Invasive Aquatic Plant surveillance in Great Lakes coastal areas: Sampling design, field procedures, data entry and analysis, and applications

Andrew J. Tucker[1], Gust Annis [2], Erick Elgin[3], W. Lindsay Chadderton [1], Joel Hoffman[4]

[1] The Nature Conservancy, 721 Flanner Hall, University of Notre Dame, IN, 46556

[2] The Nature Conservancy, 101 E. Cesar E. Chavez Ave, Lansing, MI 48906

[3] Michigan State University Extension, 160 Agriculture Hall, East Lansing, MI 48824

[4] USEPA Great Lakes Toxicology and Ecology Division, Duluth, MN

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Executive Summary

We outline a surveillance monitoring protocol designed to quantitatively assess survey performance for detection of the entire aquatic plant community, including rare and potentially invasive aquatic plants, in Great Lakes coastal areas (ports, harbors, drowned river mouths, estuaries). This protocol employs a probabilistic, random sampling design, with sample effort allocated proportionally towards shallow habitats (< 6m water depth) or areas with high predicted species richness. Species richness values at the sample-unit scale for any given site are predicted from a forest-based classification and regression approach and are based on habitat attributes associated with plant establishment and surrogates for aquatic plant pathways of introduction, including depth, fetch, percent littoral zone, distance to boat ramps, and distance to marinas. At each sample site, the aquatic plant community is surveyed from a boat with a rake sampler (multiple rake tosses) and by visual inspection to characterize species presence at each of four evenly spaced sampling stations. Between sample-units and each sample station within a unit a visual meander survey is undertaken to increase the probability of encountering areas of high plant abundance or areas of floating or emergent plants species of interest. The protocol allows for an adaptive sampling approach and reallocation of effort, including modifying the location of sample units, to ensure areas of high interest are surveyed. Species accumulation theory is used to estimate species richness and characterize species accumulation for each survey, as well as to quantify the effort required for high-probability rare species detection at each site (a proxy for difficult-to-encounter non-native species). This document contains guidance for designing and conducting a survey, including information on developing the depth or richness surface for a site, allocating sample effort based on depth or richness, obtaining an electronic file of sample site coordinates, uploading site coordinates into a geospatial information system (using ArcCollector), conducting field work, entering, summarizing, analyzing, and reporting data, and processing voucher specimens. Final products from each survey include: 1) raw data indicating species composition and abiotic conditions (depth and secchi transparency) at each sample station and combined for each sample unit, 2) survey performance measures (including estimated species richness and proportion of the estimated richness sampled, based on the sample-based rarefaction curve), and 3) voucher specimens cataloguing species presence. All non-native species detections should be shared to the Nonindigenous Aquatic Species information resource for the United States Geological Survey (USGS NAS; https://nas.er.usgs.gov/default.aspx).

Background

In 2015, a regional collaboration focused on developing a framework for aquatic invasive species early detection monitoring in the Great Lakes was established (Chadderton et al. 2021). The collaboration identified invasive aquatic plant (IAP) surveillance as a key management gap in the Great Lakes basin. Out of 144 species that were assessed as having the greatest potential to arrive, establish, and cause impacts in the Great Lakes, nearly half (65) were aquatic plants (Davidson et al. 2021). The locations representing the nexus of pathways most likely to introduce these highest risk IAP included various ports, estuaries, embayments, and drowned river mouths across the basin (Tucker et al. 2020). Many of these coastal sites are relatively large (~10 km^2) and complex, with a diversity of open water habitats (shallow, deep, exposed, sheltered, rocky, sandy, etc.).

As part of a funding opportunity (F16AS00090) from US Fish and Wildlife Service (FWS) to the Michigan Department of Environment, Great Lakes, and Energy (EGLE), on behalf of the Great Lakes states, The Nature Conservancy (TNC) and Michigan State University (MSU) Extension were contracted to develop and implement a survey design and sampling methods for early detection of IAP in the coastal areas of the Great Lakes that had been identified as high risk points of introduction. The project, "Aquatic plant survey methods development and site assessment," was included as one of four objectives in the FY17 *Interstate Aquatic Invasive Species Prevention, Early Detection, and Response* proposal to FWS.

In 2017, The Nature Conservancy reviewed existing state and regional aquatic plant survey methods and developed a pilot survey design and sampling methods protocol (Tucker 2017). The draft protocol was implemented in summer 2017 at three sites that were identified as priorities for aquatic plant surveillance based on predicted high risk of invasive aquatic plant introduction. The three sites were Milwaukee (WI), St Joseph River/Benton Harbor (MI), and Cleveland (OH). Based on results from 2017 surveys, the pilot survey design and sampling protocol was refined. Three additional surveys, using the adapted protocol, were conducted in summer 2018, including a second survey at Milwaukee (WI) and surveys on the Detroit River (Gibraltar, MI) and in Saginaw Bay/River (MI). Project results were provided to EGLE in a March 2019 final report (Tucker et al. 2019).

In 2019, additional aquatic plant surveys were conducted, including a third survey at Milwaukee and a second round of sampling at Detroit River and Cleveland. The survey design for the 2019 surveys allocated survey effort proportionally to the most species rich areas, using a surface created in GIS based on kriging of species richness measures from previous surveys at each site. These surveys were part of the *Interstate Aquatic Invasive Species Early Detection and Response for 2018-2020* project (F18AS00106) with funding from FWS to EGLE. A manuscript, describing the survey method and project results has been prepared for publication (Tucker et al. *In review*).

The sample design protocol outlined in this document is the outcome of the project activities described above. The protocol offers a useful starting point for baseline IAP surveillance at high priority coastal sites across the basin.

Survey objective

The survey design and sampling methods described here allow the user to characterize species richness and to estimate detection rates for the full aquatic plant community at the scale of an entire port, embayment, or estuary (i.e. coastal area). The survey design is based on protocols developed by US Environmental Protection Agency and FWS for fish and invertebrate sampling in Great Lakes ports (Trebitz et al. 2009, Hoffman et al. 2011, Hoffman et al. 2016, Harris et al. 2018). The sampling methods are adapted from aquatic vegetation survey protocols used by various state natural resource management agencies and from an approach described by Yin et al. (2000) for the US Geological Survey Long Term Resource Monitoring Program. Great Lakes coastal areas where high risk IAP are expected to arrive and establish vary in size (400-800 hectares) and consist of a mixture of habitat types. Some sites are mostly shallow (<4 m water depth on average) and transparent with a mix of natural shoreline or engineered shoreline with docks, while other sites are relatively deep (maximum depth 10 m or more) and turbid, with largely hardened waterfronts and a few large marinas. IAP are similarly diverse and represent a full range of growth forms, including emergent grasses (e.g. Phragmites), to submerged macroalgae (e.g. Starry stonewort), to free-floating lilies (e.g. European frog-bit). The protocol employs visual meander and rake toss methods that facilitate sampling of all plant growth forms across the varied habitats of Great Lakes coastal areas.

Sampling emphasizes shallow areas (< 6m) or areas of high predicted species richness based on depth or species richness layers created with geographic information system (GIS) software. The depth layer can be digitized from nautical charts for the coastal area, with depths subsequently assigned to each $100m^2$ sample-unit in the sample frame. The species richness surface is derived from a forest-based classification and regression method that uses five key habitat variables to predict richness for each of the $100m^2$ sample-units (depth, fetch, precent littoral area, minimum distance to boat launch, and minimum distance to marina). These five habitat variables predict species richness with high accuracy (based on previous surveys at representative Great Lakes coastal sites; Tucker et al. *In review*). All entry points (e.g., boat ramps) are also sampled. GIS expertise is recommended to facilitate the survey design process.

In summary, the protocol has the following attributes:

- Quantitative and probabilistic (to facilitate evaluation of detection rates and to decrease the likelihood that some habitats are not sampled at all or with little sampling effort)
- Targets multiple habitat types to detect the full range of watch list species (by exploiting the habitat preferences and life histories of targeted priority species, e.g. submerged vs. emergent vs. floating)
- Targets habitats most likely to support plants, native and non-native (i.e. shallow or species rich sites and points of entry; to increase survey efficiency and limit sampling effort in areas likely devoid of plants)
- Employs more than one sampling method (i.e. rake toss and visual inspection, to exploit method-specific differences that facilitate adequate sampling of various habitats and plant growth forms)
- Adaptive sampling approach that allows for relocation of sample units or sampling stations during the survey based on the observation of potential species or plant communities of interest while meandering between sample units and stations within a unit (to increase probability of encountering areas of high plant abundance and areas of floating or emergent plants that may include invasive species).
- Spatial data preserved and can be mapped for both the managers use and for clearly communicating distributional data with other stakeholders

Please note: this protocol offers a useful starting point for IAP surveillance at Great Lakes coastal areas. Previous surveys implementing this protocol indicate detection rates in the neighborhood of 60-90% with additional survey effort required for 95% or more detection rates (i.e., high confidence detection of rare, including potentially invasive, aquatic plants). Depending on survey results, additional surveillance at a site may be warranted and survey design can be further refined based on detection patterns.

Survey design

Sampling sites

This method employs a quantitative and probabilistic survey design and allocates effort proportionally to shallow areas or areas with highest predicted plant richness. Our methods are tailored for use with ArcGIS software, but the approach we describe could be adapted for other GIS software.

1) Sample frame –
    a. Priority Great Lakes coastal areas (9km x 9km) with highest predicted risk of IAP introduction are identified in Tucker et al. 2020 and available as an interactive risk map with downloadable metadata here.
    b. When a sample site (coastal area) is identified for sampling, the boundaries of the sample site (sample frame) should be digitized in ArcGIS. We recommend sample frame does not exceed ~800 hectares (2000 acres). Sample frame should be constrained to the open water areas of the site. We recommend using an air photo and/or navigational chart to digitize the water surface and zoom in to a scale of at least 1:10,000 when digitizing.
2) Sample units –
    a. Sample units ($100m^2$) are overlaid within the sample frame. In ArcGIS, create a regular grid of 100x100m polygons using the Grid Index Features tool that covers the entire sample frame.
    b. For sampling, four evenly spaced stations within each sample unit will be selected (see below, *collecting and recording field data*)
    c. For analysis, the species data collected from each of the 4 sampling stations are combined (see below, *entering data electronically*)
3) Sampling design- We outline two methods below that can be used to select sample units. The methods are based on results from previous surveys that show survey performance is improved by targeting shallow habitats (<6m) or habitats with high predicted species richness (based on a few key habitat variables). In areas with contrasting deep and shallow habitats a survey design based solely on available depth data is a practical starting point for allocating survey effort (see Method 1). If additional planning resources are available and especially when sample locations are uniformly shallow, we recommend allocating survey effort based on the predicted species richness surface (see Method 2).
    a. Method 1- Allocating effort based on depth only
        i. In ArcGIS, digitize a data layer for water depth (meters). If detailed water depth digital data is not available digitize depth sounding points from the digital NOAA raster nautical navigational charts covering the study area; these charts are also available through ArcGIS Online and can be added to desktop GIS or ArcGIS Online maps. Use the Kriging tool in ArcGIS to create a raster of water depth with a 1m cell size using the digitized depth points as input.
        ii. In ArcGIS, use the "create spatially balanced points tool" to randomly select up to 75 sample units for sampling. At least 75% of the randomly selected sample units should be selected from areas where average sample unit depth is <6m; the remaining sample units should be allocated to sample units >6m depth and to any points of entry (i.e. boat ramps).
    b. Method 2 - Allocating effort based on predicted species richness
        i. In ArcGIS, digitize or create data layers for each of the following habitat variables
            1. Water depth (meters). As described in Method 1.
            2. Maximum fetch (meters, from sample unit edge). Use the ArcMap Fetch Model tool (Rohweder at al. 2012) to compute fetch for the eight

cardinal directions using a 1m cell size. Attribute each sampling unit with the maximum fetch value.

