Invasive aquatic plant surveillance best practices meeting summary

PREAMBLE

This document provides a summary of presentations and discussion from two workshops convened in February and May 2023 to engage aquatic plant management professionals on best practice guidance for invasive aquatic plant (IAP) surveillance for inland lakes, with an emphasis on Great Lakes regional case studies and perspectives. Attendees shared information on existing survey protocols, survey design principles, and emerging technologies for early detection of IAP.

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INVASIVE AQUATIC PLANT SURVEILLANCE BEST PRACTICES WORKSHOPS – FEB 7 & MAY 19, 2023

Overview:

As the extent and impact of IAP in inland lakes continues to grow, state agencies have expressed a need for the development of best practice guidance for IAP surveys in inland lakes. To help meet this need, The Nature Conservancy and the Great Lakes Commission (GLC) convened two workshops (Feb 7 & May 19, 2023) to review current IAP early detection methods in use across the region and to solicit feedback from regional experts around potential best practices for early detection surveillance efforts. The workshops were made possible with funds provided by the US Fish and Wildlife Service, through a Great Lakes Restoration Initiative Aquatic Invasive Species Interjurisdictional Grant to Great Lakes States and Tribes for Fiscal Year 2020 (F20AS00047).

The workshops centered on presentations from IAP surveillance practitioners and researchers and covered a variety of survey design approaches and sampling methods. Facilitated group discussions focused on the strengths and limitations of each survey type. Topics considered included: survey objectives, survey performance and data reliability, the scope of plant growth forms that are targeted, and effort and cost to implement. This document provides a brief summary of each presentation and captures key points from group discussions. Where possible, a web link to each presentation is provided. This document and presentations have been archived on the GLC EDRR website. Citations to relevant publications, reports, and other resources are also provided, either in the meeting summary or in Appendix A. A "best practice guidance document" and an "annotated bibliography" of surveillance methods and relevant resources are also in preparation. All of these products are intended as resources that can be used to tailor early detection efforts in each state and to advance more effective and efficient IAP management regionally.

The workshops were attended by more than 60 participants, representing every Great Lakes state and province, various federal and tribal agencies, non-profits, and other agencies. A list of attendees and contact information is provided in the Appendix B.

FEBRUARY 7, 2023

1. AGENCY PERSPECTIVES AND REGIONAL SURVEILLANCE PROJECTS

1.1. WISCONSIN

PRESENTER: Maureen Kalscheur, *WI DNR*

PRESENTATION: Available HERE

SUMMARY: Wisconsin DNR implements various survey methods for early detection of IAP and to monitor aquatic plant communities. This presentation briefly described each method and provided an overview of the survey design, unique elements, objectives, outputs, and trade-offs for each method. Outstanding questions related to each method were also briefly discussed.

ADDITIONAL DETAILS:

- Survey type: Point intercept
    - Overview:
        - The recommended method for baseline monitoring of aquatic plant communities in WI. The method is appropriate for lakes that vary in depth, size, region, shoreline complexity, and vegetation distribution.
    - Design:
        - A geo-located grid of evenly spaced points, based on lake size, estimated littoral zone area, and shoreline complexity
    - Unique Elements
        - Systematic, quantitative and replicable
        - Collects data on native and invasive plants
        - Design allows for statistical analysis and comparison of plant communities within a lake across survey years as well as among different lakes
    - Objectives:
        - Quantitatively assess native and invasive macrophyte spatial distribution and relative abundance (i.e., rake fullness ratings)
    - Outputs:
        - Frequency of occcurence, species richness, relative abundance and maximum depth of plant colonization
        - Water depth and substrate type at each site
        - Qualitiative species list
        - Plant voucher specimens
        - Geo-referenced species data
        - A boat survey "meander" list is also recorded to capture any incidental plant species observed in the lake but which were not recorded on the rake or visually observed within 6' of the rake sampling location (this was not included in the meeting PPT)
    - Tradeoffs:
        - Time to complete the survey varies based on lake size, water clarity, and plant density. A minimum of 100 sampling points are recommended and crews can typically sample 20 – 30 points per hour.

    - High level of plant taxanomic knowledge required
    - Recommended sampling window is July-Aug, which may result in missing early season IAP species (such as curly-leaf pondweed)
  - Outstanding questions:
    - How often should lakes be surveyed? The current recommendation is to sample every five years for "baseline" data, but more frequent sampling may be required for early detection or to evaluate management activities.
- Survey type: Rapid Macrophytes Habitat Assessment
  - Overview:
    - This method was developed as a rapid assessment approach to document presence/absence of a limited number of target species, as part of the US EPA National Lakes Assessment. The method is no longer regularly implemented within WI DNR.
  - Design:
    - Transect based design using a rake to sample macrophytes at individual points, stratified by lake depth
    - Transects (min=10) are arranged perpendicular from shore and evenly distributed along the shoreline
  - Unique elements:
    - Facilitates rapid assessment of macrophyte biotic condition (i.e., habitat) and collects data on target species and growth forms present
  - Objectives:
    - Rapid assessment of plant habitat and target species presence/absence
  - Outputs:
    - Presence/absences of IAP and macrophytes (lumped by growth form); number of morphologically distinct species
  - Trade-offs:
    - Only 8 IAP species targeted
    - Low spatial coverage
    - Requires a low level of plant taxonomic knowledge
    - Relatively quick to implement (~1-3 hours per lake)
- Survey type: AIS early detection
  - Overview:
    - This is the standard method employed by WI DNR for lakewide IAP early detection efforts. It uses a combination of rake tosses and snorkel meanders at points of entry and target locations, along with a lakewide boat meander to document presence/absence of IAP.
  - Design:
    - Uses snorkeling, rakes, nets, and boat meander to search for IAP lakewide.
    - All points of entry (i.e., boat/canoe launches) plus five target sites. These sites include areas of disturbance or unique features that might be suitable for target species
    - Visual shoreline meander of all habitats
  - Unique elements:
    - Specifically targets invasive plants and invasive animals
    - Includes D-net sampling and veliger tows capable of detecting invasive aquatic animals.

        - Collect photographs/specimens.
        - Submit voucher specimens to State herbarium and State Zoological Museum
    - Objectives:
        - Early detection of AIS
        - Initially developed to implement on 200 lakes each year for 5 years to assess the rate of AIS spread
    - Outputs:
        - Presence/absence and relative abundance of AIS (entered in DNR database and shared on webpage)
    - Trade-offs:
        - Takes ~2 hours per mile of shoreline
        - Nearshore (including wetland species) are typically not detected if the lakewide meander is not completed.
        - Focuses primarily on access points, other points of pathway entry (i.e., camps, beaches, fishing peers, etc.), and unique habitat features that could be suitable for various species (i.e., substrate types, water flow).
        - Qualitative sampling
    - Outstanding questions:
        - How comprehensive is this survey method for early detection (i.e., what is the incidence of failure to detect IAP when they are present at a site)? This is difficult to evaluate because of the qualitative nature of the survey.
        - Proposing to scale number of target sites to waterbody size.
- Survey type: <u>AIS snapshot day</u>
    - Overview:
        - A community led surveillance effort that enlists volunteers to conduct surveillance as part of a single day "blitz" at "high risk" lakes. Volunteers are trained by DNR/partners staff and sent out to survey at points of entry.
    - Design:
        - Rake toss and visual surveys at points of entry in lakes deemed probable locations for IAP introduction
    - Unique elements:
        - Volunteers collect samples/photographs and return to host training site for verification
    - Objectives:
        - Early detection of IAP
    - Outputs:
        - Presence/absence and relative abundance of IAP
    - Trade-offs:
        - Limited spatial extent of survey efforts (i.e., only at points of entry and within 30 minutes of training site)
- Survey type: <u>Citizen Lake Monitoring Network</u>
    - Overview:
        - Community based surveillance program led by DNR that enlists and trains interested riparians to conduct surveillance along their lakeshore.
    - Design:
        - Volunteers monitor their shoreline for presence/basence of IAP; methods and spatial extent of survey vary

