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Protocol considerations for aquatic plant seed bank assessment

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ABSTRACT

Many procedures have been developed to assess seed bank characteristics, and most can be grouped into either of 2 categories: seedling emergence assays or seed separation from substrate. These procedures are particularly useful in predicting vegetation recurrence and determining need for restoration or control strategies through estimates of seed bank attributes (e.g., species composition, density, distribution, and viability). However, due to the lack of a standard protocol for seed bank investigations, researchers must select appropriate methods based on knowledge of different techniques and their potential to accomplish research goals. Here, we review a range of procedures for aquatic/wetland seed bank assessment with regard to difficulty in use, test reliability and accuracy, and laboratory resource (time, space, and labor) requirements. Information to improve sample collection and analysis is provided, and research to enhance seed/seedling identification and knowledge of germination requirements is advised.

Key words: emergence, extraction, restoration, seedling, separation, submersed vegetation.

INTRODUCTION

Interest in reversing declines in aquatic vegetation imposed by ecosystem disturbance (e.g., drought, eutrophication, flooding, disease, overgrazing, and herbicide treatment) have prompted greater focus on aquatic plant seed banks as a possible source of vegetation recovery. These losses in vegetation often require resource managers to decide whether to rely on naturally occurring propagules or transplants to revegetate affected areas. In such cases, examination of the propagule/seed bank can provide useful information on plant species present, their relative abundance in the substrate, and their likelihood to regenerate (van der Valk and Davis 1978, 1979, Leck and Graveline 1979, Haag 1983, Grillas et al. 1993, Crosslé and Brock 2002). Seed bank assays are helpful in revealing where desirable and undesirable species may occur and in determining whether preferred species are absent or are present in low densities. With sufficient forewarning regarding seed bank content, resource managers can take the necessary steps to ensure that cost-effective, environmentally compatible methods for restoration (or control) are implemented in the field. For example, if a formerly vegetated site has an abundant seed reserve of favorable species, then the most efficient way to restore vegetation would be through seed bank recruitment. However, to successfully exploit contributions from seed banks, the potential for regrowth of different species needs to be determined using methods that meet required levels of accuracy and that consider the time, experimental space, and labor available.

While knowledge of seed bank properties (e.g., species content, viability, distribution, density) can aid in predicting vegetation recurrence, obtaining reliable data through seed bank sampling can be exceptionally difficult. One reason is that seed distribution is often patchy, resulting in high statistical variance, particularly with small sample size (n value) and small-scale sampling (Thompson 1986, Bigwood and Inouye 1988, Hammerstrom and Kenworthy 2003). Another reason is that life history patterns may require sampling strategies that address seasonal fluctuations in plant abundance, flowering, seed set, and deposition (Thompson and Grime 1979, Britton and Brock 1994, Hammerstrom and Kenworthy 2003). Furthermore, to assess plant species richness, seed bank samples may need to be exposed to different environmental conditions to break seed dormancy and maximize germination (Baskin and Baskin 1998, Nishihiro et al. 2004a, 2004b). Other considerations involve seed/seedling size and taxonomic identification because aquatic plant seeds and seedlings are usually small and scientific literature provides little detail concerning their morphologies (McFarland, pers. observ.).

Methods used to quantify seed bank properties generally fall into two categories: seedling emergence assays and seed separation or extraction from substrate. Because emergence methods attempt to germinate as many seeds as possible, they may be applied in combination with pretreatments (such as cold-stratification and/or drying and re-wetting) to break seed dormancy (Thompson and Grime 1979, Fenner 1985, Baskin and Baskin 1998). While these procedures can broadly estimate germinable seeds in the reserve, they may underestimate the total seed bank if seeds of some species fail to germinate. In contrast, separation procedures exploit differences in seed size, shape, and density to facilitate removal from the substrate and direct counting of each seed as an individual (Malone 1967, Grillas et al. 1993, Jarvis and Moore 2008). As with seedling emergence methods, separation procedures have strengths and weaknesses, knowledge of which can aid in appropriate and effective method selection.

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The purpose of this paper is to review procedures for aquatic/wetland seed bank assessment with attention to (a) test reliability and accuracy, (b) level of difficulty in use, and (c) utilization of time, laboratory space, and labor. Advantages and disadvantages of each procedure are summarized for selected emergence (Table 1) and separation (Table 2) methods. Ecological considerations for seed bank sampling are also addressed, along with recommendations for research to improve assessment practices.

SEEDLING EMERGENCE PROCEDURES

Seed bank studies have been reported for well over a century, mostly employing emergence procedures to investigate regrowth of terrestrial vegetation such as grassland pastures (Young et al. 1981, Bakker et al. 1991, 1996), agricultural lands (Raynal and Bazzaz 1973, Archibold and Hume 1983), prairies (Archibold 1981, Abrams 1988), and forests (Frank and Stafford 1970). Far fewer studies have examined aquat-

TABLE 1. SEEDLING EMERGENCE METHODS.

Description	Advantages	Disadvantages
Direct Application[a]		
Each container is filled with a substrate sample spread 2 cm deep or less, either directly or on top of a selected rooting material (e.g., potting soil, sand, perlite)	1. Relatively simple to set up and appropriate for large-scale studies	1. Delay encountered between sampling and obtaining results
These are then placed in greenhouse or growth chamber facility under conditions promoting seed germination	2. Can examine greater sample volumes than are feasible with separation methods	2. Seed banks of some species may be underestimated due to lack of germination
As seedlings emerge, they are counted, identified, and removed; if unidentified, they are transplanted and allowed to mature for taxonomic purposes	3. Can be applied using a wide variety of substrate types	3. May require large blocks of time and space to conduct
	4. Species counts and identification may be easier because plants are the source of information rather than seed alone	4. Transplanting and care of seedlings can be labor intensive
Bulk Reduction[b]		
Substrate samples are washed through coarse and fine mesh sieves, the latter with a mesh small enough to collect expected small-seeded species	1. Similar to those above	1. Disadvantages similar to but not as great as items 1 to 3 above
When sieving is completed, the samples are spread 3 to 5 mm deep on a selected rooting material and provided conditions conducive to germination	2. Compared to direct application, can allow greater numbers and volumes of samples to be evaluated; thus, may improve results of statistical analysis	2. Organic substrates likely to be more difficult to process
	3. Can decrease greenhouse/growth chamber space requirements	3. Special care must be taken to avoid seed loss during sieving
	4. Increases ability to assess uncommon and rare species	4. Labor to care for seedlings may increase due to emergence of greater numbers of seedlings
	5. Can reduce time required for seedlings to emerge	
Substrate Saturation[c]		
Substrate samples are sieved for bulk reduction (see above), then spread no more than 5 mm deep above a ~5.0cm layer of chosen rooting medium (e.g., potting soil mixed with sand).	1. Particularly efficient for assessment of emergent seed species	1. Seedlings of submersed species will need to be transplanted immediately to avoid drying soon after emergence from substrate
Water is provided and maintained at a level from 0 to 1 cm below the sample surface, which is presumed to be sufficient for germination of submersed and emergent plant groups	2. Fewer samples will need to be processed because only one water level (i.e., saturated substrate) is applied	2. A consistent level of saturation must be maintained; failure to do so for all samples would confound study results
	3. Maintains advantages of bulk reduction	

References:
[a]Thomas and Grime 1979; Bigwood and Inouye 1988; Gross 1990.
[b]Ter Heerdt et al. 1996.
[c]Boedeltje et al. 2002.

TABLE 2. SEPARATION METHODS.

Description	Advantages	Disadvantages
Flotation using Inorganic Salts[a]		
Each substrate sample is combined with a mineral salt solution (e.g., K_2CO_3, Na_2CO_3, ZnCl, or $CaCl_2$) having a density sufficient to float seeds to be separated from substrate particles	1. Unlike seedling emergence methods, seed counts are performed directly and are not affected by differences in requirements for germination	1. Seed bank can be overestimated if not corrected for dead seeds; chemical impact on viability needs to be checked
	2. Materials used in this procedure are relatively harmless and inexpensive	2. Separation from organic material may be difficult, especially if the seeds are tiny and inconspicuous
	3. Delay between sampling and obtaining results is usually reduced	3. Difficult to use in determining seed bank composition because the specific gravity at which seeds float may vary by species
	4. Space requirement not as great as for seedling emergence methods	4. Repeated washings and transfers may be required, increasing the risk of losing seeds
Flotation using Organic Liquids[b]		
Each substrate sample is combined with an organic liquid compound (e.g., C_2Cl_4, and C_2HCl_3) with a density to float seeds for subsequent removal	1. Advantages similar to items 1, 3, and 4 above	1. Disadvantages similar to items 1 to 4 above
		2. Raises health concerns regarding ventilation, flammability, and toxicity
Sieving by Hand[c]		
Small sample of substrate (~50 g) is washed with water through one or more sieves (e.g., ranging from ~1.5 to 0.015-mm) to remove substrate particles while collecting seeds	1. Advantages similar to items 1 to 4 above	1. May overestimate potential flora if viability is not determined
	2. Can give almost 100% recovery for large (>1.5 mm dia) seeds	2. Separating seeds from organic materials may be time-consuming and difficult
	3. Little if any impact on seed viability	3. Determining species composition can be challenging when numerous species with different seed sizes occur in the samples
Elutriation[d]		
Each substrate sample is placed into an automatic washing and separation system containing several sieves decreasing in mesh size (e.g., 0.71, 0.425, and 0.243 mm) to collect seeds while removing unwanted particles	1. Advantages similar to those listed for sieving by hand (items 1 to 3 in previous block)	1. Viability testing is needed to correct seed density estimates
	2. Well-suited for quantifying large (>2.0 mm dia) or easily recognizable seeds	2. May not provide reliable estimates for seeds<~0.5 mm dia
Hand-sorting and Identification[e]		
Usually performed under magnification after flotation and/or sieving; seeds are separated from residual particulates and identified to species	1. Clarification of seed image for direct counts and identification	1. Requires meticulous cleaning of sample material; can be expensive and labor intensive
	2. Seed can be examined to determine if "apparently viable"	2. Identification to species may be difficult based on seed alone

