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Propagation methods of submersed, emergent, and floating plants for research

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INTRODUCTION

Propagation of aquatic plants is the first step in initiating a research trial or establishing a population of plants for future experimentation. Although invasive and native aquatic plants (macrophytes) can quickly occupy an aquatic system with little to no assistance from humans, wildlife, or other means, achieving plant establishment in an artificial or closed system (i.e., tanks or aquaria) when it is desired may be difficult. Prior to conducting a laboratory, growth chamber, greenhouse, or mesocosm trial, utilizing healthy aquatic plants is key to a successful trial. Establishing plants in a conducive environment where healthy growth is promoted is necessary for plant establishment and ultimately a successful research trial. Knowing how to propagate and culture aquatic plants will save valuable time and minimize or eliminate wasted efforts for new/first-time aquatic researchers. This chapter will provide useful information on how and how not to propagate submersed, floating, and emergent aquatic plants.

PROPAGATION

Submersed plants

In general, submersed (or submerged) aquatic vegetation (SAV) can be more time consuming to establish under experimental conditions compared to free-floating and emergent species. Submersed plants are most often rooted in sediments, with the majority of the plant body at or below the water surface. If plants are removed from water for an extended period of time, desiccation will occur because leaves and stems of SAVs typically have a limited or no cuticle. When collecting these plants from a field location for use in experimental systems, it is important to keep SAVs in water or wrapped in wet paper towels during transportation to the laboratory to avoid desiccation. Examples of SAVs used for aquatic research include: hydrilla (*Hydrilla verticillata*), Eurasian watermilfoil (*Myriophyllum spicatum*), eelgrass (*Vallisneria americana*), fanwort (*Cabomba caroliniana*), coontail (*Ceratophyllum demersum*), southern naiad (*Najas guadalupensis*), Egeria (*Egeria densa*), and pondweeds (*Potamogeton* spp.).

Substrate. Although some SAVs may be lightly rooted or suspended in the water column (e.g., coontail), most SAV species thrive when rooted into the substrate (i.e., hydrosoil, soil, or sediment). Submersed plants can obtain nutrients from the substrate or water column. The sediment is important for anchoring plants as well as providing a source of nutrients. Soil bulk density can influence the growth of aquatic plants because high or low sediment densities can result in nutrient deficiencies. In general, fine-textured, porous, or noncompacted substrates with low to moderate organic material such as topsoil allows for healthy root development and nutrient availability. Sand can be used for culturing SAVs, but is typically infertile, and nutrients may diffuse into the water column. Premixed commercial media such as Black Kow®, Earthgro®, and other topsoils are readily available in individual bags or in bulk. Potting or gardening soils can also be used as an alternative, but these media may contain additives such as fertilizer, rocks, bark, pine straw, and other undesirable debris that may hinder plant development or ease of harvest. If a commercial potting media is selected, products with premixed slow-release fertilizers should be avoided because the amount of nutrients, consistency, or location within the unit is predetermined by the manufacturer and uncontrollable by the researcher.

In addition to commercially purchased media, lake sediment containing sand, silt, clay, muck, organic matter, or combinations of these soil types can be extracted from field sites. Lake sediments, especially those that have an established population of SAVs, typically provide most, if not all, the necessary nutrients. Unfortunately, collecting this material in the field is labor intensive, time consuming, and requires additional storage at the research facility. In addition, field-collected sediments may contain undesirable plant seed and/or propagules that can germinate in your experimental system, and as a result confounding and compromising your stock population and future experiments. Field sediment can be useful for establishing a stock culture, but should be avoided for most research trials since it is more unreliable than commercial soil with fertilizers and may add a confounding factor (i.e., toxic elements, weed seeds, pH altering, etc.) that could be unaccounted for in the research results. Local nurseries or soil excavation companies may deliver topsoil, sand, or other potting media by the truckload to your facility. If self-mixing these ingredients to create your own substrate in-house, start with a ratio of 50 : 50 sand : soil and adjust accordingly to improve nutrient release, root development, and other variables.

Containers. The size and type of tanks and growth containers (pots or planting containers) varies depending on the type and duration of your experiment, but should be large enough to accommodate the species being propagat-

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ed. Large fiberglass or polyethylene tanks (≥ 500 gallons) are very durable and allow the plants to grow unimpeded, similar to field conditions. However, outfitting your facility with several of these cumbersome tanks can become costly. Using smaller Rubbermaid garbage cans (20 + gallons) or stock tanks (50 to 300) is economical and still permits desirable growth. If herbicides are being evaluated in the experiment, the quality, density, and/or type of plastic is important, because some herbicides or other chemicals can bind temporarily or permanently to the tank or pot. Material such as high-density polyethylene (HDPE) should be considered, because some herbicides may bind to low-density plastics, glass or plexiglas, which may prevent you from achieving the target herbicide concentration in the water column. Consequently, unknown lower concentrations may alter results of the experiment. In addition, herbicide binding in the initial trial may lead to future unintended release of chemical into the water column that may result in undesirable plant injury in a future trial.

