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Research Article

Veliger presence in residual water – assessing this pathway risk for Minnesota watercraft

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Abstract

Zebra mussels (*Dreissena polymorpha*) have moved to many inland waters across the U.S. and trailered recreational watercraft are suggested as a significant pathway for spread. Uncertainty exists on whether veligers in residual water (water remaining in boats after draining) are capable of creating new infestations. Over 100 samples of residual water from boats which were exiting Minnesota lakes with established zebra mussel populations were collected in July and August over three boating seasons (2013–2015). The majority of the boats were sport fishing boats, with most of the samples coming from live wells and bilges. Very few veligers were found in these samples, with over 90% of the samples containing 5 or fewer veligers, and 70% had zero veligers. Residual water volumes were generally less than 400 ml, and there was no correlation between volumes of water and numbers of veligers. Due to factors such as low veliger density, dispersion and high veliger mortality, residual water may present a low risk for spread of this invasive species.

Key words: veliger, *Dreissena polymorpha*, watercraft, live well, residual water, transport

Introduction

Zebra mussels (*Dreissena polymorpha* Pallas, 1771) were first discovered in North America in Lake Erie in 1986 (Carlton 2008) and spread rapidly throughout the Great Lakes. By the end of 2010, they had moved to over 600 inland lakes in 26 states (Benson 2014). Many natural resource managers and biologists suggest that trailered recreational watercraft present a primary pathway for movement to unconnected waters (Rothlisberger et al. 2010; Johnson et al. 2001; Johnson and Padilla 1996). Life history stages of *D. polymorpha* provide two distinct means of transport on trailered boats. Juvenile and adult mussels can attach to solid surfaces (boat transoms, trim tabs, entangled aquatic vegetation, etc.) and may drop off in a new waterbody. The larval stage (veliger) could be transported in any water taken onboard (such as live wells, bilges and other water-holding compartments) and may be discharged into different waters. While attached zebra mussels can be visible, the veliger stage is microscopic and planktonic; thus the potential level of risk from transport of infested water is not easily determined. Two recent studies modeled potential veliger transport and suggested high numbers of veligers could be moved (Choi et al. 2013; Dalton and Cottrell 2013) in water inside of boats, but these results were not based on field collected samples.

Recent legislation in Minnesota requires all boaters to remove drain plugs and drain any water from a boat prior to leaving the access area, and leave the drain plugs out during transport. However, boaters and lakeshore residents have still raised questions and concerns over the water remaining in a boat after these actions are taken. Some water often remains ("residual water") in live wells, bilges and other compartments after draining, but there is little data documenting the number of veligers present in this water. Because of the uncertainty, a field study was conducted during 2013–2015 that collected residual water from boats and examined these samples for veligers.

The field study also included an assessment of the vertical distribution of veligers during summer in an infested lake. Because a water-column vertical tow is typically used to assess veliger densities, we sampled veliger densities at different depths to see if veligers were uniformly distributed throughout the water column.

Table 1. Lakes in Minnesota where residual water samples were collected (2013–2015), counties and UTM location, and year when zebra mussels were first reported and confirmed.

Lake	County	UTM coordinates	Year infestation found
Prior	Dakota	468070, 4953809	2009
Minnetonka	Hennepin	456451, 4976800	2010
Mille Lacs	Mille Lacs	449234, 5121632	2005
Gull	Cass	397285, 5145068	2010
Le Homme Dieu	Douglas	318098, 5088982	2009
Carlos	Douglas	316839, 5092519	2009
Crystal	Ottertail	274077, 5166703	2010
Pelican	Ottertail	268722, 5176202	2009

Methods

Minnesota Department of Natural Resources (MN DNR) Watercraft Inspectors are stationed on many infested waterbodies in Minnesota, where they inspect boats entering and exiting at public access sites. These inspectors on eight different infested lakes across the state (Table 1) during multiple dates in July and August 2013–2015 asked exiting boaters if samples of residual water could be collected after watercraft were drained. All eight lakes had well established reproducing zebra mussel populations. The time frame when samples were collected covers when veliger densities typically peak in Minnesota waters. Boats were classified as fishing, runabout, wakeboard, or personal watercraft (PWC) and the location of the sample as live well, bilge, motor, ballast tank, or PWC footwell. Samples were collected from all areas containing water using 60 cc plastic large barrel syringes which had the tip cut to accommodate all potential veliger sizes. Rubber tubing of different lengths was attached to the tip to permit the inspectors to reach into compartments. All the water left in any particular area was removed and placed into recloseable bags and refrigerated for 2–5 days at 4–5^{o} C until they were transferred to the MNDNR biology laboratory for analysis. The tubing was cleared after each sample by detaching from the syringe, filling the syringe with air, reattaching the tubing and depressing the plunger rapidly to blow air through the tubing expelling any material or water droplets left.

