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Designing and using phenological studies to define management strategies for aquatic plants

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INTRODUCTION

Aquatic plants are vital components in freshwater systems, as they form the base of the food web and are responsible for much of the primary production in water bodies. However, in many locations throughout the world, these native plant communities are being invaded by nonnative species that are introduced from other areas of the globe. Once there is a perceived impact to the water resource, management plans are often developed and initiated to manage the nonnative species. Management approaches have generally been reactionary attempts to mitigate the perceived problem. In some instances, these approaches are based on trial and error or influenced by public opinion and/or user groups; therefore, little attempt is made to understand the factors that are influencing the invasion or why the management approaches may or may not be successful. One such factor that can influence plant response to management techniques is the basic biology of the target plant. Nichols (1991) stated that a biological basis for management is required in that

> All control techniques stress the plant. In the past, the timing of control efforts was based on the demands of the user, not the life cycle of the target species. To increase efficacy, the stress must be maximized. Research that concentrates on resource accumulation and allocation for nuisance plants under a variety of conditions is needed so that treatments can be timed to stress species when they are most vulnerable. This may be at a point when their energy reserves are low, when they are most receptive to the uptake of chemicals, or at some other critical point in their life cycle. Easily recognizable indicators of physiological status are needed to properly time management efforts.

The basis for gaining an understanding of the target plant's life cycle is phenology. Phenology is defined as the study of the seasonal timing of critical life stages in plants, whereby the allocation of biomass and other resources such as carbohydrates are fundamental aspects during these life stages. In most cases, aquatic plants will display distinct seasonal patterns in biomass and carbohydrate allocation, wherein storage peaks and then is depleted after plant growth has occurred (Madsen 1991). Understanding these annual growth cycles will allow for the determination of seasonal reductions in energy reserves. A number of phenological studies have been conducted on aquatic plants over the years. Yet few have tried to time management practices to coincide with seasonal low points in biomass or stored carbohydrates, thereby exploiting reduced energy reserves to enhance efficacy (Madsen 1997a).

Knowing when and where resources are being stored in aquatic plants can offer insights into the efficacy of management options, and the potential regrowth capability of plants after management techniques have been implemented. Therefore, the purposes here are 1) to describe how to design and conduct phenology studies in both the mesocosm and field, and 2) to discuss using phenological data to target vulnerable times in nonnative species.

DESIGNING A PHENOLOGICAL STUDY

Mesocosm studies

When designing a phenology study under mesocosm settings, it is important to consider the species that will be evaluated, its growth form (submersed, floating, emergent), and the length of time the study will be conducted. This information will determine the size of the tanks needed to grow the target species best, the number of tanks needed, and how many plants will be needed for sampling purposes throughout the study. Tanks ranging from 378 to 5,600 L work well for these types of studies. The 378-L (length 135 cm × width 79 cm × height 64 cm) tanks work well for rooted submersed plants, as these species will be planted in pots and these pots can be removed during each sampling interval. However, longer-term studies (> 1 yr) would require a greater number of the 378-L tanks in order to have enough plant material for routine harvesting. In general, eight pots (3.78 L, 15.2-cm diameter) of submersed plants can be placed into a 378-L tank and not be overcrowded. Overcrowding can cause intraspecific competition as the plants grow and may impact biomass determinations over time. Moreover, larger or smaller pots will affect the overall number of plants that will fit into a tank.

Conversely, using larger tanks gives a more realistic growth environment (greater area and volume) and a smaller number of tanks are needed to support biomass sampling. A smaller number of tanks also require less space to conduct the study. In 5,600-L tanks approximately 75 to 100 pots (3.78 L, 15.2-cm diameter) can be placed into each tank. Because phenology studies are long-term studies, all rooted plants should have ample nutrients and a source of

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inorganic carbon to support growth. In order to observe any trends in life-history characteristics, it is recommended that a study be carried out for 2 to 3 yr. For most submersed plants the 3.78-L pot is large enough to prevent the need for repotting. If species are studied that have a large rhizome structure, like waterlilies, the plants will likely need to be repotted each year. It is also recommended that environmental data be recorded throughout the study. Factors such as light intensity, photoperiod, ambient temperature, water temperature, and nutrients would impact the growth of aquatic plants.

