STANDARD OPERATING PROCEDURES

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Decontamination of SCUBA diving equipment and underwater gear after diving in waters containing zebra / quagga mussels (*Dreissena polymorpha / D. bugensis*).

This Standard Operating Procedure (SOP) is intended to provide a step-by-step procedure for the decontamination of SCUBA diving equipment after its use in dreissenid mussel infested waters. Current information regarding geographic areas of dreissenid mussel infestations is available from the U. S. Geological Survey website of Non-indigenous Aquatic Species (NAS), at the following address: http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/

Overview:

In order to avoid the spread of dreissenid mussels via SCUBA diving gear to non-infested waters, decontamination protocols must be designed and implemented. Two different methods are described in this text, one requiring the drying of gear and the other requiring the use of a commercially available chemical. Either method may be used to decontaminate dive gear and both require the gear be carefully examined, and cleaned of adult stage mussels, prior to decontamination.

A description of zebra mussel life history and habitat requirements is provided for reference (Appendix A).

Responsibility:

All USGS procedures for the decontamination of SCUBA equipment will strictly adhere to and follow all USGS requirements. All USGS authorized divers will be responsible their dive gear and underwater equipment.

Dive Gear and Equipment:

Wetsuits and Drysuits
Buoyancy Compensators
Tanks (including boots and protective mesh)
Regulators and gauges
Mask, Fins, Snorkel
Cameras and video equipment
Sampling devices

METHOD 1 – Procedures for the decontamination of gear using washing and air drying:

All dive gear and equipment used in dreissenid mussel infested waters must be inspected carefully and rid of adult mussels prior to washing.

All dive gear and equipment should washed by thoroughly soaking and rinsing with warm, chlorinated tap water. Hot water of over 120° F (Apeks / Aqua Lung regulator owner's manual), should not be used as it may damage certain temperature–sensitive gear. Buoyancy compensators must be flushed internally with warm tap water and dried completely using standard procedures as recommended by the manufacturer. Commercial dive gear cleaners, such a wetsuit shampoos, may also be beneficial as a disinfectant and may be used in the washing process. Drains in wash facilities must be attached to a source for wastewater treatment (municipal sewer).

All dive gear and equipment must be completely dry for a period of at least 24 hours prior to use in waters where there is no dreissenid mussel infestation. Note: wetsuit seams should be closely inspected to insure that the material is completely dry.

METHOD 2 – Procedures for the decontamination of gear using chemical application

The chemical Virkon, produced by DuPont, has been tested and found to be effective at killing both adult and veliger stage dreissenid mussels, when applied to gear that has been exposed to mussel infested waters (Moffit et al. 2015), with little environmental risk (Stockton and Moffitt 2013). Depending on the concentration used, Virkon can be effective at inducing mortality in both adult and veliger stages of dreissenid mussels.

This method describes the use of a 0.5% concentration solution of Virkon and is to be applied to equipment for the decontamination of dreissenid mussels in the veliger stage. All dive gear and equipment must be completely devoid of adult mussels. Any equipment that is found to have adult mussels should be soaked in a higher concentration of Virkon.

Following the DuPont manufacturers safety guidelines, mix a 0.5% solution of Virkon RelyOn (one 5 g. tablet / Liter of water).

CAUTION MUST BE USED WHEN EXPOSING METAL PARTS TO VIRKON SOLUTIONS AND EXPOSURES SHOULD NEVER EXCEED MORE THAN TEN MINUTES. METAL PARTS MUST BE RINSED WITH FRESH WATER IMMEDIATELY AFTER TREATMENT.

RECOMMENDED PROTOCOL FOR THE DECONTAMINATION OF THE INTERIOR OF BUOYANCY COMPENSATION DEVICES, TO BE USED FOR DREISSENID MUSSEL VELIGERS

Note: The following procedure can only be done with buoyancy compensation devices (BCD's) that have removable dump valves.
Following the DuPont manufacturers guidelines, mix a 0.5% solution of Virkon RelyOn (one 5 g. tablet / Liter of water). Unscrew one of the dump valve covers, remove the valve assembly and pour in the sanitizing solution. Screw the cover and valve assembly back on to the BCD, inflate the BCD, and gently rotate the BCD, in all directions, to ensure the solution has reached all of the internal parts of the BCD. Allow the BCD to sit for 10 minutes. After 10 minutes, immediately flush the BCD two times with fresh water, removing the dump valve assembly, adding about a gallon of water, sloshing and turning upside down, and dumping the waste water via the exhaust hose, to ensure it is thoroughly rinsed. Do one final rinse, filling the inside of the BCD with water and removing the dump valve to release the water. Dispose of waste solution according to local regulations.