References:
[a]Barbour and Lange 1967, Malone 1967, Hayashi and Numata 1971, Fekete 1975, Hayashi 1975, Roberts and Ricketts 1979.
[b]Jones and Bunch 1977, Lock and Butler 1977, Roberts 1981.
[c]Poiani and Johnson 1988, Jarvis and Moore 2008.
[d]Gross and Renner 1989, Gross 1990.
[e]Malone 1967, Ter Heerdt et al. 1996.

ic/wetland seed bank dynamics, although as early as the 1800s Darwin (1859) used seedling emergence, albeit rudimentarily, to count viable seeds in sediment from a pond. He wrote, "I took in February, three tablespoons of mud from each of three different points, beneath the water... and kept [the mud] in my study for six months, pulling up and counting each plant as it grew." Seedlings emerged—to Darwin's surprise—in relatively large numbers (537 total) and were of many species, all from samples totaling less than 200 mL. Notably, despite Darwin's early seed bank discoveries, emergence procedures were seldom used in aquatic/wetland seed bank investigations until the 1970s (van der Valk and Davis 1976, 1978, and 1979, Keddy and Reznicek 1982, 1986, Haag 1983, Nicholson and Keddy 1983, Leck and Simpson 1987, Kautsky 1990, Kimber et al. 1995, de Winton et al. 2000, Boedeltje et al. 2002, Peterson and Baldwin 2004).

Direct Application

In studies applying seedling emergence procedures, substrate samples are usually spread thinly in test trays, aquaria, or other wide-mouthed containers (Figure 1). These are then placed in a growth chamber or greenhouse facility maintaining conditions known or expected to promote seed germination (Table 1). Prior to setup, it may be necessary to sieve or hand pick through collected substrates to remove debris and vegetative propagules (rhizomes, stolons, stem fragments, tubers, and turions) that could confound growth initiated from seed. Some researchers have reported using absorbent paper (Finlayson et al. 1990), perlite (Skoglund and Hytteborn 1990), vermiculite (Neff et al. 2009), or sand (Leck and Simpson 1987) beneath the sample layer to adjust moisture and enhance rooting of seedlings. Others have utilized a nutrient medium to support seedling growth to a more mature stage by spreading potting soil (van der Valk and Davis 1978) or potting soil mixed with sand and peat (Keddy and Reznicek 1982) prior to spreading the substrate sample layer. Once the seedlings have emerged, they can be transplanted to larger containers of suitable substrate (e.g., fine-textured inorganic sediment; Smart and Barko 1985; Figure 2) and allowed to grow and produce flowers and seeds for identification to species. This is an especially good option for seedlings that might otherwise perish on thin layers of substrate in test containers with no additional nutrients.

The sample depth chosen for the assay is a key consideration because seedling emergence can be slowed or diminished with increasing burial depth (Holm 1972, Stoller and Wax 1973, 1974, Baskin and Baskin 1985 and literature therein, Hartleb et al. 1993, Ter Heerdt et al. 1996, Peterson and Baldwin 2004, Ailstock et al. 2010a). A seed contains only a certain amount of chemical energy reserve (e.g., carbohydrate and lipid); therefore, to survive after germination, it must produce leaves for photosynthesis when it reaches the substrate surface (Benvenuti et al. 2001). Many authors have shown that germination can be inhibited by burial due to the absence of light and insulation from other environmental cues (Brenchley and Warington 1930, Kropá 1966, Galinato and van der Valk 1986). The deeper a seed is buried, the less exposed it will be to light, aeration, and variations in temperature (Fenner 1985, Grime et al. 1988, Benvenuti et al. 2001 and references therein). Because many wetland and aquatic plant species produce tiny seeds, a sample depth of 2 cm or less is commonly applied in emergence assays (Haag 1983, Leck and Simpson 1987, Poiani and Johnson 1988, Hartleb et al. 1993, Ter Heerdt et al. 1996, Boedeltje et al. 2002, Peterson and Baldwin 2004).

Figure 1. Set-up showing direct application of seedling emergence method in an environmental growth chamber at the US Army Engineer Research and Development Center (ERDC), Vicksburg, Mississippi.

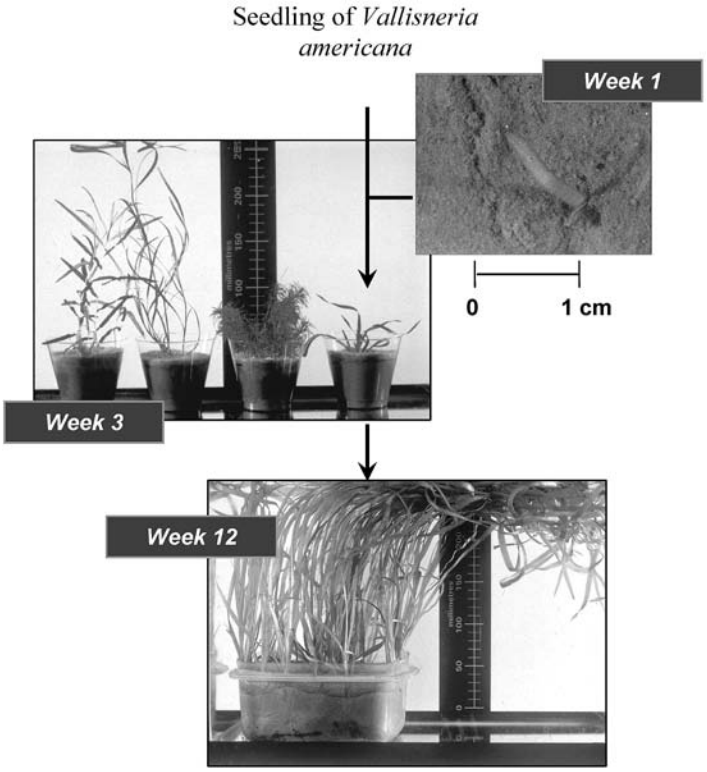

Figure 2. *Vallisneria americana* seedling after 1 week in seed bank assay, then at 3 and 12 weeks after transplanting in an environmental growth chamber, ERDC.

If the goal of the investigation is to determine seed bank content of a particular species, then conditions provided during the study should match, to the extent achievable, the germination requirements of that species. Conversely, if the goal is to determine overall seed bank species diversity, then the study should attempt to maximize the number of species and individual seedlings recruited. A complete accounting of each species may require special conditions to break dormancy, and these should be incorporated into the study design to mimic conditions that exist in nature (e.g., cold stratification, scarification, aeration, fluctuating temperature, darkness, and/or drying and rewetting). In some cases, both saturated and submersed conditions may be needed because the requirements may vary among species with different growth habits or adaptations. Although information is yet incomplete, a number of references are available (Muenscher 1936, Baskin and Baskin 1998 and references therein,

Ailstock and Shafer 2006, Hay et al. 2008, Jarvis and Moore 2008, Ailstock et al. 2010b) that describe conditions for germination of certain aquatic and wetland plant species in the laboratory, such as wild celery (*Vallisneria americana* Michx.), water stargrass (*Heteranthera dubia* [Jacq.] MacMillan), rush (*Juncus* spp.), spikerush (*Eleocharis* spp.), pondweed (*Potamogeton* spp.), and nodding waternymph (*Najas flexilis* [Willd.] Rostk. & Schmidt); these references could be helpful in designing seed bank investigations needing to recruit species whose germination requirements have already been determined.

A common problem of large-scale studies using emergence techniques is that they can require large blocks of time and space and can be labor intensive in the care of seedlings (Table 1). Thus, the size of a greenhouse or growth chamber may restrict the number of samples that can be observed as well as the area over which assay containers can be placed. These problems often intensify where the same facility must be used to transplant and house seedlings to allow them to grow for identification purposes.

The time required for seedlings to emerge depends on the seed bank component being recruited, depth of seeds in the substrate sample layer, and climate region from which the samples were taken. Haag (1983) and Kimber et al. (1995) studied seedling growth from the transient (i.e., short-lived; viable for <1 yr) component of lake seed banks and showed that under favorable conditions, emergence of new seedlings was negligible after the first 4-6 weeks. Usually, in studies such as theirs involving seed banks in temperate regions, the samples are observed approximately 2-5 months to ensure that seedling emergence has essentially ceased (Thompson and Grime 1979, van der Valk et al. 1992). In contrast, for samples from tropical or subtropical areas with characteristically longer growing seasons, 6-9 months may be needed for germination, though wide variability in timeframe exists in the literature (Williges and Harris 1995, Leeds et al. 2002, Harwell and Havens 2003, Hanselman et al. 2005). Quantifying the persistent (i.e., long-lived; viable for 1 yr) component of a seed bank requires even longer observation, which, according to Baskin and Baskin (1998), should extend at least through the end of a second or subsequent germination season. They further suggest that the samples be placed in a nonheated greenhouse or similar shelter to experience seasonal fluctuations in temperature and lighting over a period of several years.

