Center for Lakes and Reservoirs

AIS Early Detection Monitoring Methods

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revised for 2024

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Sampling Procedures

Crayfish Trapping

Targeted organisms: Crayfish

Equipment: Crayfish traps

Protocol: Set out crayfish traps before doing any other sampling or actions at each waterbody, as traps need to sit for as long as possible within the water to trap crayfish (at least 2 hours, ideally overnight, but not longer than that, as crayfish can escape the trap once they've eaten the bait).

1. Open a can of catfood ¼ of the way, which will allow for the smell to enter the water, but will limit how much or how quickly crayfish can access the bait.
2. Place the can of catfood within the trap, then secure the trap together.
3. Throw the trap into the water, and secure the rope to a hard object (a rock or sturdy plant).
4. Leave the trap for 2-10 hours, then check it for crayfish. If there are crayfish, count them, identify them, and take photos of a specimen of each present species.

Data recording: On the datasheet, record the type of survey (crustacean trapping survey), the GPS coordinates, the date, the length of time the trap was left out, whether there were or weren't crayfish, and the number of individuals of each species present. If setting up the trap for overnight sampling, save the datasheet as a draft, then go back and edit it to add in an end time in the morning.

Take a photo of the crayfish, and write a description in the notes, so that it can be ID'd by someone at the lab. Be sure to get multiple angles, and a good image of the claws, back, and side of the crayfish. You can take a sample to the lab, but often ethanol degrades the color of the carapace so much that it isn't helpful.

Veliger Sampling

Targeted organisms: zebra and quagga mussels, Asian clams

Equipment: 30 cm diameter 64-micron mesh plankton net, weight, demarcated rope, pre-labeled 500 ml sample bottles filled with 350 ml Tris Buffered ethanol, GPS, notebook, tape measure, watch or other timer, stick or other natural item for assessing flow velocity.

General towing protocols:

- Our aim is to get at least a total of 30m of tow length, whether this be horizontal, vertical, or length calculated from flowing water.
    - California's veliger tow methodology (here) recommends attempting to filter at least 1000L of water through the nets. With 30cm diameter nets, this can be attained through a 14.3 m total tow length. 0.07 m^2 (net opening area) * 14.3 m (length)) per sample bottle) = 1.0 m^3 * 1000 L/m^3 = 1000 L .
    - CLR is aiming for a little more than 2x the above tow length, 30m. CLR also assumes a 30% net inefficiency (user error and clogging). This results in: 0.07 m^2 (net opening area) * 30 m (length) * 0.7 (net efficiency) = 1.47 m^3 * 1000 L/m^3 = 1470 L
        - The Survey123 datasheet will tell you the total distance of your tows, simply look into the history of your datasheet to see if you've reached at least 30m total tow length (tow length for the entire composite bottle is at least 30m). Sometimes, this 30m length is impossible to reach due to turbid conditions, if this happens, keep the sample. Shorter lengths help reduce net clogging, so if the water is very turbid, try doing more and shorter individual tows.
        - Keep in mind that sometimes you'll be using more than one sample bottle per waterbody, make sure that you keep track of the sample bottle ID's, and only add together volume values pertaining to the same sample bottle ID.
- Take samples from the boat if you're able to launch and if the water is not too turbid/full of algae (if you're unable to tow from the boat for at least 20 seconds without the net clogging)
    - Take a mix of vertical and horizontal tows relatively close to the docks if the water depth is >10ft, and you are able to get a good sample doing so.
        - Take at least 1 horizontal tow per waterbody near the busiest dock or marina
    - If unable to launch, or unable to get a good sample from the boat, take shorter horizontal tows from the dock or along the shore
    - If the water is flowing, either anchor the boat, motor against current while sampling, or take stationary samples from a bridge/dock/shallow sections.

- If there's more space in the bottle after reaching the desired filtration value, great, take additional tows at various locations until you fill the bottle to the shoulder.
- Sometimes, the net will become clogged with material (the walls of the net will appear brown or green, as material has stuck to it). Or the liquid within the net will be an opaque brown or green color, and will not reduce down quickly when shaken.
    - A clogged net will not filter water well, as water will be unable to move through the mesh, and will instead be pushed out through the opening again by other water. A clogged net will also make it hard to filter the desired amount of water in that it's more difficult to condense the sample down.
    - **To deal with this, reduce the length of each tow and increase the number of tows you composite together into a single sample bottle (so instead of 1 30m tow, try 5 6m tows, or 4 8m tows).**
    - If you can't reach the ideal minimum length / filtration value, do take whatever amount of sample you can get, just take down a note that there was too much algae or sediment to reach the desired filtration value.

- *Sampling:*
    1. Securely attach cod end piece, marked rope, and weight/anchor to the net. The weight/anchor should hit below the cod end of the net to avoid damaging the cod end while in use.
    2. Collect samples as outlined in below sections referring to different types of veliger tows.
    3. Rinsing:
        a. Retrieve the net, wash material down the inside of the net into the cod end by quickly pulling the net vertically out of the water a few times (until most algae and debris is washed downward to the cod end), without submerging the top opening of the net.
        b. With the cod end still attached to the net, swirl the cod end to concentrate the filtered material enough that the cod end can be removed without spilling the sample.
        c. Gently swirl the cod end so water washes through the side windows until the sample is concentrated.
        d. Remove the cod end and pour the concentrate into the labeled sample bottle.
        e. Replace the cod end and re-rinse material on the net into the cod end by repeating steps 1-4.
        f. Concentrate the remaining sample within the cod end by holding the cod-end in the water up to the top of the windows (but not submerging the top of the cod end), then repeat steps 3-4.
        g. The sample bottle should be filled only to the shoulder. If there is still room in the bottle, collect a new tow sample(s) from the same waterbody

(or site if the waterbody is a river), and composite it into the same bottle. You can composite veliger tows of different types (horizontal + vertical + stationary).

h. **Keep the sample bottle out of the sun and heat (within a cooler with ice)**.

