FWP.MT.GOV THE OUTSIDE IS IN US ALL.

Aquatic Invasive Species Early Detection and Monitoring Program

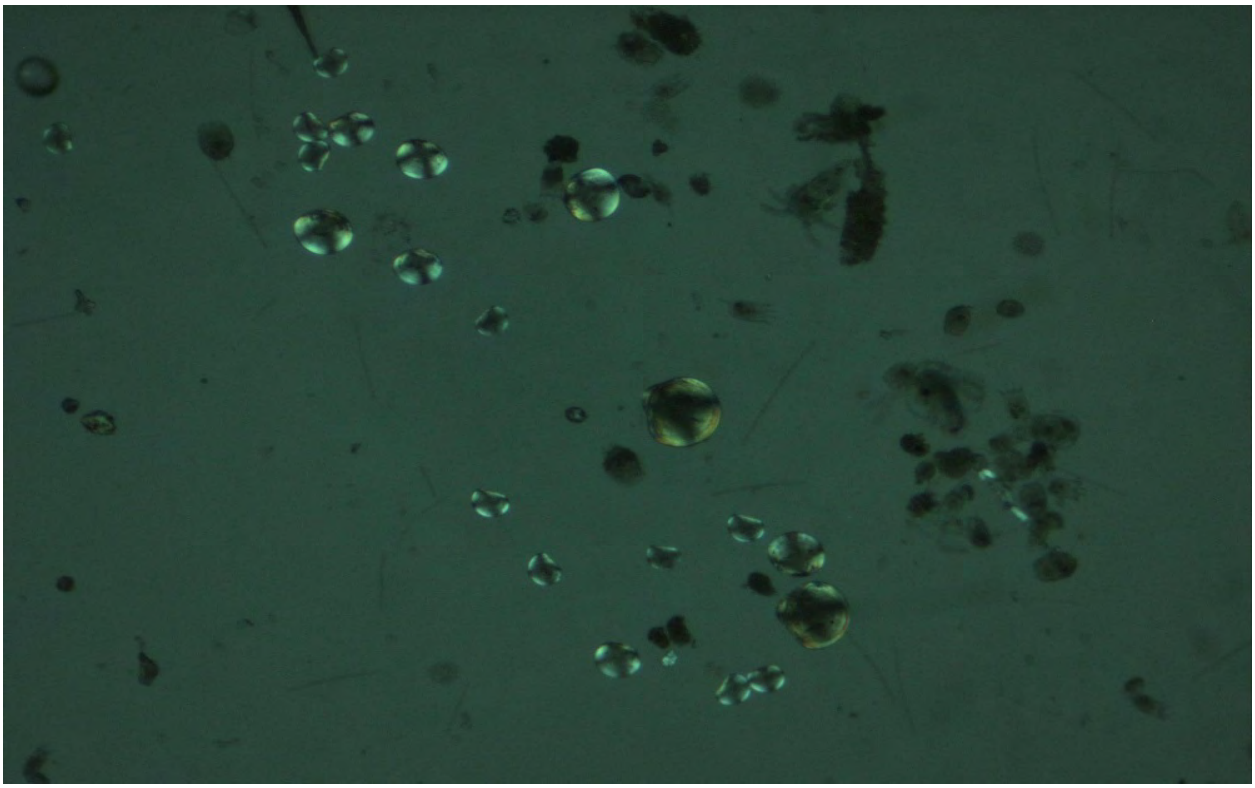

Figure 1: Dreissenid mussel veligers under cross polarized light (4.5x magnification).

Laboratory Standard Operating Procedures Spring 2019

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Contents

Purpose

The purpose of this document is to give clear, concise instructions for receiving, processing and reporting results from water samples sent to the Montana Fish, Wildlife & Parks (FWP) Aquatic Invasive Species (AIS) early detection laboratory. Montana Fish, Wildlife and Parks Aquatic Invasive Species Laboratory processes plankton samples for the agency, in-state partners, and Missouri River Basin states. The primary purpose of the lab is to detect new populations of larval Dreissenid mussel (zebra and quagga mussel veligers) in plankton samples. However, the lab also identifies Asian clam larvae in Montana samples.

Mussel Biology

Early detection of invasive species requires the detection of an often-rare species. Early detection of a rare species in an aquatic environment is often difficult. Montana utilizes the plankton sampling technique to try to locate populations of invasive mussels early since often the free-floating (planktonic) larvae of invasive mussels (veligers) are easier to find than a population of adult mussels which attach to substrate. This is the primary early detection technique of adult invasive mussels. Of the most commonly used techniques in early detection of invasive mussel veligers, this technique has proven to be the most reliable (Frischer, Kelly, & Nierzwicki-Bauer, 2012). In order to better understand why this technique is useful, one must understand the biology of invasive mussels.

Quagga mussels (*Dreissena bugensis*) and zebra mussels (*Dreissena polymorpha*) are freshwater, bivalve (2-shelled) mollusks that have invaded North American waters. Both species are similar and hard to distinguish in how they look and live, yet both pose similar, serious threats to waters they are introduced into. They are both

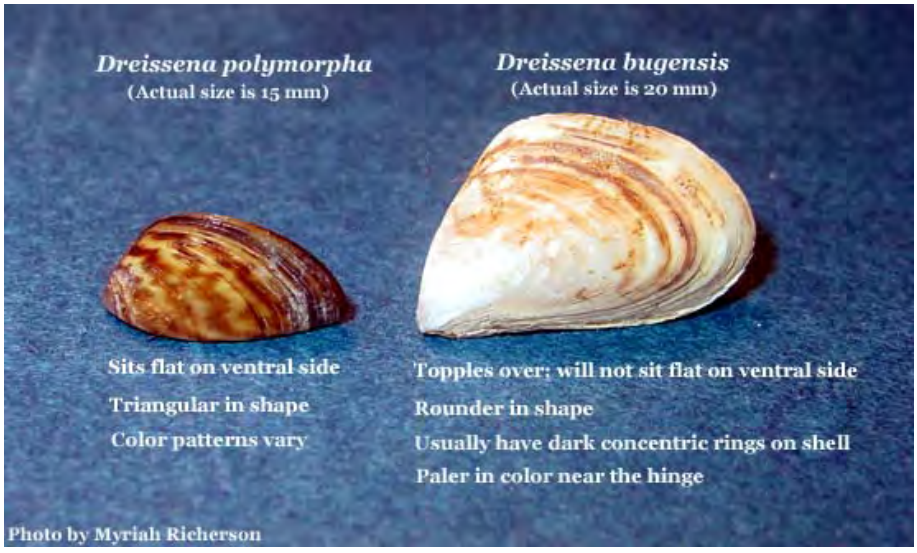


Figure 2: Side by side comparison of adult zebra (left) and quagga mussel (right). USGS.

destructive, invasive aquatic species that can grow to about an inch in diameter. Their larval stage is microscopic and cannot be seen without the aid of a microscope. Adults attach to mostly hard surfaces (using byssal threads) and often have black, cream, or white bands and sometimes have dark rings on their shells almost like stripes. No native species of mussel or clam in Montana attaches to surfaces.

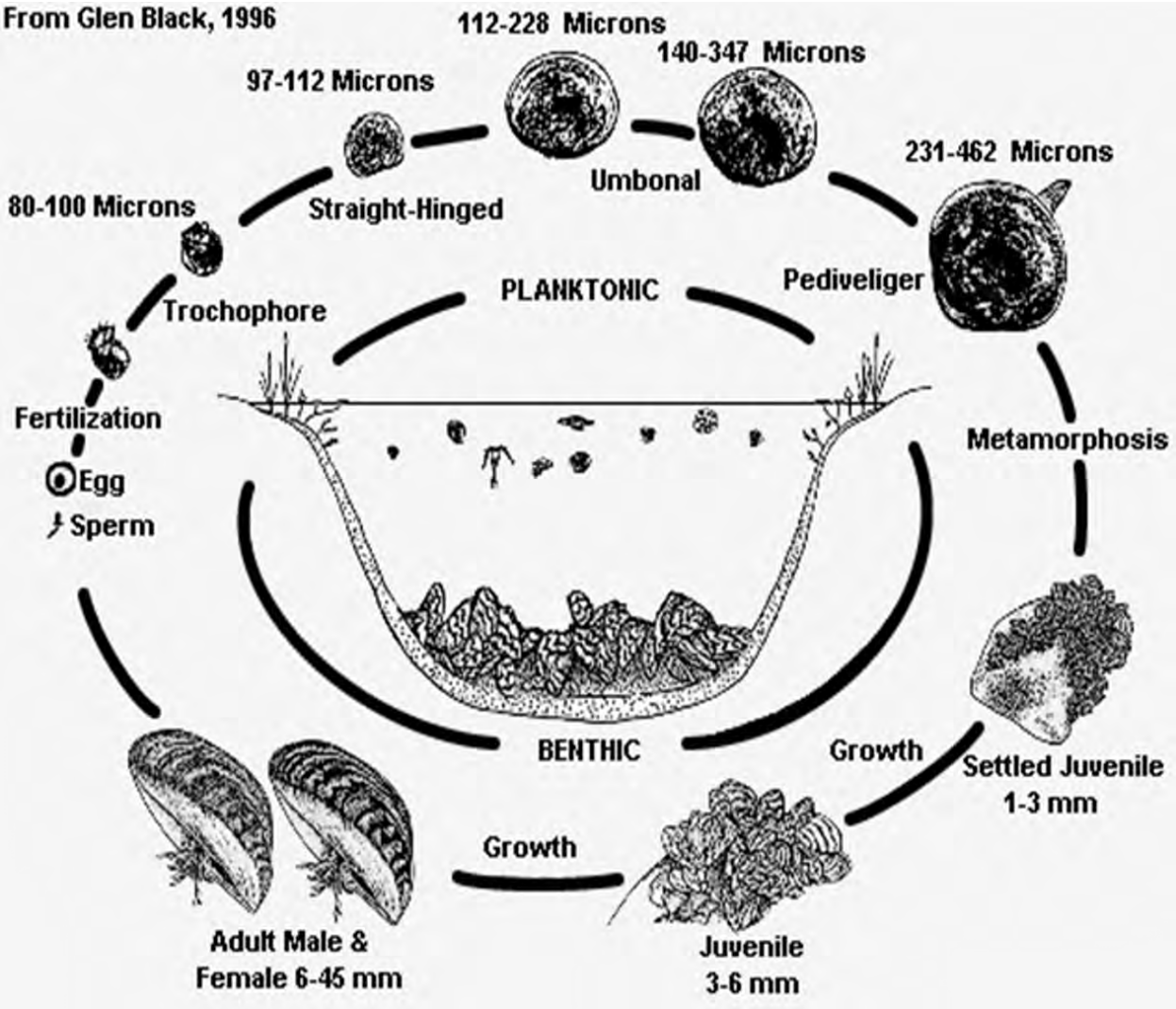


Figure 3: Dreissenid mussel reproductive cycle and development.

Quagga mussels are native to the Dneiper River drainage of the Ukraine and Zebra mussels are native to the Caspian, Black and Azov seas of Eastern Europe. Both species were discovered in the Great Lakes in the late 1980's. It is believed they arrived in North America via ballast water discharge releasing their microscopic larval stage. From the Great Lakes, these mussels move overland primarily through human related activities. Their larval stage also can move passively downstream in a system. They

attach to hard surfaces and can survive out of water for up to week (depending on weather conditions). Their microscopic larvae can be transported in bilges, ballast water, live wells, or any other equipment that holds water.

Both species of mussels are filter-feeders that consume large portions of the microscopic plants and animals that make up the base of the aquatic food web. The removal of these organisms from the food web can cause a shift in the native species in the system and the disruption of the ecological balance of the lake or reservoir. Quagga mussels are more efficient at it than Zebra mussels.

They often settle in massive colonies that can block water intake and affect municipal water supply and agricultural irrigation and power plant operation. In the United States, congressional researchers estimated that zebra mussels alone cost the power industry $3.1 billion in the 1993-1999 period, with their impact on industries, businesses, and communities more than $5 billion. Management costs are enormous, particularly for industrial raw water users like power stations and water supply agencies.

If adults attach and grow on the outside or inside of boats, they can restrict cooling systems, ruin motors, increase drag on the bottom of a boat, reducing speed and wasting fuel, jam steering equipment, and require scraping and repainting of boat bottoms. They can also damage other aquatic recreational equipment, such as colonizing docks, boat ramps, piers, pilings, water intakes and fish screens.

These invasive mussels can live for 3-5 years and can release 30,000 – 40,000 fertilized eggs in a breeding cycle and one million fertilized eggs in a year. Their potential for spread is very high once introduced. Once established, eradication is next to impossible although many control technologies are being researched.

Male and female sexes of mussels are separate, so you need both a male and a female to reproduce in a system. See Figure 2 below. Fertilization occurs in the water column – both sexes release sperm and eggs into the water column. After fertilization, the larval stage of the mussel develops (called a veliger). This stage is free-floating in the water column and does not swim and stays in the water column for up to 3-4 weeks. These stages are microscopic and cannot be seen by the naked eye. Therefore, moving water is a large concern. The end stage of the veliger is called a pediveliger and this is when the veliger grows a "foot," settles out of the water column, and crawls along the bottom to find a place to attach – they prefer harder attachment sites. This stage also lasts up to 3 weeks. Once attached, they grow into the more familiar mussel shape and just look

like, small juvenile mussels. This is called the plantigrade stage. They can be seen by the naked eye at this stage. This stage lasts up to 4 weeks. As a juvenile mussel, they will continue to grow for a few weeks more (up to 5) based on water quality and temperature and can reach sizes up to two inches in diameter but averaging one inch. Females are generally sexually mature after one year. Spawning is limited by water temperature; the ideal temperature for spawning occurs when temperatures rise and stay above 48˚F. Therefore, most of the activity in the Montana AIS lab occurs in the summer months. Optimal larval development occurs at even warmer temperatures: about 68-72˚F.

It is good to keep in mind that Quagga mussels in general can tolerate lower temperatures, and no one really knows how either species will behave in Montana waters. There really are no comparable systems with Dreissenid invasions.

They have few natural predators in North America. It has been documented that several species of fish and diving ducks have been known to eat them, but these species are not an effective control. In some cases, the mussels concentrate botulism toxin causing bird die offs.

In general, they can be found at any depth. Quagga mussels can be found deeper than zebra mussels.

- Zebra mussels are typically found from just below the surface to about 12 meters (40 feet).
- Quagga mussels are typically found at any depth if oxygen is present
- Both species prefer to avoid light and are usually found in shaded areas or below the depth that light penetrates water.
- In Montana, winter drawdown and freezing work in our favor. Neither species can survive freezing.

Water Quality Limiting Factors:

- Temperature tolerance in general is 33-86˚F.
- Salinity needs to be low: <5 PPT.
- Calcium levels need to be high: >25mg/L.
- pH needs to be high: in the range of 7.4-9.5.
- Oxygen: both species can temporarily survive low oxygen concentrations.
    - Zebra mussels need >25% of full oxygen saturation to grow and reproduce.

- Quagga mussels can tolerate low oxygen concentrations better than zebra mussels.
- Water velocity: needs to be low, <2 m/sec.
- Substrate: both species prefer hard surfaces.
  - Quagga mussels can tolerate living in soft sediments, but zebra mussels seldom do.

Larval Life Cycle Stages

It is important to understand larval growth stages when looking for veligers. There are four shelled larval stages: D-shaped or straight-hinged, umbonal, pediveliger, and plantigrade. These larval stages are defined based on hinge development, shell shape, shell size (Tables 2 and 3), and the presence or absence of a foot and velum. Determining the type of veliger is the first step towards identification and detailed procedures are provided by Nichols and Black (1993). Corbicula veligers are found only in the first two larval stages within plankton before they become too heavy and settle out of the water column. See Appendix J for a key to the identification of veligers from Nichols and Black, 1993.

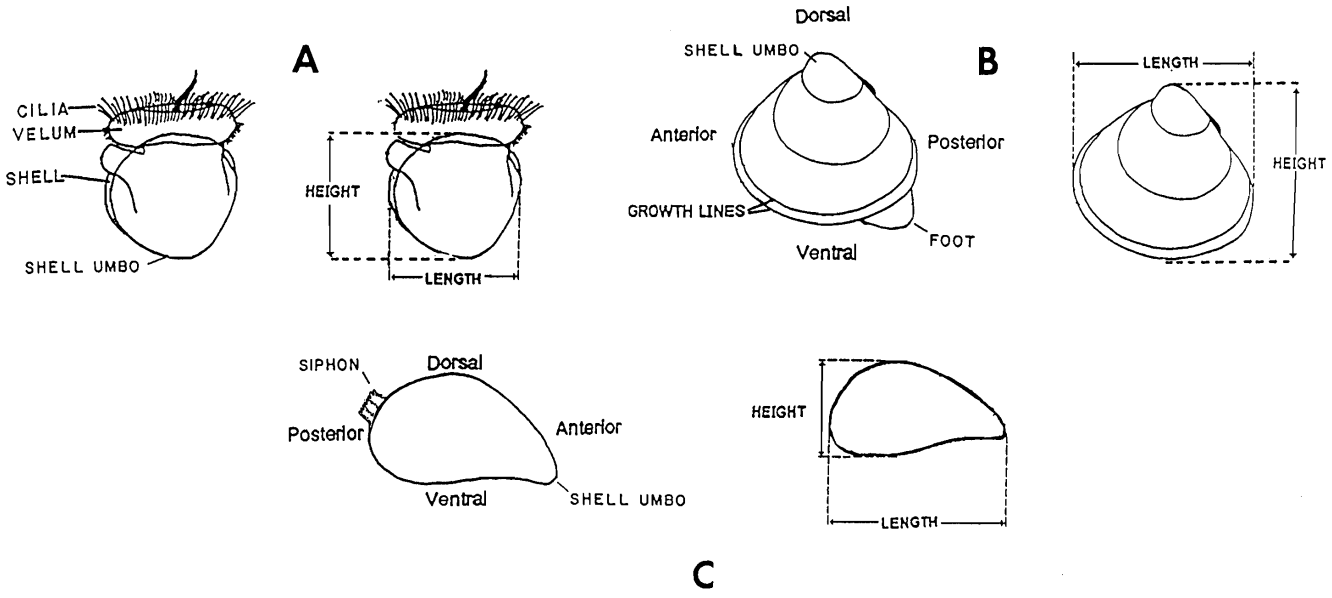


FIG. 1. (A) Veliger. (B) Clam-shaped larvae and juveniles. (C) Mussel-shaped juveniles.

Figure 4: Veliger anatomy (Nichols & Black, 1993).

D-shaped (straight-hinged) larval stage

This first shelled larval stage is most commonly encountered in plankton samples. The straight-hinge makes entire shape appear as a "D". Corbicula veligers are much larger than Dreissenids (Tables 2 and 3). Faint growth lines may be visible with Dreissenids. Distinct growth lines are present on Corbicula, and there may be secondary lines that run perpendicular to the growth lines that may give a pleated appearance.

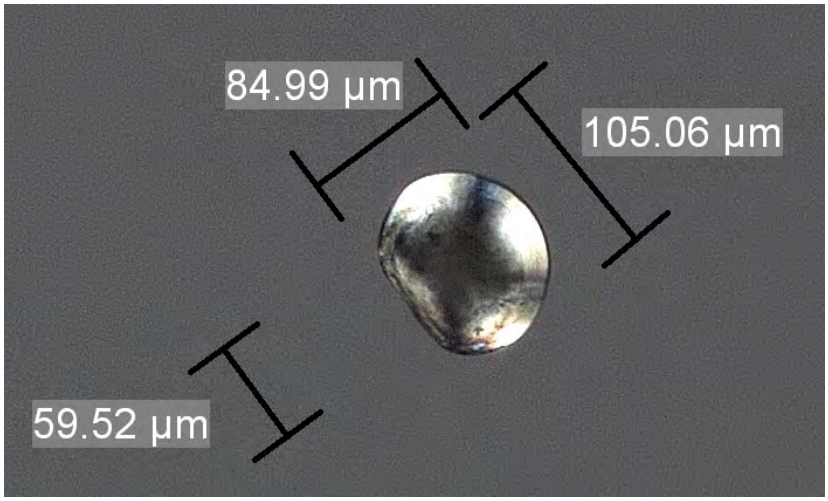


Figure 6: *D. bugensis* (D-shaped)

Umbonal larval stage

The second larval stage is characterized by an increase in growth and the development of a clam-shaped shell. The clam shaped shell is due to the development of an umbo, which protrudes from the center of the hinge line. During the umbonal stage, however, the umbo is low and rounded and does not protrude above the shell line. Growth lines may be visible with Dreissenids. Very distinct growth lines and perpendicular lines in Corbicula.

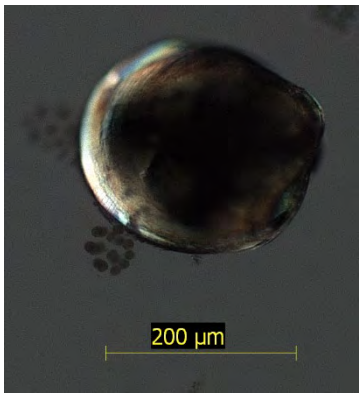


Figure 5: *D. bugensis* round shape with umbo development

Pediveliger larval stage

Corbicula not present in plankton in this or later stages. Pediveliger Dreissenid veligers have well developed and prominent umbo, clam-shaped. Quagga veligers may be slightly asymmetrical. Shell is transparent, growth rings should be visible.

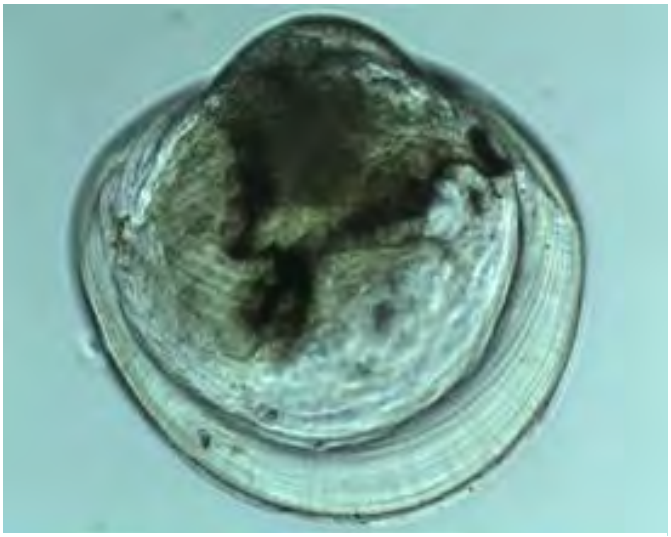


Figure 7: D. bugensis pediveliger (247 µm length).

Plantigrade larval stage

Corbicula not present in plankton. Dreissenid veligers begin to exhibit the mussel-shape. The axis of growth changes during this stage (shell shape changes from roundish to elongate). Early

plantigrade veligers are clam-shaped with unequal shoulders and a bump on one shoulder. The shell is more mussel-shaped in late plantigrade veligers.

Lab Methods

Montana utilizes cross polarized light microscopy (CPLM) as one of its primary early detection techniques according to widely accepted standards set by the Western Regional Panel (Western Regional Panel on Aquatic Nuisance Species, 2018) and (Western Regional Panel on Aquatic Nuisance Species, 2018). Cross-polarized light is one of the simplest and most efficient methods for distinguishing between items found in planktonic samples. Bivalve larvae are one of the few birefringent (optical property of a material having a refractive index that depends on the polarization and propagation direction of light) objects found in plankton samples. Polarized light is used to quickly detect mineralized material (such as a shell) in the sample. Larvae are birefringent due to the crystalline calcite structure of the larval shell and glow as bright spots under polarized light (Figure 1). Because of the arrangement of the calcite crystals, portions of the shell in line with the axes of the cross-polarizing filters do not reflect the light and thus the veligers appear with a small, glowing Maltese cross. A Maltese cross is a cross with arms of equal length that broaden from the center and have their ends indented in a shallow "V" shape. Corbicula larvae, other Dreissenid larvae, ostracods (seed shrimp, a type of microscopic, freshwater crustacean), and glochidia (the microscopic larval stage of Montana's native Unionidae and Margaritiferidae mussels) must be distinguished based on morphology, behavior, size, shape, or other features. However, given the quantity of extraneous material that may be present in a plankton sample, cross-polarized light provides a simple way to narrow the range of possibilities. (USACE, 2001)

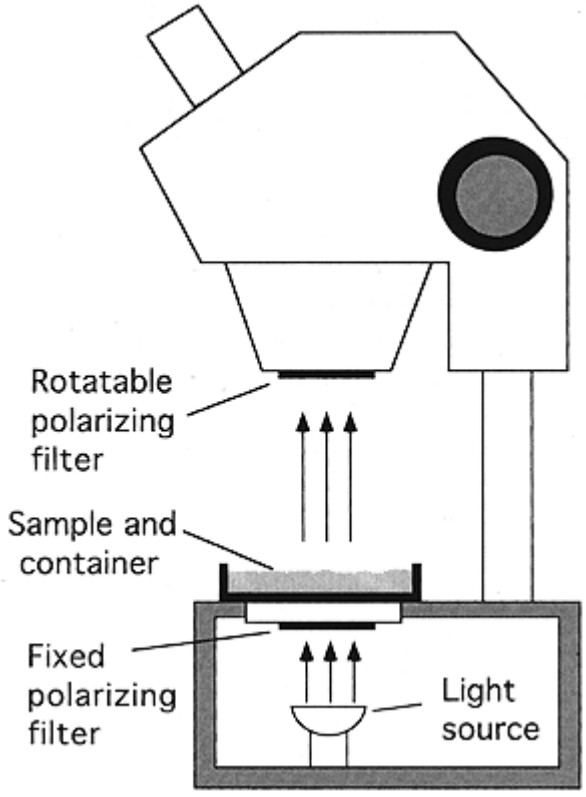


Figure 8: Cross polarized light microscopy schematic

Most samples processed in the lab are preserved using 95% ethanol (ETOH), though live samples may also be viewed. Some larval characteristics cannot be seen with preserved samples (such as the presence of a foot or siphon). Montana FWP requires all preserved samples have a final concentration of ETOH at 70%.

Trained technicians use visual identification to differentiate between invasive species and commonly occurring native species and inorganic particles. If a suspect veliger is detected, that sample may be sent to a partner lab for genetic analysis, but that testing does not occur in-house. CPLM is critical to quickly locate bivalves in a plankton sample.

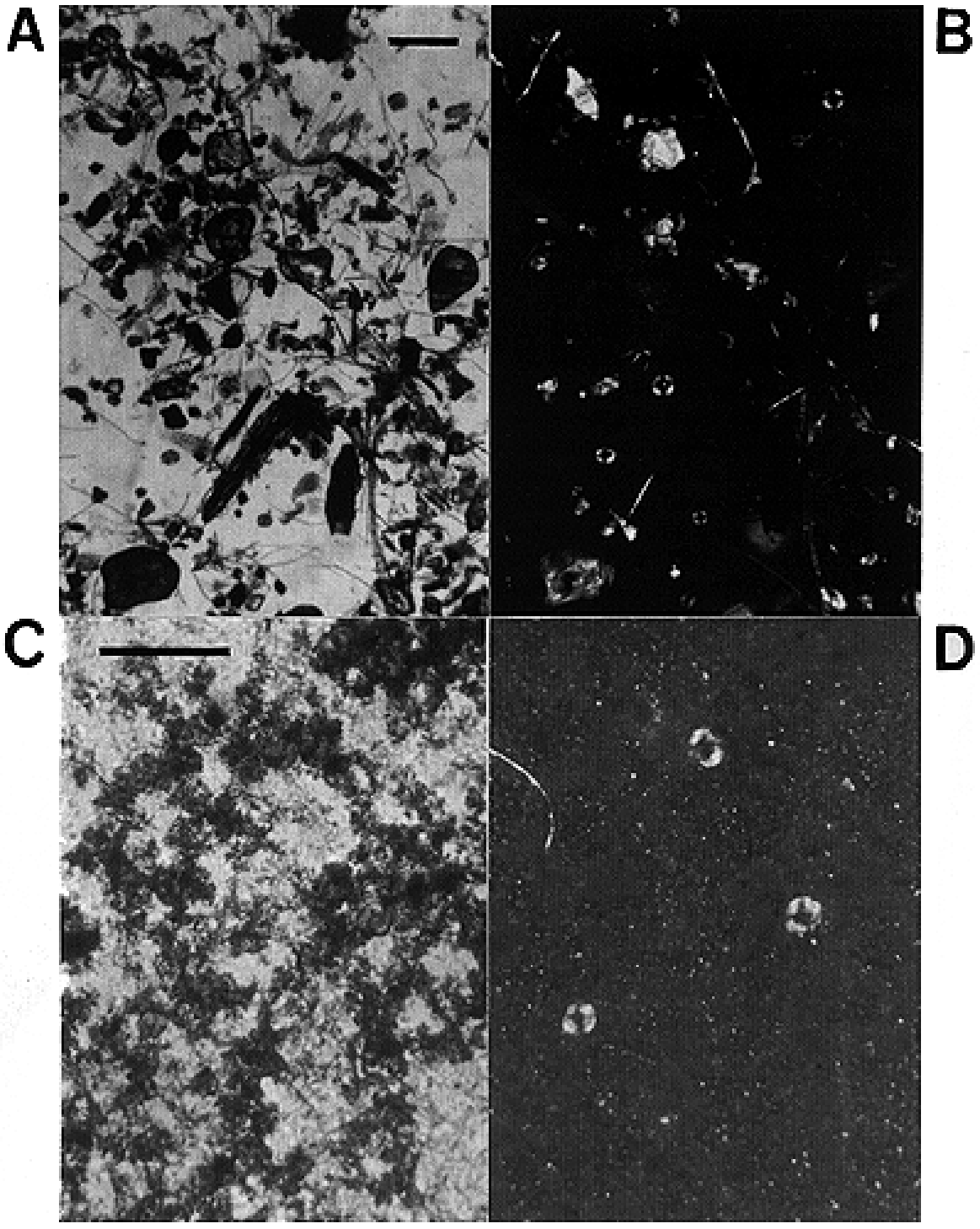


Figure 9: Two plankton samples viewed under polarized light (a. and c.) and under cross-polarized light (b. and d.) to illustrate the enhanced visualization of bivalve larval when using these techniques.

a**. and b.: 'cluttered' nearshore plankton sample with four veligers present; scale bar = 500mm.**

c and d." A silty sample with three veligers present; scale bar = 500mm.

