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Scaling studies for submersed aquatic plant management research

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INTRODUCTION

There is a certain irony to providing guidance for growing healthy aquatic plants for use in trials that are ultimately designed to kill these plants. Nonetheless, if you can't grow them, you can't kill them, and the quality of the study is directly related to pretreatment condition of the plants. In experiences gained over the past 30 yr, there are some key considerations for conducting submersed aquatic plant management research. These considerations have been broken down into the following: 1) available research facilities, 2) sources of water and sediment, 3) plant growth and treatment timing, 4) study duration, and 5) interpretation of data across a broad range of scales. The objective of this chapter is to help investigators new to the field of aquatic plant management avoid basic mistakes and consider potential strengths and weaknesses of research conducted at various scales (Table 1). Aquatic plant managers rely on technical recommendations developed through sound science to plan and execute an environmentally compatible operational program. While herbicides typically provide consistent and efficacious results in treating submersed vegetation, these treatments are inherently expensive and evoke sharp scrutiny from the public, and their failures in high-profile water bodies can be unforgiving.

It is a rare occurrence that a single study, at any scale, yields enough information to provide sound guidance for control of submersed plants in operational settings. Data feedback loops between studies conducted at various scales are important for investigators to recognize, and can be used to refine real-world treatment recommendations. In essence, small laboratory or mesocosm studies can provide useful information for planning and conducting field trials, and field trials can provide data and results that allow us to ask better questions when conducting additional small-scale studies. Many of the concentration and exposure time studies that have been conducted through the years have followed this logic and have led us to a much better understanding of several aquatic herbicides. While this approach often takes multiple iterations between laboratory and field, it results in an improved ability to predict what is likely to happen at an operational scale.

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RESEARCH FACILITIES

Small or benchtop growth chambers

One of the benefits of conducting research on invasive submersed plants such as hydrilla (*Hydrilla verticillata* L.f. Royle) or Eurasian watermilfoil (*Myriophyllum spicatum* L.) is having the ability to work with whole plants at the small growth-chamber scale. In fact, most submersed aquatic plants can be grown from apical cuttings or progagules (turions, tubers) that require limited space. The use of growth chambers requires a limited investment in infrastructure and studies can be conducted year round as long as you have access to the source plants. In a growth chamber setting (e.g., Percival, Conviron), nonrooted apical shoot cuttings (2 to 20 cm) can be grown in a liquid nutrient medium (e.g., Hoagland's solution) in studies to evaluate growth response to various herbicides or other stressors (Figure 1). These studies need to be conducted over a fairly short term (1 to 4 wk) and there are several limitations to the data generated. In these types of studies, plant cuttings are highly sensitive to contact herbicides such as diquat or copper, while response to slow-acting herbicides such as fluridone, penoxsulam, or bispyribac-sodium can often be very subtle under these conditions. This response can lead to a bias in predicting that fast-acting contact herbicides are more effective than the slow-acting enzyme inhibitors. In addition, growth of untreated reference (control) plants can be minimal, and interference from algae often becomes a confounding factor the longer these studies run. Nevertheless, these types of studies can be used to determine herbicide concentrations at which a given plant responds. They are particularly good at discerning a lack of herbicidal activity on target and nontarget plants, but when they detect activity, they do not always scale up as one might predict. The most common mistakes in conducting growth chamber studies include 1) running studies too long, 2) asking too many research questions using such a simple setup, 3) extrapolating results directly to the field without necessary caveats and/or without producing results from additional follow-up or field verification studies, and 4) not understanding the influence that limited growth of the untreated reference plant can have on interpretation of the data. These laboratory studies are often more valuable if you have a question resulting from an observation following an operational or experimental treatment in the field.

In some cases, there may be an advantage to working with rooted plants in small growth chambers. Hydrosoil adds a level of complexity, but generally results in much better sustained growth of the untreated reference plants. These

Table 1. Pros and cons of using different research scales to evaluate herbicides.