Horizontal dock tow protocol: If the water isn't flowing, you can't launch the boat, or the water is so turbid or full of algae that a boat survey wouldn't work, then perform a horizontal tow along a dock or other walkable surface.

1. Use a tape measure to measure the length you will tow along.
2. Lower the weight and net into the water at one end of that section and walk the net to the other end, turn, and return to where you started.
3. Repeat depending on the length of the section and the amount of material in the water, aiming for at least a total length of 30m if possible.
4. Process the collected samples as outlined in the General Protocol above.

- *Data recording:*
    - On the datasheet record the GPS coordinates at the start and end of the tow, date, time at start and end of tow, approximate depth of the net during the tow, total length of tows collected (e.g. back and forth along a 30m dock would be a 60m tow), unique bottle ID, number of tows composited.
    - If an organism is found on the net or cod end, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Horizontal boat tow protocol: If a longer boat tow makes sense (water is not too turbid or full of algae), you are able to launch the boat, and the water is still, you can perform a horizontal tow off the boat. Keep an eye on the position of other boats when taking horizontal tows, and ensure no other boats get close to the net/line while they are out to avoid damage.

1. Put the net anchor and the net into the water, being careful to make sure the net ends up behind the motor, and the rope and net are both away from the propeller. It can be helpful to get the boat moving very slightly forward, then changing to neutral, before putting the net into the water and taking the start time and start point.
2. Take the start time and start point when the opening of the net enters the water.
3. Move the boat forward at idle speed, towing the net in a straight line for 2-5 minutes, depending on how turbid or full of algae the water is. The depth the net sits is measured based on the angle, and the amount of line out. Ideally, samples should be taken near

the thermocline, which can be determined with a YSI depth profile before sampling, or previous YSI profiles. Retrieve the net and process as in the General protocol. Be extremely careful with the net and line near the boat motor.

4. Take additional samples as needed until the sample bottle is full to the shoulder.

- *Data recording:*
    - On the datasheet, record the GPS coordinates of the start and end of the tows, date, time at start and end of tow, length of line out during the tow, approximate angle the line enters the water when moving, depth of the net, unique bottle ID, and number of tows composited.
    - If an organism is found on the net or cod end, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Stationary tow protocol: If the water at your sampling location is flowing at a rate which would move through the net well (water pulls the net open or downstream), then you can hold the net stationary in the current to collect a sample. You can also do this from a boat by motoring against the flow of water until stationary.

1. Estimate the velocity of the water by taking the time elapsed per distance traveled by any floating object. For example you can time the movement of a stick along a measured distance along a dock, from the bow to the stern of the boat (the Klamath is 17 ft, and the Smokercraft is 12 ft long), or along a section of shore that you measure with a measuring tape.
2. Lower the anchor and net into the moving water to mid water depth and hold in place for about 2 minutes (longer for clear waters, or shorter for turbid water), retrieve net.
3. Process the collected samples as outlined in the General Protocol above.

- *Data recording:*
    - On the datasheet record the GPS coordinate of the tow, date, time at start and end of tow, approximate depth of the net during the tow, unique bottle ID, number of tows composited, and the estimated water velocity.
    - If an organism is found on the net or cod end, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID it if possible, can use uncertain terms such as "poss., prob., suspected" if not 100% sure, but have some idea. Add a detailed description into the relevant survey123 note column.

Vertical tow protocol: If the water is deep (>10 ft), and not flowing, then a vertical tow should be done. This type of tow can be done from either a boat or a dock, but the water depth is more likely to be deeper than 10 ft when on the boat further away from the dock. You can perform a

vertical tow off the boat by either anchoring or holding the boat steady in place (motoring slightly to stay in the same spot).

1. Lower the weight and net vertically to 30 m or until the anchor hits the bottom of the waterbody, whichever is shallower. You can track the movement of the net and anchor on the depth sounder. You need to avoid the net opening scraping the sediment at the bottom of the waterbody.
2. Slowly retrieve the net, process the samples as outlined in the General Protocol above.

- *Data recording:*
    - On the datasheet, record the GPS coordinate of the tow, date, time at start of the tow, depth (length) of the tow(s), total length of tows collected, unique bottle ID, number of tows composited.
    - If an organism is found on the net or cod end, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Other details: **Reference your schedule or trip packet for waterbody-specific information about how many bottles you need to fill, and where to fill them.**

eDNA sampling

When to use: We have a limited number of eDNA filters available for the field season, so only take eDNA samples when given instructions to do so. eDNA samples will be taken at some of our higher priority lakes which are too cold early in the season, and draw down too much later in the season for veliger samples to be taken effectively.

Do a blank sample for the waterbody (not per site) before taking eDNA test samples from the waterbody. A blank involves all of the below steps, except using DI water from within a container, not the waterbody itself. After the blank sample is completed, you can run a test sample with the steps below, using water from the site. Only one blank needs to be produced per waterbody, but always before the test samples are taken.

Protocol:

- Attach the two pieces of the transect pole together. Attach the long end of the transect pole tubing to the IN port on the side of the eDNA backpack (remove the red cap). Attach another, shorter, piece of tubing to the OUT port on the other side of the backpack. You can handle these sections of tubing with your bare hands, it doesn't matter if they touch the ground or get somewhat dirty. Just avoid getting dirt inside the tubing, as it will end up interfering with the eDNA backpack pull pressure, and would end up within the eDNA backpack during sampling and storage.