- Unique elements:
  - Leverages local knowledge and expertise to detect changes in aquatic plant community, including new IAP
- Objectives:
  - Primarily early detection, but can contribute knowledge on other changes in plant community
- Outputs:
  - Presence/absence and relative abundance of IAP, or other invasive species
- Trade-offs:
  - Potential for false negatives (if volunteers fail to detect or only sample limited section of shoreline); surveillance is not necessarily targeted to high risk areas

- Discussion
  - How many staff are devoted to early detection efforts in WI?
    - DNR staff works closely with county partners. Approximately 20 staff throughout the state are conducting early detection surveys.
  - What software/equipment is used to collect information during surveys?
    - Data collection is primarily paper/pencil. Survey 123 has been trialed but is not the preferred method.
  - How many or what percentage of lakes are surveyed using these methods each year? How do you rotate or prioritize surveys?
    - About 100 lakes are sampled each year with the point intercept surveys. At least 30 lakes are sampled each year with the early detection protocol (lakewide meander).
    - Priority is normally given to lakes where lake associations can subsidize the cost of the survey or for lakes where invasion potential is high (e.g., following up on IAP reports).
  - Half of IAP detections in the state are coming from volunteer community-based surveys.
  - Are you finding new detections with PI surveys?
    - Yes, e.g., SSW was found in Wind Lake, Racine Co.

1.2. MINNESOTA

PRESENTERS: Wendy Crowell*, MN DNR &* Megan Weber*, UMN Extension & MAISRC*

PRESENTATIONS: Available HERE & HERE

SUMMARY: Minnesota DNR implements various survey methods for early detection of IAP and to monitor aquatic plant communities. This presentation briefly described each method and advantages/disadvantages of each. Megan Weber provided an overview of Starry Trek, a community-based surveillance program for early detection of starry stonewort.

ADDITIONAL DETAILS:

- Targeted meander survey
  - Done by boating in a zigzag pattern throughout littoral zone, making visual observation and sampling with rakes. Supplemented by sonar (to locate stands of plants for sampling).
- SCUBA and snorkel survey
  - Done in small areas (boat landings) or if responding to a report of IAP at a particular location

    - Not ideal in turbid water or where plants are abundant
- Delineation survey
    - Primarily used to gather information on location, extent, and species composition of aquatic plant beds (e.g., to inform permitted management activities).
    - This is a boat based method with rake tosses. Can be done in a targeted area or as a lakewide survey.
- Point intercept survey
    - Primarily used to monitor efficacy of IAP management efforts (including non-target impacts).
    - Provides species presence/absence and frequency of occurrence data, that can be used to determine if additional areas have become infested and to compare trends year-to-year.
- Sediment propagule surveys
    - Used in concert with PI surveys to understand management impacts on seed bank (e.g., randomly sampling a subset of PI points with ponar dredge to look for curly leaf pondweed turions).
- Key questions:
    - Given the available time and funds for staff work, what it the best use of our time?
    - What is the trade-off between searching in a known area versus searching in other areas?
- Starry Trek
    - A program of the University of Minnesota Aquatic Invasive Species Research Center & UMN Extension
    - Prompted by discovery of starry stonewort (SSW) in MN in 2015
    - Similar to WI AIS Snapshot Day; volunteers convene at a host site for training on sampling methods and are then directed to area lakes to conduct early detection surveillance at public access points of entry.
    - Lakes are selected based on a combination of local volunteer knowledge and predicted risk from ecological niche models; Muthukrishnan, R., Sleith, R. S., Karol, K. G., & Larkin, D. J. (2018). Prediction of starry stonewort (Nitellopsis obtusa) invasion risk in upper Midwest (USA) lakes using ecological niche models. *Aquatic Botany*, *151*, 43-50.
    - Sampling occurs in August, to overlap SSW late season phenology; Glisson, W. J., Muthukrishnan, R., Wagner, C. K., & Larkin, D. J. (2022). Invasive Nitellopsis obtusa (starry stonewort) has distinct late-season phenology compared to native and other invasive macrophytes in Minnesota, USA. *Aquatic Botany*, *176*, 103452.
    - Detection sensitivity varies with sampling intensity; using Starry Trek protocol, in lakes where SSW is present a single rake toss results in about 50/50 chance of detection, whereas 5 rake tosses increases detection sensitivity to 95%
    - Up to 300 lakes are sampled annually.
    - 63 new AIS reports by way of Starry Trek, including 20% of Minnesota's new SSW detections

1.3. MICHIGAN

PRESENTER: Billy Keiper, *EGLE*; Erick Elgin & Jeremy Hartsock, *Michigan State Univ Extension*

PRESENTATION: Available HERE

SUMMARY: Michigan provided an overview of their aquatic plant surveillance programs including surveys that are 1) ongoing (annual monitoring with long term funding support), 2) intended to advance monitoring (building capacity and understanding), 3) short-term (1 or 2 year duration), and 4) conducted for regulatory purposes (e.g., to evaluate regulated activities and inform decisions).

ADDITIONAL DETAILS:

- Ongoing monitoring:
    - Inlands Lakes AIS Surveillance (Keiper)
        - Michigan's routine early detection method, employed since 2014. Uses a combination of methods including, 1) boat meander survey, 2) rake tosses, 3) shoreline wading, and 4) snorkeling (30 min).
        - Documents all native and IAP taxa, but survey is qualitative in nature and can miss species.
        - Over 100 lakes surveyed since 2014. About 10-20 lakes surveyed each year
    - Citizen Science: Exotic Aquatic Plant Watch (Elgin)
        - Part of a statewide program, dedicated to early detection and education
        - Training: Volunteers attend annual trainings (virtual and in-person) and are taught to identify probable invaders and watchlist species, notably European frogbit, starry stonewort, hydrilla, Eurasian watermilfoil, and curlyleaf pondweed
        - Methods: Volunteers survey along transects placed perpendicular to the shore in areas of high invasion risk (e.g., public access); with rake tosses and visual observation at shallow, mid-depth and deep extent of littoral zone. IAP are documented. Photos are taken for verification by professionals. Data is shared to MiCorps database and MISIN.
        - Benefits: Landscape level monitoring (84 lakes participated in 2022, from across the state), creates an educated population that can be vigilant while on or around lakes, and facilitates rapid response actions.
        - Shortcomings: Relatively limited effort and spatial extent mean the survey may miss rare species, and non-professionals may misidentify species (mitigated by expert verification).
- Advancing aquatic plant monitoring
    - Using point intercept to monitor European frogbit (EFB) and plant community changes: a case study in Pentwater Lake
        - EFB first detected in 2019; wanted to document any change in EFB population and related changes in aquatic plant community
        - Used a 60M grid to quantify macrophyte inter-annual variation and quantify EFB control efforts; sampling on grid with rake tosses; data was collected in ArcCollector in 2020 and 2021; results are forthcoming
        - Developed species detection curves to estimate effort required for detection based on lake size and shoreline complexity; roughly 100-300 sample points per lake was considered adequate (for lakes 1000 – 7000 littoral acres)
        - Work has been expanded to 20 other lakes in the state (10 with EFB and 10 without); surveys occurred in 2022 and will be repeated in 2023; sampling on these lakes includes point intercept and meander surveys; the data from the surveys will be used to assess trade-off between repeatability and detectability
    - On developing an aquatic macrophyte survey protocol for Michigan inland lakes

