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Short Communication

Livewell flushing to remove zebra mussel (*Driessena polymorpha)* veligers

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Abstract

The prevention of the spread of the zebra mussel is of great concern in many places in North America. The cost of constructing wash stations that provide hot water at high pressure precludes their application as an easy decontamination method. Therefore, the ability to use a garden hose to flush veligers remaining in residual water from the livewell of a boat was examined. Although flushing was not found to be completely effective, more than 90% of veligers were removed after 150 s of flushing.

Key words: invasive species prevention, boat decontamination, livewell, zebra mussel, veligers

Introduction

The zebra mussel, *Driessena polymorpha* (Pallas, 1771), continues to spread across North America almost three decades after its first documented presence in the Great Lakes in the 1980s (Benson 2014; Carlton 2008). This continuous movement is often attributed to the overland transport of trailered watercraft that contain larval mussels, called veligers (Johnson et al. 2001). Watercraft decontamination protocols have been developed by various state and regional agencies and workshops to address watercraft that are suspected of harboring zebra mussel veligers (e.g., DiVittorio et al. 2012). The recommended and most used form of decontamination is hot water/high pressure spray (Comeau et al. 2011; DiVittorio et al. 2012; DiVittorio 2015; Zook and Phillips 2015). This method uses water heated to temperatures of 140 °F applied for 10 s to kill zebra mussels (Morse 2009; DiVittorio et al. 2012). Due to the cost of constructing a hot water spray station (i.e. over $30,000 [Jensen 2009]), a less expensive alternative to remove any veligers that remain in a trailered watercraft could encourage more boaters to treat their watercraft because it is more accessible. As a result, more boats will be decontamintated. Due to the possibility that veligers remaining in the livewell or bilge area of a watercraft can be introduced into uninfested waters once the boat is launched, flushing livewells and bilge areas with "clean" water could be an important prevention action. In the present study, the ability of a garden hose to flush the contents of a watercraft's livewell for a short time frame was investigated as a potential veliger removal tool.

Methods

Veliger collection

Zebra mussel veligers were collected from the top 3 m of the water column of Otsego Lake, NY using horizontal tows (about 750 m per tow) with a 63 µm plankton net from 25 August to 02 September 2015. The contents of the net were placed into a 1 L bottle. Multiple tows (10–15) were performed until the bottle was at least 75% full, then they were taken to the main laboratory at the State University of New York at Oneonta's Biological Field Station located minutes away from the sampling sites. Once back at the lab, the contents of the bottle were concentrated down to 100 mL of liquid using a cup with a bottom of 63 µm net material. A Sedgewick-rafter cell was

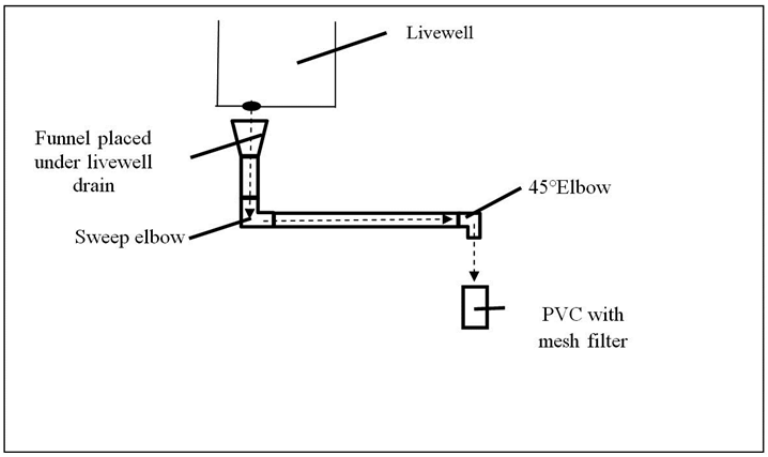

Figure 1. Diagram of water flow and capture during livewell flushing. Pipe was supported at the sweep elbow and just before the 45° elbow (not shown in diagram). The horizontal section of pipe had a drop in elevation of approximately 1″ for every 12″ of pipe (~ 2.5 cm per 30 cm of pipe).

used to count the number of veligers in 1 ml of concentrated sample water using cross-polarized light (CPL) microscopy. This was repeated two more times and the average number of veligers per milliliter was calculated. Veligers were collected in the morning and were only used for replicates during the same day experiment.

