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Standard Protocols for Monitoring and Sampling Zebra Mussels

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Contents

1 Introduction 1
1.1 Purpose of This Document 1
1.2 How to Use This Document 1
1.3 Need for Zebra Mussel Monitoring Information 1
1.4 Definition of and Need for Standard Sampling 2
1.5 Early Detection of Zebra Mussels 2

2 Zebra Mussel Biology 3
2.1 Morphology 3
2.2 Reproduction and Growth 3
2.3 Distribution 4

3 Sampling Veligers 5
3.1 Outline 5
3.2 General Comments 5
3.3 Sampling Period 5
3.4 Equipment 6
3.5 Open Water 6
Sample site, vertical plankton tow, oblique plankton tow, pumped sample, scouring pad samplers
3.6 Flowing Water 7
Sample site, collection procedure
3.7 Water Intakes 7
3.8 Collection of Ancillary Data 7
3.9 Counting Veligers 8
Veliger identification, presence/absence data, detection limit, veliger densities, equipment, counting procedure for plankton tow, counting procedure for scouring pad sampler, calculation of veliger densities

4 Sampling Settling Juveniles 11
4.1 Outline 11
4.2 General Comments 11
4.3 Sample Site 12
4.4 Equipment 12
4.5 Collection Procedure 13
PVC plates, slide rack, multiplate samplers
4.6 Counting Procedure 14
Slides, plates
4.7 Collection of Ancillary Data 14
4.8 Data Reporting: Biomass vs. Density 15

5 Sampling Adults 16

5.1 Outline 16

5.2 General Comments 16

5.3 Sample Site 16

5.4 Equipment 16

5.5 Collection Procedures Using Quadrat Frame 17

Procedure 1 (high adult densities), procedure 2 (low adult densities), procedure 3 (low densities, loose, irregular substrate)

5.6 Collection Procedures Using a Ponar Grab 17

5.7 Collection of Ancillary Data 17

5.8 Counting Procedure 18

6 Reporting Results 19

Zebra Mussel Sighting Report Form 20

U.S. Fish and Wildlife Service Nonindigenous Species Database Form 21

7 Acknowledgments 22

8 Literature Cited 23

Appendixes

I Distinguishing Adult *Dreissena polymorpha* and *Mytilopsis leucophaeata* (Reprinted from MacNeill 1991) 24

II Detection of Zebra Mussel Veligers in Plankton Samples Using Sugar Solution (Reprinted from Schaner 1990) 25

III Collector for Veliger and Drifting Postmetamorphic Zebra Mussels (By André Martel) 28

IV Equipment and Chemicals 31

V Forms and Labels 32

VI Conversion Tables 38

1 Introduction

The accidental introduction of zebra mussels (*Dreissena polymorpha*) into the Great Lakes in 1986 is predicted to have significant effects on the aquatic ecosystem and on human water users. These mussels' high fecundity, rapid growth, and ability to attach strongly to any hard substrate have already caused many problems, including blockage of water intake pipes and fouling of docks and boat hulls. Because zebra mussels are highly efficient filter feeders, they threaten to deplete the population of microorganisms that are the base of the aquatic food web. Excess ingested food is excreted in pseudofecal bundles, which can reduce benthic dissolved oxygen during decomposition.

Information about the spread of zebra mussels is critical for industries and public utilities concerned about water intake systems and for fisheries agencies concerned about managing the Great Lakes ecosystem. Water users need an early warning of the arrival of zebra mussels to their area so they can be prepared to handle biofouling problems. Biologists interested in the impact of zebra mussels on the ecosystem likewise need to know when the mussels have arrived in their study area. Population density data can be used to track the movement of zebra mussels, to determine what environmental factors influence local population densities, and to determine the need for and efficacy of control measures. In some areas, mussel densities may never reach levels high enough to affect water users or the local environment. Understanding the effect of zebra mussels on the environment, as well as the effect of the local environment on the zebra mussels, requires monitoring zebra mussel densities and growth rates.

1.1 Purpose of This Document

As zebra mussels have spread, so has the need for monitoring programs initiated by agencies, researchers, and industries around the Great Lakes and inland waters. For each of these groups to develop a sampling protocol *de novo* would involve a huge duplication of effort. Even simply determining the presence or absence of these mussels can be facilitated by the use of established methods that minimize sampling effort while maximizing information gain. Comparisons of data among sampling stations or within studies may be unfeasible unless the same monitoring protocols are used at each site. The purpose of this document, therefore, is threefold:

1. To document methods that fit broad user needs for information and are known to be effective for sampling zebra mussels. Among the numerous sampling methodologies that have been developed for zebra mussels in North America over the past few years, those included in this volume were chosen on the basis of the following criteria:
   - Equipment that is inexpensive, readily obtainable, and simple to build and deploy.
   - Techniques that can be used in a variety of field situations.
   - Straightforward, rapid data collection and analytical methods.
2. To provide a zebra mussel sampling manual for people who are not familiar with or trained in biological sampling techniques.
3. To describe standard methods that can be used to collect data that are comparable between sites.

1.2 How to Use This Document

There are no absolute rules for how to design a zebra mussel monitoring program. Individuals or agencies must decide which sampling methods to use based upon their own clearly defined information needs. Too often, much time and effort has been expended to gather information later found to be useless. Many information needs may not require either interstudy comparability ("standardized" methods) or highly quantitative data. For example, careful, quantitative sampling is unnecessary when monitoring to detect the first arrival of mussels in an area. The need in this case for presence/absence data (see below) is most appropriately met by low-effort, high-volume sampling.

To some extent, therefore, the use of the word *standard* in the title of this document is misleading. Several methods known to work for sampling zebra mussels are represented, as are the pitfalls of each method. It is up to the investigator to determine which of these methods fit his or her information needs, budget, time constraints, equipment availability, and technical expertise. Use of this document does not obviate the need for careful thought to determine what information is required.

1.3 Need for Zebra Mussel Monitoring Information

The need for monitoring is most apparent at the edge of the zebra mussel range, where early warning of the mussels' arrival is important for water users. A common misconception is that monitoring serves no useful purpose once the mussels are established at a site. Monitoring is probably needed most at industrial sites and public utilities concerned about infestations of zebra mussels. Responsible, economically effective mussel control requires continued monitoring of local population levels. As an obvious example, treatments to prevent veliger settlement are wasted if applied before or after veligers are present. Only periodic monitoring will indicate when such treatments should begin and end. In addition, monitoring may reveal sites at which control of mussels is unnecessary. In previous studies, monitoring within areas of high zebra mussel densities has revealed that some sites, for reasons not yet understood, remain relatively free of zebra mussels without control measures. Finally, almost every zebra mussel population in Europe underwent a population

decline 5 to 10 years after invasion of a new area (Walz 1973, 1975). Only by monitoring local populations can the occurrence of this decline be noticed. The timing of the population decline is particularly important for biologists conducting long-range population studies of zebra mussels and their effects on native species.

1.4 Definition of and Need for Standard Sampling

Fundamentally, any protocol used by consensus can be a standard. An ideal standard protocol fits broad user needs for information and is easy to use. Standardization is most important for individuals who plan to compare their data with results from other studies or from other years within the same study. These individuals include scientists, biological consultants, fisheries managers, and individuals from industries and public utilities. Standard methods should never be used, however, at the cost of data quality. Priority should be given to the ability to replicate results within a study, and then to comparability among studies.

Zebra mussel settlement, growth, and density appear to be affected by light, temperature, depth, currents, substrate composition, substrate texture, substrate type, pH, ionic concentrations, and local fauna. For example, because of substrate preferences, equal numbers of juveniles measured on different settlement plate materials may not reflect equal population densities. Use of a standard sampling protocol is thus essential for comparing data among sites and between studies. Reporting ancillary data such as temperature, depth, and substrate types is equally important.

1.5 Early Detection of Zebra Mussels

Personnel at power plants, water treatment facilities, marinas, and other water-use areas may need to know as soon as possible that zebra mussels have arrived in their area. Identification of the "best" method for early detection of zebra mussels is problematic. The first life stage likely to colonize a new area is the planktonic veliger, which can drift into new areas or be transported in bilge water. Adults may also colonize new areas by being transported on boat hulls. Sampling for veligers is fairly likely to give "false negative" results because the distribution of veligers is highly clumped. Sampling of settled juveniles more reliably indicates the incipient formation of a local zebra mussel population, but this requires a sensitive method to maximize early detection of these microscopic animals. Although concrete blocks are commonly used as a readily available substrate on which settled juveniles can be detected, the juveniles may be settled for several weeks before becoming visible against the blocks' coarse texture. In contrast, newly settled juveniles are highly visible on a smooth settlement plate, which can be examined under a microscope.

It is impossible to accurately predict which type of sampling will produce the first evidence of zebra mussels in a new area. At several sites where all three life stages were monitored, either veligers, settled juveniles, or adults were detected several weeks before the other stages were found. Therefore, early warning of the presence of zebra mussels can best be achieved by a combination of plankton sampling, placement of settlement plates, and regular examination of surfaces for settled adults. Veliger sampling will be most effective in areas where the veligers may be carried from a distant location by a mild current. Settled juveniles can best be detected by examining the mussels' preferred substrates, such as polyvinyl chloride (PVC) (Figure 1; Walz 1973, 1975). Adults are most likely to be found in dark areas, in corners or crevices, and in areas with a gentle current (not more than 2 m/sec). Because the adults can only attach to hard substrate, in muddy areas they will be found attached to embedded rocks, native mussels, or crayfish.

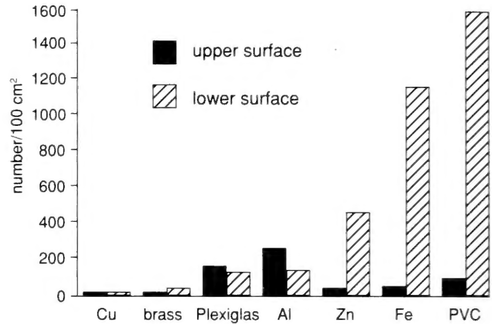

Figure 1. Numbers of settled zebra mussel juveniles on 10 cm x 10 cm plates of various substrate materials (from Walz 1973).

Sampling to detect new infestations does not require quantitative data. The most important aspect of this type of sampling is to maximize the probability of detection with minimal effort. Sampling a large volume of water for veligers, even if the volume cannot be readily quantified, is more valuable than spending time trying to carefully measure 200 liters. After zebra mussels are sighted, the monitoring program should switch to quantitative methods.

Most first sightings of mussels in new areas have been made by the informed public—that is, by people who were aware of the zebra mussel problem from the news or state agency educational bulletins or by water intake personnel aware of potential infestations. These people have reported mussels found on boats, docks, rocks collected for aquaria, and commercially harvested native mussel shells. Ordinary citizens are an especially effective monitoring resource because they greatly exceed, in time and effort spent on and near the water, the number of trained biologists who can be deployed in the field. Thus, the most effective programs to monitor the spread of zebra mussels are those that involve public education. Public education also has the benefit of helping to prevent the accidental spread of the mussels. Nonetheless, all sightings by nonbiologists must be confirmed by trained personnel who are thoroughly familiar with the zebra mussel and similar species. Most of the state natural resource agencies and Sea Grant program offices around the Great Lakes have produced information pamphlets about the zebra mussel. These could be distributed with a reporting form (see example in section 6) to marinas, bait shops, water-based industries, commercial shell fishermen, etc., to elicit information on new sitings and thus enhance an early warning detection program.

2 Zebra Mussel Biology

The life history of zebra mussels is described in detail, with an annotated bibliography of European literature, in Mackie et al. (1989). The purpose here is to summarize the biology of the zebra mussel to point out salient features that affect sampling.

2.1 Morphology

Zebra mussels are freshwater bivalves (family Dreissenidae) native to the Black and Caspian seas. The name *polymorpha* (many forms) indicates the large variability in many of their characteristics. Adult mussels have a distinctively shaped shell (Figure 2) that is variably banded with black or brown and cream stripes. Most have jagged, lateral stripes; some have single longitudinal bands; and all-cream or all-black individuals have been found (Figure 2). The shell shape is diagnostic for identification.

Two other species in the family Dreissenidae are present in North America and could be confused with *D. polymorpha*. The native false dark mussel, *Mytilopsis leucophaeata*, is found in brackish, estuarine waters, the upper Mississippi and Hudson rivers, the lower Tennessee River, and in the Ohio River below Cincinnati. A key to distinguishing between this mussel and the zebra mussel has been presented by MacNeill (1991) and is reprinted as Appendix I of this publication. If there is any doubt about correct identification, a mollusc taxonomist should be consulted.

In 1991, a second exotic dreissenid mussel was found in Lake Ontario (E. Marsden and B. May, Cornell University, unpublished data). At the time of publication, the species identity of this second exotic had not been established. The second species, given the working name "quagga mussel," is distinguished from *D. polymorpha* by the marked absence of a sharp angle between the dorsal and ventral surfaces (Figure 3). Confirmation of identification requires genetic analysis. As of late 1991, the distribution of the quagga mussel appeared to be limited to Lake Ontario and the Erie Canal.

2.2 Reproduction and Growth

Adult zebra mussels reach a maximum length of 4 cm. They usually live three to five years, though some survive as long as nine years in Europe. These mussels typically mature sexually in the second year of life, but in Lakes Erie and St. Clair they generally mature in their first year. Females can mature at 7 mm and males at 6 mm; individuals larger than 10 mm are usually mature (Smirnova 1990; J. Nichols, Great Lakes Fishery Laboratory, personal communication).

The sexes are separate, and gametes are released synchronously into the water column for external fertilization. Reproduction begins when the water temperature remains above 10°C (50°F) for one to two weeks. Spawning is stimulated by water temperatures of 12°C (54°F) and by the presence of gametes in the water. Nonetheless, veligers have been found in the plankton when spring water temperatures were still as low as 8.5°C (Joe Leach, Ontario Ministry of Natural Resources, personal communication). Females can spawn throughout the year at 8- to 10-week intervals in warm-water areas (Nichols and Kollar 1991). Individual females usually release 30,000 to 40,000 eggs each year during a period of several weeks. As many as 1,000,000 eggs per female per year have been noted (Walz 1978).

In Lake Erie, two spawning peaks have been noted, in late July and late August (Garton and Haag 1990). The egg hatches within a few days to release a veliger 40–70 µm in diameter. The veliger is a ciliated, free-swimming planktonic stage that is readily transported in water currents. Veliger densities as high as 400,000/m^3 have been observed in Europe (Smirnova 1990), and densities of 1,000,000 veligers/m^3 have been observed in Lake Erie.