- Open the battery compartment of the controller (you'll need a phillip's head screwdriver), being careful to not completely remove the screw, as it's easily lost. Put batteries into the controller for the transect pole (look at the image at the bottom of the battery case, as the direction of the batteries does not correspond to the direction of the spring as is typical). Screw the battery compartment closed securely. You can also use the eDNA backpack without the remote control, by flipping the power switch on the eDNA backpack screen and using the buttons next to the screen. **Be careful** when pressing the buttons on the eDNA backpack, only press the START and STOP buttons, not RESET, as the RESET button will delete the current entry.
- First, do the blank sample, following steps **a-k**, putting the tubing into **DI water**. This will pull water we know is free of residues, particulates, and zebra and quagga mussels into the filter pack. This allows us to make sure there is no sampling contamination in the field or by the lab during analysis.
- Then, do the test sample, following steps **a-k**, putting the tubing into the waterbody itself. This will pull water we **don't** know is free of zebra and quagga mussels through the filter pack.
    - **Put gloves on**, open a self-preserving eDNA filter pack bag, carefully grab the yellow side of the filter pack, do **not** touch the tubing within the bag, and do **not** touch the white end of the filter pack until that tubing is on it. Push the white side of the eDNA filter pack onto the tubing by holding the tubing with the bag (do **not** touch the tubing with even your gloves). The tubing found within the filter pack bag, and which is then applied to the white end of the filter pack is the only thing that is upstream of the filter pack itself, and handling of the tubing can result in contamination of the sample.
    - You can use two fingers on the white end of the filter pack to stabilize it as you clamp the transect pole to the yellow end of the filter pack (on the shoulder of the yellow side). Do **not** touch the tubing on the white end end of the filter pack.
    - Then, you can use two fingers on the white end of the filter pack to stabilize as you push the transect pole tubing onto the yellow end of the filter pack. Do **not** touch the tubing on the white end of the filter pack.
    - Place the tubing attached to the white end of the filter pack into the water where you intend to take your sample.
    - Start the backpack up when the tube is in the water, and keep it in the water until you get a notification sound. Ensure that the tubing stays in the water but that the filter pack is not submerged.
    - Three beeps notify you that the filter is clogged. The filtration rate is likely to drop below 0.1 L/min, which is the minimum that the eDNA backpack can measure.
        i. If this occurs, before the 2 beep warning, you will have filtered less than the desired 2L amount.

1. If you decide to do multiple filter packs to reach at least the 2L goal amount, you will end up repeating all steps until you reach at least 2L total (but you may as well take as much sample as you possibly can), between multiple filter packs (**not available for 2023 or 2024**).
2. **You can also accept the results of the filtration step, and end the sample when the eDNA backpack reads below 0.1 L/min, or gives you the filter clogged warning and 3 beeps. Simply record the value that it actually did sample, even if that is not the ideal amount (do this for now). When the eDNA backpack stops filtering, remove the tubing from the water and skip to step i.**

- Two beeps occur shortly before the amount of water pulled through the tube reaches 2 L, the target value (but **keep running the backpack for as long as possible, until the eDNA backpack gives 3 beeps (clogged sample)**). We want to maximize the amount of sample that we collect within the filter pack, and if conditions are good enough to get us beyond 2L, that is better than our goal value.
- Quickly turn the pole over so the filter pack's tube is facing upward, then tilt the pole back and upward so that gravity and the backpack's pump pull the rest of the water down through the filter and past it toward the backpack. The extra water that was in the front tube is what will be needed to get the total to at least 2 L. At this point, **you need to run it for another minute while it is elevated in the air, so that the pressure continues to pull water from the filter pack, drying it out so it can preserve itself.**
- Remove the tubing by grabbing it through the filter pack bag, discard tubing. Place the desiccated filter pack back into its bag with a silica gel pack (to further dry it out), close the bag, and label the bag. Bags should be labeled with: whether the sample is a blank filter pack or a test filter pack, waterbody, site, date, Sample ID # (and log ID): the number of the entry for the sample within the eDNA backpack. Place the bag into a clean, new ziplock and then into a container with ice for transport.

- *Data recording:*
  - On the datasheet record the sample ID (the number at the top of the eDNA backpack screen), if this was a blank or test sample, and the exact liters of water sampled.
  - When you get back to the lab, use the 32GB USB flash drive **for the backpack you took into the field (each backpack has its own designated flash drive, to prevent overwriting and losing data from the other backpack)** to pull the files from the eDNA backpack as a data backup. You simply plug the flash drive into

the USB-A slot on the backpack (on the front, covered by a twist cap), while the backpack is on, and press the button corresponding to “download all files”.

Other details:

- The unit can’t measure flow rates under 0.1 L/min. Refer to above steps for instructions on what to do if the filter clogs.
- The pressure should top out at 12 psi, higher psi values can destroy the cells.
- **Be careful** to only press the blue button on the eDNA backpack for start/stop NOT reset.
- Never touch the filter pack without gloves.
- Never touch the clear tubing included with the filter pack **at all**, even with gloves. The tubing is the only thing upstream of the filter, and touching it could contaminate the sample.
- The battery and the screen are resistant to light rain only, and should not be exposed to anything more than that. The screen’s casing is very water resistant/proof only if the window is closed securely. Minimize the amount of time that the window is open if there is more than light rain. Be careful not to put the backpack within water, as the battery is on the **bottom** of the backpack.

YSI Water Chemistry Sampling

Targeted metrics: Temperature, Conductivity, pH, ORP (oxidation-reduction potential), ODO (Dissolved oxygen), depth, etc.