Bulk Reduction

To improve accuracy and minimize use of laboratory resources, Ter Heerdt et al. (1996) modified emergence procedure by reducing bulk or volume of the substrate (Table 1). They washed each sample with water first through a coarse (~4.0 mm) mesh sieve to remove vegetative structures and coarse debris; they then rinsed again using a fine (~0.2 mm) mesh sieve to remove silt and clay while retaining the smallest seeds of expected species. This procedure reduced the volume of clay, peat, and sandy substrates by 85, 70, and 55%, respectively, and so reduced the amount of required greenhouse space. Sieving the samples and spreading them thinly (3 to 5 mm deep) on a layer of sterilized potting compost increased plant species and seedling numbers, and decreased the time required for seedlings to emerge. Essentially no new seedlings emerged from the sieved samples after 3 to 6 weeks, whereas, based on their extrapolations, leaving the samples unsieved would have prolonged emergence by 4 to 6 months (due to deeper burial). Hand-sorting through the remainder of the samples after seedlings ceased to emerge showed that, in the sieved samples, 88 to 100% of the viable seeds had germinated. In the un-sieved samples, germination percentages were significantly lower, ranging from 33 to 88% of the viable seeds.

Substrate Saturation

Emergence procedures often require at least 2 water-level treatments (i.e., saturated and submersed) to support germination and growth of seedlings with different water-level requirements. However, Boedeltje et al. (2002) reported that for studies targeting viable seeds of emergent and submersed plants, the only water-level treatment that may be needed is substrate saturation. In their study, emergence was significantly greater for seedlings of both groups occurring on saturated substrate (water 0-1 cm below the substrate surface) than on substrate that was shallowly submersed (water 2-3 cm deep above the substrate surface; Table 1). Their observations agree somewhat with those of other researchers, such as van der Valk and Davis (1978) and Leck and Simpson (1987). Urging some caution, we suggest that they be considered in view of the following: (a) germination requirements for all plant species are not fully understood (Bernhardt et al. 2008), and (b) seeds of many species germinate in larger numbers when the water level is above that of the substrate (Baskin and Baskin 1998 and literature therein). Furthermore, submersed species that emerge as seedlings under saturated conditions would need to be transplanted to waters of sufficient depth to avoid injury or death by desiccation. Therefore, before implementing Boedeltje et al. (2002) methods, especially on a large scale, it would be prudent to conduct preliminary trials to determine which species to anticipate and to apply the appropriate water level(s) in the assay.

SEED SEPARATION PROCEDURES

Flotation

Flotation provides a means of separating seeds from substrate particles, usually by applying different salt solutions or organic liquid compounds (Table 2). This procedure operates on the premise that liquids with the proper specific gravity will allow substrate particles to settle to the bottom, while seeds are buoyed to the surface where they can be removed. A major disadvantage of this procedure is that it can be difficult when used on highly organic substrates because organic particles tend to float along with seeds (Roberts 1981). In addition, the chemicals used for seed flotation may negatively affect viability to an extent dependent on the length of exposure (Numata 1984, Buhler and Maxwell 1993, Tsuyuzaki 1993, 1994), reactive nature of the compound (Roberts 1981, Hammerstrom and Kenworthy 2003) and seed coat

condition (Mohamed-Yasseen et al. 1994). Flotation may not be practical for studies of seed bank species composition because the specific gravity at which seeds float tends to vary among species (Gross 1990; Table 2). To separate seeds by species would likely require frequent washings and transfers that increase the chance of seed loss and associated error (Gross 1990). Thus, while flotation may be sufficient to assess distribution of a few important seed species, it may be unreliable and/or inefficient for predicting species diversity of a seed bank community.

Solutions of mineral salts, such as potassium carbonate (Hayashi and Numata 1971, Buhler and Maxwell 1993), calcium chloride (Roberts and Ricketts 1979), sodium carbonate (Hayashi 1975), sodium metaphosphate (Grillas et al. 1993), and zinc chloride (Fekete 1975) are preferred agents in attempts to retrieve seeds by the flotation method. These solutions are relatively harmless, unlike some organic liquid compounds used for the same purpose (e.g., perchloroethylene, trichloroethylene, and trichloroethane) that raise concerns regarding flammability, toxicity, and ventilation (Jones and Bunch 1977, Lock and Butler 1977; Table 2). Mineral salts are also inexpensive, and some can serve as dispersants to break down clayey substrates before implementing flotation procedures. For instance, Malone (1967) used a combination of sodium hexametaphosphate and sodium bicarbonate to disperse fine substrate particles to ensure efficient extraction of seeds by flotation using a magnesium sulphate solution.

Sieving by hand

Another means of separating seeds from substrate is by washing through one or more sieves (Table 2). Jarvis and Moore (2008) sieved clayey-sand samples through a 0.5 mm mesh for seeds of wild celery, which once collected, were counted and tested for viability using tetrazolium staining (Lakon 1949, Grabe 1970, Leist and Krämer 2003). In an earlier study, Poiani and Johnson (1988) sieved 50 g samples of substrate after finding flotation to be unreliable to collect large (>1.5 mm) seeds, as produced by bulrush (*Schoenoplectus* spp.). They mixed each sample thoroughly with water and poured the solution through a set of four sieves with pore sizes that decreased sequentially from 2.0 to 0.0149 mm. This method proved to be effective in separating out seeds of many species, as well as organic debris and sand fractions, while washing away silt and clay. After sieving, they washed the samples into aluminum pans and allowed them to air-dry before examining under a microscope to identify and count the seeds.

Elutriation

The elutriation system by Gross and Renner (1989) provided reliable estimates of seed densities for species with weights from 0.06 to 9.8 mg and diameters >0.5 mm (Table 2). Elutriation was particularly effective for large (>2.4 mm) seeds and had no significant effect on seed viability. Substrate samples up to 60 g took approximately 15 min to elutriate, while separating, classifying and counting seeds was somewhat more labor intensive (averaging 20-30 min).

Gross and Renner (1989) mechanized separation of seed from substrate by modifying the hydropneumatic elutriator (Smucker et al. 1982) used to estimate fine root production. Originally designed by Smucker et al. (1982), the system included washing and elutriating chambers, a transfer tube, and a size-graded set of two sieves with 0.840 and 0.420 mm mesh screens. For seed bank quantification, Gross and Renner (1989) replaced the two-sieve set with a series of three sieves with screens having pore sizes of 0.71, 0.425, and 0.243 mm mesh. After washing, they removed the sieves and transferred sample contents into Buckner funnels lined with filter paper and mounted on a vacuum manifold to remove excess water. Filter paper and residue in each funnel were then placed into Petri dishes to air-dry for 24 h, or oven dry at 35 C for 3 to 4 h.

Hand-sorting and Identification

After washing and sieving to reduce the amount of substrate in the samples, seeds can be hand-sorted and picked from residuals either directly or after air-drying. Hand-sorting is best performed using a binocular stereomicroscope or a large illuminated magnifier mounted on a laboratory countertop (Roberts 1981). Seed identification to species level requires a detailed taxonomic key, but for many aquatic and wetland plant species, seed descriptions in the literature are very limited. Some references that may be helpful include Montgomery (1977), Godfrey and Wooten (1979, 1981), Martin and Barkley (2000), and Crow and Hellquist (2000a, 2000b); in addition, seed reference collections from the study area may be useful for verification.

VIABILITY TESTING

Because separation procedures alone do not address seed viability, additional methods may be needed to quantify viable seeds following isolation from substrate. A seed bank can be overestimated if a significant number of seeds are dead yet are included in the total seed count without viability being determined. Germination tests are most effective when applied to a limited number of species whose germination requirements are known and a sufficient number of seeds can be examined (e.g., three replicates each consisting of 50 seeds [Baskin and Baskin 1998]). However, these tests can become quite complicated if multiple species have different germination requirements and the viability of seeds of each species needs to be evaluated.

Other methods of testing viability that have been used with varying degrees of success include direct examination of the seed embryo (e.g., white embryos are usually viable; gray, yellow, or brownish embryos are probably dead [Baskin and Baskin 1998]) or testing with the chemical 2,3,5-triphenyl tetrazolium chloride (TTC) as a viability indicator (Malone 1967, ISTA 1999, Peters 2000). When seeds are viable, their embryos release hydrogen ions during respiration, which react with TTC causing this indicator to turn pink or red. This form of testing, tetrazolium (TZ) staining, can work for both dormant and nondormant seeds (Flemion and Poole 1948), but similar to direct examination of seed embryos, it may be inefficient when the seeds are minuscule (Flemion and

Poole 1948, Madsen and Boylen 1988, Hartleb et al. 1993, Baskin and Baskin 1998). Another option is to record the seeds as "apparently viable" when they appear to be intact and resist gentle pressure from a pin or forceps (Hayashi et al. 1978, Roberts and Ricketts 1979, Bakker et al. 1996, Marion and Orth 2010). Carretero (1977) reported that of the intact, apparently viable seeds of four species, about 71 to 88% germinated when viability was tested in the laboratory (per Roberts 1981). Assessments of apparently viable seeds may be adequate for many purposes, but if seeds are small, the method may present limitations similar to those of TTC and direct examination of embryos.

Seed fall velocity for marine eelgrass (*Zostera marina* L.) was recently found to be linked to seed viability evaluated as the production of seedlings. Marion and Orth (2010) reported that for this species seedlings were produced by 89% of seeds that fell in seawater (at 20 ppt), at a rate of 5.5 cm/sec or faster. Among seeds that fell slower than 5.0 cm/sec, only 14% produced seedlings, and 95% were determined to be of poor quality (i.e., soft or structurally degraded). Whether fall velocity is a reliable predictor of seed viability in other species is unknown, but based on results from Marion and Orth (2010), further investigation is warranted.

