Occurrence and Survival of Zebra Mussel (*Dreissena polymorpha*) Veliger Larvae in Residual Water Transported by Recreational Watercraft

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Abstract

Zebra mussels (*Dreissena polymorpha*) are an aquatic invasive species (AIS) that have spread to many waterbodies in North America and transient recreational watercraft are one of the primary pathways of spread. Best management practices for reducing the risk of spreading AIS include draining all water from watercraft before leaving a water body, but removing all water is impractical. Uncertainty exists about whether zebra mussel larvae (veligers) could reside within the “residual water” that remains after draining and survive overland transport to a new water body. At two Minnesota, USA water bodies (Gull Lake and Lake Minnetonka) from July – August (2016-2017) we collected over 300 samples of residual water from recreational watercraft; compartments included ballast tanks, live wells, sterndrive engines, and others. Roughly half (48%) of these samples contained no veligers and the majority (75%) contained five or fewer. Sterndrive engines and ballast tanks ranked 1st and 2nd for volumes of residual water (median of 4945 and 2650 milliliters, respectively), Ballast tank samples contained the largest median number of veligers per sample (247) and sterndrive engines the highest maximum number of veligers (about 4500 for 2 out of 38 engines sampled). We conducted laboratory experiments on veliger survival in residual water of live wells due to the high frequency of fishing boats moving between water bodies, and ballast tanks given their high likelihood of containing veligers. We exposed live well samples to 20°, 27°, 32°, and 38°C air temperature and ballast tanks to 20° and 32°C. For veligers in live well residual water, we observed $\geq$ 95% mortality after 5 hours of exposure at all temperatures. These same levels of mortality were reached more slowly in ballast tanks ($\geq$ 95% mortality at both temperatures achieved at 48 hours). Additional prevention steps should be taken (e.g. using hot water) to reduce the risk of transporting living veligers in residual water.

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Introduction

Zebra mussels (*Dreissena polymorpha*) are an aquatic invasive species first discovered in North America in Lake Erie in 1986 (Carlton, 2008). They are filter-feeding bivalves with adults ranging from 12 to 40 mm in length (Sprung, 1993). While adult mussels are semi-sessile, attaching to any submerged hard surface with byssal threads, they also spend approximately their first month of life in plankton as microscopic larvae called veligers.

Zebra mussel veligers are found in plankton when surface water temperatures rise above 12°C and adults start to spawn (Sprung, 1993). In Minnesota (MN), and many other locations, this coincides with an increase in recreational watercraft use which continues throughout the summer months. Recreational watercraft come in a variety of shapes, sizes, and uses, but all are capable of transporting water away from water bodies in various types of compartments and engines.

This study examines water collected from boat compartments to determine if veligers are present and how long they are able to survive. This study is comprised of two phases between 2016 and 2018. Chapter 1 covers phase one in which we collected residual water from recreational watercraft leaving two zebra mussel infested water bodies. The second phase, covered in chapter 2, consists of residual water experimental trials in which we examined survival of zebra mussel veligers in residual water exposed to varying air temperatures, over a range that they often experience in Minnesota during summer months of zebra mussel reproductive activity.

Chapter 1: Zebra Mussel Veliger Presence in Residual Water

Introduction

Zebra mussels (*Dreissena polymorpha*) are an aquatic invasive species originally discovered in North America in Lake Erie in 1986 (Carlton, 2008) that continues to spread throughout the Great Lakes region and, along with their congener the quagga mussel (*D. rostriformis*) to waters in the western United States. Zebra mussels were discovered in Minnesota (MN) in 1989 in Duluth/Superior Harbor on Lake Superior, with further discoveries in MN reaches of the Mississippi River in 1992, and in MN inland lakes starting in 2003. As of 2018 Minnesota has confirmed the presence of zebra mussels in 196 water bodies, and designated an additional 150 water bodies as infested due to connections to upstream water bodies that are confirmed to be infested (MNDNR, 2018). Overland transport of adult mussels by recreational watercraft and equipment related to boating (e.g. trailers, docks, boat lifts) is viewed as the primary route for spread (Johnson et. al, 2001). As adults, zebra mussels are able to attach to hard surfaces (e.g. boat hulls) with specialized byssal threads, and are able to survive for up to 22 days out of the water in optimal conditions (10°C, 95% relative humidity (Ussery and McMahon, 1995)).

