BENCH-SCALE TECHNICAL REPORT

TESTS OF THE AMGLO KEMLITE BENCH-SCALE BALLAST WATER MANAGEMENT SYSTEM

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ABSTRACT

This technical report presents findings from bench-scale tests evaluating the performance of the Amglo Kemlite Bench-Scale Ballast Water Management System (BWMS), hereafter Amglo Kemlite BWMS, developed by Amglo Kemlite Laboratories Inc. of Bensonville, Illinois. The Amglo Kemlite BWMS applies a patented light separation technology to treat ballast water with pulsed poly-chromatic light. The Amglo Kemlite BWMS is a bench-scale prototype with a single production-sized lamp and power supply installed in an appropriately sized unit for laboratory evaluation.

Researchers began conducting the bench-scale evaluation beginning in October 2017 and ending in February 2019 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UWS) in Superior, Wisconsin, USA. The test apparatus consisted of a pump and a single production-sized xenon flash lamp housed in a stainless-steel pipe, power supply and associated sample water valves and flow meters. Two different xenon flash lamps were tested, both at two frequencies. The pump drew experimental water from a 1000 L plastic influent tank at 10 gpm, through the stainless-steel pipe housing the xenon flash lamp and out of the system through an outflow tube.

Dose effectiveness testing was completed with the algae, *Selenastrum capricornutum*, microbes, *Escherichia coli* and *Enterococcus faecium*, and the zooplankton, *Eucyclops* sp. and *Daphnia magna* ephippia, in two water types. The system was found to be highly effective in laboratory water for all species. In challenge water, the results were more variable.

The Amglo Kemlite BWMS tests with *S. capricornutum* (with one lamp and one frequency) were repeated in parallel with an UV water purification system meeting the standard NSF/ANSI 55 Class A Device designed to run at 20 gpm, hereafter referred to as UV water purification system. Both systems were tested at 10 gpm. The treated algae were analyzed with the traditional microscopic counting method as well as the Most Probable Number method to examine if there was an effect on viability of cells in addition to mortality.

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1 INTRODUCTION

A major focus area of the Lake Superior Research Institute's Great Waters Research Collaborative (LSRI-GWRC) is providing unbiased, independent data in support of the accelerated development of technologies having the potential for preventing the introduction and/or controlling the spread of non-indigenous organisms within the Laurentian Great Lakes. This report details the results of the LSRI-GWRC bench-scale evaluation of the Amglo Kemlite BWMS. Developed by Amglo Kemlite Laboratories Inc. of Bensonville, Illinois, the Amglo Kemlite BWMS applies a patented light separation technology to treat ballast water with poly-chromatic light. The technology employs high-energy, pulsed xenon flash lamps. The Amglo Kemlite BWMS is a bench-scale prototype with a single production-sized lamp and power supply installed in an appropriately-sized unit for laboratory evaluation.

This technical report presents qualitative findings from bench-scale tests of the Amglo Kemlite BWMS which took place from October 2017 to February 2019 at the LSRI of UWS in Superior, Wisconsin, USA. Test objectives included determining biological effectiveness for two lamps operated at two frequencies each in two water qualities.

Additionally, the Amglo Kemlite BWMS was tested in parallel with a UV water purification system at the request of Amglo Kemlite representatives. Tests were conducted only with *S. capricornutum*. The parallel tests were conducted in both lab water and challenge water with only one lamp and one frequency of the Amglo Kemlite BWMS. The UV water purification system was operated at one-half its rated throughput capacity.

2 TEST METHODS

2.1 TEST PLAN AND SOPS

A TQAP (Test Quality Assurance Plan) and standard operating procedures (SOPs) were used to implement all test activities. These procedures facilitate consistent conformance to technical and quality system requirements and increase data quality. The TQAP details sample and data collection and analysis, sample handling and preservation, data quality objectives, and the QAQC requirements. The SOPs followed throughout testing are described in the methods section and listed in the References section of this report.

2.2 BALLAST WATER MANAGEMENT SYSTEM AND EXPERIMENTAL APPARATUS

The Amglo Kemlite BWMS evaluated by LSRI-GWRC is in the proof-of-concept stage of development. The Amglo Kemlite BWMS consists of a single production-sized xenon flash lamp (two different flash lamps were tested during these bench-scale evaluations), charge unit (Figure 1), power supply, centrifugal pump, treatment tank and associated sample water valves and flow meters (Figure 2). Prior to conducting testing with zooplankton, the centrifugal pump was replaced with a flexible impeller pump which caused less mortality to the larger organisms.

Amglo Kemlite BWMS employs pulsed xenon poly-chromatic light technology, rather than the current industry standard of constant UV supplied by mercury halide lamps. A full-scale model, currently under development by Amglo Kemlite Laboratories Inc., would have a pre-filter followed by lamp treatment (i.e., high-energy pulsed poly-chromatic xenon flash lamps encased in a quartz cooling jacket), which would be employed as a one-pass treatment (i.e., treatment inline on uptake). The proposed mode of action of the pulsed poly-chromatic light technology is destruction of cellular structure resulting in immediate mortality upon exposure. The Amglo Kemlite BWMS evaluated through the bench-scale testing described here, consisted of one full-sized lamp. The scaled-up, commercial BWMS would consist of multiple lamps and would be integrated into other BWMS technology.

The unit subject to testing was fitted with two alternative lamps, both of which contain only inert xenon gas. The lamps were marked with an identifier, i.e., either Q 100 lamp or T 100 lamp, as the differences in the lamps were non-visual. Each lamp was tested at both 5 Hz and 10 Hz with voltage for both set at 2300V.

The pump drew experimental water from the 1000 L influent tank at a rate of 10 gpm, through the Amglo Kemlite treatment tank and into the 1000 L effluent tank. Effectively, this test unit installation simulated a single-pass, flow-through ballast water treatment during ballast water uptake. The influent tank served as a reservoir for experimental water types and microbe and algae test organisms. Control and treatment samples were withdrawn from ports designed for this purpose on the end of the treatment tank. During zooplankton testing, the organisms were introduced to the treatment tank via a zooplankton dosing apparatus and sampled from an effluent line which was discharged into a 35 µm mesh zooplankton net with a solid cod end. Following sample collection, all treatment and control sample water replicates, were enumerated and placed into environmental chambers (to mimic an approximate 48-hour hold time following treatment on uptake) with temperature and light cycles designated by the appropriate standard operating procedure (SOP).

At the request of Amglo Kemlite, GWRC conducted side-by-side experiments to definitively determine the effectiveness of both the Amglo Kemlite treatment system and a commercially-available UV water purification system on the green algae, *S. capricornutum* in lab water and amended lab water. The objective of this set of dose-effectiveness testing was to compare effectiveness of a technology claiming to kill exposed organisms through cell lysis (i.e., Amglo Kemlite Module) to that of a technology claiming to render exposed organisms non-viable through nucleic acid damage (i.e., UV water purification system) by analyzing treated samples using the 14-day most probable number MPN method of autotroph analysis in addition to the staining method traditionally employed in bench scale testing at LSRI.

Figure 1. Amglo Kemlite Charge Unit

Figure 2. Amglo Kemlite Treatment tank, power supply and pump.

2.3 EXPERIMENTAL WATER PREPARATION

Bench-scale tests evaluating the Amglo Kemlite BWMS were conducted in LSRI laboratories equipped with adequate ventilation, electrical connections, and climate control. Three experimental water types were prepared as follows:

Laboratory Water (LW): Prior to each test, the 1,000 L influent tank was filled with 1,000 L of LW via hot and cold taps located in the LSRI Wet Lab. The LW is municipal water from the City of Superior, Wisconsin (sourced from Lake Superior), that is passed through an activated carbon column in order to remove the majority of the chlorine. The remaining residual chlorine is removed through injection of sodium sulfite, and the resulting total residual chlorine concentration is below the limit of detection (i.e., < 3 µg/L Cl_2). Typically, LW has a very low concentration of organic carbon and suspended solids, and a very high UV transmittance.

Amended Laboratory Water (LW-TMH): Prior to each test, the 1,000 L influent tank was filled with 1,000 L of LW via hot and cold taps located in the LSRI Wet Lab. While the tank was filling, LW-TMH was prepared by amending the LW with 12 mg/L pre-sterilized Fine Test Dust, 12 mg/L pre-sterilized Micromate™, and

20 mg/L humic acid. The amended water was mixed thoroughly in the influent tank using a canoe paddle until no visible clumps of Fine Test Dust or Micromate™ remained and a homogenous solution was achieved. Typically, LW-TMH is used to achieve challenge conditions similar to those stipulated in the U.S. Environmental Protection Agency (USEPA) Environmental Technology Verification (ETV) Program's Generic Protocol for the Verification of Ballast Water Treatment Technology, version 5.1 (USEPA, 2010).

Performance Control Water (PCW): The use of PCW is a quality control measure. It is the optimal culture water for the species being tested; therefore, it will vary for each dose effectiveness test conducted. The purpose of the PCW group is to provide information on the health of the test organisms. The PCW for each test organism is:

- LW: *Eucyclops sp.* and *S. capricornutum*
- Moderately-Hard Reconstituted Water (MHRW): *Daphnia magna* ephippia
    - Prepared following LSRI/SOP/AC/37 – *Preparation of Moderately-Hard Reconstituted Water for Use in Amphipod and Cladoceran Culturing*.
- Tryptic Soy Broth (TSB): *E. coli*
    - Prepared following manufacturer instructions and LSRI/SOP/SA/68 – *General Microbiology Procedures and Guidelines*.
- Brain Heart Infusion Broth (BHB): *E. faecium*
    - Prepared following manufacturer instructions and LSRI/SOP/SA/68 – *General Microbiology Procedures and Guidelines.*

2.4 BWMS INSTALLATION AND COMMISSIONING

The Amglo Kemlite BWMS was delivered and installed with the assistance of Amglo Kemlite representative George Hutchinson on October 18, 2017. LSRI staff members, Kimberly Beesley and Christine Polkinghorne received hands-on training on the operation of the BWMS and were informed of the recommended operating conditions, safety measures required during operation, and taught how to change lamps on the BWMS. The Amglo Kemlite BWMS was operating at an acceptable level upon completion of the installation and details were recorded on GWRC/FORM/22 – *Bench-Scale Ballast Water Management System (BWMS) Installation Acceptance Form* on October 18, 2017.

2.5 EXPERIMENTAL DESIGN AND TEST METHODS

Dose effectiveness tests measured treatment effects of the Amglo Kemlite BWMS on organisms traditionally used for laboratory toxicity testing. All organisms used during the dose effectiveness tests were from in house cultures with the exception of *D. magna* ephippia (purchased from ebpi, Environmental Bio-detection Products, Inc., Mississauga, Ontario, Canada). The overall experimental design is outlined in Table 1. Type and Numbers of Algae, Bacteria, and Zooplankton Analyzed in Dose Effectiveness Experiments using the Amglo Kemlite BWMS. Tests were conducted in both LW and LW-TMH at 25^{o}C ± 3^{o}C. Prior to test initiation, stock solutions of test water were prepared as described in Section 2.2. Samples were collected from the stock solutions and water chemistry measurements were made on the samples.

Table 1. Type and Numbers of Algae, Bacteria, and Zooplankton Analyzed in Dose Effectiveness Experiments using the Amglo Kemlite BWMS.

Major Taxonomic Group	Type	Species	Exposure Solutions by Water Quality	Number of Organisms per Exposure/Control	Number of Replicates per Exposure/Control
Algae	Green alga	*Selenastrum capricornutum*	Untreated LW and LW-TMH LW and LW-TMH treated using Lamp Q at 5 Hz and 10 Hz LW and LW-TMH treated using Lamp T at 5 Hz and 10 Hz Untreated PCW	200,000 ± 20% cells/mL	3, plus 1 sacrificial replicate for measuring chemistry
Bacteria	Gram-negative	*Escherichia coli*		>10,000 MPN/mL	5
	Gram-positive	*Enterococcus faecium*		>10,000 MPN/mL	5
Zooplankton	Adult copepods	*Eucyclops* sp.		10	Minimum of 3 plus 1 sacrificial replicate for chemistry measurements
	Ephippia (resting eggs)	*Daphnia magna*		15	Minimum of 3 plus 1 sacrificial replicate for chemistry measurements

Once the influent tank was filled with the appropriate water type and the temperature was adjusted to 25°C ± 3°C, the Amglo Kemlite system was turned on and the lamp was powered on to simmer mode for the 5 and 10 Hz treatment samples. The pump was primed and turned on to a flow rate of 10 gpm to fill the Amglo Kemlite treatment BWMS with water. During this period, the water from the influent tank was recirculated by placing the effluent hose in the influent tank. Once the Amglo Kemlite BWMS was full, the pump was shut off (except in the case of zooplankton testing) and the effluent hose was moved to the effluent tank.

For the *S. capricornutum* and microbial (*E. coli* and *E. faecium* tested simultaneously) tests, the test organisms were added directly to the influent tank after filling the Amglo Kemlite BWMS. The dose effectiveness tests using green algae were conducted by spiking the influent tank water with *S. capricornutum* from 4- to 8-day old cultures to achieve approximately 200,000 cells/mL (LSRI/SOP/GWRC/11 – *Assessing Bench-Scale Dose Effectiveness of Potential Ballast Water Treatment Processes on* Selenastrum capricornutum). For microbial dose effectiveness tests, a 1 L whole water sample was collected from the influent tank prior to spiking to verify the absence of *E. coli* and *E. faecium* in LW and LW-TMH. The influent tank water was spiked with *E. coli* and *E. faecium* to bring the influent tank density of each to greater than 1,000,000 most probable number (MPN)/100 mL (LSRI/ SOP/GWRC/14 – *Assessing Antimicrobial Effectiveness*.

Following addition of the testing organisms, the influent tank water was manually mixed for two minutes using a canoe paddle and the following water quality parameters were measured (and in PCW stock solution): temperature, dissolved oxygen (DO), pH, and specific conductivity. Throughout the control and treatment operations of the algae and microbe tests, the influent tank water was manually mixed using a canoe paddle approximately every three minutes in order to ensure the organisms were homogeneously mixed in the intake water (SOP/LSRI/GWRC/18 - Exposing Test Organisms to Potential Ballast Water Treatment Processes using a Bench-Scale Flow-Through System).

For *S. capricornutum* tests, three replicate samples were collected prior to the start of the control/treatment operation to document the initial density. For microbial tests, three replicate samples were collected: one at the start of the control/treatment operation, one in the middle and one at the end. The influent tank replicate samples were disposed of following the initial density determination. Untreated LW and LW-TMH served as the control.

To initiate testing with *S. capricornutum* and microbes, the lamp was set to flash mode at either 5 Hz or 10 Hz. Water was pumped from the influent tank into the effluent tank through the Amglo Kemlite BWMS at a flow rate of 10 gpm. Microbe control and treatment samples were collected simultaneously at 1 minute 45 seconds, 3 minute 30 seconds, 5 minutes 15 seconds, 7 minutes and 8 minutes 45 seconds after initiation of the flash (Figure 3). Water chemistry (temperature, pH, DO and conductivity) was measured at the initial, middle and end time point. The microbial samples remained in the 1 L bottles they were collected in for the duration of their hold time. Subsamples of each microbial sample were enumerated immediately (0-6 hours, hrs.) as described in Section 2.6. Samples bottles were then placed in a shaking incubator (100 rpm) at 25^{o}C ± 3^{o}C without light for the 48-hour duration of the test to determine delayed mortality. Subsamples were collected at 24 and 48 hrs. after treatment and enumeration was completed. Triplicate sterile test water samples (method blanks) and PCW samples were also enumerated and incubated coincident with the control and treatment samples.

S. capricornutum samples were collected after three, six and nine minutes of pumping. A 1 L control and 1 L treatment sample were collected simultaneously at each time point (Figure 3). A chemistry control and treatment replicate were collected immediately after the 9-minute samples were collected. These samples were used for measuring water chemistries at 24 hrs. and were not carried through to 48 hrs. For the algae test, one 150 mL aliquot from each 1 L sample was poured into a 300 mL Erlenmeyer flask fitted with a foam plug. At each sampling point, temperature, pH, DO and conductivity were measured. Algae samples were placed in an incubator at 25^{o}C ± 3^{o}C without light for the 48-hour duration of the test. Samples were collected at 0 hrs., 24 hrs. and 48 hrs. after treatment and live/dead counts were made using the microscopic method described in Section 2.7.1.

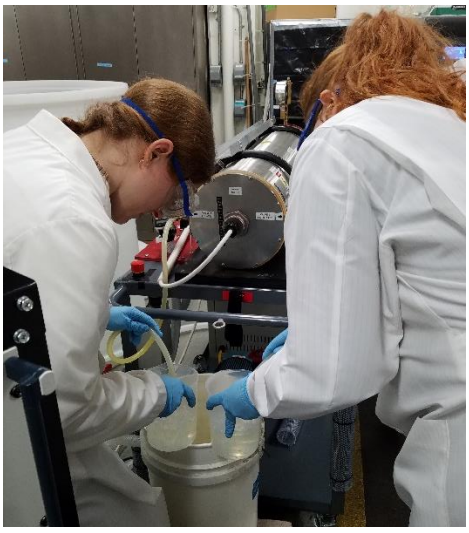

Figure 3. Collection of Algae and Microbe samples was made via control and treatment sample ports on the Amglo Kemlite treatment tank.

