# San Diego Regional Dreissena Mussel Response and Control Plan

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**Prepared by:**
**The San Diego Regional Quagga Mussel Working Group**
**April 2008**

San Diego County Water Authority

Helix Water District

Sweetwater Authority

Escondido City of Choice

The City of San Diego Water Department

City of Poway

Resources Agency California Department Fish&Game

Vista Irrigation District

The Metropolitan Water District of Southern California

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# Introduction

The San Diego Regional Dreissena Mussel Response and Control Plan (plan) is a comprehensive plan that provides guidance to San Diego County water agencies by defining related issues, consolidating available best practices, and communicating research and development opportunities. The plan complies with current regulatory requirements and will be updated quarterly or as information changes to maintain a coordinated response and continued compliance. Applicable regulations are available in Appendix A.

The plan includes descriptions of common tasks applicable to all stages of response, general guidelines for specific response activities in a given stage, and general guidelines for decision making when a particular situation has not been directly addressed by this plan. This plan will also attempt to define agency roles and transfer points to form a more uniform response.

This plan is focused on operational control measures. Any strategic planning activities will be identified through the practical implementation of the plan, but will be addressed separately. Ideally, this plan will prompt improvements in response timing, technology, organizational development, permitting efficiencies, funding mechanisms, outreach strategies, and other tools that in turn will allow the plan to evolve. *Agencies can fulfill California Department of Fish and Game requirements by issuing a compliance document that indicates control measures they are choosing to implement to protect against spread of this invasive species to non-infested water bodies.*

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## Background

Quagga (dreissena rostriformis bugensis) and Zebra (dreissena polymorpha) mussels are small freshwater bivalve mollusks native to the Ukraine. These mussels arrived in the Great Lakes region in the late 1980's via ballast water discharged from transoceanic ships. Within the United States and in other areas of the world, they are considered an invasive species with significant impact to the ecosystem.

Quagga and Zebra mussels are highly mobile in the larval or veliger stage, floating close to the water's surface for up to five weeks before settling and attaching. Mussels are able to attach to almost any solid or semi-solid surface. Common examples of attachment points include boat hulls and trailers, docks, nets, water conveyance pipes and facilities, muddy banks, and other aquatic life. The mussels remove large amounts of plankton and suspended particles from the water, decreasing the food available for fish and other consumers. This food chain disruption can cause great change to aquatic communities. Water intake structures, such as pipes and screens can clog, disrupting supplies of drinking, cooling, processing, and irrigation water. The mussels may heavily impact recreation-based activities when docks, break walls, buoys, boats, and beaches become colonized.

Presence of the invasive Quagga mussel has been verified in water bodies supplied by the lower Colorado River, beginning in January 2007. First identification of mussel presence in the San Diego region occurred in August 2007. A map indicating reservoir status and an invasion timeline are presented in Appendix B.

## Regulations and Regulatory Controls

### California State Assembly Bill 1683

AB-1683 was sponsored by Assemblywoman Lois Wolk and signed by Governor Arnold Schwarzenegger on October 14, 2007. This bill is aimed at controlling the spread of Quagga mussels within the state of California and authorizes California Department of Fish and Game (CDFG) to inspect and quarantine infected boats, close recreational facilities, and restrict access to lakes. It also authorizes public and private water agencies to maintain control of inspections, site restrictions and other control efforts by completing a control/eradication plan.

These requirements have been incorporated into Fish and Game Code beginning with Section 2300. Refer to Appendix A to view text of the adopted bill.

### California State Assembly Bill 2065

AB-2065, sponsored by Assemblywoman Loni Hancock, was introduced in February 2008 and proposes further modifications to Fish and Game Code for the addition of State mandated mussel monitoring and control plans. Text of the initial bill can be viewed in Appendix A.

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## Situational Awareness

It is crucial to become fully aware of mussel presence status, the threat of growth or new infestation, and effect on daily operations. Awareness is the first step to defining appropriate initial and sustained response activities and provides options to decision makers when determining acceptable agency risk. Situational awareness is not a one-time event; it must be continually maintained as part of an agency's control program. Elements of situational awareness include:

- Vulnerability analysis
- Baseline system status
- Monitoring
- Reports/recordkeeping
- Outreach

## Vulnerability Analysis and Baseline System Status

A vulnerability analysis marries specific system understanding with knowledge of mussel environments. For example, if an agency knows small pipes within the water treatment facility carry untreated water for pump motor cooling, and that water conditions support mussel colonization, it would know those pipes are vulnerable. If a continuous chlorine injector is located prior to the point at which that water is drawn, that same piping could be understood to be less vulnerable to mussel colonization.

Documentation of a baseline condition may be accomplished by physical inspection, observation of changes in standard operating characteristics, such as pressure or temperature changes, indicate mussel population is Having physically inspected the pipes or by evaluating if there were telling changes in operating characteristics, such as pressure changes, an agency would understand the baseline system status for that location. This would in turn provide the basis for development of monitoring and control options. If populations exist, a routine cleaning regimen may be developed. If no population is found, preventative measures could be installed to reduce or eliminate the vulnerability.

A sample vulnerability analysis checklist is available in Appendix C. Additional references are available in Appendix A as well as in the Websites and References sections at the end of this plan.

## Monitoring

### Priority

CDFG has established a priority for reservoir sampling within San Diego County as follows:

1. Water bodies supplied by Colorado River Aqueduct water
2. Water bodies allowing private boats or recreational fishing
3. All other waterways

### Methods

A monitoring program includes ongoing sampling in appropriate areas to establish the presence or absence of veliger and adult mussels. Though a variety of methods exist, agencies should follow recommended standards established for use in the San Diego region. Methods include:

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- Substrate evaluation
- Plankton tow – evaluated by PCR or microscopy

Substrates are an effective, low cost tool to assess presence and growth of mussels in a specific location. They can be used alone or in combination with laboratory testing methods. Though mussels will settle on a variety of materials, standardization of substrate materials, construction, and inspection timing allows agencies to more uniformly gather scientific data valuable in improving future tactics for control and eradication.

Recommended standard substrate materials, construction, and use are described in Appendix F.

The plankton tow process, PCR methods, and microscopy evaluation methods are also available in Appendix F.

### Monitoring Colonization

Once mussel presence is established, efforts shift to monitoring the rate of colonization and the ability of the adult mussel to reproduce. Monitoring objectives are summarized in the table below:

Table 1 – Monitoring Objectives

| Veligers | Adult Mussels |
|---|---|
| • Early warning of spawning events<br>• Track changes in reproductive status<br>• Develop management strategies using data gathered<br>• Evaluate management strategy effectiveness | • Locate optimal habitats<br>• Identify extent of infestation<br>• Track expanding populations<br>• Determine in-situ growth rates<br>• Minimize maintenance requirements<br>• Evaluate control measure effectiveness |

## Reports/Recordkeeping

### State and Federal Reporting

Agencies are required to report dreissenid occurrence in the state through CDFG and at the federal level through USGS. This reporting enables agencies to track population distribution and may provide background for networking to develop additional control methods.

### Regional or Local Reporting

Agencies within a region or locale can benefit from sharing both positive and negative test results. Reporting of negative test results is not regulated, but is encouraged to gain a more complete picture of regional activity and support increased interaction between agencies with a common interest or water supply. Evaluation of these results can aid in development of future control tactics and standards.

Contact lists and a flow chart for notifications are available in Appendix G.

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## Outreach

### Untreated Water Users

The untreated water user is at risk for damaging system components as well as spreading an infestation beyond their system if not well informed and prepared. Examples of untreated water users and outreach opportunities are:

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Table 2 – Outreach opportunities by user type

| User Type | Outreach Opportunity |
|---|---|
| Reservoir/recreation area | • Boat inspection techniques<br>• Bait and tackle precautions<br>• Other materials in contact with water |
| Agriculture and Aquaculture | • Inspection and control methods for pipe fouling<br>• Effect on crops and hatcheries |
| Firefighters/hydrant | • Maintenance tips for inspection pre/post use<br>• Limiting transfer potential during firefighting activities |

Links to informative websites and outreach materials appear in Appendix D and in the Websites section at the end of this plan. A well-informed consumer should understand their personal responsibility for spread control as well as their supplier's commitment to action.

**Water Agency Staff**

Select water agency staff involved in Operations and Maintenance activities should understand:

- Control procedures during maintenance activities
- Disinfection procedures for mobile equipment
- Physical identification and classification

All water agency staff should be generally informed, as they are ambassadors to the public. Outreach to general staff may include:

- Distribution of available public pamphlets
- Periodic status updates (email or presentation)
- Instructions on how to report suspicious activity

Some staff outreach materials are located in Appendix D.

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## Prevention

Where possible, priority should be assigned to preventing mussel colonization or spread. Prevention activities can include:

- Public outreach campaigns
- Establishing watercraft entry restrictions and intercepting contaminated vessels
- Reservoir closure
- Installation of chemical injection points within a delivery system

Most water bodies within San Diego County are supplied by Colorado River Aqueduct water which has tested positive for veliger stage mussels from its source. Limited prevention opportunities may exist in these reservoirs. Prevention for this region is focused on eliminating spread to local streams and rivers or to other disconnected water bodies.

Boating inspection and reservoir closure guidelines are found in Appendix H.

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## Spread Control and Population Management

When mussel presence is established in a body of water or system, control measures should be considered and implemented. The cost/benefit questions are:

- What is an acceptable or manageable population density?
- What actions will be taken when densities increase?

Control measures can be categorized as regulatory, cultural, physical/mechanical, biological, and chemical. Examples of control measures are shown in the table below:

Table 3 – Control measure categories and types

| Category | Control Measure |
|---|---|
| Regulatory | • Waterway closure |
| Cultural | • Exposure and desiccation<br>• Settling prevention (flow velocity, coatings)<br>• Anoxia<br>• pH management |
| Physical/Mechanical | • Screens and filters<br>• Scraping/power washing<br>• Dredging/suctioning |
| Biological | • Predatory fish<br>• Pathogens |
| Chemical | • Conventional chemicals<br>• Biorational chemicals<br>• Metallic Salts (limited) |

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# Glossary

## Acronyms

| | |
|---|---|
| CDFG | California Department of Fish and Game |
| DNA | Deoxyribonucleic acid |
| HACCP | Hazard Assessment and Critical Control Points |
| MWD | Metropolitan Water District |
| NAS | Non-indigenous Aquatic Species |
| NPDES | National Pollutant Discharge Elimination System |
| PCR | Polymerase Chain Reaction |
| RWQCB | Regional Water Quality Control Board |
| THM | Trihalomethanes |
| USGS | United States Geological Survey |
| WTP | Water Treatment Plant |

## Terms

**Anoxia** – An oxygen deficiency reaching the tissues of the body/organism of such severity as to result in permanent damage.

**Dreissena** – A division of bivalve shells, including the marine mussels, in which the two adductor muscles are very unequal. (Ref: Webster's Revised Unabridged Dictionary)

**Dreissena Polymorpha** – (D. Polymorpha) Scientific name for the zebra mussel.

**Dreissena Rostriformis Bugensis** – (D. Bugensis) Scientific name for the quagga mussel.

**Eradication** - The complete absence of the dreissena mussel, in any life stage, at a particular location over a defined period of time. In other areas of the country, it is signified by an absence exceeding one mussel's life cycle. Eradication parameters have not yet been defined within the San Diego region.

**Microscopy** - Evaluation under a microscope. Cross polarization techniques are used for purposes of identifying specific traits evident in dreissena mussels. This method can be used prior to initial confirmation of mussel presence; however, it is most useful when evaluating progress of a known infestation.

**Pediveliger** – The first post-veliger larval stage of the mussel associated with development of a "foot" marking a change in behavior that allows the animal to both swim and crawl on surfaces. Dreissena pediveligers range in size from approximately 231 to 462 microns.

**Plankton Tow Method** – A method of sample gathering most often used in a reservoir or other large body of water. The process involves filtering volumes of water to obtain a concentrated sample which can then be evaluated using PCR or microscopy.

**Polymerase Chain Reaction (PCR)** – A technique in molecular biology by which a small fragment of DNA can be rapidly cloned, or duplicated, to produce multiple DNA copies. PCR is most useful prior to initial confirmation of mussel presence. Mussel identification is made by comparison against specific genetic markers.

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**Secchi disk** – The Secchi disk is used to measure water clarity. It is lowered into the lake until the observer loses sight of it. The disk is then raised until it reappears. The depth of the water where the disk vanishes and reappears is the Secchi disk reading. The depth level reading on the measuring tape at the surface level of the lake is recorded to the nearest foot. Instructions for making a secchi disk are at http://dipin.kent.edu/makedisk.htm.

**Thermocline** - A layer within a body of water or air where the temperature changes rapidly with depth. This layer is correlated with oxygen carrying capacity and water movement.

**Veliger** – An early larval stage mollusk that has developed the velum, a ciliated swimming organelle. Dreissena veligers range in size from approximately 97 microns to 347 microns.

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## References

This section to be added prior to publishing. It will indicate references used in the development of this plan.

100th Meridian Columbia River Basin Team has developed a final working draft of their interagency response plan. It shows containment, control and eradication options that have been referenced within this plan. The current draft can be viewed at http://100thmeridian.org/ActionTeams/Columbia/RapidResponse/CRB%20Dreissenid%20Rapid%20Response%20Plan%202-6-08.pdf

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## Websites

**100th Meridian Initiative** is a cooperative effort between state, local, and federal agencies to 1) prevent the spread of Zebra mussels and other aquatic nuisance species into the western United States and 2) monitor and control zebra mussels and other aquatic nuisance species. More information about the 100th Meridian Initiative is available on the http://100thmeridian.org/ website.

**Aquafornia** – Southern California water blog consolidates water related news articles and provides related commentary at http://aquafornia.com/archives/category/invasive-species/.

**California Department of Fish and Game** website www.dfg.ca.gov/invasives/quaggamussel contains information including the scientific advisory report, brochures and posters, and Quagga mussel response across the state.

**California Department of Food and Agriculture** website http://www.cdfa.ca.gov/invasives/# contains presentations and information regarding invasive plant and animal species.

**California Department of Water Resources** (DWR) website http://www.water.ca.gov/. Of special interest may be the link to DWR's Water Data Library http://www.wdl.water.ca.gov/

**Casitas MWD Vessel Survey:**
http://www.lakecasitas.info/documents/QuaggaDeclaration-121807.pdf

**Cornell University** website has a section within their environmental impact statement for lake source cooling which covers maintenance procedures such as pipeline pigging, manual cleaning, and use of coatings at http://www.utilities.cornell.edu/utl_lsceis_mussels.html.