Example 1. Conducting a phenology study with the use of curlyleaf pondweed (*Potamogeton crispus*) in a mesocosm (adapted from Turnage et al., in press)

Curlyleaf pondweed turions were collected from a field population and transported to the mesocosm facility. Turions were floated in a shallow container filled with water until sprouting occurred. Two sprouted turions were planted into 3.78-L containers filled with sediment. The sediment was amended with Osmocote® fertilizer (19–6–12) at rate of 2 g/L of soil to maintain plant growth. A total of 72 potted containers of curlyleaf pondweed were placed into each of three 5,600-L mesocosms for a total of 216 pots. Plants were allowed to grow for 1 mo prior to the first harvest to ensure establishment had occurred. Each month for 24 mo, three containers were harvested from each tank. The numbers of turions were recorded, all turions were harvested, and then both above- and belowground biomass were harvested from each container. Plant tissues were washed to remove dirt and debris, placed into labeled paper bags, and dried at 70 C for at least 48 h. After drying, plant tissues were weighed. Day length and water temperature were recorded for the duration of the study.

Once data have been collected and processed, biomass can be graphed over time to determine growth trends throughout the year. Similarly, figures can be made using each plant structure (i.e., above- and belowground biomass, tubers, turions, submersed leaves, floating leaves, roots, rhizomes, stolons, and inflorescences). Doing this will allow for the determination of specific times when biomass has peaked or when the plant is senescing; additionally, these types of data can aid in determining which plant structures are important for nutrient and/or carbohydrate storage. Statistical models can be used to determine correlations or relationships between plant data and any environmental data that were collected. Using statistical models in this way will allow for the determination of which environmental factors are important at different times of the year and to which plant structure. These data can be useful in the development of management strategies to target weak points in the plant's life history.

Field studies

Mesocosm studies are generally easier to conduct, are easier to control, and have less variability; though some of the realism that can be gained in conducting phenology studies on field populations can be lost in mesocosm studies. Field studies are harder to conduct because of logistics, cost, permitting, landowners, weather, travel, availability of equipment, etc., though field studies will yield more accurate data with respect to the phenology of the target species because samples are being collected from natural populations.

When designing a field study, it is important to understand what species will be sampled and in what geographic areas the sampling will occur. Studies conducted in warmer climates will facilitate sampling once per month all year. However, studies conducted in colder climates may only allow for sampling during the growing season, in which sampling would occur every other week, or every 3 wk, instead of monthly. In the case of curlyleaf pondweed, where growth begins in late fall and continues through the winter months, sampling through the ice would be necessary. Unless there are specific questions regarding potential differences between biotypes or haplotypes, where geographic distance would be important, it is recommended to choose sampling locations that are close enough to one another so that they can be sampled in 1 or 2 d. This would be especially important if the sampling interval is every other week.

In general, 3 to 5 sampling locations are sufficient for a field phenology study. This will ensure that if something were to happen to one of the locations there will still be sufficient locations for sampling to be representative of the true population. Sample locations should be large enough so that destructive sampling will not adversely impact the population over time. Or, if remote sensing is being used, the area of the sampling location should be conducive to the resolution of the imagery being used. Depending upon the species, an adequate sampling area for destructive sampling can range from 0.1 to 1 ha. If lakes or reservoirs are being sampled, multiple locations within the water body can be used for phenology, though there should be adequate distance between the locations, such as sampling in different coves or bays. Sample locations can be arranged inside larger plant beds; for example, a 1-ha plot could be established inside a 15-ha plant bed. Sampling locations should be established in areas that do not receive a lot of disturbance or human use. High-traffic areas will adversely impact plant growth and sampling, and could result in equipment damage especially if environmental sensors are deployed long term. It is recommended to deploy sensors in each sample location to record at least water temperature at regular intervals throughout the study.