Vertical veliger distribution was examined in Prior Lake, Dakota County, Minnesota on four dates in 2014 (27 June, 10 July, 24 July, 8 August). This lake has been infested with zebra mussels since 2009; it supports an abundant population. Two sites were sampled in the northeast part of the lake, approximately 55 m apart, where high zebra mussel settlement and attachment have been observed. At each site samples were collected from sequential 0.6-m thick depth zones from the surface to 3.0 m depth, then 1.5-m thick depth zones from 3.0 m to 9.1 m depth. In addition, a single full column tow was taken from 9.1 m to surface. The 0.6-m depth zones were sampled in the surface waters as the assumption was made that these veligers would be most available to be entrained in any water taken on board recreational Minnesota watercraft. Veligers in deeper layers were less likely to be encountered by watercraft and thus these zones were larger (1.5-m). All depth zones were sampled with vertical tows collected with a 20 cm mouth, 80 micron mesh standard closing plankton net (Aquatic Research Instruments, Hope, Idaho). Samples were preserved in ethyl alcohol and processed within 2 days of collection.

In the laboratory, samples were poured into graduated beakers to measure water volume, and the sample bag was thoroughly rinsed into the beaker to wash off any veligers or other material that might remain. The sample was then poured through 80 micron mesh Nytex and the beaker rinsed into the Nytex to concentrate the material. Concentrated samples were then rinsed with water into clean beakers and diluted as necessary depending on the amount of material in the sample. Samples were examined with cross-polarized light to detect veligers (Johnson 1995). Entire depth zone samples were counted, due to small amounts of material and veligers. Full column tows were subsampled, and back calculated to give sample counts. Counts were converted to densities (veligers/liter) for each sample.

Results

A total of 113 samples were collected over three boating seasons from 2013 through 2015. Nearly all samples came from fishing boats (101) with sample totals from other watercraft nearly evenly split—runabout (5), wakeboard (4), PWC (3). Locations of the samples from fishing boats were predominantly

live wells (64), followed by bilges (27) and motors (10). Samples from the non-fishing boats came from bilges (4), motors (4), PWC footwells (3) and a ballast tank (1).

Numbers of veligers per sample ranged from 0 to 217, with a mean of 3.9 and a median of 0. Seventy percent of the samples contained 0 veligers, with another 23% having ≤ 5 veligers (Figure 1). Only three samples contained more than 15 veligers (54, 65, 217). Water volumes also showed a large range, from 3 to 900 ml, with a mean of 180 ml and 120 ml as the median. Nine samples contained more than 400 ml (Figure 2). There was no correlation (Pearson Product Moment correlation, $p > 0.050$, SigmaPlot 13.0) between numbers of veligers and water volumes for samples (Figure 3).

Veligers were not uniformly distributed in the water column (Figure 4A, B), with peak densities at different depths depending on sample date. While these depths varied across the sample dates, the shallowest depth showing peak densities was in the 0.6 to 1.2 m depth zone, and often the highest densities were found deeper in the lake. For each sample date, the densities for all layers were compared between sites. Densities between the two sample sites were significantly different (Mann-Whitney Rank Sum test, $p = 0.017$, SigmaPlot 13.0) for the first sample date (27 June) but were not significantly different for three later dates.

Discussion

The number of veligers found in residual water from drained boats was very low, with the majority of samples containing fewer than 5 veligers. Many samples had no veligers at all, despite having come from lakes with well-established zebra mussel populations. With abundant reproduction occurring in these lakes, the risk of taking in veliger-infested water could be expected to be very high or at a maximum. Despite this, few veligers were found in most of the samples. It could be that veligers were missed in the sampling or lost in laboratory processing. However, all water was collected from any compartment; thus, the possibility that significant numbers of veligers would remain in a particular area was unlikely. Additionally, it seems unlikely that only a few veligers would be found in the water sampled while higher numbers would be left behind. Processing samples in the laboratory included rinsing sample bags as well as washing down the mesh used in concentration, so again it was unlikely that significant numbers of veligers would have been lost in these procedures. The samples were not preserved,

