Final Technical Report

# 2012 Localized Treatment of Copco Cove in Copco Reservoir Using Environmentally Safe Algaecide

Prepared for



Prepared by:



July 2013

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#### 1.0 INTRODUCTION

On February 18, 2010, the United States, the States of California and Oregon, PacifiCorp, regional Native American tribes, and a number of other stakeholder groups signed the Klamath Hydroelectric Settlement Agreement (KHSA). The KHSA lays out the process for additional studies, environmental review, and a determination by the Secretary of the Interior regarding whether removal of four dams owned by PacifiCorp on the Klamath River (i.e., J.C. Boyle, Copco 1, Copco 2, and Iron Gate dams) will advance restoration of the salmonid fisheries of the Klamath Basin and is in the public interest (which includes effects on local communities and tribes).

The KHSA includes provisions for interim operation of the dams and mitigation activities prior to potential removal of the hydroelectric facilities. One such provision—titled Interim Measure 11: Interim Water Quality Improvements—emphasizes water quality improvement projects in the Klamath Basin during the interim period.

Watercourse Engineering, Inc. (Watercourse), under contract to PacifiCorp, conducted a series of bench studies (in 2008, 2009, and 2011) to assess the potential use of algaecide as part of an overall algae management strategy in Copco and Iron Gate reservoirs on the Klamath River (Deas *et al.*, 2009; Deas *et al.*, 2012). Bench studies consisted of laboratory-based testing on collected samples of site-specific reservoir water to assess the effectiveness of algaecide application at different dosing conditions. A copper-based algaecide, Algimycin PWF, and hydrogen peroxide-based algaecide, GreenClean PRO, were tested. However, the hydrogen peroxide-based algaecide is the only algaecide being considered for actual reservoir application because it is environmentally safe. Hydrogen peroxide is non-persistent and there is no bioaccumulation or sediment accumulation of the product because it degrades into water and oxygen. Further information on these tested algaecides is provided below in section 2.2.

The series of bench studies indicated algaecide could be effective in improving water quality by reducing algal concentrations and associated microcystin levels (microcystin is a toxin that can be produced by blue-green algae species). However, experiments in controlled environments have inherent limitations and their results can be difficult to extrapolate to applications in natural settings. As such, in September 2012, a limited pilot application of environmentally safe hydrogen peroxide-based algaecide was conducted in Copco reservoir in order to evaluate the algaecide's effectiveness under the natural conditions of the reservoir (e.g., wind factors, advective influences, etc.). The 2012 pilot test application in Copco reservoir addressed several objectives:

- Define the necessary steps and activities associated with an algaecide application.
- Identify the effect of algaecide application on nutrient levels in an open reservoir.
- Determine the impact of algaecide application on microcystin concentrations.
- Evaluate the effectiveness of algaecide in reducing algal cells by observing the response of chlorophyll *a* and cyanobacteria species to the algaecide application.

The initial objective of defining the necessary steps and activities associated with an algaecide application proved to be a valuable aspect of the pilot study. These steps included developing an application and monitoring plan, fulfilling permit requirements, identifying an algaecide supplier and professional applicator, addressing product delivery, conducting the in-reservoir application and monitoring program, and submitting required regulatory reporting, among others. The other objectives identified above are discussed further in sections 4 and 5 of this report. Addressing these objectives will help decision makers to evaluate the use and effectiveness of algaecide application as a water quality management strategy within Klamath River reservoirs, and to guide design of potential future algaecide applications.

This report is organized into several sections. Section 2 includes background information of conditions in the Klamath Basin, the use of algaecide treatment as a possible management strategy to reduce public health exposure, and previous algaecide studies. Section 3 describes methodology, including project location, algaecide application procedures, and sampling procedures. Section 4 describes experiment results, followed in section 5 by a discussion based on these results. Section 6 describes economic considerations or costs related to algaecide applications. Section 7 summarizes conclusions and provides several recommendations for future consideration.

# 2.0 BACKGROUND

Algae are a key component of aquatic systems, playing a vital role in food webs and producing oxygen through photosynthesis. However, excessive and/or persistent phytoplankton blooms can impair water quality. Algae can cause taste and odor problems in drinking water and can produce toxins that affect wildlife, livestock, or humans via contact or ingestion. Algae can also present filter clogging challenges in water treatment and irrigation facilities and lower the aesthetic appeal and recreational use of surface waters. In addition, when toxins are involved, reservoirs and other surface waters may be posted with public health warnings, as has been the case with Copco and Iron Gate reservoirs and portions of the Klamath River.

# 2.1 Algae Production Effects in the Klamath River

The Klamath River is nutrient-enriched, due to large loads of nutrients and organic matter to the river from hypereutrophic Upper Klamath Lake and other upstream sources. These nutrients help to cause seasonal algae blooms in the reservoirs along the Klamath River, including Copco reservoir. Extensive seasonal algae standing crop have known direct effects on key water quality constituents in lakes and reservoirs, including dissolved oxygen (DO), pH, and nutrients, among others (Horne and Goldman, 1994). The algal community in Copco reservoir consists of diatoms, golden-brown algae, green algae, dinoflagellates, cryptomonads, microflagellates, and cyanobacteria (blue-green algae). Diatoms and cyanobacteria typically make up the vast majority (by biovolume) of the algal community in Copco reservoir. The seasonal succession of phytoplankton typically progresses from diatoms in the spring (May), followed by cyanobacteria dominance in the summer (July through early- to mid-September) (Figure 1). Inter-annual variations are typical, as is the timing of the onset and decline of algae blooms (see Raymond, 2008; 2009; 2010).

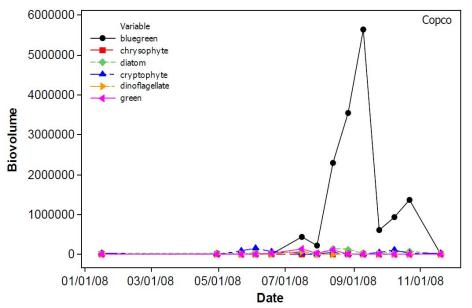


Figure 1. Seasonal Changes in Biovolume (µm<sup>3</sup>/mL) of Various Algal Groups in Copco Reservoir

Cyanobacteria are of particular concern in reservoir management because they can produce undesirable toxins, including the hepatotoxin microcystin, which can, at a sufficient dose, affect the liver of animals. Cyanobateria that can produce microcystin are *Microcystis aeruginosa*, *Anabaena flos-aquae*, *Planktothrix (Oscillatoria)*, *Nostoc*, *Hapalosiphon*, *Anabaenopsis*, *and Pseudoanabaena* (WHO, 1999; Oudra et al. 2002).

Characteristics of cyanobacteria that make their management challenging include the ability of these species to tolerate elevated water temperatures, reproduce at high rates, regulate their buoyancy, and, for certain species, the ability to fix atmospheric nitrogen. These characteristics can combine to create intensive bloom conditions for cyanobacteria populations. Heterogeneous (or "patchy") distributions, accumulation of shoreline mats, wind driven accumulations, variability in toxin production, and other factors contribute to the management challenge.

# 2.2 Algaecides

Algaecides are a common technique for management and control of overabundant algae in ponds, lakes, and reservoirs (Cooke et al., 2005). Algaecides kill algae either by direct toxicity or through metabolic interference. Algaecide treatment can provide rapid removal of algae from the water column, sometimes resulting in dramatic short-term reductions in algal standing crop and improvements in water clarity. Application frequency is a function of the lake or reservoir management objective (e.g., nuisance, taste and odor issues, toxin management, recreation, etc.) and the type of algaecide used. In certain cases, algaecides are applied annually but are, more typically, applied several times throughout periods of algae growth to prevent or reduce algal blooms. There are various types of algaecides available commercially. Algaecides that have been identified as being potentially useful for water quality improvements in the Klamath River fall into two major categories: copper-based and peroxide-based. The two algaecides that were tested in the 2009 bench study were Algimycin PWF (a copper-based algaecide) and GreenClean PRO (an oxidizer). In the 2011 bench study, a liquid version of GreenClean PRO, called GreenClean Liquid, was tested. In the 2012 in-situ pilot application, GreenClean Liquid was used. Other commonly used algaecides are discussed in Deas *et al.* (2009).

# 2.2.1 Copper-based Algaecide

In past years, copper has been the most widely used algaecide (Wagner, 2004; Cooke et al., 2005). The active ingredient in copper-based algaecide is the copper ion, which inhibits photosynthesis and may affect nitrogen metabolism. Compared to other forms of algae, cyanobacteria species appear to be more sensitive to copper. The effectiveness of copper-based algaecides is dependent on alkalinity, dissolved solids content, suspended matter, and water temperature and appears to be enhanced by exposure to sunlight.

In the 2009 algaecide bench study, a copper-based algaecide, Algimycin PWF, was tested (Deas *et al.* 2009). Algimycin PWF, made by Applied Biochemists, is based on copper citrate chelates and copper gluconate chelates (http://www.appliedbiochemists.com/). The algaecide is a solution with 62 grams/liter of copper. Applying different doses of Algimycin PWF can target select types of algae including planktonic, filamentous, and rooted forms. Additional discussion on the effectiveness and applicability of Algimycin PWF can be found in Deas *et al.* (2009).

# 2.2.2 Peroxide-based Algaecide

Various studies have shown that peroxide-based algaecide is potentially a safer and equally-effective alternative to copper (Drábková *et al.* 2007; Barrington and Ghadouani, 2008). Hydrogen peroxide is non-persistent and there is no bioaccumulation or sediment accumulation of the product because it degrades into water and oxygen (Ding *et al.*, 2012; EPA, 2012). Furthermore, hydrogen peroxide exists naturally in lacustrine environments where it is generated photochemically from organic products in the presence of sunlight (Cooper and Zika, 1983; Scully *et al.*, 1995; Scully *et al.*, 1996). Given these considerations, hydrogen peroxide is environmentally benign (Antoniou *et al.*, 2005; Qian *et al.*, 2012).

The mechanism by which hydrogen peroxide breaks down cyanobacteria has been the subject of several studies in recent years. When applied, hydrogen peroxide acts as an oxidizing agent that inhibits algal growth by altering algal physiological and biochemical processes (Samuilov *et al.*, 2004; Qian *et al.*, 2010; Qian *et al.*, 2012). Additionally, Ross *et al.* (2006) established that hydrogen peroxide addition elicited caspase activity in *Microcystis aeruginosa*. More recently, Ding *et al.* (2012) observed that hydrogen

peroxide induces apoptotic-like programmatic cell death<sup>1</sup> (PCD) in *Microcystis* aeruginosa.

Recent studies have observed changes in physiological parameters of algae associated with the introduction of hydrogen peroxide. These include changes in algal mortality, chlorophyll content, cellular soluble protein, and photosynthetic activity (Drábková et al., 2007; Hong et al., 2008; Pan et al., 2008). In addition, Qian et al. (2010) demonstrated that hydrogen peroxide inhibited carbon assimilation thereby inhibiting algal growth. Furthermore, hydrogen peroxide also decreases the levels of photosynthetic pigments: chlorophyll a, phycocyanobilin (PC), allophycocyanin (APC), and phycoerythrin (PE) (Qian et al., 2010). These pigments capture light energy necessary for photosynthesis, and so reduction of their levels inhibits algal growth. Another way that hydrogen peroxide inhibits growth is by changing the rhythms of cyanobacterial clock genes. Many physiological and metabolic activities that occur, such as cell division, nitrogen fixation, photosynthesis, carbon uptake and the biosynthesis of secondary metabolites are controlled by these rhythms. Hydrogen peroxide has been observed to affect circadian rhythms in cyanobacteria. Some observed impacts are declines in solar energy utilization and the synthesis of carbohydrates and high energy molecules, which are necessary for cyanobacterial growth (Qian et al., 2012). Qian et al. (2010) also showed that hydrogen peroxide reduces or inhibits the production or synthesis of microcystin. Finally, hydrogen peroxide can also destroy toxins that are released upon the lysis of cyanobacterial cells (Svrcek and Smith, 2004).

