

Laboratory studies for prediction of responses of algae to algaecides *in situ*

ALYSSA J. CALOMENI, TYLER D. GEER, AND JOHN H. RODGERS, JR.*

INTRODUCTION

Appropriately designed laboratory studies can be widely applicable and are often used for their predictive capabilities. One such application for these laboratory studies has been successful prediction of effective treatment strategies for algae *in situ* (see Duke 2007, Bishop and Rogers 2011, Calomeni et al. 2015, Iwinski 2016, Geer et al. 2017, for specific examples of this approach). Algae become problematic in freshwater resources when algal population densities or concentrations of secondary compounds [e.g., 2-methylisoborneol (MIB), geosmin, microcystins, and prymnesins] exceed threshold levels, triggering algaecide treatments at the site where the algae are growing (Getzinger et al. 2014). Algae can be problematic in aquatic systems with a range of biological (e.g., algal species, strains), physical [e.g., size (spatial dimensions), lotic, lentic] and chemical characteristics (e.g., pH, alkalinity, hardness, conductivity, total suspended solids). Due to the multifaceted character of algal issues, preliminary predictions of algal responses using laboratory studies can decrease associated uncertainty regarding algal responses to algaecide applications *in situ*. The current approach for predictive laboratory studies is informed by decades of development.

Historically, algal studies have attempted to use one or more unialgal cultures grown in laboratory-formulated media (e.g., Fitzgerald and Faust 1963, Miller et al. 1978) to predict algal responses to exposures (e.g., chemical stimuli such as algaecides or nutrients) in natural systems. Results from previous algal studies have demonstrated that 1) different algae (e.g., species, strains) do not respond similarly to the same exposure, 2) exposures of the same concentration of different algaecides result in disparate algal responses, and 3) algae respond divergently in different exposure waters (e.g., culture media versus water collected from an aquatic system). Questions subsequently arose pertaining to 1) the utility of responses of one or a few unialgal cultures to predict responses of natural algal assemblages, and 2) differences in responses of algae to compounds in laboratory-formulated media relative to water collected from an aquatic system of interest. A current fundamental premise for predictive algal studies is that algaecide exposures in the laboratory and *in situ* must be equal and comparable for similar and thus predictable responses of algae to these exposures. To address issues

associated with historical laboratory studies, a site-specific approach (Figure 1) is used.

This manuscript outlines requisite considerations and methods to design, interpret, implement, and translate results from predictive laboratory studies for use *in situ*. This information is outlined in the following sections 1) problem identification/definition, 2) study design, 3) laboratory studies, 4) laboratory to *in situ* translation, 5) corroboration of laboratory and *in situ* studies (Figure 1), and 6) case study.

PROBLEM IDENTIFICATION/DEFINITION

Problem identification/definition is the initial step of the laboratory approach for *in situ* predictions and provides the premise for subsequent decision making. In this step, the algal issue and source (e.g., algal species, strain, assemblage) is identified and the extent of the algal issue is characterized (e.g., spatial, temporal, magnitude). In this initial step, the question being asked in this study is discerned and focused.

For density-dependent algal issues, the problematic alga might be readily apparent. For algal issues stemming from production of secondary compounds, the compound might be problematic at a location independent and distant from the algal growth (Isaacs et al. 2013, Geer et al., 2017). For example, taste and odor (T&O) compounds (e.g., MIB and geosmin) and toxins (e.g., microcystins) can be measured at a drinking water intake although the source might not be in the vicinity of the intake. This can occur because these compounds are readily soluble and disperse within the water column away from areas where they are produced. Some knowledge of water movement or hydrodynamics is important in diagnosing these situations. In situations in which the proximity of the algal issue and the putative producer(s) are unknown, strategic sampling will be required to accurately discern the causative source.

Evidence for putative producer(s) can include visible algal growths, scums, or mats. Algae, because of their light requirements, are typically located within the photic zone; therefore, surveys of putative producer(s) should begin within this area. Algae might be evenly distributed within the water column, “layered” within the water column, associated with the benthic environment, or growing on submerged structures (e.g., submerged trees, sediments, filters, booms, bridges). Samples of algae collected from numerous locations within the impacted water resource can then be analyzed for the problematic constituent. Some compounds are originally endotoxins (i.e., contained within cells) and might be present at higher concentrations within the algae. Another line of evidence to support identification

*First and second authors: Graduate Research Assistants, third author: Professor, Department of Forestry and Environmental Conservation, Clemson University, 261 Lehotsky Hall, Clemson, South Carolina 29634. Corresponding author's e-mail: acalome@g.clemson.edu.

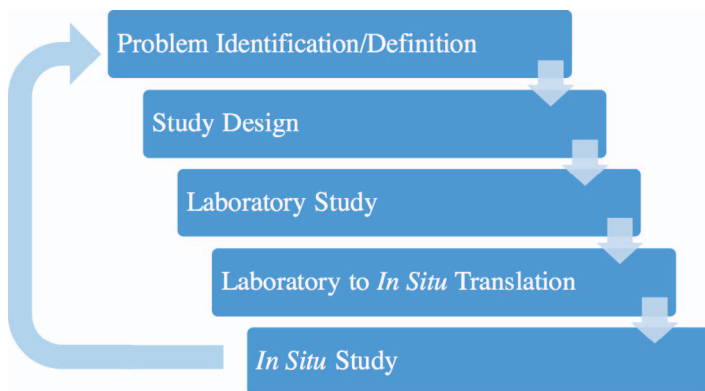


Figure 1. Outline for laboratory to *in situ* approach.

of putative producer(s) of a problematic compound includes literature searches for algal genera or species that have been documented as a source (Wehr and Sheath 2002, Graham et al. 2016, Paerl et al. 2016).