With regard to pots or cups, using a growth container with no holes is ideal for submersed vegetation, because the substrate can fall through the holes of the container. This material can impede growth, because suspended particles can block light and encourage algal growth because of nutrient release into the water column. Often, pots with predrilled holes are the most readily found, and are cheaper than pots without holes. If this is the case, placing heavy duty paper towels at the bottom or stacking two pots off-center can minimize or eliminate substrate or fertilizer leakage. With regard to pot size and shape, choose a size or shape that is conducive to the tanks and experiment needs. Round pots can be purchased in bulk locally or via internet vendors. Typically, round pots with a volume of 1 to 8L are sufficient to hold ample plant material, but larger-sized pots reduce the amount of space for additional replications or stock plants. Nontraditional containers such as plastic cups or glass beakers/flasks are not typically recommended unless a growth chamber or other small-scale trial is being conducted. Glassware or cups are usually more expensive and require extra care to ensure long-term use.

Nutrients. Once a potting media or sediment is selected, nutrients should be added to the sediment (or pot) and/or water column to encourage plant growth. All-purpose, inorganic, slow-release fertilizers (polymer-coated controlled-release fertilizers) such as Osmocote® are ideal for SAVs, because these products provide macro-, secondary, and micronutrients at low rates over an extended period of time (weeks to months). Nutrients from these products are released relatively slowly, and fertilizers vary in the amount or percentage of nitrogen, phosphorus, potassium, and other essential nutrients, but generally meets the plant's requirements for shoot and root development/health. Previous research has determined sediments are the primary source for nitrogen, phosphorus, iron, manganese, and micronutrients, whereas water provides calcium, magnesium, sodium, potassium, sulfate, and chloride (Barko et al. 1991). Slow-release fertilizers should be thoroughly mixed into the sediment or placed at the bottom of the pot/container at a rate of 1 to 2 g fertilizer kg^{-1} soil. Regardless of fertilizer type, products containing nitrogen should not be added directly to the water column, because excessive nitrogen can result in an algal bloom. In addition, a thin layer (1 to 2 cm) of silica, masonry, or builder's sand should be added to the sediment surface after planting to minimize dispersal of sediment and nutrients into the water column, which may lead to an algal bloom.

Water-soluble fertilizers (i.e., Miracle-Gro® and Peters®) instantaneously provide the same nutrients to the water column for shoot uptake as the controlled-release products; however, if the incorrect rate/concentration is applied to the water column, these rapid-release formulations can cause a "fertilizer burn" (scorch) and/or injure appendages because of excessive salts (i.e., nitrogen) and osmotic stress. During the first 2 wk after establishment, SAVs are typically stressed from transportation and placement in a new environment, slow growing, and often fail to utilize most nutrients in the water column. As a result, underutilization of water-soluble products by new propagules may result in an algal bloom. Even under ideal conditions, algae will be difficult to prevent and may become present in the experimental containers throughout the experiment. Planktonic or filamentous algae can limit SAV growth by depleting available oxygen (hypoxia), shading out the desired species, or consuming available nutrients. Because of differences in water quality (pH, conductivity, alkalinity, etc.) and individual species needs, fertilizer type and rate should be tested prior to large-scale use. For example, start off with a small quantity (1 tsp) of a low-rate fertilizer (Miracle-Gro 24–8–16 or Osmocote 15–9–12) and monitor plant growth, injury, and algal growth to determine if this product and rate are suitable. Product or rate adjustment may be required to obtain optimal growth. In addition, some fertilizers have a lower water solubility, necessitating extra agitation or a longer period of time before these products are available for plant uptake. As a result, plants may become chlorotic or weak before the nutrients are obtainable.