        - In 2021, MSU was awarded funding to categorize plant communities in inland lakes using a modified point intercept protocol
        - The goal is to survey 80-100 lakes and provide baseline information on macrophyte communities
        - Protocol calls for approximately 100 points in littoral zone (0-25 ft); approx. 150 points for lakes > 1,000 acres; approx. 300 points for lakes > 10,000 acres. At each point, 2 rake tosses with species detections recorded for each rake toss and sample point
        - Results (to date); 20 lakes sampled in 2022; 100 points is enough to characterize the community; at 20 points, detected 50% of predicted species richness; unclear if 0-25 feet is adequate (e.g., SSW detected deeper than 25 ft.); a database of species detections is being generated and will be available upon request.
        - Strengths: point intercept works well for visualizing dominant plant distributions; 2 rake tosses per point allows an unbiased estimate of occurrence when detection < 100%; PI allows for robust estimation of precision of occurrence and species accumulation curves to evaluate effectiveness of sampling
        - Shortcomings: For easily visible plants, method may not be the most time efficient approach; Larger lakes have an increased distance between points; additional sampling in 2023 will help to answer questions about optimal number of sampling points needed to adequately characterize the plant community and how deep to sample.
- Short term survey efforts
    - Early detection in the Manistee National Forest
        - 30-minute timed snorkel survey, similar to TNC/Notre Dame NFWF project
        - Survey data was summarized and shared to regionally clustered lakes to help prevent the spread between lakes
        - Benefits: Detects rare species better than the rake toss method; Rapid; Outreach may improve prevention of secondary spread
        - Tradeoffs: Requires getting in the water; Outreach takes time
    - Michigan DNR boating access site survey
        - Goal: monitor for IAP at public boat ramps and estimate abundance
        - Rapid shoreline rake toss method with visual observation (50 ft on either side of boating access)
        - Sampled 157 lakes in one season, but detections are limited to points of entry and may miss deeper and rare occurrences
- Informing management and regulatory decisions
    - In some instances, pre- and post-treatment monitoring of the aquatic plant community are now required as a condition of permitted management activity
- Discussion: How do you choose between PI verses point entry meander?
    - For now, PI is used for evaluating impacts over time and impacts on native. Meander snorkel is more for early detection.

1.4. QUEBEC

PRESENTER: Marie-Ève Tousignant, *MELCCFP*

PRESENTATION: Available upon request

SUMMARY: Quebec developed tools for early detection surveillance on lakes by volunteers and local organizations. This presentation described the protocols, including protocol application levels, sampling design, sample methods and implementation.

ADDITIONAL DETAILS:

- "Protocols on alien invasive plant detection in the lakes of Quebec" was published in 2016- only available in French; the protocol is based on the "Invasive aquatic Plant Screening survey and mapping procedure" developed by Maine; other published tools available (e.g, plant ID guide)
- Objective: Detection of invasive aquatic plants through citizen science monitoring effort (often trained and supported by professionals) and by local organizations.
- Protocol application levels (dependent upon # of volunteers and lake size)
    1. Limited
    2. Points of entry (±100m)
    3. Points of entry + suitable habitats
    4. All shoreline and littoral zone
- Protocol recommends that lakes be separated into sectors
    - This is not always necessary but useful in large lakes and helps divide the work among teams
- Sampling design: survey is primarily by boat meander (with visual observation and rake tosses), but point intercept survey (with rake tosses) is recommended for turbid waters
- Sampling method and tools: Visual (aquascope); rake sampling; Photos and samples are taken from suspicious plants and submitted for verification
- Outputs: P/A of species and central coordinates; Reporting via the citizen reporting tool, Sentinelle (Available to the public as a mobile app and on the web – only in French); Eurasian watermilfoil is the primary target for most volunteer groups
- Because detection is mainly carried about by volunteers or local organization, there is not a complete portrait of lakes across the province
- Future goals:
    - Increase detection efforts (they will have increased funding)
    - Increase spatial coverage (potentially through more targeted surveillance at high risk lakes)
    - Update list of target species and best practice protocols
- Currently, training has only been available in one region, but there is a desire to expand the program across the province

1.5. NEW YORK

PRESENTER: Lindsay Yoder, *NYSDEC*

PRESENTATION: Available HERE

SUMMARY: New York conducts surveillance through multiple state agencies as well as a state-funded network of regional partnerships for invasive species management (PRISMs). This presentation provided an overview of the primary methods used across the state for early detection and other aquatic plant monitoring applications, including an overview of rake toss, visual, and biomass survey methods. Regional and statewide site prioritization efforts were also described.

ADDITIONAL DETAILS:

- Unlike other states, most management and monitoring for AIS in New York is being done at a regional level
- Data collection varies between program and region; All data, regardless of program, is being input into iMapInvasives, the statewide invasive species repository managed by the New York Natural Heritage Program.
  - Target species lists also vary between regions, with each PRISM guiding their regions' priorities as suggested by the New York State invasive species tiered rankings
- In New York, the consensus is to utilize a combination of surveillance techniques, depending on the context and management goal
- Rake Toss surveys
  - Based on the Point Intercept Rake Toss Relative Abundance Method (PIRTRAM)
  - The method calls for 2 rake tosses at each pre-determined sample location (on a grid; grid size varies across programs or based on target. E.g., 100 m grid in lakes without hydrilla, but 25 m grid when hydrilla is present)
  - Some programs use a buffer zone of visual estimates around each grid point
  - Finger Lakes PRISM also samples areas surrounding boat launches
  - Pros: objective sampling, quantitative data, efficient whole-lake monitoring capable of early detection and providing long term plant community data, low cost
  - Cons: generally ignores visual observations, requires some technological skill/software, not appropriate for true biomass estimates, destructive
- Meander (Non-systematic method)
  - Involves meandering through littoral zone to document the extent of aquatic plant beds (by visual observation and with rake tosses)
  - Pros: Low-effort on front end, easiest and most useful for early detection of floating/ emergent species
  - Cons: subjective, typically non-repeatable, can be significantly more effort than point intercept method (i.e., to accurately map extent of plant beds), destructive
- Visual methods
  - Often used in conjunction with meander survey
  - Best suited for early detection of floating and emergent vegetation
  - Pros: cheap, easy, fast, highly effective, nondestructive
  - Cons: subjective, should not be the only tool for submerged vegetation
- Snorkling/SCUBA: NYS DEC, FLPRISM
  - Meander across specific depths at/near target areas
  - Has proven most uesful for early detection of hydrilla
  - Pros: More thorough than perforimg rake tosses, higher probabaility of finding target
  - Cons: Time consuming, exspensive, not quanitative
- Underwater ROV: SLELO, NYS DEC
  - Deployed from dock or boat near target area
  - Pros: Quick snapshot of plant community, best for low abundance areas, may be useful for ED depending on visbility, long-term cost, nondestructive
  - Cons: highly dependent on visibility, difficult to navigate/ID plants if canopy is dense
- Quadrat sampling (for biomass): NYS DEC Region 9
  - Record overall and individual species % cover, average plant height, substrate type, quantitative
  - Species identified via snorkel or viewscope