**National Park Service** (NPS) has a quagga informational website at http://www.nature.nps.gov/water/Quagga/. In addition the NPS has published a Quagga/Zebra Mussel Infestation Preventions and Response Planning Guide:
http://www.nature.nps.gov/water/Quagga/QuaggaPlanningGuide_ext.pdf
http://www.nature.nps.gov/water/Quagga/QuaggaGuideAPPENDICES_ext.pdf

**Seagrant** Non Indigenous Species website contains research papers and publication links on zebra and quagga mussels. http://www.sgnis.org/index.htm

**U.S. Department of Agriculture** National Agricultural Library http://www.invasivespeciesinfo.gov/aquatics/quagga.shtml

**U.S. Fish and Wildlife Service** website http://www.fws.gov/invasives/ has references to standard operating procedures in use by other states and agencies. It also includes a link to a development wizard for creating a Hazard Assessment Critical Control Plan (HACCP) A particularly good reference is the decontamination protocol prepared by the State of Oklahoma http://haccp-nrm.org/documents/okwrb.pdf.

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Figure B-1: Lower Colorado River - Positive Sample Locations
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**Appendix D - Outreach guidelines/materials**
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**Appendix G – Contacts**
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**Appendix H – Boating and Recreational Activities**
Non-infested Water Bodies
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**Appendix I - Summary of Zebra Mussel Eradication and Control Options**

Thermal Shock
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Table I-1 Number of days to 100% mortality of adult zebra mussels aerially exposed to different levels of relative humidity and air temperature.

Benthic Mats
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Matrix of Eradication and Control Options by Waterbody Type

**Appendix J- Filtering Process and Types**

**Appendix K - Development Opportunities**

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# Appendix A – General Reference Information

## Dreissena mussel life stages

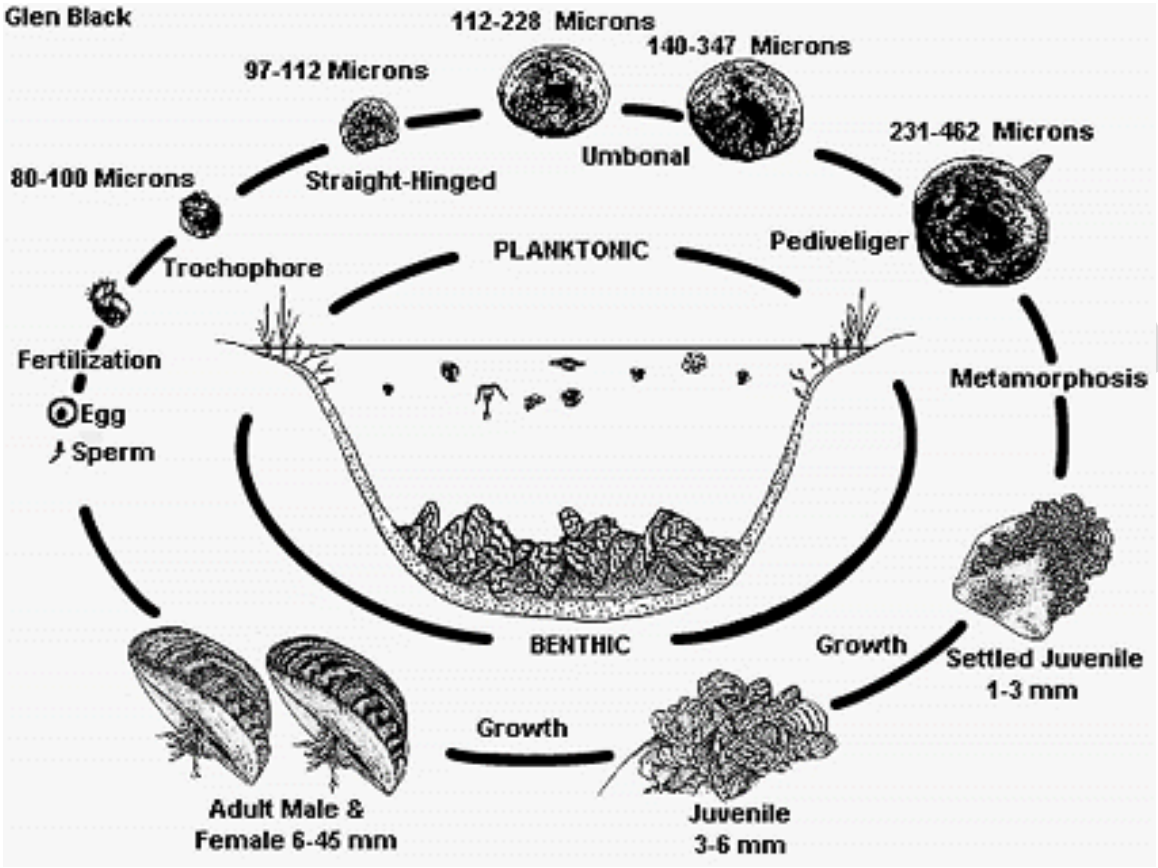


Figure A-1

E-file: http://el.erdc.usace.army.mil/zebra/zmis/zmishelp4/veliger_stages.htm

**Regulations**

## Assembly Bill No. 1683

CHAPTER 419

An act to amend Section 103 of, to amend the heading of Chapter 3.5 (commencing with Section 2300) of Division 3 of, and to add and repeal Section 2301 of, the Fish and Game Code, relating to fish and game, and declaring the urgency thereof, to take effect immediately.

[Approved by Governor October 10, 2007. Filed with Secretary of State October 10, 2007.]

LEGISLATIVE COUNSEL'S DIGEST

AB 1683, Wolk. Fish and game: dreissenid mussels.

(1) Existing law provides for the compensation of members of the Fish and Game Commission, including their actual and necessary expenses. Existing law requires the commission, on or before July 1, 2000, to submit certain recommendations to the Legislature relating to the commission.

This bill would delete that obsolete recommendation requirement.

(2) Existing law generally prohibits the sale, possession, importation, transportation, transfer, live release, or giving away without consideration, of the saltwater algae of the Caulerpa species, and subjects any person who violates that prohibition to a civil penalty of not less than $500 and not more than $10,000 for each violation.

This bill, except as otherwise authorized by the Department of Fish and Game, would prohibit a person from possessing, importing, shipping, or transporting in the state, or from placing, planting, or causing to be placed or planted in any water within the state, dreissenid mussels. The bill would authorize the Director of Fish and Game or his or her designee to conduct inspections of specified conveyances that may carry or contain adult or larval dreissenid mussels; order that areas in a conveyance that contain water be drained, dried, or decontaminated pursuant to procedures approved by the department; impound or quarantine conveyances for up to 5 days or the period of time necessary to ensure that the dreissenid mussels can no longer live on or in the conveyance; and conduct inspections of waters of the state and facilities located within waters of the state, that may contain dreissenid mussels and, if those mussels are detected or may be present, order the closure of the waters or facilities to conveyances or otherwise restrict access to the waters or facilities, with the concurrence of the Secretary of the Resources Agency. The bill would require the director or his or her designee to take specified action with respect to conveyances removed from, or introduced to, those waters and facilities. The director could authorize other state agencies to exercise this authority upon a prescribed written determination by the director. The bill would exempt a public or private agency that operates a water supply system from those enforcement activities,

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if the operator of the facilities has prepared and implemented a prescribed plan to control or eradicate dreissenid mussels. The bill would require that the discovery of dressenid mussels be immediately reported to the department. A person who violates or resists, delays, obstructs, or interferes with the implementation of these provisions would be subject to a penalty, in an amount not to exceed $1,000, that is imposed administratively by the department. The department would be authorized to adopt regulations to carry out these provisions. Because, under existing law, a violation of these provisions would be a crime, the bill would impose a state-mandated local program. These provisions would be repealed on January 1, 2012.

(3) The California Constitution requires the state to reimburse local agencies and school districts for certain costs mandated by the state. Statutory provisions establish procedures for making that reimbursement.

This bill would provide that no reimbursement is required by this act for a specified reason.

(4) This bill would declare that it is to take effect immediately as an urgency statute.

*The people of the State of California do enact as follows:*

SECTION 1. The Legislature finds and declares all of the following:

(a) Mussels of the genus Dreissena (dreissenid mussels) are a harmful, highly invasive species, and not native to California.

(b) Dreissenid mussels, which include both zebra and quagga mussels, were first discovered in the United States in the Great Lakes in or around 1988. This infestation has caused billions of dollars in costs to public agencies and private industry. Dreissenid mussels have been detected in Lake Mead on the Arizona-Nevada border and in Lake Havasu in California.

(c) Dreissenid mussels can readily move from place to place as free-swimming larvae and adults, and reproduce rapidly and in large numbers. Dreissenid mussels can severely hinder the delivery of water due to the mussels' capacity to clog or foul pipes, pumps, and other water intake structures, water delivery systems, powerplant intakes, cooling systems, and fish screens. Dreissenid mussels damage the hulls, propellers, and motors of boats and other watercraft.

(d) Dreissenid mussels alter the natural food web of aquatic ecosystems. By filtering planktonic algae and other nutrients that are the primary base of the food chain from the water, dreissenid mussels can cause the decline or extirpation of native species, and otherwise disrupt the ecological balance of infested water bodies.

(e) Dreissenid mussels' sharp-edged shells along swimming beaches can be a hazard to unprotected feet.

(f) In order to protect and preserve the health and safety of the people of the State of California, its economy, and its fish and wildlife resources, it is the intent of the Legislature to establish an effective program to prevent additional dreissenid mussels from entering the state, to prevent dreissenid

mussels from being introduced into any water in California where they currently do not exist, and to detect and destroy dreissenid mussels anywhere in the state. For this purpose, there has been developed a Quagga Mussel Incident Strategic Plan.

SEC. 2. Section 103 of the Fish and Game Code is amended to read:

103. (a) Each of the commissioners shall receive one hundred dollars ($100) for each day of actual service performed in carrying out his or her official duties pursuant to law, but the amount of this compensation shall not exceed for any one commissioner the sum of five hundred dollars ($500) for any one calendar month. In addition to this compensation, the commissioners shall receive their actual and necessary expenses incurred in the performance of their duties.

(b) The compensation and expenses provided in this section shall be paid out of the Fish and Game Preservation Fund.

SEC. 3. The heading of Chapter 3.5 (commencing with Section 2300) of Division 3 of the Fish and Game Code is amended to read:

CHAPTER 3.5. AQUATIC INVASIVE SPECIES

SEC. 4. Section 2301 is added to the Fish and Game Code, to read:

2301. (a) (1) Except as authorized by the department, a person shall not possess, import, ship, or transport in the state, or place, plant, or cause to be placed or planted in any water within the state, dreissenid mussels.

(2) The director or his or her designee may do all of the following:

(A) Conduct inspections of conveyances, which include vehicles, boats and other watercraft, containers, and trailers, that may carry or contain adult or larval dreissenid mussels. Included as part of this authority to conduct inspections is the authority to temporarily stop conveyances that may carry or contain adult or larval dreissenid mussels on any roadway or waterway in order to conduct inspections.

(B) Order that areas in a conveyance that contain water be drained, dried, or decontaminated pursuant to procedures approved by the department.

(C) Impound or quarantine conveyances in locations designated by the department for up to five days or the period of time necessary to ensure that dreissenid mussels can no longer live on or in the conveyance.

(D) (i) Conduct inspections of waters of the state and facilities located within waters of the state that may contain dreissenid mussels. If dreissenid mussels are detected or may be present, the director or his or her designee may order the affected waters or facilities closed to conveyances or otherwise restrict access to the affected waters or facilities, and shall order that conveyances removed from, or introduced to, the affected waters or facilities be inspected, quarantined, or disinfected in a manner and for a duration necessary to detect and prevent the spread of dreissenid mussels within the state.

(ii) For the purpose of implementing clause (i), the director or his or her designee shall order the closure or quarantine of, or restrict access to, these

within the system. The approved plan shall contain the following minimum elements:

(A) Methods for delineation of infestation, including both adult mussels and veligers.

(B) Methods for control or eradication of adult mussels and decontamination of water containing larval mussels.

(C) A systematic monitoring program to determine any changes in conditions.

(D) The requirement that the operator of the water supply system cooperate with the department to update or revise control or eradication measures in the approved plan to address scientific advances in the methods of controlling or eradicating mussels and veligers.

(2) Paragraph (2) of subdivision (a) does not apply to the operation of water delivery and storage facilities for the purposes of providing water supply if the operator of the facilities has prepared and implemented a plan to control or eradicate dreissenid mussels in accordance with paragraph (1). The department may require the operator of a facility to update its plan, and if the plan is not updated or revised as described in subparagraph (D) of paragraph (1), the department may engage in the actions described in paragraph (2) of subdivision (a).

(e) Any entity that discovers dreissenid mussels within this state shall immediately report the discovery to the department.

(f) (1) In addition to any other penalty provided by law, any person who violates this section, any verbal or written order or regulation adopted pursuant to this section, or who resists, delays, obstructs, or interferes with the implementation of this section, is subject to a penalty, in an amount not to exceed one thousand dollars ($1,000), that is imposed administratively by the department.

(2) A penalty shall not be imposed pursuant to paragraph (1) unless the department has adopted regulations specifying the amount of the penalty and the procedure for imposing and appealing the penalty.

(g) The department may adopt regulations to carry out this section.

(h) This section shall remain in effect only until January 1, 2012, and as of that date is repealed, unless a later enacted statute, that is enacted before January 1, 2012, deletes or extends that date.

SEC. 5. No reimbursement is required by this act pursuant to Section 6 of Article XIII B of the California Constitution because the only costs that may be incurred by a local agency or school district will be incurred because this act creates a new crime or infraction, eliminates a crime or infraction, or changes the penalty for a crime or infraction, within the meaning of Section 17556 of the Government Code, or changes the definition of a crime within the meaning of Section 6 of Article XIII B of the California Constitution.

SEC. 6. This act is an urgency statute necessary for the immediate preservation of the public peace, health, or safety within the meaning of Article IV of the Constitution and shall go into immediate effect. The facts constituting the necessity are:

In order to minimize the adverse impacts caused by dreissenid mussels, including impacts on water service deliveries, watercraft, recreational swimmers, and aquatic ecosystems, as soon as possible, it is necessary that this act take effect immediately.

