Watershed Management Experiential Learning for USDA Careers Internship (May 2012—May 2013)

Thermocline stability-induced control of a freshwater cyanobacterial bloom: hypereutrophic Mediterranean-climate Pinto Lake (Watsonville, CA)

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Project Outcomes

This project was successful in meeting its goals of further deepening my skillset and experience in project and data management necessary for work in an agency such as the USDA, while also producing important baseline data for resource managers and scientists alike.

In taking on this project, I further increased my proficiency in many important laboratory and field methods used in watershed sciences, limnology, molecular biology, and microbial ecology. I learned how to take this environmental data and perform statistical analyses to interpret and present this data. This internship has greatly furthered my career skills, preparing me to collect, process, and interpret data derived from environmental water samples.

I believe that based on the experience that I have gained that I would be equipped to work with a range of agencies within the USDA, including the ARS, NRCS, and USFS. I would like to continue performing research in the environmental and ecological sciences, using the molecular and ecological toolkit I have developed over this internship period.

Going forward, I hope to deepen my skillset in more specialized molecular techniques, such as optimizing qPCR protocols, and gaining more microbiological techniques as well. I would recommend that future research continue following the sampling design as described, while looking more at the role of nutrient loading from surface water and groundwater in promoting CB in Pinto Lake, while highlighting the importance of thermocline and zooplankton grazing in regulating CB biomass in this hypereutrophic lake.
ACKNOWLEDGMENTS

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**Abbreviations and definitions**

CB= cyanobacteria (formerly know as 'blue-green algae'). Prokaryotic phytoplankton many of which are capable of controlling their vertical movement through the water column, and some of which are capable of diazotrophy (nitrogen-fixation).

CWP= 'Clear water phase'. Defined by the Plankton Ecology Group as a period of intense productivity followed by severe grazing (generally by copepods and cladocerans, especially *Daphnia*) in early Spring, leading to large increases in water clarity and drops in chlorophyll *a*.

DO= Dissolved oxygen (mg/L). Provides a measure of phytoplankton productivity and anoxia.

DOC= Dissolved organic carbon. Dissolved (passing through a 0.45μm filter) fraction of carbon found in water. Also called total organic carbon (TOC).

MCs= Microcystins. A family of hepatotoxic peptides (>80 variants) produced by a range of cyanobacteria, including: Chroococcales (*Microcystis*, *Woronichinia*, etc.), Oscillatorales (*Oscillatoria*, *Pseudanabaena*), Nostocales (*Anabaena*).

N:P ratio= Nitrogen to phosphorus ratio. Nitrogen from ammonia and nitrate are added and divided by the soluble reactive phosphorus in each sample. Used by researchers to determine limiting nutrients, usually N:P<16:1 defined as having nitrogen limitation, above this, phosphorus-limited.

PAR= Photosynthetically active radiation. Light concentration measured in units of Mol·m⁻²·s⁻¹. Light which may be used for photosynthesis, measured at the surface and throughout the water column to determine the average extinction coefficient. Also used to describe light at the surface (incident PAR).

Secchi depth= Depth at which a black and white disk can be lowered at which it can no longer be distinguished. Provides a coarse but reliable measure of incident light, water turbidity, and chlorophyll.

Specific conductivity= Measure of a solution's ability to conduct electricity (µS/cm). Influenced by dissolved salts concentration, including nutrient salts (nitrogen, phosphorus, sulfur, etc).

SRP= Soluble reactive phosphorus. Phosphorus concentration (ppm) determined from dissolved fraction filtered through a 0.45 um filter.

TP= Total phosphorus. Includes dissolved and particulate fractions of whole-water samples, processed by the persulfate digestion method in this study.

TSI= Trophic state index. Definition after Carlson (1977), relating Secchi depth, total phosphorus, and chlorophyll to classify lakes trophic status (productivity).
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Introduction and background

Freshwater supply and quality is an issue of continued and emerging importance, especially in Mediterranean-climate regions around the world. According to climate models developed by the Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment (2004), Mediterranean-climate regions are expected to have decreases in annual precipitation along with large increases in mean annual temperature. These changes, along with increasing population and agricultural needs for water will increase demands on an overtaxed water system in these regions (Cloern et al. 2011). Additionally, climate records indicate that the 20th century was wetter than average, with the current three-year 'record-drought' conditions being mild compared with historical 'megadroughts' lasting 200 years or more (Cook et al. 2004).

Limited supplies of freshwater resources have led to efforts of damming and impoundment of freshwaters worldwide to increase freshwater resources for public, industrial, and agricultural use. These efforts have largely been successful in the last century or more of water resource management, putting aside ecological impacts to freshwater communities of such inadvertent ecosystem engineering. With early indications of decreased snowpack reliability in California, it seems likely that reservoirs will only increase in prominence for water supply solutions. However, in the face of climate change and anthropogenic nutrient loadings, there has been increased reporting of toxic cyanobacterial bloom (CB) events worldwide (Paerl and Huisman 2009; O'Neil et al. 2012) which can impair surface water quality, threatening freshwater reservoirs and the populations depending on them.

The threat to water quality posed by CB is not just theoretical, for instance a major CB event forced officials to cut off the two million residents surrounding the Lake Tai region in China from municipal water for a week in 2011. Similar actions were taken in Toledo, Ohio on the shores of Lake Erie, when a major CB tainted water entering treatment plants and forced a shutoff. Here in California, a major bloom at the 6th largest freshwater reservoir (San Luis Reservoir) increased costs of treating intake water and clearing filtration systems, leading to shut-downs in many water systems during the summer of 2013. Another key recreational, agricultural, and residential reservoir, Clear Lake, has had recurrent, dense CB for at least decades, which have caused aesthetic problems in addition to lost tourism income and increased water treatment costs (Richerson et al. 1994).