    - Pros: Higher probability of locating target (if present at sample location)
    - Cons: highly dependent on visibility, can be extremely time consuming depending on hydrology, difficult to navigate/ID plants if canopy is dense
- Hydroacoustics
    - Use of SONAR and Biobase software to generate heat maps for vegetative cover, bottom hardness, bathymetry
    - Traverse whole lake or littoral zone in a zig-zag pattern
    - Best used to assist with site selection (for additional sampling by rake, visual, etc.)
    - Pros: software free for many, determine site feasibility in real-time /eliminate unnecessary sampling points, nondestructive
    - Cons: cannot distinguish between species, labor intensive (time, data, processing)
- eDNA
    - Historically more geared toward animals, but expanding into plants
    - NYSDEC working to develop eDNA lab/protocols; hydrilla is a priority target for this application; SLELO and APIPP have conducted eDNA surveillance targeting EWM, hydrilla, and starry stonewort
- Prioritization and site selection
    - At a regional level, prioritization matrices have been created (based on criteria such aspresence of rare, threatened and endangered species, public access types, environmental justice areas and proximity to or the presence of existing aquatic invasive species
    - Programs can utilize results for the NYS watercraft inspection steward program
    - Some programs designate and focus surveillance at Highly Probable Areas within Priority Conservation Areas (PCA)
    - A within lake vulnerability analysis was developed by APPIP, which identifies areas of probable introduction based on distance to shoreline and forest as well as impervious surface presence and agricultural land use
- In an area where you have low information, how do you pick methods for ED?
    - NYS DEC uses point intercept and combines with visual (using the buffer zone)
    - In the Adirondacks, community science visual surveys help cover the large amount of area and can follow-up areas with professional staff
- When recording the data from the 100ft visual buffer (at point intercepts), do you record everything you find?
    - Varies between programs but for Western NY program they record all native and invasive species. They use a survey 123 form to document plants outside the direct rake toss in the same form.
- For site selection, they have also looked a lot of boater use and prioritize “hot spot” lakes that are being utilized a lot

1.6. OHIO

PRESENTER: Mark Warman, *Cleveland Metroparks*

PRESENTATION: Available HERE

SUMMARY: Ohio DNR largely relies on contractors and partners to conduct IAP surveillance activities in the state. This presentation highlighted the various early detection efforts implemented by

Cleveland Metroparks staff, under the auspices of Ohio DNR. Techniques discussed included, on shore visual, wader, kayak, and boat meander surveys, and community based surveillance efforts.

ADDITIONAL DETAILS:

- Ohio DNR staff may observe IAP as part of other agency surveys (e.g., fish surveys) or solicit reports from the general public, but for IAP their primary role is to facilitate EDRR efforts through other partners; there are no DNR staff dedicated to IAP early detection
- Cleveland Metroparks receives money from the state of OH for EDRR, focused on the Lake Erie watershed, and with some surveillance in the Ohio river watershed
- For CLE Metroparks, most surveys are targeted meanders
  - Visual surveys with rake tosses focused on sheltered bays, boat launches, fishing piers, & places of human interaction
  - Most surveys are in 12ft or shallower (max 30ft)
  - Increased survey effort if hydrilla or other priority IAP are detected (e.g., point intercept surveys to document extent and abundance)
- Walking the banks (visual assessment)
  - Used for small, isolated waterbodies
  - Pro: Quick, field many questions from public
  - Con: may not be able to walk entire margin
- Wader Survey:
  - Pros: Thorough, often observes species at small population sizes
  - Con: labor intensive, potentially hazardous
- Kayak:
  - Used for medium water bodies and nooks and crannies of large systems
  - Pro: Reach shallow water, emergent plant zones
  - Con: Fewer acres than boating, difficult to do rake tosses from
- Boating:
  - Used for large lakes (> 75 acres)
  - Pros: Survey many acres quickly
  - Cons: requires coordination (e.g., often borrowing boat)
- July-Sept is when most surveys are conducted
- About 100-200 sites surveyed each year (1 full time and 2 seasonal staff)
- Seasonal staff are trained to identify IAP but also native species; herbarium vouchers are used for training
- IAP detections are submitted to USGS
- Community based and other “passive” surveillance efforts
  - CLE metroparks monitors alerts on iNaturalist for target areas; has resulted in new IAP detections (e.g., water lettuce)
  - About 20 IAP “early detection kits” distributed by CLE metroparks and in circulation among OH state agency staff
- Questions they hope to answer:
  - How to improve methods with limited staff?
  - How to balance visiting more sites and performing thorough surveys?
  - How to engage with other agencies? Which community data collection method is best?

1.7. NFWF & GREAT LAKES PORTS

PRESENTERS: Andrew Tucker & Lindsay Chadderton, *The Nature Conservancy*

PRESENTATION: Available HERE

SUMMARY: This presentation provided an overview of two separate IAP early detection projects, 1) a GLRI funded project focused on developing and refining a survey design and sampling method for IAP early detection in high risk Great Lakes ports (>1000 acres), and 2) a National Fish and Wildlife Foundation funded project that compared efficacy of a rapid assessment rake toss method to a snorkel meander at points of entry.

ADDITIONAL DETAILS:

- GLRI IAP detection in GL ports
    - Phase one: Aquatic plant survey methods development and site assessment; published, Tucker, A. J., Annis, G., Elgin, E., Chadderton, W. L., & Hoffman, J. (2022). Towards a framework for invasive aquatic plant survey design in Great Lakes coastal areas. *Management of biological invasions: international journal of applied research on biological invasions*, *13*(1), 45.
    - Phase two: Invasive aquatic plant surveillance in New York Great Lakes ports (funded by NYSDEC AIS state management funds from GLRI)
    - Project goals: Develop an aquatic plant surveillance strategy to sample large GL coastal high risk sites; Apply and refine the surveillance protocol at priority sites
    - Survey designs are a probabilistic (which facilitates inferences about the places that are not sampled), and spatially explicit (which allows us to explore spatial drivers of detection and hone in on risky areas)
    - Sampling went to water's edge (not a wetland survey)
    - Maximum of three sampling days per site
    - Initial surveys covered the entire port, but most plants were found in shallow areas (<4m), so subsequent surveys focused on shallow sites and on species rich areas (based on a model of habitat features associated with high observed richness)
    - Sampling was boat-based survey with rake tosses and visual assessment
    - 100-m grid was overlaid on port and then sample locations were randomly selected in ArcGIS; in the field, the four corners of each grid were sampled; Rakes were tossed at each sample station a minimum of 4 times (from each side of the boat) and then until no new species were detected
    - Species accumulation curves were developed from rarefaction using survey data (species observed at each sample grid)
    - Targeting shallow sites (<4m) increased species richness (with less effort) compared to totally random survey across entire site
    - A single 3 day survey detects 75-95% of estimated species richness; models suggest that hitting the 100% target would require multiple 3-day trips
    - Strengths:
        - Targets a range of "hotspots". Richness (which may be a proxy for IAP presence/absence) is often but not always highest at points of entry