To combat an algal problem, inorganic nutrient solutions such as Smart & Barko (Smart and Barko, 1985), Hoagland's growth media (Hoagland and Arnon 1950), and Andrew's medium are ideal for mixing into the water column to supplement root-applied slow-release fertilizers and limit algal blooms. Smart & Barko also provides a source of bicarbonate, which is utilized by SAVs for photosynthesis in the water column. If Hoagland's is used for plant propagation, sodium bicarbonate ($NaHCO_3$) or bubbled carbon dioxide (CO_2) should be supplemented to provide a source of CO_2. Another way to limit unused nutrients is to increase the number of propagated plants per pot or place more pots per tank/container when initially establishing a plant population. Additional elements/nutrients such as iron or ammonium sulfate could be added to the water column or sediment, respectively, especially when reverse osmosis (RO) or deionized (DI) water is used that is stripped of these essential elements. Plants that are iron deficient will become chlorotic or pale in color within a few days after establishment, and this symptom is generally associated with water that has been stripped of nutrients and/or sediment that fails to provide sufficient iron.

Water source and water quality. The condition and source of water is more critical for SAVs than floating and emergent species. Because SAVs have limited or no leaf cuticle, cells are able to readily absorb water, nutrients, and gases directly from the surrounding water. Unconditioned water from a municipal, well, or pond source may contain elements such as magnesium, calcium, chlorine and iron that are toxic or at undesirable concentrations, or may contain suspended sediments that may hinder plant growth. Most of the aforementioned elements will likely slow growth or precipitate on the leaves; however, chlorine will severely injure and could kill the plants rather quickly. Therefore, selecting a chlorine-free water source will aid in quickly establishing plants and ultimately keeping them flourishing for a prolonged period of time. Plants that are cultured in environmental growth chambers and greenhouses can be grown in DO or RO water, which is water that has been filtered through a purification system to remove impurities. If DO or RO water is used, Hoagland's solution (plus sodium bicarbonate) is essential because nutrients have been completely removed from the water and SAV growth would be completely dependent on sediment applied fertilizers. If a filtered water system is unavailable or not required, well or pond water can be utilized, but these sources should be laboratory tested for beneficial and toxic nutrients prior to plant establishment.

The water pH at which a particular plant thrives will vary depending on the species. Species such as hydrilla thrive when the pH of the water is > 8.0, whereas fanwort grows best when the water is more acidic (4.0 to 6.0). Established hydrilla can naturally alter (increase) the water pH by utilizing CO_2 and HCO_3 to suit its needs. The pH can be artificially and safely manipulated in by a variety of ways. Regardless, if lowering or raising the pH, the additive must be safe to plants and should be slowly introduced to the water to prevent an extreme or permanent change in pH that can only be fixed by adding another chemical or starting over (if possible). Lowering the water pH can be achieved by adding acid (acetic or hydrochloric), sulfur, alum, peat moss, or bubbling CO_2. The use of acids are generally rapid but not long-term solutions, since the pH will rebound within a few hours or days, whereas sulfur, alum, and peat moss may offer days to weeks of lowered pH. The key is to use an additive that is buffered and will keep the pH stable for an extended period of time, which is important for those species that cannot keep the pH low. On the contrary, there are fewer options to increase the water pH rapidly and safely. Nutrient solutions such as Smart & Barko and MES [2-(N-morpholino)ethanesulfonic acid] can alter the pH and provide long-term nutrients.

Over time, the water may become depleted of nutrients and/or stagnant, especially if the plants remain in the same tanks for several weeks or months without the addition of any nutrients. Adding a small amount of nutrients or fertilizer directly into the pots/container can improve plant health, especially for mature/established plants. If required, the water can be exchanged as needed or plants moved to new tanks to encourage growth as well; however, this is generally not required if nutrient solutions such as Smart & Barko and water are continuously added to plants cultured in laboratory or growth chamber settings. On the contrary, plants grown in a mesocosm setting may receive water from rainfall or can receive periodic or continuous water via a flow through system from a well, pond, or other source. These sources will limit stagnant conditions and/or provide nutrients. Slow-release fertilizers will provide nutrients for an extended period of time, whereas water-soluble fertilizers are typically added every 4 to 8 wk. Regardless of fertilizer choice, unhealthy plants in the stock tanks or reference tanks (in the research trial) will slowly exhibit deficiency symptoms (chlorosis, stunted/suspended growth, necrosis, etc.) whenever additional fertilizer is required.

An alternative to exchanging water or moving plants to new tanks is bubbling air through aquarium air stones. This technique provides circulation, minimize stagnation, and is low maintenance. Water and nutrient exchange requirements will be dependent on the source of plants. Stock tanks that are not being used for research trials have the luxury of water/nutrient exchange on an as needed basis. Conversely, plants exposed to a long-term herbicide treatment or biological control agent must remain undisturbed or with minimal influence to dilute the chemical or living organism.