Once the putative source of the algal issue is identified, the magnitude of the algal issue needs to be quantified. This analysis includes measurements of density and spatial extent as well as temporal attributes. Adaptive cluster sampling is a sampling technique (Thompson 1990) that includes collecting random samples to initially identify the location of putative problematic algae. Additional samples are then collected to further delineate the extent of the algal issue. Planktonic or “free floating” algal samples can be collected with Kemmerer, Niskin/Nansen, or Van Dorn sampling devices as well as plankton nets (APHA 2012). Benthic algal samples can be collected with Ekman, Ponar, or Petersen Dredges (Lind 1974), as well as rakes (Kenow et al. 2007), or scraped from surfaces (e.g., rocks, woody debris, and man-made structures) (Sládečková 1962).

The final step of the problem identification stage is resolving and outlining treatment goals. By defining treatment goals, the measurement of “success” can be characterized. Because resources (e.g., economic, logistic) are ultimately limited, treatment goals need to be defined within terms that are feasible and maximize the opportunity for success. For example, for treatment of T&O compounds in the context of potable water, decreasing compound concentrations in the intake water should be targeted as opposed to attempting to eradicate the problematic algae within a lake that might encompass a large area and support several uses within that water resource.

Measurements of treatment success are a function of the algal issue and are site- and situation-specific. For density-related problems, this can include a decrease in algal mass or surface area coverage so that designated uses of the water resource can be restored (e.g., navigation, irrigation, property value). For compound-producing algae, the treatment goal might include decreasing the density of algae so that there are consequent decreases in compound concentrations to less than a specific threshold. For T&O compounds, the threshold might be the lowest concentration that people or consumers are able to detect. People are relatively sensitive to T&O compounds (e.g., MIB and geosmin) and can detect (through olfaction or taste)

concentrations of approximately 5 to 10 ng L⁻¹ (Suffet et al. 1995). For toxins, the threshold could be a human health standard or guideline (e.g., drinking, recreation; WHO 2003, USEPA 2015) for consumption or contact recreation.

STUDY DESIGN

The concept of laboratory studies providing predictions of *in situ* results is based on fundamental concepts of ecotoxicology. These concepts include: 1) exposures precede responses, and 2) exposures are predictive of responses. Thereby, if laboratory studies replicate algaecide exposures (in terms of concentration, duration, and formulation) to an alga that occurs *in situ*, laboratory measurements will be predictive of that alga’s response. An additional implicit assumption of this approach is that the organisms studied in the laboratory are the same as the organisms *in situ* in terms of sensitivity.

To capture an exposure in the laboratory that is analogous to the one that occurs *in situ*, one needs to appropriately replicate (in the laboratory) aspects of the specific aquatic environment or exposure modifying factors that influence that exposure. Many site-specific factors that influence chemical exposures can be incorporated into laboratory studies by using site-collected water instead of a laboratory-formulated medium. Factors that can influence chemical exposures (i.e., exposure-modifying factors) vary depending on the algaecide used. The site-specific factors that alter chemical exposures for a specific algaecide should be replicated in the laboratory. In the United States, the Environmental Protection Agency (USEPA)-registered algaecide active ingredients currently include copper-based products, peroxy-compounds, endothall, and diquat. These compounds are susceptible to different *in situ* exposure-modifying factors. In the case of copper-based algaecides, hardness, alkalinity, pH, and conductivity can alter the activity of applied copper. Peroxy-compounds are oxidants and can oxidize other organic material in addition to targeted algae and deplete activity. Organic matter of algal origin, as well as humic and fulvic acids can influence peroxide exposures (Geer 2016). Endothall is degraded biologically (Reinert et al. 1986), and different microbes, nutrient concentrations, temperatures, and oxygen concentrations at sites can influence the rate of microbial degradation, and subsequently, exposures. Diquat can sorb to suspended sediments and organic matter (USEPA 1995), decreasing the activity of this algaecide available to affect algae.

Algal sensitivities to algaecide exposures range widely, sometimes one to two orders of magnitude (Fitzgerald and Faust 1963, Calomeni et al. 2014, Geer et al. 2016). Additionally, the measured response of a single algal species can decrease an order of magnitude with an incremental increase (by an order of magnitude) in cell density (Geer 2016). Therefore, representative samples of the putative producer(s) of problematic compounds (e.g., toxins or T&O compounds) are needed for predictive laboratory studies. From the previous problem identification/definition step, representative samples of the problematic algae are defined so that samples can be collected and tested in laboratory

studies. Representative samples are those that accurately capture “the problem” in terms of algal genera/species/strain present as well as algal density for planktonic algae and algal mass for benthic algae.