Sample Receiving

All samples that are received in the lab will be prioritized. In addition, the pH will be tested to ensure that the samples will be stable on the shelves.

Prioritizing Samples

All incoming samples will be processed in the order received and according to their assigned risk category. In past years all samples have been processed in the order in which they were received. The risk prioritization model developed after larval Dreissenid mussel detections in 2016 will be utilized to assign risk to the incoming samples. This prioritization is due to an increase in sample volume. For more information on this model, refer to the FWP AIS Early Detection and Monitoring Protocols. This sample prioritization model will result in faster turnaround time for high priority samples. All incoming samples will be assigned one of the categories shown in Table 1. Then, as samples come in, the highest priority samples will be processed first. The goal of this prioritization is to process high priority samples faster, which may make lower priority samples have slower process times.

Table 1: Risk assignment categories for incoming samples.

Risk Category	Invasion Potential Matrix Value
Risk 5 and Hatcheries	5
Risk 4	4
Risk 3 Lake	3a
Risk 3 River	3b
Out of State Samples	3c
Risk 2	2
Risk 1	1

Determining pH

Sample preservation is one of the most important factors in the detection of a veliger from a raw water sample. Samples with a pH below 7 can partially dissolve the veliger shell making the shell undetectable by CPLM. Raw water samples can become acidic over time, due to the addition of alcohol used to preserve the sample. Buffering the sample with a baking soda solution stabilizes the pH and preserves the veliger shell for easier detection by microscopy. Samples should be tested upon arrival in the lab using a pH meter to test that pH levels are at or above 7. If sample pH is lower than 7, then buffer solution should be

added to bring it up to the appropriate level. For instructions on using a pH meter, refer to Appendix G.

Sample Processing

Filtering a Sample

Each sample is filtered using two separate filters. See Appendix G for pictures of this process.

- Measure half of total sample volume.
- Homogenize (shake) sample to suspend sediment at bottom.
- Pour half the volume of the sample bottle through 210 µm filter.
- Use DI water to rinse the contents of the 210 µm filter.
- Check contents of 210 µm filter for invasive waterfleas.
- If no waterfleas in 210 filter, dispose of contents.
- Pour the fluid that passed through the 210 µm filter (filtrate) through the 35 µm filter.
- Rinse the contents of the 35 µm filter with DI water.
- Invert the 35 µm filter over a petri dish and rinse the contents of that filter into the Petri dish.
- The contents of the Petri dish will be examined under a dissecting microscope.

Glass petri dishes must be used because the light can pass through them without interfering with the cross-polarizing lenses of the microscope. Plastic dishes should not be used.

To reduce the chances of sample cross-contamination, each Montana waterbody has assigned filters that are only used for that specified waterbody. Each state will also have its own designated set of filters. See Appendix H for a complete list of filter assignments. Filters are stored in a filing cabinet in alphabetical order with the state abbreviation and filter size marked with a sharpie on the top left side.

Sample Set Up

It is important to have a routine regarding sample processing and set-up. Having a routine will reduce the likelihood of human error in forgetting a step or causing cross contamination. It also helps to ensure a clean workspace.

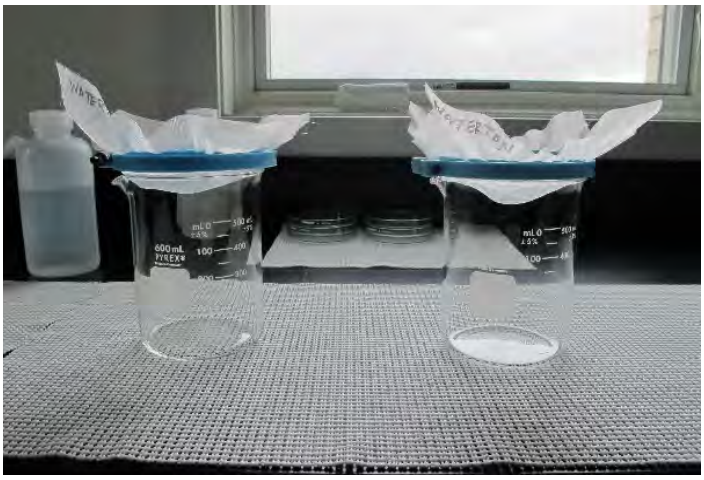


Figure 10. Filtering setup for samples

- Set up 2, clean, 500 ml beakers with filters and embroidery hoops for 210 µm and 35 µm filter
    a. Ensure the filters with hoops create a dip so that the solution you pour through does not touch the edge of the embroidery hoop. To do this, use a clean tool or gloved finger to gently push on the center of the filter cloth before tightening the embroidery hoop.
- Using a graduated cylinder measure half of the total sample size by pouring half of the sample into the graduated cylinder. To measure sample volume, Nalgene sample bottles can also be used as well as the contents of the first beaker, before rinsing. This number should be recorded in the sample log book.
- Pour half of total sample size through 210 µm filter.
- Rinse content of 210 µm filter with Deionized water, using a wash bottle, to force organisms through filter.
- Pour sample filtrate through 35 µm filter and completely rinse beaker through filter (35 µm) with Deionized water using a wash bottle.
- Rinse contents in this filter with Deionized water, using a wash bottle, to concentrate it
- Flip over filter (35 µm) and rinse contents into Petri dish (more than one dish may be necessary depending on sediment/ algae concentration).

Sample Analysis

- Set magnification at 2.5x-3x when viewing slide and searching for suspect organisms.
- Use full magnification (4.5x) when needed to view suspect object or take photographs
- Focus eyepieces using the larger focus adjustment knobs located closer to the microscope post.

Figure 11: The "arrow" on a Petri dish

- While looking through the scope turn Cross-polarized dial until maximum Cross polarized effect is observed
- Place petri dish onto the microscope stage with petri dish arrow facing directly away from you. This you will use as a landmark to keep the Petri dish oriented in the same direction during the search (Figure 12).

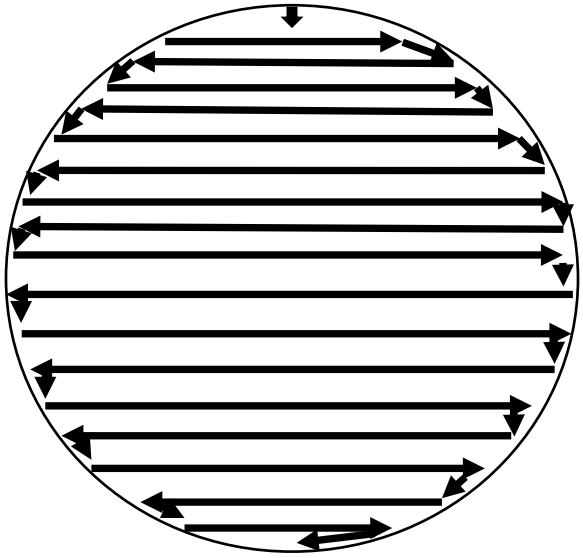


Figure 12: Example of Petri dish search pattern.

- To analyze the sample, move petri dish side to side always keeping the arrow (See Figure 11) facing directly away from you and always oriented at the top of the dish no matter where it is moved.
- Some tips to ensure your search of the Petri dish is methodical and thorough is to choose an object every time you change direction at the bottom of your field of view and to keep that object in view as you move the dish down. The idea is like mowing a lawn, in that there should always be a little overlap, but as little as possible, to reduce any chances of having gaps.
- Searching oligotrophic (lower productivity) is generally easier and quicker than productive (eutrophic) waters because there is less phytoplankton and zooplankton interfering with the mussel veligers. More time should be spent on eutrophic waters vs. oligotrophic.

If you find a suspect

All suspect organisms will be measured using an ocular micrometer.

- Measure any suspects at 4.5x magnification on the dissecting microscope.

- Count ticks on ocular micrometer and multiply by 22.22 for measurement. So, number of ticks x 22.22 = length of organism in µm.
    a. See Appendix I for information on how to measure objects using either microscope.
- Take photos of suspect with both the dissecting and compound scope (10-20x minimum); record drawing, size, any detail and photo name in Veliger Log (See Appendix D for Veliger Log Details).
- Any suspect veliger(s) found are photographed and verified with each microscope's camera. A compound microscope will be used to examine and photograph the specimen in closer detail. Higher levels of magnification on the compound microscope are used to inspect suspect objects for characteristics and morphological features. See Appendix I for information regarding calibration of the ocular micrometer.

The Transfer

When a suspect organism is found, the lab technician will transfer the organism from the Petri dish to a glass slide using a micropipette before being examined under the compound microscope. This is a difficult task and new lab technicians should practice this technique prior to processing samples. A micropipette is necessary because of the extremely small size of the organism being transferred and to measure and deliver accurate volumes of liquid so each transfer can be done with an exact amount of liquid. A micropipette is used to transfer small amounts of liquids (less than 1 mL). The organism may also be transferred from petri dish or slide into a small glass vial to be shipped to a different lab for further analysis.

Step 1: Loading the tip:

Load a clean micropipette tip onto the micropipette. The tip is just placed onto the micropipette by firmly pressing it onto the end. The lab is currently using yellow, 1-200 µL sized tips. The micropipette is a Fisherbrand® Finnpipette® and is set to 25 µL.

Step 2: Loading the sample:

The plunger of the micropipette will stop at two different positions when it is depressed. Push the plunger down slowly to the point of first resistance. This is the volume load. While holding the plunger at the load set point, put the tip into the solution so that it is immersed just enough to cover the end (3-4mm), not as deep as possible. Slowly release the plunger to draw up liquid including suspect organism, making sure to keep the tip immersed.

Remember:

- Never draw up more liquid than the pipette indicates.
- Always hold the pipette as indicated in Figure 13. Never hold the pipette so the tip is above the pipette.
- Always use the smallest size volume you can to transfer organism.
- If using the micropipette for DNA samples, filter tips should be used.
- Avoid drawing up bubbles in the tip by immersing entire tip in liquid when drawing up sample.

Figure 13: Micropipette

Step 3 Transferring the sample:

The second stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with the body of the pipette. This second stopping point is used to completely discharge the solution from the tip. You should not reach this second stopping point when drawing the liquid into the pipette, only when expelling the last drop.

To deliver the volume:

- Place the tip into the glass slide or the glass vial.
- Touch the end of the tip to a surface before depressing the plunger (the surface tension of the liquid will help to draw out the organism into the container or slide).
- Depress the plunger to the point of initial resistance.
- Wait 1 second.
- Continue to press the plunger all the way to the bottom to expel all liquid.
- Draw the tip away from the liquid while still maintaining contact with the glass surface.
- Without releasing the plunger, withdraw the tip when away from the liquid.

Taking a Photo

Photos are taken at each step during the process in case the suspect organism is lost during transfer.

- Adjust the compound microscope to 10X magnification to find the suspect and take a photo.

- Change to 20X magnification (minimum) and take a photo (make sure water level isn't too high when changing magnification).
    - When using a compound microscope, cover slides are not used to eliminate the risk of crushing a sample, so care must be taken to ensure lens does not touch the sample.
- Change to 40X magnification to see if a higher quality photo can be taken at this magnification.
- When photography is complete and pictures are saved, remove slide from compound microscope.
- Put slide in an empty petri dish bottom or cover (be sure slide lays flat in dish).
- Add a few milliliters of DI water to slide with suspect to increase the volume of the drop but not flood the slide. This should be enough liquid to use the micropipette to pick up the suspect organism.
- Prepare a small glass vial with lid to transfer suspect.
- Put slide in petri dish under dissecting microscope.
- Find suspect and using micropipette, transfer it to a glass vial as described above.
- Touch micropipette tip to bottom or side of vial before dispensing.
- Find suspect in vial using dissecting microscope (easiest to see with Cross polarized light).
- Add ethanol to vial so vial is approximately half full.
- Seal tightly and use electrical tape around lid.
- Put identification number on vial and record this in veliger log
- Identification number should be written as follows: State abbreviation/number of samples/dates received. So, for Montana samples with a batch of 21 received on August 21st, 2018, ID number should read: MT21082118.
- Retain second half of sample.
- Store both in refrigerator.
    - Notify lab manager, Stacy Schmidt or appropriate proxy.
- If Stacy is working in the field, email or text may be best.

- Put photographs on jump drive or network. Hand deliver along with photocopy of veliger log notes to Stacy Schmidt.
- Further instruction may then be given on what to do with remaining half of sample (for example: process it, send to another microscopy lab, send to a lab to run polymerase chain reaction (PCR) on it, etc.).

Figure 14. Dreissenid mussel under cross polarized light

Things to Look For

Dreissenid veligers have a shell made of calcium carbonate crystals that have a special optical property called birefringence. When viewed under polarized light, veligers produce a unique light signature which causes them to "glow". This birefringent property allows us to screen samples with the use of a dissecting microscope. When analyzing a sample using cross polarization, the first thing to look for is the "glow" and maltese cross coming from the organism in question. These features are a key characteristic shared by only a few organisms including dreissenid mussels. Next look at the overall shape and size of the specimen. Information on discerning different species is given in the appendix.

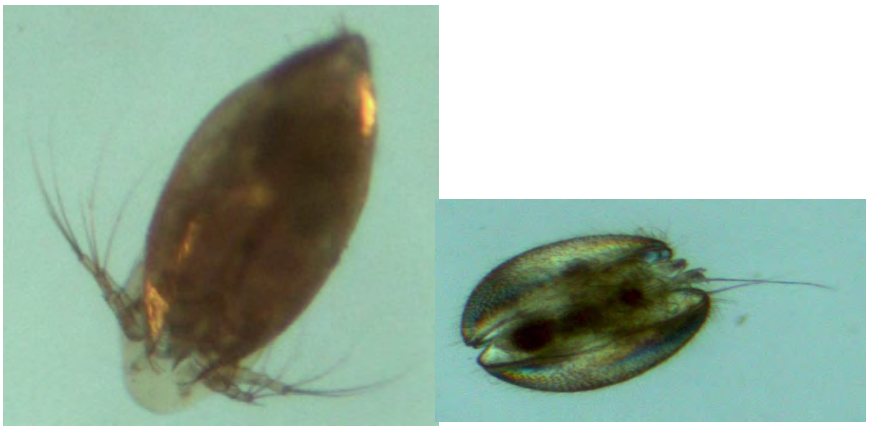


Figure 16: Dorsal (left) and ventral (right) view of a live ostracod.

Ostracods, a type of common zooplankton also known as seed shrimp are often found in plankton samples and are easily confused with larval mussels to the untrained eye. Ostracods can be separated from bivalve larvae in preserved samples by examining the edge of the shell under higher power. Ostracod shells have ornamentation (spines) at the anterior and posterior edge. In live samples, separation is easy, since ostracods have legs. Ostracods are typically larger and have a shape like a jellybean. (USACE, 2001) Ostracods also tend to have a "pitted" surface. Though the surface of the shell may be difficult to see without higher magnification and the right angle of light.

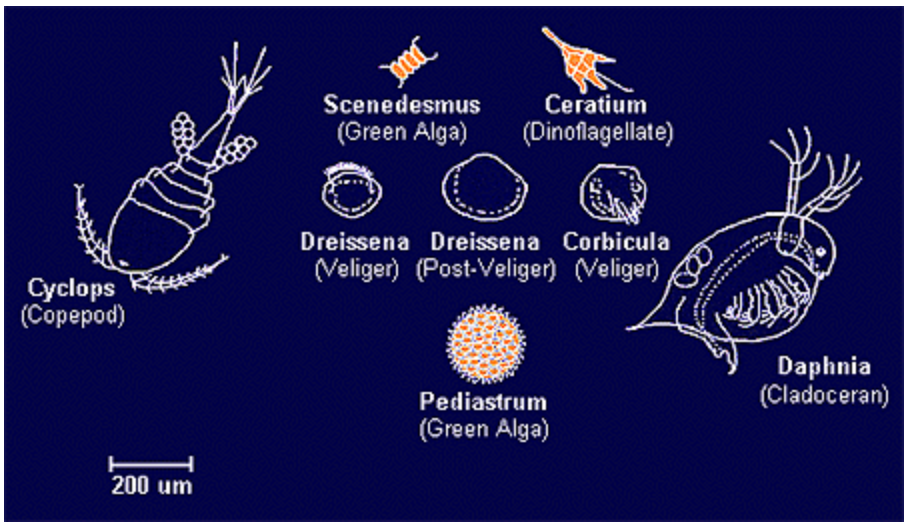


Figure 15: Size comparison of common zooplankton.

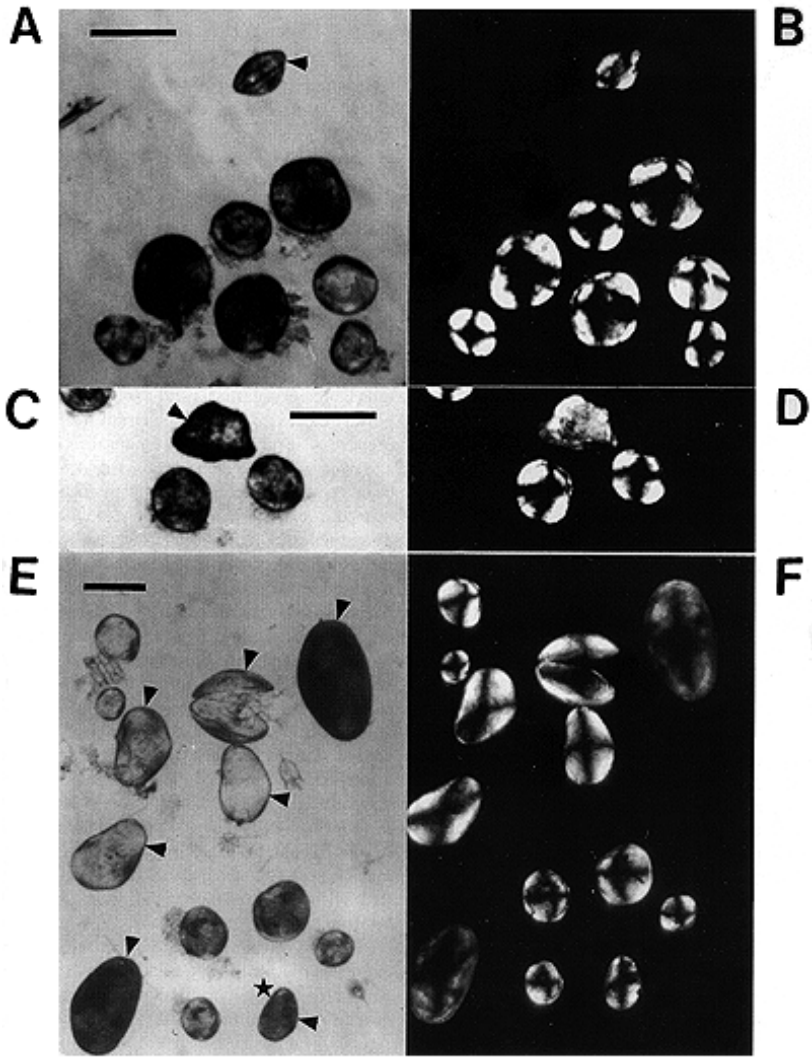


Figure 17: Various plankton samples seen under polarized light (a., c., and e.) and under cross-polarized light (b., d., and f.) (Polarized light was used instead of unpolarized light because the use of nonpolarized light would have required the repetitive removal and installation of the polarizing filters.)

Zebra mussel veligers without extraneous material are shown in a. and b. Note that the distinctive 'Maltese cross' pattern of birefringence of veligers on their sides is not evident when the veliger is viewed edge on (arrowhead); scale bar = 200mm.

c. and d.: veligers and sand grain (arrowhead). Note that the sand grain does not produce the 'Maltese cross' pattern. Sand grains are usually multi-colored, while veligers are always white; scale bar = 200mm.

e. and f.: mixed collection of veligers and ostracods (arrowheads). Note that size ranges of veligers and ostracods overlap (e.g. star) and that empty ostracod 'shells' (lighter individuals in e.) are more birefringent, and thus more like veligers, than whole animals due to the lack of interfering body tissue. Certain morphological

features that distinguish veligers from ostracods are not visible at this magnification, e.g., shell ornamentation; scale bar = 200mm. (USACE, 2001)

Verification Process

If a sample contains a suspect Dreissenid veliger, the lab technician captures digital images (both from the dissecting microscope and compound microscope). The lab technician, lab manager, and another FWP designated expert will examine the images, and when possible, the suspect under the microscope. It is always best for an expert to see the suspect organism under the microscope so it can be manipulated and looked at from different angles.

If the sample is still suspected to be a Dreissenid veliger, the digital images are sent to two independent labs/experts for verification. The independent labs used for verification identify positive Dreissenid samples on a regular basis. The remaining half of a sample may be processed either internally or by another lab (for both microscopy and/or PCR) if considered suspect. A sample is positive if any zebra or quagga mussel veligers are found no matter the quantity (after independent verification using both photographic evidence and the remaining half of suspect sample). This method is not one hundred percent accurate, but it is very useful in early detection. Accuracy is reduced when samples are not preserved correctly, or when there is a large amount of detritus in the sample. If samples are negative (veligers are undetected), the lab technician will notify the contact person when the samples are completed. If suspect veligers are found, the contact person will be notified by the lab manager and provided with the photos of the suspect organisms as well as the internal explanation on species and age.

Determining Species

Although morphological features are sometimes difficult to distinguish, it is often possible to determine species of a Dreissenid mussel at the larval stage. The primary characteristics that are used to determine species are hinge length, shell length/height ratio, shape, and size (Nichols & Black, 1993). Also, characteristics such as the presence or absence of a foot or velum in live veligers. The development of internal organs and taxonomic features are also used; however, this usually requires the use of a high-powered dissection or a scanning electron microscope.

A significant difference between Dreissenids and Asian clams and native pea clams is where larval development occurs. In native pea clams and invasive Asian clams, embryological development occurs within the female clam (brood pouch). Larval

development of Dreissenid mussels occurs exclusively in the water column. Pre-shell identification of species is nearly impossible using light microscopy. Sperm size in quagga mussel is about 9-12 µm, while zebra mussel sperm size ranges from 7-9 µm. All the following morphological feature information is from the zebra mussel information system. (USACE, 2001)

Morphological Characteristics

There are many morphological features that should be considered when determining species. Not all features may be used every time, but each feature that can be identified should be used to add to the evidence used in making an identification.

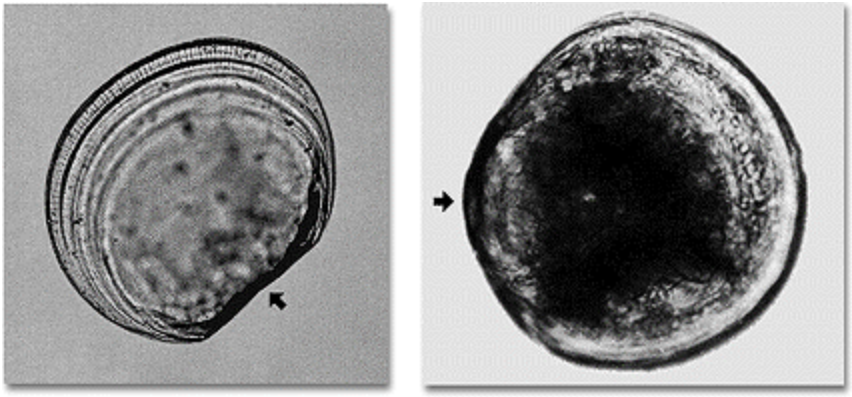
A larval size greater than 500 µm is generally too large to be found in the water column (when that size is reached, they are too heavy to remain in the water column and settle out). This is the reason why older Asian clams are not usually found in this type of sampling.

Margin Appearance

Look at the margin (or outline) of the object in question. If the margin is smooth and regular, it could be a veliger. If the object has an irregular and/or jagged margin, the object is likely inorganic material.

Veliger Umbone Appearance

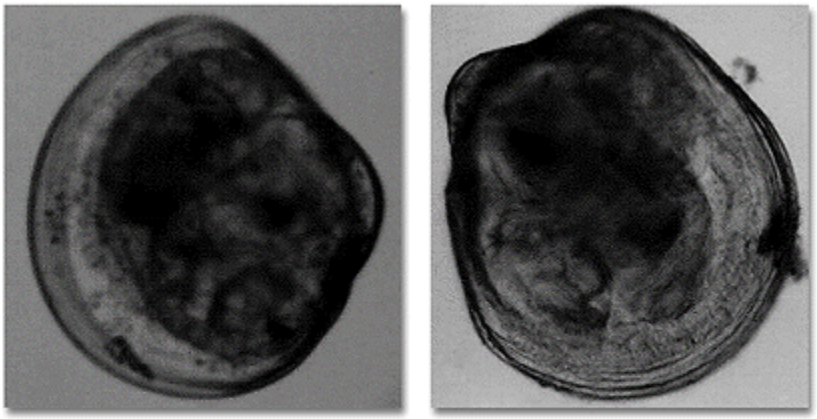
Two basic shapes of the hinge line are recognized including a straight or sway-backed form (shell is decidedly D-shaped), and one that is rounded with a protruding bump or umbo in the center. Knowing the shape of the hinge line is the first step in identifying one of the four basic types of shelled larvae or veligers. These include the straight-hinge form (d-shaped), umbonal form, pediveliger, and plantigrade. These basic types of shelled larvae are separated based on hinge development, shell shape, shell size, and the presence or absence of a foot and velum. Knowing the shelled larval type can greatly aid in the identification of the veligers. (USACE, 2001)



Straight Rounded

Shell Appearance

The appearance of the face of the shell (i.e., the entire lateral side of the valve or shell halve) is important in distinguishing the basic types of shelled larvae or veligers. This character distinguishes those shelled larvae in which the entire lateral side of the valve is only slightly asymmetrical (or clam-shaped) from those in which the entire lateral side of the valve is very asymmetrical or lopsided, as in the typical mussel shape. (USACE, 2001)



Slightly Asymmetrical "Basic Clam Shape" Very Asymmetrical

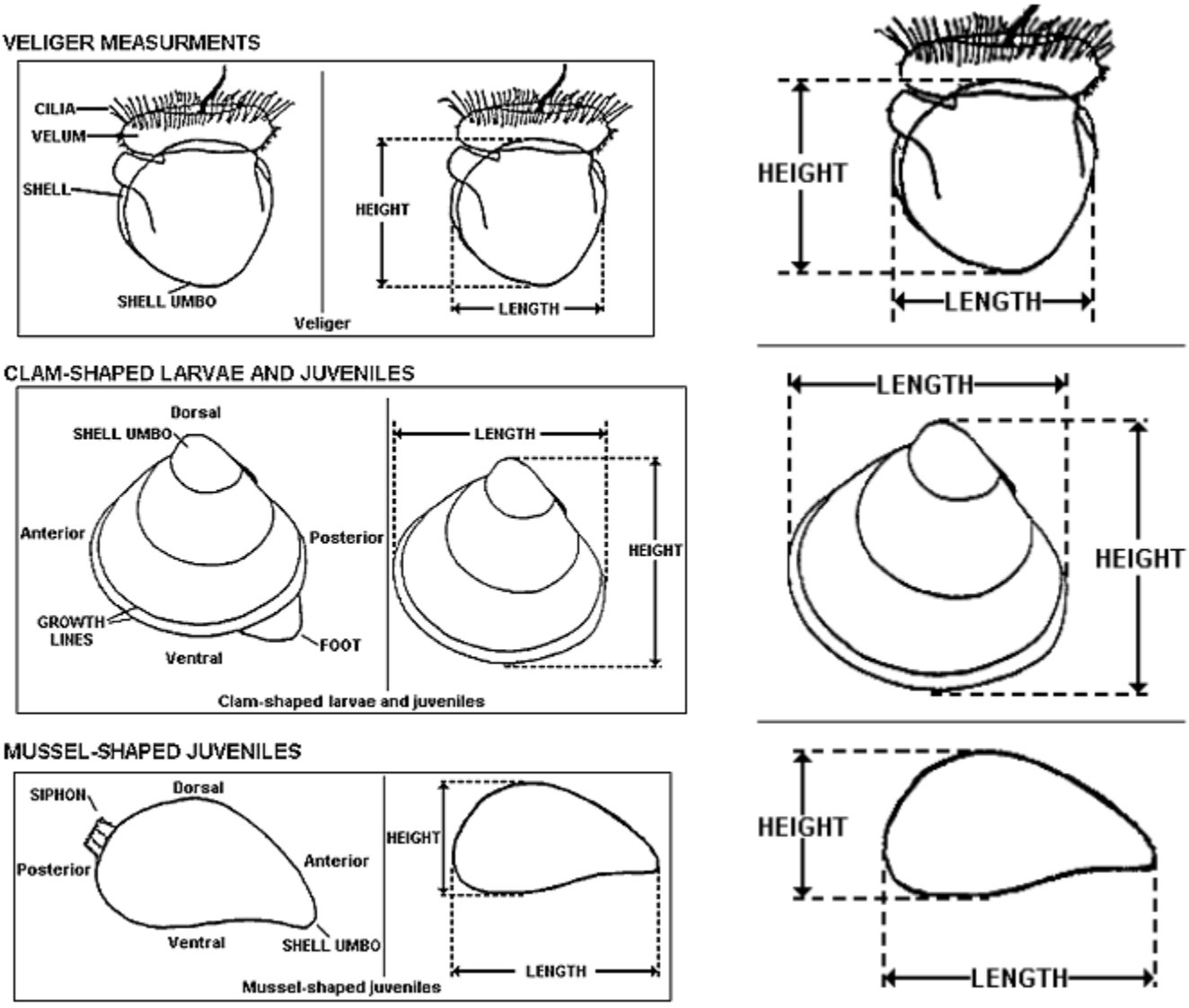
Shell Length and Height

Measurement of length and height in Asian clams and Dreissenids is complicated by the fact that there is a distinct change in body orientation of Dreissenids as they mature. The original "anterior" portion of the shell in immatures slows in growth and subsequently forms the "ventral" surface of the adult. Thus, a major change occurs in what is conceived as the length and the height.