Plant selection. Regardless if the plants are field collected or obtained from stock/culture tanks, plants with healthy apical meristems (i.e., shoot apex or tips) or growing points should be selected for propagation of hydrilla, milfoils (*Myriophyllum* spp.), and other SAV species that produce new growth at the point furthest from the substrate. Propagating plants from stem fragments with missing meristems will grow at a much slower rate and may not survive. Use a ruler or other measuring device when selecting and cutting apical stem segments to maintain consistent size. Typically, apical meristems cut to lengths of 10 to 20 cm is sufficient; however, longer apical stem segments can be used. Regardless of stem length, plants of the same size should be selected to promote uniformity so that plants reach maturity at the same time. Also, the number of plants/stems per pot will depend on the size of the pot or planting container. Adding too many stems to a smaller pot can cause overcrowding, whereas using only one stem in a larger pot can require a longer period to achieve the desired level of biomass. If propagating eelgrass or other SAVs that are planted with preexisting roots or produce runners and/or rhizomes, planting one or two ramets or plantlets will be sufficient for establishing these species. On the contrary, hydrilla, milfoil spp., coontail, pondweed spp., or other plants can be planted with 3 to 6 stems per pot, but stem quantity will depend on pot size. There are no finite guidelines for the number of plants required per pot, but previous publications in the Journal of Aquatic Plant Management can provide insight into planting details for various laboratory, greenhouse, and mesocosm trials (Getsinger et al. 1994, Netherland and Getsinger 1995, Glomski and Netherland 2008).

An alternative to using stem segments to start a population would be to use seeds or tubers. Hydrilla, eelgrass, or sago pondweed (*Stuckenia pectinata*) tubers can be used to initiate a population, because plants will start at the same size and reach maturity at approximately the same time. Hydrilla tubers, which are actually subterranean

turions, can be collected from the substrate of plants cultured in stock tanks or field collected. The latter is very labor intensive and time consuming, but could provide an ample supply, because hydrilla produces up to 6,000 tubers per m^2 (Sutton et al. 1992). Sago pondweed tubers and the seeds of other annuals and perennial SAVs can be purchased from various vendors on line and throughout the United States. If starting plants from tubers, you should select tubers of similar weight and equal size to reduce some of the variability. Tuber weight and size can vary substantially; therefore, if you require uniformity in your experimental design, you should eliminate this source of variation initially. Previous research has been carried out by Slade et al. (2008).

Planting. After preparing sediment and water and selecting plants, planting can commence. Depending on the moisture content of the sediment, a small amount of water may be necessary to make openings in the substrate for ease of planting and to prevent injury or stem breakage. Insert stem apices approximately 5 cm into the substrate, then push sediment around the stem to secure it in place. Continue adding stems or plants until the desired quantity has been reached. If planted correctly, the growing point or apical should be pointing upward. If establishing a species with preexisting roots, the roots should be planted similarly, but do not bury the stems too deep. Regardless of plant type, apply a 1 to 2–cm layer of masonry, play, or silica sand over the sediment to limit nutrient exchange from the pot and aid in plant anchorage.

Place the pots/cups into the tanks, aquaria, or other experimental unit immediately to prevent desiccation. Similar to the number of stems planted in each pot, the number of pots will depend on tank size, replication, and other experiment requirements. Because of stress from transportation and planting, the newly established plants may require an extended period of time (1 to 3 wk) before stems elongate, leaves form, and roots develop. It is important to be patient during this time and resist the urge to discard the plants and start over. If there is no noticeable growth via elongation and/or development of appendages within approximately 4 wk after establishment, the trial should be re-initiated. A few individual stems or pots of plants may die or fail to grow properly, but complete failure is highly unlikely.

If seeds or tubers are chosen as the method of plant propagation, similar methods can be used to initiate a plant population. Prior to planting, the seeds can be submerged into a bleach solution (ca. 5%) to reduce periphyton levels or kill other unwanted algae, bacteria, or detritus. After the material has been briefly surface sterilized ($<$ 30 min), place the seed or tuber 2 to 4 cm below the sediment of the preprepared pot and cover with sand. More than one seed or tuber can be planted per pot, especially for plants with small seeds/tubers and plants that are small. An alternative method is to place tubers in water for 1 to 2 wk, then select germinated tubers of similar size. Finally, transplant the germinated the tubers into pots with soil. This method may increase uniformity and success rate.