Once the sample locations have been established and data sensors deployed, plant sampling can commence. Again, the species being sampled will dictate how and what sampling device to use. The size of the sampling device will in turn dictate how many samples will need to be collected in each location. There will be a trade-off in both collection and processing time relative to the size of the samples collected. For example, a small number of large samples (0.5 to 1 m^2) can be collected, but it will take more time to collect and to process those samples, whereas a greater number of small samples (0.01 to 0.1 m^2) will take less time to collect and to process (Downing and Anderson 1985, Madsen 1993a, Madsen and Wersal 2017). Typically, 8 to 10

samples are needed per site if larger samples are collected. If a 0.1-m^2 sampling device is used, then 15 samples are often sufficient, and 30 samples are needed if a 0.01-m^2 device is used.

Floating plant species such watermeal (*Wolffia* sp.) and duckweed (*Lemna* sp.) can be sampled with the use of a 0.002-m^2 PVC sampling device (Wersal and Madsen 2009). Giant salvinia (*Salvinia molesta*) can be harvested with the use of a 0.01 to 0.05-m^2 quadrat. Species such as water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) can be adequately sampled with the use of a 0.1-m^2 quadrat. Emergent species such as bulrush (*Schoenoplectus* sp.), cattails (*Typha* sp.), and flowering rush (*Butomus umbellatus*) can be sampled with the use of quadrats and divers, though nondestructive metrics such as plant height, culm diameter, or remote sensing may be easier for aboveground estimates. If belowground structures are required, then a coring device or Ekman dredge would be needed. Submersed species such as curlyleaf pondweed, Eurasian watermilfoil (*Myriophyllum spicatum*), and hydrilla (*Hydrilla verticillata*) can be harvested with the use of a PVC coring device (Madsen et al. 2007), diver-harvested quadrats, or the spinning rake method (Johnson and Newman 2011). All biomass sampling should occur randomly within each sampling location unless, as in the case of rooted plants, a depth gradient is important; in this case transects can be established inside the sample location in order to capture depth effects on plant growth.

Example 2. Conducting a phenology study with the use of parrotfeather (*Myriophyllum aquaticum*) in the field (adapted from Wersal et al. 2011)

Parrotfeather biomass was harvested every month from four locations in Mississippi from January 2006 to December 2007. The size of the field locations ranged from approximately 0.1 to 15 ha; however, samples were harvested from only 0.1 to 0.2 ha of each location that contained parrotfeather. At each location, 30 biomass samples ($n = 1{,}880$) were randomly harvested with the use of a 0.018-m^2 PVC coring device (Madsen et al. 2007). Samples were placed in individually labeled plastic bags and stored in a cooler for transit back to the lab.

Biomass samples were transported to the lab, where they were rinsed to remove sediment and debris, and then divided into four categories: emergent shoots, submersed shoots, stolons, and sediment roots. Emergent shoots were separated by cutting the shoots at approximately the third node below the last whorl of emergent leaves. Stolons were considered the horizontal growth below the emergent shoots as both emergent biomass and adventitious roots grew from the nodes along the stolons. Adventitious roots were left on stolons and were incorporated into stolon biomass. Plant structures were placed into labeled paper bags and dried for at least 72 h at 70 C in a constant-temperature oven. Once dry, plant samples were weighed to determine dry biomass in g m^{-2} for each month.

During all harvest times, water depth was recorded with the use of a PVC rod with centimeter delineations for each sample, at all locations, prior to collecting a core. In addition to water depth, pH, and conductivity were recorded once at each site every month with a multiprobe device. Measurements were made directly adjacent to plant stands. A temperature probe was deployed at each of the four harvest locations to record water temperature in 1-h intervals for the 2 yr of sampling. Light profiles in 25-cm increments from the water surface to the bottom sediment were determined monthly at each harvest location with the use of a light meter equipped with an ambient and submersible probe. Incident and submersed light readings were used to calculate percent light transmittance through the water column.