In the 2008 bench study, two hydrogen peroxide-based algaecides, GreenClean PRO and PAK-27, were tested in a bench-top setting with water samples taken from Copco reservoir. In 2009, further bench tests were performed to investigate the effects of higher dosages and re-application of GreenClean PRO on the algae species present in Copco reservoir. The performance of the liquid version of GreenClean PRO, GreenClean Liquid, was tested in 2011. Findings from these studies can be found in Deas *et al.* (2012). Based on the results of these previous studies, GreenClean Liquid was chosen for the 2012 in-reservoir experiment.

GreenClean Liquid, like GreenClean PRO, is produced by BioSafe Systems, LLC, and is a hydrogen peroxide-based alternative to copper-based algaecides and algaecides with other toxic chemicals as their active ingredient. In California and Oregon, there are no known runoff or usage restrictions associated with the use of GreenClean Liquid, which utilizes sodium carbonate peroxyhydrate (SCP) as its active ingredient. SCP is a stabilized form of hydrogen peroxide that is paired with peroxyacetic acid (PAA). PAA is a compound made up of hydrogen peroxide and acetic acid. This compound is an activated form of hydrogen peroxide and acts as a more stable and powerful oxidizer (Larose et al., 2008). The combination of hydrogen peroxide and PAA causes an oxidation reaction that breaks down or damages algae cell walls (Knox, 2009). The reaction works quickly (seconds to minutes), reducing the likelihood of mutational

<sup>&</sup>lt;sup>1</sup> Apoptotic-like PCD is a pattern of cell death affecting single cells, marked by shrinkage and fragmentation of the cell into membrane-bound bodies that are eliminated by phagocytosis (ingestion by other cells, such as microphages).

resistance. As the reaction takes place, hydrogen peroxide and PAA break down into natural compounds: water, oxygen and elements of organic acids (Larose et al., 2008; EPA, 2012). Like hydrogen peroxide, PAA does not persist in the environment (Knox, 2009). Further, the concentration of PAA in GreenClean Liquid is extremely low (on the order of 0.0000033-0.000083 molar for the manufacturer's listed range of application rates) (V. Choppakatla, pers. comm.).

# 2.2.3 Consideration of Potential Algaecide Effects

Use of algaecides can cause temporary effects on non-target plants, but recovery of those plant communities is usually rapid (Wagner, 2004). Oxygen depletion in the water column can follow algaecide application due to the decomposition of dead algae, but associated fish kills are rarely observed with the use of current algaecides (Wagner, 2004). Because cyanotoxins are stored intracellularly, algaecide treatments could lead to releases of toxin to surrounding waters (Kenefick *et al.*, 1993). Jones and Orr (1994) and Touchette *et al.* (2005) presented studies demonstrating microcystin release when *Microcystis aeruginosa* blooms were treated with algaecides. This release following treatment can be quite rapid (i.e., within minutes), depending on the algaecide application quantity (Jones and Orr, 1994). Nevertheless, the World Health Organization (WHO) (2007) indicated that more than 95 percent of the microcystin is contained within healthy cells and that, although dying and decaying cyanobacteria release microcystin to the water, biodegradation is typically sufficient to avoid high concentrations of microcystin. However, WHO (2007) also states that artificial lysing of the cell may increase dissolved algal toxin concentrations in the water.

As discussed in the Introduction, the algaecide studies conducted to date (Deas et al. 2012 and as reported herein) have included objectives aimed at defining both the benefits and potential impacts of algaecide use. As such, in addition to assessing the effectiveness of potential algaecide treatment in reducing algal standing crop, the studies have also assessed the effects of algaecide application (such as described above) on nutrient and microcystin concentrations in the surrounding water. Study results pertaining to these various objectives are described further in sections 4 and 5 of this report.

# 3.0 METHODOLOGY

This section describes the project location, algaecide application procedure, and the sampling methods associated with the in-situ pilot application of GreenClean Liquid, which utilizes SCP as its active ingredient, conducted in Copco Cove in September, 2012.

The objective of this study was to define the necessary steps and activities associated with an algaecide application in a reservoir setting and to assess the immediate effects of algaecide application. These steps included developing an application and monitoring plan, identifying an algaecide supplier and professional applicator, and conducting the inreservoir application and monitoring program. These steps are presented herein. An assessment of the long-term efficacy of algaecide in controlling algae concentrations in a local area, such as a cove, was not an objective of this pilot project.

# 3.1 Project Location

Copco Cove (Figure 2) was selected as the project location based on its size, accessibility, and the amount of algae observed on the water surface on September 5, 2012 (the day prior to the algaecide application). Conducting the experiment in Copco Cove utilized the natural shape of the cove to limit water movement and exposure to wind.

Figure 2. Aerial Photo of Copco Reservoir that Includes the Location of Copco Cove and Mallard Cove



# 3.2 Algaecide Application Procedures

The algaecide used for the project was GreenClean Liquid (EPA Registration No. 70299-2), which is manufactured by BioSafe Systems, LLC (BioSafe). Algaecide application was performed by Clean Lakes, Inc. (CLI) on September 6, 2012. The application of algaecide was conducted in compliance with:

- California Department of Pesticide Regulation (DPR).
- State Water Resources Control Board (SWRCB) Water Quality Order No. 2004-0009-DWQ, which is the Statewide General National Pollutant Discharge Elimination System Permit for the Discharge of Aquatic Pesticides for Aquatic Weed Control in Waters of the United States (General Permit No. CAG990005).

GreenClean Liquid, contained in four 55-gallon drums, was delivered to the Pacific Power facility in Yreka, California on the morning of September 6, 2012. CLI staff loaded the drums onto CLI's truck for delivery to the Mallard Cove boat ramp. At the

boat ramp, CLI staff transferred GreenClean Liquid from the delivery drums to the treatment vessel using a closed system algaecide transfer procedure.



Figure 3. Photo at the Mallard Cove Boat Ramp Prior to Algaecide Application

Algaecide application at Copco Cove began at 13:00. Treatment was completed approximately one hour later. CLI utilized a LittLine<sup>®</sup> Littoral Zone Treatment vessel for algaecide application. Algaecide was applied to the upper 4 feet (1.2 meters) of the water column. In Copco Cove, 4.7 acres of reservoir surface was treated with 220 gallons of GreenClean Liquid.



Figure 4. Photo of Treatment Vessel used for Algaecide Application

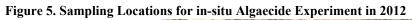
After algaecide application, empty algaecide drums were triple rinsed into the application vessel's pesticide tanks. Rinsed drums were then transported by CLI to their disposal facility in Martinez, CA for removal per DPR regulations.

3.3 Sampling Methods

Grab samples and physical measurements were collected at eight locations, which include three control sites (identified with a "C") located outside the treatment area to represent untreated conditions, and five treatment sites (identified with a "T") located within the treated area (Figure 5). Control sites were located approximately 500 feet from the treatment area so that they would be unaffected by algaecide application. Hydrogen

peroxide, the active ingredient in the algaecide, was collected at 3 treatment locations (T1, T2, and T3). At each location, samples were collected at two depths: near surface (0.1 m depth) and subsurface (1.0 m depth). The shaded region in Figure 5 represents the approximate treatment area within Copco Cove.

The sampling locations were identified using a Garmin Oregon<sup>®</sup> 450 Geographic Positioning System (GPS) prior to pre-treatment sampling. The coordinates were recorded in the GPS and later used to position the boat when samples were taken. A summary of the sampling location coordinates are included in the attached Appendix A. This procedure ensured consistent repositioning at each sampling location on the reservoir where pre-treatment (representing background conditions), post-treatment (immediately following treatment), and next day (post-event) samples were collected.

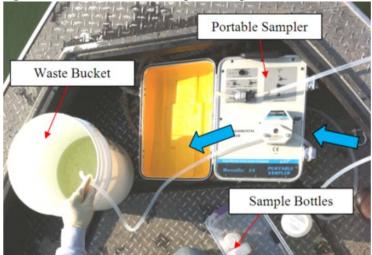




Pre-treatment samples were collected between 08:25 and 09:45 on September 6, 2012. Post-treatment samples were collected between 13:00 and 14:50 on September 6, 2012. Post-event (next day) samples were collected between 07:50 and 09:15 on September 7, 2012. All the sampling times are summarized in the Appendix A (Table A-2).

Samples were collected from a boat using a Cole-Parmer Masterflex<sup>®</sup> E/S portable sampler, which is a variable speed peristaltic pump used in conjunction with a <sup>1</sup>/<sub>4</sub> inch hose to draw water from 0.1 m and 1.0 m depths. At the start of sample collection, for each location and each depth, the hose was rinsed with environmental water by running the pump for 1 minute before taking samples. Following the environmental rinse, prepared sample bottles were filled. The set-up of the portable sampler is shown in Figure 6. The blue arrows indicate direction of water flow.

Figure 6. Photo of Portable Sampler Set-up



At each sampling location and depth, grab samples were collected for subsequent laboratory analysis of nutrients, microcystin, cyanobacteria (blue-green algae), species enumeration, and chlorophyll *a*. Hydrogen peroxide samples were also collected at locations T1, T2, and T3. In addition to these grab samples, measurements of water temperature, dissolved oxygen (DO), pH, turbidity (triplicate), Secchi depth, and total depth were taken at each location.

Laboratory analysis of samples were performed for total nitrogen (TN), nitrate and nitrite (NO3+NO2), nitrite (NO2), ammonia (NH4), total phosphorus (TP), orthophosphate (PO4), dissolved organic carbon (DOC), chlorophyll *a*, microcystin, and hydrogen peroxide. Samples were delivered directly to the Biogeochemistry Laboratory (P.I. Dr. Randy Dahlgren) at University of California, Davis within 48 hours. Samples analyzed for microcystin were frozen and shipped overnight to Tamarack Environmental Laboratories, LLC, Washington, Michigan. Hydrogen peroxide samples were transported to Davis, California and then by courier to the McCampbell Analytical, Inc. laboratory in Pittsburg, California within the 7-day holding time. All samples were stored and transported or shipped on ice. Laboratory information associated with each constituent is included in Table 1.

Constituent	Units	Method	Preservative	MDL <sup>a</sup>	RL <sup>a</sup>	Laboratory
TN	mg/l	NEMI <sup>b</sup> I-4650-03	None	0.01	0.02	Biogeochemistry Laboratory, U.C. Davis
NO3+NO2	mg/l	Nitrate via V(III) reduction <sup>c</sup>	None	0.005	0.01	Biogeochemistry Laboratory, U.C. Davis
NO2	mg/l	Nitrate via V(III) reduction	None	0.002	0.01	Biogeochemistry Laboratory, U.C. Davis
NH4	mg/l	SM <sup>d</sup> 4500-NH3 F	None	0.005	0.01	Biogeochemistry Laboratory, U.C. Davis
ТР	mg/l	NEMI I-4650-03	None	0.01	0.01	Biogeochemistry Laboratory, U.C. Davis
OPO4	mg/l	SM 4500-P E	None	0.001	0.005	Biogeochemistry Laboratory, U.C. Davis
DOC	mg/l	EPA 415.3	None	0.1	0.1	Biogeochemistry Laboratory, U.C. Davis
Chlorophyll <i>a</i>	µg/l	EPA 445.0	None	1ppb	1 ppb	Biogeochemistry Laboratory, U.C. Davis
Microcystin	mg/l	ELISA <sup>f</sup>	None	0.16	n/a	Tamarack Environmental Laboratory
Hydrogen Peroxide	mg/l	Titanium Sulfate/ Spectrophotometric <sup>g</sup>	None	n/a	1.0	McCampbell Analytical, Inc.