In this regard, for purposes of immature identification, conventional measurements are used instead of purely morphological measurements.

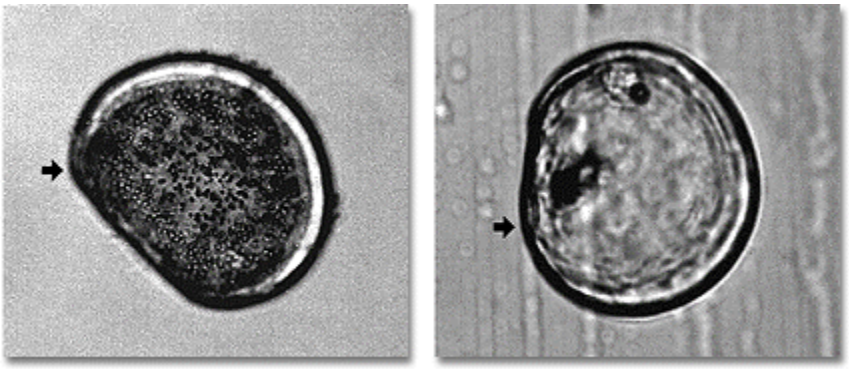
Therefore, in Asian clams and clam-shaped Dreissenids, height is defined as the distance from umbone, or middle of the hinge line, to the opposing valve margin, and length is measured perpendicular from the height line.

In contrast, in mussel-shaped plantigrade and juvenile Dreissenids, length is defined as the distance from the umbo to the opposite shell margin, and height is measured perpendicular to the length line. (USACE, 2001)



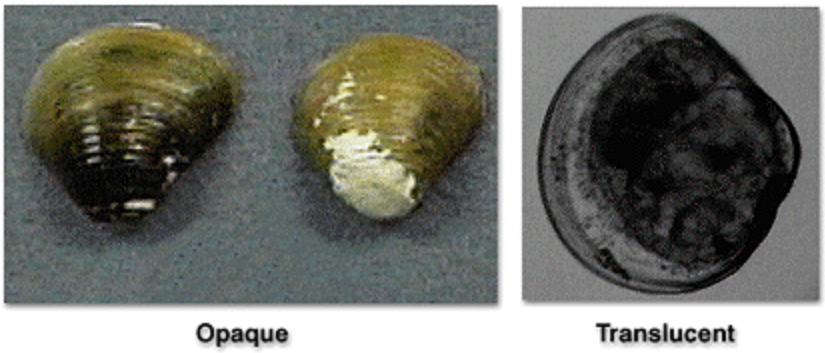
Shoulder Shape

The shoulder is the section of the shell margin just below the ends of the hinge. The shoulder shape is an important characteristic in the straight-hinged stage of Dreissenids. In *Dreissena polymorpha* (zebra mussels) the shoulders have an angular shape, but in *Dreissena bugensis* (quagga mussels) they have a rounded shape. (USACE, 2001)



Shell Transparency

During and after the pediveliger stage, the Asian clam shell is opaque (i.e., one is unable to see through the shell). The zebra and quagga mussel shells are translucent (i.e., one can see through the shell, and in live specimens, the body parts are visible). (USACE, 2001)



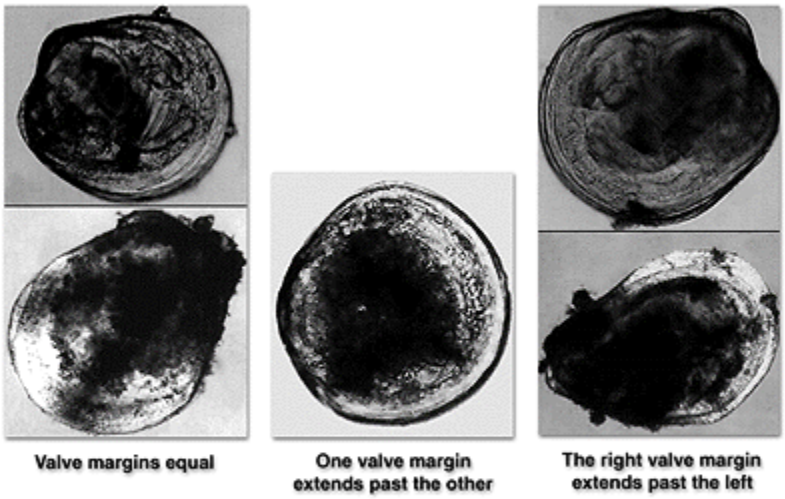
Comparison of Valve Margins

Valve margin comparison is an important characteristic in the identification of Dreissenids. Three comparisons are noted, as follows:

1. Shell margins are equal, as in *Dreissena polymorpha*.
2. One shell margin extends past the other, as in umbonal pediveligers and plantigrade larvae of quagga mussels.

3. The right valve margin extends past the left, as in quagga pediveligers of all ages.

NOTE: In the first two cases, it is very difficult to see these characteristics. (USACE, 2001)

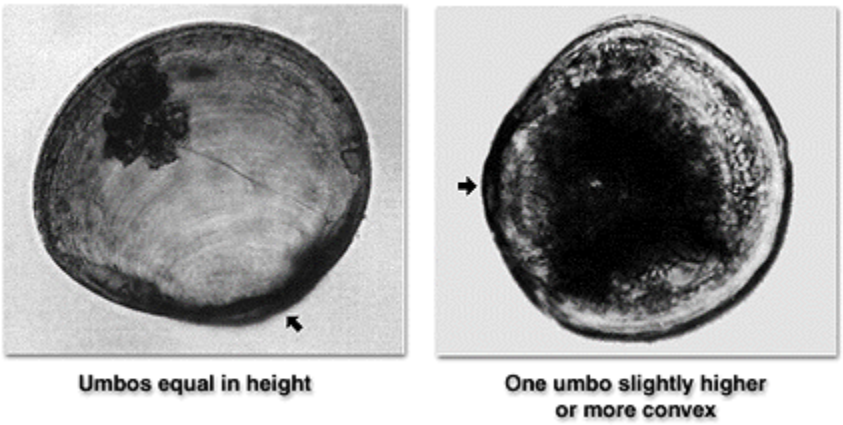


Umbone Comparison

Comparing the height and convexity of the umbo is important in Dreissenid identification. Two forms are recognized, as follows:

1. Umbos are equal in height as in *Dreissena polymorpha*.
2. Umbos are slightly higher or more convex in shape.

Cases where the umbos are unequal in height are usually very difficult to observe using standard light microscopy. (USACE, 2001)

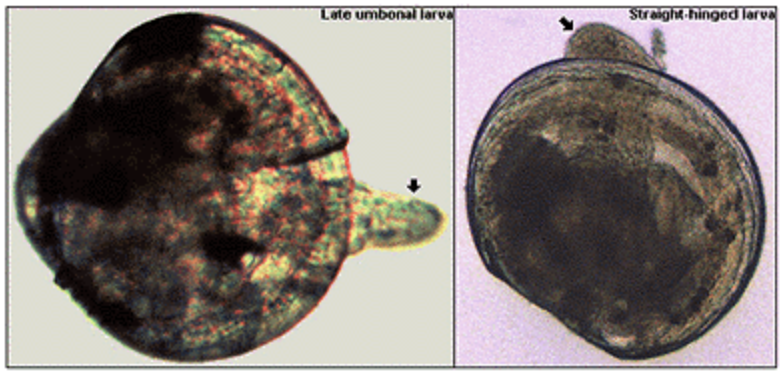


Siphons

Siphons are tubular, muscular structures formed by partial fusion of the posterior mantle margins in many bivalves. The presence or absence of siphons differentiates Dreissenids (zebra and quagga mussels) and *Corbicula fluminea* (Asian clam) in the pediveliger stage. Siphons are absent in the Dreissenids at this stage but are present in *C. fluminea*. Siphons should not be used in identifying preserved samples.

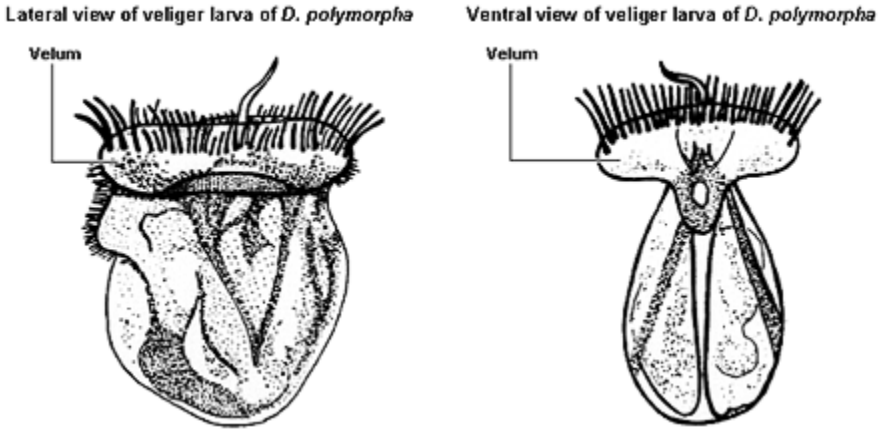
Foot

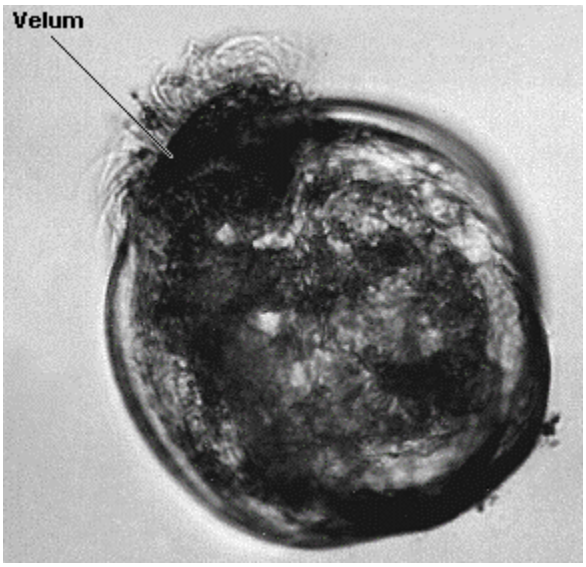
The presence or absence of a foot is important in distinguishing the four types of shelled larvae. The foot is a muscular organ that extends from between the valve halves and is used for locomotion. The foot is best identified in live samples versus preserved samples.



Velum

The presence or absence of a velum (large, ciliated lobe used for swimming, feeding and gas exchange) is important in distinguishing the four types of shelled larvae. The velum is usually covered with fine cilia and is located opposite the umbo. The velum is shed earlier in the development process for Asian clams than for Dreissenids. The presence or absence of a velum us usually not a distinguishing feature for preserved samples.

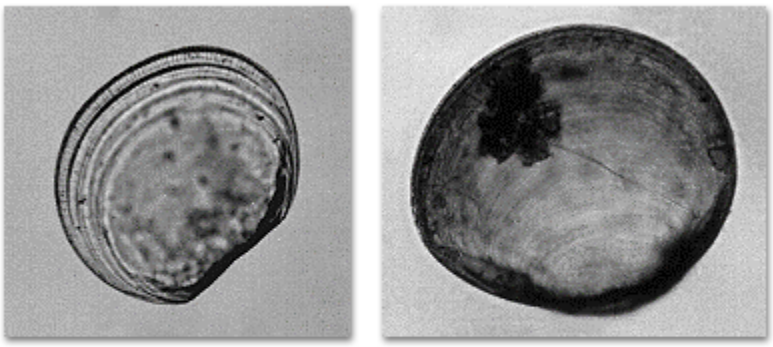




Growth Rings and Secondary Lines

The presence of growth rings is important in distinguishing Asian clams from Dreissenids. Growth lines are striations in the shell that are produced as the shell grows. In Asian clams they are typically quite pronounced, especially in the umbonal stage. In comparison, the umbonal stage of Dreissenids has growth rings that are less pronounced or weak.

In addition to growth rings, there are often secondary striations or lines running parallel to the growth rings. These are present in the umbonal stage of Asian clams. When secondary lines are present, they give the shell the appearance of being pleated.



Present - well-defined

Not well-defined or absent

Size Tables

Size of the veliger may aid in determination of species as well as age of the larvae.

Table 2: Length ranges for different freshwater veligers.

	Straight-Hinged or D-Shaped	Early Umbonal	Older Umbonal	Pediveliger	Plantigrade
Zebra Mussel	97-112	112-228	140-347	231-462	> 340
Quagga Mussel	39-71	39-71	120-221	150-228	222-410
Asian Clam	> 195	> 280		> 500	

Table 3: Size ranges for different freshwater veligers.

		Length (µm)	Height (µm)	Hinge Length (µm)
Zebra	D-shaped	70-160	64-105	64-77
	Umbonal	106-280	89-191	65-79
	Pediveliger	136-347	111-297	70-101
	Plantigrade	158-500	189-367	-
Quagga	D-Shaped	39-160	32-118	24-63

	Umbonal	111-260	98-202	31-62
	Pediveliger	141-300	129-177	43-76
	Plantigrade	136-410	119-390	-
Corbicula	D-shaped	175-380	165-246	-
	Umbonal	380-500	340-472	-

Equipment Decontamination

All equipment will be decontaminated for laboratory purposes after each use. All glassware, filters and hoops are washed in hot, soapy water, rinsed, soaked in bleach (10% solution for 15 minutes) after each use. All filters are also soaked overnight in a 5% acetic acid solution (vinegar) after each use and then rinsed and dried before storage.

- Gloves should be worn when using chemical solutions.
- Wash all filters (35 µm/210 µm), hoops and glassware with hot, soapy water (most residential and commercial water heaters are set to 120° F), and then rinse with clean water, soak in a 10% bleach solution and then rinse again and allow to dry after each sample to avoid cross contamination.
- Soak all filters in a 5 % acetic acid (household vinegar) at least 2 hours but maximum 24 hours to dissolve any remaining biological material.
- Continually keep work surfaces clean by wiping with chemical wipes and/or cleaning with bleach and change dishwater after each water body, or when dishwater starts to cool.
- Clean out and rinse sinks thoroughly when changing dishwater.

Sample Bottles and Beakers

All Sample Bottles and beakers will be decontaminated between uses to prevent cross contamination.

- Rinse all Sample Bottles and Beakers with hot tap water (120^{o}f)
- Wash with Soapy, hot tap water and scrub with brush.
- Soak in 10% bleach solution for at least 15 minutes.
- Wash in dishwasher under light setting (113min) with regular dishwashing detergent
- Soak in 5% acetic acid (household vinegar) for a minimum of 2 hours.
- Rinse with tap water
- Air dry

References

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Western Regional Panel on Aquatic Nuisance Species. (2018, October 2). Laboratory Standards for Dreissena Veliger Analysis.

Appendix A- Materials

- 500 mL Pyrex glass beakers
- Graduated cylinder (100-500 mL)
- Petri dishes (tops and bottoms)
- Glass microscope slides
- Filter/bolting cloth
    - 35 and 210 µm, cut into 6" squares
- Filtering hoops (5-inch embroidery hoops, plastic)
- Microscope: compound and dissecting
- Ocular and stage micrometer
- Microscope camera and software
- computer
- Deionized water
- Wash bottles for DI water
- Micropipette
- Micro pipette tips
- Bleach
- Dish Soap
- Vinegar
- Baking Soda
- PH Strip/meter
- Log notebook (stitched composition notebook), preferably gridded
- Computer access state network (Fish folder)
- Drain board
- Drying mat material
- Peg Board
- Dishwasher
- Scrub brushes to wash bottles and glassware
- Gloves

Appendix B- Definitions

1. Microscope

Dimmer switch: Switch on the microscope used to turn on illuminator
Illuminator: Light source for the microscope
Pillar collar: Post located on the back of the microscope which holds the ocular and objective
Objective: Lens closest to the specimen
Zoom control knob: Controls the amount of magnification of the subject
Focusing knob: Moves the objective up and down to bring specimen into focus
Fine focusing knob: Used to gain sharper focus
Stage: Platform which holds specimen
Brightfield light: Produces a dark image on a brighter background
Cross-polarized light: Polarizing filters in the light path creating birefringence for certain organisms
Transmitted light: Light which is projected from an illuminator

2. Morphological Feature Names

Maltese cross: Black cross that appears when veligers are viewed through cross polarized light.
Velum: Ciliated structure used for swimming and feeding.
Hinge: Point at which the two points of the bivalve shell are joined.
Hinge lines: Length of the hinge.
Valve: One half, or section, of the bivalve shell; the two valves are joined along the dorsal surface by a
ligament. When the bivalve is viewed with the hinge up and the anterior end directed away from the
observer, the left valve is the shell half to the observer's left and the right valve is the shell half to
the observer's right.
Shoulder areas: Section of shell margin adjacent to the ends of the hinge.

Appendix C- Microscope Information

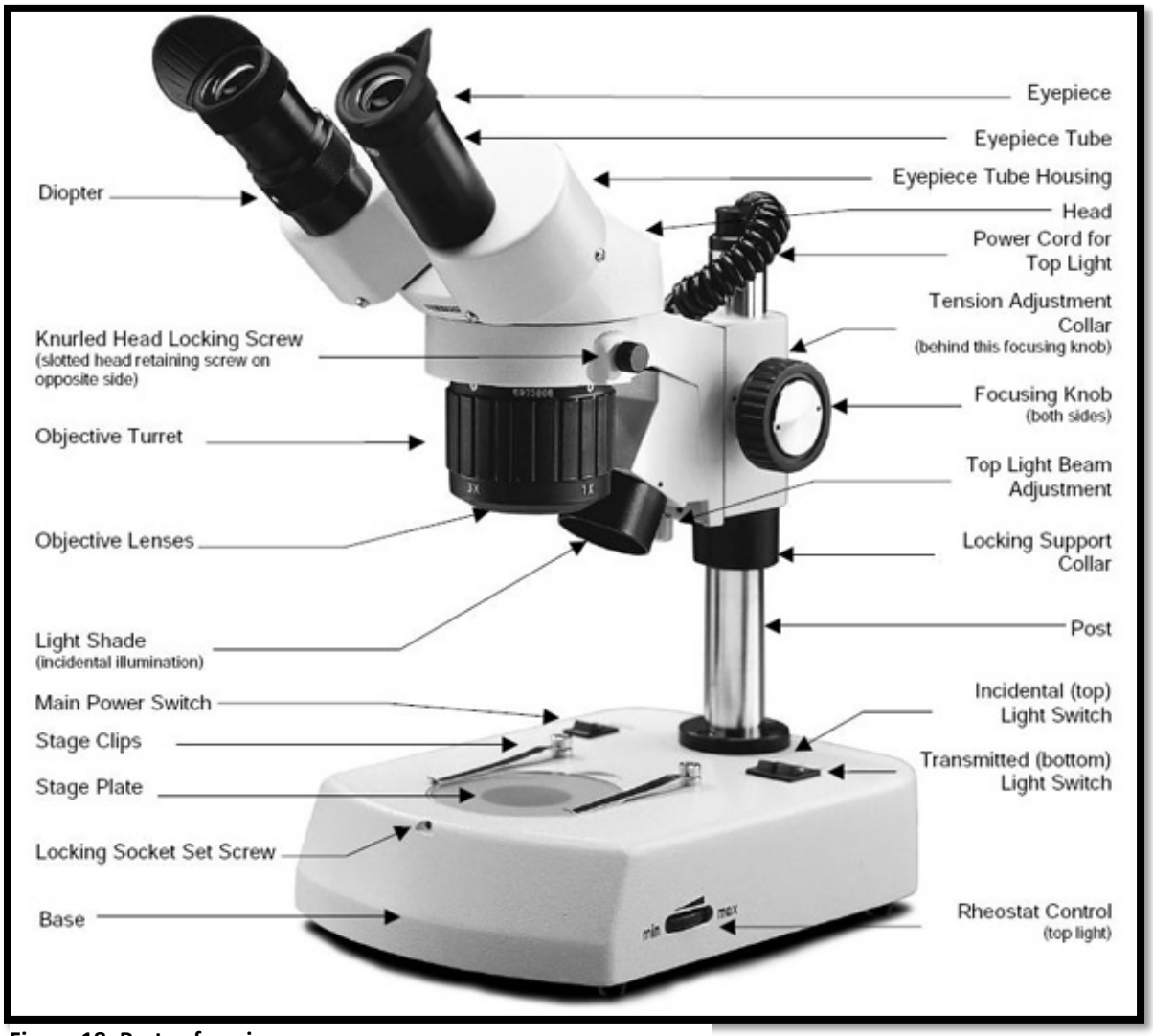


Figure 18: Parts of a microscope

Calibration

1. Refer to instruction manual of specific microscope for calibration instructions.
2. Adjust eyepiece to your comfort level.
3. Set magnification to highest magnification by using zoom control knob (dissecting microscope). This knob should be marked with magnification (0.67-4.5x).
4. Use focusing knob to focus sample until clear.
5. Set magnification to lowest magnification using zoom control knob.
6. Using right eye, look through the right eyepiece and turn the eyepiece diopter until sample is clear.

7. Repeat with left eyepiece.
8. Sample should now be clear for all magnifications. Repeat if necessary.

Replacing a light bulb

1. Turn off selector switch and dimmer switch.
2. Unplug microscope from electrical outlet.
3. Locate where light bulb is housed and open housing.
4. Some microscopes need a screwdriver to access light bulb housing.
5. Using Kimwipe, carefully remove old light bulb from illuminator.
6. Dispose of bulb in the trash receptacle.
7. Using Kimwipe, take new light bulb from box and place the two wires coming out from it in the holes in which the old bulb came from.
8. Once the new bulb has been installed, replace cover to light bulb housing.
9. Turn microscope back to its original position, reconnect plug to electrical socket and test by turning on the microscope.

Dissecting Scopes: Olympus SZX2/Zeiss Motic SMZ-171

1. Remove cover from microscope and place Petri dish containing sample in the center of the microscope staging area.
2. Turn selector switch, located in the back of the microscope, to the on position.
3. Turn dimmer switch, located next to selector switch, to desired light intensity.
4. Turn zoom control knob, located on the zoom body, to desired magnification.
5. Use both the focusing and fine focusing knobs until sample is in focus.
6. To change between cross-polarized and non-cross polarized light, turn the cross polarizer on the bottom of the stage.

https://www.manualslib.com/manual/662757/Olympus-Szx16.html
http://www.mrclab.com/data/products/SMZ-171-BL_OPR.pdf

Compound Scope- Olympus BX41

1. Remove cover from microscope and place the sample in the center of the microscope staging area between the two clips by pulling them apart.
2. Turn dimmer switch to desired light intensity.
3. Use both the focusing and fine focusing knobs until sample is in focus.
4. If non-cross polarized light is needed to view sample, turn the cross polarizer on the bottom of the stage.
5. Different objectives can be used with these microscopes to zoom in or zoom out of the image
6. These can be changed by twisting the objective piece to the desired objective.
7. To move the sample, use stage dials located on the right side underneath the stage to move the stage while viewing sample.

https://www.manualslib.com/manual/795358/Olympus-Bx41.html

Digital Cameras

Lumenera Infinity 2/Infinity Analyze

The INFINITY ANALYZE application package is designed to work exclusively with the Lumenera INFINITY camera models. It provides all the functions necessary to control all available camera settings, for both the live video preview and the capture of still images. In addition, ANALYZE provides a comprehensive set of measurement and analytical operations useful in many clinical, laboratory, and inspection operations. (Lumenera Corporation, 2018)

The manual for the infinity 2 camera can be found here.

https://www.microscopeworld.com/images/Manuals/Infinity-Analyze-Manual.pdf

FPI-HDCAMMEASURE

Chapter 1 Introduction

Advanced Design

With the amazing color fidelity, the HDvCAMMEASURE provides a perfect solution for high definition digital imaging. To meet the customer's individua requirements, the flexible parameter settings allow you to quickly get high resolution live images easily and freeze the screen to simply observe details.

The HDvCAMMEASURE inherits all the advantages of the first generation HDMI cameras and significantly improves both the hardware and software, providing a more fluent visual experience and intuitive user interface.

To get more information about the HDvCAMMEASURE camera, please read this document

Chapter 2 System Standard Items

One HDvCAMMEASURE camera,

One 12V/2A power adapter,

One HDMI cable (2-meters length),

One SD card (16G capacity, class 10),

One mouse (with 1.5-meters cable),

One USB2.0 cable (gold plated connector).

1. **USB interface:**

A: Connect a mouse to the USB port. Use the mouse to control the camera directly.

B: Connect the USB port to PC to make the camera work as a **Driver-Free** camera. Use TCapture software to control it (similar as using the other USB cameras).

Chapter 3 HDMI Operation

Step 1. Connect the camera.

1. Plug in the 12V/2A power supply. Use HDMI cable to connect the camera to the monitor. Press and hold ON/OFF key until blue light is on.

2. Connect the mouse to the USB port. Move the cursor to get the settings on the screen.

3. Insert the SD card. Capture images or videos to the SD card.

Step 2. Move the cursor to the left of the screen.

When move the cursor to the left of the screen, 'Capture' and 'Setting' icons will appear (See image on the left hand side). Click 'Setting' to get parameter setting menu (See image on the right).

Note: No driver installation is needed to connect the camera to a PC via USB port.

2. **HDMI interface:**
Use the HDMI cable to connect the camera to the monitor. Image data is transferred and displayed on the monitor according to the HDMI protocol.

3. **Power interface:**
Please use the provided 12V/2A power supply. When power is plugged in, the red light is on. When switch on the camera, blue light is on.

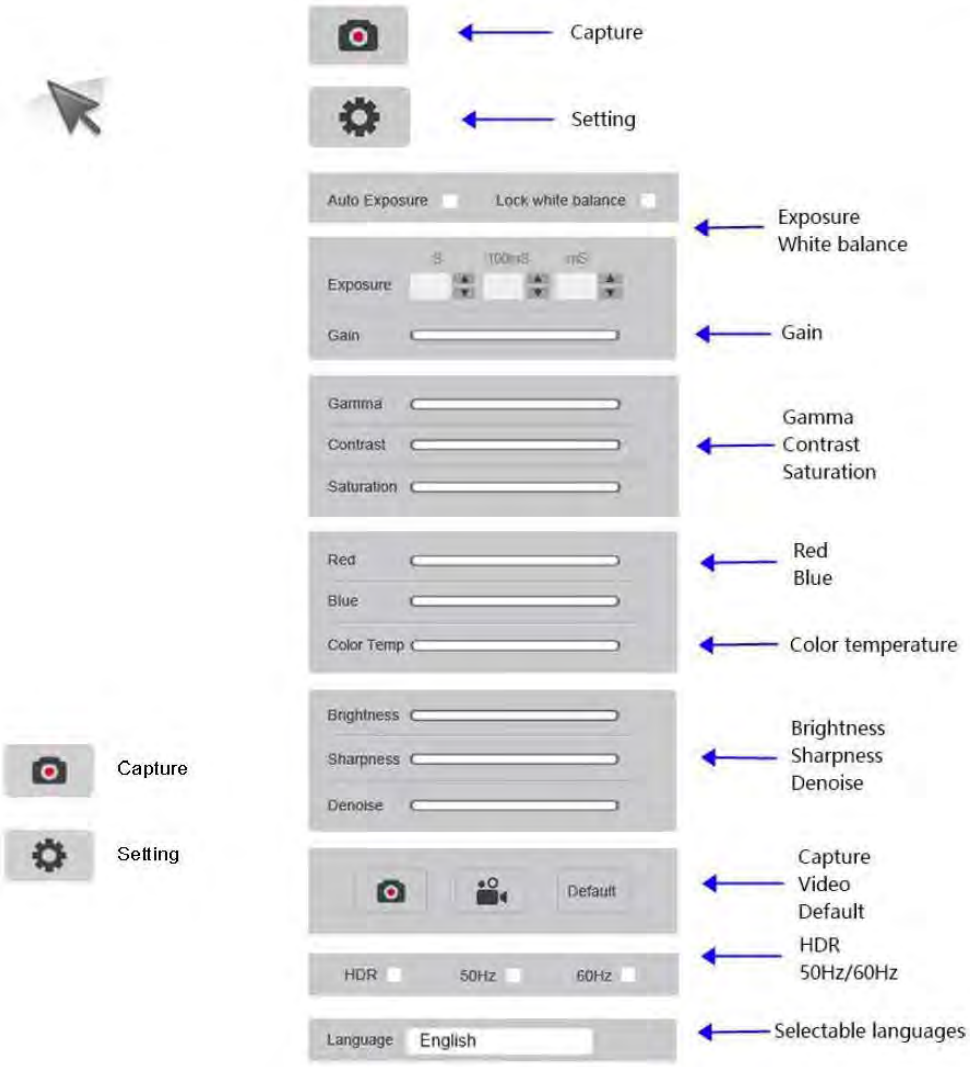
4. **ON/OFF key:**
Press and hold ON/OFF key until the blue light is on or off to turn on or off the the camera.

5. **SD card:**
To get faster and more stable data transfer, recommend to use Class10 SD card.

6. **C mount:**
Standard C-mount optical port.