	Pros	Cons
Growth chamber		
Nonrooted tips	• Small space requirements and relatively inexpensive set-up costs • Rapid evaluation of herbicide action • Highly controlled and repeatable environment	• Overestimates contact herbicides while underestimating enzyme inhibitors • Slow growth confounds comparison with UTC[1] • Algal interference • Tendency to run studies too long and ask more question than can be addressed given the simple setup
Rooted plants	• See comments above • Rooted plants generally grow much better in culture • Ability to better compare growth differences between UTC and treated plants	• See comments above • Fast-growing plants can rapidly reach carrying capacity of small flasks • Study setup and harvest is more time consuming when compared to studies using free-floating apical tips
Mesocosm		
Indoor facilities	• Some systems designed for tight control over environmental variables (e.g., temperature, light, no precipitation, etc.) • Larger tanks allow for increased plant growth prior to treatment • Studies can run for several weeks (6 to 9 wk) • Can conduct studies at any time of year as long as you have access to healthy tips	• Higher costs for systems that allow environmental control • Lower-cost systems can result in inadequate conditions for growing plants (e.g., light intensity, light quality) • Rapid plant growth can result in plants reaching carrying capacity early in life cycle of the study (e.g., UTC may reach maximum biomass one-third of the way through the study
Outdoor facilities	• Use of large tanks prevents plants from rapidly reaching carrying capacity • Studies can be conducted from weeks to several months • Can evaluate multiple plant species in a single tank • Studies are conducted at a time of year when operational treatments are typically applied	• Large mesocosm systems with control over flow can be quite costly to build • Study setup and data collection can be time consuming • Study design is generally limited by the number of tanks available
Ponds	• Good for demonstration of concepts tested at a smaller scale • Simulation of "real-world results" • Pond facilities allow for evaluation of Experimental Use Permit products, experimental products, or new concepts with an existing product • Static ponds are good for aquatic herbicide dissipation trials	• Ponds are highly variable (each has its own "personality") and it is quite difficult to replicate treatments with SAV[1] • A pond study with a UTC and one treatment requires a minimum of six ponds if the researcher wants to replicate; there are few sites where this can be done • Data collection is labor intensive and usually requires sampling from a boat or wading to discrete sample sites

[1]UTC = untreated control; SAV = submersed aquatic vegetation.

plants can also be started from small cuttings and when placed in favorable conditions will generally begin producing roots within 7 to 10 d. Since many submersed plants readily develop adventitious root tissue from shoots, plants cultures can become well rooted in the artificial sediment or hydrosoil. Finding a consistent substrate has proven to be one of the bigger challenges associated with working on rooted plants at this small scale. While some soils that can be purchased commercially from garden centers can work very well, others will not grow healthy aquatic plants. The consistencies between brands and stores are not necessarily identical. There are artificial sediments that can be constructed (OECD 2014); if you have access to a true sediment source, you should collect enough material to conduct several studies. Long-term storage is not recommended, but you can run several studies in these small growth chambers over a relatively short period of time. The objective in these studies is to grow healthy plants over a short term (3 to 8 wk). Therefore, any substrate that results in growth of healthy plants is adequate for use in herbicide trials. In general, you can add soil at a 1 : 10 or 1 : 20 ratio to the water volume (e.g., 100 or 200 ml of sediment to 2 L of water). We place the soil in a small beaker, add the plants, and then place this unit into the larger study container with liquid medium. You should plan on allowing 7 to 10 d for root formation. Once these plants take root, rapid growth of shoots should ensue. Treatment during periods of rapid growth can readily distinguish between active herbicides and nonactive products or concentrations. While these studies tend to provide a more realistic scenario (treating actively growing rooted plants), the same caveats noted above generally apply in the interpretation of results and applicability of performance in the field. Given the use of rooted plants, these studies can be run longer; however, issues with rapidly reaching carrying capacity of the flasks can result with several of the faster-growing plants. The value of these studies is the small space requirement, rapid turnaround, ability to evaluate numerous treatments (with adequate replication), and ability to evaluate multiple species.

Mesocosm facilities

Mesocosms can be highly technical systems that have been built to allow control of water temperature, photoperiod, and flow rates, or they can be a simple series of tubs placed in a vacant space (Figure 1). Generally, the complexity of the system is related to the types of research questions that can be addressed. Mesocosms can be setup in greenhouses, under polyhouses, or outdoors. One key to growing submersed plants outdoors is to provide a shade cover for the tanks. Uncovered tanks will absorb sunlight

Figure 1. Pictures of different research scales for evaluation of aquatic herbicides and other management methods.

and often heat up the water beyond levels optimal for submersed plant growth (e.g., 35 to 40 C). In a southern state such as Florida, tanks can be left uncovered in the fall, winter, and early spring, and subsequent heating of the water can be a positive attribute, but most outdoor tank studies conducted from May to October need to be shaded. We have found that 30 to 50% shade is adequate for most submersed species.

