COLORADO DEPARTMENT OF NATURAL RESOURCES

COLORADO PARKS AND WILDLIFE

State Invasive Species Program

Aquatic Nuisance Species Sampling and Monitoring Protocol

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Introduction

Colorado Parks and Wildlife (CPW) is the lead agency for statewide aquatic nuisance species (ANS) sampling and monitoring in Colorado, in cooperation with various federal, state, and local governments, non-governmental organizations, and private entities.

ANS sampling formally began in 2004. It was focused on collecting baseline data on all species present at survey locations while specifically checking for over 35 animal and plant invasive species. Following the initial discovery of zebra mussels in Colorado in January 2008, the ANS sampling and monitoring program was redesigned to focus on zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) early detection.

The Colorado ANS Sampling and Monitoring Protocol Manual (Manual) describes standard procedures for zebra and quagga mussel early detection, as well as procedures for other high priority ANS and baseline data surveys. The Manual combines protocols developed by CPW, U.S. Bureau of Reclamation (Reclamation), and Pisces Molecular. In addition, the Manual incorporates recommendations provided by the *Colorado Blue Ribbon Panel Report on ANS Sampling and Monitoring* (February 2009) and the *Interagency Dreissena Monitoring Plan for Western Waters* (100th Meridian Initiative, May 2009).

Sampling and monitoring play a critical role in ANS management. Regular sampling is necessary to detect the initial introduction of an invasive species into a water body. Early detection results in greater potential for containment or eradication, ultimately minimizing negative impacts and financial burden from ANS. Post-detection monitoring provides insight into ANS population persistence and how the water body's ecosystem changes as a result of the invasion. Furthermore, monitoring provides information on impacts to water infrastructure, recreation, and other human uses of the site. For most ANS, there are currently few, if any, effective control measures. Monitoring programs can contribute data to research and development projects focused on the effectiveness of new control strategies. Sampling and monitoring are integral to understanding the size and nature of an ANS infestation, and are valuable tools when planning the future of a site.

CPW encourages our partners to assist with ANS sampling and monitoring on their waters. Colorado state regulations (#800-807) require all persons conducting sampling for ANS, including zebra and quagga mussels, to be permitted by CPW. Due to the prohibited nature of ANS, the Special Collections Permit for wildlife does not apply. Any entity intending to sample for ANS must submit a written request to the state Invasive Species Program. The permit request must then be approved by the Director of CPW.

This Manual will help ensure that all ANS sampling and monitoring performed in Colorado is consistent and based in sound science. All permitted entities, including CPW employees, must follow the protocols detailed in this Manual for sample collection and preservation, data documentation, and equipment decontamination.

Aquatic Nuisance Species Survey Protocols

Zebra and Quagga Mussels

The zebra and quagga mussel sampling protocol is used for both early detection and long-term monitoring. The protocol includes sampling techniques aimed at three stages of the mussels' life cycle: veliger, settler, and adult.

Mussel veligers (i.e. free-floating microscopic larvae) are sampled via plankton tows. Plankton tow samples are analyzed for veligers using both visual and molecular techniques. Settlers (i.e. juveniles) and adult mussels are sampled via substrate samplers and other direct observations such as shoreline surveys, SCUBA diving, etc. Note: Dive survey protocols may be found in the CPW Invasive Species Program manual "CPW SCUBA Diving Procedures for ANS Surveys". Secondarily to mussel sampling, various types of water quality data (e.g. profile readings recorded in the field, water chemistry samples for laboratory analysis, etc.) are collected to provide insight into aquatic habitat conditions.

As of **February 1, 2014**, CPW has adopted the Western Regional Panel's Building Consensus guidelines for classifying water bodies for zebra or quagga mussels:

- *Not sampled or Unknown* – Waters that have not been sampled for Dreissenid mussels.
- *Negative* – Sampled waters with no evidence of Dreissenid mussel presence, or has met the timelines for de-listing.
- *Inconclusive* – Waters which have not met the minimum criteria for detection (eDNA).
    - There are no waters inconclusive for quagga or zebra mussels in Colorado.
- *Suspect* – Waters which has met the minimum criteria for detection once.
    - There are no waters suspect for quagga or zebra mussels in Colorado.
- *Positive* –Waters that meet the minimum criteria for detection more than once.
    - Pueblo Reservoir is the only positive water for quagga mussels in Colorado.
    - There are no waters positive for zebra mussels in Colorado.
- *Infested* – Water which has a reproducing and recruiting established populations.
    - There are no infested waters in Colorado.

Minimum Criteria for Detection

In order to declare a water body *Suspect or Positive* for zebra or quagga mussels, Colorado state regulation (#806 D) requires that veligers be identified through the testing process described under "Multi-Phase Identification of Zebra/Quagga Veligers", below. Mussel settlers or adults must be positively identified by two mollusk experts.

Multi-Phase Identification of Zebra/Quagga Veligers

(e.g. Minimum Criteria for Detection)

Per Colorado state regulations, plankton samples must be analyzed for *Dreissenid* veligers using the following multi-phase testing process.

1. Visual identification using cross-polarized light microscopy.
2. If visual analysis detects one or more positive or suspect veligers, the sample must then undergo molecular analysis via polymerase chain reaction (PCR).
3. If the PCR analysis is positive for zebra or quagga mussel DNA, then the sample must undergo gene sequencing.

All three tests must be positive on the same sample in order for one sample, and water body, to be declared *Suspect* for zebra or quagga mussels. If more than one sample meets the multi-phase identification, the water body will be declared *Positive* for zebra or quagga mussels.

Every plankton tow sample collected in Colorado (either by CPW or our partners) for *Dreissenid* veliger cross polarized light microscopy analysis must be sent to the CPW Aquatic Animal Health Lab at 122 East Edison Street, Brush, CO 80723. As of 2014, CPW also sends split samples from waters that have previously had a detection of zebra or quagga mussels to the Montana Fish, Wildlife and Parks' Aquatic Invasive Species Lab for cross-polarized light microscopy, as part of the Missouri River Basin Team.

De-Listing Timelines for Detected Waters

- *Inconclusive* – 1 year of negative testing including at least one sample taken in the same month of subsequent year as the positive sample (accounting for seasonal environment variability) to get to undetected/negative.
- *Suspect* – 3 years of negative testing to get to undetected/negative.
- *Positive* – 5 years of negative testing to get to undetected/negative.
- *Infested* – Following a successful eradication or extirpation event including a minimum of 5 years post-event testing/monitoring with negative results.

History of Zebra and Quagga Mussel Detections in Colorado

- Pueblo Reservoir State Park tested positive for one zebra mussel veliger in 2007 and for quagga mussel veligers in 2008, 2009 and 2011.
- Granby Reservoir, Grand Lake, Shadow Mountain Reservoir, Willow Creek Reservoir, Tarryall Reservoir and Jumbo Reservoir all tested positive for one quagga mussel veliger in 2008. Grand Lake also tested positive for a zebra mussel veliger in 2008. There have been no verified detections at any of these waters since.
- Blue Mesa Reservoir tested positive for quagga mussel eDNA in 2009, 2011 and 2012 by the U.S.Bureau of Reclamation. There was no detection from 2013-2015.

Sampling Frequency

Sampling frequency was originally based on a risk assessment completed in June 2008 (Appendix A). The risk assessment was updated by Invasive Species Program Staff in 2014 based on risk of introduction by recreational watercraft (only for reservoirs with existing WID stations) and an in depth habitat analysis conducted by the Colorado Department of Health and the Environment titled, *Suitability of Colorado Lakes as Habitat for Invasive Mussels*. Sampling frequency now includes 8 very high risk, 8 high risk, 9 medium risk, 4 low risk and 15 very low risk waters. The 114 waters not analyzed in 2013 were assessed and adjusted in 2016 by the ANS Program. Sampling frequency may be altered by the Invasive Species Coordinator based on current priorities or needs, staff, and funding.

- Waters that are *Positive, Suspect or Inconclusive* for zebra/quagga mussels = Lake Pueblo
    - Plankton tows:

      Each water body must be visited every 3 weeks when water temperatures are above 10°C, i.e. when mussel spawning is most likely to occur. However, there is some research indicating quagga mussels are capable of spawning at water temperatures near 5°C (Roe and MacIsaac 1997).

      On each visit, collect 5 tows at each pre-set sampling location. All tows from the same location may be combined into a single sample bottle. Refer to the plankton tow protocol on page 7.

      FlowCam samples In addition to the zebra/ quagga veliger tows, collect one additional tow, twice a year (May and September) for *De-Listed, Positive and Suspect* from each plankton sampling site for fluid imaging analysis (i.e. FlowCam). ***Also collect one additional tow at each tow site on all other water bodies once for 2016***. Refer to FlowCam plankton tow procedure on page 11.

      Dam outlet samples (*Positive* and *Suspect* waters only): Establish a plankton sampling location below the dam near the outlet. Collect plankton tows from this site each time the reservoir is visited.

    - Substrate samplers:

      Substrates are deployed at the same sampling locations where plankton tows are collected (i.e. 4 or more substrate samplers). Check all substrates on every visit to the water body. Refer to the substrate protocol on page 12.

    - Shoreline surveys:

      Conduct 2 shoreline surveys each time you visit the reservoir. Refer to the shoreline survey protocol on page 15. Note: Presence of adult zebra/quagga mussels on shorelines indicates an advanced population. If adults or settlers are found, you must call Elizabeth immediately.

    - Water quality profiles (positive, suspect or inconclusive continued):

      Collect a water quality profile at the deepest sampling location where plankton tows are collected, on every visit to the water body. Refer to the water quality sampling protocol on page 34.

- Waters that are *Very High Risk or De-Listed* for zebra/quagga mussels
    - Plankton tows:

Each water body must be visited at least every 4 weeks when water temperatures are above 10°C.

On each visit, collect 5 tows at each pre-set sampling location. All tows from the same location may be combined into a single sample bottle. Refer to the plankton tow protocol on page 7.

- Substrate samplers:

  Substrates are deployed at the same sampling locations where plankton tows are collected (i.e. 4 or more substrate samplers). Check all substrates on every visit to the water body.

- Shoreline surveys:

  Conduct 2 shoreline surveys each time you visit the reservoir. Refer to the shoreline survey protocol on page 15. Note: Presence of adult zebra/quagga mussels on shorelines indicates an advanced population. If adults or settlers are found, you must call Elizabeth immediately.

- Water quality profiles:

  Collect a water quality profile at the deepest sampling location where plankton tows are collected, on every visit to the water body.

- Waters that are *High Risk* for zebra/quagga mussels
  - Plankton tows:

    Each water body must be visited at least every 5 weeks when water temperatures are above 10°C.

    On each visit, collect 5 tows at each pre-set sampling location. All tows from the same location may be combined into a single sample bottle. Refer to the plankton tow protocol on page 7.

  - Substrate samplers:

    Substrates are deployed at the same sampling locations where plankton tows are collected (i.e. 4 or more substrate samplers). Check all substrates on every visit to the water body.

  - Shoreline surveys:

    Conduct 2 shoreline surveys each time you visit the reservoir. Refer to the shoreline survey protocol on page 15. Note: Presence of adult zebra/quagga mussels on shorelines indicates an advanced population. If adults or settlers are found, you must call Elizabeth immediately.

  - Water quality profiles:

    Collect a water quality profile at the deepest sampling location where plankton tows are collected, on every visit to the water body.

- Waters that are *Medium* risk for zebra/quagga mussels
  - Plankton tows:

Each water body must be visited at least twice per year when water temperatures are above 10°C.

On each visit, collect 5 tows at each pre-set sampling location. All tows from the same location may be combined into a single sample bottle. Refer to the plankton tow protocol on page 7.

- Substrate samplers:

  Substrates are not deployed at *Medium* risk waters.

- Shoreline surveys:

  Conduct 2 shoreline surveys each time you visit the reservoir. Refer to the shoreline survey protocol on page 15. Note: Presence of adult zebra/quagga mussels on shorelines indicates an advanced population. If adults or settlers are found, you must call Elizabeth immediately.

- Water quality profiles (medium risk continued):

  Collect a water quality profile at the deepest sampling location where plankton tows are collected, on every visit to the water body.

- Water bodies that are *Low* risk for zebra/quagga mussels
  - Plankton tows:

    Low risk waters are sampled based on available resources. The goal of the Invasive Species Program is to sample all *Low* risk waters at least once per year and to collect 5 tows at each pre-set sampling location. All tows from the same location may be combined into a single sample bottle. Refer to the plankton tow protocol on page 7.

    Note: Plankton tow samples are collected at *Low* risk waters that have no boating or only non-motorized boating. This is due to the connectivity of Colorado waters and/or agreements with our partners.

  - Substrate samplers:

    Substrates are not deployed at *Low* risk waters.

  - Shoreline surveys:

    Conduct 2 shoreline surveys each time you visit the reservoir. Refer to the shoreline survey protocol on page 15. Note: Presence of adult zebra/quagga mussels on shorelines indicates an advanced population. If adults or settlers are found, you must call Elizabeth immediately.

  - Water quality profiles:

    Collect a water quality profile at the deepest sampling location where plankton tows are collected, on every visit to the water body. If there is no boat launch or dock, and you are conducting a horizontal plankton tow from the shore, then wade out into the water to collect the water quality profile. In water that is less than 1 meter deep, collect one profile reading just under the surface.

Plankton Tows

This plankton tow procedure is designed to collect zebra and quagga mussel veligers. The plankton tow net must have a mesh pore size of 64-65µm.

For each *De-Listed*, *Positive* or *Suspect* water body, label one plankton tow net to be used exclusively at that water body. Do not use the net at any other water!

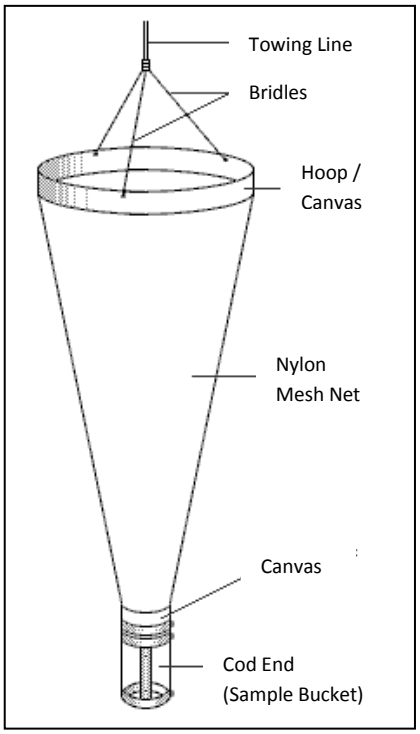


Diagram of a standard plankton tow net. ("Plankton Sample Collection Protocols for Dreissenid Veliger Early Detection Monitoring", 100th Meridian Initiative, 2009)

Sampling Locations

If plankton tows were collected at the water body in previous years, re-sample the established site(s). If plankton tows have not been previously collected at the water body, first refer to the sampling frequency on pp. 4-6 and then choose appropriate locations according to the guidelines below.

- Use maps to locate potential sampling sites. Area maps give you an overview of the water body and public access points. Bathometric maps show depths and the location of the river current through the water body.
- Sample in areas that may pose a high risk for mussel introductions, such as inlets and boat access areas (e.g. ramps, docks, marinas, etc.). Statistical analysis notes that the probability of veliger detection at "marina" sites is ~72%, compared to ~52% at "non-marina" sites ("Probability of Veliger Detection", Zehfuss, 4/12/2010).
- Sample in areas where water is being pulled out of the lake/reservoir, such as near dam outlets, and areas where the usual wind patterns push water to the shoreline (i.e. downstream and downwind). Mussel veligers are commonly pulled toward these areas.
- Conduct tows in both open water and near shore. Try to select the number and location of sites in such a way to represent the entire water body.

Before Going Out to the Field

- Make sure you are familiar with the zebra/quagga mussel sampling requirements, frequency, locations, etc. for the water bodies that you will be visiting. Review historic data and notes (e.g. the ANS sampling database, AAHL records, GIS records, reports, field notes, and photos). Save the coordinates for existing sampling sites in your GPS unit to help you navigate to those sites.

- Review maps to become familiar with the water body, public access points, restricted areas, and land ownership boundaries.
- Talk to Invasive Species Program and AAHL personnel, area biologists, and site staff to get current information on access and conditions at the water body.
- Check to be sure you have all needed field equipment and supplies (Appendix C). Inspect equipment to be sure it is in proper working order (e.g. make sure plankton net and cod end screens do not have tears, be sure you have all boat safety equipment, etc.)