SEED BANK SAMPLING

Many researchers in seed bank ecology have emphasized the value of performing preliminary investigations to obtain essential baseline data (Roberts 1981, Leck et al. 1989, Ter Heerdt et al. 1996). Even when such studies are not feasible, it is desirable to collect enough information to determine if modifications in sampling would result in meaningful statistical gains (Roberts 1981). During the first year, it would be advantageous to sample selected sites to determine which species to expect and their early identification characteristics (Hutchings 1986, Ter Heerdt et al. 1996). A preliminary study would also aid in determining suitable sampling and processing techniques, and obtaining information on species phenologies, germination requirements, and contributions from neighboring habitats (Leck et al. 1989).

Sampling methods should be selected according to the study objectives, with consideration of other factors involving resource (time, space, labor, and equipment) availability and expected seed distribution. For many projects, precise estimates of seed density may not be needed, and estimates of the relative abundance of each species will often be sufficient (van der Valk et al. 1992). This would apply, for instance, where the aim is to determine whether a species may be present from one year to the next, or to assess changes in relative abundance of different species over a given period. A list of species in the seed bank is usually enough to ascertain which desirable (or undesirable) species are present (van der Valk et al. 1992), and the completeness of the list can be determined using a species-area curve (Forcella 1984).

When a more detailed study is required, data analysis may become more complicated because seed distribution is rarely normal, and normality often cannot be achieved by applying data transformations (Thompson et al. 1997, Benoit et al. 1989, Ambrosio et al. 1997). In general, systematic and random core sampling are applied when homogeneity is expected (Kellman 1974, Moore and Wein 1977, Hill and Stevens 1981, Kramer and Johnson 1987), but because seed distribution is usually clumped or aggregated, stratified core sampling may be more appropriate. Stratified sampling is advantageous because separate mean seed densities can be calculated for different parts (or strata) of the seed bank representing different areas of interest, such as vegetation zones, hydrologic regimes, site elevation, sediment nutrient availability, and depth in substrate (Sampford 1962, Poiani and Johnson 1988, Williges and Harris 1995, de Winton et al. 2000, Price et al. 2010). Greater precision can be achieved with greater distinction among strata because the distribution of units within each stratum tends to be more even (Sampford 1962). For further information on sampling methods for spatial pattern analysis of seed banks, see Mueller-Dombois and Ellenberg 1974, Bigwood and Inouye 1988, Benoit et al. 1989, Lavorel et al. 1991, Dessaint et al. 1996, and Ambrosio et al. 1997.

Although the optimal number of samples to study a seed bank has been extensively investigated (Champness 1949, Dospekhov and Chekryzhov 1972, Bigwood and Inouye 1988, Dessaint et al. 1996, Ambrosio et al. 1997), no widely-accepted sample number or sampling unit exists. The general consensus is that, for a given volume of substrate, it is better to collect a large number of small samples than to collect a small number of large ones. The volume of substrate should be large compared to the size of the seeds (Sampford 1962) and should contain the uppermost portions of the depth profile because it usually contains seeds in greater densities (Nicholson and Keddy 1983, Gunther et al. 1984). For aquatic/wetland seed bank studies, core sample dimensions often range from 5 to 10 cm both in diameter and depth (Haag 1983, Poiani and Johnson 1988, Kimber et al. 1995, van der Valk and Rosburg 1997, Harwell and Havens 2003), but this depends greatly on study objectives.

Bigwood and Inouye (1988) found sampling precision to be greatly improved either by taking as many small samples as practical, or by taking smaller subsamples from quadrats and "pooling" them (i.e., one pooled sample per quadrat). Both strategies are effective in reducing statistical variance, and the choice between them is often a matter of the physical condition of the study area. For example, areas that have rocky substrates or large masses of roots may be more easily evaluated using a small corer to pool subsamples, while sampling with a larger corer may not be practical (Bigwood and Inouye 1988, van der Valk et al. 1992). Pooling can also reduce the number of samples needed to be processed, which for large-scale seed bank studies could be more cost-effective.

Elliott (1977) described how to estimate the number of samples required for a given level of precision using equations for estimating the density of benthic invertebrate populations. His descriptions are relevant to seed banks because invertebrates are distributed in patterns similar to the distribution of seeds and are thus pertinent to seed bank sampling strategies (Benoit et al. 1989). A simplified version of Elliot's equations for normal, Poisson, and negative binomial distributions has been reported by Thompson et al. (1997) in the following sequence:

1) For the most basic case, where seed distribution is expected to be normal:

Precision (D) = standard error/mean

$D = s/\sqrt{n})/x$

$D = (\sqrt{(s^2/n)})/x$

$D\,x = \sqrt{(s^2/n)}$

$D\,x = \sqrt{s^2}/\sqrt{n}$

$n\,D^2\,x^2 = s^2$

Thus, the number of sampling units in a random sample is given by:

$n = s^2/D^2\,x^2$

So, for a standard error of 20%

$n = s^2/0.2^2\,x^2$

$n = 25\,s^2/x^2$

2) However, if a Poisson distribution applies, then n depends only on the mean; thus (for a standard error of 20% of the mean):

$n = 25/x$

3) Where a negative binomial distribution applies, then (for a standard error of 20% of the mean):

$n = 25\,(1/x + 1/k)$

where:

n = number of samples required

s^2 = sample variance

x = mean density

k is the exponent in the binomial expansion $(q - p)^k$

and can be estimated by:

$k = x^2/(s^2 - x)$

In each of the above cases, the number of samples required depends on the known or anticipated mean density; thus, compared to a densely populated species, one that is thinly distributed would require more samples to achieve a given level of precision. This agrees with results obtained by Benoit et al. (1989) who found that sampling variance could be reduced in half by doubling the sample number; however, the relationship between variance and sample number was nonlinear. For example, their calculations showed that a minimum of 60 core samples (1.9 cm dia × 15 cm deep) would be needed to characterize the seed bank of lambsquarters (*Chenopodium album* L.), a species occasionally found in wetlands; but beyond 75 samples, the potential increase in precision did not justify the effort to collect additional samples. Note that because of their chosen sampling design, the number of samples required for their study might have been smaller had pooled sampling been employed (Bigwood and Inouye 1988). For further guidance concerning procedures to determine appropriate sample number, see Dessaint et al. (1996), Ambrosio et al. (1997), and Mickelson and Stougaard (2003).

The timing of sample collection is an important consideration because seed densities can vary from year to year and from season to season. If samples are taken at the same time over consecutive years, long-term changes in the seed bank can be quantitatively evaluated. If the aim is to determine changes over an annual growth cycle, however, more frequent sampling should be conducted (Roberts 1981). To detect peak densities of germinable seeds, the best time for seed bank sampling is at or just before the beginning of the growing season in early spring (Leck and Simpson 1987, Leck et al. 1988, 1989). Sampling at that time would allow the seed bank to undergo cold stratification naturally in winter, thereby avoiding the need to chill to break dormancy after samples are brought to the laboratory.

CONCLUSIONS AND RECOMMENDATIONS

For practical assessment of seed bank characteristics, our review of the literature suggests that in most cases emergence methods can provide much useful information. Viable seeds of species of interest that remain in the substrate after germination has ceased may be detected by separation procedures followed by viability testing. When applied alone for comprehensive purposes, separation procedures can be exceedingly laborious and can overestimate the total seed bank if nonviable seeds are included; these procedures are most effective when applied to seeds of a few species or where seeds are readily detectable due to size, color, or some other physical quality. For many community-level studies, emergence procedures are more efficient and may be enhanced by bulk reduction to offset time and space limitations.

A preliminary greenhouse or growth chamber study to determine what species to expect as well as their morphologies as seedlings could be advantageous for identification. Conditions to promote seed germination and reduce time required for seedlings to emerge can be determined through experimentation and/or a literature search. Other issues that should be resolved in the preliminary study include:

- sample collection (e.g., time, location, pattern, sample number and dimensions) and processing techniques
- requirements for set-up in growth facilities
- seed separation and viability testing
- seed/seedling identification methods

Currently, a large body of information exists on germination and initial growth of the seeds of emergent plant species under flooded and nonflooded conditions (e.g., Baskin and Baskin 1998 and references therein). However, further studies are needed to improve seed bank study design by elucidating responses of seeds and seedlings of submersed plants to different water-level (including nonflooded) treatments (see Boedeltje et al. 2002). This information would help to clarify the minimum number of water levels that can be used in seed bank studies seeking to recruit seedlings from different habitat groups.

The present lack of taxonomic keys to classify aquatic/wetland seeds and seedlings is a major obstacle to early identification to species level. Thus, additional time and effort to allow seedlings to grow to maturity may be necessary in situations where species identities have remained undetermined over the course of the assay. Alternatively, identification through DNA fingerprinting may offer a possible solution as it requires only small amounts of plant tissue and can be faster and more precise than morphological characterization (Joel et al. 1998, Kress et al. 2005, Rahman 2007, CBOL Plant Working Group 2009). Recent advances in DNA fingerprint-

ing are making it more effective and affordable (Cameron et al. 2006), but while its application is generally increasing, its use in plant research is still in its infancy. Future research on the taxonomy of wetland and submersed aquatic plants should attempt to advance species identification through seed/seedling keys and DNA databases.

Seed fall velocity was recently confirmed to be a meaningful predictor of the viability of marine eelgrass seeds based on production of seedlings (Marion and Orth 2010). This finding represents a new approach to viability testing that needs further study to adapt to seeds of other species. Given that seed size, shape, and density may exert significant physical effects, relationships of these factors to seed fall velocity in different species should be evaluated.