Pest management. Invertebrates, especially insects and snails, are very detrimental to aquatic plants if left unmanaged. These herbivores are relatively small and may go unnoticed for an extended period of time. Aquariums and tanks are a suitable environment for invertebrates to thrive in because these systems are closed off and self-contained. Because of the limited amount of vegetation present in these containers, the undesirable species will rapidly use the plants as a food source or for reproduction purposes. Prior to propagating, plants should be thoroughly rinsed to remove unwanted pests in the egg, larval, pupal, or adult growth stage. Plant-feeding insects, including those in orders Coleoptera, Diptera, Lepidoptera, and Trichoptera, are highly problematic to SAVs cultured in mesocosms, greenhouses, and growth chambers and include *Paraponyx* and *Hydrellia* spp.

Insecticides and molluscicides can be beneficial to provide initial and/or sustained control of hitchhikers or later introduced pests. However, despite some of these chemicals being highly efficacious against insects and snails, products including malathion, carbaryl, and niclosamide can be toxic to plants if applied at higher doses. Because the susceptibility of plants to insecticides and molluscicides will vary by species, low concentrations should be administered to a small group of plants prior to application to the entire population. Alternatively, products such as Bt (*Bacillus thuringiensis*) and temephos are less phytotoxic to aquatic plants, but the control spectrum is limited. Regardless of product choice, the water should be treated immediately to eliminate pests that traveled with the propagated plant, and it should be treated periodically to eliminate any new introductions that could arrive at a later date. Due to the potential negative impacts of insecticides, careful consideration should be used before administering these products to the plants in a research trial. Test these products on plants cultured in stock tanks prior to application during the experimental phase of the project. An alternative to chemical control is to stock low densities of mosquito fish (*Gambusia affinis*) (Moyle 1976), sunfish (*Lepomis macrochirus*), loaches (more than one genera) or other small vertebrates. These organisms should be stocked in low densities to avoid consumption, harborage, or damage to the plants you are culturing.

Intangibles. In addition to the amending or altering the sediment, water, or nutrients to encourage healthy growth, there are some additional tips that may aid in successful SAV establishment. Air bubblers can be placed in each experimental unit or stock tank to facilitate water circulation and prevent stagnation. If the tanks or tanks where the SAVs will be cultured are clear, opaque, or light in color (i.e. white), excessive light can penetrate the unit at all sides and encourage lateral or bushy plant growth. Wrapping the sides of experimental tanks (i.e. glass aquaria) in dark plastic or painting the outsides of the container with a dark color will simulate a pond or lake environment and encourage vertical plant growth towards the light, which is more natural and important for species such as dioecious hydrilla and milfoils.

Aquatic plants use light, carbon dioxide, and water to produce food for photosynthesis. In particular, light quantity and quality will influence growth. Plants grown outdoors in mesocosms or in greenhouses rely on natural

sunlight unless artificial lights such as ultraviolent, fluorescent, or incandescent bulbs are supplemented during short days. High levels of sunlight can be beneficial during the initial establishment of SAVs. However, high quantities of light can also result in "bleaching" of the apical meristem once plants reach the surface of the water. Growing plants under shade cloth, netting, or other light-restricting canopies that reduce sunlight by 30 to 50% can protect the leaves and stems growing at the water surface. Regardless of light-restricting material utilized, plants shouldn't be grown under > 70% shade, because slow or inhibited growth can occur, which may alter research results.

Growth chambers are typically equipped with the aforementioned artificial lights, and the quantity can be adjusted electronically, so injury from too much light is not a problem. The problem is usually associated with not replacing older light bulbs that are producing low light intensities. Regular maintenance and bulb replacement can aid in healthy plant growth. Digital light meters can provide rapid light measurements that can be used to keep plants healthy. High-pressure sodium, metal halide, fluorescent, or other bulbs that emit the appropriate amount of light are necessary for optimal growth. Although full sunlight is 2,000 μmole m^{-2} s^{-1}, most aquatic plants require significantly lower levels and can thrive at 250 to 1,000 μmole m^{-2} s^{-1} under closed conditions. In addition, the photoperiod (i.e., light : dark cycle) should be adjusted to accommodate plants grown under the appropriate seasonal conditions. For example, the light : dark cycle should be 14 : 10 h or 12 : 12 h for warm weather or summer-like conditions, whereas natural or simulated winter conditions require a shorter day length. Regardless of desired photoperiod, utilizing a system with automatic on/off lighting capabilities can enhance plant growth and will be easier for the researcher to maintain.

In addition to SAVs requiring dissolved macro or micronutrients, these species require dissolved inorganic carbon (DIC), which includes carbon dioxide, carbonate, and other forms of carbon (Madsen and Owens 2000). The availability of carbon in the water column and diffusion of this element from the air into the water column is limited (Barko et al. 1986); therefore, some SAVs utilize bicarbonate as a carbon source in the water (Raven 1970, Bowes and Salvucci 1989). Fortunately, most of these nutrients can be obtained from the substrate or supplemented directly into the water column via fertilizer or nutrient solution (Madsen and Owens 2000).