Vertical Plankton Tow Procedure

1. Anchor your boat at the sampling site and make sure the boat is not drifting. Examine the tow net to be sure the cod end is secure and the towing line is ready to be deployed.
2. Calculate the appropriate tow length.
    a. Determine the water depth (in meters) using your watercraft depth finder or water quality profile meter (e.g. hydrolab, YSI, In-Situ).
    b. Subtract 0.5 meter from the depth. This prevents you from hitting the bottom with the tow net and capturing too much sediment in the plankton sample. A large amount of sediment interferes with sample analysis.
    c. Finally, subtract the length of the tow net to determine how much line to deploy. (You want the cod end to be 0.5 meter off the bottom. The tow line usually measures from the net opening, not from the cod end).
3. Lower the tow net to the calculated depth (Tip: If air pockets occur, lower the net slowly with the opening tilted to the side). As previously mentioned, be careful not to hit bottom with the net. If you hit bottom, raise the net, thoroughly clean out the net and cod end in the lake, and repeat the tow. Keep the net submerged at the calculated depth for 30 seconds.
4. Slowly pull up the net using a hand-over-hand technique, no faster than 0.5 meter per second. Raise the net until the opening is out of the water but the cod end is still submerged. Keeping the opening out of the water, pulse the net up and down several times as you finish pulling it up – this flushes the plankton into the cod end.
5. Carefully unscrew the cod end from the net without spilling any of the collected sample. You can concentrate the plankton sample by gently swirling the cod end and allowing some of the water to drain out through the mesh. Pour the plankton sample into a leak-proof polyethylene bottle (e.g. Nalgene). Thoroughly rinse the cod end several times using a spray bottle filled with distilled water. Pour the rinse water from the cod end into the sample bottle. Be sure to rinse out the cod end after each tow.
6. Repeat the vertical tow procedure in order to collect replicate samples at the same location (refer to the sampling frequency on pp. 4-6). All zebra/quagga veliger tows from the same sampling site may be composited into a single bottle.
7. Follow the procedure for processing a plankton sample on page 11.
8. Record a GPS point at the tow location. Enter all data into the Trimble GPS data dictionary and complete a Plankton Tow paper datasheet (Appendix B). Always record all data in the field while

you are collecting the plankton tow. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

Oblique/Horizontal Plankton Tow Procedure

Vertical plankton tows are preferred because they sample the entire water column. Oblique or horizontal tows should only be done in areas that are not deep enough to conduct an effective vertical tow (e.g. less than 4 meters deep) or in moving water. In moving water, conduct a horizontal tow if the water is deep enough and wide enough to not damage the net. Otherwise, hold the net beneath the surface and allow the water to flow through it. Record the amount of time that you held the net in the water.

1. If you are conducting the tow from a boat, anchor/secure the boat at the sampling site and make sure the boat is not drifting. If conducting the tow from a stationary position (e.g. dock, shore), make certain that you have stable footing. Examine the tow net to be sure the cod end is secure and the towing line is ready to be deployed.
2. Hold the net by the hoop (i.e. the metal ring that holds the net mouth open) using thumb and forefinger. Make large loops with the line and hold it loosely in the same hand that is holding the net. Firmly grasp the other end of the line with your free hand.
1. Throw the net using a sidearm-style, opening your hand upon release to allow the line to feed out with the net.
2. Allow the net to sink into the water to within 0.5-1 meter of the bottom. Your goal is to pull the net on a diagonal path from bottom to top, sampling as much of the water column as possible without letting the net or cod end hit the bottom. If an air bubble gets trapped in the net, retrieve the net and start again.
3. Slowly pull in the net using a hand-over-hand technique, no faster than 0.5 meter per second. Keep the net and cod end off the bottom to avoid both collecting debris and snagging the net. If you hit bottom, carefully pull in the net, thoroughly clean out the net and cod end in the lake, and repeat the tow.
4. Raise the net until the opening is out of the water but the cod end is still submerged. Keeping the opening out of the water, pulse the net up and down several times as you finish pulling it up – this flushes the plankton into the cod end.
5. Carefully unscrew the cod end from the net without spilling any of the collected sample. You can concentrate the plankton sample by gently swirling the cod end and allowing some of the water to drain out through the mesh. Pour the plankton sample into a leak-proof polyethylene bottle (e.g. Nalgene). Thoroughly rinse the cod end several times using a spray bottle filled with distilled water. Pour the rinse water from the cod end into the sample bottle. Be sure to rinse out the cod end after each tow.
6. Repeat the oblique/horizontal tow procedure in order to collect replicate samples at the same location (refer to the sampling frequency on pp. 4-6). All zebra/quagga veliger tows from the same sampling site may be composited into a single bottle.
7. Follow the procedure for processing a plankton sample on page 11.

8. Record a GPS point at the tow location. Enter all data into the Trimble GPS data dictionary and complete a Plankton Tow paper datasheet (Appendix B). Always record all data in the field while you are collecting the plankton tow. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

FlowCam Sample Procedure

1. At *De-Listed, Positive* and *Suspect* waters, collect one additional plankton tow from each site, twice a year (May and September), where you collect zebra/quagga veliger tows. This tow will be analyzed using fluid imaging (i.e. FlowCam). Note: You do not need to collect a FlowCam sample from the sampling location below the dam outlet.
2. ***Also collect one additional tow at each tow site on all other water bodies as indicated on the crew schedules.***
3. The FlowCam tow should be the same type (vertical or oblique/horizontal) and length as the zebra/quagga veliger tows that you collect from the sampling location.
4. Do not composite the FlowCam tow with the zebra/quagga veliger tows – pour it into its own sample bottle. Be sure to clearly label the bottle as "FlowCam". Follow the procedure for processing a plankton sample on page 11.
5. Record a GPS point for the FlowCam plankton sample. Enter all data into the Trimble GPS data dictionary and note "FlowCam" in the comments. Complete a Plankton Tow "FlowCam" paper datasheet (Appendix B). Always record all data in the field while you are collecting the plankton tow. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

Plankton Sample Processing (not FlowCAM)

After you have finished conducting all plankton tows at the sampling site and have combined samples into a bottle, then process the composite sample as described below.

1. On each bottle, mark the water level with permanent ink. Add an appropriate volume of ethanol to get a 25% EtOH final solution. Visual estimate does not have to be exact. Mark the level of added ethanol on the sample bottle with permanent ink.
2. Add a pinch of baking soda to each sample bottle to buffer the pH – This helps prevent degradation of the veligers' calcium carbonate shells ("Effects of pH, Temperature, Ethanol Concentration, and Buffer on Persistence of Preserved Quagga Mussel Veliger Shells", Meara, Hosler, et.al., 2010).

3. Label the outside of all sample bottles with the water body name, sample location name and coordinates, CPW Unique ID, date, collector's name/initials, tow data (e.g. vertical or horizontal, number of tows, and length), and the % EtOH.
4. Secure the bottle cap with electrical tape to help prevent leakage. Place the bottle in a resealable plastic bag (e.g. Ziploc). Package all sample bottles in a cooler with ice packs and keep refrigerated.
5. After you have proofed and corrected your field data, make copies of the paper datasheets. Fill out an Aquatic Specimen Collection Report (Appendix B) listing the samples. The report and copied datasheets are sent to the lab with the samples.
    - Use one Aquatic Specimen Collection Report per water body (occasionally you may need more than one page to list all the plankton samples from a particular water).
    - If a composite plankton sample filled more than one bottle (i.e. both bottles have the same CPW Unique ID), record the data on one row of the report and note that there are 2 bottles.
    - For split samples, fill out one report for each lab. For AAHL, you may simply record the CPW Unique ID for the sampling site description.

    Refer to the "Shipping" section of the Specimen Collection Protocols (Appendix D) for instructions on mailing plankton samples.

Substrate Samplers

Substrates samplers are deployed at various locations within a water body and monitored for presence/ absence of attached settlers or adult zebra/quagga mussels. Refer to the sampling frequency on pp. 4-6.

Sampling Locations

In general, deploy substrate samplers at the same locations where plankton tows are collected. If substrate samplers were deployed at the water body in previous years, re-sample the established site(s). If plankton tows and substrate sampling have not been previously conducted at the water body, first refer to the sampling frequency on pp. 4-6 and then the sampling location guidelines for plankton tows on page 7. Deploy substrates as described below.

- Deploy substrate samplers in areas that may pose a high risk for mussel introductions (e.g. marinas, boat ramps, etc.). At these types of sites, you can generally tie the substrate directly to the dock.
- Deploy substrate samplers in areas where mussel veligers are most likely to settle out of the water column (e.g. areas where water is being pulled out of the lake/reservoir, such as near dam outlets, and areas where the usual wind patterns push water to the shoreline). These locations are usually in open water locations where the substrate must be tied to a buoy.
- Obtain permission from the water body manager before deploying substrate samplers. Do your best to place substrates so that they are out of the way of boat propellers, jet skis, fishing lines, etc. Do not attach substrates to existing navigational or warning buoys.

Substrate Sampler Construction

Substrate samplers are constructed of dark polypropylene rope and dark plastic objects (e.g. PVC or ABS pipe, tile, mesh, etc.) that form an artificial substrate for potential mussel attachment. Note: It is suggested to use dark-colored materials instead of light-colored materials because it is believed that mussels tend to avoid light. The substrate sampler is anchored with weights to the bottom of the water body and attached at the water's surface to a buoy or other structure (e.g. tied to a dock).

Substrate samplers are assembled in the field based on the water depth at the sampling site. Before you go into the field, check to be sure that you have all needed substrate materials (Appendix C). **Always keep substrate materials on your boat in case you need to replace or repair substrate samplers.**

Substrate Deployment Procedure

1. Assemble the substrate sampler.
    a. Use a concrete anchor, or build an anchor by wrapping large rocks with chicken wire. Secure the chicken wire with zip ties.
    b. Securely tie the rope to the anchor. Use your boat's depth finder to estimate how much rope you need to deploy. **If you expect the water level to rise, be sure to add extra rope so the buoy will remain on the surface. Add a bullet weight about ¼ of the way down from the buoy so the slack of the rope will stay under water.**
    c. In general, attach one substrate medium (e.g. PVC pipe) in approximately the lower third of the rope's length. For deeper sites, you can add multiple pieces of substrate media. Tie each object securely to the rope or attach with zip ties.
    d. Securely tie the rope at the water's surface to the buoy or infrastructure. At the top of the rope, use zip ties to attach a tag labeled "CPW Mussel Trap. Please Do Not Disturb". Write the water body name, the CPW Unique ID, and the ANS hotline phone number (303-293-6531) on all substrate tags. If using a buoy, label the buoy with a CPW decal.
2. Record a GPS point at the substrate deployment location. Enter all data into the Trimble GPS data dictionary and complete the deployment section of a Substrate paper datasheet (Appendix B). Always record all data in the field while you are deploying the substrate. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet in a binder that you keep with you while sampling, so that you can record substrate check data throughout the field season.

Substrate Check Procedure

Check all substrate samplers every time you visit the water body.

1. Use your GPS unit as needed to navigate back to the substrate sampler's location. If you cannot relocate the substrate, search the area to see if it has drifted.
2. Wear gloves and carefully pull the substrate sampler out of the water. Visually examine all parts of the substrate, including the rope and all surfaces of the substrate media. Use a magnifying glass if one is available. Feel for sandpaper-like bumps that do not brush off easily (i.e. recently settled mussels). Do not clean off the substrate; mussels prefer to settle on materials with biofilm.
3. If you find suspect ANS on the substrate sampler, collect a specimen. To collect a specimen, remove the entire substrate medium (e.g. PVC pipe and/or rope) from the sampler and place it into a sample bottle. Preserve the specimen with 70% ethanol. Be sure to properly label the bottle. Refer to the Specimen Collection protocols (Appendix D) for complete instructions. Note: You do not have to record an ANS Point in your GPS unit for suspicious bumps on substrate samplers. The GPS location of the substrate check will be recorded with notes, and if the lab determines the sample is positive, the GIS and database records will be updated at that time.

4. If you collect a specimen, attach a new piece of substrate medium to the rope. **If you discover that a substrate is missing or damaged, then replace or repair the substrate.**

5. Record a GPS point for the substrate check. If the substrate sampler has drifted from its deployment location, record the point at the substrate's current location and then move it back to where it was deployed. Enter all data into the Trimble GPS data dictionary. On the paper datasheet corresponding to the substrate, complete one line under "Substrate Checks". Record all data for each substrate check, regardless of whether or not you collect specimens, to provide presence/absence data. Always record all data in the field while you are checking the substrate.

   At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data.

6. After examining the substrate and recording data, replace the substrate back into the water.

Substrate samplers remain deployed for the entire sampling season (typically from about May through November). Most substrate samplers are removed from the water body at the end of the sampling season and brought back to the CPW Invasive Species Program. At some waters, if the substrate sampler is tied to a permanent dock that remains in the water year-round, then the substrate may be left in place over the winter. On the final check of the year, remember to record that the substrate will remain deployed (Note: Substrate samplers that remain deployed over the winter retain their CPW Unique ID from year to year.)

Shoreline or Stream Surveys

Qualitative surveys of shorelines (lentic systems, e.g. lakes, reservoirs, ponds) and streams (lotic systems, e.g. rivers, creeks, ditches) are used for several purposes:

1. To gather baseline data on species presence or absence in aquatic environments.
2. To locate populations of ANS including mollusks, crayfish, and plants.
3. To monitor known populations of ANS. (Note: Quantitative protocols may also be implemented to monitor specific ANS populations.)

Sampling Frequency

Refer to the zebra/quagga mussel sampling frequency on p. 4-6. Additional shoreline or stream surveys may be undertaken as directed by Invasive Species Program supervisors, based on current priorities, staff, and funding.

Sampling Locations

The goal of shoreline/stream surveys is to examine as much of the waterline as possible. In a given year, it is generally better to try to survey more areas at the water body, rather than to survey the same location on every visit. Repeat surveys of these locations will be conducted in future years to obtain long-term data.

If shoreline/stream surveys were conducted at the water body in previous years, then re-survey the established site(s). If shoreline/stream surveys have not been previously conducted at the water body, or if you are surveying new areas of the water body, then choose appropriate locations according to the guidelines below.

- Use area maps (e.g. Colorado Atlas & Gazetteer, Forest Service maps, Bureau of Land Management maps, etc.) to locate potential survey areas. Maps give you an overview of the water body, surrounding terrain, public land boundaries, and site access. This can help you break large water bodies into manageable zones for surveying (i.e. zones should be large enough to be effective, but small enough to transect and accomplish your goals).
- Survey in areas that possess the habitat requirements of your target species.
- Focus surveys in areas that may pose a high risk for ANS introductions, such as areas of high recreational use (e.g. shoreline fishing areas, boat ramps, docks, marinas, hand-launch sites, river access points, day-use areas, campgrounds, etc.). However, you should also survey undisturbed areas in order to collect baseline presence/absence data for the water body.
- Surveys for zebra/quagga mussels should include areas where water is being pulled out of the lake/reservoir, such near dam outlets, and areas where the usual wind patterns push water to the shoreline (i.e. downstream and downwind). Mussel veligers are commonly pulled toward these

areas and may subsequently attach to the substrate. If possible and safe, examine rocks along the dam and near the outlet structures.

Before Going Out to the Field

- Make sure you are familiar with the sampling requirements, frequency, locations, etc. for the water bodies that you will be visiting. Review historic data and notes (e.g. the ANS sampling database, AAHL records, GIS records, reports, field notes, and photos). Save the coordinates for existing sampling sites in your GPS unit to help you navigate to those sites.
- Review maps to become familiar with the water body, public access points, restricted areas, and land ownership boundaries. It is illegal to collect specimens on protected areas and private land unless permission is granted beforehand. Most protected areas are well-marked. Many private lands are fenced or have "No Trespassing" signs, but it is the responsibility of the person accessing the water body to know public/private land boundaries. If you need to access private land, obtain permission in advance. The District Wildlife Manager (DWM) is often the appropriate person to contact private land owners.
- Talk to Invasive Species Program and AAHL personnel, area biologists, DWMs, and site staff to get current information on access and conditions at the water body. These people may also be able to provide you with information on known ANS populations or suggest areas where you should survey.
- Study the unique characteristics of the target species to learn/refresh your field identification skills, and review the species habitat requirements.
- Check to be sure you have all needed field equipment and supplies (Appendix C). Inspect equipment to be sure it is in proper working order.

Survey Technique

When surveying, employ the following techniques. Try to spend enough time surveying the area to compile a representative inventory of the aquatic species (both animal and plant).

Visually scan the ground and aquatic substrate for organisms: turn over rocks and logs, search crevices and undercut banks, inspect vegetation and roots, etc. Use equipment such as a dip net, wire mesh sieve, plant hook, and/or kick net to aid your search. Take photos and collect specimens as appropriate to document your observations (refer to the survey procedure below for detailed guidelines on collecting specimens).

When you pick up or roll over substrates to examine them, try to replace them back in their original position. In sensitive habitat (e.g. springs) or when handling sensitive organisms (e.g. amphibians), it is a good idea to avoid wearing chemicals such as sunscreen & insect repellent on your hands and forearms.

- Adult Mollusk Survey Techniques
    - Search for adult mollusks on solid substrates (e.g. rocks, logs, vegetation, roots, floating debris) in and adjacent to the water body. Note: Adult zebra/quagga mussels attach to

almost any available hard surface, natural or man-made. Look, feel with your hands, or scrape with a sieve in undercut banks and emergent vegetation. Carefully remove all specimens from substrates with forceps. Alternately, use a stiff-bristle nylon brush to gently sweep specimens off the substrate into a white pan containing about ½ inch of water. Search the pan (using a magnifying lens if available) and pick out all mollusks with forceps.

- In soft or silty substrates (e.g. sand and mud), search for mollusks by collecting scoops of substrate using a wire mesh sieve. Let the water drain from the substrate, then dump the contents of the sieve into a white pan. Search the pan (using a magnifying lens if available) and pick out all mollusks with forceps.

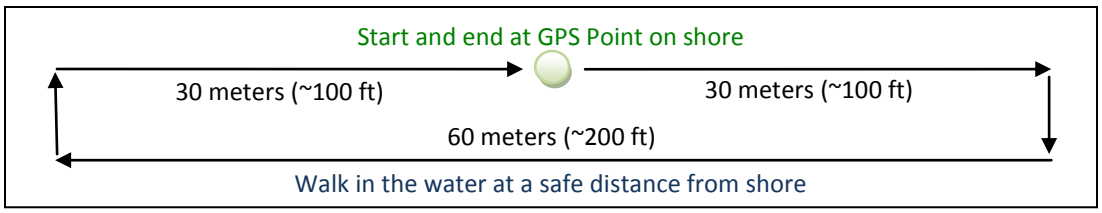
- Crayfish Survey Techniques
  - Search for crayfish in areas of slower flowing water. Crayfish can be captured by hand, with a long-handled dip net, or by using a kick net. Refer to the detailed crayfish survey techniques beginning on page 23.
- Aquatic Plant Collection
  - Collect aquatic plant specimens in accordance with the survey procedure below and the aquatic plant specimen collection protocol beginning on page 26.