    - Detection method appears to be relatively efficient compared to more systematic point-intercept sampling (although more comparisons need to be done)
    - Covers ground efficiently (including potential for IAP observation during meander from one sample grid to the next)
    - Facilitates adaptive optimization (can fill in coverage gaps and are building a robust data set)
  - Limitations
    - Requires taxonomic expertise
    - Survey design requires GIS proficiency
    - Questions of how much is enough from an AIS detection perspective (i.e., is 90% detection good enough?)
    - Models assume “rare” species are a good proxy for IAP. Is this a sound assumption?
    - Capable of detecting all growth forms but might be overkill if you have species specific targets in mind, especially for floating or emergent species
- NWFW point of entry methods comparison
  - This was started in 2009/10; prompted by the detection of hydrilla in Lake Manitou (IN) and to test and refine IAP surveillance methods and to inventory plants and record presence of dressenid mussels and mystery snails
  - shore-based rake surveys
    - IN method = 25 rake tosses around boat landing
    - IL method = 6 rake tosses at boat landing
    - NFWF method = rRepeated rake tosses (until no new species detected) at up to 6 locations (at 5m intervals from landing)
  - 30 min snorkel survey
    - 30-minutes of searching areas around the boat landing
    - Collecting all plant species observed as well as introduced mollusks
    - Accompanied by kayaker (for safety) and to collect emergent plants and physico-chemical data
  - Plants were returned to lab and identified by experts
  - Results:
    - snorkel survey method detected more species than rake toss methods, but each method collected unique species (i.e., snorkeling detected, on average, ~80% of the total species richness at each site)
    - Diver experienced mattered, experienced divers were able to ID more species, although NWFW dive crews’ proficiency increased over time
  - Pros:
    - 30-min snorkel time appears sufficient to collect most common species
    - Possible to cover multiple sites in a day
    - Snorkeling boat ramps provides a cost-effective rapid survey methods that is suitable for surveillance and AIS detection
  - Cons:
    - Limited spatial coverge
    - Assumes boat ramp areas are of greatest risk

- Assumes rare natives are good surrogate for AIS- results possibilty overestimate detection sensitivty
- Requires good visbility and safe conditions

1.8. GROUP DISCUSSION & EASY RETRO BOARD

SUMMARY: Workshop participants were asked to add content to an “EasyRetro” board, on strengths, limitations, uncertainties, and general comments, for each of 7 survey design methods (point intercept, line transect, meander, randomized, point of entry, POE + meander, community led). It was noted that the EasyRetro content would inform development of a “decision matrix” as part of a best practice guidance document. General discussion followed.

- **Discussion**
  - Q: Is a meander survey repeatable? If there are guidelines to meander (point of entry, etc.) – could that provide some better tracking/objectivity
    - R: Making a meander “prescribed” can take away from the value of that method. Likely not repeatable over time
    - R: From a data point of view, if you break up a meander into segments (targeted vs haphazard/random), take note of time, etc. that may help with standardization
  - Q: Do we have any data on return on investment for these different survey methods in two dimensions? First would be effort metrics like staff hours, area covered, cost, etc. And the second dimension would be outcomes (i.e. the number of early detections per survey method)?
    - R: Brian Greene (TNC, APIPP) has some anecdotal numbers on this, but it would be nice to have this on a larger scale
  - Q: What data do we have to help inform one data design over another?
    - R: Limited. A matrix would be useful to display the tradeoffs
  - Q: Are we more concerned with missing plants or are we more concerned about thinking we are seeing effects that are not actually there? (Type 1 vs type 2 error)
    - R: In a surveillance sense, a false negative would be worse
  - Q: For the detections received from citizen science/ opportunistic public reports, are they truly new infestations/ early detections being found?
    - Depends on how present the species is. For SSW, yes it has been really helpful. For CLPW, not so much
    - Opportunistic public reports are quite different than formal public monitoring programs
    - Brittany Rogers: In my region we typically see reports for species that are already known from an area
    - In the Adirondacks we have examples of both. Some are early detections of small populations and others are reports for waterbodies that haven't been surveyed before and have established populations
    - Maureen K: We require photos for all new detections and specimens of certain species. It's worked really for us to streamline verification
    - Jo Latimore: We require our volunteers to take photos and we give them laminated white photo backgrounds with scales that they can use for photos: https://micorps.net/wp-content/uploads/2021/04/EAPW_Photography-Guide-2-sided_2019.pdf

2. REMOTE SENSING APPLICATIONS

2.1. OVERVIEW OF REMOTE SENSING FOR EARLY DETECTION

PRESENTER: Margaret Kalacska, *McGill University*

PRESENTATION: Available upon request

SUMMARY: This presentation provided participants with an overview of essential concepts in remotely sensed data and perspectives on the utility of remote sensing applications for early detection of IAP. Dr. Kalacska also shared results from remote sensing for IAP in St. Lawrence River and for Phragmites.

ADDITIONAL DETAILS:

- Types of remotely sensed data include photograph (film/digital), multispectral, hyperspectral, and active remote sensing
- Certain data sets lend themselves to different questions, based on spatial information (i.e., how fine or coarse scale is the data spatially) and information content (i.e., how rich is the dataset; how many layers of information are available)
- On the spectrum of data detail and content, we don't need to gather overly complicated data if we don't need it for analysis (detection to qualification scale); but if we want to identify something unique, e.g., IAP to species level, then we really need to look at data that has more information content rather than less.
- New, hyperspectral datasets are potentially able to differentiate species/genera – e.g., limiting to shorter wavelength, increased water penetration
- For each pixel, high information data can be separated into different bands of light and qualified based on species knowledge and (light) reflectiveness of that species
- Remote sensing is severely limited by water depth, even when water is clear. Plants do not reflect as much light. Particularly in aquatic environments, much of the light is re-directed to somewhere other than the remote sensing tool
- Case study in Long Sault Parkway, Quebec, drone imagery with 1m x 1m pixel resolution was able to distinguish individual species with ~80% accuracy, but
  - Differentiation is more apparent in peak growing season than late season
  - Difficult to differentiate mixed species compared to monotypic stands within a pixel
- Most publicly available datasets do not currently contain the layers necessary to make genera or species differentiation. Satellites get bigger areas, but there's no getting around... If you want individual plant level, the satellites are not there yet
- Smaller pixels are recorded from airborne vehicles, like planes or drones, compared to satellites
  - Specialized data sets are designed to record data with the layers required for assessment
- Floating plants on satellites? The challenge with satellite is the pixel size – 1 pixel is about 30 x 30 m
- Remote sensing is best for relatively shallow and clear water, and to complement traditional survey methods

2.2. REMOTE SENSING – CASE STUDY #1

PRESENTER: Amanda Grimm, *Great Lakes Commission*

PRESENTATION: Available HERE

SUMMARY: This presentation provided participants with an overview and comparison of remote sensing methods used to distinguish emergent and submerged invasive aquatic vegetation from other plant species. Amanda presented two case studies showing the utility of UAV's/aerial imagery and commercial satellite imagery for distinguishing *Phragmites* and Eurasian watermilfoil.

ADDITIONAL DETAILS:

- The three options for remote sensing of aquatic invasive plant species when considering multispectral remote sensing include moderate-resolution satellite (e.g., Landsat), high resolution satellite, UAV-borne sensors/aerial imagery.
- For early detection of small IAP patches, high resolution imagery from commercial satellites or UAV's/aerial imagery is required.
- Case Study 1 focused on delimiting the extent of *Phragmites* across the Great Lakes region.
    - *Mapping and Monitoring of Invasive Phragmites in the Coastal Great Lakes Using Remote Sensing Data from Multiple Platforms* funded by USGS Great Lakes Science Center (PI: Laura Bourgeau-Chavez)
    - Regional scale mapping involved a combination of Landsat8 and PalSar synthetic aperture radar classification (5-meter pixels).
    - Individual site monitoring (e.g., to quantify efficacy of Phragmites management activities) involved WorldView-2 classification (1-meter pixels) which picks up smaller differences to better track changes in a single stand.
    - Small UAV's/drones were used to map smaller patches of *Phragmites* as part of the *Phragmites* Adaptive Management Framework (PAMF).
    - Object based image analysis (OBIA) using eCognition was used to classify to species level
    - Object based classification clusters individual pixels into contiguous shapes, which allows the user to classify vegetation patches based not just on the spectral values of the individual pixels, but the characteristics of the segments as well their size and shape and texture and context
    - UAV images were processed in Agisoft which aligns the images to create orthophoto mosaics georeferenced to the site location (3cm pixels). A digital surface model (DSM) of elevation was created (13cm pixels) which can be a helpful tool for identifying emergent vegetation.
    - These methods are capable of quantifying *Phragmites* survival and treatment efficacy.
- Case Study 2 focused on delimiting Eurasian watermilfoil using OBIA (PI Colin Brooks, Michigan Tech Univ)
    - UAV drones with custom sensors were used to collect data in the Keweenaw Waterway in Michigan's upper peninsula (relatively low water clarity) and the Les Cheneaux Islands near Lake Huron (high water clarity).