3. Minimum distance to a boat launch (meters, from sample unit edge). Use the CostDistance tool in ArcMap to compute the distance to the nearest public boat launch using a 1m cell size.
4. Minimum distance to marina (meters, from sample unit edge). Use the CostDistance tool in ArcMap to compute the distance to the nearest marina using a 1m cell size.
5. Percent littoral. For each sample unit, reclassify depths of less than 6ft (1.8m) as littoral zone and calculate the percent of the sample unit comprised on littoral zone depth water.

ii. In ArcGIS, use forest classification and regression method to create a predicted species richness surface for each sample unit (i.e. the Forest Based Classification and Regression tool in ArcGIS Pro, vers 2.8).

iii. In ArcGIS, use the "create spatially balanced points tool" to randomly select up to 70 sample units for sampling. Probability weights based on richness are assigned to each sample unit and the "create spatially balanced points" tool in ArcGIS is used to randomly select 70 sample units across the whole site (i.e. 90th percentile = 0.6, 80th percentile = 0.5, 70^{th} percentile = 0.4, 60^{th} percentile = 0.3, 50^{th} percentile = 0.2, $<50^{th}$ percentile = 0.1)

iv. In ArcGIS, also note location of any points of entry (i.e. boat ramps). If the ramps or an adjacent sample unit have not been randomly selected, add all points of entry to the sample list.

4) Sources of error or uncertainty

a. Key sources of error or uncertainty relate to the a priori predictions of suitable plant habitats within the survey area that is used to assign sampling locations and the failure to detect plant species either because of misidentification or lack of sampling effort. Here we recommend a simple model based on a few easily accessible abiotic variables (e.g., depth, fetch, percent littoral) to predict suitable plant habitat so survey effort can be weighted towards those habitats. By their very nature, model predictions will not be 100% accurate. We have used a relatively small set of sites to train the models, a limited number of abiotic variables (that are in themselves models of physical variables with their own inherent errors) that are simple proxies for complex ecological relationships that vary across space and time (see Tucker et al. *In review*). Thus, it is important to employ an adaptive sampling approach when conducting the survey. To mitigate the possibility of not detecting a species that is present at a site (including a novel IAP), the survey team should be willing to modify the location of sample units during a survey (e.g., based on the observation of potential species or plant communities of interest during the boat meander) or to modify the survey design based on the results from an initial survey to account for unexpected patterns in plant detection (e.g., presence of plants at sites >6m water depth). Employing a qualified plant taxonomist is critically important to avoid detection errors, especially given differences between congeners can be subtle and easily missed. Finally, the analytical approach recommended (i.e. sample-based rarefaction) is critically important to quantify the potential failure to detect species and should be used to inform additional sampling effort. Rarefaction provides a measure of the accuracy of a survey, namely confidence intervals around the estimates of species richness for a given survey effort, and also facilitates estimates of the additional effort required to detect most (95%) or all (100%) of the species present at a site.

Field methods and sample processing

*Field Gear**

Necessary equipment:

- Appropriate boat and all equipment required by law
  - Recommended: 16' – 20' flat bottom boat with elevated bow platform.
- Weighted sampling rakes attached to 50-ft (~15m) rope (x2)
  - see Appendix 1
  - Double-headed 14 tine rake heads
  - Recommend 3/8" braided floating polypropylene rope
- Tablet (e.g. iPad) and accessories for data collection
  - See Appendix 2
  - External Bluetooth GPS (e.g. Dual XGPS)
  - Portable power bank (charger) with USB ports
- Depth sounder (e.g. HawkEye DepthTrax 1H, or as part of a sonar transducer)
- For voucher specimens
  - Sealable storage bags (ziplocs)
  - Absorbent paper towels
  - Waterproof voucher sample labels (or Sharpie to label bags)
  - Cooler (w/ ice)
  - Plant press
    - Blotter paper recommended to improve drying

Optional equipment:

- 8 in x 11 in white sorting trays for aiding in plant identification
- Sonar to aid in locating vegetation for rake tosses (e.g. Garmin Striker 4 Portable Bundle, Olathe, KS USA)
- Secchi disk (to estimate euphotic zone)
- Polarized sunglasses
- Thermometer to record water temperature
- Plant guide(s) to aid in plant identification and species watch lists, e.g.
  - Crow GE, Hellquist CB (2000a) Aquatic and wetland plants of northeastern North America. Vol. 1. Pteridophytes, gymnosperms and angiosperms: dicotyledons. University of Wisconsin Press, Madison, Wisc.
  - Crow GE, Hellquist CB (2000b) Aquatic and wetland plants of northeastern North America. Vol. 2. Angiosperms: monocotyledons. University of Wisconsin Press, Madison, Wisc
  - Skawinski, P (2019) Aquatic Plants of the Upper Midwest. Wausau, WI.
  - Borman et al. (1997) Through the Looking Glass: A field guide to aquatic plants. Second Edition. Stevens Point, WI. Wisconsin Lakes Partnership.
- Hand lens to aid in plant identification
- Hard copy maps of sample frame and sample units
- Standard garden rake (for collecting plants observed during visual inspection in shallow water)
- GPS receiver and hard copy data sheets (with sample unit coordinates) in case of technology failure

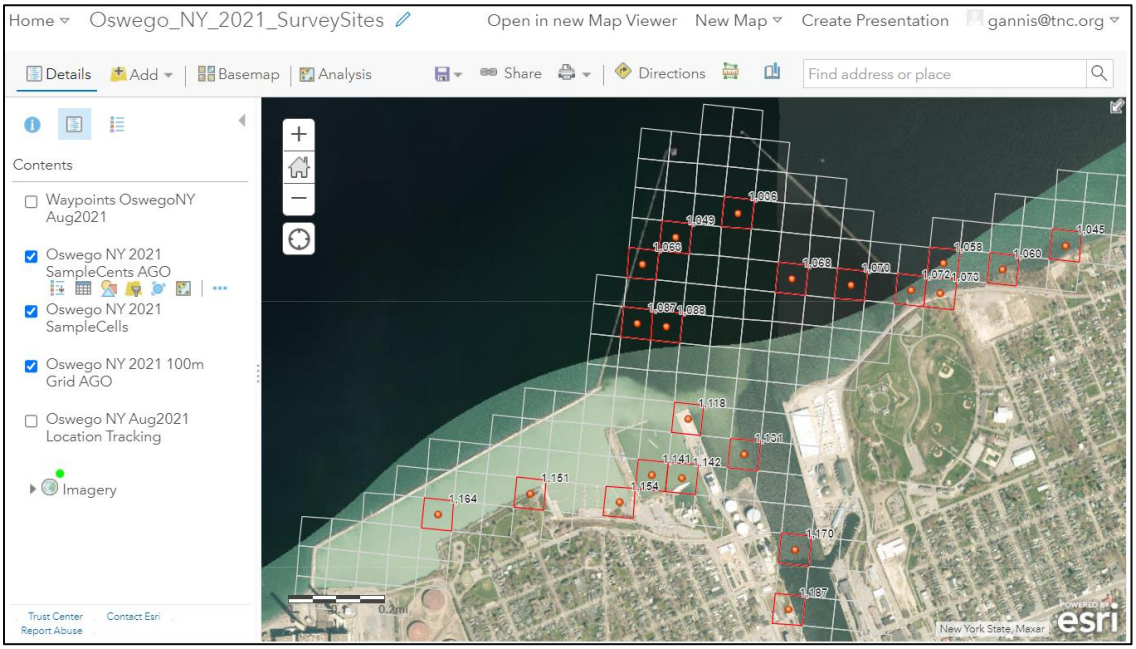
* Use of product names does not imply any affiliation with or endorsement by them.

Preparing for data collection

A tablet (iPad) and external bluetooth GPS are recommended for data collection and to navigate to sample sites. We use an ESRI mobile monitoring application, ArcGIS Collector, for data collection. In 2021 ESRI will be retiring Collector and moving to FieldMaps. The workflow we describe here can be adapted for use with FieldMaps or other mobile monitoring applications. The data fields and map extent for the sample location(s) of interest are created in ArcGIS, published on ArcGIS Online and accessed in the field via the ArcCollector app. An ArcGIS Online (AGO) subscription is required (https://tnc.maps.arcgis.com/).

Creating the Collector map and data fields:

1) Create empty waypoints geodatabase to use in AGO and Collector for recording field data. Include the fields and domains described in Appendix 4.
2) Upload waypoints to AGO. Enable editing and offline use.
3) Upload geodatabase or shapefile of the sampling grid (100m grid) that includes the 'SampGridID'. Enable offline use.
4) (Optional) Upload the grid cells of the specific sample locations (grid cells identified for sampling) that includes the 'SampGridID.' Enable offline use.
5) Upload centroids of the specific sample locations (grid cells identified for sampling) that includes the 'SampGridID.' Enable offline use.
6) Create a tracking layer in AGO. Enable offline use.
7) Create a new map in AGO and populate with resulting layers from steps 2-6 above, as in image below. Enable offline use. Use air photo imagery as the base map. Label the sample centroids using 'SampGridID.' This map will be used in Collector and the SampGridID numbers are useful for navigating and recording in the Waypoints layer as data is collected.



Navigating to sample sites

Two iOS applications and a Bluetooth device are used:

1) ArcCollector (Collector) – This is an ESRI mapping application that will be used to collect map, photo, and field visit information. This can be accessed while offline and at field sites.
2) Dual GPS Status Tool – The app that collects to the Bluetooth GPS, and lets you know when you have a signal and the strength of the signal.
3) Dual Bluetooth GPS – This will GREATLY improve accuracy of GPS signal and location for photo points while monitoring in Collector.

Prior to Field Visit

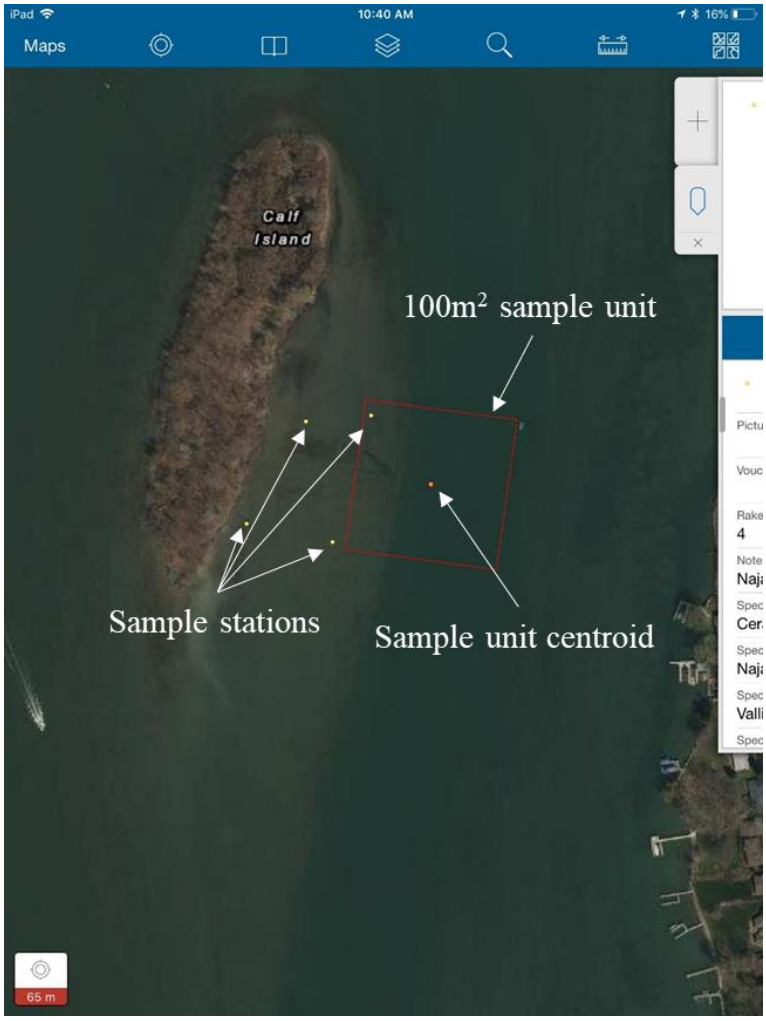
1) Charge Dual GPS and tablet
2) Ensure tablet and apps are functioning properly
3) Use Collector to download a new map extent (i.e. data collection area)
    a. Ensure device is connected to the internet and then open the Collector app
    b. Select "ArcGIS Online" and enter login details
    c. Download a map of the area you will be sampling (i.e. map for offline use in the field)
        i. Navigate to the appropriate map and select the download button
        ii. Select the work area – this will be the extent of the downloaded area so ensure that the entire sample area is selected.
        iii. Select the "Map detail" – this will influence how far you can zoom in on the sample area and the resolution of the satellite image while in the field. 8 MB zoom provides adequate resolution without using excessive space on the device or requiring long download time.
        iv. Select "Download" in the top right corner.

