Guideline for Field Collecting and Preserving Sphaeriidae Clams, for DNA and Taxonomic Research (First Draft).

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(Photo on cover page; Photograph of Pisidium idahoense taken by Adam Frankiewicz.)

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Goals

The objective of this sampling protocol is to provide a guideline for collecting and preserving Sphaeriidae clams for future taxonomic and DNA analysis. This protocol will give a brief description of the ideal habitats for clams, equipment and methods for qualitative sampling, how to identify specimens in the field, and the different ways to preserve and transport specimens for DNA analysis.

While the goal is to collect as many species of Sphaeriidae as possible, for research purposes there is a special interest in collecting *Sphaerium simile* and *Sphaerium striatinum,* which will be discussed on pages 8-9.

Sphaeriidae General Description

Sphaeriidae, commonly called fingernail, pea, or pill clams, are the smallest and most poorly understood freshwater bivalves. Their shell length ranges from < 3 mm in the smallest species (*Pisidium punctatum*) to approximately 25 mm for the largest (*Sphaerium simile*). Because of their small size and cryptic nature, they may be overlooked during field collection or mistaken as pebbles or plant seeds. But with practice and patience, the field crew can separate clams from other debris by looking for: 1) an object that is not transparent (similar to a grain of sand), 2) is hard like a pebble (though some species have delicate shells), and 3) has smooth sides with either very distinct or fine striations. The specimen should also have a distinct line that separates the two shells. For mature specimens, the shell will have a hump called an umbone on the dorsal portion of the shell (Figure 1).

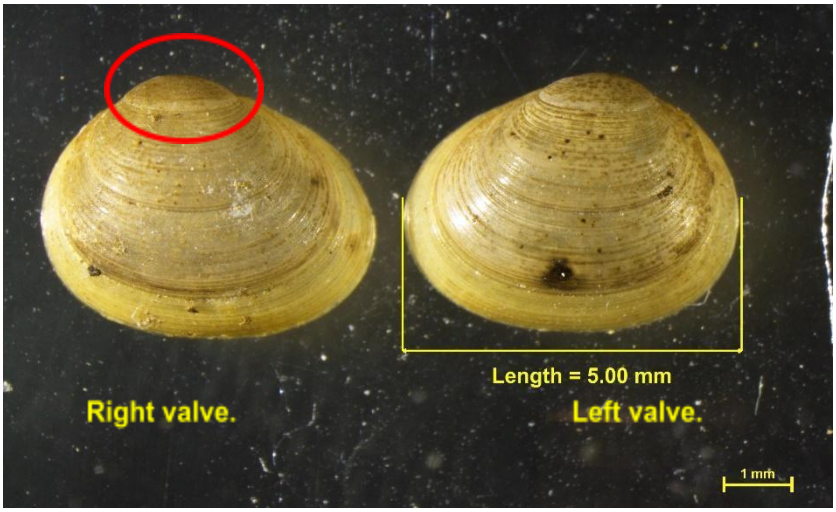

Figure 1, Is a photograph of *Pisidium adamsi* right and left valves, the red circle shows the location of the umbone. Photo by A. Frankiewicz.

Sphaeriid shells are usually rounded or oval, but some species have more of a trigonal or tetragonal shape. Shell coloration ranges from pale yellow or white to chestnut or a dark brown tint. Soft anatomy inside the shell will take on the coloration of the water or food from where the specimen was collected. For example, I have found clams from vernal pools that appear red or orange despite their shell naturally being white or pale yellow. Many of the paler color clams can be spotted by a distinctive orange tint at the umbone; this is due to internal coloration of the gonads and can make them easier to separate from other material such as plant seed or sand grains.

Sphaeriidae Habitat

Sphaeriidae can be found in almost any type of aquatic habitat, from large lakes and rivers to springs, bogs, and vernal pools. Generally, the greatest diversity can be found in lakes, ponds, and small rivers. Other habitats, such as vernal pools or small creeks, are usually colonized by a small number of species, but may contain unique species such as *Sphaerium occidentale.* Like mussels, Sphaeriidae are burrowers, and thus only a few species are found in areas with coarse sediment like rocks, gravel, and hard sand (such as the invasive *Pisidium amnicum*). But unlike mussels, Sphaeriidae will not burrow very deep into the sediment (< 3 cm). Most sphaeriid species prefer living in detritus or in finer sediment such as silt, clay, mud, or muddy sand. For example in vernal pools I found more clams on top of the leaf litter than in the sediment, but in some lakes I found the opposite where more clams were in the sandy sediment than in the detritus.