Furthermore, CB have the potential to produce a range of toxins, including alkaloid (anatoxin, cylindrospermopsin, saxitoxin) and peptide toxins (microcystin, BMAA) (Cox et al. 2005). Additionally, the sheer effect of large accumulations of cyanobacterial cells can lead to aesthetic impact (scums and odor), exclusion of more palatable green algae and diatoms, pH and dissolved oxygen fluctuations leading to fish kills, as well as increased TOC which can exacerbate internal loading processes.

Pinto Lake, a small (< ½ km²), shallow hypereutrophic lake located near the coast of the Monterey Bay in Watsonville, CA. Historic alterations include installation of a small dam in 1948 and again in 1955 to allow construction of Green Valley Road, as well as application of piscicide (rotenone) in the 1960s to control invasive carp species (Gordon 1966), annual stocking of trout by the CDFW, and state-high concentrations of DDT in fish, as well as high levels of PCBs (Gordon 1966; Mayers 2001; SWAMP, 2009). Seasonal pumping and draw-down for agricultural use was regular in the history of this lake.

Historical sediment loading from periods of logging as well as land-clearing for agriculture have left Pinto Lake with a high level of nutrients in sediments and also contributed to infill which rapidly reduced the depth of the water column (Plater et al. 2006). Along with increased runoff from
burgeoning urbanization in the watershed, which increases potential erosion in the steep, erodible soils of this watershed. There also exists the unquantified potential for groundwater loading through leaky septic systems. The installation of the dam (1948-1955) also decreased the average depth of the lake by flooding upper tributary arms of the lake. Increased sedimentation in the 1950s from agriculture also continues to impact water quality at the lake (Mayers 2001).

Pinto Lake is a valuable recreational resource to residents and surrounding communities, offering boating, fishing, and birding opportunities (CoW 1994). However, aquatic uses are impaired, with swimming being prohibited by city council resolution, and boating requiring waivers against cyanotoxin exposure in the event of capsize.

For at least a decade, the lake has experienced dense, recurrent CB since at least 2005, impairing the aesthetic value of the lake, as well as reducing value to wildlife. These have impaired the lake by virtue of seasonal high pH, low DO, high chlorophyll and 'unnatural scum and foam' (blooms), and hepatotoxic cyanotoxins (microcystin) (SWRCB 2010). These cyanobacterial blooms can drastically alter aquatic food webs, through shading out of other phytoplankton during blooms, as well as through reduced food value to many planktivores.

Dominant CB species at Pinto Lake include *Anabaena*, *Aphanizomenon*, *Microcystis* (and allied Chroococales), as well as *Oscillatoria*. These species can form dense surface accumulations, and are generally believed to be resistant to grazing. Cyanotoxins (toxins produced by CB) documented at the lake include anatoxin, and microcystin LR. Microcystins (the toxin of interest in this study) are hepatotoxic peptides which are toxic in the ppb range, and have had a WHO limit of 1 ppb for drinking water, and preliminary recreational levels of 0.8 ppb set by the state of California (Cal EPA 2012).

In this study, we focus on the role of abiotic and biotic factors in determining the timing and concentration of microcystin levels in the lake over a two-year period from 2012-2013. These factors include (among others) nutrient levels, thermocline strength, specific conductivity, pH, dissolved oxygen, and CB cell counts. Sampling was conducted on continuous weekly basis from April 2012-December 2013.

We use non-parametric (Spearman Rank) correlation to determine the relationship between variables over time. We then performed Principal Components Analysis (PCA) to display associations between key variables and toxicity. Finally, we generated a generalized linear model (GLM) for 2012 and 2013 to model (and potentially predict) the response of toxicity to various predictor variables.

As a brief characterization of the differences between years, 2012 was a period containing highly toxic, dense CB, while 2013 had a much lower bloom density and toxin production. We put forth that the reason for these contrasting observations is due to displacement of thermocline in 2012, releasing CB from nutrient limitation. The year 2013 had a strong, nearly undisturbed thermocline which induced raised N:P ratios, limiting CB density overall, and thus lowering toxicity during that year.
Results

Summary statistics comparing the 2012 and 2013 bloom seasons are presented below (Table 1). It is important to note that the yearly minima (0, 3.0·10⁵) in CB count for 2013 were observed on consecutive sampling dates in May, while zero counts in 2012 were only observed in January. Chlorophyll $a$ was much greater in 2012 than in 2013, with a greater mean and range.

Table 1. Summary statistics for 2012-2013 (Dock)

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<td>MC (ppb)</td>
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<td>SpCond (μS/cm)</td>
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Biotic parameters

Cell counts

We tracked the concentrations of bloom-forming CB species (*Oscillatoria*, *Anabaena*, *Aphanizomenon*, and *Microcystis*) using the modified Utermohl protocol with an inverted-scope. Counts were averaged over each month of the study period. *Aphanizomenon* was the taxa with the highest overall abundance in both 2012-2013 averaging $4.6 \cdot 10^7$ and $8.6 \cdot 10^4$ (cells·ml$^{-1}$) in 2012 and 2013, respectively. It is important to note that the sample size for 2013 exceeds that of 2012 by ~50% (n=23, 36 respectively) and may therefore be more informative at this time with respect to trend analysis.

An aspect of note is that the late 2012 bloom extended almost entirely into 2013 (Figure 1), with the *Microcystis* bloom extending into February, being replaced by an early bloom of *Anabaena* in March 2013 (data not shown). This extended, nearly continuous, year-round CB is not a typical phenomena for this lake, where blooms generally dissipate by December or January, at the latest for years 2009-2012. This period of low or absent CB usually extends until April as may be observed in 2012, but not 2013.