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CALIFORNIA LEGISLATURE—2007–08 REGULAR SESSION

# ASSEMBLY BILL No. 2065

**Introduced by Assembly Member Hancock**

February 19, 2008

An act to add Section 2302 to the Fish and Game Code, relating to invasive aquatic species.

LEGISLATIVE COUNSEL'S DIGEST

AB 2065, as introduced, Hancock. Invasive aquatic species.

(1) Existing law, until January 1, 2012, generally prohibits a person from possessing, importing, shipping, or transporting in the state, or from placing, planting, or causing to be placed or planted in any water within the state, dreissenid mussels, and authorizes the Director of Fish and Game or his or her designee to engage in various enforcement activities. Existing law exempts a public or private agency that operates a water supply system from those enforcement activities, if the operator of the facilities has prepared and implemented a prescribed plan to control or eradicate dreissenid mussels.

This bill would require a water district, as defined, that owns or manages a waterway to develop and implement a monitoring and control program to prevent the introduction of nonnative mussel species, thereby imposing a state-mandated local program.

(2) The California Constitution requires the state to reimburse local agencies and school districts for certain costs mandated by the state. Statutory provisions establish procedures for making that reimbursement.

This bill would provide that, if the Commission on State Mandates determines that the bill contains costs mandated by the state, reimbursement for those costs shall be made pursuant to these statutory provisions.

99

Vote: majority. Appropriation: no. Fiscal committee: yes. State-mandated local program: yes.

*The people of the State of California do enact as follows:*

1 SECTION 1. Section 2302 is added to the Fish and Game Code,
2 to read:
3 2302. A water district that owns or manages a waterway shall
4 develop and implement a monitoring and control program to
5 prevent the introduction of nonnative mussel species. As used in
6 this section, "water district" has the same meaning as defined in
7 Section 20200 of the Water Code.
8 SEC. 2. If the Commission on State Mandates determines that
9 this act contains costs mandated by the state, reimbursement to
10 local agencies and school districts for those costs shall be made
11 pursuant to Part 7 (commencing with Section 17500) of Division
12 4 of Title 2 of the Government Code.

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## General Response Checklist

*Note: This list is not ordered for sequential completion*

- ❑ Gather information on sighting from original reporter; verify it's not a hoax.
- ❑ Obtain verification of physical sample by recognized expert(s).
- ❑ Once report is confirmed by recognized expert(s), notify primary response contacts.
- ❑ Verify all critical notifications were completed.
- ❑ Once secondary confirmation occurs, notify other interested parties.
- ❑ Determine geographic extent of infestation.
- ❑ Survey nearby water bodies with vulnerability to the same vectors.
- ❑ Evaluate/collect data on density, age, size demography of infestation.
- ❑ Identify potentially affected facilities.
- ❑ Convene initial meeting of all relevant response organizations.
- ❑ Select a lead coordinator for coordinating management activities.
- ❑ Establish a communications system (media briefings, etc.).
- ❑ Develop estimates for staffing needs, facilities and equipment, and funding.
- ❑ Identify potential sources for staffing, facilities, equipment, and funds.
- ❑ Secure commitments for needed staff, facilities and equipment, and funds.
- ❑ Evaluate vectors for subsequent dispersal of initial infestation.
- ❑ Based on risk evaluation, restrict dispersal pathways where feasible.
- ❑ Decide if eradication is possible based on analysis of population dynamics and pathways of spread.
- ❑ If eradication is attempted, select appropriate method(s).
- ❑ If eradication is not possible, develop control objectives and select/design appropriate control measures.
- ❑ Obtain relevant permits and regulatory agency concurrence.
- ❑ Design and implement public and media outreach associated with eradication/control plans.
- ❑ Implement eradication or control strategies.
- ❑ Design a monitoring program to evaluate:
  - Quagga mussel populations
  - Associated ecological and economic impacts
  - Effectiveness of management interventions
  - Negative consequences of management interventions (including compliance monitoring for permit obligations).
- ❑ Carry out monitoring activities in coordination with other field operations.
- ❑ Disseminate findings on easily accessible coordinated database.
- ❑ Revise existing plans/guidelines to make improvements.
- ❑ Determine the need, amount, and sources for long-term funding.

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# Appendix B – Lower Colorado River Sampling Status

## Table B-1: Quagga Mussel Timeline

| Date | Location | Comments |
|---|---|---|
| 6 Jan 07 | Lake Mead, NV | Announced by California Department of Fish and Game (CDFG) |
| 17 Jan 07 | Lake Havasu | Divers first discover mussels in Metropolitan Water District's system |
| Jan 07 | Diamond Valley Lake & Lake Skinner | Dive surveys conducted with no mussels found |
| 26 Jul07 | Lake Mathews Inlet | Presence verified throughout the 242-mile Colorado Aqueduct system, including portions of the 13-mile San Jacinto Tunnel, and at the inlet channel at Lake Mathews |
| Feb 07 | | MWD initiated Phase I of the Quagga Mussel Control Program, incorporating enhanced detection, surveillance, and mitigation strategies |
| 2 Aug 07 | Lake Skinner | |
| 13 Aug 07 | San Vicente Reservoir | Veligers identified using microscopy |
| 21 Aug 07 | Lake Dixon | Adults found at boat dock by City of Escondido |
| 13 Sep 07 | Lake Murray | Veligers identified using microscopy |
| 20 Sep 07 | Lower Otay | Veligers and adult population identified |
| Dec 07 | Lake Miramar | Adults identified by dive teams |
| 29 Jan 08 | SD-1 Weir, SD-6 Weir, and TOFRS | Veligers identified using PCR |
| 29 Jan 08 | Olivenhain Pipeline | Adults found during inspection of inactive portion of untreated pipeline |
| 30 Jan 08 | El Capitan Reservoir | Veligers identified at inlet to Reservoir |
| 21 Feb 08 | SD-1 Weir | No adults found during internal structure inspection |
| 22 Feb 08 | Sweetwater Reservoir | Negative for veligers during regular sampling (however, veligers have been identified in WTP intake water) |
| 11 Mar 08 | Olivenhain Reservoir | Single juvenile mussel found on coupon at I/O tower. Additional mussels located in subsequent checks. |
| 18 Mar 08 | Helix WD Pipeline | Quantity of adult mussels located in pipeline between Lake Jennings and Levy WTP |
| 24 Mar 08 | Lake Miramar | Divers found 2 adult mussels on outlet tower |
| 24 Mar 08 | San Vicente Reservoir | Single adult mussel (7 mm) located on rope near treated water inflow at 22 feet deep |

Updated April 8, 2008

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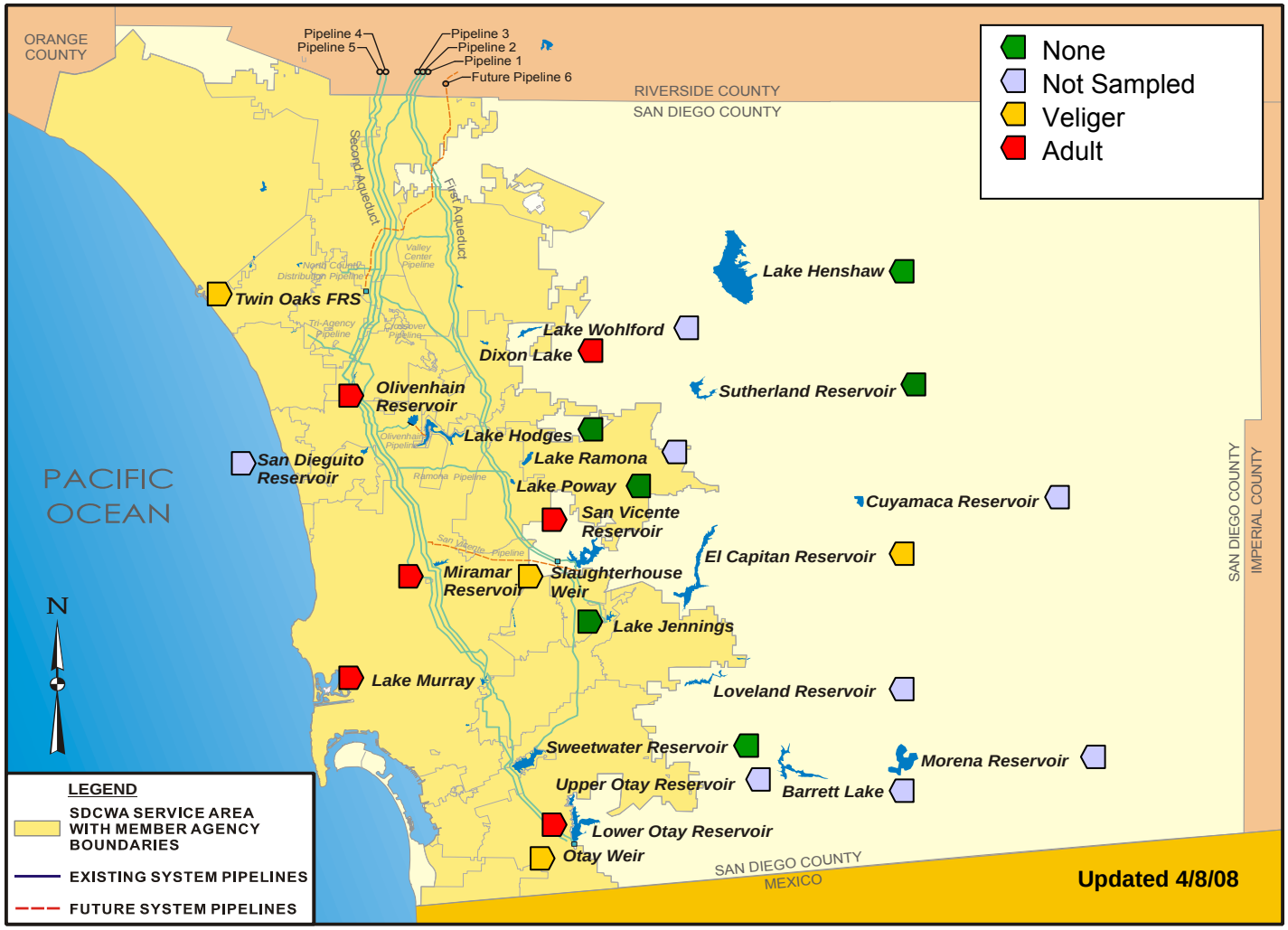
**Figure B-1: Lower Colorado River - Positive Sample Locations**

## Figure B-2: San Diego County Reservoir Sampling Status



# Appendix C - System Vulnerability Analysis

## Environmental factors affecting mussel growth and survival

Growth rates of dreissenids are influenced by several environmental factors including water chemistry, food concentration, water current and sediment characteristics. Under optimal conditions dreissenids can grow .21mm/day (Ackerman 1994), attaining sexual maturity in approximately 40 days under optimal conditions. Calcium, alkalinity, total hardness, pH, dissolved oxygen and temperature are all important factors in the survival and growth of dreissenids.

*D. polymorpha* juveniles show initial growth at calcium concentrations between 8.5 and 11 mg $Ca^{2+}$/ L, alkalinity levels above 17.1 mg $CaCO_3$/L and total hardness levels greater than 31 mg $CaCO_3$/L (Hincks and Mackie 1997) with maximum growth rates occurring at 32 mg $Ca^{2+}$/ L calcium, 65 mg $CaCO_3$/L alkalinity and 100 mg $CaCO_3$/L total hardness. In general, *D. polymorpha* adults inhabit waters with calcium concentrations greater than or equal to 15 mg $Ca^{2+}$/ L and populations become dense at concentrations greater than or equal to 25 mg $Ca^{2+}$/ L (Hincks and Mackie 1997). The pH concentration can be limiting to dreissenids above 10 and below 7.4. pH values below 7.4 are lethal to veligers with a pH value of 8.0 needed for infestation (Hincks and Mackie 1997).

Dreissenids are highly intolerant of oxygen deprivation but *D. bugensis* is more hypoxia tolerant than *D. polymorpha* (McMahon 1996).

Dreissenids inhabit a wide range of temperatures in North America. Both dreissenids are found in the Great Lakes at temperatures less than 5°C. Populations of *D. polymorpha* in the lower Mississippi are thriving where temperatures reach and exceed 30°C and fall below 15°C for a few winter months (McMahon 1996). Thorp et al. (2002) found that *D. bugensis* survived high temperatures better than *D. polymorpha* and both survived in outdoor tanks where temperatures occasionally exceeded 30°C with a maximum of 32°C.

Table C-1. Summary of Environmental Factors needed for veliger survival and adult growth.

| | Minimum Levels for Veliger Survival | Optimal Levels for Infestations to Occur |
|---|---|---|
| **Calcium mg/L** | 20 | > 25 |
| **Alkalinity mg/L** | 40 | ~65 |
| **Total Hardness mg/L** | 64 | ~100 |
| **pH** | > 7.4 | 8-8.5 |
| **Temperature °C** | 0 | 17-23 |

References:

Ackerman, J.D., B. Sim, S.J. Nichols and R. Claudi, 1994. A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. Can. J. Zool. 72:1169-1179.

Hincks, S.S. and G.L. Mackie, 1997. Effects of pH, calcium, alkalinity, hardness, and chlorophyll on the survival, growth, and reproductive success of zebra mussel (*Dreissena polymorpha*) in Ontario lakes. Can. J. Fish. Aquat. Sci. 54:2049-2057.

Mackie, G.L. and , 1996. Comparative Biology of Zebra Mussels in Europe and North America: An Overview. Amer. Zool. 36:244-258.

McMahon, R.F., 1996. The Physiological Ecology of the Zebra Mussel, *Dreissena polymorpha*, in North America and Europe. Amer. Zool. 36:339-363.

Thorp, J.H., J.E. Alexander and G.A. Cobbs, 2002. Coping with warmer, large rivers: a field experiment on potential range expansion of northern quagga mussels (*Dreissena bugensis*). Freshwater Biology. 47:1779-1790

## System Vulnerability Checklist

A system vulnerability checklist is currently under development and planned for addition prior to publishing. An alternative to the traditional vulnerability assessment is to complete a Hazard Assessment and Critical Control Points (HACCP) for the affected system. Samples and a HACCP wizard are available at http://www.haccp-nrm.org/.

A research proposal has been submitted to AwwaRF (April 2008) for consideration of a standardized vulnerability analysis relative to dreissenid mussels in western waters/climates.