Dedicated indoor growth chambers of a walk-in size (see Bibliography) can result in tight control over temperature, photoperiod, and precipitation, and good growth has been observed. As long as you have a source of healthy plants or propagules, these studies can be conducted at any time of year. In some cases, plants can be grown to mimic field-level life cycle stages and biomass. With this said, we have seen attempts to create "indoor growth rooms" in buildings and warehouses, and while some have resulted in good plant growth, most are plagued by the inability to generate adequate light quantity and light quality for good submersed plant growth. These plants are often spindly and fail to produce much in the way of canopies. Improvements in indoor lighting for the greenhouse industry have been made in recent years, and the ability to purchase affordable lights designed to grow plants makes it more feasible to set up mesocosm facilities. For submersed plants a light intensity between 200 and 800 µmol m^{-2} s^{-1} provides consistent growth of most species we have tested.

Another key factor in conducting successful submersed plant studies is amending the sediment with a proper level of fertilizer to provide required sediment nutrients. Experimental plants are easily lost with overfertilizing, and a key rule of thumb is to not try to extend the life of the study by loading the sediment with excessive fertilizer. Excess fertilization will simply burn the roots, and while the

Figure 1. Continued.

plants can survive, growth will be stunted and you are likely to have problems with algae growth. Subsequent plant biomass data is likely to be confounded. It is critical to time herbicide applications before plants start to decline in growth. As in terrestrial settings, herbicides are most active when plants are in healthy growth phases. Plants that are stressed or plants in senescence can confound studies with an objective of determining herbicide activity.

In some instances, poor plant growth may be due to the local water quality. If you use local tap water, let it cure for a few days for the residual chlorine to be removed. Depending on the precision demanded by the study, the water medium can be treated by reverse osmosis and reconstituted with desired ions (see Smart and Barko 1985). At a minimum, source water should be tested to determine the ionic content, alkalinity, and hardness. While most submersed plants and herbicides perform across a wide range of water chemistry, there are some species where growth is highly influenced by water chemistry.

If there are typical banes to mesocosm research with submersed aquatic macrophytes, first in line would be confounding by filamentous algae. Generally there is little problem with phytoplankton, and so simply flowing water through the system until the bloom is removed is an easy remedy. However, controlling filamentous algae growth in study tanks without a corresponding injury to the macrophyte of interest is still a puzzling issue. If filamentous algae growth is compromising a study, manual removal of the algae is likely the best remedy. In many cases, a single effort to remove the algae provides control through the entire study. Early detection and rapid response to limit algae growth in early phases of a study are well-advised measures, as separating algae from harvested macrophyte tissue is tedious and time consuming.

Another consideration for submersed plant studies is the potential for insect herbivory. Insects can do enormous damage in a confined tank over a short period of time. Insects often attack just a few study tanks and the researcher is left to consider using an insecticide only on those affected tanks or just treating all tanks in a prophylactic manner. Unfortunately, it is likely too late to salvage the study tank once you make note of significant insect herbivory. When used at an appropriate rate, deleterious effects of insecticides (Abate, Bug B Gone, etc.) on experimental plants have not been observed. If possible, avoid having insecticide and herbicide in the water at the same time. For some studies, weekly treatment with insecticides is simply part of the protocol. Successful insect control has been achieved in