A key insight gained from the Spearman correlations was that *Aphanizomenon* was strongly positively associated with thermocline strength in 2012 ($\rho = 0.79$), and less so in 2013 ($\rho = 0.35$) (Tables 2 and 3). Changes in deltaT appear to have a strong direct effect on *Aphanizomenon* density in 2012, and to a smaller degree in 2013. It would appear that though thermocline strength was stronger in 2013, *Aphanizomenon* did not attain as high density as 2012. This may be explained by multiple factors, including the two of the clear water phases (late May and late June), and via thermocline disruption (late July-early August and late October) both of which appeared to prevent *Aphanizomenon* from attaining mean density comparable to 2012 ($\mu_{2012} = 4.6 \cdot 10^7$; $\mu_{2013} = 8.6 \cdot 10^4$ cells·ml$^{-1}$). Figure 2 (below) demonstrates the strong correspondence between *Aphanizomenon* density and thermocline strength (deltaT), which had not previously been documented for this lake.
Figure 2. Correspondence between thermocline development and *Aphanizomenon* dominance in 2012-2013
Toxin concentration

Intracellular MC concentrations for the 2012-2013 bloom seasons are plotted below. Average toxins were higher in 2012 than 2013 ($\mu_{2012} = 39.4$ ppb, $\mu_{2013} = 0.88$). Clear periods of elevated toxin appear in 2012 were: (1) June, (2) late July-early August, (3) late September, with a secondary, smaller peak in toxicity from (4) October-December 2012. (1) Mid-March-Early April, (2) Mid-April-Early May, (3) Late May-Early July, and secondarily from (4) early November to late December 2013.

Periods of elevated toxicity in 2013 were: (1) early April, (2) early May, (3) mid-June, (4) early July and secondarily from (5) mid-October to mid-December.

Though timing of toxin peaks was shifted about two months earlier in 2013 than 2012, the years shared the late October to late December secondary peak in toxicity. This secondary peak also appeared reliably in previous years (2009-2011), along with a peak in Microcystis density. Therefore it may be surmised that a late summer to early Winter peak in toxicity may be reasonably expected in every year.
Chlorophyll a

Chlorophyll a concentrations (ppb) at the Dock and averaged open-water samples are plotted below. When comparing these graphs, it is crucial to keep in mind that Dock concentrations of phytoplankton, especially bloom-forming cyanobacteria are highly influenced by wind direction and strength in the period preceding sampling, and therefore are less robust to this stochastic phenomena than averaged open-water chlorophyll a. These graphs clearly illustrate high winter concentration (diatom and green algae bloom) in January 2012, which declines with declining nutrients and a stabilizing water column in March and April 2012.

Chlorophyll concentrations rapidly intensified in April and May 2012 (150 ppb), then drops off quickly in early to mid-June (<5 ppb). This dropoff in chlorophyll will need to be explored further in future work. By late June chlorophyll again attained high concentrations sustained high chlorophyll levels (~110 ppb). In early July, however, there was another sustained drop in surface average chlorophyll (<60 ppb) through the remainder of July.

By August, chlorophyll increased again to 100 ppb, remaining above 80 ppb until August 8\textsuperscript{th}. Chlorophyll remained below 45 ppb until the end of the month. By September, chlorophyll quickly rose to the 2012 bloom season peak (>250 ppb) near the end of September. By late September to mid-October, chlorophyll concentrations rapidly cycled between 100 and 150 ppb.

At the end of October, chlorophyll declined to around 60 ppb, remaining at this level until early-November. By mid November, chlorophyll increased to just over 200 ppb, then falling through early December to 16 ppb. Finally mid-December saw a gain in chlorophyll to ~100 ppb, which fell by the end of December to 14 ppm.

Winter minima in 2013 is visible, though based on cell counts it may be that Microcystis density had been overestimated for January 2012 and February 2013. Following the winter minima, chlorophyll rose in April to >60 ppb, continuing to elevate until late April ~200 ppb. There was a sharp drop-off by the end of April, a phenomena which is likely representative of a clear-water phase (CWP), discussed in detail further in the context of DOC observations below.

Chlorophyll rebounded from this CWP to its open-water maxima for 2013 in early May (240 ppb), but by late May, there was a second CWP, which again depressed chlorophyll a concentrations (under 35 ppb) until nearly mid-June. By mid-June, chlorophyll levels again built up to high levels ~200 ppb, before plunging in late June to ~15 ppb in another CWP.

Chlorophyll levels rebounded again to ~80 ppb, fell for three straight weeks (through late July), until finally rising again to >80 ppb at the end of July followed by frequent swings between 12 and 30 ppm until a late season peak of ~40 ppb at the end of October, whereupon chlorophyll levels varied between 13 and 26 ppb until the end of the year.
Figure 5. Averaged open-water (B1-B3) chlorophyll $a$ concentration
Abiotic parameters

Nutrients

**Dissolved N:P ratio**
Nitrogen to phosphorus ratio has been considered a key parameter in water quality monitoring, especially in eutrophic waters, where low ratios have been associated with CB. N was calculated as a composite (ammonia + nitrate). Mean N:P ratio was higher in 2013 than 2012 ($\mu = 15.2$, 12.3; CV = 1.47, 1.36) indicating a possible role for phosphorus as a possible limiting resource to growth in that year.

Figure 6. N:P ratio average across surface samples (B1-B3)
Figure 7. Ammonia concentrations (average ppm) in open-water (B1-B3) samples

Figure 8. Nitrate concentrations (average ppm) in open-water (B1-B3) samples

Figure 9. Soluble reactive phosphorus (SRP) concentrations (average ppm) of open water (B1-B3)
Hydrographical parameters

Secchi/PAR

Correlation between Secchi depth and PAR is displayed below. There is good agreement between these data, however, PAR is a more precise tool for prediction of cyanobacterial biomass and overall toxicity.

![Figure 10. Extinction coefficient (average between 0-3m) vs. Secchi depth relationship](image)

Figure 10. Extinction coefficient (average between 0-3m) vs. Secchi depth relationship

To see whether extinction coefficient in open water was predictive of the amount of chlorophyll \(a\) in the water column, we plotted this data against chlorophyll \(a\) at the Dock (Figure 16). It appears that there are two distinguishable clouds of data, with values of chlorophyll \(a\) (>100 ppb) being more scattered with regard to extinction coefficient. Note that only data used in statistical analyses is presented here.

