

## **DRAFT – *Microcystis* and POD white paper**

### **Introduction**

The goal of this report is to summarize the significance and potential negative impacts the recent blooms of *Microcystis aeruginosa* present to the Delta. *Microcystis* was initially observed in bloom forming surface scums in the late 1990's when it was localized. Currently the blooms extend over wide regions of the Delta, from salinities ranging 0.1-18 ppt. It was found from Suisun Bay to the freshwater habitat of upstream rivers (Lehman 2005). This presence and expanding distribution is likely having an impact to the pelagic foodweb.

Potential factors contributing to the recent large-scale, Delta-wide decline of open water planktonic organisms include a consideration of impacts caused by the dramatic proliferation of *Microcystis* within the Delta. As such, the following sections introduce the different factors and processes associated with blooms of toxic *Microcystis*. Included here is a literature based assessment of *Microcystis* impacts to all levels of the foodweb, focusing on zooplankton and fish, the mechanisms underlying such impacts, the current state of the Delta bloom, what this means for the growth and reproduction of pelagic organisms of concern, and finally a set of recommendations of future research to better understand the problem and possible solutions for management. The most urgent goal is to understand if and how the co-occurring blooms and decline in pelagic organisms relate to each other.

Both the type and extent of marine and freshwater phenomena now commonly called Harmful Algal Blooms (HAB's) have extraordinarily expanded. HAB's now globally threaten water quality, potential human uses like drinking water consumption and fishing, and fundamental ecological functions (Paerl 1988, Anderson and Garrison 1997, Stahl-Delblanco et al, 2003). *Microcystis aeruginosa* is a colony forming cyanobacteria (bluegreen algae), one of the most common species of cyanobacteria worldwide, and is dominant in most eutrophic and hyper-eutrophic waters (Christoffersen 1996). Its toxicity comes from microcystins, a class of hepatotoxins produced by *Microcystis* cells that cause severe liver damage and tumor formation, along with other compounds such as microviridin (Dawson 1998, Rohrlack et al, 2003).

In recent years there has been an increase in the abundance and distribution of the toxic cyanobacteria *Microcystis* blooms in the upstream portion of the San Francisco Estuary known as the Delta (Lehman 2005). The first Delta-wide toxic bloom was monitored in 2003 (Lehman et al, 2004). Majority of the *Microcystis* found throughout the Delta during a comprehensive sampling campaign in October 2003 contained the variant of the toxic strain microcystin LR (Lehman et al, 2004). While the overall toxicity of the Delta population has not been documented, highest toxin concentrations were in the Sacramento River sites, while highest biomass was at the Central Delta stations, lake-like flooded islands, including agricultural channels diverting water to storage reservoirs and the State Water project (Lehman et al, 2004).

## Toxicity

Toxic strains of *Microcystis*, *Anabaena*, and *Oscillatoria* produce microcystins (a class of heptacyclic peptide toxins), which are classified according to their target organs. Microcystins are hepatotoxins, as they mainly injure the liver, though tumor formation is also reported (Carmichael, 1996). Microcystins inhibit protein phosphatases 1 and 2A, causing liver damage in a wide variety of animals, and negatively affecting certain plants, algae, and protozoa. There are over 60 different strains of microcystins identified (Best et al 2003).

There are several studies that look at toxic effects of microcystins on different animals (Christoffersen 1996). The main pathway for toxin exposure is ingestion, though toxins are also released into the water when algal cells rupture through death. Experiments focusing on zooplankton typically involve large cladocerans such as *Daphnia*. Toxic strains of *Microcystis* inhibit feeding and growth in most species of *Daphnia*, but the resulting patterns are complex beyond that (DeMott 1999, Lurling 2003). There are other compounds that are toxic to zooplankton, such as lipopolysaccharide (LPS) endotoxins that occur near the cell surface of all gram negative bacteria including cyanobacteria (Best et al 2003). Further, it is difficult to distinguish between inhibition caused by feeding inhibition vs. direct toxicity.