Free-floating plants

Floating aquatic plants grow on the water surface. Because a substantial portion of the stems, shoots, leaves, flowers, etc. are situated out of the water, desiccation is less of a problem compared to SAVs, and is only an issue if the plants are allowed to remain out of water for an extended period of time and the roots dry out. Examples of free-floating plants include water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), duckweed (*Lemna* spp.), water fern (*Azolla caroliniana*), giant salvinia (*Salvinia molesta*), and common salvinia (*S. minima*).

Substrate. Floating plants obtain nutrients directly from the water column through roots or other appendages located at or below the water and do not require substrate for survival or anchorage. If desired, sediments or soil can be added to the bottom of the experimental container/tank to provide nutrients, buffer pH, or alter water quality.

Nutrients. Nutrients can be applied as slow or rapid release fertilizer directly to the water column. Because free-floating plants are capable of conducting photosynthesis and taking carbon through emergent leaves instead of in the water column, algal influence on emergent species is less severe compared to SAVs and applying either type of fertilizer is acceptable. Specific details on advantages and disadvantages of each type of fertilizer can be found in the "Nutrients" subsection under "Submersed plants." Most commercially available fertilizers containing essential macro, secondary, and micro trace elements can be used to establish and provide long-term stability of floating plants including but not limited to water hyacinth, water lettuce, duckweeds, and water fern. Although the rate of slow-release fertilizer will vary among plants and water quality/chemistry, 0.125 g/L (ca. 0.5 teaspoons/20 gallons) is a good rate to start with when culturing floating plants. Compared to most floating species, giant and common salvinia require different levels of nutrients to promote plant growth. Typically, fertilizers high in nitrogen (28 to 49% N) are beneficial to these floating ferns, but can become limited, bound, or toxic in the water column if applied to water with a high pH (>7.5). Regardless of plant species, fertilizer additions may be required through the duration of your experiment to encourage growth and prevent nutrient deficiency.

Water and water quality. Similar to submersed vegetation, the source and quality of water is important to culturing and establishing floating species. If possible, the water should be free of toxic elements, suspended particles, and other detrimental additives. Most plants, including water hyacinth, water lettuce, duckweed, and water fern, can tolerate water with a pH ranging from 6.0 to 9.0 and do not require amending. Conversely, giant and common salvinia require acidic water (pH < 7.0) and both thrive when the pH is 5.0 to 6.0. If cultured under basic conditions, growth will be halted and plant discoloration (light green or brown) will occur. If the local water has a pH > 7.5, utilize rainwater or artificially lower the pH with peat moss, pine straw, hay, or acid (acetic or hydrochloric) to achieve a desirable pH. If algal blooms are problematic, especially in nutrient-rich waters, dyes can be added to the experimental containers prior to plant propagation. These dyes or colorants inhibit algae growth by filtering out wavelengths critical for photosynthesis (i.e., prevent necessary sunlight from reaching algae) (Glomski and Netherland 2005). As a result, floating plants are able to utilize available nutrients without algae competition until they are able to cover the surface of the containers completely.

Plant selection and planting. Healthy plants of equal number and size should be selected when establishing free-floating aquatic plants. The propagation of floating species is often

accomplished by vegetative propagules, because most floating plants produce limited or no viable seed and this method is easy; the plants establish rather quickly. Water hyacinth or water lettuce are often found growing in large continuous mats of parent and daughter plants (i.e., clones of the parent plants), which make collecting from field locations easy. Once the plants have been collected, break or cut off individual parent/daughter plants from each other at the connecting stolon. The number of plants per tank will be dependent on how much material is required for the research and how fast you need the plants to establish or reach maturity. If the tank is crowded initially, competition will ensue and rapid plant growth will generally occur. Once maximum horizontal growth or capacity has been achieved, stem and leaf elongation (i.e. vertical growth) will occur.

Small floating species such as duckweed, water fern, or watermeal are difficult to measure or count quantitatively because of their small size, which can be problematic when a large quantity are required to initiate a trial. If small containers and small quantities are required, counting can be accomplished, but is often time consuming and not accurate. An alternative to counting a large population of these small species can be achieved by collecting the plants in a fine mesh net, colander/sieve, or other collection device with small holes. Excess water should be removed by paper towel, salad spinner, or dripping dry for an extended period of time. After removing excess water, weigh the plants on a digital scale with two or three decimal places to obtain a specific amount of plant material or desired fresh weight. Although fresh weight is not as accurate as dry weight, counting plants, or cutting plants to the same stem length, this method provides a suitable alternative when establishing small floating aquatic plants. An additional method (also less accurate) to establish small floating species is to use a small scoop (teaspoon or tablespoon) or cup and fill with the desired plants to estimate an equal starting population. Although the exact quantity of plants will vary in each experimental container, this method provides a rapid technique for initiating a trial or setting up a stock population.