    - A traditional backpack spectrometer and portable spectrometers (attached to a drone or the boat) were used to obtain spectral profiles and determine if Eurasian watermilfoil was distinguishable from other submerged vegetation.
    - Hyperspectral data can differentiate invasive milfoil from other vegetation but when resampling to obtain data similar to multispectral sensors, the milfoil is not distinguishable because much of the signal is lost to the water column.
    - Two multispectral cameras were used: a tetracam (6 imaging sensors – 490 -900 nm bands) and VISNIR (4 band sensors – RGB and near infrared).
        - Tetracam imagery could distinguish between invasive milfoil and other plants (including native milfoil) on sunny days with clear water but once conditions/clarity decreased distinctions could not be made.
        - VISNIR produced similar results, but is limited by water depth and overall less sensitive to species differences.
        - Marker buoys were helpful in serving as referencing points for the imaging software.
    - Field collection procedures used point intercept methods with a variety of vegetation and water collections methods (rake toss, twist rake sampling, sonde, LI-COR light meter) to characterize the water, vegetation, and light levels.
- Overall similarities and differences arise comparing commercial satellites and UAV platforms.
    - UAV/aerial imagery have a much finer resolution which is useful for an object-based approach but have high upfront costs.
    - Commercial imagery is expensive but can be obtained by educational or federal entities for a low to zero cost. With this imagery, larger areas can be covered, and it requires less processing time, but it is also less flexible and is limited by environmental conditions especially for submerged vegetation.

2.3. REMOTE SENSING – CASE STUDY #2

PRESENTER: Cass Stabler, Elizabeth Miller & Tricia Workman, *Parks Canada Agency*

PRESENTATION: Available [HERE]

SUMMARY: Water soldier was first detected in the Trent-Severn Waterway (TSW) in 2008. This presentation provided an overview of efforts by Parks Canada to conduct early detection surveillance for water soldier in the TSW using imagery acquired during drone flights, especially to target areas where access for more conventional surveillance was limited.

ADDITIONAL DETAILS:

- From 2008 to 2020 water soldier spread from upstream reaches of the Trent River (near Lake Seymour) into the Bay of Quinte. Efforts to eradicate water soldier have been hampered by presence of persistent populations in in accessible areas. Drone flights were deployed to aid in detecting remnant and new populations of water soldier in these areas.
- In 2020, drone flights covered ~25 ha; Drone imagery was acquired from flights with Drone Deploy subscription and analyzed in ArcMap
- In 2021, Parks Canada partnered with ECCC and MECP to deploy larger drones and acquire multispectral imagery; covered ~250 ha; drone imagery was acquired from Drone2 Map

- In 2022, PC and MECP covered ~160 ha; purchased Pix4D for imagery stitching
- Water soldier has a unique morphology that makes it possible to identify from aerial imagery, but depending on conditions of imagery, the technique is not always easy to apply
- Desktop identification was very time consuming, need to zoom in on images (1 to 50 zoom; particularly for individual specimens and when in mixed stands; identifying to individual plant requires resolution to 1 or 1.5 cm)
- Drones can be flown beyond the edges of traditional grid (point intercept) monitoring boundary
- Data software: ArcGIS Drone2Map (not a good product), Drone Deploy (expensive), Pix4D (good product)
- Challenges:
  - Landowner permissions to fly
  - Flying from water/boats
  - Splitting time between UAV boat flying and traditional surveys
- Given the time intensive nature of manual imagery analysis, PC will be looking to automate the process (including use of multi spectral camera and object based imagery analysis)
- While it is really geared towards DJI cameras, the software DJI Terra has done wonders in some cases where Pix4d fails.

2.4. GROUP DISCUSSION

SUMMARY: Workshop participants were asked to identify the species for which development of remote sensing tools would be a high priority (EasyRetro). General discussion followed.

- Q: How do these approaches compare in terms of cost or personnel requirements to some of our other survey approaches?
  - R: For the some of the newest drone based equipment we're looking at a range of five to 18,000 Canadian dollars for initial purchase price (for equipment that provides geotagging information so over time you don't have to do a lot of manual georeferencing which saves on labor, but costs a bit more upfront). Quality satellite imagery is not free. Landsat imagery are free, but that's not really suitable for these kinds of questions. So you're still looking at 500 to a thousand or more dollars per scene.
  - R: In the US, if you're buying, for example, digital globe commercial imagery at the the regular rate, it tends to be $20 to $60.00 per square kilometer. There are a few programs for getting that kind of imagery at a lower cost or for free (e.g., Civil Applications Committee, available to federal agencies and collaborators by requesting access to their giant archive of commercial imagery).
  - R: One perspective is to ask yourself, Is remote sensing already happening for the species that you're interested in? If not, start small before determining if it's worth investing in
  - R: Sites that are inaccessible via canoe are good candidates for aerial imaging…this is where drones really shine and could be worth the investment; but the method may not be as cost efficient for areas that are easier to access
- Q: What has been Michigan's experience with drones?

    - R: We've funded a number of projects to look at European frog bit detection with drones. Overall, the separation of frog bit in particular from lookalikes (e.g., water lilies) with spectral imaging really didn't seem very effective.
- Q: In people's experience, do floating-leaved AIS tend to appear where there was no floating-leaved vegetation before? Or do they tend to replace other floating-leaved vegetation? Thinking of RS simply for detecting beds of floating veg where none was there before.
    - R: There is evidence of treated Phragmites giving way to new frogbit populations, emergent to floating
- Q: other comments
    - R: May be helpful to also identify plants based on shape, rather than light reflection for distinct species (e.g., water soldier). But a key for the object based imagery analysis is to ensure you have enough variability in your training data sets to capture all the variations to ensure accurate predictions
    - R: Consideration of use in participatory science? Many members of the public own and use drones. There are examples of this kind of application for monitoring seals on Sable Island (see Appendix). It would be important to develop some best practices around methods (e.g., appropriate flying height for the species that you're focused on, angles of data collection, how much overlap do you want the between images, etc.). Also, one of the biggest downfalls of remote sensing is the lack of really good field data to help build the models, and the model is only as good as the actual outdoor, boots on the ground data for groundtruthing. Leveraging the enthusiasm of the citizen scientists to help collect that database of the ground truth data could really help with interpretation from any of the data sets that we've seen.

MAY 19, 2023

1. eDNA SURVEILLANCE FOR AQUATIC PLANTS

1.1. OVERVIEW AND PERSPECTIVES

PRESENTER: Chris Wilson & Stephanie Coghlan, *Ontario Ministry of Natural Resources and Forestry*

PRESENTATION: Available HERE

SUMMARY: This presentation discussed eDNA approaches used for detecting aquatic plant species along with challenges and research gaps for future work in this field. Additionally, results from a case study in Ontario describe the process for developing and testing aquatic plant eDNA metabarcoding methods.