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# Appendix D - Outreach Guidelines/Materials

## General public

**California Dept of Fish & Game**

"What boaters can do to stem the spread of invasive quagga/zebra mussels"
http://www.dfg.ca.gov/invasives/quaggamussel/docs/quagga_boaters.pdf

"Quagga/Zebra Mussel Fact Sheet"
http://www.dfg.ca.gov/invasives/quaggamussel/docs/quagga_factsheet.pdf

"Frequently asked questions Quagga/Zebra mussels"
http://www.dfg.ca.gov/invasives/quaggamussel/docs/quagga_FAQ.pdf

## Staff training materials

Links to potential training materials are available in the Website section. Specific training materials will be listed after further review and evaluation of applicability.

WA procedure for sampling and field identification guide to be inserted here prior to publishing.

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# Appendix E - Zebra Mussel and Quagga Mussel Identification

Quagga mussels are frequently confused with the Zebra mussel (Dreissena polymorpha). Zebra mussels are also an invasive species; however the Zebra mussel has not been identified as invading California water at this date. The comparison between species is shown in Table 2.

**Table E-1**
**Comparison between Zebra Mussel and Quagga Mussel**

*Zebra mussel*

*Quagga mussel*

| | ZEBRA MUSSEL | QUAGGA MUSSEL |
|---|---|---|
| **Shell** | Triangular shape, byssal (ventral) side flat. Obvious ridge between side and bottom | Rounder sides, byssal side rounded. ridge lacking |
| **Color** | Variable colors and patterns, usually dark | Pale near hinge, dark concentric rings on the shell |
| **Byssal** | Large groove in middle of flat side; allows tight hold on rocks | Small byssal groove near the hinge |
| **Depth in Lake** | 3 to 98 feet (1-30 m), rarely found below 50 feet (15 m) | 3 to 351 feet (1-107 m), commonly found down to 98 feet (30 m) |
| **Temperature** | 32° to 86°F (0° to 30°C) | 32° to 86°F (0° to 30°C) |
| **Tolerance** | 54° to 68°F (12° to 20°C) preferred | 39° to 68°F (4° to 20°C) preferred |
| **Reproductivity Temperature** | Young present at 57 ° to 68°F (14° to 20°C) | Young present as low as 46°F (8°C) |

The Quagga mussel topples over and will not sit flat on a vertical surface, is asymmetrical with no straight midventral line, lacks ridges, is rounder in shape, usually has dark concentric rings on shell, and is paler in color near the hinge. The Zebra mussel sits flat on ventral side, is triangular in shape, has obvious ridges, is bilaterally symmetrical, joining together in a midventral line, and the color patterns vary.

Quagga mussels tend to colonize over top of the Zebra mussel, smothering them, and eventually reduce or eliminate the Zebra mussel colony.

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**Credits:**

- USGS Species Fact Sheet
- Seagrant Pennsylvania and PennState Erie, The Behrend College, Aquatic Invasive Species of Pennsylvania
- United States Department of Agriculture, National Agricultural Library, National Invasive Information Center

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# Appendix F – Sampling/Testing Methods

## General Sampling Information

Sample collection and monitoring should emphasize areas where introductions are most likely to occur. These include raw water inflows, marinas, boat ramps and other access points, especially areas with high boat traffic.

## Substrate Evaluation

A variety of substrate assemblies exist for monitoring dreissena mussel presence and population growth. Within the San Diego region, a standard method is recommended using black ABS pipe for construction. Black ABS pipe is understood to be particularly attractive to quagga mussels.

Once veliger larvae or settled mussels are found, a plate substrate method may be substituted in order to gather population dynamics data for the mussel (density measurements).

**Materials and construction**

Presence model

6 each - 1½ inch diameter x 6 inch black ABS pipe
5 each – ¾ inch diameter x 6 inch PVC
?? feet ?/? inch diameter poly rope
Cinder block or concrete weight
Location buoy (optional)

1. Drill 15 each ½ inch holes in each larger black ABS pipe with one set of holes straight through the middle.
2. Assemble by alternating placement of large and small diameter pipes. Large pipes are placed horizontally and small pipes placed vertically.
3. Rope placement should be such that the top piece of pipe is submersed at XX feet. Knot to secure at ends.
4. Attach devise to a launch ramp. In deeper water, anchor with a cinder block or concrete weight and attach a location buoy at the water surface.

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Population dynamics model

4 each 1/16 inch thick, 6 x 6 inch PVC plates
?? feet ?/? inch diameter poly rope
Cinder block or concrete weight
Location buoy (optional)

1. Drill ?? each ½ inch hole(s) in each plate.
2. Assemble as shown in diagram. (To be added.)
3. Rope and plate placement should be such that the upper plate is below the average Secchi disk reading for the water body and the bottom plate is above the top of the thermocline.
4. Attach devise to a launch ramp. In deeper water, anchor with a cinder block or concrete weight and attach a location buoy at the water surface.

**Sample Inspection**

Allow substrate layers to partially dry and then rub fingers over surfaces to feel for the sandpaper-like presence of quagga mussel shells.

Presence model – Inspect pipes monthly for presence.

Population dynamics model - Inspect plates at least once every two months, recording volume and sizes of settled mussels.

**Considerations for Placement**

Juvenile settlement is affected by substrate type, substrate texture, depth, light, water currents, proximity to other mussels or adjacent surfaces, and ionic concentrations. Juveniles prefer dark areas, such as the undersides of suspended substrates, with currents that will transport food organisms.

**Reporting Procedures**

Follow reporting procedures indicated in Appendix G.

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# Plankton Tow Technique

### Scope and Application

This procedure is a modified version of that obtained from The Center for Lakes and Reservoirs at Portland State University as supplied by Dan Daft, Biologist, City of San Diego.

### Summary of Method

The plankton net is lowered 20 feet through the water column and allowed to rest at this depth 60 seconds before being raised slowly back to the surface. The sample is then condensed and stored in a labeled container. Employees should be trained by experienced personnel before attempting this sampling.

### Apparatus and Equipment

- Plankton net (simple, conical plankton-tow net, 63 µm pore size, 0.25 m diameter net opening, removable, weighted cod-end piece)
- Line for deploying the net (8 m or about 25 feet long)
- Sample container (polyethylene material, 50 to 500 mL volume, screw lid)
- Field sheets and pen/ pencils
- Tweezers or small spatula (recommended)
- Measuring tape or ruler (optional)
- Permanent marker (optional)

### Optional Assembly and Parts List (US Bureau of Reclamation)

*Information from Carol Welsh, Aquatic Biologist*
*Wildlife Supply Company*
*Updated August 2007*

The Bureau of Reclamation uses the following custom net configuration, consisting of 5 parts:

Larger net:
7-E50 Ring & Bridle, 20 inch (500 mm) $62.50
30-E28-1:4 Plankton Net, 20 inch (500 mm), 63 micron mesh, 1:4 ratio (~80 inches) $284.00
48-D80 Adaptor, 3-1/2 inch $79.00
47-F28 Dolphin Bucket, 1000 ml $134.00
47-F99 Guard Rails for Dolphin Bucket $36.00 (These are optional)

Shorter net:
30-E28 Plankton Net, 20 inch (500 mm), 63 micron mesh, 1:3 ratio (~60 inches) $185.00
Same ring, bridle, adaptor, bucket, guardrails and flowmeter

Smaller plankton tow net for using on a pipe or when pumping water:
426-A28 Turtox Plankton Tow Net, 8 inch ring (200 mm), 30 inch length $109.00
It comes with 125 ml plastic bottle, hose and pinch clamp. Extra bottles are available.

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Flowmeter:
39-B10 Mechanical Flow Meter $389.95

**Sample Preservation**

If the sample cannot be analyzed within one week, it must be preserved. Preserve samples in a 70% ETOH solution immediately after collection to ensure sample integrity. To make a 70% solution in the sample container note the volume of sample in the container and then add 3 times the volume of 95% ETOH to the sample. For example, if your sample bottle contained 1 inch of sample, you would add 3 inches of 95% ETOH so that the sample bottle contained 4 inches of combined sample and preservative. Do not fill the sample bottle more than ¼ full of sample to avoid spillage when adding the preservative. A measuring tape or ruler may be placed alongside the sample container to estimate the volumes.

**Decontamination**

Field equipment must be decontaminated to prevent transfer of organisms within and between systems. The plankton net, cod-end piece and rope assembly are cleaned by first washing with freshwater and then decontaminating them with 5% acetic acid solution (white vinegar). The clean sampling equipment should be rinsed and allowed to dry before reuse.

**Sample Procedure**

Collect a minimum of four plankton tows at each site and combine into one sample container. More than four plankton tows may be collected to increase the likelihood of collecting veligers. Collect each plankton tow in a different area of the site to further increase the likelihood of collecting veligers. Figure F-1 depicts plankton tows conducted at a site.

**Vertical Plankton Tow**

1. Prepare the net by securing the cod-end piece to the end of the net. Securely attached the tow line to the top ring of the plankton net and secure the other end of the line to the boat.
2. Lower the net 6.1 m (20 ft) below water surface, or to 1 m above the sediment and/or thermocline. Record the depth the net is lowered.
3. Keep net at this depth for 60 seconds and then manually retrieve using a hand-over-hand technique at a rate of 0.5 m/s (1.5 ft/ s). Slow and steady retrieval is the key to collecting a good plankton tow. Retrieval to the surface should take approximately 10 seconds.
4. Rinse the net by raising it so that the cod end of the net is at the water surface. Rinse organisms into the cod end of the net by lowering the net back into the water, keeping the opening above the water surface. Repeat this procedure several times to ensure that all the organisms inside the net are in the cod end.
5. A squirt bottle, filled with either tap water or water from the lake or river, can be used to squirt down the sides of the net. Spray the outside of the net starting at the mouth to rinse organisms into the cod end.
6. Carefully remove the cod-end piece without spilling collected water and plankton. Condense the sample as much as possible by swirling the cod-end piece. You may need to use tweezers or a spatula to gently clear the mesh netting in the cod-end piece to allow the water to filter through.

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7. Lower the cod-end-piece (separated from the plankton net) into the water, keeping the opening above the water surface. Condense the sample again and pour into the sample container. Repeat this procedure until the cod-end piece appears clean.
8. It is important to record the number and length of tows so that the quantity of water sampled can be determined.
9. The quantity of water sampled is determined using the formula below, assuming a net filtering efficiency of 100% (i.e. no clogging). If clogging occurs, a pressure wave develops, and water will be forced to the surface prior to the net emerging from the water. If clogging occurs, first try reducing the depth of the tow. If it still occurs, estimate the net filtering efficiency and multiply the corresponding percent by the maximum volume of water filtered (e.g. 80% filtering efficiency means 0.80 x $V_m$).

   Maximum volume of filtered water, $V_m$ is

   $$V_m = \pi * r^2 * d$$

   Where:
   r = radius of the net opening (0.30 m)
   d = depth to which the net is lowered (6.1 m)

   *Example:*
   *If the reservoir group completed four (6.1 m) vertical tows at San Vicente Reservoir, what is the total volume of water filtered through the plankton net? (The radius of the plankton net used is 0.30 m)*

   $V_m = 3.14*(0.30)^2*6.1 = 1.7239\ m^3 \times 1000\ L/m^3 = 1724\ L * 4$ (# of tows) = 6896 L

**Horizontal Plankton Tow**

Horizontal plankton tows are taken near shore in depths that are too shallow to collect a vertical tow. A weight (1-2 kg or 2-4 lbs) is attached to the rope at the bridle in front of the net opening and possibly at the cod end piece to keep the net from surfacing while performing the tow. The net is thrown into the water and allowed to sink. Slowly pull the net back to you at a slow and steady rate as described above. Keep the net off the sediment to avoid both snagging and collecting debris. Note the distance that the net is towed through the water and record. Repeat techniques used for vertical plankton tows to concentrate organisms into the cod end of the net.

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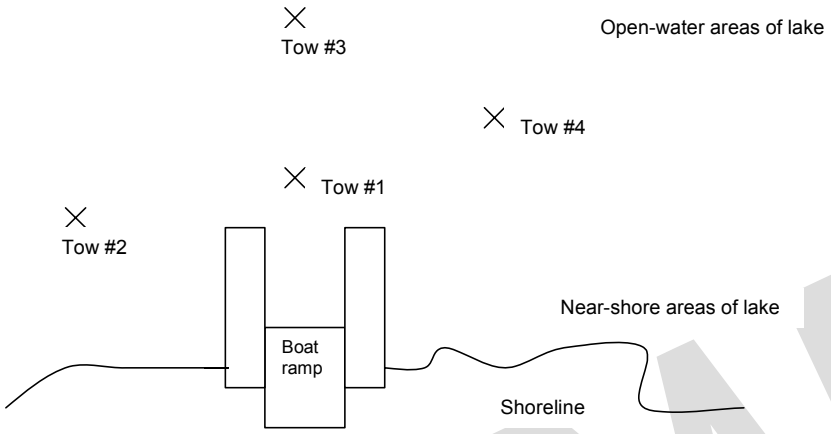


Figure F-1: An example of plankton collection at a site location.

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## PCR Methods

MWD has developed new primers for quagga mussel identification using PCR; however, they are not published for general use. Contact MWD for further information if your agency is developing in-house capabilities for PCR evaluation. Two agencies are known to provide mussel detection using PCR for a fee. These are US Bureau of Reclamation (see Websites) and UCSD/Scripps Institute of Oceanography. Contact the San Diego County Water Authority to obtain a copy of the current contract with UCSD. It contains a public agency clause which can be used by other public agencies within the State of California.

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## Microscopy methods

(Borrowed with permission from Steve Wells, Portland State University, draft Work Plan)

**Analytical Method.**

Veliger Analysis Equipment

- Compound microscope with ocular micrometer, polarizing kit and trinocular mount
- Digital camera, camera mount and software
- Sedgewick-Rafter cell
- Cover slips
- Disposable pipettes
- Vinegar
- Centrifuge and centrifuge vials
- 63 and 500 micron mesh
- Dissecting tools
- Computer

Microscope Setup

Plankton is analyzed under 40x to 100x total magnification using a standard compound microscope with polarized light. Initial scanning is performed under 40x total magnification and 100x total magnification is used to make shell measurements and inspect for specific markings (e.g. growth lines, eyespots). Plankton can be analyzed with dissecting/ stereo microscopes but the total magnification is generally limited to 40x. Cross-polarization is done with a polarization kit or with two pieces of polarizing film. Cross-polarization can be done partially, which allows more light waves into the field background and this increases the visibility of non-birefringent objects.