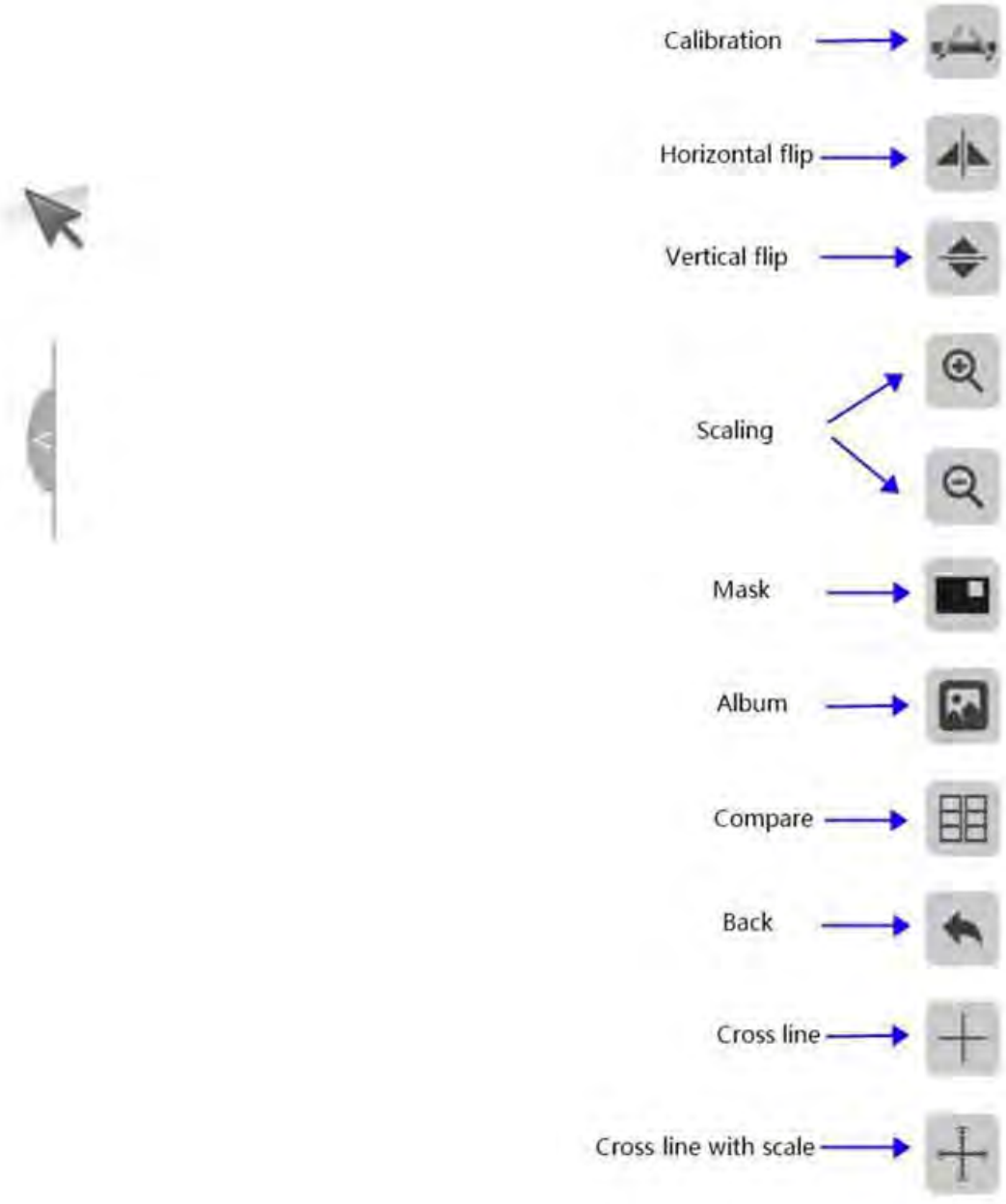
7. **Anti-dust seal:**
Please remove the seal when first time use the camera.

The seal was placed on the camera optical port when it lefi the factory. It is used to avoic the dust accumulating during the transport.

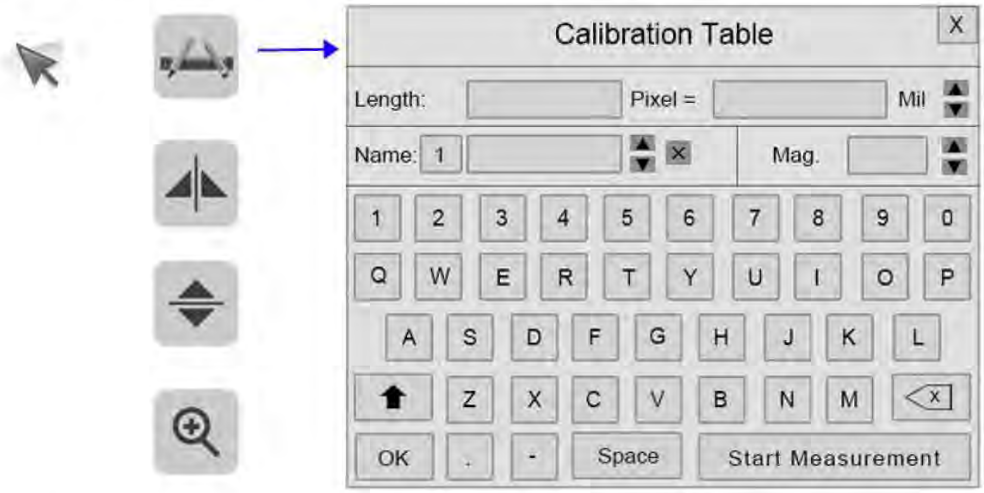


Selectable languages, includes English, Chinese, German, Italian, French, Japanese, Korean.

Step 3. Move the cursor to the right of the screen.



Step 4. How to do calibration and measurement in HDMI mode:



Basic steps:

1. Click on the calibration icon  to get calibration table.

2. Move the cursor out of the calibration table to start the calibration.

1) The live image should be the calibration slide or some know dimensions specimen at this moment.

2) Draw a line to get a reference length, and enter the length value in calibration table

Five units options available: MIL, CM,MM,UM and INCH.

3. Select the objective magnification in 

, and it can also fill in one extra group Mag. value if needed.

1)This data just need to be entered if the user will use the same camera to get images at different objective settings and do the measurement for them.

In this case, no need to create calibration files for images at all the objective settings, just change "Mag." to get the corresponding calibration file.

2)If only need to use one objective in application, select "N/A"

3)If can not find suitable objective magnification from list, it can use keyboard to fill in one extra group mag. value directly.

4. Enter a name for the newly created calibration file 

And it allows to create 10 groups calibration flies.

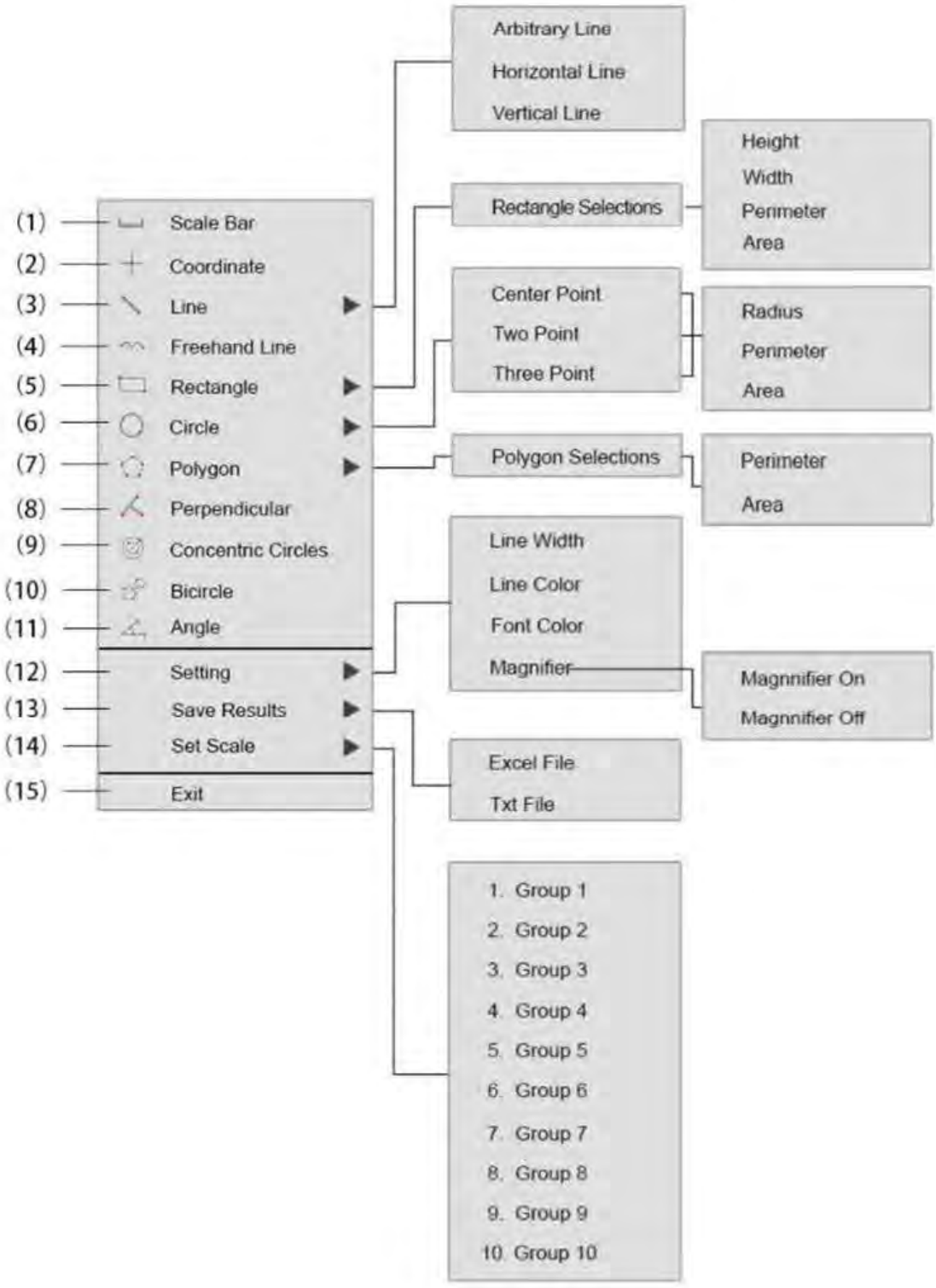
5. Click 

to complete the calibration settings.

6. Click 

to go to the image measurement page.

7. Right-click anywhere on the live image to get the measurement menu.



1	Scale Bar	On/off the scale bar on the picture
2	Coordinate	Get the coordinates of the selected points
3	Line	Arbitrary, horizontal and vertical line measurement.
4	Freehand Line	Get a freehand line length.
5	Rectangle	Measure rectangle perimeter and area. Selectable to have height, width, perimeter or area data.
6	Circle	**Center Point:** Use center point and point on the circle to draw a circle **Two Point:** Draw a circle according to the diameter. **Three Point:** Use 3 points on the circle to draw a circle. Selectable to have radius, perimeter or area data.
7	Polygon	Measure polygon perimeter and area. Selectable to have perimeter or area data.
8	Perpendicular	Measure the perpendicular length.
9	Concentric circles	Get the diameters of the two concentric circles.
10	Bicircle	Get the distance between two circles' center points.
11	Angle	Measure the angle.
12	Setting	1)Set the measurement line width, color and font color. 2)**Magnifier On/off:** Switch on/off the magnifier. When the magnifier is activated, the cursor located area will be zoomed in and placed at the corner to help accurately locate the measurement point.
13	Save Results	Select to export the measurement results to an excel or text file. The exported results will be saved in the *SD card\MEASURE folder*.
14	Set Scale	It allows to create 10 groups calibration files in calibration table, and the user can select 10 groups files to do measurement.
15	Exit	Exit the measurement.

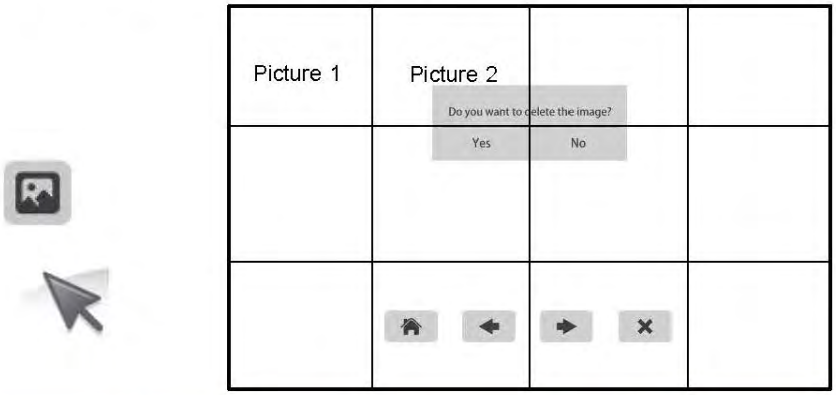
Note:

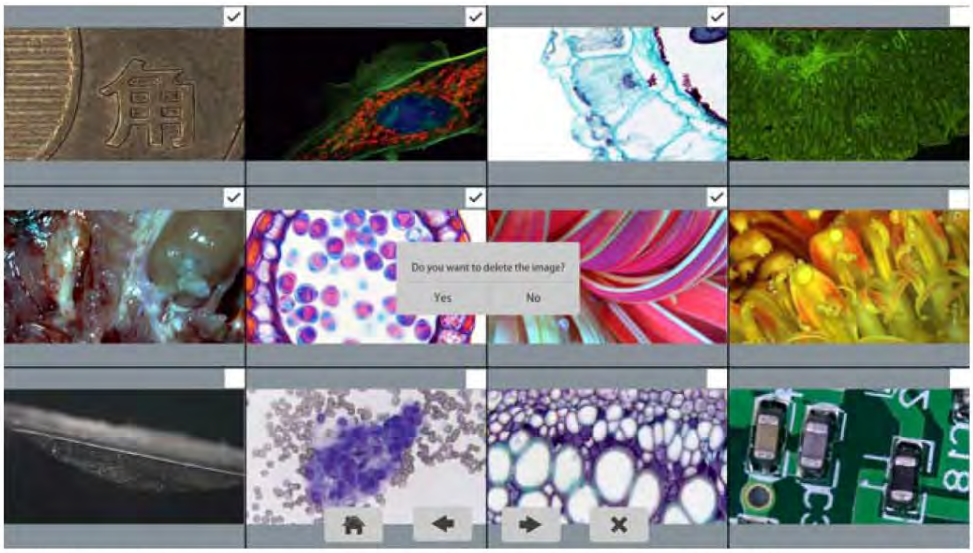
All the measurements on the live image will be removed when exit the measurement.

The measurement result can be saved on the captured images when click capture button.

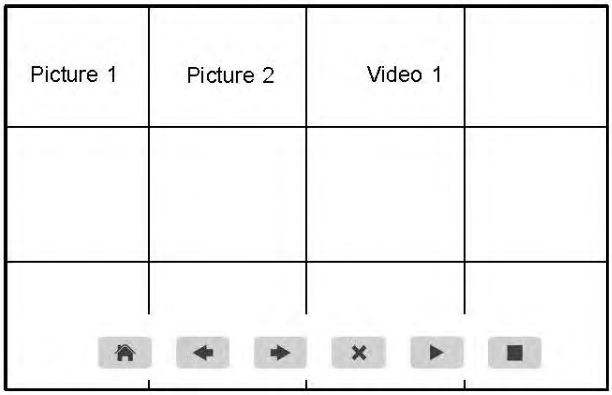
Step 5. Check the photo album and videos

1. Check the album and delete the pictures.

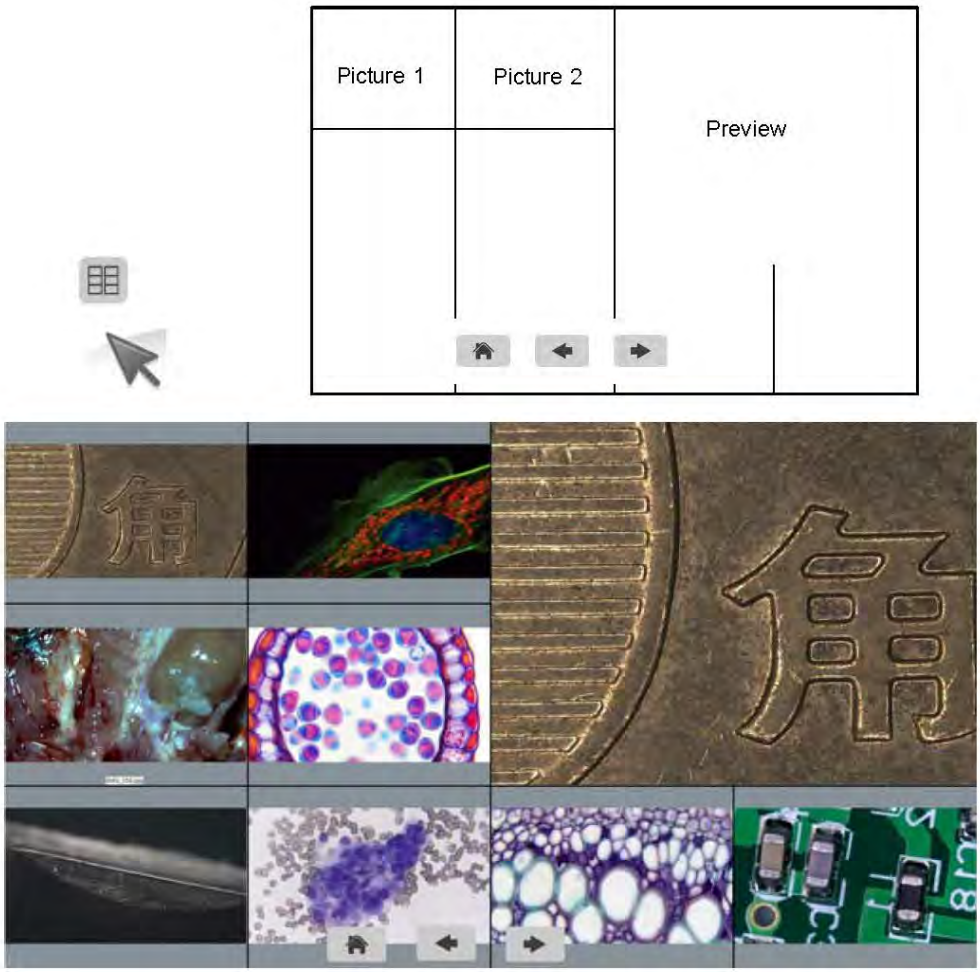




2. Check the video and delete the video. To delete the video successfully, be sure the video is not in use.



Step 6. Compare preview with the captured images.



Chapter 4 Connect to a PC

1. Use USB cable to connect the camera to the PC.
2. Plug in the 12V/2A power supply. Press and hold the ON/OFF key until blue light is on to turn on the camera.
3. No driver installation is needed when connected to PC. Install TCapture to adjust parameters and acquire images.

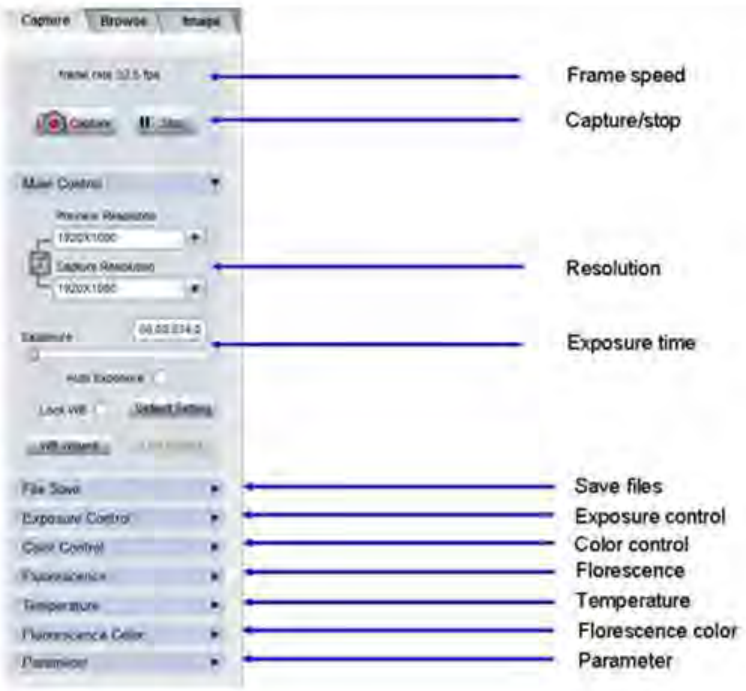
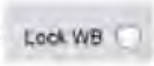


Fig.4-1. The left side of TCapture

(1) Start the TC. The parameter settings are shown on the left side of the software. See Fig.4-1.

Note: When use 'Lock WB' 

, it takes 3 seconds to make sure the camera finishes the initialization.

(2) Switch the 

in 'Exposure Control' tab to get different frame rate. In Normal mode, the image quality is better than High mode. To get faster frame rate, please select High mode.

(3) Functions 'Fluorescence', 'Temperature Control' and 'Fluorescence Color are not available for HDvCAMMEASURE. These functions are gray out when HDvCAMMEASURE camera is attached.

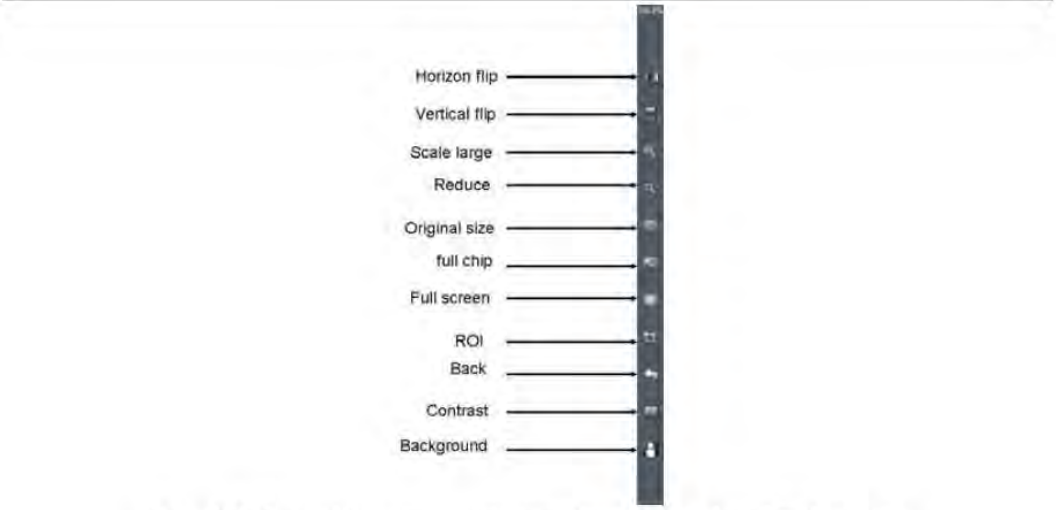


Fig.4-2. Function shortcuts on the right side of TCapture

Fig.4-3. Captured image thumbnails

(4) Users can double click on one image thumbnail to display it. Click on the little triangle button to get more captured image thumbnails.

(5) Further more, you also can select one or more images and delete them.

Fig.4-4. Imaging processing

Fig.4-5. measurement

(6) Click on Image tab, get the image processing functions (Fig.4-4).

(7) Provide Focus stacking, HDR, Fluorescence Combination functions etc. Note: When camera is disconnected, all the image processing functions will be gray out.

(8) Click on Measure tab to get measurement functions (Fig.4-5).

(9) It is allowed to apply measurements to the live and still images. To get more details about measurement, please read the TCapture software manual.

If have any questions, please Click on 'Help' to get support information.

Cleanliness

When the camera is NOT in use, please screw in the dustproof cap to avoid dust from the environment accumulating on the optical port.

If dust is accumulated on surface of the optical port, we recommend using a blower bulb to blow away the dust first. If dust is still there, please use a very soft lint free cloth (Micro Fiber cloth) with absolute ethyl alcohol or similar cleaning agent to gently clean the surface.

If dust is found inside the camera, please DO NOT open the camera case. Please contact our support team to get further advice.

Maintain

Only the manufacturer has the right to open the camera case for maintenance. If repair is needed, please contact your vendor for assistance.

CAUTION: Please DO NOT open the camera. Dust and moisture is easily introduced into the sealed camera body if it is opened by unauthorizec personnel. Any sensor scratch or moisture issue brought by opening the camera case by unauthorized personnel is not covered by the warranty.

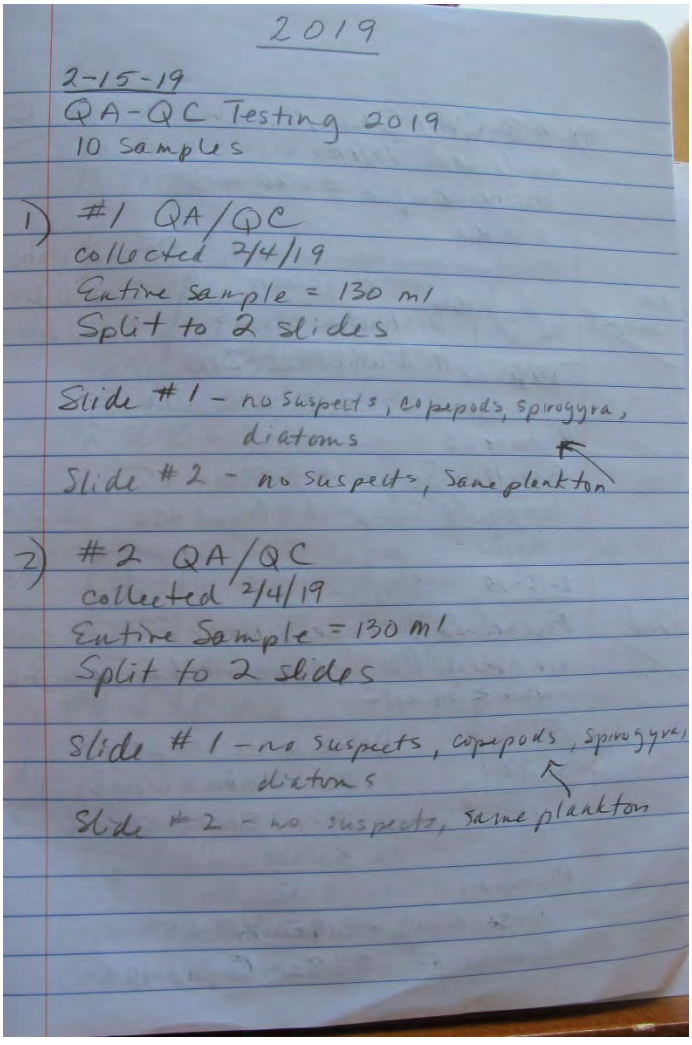
Contact information

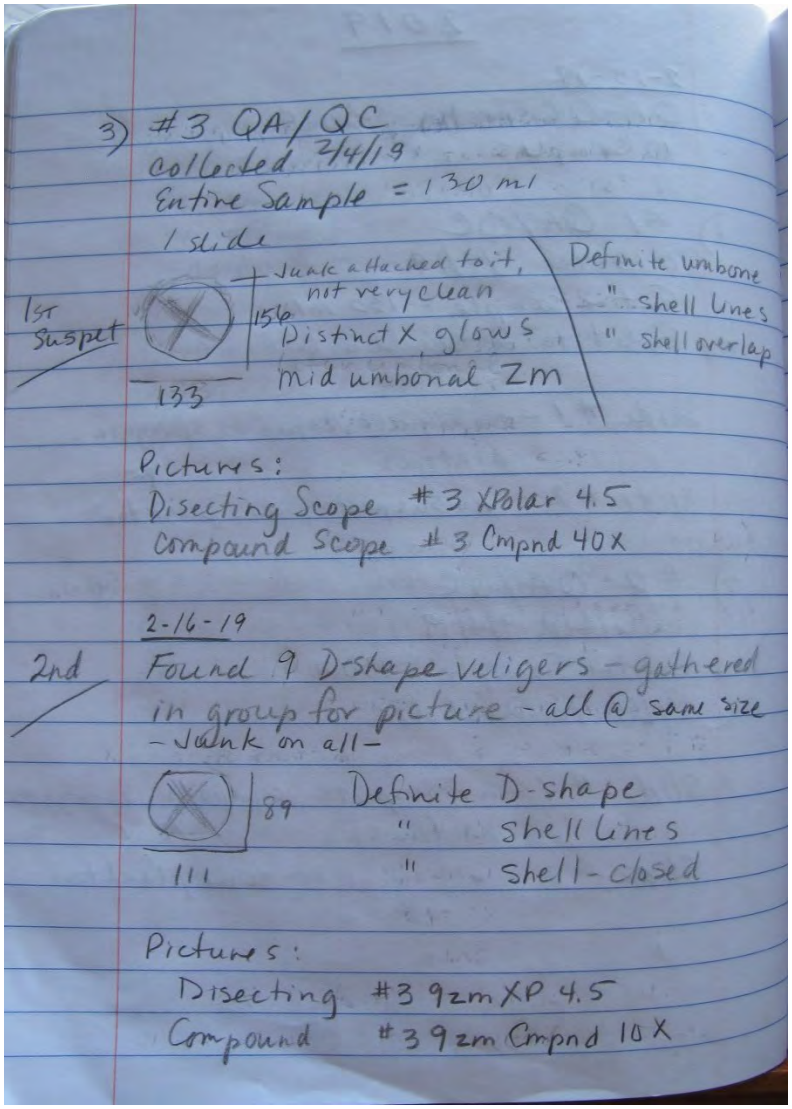
Focus Precision Instruments
Joel Ash
952-380-3696
joel@focuspi.com

Appendix D- Veliger Log Notebook

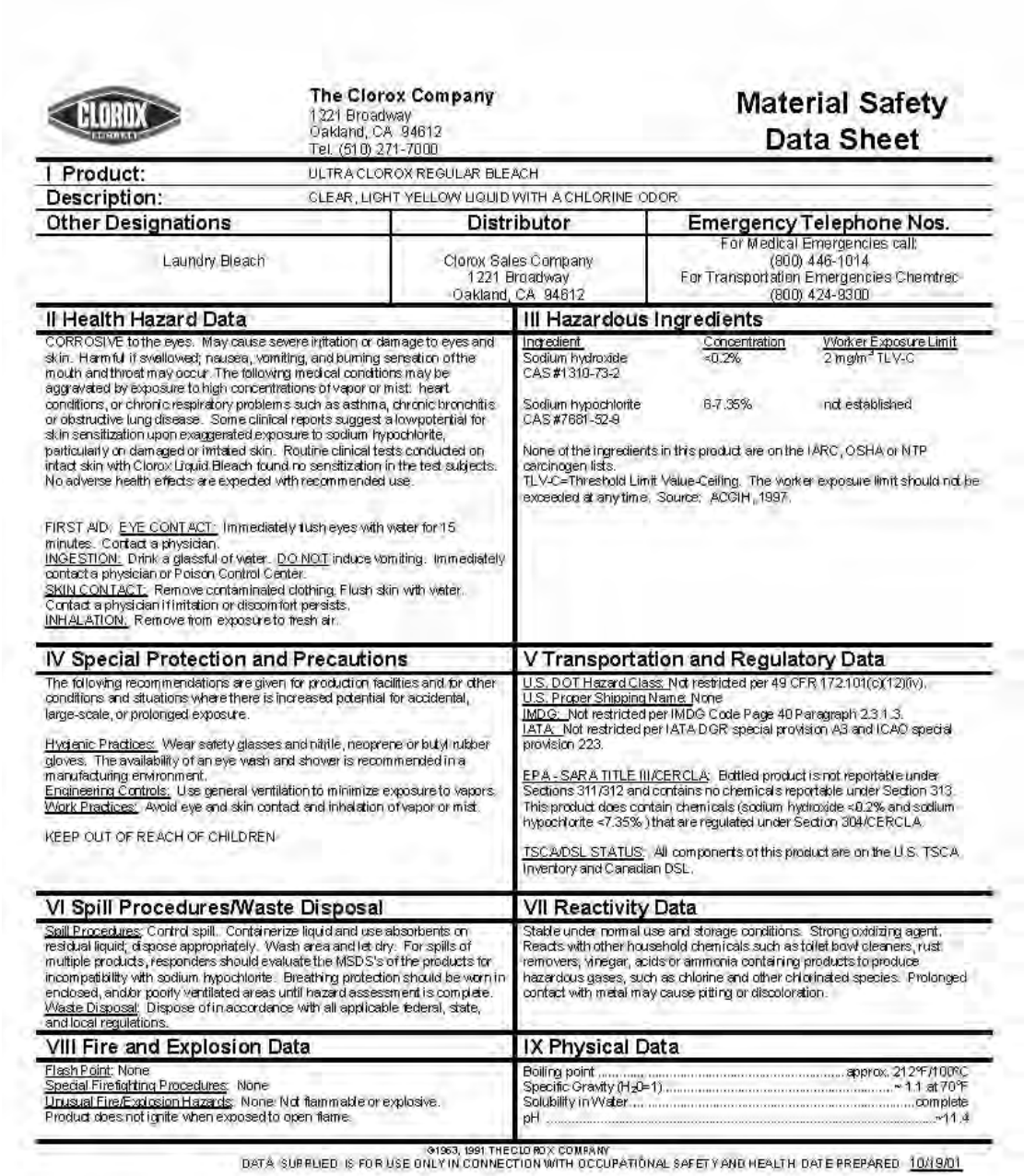
All processed samples are recorded into the lab technician's sample log notebook Record waterbody, date collected, date processed, total sample size, sample size processed, all results/findings, pictures of suspect organisms.