In 8 to 15 days, the veligers grow to 150–250 µm and develop a clamlike shell. As the shell develops, the ciliated velum is lost, and the veliger becomes too heavy to swim and settles onto the substrate. Settlement may occur at a range of sizes (Lewandowski 1982); in the Great Lakes, settling juveniles are generally 180–250 µm (G. Mackie, University of Guelph, personal communication), although individuals up to 2 mm have been found in the plankton (see Appendix III).

The settled juveniles, also referred to as postveligers or spat, initially have a round, symmetrical shell. Within a few days the juveniles begin to elongate into their adult shape and develop pigmentation in the shell. If settlement occurs on a hard substrate, the juveniles may crawl around for several days before extruding gluelike fibers called byssal threads to attach to the substrate (Lewandowski 1982). The juveniles prefer dark areas, such as the underside of suspended surfaces, with currents that will transport food organisms. In studies by Walz (1973), settlement on the undersurface of test plates was more than an order of magnitude greater than that on the upper surface (Figure 1). Settlement is inhibited in currents greater than 2 m/sec (6.6 ft/sec), and feeding slows in currents of 1–1.5 m/sec. Textured substrates appear to provide a good surface for attachment. The juveniles aggregate in cracks and corners and adjacent to each other. Photographs of the various juvenile stages are shown in Hopkins (1990).

Although attached zebra mussels are difficult to displace, due to the strength of the byssal attachment, they can voluntarily detach from the substrate and move around using their muscular foot. Juveniles can also disperse by drifting on mucus threads (Prezant and Chalermwat 1984)

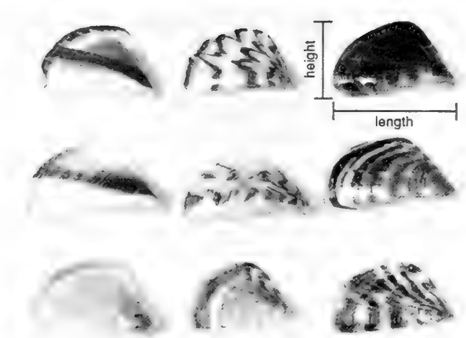

Figure 2. Adult zebra mussels, showing range of shell patterns and axes used for length and height measurement.

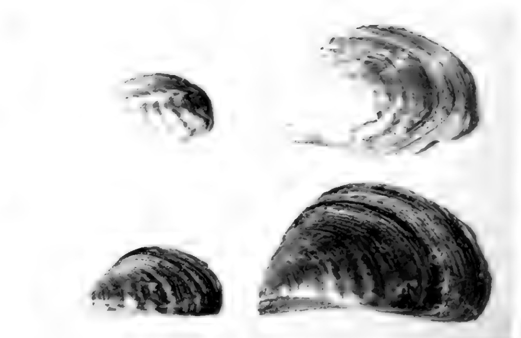

Figure 3. Comparison of the shell shape of zebra mussels (below) and quagga mussels (above).

or by displacement during storms (see Appendix III). Thus, it is not unusual to suddenly find adult mussels in an area presumed to be free of mussels. Live mussels that have accidentally or purposefully become detached will readily reattach to the substrate by producing new byssal threads. In dense colonies mussels are often anchored in place by their neighbors.

Settled juveniles may grow 5–20 mm in their first year of life, and under optimal conditions they can grow up to 0.21 mm/day (J. Nichols, personal communication). They are highly efficient filter feeders, removing any particles between 15 and 450 µm from the water column (Sprung and Rose 1977). Items that are not suitable for ingestion are aggregated into a mucoid ball and ejected as pseudofeces. Zebra mussel colonies can create sizeable accumulations of humus due to the production of this pseudofecal matter.

A brief word on life cycle terminology is appropriate here. The nomenclature for early life history stages of zebra mussels is complex. Many terms, such as trocophore, pediveliger, and plantigrade veliger, are primarily useful for biologists who need exact descriptors of particular life stages. For the general purposes of zebra mussel monitoring (and ease of discussion in this document), the life cycle can be divided into three stages: veligers, settled juveniles, and adults. Veligers are planktonic and smaller than approximately 250 µm. Settled juveniles, as the term suggests, have settled onto a substrate, but they may still be highly motile on the substrate. The term *adult* usually denotes a reproductively mature individual; however, because the reproductive status of a mussel is not readily apparent without dissection, the term will be used here to indicate a settled mussel easily identified with the naked eye (i.e., longer than 2 mm).

2.3 Distribution

Veligers can be found at depths of 0 to 10 m, and their maximum density occurs at depths of 3 to 7 m (10–23 ft) along the perimeter of lakes (Mackie et al. 1989). The juveniles tend to be clumped rather than randomly distributed through the water column. Veligers may undergo a diurnal vertical migration, remaining in deeper water (8–10 m) during the day and ascending in the water column during the night (Zhadanova and Gusynskaya 1985, Mackie et al. 1989). Adult zebra mussels can be found as deep as 55 m (180 ft), but the depth of maximum abundance is usually 2–4 m (6–13 ft; Mackie et al. 1989). Maximum densities have occurred in some European lakes at depths of up to 15 m (49 ft).

3 Sampling Veligers

3.1 Outline

- Sample at a minimum of three sites in open water at least 1 km (0.6 miles) apart; sample at a minimum of two sites in flowing water, one in center of current and one near edge.
- Sampler: 63- or 64-µm-mesh plankton net with 30-cm-diameter opening, 1:3 diameter:length bias; or 12-liter Schindler-Patalas trap.
- Method: open water—vertical tow from 3 m depth or 0.5 m above the bottom, or oblique tow starting at 3 m; flowing water—pump or pour 200 liters (53 gallons) through net, less in highly eutrophic waters.
- Sampling interval: once per week while water temperature is above 10°C.
- Preserve sample in 5% buffered sugar formalin or ethanol (see Appendix IV).
- Count veligers in five subsamples from each sample.
- Report mean number of veligers/m^3 and variance.
- Ancillary data: water temperature at depth of sample, Secchi disk depth, direction and rate of current.
- Optional data: water temperature at 3, 5, and 10 m; adjacent substrate type; Ca^{++} concentration; organic carbon and chlorophyll concentrations; identification and enumeration of other planktonic organisms in samples.

3.2 General Comments

Veligers are microscopic, planktonic organisms. To estimate their abundance in the water column, they must first be concentrated by sieving a known volume of water through a plankton net. The net mesh must be sufficiently small to retain the veligers. The distribution of veligers can be highly nonrandom in time and in space due to the synchronous release of eggs within a colony (Stanczykowska 1964 and references therein). Consequently, there is a high probability that a single sample from a single location will not contain veligers, even if veligers are present in an adjacent area or were present one week previously (Figure 4). The challenge is to determine the optimal intervals of distance and sampling frequency that will maximize the probability of finding veligers while minimizing the sampling effort. Replicate samples must be taken to estimate the variance in the veliger densities. Otherwise, a single sample taken by chance within a clump of veligers could yield a significant overestimation of veliger abundance. A large number of small samples will give better results than subsampling a few large samples.

To maximize the potential to detect veligers in a new area, use of a pump or Clarke-Bumpus sampler to filter large volumes of water from several depth strata is optimal. Pumped samples are also the most quantitatively accurate samples because of the ease of measuring the volume of water that passes through the net. Plankton net flowmeters are notoriously inaccurate (except for the very expensive models). Time spent on quantifying densities and ensuring replication, however, is less important than sampling large volumes of water in multiple areas.

Veliger densities may range over several orders of magnitude. When densities are low, large volumes of water must be sampled to detect the veligers. One method to sample large volumes is to pump water slowly through a plankton net for several hours. When veliger densities are high, other planktonic organisms will also be abundant. The volume of water sampled should therefore be reduced to facilitate counting the veligers and to avoid clogging the plankton net. The sampling volumes recommended below should be adequate for detecting and counting veligers until densities become very high (>10,000/m^3). If net clogging becomes a problem or veliger densities are too high to count without diluting the sample, reduce the sample volume. Record the volume sampled to estimate veliger density.

Remember that veligers clinging to plankton nets can be readily transmitted to an uninfected body of water. *Disinfect all water-contact equipment when transferring equipment between infested and noninfested sites.* Disinfect by rinsing with ethanol or formalin in a ventilated area or by drying equipment thoroughly. A 5% chlorine rinse can be used for most pieces of equipment but may harm the Nitex mesh of a plankton net.

3.3 Sampling Period

Zebra mussels release their gametes (eggs and sperm) after the water temperature has been maintained at 12°C (54°F) for one to two weeks, though exceptions to this pattern have been noted (Joe Leach, personal communication). This temperature normally occurs in mid-May in the lower Great Lakes but can vary considerably from year to year and between different locations. Therefore, the water temperature should be monitored starting in the early spring. Sampling the water column for veligers is usually unnecessary until the local water temperature has reached 10°C (50°F).

Sampling frequency depends upon the cost of each sampling trip versus the need to have frequent estimates of veliger densities. Veligers are present in the water for approximately one month after each mass spawning event, so the density of veligers over time at a given site probably approximates a standard curve (Figure 4). Samples taken every two weeks could miss the peak density and thus underestimate maximum veliger counts. Again, decisions about the frequency of sampling required must be based on an assessment of information needs.

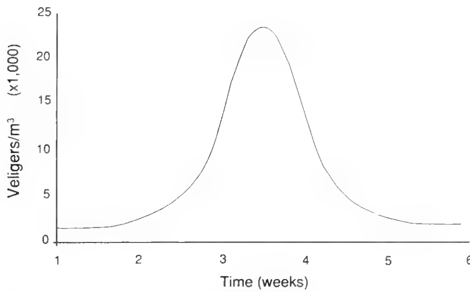

Figure 4. Possible distribution of zebra mussel veligers over time at a given location. Note that sampling once every two weeks could miss the peak veliger density.

3.4 Equipment

- One plankton net with a 30-cm-diameter opening, 63- or 64-µm mesh (= size 25; 200 meshes per inch), and 1:3 bias. Attach a wide-mouth mason jar screw lid rim into the end of the net using a hose clamp. Alternatively, a mesh-lined plankton bucket can be used. Attach small lead weights to the hose clamp to ensure rapid sinking of the net. A 12-liter Schindler-Patalas trap can be used for shallow water samples.
- (Optional) Electronic or digital flowmeter mounted in the mouth of the net. If the net clogs at all during sample collection, the flowmeter will give a better estimate of how much water actually flowed through the net than could be derived from calculations using the length of the tow. However, only the expensive flowmeters (>$200 U.S.) tend to be very reliable.
- One case of wide-mouth 1-pint mason jars with lids.
- Eight liters (2 gallons) of 10% buffered sugar formalin or ethanol.
- (Optional) Sieve made from a 250-ml plastic beaker with the bottom cut off and replaced with 64-µm plankton net mesh glued across the bottom.
- Squirt bottle or water pump.
- Clipboard, pencils, standard forms, and sample labels (see Appendix V).
- Thermometer or temperature probe.
- Secchi disk, 20 cm diameter.

3.5 Open Water

Sample Site

Sample sites should be chosen to maximally represent the various strata that affect veliger distribution or the logistics of sampling. Such strata include inshore areas, offshore areas, inlets, outlets, sheltered bays, areas of high current, areas with high boat traffic, etc. Sampling results from one stratum cannot necessarily be used to infer veliger distributions in other strata. Maintaining the same sites in each stratum is less important than sampling as many strata as possible.

Where possible, nearshore sites should be at least 5 m deep to avoid collecting sediment in the plankton net. Net clogging can also be avoided by sampling over hard substrates or by sampling after a period of clear weather when disturbed sediments have had time to settle.

The Schindler-Patalas plankton sampler is a good alternative to a plankton net for shallow water samples. Because it is more expensive, more delicate, and somewhat more complicated to deploy than a plankton net, its use is not described here. Investigators familiar with the Schindler-Patalas sampler are encouraged to use it.

Vertical Plankton Tow

1. Prepare the net by screwing a wide-mouth 1-pint mason jar or plankton bucket into the jar lid attached to the end of the net.
2. Record the sample number, date, time, station identification, and bottom depth on the sample form. Label sample jars (see example, Appendix V).
3. Drop the net to 3 m depth or to 0.5 m above the bottom, whichever is shallower. Retrieve the net by pulling it vertically through the water column with a steady, unhurried hand-over-hand motion. Retrieval to the surface should take approximately 10 seconds (0.5 m/sec).
4. Calculate the volume of water sampled as follows:

   $$\text{volume sampled} = \pi \times \text{length of tow} \times \text{radius of net opening}^2$$

5. Wash down the sides of the net *from the outside* (to avoid adding additional organisms to the sample) to wash any organisms on the netting into the jar. Washing can be done with a water pump, hand bilge pump, hand pumped fire hose, squirt bottle, or with water in any small, clean container. When the net is clean, carefully remove the jar.
6. Drain the jar to one-half full by pouring its contents through a section of the plankton net mesh. Wash any sample accumulated on the mesh into the jar using a squirt bottle. Alternatively, use the sieve described under "Equipment" by pouring excess sample through the sieve. Flush the sieve into the sample jar.
7. Preserve the sample by filling the sample jar with 10% buffered sugar formalin. Invert the jar several times to ensure mixing. This will dilute the preservative to the required 5%. Alternatively, add 95% ethanol to a one-third full jar. (Note: Use of other preservatives such as Lugol's solution will damage the veligers and hinder identification.)
8. After use, rinse the plankton net to remove any remaining organisms and allow the net to dry to kill any remaining veligers.

If observation of live specimens is required, divide the fresh sample into two equal parts after shaking to evenly distribute the contents in the water. Keep one subsample cool to increase the longevity of the organisms, and preserve the other subsample by adding buffered sugar formalin or ethanol.

Oblique Plankton Tow

Large-volume sampling for presence/absence data, or where veliger densities are extremely low, can be

accomplished by doing multiple vertical plankton tows, pumping water through a net (see below), or with an oblique plankton tow. An oblique plankton tow consists of setting the net at 3 m (or 0.5 m above the bottom if water depth is less than 3 m) and pulling it to the surface while towing it behind a boat. The boat speed must be slow (approximately 2 knots). Problems that may be encountered when doing an oblique tow include snagging the net on the bottom, clogging the net with disturbed sediments, and estimating the volume of water sampled. Measuring the volume of water sampled requires a flowmeter; however, as mentioned above, even a flowmeter may not accurately measure the volume of water sampled. Even when veligers are absent, other plankton may be abundant, and the net is likely to clog rapidly. A horizontal tow, in which the net is kept just below the surface, can also be used. The volume of water sampled can then be calculated from the tow distance, estimated from the boat speed.