*Daphnia* are known to limit food intake in the presence of toxic algae. Since they cannot select between individual food items, reduced ingestion in the presence of toxic algae comes at the overall cost of reduced food intake. This makes nutritional acclimation important because zooplankton that are previously well fed can reduce ingestion when confronted with toxic strains without a high cost (DeMott 1999). Sensitivity to microcystins is species specific. A comparison of 13 cladocerans revealed that *Daphnia magna* and *Daphnia pulex* show the strongest feeding inhibition, while *D. pulicaria* and *Bosmina longirostris* were less inhibited (Lampert 1982, mentioned in DeMott 1999). Tolerance to toxins such as microcystins has been demonstrated for *Daphnia*. Specifically, previous exposure to toxic *Microcystis* improved survival, growth and reproduction (Gustafsson and Hansson 2004).

Zooplankton reproduction is another variable that is affected by presence of toxic strains of *Microcystis*. *Daphnia* typically produce high clutch size with smaller individual offspring during high food levels. Allocation of resources to reproduction versus growth depends on environmental conditions. Research shows that *Daphnia* exposed to high microcystin concentrations were barely able to reproduce and produced smaller clutches at lower concentrations. The presence of *Microcystis* apparently increased the portion of resources allocated for reproduction (Reinikainen et al, 1999).

Colonial *Microcystis* has a protective mucus glycocalyx layer that makes it impermeable to the digestive enzymes of fish. If fish ingest *Microcystis* directly, the ingested cells should travel the alimentary canal without being lysed and releasing their toxins (Carbis et al. 1997). As mentioned earlier, *Microcystis* cells have endotoxins of the surface, which cause gastroenteritis in mammals and likely irritates fish also (Carbis et al. 1997). Additionally, microcystins are readily adsorbed to surfaces, similar to P adsorption (Zimba, per com). This means that ingestion of zooplankton from waters exposed to microcystin could result in ingesting the toxin, even if the zooplankton do not have much of it in their tissue.

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## Foodweb impacts

The impact to foodweb interactions comes from two factors: toxicity and food quality. Exposure to microcystins can either kill organisms directly, or weaken resistance to bacterial/viral infections and disturbance (Christoffersen, 1996). *Microcystis* blooms pose a threat to aquatic ecosystems at every level via direct or indirect impacts, including heterotrophic bacteria, phytoplankton, zooplankton, benthic invertebrates, and fish (Christoffersen, 1996, Lurling, 2003). Direct impacts (function of *Microcystis* biomass and toxicity combined) are a result of toxicity from exposure or ingestion and reduced food intake.

Indirect impacts arise from *Microcystis* affecting the overall food quality for zooplankton, species interactions, and bio-accumulation. Several studies indicate that cyanobacteria are an unsuitable food source for zooplankton due to their colonial aggregation, toxin production, and/or their nutritional inadequacy (Carmichael 1996, Hessen et al, 2005). *Microcystis* is no exception, and its impact to ecosystems is a function of both toxicity and reduced ingestion.

Zooplankton are either killed by microcystins, or show reduced feeding, growth, and reproduction even in the presence of non-toxic strains (Kirk and Gilbert, 1992). *Daphnia* seem to tolerate microcystins better than copepods: the lethal dose for *Daphnia* spp. were between 10-21 µg microcystin LR/ml, which is 20-40 times higher than those for copepods (Christoffersen, 1996). Some rotifer species are able to survive on a diet of toxic *Microcystis* under laboratory conditions (Fulton and Paerl, 1987, mentioned in Christoffersen, 1996). Most pronounced effects can be expected during the breakdown of *Microcystis* blooms, when ambient water concentration of dissolved microcystin is highest (Christoffersen, 1996).

There is some debate regarding the negative effects of *Microcystis* on zooplankton. Recently it has been suggested that while *Daphnia* avoids feeding on live cells, it may feed on decomposed ones. Park et al showed that heterotrophic nano-flagellates improved the fatty acid content of decaying *Microcystis* cells, thereby increasing its food quality for zooplankton (2003). If true, this would imply that while zooplankton avoid live colonies; decomposing *Microcystis* could still be used as a source of food.