In addition to biomass and environmental data, seasonal starch allocation was determined in parrotfeather tissues. For each set of 30 biomass samples at each location in a given month, and for each tissue type, dried biomass was combined into 3 bulked samples comprising 10 samples each. Combining samples ensured that adequate tissue mass was available for analytical techniques, and to reduce the number of tissue analyses required. The bulked samples were ground with a grinding mill to pass through No. 40 mesh screen (0.42 mm). Approximately 50 mg of the ground sample was transferred into plastic centrifuge tubes for storage and preparation for starch analysis. Starch extraction and determination was conducted with the use of a commercially available starch assay kit (SA-20) from Sigma Aldrich.

Monthly averages for biomass, percent starch, and environmental variables were computed for each site and analyzed together. Data were analyzed by fitting mixed models to determine potential relationships between environmental factors and parrotfeather biomass and percent starch. Total, emergent shoot, submersed shoot, stolon, and root biomass were included as dependent variables. Water temperature, water depth, incident light, light transmittance, pH, and year were included as the independent variables in all models. Site and site $*$ year interaction term were included as random effects in the model to account for their influence on the results. All terms included in the analyses were linear. Data are reported as means ($\pm$ 1 standard error [SE]) and analyses were conducted at a $P < 0.05$ significance level. Data are displayed graphically over time to show trends in growth (Figure 1), or biomass allocation by plant structure (Figure 2).

FACTORS THAT CAN IMPACT DATA COLLECTION AND INTERPRETATION

It is important to remember that the experimental or sampling design will have a profound effect on the overall outcome of the study, and will impact the conclusions offered. If representative populations are not chosen for sampling, or too few populations are sampled, there may be too much variability to capture any relationships that may be occurring or too few data to develop any trends over time. If too few populations are sampled, false conclusions may result because anomalies that occurred in a sample site may not be representative for the species or region. Sample sites (populations) should be selected so that if something happens to one site the entire study is not impacted. For

