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Author(s): Nathan S. Holoubek Jason M. Goeckler Benjamin R. Smith and David R. Edds

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Comparison of zebra mussel veliger laboratory enumeration and sampling techniques

Nathan S. Holoubek¹, Jason M. Goeckler², Benjamin R. Smith¹ and David R. Edds¹

1. Department of Biological Sciences, Emporia State University, Emporia, Kansas 66801

2. Kansas Department of Wildlife, Parks, and Tourism, Emporia Research Office, Emporia, Kansas 66801 Corresponding author: Nathan.Holoubek@Wisconsin.gov

Zebra mussel (*Dreissena polymorpha*) invasion greatly impacts aquatic systems, with economic and ecological consequences. Monitoring zebra mussel density and reproductive timing are essential for effective detection, management, and control of this invasive species. Veligers, the free-floating planktonic life stage of zebra mussels, are often monitored to estimate reproductive effort and as a first indicator of zebra mussel invasion. Although many veliger monitoring methods exist and vary greatly in cost and effort, no study has compared results yielded by different sampling techniques. We compared estimated veliger densities collected with offshore vertical plankton net tows and oblique plankton net tows taken from shore from April through October 2011 in Marion Reservoir, Kansas. Secondly, we compared enumeration results returned by an established laboratory and a laboratory that had recently begun enumerating veligers. Using ANOVA testing, we found no significant difference between sampling methods or laboratories. We recommend using oblique plankton net tows taken from shore because of their potential to reduce monitoring costs compared to other sampling techniques by reducing equipment, time, and manpower expenses.

Keywords: zebra mussel, *Dreissena polymorpha*, veliger, sampling method, plankton, enumeration

INTRODUCTION

The zebra mussel (*Dreissena polymorpha*), an invasive species, has numerous and dramatic economic impacts on the water bodies it invades. For example, its invasion into North American waters has had negative impacts on many manmade structures and utilities where adult mussels occlude industrial and municipal piping, resulting in high costs associated with control, prevention, and maintenance (LePage 1992; Claudi and Mackie 1994). Zebra mussels have been linked to decreases in dissolved oxygen, which disrupts municipal utilities that require high dissolved oxygen for waste assimilation (Effler et al. 1996).

Native mussels (order Unionoida) have experienced or are expected to experience declines due to zebra mussel invasion

(Ricciardi, Neves and Rasmussen 1998; Schloesser et al. 2006), in part because they directly colonize native mussels (Fig. 1) and greatly decrease phytoplankton levels in waters they invade (Effler et al. 1996; Caraco et al. 1997). Additionally, game fish feeding directly on plankton at some point in their life cycle, such as yellow perch (*Perca flavescens*) and walleye (*Sander vitreus*), may be negatively affected by decreased phytoplankton associated with zebra mussel invasion (MacIsaac 1996). Zebra mussel consumption contributes to an increased contaminant burden in scaup (*Aythya* spp.), long-tailed ducks (*Clangula hyemalis*) (Ross et al. 2005), and tufted ducks (*Aythya fuligula*), which has negatively affected nesting behavior and increased embryo mortality (de Kock and Bowmer 1992). In several locations in Kansas, deceased redhead ducks (*Aythya americana*) have been found with stomachs



Figure 1. Native unionid mussel infested with zebra mussels. (Photo by B. R. Smith)

full of zebra mussels, while toxicology analysis found nothing unusual (J. Goeckler, pers. obs.).

Veligers (Fig. 2) are the free-floating planktonic stage of zebra mussels, living in the water column for 1 to 5 weeks before settling and attaching to substrate (Ludyanskiy, McDonald and MacNeill 1993). Veliger sampling provides essential information on the timing and extent of zebra mussel reproduction and infestation levels (Kraft 1992). Veligers primarily move with water flow, making assessment of reproductive effort in lakes especially important when downstream infestation is a concern (Neumann, Borchering and Jantz 1992; Claudi and Mackie 1994; Stoeckel et al. 2004). Sampling methods include performing vertical, horizontal, and oblique tows with plankton nets and straining with pumps (Kraft 1992; Claudi and Mackie 1994). Veligers are often found to be equally distributed vertically throughout the water column (except when oxygen is low) due to wind mixing (Fraleigh et al. 1992; Riessen, Ferro and Kamman

1992). Significantly higher veliger densities were found by Riessen, Ferro and Kamman (1992) at inshore sampling stations compared to offshore sampling stations in Lake Erie, indicating horizontal distribution may not be equal. Although methods differ in time, equipment, and personnel expenditures, there has been no study of their equivalence in density estimation.

