Detection of Zebra Mussel Veligers in Plankton Samples Using Sugar Solution.

Ted Schaner

Ontario Ministry of Natural Resources
Lake Ontario Fisheries Unit
R.R. # 4, Picton, Ontario, K0K 2T0

Lake Ontario Fisheries Unit 1990 Annual Report, LOA 91.1 (Chapter 6)

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.

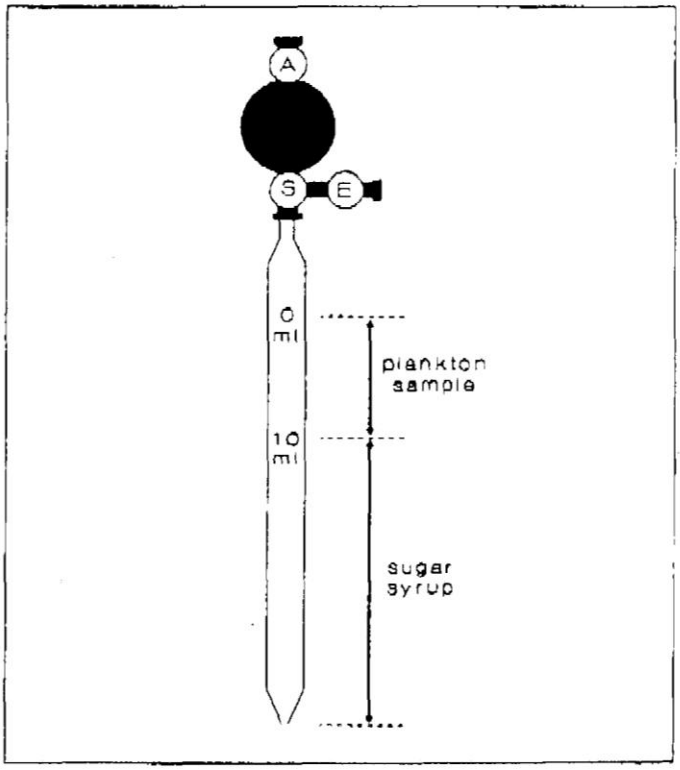

FIG. 1. The settling apparatus.

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

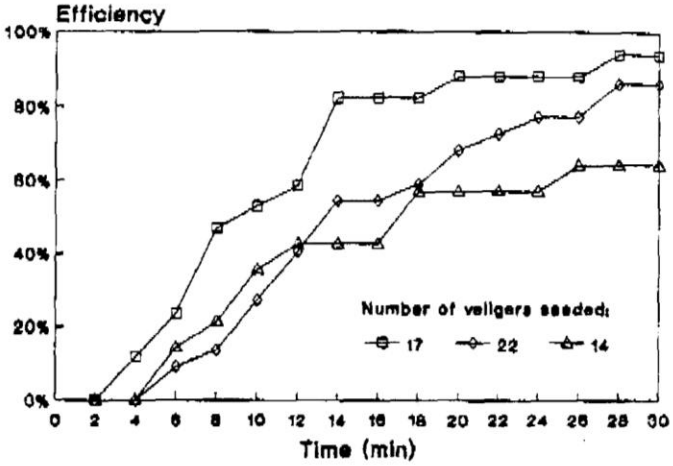

FIG. 2. Settling rates measured by allowing exactly known numbers of veligers to settle through the sugar column in almost complete absence of other organisms. Counts were made at 2 min intervals, and are expressed as cumulative (over time) percentages of the starting numbers.

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.

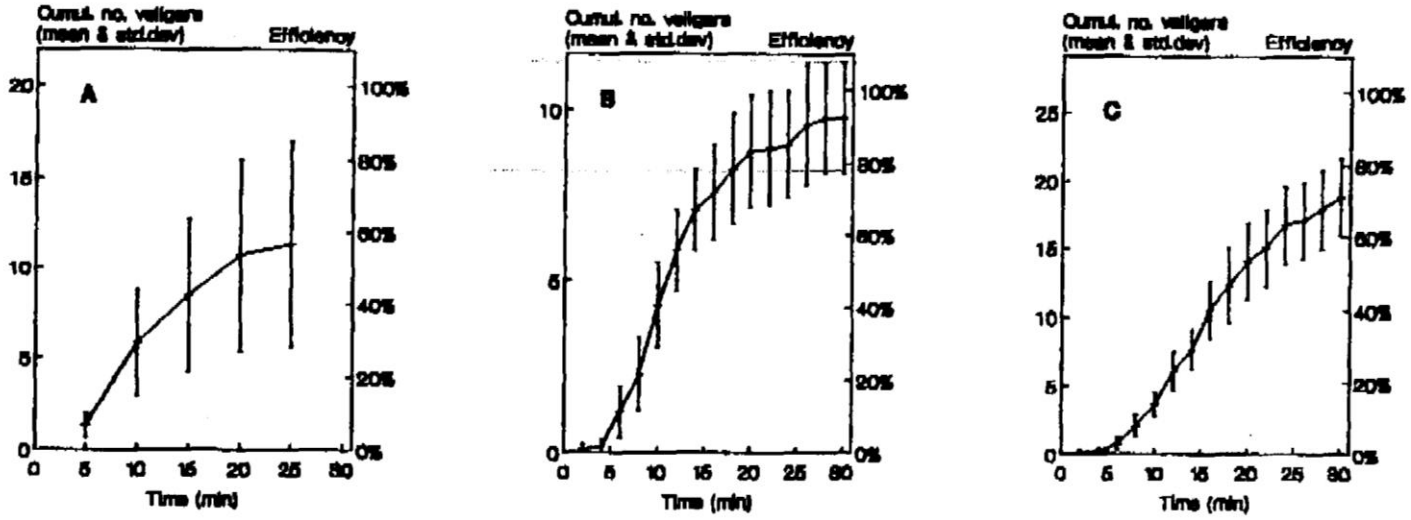

FIG. 3. Settling rates of veligers, measured in samples that included other plankton organisms. Each graph represents several replicate runs, and the mean cumulative (over time) count is shown. The replicates were taken from known concentrations of veligers, but the exact number of veligers in each replicate 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.

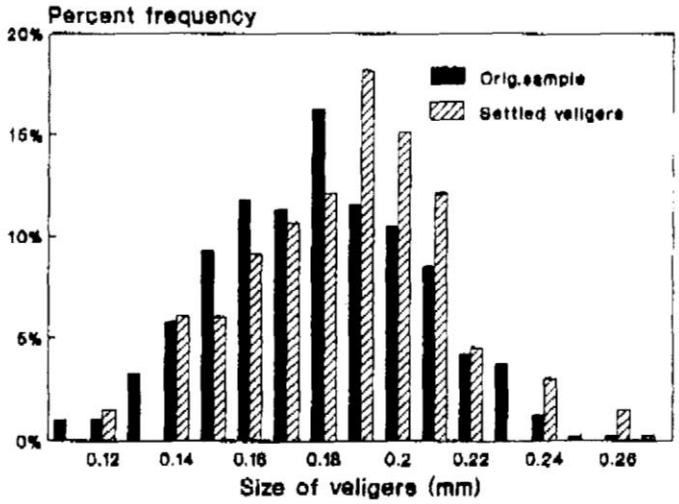

FIG. 4. Size selectivity of the settling process: size frequency distribution of introduced sample (400 veligers measured, sample from Fig. 3A) contrasted with distribution of veligers settled within 25 min (66 veligers pooled from six replicate runs).