Equipment: YSI handheld, YSI cable (20 to 50m), ProDSS Conductivity and Temperature sensor, ProDSS ODO Optical Dissolved Oxygen sensor, ProDSS pH/ORP Sensor, pH 5 buffer solution, pH 7 buffer solution, pH 10 buffer solution, conductivity standard solution

Protocol:

Right before you intend to use the YSI for the first time during a given day, calibrate the specific conductance:

1. Rinse the probes **twice** with a small amount of conductivity solution.
2. Fill the blue case with conductivity solution until the probes are covered.
3. On the YSI handheld, enter the calibration solution concentration value (100 µ/S) into the calibration value field.
    a. If the probe is reading around half the calibration solution value, then ensure that the probes are clean, try shaking the liquid around to remove air bubbles, and try filling the blue container with additional calibration solution.
        i. If still not working, contact supervisor.
    b. If the probe is reading ~100 µ/S, wait a minute or two until the current reading seems to have stabilized, then hit “accept calibration”.

4. Dispose of the calibration solution.

Next, calibrate the YSI pH sensors using both the pH 7 and pH 10 buffer solutions. To do this:

1. Put a small amount of pH7 solution into the blue casing (screw on to enclose the sensors on the cable).
2. Gently shake the solution onto the probes to wash them with the calibration solution, then dispose of the rinse solution.
3. Then, put more solution into the case until the sensors are covered, allow it to sit for a minute, then calibrate the pH on the handheld and accept the post-calibration value (the handheld will automatically adjust the post-calibration value based on what solution is on the sensors, and the temperature so it might be slightly of from 7 (i.e. 7.04) - that's okay. Dispose of the rinse solution..
4. Repeat steps 1-3 for pH 10 (again, the handheld will automatically update the post-calibration value to 10). After you hit accept, a new pH calibration will appear- select "finish calibration".

Right before you use the YSI at a given waterbody, calibrate the DO% by putting a small amount of water into the blue YSI case, allow the air to saturate with water, then set the DO% post-calibration value to 100% (for 100% water-saturated air).

When done sampling at a waterbody, store the YSI sensors with a small amount of water (to saturate the air within the blue case). When done sampling for the entire day, store the YSI sensors with a small amount of water mixed with a small amount of pH 5 calibration solution. The pH 5 calibration solution is important for preservation of the pH probe, to prevent the stripping of ions from the bulb. The water is important to prevent the drying out of several sensors, as drying out can cause them to lose functionality permanently.

- If unable to launch the boat to take a depth profile, simply take a reading just below the water at the end of a dock or otherwise where you are able to.
- If able to launch the boat, take a full depth profile at the deepest point of the waterbody, to the depth you are able to (depending on waterbody depth and cable length).
    - YSI readings should be taken at every 1 m of depth until you reach the thermocline, then every 1m for a few additional meters (3 additional meters / readings is sufficient), then every 5m of depth until you reach the depth right before the bottom of the waterbody or the maximum depth of the YSI cable.
        - If the waterbody is shallow (<15m depth), then simply take YSI readings at every 1m increment until you reach the bottom of the reservoir or the maximum cable depth, without changing to every 5m.
- To take a YSI reading, simply turn on the handheld, and place the YSI sensors into the water to the desired depth, allowing the sensor to sit at that depth for a few minutes

until the reading stabilizes. Make sure to hit “enter” on the handheld in order to take a digital copy of the YSI readings (you should hear a small beep when you do this).

Data recording:

- Readings should be captured by pressing the capture button on the YSI handheld, and also by writing reading information down on rite-in-the-rain paper, and within the survey123 datasheet (be sure to take a GPS point).

Secchi water clarity

Target measure: Water clarity

Equipment: Secchi disk on measuring tape, survey123 datasheet

Protocol: This information is only possible to retrieve if the boat has been launched.

1. Navigate to the deepest point of the lake or reservoir, either anchor the boat or keep the boat stationary.
2. Slowly release the Secchi disk into the water on the shady side of the boat (so the glare from the sun doesn’t interfere with visibility) to the depth at which it cannot be seen anymore
3. Move the disk up and down to pinpoint the most accurate and deepest depth at which any part of the disk can be seen.
4. Record the depth at which you can barely see the Secchi disk, don’t tell anyone your value.

Data recording: Take note of the depth value (meters), record the value in survey123 without sharing it with the other people, and have the other people in the boat repeat the procedure and record their values down. Record the average of the team’s recorded Secchi depth values in the survey123 datasheet.

Shoreline Zig-Zag Surveys

Targeted organisms: zebra and quagga mussels, New Zealand mudsnails, mystery snails, Asian clams

Equipment: waders, shoes, or boots for wading, life jacket, hand lens, GPS, notebook, sampling bags or bottles. Old tennis shoes work well, careful with open toed sandals as you’ll be wading among slippery rocks.

Protocol: Perform a shoreline survey if it’s safe, and there are rocks or other hard substrates (other than docks) that molluscs could attach to. Structures can be included in shoreline surveys

if they aren't extremely large (<10m long), but if they are >10 m long, or are an intentional artificial substrate, enter them as their own distinct Dock/Substrate survey type. If there's a boat ramp, include it within the shoreline. If a waterbody is drawn down, or it's treacherous to wade in the water, perform a shoreline on the dry shore where it's safe to walk (even if there are no rocks or other substrates).

- **Shoreline walks are conducted for at least 30-m in each direction from an access point for a minimum of 30 minutes of survey effort.** If accessible shorelines are shorter than this, still conduct a survey for as far/long as possible. Conduct surveys along the shoreline in a zig-zag pattern at wadable depths (0 to about 0.5-m).
- Pick up hard substrates (rocks, wood) every few steps and inspect for target organisms, if rocks or hard substrates are too large to pick up, feel them for organisms.
- Don't worry about aquatic vegetation while doing this survey, as vegetation will be addressed along the transect with a different type of sample (Daubenmire and/or Rake-fullness).
- Count the number of individuals of each invertebrate species encountered up to 20, if there are over 20, ballpark the number.
    - Place a couple example individuals of each species found in a small sample container (it can be the same sample container used for other invertebrate samples from the same waterbody during the same day).
- All samples should have a unique label corresponding to the waterbody sampled, date, time, composite sample # (to tie it to the survey123 surveys). If the waterbody is a river, however, have different sample bottles for each site visited (as opposed to for each waterbody).
- Put enough ethanol into the sample to cover organisms, and place the sample in a cooler on ice.