Initial tests run with zooplankton had high mortality and low recovery of organisms in the controls as well as the treatments. This led to changes in the test plan. First, the centrifugal pump supplied with the Amglo Kemlite system was replaced with a flexible impeller pump to decrease mortality. Testing was initially planned on neonates of *D. magna*. Mortality remained high for *D. magna* with the flexible impeller pump, so *Ceriodaphnia dubia* were tested for feasibility. Mortality was equally high. Both *D. magna* and *C. dubia* are cladocerans, and mortality seemed to be caused by the pump introducing air underneath their carapace, causing the organisms to become trapped in the surface tension of water, leading to death. The cladocerans were eliminated from the test plan (LSRI, 2017).

Trial runs were conducted with *Eucyclops* and *D. magna* ephippia. While immediate mortality was not an issue, recovery of organisms was low. This was seemingly due to the drain of the system's treatment tank being several centimeters above the bottom of the tank. It was determined that to effectively test the zooplankton and not have concerns about carry over from different frequencies and the controls, the end cap of the tank would be removed between each trial and the tank would be rinsed into a bucket, which would then be filtered through the cod end of the 35 µm net used to collect organisms from the treatment. To ensure that organisms weren't lost when the end cap was removed, a plastic apron was designed to direct the water from the treatment tank into a bucket.

While algae and microbe testing for control and both frequencies of one lamp could be conducted using one tank of water, three tanks of water were used to conduct the same tests for zooplankton to ensure that the organisms from an exposure were not mixed with those from another exposure. Prior to initiation of each test cycle, just after the influent tank was filled with either LW or LW-TMH, a water sample was

Figure 5. Zooplankton dosing bucket with hose leading to influent line of treatment tank.

Figure 4. Effluent/Sampling tank with hose leading to collection net with solid 1 Liter cod end attached.

taken from the influent tank to ensure that no detectable chlorine was present in the water, as chlorine is toxic to zooplankton. Chlorine levels were not detected at the initiation of any of the zooplankton tests.

If conducting an experimental exposure, the lamp simmer mode was turned on and the pump was run at a rate of 10 gpm for approximately two minutes, or until the treatment BWMS was full of water. This was indicated by water coming out of the air release valve on top of the treatment BWMS. During this initial period, the water was recirculated into the influent tank. The effluent line was moved into the effluent/sampling tank and the lamp was set to flash mode at the frequency being tested. After approximately 3 minutes, 150 *Eucyclops* or 150 ephippia were passed through the treatment BWMS via the zooplankton dosing apparatus (Figure 44). Organisms were collected in the cod end of a 35 µm plankton net. Treated water was collected from the effluent container in which the cod end of the net was suspended. Water chemistry analysis was done in the treated water and the water was placed into 300 mL beakers in 200 mL aliquots. The pump continued to flow at 10 gpm until the influent tank reached a level of 600 L, at which time the flow rate was increased to approximately 16 gpm to flush any remaining organisms out of the treatment BWMS and into the plankton net. Once the volume of the influent tank reached approximately 50 L, the pump was shut off. The Amglo Kemlite tank was drained into a collection bucket and all water collected was concentrated in the 35 µm plankton net. Once all of the water was drained, the end plate of the treatment BWMS was removed, the water that remained in the tank was directed to a bucket using a plastic "apron" (Figure 2, on left side). The treatment BWMS was rinsed into the bucket and all rinse water was concentrated through the 35 µm plankton net. The organisms were rinsed into the bottom of the plankton net using filtered water that collected in the 166.5 L effluent/sampling tank (Figure 55). The live organisms collected were divided into multiple pseudoreplicates (minimum of 3 for *Eucyclops* and 5 for ephippia), consisting of either 10 *Eucyclops* (LSRI/SOP/GWRC/9) or 15 ephippia (LSRI/SOP/GWRC/15), and held in treated water for 48 hrs. as described in the dose effectiveness SOPs. The treatment BWMS was reassembled and the influent tank was filled with lab water. The cycle was repeated twice during one day (to complete a control and the other frequency for a lamp). When the control cycle was run, the lamp was not on. The order of frequencies and control was randomly chosen daily. In order to determine delayed mortality on the test organisms exposed to the Amglo Kemlite BWMS, all of the test organisms were held for a period 48 hrs. in the dark at 25°C ± 3°C with no renewal of test water (Table 2). During the 48 hrs. following exposure, the temperature, DO, conductivity and pH were measured at 24 hrs. in the Chemistry replicates and 48 hrs. in all replicates. Following the 48-hour exposure, the *D. magna* ephippia were gently filtered through a 35 µm mesh sieve, rinsed into a hatching tray using PCW, and held for a period of 72 hrs. in continuous light (6000 lux) to assess viability of the resting eggs. The number of *D. magna* that emerge from the ephippia were counted at 72 hrs.

Table 2. Treatment Conditions for Algae, Bacteria, and Zooplankton Analyzed in GWRC Dose Effectiveness Experiments using the Amglo Kemlite BWMS.

Experimental Water Qualities	Organism Types	Exposure Volume per Replicate (mL)	Exposure Duration (hrs.)	Light: Dark Cycle (hrs.)	Temperature (°C)
LW and LW-TMH	Green alga (*S. capricornutum*)	150	48	0:24	25 ± 3
	Copepods (*Eucyclops spp.*)	200			
	Escherichia coli, Enterococcus faecium	750 - 1000			
	Ephippia (Resting Eggs)	200	48 (exposure time); 72 (hatching time)	0:24 (exposure); 24:0 (hatching)	25 ± 3 (exposure); 21 ± 1 (hatching)

Table 3 outlines the dates on which tests were conducted, the water type used for each test, which lamp was tested at what frequency and the organism exposed during the test.

Table 3. Dates, Water Type, Lamp, Frequency and Organisms tested during Amglo Kemlite Bench-Scale Testing.

Date	Water Type	Lamp	Frequency (Hz)	Organisms
1 November 2017	LW	Q	0, 5, 10	*S. capricornutum*
15 November 2017	LW	T	0, 5, 10	*S. capricornutum*
13 December 2017	LW-TMH	T	0, 5, 10	*S. capricornutum*
19 December 2017	LW	T	0, 5, 10	*E. coli, E. faecium*
3 January 2018	LW-TMH	Q	0, 5, 10	*S. capricornutum*
9 January 2018	LW	Q	0, 5, 10	*E. coli, E. faecium*
17 January 2018	LW-TMH	Q	0, 5, 10	*E. coli, E. faecium*
23 January 2018	LW-TMH	T	0, 5, 10	*E. coli, E. faecium*
9 October 2018	LW	Q	0, 5, 10	*D. magna* ephippia
10 October 2018	LW	T	0, 5, 10	*D. magna* ephippia
15 October 2018	LW	Q	0, 5, 10	*Eucyclops sp.*
7 November 2018	LW	T	0, 5, 10	*Eucyclops sp.*
13 November 2018	LW-TMH	Q	0, 5, 10	*D. magna* ephippia
28 November 2018	LW-TMH	T	0, 5, 10	*D. magna* ephippia
2 January 2019	LW-TMH	T	0, 5, 10	*Eucyclops sp.*
9 January 2019	LW-TMH	Q	0, 5, 10	*Eucyclops sp.*
16 January 2019	LW	Q	0, 10	*S. capricornutum*
29 January 2019	LW-TMH	Q	0, 10	*S. capricornutum*

2.6 STAINING VS MPN METHOD

To examine viability of the green algae *S. capricornutum* following treatment by the Amglo Kemlite system and the UV water purification system (Figure 6), side by side tests were conducted. As in the dose effectiveness tests with Amglo Kemlite, the 1000 L influent tank was filled with either lab water or LW-TMH at 25°C ± 3°C. The influent tank water was spiked with *S. capricornutum* from 4- to 8-day old cultures to achieve approximately 200,000 cells/mL. Three replicate samples were collected prior to the start of treatment to verify the initial density of the test organism population.

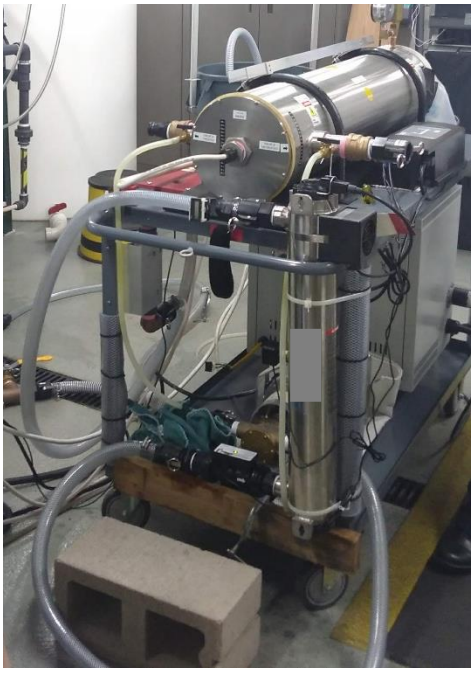

Figure 6. UV water purification system attached to the Amglo Kemlite BWMS cart.

Amglo Kemlite Lamp Q was set to flash mode at 10 Hz. Water was pumped from the influent tank into the effluent tank through the Amglo Kemlite BWMS at a flow rate of 10 gpm. After three minutes of pumping, two, 1 L treatment samples were collected, one of which was used for chemistry measurements and traditional microscopic counting methods described in Section 2.9.1 and the second of which was used for the MPN method of enumeration (Section 2.9.2). Treatment samples were also collected at 5 minutes and 7 minutes after initiation of pumping. A treatment replicate for chemistry analysis at time 24 hrs. was collected immediately after the 7-minute samples were collected. One 150 mL aliquot from one of the 1 L samples at each time point was poured into a 300 mL Erlenmeyer flask fitted with a foam plug. The second 1 L sample was carried through the MPN enumeration process and incubated for 14 days at 25°C ± 3°C (Petri, 2015). The MPN of each replicate sample, plus 95% confidence intervals, was determined using a publicly-available MPN calculator (http://www.wiwiss.fu-berlin.de/fachbereich/vwl/iso/ehemalige/wilrich/index.html).

Following treatment with the Amglo Kemlite system, the hoses connecting the Influent Tank to the Amglo Kemlite system were moved to the UV water purification system. The pump was turned on at a flow rate of 10 gpm and water was run through the system until the lights on the UV water purification system indicated it was operating optimally. With LW-TMH, the sensor which indicates the UV dose is adequate remained red at this point due to the low percent transmittance of the water. The test was continued

using the LW-TMH water. Once the lights indicated the system was operating correctly, treatment samples were collected at 3, 5 and 7 minutes and handled as the Amglo Kemlite samples were handled.

Three control samples were collected from the Influent Tank: one prior to initiation of testing, one following treatment with Amglo Kemlite and one following treatment with UV water purification system. Additionally, three PCW samples were prepared and carried throughout the 48-hour test period to ensure that the organisms used for the test were healthy.

2.7 WATER QUALITY

Water quality measurements were made throughout the duration of the Amglo Kemlite BWMS testing period and involved determination of total suspended solids (TSS), percent transmittance (%T), particulate organic matter (POM), non-purgeable organic carbon (NPOC) and dissolved organic carbon (DOC), total alkalinity, total hardness, DO, temperature, specific conductivity, and/or pH, depending on the specific type of test being conducted. TSS analysis was conducted according to LSRI/SOP/SA/66 – *Analyzing Total Suspended Solids (TSS), Particulate Organic Matter (POM), and Mineral Matter (MM)*. Briefly, accurately measured sample volumes (± 1%) were vacuum filtered through pre-washed, dried, and pre-weighed glass fiber filters (i.e. Whatman 934-AH). After each sample was filtered it was dried in an oven and brought to constant weight. TSS values were determined based on the weight of particulates collected on the filter and the volume of water filtered. The residue from the TSS analysis was ignited to a constant weight at 550°C in a muffle furnace. The concentration of POM was determined by the difference of the dry weight of the particulates on the filter before and after ignition (the mass lost to combustion).

%T sample analysis was conducted according to LSRI/SOP/SA/65 – *Determining Percent Transmittance (%T) of Light in Water at 254 nm*. For analysis of the filtered aliquot, an appropriate volume of sample was filtered through a glass fiber filter (i.e. Whatman 934-AH). A Perkin Elmer Lambda 35 UV-Vis spectrophotometer was used to measure %T of the unfiltered and filtered sample aliquots. Deionized water was used as a reference to adjust the spectrophotometer to 100%T, and then each unfiltered and filtered sample aliquot was measured in a pre-rinsed sample cuvette with a 1 cm path length.

NPOC/DOC analysis was conducted according to LSRI/SOP/SA/47 – *Measuring Organic Carbon in Aqueous Samples* on a Shimadzu Model TOC-L Total Organic Carbon Analyzer. Before analysis, the samples were acidified to a pH < 2 with concentrated hydrochloric acid (HCl; ~ 0.2% v/v). Samples were then purged with high purity air to remove the inorganic carbon and purgeable organic carbon and injected into the analyzer. Samples amended with Micromate (i.e., LW-TMH) were sonicated for a minimum of 30 minutes with a stir bar and stirred continuously on a stir plate while being manually injected into the instrument. An organic carbon stock solution which had a concentration of 1,000 mg/L carbon was used to prepare a working standard of 50 mg/L C which was also acidified to a pH < 2 with concentrated HCl. The standard was used to generate a calibration curve which was then used to determine the concentration of organic carbon in the samples.

Analysis of total hardness was conducted using the Ethylenediaminetetraacetic acid (EDTA) titrimetric method through manual titration according to the method described in LSRI/SOP/GLM/02 – *Procedure for Measuring Total Hardness*. Total hardness is reported as mg/L $CaCO_3$. Analysis of total alkalinity was conducted using the sulfuric acid titrimetric method through manual titration and according to the method described in LSRI/SOP/GLM/01 – *Procedure for Measuring Alkalinity*. Total alkalinity is reported as mg/L $CaCO_3$.

DO analysis was conducted using a Hach LDO HQ30d Dissolved Oxygen meter and LDO101 electrode, which was calibrated daily following LSRI/SOP/GLM/30 – *Calibrating, Maintaining and Using the HQ30d and HQ40d Meter and LDO101 Optical Electrode to Measure Dissolved Oxygen in Water Samples.* Temperature was measured using a Fisher digital thermometer that was calibrated quarterly following LSRI/SOP/GLM/17 – *Procedures for Thermometer Verification and Calibration*. Specific conductivity was measured using an Oakton Model CON 110 Conductivity/TDS/Temperature Meter that is calibrated on a monthly basis following LSRI/SOP/GLM/26 - *Procedures for Calibrating and Using the Oakton CON 110 Conductivity/TDS/Temperature Meter*. Its accuracy was also verified daily prior to sample analysis using a Daily Check Standard (0.0100M potassium chloride). pH analysis was conducted using an Orion 3 Star meter and Orion 8157BNUMD pH probe. Both instruments were calibrated daily following LSRI/SOP/GLM/05 – *Procedure for pH Meter Calibration and pH Measurement for Ballast Treatment Systems Utilizing pH as the Active Substance*. A check buffer of 8.00 was also measured after calibration to verify the accuracy of the calibration.

2.8 BACTERIAL ENUMERATION

From each whole water sample, subsamples were collected at designated analysis periods (0-6 hrs., 24 hrs., and 48 hrs.) and placed in sterile 120 ml sample vessels. *E. coli* and *Enterococcus* were enumerated according to LSRI/*SOP/SA/56 – Detection and Enumeration Total Coliforms and E. coli Using IDEXX Colilert®* and LSRI/SOP SA/62 *Detection and Enumeration of Enterococcus using IDEXX Enterolert®* The Colilert® assay has a detection limit for *E. coli* of 1 most probable number (MPN) per 100 mL, and the Enterolert™ assay can detect *Enterococcus* at 1 MPN/100 mL. Both tests use Defined Substrate Technology® (DST) in which the bacteria metabolize the enzymes in the specific media causing the sample to fluoresce. Results are given as most probable number (MPN), a common method of obtaining quantitative data on concentrations of discrete items from positive/negative (incidence) data, and in this case correlates well with colony forming units (CFU). Microbial density (as MPN/100 mL) values over the 48 hour test period were calculated and reported.

2.9 ALGAL ENUMERATION

2.9.1 MICROSCOPIC COUNTS

Whole water algae samples collected at 0 (analyzed at 0, 24 and 48) hrs. were analyzed by staining a subsample of *S. capricornutum* cells from each sample with the vital stain SYTOX® Green. The *SOP GWRC/11* was followed for staining and counting. Counting was conducted by enumerating the number of live and dead cells within a known area using a compound microscope equipped with epifluorescence able to excite samples at 450-490 nm under 400x magnification.