ADDITIONAL DETAILS:

- eDNA is cellular and tissue debris that is shed from organisms into the water and we can use eDNA “as molecular smoke alarm” to detect aquatic species.
- A positive eDNA detection is useful for determining where to concentrate sampling efforts and serves as an early warning system.
- Active/targeted surveillance can be used for detecting a particular species of interest/concern.
- Passive surveillance (community eDNA/ metabarcoding) can be used to broadly characterize community composition.
- Both approaches must consider quality of the assay being used is (i.e., what can it find, and will it find what you’re after), how sensitive is the assay (i.e., what’s the smallest amount of DNA that the assay can detect), and what is the spatial and temporal sensitivity of the assay.
- It is difficult to quantify organism abundance with eDNA.
- It is hard to guarantee presence from a positive detection, therefore rigorously tested methods are needed to argue presence. This includes using an assay with carefully designed primers, standardizing design, testing, and reporting protocols, and tool validation before field sampling. Additionally, the sampling design must reflect the hypothesis in question.
- In 2021 the Canadian Standards Association (CSA) released a standard eDNA guidance document outlining processes for lab accreditation and result validation.
- Current challenges for aquatic plant eDNA detection include limited availability of baseline sequence data for eDNA barcoding when compared to animals. The stationary nature of most aquatic plants makes them harder to detect. Additionally, plant shedding coincides with seasonality and is therefore not constant and dormant life stages of plants don’t shed DNA.
- *Scriver et al. 2015* developed eDNA markers for 7 different aquatic plant species (Water solider, Eurasian watermilfoil, Carolina Fanwort, Parrotfeather, Water hyacinth, Water lettuce, Yellow floating heart).
    - Scriver, M., Marinich, A., Wilson, C., & Freeland, J. (2015). Development of species-specific environmental DNA (eDNA) markers for invasive aquatic plants. *Aquatic botany*, *122*, 27-31.
- Water solider is of particular interest in Ontario where two infestations have occurred, in the Black River and Trent River.

    - Pre-herbicide treatment, eDNA sampling in Trent River resulted in strong detections upstream and low detections downstream. After herbicide treatment stronger detections occurred downstream and in Lake Ontario with presence confirmed in 2022.
    - The herbicide treatment had a big effect on detections due to living colonies experiencing post treatment decay and downstream movement.
    - Collaborations of several groups are working on Water solider removal. Additionally, groups are expanding survey area and adding Water chestnut and *Hydrilla* to monitoring efforts.
    - Community AIS detection kits have been created.
- Aquatic plant metabarcoding (Stephanie Coghlan).
    - For plants there isn't one "go-to" barcoding region, so Stephanie tested two that have shown promise (rbcL and ITS2).
    - To provide a detection baseline, primers were tested using mock plants communities.
    - The rbcL primer identified the largest number of aquatic plant species.
    - The tested assays returned species and genus identification across wide range of plant taxa.
    - The assays were tested at pilot sites in Black and Trent Rivers (Ontario) - 10 water samples collected at each location. From those tests, expected and unexpected detections occurred including some invasives.
    - Follow up work has focused on testing the best performing assay at 44 more sites and including more replicates.
    - Challenges for successful passive surveillance (metabarcoding) with eDNA include plant hybridization, limited insight on abundance, limited species level identification, and having to rely on incomplete/error-free databases.
    - But the tool can help to monitor biodiversity over time (i.e., before and after invasion) and it has potential as a good tool to inform early detection.
- eDNA resources include GEN-FISH, GLFC Science Transfer eDNA, CSA Group, and eDNAtlas.

1.2 . eDNA METABARCODING TO CHARACTERIZE PLANT COMMUNITIES IN MICHIGAN'S INLAND LAKES

PRESENTER: Kim Scribner (w/ contributions from John Robinson), *Michigan State University*

PRESENTATION: Available HERE

SUMMARY: This presentation discussed methods, applications, and lessons learned from single and multiple species eDNA sampling for aquatic species. A case study from eDNA sampling in 22 Michigan lakes was presented along with future considerations and recommendations for eDNA use, especially for IAP.

Cellular materials containing DNA from all aquatic unicellular and multi-cellular organisms, including plants and vertebrates reside in lentic and lotic environments, and can be used to characterize species presence/absence and community composition. Entire communities can be enumerated by interrogation of environmental (e)DNAs using taxon-specific polymerase chain reaction (PCR) primers. In this presentation we discuss the nuances of eDNA sampling in freshwater aquatic realms. We discuss the importance of survey design (e.g., sample number, depths, spatial dispersion) to account for factors affecting eDNA distribution in lakes, and discuss the importance of using antiseptic sampling techniques to minimize contamination among samples. For single species survey applications, we discuss concepts of PCR survey sensitivity and species specificity. The presentation also highlights empirical data from multi-species or metabarcoding surveys of 22 inland Michigan lakes. We highlight considerations for creation of DNA sequence baseline data bases including interrogation of database archives for species presence/absence. We provide an overview of characteristics of useful PCR primers for multiple species interrogation and discuss options for development of 'pipelines' to assign sequences obtained from metabarcoding to user-assigned classifications. We present data based on 'traditional' fisheries gear estimating lake fish and plant community composition that contrast eDNA survey results. Results show eDNA sampling identified more species, especially rare species that included aquatic invasive species (AIS). Using occupancy modeling we determined that AIS detection probabilities from eDNA surveys were comparable to or higher than those estimated for traditional gears. We demonstrated novel applications of remotely sensed lake and surrounding terrestrial land cover data to develop predictive models for estimating fish and plant species richness. The area of upstream lakes, which we used as a measure of aquatic connectivity, was predictive of fish AIS sequence abundance. The number of fish species detected was positively related to both the area of upstream lakes and the size of the sampled lake. For plants, species richness declined as a function of Julian date of sampling. The proportion of all sequencies assigned to plant AIS was associated with lake area. We demonstrated the use of 'heatmaps' for species richness and relative sequence abundance for AIS to highlight the ability of multi-species metabarcoding to provide managers with spatially explicit representation of community diversity and AIS relative sequence abundance and highlighted downstream applications. Finally, we provide recommendations for design and implementation of plant eDNA studies. Timing of sampling and lake size were likely responsible for large proportions of sequences from non-targeted terrestrial and wetlands plant taxa. Development of plant PCR primers that would provide greater resolution to the species level would be important for Great Lakes region interrogation of plant AIS because of the number of congeneric native and AIS species that are currently not resolvable genetically.

ADDITIONAL DETAILS:

- eDNA methodologies include obtaining an environmental sample, amplifying the DNA using PCR, and screening that DNA for particular species of interest. Screening can be for a single species (binary (yes/no) or a measure of relative abundance), or for multiple species (community composition and relative abundance of each species).
- Considerations for eDNA sampling include lake area (i.e., the larger the lake, the more samples are needed), the depth profile (i.e., surface, or benthic samples depending on where the target organism(s) resides), terrestrial sources mixing in (i.e., pollen), seasonality (i.e., litter input from falling leaves), and filter characteristics (i.e., dirty water needs a larger pore size).
- Factors that affect eDNA detection include prevailing winds, seiches, waves, and current patterns.
- Whether in the field or the lab, antiseptic methods and verification are needed for good eDNA QA/QC (i.e., always have a distilled water control, wear gloves, bleach equipment, etc). This is important to emphasize with citizen science eDNA work.
- Typically, mitochondrial or chloroplast DNA is amplified because the closed circular DNA is less prone to degradation than chromosomal DNA.