Separation procedures that require the use of chemical and mechanical (sieving) techniques always run the risk of injuring seeds and diminishing seed viability. While sieving may damage seeds that are fragile or of a particular morphology (e.g., pointed as opposed to round), it may improve germination through impacts on the seed coat that break physical dormancy (Bewley and Black 1994, Mohamed-Yasseen et al. 1994). Flotation procedures may injure seeds because of the chemicals that are used and, as with sieving techniques, the severity of damage depends on seed coat structure and duration of exposure (Roberts 1981, Tsuyuzaki 1993, 1994, Hammerstrom and Kenworthy 2003, Smith et al. 2003). It is therefore advisable that the impacts of separation procedures be considered to avoid confounding estimations of seed bank viability.

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Distribution, interspecific associations and abundance of aquatic plants in Lake Bisina, Uganda

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ABSTRACT

A survey of floating-leafed and submersed aquatic plants was conducted in Lake Bisina, Uganda. Seven of the species collected were not previously reported from Lake Bisina, including *Stuckenia pectinata* (L.) Börner, *Utricularia reflexa* Oliv., *Utricularia foliosa* L., *Caldesia parnassifolia* (L.) Parl., *Wiesneria filifolia* Hook. f., *Brasenia schreberi* J. F. Gmel., and a multicelluar algae, *Chara* sp. Examination of pairwise associations between plant species revealed that the *Chara* sp. was negatively associated with *Najas horrida* ex Magn., *Nymphaea caerulea* Savigny, and *Utricularia reflexa* Oliv., which was likely due to differences in habitat requirements. A strong, positive association between *N. caerulea* and *U. reflexa* may have been due to niche similarities, but may also indicate a commensal relationship with *U. reflexa* performing well under shaded conditions provided by *N. caerula*. *Hydrilla verticillata* was the only species associated with water clarity, with abundance increasing as turbidity increased. This study provides new baseline information on the diversity, distribution, and interspecific associations of floating-leafed and submersed aquatic plants in Lake Bisina, Uganda, that will be useful for comparison with future biological studies.

Key words: aquatic plants, hydrilla, macrophytes, shallow lake.

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INTRODUCTION

Aquatic plants are central members of wetland communities that provide food and shelter, directly or indirectly, for many organisms; erosion control; oxygen enrichment of water through photosynthesis; nutrient cycling; absorption of pollutants (Cook 1974, Halls 1997); reduction of sediment suspension; refuges for zooplankton from fish grazing; and suppression of algal growth by competing for nutrients and light and the release of allelopathic substances (Takamura et al. 2003). Aquatic plant distribution, abundance, and vigor are influenced by abiotic factors including water temperature, pH, dissolved oxygen, nutrient levels, turbidity (Squires et al. 2002), sediment type, water and wind currents, depth, and changes in water levels (Wetzel 1983). Biotic components of a given water body, such as the presence and density of herbivorous fish (Hanson and Butler 1994), insects, mollusks, diseases (fungi, nematodes), plant-to-plant interactions, and movement of vegetative portions of aquatic plants by man (e.g., plant parts entangled in fish nets) also influence the distribution and abundance of aquatic plants.

Lake Bisina (N 2.649, E 33.970) is part of the Kyoga lakes system in central Uganda. The Kyoga system receives inflow from Lake Victoria through the Nile River and from streams arising on the slopes of Mount Elgon to the east. The system includes several lakes connected by large areas of papyrus swamp (Green 2009). Lake Bisina is east and upstream of the larger Lake Kyoga, through which the Nile flows. Bisina receives its fluvial input from streams originating on Mount Elgon to the east and the Apendura River to the north. Slightly higher (1041 m a.s.l.) than Lake Kyoga (1026 m), it outflows gently through its western, swampy margins toward Kyoga. As such, Bisina is not directly influenced by either the Nile or the downstream Kyoga-system lakes. Lake Bisina grows and shrinks depending on rainfall, but in a typical year covers about 150 km^2 with a mean depth of 3 m (Vanden Bossche and Bernacsek 1990). It is a designated wetland of international importance (RAMSAR site) because of its unique biodiversity and support of rare and endangered species (Byaruhanga and Kigoolo 2005). The lake can be divided into 3 zones based on the aquatic macrophyte flora: the submersed aquatic plant zone, the Nymphaea zone, and the emergent plant zone.

Katende (2004) examined the species diversity of aquatic macrophytes in Lake Bisina but provided no information on the distributions or interspecific associations of plants in the lake. As part of a project to search for insect herbivores of *Hydrilla verticillata* (Overholt and Wheeler 2006), the aquatic plant communities of several water bodies in Uganda, Kenya, and Burundi were surveyed, including Lake Bisina. Observations during sampling expeditions indicated that the aquatic plant species in Lake Bisina exhibited clear and consistent spatial patterns. The objective of this study was to describe and examine those patterns and attempt to explain the patterns based on water depth, physico-chemical water conditions, and inter-relationships between plants.

MATERIALS AND METHODS

Plant samples

Sampling was conducted in Lake Bisina on 7 occasions between February 2008 and August 2009. Locations for sampling were selected by traversing the lake along several transects and stopping at approximately 1 km intervals to collect samples. On three occasions (April 2008, January 2009, and April 2009) an effort was made to sample as much of the lake as possible by following 5 to 7 transects and revisiting many of the same sites. However, due to a lack of specific landmarks, drift of the boat during sampling, and inherent inaccuracy of the GPS units (30 m), it proved impossible to sample the exact same sites repeatedly. On the other four occasions, sampling was targeted to cover isolated bays and the extreme eastern and western portions of the lake. In total, 199 locations were sampled with 11 to 46 locations visited on each sampling occasion. At each sampling location, vegetation was collected from a boat by throwing a grappling hook attached to a rope and then dragging the hook along the bottom until it was located below the boat. The hook was then slowly lifted to the surface and the collected vegetation was placed on the bottom of the boat. At each location, the grappling hook was thrown three times, each time in a different direction. Plant species were recorded when known, or a voucher sample was collected and pressed for later identification. Water depth was measured at each location using a weighted rope, and water clarity was measured on three of the sampling dates (June 2008, January 2009, and April 2009) with a Secchi disk. The relative abundance of each plant species in each sample of three grappling-hook throws was estimated using a DAFOR scale (Hurford 2006) with scoring modified as follows: 5 (dominant) ≥76% of the estimated biomass of a sample, 4 (abundant) = 51-75%, 3 (frequent) = 26-50%, 2 (occasional) = 5-25%, 1 (rare) = >0 and <5%, and 0 = absent.

Water samples

Water samples were taken from each sampled location on two occasions, January and April 2009, representing the dry and rainy seasons, respectively. Samples were collected in 2L PVC Van Dorn Bottles at approximately 30 cm depth and then transferred to clean plastic 2 L bottles sealed with a lid. The samples were maintained in a cooler on ice for less than 48 h prior to analyses. Chemical analyses for orthophosphates, nitrates, and chlorophyll *a* were based on methods described by Stainton et al. (1977). Dissolved oxygen, pH, and temperature were measured using a portable water quality meter (Fisher Scientific Accumet®).

Data analyses

The frequency of occurrence (incidence) of each plant species was determined by dividing the number of locations where the plant was found by the total number of locations sampled on each sampling occasion. Average incidence was the mean value of incidence of the seven sampling occasions. The abundance of each plant was derived in a similar manner by summing the abundance of each plant on each sampling occasion and dividing by the number of locations sampled. Confidence intervals (95%) were constructed around the mean of incidence and abundance of each species (mean ± SE × 0.994; Payton et al. 2003). An index of prevalence (*Ip*) was calculated for each plant species as the

product of each plant's incidence and abundance (Zhou et al. 2003) as follows:

$$Ip = \left[\frac{Fo}{N}\right]\left[\frac{S}{N}\right]$$

where *Fo* = the number of locations a species was found, *N* = the number of locations sampled, and *S* = the sum of the abundance values (DAFOR scores) for a species. Because *Ip* is the product of two terms, a 95% confidence interval for the mean *Ip* could be calculated using a statistical inference method provided by Buonaccorsi and Liebold (1988). Incidence, abundance, and prevalence were compared between species by examining overlap between 95% confidence intervals (Payton et al. 2003).

The relationships of water depth and water clarity (Secchi reading) to abundance of each species were examined with linear regression. The frequency distributions of the eight most prevalent plants in 0.5 m wide depth classes were plotted to illustrate vertical distributional differences in relation to water depth.

Presence-absence data at all sampling locations for all pairwise combinations of the 10 most abundant plant species were analyzed using a chi-square test with Yate's continuity correction to determine whether certain species tended to occur together or apart more often than expected by random chance (Turner et al. 2004). Chi-square probabilities were adjusted for the number of chi-square tests using the Dunn-Sidak equation, as follows:

$$\propto = 1-(1-\propto')^{k}$$

where α = adjusted P-value, $\propto'$ = calculated P-value, and k = number of chi-square tests (Sokal and Rohlf 1995). Yule's V was used to examine the strength and direction of the association of pairs of species with statistically significant (adjusted $P < 0.05$) chi-square values. Yule's V ranges from +1 for absolute positive association (two plants only occur together) to -1 (two plants never occur together; Turner et al. 2004).