Survey Procedure

1. Assess the area – Upon arriving at the water body, locate the appropriate public access for the survey sites. Scan the area and carefully evaluate the shore or stream bank for safety. Use common sense to consider factors such as bank steepness and stability, amount of vegetation, rocks, water depth, etc. For streams, survey only in areas where you can safely wade. High velocity and/or high volume currents are dangerous, particularly during spring runoff, and should be avoided. Do not enter streams if you have any safety concerns.

2. Conduct the survey – Begin by recording a GPS point at your starting location on the shore or stream bank. The GPS point should be in the center of the survey area and is representative of 30 meters (~100 ft) in both directions. After the point is stored in the GPS unit, survey along the shoreline or stream bank for approximately 30 meters. Walk a safe distance into the water and survey in the opposite direction, passing your GPS point and continuing for an additional 30 meters. Complete the survey by returning to the shore/bank and proceeding back to the GPS point where you began.

   The survey may be conducted by two (or more) people walking in opposite directions from the starting point and then meeting back in the middle. In complex habitat, it may be beneficial for a crew to proceed together through the survey area but divide up tasks: one person examines rocks, one person searches vegetation and woody debris, one person sifts sediment, etc. Alternately, one person could do the searching while one person records data and handles specimens.



3. Collect specimens as appropriate, or as directed by Invasive Species Program supervisors – In general, if a species has been previously documented at a site, then you probably do not need to collect it. However, be sure to collect any organism that you cannot 100% positively identify.

   If you detect a known or suspect ANS at a water body where it has not been previously documented, then it must be collected and sent for expert identification. All specimens are sent to AAHL unless specifically directed otherwise (e.g. plant specimens are sent to Elizabeth Brown). Remember, if you are unsure whether or not an organism is an ANS, err on the side of caution and collect it for identification. Notify the Invasive Species Program supervisors and AAHL of your observations as soon as possible.

   - When you detect a known ANS, finish the shoreline/stream survey and then follow the procedure for ANS Population Mapping on page 19.

   In addition, collect all Form 1 male crayfish. If you do not capture any Form I male crayfish, then you may preserve a selection of the crayfish that you did capture (Form 2 males, females, and/or juveniles). Collect all milfoil plants unless it is a known exotic or hybrid milfoil population. Collect a representative sample of the observed mollusk species, especially if it is the first time the location has been surveyed.

   Place specimens in a sample bottle and preserve with 70% ethanol (you may use the same sample bottle for all mollusk specimens from one survey location). Be sure to properly label the bottle. Refer to the Specimen Collection protocols (Appendix D) for complete instructions.

4. Record data – Enter all data into the Trimble GPS data dictionary and complete a Shoreline/ Stream Survey paper datasheet (Appendix B). Record all data for each survey, regardless of whether or not you collect specimens, to provide presence/absence data. Always record all data in the field while you are performing the survey. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

ANS Population Monitoring and Mapping

The ANS population monitoring and mapping protocol utilizes qualitative survey techniques (similar to the standard shoreline/stream survey) to search for organisms, with the goal of mapping the extent of the population. This protocol is used in the following circumstances.

- Detection of ANS during another type of survey.
    - Collect specimens and map the population to the best of your ability, with consideration for time constraints and your level of certainty regarding the identification (i.e. if your schedule is flexible and you are certain of your ID, then try to completely map the population). Follow the mapping procedure to collect an ANS Point or Polygon for the population, in addition to the point that you recorded for the original survey (you might have some members of your crew finish the original survey while others map the ANS population). Note: You do not have to record an ANS Point for "suspicious bumps" on zebra/quagga mussel substrate samplers.
    - As soon as possible, notify the Invasive Species Program and AAHL supervisors that you found an ANS and report on your survey/mapping activities.
    - If the specimen is confirmed to be ANS by expert identification, then more extensive mapping surveys will be scheduled to determine the full extent of the population. A different field crew or supplementary personnel may be used to complete these surveys.
- Monitoring a known ANS population.
    - Monitoring and mapping surveys of known ANS populations may be scheduled annually in addition to regular sampling activities. Furthermore, various quantitative studies may be conducted for specific ANS populations (e.g. density surveys, complete aquatic plant inventories, etc.). The frequency of these surveys will be determined by Invasive Species Program supervisors based on current priorities, staff, and funding.

Before Going Out to the Field

"Do your homework" in a similar manner as described for shoreline/stream surveys.

- Before visiting a known ANS population, review any applicable management plans and historic data for the site (e.g. the ANS sampling database, AAHL records, GIS records, reports, field notes, and photos). Save the site coordinates in your GPS unit to help you navigate back to the locations. Print previous population maps, annotated/labeled with relevant information and data, and take them with you into the field for reference. Maps are also used for hand-drawing the current extent of the population to create a paper backup for your GPS unit (or in the event that your GPS unit is not working).
- Review maps (e.g. Colorado Atlas & Gazetteer, Forest Service maps, Bureau of Land Management maps, etc.) to become familiar with the water body, public access points, and land

ownership boundaries. If you need to access private land, obtain permission in advance. The District Wildlife Manager (DWM) is often the appropriate person to contact private land owners.

- Talk to Invasive Species Program and AAHL personnel, area biologists, DWMs, and site staff to obtain current information on the ANS population, conditions at the sampling location, and access.
- Refresh your field identification skills for the target species and review its habitat requirements.
- Check to be sure you have all needed field equipment and supplies (Appendix C). Inspect equipment to be sure it is in proper working order.

Survey Procedure

In general, employ survey techniques similar to those used in the standard shoreline/stream survey to search for organisms: examine rocks and vegetation, check undercut banks, sift sandy substrate, etc. For some ANS, you might follow a species-specific survey protocol (e.g. crayfish trapping or aquatic plant inventory). The goal is to determine the complete extent of the population and map it as accurately as possible.

When monitoring a water body with known ANS populations, thoroughly survey all sites where ANS were previously recorded. Map the current extent of the population and record your observations at every site. Current data will be compared to previous surveys to determine if there is a change in population dimensions or density (larger or smaller). In addition, keep an eye open for any new populations at the water body. As time and priorities permit (or as directed by the Invasive Species Program supervisor), survey areas where ANS have not been found, but that may have a high risk for population spread or introduction (e.g. adjacent, downstream, downwind, or high recreational use).

Fine Mapping (FM) – Refers to spatial data collection by GPS unit. FM is preferred because it is the most accurate method of spatially defining a location. Data collected with a GPS unit is subsequently uploaded into mapping software such as ArcGIS.

Coarse Mapping (CM) – Refers to spatial data collection by hand-drawing the ANS population onto a printed map. CM may be used when a GPS signal is unavailable in a particular location, if your GPS unit is not working, or to create a paper backup of GPS data. Draw a point or polygon on the printed map and record field data on a paper datasheet. Later digitize the hand-drawn point/polygon into GIS and enter data into the attribute table.

Mapping Procedure

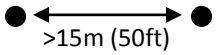
This population mapping procedure is based on state weed mapping standards (refer to the Colorado State Parks weed inventory protocol).

- Begin by searching the area to determine (as well as possible) the full extent of the ANS population. Take time to locate all ANS in the area to the best of your ability. Often it is helpful to mark the population boundaries in some way, such as with flagging. This will enable you to record an accurate point or polygon.

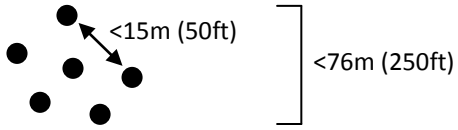
- Use the following rules to determine how to map the ANS occurrence.
    - Rule #1 – Populations are defined as being separated by 15 meters (50 ft) or more. Each population is mapped separately.
    - Rule #2 – If the area occupied by a population is less than 76 meters (250 ft) in diameter, then record an ANS Point. If the area occupied by a population is more than 76 meters (250 ft) in diameter, then record an ANS Polygon.

  For example:

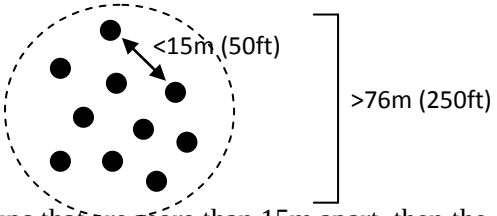
    - If you have two individuals that are more than 15m apart, then the individuals are considered two distinct populations and are mapped as two points.



    - If you have a group where all individuals are less than 15m apart, then the group is considered one population. Map the population as either one point or one polygon.
        - If the group is less than 76m in diameter, then it is mapped as a point.



        - If the group is more than 76m in diameter, then it is mapped as a polygon.



    - If you have two groups that are more than 15m apart, then the groups are considered two distinct populations and are mapped separately. Each group is mapped as a point or polygon based upon its diameter (e.g. two points, one point and one polygon, or two polygons).

- Map the population(s) as appropriate.
    - If you record an ANS Point, mark the coordinates at the exact location of the individual or as close to the center of the population as you can estimate. Record the observed population dimensions in the Trimble GPS data dictionary and/or on a paper datasheet.
    - If you record an ANS Polygon, mark the population perimeter as accurately as possible. When time constraints or field conditions (e.g. weather, deep water) prevent you from precisely mapping the entire population perimeter, then note in the comments that the polygon width may not be accurate. Note: If the population was previously recorded as a

point but has grown to more than 76 meters (250 ft) in diameter, then map the current extent of the population as a polygon.

  - To create a paper backup for your GPS unit, or if your GPS unit is not working, draw the population as accurately as possible onto a printed map of the area. Label and annotate the map in enough detail that someone else could find the location again.

- Collect specimens as appropriate, or as directed by Invasive Species Program supervisors. In general, you only need to collect specimens from newly detected ANS populations. If you are surveying a known ANS population that has been monitored/mapped in previous years, then you typically do not need to collect additional specimens.

  Place specimens in a sample bottle and preserve with 70% ethanol. Be sure to properly label the bottle. Refer to the Specimen Collection protocols (Appendix D) for complete instructions.

- Enter all data into the Trimble GPS data dictionary and complete an ANS Population Monitoring paper datasheet (Appendix B). Record all data for each survey, regardless of whether or not you collect specimens, to provide presence/absence data. Always record all data in the field while you are performing the survey. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

New Zealand Mudsnail Density Studies

The initial purpose of this density study is fully document the New Zealand Mud Snails (NZMS) population(s) including describing in detail the population extent, density, connectivity, and native or exotic competitors. The secondary purpose is to establish a baseline to begin long term population monitoring of NZMS populations in Colorado's reservoirs or streams/rivers. Each year this protocol should be repeated at positive waters where NZMS are found to keep a continuous record of establishment and population changes over time. The end goal is to gain a better understanding of NZMS reproduction rates, ecology and determination of the ecological and social impacts from invasion.

Protocol

Prepping and Equipment

To start, waders, bug spray, and sunscreen must be put on for comfort; you will be in the water for hours at a time. Water must also be carried to prevent dehydration. If a $0.0625m^2$ counting tote is not available, create one by cutting a $0.0625m^2$ square hole into the bottom of a standard size tote and use that for your counting area. Or create a plot using PVC pipe. You will also need white collection pans to place the snails for counting. A GPS will also be needed to collect coordinates and record counts. A write in the rain note book will also be helpful to keep tally and is good just in case it's dropped in the water.

Equipment Checklist

- $0.0625m^2$ quadrat
- White observation pans
- #14 (1.41mm) sieve*
- #20 (0.85mm) sieve
- 4 collection cups
- Forceps
- Ruler
- GPS unit and charger (may need solar charger and mifi also)

- Notebook and pencil
- Waders and boots
- Water, sunscreen, bug spray, hat, uniform

* A 1.41mm sieve is used to separate adults from juveniles because NZMS reach reproductive size around 3mm in length. A mudsnail that is 3mm in length has a width of ~ 1.5mm, so a 1.41mm mesh should capture adults and allow juveniles to wash through

Sampling Procedure

Locate the previous year's data and create field maps to help guide you and to serve as a back-up if the GPS unit malfunctions. Assuming that the population is not new and you have data from previous years that indicate the "start" and "stop" of the population, you will begin at the most upstream recorded location and work downstream. Public access points (primarily for anglers) should be checked upstream and downstream of the known population (outside of the start and stop locations) to ensure that NZMS haven't been moved greater distances by people within the same watershed.

Population Mapping

Goal 1 – Documenting the Full Extent of the NZMS Population:

The goal is to determine or to verify previously documented "start" and "stop" locations for the most upstream and downstream points of the population in a flowing water setting or the shoreline boundaries of the population in a reservoir setting..

Locate the most upstream point using your GPS unit (via coordinates) or map and navigate to 100m upstream from it. This will be your start point to conduct shoreline surveys. Follow the sampling manual's protocol for conducting shoreline surveys, including shoreline GPS points, and if you find NZMS here, continue to work downstream (stopping every 100m to survey) (you can move upstream to determine the new "start" after cleaning waders and gear, possibly another day).

Once NZMS are found, switch to the Sampling Manual's protocol for known ANS populations. Moving downstream, stop every 100m and take an ANS Point in the GPS unit. Document the presence or absence of NZMS and record data such as relative density. Repeat this process until the "stop" is found.

NOTE: Clean waders and all gear before moving outside the known population.

If you began finding NZMS further upstream than was previous documented, you must go back and find the new "start" of the population. Attempt to begin so far upstream there will be no NZMS and work your way into the known population. If you find NZMS upstream, and have to go farther upstream, you will have to clean your waders and gear in between each survey point to avoid moving NZMS to new locations outside the current population.

NOTE: Always be on the lookout for other ANS or invasive species! If encountered, stop mapping the NZMS and take the time to map the new invader. Text Elizabeth when you can.

Density Plots

Goal 2 - Setting New Density Plots for Long Term Population Monitoring:

The goal is to set plots evenly spaced throughout the known NZMS population in areas of high density, low density and areas that have no NZMS present.

Assuming that the population is not new and you have data from previous years that indicate the "start" and "stop" of the population, you will begin at the most upstream record location and work downstream. Once the NZMS are found, walk 3m into the water and place the quadrat all the way at the bottom. If it is too deep, walk back towards shore to where you can successfully reach to the bottom. On shore, tightly secure the #14 sieve on top of the #20 sieve.

Collect everything in the $0.0625m^2$ area and place in the #14 sieve. Submerge sieves in water so that the rim of sieve #14 is always above water. Sift out fine sediment and debris. Place the remaining contents of each sieve in separate white observation pans. Remove all NZMS from #14 sieve and place in separate cups corresponding to life stage (alive/dead). Snails from the #14 sieve will comprise our "adult" count. Next, remove all NZMS from #20 sieve and place in cups corresponding to life stage (alive/dead). Snails from the #20 sieve will comprise our "juvenile" count. Life stage should be determined by the color of the snail. All white snails are dead and can be found hollow. Live snails are dark brown or black. Some snails will be a mixture of dark and white (opaque) use your best judgment to determine whether a snail is dead or alive. Once all snails have been removed from the sieves and placed in assigned cups, designate two or three people to count and sort the snails. Start counting snails in the cups labeled dead first--that way the snails in the cups marked alive have time to sit still and move around, which will help you distinguish the live snails from the dead ones.

While some people are counting, others can record NZMS totals in the GPS and collect samples for AAHL and Pisces. Any other native or invasive mollusks or plants that are found within the 0.0625 m^2 area should be recorded in the comments section of the GPS point. Once complete, move downstream per the above protocol for Goal 1 and repeat the same procedure. Keep walking the shoreline or floating the river, repeating the procedure until NZMS are no longer found under the ANS protocol. Once the farthest downstream end of their distribution is found, clean your waders and boots and gear before entering uninfected waters.

Sampling Checklist for Setting Plots:

- Find start location from previous year's data.
- Walk out 3m and place tote in water; if too deep walk back towards shore and find reasonable depth.
- Stack #14 sieve on top of #20 sieve on shore.
- Collect everything in the 0.0625m^2 area and place in #14 sieve.
- Making sure both meshes are tightly connected, submerge in water and sift fine sediment out of sieves.
- Remove all mudsnails from sieves and place in cups corresponding to life stage.
- Count all NZMS and record totals in the GPS unit.
- Count all native snails and note any aquatic plants
- Record GPS coordinates and totals in the notebook or field data sheet as a back-up.
- Repeat procedure in sites within the known population that have high and low densities.
- Repeat procedure in sites within the known population that have no NZMS, if any.
- Repeat procedures (after full decontamination) in areas outside the known population with no NZMS for control.

Equipment Decontamination

Equipment decontamination is mandatory to avoid transporting ANS (animals, plants, and pathogens) between water bodies and sampling locations. All sampling equipment must be fully decontaminated in accordance with state protocol between each water body. In certain situations (e.g. mapping ANS populations, surveying in areas with New Zealand mudsnails, etc.) all equipment must be fully decontaminated between each sampling location so that ANS are not spread.

CPW ANS Staff are NOT permitted to use felt sole waders.

Keep all waders, boots, equipment and gear that come into contact with the water free of mud, plants, and organic debris in between each and every use. Unknowingly moving a species from one body of water to another, even within different stretches of the same river, can start a domino effect of invasion, causing irreversible ecological damage. It is especially important to keep waders clean.

FIRST - Staff MUST scrub the bottom of boots or waders with a brush and remove all mud, plants, and organic materials in between each and every use.

SECOND - Staff MUST also perform ONE of the following options before going into the next body of water:

OPTION 1
Submerge waders and gear in a large tub filled with a mixture of 6 ounces per gallon quaternary ammonia-based institutional cleaner (such as Super HDQ Neutral) and water for at least 10 minutes, scrubbing debris from the gear, and visually inspecting the gear for snails before rinsing. Follow all precautionary label instructions! Rinse water must be from a New Zealand mudsnail-free source (to avoid re-infection), and the chemical bath must be properly disposed of, away from the water body.