While the staining method worked well in LW and provided clear definition between live and dead cells, there was an issue with LW-TMH water. Although the efficacy of the stain was verified by a heat-kill test prior to testing, the treated cells in the LW-TMH did not show the characteristic appearance of dead cells (entirely green, green nucleus, colorless), rather, some of the treated cells looked less red than other cells. This may be due to the TMH itself displaying some fluorescent behavior, although it seems more likely that the treatment in combination with the TMH caused matrix interference with the previously successful staining method.

To definitively determine the effectiveness of the Amglo Kemlite BWMS in LW-TMH, the MPN autotroph procedure was conducted.

2.9.2 MPN METHOD

Sterile technique, media, and glassware were used throughout the MPN autotroph procedure. Millipore water was used to prepare EPA Media. The media consisted of five components, which were sterile filtered through 0.2 µm sterile syringe filter prepared according to LSRI/SOP/AT/45 – *Conducting a Chronic Whole Effluent Toxicity Test with S. capricornutum*.

The method described in Petri, 2015 was followed to conduct the MPN method. Aseptic techniques were used throughout the entire MPN autotroph procedure to prevent contamination of subsamples, tubes, and media. Triplicate subsamples of the EPA media (Method Blanks) were prepared for each test initiation day. During testing with LW-TMH water, the method blanks were prepared by adding 1.5 mL of LW-TMH to 7.5 mL EPA media.

Serial dilutions for each subsample were prepared according to cell-density group listed in Table 4. First, a 10 mL aliquot of each 50 mL subsample was transferred to a sterile 16-mm borosilicate glass tube with cap. The undiluted sample is referred to as 10^0 in Table 4 below. Dilutions of each subsample were prepared by transferring 1 mL of subsample to a sterile 16-mm test tube containing 9 mL of EPA Media to create a 10^{-1} dilution. The 10^{-1} tube was vortexed and 1 mL of 10^{-1} dilution was transferred to a sterile 16-mm test tube containing 9 mL of EPA Media to create a 10^{-2} dilution. The assumption was made that the treatment systems were effective, so the 10^0, 10^{-1}, 10^{-2} dilutions were prepared for the treated samples. The 10^{-2} tube was vortexed and 1 mL of 10^{-2} dilution was transferred to a sterile 16-mm test tube containing 9 mL of EPA Media to create a 10^{-3} dilution. Finally, to create a 10^{-4} dilution (for control samples only), the 10^{-3} tube was vortexed and 1 mL of 10^{-3} dilution was transferred to a sterile 16-mm test tube containing 9 mL of EPA Media. The 10^{-5} and 10^{-6} dilutions were made by serially diluting the prepared 10^{-4} dilution.

Table 4. Dilutions and MPN Matrices for Cell-Density Groups Analyzed in Fresh Water via Autotroph Method.

Cell-Density Group	Live Cell Density (cells/mL)	Dilutions	Range of Matrix Resolution (Cells/mL)
Control Samples	200,000	10^{-4}, 10^{-5}, 10^{-6}	**2,000 – 1,600,000**
Treated Samples	10	10^0, 10^{-1}, 10^{-2}	**0.2 – 160**
Method Blank	**0**	**10^0**	**NA**

From the most dilute tube within each group, the MPN array was filled by making five individual 1.5-mL transfers to fill five replicate tubes containing 7.5 mL (HD) of EPA Media. This was repeated for each dilution created.

The fluorescence of each replicate analysis tube was measured by first vortex mixing the sample tube, then pipetting 0.5 mL sample into the cuvette to rinse the cuvette, disposing of the rinse water, and then pipetting 1.5 mL sample into the cuvette to obtain a fluorescence reading (described in more detail in LSRI/SOP/SA/72 - *Measuring in vivo Chlorophyll a using the Trilogy Laboratory Fluorometer*). Results were recorded on pre-printed datasheets. All replicate analysis tubes for the MPN validation using source water organisms were incubated at the sample collection temperature 25 ± 2.5°C and a 16:8 hour light cycle (343.5 – 1030.5 FC).

After 14 days incubation, fluorescence was measured in each replicate analysis tube as described above and recorded again. The fluorescence values from day 0 and day 14 were entered into a spreadsheet. The values with positive fluorescence on day 14, when corrected for blank fluorescence, were compiled and the information was entered into an MPN calculator to determine the MPN of cells at the time of test initiation (Jarvis, 2017).

2.10 DEVIATIONS

During the course of conducting biological effectiveness testing with the Amglo Kemlite BWMS, there were several deviations that occurred from the TQAP. Those deviations are listed in Table 5 along with corrective actions that were taken as a response to the deviation and perceived impact of the deviation on the test results.

Table 5. Deviations encountered during Amglo Kemlite bench scale testing.

Test Date(s)	Description and Root Cause of Deviation or Quality Control Failure	Description of Corrective Action(s)	Describe the Impact on the Project/Test	Do the Data Need to be Qualified? (Y/N)	Analyst Name
10/9/18, 10/10/18	Minimum of hatch rate (>25%) was not achieved in control exposures	Ordered new lot of ephippia, will conduct hatch rate test upon receipt of each new batch of ephippia prior to conducting dose effectiveness testing.	Tests were repeated. After repeating, hatch rate was not within acceptance range of SOP. Literature review and historical data from LSRI indicated hatch rate should be revised in the SOP.	N	Christine Polkinghorne
12/13/17, 1/3/18	The *S. capricornutum* count in the initial tank counts was higher than the acceptable range	Perform further counts of initial inoculum to ensure calculations of amount of inoculum to add to influent tank are as accurate as possible	Minimal, for both days the average of the control and treatment samples was <3% outside of the acceptable range	N	Christine Polkinghorne
11/13/18	The wrong indicator packet was added to the hardness samples resulting in the loss of the PCW and Control end of test hardness data.	Double check that the packet label is the correct one for the analysis.	Minimal, the treatment samples hardness values were measured.	N	Deanna Regan
11/28/18	Incubator temperature was not recorded at 24 and 48 hour points during hatching portion of ephippia test.	Reread Ephippia SOP with special attention to required times to take temperature readings.	Minimal, Min/Max reading was 19.1°C to 25.3°C during that period. The 25.3°C was likely due to forgetting to reset	N	Christine Polkinghorne

Test Date(s)	Description and Root Cause of Deviation or Quality Control Failure	Description of Corrective Action(s)	Describe the Impact on the Project/Test	Do the Data Need to be Qualified? (Y/N)	Analyst Name
			thermometer when the temperature of the incubator was adjusted to 21°C.		
1/9/19	PCW sample changed color almost immediately on addition of small amount of titrant. This could be the result if the burette tip was not rinsed fully and solid precipitate of the titrant gets added to flask.	Analyzed duplicate PCW sample as the sample and collected another sample as a duplicate since the PCW samples had already been dumped. Ensure the burette tip gets rinsed into waste before titrating each sample.	Minimal, still had PCW sample to measure.	N	Deanna Regan
11/1/17, 12/13/17, 12/19/17, 1/3/18, 1/9/18, 1/17/18, 1/23/18, 10/15/18, 11/13/18, 11/28/18, 1/2/19, 1/9/19, 1/29/19	%T was not within acceptable range listed in the test plan.	Water quality acceptance criteria for %T revised based on historical data.	Minimal, values are within revised acceptance limits for %T.	N	Deanna Regan
11/15/17, 1/3/18, 1/9/18, 1/17/18, 1/2/19	NPOC was not within acceptable range listed in the test plan.	Water quality acceptance criteria for NPOC revised based on historical data.	Minimal, values are within revised acceptance limits for NPOC.	N	Deanna Regan
11/1/17, 11/15/17, 12/13/17, 12/19/17, 1/3/18, 1/9/18, 1/17/18, 1/23/18, 11/13/18, 11/28/18	DOC was not within acceptable range listed in the test plan.	Water quality acceptance criteria for DOC revised based on historical data.	Minimal, values are within revised acceptance limits for DOC.	N	Deanna Regan
12/13/17, 1/3/18, 1/17/18, 1/23/18,	POM was not within acceptable range listed in the test plan.	Water quality acceptance criteria for POM revised based on historical data.	Minimal, values are within revised acceptance limits for POM.	N	Deanna Regan

Test Date(s)	Description and Root Cause of Deviation or Quality Control Failure	Description of Corrective Action(s)	Describe the Impact on the Project/Test	Do the Data Need to be Qualified? (Y/N)	Analyst Name
11/13/18, 11/28/18, 1/2/19, 1/9/19, 1/29/19					

Following the Amglo Kemlite testing and review of the water quality data, a review of historical data collected during bench-scale and land-based testing was undertaken. The review led to revised water quality acceptance limits which are shown in Table 6.

Table 6. Revised Reference Limits for Water Types Prepared for GWRC Bench-Scale Evaluation.

Parameter	Units	Water Type	Acceptable Range for Initiating Bench-Scale Testing
Temperature	°C	LW	22 - 28
		LW-TMH	
pH	NA	LW	6.5 - 9.0
		LW-TMH	
Specific Conductivity	µS/cm	LW	120-170
		LW-TMH	120-170
Salinity	ppt	LW	< 1
		LW-TMH	
Dissolved Oxygen (DO)	mg/L	LW	4 - 12
		LW-TMH	
Total Suspended Solids (TSS)	mg/L	LW	Less than reporting limit
		LW-TMH	11.9 - 30.3
Particulate Organic Matter (POM)	mg/L	LW	Less than reporting limit
		LW-TMH	4.1-12.1
Dissolved Organic Carbon (DOC)	mg/L	LW	Less than detection - 2
		LW-TMH	4.4-6.8
Non-Purgeable Organic Carbon (NPOC)	mg/L	LW	Less than detection - 2
		LW-TMH	5.1-13.1
Percent UV Transmittance at 254 nm (%T)	%	LW	93.0-100 (filtered and unfiltered)
		LW-TMH	25.5-35.5 (filtered and unfiltered)

3 AMGLO KEMLITE BWMS OPERATIONAL PERFORMANCE

During the testing period, several operational issues occurred with the Amglo Kemlite BWMS. Each time there was a performance issue with the Amglo Kemlite system, it was documented on either *GWRC/FORM/22-Bench-Scale Ballast Water Management System (BWMS) Installation Acceptance Form* or *GWRC/FORM/28 – Bench-Scale Technology Testing Form: Documentation of Technology Operability Issue*.

On September 18, 2018, during conduct of a *Eucyclops* effectiveness test in LW with Lamp T, a loud popping noise was heard coming from the system followed by several more pops and sparks 8-12" high shooting out of the positive anode side of the connection box. All electricity was disconnected and the LW was drained from the system. The wires entering the connection box appeared rusty and the anode end of the bulb appeared as if the seal had deteriorated, allowing water to enter the braided wire at the anode connection point. Testing was suspended. Four new flash bulbs were delivered by Amglo Kemlite Laboratories and testing continued. On October 9, 2018 sparks were again given off by the system. Upon inspection by LSRI-GWRC staff it was determined that the newly delivered bulbs were not sealed completely. The bulbs were resealed by LSRI-GWRC staff with material provided by Amglo Kemlite, and testing continued.

During testing on December 5, 2018 with Lamp Q in LW-TMH, the system had been functioning at 5 Hz for approximately 14 minutes when the flashing quit and an error message was displayed on the top charger unit that read "Power inverter" followed by a message saying "Power not correct". The system would not restart with either lamp. The UW-Superior campus electrician inspected the electrical connections and did not find any issues, but the system would not function. An Amglo Kemlite representative inspected the transformer and found scorch marks on the wires, and one wire was completely disconnected from the system. The damaged connections were repaired by the Amglo Kemlite representative and the system was tested. No further issues were identified and the trials continued.

4 RESULTS

Findings from the Amglo Kemlite biological effectiveness tests, which were conducted on one species of algae, two species of bacteria and two species of zooplankton in both LW and LW-TMH with two lamps (Q and T) at two frequencies (5 and 10 Hz) are presented in the following subsections. Also presented are the results of testing the algae, *S. capricornutum*, in both LW and LW-TMH with the Amglo Kemlite BWMS (Lamp Q, 10 Hz) and the UV water purification system.

4.1 GREEN ALGAE (*SELENASTRUM CAPRICORNUTUM*)

As detailed in the "Methods" section, water chemistry and water quality were measured on stock solutions of the water prior to initiation of testing. Water chemistry was additionally measured during the testing period and daily during the 48-hour exposure period. The results of water chemistry measurements taken during the tests with *S. capricornutum* are shown in Table 7. Water chemistry measurements of the stock solutions met the water chemistry acceptance requirements listed in the test plan (LSRI, 2017). In general, temperature increased immediately following treatment. For example, in the LW control the water temperature increased from 25.7°C to 26.2°C as a result of running the pump. This 0.5°C difference was markedly increased as a result of running the Amglo Kemlite system as water temperature increased by 3.5°C at 5 Hz and 5.7°C at 10 Hz. The pH and DO both increased from immediately following treatment to 48 hrs. Conductivity was not affected by the treatment process.

Table 7. Average (minimum, maximum) Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with Amglo Kemlite BWMS involving *S. capricornutum* in LW, LW-TMH and PCW at 25°C ± 3°C. Stock solutions are measured prior to the start of the test and do not have average values. NM = not measured.

Water Type	Lamp	Exposure		Incubation Time Post-Treatment (Hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
LW	Q	Stock		-	25.7	6.90	5.1	145.0	51.5	49.0
		PCW		0	25.4	7.35	6.9	167.4	55.5	56.1
				24-48	25.0 (25.0, 25.0)	7.88 (7.85, 7.92)	8.4 (8.3, 8.5)	169.0 (168.1, 169.8)	NM	NM
		5 Hz	Control	0	26.2 (26.1, 26.3)	7.08 (7.03, 7.12)	5.8 (5.7, 5.9)	145.5 (144.7, 146.5)	NM	NM
				24-48	25.1 (25.0, 25.1)	7.95 (7.95, 7.96)	8.3 (8.2, 8.4)	146.5 (145.2, 147.7)	NM	NM
			Treatment	0	29.2 (28.9, 29.5)	7.10 (7.06, 7.16)	5.7 (5.6, 5.9)	145.6 (145.3, 145.7)	NM	NM
				24-48	25.0 (24.9, 25.0)	7.98 (7.95, 8.02)	8.2 (8.1, 8.3)	146.4 (146.3, 146.5)	NM	NM
		10 Hz	Control	0	26.2 (26.2, 26.2)	7.20 (7.14, 7.25)	5.9 (5.8, 6.0)	145.2 (144.8, 145.5)	NM	NM
				24-48	25.0 (24.9, 25.0)	7.99 (7.98, 8.01)	8.2 (8.1, 8.3)	146.0 (145.3, 146.7)	NM	NM
			Treatment	0	31.4	7.19	5.7	146.0	NM	NM

Water Type	Lamp	Exposure		Incubation Time Post-Treatment (Hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
					(31.0, 31.8)	(7.16, 7.24)	(5.6, 5.7)	(145.6, 146.2)		
				24-48	25.0 (24.9, 25.0)	8.01 (8.00, 8.03)	8.2 (8.1, 8.3)	146.5 (146.4, 146.5)	NM	NM
LW	T	Stock		-	25.6 (25.5, 25.6)	7.01 (6.87, 7.20)	6.0 (5.8, 6.2)	145.7 (145.0, 146.8)	47.9	41.4
		PCW		0	25.0	7.32	6.9	196.4	63.4	66.9
				24-48	25.1 (24.9, 25.2)	8.14 (7.97, 8.23)	8.1 (7.9, 8.3)	197 (194.3, 199)	NM	NM
		5 Hz	Control	0	26.2 (26.1, 26.2)	7.19 (7.16, 7.21)	6.1 (6.0, 6.1)	144.1 (143.0, 145.1)	NM	NM
				24-48	25.1 (24.9, 25.2)	8.10 (8.04, 8.14)	8.2 (8.1, 8.3)	146.1 (142.3, 149.1)	NM	NM
			Treatment	0	29.0 (28.8, 29.2)	7.19 (7.16, 7.21)	6.1 (6.1, 6.2)	145.0 (144.4, 145.4)	NM	NM
				24-48	25.1 (24.8, 25.2)	8.09 (8.05, 8.13)	8.1 (8.0, 8.2)	148.4 (144.7, 155.4)	NM	NM
		10 Hz	Control	0	26.1 (26.0, 26.2)	7.23 (7.15, 7.29)	6.0 (5.9, 6.2)	143.9 (143.0, 145.5)	NM	NM
				24-48	25.1 (24.9, 25.2)	8.12 (8.07, 8.15)	8.1 (8.1, 8.3)	145.3 (143.8, 145.9)	NM	NM
			Treatment	0	31.9 (31.6, 32.0)	7.21 (7.17, 7.23)	6.1 (6.0, 6.2)	145.1 (144.3, 145.5)	NM	NM
				24-48	25.1 (25.0, 25.1)	8.12 (8.08, 8.15)	8.1 (8.0, 8.2)	145.9 (145.0, 146.4)	NM	NM
LW-TMH	T	Stock		-	25.0 (24.9, 25.2)	6.91 (6.81, 6.99)	6.0 (5.8, 6.2)	125.9 (124.3, 128.1)	43.8	49.0
		PCW		0	24.4	7.42	7.1	155.0	49.0	55.3
				24-48	24.8 (24.6, 25.3)	8.09 (8.04, 8.13)	8.2 (8.1, 8.3)	149.9 (147.7, 153.9)	NM	NM
		5 Hz	Control	0	25.7 (25.6, 25.7)	6.87 (6.76, 6.97)	6.3 (6.2, 6.4)	125.4 (125.2, 125.7)	NM	NM
				24-48	24.7 (24.5, 25.2)	8.03 (7.99, 8.09)	8.1 (8.1, 8.2)	124.4 (122.8, 125.5)	NM	NM
			Treatment	0	29.0 (29.0, 29.1)	6.90 (6.81, 6.96)	6.2 (6.2, 6.2)	126.1 (125.4, 126.4)	NM	NM
				24-48	24.7 (24.6, 25.1)	8.03 (7.99, 8.08)	8.1 (8.0, 8.1)	125.5 (124.5, 126.3)	NM	NM
			Control	0	25.6	6.90	6.3	126.8	NM	NM

