APPENDIX A: The General Biology of Dreissenids

Dreissena polymorpha (Pallas 1771), the zebra mussel, and *D. rostriformis bugensis* (formerly *D. bugensis* [Andrusov 1897]), the quagga mussel, are freshwater mussels (Mollusca: Bivalvia) from the family Dreissenidae. Both species originate from the Ponto-Caspian region of Eastern Europe/Western Asia and were likely to be transported to the United States unintentionally by large, transoceanic ships. The zebra mussel was first discovered in North America at Lake St. Clair, MI in June 1988[1] while the first North American appearance of the quagga mussel was in the Erie Canal, NY in August 1991[2].

External Morphology

Although similar in many respects, the zebra and quagga mussel are distinguishable from one another by external morphological characteristics. The ventral surface of the zebra mussel shell is flattened (although occasionally the anterior apex is pointed in a downward fashion) or slightly arched such that when placed on a flat surface the shell will stand more-or-less upright. In contrast, the ventral margin of the quagga mussel shell is typically convex and will lean when placed on a flat surface[3]. Additionally, the mid-ventral line of the zebra mussel is generally linear, while that of the quagga mussel is typically curved, or s-shaped[4] (Figure A1). Finally, the quagga mussel shell is often thinner[5], and may have overlapping shell valves[6].

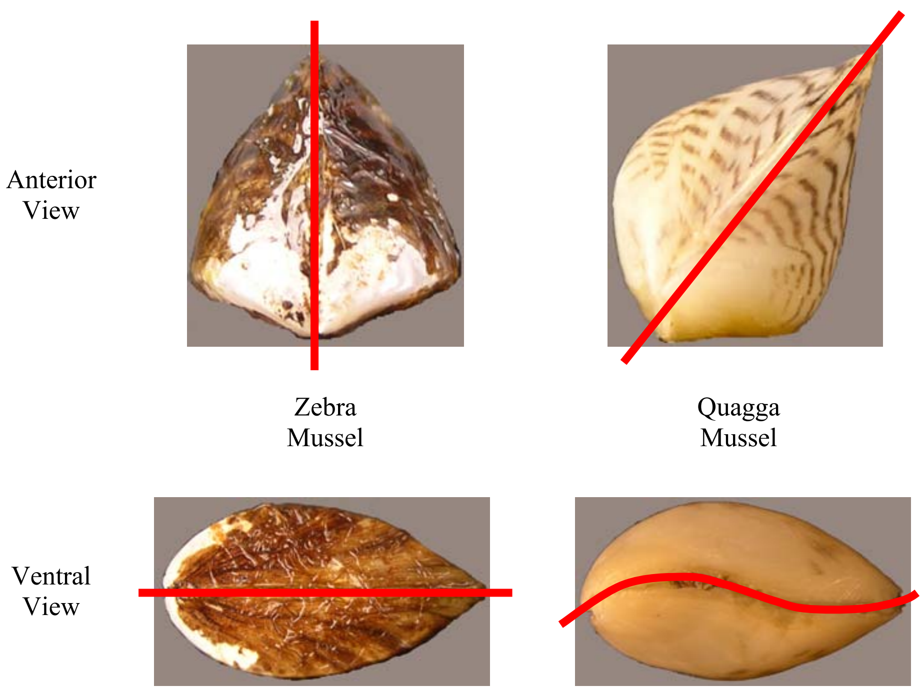

Figure A1. External morphology of *Dreissena polymorpha*, the zebra mussel, *and D. rostriformis bugensis*, the quagga mussel.

Physiological Tolerances

Physiological tolerances derived from experimental testing often vary widely as a result of differences in experimental technique and source of the experimental animals. It is for this reason that physiological tolerances should be considered approximate values and not absolute. The tolerance of zebra and quagga mussels to salinity, temperature, calcium content, pH, and dissolved oxygen have been studied extensively and have recently been reviewed by the San Francisco Estuary Institute[7]. A summary of this extensive review is presented below.

Zebra mussels are believed to be more tolerant of saline conditions when compared to quagga mussels. Reported zebra mussel tolerances to salinity vary widely (0.4 to 18 ppt) and may be dependant on a number of factors including temperature, ionic makeup, and salinity stability. While salinities < 3 ppt are generally preferred, zebra mussels in the Aral Sea maintain dense populations at salinities up to 10 ppt and are present at salinities up to 12 ppt. Zebra mussels in North America appear less tolerant and may have an upper salinity tolerance of approximately 6 ppt. Quagga mussels may be less tolerant with an upper salinity tolerance of 3.5 to 5 ppt.