Effects on the foodweb are transmitted through bioaccumulation and by changes in community structure. Bioaccumulation extends the toxin to higher trophic levels such as fish. Changes in community structure result from *Microcystis* altering the dominant herbivores. This can be a shift in zooplankton species, from large cladocerans to smaller zooplankton, or a shift from planktonic secondary production to benthic production. Overall, it has the ability to reduce the efficiency of pelagic foodwebs (Vanderploeg et al. 2001).

*Microcystis* is known to interact with the foodweb at several levels, and feedback mechanisms can promote its proliferation. In several lakes, including the great lakes region, *Microcystis* blooms followed invasions of Zebra mussels. This was especially surprising because the *Microcystis* came after the famous reduction in P-loading, intended to control eutrophication. Research revealed that Zebra mussels promote *Microcystis* blooms by filtering and ingesting all algae, while ejecting *Microcystis* back into the water column as pseudofeces. As such, the selective filtering of Zebra mussels leads to *Microcystis* dominance (Vanderploeg et al. 2001).

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Studies also are beginning to reveal mechanisms that *Microcystis* impact fish. Feral carp in Australia living in lakes dominated by *Microcystis* showed sublethal toxic effects in the stomach and intestine. Because carp do not secrete acid into the alimentary canal, their entire gut is neutral. Efficient digestion of *Microcystis* cells require an acidic environment, this feature of carp may provide them some protection against *Microcystis* (Carbis et al. 1997). However, hepatic lesions were present in more than 50% of the fish examined in the study, and provides evidence that even in species like carp, *Microcystis* ingestions inflicts sub-lethal injury. Further, these results indicate that fish exposed to *Microcystis* can be a source of toxins, with microcystin and its breakdown products concentrating in the liver (Carbis et al. 1997).

In another study, Zimba et al. linked catfish mortality to *Microcystis* ingestion (2001). The study site was a commercial catfish production pond, where dominant blooms of *Microcystis* are common. A rapid decline in temperature was noted in the 10 days before fish mortality was observed. This temperature shift coincided with increased toxin production in *Microcystis*, by shifting from low toxicity microcystins (RR, YR) to more toxic ones (LR). Authors suggest that rapid temperature declines could result in increased toxin production over large areas (Zimba et al. 2001).

Based on literature reviewed above, the trophic structure of foodwebs dominated by *Microcystis* is likely to be altered in order to accommodate species that are tolerant towards its effects. Over the last three decades, phytoplankton biomass has declined delta-wide, and the phytoplankton community has shifted towards species that have less nutritional value to zooplankton (Jassby, 2002). It is not clear why *Microcystis* is on the rise while other phytoplankton species are in decline. Today, relatively scarce good-quality algal food resources often limit zooplankton growth in the Delta (Mueller-Solger et al 2002). If available high quality food at the base of the food chain (phytoplankton) is declining, and *Microcystis* blooms are on the rise, this could be a profound threat for the ecosystem. As the fraction of non-ingestible, low food quality or toxic algae increase, there is effectively less 'food' for higher trophic levels. As mentioned earlier, in addition to its toxic effects on zooplankton, *Microcystis* has been shown to be non-ingestible and of low nutritional value (Lurling, 2003). It can thus reduce the fraction of available food biomass to higher trophic levels in the ecosystem in the Delta. This could mean a shift towards the benthic foodweb, where decomposition of *Microcystis* fuels the microbial foodweb.

### **Life-cycle**

The annual life cycle of *Microcystis* has been documented extensively (Reynolds 1981, Brunberg and Blomqvist 2003). Planktonic populations originate from the overwintering cysts in sediments. Recruitment to the water column is triggered by increases temperature and light combined with anoxic conditions in sediments (Stahl-Delbanco et al, 2003). Benthic colonies are near neutral buoyancy, and photosynthesis prompts positive lift (Reynolds 1981). Although recruitment from sediments has been shown under oxic conditions, this does not exclude that sediments themselves may provide anoxic microhabitats (Brunberg and Blomqvist, 2003).