Equipment list

- Dip nets
- Round pointed shovel (optional but useful)
- Drag dredge (optional, if available)
- Sorting sieve with a 500 µm mesh (a fine metal cooking sieve can also work)
- Flat tubs or tray for picking
- Disposable plastic pipette with opening of >5 mm
- Forceps with soft or curved tip
- Vials ranging from 5 ml to 30 ml or larger, depending on the size and number of specimens
- Cotton, wool or other soft absorbent material to place in vial
- Cooler (for storage of specimens)
- Foam vial holder (needs to fit in cooler)
- Ethanol (95% or higher ONLY)

Methods for Collecting and Preserving Specimens

This protocol focuses on qualitative sampling techniques using dip nets and shovels. If available, a drag dredge can be used for collecting in depths > 5 feet. Sphaeriidae, like all bivalves, are found primarily near the surface of the substrate in aquatic habitats. However, some can also be found in other zones clamped onto debris or submerged plants. When collecting sediment in water of depths 2 ft or less, a round pointed shovel or dip net can be used to skim the surface of the substrate. When collecting from depths deeper than 2 ft, or when the sediment at the site is very soft, the dip net should be used. Collecting with the dip net follows similar protocol as collecting for other benthic invertebrates except field collectors may have to skim deeper into the substrate than typical. When using a drag dredge to collect samples from depths deeper than 5 ft (usually being dragged behind a boat), samples are emptied into a tub. I prefer using a dip net and scraping the surface of the sediment or leaf litter; you shouldn't need to dig too deep with the net.

Once sediment samples are collected, the contents should be carefully sieved through a 500 µm mesh sieve (or fine metal cooking sieve). Once samples are cleaned, specimens can either be picked from the sieve or the contents can be placed in a picking tub. Note that I find it easier to pick Sphaeriidae when using as little water as necessary; they tend to stand out more when they are semi-dry and picking them out of water can be frustrating.

When picking, specimens must be handled with care! The shell is very important for taxonomic identification and any damage to the shell will make it difficult to key out a species. Large specimens such as *Sphaerium* or *Musculium* can be collected by hand, but for most clams, use

forceps with a soft or curved tip. Most dissection forceps are also acceptable, but remember to be careful to only put enough pressure to hold the specimen. I also found a small fine paint brush also works very effectively when collecting the specimens when they are semi-dry. To collect smaller clams safely and effectively, use a disposable plastic pipette with an opening of 5 mm or larger. If the specimen is to be kept cool then pipetting excess water or other material is not only acceptable but could also help the specimen stay alive longer. But if putting the specimen in ethanol try to avoid pipetting extra water which could risk diluting the preservative and jeopardizing the DNA preservation.

Sphaeriidae should be preserved and transported one of two ways to allow future DNA work: 1) placing specimens in 95% ethanol, OR 2) freezing the specimens (PREFERRED). When using 95% ethanol, remove any excess material or vegetation. Excess organic material and water will dilute the ethanol and compromise preservation. Replace the ethanol at the end of the collecting day (or when needed). When freezing, clams should be placed in vials with a small amount of water and soft material—such as wet cotton wool, grass, or moss—to help prevent fragile shells from being damaged when transported. Label vials and place them into a foam vial holder or appropriate container that can protect and hold the vials for transport. Place the container holding the live specimens into a cooler with ice at a temperature roughly around 5°C (41°F) or lower; this will keep the specimens alive for transport. Most species of Sphaeriidae can survive for several weeks or even months in cold temperatures; although it is worth noting that others may not have the tolerance for such conditions and will perish. Regardless of their survivability, the cold temperature will aid in maintaining the integrity of the specimen's DNA until they can be processed in the lab or placed in a freezer for storage and later transport. These specimens should be frozen as soon as is practicable and then must be kept frozen until they are shipped to me.

Although ethanol is an easy way to preserve specimens, problems do arise because of improper preservation and the tendency for clam muscles to stiffen. This stiffness makes it challenging to open the shell for identification. Because of these issues, the field crew is encouraged to use the freezing method for at least some of the specimens found when collecting.

It is acceptable to put multiple specimens in one vial, but be considerate of the size of the vial, number of individual clams that will be put into the vial, and the size of the clams. Vial sizes may fluctuate, but generally keep to no more than 50 small specimens (1 – 6mm) per vial, < 20 medium specimens (7 – 15mm) per vial, and < 10 larger specimens per vial. If preserved with 95% ethanol, biotic material should make up no more than 40% of the vial with the rest filled completely with ethanol to ensure preservation.