![Figure 11. Open-water extinction coefficient versus chlorophyll \(a\) (Dock)](image)

Figure 11. Open-water extinction coefficient versus chlorophyll \(a\) (Dock)

Because the data were scattered in distribution, we decided to log the chlorophyll \(a\) concentration. To test the predictive power of PAR against chlorophyll \(a\), we performed a simple least-squares regression (below). There appeared to be good agreement \(\left(r^2 = 0.56\right)\) between log\(_{10}\) chlorophyll \(a\) and extinction coefficient, in line with expectations. This means that higher chlorophyll concentrations, are associated with higher extinction coefficients, and therefore shallower photic depth. It may also be worth
exploring seasonality of the data, correlations with CB cell density in the water column, as well as deviations between extinction coefficient and Secchi depth.

Figure 12. Open-water extinction coefficient versus $\log_{10}$ chlorophyll $a$ (Dock)

**PAR**

Incident PAR intensities (above water surface) at each sample visit are plotted below (Figure 18). It is very important to keep in mind that surface PAR (unlike extinction coefficient) is a highly stochastic measurement, entirely dependent on a single data point dictated to a large degree by time of visit, reflectivity of the lake surface, cloud cover, and overall light intensity. Data are missing for the Winter (2012-2013) due to equipment failure and repair time. Nonetheless, it appears that there are no significant differences between 2012 and 2013 PAR means ($\mu_{2012} = 2134$, $\mu_{2013} = 2180$).

Figure 13. Incident PAR at lake surface (2012-2013)
Secchi depth

Secchi depth is calculated as the depth at which a black and white disk is lowered into the lake until the depth at which the difference between colors is no longer distinguishable. Secchi depths are influenced by incident light, water color, dissolved organic matter, and suspended particulate matter (including phytoplankton). Overall Secchi depth is used as a proxy for water clarity or overall phytoplankton productivity.

Conspicuous trends include high Secchi values (1.4-3m) during the cool, nutrient-rich months (Jan-Mar) coinciding with rainfall, decreasing. An important ecological observation is the existence of a dramatic clearing of the water column in late April 2013, as well as a smaller increase in Secchi depth in June 2013.

Figure 14. Secchi depth for 2012-13 at Buoy 2.
**DeltaT (thermocline)**

Thermocline was calculated using a simple measure of difference in temperature (DeltaT) between surface and bottom of the water column (Miller et al. 2013). Weekly observations as well as average DeltaT are plotted below.

![Graph showing DeltaT changes over 2012-2013](image)

Figure 15. Detail of thermocline (deltaT) changes over 2012-2013 with mean value line plotted

There are clear differences in thermocline strength, onset, and duration between 2012 and 2013 ($\mu_{2012} = 4.4^\circ C$, $\mu_{2013} = 5.9^\circ C$).

Comparisons of surface, bottom and difference in temperature (deltaT) are plotted below. Differences can be noted between when temperatures between the surface and the bottom of the lake diverge, as well as the overall stability in bottom temperatures in 2013 compared to 2012. Periods during which bottom temperature increased noticeably coincided with increases in surface nutrient concentrations, thus lending support to the idea that thermocline controls surface nutrient concentrations during the dry months.

![Graph showing temperatures at surface and bottom](image)

Figure 16. Temperatures at surface and bottom, with difference (deltaT) plotted
Benthic dissolved oxygen (DO)

A time-series plot displaying oxygen at the bottom of the lake is plotted below. There are clear differences between 2012 and 2013, with a higher average oxygen percent saturation for 2012 versus 2013 ($\mu_{2012} = 30.6\%$, $\mu_{2013} = 11.9\%$). Low benthic dissolved oxygen has been demonstrated to be key in nutrient cycling processes from bottom sediments. However, the particularly robust thermocline of 2013, as demonstrated in the section on deltaT above, prevented mixing with bottom waters, which can be clearly seen in Figure 27, with no disturbance of the anoxic bottom waters from February until mid-October.

Figure 17. Dissolved O$_2$ (percent saturation) at lake bottom (2012-2013)
Spearman rank correlation results

In order to better understand the relationship between measured variables at the lake to CB biomass and toxin potential, we performed a non-parametric statistical analysis (Spearman Rank Correlation). Years were analyzed separately based on the *a priori* knowledge that 2013 bloom progressed in a significantly different fashion to 2012 and previous study years.

Results of the Spearman Rank Correlation statistical analyses for 2012 and 2013 are presented below in table form. Key differences in water quality were noted between 2012 and 2013. As discussed earlier, MCs were higher in 2012 than 2013 (μ2012 = 39.4 ppb, μ2013 = 0.88ppb) We set the significance level at p≤0.10 prior to analysis based on high natural variability (noise) inherent to ecological and population monitoring data.

A few key correlations to highlight in the 2012 dataset are the strong association between *Microcystis* and toxin (p=0.01; ρ=0.53), which was expected from previous years' experience, as well as literature observations. *Microcystis* was also strongly correlated to both specific conductivity, as well as benthic DO (p<0.003, 0.02; ρ=0.62, 0.50). These measures themselves were strongly associated with disruption in the thermocline allowing oxygen to reach the benthos and increase dissolved nutrients (and thus specific conductivity) in surface waters, allowing *Microcystis* populations to grow. Changes in conductance have been used as predictor variable in models of *Microcystis* density in Lake Tai, China (Tao et al. 2012).

Interestingly, both *Aphanizomenon* and *Anabaena* were also correlated to toxin (p<0.01, 0.05; ρ=0.55, 0.42). These correlations may be indicative of the dynamic nature of surface nutrients and thermocline during this year, which induced rapid changes in taxa and relative percent composition such that all CB appeared to be correlated to MC production in this year.