- For single species detection, primers must amplify only when matching the species of interest.
  - Specificity minimizes false positives by establishing confidence that the target species is being amplified.
  - Sensitivity minimizes false negatives by amplifying DNA when it is present.
    - Limits of detection (LOD) is the lowest copy number where 95% of replicates per concentration were positive.
    - Limits of quantification (LOQ) is the lowest concentration of target DNA that can be quantified within an assay.
  - Protocols should contextualize environmental conditions and test for contamination.
- Metabarcoding for multiple species uses "universal primers" that are faithful to all potential taxa of interest to gather community information.
- Assembling a DNA sequence baseline involves determining expected species observations using data repositories (e.g., VertNet, GBIF, ICUN Redlist, USGS, PRISM) and obtaining a marker of choice. Then, a sequence baseline is developed by using either sanger sequencing or database mining (e.g., GenBank or NCBI) and a bioinformatic analysis takes raw and sequencing primers and puts them all into a database (e.g., *Mothur*).
- Michigan State University eDNA metabarcoding projects
  - Sampled 22 Inland lakes in Michigan between 2016-2018; primary target was fish community but samples were later analyzed for aquatic plants
  - Michigan representative lakes were chosen to proportionally represent based on area, depth, development, connectivity, and usage. Lakes with fish surveys were prioritized.
  - At each lake a Smithroot backpack sampler was used to take 30-50 eDNA samples (both surface and benthic) with negative controls in the field and lab.
  - For Fish,
    - eDNA detected more fish species and had less inter-sample variation when compared to traditional gears (e.g., seines).
    - eDNA metabarcoding was as good if not better than traditional samples and mock communities showed a tight correlation between expected and observed read proportions.
    - eDNA sampling showed that species richness and AIS prevalence increases proportionally with the size of lake and associated wetland area.
    - The number of AIS were proportional to the amount of developed land.
    - Spatial information of species presence collected with eDNA were used to create species heatmaps at the lake scale.
  - For Plants,
    - Species richness for plant communities varied across lakes and 48-77% of detected species were from terrestrial habitats.
    - Plant species richness was best predicted by Julian Day sampling with early season producing higher species richness.
    - There were 3-24 invasive plants detected per lake and eDNA was able to detect more invasive species when compared to traditional surveys.
    - The non-native plant representation was 14.6% of all sequence reads.
    - Likely due to terrestrial signal interference, species richness was higher earlier in the year showing inconsistencies with aquatic species growth.

- General observations,
    - Marker choice is critical and databases for them need to be continually refined.
    - Correlations between species richness are related to both biotic and abiotic factors.
    - False positives may occur due to resolution issues and moving forward, markers are needed with species-level resolution.
    - eDNA sampling is best paired with other approaches.
    - Plant specific primers are needed for database development.

1.3. GROUP DISCUSSION

- Q: Is more extensive sampling work done by Chris Wilson's crew going to be qPCR or metabarcoding focused?
    - Chris and his crew plan to do both – surveillance targeting water soldier will continue (to inform prevention actions) and they will be doing community eDNA to develop baseline biodiversity information.
- Q: What are some challenges for metabarcoding?
    - Metabarcoding challenges include the need for DNA primers to amplify the target across a range of species and generally there's no one best tool to do this, therefore a balance must be found between taxonomic breadth and resolution and multiple assays may be needed to focus in and get better resolution.
- Q: Is qPCR more sensitive than metabarcoding?
    - qPCR is at least an order of magnitude more sensitive but in absence of a single species marker, metabarcoding is a good choice.
    - Its advantageous to take metabarcoding approach initially and if a species of concern shows up, follow up with a targeted qPCR approach that has greater specificity and sensitivity
    - When targeting as many species as possible with metabarcoding, the universal primers could cause preferential amplification for species the primers anneal to more specifically
- Q: When separating terrestrial and aquatic species, when is species richness highest for aquatic plants?
    - Likely there is a higher number of aquatic species later in the year, but this may be impacted by lake turnover and there is still a lot unknown.
    - Future work should sample the same lake multiple times throughout the year.
- Q: What is the number of replicates needed and the costs of that?
    - There is no one magic number, it depends on your question, the area you want to sample and what degree of certainty you want (higher certainty means more sampling is needed).
- Q: Are benthic or surface samples more specific?
    - For fish and mussels similar communities were found at both depths.
- Q: What differences exist between lentic and lotic systems for eDNA sampling?
    - The dilution effect is different in both environments.
    - For flowing waters depth is not a concern so usually only a surface sample is needed and a natural transect design is present as the water is moving by. However, in lentic systems depth is likely important as is wind direction and thermal stratification may have impacts.

    - More research is needed to understand seasonality of eDNA sampling in all systems but specifically in river/flowing systems.
    - For flowing habitats transect sampling can help localize an organism's source as stronger signals are detected while traveling upstream.
- Q: Does RNA provide a means of detection?
    - The rate of degradation for RNA is much faster than DNA so it will more difficult to find in the environment (than DNA).
    - Also, RNA data availability is lagging behind DNA, so it will require more lab time (to develop primers, etc.)
- Q: Under what circumstances is eDNA preferred for plants when compared to traditional tools?
    - eDNA can be an effective early detection tool, especially as a screening tool to indicate that there may be a species of concern present (e.g., based on some evidence of a species or taxon signature detected), that might otherwise be overlooked by traditional surveys
    - eDNA can help to direct traditional surveillance efforts to most probably areas of concern

APPENDIX A – REFERENCES & RESOURCES

Community-led surveillance resources:

1) Sampling rake design:

   https://micorps.net/wp-content/uploads/2017/12/CLMP-AqPlant-SamplingRake.pdf
   https://z.umn.edu/AquaticPlantSampling

2) Starry Trek:

   Starry Trek Monitoring Protocols Handout: z.umn.edu/AISMonitoring
   Starry Trek Monitoring Protocols Video: https://youtu.be/sgJRKkC_4eQ

3) NYS DEC 3-tiered system for aquatic plant monitoring (Kishbaugh, Lord, and Johnson 2006)

   http://www.eaglelake1.org/archives/documents/plant_surveys/2006%20aquatic%20plant%20monitoring%20guidelines.pdf

4) Maine Invasive Aquatic Plant Screening and Mapping Survey Procedures:

   IAP-Survey-Procedures.pdf (lakestewardsofmaine.org)

5) MiCorps photo backgrounds (laminated and distributed to volunteers):

   https://micorps.net/wp-content/uploads/2021/04/EAPW_Photography-Guide-2-sided_2019.pdf

Remote sensing resources:

1) Papers with additional details on Phragmites & Eurasian Watermilfoil mapping:

   https://www.mdpi.com/2072-4292/13/10/1895/pdf
   https://www.mdpi.com/2072-4292/14/10/2336/pdf

2) Community-based surveillance by remote sensing

   Counting the seals of Sable Island: A new Parks Canada citizen science project - Sable Island National Park Reserve
   https://geonadir.com/

Other:

1) Re: number of samples needed to locate uncommon species, see pgs. 59-60 in, Perleberg, D., P. Radomski, S. Simon, K. Carlson, and J. Knopik. 2015. Minnesota Lake Plant Survey Manual, for use by MNDNR Fisheries Section and EWR Lakes Program. Minnesota Department of Natural Resources. Ecological and Water Resources Division. Brainerd, MN. 82 pp. and appendices

APPENDIX B – ATTENDEES

Name	Affiliation	email	7-Feb	19-Mar	Presenter
Alwin, Thomas	Michigan EGLE	AlwinT@michigan.gov		X	
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