Water Type	Lamp	Exposure		Incubation Time Post-Treatment (Hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
		10 Hz			(25.4, 25.7)	(6.80, 7.02)	(6.2, 6.3)	(125.4, 130.2)		
				24-48	24.8 (24.7, 25.1)	8.04 (7.99, 8.11)	8.1 (8.1, 8.2)	125.2 (125.0, 125.3)	NM	NM
			Treatment	0	32.2 (31.9, 32.4)	6.95 (6.83, 7.03)	6.1 (6.0, 6.2)	126.2 (125.6, 126.8)	NM	NM
				24-48	24.8 (24.6, 25.2)	8.06 (8.01, 8.09)	8.0 (8.0, 8.1)	125.6 (125.1, 125.9)	NM	NM
LW-TMH	Q	Stock		-	25.2 (25.1, 25.4)	7.20 (7.15, 7.25)	7.4 (7.3, 7.5)	139.5 (139.2, 139.9)	51.8	50.9
		PCW		0	24.8	7.30	6.5	164.7	57.7	59.7
				24-48	25.1 (25.1, 25.1)	8.11 (8.06, 8.16)	8.4 (8.3, 8.4)	164.8 (164.4, 165.4)	NM	NM
		5 Hz	Control	0	25.9 (25.9, 26.0)	7.24 (7.22, 7.26)	7.5 (7.4, 7.6)	139.6 (139.3, 139.8)	NM	NM
				24-48	25.1 (25.1, 25.1)	8.10 (8.09, 8.11)	8.3 (8.3, 8.3)	139.3 (139.0, 139.5)	NM	NM
			Treatment	0	28.8 (28.7, 28.9)	7.24 (7.23, 7.25)	7.3 (7.2, 7.4)	140.0 (139.6, 140.5)	NM	NM
				24-48	25.1 (25.1, 25.1)	8.09 (8.06, 8.11)	8.3 (8.3, 8.4)	139.0 (138.6, 139.3)	NM	NM
		10 Hz	Control	0	25.8 (25.7, 25.8)	7.28 (7.27, 7.30)	7.5 (7.4, 7.5)	140.2 (140.0, 140.4)	NM	NM
				24-48	25.1 (25.0, 25.1)	8.11 (8.09, 8.13)	8.3 (8.3, 8.3)	138.9 (138.8, 139.1)	NM	NM
			Treatment	0	31.1 (30.8, 31.3)	7.26 (7.24, 7.27)	7.2 (7.2, 7.2)	140.4 (140.0, 140.6)	NM	NM
				24-48	25.1 (25.1, 25.1)	8.10 (8.09, 8.12)	8.3 (8.3, 8.3)	138.6 (136.2, 139.5)	NM	NM

Water quality measurements taken on stock solutions at the initiation of tests with *S. capricornutum* are presented in Table 8. Total suspended solid values were within acceptance limits for all tests. The percent transmittance was not within the acceptance limits listed in the TQAP during the testing in LW with Lamp Q and in LW-TMH with Lamps Q and T (Table 5). NPOC was not within the acceptance limits listed in the TQAP during the testing in LW with Lamp T and testing in LW-TMH with Lamp Q (Table 5). DOC was not within the acceptance limits for any of the tests and POM was not within the acceptance limits for the tests in LW-TMH with Lamps Q and T (Table 5). The acceptance limits were revised following the testing to be comprised of ranges measured historically at LSRI during bench scale testing. Following the revision of

acceptance limits, all values with the exception of DOC and POM in the LW-TMH test with Lamp Q were within the acceptance range.

Table 8. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the Amglo Kemlite BWMS involving *S. capricornutum* in LW, LW-TMH and PCW at 25°C ± 3°C.

Stock Solution Water Type	Lamp	Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered/Unfiltered (%)	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
PCW (LW)	Q	<1.25	96.8 / 96.3	1.3[J]	1.2[J]	<1.25	<1.25
LW		<1.25	96.8 / 96.5	1.3[J]	1.5[J]	<1.25	<1.25
PCW (LW)	T	<1.25	97.1 / 97.0	1.3[J]	1.3[J]	<1.25	<1.25
LW		<1.25	97.3 / 97.4	1.6[J]	1.6[J]	<1.25	<1.25
PCW (LW)	T	<1.25	97.2 / 97.0	1.4[J]	1.5[J]	<1.25	<1.25
LW-TMH		20.6	28.7 / 26.7	9.2	6.3	7.8	12.8
PCW (LW)	Q	<1.25	96.8 / 96.3	1.8[J]	1.6[J]	<1.25	<1.25
LW-TMH		28.8	28.8 / 25.1	10.6	6.9	13.5	15.3

J = Value between limit of detection (0.69 mg/L) and limit of quantitation (2.3 mg/L).

Initial counts of *S. capricornutum* in the influent tank were targeted to be 200,000 cells/mL ± 20%. Initial cells count in each test are shown in Table 9. Both tests in LW-TMH had influent tank concentrations higher than the acceptance range. For the test with lamp T, the concentration in the influent was just above the acceptance range, and the average concentration in the control and treatment samples was 3% higher than the acceptable starting concentration. For the test with lamp Q in LW-TMH, although the influent tank concentration was higher than the acceptable range, the average of the control and treatment tanks was 2% higher than the acceptable starting concentration. The tests were conducted because of the time involved in growing and preparing the algae for testing. The higher concentration is believed to have a minimal impact on the testing.

Table 9. Initial *S. capricornutum* Concentrations in Influent Tank during Amglo Kemlite testing.

Water Type	Frequency (Hz)	Lamp	Influent Tank Concentration (cells/mL)
LW	0, 5, 10	Q	210,265 ± 16,817
LW	0, 5, 10	T	231,746 ± 32,400
LW-TMH	0, 5, 10	T	243,333 ± 33,185
LW-TMH	0, 5, 10	Q	274,074 ± 12,239

For the PCW and Pump Control mortality was negligible in both water types, with both lamps and at both frequencies, indicating the organisms were in good health and the pump did not cause mortality (Table 10). In LW, both lamps at both frequencies caused 100% mortality within 24 hrs. following treatment. In LW-TMH, with both lamps and frequencies there was mortality, but results were inconclusive using the proposed vital stain method of analysis to identify live versus dead cells. Many of the cells did not appear to have the characteristics defined as indication of death (i.e., green in its entirety, green nucleus, colorless). Rather, some cells just appeared a distinctly lighter red color. The stain was verified using a heat kill method in LW-TMH. It is likely that there was some matrix interference caused by interaction between the stain, LW-TMH and the treatment method causing the difficulty with definitively identifying cells as live or dead.

In cases where the 24-hour mortality appears to be higher than the 48 hour mortality, this is the result of an extremely low number of dead cells counted (i.e., one or two cells) at 24 hrs. Because the 24- and 48-hour counts are conducted on individual samples, it is possible that there could be dead individuals in one sample and not in another. There is not an appreciable difference in mortality from 24 to 48 hrs. in these cases.

Table 10. Percent Mortality (± Standard Deviation) of *S. capricornutum* during Dose Effectiveness Testing with the Amglo Kemlite BWMS.

Water Type	Frequency (Hz)	Lamp	Exposure	0 Hour % Mortality	24 Hour % Mortality	48 Hour % Mortality
LW	5	Q	Performance Control	0 ± 0 (n=4)	1.2 (n=1)	0 (n=1)
			Pump Control	0.5 ± 0.54 (n=4)	0 (n=1)	0 (n=1)
			Treatment	100 ± 0 (n=4)	100 ± 0 (n=4)	100 ± 0 (n=3)
LW	10	Q	Performance Control	0 ± 0 (n=4)	1.2 (n=1)	0 (n=1)
			Pump Control	0.5 ± 0.64 (n=4)	0 (n=1)	0 (n=1)
			Treatment	100 ± 0 (n=4)	100 ± 0 (n=4)	100 ± 0 (n=3)
LW	5	T	Performance Control	0 ± 0 (n=4)	0.2 ± 0.47 (n=4)	0 (n=1)
			Pump Control	0.2 ± 0.43 (n=4)	0.3 ± 0.5 (n=4)	0 (n=1)
			Treatment	98.6 ± 1.2 (n=4)	100 ± 0 (n=4)	100 ± 0 (n=3)
LW	5	T	Performance Control	0 ± 0 (n=4)	0.2 ± 0.47 (n=4)	0 (n=1)
			Pump Control	0.4 ± 0.48 (n=4)	0 ± 0 (n=4)	0 (n=1)
			Treatment	99.8 ± 0.45 (n=4)	100 ± 0 (n=4)	100 ± 0 (n=3)
LW-TMH	5	Q	Performance Control	0.2 ± 0.43 (n=4)	0 (n=1)	0 ± 0 (n=3)
			Pump Control	0 ± 0 (n=4)	0 (n=1)	0 ± 0 (n=3)
			Treatment	0.2 ± 0.42 (n=4)	0 (n=1)	52.3* ± 3.54 (n=3)
LW-TMH	10	Q	Performance Control	0.2 ± 0.43 (n=4)	0 (n=1)	0 ± 0 (n=3)
			Pump Control	0 ± 0 (n=4)	0 (n=1)	0 ± 0 (n=3)
			Treatment	0.7 ± 0.44 (n=4)	1 (n=1)	81.9* ± 7.91 (n=3)
LW-TMH	5	T	Performance Control	0.2 ± 0.42 (n=4)	0 (n=1)	0.7 ± 0.6 (n=3)
			Pump Control	0 ± 0 (n=4)	0.9 (n=1)	0 ± 0 (n=3)
			Treatment	0 ± 0 (n=4)	0 (n=1)	0 ± 0 (n=3)

Water Type	Frequency (Hz)	Lamp	Exposure	0 Hour % Mortality	24 Hour % Mortality	48 Hour % Mortality
LW-TMH	10	T	Performance Control	0.2 ± 0.42 (n=4)	0 (n=1)	0.7 ± 0.6 (n=3)
			Pump Control	0 ± 0 (n=4)	0 (n=1)	0 ± 0 (n=3)
			Treatment	0 ± 0 (n=4)	0 (n=1)	61.8* ± 9.3 (n=3)

*Indicates samples where Sytox Green stain did not provide definitive indication of cells being alive or dead.

4.2 MICROBES (*ESCHERICHIA COLI* AND *ENTEROCOCCUS FAECIUM*)

Water chemistry was measured on stock solutions of the water prior to initiation of testing with microbes. Water chemistry was also measured during the testing period and daily during the 48 hour exposure period. The results of water chemistry measurements made during the tests with *E. coli* and *E. faecium* are presented in Table 11. Water chemistry measurements in the stock solutions met the water quality criteria defined in the test plan. As seen during green algae testing, water temperature was highest immediately following treatment as the treatment process produces heat. DO values in both PCW types were <1 mg/L at 24 and 48 hrs., due to high growth rates of microbes. The pH and DO both increased from immediately following treatment to 48 hrs. Conductivity was not affected by the treatment process.

The LW test done with Lamp Q was run at 37°C in addition to 25°C. This was done because 37°C is the optimal temperature for both *E. coli* and *E. faecium*, so the experiment was aimed at determining if running at 37°C led to higher control survival. There was no substantial difference in control survival at 25°C relative to 37°C, therefore, the remaining tests were conducted only at 25°C.

Table 11. Average (minimum, maximum) Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with Amglo Kemlite BWMS involving *E. coli* and *E. faecium* in LW, LW-TMH and PCW at 25°C ± 3°C. Stock solutions are measured prior to the start of the test and do not have average values. BHB= Brain Heart Infusion Broth, TSB= Tryptic Soy Broth, NM = not measured. Time is Incubation Time Post-Treatment.

Water Type	Lamp	Exposure		Time (hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
LW	T	Stock		-	24.6 (24.5, 24.7)	7.03 (6.89, 7.21)	6.7 (6.6, 6.7)	123.5 (121.6, 126.0)	44.8 (44.6, 45.0)	46.4 (45.8, 47.0)
		PCW		0	-	-	-	-	-	-
				24-48	24.4 (24.3, 24.4)	6.38 (6.18, 6.51)	0.2 (0.2, 0.2)	13818 (13440, 14010)	NM	NM
		5 Hz	Pump Control	0	25.2 (25.1, 25.2)	7.08 (7.04, 7.11)	6.8 (6.8, 6.9)	123.6 (123.3, 123.8)	NM	NM
				24-48	24.3 (24.0, 24.6)	7.83 (7.59, 7.97)	7.9 (7.7, 8.3)	127.0 (126.2, 128.0)	NM	NM
			Treatment	0	28.1 (27.8, 28.3)	7.11 (7.08, 7.13)	6.8 (6.7, 6.9)	123.9 (123.7, 124.1)	NM	NM
				24-48	24.0 (23.5, 24.4)	7.88 (7.68, 7.99)	7.9 (7.8, 8.2)	124.4 (122.1, 125.6)	NM	NM
		10 Hz	Pump Control	0	25.2 (25.0, 25.5)	7.13 (7.09, 7.14)	6.6 (6.6, 6.7)	124.2 (122.8, 125.0)	NM	NM
				24-48	24.1	7.83	8.2	125.9	NM	NM