Using the device during the Field Visit (No internet connection needed):

1) Connect external Bluetooth GPS using the GPS app (see Appendix 3)
2) Open Collector to begin navigating to sample sites and collecting data
    a. Select the map of interest from the "On Device" tab
    b. If the GPS is enabled and tracking, your current location is indicated by a blue dot. Sample units will be visible in the map frame.
    c. Use the Collector map and GPS to navigate to sample units.

Sampling plants and recording data

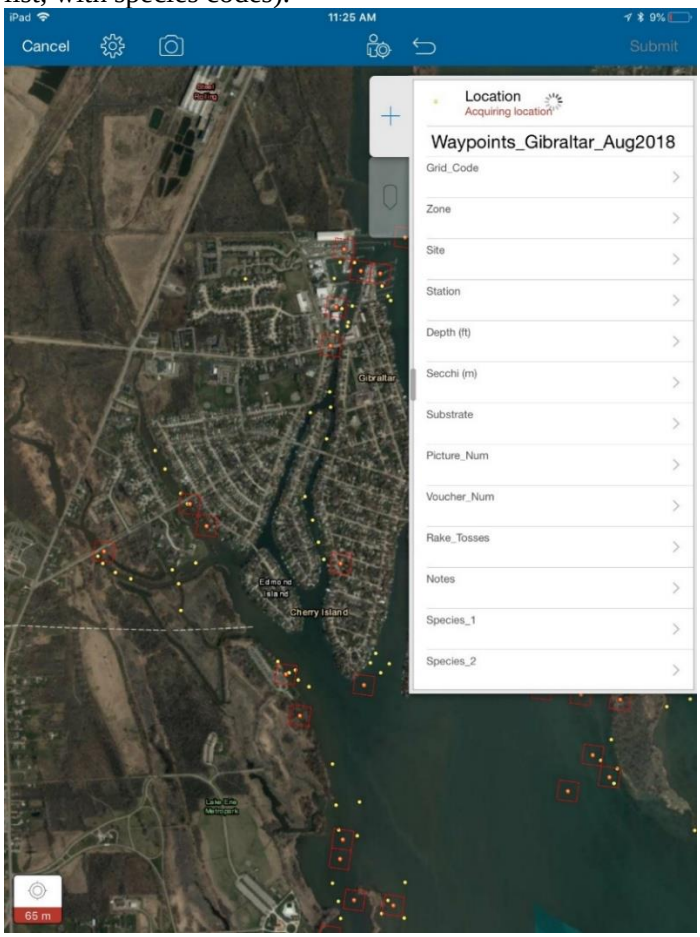
1) In general, four evenly spaced and discrete "sample stations" are sampled within each sample unit (e.g. the four corners of the sample grid).
    a. As the objective of the survey is early detection of invasive aquatic plants, the exact location of sampling stations at each sample unit can be adjusted to maximize the potential to detect plants during rake tosses or visually, as in the image below.
    b. In deep or turbid areas where lake bottom is not visible, a transom mounted sonar unit can be used as an aide to identify sampling stations within the sample unit (based on potential presence of macrophytes from sonar imaging).



2) At each of the four sample stations, aquatic plants are collected with rakes or observed visually and recorded. The survey shall include all submerged, floating, and emergent taxa.
    a. For rake sampling,
        i. At each station the rake is tossed 5 – 10 meters from each side of the boat (i.e. four rake tosses), allowed to sink to the lake bottom, and slowly retrieved. *Note*: the standing end of the rake rope should be secured to the boat before being tossed into the water for sampling.
        ii. Plants are identified to species in the field. *Note:* Removing the plant material from the rake and observing over a white sorting tray can aid with sorting and identification.
        iii. When specimens cannot be identified in the field (e.g. when identification requires scrutiny of morphological features under a microscope) the plant must be vouchered and identified to species (or lowest possible taxonomic group) in the laboratory. See below, "Plant identification and voucher collections" for more detail.
        iv. In instances where the fourth rake toss results in collection of a species not observed in the previous three tosses, subsequent rake tosses are made into areas not previously sampled until no new species are collected.
        v. Retain a plant specimen of every species for vouchering, see below "Plant identification and voucher collections" for more detail.

b. For visual observation,
    i. All submerged, emergent, or free-floating plants within approximately 10m of boat are identified to species and recorded, except that observations are only made from the waterline lakeward to the maximum depth at any sampling station and plants are only recorded when they are observed in water (i.e. riparian or wetland plants above the waterline are not included in the survey). *Note:* A hand rake can be used to collect plants observed visually for vouchering or for closer inspection.
c. Visual meander between and within sample units.
    i. When moving between sample units or sampling stations, observe the surrounding area for any high-risk plants (e.g. water hyacinth). If a high-risk species is observed, adjust the nearest sample site or unit to include the high-risk occurrence.

3) To record the data,
    a. Once the boat has arrived at a sample station, use the tablet to record data in Collector.
    b. In the map view, click the "+" sign next to the heading "Collect a new feature." Then, select "New Feature."
    c. Select the "target" icon in the top banner to drop a point with lat/long location for the station.
    d. In the dropdown menu on the right of the screen, select each data field to record other relevant data, including species records (see image below; and see Appendix 4 for a description of the data fields and Appendix 5 for a recommended pre-populated species list, with species codes).



e. To include photos, follow these steps:
    i. Select the camera icon in the top banner
    ii. Select Add
    iii. Select Take Photo or Video (you can take up to 5 photos per station)
    iv. Take Photo
    v. Select “Use Photo” or “Retake”
    vi. Select “Done” or “Add” to add an additional photo (repeat steps iv to vi)
f. Once all data fields have been entered and photos taken, select “Submit”
g. To edit data or photos at a station,
    i. Highlight the station of interest by tapping the point on the map viewer
    ii. Select the “box with arrow” icon
    iii. Select “Edit” from the dropdown menu that appears
    iv. Edit the data field(s) as needed
    v. When complete, select “Update”

Plant identification and voucher collections

This section is adapted from, Hauxwell, J., Knight, S., Wagner, K., Mikulyuk, A., Nault, M., Porzky, M., & Chase, S. (2010). Recommended baseline monitoring of aquatic plants in Wisconsin: sampling design, field and laboratory procedures, data entry and analysis, and applications. *Available from the Wisconsin Department of Natural Resources, Madison, WI. PUB SS-1068*.

Plant Identification

1) Plants should be identified to species whenever possible. Certain genera, including *Carex*, *Sparganium*, and *Sagittaria* must be flowering and/or fruiting to confirm identification and may not be identifiable to species without these parts.

2) Non-angiosperms such as *Chara* or *Nitella* are identified to genus only, except for starry stonewort (*Nitellopsis obtusa*). Often, *Isoetes* can be identified to species by looking at spores, if present. Filamentous algae is not included in the survey. Aquatic moss is identified to genus or can be labeled as "Freshwater Moss".

3) If a plant cannot be identified in the field, place the specimen in a re-sealable bag with a separate voucher label and return the specimen back to the lab to verify the identity. The label should include the collection date, the site name, the sample site number, and a unique identifier (e.g. voucher #). In Collector, note the voucher # and include any other relevant detail in the "Notes" field for that sample station.

4) In the lab, try to identify the plant using plant identification keys and a stereo microscope. If you are still uncertain of the identity of the plant, contact a DNR biologist or other botanical expert in your region to help with identification. Do not send specimens to an expert until you notify them of your intended shipment and they have instructed you to do so. Once the plant is identified, record this information so that the correct identification is used during data entry.

Voucher collections

1) A voucher specimen must be retained and prepared for each plant species collected at a site. You can often use plants collected on the rake as vouchers. However, if the sample is of poor quality or lacks reproductive structures, attempt to collect a better specimen. If a better specimen is unavailable, voucher and press what you are able to collect. Remember that the more material collected, the easier identification will be. Whenever possible, collect at least two specimens, and include reproductive material such as seeds, flowers, fruit, roots, etc. If a species identification cannot be resolved in the field, retain a representative specimen and return it to the lab for species identification.

2) Wet a paper towel square and fold it around your specimen. Place the voucher plant into a re-sealable plastic bag with a waterproof voucher label. The voucher label should include the species name, or in the case of unknown species, a unique identifier, the date, the site name, the collector initials, and the sample site. Note the voucher number in Collector during data collection at the sample station where the voucher is retained. Additional information about habitat or co-occurring species may also be included on the tag or in the "Notes" data field in Collector. Place all specimens in a cooler with ice for transport to the lab. See below, "Pressing Plants" for instructions once back at the laboratory.

Preparation of voucher specimens

"Floating" Specimens

Because most aquatic plants, especially finely dissected specimens, tend to stick to paper as they dry, it is usually better to "float" the plant directly onto herbarium paper. However, if the plant is large and robust, or not entirely aquatic (such as bulrushes, emergent sedges or pickerelweed) you can press the plant in newsprint.

1) Use a pencil to label the mounting paper with the plant name, geographic location, date collected, and collector name. Mount only one species per sheet, and do not cut herbarium sheets in half.
2) Carefully rinse the plant so it is free of epiphyton, silt, and other debris.
3) Fill a sink or tray with about one inch of water. Slip the labeled mounting paper into the water.
4) Float the plant in the water and arrange it onto the sheet.
5) If the plant has fine leaflets, such as water milfoil or bladderwort, cut off one leaf and display it floated out onto the paper so that leaflet characteristics can be readily observed.
6) The plant may be bent into a "V" or "W" or curled shape to fit on the sheet.
7) Slowly lift the paper out of the water by one end. Keeping the plant in place, let the water slowly drain off.
8) Use a toothpick or probe to spread out plant parts for better display, making sure to expose identifiable characteristics such as stipules, sheaths, or seeds.

Pressing Specimens

1) Place the specimen sheet inside folds of newspaper. Place the newspaper between two sheets of blotting paper, and the blotting paper between two sheets of corrugated cardboard.
2) Place multiple specimens in a plant press. Use rope or straps to compress plants to keep specimens flat as they dry.
3) Place the press somewhere warm and dry. Placing the press on its long edge on top of a ventilated aluminum or aluminum-lined box containing incandescent light bulbs allows for quick drying. Remove plants after several days when they are thoroughly dry.

Suggested Herbarium Materials

Herbarium and science supply businesses such as the Herbarium Supply Company (www.herbariumsupply.com; 800-348-2338) sell many herbarium products including mounting paper, plant presses, blotting paper, and cardboard spacers. When ordering herbarium mounting paper, look for acid-free, non-glossy, 100% rag, and heavy or standard weights.

Preparing Dried Specimens for Shipment to an Herbarium

1) Herbarium selection. Voucher specimens should be sent to the correct state/ regional herbarium. Please notify the herbarium of your intention and wait for confirmation and instructions before sending plants.

2) Package specimens. Note some herbariums will have slightly different requirements. General Instructions: Place each dried specimen with information clearly marked on the newsprint or mounting paper in the fold of a single sheet of newspaper and place all of the newspaper/specimens between two pieces of cardboard. Tie or rubber band the cardboard bundle together and put it into a padded envelope or a box. As long as the package is going to or from an educational institution, a special 4th class mailing rate called "Library Rate" can be used.