An important distinction for design of laboratory studies is the difference between concentration (e.g., mg L⁻¹) and dose (e.g., mass of active ingredient/mass of algae). A fundamental concept of ecotoxicology is that ultimately the response of an organism depends on the amount of a constituent such as an algaecide in and on the organism (i.e., dose). This is fundamentally different from an algaecide concentration in the aqueous phase. For example, if you have a problematic planktonic algal density of 1×10^5 cells ml⁻¹ and 1,000 µg Cu L⁻¹ was applied, the maximum achievable dose of copper on a per cell basis would be 0.01 µg cell⁻¹, if all of the applied copper is partitioned to algal cells. If the problematic density was 1×10^6 cells ml⁻¹ and the same copper concentration was applied, the achievable dose of copper would be 0.001 µg copper cell⁻¹ (an order of magnitude decrease). As mentioned previously, the difference in dose under these two situations likely will result in approximately an order of magnitude difference in response. This would translate into a significant decline in performance of an algaecide in the field. This distinction is especially important when considering benthic algae because the mass of benthic algae is typically large (relative to planktonic algae) in the vicinity of the sediment–water interface. For benthic algae, it is particularly important to deliver the algaecide to the sediment–water interface.

Under some scenarios, one might want to adjust the density or mass of algae artificially in the laboratory to more accurately simulate the density of the algae during the time of treatment *in situ*. While these laboratory experiments are being conducted and appropriate paperwork (e.g., permits and contracts for application) for treatment is completed, the algal density *in situ* might be altered (e.g., growth of algae as water temperatures increase from Spring to Summer or senescence of algae as water temperatures decrease from Summer to Fall). To adjust algal densities in the laboratory to account for differences in algal density *in situ*, the laboratory temperature can be altered to stimulate or depress algal growth representative of *in situ* conditions. Alternatively, site water without algae (e.g., filtration, centrifugation) can be added or removed, although care must be taken to maintain the viability of the algae during these alterations.

LABORATORY STUDIES

Laboratory studies can be used to ask what the responses of algae from a site are to algaecide exposures (e.g., timing of response and extent of response as well as diagnostic symptoms of exposure to the algaecide). Depending on the specific situation, these exposures can consist of different algaecide formulations, concentrations, or exposure durations. A strategic review of algal laboratory experiments designed to ask questions about responses *in situ* was performed. Experimental methods in these peer reviewed publications are presented in Table 1.

Experimental methods for each laboratory evaluation might need to be adjusted for site specific conditions or questions. Generally, experimental chambers are less than or equal to 200 ml (Table 1, Duke 2007). The volume of the experimental chambers must be sufficient to contain a representative quantity of algae. Based on previous research, ≤ 100 ml is an appropriate volume for experimental chambers containing algae that are relatively homogenous (i.e., planktonic algae; Duke 2007). The volume of the experimental chambers might have to be increased for heterogeneously distributed algae (i.e., an algal assemblage containing many different algae). Appropriate durations to observe exposures of algae are typically ≥ 72 h (Duke 2007). However, algal responses might take longer to manifest for dense masses of algae (i.e., benthic algae), and observations might have to be continued for ≥ 7 d. Typically, exposures are conducted at 20 to 25 C (Table 1, USEPA 2002, Duke 2007) and experimental chambers can be agitated, either by hand or using a mechanical shaker (USEPA 2002, Duke 2007).

An example of an experimental design is presented in Figure 2. The objective of this experiment was to measure responses of *Microcystis aeruginosa* from a farm pond to a series of concentrations of a copper-based algaecide. *Microcystis aeruginosa* and site water were collected from the pond. Algaecide exposures consisted of a series of chelated copper concentrations in eighteen 250 ml beakers. In this example experimental design, five algaecide concentrations and an untreated control were used with three replicates per exposure (i.e., experimental treatment) (Figures 2 and 3).

Algal responses to exposures can be modeled by a sigmoidal or S-shaped relationship (Figure 3). The S-shape occurs because there are relatively “low” concentrations of algaecide in which an algal response cannot be measured relative to an untreated control (black rectangle) and there will be relatively “high” algaecide concentrations that result in a maximum response (red rectangle) (Figures 2 and 3). Because site-specific algae and water were used in this experiment, the S-shaped exposure–response relationship provides a model for this specific site and algal issue and can be used to predict the responses of this strain of *Microcystis aeruginosa* (y-axis) to algaecide concentrations (x-axis).

The algal response measured as a consequence of the algaecide exposure needs to be carefully considered (Calomeni and Rodgers 2015). If an assemblage of algae is the putative source of the algal issue, algal responses that will capture changes in the assemblage can be used, including pigment concentrations (e.g., chlorophyll *a* and phycocyanin) or mass (Calomeni et al. 2014). If there is one alga that is the putative source of the algal issue (e.g., *Microcystis* as the source of production of microcystins or *Prymnesium* as the source of prymnesins), specific measures of algal responses are necessary. Specific algal response measures include parameters such as cell density because light microscopy can be used to identify and enumerate a specific alga or multiple algae. For planktonic algae, the aforementioned response measures are typically expressed on a per liter basis. For benthic algae, responses are expressed per mass or surface area (Weitzel 1979). When responses are expressed on a mass basis (e.g., cells of

TABLE 1. SUMMARY OF EXPERIMENTAL METHODS FOR LABORATORY STUDIES DESIGNED TO PREDICT RESPONSES OF ALGAE TO ALGAECIDES *IN SITU*. MIB INDICATES 2-METHYLISOBORNEOL.