Moffitt, Christine M, Barenburg, Amber, Stockton, Kelly A., and Watten, Barnaby J., 2015, Efficacy of two approaches for disinfecting surfaces and water infested with quagga mussel veligers: CRC Press, p. 467-477.

Stockton, K. A., and C. M. Moffitt. 2013. Disinfection of three wading boot surfaces infested with New Zealand mudsnails. North American Journal of Fisheries Management 33:529-538.

Appendix A

Life History and Ecological Requirements of the Zebra Mussel - North American Experience Through 1992

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The rapid spread of zebra mussels (*Dreissena polymorpha*) across the United States is due to their ability to grow and reproduce in a wide range of environmental conditions, coupled with a free-living, planktonic larvae (veliger). When zebra mussels were first discovered in the United States, predictions concerning their habitat requirements were based on the European experience with these bivalves. However, zebra mussel populations in this country have consistently exceeded all expectations and predictions as to how fast they could grow, reproduce, and expand their range. Although many research projects are currently underway to delineate the ecological needs of zebra mussels in the United States, much of these results are not yet published.
The information presented below represents what is currently known about the life history and ecological requirements of zebra mussels. The primary purpose of this information is to emphasize specific features that increase the risk of accidental escape of zebra mussels from research facilities. Data from both on-going research and findings presented in the European literature has been used, although as mentioned earlier, European results have not always been applicable here. The recent discovery of the second type of Dreissenidae, the **quagga**, may complicate the situation since the ecological needs of this mussel are unknown. Based on available information and experience, we have assumed that the basic environmental needs of quaggas are similar to those of zebra mussels.

ADULT MUSSELS: Life History

Mobility. Mussels less than 15 mm in length are very mobile, capable of crawling, drifting, and floating for some time in the water column. Movement is believed to be in response to environmental conditions.
Risk Assessment: Severe. Mussels will crawl into any small crack or crevice, into filter floss, water intake systems, and even up out of the water. The narrowness of their shells enables mussels to pass through small openings. For example, 5-mm-long mussels have been known to crawl through 0.5-mm mesh netting. Extra precautions are needed to prevent contamination of all equipment that is in contact with zebra

mussels or water in which zebra mussels are known to be present. Do not assume that netting or coarse filters can prevent escape of small mussels.

Reproduction. Zebra mussel fertilization is external, and spawning can continue over a period of several weeks. Mussel reproduction starts when water temperatures are above 12C. In most temperate regions, water temperature limits the spawning season to May through September. However, reports from Russia and laboratory studies conducted in this country indicate that spawning continues year-round in areas where water temperatures remain above 12 C. About 10-15% of zebra mussels will reach sexual maturity at a ventral shell length of 2-3 mm. Most become sexually mature at a ventral shell length of 6 mm.
Risk Assessment: Severe. Laboratory colonies held at water temperatures above 12C can and will spawn continually, increasing the risk of veligers being present in all wastewater.

Food Supply. Mussels are filter feeders and were initially reported by the Europeans to feed and survive only on live algae. However, research done in this country indicate that zebra mussels consume all types of food, including detritus and zooplankton, as well as their own young, and can therefore grow during periods of time when live algae are unavailable. Also note that mussels can survive for up to 11 months without food under laboratory conditions at 4 C.
Risk Assessment: Moderate. Mussels can colonize areas where live algae is limited or areas where the food supply is intermittent (such as drainage pipes).

Growth. Juvenile mussels are capable of rapidly growing to sexual maturity. Juvenile mussels average only 0.4 mm in ventral shell length just after undergoing metamorphosis, and under optimal conditions can reach 13 mm in less than 3 months. Growth begins when water temperatures are over 3C.
Risk Assessment: Low. Small mussels will grow to sexual maturity under laboratory conditions even if held at less than 10 C, although spawning has not been reported at such temperatures.

ADULT MUSSELS: Special Handling Problems.

Handling small mussels. Juvenile mussels (less than 1 mm long) are difficult to detect visually without using a microscope. The easiest way to determine if these mussels are present under field conditions is to feel them--they feel like sand grains. They also "stick" to everything, lodging under fingernails, in net handles, on clothing, etc., increasing the risk of accidental release. Extra precautions should be taken to insure proper "decontamination" of all gear, etc. that may have been exposed to juveniles less than 1 mm in shell length.