While much research has focused on overland transport of adult mussels (as well as non-native plants) between water bodies via recreational watercraft and associated equipment (Johnson and Padilla, 1996; Johnson et al. 2001; Rothlisberger et al. 2010), much less is understood about the contribution of the zebra mussel larvae, known as veligers. Many US states, including Minnesota, have implemented laws that require watercraft users to clean and drain their equipment prior to transporting watercraft to new water bodies in an attempt to reduce the spread of aquatic invasive species.

Varying sizes and life-stage characteristics of plant and animal species can make it difficult for recreational watercraft users to be certain that they have successfully removed all plant and animal material, and complex watercraft systems make it impossible for users to fully drain the water from all parts of a watercraft prior to leaving a water access-site. For zebra mussels, efforts to fully drain watercraft are targeted mainly at veliger larvae suspended within surface water that is brought into boats and motor compartments, posing a risk of introduction of larvae into the next-visited water body—if larvae can persist and survive draining attempts.

Zebra mussels are highly fecund, and it is possible for a single female to produce from 250,000 to more than 1 million eggs each spawning season (Stoekel et al., 2004; Sprung, 1991). Zebra mussels have a planktonic veliger larval stage, which is common for bivalves and other animals in marine environments, but in freshwater ecosystems native mussel species do not produce veliger larvae (Sprung, 1993). Veligers develop within the water column and can range in sizes from 70 µm -300 µm (Sprung, 1989; Walz, 1973). Zebra mussel veligers are present at the greatest densities during warm summer months in Minnesota waters, which overlaps with the busiest period of boater movement in Minnesota (MNDNR Watercraft Inspection Data, Retrieved from: ftp://ftp.dnr.state.mn.us/pub/eco/watercraft_insp/). At their largest size (about 0.3 mm or 1/100th of an inch long), veligers are too small for boaters to see while completing a watercraft inspection and have been found repeatedly in the small amounts of water. This remaining water —termed “residual water”— remains inside bilge areas, live wells, engines, and other water holding areas even after operators have reasonably attempted to drain them (Montz et al., 2016; Campbell, 2016).

Minnesota has the most boat registrations per-capita in the United States (US Coast Guard, 2017). With 2,977 public water accesses, there are many opportunities for recreational boaters to freely move between water bodies. Many Minnesota statutes address the movement of recreational watercraft on public roads, including the need to remove all plants and invasive animals, and drain all residual water by opening or removing drain plugs. Compliance with these laws is high, with over 94% of boats arriving at watercraft inspection stations without observable statute violations (MNDN Watercraft Inspection Data, 2018. Retrieved from: ftp://ftp.dnr.state.mn.us/pub/eco/watercraft_insp/).

Even with high boater compliance, multiple different spread mechanisms could be responsible for the increasing frequency of new zebra mussel infestations in MN. Not all equipment that enters Minnesota water bodies is required to be inspected, and equipment such as boat lifts, docks, or swim rafts are installed in the water away from public water accesses where inspections typically occur. Residual water present in watercraft, even when inspected properly, could also pose a risk of transporting live veligers that could be introduced at the next location.

Only a few studies have looked specifically at residual water movement as a potential vector for zebra and quagga mussel veliger transport (Montz et al., 2016; Campbell 2016; Dalton 2013). My study expands on the previous research of Montz and Campbell, both of whom examined residual water in Wisconsin and Minnesota. During Montz's study Minnesota DNR staff collected 113 residual water samples from eight zebra mussel infested lakes during the 2013 - 2015 boating seasons. Most of the samples came from live wells and bilge areas (n=91), with few samples from other compartments such as ballast tanks (n=1). Campbell collected samples from 13 wakeboard boats in Wisconsin in September and October 2013 to determine if ballast tanks were transporting water. The sampled ballast tanks had a mean of 31.7 liters of residual water, and further sampling by MN DNR biologists determined that two of the samples contained veligers (9 and 47 veligers).