Data recording:

- On the datasheet, record the GPS coordinates at start and end of surveyed area, date, time at start and end of survey, and the unique label ID for the date and site of the sample.
- If an organism is found, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Dock and Other Structure Surveys

Targeted organisms: zebra and quagga mussels, New Zealand mudsnails, Mystery snails

Equipment: kneepads, lifejacket (for cushion and safety), hand lens, GPS, long tape measure, notebook.

Protocol: Perform a dock or other structure patdown if the dock or other structure is within the water. Substrates can be included in shoreline surveys if they aren't extremely large (<5m long). However, if a substrate is >5 m long, or is an intentional artificial substrate, enter it as a separate Dock/Substrate survey type.

- Carefully feel along floats and supports underneath a dock, mooring, buoy, or other hard structure. Use a patting motion to feel for any rough surfaces or organisms rather than a swiping motion to protect yourself from cuts and scrapes.
- Survey the entire dock or other substrate, and **spend 30 minutes total surveying** if there is enough substrate length, or if multiple docks are available.
    - Submit each individual dock or structure surveyed as its own distinct Dock/substrate survey.
- The length and width measurements of the docks are important for tracking survey efforts, be sure to get accurate GPS coordinates, and take down these values within the notes as well.
- Treat any suspect samples in the same way as you would with other samples. Count the number of individuals of each species encountered up to 20, if there are over 20, ballpark the number.
- If there are plants on the dock itself, or floating nearby, ID the plant species and include within the dock survey.
    - If there's enough vegetation to collect with the rake, perform a rake survey on that section of plants instead.

Data recording:

- On the datasheet record the GPS coordinates at start and end of survey area, date, time at start and end of survey, total length of dock surveyed (if both sides of a 30m dock are surveyed, the total survey length would be 60m), and the unique label for the date and site collected.
- If an organism is found, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Benthic Ponar Grabs

Targeted organisms: zebra and quagga mussels, New Zealand mudsnails, Mystery snails, Asian clams

Equipment: petit ponar dredge, sieve, gloves, hand lens, GPS

Protocol: Take at least one traditional ponar grab (using the petit ponar dredge), and potentially more ponar grabs (using the shovel), if the lake substrate consists of finer materials (smaller than sand). Sand will not go through the mesh of the sieve.

1. Deploy Ponar dredge from a dock, boat, or while wading in the water, to a depth of between 1 and 5 meters.
2. Deposit dredge material in the sieve.
    a. The ponar does not work well in cobble or larger rock, so you may have to deploy the ponar multiple times and composite it together in the sieve to obtain a suitable sample.
    b. Take note of how much material you end up with after doing this (how full the sieve is).
3. Take note of how deep the ponar reached (measure the amount of wet rope attached to the ponar.
4. Move the sieve around in the water and manually agitate sediment to remove fine silt.
5. Carefully inspect dredge material for organisms. It helps to leave the sieve alone for a few minutes so organisms begin moving around again.
6. Count the number of individuals of each species encountered up to 20, if there are over 20, ballpark the number.

Samples/Data recording:

- On the datasheet, record the GPS coordinates, date, time, approximate depth(s) of ponar grab(s) at the site, number of grabs collected, type and count of snails, mussels, and plants, and a unique label for any sample collected.
- If an organism is found, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Daubenmire & Rake Toss and Rinse

Targeted organisms: zebra and quagga mussels, New Zealand mudsnails, Mystery snails, aquatic plants

Equipment: Double-sided thatch rake on a pole or rope, bucket, sieve, hand lens, GPS.

Protocol: Only perform rake tosses and Daubenmire if there is some vegetation within the water at a location. Look along the dry shore for emergent vegetation. A rake-toss can be performed from the boat if there is vegetation along the veliger tow transect.

- If there is suspicious emergent vegetation (out of the water along the shore or up on the banks):

1. Take a rake survey in survey123 (no need to actually toss a rake), noting their location and details.

- If there is aquatic vegetation along the walked shoreline transect, or the boat veliger-tow transect:
    1. Generate 3 random points along the transect length using app on tablet
        a. Use the value as a proportion of the transect (0% to 100% length of the transect), round up to the nearest half meter.
            i. For example, if the transect is of length 125 m, and you randomly generate the numbers: 33, 41, 71, then:
                1. 0.33 * 125 = 41.25 -> 41.5m along transect
                2. 0.41 * 125 = 51.25 -> 51.5m along transect
                3. 0.71 * 125 = 88.75 -> 89m along transect
    2. Take Daubenmire and/or rake surveys at those points.
        i. If points are along the shoreline transect, perform a daubenmire cover estimate before performing the rake survey at that point.
        ii. If points are along the previous boat veliger-tow transect, only perform a rake survey at that point.
        iii. If there is no vegetation at those points, do 3 rake/daubenmire surveys at the nearest locations which have vegetation.

Daubenmire: If the water is clear enough to see plants along the shoreline well, perform a Daubenmire survey.

- Hold the Daubenmire frame over the sampling point, and note what percentage cover is filled by vegetation.
    - Note this value generally, and for the individual species.
    - To avoid influencing the sample point, take plants from around the daubenmire frame to ID.