Water Type	Lamp	Exposure		Time (hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
					(23.9, 24.3)	(7.58, 7.98)	(8.2, 8.2)	(125.2, 126.6)		
			Treatment	0	30.8 (30.5, 31.0)	7.16 (7.11, 7.18)	6.7 (6.6, 6.7)	124.8 (123.8, 125.5)	NM	NM
				24-48	24.0 (23.7, 24.3)	7.90 (7.73, 7.99)	8.2 (8.1, 8.3)	125.2 (124.2, 125.8)	NM	NM
LW	Q	Stock		-	25.2 (25.0, 25.5)	7.33 (7.21, 7.43)	8.7 (8.2, 9.0)	136.5 (135.4, 137.7)	47.2 (46.2, 48.2)	50.1 (50.1, 50.1)
		TSB PCW	Stock	0	22.2	7.11	8.8	12870	NM	NM
			25°C	24-48	23.7 (22.9, 24.5)	6.74 (6.45, 8.16)	0.2 (0.2, 0.2)	14175 (13940, 14410)	NM	NM
			37°C	24-48	26.7 (24.7, 28.7)	7.55 (7.27, 8.55)	2.5 (0.1, 4.9)	14660 (14500, 14820)	NM	NM
		BHB PCW	Stock	0	21.5	7.28	8.9	13150	NM	NM
			25°C	24-48	23.8 (23.2, 24.3)	6.84 (6.82, 6.87)	7.6* (0.6, 14.5*)	13520 (13250, 13790)	NM	NM
			37°C	24-48	26.8 (24.4, 29.1)	6.36 (6.36, 6.36)	6.7 (5.0, 8.3)	14320 (14140, 14500)	NM	NM
		5 Hz	Pump Control	0	25.5 (25.4, 25.6)	7.36 (7.34, 7.38)	8.5 (8.4, 8.6)	135.8 (135.7, 136.0)	NM	NM
				24-48	24.2 (24.1, 24.4)	7.86 (7.69, 7.97)	8.1 (8.0, 8.2)	141.8 (137.6, 152.1)	NM	NM
			Treatment	0	28.0 (27.8, 28.2)	7.36 (7.34, 7.38)	8.4 (8.3, 8.4)	135.7 (135.3, 136.2)	NM	NM
				24-48	24.1 (23.9, 24.6)	8.03 (7.94, 8.08)	8.1 (8.0, 8.1)	136.9 (136.6, 137.4)	NM	NM
		10 Hz	Pump Control	0	25.5 (25.4, 25.6)	7.37 (7.35, 7.40)	8.3 (8.1, 8.4)	136.1 (135.9, 1364)	NM	NM
				24-48	24.0 (23.8, 24.4)	7.79 (7.56, 8.04)	8.0 (8.0, 8.1)	136.9 (136.4, 137.4)	NM	NM
			Treatment	0	30.1 (29.7, 30.5)	7.36 (7.31, 7.39)	8.1 (8.0, 8.2)	136.1 (135.8, 136.3)	NM	NM
				24-48	23.9 (23.5, 24.7)	8.05 (8.00, 8.09)	8.1 (8.0, 8.1)	136.8 (136.1, 137.2)	NM	NM
LW-TMH	Q	Stock		-	24.8 (24.7, 24.9)	7.20 (7.12, 7.27)	8.4 (8.1, 8.6)	137.2 (134.2, 139.0)	51.0 (50.2, 51.8)	52.7 (51.3, 54.1)
		TSB PCW		0	22.6	7.17	3.8	12340	NM	NM
				24-48	26.2 (25.6, 27.0)	6.24 (6.04, 6.39)	0.1 (0.0, 0.2)	13598 (12990, 13830)	NM	NM
		BHB PCW		0	23.4	7.33	4.2	12410	NM	NM
				24-48	25.9 (25.3, 26.6)	6.29 (6.24, 6.31)	0.3 (0.0, 0.5)	13168 (12910, 13300)	NM	NM
		5 Hz	Pump Control	0	25.4 (25.4, 25.4)	7.28 (7.26, 7.29)	8.1 (8.1, 8.2)	137.0 (135.5, 138.7)	NM	NM
				24-48	25.7 (24.6, 26.2)	7.89 (7.82, 7.95)	8.1 (8.0, 8.2)	141.0 (136.2, 149.9)	NM	NM
			Treatment	0	28.1 (27.8, 28.3)	7.32 (7.29, 7.34)	8.0 (8.0, 8.0)	136.9 (136.3, 137.5)	NM	NM
				24-48	25.3 (24.7, 25.9)	7.95 (7.84, 8.01)	7.8 (7.4, 8.1)	137.8 (137.5, 138.2)	NM	NM
		10 Hz	Pump Control	0	25.4 (25.3, 25.5)	7.32 (7.29, 7.36)	8.1 (8.0, 8.1)	138.1 (137.6, 138.9)	NM	NM
				24-48	25.7	8.01	8.0	138.0	NM	NM

Water Type	Lamp	Exposure		Time (hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
					(24.7, 26.4)	(7.96, 8.06)	(7.9, 8.1)	(137.7, 138.2)		
			Treatment	0	30.8 (30.8, 30.8)	7.33 (7.32, 7.34)	7.8 (7.7, 7.9)	137.4 (137.0, 137.9)	NM	NM
				24-48	26.0 (24.7, 26.4)	8.05 (8.04, 8.07)	7.9 (7.8, 8.1)	137.7 (137.3, 137.9)	NM	NM
LW-TMH	T	Stock		-	25.1 (24.7, 25.4)	7.01 (6.97, 7.10)	4.8 (4.5, 5.2)	128.0 (124.5, 132.5)	40.2 (36.2, 44.2)	46.0 (45.4, 46.6)
		TSB PCW		0	23.1	7.13	7.00	12400	NM	NM
				24-48	23.7 (23.5, 23.8)	6.21 (5.92, 6.40)	0.1 (0.1, 0.2)	13585 (13280, 13710)	NM	NM
		BHB PCW		0	23.2	7.28	6.6	12570	NM	NM
				24-48	23.7 (23.6, 23.8)	6.34 (6.19, 6.90)	0.7 (0.5, 1.0)	13130 (12860, 13260)	NM	NM
		5 Hz	Pump Control	0	25.9 (25.8, 25.9)	7.04 (7.02, 7.05)	5.2 (5.1, 5.3)	128.4 (127.5, 129.8)	NM	NM
				24-48	24.2 (24.1, 24.3)	7.78 (7.62, 7.96)	8.4 (8.4, 8.5)	127.4 (126.8, 128.5)	NM	NM
			Treatment	0	29.0 (28.8, 29.1)	7.07 (7.06, 7.07)	5.3 (5.3, 5.3)	126.8 (126.4, 127.2)	NM	NM
				24-48	24.2 (24.1, 24.3)	8.01 (7.94, 8.04)	8.3 (8.2, 8.3)	126.4 (125.9, 127.0)	NM	NM
		10 Hz	Pump Control	0	25.8 (25.8, 25.8)	7.10 (7.08, 7.12)	5.4 (5.2, 5.5)	132.2 (127.1, 142.0)	NM	NM
				24-48	24.3 (24.0, 24.5)	7.96 (7.86, 8.05)	8.3 (8.2, 8.3)	127.7 (127.4, 128.2)	NM	NM
			Treatment	0	31.6 (31.4, 31.7)	7.10 (7.09, 7.12)	5.5 (5.4, 5.5)	127.4 (127.1, 127.7)	NM	NM
				24-48	24.2 (24.1, 24.4)	7.96 (7.89, 8.04)	8.2 (8.1, 8.2)	126.7 (126.3, 127.0)	NM	NM

*Readings rapidly increased at the 48 hour time point.

Water quality measurements were made on stock solutions prior to the initiation of the microbe tests (Table 12). TSS values were within the acceptance range for all tests with microbes. Percent Transmittance and DOC values were not within the acceptance range listed in the TQAP for any of the tests (Table 5). NPOC was not within the acceptance range listed in the TQAP for the tests in LW and LW-TMH with Lamp Q (Table 5). POM was not within the acceptance range listed in the TQAP for the tests in LW-TMH with Lamps Q and T (Table 5). The acceptance limits were revised following the testing to be comprised of ranges measured historically at LSRI during bench scale testing. Following the revision of acceptance limits, all values were within the acceptance ranges prior to addition of the inoculum.

Table 12. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the Amglo Kemlite BWMS involving *E. coli* and *E. faecium* in LW and LW-TMH at 25°C ±3°C.

Stock Solution Water Type	Lamp	Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered/Unfiltered (%)	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
LW	T	<1.25	89.9 / 90.0	1.3[J]	1.6[J]	<1.25	<1.25
LW after inoc.		<1.25	89.9 / 90.0	2.8	2.8	<1.25	<1.25
LW	Q	<1.25	96.9 / 96.4	1.9[J]	1.5[J]	<1.25	<1.25
LW after inoc.		<1.25	95.9 / 95.5	2.8	2.6	<1.25	<1.25
LW-TMH	Q	18.4	29.5 / 26.4	9.4	6.0	6.8	11.6
LW-TMH after inoc.		20.4	29.3 / 26.2	10.7	7.1	7.4	13.0
LW-TMH	T	18.1	29.0 / 26.5	8.9	6.3	6.6	11.5

J = Value between limit of detection (0.69 mg/L) and limit of quantitation (2.3 mg/L).

Results of dose effectiveness tests involving *E. coli* and *E. faecium* in LW and LW-TMH, for both lamps Q and T at the two frequencies (5 Hz. and 10Hz.) are shown in Table 13. Initial concentrations (0 hour) of both species in the influent tank and in the Pump Control replicates were > 1,000,000/100 mL for all tests indicating initial target concentrations were achieved. Concentrations of E. coli and E. faecium in the Pump Controls remained relatively stable throughout the 48-hour test period, indicating that there was no substantial mortality caused by the pump alone. Growth occurred in all performance control replicates (PCW-TSB and PCW-BHB) for both *E. coli* and *E. faecium* indicating that test organisms were healthy and adequate for testing.

For the Amglo Kemlite tests using LW, none of the 0-hour treatment subsamples analyzed, for either lamp or frequency, had any detectable *E. coli* or *E. faecium*. At 24 hrs. after treatment however, two of the five Lamp T- 5 Hz Treatment replicates had detectable *E. coli* densities and one of the 48 hour replicates had detectable *E. coli* densities giving averages of 47.1 MPN/100 mL at 24 hrs. and 397.7 MPN/100 mL at 48 hrs. (still a 99.99998% reduction from initial control numbers). Results were nearly the same for the lamp T -10 Hz replicates which had an average of 83.3 MPN/100 mL at 24 hrs. and 796.8 MPN/100 mL at 48 hrs. (99.991% reduction from initial control numbers). There was no evidence of procedural contamination as diluent blanks and procedural blanks for all analysis periods were below the LOD. Therefore, the only plausible explanation is re-growth of the minimal bacterial cells present in the 1 L treated sample. Regrowth was not observed for *E. coli* or *E. faecium* in LW treated with Lamp Q at either frequency tested; concentrations of both bacteria species remained below detection during the entire 48-hour test period.

For the tests using LW-TMH, the samples treated with Lamp Q-5 Hz were the only ones in which no *E. coli* was detected at 0, 24 or 48 hrs. The remaining treatment groups had *E. coli* and *E. faecium* detected in at least one of the replicates analyzed and densities ranged between <1 MPN/100 mL and 5906 MPN/100 mL for *E. coli* and between <1 MPN/100 mL and 68.6 MPN/100 mL for *E. faecium*.

Overall, the Amglo Kemlite BWMS was 99.99% effective in killing both bacterial species in both LW and LW-TMH. All samples analyzed *immediately after treatment* with the Amglo Kemlite BWMS, regardless of lamp or frequency used for treatment, revealed a log reduction of at least 5 $\log_{10}$ units for both *E. coli* and *E. faecium* and in a compliance situation would have been below the Ballast Water Discharge Standard set forth by the U.S. EPA and U.S. Coast Guard.

Table 13. Average Density (MPN/100mL) ± Standard Error of the Mean of Bacteria *E. coli* and *E. faecium* During Biological Effectiveness Tests of the Amglo Kemlite BWMS.

Water Type	Frequency (Hz)	Lamp	Species	Exposure	0 Hour	± SEM	24 Hour	± SEM	48 Hour	± SEM
Lab Water	5	Q	*E. coli*	Influent Tank (n=3)	5.6E+06	4.7E+05	NA			
				Performance Control (n=5)	8.9E+06	2.0E+06	1.1E+12	2.6E+11	7.1E+11	7.3E+10
				Pump Control (n=5)	7.7E+06	6.7E+05	1.5E+08	1.1E+08	1.4E+06	2.8E+05
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
			E. faecium	Influent Tank (n=3)	3.2E+06	3.5E+05	NA			
				Performance Control (n=5)	6.2E+06	9.1E+05	9.3E+10	1.8E+10	3.2E+09	2.1E+09
				Pump Control (n=5)	3.3E+06	3.1E+05	2.5E+06	3.2E+05	1.2E+05	2.1E+04
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
Lab Water	10	Q	*E. coli*	Pump Control (n=5)	9.1E+06	1.6E+06	6.4E+07	1.5E+07	2.1E+06	3.8E+05
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
			E. faecium	Pump Control (n=5)	3.1E+06	3.1E+05	3.0E+06	3.1E+05	1.3E+05	1.8E+04
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
Lab Water	5	T	*E. coli*	Influent Tank (n=3)	8.3E+06	3.2E+05	1.5E+08	2.8E+07	* 2.0E+06	6.1E+05
				Performance Control (n=3)	1.3E+07	3.2E+05	> 2.4E+09	NC	> 2.4E+07	NC
				Pump Control (n=5)	1.1E+07	2.2E+06	8.8E+07	2.5E+07	* 1.7E+06	4.5E+05
				Treatment (n=5)	< 1.0	0.0	* 47.1	29.1	* 397.7	397.2
			E. faecium	Influent Tank (n=3)	5.1E+06	9.5E+05	5.3E+06	5.9E+05	1.7E+05	3.0E+04
				Performance Control (n=3)	1.1E+07	3.4E+06	> 2.4E+07	NC	3.6E+05	2.6E+05
				Pump Control (n=5)	5.4E+06	7.5E+05	5.8E+06	8.3E+05	1.5E+05	6.7E+03
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
Lab Water	10	T	*E. coli*	Pump Control (n=5)	1.5E+07	3.0E+06	1.1E+08	3.9E+07	* 8.9E+06	6.6E+06
				Treatment (n=5)	* < 1.0	0.3	* 83.3	44.8	* 796.8	344.3
			E. faecium	Pump Control (n=5)	7.1E+06	8.3E+05	4.2E+06	5.7E+05	1.3E+05	2.9E+04
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
LW-TMH	5	Q	*E. coli*	Influent Tank (n=3)	9.0E+06	9.3E+05	NA			
				Performance Control (n=5)	1.3E+07	8.8E+05	2.5E+11	2.9E+10	1.9E+11	1.5E+10
				Pump Control (n=5)	8.0E+06	8.6E+05	5.6E+07	7.4E+06	1.8E+06	5.9E+05
				Treatment (n=5)	< 1.0	0.0	< 1.0	0.0	< 1.0	0.0
				Influent Tank (n=3)	3.1E+06	1.8E+05	NA			

Water Type	Frequency (Hz)	Lamp	Species	Exposure	0 Hour	± SEM	24 Hour	± SEM	48 Hour	± SEM
			E. faecium	Performance Control (n=5)	4.6E+06	3.4E+05	1.0E+11	5.2E+09	1.0E+11	9.6E+09
				Pump Control (n=5)	3.6E+06	5.3E+05	3.0E+06	3.7E+05	2.6E+04	4394
				Treatment (n=5)	* 68.6	39.9	5.1	1.8	3.5	1.4
LW-TMH	10	Q	*E. coli*	Pump Control (n=5)	9.4E+06	8.3E+05	6.3E+07	6.3E+06	1.8E+06	2.0E+05
				Treatment (n=5)	* 10.9	10.3	* 3988	3194	* 5906	5070
			E. faecium	Pump Control (n=5)	3.4E+06	5.2E+05	4.4E+06	1.9E+06	3.1E+04	2246
				Treatment (n=5)	* 25.0	22.2	* 16.3	14.9	* 18.3	16.4
LW-TMH	5	T	*E. coli*	Influent Tank (n=3)	6.9E+06	6.3E+05	NA			
				Performance Control (n=5)	9.4E+06	1.0E+06	2.0E+11	2.3E+10	2.9E+11	3.7E+10
				Pump Control (n=5)	8.2E+06	5.2E+05	7.8E+07	3.4E+07	1.4E+06	6.2E+04
				Treatment (n=5)	< 1.0	0.0	* 38.1	37.3	* 46.6	45.4
			E. faecium	Influent Tank (n=3)	3.0E+06	2.5E+05	NA			
				Performance Control (n=5)	5.1E+06	6.3E+05	1.2E+11	4.3E+09	8.4E+10	1.1E+10
				Pump Control (n=5)	3.7E+06	4.0E+05	2.4E+06	2.2E+05	1.8E+04	2305
				Treatment (n=5)	* 39.6	9.2	5.3	1.9	2.4	0.5
LW-TMH	10	T	*E. coli*	Pump Control (n=5)	8.5E+06	5.6E+05	7.0E+07	1.9E+07	1.7E+06	2.2E+05
				Treatment (n=5)	20.3	18.3	* 7756	7201	** 1013	495
			E. faecium	Pump Control (n=5)	4.1E+06	1.7E+05	n=4 2.4E+06	3.0E+05	1.7E+04	2416
				Treatment (n=5)	* 24.2	21.6	* 16.4	14.8	* 1.1	0.5

NA - Indicates no analysis conducted at 24 or 48 hrs.

NC - Not calculable - one or more replicate was greater than range of Quanti-tray (>2419.6 MPN/100mL) for all dilutions analyzed

**Indicates that one or more of the replicate values were < LOD; Half of LOD was used to calculate average of replicates and SEM*

***Indicates that one or more of the replicate values were < LOD and one or more replicate was greater than range of Quanti-tray (>2419.6 MPN/100mL); LOD = 1MPN/100mL and 2419.6 were used to calculate average of replicates and SEM*

4.3 ZOOPLANKTON

4.3.1 ADULT EUCYCLOPS

Water chemistry was measured in stock solutions prior to testing the Amglo Kemlite system with the zooplankton *Eucyclops sp*. Three stock values are listed for 0, 5, and 10 Hz because three different tanks of water were used during the zooplankton testing, as opposed to one tank being used for all three (0, 5, and 10 Hz) for the algae and microbe testing. Results of the values obtained are found in Table 14. As

measured during both the algae and bacteria effectiveness testing, temperatures were highest immediately after treatment (Time 0). The Amglo Kemlite system did not have substantial effect on pH, DO or conductivity values.

Table 14. Average (minimum, maximum) Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with Amglo Kemlite BWMS involving *Eucyclops sp.* in LW, LW-TMH and PCW at 25°C ± 3°C. Stock solutions are measured prior to the start of the test and do not have average values. NM = not measured.