The correlation between toxin and N:P ratio (p=0.02; ρ=-0.53) is especially notable, since *Aphanizomenon* is also negatively correlated to N:P ratio (p=0.005; ρ=-0.56). This means that periods when N:P ratios are high are associated with periods of reduced *Aphanizomenon* dominance, as well as reduced toxin potential. A similar relationship exists between toxin and pH as well as *Aphanizomenon* and pH (p<0.001, 0.004; ρ=0.60, 0.64).
Table 2. Spearman rank correlation results (2012); significant p≤0.10 level= **Bold**; significant at  p≤0.05 level= *Bold italics*

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<th>Parameter</th>
<th>Log ana</th>
<th>Log alpha</th>
<th>Log micro</th>
<th>Chl</th>
<th>deltaT</th>
<th>Secchi</th>
<th>PAR</th>
<th>DO surface</th>
<th>TOC</th>
<th>TP</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>SRP</th>
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Some key results from correlation analysis of the 2012 dataset are between parameters related to thermocline with those factors controlled by thermocline, which in turn are correlated to determinants of toxin production. One such association exists between deltaT and SRP (p<0.0001;  p=-0.64). This correlation provides support for the hypothesis that 2013 was a year in which CB was at least partially limited by SRP concentrations. This association is demonstrated in the relationship between N:P ratio and *Microcystis* (p=0.002;  p=-0.49) which was not present in 2012. Additionally, deltaT in 2013 was strongly negatively correlated with SRP in 2013 as well (p<0.0001;  p=-0.64).

Table 3. Spearman rank correlation results (2013); significant p≤0.10 level= **Bold**; significant at  p≤0.05 level= *Bold italics*
Streamflow and nutrient concentrations

**Tributary nutrient loading**

Potential nutrient loading from the watershed was calculated through sampling of the four major tributaries of Pinto Lake. These include: Todos Santos Creek (TS), Cal Conservation Corps (CCC), Amesti (AM), and Pinto Creek (PC). Most of these creeks were intermittent in discharge, with CCC and TS being the most frequently flowing tributaries. These stream nutrient loads are not displayed due to paucity of data.

Potential load for each nutrient measured was calculated as:

\[
\text{Load (g·day}^{-1}) = \left(\text{Concentration (mg·L}^{-1}) \cdot \text{Discharge (L·day}^{-1})\right) \cdot 10^{-3}
\]

Samples were collected during each regular sampling date, and therefore did not take into account rising or falling limb of streamflow. Tributary nutrient loading for 2012 were not calculated due to a combination of lack of flow and absence of flow data.
Figure 18. Nutrient loading at CCC Creek in grams·day$^{-1}$

Figure 19. Nutrient loading at TS Creek in grams·day$^{-1}$ (note: log$_{10}$ y-scale)
Phosphorus load was calculated using total phosphorus (TP) data. Estimates of phosphorus load for April 2014 may be underestimated because TP has not yet been determined for these dates because they fell outside of the study period. Typically, TP:SRP ratio ranges between one to three.

Counter-intuitively, increased flows were associated with decreases in proportion of particulate phosphorus (PP = TP-SRP) for CCC and TS. There was no clear association between flow and ammonia concentration for TS, however, increased flow was associated with higher nitrate concentrations for this tributary. The same was true for CCC, except that the positive association between nitrate concentration and flow was not as strong as for TS.

Based on these load analyses, it appears that though flow at TS is more intermittent than at CCC, a combination of higher total discharge and turbidity (due to high suspended sediment concentration) led to higher amounts of load (especially that of phosphorus and ammonia) during precipitation events, leads to higher relative contribution of TS relative to CCC.

Nutrient concentrations at the other tributaries (AM, PC) are presented below. Again, loads were not calculated for these tributaries owing to time constraints for streamflow velocity measurements in the case of Amesti, and diffuse flows at Pinto Creek. Based on the data, it appears that any potential loading is likely dominated by nitrate for these tributaries, as it exceeded 1 ppm on several occasions. Amesti also demonstrated potential TP loading, (TP = 1 ppm) in December 2012.
Principal Components Analysis (PCA)

Results of PCA analyses for 2012 and 2013 are presented below. Briefly, PCA is a statistical method using basic matrix algebra to transform multiple variables in a way that reduces the number of dimensions while highlighting covariance between variables. In other words, it allows visualization of correlations which may otherwise be hidden in a dataset. Line length indicates strength of relationship between variables, while direction indicates sign of relationship. For example, in 2012, *Microcystis* cells were strongly correlated to specific conductivity, while toxin was inversely proportional to N:P ratio.

In performing the PCA, we attempted to only include variables which likely had a mechanistic impact on toxin potential, while also not being directly correlated to each other.

![Correlation biplot](image)

Figure 20. PCA of biotic and abiotic factors associated with MC toxin at Pinto Lake in 2012

Principal components (axes) 1 and 2 explain 35 and 23% (cumulative proportion=58%) of variability in the dataset respectively. The remaining two axes (not shown) explain an additional 28% of variability in the 2012 dataset.

In 2012, *Microcystis* has a strong relationship with specific conductivity, a scenario that is likely due to the relationship between disturbance in the thermocline and increases in surface water conductance in this year. Toxin was also negatively associated with N:P ratio, meaning that at points when phosphorus is more depleted relative to nitrogen, toxin potential is higher.
Principal components (axes) 1 and 2 explain 41 and 21% (cumulative proportion=62%) of variability in the dataset respectively. The remaining two axes (not shown) explain an additional 24% of variability in the 2013 dataset.

Between years, it is of note that toxin is inversely related to N:P ratio, in line with the Spearman Rank correlation analysis. In this year the PCA makes clearly evident that *Microcystis* is largely associated with decreases in light penetration (PAR), while also being strongly inversely proportional in magnitude to increased N:P ratios.

![Correlation biplot](image)

Figure 21. PCA of biotic and abiotic factors associated with MC toxin at Pinto Lake in 2013
**Generalized linear model**

We assembled generalized linear models with a Poisson regression for prediction of MC concentration at Pinto Lake using stepwise (backward and forward) AIC for the 2012 and 2013 bloom seasons. Again, we made *a priori* decisions on which variables to include (using relevant literature, experience, and observations) before regression analysis to avoid data mining.

Model predictions versus observations are plotted below.

