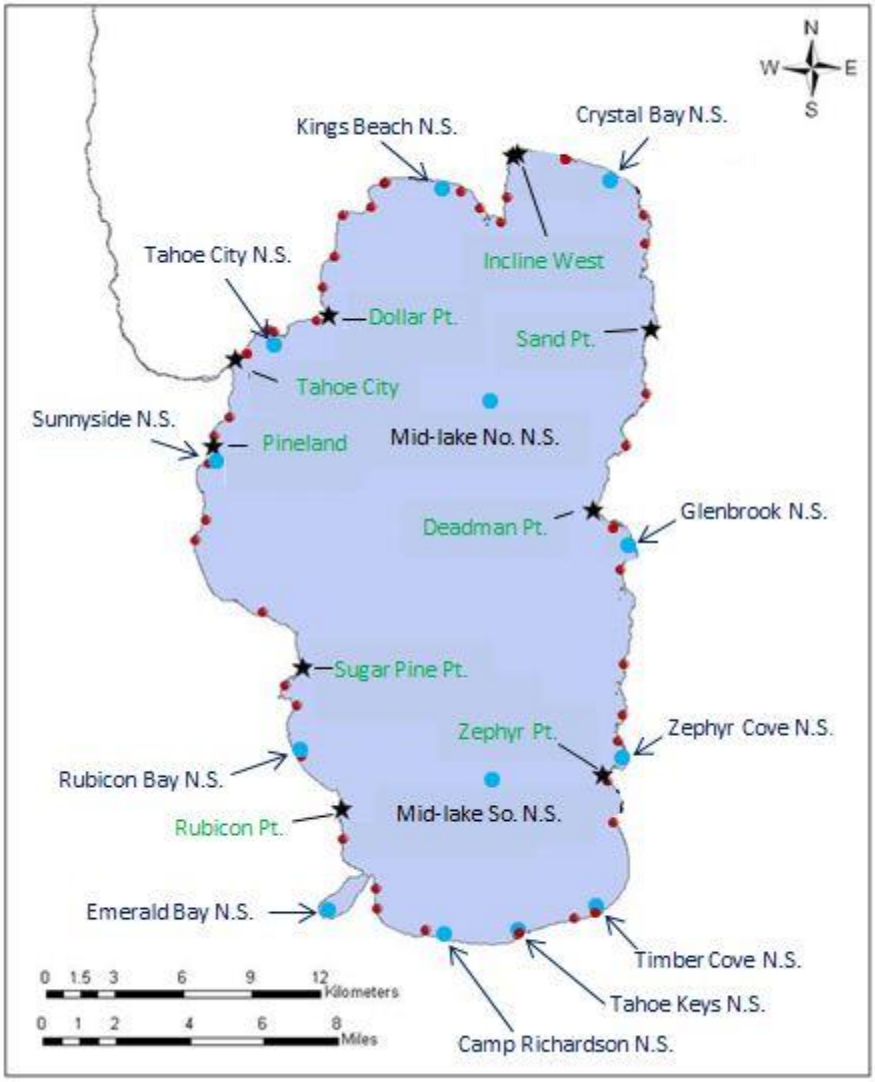

Figure 1. Map showing locations of AGP nearshore stations (light blue dots), routine periphyton monitoring stations (green text, black stars) and spring synoptic periphyton stations (red dots).

Table 1. Description of AGP and phytoplankton monitoring sites.

Site	Coordinates	Site Description	Water Depth at Station
Nearshore Sites			
Sunnyside	N39 07.805 W120 09.216	~ 15 m from first pier just north of Ward Cr.	~ 3m
Tahoe City	N39 10.808 W120 07.173	~18-27 m outside of entrance to Tahoe City Boat Ramp area and pier	~2.5m
Kings Beach	N39 14.179 W120 02.207	~ 70 m from shore, offshore of "Lake Point Pier" slightly east of "Heritage Cove" condominiums	~ 2m
Crystal Bay	N39 14.258 W119 56.798	~45 m offshore of mouth of Incline Cr., Crystal Bay	~2.5m
Glenbrook	N39 05.371 W119 56.489	~ 15 m from right side "T" of old pilings, near piling at boundary of swim area,~70 m from shore, Glenbrook	~2.5m
Zephyr Cove	N39 00.512 W119 56.993	Off first set of beach stairs north of Zephyr Cove pier, ~27 m outside of swim area boundary, ~90 m from shore.	~2.5m
Timber Cove	-	~45-70 m northwest of end of Timber Cove pier	~2m
Tahoe Keys Nearshore	N38 56.423 W120 00.574	~70 m offshore of lake-side pier at Tahoe Keys, (Note-site for AGP#1 was ~115 m further offshore)	~1.5-2m
Camp Richardson	N38 56.531 W120 03.383	Adjacent to end of Camp Richardson pier	2-3m
Emerald Bay	N38 57.187 W120 06.367	Adjacent to either the pier or near north edge of swim area boundary, both near Vikingsholm	~4-5m
Rubicon Bay	N39 00.875 W120 06.840	~70 m offshore of pier in shallow area	~2-3m
Mid-lake Sites			
Mid-lake North	N39 09.255 W120 00.478	Location of TERC MLTP station in north mid-lake, approx. 10.5 km east of Tahoe City	>450m
Mid-lake South	N38 59.641 W120 00.080	South mid-lake approximately 6.5 km north of Pope Beach.	>400m

In the AGP experiment, lake water from each site is divided into duplicate flasks and incubated under controlled light (CW fluorescent light with intensity ~ 74 µ E m^{-2} sec^{-1}), standard light cycle (i.e. 16 hour light, 8 hour dark) and at ambient lake temperature.[2] Algal biomass changes are measured by tracking *in vivo* chlorophyll *a* fluorescence in water from the flasks throughout the experiment using a Turner Designs 10AU fluorometer (configured for *in vivo* and extractable chlorophyll *a* measurement). On one or more days of the experiment, typically near the growth peak, subsamples are also filtered for later chlorophyll *a* extraction and analysis. Equations relating *in vivo* fluorescence measurements to extracted chlorophyll *a* are determined. The

[2] These methods differ slightly from the early DWR studies with respect to: lighting (DWR used a light intensity of 700 foot candles or ~91 µ E m^{-2} sec^{-2}) and temperature (DWR used a constant temperature of 20° C) However, we think incubation at 20° C might adversely affect some cold water species represented in the winter community.

equations may then be used to calculate chlorophyll *a* on days when *in vivo* fluorescence peaks and extracted chlorophyll *a* was not measured. The peak chlorophyll *a* value achieved during the assay is considered the Algal Growth Potential (AGP).

Extracted chlorophyll *a* is analyzed fluorometrically using a Turner Designs 10AU fluorometer, calibrated with pure chlorophyll *a* from *Anacystis nidulans* algae. Frozen sample filters containing algae are thawed and extracted overnight at 4°C, in 100% methanol, then fluorescence before and after acidification with 0.05ml of 0.3N HCl is measured. Chlorophyll *a* and pheophytin concentrations are determined using the following equations:

Chlorophyll *a* (µg/l) = $(r/(r-1)) \times (R_b-R_a) \times V_{ex}/V_{fil}$
Pheophytin (µg/l) = $(r/(r-1)) \times (rR_a-R_b) \times V_{ex}/V_{fil.}$
R_b= Fluorescence of sample extract before acidification (minus) fluorescence of filter blank
R_a= Fluorescence of sample extract after acidification (minus) fluorescence of filter blank
V_{fil}= Volume of lake water filtered (Liters), usually 0.1 L
V_{ex} = Volume of methanol used for extraction (Liters), usually 0.005L
r = mean of R_b/R_a values for a range of pure chlorophyll standards.
(r = 2.369 for current calibration)

Additional field and lab data collected for these experiments includes: lake surface water temperature at time of collection; background fluorescence of the initial water collected (fluorescence of GF/F filtered water); and results of chemical analysis of N and P in the initial lake water (not part of contracted work; however, this was done to provide supplementary information for all four AGP assays completed during 2014-2015).

AGP Assay Results July 2014-June 2015:

AGP assay tests were done on lake water collected 8/29/14, 12/9/14, 2/26/15 and 5/26/15. Table 2 provides results for initial in lake water samples, and Algal Growth Potential (AGP) chlorophyll *a* concentration of the samples. Figures 2a-2d present the results graphically. Detailed summaries of AGP bioassay data are also presented in Appendix 1.

Site to site differences in AGP were apparent in 3 of 4 of the assays completed during 2014-2015. In this year's report we briefly summarize the results of individual assays, report on interesting observations from this set of assays, and provide an initial evaluation of 2014 nearshore AGP relative to the Lahontan standard for AGP.

Table 2. Initial chlorophyll *a* (µg/l) and Algal Growth Potential (AGP) chlorophyll *a* (µg/l) for four bioassays completed in 2014 and 2015.

Nearshore:	8/29/14		12/9/14		2/26/15		5/26/15	
	Initial Chl *a*	AGP Chl *a* ± s.d.	Initial Chl *a*	AGP Chl *a* ± s.d.	Initial Chl *a*	AGP Chl *a* ± s.d.	Initial Chl *a*	AGP Chl *a* ± s.d.
Sunnyside	.19	.42 ± .07	.52	.52	.52	.71 ± .04	.28±.01	.44 ± .02
Tahoe City	.41	.82 ± .05	.46	.46	.35	.62 ± .01	.63±.00	.78 ± .04
Kings Beach	.40	.48 ± .03	.45	.45	.43	.83 ± .03	.29	.44 ± .01
Crystal Bay	.17	.43 ± .02	.61	.61	.59	.84 ± .02	.27±.01	.43 ± .02
Glenbrook	.23	.40 ± .01	.46	.46	.42	.97 ± .04	.25±.01	.35 ± .00
Zephyr	.18	.61 ± .02	.34	.39 ± .02	.33	.94 ± .01	.27±.01	.46 ± .05
Timber Cove	.11	.65 ± .01	.31	.39 ± .04	.17	1.08 ± .09	.09±.01	.88 ± .01
Tahoe Keys	.20	.56 ± .03	.53	.53	.37	.90 ± .02	.23±.01	.39 ± .01
Camp Rich.	.18	.45 ± .01	.43	.43	.48	.75 ± .01	.27±.02	.43 ± .00
Emerald Bay	.23	.39 ± .02	.52	.52	.98	.98	.49±.00	.52 ± .01
Rubicon Bay	.16	.44 ± .08	.38	.38	.76	.76	.33±.02	.38 ± .02
Mid-Lake:								
Mid-lk No.	.15	.44 ± .002	.53	.53	.63	.67 ± .03	.22	.33 ± .02
Mid-lk So.	.17	.37 ± .04	.43	.43	.62	.76 ± .02	.19±.01	.24 ± .01

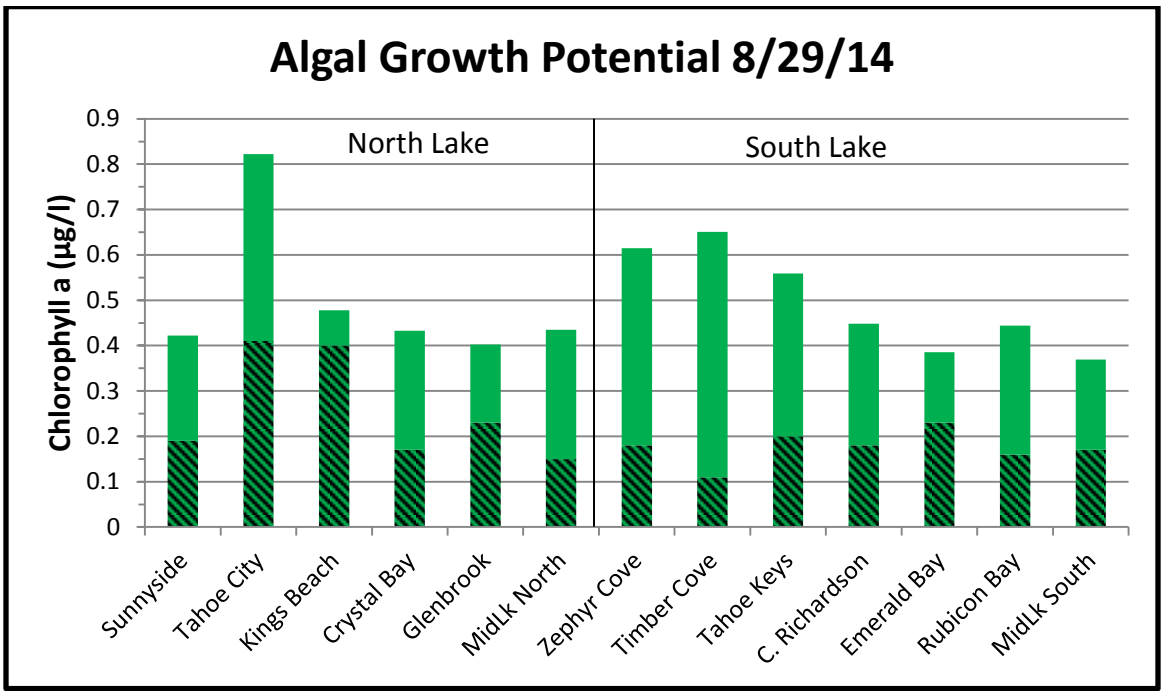

Figure 2a . Algal growth potential for samples collected 8/29/14 (total height of bar), the initial chlorophyll *a* concentration in samples at start of bioassay is indicated by shading.

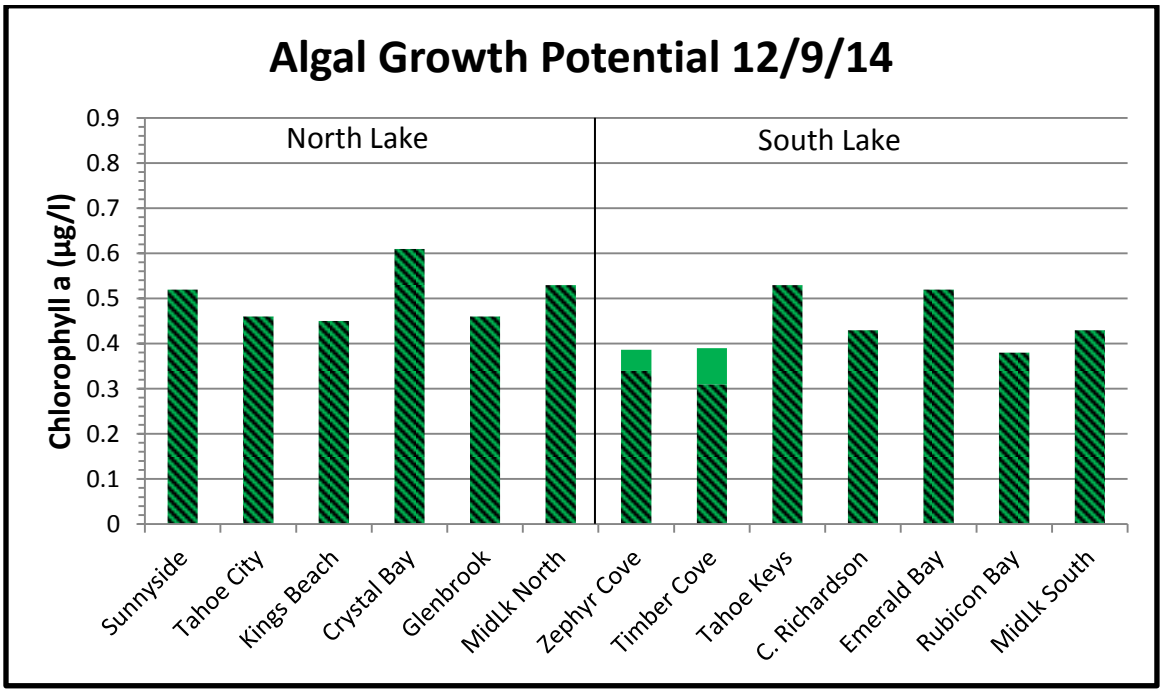

Figure 2b. Algal growth potential for samples collected 12/9/14 (total height of bar), the initial chlorophyll *a* concentration in samples at start of bioassay is indicated by shading.

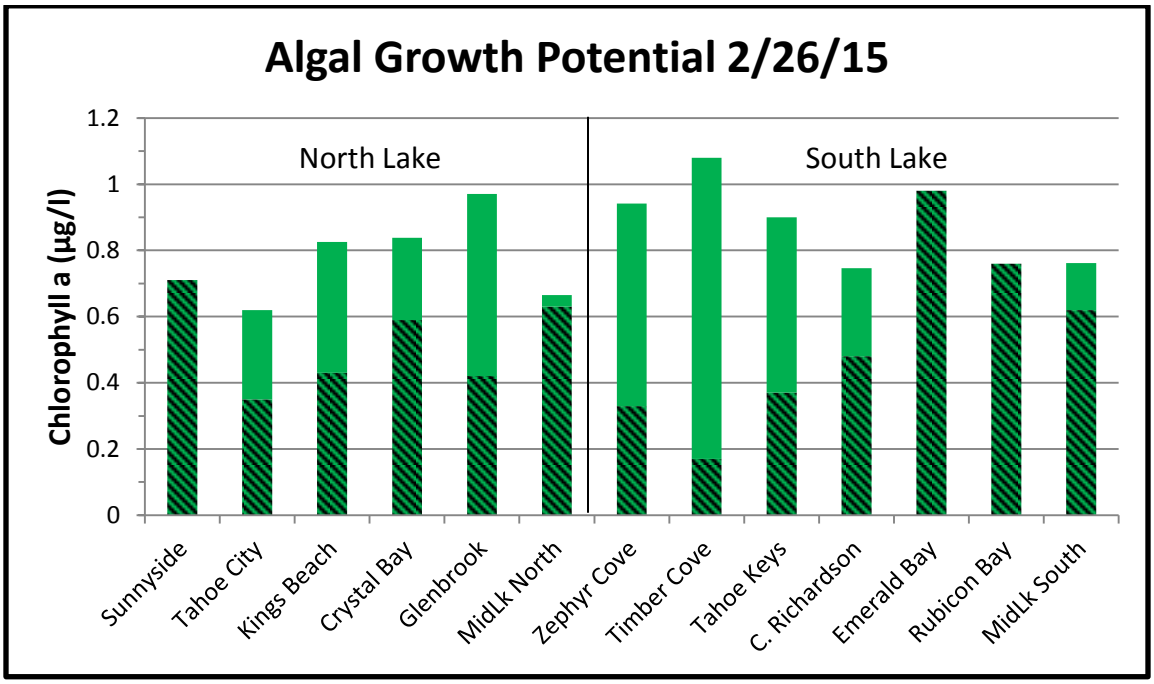

Figure 2c. Algal growth potential for samples collected 2/26/15 (total height of bar), the initial chlorophyll *a* concentration in samples at start of bioassay is indicated by shading.

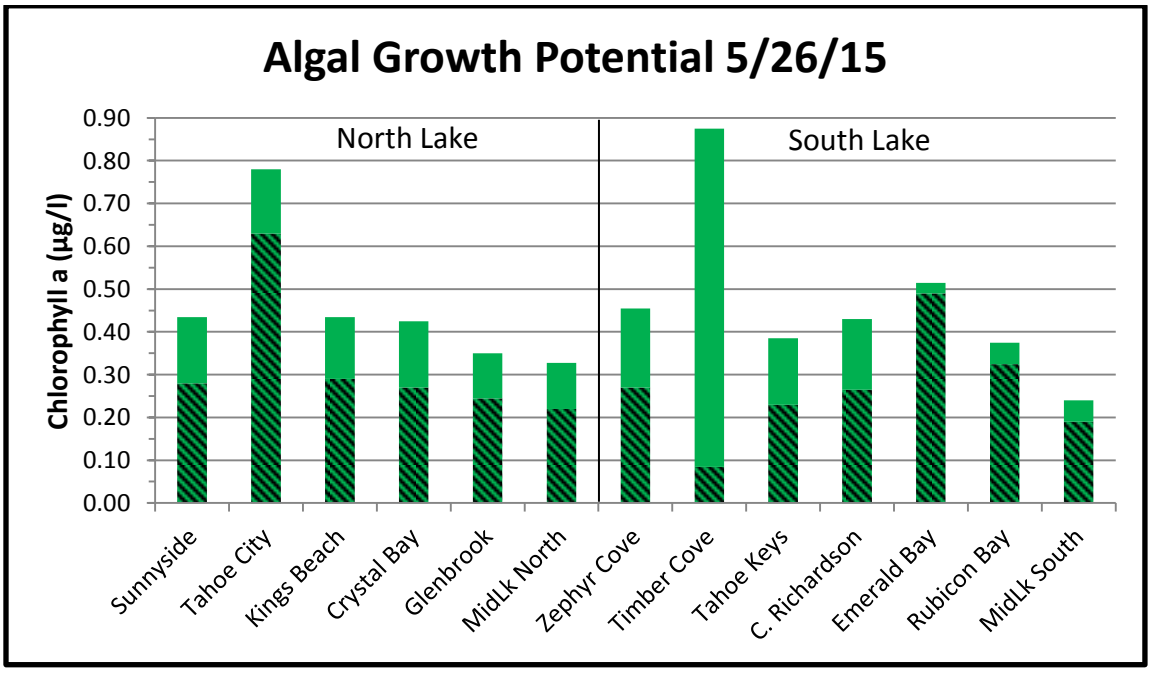

Figure 2d. Algal growth potential for samples collected 5/26/15 (total height of bar), the initial chlorophyll *a* concentration in samples at start of bioassay is indicated by shading.

Brief Summary of Results by AGP Assay:

AGP Assay #5 (8/29/14)
This was a late summer sampling. Lake surface temperature was still very warm and ranged between 17-19 °C. Lake chlorophyll *a* concentrations were generally low at most sites (between 0.1 to 0.25 µg/l) with only Tahoe City and Kings Beach having moderate chlorophyll *a* near 0.40 µg/l. Initial levels of NO_3-N, NH_4-N, SRP and TP for each assay are reported below in Tables 3a-3d. Initial levels of NO_3-N and SRP were low, but measurable, with no substantial site to site differences. TP was slightly to moderately elevated at most sites and ranged from 5-40 µg/l. TP was highest at the Timber Cove site. NH_4-N was slightly elevated (range 4-9 µg/l) at the sites. The highest AGP was measured at Tahoe City (Chlorophyll *a* = 0.82 µg/l) in the north lake region and at three south shore sites (Zephyr Cove, Timber Cove and Tahoe Keys) with AGP chlorophyll *a* ranging between 56-61µg/l. AGP at the other sites were close to the mid-lake AGP (i.e. near 0.40 µg/l).

