DRAFT REPORT

Algal toxins bioassessment – Clear Lake, July/August 2010

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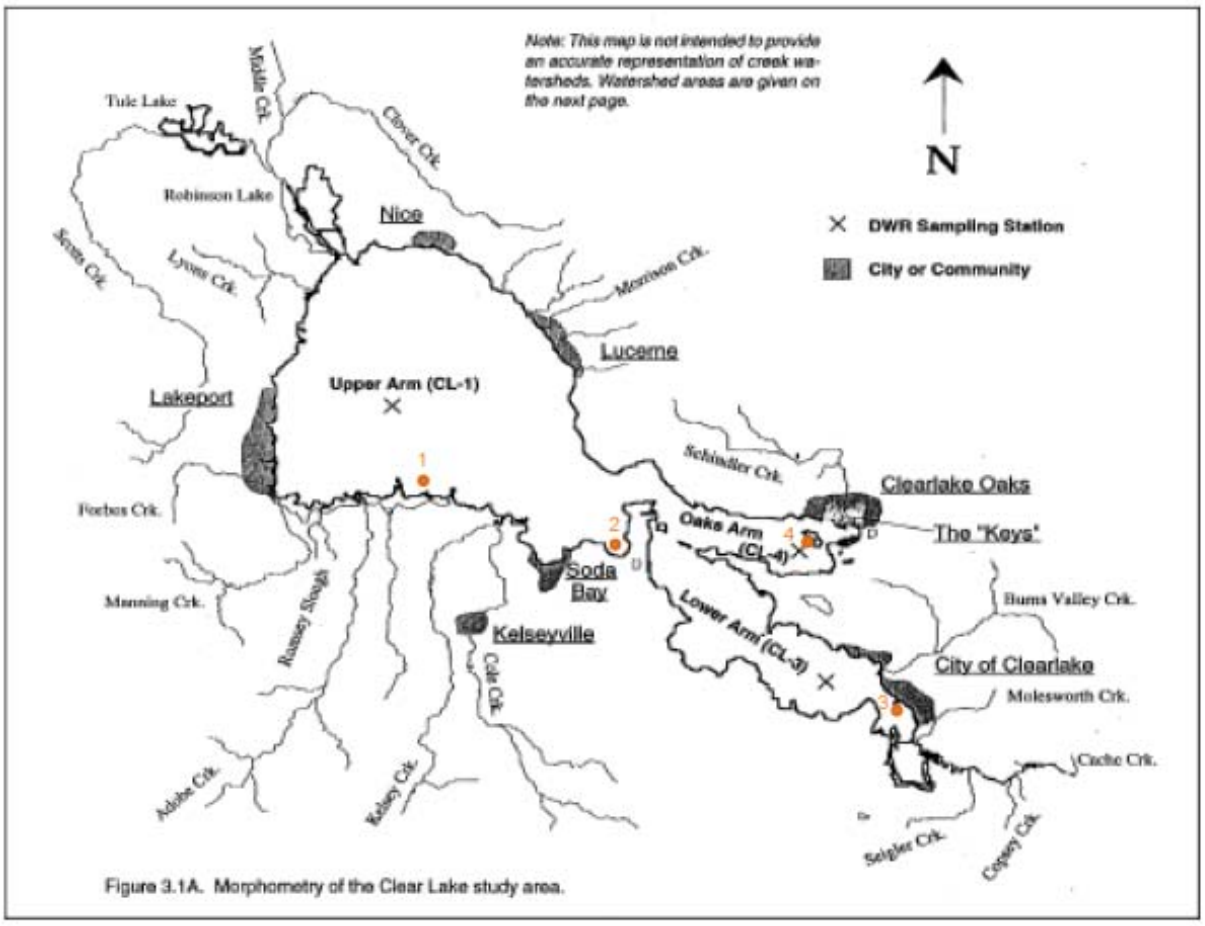

Figure 1: Study are map. CL-1, CL-2 and CL-3 discrete monitoring stations are historical DWR monitoring stations (Richerson 1994). They were also used for a toxicology study performed in 1990 (ODWSESP 1991). The stations 1, 2, 3, 4 are continuous monitoring stations (SPATT samples, HOBOS) and are located at coastal buoys (county owned). Discrete samples were also collected monthly at these stations

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The following draft report describes the results from a series of monthly investigations into the toxicity of cyanobacteria in Clear Lake, California. Individuals from the Lake County Water Resources Department and the University of California, Santa Cruz all redirected efforts to gather samples, carry out assays, and/or interpret results. The findings can be summarized as follows:

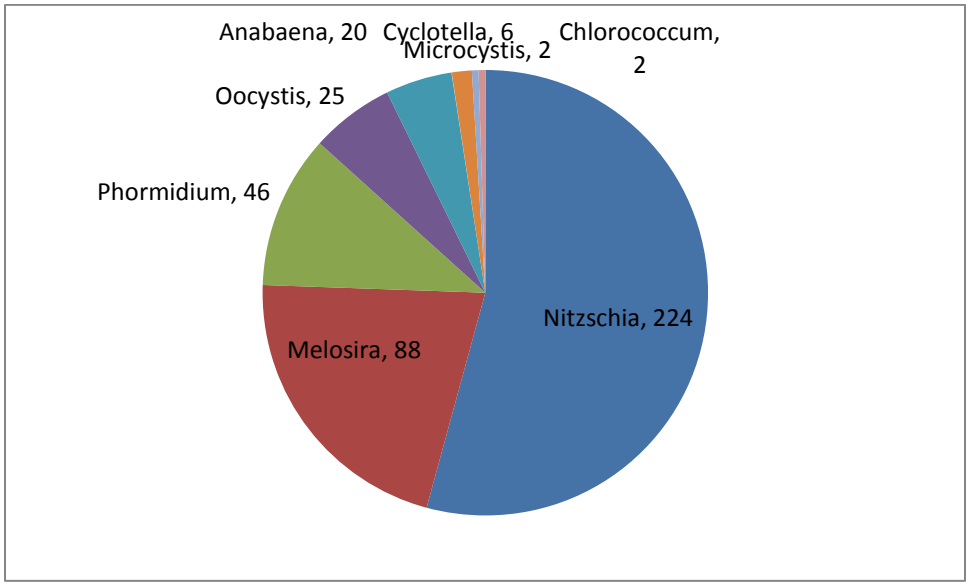

Figure 2: Algal composition a bloom sample collected in August 2010 at station 5B located in the northern Lower Arm. The bloom was dominated by: *Nitzschia* (diatoms), *Melosira* (diatoms), *Phormidium cincinnatum* (aka *Lyngbya cincinnati*, cyanobacteria), *Oocystis* (green alga), *Anabaena spiroides* (cyanobacteria) and *Microcystis aeruginosa* (cyanobacteria). Note: *Microcystis* is a floating species and does not settle to the bottom of a Utermohl chamber. Therefore the number of *Microcystis* enumerated in the sample is underestimated.

Presence of toxic cyanobacteria in Clear Lake

Thirty-one surface water samples were collected from Clear Lake for algal toxicology analysis at seven stations (Figure 1). One sample collected in August at the epicenter of the bloom (northern part of Lower Arm, station 5B) was sent to EcoAnalysts, Inc. for taxonomy analysis. Cyanotoxin concentrations (microcystins, anatoxin-a and saxitoxins) were analyzed using ELISA (microcystins, saxitoxins) and LC/MS (microcystins, anatoxin-a) analysis at the University of California, Santa Cruz. Some pictures of the bloom are included in the appendix section of this report.

Taxonomy results indicate that the algal bloom assemblage was composed of a mixed assemblage of diatoms, green algae and cyanobacteria. The cyanobacteria comprised a significant fraction of the algal assemblage and included several potentially harmful cyanobacteria such as: *Aphanizomenon* (June samples), *Microcystis aeruginosa* (mostly June and September), *Anabaena spiroides (throughout summer)*, and *Lyngbya cincinnati* (aka *Phormidium cincinnatum*, throughout summer). In the sample collected in August 2010 in the northern section of the Lower arm (station 5B, epicenter of the bloom) and analyzed by Ecoanalyst, Inc., *Lyngbya cincinnati* and *Anabaena spiroides* were the two dominant cyanobacteria species (Figure 2).