Algae (type)	Algal mass (g)	Exposure volume	Temperature (C)	Light	Agitation	Experiment duration (days)	Algal response (units)	Citation
<i>Lyngbya</i> (Benthic)	0.1	200 ml site water in 250 ml beakers	21–25	16:8 h light:dark with “cool white” fluorescent lighting	Beakers swirled daily by hand	7	Chlorophyll <i>a</i> concentration ($\mu\text{g g}^{-1}$ of <i>Lyngbya</i>)	Duke 2007
<i>Lyngbya</i> (Benthic)	0.35	200 ml site water in 250 ml beakers	22–25	16:8 h light:dark with “cool white” fluorescent lighting	Not Specified	7	Wet weight (g) and chlorophyll <i>a</i> concentration ($\mu\text{g g}^{-1}$ of <i>Lyngbya</i>)	Bishop and Rodgers 2011
<i>Lyngbya</i> (Benthic)	0.5	200 ml site water in 250 ml beakers	Not Specified	Not Specified	Not Specified	7 and 14	Wet weight (g) and chlorophyll <i>a</i> concentration ($\mu\text{g g}^{-1}$ of <i>Lyngbya</i>)	Calomeni et al. 2015
<i>Oscillatoria</i> , <i>Anabaena</i> , <i>Planktothrix</i> , <i>Tabellaria</i> and <i>Fragilaria</i> (Benthic)	0.5	200 ml site water in 250 ml beakers	21–25	18:6 h light:dark with “cool white” fluorescent lighting	Not Specified	4 and 7	Cell density (cells m^{-2}), phycocyanin ($\mu\text{g m}^{-2}$), chlorophyll <i>a</i> concentration ($\mu\text{g m}^{-2}$) and geosmin and MIB (ng L^{-1})	Geer et al. 2017
<i>Microcystis aeruginosa</i> (Planktonic)	Not Specified	200 ml site water in 250 ml beakers	19–23	18:6 h light:dark	Not Specified	4	Cell density (cells ml^{-1}), chlorophyll <i>a</i> concentration ($\mu\text{g L}^{-1}$) and microcystin-LR ($\mu\text{g L}^{-1}$)	Iwinski 2016

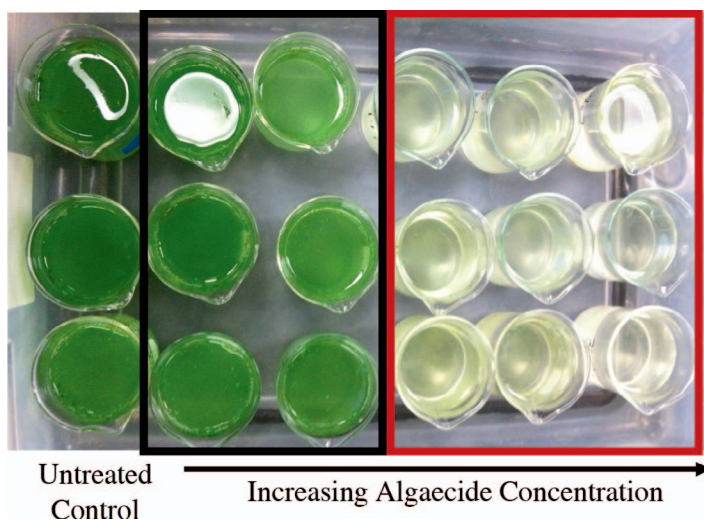


Figure 2. Example of an algae study with the objective of identifying an effective concentration to decrease the density of *Microcystis aeruginosa*. Based on macroscopic visual observations, the exposure within the black rectangle did not result in a discernable algal response relative to the untreated control. The exposures within the red rectangle resulted in a discernable algal response.

putative producer(s) g^{-1} periphyton) the potential that the mass of the periphyton could decrease following treatment could hinder the ability to measure a response in the putative producer(s). Responses expressed in terms of surface area (e.g., cells of the putative producer(s) m^{-2}) can be used to correct for changes in the periphyton mass, although there might be greater variability (*in situ*) for measurements expressed on an areal basis (per m^2). Visual observations can also be useful to distinguish effective algaecide treatments in laboratory studies. For waters that contain dissolved organics (i.e., humic and fulvic acids), a “no algae” control can be useful to discern an effective algaecide treatment.

Algal responses can be discerned statistically. Analysis of variance (ANOVA) can be used to discern if differences exist among treatments. Then additional analyses such as *t*-tests, linear contrasts, and all pair-wise comparisons can be used to identify specific treatments that result in different algal responses. Algaecide concentrations that are not statistically different from the untreated control are termed no observed effect concentrations (NOEC). The lowest concentration tested that is significantly different from the untreated control is the lowest observed effect concentration (LOEC). As mentioned previously, algal responses typically follow an S-shaped relationship with increasing exposures. Statistical analyses such as probit and logit allow percent algal responses corresponding with a specific algaecide concentration to be discerned (e.g., effect concentration corresponding with a 50% decrease in algal response = EC_{50}).