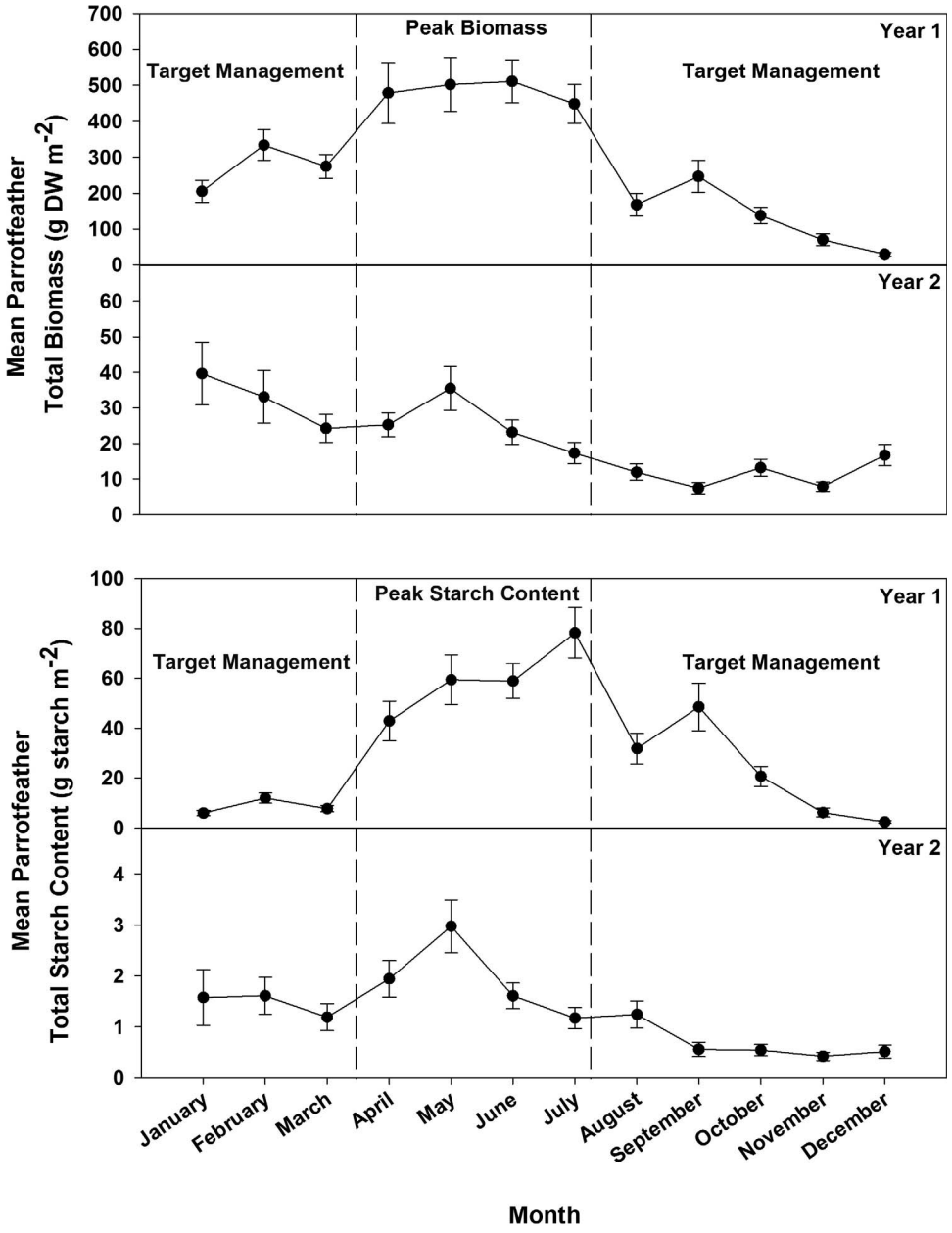

Figure 1. Seasonal phenology and starch allocation in parrotfeather, identifying weak points representing either low biomass or starch accumulation.

example, if all of the sites are located in shallow ponds, these areas are prone to drying up over the course of a hot dry summer, thereby affecting the plants in those areas and impacting the study.

If sampling effort (number of samples taken) or sampling intensity (how often samples are collected) are too low or do not occur enough, it will result in the missing of major life-history trends. Collecting too few samples per unit area will result in highly variable data and reduce the ability of accurately developing trends in life-history characteristics over time. If samples are not collected often enough, seasonal trends may be missed with respect to important phases in the plants' life cycle (i.e., flowering, tillering, seed set, tuber production, etc.).

Additionally, as genetic analyses become more commonplace in aquatic plant management, it will undoubtedly become imperative to have an understanding of which biotype, haplotype, or genotype is being targeted. Phenotypic responses can be influenced by both environmental and genetic factors. For example, consider Eurasian watermilfoil and hybrid water milfoil (Eurasian watermilfoil × northern watermilfoil [*Myriophyllum sibiricum*]); although individuals are from the same species, genetically they are different. The hybrid milfoil has genetic information from both parental types, and therefore, may have a phenology that is different from each parent. If a study has three sample populations of Eurasian watermilfoil and one sample population of hybrid milfoil, the results may not