AGP Assay #6 (12/9/14)
This was an early winter sampling. Lake surface temperature was still relatively warm for the time of year (8.0-9.0 °C). Some rain and snow occurred 12/2-12/4/14, however no large precipitation events preceded the sampling. Lake chlorophyll *a* concentrations showed slight variations among the sites ranging from 0.31 µg/l at Timber Cove to 0.61 µg/l at Crystal Bay). AGP levels were the same as initial lake chlorophyll *a* concentrations at most sites (i.e. most sites showed no increase in chlorophyll *a* during the assay). Two sites Timber Cove and Zephyr Cove showed slight increases in chlorophyll *a* during the test. However the AGP for all sites were close to values observed at the two mid-lake stations (i.e. 0.43 at the South Mid-lake station and 0.53 µg/l at the North Mid-lake station). Initial N and P levels in samples were all low and showed no obvious patterns relative to chlorophyll *a* or AGP at the sites.

AGP Assay #7 (2/26/15)
This was a late winter/early spring sampling. Lake surface temperature was likely just beginning to warm and ranged from 6.0-7.0°C. The strongest storm of the year had occurred Feb. 6-9 contributing substantial rain and snow. Strong N-NE winds Feb. 21-23 preceded sampling for this AGP test. Initial lake chlorophyll *a* concentrations varied between sites (i.e., chlorophyll ranged from a low of 0.17 µg/l at Timber Cove to a high of 0.98 µg/l at Emerald Bay, with the mid-lake stations having a chlorophyll *a* concentration of 0.62-0.63 µg/l). Most sites showed growth during the AGP test, and all nearshore sites ultimately had an AGP either close to or greater than the nearest mid-lake sampling site. The highest AGP was measured for the Timber Cove sample (1.08 µg/l), which is notable as this site had the lowest initial chlorophyll *a* concentration. Zephyr Cove, Tahoe Keys and Emerald Bay AGP (chlorophyll range 0.90-0.98 µg/l) were also above the mid-lake South AGP of 0.76 µg/l. Kings Beach, Crystal Bay and Glenbrook sites had the highest AGP in the north portion of the lake ranging from 0.83-0.97 µg/l, all greater than AGP of the mid-lake north site (0.67 µg/l). Again, Initial N and P levels in samples were all low and showed no obvious patterns relative to chlorophyll *a* or AGP at the sites. There was no strong increase in surface NO_3-N which in some years occurs associated with lake mixing.

AGP Assay #8 (5/26/15)

This was a late spring/early summer sampling. Lake temperature was warming and ranged from 11.0-15.0°C. The timing of this sampling was at the end of a very meager snowmelt runoff, however the two weeks preceding sampling had periods of rain and snow (including some areas with thunderstorms the day before sampling, e.g., the Sunnyside/Ward Cr. area). Initial lake chlorophyll *a* levels were relatively similar and relatively low at most sites ranging between 0.19-0.33 µg/l. Notable exceptions were Timber Cove, which once again had the lowest chlorophyll *a* (0.09 µg/l) and moderately high levels at Emerald Bay (0.49 µg/l) and Tahoe City (0.63 µg/l). All sites showed increases in chlorophyll *a* during the AGP test. Timber Cove once again showed substantial growth from a very low initial chlorophyll level, and had the highest AGP (0.88 µg/l) which was 3.67 times the AGP level at mid-lake south (0.24 µg/l). All other nearshore site AGP levels in the southern lake region were also higher than the mid-lake south AGP level. Along the north shore, Tahoe City had the highest AGP (0.78 µg/l), with Sunnyside, Kings Beach and Crystal Bay AGP (ranging from 0.43-0.44 µg/l), also higher than the mid-lake north AGP (0.33 µg/l). Levels of N and P were very low in the initial lake water samples collected. NO_3-N levels were non-detectable at many of the sites. TP was slightly elevated at many of the sites (ranging from 2-13 µg/l), NH4-N was slightly elevated (8µg/l) only at the Timber Cove site.

Table 3a. Initial NO_3-N concentrations in lake samples collected for AGP bioassays.

	NO_3-N	NO_3-N	NO_3-N	NO_3-N	Mean	Std. Dev.
	8/29/14	12/9/14	2/26/15	5/26/15		
Sunnyside	4	2	1	0	2	2
Tahoe City	3	2	1	2	2	1
Kings Beach	3	1	2	0	2	1
Crystal Bay	2	2	3	1	2	1
Glenbrook	3	1	4	0	2	2
Mid-lake North	3	2	2	1	2	1
Zephyr Cove	3	2	4	1	2	1
Timber Cove	3	2	4	0	2	2
Tahoe Keys	3	1	2	2	2	1
Camp Richardson	3	1	2	0	2	1
Emerald Bay	3	3	1	0	2	2
Rubicon Bay	3	2	1	0	2	1
Mid-lake South	2	2	2	0	2	1

Table 3b. Initial NH_4-N concentrations in lake samples collected for AGP bioassays.

	NH_4-N	NH_4-N	NH_4-N	NH_4-N	Mean	Std. Dev.
	8/29/14	12/9/14	2/26/15	5/26/15		
Sunnyside	4	1	3	4	3	1
Tahoe City	8	4	5	4	5	2
Kings Beach	8	3	4	4	5	2
Crystal Bay	7	3	3	4	4	2
Glenbrook	9	2	2	4	4	3
Mid-lake North	9	3	3	4	5	3
Zephyr Cove	7	3	2	4	4	2
Timber Cove	6	4	4	8	6	2
Tahoe Keys	4	3	4	5	4	1
Camp Richardson	6	3	3	5	4	2
Emerald Bay	4	3	4	5	4	1
Rubicon Bay	4	3	4	5	4	1
Mid-lake South	5	3	3	4	4	1

Table 3c. Initial SRP concentrations in lake samples collected for AGP bioassays.

	SRP	SRP	SRP	SRP	Mean	Std. Dev.
	8/29/14	12/9/14	2/26/15	5/26/15		
Sunnyside	1	2	1	1	1	1
Tahoe City	2	1	2	2	2	1
Kings Beach	2	1	2	2	2	1
Crystal Bay	2	1	2	2	2	1
Glenbrook	2	1	1	1	1	1
Mid-lake North	2	1	1	1	1	1
Zephyr Cove	3	3	1	1	2	1
Timber Cove	2	3	1	1	2	1
Tahoe Keys	2	2	1	1	2	1
Camp Richardson	2	2	1	1	2	1
Emerald Bay	1	2	1	1	1	1
Rubicon Bay	1	2	2	1	2	1
Mid-lake South	2	3	1	2	2	1

Table 3d . Initial TP concentrations in lake samples collected for AGP bioassays.

	TP	TP	TP	TP	Mean	Std. Dev.
	8/29/14	12/9/14	2/26/15	5/26/15		
Sunnyside	27	5	2	11	11	11
Tahoe City	5	8	3	9	6	3
Kings Beach	18	6	3	10	9	7
Crystal Bay	30	6	3	10	12	12
Glenbrook	22	7	4	10	11	8
Mid-lake North	17	6	3	2	7	7
Zephyr Cove	25	4	3	10	11	10
Timber Cove	40	3	3	9	14	18
Tahoe Keys	30	6	3	13	13	12
Camp Richardson	17	3	3	9	8	7
Emerald Bay	12	5	3	10	8	4
Rubicon Bay	23	6	3	9	10	9
Mid-lake South	20	5	3	7	9	8

Summary observations from the 2014-2015 AGP Bioassays

1. During early and late summer AGP tests the Tahoe City site had noticeably higher AGP levels than other north shore sites. This site is near the Tahoe City Boat ramp and also near Star Harbor which receives inflow from Burton and Polaris creeks.
2. Timber Cove along the South Shore had the highest AGP among South Shore sites in early and late summer AGP tests as well as the early spring test. This was particularly notable since initial chlorophyll levels at this site were consistently the lowest of all sites. The algae at this site appear to have significant capacity for growth during those periods. This site may be influenced by several potential nutrient sources including: nearby flows from the Upper Truckee/Trout Cr. watersheds, and nearby urban inputs. This site is also an area with substantial Asian clam presence (which may contribute nutrients through excretion), as well being an extensive shallow shelf area where bottom sediments may be stirred up by wind/wave activity, boating activity and human activities in the nearshore potentially bringing associated nutrients into the water column. The fact that initial chlorophyll *a* levels were consistently the lowest at Timber Cove during the past year, also makes this site particularly interesting. Some factor or combination of factors resulted in relatively low initial chlorophyll *a* there. Some possible explanations include: (a) effects of high light/UV over the shallow shelf; (b) competition for nutrients from bacteria and benthic algae; (c) predation on the phytoplankton by other organisms (i.e., zooplankton and possibly Asian clams); (d) differing levels of nutrient enrichment which favor differing species composition; and (e) possibly the presence of inhibitory substances in the water. The potential for Asian clams to have some impact is discussed further in the phytoplankton section below.

3. The initial chlorophyll *a* level from sampling sites did not necessarily always relate to the Algal Growth Potential during this period. Some sites with very low initial chlorophyll concentration had high potential for growth (i.e. Timber Cove), while several sites with high initial chlorophyll showed little potential for additional growth (i.e. Emerald Bay on 2/26/15).
4. Nutrient levels did not show obvious site to site trends similar to AGP or initial chlorophyll *a* although some variation in nutrient levels was observed. TP tended to be somewhat elevated at most sites in the bioassays in Aug. 2014 and June 2015. The highest TP was measured at Timber Cove in Aug. 2014. NH_4-N was slightly elevated at most sites also in Aug. 2014. NH_4-N was also slightly elevated relative to other sites in May 2015 at Timber Cove. Otherwise, the nutrient levels at sites tended to be fairly uniform and low. The nutrients present in lake water are subject to rapid biological uptake, and may not show large variations from site to site.

Levels of AGP in 2014 and the Lahontan AGP Standard

The Lahontan standard for AGP states that mean annual AGP at a site should not be greater than two times the mean annual AGP at a mid-lake reference station. The four AGP tests for 2014 provide the first opportunity to make a determination whether any of the sites violated the standard for a complete calendar year of data. However, there is uncertainty regarding the analytical approach used to assess conditions relative to the Lahontan standard . We used four different analytical approaches, and the results are presented in Table 4. For each site two means were calculated: (1) the mean annual AGP using data for all four dates; (2) the mean for June and August 2014 AGP data[3]. These means in turn were divided by either the mean AGP from both mid-lake reference sites for the same period or the mean AGP for the mid-lake reference site nearest to the nearshore site.

The results of these analyses indicated the following:
There were <u>no violations</u> of the Lahontan Standard based on the following calculation methods:

1. The mean annual AGP for nearshore sites collected on all four dates during 2014 divided by the mean of AGP levels from both mid-lake reference sites during the same period.
2. The mean annual AGP for nearshore sites collected only June and August, 2014 divided by the mean of AGP levels from both mid-lake reference sites during the same period.
3. The mean annual AGP for nearshore sites collected on all four dates during 2014 divided by the mean of AGP levels for only the nearest mid-lake reference site during the same period.

<u>There was one violation</u> of the Lahontan Standard when the following method was used:

1. The mean annual AGP for nearshore sites collected for only June and August (mean summer AGP) during 2014 divided by the mean of AGP levels for only the nearest mid-lake reference site during the same period.

[3] Note, for several years in the DWR's 1970's AGP studies, evaluations relative to the Lahontan standard for the calendar year were based on the mean of data from AGP bioassays done in May and August. Therefore we included a similar comparison with our data except using June and August data. We also did a comparison using all the data for 2014 to see if different conclusions are obtained using data from all seasons.

Using this method, where the mean summer AGPs were divided by the mean of the AGP for the nearest mid-lake site showed Tahoe City just exceeded the Lahontan standard.

With completion of an additional year of data in 2015, we may be able to provide more guidance into which method is most useful in assessing conditions relative to the Lahontan standard. Based on the 2014 data, Tahoe City may be an area to monitor further. Although the Timber Cove site did not exceed the Lahontan AGP standard, it appears to be a site with generally higher algal growth potential, and also deserves close observation.

Table 4. Summary of Algal Growth Potential (AGP) test results by site during calendar year 2014. Mean AGP levels were calculated for both the full year (Mean Annual) and the summer period (June-Aug.). These values were then divided by either: the mean annual mid-lake AGP; the mean June-Aug. mid-lake AGP value; the mean of the nearest mid-lake station annual AGP values; mean of the nearest station mid-lake June-Aug AGP values. There were no violations relative to the Lahontan Standard for AGP (i.e. no annual mean for a site was more than twice the annual mean at a mid-lake site) when all annual data were used and the reference mid-lake AGP used was the mean of both north and south values. However, when the nearest mid-lake station data was used as the reference mid-lake value and only summer data was used, one site (Tahoe City) had a value which was more than twice the mean June-Aug. value for a nearby mid-lake site.

	AGP Feb. 20 2014	AGP June 9 2014	AGP Aug. 29 2014	AGP Dec. 9 2014	Annual Mean AGP	June-Aug. Mean AGP	Site Annual Mean AGP/ Mean Annual ML AGP	Site Jun-Aug Mean/ Mean Jun-Aug. ML AGP	Site Annual Mean AGP/ Nearest ML Station Mean Annual AGP	Site Jun-Aug Mean AGP/ Nearest ML Station Mean Jun-Aug AGP
Sunnyside	.63	.69	.42	.52	0.57	0.56	1.04	1.35	1.08	1.59
Tahoe City	.69	.61	.82	.46	0.65	0.72	1.19	1.73	1.23	2.04*
Kings Beach	.87	.37	.48	.45	0.54	0.43	1.00	1.03	1.03	1.21
Crystal Bay	.81	.39	.43	.61	0.56	0.41	1.03	0.99	1.07	1.17
Glenbrook	.79	.44	.40	.46	0.52	0.42	0.96	1.02	1.00	1.20
Zephyr Cove	.96	.50	.61	.39	0.62	0.56	1.13	1.35	1.09	1.17
Timber Cove	1.09	.50	.65	.39	0.66	0.58	1.21	1.39	1.17	1.21
Tahoe Keys	1.08	.65	.56	.53	0.71	0.61	1.30	1.47	1.25	1.27
Camp Rich.	.83	.83	.45	.43	0.64	0.64	1.17	1.55	1.13	1.35
Emerald Bay	.77	.69	.39	.52	0.59	0.54	1.09	1.31	1.05	1.14
Rubicon Bay	.61	.26	.44	.38	0.42	0.35	0.78	0.85	0.75	0.74
<u>Mid-Lake:</u>							No Violations	No Violations	No Violations	*T.City >2X Jun-Aug Nearby ML Mean
ML No.	0.87	.26	.44	.53	0.53	0.35				
ML So.	0.87	.58	.37	.43	0.56	0.48				
Mean ML	0.870	0.420	0.405	0.480	0.544	.413				

Section II. Enumeration and Identification of Phytoplankton

Lake Tahoe water quality, its clarity and color, are resources of value. It is through constant vigilance, limiting human impacts and education that these resources continue to attract worldwide attention. Lake Tahoe is classified as an ultra-oligotrophic lake by limnologists (Elser et al., 1991; Jassby et al., 1992). By definition, the waters have limited nutrient concentrations, making the environment challenging for plants and animals to survive. Planktonic plants, called phytoplankton, are the foundation of the biological food web, and are highly responsive to changes in the type and concentration of nitrogen-based and phosphorous-based nutrients. If changes occur in lake water quality, the phytoplankton are among the first indicators of that change. The abundance or numbers of the cells will change, the biodiversity may change, and these changes may trigger changes in other parts of the food web. The appearance of new and/or undesirable phytoplankton species may also be foretelling of qualitative water quality problems such as water color change or possibly odors. With increased interest in the state of the nearshore, this project task looks at phytoplankton in the nearshore around the lake.

This is the second year of sampling the phytoplankton community in near-shore stations around the circumference of Lake Tahoe. With two years of data, we are beginning to discern patterns between stations even though the annual weather patterns have been challenged by drought. Monitoring of phytoplankton bio-volume, abundance, diversity and the community assemblage have all been emphasized. These four parameters, over time, should give a good characterization of the near-shore sites. In the past year, eleven near-shore sites and two open water (mid-lake) sites were sampled quarterly for phytoplankton identification and enumeration. Using light microscopy, differential interference contrast at 630X magnification, cells were counted and identified to species level when possible following established TERC protocol (see Appendix 2). Periodically cell dimensions were measured and photos taken for documentation. Results are reported as cell numbers and biovolume for each species or taxon.

For biologists, the measurement of biomass is a critical parameter. It helps to characterize an environment by how much mass (weight) of living matter is present in a defined region. In aquatic ecosystems, while it is possible to weigh macro-organisms like crayfish and insects, it is nearly impossible to weigh phytoplankton with accuracy because of their small size. Instead, volume is a valid substitute for mass. Individual algal cell dimensions are measured and their volumes are calculated using a variety of volumetric equations to mimic their shape. This parameter is called the cell bio-volume.

For each of the near-shore stations, total algal bio-volume was calculated for every sample date (Figure 3). Phytoplankton have successional patterns in abundance and community composition throughout the year. Each sampling date generally shows that similar groups of phytoplankton are found at many of the stations. However, between dates, the colors in the figure

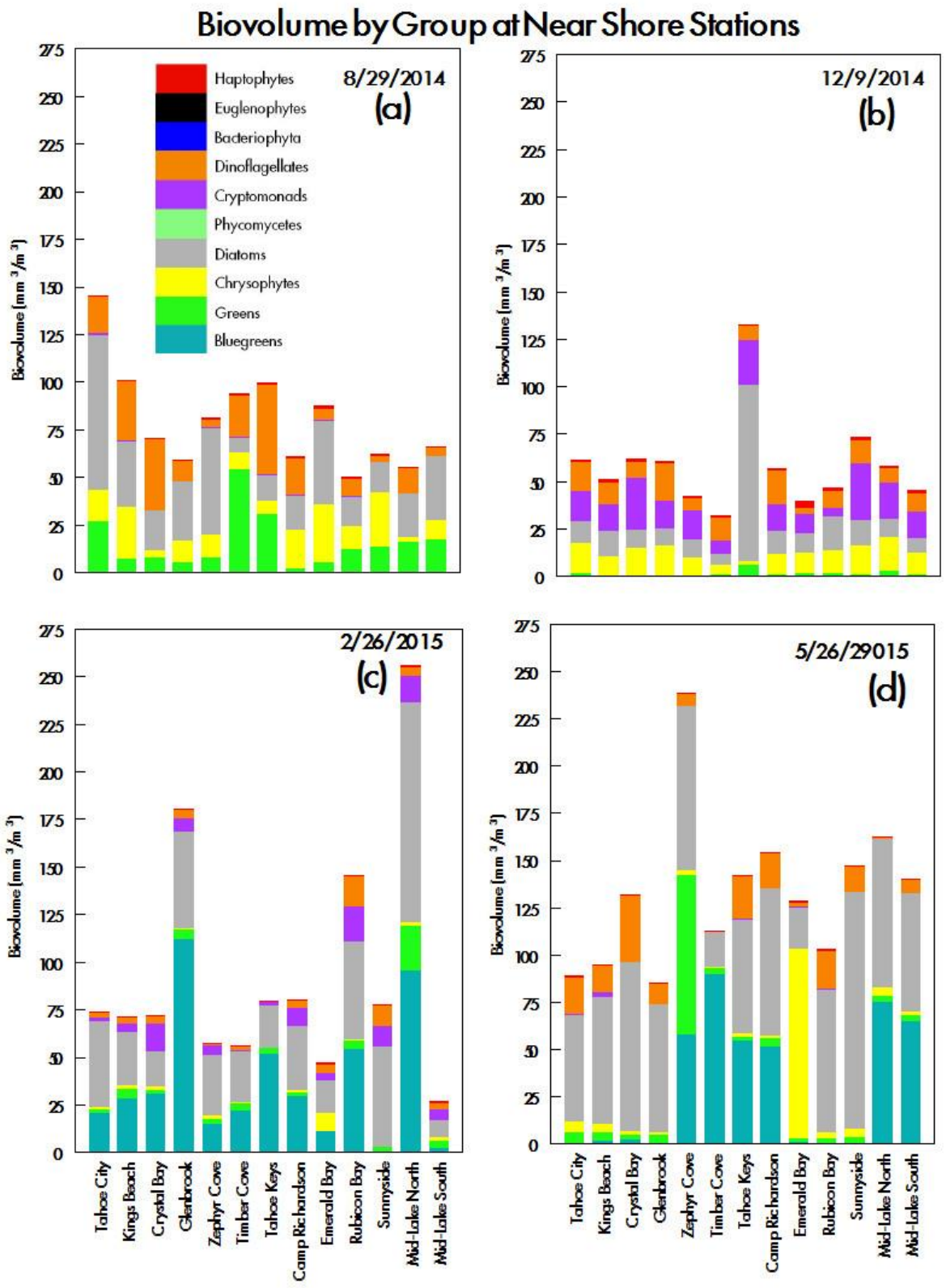

Figure 3. Biovolume by phytoplankton group and sampling date 8/29/14, 12/9/14, 2/26/15 and 5/26/15.

(phytoplankton taxonomic groups) change as new successional communities are established. The highest total bio-volume of the year was seen in May 2015 (Fig. 3-***d***), with some exceptions. Typically spring and summer are the height of phytoplankton growth activity. August 2014 (Fig. 3-***a***) had exceptionally low phytoplankton bio-volume at nearly all stations. December 2014 sampling (Fig. 3-***b***) showed the lowest total annual bio-volume at many of the near shore sites. Surprisingly, the total phytoplankton bio-volume was quite high by February 2015 (Fig. 3-***c***). This was quite different from the results found a year earlier, in February 2014 when biovolume was low lake-wide. It's possible the increased biovolume in February 2015 may have been a response to the hydrologic and weather conditions in the new year. There was generally little precipitation and input of nutrients with runoff until a relatively strong rain event Feb. 6-9.