OPTION 2
Spray or soak waders and gear with 140º Fahrenheit water for at least 10 minutes.

OPTION 3
Dry your waders and equipment completely for a minimum of 10 days in between each use (remember that mudsnails can survive several days out of water).
NOTE: This option is not available to staff due to the high frequency of ANS work.

OPTION 4
Place waders and boots in a freezer overnight.
NOTE: This option is typically not available to staff due to the rustic overnight nature of ANS work.

Crayfish Survey Techniques

Fishing / Hand Capture

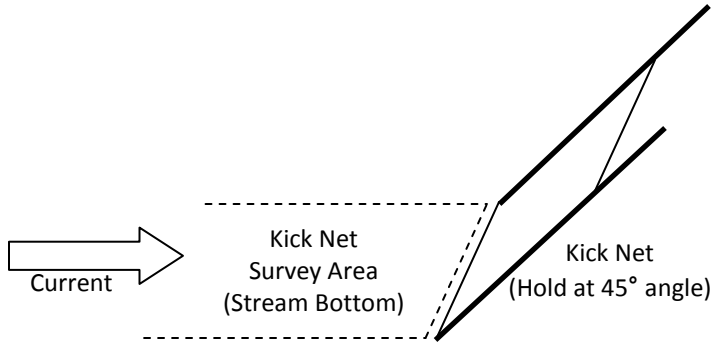
Crayfish "fishing" methods (hand capture, dip net, or baited line) may be used as survey techniques for shoreline/stream surveys or ANS population monitoring.

- Visually search for crayfish in areas of slower flowing water such as shallows, behind large rocks and logs, and along rocky dams.
- Capture crayfish by hand (grasp the thorax with your thumb and forefinger) or with a long-handled dip net. A fishing pole or string baited with a piece of meat can also be effective. However, a fishing pole must be attended and is not really necessary. In addition, if the bait is not pulled back in carefully, the crayfish will drop off before you can collect it.
- Although you can search for crayfish any time, it is often most effective at dusk when crayfish tend to be more active. You can shine a strong light into the water to help attract crayfish to the area. If you are searching for crayfish during the day when they may be more wary, a long-handled dip net is especially useful so that you don't have to sneak in so close to capture them.
- Collect specimens as appropriate, or as directed by Invasive Species Program supervisors (refer to the crayfish specimen guidelines, below).
- Record all data.
- If you are using a net or other equipment, clean and decontaminate it before using it again.

Kick Net

A kick net may be used as a survey technique for shoreline/stream surveys or ANS population monitoring. This procedure describes standard kick net protocol (refer to RiverWatch or the US EPA "Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers"). Use a 1x1 meter square kick net with 500 µm mesh.

- Sampling with a kick net should take place in stream riffle areas with moderate water flow (i.e. moving current but still safely wadeable) and rock cobble substrate. These areas provide good habitat with plenty of oxygen for benthic invertebrates.
- Kick nets require two people: one person to hold the net and a second person to flush invertebrates.
    1. The person holding the net enters the water downstream from where the kick net will be placed. Position the net perpendicular to the stream flow. Work the bottom of the net into the gravel/cobble so that it is slightly below the level of the substrate. This anchors the bottom of the net and prevents invertebrates from escaping. Hold the net at a 45° angle.



2. The second person enters the water several meters upstream and flushes invertebrates into the net (start upstream and work down toward the net). Pick up larger rocks from the stream bed but hold them underwater. Scrub the rock surface with your fingers to dislodge invertebrates. Return rocks to their original locations. Kick, shift, and stir the substrate with your feet in the entire survey area.

3. Pick up the net using both people. The kicker should grab the bottom of each pole. In coordination with the person holding the top of the net, lift the net out of the water with an upstream scooping motion. Do not allow the top of the net to go underwater or you may lose part of the sample. Carry the net to the stream bank to remove, identify, and collect specimens.
4. Collect specimens as appropriate, or as directed by Invasive Species Program supervisors (refer to the crayfish specimen guidelines, below).
5. Record all data.
6. When you are done, wash the net in the stream to remove any debris. Decontaminate and completely dry the net before storage.

Trapping

Crayfish trapping is considered a distinct survey type (rather than technique that may be employed on a standard shoreline/stream survey). Record a crayfish survey GPS point and collect data at every location where you set a crayfish trap.

- Place crayfish traps in areas with slower flowing water: in the shallows, behind large rocks and logs, etc. Dams, especially those with many rocks, are also good locations for setting traps.
- Bait traps with chicken, pork, hot dogs, or other fatty/oily meat. Small pop-top cans of cat food work well as bait. They are cheap and easy to carry around; they do not leak juices and do not need refrigeration. To bait a crayfish trap with canned cat food, just open the can and place it in the trap.
- In general, you should leave crayfish traps out overnight. Check traps daily. Do not exceed 48 hours between checks.
- Remove all crayfish from the trap. Collect specimens as appropriate, or as directed by Invasive Species Program supervisors (refer to the crayfish specimen guidelines, below).
- Record all data (refer to the crayfish survey data instructions, below).

- When you are done, dispose of used bait in a trash receptacle. Rinse the traps to remove any debris. Decontaminate all traps before using them again.

Quadrats

Crayfish quadrat sampling is a distinct survey type. Use the crayfish quadrat protocol developed by Pat Martinez, CPW Aquatic Biologist (refer to his slide presentation "Quadrat method for sampling the crayfish *Orconectes virilis* population in the middle Yampa River, Colorado").

Specimens

Preserve all Form 1 male crayfish as specimens. If you do not capture any Form I male crayfish, then preserve a selection of the crayfish that you did capture (Form 2 males, females, and/or juveniles). Place specimens in a sample bottle and preserve with 70% ethanol (you may use the same sample bottle for all crayfish specimens from one survey location). Be sure to properly label the bottle. Refer to the Specimen Collection protocols (Appendix D) for complete instructions.

Release the other crayfish back into the water body from which they were caught. If you are west of the Continental Divide, where there are no native crayfish, then crayfish may be killed instead of released.

If you detect a suspect rusty crayfish (*Orconectes rusticus*), then it must be collected and sent for expert identification at AAHL. Notify the Invasive Species Program supervisors and AAHL of your observations as soon as possible. Follow the procedure for ANS Population Mapping on page 19. Note: Crayfish are very difficult to identify down to species, so usually you can wait for expert ID confirmation from AAHL before undertaking intensive trapping efforts. However, if your field schedule allows, it is a good idea to set some additional crayfish traps or spend some extra time hand-capturing while you are in the area.

Data

Enter all data into the Trimble GPS data dictionary and complete a Crayfish Survey paper datasheet (Appendix B). Record all data for each survey, regardless of whether or not you collect specimens, to provide presence/absence data. If you were unsuccessful at capturing crayfish, but observed evidence of crayfish presence at the site (e.g. carapaces, claws, burrows, etc.) remember to record your observations in the survey comments. Always record all data in the field while you are performing the survey.

At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

Aquatic Plant Specimen Collection

It is expected that sampling crews will document native and exotic submerged and floating plant species when performing all other sampling or monitoring tasks. However, field identifications are not definitive and specimen must be collected for expert identification by Elizabeth Brown in the CPW Plant Lab. It is the goal of the Invasive Species Program to document the distributions of both native and exotic macrophytes over time in Colorado's public waters, along with mollusks, crayfish and zooplankton.

The following is required of ANS sampling and monitoring staff:

- Native or exotic aquatic plant specimen must be collected, no matter how limited, when fragments are floating near a tow, substrate or other sampling site. The goal will be for crews to collect specimen that have all plant parts needed for taxonomic identification - roots, stems, leaves, fruits, flowers, etc.
    - After identification in the CPW Plant Lab, the specimen will be pressed and submitted to the Kathryn Kalmbach Herbarium of Vascular Plants (KHD) at the Denver Botanic Gardens.
    - Once a specimen is identified and the voucher is submitted to KHD, it will be added to the species list for that water body and no further collection will be warranted unless explicitly requested by Elizabeth Brown.
- Detections of known or suspect invasive aquatic plants that have not been previously documented at a water body must be collected and the specimen sent to the CPW ANS Lab for expert identification and to comply with the invasive species regulatory process.
    - Please email or text Elizabeth if you think you have a listed prohibited plant species detection so that she can respond within required timeframes.
    - If you suspect the plant is an exotic or hybrid milfoil species (*Myriophyllum* sp.), then it is required that two specimens are collected – one for expert visual identification and one for genetic analysis. Both specimen must be submitted to the CPW Plant Lab.

Follow the procedures below to collect aquatic plant specimens. At this point everyone will collect using the preserved specimen method unless instructed to do otherwise. Then follow the procedure for ANS Population Mapping on page 19.

Specimen Collection for Visual Identification

Eurasian watermilfoil being collected by hand.

Specimen Collection

When selecting which plants to collect, it is best to choose plants that have fresh meristematic growth as opposed to "floating fragmetns" that may not be growing. Plants should have minimal amounts of periphyton or other debris covering them.

It is preferable to collect plants by hand to ensure that the entire

plant is collected, including roots and any flowering or fruiting parts. These structures are sometimes necessary for identification (depending on the species) and for creating morphological voucher specimens for submission to a herbarium. However, most plants can be visually identified from a fragment if that is all that is available. If you cannot collect an entire rooted plant by hand, it is acceptable to use a grappling hook or plant rake to collect plants from deep water, or to use a net to collect floating fragments.

Grappling hook with aquatic plants.

In general, collect 1-3 individuals of the plant species, per water body. For milfoils, be aware that the different species do co-habitat and hybridize. If you notice distinct differences between the various milfoil beds within a single water body, it is recommended that you collect 1-3 individuals from each bed.

Be sure to mark the location coordinates for the plants that you collect in your GPS unit.

Specimen Preparation

There are three options for specimen preparation and they are all acceptable methods: dried voucher, preserved specimen or jelly roll. The three preparation protocols are described below in order of preference.

1. Preserved Specimen –

   NOTE: All crews should send plants in to Elizabeth as preserved specimen.

   a. Place the plant into a large specimen bottle with 70% EtOH. Do not put anything else into the bottle (e.g. paper label, other specimens, etc.)

   b. Tightly close the bottle cap and wrap it with electrical tape to prevent leakage.

   c. Use a permanent marker to clearly label the outside of the bottle.

   d. Place the bottle into a re-sealable plastic bag.

   e. Use a permanent marker to clearly label the outside of the plastic bag, in case the bottle leaks inside the bag and the bottle label is not legible. In addition, it is a good idea to write the information in pencil on a piece of waterproof paper and include the paper inside the plastic bag. Note: Labeling redundancy is necessary because 70% EtOH washes off permanent marker from many surfaces.

   f. Specimens preserved with 70% EtOH do not need to be refrigerated.

2. Dried Voucher (only if requested by Elizabeth)

   a. Remove any debris that covers the plant by gently agitating it under water.

b. Lay the plant out in a sink or tub filled with water. Arrange the plant as if it was growing in that water body.

c. Slide a piece of herbarium paper into the water and under the plant.

d. Raise the paper up so that the water runs off the edges and the plant sticks to the paper with leaves and structures spread out (as they would be positioned if the plant remained in the water). Most aquatic plants will clump when out of water and this method ensures that all morphological features are visible for identification after the specimen is dried.

e. Place the wet paper with the plant stuck to it onto a cookie cooling rack (or similar location) to fully dry.

f. Once dry, place the paper with the specimen into a large re-sealable plastic bag that is clearly labeled. Alternately, place a piece of wax paper over the dried plant and then put it between two pieces of newspaper. Place this package into a padded envelope that is clearly labeled.

g. Dried specimens do not need to be refrigerated.

3. Jelly Roll (refer to photos below - only if requested by Elizabeth)

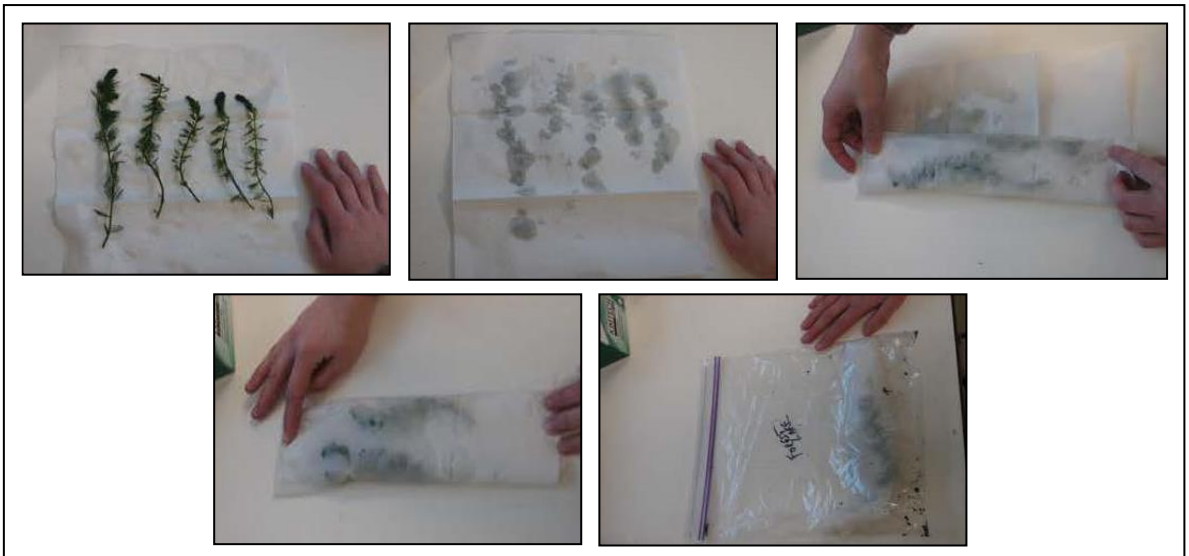
a. Remove any debris that covers the plant by gently agitating it under water.

b. Lay the plant out on a damp (NOT wet) paper towel. Note: you can typically fit 5-10 specimens on a standard size paper towel.

c. Place another damp (NOT wet) paper towel on top of the plants. Gently roll up the paper towels and plants, like a jelly roll.

d. Place the paper towel roll in a clearly labeled gallon-size re-sealable plastic bag.

e. <u>Refrigerate</u> this specimen until it is ready to be shipped.



"Jelly Roll" method of aquatic plant specimen preparation

Specimen Collection for DNA Identification

Only if requested by Elizabeth.

This protocol is paraphrased from John Wood at Pisces Molecular, LLC.

It is important to keep the plant specimen free from contamination by other plants. Genetic analysis to confirm species identification relies on minute amounts of DNA that can easily be transmitted between specimens via forceps, fingers, plastic bags, and other plant species in the same specimen bottle or water sample. This procedure reduces the risk of contaminating plant specimens collected for genetic analysis.

1. Sterilize your forceps by dipping them in a bottle of 70% EtOH and then heating with a lighter to carbonize any DNA.
2. Do not remove the plant from the water body. Use the sterilized forceps to pull a leaflet off the plant. Place the leaflet into a specimen bottle and preserve with 70% ethanol. Do not put anything else into the specimen bottle (e.g. paper label, other specimens, etc.).
3. Tightly close the bottle cap and wrap it with electrical tape to prevent leakage.
4. Use a permanent marker to clearly label the outside of the bottle.
5. Place the bottle into a re-sealable plastic bag.
6. Use a permanent marker to clearly label the outside of the plastic bag, in case the bottle leaks inside the bag and the bottle label is not legible. In addition, it is a good idea to write the information in pencil on a piece of waterproof paper and include the paper inside the plastic bag. Note: Labeling redundancy is necessary because 70% EtOH washes off permanent marker from many surfaces.
7. Specimens preserved with 70% EtOH do not need to be refrigerated.

Dried Voucher Plant Specimen Labeling and Shipping

- Label all specimens with as much of the following information as you know.
    - County and/or city
    - Water body name and CPW water code
    - Collection location name and coordinates (NAD83 CONUS).
        - If no coordinates are available, then include a detailed description of the collection location.
    - CPW Unique ID
    - Collection date
    - Collector contact information: name, organization, phone number, and email
    - % EtOH (if applicable)
    - Any other relevant comments

Sample Herbarium Specimen Label

COMMON NAME: Eurasian watermilfoil

- STATE: Colorado; COUNTY: Jefferson; CITY: Westminster
- WATER BODY: Standley Lake Reservoir
- LOCATION: North side of west boat ramp
- GPS COORDINATES: ######E, ########N (UTM, NAD83 CONUS, Zone 13)
- QUAD MAP: Black Hawk
- T2S, R72W, Sec 14
- ELEVATION: 6360 ft
- CPW UNIQUE ID: 04-33153-001-09
- DATE: June 8, 2009
- COLLECTOR: Ellen Hayes, Elizabeth Brown – CPW Invasive Species Program
- IDENTIFIED BY: Elizabeth Brown, CPW Invasive Species Coordinator, 303-291-7362

- Prepare specimens using one of the methods described above. Do not ship plants in lake, pond, or stream water.
- If available, pack the specimens in an insulated package (e.g. plastic or foam cooler). If available, include cold packs to keep the specimens cool in transit.
- If possible, ship overnight.
- Send all plant specimens to Elizabeth Brown at CPW Invasive Species Program, 6060 Broadway, Denver, CO, 80216.
- If genetic analysis is needed to confirm the visual identification, then Elizabeth Brown will send the additional specimen that was collected for DNA testing to Pisces Molecular.
- Email Invasive.Species@state.co.us to notify the Invasive Species Program that a specimen is being shipped.