According to the literature, Clear Lake has been seriously impaired by seasonal blooms of scum-forming cyanobacteria (Richerson 1994). Over the entire 23 year period for which the DWR phytoplankton taxonomy data record summarized in the Richerson's report (1994), the three most common scum-forming cyanobacterial genus were *Anabaena*, *Aphanizomenon* and *Microcystis. Microcystis* blooms have been documented in both the Oaks Arm and the Lower Arm of Clear Lake in the mid 1970's with the largest blooms recorded by DWR occurring in the Lower Arm in 1991 (Richerson 1994). *Aphanizomenon* showed similar trends and was the most important scum former for most years up to 1985, when it suddenly became much less important (Richerson 1994). *Anabaena* was never responsible for blooms on the scale of *Microcystis* and *Aphanizomenon* in any arm but was a common component of the midsummer scums throughout the record, becoming much more important after 1980 before diminishing as the drought progressed (Richerson 1994). *Lyngbya* was not observed until 1984 (Richerson 1994) and the first large bloom event dominated by this mat-forming filamentous cyanobacterium was not observed until 2009 (Tom Smythe, *pers. com*.). Please note that the DWR taxonomy data records alternatively identified this cyanobacterium as *Phormidium,*

Lyngbya or *PlanktoLyngbya* so the historical records might be misleading with regard to the presence/abundance of this cyanobacterium.

Taxonomists from Ecoanalysts, Inc. identified the dominant mat-forming cyanobacterium from August 2010 mat sample as *Lyngbya Cincinnati* (synonym: *Phormidium cincinnatum*). Although some freshwater *Lyngbya* species can produce the cyanotoxins saxitoxin, dedromoaplysiatoxin and lyngbyatoxin-a (Onodera et al. 1997, Puscher and Humbert 2007), the literature contains no information with regard to the toxicity of this specific *Lyngbya* strain. Over the course of our study, we did not detect any saxitoxin or its analogues (potent neurotoxins responsible for the human poisoning syndrome called paralytic shellfish poisoning) in any of the Clear Lake surface water samples (see below). Lyngbyatoxin-a (a dermatoxin causing "swimmer itch") wasn't monitored as part of this study but LC/MS preliminary results indicate that a compound with similar chemical properties was present in some discrete surface samples. Our future toxicology studies will attempt to determine the toxicity of this filamentous cyanobacteria strain using LC/MS and a lyngbyatoxin-a standard acquired from SCRIPPS (Dr. Jensen). Indeed, although no swimmer itch appear to have been reported among swimmers in summer 2010, 8 cases of "swimmer's itch" were reported on June 19 of 2010 and may have been the result to exposure to dermacyanotoxins such as lyngbyatoxin-a (ODWSESP 1991). These cases were then diagnosed as schistosome dermatitis but mot cercaria were found in the swimming area or elsewhere in Clear Lake. On the day of the outbreak, no cyanobacteria were found in the swimming area water samples but cyanotoxins. However, cyanobacteria cell counts can underestimate the risk of cyanotoxin poisoning because cyanotoxins may persist in the water after a bloom has subsided.

The dominant *Anabaena* species growing in Clear Lake during summer 2010 was identified as *Anabaena spiroides*. It was the second most abundant cyanobacterium in the mat sample collected in August 2010. Compiled literature evidences suggest that this scum-forming filamentous cyanobacterium species can produce at least two types of toxins: anatoxin-a and microcystins (Puschner and Humbert 2007). Microcystins are nonribosomal peptide toxin that inhibits protein phosphatases in a broad range of eukaryotes from zooplankton to humans. These potent hepatotoxins are capable of causing liver failure and acting as tumor-promoters (Falconer 1991, Carmichael 1995, Chorus 2001, Grosse et al. 2006). Microcystins constitute the larger group of cyanotoxins with over 80 variants identified to date. Microcystin-LR is the most common congener of the series. Most microcystins display a LD-50 (intraperitoneal) ranging between 50 and 100 µg/kg in mice although Microcystin-RR are slightly less toxic with a LD-50 of 600 µg/kg in mice (Rinehart et al. 1994). Anatoxin-a(s) is a highly toxic neurotoxin structurally similar to an organophosphorus pesticide, with a LD-50 (intraperitoneal) in rats of about 20 µg/kg and 31 µg/kg in mice, respectively (Cook et al. 1988, Falconer 1996).

Microcystis spp. are bloom-forming single-celled, colonial, freshwater cyanobacteria and have been observed world-wide. In summer 2010, *Microcystis* colonies sizes varied from a dozen to several hundred of cells in the surface water of Clear Lake and were observed throughout the summer. They were not the most abundant components of the cyanobacteria mat from June-August but their relative abundance increased as compared to the other genera in September 2010. *Microcystis aeruginosa* strains can produce a multiple of toxins in addition to microcystin (Okino et al. 1995, Namikoshi and Rinehart 1996). Some *Microcystis aeruginosa* strains may also produce anatoxin-a (Park et al. 1995) and microviridins (Okino et al. 1995). Most microviridins show inhibitory activities against serine-type proteases. One of the peptide isoforms, microviridin J, has been shown to inhibit the molting process of *Daphnia* (small, planktonic crustaceans), and ultimately leading to death (Rohrlack et al. 2004).