Preferably, specimens should be shipped in a foam cooler with ice packs (if shipping frozen specimens) or a standard shipping box (if in ethanol) and protective packaging (foam, bubble

wrap,...etc,.) to keep the vails from breaking in transport. Vials should also be placed in a secondary container such as a ziplock bag or small box encasing all vials to prevent breakage during transportation and shipment. Specimens preserved in ethanol don't necessarily need to be in a foam cooler, but if they can be shipped this way, the ice will help preserve the body for DNA analysis and thus transporting the specimens cool is still encouraged. Specimens are to be shipped to the Natural Resources Research Institute (NRRI) at 5013 Miller Trunk Hwy, Hermantown, MN 55811, Attn: Dr. Valerie Brady or Robert Hell. Please contact us and we can provide a prepaid shipping label (vbrady@d.umn.edu). We will need to know the size (dimensions) and weight of the box being shipped.

Identification of Sphaeriid Genera

There are an estimated 39 species of Sphaeriidae in North America, and 34 of those species are found around the Great Lakes region. The family is split into 4 genera: *Sphaerium*, *Musculium*, *Pisidium* (Figure 2), and the fourth, *Euperinae,* is only found in the southern United States. Genera can be separated in the field using the following descriptions and images. The descriptions of genus and habitat preferences are based on personal experience and notes from Mackie (2007) and Herrington (1962).

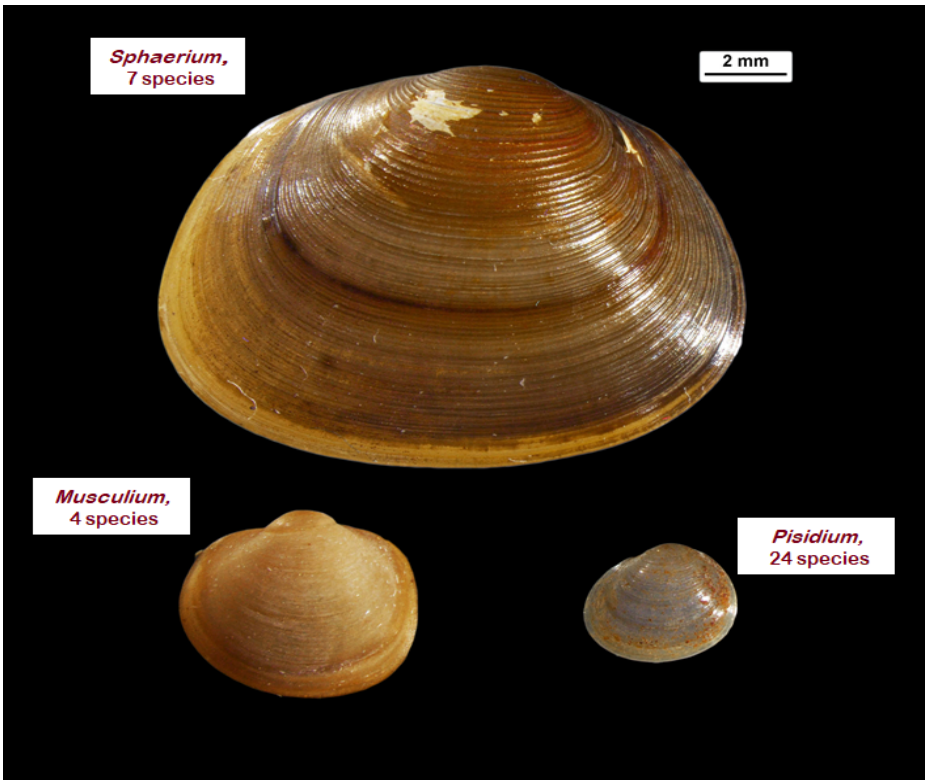

Figure 2: Photographs showing the 3 genera found in the Great Lakes, created by A. Frankiewicz.

***Sphaerium*:** Adults are easy to moderately easy to spot in samples because of their large size, with adults ranging from 7 – 25 mm in length. Depending on the species, shell thickness can range from thick to thin, with either fine or coarse striations. Coloration ranges from yellow to chestnut to dark brown (Figure 3 and 4).

Figure 3. *Sphaerium rhomboideum*, by A. Frankiewicz.

Figure 4. *Sphaerium simile* (top) and *Sphaerium striatinum* (bottom) next to a penny, by A. Frankiewicz.