Aquaculture Facility (Hatchery) Inspection

Aquaculture facility (hatchery) inspections for ANS include qualitative visual and tactile encounter surveys, and at some facilities, may include plankton tows. Hatchery inspections are conducted for several purposes:

- To gather baseline data on species presence/absence in aquaculture facilities.
- To locate populations of ANS within these facilities.
- To monitor any known populations of ANS within these facilities. (Note: Quantitative protocols may also be implemented to monitor specific ANS populations.)
- To provide evidence that the inspections have been performed through completion of an Aquaculture Facility (Hatchery) Inspection datasheet.

Inspection Frequency

State hatcheries are inspected annually, unless directed otherwise by Invasive Species Program supervisors. Repeat inspections within the same calendar year are required if the initial inspection was not completed, was performed at a time not optimal for ANS detection, or when additional specimens of a suspect ANS need to be collected.

Plankton tows are typically only performed at Pueblo Hatchery. These plankton tows are conducted by the regular ANS Sampling Crew when they sample Pueblo Reservoir, rather than being collected by the hatchery inspector.

Before Going Out to the Field

- Contact the facility manager well in advance to schedule the inspection. Most inspections will take the majority of a day and may require touring the hatchery with a staff member to familiarize yourself with the facility. Before your visit, find out where to park your vehicle and where to meet the manager or staff.
- Review facility maps if available.
- Review historic data and notes (e.g. the ANS sampling database, AAHL records, GIS records, reports, field notes, and photos) related to the facility and nearby water bodies.
- Study the unique characteristics of the target species to learn/refresh your field identification skills, and review the species habitat requirements.
- Check to be sure you have all needed equipment and supplies (Appendix C). Inspect equipment to be sure it is in proper working order. When surveying a facility (as opposed to nearby waters), do not bring in your own waders or dip nets. If you need to use these items, obtain them from the hatchery manager.

- Always remember to decontaminate knee-high rubber boots between every facility. Refer to the Equipment Decontamination protocols on page 40.
- Thoroughly wash (hot water and soap) and rinse your vehicle at a car wash between facilities, especially if you are traveling directly from one facility to another.

Inspection Procedure

1. Upon arriving at the facility, introduce yourself to the manager and any available staff. Briefly describe the purpose of your visit. Talk to the manger or staff to find out if there are any known ANS populations in or near the facility, and to determine areas where ANS introduction to the facility might occur. Ask if they have observed any invertebrates within their system.

2. If available, review a map of the facility to obtain an overview of the areas needing inspection (most facilities have these maps). It is preferable for a staff member to accompany you around the facility to show you the different areas (e.g. water diversions, spring line collection boxes, hatchery and nurse areas, raceways, ponds, settling ponds, and effluent discharge points). Otherwise, get directions on locating all these areas on your own. Be sure to use any foot baths present in the facility. If requested, use the facility's rubber boots/waders, especially when entering quarantine/isolation areas.

3. Begin the inspection in the "cleanest" portions of the facility, such as the hatch house and incoming water supply. Move downward through the system to nurse areas, raceways, and rearing ponds. Finally, examine the settling pond and effluent areas. Visits to waterways above and below the facility are desirable, but should be performed after the facility itself is inspected.

4. At each site within the facility, check accessible ends of open pipes, walls of egg/fish containers (e.g. walls of inlet boxes, troughs, tanks, raceways, etc.), and any screens on which ANS might attach or collect (e.g. small mesh screens placed to collect debris or invertebrates at sites of incoming water). Pay special attention to interchanges between raceways as well as areas immediately behind tail screens, dam boards inside waterfalls, and any other places without fish. Use disposable shoulder-length gloves when reaching into the water. Remove and discard gloves between tanks, troughs, and raceways to prevent cross-contamination, not only of ANS but also of any potential viral/bacterial fish diseases.

   Snails, particularly New Zealand mudsnails (*Potamopyrgus antipodarum*), are most numerous from 15 centimeters (6 inches) above the raceway floor level up to about 45 centimeters (18 inches) above floor level. Snails are very difficult to feel on coarse concrete. It is more effective to gather material using a strainer (reinforced aquarium net – use the net supplied by facility staff) and then visually inspect.

5. At settling ponds and water bodies above/below the facility, perform a Shoreline or Stream Survey to look for target invertebrates or evidence of their presence (e.g. crayfish exoskeletons, mollusk shells, etc.). Refer to the Shoreline/Stream Survey protocol starting on page 15. Turn over rocks and examine coarse woody debris. Dig into any sandy substrates with a sieve. If you

have a field partner, you can use a kick net to search for crayfish. If you are alone, use a long-handled dip net or hand-capture crayfish. You can also set crayfish traps – Refer to the Crayfish Survey Techniques starting on page 23. Crayfish traps may be set the day before you perform the facility inspection, but be sure boots and equipment are thoroughly decontaminated before entering the facility.

6. Collect specimens as appropriate, or as directed by Invasive Species Program supervisors – In general, if a species has been previously documented at a site, then you probably do not need to collect it. However, be sure to collect any organism that you cannot 100% positively identify.

   If you detect a known or suspect ANS at a facility or water body where it has not been previously documented, then it must be collected and sent for expert identification. All specimens are sent to AAHL unless specifically directed otherwise (e.g. plant specimens are sent to Elizabeth Brown). Remember, if you are unsure whether or not an organism is an ANS, err on the side of caution and collect it for identification. Notify the Invasive Species Program supervisors and AAHL of your observations as soon as possible.

   - When you detect a known ANS, finish initial survey and then follow the procedure for ANS Population Mapping on page 19.

   Place specimens in a sample bottle and preserve with 70% ethanol (you may use the same sample bottle for all mollusk specimens from one survey location). Be sure to properly label the bottle. Refer to the Specimen Collection protocols (Appendix D) for complete instructions.

8. Complete an Aquaculture Facility (Hatchery) Inspection datasheet and sign. As appropriate, record all data from shoreline/stream surveys, crayfish surveys, plankton tows, and ANS population mapping into the Trimble GPS data dictionary and on the corresponding paper datasheet (Appendix B). Record all data for each survey, regardless of whether or not you collect specimens, to provide presence/absence data. Always record all data in the field while you are performing the survey. At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. Submit completed datasheets to the AAHL.

Water Quality Sampling

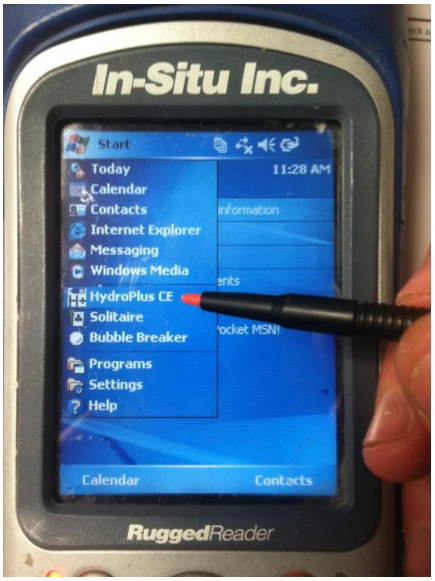
Water quality sampling provides data on habitat suitability for ANS. In general, Secchi depth and water quality profiles are recorded every time you conduct a plankton tow for zebra/quagga mussel veligers (except in flowing water, i.e. below the dam outlet). Refer to the zebra/quagga mussel sampling frequency on pp. 4-6.

BEFORE YOU ATTEMPT ANY WATER QUALITY READINGS BE SURE TO CALIBRATE YOUR EQUIPMENT FIRST, AND ON A DAILY BASIS IN ORDER TO MAINTAIN ACCURACY

Sonde Calibration

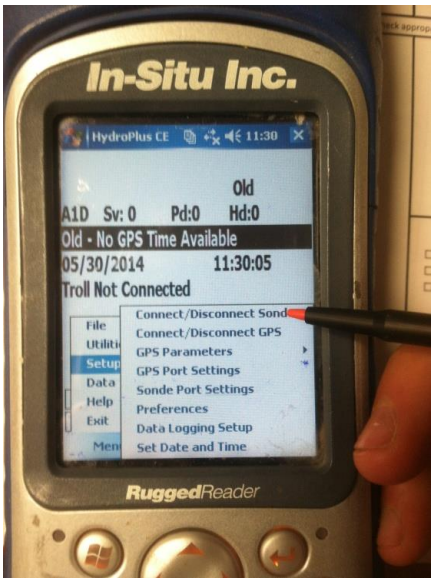
Successful water quality readings depend on a daily calibration of the electronic equipment involved. The Sonde is a highly sensitive device, and requires a brief calibration session each and every morning. In order to calibrate, kits have been provided which contain three 1000mL bottles of calibration liquid (**yellow pH7, blue pH10 and Conductivity 1413**), an empty 1000mL bottle, a calibration cup, pH filling solution, pH storage solution and an additional set of directions.

1. Connect the Sonde and Rugged Reader's cables, with tight fittings at each connection. Remove the Guard and pH storage solution container from the probe, and rinse the device thoroughly, twice, with de ionized water. Pull out the calibration kit, and rinse out the calibration cup, twice, with de ionized water. This double rinsing process will be repeated between each sensor calibration, as well as before and after calibration itself.

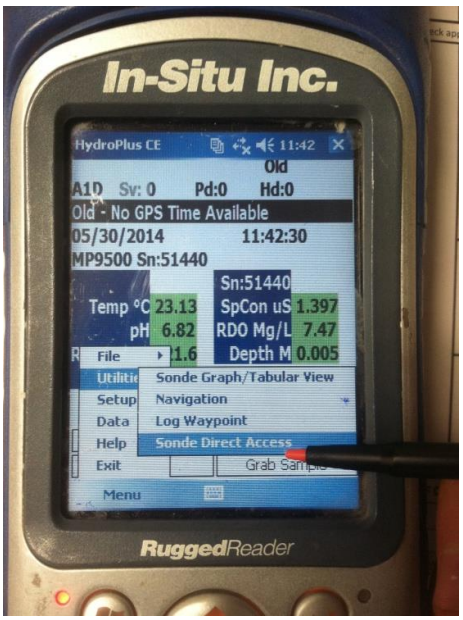


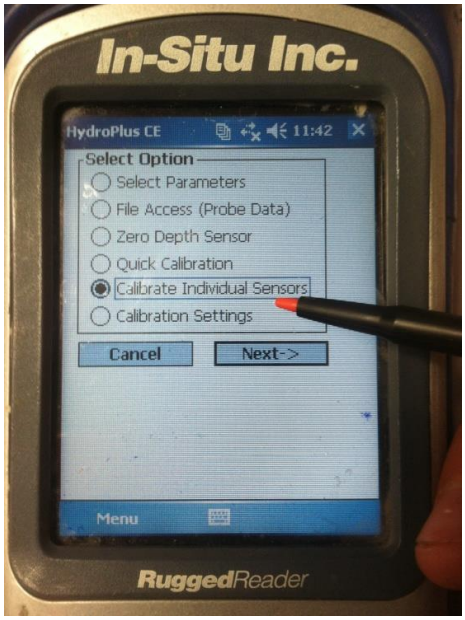
2.

3. Activate the Rugged Reader and access Hydroplus CE from the Start Menu. From the Hydroplus home screen press "**Menu**", then "**Setup**", and click "**Connect Sonde**". This will let the Rugged Reader and Probe connect to one another.

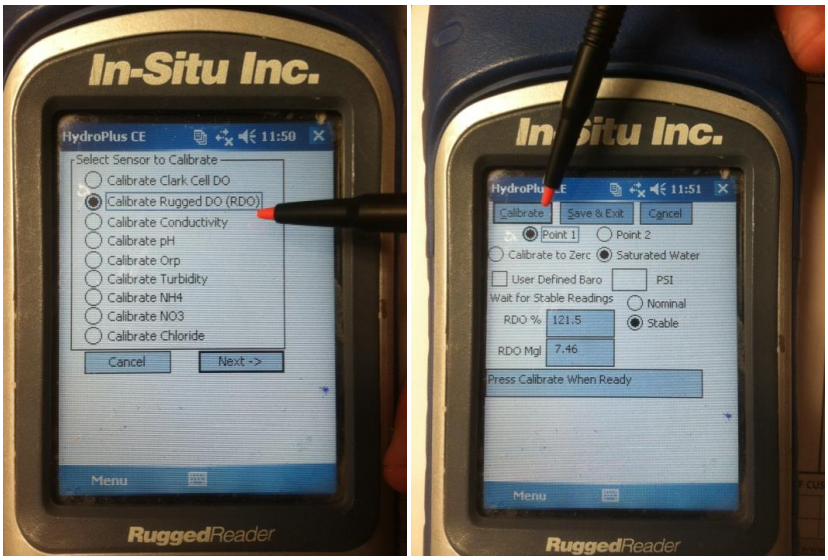


4. To start calibrating, click "**Menu**", followed by "**Utilities-Sonde Direct Access**". Next, "**Calibrate Individual Sensors**". This series will bring the user to a list of sensors to be calibrated, and is the main calibration menu. Dissolved Oxygen is the first sensor to calibrate, and it requires the probe cup and the empty bottle.



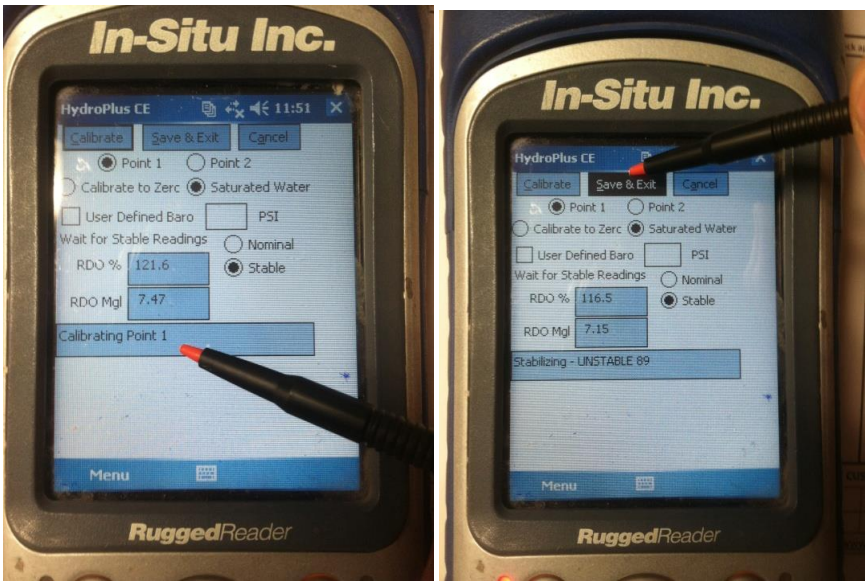


5. Fill the empty bottle with water from the site (**do not use tap water**), seal the bottle, and begin to shake vigorously for two minutes. This will hyper oxygenate the water within, allowing a saturated reading. Within the calibration menu, click "**RDO (Rugged Dissolved Oxygen)**", then "**Next**". This will open the menu for Dissolved Oxygen calibration. Pour the shaken water into the probe cup, place the probe into the cup, and access the RuggedReader. Click "**Saturated**", ensure "**Stable**" has been checked, followed by "**Calibrate**" and hold the probe in the cup as the calibration takes place.



6. The calibration process takes approximately 90 seconds and as the Rugged Reader counts down it will let you know of its progress. Its progress registers as three stages, Unstable, Nominal, and Stable. Having Stable checked in the previous step ensures that the Reader will calibrate until its variability has been reduced to a minimum. Generally, calibration failure occurs when the Reader is unable to progress to Stable readings, and merely reads at Nominal.

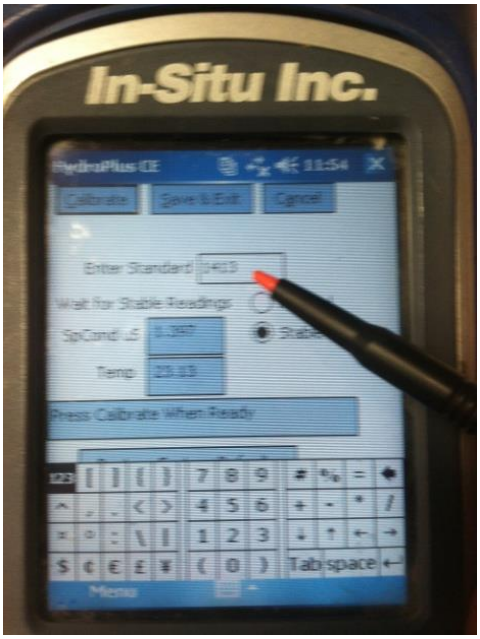
7. Upon the countdowns conclusion, either success or failure will occur. In the event of failure, repeat the above process (steps 4-5), sometimes this equipment takes multiple calibrations for a success. In the event of success click "**Save and Exit**", and a calibration report is brought forth. Click "**Okay** or **Skip**" unless something of note occurred, such as the event of total calibration failure, in which case, add a note, and then save the calibration.



8. While the Rugged Reader saves the calibration, the user is brought to the Hydroplus home screen. Before advancement to the other sensors, rinse out the probe cup and rinse off the probe with de ionized water, rinse them a second time, and pour out the 1000mL bottle. Place this bottle with other equipment to be decontaminated before the next body of water. Dissolved oxygen has now been calibrated.

9. Next, Conductivity will be calibrated. Re-rinse the probes, and fill the calibration cup with conductivity 1413 solution. Re-access the calibration menu by clicking "**Menu-Utilities-Sonde Direct Access-Calibrate Individual Sensors**", the exact series of prompts followed in step 4.