We conducted a post-hoc study to compare two zebra mussel veliger sampling methods: vertical plankton tows from an offshore boat (vertical tows) and oblique plankton tows from shore (oblique tows). The methods differed in three ways: direction of tow, location in water body, and calculation of water volume sampled. We expected that vertical tows and oblique tows would provide the same veliger density estimates per sampling session. We also compared enumeration results returned by two laboratories, one experienced (established) and one which recently began enumerating zebra mussel veligers (new). This was done to assess

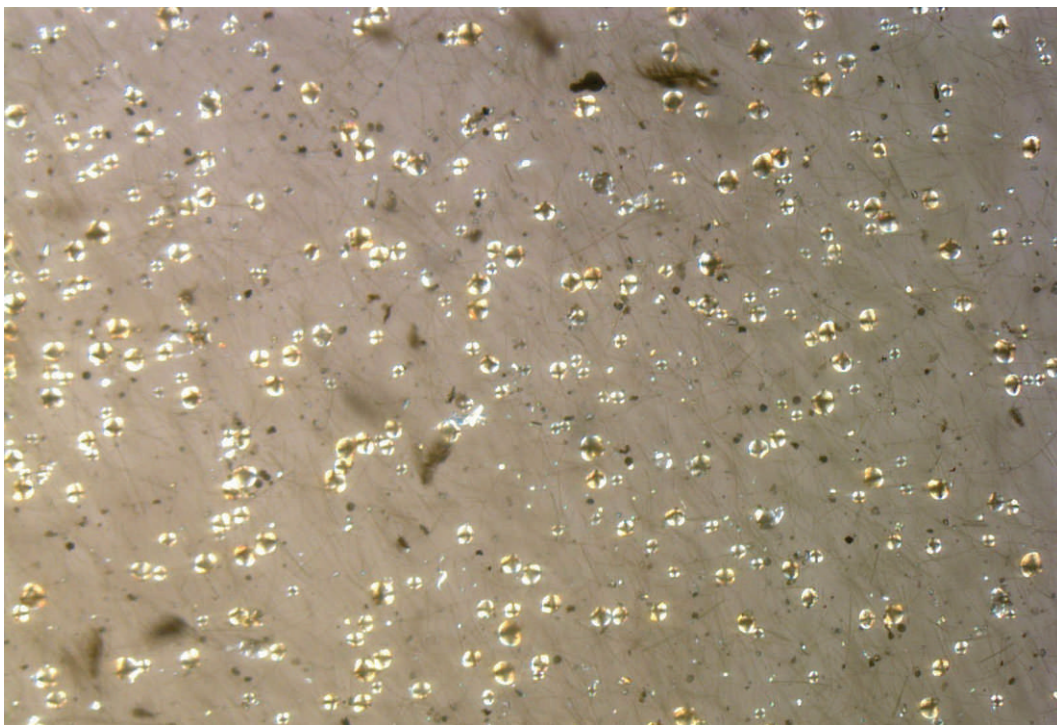


Figure 2. Cross-polarized light microscopy illuminates a particularly dense assemblage of zebra mussel veligers ranging in maturity from the young D-stage to the pediveliger stage that occurs just prior to settling on firm substrate. Photo by Chad Boeckman, used with permission.

possible variation in our sampling methods comparison that could be due to experience at the laboratory level. We compared laboratories by sending samples drawn from the same time and place (using the same method) to both laboratories to identify potential variation in veliger enumeration results.

METHODS

Most data used in this study were collected for invasive species monitoring by the Kansas Department of Wildlife, Parks, and Tourism (KDWPT). The exception is the oblique tow data, which were a portion of a dataset collected for separate research through KDWPT and Emporia State University.

Study reservoirs - For the laboratory comparison, four Kansas reservoirs were sampled in 2011: Marion, Cheney, Wilson, and Milford. Marion Reservoir (2509 ha) was

created in 1968 by damming the Cottonwood River, Cheney Reservoir (3868 ha) was created in 1965 by damming the Ninnescah River, Wilson Reservoir (3661 ha) was created in 1964 by damming the Saline River, and Milford Reservoir (6561 ha) was created in 1967 when the Republican River was dammed. All reservoirs sampled were known to contain zebra mussel populations (USGS 2012). Marion Reservoir alone was utilized for the sampling techniques comparison.