To determine whether plant distribution data could be pooled over sampling dates, it was necessary to examine whether plant distributions changed over time. Interpolated density surfaces were generated for the five most prevalent plant species (*N. careula*, *H. verticillata*, *P. schweinfurthii*s *N. horida*, and *C. demersum*) for three sampling dates (April 2008, January 2009, and April 2009) using the Inverse Density Weighted (IDW) method in the Geospatial Analyst of ArcMap 9.2 (ESRI; Redlands, CA). The IDW method makes predictions at locations not sampled from weighted averages of nearby known values, giving closer values more influence on the predicted value than those that are farther away. The three dates used for comparisons were selected because samples on those dates were spread more or less evenly throughout the lake. On other dates, sampling was in more limited areas of the lake, and thus interpolated surfaces would not have been representative. Matrices (each cell = 34.1 m × 34.1 m) of the interpolated surfaces were exported from ArcMap 9.2 and compared between sampling dates (within plant species) using Mantel tests (Sokal and Rohlf 1995) with 100 permutations in R statistical software (R Development Core Team 2004). In total, 15 Mantel tests were conducted; three (April 2008 vs. January 2009, April 2008 vs. April 2009, and January 2009 vs, April 2009) for each of the five plant species. Interpolated abundance surfaces of the five most prevalent plants were generated from data pooled over all sampling dates once it was determined that distributions were highly correlated between sampling dates (see results).

Physico-chemical parameters (temperature, pH, conductivity, phosphates, nitrates, and chlorophyll *a*) were compared between the two sampling occasions using a t-test.

RESULTS AND DISCUSSION

The depth of Lake Bisina at sampling locations ranged from 0.6 to 5.0 m with an average depth of 2.89 m (±0.6 SE; Figure 1). Of the 199 locations sampled, at only four were no plants found, three of which were in the deepest part of the lake (4.9 to 5.0 m), and the fourth had a rocky bottom. A total of 15 vascular plants and one multicellular green algae, *Chara* spp., were collected. The majority of species (11) were submersed, and the other five were floating-leafed plants (Table 1). The index of prevalence, which combines incidence and abundance, indicated that *N. caerulea* was by far the most prevalent floating-leaf plant (Figure 2). The other four floating-leaf plants, *Nuphar* spp., *N. lotus*, *C. parnassifolia*, and *B. schreberi*, had very low indices of prevalence and could be considered rare. *Najas horrida* was the most prevalent submersed plant, followed by *C. demersum* and *P. schweinfurthii*. *Hydrilla verticillata* was less prevalent than *C. demersum*, but not different from *P. schweinfurthii*. *Utricularia reflexa, O. ulvifolia*, and *Chara* sp. were of lesser prevalence, and the remaining species had very low prevalence.

The abundances of six of the plants were significantly related to water depth (Table 2), although the coefficients of determination for all relationships were low, indicating that the relationships may not have been linear and/or factors other than depth also influenced the abundance of these plants. *Chara* sp. was positively associated with water depth, whereas the abundances of the other five species (*C. demersum*, *H. verticillata*, *O. ulvifolia*, *N. caeruleai*, and *U. reflexa*) were negatively related to depth. Based on the coefficient of determination (R^2), the abundance of the floating-leafed plant *N. caerulea* was most strongly related to depth, with abundance decreasing 0.95 on the DAFOR scale for each meter increase in depth. The frequency distributions at different depth classes of the eight most commonly collected plants (Figure 3) show that *Najas horrida*, *C. demersum*, *P. schweinfurthii*, and *H. verticillata* occurred at a similar range of depths from about 0.9 to 4.5 m, with the highest incidence from 3 to 3.5 m. *Ottelia ulvifolia*, *N. caerulea*, and *U. reflexa* tended to occur in shallow water, and *Chara* sp. only occurred in deeper areas of the lake from 3.15 to 4.34 m (Figure 3).

Hydrilla verticillata was the only species in which abundance was related to water clarity (Secchi reading). Abundance of *H. verticillata* decreased as water clarity increased ($F_{1,83} = 12.57$, $P = 0.0006$, $R^2 = 0.11$). Mean water clarity was higher in January (2.09 m) and April 2009 (1.92 m) than in June 2008 (1.63; $F_{2,82} = 5.75$, $P = 0.004$).

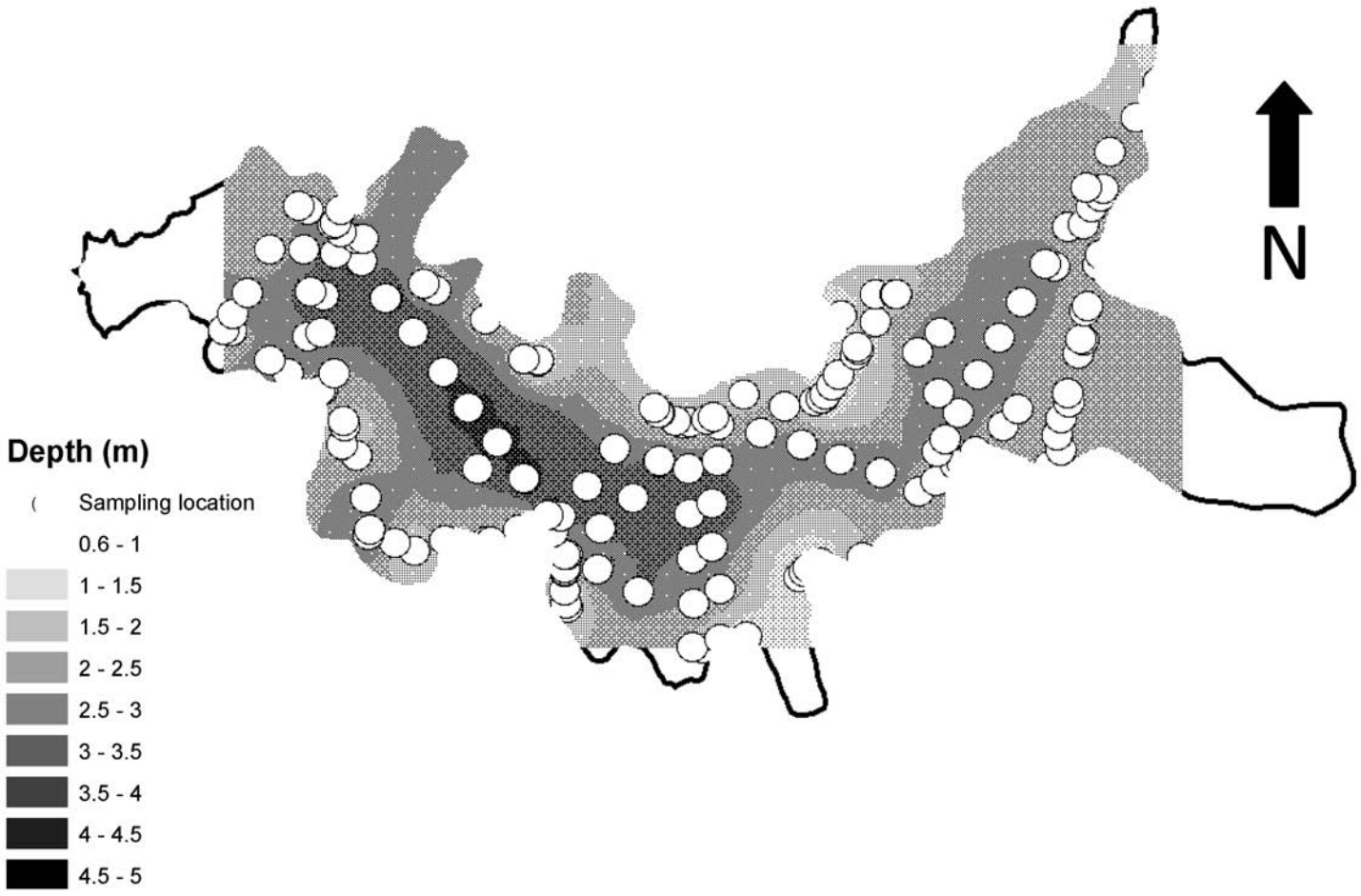

Figure 1. Sampling locations and interpolated water depth in Lake Bisina, Uganda.

Examination of associations of all possible pairwise combinations (36) of plant species that were encountered more than three times revealed that seven pairs of plants were either found to co-occur more often than expected by random chance or were found to occur separately more often than expected (Table 3). The strongest positive association was between *N. caerulea* and *U. reflexa*, and the strongest negative association was between *N. horrida* and *Chara* sp.

The Mantel tests to examine whether plant distributions of the five most prevalent plants were correlated between sampling dates were all highly significant (Mantel r ranging from 0.602 to 0.903, $P < 0.01$ in all cases), indicative that distributions did not change over time. Therefore, data from all sampling occasions were pooled to create plant abundance surfaces (Figure 4). *Najas horrida* was most abundant in the far western part of the lake but also occurred throughout the lake except in the deepest parts. *Ceratophyllum demersum* was found in protected bays and was most abundant along the northwestern shore. *Nymphaea caerulea* also tended to be most abundant in bays. *Hydrilla verticillata* density was highest at one location along the eastern-central part of the north shore of the lake and, to a lesser extent, along the central southern shore. *Potamogeton schweinfurthii* was most abundant in the western part of the lake, but not near shore, and also along a southwest to northeast axis on the eastern side of the lake.

Water temperature was about one degree higher in April 2008 than in January 2009, and conductivity was lower in the April than in the January sample (Table 4). Phosphates, pH, nitrates, and chlorophyll *a* were not different between the two sampling occasions. Total dissolved solids (TDS) were only measured in January 2009, and dissolved oxygen (DO) and redox were only measured in April 2008, and thus no comparisons were made. All measurements are included here to provide data for comparison with other East African lakes and for future studies on Lake Bisina.