Data management and analysis

Uploading field data

Upload data from Collector (immediately following field visit)

1) *Note:* Tablet must be connected to Internet
2) Open Collector and ensure that you are logged in to your ArcGIS Online account
3) Go to the maps "On Device" page
4) For the map of interest, Select the "cloud" icon to upload data. The number in the red circle indicates the number of stations for which data has not yet been synced to ArcGIS Online.
5) Allow the app to sync the data (the red circle will disappear when the data has been successfully uploaded to ArcGIS Online)

Entering data electronically (to Excel workbook)

Field collected data are stored to the tablet and then uploaded to ArcGIS Online as described above. From AGO (or desktop ArcGIS) raw data can be downloaded as an Excel worksheet and copied to the "RAW DATA" worksheet in the Aquatic Plant Survey Data Workbook, which is a separate Microsoft Excel file that contains multiple worksheets to facilitate data summary and analysis.

Workbook Descriptions and Instructions
The Aquatic Plant Survey Data Workbook contains seven worksheets:

1) READ ME
   Provides a brief description of the other worksheets included in the workbook

2) RAW DATA
   a. The worksheet shows a sample of the raw data file that is generated from ArcGIS Online.
   b. Replace the sample data in the RAW DATA worksheet by copying and pasting the field data from the sample location of interest.
   c. Save the workbook with a new unique identifier (e.g. Aquatic Plant Survey Data_site name_(mm yyyy).xls)

3) RAW DATA ADJUST
   a. Usually, there will be some plant species that cannot be identified during field collections. In those instances, the species' ID may be provisionally entered in the Collector app but will need to be revised in the final dataset after the species (or lowest taxonomic level) ID is confirmed in the laboratory.
   b. Copy the data from the RAW DATA worksheet and use this worksheet to manually update species' IDs as needed. We recommend using a separate font color or highlighting the cell to indicate where changes occurred. Inserting a new "comments" field (column) may also be helpful if any additional notes regarding the changes are needed.
   c. If no corrections are necessary, add a note to that effect in cell A1.

4) PIVOT TABLE
    a. This worksheet is used to create a pivot table with multiple consolidation ranges to pull out a list of unique species found across the four sample stations that comprise each sample unit.
    b. Copy the data in the "SampGridID" column and "Species_1" through "Species_30" columns (including column headers) from the "RAW DATA ADJUST" worksheet and Paste over the sample data into cell A3 on the "PIVOT TABLE" worksheet. Ensure any excess sample data is deleted. Also delete the sample data in column 'AG'.
    c. In the "PIVOT TABLE" worksheet, click any cell in the data array, and press "Alt+D" keys, then press the "P" key immediately to open the "PivotTable and PivotChart Wizard." Note you may have to press P a second time to open the wizard.
    d. Choose "Multiple consolidation ranges" in the wizard
    e. Click the "Next" button and check "Create a single page field for me" option in wizard step2. Click the "Next" button again
    f. Click to select the data range and then click and drag to select all the data in the worksheet (including column headers),
    g. After selecting the data range, click "Next", in the wizard step 3. Insert the pivot table on the existing sheet into cell 'AG1'
    h. Click "Finish" to complete the wizard. The Pivot Table Fields pane should appear.
    i. Initially, all fields in the pane will be selected with a checkmark. Unselect all fields.
    j. Drag "Row" and "Value" into the rows field in the bottom left of the pane. This will create the list of unique species found in each sample unit (i.e. an aggregate list of every species found across the four sample stations in each sample unit). The species are automatically sorted A to Z within each sample unit. You can use these lists to manually populate the "Entry" tab with 1s (if detected in the sample unit) or 0s (if not detected) as appropriate for every species by sample unit
    k. Note: if you move only the "value" into the row field, a full list of all spp detected from all sample units will be generated

5) ENTRY
    a. Information on species presence or absence in each sample unit from the summary report in the "PIVOT TABLE" worksheet is manually entered here to create a sample-based data matrix that can be saved as a tab-delimited file for use with EstimateS (see Rarefaction and Survey Performance below).
    b. After station level species detections have been summarized by sample unit (see "PIVOT TABLE"), the user can manually enter species data by sample unit into this worksheet.
        i. Enter (or copy and paste) the sample unit values in row 'A' of the 'ENTRY" worksheet (starting in cell 'A4'). The sample units can be found in column 'AG' of the "PIVOT TABLE" worksheet.
        ii. Then, for each of the species listed in row 3, enter a "1" in the corresponding sample unit row if that species was detected in the sample unit (i.e. present). For example, the "1" in cell G12 (in the 'ENTRY' tab of the sample Aquatic Plant Survey Data Workbook) shows that *Ceratophyllum demersum* was detected at sample unit 1131.
    c. After all presence data has been entered (i.e. "1"s), highlight the data range of interest and use the "replace all" function to autofill zeroes in blank cells for those species not detected in a sample unit (i.e. absent).

6) STATS
    a. Summary statistics based on the "ENTRY" data are reported in this worksheet.

b. Overall summary statistics include: number of sites visited, number of sites with plants, species richness, and non-native richness.
c. Species-level summaries include: frequency of detection (which can be used to summarize site occupancy for each species), and whether or not a species is non-native (to the basin) or qualifies as a "rare" species based on detection levels from the survey (i.e. detected in < 5% or <20% of samples)

7) MAX DEPTH
   a. Provides a graphical look at plant detections by depth. The number of sample units where plants were detected is shown for each 1 foot depth bin (up to 30').

Save the data file with a unique name (including date and sample location).

Rarefaction and survey performance

For analysis, species incidence data for each sample unit is aggregated from the four stations in each sample unit (e.g. see "ENTRY" in the Aquatic Plant Survey Workbook). The species-effort relationship (i.e., a species accumulation curve) is used to estimate species richness and characterize species accumulation on a sample-basis for each survey, as well as to quantify the effort required for high-probability rare species detection at each site (a proxy for difficult-to-encounter non-native species). Data are analyzed after Hoffman et al. (2011).

The species-effort relationship describes how much search effort (whether measured as the number of samples or the number of individuals encountered) is required to obtain a given number of species. Generally, there is a positive correlation between sampling effort and species richness such that an increasing amount of effort yields an increasing number of species. It is not linear, however. The rate of species acquisition slows with increasing effort because rare species require increasing effort to find (Gotelli and Colwell 2001). That is, rare species require relatively more effort to detect than common species. Whereas a survey encounters species in a sequence that depends on which sites are sampled first, the average increase in species with each additional sample taken can be obtained by randomizing site order (see Hoffman et al. 2011 for technical details). By plotting the average number of species detected (y-axis) as a function of effort (i.e., sample size or number of plants grabs on the x-axis), the asymptote of this relationship is an estimate of the "true" number of species in a given search area. This number is the total species richness.

Calculating key performance metrics from the species-effort relationship

A nonparametric estimator is used to estimate total species richness (S_{est}) based on sample-based plant abundance or incidence data. The S_{est} values ("Chao2") are calculated using EstimateS v8.20 software (Colwell 2006). Chao 2 is an incidence-based estimator (i.e., presence or absence of a species), so the way to interpret the software output is that this is the likelihood of detecting a new species (of any number within a sample) given a specific level of effort. As such, it recognizes well the inherent spatial patchiness to plant distributions. Note that it is a more conservative estimate than "Chao 1," which is an abundance-based estimate of total species richness (S_{est}). Following Colwell (2006), bias-corrected Chao2 values are reported unless there is substantial heterogeneity among species in their probabilities of detection (incidence-based coverage estimator of species richness coefficient of variation (CV) > 0.5), in which case the uncorrected Chao2 value should be reported.

To quantify the effort required for high-probability nonnative species detection, estimates of the number of samples required to obtain a near-total (95%) or total (100%) census of the plant species present should be derived. S95% and S100% values are calculated using the nonparametric method proposed by Chao et al. (2009), which calculates the number of samples required to detect some proportion of the asymptotic sample-based species richness estimator, Sest. When using the Excel worksheet calculator provided by Chao et al. (2009), to align with the Chao 2 estimator, use the incidence-based calculator. Because the species-effort relationship is asymptotic, expect that the effort required to sample 95% of the total species richness will be substantially less than that required to sample 100% of the total species richness.

Reporting

A summary of survey methods and results should be provided to the local AIS coordinator(s) where the work was performed. At a minimum the report should include the following information:

- Survey date(s)
- Brief summary of survey design and sampling methods, including any details for data analysis
- Survey results, including,
    - the number and location of sample units selected for sampling (e.g. a map or table of latitude and longitude for sample units)
    - A list of species detected
    - Survey performance metrics (including observed and estimated richness and S95% or S100% values from rarefaction, as described above)
- Any noteworthy observations, especially detection of new non-native species or rare native species

Other information to consider adding to the report includes,

- Sample-based rarefaction curves
- Species occupancy summaries (i.e. the percentage of sample units where each species was detected)
- Frequency histogram showing number of species detected by sample unit
- Scatter plot of species detected by depth
- Maps of species richness and rarity measures across the site
- Etc.

Some of the above information will be available for each site after data are entered in the "Aquatic Plant Survey Data Workbook." A sample report is provided (Appendix 6).

All non-native species detections should be shared to the Nonindigenous Aquatic Species information resource for the United States Geological Survey (USGS NAS; https://nas.er.usgs.gov/default.aspx).

Reporting of novel incursions of any Great Lakes' surveillance list species (see 'Table 6' in Davidson et al. 2021) should follow protocols outlined in the communication guidance included in the Great Lakes' Basin AIS Response Framework (Chadderton et al. 2021b).

Personnel requirements and training

At a minimum, a crew of three people is necessary to conduct the survey (a crew of four or five personnel will increase efficiency, especially for plant collection and processing). The crew shall consist of:

- a boat operator, (1)
- an aquatic plant taxonomist, (1)
- a field technician(s) (2-3)
    - Data recorder
    - Other- rake toss/plant sort

The *boat operator* shall meet all state requirements to safely operate the survey vessel and should be proficient in general boating operations and knowledge. Familiarity with the area to be surveyed is ideal. The boat operator shall ensure that the vessel is registered in accordance with relevant local and federal regulations and that the vessel complies with all local, state, and federal safety requirements, including life jackets for all passengers onboard, safety kit (including flares and sound producing devices), throwable floatation devices, and a redundant power source (e.g. oars or a redundant engine). The boat operator shall ensure that at least one dependable means of communication is available (e.g. VHF radio or mobile phone). An anchor and anchor line suitable for the vessel is also recommended. Preparing and filing a float plan is recommended (indicating the personnel on board, the location and expected time of departure and return, and emergency contacts).

The *plant taxonomist* is responsible for field identification of all specimens collected and for the collection, maintenance, and deposition of all voucher specimens. Aquatic plant identification experience is required for rapid field identification. The plant taxonomist will assist in collection and sorting of plants.

The *field technician* will assist in collection and sorting of plants as needed and is primarily responsible for data recording (using the tablet). Experience with Collector for ArcGIS or other mobile data collection applications is recommended.

It is the responsibility of each crew member to assess the condition of the vessel, the weather and the water, and to refuse to embark if, in their judgment, conditions are unsafe or unfavorable for conducting the survey. In an effort to mitigate the spread of local or regional invasive species all hulls should be thoroughly cleaned when operating in different bodies of water or in regions where invasive species are a concern. Local and regional biosecurity measures and standards should be followed for all vessels.

Other personnel with expertise in GIS and data analysis will be helpful to facilitate survey design and reporting elements, including:

- a GIS specialist
- data analyst

The *GIS specialist* will create maps to direct sampling and should be familiar with ArcGIS and ArcGIS Online (or similar GIS mapping software). Experience with mobile data collection apps and geoprocessing tools, including geostatistical analysis and sample network design as well as spatial statistics and modeling spatial relationships is recommended.

The *data analyst* should be familiar with species accumulation theory and statistical methods for estimating species richness from biotic sampling data, including nonparametric rarefaction and Chao richness estimators. Familiarity with EstimateS software is recommended.

Operational requirements

Timing of sampling

Surveys should be conducted between late July and mid-September. Plant biomass and density will vary seasonally by species, but this period offers greatest potential for detecting presence of the full range of growth forms and species including a number of high-risk invasive species (e.g. water hyacinth and water lettuce; EGLE 2020).