As mentioned in last year's report, some stations were impacted by the presence of benthic periphyton and potentially metaphyton (collectively referred to as the 'benthic bias'), which do not typically 'live' in the plankton. Sites with rocky substrates and/or piers may be covered with periphyton, often diatoms. These algae may become detached from the substrate and mix into the water column during turbulence or wave activity. The metaphyton (algae that does not adhere to a substrate but are also not truly planktonic and often composed of filamentous green algae along the south shore) may also be suspended in the water column for periods. The benthic algae cells are large and their presence in a sample can sway the bio-volume numbers significantly. In August 2014 (Fig. 3 ***a***), for example, Tahoe Keys and Timber Cove had high bio-volumes of green algae (*Zygnem*a and *Spirogyra*). In both cases, benthic filamentous strands of cells contributed to increase the green color on the August 2014 graph. Tahoe City and Kings Beach were also impacted to a somewhat lesser degree, by the presence of benthic diatoms. In December, 2014 (Fig. 3 ***b***) Tahoe Keys was once again subject to benthic bias, which contributed to the high diatom bio-volume, while in February 2015 (Fig. 3 ***c***), this station had no notable benthic cells in the samples. By May 2015 (Fig.3 ***d***) the very visible green algae (*Mougeotia*), in Zephyr Cove, were responsible for this anomaly. It is debatable if benthic algae should be considered part of the plankton, by their mere presence, or if they should be excluded from the data. We have chosen to include them but not to place too much importance on the overall total bio-volume numbers at that particular station. In the future, if benthic cells become more predominant in the plankton, a protocol should be developed to interpret the phytoplankton data appropriately.

Comparisons between stations within the same sampling date, for total bio-volume (bar height) and the palette of colors (community assemblage) show differences between stations. What is remarkable is that collectively the near shore sites generally do not differ very much from the two control stations at mid-lake. The near-shore sites can have higher nutrient content both from benthic re-suspension and their proximity to allochthonous water inflows. One would expect to see higher total bio-volumes at the near-shore in relation to the middle of the lake (Lewis 2002).

Blue-green algae, in February and May 2015 greatly influenced the total bio-volume at many stations. There was only one species of blue-green, *Aphanothece sp*., a very small (3µm) solitary cell which has the capacity to fix nitrogen from the atmosphere. *Aphanothece sp*. has been present in the past but its abundance this year was remarkable. These cells prefer high light, low

nitrogen, warmer temperatures and sources of inorganic carbon to enhance their ability of aerobic nitrogen fixation (Reddy et al., 1993). The algal cells can be present without fixing nitrogen, since they have the ability to photosynthesize, but their abundance is indicative of waters which lack nitrogen. In May 2015 the blue-greens were seen predominantly at the South Tahoe stations with the Mid-lake North station being the only station in the north also having them. The unusual high abundance of *Apanothece sp*. certainly has implications on the biology and clarity of the lake, but very little can be said about the implication for the near-shore stations in particular. One question that arises is if these blue-greens are fixing nitrogen, how does that affect the nitrogen budget both regionally and at each of the near shore stations? Typically picoplankton, a term which describe these tiny cells, are not enumerated but when their abundance becomes noticeable at any one station, an effort is made to quantify them at all the stations for any one sampling date. Bioassays could be used to determine if *Aphanothece sp*. are fixing nitrogen. The cells should grow in abundance, even without nutrient additions, if they are creating dissolved nitrogen.

The other odd occurrence seen in February was the dominance of a small centric diatom, *Cyclotella gordonensis*, which typically is seen only during summer stratified months of July and August. These cells are excellent competitors during low nutrient, high light and warmer temperature conditions (Winder and Hunter, 2008 and Winder et. al. 2009). Their habitat preferences suggest all the stations in February, at shallow depths were stable and nutrient deficient, which would be a consequence of little precipitation runoff and mixing. The presence of *Cyclotella sp*. was a lake-wide event, unusual for February, but not useful in discerning differences among near-shore stations.

The one consistent 'outlier' station, in terms of phytoplankton community, was Emerald Bay. This past year Emerald Bay had relatively low total bio-volume, but the community composition was often entirely different from the larger body of water in Tahoe. August 2014 in Emerald Bay was lacking the abundance of dinoflagellates that were dominant in the larger lake area. In February, 2015, Emerald Bay had fewer blue-greens and in May, 2015 Emerald Bay had its own phytoplankton bloom of Chrysophytes, not seen anywhere else in the lake.

Total algal abundance (Figure 4) shows dramatic differences between seasons. Abundance was generally lower in August and December, 2014 and diatoms were the dominate algal group at these times (Figs. 4 a and 4 b). February (Fig. 4 ***c***) and May (Fig. 4 ***d***) 2015 sampling results show the impact of blue-green algae on cell numbers. The differences between stations are largely obscured because of the widespread large numbers of blue-green algae. Indeed, if blue-green algae were removed from the graphs, all the stations would appear very similar, with the exception of Emerald Bay in May, 2015.

One interesting observation at the species level was seen in Timber Cove, both in February and May, 2015. The dominant diatom on both of those dates, at nearly all stations was *Cyclotella gordonensis*. It is a small centric diatom, about 5µm in diameter. The abundance of *Cyclotella sp*. between near shore stations was fairly consistent, 250,000-350,000 cells/l. At Timber Cove, however, the numbers of *Cyclotella sp*. (85,000 cells/l), were less than half the value of neighboring sites. At this near shore site the bottom topography is a shallow shelf extending

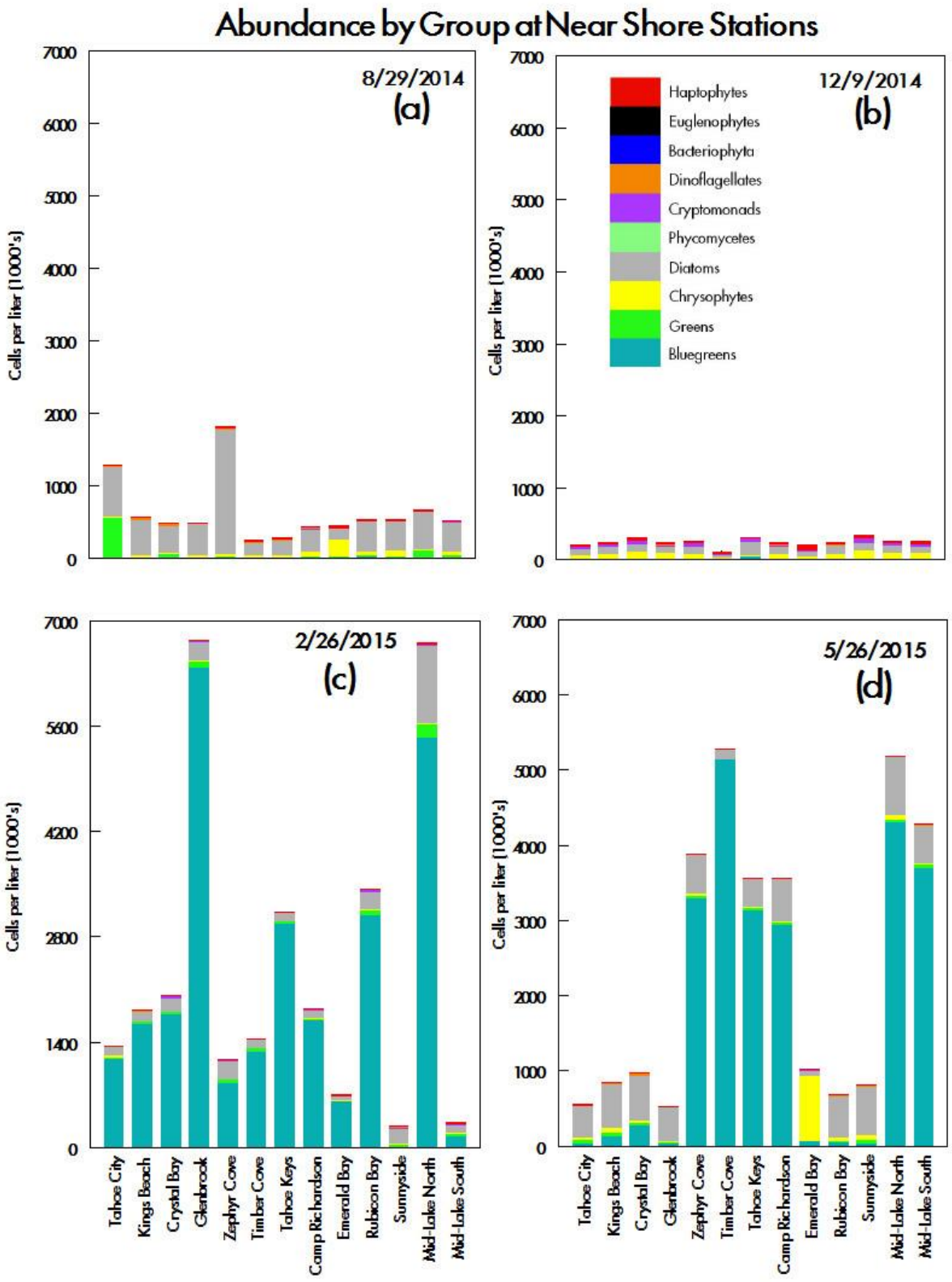

Figure 4. Abundance by phytoplankton group 8/29/14, 12/9/14, 2/26/15 and 5/26/15.

from the beach outward for quite a distance. There are a number of Asian clams (*Corbicula fluminea*) in the sandy bottom substrate. It is possible that Asian clams are having an impact on the shallow water column in this area, filtering out phytoplankton as a food source from the ambient water (Boltovskoy et. al. 1995). Asian clams have the ability to both filter feed on material in the water column and pedal feed on deposited material in the sediments. Filtration rates for Corbicula sp. are highest with particles 3-5 µm, exactly the same size class as the abundant Cyclotella cells in Lake Tahoe. When clams densely populate a near-shore area, they can potentially filter large volumes of water (Way et. al. 1990). However there could also be other reasons for the lower levels of *Cyclotella* at Timber Cove. One alternative explanation is that it is also possible greater nutrient enrichment at this site favored other algal species over *Cyclotella gordonensis*, which competes well in very low nutrient conditions - note that NH_4-N was slightly higher (8 µg/l at Timber Cove on 5/26/15 compared to 4-5 µg/l at all other sites). The fact that this site had higher AGP seems to support greater nutrient enrichment. However, the observation that initial chlorophyll *a* was the lowest of all sites at this site seems to counter the idea of greater enrichment. Other factors may also have contributed to the reduction. Additional study would be required to determine if the presence of Asian clams contributed to the reduction in *Cyclotella*.

Species richness and distribution equity were looked at this year (Figure 5) and provide some indication of the diversity of species among sites. The species richness (blue colored bar) is the actual number of species counted in each sample. The equity (green colored bar) is an indicator of how evenly distributed the algal population was among the various species. Shared dominance among the species (in terms of cells numbers) increases with the height of the green bar. The greatest equity in the phytoplankton communities was seen in December (Fig. 5 ***b***). The cell abundance was low but the cells which were present, shared dominance. In contrast, in February (Fig. 5 c) and May, 2015 (Fig. 5 d) the equity index was low and the community was dominated by only one or two species. As for individual stations, Tahoe City, on each sampling date had the greatest number of species identified. However, the shared distribution among species was generally low. This reflects the presence of benthic diatoms, discussed earlier, with only a few cells per species. The bulk of the phytoplankton at Tahoe City was usually confined to several dominant species.

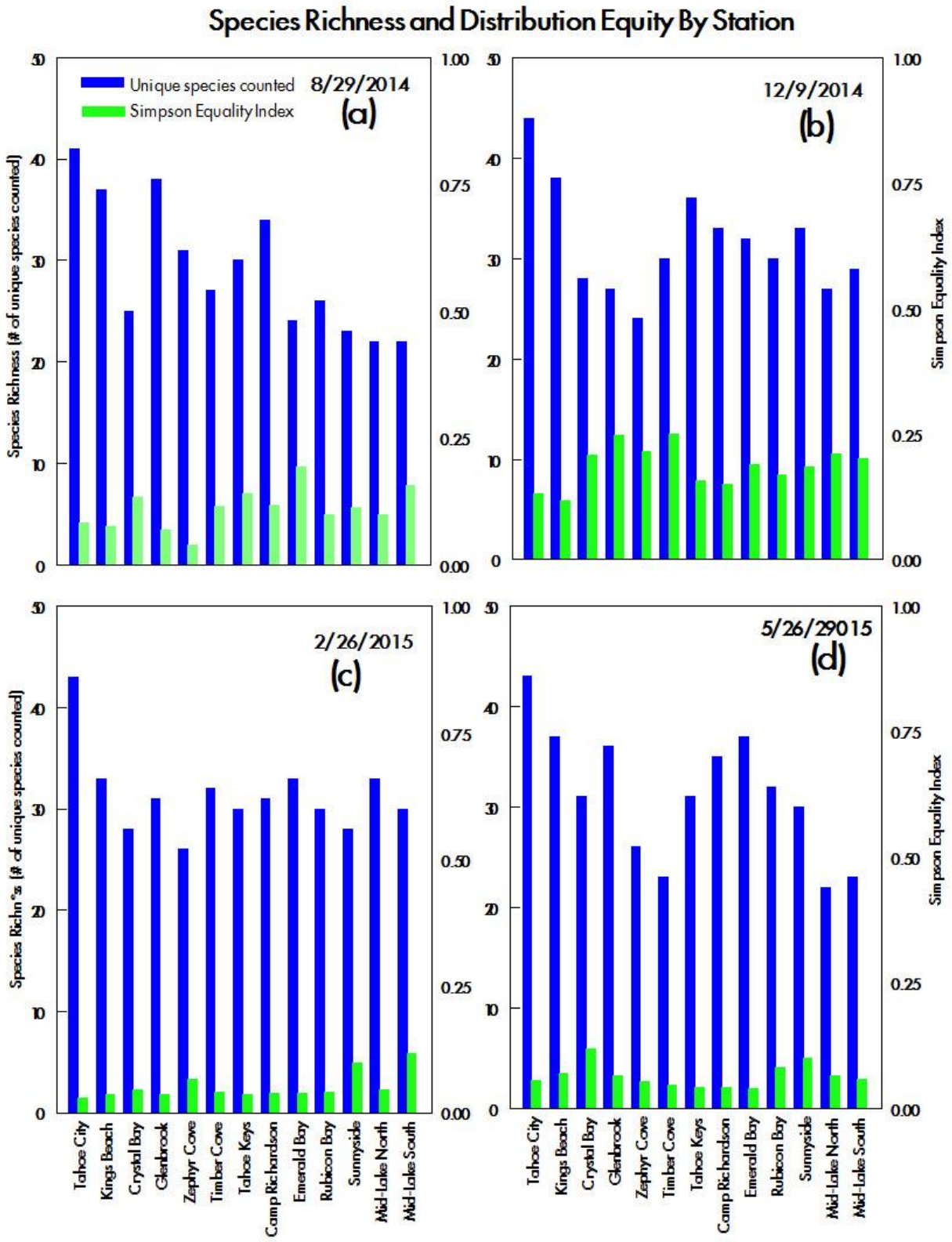

Figure 5. Species richness and distribution equity at four points in time in 2014 and 2015.

Section III. Periphyton Results

The purpose of the periphyton monitoring task is to assess the levels of nearshore attached algae (periphyton) growing around the lake. As with phytoplankton, nutrient availability plays a large role in promoting periphyton growth. The amount of periphyton biomass can reflect local nutrient loading and also be affected by long-term environmental changes. Periphyton biomass is considered an important indicator, which together with nearshore chlorophyll, phytoplankton and macrophyte metrics provide information on the trophic status of the Lake Tahoe nearshore. Trophic status in turn, along with nearshore clarity, community structure and conditions for human health are considered primary indicators of nearshore condition or health as outlined in the Lake Tahoe nearshore monitoring framework (Heyvaert et al., 2013).

Periphyton grows in the littoral (shore) zone of Lake Tahoe, which may be divided into the eulittoral zone and the sublittoral zone, each with distinct periphyton communities. The eulittoral zone is the shallow area between the low and high lake level and is substantially affected by wave activity. Substrata within this region desiccate as the lake level declines, and periphyton must recolonize this area when lake level rises. The sublittoral zone extends from the bottom of the eulittoral to the maximum depth of photoautotrophic growth. The sublittoral zone remains constantly submerged and represents the largest littoral benthic region of Lake Tahoe.

The algal community in the eulittoral zone is typically comprised of filamentous green algae (i.e., *Ulothrix sp., Zygnema sp.*) and stalked diatom species (i.e., *Gomphoneis herculeana*). The attached algae in the eulittoral zone display substantial growth resulting in rapid colonization of suitable areas. These algae are able to take advantage of localized soluble nutrients, and can establish a thick cover over the substrate within a matter of months. Similarly, this community rapidly dies back as nutrient concentrations diminish and shallow nearshore water temperatures warm with the onset of summer. The algae can slough from the substrate and disperse into the open water, or wash onto the shore. In areas where biomass is high, the slimy coating on the rocks, and sloughed material that accumulates along shore can be a nuisance. The eulittoral zone periphyton has a substantial influence on the aesthetic condition of the shorezone. It is the strong response of eulittoral periphyton to localized nutrient inputs that lends particular value to monitoring this community as an indicator of localized differences in nutrient loading.

The sublittoral zone is made up of different algal communities down through the euphotic zone. Cyanophycean (blue-green) algal communities make up a substantial portion of the uppermost sublittoral zone. These communities are slower growing and more stable than the filamentous and diatom species in the eulittoral zone.

Stations and Methods

Nine routine stations were monitored during Nov. 2014- June, 2015 (Rubicon Pt., Sugar Pine Pt., Pineland, Tahoe City, Dollar Pt., Zephyr Pt., Deadman Pt., Sand Pt and Incline West). These nine sites are located around the lake (Figure 1 presents a map of locations and Table 5 provides coordinates of locations) and represent a range of backshore disturbance levels from relatively undisturbed land (Rubicon Point and Deadman Point) to a developed urban center (Tahoe City).

Table 5. Locations of Routine Periphyton Monitoring Stations

SITE NAME	LOCATION
Rubicon	N38 59.52; W120 05.60
Sugar Pine Point	N39 02.88; W120 06.62
Pineland	N39 08.14; W120 09.10
Tahoe City	N39 10.24; W120 08.42
Dollar Point	N39 11.15; W120 05.52
Zephyr Point	N39 00.10; W119 57.66
Deadman Point	N39 06.38; W11957.68
Sand Point	N39 10.59; W119 55.70
Incline West	N39 14.83; W119 59.75

A detailed description of the sample collection and analysis procedures is given in Hackley et al. (2004). Briefly, the method entails collection while snorkeling of duplicate samples of attached algae from a known area of natural rock substrate at a depth of 0.5m, using a syringe and toothbrush sampler. These samples are transported to the laboratory where the samples are processed and split, with one portion of the sample analyzed for Ash Free Dry Weight (AFDW) and the other portion frozen for later analysis of chlorophyll *a* concentration (both AFDW and chlorophyll *a* are used as measures of algal biomass). We also measure average filament length, percent algal coverage, and estimate the visual score in field observations. The visual score is a subjective ranking (1-5) of the level of algal growth viewed underwater (as well as above water for a portion of the data), where 1 is least offensive appearing (usually natural rock surface with little or no growth) and 5 is the most offensive condition with very heavy growth.

Results

Monitoring at Routine Sites

In this report we summarize the data collected during the period November, 2014 to June, 2015. Nine routine sites were sampled. All sites were sampled five times during the period. Three of the five samplings were made between late February and May when spring periphyton biomass typically peaks, with additional sampling circuits made during fall 2014 and early summer 2015. Table 6 presents the results for biomass (chlorophyll *a* and Ash Free Dry Weight (AFDW)) and field observations (visual score, average filament length, percent algal coverage, biomass index and basic algal types) at the nine routine periphyton sites for the period November 2014 through June, 2015. The results for periphyton chlorophyll *a* biomass are also presented graphically in Figures 6 (a-i) together with earlier data collected since 2000. Figure 7 presents a graph of lake surface elevation and 0.5m sampling elevation Jan. 2000-Sept., 2015.

Water Year 2015 Patterns of Periphyton Biomass

WY 2015 was similar to WY 2014 in that generally low to moderate periphyton biomass was measured at the routine monitoring sites. Sites along the northwest shore (Pineland, Tahoe City and Dollar Pt.) ranged from low to moderate chlorophyll *a* biomass during WY2015 (i.e. Pineland chlorophyll *a* ranged from 15.88 to 47.49 mg/m^2, Tahoe City ranged from 5.63 to 35.64 mg/m^2 and Dollar Pt. ranged from 11 to 39.55 mg/m^2). Along the southwest shore, Rubicon Pt.

ranged from 18.90 to 47.82 mg/m^2. At Sand Pt. along the east shore, chlorophyll *a* gradually increased through the WY from 17.86 to 34.57 mg/m^2. At the remaining routine sites, chlorophyll remained relatively low (i.e. Sugar Pine Pt. ranged from 9.12 to 16.82 mg/m^2, Zephyr Pt. ranged from 10.58 to 18.55 mg/m^2 and Deadman Pt. ranged from 16.02 to 28.15 mg/m^2).

The relatively low biomass levels at sites in the northwest portion of the lake (Pineland, Tahoe City and Dollar Pt.) are interesting to note. These sites showed smaller spring biomass peaks than seen in previous years. It was likely this was a response to the continued below average precipitation and low inputs of nutrients to the lake's surface waters. WY 2015 was the fourth WY in a row of lower than average precipitation. Precipitation at Tahoe City in WY 2015 was only 52% of normal (www.cnrfc.noaa.gov/monthly_precip.php). There were infrequent storms with only one strong event which occurred Feb. 6-9, 2015 as significant rain with some snow. A very small snowpack accumulated for the WY which resulted in a very light spring snowmelt. Contribution of nutrients from stream and urban runoff was likely reduced compared to "wetter" years. Other factors may also have contributed. There was little early season precipitation to provide nutrients for initial periphyton growth, and storms were relatively infrequent potentially reducing the level of lake mixing and upwelled nutrients contributed to surface waters. The ongoing below average precipitation may have also resulted in reduced subsurface and groundwater inputs to the lake.

The lake level was extremely low during WY 2015, which had an impact on the predominant algae observed during this period. Lake surface elevation was below the natural rim (6223.00 ft.) for the majority of WY 2015 and the 0.5m sampling depth was 1.64 ft. (or 0.5m) below this. Sampling at 0.5m resulted in the collection of algae from a Cyanophycean (blue-green algae) zone of periphyton growth at most sites. The blue-green algae are a stable, slow-growing community typically found in the sublittoral zone, 1-2m deep, under more "normal" lake levels, but were located near the surface under the extremely low lake levels in 2015. Filamentous green algae were observed growing in association with the blue-green algae at many sites, particularly along the north, southwest and east shores. Stalked diatoms were observed primarily along the west and northwest sites this year (Sugar Pine Pt., Pineland, Tahoe City and Dollar Pt.). Tahoe City was the only site that appeared to have relatively little blue-green algae at 0.5m.

