Invasive Mussel Biobox Monitoring Protocol*

*This protocol was adapted from the California Department of Fish and Wildlife's protocols for Aquatic Invasive Species Monitoring at CDFW Hatcheries.

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Description of Quagga, Zebra, and Golden Mussels

The quagga mussel, *Dreissena rostriformis bugensis*, and the zebra mussel, *Dreissena polymorpha*, are small, invasive mussels found only in freshwater. They look very similar to each other. They commonly have alternating light and dark brown stripes, but can also be solid light brown or dark brown. They have 2 smooth shells that are shaped a bit like the letter "D". These mussels are usually less than 2 inches in length. In new populations, most mussels are young and therefore very small (under ¼ -inch long).

The golden mussel, *Limnoperna fortunei*, are another species of small invasive mussels that can be found in both brackish and freshwater. The golden mussel also has two smooth and shiny shells but their shells are more elongated than quagga and zebra mussels. Their shells range in color from dark brown to gold. They can reach up to 1 ¾ inches long.

Quagga Mussel
Dreissena bugensis

- Shell: D-shaped and triangular; thin, fragile; smooth or shallowly ridged; solid light to dark brown or dark concentric rings; paler near hinge
- Attaches to hard and soft surfaces

Zebra Mussel
Dreissena polymorpha

- Shell: D-shaped and triangular; thin, fragile; smooth or shallowly ridged; solid light to dark brown or striped
- Attaches to hard surfaces

Figure 1 Color variation in quagga and zebra mussels

Golden Mussel
Limnoperna fortunei

- Shell: Elongated shell; thin, fragile; smooth and shiny; dark brown to gold in color
- Attaches to hard and soft surfaces

Quagga, zebra, and golden mussels are freshwater mussels that can physically attach onto hard substrates. Like the mussels found clinging to the rocks along the California coastline, quagga, zebra and golden mussels attach onto hard surfaces (e.g. pipes, screens, rock, logs, boats, etc.). They form colonies made up of many individuals attached onto an object and even onto each other. Small newly settled mussels feel like gritty sandpaper when attached to a smooth surface. Larger mussels will feel coarser (like a small pebble or sunflower seed) or be visually apparent.

Other Organisms Mistaken for Invasive Mussels

Asian clam, *Corbicula fluminea*

People often mistake the very common Asian clam (also introduced) for quagga, zebra, and golden mussels. The Asian clam is widespread and abundant in California. It is brown and has ridges in concentric rings on its shells. The shells of older clams or of dead clams are white at the hinge (where the two shells join together). These clams do not attach onto surfaces. They live in mud or sand.

Figure 2 Asian Clam with concentric rings on its shells

Snails and freshwater limpets

Small snails and freshwater limpets cling to hard substrates and can be mistaken for small juvenile mussels. They are similar in color and size to small quagga, zebra, and golden mussels. Snails have a spiral shape. Limpets have one shell and are flat. Quagga, zebra, and golden mussels attach on the edge of their shell and stick up and away from the surface.

Figure 3 Left: Close up of a snail. Middle: Snails on a rock. Right: Close up of limpets.

New Zealand mudsnails, *Potamopyrgus antipodarum*

California's waters are home to many species of small, native freshwater snails. However, the New Zealand mudsnail is an invasive snail that is similar in appearance and of great concern in California for its potential impacts to trout and salmon and the food sources they rely on. New Zealand mudsnails are parthenogenic, meaning that they reproduce clonally and do not require a second individual for reproduction. All mudsnail populations in the U.S. are believed to consist solely of females, which are born with embryos in their reproductive tract. Hence, introduction of a single snail can create a large population in a very short amount of time.

Figure 4 Dense colony of New Zealand mudsnails attached to the underside of a rock.

New Zealand mudsnails have a single shell that is elongated and spiraled, with 5-7 spirals when fully grown. Their size ranges from microscopic to ¼" long and their color ranges from light to dark brown. New Zealand mudsnails require expertise to accurately identify. Any snail with a whorled shell smaller than ¼" should be forwarded to CDFW's New Zealand mudsnail expert for identification. Contact your CDFW Regional Scientist for guidance on providing a specimen.

For a map of known occurrences in California, visit the US Geological Survey(USGS) Nonindigenous Aquatic Species webpage.

Figure 5 Left: Dead New Zealand mudsnail on metric ruler (5mm = 1/4 inches). Right: Live New Zealand mudsnail showing operculum and spirals (1-5).

Monitoring for AIS Settlement within Bioboxes

This method is suitable for detection of golden mussels, quagga mussels, zebra mussels, and New Zealand mudsnails.

Bioboxes are flow-through systems designed specifically to sample for the larval/settlement stage of golden mussel, quagga mussels, zebra mussels and New Zealand mudsnails. Microscopic larvae are suspended in the water, and upon reaching settlement stage, attach to surfaces. The biobox provides suitable conditions (surface and flow) for this to occur. Flow rates greater than 5 feet/sec inhibit mussel and mudsnail settlement, so a flow-through system must not exceed this velocity.