Zebra and quagga mussels adults are cold tolerant and can survive prolonged exposure to 0 °C so long as their surrounding waters do not freeze. Zebra and quagga mussels are generally intolerant of elevated temperatures beyond 30 °C and that the apparent upper thermal limit is dependent on long term acclimatization and short term acclimation. Water temperatures above 25 °C will lead to the eventual starvation of adult mussels. Water temperatures above 10 – 12 °C are needed to achieve spawning although 18 °C is likely to be optimal while temperatures above 24 °C will negatively impact mussel reproduction.

While there is little information regarding the necessary calcium threshold for quagga mussel survival, adult zebra mussels can tolerate calcium levels ≥10 mg l^{-1} although higher concentrations are preferred. Adult dreissenid mussels require neutral or moderately basic water with a pH range of 6.5 – 9.4 although the limits for reproduction could be much narrower (pH 7.4 – 9.4).

Zebra and quagga musses are generally intolerant of low oxygen concentrations (hypoxia/anoxia). Adult zebra mussels require oxygen concentrations to remain above 2 to 4 mg l^{-1} for long term adult survival while quagga mussels may tolerate oxygen concentrations as low as 1.5 mg l^{-1}.

Compared with native freshwater bivalves, zebra mussels are intolerant of aerial exposure, and under hot, dry conditions (30 °C and 0% relative humidity) suffer 100% mortality within 2 d; while under cooler, humid conditions (10 °C and 80% relative humidity) 100% mortality may require 17 d. Zebra mussels can survive more than 1 month out of water in cold, humid climates (5 °C and 100% relative humidity).[8]

Life History

Dreissenid mussels are dioecious (separate sexes) with external fertilization. A single dreissenid female may produce upwards of 1 million eggs in a single reproductive event[9]. A fertilized egg develops through a brief trochophore larvae stage before growing a ciliated velum and secreting a straight-hinged shell (D-shaped veliger). The development of a thicker shell initiates the veliconcha which is the last obligately planktonic larval stage. The development of a foot and byssal apparatus identifies the pediveliger stage which settles and attaches to a hard substrate. The plantigrade larva represents a settled individual that has not yet developed into a juvenile mussel with a fully-developed feeding apparatus. Juvenile mussels are >1 mm in length and do not become adult mussels until the reach sexual maturity at >5 mm[10]. The time required for a fertilized egg to develop into a juvenile mussel can vary widely (8 to 240 d)[11] and is dependent on food availability and temperature[12].

Feeding and Attachment

Adult zebra mussels exhibit a diurnal pattern in filtering activity and appear to be able to handle particles ranging from 10 to 150 µm with equal efficiency. A 2 cm long adult mussel is capable of clearing 250 ml in 1 hr during the day and 184 ml in 1 hr at night. Thus, on average a large mussel may be capable of removing the phytoplankton from more than 5 L of water per day[13] although it is generally accepted that a single mussel probably filters approximately 1 L per day. When populations densities are exceedingly high zebra mussels may be capable of filtering the entire volume of a lake within a single day.

Unique to freshwater mussels of North America, dreissenids have the ability to attach themselves to hard substrata via proteinaceous byssal threads. The ability of the byssus to produce threads increases with increasing temperature and is capable of producing ≥10 threads per day at 30 °C[14], although thread production slows considerably once a firm attachment has been made[15].

Invasion History

The spread of zebra mussels throughout North America has been rapid, especially through interconnected waterways in part due to the planktonic nature of zebra mussel larval stages. Within one year of being detected in Lake St. Clair in 1988 there were reported sightings of zebra mussels in all of the Great lakes and by 1991 they had made their way into the Illinois and upper Mississippi River. To date, zebra mussels currently inhabit a vast number of freshwater rivers and lakes throughout the eastern half of the United States and have recently crossed the continental divide and begun to spread across the western United States (Figure A2). The spread of quagga mussels since their initial discovery in the Erie Canal, NY in 1991 has been considerably slower although they have begun to replace zebra mussels in the lower Great Lakes and are spreading throughout the southwest after being discovered in Lake Mead in January of 2007 (Figure A2).

The downstream transport of planktonic veligers limits how effectively the spread of dreissenids can be controlled among interconnected waterways. However, upstream and overland dispersal can be reduced by eliminating mussels attached to boat hulls and other aquatic vessels or equipment, the primary vector responsible for the transport of dreissenids to otherwise unreachable areas.