Staff time estimates (for a single survey)

Permitting (0.5 day) – Permits are generally not required for aquatic plant surveillance. However, if the surveillance activity is funded or authorized by a US federal agency (e.g. US Fish and Wildlife Service), then under Section 7 of the Endangered Species Act project staff must request an informal consultation with the federal agency to assess whether the proposed activity will affect a listed endangered or threatened species. Note: if the agency determines that the action is likely to adversely affect a listed threatened or endangered species, then a formal consultation process will ensue. The formal consultation process may last up to 90 days. Some jurisdictions may require a permit to collect any regulated or prohibited plant species (which includes some invasive aquatic plants). Check with the relevant management agency to confirm permit requirements.

Survey design and mapping prep (2 days) - Survey design includes but is not limited to creating the site polygon, overlaying the sample grid, digitizing depth and other habitat layers, creating a species richness surface, and selecting spatially balanced sample points, etc.. Creating a map and data fields for mobile data collection will require additional time.

Logistics/planning (1 day) - This includes coordinating with local jurisdictions to arrange for operational support, purchasing materials (for sampling), planning travel, etc.

Site survey (1-3 days) - For small sites (<100 hectares) it is possible that a survey may be completed in a single day. Generally, surveys will require several days. A 3-day survey should reasonably be able to sample up to 70 sample units, with crews spending 10-20 minutes at each sample site. Sampling time and survey extent may vary depending on the following factors:

- Distance between sample sites & ease of navigation
- Weather (i.e. wind, rain, etc.)
- Rake fullness (high biomass reduces the number of sites that can be sampled, since each rake toss must be processed to ensure detection of all species collected)

Plant vouchering (1-2 days) - Including identification, pressing, drying, and shipment of collected plants to herbarium.

Data analysis (1-3 days) - Including data clean-up and validation (e.g. voucher cross-checks), data summary statistics, and richness estimation.

Reporting (1 day) - Including preparing site reports for management agencies.

Other (as needed) – Including grant reporting, information sharing at professional meetings, etc.

Total time estimate: 7.5 - 12.5 days per site (not including “other” tasks)

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Appendices:

Appendix 1- constructing a rake sampler

To make the rake sampler we removed the handles from two standard 14-tine bow rakes (available at most hardware stores) and then connected the rake heads bar-to-bar to form a double-sided rake head, approximately 14” wide. The rake heads can be welded together or connected with hose clamps (as in Figure A1). In order to ensure a rapid vertical descent to lake bottom, a light weight (< 5lb) can be attached to the rake head, away from the tines. Insert an eye bolt into one of the rake heads and attach at least 50’ of 3/8” braided floating polypropylene rope (or similar) to the eye bolt with a secure knot.

Figure A1. *Double-headed rake sampler*

Appendix 2- Tablet and accessories for mobile monitoring

Item	**Estimated Cost**	**Specifications**
iPad	$450	iPad mini 4 Wifi, 32GB
Screen protector	$11	Screen protector compatible with Nuud Case
waterproof case	$60	Lifeproof Nuud case
External Bluetooth GPS	$100	GPS
Keyboard (optional)	$38	Keyboard

Total cost $650

Appendix 3- External GPS user instructions

Online Specs and link to Manual: http://gps.dualav.com/explore-by-product/xgps150a/

1) External GPS is NOT waterproof! Place the unit in a plastic bag if raining.
2) Charge the GPS prior to field use.
3) In the field, connect GPS to iPad using Bluetooth
    a. Hold the power button until the Bluetooth light blinks blue (Figure A2; blinking means its searching for a device to connect to).
    b. On iPad, open settings. Select Bluetooth ON and wait until "My Devices picks up ""XGPS150-312D63"
4) Start the GPS
    a. On iPad open Dual GPS app – this will activate the GPS. On the GPS, the green light next to "GPS" will flash while searching for satellite signal.
    b. In the app, you will see the GPS battery life and the satellite configuration. You can also see Lat and Long (Figure A2).
    c. Once the GPS is connected to satellites, the green light will stop flashing.
    d. Open Mapping App and begin working.

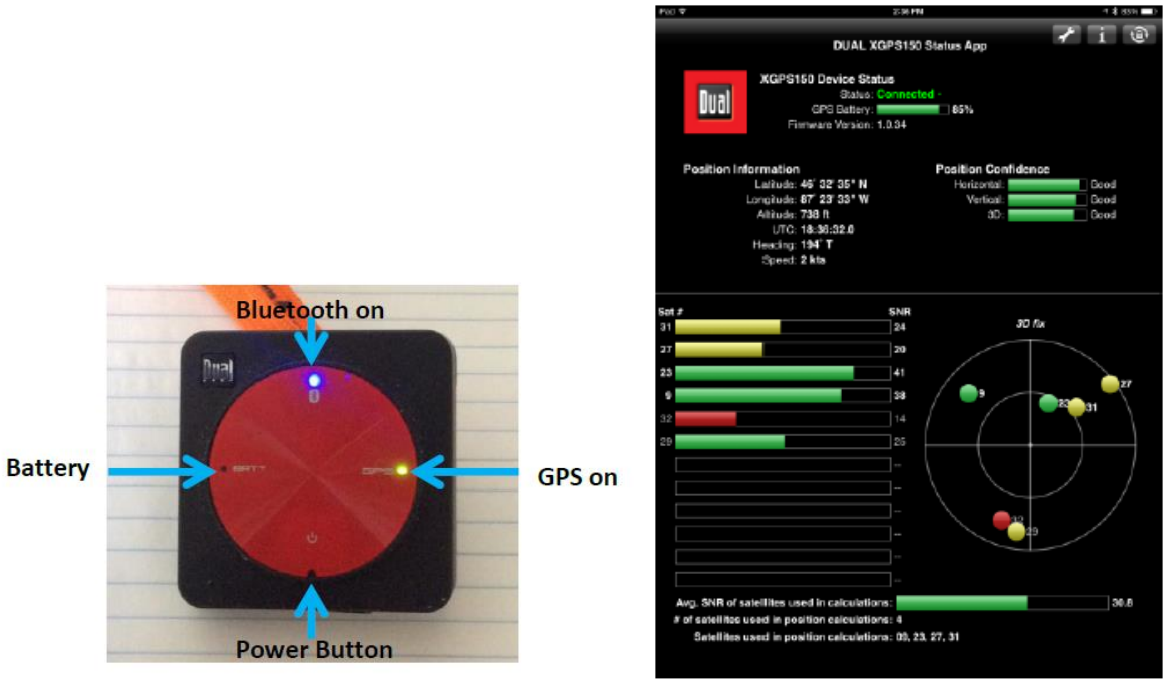

Figure A2. Dual XGPS receiver (left) and application interface (right).

Appendix 4- Collector data fields

Data field	Type	Length	Description
SampGridID	Double	-	numeric
Sample_Station	Integer	-	numeric
Lat		-	Added after the field records are entered using desktop GIS
Long		-	Added after the field records are entered using desktop GIS
Depth_ft	Single	-	Depth in feet
Secchi_m	Single	-	Depth in meters
Substrate	String	50	Dropdown (mud, silt, sand, cobble, gravel. boulder, bedrock, other)
Picture_Num	Integer	-	Picture number
Voucher	String	256	Record species being vouchered
Rake_Tosses	Small Integer	-	Number of rake tosses performed
Rake_Fullness	String	20	Dropdown ((0) none, (1) few, (2) moderate, (3) abundant)
Notes	String	250	Open ended for recording notes.
Species_1	String	50	Dropdown choices (see spp code list, Appendix 5)
…	String	50	Dropdown choices (see spp code list, Appendix 5)
Species_30	String	50	Dropdown choices (see spp code list, Appendix 5)
CreationDate	Date	8	Auto-generated
StartTime	Date	8	Auto-generated
EndTime	Date	8	Auto-generated
Creator	String	128	Auto-generated
EditDate	Date	8	Auto-generated
Editor	String	128	Auto-generated

Appendix 5 – Species names

Ceratophyllum demersum (CEDE4)
Elodea canadensis (ELCA7)
Elodea nutalii (ELNU)
Heteranthera dubia
Myriophyllum spicatum (MYSP2)
Potamogeton crispus (POCR3)
Potamogeton foliosus (POFO3)
Potamogeton richardsonii (PORI2)
Stukenia pectinata (STPE 15)
Vallisneria americana (VAAM3)
Achyranthes japonica (ACJA)
Alisma plantago-aquatica
Alliaria petiolata (ALPE4)
Allisma spp. (ALISM)
Azolla spp. (AZOLL)
Bidens beckii (BIBE2)
Bolboschoenus fluviatilis
Brasenia shreberi (BRPE2)
Butomus umbellatus (BUUM)
Cabomba caroliniana (CABOM)
Cardamine impatiens (CAIM)
Carex spp
Chara spp. (CHARA)
Cynanchum louiseae (CYLO11)
Dioscorea oppositifolia (DIOP)
Drepanocladus spp
Egeria densa (EGDE)
Eichhornia crassipes (EICR)
Elatine minima (ELATI)
Eleocharis robbinsii (ELRO)
Eleocharis spp. (ELEOC)
Equisetum spp. (EQUIS)
Euphorbia esula (EUES)
Fontinalis spp. (FONTI)
Heracleum mantegazzianum (HEMA17)
Humulus japonicus (HUJA)
Hydrilla verticillata (HYVE3)
Hydrocharis morsus-ranae (HYMO6)
Hydrocotyle umbellutus (HYDRO2)
Iris (psuedacorus)
Lemna minor (LEMI3)

Lemna spp. (LEMNA)
Lemna trisulca (LETR)
Lemna turionifera (LETU)
Ludwigia spp. (LUDWI)
Lysimachia spp. (LYSIM)
Lythrum salicaria (LYSA2)
M. spicatum x M. sibiricum
Marsilea quadrifolia (MAQU)
Microstegium vimineum (MIVI)
Myriophyllum aquaticum (MYAQ2)
Myriophyllum heterophyllum (MYHE2)
Myriophyllum sibiricum (MYSI)
Myriophyllum tenellum (MYTE)
Myriophyllum spp. (MYRIO)
Najas flexilis (NAFL)
Najas guadalupensis (NAGU)
Najas marina (NAMA)
Najas minor (NAMI)
Najas spp. (NAJAS)
Nelumbo lutea (NELU)
Nitella spp. (NITE)
Nitellopsis obtusa (NIOB)
Nuphar advena (NUAD)
Nuphar spp. (Nuphar_spp)
Nuphar variegata (NUVU)
Nymphaea odorata (NYOD)
Peltandra virginica
Persicaria amphibia
Phalaris arundinacea (PHAR3)
Phragmites australis (PHAU7)
Pistia stratiotes (PIST2)
Polygonum amphibium (POAM8)
Polygonum cuspidatum (POCU6)
Polygonum perfoliatum (POPE10)
Pontederia cordata (POCO14)
Potamogeton amplifolius (POAM5)
Potamogeton diversifolius (PODI)
Potamogeton epihydrus (POEP2)
Potamogeton friesii (POFR3)
Potamogeton gramineus (POGR8)
Potamogeton illinoensis (POIL)

Potamogeton natans (PONA4)
Potamogeton nodosus (PONO2)
Potamogeton perfoliatus
Potamogeton praelongus (POPR5)
Potamogeton pulcher (POPU6)
Potamogeton pusillus (POPU7)
Potamogeton robbinsii (PORO2)
Potamogeton zosteriformis (POZO)
Potamogeton spp. (POTA)
Proserpinaca pectinata (PRPE)
Ranunculus aquatilis
Ranunculus longirostris (RACO2)
Riccia fluitans
Riccia spp. (RICCI)
Ruppia maritima (RUMA5)
Sagittaria graminea
Sagittaria latifolia
Sagittaria rigida
Sagittaria spp. (SAGIT)
Schoenoplectus pungens (SCPU)

Shoenoplectus acutus (SCAC3)
Shoenoplectus americanus (SCAM6)
Shoenoplectus subterminalis (SCSU10)
Shoenoplectus validus (SCTA2)
Sparganium spp. (SPARG)
Spirodela polyrhiza (SPPO)
Stukenia filiformis (STFI)
Stukenia spp. (STUCK)
Typha spp. (TYPHA)
Utricularia intermedia (UTIN2)
Utricularia macrorhiza
Utricularia minor (UTMI)
Utricularia purpurea (UTPU)
Utricularia vulgaris (UTMA)
Utricularia spp. (UTRIC)
Wolffia spp. (WOLFF)
Zannichellia palustris (ZAPA)
Zizania spp. (ZIZAN)
Other

Appendix 6- Sample report

Memo:

To: Bob Wakeman (Wisconsin DNR)

From: Andrew Tucker, Gust Annis, Lindsay Chadderton (The Nature Conservancy, Great Lakes Project), and Erick Elgin (MSU extension)

Date: March 29, 2019

Subject: Port of Milwaukee 2018 aquatic plant survey results

Background

This memo reports on the methods and results of an aquatic plant survey conducted in Milwaukee harbor and connected waterways (Milwaukee River, Menomonee River, and Kinnickinnic River) between August 7 - 9, 2018. The survey was undertaken as part of a funding opportunity (F16AS00090) from US Fish and Wildlife Service (FWS) to the Michigan Department of Environmental Quality, on behalf of the Great Lakes states. Milwaukee was one of three high priority sites surveyed in 2018 under the "Aquatic plant survey methods development and site assessment" objective included as part of FY17 funding from FWS for the Interstate Aquatic Invasive Species Prevention, Early Detection, and Response project. This is the second survey conducted in Milwaukee under this funding opportunity. Results from a survey conducted September 2017 were reported in a memo dated July 5, 2018.