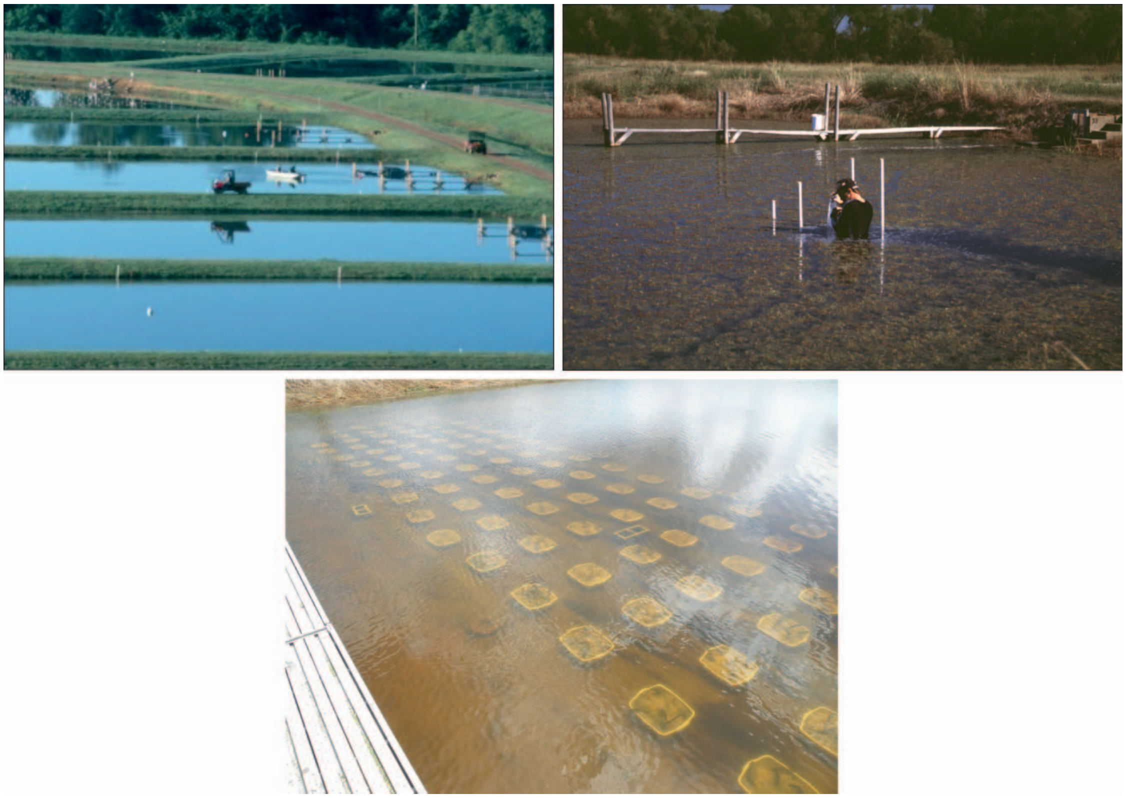

Figure 1. Continued.

some cases by stocking both culture and study tanks with insect-foraging fish such as *Gambusia* or small bluegill (*Lepomis macrochirus* Rafinesque, 1810). During the course of a long-term mesocosm study, you are likely to observe high numbers of snails or tadpoles in some of the tanks—even in an indoor setting, as egg masses of these species can already be attached to shoot cuttings. These organisms are generally not found to be disruptive to macrophyte studies, but there are certain herbivorous snails that could confound study results. In general, our overall observations are that snails and tadpoles feed on epiphytes and decaying plant material, so they likely serve to keep the mesocosm system "clean."

Tank size is another consideration when conducting a mesocosm trial. Plants can rapidly reach carrying capacity in small tanks, and this will affect the growth rate, onset of plant senescence, and potential response to herbicide treatment. In general, it is best to limit the length of the study to a few weeks in smaller mesocosm tanks (50 to 200 L). Use of larger tanks (e.g., 6,700-L tanks in Lewisville, TX) can provide adequate space to grow plants for several months. In this case, you can plant your target community (potentially with multiple plant species) in the preceding summer or fall, allow the plants to establish, and treat the following spring. We have found treatment of well-established submersed plants during a time of year consistent with operational management provides data that is highly predictive of field results. Two potential disadvantages to using large tanks are that the study design is usually limited by the number of tanks, and the amount of labor required to setup, maintain, and harvest big tanks is substantial. Therefore it is very important to go into these studies with questions that were derived by testing at other scales (both lab and field).