Collecting *Sphaerium simile* and *Sphaerium striatinum*: As mentioned above, while it is important to collect as many sphaeriid species as possible, the main focus of my current research is to collect two specific species: *Sphaerium simile* (Figure 5a) and *Sphaerium striatinum* (Figure 5b). Luckily both species are large (for a sphaeriid), with adult shell lengths ranging from 10-18 mm, although *S. simile* can reach up to 25 mm (Mackie, 2007). Both species also share similar habitats (lakes, rivers, creeks, and large ponds). They prefer shallows (<1 - 2 m) but can also be found down to depths of 6 m. *S. striatinum* can be found in a wide range of substrate (mud, sand, gravel, silt, organic matter) and vegetation, while *S. simile* prefers specific conditions. In lakes and large ponds, a large number of *S. smile* can be found in mud, sand, or both, and with submersed macrophytes (*Ceratophyllum, Myriophyllum,* or *Potamogeton* are common, Mackie, 2007). In rivers or creeks, *S. smile* can be found in eddies with thick organic ooze or fine sand and silt.

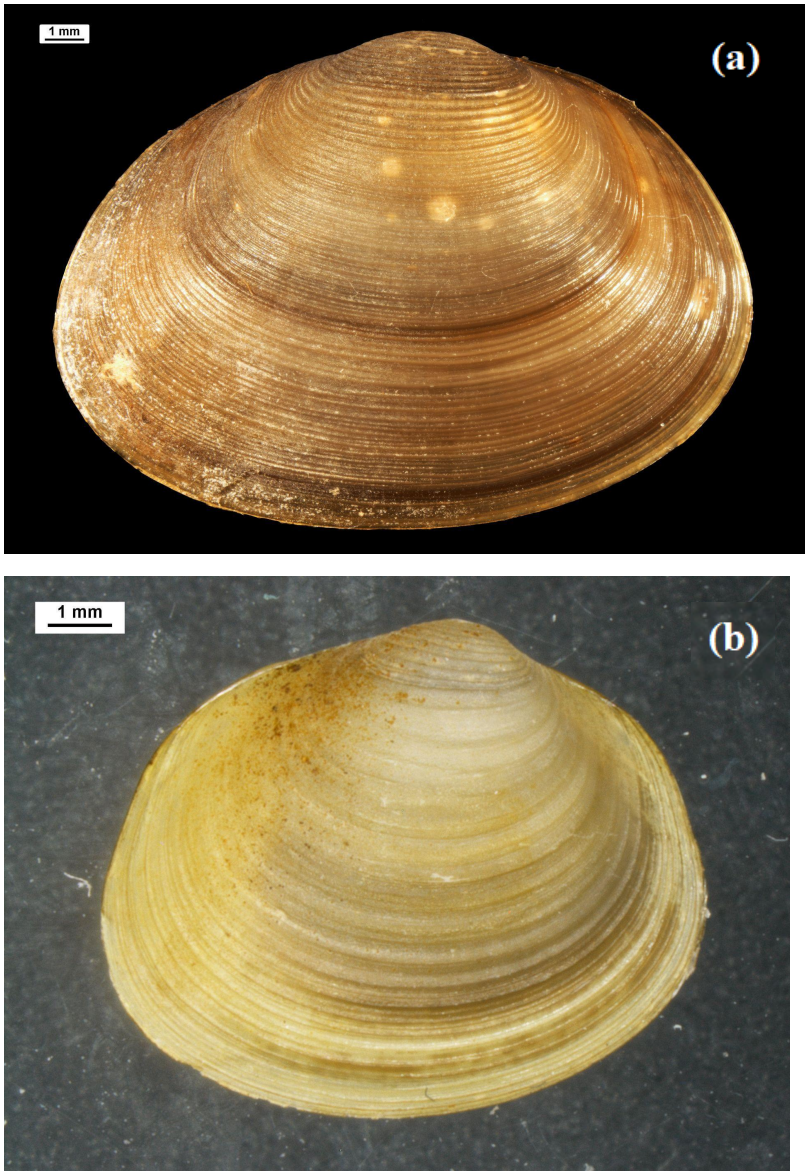

Figure 5. (a) *Sphaerium simile*, (b) *Sphaerium striatinum*, by A. Frankiewicz.

