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TECHNICAL UPDATE

Date: [redacted]

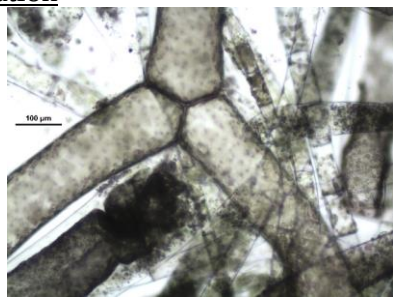
To: [redacted]
Attn: [redacted]

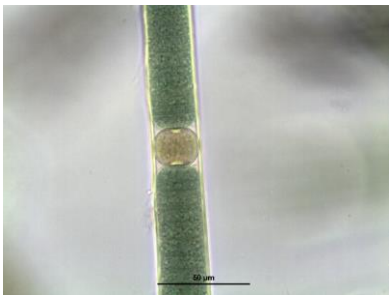
SAMPLE(s): "Lake #6"
Received: [redacted]
Analyzed: [redacted]
Sample analyzed by Benjamin Pankratz and Deborah Lee, Microbiologist, AQUAFIX

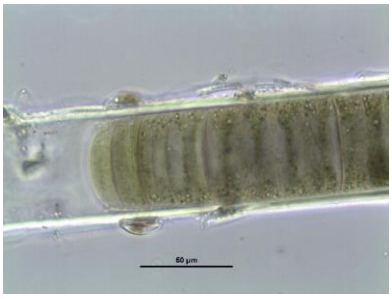
Problem(s): Perform water quality analysis and identify algae and/or plants.

Microscopic Observation
Sample "Lake #6"
Algae taken off plants in sample

Wet mount unstained viewed under 400X magnification

A 

B 

C 

Water Quality Analysis
Sample "Lake #6"
Sample filtered though 0.45um pore membrane

[redacted]	Lake #6
Conductivity	553.3 uS
Salinity (NaCl)	0.249 ‰
Total Dissolved Solids	378.9 ppm
Oxidation Reduction Potential	195 mV
pH @ 18.0C	8.25
NH_3	0.0 ppm
NO_2^-	0.001 ppm
NO_3^-	0.0 ppm
PO_4^{3-}	0.01 ppm
Total Iron (undigested sample)	0.05 ppm
SiO_2	0.082 ppm
Total Hardness	280 ppm as CaCO3
Calcium Hardness	210 ppm as CaCO3
Magnesium Hardness	70 ppm as CaCO3
Total Alkalinity	220 ppm as CaCO3
Phenolphthalein Alkalinity	0.0 ppm as CaCO3