Presence of cyanotoxins in Clear Lake

Previous studies suggest that the possibility that a cyanobacterial bloom may be toxic is more than 50% (Olson 1964). The ability to produce cyanotoxins may be regulated both by environmental factors and genotype (Kaebernick et al. 2000, Downing et al. 2005, Kardinaal et al. 2007) but these links are not well understood. Although recurrent cyanobacteria blooms have plagued Clear Lake for the past century, there is only one toxicology survey available to date that was conducted by the Office of Drinking Water and the Special Epidemiological Studies Program in 1990 (ODWSESP 1991). This study was focused on microcystin toxin only and did not examine other cyanotoxins. Results from this study reported large temporal and spatial variability as well as trace level (2.6×10^{-3} µg/L) of this cyanotoxin in one surface water sample collected in a swimming area of the lake and measured via immunoassay/ELISA (ODWSESP 1991). Among the 20 algae samples analyzed for toxicity using mouse bioassay, only two samples collected in Oaks arm (station CL-4) on June 28, 1990 were reported toxic. The most toxic of these samples contained 3.4 mg hepatotoxin/g of lyophilized algae (ODWSESP 1991). Based on these results, the study concluded that the oral NOAEL (No observed adverse effect level) for a 60 kg (ca. 132 lbs) adult would need to ingest 3.17 lbs of wet algae from Clear Lake.

Results from our study (LC/MS analyses) confirm a low level of microcystins, below the recreational advisory limit of 8 µg/L, however microcystin concentrations exceeded the WHO advisory limit for drinking water (1 µg/L) in several samples (Table 1). There were discrepancies between the ELISA and the LC-MS results. We believe that these discrepancies results from other compounds present in the water that could interfere with the ELISA kit optical reading and are working on modifying our methods to solve the issue (e.g. dilution, filtration at smaller pore size). Some compounds are also interfering with the detection of Microcystin-YR on the LC-MS and therefore we did not add this variant to the table below. The LC-MS results are

Table 1: Levels of cyanotoxins (µ/L) as measured by LC/MS (total microcystins –MC- and individual variants, anatoxin-a -ANA) and ELISA (saxitoxin –PSP-).

Date	Station	Name	MC-RR	MC-YR	MC-LR	MC-LA	Total MC	ANA	PSP
6/23/2010	Stn 1	Lakeport	0	NA	0	0		0	0
6/23/2010	CL1	Upper Arm	0	NA	0	0		0	0
6/23/2010	Stn 2	Horseshoe bend	0	NA	0	0		0	0
6/23/2010	Stn 3	Man	0	NA	0	0		0	0
6/23/2010	CL3	Lower Arm	0	NA	0	0		0	0
6/23/2010	Stn4	Rattlesnake island	0	NA	0	0		0	0
6/23/2010	CL4	Oaks Arm	0	NA	0	0		0	0
7/22/2010	Stn 1	Lakeport	0.617	NA	2.998	0	**3.615**	0	0
7/22/2010	CL1	Upper Arm	0	NA	0	0		0	0
7/22/2010	Stn 2	Horseshoe bend	0	NA	0	0		0	0
7/22/2010	Stn 3	Man	0	NA	0	0		0	0
7/22/2010	CL3	Lower Arm	0	NA	0	0		0	0
7/22/2010	Stn4	Rattlesnake island	0	NA	0	0		0	0
7/22/2010	CL4	Oaks Arm	0	NA	0	0		0	0
7/22/2010	Stn 5	north lower arm	0	NA	0	0		0	0
7/22/2010	Stn 6	Horseshoe bend	0	NA	0	0		0	0
8/16/2010	Stn 1	Lakeport	0	NA	0	0		0	0
8/16/2010	CL1	Upper Arm	0	NA	0	0		0	0
8/16/2010	Stn 2	Horseshoe bend	2.586	NA	0	0	**2.586**	0	0
8/16/2010	Stn 3	Man	0	NA	0	0		0	0
8/16/2010	CL3	Lower Arm	0	NA	0	0		**0.52**	0
8/16/2010	Stn4	Rattlesnake island	0	NA	3.193	0	**3.193**	0	0
8/16/2010	CL4	Oaks Arm	2.265	NA	0	0	**2.265**	0	0
8/16/2010	Stn 5B	north lower arm	0	NA	0	0		**7.78**	0
9/9/2010	Stn 1	Lakeport	0	NA	1.73	0	**1.73**	0	0
9/9/2010	CL1	Upper Arm	0	NA	0	0	0	0	0
9/9/2010	Stn 2	Horseshoe bend	0	NA	0	0	0	0	0
9/9/2010	Stn 3	Man	0	NA	0	0	0	0	0
9/9/2010	CL3	Lower Arm	0	NA	0	0	0	0	0
9/9/2010	Stn4	Rattlesnake island	0	NA	0	0	0	0	0
9/9/2010	CL4	Oaks Arm	0	NA	0	0	0	0	0