Ultimately, the approach for identifying an effective algaecide treatment is based on weight of evidence (Calomeni et al. 2014). In this approach, the greatest weight in terms of decision making is given to the measure of the algal issue. From the problem identification/definition step, measures of success were identified. These same parameters

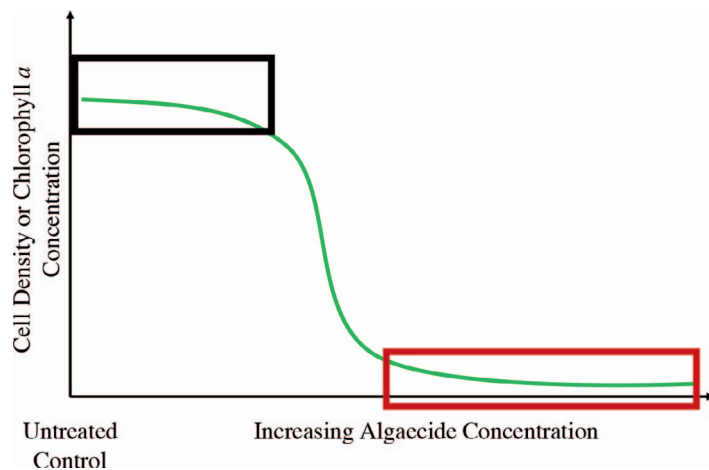


Figure 3. S-shaped exposure-response model for algae exposed to algaecides corresponding with Figure 2. The black box captures the portion of the curve in which a difference in algal response cannot be discerned relative to the untreated control. The red box indicates the maximum algal response measured. Responses (e.g., no observed effect concentrations [NOEC], lowest observed effect concentration [LOEC], effect concentration corresponding with a specified percent decrease in algal response [EC_x]) can be discerned using statistical analyses.

can be measured in the laboratory following algaecide exposures. For example, with a density-dependent issue, success is defined as a decrease in mass or algal density sufficient to regain use of the critical water resource. For a density-independent issue, success is a decrease in toxin or T&O compound concentration (e.g., microcystins, geosmin concentration). Knowledge of the fate processes that influence these compounds (e.g., microcystins, geosmin) might be important. For example, a fate process that results in decreasing aqueous concentrations of microcystins and geosmin is dilution *in situ*. Dilution rates are relatively small in a 250 ml beaker relative to a lake, pond, and river. Measured concentrations of microcystins and geosmin in beakers are therefore likely conservative (i.e., overestimates) relative to *in situ* concentrations.

LABORATORY AND *IN SITU* TRANSLATION

For laboratory to *in situ* translation, additional exposure-modifying factors might need to be considered. As mentioned previously, the premise behind laboratory experiments for predictions *in situ* is that algaecide exposures in the laboratory and *in situ* are comparable. If the results from a laboratory experiment are obtained and communicated promptly, the lowest algaecide concentration that achieved control of the target algal population in the laboratory experiment can be applied to the target alga in the water resource. However, additional exposure-modifying factors can come to bear *in situ* that were not factored into the original laboratory experimental design. Factors that can decrease exposures of active ingredients include dilution of algaecide, suspension of particulate and dissolved solids from wave action, and sorption of the algaecide by the sediment phase or particulates. Factors that can alter doses of algaecide include shifts in algal density *in situ* relative to the laboratory. Algal density can shift as algae

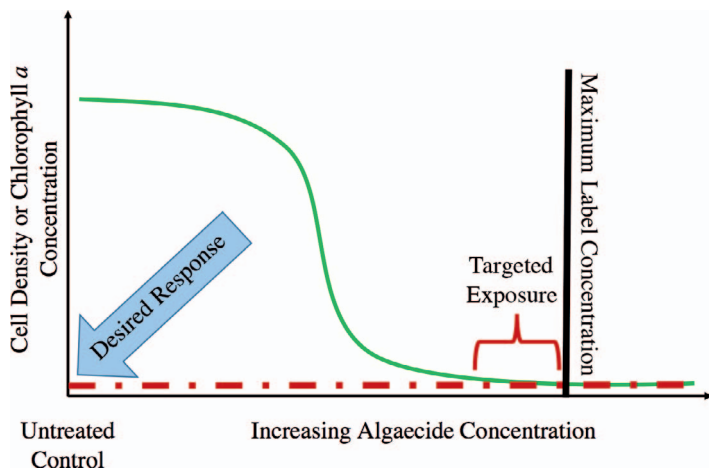


Figure 4. Example of the use of the S-shaped exposure-response model to discern a targeted copper concentrations for application *in situ*. In this example, concentrations less than and exceeding the maximum label concentration resulted in a desired algal response at the site. The targeted exposure *in situ* is therefore less than the maximum label concentration but still results in the desired response of the targeted algae.

grow in the aquatic system during the time required to obtain and communicate the data from the laboratory study. Additionally, algae such as *Microcystis aeruginosa* can move with wind and water, resulting in rapid changes in spatial distributions and densities within the water body. Using the exposure-response model obtained from the laboratory study, targeted algaecide concentrations *in situ* can be increased or decreased depending on the site-specific conditions at the time of treatment, acceptable or anticipated risks to nontarget species, and algaecide label or regulatory restrictions (Figure 4).