Concentration, Extraction, Analysis and Storage

Plankton samples are concentrated and filtered to reduce sample volume. Batch centrifugation is done at 1000 RPM for 20 minutes in centrifuge vials. The sample volume is reduced with 500 micron filters. The sample that passes through the 500 micron filter is centrifuged again and the sample volume is further reduced with 63 micron filter tubes. The filter tube is inserted into the centrifuge vial so that the tip remains above the concentrated sample at the bottom of the centrifuge vial. Supernatant is removed from the inside of the filter tube using a disposable pipette. The filter tube is then rinsed with clean water so that the rinse-water is returned to the centrifuge vial to retain organisms that became stuck to the filters.

The sample is analyzed in a Sedgewick-Rafter cell with successive aliquots that are taken from the bottom of the centrifuge vials using pipettes. A cover slip is placed on the empty Sedgewick-Rafter cell diagonally, leaving two opposing corners open. One mL of sample is added into one corner with a pipette and trapped air bubbles are forced out of the opposite corner. Once the Sedgewick-Rafter cell is full, the cover slip is slid into position so that the chamber is covered. Wipe the Sedgewick-Rafter cell with a cloth to remove dust, moisture and oil and place onto microscope stage.

The Sedgewick-Rafter cell is scanned by moving through the fields of each row and repeating for all the rows; there are approximately 5 rows within a Sedgewick-Rafter cell

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under 40x total magnification. The time required for analyzing one sample depends upon the sample volume, density of plankton, the quantity of birefringent organisms and other factors but 130 minutes is a rule-of-thumb. Veligers and unknown objects are measured and photographed. Measurements (shell length, shell height and hinge length) are recorded to the nearest micrometer (µm) with an ocular micrometer. Shell height is measured from the umbo or center of the hinge line to the opposing margin of the shell (Figure 1C) and length is measured perpendicular to this axis for clam-shaped larval stages (Figure 1B), which include all larval stages for Asian clams and the D-shaped, umbonal and pediveliger stages for dreissenids (Nichols and Black 1994; Johnson 1995). During metamorphosis, the axis of shell growth for dreissenids changes and so for the plantigrade and juvenile dreissenids, shell length is measured from the umbo to the opposing shell margin and height is measured perpendicular to this axis. Shell width is measured from the center of the face of one valve straight through the body to the center of the face on the opposing valve and this applies for all life stages of both dreissenids and corbiculids.

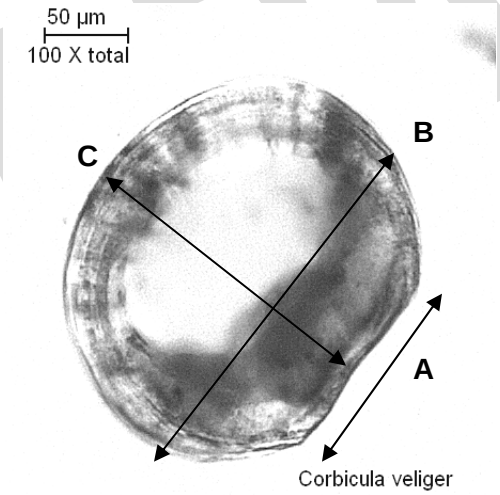


Figure 1: D-shaped veliger showing axes for measuring hinge length (A), shell length (B) and shell height (C) for clam-shaped larvae.

Samples are stored at the testing laboratory after analysis. Each aliquot is returned to the original sample container (e.g. 250 mL bottle). Aliquots containing veligers or something questionable, however, are stored in separate 50 mL bottles and held for cross-validation. Samples are stored in a locked room within the laboratory. Samples preserved in 70% or greater ETOH may be discarded into sewage treatment system. Samples preserved with formalin are hazardous waste materials and must be discarded appropriately. Living organisms cannot be discarded into the wastewater treatment system and must first be preserved in a minimum of 70% ETOH.

Laboratory Decontamination

Laboratory equipment is decontaminated between samples to prevent cross-contamination. The Sedgewick-Rafter cell, pipettes, slide covers, centrifuge vials and filter tubes are decontaminated using vinegar. Vinegar dissolves calcite in the shells of veligers and ostracods. Pipettes, filter tubes and centrifuge vials are soaked for a minimum of two hours in vinegar and rinsed in tap water. Sedgewick-Rafter cells and slide covers are immersed in vinegar, rinsed in fresh water and towel-dried. If veligers

are found in a sample, the clean Sedgewick-Rafter cell is scanned prior to adding a new sample.

Caveats of Cross-Polarization Microscopy

Veligers can be distinguished from inorganic birefringent objects in plankton samples with several features. Crystals in sand grains and other inorganic molecules are birefringent but do not exhibit a “maltese cross” and are generally multi-colored. Additionally, the shape and margin of these objects are irregular. Veligers have a distinct shape and margin and usually produce a “maltese cross” under full and partial polarization (Figure 2).

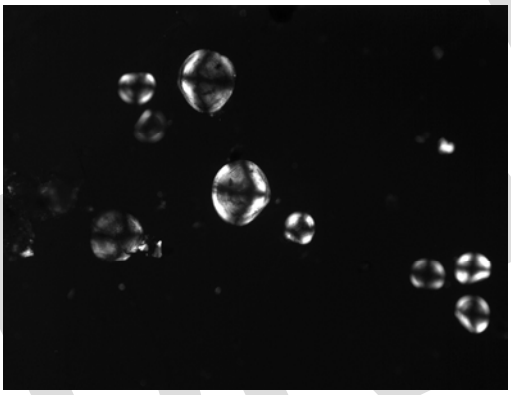

Figure 2: Veligers of D. polymorpha showing the “maltese cross” for various larval stages under full cross-polarization at total magnification 100x.

Veligers can be separated from other birefringent organisms using several features. Ostracods are common, small crustaceans that exhibit birefringence similar to bivalves and many produce a “maltese cross”. Ostracods are separated from bivalve veligers based on shell shape and size, appendages, eyespots and shell ornamentation (Johnson 1995). Bivalve larvae are either round or oval and the hinge is straight or with the umbo (Nichols and Black 1994). Ostracods are oval or “bean-shaped” and the hinge is curved (Figure 3) (Johnson 1995). The shape of the “maltese cross” produced by ostracods appears stretched along one axis while the “maltese cross” produced by bivalves is generally symmetrical with 90o angles (Figure 3). Ostracods are generally much larger than dreissenid larvae (e.g. ostracods: 300 – 1000 µm; dreissenids: 60 – 500 µm) (Johnson 1995). Ostracods have antennae, although these are small and generally difficult to see; bivalves have no antennae, although they sometimes have a velum (Johnson 1995). The velum is difficult to see in preserved bivalves. Bivalves never have an eyespot while these are often present in ostracods; appearing as a dark circle, usually near the shell margin (Johnson 1995). Veligers have a velar pigment close to the shell margin but the velar pigment, if visible, is generally crescent-shaped. The velar pigment may not be visible in preserved veligers. The shells of bivalves have no ornamentation while ostracods usually exhibit a pitted valve surface (Figure 3) (Johnson 1995).

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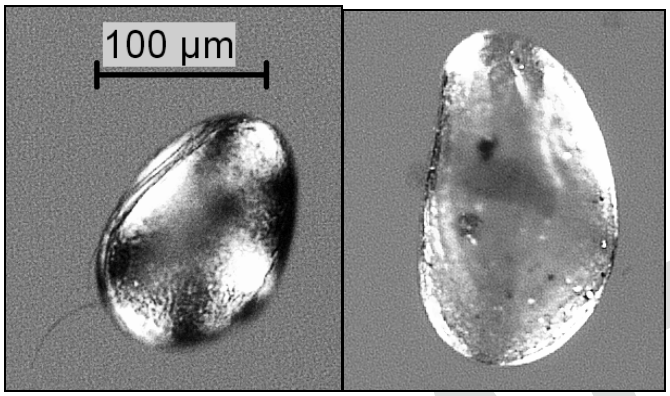


Figure 3: The stretched “maltese-cross” produced by an ostracod and the pitted valve surface is shown on the left and the oval shape is shown on the right.

C. fluminea is common in North America (Hornbach 1992) and it produces veligers that may be confused with dreissenid veligers. C. fluminea veligers are birefringent but they are different from dreissenid veligers. C. fluminea veligers are released as pediveligers and inhabit the benthos but they can be entrained within the seston and sampled with plankton nets. Dreissena and C. fluminea veligers are primarily differentiated by shell measurements and features (e.g. secondary growth lines). Dreissenid veligers are smaller compared to those of C. fluminea in all larval stages (Table 1). C. fluminea veligers have large, D-shaped valves when they are released (Figure 4) (Hu et al. 1994). Aldridge and McMahon (1978) reported that the mean shell length of a C. fluminea veliger was 200 µm (S.D. = 10, range = 175 to 225, n = 30) at release from adults caught in a Texas reservoir. Identification based only on size is difficult when comparing veligers of different ages and other features such as growth lines are used for identification as well as size. The C. fluminea, D-shaped veliger should have visible growth rings whereas it is unlikely dreissenids would have visible growth rings during the D-shaped larval stage (Nichols and Black 1994). In the D-shaped stage C. fluminea may have secondary lines that are perpendicular to the growth rings and D. polymorpha will generally not (Figure 4) (Nichols and Black 1994). C. fluminea umbonal veligers have prominent growth and secondary lines (Nichols and Black 1994).

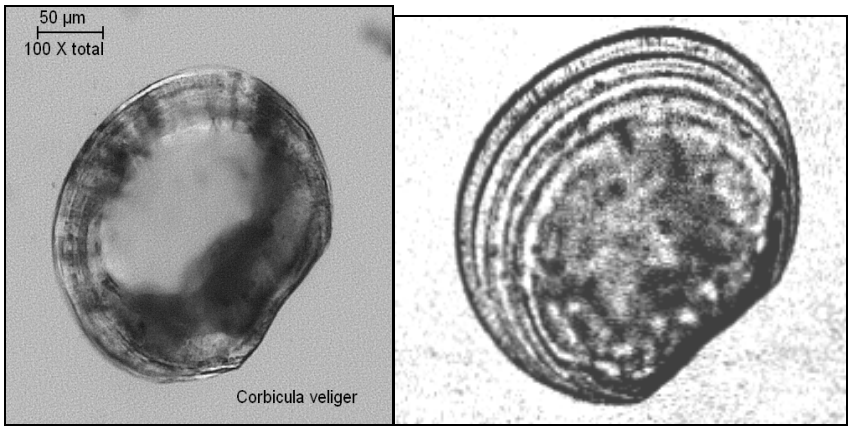


Figure 4: D-shaped C. fluminea veligers showing the prominent growth lines and secondary lines.

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Early detection of dreissenids is the primary objective of the laboratory but D. polymorpha and D. bugensis veligers can be differentiated. Nichols and Black (1994) provide a detailed identification key. The length/ height ratio of the shell and hinge length are used to separate D. bugensis and D. polymorpha in the D-shaped larval stage. D. polymorpha have a higher ratio (i.e. elongated shell length) and a longer hinge (Nichols and Black 1994). D. polymorpha's mean shell height is 80% of the shell length and the hinge length is 64 µm or greater (Nichols and Black 1994). D-shaped D. bugensis veligers are more round compared to D. polymorpha. The mean shell height is 90% of the shell length and the hinge length is less than 64 µm (Nichols and Black 1994). Freshwater dreissenid veligers early in the umbonal larval stage are separated based on the length/ height ratio of the shell, with D. polymorpha being more elongated compared to D. bugensis (Nichols and Black 1994). Freshwater dreissenid veligers late in the umbonal larval stage are separated based on valve margins and umbo heights. D. polymorpha have umbos of equal height and the valve margins do not overlap (Nichols and Black 1994). D. bugensis in the late umbonal stage have umbos and valve margins of unequal size (Nichols a and Black 1994). Freshwater dreissenids in the pediveliger and plantigrade larval stages are separated on the same features used in the late umbonal stage (i.e. valve margins and umbos).

It is difficult to separate freshwater dreissenid veligers in the umbonal larval stage. Features used to differentiate these dreissenids early in the umbonal larval stage become inconsistent late in the umbonal larval stage and the features used late in the umbonal stage are not present early in this stage (Nichols and Black 1994). As D. polymorpha umbonal veligers grow, they become more round in shape and D. bugensis are round throughout this stage. Additionally, the shell characteristics used to differentiate older D. bugenis umbonal veligers are less prominent than in the pediveliger and plantigrade larval stages.

Veliger identification is more difficult in marine and estuarine settings because veligers of many molluscan species are commonly found and there is greater diversity of planktonic organisms. Mytilopsis leucophaeata (dark false mussel) is a native dreissenid in brackish and estuarine waters of North America that can be confused with D. polymorpha and D. bugensis. M. leucophaeata adults occur occasionally in freshwater environments and freshwater dreissenids (D. polymorpha and D. bugensis) may occur in brackish waters (Conn et al. 1993). Conn et al. (1993) is the best guide to differentiate Dreissena and Mytilopsis veligers. Discriminating between Mytilopsis and Dreissena veligers in the D-shaped stage is difficult to impossible (Conn et al. 1993). Size at any given stage, is not a reliable characteristic for distinguishing Mytilopsis and Dreissena (Conn et al. 1993). The larval hinge structure of dreissenids is distinct from other species in that it has numerous, small denticles (Hu et al. 1994) but these features are visible using a scanning electron microscope. The general shapes differ between genera, with Dreissena D-shaped and umbonal veligers being more ovoid compared to the more rounded Mytilopsis. The shell valves of straight-hinge and umbonal stages of M. leucophaeata are more rounded than Dreissena. The anterior shoulder of M. leucophaeata differs only slightly from the posterior shoulder during the later umbonal stage, while the anterior shoulder of Dreissena is markedly longer and lower than the posterior shoulder (Conn et al. 1993). The overall shapes of the two genera differ markedly in the pediveliger, plantigrade and juvenile stages, with the umbo and anterior shoulder of Mytilopsis being far more pronounced than for Dreissena (Conn et al. 1993).

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Quality Control

Each laboratory that uses these protocols is required to operate a quality assurance program and this document does not address all safety issues associated with the laboratory use. The laboratory demonstrates its ability to identify and count dreissenid veligers with matrix spiked samples (MS) and laboratory control samples (LCS). Precision and accuracy studies are done for initial certification and annually thereafter and also after modifying methods and or changing equipment or personnel. If the change will affect the method detection limit (MDL), the laboratory is required to demonstrate that the MDL is lower than the MDL in this method. Changes that degrade method performance are not allowed. The laboratory is responsible for maintaining a safe work environment and OSHA regulations regarding the safe handling of the chemicals specified in this method are in Appendix B.