considered more accurate than ELISA (high-throughput assay) and therefore, these are the results that we will keep in the database. All samples are expressed in ppb (µg/L). The microcystin levels measured in Clear Lake during Summer 2010 are much lower than that reported other local lakes with similar cyanobacteria biomass (e.g. Pinto Lake, Miller et al. 2010).

Anatoxin-a was detected in two of the samples collected in the lower arm in August 2010 (CL-3 and station 5B, table 1). At both collection sites, anatoxin-a was well below the suggested action levels for recreational use (50 µg/L, OEHHA/EPA 2009).

Saxitoxins were below the detection limit or not present in the lake surface water (using Abraxis ELISA kits only).

Other cyanotoxins (e.g. lyngbyatoxin-a) might have been present in the surface water but we did not have a standard and therefore cannot quantify the compound or validate that it is actually lyngbyatoxin-a. Based on the LC-MS results, the mass seems to match lyngbyatoxin-a but the level appear low. We have acquired a lyngbyatoxin-a pure extract from SCRIPPS (Dr. Jensen) and will attempt to calibrate the LC/MS to measure this toxin during Summer 2011.

As noted in the toxicological report from 1991, cyanotoxin concentrations in Clear Lake display a high variability in space and time. Except for September 2010 samples, Upper arm is usually the least toxic as compared to the Lower Arm and Oaks Arm. The possible sources of variation that may be responsible for this variability in cyanotoxin concentrations are:

(i) *Variability in cyanobacterial biomas*s. A higher biomass of cyanotoxin producers may result in higher toxin concentrations. Indeed, the cyanobacterial biomass was usually lower in the Upper Arm as compared to the Lower Arm and Oaks Arm. The growth of cyanobacteria can be influenced by several environmental drivers in lakes that have not been identified and previous studies have pointed out the influence of prominent winds on the accumulation of cyanobacteria in the Lower Arm and Oaks Arm (Richerson 1994).

(ii) *Physiological variability*. Cyanotoxin production is affected by several environmental factors (nutrient availability, light conditions, and temperature) as demonstrated by controlled laboratory experiments. We did not observe any significant correlations between cyanotoxin concentrations and nutrient concentrations. The lack of significant correlation might be related to a low amount of samples presenting detectable levels of toxins.

(iii) *Variability in cyanobacterial species and genotype composition.* The cellular content and diversity of cyanotoxins vary among species and even among different genotypes within the same species. As a result, changes in the species composition of cyanobacteria, and also changes in genotype composition within the same species may lead to fluctuations in cyanotoxin concentration. During Summer 2011, we will collect samples for molecular analysis

in the attempt to identify the potentially harmful cyanobacterial strains as well as to determine their toxigenicity (presence/absence of toxic genes).

Because toxins concentrations vary greatly on a spatiotemporal scale, we also monitored the cyanotoxins using the SPATT (Solid Phase Adsorption Toxins Tracking) methodology which is a modification of a method originally developed for marine lipophilic toxins by Dr Kudela (UCSC) for continuous toxin tracking by passively absorbing dissolved toxins from the water column. SPATT devices were attached at buoys located at the continuous monitoring stations (maps). Unfortunately, we weren't able to attach the SPATT devices in the proximity of the surface and therefore, the results presented here are representative of deep (1-2 m) rather than sub-/surface (0.1-0.2m) waters. SPATT samples were analyzed for microcystin and anatoxin-a detection using LC/MS as described above and the daily cyanotoxin production was computed (Table 2). Microcystis LA was the most abundant variant at the SPATT depth although it wasn't detected in the discrete surface water samples. No anatoxin-a toxin were detected in the SPATT. These results confirms the spatio-temporal heterogeneity of the cyanotoxins concentration and speciation, not only horizontally (e.g. between stations) but also with depth. It could be possible that cyanobacteria from the mat might be producing different toxins than the cyanobacteria growing in the water column. Another hypothesis would be that discrete samples collected at a given time may not representative of the cyanotoxin cocktail due to the influence of mixing (winds, currents) while SPATT integrate these variations. We will attempt to maintain the SPATT devices in the surface of the the water column next summer to allow a better comparison between the continuous toxin measurements and the discrete toxins measurements.