Several factors determine the amount of recruitment: number of colonies accumulated in sediments, cyst survival, and conditions favoring recruitment (Brunberg and Blomqvist 2002). Darkness, coupled with low temperatures, and hypoxia have been shown to enhance cyst survival in sediments (Brunberg and Blomqvist 2002). Cysts need photosynthesis (light) to gain positive buoyancy

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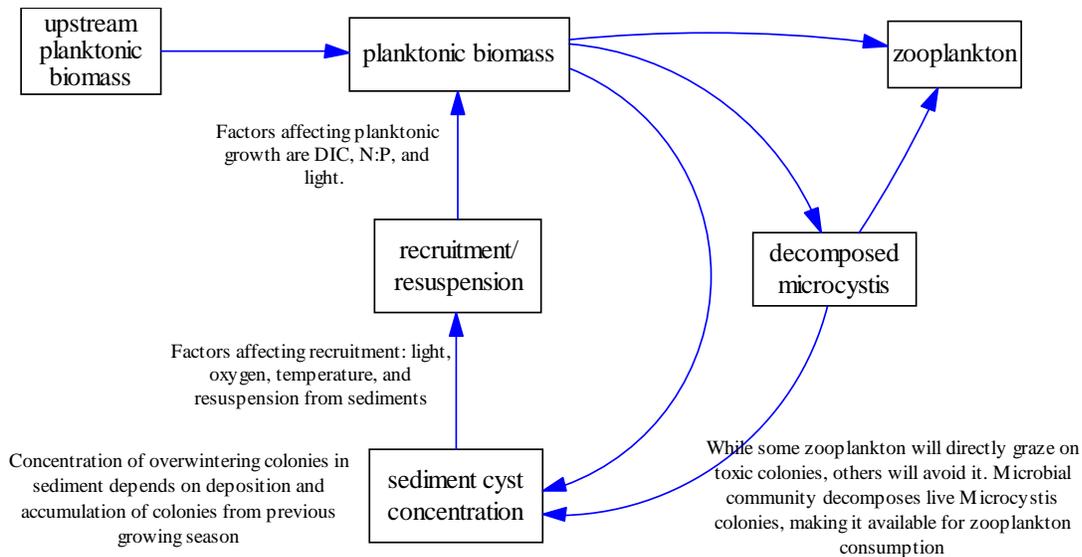
(Reynolds 1981, Tsujimura et al, 2000). Shallow sediments are important inocula for the recruitment of *Microcystis* because recruitment of benthic colonies has also been shown to be a result of passive re-suspension or bio-turbation, rather than active buoyancy control (Verspagen et al, 2004). High nutrient concentrations combined with low N:P ratios (8:1 by mass) favor maximum recruitment (Stahl-Delbanco et al, 2003). Overall, planktonic blooms originate from only a small fraction of the benthic colonies that rapidly reproduce once they are resuspended in the water column.

Once in the water column, *Microcystis* colonies rapidly multiply and tend to concentrate at the water surface, though strong migrations as a result of the ability to regulate buoyancy are well known (Reynolds 1981). Reynolds found that the relative depth of the euphotic zone (secchi depth) compared to the epilimnion predicted the position of *Microcystis* colonies in the water column (1981). Incidence of surface blooms was associated with conditions where the epilimnion was deeper than the euphotic zone. Similarly, downward migrations occurred when the euphotic depth exceeded the epilimnion. In other words, a turbid epilimnion (where epilimnion > euphotic zone) favors positively buoyant colonies.