My study builds on previous research by focusing on two water bodies, each among the most heavily used and highly interconnected to other destination water bodies, and each with large populations of veligers during peak veliger production months. The goal of this study was to increase the total number of residual water samples, and to increase the number of compartment types sampled across a range of vessel types that are trailered between recreational lakes in the state. By doing so, I aimed to determine the volumes of residual water and numbers of veligers found in different compartment types, to determine whether particular compartments are more likely to contain veligers and to estimate the numbers of veligers transported per trip. These results can be used to better estimate the likelihood that different types of trailered watercraft are capable of moving zebra mussel veligers away from zebra mussel infested water bodies.

Materials and Methods

For the purposes of this study, Minnesota Department of Natural Resource (MN DNR) watercraft inspectors at two high-use Minnesota water bodies were tasked with collecting residual water from a subset of watercraft leaving the access sites. Minnesota watercraft inspectors are trained to use both visual and tactile means to inspect watercraft and associated equipment for invasive species, aquatic plants, and water. Lakes Minnetonka (Hennepin County, DOW number 27013300) and Gull (Cass and

Crow Wing Counties, DOW number 11030500) were chosen due to large zebra mussel infestations that have been present since 2010 (MN DNR, 2018) and are used for multiple forms of aquatic recreation (fishing, touring, wake boarding, etc.). Watercraft Inspection Program data show these two lakes to be among the 10 lakes that are most connected by boater traffic to other water bodies in the state (MNDNR Watercraft Inspection Data, Retrieved from: ftp://ftp.dnr.state.mn.us/pub/eco/watercraft_insp/). Watercraft were classified by type (fishing, Jon boat, pontoon, personal watercraft (PWC), runabout, wakeboard boat). Residual water samples were collected at public water accesses by DNR inspection staff, and at a Lake Minnetonka marina (Tonka Bay Marina) by professional mechanics and research staff (Adam Doll and Rosie Daniels).

Water samples were collected between June and August in 2016 and 2017 at 7 accesses (Table 1). Veliger density data from 10 lakes distributed throughout MN (including Minnetonka and Gull), estimated from triplicate plankton tows at multiple sites throughout each lake, show pronounced variation in veliger densities across summer months, with mid-summer peaks in density (McCartney and Mallez 2018 and unpublished). These data show that the dates on which residual water samples were collected bracket the times of maximum veliger density in the water column. Some lakes that were sampled in 2014 and 2015 (and a few in 2016: McCartney unpublished) show that the magnitude and timing of veliger density maxima can shift annually, but the peak still remains within the time window sampled by the DNR watercraft inspection staff in the present study.

Watercraft inspectors approached boaters and asked for voluntary participation in this research project. One compartment (sampling location) was selected on each boat by watercraft inspection staff with approval by the boat owner. Inspectors had less success gaining access to hard-to-reach compartments, and as a result, fewer samples were collected from internal compartments such as bilge areas and ballast tanks. Inspection staff attempted to obtain samples from a broad range of compartment types, and to balance sampling across a variety of different makes and models of recreational watercraft.

Samples were collected from the following watercraft compartments: ballast tank, bilge, foot well (PWC), live well, splash well. Residual water was collected using 60 cc polypropylene syringes. Tygon® tubing was attached to the syringe tip to aid collection efforts in hard-to-reach areas. Syringes and tubing were cleared with filtered lake water after each sample was drawn to remove any organic material and pumped until no water was left in the system.

Additional samples were collected from outboard motors and PWC jet engines. Watercraft inspection staff used 5-gallon oil drain pans (Hopkins FloTool, Emporia, KS) to catch residual water that remained inside engines after the equipment was pulled from the water. Outboard motor water was collected by lowering the motor to the upright “run” position which allows all remaining water to drain from the intake in the motor’s lower unit. PWC engine water was collected by running the engine for a few seconds to collect any remaining water.