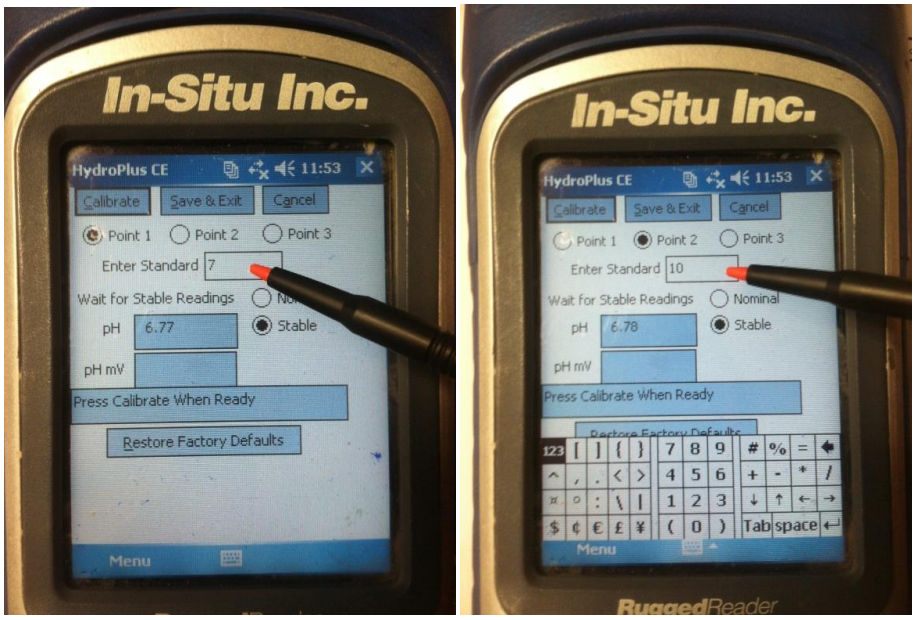
10. Once returned to the Calibration menu, click "**Calibrate Conductivity**" and place your probe into the filled calibration cup. The Conductivity screen will request a standard measurement. Enter 1413 (uS/cm), then make sure Stable is selected before clicking "**Calibrate**". From here, steps 5 and 6 can be repeated as the Rugged Reader's calibration process occurs.



11. Upon a successful calibration, pour the conductivity solution back into its appropriate 1000mL container, and rinse the probe and calibration cup twice. Conductivity has now been calibrated, and the process for calibrating pH can begin.

12. To start calibrating pH, pull out the bottles of yellow pH7 solution and blue pH10 solution and keep them both nearby. Fill the calibration cup with pH7 solution. Re-access the calibration menu by clicking "**Menu-Utilities-Sonde Direct Access-Calibrate Individual Sensors**", the exact series of prompts followed in step 4.

13. Once returned to the Calibration menu, click "**Calibrate pH**" and place your probe into the filled calibration cup. From here, the Reader will ask whether or not this is a two or three step calibration. Click "**Traditional Calibration- 2 Point**", and check the bullet for the first point. Enter the standard of 7, and click "**Calibrate**". Allow the 90 seconds for calibration, and repeat in the event of a failure.



14. After a successful pH7 calibration, pH10 will need to calibrate next. Click the bullet for the second calibration point, and enter 10 for the pH. Now, pour the pH7 back into the pH7 solution, rinse the probe and calibration cup twice, and pour pH10 into the cup. Place the probe into the cup, and click "**Calibrate**". Again, the Rugged Reader will give a 90 second countdown to calibration.

15. Upon the countdowns conclusion, either success or failure will occur. In the event of failure, repeat the process, sometimes this equipment takes multiple calibrations for a success. In the event of success, click "**Save and Exit**", and a calibration report is brought forth. Click "**Okay** or **Skip**" unless something of note occurred, such as the event of total calibration failure, in which case, add a note, and then save the calibration.

16. Pour the pH10 solution back into the 1000mL bottle. Rinse off the probe and calibration cup twice with de ionized water. Re-affix the pH storage solution cup to the pH probe, and re-attach the probe guard. Pack up the contents of the calibration kit, which should include the three 1000mL calibration solutions and the calibration cup. **DO NOT KEEP THE EMPTY BOTTLE IN THE KIT FOLLOWING USAGE OF LOCAL WATERS. DECONTAMINATE THIS BOTTLE BEFORE THE NEXT CALIBRATION**. De-activate the Rugged Reader, disconnect and store the cable components. The water quality equipment has now been calibrated, and is ready for one day's use. **REPEAT THIS CALIBRATION PROCESS ONCE EACH DAY PRIOR TO ANY WATER QUALITY MEASUREMENTS**.

GPS and Sampling Data

Record a GPS point at the water quality sampling location. Enter all data into the Trimble GPS data dictionary and complete a Water Quality paper datasheet (Appendix B). Always record all data in the field on paper while you are collecting handheld data.

At the end of the work day or end of the week when you are at a location with internet access, upload all of your GPS data and input it into the ANS Sampling Database. Do not let more than a week go by before uploading GPS data. File the paper datasheet as backup documentation.

Secchi Depth

Secchi depth is a measure of water clarity (transparency) and is calculated using a Secchi disk. Changes in Secchi depth are related to changes in water clarity due to algae populations, silt loads, or water color.

This procedure describes standard Secchi depth measurement (refer to the North American Lake Management Society "Secchi Dip In" website or the US EPA volunteer lake monitoring manual).

Secchi Disk (photo from http://www.the-macc.org/watershed/water-quality/)

General Instructions

- Try to take Secchi disk readings when the water is calm, sky is clear, and winds are calm to breezy (no whitecaps).
- The angle of the sun affects your ability to see the Secchi disk, thus it is ideal to take readings between 9am and 3pm.
- Do not wear sunglasses.
- Store the Secchi disk in a dry location.

Secchi Disk Procedure

1. Anchor your boat at the sampling site and make sure the boat is not drifting. Examine the Secchi disk and measuring line to be sure it is ready to be deployed.

2. Deploy the Secchi disk on the shady side of the boat. Slowly lower the disk into the water until you can no longer see it. Read the depth from the tape measure (in tenths of meters) at the water surface. Record this depth as the Lowering Depth (L).

3. Lower the Secchi disk another meter or so. Slowly pull up the disk until you see it reappear. Read the depth from the tape measure (in tenths of meters) at the water surface. Record this depth as the Raising Depth (R).

4. Average the Lowering Depth and the Raising Depth to calculate the final Secchi Depth.

   (L + R) / 2 = Secchi depth (e.g. If L=3.2m and R=3.4m, then Secchi Depth=3.3m)

Logging Water Quality Profiles

Water quality profiles are collected in the field using a sonde (water quality monitoring instrument, e.g. Hydrolab or YSI). The profile includes measurements of various parameters (e.g. water temperature, pH, dissolved oxygen, conductivity, turbidity, etc.) recorded at .5, 1 and/or 5 meter depth intervals from the surface to the bottom of the water body.

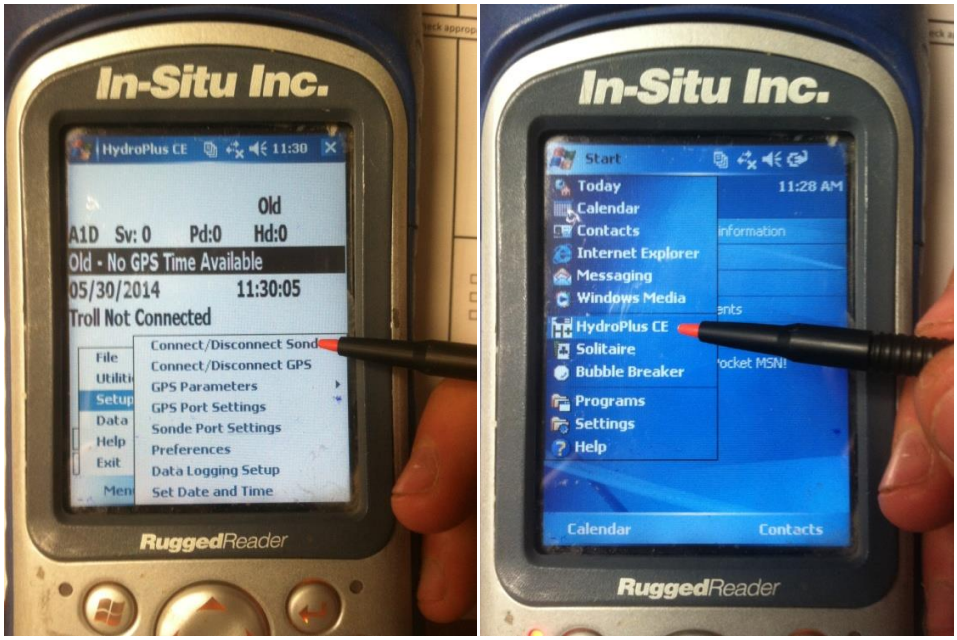
Water Quality Profile Procedure - Sonde Unit

Setting Up:

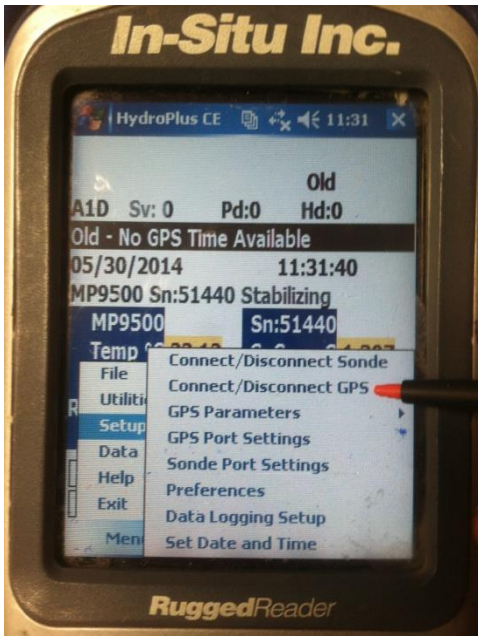
1. Anchor your boat at the sampling site and make sure the boat is not drifting.

2. Affix the Sonde and Rugged Reader to their respective cables. Ensure all connections are tight. Remove the probe guard and pH solution cup. Rinse the probe with de-ionized water, then re-attach the probe guard. Now, rinse the probe and guard with de ionized water. Leave the pH solution cup in a safe place until after your sample. Lower the Sonde into the water and then turn on the instrument.

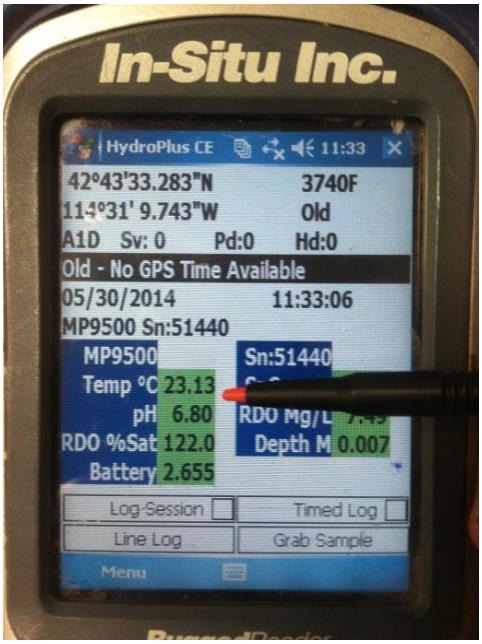
3. Activate the Rugged Reader and access Hydroplus CE from the Start Menu. From the Hydroplus home screen press "**Menu**", then "**Setup**", and click "**Connect Sonde"**. This will let the Reader and Probe connect to one another



4. Once connected, the GPS system will now need to connect to satellites. Press "**Menu**", then "**Setup**", followed by "**Connect GPS**"



5. With the GPS locked in (it will give you coordinates as shown in the image below) place your probe into the water, and allow time for its measurements to stabilize (stable readings will appear as green)



6. While the probe stabilizes, begin to fill out the Lake Water Quality Assessment Date Sheet. Follow the example shown below, start by including the Lake or Water Body, the Site Name (Station #) and the initials of the sampling crew involved, as well as the Date and Start time. Fill out the final Secchi Depth and utilize the boats depth finder in order to determine the maximum

depth of the site. Mark this down, as well as the weather and surface conditions in the "Notes" section. **IMPORTANT**: be sure to mark down the CDOWID in this section as well.

COLORADO DEPARTMENT OF PUBLIC HEALTH & ENVIRONMENT – Lake Water Quality Assessment

TOP SAMPLE LABEL

BOTTOM SAMPLE LABEL

Lake *GRANBY RESERVOIR*

Station # *DAM* Samplers *JSTNB SA*

Date *08 / 14 / 14* Start Time *11 : 35* End Time *12: 05*

Latitude *40.14928167* Longitude *-105.86594823*

Datum (if not WGS84) _____ Secchi Depth (m) *2.9* MaxDepth (m) *38*

Bottom Sample Depth Range (m) *5* Chl a Volume Filtered (mL) *750*

Notes (surface conditions, weather, blooms, etc) *Cloudy, windy, cool, with passing showers. Choppy.*

05-66969-051-14

Depth m	Temp	DO	Cond	pH	Depth m	Temp	DO	Cond	pH
0	19.64	6.85	58.45	7.58	25	7.17	6.08	65.49	7.09
0.5	19.65	6.87	58.45	7.60	30	7.05	6.07	65.78	7.09
1	19.55	6.85	58.41	7.59	35	6.96	6.02	66.04	7.10
1.5	19.39	6.87	58.20	7.60	40				
2	19.31	6.92	58.12	7.53	45				
2.5	19.31	6.92	58.12	7.58	50				
3	19.29	6.88	58.13	7.60	55				
3.5	19.26	6.85	58.03	7.60	60				
4	19.24	6.92	58.01	7.58	65				
4.5	19.23	6.80	58.01	7.62	70				
5	19.22	6.81	57.91	7.61	75				
6	19.13	6.77	56.97	7.60	80				
7	16.43	5.51	59.38	7.13	85				
8	16.23	5.40	59.87	7.10	90				
9	15.73	5.24	59.59	7.08	95				
10	15.40	5.28	58.95	7.07	100				
11	13.68	5.38	59.70	7.07					
12	11.96	5.64	57.03	7.06					
13	11.45	5.69	57.31	7.06					
14	10.64	5.80	57.67	7.08					
15	9.86	5.87	59.24	7.08					
16	9.52	5.94	60.10	7.08					
17	8.92	5.86	61.88	7.08					
18	7.72	5.94	64.79	7.08					
19	7.49	5.95	65.11	7.08					
20	7.49	5.96	65.09	7.06					
21	7.43	5.93	65.09	7.05					
22	7.38	5.87	65.27	7.07					
23	7.28	5.89	65.31	7.07					
24	7.21	5.97	65.43	7.08					

DUPLICATE LABEL

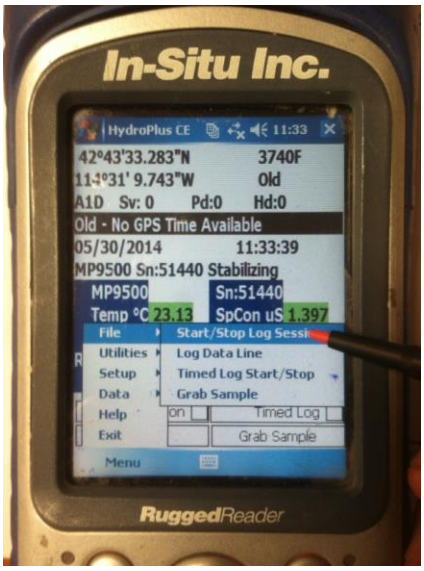
BLANK LABEL

Surface Recheck Temp: *19.90* DO: *6.87* Cond: *58.56* PH: *7.75*

7. For the time being, ignore the latitude and longitude (more on that later) and determine your Bottom Sample Depth Range. As shown on the example below, the depth between readings varies based on the depth itself. From the surface to 5m deep, readings are to be taken every .5m. Between 5m and 25m readings are to be taken every meter, and beyond 25m deep, every 5m. The Bottom Range is important, for if a site is 47m deep, the Bottom Range means that the deepest reading will be taken at 45 meters, rather than the full depth of the site.

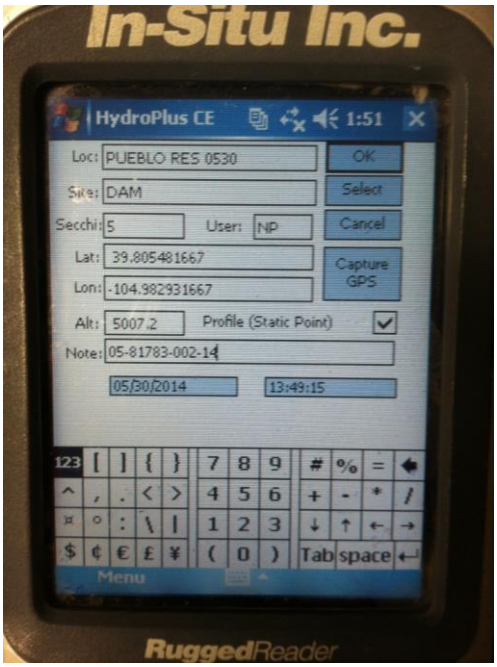
Logging:

8. With the documentation prepped to go, the probe readings should be stable. In order to begin logging, a water body file must be created. Press **"Menu**", press **"File**", then click **"Start/Stop Log Session**"

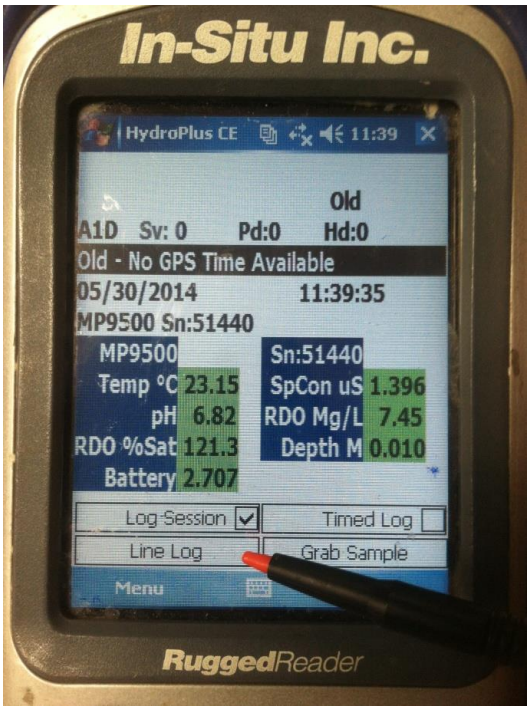


9. The Reader will open a file selection menu. Press "**New**" every time, even if this is the second time at a site and the old file is still in the Reader. This will bring the user to a prompt for background information, most of which has just been written down on the handwritten backup. Fill out the form accordingly.