Table 1. Laboratory Methods, Method Detection Limits (MDL), and Reporting Limits (RL), as Applicable

<sup>a</sup> Units are in mg/l unless otherwise specified.

<sup>b</sup> National Environmental Methods Index

<sup>c</sup> This method was developed by UC Davis Department of Land, Air and Water Resources (Doane and Horwath, 2003)

<sup>d</sup> Standard Methods

<sup>e</sup> Environmental Protection Agency

<sup>f</sup> USEPA Region 9 SOP 1305 (Envirologix ELISA method)

<sup>g</sup> This method is from Industrial and Engineering Chemistry Analytical publication, "Colorimetric Determination of Hydrogen Peroxide" (Eisenberg, 1943).

# 4.0 RESULTS

The water quality sample results (including nutrients, microcystin and hydrogen peroxide) and algal response from the September 2012 test application of algaecide in Copco Cove are summarized below, and the discussion of these results is presented in Section 5. Measurements for water temperature, dissolved oxygen, pH, turbidity, Secchi depth, and total water depth are included in Appendix A.

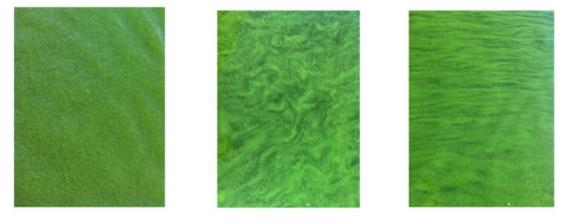
4.1 Visual Observations

Visual observations indicated high algal densities throughout the pilot project period. Weather conditions were calm and clear. Wind speed was low throughout the sampling effort. There were no visible sheens, and clarity was limited due to algal densities (7). However, the distribution of algae varied (8).

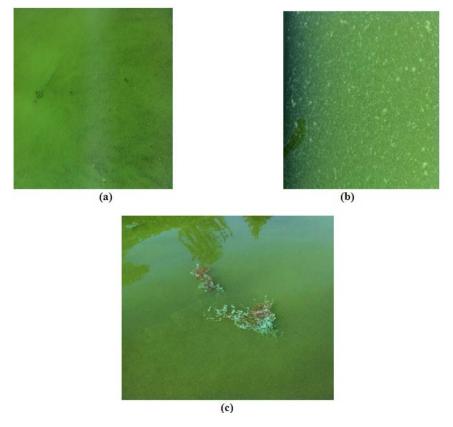


Figure 7. Copco Cove Treatment Area (Left) and Shoreline Area Within Cove (Right)

Figure 8. Surface Algae Conditions in the Project Site Prior to Algaecide Application



At the time of the treatment (post-treatment sampling), high algal densities were evident in the near surface waters (Figure 9a). Subsequent to treatment, clarity was improved (Figure 9b). While algae were still present, accumulations of algae in surface waters were dispersed and in small clumps (assumed to be dead algae) that tended to accumulate along the shore, or on macrophytes or other material in the water (Figure 9c). Post-event visual conditions were similar to pre-treatment conditions. Figure 9. Lake Surface Algae Conditions (a) Prior to Algaecide Application, (b) After Algaecide Application, and (c) Accumulations of Dead Algae After Algaecide Application



#### 4.2 Water Quality

Water quality grab samples were collected at all control and test locations before treatment ("pre-treatment"), shortly after treatment ("post-treatment"), and approximately one day after treatment ("post-event"). Pre-treatment and post-treatment sampling was carried out at approximately the same time on each day to provide for a direct comparison. For each sampling period, average values of the control samples and the treatment samples were calculated. These averages, at multiple depths, are presented in tabular and graphical form herein to highlight the effects of algaecide treatment. All field data are included in Appendix A.

#### 4.2.1 Nitrogen

Total nitrogen (TN) concentrations for all control and treatment sites are shown in Figure 10. Average concentration of control and treatment area pre-treatment, post-treatment, and post-event total nitrogen concentrations, including percentage change between preand post-treatment, and pre-treatment and post event sampling are shown in Table 2.

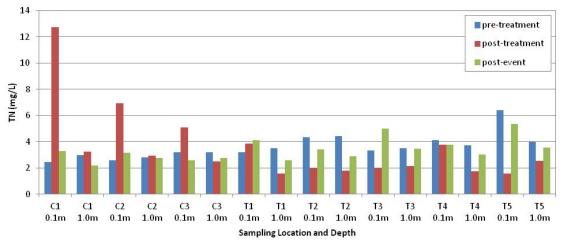


Figure 10. Total Nitrogen (TN) Concentrations in all Sampling Locations and Depths

 Table 2. Summary of Average Total Nitrogen (TN) Response for 0.1 Meter, 1.0 Meter, and All

 Depths for Both Control and Treatment Area Samples

	TN (mg/l)				
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	2.67	7.63	2.87	185.4%	7.2%
Treatment (0.1)	3.18	3.20	2.96	0.5%	-7.0%
Control (1.0)	4.04	1.93	3.77	-52.1%	-6.7%
Treatment (1.0)	4.34	2.36	3.84	-45.7%	-11.6%
Control (all depths)	3.36	4.78	3.32	42.5%	-1.1%
Treatment (all depths)	3.76	2.78	3.40	-26.2%	-9.7%

Post-treatment reduction in average total nitrogen (TN) concentration was observed at 1.0 meter depth, while little variation was observed in post-treatment for surface samples (0.1 meter). One day later, average TN levels at all depths returned to approximately pretreatment levels. The control samples taken near the reservoir surface (at 0.1 m depth) showed a notable increase in average TN concentrations when post-treatment event samples were collected. This increase is likely related to higher algae concentrations in near-surface waters during mid-day periods, compared to pre-treatment control sampling performed earlier in the morning. As discussed further below, similar increases in nearsurface control samples occurred for TP, microcystin, and chlorophyll a, providing additional evidence of increased algae production in near-surface waters during posttreatment sampling. If increased algae production near the surface at control sites was indicative of Copco Cove overall during post-treatment sampling, the results from posttreatment samples obtained at the near-surface test locations (at T1-T5) may be conservative; that is, the effect of treatment may be greater than indicated by direct comparison of post-treatment to pre-treatment levels (Table 2). Average TN levels in samples taken the next day (post event) at the near-surface control locations (0.1 meters) showed concentrations similar to pre-treatment levels. Average TN concentrations for

control samples taken at 1.0 m depth remained relatively stable throughout the study period.

All nitrite (NO2) concentrations were non-detectable, and so the combined nitrate and nitrite concentrations (NO3+NO2) are presented herein. NO3+NO2 concentrations for all control and treatment sites are shown in Figure 11. Average concentration of control and treatment area pre-treatment, post-treatment, and post event NO3+NO2 concentrations, including percentage change between pre- and post-treatment, and pre-treatment and post event sampling are shown in Table 3.

For surface samples, reductions in average NO3+NO2 concentrations were observed after the addition of GreenClean Liquid, but this average decrease was not observed at the 1.0 meter depth. In all control samples at the surface, decrease in average NO3+NO2 concentration was observed. As discussed above, naturally-higher algae production likely was present near the surface during the mid-day period, which could explain the notable decrease in NO3+NO2 levels in the post-treatment samples for control sites. This natural decrease in NO3+NO2 levels during post-treatment sampling, if also indicative of Copco Cove overall, suggests that the effect of treatment on NO3+NO2 levels at test locations may be less than indicated by direct comparison of post-treatment to pre-treatment levels.

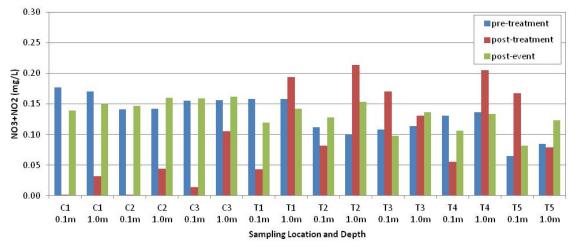


Figure 11. Nitrate and Nitrite (NO3 + NO2) Concentrations at All Sampling Locations and Depths

 Table 3. Summary of Average Nitrate And Nitrite (NO3+NO2) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

	NO3+NO2 (mg/l)				
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	0.163	0.012	0.145	-92.8%	-10.6%
Treatment (0.1)	0.154	0.080	0.149	-47.9%	-3.5%
Control (1.0)	0.106	0.155	0.126	46.0%	18.8%
Treatment (1.0)	0.106	0.127	0.116	20.0%	9.5%

	Ň	103+NO2 (mg	/I)		
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (all depths)	0.135	0.084	0.136	-37.9%	1.0%
Treatment (all depths)	0.130	0.104	0.132	-20.2%	1.8%

Table 3. Summary of Average Nitrate And Nitrite (NO3+NO2) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

Ammonia (NH4) concentrations for all control and treatment sites are shown in Figure 12. Average concentration of control and treatment area pre-treatment, post-treatment, and post event NH4 concentrations, including percentage change between pre- and post-treatment, and pre-treatment and post event sampling are shown in Table 4. Average ammonium (NH4) concentrations at all depths were uniformly reduced after algaecide application. The next day, a rebound in average NH4 levels was observed, but these increases did not reach pre-treatment levels from the previous day.

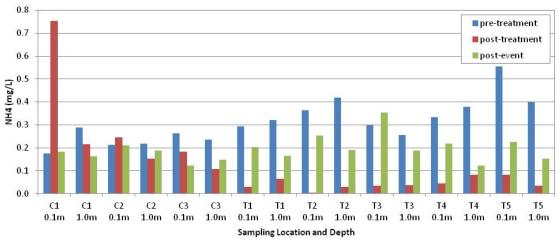


Figure 12. Ammonia (NH4) Concentrations at All Sampling Locations and Depths

 Table 4. Summary of Average Ammonium (NH4) Response for 0.1 Meter, 1.0 Meter, and All

 Depths for Both Control and Treatment Area Samples

	NH4 (mg/l)			_	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	0.22	0.40	0.18	79.9%	-17.8%
Treatment (0.1)	0.27	0.11	0.16	-59.9%	-38.1%
Control (1.0)	0.36	0.02	0.27	-93.7%	-26.5%
Treatment (1.0)	0.38	0.06	0.18	-85.6%	-52.8%
Control (all depths)	0.29	0.21	0.23	-27.1%	-23.1%

	NH4 (mg/l)			_	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Treatment (all depths)	0.33	0.08	0.17	-75.0%	-46.8%

Table 4. Summary of Average Ammonium (NH4) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

#### 4.2.2 Phosphorus

Total phosphorus (TP) concentrations for all control and treatment sites are shown in Figure 13. Average concentration of control and treatment area pre-treatment, post-treatment, and post event TP concentrations, including percentage change between preand post-treatment, and pre-treatment and post event sampling are shown in Table 5. Average post-treatment reductions from pre-treatment levels in total phosphorus (TP) were observed for 1.0 meter depth, but not at the surface. One day later, average TP levels at most test locations and depths returned to approximately pre-treatment levels. The control samples (at C1, C2, and C3) taken near the reservoir surface (at 0.1 m depth) showed a notable increase in TP concentrations when post-treatment samples were collected. This increase is likely related to naturally-higher algae production near the surface during post-treatment control sampling (as discussed above regarding a similar trend for TN). This natural increase in TP levels during event (post-treatment) sampling, if also indicative of Copco Cove overall, suggests that the effect of treatment on TP levels at test locations may be greater than indicated by direct comparison of post-treatment to pre-treatment levels.