Description of sampled compartments

Ballast tank: Water holding devices typically found on wakeboard boats to add weight to the watercraft and increase the size of the wake the boat creates. Tanks come in a variety of shapes and sizes and are typically hard plastic or soft vinyl.

Bilge: The lowest compartment of a watercraft where the bottom curves up and meets the sides.

Foot well (PWC): The places on a personal watercraft where the operator and riders position their feet.

Jet engine (PWC): Propulsion system for personal watercraft. A pump containing an impeller draws water up from beneath the PWC and pushes it out the back, generating thrust.

Live well: Water holding compartments found on fishing boats that are used to keep fish alive.

Outboard: An external watercraft propulsion system that is attached to the back of a watercraft.

Splash well: An area at the back of a watercraft where an outboard motor is attached.

Sterndrive engine: Sterndrive engines are a combination between inboard and outboard engine types. The engine is located inside the watercraft, with a lower unit that extends from the back of the watercraft down below the waterline. Water enters the engine through the lower unit and keeps the engine at operating temperature.

Collections from sterndrive engines

Residual water from sterndrive engines was collected with the assistance of certified marine mechanics at Tonka Bay Marina, which is located on Lake Minnetonka. The marina stores watercraft in buildings called “dry-stacks”. Boats held in dry-stack are stored on racks inside a covered building and placed into the water at the customer’s request. Boats were chosen for sampling if they were operated on Lake Minnetonka within the previous 24-hour period. Sterndrive engines were sampled by draining the engine block into small cups and transferring the water to 5-gallon buckets. All watercraft were out of the water during the sampling process to avoid any possible siphon effects from the lake (i.e. water being pulled in through the intake as the block was being drained).

Collections from ballast tanks

Ballast tanks on wakeboard boats are difficult to access (hard tanks cannot be feasibly sampled, and samples can be obtained only from “soft” ballast tanks or bags using appropriate tools), so as a result, watercraft inspectors were only able to collect samples from six ballast tanks at accesses, and an additional two ballast tanks with the assistance of marina staff. To increase sample sizes from ballast, an additional ballast tank (Fly High Pro X Series 370lb, Fatsac, Milwaukee WI) was purchased and fit to a single test boat, which was operated to fill and sample the tank repeatedly. The ballast tank was placed inside a runabout boat (Crownline 180 BR) and filled using a centrifugal (Fly High Pro X Tsunami) pump, which has a fill/drain rate of 1,200 gallons per hour. An additional 25 samples were collected with this ballast tank and pump over seven days in July and August of 2017.

The centrifugal pump was submersed to a depth of 1 meter for each sample, and samples were collected at lake depths ranging from 2.5 meters to 22 meters in the lower basin of Lake Minnetonka. The pump was placed in the lake at a depth of 1 meter and ran until the ballast tank was filled. The tank was then drained by reversing the pump assembly and attaching the pump directly to the tank. This was done in a manner similar to operators, who typically pump water from ballast tanks prior to departure from a lake, to reduce the weight of tank ballast when trailering to a new water body. To simulate the conditions of ballast tanks on wakeboard boats and the water that remains inaccessible to the user, the ballast tank was drained until the pump was no longer able to effectively pump water out of the ballast tank. The water still remaining in the ballast tank at this stage was then drained into a 5-gallon bucket and treated as residual water for the purposes of this study.

Residual water sample processing and analysis

Residual water sample volumes were measured, and water was concentrated and preserved at the collection site (public water accesses, marina, Fatsac ballast samples). Samples were first poured into graduated cylinders to measure volume, and then filtered through 80-micron Nytex® mesh and collected in Nalgene bottles. Absolute ethanol was added to a final concentration of 70%, and samples were stored in a fireproof container until analysis could be completed. In the laboratory, samples were filtered through 80-micron Nytex® and rinsed with filtered lake water into a petri dish. The sample bottle and Nytex were rinsed with additional filtered lake water to wash off any remaining veligers or other organic material. Samples were examined under cross-polarized light to detect and count the veligers present (Johnson, 1995).