**Model 2012**

\[
\log_{10}(MCs) = -12.5 + 0.30 \cdot \log_{10}(\text{Micro}_\text{Cells}+1) + 0.27 \cdot (\text{DeltaT}) + 2 \cdot (\text{pH}) - 0.06 \cdot (\text{N:P})
\]

Figure 22. Agreement between predicted and observed toxicity (2012) of the generalized linear model (GLM)
Model 2013

\[ \log_{10}(MCs+1) = -0.3 + 0.22 \cdot \log_{10}(\text{Micro\_Cells}+1) + 0.30 \cdot \text{ExtinctionCoefficient} \]

Figure 23. Agreement between predicted and observed toxicity (2013) of the generalized linear model (GLM)

It is clear that toxin model fit for 2012 is superior to that of 2013. Reasons for this include a greater range of toxin values (especially non-zero values) and cell counts for 2012. Additionally, 2013 had evidenced four CWP which disrupted bloom progression, and would by necessity disrupt any model predictions based primarily on Microcystis density. While toxin was overestimated during several periods in 2013, the model did correctly predict most of the detectable toxin observations for that year.

We also developed a simpler model for use by the City of Watsonville for practicability and use for typical years (2012), as well as possible cases of future years similar to 2013.
Simplified model (2012):

\[ \log_{10}(MCs+0.7) = -6.5 + 0.29 \cdot \log_{10}(Micro_{\text{Cells}}+1) + 0.34 \cdot PAR + 1.04 \cdot pH \]

Figure 24. Agreement between predicted and observed toxicity (2012) of the simplified generalized linear model (GLM)

This simplified model provides good agreement between predicted and observed log MCs, albeit more underestimates in toxicity.

Simplified model (2013):

\[ \log_{10}(MCs+0.7) = -1.62 + 0.53 \cdot \log_{10}(Micro_{\text{Cells}}+1) + 0.004 \cdot \text{chlorophyll } a \]

Figure 25. Agreement between simplified model of toxicity and observations (2013)

While this simplified model underestimated toxin in several instances, again it does provide some certainty in the periods during which toxicity may be expected, if not the exact magnitude of that toxicity. Therefore, this model provides information on periods when the lake should be monitored more closely, as well as helping to focus public health efforts during those periods.
Discussion/Recommendations

Comparisons between 2012 and 2013

CB density, chlorophyll and toxin concentrations were lower overall for 2013 compared with 2012. Surface water temperature were higher during the bloom period in 2013, while SRP was significantly lower. Additionally, while peak Microcystis density occurs in the Autumn months, peak toxicity is coincident with the Spring and Summer months.

These results highlight the question of which are the key driving factors behind cyanobacterial biomass and toxicity in this hypereutrophic lake. Based on the results of the statistical analysis, Microcystis biomass had a strong negative correlation with increasing N:P ratio in 2013, but not 2012. These results may point to the effect of relatively nutrient-replete growing conditions in the lake in 2012 as compared to 2013. This relative nutrient limitation in 2013 was in turn probably a result of early onset of a strong thermocline, which was undisturbed until the late summer months.

Further, it appears that there are distinct temporal patterns in toxin production which may themselves serve as strong predictors of toxin potential at the lake. These include pH, which was itself strongly correlated to Aphanizomenon density in both years. High pH is itself generated when water column CO2 is depleted through photosynthetic production, largely controlled by Aphanizomenon in both years.

Role of grazing in limiting toxic potential of CB

Preliminary data show support for the coupled role of high thermocline strength and strong periodic zooplankton grazing in limiting CB in 2013.

Both of these results are surprising given the relative shallowness of the lake, as well as the key role of herbivory which Daphnia and other grazing crustaceans appeared to fill in 2013 as compared to previous years (including 2012). This is in contradiction to some studies which showed that high concentrations of CB and MCs severely limit the ability of Daphnia to feed and reproduce (Carpenter et al. 2001).

However, according to Chislock et al. (2013), previously-adapted genotypes of Daphnia were able to graze down high levels of toxic cyanobacteria (100 ppb MCs) by 80% in large in-situ enclosure experiments. Additionally, Sarnelle and Wilson (2005) were able to demonstrate adaptation of Daphnia pulicaria to toxic cyanobacteria in the laboratory. Finally, Hairston et al. (1999) isolated and hatched Daphnia ephippia isolated from different sediment depths (and hence depositional period) in a lake for which a record of the differing concentrations of cyanobacteria and toxins were known. Hairston and colleagues were able to demonstrate that Daphnia originating from periods when cyanobacterial blooms and toxin were common were more able to resist negative effects from cyanotoxin exposure, thus demonstrating the role of genotype and pre-adaptation in Daphnia response to toxic CB.

The Plankton Ecology Group (PEG) model (Sommer et al. 1986) predicts an early spring bloom of phytoplankton, closely followed by a short-lived bloom of zooplankton grazers. This bloom of grazers is said to produce a “clear-water phase” (CWP), which dramatically reduces phytoplankton density to levels which increase water clarity, while also limiting their own population through over-exploitation of food resources (Sommer et al. 1986). CWP also may occur in the autumn months as well, owing to decreased fish predation on zooplankton, and increasing algal production from due to increased nutrient
availability caused by mixis.

Climate change has been hypothesized to strongly influence phytoplankton and lake trophic dynamics as it occurs. Effects on phytoplankton include earlier bloom of green algae and diatoms, due to early heating of the water column and associated increases in growth rate at higher temperatures (Edwards and Richardson 2008). This temporal shift due to higher spring temperatures appeared to be the case in March 2013, with the appearance of total CB in densities not normally seen until April or May, as well as temperatures in surface waters not attained until mid April in 2012.

In line with thermal and phytoplankton observations, Winder and Sommer (2004) found evidence for the advancement of the spring diatom bloom as well as thermal stratification by over 20 days. In contrast to their findings, as well as those of Edwards and Richardson (2008) however, it would appear that in 2013, the Daphnia bloom was especially well-timed and not experiencing the “temporal mismatch” predicted by changing climate and warmer springs. These findings may be a result of surface temperatures not reaching a critical low, allowing, according to Sommer et al. 2012 “ only overwintering active populations of zooplankton or early emergence from diapause [yielding] substantial grazing pressure during the onset of the phytoplankton spring bloom”.