Methods

Survey design generally followed recommendations from Trebitz et al. (2009) and Hoffman et al. (2016), including random selection of sample sites to facilitate evaluation of detection rates and optimization of sampling efficiency. Sample units (100 m^{-2}) were overlaid on the Milwaukee Port and rivers in ArcGIS. In the 2017 survey, sample units were allocated across four sample zones (inner harbor, outer harbor, marinas, rivers) with the aim of surveying at least 10% of grid cells in each sample zone. Ninety-four percent of all plants detected in the September 2017 were collected from sites less than four meters deep. For the 2018 survey a stratified survey design was employed for sample unit selection with grid cells in Milwaukee harbor and tributaries attributed as shallow (<4m) or deep (>4m) based on cell centroids overlaid on depth layers created in ArcGIS 10.5.1. Sample units (i.e. grid cells based on cell centroids) were randomly selected using the Create Spatially Balanced Point tool in ArcGIS, with approximately 3:1 ratio of shallow to deep sites (Figure 1).

The survey was conducted between August 7 – 9, 2018. Sixty-five grid cells (of 82) were sampled. At each grid cell, plants were collected with rakes or observed visually and recorded. The rakes were double headed 14-tine rake heads attached to approximately 50 feet of 3/8" braided polypropylene rope. The survey targeted submerged, floating, and emergent taxa. All observations were from the shoreline lakeward to the maximum depth in any grid cell. The shoreline was defined as the land/water interface (i.e. the boundary between aquatic and riparian or wetland conditions). Plants were only recorded when observed in water. In general, four evenly spaced and discrete stations were sampled within each grid cell (e.g. the four corners of the grid cell). In some deep and/or turbid areas, where river/harbor bottom was not visible from the boat, a transom mounted sonar unit (Garmin Striker 4 Portable Bundle, Olathe, KS USA) was used as an aide to identify sampling stations within the grid cell (based on potential presence of

macrophytes from sonar imaging). At each station a rake was tossed 5 – 10 meters from each side of the boat (i.e. four rake tosses), allowed to sink to the river/harbor bottom, and slowly retrieved. In instances where the fourth rake toss resulted in collection of a species not observed in the previous three tosses, subsequent rake tosses were made until no new species were collected. In this way, sampling effort at each grid cell was relatively uniform.

Plants were identified to species in the field by a trained taxonomist (Erick Elgin, Michigan State University Extension). When specimens could not be identified in the field (e.g. when identification required scrutiny of morphological features under a microscope) the plant was vouchered and identified to species (or lowest possible taxonomic group) in the laboratory. The total number of species collected at each sample location was tallied for data analysis. Water depth at each sample location was recorded from the boat mounted sonar unit. GPS coordinates, plant species observed, and water depth at each sample station were recorded electronically in the field to an iPad (connected to a Bluetooth enabled GPS receiver) using Collector for ArcGIS.

Descriptive statistics (e.g. species richness mean, median, range) for each site and depth zone were compiled. Species richness (+/- 95% confidence intervals) and sampling completeness statistics were estimated from sample-based rarefaction using a nonparametric estimator (Chao et al. 2009) in EstimateS (v8.20; Colwell 2013). For analysis, a "sample" is equivalent to a grid cell, and species richness for each sample was the aggregate from the four stations in each grid. Depth for each sample was the average depth from across the four stations. Species richness and rarity measures were mapped in ArcGIS 10.5.1.

Results

Twenty-six unique species (or lowest identifiable taxonomic unit) were collected from 65 grid cells sampled over an area of nearly 2100 acres. In 2017, fifteen species were collected from 73 grid cells (Figure 2, Table 1). Four taxa could only be identified to genus level (Drepanocladus, Iris, Typha, and Nitella spp.). Six species are non-native to Lake Michigan but all are widely established in the Great Lakes basin (Table 1).

For nearly one-third of sample stations (84 of 262), sampling failed to collect any plants (Figure 3). Maximum richness for a single sample station was seven species. In general, plants were more frequently detected in shallow versus deep sites (Figure 4). On average, the number of species detected in shallow sites was three times greater than in deep sites (Figure 5). No plants were found at depths greater than 20' (Figure 6). Species rich sites (>5 species) were concentrated in the South Shore Marina, McKinley Harbor, Summerfest Lagoon, and in the more upstream reaches of the Milwaukee and Kinnickinnic Rivers (Figure 7a). The Milwaukee and Kinnickinnic Rivers were also where a majority of rare species were encountered (i.e. species surveyed from less than 5% of survey sites; Figure 7b).

Species based rarefaction predicts species richness for Port of Milwaukee is thirty-one species (95% CI [27, 50]), which suggests that the August 7 – 9 sample effort detected just under 85% of the true richness (i.e. 26 of 31 species). However, richness estimates vary substantially across the two depth strata, with predicted richness at shallow sites 58% higher than at deep sites (38 vs. 24 species respectively; Table 2). Consequently, shallow sites (i.e. < 4m) appear to have been under-sampled. For example, the species-effort relationship suggests that 62 additional grid cells would need to be sampled to detect 95% of the true species pool at deep sites whereas 210 additional samples are needed to detect 95% of the predicted species at shallow sites. Future sampling that targets shallow sites with the highest observed richness and rarity measures could facilitate a more robust and efficient survey.

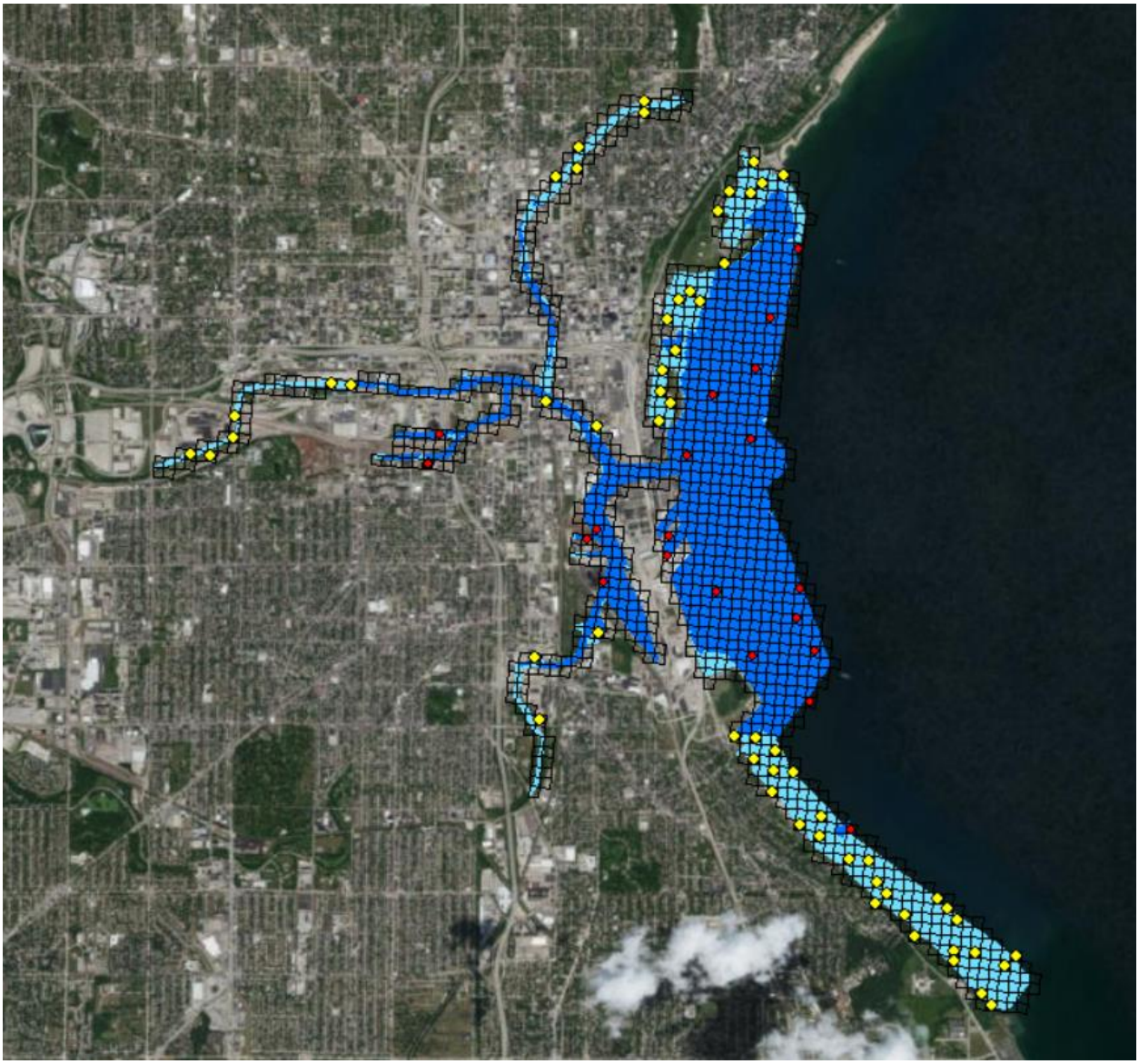

Figure 1. Survey area for Port of Milwaukee showing randomly selected sampling units (yellow = shallow; red = deep).