Locations

If using bioboxes, one will be installed where raw water enters the facility and, if feasible, at each (if more than one) hatchery outflow, prior to discharge. Bioboxes are not needed on water drawn directly from a well. Bioboxes should be placed on a stable surface adequate to support its weight. If the water temperature inside the biobox is more than 2°F above the hatchery water temperature, then the biobox must be shaded. Bioboxes should be in areas that will not be damaged by water if the box were to overflow. Individual hatcheries may need to modify the biobox during installment to adequately meet all flow and temperature requirements. Infrastructure modifications may be needed to connect the biobox to an intake or outflow system.

Monitoring frequency

Bioboxes should be checked regularly to ensure they are operating correctly and maintaining the appropriate flow rate. A visual and tactile (touch) examination is conducted monthly.

Requirements for Biobox Design

- Minimum internal volume of 12 gallons
- Flow rate of 1.32 gallons/minute

Biobox Materials and Parts List

The following design specifications meet the biobox requirements above.

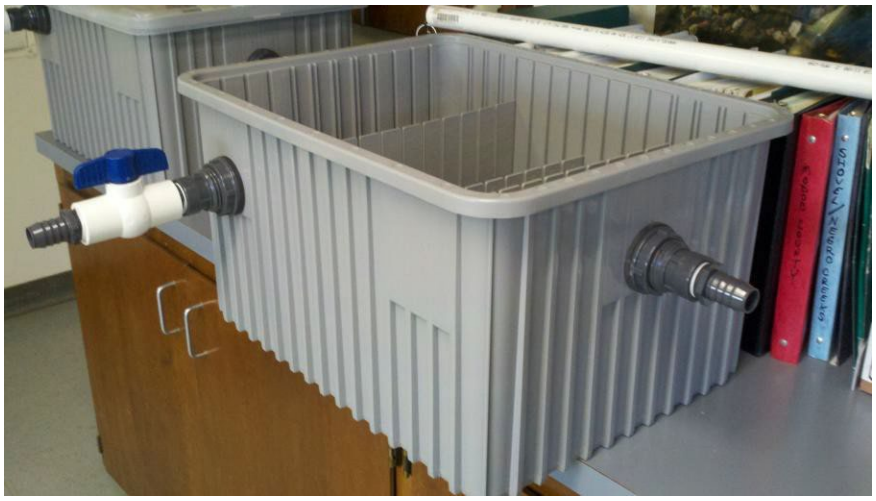

Figure 6 Biobox consisting of a grey bin with an inflow valve, an outlet spigot, and removable divider plates.
Designed by Jody Rightmire, CDFW Yreka Screen Shop.

Quantity of materials and parts required to construct a single biobox

Material/Part	Quantity
1" PVC Ball Valve Female threaded ends, quarter turn design	1
Nipple TBE SCH 80 1" x close PVC	1
1" PVC 90-degree elbow slip x slip SCH 40	1
1" pipe x MIPT PVC insert male adapter	2
1' PVC Tank adapter SOCXFPT NPRN gasket	2
1' x 2" (length) SCH 40 PVC pipe	1
22 x 17 x 12" grey storage bin with dividers	1
Snap F/DC3000 storage bin cover	1
Short Divider F/DC3080 (sold in 6 pk)	3
ER308L 3/32 x 36" TIG welding rod	1
1/2 " bolt size medium flat washer 18-8 stainless/steel	6

The plates slide down into "grooves" on either side of the interior walls of the box (Figure 7) and water flows over and under the plates as it passes through the box. Plates are kept submerged with stainless steel wire and washers that allow for removal when inspecting the plates. Flow into the box is regulated by a valve on the incoming water line. The outlet is an overflow pipe that ensures the water level in the box remains at a constant level. All interior surfaces and plates are roughed up with fine (150-180 grit) sandpaper to maximize suitability for settlement.

Figure 7 Interior view of biobox plates that provide suitable surfaces for mussel/mudsnail settlement.

Monitoring procedure

To inspect a biobox, begin by closing the inflow valve. One at a time, carefully remove each plate. Do not set the plates down as small or delicate organisms could be crushed. Hold the plate over a separate container to catch any dislodged organisms and visually inspect it. Use a magnifying glass if necessary. Next, gently run fingers over the plates to feel for any organisms. Very small golden, quagga or zebra mussels may be more easily felt than seen. Do not leave the plates out of the water so long that they dry; examine and return to the water immediately if no suspect organisms are found.

When finished with the first plate, reinsert it and inspect the remaining plates the same way. Also examine the inner walls of the biobox. If the walls are transparent, look in from the outside. If not, view from above. Next, gently run fingers over the walls as with the plates. When finished, open the valve to resume appropriate flow.

Specimen Collection

If you suspect you have found a mussel, immediately contact the appropriate CDFW Regional Scientist. To aid identification, first take a close-up digital photograph of each specimen. Next, collect the specimen(s) and place in a vial with 70% ethanol. Label the vial with location, date, and name of collector. If ethanol is not available, place the sample in a rigid container (to prevent crushing) without water, label, and refrigerate. E-mail the photos to your CDFW regional scientist and they will attempt to identify the specimens from the photographs, but may request the actual specimen(s) to make a positive identification.

CDFW Regional Scientist Contacts

For the current list of CDFW's Regional Scientists and their contact information, please visit CDFW's invasive mussel webpage, or download the contact list.