In 2013, it appeared that the zooplankton bloom was well-timed relative to the phytoplankton bloom, which was itself early relative to PEG model predictions, but in accordance with predictions of altered phenology by researchers in climate biology (Sommer and Lengfellner 2008; Richardson 2008). According to Winder and Schindler (2004), climate change has been anticipated to possibly cause a mismatch between zooplankton grazers, particularly Daphnia and other planktonic crustaceans. However, according to our observations of early and sustained onset of strong stratification, early phytoplankton bloom with associated zooplankton and CWP, this was not the case in Pinto Lake.

Interestingly, one of the major clear-water phases late May 2013 associated with a high amount of DOC coincided with low dissolved O$_2$ (26% sat. at surface), as well as observations of very high densities of copepod and Daphnia grazers, as well as hundreds of dead minnows at the dock. There was also a large increase in the amount of ammonia (0.05 to 0.79 ppm) and large decrease in chlorophyll (~200 ppb to 1 ppb) from the previous sampling dates to this one. According to Lampert (1977), between 10-17% of DOC in phytoplankton cells may be released during the feeding process of Daphnia during peak grazing events (when all primary production is being consumed).

It may therefore be hypothesized that DOC and other nutrients (including ammonia) is released from phytoplankton cells by grazing Daphnia (through inefficient feeding and faeces). Further, it may be that this DOC increased to biological oxygen demand (BOD) in the lake, thus consuming oxygen in the water column. Along with decreases in dissolved O$_2$, increases in ammonia may have caused a die-off in the most vulnerable fish populations (i.e. young-of-year minnows). It may be release from YOY fish predation which allowed Daphnia and other zooplankters to persist into the summer, as predicted by Scheffer et al. (1997).

Strong stratification may have also limited key micronutrients (Fe, S) from diffusion to the photic zone from benthic sediments, thereby limiting overall cyanobacterial biomass in 2013. This hypothesis may be tested in future study using ion chromatography on archived (frozen) filtered samples.

PAR, and to a lesser degree Secchi depth, may be indicative of overall toxicity because they provide a more representative measure of the overall density of MC-producing cells (i.e. Microcystis) in conjunction with cell counts from surface sampling. There are several compelling studies which point
to *Microcystis* density itself as being a possible factor in up-regulation of MC production (Wood et al. 2011). Additionally, mean colony size has been positively correlated with increased proportion of microcystin-producing genotypes, as well as per-cell microcystin content (Kurmayer et al. 2003).

**Tributaries and nutrient loading**

Based on the watershed study, it appears that the major source of nutrients does appear to be internal loading. Nutrients derived from surface flows over time will, however become important to any future nutrient remediation projects at the lake, including biomanipulation, alum application or selective dredging.

Based on the load data, it would appear that riparian restoration and installation of sediment retention basins along the TS corridor would be the most effective use of resources, given the relatively high phosphorus concentrations, as well as suspended sediments at high flows from this subwatershed. Additionally, the TS arm of the lake appears to be the most impacted by aggradation, which is also in line with observed high peak-flow suspended sediment concentrations at this tributary.

**Future work**

Because of the strong agreement between both simple and more complex models of MC toxin potential in 2012, I would recommend that we apply this model to previous years' data as well as performing cross-validation estimates of predictive accuracy for this model to test whether it is robust to external sample points.

In exploration of chlorophyll *a* trends, it was discovered that Microcystis biomass may have been overestimated for the months of January and February. This possibility will need to be explored through increased counts (at least three per month) for 2012, which will be performed in future work. It is highly probable that this will increase precision in toxin model predictions, since *Microcystis* is the strongest predictor of MCs. Additional cell counts and toxin determinations will be made for 2012 to better determine which factors are responsible for *Microcystis* abundance and toxicity on a per-cell quota basis.

Production of a GLM for *Microcystis* density would be possible using the dataset created for this report, and would be valuable for predicting conditions under which potentially toxic blooms are most likely to form.

More sophisticated measures of nutrient loading from internal cycling may be produced given that nutrients have been determined from the surface to benthos of the lake during regular sampling dates.

Finally, and perhaps most compelling would be to perform an analysis of zooplankton abundance using preserved refrigerated samples for those periods which have been identified as CWP to find evidence to support or reject the hypothesis of CWP being due to zooplankton pressure in 2013.
Citations


Kurmayer R, Christiansen G, Chorus I. 2003. The abundance of microcystin-producing genotypes


Appendix A. Additional figures

Fig 1. Map of Pinto Lake, sampling buoy, and approximate tributary sample locations (Dock, B1-B4)
Fig 2. Mixed bloom with >30ppb MCs (Dock, October 2012)
Figure 3. Agarose gel electrophoresis of cyanobacterial 16SrRNA gene (450bp) with 500 bp ladder

Figure 4. Agarose gel electrophoresis of cyanobacterial mcyB gene (320 bp) with 500 bp ladder
Figure 5. Clear water phase at Pinto Lake, April 2013 (note: many YOY minnows on right)

Figure 6. Thousands of *Daphnia* in macrozooplankton bloom concurrent with clear water phase, April 2013
Macro-zooplankton counts

Figure 7. Autumn bloom of macro-zooplankton with Secchi depth and CB counts (Dock)

Figure 8. Hundreds of macro-zooplankton per liter on a glass fiber filter (May 2013)
Figure 9. Detached *Oscillatoria* mats
Figure 9. Detached *Oscillatoria* mats

Figure 10. Todos Santos Creek discharge (April 2014)
Figure 11. Preliminary watershed land use/land cover analysis based on National Land Cover Database (2006)
Figure 12. Pinto Lake outlet during CB (December 2012)
Table 1. Trophic state index calculations (after Carlson 1977)

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Appendix B: Methods

A. CHAB and water quality monitoring

We measured *in situ* environmental parameters by calibrated DS5X sonde (Hach Environmental), LI-250 light meter and LI-193 quantum sensor (LI-COR), and Secchi disk. Water quality parameters are photosynthetically active radiation (PAR), pH, specific conductivity, DO, depth, and water temperature through the water column at Buoy 2 at Pinto Lake.