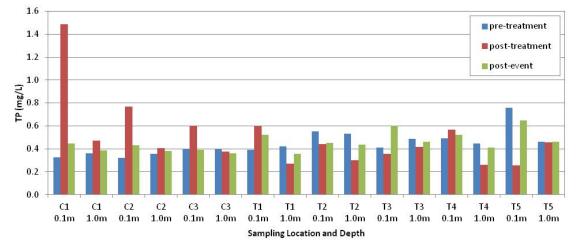


Figure 13. Total Phosphorus (TP) Concentrations at All Sampling Locations and Depths

		TP (mg/l)			
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	0.33	0.91	0.42	170.7%	25.4%
Treatment (0.1)	0.39	0.45	0.40	15.1%	2.9%
Control (1.0)	0.50	0.36	0.50	-26.9%	-0.6%
Treatment (1.0)	0.53	0.39	0.50	-25.9%	-5.2%
Control (all depths)	0.42	0.64	0.46	52.5%	9.8%
Treatment (all depths)	0.46	0.42	0.45	-8.5%	-1.8%

Table 5. Summary of Average Total Phosphorus (TP) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

Orthophosphate (PO4) concentrations for all control and treatment sites are shown in Figure 14. Average concentration of control and treatment area pre-treatment, post-treatment, and post event PO4 concentrations, including percentage change between pre-and post-treatment, and pre-treatment and post event sampling are shown in Table 6. After treatment, increases in average orthophosphate (PO4) concentrations were observed at both experiment depths. One day later, average PO4 levels declined to concentrations slightly below pre-treatment levels. Control samples remained fairly constant throughout the experiment, with the next day samples (post-event) illustrating slight decreases in PO4 concentrations.

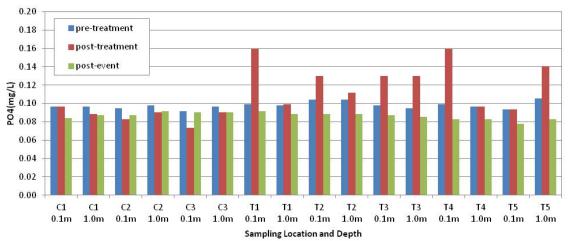


Figure 14. Orthophosphate (PO4) Concentrations at All Sampling Locations and Depths

		PO4 (mg/l)				
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change	
Control (0.1)	0.096	0.089	0.086	-7.0%	-10.2%	
Treatment (0.1)	0.097	0.102	0.090	6.0%	-6.4%	
Control (1.0)	0.102	0.124	0.088	21.6%	-13.6%	
Treatment (1.0)	0.098	0.124	0.082	26.7%	-16.0%	
Control (all depths)	0.099	0.107	0.087	7.8%	-11.9%	
Treatment (all depths)	0.097	0.113	0.086	16.4%	-11.2%	

 Table 6. Summary of Average Orthophosphate (PO4) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

#### 4.2.3 Dissolved Organic Carbon

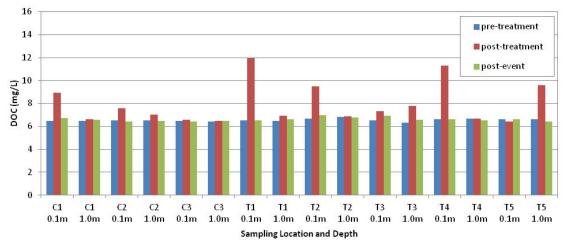
Dissolved organic carbon (DOC) concentrations for all control and treatment sites are shown in

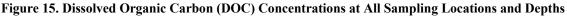
Figure 15. Average concentration of control and treatment area pre-treatment, posttreatment, and post event DOC concentrations, including percentage change between preand post-treatment, and pre-treatment and post event sampling are shown in Table 7. Average dissolved organic carbon (DOC) concentrations increased with the addition of GreenClean Liquid. One day later, DOC levels decreased to pre-treatment levels. DOC at control sites remained relatively constant throughout the study period.

		DOC (mg/l)		_	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	6.49	7.71	6.57	18.8%	1.3%
Treatment (0.1)	6.48	7.78	6.51	20.1%	0.4%
Control (1.0)	6.67	7.90	6.89	18.4%	3.3%
Treatment (1.0)	6.59	8.35	6.55	26.7%	-0.6%
Control (all depths)	6.58	7.80	6.73	18.6%	2.3%
Treatment (all depths)	6.54	8.07	6.53	23.4%	-0.1%

 Table 7. Summary of Average Dissolved Organic Carbon (DOC) Response for 0.1 Meter, 1.0

 Meter, and All Depths for Both Control and Treatment Area Samples.





#### 4.2.4 Microcystin

Microcystin concentrations for all control and treatment sites are shown in Figure 16. Average concentration of control and treatment area pre-treatment, post-treatment, and post event microcystin concentrations, including percentage change between pre- and post-treatment, and pre-treatment and post event sampling are shown in Table 8. Reductions in average microcystin concentrations following treatment were observed at both experiment depths. One day later, microcystin levels returned to approximately pretreatment levels. Control samples that were taken near the reservoir surface experienced a sharp increase in microcystin concentrations at the time when event samples were collected. As discussed above for TN and TP, this increase is likely related to naturallyhigher algae production near the surface during event (post-treatment) control sampling. This naturally-higher algae production, if indicative of Copco Cove overall, suggests that the effect of treatment on microcystin levels at test locations may be greater than indicated by direct comparison of post-treatment to pre-treatment levels. Microcystin levels in the control samples returned to pre-treatment levels the next day.

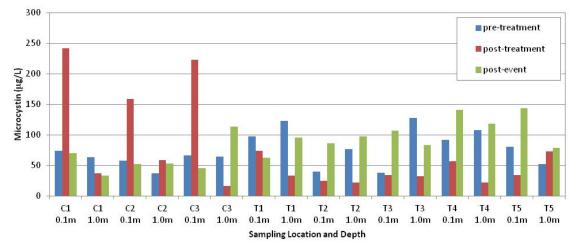


Figure 16. Microcystin Concentrations at All Sampling Locations and Depths.

	Μ	licrocystin (mg	g/l)		Pre-treatment to Post-Event % Change
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	
Control (0.1)	65.8	207.7	56.0	215.8%	-14.9%
Treatment (0.1)	69.3	44.7	107.9	-35.5%	55.7%
Control (1.0)	54.6	37.2	66.4	-31.8%	21.5%
Treatment (1.0)	97.3	36.5	94.5	-62.5%	-2.8%
Control (all depths)	60.2	122.5	61.2	103.5%	1.6%
Treatment (all depths)	83.3	40.6	101.2	-51.3%	21.5%

 Table 8. Summary of Average Microcystin Response for 0.1 Meter, 1.0 Meter, and All Depths for

 Both Control and Treatment Area Samples

#### 4.2.5 Hydrogen Peroxide

Samples were analyzed for hydrogen peroxide at T1, T2, and T3. Sampling results indicate that hydrogen peroxide was non-detect in the pre-treatment and post-event sampling, and was non-detect in four of the six samples collected during post-treatment sampling (Table 9). The remaining two samples indicated low hydrogen peroxide concentrations.

		Ну	Hydrogen Peroxide (mg/L)			
Location	Depth (m)	Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	Post-event (9/7/2012)		
T1	0.1	ND	4.0	ND		
T2	0.1	ND	7.0	ND		
Т3	0.1	ND	ND	ND		
T1	1.0	ND	ND	ND		
T2	1.0	ND	ND	ND		
Т3	1.0	ND	ND	ND		

Table 9. Summary of Hydrogen Peroxide Analysis Results

\*ND means not detected at or above the reporting limit. Reporting limit is 1 mg/L.

#### 4.3 Algal Response

Algae response to the treatment was measured by analysis of chlorophyll *a* concentrations and enumeration of cyanobacteria (blue-green algae) species from the collected samples.

#### 4.3.1 Chlorophyll a

Chlorophyll *a* concentrations for all control and treatment sites are shown in Figure 17. Average concentration of control and treatment area pre-treatment, post-treatment, and post event chlorophyll *a* concentrations, including percentage change between pre- and post-treatment, and pre-treatment and post event sampling are shown in Table 10. Reductions were observed in average chlorophyll *a* concentrations at all depths. The next day, a rebound in chlorophyll *a* levels was observed, but concentrations did not reach the pre-treatment levels from the previous day. Control samples that were taken near the reservoir surface experienced a sharp increase in average chlorophyll *a* concentrations in the post-treatment samples that was not observed in the samples within the treated area. As discussed above for TN, TP, and microcystin, this increase is likely related to naturally-higher algae production near the surface during event (post-treatment) control sampling. Chlorophyll *a* levels in these samples returned to pre-treatment levels the next day.

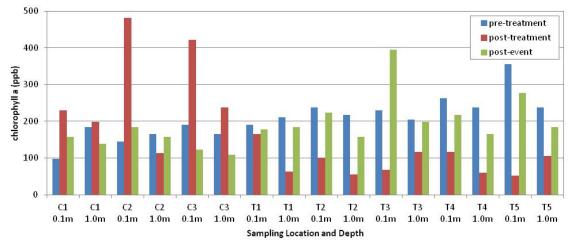


Figure 17. Chlorophyll *a* Concentrations at All Sampling Locations and Depths

Table 10. Summary of Average Chlorophyll *a* Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

	Ch	lorophyll a (p	pb)		
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	144.3	377.5	155.3	161.6%	7.6%
Treatment (0.1)	255.5	100.4	258.1	-60.7%	1.0%
Control (1.0)	171.2	182.7	135.0	6.7%	-21.2%
Treatment (1.0)	221.2	80.0	177.8	-63.8%	-19.6%
Control (all depths)	157.7	280.1	145.1	77.6%	-8.0%
Treatment (all depths)	238.4	90.2	217.9	-62.2%	-8.6%

#### 4.3.2 Cyanobacteria (Blue-Green Algae)

In the 2011 algaecide study (Deas *et al.* 2012), six types of algae species groups were identified in water samples taken from Copco reservoir: cyanobacteria, chrysophyte, cryptophyte, diatom, dinoflagellate, and green. In this study, algae species enumeration was limited to cyanobacteria since this is the algae species group of concern for toxin production. Algae species densities for *Aphanizomenon flos-aquae* (APFA), *Microcystis aeruginosa* (MSAE), and *Pseudoanabaena sp.* (PSAB) were analyzed. APFA, MSAE, and PSAB concentrations for all control and treatment sites are shown in Figure 18 through Figure 20, respectively. Average APFA, MSAE, and PSAB concentrations of control and treatment area pre-treatment, post-treatment, and post event concentrations, including percentage change between pre- and post-treatment, and pre-treatment and post event sampling are shown in Table 11 through Table 13, respectively.