Spiked measurements

One blind matrix spiked sample (BMS) is made for every batch of 20 samples. BMS are made by adding less than five dreissenid veligers into a 50 mL dreissenid-free matrix of plankton, preservative and inorganic debris placed into centrifuge vials and assigned random tracking numbers within the batch of samples. BMS are continuously made from samples analyzed previously that are known to be devoid of dreissenids.

Laboratory control samples (LCS) improve the accuracy of analysis by providing reference samples for comparison. LCS are made for C. fluminea and D. polymorpha and D. bugensis veligers. C. fluminea veligers are collected from clams held in aquaria at the testing laboratory. D. polymorpha veligers in the planktonic larval stages are obtained from the El Dorado Reservoir, KS using a 63 micron plankton net. D. bugensis veligers in the planktonic larval stage are obtained from the lower Colorado River using a 63 micron plankton net. LCS are made by adding veligers to a solution of tap water and ETOH (1:3 ratio, respectively).

Instrument Maintenance and Calibration

Microscope and camera equipment requiring set-up and calibration is done by a certified Leica®, or equivalent, technician. The testing laboratory staff performs microscope and camera equipment maintenance in accordance with manuals. No other laboratory equipment requires calibration. Equipment used in the collection of ancillary field data is inspected for cleanliness and needed repairs and calibrated according to equipment manuals provided by suppliers prior to use. At the end of each field day, equipment is thoroughly washed with fresh water and set out to dry prior to storage. Small holes in plankton net are sealed with clear fingernail polish and larger holes are patched with 63 µm mesh material. Damaged equipment that cannot be satisfactorily repaired is replaced.

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**Data Assessment and Management**

Data Quality Assessment

The percent recovery of BMS is used to evaluate the accuracy of analysis. Percent recovery is affected by the particular sample matrix, laboratory protocols (e.g. sample concentration and sample volume reduction) and analyst error. Repeated analysis of matrix spiked samples is done for initial laboratory certification and annually thereafter to estimate the precision of methods with the relative standard deviation. Equations for calculating percent recovery and relative standard deviation (RSD) are provided in Section 3.1.1.

The acceptable percent recovery for BMS is 80%. If the percent recovery for the BMS is less than 80%, the sample concentration, volume reduction and analysis are repeated for the entire 20-sample batch including the BMS. This action is recorded in the laboratory log. If reanalysis is not possible (insufficient sample volume) this must be recorded with data results. The analyst is allowed to repeat procedures once for every batch of samples. If percent recovery is 80% or greater following repeated procedures, no further action nor documentation is required. If the percent recovery is still less than 80% following the repeated procedures, the data is presented with qualifiers. Data qualifiers must include the percent recoveries for both BMS and the modifications that were made to the procedure.
The method detection limit is limited by the volume of water sampled, the volume of the concentrated sample and the number of subsamples examined. The detection limit for a sample collected using these protocols (i.e. four tows from 6 m depth using net 0.25 m wide) would be

$$\frac{(1 \text{ veliger/ total subsample vol. [mL]})^* \text{ vol. concentrated sample (mL)}}{\text{vol. of water sampled (mL)}}$$

$$= \frac{(1 \text{ veliger/ 10 mL}) * 200 \text{ mL}}{4{,}800 \text{ L}} = 0.003 \text{ veligers/ L}$$

The detection limit is conservative because the entire filtered sample is examined. Twenty mL of sample are generally analyzed following centrifugation and filtering for a 250 mL sample.

Equations

Percent recovery (% recovery) is calculated from the following equation:
% recovery = $100 * (S – U)/C_{sa}$
where: S = measured concentration in spiked aliquot
U = measured concentration in unspiked aliquot
$C_{sa}$ = actual concentration of spike added

Relative percent difference (RPD) is calculated from the following equation:
$RPD = (C_1 - C_2)/ [(C_1 + C_2)/ 2] * 100$

where: $C_1$ = larger of the two observed values
$C_2$ = smaller of the two observed values

Relative standard deviation (RSD) is calculated from the following equation:

$$RSD = 100 * (s/ y)$$

where: s = standard deviation
y = mean of replicate analyses

Standard deviation is defined as follows:

$$s = \sqrt{[ \sum^{n}_{i=1} (yi - m)^2 / (n-1)]}$$

where: s = standard deviation
yi = measured value of the ith replicate
m = mean of replicate measurements
n = number of replicates

Tracking Data

Samples are assigned tracking numbers. Tracking numbers are assigned after samples have been transferred from the original container into centrifuge vials. The tracking number is the only label recorded on centrifuge vials. The tracking number is also recorded on the corresponding original sample container. Tracking numbers consist of two sets of numbers that are separated with an underscore (e.g. _ ). The first number is a six-digit number corresponding to date of collection (e.g. 09/26/06 = 092606) and is followed with the sample number. The sample number is a consecutive number assigned to each sample as they are processed (e.g. 45, 46, 47…) and the starting point for sample numbers is determined haphazardly.

All data generated in the laboratory is transferred into an electronic format and stored in the project database (Microsoft Access® version 2003) that is maintained at the testing laboratory. Database fields include tracking number, date of collection, date of analysis, water body name, site location name, GPS coordinates for site location (degrees with decimal, North American Datum of 1983 or NAD83), collector's name, collector's contact information, analyst's name, results of analysis, percent recovery of BMS and data qualifiers.

All updates, revisions, modifications, calibrations, equipment repairs and data are recorded on field and laboratory log sheets that are kept at the testing laboratory. Laboratory log sheets are maintained in a bound laboratory notebook that remains in the laboratory. Field log sheets are compiled and stored at the testing laboratory. A hard and an electronic copy of this document are stored in the laboratory at all times. A new hard copy is generated following any updates or revisions that cannot be manually added to the existing hard copy.

Data Validation

Positive results for the presence of dreissenid veligers, excluding BMS, are verified with inter-laboratory comparisons. Digital photographs and shell measurements are sent to two separate laboratories for verification. Sample splits are sent to these laboratories if requested. Laboratories that perform the verification are trained in dreissenid identification and operate a quality assurance program. The testing laboratory, its protocols and quality assurance program and copies of field and laboratory log sheets are available for inspection and review

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## Other Adult Mussel Inspection Methods

### Physical Inspection

Physical inspection that is not covered by other listed methods may include perimeter discovery such as

- Surface inspection of docks and buoys in a reservoir or lake
- Surface inspection of rocks and loose materials at water's edge

In addition, significant increases in water clarity may be an indicator of mussel presence or growth and should be further inspected if observed.

A sample surface survey form has been prepared by Department of Water Resources and can be found at http://www.des.water.ca.gov/docs/datasheet%20-%20surface%20survey.pdf.

### ROV

A remotely operated vehicle can be used for inspection when diving or other physical inspection is not available. An ROV can descend into a water body typically up to 300 feet deep and provides video documentation of underwater conditions. Units may be available which collect physical samples that can be brought to surface for further inspection. Vendors available for ROV inspection are listed in Appendix G under Resources.

### Diving

Diving is useful for both inspection and maintenance activities. Diving parameters are defined within the certification process and are not listed in this document. Training should be conducted for divers in identification of mussels and desired collection procedures. A sample diver survey form has been prepared by Department of Water Resources and can be found at http://www.des.water.ca.gov/docs/datasheet%20-%20diver%20survey.pdf.

USGS has published a standard operating procedure for "Decontamination of SCUBA diving equipment and underwater gear after diving in waters containing zebra mussels and other exotic species of Dreissenidae". The procedure may be useful in developing agency specific decontamination procedures for operations and maintenance staff. It can be viewed at http://www.glsc.usgs.gov/_files/ZMSOP.pdf.

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# Appendix G – Contacts

## Process for Reporting Results

The notification list is intended to guide agencies when reporting test results or sampling observations within the San Diego region. These contacts are responsible for any internal contacts within their organization that are not included in this list.

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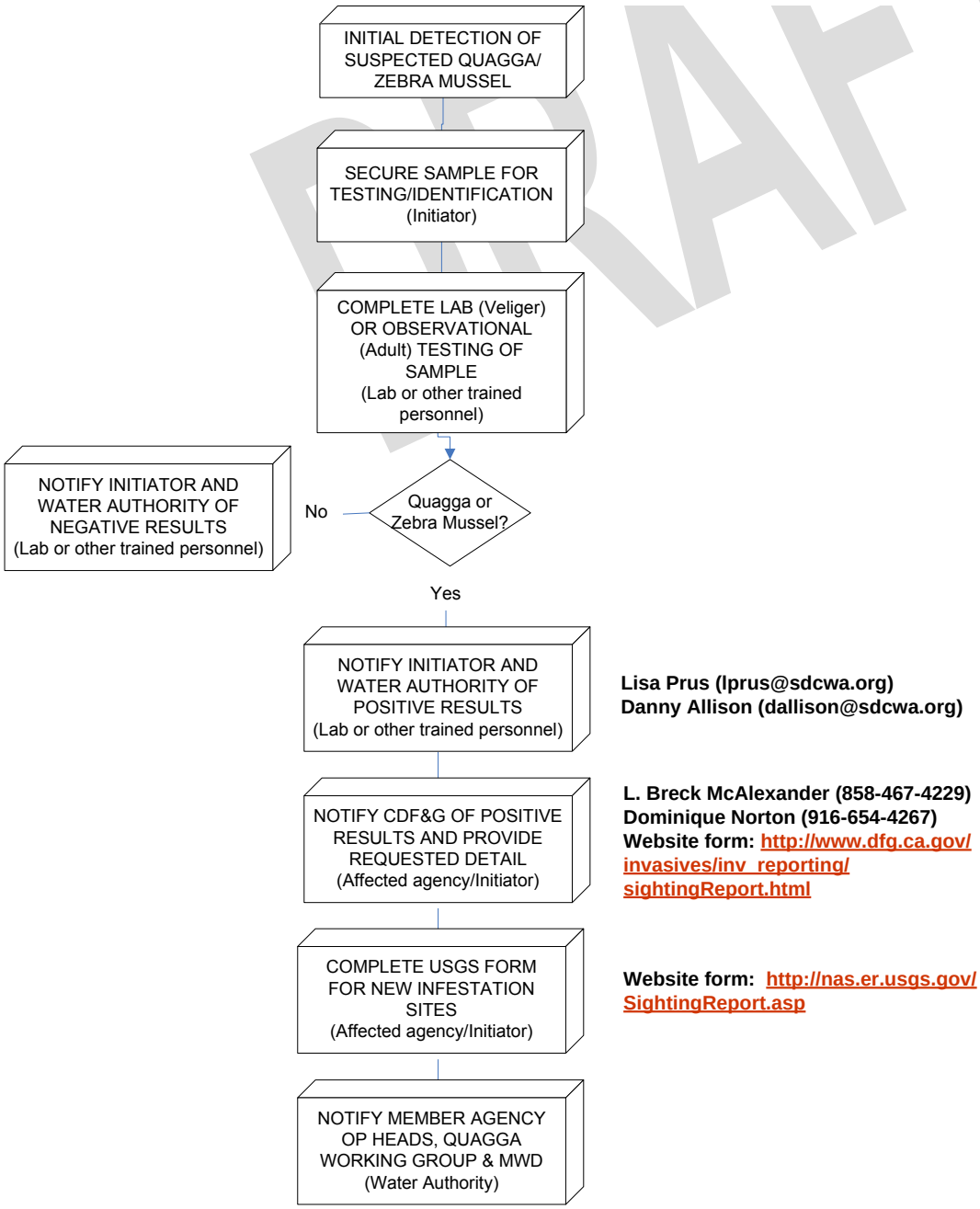
### QUAGGA/ZEBRA MUSSEL NOTIFICATIONS



## Contacts

| **Initial Notifications: Notify each of these agencies within 24 hours or as soon as practical after confirmation of test results or physical observation.** | | | | |
|---|---|---|---|---|
| **Agency** | **Name/position** | **Office Phone** | **Cell phone/ After hours** | **Email** |
| California Department of Fish & Game | L. Breck McAlexander, Environmental Scientist | (858) 467-4229 | (858) 437-4177 | lmcalexander@cdfg.ca.gov |
| | Dominique Norton | (916) 654-4267 | | dnorton@cdfg.ca.gov |
| | **Website Reporting Required for new sites: http://www.dfg.ca.gov/invasives/inv_reporting/sightingReport.html** | | | |
| US Geological Survey | **Website Reporting Required for new sites: http://nas.er.usgs.gov/SightingReport.asp** | | | |
| San Diego County Water Authority | Lisa Prus, Supv Mgmt Analyst | (760) 233-3252 | (619) 993-2431 | lprus@sdcwa.org |
| | Dan Allison, Operations Supervisor | (760) 233-3279 | | dallison@sdcwa.org |

*Note – This list is based on regional notification protocol. Other notifications may be required and are managed by those agencies directly. Additional contacts may be added as required.*

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## Contacts (Continued)

**Other Notifications: Notify members of the Regional Quagga Mussel Control Group via e-mail as soon as practical after confirmation of test results or physical observation.**

| Name | Title | Agency | Email | Phone |
|---|---|---|---|---|
| Dwayne Maxwell | Senior Fishery Biologist Supervisor | CA Dept. of Fish & Game | dmaxwell@dfg.ca.gov | (562) 342-7152 |
| Sean Sterchi | District Engineer | CA Department of Public Health | ssterchi@cdph.ca.gov | |
| Jay Cowan | Ranger Supervisor | City of Escondido | jcowan@escondido.org | (760) 839-6398 |
| Richard Walker | Deputy Utility Mgr. | City of Escondido | rwalker@escondido.org | (760) 839-5460 |
| Tony Smock | Lakes Superintendent | City of Escondido | tsmock@ci.escondido.ca.us | (760) 839-4240 |
| John Walters | Utility Systems Supervisor | City of Poway | jwalters@ci.poway.ca.us | (858) 668-4754 |
| Kevin O'Reilly | Water Plant Supervisor | City of Poway | koreilly@ci.poway.ca.us | (858) 668-4752 |
| Stephanie Lacombe | Water Treatment Plant Operator III | City of Poway | slacombe@ci.poway.ca.us | (858) 668-4751 |
| Tom Howard | Public Works Utilities Manager | City of Poway | thoward@ci.poway.ca.us | |
| Dan Daft | Biologist | City of San Diego | ddaft@sandiego.gov | (619) 980-9795 |
| Dana Chapin | Water Prod. Superintendent | City of San Diego | dchapin@sandiego.gov | (619) 668-3233 |
| Nelson Manville | Water Program Supervisor | City of San Diego | nmanville@sandiego.gov | (619) 668-2013 |
| Jeff Pasek | Watershed Manager | City of San Diego | jpasek@sandiego.gov | |
| Jim Fisher | Systems Operations Manager | City of San Diego | jfisher@sandiego.gov | |
| Joe Young | OP Manager | Helix Water District | Joe.young@helixwater.org | |
| Mark Umphres | Director of WQ and System Operations | Helix Water District | Mark.umphres@helixwater.org | |