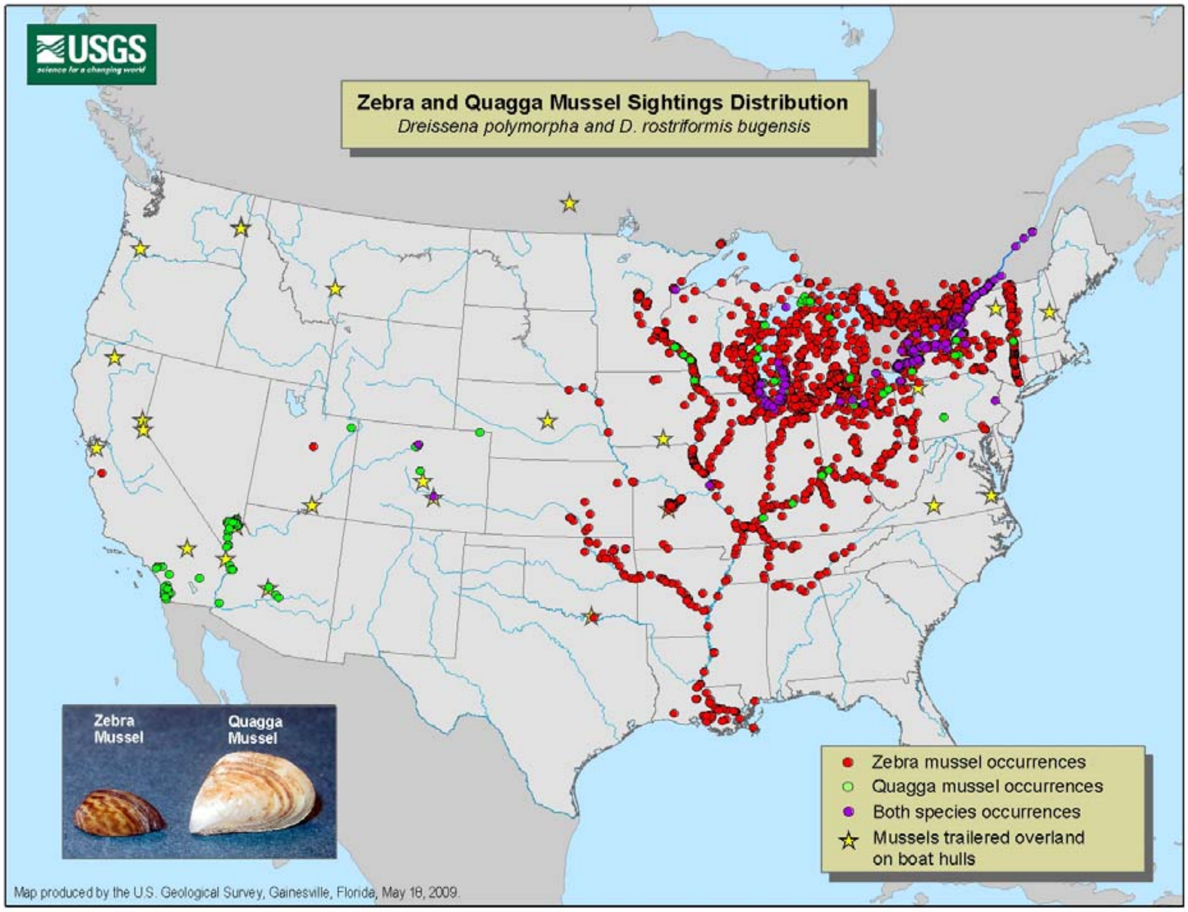

Figure A2. The distribution of zebra and quagga mussels in the United States as of April 2009. Real-time maps are available at http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/

APPENDIX B: Early Detection and Monitoring

The 100th Meridian Initiative conducted a technical workshop in January 2009 in Denver, Colorado, hosted by the U.S. Bureau of Reclamation. The workshop addressed four primary objectives 1) to identify best practices for the detection at low concentrations the presence of quagga/zebra mussels larvae in plankton samples, 2) identify current monitoring and detection programs utilized by different western states and regions, 3) attempt to develop a consensus concerning the determination of whether quagga/zebra mussel larvae are present in a body of water, and 4) to develop specific recommendations for the working group charged with developing a comprehensive quagga/zebra mussel early detection and monitoring program for the western region during the 2009 season. Participants of the 100th Meridian Initiative's technical workshop provided the following recommendations based on discussion where at least a majority consensus was reached.