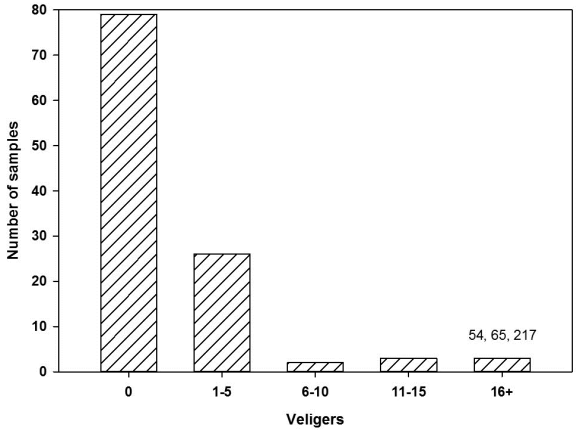

Figure 1. Numbers of veligers in residual water samples (n = 113), 2013–2015 (numbers above right bar are veliger counts from three highest samples.

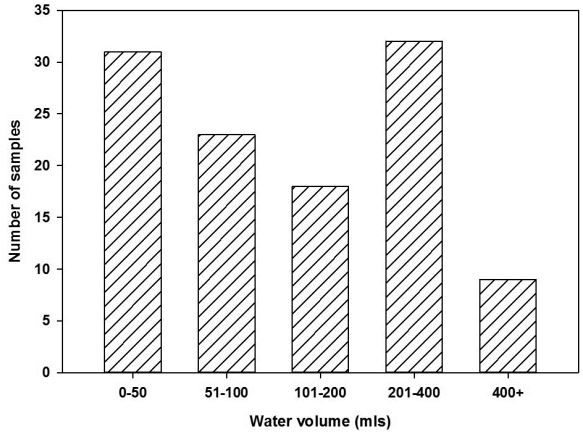

Figure 2. Volumes of water in residual water samples (n = 113), 2013–2015.

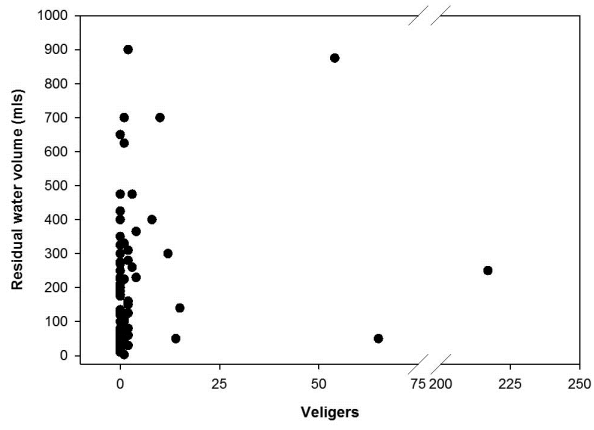

Figure 3. Water volumes and veliger counts from residual water samples (n = 113), 2013–2015.

Table 2. Summer veliger densities in selected lakes in Minnesota. Numbers in monthly columns are "date; **density"** (in numbers/liter). Densities are from vertical plankton tows. * - non-study lake

Lake	Year	June	July	August
Minnetonka (Lower Lake N)	2013	11th; **0.11**	16th; **5.10**	13th; **4.49**
Minnetonka (Grays Bay)	2013	11th; **0.00**	16th; **14.67**	13th; **3.34**
Minnetonka (Lower Lake N)	2014	5th; **0.01**	8th; **36.97**	5th; **2.56**
Minnetonka (Grays Bay)	2014	5th; **0.11**	8th; **36.81**	5th; **10.76**
Carlos	2014	25th; **1.93**	24th; **14.27**	28th; **0.30**
Pelican *	2015	24th; **29.16**	22th; **54.42**	17th; **16.73**

but kept refrigerated. In many samples, other biota including small Naididae oligochaetes, ostracods, copepods, and cladocerans were observed and often these were moving about, suggesting that conditions in the sample bags were sufficient for survival of these organisms and likely for veligers as well. Thus, it is unlikely that any veligers were lost due to death and decomposition in the samples. No testing was done to determine viability of veligers, but visceral masses observed inside appeared intact.