For laboratory analyses, surface samples were taken using one liter wide-mouth amber glass bottles, with discrete, 1-meter interval depth samples (2012) and 1.5 m interval (2013) collected using van Dorn depth sampler to the lake bottom. Samples were stored in an insulated cooler to protect from heat and light until processing (within 24 hours) and analysis within appropriate holding times.

Sample processing took place as follows, using the 1L of gently homogenized sample:

- 50 ml of unfiltered sample transferred to 50ml polypropylene cylindrical centrifuge tubes (Falcon tubes) with 7-9 drops of Lugol’s iodine solution to preserve, settle, and stain cyanobacterial cells, with storage at 4°C for at least 24 hours prior to cell counts.
- 100ml sample poured into a polycarbonate bottle, acidified using 1-2 drops of sulfuric acid, and prior to total phosphorus (TP) determination.
- 250ml is filtered through a 0.7 μm glass fiber filter (FisherBrand) and the filter placed in a foil-wrapped 15ml Falcon tube filled with 90% acetone (Holm-Hansen and Riemann, 1978) and stored at -20°C prior to chlorophyll *a* determination.
- 250ml is filtered through a 0.7 μm glass fiber filter (FisherBrand) and the filter placed in a foil-wrapped 5ml glass collection tubes (FisherBrand) stored at -20°C before toxin analysis using enzyme-linked immunosorbant assay (ELISA) (EnviroLogix).
- 200 ml of filtrate from chlorophyll *a* and cyanotoxin filtering is filtered again through 0.45um polycarbonate filters with 125ml stored in polycarbonate bottles stored at -20°C prior to dissolved nutrients analysis and 75ml acidified and stored in glass bottles at 4°C prior to dissolved organic carbon analysis.

We enumerated cyanobacterial cells by microscopic enumeration using the rapid Utermöhl method. This method required collection of the 1ml cell concentrate from the 50ml Lugol’s preserved sample. Sub-sample (1ml) was pipetted from the bottom pellet and placed in a Sedgwick-Rafter (counting cell) with a glass cover slip. Using an inverted microscope (Olympus IX71) and associated software, 12 fields in a grid pattern were photographed. We then quantified lengths and areas (for filamentous cyanobacteria and colonies of single cells, respectively) with minimum dilutions made when cells exceed counting capacity. Count data were entered into a spreadsheet of cell measurements to estimate total cyanobacterial cell count in cells per milliliter using the “natural unit method” into formulas derived from AWWA standard methods (AWWA 2005).
Cyanotoxin determination for microcystins (MC) were made using enzyme-linked immunosorbant assay (ELISA) on select surface samples. Two samples for ELISA analysis were selected for each sample date, with one Dock sample and an open-water sample (data not reported). Samples were run in duplicate wells with appropriate blanks and positive and negative controls.

Macro-zooplankton were quantified on a 0.70 um glass fiber filter and counted by eye.

B. Molecular Analyses
Molecular analyses include evaluation of the presence of microcystin synthetase (mcyB and mcyE) genes by polymerase chain reaction (PCR), targeting general microcystin cyanotoxin gene fragment (mcyB) and taxa-specific toxin gene fragment (mcyE) for distinguishing toxin gene presence between Microcystis and Anabaena. PCR results will help determine the diversity and toxicity of MC-producing cyanobacteria in Pinto Lake.

I performed PCR on selected surface samples using previously established primers to amplify cyanobacteria-specific 16S rRNA gene (Nübel et al. 2002) and microcystin-synthetase gene mcyB and mcyE (Nonneman and Zimba 2002; Rantala et al. 2006). I used 16S rRNA gene primers to detect a broad array of cyanobacteria in Pinto Lake as well as detect possible (naturally-occurring) PCR inhibitors in environmental samples. PCR primers targeting 16S rRNA gene include reverse sequences with degeneracy in the sequence to optimize binding to the majority of cyanobacterial 16S rRNA gene (Nübel et al. 2002). We also used mcyB primers to ascertain potential for microcystin production. The mcyE primer is used to differentiate between taxa (Microcystis and Anabaena) producing the toxin genes. Each PCR reaction was paired with a positive control (purchased cyanobacteria culture) and a negative control (sterile molecular-grade water) to rule out contamination or PCR inhibition.

Cyanobacterial DNA was extracted from cell samples collected on polycarbonate (0.2μm pore size; FisherBrand) filters. Cyanobacteria extraction was performed using the Qiagen DNeasy Plant Kit (Germantown, MD) with additional bead-beating, freeze-fracture and proteinase-K digestion to ensure cell lysis. Resulting DNA were held at -20˚C before PCR. PCR reactions contained a final concentration of 1x PCR buffer, 2 mmol·l⁻¹ MgCl₂ buffer, 800 μmol·l⁻¹ deoxynucleoside triphosphates (dNTPs), 0.4 μmol·l⁻¹ each of forward and reverse primers, 0.5 U taq (Fisher Bioreagents) and 50-200 ng DNA template or 25μl 2x Brilliant PCR Master Mix (Agilent Technologies) containing, 0.4 μmol·l⁻¹ each of forward and reverse primers and 50-200 ng DNA template (Ouellette et al. 2006).

Examination of PCR products was via gel electrophoresis using 2% agarose, 1X TAE buffer stained with 1μL ethidium bromide (10 mg/mL) and 100 bp DNA ladder. UV visualization of bands will take place in a UVP bench top UV illuminator (Upland, CA). The PCR amplifications of extracted DNA will result in a 450 bp 16S rRNA gene (Vaitomaa et al. 2003).