However, research also indicates that artificially mixing which eliminates any stratification can prevent *Microcystis* blooms, even if the euphotic depth is constant (Visser et al, 1996). The authors suggest that mixing reduces the ability of *Microcystis* to maintain positive buoyancy, and thus reduces its photosynthesis. However, it is not clear whether mixing itself is the mechanism responsible for the reduced ability of *Microcystis* to remain in the euphotic zone. Mixing also increases the CO<sub>2</sub> concentration, resulting in lower pH levels. Lower pH has been shown to favor eukaryotic algae over the prokaryotic cyanobacteria. Further, the formation of surface scums has been linked to low levels of CO<sub>2</sub> in the water (Paerl 1983). *Microcystis* colonies appear to concentrate at the surface in scums to take advantage of atmospheric CO<sub>2</sub> when there is not enough in the water (Paerl 1983). As such, mixing probably does not directly affect buoyancy but rather affects pH, CO<sub>2</sub> concentration, and possibly other nutrients, which then impact buoyancy and *Microcystis* growth.

As temperatures begin to fall at the end of the growing season, surface colonies begin to accumulate carbohydrate reserves, and lose buoyancy, thereby returning them to the bottom sediments to over-winter. There they can survive several years under cold, dark, and hypoxic conditions (Reynolds 1981).

**Figure 1:** Conceptual model of the lifecycle of *Microcystis* in the Delta, and its interaction with the foodweb. Factors affecting the life cycle and flow of carbon are indicated. Life cycle begins each year with overwintering colonies in sediments (cyst) being recruited into the water column as a result of active buoyancy control and passive resuspension from turbulent mixing. Once in the water, rapid growth of colonies occur. Toxic colonies are grazed by some zooplankton, while decaying colonies are grazed by others. Life cycle is completed with the deposition of colonies back to the sediments.



## Conclusion and Recommendations

*Microcystis* is an emerging problem that appears to be intensifying each year. Since this is an emerging problem that is likely to get worse, it is crucial to get a better picture of the onset of a HAB event that could potentially affect. As such, it is a unique opportunity to observe the onset of HAB's in the Delta. From an applied point, any information about the reasons that result in blooms of toxic *Microcystis* will help guide management decisions in the Delta. It is now generally accepted that toxic cyanobacteria are able to alter community structure and function by limiting the abundance of certain zooplankton species affected by toxins and changes in food quality/quantity. Since *Microcystis* is on the rise in the Delta, it must be considered as a potential factor that is contributing to the observed decline in planktonic organisms. Some critical questions to resolve this role in POD are listed below.

Most work on *Microcystis* comes from eutrophic lakes throughout the world. The recent expansion of colonial scums in the Delta presents a unique problem because estuarine studies of *Microcystis* are rare and difficult to assess. Such a lack implies the need to study this bloom much more closely, to identify its role in the ecosystem. The Delta is notoriously turbid, and has complex hydrology. These physical traits probably affect the abundance and distribution of *Microcystis*, and are an example to how the Delta is a different case from the lake blooms. Lake studies provide several key potential processes through which *Microcystis* is affecting the foodweb. To find out how *Microcystis* relates to the POD, these isolated processes must be analyzed and tested in the context of Delta specific conditions and organisms.

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We recommend that as a first step, the impact of *Microcystis* on target zooplankton species should be assessed by laboratory studies. Because copepods can select their food, results will be different from most studies, which look at *Daphnia*. The interplay between food quality, quantity and toxicity will be key to understand and extract the processes behind observed effects. Secondly, we must relate the morphology of *Microcystis* to its effects. Are larger colonies worse or better for zooplankton. When are the colonies most toxic? There are available methods to isolate the effects of toxicity from nutritional inadequacy. This is important when considering if zooplankton are starving in order to avoid eating *Microcystis*. Field sampling will compliment these experiments by revealing toxin accumulation and nutritional status of Delta zooplankton.

- 1) How are target copepods affected by presence of toxic *Microcystis*?
- 2) Does *Microcystis* morphology change throughout space and time, and does morphology differentially impact zooplankton?
- 3) Is there a threshold toxicity/biomass after which community change occurs?
- 4) How does *Microcystis* affect the nutritional state of zooplankton and fish?
- 5) What is the fate and transport of *Microcystis* carbon in the Delta foodweb; aka what happens to *Microcystis* biomass?