The majority of plants found in the survey were previously reported to occur in Lake Bisina (Katende 2004), and two of the plants were probably reported by Katende but under different names (*P. schweinfurthii* as *P. schweintanithi* and *N. caerulea* as *N. noudian*). However, several of the plants recovered were not previously reported from Lake Bisina, including *S. pectinata*, *U. reflexa*, *U. foliosa*, *W. filifolia*, *C. parnassifolia*, *B. schreberi*, and the *Chara* sp. The intensity of sampling in Katende's (2004) study is not entirely clear, but it appears that plants were collected on one occasion at an unreported number of sites, as opposed to the 199 locations sampled over 18 months in our study. Additionally, it is evident from the diversity of species reported by Katende (2004) that many of his samples were collected in emergent vegetation in swampy areas bordering the lake, whereas all our samples

TABLE 1. GROWTH HABITS AND GEOGRAPHIC DISTRIBUTIONS OF PLANT SPECIES COLLECTED IN LAKE BISINA, UGANDA, FEBRUARY 2008 TO AUGUST 2009.

Plant species	Family	Growth habit	Native range	Reported from Uganda	Reported from Lake Bisina
Najas horrida Magn.	Hydrocharitaceae	submersed	Africa to Sinai[1]	Triest 1989	Katende 2004
Hydrilla verticillata (L.f.) Royale	Hydrocharitaceae	submersed	Eastern Europe to Asia, Uganda to North Zambia[1]	Simpson 1989	Katende 2004
Ottelia ulvifolia (Planch.) Walp.	Hydrocharitaceae	submersed	Tropical and South Africa, Madagascar[1]	Simpson 1989	Katende 2004
Potamogeton schweinfurthii A. Benn	Potamogetonaceae	submersed	Africa, Mediterranean Islands, Arabian Peninsula[1]	Symoens 2006	Katende 2004[7]
Stuckenia pectinata (L.) Börner	Potamogetonaceae	submersed	Cosmopolitan[1]	Symoens 2006[4]	no
Potamogeton richardii Solms	Potamogetonaceae	submersed	Cameroon, Eritrea to South Africa, Madagascar[1]	Symoens 2006	Katende 2004
Ceratophyllum demersum L.	Ceratophyllaceae	submersed	Cosmopolitan[1]	Wilmot-Dear 1985	Katende 2004
Nymphaea caerulea Savigny	Nymphaeaceae	floating-leafed	Africa, Arabian Peninsula[2]	Verdcourt 1989[5]	Katende 2004[8]
Nymphaea lotus L.	Nymphaeceae	floating-leafed	Africa, Southeastern Europe[2]	Verdcourt 1989	Katende 2004
Nuphar spp.	Nymphaeceae	floating-leafed	—	—	—
Utricularia reflexa Oliv.	Lentibulariaceae	submersed	Africa[3]	Taylor 1973	No
Utricularia foliosa L.	Lentibulariaceae	submersed	Africa, America[3]	Taylor 1973	No
Wiesneria filifolia Hook.f.	Alismataceae	submersed	Uganda to North Botswana, Madagasca[1]	WCSPF[1]	No
Caldesia parnassifolia (L.) Parl.	Alismataceae	floating-leafed	Europe, East Africa, Madagascar, China to Queensland[1]	Carter 1960[6]	No
Brasenia schreberi J.F. Gmel.	Cabombaceae	floating-leafed	Africa, Temperate and Tropical Asia, Eastern Australia, America[1]	Verdcourt 1971	No
Chara sp.	Characeae	submersed	—	—	No

[1]World Checklist of Selected Plant Families 2010
[2]Global Biodiversity Information Facility 2010
[3]Taylor 1989
[4]as Potamogeton pectinatus
[5]as *Nymphaea nouchali* var. *caerulea*
[6]as *Caldesia reniformis*
[7]likely included in Katende 2004 as *Potamogeton schweintanithi*
[8]likely included in Katende 2004 as *Nymphaea noudian.*

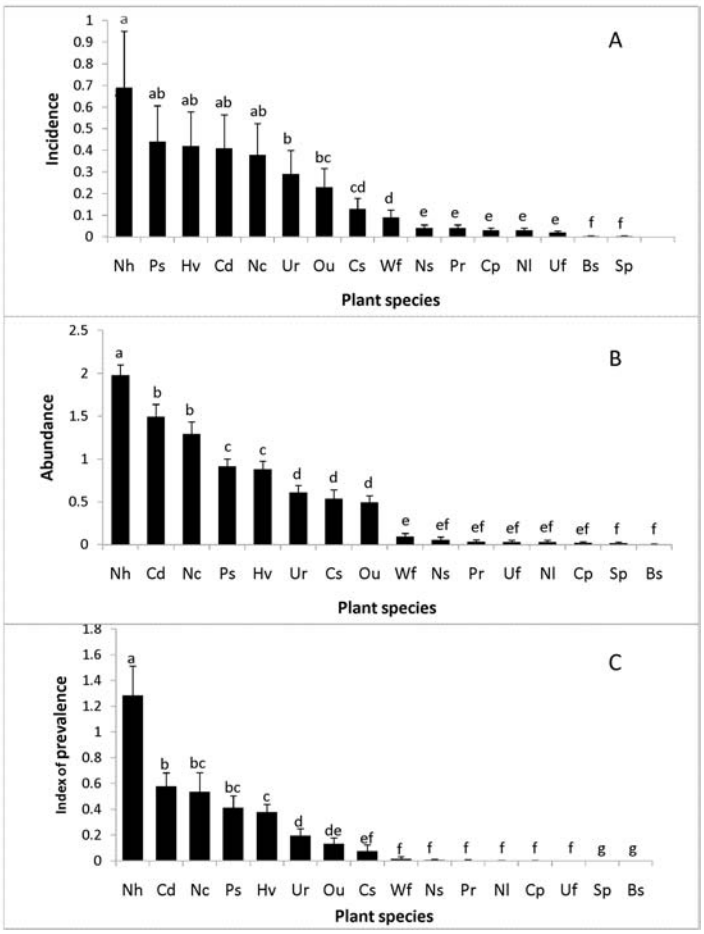

Figure 2. Incidence (A), abundance (B) and prevalence (C) of plants collected in Lake Bisina, Uganda, February 2008 to August, 2009. Bars capped by the same letter are not statistically different (P > 0.05). Nh = *Najas horrida*, Cd = *Ceratophyllum demersum*, Nc = *Nymphaea caerulea*, Ps = *Potamogeton schweinfurthii*, Hv = *Hydrilla verticillata*, Ur = *Utricularia reflexa*, Ou = *Ottelia ulvifolia*, Cs = *Chara* sp., Wf = *Wiesnera filifolia*, Ns = *Nuphar* sp., Pr = *Potamogeton richardii*, Nl = *Nymphaea lotus*, Cp = *Caldesia parnassifolia*, Uf = *Utricularia foliosa*, Sp = *Skuckenia pectinata*, Bs = *Brasenia schreberi*.

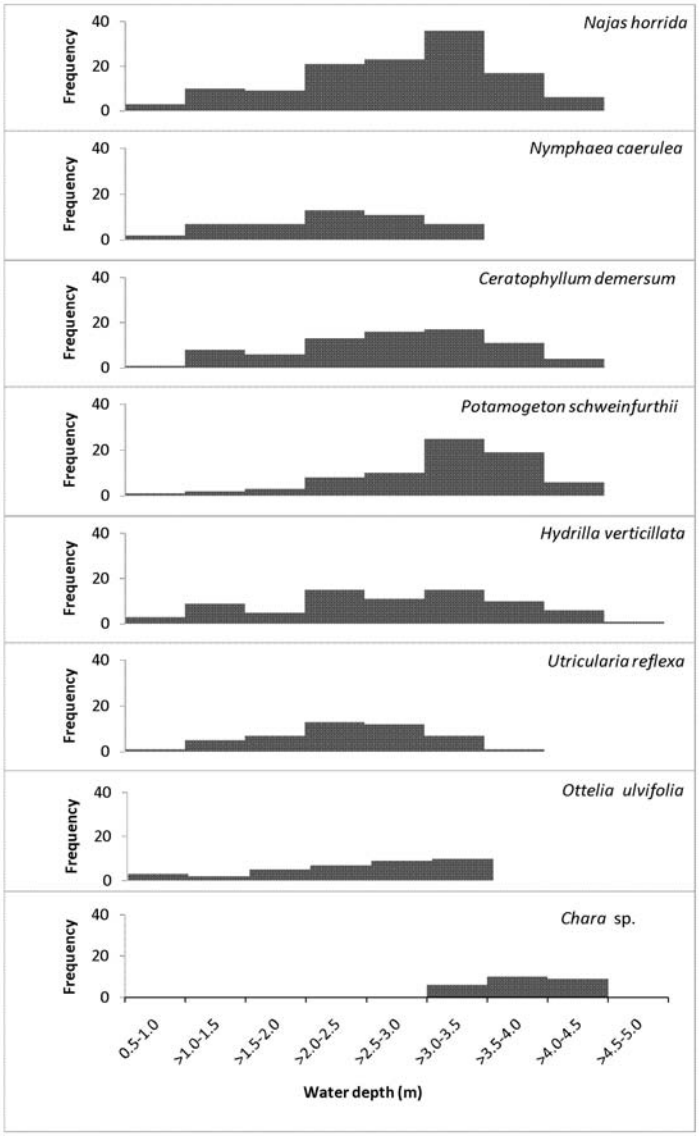

Figure 3. Frequency distributions at different depth classes of the eight most prevalent aquatic plants in Lake Bisina, Uganda.

were collected from the lake proper. Katende (2004) did not report any *Chara* spp. from Lake Bisina or any of the other nine lakes included in his study, although he may have ignored the algae because his study focused on vascular plants. The only reports of *Chara* spp. in Uganda lakes are from Lake Bunyonyi in southwestern part of the country (Denny 1971, 1973). Thus, with the greater intensity and focus of our sampling, it is not surprising that several of the less common submersed and floating-leafed aquatic species in Lake Bisina were found in the present study.