Temporal variation in veliger abundance could have resulted in reduced veliger numbers in infested lakes. However, sampling by the MN DNR for veligers in various lakes over the past decade or more of inland zebra mussel invasion in Minnesota has shown that veliger densities often occur in at high levels during the summer, generally from early July through mid-August (Figure 5, Table 2). Other veliger monitoring has shown similar patterns (M. Rufer, pers communication). In some years, depending on spring temperatures, veligers may show highest abundances in late June. However, high densities in late June will still provide high veliger numbers into early or even middle July. Thus, the collection of residual water during July and August would fall into the time frame for high or peak veliger abundances in Minnesota waters.

One factor that may explain the low numbers of veligers in this study is the low volume of residual water present from drained boats. The mean of 180 ml of residual water in this study is substantially lower than estimated volumes of residual water used for veliger risk calculations in two previous studies. Choi et al. (2013) used a volume estimate of 8 L based on some Utah aquatic invasive species (AIS) biologists' observations, while Dalton and Cottrell (2013) used a volume of 4 L based on data from Colorado and Utah resource agencies. Thus, an emphasis on thorough draining of fishing boats is important, as this action minimizes residual water volume and, thus, veliger transport risk. However, the lack of correlation of veliger abundance with water

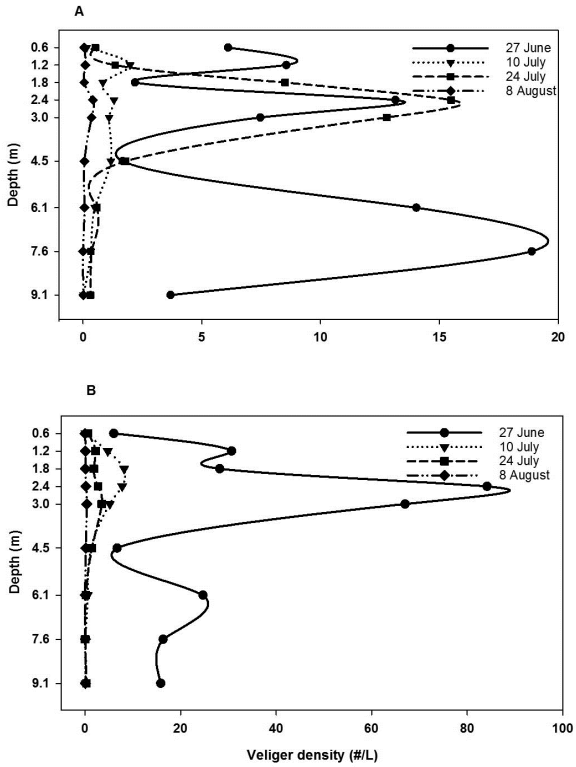

Figure 4. Vertical distribution of veligers during summer in 2014 at two sites (A: site 1, B: site 2) in Prior Lake, Dakota County, Minnesota.

volumes suggests the possibility that larger volumes of water do not necessarily mean more veligers will be present. Sampling of more boats from one specific lake at one time might help determine if this lack of correlation is a result of sampling boats over several weeks at different lakes. However, that information is currently lacking, and our results, combined with the unequal vertical distribution of veligers argues that higher residual water volumes does not necessarily mean higher numbers of veligers are also being transported.

Another factor may be related to the distribution of veligers in the water column. Water in live wells and the boat bottom or bilge originates, for the most part, as water from the upper surface of the lake. However, sampling in Prior Lake showed that veligers were not uniformly distributed in the water column, and highest densities were often found at deeper depths. Dalton and Cottrell (2013) suggested that veligers could be stirred upward by wave action or boat use, and may be found evenly distributed throughout the water column. However, Fraleigh et al. (1993) reported that vertical distribution of veligers was variable in western and central Lake Erie. Veligers were absent from warmer surface waters in July in the central basin, while vertical distribution in the western basin was related to wind driven mixing. Wind-driven mixing in inland lakes may impact distribution in the epilimnion, depending on wind strength and direction. However, our vertical veliger density data suggest that veligers were not mixing uniformly throughout the water column on our sample dates; in both sites, veligers showed distinct peaks in densities at varying depths. Thus, water taken from near the surface may well have few veligers compared to deeper depths, and mean veliger densities calculated from whole column tows may not provide an accurate estimate of veliger risk.