Pest management. Insects, and to a lesser degree snails, can decrease or eliminate a population of floating plants, especially in closed conditions (i.e., greenhouses) where no other food sources exist. The water hyacinth weevil (*Neochetine eichhorniae*), water lettuce weevil (*Neohydromonus affinis*), salvinia stem borer (*Samea multiplicalis*), aphids (*Aphis* spp.), white flies (various members of the family Aleyrodidae), spider mites (various members of the family Tetranychidea) and other insects are easy to locate on floating plants, because the majority of the plant's biomass is found outside of water. Insecticides such as malathion, carbaryl, niclosamide, Bt, temephos, or other contact and systemic treatments should be applied regularly. In addition, products with different modes of action should be rotated to eliminate the development of insecticide resistance. As previously described in the "Pest management" subsection for SAVs, plant susceptibility to insecticides will vary, so test out new products on small groups of plants prior to application to the entire population.

Emergent plants

Emergent aquatic plants are rooted in sediments with the majority of their stems, leaves, and flowers extending partially or fully out of the water. Emergent plants such as maidencane (*Panicum hemitomon*), torpedograss (*Panicum repens*), jointed spikerush (*Eleocharis interstincta*), giant bulrush (*Schoenoplectus californicus*), duck potato (*Sagittaria lancifolia*), and pickerelweed (*Pontederia cordata*) are often referred to as shoreline plants, and are typically found growing in water that is 4 to 5 ft deep or less. Emergent species are cultured similarly to SAV, but require less maintenance.

Substrate. Emergent aquatic plants rely on the substrate for nutrients and anchorage. Similar to SAVs, emergent species can grow in a variety of substrates/sediments including sand, potting soil, topsoil or other high-quality medium. See the "Substrate" subsection for SAVs for more specific details on choosing a potting media. Because emergent plants have a significant portion of their biomass out of the water, the substrate must be sturdy/firm to support the plant. Using pots with holes in the bottom is optional and not necessary, but does allow for root expansion, especially if larger and more robust species such as bulrush spp. (*Schoenoplectus* spp.) or spatterdock are planted into smaller pots.

Nutrients. Ideally, fertilizer should be placed at the bottom of the pot if there are no holes or mixed in the sediment if the pot has holes for optimal emergent plant growth. Slow-release fertilizers, as previously described in the SAV *Nutrient* subsection, are ideal for providing long-term nutrients. Rapid-release products can be applied at low rates to the water column and absorbed by plant appendages above and below the substrate, but nutrient absorption is more beneficial if placed in the substrate to limit algae growth.

Water and water quality. Similar to SAVs and floating plants, emergent plants should be cultured in water that is free of toxic elements, suspended particles, and other detrimental additives. Most emergent plants can tolerate water with a pH of 6.0 to 9.0 and often below and above this range. Culturing media or nutrient solutions (e.g., Smart & Barko or Hoagland's) are not as critical when establishing emergent species compared to floating or submersed plants. Emergent plants primarily acquire nutrients from the sediment and are able to use CO_2 from the air for photosynthesis.

Plant selection and planting. Emergent aquatic plants can be field collected for use in laboratory experiments, but this method is time consuming and labor intensive. An alternative is to purchase these species from a commercial nursery, but care must be taken to ensure they are not misidentified or hybrids. Using the wrong species will provide false/inaccurate results and impact your research. Seek out a reputable nursery that has been selling plants commercially for an extended period of time and list plants by scientific and common name. Also, if possible, visit the vendor to see which species are available for purchase.

Nursery stock plants are typically small/immature, similar in size, and suitable for propagating a new population. Irrespective of source and size of material, you should use plants that have healthy stems/leaves and sizable roots,

otherwise the plant will require an extended period of time to establish. Once the substrate and fertilizer are placed in the pot, make a sizable hole that is big enough for the roots and other belowground material. Because of the eventual size of emergent plants, it is recommended that only one plant per pot be placed. Completely cover the roots with substrate to firmly anchor the plant in each container. The sediment should then be covered with a 1 to 2–cm layer of sand (masonry, play, or silica) to limit nutrient exchange from the pot and aid in plant anchorage.