Reductions were observed in average APFA density at all depths. The next day, a rebound in APFA counts was observed at both experiment depths, but these increases did not reach pre-treatment concentrations from the day before. Some patterns in APFA density are discernible in the control samples: 1) in the control samples taken in the morning, average APFA density at each location is similar at both sampled depths (0.1 m and 1.0 m); and 2) in the afternoon, APFA density was higher at the surface.

	APFA (1000 cells/ml)				
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	216.5	450.9	245.8	108.2%	13.5%
Treatment (0.1)	345.0	65.1	195.7	-81.1%	-43.3%
Control (1.0)	239.4	92.7	106.6	-61.3%	-55.5%
Treatment (1.0)	294.5	86.3	174.6	-70.7%	-40.7%
Control (all depths)	228.0	271.8	176.2	19.2%	-22.7%
Treatment (all depths)	319.8	75.7	185.1	-76.3%	-42.1%

 Table 11. Summary of Average Aphanizomenon flos-aquae (APFA) Response for 0.1 Meter, 1.0

 Meter, and All Depths for Both Control and Treatment Area Samples

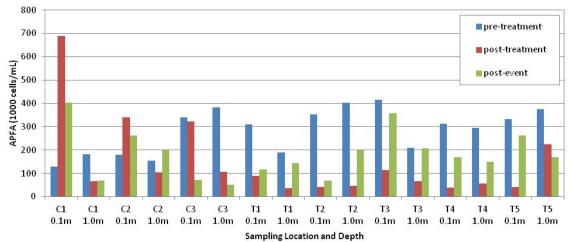


Figure 18. Aphanizomenon flos-aquae (APFA) Density (1,000 cells/ml) at All Sampling Locations and Depths

No discernible pattern was observed in the MSAE density results.

Figure 19. *Microcystis aeruginosa* (MSAE) Density (1,000 cells/ml) at All Sampling Locations and Depths

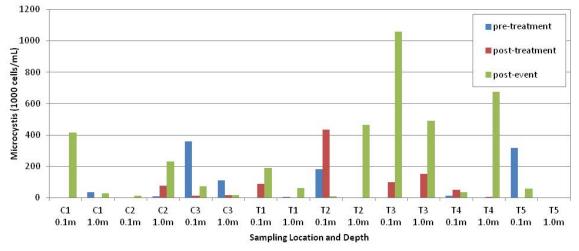


Table 12. Summary of Average *Microcystis aeruginosa* (MSAE) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples.

	MSAE (1000 cells/ml)			_	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	12.1	0.0	152.8	-100.0%	1160.2%
Treatment (0.1)	96.3	40.3	114.7	-58.1%	19.1%
Control (1.0)	61.2	179.3	511.7	193.0%	736.2%
Treatment (1.0)	66.2	42.1	251.8	-36.4%	280.3%

Control (all depths)	36.7	89.6	332.2	144.5%	806.3%
Treatment (all depths)	81.2	41.2	183.2	-49.3%	125.6%

Reductions were observed in average *Pseudoanabaena sp.* (PSAB) density at both experiment depths. Overall rebounds in *Pseudoanabaena sp.* levels were observed on the subsequent morning.

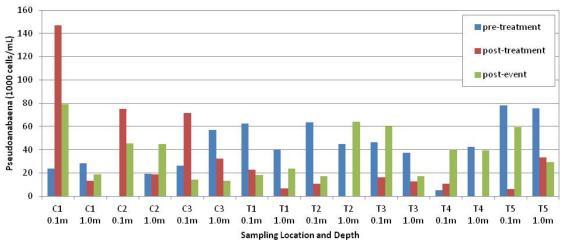


Figure 20. Pseudoanabaena Sp. Density (1,000 cells/ml) at All Sampling Locations and Depths

Table 13. Summary of Average Pseudoanabaena sp. (PSAB) Response for 0.1 Meter, 1.0 Meter, and
All Depths for Both Control and Treatment Area Samples

	PSAB (1000 cells/ml)			_	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	Pre-treatment to Event % Change	Pre-treatment to Post-Event % Change
Control (0.1)	27.9	78.3	47.6	181.0%	71.0%
Treatment (0.1)	40.8	30.4	22.8	-25.4%	-44.2%
Control (1.0)	51.6	13.5	47.1	-73.8%	-8.8%
Treatment (1.0)	47.6	14.8	37.2	-68.8%	-21.9%
Control (all depths)	39.7	45.9	47.3	15.6%	19.2%
Treatment (all depths)	44.2	22.6	30.0	-48.8%	-32.2%

# 5.0 DISCUSSION

As described above in the Introduction, the objectives of the 2012 pilot application of environmentally safe algaecide in Copco Cove focused on several technical objectives, including identifying the effects of peroxide-based algaecide application on nutrient concentrations, microcystin levels, and algal response. These technical objectives were successfully studied in the 2011 bench-top tests (Deas et al. 2012). During the 2012 pilot algaecide study, the same technical objectives were studied in an in-situ reservoir setting.

#### 5.1 Nutrients

Effects of algaecide application on inorganic nitrogen were consistent with expected impacts of a peroxide-based algaecide that functions as a strong oxidizing agent. Overall, post-treatment NO2+NO3 concentrations were higher than pre-treatment levels at most test locations and depths. While NO2+NO3 levels did not consistently increase with the application of GreenClean Liquid, notable NO2+NO3 reductions in control samples (from 32 to 99 percent) were likely related to naturally-higher algae production at the time that event samples were taken. This naturally-higher algae production, if indicative of Copco Cove overall, suggests that the effect of treatment on NO2+NO3 levels at test locations may be less than indicated by direct comparison of post-treatment to pre-treatment levels.

In contrast, NH4 levels were uniformly reduced at all the test locations and depths (from 79 to 99 percent). Further, NH4 concentrations in control samples were also higher, confirming that application of GreenClean Liquid resulted in NH4 concentrations in the reservoir below pre-treatment levels. These changes in inorganic nitrogen levels were likely due to the oxidizing action of GreenClean Liquid.

Increases in inorganic phosphorus were observed at all test locations and depths. PO4 concentrations increased up to 60 percent with the addition of GreenClean Liquid. In the control samples, PO4 levels remained fairly constant throughout the experiment. These increases in PO4 levels that correspond with algaecide addition were likely due to release of PO4 from algal cells and possibly due to substantially reduced algal uptake of PO4 from the water column following treatment.

DOC concentrations increased at some test locations and depths with the addition of GreenClean Liquid, but DOC levels returned to pre-treatment levels the next day. While overall changes in DOC were small for most samples, larger changes in certain samples could be related to release of DOC into the water column from algal cell lysis or substantially reduced algal uptake of DOC<sup>2</sup> from the water column due to reduced algae standing crop.

Reductions in TN and TP concentrations were observed at most test locations and depths. Moreover, for both of these constituents, control samples taken from the surface (0.1 m) show a marked increase. The latter phenomenon was also observed in chlorophyll *a* and cyanobacteria species analyses results, thus suggesting that these TN and TP increases in the control samples were due to naturally-higher algae production near the water surface

<sup>&</sup>lt;sup>2</sup> In addition to inorganic carbon assimilation, numerous algal and cyanobacterial taxa have been described as potential consumers of DOC (Vincent and Goldman, 1980; Ellis and Stanford, 1982). DOC uptake is not a dominant strategy in phytoplankton nutrition, but the ability to utilize DOC may play a role in intraspecific competition with other phytoplankton and succession of populations, including in eutrophic reservoirs (Znachor and Nedoma, 2009). Amino acid and peptide components of DOC can serve as alternative N or C sources to varying degrees by phytoplankton, including cyanobacteria (Berman, 1997; Berman and Chava, 1999). The importance of DOC uptake by phytoplankton generally increases with decreasing light availability. However, in the case of cyanobacteria, DOC uptake can take place at any level of irradiance (Kirkwood et al., 2003).

at the time (early afternoon) when event samples were collected. In other words, a large fraction of total nutrients in Copco reservoir were made up of organic nutrients. Consequently, upon the introduction of GreenClean Liquid into the system, TN and TP levels declined as algae production declined and damaged or dead algae were removed (e.g., settled) from the water column.

Even though certain inorganic nutrient forms (e.g., NO2+NO3, PO4) increased in concentration in the post-treatment samples, the period of data collection was too short to determine if these increased inorganic nutrients led to increased algae growth. However, following algaecide application, it is expected that there would be a delay before algae production returns to background (pre-treatment) levels in the treated area. This is because the algaecide kills and damages algae cells (as indicated both from TN and TP reductions as well as marked reductions in chlorophyll *a*), and a return to background algae production levels would be delayed for a period of time until re-growth or wash-in of algae could compensate for the loss of algae cells from the water column in the treated area. Further, while treatment may release inorganic nutrients available for uptake by remaining algae, regrowth would be limited since there would be a smaller amount of surviving algae present following treatment. Finally, from a mass balance perspective, the release of algal nutrients as a result of treatment cannot lead to a larger amount of algae.

# 5.2 Microcystin

GreenClean Liquid reduced microcystin concentrations by 11 to 80 percent at 9 of the 10 test locations (not including T5 at 1.0 m). Further, in control samples taken from the reservoir surface, microcystin concentrations increased sharply (between 176 percent and 236 percent) between pre-treatment sampling and the time when event samples were collected.

A key question at the outset of this study was whether algaecide treatment might cause an increase in microcystin due to release from dead and lysed *Microcystis* cells. However, the observed treatment-related reductions in microcystin levels indicate that the algaecide-related destruction of cyanobacteria did not result in increased concentrations of microcystin in the reservoir. These results indicate that algaecide application is effective in reducing microcystin concentrations and does not cause microcystin concentrations to increase above initial concentrations following treatment. These results are consistent with the results observed in prior studies (Deas et al. 2012).

# 5.3 Algal Response

On average, chlorophyll *a* concentrations were reduced by 60 percent after algaecide treatment, thus indicating that GreenClean Liquid was able to effectively damage or kill algal cells and reduce cyanobacteria populations in the reservoir. Additionally, in control samples taken from the reservoir surface, chlorophyll *a* concentrations increased sharply (68 percent to 296 percent) between pre-treatment sampling and the time when event samples were collected. This naturally-higher algae production, if indicative of Copco Cove overall, suggests that the effect of treatment on algae reductions at test locations

may be even greater than indicated by the direct comparison of post-treatment to pretreatment levels.

Application of GreenClean Liquid also shows a noticeable and consistent reduction in other cyanobacteria species such as *Aphanizomenon flos-aquae* and *Pseudoanabaena sp.* Given these reductions, it can be concluded that GreenClean Liquid was able to effectively damage or kill algal cells of those species.

However, *Microcystis* results did not follow a discernible pattern, and in several of the samples, *Microcystis* was not detected. In the Klamath River, microcystin is commonly associated with *Microcystis aeruginosa*, but in this pilot study there appeared to be little correlation between the microcystin concentrations and *Microcystis* cell counts in the analysis results. However, changes in *Pseudoanabaena sp* levels seem to follow the pattern of microcystin concentrations. *Pseudoanabaena sp*. found at other water bodies have been observed to produce microcystin (Oudra *et al.*, 2001; Olvera-Ramirez *et al.*, 2010). While similar response can also be observed in *Aphanizomenon flos-aquae* samples, previous studies have shown that the *Aphanizomenon flos-aquae* species in the Klamath River basin is considered non-toxic, and they are even used as food supplements (Carmichael *et al.*, 2000 (Upper Klamath Lake specific); Li *et al.*, 2003; Saker *et al.*, 2005). Further, microcystin levels may be the relic of past blooms, or water transported into the study area from in-reservoir circulation (e.g., wind driven currents), or other factors. Given these considerations, further study is recommended to determine the cyanobacteria species responsible for the occurrence of microcystin in Copco Reservoir.