Sampling - Samples for the laboratory comparison were collected by KDWPT technicians in 2011 using offshore vertical tows from a boat. Three samples were drawn per lab during each sampling event at each site. A Wildco® (Yulee, Florida) 63 μm mesh, 20.32 cm diameter plankton net was lowered to the substrate on an attached rope (with distance increments marked) and slowly drawn to the water surface. Distance sampled was recorded to

Reservoir	Month (2011)	Established	New
Cheney (n=12)	May	5.65 ± 0.90	5.72 ± 0.84
Marion (n=18)	May	2.47 ± 1.04	2.59 ± 0.90
	August	3.86 ± 2.00	3.65 ± 1.79
Milford (n=12)	June	1.75 ± 0.52	3.04 ± 0.93
Wilson (n=27)	June	64.83 ± 16.52	78.94 ± 25.02
	August	34.20 ± 20.65	22.59 ± 3.12
	October	3.72 ± 0.62	3.11 ± 0.64
Overall (n=69)		15.62 ± 5.45	15.88 ± 6.05

Table 1. Mean (\pm SE) density estimates of zebra mussel veligers (veligers/L) per month for four Kansas reservoirs, enumerated by an established laboratory experienced in veliger enumeration (Established) and by a laboratory new to veliger enumeration (New). n=total number of samples drawn for each laboratory per reservoir.

the nearest meter and multiplied by area of the net mouth to calculate volume of water sampled. Veligers were washed down the net into a sample bottle using distilled water, and specimens were preserved in 70% isopropyl alcohol.

Samples were drawn for each laboratory in alternating order (i.e., one for the established laboratory, one for the new laboratory, one for the established laboratory, etc.). Three sites were sampled within Marion and Wilson reservoirs, whereas 4 sites were sampled within Cheney and Milford reservoirs. Marion Reservoir was sampled on May 16 and August 16, Cheney Reservoir on May 26, Wilson Reservoir on June 23, August 18, and October 13, and Milford Reservoir on June 23, for a total of 69 samples for each laboratory from 14 sites.

For comparing sampling techniques, we used veliger data from April 2011 through October 2011. For vertical tows, KDWPT technicians collected samples by boat as described previously. Hypoxic zones in which veligers would not exist were not sampled, the greatest depth sampled was 5 m and the lowest measured dissolved oxygen level was 1.0 mg/L. Marion Reservoir was sampled near the middle of each

Month (2011)	Oblique Tows	Vertical Tows
April	0.60 ± 0.44	0.02 ± 0.02
May	5.53 ± 2.81	2.47 ± 1.04
June	3.29 ± 0.55	1.70 ± 0.21
July	8.06 ± 1.96	5.64 ± 4.32
August	1.05 ± 0.38	3.86 ± 2.00
September	4.71 ± 2.45	0.24 ± 0.06
October	0.84 ± 0.43	0.02 ± 0.01
All months	3.44 ± 1.35	1.99 ± 1.27

Table 2. Mean (\pm SE) density estimates of zebra mussel veligers (veligers/L) from nine tows per month using both oblique tows from shore (Oblique) and vertical tows from offshore (Vertical) in 2011 in Marion Reservoir, Kansas.

month at three sites: one near the inlet, one near the midpoint, and one near the outlet.

We collected shore samples by oblique tow from Marion Reservoir in April through October 2011 using similar methods, with the following differences. Three shore sites were selected: one each near the inlet, midpoint, and outlet of the reservoir. All shore sampling sites were located on the northeast side of the reservoir, which received the prevailing winds. A Wildco® plankton net (identical to the one used for vertical tows) was tossed with an attached 6 m rope and drawn obliquely through the water column, beginning near the substrate and ending at the water surface. Three samples were drawn from each site per month. Sampling was conducted on day 24 of each month, \pm 5 days. Veligers were collected from nets and preserved in a manner identical to those in vertical tow samples. The linear distance of water sampled was measured using a General Oceanics, Inc. (Miami, Florida) mechanical flow meter (model 2030R) fixed to the mouth of the net and multiplied by the area of the net mouth to obtain volume sampled.

Sample examination - For comparison between laboratories, samples were sent to one established laboratory and one new laboratory. Both laboratories used cross-polarized microscopy (Johnson 1995) and a segmented Petri dish to enumerate veligers.

Oblique tow samples were transported to Emporia State University for examination and enumeration. Veligers were identified as zebra mussels (Nichols and Black 1994) and counted using a cross-polarized microscope (Carl Zeiss MicroImaging, LLC., Thornwood, New York) and a Wildco® zooplankton counting wheel (Wards Natural Science, Rochester, New York). Vertical tow samples used in the techniques comparison were sent to the same established laboratory used in the laboratories comparison for enumeration. The same techniques as above were used except that a segmented Petri dish was used instead of a zooplankton counting wheel.