CORROBORATION OF LABORATORY AND *IN SITU* STUDIES

Because the laboratory model is constituted by measures of exposures and responses, measures of exposures and responses are also necessary *in situ* for comparison or corroboration (Figure 5). Targeted exposures alone are insufficient measures of exposures in an aquatic system because exposures *in situ* are modified by a number of site-specific characteristics (e.g., dilution, sorption). Additionally, exposures are not limited to concentration alone. Exposures also encompass the temporal duration in which the targeted algae are exposed.

To measure *in situ* exposures, replicate samples are collected in proximity to the problematic algae that are treated with an algaecide. Careful sampling is necessary to ensure accurate measurement of algaecide exposures because algae and algaecide treatments are typically heterogeneously distributed within a water column. The goal for measurement of algaecide exposures is to capture a representative sample of concentrations of the active ingredient influencing the target alga. If the target alga is associated with the benthic environment, and samples for analysis of algaecide concentrations are collected at the water-air interface, measures of exposures are likely

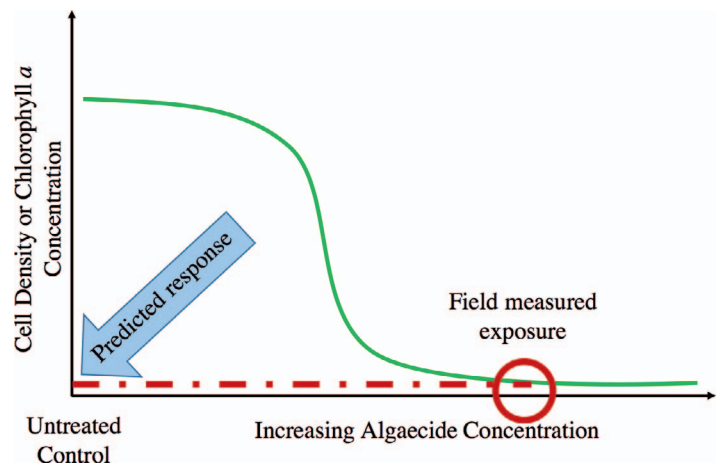


Figure 5. Use of a laboratory model for *in situ* predictions. Based on fundamental concepts of ecotoxicology, exposures are predictive of responses. In this example, the same exposure, regardless of the study being conducted in the laboratory or *in situ* should result in the same predicted response (i.e., inverse prediction).

inaccurate. The number of samples collected depends on the heterogeneity and size of the site. Replicate samples should also be collected for analysis through time following the initial application to discern the duration of exposure.

Exposure and response data measured in the laboratory and *in situ* can be used to interpret the outcome of algaecide applications. Interpretation of these data involves comparisons between the laboratory exposure-response model and data collected *in situ*. There are four potential overall outcomes of these comparisons (Figure 6). Approaches to address potential inconsistencies in comparisons of exposures and responses between the laboratory and *in situ* include 1) conducting another laboratory experiment with representative algal samples collected from the site, 2) altering the sampling plan to collect representative measurements of algaecide exposures, or 3) adjusting the algaecide treatment to account for additional exposure modifying factors *in situ* (e.g., dilution). Because the laboratory model was designed for this specific alga or algal assemblage, if the problematic algae at the site shift (as a result of treatment or through time), a different laboratory model might need to be developed.

CASE STUDY

A laboratory study was designed using this approach in Hartwell Lake, Anderson, SC to predict algal responses to sodium carbonate peroxyhydrate algaecide exposures *in situ* (Huddleston et al. 2015, Geer et al. 2017). The impetus for this study was seasonal (May through November) taste and odor issues associated with potable drinking water. The first step was identification (i.e., problem identification/definition) of the putative source of the taste and odor compounds. Strategic sampling was conducted by collecting water and sediment samples in the vicinity of the drinking water intake (Huddleston et al. 2015). When odor was detected (via olfaction) samples were collected outward from the drinking water intake to delineate the spatial

