PROTOCOLS

PLANKTON TOW PROTOCOLS

The horizontal plankton tow technique is typically performed from the convenience of a boat dock or shoreline, but can also be achieved from a boat. Our recommended plankton tow protocol focuses on collecting a sample from a large volume of water to capture target dreissenid veligers and/or their environmental DNA (eDNA). The plankton tow net allows the filtering of thousands of liters of water through fine pore size mesh in a dolphin bucket, which captures planktonic larvae and other compound particles. The material in the dolphin bucket is then preserved in a provided sample bottle with 95% ethanol and transferred to a lab for PCR, microscopy analyses, or both.

Horizontal Plankton Tow Protocol:

1. Put on a clean pair of latex or nitrile gloves.
2. From an appropriate surface (boat dock, seawall, shoreline), measure out a length of 100 meters to be sampled. If the shore to be measured is shorter than 100 meters, doubling back to a total of 100 linear meters is acceptable.
3. At the start of the transect, proceed to dip the net until the net is completely submersed under water with the net's rim being vertical. At a slow and steady pace continue on for the 100 meters. The pace should be fast enough that the water filters through the net but slow enough as to not let water back flush out of the net. Prevent large air bubbles from getting into the net and bucket, as this prevents proper collection.
4. At the end of 100 meters, swiftly lift the net straight up out of the water and hold until most of the water drains from the net through the dolphin bucket.
5. Quickly sweep the net opening through the water 2 more times to flush all organic material on the net sides down through the dolphin bucket.
6. Remove the dolphin bucket, and remove excess water by tapping water out through the screen.
7. Using the ethanol squirt bottle, dislodge as much organic material as possible with a small amount of ethanol from the screens to the insides of the dolphin bucket. Use the provided %95 EtOH preservative. Avoid using denatured EtOH or isopropyl alcohol as these may interfere with analysis and may cause health concerns.
8. Swish the contents to mix and transfer as much organic material as possible to the 50 mL sample bottle.
9. Store tubes upright, covered and cool - preferably on ice. Do not freeze plankton samples. Freezing damages shells and reduces detection sensitivity.
10. Record sample information (GPS location, sample #, date, sampler, lake name) on data forms and bottle labels. Sample vials should be pre-labeled whenever possible to avoid cross-contamination, and carefully wrapped with clear packing tape. Ethanol will cause any label ink to bleed, and the packing tape protects the ink.
11. Rinse all field and personal gear well before leaving the site.
12. To decontaminate the net and dolphin bucket, soak them in a 50% vinegar (acetic acid) solution for at least 4 hours, mixing often. Acetic acid is a safe way to dissolve the dreissenid shells, as remnant shells may lead to false positives.
13. Rinse net and dolphin bucket with copious amounts of water to free of excess vinegar.

The sampling data provided to us on your data form will be entered into Montana Fish, Wildlife & Park's AIS and water quality monitoring application (Survey 123 APP) which facilitates accurate and consistent data collection amongst participating groups. The sample analysis and location information is posted to the FWP GIS and Montana Heritage Program websites where partners and the public are able to view their sample collection data and the results from any early detection samples that were collected and submitted in conjunction with the app. This is part of a larger goal to increase early detection monitoring on lakes throughout the state.

AQUATIC INVASIVE SPECIES (AIS) VISUAL MONITORING PROTOCOLS

Please spend some time reviewing AIS information online and in the provided AIS guide before going into the field. Montana Fish, Wildlife & Parks' (MTFWP) website has a complete list of aquatic invasive species found in Montana.

When doing visual or tactile observations for zebra or quagga mussels, inspect rocks near the shorelines, dock pilings, retaining walls and other hard surfaces that could provide a place for mussels to attach. Please attempt to get a GPS location. If you suspect a positive sighting, please contact MTFWP immediately (Region 1 at 752-5501. They will then coordinate with partner agencies to further evaluate the situation. Photos are helpful as well.

WATER QUALITY MONITORING PROTOCOL

Sampling timing and location

Lake data measurements pertaining to physical conditions and visual observations are among the most important parameters that can be monitored for lake health trends. This includes temperature, Secchi disk depth, and the presence/absence of AIS. Qualitative atmospheric and water condition data can also be recorded. Ideally lakes are sampled at a designated mid-lake, deep location twice per month, at roughly two week intervals. Midday (11 am – 3 pm) is the best time to insure optimum sunlight penetration, regardless of cloud cover. Within that four-hour period, random times for observations are preferable to always using the same exact time. GPS coordinates should be recorded for each monitoring site, and data should be collected consistently at the same location.

SAMPLING PARAMETERS

Water Temperature

Water temperature plays a significant role in the stratification of lakes. Most of the lakes in northwest Montana are *dimictic*, meaning they mix twice per year. Water is most dense around 39ºF and is less dense at temperatures above and below 39ºF. After the ice melts off in the spring, the surface temperature of a lake begins to increase until it reaches 39ºF. When this happens, the surface water sinks and mixes with the water below it. This is known as *spring turnover*. In the fall, the same thing happens only the water is cooling to 39ºF. Some program lakes are polymictic, meaning they may mix and stratify multiple times a year based on meteorological conditions.

Water temperature and dissolved oxygen are inversely related. As water temperature goes up, the amount of oxygen goes down. Oxygen is produced through plant metabolism (*photosynthesis*), and is consumed during respiration and decomposition. Oxygen in lake water is also influenced by wind and wave action through weather events and the exposure of surface water to the atmosphere. An adequate supply of dissolved oxygen (DO) in lake water is essential to fish and other aquatic life forms.