The possibility of establishing a new zebra mussel infestation from discharge of veligers in residual water remains unknown. However, there are a number of factors that suggest that this pathway of veliger movement may not be effective. First, mortality from egg to successful settlement for veligers has been reported to be very high, up to 90% in laboratory rearing, while Mackie and Schloesser (1996) reported 90–99% mortality in the settling stage. If, like Dalton and Cottrell (2013), we apply an estimate of 90% mortality to the veliger counts found in the residual water samples, most samples will result in less than 2 live veligers, and even the highest count (217) would result in 22 live veligers. Thus, natural mortality may substantially reduce or even eliminate surviving veligers moved in residual water.

This mortality rate could even rise higher depending on transport of the boat and length of time it remains out of water. Both Johnson et al. (2001) and Kelly et al. (2013) suggested that conditions in small volumes of water in such areas as live wells and bilges could reduce survival, due to higher temperatures, lower dissolved oxygen and contaminants such as fuel or oil that can be found in the bilge. Additionally, small volumes of water create other adverse conditions. Snider et al. (2014) found survival times of 20+ hours for quagga mussel (*Dreissena bugensis* Andrusov, 1897) veligers in small water volumes and droplets in static laboratory conditions. However, water in a trailered boat is likely to experience turbulence, due to travel on roads. Thus, veliger survival may be further compromised in normal transport.

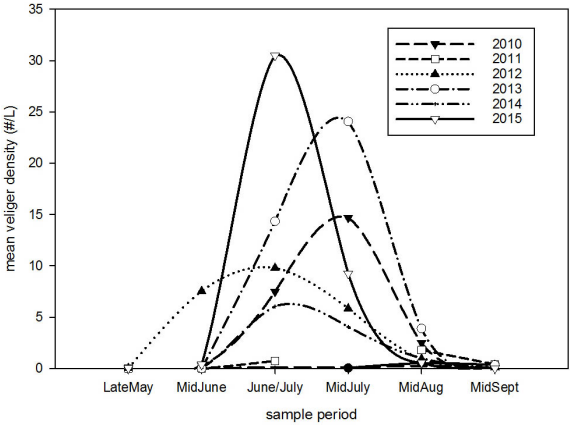

Figure 5. Mean veliger densities from nine samples per sample period in Mille Lacs Lake, Minnesota, 2010–2015.

Researchers have suggested that there are substantial difficulties in establishing new viable zebra mussel populations from veliger introductions. Johnson and Padilla (1996) stated that veligers would be dispersed widely after discharge into a new waterbody since veligers may spend up to a month before settlement (Nichols 1996). Thus, it is not likely that they would settle close enough together for successful reproduction. Dalton and Cottrell (2013) suggest that mussels settled only a few feet from one another may not be able to reproduce. Johnson et al. (2001) suggested that incidental releases of small numbers of veligers would not likely result in enough aggregated adults to successfully colonize a new waterbody. It might be argued that while each boat has few veligers, multiple boats could add up to sufficient densities of this life stage. However, each boat would better be viewed as an individual event; actions that influence when, how or how much residual water that is transported to a lake is actually discharged are likely to vary from boat to boat. The possibility that enough boats would release enough veligers at the same area and time to settle in sufficient density is extremely small. Similarly, the possibility that veligers released in different areas of the lake would end up successfully settling in close proximity is also very low. The possibility exists that some launch areas (such as marinas or small enclosed bays) could potentially prevent dispersion of veligers throughout the lake. However, boats being launched do not necessarily pump out live wells and bilges

immediately after launch. Thus, introduction of veligers in these areas may well be limited. These areas argue again for thorough draining of watercraft, as minimal residual volumes would be less likely to be discharged quickly after launch.

It should be noted that most of the samples collected came from boats classified as "fishing boats" by the watercraft inspectors. There are other boat types that present unique water issues. For example, ballast or wakeboard boats pump large quantities of water into holding tanks (potentially over 300 L) to produce wakes for recreation. Such tanks may not fully drain, and could represent larger volumes of residual water. Samples collected in Wisconsin from wakeboard boats during fall of 2013 (Campbell et al. 2016) found that volumes of water left in tanks ranged from 1–87 L. However, despite large volumes, veliger counts were zero for most samples, and the highest count was 47. The two samples that had veligers had the lowest volume of residual water and the second highest. Thus, it appears that for their samples, similar to results from this study, volume of residual water has no relationship with numbers of veligers in this residual water. It is important to note that the timing of samples may not have coincided with zebra mussel reproductive events and the origins of samples are unknown. More work on residual water in such boats, especially from boats that are known to be from zebra mussel positive waters during zebra mussel reproductive events, would be beneficial in assessing risk of veliger movement in this pathway.

