Report

Colonization and Population Growth of Zebra Mussels in Reservoirs of the Housatonic River Basin.

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July 7, 2015

Executive Summary

Zebra mussels (*Dreissena polymorphya*) have inhabited the upper reaches of the Housatonic River at least since 2009 and the impoundments of the lower Housatonic River at least since 2010. In 2011 we began a study to investigate the concentrations of larvae in the impoundments, how the populations of larvae and adults change over time, and from which source they might have come.

As a continuation of this study, in 2013 and 2014, samples were taken from Lakes Candlewood, Lillinonah, and Zoar, the Housatonic River which connects them, and two brooks and a pipe from bodies of water known to contain populations of mussels. Samples were examined for zebra mussel larvae (veligers) using cross polarization microscopy (CPM) and polymerase chain reaction (PCR) assays. In addition, colonization of adults was examined in Lakes Candlewood, Lillinonah, and Zoar and changes in zooplankton density were studied in Zoar.

To date no zebra mussels—veligers or adults— have been found in Candlewood Lake. The danger of future introduction, however, is very real. In contrast, populations of veligers are increasing exponentially in Lillinonah and Zoar. As the density of veligers increases the numbers of zooplankton have clearly decreased.

Numbers of adult mussels settling on artificial substrates greatly increased from 2013 to 2014. While the maximum shell size was found to be smaller than those in other studies (our mussels were only a few months old), the growth rate is similar to that found in other reservoirs.

Laurel Lake in Lee, MA is a possible source of zebra mussels in the Housatonic River through two routes. Laurel Brook, which flows intermittently from lake to river was seen to contain living veligers, as was the pipe that flows constantly from Laurel Lake to Housatonic River by way of the Eagle Paper Mill in Lee. East and West Twin Lakes in Salisbury, CT have populations of zebra mussels. These lakes drain into the Housatonic through Shenob Brook. On its way from lake to river, this brook temporarily widens into an area of wetland, which is not the preferred habitat of zebra mussels. Although veligers were found in Shenob Brook, their numbers were very low and the possibility that this brook is a significant source of veligers downstream is doubtful.

Although a path for the colonization of the Housatonic impoundments from Laurel Lake clearly exists, our preliminary DNA analysis suggests that the zebra mussels found in Lillinonah and Zoar are more closely related to those in Lake Champlain. Caution must currently be used in interpreting these data because of

the low sample size. Analyses of additional samples might clarify questions of the source of mussels in the Housatonic.

The contrast between the small numbers of veligers found in the Housatonic River upstream of Lakes Lillinonah and Zoar and the very high densities in the lakes themselves suggests that the mussel populations in the lakes are successfully reproducing. The proportion of adult mussels spawned locally is difficult to judge, however, due to our insufficient understanding of water resident times in Lillinonah and Zoar.

Introduction

In 2009 zebra mussels (*Dreissena polymorphya*) were reported in Laurel Lake (Lee, MA), its outlet Laurel Brook, and below the confluence of Laurel Brook and the Housatonic River in western Massachusetts (Biodrawversity, 2009). In 2010 the CT DEEP reported small colonies in Lakes Lillinonah and Zoar which are impoundments of the lower Housatonic River, , and that are some 80 miles downstream from the confluence of Laurel Brook and Housatonic River. Additional research that year suggested that the populations in Lake Zoar had not been established for more than one year while the smaller number of individuals at Lake Lillinonah may have been established prior to that (Biodrawversity, 2011)

In 2011 and 2012 we reported on results from monitoring efforts for zebra mussel veligers in Lakes Candlewood, Lillinonah, and Zoar using cross polarization microscopy (CPM) and polymerase chain reaction (PCR) assay. Modest increases were observed in veliger concentrations at two sites in both Lakes Lillinonah and Zoar from 2011 to 2012 while no veligers were observed in samples collected from one site on Candlewood Lake in either year. Results of PCR analyses for the presence of zebra mussel DNA closely corroborated results from CPM analyses. Below we report on results of CPM and PCR analyses conducted on samples collected from the three impoundments in 2013 and 2014.

The Twin Lakes (Salisbury, CT) are also known to harbor populations of zebra mussels (Biodraversity, 2009; Also: CT DEEP 2015 Angler's Guide). These lakes also feed streams that eventually drain into the Housatonic River. During the summer of 2014, we collected plankton samples from these streams and from the Housatonic River. Our primary objective was to gather initial data relating to the possibility of Schenob Brook as a vector by which zebra mussels of the Twin Lakes population may reach the Housatonic River in Connecticut. A secondary objective was to confirm Laurel Brook as a possible vector by which Laurel Lake zebra mussels reach the Housatonic River.

At the May 28, 2014 meeting of the Candlewood Lake Authority's Invasive Species Task Force researchers from Western Connecticut State University shared preliminary PCR data suggesting that the zebra mussel populations in Lillinonah and Zoar were more closely related to the populations from Laurel Lake than they were to populations from Lake Champlain. As a result, an experiment was conducted to determine if live veligers could be observed at the end of the pipe originating from Laurel Lake that flows to the Housatonic River *via* the abandoned paper mill in Lee, MA. Results of that investigation are reported below.

Since 2012, the concerns about colonization and potential for colonization have escalated. Based on anecdotal information and observations, the colonization at Lakes Lillinonah and Zoar dramatically increased with many hard substrates becoming covered with adult mussels by the fall of the year (see Figure 1). In 2014 the CT DEEP issued temporary authorization to FirstLight Power Resources to conduct studies to evaluate the effectiveness of zebra mussel control treatments including the application of *Zequanox*, carbon dioxide, licensed molluscicides, and quaternary or tertiary amines at the Stevenson Hydro Dam on Lake Zoar. This was a clear indication of a present or future concern about infrastructural and economic damages due to colonization of adult zebra mussel in the lower Housatonic River.

Figure 1. Photographs from Lake Zoar provided to the Candlewood Lake Authority showing single season colonization on a small aluminum boat in 2014. The image on the right provides a close up of colonization depicted in the image on the left.

Growing concerns over the increasing adult zebra mussel populations in Lakes Lillinonah and Zoar led to a monitoring effort in 2013 in those impoundments and Candlewood Lake using artificial substrates. In 2014 the artificial substrate monitoring effort for adult zebra mussels was expanded in Candlewood Lake. Results from that program are provided below.

Impacts on lake ecology due to colonization by zebra mussels include declines in the zooplankton community (Pace et. al. 1998) due to the adult mussels' efficiency in filtering water for food. Starting in 2013 analyses of the copepod, cladoceran, and rotifer communities in Lake Zoar were performed to assess

changes in the zooplankton community structure. Results from analyses of samples collected in 2012, 2013, and 2014 are provided below.

This research initiative began in 2011 as a collaborative effort between faculty and students at Western Connecticut State University and staff at the Candlewood Lake Authority with funding from the Connecticut Department of Energy and Environmental Protection. In addition, members of the Candlewood, Lillinonah and Zoar communities have contributed in various ways including providing transportation on Lakes Lillinonah and Zoar for veliger sample collections and deployment artificial substrates to monitor adult growth in all three impoundments. Additional funding was also provided by the Goldring Family Foundation, the Lake Lillinonah Authority, the Lake Zoar Authority, and the Woman's Club of Danbury / New Fairfield.

Zebra Mussel Veligers in Reservoirs

Sampling

During the summer of 2013 and 2014 (Table 1), plankton samples were collected from Lakes Candlewood, Lillinonah, and Zoar. Lake samples were taken using zebra mussel veliger nets (63 μm mesh, 50 cm diameter, 200 cm long with a 500 ml bucket). To avoid cross-contamination of veligers and DNA among the lakes, a separate net was used exclusively in each of the three lakes. Between sampling dates the nets were soaked in a bleach solution to kill any veligers clinging to the nets, as well as to destroy any residual DNA. All sampling nets were purchased from Aquatic Sampling Company (no longer in business).

Table 1. The number samples collected and counted from Lakes Candlewood, Lillinonah, and Zoar in 2013 and 2014.

	Samples collected	Samples counted
2013		
Candlewood Lake	12	12
Lake Lillinonah	31	23
Lake Zoar	27	19
2014		
Candlewood Lake	9	6
Lake Lillinonah	27	16
Lake Zoar	30	21

Candlewood samples were collected near the boat barrier separating the aqueduct where the intake structure is located from the lake proper near Lynn Deming Park in New Milford, CT. As in previous years, samples were collected in Lake Lillinonah at a site designated as L15 by Ethan Nedeau (Biodrawversity, 2011). Unlike previous years, in 2013 and 2014, site L10 (a cove site) was replaced by a site south of the Route 133 bridge crossing at the Friends of Lake Lillinonah GLEON Buoy location. This site, located in the natural river channel, is referred to as site LX in this report. The Zoar samples were taken at sites designated as Z9 and Z11 by Nedeau (Figure 2).

All lake samples were taken as 5-meter vertical tows from boats and each tow filtered 1157 L. For each sample, the plankton was divided into three 125-ml bottles. Absolute ethanol was added to bring the concentration to 70%. Each bottle was labeled with the sample number plus an A (microscopy), B (PCR), or C (reserve). In the lab, samples were stored in a refrigerator until processing. Three plankton samples were collected at each site on each sample date.

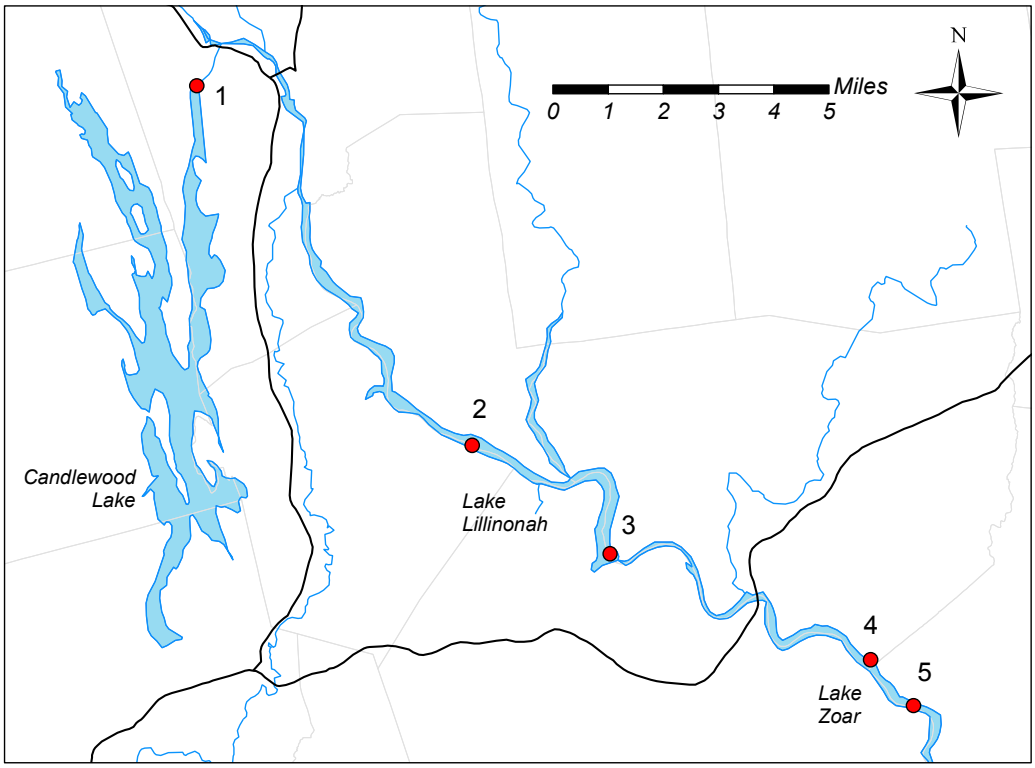

Figure 2. Locations of sampling sites: (1) Candlewood, (2) Lillinonah LX, (3) Lillinonah L15, (4) Zoar Z9, and (5) Zoar Z11.