Water Type	Lamp	Exposure		Time in Hrs.	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
LW	Q	Stock	Control	-	24.1	7.64	7.8	144.7	50.8	54.8
			5 Hz	-	24.2	7.64	6.8	145.1	52.0	56.8
			10 Hz	-	25.8	7.61	7.0	147.0	52.0	57.6
		PCW		0	24.5	7.70	6.6	155.9	58.8	59.2
				24-48	24.2 (23.9, 24.5)	8.11 (7.83, 8.18)	8.4 (8.4, 8.4)	179.1 (167.4, 192.6)	64.0	45.2
		Control		0	24.3	7.70	7.8	144.7	52.4	57.2
				24-48	23.6 (22.8, 24.1)	8.11 (7.98, 8.15)	8.4 (8.3, 8.5)	162.5 (150.4, 174.9)	62.0	66.0
		5 Hz Treatment		0	26.7	7.61	7.0	143.7	51.6	59.6
				24-48	24.1 (24.0, 24.2)	8.08 (8.04, 8.14)	8.3 (8.2, 8.4)	151.0 (148.7, 153.2)	54.0	59.6
		10 Hz Treatment		0	30.1	7.51	7.1	144.9	52.0	58.0
				24-48	24.1 (24.0, 24.2)	8.08 (8.02, 8.13)	8.4 (8.3, 8.4)	156.3 (152.9, 159.6)	59.6	60.8
LW	T	Stock	Control	-	25.4	7.57	8.9	130.3	48.0	51.2
			5 Hz	-	23.8	7.50	8.8	129.4	48.8	50.8
			10 Hz	-	24.1	7.48	8.4	128.7	48.8	52.4
		PCW		0	24.1	7.47	8.7	134.3	48.0	52.4
				24-48	23.8 (23.1, 24.3)	8.09 (7.87, 8.17)	8.6 (8.5, 8.7)	151.5 (142.2, 158.6)	58.0	56.0
		Control		0	25.3	7.63	8.8	131.3	54.4	49.2
				24-48	23.9 (23.1, 24.3)	8.08 (7.92, 8.17)	8.4 (8.4, 8.5)	146.4 (136.9, 151.3)	56.4	59.6
		5 Hz Treatment		0	26.6	7.64	8.7	126.8	47.6	53.2
				24-48	23.9 (23.7, 24.2)	8.04 (8.02, 8.07)	8.5 (8.4, 8.5)	142.5 (137.4, 147.5)	52.4	57.2
		10 Hz Treatment		0	28.9	7.70	8.2	127.7	48.8	51.2
				24-48	24.0 (23.6, 24.4)	8.06 (8.04, 8.08)	8.4 (8.3, 8.5)	144.9 (137.0, 152.8)	52.0	59.6
LW-TMH	T	Stock	Control	-	24.4	7.62	9.8	140.7	52.2	54.8
			5 Hz	-	24.0	7.62	9.6	140.1	48.6	54.0
			10 Hz	-	23.8	7.57	8.9	138.0	50.2	54.8
		PCW		0	24.0	7.81	8.7	141.1	50.2	52.4
				24-48	24.4 (23.9, 24.9)	8.10 (8.06, 8.15)	8.3 (8.2, 8.4)	156.6 (150.9, 166.2)	59.0	57.6
		Control		0	24.4	7.74	9.4	140.2	54.2	56.0
				24-48	24.0 (23.3, 24.3)	8.01 (7.95, 8.07)	8.3 (8.2, 8.4)	154.0 (144.5, 164.9)	55.8	58.4
		5 Hz Treatment		0	27.0	7.68	9.1	140.1	51.0	54.8
				24-48	24.1 (23.8, 24.4)	7.97 (7.83, 8.05)	8.3 (8.3, 8.3)	152.1 (142.4, 158.0)	56.6	60.0
		10 Hz Treatment		0	28.7	7.61	8.4	139.0	51.4	54.0
				24-48	24.4 (24.1, 24.7)	8.00 (7.95, 8.08)	8.2 (8.1, 8.2)	153.2 (148.8, 160.9)	53.8	60.0
	Q	Stock	Control	-	24.5	7.71	8.9	142.5	52.2	54.4

Water Type	Lamp	Exposure		Time in Hrs.	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
LW-TMH			5 Hz	-	24.6	7.69	9.5	138.6	48.6	52.4
			10 Hz	-	24.1	7.70	9.3	134.0	50.2	55.6
		PCW		0	24.5	7.83	9.1	143.9	52.2	53.6
				24-48	24.2 (23.3, 24.8)	8.09 (7.97, 8.13)	8.6 (8.5, 8.7)	158.1 (147.5, 165.0)	61.0	58.8
		Control		0	24.7	7.74	9.3	138.9	51.4	55.6
				24-48	23.6 (23.1, 24.1)	8.04 (7.96, 8.08)	8.6 (8.5, 8.6)	166.3 (149.9, 186.9)	63.1	63.6
		5 Hz Treatment		0	27.3	7.66	9.0	139.3	52.2	55.2
				24-48	23.9 (23.7, 24.1)	8.07 (8.03, 8.11)	8.5 (8.4, 8.5)	152.2 (146.5, 155.5)	60.6	62.4
		10 Hz Treatment		0	29.9	7.67	8.6	136.7	50.6	58.4
				24-48	23.9 (23.4, 24.4)	8.04 (7.98, 8.12)	8.3 (8.1, 8.5)	154.8 (151.0, 157.6)	58.2	64.0

Water quality was measured in stock solutions prior to testing the Amglo Kemlite system with the zooplankton *Eucyclops sp*. Three stock values are listed for 0, 5, and 10 Hz because three different tanks of water were used during the zooplankton testing, as opposed to one tank being used for all three (0, 5, and 10 Hz) for the algae and microbe testing. The measured values are presented in Table 15. Percent transmittance was not within the acceptance range listed in the TQAP during the tests in LW with Lamp Q or in either test in LW-TMH (Table 5). NPOC was outside of the acceptance range in the LW-TMH test with Lamp T (Table 5). POM was outside of the acceptance range in both LW-TMH tests (Table 5). The acceptance limits were revised following the testing to be comprised of ranges measured historically at LSRI during bench scale testing. Following the revision of acceptance limits, all values were within the acceptance ranges.

Table 15. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the Amglo Kemlite BWMS involving *Eucyclops sp*. in LW and LW-TMH at 25°C ±3°C.

Lamp	Stock Water Type		Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered/Unfiltered (%)	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
Q	PCW (LW)		<1.25	100.1 / 100.3	<0.69	<0.69	<1.25	<1.25
	LW	0 Hz	<1.25	100.6 / 100.3	<0.69	0.73[J]	<1.25	<1.25
		5 Hz	<1.25	100.6 / 100.4	<0.69	<0.69	<1.25	<1.25
		10 Hz	<1.25	100.4 / 100.3	<0.69	<0.69	<1.25	<1.25
T	PCW (LW)		<1.25	99.3 / 99.2	<0.69	<0.69	<1.25	<1.25
	LW	0 Hz	<1.25	99.2 / 99.2	<0.69	<0.69	<1.25	<1.25
		5 Hz	<1.25	99.1 / 99.2	<0.69	<0.69	<1.25	<1.25
		10 Hz	<1.25	99.3 / 99.2	<0.69	<0.69	<1.25	<1.25
T	PCW (LW)		<1.25	99.0 / 99.1	<0.69	<0.69	<1.25	<1.25
	LW-TMH	0 Hz	17.9	27.8 / 25.3	7.8	5.7	6.9	11.0
		5 Hz	20.5	29.7 / 27.0	8.0	5.1	9.9	10.6
		10 Hz	18.0	30.0 / 27.4	11.6	5.4	7.9	10.1
Q	PCW (LW)		<1.25	99.0 / 98.9	<0.69	<0.69	<1.25	<1.25
	LW-TMH	0 Hz	20.4	29.7 / 26.9	8.2	5.0	7.5	12.9
		5 Hz	22.0	29.6 / 26.8	7.6	5.1	8.9	13.1
		10 Hz	20.3	29.9 / 27.0	8.0	4.6	8.8	11.5

J = Value between limit of detection (0.69 mg/L) and limit of quantitation (2.3 mg/L).

Table 16 displays the treatment effect of the Amglo Kemlite system on *Eucyclops sp*. Low percent mortality in PCW indicates the organisms used for testing were healthy. Pump mortality in controls ranged from a low of 16% in LW to a high of 45.3% in LW-TMH; treatment mortality is the combination of mortality resulting from both the pump and the treatment process. In LW, both Lamps Q and T at 10 Hz

had mortality greater than 98% by 48 hrs. Mortality was lower at 5 Hz with Lamp Q, but still exceeded 89%. In LW-TMH, the effectiveness of the treatment was markedly lower, with percent mortalities being less than 60% for both lamps and both frequencies. The highest mortality in LW-TMH was observed with Lamp T at 10 Hz (58.7%). Comparing the percent mortality immediately following treatment to the percent mortality 48 hrs. after treatment indicates that there was no delayed mortality. The same trend follows for organisms exposed to the pump only; no delayed mortality occurred over the 48-hour holding time. These results suggest that the mode of action of the treatment process occurs instantaneously during flow-through exposure to the light, and there does not appear to be mortal injury to the organisms that survive the treatment process.

Table 16. Treatment Effect of Amglo Kemlite BWMS on *Eucyclops* sp. No mortality values are reported for PCW samples because they do not go through the treatment system and are used to ensure that the organisms were healthy.

Test Water	Lamp	Treatment/ Frequency	Percent Mortality Immediately Following Treatment	Percent Mortality 48 hrs. after Treatment
Lab Water	Lamp Q	PCW	-	0.7
		Control	16.0	16.0
		5 Hz	94.0	94.0
		10 Hz	97.3	98.7
Lab Water	Lamp T	PCW	-	0.0
		Control	39.3	39.3
		5 Hz	88.0	88.7
		10 Hz	98.7	99.3
LW-TMH	Lamp Q	PCW	-	0.7
		Control	25.3	26.0
		5 Hz	44.7	48.0
		10 Hz	50.7	52.7
LW-TMH	Lamp T	PCW	-	0.0
		Control	44.7	45.3
		5 Hz	50.7	51.3
		10 Hz	54.7	58.7

4.3.2 *DAPHNIA MAGNA* EPHIPPIA

Water chemistry was measured in stock solutions prior to testing the Amglo Kemlite system with the zooplankton *D. magna* ephippia. Three stock values are listed for 0, 5, and 10 Hz because three different tanks of water were used during the zooplankton testing, as opposed to one tank being used for all three (0, 5, and 10 Hz) for the algae and microbe testing. Results of the values obtained are found in Table 17. As observed in previous testing, Temperatures were highest immediately after treatment (Time 0). The Amglo Kemlite system did not have substantial effect on pH, DO or conductivity values.

Table 17. Average (minimum, maximum) Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with Amglo Kemlite BWMS involving *D. magna* ephippia in LW, LW-TMH and PCW at 25°C ± 3°C. Stock solutions are measured prior to the start of the test and do not have average values. NM = not measured.

Water Type	Lamp	Exposure		Time in Hours	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
LW	Q	Stock	Control	-	24.1	7.64	7.8	144.7	50.8	54.8
			5 Hz	-	24.2	7.64	6.8	145.1	52.0	56.8
			10 Hz	-	25.8	7.61	7.0	147.0	52.0	57.6
		PCW		0	24.5	7.70	6.6	155.9	58.8	59.2
				24-48	24.2 (23.9, 24.5)	8.11 (7.83, 8.18)	8.4 (8.4, 8.4)	179.1 (167.4, 192.6)	64.0	45.2
		Control		0	24.3	7.70	7.8	144.7	52.4	57.2
				24-48	23.6 (22.8, 24.1)	8.11 (7.98, 8.15)	8.4 (8.3, 8.5)	162.5 (150.4, 174.9)	62.0	66.0
		5 Hz Treatment		0	26.7	7.61	7.0	143.7	51.6	59.6
				24-48	24.1 (24.0, 24.2)	8.08 (8.04, 8.14)	8.3 (8.2, 8.4)	151.0 (148.7, 153.2)	54.0	59.6
		10 Hz Treatment		0	30.1	7.51	7.1	144.9	52.0	58.0
				24-48	24.1 (24.0, 24.2)	8.08 (8.02, 8.13)	8.4 (8.3, 8.4)	156.3 (152.9, 159.6)	59.6	60.8
LW	T	Stock	Control	-	25.4	7.57	8.9	130.3	48.0	51.2
			5 Hz	-	23.8	7.50	8.8	129.4	48.8	50.8
			10 Hz	-	24.1	7.48	8.4	128.7	48.8	52.4
		PCW		0	24.1	7.47	8.7	134.3	48.0	52.4
				24-48	23.8 (23.1, 24.3)	8.09 (7.87, 8.17)	8.6 (8.5, 8.7)	151.5 (142.2, 158.6)	58.0	56.0
		Control		0	25.3	7.63	8.8	131.3	54.4	49.2
				24-48	23.9 (23.1, 24.3)	8.08 (7.92, 8.17)	8.4 (8.4, 8.5)	146.4 (136.9, 151.3)	56.4	59.6
		5 Hz Treatment		0	26.6	7.64	8.7	126.8	47.6	53.2
				24-48	23.9 (23.7, 24.2)	8.04 (8.02, 8.07)	8.5 (8.4, 8.5)	142.5 (137.4, 147.5)	52.4	57.2
		10 Hz Treatment		0	28.9	7.70	8.2	127.7	48.8	51.2
				24-48	24.0 (23.6, 24.4)	8.06 (8.04, 8.08)	8.4 (8.3, 8.5)	144.9 (137.0, 152.8)	52.0	59.6
LW-TMH	T	Stock	Control	-	24.4	7.62	9.8	140.7	52.2	54.8
			5 Hz	-	24.0	7.62	9.6	140.1	48.6	54.0
			10 Hz	-	23.8	7.57	8.9	138.0	50.2	54.8
		PCW		0	24.0	7.81	8.7	141.1	50.2	52.4
				24-48	24.4 (23.9, 24.9)	8.10 (8.06, 8.15)	8.3 (8.2, 8.4)	156.6 (150.9, 166.2)	59.0	57.6
		Control		0	24.4	7.74	9.4	140.2	54.2	56.0
				24-48	24.0 (23.3, 24.3)	8.01 (7.95, 8.07)	8.3 (8.2, 8.4)	154.0 (144.5, 164.9)	55.8	58.4
		5 Hz Treatment		0	27.0	7.68	9.1	140.1	51.0	54.8
				24-48	24.1 (23.8, 24.4)	7.97 (7.83, 8.05)	8.3 (8.3, 8.3)	152.1 (142.4, 158.0)	56.6	60.0
		10 Hz Treatment		0	28.7	7.61	8.4	139.0	51.4	54.0
				24-48	24.4 (24.1, 24.7)	8.00 (7.95, 8.08)	8.2 (8.1, 8.2)	153.2 (148.8, 160.9)	53.8	60.0
LW-TMH	Q	Stock	Control	-	24.5	7.71	8.9	142.5	52.2	54.4
			5 Hz	-	24.6	7.69	9.5	138.6	48.6	52.4
			10 Hz	-	24.1	7.70	9.3	134.0	50.2	55.6
		PCW		0	24.5	7.83	9.1	143.9	52.2	53.6
				24-48	24.2 (23.3, 24.8)	8.09 (7.97, 8.13)	8.6 (8.5, 8.7)	158.1 (147.5, 165.0)	61.0	58.8
		Control		0	24.7	7.74	9.3	138.9	51.4	55.6

Water Type	Lamp	Exposure	Time in Hours	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
			24-48	23.6 (23.1, 24.1)	8.04 (7.96, 8.08)	8.6 (8.5, 8.6)	166.3 (149.9, 186.9)	63.1	63.6
		5 Hz Treatment	0	27.3	7.66	9.0	139.3	52.2	55.2
			24-48	23.9 (23.7, 24.1)	8.07 (8.03, 8.11)	8.5 (8.4, 8.5)	152.2 (146.5, 155.5)	60.6	62.4
		10 Hz Treatment	0	29.9	7.67	8.6	136.7	50.6	58.4
			24-48	23.9 (23.4, 24.4)	8.04 (7.98, 8.12)	8.3 (8.1, 8.5)	154.8 (151.0, 157.6)	58.2	64.0

Water quality was measured in stock solutions prior to testing the Amglo Kemlite system with the zooplankton *D. magna* ephippia. Three stock values are listed for 0, 5, and 10 Hz because three different volumes of water were used during the zooplankton testing, as opposed to one tank being used for all three (0, 5, and 10 Hz) for the algae and microbe testing. The measured values are presented in Table 18. Percent transmittance, DOC and POM were not within the acceptance range listed in the TQAP during either test in LW-TMH. The acceptance limits were revised following the testing to be comprised of ranges measured historically at LSRI during bench scale testing. Following the revision, all values were within the acceptance ranges.

Table 18. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the Amglo Kemlite BWMS involving *D. magna* ephippia in LW and LW-TMH at 25°C ±3°C.