Livewell flushing

The livewell of a 16 foot aluminum johnboat was used for all livewell flushing tests. The plug was placed into the livewell (Figure 1) and filled with ~22 L of veliger-free well water (about ¾ full). Then enough concentrated veliger solution with known quantified number of veligers was added to bring the veliger density in the livewell to 10–200 veligers/L which is within the range of Otsego Lake veliger abundance (Horvath 2012). After waiting for 30 s to let the veligers distribute throughout the livewell, the plug was removed and the livewell was drained. After 3 min, a funnel and PVC pipe drain were placed under the livewell drain. At the terminal end of the drain pipe, a 45° elbow directed contents of the pipe towards the ground. A piece of 3″ PVC was set below the end of the drain pipe with a 63 µm mesh inside a coupler, across the pipe, so that the netting remained tight. The livewell was then flushed using a sprayer with a FIMCO HighFlo Gold Series pump (Model 5277981; 60 PSI) that ran on a 12-volt battery, equipped with a garden hose attachment on the end that was set on the "Stream" setting for a total of 7 min. During that time, every 30 s the 3″ pipe piece was removed and another 3″ pipe with filter was placed under the drain pipe until the 5 min point. The final piece of 3″ pipe was placed under the drain pipe for the final 2 min of flushing and for 3 min of draining following the flushing. When performing the flushing of the livewell, the water was sprayed around the sides of the livewell and along the bottom first. Thus, every surface of the livewell was sprayed. The water was sprayed on the sides of the livewell in either a clockwise or counter-clockwise direction to promote the water flowing to the drain and to avoid splashing that could move veligers to areas where they were not reachable by the flushing spray. Each of the pipe pieces was triple back-flushed into a 250 mL beaker and there was no water lost during pipe/filter switches. The contents of the beaker were then poured into a veliger holding device (VHD). The VHDs were constructed by drilling a one-quarter inch hole in the cap of a 15 mL centrifuge tube, removing the screw-on cap and placing 63 µm net material over the end of the tube and putting the cap back on, and finally cutting the pointed end of the centrifuge tube off. This design allowed the veligers to be constrained in a small area so that it would be easy to recollect them for preservation. The VHDs were placed in a test tube rack so that only about 2 mL of water was inside the holding device. A pipette was used to collect the contents of the VHD and place them into a 15 mL centrifuge tube. Then 3 mL of 70% ethanol was added to the centrifuge tube to preserve the contents for analysis of veliger presence at a later date. Each tube was labelled with the replicate number and the interval in the flushing time. A 25% distilled white vinegar solution was then poured into the sides of the livewell so that all surfaces in the livewell were contacted by the vinegar solution. It was allowed to soak for 10 min [this solution caused the shell of veligers to be dissolved during other testing (Davis et al. 2015)] to ensure that veligers left behind by one replicate were not counted for the following replicate. Following

Table 1. Number of veligers observed in samples taken at intervals during livewell flushing and average number of veligers observed per interval.