Two sites along the northeast shore (Sand Pt. and Deadman Pt.) showed slightly increased biomass relative to levels in 2012 and 2013. This was likely a consequence of sampling within the zone of thicker blue-green algal growth in 2015. With a much lower lake level in 2015, sampling was done in the area where the substrate had been submerged for many years (since previous record low lake levels in the early 1990's) and blue-greens were well established.

Table 6. Summary of eulittoral periphyton chlorophyll *a* (Chlor.*a*), Ash Free Dry Weight (AFDW), visual score from above and below water, average filament length, percent algal cover, and predominant algal types estimated visually underwater (where SD= stalked diatoms; FG= filamentous greens; CY= blue-green algae) for routine periphyton monitoring sites during November, 2014-June, 2015. Note for chlorophyll *a* and AFDW, n=2 unless otherwise indicated (i.e. two replicate samples were taken and analyzed). Visual score is a subjective ranking of the aesthetic appearance of algal growth ("above" viewed above water; "below" viewed underwater) where 1 is the least offensive and 5 is the most offensive. Biomass Index is Filament Length times percent Algal Cover. Also,"NA" = not available or not collected; "NES" = not enough sample for analysis; "Var." = variable amount of cover. Sampling depth and corresponding sampling elevation are also indicated. *Note: Dollar Pt. 11/11/14 samples contained significant sand, chlorophyll a highly variable likely due to sand content, there may have been algae mixed in with sand, the chlorophyll at Dollar on this date was considered anomalous and not used, (see Section IV QA/QC, for more explanation).

Site	Date	Sampling Depth/Elev. (m/ ft.)	Chlor. *a* (mg/m^2)	Std Dev (mg/m^2)	AFDW (g/m^2)	Std Dev (g/m^2)	Above Visual Score	Below Visual Score	Avg. Fil. Length (cm)	Algal Cover (%)	Biomass Index	Algal Type
Rubicon Pt.	11/11/14	0.5/6221.05	18.90	4.40	26.23	3.86	NA	NA	1.0	70	0.70	CY,FG
	2/20/15	0.5/6221.20	30.17	4.34	30.93	4.52	3	3	0.7	95	0.67	CY
	3/19/15	0.5/6221.19	26.71	0.95	26.88	3.31	2	3	0.5	100	0.50	CY,FG
	4/9/15	0.5/6221.14	26.76	3.36	30.71	4.37	3	3	0.6	90	0.54	SD,CY,FG
	6/16/15	0.5/6221.35	47.82	2.05	50.68	5.45	3.5	3.5	1.2	95	1.14	CY,FG
Sugar Pine Pt.	11/11/14	0.5/6221.05	15.84	3.44	17.69	6.68	2	2	0.3	90	0.27	CY,FG
	2/20/15	0.5/6221.20	16.82	0.10	19.98	2.14	1	2	0.2	90	0.18	SD,CY
	3/19/15	0.5/6221.19	14.85	0.94	17.02	2.28	2	2	0.2	60	0.12	SD,CY
	4/9/15	0.5/6221.14	12.37	6.36	15.61	9.88	NA	2	0.2	40	0.08	SD
	6/16/15	0.5/6221.35	9.12	3.88	14.27	5.74	3	2	0.1	70	0.07	CY
Pineland	11/11/14	0.5/6221.05	25.15	6.36	33.72	11.05	2	2	0.5	70	0.35	SD,CY
	2/20/15	0.5/6221.20	47.49	15.11	45.81	6.10	2	3	1.0	48	0.48	SD,CY
	3/19/15	0.5/6221.19	18.19	10.10	12.67	5.01	2.5	3.5	1.5	60	0.90	SD,CY
	4/9/15	0.5/6221.14	32.07	14.96	31.75	9.90	2.5	3	1.4	45	0.63	SD,CY
	6/16/15	0.5/6221.35	15.88	2.83	11.81	(n=1)	2	3	0.3	48	0.14	CY,FG
Tahoe City	11/11/14	0.5/6221.05	5.63	1.05	8.17	0.00	NA	NA	NA	NA	NA	NA
	2/20/15	0.5/6221.20	32.19	0.91	29.98	(n=1)	2	2	0.2	80	0.16	SD
	3/19/15	0.5/6221.19	35.64	1.27	53.38	7.23	2	3	1.4	25	0.35	SD
	4/9/15	0.5/6221.14	16.98	1.54	22.00	1.01	NA	3	0.5	35	0.18	SD
	6/16/15	0.5/6221.35	12.09	(n=1)	15.54	2.03	2.5	2.5	0.3	60	0.18	SD

Site	Date	Sampling Depth/Elev. (m/ ft.)	Chlor. *a* (mg/m^2)	Std Dev (mg/m^2)	AFDW (g/m^2)	Std Dev (g/m^2)	Above Visual Score	Below Visual Score	Avg. Fil. Length (cm)	Algal Cover (%)	Biomass Index	Algal Type
Dollar Pt.	11/11/14	0.5/6221.05	-	-	25.14*	(n=1)	2	2	0.3/0.1	30/80	0.14	CY,FG
	2/20/15	0.5/6221.20	11.00	(n=1)	10.62	2.14	2	2	0.1	90	0.09	SD,CY,FG
	3/19/15	0.5/6221.19	12.11	1.66	9.37	0.75	3	3	0.8	70	0.56	SD
	4/9/15	0.5/6221.14	39.55	13.50	18.49	5.83	3	2	0.1	70	0.07	SD,CY
	6/16/15	0.5/6221.35	12.37	0.60	14.03	0.96	3	3	0.6	47	0.28	CY,FG
Incline West	11/11/14	0.5/6221.05	30.17	2.40	42.19	2.00	NA	3	0.2	90	0.18	CY,FG
	2/20/15	0.5/6221.20	22.92	2.87	35.20	1.55	2	3	0.3	95	0.29	CY
	3/19/15	0.5/6221.19	40.12	0.81	58.46	1.42	3	3	0.5	80	0.40	CY,FG
	4/23/15	0.5/6221.09	18.98	5.90	36.99	15.20	3	3	1.0	90	0.90	CY,FG
	6/16/15	0.5/6221.35	37.77	3.32	62.41	3.05	3	3	1.1	90	0.99	CY,FG
Sand Pt.	11/11/14	0.5/6221.05	17.86	0.58	22.75	0.31	3	3	0.2	80	0.16	CY,FG
	2/20/15	0.5/6221.20	19.95	0.97	31.46	3.22	2	3	0.3	100	0.30	CY,FG
	3/19/15	0.5/6221.19	25.19	0.59	33.06	3.88	3	3	0.6	80	0.48	CY
	5/19/15	0.5/6221.24	25.56	3.26	35.65	0.22	3	3	0.8	80	0.64	CY,FG
	6/16/15	0.5/6221.35	34.57	9.40	49.48	11.41	2	3	1.2/0.5	20/90	0.59	CY,FG
Deadman Pt.	11/11/14	0.5/6221.05	22.19	0.71	31.81	7.85	3	3	0.3	90	0.27	CY
	2/20/15	0.5/6221.20	16.02	3.85	26.72	2.13	2	2	0.2	80	0.16	CY
	3/19/15	0.5/6221.19	28.15	4.57	43.82	8.50	3	3	0.4	88	0.35	CY
	5/19/15	0.5/6221.24	24.78	2.96	39.81	3.08	3	3.5	0.5	80	0.40	CY,FG
	6/16/15	0.5/6221.35	25.72	4.04	46.10	3.44	3	3	0.6	90	0.60	CY/FG
Zephyr Pt.	11/11/14	0.5/6221.05	13.91	2.15 (n=3)	13.25	4.37 (n=3)	3	2	0.1	70	0.07	CY,FG
	2/20/15	0.5/6221.20	12.53	1.22	12.92	1.74	2	2	0.3	80	0.24	SD
	3/19/15	0.5/6221.19	12.01	3.94	13.36	6.52	2.5	2.5	0.3	60	0.18	CY,FG
	5/19/15	0.5/6221.24	10.58	1.12	14.81	0.18	3	3	0.8/0.1	50/80%	0.43	CY,FG
	6/16/15	0.5/6221.35	18.55	(n=1)	23.40	(n=1)	3	3	0.9/0.1	50/60	0.46	CY,FG

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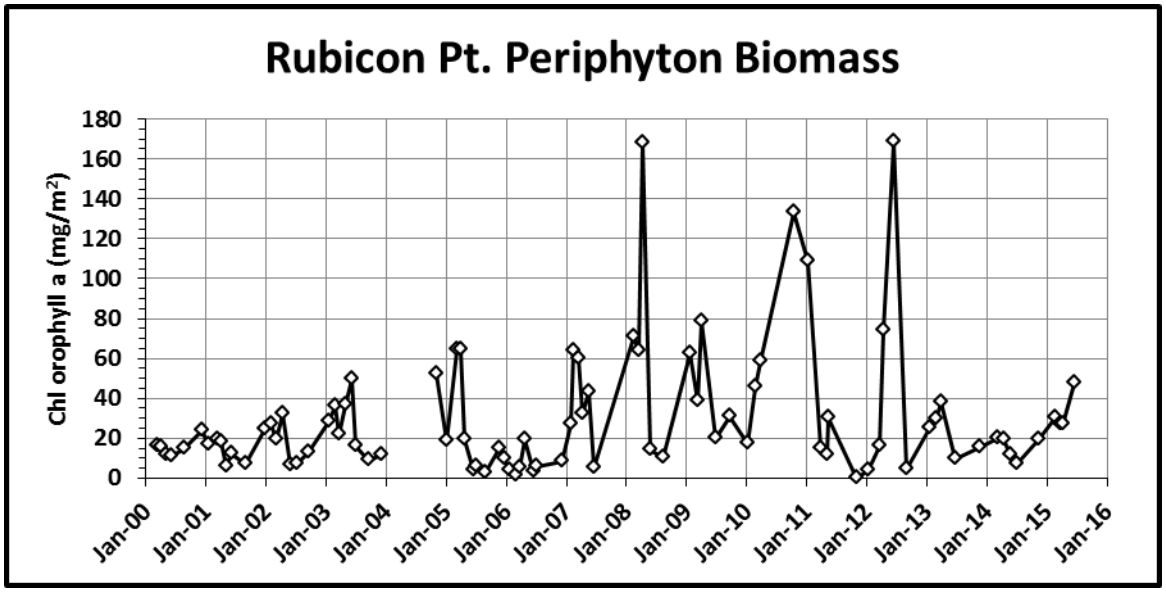

Figure 6 a. Rubicon Pt. periphyton biomass (chlorophyll *a*) 2000-2015.

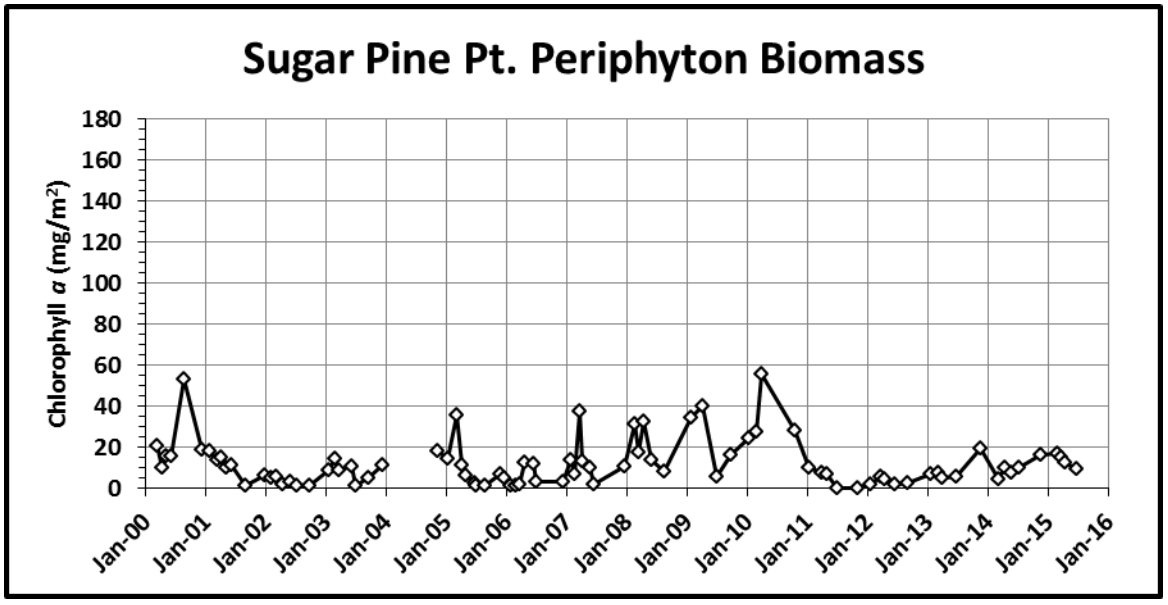

Figure 6 b. Sugar Pine Pt. periphyton biomass (chlorophyll *a*) 2000-2015.

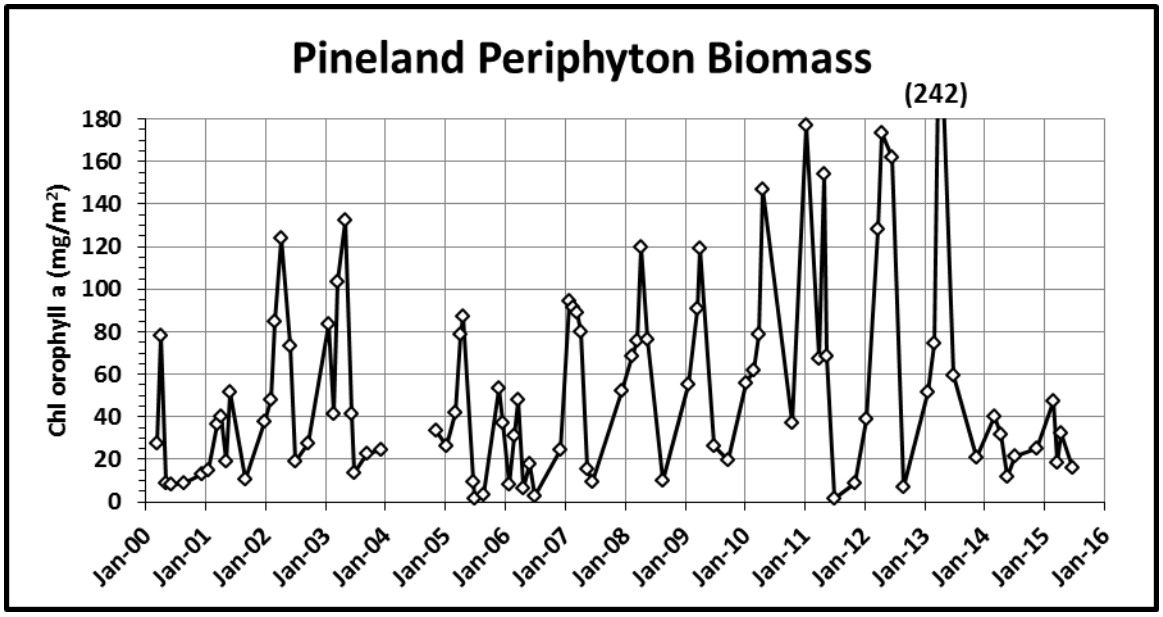

Figure 6 c. Pineland periphyton biomass (chlorophyll *a*) 2000-2015.

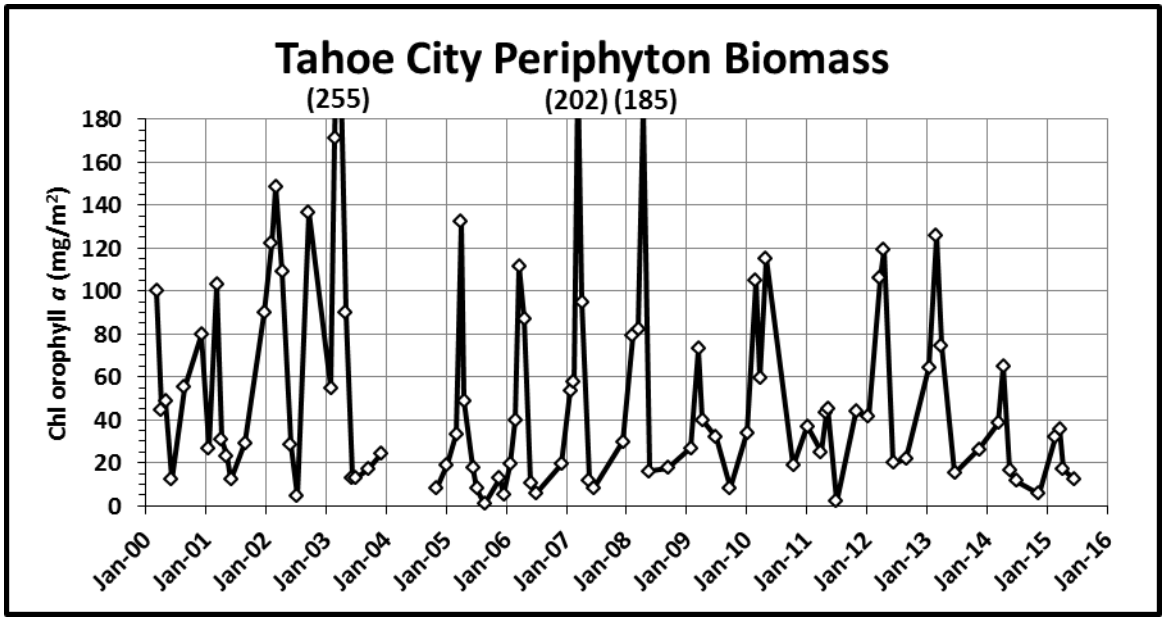

Figure 6 d. Tahoe City periphyton biomass (chlorophyll *a*) 2000-2015.

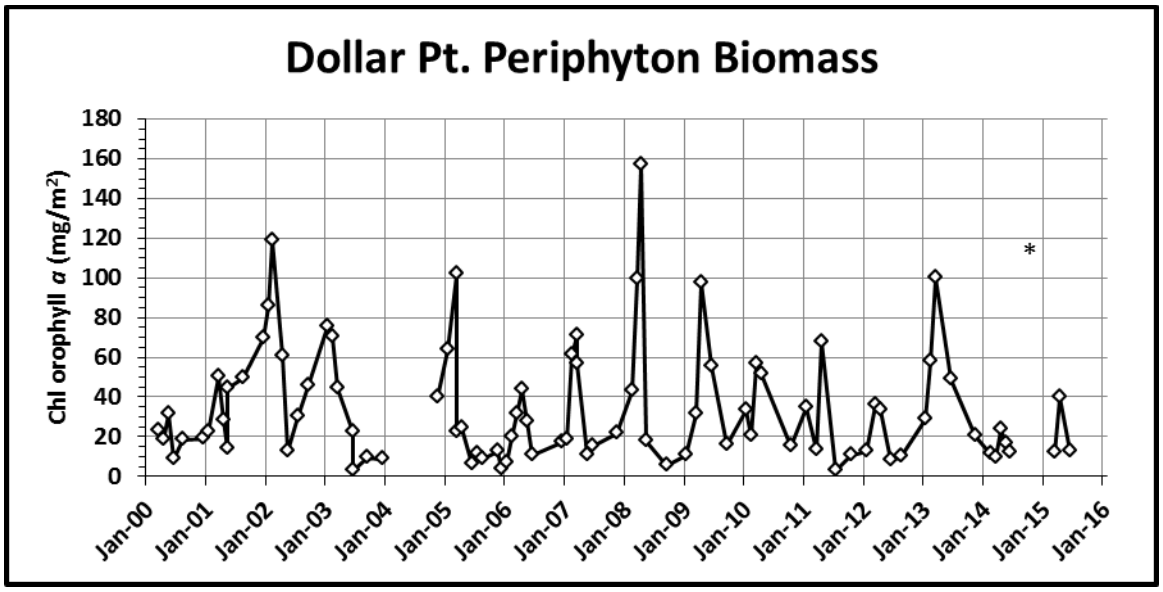

Figure 6 e. Dollar Pt. periphyton biomass (chlorophyll *a*) 2000-2015. *Note- 11/11/14 Dollar Pt. samples had much sand associated with them and the chlorophyll was high with high variation. In contrast, another biomass indicator AFDW was low and more consistent with levels before and after 11/11/14 date. The chlorophyll data for 11/11/14 was considered anomalous and not included in the long-term data (see Quality Assurance section for additional explanation).

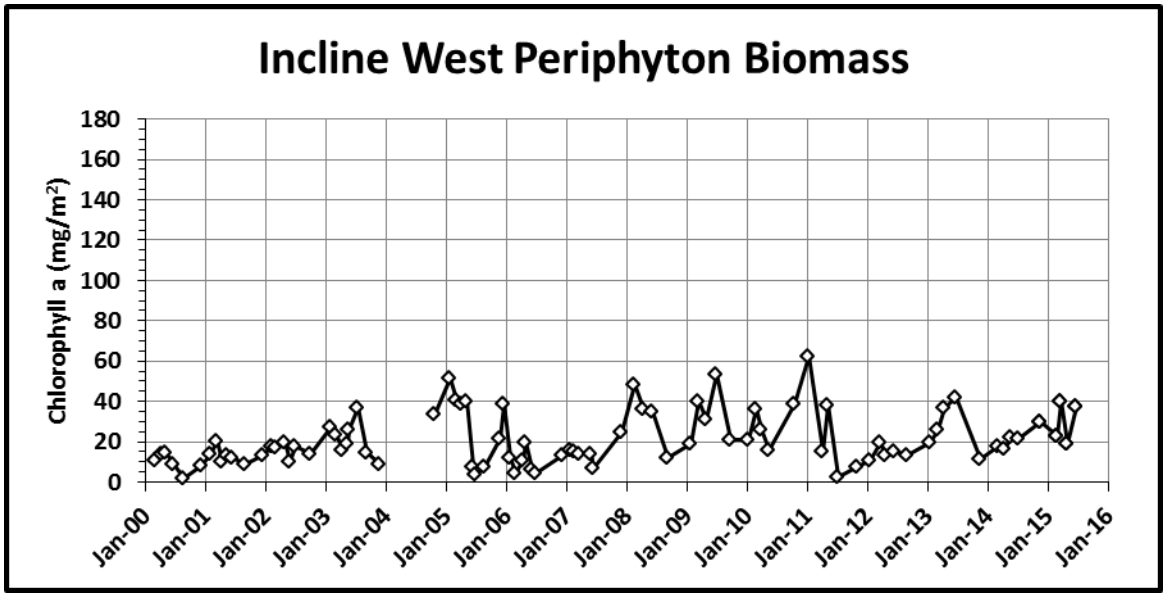

Figure 6 f. Incline West periphyton biomass (chlorophyll *a*) 2000-2015.