The data from the notebook is entered into an online spreadsheet as well as an online database. This data should be entered daily.





Appendix E- Hazards



The Clorox Company
1221 Broadway
Oakland, CA 94612
Tel. (510) 271-7000

Material Safety Data Sheet

I Product: ULTRA CLOROX REGULAR BLEACH

Description: CLEAR, LIGHT YELLOW LIQUID WITH A CHLORINE ODOR

Other Designations	Distributor	Emergency Telephone Nos.
Laundry Bleach	Clorox Sales Company 1221 Broadway Oakland, CA 94612	For Medical Emergencies call: (800) 446-1014 For Transportation Emergencies Chemtrec (800) 424-9300

II Health Hazard Data

CORROSIVE to the eyes. May cause severe irritation or damage to eyes and skin. Harmful if swallowed; nausea, vomiting, and burning sensation of the mouth and throat may occur. The following medical conditions may be aggravated by exposure to high concentrations of vapor or mist: heart conditions, or chronic respiratory problems such as asthma, chronic bronchitis or obstructive lung disease. Some clinical reports suggest a low potential for skin sensitization upon exaggerated exposure to sodium hypochlorite, particularly on damaged or irritated skin. Routine clinical tests conducted on intact skin with Clorox Liquid Bleach found no sensitization in the test subjects. No adverse health effects are expected with recommended use.

FIRST AID: EYE CONTACT: Immediately flush eyes with water for 15 minutes. Contact a physician.
INGESTION: Drink a glassful of water. DO NOT induce vomiting. Immediately contact a physician or Poison Control Center.
SKIN CONTACT: Remove contaminated clothing. Flush skin with water. Contact a physician if irritation or discomfort persists.
INHALATION: Remove from exposure to fresh air.

III Hazardous Ingredients

Ingredient	Concentration	Worker Exposure Limit
Sodium hydroxide CAS #1310-73-2	<0.2%	2 mg/m^3 TLV-C
Sodium hypochlorite CAS #7681-52-9	6-7.35%	not established

None of the ingredients in this product are on the IARC, OSHA or NTP carcinogen lists.
TLV-C=Threshold Limit Value-Ceiling. The worker exposure limit should not be exceeded at any time. Source: ACGIH, 1997.

IV Special Protection and Precautions

The following recommendations are given for production facilities and for other conditions and situations where there is increased potential for accidental, large-scale, or prolonged exposure.

Hygienic Practices: Wear safety glasses and nitrile, neoprene or butyl rubber gloves. The availability of an eye wash and shower is recommended in a manufacturing environment.
Engineering Controls: Use general ventilation to minimize exposure to vapors.
Work Practices: Avoid eye and skin contact and inhalation of vapor or mist.

KEEP OUT OF REACH OF CHILDREN

V Transportation and Regulatory Data

U.S. DOT Hazard Class: Not restricted per 49 CFR 172.101(c)(12)(iv).
U.S. Proper Shipping Name: None
IMDG: Not restricted per IMDG Code Page 40 Paragraph 2.3.1.3.
IATA: Not restricted per IATA DGR special provision A3 and ICAO special provision 223.

EPA - SARA TITLE III/CERCLA: Bottled product is not reportable under Sections 311/312 and contains no chemicals reportable under Section 313. This product does contain chemicals (sodium hydroxide <0.2% and sodium hypochlorite <7.35%) that are regulated under Section 304/CERCLA.

TSCA/DSL STATUS: All components of this product are on the U.S. TSCA Inventory and Canadian DSL.

VI Spill Procedures/Waste Disposal

Spill Procedures: Control spill. Containerize liquid and use absorbents on residual liquid; dispose appropriately. Wash area and let dry. For spills of multiple products, responders should evaluate the MSDS's of the products for incompatibility with sodium hypochlorite. Breathing protection should be worn in enclosed, and/or poorly ventilated areas until hazard assessment is complete.
Waste Disposal: Dispose of in accordance with all applicable federal, state, and local regulations.

VII Reactivity Data

Stable under normal use and storage conditions. Strong oxidizing agent. Reacts with other household chemicals such as toilet bowl cleaners, rust removers, vinegar, acids or ammonia containing products to produce hazardous gases, such as chlorine and other chlorinated species. Prolonged contact with metal may cause pitting or discoloration.

VIII Fire and Explosion Data

Flash Point: None
Special Firefighting Procedures: None
Unusual Fire/Explosion Hazards: None. Not flammable or explosive. Product does not ignite when exposed to open flame.

IX Physical Data

Boiling point ..... approx. 212°F/100°C
Specific Gravity (H_2O=1) ..... ~ 1.1 at 70°F
Solubility in Water ..... complete
pH ..... ~11.4

DATA SUPPLIED IS FOR USE ONLY IN CONNECTION WITH OCCUPATIONAL SAFETY AND HEALTH. DATE PREPARED 10/19/01

Complies with EC no. 1907/2006
Date of Issue: 01/20/2006
Date of Revision: 05/01/2018

Safety Data Sheet (SDS)

Section 1: Chemical Product and Company Identification

Cat # 190 Proof - 2816, 2816G, 2801, 2801G, 2805, 2705SG, 2705M, 2855, 2855M

Part Name: Decon's Ethanol, 190 Proof

Emergency Telephone Numbers
US Chemtrec: (800) 424-9300
Canada: (703) 527-3887

Supplier: **Decon Laboratories Inc.**
460 Glennie Circle King of Prussia, Pa 19406
SDS Telephone # (610) 755-0800

Identified uses: Laboratory use

Email Contact: cveloski@deconlabs.com

Section 2: Hazards Identification:

GHS Classification

Flammable Liquids, Category 2 H225
Eye Irritation, Category 2A H319
Full text of H-phrases: see section 16

Signal Word: DANGER

Hazard and Precautionary Statements

H225 Highly Flammable liquid and vapor.
H319 Causes serious eye irritation.
P210 Keep away from heat, sparks, open flames and hot surfaces - no smoking.
P233 Keep container tightly closed.
P240 Ground/bond container and receiving equipment.
P241 Use explosion-proof electrical/ventilating/lighting equipment.
P242 Use only non-sparking tools.
P243 Take precautionary measures against static discharge.
P264 Wash thoroughly after handling.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303/361/353 IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Rinse skin with water.
P305/351/338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing.
P337+313: If eye irritation persists get medical advice/attention.
P403 + P235 Store in a well-ventilated place. Keep cool.
P243 Take precautionary measures against static discharge.
P241 Use explosion-proof electrical/ventilating/lighting equipment.
P370 + 378 In case of fire: Use appropriate extinguishing media (See Section 5)

P403 + P235 Store in a well-ventilated place. Keep cool.
P501 - Dispose of contents/container in accordance with local, regional, national, territorial, provincial, and international regulations

Other Hazards

Other Hazards Not Contributing to the Classification: Flammable vapors can accumulate in head space of closed systems.

Unknown Acute Toxicity (GHS-US) Not available

NFPA Rating

Hazard Ratings:
These ratings are Decon Laboratories Inc.'s own assessments of the properties of the material using the ANSI/NFPA 704 Standard. Additional information can be found by consulting in the NFPA published ratings lists (List 325 and list 49).

If no data is listed the information is not available

Health 1 Flammability 3 Reactivity 0

Section 3: Composition/ Information on ingredients

Note: Items listed with a CASRN number have no CAS# available

Mixture

Name	Product identifier	% (w/w)	GHS-US classification
Ethyl alcohol	(CAS No) 64-17-5 (EC no) 200-578-6	92.3 - 94.6	Flam. Liq. 2, H225 Eye Irrit. 2A, H319
Water	(CAS No) 7732-18-5 (EC no) 231-791-2	5.4 - 7.7	Not classified

Section 4: First Aid Measures

Description of First Aid Measures

General: Never give anything by mouth to an unconscious person. If exposed or concerned: Get medical advice/attention.

Inhalation: When symptoms occur: go into open air and ventilate suspected area.

Skin Contact: Remove contaminated clothing. Rinse immediately with large amounts of water. Call a

POISON CENTER or doctor/physician if you feel unwell. Wash contaminated clothing before reuse.

Eye Contact: Rinse cautiously with water for at least 15 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Obtain medical attention.

Ingestion: Get medical advice and attention if you feel unwell. Rinse mouth. Do NOT induce vomiting.

Most Important Symptoms and Effects Both Acute and Delayed

General: Causes serious eye irritation.

Inhalation: Prolonged exposure to liquid may cause a mild irritation.

Skin Contact: Repeated or prolonged skin contact may cause dermatitis and defatting.

Eye Contact: Causes serious eye irritation. Symptoms may include: Redness, pain, swelling, itching, burning, tearing, and blurred vision.

Ingestion: Ingestion of this product is extremely harmful to human health. Nausea and vomiting, higher exposure causes unconsciousness.

Chronic Symptoms: None expected under normal conditions of use.

Indication of Any Immediate Medical Attention and Special Treatment Needed

If medical advice is needed, have product container or label at hand.

Section 5: Fire-Fighting Measures

Extinguishing Media

Suitable Extinguishing Media: Alcohol-resistant foam, carbon dioxide, dry chemical, water spray, fog.

Unsuitable Extinguishing Media: Do not use a heavy water stream. A heavy water stream may spread burning liquid. Water may be ineffective because it may not cool material below its flash point.

Special Hazards Arising From the Substance or Mixture

Fire Hazard: Highly flammable liquid and vapor.

Explosion Hazard: May form flammable/explosive vapor-air mixture. When mixed with air and exposed to an ignition source, flammable vapors can burn in the open or explode in confined spaces. Being heavier than air, vapors may travel long distances to an ignition source and flash back. Runoff to sewer may cause fire or explosion hazard.

Reactivity: Reacts violently with (strong) oxidizers: (increased) risk of fire/explosion.

Advice for Firefighters

Precautionary Measures Fire: Exercise caution when fighting any chemical fire. Under fire conditions, hazardous fumes will be present.

Firefighting Instructions: Use water spray or fog for cooling exposed containers. Prevent fire-fighting water from entering environment.

Protection During Firefighting: Do not enter fire area without proper protective equipment, including respiratory protection.

Hazardous Combustion Products: Carbon oxides (CO, CO_2).

Reference to Other Sections

Refer to section 9 for flammability properties.

Section 6: Accidental Release measures

Personal Precautions, Protective Equipment and Emergency Procedures

General Measures: Use special care to avoid static electric charges. Keep away from heat, sparks, open flames, hot surfaces. – No smoking. Avoid all eyes and skin contact and do not breathe vapor and mist.

For Non-Emergency Personnel

Protective Equipment: Use appropriate personal protection equipment (PPE).

Emergency Procedures: Evacuate unnecessary personnel.

For Emergency Personnel

Protective Equipment: Equip cleanup crew with proper protection. Use appropriate personal protection equipment (PPE). **Emergency Procedures:** Upon arrival at the scene, a first responder is expected to recognize the presence of dangerous goods, protect oneself and the public, secure the area, and call for the assistance of trained personnel as soon as conditions permit. **Environmental Precautions**

Prevent entry to sewers and public waters. Notify authorities if liquid enters sewers or public waters.

Methods and Material for Containment and Cleaning Up

For Containment: Contain any spills with dikes or absorbents to prevent migration and entry into sewers or streams. **Methods for Cleaning Up:** Absorb and/or contain spill with inert material, then place in suitable container. Do not take up in combustible material such as: saw dust or cellulosic material. Use only non-sparking tools.

Reference to Other Sections

See Heading 8. Exposure controls and personal protection.

Section 7: Handling and Storage

Precautions for Safe Handling

Additional Hazards When Processed: Handle empty containers with care because residual vapors are flammable.

Hygiene Measures: Handle in accordance with good industrial hygiene and safety procedures. Wash hands and other exposed areas with mild soap and water before eating, drinking, or smoking and again when leaving work.

Conditions for Safe Storage, Including Any Incompatibilities

Technical Measures: Proper grounding procedures to avoid static electricity should be followed. Ground/bond container and receiving equipment. Use explosion-proof electrical, lighting, ventilating equipment.

Storage Conditions: Store in a dry, cool, and well-ventilated place. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Keep container closed when not in use. Keep

in fireproof place.

Incompatible Materials: Strong oxidizing agents, acids, alkali metals, ammonia, hydrazine, peroxides, sodium, acid anhydrides, calcium hypochlorite, chromyl chloride, nitrosyl perchlorate, bromine pentafluoride, perchloric acid, silver nitrate, mercuric nitrate, potassium-tert-butoxide, magnesium perchlorate, acid chlorides, platinum, uranium hexafluoride, silver oxide, iodine heptafluoride, acetyl bromide, disulfuryl difluoride, tetrachlorosilane + water, acetyl chloride, permanganic acid, ruthenium (VIII) oxide, uranyl perchlorate, potassium dioxide.

Specific End Use(s)

Solvent.

Section 8: Exposure Controls / Personal Protection

Control Parameters

For substances listed in section 3 that are not listed here, there are no established Exposure limits from the manufacturer, supplier, importer, or the appropriate advisory agency including: ACGIH (TLV), NIOSH (REL), OSHA (PEL), Canadian provincial governments, or the Mexican government.

Ethyl Alcohol (64-17-5)		
Mexico	OEL TWA (mg/m^3)	1900 mg/m^3
Mexico	OEL TWA (ppm)	1000 ppm
USA ACGIH	ACGIH STEL (ppm)	1000 ppm
USA ACGIH	ACGIH chemical category	Confirmed Animal Carcinogen with Unknown Relevance to Humans
USA OSHA	OSHA PEL (TWA) (mg/m^3)	1900 mg/m^3
USA OSHA	OSHA PEL (TWA) (ppm)	1000 ppm
USA NIOSH	NIOSH REL (TWA) (mg/m^3)	1900 mg/m^3
USA NIOSH	NIOSH REL (TWA) (ppm)	1000 ppm
USA IDLH	US IDLH (ppm)	3300 ppm (10% LEL)
Alberta	OEL TWA (mg/m^3)	1880 mg/m^3
Alberta	OEL TWA (ppm)	1000 ppm
British Columbia	OEL STEL (ppm)	1000 ppm
Manitoba	OEL STEL (ppm)	1000 ppm
New Brunswick	OEL TWA (mg/m^3)	1880 mg/m^3
New Brunswick	OEL TWA (ppm)	1000 ppm
Newfoundland &	OEL STEL (ppm)	1000 ppm
Nova Scotia	OEL STEL (ppm)	1000 ppm
Nunavut	OEL STEL (mg/m^3)	2355 mg/m^3
Nunavut	OEL STEL (ppm)	1250 ppm
Nunavut	OEL TWA (mg/m^3)	1884 mg/m^3
Nunavut	OEL TWA (ppm)	1000 ppm
Northwest Territories	OEL STEL (mg/m^3)	2355 mg/m^3
Northwest Territories	OEL STEL (ppm)	1250 ppm

Northwest Territories	OEL TWA (mg/m³)	1884 mg/m³
Northwest Territories	OEL TWA (ppm)	1000 ppm
Ontario	OEL STEL (ppm)	1000 ppm
Prince Edward Island	OEL STEL (ppm)	1000 ppm
Québec	VEMP (mg/m³)	1880 mg/m³
Québec	VEMP (ppm)	1000 ppm
Saskatchewan	OEL STEL (ppm)	1250 ppm
Saskatchewan	OEL TWA (ppm)	1000 ppm
Yukon	OEL STEL (mg/m³)	1900 mg/m³
Yukon	OEL STEL (ppm)	1000 ppm
Yukon	OEL TWA (mg/m³)	1900 mg/m³
Yukon	OEL TWA (ppm)	1000 ppm

Exposure Controls

Appropriate Engineering Controls: Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure. Provide sufficient ventilation to keep vapors below permissible exposure limit. Gas detectors should be used when flammable gases/vapors may be released. Proper grounding procedures to avoid static electricity should be followed.
Ensure all national/local regulations are observed.

Personal Protective Equipment: Protective clothing. Gloves. Protective goggles. Insufficient ventilation: wear respiratory protection.

Materials for Protective Clothing: Not available

Hand Protection: Wear chemically resistant protective gloves.

Eye Protection: Chemical goggles or safety glasses.

Skin and Body Protection: Use chemically protective clothing.

Respiratory Protection: If exposure limits are exceeded or irritation is experienced, approved respiratory protection should be worn.

Other Information: When using, do not eat, drink, or smoke.

Chemical-resistant gloves should be worn whenever this material is handled. The glove material has to be impermeable and resistant to the product. Gloves should be removed and replaced immediately if there is any indication of degradation or chemical breakthrough. Rinse and remove gloves immediately after use. Wash hands with soap and water. All glove recommendations presume that the risk of exposure is through splash and not intentional immersion of the hands into the product. Since glove permeation data does not exist for this material, no recommendation for the glove material can be given for the product. Permiation data must be obtained from the glove manufacturer to determine if the glove is suitable for the task.

Section 9: Physical and Chemical Properties

Information on Basic Physical and Chemical Properties

Physical state		Liquid
Appearance		Colorless, clear, volatile liquid
Odor		Alcohol
Odor Threshold	:	Not available
pH	:	Not available
Evaporation Rate	:	Not available
Melting Point	:	Not available
Freezing Point	:	-114°F (-173°F)
Boiling Point	:	78 °C (172.4 °F)
Flash Point	:	12.8 °C (55 °F) CC
Auto-ignition Temperature	:	Not available
Decomposition Temperature	:	Not available
Flammability (solid, gas)	:	Not available
Lower Flammable Limit	:	3.3 % for Ethanol
Upper Flammable Limit	:	19 % for Ethanol
Vapor Pressure	:	44.6 mm Hg @ 20°C (68°F)
Relative Vapor Density at 20 °C	:	1.59 for Ethanol
Relative Density	:	0.8157 - 0.814
Specific Gravity	:	Not available
Solubility	:	Water: Completely
Partition Coefficient: N-Octanol/Water	:	Not available
Viscosity	:	Not available
Explosion Data – Sensitivity to Mechanical Impact	:	Not expected to present an explosion hazard due to mechanical impact.
Explosion Data – Sensitivity to Static Discharge	:	Static discharge could act as an ignition source.

Section 10: Stability and Reactivity:

Reactivity:	Reacts violently with (strong) oxidizers: (increased) risk of fire/explosion.
Chemical Stability:	Stable at standard temperature and pressure.

Possibility of Hazardous Reactions: Hazardous polymerization will not occur

Conditions to Avoid: Direct sunlight. Extremely high or low temperatures. Open flame. Ignition sources.

Incompatible Materials: Strong oxidizing agents, acids, alkali metals, ammonia, hydrazine, peroxides, sodium, acid anhydrides, calcium hypochlorite, chromyl chloride, nitrosyl perchlorate, bromine pentafluoride, perchloric acid, silver nitrate, mercuric nitrate, potassium-tert-butoxide, magnesium perchlorate, acid chlorides, platinum, uranium hexafluoride, silver oxide, iodine heptafluoride, acetyl bromide, disulfuryl difluoride, tetrachlorosilane + water, acetyl chloride, permanganic acid, ruthenium (VIII) oxide, uranyl perchlorate, potassium dioxide.

Hazardous Decomposition Products: Carbon oxides (CO, CO_2).

Section 11: Toxicological Information

Information on Toxicological Effects - Product

Acute Toxicity: Not classified

LD50 and LC50 Data:

Ethyl Alcohol, 200 Proof (64-17-5)	
LC50 Inhalation Rat	124.7 mg/l/4h
Ethyl Alcohol, 200 Proof (64-17-5)	
OSHA Hazard Communication Carcinogen List	In OSHA Hazard Communication Carcinogen list.

Skin Corrosion/Irritation: Not classified

Serious Eye Damage/Irritation: Causes serious eye irritation.

Respiratory or Skin Sensitization: Not classified **Germ Cell Mutagenicity:** Not classified **Teratogenicity:** Not classified

Carcinogenicity: Not classified

Specific Target Organ Toxicity (Repeated Exposure): Not classified

Reproductive Toxicity: Not classified

Specific Target Organ Toxicity (Single Exposure): Not classified

Aspiration Hazard: Not classified

Symptoms/Injuries After Inhalation: Prolonged exposure to liquid may cause a mild irritation.
Symptoms/Injuries After Skin Contact: Repeated or prolonged skin contact may cause dermatitis and defatting.
Symptoms/Injuries After Eye Contact: Causes serious eye irritation. Symptoms may include: Redness, pain, swelling, itching, burning, tearing, and blurred vision.
Symptoms/Injuries After Ingestion: This product is adulterated to prevent ingestion. Ingestion of this product is extremely harmful to human health. nausea and vomiting, higher exposure causes unconsciousness.
Chronic Symptoms: None expected under normal conditions of use.

Information on Toxicological Effects - Ingredient(s) LD50 and LC50 Data:

Ethyl alcohol (64-17-5)	
LD50 Oral Rat	10470 mg/kg
LD50 Dermal Rat	20 ml/kg
LC50 Inhalation Rat	124.7 mg/l/4h

Ethyl alcohol (64-17-5)	
OSHA Hazard Communication Carcinogen List	In OSHA Hazard Communication Carcinogen list.

Section 12: Ecological Information

Toxicity

Ecology - General: Readily biodegrades. Evaporates to moderate extent. Does not bioaccumulate.

Ethyl alcohol (64-17-5)	
LC50 Fish 1	12.0 - 16.0 ml/l (Exposure time: 96 h - Species: Oncorhynchus mykiss [static])
EC50 Daphnia 1	9268 - 14221 mg/l (Exposure time: 48 h - Species: Daphnia magna)
LC 50 Fish 2	> 100 mg/l (Exposure time: 96 h - Species: Pimephales promelas [static])

Persistence and Degradability

Ethyl Alcohol (64-17-5)	
Persistence and Degradability	Not established. May cause long-term adverse effects in the environment.

Bioaccumulative Potential

Ethyl Alcohol (64-17-5)	
Log Pow	-0.32
Bioaccumulative Potential	Not established.

Mobility in Soil Not available

Other Adverse Effects

Other Information: Avoid release to the environment.

Section 13: Disposal Considerations

Waste Disposal Recommendations: Dispose of waste material in accordance with all local, regional, national, provincial, territorial and international regulations.

Additional Information: Handle empty containers with care because residual vapors are flammable

Section 14: Transportation Information

In Accordance with DOT

Proper Shipping Name : ETHYL ALCOHOL SOLUTIONS
Hazard Class : 3
Identification Number : UN1170
Label Codes : 3
Packing Group : II
ERG Number : 127

Note:
Exemptions apply for small pack sizes.

In Accordance with IMDG

Proper Shipping Name : ETHYL ALCOHOL SOLUTIONS
Hazard Class : 3
Identification number UN1170
Packing Group : II
Label Codes : 3
EmS-No. (Fire) : F-E
EmS-No. (Spillage) : S-D

In Accordance with IATA

Proper Shipping Name : ETHYL ALCOHOL SOLUTIONS
Packing Group : II **Identification Number** : UN1170 **Hazard Class** : 3
Label Codes : 3
ERG Code (IATA) : 3L

In Accordance with TDG

Proper Shipping Name : ETHYL ALCOHOL SOLUTIONS
Packing Group : II
Hazard Class : 3
Identification Number : UN1170

Label Codes : 3

Section 15: Regulatory Information

Ethyl Alcohol, 200 Proof (64-17-5)	
Listed on the United States TSCA (Toxic Substances Control Act) inventory	
SARA Section 311/312 Hazard Classes	Fire hazard Immediate (acute) health hazard
Ethyl alcohol (64-17-5)	
Listed on the United States TSCA (Toxic Substances Control Act) inventory	
Water (7732-18-5)	
Listed on the United States TSCA (Toxic Substances Control Act) inventory	

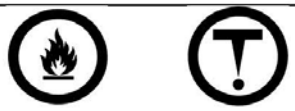
US State Regulations:

State or local regulations
U.S. - Massachusetts - Right To Know List
U.S. - New Jersey - Right to Know Hazardous Substance List
U.S. - Pennsylvania - RTK (Right to Know) List

Ethyl alcohol (64-17-5)
U.S. - Massachusetts - Right To Know List
U.S. - New Jersey - Right to Know Hazardous Substance List
U.S. - Pennsylvania - RTK (Right to Know) List

Canadian Regulations

Ethyl Alcohol, 200 Proof (64-17-5)	
Listed on the Canadian DSL (Domestic Substances List) Listed on the Canadian	
YesIDL Concentration 0.1 %	
WHMIS Classification	Class B Division 2 - Flammable Liquid Class D Division 2 Subdivision B - Toxic material causing other toxic effects



Ethyl alcohol (64-17-5)	
Listed on the Canadian DSL omestic (D Listed on the Canadian Substances	
IDL Concentration 0.1 %	
WHMIS Classification	Class B Division 2 - Flammable Liquid Class D Division 2 Subdivision B - Toxic material causing other toxic effects
Water (7732-18-5)	
Listed on the Canadian DSL omestic Substances List)	
WHMIS Classification	Uncontrolled product according to WHMIS classification criteria

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the SDS contains all of the information required by CPR.

Section 16: Other Information

Date of Issue: 01/20/2006
Date of Revision: 05/01/2018

Other Information : This document has been prepared in accordance with the SDS requirements of the OSHA

GHS Full Text Phrases:

Eye Irrit. 2A	Serious eye damage/eye irritation, Category 2A
Flam. Liq. 2	Flammable liquids, Category 2
H225	Highly flammable liquid and vapor
H319	Causes serious eye irritation

End of Safety Data Sheet

Sodium Bicarbonate

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules And Regulations
Revision Date: 03/12/2015 Date of Issue: 03/12/2015 Supersedes Date: 03/09/2012 Version: 1.0

SECTION 1: IDENTIFICATION

Product Identifier

Product Form: Substance

Product Name: Sodium Bicarbonate

CAS No: 144-55-8

Formula: $NaHCO_3$

Synonyms: Baking Soda

Intended Use of the Product

Food Ingredient, Pharmaceutical, Household and Personal Care Product, Water Treatment, General Industrial Use.

Name, Address, and Telephone of the Responsible Party

Company

Church & Dwight
500 Charles Ewing Blvd
Ewing Township, NJ 08628
T 1-800-524-1328
www.churchdwight.com

Emergency Telephone Number

Emergency Number : For Medical Emergency: 1-888-234-1828, For Chemical Emergency: 1-800-424-9300 (CHEMTREC)

SECTION 2: HAZARDS IDENTIFICATION

The consumer variant of this product is labeled in accordance with regulations administered by the Consumer Product Safety Commission (CPSC) and the Food and Drug Administration (FDA). The use pattern and exposure in the workplace are generally not consistent with those experienced by consumers. The requirements of the Occupational Safety and Health Administration applicable to this SDS differ from the labeling requirements of the CPSC and FDA, and as a result, this SDS may contain additional health hazard information not pertinent to consumer use and not found on the product label.

Classification of the Substance or Mixture

Classification (GHS-US) Not classified

Label Elements

GHS-US Labeling No labeling applicable

Other Hazards Exposure may aggravate those with pre-existing eye, skin, or respiratory conditions. Prolonged contact with dust can produce mechanical irritation.

Unknown Acute Toxicity (GHS-US) Not available

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

Substances

Name : Sodium Bicarbonate

CAS No : 144-55-8

Name	Product Identifier	% (w/w)	Classification (GHS-US)
Sodium bicarbonate	(CAS No) 144-55-8	100	Not classified

SECTION 4: FIRST AID MEASURES

Description of First Aid Measures

General: Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice.

Inhalation: When symptoms occur: go into open air and ventilate suspected area.

Skin Contact: Brush off loose particles from skin. Rinse immediately with plenty of water. Obtain medical attention if irritation develops or persists.

Eye Contact: Rinse cautiously with water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Continue rinsing. Obtain medical attention if irritation persists.

Ingestion: Rinse mouth. Do NOT induce vomiting. Seek medical attention if a large amount is swallowed.