The newly established plants should be placed immediately in a small volume of water to cover the substrate surface and prevent plant desiccation. Although emergent plants can die rather quickly from lack of water, placement in water that is too deep and above the longest leaf can be detrimental as well. Water-level manipulation is critical for a newly established emergent plant. If using pots with holes in the bottom, there should be enough water to keep the roots and substrate saturated for the first couple of weeks after planting. However, if plants are established in pots without holes, the water level will need to be positioned just above the top of the pot to keep substrate and roots saturated. Regardless of pot type, increase the depth of water slowly (ca. 1 to 2 more inches per week) over to time to allow stems and leaves to remain out of the water to prevent the plant from "drowning." The increase in water depth will be dependent on the growth and elongation of each individual plant species, time of year, maturity, etc. To date, no water-level tolerance data has been collected; therefore, all species should be "coddled" until mature and ready for research. If the water level is properly placed initially and slowly raised over time, the roots will develop and anchor the plant.

The time required for emergent plants to reach maturity or maximum height will be dependent on species as well as initial plant size. After establishing the emergent plant in a pot, do not cut, trim, or remove healthy vegetation (stems or leaves). Young/immature or newly established plants often possess a limited quantity of leaves and stems when first established. The leaves and stems are required for growth and photosynthesis; thus removing these appendages may delay growth or result in plant death.

Pest management. Emergent aquatic plants should be regularly treated with insecticides in a similar manner as floating plants. See the "Pest management" subsection in the "Floating plants" section for more specific details and products.

Rooted floating-leaved plants

Floating-leaved plants such as the floating hearts (*Nymphoides* spp.), waterlilies (*Nymphaea* spp.), spatterdock (*Nuphar advena*), and American lotus (*Nelumbo lutea*) are rooted in sediment, but have leaves that float on the water surface. Some of these species produce roots and/or daughter plants (i.e., clones) from the parent plant's floating leaves.

Substrate. Similar to emergent and submersed aquatic plants, the roots should be anchored into substrate for nutrient extraction and anchorage. Floating-leaved species can grow in a variety of substrates/sediments, including sand, potting soil, and topsoil. See the "Substrate" subsection in SAVs for more specific details on choosing a potting media.

Nutrients. Nutrients should be administered into the substrate for root uptake, but can also be supplemented directly to the water column for uptake by daughter plants located outside of the pots. Slow-release fertilizers provide essential nutrients at low doses over an extended period of time, whereas water soluble or rapid-release fertilizers are instantaneously available for the plants. As previously discussed in the "Nutrients" subsection in "Submersed aquatic vegetation," water-soluble products may cause a "fertilizer burn" if too high of a concentration is applied or result in an algal bloom or if the plants are unable to utilize the nutrients fast enough.

Water and water quality. Floating-leaved species have similar water and water quality requirements as emergent and free-floating plant species; therefore, see the previous "Water and water quality" subsections for further details.

Plant selection and planting. Plants classified as floating leaved can be field collected or purchased from a plant nursery. Although it is considerably more cost efficient to travel to a field site, finding floating-leaved plants such as spatterdock, lotus, lilies, and other species with root structures not anchored to the substrate and/or of consistent size will be time consuming. However, several species can be found free-floating with exposed roots or daughter plants, as well as being found in shallow areas where roots can be dug up easily. If a field site containing the desired plant is unavailable, the best approach is to purchase plants directly from a nursery. The quantity and size of plants can be requested and will arrive with roots in a timely manner. After plant selection, place the plant into the prepared substrate and pot. Fill the hole with enough sediment to cover the roots and then place a 1 to 2–cm layer of sand on top. Immediately place the potted plant into water. Similar to emergent species, a floating-leaved plant (i.e., spatterdock) should not be submerged into deep water for the first few days. The water level should be maintained at the surface of the leaf and slowly increased as the plant grows/elongates. See the subsection on "Plant selection and planting" under "Emergent plants" for further details.

An alternative to starting with young or mature plants is to start the population from seed. Similar to emergent plants, place the floating-leaved plant seed into a pot partially filled with substrate and cover with additional substrate and sand cap. Despite immediate germination by many species/seeds, plants including lotus must have seeds scarified prior to placing into the substrate. Scarification involves removing the outer coat by sandpaper or other rough surface to allow water penetration and seedling germination. An alternative to planting scarified lotus seed directly into the substrate is to float seeds for several days and only select germinated seeds. This approach can greatly increase planting success.

Pest management. Emergent aquatic plants should be regularly treated with insecticides in a similar manner as floating plants. See the "Pest management" subsection in "Floating plants" for more specific details and products

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