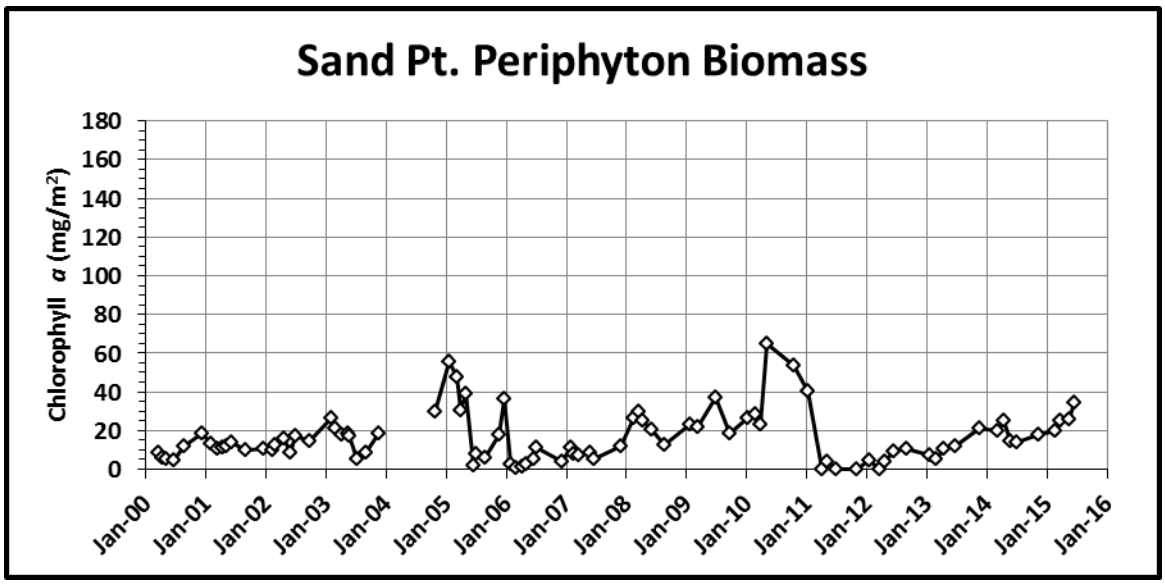

Figure 6 g. Sand Pt. periphyton biomass (chlorophyll *a*) 2000-2015.

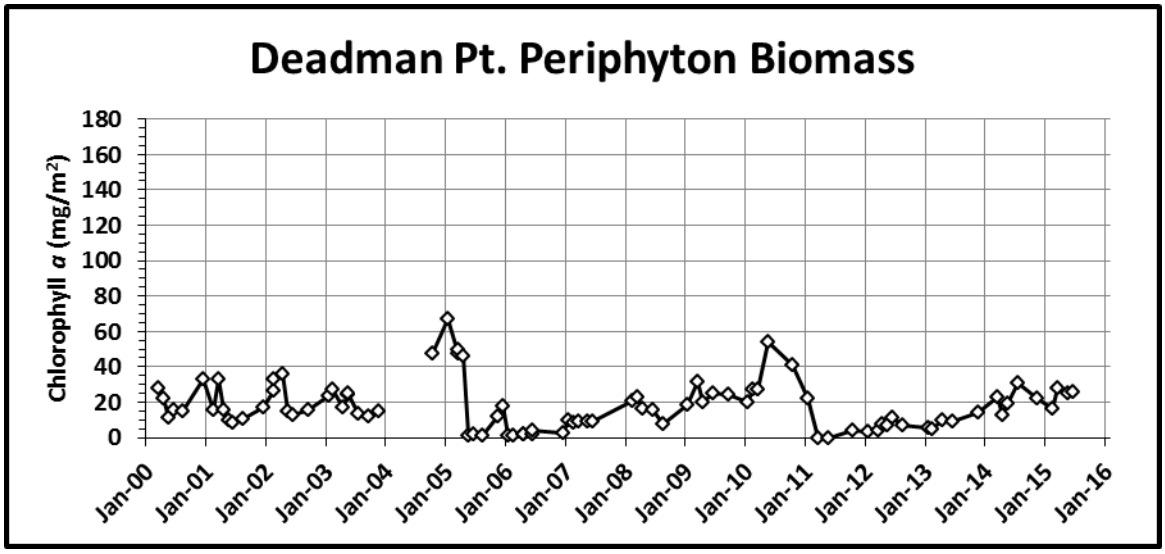

Figure 6 h. Deadman Pt. periphyton biomass (chlorophyll *a*) 2000-2015.

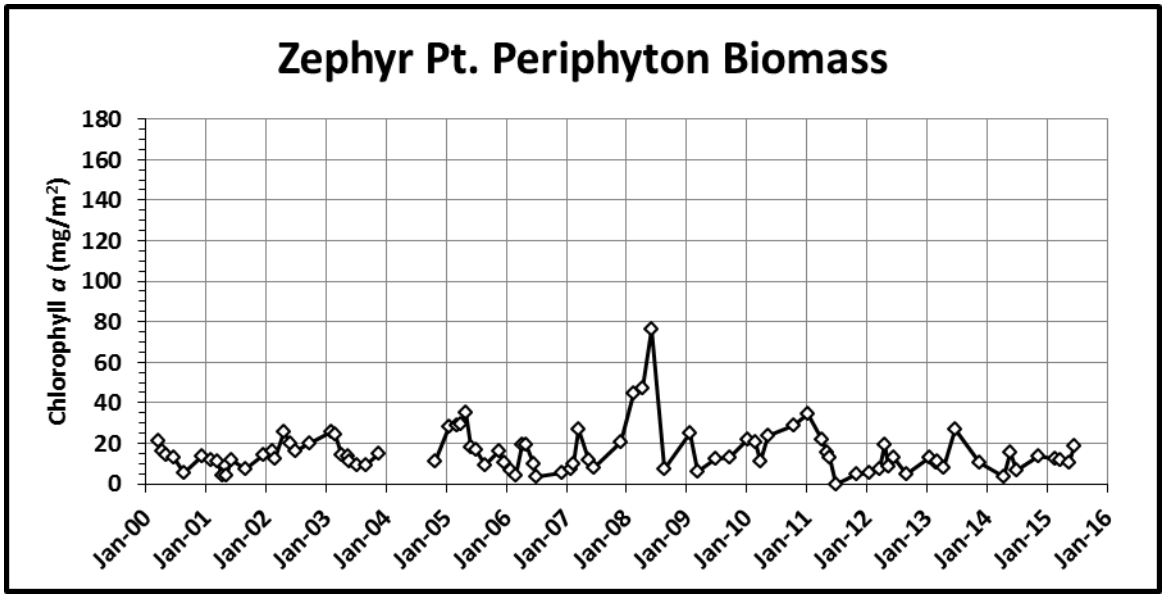

Figure 6 i. Zephyr Pt. periphyton biomass (chlorophyll *a*) 2000-2015.

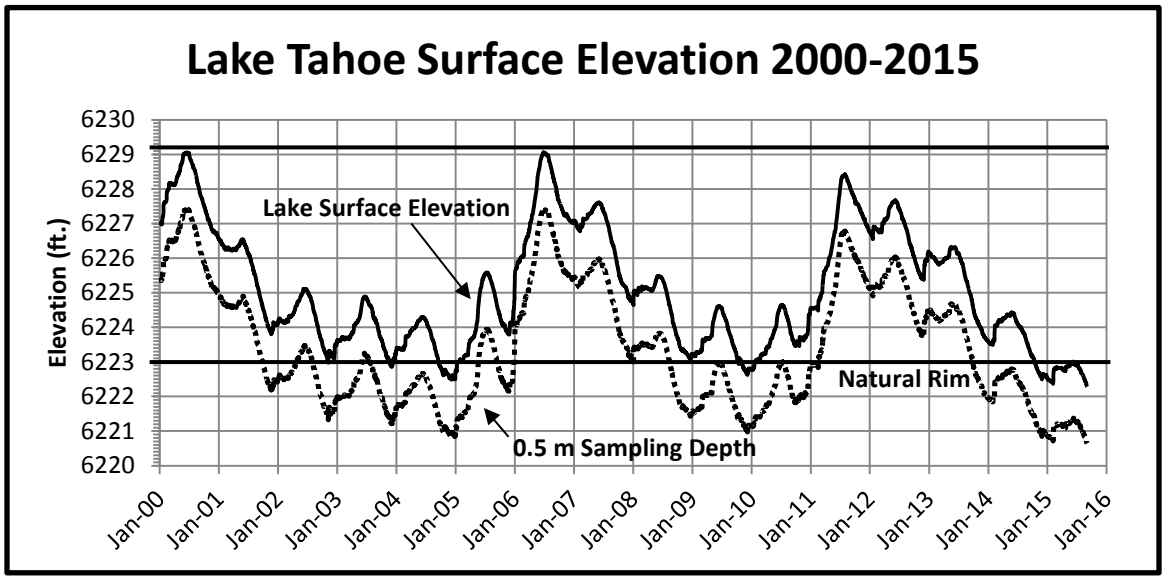

Figure 7. Fluctuation in Lake Tahoe surface elevation 1/1/00-9/1/15. Periphyton samples were typically collected during the period from natural rock substrata at a depth of 0.5m below the water surface. The 0.5m sampling depth (shown as a dotted line) fluctuates with the lake surface elevation. The elevation of the natural rim of Lake Tahoe is 6223 ft. The top 6.1 ft. of the lake above the natural rim (to 6229.1 ft.) is operated as a reservoir. Lake level data is from USGS web site (http://nwis.waterdata.usgs.gov) data and includes provisional data in 2015).

Annual Maximum Biomass

Figure 8 presents the maximum periphyton chlorophyll *a* biomass for water years 2012-2015. Several interesting observations emerge:

- Annual maximum chlorophyll *a* levels in 2015 were relatively low among all sites ranging from 16.82 mg/m^2 at Sugar Pine Pt. to 47.49 mg/m^2 at Pineland. It is noteworthy that sites along the northwest shore (Pineland, Tahoe City and Dollar Pt.) did not show substantially greater peak annual biomass than other routine sites as they have in many previous years. It is likely lower overall nutrient inputs contributed to lower biomass in 2014 and 2015.

- It is apparent that a "drier than normal year" doesn't necessarily always equate to a low periphyton year. WY 2012 and 2013 were years of lower precipitation, yet annual maximum biomass was quite high at Pineland and Tahoe City in 2012 and 2013 and at Rubicon Pt. and 2012 and Dollar Pt. in 2013. The timing of when precipitation occurs during a year, carryover conditions from the previous year (i.e. the degree of soil saturation and ground water levels), lake level and other factors may also play a role in determining the biomass level in any year. WY 2012 followed an extremely wet year in 2011. WY 2013 started out very "wet" with much precipitation occurring in Nov. and Dec. however very dry conditions prevailed the rest of that WY. It is possible these conditions contributed to heavier periphyton growth in 2012 and 2013 despite those years being drier than usual.

- Biomass increased slightly in 2014 and 2015 at several sites (i.e. Sand Pt., Deadman Pt. and Sugar Pine Pt.). This was likely a consequence of sampling in zones of greater blue-green algae at these sites, due to lower lake levels in 2014 and 2015.

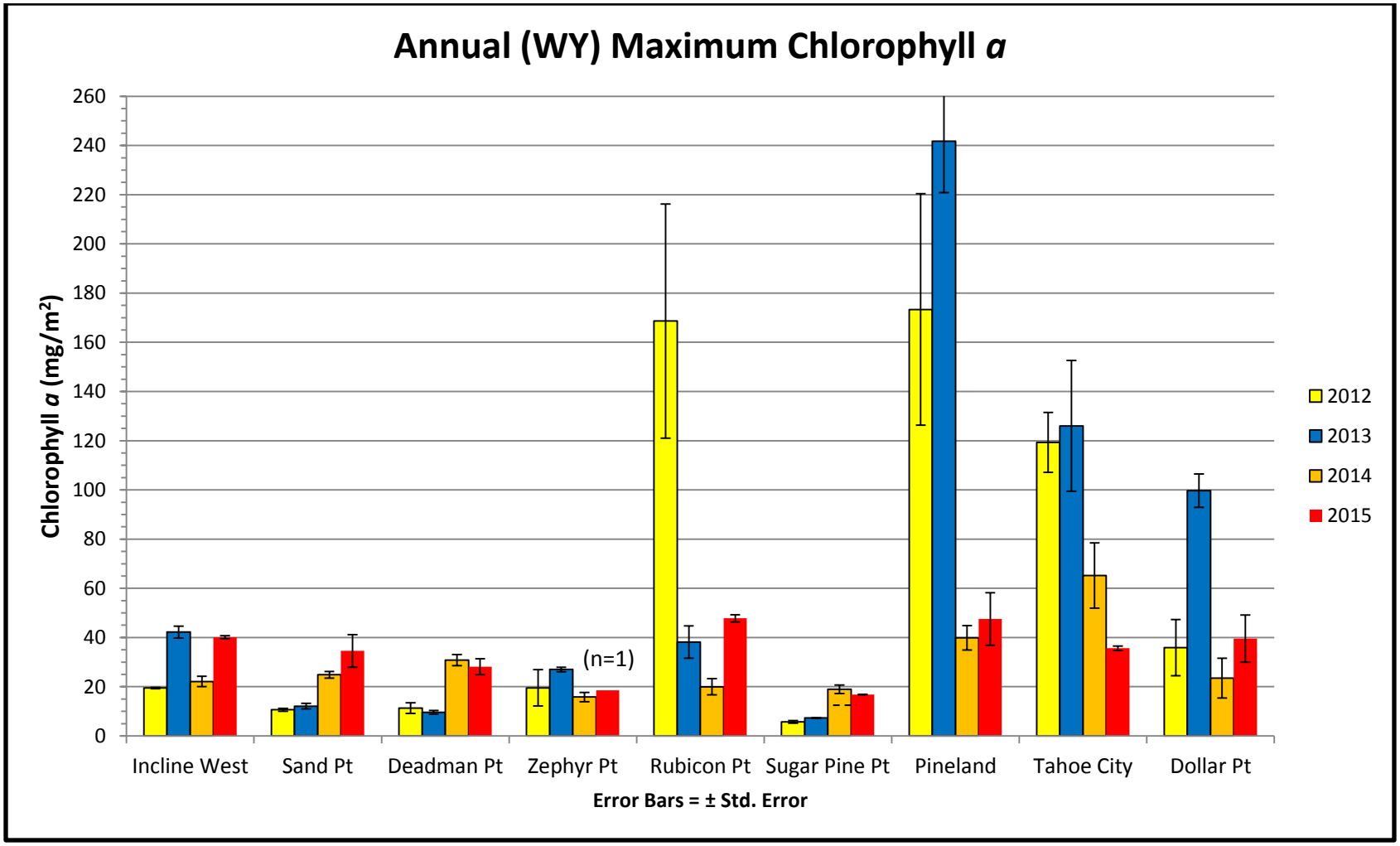

Figure 8. Maximum periphyton chlorophyll *a* for Water Years 2012-2015 at the nine routine periphyton monitoring sites at 0.5m Note: n=1 for Zephyr Pt. maximum value in 2015. Note, the 2015 WY peak at Sugar Pine Pt.occurred in Nov. 2014, the 2015 spring peak biomass for this site (which was lower) is indicated by dashed line.

Spring Synoptic Monitoring 2015

An additional 40-45 sites are monitored once each spring to provide information on the distribution of biomass between the nine routine sites around the lake. Monitoring of these additional sites is timed as much as possible to occur with the peak spring biomass. This "spring synoptic" sampling provides essentially a "snapshot picture" of the distribution of periphyton biomass around the lake. Since peak periphyton growth does not necessarily occur at the same time at all sites around the lake, this synoptic monitoring may catch some sites prior to or following their peak biomass.

2015 Chlorophyll *a* to Periphyton Biomass Index Relationship

At all spring synoptic sites, a "Periphyton Biomass Index (PBI)" value was determined for each date to approximate the level of biomass present. The PBI was developed to provide a means to rapidly estimate the level of periphyton biomass without collection of samples at every site. Measurements of average algal filament length and percent coverage of algae over rocks at 0.5m were made while snorkeling. The Biomass Index was calculated by multiplying the average filament length (cm) of the periphyton by the estimate of percent coverage of algae over the rock. At a portion of the sites, biomass samples were also collected for measurement of chlorophyll *a* and AFDW, to check the relationship between measured biomass and periphyton biomass index. Higher PBI should be indicative of more material over the rock surface. TERC has been making measurements of PBI during spring synoptics since 2003. Table 7 presents the names and locations of these synoptic sites included in 2015.

Table 7. Periphyton Spring Synoptic monitoring locations.

SITE DESIGNATION	WEST SHORE	LOCATION
A	Cascade Creek	N38 57.130; W120 04.615
B	S. of Eagle Point	N38 57.607; W120 04.660
C	E.Bay/Rubicon	N38 58.821; W120 05.606
D	Gold Coast	N39 00.789; W120 06.796
E	S. Meeks Point	N39 01.980; W120 06.882
F	N. Meeks Bay	N39 02.475; W120 07.194
G	Tahoma	N39 04.199; W120 07.771
H	S. Fleur Du Lac	N39 05.957; W120 09.774
I	Blackwood Creek	N39 06.411; W120 09.424
	Kaspian Pt.	(Point near Elizabeth Dr.)
J	Ward Creek	N39 07.719; W120 09.304
K	N. Sunnyside	N39 08.385; W120 09.135
L	Tavern Point	N39 08.806; W120 08.628
TCT	Tahoe City Tributary	(adjacent to T.C. Marina)
M	TCPUD Boat Ramp	N39 10.819; W120 07.177
N	S. Dollar Point	N39 11.016; W120 05.888
O	S. Dollar Creek	N39 11.794; W120 05.699
P	Cedar Flat	N39 12.567; W120 05.285
Q	Garwood's	N39 13.486; W120 04.974
R	Flick Point	N39 13.650; W120 04.155
S	Stag Avenue	N39 14.212; W120 03.710
T	Agatam Boat Launch	N39 14.250; W120 02.932
	EAST SHORE	
E1	South side of Elk Point	N38 58.965; W119 57.399
E2	North Side of Elk Point	N38 59.284; W119 57.341
E3	South Side of Zephyr Point	N38 59.956; W119 57.566
E4	North Zephyr Cove	N39 00.920; W119 57.193
E5	Logan Shoals	N39 01.525; W119 56.997
E6	Cave Rock Ramp	N39 02.696; W119 56.935
E7	South Glenbrook Bay	N39 04.896;W119 56.955
E8	South Deadman Point	N39 05.998; W119 57.087
E9	Skunk Harbor	N39 07.856; W119 56.597
E10	Chimney Beach	N39 09.044; W119 56.008
E11	Observation Point	N39 12.580; W119 55.861
	NORTH SHORE	
E12	Hidden Beach	N39 13.263; W119 55.832
E13	Burnt Cedar Beach	N39 14.680; W119 58.132
	Incline Condo	N39 14.90; W119 59.63
	Old Incline West	(100 yds No. Incline West)
E14	Stillwater Cove	N39 13.789; W120 00.020
E15	North Stateline Point	N39 13.237; W120 00.193
E16	Brockway Springs	N39 13.560; W120 00.829
E17	Kings Beach Ramp Area	N39 14.009; W120 01.401
	SOUTH SHORE	
S1	Tahoe Keys Entrance	N38 56.398; W120 00.390
S2	Kiva Point	N38 56.555; W120 03.203
	Timber Cove Rocks	Rocks west T. Cove Pier

Figure 9 shows the association between PBI and chlorophyll *a* for biomass samples collected during the spring synoptic 2015. Chlorophyll *a* samples were collected at 21 of 54 sites. The linear relation between chlorophyll *a* and PBI had an R^2 value of 0.31. This year there appeared to be quite a bit of variation in the lower range of chlorophyll a (i.e. between 0-30mg/m^2). The association between chlorophyll and PBI was not as strong as last year when $R^2 = 0.57$. Figure 10 below presents the association between chlorophyll *a* and PBI for spring synoptic data 2012-2015, which have a much broader range of PBI and chlorophyll levels. R^2 for the association between PBI and chlorophyll

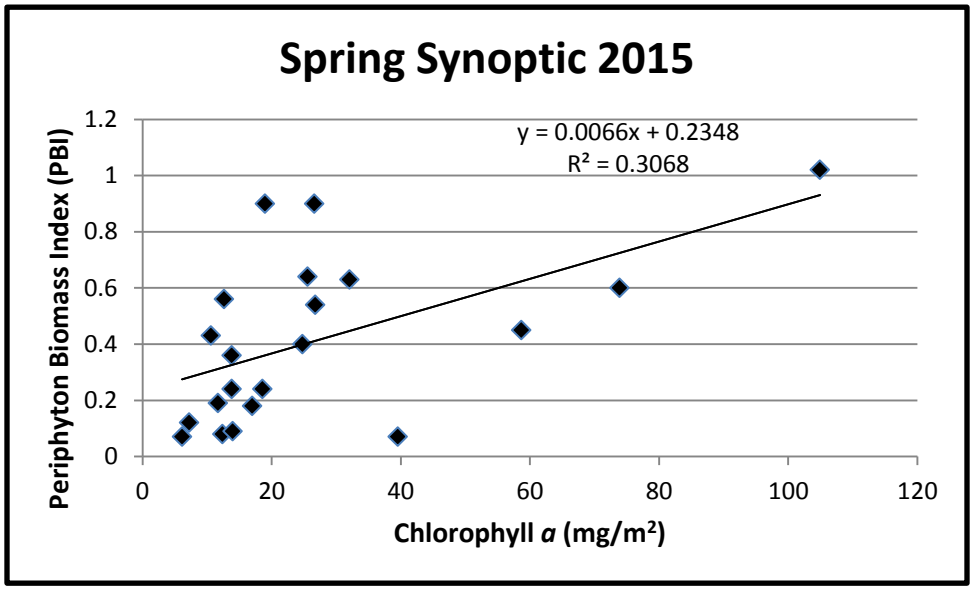

Figure 9. Relation between periphyton chlorophyll *a* and Periphyton Biomass Index for sites where both were measured during the 2015 spring synoptic survey.