Most Important Symptoms and Effects Both Acute and Delayed

General: None expected under normal conditions of use.
Inhalation: Prolonged inhalation of dust may cause respiratory irritation.
Skin Contact: Skin contact with large amounts of dust may cause mechanical irritation.
Eye Contact: Contact may cause irritation due to mechanical abrasion.
Ingestion: Large doses may produce systemic alkalosis and expansion in extracellular fluid volume with edema.
Chronic Symptoms: None expected under normal conditions of use.

Indication of Any Immediate Medical Attention and Special Treatment Needed

If exposed or concerned, get medical advice and attention.

SECTION 5: FIRE-FIGHTING MEASURES

Extinguishing Media

Suitable Extinguishing Media: Use extinguishing media appropriate for surrounding fire.
Unsuitable Extinguishing Media: For surrounding fire: Use of heavy stream of water may spread fire.

Special Hazards Arising From the Substance or Mixture

Fire Hazard: NOT FLAMMABLE . Under fire conditions, hazardous fumes will be present.
Explosion Hazard: Product is not explosive.
Reactivity: Hazardous reactions will not occur under normal conditions.

Advice for Firefighters

Precautionary Measures Fire: Wear self-contained breathing apparatus when entering area unless atmosphere is proved to be safe.
Firefighting Instructions: Exercise caution when fighting any chemical fire.
Protection During Firefighting: Do not enter fire area without proper protective equipment, including respiratory protection.
Hazardous Combustion Products: Carbon oxides (CO, CO_2). Sodium oxides.

Reference to Other Sections

Refer to section 9 for flammability properties.

SECTION 6: ACCIDENTAL RELEASE MEASURES

Personal Precautions, Protective Equipment and Emergency Procedures

General Measures: Handle in accordance with good industrial hygiene and safety practice. Do not breathe dust or fumes. Avoid skin and eye contact.

For Non-Emergency Personnel

Protective Equipment: Use appropriate personal protection equipment (PPE).
Emergency Procedures: Evacuate unnecessary personnel.

For Emergency Personnel

Protective Equipment: Equip cleanup crew with proper protection.
Emergency Procedures: Ventilate area.

Environmental Precautions

Prevent entry to sewers and public waters. Avoid release to the environment.

Methods and Material for Containment and Cleaning Up

For Containment: Contain and collect as any solid.
Methods for Cleaning Up: Clean up spills immediately and dispose of waste safely. Avoid generation of dust during clean-up of spills. Keep in suitable, closed containers for disposal. Contact competent authorities after a spill.

Reference to Other Sections

See heading 8, Exposure Controls and Personal Protection.

SECTION 7: HANDLING AND STORAGE

Precautions for Safe Handling

Additional Hazards When Processed: When heated, material emits irritating fumes.
Hygiene Measures: Handle in accordance with good industrial hygiene and safety procedures. Wash hands and other exposed areas with mild soap and water before eating, drinking, or smoking and again when leaving work.

Conditions for Safe Storage, Including Any Incompatibilities

Storage Conditions: Store in a dry, cool and well-ventilated place. Keep container closed when not in use.
Incompatible Materials: Acids. Water. Lime.
Storage Temperature: < 65 °C (150 °F)

Specific End Use(s) Food Ingredient, Pharmaceutical, Water Treatment, General Industrial Use

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

Control Parameters

Particulates not otherwise classified (PNOC)		
USA ACGIH	ACGIH TWA (mg/m^3)	3 mg/m^3 Respirable fraction 10 mg/m^3 Total Dust
USA OSHA	OSHA PEL (TWA) (mg/m^3)	5 mg/m^3 Respirable fraction 15 mg/m^3 Total Dust
Alberta	OEL TWA (mg/m^3)	10 mg/m^3 (total)
British Columbia	OEL TWA (mg/m^3)	10 mg/m^3 (total dust)
Manitoba	OEL TWA (mg/m^3)	10 mg/m^3 (inhalable particles, recommended)
New Brunswick	OEL TWA (mg/m^3)	3 mg/m^3 (particulate matter containing no Asbestos and <1% Crystalline silica, respirable fraction)
Newfoundland & Labrador	OEL TWA (mg/m^3)	10 mg/m^3 (inhalable particles, recommended)
Nova Scotia	OEL TWA (mg/m^3)	10 mg/m^3 (inhalable particles, recommended)
Nunavut	OEL TWA (mg/m^3)	5 mg/m^3 (respirable mass)
Northwest Territories	OEL TWA (mg/m^3)	5 mg/m^3 (respirable mass)
Ontario	OEL TWA (mg/m^3)	10 mg/m^3 (inhalable)
Prince Edward Island	OEL TWA (mg/m^3)	10 mg/m^3 (inhalable particles, recommended)
Québec	VEMP (mg/m^3)	10 mg/m^3 (including dust, inert or nuisance particulates; containing no Asbestos and <1% Crystalline silica-total dust)
Saskatchewan	OEL STEL (mg/m^3)	20 mg/m^3 (insoluble or poorly soluble-inhalable fraction) 6 mg/m^3 (insoluble or poorly soluble-respirable fraction)
Saskatchewan	OEL TWA (mg/m^3)	10 mg/m^3 (insoluble or poorly soluble-inhalable fraction) 3 mg/m^3 (insoluble or poorly soluble-respirable fraction)

Exposure Controls

Appropriate Engineering Controls: For occupational/workplace settings: Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure. Ensure adequate ventilation, especially in confined areas. Ensure all national/local regulations are observed.

Personal Protective Equipment: For occupational or bulk quantities: Gloves. Safety glasses. Dust formation: dust mask.

Materials for Protective Clothing: For occupational or bulk quantities: Chemically resistant materials and fabrics.

Hand Protection: For occupational or bulk quantities: Wear chemically resistant protective gloves.

Eye Protection: For occupational or bulk quantities: Chemical goggles or safety glasses.

Respiratory Protection: Use a NIOSH-approved respirator or self-contained breathing apparatus whenever exposure may exceed established Occupational Exposure Limits.

Other Information: When using, do not eat, drink or smoke.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

Information on Basic Physical and Chemical Properties

Physical State	:	Solid
Appearance	:	White, crystalline powder
Odor	:	None
Odor Threshold	:	Not available
pH	:	8.2 (1% Solution)
Evaporation Rate	:	Not available
Melting Point	:	Not available
Freezing Point	:	Not available
Boiling Point	:	Not available
Flash Point	:	Not available

Auto-ignition Temperature	:	Not available
Decomposition Temperature	:	Not available
Flammability (solid, gas)	:	Not available
Upper/Lower Flammable Limit	:	Not available
Vapor Pressure	:	Not available
Relative Vapor Density at 20 °C	:	Not available
Specific gravity / density	:	62 lb/ft^3
Specific Gravity	:	Not available
Solubility	:	Water: 8.6 g/100ml @ 20 °C (68 °F)
Partition Coefficient: N-octanol/water	:	Not available
Viscosity	:	Not available
Explosion Data – Sensitivity to Mechanical Impact	:	Not expected to present an explosion hazard due to mechanical impact.
Explosion Data – Sensitivity to Static Discharge	:	Not expected to present an explosion hazard due to static discharge.

SECTION 10: STABILITY AND REACTIVITY

Reactivity: Hazardous reactions will not occur under normal conditions.

Chemical Stability: Decomposes slowly on exposure to water (moisture).

Possibility of Hazardous Reactions: Hazardous polymerization will not occur.

Conditions to Avoid: Exposure to moisture or moist air. Temperatures above 150°F (65 °C).

Incompatible Materials: Acids. Water. Lime.

Hazardous Decomposition Products: None known. At high temperature may liberate toxic gases.

SECTION 11: TOXICOLOGICAL INFORMATION

Information on Toxicological Effects - Product

Acute Toxicity: Not classified

LD50 and LC50 Data:

Sodium Bicarbonate	
LD50 Oral Rat	7.3 g/kg
LC50 Inhalation Rat	> 4.7 mg/l/4h

Skin Corrosion/Irritation: Not classified [**pH:** 8.2 (1% Solution)]

Serious Eye Damage/Irritation: Not classified [**pH:** 8.2 (1% Solution)]

Respiratory or Skin Sensitization: Not classified

Germ Cell Mutagenicity: Not classified

Teratogenicity: Not classified

Carcinogenicity: Not classified

Specific Target Organ Toxicity (Repeated Exposure): Not classified

Reproductive Toxicity: Not classified

Specific Target Organ Toxicity (Single Exposure): Not classified

Aspiration Hazard: Not classified

Symptoms/Injuries After Inhalation: Prolonged inhalation of dust may cause respiratory irritation.

Symptoms/Injuries After Skin Contact: Skin contact with large amounts of dust may cause mechanical irritation.

Symptoms/Injuries After Eye Contact: Contact may cause irritation due to mechanical abrasion.

Symptoms/Injuries After Ingestion: Large doses may produce systemic alkalosis and expansion in extracellular fluid volume with edema.

Chronic Symptoms: None expected under normal conditions of use.

SECTION 12: ECOLOGICAL INFORMATION

Toxicity No additional information available

Sodium Bicarbonate	
LC50 Fish 1	7100 mg/l Bluegill
EC50 Daphnia 1	4100 mg/l
LC 50 Fish 2	7700 mg/l Rainbow Trout
Sodium bicarbonate (144-55-8)	
LC50 Fish 1	8250 - 9000 mg/l (Exposure time: 96 h - Species: Lepomis macrochirus [static])
EC50 Daphnia 1	2350 mg/l (Exposure time: 48 h - Species: Daphnia magna)

Persistence and Degradability Not established
Bioaccumulative Potential Not established
Mobility in Soil Not available
Other Adverse Effects
Other Information: Avoid release to the environment.

SECTION 13: DISPOSAL CONSIDERATIONS

Waste Disposal Recommendations: Dispose of waste material in accordance with all local, regional, national, provincial, territorial and international regulations.

SECTION 14: TRANSPORT INFORMATION

In Accordance with DOT Not regulated for transport
In Accordance with IMDG Not regulated for transport
In Accordance with IATA Not regulated for transport
In Accordance with TDG Not regulated for transport

SECTION 15: REGULATORY INFORMATION

US Federal & International Regulations

Sodium Bicarbonate (144-55-8)
Listed on the AICS (Australian Inventory of Chemical Substances)
Listed on the Canadian DSL (Domestic Substances List)
Listed on IECSC (Inventory of Existing Chemical Substances Produced or Imported in China)
Listed on the EEC inventory EINECS (European Inventory of Existing Commercial Chemical Substances)
Listed on the Japanese ENCS (Existing & New Chemical Substances) inventory
Listed on the Korean ECL (Existing Chemicals List)
Listed on NZIoC (New Zealand Inventory of Chemicals)
Listed on PICCS (Philippines Inventory of Chemicals and Chemical Substances)
Listed on the United States TSCA (Toxic Substances Control Act) inventory

US State Regulations

Neither this product nor its chemical components appear on any US state lists.

Canadian Regulations

Sodium bicarbonate (144-55-8)	
Listed on the Canadian DSL (Domestic Substances List)	
WHMIS Classification	Uncontrolled product according to WHMIS classification criteria

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the SDS contains all of the information required by CPR.

SECTION 16: OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION

Revision Date : 03/12/2015
Other Information : This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200.

Party Responsible for the Preparation of This Document

Church & Dwight
500 Charles Ewing Blvd
Ewing Township, NJ 08628
T 1-800-524-1328

This Product Safety Data Sheet is offered solely for your information, consideration and investigation. Church & Dwight Co., Inc. provides no warranties; either expressed or implied, and assumes no responsibility for the accuracy or completeness of data contained herein. Church & Dwight Co., Inc. urges persons receiving this information to make their own determination as to the information suitability for their particular application.

SAFETY DATA SHEET

This SDS complies with 29 CFR 1910.1200 (Hazard Communication Standard)
IMPORTANT: Read this SDS before handling & disposing of this product. Pass this information on to employees, customers, and users of this product.

1. Identification

1.1. Product identifier

Product Identity	Vinegar Floor Neutralizer
Alternate Names	Vinegar Floor Neutralizer
Product Code	119-01

1.2. Relevant identified uses of the substance or mixture and uses advised against

Intended use	Cleaner
Application Method	See Label Instructions

1.3. Details of the supplier of the safety data sheet

Company Name	Diamond Products Inc. 1216 Bozeman Ave. Helena, MT 59601

Emergency

24 hour Emergency Telephone No.	Infotrac: 1 800-535-5053 Emergency: (406) 449-6570
Customer Service: Diamond Products Inc.	(406) 449-6570

2. Hazard(s) identification

2.1. Classification of the substance or mixture

Skin Corr. 2;H315 Causes skin irritation.
Eye Dam. 2A;H319 Causes serious eye irritation
Aquatic Acute 3;H402 Harmful to aquatic life

2.2. Label elements

Using the Toxicity Data listed in section 11 and 12 the product is labeled as follows.

Warning

H315 Causes skin irritation.
H319 Causes serious eye irritation
H402 Harmful to aquatic life
[Prevention]:

P264: Wash thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
[Response]:
P302+352: IF ON SKIN: Wash with plenty of water.
P305+351+338: IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P337+313: If eye irritation persists get medical advice/attention.
P332+313: If skin irritation occurs: Get medical advice/attention.
P362+364: Take off contaminated clothing and wash it before reuse.
P391 Collect spillage.
[Storage]:
No GHS storage statements
[Disposal]:
No GHS disposal statements

3. Composition/information on ingredients

This product contains the following substances that present a hazard within the meaning of the relevant State and Federal Hazardous Substances regulations.

Ingredient/Chemical Designations	Weight %	GHS Classification	Notes
Acetic Acid **CAS Number: 0000064-19-7**	1.0 - 10	Flam. Liq. 3;H226 Skin Corr. 1A;H314 Eye Dam. 1;H318 Skin Sens. 1;H317 Aquatic Acute 3	[1][2]

In accordance with paragraph (i) of §1910.1200, the specific chemical identity and/or exact percentage (concentration) of composition has been withheld as a trade secret.
[1] Substance classified with a health or environmental hazard.
[2] Substance with a workplace exposure limit.
[3] PBT-substance or vPvB-substance.
*The full texts of the phrases are shown in Section 16.

4. First aid measures

4.1. Description of first aid measures

General	In all cases of doubt, or when symptoms persist, seek medical attention. Never give anything by mouth to an unconscious person.
Inhalation	Remove to fresh air, keep patient warm and at rest. If breathing is irregular or stopped, give artificial respiration. If unconscious place in the recovery position and obtain immediate medical attention. Give nothing by mouth.
Eyes	Irrigate copiously with clean water for at least 15 minutes, holding the eyelids apart and seek medical attention.

Skin	Remove contaminated clothing. Wash skin thoroughly with soap and water or use a recognized skin cleanser.
Ingestion	Do NOT induce vomiting. Dilute product by giving large quantities of water or milk. Call your nearest poison control center for further action and seek medical attention immediately.

4.2. Most important symptoms and effects, both acute and delayed

Overview	Exposure to solvent vapor concentrations from the component solvents in excess of the stated occupational exposure limits may result in adverse health effects such as mucous membrane and respiratory system irritation and adverse effects on the kidneys, liver and central nervous system. Symptoms include headache, nausea, dizziness, fatigue, muscular weakness, drowsiness and in extreme cases, loss of consciousness. Repeated or prolonged contact with the preparation may cause removal of natural fat from the skin resulting in dryness, irritation and possible non-allergic contact dermatitis. Solvents may also be absorbed through the skin. Splashes of liquid in the eyes may cause irritation and soreness with possible reversible damage. See section 2 for further details.

5. Fire-fighting measures

5.1. Extinguishing media

Water or other normal extinguishing media.

5.2. Special hazards arising from the substance or mixture

Hazardous decomposition: Thermal decomposition may yield carbon dioxide and/or carbon monoxide.

5.3. Advice for fire-fighters

Wear self-contained breathing apparatus

ERG Guide No. —

6. Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

Put on appropriate personal protective equipment (see section 8).

6.2. Environmental precautions

Do not allow spills to enter drains or waterways.
Use good personal hygiene practices. Wash hands before eating, drinking, smoking or using toilet. Promptly remove soiled clothing and wash thoroughly before reuse.

6.3. Methods and material for containment and cleaning up

Small spills: Mop up with water.
Large spills: Absorb with inert material and place in suitable containers.
Dispose of in accordance with local, state and federal regulations.

7. Handling and storage

7.1. Precautions for safe handling

See section 2 for further details. - [Prevention]

7.2. Conditions for safe storage, including any incompatibilities

Handle containers carefully to prevent damage and spillage.
Incompatible materials: Strong oxidizing agents
Keep product containers cool and dry.
See section 2 for further details. - [Storage]:

7.3. Specific end use(s)

No data available.

8. Exposure controls and personal protection

8.1. Control parameters

Exposure

CAS No.	Ingredient	Source	Value
0000064-19-7	Acetic Acid	OSHA	TWA 10 ppm (25 mg/m3)STEL N/A
		ACGIH	TWA: 10 ppm STEL: 15 ppm
		NIOSH	TWA 10 ppm (25 mg/m3)
		Supplier	No Established Limit

Carcinogen Data

CAS No.	Ingredient	Source	Value
0000064-19-7	Acetic Acid	OSHA	Select Carcinogen: No
		NTP	Known: No; Suspected: No
		IARC	Group 1: No; Group 2a: No; Group 2b: No; Group 3: No; Group 4: No;

8.2. Exposure controls

Respiratory	If workers are exposed to concentrations above the exposure limit they must use the appropriate, certified respirators.
Eyes	Protective safety glasses recommended
Skin	Not normally required.
Engineering Controls	Provide adequate ventilation. Where reasonably practicable this should be achieved by the use of local exhaust ventilation and good general extraction. If these are not sufficient to maintain concentrations of particulates and any vapor below occupational exposure limits suitable respiratory protection must be worn.
Other Work Practices	Use good personal hygiene practices. Wash hands before eating, drinking, smoking or using toilet. Promptly remove soiled clothing and wash thoroughly before reuse.

See section 2 for further details. - [Prevention]:

9. Physical and chemical properties

Appearance	Clear Liquid
Odor	Vinegar
Odor threshold	Not Measured
pH	2.4 at 6%
Melting point / freezing point	Not applicable
Initial boiling point and boiling range	210 - 220°F

Flash Point	Non-flammable
Evaporation rate (Ether = 1)	Not available
Flammability (solid, gas)	Not Applicable
Upper/lower flammability or explosive limits	**Lower Explosive Limit:** Not applicable **Upper Explosive Limit:** Not applicable
Vapor pressure (Pa)	11.4
Vapor Density	2.07 (Air = 1.0)
Specific Gravity	1.05 g/ml
Solubility in Water	Complete
Partition coefficient n-octanol/water (Log Kow)	Not Measured
Auto-ignition temperature	Not applicable
Decomposition temperature	Not available
Viscosity (cSt)	Not available
VOC Content	Not available

9.2. Other information
No other relevant information.

10. Stability and reactivity

10.1. Reactivity
Hazardous Polymerization will not occur.
10.2. Chemical stability
Stable under normal circumstances.
10.3. Possibility of hazardous reactions
No data available.
10.4. Conditions to avoid
No data available.
10.5. Incompatible materials
Strong oxidizing agents
10.6. Hazardous decomposition products
Thermal decomposition may yield carbon dioxide and/or carbon monoxide.

11. Toxicological information

Acute toxicity

Ingredient	Oral LD50, mg/kg	Skin LD50, mg/kg	Inhalation Vapor LC50, mg/L/4hr	Inhalation Dust/Mist LC50, mg/L/4hr	Inhalation Gas LC50, ppm
Acetic Acid – (64-19-7)	90,000.00, Rat	No data available	5620 ppm Mouse	No data available	No data available

Note: When no route specific LD50 data is available for an acute toxin, the converted acute toxicity point estimate was used in the calculation of the product's ATE (Acute Toxicity Estimate).

Classification	Category	Hazard Description
Acute toxicity (oral)	—	Not Applicable
Acute toxicity (dermal)	—	Not Applicable
Acute toxicity (inhalation)	—	Not Applicable
Skin corrosion/irritation	2	Causes skin irritation.
Serious eye damage/irritation	2A	Causes serious eye irritation
Respiratory sensitization	—	Not Applicable
Skin sensitization	—	Not Applicable
Germ cell mutagenicity	—	Not Applicable
Carcinogenicity	—	Not Applicable
Reproductive toxicity	—	Not Applicable
STOT-single exposure	—	Not Applicable
STOT-repeated exposure	—	Not Applicable
Aspiration hazard	—	Not Applicable

12. Ecological information

12.1. Toxicity
No additional information provided for this product. See Section 3 for chemical specific data.
Aquatic Ecotoxicity

Ingredient	96 hr LC50 fish, mg/l	24 hr EC50 crustacea, mg/l	ErC50 algae, mg/l
Acetic Acid – (64-19-7)	79.00, Fathead Minnow	47.00, Daphnia	No Data Available

12.2. Persistence and degradability
Biodegradable
12.3. Bioaccumulative potential
Biodegradable, no accumulation expected.
12.4. Mobility in soil
No data available.
12.5. Results of PBT and vPvB assessment
This product contains no PBT/vPvB chemicals.
12.6. Other adverse effects
No data available.

13. Disposal considerations

13.1. Waste treatment methods
Observe all federal, state and local regulations when disposing of this substance.

14. Transport information

	DOT (Domestic Surface Transportation)	IMO / IMDG (Ocean Transportation)	ICAO/IATA
14.1. UN number	Not Applicable	Not Regulated	Not Regulated
14.2. UN proper shipping name	Not Regulated	Not Regulated	Not Regulated
14.3. Transport hazard class(es)	**DOT Hazard Class:** Not Applicable	**IMDG:** Not Applicable **Sub Class:** Not Applicable	**Air Class:** Not Applicable
14.4. Packing group	Not Applicable	Not Applicable	Not Applicable

14.5. Environmental hazards
IMDG Marine Pollutant: No
14.6. Special precautions for user
No further information

15. Regulatory information

Regulatory Overview	The regulatory data in Section 15 is not intended to be all-inclusive, only selected regulations are represented.
Toxic Substance Control Act (TSCA)	All components of this material are either listed or exempt from listing on the TSCA Inventory.
WHMIS Classification	Not Regulated
US EPA Tier II Hazards	**Fire:** No **Sudden Release of Pressure:** No **Reactive:** No **Immediate (Acute):** Yes **Delayed (Chronic):** No

EPCRA 311/312 Chemicals and RQs:
Acetic Acid (5000 lbs.)
EPCRA 302 Extremely Hazardous:
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
EPCRA 313 Toxic Chemicals:
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Carcinogens (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Developmental Toxins (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Female Repro Toxins (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Male Repro Toxins (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
New Jersey RTK Substances (>1%):

To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Pennsylvania RTK Substances (>1%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.

16. Other information

The information and recommendations contained herein are based upon data believed to be correct. However, no guarantee or warranty of any kind, expressed or implied, is made with respect to the information contained herein. We accept no responsibility and disclaim all liability for any harmful effects which may be caused by exposure to our products. Customers/users of this product must comply with all applicable health and safety laws, regulations, and orders.

The full text of the phrases appearing in section 3 is:

H226 Flammable liquid and vapor.

H314 Causes severe skin burns and eye damage.

H317 May cause an allergic skin reaction

H318 Causes serious eye damage.

This is the first version in the GHS SDS format. Listings of changes from previous versions in other formats are not applicable.

The information herein is presented in good faith and believed to be correct as of the date hereof. However, Diamond Products, Inc., makes no representation as to the completeness and accuracy thereof. Users must make their own determination as to the suitability of the product for their purposes prior to use. No representations or warranties, either express or implied, of merchantability, fitness for a particular purpose or of any other nature with respect to the product or the information herein is made hereunder. Diamond Products, Inc., shall in no event be responsible for any damages of whatsoever nature directly or indirectly resulting from the publication or use of or reliance upon information contained herein.

End of Document

Buffer Solutions:

pH 4, pH 7, pH 10, UN-No Not Regulated, Hazard Class-Not Regulated
Handling: Wear personal protective equipment. Ensure adequate ventilation. Do not breathe vapors or spray mist. Avoid contact with skin, eyes and clothing.
Storage: Keep containers tightly closed in a dry, cool and well-ventilated place.
Engineering Controls: Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.
Personal Protective Equipment: Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Skin/Clothing: Wear appropriate protective gloves and clothing to prevent skin exposure. Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced (MSDS. No C2948. 2006). (Carmon & Hosler, 2013)

Glassware

In the case of broken glassware, obtain a dustpan and broom and sweep up the pieces. Discard them in the container marked 'Recycled Glass' in the lab. Do not handle broken glass by hand, if it can be avoided. Broken glassware with chemical residue should either be cleaned (if there is a way to do so safely) or placed into the trash container. Only place clean broken glass in the 'Recycled Glass' container. Glass pipettes are rinsed and disposed of in the 'Recycled Glass' container after each sample. (Carmon & Hosler, 2013)

Appendix F: Deionized water use and filtration system.

The Montana AIS laboratory uses deionized water to reduce the chances of sample contamination. The lab is equipped with its own filtration system (Figure 19).

DI water is generally used for:
cleaning reusable equipment
making solutions
rinsing filter cloth

DI water is not used for:
general lab cleaning
analytical chemistry procedures

Instructions on using the DI water filtration system:
Use the black knob on the right-hand side of the machine to fill the beaker and use the beaker to fill containers. Knob should be turned counter clockwise to open and clockwise to close.
Change filters as instructed in system manual to keep the system working properly.

Figure 19: Deionizing water filtration system

Appendix G: pH Meter

Table of Contents

1 Introduction

Thank you for purchasing this high quality METTLER TOLEDO laboratory meter. With the FiveGo™ portables for pH, conductivity, and DO measurement, we wish to simplify your measuring process and your workflows.

The FiveGo™ portables are much more than just a series portable meters with an excellent price/ performance ratio. The meters offer a number of user-friendly features, including

- **Waterproof operation**
  The IP67 waterproof rating that allows free operation in wet or damp environments
- **Optimized ease of use**
  Simple menus for quick and easy operation
- **Excellent ergonomics**
  Handle the instrument with comfort and ease

2 Safety Measures

2.1 Definition of signal warnings and symbols

Safety notes are marked with signal words and warning symbols. These show safety issues and warnings. Ignoring the safety notes may lead to personal injury, damage to the instrument, malfunctions and false results.

Signal words

WARNING	for a hazardous situation with medium risk, possibly resulting in severe injuries or death if not avoided.
CAUTION	for a hazardous situation with low risk, resulting in damage to the device or the property or in loss of data, or minor or medium injuries if not avoided.
Attention	(no symbol) for important information about the product.
Note	(no symbol) for useful information about the product.

Warning symbols


General hazard


Toxic substance


Inflammable or explosive substance

2.2 Product specific safety notes

Your instrument represents state-of-the-art technology and complies with all recognized safety rules, however, certain hazards may arise in extraneous circumstances. Do not open the housing of the instrument; it does not contain any parts that can be maintained, repaired or replaced by the user. If you experience problems with your instrument, contact your authorized METTLER TOLEDO dealer or service representative.

Intended use

This instrument is designed for a wide range of applications in various areas and is suitable for measuring pH.

The use therefore requires knowledge and experience in working with toxic and caustic substances.

The manufacturer shall not be held liable for any damage resulting from incorrect usage divergent to the operating instructions. Furthermore, the manufacturer's technical specifications and limits must be adhered to at all times and in no way exceeded.

Location

The instrument has been developed for indoor operation and may not be used in explosive environments.

Use the instrument in a location which is suitable for the operation, protected from direct sunlight and corrosive gases. Avoid powerful vibrations, excessive temperature fluctuations and temperatures below 0 °C and above 40 °C.

After use, place the instrument back in the carrying case to reduce instruments exposure to UV radiation and prolong material quality and appearance.

Protective Clothing

It is advisable to wear protective clothing in the laboratory when working with hazardous or toxic substances.

A lab coat should be worn.

Suitable eye protection such as goggles should be worn.

Use appropriate gloves when handling chemicals or hazardous substances, checking their integrity before use.

Safety notes

⚠ WARNING

Chemicals

All relevant safety measures are to be observed when working with chemicals.

a) Set up the instrument in a well-ventilated location.

b) Any spills should be wiped off immediately.

c) When using chemicals and solvents, comply with the instructions of the producer and the general lab safety rules.

⚠ WARNING

Flammable solvents

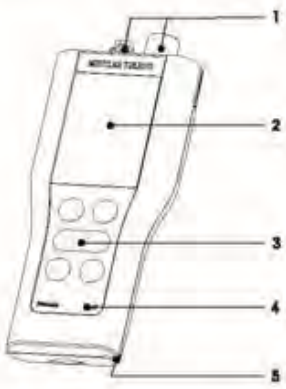
All relevant safety measures must be observed when working with flammable solvents and chemicals.

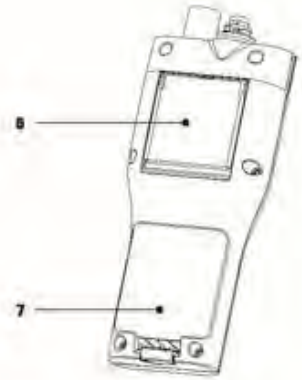
a) Keep all sources of flame away from the workplace.

b) When using chemicals and solvents, comply with the instructions of the producer and the general lab safety rules.

3 Design and Function

3.1 Overview



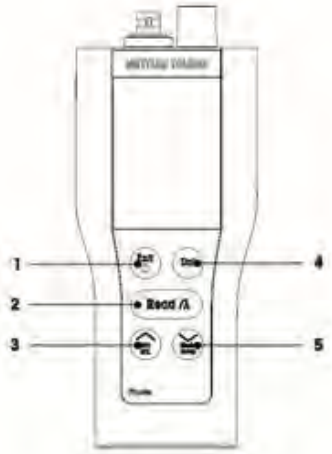


1 Sensor connections
2 Display
3 Keypad
4 Type label
5 Slot for wrist strap
6 Table top stand
7 Battery compartment

3.2 Sensor connections

1 RCA (Cinch) socket for temperature input
2 BNC socket for mV/pH signal input

3.3 Keypad



	Key	Naming	Press and release	Press and hold
1	Exit	On / Off / Exit	• Switch meter on • Back to measurement screen	• Switch meter off
2	Read /Δ	Read / Endpoint format	• Start or endpoint measurement • Confirm setting	• Turn auto endpoint on or off
3	STO RCL	Store / Recall	• Store current reading to memory • Increase value during setting • Scroll up through the memory	• Recall stored data
4	Cal	Calibration	• Start calibration	• Recall calibration data
5	Mode Setup	Mode / Setup	• Decrease value during setting • Scroll down through the memory	• Enter setup mode

3.4 Display and icons

When turning on the instrument, the startup screen appears for 3 seconds. The startup screen shows all icons which can appear on the display. In the following table you find a short description about these icons.