Pumped Sample

Sampling with a pump is useful in shallow water and other areas where disturbed sediments or plankton blooms may clog a plankton net. Pumping is also the best method for sampling in large rivers, where currents and high plankton densities prohibit use of plankton tows. In strong currents, a weight may be needed to hold the pump or pump hose at the desired depth. By pouring or pumping water through the net, the flow of water through the net can be controlled to prevent overflow and consequent overestimation of the volume of water sampled. Water-use facilities may choose to pump a sample of water through a plankton net in preference to deploying the net in a water intake. For on-shore samples, the plankton net can be suspended by its rim in a 55-gallon drum fitted with an outflow tube near the bottom. Water is poured or hosed through the net until the drum is filled to a known volume, and then the drum is emptied through the outlet. This procedure can be repeated as many times as needed until the desired volume has been sampled.

Note that both centrifugal and diaphragm pumps can be used for sampling veligers. Field data from several sources indicate that even high-velocity/small-orifice pumps do not appear to destroy a significant proportion of veligers. Installing a screen, such as fiberglass window screening, over the pump opening is advisable to prevent the pump from clogging with debris.

Scouring Pad Samplers

These samplers, described by Martel in Appendix III, provide an integrated plankton sample over time and thus sample more water than can be accessed by a single plankton tow. They are broadly applicable for zebra mussel sampling but are especially useful in areas where severe weather or vandalism are problems.

1. Assemble three samplers and deploy with the top sampler at a depth of 3 m.
2. Retrieve sampler 24 hours after deployment. Place sampler in water or preservative for transport to the laboratory.
3. Repeat the procedure once per week, or as desired.

3.6 Flowing Water

Sample Site

Samples should be taken from at least two sites, one near-shore and one in the open current. Slower currents near the shore will be more conducive to veliger settlement, whereas veligers in the center of the current will be carried downstream. Take care to avoid areas where silt and debris may clog the net. Mark the sites using landmarks or stakes so that the same sites can be used repeatedly.

Collection Procedure

Use the procedures described under "Pumped Samples" or "Scouring Pad Samplers," above.

3.7 Water Intakes

Water intake samples will primarily be used by individuals concerned with veligers infesting water works or water cooling stations. When information on the efficacy of control measures is required, set up two sampling stations, one at the water intake upstream of the control initiation point (e.g., chlorine injection) and one at a point within the plant at which veligers must not be found.

Sampling can be done using any of the three methods described above. A vertical plankton tow can be used in a wet well or settling tank, a plankton net can be deployed near an intake pipe so that water flows through the net, or water can be pumped or poured through a suspended net. Turbulence is a problem for within-plant sampling; care must be taken to locate sampling devices to minimize damage due to high flow. Vertical plankton tows are frequently unfeasible because turbulence may drag the net sideways, impeding estimation of the volume of water passing through the net. Many plants have installed bioboxes to monitor settling juvenile mussels. Results from some bioboxes, however, have been equivocal: mussels have settled elsewhere within a plant, but not in the biobox. Bioboxes are clearly useful for straightforward monitoring, but their efficiency should not be assumed under all circumstances.

3.8 Collection of Ancillary Data

At each sample site:

1. Measure water temperature at the sample depth. In turbulent water, the surface and sample depth temperatures may be the same, in which case surface temperatures can be used. Report water temperatures in degrees Celsius.
2. Measure and record the Secchi disk depth. Attach a Secchi disk to a line and lower it into the water until the white quarters are no longer visible. The depth at which the white disappears is the Secchi disk depth. For accurate estimation of depth, the line must be vertical when the measurement is taken; additional weight may be necessary to hold the disk down in a current.
3. Record the depth at which the sample was taken.
4. Record the direction and velocity (m/sec) of local currents. In open water, this will vary according to weather patterns. In intake pipes, current is equivalent

to the intake velocity. For flowing water samples, carefully measure the current at each of the sample sites. If possible, also measure Ca^{++} concentration, dissolved oxygen, chlorophyll content of the water, and organic carbon using American Public Health Association (APHA) standard techniques (APHA 1989).

3.9 Counting Veligers

Veliger Identification

Veligers collected in the plankton tow will usually range from 40 to 250 µm, although mussels up to 2 mm long may be found (Appendix III; Lois Deacon, Ontario Ministry of Natural Resources, personal communication). Veligers can generally be observed only by using a microscope with at least 40x magnification, and preferably 50–100x magnification. Photographs of veligers at various life stages are shown in Hopkins (1990); a sketch of a veliger, as well as of common plankton of similar size, is shown in Figure 5. Post-trochophore larvae (≥ 60 µm) can be readily identified because they look like microscopic clams. Native clams, except for the false dark mussel, have a glochidia larva, which is not planktonic and has a readily distinguishable flagellum or whiplike appendage protruding from the shell.

The only organisms that could be confused with zebra mussel veligers are the veligers of the introduced Asian clam, *Corbicula fluminea*, and the false dark mussel, *Mytilopsis leucophaeata*. *Corbicula* are found as far north as western Lake Erie and southern Lake Michigan (Scott-Wasilk et al. 1983, White et al. 1984). The most northern populations are usually associated with warm-water effluents from power plants. In areas where the species overlap, differentiation of *Corbicula* and *D. polymorpha* veligers may be difficult or impossible; the primary distinction is the slightly flattened hinge in the *Corbicula* veliger, which is larger than the D-shaped *Dreissena* veliger (Figure 5). *M. leucophaeata* occurs in brackish, estuarine waters and in the upper Mississippi and Hudson rivers. Correct identification of veligers is painstaking and requires extensive experience; basically, the three veliger types should be considered indistinguishable.

Until veligers are seen and identified for the first time, anxiety about misidentification can be great. The best solution is to obtain a sample of known veligers from someone familiar with zebra mussels; however, be aware that supplying such samples can place a considerable burden on the relatively few investigators and consulting firms who make regular collections of veligers. Potentially confusing components of the plankton can be eliminated rapidly by a mental review of the following characteristics: veligers lack legs, antennae, eye-spots, or stalklike appendages; they have a crisp outline (many rotifers seem to have a fuzzy outline, even if their stalk is not visible); and a dark line is usually apparent within the shell. To assure correct identification, use a probe to turn the veliger on its side so that the two shells are clearly apparent.

Cross-polarized light can be used to facilitate detection and identification of veligers. Cross-polarization is accomplished by using a microscope with a polarizing filter above and below the sample. One filter is rotated until the only light passing through both filters is that which is refracted by certain substances. Veligers appear irridescent under cross-polarized light (E. Marsden, unpublished observations; Johnson 1992).

Newly hatched trochophore larvae may lose several features such as the cilia and velum during preservation. If identification of this brief stage is required, observation of live veligers is advisable. Veligers can be kept alive for up to a week if the sample is placed in an ice chest immediately after collection and is maintained at about 5°C.

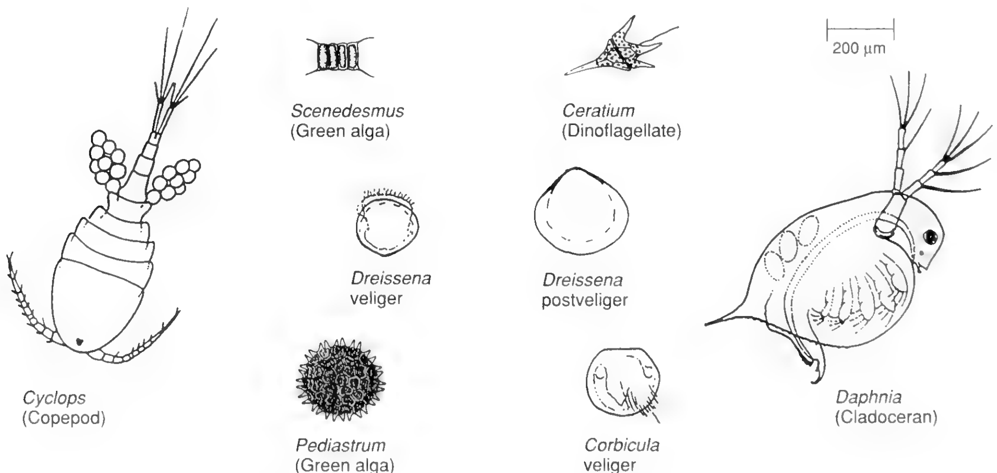

Figure 5. Comparison of zebra mussel veligers with other common planktonic organisms.

Presence/Absence Data

Prior to the first detection of zebra mussel veligers in a local area, portions of each sample can be pooled to look for veligers. Large volumes of water can be scanned by pouring 25 ml into a Petri dish and examining the dish under a dissecting microscope. Once veligers are seen in one of these pooled samples, go back and count the veligers in each original sample from that date to obtain quantitative data. To maximize the probability of detecting veligers in a plankton sample, veligers can be concentrated in the sample by either of two methods:

1. Add a few drops of ethanol to the fresh (unpreserved) sample. This will slightly anesthetize the veligers, which will sink to the bottom. A drop of water can then be pipetted from the bottom of the sample jar and placed on a microscope slide for observation. Other narcotizing agents include magnesium chloride, MS-222, CO_2, and chlorotone.
2. Use Schaner's sugar solution method described in Appendix II.

These methods should only be used for rapid detection of the presence of veligers; they should not be used if the density of veligers is to be calculated.

Detection Limit

Your ability to detect veligers is limited by the volume of water sampled, the volume of the concentrated sample, and the number of subsamples examined. For example, if you sampled 200 liters, concentrated the sample to 200 ml in the sample jar, and then counted five 1-ml subsamples, then the lowest detection limit (one veliger) would be

$$\frac{\text{(1 veliger/total subsample vol.[ml]) x vol. conc. sample (ml)}}{\text{vol. water sampled (ml)}}$$

$$= \frac{\text{(1 veliger/5 ml) x 200 ml}}{\text{200 liters}} = \text{0.2 veligers/liter}$$

If a lower detection limit is desired, as in areas where imminent infestation is predicted, more subsamples should be examined. For perspective, the first sighting of veligers at one location in Ontario was at densities of 0.002 veligers per liter (Lois Deacon, personal communication).

Veliger Densities

Veliger densities may vary by several orders of magnitude among sample sites. Densities as high as 1,000,000/m^3 (1,000/liter) have been reported in Lake Erie (Joe Leach, personal communication). A vertical tow sample from 3 m in this density of veligers would collect

depth x π x radius of net mouth^2 = vol. of water sampled
3 m x 3.14 x $(0.15 \text{ m})^2 = 0.212 \text{ m}^3$
vol. of water sampled x veliger density = # veligers in jar
0.212 m^3 x (1,000,000 veligers/m^3) = 212,000 veligers

If 1-ml subsamples are taken from 200 ml of concentrated sample to count veligers, each will contain

in sample/vol. of sample jar (ml) = #/unit vol.
212,000 veligers/200 ml = 1,060 veligers/ml

Counting this many animals under a microscope is not only time consuming but also difficult to do accurately. An efficient counting protocol will minimize the number of organisms to be counted while also minimizing the variance among samples. Counting precision can only be estimated by counting replicate subsamples, then calculating the variance among the counts. To minimize variation among subsamples, each subsample count should include approximately 60 individuals. If there are extremely few veligers in the sample, so that some 1-ml subsamples have no veligers at all, examine the total contents of 10 ml of sample (= 10 Sedgewick-Rafter cells). The sample can be further concentrated by sieving through 63-µm mesh.

Equipment

- Stereomicroscope (dissecting microscope) with magnification to at least 40x, preferably to 50–100x.
- Ocular micrometer for microscope if veliger size measurements are required.
- Sedgewick-Rafter counting cell (Figure 6) or plankton wheel.
- Disposable Pasteur pipettes and rubber suction bulbs; calibrate pipettes by marking them at 1 ml volume.
- If available: magnetic stirrer, Hensen-Stempel pipette.
- Dissecting probes.

Counting Procedure for Plankton Tow

1. Mix the sample completely by swirling the jar or using a magnetic stirring plate. Remove 1 ml of sample from the center of the jar using a Pasteur pipette or Hensen-Stempel wide-bore pipette. Place a cover glass diagonally across the Sedgewick-Rafter cell. Fill the cell slowly until the water is evenly in contact with the cover slip. As the water comes in contact with the cover slip, the slip will swivel until it covers the cell. You may wish to dispense with the cover slip if you need to manipulate organisms on the slide.
2. Examine the slide to familiarize yourself with common organisms in the sample, and get a feel for scale and for nonveliger shapes. At this point, evaluate whether the sample needs to be concentrated or diluted. High plankton densities may obscure your ability to see veligers; low densities decrease your chances of finding veligers.
3. Scan the Sedgewick-Rafter cell at 50x to detect veligers. If necessary, verify veliger identification at higher magnification.

   Low veliger densities. Count the number of veligers in the entire Sedgewick-Rafter cell by scanning evenly back and forth over the cell. Count the cell at least twice, or until the same total is reached twice.

   High veliger densities. If the samples are crowded with veligers and other planktonic organisms, you can dilute the sample to reduce veliger densities (record the dilution!). Counting large numbers of veligers in the Sedgewick-Rafter cell can be simplified by placing a grid under the cell so that the veligers can be counted in smaller units. A reverse grid (white lines on black background) is preferable because the pale veligers are more highly visible against a dark background.

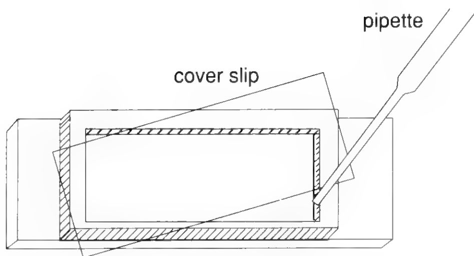

Figure 6. Sedgewick-Rafter cell for counting veligers.

4. Repeat the procedure for a total of five samples from each sample jar. Record each count separately on the sample form.