- The Western Region Should Include All Western States and Provinces – For the purpose of a regional early detection and monitoring program the "western region" should include the 19 states participating in the Western Regional Panel as well as the countries of Canada and Mexico and their constituent provinces/states. Communication with other regions should be actively sought. Lack of participation by any component to the West could lead to further invasions.

- Monitoring Should Include Substrate Sampling – Although substrate sampling may not be the most effective method for early detection, substrate sampling has provided the first evidence of some quagga/zebra mussel invasions. Substrate sampling should be continued. Samplers should be as simple as possible and maximize surface area and "edge" habitat. Samplers should be placed in areas thought to be at high risk and extend from the surface to the bottom with samplers at 10' depth intervals.

- Corroborated Detection by Microscopy and PCR-based Assay**s** – By an almost unanimous vote, quagga or zebra mussel veligers are considered to be present if such presence can be confirmed in a plankton samples by at least one authenticated cross-polarized microscopic analysis and one polymerase-chain-reaction (PCR) assay.

- Dual Confirmation – By an approximate two-thirds majority, quagga/zebra mussel veligers are considered to present in a plankton sample if the presence is confirmed by two qualified microscopists and/or by two different PCR assays.

- Common Language – There was a consensus that a common set of terminology describing graded levels of infestation was needed. In order to avoid confusion and ambiguity, common terminology should be clearly defined and used accordingly. This common language will be defined in the 100th Meridian Initiative's 2009 Detection and Monitoring Plan (http://100thMeridian.org).

- Standardization and Quality Control – A standardized quality control and training program for labs involved in both PCR and microscopy veliger detection assays should be established.

- Evaluation of Effectiveness for Detection and Monitoring – Research evaluating the effectiveness in PCR and microscopy protocols should be continued.

- Preparation for Rapid Response – Impacts generated by quagga/zebra mussels include loss of recreational opportunities, water shortages, increased maintenance costs, damage to goods and equipment, power interruption, and irreparable ecological degradation. Meanwhile, even the most effective prevention programs are unlikely to prevent all invasions. However, if incipient populations of an unwanted species are detected early, rapidly coordinated responses may have an otherwise unavailable opportunity to eradicate or contain the unwanted species before such populations have increased or spread to an unmanageable extent. Until recently, quagga/zebra mussels have eluded early detection and have typically become well established prior to initial discovery to the extent that eradication and spread prevention has been technically and/or fiscally impractical or impossible. However, with the development and application of early detection methodologies using plankton tows, incipient populations of quagga and zebra mussels have been detected in the West over the last two years. Specifically, the large-scale applications of cross-polarized microscopy and polymerase-chain-reaction (PCR) based genetic assays are more capable of detecting the presence of quagga/zebra mussel veligers, while more traditional methods such as substrate sampling, usually detect adult or established populations. However, the existing laboratories that process either light microscopy or PCR assays, or both, are not able to do so regularly with sufficient turn-around time. In 2008, some samples required more than six months for sample handling, processing, and analysis.

APPENDIX C: Rapid Response

Capacity to conduct a rapid and effective response to an incipient introduction is gaining emphasis in the West as a second line-of-defense to stop an invasion if prevention efforts fail. A variety of guidance materials now exist to guide the development of aquatic invasive species rapid response plans, such as a template developed by the Western Regional Panel. Some states are drafting general rapid response plans and policies within their overall AIS management plans. In some cases, state or regional rapid response plans specific to zebra and quagga mussels are in place. For example, the Columbia Basin Team of the 100th Meridian Initiative has completed a rapid response plan for the Columbia/Snake River watershed. This plan incorporates the National Incident Management System within its organizational framework, and outlines step-by-step actions that should be implemented in the event that quagga or other dreissenid mussels appear in Columbia Basin waters. Similarly, the National Park Service has developed a broad mussel response plan at the national scale.