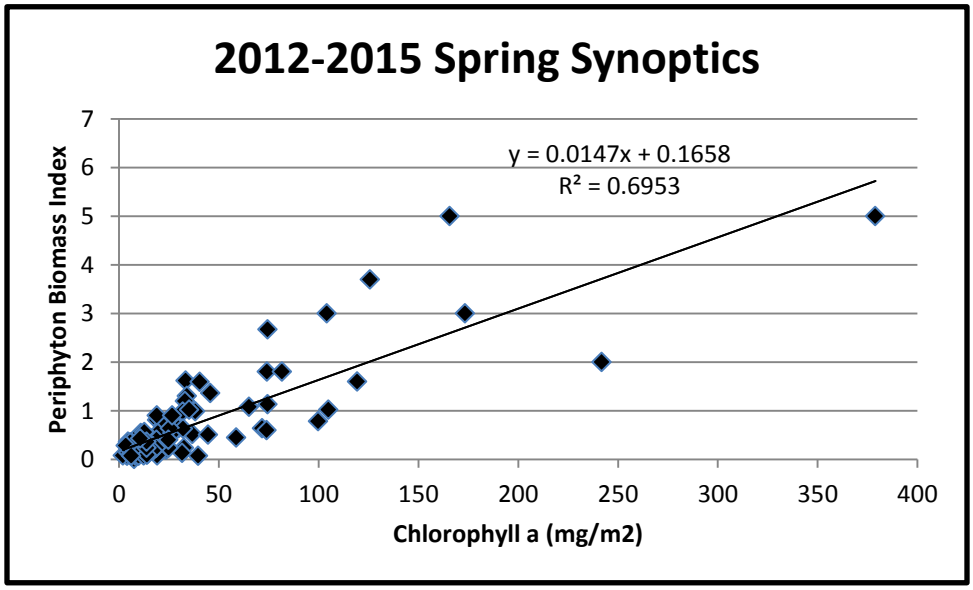

Figure 10. Relation between periphyton chlorophyll *a* and Periphyton Biomass Index for sites where both were measured during the 2012 to 2015 spring synoptic surveys.

was good for this 3-year period (R^2 =0.70) for the linear equation describing the association between chlorophyll (X) and PBI (Y): Y= 0.0147X + 0.1658. This was similar to the relationship for spring synoptic data observed in an earlier period (2008-2011) where (y=0.0152X + 0.2551; R^2 =0.71) presented in the Lake Tahoe Nearshore Monitoring and Evaluation Report (Heyvaert et al., 2013).

The variation in the PBI to Chlorophyll relationship this year may have related to variation in the levels of live algae in the periphyton. The PBI measurement utilizes filament length (or thickness of the algae) and % coverage to estimate the level of biomass at a site. The periphyton coating a rock may contain variable amounts of live and dead algae, as well as non-living organic mat and stalk material. Chlorophyll *a* is primarily associated with living algal material. Areas of periphyton with similar thickness and % coverage, yet different amounts of live algae could have different levels of chlorophyll *a* for the same measured PBI. For the 2015 data, the association between PBI and AFDW (which provides an estimate of the total organic matter) was in fact slightly better (R^2 = 0.43) than that between PBI and chlorophyll (R^2 =0.31) (Figure 11). The weak association between the 2015 PBI and chlorophyll data is a reminder that PBI is not a perfect surrogate for chlorophyll *a*. PBI probably cannot discern between very small differences in chlorophyll biomass. We would recommend chlorophyll *a* be used in preference to PBI when available.

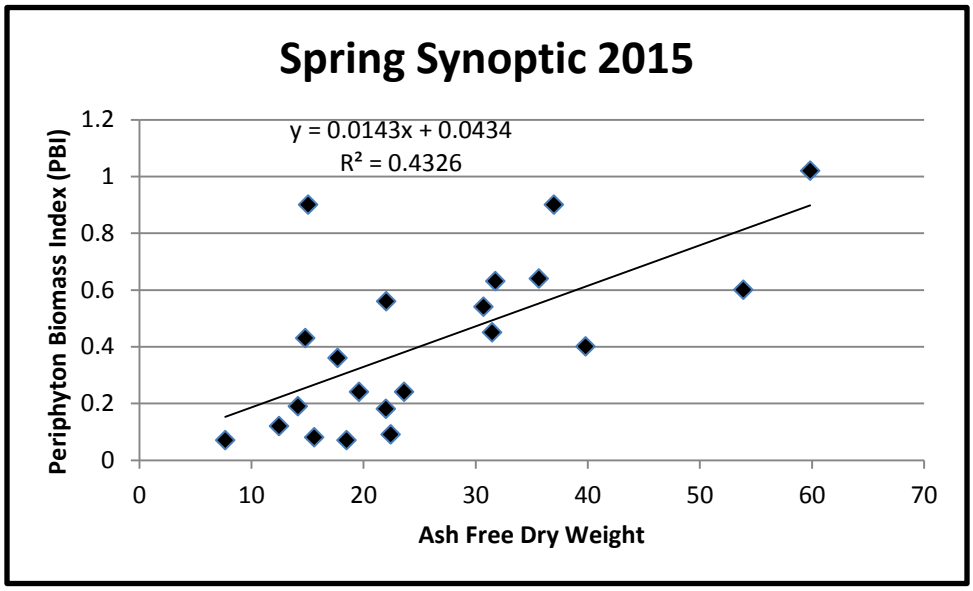

Figure 11. Relation between periphyton Ash Free Dry Weight and Periphyton Biomass Index for sites where both were measured during the 2015 spring synoptic survey.

Results of Spring Synoptic Monitoring 2015

The PBI and chlorophyll *a* data were used to prepare the map of synoptic biomass for spring 2015 (Figure 12). This map shows distribution of biomass around the lake during April 6 – May 19, 2015. 45 sites were monitored in addition to the nine routine sites (total number of sites sampled = 54). Data collected for the 2015 spring synoptic survey are summarized in Table 8. This synoptic monitoring was timed as much as possible to correspond to peak spring periphyton growth in the lake. Based on a comparison of data throughout the year at routine sites and observations in the field, it appeared the synoptic survey occurred slightly after the peak at some sites (e.g., Pineland, and South Shore sites) and slightly before the peak at some east shore sites (e.g., Sand Pt. and Zephyr Pt.).

Generally light biomass (indicated by the two shades of green, in the map) with some areas of slightly heavier growth interspersed (indicated by yellow shading) was observed along the shoreline during the spring synoptic in WY 2015. Chlorophyll *a* levels were below 35 mg/m^2 at most of the sites where samples were taken. Three sites (Dollar Pt., Timber Cove and So. Fleur du lac) had moderate chlorophyll *a* ranging from 40-74 mg/m^2 and the Tahoe City Tributary site had high chlorophyll a (105 mg/m^2). The Tahoe City Tributary site was the only site 2015 with PBI > 1 (PBI=1.02).

Predominant algae types at sites consisted of either blue-greens, stalked diatoms or filamentous green algae or a combination of these types. With the lowered lake level in 2015, blue-green algae were prominent in the algal community at 0.5m at a majority of sites. Stalked diatoms and/or filamentous green algae were also present at many sites.

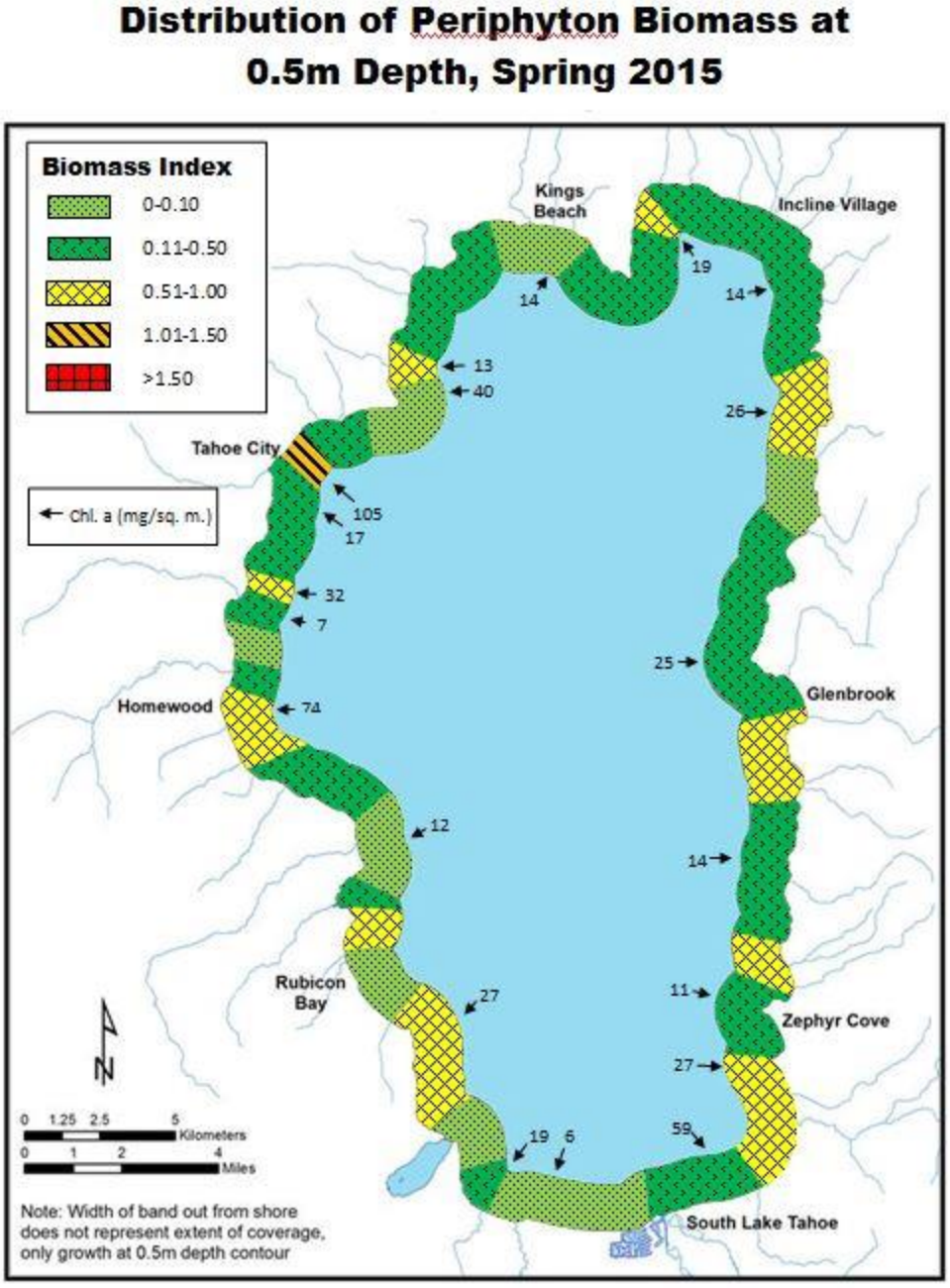

Figure 12. Distribution of periphyton biomass measured during the spring synoptic 2015 (April 6-May 19, 2015). Shading indicates levels of biomass measured using a rapid assessment method: Periphyton Biomass Index (PBI). (PBI = Avg. Filament Length x % Area Covered with Algae). Levels of periphyton chlorophyll *a* measured at selected sites are also shown (black numbers and arrows).

Table 8. Summary of 0.5m periphyton chlorophyll *a*, Ash Free Dry Weight (AFDW), visual score, avg. filament length and percent algal coverage, predominant algae present based on visual observations while snorkeling (FG=filamentous greens; SD=stalked diatoms; CY= blue green algae), for routine sites (shaded) and Spring Synoptic survey sites during April 6-May 19,2015. Note for chlorophyll *a* and AFDW, n=2 unless otherwise indicated. Visual score is a subjective ranking of the aesthetic appearance of algal growth (viewed underwater) where 1 is the least offensive and 5 is the most offensive. Biomass Index is filament length times percent algal cover. "NA" = not available or not collected; "NES" = not enough sample for analysis. Sampling depth and corresponding sampling elevation are also indicated.

Site	Site Name	Date	Sampling Depth/Elev. (m/ ft.)	Chl a (mg/m^2)	Std Dev (mg/m^2)	AFDW (g/m^2)	Std Dev (mg/m^2)	Above Visual Score	Below Visual Score	Fil. Length (cm)	Algal Cover. %	Biomass Index	Algal Type
A	Cascade Creek	4/9/15	0.5/6221.14	18.58	0.82	19.59	1.32	2	2	0.3	80%	0.24	CY,FG
B	S. of Eagle Point	4/9/15	0.5/6221.14					2	2	0.1/0.3	80/5%	0.09	CY,FG
C	E.Bay/Rubicon	4/9/15	0.5/6221.14					3	4	1.0	95%	0.95	CY,FG
	Rubicon Pt.	4/9/15	0.5/6221.14	26.76	3.36	30.71	4.37	3	3	0.6	90%	0.54	SD,CY,FG
D	Gold Coast	4/9/15	0.5/6221.14					1	2	0.4	20%	0.08	SD
E	S. Meeks Point	4/9/15	0.5/6221.14					3	3	0.8	90%	0.72	CY,FG
F	N. Meeks Bay	4/9/15	0.5/6221.14					2	3	1.5	30%	0.45	SD
	Sugar Pine Pt.	4/9/15	0.5/6221.14	12.37	6.36	15.61	9.88	NA	2	0.2	40%	0.08	SD
G	Tahoma	4/9/15	0.5/6221.14					2	2	0.2	90%	0.18	CY,SD
H	S. Fleur Du Lac	4/9/15	0.5/6221.14	73.87	31.28	53.88	12.54	3	3	0.8	75%	0.60	SD,CY
I	Blackwood Creek	4/9/15	0.5/6221.14					2	3	0.4	37%	0.15	SD
	Kaspian Pt.	4/9/15	0.5/6221.14					2	2.5	0.1	50%	0.05	CY
J	Ward Creek	4/9/15	0.5/6221.14	7.23	2.89	12.46	(n=1)	2	3	0.2	60%	0.12	SD
	Pineland	4/9/15	0.5/6221.14	32.07	14.96	31.75	9.90	2.5	3	1.4	45%	0.63	CY,SD
K	N. Sunnyside	4/9/15	0.5/6221.14					2	2.5	0.3	18%	0.05	SD
L	Tavern Pt.	4/9/15	0.5/6221.14					1.5	2	0.1	55%	0.06	CY
	Tahoe City	4/9/15	0.5/6221.14	16.98	1.54	22.00	1.01	NA	3	0.5	35%	0.18	SD
TCT	Tahoe City Trib.	4/13/15	0.5/6221.14	104.93	39.94(n=3)	59.84	18.08	4	4	1.5	68%	1.02	SD
M	TCPUD Boat Ramp	4/13/15	0.5/6221.14					2	2	0.3	38%	0.11	SD
	Lake Forest	4/13/15	0.5/6221.14	11.67	0.23	14.14	1.88	3	3	0.5	37%	0.19	SD,CY
N	S. Dollar Pt.	4/9/15	0.5/6221.14					2	2	0.1	52%	0.05	SD,CY
	Dollar Pt.	4/9/15	0.5/6221.14	39.55	13.50	18.49	5.83	3	2	0.1	70%	0.07	SD,CY
O	S. Dollar Creek	4/23/15	0.5/6221.09	12.62	1.90	22.02	3.63	2.5	3	0.7	80%	0.56	SD,CY,FG
P	Cedar Flat	4/23/15	0.5/6221.09					3	3	0.1/1.0	70/40%	0.43	CY,FG
Q	Garwood's	4/13/15	0.5/6221.14					2	2.5	0.4	60%	0.24	SD,CY
R	Flick Point	4/23/15	0.5/6221.09					2.5	2.5	0.5	50%	0.25	SD
S	Stag Avenue	4/13/15	0.5/6221.14					NA	2	0.1/0.4	90/50%	0.24	SD,CY,FG

Site	Site Name	Date	Sampling Depth/Elev. (m/ ft.)	Chl a (mg/m^2)	Std Dev (mg/m^2)	AFDW (g/m^2)	Std Dev (mg/m^2)	Above Visual Score	Below Visual Score	Fil. Length (cm)	Algal Cover. %	Biomass Index	Algal Type
T	Agatam Boat R.	4/13/15	0.5/6221.14					NA	1.5	0.1	30%	0.03	SD,CY
E17	Kings Beach	4/23/15	0.5/6221.09	13.95	2.16	22.44	1.36	1.5	2	<0.1	90%	<0.09	SD,CY
E16	Brockway Springs	423/15	0.5/6221.09					2	2	0.3	90%	0.27	SD,CY
E15	No. Stateline Point	4/23/15	0.5/6221.09					NA	2	0.4	70%	0.28	SD,CY
E14	Stillwater Cove	4/23/15	0.5/6221.09					2	3	0.6	80%	0.48	CY,FG
	Old Incline West	4/23/15	0.5/6221.09					3	3	0.7	90%	0.63	SD,CY,FG
	Incline West	4/23/15	0.5/6221.09	18.98	5.90	36.99	15.20	3	3	1.0	90%	0.90	CY,FG
	Incline Condo	4/23/15	0.5/6221.09					2	3	0.1/1.0	80/30%	0.35	CY,FG
E13	Burnt Cedar Beach	4/23/15	0.5/6221.09					2	2.5	0.1/0.5	70/30%	0.22	NA
E12	Hidden Beach offsh.	4/23/15	0.5/6221.09					2	2	0.3	60%	0.18	CY
	Hidden Beach insh.	4/23/15	0.5/6221.09	13.79	0.13	23.64	2.18	2	2	0.3	80%	0.24	CY
E11	Observation Point	5/19/15	0.5/6221.24					2.5	2.5	0.3/0.1	15/75%	0.11	CY,FG
	Sand Pt.	5/19/15	0.5/6221.24	25.56	3.26	35.65	0.22	3	3	0.8	80%	0.64	CY,FG
E10	Chimney Beach	5/19/15	0.5/6221.24					2	2.5	0.1	71%	0.07	SD,CY
E9	Skunk Harbor	5/19/15	0.5/6221.24					3	3	0.6/0.1	30/70%	0.22	CY,FG
	Deadman Pt.	5/19/15	0.5/6221.24	24.78	2.96	39.81	3.08	3	3.5	0.5	80%	0.40	CY,FG
E8	So. Deadman Point	5/19/15	0.5/6221.24					3.5	3.5	0.6	70%	0.42	CY,FG
E7	So. Glenbrook Bay	5/19/15	0.5/6221.24					3	3	0.3/1.2	80/30%	0.51	CY,FG
E6	Cave Rock Ramp	5/19/15	0.5/6221.24	13.81	1.00	17.69	3.04	2	2	0.4	90%	0.36	SD,CY,FG
E5	Lincoln Park	5/19/15	0.5/6221.24					1	2	0.3	40%	0.12	CY,FG
E4	No. Zephyr Cove	5/19/15	0.5/6221.24					2.5	3	1.4	50%	0.70	SD,FG
E3	So. Zephyr Pt.	5/19/15	0.5/6221.24					2	2	0.3	70%	0.21	SD,CY
	Zephyr Pt.	5/19/15	0.5/6221.24	10.58	1.12	14.81	0.18	3	3	0.8/0.1	50/80%	0.43	CY,FG
E2	No. Elk Pt.	5/19/15	0.5/6221.24					NA	2	0.5	90%	0.45	SD,CY
E1	So. Elk Point	5/19/15	0.5/6221.24	26.61	9.48	15.06	3.85	4	4	1	90%	0.90	SD
	Timber Cove Rock	4/6/15	0.5/6221.13	58.66	20.24(n=3)	31.47	8.21(n=3)	NA	4	1.5	30%	0.45	SD
S1	T. Keys Entrance	4/6/15	0.5/6221.13										
S2	Kiva Point	4/6/15	0.5/6221.13	6.12	0.36	7.67	0.67	2	2.5	0.35	20%	0.07	SD,FG

Some of the sites evaluated in previous reports that typically have higher biomass in the spring (i.e. Ward Cr. mouth, Pineland, Tahoe City, Tahoe City Tributary and South Dollar Cr.) have shown different trends for biomass the last three years. Figures 13 a,b present chlorophyll *a* and PBI levels for these sites the past three years during spring synoptic surveys.

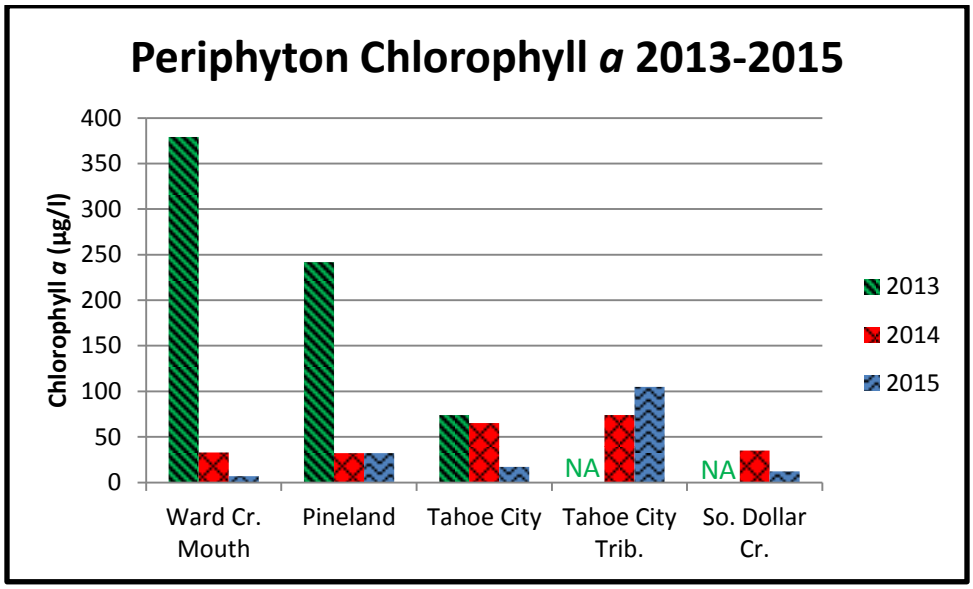

Figure 13 a. Comparison of patterns for Chlorophyll a at some of the typically heavier periphyton biomass sites during spring synoptics 2013-2015.