Microscopy

Our procedure for examining samples microscopically is mostly unchanged from 2011 and 2012. Samples to be examined with CPM (Johnson, 1995) were first removed from refrigeration and allowed to reach room temperature. A large 77 mm photography-grade polarizing filter was placed on the transparent glass plate on the stage of an Olympus SZ-ST stereo zoom microscope. The Olympus TL3 light source was directed up through the large polarizer on the stage. Small aliquots of sample were poured from one of the 125-ml bottles labeled A into a glass Petri dish (60 x 15 mm) and then set on top of the large polarizing filter. A smaller 62 mm photography-grade polarizing filter was seated on top of the Petri dish and rotated until the light oriented by the large filter was blocked by the small filter, resulting in a darkened field of view as seen through the ocular lens. Under these conditions only items containing optically anisotropic crystals such as calcite or quartz appear bright against the dark field. The concentric arrangement of the crystals within the shell or carapace of some organisms (such

as zebra mussel veligers) makes the shells appear as glowing Maltese crosses (Johnson, 1995). Such objects are termed "birefringent", and include the veligers of some bivalves including zebra mussels, ostracods, and some phytoplankton.

After cross polarization was achieved, the top filter was left in place and the sample was examined by carefully moving the lower filter by hand, either up or down or side to side along the transparent glass plate, while making observations at a magnification of 250X until the entire surface area (28 cm^2) of the Petri dish was examined for veligers. Birefringent objects, including veligers and ostracods, were examined at higher power (400X) with and without cross polarization. Observations were made without cross polarizing light simply by removing the top filter in order to see diagnostic anatomical features (e.g. eyespots and appendages of ostracods).

After examining the contents of the sample, the small polarizing filter was removed and the contents of the Petri dish were emptied into a small beaker. The Petri dish was rinsed with ethanol into the beaker and refilled with a new aliquot from the sample. The procedure was repeated until the entire sample was analyzed. Completed samples were returned to the original 125-ml bottle. For samples containing especially large amounts of plankton, smaller aliquots were used and sometimes diluted with additional ethanol.

Due to the significant increase in the number of veligers found in 2013 and especially 2014 samples, the counts from some samples were estimated from 10 ml or 20 ml subsamples. For example, sample 14-264 A from Lake Lillinonah was estimated to contain 47,013 veligers (121.5 per L) based on the 3761 counted in a 10 ml subsample.

Results

The time-consuming nature of counting veligers and the vast increase in their numbers limited the number of samples we could process. This, in turn, limits the statistical conclusions that we can draw from the data. Tabular and graphical presentations of the data do, however, make clear some broad trends.

First, we have no evidence that there are zebra mussel veligers in Candlewood Lake. Repeated sampling over four years has not turned up a single individual. In contrast to Candlewood, numbers of veligers in Lillinonah and Zoar have increased greatly. Average density in Lake Lillinonah in both 2013 (Table 2) and 2014 (Figures 3) peaked in early July. In Lake Zoar, veligers peaked in late July in 2013 (Table 2) and in late June in 2014 (Table 2, Figure 4). Over the period of 2011 to 2014 the veliger densities of Lillinonah and Zoar have increased exponentially

(Figure 5), i.e. the growth in in Lillinonah ($R^2 = 0.95$) and Zoar ($R^2 = 0.94$) closely fit an exponential model.

Table 2. Average veliger densities (per Liter) 2013 and 2014 in lakes Lillinonah and Zoar.

	Lillinonah 2013	Lillinonah 2014	Zoar 2013	Zoar 2014
***circa* June 1**	0.002	1.36	0.077	4.45
***circa* June 15**	0.003	5.35	0.106	44.80
***circa* July 1**	0.001	59.82	0.261	38.05
***circa* July 15**	0.001	5.30	0.003	26.73
***circa* August 1**	0.0004	3.15	0.016	4.65

Effects of Zebra Mussels on Zooplankton in Reservoirs

The negative effect of zebra mussels on zooplankton abundance has been well documented. Pace et. al. (2010) in a long-term study of the Hudson River found that zooplankton biomass declined approximately 50% after the zebra mussel invasion, especially citing the effects on rotifers and copepod nauplii. Wong et. al. (2003) also studied the Hudson River and found a similar decline in biomass, again especially noting the decline in rotifers. Most importantly, Higgins and Vander Zanden (2010) performed a meta-analysis of published studies on the effects of zebra mussels in freshwater ecosystems. For pelagic organisms in rivers and lakes they found a 44-77% decline in zooplankton biomass; smaller bodied zooplankton such as rotifers and copepod nauplii declined 70-85% while adult copepods and cladocerans declined 56-67%.

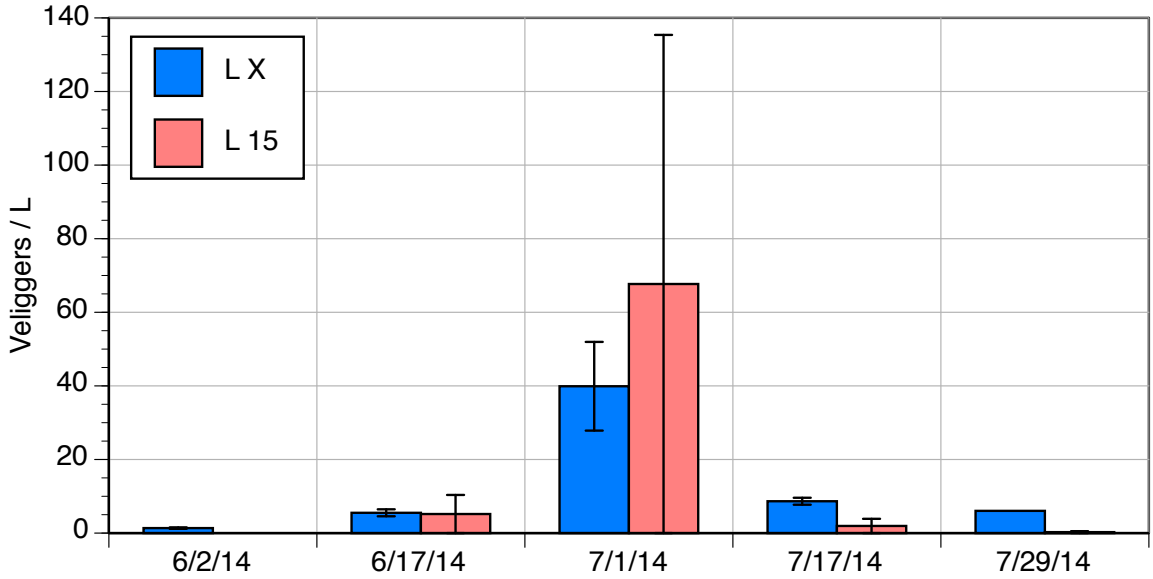

Figure 3. Mean veliger counts per liter in Lake Lillinonah samples, 2014. Error bars represent standard error.

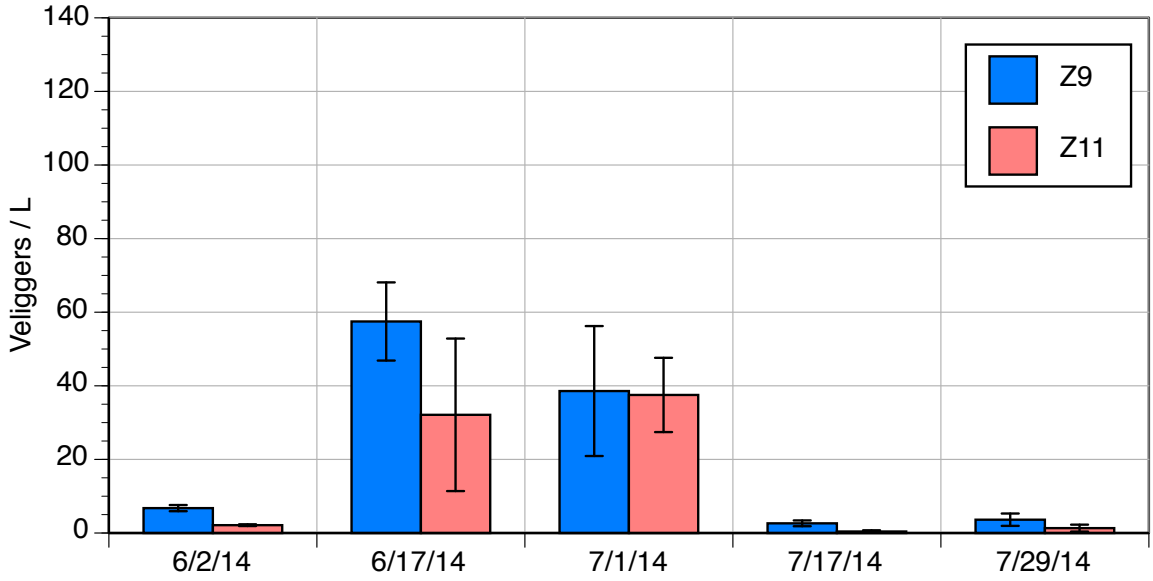

Figure 4. Mean veliger counts per liter in Lake Zoar samples, 2014. Error bars represent standard error.

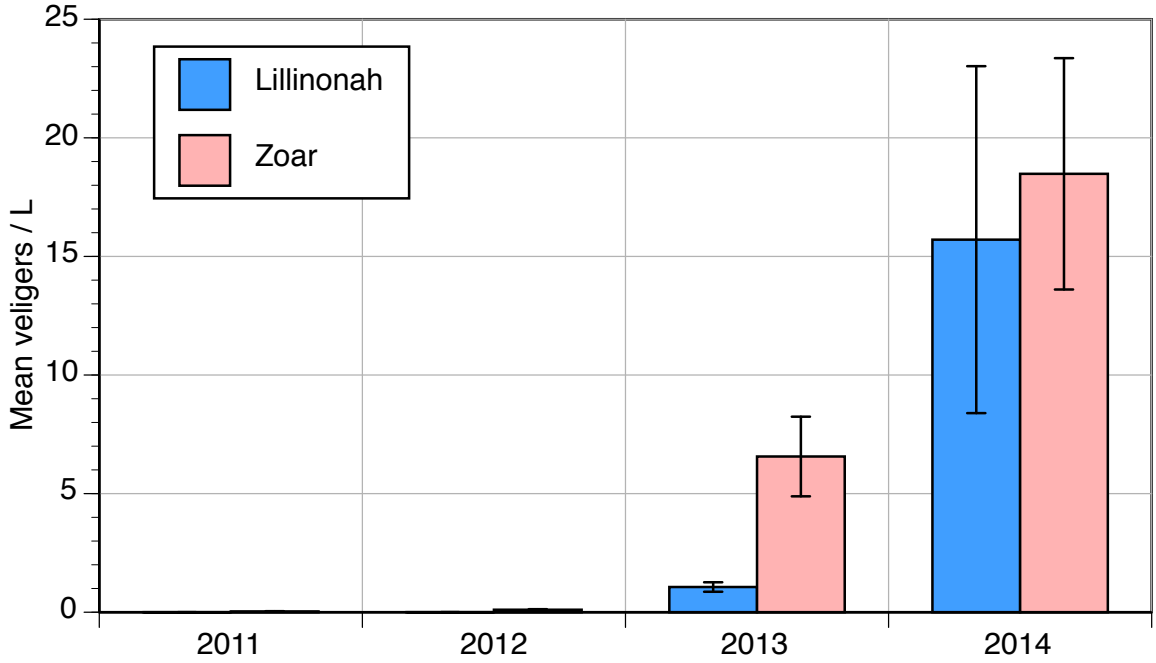

Figure 5. Mean veliger counts per liter in samples from lakes Lillinonah and Zoar, 2011–2014. Error bars represent standard error.