Planning is only the first step in rapid response preparedness. There are additional efforts in the West to enhance the ability to respond via training and other strategies, including:

- Identifying and securing emergency response funding pools
- Defining internal and external notification lists and processes
- Providing Incident Command System training to aquatic invasive species specialists and others likely to participate in response activities
- Developing advance intergovernmental cooperative agreements.
- Defining federal, state and local agency roles and responsibilities
- Developing systems to quickly hire personnel to complete response tasks
- Holding drills and exercises to test and enhance ability to implement plans

Only a small number of Western waters are covered by rapid response plans and the capacity to implement those plans. If zebra/quagga mussels were detected in uninfested watersheds in the West, most associated jurisdictions do not yet have a strategy or defined roles and responsibilities to guide a response. As a result, response is unlikely to be rapid and eradication efforts will be inefficient, and odds are that a full-scale invasion will likely result due to the uncoordinated response. In locations where response plans are in place, the lack of guaranteed funding for response significantly limits the likelihood the plan can be implemented in a timely fashion. The ability of governmental entities to secure funds, hire employees, make purchases and get boots on the ground in quick order is greatly limited. Policy constraints, including unresolved questions about short-term environmental impacts associated with certain eradication techniques, also limit the existing state of response preparedness in the West. There are very few individuals trained and available to support a zebra/quagga mussel rapid response. Similarly, there are gaps in the availability of effective response methods and associated supplies and equipment. Roles and responsibilities also need to be defined, funded and supported at the agency level in advance, versus at the ground level during an incident response. A rapid response plan ideally should be created prior to an invasion. There are numerous templates, including one by the Western Regional Panel and one by the 100th Meridian Initiative Columbia River Basin Team, outlining what components should be included in a rapid response plan.

APPENDIX D: Controlling Established Populations

The efficacy of a wide variety of macrofouling mitigation methods have been experimentally tested against dreissenid mussels. A majority of the methods explored have concentrated on the elimination of mussels from submerged structures and raw water systems of industrial plants. Much less research has been performed on control methods for treating open waters and transport vectors such as boats and trailers. A summary of common control methods for open water systems and their costs (when estimable) are summarized below.

Oxidizing chemicals are successful in mitigating mussels from raw water systems. Chlorine and chlorinated compounds such as sodium hypochlorite are the most widely used compounds to control mussel fouling in North America and Europe. Continuous treatment of zebra mussels at 20 °C with sodium hypochlorite at 2.50 mg/L and 5.00 mg/L will induce 100% mortality in 360 h and 264 h, respectively. The efficacy of mussel mitigation from chlorination is significantly affected by temperature, with increasing temperature decreasing the duration of exposure necessary to induce high levels of mortality. Adult zebra mussels exposed to a residual chlorine level of 0.5 mg/L will experience 95% mortality within 20 d when acclimated to 30 °C but may take as long as 60 d when acclimated to 5 °C, indicating that treatment during winter months will take much longer to be effective. Intermittent chlorination (4 h on, 4 h off) is completely ineffective in treating adult zebra mussels, who close their valves during treatment and display normal filtration and respiration during treatment breaks. Alternative oxidizing chemicals such as bromine and ozone are also effective in controlling zebra mussel fouling however they may be less effective that chlorination or may be prohibitively expensive to utilize. Annual chlorination costs (not including installation of equipment) generally range from $100k to $150k for an average sized power generation facility.

Thermal treatment is a widely used method to mitigate dreissenid mussel fouling from raw water systems. Exposure of submerged adult mussels to 37 °C will induce 100% mortality within 1.25 h or within 24 h at 34 °C. The installation of recirculation systems will be negligible to total plant construction costs if they are performed during the original construction phase although retrofitting existing power plant recirculation systems can be extremely costly. However, if installed, thermal recirculation costs approximately sixteen times less than chlorination on a per treatment basis with costs of $6K and $100K, respectively.

Dreissenid mussels are particularly susceptible to high levels of potassium chloride (KCl) when compared to many other freshwater animals. When compared side by side with 18 other biocides (including molluscicides) it was the most selective toward zebra mussels and had little effect on two species of fish and a native mussel. Similarly, zebra mussels are more susceptible to KCl than two species of freshwater cladocerans although other freshwater unionid clams may be equally intolerant of KCl, especially during chronic exposure. Potassium chloride was used in the only successful dreissenid mussel eradication effort to date at the Millbrook Quarry, Virginia at a cost of $370k. Complete dreissenid eradication was achieved by treating the entire lake with 100 ppm KCl, with residual KCl providing an additional 30+ years of protection from future dreissenid mussel invasion. It is worth noting however that the lake at Millbrook Quarry was small with no surface flow and limited groundwater attachment to adjacent water bodies. Additionally, the lake was man-made and contained only introduced wildlife, none of which were sensitive molluscan species. It is unlikely that KCl would be as effective and environmentally safe in larger, interconnected water bodies, or at sites where the long term exposure of KCl would be detrimental to native wildlife.