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## Contacts (Continued)

| Other Notifications (continued): Notify members of the Regional Quagga Mussel Control Group via e-mail as soon as practical after confirmation of test results or physical observation. | | | | |
|---|---|---|---|---|
| **Name** | **Title** | **Agency** | **Email** | **Phone** |
| Bill Taylor | Limnologist | Metropolitan Water District | wtaylor@mwdh2o.com | |
| Ricardo De Leon | Microbiology Unit Manager | Metropolitan Water District | Ricardo_deleon@mwdh2o.com | |
| Cor Shaffer | | Santa Fe Irrigation Dist. | cshaffer@sfidwater.org | (858) 607-7611 |
| Tim Bailey | Water Quality Analyst | Santa Fe Irrigation Dist. | tbailey@sfidwater.org | (858) 602-7821 |
| Gary Stine | O & M Manager | SDCWA | gstine@sdcwa.org | (760) 233-3205 |
| Don Thompson | Director of Water Quality | Sweetwater Authority | dthompson@sweetwater.org | |
| Mark Hatcher | Lab Supervisor | Sweetwater Authority | mhatcher@sweetwater.org | (619) 409-6813 |
| Scott McClelland | WT Superintendent/Prin. Engineer | Sweetwater Authority | smcclelland@sweetwater.org | |
| Ron Burton | Professor/Director of Lab Services | UCSD/ SIO | rburton@ucsd.edu | (858) 822-5784 |
| Nerida Wilson | Postdoc | UCSD/ SIO | ngwilson@ucsd.edu | |
| Gary Moy | Research Associate | UCSD/SIO | gmoy@ucsd.edu | (858) 534-7827 |
| John Spangler | Lab Director | Vista Irrigation District | jspangler@vid-h20.org | (760) 597-3143 |
| Don Smith | Director of Water Resources | Vista Irrigation District | dsmith@vid-h20.org | (760) 597-3168 |

*This list represents members of the San Diego Regional Quagga Mussel Control Group. All members may not be mentioned here. Members will be contacted via the approved e-mail notification list or by special request.*

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## Resources

| Resource | Provider | Contact | Cost |
|---|---|---|---|
| Dive services<br>ROV inspection<br>ROV training | Above & Below the H2O<br>2325 25th Ave, Sacramento, CA 95822 | Richard Faulk<br>(916) 457-1413<br>mailto:rfaulk@aboveandbelowtheh2o.com | $1,850 - $7,000 /day (Divers)<br>$1,450 - $2,500 /day (ROV) |
| Lab testing<br>Research assistance | UCSD/Scripps Institution of Oceanography | Ron Burton<br>(858) 822-5784<br>rburton@ucsd.edu | $225 PCR<br>$125 Microscopy<br>$50 Microscopy Quantification (add'l) |
| ROV<br>Dive services<br>Research assistance | Scripps Institution of Oceanography | Rudy Morrello<br>(858) 822-4034<br>Russ Moll, CA Seagrant Program<br>(858) 534-4440 | Unknown |
| Information relative to ROV inspection | SPAWAR | Rich Arrieta<br>(619) 553-1968 | No Cost |
| ROV inspection | Ocean Innovations<br>7709 Prospect Place<br>La Jolla CA. 92037 | Brock Rosenthal<br>(858) 454-4044<br>brock@o-vations.com | $850 - $1,200 |
| ROV internal pipeline inspection (>36 inch) | RedZone<br>621 N. Naomi St.<br>Burbank, CA 91505 | David Vadgely<br>(818) 843-3318<br>dvadgley@redzone.com | $5,000 - $20,000/day |
| ROV inspection | Remote Ocean Systems, Inc.<br>5618 Copley Drive<br>San Diego, DAA 92111 | Beto Campos<br>(858) 565-8500 ext. 109 | Unknown |
| ROV purchase | JW Fishers Mfg<br>1953 County Street<br>Taunton, MA | Chris Combs<br>(800) 822-4744 | Sold unit to City of San Diego |
| Divers<br>Sampling/Analysis<br>ROV inspection | SAIC<br>9455 Townecentre Drive MS W-2<br>San Diego 92121 | John Evans, (858) 826-7476<br>evansj@saic.com<br>George Seeley, (858) 514-5420<br>george.seeley@saic.com | $5,000 / day divers<br>$ Unknown Sampling<br>$ Unknown ROV |

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| Resource | Provider | Contact | Cost |
|---|---|---|---|
| Divers | Procinctu Group<br>3405 Lakeview Drive<br>Spring Valley, 91977 | Jack Downes<br>951-235-6942 | $1,500 for ½ day<br>$6,000 for full day |
| Cleaning stations | Hydro Engineering, Inc.<br>865 W. 2600 S.<br>Salt Lake City, UT 84119 | http://www.hydroblaster.com/4picsMarinaapplications.htm<br>Check website for contact names and numbers.<br>Main line: (800)247-8424 | |
| Dewatering Tanks | Baker Corp.<br>310 S. Twin Oaks Valley Rd.<br>Suite 107-382<br>San Marcos, CA 92078 | Patrick Thurman<br>(760) 750-1985<br>Pthurman@BAKERcorp.com | |
| Bag Filtration Systems | Rain for Rent<br>6400 Fischer Road<br>Riverside, CA 92508 | Matthew Stanton<br>Cell (760) 275-1138<br>Office (951) 653-2171<br>mstanton@rainforrent.com | |

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# Appendix H – Boating and Recreational Activities

At this time, there is no universal system in place to track and certify decontamination of boats or other possible mussel transfer mechanisms. Reservoir owners and operators should tailor a plan that can work effectively within their system's constraints.

- Control/oversight of boat ingress is paramount for operators of non-infested water bodies.
- Control/oversight of boat egress is paramount operators of infested water bodies.

## Non-infested Water Bodies

Agencies have two basic options to reduce the threat of contamination. Those are:

1. Control and manage transfers via potentially infested vectors such as boats or live bait.
2. Close the water body to private boating use.

### Boater Evaluation

Options for inspection by staff at non-infested water bodies include:

- If the ingress of boaters can be managed, boats may be inspected per standard risk assessment procedures and let through if judged "low risk". If decontamination equipment is available, higher-risk boats may be shunted there and cleaned prior to allowing entry.

- Quagga-sniffing dogs have been trained by the Department to inspect for quagga mussel infestation on boats and trailers. At this time, they are capable of finding adult mussels only. The cost for training a dog and handler is less than $10k. These dogs could be used for inspecting boats entering non-infested water bodies and confirming the effectiveness of de-contamination procedures after leaving infested waters.

## Infested Water Bodies

Owners and operators of infested water bodies must quarantine the recreational use of those waters until adequate measures to eliminate the spread of mussels are in place.

Tactics to eliminate or reduce the potential for quagga spread include the following:

- Incorporate public education that emphasizes reducing the risk of spreading mussels to non-infested water bodies. This may include signage to display status of reservoir, handouts, permanent bulletin boards with personal/public control procedures, or other educational items.

- Install cleaning stations at infested reservoirs that are capable of decontaminating small and medium-sized equipment including jet skis, inflatables, boots, canoes, and floatation devices that have come into contact with reservoir water. If the stations will be self-serve, steps must be taken to minimize potential danger to the public. Steps may include providing non-scalding water, or 1ppm chlorine solution, or a concentrated saltwater solution,

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and stiff brushes. Instructions, including drying time or recommended quarantine periods, should be provided at all self-serve stations.

- Require decontamination of moored boats prior to departure and post signage.

**Decontamination Techniques**

Conspicuous signage should indicate that after leaving and before entering any other fresh-water body, all vessels must comply with the following:

Drain. Dry. Clean.

- Empty and dry any buckets. Do not re-use any suspect bait.
- Drain any water through the vessel's hull plug, and ensure the area is dry.
- Drain all water from ballast tanks.
- Drain and dry all water from lower outboard unit (open cooling systems).
- Clean and dry any live-wells aboard the vessel.
- Decontaminate any equipment (including boat trailers) that had exposure to water if an adequate drying time has cannot been achieved.

Cleaning stations

Cleaning stations staffed by trained personnel may also be used to decontaminate vessels. General requirements and instructions for decontamination can be made available upon request.

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# Appendix I - Summary of Zebra Mussel Eradication and Control Options

***This information must be validated for Quagga mussels.***

Except as noted, this material is from: Messer, C. and T. Veldhuizen. 2005. Zebra Mussel Early Detection and Public Outreach Program Final Report. Report for California Bay-Delta Authority and US Dept of the Interior, Fish and Wildlife Service. CBDA Project No.99-F07, Zebra Mussel Detection and Outreach Program. 278 pp. to become available at http://www.des.water.ca.gov/zmwatch/.

## Thermal Shock

Hot water treatment can kill zebra mussels. Temperatures of 37°C and above are lethal to zebra mussels. Depending upon acclimation temperature, zebra mussels will die in about 1 hour. At winter acclimation temperatures (5 to 10°C), temperatures of 33°C and above will kill zebra mussels within 13 hours. For further information, see Table 1 in McMahon, et al.[1]

[1] McMahon R.F., Ussery T.A., Miller A.C., Payne B.S. 1993. Thermal tolerance in zebra mussels (Dreissena polymorpha) relative to rate of temperature increase and acclimation temperature. Proceedings of the Third International Zebra Mussel Conference. EPRI TR 0102077:4-97 - 4-118, 22 pages.

## Freezing

Adult zebra mussels die when aerially exposed to freezing temperatures for varying lengths of time. Populations can be controlled by winter-time dewatering and exposing zebra mussels to freezing air temperatures. Zebra mussels die in 2 days at 0°C and at minus 1.5°C, in 5 to 7 hours at minus 3°C, and in under 2 hours at minus 10°C. Duration to mortality is less for single mussels than for clustered mussels.

Research conducted by Dr. R.F. and T.A. Ussery (in Payne2). 2 Payne, B.S. 1992. Freeze survival of aerially exposed zebra mussels. US Army Corps of Engineers Waterways Experiment Station Technical Note ZMR-2-09.

## Oxygen Starvation

Oxygen is removed from the water by cycling it through oxygen-starving pumps. The developer claims the equipment can cycle 200 million gallons of water. This technology was developed by Wilson J. Browning of Amark Corp, Norfolk County, VA. Another method of removing oxygen is to add oxygen scavenging chemicals, such as sodium-meta-bisulfite and hydrogen sulfide gas (USACE-ZMIS). Zebra mussels are able to tolerate oxygen deprivation for up to 2 weeks, provided ambient temperatures are low enough (USACE-ZMIS).

## Desiccation

Instantaneous mortality occurs at 36°C. Temperatures over 32°C are lethal within 5 hours. At temperature below 30°C, time to mortality is dependent upon relative humidity. Temperature is positively related and humidity is negatively related to adult zebra mussel mortality. As humidity increases and temperature decreases, survivorship increases (Table 1). Aerial exposure of zebra mussels during summer months, when temperatures exceed 25°C, will result in 100% mortality in 2.1 days. During winter months, 100% mortality will take longer, depending upon the relative humidity.

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Desiccation is a viable option for eradicating zebra mussels from areas that can be dewatered for several days. Alternatively, desiccation can also act as a population control method in areas that can not be completely dewatered. For example, reservoir levels can be lowered (at least 30 vertical feet) to expose zebra mussel inhabiting shallow water. The majority of the zebra mussel population inhabits shallow water within 2 to 7 m below the surface, with moderate to low densities up to 50 m. Colonization is dependent upon water temperature, oxygen content, and food availability. They tend to colonize above the thermocline.

Table I-1. Number of days to 100% mortality of adult zebra mussels aerially exposed to different levels of relative humidity and air temperature.

| | Days to 100 % Mortality at Air Temperature, °C | | |
|---|---|---|---|
| **Relative Humidity, %** | **5** | **15** | **25** |
| **95** | 26.6 | 11.7 | 5.2 |
| **50** | 16.9 | 7.5 | 3.3 |
| **5** | 10.8 | 4.8 | 2.1 |

[3] Payne, B.S. 1992. Aerial exposure and mortality of zebra mussels. US Army Corps of Engineers Waterways Experiment Station Technical Note ZMR-2-10.

## Benthic Mats

Researchers from the Rensselaer Polytechnic Institute in New York are investigating the use of benthic mats that would cover the sediment and zebra mussels, and smother the mussels. Research is planned to occur in Lake George, NY.

## Predation

The relatively soft shells of zebra mussels and their exposure (on substrates as opposed to buried in sediment) make them vulnerable to predation. Possible predators of adult mussels are some species of carp, catfish, bullhead, sucker, sunfish, sturgeon, crayfish, and muskrats. A possible predator of veligers is the American shad. However, there is no evidence of predation control in the Great Lakes, Ohio River, and Poland. There is some evidence of population reduction in the Hudson River. Despite the lack of clear evidence of population control through predation, it is recommended that harvest of predatory species in infested waterbodies be stopped.

## Chemical Treatment

The most susceptible life stages to chemical treatment are post-spawned mussels that are in a low energy state, and veligers and pediveligers that have undeveloped shells. There are three general categories of chemicals used to treat zebra mussel infestations: metallic salts, oxidizing biocides, and nonoxidizing biocides. Application rates and duration data for these compounds come from laboratory studies, power plants, and water treatment plants.

Metallic salts, electrolytically dissolved metallic ions, are effective on adult mussels because of the incomplete sealing of their shells. The required exposure time for most metallic ions ranges from 5 to about 48 hours.