# 6.0 ECONOMIC CONSIDERATIONS

The cost of algaecide treatment is an important consideration for assessing the role that algaecide application may play in a management program aimed at improving water quality conditions in the hydroelectric project reservoirs, and perhaps elsewhere in the Klamath Basin. GreenClean Liquid costs discussed below are based on input from BioSafe staff and include product and delivery costs. Application costs discussed below are based on input from Clean Lakes, Inc., the recommended applicator for the product and the applicator used in this pilot study. There are other manufacturers of SCP-based products and other applicators, and costs may vary from those described below with other suppliers and applicators. While costs may vary from these estimates, these costs are assumed to be a representative estimate of product and application costs for relatively small treatment areas (e.g., 10's of acres). Treatment of larger areas would result in reduced unit costs as a result of volume product purchases and scaling efficiencies related to mobilization and application costs. All costs below are stated in 2013 dollars.

Cost estimate summaries were developed for a range of potential treatment application rates appropriate for low, medium and high algal densities. All estimates were based on treatment of the upper 4 feet of a 10 acre portion of the reservoir. The cost of GreenClean Liquid ranges from \$20 to \$28 per gallon (including shipping and applicable taxes), depending on the amount purchased (J. Kline, BioSafe, pers. comm.). A cost of \$25 per gallon was used for this assessment. The estimated cost of algaecide application, including product, delivery, mobilization, and staffing for a single application, is

provided in Table 14. For multiple applications an approximate cost can be estimated by multiplying these costs by the number of applications performed within a season. However, multiple treatments may result in reduced costs due to volume discounts on algaecide.

Algae Density	Application Rate (ppm)	Volume Algaecide (gallons)	Algaecide Cost	Application Cost*	Total Cost per Treatment
Low	3.0	144	\$3,600	\$5,880	\$9,480
Medium	5.0	240	\$6,000	\$5,880	\$11,880
High	10.0	480	\$12,000	\$5,880	\$17,880

Table 14. Cost Estimate Summary for Potential Algaecide Treatment of Lake Surface Area of 10 Acres, 4-Foot-Deep Treatment (Total Volume 40 Acres) for Low (3 ppm), Medium (5 ppm), and High (10 ppm) Algae Densities

\*Estimated application costs were provided by Tom McNabb (Clean Lakes, Inc.). Costs subject to change.

# 7.0 CONCLUSION AND RECOMMENDATIONS

The 2012 pilot study of the application of environmentally safe algaecide in Copco reservoir was designed based on information developed from previous bench-scale studies conducted in 2008, 2009, and 2011 (Deas et al. 2009; Deas et al. 2012). Overall, the results of the 2012 pilot application study indicated that GreenClean Liquid is effective in reducing blue-green algae in the reservoir environment and reducing microcystin concentrations.

Response patterns of TN and TP concentrations mirrored the response of chlorophyll *a*, *Aphanizomenon flos-aquae*, and *Pseudoanabaena sp.* indicating that a large component of total nutrients are in their organic form at the time of treatment. Further, reductions in these constituents show that the application of the hydrogen peroxide-based algaecide is effective in killing algal cells and reducing their overall levels. In addition, algaecide treatment led to modest increases in NO2 + NO3 and PO4 concentrations. These increases are a consequence of reduction in algal uptake or release of inorganic nutrients from algal cell death and lysis. However, ammonium levels were reduced. GreenClean Liquid application was also shown to reduce microcystin levels within the treated area of the reservoir.

Based on the 2012 findings, as well as previous algaecide experiments, recommendations for future work include:

• Assessment of optimal algaecide application timing. The 2012 pilot study occurred in early September, when algae standing crop within Copco reservoir had already developed to a level at which a high algaecide application rate was recommended by the applicator. An earlier application of algaecide would allow for an assessment of the effectiveness of algaecide in preventing the development of a large standing crop that adversely affects reservoir water quality conditions and thus requires higher algaecide application rates to subsequently control. In addition, the timing of

algaecide application during a 24-hour period could also be evaluated. This could potentially indicate whether applications might be more effective during certain times of the day.

- Assessment of optimal algaecide application rates. The algaecide manufacturer recommends various application rates depending on the algal density. For the 2012 pilot application in Copco Cove, the application rate used was 9.7 ppm, because high algal density was assumed and ultimately observed in Copco Cove during the September application. An assessment of the efficacy of different application rates would identify the optimal and most cost-effective application rates for differing algal bloom conditions.
- Assessment of the effects of algaecide over time. The 2012 pilot application in Copco Cove was intended only to assess the immediate efficacy of algaecide treatment and to determine whether algaecide application would result in increased microcystin levels. Ongoing control or management of algal levels in Copco reservoir following the application was not an objective and there was no control to prevent reentrainment of algal cells into the treatment area. However, information obtained from this study will be useful for estimating requirements and costs for potential future applications of the algaecide for more persistent control of algal conditions in localized reservoir areas where algae reduction and management is desirable (e.g., recreational and high public use areas). Potential future algaecide applications in localized areas of the reservoir might also be used in conjunction with methods to enclose the treated area and limit re-population of algae by wash-in (lateral advection) from other untreated areas of the reservoir or from deeper depths via buoyancy regulation – both processes that can reintroduce algae into a treated volume of water.
- **Monitoring.** The monitoring completed in the 2012 pilot study identified that sample results from within the treatment area were consistent and that a control sample or samples were important to assess the effects of the algaecide application. For potential future studies, a balance of resources and appropriate level of monitoring should be identified to (a) meet applicable regulatory requirements, and (b) effectively characterize conditions in treated and untreated areas.
- **Microcystin producing cyanobacteria.** Further studies should explore algae species, other than, but including, *Microcystis* and *Pseudoanabaena Sp.*, and possibly other species through selected sampling, to explore the relationship between microcystin concentrations and algal species presence to better understand the relationships between algal species composition and microcystin concentrations.

Overall, the 2012 pilot study in Copco Cove demonstrated that algaecide application effectively reduced algal concentrations, reduced microcystin concentrations, and reduced algal biomass as measured by chlorophyll *a*. However, constituent concentrations returned to pre-treatment levels in samples collected a day following treatment. While certain inorganic nutrients increased following algaecide application, these increases likely would not lead to larger algae standing crop, and the reduction in total forms of nitrogen and phosphorus suggest that post-treatment standing crop would be reduced. These findings indicate that a hydrogen peroxide-based, environmentally-safe algaecide could be a useful management tool to reduce algal production and

associated algal toxins if treatment persistence could be achieved in an economical manner.

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# **Personal Communications**

- Jeff Kline, BioSafe Systems, LLC. April 29, 2010; July 19, 2012; October 9, 2012; January 28, 2013.
- Vijay Kumar Choppakatla, PhD, BioSafe Systems, LLC. July 19, 2012; September 11, 2012; October 9, 2012, February 25, 2013.
- Judy Westrick, PhD, Tamarack Environmental Laboratories, LLC. September 24, 2012; December 17, 2012; January 10, 2013.

Tom McNabb, Clean Lakes, Inc. September 1, 2012; January 28, 2013.

# APPENDIX A **Summary Tables of Sampling Locations and Data**

This appendix contains summary tables for sampling location coordinates, sampling times, dissolved oxygen (DO) measurements, DO saturation, water temperature, turbidity, secchi disk readings, reservoir depth, and field data measurements.

# A.1 Sampling Location Coordinates

Each sampling location was identified using a Garmin Oregon<sup>®</sup> 450 Geographic Positioning System (GPS) prior to pre-treatment sampling (Table A-1). The coordinates were recorded in the GPS and later used to position the boat when subsequent samples were collected. This procedure ensured that the location of the pre-treatment, post-treatment, and post-event sampling would consistent.

Sampling Location	Coord	linates
C1	41°59'4.84"N	122°19'44.13"W
C2	41°59'2.47"N	122°19'44.29"W
C3	41°58'59.89"N	122°19'44.48"W
T1	41°59'3.82"N	122°19'48.85"W
T2	41°59'2.75"N	122°19'49.09"W
Т3	41°59'1.34"N	122°19'49.28"W
Τ4	41°59'3.41"N	122°19'50.16"W
T5	41°59'2.11"N	122°19'50.53"W

Table A-1. Coordinates of Sampling Locations

# A.2 Sampling Times

Sampling occurred at three times: the morning prior to application ("pre-treatment"), immediately after application ("post-treatment") and the following morning ("post-event"). Sampling times for each location and depth are summarized below.

Location	Depth (m)	Sampling Time				
		<u>Pre-treatment</u> (9/6/2012)	<u>Post-treatment</u> (9/6/2012)	<u>Post-event</u> (9/7/2012)		
C1	0.1	8:26	13:03	7:51		
C2	0.1	8:41	13:12	8:02		
C3	0.1	8:46	13:18	8:14		
T1	0.1	8:57	13:52	8:24		
T2	0.1	9:05	14:04	8:35		
Т3	0.1	9:17	14:14	8:45		
Τ4	0.1	9:25	14:23	8:58		
Τ5	0.1	9:34	14:30	9:10		
C1	1.0	8:30	13:05	7:56		
C2	1.0	8:43	13:13	8:08		
C3	1.0	8:50	13:22	8:17		
T1	1.0	9:00	13:55	8:26		
T2	1.0	9:09	14:07	8:38		
Т3	1.0	9:20	14:16	8:48		
Τ4	1.0	9:28	14:24	9:02		
T5	1.0	9:38	14:33	9:12		

Table A-2. Summary of Sampling Times

#### A.3 Dissolved Oxygen

Dissolved oxygen levels were in excess of water quality criteria at all sampling times (Table A-3). Morning samples (pre-treatment and post-event) indicated supersaturation (Table A-5), with mid-day samples (event) indicating higher DO concentrations (particularly in near surface waters) in response to algal photosynthesis. Control sites had the highest post-treatment sampling DO concentrations, while treatment sites had lower concentrations in comparison, but were still well above water quality standards. DO readings were collected with an In-Situ, Inc., Troll 9500 Professional (#45654) water quality probe.

Location	Depth (m)	Dissolved Oxygen (mg/L)				
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	Post-event (9/7/2012)		
C1	0.1	13.15	18.70	12.85		
C2	0.1	13.44	20.96	12.56		
C3	0.1	13.19	20.04	12.64		
T1	0.1	13.20	16.09	12.98		
T2	0.1	13.63	14.72	13.66		
Т3	0.1	13.53	14.60	14.25		
T4	0.1	13.29	14.61	13.10		
T5	0.1	14.14	13.43	14.61		
C1	1.0	13.08	13.73	12.60		
C2	1.0	13.33	15.19	12.49		
C3	1.0	13.16	14.57	12.66		
T1	1.0	13.00	13.55	12.90		
T2	1.0	13.53	12.81	12.93		
Т3	1.0	13.40	13.05	12.67		
T4	1.0	13.10	12.60	12.90		
T5	1.0	13.73	13.29	12.69		

Table A-3. Summary of Dissolved Oxygen Measurements

\*The DO measurements have an accuracy of  $\pm 2$  percent of reading or  $\pm 0.2$ mg/L, whichever is greater.