Sampling

As previously described, water samples were collected from two different locations on Lake Zoar on six different dates during the summers of 2012 and 2013 (n =12 samples each) and five different dates during the summer of 2014 (n = 10 samples). The relative abundance of zooplankton in each sample was measured by the following method:

Each sample bottle was mixed using a consistent method. Then, a portion of the bottle's contents was taken by pipette from the center of each bottle and six drops were placed in each of two depression slides. Each depression slide was completely examined using an Olympus CX31 compound microscope and the number of the major groups of zooplankton (Cladocera, Copepoda, Rotifera) were tallied. The Cladocera were classified as to Family (predominantly Bosminidae and Daphniidae) and the Copepoda were classified as to Order (predominantly Calanoida, Cyclopoida and their nauplius larvae). The major types of Rotifera were also noted (Keratella, Kellicottia, Polyartha, other members of the Orders Ploima and Ploimida). However, for the purposes of this publication only the total number of Cladocera, Copepoa and Rotifera were analyzed.

Results

A simple regression analysis was performed on the data from each of the three groups of zooplankton. In all three cases there was a highly significant decline in the relative abundance of zooplankton from 2012 to 2014 (Table 3).

These data therefore clearly support the results of previous studies (cited directly above) concerning the effect of zebra mussels on zooplankton abundance. Indeed, the decline in the present study in the relative abundance of cladocerans (87.6%), copepods (88.7%) and rotifers (73.6 %) match or exceed the declines previously described in the published literature.

Table 3. Relative abundance of three types of zooplankton in Lake Zoar in the summers of 2012, 2013 and 2014.

Types of zooplankton	**Mean number of individuals per sample ± standard error**			**Regression analysis**
	2012	2013	2014	
Cladocera	83.9 ± 14.4	32.7 ± 12.7	10.4 ± 42.4	$p < 0.0001$ F = 26.53
Copepoda	59.1 ± 22.4	31.2 ± 9.5	6.7 ± 9.2	$p < 0.0001$ F = 26.62
Rotifera	122.7 ± 104.6	71.3 ± 23.2	32.4 ± 50.1	$p < 0.005$ F = 9.18

Adults in the reservoirs

Method

Artificial substrates were placed in each lake to monitor colonization by adult mussels. The substrates provide suitable locations for mussels to attach themselves as they become adults. Each substrate (Figure 6) consisted of four Masonite® plates 1/8" (3.2 mm) thick in graduated sizes 31.5 cm^2, 25.4 cm^2, 20.3 cm^2, and 15.2 cm^2. Each square had and a hole in the center. PVC spacers were placed between plates and all pieces connected by a stainless steel eyebolt with the eye above the largest plate. A small lead weight was attached to the underside of the smallest plate. An aluminum identification tag was attached to the eye. Each sampler was suspended by rope to a depth of 1.8 m from docks owned by lake residents willing to participate in the study.

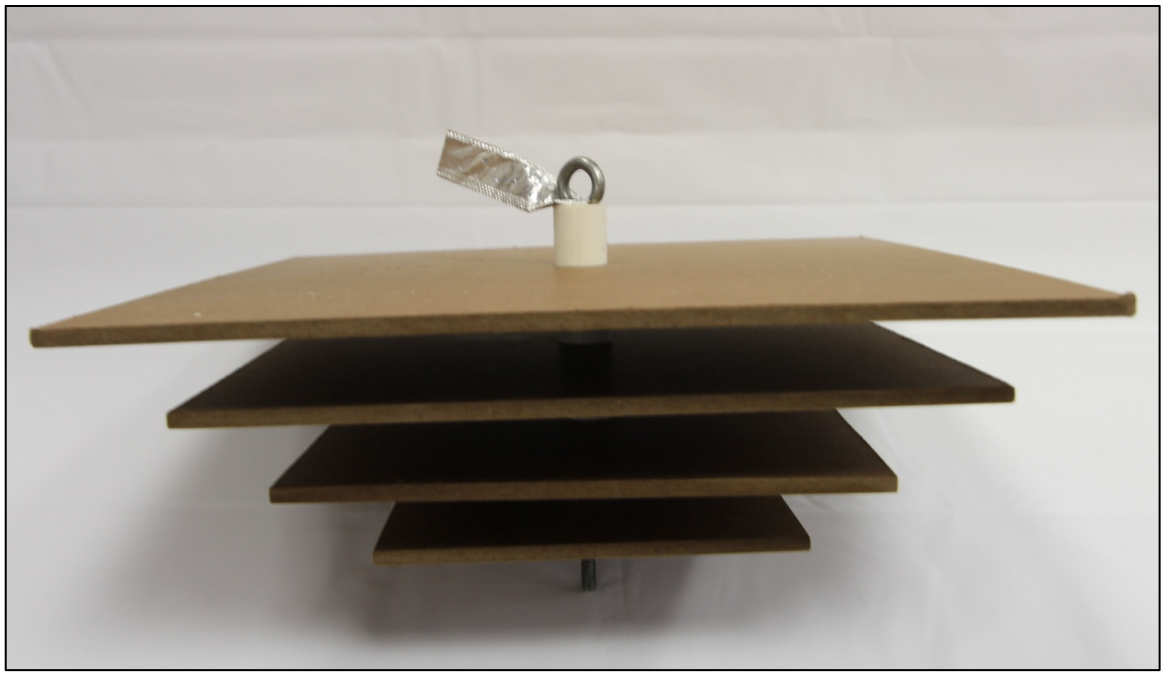

Figure 6. Artificial substrate used to monitor colonization of adult zebra mussel in reservoirs.

In 2013, 25 substrates were placed in Lake Lillinonah and 8 in Lake Zoar in late June. Of these, 12 substrates from Lillinonah and two from Zoar survived to be retrieved in late October (Figure 7). In 2014, 7 substrates survived to be retrieved from Lake Lillinonah. At three sites, substrates were recovered both years. The attached mussels were scraped from both sides of each plate and preserved in alcohol. The shell of each mussel was later measured and counted.

In 2013 three substrates were deployed in Candlewood Lake: one of a navigational buoy in Lattin's Cove, one off a regulatory speed buoy in Squantz Cove, and one off the boat barrier near the outlet structure at the north end of the New Milford arm. In 2014, 16 substrates were place in Candlewood Lake as shown in Figure 8. No adult mussels were found attached to any plate placed in Candlewood, either year.

Figure 7. Locations of artificial substrates in lakes Lillinonah and Zoar, 2013.

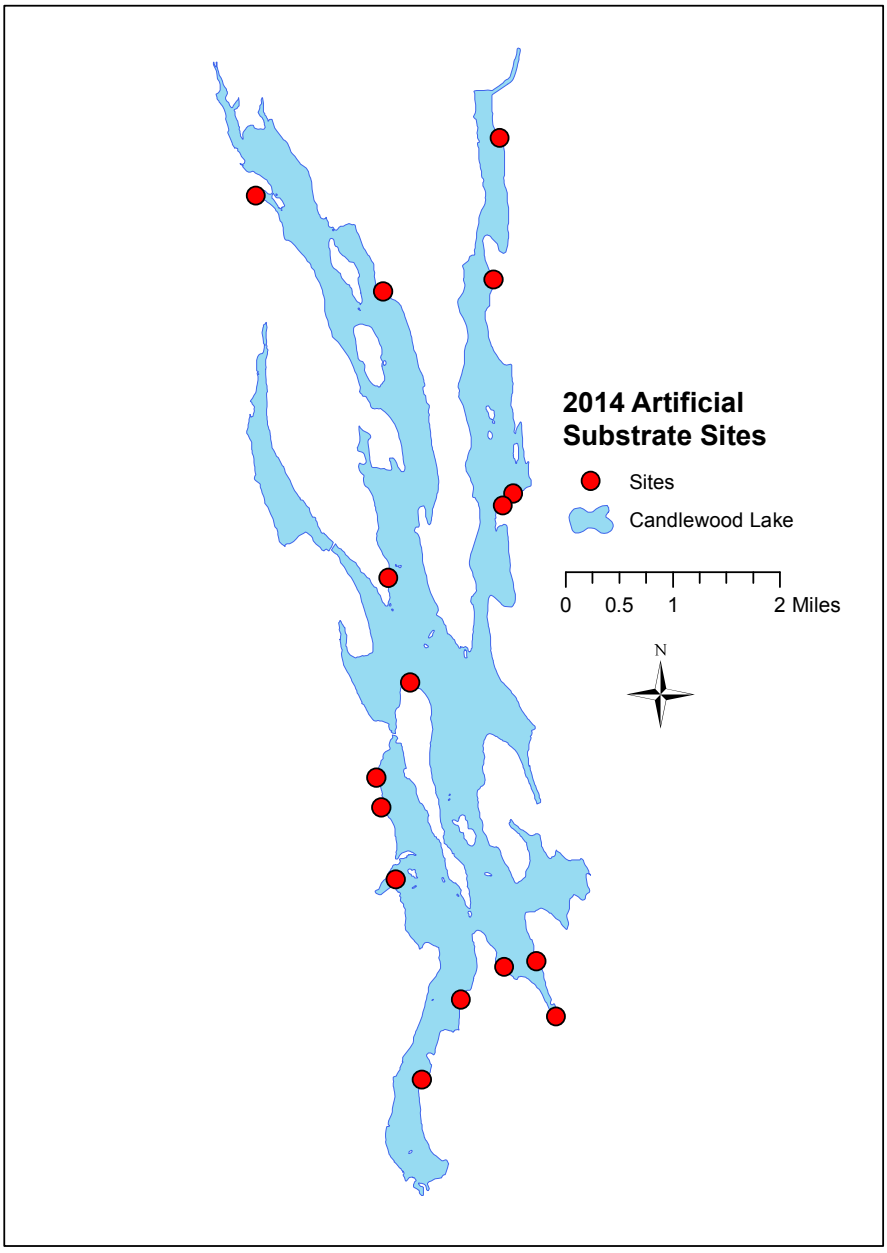

Figure 8. Locations of artificial substrates in Candlewood Lake, 2013 and 2014.

Results

In 2013, 6 of the 12 recovered substrates in Lillinonah contained 10 adult mussels or fewer (Figures 9 and 10). The substrate with the highest number (116) was L 13, and was located in the main channel of the lake. The five substrates located in the Shepaug arm and the Pond Brook arm contained from one to 13 adult mussels. The two substrates recovered from Lake Zoar (Figure 11) in 2013 contained 120 and 466 mussels.

Those sites in Lillinonah where substrates were recovered in both years were compared. At site L 11, the adults increased from 29 in 2013 to 1276 in 2014, at L 13 from 116 to 869, and at L 14 from 13 adults to 2428 (Figures 12–14.)