***Musculium*:** These are easy to moderately difficult to spot due to their small to medium sizes (adults ranging from 7 – 15 mm in length) and their very thin shell, which makes them appear semi-transparent with a slight brown tint. **Care should be taken when handling to avoid breaking the shell!** Their most distinctive feature is the umbone, which is separated from the rest of the shell by a distinct sulcus or ridge which forms a cap on the top of the shells (Figure 6 and 7). It should be noted that the cap is common in specimens found in organic/detritus material but sometimes not present on specimens found in lakes and rivers with sandy sediments (Figure 8).

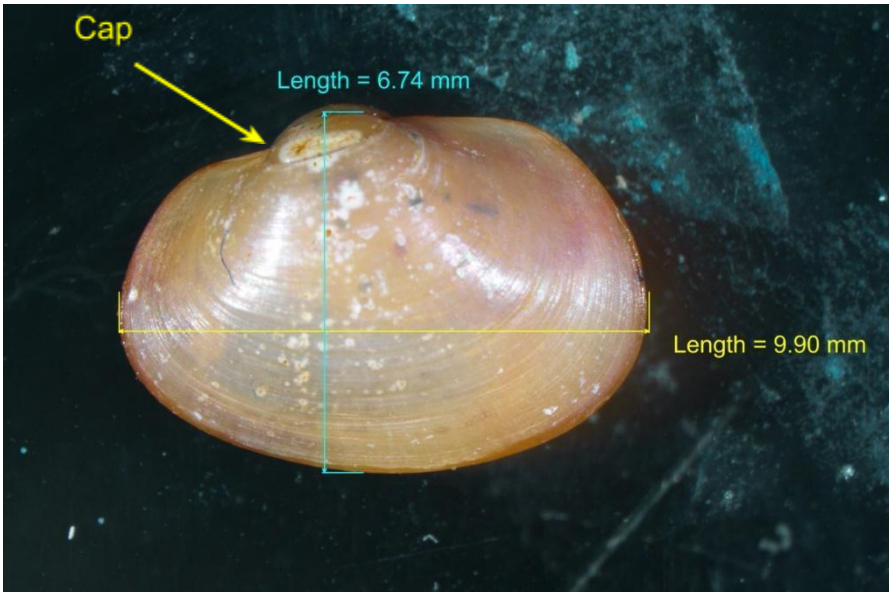

Figure 6. *Musculium transversum*. Arrow indicating the shell cap., Photo taken by A. Frankiewicz.

Figure 7. *Musculium lacustre* on a cattail leaf. Photo by M. Leppin (2009).

Figure 8. Capless *Musculium transversum* from St. Louis River, MN, Photo taken by A. Frankiewicz.

***Pisidium*:** Although some species of *Pisidium* can be easily spotted (such as *P. amnicum*), most are very difficult to find in collected samples due to their small size (adults ranging from 2 – 7 mm) and sometimes pale shell coloration. They are often mistaken for pebbles or plant seeds. Thus, **field crews must take caution and care when sorting through small material**. Most species have thick shells with distinctive striation, but a few species have thin shells with fine striae. Shell coloration ranges from pearl white or transparent to dark brown (Figure 9 - 11).

Figure 9. *Pisidium ventricosum* resting on a man's index finger, photo taken by L. Clarfeld (2014).

Figure 10. Photographs of *Pisidium dubium*; photo by A. Frankiewicz.

Figure 11. Photographs of *Pisidium adamsi*; photo by A. Frankiewicz.

Corbiculidae

Although Corbiculidae (Asian clams) are not targeted for this research, some argue that they should be placed in a superfamily with Sphaeriidae (Corbiculacea), but DNA test have concluded that the two families are in fact not sister family (Graf, 2013). Regardless, if any Corbiculid are found they should be documented and collected.

The invasive Corbiculidae, genus *Corbicula,* has two species that were possibly introduced in North America, *C. fluminea* and *C. fluminalis*. While both are described species, the taxonomy of Corbiculid is in disarray and thus the only really confirmed species in North America is *C. fluminea.* Corbiculid are very easy to spot in samples and to separate from Sphaeriidae due to their size (adults ranging from 13 – 30 mm in length) and triangular-shaped shell. Adult shell height usually = length. The shells are thick, with very distinctive striations and have a light to dark brown coloration (Fig.7.)