10. **First**, **be sure you have already taken your Secchi measurement and record it here as you cannot reenter it later.** In the (Loc:) field write the name of the water body excluding Reservoir and Lake and add the month and day to the end as shown here (**PUEBLO 0710**). Next, **enter the CDOWID into the notes section**, these extra steps will ensure further accuracy within the data and databases. Finally, establish the location. First check "**Profile (Static Point)**" so that the GPS is not attempting to read a new location for every depth. Then, click "**Capture GPS**" to fill out your Lat and Long. Now, fill out the Lat and Long on the paper document, and press "**Ok**"



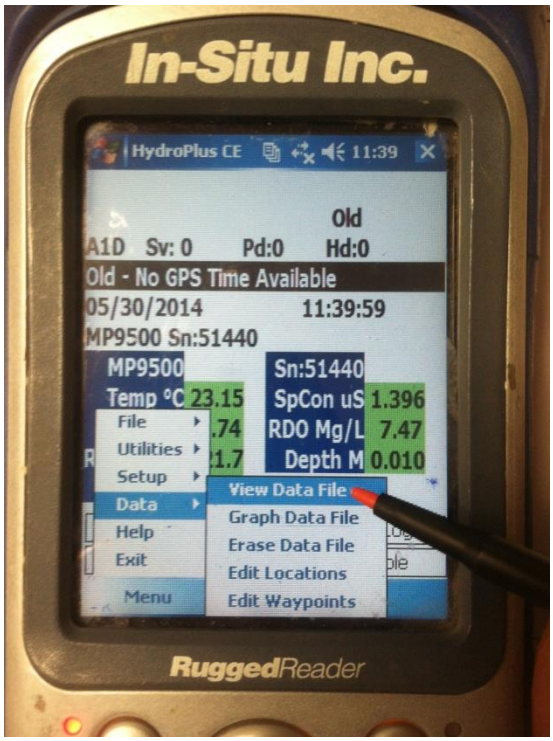
11. The Reader will then take you to the main screen, and now the "**Log Session**" box has been checked. **DO NOT UNCHECK THIS BOX**. The Reader is now ready to log. Using the depth measurement, drop the probe to the appropriate depth, and press "**Line Log**" to capture the readings at that particular depth. Keep an eye on the data, as certain measurements may have unstable readings. Unstable readings are acceptable, however stable is highly preferred (use your judgment, as weather conditions may make stable readings difficult to come by)

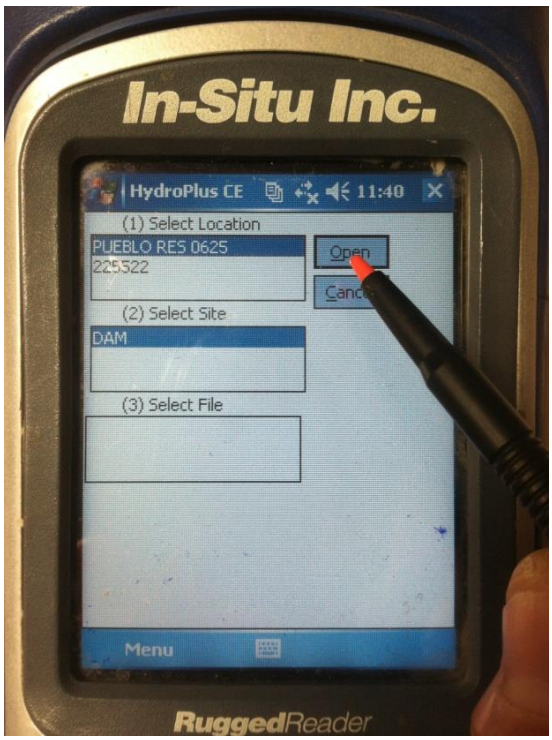


12. Then, slowly lower the probe to the next depth, press "**Line Log**" once the readings have stabilized and repeat until you have reached your maximum measuring depth. Enter this line, and begin to reel in the probe but **DO NOT UNCHECK THE** "**Log Session**" **BOX**. Once the probe is at the surface again press "**Line Log**" one final time for a surface recheck, then "**Log Session**" may be unchecked to close out the session.

13. Remove the probe guard, and thoroughly rinse submerged equipment twice with de-ionized water. Affix the pH solution cup back onto its respective probe (ensure the cup is filled with pH storage solution), re-attach the probe guard and disconnect the cables.

14. As soon as possible, re-open the file by accessing "**Menu-Data-View Data File**" and transcribe your data from the Rugged Reader to the paper backup. File the paper backup with the rest of the week's documentation.





15. Store the instruments in the office between sampling weeks. **THIS EQUIPMENT IS EXTREMELY SENSITIVE! AVOID OVEN-LIKE CAR CONDITIONS AS ONE WOULD WITH ICE CREAM CAKE, A PUPPY OR A BABY!**!!

Water Samples for Laboratory Analysis

Depending on Invasive Species Program priorities and resources, water samples may be collected for laboratory water chemistry analysis. This protocol was developed by the Colorado Department of Public Health (CDPHE). The water sample collection process utilizes the Colorado Department of Public Health and Environment laboratories to create accurate water body chemistry measurements and analysis. As a result of this co-operative research, much of the materials required and information collected will be used by multiple programs, not just the Invasive group, therefore, accuracy and care must be maintained at all times.

Materials: Several large brown water collection pouches, PVC water collection straw with valve and plug, funnel, Water Quality Bottle Kit (Provided by CDPHE) which contains six bottles, chlorophyll filter apparatus (includes pump, magnetic cup, filter/stopper and erhynmeyer flask), forceps, tinfoil, cellulose filters, barcode label sticky sheets and a sulfuric acid dropper.

Water Quality Panel Kit: This kit contains six bottles provided by the CDPHE labs for the specific usage of water quality measurement. Some of these bottles contain sulfuric acid within them already, and are not to be filled to their entirety, so that altitude and potential freezing do not disrupt bottle integrity. Each kit contains

- Three 250mL bottles. One for metals analysis, a neutral bottle, and a bottle for nutrient analysis. **THE NUTRIENT ANALYSIS BOTTLE CONTAINS SMALL AMOUNTS OF SULFURIC ACID, WHICH IS INCREDIBLY CORROSIVE TO SKIN AND CLOTHING. HANDLE THIS BOTTLE WITH CARE AND DO NOT OVERFILL.**
- Two 50 mL centrifuge bottles.
- One small, brown 125 mL bottle for nitrate analysis (**may also contain sulfuric acid)**

Collecting Samples: Once a year physical water samples will be required from each water quality site. Immediately following the water quality profiling, remain anchored and prepare by pulling out one water quality kit, one collection bag, the PVC straw, and the rest of the necessary materials listed above.

1. Begin by using the PVC straw to collect a large sample of water. Open the valve, and remove the plug. Slide the straw vertical into the water, and place the plug in the top. Physics will then hold the water in place (much like placing your thumb on the straw in a drink) while you pull the straw up and out of the water. However, as there may not be a perfect seal, close the valve immediately as the straw exits the water.

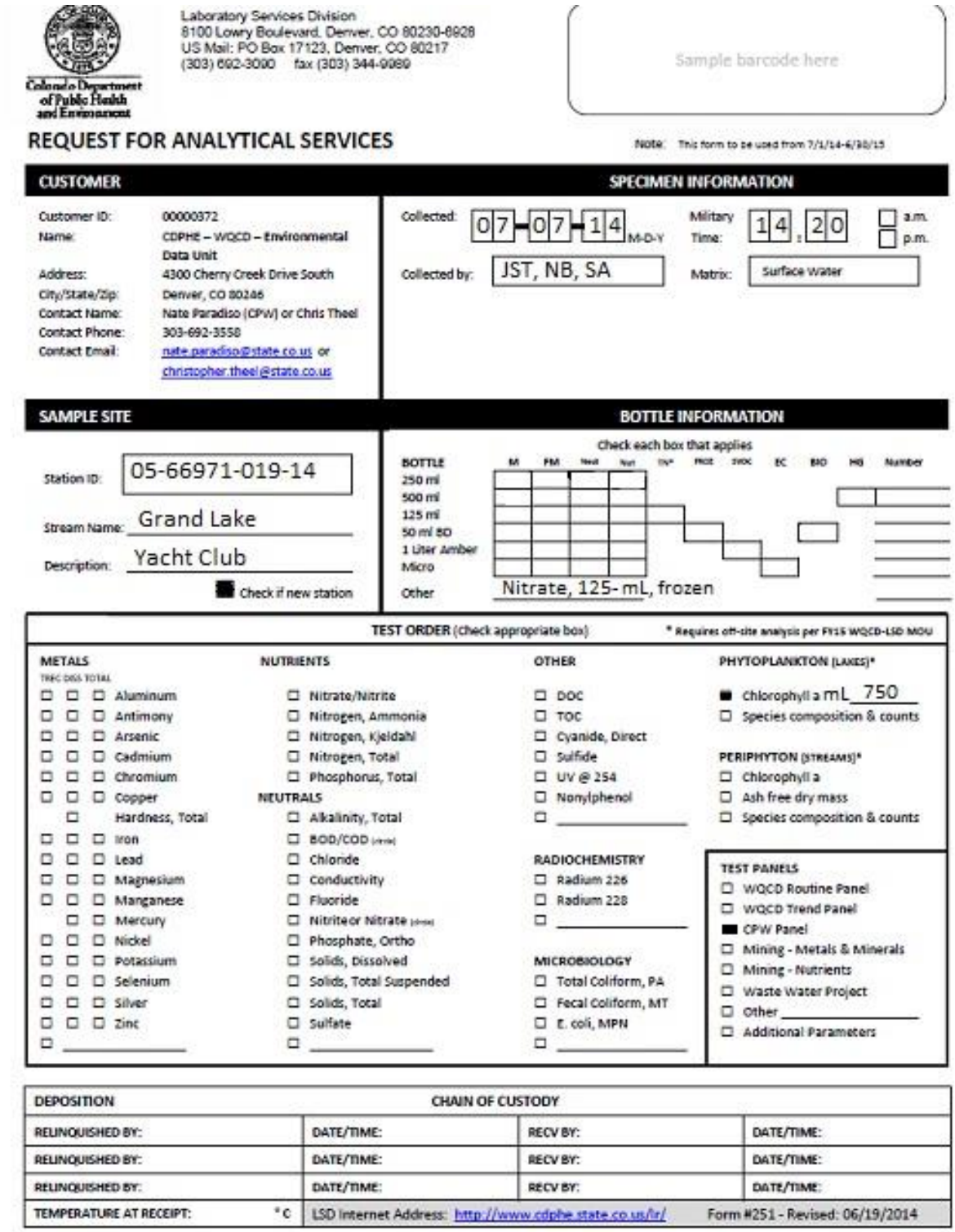
2. Next, affix the valve end of the PVC over the opening of a brown water quality collection bag with a funnel, and proceed to fill the bag up with water by pulling the plug with some assistance from another member of the sampling crew. Once filled, seal the bag, and shake vigorously for approximately ten seconds. This process creates a mixed sample of a vertical water profile, as opposed to merely skimming available resources from the top. This surface water could potentially have different chemical composition than waters merely 15cm deeper. Therefore, the PVC collection method is considered ideal for water quality profiles.

3. With the filled bag and assistance from the funnel, fill up 5 of the 6 water quality bottles provided by CDPHE. Leave one centrifuge bottle empty, but fill the rest of the bottles **MOSTLY** full, but be sure to leave some air in the sample.

4. Stick one barcode sticker to each of the bottles, as well as one to the handwritten profile datasheet and add **SIX** drops of sulfuric acid (from the dropper) to the contents of the **BROWN 125mL** bottle. This will extend the sample's longevity.

5. Next, proceed to fill out the Water Sample datasheet, provided by CDPHE. Begin by placing one of the sticky barcodes to the appropriate space (be sure to use the stickies from the same column, with the same number), and advance to the Specimen Information section directly below. **NOTE:** It is extremely helpful for the purposes of shipping and data that you keep a side sheet, or mark down directly onto the barcode sheet which barcode # corresponds with which body of water, and/or CDOWID. As this may be your last barcode, be sure to make this note before it becomes too late!

6. Within the specimen section, fill out the date, time, and initials of the sampling crew involved. Then, check the box marked "CPW Lakes Panel", and look to the Sample Site section. Within here, fill out the Station ID slot with the site's CDOWID, the Stream Name with the current water body, and the description with the site's name. Look below to the Test Order section, under "Phytoplankton (Lakes)", check off "Chlorophyll A", and under "Test Panels" check "CPW Panel".



Colorado Department of Public Health and Environment

Laboratory Services Division
8100 Lowry Boulevard, Denver, CO 80230-6928
US Mail: PO Box 17123, Denver, CO 80217
(303) 692-3090 fax (303) 344-9089

Sample barcode here

REQUEST FOR ANALYTICAL SERVICES

Note: This form to be used from 7/1/14-6/30/15

CUSTOMER

Customer ID:	00000372
Name:	CDPHE – WQCD – Environmental Data Unit
Address:	4300 Cherry Creek Drive South
City/State/Zip:	Denver, CO 80246
Contact Name:	Nate Paradiso (CPW) or Chris Theel
Contact Phone:	303-692-3558
Contact Email:	nate.paradiso@state.co.us or christopher.theel@state.co.us

SPECIMEN INFORMATION

Collected: 07-07-14 M-D-Y

Military Time: 14:20 ☐ a.m. ☐ p.m.

Collected by: JST, NB, SA

Matrix: Surface Water

SAMPLE SITE

Station ID: 05-66971-019-14

Stream Name: Grand Lake

Description: Yacht Club

■ Check if new station

BOTTLE INFORMATION

Check each box that applies

BOTTLE	M	FM	Nnut	Nutr	UV*	PHOS	SWOC	EC	BIO	HG	Number
250 ml											
500 ml											
125 ml											
50 ml BD											
1 Liter Amber											
Micro											
Other	Nitrate, 125- mL, frozen										

TEST ORDER (Check appropriate box) * Requires off-site analysis per FY15 WQCD-LSD MOU

METALS

TREC DISS TOTAL

- ☐ ☐ ☐ Aluminum
- ☐ ☐ ☐ Antimony
- ☐ ☐ ☐ Arsenic
- ☐ ☐ ☐ Cadmium
- ☐ ☐ ☐ Chromium
- ☐ ☐ ☐ Copper
- ☐ Hardness, Total
- ☐ ☐ ☐ Iron
- ☐ ☐ ☐ Lead
- ☐ ☐ ☐ Magnesium
- ☐ ☐ ☐ Manganese
- ☐ ☐ Mercury
- ☐ ☐ ☐ Nickel
- ☐ ☐ ☐ Potassium
- ☐ ☐ ☐ Selenium
- ☐ ☐ ☐ Silver
- ☐ ☐ ☐ Zinc
- ☐ _____

NUTRIENTS

- ☐ Nitrate/Nitrite
- ☐ Nitrogen, Ammonia
- ☐ Nitrogen, Kjeldahl
- ☐ Nitrogen, Total
- ☐ Phosphorus, Total

NEUTRALS

- ☐ Alkalinity, Total
- ☐ BOD/COD (circle)
- ☐ Chloride
- ☐ Conductivity
- ☐ Fluoride
- ☐ Nitrite or Nitrate (circle)
- ☐ Phosphate, Ortho
- ☐ Solids, Dissolved
- ☐ Solids, Total Suspended
- ☐ Solids, Total
- ☐ Sulfate
- ☐ _____

OTHER

- ☐ DOC
- ☐ TOC
- ☐ Cyanide, Direct
- ☐ Sulfide
- ☐ UV @ 254
- ☐ Nonylphenol
- ☐ _____

RADIOCHEMISTRY

- ☐ Radium 226
- ☐ Radium 228
- ☐ _____

MICROBIOLOGY

- ☐ Total Coliform, PA
- ☐ Fecal Coliform, MT
- ☐ E. coli, MPN
- ☐ _____

PHYTOPLANKTON (LAKES)*

- ■ Chlorophyll a mL 750
- ☐ Species composition & counts

PERIPHYTON (STREAMS)*

- ☐ Chlorophyll a
- ☐ Ash free dry mass
- ☐ Species composition & counts

TEST PANELS

- ☐ WQCD Routine Panel
- ☐ WQCD Trend Panel
- ■ CPW Panel
- ☐ Mining - Metals & Minerals
- ☐ Mining - Nutrients
- ☐ Waste Water Project
- ☐ Other _____
- ☐ Additional Parameters

DEPOSITION		CHAIN OF CUSTODY		
RELINQUISHED BY:	DATE/TIME:	RECV BY:	DATE/TIME:	
RELINQUISHED BY:	DATE/TIME:	RECV BY:	DATE/TIME:	
RELINQUISHED BY:	DATE/TIME:	RECV BY:	DATE/TIME:	
TEMPERATURE AT RECEIPT: °C	LSD Internet Address: http://www.cdphe.state.co.us/lr/		Form #251 - Revised: 06/19/2014	

Water Quality Sampling

Chlorophyll and Algal Toxin (Microcystins) Samples

Depending on Invasive Species Program priorities and resources, samples may be collected for algal toxin analysis, and/or for chlorophyll analysis. This protocol was developed by Davine Lieberman (Reclamation) and has not been implemented since her project concluded in 2010.