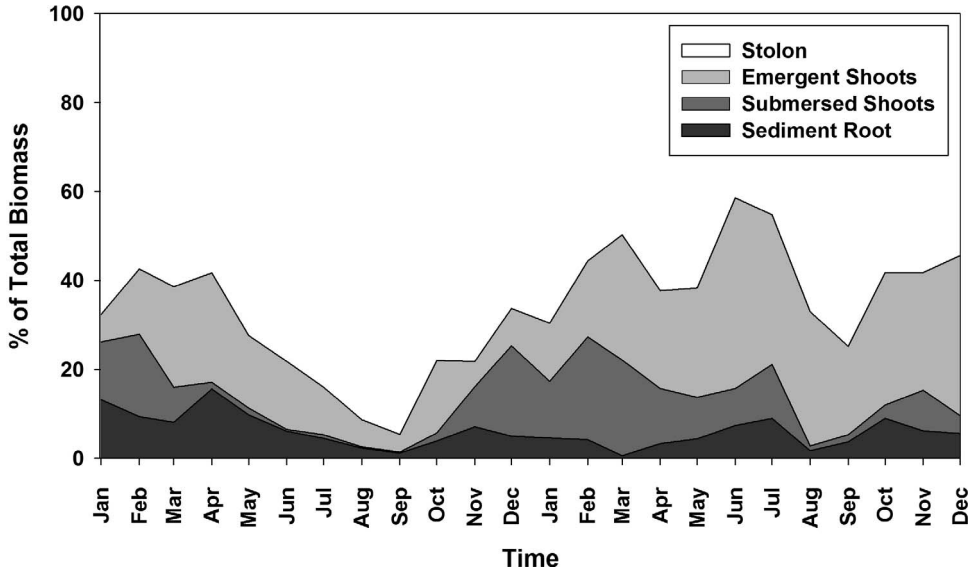

Figure 2. Seasonal biomass accumulation by plant structure for parrotfeather collected from field locations.

be representative of either genotype, especially if phenological timing of key life-history characteristics are different, and thus the interpretation of observed relationships will not be correct.

There are a number of abiotic and biotic factors that can impact the overall results of a phenological study. Because these studies are conducted outdoors, environmental factors will affect plant growth, sampling times, site accessibility, etc.; therefore, there is inherent variability in phenological studies that is difficult to address with many statistical models. Phenological studies are important to discover trends in a population over time accurately, and to elucidate major points in a plant's life cycle. Depending upon the experimental design, statistical models can be used to show relationships between plant growth (biomass, length, carbohydrates) and other factors. An understanding of these limitations when designing a study will serve to help avoid overextrapolating data at the conclusion of the study.

USING PHENOLOGICAL DATA TO TARGET MANAGEMENT APPROACHES

Phenological studies are common in aquatic plant ecology and offer baseline data on the growth patterns of target species (Table 1). These data are necessary when developing management strategies to manage invasive species better and potentially gain greater selectivity for nontarget species. The practical application of this strategy will be dependent upon knowing the phenological cycle and important modes of reproduction of the target plant, and timing management to that cycle. The target plant and its phenology will also influence what management technique is chosen. Management techniques can be targeted to occur during weak points in a plant's life cycle, when it is presumed there would be little stored energy remaining to reinitiate growth, or decreased ability to produce and reallocate new energy stores.

Understanding this dynamic can be beneficial in managing aquatic plants such as Eurasian watermilfoil. Repeated harvesting of aboveground biomass can interrupt carbohydrate storage and allow for greater success in long-term management of milfoil. Timing herbicide applications to target critical points in the life cycle when carbohydrates and biomass are at their lowest can reduce the ability of the plant to recover later in the season. Spring herbicide applications on curlyleaf pondweed will control early-season growth prior to the sprouting of most native species, thereby gaining selectivity (Figure 3). Early-season treatment will also target curlyleaf pondweed during a time when biomass is lower, plants are smaller and actively growing, and turions have not yet been produced. Reducing turion production will reduce year after year recruitment by managing the propagule bank. Furthermore, if early-spring herbicide applications are not feasible, it may be beneficial to wait and treat curlyleaf pondweed in the fall once turion sprouting has initiated. This would allow for targeting a greater proportion of the population that would be contributing next year's recruitment, and most native species have senesced by this time (Figure 3).

Current management strategies for the riparian grass phragmites (*Phragmites australis*) include applying herbicides in the fall to get better translocation of systemic herbicides into belowground tissues as phragmites reallocates its resources from aboveground to belowground tissues. It should be noted that biomass and culm density at this time would be at maximum levels and therefore herbicide coverage would not be optimal, and plants could be missed during fall applications. Therefore, management during the early spring and summer (March through June) would target low points in starch reserves, and target shorter, less-dense plants, making herbicide applications easier.

Much of our understanding of phenological cycles and how they affect aquatic plant management has been gained through smaller-scale studies. However, these studies have

Table 1. Studies that evaluated phenology and/or resource allocation in aquatic plants. The list is not exhaustive, but meant to be a guide to studies that have been conducted under both small- and large-scale scenarios and contain citations for other phenological studies.