Counting Procedure for Scouring Pad Sampler

1. Rinse each sampler three times by forcing a jet of water (from a hose or spray bottle) through the pad, working the spray across the entire pad. Rinse into a shallow pan. Check efficiency of rinsing by noting how many organisms are removed from the pad by a fourth rinse. This check will become unnecessary with practice.
2. Sieve the collected water, including liquid from the container used for transportation, through a 63-µm-mesh sieve, until a workable volume of liquid is achieved. Preserve with ethanol or buffered sugar formalin, unless observation of live juveniles is desired.
3. Subsample, if necessary, by serially diluting the sample into equal volumes. Check that the split is equal by examining the density of organisms in more than one subsample. Place successive volumes of sample in a Petri dish and count all the juveniles in the dish using a dissecting microscope; repeat until the entire sample or subsample has been processed. Counting can be facilitated by placing graph paper underneath the Petri dish and scanning rows of squares.
4. If size measurements are needed, measure the length of approximately 50 juveniles. Choose juveniles randomly by selecting a subset of the Petri dish (e.g., the right half) from which to measure juveniles.
5. Report data as the number of juveniles collected per cubic centimeter of pad per day deployed. For example: 350 juveniles collected in a 10 cm x 10 cm x 5 mm pad in 24 hours = 350/50 cm^3/day = 7/cm^3/day.

Calculation of Veliger Densities

Determine the number of veligers per liter of lake water as follows:

veliger density = # in sample jar/vol. of water sampled,
where # in sample jar = (#/unit vol.) x vol. of sample

The number of veligers per milliliter of sample (number per unit volume) is the number counted in a Sedgewick-Rafter cell, multiplied (if necessary) by the number of dilutions used; for example, multiply by 2 if the sample was diluted by one-half.

The volume of lake water sampled during a vertical tow is calculated as follows:

depth of tow x π x radius of net mouth^2 = vol. of water sampled
3 m x 3.14 x $(0.15\ m)^2 = 0.212\ m^3$

Calculate the mean number of veligers per cubic meter by averaging over the five samples. Calculate the variance among the samples using the following equation:

$$s^2 = \frac{\sum(x-\bar{x})^2}{n-1}$$

where x = number of veligers in each of the five samples; $\bar{x}$ = the mean number of veligers in the five samples; and n = the number of samples = 5

Some people prefer to report veliger densities as number per liter. To convert from veligers per cubic meter to veligers per liter, multiply the number of veligers by 0.001.

4 Sampling Settling Juveniles

4.1 Outline

- Locate sample at sites of interest.
- Sampling plates: 15-cm-square PVC plates, slide rack containing microscope slides, or multiplate sampler.
- Deploy settlement plates, slide rack, or multiplate sampler at 3 m. Retrieve and replace plate, slides, or multiplate sampler at each sampling interval.
- Sampling interval: once per week while water temperature is above 10°C.
- Preserve slides and plates in buffered sugar formalin or ethanol.
- Scrape one side of each plate clean with razor; count settled juveniles in 1-cm squares on each plate until >60 juveniles have been counted, or the entire surface of the plate has been examined.
- Calculate surface area counted on each plate; report data as number of juveniles per square meter and volume of juveniles per square meter.
- Ancillary data: Water temperature at surface, Secchi disk depth, direction and rate of current.
- Optional data: Water temperature at 3, 5, and 10 m; adjacent substrate type; Ca^{++} concentration; organic carbon and chlorophyll concentrations; identification and enumeration of other organisms settled on slides.

4.2 General Comments

Juvenile settlement is affected by substrate type, substrate texture, depth, light, water currents, proximity to other mussels or adjacent surfaces, and ionic concentrations. Figure 1 gives an indication of how dramatically settling densities can be affected by substrate material. Settling veligers select substrate both during and after settlement. The settled juveniles can crawl up to 3.8 cm/hr for several days before making a permanent attachment to the substrate (Lewandowski 1982). They may be seeking a better surface than the one on which they originally settled. Because juveniles may move off a sampling plate after settlement, the numbers of juveniles estimated may be affected by the period of plate immersion. Juvenile counts will also be affected by the following factors:

- Sampling plate orientation. If the plate is horizontal, different numbers of veligers will settle on the upper and lower surfaces, due to light avoidance. Juvenile settlement will also be different on horizontal versus vertical surfaces.
- Edge effects. Juveniles appear to be thigmotaxic: they aggregate near corners or each other.
- Turbulence. Juvenile settlement appears to be inhibited in areas of high turbulence.
- Biofilm. Juveniles tend not to settle on "fresh" surfaces that have not acquired a microscopic biofilm; some species of algae and diatoms may also inhibit settlement (C. Brousseau, Ontario Ministry of Natural Resources, personal communication).

For presence/absence sampling, the sampling substrate should be one that is favored by settling mussels. Juveniles prefer to settle on horizontal, shaded surfaces with a lot of surface irregularity—that is, corners and crevices. Roughened surfaces and PVC are also favored by settling mussels. One of the simplest techniques for monitoring is to suspend concrete blocks in the water; although the earliest settlement stages will not be noted against the coarse texture of the block, larger juveniles can be seen on the block and on the line that holds it.

Microscope slides are optimal standard settling plates because (1) they are readily available, (2) they are uniform from source to source, (3) they are inexpensive and disposable, (4) juveniles are readily observed against a smooth, uninterrupted background, and (5) slides can be examined under a microscope if detection of the smallest settled juveniles is important. Lewandowski (1982), D. Garton (Ohio State University, personal communication), and others report good settlement of juveniles on slides. Microscope slides are also an accepted standard for limnological studies involving settlement (Lind 1979, APHA 1989). The smallness of microscope slides relative to other samplers does not affect the settlement of juveniles; the surface available to examine is merely reduced. Slides do present difficulties, however, because of their fragility; this can be overcome by combining sampling methods, as suggested under "PVC Plates," below.

Whatever type of settlement plate is used, vertical deployment is preferable to horizontal for several reasons: (1) sediment and organic matter will settle on the upper surface of horizontal plates, making it difficult to discern settled juveniles; (2) use of horizontal surfaces adds the onus of having to mark which surface was on top, and then counting both surfaces; (3) juveniles that land on the upper surface of a plate tend to migrate to the lower surface (Lewandowski 1982), so the counts on the upper and lower surfaces must somehow be integrated; (4) horizontal plates will tend to "kite" in currents, thus changing the depth of the sampler and increasing abrasion on the lines. Fewer *Corbicula* juveniles may be found on vertical surfaces because they generally secrete only a few byssal threads for attachment and may fall off these surfaces.

Of the various methods and substrates that have been used to sample settling juveniles, three are described here because they fit the criteria of ease of construction, ease of use, and applicability in diverse field situations. Juvenile settlement on any of the samplers is enhanced if the plates have been "prepared" by immersion in fresh water for one to two weeks, during which time the plates become colonized by a microscopic layer of biological material. Obviously, the longer the samplers are immersed, the larger the juveniles that will have settled on the plates. The immersion time should be chosen to balance how rapidly

information is needed with the increased ease of seeing and counting larger juveniles.

4.3 Sample Site

Choose sites based on the comments on strata in section 3.5. Avoid sites near chemical discharge points, which could adversely affect mussel settlement. Also avoid sites in the path of boat traffic or near popular fishing areas, where samplers could be inadvertently snagged. Label each sampler, as needed, with your agency's name, a sign stating "Scientific Equipment—Do Not Disturb," and a contact number or address in case the sampler is inadvertently damaged or washed ashore.

Deployment of artificial substrates can be the most difficult part of zebra mussel sampling because the apparatus may be vulnerable to storms, currents, turbulence, and vandalism. If you are monitoring in the open waters of the Great Lakes, or in areas of high human activity, expect to lose several samplers, no matter how well you have anchored or protected them. Losses may be reduced if you follow the following suggestions:

- Choose a protected site, such as a bay.
- Deploy replicate sets at each site whenever possible.
- Use at least 1/4-in. line for buoys, anchor attachments.
- Wherever line may chafe, such as where it runs through a buoy ring or where it is attached to a cinder block anchor, protect the line with a rubber sleeve such as garden hose or Tygon tubing, or use chain.
- Use twice as much weight for an anchor as seems necessary. Three cinder blocks chained together is a minimal anchor for areas where waves may reach 1–2 m high.
- Maximize the scope on the buoy (i.e., length of line relative to water depth). Use at least a 5:1 to 7:1 ratio of line to depth.
- Deploy a lighter, secondary anchor between the sampler and the main anchor, approximately 3 m (10 ft) from the main anchor (Figure 7). This light anchor acts as a shock absorber to reduce sudden shock loads on the main anchor and buoy attachment. Alternatively, use a satellite buoy (Figure 7).

Near-shore monitoring can take advantage of fixed structures such as piers, docks, and buoys (if permission is sought first!) to attach sampling equipment. Remember, however, that shore access increases the probability that your equipment will be vandalized. Samplers can be deployed under a submerged buoy to avoid wave and human damage. During deployment, run a line from the anchor to shore; during retrieval, the line can be followed out from shore to the sampler using a gaff to hold the line. In areas where water levels may vary considerably due to floods, water draw-downs, etc., use of fixed structures to deploy samplers is inadvisable because settling juveniles will not tolerate repeated drying periods.

4.4 Equipment

- Settlement sampler of choice.
- 2.5 x 7.5 cm microscope slides.
- Indelible pen or paint for labeling slides.
- 2–3 liters 5% buffered sugar formalin or ethanol.
- Ropes, anchors, and buoys to suspend slide rack at desired depth.

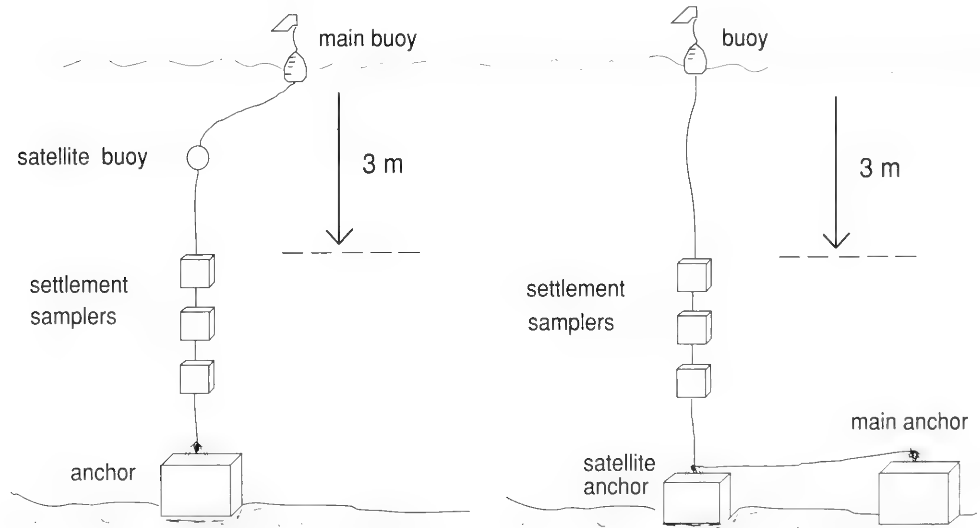

Figure 7. Suggested methods for deployment of settlement plates.

- Razor for scraping slides.
- Clipboard, pencils, standard forms, and sample labels (Appendix V).

4.5 Collection Procedure

PVC Plates

1. Attach three 15-cm-square PVC plates in series (see Figure 8). (Note: Plexiglas, which is often used for settlement plates, has two major disadvantages: it dissolves slightly in ethanol, and its transparency may discourage juvenile settlement. Gray PVC is preferable to white because of the preference of zebra mussel juveniles for dark surfaces.) Attach a microscope slide to each plate using a binder clip or similar device.
2. Deploy the plates so that the upper plate is suspended at a depth of 3 m. Deploy the plates at least one week before you anticipate settling may begin, in order to condition the plates.
3. Once per week, or at longer, regular intervals determined by your sampling plan, remove the bottom plate and put a fresh plate between the remaining two plates. Thus, each plate will have been in place for two weeks (one week for conditioning) before retrieval, except for the first plate retrieved each season. Place the retrieved plate in a container with buffered sugar formalin or ethanol, such that the sides of the plate are protected from accidental scraping. A plastic food container in which the plate is supported diagonally works well. Handle the plates by the sides to avoid damaging settled juveniles. If observation of live juveniles and other settled animals is desired, place the plate in water for transportation and keep it cool. Examine "fresh" plates within a day of collection to avoid decomposition of the settled animals. On the next sampling date, the new bottom plate is retrieved, having been in place for two weeks. At the end of the sampling period, remove the top plate to estimate the seasonal accumulation of juvenile mussels.

Slide Rack

Periphyton sampling racks that hold a number of microscope slides can be purchased ready-made (Figure 9; see Appendix IV). The interval between sampling dates should be determined by access to the sample site. Weekly sampling, suggested here, is ideal but may not be practical at distant sites. Most important is to sample at regular intervals.

1. Label microscope slides using an indelible marker. Place slides into the rack.
2. Deploy the rack so that it is suspended at a depth of 3 or 5 m. A choice of depths is given because one or the other depth may be inaccessible at some sites. Ideally, suspend a rack at each depth from the same buoy-anchor line. Deploy the rack at least two weeks before settling may begin, in order to condition the slides. Place the rack so that the slides are vertical.
3. Once per week remove five slides (every alternate slide) from the rack and place them in a slide box. Immerse the slide box in 70% ethanol or buffered sugar formalin. Handle the slides by the sides to avoid damaging settled mussels. Place five new slides in the rack. On the next sampling date, remove the next set of slides. This way, each slide will be in place for two weeks before collection.
4. Once per month, remove two slides that have been in place since the rack was deployed. These slides permit observation of long-term settlement and will not be replaced.
5. Prior to examining the slides, scrape one side clean with a razor blade. Avoid "selecting" which side to clean based on the number of mussels observed. For example, always scrape the unlabeled side.

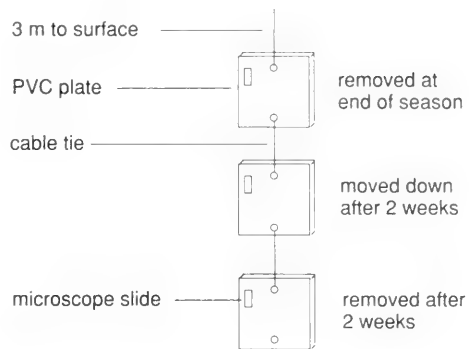

Figure 8. Vertical PVC plates for collecting settling zebra mussel juveniles.

Multiplate Samplers

Multiplate samplers (Figure 10) have the advantage that, like microscope slides, they are traditionally used for periphyton sampling. They also can be designed to conveniently fit into a mason jar for preservation and shipping. Their major disadvantages are the horizontal orientation of the plates and the need to retrieve an entire sampler for disassembly to collect data. Multiplate samplers are commonly constructed of tempered hardboard, but they can also be manufactured from PVC. The design suggested by the U.S. Fish and Wildlife Service (William Mason, personal communication) consists of eight PVC and two hardboard disks, 7.5 cm in diameter and 0.3 cm thick (Figure 10). The disks are held together on a 17-cm length of threaded rod. The spacers can be cut lengths of small-diameter PVC tubing or stacks of plastic or stainless steel washers. A turnbuckle is then screwed onto either end of the threaded rod to provide attachment points for deployment lines.