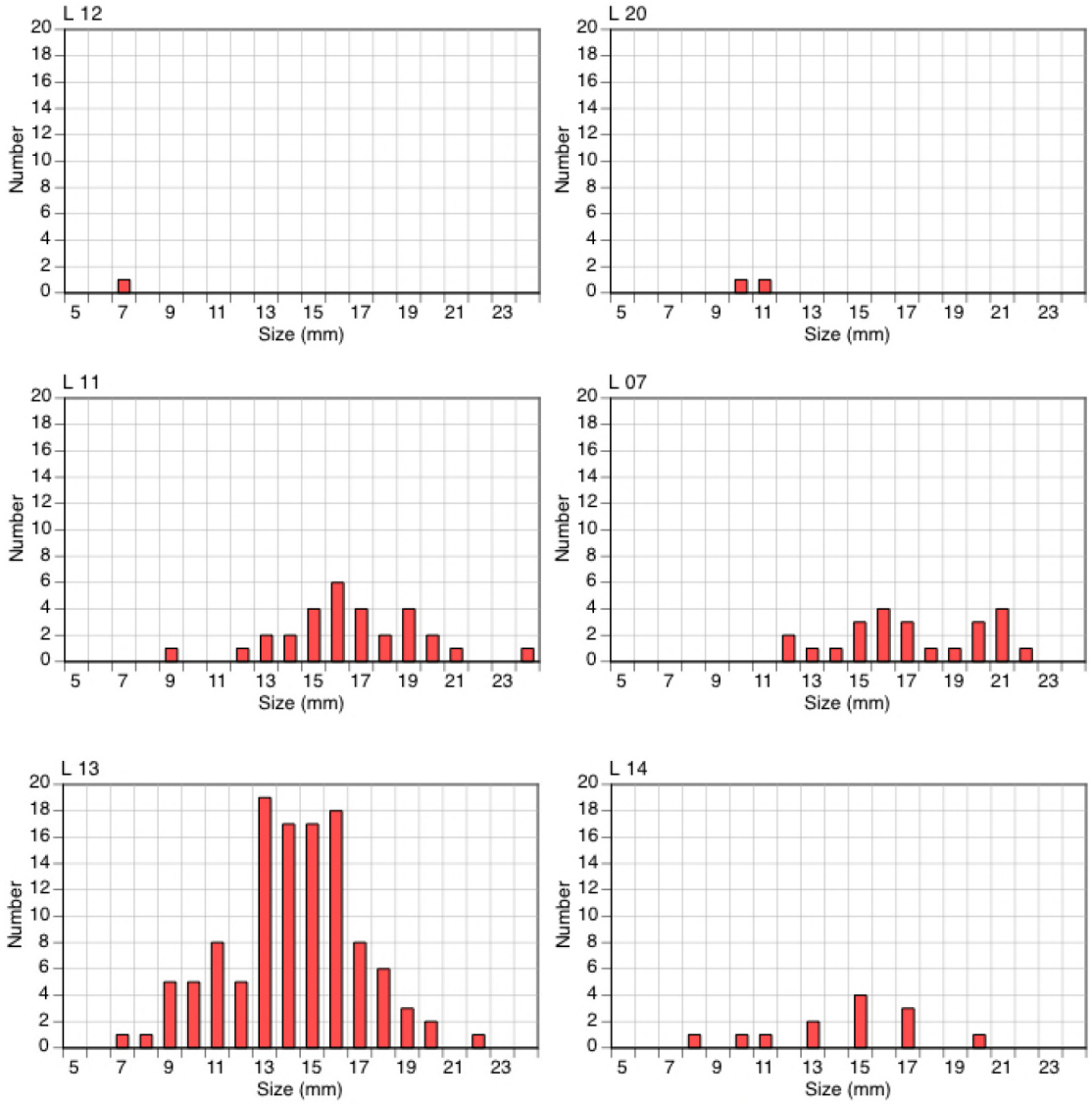

Figure 9. Size frequency histograms from adult mussels attached to artificial substrates in Lake Lillinonah, 2013.

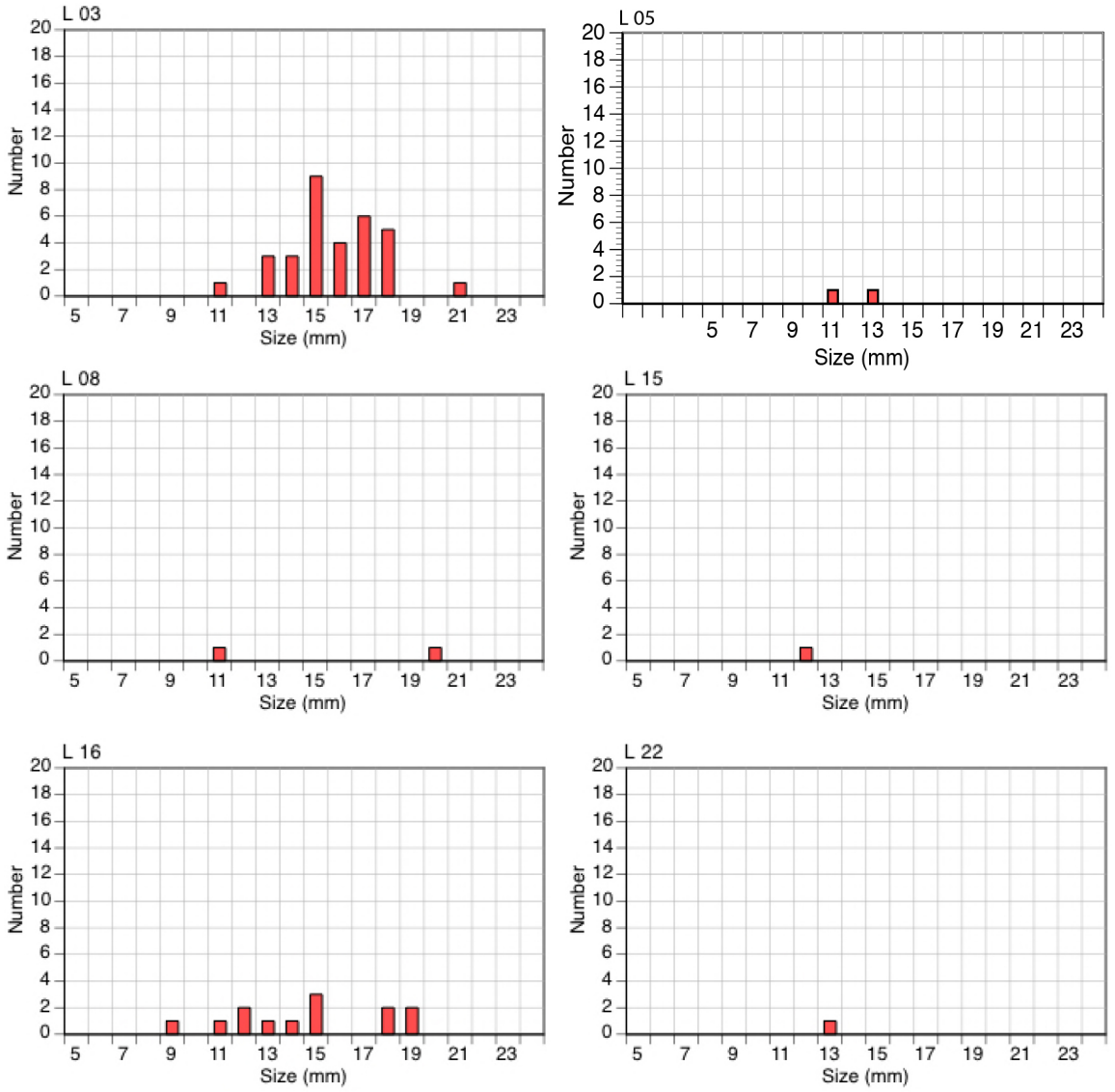

Figure 10. Size frequency histograms from adult mussels attached to artificial substrates in Lake Lillinonah, 2013.

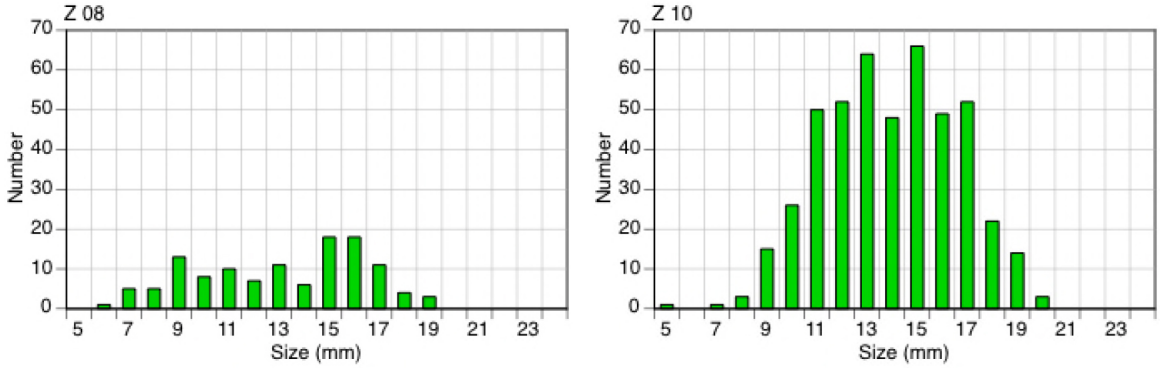

Figure 11. Size frequency histograms from adult mussels attached to artificial substrates in Lake Zoar, 2013.

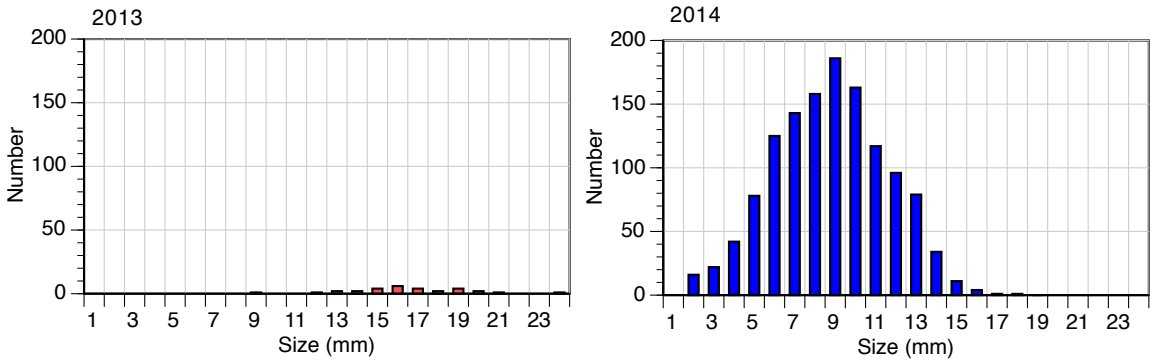

Figure 12. Comparison of size frequency distribution in 2013 and 2014 on artificial substrates at site L 11 (Lake Lillinonah).

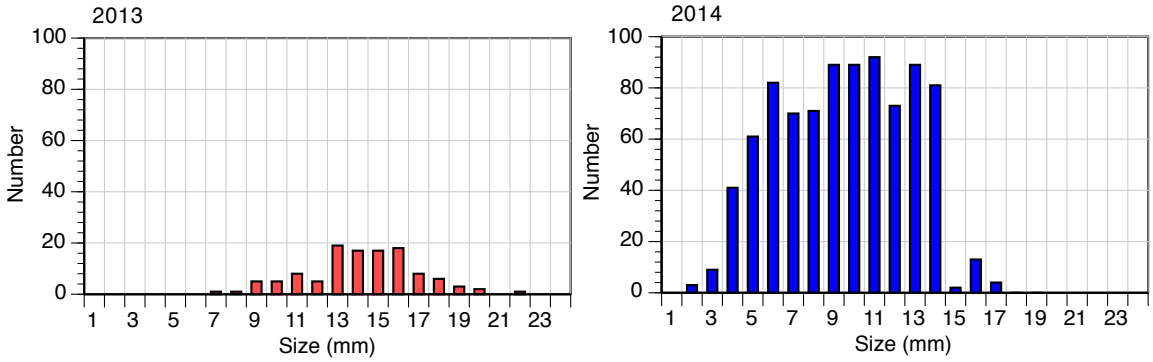

Figure 13. Comparison of size frequency distribution in 2013 and 2014 on artificial substrates at site L 13 (Lake Lillinonah).