Pond trials

Treatment of individual ponds to determine efficacy of a product or use rate can provide valuable information to both practitioners and researchers (Figure 1). The problem with using ponds when conducting aquatic plant research is that each pond rapidly develops its own biological history and "personality." For instance, if you dig 10 ponds at the same site and use the same source water, at the end of the season it is quite possible that in the absence of management, three of the ponds will support a phytoplankton bloom, three ponds will support macrophytes, two will support filamentous algae, and the other has problems holding water. Subsequently, the fish and invertebrate communities that develop under these conditions can also be very different. As time goes on, some ponds will remain fairly stable, while others will swing greatly between macrophytes, phytoplankton, and filamentous algae. We have tried planting target plants in drawn-down ponds, but getting equivalent levels of growth of the target species of interest across multiple ponds is difficult. Furthermore, assessment of efficacy is challenging due to the uneven nature of the growth across ponds. We are often left with visual assessment as the main metric. While visual assessments can be a useful to compliment "hard" data (plant density, biomass, etc.), using the "eye test" alone to recommend field treatments is risky. All of the factors noted above make treatment replication in ponds problematic. Ponds have played a valuable role for conducting herbicide dissipation trials, but have yielded limited publications when using them for replicated efficacy trials on submersed vegetation. If the project requires replication, it is better to use surface-to-bottom curtains to separate a single pond into two cells than to compare two different ponds. This creates an untreated control side and a treated side that were presumably very similar prior to application of the management technique. Another factor that is often underestimated is the amount of effort and time dedicated to data collection in a pond study. Given the often confounding data that pond studies produce, developing a practical sampling strategy on the front end is highly recommended. Recent research using large containers with established submersed plants placed into ponds has proven to be effective for short-term efficacy evaluations in in southern Florida. This approach allows for evaluating efficacy in a more natural setting, but it is quite labor intensive.

While ponds do not easily lend themselves to replicated trials for publication, they have been invaluable for demonstration of efficacy or of a novel treatment strategy prior to making field recommendations at a larger scale. Most of the recently registered herbicides (2005 to 2014) were evaluated at the pond scale for efficacy and selectivity. In some cases multiple ponds were treated with a selected herbicide and biomass data were collected (e.g., Lewisville Aquatic Ecosystem Research Facility); however, treatment rates were not replicated. In other cases, private applicators and registrants in conjunction with third-party researchers (i.e., academia and government agencies) will evaluate a new strategy on ponds to determine if effective control can be achieved. This type of coordinated effort can occur all around the country. As noted, these demonstrations have been highly valuable in determining whether to proceed to the field, but they have also been valuable in formulating better research questions for replicated testing at the mesocosm scale.

While pond sites have yielded limited success when used for replicated efficacy trials on submersed vegetation, they play an important role in herbicide fate and dissipation trials. Typically, these sites utilize constructed ponds that are similar in morphometry, i.e., size, depth, volume and gross physical characteristics, and they support an active and sustainable biological community. The fate and dissipation trials are usually conducted in two or three of these similar ponds per trial, and may be conducted in different regions of the United States if the pesticide tested is being considered for a national U.S. Environmental Protection Agency (USEPA) Section 7 label. In addition, pond studies may be part of broader national trials under a USEPA Experimental Use Permit Section 5 label. To insure rigorous treatment of sampling and analyses, fate and dissipation studies are conducted under the required USEPA good laboratory practices (GLP) guidelines or GLP-like protocols. These data-rich studies provide information that is used to characterize the fate and dissipation of herbicides in water, sediment, and organisms such as fish and shellfish that are held in specifically designed cages deployed in the ponds. And results are critical for development of valid field studies that are needed to refine aquatic use restrictions on labels. In-house technical reports and open literature manuscripts are produced from the data, which are used to support the registration of aquatic herbicides with the USEPA and/or state regulatory agencies.

Field scale

Field evaluations are discussed in a subsequent chapter and will not be presented here. It is worth reiterating that data developed from field trials, and subsequent observations, can lead to posing germane research questions best evaluated in the smaller scale trials discussed above. Environmental complexity can be reduced in these smaller systems to allow the researcher to address specific interactions of interest.

Linkage of multi-scale studies to operational control

Clearly, the foundations for developing environmentally compatible use patterns to manage submersed plants with herbicides rests in the linkage of properly designed and scientifically sound studies of various scales. These studies cover a broad range of venues, including growth chambers, mesocosms, ponds, and field-verification sites. All of these investigative scales have assets and pitfalls. If these attributes are not carefully considered by investigators, research results might not reflect the true nature of herbicide–plant interactions, leading to faulty recommendations for field applications. However, the development of strong data sets derived from linking such studies will help ensure successful operational herbicide treatments.

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