1. Deploy two multiplate samplers at a depth of 3 m at each site. Deploy the samplers at least two weeks before you anticipate settling may begin, in order to condition the plates.

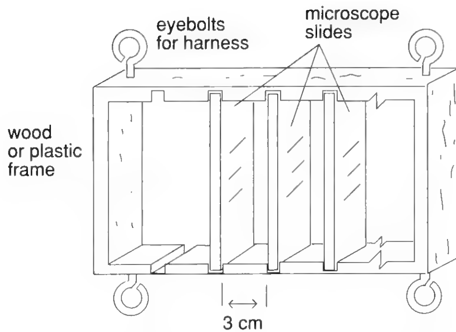

Figure 9. Slide rack for collecting settling zebra mussel juveniles.

2. Retrieve one sampler every two weeks, replacing it with a new sampler; or use longer, regular intervals as dictated by your sampling plan.
3. Remove turnbuckles, then insert entire sampler into a mason jar and fill with 70% ethanol or 5% buffered sugar formalin.

Loss of some settled juveniles from the plates after retrieval is inevitable and difficult to quantify. Juveniles that have settled on a surface but not yet attached themselves with byssal threads are especially vulnerable to displacement. Juveniles that have fallen off inside the container used to transport slides or settlement plates can be counted by sieving the preservative through plankton netting. The mesh size should be slightly smaller than the smallest juveniles you wish to count. If the proportion of "lost" juveniles to total juveniles is consistent, a figure can be obtained and used subsequently to estimate juvenile loss. Juveniles lost during plate retrieval may be impossible to estimate unless the sampling plates are placed in a bag *in situ* before retrieval. Whether the effort is worth the result depends, again, upon the information needed.

4.6 Counting Procedure

When counting juveniles settled on plates, do not count near the edges of the plates where mussels are likely to have been dislodged during handling. Once the settled juveniles have begun to develop pigment and assume their adult shape, the only other organisms likely to be confused with them are ostracods. In live samples, ostracods are readily distinguished when they scuttle rapidly across the settlement plate; dead ostracods often fall off settlement plates. Ostracods are bean-shaped, often have fine hairs on the shell, and vary from 0.5 to 3 mm. Close examination and manipulation with a probe will reveal the presence of leglike appendages or antennae.

Slides

1. In the laboratory, gently scrape one surface of each slide clean using a razor blade. Place this side down on the dissecting microscope stage. Place a piece of graph paper with 1-cm^2 divisions under the slide.
2. Scan the plate or slide at 30–40x to find settled juveniles; confirm identification by turning the organism on its edge so that the two shells can be seen. Encrusted algae and other organisms may need to be probed and teased apart to detect newly settled zebra mussels. Newly settled juveniles will look like planktonic veligers; they will be white and approximately round, with a distinct umbo. As the settled juveniles grow, they will begin to elongate into the adult shape. Dark stripes generally begin to appear on the shell after the shell has begun to elongate.
3. Count the juveniles in each of five 1-cm squares that were previously marked on the graph paper to randomize the counts. Include juveniles on the upper and right-hand lines; ignore those lying on the lower and left-hand lines. Record the total number of juveniles in all five squares; repeat the count for the other microscope slides. Avoid the area that was covered by the clip. If juvenile densities are very high, count only as many squares as needed to reach a total of approximately 60 animals. Record the number of squares counted. If juvenile densities are very low, count the entire surface of the plate.
4. If a microscope is unavailable, count juveniles in five 1- cm squares using the naked eye. Use of a dark background, divided into a metric grid, will assist the count.
5. If size measurements are required, determine what size square will contain approximately 50 animals. This may be one 1-cm square, or it may be five 1-cm squares on each of two slides, depending on how densely the veligers have settled. Measure the length of all animals in the selected area using an ocular micrometer.
6. Do not reuse the slides. Even if the slides are scraped clean, their surface will be different (to a juvenile) than that of a fresh slide.

Plates

The plates can be processed in the same manner as the slides, except that a grid cannot be used beneath the opaque PVC. The surface of the PVC can be subdivided for counting by lightly drawing lines through the accumulated biofilm (scratches in the PVC will affect subsequent settlement of the juveniles!). Alternatively, 1-cm strips across the plate can be isolated by scraping away material on either side; juveniles within the strip can then be counted in 1-cm blocks. Clean plates by scraping with a razor blade, then washing. PVC plates can be reused, but

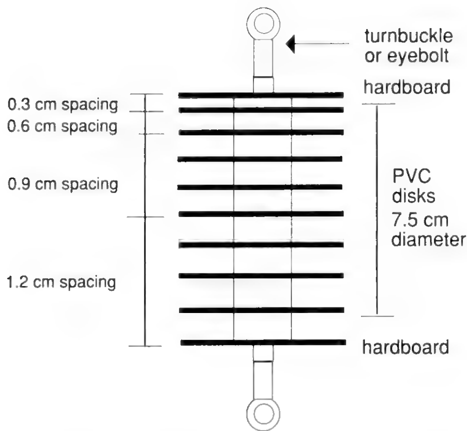

Figure 10. Multiplate sampler for collecting settling zebra mussel juveniles.

standardization of the texture of these plates is probably impossible, especially because the composition of the plastic itself varies slightly among manufacturers.

4.7 Collection of Ancillary Data

At each sample site:

1. Measure water temperature at the sample depth. In turbulent water, the surface and sample depth temperatures may be the same, in which case surface temperatures can be used. Report water temperatures in degrees Celsius.
2. Measure and record the Secchi disk depth.
3. Record the depth at which the sample was taken. If possible, also measure Ca^{++} concentration, chlorophyll content of the water, and total organic carbon using APHA standard methods (APHA 1989). In the laboratory, you can identify and count other settled organisms on the slides.

4.8 Data Reporting: Biomass vs. Density

Reports of high densities of settled juveniles or adult mussels are impressive and attractive to the popular press, but they are not very meaningful unless accompanied by size measurements. For example, 100,000 newly settled mussels per square meter may only constitute a monolayer 1–2 mm thick, whereas a similar density of 2- to 3-cm adults would form an encrustation over 10 cm thick. Unfortunately, acquisition of accurate biomass data requires time and specialized equipment and is beyond the scope of most monitoring programs. Details of dry-weight, wet-weight, and live-weight biomass measurements are covered by McCauley (1984) and need not be repeated here. Length and volume measurements may be the most accessible data with which to estimate biomass for most investigators. Ideally, a random subset of 50–100 animals of each age cohort from each sampling location and date should be measured. Minimally, the largest and smallest mussels should be measured, and the median size should be estimated by examination of the whole group. The relationship between free dry weight and shell length can be estimated using the equation of Bij de Vaate (1991); however, this equation requires calibration with field data. A more useful figure for general comparisons is the volume of mussels per unit area. Volume can be estimated using the displacement method: place the mussels from a known area in a beaker, fill the beaker with water to just above the mussels, then subtract the volume of water added from the total volume in the beaker. Report data as milliliters of veligers per square meter.

5 Sampling Adults

5.1 Outline

- Locate sample sites in areas of interest.
- Sampler: 1-m quadrat square divided into 10-cm squares, or smaller quadrat square; coring device.
- Sampling interval: at investigator's discretion.
- Quadrat method: for multilayer colonies, select a random location, take a core sample of known area, count all adults within core; for low-density colonies, count mussels in 10-cm-square areas until at least 60 have been counted.
- Grab method: use ponar grab to collect three replicate samples of bottom substrate.
- Estimate percent cover by zebra mussels within local area.
- Preserve adults in ethanol.
- Report data as number of mussels per square meter and volume of mussels per square meter.
- Ancillary data: water temperature at surface and sampling depth, Secchi disk depth, direction and rate of current, description of substrate type.
- Optional data: Ca^{++} concentration, organic carbon and chlorophyll concentrations, size of adults in 5-mm intervals.

5.2 General Comments

Sampling of adult zebra mussels provides information on their settlement and growth rates in a local area. The sampling protocol is intended for enumeration of animals large enough to be visible to the naked eye. Adults can also be counted on substrates such as smooth tiles that have been left in the water for several months. The substrate on which the adults are counted must be noted. Substrate material and texture will affect adult densities. Material texture will also influence accuracy of counting the smallest individuals, which may be lost against a high-relief background.

Sampling adult zebra mussels *in situ*, on natural substrates, usually requires scuba divers. Fixed sites such as water intake structures can only be sampled *in situ*. Mussel densities on natural substrates can be measured using a ponar grab, but ponar grabs do not sample bedrock or large cobble substrates well because the grab cannot close over large rocks. Sampling adults on natural substrates may not provide data that are comparable from site to site, due to differences in substrate type. For many purposes, use of artificial substrates will be simpler than sampling on natural substrates. For example, adult densities can be measured on concrete blocks or multiplate samplers placed in the water in the early spring. This measurement provides a good estimate of adult densities on nearby concrete structures; it also allows comparison of densities between different bodies of water that may not have similar natural substrates.

Much data can be acquired with the assistance of local dive clubs, which are often more than willing to find an excuse to dive. In large river systems, commercial shell fishermen have greater and more frequent access to the bottom substrates than anyone else and are therefore a valuable source of data. As noted in the introduction, these resources are invaluable for extensive monitoring.

5.3 Sample Site

Choice of sites to calculate adult densities will depend upon the questions of interest to the investigator. Note that adult densities will be dramatically affected by sampling location. Adult mussels can only settle on hard substrate and tend to prefer dark areas, corners, crevices, and other zebra mussels. These factors must be noted to avoid bias when sampling. As with sampling of veligers and juveniles, the various types of substrate in the sampling area should each be sampled to avoid bias. A report of adult zebra mussel densities must be accompanied by an estimate of the area represented by the measurement. For example, if you counted an equivalent of 10,000 zebra mussels on a unionid mussel isolated on a muddy bottom, report the average number of unionid mussels per square meter. Artificial substrates such as concrete blocks should be deployed in areas where they are unlikely to be interfered with by curious passersby or vandals and where currents or turbulence will not result in loss of the substrates.

5.4 Equipment

- Small (15-cm-square) ponar grab and a no. 30 sieve; or a quadrat frame.
- Quadrat frame. This can be purchased ready-made or can be easily constructed. The size of the quadrat square depends on the population densities of mussels. A 1-m square may be necessary to count reasonable numbers of mussels in areas of very low density ($<100/m^2$), whereas a 10-cm square may be sufficient in high-density areas. A quadrat square for use by divers can be constructed of 1-in. PVC tubing and right-angle connectors. Drill small holes in the frame to release trapped air so that the frame will be negatively buoyant. On a 1-m quadrat square, mark the edges at 10-cm intervals. String wire or line across the quadrat, tied at opposite edges, to subdivide the frame into 10-cm squares.
- "Coring" device 8–12 cm in diameter. This is not a true corer but a device for outlining a discrete, measureable area of hard substrate in which mussels can be counted. The device can be constructed from an aluminum food can opened at both ends. Cut the rim off one end to leave a sharp edge. A dowel passed through two holes at the upper end of the can will

serve as a handle. Measure and record the opening diameter.
- Fine-mesh bags of material such as cheesecloth (pantyhose material has also been used effectively).
- Large zip-lock bags.
- 2–3 liters of ethanol.
- Razor blades (the type used in paint scraping, mounted in a handle or having one blunted edge) and paint scrapers.
- Hammer.

5.5 Collection Procedures Using Quadrat Frame

Procedure 1 (high adult densities, >10,000/m² or multi-layer colonies)

1. Randomly place the coring device on the surface to be sampled. Decide on your method for randomizing *before sampling* to avoid bias. For example, pick a square in the quadrat frame, drop the frame so that it lands at random, then place the corer within the selected square. Using whatever force is necessary (this is where the hammer may come in handy), push the device into the colony of mussels until its edges are firmly in contact with the substrate.
2. Using a razor blade or paint scraper, remove mussels from around the device until there are none within several centimeters of the device.
3. Remove the device. If there is more than a single layer of mussels, measure the height of the isolated plug of mussels in centimeters. Carefully, to avoid damaging the animals, use a razor blade to scrape the mussels into a mesh bag. At the surface, transfer the sample to a zip-lock bag and add ethanol to preserve the mussels.
4. Repeat the procedure until five samples have been collected in separate bags. This procedure can also be used to estimate densities of mussels on introduced substrates such as tiles or on rocks brought to the surface by divers.

Procedure 2 (low adult densities, <10,000/m² or single-layer colonies)

1. Place the 1-m quadrat frame against the substrate to be sampled. Minimize inadvertent "selection" of the area to be sampled, which will result in biased data. This can be accomplished by dropping or throwing the frame onto a horizontal substrate so that it lands at random. For vertical substrates, one method is to keep your eyes closed, move parallel with the vertical surface, then place the quadrat frame against the surface. Do not decide to reposition the frame because there are "too few" mussels within it; replication will sample both high and low densities to give a representative average and variance.
2. Estimate how many 10-cm squares you will need to count to find a total of approximately 50 mussels. Select the required number of squares randomly before placing the quadrat on the substrate. For example, number the squares from 1 to 100, then use a random number table to select the squares by number.
3. Count the number of mussels in each of the selected 10-cm squares.
4. Remove the mussels from each of the selected squares using a razor blade; place the mussels in a zip-lock bag and cover them with ethanol.
5. Make five replicate counts (i.e., counts from five placements of the quadrat square, with the same number of small squares examined in each).
6. Calculate the total area counted in each 1-m quadrat square (= number of small squares counted x 100 cm²). Calculate the adult density as follows:

$$\text{adults/m}^2 = \frac{\text{\# of mussels counted x 10,000}}{\text{area counted (cm}^2\text{)}}$$

Procedure 3 (low densities, loose, irregular substrate)

1. Place the 1-m quadrat square against the substrate as described above.
2. Estimate how many 10-cm squares you will need to count to find a total of approximately 50 mussels. Randomly select the required number of squares before placing the quadrat on the substrate. For example, number the squares from 1 to 100, then use a random number table to select the squares by number.
3. Within the selected squares, remove rocks or other particles to which mussels are attached. Remove only those particles that have mussels attached and that have 50% or more of their mass within the sample square. Place all material collected into a zip-lock bag and add ethanol to cover the sample.