The extremely low numbers of veligers collected from live wells and bilge areas in this study suggest that current regulations in Minnesota (draining of all water, removal of drain plug during transport) are serving as an effective management action in reducing the spread of zebra mussel veligers from a typical fishing boat. These data argue that this residual water does not present a high level of risk. While resource managers and aquatic invasive species biologists would prefer to have no water and no veligers moved, actions to prevent zebra mussel spread must be simple and easy enough to be widely used by the recreational boating public. The efficacy of these draining regulations appears to support their use.

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References

Benson AJ (2014) Chronological history of zebra and quagga mussels (Dreissinidae) in North America, 1988–2010. In: Nalepa TF, Schloesser DW (eds), Quagga and Zebra mussels: Biology, Impacts and Control 2nd edn. Taylor and Francis Group, Florida, pp 9–31

Carlton JT (2008) The zebra mussel *Dreissena polymorpha* found in North America in 1986 and 1987. *Journal of Great Lakes Research* 34: 770–773, http://dx.doi.org/10.1016/S0380-1330(08)71617-4

Campbell TC, Verboomen T, Montz G, Seilheimer T (2016) Volume and contents of residual water in recreational watercraft ballast systems. *Management of Biological Invasions* 7: 281–286, http://dx.doi.org/10.3391/mbi.2016.7.3.07

Choi WJ, Gerstenberger S, McMahon RF, Wong WH (2013) Estimating survival rates of quagga mussel (*Dreissena rostriformis bugensis*) veliger larvae under summer and autumn temperature regimes in residual water of trailered watercraft in Lake Mead, USA. *Management of Biological Invasions* 4: 61–69, http://dx.doi.org/10.3391/mbi.2013.4.1.08

Dalton LB, Cottrell S (2013) Quagga and zebra mussel risk via veliger transfer by overland hauled boats. *Management of Biological Invasions* 4: 129–133, http://dx.doi.org/10.3391/mbi.2013.4.2.05

Fraleigh PC, Klerks PL, Gubanich G, Matisoff G, Stevenson RC (1993) Abundance and settling of zebra mussels *(Dreissena polymorpha*) veligers in western and central Lake Erie. In: Nalepa TF, Schloesser DW (eds), Quagga and Zebra mussels: Biology, Impacts and Control 2nd edn. Taylor and Francis Group, Florida, pp 129–142

Kelly NE, Wantola K, Weisz E, Yan ND (2013) Recreational boats as a vector of secondary spread for aquatic invasive species and native crustacean zooplankton. *Biological Invasions* 15: 509–519, http://dx.doi.org/10.1007/s10530-012-0303-0

Johnson LE (1995) Enhanced early detection and enumeration of zebra mussel (*Dreissena* spp.) veligers using cross-polarized light microscopy. *Hydrobiologia* 312: 139–146, http://dx.doi.org/10.1007/BF00020769

Johnson LE, Padilla DK (1996) Geographic spread of exotic species: ecological lessons and opportunities from the invasion of the zebra mussel *Dreissena polymorpha*. *Biological Conservation* 78: 23–33, http://dx.doi.org/10.1016/0006-3207(96)00015-8

Johnson LE, Ricciardi A, Carlton JT (2001) Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. *Ecological Applications* 11: 1789–1799, http://dx.doi.org/10.1890/1051-0761(2001)011[1789:ODOAIS]2.0.CO;2

Mackie GL, Schloesser DW (1996) Comparative biology of zebra mussels in Europe and North America: an overview. *American Zoologist* 36: 244–258, http://dx.doi.org/10.1093/icb/36.3.244

Nichols SJ (1996) Variations in the reproductive cycle of *Dreissena polymorpha* in Europe, Russia, and North America. *American Zoologist* 36: 311–325, http://dx.doi.org/10.1093/icb/36.3.311

Rothlisberger JD, Chadderton WL, McNulty J, Lodge DM (2010) Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. *Fisheries* 35: 121–132, http://dx.doi.org/10.1577/1548-8446-35.3.121

Snider JP, Moore JD, Volkoff MC, Byron SN (2014) Assessment of quagga mussel (*Dreissena bugensis*) veliger survival under thermal, temporal and emersion conditions simulating overland transport. *California Fish and Game* 100(4): 640–651