Replicate	Concentration (veligers/L)	Seconds after start of flushing of livewell										
		30	60	90	120	150	180	210	240	270	300	600
1	10	42	0	0	1	2	1	1	2	5	1	6
2	20	127	14	18	19	7	3	2	1	0	1	0
3	200	624	133	68	84	13	1	1	1	1	0	2
4	200	222	68	105	47	5	9	7	2	2	12	12
5	200	18	26	146	118	139	8	2	1	1	1	1
6	200	394	77	35	12	6	4	3	6	0	5	8
7	200	374	104	20	4	8	1	6	9	2	3	2
8	200	44	42	45	24	20	5	3	5	8	6	10
9	200	321	84	120	51	19	10	7	8	2	2	6
10	200	94	85	61	43	17	3	1	2	2	1	2
11	200	307	122	63	44	39	8	5	5	4	1	1
12	200	137	91	67	67	15	10	4	4	2	1	2
13	200	281	132	93	54	34	10	1	3	5	3	2
14	200	17	9	10	2	5	4	0	1	4	2	1
15	200	284	72	82	18	24	6	5	4	6	6	7
16	200	219	48	77	69	25	7	0	4	2	5	1
17	200	265	54	82	51	15	2	1	4	2	0	8
18	200	225	107	36	14	13	9	4	6	0	4	6
19	200	188	103	58	33	33	9	0	1	2	7	2
20	200	167	73	17	39	32	3	4	6	6	5	8
21	200	324	48	77	32	34	6	4	2	5	0	12
22	200	100	83	25	48	18	7	4	4	5	2	3
23	200	258	104	62	37	20	5	5	4	2	4	10
24	200	253	63	79	37	37	7	4	0	3	2	9
25	200	275	58	31	43	27	8	3	4	6	5	1
26	200	163	74	50	50	29	3	5	5	0	6	4
27	200	325	102	33	65	16	6	2	5	2	1	6
28	200	224	103	32	26	10	2	0	2	3	0	5
29	200	283	50	22	36	36	9	3	1	3	7	6
30	200	159	47	80	44	38	3	5	3	1	0	2

Table 2. Average percentage of veligers observed at intervals after livewell flushing began.

	Time after livewell flushing began (s)										
	30	60	90	120	150	180	210	240	270	300	600
Average percentage of veligers observed	49.0%	16.3%	12.9%	9.2%	5.9%	1.5%	0.8%	1.0%	1.1%	0.9%	1.5%

the soaking period, the livewell was flushed twice by filling the plugged livewell with ~22 L of water and then draining. A total of thirty replicates were performed over the course of 8 days from 25 August to 02 September. Any equipment that could have had contact with veligers during a replicate was rinsed three times with veliger-free well water before the next replicate was started.

Preserved sample analysis

The bottom 1 mL of sample in the centrifuge tube was removed with a pipette with the tip cut and placed into a gridded Sedgewick-rafter cell. The sample was then examined under cross-polarized light microscopy and the number of veligers present was counted and recorded. This was repeated two more times for each preserved sample to count all veligers (any veliger in the centrifuge tube would settle to the bottom of the tube once they died from the ethanol added to the sample). Between sample enumerations, the Sedgewick-rafter cells and cover slips were rinsed with veliger-free water and dried.

Statistical analysis

The number of veligers per replicate observed in each time interval sample was converted to the percentage of the total veligers observed in the replicate. Because the test was performed as a series

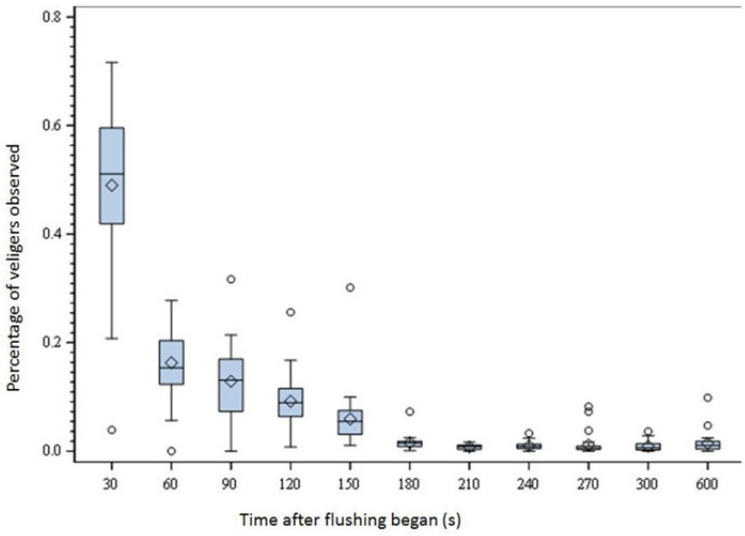

Figure 2. Box-Whiskers plot for veligers identified in each time interval as a percentage at different intervals after livewell flushing began.

of time intervals, the nonparametric Kendall's tau correlation coefficient was calculated to investigate the relationship between the time of flushing and the percentage of veligers removed. Analysis of variance (ANOVA) and posthoc multiple comparisons (Student-Newman-Keuls (SNK) Test) were performed to see if veliger removal percentage at these time intervals were significantly different from each other. Arcsine square root transformation of the percentage of veliger removal was conducted prior to ANOVA since it is a standard procedure when analyzing proportional data (Warton and Hui 2011). All statistics were performed using SAS (Version 9.3 SAS Institute Inc., Cary, NC). The level of significance was set at $\alpha = 0.05$.