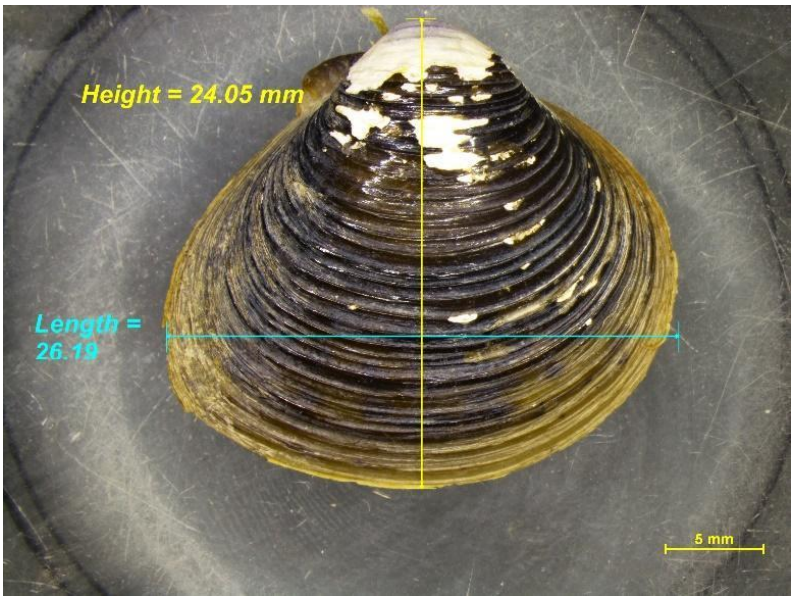

Fig. 8. *Corbicula fluminea*, photo by A. Frankiewicz.

References

- **Photographs:**

Clams on ruler or leaf: https://hiveminer.com/Tags/sphaeriidae/Recent

Photo of clam on finger: https://www.inaturalist.org/observations/645491

- **Articles and Books:**

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Spencer, K. L., Boehler, J. A., & Krieger, K. A. (2016). Gene Sequences as a Tool for Identifying Native and Non-indigenous Sphaeriid Clams in Lake Erie. 1-29.

Collecting Sphaeriidae Field Sheet

By Adam Frankiewicz
Date 01/31/2018, Revised 04/29/2022

1. Collecting samples: samples can be collected by either using a shovel or dip net to skim the surface of the sediment, digging in roughly 1 to 3 inches into the substrate.

    a. Use a dip net or shovel for shallow water (depth of <1 - 6 ft).

    b. If available, a drag dredge should be used when the water depth is deeper than 5 feet.

    c. Be aware when collecting for other inverts that some clams can also be found in plant vegetation, so it is recommended to sample as many habitats as possible.

2. Sorting samples: Collected samples need to be sieved through a 500 µm mesh sieve or fine metal cooking sieve. This will remove materials such as muck, silt, organic debris, and other fine particles.

3. Pick specimens: **Please handle specimens with care!** Some species of clam are very delicate and may crack if too much pressure is applied.

    a. Use your finger to collect larger specimens. You can use thin nitrile gloves, but you want to feel the clam in your finger so you know how much pressure you are applying.

    b. Smaller clams can be picked with soft or curved tip forceps (just remember to put just enough pressure to hold the specimen). A fine tip paint brush can also be useful in collecting clams without damaging the shell.

c. A disposable plastic pipette with a large opening (> 5 mm) works very well for collecting smaller specimens. If the specimen is to be placed in ethanol, avoid putting excess water into the vial if ethanol is being used for preservation.

4. Preserving and transporting: Specimens should either be preserved in 190 proof ethanol (95%) or placed in vials with a little water and chilled until they can be frozen. If possible, please chill and then freeze specimens instead of using ethanol. The ethanol method is only preferred if freezing is not an option or if the specimen is damaged.

a. It is acceptable to put multiple specimens in one vial. Vials should be no more than 40-50% full of specimens/biotic material.

b. When using ethanol, the vial must be filled completely (no air bubbles) to protect the specimens. In addition, biotic material should make up no more than 40% of the vial. Ethanol in vial should be replaced with clean ethanol after a day or if needed; you can tell if it needs clean ethanol if it's not clear or the alcohol has lost its odor.

c. When freezing, clams should be placed in a vial with a small amount of water (1/10 the volume of the vial) and something that is soft that can protect the clam shell when transporting such as wet cotton wool, wet grass, or moss (moss is supposedly the best option). Vials should then be placed into a foam vial holder or some other container that can protect the vial while transporting. Containers should be placed in coolers with temperature roughly around 5°C (41°F) or colder. As soon as possible, the vials should be placed into a freezer and kept frozen to protect the DNA.

5. Email Adam Frankiewicz (fran1075@d.umn.edu) and Valerie Brady (vbrady@d.umn.edu) to pay for shipping charges.

6. THANK YOU!