Rake-fullness:

1. Toss the double-sided thatch rake into beds of aquatic plants at the sample point.
2. Let the rake sink to the bottom and slowly retrieve it. Be careful to avoid snags or other obstacles that the rake will catch on.
3. Perform a rake-fullness ranking of the overall abundance of plants on the rake (example in the diagram below).
4. Remove vegetation from the rake, place vegetation into a bucket, rinse thoroughly with water, and pour the water (but not the vegetation) into the sieve.
5. Once rinsed, sort the vegetation into piles based on their species, and use the ranking system for the % composition of each species.
6. Count the number of individuals of each invertebrate species encountered up to 20, if there are over 20, ballpark the number.

Samples/Data recording:

- On the datasheet record the GPS coordinates, date, time, approximate depth(s) of tosses, and the number of tosses collected. Record the Daubenmire and Rake-fullness information in Survey123.
- If an organism is found, take a sample of the organism back to the lab, and if it's a large organism, take a photo. ID them if possible. You can use uncertain terms such as "poss., prob., sus." if you're not 100% sure, but do have some idea. Add a detailed description into the relevant survey123 note column.

Rake Fullness:

- Ratings should be represented as percentage fullness (0-1% (1 or 2 sprigs of plant material)), 5-25% (similar to "rating 1" (which we won't be using) below)), 25-50% (similar to "rating 2" (which we won't be using) below)), 50-75% (somewhere between rating 2 and rating 3 below), 75-95% (similar to "rating 3" (which we won't be using) below)), 100% (similar to "rating 3" but with leftover plant material which falls off the rake)

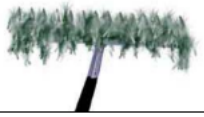
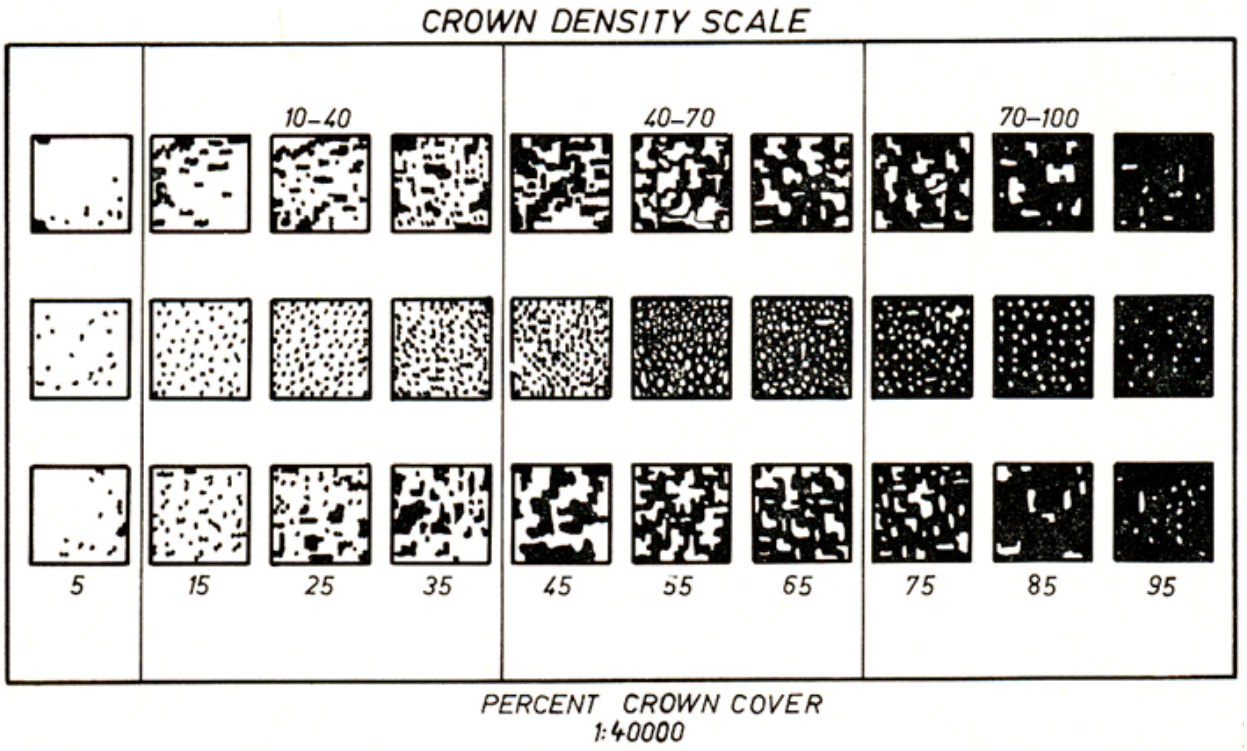
Fullness Rating	Coverage	Description
1		Only few plants. There are not enough plants to entirely cover the length of the rake head in a single layer.
2		There are enough plants to cover the length of the rake head in a single layer, but not enough to fully cover the tines.
3		The rake is completely covered and tines are not visible.

Figure example from Hauxwell et al. 2010.

Daubenmire Cover:

Daubenmire Cover Class	Class color	Range of Coverage	Rake-fullness example	Daubenmire example
1	Blue	0.1% - 5%		
2	Green	5%-25%		
3	Yellow	25% - 50%		

4	Red	50% - 75%		
5	-	75%-95%		
6	-	95%-100%		



Kleinn C. 2000. Estimating metrics of forest spatial pattern from large area forest inventory cluster samples. Forest Science 46(4):548-557.

Calcium sampling

Equipment: 500 mL brown Nalgene bottles, 125 mL labeled white Nalgene sample bottles, 1x 47mm filter funnel, 2x 25mm filter funnel, 2x hand pumps, 2x glass sidearm flasks, forceps/tweezers, masking tape, sharpie, nitric acid, packs of round filters (two sizes), DI water

Protocol: Perform this grab sample at every waterbody you visit, and every site for rivers.