Results

Among all times veliger concentration was measured, there was an average of 440.7 veligers identified per replicate (N = 30, Standard Deviation = 171.7, Table 1). The first 30 s after flushing began had the greatest percentage of total removed veligers (Table 2, Figure 2). There was a significant correlation between the time after flushing began (i.e. flushing time) and the percentage of veligers that were removed (Kendall's tau correlation coefficient = −0.632, $p < 0.0001$, N = 330). There were significant difference in veliger removal rate at different time intervals (ANOVA, $F = 171.1$, $p < 0.0001$) and multiple comparison test (SNK) shows the following order: 30 s > 60 s > 90 s > 120 s > 150 s > 180 s = 600 s = 240 s = 270 s = 300 s = 210 s.

Discussion

The correlation between the time of flushing and the percentage of veligers observed indicated that as time of flushing increased, the percentage of veligers observed decreased significantly. However, our study also found that a 5 min interval is not sufficient to remove all veligers from the livewell of a boat. There was an average of 4.8 veligers observed in the final sample of each replicate. Only one replicate had 0 veligers in the final sample while 4 replicates had 10 or more veligers in the final sample. The replicate with no veligers was one of the two replicates with a lower concentration. Veligers remaining in the livewell after 5 min of flushing could be transported and introduced into another body of water. A longer flushing period could result in the removal of more veligers (Figure 2) but is not likely to be performed by watercraft owners. Jensen (2009) found that the majority of boat owners (69%) were willing to spend 5 min to get their boat washed; however, only 26% of boat owners were willing to spend 10 min at a boat wash station. Under natural conditions, quagga mussel veliger densities in Lake Mead typically are below 40 veligers/L (Gerstenberger et al. 2011); however, peak densities of 200 veligers/L of zebra mussel veligers have been found in western Lake Erie (Garton and Haag 1993) or even higher than 200 veligers/L was occasionally reported in Otsego Lake (Horvath 2012). The concentration of veligers tested here represent higheer concentrations seen during an entire spawning season, which may only occur for a period of a few days. It is likely that

watercraft would encounter lower densities of veligers which may be more easily removed by flushing.

During the first 150 s of flushing, greater than 90% of veligers were removed. This short period of flushing can remove a large proportion of the veligers that are left in the livewell, which can greatly reduce the likelihood of spread when no other decontamination method is available for use. It is reported that the mortality rate of dreissenid mussel veligers is very high (from 90% to 99%) from hatching to successful settlement under laboratory culture conditions (Mackie and Schloesser 1996; Sprung 1987). In addition, the disturbance from flushing itself will also be a problem for veligers. Taking all these into consideration, a significantly reduced number of veligers can help minimize the spread of zebra mussels because it decreases the likelihood that veligers will settle close enough to each other for reproduction to occur (Dalton and Cottrell 2013). However, currently we don't know if there is a threshold of the number of veligers below which reproduction will not occur.

It is recommended that 150 s is needed for flushing veligers in the livewell because more than 90% of them can be removed. Effectiveness of livewell flushing may vary dependent upon the density of veligers and the species of veligers involved. Additional studies using lower veliger densities and quagga mussel veligers are recommended. Comparisons between the effectiveness of hot water and ambient temperature flushing could also be performed to examine whether hot water treatment is able to remove the rest of the 10% of the veligers when treated with water with ambient water temperature. Moreover, effectiveness of flushing potential veligers remaining in different configurations of livewells, as well as the bilge area of a watercraft needs to be investigated in the future.

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