Microcystins are cyclic heptapeptide toxins produced by cyanobacteria (blue-green algae). Not all blue-green algae produce microcystins, but blue-green algae must be present for microcystins to be present.

Microcystins are responsible for periodic poisonings of humans and livestock from drinking fresh water where the blue-green algae are endemic. The toxins are selective for liver cells (hepatotoxic) and inhibit the proteins (phosphatase 1 and 2A) that control sodium secretion. These toxins have also been demonstrated to promote liver tumors. In 1996, microcystins caused 26 deaths in Brazil when contaminated reservoir water was used at a dialysis clinic (Jochimsen et.al. 1998).

There is also a connection between zebra mussels and blue-green algae. Once zebra mussels become established in a water body, there appears to be a greater presence of blue-green algae and therefore, increased levels of microcystins (Vanderploeg et.al. 2001, Raikow et.al. 2004, etc.).

Reclamation is testing for Microcystin LR algal toxin, which is produced by blue-green algae including *Microcystis spp*., *Anabaena spp*., *Nodularia spp*., and *Oscillatoria spp*.

Sample Collection

The sample collection process for the both of these is rather similar. Collect 1000 mL (1 L) of water from the lake/reservoir (using the PVC straw collection method described in the previous section) at the same sampling locations where you measure secchi depth and log water quality profile data (i.e. same sampling location where you conduct zebra/quagga mussel plankton tows).

In addition, if you ever observe signs of an algal bloom, collect an algal toxin sample. Blue-green algae could be present in water that is visibly discolored or that has surface scums. Colors include shades of green, blue-green, yellow, brown, or red. Water affected by blue-green algae blooms is sometimes so strongly colored that it can develop a paint-like appearance. Wear disposable shoulder-length exam gloves when collecting samples from an algal bloom to protect your skin.

In the Trimble GPS data dictionary for your water quality point, and on the Water Quality paper datasheet, be sure to mark that you collected an algal toxin sample.

Collection Procedure

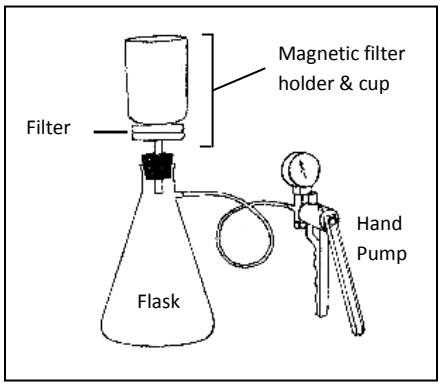


Diagram of filter apparatus (Adapted from http://www.umass.edu/tei/mwwp/chloro.html)

1. Assemble the filter apparatus: Use a 1 L Erlenmeyer flask with side-arm. Attach tubing to the flask side-arm, and then attach a hand pump to the tubing. Attach the magnetic 750 mL plastic cup to the flask. Place a Whatman 934-AH, 47mm, Glass Fiber filter between the cup and magnetic holder.

2. Use the PVC straw and bag collection method previously described to collect enough water for a sample. In an algal bloom, using less water will be effective, whereas incredibly clear water may need more water to create a sample. Somewhere between 350 mL and 2000 mL will be effective, use sampler judgment to determine the amount of water needed but **the amount filtered must be recorded**

3. Pour the sample water into the top of the filter apparatus. Use the hand pump to pull the water through the filter into the flask. Continue to pump until the filter is completely dry. Remove the top of the apparatus from the flask, and discard the filtrate (water).

4. Leave the filter on the pump apparatus, or remove it and set on aluminum foil, to allow the filter to dry for a few more minutes. The filter must be totally dry before packaging.

5. Fold the filter in half and blot on a paper towel. Wrap the dry filter in aluminum foil and then place it into the final empty centrifuge bottle provided from CPDHE. Indicate on the Profile datasheet the volume of water filtered, and affix the final barcode sticky to the vial. Place all bottles back into the kit provided, and place on ice as quickly as possible.

THE WATER CHEMISTRY SAMPLES ARE INCREDIBLY TIME SENSITIVE AND WILL NEED TO BE PLACED ON ICE IMMEDIATELY. AS SAMPLING CREWS ARE IN THE FIELD FOR EXTENDED PERIODS OF TIME, TAKE THIS INTO ACCOUNT AND PLAN ACCORDINGLY. BUY ICE EARLY AND OFTEN, ESPECIALLY DURING SUMMER MONTHS, OR YOU MAY END UP SAMPLING AGAIN.

HOBO Pendant Data Standard Operating Procedure

Purpose

To collect surface water temperature at waterbodies that have WID stations in the Very High to Medium risk category. The HOBO will log data all year and will be removed at the end of the season.

HOBO Launch Procedure

1. Ensure the *HOBO Pendant Data HOBO* is ready to be deployed by downloading the correct reading software to your computer (HOBOware) from this website.
   http://www.onsetcomp.com/hoboware-free-download.
   Select the "**Download for Windows**" button.

2. Once downloaded open the HOBOware and plug in the provided *Optic USB Base Station and Coupler* or *the Waterproof shuttle* to the computer.
3. Plug in the HOBO to the other end of the coupler by aligning the rigid edge. The HOBO communicates with the base station/shuttle via infrared light, thus allowing HOBOs to be completely sealed and waterproof.
4. Once the computer recognizes the device click the "**Launch device"** button located in the top left. From this screen you can you view the HOBO status, sensors, and deployment.
5. From the "**Deployment**" section change the settings as below. ***Leave everything else as is.***
   a. Name: Full Waterbody name, no spaces. (***VallecitoReservoir***)
   b. Logging Interval: **15min**
   c. Start Logging: **Using Coupler**
6. Click the "**Coupler Launch**" button and now remove the HOBO from the coupler by pulling straight out.
7. The HOBO will now have a blinking red light every **8 seconds** indicating it is ready to be activated in the field. Repeat this process for all HOBO's being deployed during that week.
8. Once all HOBO's are ready to be launched pull the coupler from the base station to take with you into the field. **You will use the coupler to activate the HOBO by placing it back into the coupler**. **Activation should be done on boat seconds before placing in the water!** *****IMPORTANT***** The activation is triggered by a magnet located on the coupler itself. Please keep the coupler and any other magnets away from the HOBO's to prevent premature activation

HOBO Deployment Procedure

1. Materials needed:
    a. **HOBO Pendant Data HOBO**
    b. **Coupler**
    c. **H-Bomb PVC Housing**
    d. **Heavy Duty Zip Ties**
    e. **Tape Measure**
    f. **Heavy Anchor**
2. Navigate to the WQ sampling location of your water body. You will be placing the HOBO on the substrate buoy at this location.
3. Pull up the existing substrate and attach either a larger anchor "rock" or additional weight to help the HOBO from disappearing. The anchor should be at least twice as heavy as our normal size anchors.
4. Make you are using the most current data dictionary (**ANS_2015_TSv15**). Record the appropriate GPS data in the **HOBODeployment** feature of the data dictionary. **This will include the serial number located on the inside of the HOBO on a white sticker above the battery.** Give the HOBO a unique ID starting with 05 but keep it unique with the running number.
5. Activate the HOBO by placing it into coupler for **3 seconds**. Pull out and confirm the light is **blinking every 4 seconds.**
6. Place the HOBO into the H-Bomb PVC Housing and attach it to the substrate **2 meters** down from the buoy. Please use a tape measure to get an accurate depth.
7. The H-Bomb PVC Housing is weighted and will act as a plumb bob to keep slack on the line below water as the water lever fluctuates and to keep the HOBO at a constant depth of 2 meters.
8. **The HOBO has now been deployed!**

HOBO Check and Shuttle Download

Preparation for Field

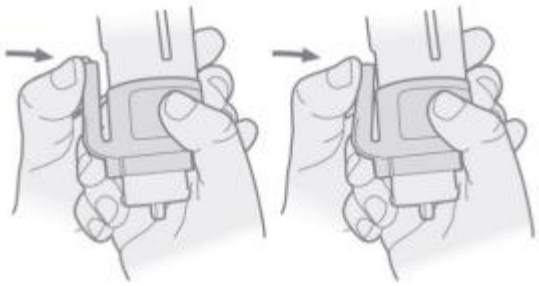
1. You will need the *HOBO Waterproof Shuttle* to download data off the *HOBO Pendant Data HOBO* when in the field.
2. Before going in the field you must launch the Shuttle in HOBOware on the computer. Use the appropriate USB cable. Unscrew the center cap on the shuttle. If the cap is too tight to loosen by

hand, insert a screwdriver through the lanyard hole and rotate counterclockwise until the cap is loosened.

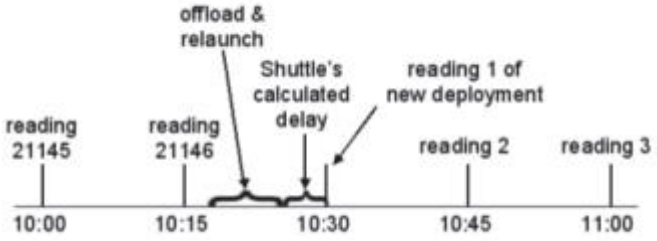
3. Open HOBOware. Once the computer recognizes the device click the "**Launch device"** button located in the top left. Select the appropriate device from the pop-up menu.
4. From this window you will be able to manage all files on the Shuttle.
5. HOBOware will automatically sync and prepare for launch but make sure the *Shuttle* clock is synced with the computer by clicking the "***Sync Shuttle Clock****"* button.
6. The shuttle's clock is used to set the HOBO's clock at relaunch. ***For most accurate results, make sure the host computer's clock is correct before launching the shuttle. If you need to adjust the computer's clock, quit HOBOware, set the computer's clock, then reopen HOBOware and launch the shuttle. ** It is also good to use the same computer to launch the Shuttle and the HOBOs.***
7. Check to make sure the Shuttle has plenty of battery power. * If you change the batteries in the field, the HOBO's clock will stop, and the shuttle will not read out the HOBO again until you relaunch it in HOBOware.
8. When "***Shuttle Launched Successfully"*** pops up in the center of the window you are ready for the field. Click the "***Close****" button.*
9. The shuttle is now ready for data download in the field!

Data Download in the Field

1. Collect the appropriate GPS data in the **HOBOCheck** feature. It will ask if the HOBO is present and if you were able to successfully download the data.
2. You will need the Shuttle and the "*Coupler for Pendant*" to successfully download the data from HOBO.
3. Pull the substrate out of the water and remove the HOBO from the H-Bomb housing. ***Be careful not to drop or smash the HOBO as it could delete all the data!**
4. Plug the coupler into the Shuttle and plug the HOBO into the other end of the coupler. Make sure everything is firmly seated.
5. Momentarily press the coupler lever (pressing hard enough so the lever bends). The amber Transfer light should begin blinking.



6. Once amber light stops blinking the green OK light will start blinking indicating the download was successful.
7. Press the coupler lever again to stop the green OK blinking light and remove the HOBO.
8. After downloading from the HOBO, the shuttle synchronizes the HOBO's clock to the shuttle's internal clock and relaunches the logger *** The HOBO is launched with a slight delay that causes its readings to be synchronized with those of the previous deployment. It will blink at 8 second intervals until this delay is over then back to 4 second intervals.**



9. You have now downloaded the data from your HOBO**!** **Confirm the HOBO is blinking red in 4 second intervals after the delay** and place it back into the H-Bomb housing and into the water.
10. When you return to the office, copy and paste the HOBO data from your desktop into the subsequent folder on the (Q:)drive. File path here.
    Q:\Invasive_Species\A.N.S\Sampling_Monitoring\Sampling Data\HOBO 2016

Shuttle Data Offload to Computer

1. Connect the shuttle to a host computer running HOBOware. Open HOBOware.
2. Click the "***Readout"*** button on the top left and select the appropriate device.
3. The Shuttle Management window will pop-up. All files that have not be previously offloaded will be check.
4. Make sure to save them in the appropriate folder here.
   Q:\Invasive_Species\A.N.S\Sampling_Monitoring\Sampling Data\HOBO 2016

5. In the "***Datafile Name***" field add the date you are offloading to the end of the file name as shown here (WaterbodyYYYYMMDD)
6. Once all files have been renamed with the date click the "***Save Checked***" button.
7. Now you can click the ***"Launch Shuttle"*** which will remove any data on the shuttle and be ready to download data again from the next waterbody!

When returning to the water body to check the substrate please try to keep the HOBO in the water so we do not get any inaccurate readings. The HOBO will be logging data the entire season and will be removed sometime in October or November.

Watercraft and Equipment Decontamination

Equipment decontamination is mandatory to avoid transporting ANS (animals, plants, and pathogens) between water bodies and sampling locations.

Watercraft Decontamination

All sampling crew members must attend a state-sponsored Watercraft Inspection and Decontamination training class and be certified annually in both inspection and decontamination.

Sampling crew members must **fully** decontaminate their boats and trailers using a combination of hot water and high or low pressure, following the state protocol detailed in the *CPW ANS Watercraft Decontamination Manual* (2015). Sampling boats and trailers must be fully decontaminated between each water body.

Equipment Decontamination

All sampling equipment must be fully decontaminated in accordance with state protocol between each water body. In certain situations (e.g. mapping ANS populations, surveying in areas with New Zealand mudsnails, doing multiple shoreline surveys in different reaches of a watercourse, etc.) all equipment must be fully decontaminated between each sampling location so that ANS are not spread.

Decontamination Supplies

- Decontamination unit for hot water decontaminations.
- Decontamination solution for chemical decontaminations.
- Clean tap water (do not use lake or stream water) to make the decontamination solution and for rinsing equipment.
- Large tub or bin for submerging equipment.
- Stiff bristle scrub brush.
- Rubber gloves – It is recommended that you wear rubber gloves when handling quaternary ammonia.

Equipment Decontamination Locations

Conduct hot water decontaminations in a "high and dry" location, where the decontamination water will not run off into any water body. Chemical decontaminations must be conducted at least 60 meters (200 feet) away from a water body. Decontamination solutions must be properly discarded away from any water body, into a drain that goes to a wastewater treatment facility.

Note: Water quality meters should only be decontaminated with water. Chemical decontamination may damage the sonde.

Equipment Decontamination Options

- Hot water – Refer to the *CPW ANS Watercraft Decontamination Manual*.

  Note: Hot water is the preferred method of decontamination. It is preferred that equipment be submerged in hot water first, so that they may remain submerged while the boat and trailer are being decontaminated.

  - Plankton Net and Cod End
    1. Remove the cod end from the plankton net. Thoroughly rinse the net, rope, and cod end with clean water. Remove all debris, mud, and plant material.
    2. Place the net, rope, and cod end in a large tub or bin.
    3. Fill the bin with 140°F water at low pressure until all equipment is completely submerged.
    4. Do not spray the cod end directly with the decontamination wand, because it will damage the mesh screens.
  - Water Quality Meter
    1. Thoroughly rinse the cord, sonde, guard, and storage cup with clean water. Remove all debris, mud, and plant material.
    2. Place the cord, sonde, guard, and storage cup in a large tub or bin.
    3. Fill the bin with 120°F water at low pressure until all equipment is completely submerged.
    4. After decontamination, fill the storage cup with about 0.5 inch of deionized water (do not use lake water) to prevent the sonde from drying out. Attach the storage cup to the sonde.
  - Equipment - Secchi Disk, Ropes, Waders, Boots, Dip Nets, Crayfish Traps, Anchor, etc.
    1. Thoroughly rinse equipment with clean water. Use a stiff bristle scrub brush to remove all debris, mud, and plant material.
    2. Place the equipment in a large tub or bin.
    3. Fill the bin with 140°F water at low pressure until all equipment is completely submerged.

- Quaternary Ammonia (*CPW Quaternary Ammonia Compound Disinfection Protocols,* 2015)
  - Bath Disinfection Recommendations for Submersion of Small Gear and Waders:
    1. Prior to disinfection, clean debris, mud, and vegetation off of equipment and waders.
    2. Muddy disinfectant solution can lose its effectiveness and capacity to kill invasive organisms.

3. Visually inspect waders and equipment for New Zealand mudsnails and other invasive aquatic organisms prior to cleaning.
4. The recommended minimum active QAC concentration for effective disinfection is 0.4% or 4.0 ml of QAC per L of water; amount of disinfectant per gallon varies, and is dependent upon the percent active QAC in the disinfectant being used (Table 1).
5. Equipment and waders should be submerged in disinfectant solution for a minimum of 10 minutes.
6. Follow all handling instructions on disinfectant label or Material Safety Data Sheet (MSDS).Vinegar solution

- Spray Disinfection Recommendations for Cleaning off Small Gear and Waders using Disinfectant Spray:
    1. Prior to disinfection, clean debris, mud, and vegetation off of equipment and waders.
    2. Visually inspect waders and equipment for New Zealand mudsnails and other invasive aquatic organisms prior to cleaning.
    3. The recommended minimum active QAC concentration for effective spray-application disinfection is twice that for submersion disinfection, 0.8% or 8.0 ml of QAC per L of water (Table 2).
    4. Equipment and waders should be fully covered in disinfectant solution for a minimum of 10 minutes. Reapplication may be necessary if hot (evaporative) or wet conditions dilute spray solution on equipment.
    5. Follow all handling instructions on disinfectant label or MSDS.

- Drying
    - Dry equipment for a minimum of 10 days in between each use.
- Freezing
    - Place equipment in a freezer overnight between each use.

Appendices

A. Water Body Risk Assessment

B. Datasheets and Definitions

C. Field Equipment Lists

D. Specimen Collection Protocol

E. Field Activities Checklist

F. ANS Biological Information and Maps

G. ANS Laws and Regulations

H. GPS and Data