Conclusion

Based on our results, it doesn't appear that there is any significant recreational toxin exposure risks in Clear Lake surface waters. However, the microcystins levels measured during Summer 2010 were several orders of magnitude higher than that reported for the same stations for Summer 1990 (ODWSESP 1991). More research is needed to determine if the cyanotoxin level increase between the two studies is a real trend or if the discrepancies are related to the technologies used ("old" ELISA kits and mouse assay vs. "newer" ELISA kits and LC/MS).
This study also indicate that more that Clear Lake might be a cyanotoxin cocktail (microcystin, anatoxin-a and maybe other cyanotoxins). Indeed, the only toxicology report available for Clear Lake was focusing on Microcystin toxins only (ODWSESP 1991). Our future efforts will focus on expanding the range of monitored toxins in order to gain a more comprehensive understanding of this system.

Table 2:
Clear Lake SPATT samples

Analysis: LC/MS

Column: Zorbax Rapid-Resolution HT

Extraction: 50% MeOH

Analyzed For: MCY-LR, MCY-RR, MCY-YR, MCY-LA, Anatoxin-a

	----------ppb in extract----------						----------ng/g/day ----------					
Sample	[MCY-RR]	[MCY-LR]	[MCY-YR]	[MCY-LA]	[ANA-A]	Extract Vol	Duration (days)	[MCY-RR]	[MCY-LR]	[MCY-YR]	[MCY-LA]	[ANA-A]
CL072210.1	1.36	4.99	0	13.88	0	10	30	0.15	0.55	0.00	1.54	0.00
CL072210.2	0.98	5.24	0	12.29	0	10	30	0.11	0.58	0.00	1.37	0.00
CL072210.3	0	6.03	0	30.61	0	10	30	0.00	0.67	0.00	3.40	0.00
CL072210.4	0	0	0	12.52	0	10	30	0.00	0.00	0.00	1.39	0.00
CL081601.1	0	5.71	0	14.79	0	10	30	0.00	0.63	0.00	1.64	0.00
CL081601.2	0	0	0	10.1	0	10	30	0.00	0.00	0.00	1.12	0.00
CL081601.3	3.13	5.52	0	20.64	0	10	30	0.35	0.61	0.00	2.29	0.00
CL081601.4	0	3.97	0	22.5	0	10	30	0.00	0.44	0.00	2.50	0.00

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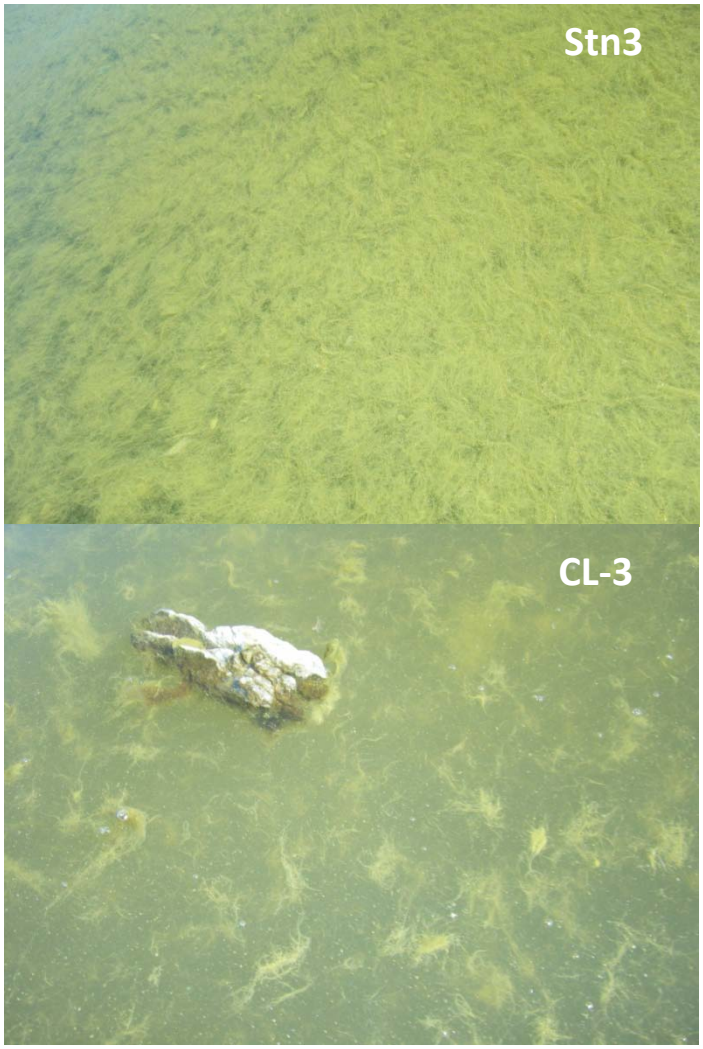
APPENDIX