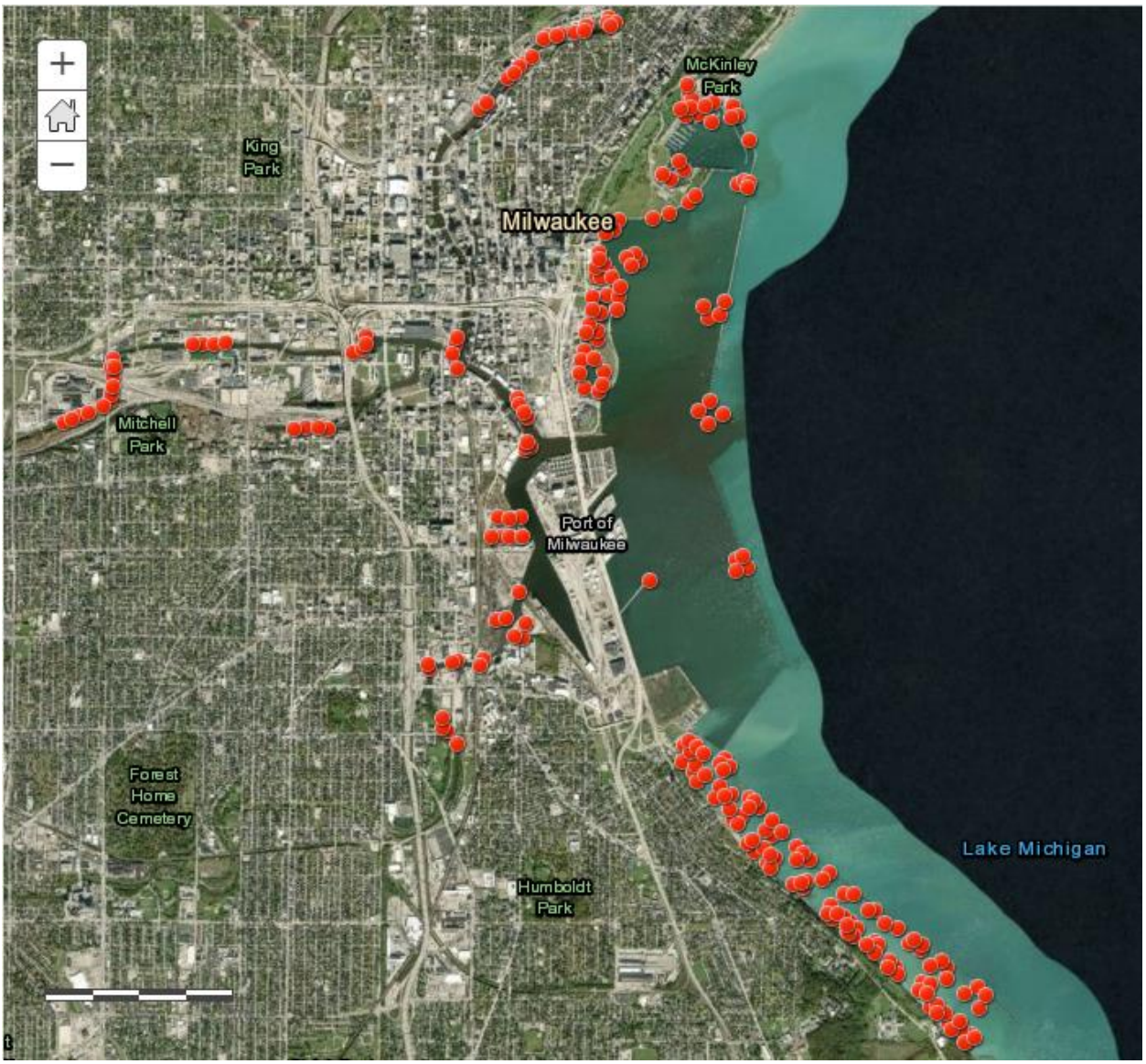

Figure 2. Survey area for Port of Milwaukee showing sampling stations (n = 262).

Table 1. Species list for Port of Milwaukee aquatic plant surveys, 2018 (Aug 7 – 9) and 2017 (Sept 12 - 14). (*) indicates non-native species. Bold fond indicates the species was found both years. Occupancy is calculated as the percentage of sample stations where the species was observed. Note: All Elodea collected in 2017 were recorded as E. canadensis, but E. nutallii may have been misidentified as E. canadensis in 2017.

	2018		2017	
	# stations encountered (of 262)	Occupancy	# stations encountered (of 301)	Occupancy
Potamogeton foliosus	98	0.37	37	0.12
Elodea nutallii	92	0.35		
Myriophyllum spicatum *	66	0.25	35	0.12
Potamogeton crispus *	60	0.23	4	0.01
Stuckenia pectinata	51	0.19	18	0.06
Potamogeton richardsonii	22	0.08	10	0.03
Elodea canadensis	20	0.08	65	0.22
Lemna turionifera	17	0.06	1	0.00
Zannichellia palustris	11	0.04		
Ceratophyllum demersum	10	0.04	3	0.01
Lythrum salicaria*	7	0.03	3	0.01
Stuckenia filiformis	7	0.03		
Zosterella dubia	5	0.02		
Spirodela polyrhiza	4	0.02		
Drepanocladus spp.	2	0.01	1	0.00
Potamogeton nodosus	2	0.01	3	0.01
Phragmites australis *	2	0.01		
Nymphaea odorata	2	0.01		
Iris (pseudacorus)? *	2	0.01		
Polygonum amphibium	1	0.00	1	0.00
Potamogeton zosteriformis	1	0.00	1	0.00
Potamogeton epihydrus	1	0.00		
Typha spp. *	1	0.00		
Polygonum amphibia	1	0.00		
Vallisneria americana	1	0.00		
Lemna minor	1	0.00		
Lemna trisulca	0	0.00	1	0.00
Nitella spp.	0	0.00	1	0.00

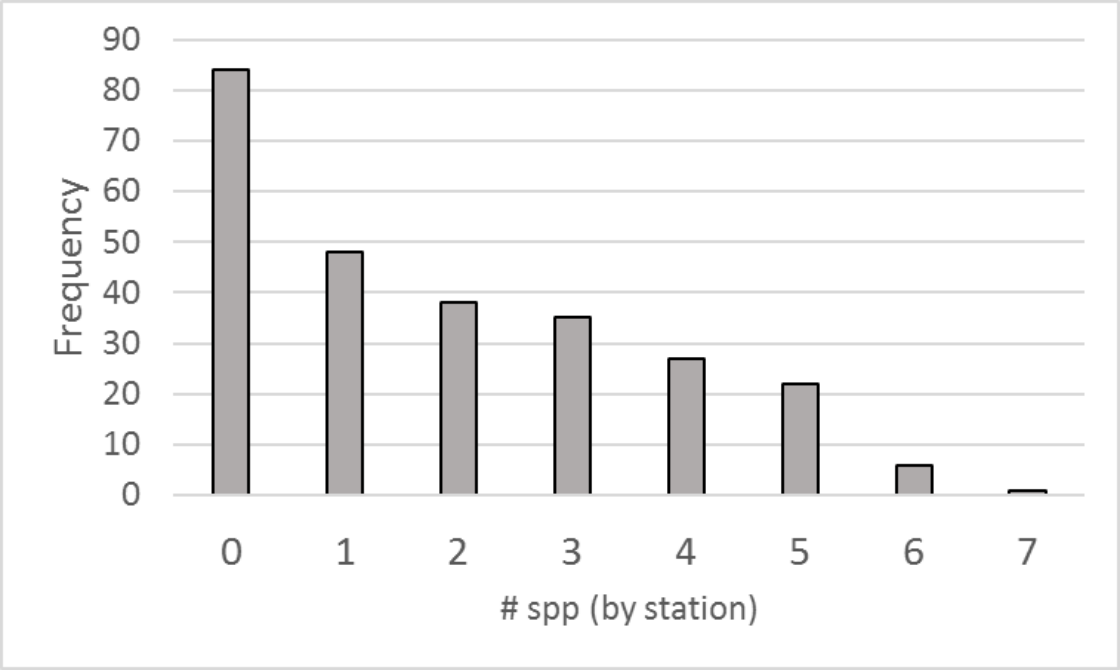

Figure 3. Frequency histogram showing the number of species collected at each sample station (e.g. zero species were collected from 84 of 262 sample stations).

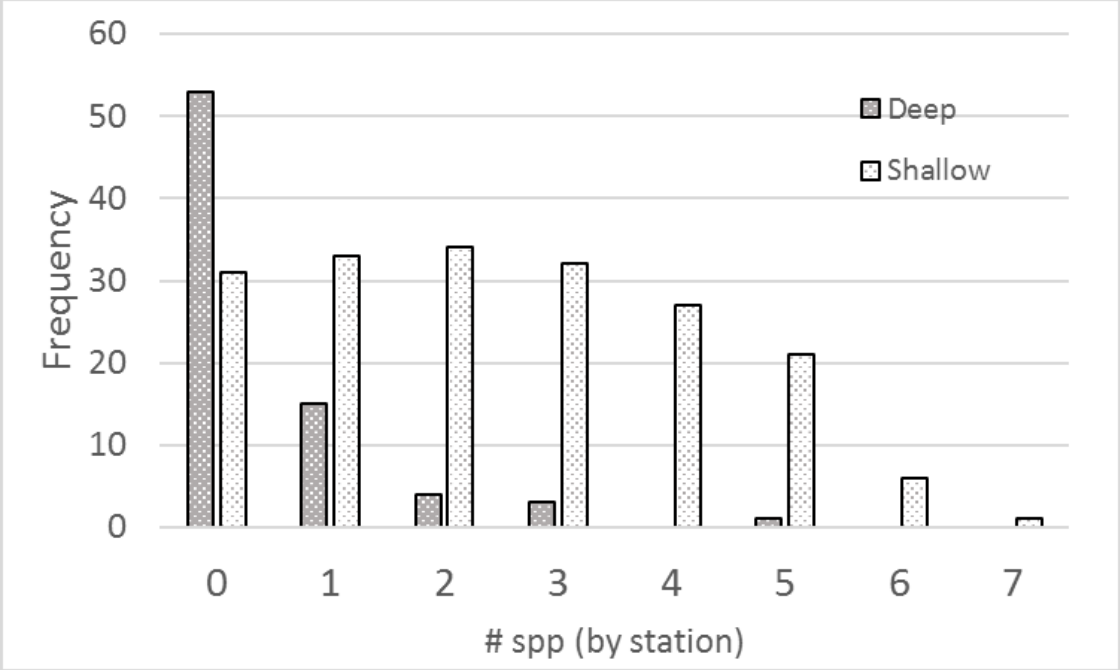

Figure 4. Frequency histogram showing the number of species collected at each sample station in shallow versus deep sites. At least one species was detected in the majority (83%) of shallow sites, whereas plants were not collected at the majority (70%) of deep sites.

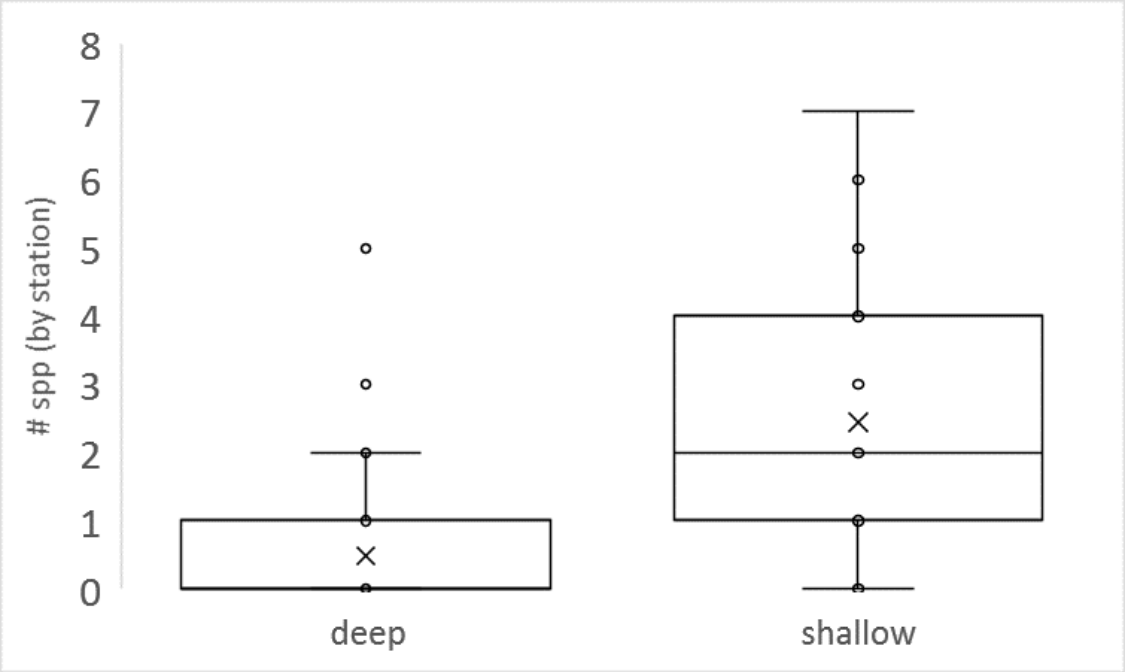

Figure 5. Box plots showing the number of species in deep versus shallow sample stations." "X" indicates the sample mean. Open circles show values from individual grids.