Taking Water Temperature

- Make sure that the measurement end of the thermometer is 18 inches from the bobber or lake surface.
- Place the thermometer in the lake and allow it to remain submerged while you are taking the Secchi disk measurement.
- Remember to attach the end of the thermometer string to the boat so it doesn't float away.
- Remove the thermometer from the water and record the temperature on the field data form.

The Secchi Disk

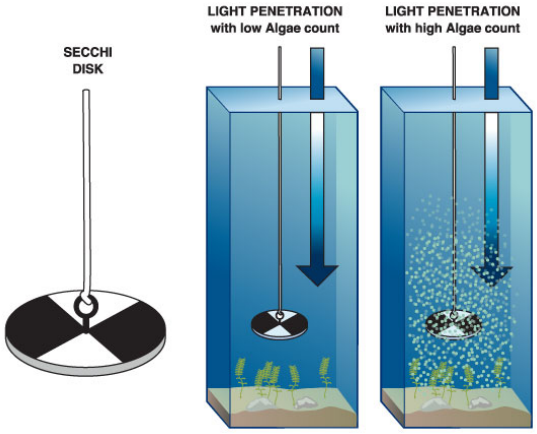
The Secchi disc is a black and white quadrant weighted disk attached to a measuring tape that is used to determine lake transparency or water clarity. The Secchi disk is one of the least expensive and easiest pieces of equipment to use for monitoring lakes. Named after Fr. Pietro Angelo Secchi, a scientific advisor to the Pope, the Secchi was first

used in the mid-19th century to measure the transparency of the Mediterranean Sea. Since then, the Secchi disk has been used to measure transparency of water bodies throughout the world.

The Secchi disk tells scientists a few things. First, a Secchi reading indicates the amount of light penetration into a lake. Because many living organisms in a lake depend on sunlight, the Secchi reading is an important indicator of the biological health of a lake. The Secchi reading is also a measure of the amount of suspended material or algae in a lake. The amount of suspended algae is important in determining the trophic status of lakes (amount of nutrients present), and repeated consistent Secchi data gives scientists an idea of water quality trends occurring in the lake.

Using a Secchi Disk

- Travel to your monitoring site and anchor the boat if possible.
- Remove your sunglasses to reduce glare. If you are wearing prescription sunglasses, please make note of that on the monitoring form.
- Use the shaded side of the boat and your body to reduce surface glare and to improve visibility.
- Lower the Secchi disk slowly into the water until it disappears.
- Record on the field data form the depth at which the Secchi disk disappears.
- Lower the disk an additional five feet then slowly retrieve the disk until it reappears.
- Record on the field data form the depth at which the disk reappears.
- Average the two depths and record the depth on the field data form.
- This process can be repeated to ensure accuracy.



AIS BACKGROUND

Zebra and quagga mussels have devastated waterways and water systems throughout the United States and were recently found in Montana reservoirs. Once established, zebra and quagga mussels are impossible to completely eradicate. They also have the ability to reproduce and spread rapidly. One female zebra mussel is capable of producing up to one million eggs per year. It is important to do everything you can to help prevent the spread of these invasive non-native mussels. Never travel from one lake to another without completely decontaminating your boat and equipment.

A Portland sampler that has been colonized by zebra mussels

Please be on the lookout for AIS, including zebra and quagga mussels, New Zealand mudsnails, Eurasian watermilfoil, curly-leaf pondweed and flowering rush. Zebra mussels reproduce when water temperatures exceed 50 degrees Fahrenheit, and the microscopic *veligers* (mussel larvae) are most abundant in late July through August.

Small zebra mussels will feel like sandpaper when you run your fingers over them. Adult zebra mussels are typically ¾" long. Zebra mussels will attach themselves to plants, rocks, boat docks or any other hard surfaces. There are native mussels that live in northwest Montana lakes; however native mussels lack the ability to attach themselves to substrates.

Eurasian Watermilfoil (EWM) is a plant that roots to the bottom of water bodies. EWM can grow up to 21 feet tall from the bottom of a lake and can spread very easily because it reproduces through stem fragmentation. This means that even a small piece of EWM the size of a thumbnail transported on a boat and launched in another lake can reproduce and spread rapidly. EWM is an especially large threat to lakes in northwest Montana because it is already present in several locations throughout Montana including Beaver Lake in Whitefish and Noxon Reservoir in Sanders County. Other AIS found locally include curly-leaf pondweed, flowering water pond lily, yellow flag iris, and flowering rush.

WHAT YOU CAN DO TO PREVENT THE SPREAD OF AIS

CLEAN. DRAIN. DRY.

CLEAN. Completely remove all mud, water, and vegetation before leaving the access area.

- Inspect your boat, trailer, and all gear. Pay attention to crevices and hidden areas.
- Remove all vegetation (by hand or sprayer).
- Remove all mud (use a pressurized power sprayer, found at most do-it-yourself car washes). The hot water kills organisms and the pressure removes mud and vegetation. No need to use chemicals or soap.
- Dispose of debris in trash or on dry land away from water or ramp.

DRAIN. Drain all water from watercraft and equipment.

- Drain or remove water from boat, bilge, live well, engine, internal compartments, and bait buckets by removing drain plugs before leaving the access area.

DRY. Aquatic invaders can survive only in water and wet areas.

- Dry your watercraft and fishing equipment thoroughly; this will kill most invasive species. The longer you keep your watercraft, trailer, waders, and other equipment outside in the hot sun between fishing trips, the better.

Help spread the word about AIS and encourage friends to follow decontamination procedures. If you suspect AIS in your lake, immediately contact Montana Fish Wildlife and Parks Region 1 at 752-5501.