Biological control methods include biopesticides, parasites, and the introduction of competitive and predator species. The introduction of non-native species to mitigate mussel fouling is currently not a viable option. Introduced species are unlikely to outcompete *Dreissenid* mussels for resources and are equally unlikely to decrease mussel abundance by predation due to the zebra mussel's high fecundity. The negative effects of the introduction of non-native species for biocontrol are difficult to determine experimentally and are often discovered after it is too late. In contrast, molluscan parasites are often highly specific, the discovery and introduction of host-specific parasites such as the ciliophorans, *Ophryoglena hemophaga* and *Conchophthirus acuminatus* of dreissenid mussels may help keep invasive populations in check. The discovery and investigation of dreissenid-specific parasites may elucidate future biological control methods with minimal impact to the environment, therefore funding of parasite research should be strongly considered. Finally, there is strong evidence that a strain of a common soil bacterium, *Pseudomonas fluorescens*, produces a toxin that is lethal to dreissenid mussels and that is highly specific as is indicated by a lack of mortality against many non-target organisms. Commercial development of this bacterial strain is currently underway and may provide an environmentally friendly treatment alternative to chlorination, although there are currently no cost estimates for treatment with *P. fluorescens*.

The control methods discussed thus far have dealt exclusively with mitigation of mussels from raw-water systems and open waters. The following control strategies are useful in controlling mussels in raw-water systems and open waters as well as on boat hulls and other dispersal vectors:

- <u>Physical Removal</u>: Perhaps the most obvious and labor intensive control method is the physical removal of dreissenid mussels by scraping, hand picking, and pressurized spray. Unfortunately, it is easy to achieve less than 100% removal of mussels using these strategies, limiting their usefulness in preventing the spread of these mussels. Scraping and hand picking mussels may be effective when infested surfaces are flat and there are no refugia containing mussels that will be overlooked. Hot-water spray has been suggested as a control method to remove mussels from recreational boats and trailers. However, recent research has indicated that short-duration contact with hot-water spray is unlikely to kill adult mussels, especially those attached to irregular surfaces where mussels may be sheltered from direct contact with the spray. High-pressure, self-contained boat washing stations cost approximately $70K-$100K. Despite the limitations of physical removal, it remains a critical part of transport vector control, as a first line of defense against the spread of dreissenid mussels.

- <u>Antifouling Paints</u>: Antifouling coatings guard against zebra mussel fouling by protecting the surface from byssal attachments. These coating have generally relied on heavy metals imbedded within the paint that release toxic ions into the water and repel macrofouling mussels. Recently, anti-fouling compounds have been discovered that are environmentally safe (Skaja, Bureau of Reclamation). Antifouling coatings are expensive (up to $100/m2 for silicone-based coatings), require recoating of surfaces as the paint is worn away, and only protect surfaces that can be coated directly with the antifouling agent. Boat hulls and raw-water intakes can both be treated with antifouling coatings, however, the inability to paint water intakes and motor of recreational watercraft limit their usefulness in preventing the overland transport of mussels on boats and trailers.

- Dessication: Zebra mussels are intolerant of aerially exposure, experiencing mortality from desiccation from durations depending on air temperature and humidity. Under hot, dry conditions specimens of D. polymorpha may suffer 100% mortality within 2 d; while under cooler, humid conditions 100% mortality may require 17 d. Zebra mussels can survive more than 1 month when emersed in frigid, humid climates . Nevertheless, mitigation of mussel fouling by emersion requires little effort by boat operators and is highly effective. If air temperatures are below freezing mussel mortality rapidly occurs within 2 d18. Boats that are permanently moored in slips will benefit from installing lifts.

- Isolation systems: Watercraft that are permanently stored in the water (i.e. slip docked) may benefit from commercially available slip water isolation systems. These systems isolate the water that surrounds the hull of a boat, creating inhospitable conditions for mussels and other fouling organisms. Although the isolated mooring systems are unlikely to keep boat hulls from being 100% mussel free, in principle they should minimize fouling of watercraft permanently moored in infested waters and are generally inexpensive (<$3k for an average boat).

The control of dreissenid mussel fouling of raw-water intake systems and the mitigation of mussel fouling on boat hulls and other transport vectors will require the combined use of several control strategies. Each control strategy has both advantages and disadvantages and may not be feasible or cost effective for each water user.

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