Startup screen

	Icon	Description
1	---	pH measurement value
2	/A / /M	Endpoint format: /A Automatic /M Manual
3		Buffer/Standard settings
4	---	Memory information
5	**Slope**	Slope is one of two quality indicators for the attached sensor and is determined during calibration.
6	**Offset**	Offset reading
7	**mV / pH**	Currently used measurement unit
8	---	Temperature information
9	**MTC / ATC**	**MTC** (Manual temperature capture) **ATC** (Automatic temperature capture)
10		Power status fully charged half charged lowly charged fully discharged
11	Err 8	Error code
12		Setup mode

	Icon	Description
13		Measurement mode
14		Calibration mode: Indicates calibration mode and appears whenever you are performing a calibration or reviewing calibration data.
15		Electrode performance Slope: 95-105% / Offset: ± 0-20 mV (Electrode in good condition) Slope: 90-94% / Offset: ± 20-35 mV (Electrode needs cleaning) Slope: 85-89% / Offset: ≥ 35 mV (Electrode is faulty)

3.5 Setup menu navigation

For general navigation in the setup menu read the following information:

- Press and hold **Setup** to enter the setup menu.
- Press **Exit** to exit the setup menu.
- Use ⌒ and ⌣ do increase or decrease values.
- Press **Read** to confirm a change.

The following parameters can be changed in the order as shown.

Parameter	Description	Range
MTC	Manual temperature setting	0.0...100.0°C / 32.0...212°F
	Buffer standard setting	B1, B2, B3, B4
°C, °F	Temperature unit	°C, °F

3.6 Measurement modes

With the F2 pH/mV meter it is possible to measure the following parameters of a sample:

- pH
- mV

To change the unit, press **Mode** on the measurement screen until the desired appears.

4 Putting into Operation

4.1 Scope of delivery

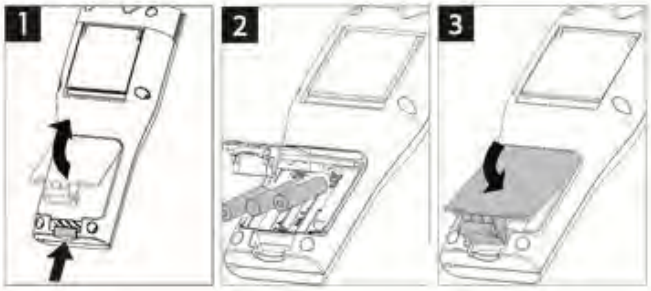
FiveGo™ F2 instrument
for pH/mV measurement

Battery LR03/AAA 1.5V
4 pcs.

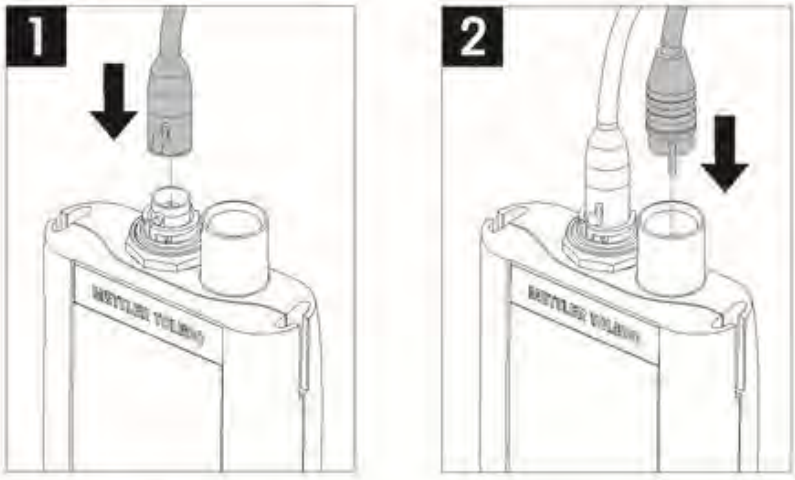
FiveGo™ electrode clip
1 pc.

CD-ROM including operating instructions

4.2 Installing the batteries



4.3 Connecting sensors

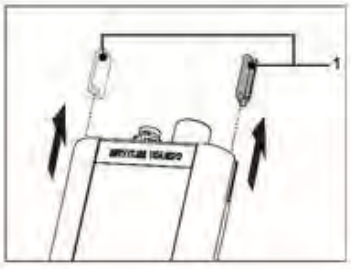


4.4 Installing optional equipment

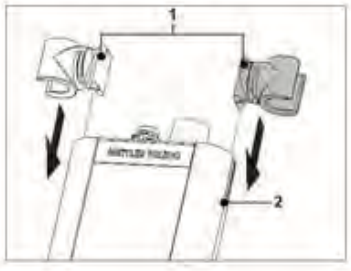
4.4.1 FiveGo™ electrode clip

For a safe placing of the electrode you can mount an electrode clip on the side of the instrument. The electrode clip is part of delivery. You can mount it on either sides of the instrument according to your preference.

- Remove the protective clips (1).

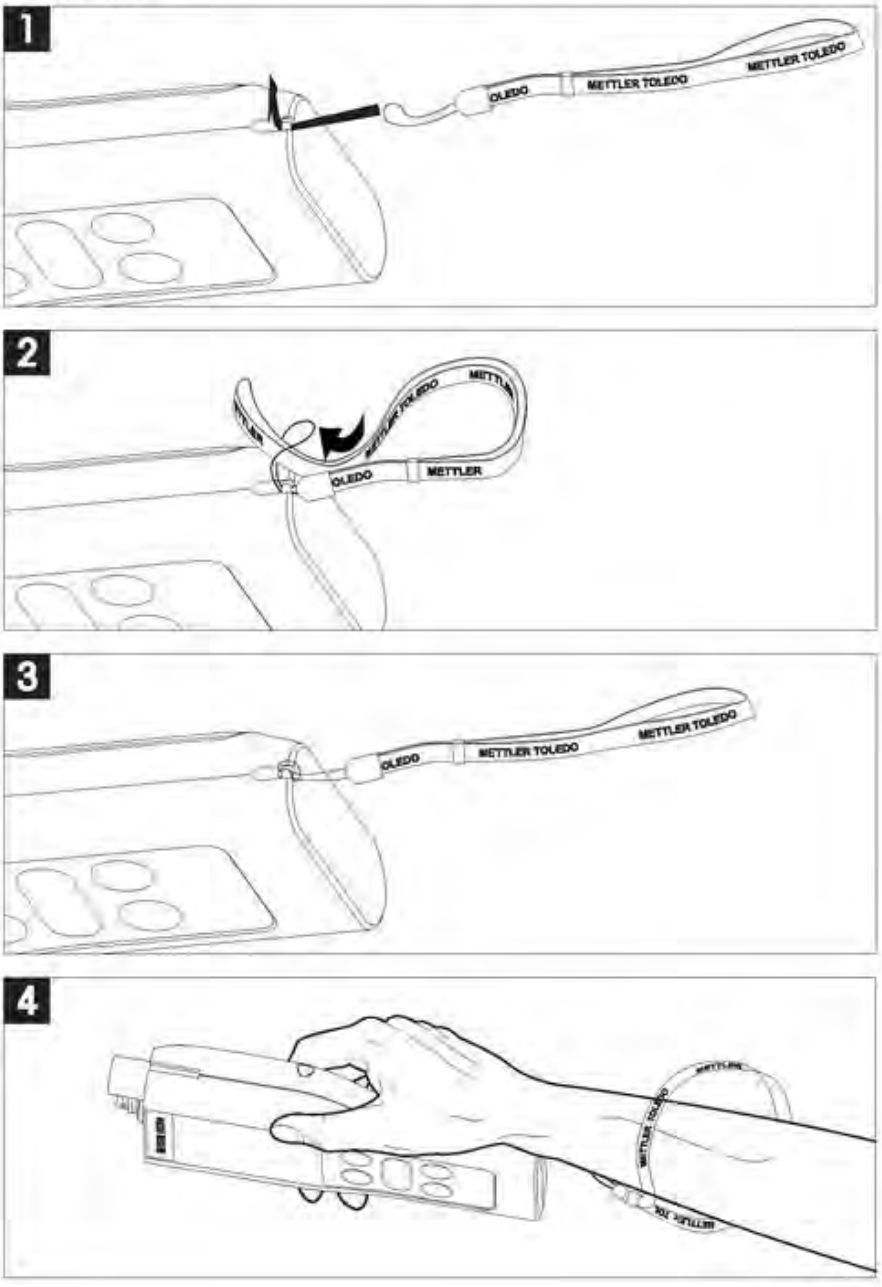


- Push the electrode clip (1) into the recess (2) of the instrument.



4.4.2 Wrist strap

For better protection against damage caused by dropping, you can mount the wrist strap as shown in the following diagrams.



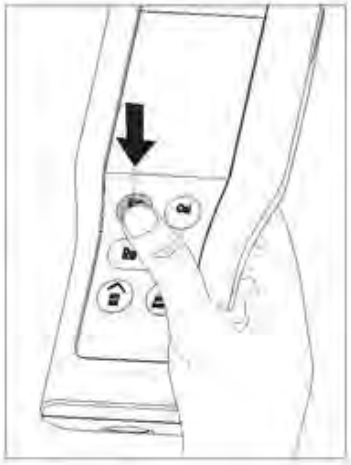
4.5 Switching the instrument on and off

1 Press and release ⏻ to switch on the instrument.
   - All segmented digital numbers and icons are displayed for 3 seconds. After that the installed software version appears (e.g. 1.00) and the instrument is ready for use.

2 Press ⏻ for 3 seconds and release to switch off the instrument.

Note

By default after 10 minutes not in use, the instrument shuts down automatically.



5 Operation of the Instrument

5.1 General settings

5.1.1 Endpoint Formats

The FiveGo™ offers two different endpoint formats, automatic and manual. To switch between the automatic and manual endpoint modes, press and hold **Read**.

Automatic endpoint

With the automatic endpoint, the measurement stops automatically as soon as the input signal is stable. This ensures an easy, quick and precise measurement.

Manual endpoint

Unlike the automatic endpoint, user interaction is required to stop the measurement reading in manual mode. To manually endpoint a measurement, press **Read**.

5.1.2 Temperature capture

Automatic temperature capture (ATC)

For better accuracy, we recommend the use of either a sensor with a built-in or a separate temperature probe. If a temperature probe is recognized by the meter, **ATC** and the sample temperature are displayed.

Note

The meter accepts NTC 30 kΩ temperature sensors.

Manual temperature capture (MTC)

If the meter does not detect a temperature probe, it automatically switches to the manual temperature mode and **MTC** appears. The entered MTC temperature is used for temperature compensation.

1 To set the MTC temperature, press and hold **Setup**.
   ⇨ The temperature value is blinking. The default setting is 25 °C.
2 Select the temperature value by using ⌃ and ⌄.
3 Press **Read** to confirm your settings.
4 Continue with buffer group selection or press **Exit** to return to measurement screen.

5.1.3 Predefined buffer groups

The buffer group is selected in the setup menu.

B1	1.68	4.01	7.00	10.01		(at 25 °C)
B2	2.00	4.01	7.00	9.21	11.00	(at 25 °C)
B3	1.68	4.00	6.86	9.18	12.46	(at 25 °C)
B4	1.68	4.01	6.86	9.18		(at 25 °C)

- After confirmation of the MTC temperature, the current buffer group is blinking.

1 Select the buffer group by using ⌃ and ⌄.
2 Press **Read** to confirm.
3 Continue with temperature unit setting or press **Exit** to return to measurement screen.

Note

It is not needed to calibrate a pH electrode with all pH values of a buffer group. Select the buffer group which contains the ones you are using for calibration. During calibration, the order in which the buffers are used is not relevant. The instrument has an auto buffer recognition function. This allows to calibrate in any order.

5.1.4 Temperature unit

The temperature unit is changed in the setup menu.

- After selection and confirmation of the predefined buffer group the temperature unit starts blinking.

1 Select the temperature unit (°C or °F) using ⌃ and ⌄.

2 Press **Read** to confirm and get back to the measurement screen.

5.2 Performing a calibration

For better accuracy, we recommend the use of either a sensor with a built-in or a separate temperature probe. If you use the MTC mode, you should enter the correct temperature value and keep all buffer and sample solutions at the set temperature. To ensure the most accurate pH reading, you should perform a calibration regularly.

The FiveGo™ pH meter allows you to run 1-,2- and 3-point calibrations. If you select your calibration buffer group from one of the four predefined groups stored in the meter, the buffers are automatically recognized and displayed during calibration (auto buffer recognition).

5.2.1 Performing a 1-point calibration

- An electrode is connected to the instrument.

1 Place the electrode in a calibration buffer.

2 Press **Cal**.

⇨ and appear on the display.
During measurement the pH value based on the previous calibration is shown. Depending on the endpoint format, the instrument stops measuring when the signal is stable (auto endpoint) or after pressing **Read** (manual endpoint).

⇨ At endpoint, disappears from the display and the pH value of the recognized buffer at measured temperature is shown.

3 If you do not want to proceed with the 2-point calibration, press **Read** to finish the 1-point calibration.
– or –
If you want to reject the 1-point calibration press **Exit**.
– or –
Proceed with next calibration point and go to Performing a 2-point calibration [▷ 19].

Note

With the 1-point calibration only the offset is adjusted. If the sensor was previously calibrated with multi-point calibration the previously stored slope will remain. Otherwise the theoretical slope (100 %) will be used.

5.2.2 Performing a 2-point calibration

- Perform the first calibration point as described in the section Performing a 1-point calibration [▷ 19].

1 Rinse the electrode with deionized water.

2 Place the electrode in the next calibration buffer and press **Cal**..

⇨ and appear on the display.
During measurement the pH value based on the previous calibration is shown. Depending on the endpoint format, the instrument stops measuring when the signal is stable (auto endpoint) or after pressing **Read** (manual endpoint). Slope and offset are then calculated.

⇨ At endpoint, disappears from the display and the pH value of the recognized buffer at measured temperature is shown.

3 If you do not want to proceed with a 3-point calibration press **Read** to finish and save the 2-point calibration.
-or-
if you want to reject the 2-point calibration, press **Exit**.
-or-
if you want to proceed with the next calibration point go to Performing a 3-point calibration.

Note

With the 2-point calibration, both slope and offset are updated and shown on the right side of the display

5.2.3 Performing a 3-point calibration

- Perform the same steps as described in Performing a 2-point calibration [▷ 19].

- Repeat steps 1, 2 and 3 of Performing a 2-point calibration [▷ 19] for the third calibration point.

5.3 Performing a measurement

5.3.1 Measurement mode

The FiveGo™ pH/mV meter offers two different reading modes: pH and mV.

- Press the **Mode** button to switch between pH and mV mode.

5.3.2 Performing a pH measurement

- An electrode is connected to the instrument.
- Make sure that the pH reading mode is selected.

1 Place the electrode in the sample and press **Read** to start the measurement.
   ⇨ The decimal point blinks.
   ⇨ The display shows the pH of the sample.
   ⇨ If the automatic endpoint is selected, and the signal has stabilized, the display freezes, $\sqrt{\underline{A}}$ appears and the decimal point stops blinking. In case the **Read** button was pressed before the automatic endpoint, the display freezes and $\sqrt{\underline{M}}$ appears.

2 If the manual endpoint is chosen, press **Read** to manually endpoint the measurement. The display freezes and $\sqrt{\underline{M}}$ appears.

Note

Press and hold **Read** to switch between the automatic and manual endpoint format.

5.3.3 Performing a mV measurement

- An electrode is connected to the instrument.
- Make sure that the mV mode is selected.
- Continue as described in steps 1 and 2 of the section Performing a pH measurement [▷ 21].

5.4 Using the memory

5.4.1 Storing a measurement result

The instrument can store up to 200 endpointed results.

- Press **STO** when the measurement has endpointed.
  ⇨ **M001** indicates that one result has been stored, and **M200** that the maximum of 200 results have been stored.

Note

If you press **STO** when **M200** is displayed, **Err 6** indicates that the memory is full. To store further data you will have to clear the memory.

5.4.2 Recalling from memory

1. Press and hold **RCL** to recall the stored values.
2. Press ⌒ or ⌣ to scroll through the stored results.
   ⇨ **MR 001** to **MR 200** indicates which result is currently displayed.
3. Press **Exit** to go back to the measurement screen.

5.4.3 Clearing the memory

1. Press and hold **RCL** to recall the stored values from memory.
2. Press **RCL** until **ALL** appears on the display.
3. Press **Read** to delete all measurement results.
   ⇨ **CLr** starts blinking on the display.
4. Press **Read** to confirm the deletion
   - or -
   Press **Exit** to cancel the deletion.

5.5 Self-diagnosis

1. Switch the meter on.
2. Press **Read** and **Cal** simultaneously until the meter displays the full screen.
   ⇨ Each icon blinks one after the other whereby you can check if all icons are correctly shown on the display.
   ⇨ After that, *b* starts to blink and 5 hardkey-icons are shown on the display.
3. Press any hardkey.
   ⇨ The specific icon disappears from the display.
4. Press each hardkey once.

⇨ When the self-diagnosis is completed successfully, **PAS** appears. If the self-diagnosis has failed, **Err 2** appears.

Note

You must press all hardkeys within 1 minute. Otherwise **FAL** appears and the self-diagnosis has to be redone.

5.6 Factory reset

Note

Loss of data!

With a factory reset all user-specific settings will be set to standard. Also all data memories will be deleted.

- The instrument is switched off.

1 Press and hold **Read**, **Cal** and **Exit** simultaneously for 2 seconds.
   ⇨ **RST** appears on the display.
2 Press **Read**.
3 Press **Exit**.
   ⇨ The instrument switches off.
   ⇨ All settings are reset.

6 Maintenance

6.1 Cleaning the housing

Note

Damage to the instrument!

Ensure that no liquid enters the interior of the instrument.

Wipe off any spills immediately.

The meter does not require any maintenance other than an occasional wipe with a damp cloth. The housing is made of acrylonitrile butadiene styrene (ABS). This material is sensitive to some organic solvents, such as toluene, xylene and methyl ethyl ketone (MEK).

– Clean the housing of the instrument using a cloth dampened with water and a mild detergent.

6.2 Electrode maintenance

- Make sure pH electrodes are always kept filled with the appropriate filling solution.
- For maximum accuracy, any filling solution that may have crystallized and encrusted the outside of the electrode should be removed with deionized water.
- Always store the electrode according to the manufacturer's instructions and do not allow it to dry out.

If the electrode slope falls rapidly, or if the response becomes sluggish, the following procedures may help. Try one of the following, depending on your sample. Run a new calibration after treatment.

Symptom	Procedure
Fat or oil build-up.	Degrease the membrane with cotton wool soaked in either acetone or a soap solution.
Membrane has dried out.	Soak the tip of the electrode overnight in 0.1 M HCl.
Protein build-up in the diaphragm.	Remove deposits by soaking the electrode in an HCl/pepsin solution.
Silver sulfide contamination.	Remove deposits by soaking electrode in a thiourea solution.

Note

- Cleaning and filling solutions should be handled with the same care as that given to toxic or corrosive substances.
- For pH electrode trouble shooting you can also turn to www.electrodes.net

6.3 Error messages

Error	Description	Resolution
Err 1	Memory access error	Reset to factory settings
Err 2	Self-diagnosis failed	Repeat the self-diagnosis procedure and make sure that you finish pressing all five keys within one minute.
Err 3	Measured values out of range	Make sure that the electrode wetting cap has been removed and the electrode is properly connected and placed in the sample solution. If no electrode is connected, put the shorting plug in the socket.
Err 4	Measured buffer temperature out of range (5 to 40 °C)	Keep the temperature within the range for calibration (5 to 40 °C).
Err 5	Offset out of range	Make sure you have the correct buffer and that it is fresh. Disconnect, clean and replace the electrode.
Err 6	Slope out of range	Make sure you have the correct buffer and that it is fresh. Disconnect, clean and replace the electrode.
Err 7	Meter cannot recognize the buffer (Wrong buffer)	Make sure you have the correct buffer and that it is fresh. Disconnect, clean and replace the electrode.
Err 8	Memory is full	Clear the memory
Err 9	Measurement data cannot be stored twice	---

6.4 Error limits

Message	Description	Range not accepted	
Err 3	Value out of range	• pH • mV • Temperature	• < 0.00 or > 14.00 • < -1999 or > 1999 • < 0 or > +100
Err 4	Buffer temperature out of range	T	< 5 °C or > 40 °C
Err 5	Offset out of range	Offset	≤ -35 or ≥ 35 mV
Err 6	Slope out of range (following cal. points)	Slope	≤ 85% or ≥ 110%
Err 7	Wrong buffer	Signal difference between two buffers	< 60 mV

6.5 Disposal

In conformance with the European Directive 2002/96/EC on Waste Electrical and Electronic Equipment (WEEE) this device may not be disposed of in domestic waste. This also applies to countries outside the EU, per their specific requirements.

Please dispose of this product in accordance with local regulations at the collecting point specified for electrical and electronic equipment. If you have any questions, please contact the responsible authority or the distributor from which you purchased this device. Should this device be passed on to other parties (for private or professional use), the content of this regulation must also be related.

Thank you for your contribution to environmental protection.

7 Product Portfolio

Meter and Kits	Description	Order No.
F2-Meter	FiveGo™ pH/mV meter without sensor	30266946
F2-Standard	FiveGo™ pH/mV meter standard kit with LE438 IP67 sensor	30266889
F2-Food	FiveGo™ pH/mV meter food kit with LE427 IP67 puncture sensor and carrying case	30266881
F2-Field	FiveGo™ pH/mV meter field kit with LE438 IP67 senor and carrying case	30266882

8 Accessories

Parts	Order No.
FiveGo™ carrying case (incl. 4 sample bottles)	30239142
FiveGo™ electrode clip (1 pc) and electrode clip covers (2 pcs.)	30239144
Wrist strap (METTLER TOLEDO)	30122304
Battery cover	30254145
Table top stand	30254146
Sample bottles (4 pcs.)	30239143
BNC shortening plug	30133643

Sensors	Order No.
LE438 IP67	30247153
LE438	51340242
LE407	51340330
LE408	51340347
LE409	51340331
LE410	51340348
LE420	51340332
LE422	30089747
LE427 IP67	30259840
LE427	51340333
ATC probe, temperature sensor	51300164

Solutions	Order No.
pH 2.00 buffer sachets, 30 x 20 mL	30111134
pH 2.00 buffer solution, 250 mL	51350002
pH 2.00 buffer solution, 6 x 250 mL	51350016
pH 4.01 buffer sachets, 30 x 20 mL	51302069
pH 4.01 buffer solution, 250 mL	51350004
pH 4.01 buffer solution, 6 x 250 mL	51350018
pH 7.00 buffer sachets, 30 x 20 mL	51302047
pH 7.00 buffer solution, 250 mL	51350006
pH 7.00 buffer solution, 6 x 250 mL	51350020
pH 9.21 buffer sachets, 30 x 20 mL	51302070
pH 9.21 buffer solution, 250 mL	51350008
pH 9.21 buffer solution, 6 x 250 mL	51350022
pH 10.01 buffer sachets, 30 x 20 mL	51302079
pH 10.01 buffer solution, 250 mL	51350010
pH 10.01 buffer solution, 6 x 250 mL	51350024
pH 11.00 buffer sachets, 30 x 20 mL	30111135
pH 11.00 buffer solution, 250 mL	51350012
pH 11.00 buffer solution, 6 x 250 mL	51350026
Rainbow sachets I (10 sachets of pH 4.01 / 7.00 / 9.21)	51302068
Rainbow sachets II (10 sachets of pH 4.01 / 7.00 / 10.00)	51302080
Rainbow bottles I (2 x 250 mL of pH 4.01 / 7.00 / 9.21)	30095312
Rainbow bottles II (2 x 250 mL of pH 4.01 / 7.00 / 10.00)	30095313
Electrolyte 3 mol/L KCl, 25 mL	51343180
Electrolyte 3 mol/L KCl, 250 mL	51350072
Electrolyte 3 mol/L KCl, 6 x 250 mL	51350080

Solutions	Order No.
HCl/Pepsin solution (removes protein contamination), 250 mL	51350100
Reactivation solution for pH electrodes, 25 mL	51350104
Thiourea solution (removes silver sulfide contamination), 250 mL	51350102

9 Technical Data

General

Power requirements	Batteries	4 x LR03/AAA 1.5 V Alkaline - or - 4 x AAA 1.2 V NiMH rechargeable
	Battery life	> 200 h
Dimensions	Height	188 mm
	Width	77 mm
	Depth	33 mm
	Weight (without batteries)	260 g
Display	LCD	3.1" Segmented LCD, b/w
Ambient conditions	Operating temperature	0…40 °C
	Relative humidity	5%…85% (non-condensing) at 31 °C, linearly descending to 50% at 40 °C
	Overvoltage category	Class II
	Pollution degree	2
	Maximum operating altitude	2000 m above sea level
	Range of application	For indoor use
Materials	Housing	ABS
	Window	Polymethyl methacrylate (PMMA)
	IP Protection class	IP67

Measurement

Parameters	pH, mV	
Sensor inputs	pH/mV	BNC, impedance > 10^{12} Ω
	Temperature	Cinch, NTC 30 kΩ
pH	Measuring range	pH 0.00...14.00
	Resolution	0.01
	Accuracy (electronic)	± 0.01
mV	Measuring range	-1'999…1'999 mV
	Resolution	1 mV
	Limits of error	±1 mV
	Units	mV
Temperature	Measuring range	0…100 °C (32…212 °F)
	Resolution	0.1 °C
	Limits of error	± 0.5 °C
	ATC/MTC	Automatic switch
Calibration	Calibration points	3
	Predefined buffer groups	4
	Automatic buffer recognition	Yes
	Calibration method	Linear
Data storage	Memory size	200

10 Appendix

B1 METTLER TOLEDO USA (Ref. 25 °C)

T [°C]	1.68	4.01	7.00	10.01
5	1.67	4.00	7.09	10.25
10	1.67	4.00	7.06	10.18
15	1.67	4.00	7.04	10.12
20	1.68	4.00	7.02	10.06
25	**1.68**	**4.01**	**7.00**	**10.01**
30	1.68	4.01	6.99	9.97
35	1.69	4.02	6.98	9.93
40	1.69	4.03	6.97	9.89

B2 METTLER TOLEDO Europe (Ref. 25 °C)

T [°C]	2.00	4.01	7.00	9.21	11.00
5	2.02	4.01	7.09	9.45	11.72
10	2.01	4.00	7.06	9.38	11.54
15	2.00	4.00	7.04	9.32	11.36
20	2.00	4.00	7.02	9.26	11.18
25	**2.00**	**4.01**	**7.00**	**9.21**	**11.00**
30	1.99	4.01	6.99	9.16	10.82
35	1.99	4.02	6.98	9.11	10.64
40	1.98	4.03	6.97	9.06	10.46

B3 JJG119 (Ref. 25 °C)

T [°C]	1.680	4.003	6.864	9.182	12.460
5	1.669	3.999	6.949	9.391	13.210
10	1.671	3.996	6.921	9.330	13.011
15	1.673	3.996	6.898	9.276	12.820
20	1.676	3.998	6.879	9.226	12.637
25	**1.680**	**4.003**	**6.864**	**9.182**	**12.460**
30	1.684	4.010	6.852	9.142	12.292
35	1.688	4.019	6.844	9.105	12.130
40	1.694	4.029	6.838	9.072	11.975

B4 JIS Z 8802 (Ref. 25 °C)

T [°C]	1.679	4.008	6.865	9.180
5	1.668	3.999	6.951	9.395
10	1.670	3.998	6.923	9.332
15	1.672	3.999	6.900	9.276
20	1.675	4.002	6.881	9.225
25	**1.679**	**4.008**	**6.865**	**9.180**
30	1.683	4.015	6.853	9.139
35	1.688	4.024	6.844	9.102
40	1.694	4.035	6.838	9.068

Appendix G: Filtering Process in Photos

Figure 20: Set up two beakers with 210 and 35 µm filters.

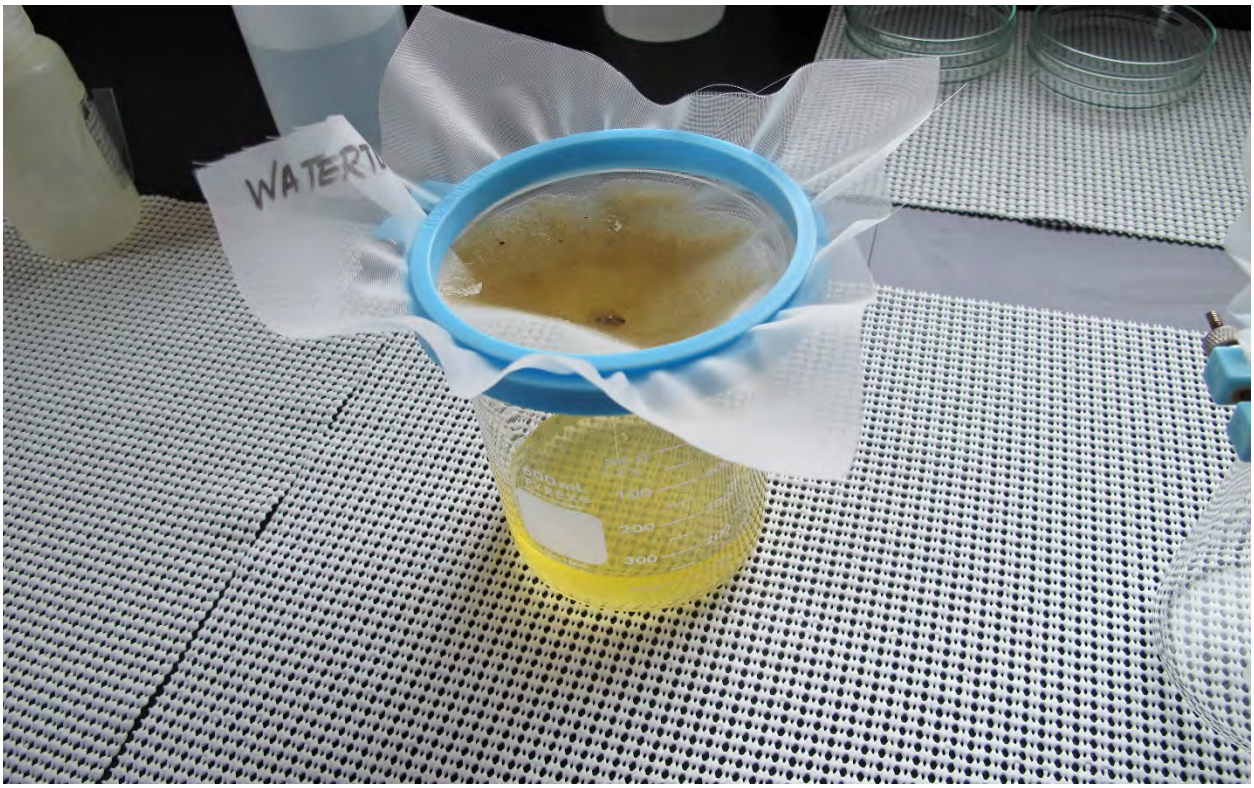


Figure 21: Measure half of the sample size (after homogenizing the sample) and pour through the 210 µm filter.