Study Citation	Plant Species	Biomass (B), Carbohydrate (C)
Best and Dassen (1987)	*Elodea nuttallii*, *Polygonum amphibium*, *Phragmites australis*	B, C
Best and Visser (1987)	*Ceratophyllum demersum*	B, C
Hodgson (1966)	*Stuckenia pectinata*	C
Kimbel and Carpenter (1981)	*Myriophyllum spicatum*	C
Luu and Getsinger (1990)	*Eichhornia crassipes*	B, C
Madsen (1993b)	*Myriophyllum spicatum*	B, C
Madsen et al. (1993)	*Eichhornia crassipes*	B, C
Madsen (1997b)	*Myriophyllum spicatum*	B, C
Madsen and Owens (1998)	*Hydrilla verticillata*	B, C
Madsen et al. (2016)	*Butomus umbellatus*	B
Marko et al. (2015)	*Butomus umbellatus*	B
Nichols and Shaw (1986)	*Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis*	B
Owens and Madsen (1998)	*Hydrilla verticillata*	B, C
Pennington and Sytsma (2009)	*Egeria densa*	B, C
Perkins and Sytsma (1987)	*Myriophyllum spicatum*	C
Robles et al. (2015)	*Eichornia crassipes*	B
Sytsma and Anderson (1993)	*Myriophyllum aquaticum*	B
Titus and Adams (1979)	*Myriophyllum spicatum*, *Vallisneria americana*	C
Tucker and DeBusk (1981)	*Eichornia crassipes*	C
Turnage et al. (in press)	*Potamogeton crispus*	B
Weldon and Blackburn (1968)	*Alternanthera philoxeroides*	C
Wersal et al. (2006)	*Stuckenia pectinata*	B
Wersal et al. (2011)	*Myriophyllum aquaticum*	B, C
Wersal et al. (2013)	*Phragmites australis*	B, C
Woolf and Madsen (2003)	*Potamogeton crispus*	B, C

provided evidence that targeting weak points in a species life history, or carbohydrate allocation cycle, can impact the effectiveness of management techniques. In operational management programs, the application of management techniques will depend upon location and environmental factors. These factors will drive management decisions as to what techniques will be used and when they can be implemented. It may not always be possible to target a specific species early in its life history, and alternative timings will need to be determined.

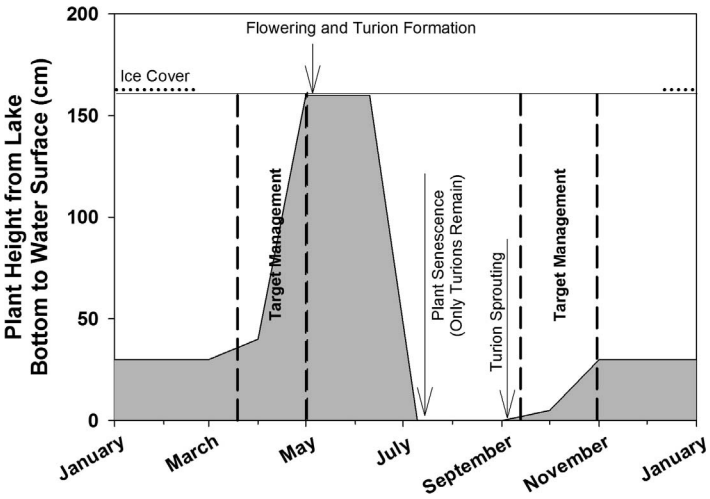

Figure 3. Conceptual diagram based on curlyleaf pondweed phenology for timing management based on seasonal phenology (adapted from Turnage et al. in press).

There are always many factors to consider when deciding upon the proper management techniques to control nonnative aquatic plants. These factors can include economic, social, and environmental issues that need to be addressed when developing a management plan. Management techniques should be site specific, based on environmental factors, and chosen to maximize control of the target species based on phenological cycles. Management decisions should be based upon the desired use and desired outcomes of the habitat being managed.

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