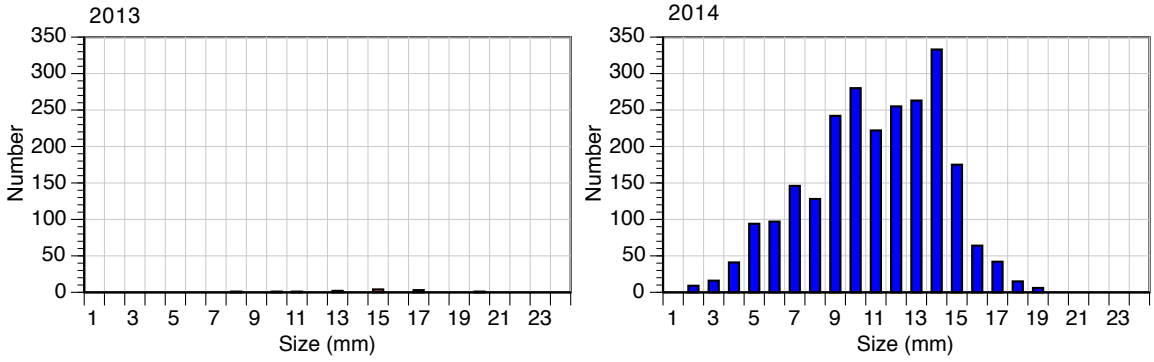

Figure 14. Comparison of size frequency distribution in 2013 and 2014 on artificial substrates at site L 14 (Lake Lillinonah).

Sampling veligers in the pipe at Lee, Massachusetts

Zebra Mussels were found in Laurel Lake in Lee, Massachusetts in 2009 (Biodrawversity, 2009). Constructed to provide water to the now-closed Eagle Paper Mill, Laurel Lake has two hydrological connections to the Housatonic River. Laurel Brook flows intermittently to the river from the overflow channel at the dam forming the lake. A pipe carries water from the lake dam approximately one km to the old mill, where it flows into the building (the filter house) that once housed the filter apparatus. Currently the pipe exits the filter house and flows freely into the Housatonic River (Figure 15).

Figure 15. The pipe carrying water from Laurel Lake empties into the Housatonic River at the old Eagle Paper Mill in Lee, MA.

Inside the filter house a valve (Figure 16) allows the controlled extraction of water from the pipe. In an effort to determine if veligers survive transit in the pipe from lake to river, three water samples were collected on July 30, 2013. Each

water sample was 50 L and each was filtered through a plankton bucket (63 μm mesh) concentrating samples to a volume of 50 – 60 mL. After remaining still for 10 minutes to allow any live veligers to recover from the turbulence of the sampling process, each filtered sample was examined for live veligers using cross polarization microscopy. Living veligers were found in all three samples at densities of 0.12, 0.32, and 0.28 veligers L^{-1}.

Figure 16. A small valve allows the sampling of water from the pipe carrying water from Laurel Lake to the Housatonic River at the old Eagle Paper Mill in Lee, MA.

Zebra Mussel Veligers in Streams and Rivers

Sampling

Laurel Lake drains into the Housatonic River via a pipe associated with the now-closed Eagle Paper Mill and intermittently via Laurel Brook (Figure 17). We sampled Laurel Brook immediately prior to its outfall into the Housatonic River in Lee, MA (Figure 17) on 17 July 2014, a day on which the stream flowing.

Schenob Brook is fed with water from the Twin Lakes, as well as wetland areas and various other lower-order streams (Figure 18). We sampled two locations in Schenob Brook on 31 July 2014, one between the Twin Lakes and a slow-flowing wetland portion of the stream, and one after the wetland area, as close to its outfall into the Housatonic River as we could get by public land (Figure 17; Figure 18).

We sampled the Housatonic River both immediately upstream and immediately downstream from the Laurel Brook outfall in Lee, MA on 17 July 2014 (Figure 17). We also sampled the Housatonic River further downstream at Boardman's Bridge in New Milford, CT on 26 June 2014 (Figure 17). We sampled a total of six sites, taking duplicate samples at each.

We collected plankton samples from each stream/river site by fastening a specially-designed plankton net with a stainless steel frame (63 μm mesh, 30 x 45 cm opening, 100 cm long with a 500 mL bucket; Figure 19) to the bottom sediment with stainless steel spikes. We measured stream/river flow rates using a Global Water Flow Probe® model FP111, and sample durations were recorded (ranging from 5 to 20 MINUTES); we estimated total sampled volumes from these data coupled with the net opening area. We set sample durations based on stream flow rate; shorter durations were required to avoid clogging the net in streams with high flow rate and substantial suspended particles. After the desired sample duration, we rinsed net contents into the sample bucket, and then into 500-mL bottles with 80% ethanol. In the laboratory, we thoroughly mixed ethanol-preserved samples and divided each evenly into three 125-ml bottles. Each bottle was labeled with the sample number plus an A (microscopy), B (PCR), or C (reserve). We stored samples in a refrigerator until processed.

Our microscopy procedure was the same as for reservoir samples and is detailed under the *Microscopy* subheading of the **Zebra Mussel Veligers in Reservoirs** section of this report. For samples containing especially large amounts of extraneous particulates, we examined 20- to 60-mL subsamples and estimated veliger densities in samples accordingly. For example, we estimated that sample 14-005 A from the downstream Housatonic River site in Lee, MA contained 132 (2.8 veligers / kL) based on the seven veligers we counted in a 20-mL subsample.

Results

Total volumes of stream/river water filtered in samples ranged from approximately 4,000 to 47,000 L, with a mean of about 22,000 L. The average number of veligers estimated in samples was 275, ranging from 0 to 1,866. For scaling purposes, we present mean veliger densities (of duplicate samples, ± standard error) in numbers per kiloliter (kL) of stream water; note that other sections of this report normalize veliger densities to liters rather than kiloliters. Veliger densities in stream/river samples were substantially lower (typically around three orders of magnitude) than were densities of veligers found in Lakes Lillinonah and Zoar.

We found no veligers in duplicate Housatonic River samples taken upstream of the Laurel Brook outfall. The highest average density of veligers was found in Laurel Brook at 254.4 (± 18.6) veligers / kL (Figure 20). Both the Housatonic River sample immediately downstream of the Laurel Brook outfall, and the Schenob Brook sample from between the Twin Lakes and the wetland area had mean veliger densities near 1.0 veliger / kL (Figure 20). Finally, the Housatonic sample taken further downstream in New Milford, CT (Boardman's Bridge), and the Schenob Brook sample downstream of the slow-flowing wetland areas had veliger densities below 0.1 veligers / kL (Figure 20).

Our detection of zebra mussels in the Housatonic River sample immediately downstream from the Laurel Brook outfall, coupled with our lack of detection in the sample immediately upstream from the outfall, suggests that veligers from Laurel Brook are getting into the Housatonic. Furthermore, this indicates that there is not likely a source further upstream in the Housatonic watershed.
Both of our sample sites in Schenob Brook had significantly lower densities of veligers than Laurel Brook (ANOVA followed by Tukey's pairwise comparisons; $F_{5,11} = 185$, $P < 0.0001$). Additionally, the density of veligers in Schenob Brook downstream of the slow-flowing wetland area was lower than that in the sample closer to the likely source of the veligers, the Twin Lakes. In fact, the low density of veligers in the post-wetland Schenob Brook site is the result of finding only a single veliger in the over 30 kL of stream water filtered by our plankton net. This suggests that veligers largely settle out, die, or otherwise do not pass through the

wetland areas. Regardless of the mechanisms involved, our data show that Schenob Brook supplies significantly fewer veligers to the Housatonic River than does Laurel Brook.

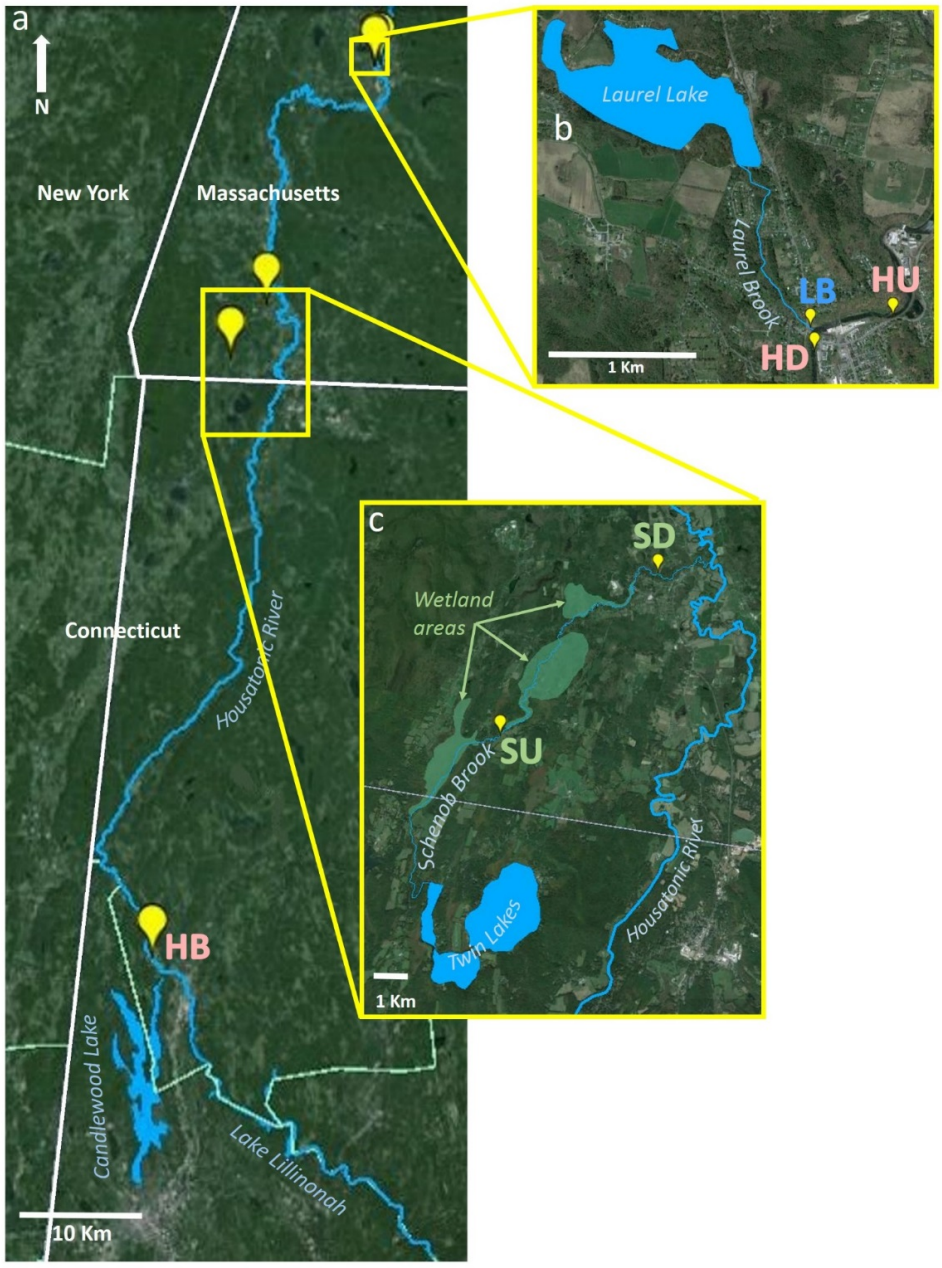

Figure 17. Sites screened for zebra mussel veligers in the Housatonic River watershed. The large map (panel a) shows the entire extent of the zebra mussel monitoring project. Inset maps (panels b, c) detail confirmed zebra mussel infested lakes and streams that drain these lakes into the Housatonic River. Site abbreviations are as follows: HB = Housatonic at Boardman's Bridge, HD = Housatonic immediately downstream from Laurel Brook outfall, HU = Housatonic upstream of Laurel Brook outfall, LB = Laurel Brook, SD = Schenob Brook downstream of the major wetland, SU = Schenob Brook upstream of the major wetland.