Statistical analysis - Veliger densities were calculated for each tow and averaged per site using all three samples drawn per site, per date. We compared laboratory results with a three factor ANOVA with factors as laboratory, month, and reservoir. We compared sampling techniques using mean veliger density per site in a two factor ANOVA, using month and technique as factors. We used a Tukey post-hoc test to further investigate significant differences among months and reservoirs. SAS version 9.2 (SAS Institute Inc. Cary, North Carolina) was used to conduct analyses. Results were considered significant at the $\alpha=0.05$ level.

RESULTS

Laboratory comparison - Laboratory results were similar (Table 1) and mean veliger density estimates were not significantly different ($F_{1,32}=0.002$; $P=0.97$), although there were differences among months ($F_{3,32}=4.16$; $P=0.01$) and reservoirs ($F_{3,32}=6.87$; $P=0.001$). June mean veliger density was significantly higher than that of October and May (Tukey $P<0.05$).

Mean veliger density in Wilson Reservoir was significantly higher than measurements in all other reservoirs (Tukey $P<0.05$). There was no interaction between laboratory and month ($F_{3,32}=0.17$; $P=0.92$), or between laboratory and reservoir ($F_{3,32}=0.003$; $P=0.9997$).

Sampling comparison - The lowest mean entire reservoir estimates (average of nine tows for that month), obtained by vertical tow sampling in both April and October, was 0.02 veligers per liter (April SE=0.019, October SE=0.007), and the highest mean estimate was 8.06 veligers per liter (SE=1.96), obtained from oblique tow sampling in July (Table 2). The mean veliger density estimate from oblique tow sampling across all months was 3.44 veligers per liter (SE=1.35). Vertical tow sampling returned a mean estimate across all months of 1.99 veligers per liter (SE=1.27). In all months except August, oblique tow sampling yielded higher (but not significantly different) veliger density estimates for the entire reservoir (Table 2).

There was no significant difference in mean veliger densities between sampling techniques ($F_{1,28}=2.43$; $P=0.13$), although there was a significant difference among months ($F_{6,28}=3.30$; $P=0.01$). Mean veliger density in July was significantly higher than mean densities in April and October (Tukey $P<0.05$). There was no significant interaction between sampling technique and month ($F_{6,28}=0.88$; $P=0.52$).

DISCUSSION

Laboratory comparison - We conclude there was no difference in mean veliger density estimates provided by the established and new laboratories. These results suggest managers could confidently select a laboratory to provide this service regardless of experience. However, we caution using a new laboratory to initially document invasion because of look-alike species (Nichols and Black 1994), which was not evaluated here.

Sampling comparison - There was no difference in veliger density estimates between oblique tows and vertical tows at the entire reservoir scale. Oblique tows could often replace vertical tows, reducing costs in equipment and labor. Given the results of our laboratory comparison we conclude that the enumeration laboratory had no effect on veliger density estimates. However, our study had several limitations. We focused on one reservoir for sampling techniques comparison, and for just one sampling season. Higher (although not statistically different) veliger densities were found from shore sampling in all months except August (Table 2). There are several factors to which this could be attributed. Two different, but both widely accepted, water volume calculation methods were used: vertical tow water volumes were calculated using distance to substrate whereas oblique tows used distance recorded by a flow meter. Reissen, Ferro and Kamman (1992) also found higher veliger densities at inshore sampling sites on Lake Erie, although that was attributed to higher adult zebra mussel concentrations near shore, which was not quantified in this study. Alternatively, higher veliger density estimations from shore oblique tows could be due to veligers being concentrated near shore by wind and waves, especially since all shore sites were located on the side of the reservoir receiving the prevailing winds. Plankton distribution is often heterogeneous and greatly affected by winds (George and Edwards 1976; Verhagen 1994), and we hypothesize that higher (but non-significant) veliger concentrations near shore were at least partially due to wind-induced currents. Further research should be conducted at a larger scale considering the potential long-term cost savings associated with oblique tows.

CONCLUSIONS

Many variables must be taken into account when choosing a sampling regime, such as goal(s) of sampling, position in the reservoir or lake, and local climate. With that in mind,

oblique tows from shore appear to be a reliable cost and time-efficient alternative to offshore vertical tows. With oblique tows from shore only one person is needed, a boat is not required which greatly reduces equipment demands, and sampling is much quicker to complete. This streamlines the sampling process, with no reduction in data quality.

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