1. Use water from the waterbody to rinse out a 500 mL brown bottle twice, at any depth. Then fill the bottle up all the way.
2. Within the survey123 datasheet, enter the 125 mL labeled white sample bottle label ID (they will have pre-assigned label numbers such as “PSU2024_CAL_#”) within the

sample ID field. Use masking tape to label the 500 mL brown Nalgene bottle with this ID as well.

3. At the end of the sampling day, you will filter all collected samples into their corresponding125 mL labeled white sample bottles.
    a. Rinse the beaker and filter with DI water before use, then make sure a good seal exists between the rubber stopper and the beaker.
    b. Place a new filter within the filtration equipment and filter ~100 ml of DI water through it, discarding the collected water.
    c. Filter ~100 ml of waterbody water through the set up and use the collected water to rinse out the 125 mL labeled white sample bottle 2x.
    d. Filter ~150 mL of waterbody water through the set up, replacing the filter as needed with a new filter.
    e. Dump the collected filtered waterbody water into the 125 mL labeled white sample bottle. Put this sample bottle on ice.

Waterbody Use surveys

Protocol: Perform this survey at every water body, either before or after completing sampling. The aim is to survey up to 3 people per waterbody per visit. If you are at a waterbody late in the day, with intent to camp at that waterbody, and there are few people around, talk to as many people as you can, then save the survey in drafts and continue in the morning.

- At each boat ramp, take pictures of the water level, and take photos of the signage present at the boat ramp.
- If this is the first visit to a waterbody, or you notice that CLR's self-service Waterbody Use flier is missing, attach a flier to the boat ramp bulletin board.
- Check bulletin boards to see if pamphlets are available for boaters and recreationists to take. If the pamphlet box is empty, place more pamphlets in the box for people to take. Pamphlets have the QR code for the waterbody use survey as well.
- Walk through the parking lot, and take note of the number of cars with and without boat trailers, and the license plate states for cars with and without boat trailers.
- Perform a survey of people at the waterbody, using the survey123 Waterbody Use survey type.
- While at the sampling location, if you notice someone arriving at or leaving the parking lot with vegetation hanging from their boat or trailer, or with a dirty boat, inform them that it is **illegal** to **launch** a boat that is dirty, and that the boat and trailer must be clean and free of vegetation before launching at a boat ramp. It is however **legal** for them to **leave** an area with vegetation on their trailer or boat, or with a dirty boat, as they are allowed to clean it at any point before launching at a new location. If you are disregarded (or are given reason to think that they may launch at another location without cleaning their boat), and especially if they attempt to launch at the current

location with a dirty boat, note their license plate information (and if safe and possible to do so, take a picture including boat registration numbers and license plate numbers), and write boat information and license plate information down. Do not interfere, but do contact the sheriff or police to provide them with that information.

Samples and Data Recording

- If you performed a Daubenmire or Rake fullness vegetation ranking, or collecting vegetation from a dock or other survey, then:
    - Get a few good-quality example pieces of vegetation of each species to take back to the lab as a sample.
        - A good-quality sample will be fresh pieces of vegetation that includes as much of the plant as possible: intact leaves, roots, rhizomes, flowers, buds, turions, etc. Get samples with as many of these features as you can.
    - Plant samples should be stored within either a plastic bag with water (either lake water or other water), or a plastic container with water.
    - Store plant samples in a cooler with ice.
- If you found invertebrates from any surveys, then:
    - Get a few good-quality example organisms of each species to take back to the lab as a sample. It's a good idea to take individuals of various sizes back to the lab as well.
        - A good-quality sample will be organisms that have unbroken shells. If you only find poor-quality samples (washed out/broken shells etc.) then still take those.
    - Invertebrates should be stored within a small hard plastic container, filled with ethanol until the tops of the organisms are covered completely.
    - Store invertebrate samples in a cooler with ice.
- Sample containers can be a composite container used for other invertebrate samples from the same waterbody during the same day. If the waterbody is a river, however, have different sample bottles for each site visited (as opposed to for each waterbody).
- All samples should have a unique label written on rite-in-the-rain paper (placed within the sample container), and written within the survey123 form. These labels should correspond to the waterbody sampled (can be abbreviated), date, composite sample #/type value. This unique identifier will allow the sample to be tied to the entry within survey123).
    - For example: LBC060123_P1 (for Lake Billy Chinook, 06/01/23, plant sample 1), or LBC060123_I1 (for Lake Billy Chinook, 06/01/23, invertebrate sample 1)
        - Usually you would end up with only one plant sample and one invertebrate sample per waterbody, but there's potential that you find

larger invertebrates, or many plants. So, it's best to include a number value.
    - You would also want to write: 06/01/23 1:20pm AN on the rite-in-the-rain label
- Species ID:
    - If the ID for a plant or invertebrate is unknown or uncertain, or the organism is a suspected invasive species based on reference to documentation, then: take photos, take thorough notes (both of rite-in-the-rain paper and survey123), then put that organism into its own bag or sample container, with a unique label and the rite-in-the-rain notes within that bag or container. You can write "suspected *Genus species*".
        - Label pictures similarly to the samples (LBC060123_*Genusspecies*_pic1). Submit pictures on return to PSU.
    - **If you suspect you've found zebra or quagga mussels, put the entire substrate they are attached to in a container (bag, bucket, etc.), take pictures, record all information on datasheets including detailed notes, and inform your supervisor as soon as possible.**

Decontamination

Inspect the boat, trailer, equipment, clothing, etc. for any aquatic plants or other organisms and dispose of them in the trash or away from shore.