Table A-4. Summary of Average Dissolved Oxygen (DO) Response for 0.1 Meter, 1.0 Meter, and All
Depths for Both Control and Treatment Area Samples

		DO (mg/l)		Pre-treatment	Pre-treatment
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	to Event % Change	to Post-Event % Change
Control (0.1)	13.3	19.9	12.7	50.1%	-4.3%
Treatment (0.1)	13.6	14.7	13.7	8.3%	1.2%
Control (1.0)	13.2	14.5	12.6	9.9%	-4.6%
Treatment (1.0)	13.4	13.1	12.8	-2.2%	-4.0%
Control (all depths)	13.2	17.2	12.6	30.0%	-4.5%
Treatment (all depths)	13.5	13.9	13.3	3.1%	-1.4%

Location	Depth (m)	DO Saturation (%)				
		<u>Pre-treatment</u> (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)		
C1	0.1	160.6	229.3	155.6		
C2	0.1	163.3	273.5	152.5		
C3	0.1	160.3	258.8	154.0		
T1	0.1	160.9	202.2	157.6		
T2	0.1	166.7	183.1	166.4		
Т3	0.1	165.7	175.7	173.9		
T4	0.1	160.2	180.0	160.0		
T5	0.1	172.7	166.6	177.3		
C1	1.0	159.8	172.4	152.7		
C2	1.0	161.5	185.2	151.0		
C3	1.0	160.8	178.4	154.1		
T1	1.0	158.9	165.4	156.3		
T2	1.0	165.2	157.6	156.1		
Т3	1.0	163.5	159.1	153.7		
T4	1.0	159.0	153.7	156.5		
T5	1.0	168.5	162.7	154.6		

Table A-5. Summary of DO Percent Saturation Measurements

\*The DO measurements have an accuracy of ±2 percent of reading or ±0.2mg/L, whichever is greater.

	D	DO Saturation (%)		_ Pre-treatment Pre-treatr	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	to Event % Change	to Post-Event % Change
Control (0.1)	161.4	253.9	154.0	57.3%	-4.6%
Treatment (0.1)	165.2	181.5	167.0	9.9%	1.1%
Control (1.0)	160.7	178.7	152.6	11.2%	-5.0%
Treatment (1.0)	163.0	159.7	155.4	-2.0%	-4.6%
Control (all depths)	161.1	216.3	153.3	34.3%	-4.8%
Treatment (all depths)	164.1	170.6	161.2	3.9%	-1.8%

 Table A-6. Summary of Average DO Saturation Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples.

# A.4 pH

pH was above the water quality standard throughout the experiment, and it is routinely above the 7.0 to 8.5 standard throughout much of the Klamath River system in summer periods and was in excess of 8.5 prior to algaecide application. pH was collected during baseline and post-event monitoring; however, equipment error led to no pH data collection during the event. Application of GreenClean Liquid is not expected to remarkably change pH (pers. comm. V. Choppakatla). This was confirmed through a review of expected pH changes as determined from BioSafe bench-top studies (BioSafe, 2009) on GreenClean Liquid, wherein distilled water, pond water, and groundwater were tested with GreenClean Liquid to determine pH response. Further, a review of pH measurements in previous PacifiCorp algaecide studies using GreenClean Liquid indicates that pH in discrete containers experienced decreases in the range of 0.03 to 0.04 pH units after algaecide application (Deas et al., 2012). Based on discussions with BioSafe, review of prior BioSafe and PacifiCorp algaecide study data, and considering the dilution of the GreenClean product within the reservoir as well as the buffering capacity of natural waters (due to alkalinity and organic matter) significant pH changes were not expected, nor were they observed in the post-event data. Further, post-event pH values results are similar to pre-treatment values. pH readings were collected with an In-Situ, Inc., Troll 9500 Professional (#45654) water quality probe.

Location	Depth (m)		рН	
		<u>Pre-treatment</u> (9/6/2012)	<u>Post-treatment</u> (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	9.38		9.34
C2	0.1	9.40		9.35
C3	0.1	9.38		9.37
T1	0.1	9.37		9.38
T2	0.1	9.42		9.44
Т3	0.1			9.49
T4	0.1	9.38		9.42
T5	0.1	9.44		9.49
C1	1.0	9.35		9.35
C2	1.0	9.40		9.36
C3	1.0	9.39		9.37
T1	1.0	9.37		9.36
T2	1.0	9.43		9.38
Т3	1.0	9.41		
T4	1.0	9.38		9.39
T5	1.0	9.44		9.39

#### Table A-7. Summary of pH Measurements

"--"no data were recorded. Error was found in event measurements. These data are thus not presented. The pH sensor has an accuracy of ±0.2 units.

# Table A-8. Summary of Average pH Response for 0.1 Meter, 1.0 Meter, and All Depths for Both Control and Treatment Area Samples

		рН		Pre-treatment	Pre-treatment
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	to Event % Change	to Post-Event % Change
Control (0.1)	9.39		9.35		-0.4%
Treatment (0.1)	9.40		9.44		0.4%
Control (1.0)	9.38		9.36		-0.2%
Treatment (1.0)	9.41		9.38		-0.3%
Control (all depths)	9.38		9.36		-0.3%

Treatment (all depths)	9.40		9.42		0.1%
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#### A.5 Temperature

There was no discernible change in water temperature related to the algaecide application. Rather, changes in water temperature were in response to daily thermal dynamics of the reservoir and atmosphere. Water temperature readings were collected with an In-Situ, Inc., Troll 9500 Professional (#45654) water quality probe.

Location	Depth (m)	Temperature (°C)			
		<u>Pre-treatment</u> (9/6/2012)	<u>Post-treatment</u> (9/6/2012)	<u>Post-event</u> (9/7/2012)	
C1	0.1	20.22	21.63	20.10	
C2	0.1	20.24	23.15	20.06	
C3	0.1	20.19	23.71	20.09	
T1	0.1	20.22	22.25	20.12	
T2	0.1	20.23	21.14	20.17	
Т3	0.1	20.27	20.35	20.22	
T4	0.1	20.24	20.80	20.15	
T5	0.1	20.35	20.61	20.18	
C1	1.0	20.20	20.30	20.08	
C2	1.0	20.24	20.38	20.05	
C3	1.0	20.19	20.32	20.09	
T1	1.0	20.22	20.32	20.11	
T2	1.0	20.23	20.24	20.10	
Т3	1.0	20.25	20.22	20.10	
T4	1.0	20.22	20.17	20.11	
T5	1.0	20.33	20.17	20.12	

 Table A-9. Summary of Water Temperature Measurements

\* The temperature/conductivity sensor has an operational range of  $-5^{\circ}$ C to  $75^{\circ}$ C in water. The results have an accuracy of  $\pm 0.2^{\circ}$ C.

Table A-10. Summary of Average Temperature Response for 0.1 Meter, 1.0 Meter, and All Depths
for Both Control and Treatment Area Samples

	Temperature (°C)			Pre-treatment	Pre-treatment	
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	to Event % Change	to Post-Event % Change	
Control (0.1)	20.2	22.8	20.1	12.9%	-0.7%	
Treatment (0.1)	20.3	21.0	20.2	3.8%	-0.5%	
Control (1.0)	20.2	20.3	20.1	0.6%	-0.7%	
Treatment (1.0)	20.3	20.2	20.1	-0.1%	-0.7%	
Control (all depths)	20.2	21.6	20.1	6.8%	-0.7%	
Treatment (all depths)	20.3	20.6	20.1	1.8%	-0.6%	

#### A.6 Turbidity

Turbidity was notably less in the treatment area following application of algaecide. Post-treatment turbidity returned to pre-treatment levels (Table A-11).

Location	Depth (m)		Turbidity (NTU)	
		<u>Pre-treatment</u> (9/6/2012)	<u>Post-treatment</u> (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	9.9	136.7	42.5
C2	0.1	32.9	80.8	20.1
C3	0.1	44.0	106.9	27.2
T1	0.1	33.1	84.5	34.6
T2	0.1	33.0	25.1	27.7
Т3	0.1	44.0	28.1	60.6
T4	0.1	31.0	42.9	32.3
T5	0.1	72.4	40.6	32.2
C1	1.0	27.3	11.4	28.7
C2	1.0	25.9	28.9	43.6
C3	1.0	29.5	47.6	11.6
T1	1.0	26.4	23.2	22.4
T2	1.0	58.2	10.1	48.4
T3	1.0	39.8	13.0	38.3
T4	1.0	35.0	17.9	33.7
T5	1.0	49.6	16.3	19.8

Table A-11. Summary of Turbidity Measurements

\*The Hach<sup>®</sup> 2100Q Portable Turbidimeter has an accuracy of  $\pm 2\%$  of reading.

Table A-12. Summary of Average Turbidity Response for 0.1 Meter, 1.0 Meter, and All Depths for	
Both Control and Treatment Area Samples	

	-	Turbidity (NTU)			Pre-treatment	
Sample Location	on Pre- Po treatment treat (9/6/12) (9/6		Post- Event (9/7/12)	<ul> <li>Pre-treatment to Event % Change</li> </ul>	to Post-Event % Change	
Control (0.1)	28.9	108.1	29.9	274.0%	3.5%	
Treatment (0.1)	42.7	44.2	37.5	3.6%	-12.3%	
Control (1.0)	27.6	29.3	28.0	6.3%	1.4%	
Treatment (1.0)	41.8	16.1	32.5	-61.5%	-22.2%	
Control (all depths)	28.2	68.7	28.9	143.4%	2.5%	
Treatment (all depths)	42.3	30.2	35.0	-28.6%	-17.2%	

#### A.7 Electrical Conductivity

Conductivity remained stable throughout the pilot study (Table A-13). Electrical conductivity readings were collected with an In-Situ, Inc., Troll 9500 Professional (#45654) water quality probe.

Location	Depth (m)	Electrical Conductivity (µS/cm)				
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)		
C1	0.1	143.8	143.2	144.6		
C2	0.1	143.2	148.8	144.6		
C3	0.1	144.0	147.9	144.5		
T1	0.1	143.9	144.5	144.6		
T2	0.1	143.5	142.7	144.6		
Т3	0.1		143.9	144.6		
T4	0.1	143.5		144.4		
T5	0.1	143.6	143.8	144.5		
C1	1.0	144.1	143.7	144.7		
C2	1.0	143.0	144.1	144.6		
C3	1.0	144.1		144.6		
T1	1.0	143.9	143.3	144.7		
T2	1.0	143.5	143.3	144.6		
Т3	1.0	143.5	142.9			
T4	1.0	143.6	143.9	144.6		
T5	1.0	143.7	143.6	144.6		

 Table A-13. Summary of Electrical Conductivity Measurements

 $^{\star}$  "--" no data were recorded. The temperature/conductivity sensor has an accuracy of ±1  $\mu S$  or ±1% of reading, whichever is greater

Table A-14. Summary of Average \$(EC) Response for 0.1 Meter, 1.0 Meter, and All Depths for Both	1
Control and Treatment Area Samples	

		EC (µS/cm)			Pre-treatment
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	<ul> <li>Pre-treatment to Event % Change</li> </ul>	to Post-Event % Change
Control (0.1)	143.7	146.6	144.6	2.1%	0.6%
Treatment (0.1)	143.6	143.7	144.5	0.1%	0.6%
Control (1.0)	143.7	143.9	144.6	0.1%	0.6%
Treatment (1.0)	143.6	143.4	144.6	-0.2%	0.7%
Control (all depths)	143.7	145.5	144.6	1.3%	0.6%
Treatment (all depths)	143.6	143.5	144.6	-0.1%	0.7%

### A.8 Depths

Secchi depth and reservoir depth readings were taken at all the locations.