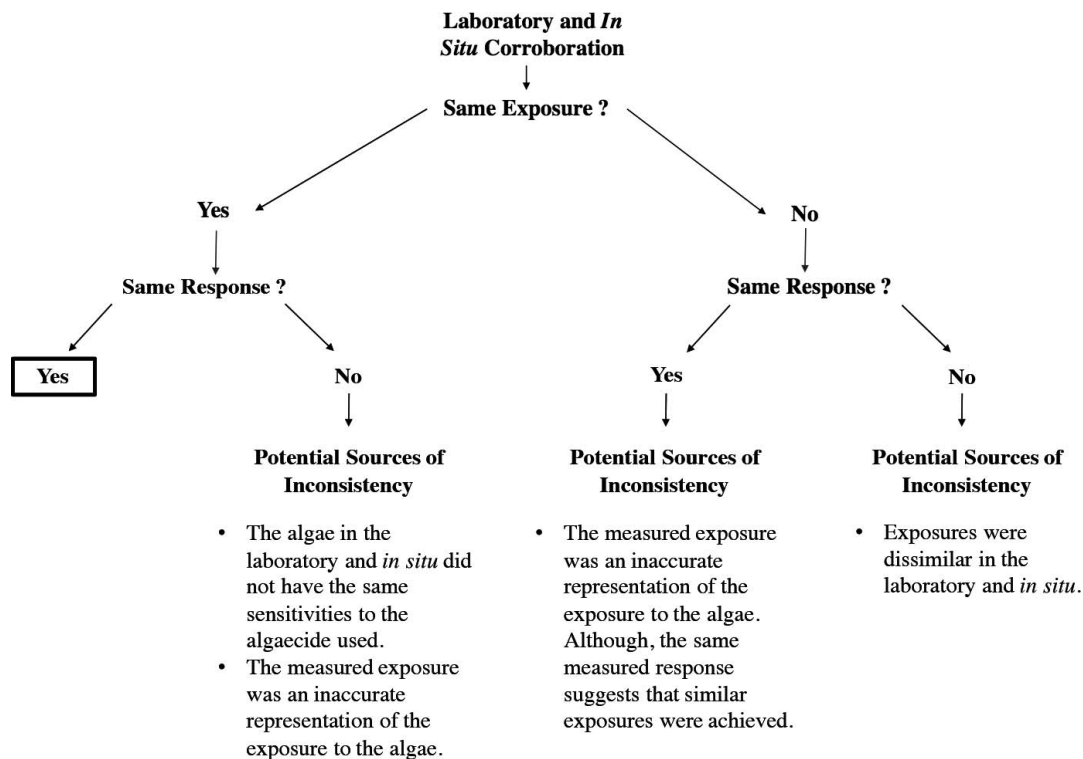


Figure 6. Potential sources of inconsistencies resulting from laboratory and *in situ* comparisons of exposures and responses.

extent of the odor. Sediment samples with strong odors were shipped to the laboratory at Clemson University and algal samples were compared with published literature to identify putative producers of the taste and odor compounds.

For this site, the main water resource use that was being influenced by the algal issue was potable water production. The taste and odor compounds at this site, geosmin and MIB, can be detected at 5 to 10 ng L⁻¹. The treatment goal is therefore a raw/source water taste and odor compound concentration less than the concentration that can be removed in the drinking water plant and still achieve detectable taste and odor compound concentrations. For example, if the treatment plant can remove 50% of the taste and odor compound concentration in raw water, the treatment goal would be less than 10 ng L⁻¹. Success would then be defined as an algaecide application that decreased densities of algae sufficiently to result in less than 10 ng L⁻¹ of geosmin and MIB in the raw water of the drinking water facility.

After the problem was identified and the treatment goal was selected, laboratory experiments were designed. Potential confounding issues associated with using laboratory water were eliminated by conducting laboratory experiments with site-collected water, algae, and sediment (Geer et al. 2017). A summary of experimental details of this experiment are presented in Table 1. To provide data that exposures were comparable between the laboratory and *in situ*, they were measured along with consequent algal responses. This experiment demonstrated the importance of using a preliminary laboratory exposure-response model to predict algal responses *in situ*.

CONCLUSIONS

Laboratory studies are used to develop site-specific predictions about algal responses to different algaecide exposures and are expressed as exposure-response models. Appropriately designed and implemented laboratory experiments (e.g., using site water and representative algal samples) result in site-specific predictions of effective treatments *in situ*. The basis for comparison between the laboratory experiment and results *in situ* are measurements of exposures and responses. Laboratory and *in situ* comparisons and subsequent adjustment of sampling or treatment plans (e.g., adaptive water resource management) can result in predictable responses of algae to algaecide exposures *in situ*. This approach provides a data-driven, site-specific, and defensible approach for selecting effective treatments *in situ*. As such, adaptive water resource management can be used to decrease risks associated with applying a greater concentration of algaecide than is needed to result in a desired algal response. Potential positive outcomes of this approach are limiting expenses, decreasing exposures, minimizing effects on nontarget species, and addressing other sources of potential risk (e.g., release of microcystin toxins).

LITERATURE CITED

- [APHA] American Public Health Association. 2012. Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, Port City Press. Baltimore, MD. 1,496 pp.
- Bellinger EG, Sigee DC. 2010. Freshwater algae: Identification and use as bioindicators. John Wiley & Sons, Ltd., Chichester, UK. 271 pp.