Clear Lake - June 2010

Picture of cyanobacteria growth observed in the surface water of the Lower Arm of Clear Lake (June 2010). The filamentous cyanobacteria were *Anabaena sp*., *Aphanizomenon sp*. and *Lyngbya Cincinnati* (*Phormidium cincinnatum*). Some macrocolonies can be seen floating in surface. Other potentially harmful cyanobacteria such as *Microcystis spp*. were also present but not as these filamentous cyanobacteria. *Picture: Cécile Mioni*

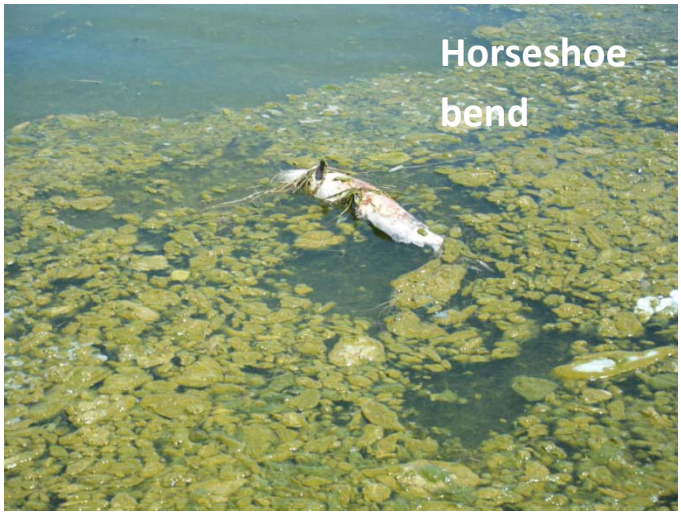
Clear Lake – July 2010



Lyngbya cincinnati was the dominating cyanobacteria present in the mat in the Lower Arm.

Some clumps were rotting in the surface of the lake, releasing some fool odor.

Picture: Cécile Mioni

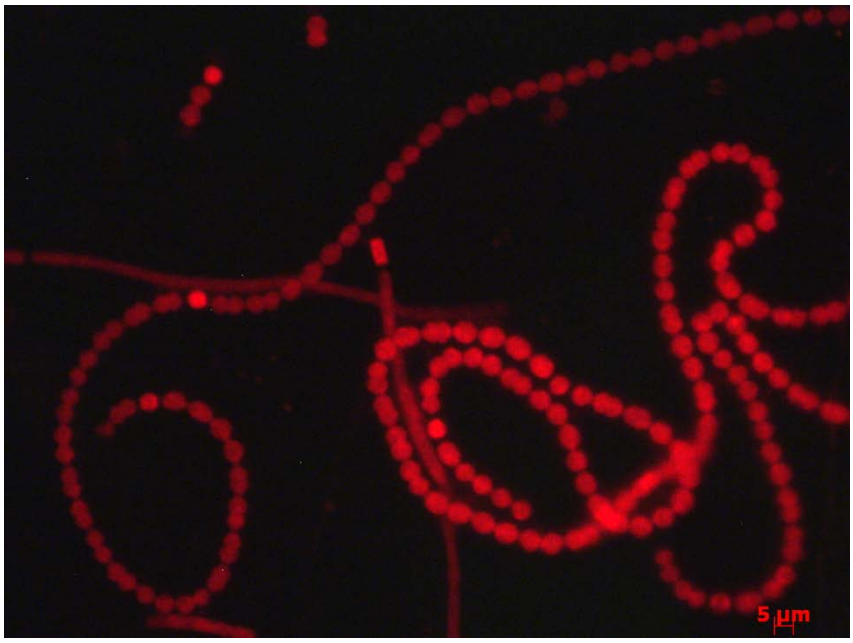


Horseshoe bend cyanobacterial scum/mat was dominated by *Anabaena* and *Lyngbya.*

We saw a couple of dead fish but we did not investigate the cause of death.