Figure 18. The photograph shows a slow flowing section of Schenob Brook as it meanders through a wetland area.

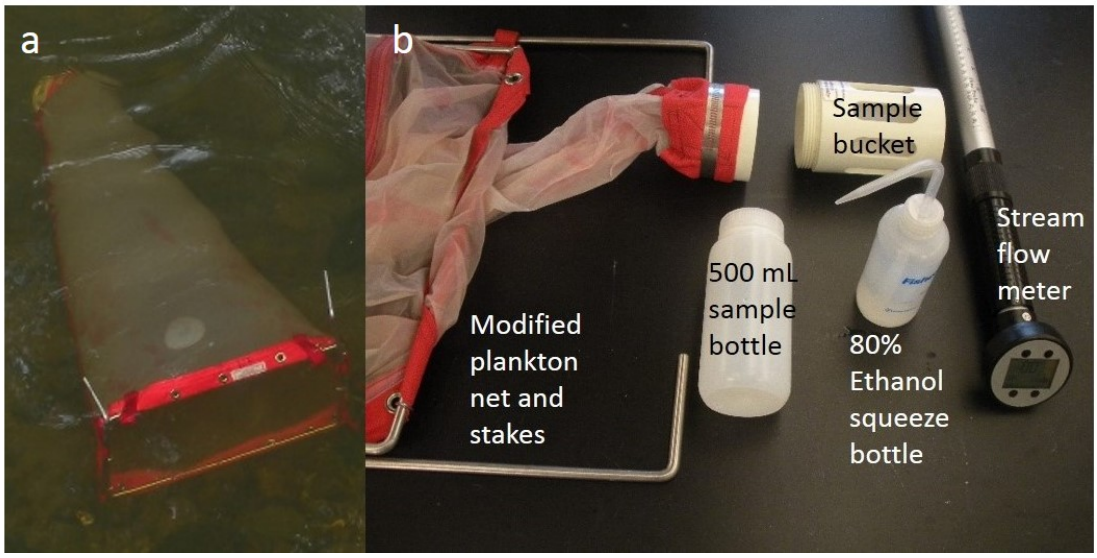

Figure 19. The photograph (panel a) shows the stream sampling apparatus with a modified plankton net staked to the stream bed. After 5 to 20 minutes (depending on stream flow) we lifted the net from the water, and rinsed its contents into the sample bucket. We the rinsed sample bucket contents into a bottle with 80% ethanol. We stored samples at approximately 4°C until sorting. The sampling equipment is also pictured (panel b).

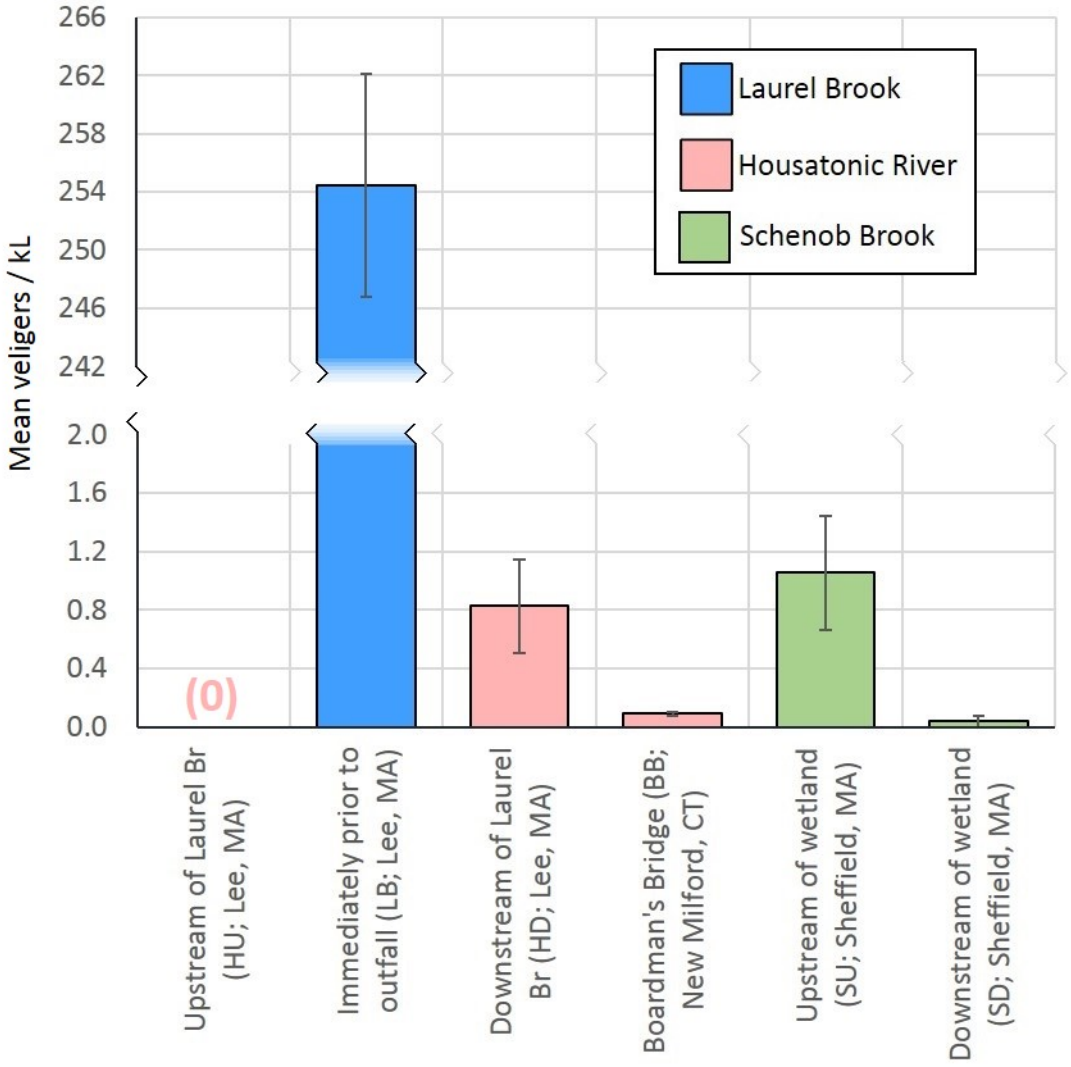

Figure 20. Average densities of veligers in samples (*n* = 2 per site). Note break and change in scale of the y-axis. Error bars denote standard error.

Dissection of Adult Zebra Mussels for DNA Isolation

Our lab was interested in isolating and purifying DNA from adult zebra mussels for various molecular studies. One concern about simply using whole body mass for DNA isolation was the possibility of contaminating DNA from food contents in the mussel's gut. Therefore, we developed a protocol for dissecting select tissues from adult zebra mussels. Furthermore, we tested the efficacy of different commercial DNA isolation kits in producing high quality, high yield genomic DNA from dissected zebra mussel tissue.

Materials & Methods
Adult zebra mussels were collected or donated from various sources — Lake Lillinonah, Lake Zoar, and Twin Lake (Connecticut), Laurel Lake (Massachusetts), and Lake Champlain (Vermont) — and stored in 70% ethanol under 4°C refrigeration until use. Adductor muscles and mantle tissue were identified (U.S. Army Corps of Engineers, 2015), dissected out, weighed, and stored frozen at –80°C until ready for processing.

Two commercial kits — MoBio UltraClean Tissue & Cells DNA Isolation Kit and Qiagen DNeasy Blood & Tissue Kit — were used to isolate genomic DNA from tissue samples; an optional Proteinase K digestion was included in the MoBio protocol. Purified DNA was quantified and assessed for purity by using spectrophotometry at A260 (ThermoScientific NanoDrop ND-1000 spectrophotometer) and by agarose gel electrophoresis in 1% agarose and 0.5X lithium-borate (LB; Faster Better Media) running buffer.

Results
Our lab had been using the MoBio UltraClean Tissue & Cells DNA Isolation Kit for purifying genomic DNA from adult zebra mussels. Gel electrophoresis and spectrophotometry analysis of the resulting DNA showed significant sample-to-sample inconsistencies in yield and purity (data not shown). Hence, we explored the use of another kit — the Qiagen DNeasy Blood & Tissue Kit — to see if it made any difference.

An adult zebra mussel collected from Lake Zoar (designated "ZAF") was used in this experiment. The adductor muscles and mantle tissue were removed, and part of the tissue was processed using the MoBio kit and the rest processed with the Qiagen kit.

Table 4 shows a comparison of the quantity and quality of genomic DNA isolated using the two kits. Though only half as much tissue was used in processing by the Qiagen kit, it yielded over 7-fold more DNA than that from the

MoBio kit. Furthermore, the quality — as assessed by spectrophotometry at 230, 260, and 280 nm wavelength — was higher in the Qiagen sample (260/280 values above 1.8 and 260/230 values above 2 are usually indicative of highly pure DNA).

To assess the size of the genomic DNA, an aliquot from each sample was separated by gel electrophoresis. As seen in Figure 21, the Qiagen sample (ZAF-Q) contained a higher concentration of DNA, with a sizable portion in the high molecular weight range. The MoBio sample also displayed high molecular weight DNA, but at a much lower concentration.

NAME	Tissue mg	DNA ng/µL	260/280	260/230	Spec Curve
ZAF – Q	26	441	2.08	3.55	nice
ZAF – M	47	58	1.82	0.96	nice

Table 4. Quantity and quality of genomic DNA isolated from zebra mussel by Qiagen (Q) and MoBio (M) tissue kits.

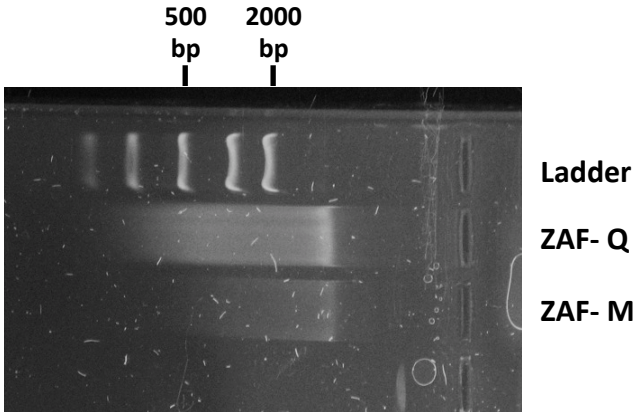

Figure 21. Gel electrophoresis of genomic DNA isolated from zebra mussel by Qiagen (Q) and MoBio (M) tissue kits.

Discussion

Overall, both the Qiagen and MoBio kit had limitations when it came to isolating DNA from very tiny tissue samples; such samples often did not produce adequate yields or quality of DNA. We prefer the Qiagen kit since the amount of tissue used in the isolation more often closely correlated with the amount of

DNA obtained at the end. There were much greater inconsistencies in yield from samples processed with the MoBio kit.

The MoBio kit uses bead-beating to physically disrupt the tissue and cells. The standard MoBio protocol suggests addition of Proteinase K with a 30 minute incubation at 60°C to assist in digesting tough tissues, and we included this step in our purification. In contrast, the Qiagen kit did not use physical disruption, but included a required Proteinase K enzymatic digestion at 56°C overnight. In our hands, the longer Proteinase K digestion recommended by the Qiagen protocol may have been key to obtaining higher yields of genomic DNA from zebra mussel tissue, and seemed to work more consistently than the bead-beating/short enzyme digestion method used in the MoBio kit.

In conclusion, we recommend using the Qiagen DNeasy Blood & Tissue Kit for consistent isolation of high-yield, high-purity genomic DNA from adult zebra mussel tissue.