Lamp	Stock Water Type		Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered/Unfiltered (%)	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
Q	PCW (MHRW)		<1.25	99.6 / 99.4	<0.69	<0.69	<1.25	<1.25
	LW	0 Hz	<1.25	99.3 / 99.4	<0.69	<0.69	<1.25	<1.25
		5 Hz	<1.25	99.3 / 99.1	<0.69	<0.69	<1.25	<1.25
		10 Hz	<1.25	98.8 / 99.0	<0.69	<0.69	<1.25	<1.25
T	PCW (MHRW)		<1.25	98.8 / 99.6	<0.69	<0.69	<1.25	<1.25
	LW	0 Hz	<1.25	98.9 / 99.6	<0.69	<0.69	<1.25	<1.25
		5 Hz	<1.25	99.2 / 99.6	<0.69	<0.69	<1.25	<1.25
		10 Hz	<1.25	98.9 / 98.9	<0.69	<0.69	<1.25	<1.25
Q	PCW (MHRW)		<1.25	99.6 / 99.6	<0.69	<0.69	<1.25	<1.25
	LW-TMH	0 Hz	21.2	28.1 / 25.6	7.4	4.6	8.9	12.3
		5 Hz	24.9	27.8 / 25.1	9.2	6.3	10.8	14.1
		10 Hz	21.8	28.8 / 26.3	8.7	5.9	9.1	12.7
T	PCW (MHRW)		<1.25	99.7 / 99.8	<0.69	<0.69	<1.25	<1.25
	LW-TMH	0 Hz	19.8	27.9 / 25.3	7.1	6.1	8.3	11.5
		5 Hz	18.8	28.0 / 25.3	8.0	5.8	7.6	11.2
		10 Hz	18.4	27.8 / 25.2	8.3	5.2	7.4	11.0

Hatch rate of *D. magna* ephippia that were passed through the Amglo Kemlite BWMS, held for 48 hours in treated water and then transferred to optimal hatching conditions for 72 hours are presented in Table 19. Hatch rates of the ephippia in PCW ranged from 9.7% to 15.3% across water types. Control hatch rates ranged from 10.1 to 16.7% in LW and LW-TMH combined. There was little effect of the Amglo Kemlite system on the hatch rates of ephippia, with the exception of amended water treated at 10 Hz with Lamp Q where there was a statistically significant (p<0.05) decrease in hatch rate relative to the control analyzed with that batch of samples. With the exclusion of Lamp Q at 10 Hz in LW-TMH, the average hatch rate of ephippia exposed to the Amglo Kemlite system was very similar to the PCW and pump control and ranged from 10.0 to 17.1%.

Table 19. Treatment Effect of Amglo Kemlite BWMS on *D. magna* ephippia. PCW samples are not exposed to the treatment system and are used to ensure that the organisms were healthy.

Test Water	Lamp	Treatment/ Frequency	Ephippia Recovered	Average Percent Hatch of Ephippia
Lab Water	Lamp Q	PCW	150/150	14.0 ± 7%
		Control	141/150	13.3 ± 8%
		5 Hz	141/150	15.9 ± 12%
		10 Hz	150/150	17.3 ± 9%
Lab Water	Lamp T	PCW	150/150	15.3 ± 9%
		Control	150/150	16.7 ± 4%
		5 Hz	146/150	15.6 ± 10%
		10 Hz	142/150	10.0 ± 7%
LW-TMH	Lamp Q	PCW	150/150	12.3 ± 9%
		Control	140/150	10.1 ± 6%
		5 Hz	143/150	14.3 ± 6%
		10 Hz	146/150	2.6 ± 4%*
LW-TMH	Lamp T	PCW	150/150	9.7 ± 9%
		Control	150/150	12.7 ± 11%
		5 Hz	129/150	14.4 ± 6%
		10 Hz	141/150	11.7 ± 7%

* Indicates a significant difference from the control (p=0.015)

4.4 STAINING VS. MPN METHOD WITH AMGLO KEMLITE AND UV WATER PURIFICATION SYSTEMS

Water chemistry measurements taken in the LW and LW-TMH tests with Amglo Kemlite (Lamp Q, 10 Hz) and the UV water purification system are shown in Table 20. The water chemistry values were within the ranges specified in the TQAP prior to test initiation. The temperature of the water treated by the Amglo Kemlite BWMS was increased relative to the control, which was observed throughout Amglo Kemlite testing. There was no impact on water temperature observed following treatment with the UV water purification system. The other parameters measured were not affected by the treatment systems.

Table 20. Average (minimum, maximum) Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with Amglo Kemlite BWMS and UV water purification system involving *S. capricornutum* in LW, LW-TMH and PCW at 25°C ± 3°C. Stock solutions are measured prior to the start of the test and do not have average values. NM = not measured.

Water Type	Lamp	Exposure	Time in Hrs.	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
LW	Q	Stock	-	24.7	7.59	9.3	139.4	50.6	52.4
		PCW	0	24.3	7.54	9.0	142.4	52.2	54.4
			24-48	25.2 (25.1, 25.3)	8.08 (8.04, 8.11)	8.4 (8.3, 8.4)	144.4 (143.5, 144.8)	NM	NM
		Control	0	24.5 (24.4, 24.7)	7.82 (7.78, 7.86)	8.9 (8.7, 9.3)	139.2 (132.5, 149.7)	NM	NM
			24-48	25.1 (25.0, 25.2)	8.09 (8.04, 8.12)	8.3 (8.3, 8.4)	138.8 (137.2, 139.6)	NM	NM
		AK Treatment	0	29.7 (29.7, 29.8)	7.43 (7.00, 7.80)	9.0 (8.9, 9.1)	138.3 (135.9, 139.7)	NM	NM
			24-48	25.1	8.11	8.3	138.8	NM	NM

				(25.1, 25.1)	(8.05, 8.14)	(8.3, 8.4)	(137.0, 139.7)		
		UV Treatment	0	24.6 (24.6, 24.6)	7.83 (7.83, 7.84)	8.9 (8.8, 8.9)	135.9 (135.6, 136.2)	NM	NM
			24-48	25.1 (25.1, 25.1)	8.10 (8.04, 8.12)	8.3 (8.3, 8.4)	138.4 (137.1, 139.5)	NM	NM
LW-TMH	Q	Stock	-	25.0	7.65	9.4	154.8	51.8	56.4
		PCW	0	24.7	7.4	8.9	140.0	52.6	52.8
			24-48	25.3 (25.0, 25.4)	8.03 (7.93, 8.08)	8.3 (8.1, 8.4)	143.1 (142.4, 144.6)	NM	NM
		Control	0	24.7 (24.6, 24.8)	7.82 (7.75, 7.86)	8.9 (8.7, 9.2)	139.2 (138.8, 140.1)	NM	NM
			24-48	25.3 (25.1, 25.4)	8.08 (8.03, 8.10)	8.2 (8.1, 8.4)	138.8 (137.4, 139.4)	NM	NM
		AK Treatment	0	30.1 (30.0, 30.2)	7.74 (7.73, 7.75)	8.7 (8.6, 8.8)	130.9 (122.1, 139.0)	NM	NM
			24-48	25.3 (25.0, 25.4)	8.05 (8.04, 8.06)	8.3 (8.1, 8.4)	138.6 (137.7, 139.2)	NM	NM
		UV Treatment	0	24.8 (24.8, 24.9)	7.84 (7.82, 7.86)	8.7 (8.6, 8.8)	138.7 (137.8, 139.1)	NM	NM
			24-48	25.3 (24.9, 25.4)	8.08 (8.03, 8.11)	8.3 (8.1, 8.4)	137.2 (136.7, 138.5)	NM	NM

Water quality measurements taken during testing taken in the LW and LW-TMH tests with Amglo Kemlite (Lamp Q, 10 Hz) and UV water purification system are shown in Table 21. The percent transmittance and POM of the water used in the LW-TMH testing were outside of the acceptance range listed in the TQAP (Table 5). The acceptance limits were revised following the testing to be comprised of ranges measured historically at LSRI during bench scale testing. Following the revision, all values were within the acceptance ranges.

Table 21. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the Amglo Kemlite BWMS and UV Water Purification System involving *S. capricornutum* in LW and LW-TMH at 25°C ±3°C.

Stock Solution Water Type	Lamp	Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered/Unfiltered (%)	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
PCW (LW)	Q	<1.25	98.7 / 99.0	<0.69	<0.69	<1.25	<1.25
LW		<1.25	98.9 / 98.9	<0.69	<0.69	<1.25	<1.25
PCW (LW)	Q	<1.25	93.7 / 93.5	0.83[J]	0.95[J]	<1.25	<1.25
LW-TMH		20.2	29.1 / 27.0	7.1	5.0	7.8	12.4

J = Value between limit of detection (0.69 mg/L) and limit of quantitation (2.3 mg/L).

As in earlier tests with Amglo Kemlite utilizing vital stain and epifluorescence microscopy, mortality in LW was 100% at time 0 (Table 22). In LW-TMH, the difficulty in determining whether cells were alive or dead with the staining method remained. An estimate of 92.3% dead in LW-TMH at 48 hrs. was made.

Table 22. Percent Mortality (± Standard Deviation) of *S. capricornutum* during Dose Effectiveness Testing with the Amglo Kemlite BWMS (Lamp Q, 10 Hz) as Determined using the Vital Stain Method of Analysis. NC=not counted.

Water Type	Exposure	% Mortality		
		0 hrs.	24 hrs.	48 hrs.
Lab Water	Performance Control	1.04 ± 0.77 n=4	0.85 n=1	2.08 ± 0.59 n=3
	Control	0.90 ± 1.05 n=4	0 n=1	1.14 ± 0.32 n=3
	Amglo Kemlite Treatment	100 ± 0 n=4	100 n=1	NC
LW-TMH	Performance Control	0.83 ± 1.12 n=4	0 n=1	8.31 ± 10.58 n=3
	Control	0 ± 0 n=4	0 n=1	0 ± 0 n=3
	Amglo Kemlite Treatment	40.07 ± 16.12 n=4	73.9 n=1	92.3 ± 2.91 n=3

The mortality with the UV water purification system was not as immediate as with the Amglo Kemlite system. At time 0 in LW, there was negligible mortality but by 48 hrs. mortality was nearly 70%. In LW-TMH, there was 0% mortality at 48 hrs. with the algae exposed to the UV water purification system (Table 23). However, the UV water purification system was not designed to be used in low transmittance water and the indicator light on the system indicated it was not operating optimally.

Table 23. Percent Mortality (± Standard Deviation) of *S. capricornutum* during Dose Effectiveness Testing with the UV water purification system as Determined using the Vital Stain Method of Analysis. NC=not counted.

Water Type	Exposure	% Mortality		
		0 hrs.	24 hrs.	48 hrs.
Lab Water	Performance Control	1.04 ± 0.77 n=4	0.85 n=1	2.08 ± 0.59 n=3
	Control	0.90 ± 1.05 n=4	0 n=1	1.14 ± 0.32 n=3
	UV Water Purification Treatment	0.44 ± 0.50 n=3	44.7 n=1	69.85 ± 6.04 n=3
LW-TMH	Performance Control	0.83 ± 1.12 n=4	0 n=1	8.31 ± 10.58 n=3
	Control	0 ± 0 n=4	0 n=1	0 ± 0 n=3
	UV Water Purification Treatment	0.21 ± 0.42 n=3	1.64 n=1	0 ± 0 n=3

The MPN results of Amglo Kemlite BWMS treatment in LW corroborated the microscopic count method (Table 24), with no positive tubes present after the 14 day incubation period. In LW-TMH, the Amglo Kemlite system showed reduced efficacy. The MPN for the Amglo Kemlite system was >160 (Figure 8). A more accurate number was not able to be determined because when doing the dilution series for the MPN method the assumption was made that the system would be effective. Based on this assumption the dilution series for 10 cells/mL was utilized.

Table 24. *S. capricornutum* MPN determined after Dose Effectiveness Testing with the Amglo Kemlite BWMS (Lamp Q, 10 Hz).

Water Type	Exposure	MPN	95% Confidence Limits	
			Lower	Upper
Lab Water	Control	920,000	296,000	2,880,000
	Amglo Kemlite Treatment	0	0	0
LW-TMH	Control	476,667	140,000	1,600,000
	Amglo Kemlite Treatment	>160	61	>490

For the algae treated with the UV water purification system in LW, the MPN value was 0.57 indicating that very few of the algae treated with the UV system were viable (Table 25). The efficacy of the UV water purification system was reduced in LW-TMH. The MPN for the UV water purification system in LW-TMH was >490 (Figure 7). A more accurate number was not able to be determined because when doing the dilution series for the MPN method the assumption was made that the system would be effective. Based on this assumption the dilution series for 10 cells/mL was utilized. All of the tubes were positive in the UV water purification treatment, resulting in an infinite result where the MPN cannot be determined.

Table 25. *S. capricornutum* MPN determined after Dose Effectiveness Testing with the UV water purification system.

Water Type	Exposure	MPN	95% Confidence Limits	
			Lower	Upper
Lab Water	Control	920,000	296,000	2,880,000
	UV water purification Treatment	0.57	0.165	2.23
LW-TMH	Control	476,667	140,000	1,600,000
	UV water purification Treatment	>490	-	-

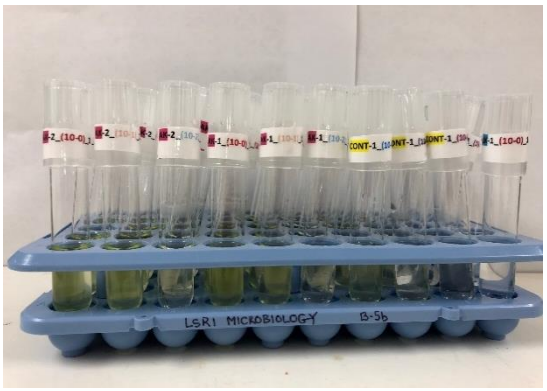

Figure 7. *S. capricornutum* tubes MPN dilution tubes 14 days after treatment with the Amglo Kemlite BWMS in LW-TMH.

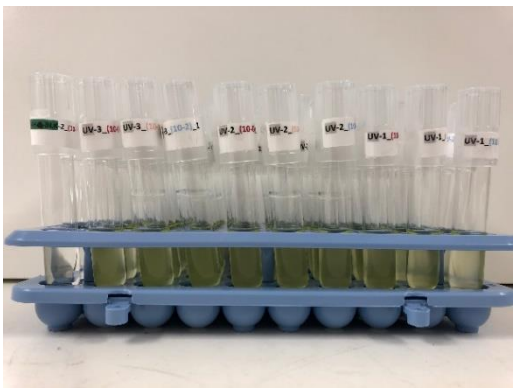

Figure 8. *S. capricornutum* MPN dilution tubes 14 days after treatment with the UV water purification system in LW-TMH.

5 QUALITY ASSURANCE/QUALITY CONTROL – DATA QUALITY OBJECTIVES

5.1 WATER CHEMISTRY AND WATER QUALITY

The data quality objectives (DQO) for water quality and chemistry analyses conducted during the evaluation of the Amglo Kemlite BWMS are summarized in Table 26. Duplicate samples were analyzed on 13.7% of the stock solutions analyzed for TSS, POM, NPOC, DOC and %T. Relative Percent Difference of duplicates for those parameters was less than 20%, therefore meeting the DQO for TSS, POM, NPOC, DOC and %T. Hardness was measured in duplicate on 18.5% of samples while alkalinity was measured in duplicate on 19.4% of the samples. The DQOs were met on both hardness and alkalinity measurements. The DQOs were also met on filter blanks analyzed for TSS/POM, NPOC, DOC and %T. To measure accuracy, NPOC/DOC spikes were prepared on the stock solutions for 13.7% of the samples and reference standards were analyzed for NPOC/DOC, TSS, POM, hardness and alkalinity. The spike recovery for NPOC/DOC and the percent difference between the nominal and measured concentrations met the DQO for accuracy. One hundred percent completeness was achieved for analysis of TSS, %T, NPOC/DOC, and alkalinity. Hardness measurements achieved 98% completeness.

Table 26. Data Quality Objectives (DQOs), Criteria, and Performance Measurement Results from Water Chemistry and Water Quality Analyses Conducted during Amglo Kemlite Dose Effectiveness testing.