Picture: Cécile Mioni

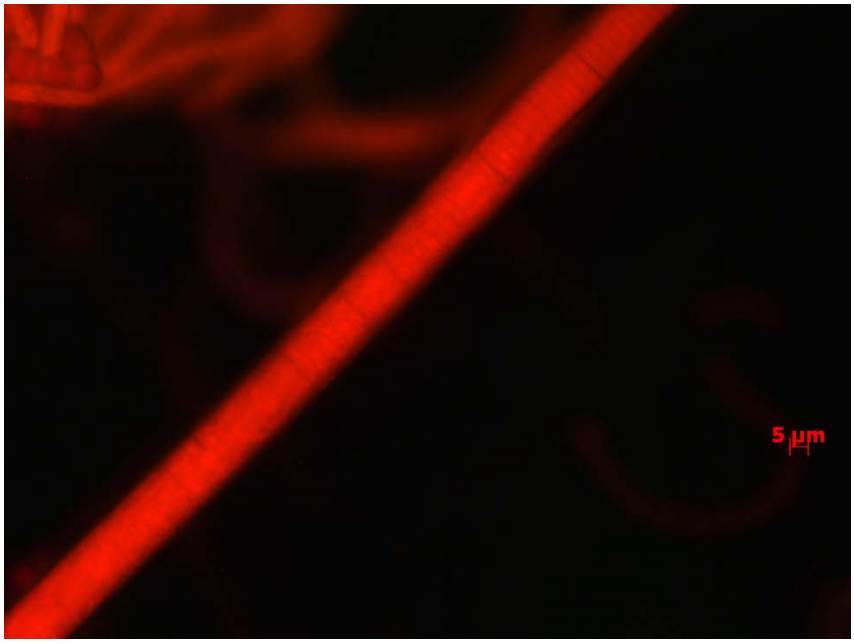
Selected micrographs (epifluorescence microscopy) of Clear Lake samples



Heterocystous *Anabaena spiroides* and *Aphanizomenon sp*.

Oaks Arm sample, station 4 (June 23, 2010)

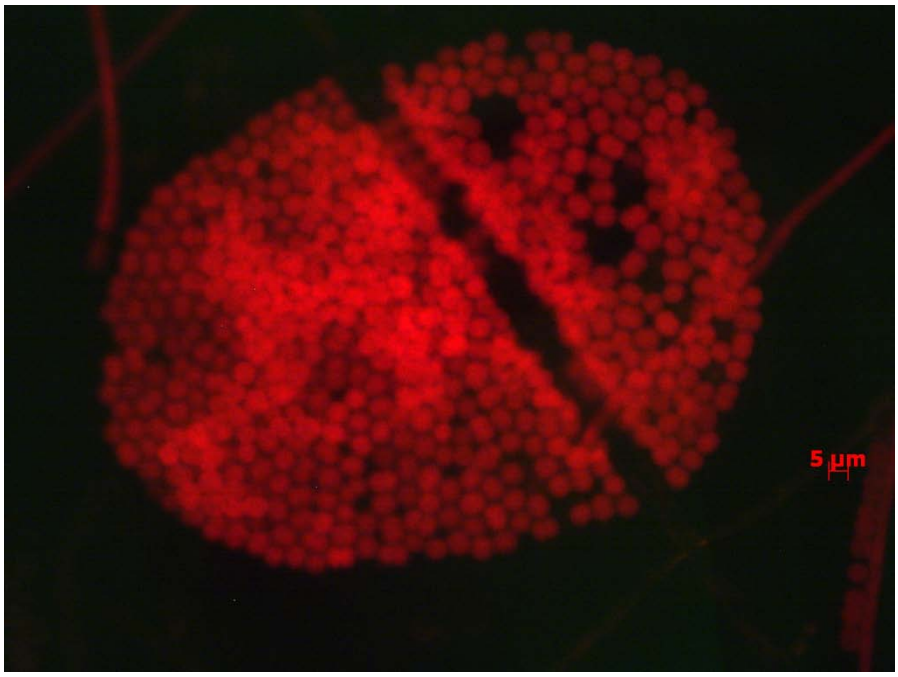
Picture: Cécile Mioni



One *Lyngbya cincinnati* filament (*Gleotrichia* filaments can also be seen on the top left corner of the image and a blurry *Anabaena* spiroides filament can be seen right by the scale).

Oaks Arm sample, station 4 (June 23, 2010)

Picture: Cécile Mioni



One *Microcystis aeruginosa* colony

Oaks Arm sample, station 4

(June 23, 2010)

Picture: Cécile Mioni