Summary

- One bottle of algae contained filamentous green algae similar to *Hydrodictyon* along with strands of *Oedogonium* and low amounts of the filamentous cyanobacterium *Scytonema*. The *Hydrodictyon* in the sample appeared damaged but this could have been due to where it was collected from. There were healthy microcolonies present in the sample.
    - *Hydrodictyon,* commonly called "water net", is a colonial planktonic green alga that forms floating mats. The cells are cylindrical and once cell is attached to two others at the ends forming a large reticulate network. Each cell can produce a cylindrical net within it which can rapidly expand upon release. This alga is commonly found in eutrophic hard water environments where it can rapidly cover the surface of a pond.
    - *Oedogonium* is green alga that forms unbranched, uniseriated filaments composed of long cylindrical cells. The cells are highly vacuolated with large reticulate, parietal chloroplast and usually many pyrenoids. Some species have a long spike on the terminal cell called a clyptra. This alga does have a thin mucilaginous coating but is generally not slimy to the touch. A definitive characteristic of this alga is the presence of rings or bands at the anterior end of vegetative cells that are formed during vegetative cell division and are the remnants of broken parietal cell walls. This alga can reproduce both asexually by fragmentation or zoospore production and also sexually resulting in the formation of oospores that are brown or red in color. *Oedogonium* is generally found attached to submersed plants or organic debris via a basal holdfast cell although some species are known to create floating mats. Most species are found in stagnant, slow-moving water, possibly containing high nitrates.
- The other bottle of algae consisted of a mat and plant fragments. The algae mats appeared to be mostly *Lyngbya* and *Scytonema*, filamentous cyanobacteria, and low amounts of filamentous green algae that were too fragmented to identify. Some of the plant fragments were similar to the alga *Chara*.
    - *Lyngbya* is a filamentous cyanobacterium that forms layered, leathery, dark colored mats that rise to the surface of ponds and lakes. These filaments are straight and uniseriated without heterocytes or akinetes and are generally very thick, greater than 5.5um wide. Rarely, the filaments display false branching. The cells are discoid and usually much wider than long and are blue-green, olive-green, yellowish, or brownish in color with many fine granules. The trichomes are generally encased in firm sheaths that are sometimes multi-layered and may be brownish in color and open at the ends of the filament. Under extreme conditions, the trichomes may leave the sheaths. Reproduction is by asexual cell division or by production of hormogonia, short motile filaments that are ejected from the sheaths and can form full filaments at a different location. *Lyngbya* is very similar in appearance to *Phormidium*, which has a thinner sticky sheath, and *Oscillatoria*, which normally lacks a sheath and has tapering apical cells. *Lyngbya* is generally found in areas of calm clear water with high light intensity and sandy bottoms in fresh or brackish water.
    - *Scytonema* is a filamentous cyanobacterium that forms dark mats on substrates that float to the surface of ponds and lakes. The filaments are uniseriated with solitary intercalary heterocytes. Heterocytes are specialized structures where nitrogen fixation, converting atmospheric dinitrogen to ammonium and/or glutamine, takes place. The vegetative cells in the filament are pale green or olive-green, with granular content, cylindrical or barrel-shaped. There is usually a sheath present that may be thin and narrow or wide and lamellate and is often yellow or brown in color due to the presence of scytonemin. *Scytonema* reproduces by asexual cell division as well as by hormogonia, which are liberated from the sheath. This cyanobacterium commonly displays false branching with the two branches extending from a break in the trichome of one filament and growing parallel to one another. Branching does not usually occur at heterocytes, as in *Tolypothrix*. *Nodularia* is similar in appearance but does not exhibit false or true branching. Many species of *Scytonema* are found growing in alkali fresh waters with high incoming

phosphorus levels. This alga commonly grows on rocks, wood, or soil and some species have sheaths that are incrusted by calcium carbonate.

- *Chara* is a macrophytic alga that looks like a simple vascular plant. Some common names include muskgrass, skunkweed, and stonewort. This alga typically grows to 10-50cm high with a "stem" that is 0.5-1.0mm in diameter. It typically appears gray or dark green and may feel gritty and smell musty when crushed. Although as an alga it has no true roots it can attach to bottom substrates by holdfast cells. The "stem" bears whorls of branchlets, between 6-16, that are cylindrical and clustered at regularly spaced nodes. These branchlets are never divided and are of equal length often with tiny thorn-like projections at the ends. The gritty feeling of this alga is due to build ups of calcium deposits on the surface of the algal cells. *Chara* reproduces vegetatively by fragmentation or sexually. The plants can be monoecius or dioecious and the oogonium is 900um long, brown or black, with five cells wrapped around in a spiral to form the coronula. The antheridium is up to 500um in diameter. *Chara* is generally found in freshwater, both soft and alkaline, and grows down to 1m in a variety of ponds, rivers, littoral pools, or slowly flowing streams. This genus of algae is highly polymorphic and many forms have been described. *Nitella* is similar in appearance to *Chara* but has symmetrically forked smooth branchlets and does not have the calcium coating or musty odor. *Tolypella* spp. have unsymmetrically forked branchlets. *Chara* may sometimes be confused with some vascular plants such as *Najas flexilus* and *Ceratophyllum demersum*, which also do not produce an odor when crushed. Raking is not an effective control of *Chara* as fragments can give rise to new plants.

- The water sample contained low nitrogen, with enough orthophosphate for blue greens algae to bloom, especially since *Scytonema* can fix nitrogen. The water was hard with high alkalinity. It is possible that the nutrient levels in the water were higher before but the plants and algae present in the lake used all of the nutrients. The nutrients could have also been used by bacteria in the water sample during transit. It could be that most nutrients may be concentrating close to the shoreline of the lake.