Oxidizing chemicals have been used by the water treatment industry for disinfection since the late 1800s and their effect on the environment is understood and documented

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(Claudi[4]). Zebra mussels can recognize oxidizing chemicals, such as chlorine, as a toxin. Oxidizing chemicals are very irritating. They work by oxidizing the gill lamellae and other parts, eventually causing death. In response to the irritation, zebra mussels expel the offending water and close their valves for several days. Periodically, they reopen their valves to "test" the water. Depending upon water temperature, respiration rate, and stored nutrient reserves, zebra mussels can remain closed and withstand exposure for many days before reopening their valves to resume respiration and feeding. Therefore, required exposure time for oxidizing biocides is usually 1 to 3 weeks.

Zebra mussels do not detect most non-oxidizing chemicals and continue to filter water. The chemical is drawn into the mussel's body and attacks the cell walls. The cells lose the ability to maintain their chemical balance, and the mussel dies. Because the mussels continue to filter, exposing themselves to the chemical, treatment with non-oxidizing chemicals can be accomplished in hours as opposed to weeks.

The most commonly used non-oxidizing compounds are proprietary molluscicides (e.g. Clamtrol, Bulab, Bayluscide). They are applied at high concentrations, and, in most cases, the water must be detoxified after treatment. These compounds are usually deactivated by releasing slurry of bentonite clay into the water. The cationic or surfactant active ingredients bind onto the clay, becoming inactive. The clay settles out of the water column and becomes part of the bed sediments. The compound is microbially degraded into nontoxic products. These chemicals are less effective at lower water temperatures, so treatment is recommended during warmer months. The chemicals are usually administered with equipment supplied by the vendors.

Non-oxidizing chemicals were used to control the Asian clam in the southeastern US (Green[5]).

Additional information on most of these chemicals, such as formula, manufacturer, and application method, is available at http://www.wes.army.mil/el/zebra/zmis/idxlist.htm.

[4] Claudi, R. and G. Mackie. 1994. Zebra mussel monitoring and control. Lewis Publishers, Inc. Boca Raton, FL.`

[5] Green, R.F. 1995. Strategies for application of non-oxidizing biocides. Proceedings of the Fifth International Zebra Mussel and Other Aquatic Nuisance Organisms Conference, Toronto, CA, February 1995: 175-181.

## Bacterial Toxin

Experimental research is occurring on a toxin produced by *Pseudomonias fluorescens*, a soil bacterium. The toxin destroys the digestive gland of zebra mussels, but reportedly does not harm fish or native mussels. Testing and manufacturing scale-up is in process. If approved by the FDA, the product may be marketed commercially as soon as 2010.

## No-Growth Materials (anti-fouling paints)

Anti fouling paints and other no-growth materials are available, though life expectancy does need to be scrutinized carefully. More information will be posted at a later date.

## Matrix of Eradication and Control Options by Waterbody Type

(From: Messer, C. and T. Veldhuizen. 2005)

| Eradication and control options for various zebra mussel waterbody infestation scenarios. | | |
|---|---|---|
| **Population Level Waterbody** | **Isolated Population** | **Widespread Population** |
| **Pond, Isolated, non-draining** | • Evaluate for natural control (e.g. winter freeze, summer desiccation)<br>• Chemically treat area and buffer zone<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Mandatory cleaning of departing vessels and equipment | • Chemically treat entire waterbody<br>• Stop water diversions, if any, and chemically treat diversion infrastructure<br>• Mandatory cleaning of all departing vessels and equipment<br>• Quarantine and/or stop all recreational uses |
| **Pond, draining** | • Chemically treat released water or prevent water release<br>• Chemically treat area and buffer zone<br>• Monitor for spread within pond and downstream<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Mandatory cleaning of departing vessels and equipment | • Minimize or prevent water release<br>• Chemically treat released water<br>• Chemically treat diversion infrastructure, if any<br>• Monitor for spread downstream<br>• Chemically treat entire waterbody<br>• Mandatory cleaning of all departing vessels and equipment<br>• Quarantine and/or stop all recreational and commercial uses |

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| Eradication and control options for various zebra mussel waterbody infestation scenarios. | | |
|---|---|---|
| **Population Level Waterbody** | **Isolated Population** | **Widespread Population** |
| **Small Reservoir** | • Minimize water releases<br>• Chemically treat released water<br>• Chemically treat area and buffer zone<br>• Monitor for spread within reservoir and downstream<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Mandatory cleaning of departing vessels and equipment | • Evaluate need to reduce reservoir volume through water releases<br>• Chemically treat released water<br>• Chemically treat diversion infrastructure, if any<br>• Monitor for spread downstream<br>• Chemically treat entire waterbody<br>• Mandatory cleaning of all departing vessels and equipment<br>• Quarantine and/or stop all recreational and commercial uses |
| **Large Reservoir** | • Reduce reservoir volume<br>• Chemically treat released water<br>• Chemically treat infested area and buffer zone<br>• Monitor for spread within reservoir and downstream<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Mandatory cleaning of departing vessels and equipment | • Chemically treat released water<br>• Monitor for spread downstream<br>• Chemically treat diversion infrastructure, if any<br>• Evaluate potential for a water level drawdown to reduce the population<br>• Evaluate ability to chemically treat entire waterbody<br>• Prevent spread to upstream waterbodies and other watersheds<br>• Quarantine and/or stop all recreational and commercial uses<br>• Mandatory cleaning of all departing vessels and equipment |

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| Eradication and control options for various zebra mussel waterbody infestation scenarios. | | |
|---|---|---|
| **Population Level Waterbody** | **Isolated Population** | **Widespread Population** |
| **River, Small Volume** | • Minimize or stop inflow and increase upstream water diversions to reduce stream volume and flow rate<br>• Install veliger settlement materials at downstream end of population<br>• Create pool conditions at downstream end of population to facilitate veliger settlement (e.g., installation of temporary weir)<br>• Treat with molluscicide<br>• Detoxify downstream of infested area<br>• Monitor for spread downstream<br>• Prevent spread to upstream waterbodies and other watersheds<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Installation of travel barrier and mandatory cleaning station for all vessels traveling upstream via waterway<br>• Mandatory cleaning of all departing vessels and equipment | • Minimize or stop inflow and increase upstream water diversions to reduce stream volume and flow rate<br>• Treat with molluscicide<br>• Detoxify downstream of infested area<br>• Monitor for spread downstream<br>• Prevent spread to upstream waterbodies and other watersheds<br>• Quarantine and/or stop all recreational and commercial uses<br>• Installation of travel barrier and mandatory cleaning station for all vessels traveling upstream via waterway<br>• Mandatory cleaning of all departing vessels and equipment |

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| Eradication and control options for various zebra mussel waterbody infestation scenarios. | | |
|---|---|---|
| **Population Level Waterbody** | **Isolated Population** | **Widespread Population** |
| **River, Large Volume** | • Minimize inflow and increase upstream water diversions to reduce stream volume and flow rate<br>• Install veliger settlement materials at downstream end of population<br>• Create pool conditions at downstream end of population to facilitate veliger settlement (e.g., installation of temporary weir)<br>• Treat with molluscicide<br>• Detoxify downstream of infested area<br>• Monitor for spread downstream<br>• Prevent spread to upstream waterbodies and other watersheds<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Installation of travel barrier and mandatory cleaning station for all vessels traveling upstream via waterway<br>• Mandatory cleaning of all departing vessels and equipment | • Prevent spread to upstream waterbodies and other watersheds<br>• Quarantine and/or stop all recreational and commercial uses<br>• Mandatory cleaning of all departing vessels and equipment<br>• Installation of travel barrier and mandatory cleaning station for all vessels traveling upstream via waterway<br>• Closure of unattended boat ramps, especially in zebra mussel-free areas<br>• Mandatory inspection/cleaning of all vessels entering zebra mussel-free waterbodies<br>• Evaluate ability to chemically treat |

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| Eradication and control options for various zebra mussel waterbody infestation scenarios. | | |
|---|---|---|
| **Population Level Waterbody** | **Isolated Population** | **Widespread Population** |
| **Estuary** | • Install veliger settlement materials at perimeter of population<br>• Divert upstream water to reduce river volume and flow rate (e.g. rock barrier)<br>• Create pool conditions at downstream end of population to facilitate veliger settlement (e.g., installation of temporary weir, tidal flow/rock barrier)<br>• Treat with molluscicide<br>• Detoxify downstream of infested area<br>• Monitor for spread<br>• Prevent spread to upstream waterbodies and other watersheds<br>• Quarantine and/or stop all recreational and commercial uses in infested area and buffer zone<br>• Installation of travel barrier and mandatory cleaning station for all vessels traveling upstream via waterway<br>• Mandatory cleaning of all departing vessels and equipment | • Eradication doubtful<br>• Implement population level control measures (e.g. salt water intrusion during spawning season and veliger settlement)<br>• Prevent spread to upstream waterbodies, other watersheds, pumping plants, and aqueducts/diversion canals<br>• Mandatory cleaning of all departing vessels and equipment<br>• Closure of unattended boat ramps, especially in zebra mussel-free areas<br>• Installation of travel barrier and mandatory cleaning station for all vessels traveling upstream via waterway<br>• Mandatory inspection/cleaning of all vessels entering zebra mussel-free waterbodies<br>• Establish regulations for ships traveling to/from ports of the Columbia River<br>• Evaluate treatment/spread prevention at all points of diversion |

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| Eradication and control options for various zebra mussel waterbody infestation scenarios. | | |
|---|---|---|
| **Population Level Waterbody** | **Isolated Population** | **Widespread Population** |
| **Water Diversions** | • If only one facility is impacted, transfer all diversions to alternate facility(ies)<br>• Drain and desiccate facilities, chemically treat standing water<br>- OR -<br>• Isolate infested area and buffer zone with temporary barriers, chemically treat<br>• Chemically treat removed water or quarantine and discharge the mussel-infected water to safe disposal area<br>• Monitor for downstream spread<br>• Mandatory cleaning of all vessels and equipment<br>• Quarantine and/or stop all recreational and commercial uses of aqueduct<br>• Retrofit facility(ies) to minimize impacts | • If only one diversion system is impacted, transfer all diversions to other facility(ies);<br>• Drain and desiccate facilities, chemically treat standing water<br>• If both facilities/water transfer infrastructure are impacted:<br>o Chemically treat water before transferring to "downstream" uses<br>o Chemically treat water before entrance into the facility(ies)<br>o Mandatory cleaning of all vessels and equipment departing facility(ies)<br>o Quarantine and/or stop all recreational and commercial uses of contaminated facilities<br>o Desiccate and chemically treat one facility and aqueduct at a time; continue diversions through alternate facility(ies)<br>• Retrofit facility(ies) to minimize impacts |

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# Appendix J- Filtering Process and Types

Agencies may conduct scheduled water releases each year to support maintenance and repair activities or for other reasons. When an infested water body will be drained to a non-infested perennial water body, control measures must be implemented to eliminate transfer of mussels.

The following steps can aid in the decision to filter discharged water:

- Obtain blueline GIS drawings for each location of untreated water release, if available.
- Evaluate volume of water and likely flow pattern to determine if potential exists for released water to enter a perennial stream or waterway.
- If potential exists, evaluate site conditions at release point to determine appropriate filtration unit or units for installation.

Several filtration options can be evaluated for use in mussel removal from infested waters. In the particular case of filtering discharge water during scheduled maintenance activities, mobile filtering units are available for rental or purchase. Two such units and their suppliers are described below.

*A maximum pore size of 150 micron is advised to extract mussels in the pedi-veliger and adult stage of development for filtering operations preceded by chlorination or other chemical treatment. If chemical treatment is not used, filters must be sized with a 40 micron or smaller pore size to remove veliger stage and larger mussels.*

A more detailed unit selection, sample installation, and operating procedure will be included at a later date. Guidelines are available from the provider for reference.

| | |
|---|---|
| **Unit Type 1** | 20 or 25-CY Vacuum sealed dewatering unit with filter blanket |
| | |
| **Approx flow rate** | Approx. 2 MGD (1400 GPM) using 150 micron filter media |
| **Dimensions** | Height 69” to 86”<br>Width 92” to 98”<br>Length 238” to 284” |
| **Specifications** | See http://www.bakercorp.com/tanks-roll-off-boxes-vacuum-boxes.asp |
| **Provider** | Baker Corporation |
| **Est. Lead time** | 3 days |

| | |
|---|---|
| **Unit Type 2** | Model BF-1000 Bag filtration unit |
| | |
| **Approx flow rate** | 1000 GPM |
| **Dimensions** | Height 63”<br>Width 48"<br>Length 48” |
| **Specifications** | See http://www.rainforrent.com/products/Filters/bf1000.htm |
| **Provider*** | Rain for Rent |
| **Est. Lead time** | 1 week |

*Note: Additional providers may be available. The unit pictured above is manufactured by Filter Specialists, Inc.

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# Appendix K - Development Opportunities

Opportunities exist to develop additional strategies and tactics to eliminate the spread of dreissenid mussels and control population growth in infested water bodies. Development will require interagency coordination for research and feasibility studies to determine parameters for practical application and return on investment. The ideas mentioned below are available for consideration, but limited support currently prohibits effective application.

## Tagging System

Working group members have proposed a tagging system, in conjunction with an Inspection Logbook (as documentation) for privately owned boats that would show a certificate of inspection as a condition of entry into a body of water that has tested negative for quagga and zebra mussels. A description of the system follows:

1. Prior to launching a vessel into a non-infected body of water, the owner/operator must have the vessel inspected and possibly decontaminated at a State approved and certified inspection station. The owner/operator of the boat will be required to maintain a logbook of such inspections.
2. After the vessel has passed inspection, a (uniquely identifiable) "tamper-proof" tag is attached to the propeller or jet drive on the vessel. The tag number, date of inspection, inspection station location, and the I.D. of the inspector are entered into the inspection logbook.
3. Prior to launching a private boat in a non-infested body of water, the vessel is checked for proper tagging. As oversight of possible tampering, such tags can be cross checked with the inspection logbook for matching identification.
4. After confirmation that the vessel has been inspected/decontaminated by an acceptable method, the tag will be removed and the vessel will be allowed to launch.
5. Upon exiting the water body, the boat operator can have the boat re-tagged and documented, so that the tag will be ready prior to the next launch.

This system will depend on approved methods to perform the inspections and any decontamination of private boats. Oversight of checking tags on boats entering the marinas (or other launch points) will be the responsibility of the owner/operator of the non-infested water body. Reservoirs/water bodies that do not institute such a system could be closed to private boating access.

Such a system could develop into a boat "passport" system that tracks the movement status of private boats by registration numbers.

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