5.6 Collection Procedures Using a Ponar Grab

1. Select three sampling sites at approximately 3 m depth that are representative of the area to be sampled.
2. Lower the cocked (open) grab into the water until it touches the bottom, close the grab by releasing the messenger or trip line, then bring the grab to the surface. Place a bucket underneath the grab as it leaves the surface to collect small organisms that may be lost as the water runs out. If the grab is brought to the surface partially open because a hard object has jammed in the opening, discard the sample and take another grab.
3. Place the full grab into the bucket and wash out the contents using a bucket of water. Slosh the grab in the water until clean. Be careful: a grab in the cocked position can be dangerous if it shuts accidentally.
4. Pour the sample into a no. 30 sieve and wash it using a twisting, sloshing motion in the water. Do not let water slosh over the rim of the sieve.
5. Preserve the cleaned material that remains in the sieve in ethanol or buffered sugar formalin.

5.7 Collection of Ancillary Data

At each sample site:

1. Measure water temperature at the surface and the collection depth. If a temperature probe is available, measure water temperature at 3, 5, and 10 m. Report water temperatures in degrees Celsius.
2. Measure and record the Secchi disk depth.

3. Record the depth at which the sample was taken. If the sample was taken in an intake pipe, record the depth and location of the inlet opening, as well as where in the pipe (relative to the opening) the sample was taken.
4. Record the type of substrate sampled—for example, broken limestone bedrock, vertical concrete pilings, silty bottom with native mussels, etc.—and the type of sampler used. The substrate can be quantitatively described by using the quadrat frame. Place the frame randomly, then record the most common substrate type (>50% of the area) in each square. Flip the frame so that it rests on a new area, then repeat the data recording. Do this for five frame counts.
5. Estimate the proportion of the area of interest that is covered by mussels. This can be done in either of two ways:

   Random distribution of mussels. Place quadrat square randomly (see above) on the substrate, then record which squares are more than half filled with mussels. The percent coverage of mussels is then equivalent to the proportion of squares counted as more than half full. For example, if 43 of the 100 squares were more than half full of mussels, then 43% of the area inside the quadrat square was covered with mussels. Repeat this for a total of five quadrat square counts.

   Clumped distribution of mussels. Measure five clumps as follows. Measure the longest axis of the clump, then the length of an axis at right angles to the long axis. Measure the average distance between clumps, or the average number of clumps per square meter.
6. If possible, also measure Ca^{++} concentration, dissolved oxygen, chlorophyll content of the water, and organic carbon using APHA standard methods (APHA 1989).

5.8 Counting Procedure

1. In the laboratory, carefully separate the mussels from each other and count them. Discard all mussels broken during the collection process and any dead mussels (i.e., empty shells). Carefully examine the shells of each mussel and remove smaller attached mussels. Record the size of the smallest mussel that you count. Measure the length of approximately 200 individuals as illustrated in Figure 2. To select the individuals for length measurement, subsample by gently breaking the colony apart before separating individual mussels, so a random subsample is achieved. A dissection microscope with 10–40x magnification may be useful for this process.
2. If possible, count the number of individuals that were alive during sampling. Dead mussels will tend to have open, empty shells. Because some closed shells may be empty, each shell needs to be opened and examined.
3. If possible, record the wet weight of the sample in grams.
4. Calculate the density of the mussels:

$$\text{density (N/m}^2\text{)} = \frac{\text{\# counted x 10,000}}{\text{area counted (cm}^2\text{)}}$$

5. Estimate the volume of the mussels in a unit area by displacement. Place the mussels in a beaker, add water to just above the mussels, then subtract the volume of water added from the total volume in the beaker. Calculate the volume of mussels per unit area as follows:

$$\text{volume (liters/m}^2\text{)} = \frac{\text{volume of mussels (ml)}}{\text{area counted (cm}^2\text{)}}$$

Note: The counts from each square within the quadrat frame are summed to obtain a total, not an average. Replication is achieved by repetitive placements of the quadrat frame, not by multiple counts of squares within the frame.

6 Reporting Results

Several extensive monitoring programs are already under way in the Great Lakes. These programs provide regional coordination of sample collection and analysis, with dissemination of information and sampling results. Results of independent monitoring can be reported to the project leader of the regional monitoring program. The U.S. Fish and Wildlife Service has been involved with tracking the distribution and status of nonindigenous aquatic species since 1977. The USF&WS database has a national scope and will be very useful in tracking zebra mussel infestation nationwide. The USF&WS reporting form is shown on page 21. Reporting new zebra mussel sightings is highly encouraged, as are summaries of seasonal monitoring. A generalized reporting form is shown on the next page.

State and National Zebra Mussel Information Centers

National	U. S. Fish and Wildlife Service Geographic Information Section National Fisheries Research Center 7920 N.W. 71st Street Gainesville, FL 32606 904-378-8181 FAX: 904-378-4956
Illinois, Indiana	Joseph O'Leary Illinois-Indiana Sea Grant Purdue University Forestry Building West Lafayette, IN 47907 317-494-0409
Michigan	John Schwartz Michigan Sea Grant Michigan State University 334 Natural Resources Building East Lansing, MI 48824 517-353-9568
Minnesota	Jeff Gunderson Minnesota Sea Grant College Program University of Minnesota-Duluth 208 Washburn Hall Duluth, MN 55812 218-726-8106
New York	Charles O'Neill, Jr. New York Sea Grant 250 Hartwell Hall SUNY College at Brockport Brockport, NY 14420-2928 716-395-2638 FAX: 716-395-2466
Ohio	Maran Brainard Ohio Sea Grant College Program The Ohio State University 1314 Kinnear Rd. Columbus, OH 43212 614-292-8949
Ontario	Chris Brousseau, Coordinator Zebra Mussel Coordination Office Ontario Ministry of Natural Resources P. O. Box 500, Maple, Ontario L6A 1S9 416-832-7275 FAX: 416-832-7177
Wisconsin	Clifford Kraft Wisconsin Sea Grant Program Bldg. ES-105 University of Wisconsin, Green Bay Green Bay, WI 54311-7001 414-465-2795

Zebra Mussel Sighting Report Form

What Are Zebra Mussels?
Zebra mussels are small brown-and-white–striped clamlike animals native to Eastern Europe. They were discovered in the Great Lakes in 1988 and were probably distributed from a freighter's ballast water picked up in a European port. They grow to about 5 centimeters (2 inches) in length and breed and spread very quickly. They are causing significant damage to water intake pipes, where they build up in large numbers and block water flow. They also foul boat hulls and may harm fish communities.

How Can You Help?
You can help monitor the spread of the zebra mussel. To report a sighting in your area, complete this form and forward it to the address indicated at the bottom of this page. Your sighting information will form part of a database that will be used to track the distribution, spread, and abundance of zebra mussels.

PLEASE PROVIDE THE FOLLOWING INFORMATION (please print):

Name: ____________________ Phone: Home ____________________

Address: ____________________ Work ____________________

____

Date of sighting: ____________________ State, county: ____________________

Name of body of water: ____________________

Distance to and name of nearest town: ____________________

Zebra Mussel Information

Approximate number of mussels found: ____________ Size range: ____________

Depth of water: ____________ River, lake, or pond?: ____________

Type of substrate: mud ___; rocks ___; mud and rocks___ ; sand ___; other (describe)____________

Substrate on which mussel(s) were found: ____________________

Additional comments: ____________________

Name and phone number of person who confirmed identification of mussel (if any)

____

Mail this form to:
Zebra Mussel Watch
(Address of local natural resource agency or state Sea Grant office)

National Fisheries Research Center
7920 N.W. 71st Street
Gainesville, Florida 32606
Telephone (904) 378-8181 FAX (904) 378-4956

NONINDIGENOUS SPECIES DATA BASE

Common Name ______________________________

Genus ______________________ Species ______________________

Subspecies ______________________ **Date Collected** ______________________

State ________ **County** ________________ Drainage Basin ________________

Location ______________________________
(please be as specific as possible)

____

____

____

Habitat Type: River/Stream _____ Natural Lake/Pond _____ Marsh/Swamp _____

Canal/Ditch _____ Man Made Reservoir _____ Estuary/Bay _____

Other Habitat ______________________________

Water Temperature ________ Salinity ______ DO _______ pH ________ Depth ______

Water Velocity ____________ Substrate ______________________

Vegetation ______________________________
(presence, absence, type, species if known)

Identified By ______________________________
(name, address, and phone number)

Collected By ______________________________
(name, address, and phone number)

Method of Collection ______________________________
(cast net, electrofishing, gill net, hook/line, rotenone,seine,trammel net,trawl,trot line ?)

Number Collected _________ Age _________ Class ____________ Size ______________
(larvae, juvenile, adult)

Method of Disposal ______________________________
(discarded, ethanol, formalin, frozen, mounted, released, tagged and released ?)

Specimen Storage ______________________________
(museum or agency name and collection number)

Comments ______________________________

____

____

____

At minimum, please provide the name or description of what you have observed, where it was observed, the date it was observed, your name and how you may be reached.

7 Acknowledgments

This document was conceived during a workshop on zebra mussel research sponsored by the Great Lakes Research Consortium and held in Syracuse, New York, on April 19–20, 1990. Funds for researching methods and preparing the document were granted by the Great Lakes Research Consortium in response to a joint proposal submitted by Ellen Marsden (then at Cornell University), Dan Molloy (New York State Museum), Alec Aitken (Queens University), and Ron Engel (State University of New York at Oswego).

The methods described here were compiled using information from numerous researchers and agency personnel already involved in zebra mussel monitoring, and from standard limnological techniques (e.g., see Lind 1979, McCauley 1984, APHA 1989). Many individuals contributed comments, reviews, and helpful discussions of the first draft of this document, and I hereby gratefully acknowledge their assistance. I would especially like to thank the following people: Chris Brousseau and Lois Deacon of the Ontario Ministry of Natural Resources Zebra Mussel Coordination Office for inviting me to participate in the development of their Fisheries Assessment Unit sampling program for zebra mussels; Dan Molloy, Brian Malone, and Jim Carlton for their extensive comments on the draft; William Mason, Bob Peoples, and Ken Pickering of the U.S. Fish and Wildlife Service for coordination with the USF&WS protocols; Michael J. Keniry for preparation of figures; Tammy Keniry and Nanette Trudeau for assisting with monitoring experiments; and the participants of the round-table discussion on monitoring techniques and results held at the Second International Zebra Mussel Conference, Rochester, New York, November 19–22, 1991. I would also like to thank Mary Balcer, Art Brooks, Thomas Ferro, David Garton, Wendell Haag, Jory Jonas, Cliff Kraft, Gerry Mackie, Dave MacNeill, Leif Marking, André Martel, Allen H. Miller, Jerrine Nichols, Hans Pearson, Charles Ramcharan, Ted Schaner, and Ray Tuttle for their generous advice and information.

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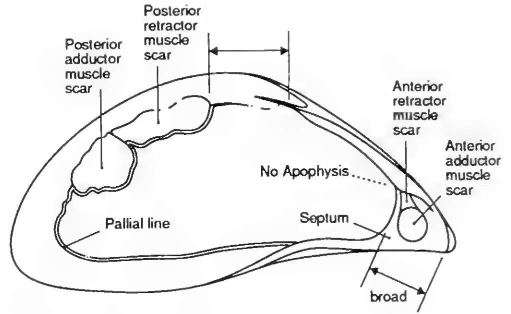
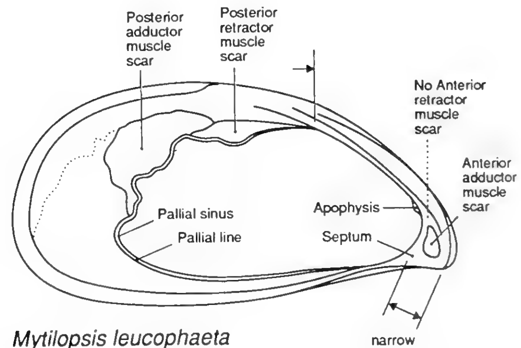
Appendix I: Distinguishing Adult *Dreissena polymorpha* and *Mytilopsis leucophaeata*

Reprinted with permission from MacNeill, D. 1991. Identification of *Dreissena* and *Mytilopsis*, part II. *Dreissena polymorpha* Information Review 2(2):9.



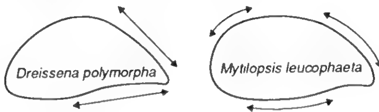
Dreissena polymorpha

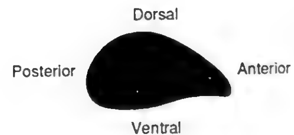
Shell structure	Dreissena	Mytilopsis
1) Internal Microscopic Features		
a) Posterior Retractor Muscle:	does not extend to anterior shell margin	extends to anterior shell margin.
b) Pallial Line:	rounded at posterior portion, no sinus.	may be invaginated forming a sinus.
c) Myophore Plate (Septum):	broad, scars of both anterior muscles present on septum. no apophysis present.	narrowed, only the anterior adductor scar present on septum. anterior retractor attached to inward facing apophysis.



Mytilopsis leucophaeta

	Dreissena	Mytilopsis
2) External Shell		
a) Shape:	more flattened at anterior margin and ventrally.	more rounded and broad laterally.





	Dreissena	Mytilopsis
b) Markings:	typically have herring-bone patterns, may be radially striped or show diffuse striping.	often have the herring-bone pattern. generally darker coloration.

Appendix II: Detection of Zebra Mussel Veligers in Plankton Samples Using Sugar Solution

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A method is presented to efficiently detect presence of zebra mussel veligers in plankton samples. The veligers can be separated from many other constituents of the sample by allowing the sample to settle through a column of sugar solution. Most of the veligers are recovered within 20 min in a few drops of liquid at the bottom of the column, allowing quick examination. The method is especially suitable for initial detection of veligers at low concentrations, but potentially it also has quantitative applications.

Introduction

In 1990, the Ontario Ministry of Natural Resources started to monitor zebra mussel (*Dreissena polymorpha*) in Lake Ontario. Since the mussel was just beginning to invade the lake, and population densities were expected to be low, we examined large volumes of water to reliably detect presence of the mussel's veliger larvae. The method described here was developed to speed up processing of the samples, and to reduce analytical costs.

Materials and methods

Plankton samples were processed through a settling apparatus consisting of a 25 ml pipette fitted with a three-way rubber pipetting bulb (Fig. 1). The pipette was partly filled with sugar solution (Table 1), and the plankton sample was introduced over the top of the solution. Planktonic organisms were allowed to settle through the sugar solution for a period of time, and were then collected from the tip of the pipette.