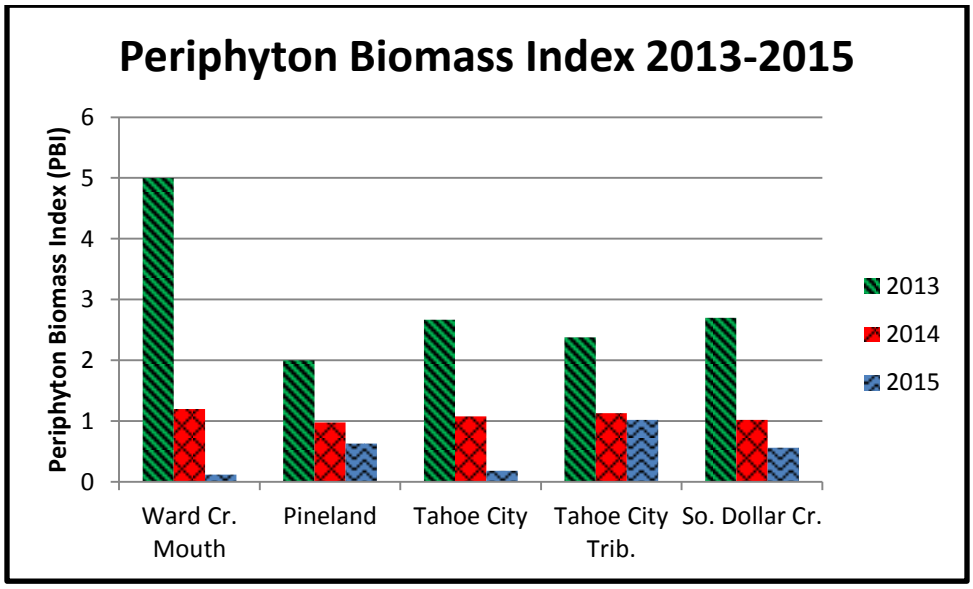

Figure 13 b. Comparison of patterns for Periphyton Biomass Index (PBI) at some of the typically heavier periphyton biomass sites during spring synoptics 2013-2015.

Ward Cr. mouth has shown a substantial decline in biomass over the period, with chlorophyll *a* decreasing from a very high level of 379 mg/m^2 in 2013 to 7 mg/m^2 in 2015. Nearby Pineland also showed a substantial decline from very high biomass in 2013 (242 mg/m^2) but chlorophyll *a* biomass was similar the past two years (32 mg/m^2). Tahoe City and South Dollar have also shown declines in biomass since 2013. However, the pattern at the Tahoe City Tributary site was different. Although biomass was higher in 2013 based on PBI, PBI was also high in 2014 and 2015. It is probable water flows and associated nutrient inputs from this creek are contributing to the elevated spring periphyton growth there. The Tahoe City tributary site had the highest overall biomass during the 2015 spring synoptic. Other sites with elevated biomass in addition to Tahoe City tributary in 2015 included South Fleur du lac, Timber Cove and Dollar Pt.

Observations of localized area of heavy periphyton growth in 2015

One additional area of heavier growth stood out during sampling in 2015. This was a site slightly west of the Garwoods spring synoptic site. Although very light biomass was found at the Garwoods site this year, very heavy growth of stalked diatoms was observed in a localized area of shoreline a short distance away in April, 2015 (Figure 14). Heavy biomass persisted at this site through the summer of 2015, with a heavy, green filamentous algae (*Cladophora sp*) dominating the biomass, but substantial stalked diatoms also persisting through the summer (Figure 15). Substantial dried, white old periphyton material coated a good portion of the backshore rocks in this area, which extends about 50-60 yards along the shore. Very little periphyton growth was observed on either side of the heavy growth as well as further out into the lake.

At this heavy periphyton growth site (west of the Garwoods site), several areas of flowing water seeping through the backshore were observed both during the spring and summer. This appears to be an area where subsurface water or groundwater is seeping through backshore sediments (which are submerged during higher lake levels). Upslope of this site, there is an ephemeral drainage. A sample of water seeping from the backshore 4/21/15 was analyzed and found to have slightly elevated levels of both nitrogen and phosphorus (NO_3-N= 86µg/l; NH_4-N=2µg/l) and phosphorus (SRP=29 µg/l). The conductivity was 176 µS. The levels of N and P in this water was similar to lower levels of nutrients found in groundwater in Upper Ward Valley (see Loeb, 1987).

The continuous flow of seepage water at this site appears to provide a very visible demonstration of the impact of localized nutrient inputs on periphyton growth. Steady inflow of water with elevated N and P is likely causing the heavy growth of periphyton immediately along shore at this site. The flow of this seepage water through the shoreline sediments, continued from spring through fall. Very little periphyton growth occurred on either side of the seepage zone. Persistent nutrient inputs concentrated in a shallow zone appear to have sustained heavy growth of stalked diatoms (*Gomphoneis herculeana).* The presence of heavy *Cladophora* growth also is indicative of nutrient enriched conditions. The *Cladophora* appeared heaviest in spring and summer and appeared to be dying back in the fall of 2015. Heavy growth of algae including Cladophora has been observed at this site in some previous years by TERC.

Figure 14. Localized area of heavy growth of stalked diatoms on 4/21/15 observed adjacent to a residential area, slightly west of the Garwoods synoptic site. The periphyton growth in this area appears to be supported by localized, and persistent seepage of subsurface water or groundwater from sediments in the backshore and along the beach, and which contains slightly elevated nitrogen and phosphorus levels.

Figure 15. Localized area of heavy growth of *Cladophora sp* with stalked diatoms also present mid-August 2015 observed adjacent to a residential area, slightly west of the Garwoods synoptic site. The periphyton growth in this area appears to be supported by localized, and persistent seepage of subsurface water or groundwater from sediments in the backshore and along the beach, and which contains slightly elevated nitrogen and phosphorus levels.

Although it appears this seepage water is groundwater, and N and P are slightly elevated the extent to which there is an anthropogenic contribution of nutrients to this water is not known. With the unusually heavy growth of periphyton observed at this site in 2015 as well as in some previous years, it would be desirable to further evaluate what is stimulating the algal growth at this location.

Section IV. Project Quality Assurance

This section provides details of the project quality assurance and quality control measures for the primary areas of study associated with this contract. The QA/QC is an explicit task (Task 2) as required in the original contract. QA/QC provides information on procedures for assuring quality in the research being done and the observation techniques or measures that are used to help verify quality data are being collected. The QA/QC details are presented for the three primary areas study below: (1) algal growth potential assays; (2) phytoplankton enumeration; (3) periphyton analyses.

1. Quality assurance and quality control for algal growth potential bioassays

(QA/QC) applied to the AGP bioassays was similar to methods used for QA/QC in algal nutrient bioassays, see: "Lake Tahoe Algal Bioassay Procedure" in Hackley et al., (2007). Avoidance of sources of contamination and factors that can compromise samples is a critical quality assurance concern in collection of AGP bioassay samples. Glassware and carboys are carefully cleaned in the lab with Liquinox soap, tap water, 0.1N HCl and deionized water. When sampling on the research boat, standard, clean limnological sampling techniques are employed to prevent contamination. After collection, samples are protected from direct sunlight and kept cool. The bioassays are typically initiated on the same day of collection. Similarly, avoidance of sources of contamination in bioassay set-up is of critical concern.

Coefficients of variation for treatment chlorophyll *a* and fluorescence replicates were determined to provide information on replicate variation in the assays. Duplicate treatments were used for assays from August 2014 to June 2015. Coefficients of variation (std. dev. /mean of replicates) were generally low for fluorescence and chlorophyll *a* replicates. The following patterns for coefficient of variation were observed:

- In AGP assay #5, coefficients of variation values for peak *in vivo* fluorescence measurements (as indicator of peak chlorophyll *a*) were relatively low ≤10% for all but two sites which had coefficients of variation between 13-14%. Coefficients of variation (CV) for chlorophyll *a* were ≤ 6% for all but three sites on Day 4, CV for two sites was 15% and one site was 22%. Replication using duplicates was fair in this experiment.
- In AGP assay #6, coefficient of variation values for *in vivo* fluorescence measurements were relatively low, ≤13% through Day 10 of the bioassay. The coefficient of variation for extracted chlorophyll *a* on Day 10 was ≤12% for all but two sites (Tahoe City and Glenbrook, which had CV of 17% and 22% respectively). Replication of fluorescence and chlorophyll *a* for treatment duplicates was fair for this AGP experiment.
- For Algal Growth Potential Bioassay #7, coefficients of variation values for *in vivo* fluorescence measurements were relatively low, generally less than 5%, with a high of 11% for fluorescence and 12% for chlorophyll *a* for single values. Replication of fluorescence and chlorophyll *a* for treatment duplicates was good for this AGP experiment.

- For AGP assay #8 coefficients of variation values for *in vivo* fluorescence measurements were relatively low, generally less than 5%, with a high of 11% for fluorescence and 14% for chlorophyll *a* for single values through day 11 of the experiment. After 14 days the coefficient of variation for fluorescence increased to 21% for one set of replicates with two other sites having coefficients of variation above 5% (9-12%). Replication of fluorescence and chlorophyll *a* for treatment duplicates was good for this AGP experiment, particularly for data collected through Day 6 near peak biomass or AGP.

2. Quality Assurance for Phytoplankton

Appendix 2 of this report provides detailed methods for phytoplankton counting and quality assurance. Quality assurance for phytoplankton enumeration focuses on careful preparation and settling of samples and multiple counts of settled samples. Counts were made along multiple strips of view of settled samples, under the inverted microscope. The replicate strip counts are a measure of precision, much like duplicate water samples provide an estimate of precision for water chemistry. Precision measures the goodness of the procedure, i.e., did the cells settle randomly in the chamber. 1 cm^2 areas of view in the settling chamber were first counted at low magnification to quantify larger cells. Then multiple counts were made at high magnification along 1 cm long strips. The data from all counted strips are combined in computation of totals for the sample. The data from individual counts of settling chamber 1 cm strips is retained in a database if needed for further analysis.

3. Quality Assurance and Quality Control for Periphyton

For QA/QC applied to periphyton monitoring see "Periphyton Quality Assurance Project Plan" in Hackley et al. (2004). Periphyton monitoring is designed to reflect the amount of attached algal biomass present in specific lake locations. There is no standard growth pattern that the collected samples can be compared to; therefore, it is assumed that the collected biomass is representative of the area in which it was collected. Assurances that collected samples are representative rely on replicate samples and expertise of the sampling personnel to place sampling tubes over sections of substrate that reflect the area's growth pattern. During periods of high standing biomass, when within site variability can be high, researchers may collect triplicate samples. The additional sample increases the statistical power of the analysis and can help account for the presence of higher variability. Collection of the triplicate sample is at the discretion of the scientist. During the study period, triplicate samples were collected for 1 of 45 routine site samples and 2 of 12 spring synoptic site samples.

One set of samples was collected which exhibited substantial variation in replicate chlorophyll *a*. This was the Dollar Pt. sample collected Nov. 14, 2014. Along with high chlorophyll *a* (mean chlorophyll *a*= 116 mg/m^2 std. dev. = 55 mg/m^2) these samples had high sand content. PBI however was very low. Figure 16 shows a comparison of

chlorophyll *a* and AFDW for samples collected from routine sites on Nov. 11, 2014. The samples from Dollar Pt. had much higher chlorophyll *a* relative to AFDW compared to the other sites. We considered the chlorophyll levels from this sample anomalously high and the data were not used.

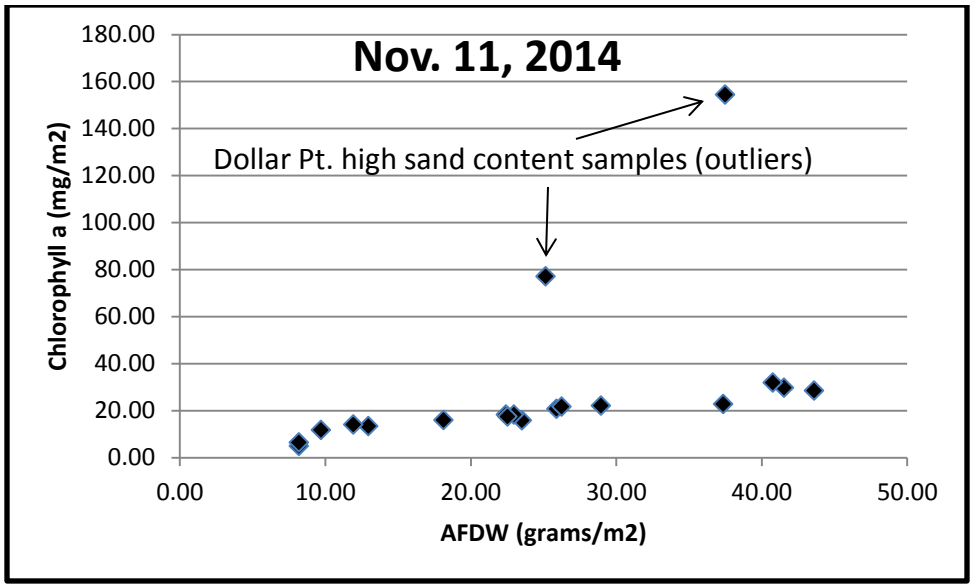

Figure 16. Dollar Pt. routine samples for November 11, 2014 had unusually high chlorophyll *a* relative to AFDW biomass compared to all other routine samples collected on this date. These samples also had very high sand content. The chlorophyll *a* levels for Dollar Pt. on this date were considered anomalous and not used.

References

Boltovskoy, D., Izaguirre, I., and Correa, N. 1995. Feeding selectivity of *Corbicula fluminea* (Bivalvia) on natural phytoplankton. Hydrobiologia 312: 171-182.

California Department of Water Resources. 1970. California-Nevada-Federal Joint Water Quality Investigation of Lake Tahoe, Fourth Annual Summary, July 1968-June 1969. Report produced by California Department of Water Resources, Central District.

California Department of Water Resources. 1971. California-Nevada-Federal Joint Water Quality Investigation of Lake Tahoe, Fifth Annual Summary, July 1969-June 1970. Report produced by California Department of Water Resources, Central District.

California Department of Water Resources. 1972. California-Nevada-Federal Joint Water Quality Investigation of Lake Tahoe, Sixth Annual Summary, July 1970-June 1971. Report produced by California Department of Water Resources, Central District.

California Department of Water Resources. 1973. California-Nevada-Federal Joint Water Quality Investigation of Lake Tahoe, Seventh Annual Summary, July 1971-Dec. 1972. Report produced by California Department of Water Resources, Central District.

California Department of Water Resources. 1974. California-Nevada-Federal Joint Water Quality Investigation of Lake Tahoe, Eighth Annual Summary, 1973. Report produced by California Department of Water Resources, Central District.

California Department of Water Resources. 1975. California-Nevada-Federal Joint Water Quality Investigation of Lake Tahoe, Ninth Annual Summary, 1974. Report produced by California Department of Water Resources, Central District.

Elser, J.J. and C.R. Goldman. 1991. Zooplankton effects on phytoplankton in lakes of contrasting trophic status. Limnol. Oceanogr. 36(1): 64-90.

Hackley, S. H., Allen, B.C., Hunter, D.A., and J.E. Reuter. 2004. Lake Tahoe water quality investigations: algal bioassay, phytoplankton, atmospheric nutrient deposition, periphyton. Final report submitted to State Water Resources Control Board, Lahontan Regional Water Quality Control Board. Tahoe Research Group, University of California, Davis.

Hackley, S. H., Allen, B.C., Hunter, D.A., and J.E. Reuter. 2007. Lake Tahoe water quality investigations: algal bioassay, phytoplankton, atmospheric nutrient deposition, periphyton, Final Report 2004-2007. Submitted to State Water Resources Control Board, Lahontan Regional Water Quality Control Board. Tahoe Environmental Research Center, University of California, Davis. 121pp.

Heyvaert, A.C., Reuter, J.E., Chandra, S., Susfalk, R.B., Schladow, S.G. and Hackley, S.H. 2013. Lake Tahoe Nearshore Evaluation and Monitoring Framework. Final Report prepared for the USDA Forest Service Pacific Southwest Research Station. 410pp.

Jassby, A.D., Goldman, C.R. and T.M. Powell. Trend, seasonality, cycle, and irregular fluctuations in primary productivity at Lake Tahoe, California-Nevada, USA. Hydrobiologia 246:195-203.

Lewis, M.R. 2002. Variability of plankton and plankton processes on the mesoscale. In: Phytoplankton Productivity, Carbon Assimilation in Marine and Freshwater Ecosystems. Eds. Williams, P.J.B., Thomas, D.N. and Reynolds, C.S. pp141-155. Blackwell Pub., Oxford, U.K.

Loeb, S.L. 1987. Groundwater quality within the Tahoe Basin. Institute of Ecology, University of California, Davis. 265p.

Reddy, K.J., Haskell, B., Sherman, D.M. and L.A. Sherman. 1993. Unicellular aerobic nitrogen-fixing cyanobacteria of the genus Cyanothece. J. Bacteriology 175:1284-1292.

Way, C.M., Hornback, D. J., Miller-Way, C. A., B. S. Payne, and Miller, A. C. 1990. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). Canadian Journal of Zoology 68: 115-120.

Winder, M. and D.A. Hunter. 2008. Temporal organization of phytoplankton communities linked to physical forcing. Oecologia 156: 179-192.

Winder, M., Reuter, J. E. and S.G. Schladow. 2009. Lake warming favors small-sized planktonic diatom species. Proc. Royal Society B. 276, 427-435.

Appendix 1. Summary of data for Algal Growth Potential Assays

Appendix 1a. Summary of field and experimental data collected for Algal Growth Potential (AGP) experiment done on Lake Tahoe water collected from nearshore and mid-lake sites on 8/29/14. Data for date of collection from various sites is shown in upper left (Date, Time, Surface Temp., Depth collected, chlorophyll *a*, selected observations). On selected dates, extracted chlorophyll *a* was measured, these values are summarized under heading "Extracted Chlor. a". Final AGP results are shown at top right of table (in bold). Initial background fluorescence (i.e. fluorescence of filtered lake water) and mean daily *in vivo* fluorescence readings during the AGP experiment are shown along bottom of table.

AGP #5 H_2O Collection 8/29/14	Date Collected	Time Collected	Lake Surface T (°C)	Collection Depth (m)	Lake Chl. *a* (µg/l)	Observations	**Extracted Chlor. *a*** AGP D4 9/2/14	**Extracted Chlor. *a*** AGP D11 9/9/14	**Final AGP Results Chl. *a* ± s.d. (µg/l)**
Nearshore:									
Sunnyside	8/29/14	13:10	NA	1	.19		.34 ± .02		**.42 ± .07**
Tahoe City	8/29/14	08:30	17.0	1	.41	Surf. Oil sheen	.74 ± .11	.36 ± .02	**.82 ± .05**
Kings Beach	8/29/14	09:30	17.5	0.5	.40		.40 ± .00		**.48 ± .03**
Crystal Bay	8/29/14	10:00	18.0	1	.17		.35 ± .01		**.43 ± .02**
Glenbrook	8/29/14	10:24	17.5	1	.23		.40 ± .01		**.40 ± .01**
Zephyr	8/29/14	10:46	17.5	0.5	.18		.62 ± .02		**.61 ± .02**
Timber Cove	8/29/14	11:15	18.5	0.5	.11	Metaphyton	.50 ± .02	.38 ± .06	**.65 ± .01**
Tahoe Keys	8/29/14	11:30	19.0	0.25	.20	Aquatic plants	.38 ± .06		**.56 ± .03**
Camp Rich.	8/29/14	11:42	18.5	1	.18		.50 ± .01		**.45 ± .01**
Emerald Bay	8/29/14	12:14	19.0	1	.23		.28 ± .00		**.39 ± .02**
Rubicon Bay	8/29/14	12:45	18.0	1	.16		.38 ± .09	.17 ± .00	**.44 ± .08**
Mid-Lake:									
Mid-lk No.	8/29/14	08:56	17.5	1	.15		.30 ± .00		**.44 ± .002**
Mid-lk So.	8/29/14	11:00	18.0	1	.17		.33 ± .01		**.37 ± .04**

Experiment Daily Fluor.	Backgrd. Fluor. GF/F Fil.	D0 Fluor. 8/29/14 17:30	D2 Fluor. 8/31/14 11:00	D3 Fluor. 9/1/14 17:05	D4 Fluor. 9/2/14 10:25	D5 Fluor. 9/3/14 14:15	D6 Fluor. 9/4/14 11:15	D8 Fluor. 9/6/14 17:50	D11 Fluor. 9/9/14 13:15
Nearshore:									
Sunnyside	.042	.188± .001	.207±.002	.231± .001	.224± .002	.245± .016	.244± .019	.261± .033	.247± .035
Tahoe City	.046	.295± .006	.379±.005	.443± .021	.395± .010	.419± .024	.391± .037	.359± .003	.313± .019
Kings Beach	.049	.270± .003	.255± .009	.275± .024	.244± .026	.286± .016	.269± .002	.286± .023	.264± .016
Crystal Bay	.034	.195± .031	.226± .019	.263± .008	.247± .015	.266± .011	.251± .009	.224± .004	.202± .002
Glenbrook	.042	.192± .002	.210± .003	.239± .006	.221± .007	.252± .003	.247± .001	.230± .018	.208± .046
Zephyr	.050	.160± .001	.236± .002	.319± .001	.318± .007	.347± .008	.331± .019	.310± .006	.307± .052
Timber Cove	.040	.151± .009	.203± .001	.299± .018	.320± .021	.365± .002	.347± .009	.345± .004	.307± .023
Tahoe Keys	.051	.209± .003	.231± .019	.282± .004	.277± .011	.323± .014	.313± .008	.275± .000	.202± .004
Camp Rich.	.038	.201± .002	.222± .004	.255± .006	.254± .004	.273± .005	.265± .000	.250± .006	.225± .016
Emerald Bay	.045	.190± .001	.228± .004	.244± .007	.232± .001	.230± .011	.218± .020	.206± .021	.182± .016
Rubicon Bay	.044	.169± .004	.211± .016	.247± .023	.242± .025	.271± .038	.250± .001	.222± .002	.171± .001
Mid-Lake:									
Mid-lk No.	.044	.169± .004	.193± .003	.227± .011	.218± .004	.231± .021	.237± .001	.267± .001	.245± .018
Mid-lk So.	.034	.178± .001	.192± .005	.204± .012	.200± .016	.223± .002	.232± .005	.237± .018	.208± .015

Appendix 1b. Summary of field and experimental data collected for Algal Growth Potential (AGP) experiment done on Lake Tahoe water collected from nearshore and mid-lake sites on 12/9/14.