- Bishop W, Rodgers JH, Jr. 2011. Responses of *Lyngbya wollei* to exposures of copper-based algaecides: The critical burden concept. Arch. Environ. Contam. Toxicol. 62:403–410.
- Calomeni AJ, Iwinski KJ, Kinley CM, McQueen A, Rodgers JH, Jr. 2015. Responses of *Lyngbya wollei* to algaecide exposures and a risk characterization associated with their use. Ecotoxicol Environ Saf. 116:90–98.
- Calomeni AJ, Kinley CM, Rodgers JH, Jr. 2014. Responses of *Planktothrix agardhii* and *Pseudokirchneriella subcapitata* to copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and a chelated copper compound (Cutrine®-Ultra). Water Air Soil Pollut. 225:2231–2246.
- Calomeni AJ, Rodgers JH, Jr. 2015. Evaluation of the utility of six measures for algal (*Microcystis aeruginosa*, *Planktothrix agardhii* and *Pseudokirchneriella subcapitata*) viability. Ecotoxicol. Environ. Saf. 111:192–198.
- Duke B. 2007. Laboratory and field responses of target and non-target species to algaecide exposures. Ph.D dissertation, Clemson University, Clemson, SC. pp. 12–49.
- Fitzgerald GP, Faust SL. 1963. Factors affecting the algicidal and algistic properties of copper. Appl. Microbiol. 11(4):345–351.
- Geer TD, Calomeni AJ, Kinley CM, Iwinski KJ, Rodgers JH, Jr. 2017. Predicting *in situ* responses of taste- and odor-producing algae in a southeastern U.S. reservoir to a sodium carbonate peroxyhydrate algaecide using a laboratory exposure-response model. Water Air Soil Pollut. 228: 53.
- Geer TD, 2016. Responses of aquatic organism to exposures of sodium carbonate peroxyhydrate. Master's Thesis. Clemson University, Clemson, SC. pp. 9–27.
- Geer TD, Kinley CM, Iwinski KJ, Calomeni AJ, Rodgers JH, Jr. 2016. Comparative toxicity of sodium carbonate peroxyhydrate to freshwater organisms. Ecotoxicol. Environ. Saf. 132:202–211.
- Getsinger K, Dibble E, Rodgers JH, Jr., Spencer D. 2014. Benefits of controlling nuisance aquatic plants and algae in the United States. Council for Agricultural Science and Technology (CAST) Commentary QTA 2014-1, Ames, IA. pp. 1–12.
- Graham, J.L., Loftin, K.A., Ziegler, A.C., and Meyer, M.T., 2008, Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs: U.S. Geological Survey Scientific Investigations Report 2008—5038, 39 p.
- Huddleston M, Rodgers JH, Jr., Wardlaw K, Geer T, Calomeni A. 2015. Adaptive Water Resource Management for Taste and Odor Control for the Anderson Regional Joint Water System. South Carolina's Water Associations, p. 41–45. www.scwaters.org. Accessed July 12, 2016.
- Isaacs DA, Brown RG, Ratajczyk WA, Long NW, Rodgers JH, Jr. Schmidt JC. 2013. Solve taste-and-odor problems with customized treatment. Opflow 39:26–29.
- Iwinski KJ. 2016. Release and degradation of microcystin-LR following exposures of *Microcystis* to copper-based algaecides. Ph.D dissertation. Clemson University, Clemson, SC. pp. 115–135.
- Kenow KP, Lyon JE, Hines RK, Elfessi A. 2007. Estimating biomass of submersed vegetation using a simple rake sampling technique. Hydrobiologia 575:447–454.
- Lind OT. 1974. Handbook of common methods in limnology. The C. V. Mosby Company, Saint Louis, MO. pp. 153–179.
- Miller WE, Greene JC, Shiroyama T. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test: Experimental design, application, and data interpretation protocol. USEPA-600/9-78-018. Environmental Protection Agency, Office of Research and Development, Corvallis Environmental Research Laboratory, Corvallis, OR. 125 pp.
- Paerl HW, Gardner WS, Havebs KE, Joyner AR, McCarthy MJ, Newell SE, Qin B, Scott JT. 2016. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae. 54:213–222.
- Reinert KH, Rodgers JH Jr., Leslie TJ, Hinman ML. 1986. Static shake-flask biotransformation of endothall. Water Res. 20:255–258.
- Sládečková A. 1962. Limnological investigation methods for the periphyton (“Aufwuchs”) community. Bot. Rev. 28(2):286–350.
- Suffet IH, Mallevalle J, Kawcynski E. 1995. Advances in taste-and-odor treatment and control. American Water Works Association Research Foundation (AWWARF) Denver, CO. 277 pp.
- Thompson JN. 1990. Adaptive cluster sampling. J. Am. Stat. Assoc. 85:1050–1059.
- [USEPA] U.S. Environmental Protection Agency. 1995. Reregistration Eligibility Decision (RED) for Diquat Dibromide. 738-R-95-016. 304 pp.
- [USEPA] U.S. Environmental Protection Agency. 2002. Method 1003.0: Green Alga, *Selenastrum capricornutum*, Growth Test; Chronic Toxicity. EPA-821-R-02-013. 35 pp.
- [USEPA] U.S. Environmental Protection Agency. 2015. Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. Office of Water 4304T. EPA-820R15100. 75 pp.
- Wehr JD, Sheath RG. 2003. Freshwater algae of North America: Ecology and classification, Academic Press, San Diego, CA. 918 pp.
- Weitzel RL. 1979. Methods and measurements of periphyton communities: A review. American Society for Testing Materials, Philadelphia, PA. pp. 14–22.
- Whitford LA, Schumacher GJ. 1984. Manual of freshwater algae. Sparks Press Inc., Raleigh, NC. 318 pp.
- [WHO] World Health Organization. 2003. Cyanobacterial Toxins: Microcystin-LR in Drinking Water. Background Document for Preparation of WHO Guidelines for Drinking Water Quality. World Health Organization, Geneva, Switzerland (WHO/SDE/WSH/03.04/57). 11 pp.