The pipette was FISHERbrand 25 ml in 1/10 (#13-665 SZ N). The outflow tip of the pipette was sanded off to the point where the inner diameter was approximately 1.5 mm. This prevented clogging of the opening with large organisms and filamentous algae. The pipette was filled with sugar solution up to the "10 ml" mark (slightly more than 15 ml of liquid or a column 195 mm tall). A 10 ml plankton sample topped up the liquid to the "0 ml" mark.

To set up the settling apparatus, the sugar solution was first drawn into the pipette using the rubber bulb. The tip was then sealed off with Parafilm, and the bulb removed. The plankton sample was introduced from the top using a syringe, and the timer was started . In a quick succession, the bulb was fitted back on the pipette, the Parafilm was removed, and any hanging drops were wiped off. Samples were periodically withdrawn from the tip of the pipette by pressing the "E" button (Fig.1) of the rubber bulb gently and slowly so that a single drop of liquid was released onto a depression slide.

Rates at which veligers settle through the apparatus were investigated by processing plankton samples, either containing known numbers of veligers, or representing batches of known veliger concentration. In all cases the samples were preserved with buffered formalin (Table 1) for at least 0.5 h before processing to allow osmotic equalization. Drops with settled organisms were collected from the tip of the pipette at 2 or 5 min intervals over a period of up to 30 min, and examined under a dissecting microscope at 25x magnification. To indicate efficiency, the numbers of recovered veligers were expressed as percentage of the number introduced into the apparatus.

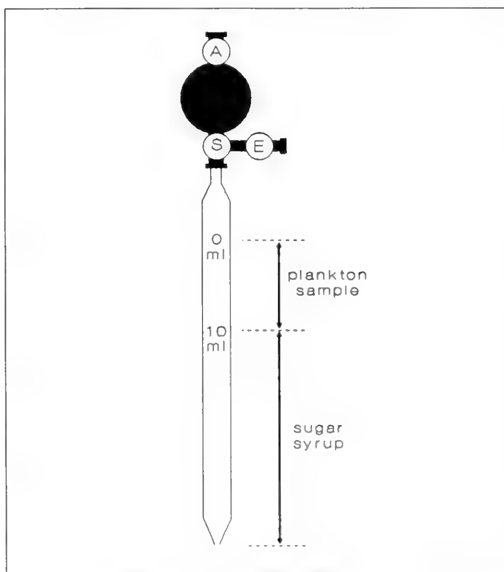

FIG. 1. The settling apparatus.

TABLE 1. Composition of preservative fluid and sugar solution used in processing of the plankton samples.

Preservative fluid:

(Modified after G.Hopkins
Ontario Ministry of Environment
Rexdale, pers. comm.)

37% formaldehyde	850 ml
Distilled water	1000 ml
Sugar	500 g
Sodium bicarbonate to raise pH to 7.0	

Combining 1 part of this solution with 4 parts of plankton sample results in approximately 4% formaldehyde concentration.

Sugar solution:

Sugar	130 g
Distilled water	400 ml

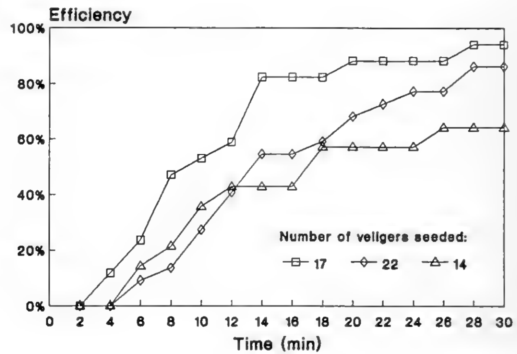

FIG. 2. Settling rates measured by allowing exactly known numbers of veligers to settle through the sugar column in almost complete absence of other organisms. Counts were made at 2 min intervals, and are expressed as cumulative (over time) percentages of the starting numbers.

Results

Veligers

The ideal settling rates and efficiencies for veligers were examined in three trials in which known exact numbers of veligers were introduced into the apparatus. Few other particles and organisms were present to interfere with veliger settling. The first veligers settled at 2 to 4 min (Fig. 2), and settling began to level off at 15 to 20 min. After 30 min the cumulative number of settled veligers represented 75-94% of the numbers used to seed the samples.

Tests with "real" plankton samples showed similar or somewhat lower efficiencies. After 20 min of settling, mean efficiencies ranged between 55 and 85%, and increased up to 90% at 30 min (Fig. 3A, B, C). The efficiencies appear to vary depending on the overall particle concentration of the introduced plankton sample. A very dense plankton sample from Nanticoke, Lake Erie, was diluted to 20 and 50% of original concentration, and a series of tests were run with each of the two batches. The more diluted batch (20%, Fig. 3B) showed efficiencies similar to ones experienced with veliger-only samples (Fig. 2), while the efficiencies with the more concentrated batch (50%, Fig. 3C) were lower, and arrivals at the bottom of the pipette leveled off only slightly during the 30 min experiments. This suggests that in highly concentrated samples the veligers are prevented from settling through the introduced sample to the top of the sugar column.

Size selectivity of the settling process was investigated using measured veligers mixed with a veliger-free plankton sample to simulate a realistic sample. Sizes of settled veligers were compared with those in the original sample. It appears that the size composition of the veligers settling through the apparatus within 25 min tended to be biased towards larger individuals (Fig. 4), though the difference from the original size frequency distribution was not statistically significant (chi-square and Kolmogorov-Smirnov tests, $p > 0.1$).

Other Organisms and Debris

The settling times for the other plankters varied. Too few experiments were performed to confidently describe the generalities. However, two observations appear reliable: 1) inorganic debris tended to settle within the first 4 min, and 2) cladocerans started arriving after approximately 15 min. At 30 min the arrivals of all types of particles began to decrease, though the liquid above the sugar column remained turbid.

Discussion

Settling the plankton samples through a column of sugar solution can be useful in detection of zebra mussel veligers. A high proportion of the veligers passed through the settling apparatus within the time window of 5 to 20 min, and allowed separation from both early-settling inorganic debris, and late-settling planktonic organisms. The bias in sizes of the settled veligers was small.

The method offers several advantages. Firstly, a bank of settling pipettes can be set up and operated simultaneously, and thus samples representing large volumes of water can be rapidly processed. In our sampling program, two people were able to process and microscopically examine samples representing 840 litres in 40 min. Fatigue is minimized since very little time is spent in microscopic examination. Various types of organisms tend to settle at various times, and if samples of settlers are taken periodically throughout the settling period, then the lesser variety of organisms in any single sample leads to easier detection of veligers. Fractions of the plankton sample can be discarded with little decrease in efficiency: discarding a drop at 3 min and then stopping the process at 20 min, will avoid sand and silt particles as well as most cladocerans, while still capturing at least half of the veliger larvae.

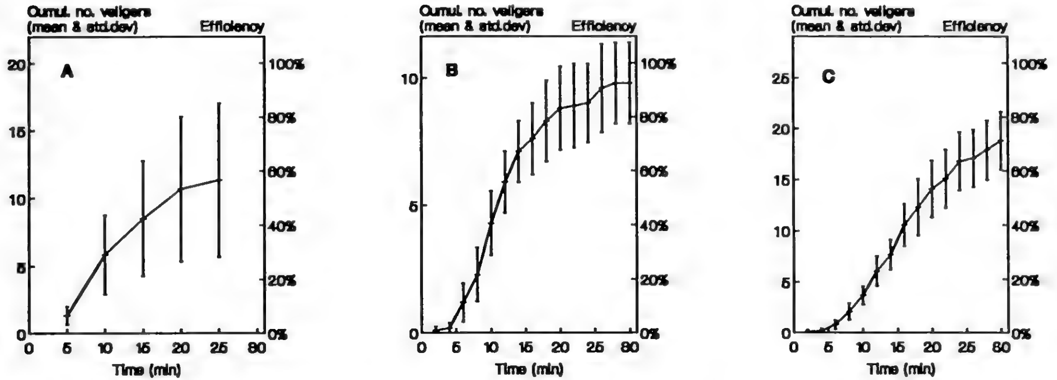

FIG. 3. Settling rates of veligers, measured in samples that included other plankton organisms. Each graph represents several replicate runs, and the mean cumulative (over time) count is shown. The replicates were taken from known concentrations of veligers, but the exact number of veligers in each replicate was not known. Therefore, some part of the standard deviation is due to variability in the starting numbers. Efficiency is the number of recovered veligers expressed as percentage of the starting numbers. **A:** Lake Erie veligers mixed with veliger-free plankton sample from Lake Ontario, six replicate trials. **B:** Plankton sample with veligers from Nanticoke, Lake Erie diluted to 20% original strength, nine replicate trials. **C:** Same as B, but diluted to 50%, eight replicate trials.

It appears that efficiency decreases when the plankton sample is too concentrated and veligers are impeded in reaching the sample-sugar interface. The data presented here indicate approximate efficiencies, and allow for rough correction. No guidelines for maximum particle concentration are given here, though establishing such guidelines would render the method more suitable for quantitative applications.

The most logical application of this method is in the initial detection of zebra mussel infestation, when veliger densities are on the order of several veligers per cubic metre. Here the drawback of incomplete enumeration is offset by the ability to examine large volumes of water.

Acknowledgments

I thank Joe Leach, Dave Lowther, and Don Lewis for providing veliger larvae and plankton samples, and Gord Hopkins for providing the formalin recipe.

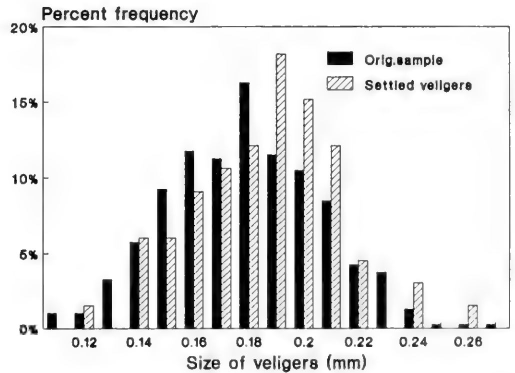

FIG. 4. Size selectivity of the settling process: size frequency distribution of introduced sample (400 veligers measured, sample from Fig. 3A) contrasted with distribution of veligers settled within 25 min (66 veligers pooled from six replicate runs).

Appendix III: Collector for Veliger and Drifting Postmetamorphic Zebra Mussels

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One of the major disadvantages of using settlement plates for collecting juvenile zebra mussels is the long period of time needed for deployment. The plates are vulnerable to storm damage and vandalism during this time, and results are not obtained for weeks to months. Plankton tows to collect veligers have the disadvantage that they sample a relatively small, contiguous unit of water. This results in a high probability of missing veligers if their distribution is patchy. The purpose of this paper is to describe a new technique for collecting planktonic zebra mussels which overcomes the disadvantages of existing techniques. The collectors used in this new technique have two main advantages over other types of artificial substrate currently used to monitor and sample zebra mussels:

1. Only a short time period is needed between deployment and retrieval in the field. High collection rates are achieved within 24–72 hours of deployment. This means that results are obtained rapidly, and the equipment has a minimal chance of being lost or damaged due to severe weather or vandalism.
2. The collection rate of both veligers and postmetamorphic stages (early juveniles) is higher than that with other settlement plates. This results in a high presence-or-absence detection rate in a short period of time. Also, because a wide size range of zebra mussels are collected, these collectors integrate the functions of both plankton nets and settlement plates.

Equipment Construction

In recent studies using the new technique, the active element of the collector was a 12 cm x 11 cm, 0.6- to 0.8-cm-thick, white nylon scouring pad. Similar collectors have been used in studies of *Mytilus* in Ireland (King et al. 1990) and in Canada (Martel, unpublished data). The scouring pads used in this study of zebra mussels were manufactured by the Fireco Company, Houseware Products, 1280 Courtney Park Dr., Mississauga, Ontario, L5T 1N6, and were purchased at a local grocery store. Similar fine-fiber scouring pads from other manufacturers may be useable, but they must be free of detergent. The pads were approximately $0.50 (Canadian) apiece. To stabilize the pads and prevent them from folding during deployment, and to permit comparison of the pads with standard Plexiglas settlement plates, two Plexiglas strips were attached to the top and bottom of the pad using hot-melt glue (Figure 1). Because they make strong joints, yellow or white glue sticks are preferable to transparent glue sticks for this purpose. The edges of the Plexiglas strips were roughened with sandpaper prior to gluing to enhance the strength of the joint. Glue was allowed to penetrate about 4 mm into the pad fibers. The strongest joint was achieved when the pad and Plexiglas were bound together while the glue was still very hot and liquified. The Plexiglas strips used in this experiment were 10 cm x 1 cm (top) and 10 cm x 5 cm (bottom) (Figure 1A). However, two similar-sized Plexiglas stabilizing strips can be used if comparison of the scouring pad with Plexiglas plates is not an objective of the study.

During testing of the scouring pads in Lake Erie, swivels were attached to the top and bottom of the collector to allow free rotation of the collector in wind-generated water currents and waves. This system permitted consistent exposure of the collector's broad surface to the water flow, thus maximizing the amount of water "sieved" by the collector and ensuring a similar orientation for all the collectors. The effectiveness of the swivel depended upon how well the swivel was centered on the collector's Plexiglas strips (Figure 1A). Newly constructed collectors were tested prior to deployment by attaching them to a line and dragging them through a large tub of water. The collectors oriented easily and consistently toward the imposed current, similar to a self-orienting windmill.

Deployment of Collectors

Collectors were deployed in series along a line, using a 3½-in. galvanized nail attached to each swivel using hot-melt glue (Figure 1A). A small notch was made 7–8 mm from the end of each nail, and the swivel ring was positioned inside the notch before gluing (Figure 1A). The head of each nail was inserted through the ply of 3/16-in. or 3/8-in. three-ply twisted polypropylene line. This system permitted rapid addition, replacement, and removal of collectors. The system was also highly reliable: no collectors were lost during multiple trials under various weather conditions in Lake Erie and on the coast of Vancouver Island. For optimal deployment, the two nails were parallel to each other and perpendicular to the line before the nail heads were inserted into the line. Several collectors were deployed on a single anchored and buoyed line. The lowest collector was at least 40–60 cm above the bottom to avoid damage and clogging with bottom silts. The distance between collectors varied according to study needs and the water depth at a particular site. After initial deployment, the collectors were checked to ensure that the nails of the collectors were still aligned in one plane and nearly parallel (Figure 1A).