AGP #6 H_2O Collection 12/9/14	Date Collected	Time Collected	Lake Surface T (°C)	Collection Depth (m)	Lake Chl. *a* (µg/l)	Observations	**Extracted Chlor. *a*** AGP D10 12/19/14	**Extracted Chlor. *a*** AGP D14 12/23/14		**Final AGP Results Chl. *a* ± s.d. (µg/l)**
Nearshore:										
Sunnyside	12/9/14	14:55	9.5	0.5	.52		.31 ± .02			**.52**
Tahoe City	12/9/14	09:10	8.0	0.5	.46	Dredging Ramp	.45 ± .10	.39 ± .05		**.46**
Kings Beach	12/9/14	10:15	9.0	0.5	.45		.34 ± .01			**.45**
Crystal Bay	12/9/14	10:40	9.5	0.5	.61		.30 ± .02			**.61**
Glenbrook	12/9/14	11:15	9.5	0.5	.46		.30 ± .05			**.46**
Zephyr Cove	12/9/14	11:35	9.0	0.5	.34		.37 ± .03			**.39 ± .02**
Timber Cove	12/9/14	12:15	9.0	0.5	.31	Macrophytes	.39 ± .04			**.39 ± .04**
Tahoe Keys	12/9/14	12:30	9.0	0.5	.53	Macrophytes	.42 ± .05	0.43 ± .04		**.53**
Camp Rich.	12/9/14	12:45	9.0	0.5	.43		.31 ± .01			**.43**
Emerald Bay	12/9/14	13:15	9.0	0.5	.52		.44 ± .04	.32 ± .01		**.52**
Rubicon Bay	12/9/14	13:50	9.5	0.5	.38		.27 ± .01			**.38**
Mid-Lake:										
Mid-lk No.	12/9/14	09:40	9.0	0.5	.53		.27 ± .02			**.53**
Mid-lk So.	12/9/14	11:55	9.5	0.5	.43		.31 ± .04			**.43**
Experiment Daily Fluorescence	Backgrd. Fluor. GF/F Fil.	D0 Fluor. 8/29/14 17:30	D1 Fluor. 12/10/14 16:00	D3 Fluor. 12/12/14 12:10	D4 Fluor. 12/13/14 12:50	D6 Fluor. 12/15/14 15:35	D8 Fluor. 12/17/14 13:45	D10 Fluor. 12/19/14 15:00	D12 Fluor. 12/21/14 14:05	D14 Fluor. 12/23/14 17:15
Nearshore:										
Sunnyside	.057	.293	.227±.008	.211± .000	.192± .005	.213± .001	.219± .009	.239± .001	.255± .004	.264± .025
Tahoe City	.063	.263	.223±.007	.197± .013	.220± .010	.245± .007	.280± .010	.292± .010	.288± .011	.311± .015
Kings Beach	.045	.243	.216± .009	.186± .013	.193± .004	.196± .004	.216± .005	.233± .001	.244± .005	.264± .002
Crystal Bay	.059	.279	.223± .008	.197± .006	.197± .004	.205± .002	.209± .020	.220± .011	.234± .006	.237± .005
Glenbrook	.056	.249	.210± .008	.186± .005	.185± .005	.189± .001	.202± .009	.220± .001	.237± .004	.257± .010
Zephyr	.054	.208	.182± .001	.174± .001	.187± .001	.209± .000	.223± .007	.248± .001	.264± .007	.274± .019
Timber Cove	.058	.209	.181± .009	.187± .001	.201± .006	.238± .006	.264± .012	.279± .013	.276± .006	.261± .007
Tahoe Keys	.067	.307	.246± .009	.226± .015	.215± .009	.222± .017	.254± .012	.279± .013	.288± .028	.286± .016
Camp Rich.	.055	.252	.212± .002	.198± .007	.198± .009	.210± .001	.215± .004	.241± .004	.247± .004	.249± .011
Emerald Bay	.093	.322	.300± .000	.303± .008	.316± .006	.336± .001	.322± .000	.330± .006	.292± .001	.255± .004
Rubicon Bay	.057	.225	.193± .003	.176± .004	.192± .009	.198± .010	.206± .001	.222± .004	.240± .015	.252± .040
Mid-Lake:										
Mid-lk No.	.056	.252	.211± .013	.176± .004	.182± .001	.182± .002	.187± .002	.207± .006	.225± .007	.244± .005
Mid-lk So.	.051	.265	.208± .006	.198± .010	.192± .001	.202± .006	.209± .006	.227± .003	.233± .004	.244± .003

Note- Used association between Corrected *In Vivo* Fluorescence (Uncorrected – Day 0 Blank) and Chlorophyll *a* to calculate Day 14 chlorophyll *a* for Zephyr Cove.

Appendix 1c. Summary of field and experimental data collected for Algal Growth Potential (AGP) experiment done on Lake Tahoe water collected from nearshore and mid-lake sites on 2/26/15.

AGP #7 H_2O Collection 2/26/15	Date Collected	Time Collected	Lake Surface T (°C)	Collection Depth (m)	Lake Chl. *a* (µg/l)	Observations	**Extracted Chlor. *a*** AGP D6 3/4/15	**Extracted Chlor. *a*** AGP D14 3/12/15		**Final AGP Results Chl. *a* ± s.d. (µg/l)**
Nearshore:										
Sunnyside	2/26/15	10:10	6.5	1.0	.52		.71 ± .04	.65 ± .07		**.71 ± .04**
Tahoe City	2/26/15	09:05	6.0	0.5-1.0	.35		.45 ± .01	.62 ± .01		**.62 ± .01**
Kings Beach	2/26/15	14:15	7.0	0.5	.43	Bottom Detritus	.62 ± .03	.77 ± .01		**.83 ± .03**
Crystal Bay	2/26/15	13:50	7.0	0.5	.59		.71 ± .00	.73 ± .04		**.84 ± .02**
Glenbrook	2/26/15	13:15	7.0	0.5	.42		.57 ± .00	.85 ± .04		**.97 ± .04**
Zephyr Cove	2/26/15	12:55	6.5	0.5	.33		.54 ± .04	.87 ± .04		**.94 ± .01**
Timber Cove	2/26/15	12:15	6.0	0.5	.17		.37 ± .01	1.08± .09		**1.08 ± .09**
Tahoe Keys	2/26/15	12:00	7.0	0.5	.37		.59 ± .01	.69 ± .01		**.90 ± .02**
Camp Rich.	2/26/15	11:45	6.5	0.5	.48		.61 ± .02	.62 ± .01		**.75 ± .01**
Emerald Bay	2/26/15	11:20	6.0	0.5	.98		.93 ± .04	.51 ± .01		**.98**
Rubicon Bay	2/26/15	10:45	6.5	0.5	.76		.71 ± .04	.59 ± .07		**.76**
Mid-Lake:										
Mid-lk No.	2/26/15	09:30	6.5	1.0	.63		.56 ± .01	.61 ± .03		**.67 ± .03**
Mid-lk So.	2/26/15	12:35	7.0	0.5	.62		.61 ± .01	.67 ± .06		**.76 ± .02**

Experiment Daily Fluorescence	Backgrd. Fluor. GF/F Fil.	D0 Fluor. 2/26/15 19:35	D1 Fluor. 2/27/15 14:05	D2 Fluor. 2/28/15 15:00	D4 Fluor. 3/2/15 13:15	D6 Fluor. 3/4/15 14:20	D8 Fluor. 3/6/15 14:30	D10 Fluor. 3/8/15 11:20	D12 Fluor. 3/10/15 13:00	D14 Fluor. 3/12/15 12:50
Nearshore:										
Sunnyside	.074	.389	.345±.004	.332± .006	.361± .002	.368± .002	.390± .012	.390± .013	.384± .018	.350± .004
Tahoe City	.079	.285	.254±.004	.289± .001	.301± .005	.295± .000	.302± .002	.292± .009	.316± .012	.307± .012
Kings Beach	.067	.298	.297± .007	.309± .005	.362± .007	.386± .015	.417± .000	.422± .012	.435± .015	.391± .008
Crystal Bay	.071	.344	.306± .006	.333± .002	.352± .009	.408± .014	.459± .012	.449± .007	.438± .004	.387± .004
Glenbrook	.080	.293	.271± .001	.292± .003	.331± .002	.388± .006	.453± .012	.474± .004	.499± .019	.445± .018
Zephyr	.080	.283	.266± .003	.283± .003	.324± .002	.368± .009	.434± .004	.471± .015	.486± .004	.456± .014
Timber Cove	.077	.171	.157± .002	.184± .000	.218± .003	.272± .004	.357± .004	.420± .016	.515± .004	.521± .027
Tahoe Keys	.084	.270	.260± .001	.278± .016	.313± .001	.368± .001	.429± .006	.441± .001	.468± .009	.407± .010
Camp Rich.	.078	.326	.312± .006	.333± .008	.362± .011	.365± .008	.407± .017	.397± .008	.400± .005	.366± .001
Emerald Bay	.112	.464	.468± .002	.474± .027	.489± .015	.462± .005	.424± .006	.363± .021	.353± .004	.307± .013
Rubicon Bay	.079	.408	.356± .011	.371± .001	.378± .006	.390± .004	.411± .002	.411± .044	.379± .016	.341± .025
Mid-Lake:										
Mid-lk No.	.074	.370	.307± .011	.304± .005	.339± .001	.356± .005	.364± .019	.342± .026	.364± .015	.325± .011
Mid-lk So.	.068	.408	.356± .006	.359± .004	.377± .011	.394± .003	.406± .004	.389± .013	.407± .011	.390± .019

Appendix 1d. Summary of field and experimental data collected for Algal Growth Potential (AGP) experiment done on Lake Tahoe water collected from nearshore and mid-lake sites on 5/26/15

AGP #8 H_2O Collection 5/26/15	Date Collected	Time Collected	Lake Surface T (°C)	Collection Depth (m)	Lake Chl. *a* (µg/l)	Observations	**Extracted Chlor. *a*** AGP D6 6/1/15	**Extracted Chlor. *a*** AGP D11 6/6/15	**Final AGP Results Chl. *a* ± s.d. (µg/l)**
Nearshore:									
Sunnyside	5/26/15	13:35	14.0	0.5	.28±.01		.44 ± .02	.32 ± .01	**.44 ± .02**
Tahoe City	5/26/15	09:10	11.0	0.5	.63±.00	5/24 T-storm, mod. turbidity	.78 ± .04	.46 ± .03	**.78 ± .04**
Kings Beach	5/26/15	09:55	11.0	0.5	.29	Slight turbidity	.44 ± .01	.29 ± .01	**.44 ± .01**
Crystal Bay	5/26/15	10:20	11.5	0.5	.27±.01		.43 ± .02	.27 ± .02	**.43 ± .02**
Glenbrook	5/26/15	10:50	11.5	0.5	.25±.01		.35 ± .00	.38 ± .03	**.35 ± .00**
Zephyr Cove	5/26/15	11:15	12.0	0.5	.27±.01		.46 ± .05	.28 ± .01	**.46 ± .05**
Timber Cove	5/26/15	11:40	13.5	0.25	.09±.01		.88 ± .01	.32 ± .04	**.88 ± .01**
Tahoe Keys	5/26/15	11:50	13.0	0.5	.23±.01		.39 ± .01	.34 ± .03	**.39 ± .01**
Camp Rich.	5/26/15	12:10	13.0	0.5	.27±.02		.43 ± .00	.39 ± .01	**.43 ± .00**
Emerald Bay	5/26/15	12:35	15.0	0.5	.49±.00		.52 ± .01	.44 ± .01	**.52 ± .01**
Rubicon Bay	5/26/15	13:05	12.5	0.5	.33±.02		.38 ± .02	.35 ± .05	**.38 ± .02**
Mid-Lake:									
Mid-lk No.	5/26/15	09:30	11.0	0.5	.22		.32 ± .00	.25 ± .01	**.33 ± .02**
Mid-lk So.	5/26/15	11:30	11.0	0.5	.19±.01		.24 ± .01	.25 ± .02	**.24 ± .01**

Experiment Daily Fluorescence	Backgrd. Fluor. GF/F Fil.	D0 Fluor. 5/26/15 17:35	D1 Fluor. 5/27/15 14:30	D3 Fluor. 5/29/15 13:15	D4 Fluor. 5/30/15 14:15	D6 Fluor. 6/1/15 14:20	D8 Fluor. 6/3/15 15:40	D11 Fluor. 6/6/15 14:40	D14 Fluor. 6/9/15 16:15
Nearshore:									
Sunnyside	.046	.289	.242±.000	.219± .001	.216± .001	.224± .002	.193± .008	.171± .004	.164± .008
Tahoe City	.053	.412	.404±.004	.357± .010	.359± .018	.346± .001	.306± .035	.267± .005	.236± .003
Kings Beach	.058	.253	.243± .014	.221± .001	.218± .004	.231± .006	.221± .004	.178± .005	.149± .013
Crystal Bay	.064	.255	.270± .004	.257± .000	.249± .001	.253± .009	.224± .002	.183± .000	.169± .004
Glenbrook	.048	.225	.213± .000	.200± .000	.203± .004	.202± .006	.195± .005	.201± .008	.223± .046
Zephyr	.053	.249	.247± .004	.237± .010	.245± .001	.247± .022	.228± .007	.177± .001	.163± .004
Timber Cove	.053	.131	.140± .009	.216± .006	.301± .003	.454± .008	.455± .004	.232± .014	.141± .001
Tahoe Keys	.054	.239	.234± .003	.232± .006	.227± .013	.242± .006	.240± .004	.213± .010	.194± .008
Camp Rich.	.047	.251	.259± .007	.237± .001	.233± .004	.244± .011	.237± .002	.219± .005	.238± .003
Emerald Bay	.069	.341	.331± .011	.308± .005	.311± .001	.321± .008	.310± .006	.316± .001	.274± .002
Rubicon Bay	.048	.243	.244± .005	.211± .004	.208± .008	.204± .010	.198± .008	.180± .008	.161± .007
Mid-Lake:									
Mid-lk No.	.048	.205	.212± .010	.196± .004	.195± .008	.183± .001	.173± .004	.155± .004	.138± .017
Mid-lk So.	.044	.196	.184± .001	.172± .003	.176± .008	.184± .008	.178± .006	.180± .009	.141± .001

Appendix 2. Phytoplankton Enumeration Standard Operation Procedure

Appendix 2. Tahoe Environmental Research Center
Standard Operating Procedure

Freshwater Phytoplankton Analysis

Introduction:

Phytoplankton are unicellular microscopic plants that live suspended in natural waters. The abundance and growth of these cells are important to the biological systems in lakes and oceans. While other methods can quantify phytoplankton cells based on physiological components, enumeration, using the microscope, is the only method to reliably identify cells.

This method is called the inverted-microscope method (Utermöhl method) which is based on the gravity sedimentation of lake samples. In a series of steps, particles are concentrated and the surrounding sample water is removed. Phytoplankton cells can then be viewed and quantitatively counted. Taxonomic identification of cells can be accomplished because cells are concentrated gently. Very little disturbance to the cell morphology has occurred due to sample handling. This method does not require expensive specialized equipment for sample concentration.

Procedures described in this document summarize the treatment of samples prior to microscopic examination, including sample preservation, storage, chamber preparation, sedimentation, counting methods and sample disposal.

Particles ranging in size from 1-300 µm can clearly be seen during microscope observation. Often it is difficult to identify cells less than 4 µm and so the method yields the best results on cells larger than this size.

Pre-treatment of Water Samples:

Analysis is run on unfiltered lake water samples. Water is drained into the sample bottles directly from the collection vessels (Van Dorn Bottles). For Lake Tahoe, 100 ml of water from each discrete depth is placed in a clean glass bottle fitted with a lid containing a Teflon liner. Approximately 1 ml of Lugol's solution is added to the bottle, as a preservative, and the samples are tightly capped. Samples can be stored at room temperature for several years, but the best counting results come from samples less than one year old. The acidic Lugol's solution will contribute to degradation over time.

Reagents:

Lugol's Solution - Store in a glass stopcock bottle, room temperature

Iodine crystals (I2) Potassium Iodide (KI)	25g
50 ml Acetic Acid	50g
Distilled De-ionized Water	50ml
	bring to 500 ml

Instruments:
Phase-Contrast Inverted Compound Microscope
Utermöhl Counting Chambers and Settling Towers

Chamber Preparation:

The sedimentation apparatus has two components, the chamber and the settling tower. Once both parts are cleaned and prepared, they are 'adhered' together to form the complete unit. Each chamber is marked with an identification tag which is unique. The towers are also marked, but it is not necessary to match the towers to the chambers.

Sedimentation chambers must be exceptionally clean. The cover glass in the base of the chamber is very fragile. It is essential that the cover glass be free of grease streaks and particulates which interfere with high magnification viewing. Extreme care must be taken when cleaning the cover glass. Wiping the glass with dish soap on a Q-tip®, rinsing with water and drying with a tissue is one option. It is sometimes helpful to use a glass cleaner, such as Windex®, and a tissue for drying, to clean the cover glass.

When the chamber is clean, it is ready for assembly. The topside Plexiglas, forming the outer collar of the settling chamber, should be thinly coated with Dow Corning® high vacuum grease. Set this component to one side and prepare the settling towers.

The settling towers must be wiped down between uses. Sometimes cells and particulates adhere to the towers and can lead to contamination of the next sample. It is helpful to use a small nylon bottle brush soaked in dish soap and water to gently cleanse the towers. Rinse with distilled water. The towers can be air dried or the water can be shaken loose from the towers before assembly. The bottom side of the Plexiglas, forming the outer collar of the tower base, should be thinly coated with Dow Corning® vacuum grease.

The tower and chamber can now be placed together. Set the settling tower on top of the chamber, moving the two pieces slightly back and forth to

adhere the two layers of vacuum grease. Wipe away excess grease from the union of the two components.

Procedure:

Sedimentation

-Record the tower *I* chamber identification numbers as well as the sample information data.

-The sample bottle should be at room temperature. Gently invert the bottle back and forth several times to dislodge cells from the bottom. After sufficient mixing, pour 100 ml (or less) of the sample water into the sedimentation unit. Cover the top of the cylindrical tower with a rubber stopper or glass cover slip. Measure the **height** (cm) of the water column from the base to the meniscus line. **Record this number for each sample.**

-The selection of an 'area' where sedimentation of particles from the sample water can occur is critical to the success of this method. The sedimentation units should be placed on a level surface. The area should be shielded from temperature changes and vibration to discourage convection currents in the towers which will adversely affect the random settling. Depending on the height of the tower, the units should be left undisturbed for at least 48 hours. If 100 ml of sample was settled, it is recommended that a full 72 hours of settling be used.

Un-settling Chambers

-After the required waiting period, the sedimentation units can now be prepared for microscope viewing. The tower (and the remaining sample water) must be removed from the chamber section. This is accomplished by sliding the tower (and water) onto an adjacent sliding plate. A cover plate of glass is used on the right side of the tower to help push the tower onto the sliding plate (to the left). The goal is to move the tower without spilling any water while simultaneously sliding a cover plate on top of the sedimentation chamber. It is important that this process be accomplished with very little disturbance to the sample in the sedimentation chamber. This skill should be practiced numerous times before 'real time' performance.

Counting Methods

-The best microscopic viewing requires that cells are visible with as much definition and resolution as possible. An inverted microscope is necessary, where viewing of the chamber is from the bottom, through the cover glass. The best optical systems for cell identification are with either phase contrast microscopy or differential interference contrast systems (DIC). The microscope should have a full range of objective powers available for use. The oculars should have a micrometer for measuring cells. It is also very helpful to have stage stops where viewing of the chamber is confined to 1cm^2.

-If quantitative counts of the cells are needed, you must track the area of the chamber that is viewed and the magnification used.

-Select a chamber and record the identification tag which links it back to the sample identification. Begin by placing the chamber on the stage, using the 10x objective (low magnification), bring the chamber bottom into focus. View the entire square centimeter of the chamber bottom as follows. Looking through the eyepieces of the microscope, move the stage so that the chamber position is in the upper right corner. Begin moving the chamber directly to the left until the stage stops. Move the chamber down one field of view and go back to the right. Continue this consecutive strip viewing until the entire area is viewed. Make sample notations about particulate concentration and size, zooplankton presence, and the relative health of the phytoplankton community. Count and identify all cells that are large and relatively rare.

-Change the objectives to higher magnification. Typically we use the 40x objective with 10x oculars. There is a 1.5x tube magnifier so the total magnification using this power is: $40 \cdot 10 \cdot 1.5 = 600X$

-At this magnification only a portion of the chamber bottom will be viewed. Counts will be done in strips (transects), where the width of the strip is the field of view and the length of the strip is 1cm. The chamber bottom is round, so the goal is to count 2-4 transects of the chamber. Transects should cross over one another at the center of the chamber. **Note the number of transects counted at this magnification.**

- Identification of cells generally requires the use of taxonomic keys.
Record phytoplankton name identification, counts, and measurements of the cells (when needed). Counting can cease when an arbitrary number of 400 cells has been recorded.

-When doubtful about the cellular identification, it is often useful to make sketches, measurements and photographs to assist colleagues who might assist at a later date.

Calculations:

-The information from the sample counts needs to be entered into the **Phytoplankton Manager** Database. This program has several modules which require information input.

-Sample module: Enter sample identification such as sample name, sample date, depth, id number, group id, sampler name, counter name and the height of the tower chamber. Sample notes can also be entered on this screen.

-Countmetrics: Enter the objectives used and the area of the chamber viewed at each magnification.

-Counts: Enter the phytoplankton identification name, number of cells counted, and the magnification used to make those counts. The program will verify eligible counts by checking against the species dictionary and the biometrics database. Problems arise when new species (previously unrecorded) are being entered or when no measurements exist for cells. The respective modules require additional information before the counts can be validated and entered.

-Sample sets: Samples can be grouped in several ways. Using the sample set module you can define an individual sample or a group of samples. Reports can be issued based on this designation.

-Reports: Various report formats are available and can either be printed or exported to other database management programs.

QA/QC Procedures:

Field Replicate samples are sometimes counted and compared for an estimate of counting precision.

References:

Phytoplankton Manual, 1978, Monographs on Oceanographic Methodology. United Nations Educational, Scientific and Cultural Org. (UNESCO), Page Brothers (Norwich) Ltd. 337pp, ISBN 92-3-101572-9

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