How to determine if mussels are dead. When mussels die, the shells remain open with body parts exposed. A dull probe can be used to touch mussel tissue to determine

if animal is alive or dead. Mussels that float when they are placed in water are not necessarily dead. Live quaggas frequently retain air in the shell valves during handling and will float for hours.
Risk Assessment: Severe. Assume mussels are alive, unless body tissue has sloughed off from the shell.

ADULT MUSSELS: Habitat Needs.

Zebra mussels are very tolerant of a wide range of environmental conditions if certain basic needs are met. The following basic needs and tolerances have been noted in Europe and in the Great Lakes region:

Calcium needs. European research indicates that mussels require 30 ppm dissolved calcium for shell growth and 50 ppm for reproduction. However, laboratory studies done in this country indicate that some growth can occur at 20 ppm and reproduction at 35 ppm. Quagga calcium needs have not been tested, but their shells are noticeably thinner than zebra mussels.

Dissolved oxygen. Oxygen needs of zebra mussels have not been documented. However, mussels have been reported from lakes in Europe where summer oxygen levels are less than 2.0 ppm.

pH. In Europe, zebra mussels usually occur in areas where the pH is over 7.5. The degree of acidity in the water that will be tolerated by zebra mussels will in part be related to calcium levels, and is at this time unknown.

Salinity. European studies indicate that zebra mussels will not live in sea water, but can tolerate estuarine conditions. However, Russian literature indicates that some of the other Dreissenidae are more salt-water tolerant than zebra mussels. At this time, salinity tolerance of the quagga mussel is unknown.

Water temperatures. Mussels can survive in temperatures ranging from below 0 to 35C, if they are submerged. Mussels exposed to the air have a much narrower temperature range (about 6-28C). To date, spawning has only been seen when water temperatures are over 12C.

Water velocity. Mussels are positively attracted to water current and will colonize areas with water velocities up to 2 meters per second.

VELIGERS: Life History.

The physical requirements necessary to insure survival of the free-living larvae or veliger are poorly understood. Much of the information available from the European literature relates to distribution and abundance data rather than physiological studies run under laboratory situations, in part due to the difficulties in handling larvae in the laboratory.

Development. When water temperatures rise above 12 C, adult mussels release eggs and sperm into the water column. After fertilization, developing embryos remain in the water column, and can drift for some distance from the parent colony. The time required to develop from egg to juvenile mussel varies according to water temperature, but averages about 2 weeks under laboratory conditions at 22C. Studies in Europe have documented the presence of veliger in the water column for up to one year. Initial size at shell formation is approximately 100 microns (some quaggas are smaller at D-shell, under 70 microns), and 300 to 450 microns at metamorphosis.
Risk Assessment: Severe. Since larvae are microscopic, their presence or absence on sampling gear or in samples cannot be determined unless examined under a microscope. Assume that veligers are present if water temperatures are over 12 C.

Mobility. Young larvae have a ciliated organ called a velum that is used for swimming. Older larvae, just before metamorphosis, also have a foot that can be used for crawling. Since the larvae are so small, they are readily picked up by water currents, and can be transported some distance.
Risk Assessment: Severe. Assume that veligers are present if water temperatures are over 12 C. Although veligers are described as planktonic, any object collected in a zebra mussel area during spawning season will have veligers of various ages crawling on it.

VELIGERS: Habitat Needs.

Very little is known about the habitat needs and food requirements of veligers. European literature describes veligers as being very intolerant of a wide range of conditions, and mortality rates of over 99% under field conditions are common. However, since specific habitat needs are not known for this life stage, assume that veligers can survive under the same conditions that are suitable for adult mussels.
Food. Veligers begin to feed just after shell formation. They are filter feeders, consuming algae, bacteria, and detritus. Initially, veligers feed off of particles less than 4 microns in size.

Settling substrate. Proper substrate must be present during the time veligers under metamorphosis, or the larvae will die. Veligers settle on filamentous material first, undergo metamorphosis, and then move to a hard substrate.

Water temperature. Veligers tolerate the same temperature regime as do the adults. Development rate is directly correlated to water temperature. Live larvae have been held at 4 C for up to one week without food.

<u>Water velocity</u>. Water velocities over 2 meters per second discourage the settling of veligers.