General decontamination:

2% Virkon Aquatic solution: 2.7 oz (76.5 g) per gallon water. Soak nets and other sampling equipment that can fit in a bucket in the Virkon solution for a minimum of 15 minutes. Spray Virkon on equipment that cannot fit in buckets, wading boots, and the hull and inside of boats and scrub with a brush to remove dirt or vegetation. If conserving Virkon is necessary, it's acceptable to thoroughly spray all items instead of dunking/soaking some. Rinse thoroughly with tap water after at least 15 minutes. Decontaminate boat motors by running Virkon through the cooling water intake for 5 seconds, soaking for 15 minutes, and then running clean tap water through the motor for two minutes.

Virkon Aquatic in the 2% solution degrades in strength over time (7 days), and should be dumped and a fresh batch made every week using dry Virkon. If a batch was mixed half-way through a field week, it can be used until the middle of the next week, at which point it should be dumped out and a new batch should be mixed using dry Virkon.

5% acetic acid solution: Undiluted white vinegar. Used for zebra quagga mussel sampling equipment (nets, ropes). After decontamination in Virkon Aquatic and thorough tap water rinse,

place mussels sampling equipment in vinegar tote for a minimum of 4 hours. Rinse thoroughly after soaking with tap water.

Net Decontamination:

- Don't leave the nets in vinegar overnight. 3 nets are provided for the field team so that this will not cause a lack of nets while sampling if two nets must be in the vinegar solution at the same time. Leaving the nets in the vinegar overnight degrades the material over time, and makes the nets more fragile and more likely to fall apart.
- If a net was used but it cannot be taken out of the vinegar at or slightly after the 4 hour mark (up to 6 hours), apply Virkon to the net for 15 minutes then rinse it, then leave it overnight or until it can be put into vinegar for 4 hours. Note on the decontamination checklist which net was NOT decontaminated, put a brightly colored clip on it, and keep it away from clean nets.
    - This applies to both in the field and when returning from a trip without enough time to fully decontaminate nets.
    - Hung up clean nets should not have any clip on them, only their designated number tag.
    - If leaving for a trip, but a net which has not been decontaminated is needed, put that net into vinegar right away.

eDNA Decontamination:

- Run 2% Virkon aquatic solution through the eDNA backpack, dip the tubing in Virkon as well, leave for 10 minutes, then rinse thoroughly with clean water - can be tap water. Don't run DI water through at this point, anything that could be spread to other waterbodies will be dead, and the tubing and eDNA backpack itself is downstream of the next filter pack and will not contaminate it.

Field Trailer and Boat Procedures

Connecting trailer to the hitch:

Step 1:	Make sure that the latch on the coupler is released (the latch should be positioned vertically).	Add Picture
Step 2:	Pull the trailer into alignment with the hitch so that the coupler on the trailer is above and slightly beyond the ball of the hitch.	Add Picture
Step 3:	Lower the front of the trailer by cranking the wheel upward. The coupler will move down and around the ball of the hitch.	Add Picture
Step 4:	After the coupler is seated, flip the latch downward so that it is horizontal and lock it with the padlock.	Add Picture
Step 5:	Take the chains and hooks connected to the trailer and connect them to the truck. One chain should cross over the other chain.	Add Picture

Step 6:	Plug the trailer's power cable into the back of the truck, and turn on the truck's hazard lights. Check that you can see the hazard lights blinking on the back of the boat.	Add Picture

Launching the boat:

1. Put everything needed from the truck into the boat.
2. Put the boat's plug in so that the boat doesn't fill with water when released.
3. Release the winch and let out enough length of the strap to unclip the boat. Unclip the boat from the trailer
4. Remove the strap from the back of the boat, unclipping it from the trailer. Put the strap into the boat.
5. Back the truck and trailer up until it is a few feet from the water's edge. Put on the parking brake.
6. Remove the trailer's power cable from the port on the back of the truck, and wrap it around the trailer. There’s potential for the trailer lights to crack or break if they come into contact with sufficiently cold water.
7. Ensure that your colleague has grabbed the bowline and walked onto the dock, or aside from the boat.
8. Re-enter the truck and back up slowly until the wheel wells of the trailer are mostly in the water, and the boat floats off the trailer.
9. Once the boat is free of the trailer and somewhat away from it, drive the truck forward and away from the boat ramp.
10. To power the boat, plug in the key inside the left side panel near the back of the boat, and give it a quarter turn.
11. Lower the engine into the water using the buttons on the side of the steering handle until the water intake is under the water line (it can go lower than this also).
12. Put the boat key in the ignition, pump the ball connected to the fuel hose, and turn the ignition key to start the engine.
13. Release the latch behind the steering handle (near the engine and the edge of the boat) so that the engine can move back and forth relatively easily.
14. Let the engine sit idling for a couple of minutes to warm up.
15. When ready, shift into forward (or backward) gear and move away from the dock.

Trailering the Boat:

1. Turn off the boat engine and raise the motor out of the water using the buttons on the side of that steering handle. Lock the engine in place using the latch behind the steering handle near the engine.
2. Back the trailer into the water until most of the wheel well is in the water
3. Grab the bowline of the boat and from the dock (or in the water near the trailer) maneuver the boat so that it is centered with the trailer as much as possible.
4. Pull the boat as far onto the trailer as possible with the bowline, as centered as possible, then release the strap using the winch at the front of the trailer. Hook the strap onto the boat and use the winch until the boat is on the trailer.
5. If the back end of the boat is not centered to the back of the trailer it can be pulled sideways until centered.
6. Hook the loose chain from the trailer into one of the holes on the bow of the boat
7. Pull forward with the truck until out of the water.
8. Attach the power cable from the trailer back to the truck.
9. Pull the plug on the boat and allow it to drain before pulling away from the ramp
10. Check the hazard lights on the boat
11. Drive away from the boat ramp and park to perform the decontamination steps.

References

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