Location	Secchi Depth (m)				
	Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)		
C1	0.81	0.15	0.63		
C2	0.80	0.51	0.68		
C3	0.72	0.30	0.76		
T1	0.63	0.38	0.75		
T2	0.50	0.90	0.52		
Т3	0.55	0.85	0.27		
T4	0.51	1.10	0.38		
T5	0.40	1.23	0.42		

Table A-15. Summary of Secchi Disk Readings

Table A-16. Summary of Average Secchi Disk Readings

	Secchi Depth (m)			Pre-treatment	Pre-treatment
Sample Location	Pre- treatment (9/6/12)	Post- treatment (9/6/12)	Post- Event (9/7/12)	to Event % Change	to Post-Event % Change
Control	0.78	0.32	0.69	-58.8%	-11.2%
Treatment	0.52	0.89	0.47	72.2%	-9.7%

Location	Reservoir Depth (m)
C1	10.8
C2	16.2
C3	21.8
T1	10.45
T2	14.5
Т3	12.5
Τ4	5.35
T5	5.5

#### A.9 Field Data

This section summarizes the field data that was collected and analyzed for the 2012 algaecide experiment.

Location	Depth (m)		TN (mg/l)	
		<u>Pre-treatment</u> (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	2.47	12.75	3.30
C2	0.1	2.97	3.22	2.17
C3	0.1	2.59	6.93	3.13
T1	0.1	2.80	2.93	2.75
T2	0.1	3.20	5.10	2.57
Т3	0.1	3.19	2.51	2.76
T4	0.1	3.21	3.88	4.13
T5	0.1	3.52	1.57	2.59
C1	1.0	4.34	2.00	3.43
C2	1.0	4.44	1.80	2.87
C3	1.0	3.33	1.99	5.00
T1	1.0	3.49	2.13	3.48
T2	1.0	4.10	3.76	3.76
Т3	1.0	3.73	1.75	3.04
T4	1.0	6.40	1.57	5.37
T5	1.0	3.98	2.55	3.53

Table A-18. Summary of Total Nutrients (TN) Results

Location	Depth (m)	NO3+NO2 (mg/l)				
		<u>Pre-treatment</u> (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)		
C1	0.1	0.177	0.002	0.139		
C2	0.1	0.170	0.031	0.149		
C3	0.1	0.141	0.002	0.147		
T1	0.1	0.142	0.044	0.160		
T2	0.1	0.155	0.014	0.159		
Т3	0.1	0.156	0.106	0.162		
T4	0.1	0.158	0.043	0.120		
T5	0.1	0.158	0.194	0.142		
C1	1.0	0.112	0.082	0.127		
C2	1.0	0.099	0.214	0.154		
C3	1.0	0.108	0.171	0.098		
T1	1.0	0.114	0.131	0.137		
T2	1.0	0.131	0.055	0.106		
Т3	1.0	0.137	0.205	0.134		
T4	1.0	0.065	0.168	0.081		
T5	1.0	0.085	0.078	0.123		

Location	Depth (m)		NH4 (mg/l)	
		Pre-treatment (9/6/2012)	<u>Post-treatment</u> (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	0.17	0.75	0.18
C2	0.1	0.29	0.21	0.16
C3	0.1	0.21	0.25	0.21
T1	0.1	0.22	0.15	0.19
T2	0.1	0.26	0.18	0.12
Т3	0.1	0.23	0.11	0.15
T4	0.1	0.29	0.03	0.20
T5	0.1	0.32	0.06	0.17
C1	1.0	0.36	0.00	0.25
C2	1.0	0.42	0.03	0.19
C3	1.0	0.30	0.03	0.35
T1	1.0	0.25	0.04	0.19
T2	1.0	0.33	0.04	0.22
Т3	1.0	0.38	0.08	0.12
T4	1.0	0.55	0.08	0.23
T5	1.0	0.40	0.03	0.15

Table A-20. Summary of Ammonia (NH4) Results

Table A-21. Summary of Total Phosphorus (TP) Results

Location	Depth (m)		TP (mg/l)	
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	0.32	1.49	0.45
C2	0.1	0.36	0.47	0.39
C3	0.1	0.32	0.77	0.43
T1	0.1	0.35	0.41	0.38
T2	0.1	0.39	0.60	0.39
Т3	0.1	0.39	0.37	0.36
T4	0.1	0.39	0.60	0.52
T5	0.1	0.42	0.27	0.35
C1	1.0	0.55	0.44	0.45
C2	1.0	0.53	0.30	0.44
C3	1.0	0.41	0.35	0.60
T1	1.0	0.48	0.42	0.46
T2	1.0	0.49	0.57	0.52
Т3	1.0	0.45	0.26	0.41
T4	1.0	0.76	0.25	0.65

T5	1.0	0.46	0.46	0.46	
Table A-22. Summary of Orthophosphate (PO4) Results					
Location	Depth (m)		PO4 (mg/l)		
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)	
C1	0.1	0.096	0.096	0.084	
C2	0.1	0.096	0.089	0.087	
C3	0.1	0.095	0.082	0.087	
T1	0.1	0.098	0.090	0.092	
T2	0.1	0.092	0.073	0.090	
Т3	0.1	0.096	0.090	0.090	
Τ4	0.1	0.099	0.159	0.092	
Т5	0.1	0.098	0.099	0.089	
C1	1.0	0.104	0.130	0.089	
C2	1.0	0.104	0.112	0.089	
C3	1.0	0.098	0.130	0.087	
T1	1.0	0.095	0.130	0.086	
T2	1.0	0.099	0.159	0.082	
Т3	1.0	0.096	0.096	0.082	
Τ4	1.0	0.093	0.093	0.078	
Т5	1.0	0.106	0.141	0.082	

Table A-23. Summary	of Dissolved Or	ganic Carbon (	(DOC) Results

		DOC (mg/l)		
		<u>Pre-treatment</u> (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	6.45	8.94	6.71
C2	0.1	6.48	6.60	6.56
C3	0.1	6.53	7.57	6.43
T1	0.1	6.53	7.00	6.48
T2	0.1	6.48	6.59	6.43
Т3	0.1	6.40	6.45	6.47
T4	0.1	6.51	11.94	6.53
T5	0.1	6.49	6.93	6.62
C1	1.0	6.66	9.49	6.99
C2	1.0	6.81	6.89	6.78
C3	1.0	6.54	7.31	6.91
T1	1.0	6.34	7.77	6.57
T2	1.0	6.64	11.29	6.61
Т3	1.0	6.69	6.68	6.52

Τ4	1.0	6.65	6.44	6.60
Τ5	1.0	6.64	9.57	6.44

#### Table A-24. Summary of Microcystin Results

Location	Depth (m)	Microcystin (mg/l)			
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)	
C1	0.1	73.5	241.5	70.0	
C2	0.1	57.5	158.7	52.4	
C3	0.1	66.3	222.9	45.5	
T1	0.1	97.7	74.1	62.6	
T2	0.1	39.4	24.9	86.0	
Т3	0.1	37.9	33.9	106.7	
Τ4	0.1	91.3	56.5	141.0	
T5	0.1	80.2	34.2	143.2	
C1	1.0	63.0	36.5	33.5	
C2	1.0	36.7	58.7	52.7	
C3	1.0	64.2	16.5	112.9	
T1	1.0	122.9	33.6	95.6	
T2	1.0	76.9	21.8	97.0	
Т3	1.0	127.1	31.8	83.5	
T4	1.0	108.0	22.0	118.2	
T5	1.0	51.5	73.1	78.3	

#### Table A-25. Summary of Chlorophyll *a* Results

Location	Depth (m)	Chlorophyll <i>a</i> (ppb)		
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	97.1	230.4	158.0
C2	0.1	144.9	480.7	184.4
C3	0.1	190.9	421.4	123.5
T1	0.1	190.9	164.6	177.8
T2	0.1	237.0	100.4	223.9
Т3	0.1	230.4	67.5	395.1
T4	0.1	263.4	116.9	217.3
T5	0.1	355.5	52.7	276.5
C1	1.0	184.4	197.5	138.3
C2	1.0	164.6	113.6	158.0
C3	1.0	164.6	237.0	108.6
T1	1.0	210.7	62.6	184.4
T2	1.0	217.3	56.0	158.0
Т3	1.0	204.1	116.9	197.5

T4	1.0	237.0	59.3	164.6
T5	1.0	237.0	105.3	184.4

Location	Depth (m)	APFA (1000 cells/ml)			
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)	
C1	0.1	130.1	689.9	403.4	
C2	0.1	178.7	340.3	262.1	
C3	0.1	340.8	322.6	71.8	
T1	0.1	310.7	89.9	118.1	
T2	0.1	353.8	42.1	69.6	
Т3	0.1	416.3	113.1	357.3	
T4	0.1	311.5	38.2	170.3	
T5	0.1	332.8	42.1	263.0	
C1	1.0	181.3	65.7	70.0	
C2	1.0	155.0	104.8	198.5	
C3	1.0	382.1	107.7	51.4	
T1	1.0	190.8	37.1	144.8	
T2	1.0	404.0	45.7	200.8	
Т3	1.0	209.1	66.6	207.8	
T4	1.0	294.2	57.2	149.7	
T5	1.0	374.5	224.7	169.8	

#### Table A-26. Summary of Aphanizomenon flow-aquae (APFA) Results

Table A-27. Summary of *Microcystis aeruginosa* (MSAE) Results

Location	Depth (m)	MSAE (1000 cells/ml)			
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	Post-event (9/7/2012)	
C1	0.1	0.0	0.0	417.2	
C2	0.1	34.6	0.0	26.7	
C3	0.1	1.7	0.0	14.4	
T1	0.1	7.8	76.3	231.8	
T2	0.1	357.8	13.3	72.4	
Т3	0.1	109.5	18.6	16.4	
Τ4	0.1	0.0	90.1	190.1	
T5	0.1	6.3	3.2	62.8	
C1	1.0	183.6	434.1	11.2	
C2	1.0	0.0	2.9	465.6	
C3	1.0	0.0	100.8	1,058.2	
T1	1.0	0.0	153.5	490.9	
T2	1.0	11.7	50.1	35.4	

Т3	1.0	0.0	4.7	673.1
T4	1.0	319.3	0.0	59.4
T5	1.0	0.0	2.2	0.0

Location	Depth (m)	PSAB (1000 cells/ml)		
		Pre-treatment (9/6/2012)	Post-treatment (9/6/2012)	<u>Post-event</u> (9/7/2012)
C1	0.1	23.9	146.7	79.0
C2	0.1	28.5	13.3	18.6
C3	0.1	31.2	74.9	45.3
T1	0.1	19.1	18.5	44.8
T2	0.1	26.0	71.6	14.1
Т3	0.1	56.8	32.5	13.2
Τ4	0.1	62.2	22.7	18.2
Т5	0.1	39.9	6.7	23.6
C1	1.0	63.7	10.5	17.1
C2	1.0	44.9	13.8	63.9
C3	1.0	46.1	16.2	60.2
T1	1.0	37.3	12.9	17.4
T2	1.0	5.0	10.7	40.3
Т3	1.0	42.2	11.2	39.4
Τ4	1.0	78.3	6.2	59.4
T5	1.0	75.3	33.1	29.5

Table A-28. Summary of Pseudoanabaena sp. (PSAB) Results