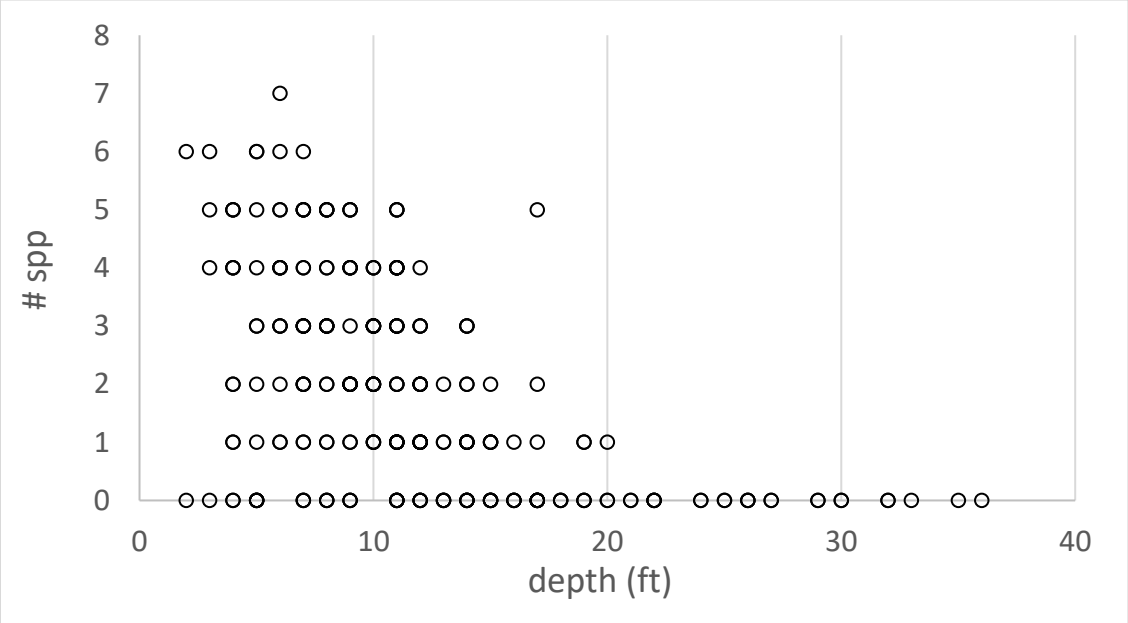

Figure 6. Scatter plot showing number of species detected at each sample station as a function of depth. Darker circles indicate depth x spp combinations with more than one observation.

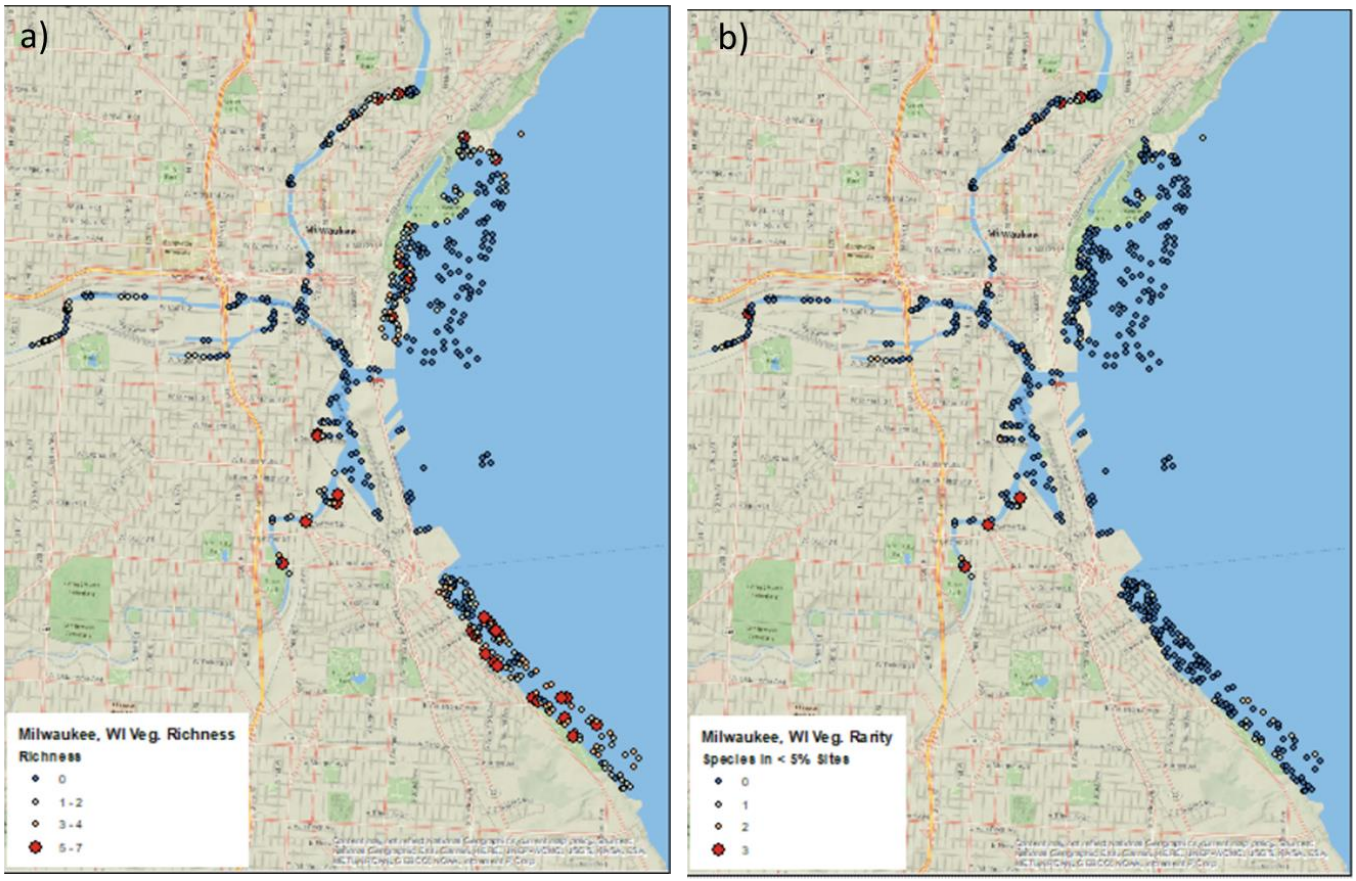

Figure 7. Species richness (a) and rarity (b) measures from August 2018 survey of Milwaukee harbor.

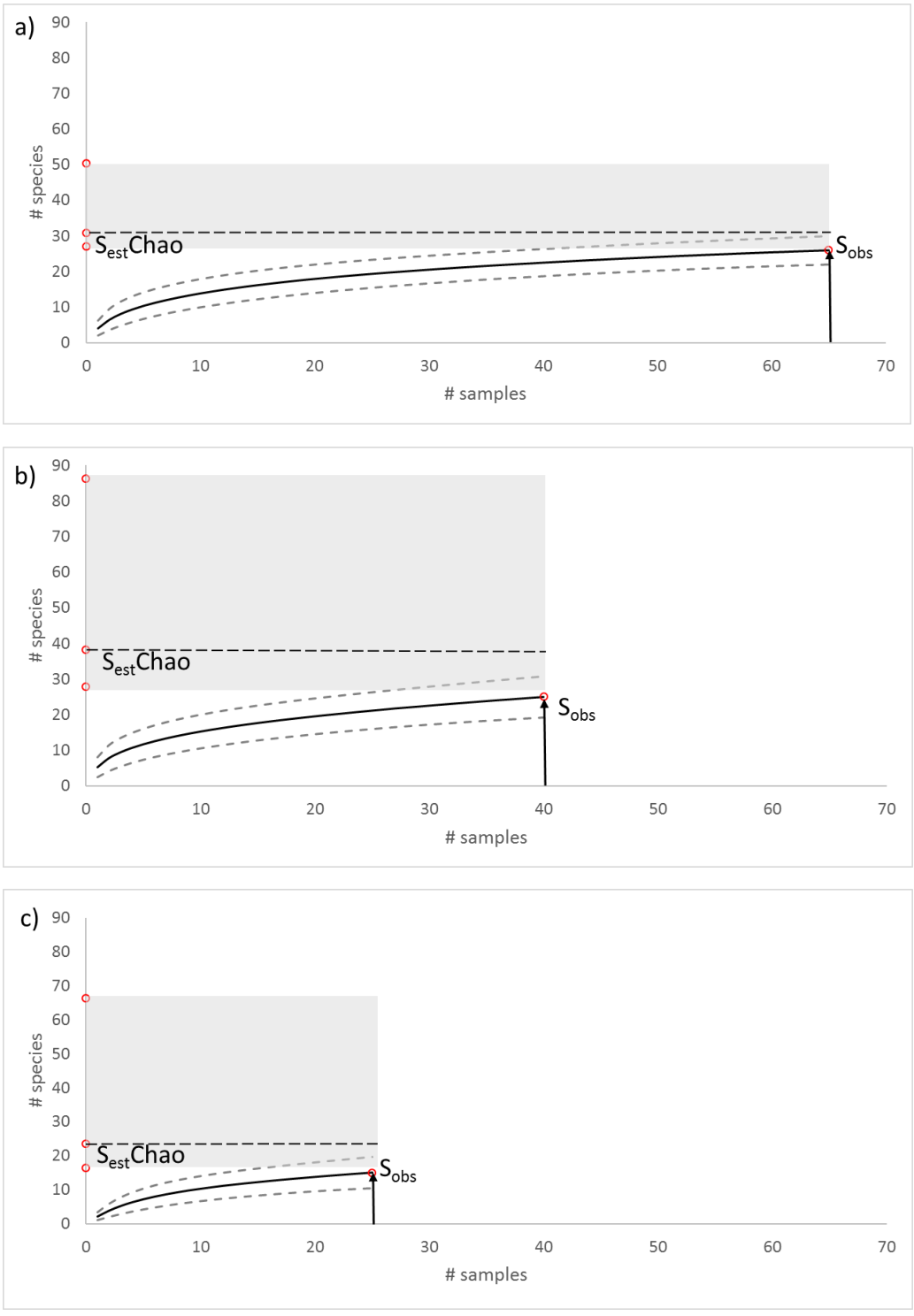

Figure 8. Sample-based species rarefaction curves (+/- 95% confidence intervals) showing the average increase in species with each additional sample taken based on a random re-ordering of sites using EstimateS software. Estimated true species richness at Milwaukee for all sites combined (a) is 31 species (95% CI [27, 50]), but richness estimates vary widely based on depth strata, (b) <4m = 38 (95% CI [28,86] and (c) >4m = 24 (95% CI [16, 66]).

Table 2. Survey data and sampling completeness statistics for Milwaukee plant survey. Number of incidences is the tally of all plants across all sites sampled. Six species for < 4m stratum and 5 species for > 4m stratum, were only detected in a single grid cell (i.e. "uniques"). One species for < 4m stratum and three species for > 4m stratum were detected in exactly two sample units ("duplicates"). S_{est} is the estimated true species pool with 95% confidence interval shown. "A'ddl S_{95} effort" is the additional number of grid cells that would need to be sampled to detect 95% of the true species pool for each depth stratum. "A'ddl S_{100} effort" is the additional number of sample units that would need to be sampled to detect 100% of the true species pool for each depth stratum.

Variable	combined	< 4m	> 4m
№ sites sampled	65	40	25
№ species detected	26	25	15
№ incidences	265	210	55
Uniques	7	9	6
Duplicates	5	3	2
S_{est} (95% CI)	31	38	24
A'ddl S_{95} effort	52	114	73
A'ddl S_{100} effort	182	299	167

References

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Trebitz, A. S., Kelly, J. R., Hoffman, J. C., Peterson, G. S., & West, C. W. (2009). Exploiting habitat and gear patterns for efficient detection of rare and non-native benthos and fish in Great Lakes coastal ecosystems. Aquatic Invasions, 4(4), 651-667.

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