Data Quality Indicator	Evaluation Process/Performance Measurement	Data Quality Objective	Performance Measurement Result	
Precision	Samples (10%) were collected and analyzed in duplicate with performance measured by average relative percent difference (RPD).	< 20% average RPD	Percentage of Samples Collected and Analyzed in Duplicate: TSS/POM: 13.7% %T: 13.7% NPOC/DOC: 13.7% Hardness: 18.5% Alkalinity: 19.4%	TSS: 9.7% ± 9.1% %TF: 0.41% ± 0.66% %TU: 0.19% ± 0.19% NPOC: 10.1% ± 12.3% DOC: 12.4% ± 12.3% POM: 16.8% ± 16.7% Hardness: 3.6% ± 3.0% Alkalinity: 10.1% ± 31.3%
Bias, Filter Blanks	%T filter blanks were prepared by filtering deionized water samples (one per analysis date)	> 98% average %T	Number of %T Filter Blanks Analyzed: 18	Filter blank (%T): 99.1% ± 2.3%
	TSS/POM filter blanks were prepared by filtering deionized water samples (one per analysis date) and then drying, weighing, ashing and weighing the filter	< 0.63 mg/L average TSS/POM	Number of TSS/POM Filter Blanks Analyzed: 18	Filter blank (TSS): < 0.63 mg/L ± 0
				Filter blank (POM): < 0.63 mg/L ± 0
	NPOC blanks were prepared by acidifying a volume of deionized water to 0.2% with concentrated hydrochloric acid	< 0.69 mg/L average NPOC	Number of NPOC Blanks Analyzed: 43	Blank (NPOC): < 0.69
	DOC filter blanks were prepared by filtering deionized water samples (one per analysis date)	< 0.69 mg/L average DOC	Number of DOC Filter Blanks Analyzed: 18	Filter blank (DOC): < 0.69
Accuracy	Samples (10%) were spiked with a total organic carbon spiking solution with performance measured by average spike-recovery (SPR).	75% - 125% average SPR	Percentage of NPOC/DOC Samples Spiked: 13.7%	NPOC/DOC: 99.7% ± 5.9%
	Performance was measured by average percent difference (%D) between all measured and nominal reference standard values.	< 20% average D	Percentage of Analysis Days Containing a Reference Standard: TSS: 94.4% POM: 16.7% NPOC: 100%	TSS: 6.2% ± 5.0% D POM: 10.6% ± 14.0% D

Data Quality Indicator	Evaluation Process/Performance Measurement	Data Quality Objective	Performance Measurement Result	
				NPOC Reference Standard 6.2% ± 4.3% D NPOC 10 mg/L Standard: 6.4% ± 2.2% D
	A hardness/alkalinity reference standard was analyzed once per bench scale test type per analyst. Performance was measured by ensuring the titrated value was within the acceptance range for the standard.	Within acceptance range (lot dependent)	Number of Analysis Days Containing a Reference Standard: 3 Number of test types: 1	Hardness: DQO met 100% of the time Alkalinity: DQO met 100% of the time
Representativeness	All samples were collected, handled, and analyzed in the same manner.	Not Applicable – Qualitative.	All water chemistry/quality samples were collected, handled, transported and analyzed in the same manner using the appropriate SOPs.	
Comparability	Routine procedures were conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The SOPs listed in the methods and references section were used for all water chemistry and water quality analyses.	
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meeting DQOs) water chemistry samples measured out of the total number of water chemistry samples collected. Performance is measured by percent completeness (%C).	> 90% C	TSS: 100%	
			%T, Filtered: 100%	
			%T, Unfiltered: 100%	
			NPOC: 100%	
			DOC: 100%	
			Hardness: 98%	
			Alkalinity: 100%	
Sensitivity	The limit of detection (LOD) and limit of quantification (LOQ) for each analyte and analytical method utilized was determined annually unless a reporting limit was used based on the amount filtered as was the case with TSS/POM.	Not Applicable	TSS/POM RL: 1.25 mg/L based on filtering 800 mL of sample	
			NPOC/DOC LOD: 0.69 mg/L; NPOC/DOC LOQ: 2.3 mg/L Determined 25 May 2017	

5.2 ALGAE TESTING

During *S. capricornutum* testing, data quality was measured by analyzing a minimum of 10% of samples in duplicate and by having a second individual conduct quality assurance counts on a minimum of 10% samples. For all testing with *S. capricornutum*, the minimum number of duplicate and quality assurance samples were met or exceeded (Table 27). For the three relative percent difference values that were greater than 50%, the cause was that in either the duplicate count or QA count one dead cell was present in analysis and not in the other. During the Lamp Q, *S. capricornutum*, LW-TMH testing there was one duplicate sample with poor RPD for the live counts. An extra duplicate analysis was done during that test to compensate for the sample that didn't meet the DQOs. This resulted in 14% of the samples being analyzed in duplicate rather than the minimum of 10%.

Table 27. Average Relative Percent Difference (RPD) for *S. capricornutum* counts conducted during Amglo Kemlite bench-scale testing.

Test Date	Duplicate or Quality Assurance Count	Percent of Samples with QA counts	DQO	Relative Percent Difference	
				Live	Dead
1 November 2017	Duplicate	10%	RPD ≤ 20%, when greater than 10	6.1	67.5
	Quality Assurance	10%		2.2	52.8
15 November 2017	Duplicate	12%		3.1	4.1

Test Date	Duplicate or Quality Assurance Count	Percent of Samples with QA counts	DQO	Relative Percent Difference	
				Live	Dead
	Quality Assurance	10%	cells of live/dead are counted	0.7	0.8
13 December 2017	Duplicate	11%		6.7	1.4
	Quality Assurance	11%		6.3	0.0
3 January 2018	Duplicate	14%		24.6	9.2
	Quality Assurance	11%		2.8	0.6
16 January 2018	Duplicate	11%		11.1	74.9
	Quality Assurance	14%		2.5	9.8
29 January 2018	Duplicate	14%		11.3	3.3
	Quality Assurance	11%		3.8	0.0

5.3 MICROBE TESTING

Data quality objectives for precision, bias and accuracy and completeness (i.e., method blanks, duplicate agreements, and quantitative positive and negative controls) were within acceptable limits for microbe testing (Table 28).

Table 28. Data Quality Objective summary for Amglo Kemlite bench-scale tests using *E. coli* and *E. faecium*.

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Precision	Samples (10%) are analyzed in duplicate – with performance measured by average relative percent difference (RPD) of all duplicate analyses.	<30% average RPD	***E. coli:*** 36 of 327 (11.0%) samples were analyzed in duplicate; Average RPD = 29.7% (±40.2%)
			E. faecium: 45 of 327 (13.8%) samples were analyzed in duplicate; RPD =24.7.8% (±26.2%)
Bias, Operator	Samples (10%) are counted by two separate analysts with performance measured by average relative percent difference (RPD) of all second counts.	<20% average RPD	***E. coli*:** 129 QA Counts; RPD =2.1% (±16.5%)
			E. faecium: 121 QA Counts; RPD =0% (±0%)
Bias, Positive Control	Qualitative positive control samples (American Type Culture Collection) are analyzed on each analysis date or IDEXX-QC samples are analyzed as a quantitative positive control at least once per ballast water treatment system test.	Results must be greater than the limit of detection.	***E. coli:*** Qualitative Positive controls >1 MPN/100 mL n=5
			E. faecium: Qualitative Positive controls >1 MPN/100 mL n=5
Bias, Negative Control	Qualitative negative control samples (American Type Culture Collection) are analyzed on each analysis date or IDEXX-QC samples are analyzed as a negative control at least once per ballast water treatment system test.	Results must be less than the limit of detection.	***E. coli:*** All Qualitative Negative controls <1 MPN/100 mL, n=2
			E. faecium: All Qualitative Negative controls <1 MPN/100 mL, n=1

Bias, Method	Sterilized water (similar matrix sample) analyzed using same method as samples on each analysis date.	Results must be less than the limit of detection.	***E. coli:*** All method blanks <1 MPN/100 mL, n=36
			E. faecium: All method blanks <1 MPN/100 mL, n=36
Bias, Diluent Blank	One per analysis day, diluent (e.g., sterile deionized water) blank run analyzed using same media as samples	Results must be less than the limit of detection.	***E. coli:*** All method blanks <1 MPN/100 mL, n=12
			E. faecium: All method blanks <1 MPN/100 mL, n=12
Accuracy	IDEXX-QC samples are analyzed as a quantitative positive control at least once per ballast water treatment system test.	*E. coli:*	***E. coli:*** All quantitative analyses within IDEXX acceptance ranges (n=3)
		7-102 MPN/100 mL	09 Jan. 2018; 19.9 MPN/100 mL
			17 Jan. 2018; 68 MPN/100 mL
			23 Jan. 2018; 47.4 MPN/100 mL
		E. faecalis:	***E. faecalis:*** All quantitative analyses within IDEXX acceptance ranges (n=3)
		74-210 MPN/100 mL	09 Jan. 2018; 115.3MPN/100 mL
			17 Jan. 2018; 172.3 MPN/100 mL
		58-212 MPN/100 mL	23 Jan. 2018; 125.9 MPN/100 mL
Representativeness	All samples are collected, handled, and analyzed in the same manner.	Not Applicable – Qualitative.	All microbial samples were collected, handled, and analyzed in the same manner (using the appropriate LSRI/GWRC SOPs).
Comparability	Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The LSRI/GWRC SOPs listed in Section 2.8 were used for all microbial analyses conducted.
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meet DQOs) microbial samples measured out of the total number of microbial samples collected. Performance is measured by percent completeness (%C).	>90% C.	***E. coli*****:** 403 of 429 samples (Control, Treatment, PCW, QA) = 94% Completeness
			E. faecium: 416 of 434 samples (Control, Treatment, PCW, QA analyses and QA Counts) = 96% Completeness
Sensitivity	The limit of detection (LOD) for the analytical method used is reported.	Dependent upon the analytical technique	***E. coli*** **LOD:** <1 MPN/100 mL
			E. faecium: **LOD:** <1 MPN/100 mL

		used. Adjusted for volume used.	

5.4 ZOOPLANKTON TESTING

During dose effectiveness testing with *D. magna* ephippia data quality was ensured by having a second individual conduct counts on a minimum of 10% of the samples. This minimum was exceeded in all of the ephippia tests (Table 29). The RPD met the DQO for all samples in the ephippia tests.

Table 29. Average Relative Percent Difference (RPD) of samples counted for *D. magna* ephippia tests done during Amglo Kemlite bench-scale tests.

Test Date	Percent of Samples with QA counts	DQO	Relative Percent Difference between counts
9 October 2018	81%	RPD ≤ 10%	0%
10 October 2018	100%		0%
13 November 2018	73%		0%
28 November 2018	73%		0.2%

During dose effectiveness testing with *Eucyclops sp.* data quality was ensured by having a second individual conduct counts on a minimum of 10% of the samples. This minimum was exceeded in all of the *Eucyclops sp*. Tests (Table 30). The RPD was 0% for all of the samples counted in duplicate.

Table 30. Average Relative Percent Difference (RPD) of samples counted for *Eucyclops* spp. tests done during Amglo Kemlite bench-scale tests.

Test Date	Percent of Samples with QA counts	DQO	Relative Percent Difference between counts
15 October 2018	40%	RPD ≤ 10%	0%
7 November 2018	39%		0%
2 January 2019	39%		0%
9 January 2019	43%		0%

6 CONCLUSIONS/DISCUSSION

Over the course of ~1.5 years, LSRI-GWRC evaluated the Amglo Kemlite BWMS, developed by Amglo Kemlite Laboratories Inc. of Bensonville, Illinois. The Amglo Kemlite BWMS as installed in LSRI's laboratory was a small-scale simulation of a single-pass, flow-through ballast water treatment process, wherein the treatment occurred on ballast water uptake and was followed by a ~48-hour hold ("voyage") time. Overall, the testing unit provided by Amglo Kemlite Laboratories Inc. operated consistently. However, there were several electrical issues that occurred during testing stemming from water entering components of the test unit that were not meant to get wet. These operational performance issues were resolved, and ultimately did not impact LSRI-GWRC's ability to evaluate this technology. These electrical issues, however, did pose a human health and safety danger to those operating the test unit.

The Amglo Kemlite BWMS was evaluated with respect to biological effectiveness to green algae, microbes, and zooplankton in two water types, LW and LW-TMH, and at two frequencies (5 Hz and 10 Hz). Deviations to LSRI SOPs and/or the LSRI-GWRC test plan that occurred during testing were minor and did not impact the results. The majority of the deviations that occurred, as described in Table 5 of this report, had to do with the need to update the test water stock solution water quality acceptance criteria listed in the test plan using the most recent data from bench-scale testing.

Throughout effectiveness testing, there was an increase in water temperature measured immediately following treatment. The 10 Hz frequency seemed to have a slightly greater impact on water temperature than the 5 Hz frequency. No other treatment-related impacts to water chemistry were measured. Qualitatively, the treatment process did not seem to impact the color, clarity, or turbidity of the treated water. Regardless of organism type or life stage, there was a reduction in treatment biological effectiveness when the Amglo Kemlite BWMS was challenged with LW-TMH water, which has reduced light transmittance in comparison to the highly transparent LW. In LW, the Amglo Kemlite BWMS was effective on single-celled and multi-cellular organisms.

Effectiveness tests with the green algae, *S. capricornutum*, showed that the Amglo Kemlite BWMS is highly effective in LW with both lamps tested at both frequencies. Mortality was immediate in the *S. capricornutum* treated in LW, with very little delayed mortality. In contrast, effectiveness was reduced in LW-TMH, but delayed mortality was observed. Ultimately, analysis was confounded by the Sytox stain reacting differently than expected in the treated LW-TMH water. To address this apparent matrix interference, the MPN method of analysis was used to definitively determine biological effectiveness in both LW and LW-TMH. These tests confirmed that *S. capricornutum* mortality was 100% in LW after treatment with the Amglo Kemlite BMWS, as the MPN method resulted in 0 cells/mL in LW. The MPN method in the LW-TMH resulted in an MPN of >160 cells/mL. All but one of the tubes was positive at the end of the 14 day MPN incubation period, indicating viability of the treated cells.

The results of testing the effectiveness of the Amglo Kemlite BWMS on two species of bacteria were very interesting. The Amglo Kemlite BWMS was highly effective in killing *E. coli* and *E. faecium* in both LW and LW-TMH. Immediately following treatment, regardless of lamp or frequency, there was a reduction in *E. coli* and *E. faecium* concentrations of at least 5 $\log_{10}$ units. In LW, bacteria regrowth was observed over the 48-hour hold time for *E. coli* treated with Lamp T at both frequencies. This regrowth was not observed for *E. coli* exposed to Lamp Q in LW at either 5 or 10 Hz. Interestingly, the regrowth that was observed for *E. coli* exposed to Lamp T did not occur for *E. faecium*, in fact, no regrowth of *E. faecium* was observed

during LW testing. This indicates that a very small number of viable *E. coli* cells remained after treatment with Lamp T in LW, and these viable cells reproduced during the holding time, whereas no viable *E. faecium* cells remained following treatment in LW. Results from testing in LW-TMH using Lamp T indicated a slightly less effective initial impact of treatment on *E. coli*, and regrowth of the remaining viable cells was again observed. When *E. coli* were exposed in LW-TMH to Lamp Q at 5 Hz the results were the same as testing conducted in LW (100% mortality and no regrowth), however, Lamp Q at 10 Hz was less effective on *E. coli* initially than it was in LW and regrowth was observed. For both lamps and at both frequencies, results for *E. faecium* testing in LW-TMH indicated a reduction in initial effectiveness compared to testing in LW, but in all cases delayed mortality was observed over the 48-hour hold time indicating initial cellular injury occurred as a result of treatment.

The Amglo Kemlite BWMS was highly effective in killing the zooplankton *Eucyclops sp*., in the effectiveness tests in LW. There was greater than 98% mortality using either lamp at 10 Hz in LW. In LW-TMH mortality was >48% with either lamp at either frequency. Mortality was slightly lower in both lamps at 5 Hz in both water types. There was no delayed mortality. The effect was seen immediately following exposure to the treatment system, rather than as a mortal injury causing delayed mortality.

There was little effect on hatch rates of *D. magna* ephippia treated with the Amglo Kemlite BWMS in either water type with either lamp or frequency. Hatch rates of ephippia were similar among all treatments. Interestingly, the only exception to this was a significant (p<0.05) decrease in hatch rate in the ephippia treated with Lamp Q at 10 Hz in LW-TMH water.

Results from this laboratory-based testing, although limited, indicate potential effectiveness of the Amglo Kemlite BWMS for treatment of freshwater ballast water. Overall, biological effectiveness seemed to be increased at increasing frequency, the exception being the microbial results. Lamp Q appeared slightly more effective than Lamp T overall, which was especially evident during microbial testing. Although there was a reduction in treatment effectiveness in water quality that would resemble that of a commercial port within the Laurentian Great Lakes, the Amglo Kemlite BWMS was still somewhat effective in this single-pass testing using LW-TMH. Based upon these results, the Amglo Kemlite BWMS, given additional research and development, could be both applicable and effective in the Laurentian Great Lakes ecosystem. There are no active substances produced as a result of the treatment process, therefore, toxicity of discharge to receiving waters is not a concern with this system. This testing did not evaluate the feasibility or cost of this technology as installed in a shipboard setting, but these data could be generated in larger-scale (land-based or shipboard) testing. Improvements in the safety of the current prototype's design need to be made to ensure operator health and safety and reduce the risk of explosion in a shipboard engine room setting.

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