Figure 22: Rinse the contents of the 210 µm filter with DI water from a wash bottle

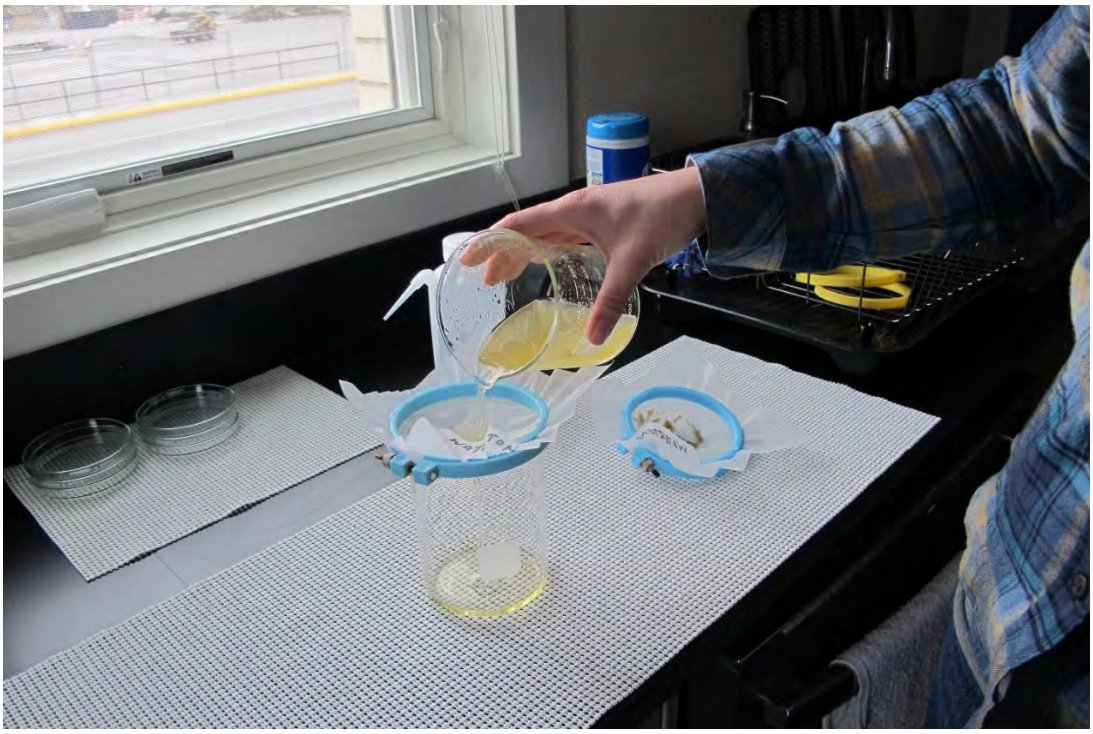


Figure 23: Pour the contents of the beaker underneath the 210 µm filter through the 35 µm filter and rinse contents of 35 µm filter with DI water. Discard contents of 210 µm filter after checking for invasive water fleas.

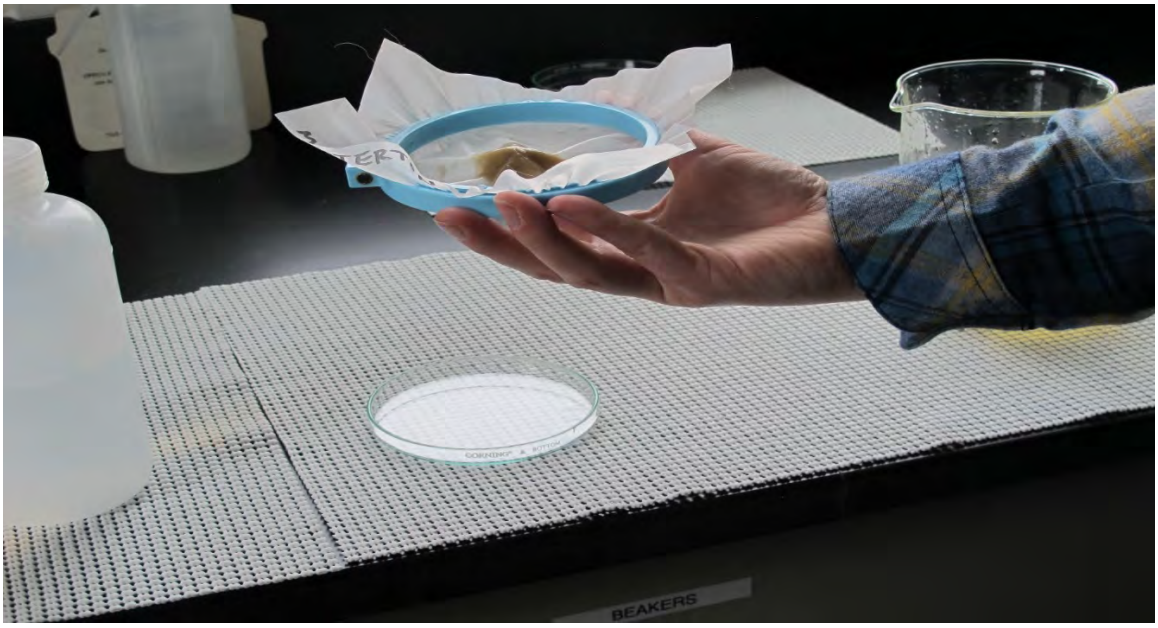


Figure 24: Use your finger to create a point in the center of the 35 µm filter then flip filter over and rinse contents into a glass Petri dish.

Figure 25: Rinse contents of 35 µm filter into glass Petri dish. More than one Petri dish should be used for samples with high algae or sediment content.

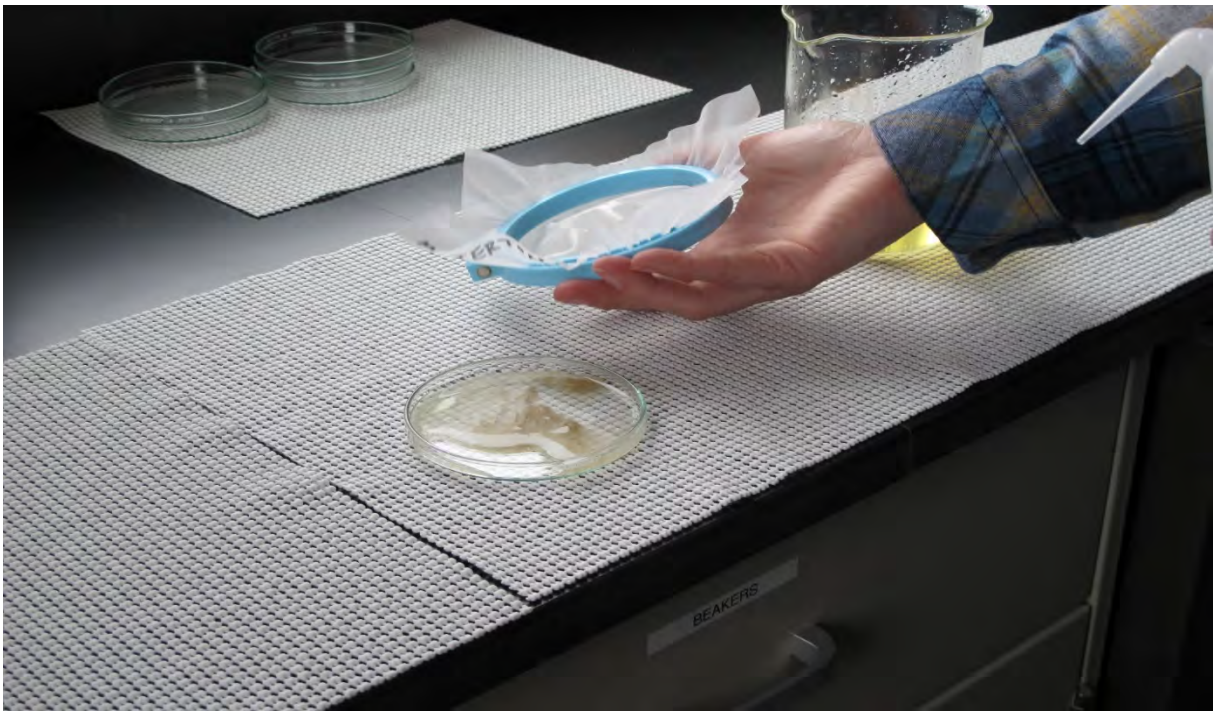


Figure 26: Use DI wash bottle and gentle agitation to separate clumps of particulate matter.

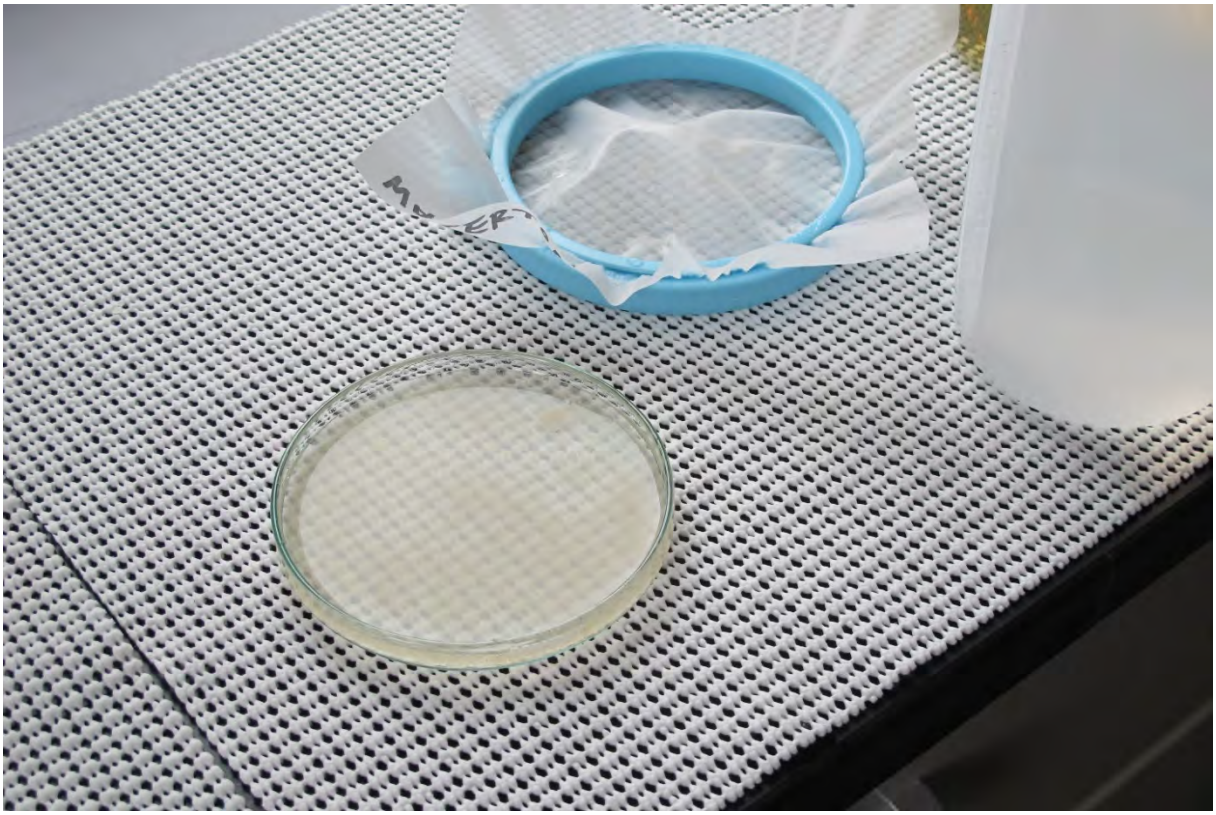


Figure 27: Filtered sample ready for processing.

Appendix H: List of assigned filters for MT and Out-of-State samples

State	Waterbody Name
MT	Abbot Lake
MT	Ackley Lake
MT	Afterbay Resevoir
MT	AMC Settling pond
MT	Anderson Resevoir
MT	Arapooish Pond
MT	Ashley Lake
MT	Ashley Lake
MT	Bailey Lake (WLI)
MT	Bailey Resevoir
MT	Bair Resevoir
MT	Base Pond
MT	Basin Creek
MT	Bean Lake
MT	Bear Creek
MT	Bearpaw Lake
MT	Beaver Creek Resevoir
MT	Beaver Impoundment (WLI)
MT	Beaver Lake
MT	Beaverhead River
MT	Big Casino Creek Reservoir
MT	Big Hole River
MT	Big Reservoir
MT	Big Sky Lake
MT	Big Spring Creek &Hatchery
MT	Big Therriault
MT	Bighorn Lake
MT	Bighorn River
MT	Birch Creek
MT	Bison Bone Reservoir
MT	Bitterroot River
MT	Blackfoot River
MT	Blacktail Meadows Kids Pond
MT	Blaine Lake
MT	Blanchard Lake

State	Waterbody Name
MT	Bluewater Creek Hatchery
MT	Bootjack Lake
MT	Boulder River
MT	Bowman Lake-GNP
MT	Boxelder Lake
MT	Bozeman Pond
MT	BR047 Reservoir
MT	Broadview Pond
MT	Brownes Lake
MT	Browns Lake
MT	Brush Lake
MT	Bull Lake
MT	Bynum Reservoir
MT	Carters Pond
MT	Castle Rock Lake
MT	Canyon Ferry Reservoir
MT	Clark Canyon Reservoir
MT	Clark Fork River
MT	Clark's Fork Yellowstone River
MT	Clearwater River
MT	Cliff Lake
MT	Compton Reservoir
MT	Cooney Reservoir
MT	Cottonwood Creek
MT	Cow Creek Reservoir
MT	Creston National Fish Hatchery (Jessup Mill Pond)
MT	Crystal Lake
MT	Cutbank Creek
MT	Daily Lake
MT	Darlington Ditch
MT	Deadmans Basin
MT	Dearborn River
MT	Delmo
MT	Dickey Lake
MT	Dollar Lake
MT	Don Reservoir
MT	Dray Reservoir
MT	Dry Fork Reservoir

State	Waterbody Name
MT	Duck Creek
MT	Duck Lake
MT	East Fork Reservoir
MT	Echo Lake
MT	Elk Lake
MT	Emerald Lake
MT	Ennis Lake
MT	Ennis National Fish Hatchery
MT	Ester Reservoir
MT	Eureka Reservoir
MT	Eyraud Lake
MT	Fish Lake
MT	Flathead Lake
MT	Flathead River
MT	Flinstone Reservoir
MT	Flynn Pond
MT	Forsman Reservoir
MT	Fourhorn Lake
MT	Foys Lake
MT	FPR Dredge Cuts/mr
MT	Freezeout Lake
MT	Frenchtown Lake
MT	Fresno
MT	Fort Peck Reservoir
MT	Gallatin River
MT	Gardener River
MT	Gartside Reservoir
MT	Gibson Reservoir
MT	Glasgow Base Pond
MT	Glean Lake
MT	Gullwing Reservoir
MT	Halfmoon Lake
MT	Handkerchief Lake
MT	Hansen
MT	Hanson-Doyle
MT	Harbor Pond
MT	Harper's Lake
MT	Harriman Trout Co.

State	Waterbody Name
MT	Harrison Lake
MT	Hauser
MT	Hebgen Lake
MT	Helena Vly Reg reservoir
MT	Holgate Reservoir
MT	Holland Lake
MT	Holter Lake
MT	Horseshoe Lake
MT	Hubbart Reservoir
MT	Hump Reservoir
MT	Hundred Dollar Pond
MT	Hungry Horse
MT	Hyalite Creek
MT	Hyalite Reservoir
MT	Indian Creek
MT	Indian Road Pond
MT	Jefferson River
MT	Jette Lake
MT	Jocko River SFH
MT	John Beck Pond
MT	Johnson Reservoir
MT	Judith River
MT	Karsten Coulee Reservoir
MT	Kolar Reservoir 1
MT	Kolar Reservoir 2
MT	Koocanusa
MT	Lake Alva
MT	Lake Elmo
MT	Lake Five
MT	Lake Frances
MT	Lake Helena
MT	Lake Inez
MT	Lake Josephine
MT	Lake Sutherlin
MT	Langen (Forsman) Reservoir
MT	Laurel Pond
MT	Lima Reservoir
MT	Lindbergh Lake

State	Waterbody Name
MT	Little Bitterroot Lake
MT	Little Boulder River
MT	Little Warm Reservoir
MT	Lost Coon Lake
MT	Lower Glasten Lake
MT	Lower St Mary Lake (Blackfeet)
MT	Luloff
MT	Madison River
MT	Marias River
MT	Mary Ronan
MT	Martinsdale Reservoir
MT	McDonald Lake-GNP
MT	McGilvray Lake
MT	McGregor lake
MT	Medicine Lake
MT	Meyers Lake
MT	Miles City Fish Hatchery
MT	Milk River
MT	Mission Lake
MT	Missouri
MT	Missouri Colter Falls Hatchery
MT	Morrison Lake
MT	Murphy Lake
MT	Murray Lake
MT	Murray Spring SFH
MT	Musselshell River
MT	Nelson Dredge-MR
MT	Nelson Reservoir
MT	Nevada Reservoir
MT	Newlan Creek Reservoir
MT	Nilan Reservoir
MT	North Polly Reservoir
MT	OJuel Lake
MT	Ostel Reservoir
MT	Paulo Reservoir
MT	Payola Reservoir
MT	Pelican Point Hatchery
MT	Peterson Lake

State	Waterbody Name
MT	Petrolia Reservoir
MT	Pishkun Reservoir
MT	Placid Lake
MT	Priest Butte Reservoir
MT	Quake Lake
MT	Rainbow Springs Trout Farm
MT	Rainy Lake
MT	Raymond Lake
MT	Red Rock Lakes
MT	Red Rock River
MT	Redwater River
MT	Regional Parks Pond
MT	River Rock Pond
MT	Rock Creek
MT	Roe River
MT	Rogers Lake
MT	Rose Creek SFH
MT	Rose Creek SFH
MT	Rosebud Creek
MT	Rosebud Lake
MT	Ross Reservoir
MT	Ruby Reservoir
MT	Ruby River
MT	Sage Brush Reservoir
MT	Salmon Lake
MT	Seeley Lake
MT	Sekokini SFN
MT	Shields River
MT	Skyles Lake
MT	Smith Lake
MT	Smith River
MT	Sophie Lake
MT	South Sandstone Reservoir
MT	Spencer Lake
MT	Spook Lake
MT	Spotted Eagle Lake
MT	Spring Meadow
MT	St. Mary -GNP

State	Waterbody Name
MT	Stillwater Lake
MT	Stillwater River
MT	Sun River
MT	Swan lake
MT	Swift Reservoir
MT	Taint Reservoir
MT	Tally Lake
MT	TenMile Creek
MT	Tetrault Lake
MT	Thompson Lakes
MT	Three Forks Pond
MT	Tiber Reservoir
MT	Tongue River Reservoir
MT	Toston Reservoir
MT	Trout Meadow
MT	Trout Pond (East Crew)
MT	Tunnel Lake
MT	Tupper's Lake
MT	Twin Lakes
MT	Two Medicine- GNP
MT	Upsata Lake
MT	Valley Reservoir VR009
MT	Van Lake
MT	Wade Lake
MT	Wapiti Reservoir
MT	Ward Reservoir
MT	Washoe Trout Hatchery
MT	Waterton Lake- GNP
MT	Wayne Edsall Pond (Bozeman Pond)
MT	Westslope Trout CO.
MT	Whitefish Lake
MT	Willow Creek Reservoir
MT	Winter Harbor pond
MT	Wood Lake
MT	Yellowstone River
MT	Yellowstone River Trout Hatchery
MT	Yellowstone water Reservoir
MT	Yellowtail Afterbay Reservoir

State	Waterbody Name
CO	Various
ID	Various
KS	Various
NE	Various
SD	Various
WY	Various
ZM Training	Various

Appendix I: Measurement calibration instructions

Use the *Magnification* command to synchronize calibration with objective switching. For correct measurement and printing, infinity analyze needs to know the sampling intervals and magnification of the digital image. Horizontal sampling interval, vertical sampling interval and magnification comprise a micrometer. Each image acquired with infinity analyze has a micrometer associated with it. This micrometer is the basis of correct measurement and printing and cannot be modified after the image has been created. Infinity analyze also maintains a system micrometer. When an image is being captured, the system micrometer is duplicated and set as the micrometer of that image. The process that sets up the system micrometer is referred to as calibration. In the simplest sense, calibration can be done for a single objective and use the *Magnification* command to adapt to different objectives or magnifications. A successful calibration will correctly set up the sampling intervals and magnification for use with that objective. When a new objective and/or some other intermediate lenses are in place, the *Magnification* command records the updated magnification and scales the sampling intervals accordingly (Lumenera Corporation, 2018). For complete instructions on synchronizing calibration with objective switching, refer to the infinity analyze user manual. (Lumenera Corporation, 2018)
https://www.microscopeworld.com/images/Manuals/Infinity-Analyze-Manual.pdf

Appendix J: Key to differentiate larvae of zebra mussels, quagga mussels and Asian clams.

From Nichols and Black, 1993.

1*a*. Larval shell absent. . . . . preshell larva (Fig. 2)
1*b*. Larval shell present 2

2*a*. Hinge line straight (shell is D-shaped) or sway-backed . . . . . straight-hinged larva (Figs. 3–5) . . . 5
2*b*. Hinge line rounded, with a protruding bump (the umbo) in the center. 3

3*a*. Umbo (bump) present, but is low and rounded, and does not protude above the shell line . . . . . umbonal larva (Figs. 6–8) . . . 7
3*b*. Umbo is well-developed and knobby, and protrudes above the shell line 4

4*a*. Face of shell valve slightly asymmetrical (clam-shaped). Foot present. Velum present or absent . . . . . pediveliger larva (Figs. 9–11) . . . 9
4*b*. Face of shell valve very asymmetrical (lopsided) or mussel-shaped. Foot present. Velum absent. Dreissenidae only. . . . . plantigrade larvae (Figs. 12–13) . . . 11

5*a*. Shell length 190–380 μm. Foot present. Velum present or absent. Hinge line straight or sway-backed. Well-defined growth lines. Numerous secondary lines perpendicular to growth rings, giving growth ring region a pleated appearance. . . . . *Corbicula fluminea* (Fig. 3)

Measurements of laboratory-bred larvae: mean length 225 μm (range 210–273 μm), height 195 μm (170–221 μm), hinge length 118 μm (100–131 μm). Measurements of field-caught larvae: mean length 240 μm (range 190–265 μm), height 199 μm (165–246 μm), hinge length 110 μm (74–129 μm). Face of shell valve usually asymmetrical and clam-shaped.

5*b*. Shell length 94–160 μm. Velum present, foot absent. Hinge line straight. Growth lines weak, if any, and no secondary lines running perpendicular to shell edge . . . . . Dreissenidae . . . 6

6*a*. Shell height usually 80% of shell length, giving shell an elongated appearance. Shoulders angular. Hinge length ≥ 64 μm . . . . . *Dreissena polymorpha*[2] (Fig. 4)

Measurements of laboratory-bred larvae: mean length 104 μm (range 97–112 μm), height 83 μm (64–92 μm), hinge length 69 μm (64–76 μm), height/length ratio 80% (66–84%). Measurements of field-caught larvae: mean length 108 μm (97–133 μm), height 72 μm (64–105 μm), hinge length 70 μm (64–77 μm), height/length ratio 81% (66–84%). Face of shell valve usually symmetrical.

6*b*. Shell height usually 90% of shell length, giving shell a rounded shape. Shoulders rounded. Hinge line usually <64 μm in length ..... *Quagga*[3] (Fig. 5)

Measurements of laboratory-bred larvae: mean length 71 μm (range 39–101 μm), height 65 μm (32–92 μm), hinge length 58 μm (24–63 μm), height/length ratio 91% (86–100%). Measurements of field-caught larvae: mean length 109 μm (89–129 μm), height 93 μm (83–118 μm), hinge length 59 μm (54–62 μm), height/length ratio 90% (85–100%). Face of shell valve usually symmetrical.

7*a*. Shell length 380–500 μm. Foot present. No velum. Well-defined growth lines with secondary lines running perpendicular to shell margin ..... *Corbicula fluminea* (Fig. 6)

Measurements of laboratory-bred larvae: mean length 405 μm (range 385–450 μm), height 362 μm (349–420 μm), hinge length 126 μm (119–156 μm). Measurements of field-caught larvae: mean length 421 μm (380–502 μm), height 398 μm (340–472 μm), hinge length 135 μm (120–176 μm). Face of shell valve usually asymmetrical.

7*b*. Shell length 120–260 μm. Velum present. No foot. Growth lines present, but not well defined. No secondary lines ..... Dreissenidae . . . 8

8*a*. Valve margins equal in size. Umbos equal in height ..... *Dreissena polymorpha* (Fig. 7)

Young umbonal larvae retain elongated shape and hinge line >64 μm as seen in straight-hinged larvae. Older umbonal larvae tend to be rounder rather than elongated, and the ends of the hinge line are difficult to distinguish. On larvae of all ages, face of shell valve may be bilaterally symmetrical, or one side or shoulder of shell may be more pointed than the other. Asymmetry is more common in older larvae. Measurements of laboratory-bred larvae: mean length 155 μm (range 140–228 μm), height 131 μm (89–178 μm), hinge length 73 μm (65–77 μm). Measurements of field-caught larvae: length 148 μm (106–209 μm), height 128 μm (92–191 μm), hinge length 69 μm (65–79 μm).

8*b*. Margin of one valve extends past the margin of the other, particularly just below shoulder line. One umbo slightly higher or more convex than the other (difficult to see) ..... quagga (Fig. 8)

Umbonal larvae retain the rounded shape and hinge line <64 μm seen in straight-hinged larvae. Older umbonal larvae are round in proportion, but ends of the hinge line are difficult to distinguish. Face of shell valve are bilaterally symmetrical in younger umbonal larvae, but one side of shell is usually more pointed than the other in older umbonal larvae. Measurements of laboratory-bred larvae: mean length 136 μm (range 111–198 μm), height 129 μm (98–191 μm), hinge length 51 μm (31–62 μm). Measurements of field-caught larvae: length 166 μm (120–221 μm), height 150 μm (99–202 μm), hinge length 56 μm (35–60 μm).

9*a*. Shell length >500 μm. Shell is clam-shaped, opaque, with prominent ridges. Foot and siphons present ..... *Corbicula fluminea* (Fig. 9)

Corbicula larvae do not change in appearance after this stage.

9*b*. Shell length 200–300 μm. Shell is clam-shaped and translucent, with visible growth rings but no ridges. Velum and foot present. Siphons absent ..... *Dreissenidae* . . . 10

10*a*. Margins of shell valves do not overlap. Umbos equal in height. ..... *Dreissena polymorpha* (Fig. 10)

Umbo knobby, prominent, and extends well above the shell margin in older pediveligers. Measurements of laboratory-bred larvae: mean length 210 μm (range 136–296 μm), height 195 μm (111–261 μm), hinge length 86 μm (70–89 μm). Measurements of field-caught larvae: length 214 μm (140–347 μm), height 213 μm (120–297 μm), hinge length 91 μm (74–101 μm).

10*b*. Parts of the right valve margin extend beyond the margin of the left. Umbos not the same height (difficult to see) ..... quagga (Fig. 11)

In pediveligers of all ages the umbo is not prominent, and it has a lower, less knobby profile than is usually seen in zebra mussels. Measurements of laboratory-bred larvae: mean length 164 μm (range 141–168 μm), height 142 μm (129–157 μm), hinge length 58 μm (43–64 μm). Measurements of field-caught larvae: length 184 μm (150–228 μm), height 158 μm (133–177 μm), hinge length 60 μm (51–76 μm).

11*a*. Margins of shell valves do not overlap. Umbos pronounced and equal in height ..... *Dreissena polymorpha* (Fig. 12)

The slope of line from umbo to dorsal peak varies from very steep (resulting in an upright, squat-looking shell) to very low (resulting in a "low-slung" shell). The type of shells were evenly mixed at all sites. Measurements of laboratory-bred larvae: mean length 280 μm (range 219–365 μm), height 215 μm (199–352 μm). Measurements of field-caught larvae: length 291 μm (231–462 μm), height 233 μm (189–367 μm).

11*b*. Margin of right valve overlaps margin of left valve. Overlap may not occur evenly around shell margin. ..... quagga (Fig. 13)

Slope of line from umbo to dorsal peak varies from very steep (resulting in an upright, squat-looking shell) to very low (resulting in a "low-slung" shell). Quaggas from Nanticoke tended to be more upright in shape and those from Presque Isle more "low-slung." Measurements of laboratory-bred larvae: mean length 167 μm (range 136–232 μm), height 139 μm (119–201 μm). Measurements of field-caught larvae: length 251 μm (222–410 μm), height 220 μm (178–390 μm).