Microsatellite Analysis of Adult Zebra Mussel Populations

The source of the zebra mussel invasion of the lower Housatonic River in Connecticut has never been confirmed. Some have hypothesized that zebra mussel veligers may have drifted the 70-odd miles downriver from a large starter community of zebra mussels in Laurel Lake, Massachusetts, near the headwaters of the Housatonic. Another possible source of the invasion is via adult zebra mussels attached to personal watercraft or veligers in bilge water following the use of boats in contaminated waterways (e.g. Hudson River, NY; Lake Champlain, VT).

To help determine the source of the Housatonic River invasion, we were interested in using DNA fingerprinting methods to perform a phylogeographic analysis of zebra mussel communities in the Northeast U.S. Molecular phylogeographical studies investigate genetic relationships between organismic populations. These genetic relationships may suggest historical avenues or processes by which these populations distributed themselves geographically (Knowles, 2009).

A variety of DNA-based methods have been used in ecological studies for the purpose of identifying species and distinguishing between related populations (Arif & Khan, 2009). We wanted to explore the use of DNA microsatellites. Microsatellites — also known as Short Tandem Repeats (STR) — are highly variable regions of DNA that can be easily screened in individual genomes; STRs

are best known as the genetic markers used in standard human DNA fingerprinting (Selkoe & Toonen, 2006). What makes pursuing microsatellites attractive is that several labs have already identified microsatellite regions for Zebra mussels and used them to assess genetic relationships between populations in Europe (Astanei, et al., 2005) and in the western U.S. and Great Lakes regions (Feldheim, et al., 2011). There have been no published microsatellite studies as of yet which examined populations of zebra mussels in western New England. Such data would contribute to our understanding of how this destructive invasive species spreads.

Materials & Methods

D. polymorpha zebra mussel genomic DNA was isolated from zebra mussel adults collected from several population sites: Lake Lillinonah, Lake Zoar, and Twin Lake (Connecticut), Laurel Lake (Massachusetts), and Lake Champlain (Vermont). Adult zebra mussels were stored in 70% ethanol under refrigeration until use. Adductor muscle and mantle tissue were dissected out for DNA isolation. Two commercial kits — MoBio UltraClean Tissue & Cell DNA Isolation Kit and Qiagen DNeasy Blood & Tissue Kit — were used to isolate genomic DNA from tissue samples. Purified DNA was quantified and assessed for purity by spectrophotometry and by agarose gel electrophoresis.

Microsatellite analysis of *D. polymorpha* was performed using microsatellite loci and PCR primers as determined by Feldheim, et al. (2011). Four loci were examined: Dpo 101, Dpo 221, Dpo 260, and DpoLB9. Gradient PCR was utilized to determine the optimal annealing temperature for each primer set. Three PCR kits were tested for use on microsatellites: Qiagen Taq PCR Core Kit, Qiagen Fast Cycling PCR Kit, and the Qiagen Type-It Microsatellite PCR Kit. PCR amplifications were conducted in a Bio-Rad iCycler.

Microsatellite alleles from each locus were amplified using the Qiagen Type-It Microsatellite PCR kit along with the appropriate fluorescent HEX- or FAM-labeled primers (0.2 μM), 0.08 mg/mL non-acetylated BSA, and approximately 15 ng of template genomic DNA. Samples were amplified using a 95°C hot start for 5 minutes. This was followed by 28 cycles consisting of a 30-sec denaturation at 95°C, a 90-sec annealing at 66°C (Dpo101 primers), 62°C (Dpo221 primers), 58°C (Dpo260 primers) or 60°C (DpoLB9 primers), and a 30-sec extension at 72°C. A 30-minute final extension at 60°C was used. Microsatellite PCR products were sized at the Yale DNA Analysis Facility on an ABI 3730xl DNA Genetic Analyzer.

Microsatellite alleles for each locus were binned and scored. To analyze the relationships among the different population sites, pairwise genetic distances were calculated using Nei's distance formula (Table 5; Takezaki & Nei, 1996). The distance matrix was used to construct an unrooted dendrogram (Figure 2; http://genomes.urv.cat/UPGMA/index.php; Garcia-Vallve, S., et al., 1999).

Results

Microsatellite PCR Optimization

DpoI101 primers were tested on a zebra mussel genomic DNA sample (LAW-3), using a gradient of eight different PCR annealing temperatures, as well as three different Qiagen PCR kits to see which produced the cleanest banding pattern, as displayed on agarose gels. An example of a microsatellite primer optimization protocol is shown in Table 5. The results of PCR optimization are shown in Figure 22. The PCR thermocycler temperature gradient ranged from 54°C to 66°C. All other temperatures and times followed the kit protocol.

The Dpo101 primers were expected to generate no more than two bands, corresponding to the two alleles at this locus; additional bands are expected to reflect non-specific amplification. As seen in Figure 22, non-specific bands were mostly eliminated at annealing temperatures above 63.9°C. To ensure that undetectable non-specific products were not present, we settled on a final annealing temperature at least 2°C above that point (i.e. 66°C).

iCycler Program	Std54to66		Fast54to66		Type54to66	
Hot Start	94°C	3 min	95°C	5 min	95°C	5 min
Cycles	30		30		28	
Denat	94°C	30 sec	96°C	5 sec	95°C	30 sec
Anneal A	66°C	30 sec	66°C	5 sec	66°C	90 sec
B	65.3		65.3		65.3	
C	63.9		63.9		63.9	
D	61.7		61.7		61.7	
E	58.6		58.6		58.6	
F	56.4		56.4		56.4	
G	55.0		55.0		55.0	
H	54.0		54.0		54.0	
Extend	72°C	1 min	68°C	15 sec	72°C	30 sec
Extension	72°C	30 min	72°C	30 min	72°C	30 min
Hold	4°C	hold	4°C	hold	4°C	hold

Table 5. PCR programs for temperature optimization of three PCR kits.
Std54to66 = Qiagen Taq PCR Core Kit; Fast54to66 = Qiagen Fast Cycling PCR Kit; and Type54to66 = Qiagen Type-It Microsatellite PCR Kit.

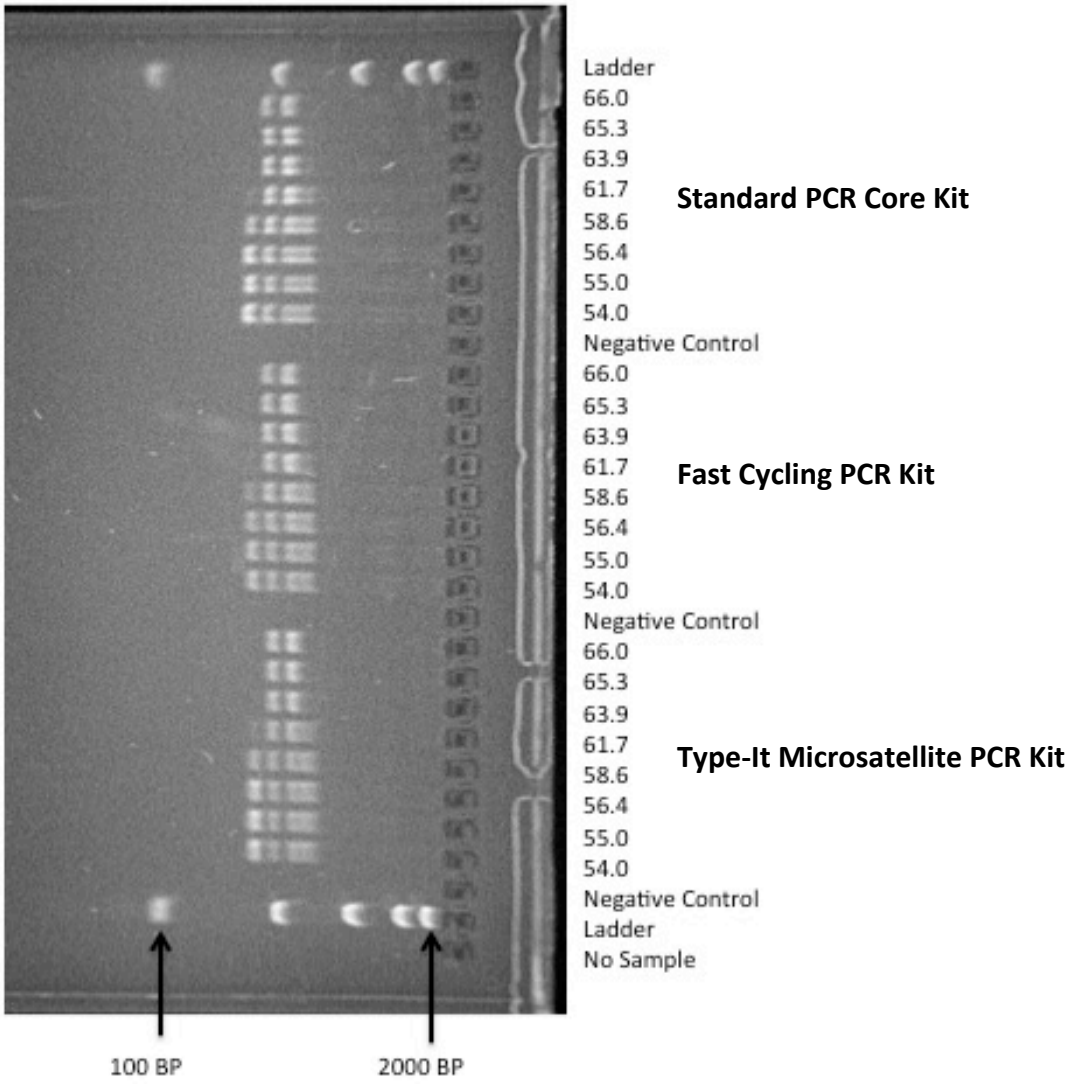

Figure 22. PCR annealing temperature optimization and kit preference for microsatellite analysis.

PCR products from a 54°C–66°C temperature gradient amplification of the Dpo101 locus in zebra mussel adult LAW-3 (Lake Lillinonah) were separated on a 3% agarose gel in lithium-borate (LB) running buffer, and stained with ethidium bromide.

Similar optimization of PCR conditions for microsatellite primer sets Dpo 221, Dpo 260, and DpoLB9 (data not shown). Optimized PCR annealing temperatures were determined to be 62°C (Dpo221 primers), 58°C (Dpo260 primers) or 60°C (DpoLB9 primers).

PCR Amplification of Microsatellite Loci in Zebra Mussels

Four different microsatellite loci were examined: Dpo101, Dpo221, Dpo260, and DpoLB9 (Feldheim, et al., 2011) in adult zebra mussels collected from several population sites: Lake Lillinonah, Lake Zoar, and Twin Lake (Connecticut), Laurel Lake (Massachusetts), and Lake Champlain (Vermont). PCR-amplified microsatellite alleles from each locus and genomic DNA were binned by size (Table 6a–6d).

Locus: Dpo101	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11	Allele 12	Allele 13	Allele 14
LA5PA	222												273	
LAW_1			231				250							
LAW_2									258					
LAW_3	222								258					
LAW_4											270		273	
LA_7						241								284
MA_5		225							258					
MA_6			231										273	
MA_8.1						240								284
TA_2	222				234									
TA_4				232				253						
VA1_5PA			231											
VA4-5					234						270			
VA6-5									258					
ZAC_2												272		284
ZAF_1										264				
ZAF_2						243						272		

Table 6a. Alleles at Dpo101 locus.
LA = Lake Lillinonah, Connecticut; MA = Laurel Lake, Massachusetts; TA = Twin Lakes, Connecticut; VA = Lake Champlain, Vermont; ZA = Lake Zoar, Connecticut. Numbers are in basepairs.