An alternative method of attachment and deployment of the scouring pad collectors consists of simply tying the two Plexiglas pieces (top and bottom of collector) to the rope using a stainless steel ring and clip system. The clip is attached to one of the three major plies of the rope (Figure 1B). This method of attachment may be used in situations where currents are weak or orientation of the collector to currents is not a concern. Also, where currents are unidirectional, the collector can be tied by the corners to a fixed point, such as a float or a wall of a large intake pipe, allowing the pad to be fully exposed to the flow. Evidently,

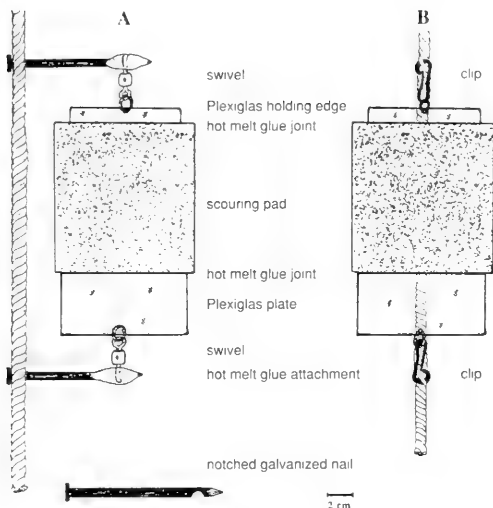

Figure 1. Diagrams of two off-bottom scouring pad collectors with attached Plexiglas stabilizing plates. Collectors are shown attached to a 5/16-in. polypropylene rope by (A) a self-orienting swivel system using a notched galvanized nail and (B) a simple clip system.

Table 1. Number of larval and postmetamorphic zebra mussels collected on scouring pad samplers and Plexiglas plates deployed at several stations near a breakwater at Wheatley, Ontario, in Lake Erie during August 1991.*

Station	Date	Collector	Plexiglas	Scouring pad
1	Aug 8	1A	0	368
		1D	0	486
2		2A	0	317
		2C	0	345
		2E	2	527
1	Aug 14	1A	0	5
		1B	0	9
		1C	0	3
		1D	0	6
		1E	0	2
4	Aug 24	3A	0	151
		3C	0	212
		4A	1	451
		4E	2	639

*Collectors were each deployed for 24 hours. Collectors were progressively deeper from A to E, with the maximum depth at 5 m. Note the high numbers of zebra mussels captured in scouring pads compared with settlement on the Plexiglas plates.

the fixation and deployment of the scouring pads can be adapted to specific situations and to the needs of the study.

To ensure that the line holding the collectors was vertical, a small, submerged buoy was attached to the line 20–30 cm below the main buoy on the surface. Alternatively, a small satellite anchor can be attached a few meters away from the main anchor. This anchor will bounce in heavy waves and acts as a shock absorber to reduce tension on the main anchor. For the brief deployment periods needed for these collectors, cinder block or brick anchors appear to be quite adequate. For deployment in highly exposed areas, an anchor made from a section of chain (e.g., 50–70 cm length of 1- to 2.5-cm-diameter link chain) offers the advantage of being extremely stable due to its high density and low profile during strong bottom surge generated by storms.

The scouring pad collectors worked best when deployed for short periods of time, between 24 hours to one week. Longer periods of time may result in fouling of the pads with detritus and planktonic organisms. The optimum period for deployment will vary according to the local turbidity (as measured by Secchi disk). Recent storms will tend to suspend volumes of sediment, particularly detritus, which may clog the collectors, although at Wheatley, Ontario, deployment during such periods usually resulted in very high numbers of zebra mussels, particularly postmetamorphic stages, in the scouring pads (Table 1). Before use, all new collectors were soaked in filtered (64 µm) lake water for 2–3 days. This prewashing assisted in conditioning the pads and removed any traces of chemicals that may have been present on the pads.

Extraction, Observation, and Preservation of Mussels
The Plexiglas portion of the collectors was examined prior to extracting zebra mussels from the pad. The pads were washed using a low-flow, fairly strong jet of cool tap water, obtained using a hose equipped with an adjustable nozzle. This pressurized jet is necessary to remove zebra mussels that have already secreted new byssal threads during the 24-hour period of deployment. Washing was done over a shallow pan, with the hose held 1 cm from the pad. Each collector was washed three times for 30 seconds each. A quick examination of the filtrate under a stereomicroscope permitted estimation of whether successive washes were removing additional material. The filtrate was passed through two filters to grade the collected material. Both filters were constructed by removing the bottom from a 250-ml plastic beaker and gluing Nitex mesh across the hole. A 500-µm and a 125-µm filter were used. After extraction, the samples were carefully examined under a stereomicroscope to determine whether zebra mussels caught in the pads were alive. This determination is important because empty shells of small juveniles (2–3 mm or less) may be resuspended by currents. In areas where zebra mussels are known to be present, the occurrence of empty shells or dead mussels may not, for instance, necessitate any immediate mitigation treatments. With a high-power bright-field stereomicroscope (40–60x), live veligers and early postmetamorphic zebra mussels can easily be distinguished by checking for distinct, well-defined internal body organs, including the foot and gill lamellae. In particular, the foot and the long cilia of the veliger's velum can be seen, either expanded or withdrawn into the shell.

After initial observation under the microscope, the samples were preserved in 70% ethanol. The collectors were then thoroughly washed with a water jet and placed in 55–60°C water for 5 minutes to ensure that any remaining mussels were killed. Boiling water will melt the hot-melt glue; ethanol is also an inappropriate disinfectant because it is a weak solvent for both the Plexiglas and the scouring pad. The collectors were dried flat, inspected for damage, repaired with hot-melt glue if needed, and then reused.

Results

During short-term deployment (24–72 hours), collection rates of zebra mussels on the pads was frequently two orders of magnitude higher than settlement on the Plexiglas plates (Table 1). More than 600 individual zebra mussels were collected in a single pad after 24 hours. Settling veligers as well as drifting juveniles from 300 to 900+ µm long were collected in the pads. Factors that may contribute to the high collection rates obtained with the pads include the following: (1) the three-dimensional complexity of the pad substrate, which offers a large surface area for settlement; (2) the large amount of water "filtered" by the porous structure of the pad; and (3) preferential settlement of larvae and early postmetamorphic stages onto structurally complex substrata compared with very smooth surfaces.

Conclusion

The scouring pad collector appeared to act as a passive plankton net by sieving a large volume of water over the entire period of deployment. A wide size range of planktonic zebra mussels were collected, including postmetamorphic stages or early juveniles. These small juveniles may have been washed off surfaces during severe weather or currents; possibly, some may have undergone intentional dispersal by drifting on mucus threads, as shown in *Corbicula* (Prezant and Chalermwat 1984), marine bivalves (Lane et al. 1985, Beukema and de Vlas 1989, Martel and Chia 1991a), and some gastropods (Martel and Chia 1991a, b). The collector therefore effectively integrates the uses of plankton nets and settlement plates. The collectors are inexpensive (less than $2.50 U.S. apiece), easy to build from readily available materials, reusable, and provide quantitative, reproducible results. They can be used for short periods of time to avoid severe weather and vandalism, and they quickly produce information about local zebra mussel levels.

Acknowledgments

I am indebted to R. Toonen for his assistance in the original design and construction of the scouring pad collectors. J. Fournier, K. Klein, J.E. Marsden, and J. Topping commented on earlier versions of the manuscript. I also want to thank the staff of the Lake Erie Fishery Station - OMNR, at Wheatley, Ontario, especially the director, Dr. S. Nepszy, as well as Dr. J. Leach, for their support and for allowing me to use the station facilities during the testing of the scouring pad collectors in Lake Erie.

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Appendix IV: Equipment and Chemicals

Reagents

A note about preservatives: Formalin (10% formaldehyde) is a traditional preservative for plankton samples. However, formalin vapor is irritating to the eyes and nose, and prolonged exposure is carcinogenic. Great care should be used to keep formalin contained and to only examine preserved specimens under an exhaust fan or in a well-ventilated area. Samples of shelled mussels (settled juveniles or adults) can be rinsed and transferred to water for the duration of the counting procedure to minimize exposure to formaldehyde. This procedure is trickier with veligers because of the probability of losing veligers during the transfer to water. Ethanol is also a commonly used preservative and is gaining popularity because it is more benign to the user than formalin. However, state and federal agencies are often required to use alcohol that has been denatured with compounds such as acetone, which are irritating to humans and may affect the sample.

Buffered sugar formalin: Dissolve 80 g of granulated sugar in 1 liter of 10% formalin. Dilute with sample by one-half to produce a 5% solution. (Note: 10% formalin is a 9:1 dilution of water and formaldehyde. Formaldehyde, as purchased, is a 37% solution in water). Buffer the formalin by adding a handful of marble chips (calcium carbonate) to each liter of solution. Sodium bicarbonate can also be used to buffer to pH 7.0

Alcohol: Use 70% ethanol or isopropyl alcohol.

Equipment Sources

Note: Identification of equipment manufacturers and distributors is provided for convenience and should not be taken as an endorsement of any kind.

Design Alliance, 114 East 8th St., Cincinnati, OH 45202. 513-621-9373 (slide racks)
Environmental Research Instruments, 70 Durham St., Guelph, Ontario, Canada, N1H ZY3
Forestry Suppliers Inc., P.O. Box 8397, Jackson, MS 39284-8397. 800-647-5368
Ernest A. Case, P.O. Box 45, Andover, NJ 07821. 201-347-1365
Limnotech, 136 Scarborough Rd., Toronto, Ontario, Canada, M4E 3M6. 416-698-0978
Research Nets, 23102 55th Ave. W., Mt. Lake Terrace, WA 98043. 206-821-7345
Robar Maching Inc., 2611 East 40th St. Chattanooga, TN 37407. 615-867-4717 (settlement racks)
Wildco Wildlife Supply Company, 301 Cass St., Saginaw, MI 48602. 517-799-8100

Estimated Sampling Costs

Prices for various critical pieces of sampling equipment are provided to assist investigators in estimating the scope and strategy for their sampling program. Cost estimates, given in U.S. dollars, are based on 1991 equipment prices.

Sampling Veligers

Plankton net 30 cm, D/L ratio 1:3, 63–64 mm mesh $200
Flowmeter >$200
Secchi disk (20-cm diameter) $50
Sedgewick-Rafter counting cell $25
Dissecting microscope $500–$5,000
Incidental costs for preservative, pipettes, clipboards, etc.

Sampling Settled Juveniles

Microscope slides ($14/gross) $0.10
Periphyton sampling rack for microscope slides (ready-made) $38
Multiplate sampler (ready-made) $17
PVC plate sampler (home-made) $5
Scouring pad sampler (home-made) $3
Incidental costs for lines, buoys, anchoring material, preservative, etc.

Sampling Adults

Ponar grab $300–$600
Incidental costs for constructing sampling frame and coring device
Use of commercial divers can run from $50 to several hundred dollars per diver and may require a minimum of three divers per dive.

Appendix V: Forms and Labels

ZEBRA MUSSEL VELIGER SAMPLING FORM

RECORDER:

date	time	sample location	sample type*	lift #	bottom depth	bottom type**	current velocity	current direction	sample depth	volume sampled	water temp.	Secchi depth

*sample type: vertical tow (vt), oblique tow (ot), pumped water (pump), flowing water (flow)
**bottom type: rocky (rky), sand, silt/mud, vegetation (veg), artificial (art), unknown (unk)

ZEBRA MUSSEL VELIGER COUNTING FORM

RECORDER: ____________________

sample location	sample date	sample number	vol. of sample	total vol. of organic material	dilution	sub-sample	# veligers per counting square					total	# veligers per ml of sample	# veligers per liter
							1	2	3	4	5			
						1								
						2								
						3								
						4								
						5								
													mean	
													S.D.	
						1								
						2								
						3								
						4								
						5								
													mean	
													S.D.	
						1								
						2								
						3								
						4								
						5								
													mean	
													S.D.	
						1								
						2								
						3								
						4								
						5								
													mean	
													S.D.	

SAMPLING FORM FOR SETTLED ZEBRA MUSSEL JUVENILES

RECORDER: ______________________________

sampler location	sampler type	deployment date/hour	retrieval date/hour	sampler depth	bottom type*	water depth	water temp.	Secchi depth	current velocity	current direction	nearest mussel colony

* bottom type: rocky (rky), sand, silt/mud, vegetation (veg), artificial (art), unknown (unk)

COUNTING FORM FOR SETTLED ZEBRA MUSSEL JUVENILES

sample location	sampler number	retrieval date	immersion time	# juven. per 1 cm square						#juven. per m2	vol. of juven. per m2	smallest mussel counted	largest mussel on slide
				1	2	3	4	5	total				

ADULT ZEBRA MUSSEL SAMPLING FORM

RECORDER: ____________________ DIAMETER OF CORER (if used): ____________________

sample location	date	subst. type*	sample depth	water temp.	Secchi depth	sample	core height	core weight	# adults per 10 cm square (for transect square use)					total # adults	adults/m2	vol. of adults/m2	smallest mussel counted	% dead mussels
									1	2	3	4	5					
						1												
						2												
						3												
						4												
						5												
														mean				
														S.D.				
						1												
						2												
						3												
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						3												
						4												
						5												
														mean				
														S.D.				

*bottom type: rocky (rky), sand, silt/mud, vegetation (veg), artificial (art), unknown (unk)

Sample labels - fill in with pencil and insert in sample container with preservative

Site:
Sample #:
Replicate #:
Date:
Time:
Collector:
Preservative:

Site:
Sample #:
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Appendix VI: Conversion Table

Convert from	Convert into	Multiply by	Reverse conversion
Centimeters	feet	0.0328	30.48
	inches	0.3937	2.54
Cu centimeters	liters	0.001	1000
	pints (US liq)	0.002	473.2
	milliliters	1	1
Liters	cu feet	0.035	28.316
	cu meters	0.001	1000
	milliliters	1000	0.001
	pints (US liq)	2.113	0.473
	gallons (US liq)	0.264	3.785
Meters	centimeters	100	0.01
	feet	3.281	0.3048
	inches	39.370	0.0254
Meters/sec	feet/min	196.85	0.00508
	km/hr	3.6	0.2778
	statute miles/hr	2.2369	0.447
Microns	inches	0.000039	25400
	millimeters	0.001	1000
Kilometers	statute miles	0.621	1.609
Sq centimeters	sq meters	0.0001	10000
	sq inches	0.155	6.4516
	sq millimeters	100	0.01
Sq meters	sq centimeters	10,000	0.0001
	sq yards	1.196	0.836

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