Table 2. Linear regressions of plant abundance on water depth of six species of aquatic plants in Lake Bisina, Uganda.

Plant species	Slope	F	P-value	Adj R^2
Ceratophyllum demersum	0.54	10.51	0.0014	0.05
Hydrilla verticillata	0.55	26.95	<0.0001	0.13
Otellia ulvifolia	0.39	18.73	<0.0001	0.09
Nymphaea caerulea	0.95	46.41	<0.0001	0.22
Utricularia reflexa	-0.45	24.22	<0.0001	0.12
Chara spp.	0.06	30.50	<0.0001	0.15

Positive or negative associations between plants can occur due to similarities or differences in habitat requirements, or to direct interactions between species (e.g., mutualism, commensalism, competition, and predation; Roxburgh and Chesson 1998). The *Chara* sp. was negatively associated with *N. horrida*, *N. caerulea*, and *U. reflexa*, and all of these associations may be due to differences in habitat requirements. *Nymphaea caerulea* and *U. reflexa* were negatively related to water depth, while *Chara* sp. was positively associated with depth. *Chara* spp. are known to often occur in deeper water than vascular macrophytes due to high shade tolerance, and may be competitively excluded in shallow water by vascular macrophytes (Kufel and Kufel 2002). The negative relation-

TABLE 3. PAIRWISE ASSOCIATIONS BETWEEN PLANT SPECIES FOUND IN LAKE BISINA, UGANDA.

Species 1	Species 2	Species 1 present/ Species 2 present	Species 1 absent/ Species 2 present	Species 1 present/ Species 2 absent	Species 1 absent/ Species 2 absent	Chi-square	P-value[1]	Yule's V
Ceratophyllum demersum	*Potamogeton schweinfurthii*	21	68	65	45	25.26	<0.0001	-0.356
Najas horrida	*Hydrilla verticillata*	73	13	71	42	11.87	0.02137	0.237
Najas horrida	*Utricularia reflexa*	49	5	95	50	12.52	0.0143	0.251
Najas horrida	*Chara sp.*	6	20	138	35	36.32	<0.0001	-0.427
Nymphaea caerulea	*Utricularia reflexa*	40	14	25	120	57.78	<0.0001	0.539
Nymphaea nouchali	*Chara* sp.	0	26	65	108	14.51	<0.0001	-0.270
Utricularia reflexa	*Chara* sp.	0	26	54	119	11.14	0.0284	-0.237

[1]P-values adjusted using the Dunn-Sidak correction. Yule's V indicates the direction (positive or negative) and strength of associations between species.

Figure 4. Interpolated surfaces of abundance of *Nymphaea caerulea*, *Hydrilla verticillata*, *Potamogeton schweinfurthii*, *Najas horrida*, *and Ceratophyllum demersum*. Abundance estimated on a scale of 0-5 (see modified DAFOR scale scoring in text).

ship between the *Chara* sp. and *N. horrida* was also probably due to partitioning of the lake by water depth, although the relationship of *N. horrida* with water depth was not statistically significant. The negative association between *C. demersum* and *P. schweinfurthii* is interesting because they both occur at similar water depths (Figure 3) but in different locations of the lake (Figure 4). *Ceratophyllum demersum* tended to be most abundant in sheltered bays, whereas *P. schweinfurthii*

Table 4. Physico-chemical parameters of water in Lake Bisina in April 2008 and January 2009.

Month /year	N	Temperature	DO mg/L	pH	TDS mg/L	Conductivity	PO_4-P (ug/L)	NO_3-N (ug/L)	Chl-*a* (ug/L)
Jan 09	38	26.5 ± 0.14 b	—	7.9 ± 0.13 a	138.5 ± 0.7	276.9 ± 1.5 a	16.1 ± 2.2 a	21.2 ± 3.1 a	5.0 ± 1.2 a
Apr 09	37	27.7 ± 0.14 a	6.8 ± 0.1	8.1 ± 0.07 a	—	254.4 ± 6.1 b	12.0 ± 1.5 a	26.3 ± 1.4 a	3.8 ± 0.3 a

was abundant in more open areas of the lake. The former species lacks true roots but anchors itself to the bottom with modified leaves (rhizoids), which may explain its occurrence in sheltered bays (Best 1980). The strong positive association between *N. caerulea* and *U. reflexa* may have been due to niche similarities but may also suggest a commensal relationship with *U. reflexa* performing well under shaded conditions provided by the floating leaves of *N. caerula*. Alternatively, *Utricularia* spp. are rootless, and thus may simply get caught on the stems of *N. caerulea*. Kateyo (2006) also mentioned a positive association between *N. caerulea* and a *Utricularia* sp. in Lake Nabugabo, Uganda.

The vertical distributions of *N.caerulea* and *H. verticillata* in relation to water depth were similar to those reported for the same plants in Lake Bunyonyi, Uganda, by Denny (1971), although *H. verticillata* was found infrequently between 4.5 and 7 m in Lake Bunyonyi, whereas it was not found deeper than 4.5 m in our study (although the deepest location sampled in the present study was 5 m). However, the vertical distribution of *C. demersum* was wider in Lake Bunyonyi (~0.2 to 7 m, mean ≈ 3.1) than in Lake Bisina (0.6 to 4.3 m, mean = 2.7 m), and the distributions of the *Chara* sp. were very different, with the Lake Bunyonyi *Chara* sp. occurring in shallow water (~0.2 to ~5.2 m, mean depth ≈ 2.1 m), whereas the *Chara* sp. in Lake Bisina was only found in deeper portions of the lake within a very narrow range of depths (3.2 to 4.3 m, mean = 3.8). This could be attributed to the presence of different *Chara* spp. in the lakes and/or environmental differences resulting in dissimilar vertical distributions.

Hydrilla verticillata was the only species associated with water clarity, with abundance increasing as clarity decreased. The area of the lake where *H. verticillata* was most abundant (Figure 4) was just offshore from a small village. During sampling in this area, women were observed washing clothes, and cattle drank by entering the water, which may provide an explanation for the elevated turbidity, either through physical disturbance of the bottom or promotion of phytoplankton and algal growth due to high nutrient levels. Interestingly, the other area where *H. verticillata* was relatively abundant on the south side of the lake was also next to a village. Van et al. (1976) compared the light requirements of three submersed macrophytes, *H. verticillata*, *C. demersum*, and *Myriophyllum spicatum* L., and found that *H. verticillata* required the least amount of light to achieve half its maximum photosynthetic rate. The same authors concluded that *H. verticillata* had a distinct competitive advantage over the other 2 species because of its superior ability to perform under low light. Underscoring its ability to fix carbon at low light levels, *H.verticillata* in Lake Tanganyika was found growing at 8 m depth off Nyanza Lac, Burundi (Copeland unpub. data) and also at 10 m depth off Kigoma, Tanzania (Eggermont et al. 2008). This reasoning may provide a possible explanation for the abundance of *H. verticillata* in turbid areas of the Lake Bisina. *H. verticillata* may out-compete other species in Lake Bisina when light is limited, or in potentially nutrient enriched conditions, but other species have an advantage in clearer or lower nutrient areas of the lake. A similar light-mediated competitive interaction between two *Potamogeton* spp. was recently reported by Spencer and Rejmánek (2010).

Hydrilla verticillata was introduced into the southern United States in the 1950s by the aquarium trade (Schmitz et al. 1991) and rapidly spread to become one of the most serious aquatic invaders in many lakes, rivers, and canals (Balciunas et al. 2002). *Hydrilla verticillata* causes problems because it grows in large, dense monocultures that interfere with water flow, recreational activities, and displace native aquatic plant communities (Langeland 1996), although the impacts of *H. verticillata* on native biodiversity have recently been questioned (Hoyer et al. 2008). This situation differs greatly from that encountered in Lake Bisina, where *H. verticillata* grows in association with several other plants (mean number of other plant species found growing at locations where *H. verticillata* occurred = 2.48 ± 1.1), has a patchy distribution (Figure 4) and is only moderately abundant compared to the other plants in the lake. The reasons for the difference in abundance of *H. verticillata* in Lake Bisina and freshwaters in the southern United States are unknown but could be due to differential pressure from biotic factors and/or differences in water quality. Florida lakes in general are less alkaline than Lake Bisina, and nutrients tend to be much higher in Florida lakes (Hoyer et al. 1996). Additionally, genetic differences may play a role, as several studies have described physiological differences between hydrilla populations (e.g., Steward 2000, Puri et al. 2007, Maki and Calatowitch 2008).

Lake Bisina is designated a RAMSAR site (wetland of international importance; Byaruhanga and Kigoolo 2005), because of its (1) unique macrophyte ecosystem, (2) support of rare and endangered organisms, (3) support of populations of plants and animals important for maintaining biodiversity of the region, (4) support for bird and animal species at a critical stage in their life cycles, (5) support of endemic fishes, and (6) importance as a spawning ground for fish. Our study provides new information on the diversity, distribution, and interrelationships of floating-leafed and submersed aquatic plants in Lake Bisina, Uganda, including several first records of species occurrences in the lake. This information, coupled with the physico-chemical water data, provide a solid baseline for comparison with future biological studies on Lake Bisina and for the design of conservation management strategies.

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