Locus: Dpo221	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6
LA5PA	153					
LAW_1	153					
LAW_2	153	157				
LAW_3				168		
LAW_4	153					
LA_7			159			
MA_5			159			
TA_2	153					
VA1_5PA	153					
VA2-5	153					
VA3-5					171	
VA4-5				168		
VA6-5						180
ZAC_1	153					
ZAC_2	153					
ZAF_1	153					
ZAF_2	153					

Table 6b. Alleles at Dpo221 locus.
LA = Lake Lillinonah, Connecticut; MA = Laurel Lake, Massachusetts; TA = Twin Lakes, Connecticut; VA = Lake Champlain, Vermont; ZA = Lake Zoar, Connecticut.

Locus: Dpo260	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11	Allele 12	Allele 13	Allele 14
LA5PA-260										263				
LA7-260	121					238								
LAW1-260													308	
LAW2-260								251					308	
LAW3-260	121								255					
LAW4-260										263		283		
LAG4-260	121													
MA5-260														398
MA6-260			131											
MA7-260	121													
MA8-260											275			
TA2-260	120						247							
TA3-260	121													
TA4-260	121													
VA15PA-260	121							251						
VA25-260				136										
VA35-260	121		132											
VA45-260	121		132											
VA65-260		126							255					
ZAC1-260	121													
ZAC2-260	121						247							
ZAF1-260														
ZAF2-260	121				226									
ZAR1-260	121		132											

Table 6c. Alleles at Dpo260 locus.
LA = Lake Lillinonah, Connecticut; MA = Laurel Lake, Massachusetts; TA = Twin Lakes, Connecticut; VA = Lake Champlain, Vermont; ZA = Lake Zoar, Connecticut.

Locus: DpoLB9	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11	Allele 12	Allele 13
LA5PA-LB9					290								
LA7-LB9				284							394		
LAG4-LB9				284									
LAW1-LB9			275	284									
LAW2-LB9				282									411
LAW3-LB9			275	282									
LAW4-LB9		272		282									
MA5-LB9		272											
MA6-LB9						295				382			
MA7-LB9	266			284									
MA8-LB9		272								382			
TA2-LB9				282									
TA3-LB9												409	412
TA4-LB9		272	278										
VA15PA-LB9				282					376				
VA25-LB9							308		378				
VA35-LB9				284		295							
VA45-LB9				284		292							
VA65-LB9		272											
ZAC1-LB9									376		394		
ZAC2-LB9			275	282									
ZAF1-LB9					291								
ZAF2-LB9				284				373					
ZAR1-LB9				284			309						

Table 6d. Alleles at DpoLB9 locus.
LA = Lake Lillinonah, Connecticut; MA = Laurel Lake, Massachusetts; TA = Twin Lakes, Connecticut; VA = Lake Champlain, Vermont; ZA = Lake Zoar, Connecticut.

Using this allelic data, a distance matrix was calculated using Nei's distance formula (Takezaki & Nei, 1996). The largest distances were observed between the Laurel Lake, Massachusetts population and populations from all the other sample sites. The shortest distance is seen between Lake Lillinonah, Connecticut, and Lake Champlain, Vermont populations. This observation is graphically displayed in the unrooted dendrogram in Figure 23.

DISTANCE MATRIX

	L	M	T	V	Z
L	0	0.29024957	0.21188806	0.19148719	0.206853379
M		0	0.70164249	0.40418001	0.570470318
T			0	0.37121733	0.286697899
V				0	0.32725968
Z					0

Table 7. Distance Matrix.
Pairwise genetic distances were calculated using Nei's distance formula. L = Lake Lillinonah, Connecticut; M = Laurel Lake, Massachusetts; T = Twin Lakes, Connecticut; V = Lake Champlain, Vermont; Z = Lake Zoar, Connecticut.

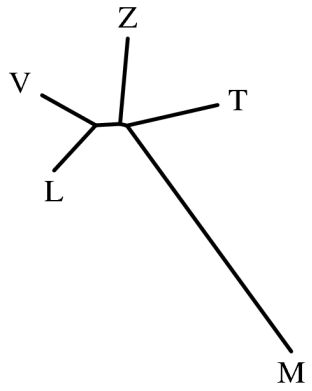

Figure 23. Unrooted dendrogram based on distance matrix.
Distance matrix values were used to construct an unrooted dendrogram (http://genomes.urv.cat/UPGMA/index.php; Garcia-Vallve, S., et al., 1999). L = Lake Lillinonah, Connecticut; V = Lake Champlain, Vermont; Z = Lake Zoar, Connecticut; T = Twin Lakes, Connecticut; M = Laurel Lake, Massachusetts.

Discussion

Our work on the phylogeographical analysis of zebra mussel populations from various Northeast waterways continues to offer clues to the origins of the current infestation in nearby Lake Lillinonah and Lake Zoar. Although our sampling size is quite limited, the data are currently showing a closer relationship to zebra mussels originating in Vermont (the Lake Champlain region) than from the Housatonic River near Laurel Lake in Massachusetts, as previously hypothesized. A larger dataset may prove otherwise, but it is quite possible that the introduction of zebra mussels into our local waterways was initiated by a contaminated recreational vessel(s) moving between lakes rather than through larval veligers transported downstream in the Housatonic River.

To test our conclusions, we will be expanding the sampling size to include recently isolated zebra mussel tissue, which has not yet undergone PCR and microsatellite analysis. We also plan to gather additional adult zebra mussels from surrounding lakes and rivers, including multiple Hudson River sites, the Twin Lakes in northern Litchfield County, Laurel Lake in Massachusetts, and Lake Champlain, if possible, in order to increase the statistical significance of the results. Besides the four microsatellite loci we currently study, several additional published loci can also be used for these studies. We plan to have these microsatellite primers prepared and optimized in coming months. Additional research into the use of other genetic markers and DNA fingerprinting methods may help in further differentiating the populations in our study.

Community Science

From the beginning of the study in 2011, local organizations and members of the community have directly participated in this project. Their help has been vital. Zebra mussels are a local and regional problem, and *community science* has proved to be a very productive model for this work. Organizations participating in and supporting this study were CT Department of Energy and Environmental Protection, Candlewood Lake Authority, Friends of Lake Lillinonah, and the Lake Zoar Authority. Fourteen members of local communities were a part of this effort; seven of these were WCSU biology alumni. Another four community participants were students at Bethel, Shepaug, and New Fairfield high schools. Their participation resulted in two entries to Connecticut State Science and Engineering Fairs in 2014 and 2015. One of these entries won special prizes in the environmental category.

Another very important result of this project has been providing field and lab experience to WCSU students. The zebra mussel project has offered opportunities in the kinds of applied ecology that our students might perform if they pursue a career in the environmental field. In fact, four students who worked with us have gone on to graduate studies. One of the academic scientists working with us is Dr. Andy Oguma, a WCSU alumnus who got his start studying milfoil weevils in Candlewood Lake as an undergraduate. Dr. Oguma went on to earn a doctorate in ecotoxicology from the University of Louisiana Lafayette. In addition to WCSU students, four undergraduates and one graduate student from other colleges and universities have volunteered in the project.

Discussion

Through the 2014 sampling season, we have found no evidence of zebra mussels in Candlewood Lake. We found no veligers or adults. The danger of future introduction, however, is very real. Boats are regularly transported between Candlewood Lake and water bodies where zebra mussels are known to exist. Ideally, boat owners would wash their boats and drain any bilge water between lakes. We have no certainty that they will do so. Of the seven public boat launches on Candlewood (two State launches and five municipal launches), *voluntary* inspections are only provided at Lattin's Landing and the Squantz Cove State launch sites, but that is generally Friday thru Monday. Voluntary inspections are not conducted at the municipal, commercial, and private community launches.

We have found veligers in the Housatonic River, albeit in very low numbers. The penstock forms a hydrologic connection between the Housatonic and Candlewood. Since adult zebra mussel gonads reach maturity at 51 °F (Churchill 2013) and are certainly capable of spawning by the mid 50s °F (Mackie & Schloesser 1996) then restricting the pumping of water from river to lake when water temperatures at Laurel Lake exceeds this temperature would preclude the entry of veligers through this route. While this has been the practice in the last several years, recent discussions by FirstLight Power with stakeholders indicated that the temperature threshold they are now considering ranges from the mid-50s to 59 °F.

In contrast, the numbers of zebra mussels in Lake Lillinonah and Lake Zoar have grown exponentially from 2011–2014. Ecological effects of this rapid increase have been seen as well. As the density of veligers increases the numbers of zooplankton have clearly decreased.

Adult mussels were encountered only in small numbers in Lakes Lillinonah and Zoar before 2013. In the autumn of 2013, lake residents began to report significant numbers of adults attached to boats, docks, and other submerged surfaces. Fortuitously, we had set out artificial substrates to detect mussel colonization earlier that summer and have been able to examine the pattern and density of colonization in Lillinonah and Zoar. In 2013, the highest densities of colonization in Lake Lillinonah were in the middle and downstream portions. Few adults were found on substrates far upstream or in the Shepaug arm. We had fewer substrates to examine in Lake Zoar, but the substrate with highest number of adults was from Zoar rather than Lillinonah. We had fewer substrates to examine in 2014 than 2013, but the average densities of adult zebra mussels (per substrate) had increase by an order of magnitude (Figures 12–14).

The average length of adult zebra mussels found on our artificial substrates in lakes Lillinonah and Zoar in 2013 was 9.2 mm. At 22 mm in length our largest mussel is below the range of maximum lengths (25–40 mm) found in published studies and summarized by Karateyev and coworkers (2006). The average growth rate we measured (0.08 mm/day) was similar to that reported (0.09 mm/day) in other studies of reservoirs (Karateyev, et al 2006).

We have been able to confirm Laurel Brook and the pipe from Laurel Lake as possible sources of veligers in the Housatonic River and colonization of the Housatonic impoundments. No evidence was found of sources above Laurel Lake. However, the preliminary molecular data suggests that Laurel Lake might not be the source of zebra mussels in the Housatonic, or the only source. If further analyses point to Lake Champlain as the ultimate source, that will also highlight the important role of boats as a vector of mussel colonization.

Given the very small numbers of veligers found in the Housatonic River, it seems likely that the resident adults of Lake Lillinonah spawned many of the veligers found there. Our observation that the 2014 peak in veliger density in the downstream impoundment (Lake Zoar; Figure 4) arrived before the peak in the upstream impoundment (Lake Lillinonah; Figure 3) suggests that the veligers in Zoar were spawned in that lake as well.

Confounding this issue, however, is the difficulty of assessing the water retention time in lakes Lillinonah and Zoar. Unlike Candlewood, Lillinonah and Zoar are impoundments of the Housatonic River itself. Water flows through these reservoirs when discharge into the reservoir is high and when electricity is generated by the dams. When water flow is very low veligers will settle near where they are spawned. When flow is high, veligers are displaced downstream. Under conditions of significant flow, adult mussel populations might contain more individuals recruited from upstream.

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Appendix 1

Mean veliger counts per liter in lake samples, 2013

Sampling date	Candlewood	Lillinonah LX	Lillinonah L15	Zoar Z9	Zoar Z11
June 21	0	0.98	0.29	0.02	0.10
July 2	0	2.35	0.03		0.88
July 17	0	1.57	0.66	0.83	6.01
July 30	0	1.58	0.06	7.89	18.31
August 13	0	1.49	0.10	5.14	13.44

Mean veliger counts per liter in lake samples, 2014

Sampling date	Candlewood	Lillinonah LX	Lillinonah L15	Zoar Z9	Zoar Z11
June 2	0	1.36		6.76	2.13
June 17	0	5.51	5.18	57.48	32.12
July 1	0	51.95	67.69	38.58	37.52
July 17	0	8.66	1.94	2.63	0.41
July 29	0	6.04	0.26	3.62	1.33

Mean veliger counts per liter in samples from lakes Lillinonah and Zoar, 2011–2014.

Year	Lake Lillinonah	Lake Zoar
2011	0.0003	0.032
2012	0.0015	0.105
2013	1.06	6.56
2014	15.7	18.5