Recommend

- Control of the various algae will depend on the location and prevalence of each type. In the mat sample, there seems to be more biomass of *Lyngbya* and *Scytonema* mixed in with fragments of plants. Perhaps this mat is occurring in the littoral zone? It is not clear if the algae that contained *Hydrodictyon* are in a different area from where the mat-formers were sampled or if it was floating on top of the same area.
- Generally, we recommend mixing AquaSticker with Hydrothol 191, the preferred algaecide for controling *Lyngbya* and *Scytonema*. Spraying PondZilla 100 may help to quickly degrading the resulting dead, decaying biomass and remove the resulting nutrients from the water column.
- It is possible that Hydrothol 191 will also be able to control *Hydrodictyon*, however we generally recommend nutrient removal instead though applications of VitaStim MD Pellets and VitaStim Summer Slam or VitaStim 6000.
- Diquat is recommended to control *Chara*, but not to be used in water with high levels of suspended sediments.

Understanding Your Lab Results

Conductivity	Desirable Pond Range 100 to 2,000 µS/cm Acceptable Pond Range 30 to 50,000 µS/cm Seawater is 50,000 to 60,000 µS/m	Conductivity is correlated with salt content. Some salt is needed for fish to maintain osmotic balance. Conductivity also gives a rough estimate of total dissolved solids (TDS) in water. Usually TDS in ppm is about half the conductivity in µS/cm.
Salinity (NaCl)	Fresh water < 0.5 ‰ Brackish water = 0.5 to 30 ‰	Measures the amount of sodium chloride in water. Salinity affects what species of algae will grow. 1 ‰ = parts per thousand
Total Dissolved Solids	Unpolluted = 17-30 ppm Polluted = 400 ppm	Total amount of solids both organic and inorganic that are in water. Includes sodium, calcium, magnesium, sulfates, orthophosphates, dissolved chemicals. Similar to conductivity.
Oxidation Reduction Potential	Done for Aquafix research purposes	Measures ability perform a chemical task such as oxidizing contaminants. Negative ORP denotes antioxidant activity; positive ORP oxidizing activity.
pH	Surface layer commonly 7 to 8.5 Bottom layer commonly 6 to 7.5 Ideal 7.2 to 7.6 Acceptable 6.8 to 8	pH in samples is artificially high due to decomposition. Field testing of pH is more accurate.
NH_3	< 0.1 ppm is considered non-polluted Lethal levels for fish vary 0.05 to 2.0 ppm	Sources ag runoff, lawn fertilizer, ducks and geese, fish, decaying organisms including plants and algae.
NO_2^-	Typically present at < 1 ppm	High concentrations require further research.
NO_3^-	< 0.05 ppm preferred	Top layer of lake may have low levels due to algae uptake. Bottom layer will be higher due to decay. Algae uses NO3 as nitrogen source for growth.
PO_4^{3-} **Orthophosphate**	< 0.02 ppm = Ideal > 0.1ppm = Plant growth is stimulated, expect problems with weed and algae growth.	Algae and aquatic plants use only the orthophosphate (PO_4) form.
Total Iron (undigested sample)	Desirable range is < 0.1 ppm	Responsible for the presence of some algae. At 0.3 ppm Pithophera will proliferate. Iron will concentrate in the bottom layer.
SiO_2	The lower, the better	Forms diatomaceous algae that are impervious to treatment.
Total Hardness	0-60 ppm = soft 120-180 ppm = hard Desirable range is 50 to 150 ppm Minimum acceptable 10 ppm	Total hardness measures calcium and magnesium concentrations. Expect reduced effectiveness of copper-based algaecides in hard water.
Calcium Hardness	Desired range 11 to 23 ppm	Calcium is used in plant cell walls.
Magnesium Hardness	Done for Aquafix research purposes	Research parameter
Total Alkalinity	Optimal is 50 to 150 ppm Acceptable 20 to 400 ppm	Measures ability to withstand changes in pH (buffering capacity). Algae often causes high pH swings.
Phenolphthalein Alkalinity	Done for Aquafix research purposes	Research parameter