Statistical analysis

Data were tested for normality with a Shapiro-Wilk test (Shapiro and Wilk, 1965). Volume per sample and veligers per sample were separately tested for normality, and both tests rejected the null hypothesis (see results, $P < 0.001$). Differences in residual water volumes among compartment types and in numbers of veligers recovered per compartment were therefore tested with a non-parametric Kruskal-Wallis rank sum test. To examine whether the likelihood of veligers being present differed between compartment types, we conducted a G-test of independence. Because the sample size was small for some of the compartment types, we applied the Williams correction for continuity and compared the corrected value of G to a χ^2 distribution with 7 degrees of freedom (Sokal and Rohlf, 1994).

Estimating Likelihood of Transport

To estimate relative veliger contributions of each compartment to the total transport by a boat of veligers to a next-visited water body, we proceeded as follows. Following previous risk estimation work (Johnson et al., 2001), we assigned a probability of transport to each compartment. Each compartment was assigned a probability that veligers were present in the residual water within the compartment P(x) based on the frequency that x number of veligers were found in residual water samples; it was assumed that all compartments contained residual water ($P_{exposure} = 1$). Compartments receive a score of “Low” if P(x) is less than .33 “Moderate” if P(x) is between .33 and .66 and “High” if P(x) is greater than .66.

To assess the combined likelihood of transporting veligers for exiting watercraft we created compartment lists for each type of watercraft (Table 5). This allowed us to sum the estimated veligers per compartment to assign an overall likelihood for each watercraft type.

Results

A total of 379 residual water samples were collected during the 2015 and 2016 boating seasons. Most of the samples collected were from fishing boats (250: Tables 2 and 3). Additional samples were collected from pontoon boats (5), personal watercraft (30), wakeboard boats (36), and runabouts (53). The primary locations sampled were dependent on boater cooperation at public water accesses; often inspection staff were given access to only the most easily accessed compartments. Areas sampled (Table 3) included: live wells (127), lower units of outboard motors (67), bilge areas (57), sterndrive engines (38), ballast tanks/bags (34), transom splash wells (28), PWC footwells (20), and PWC jet engines (8).

Volumes of residual water varied substantially among compartment types, with sterndrive engines consistently containing the greatest volumes of residual water (Figures 1 and 2). Differences in residual water volumes among compartment types were highly significant (Kruskal-Wallis H = 206.07; $P < 0.001$). Large volume compartments include sterndrive engines, ballast tanks, and outboard motors with median residual water volumes of 4945, 2650, and 292 milliliters, respectively. Small volume compartments included PWC foot wells and PWC jet engines, and bilge areas, live wells, and splash wells, with median residual water volumes of 145, 79, 120, 62, and 40 milliliters, respectively (Table 2).

The frequency at which veligers were present or entirely absent from sampled residual water varied substantially across compartment types (Table 3, $G_{adj} = 92.113$ *df* = 7, $P < 0.001$). The ranking from highest to lowest % frequency of veliger presence was as follows: ballast, sterndrive engine, outboard motor, live well, foot well, splash well, bilge and jet. Median veliger counts were higher in the large volume compartments when compared to the small volume compartments.

The number of veligers recovered per compartment also varied substantially among compartment types (Kruskall-Wallis rank sum test, H = 139.92; $P < 0.001$). Ballast tanks had the largest median and sterndrive units had the largest maximum values (Table 2, Figure 3, Figure 4). We further split compartment types into two categories, large volume (Ballast, Sterndrive, Outboard) and small volume (Live well, Foot well, Splash well, Bilge, Jet). Compartments that held more residual water on average tended to have higher veliger counts. Similarly, the likelihood of finding zero veligers in a sample was greatest for small volume compartments.

Ballast tanks contained the largest median number of veligers with 247. The median number of veligers for sterndrive engines was 13, followed by one for outboard motor lower units. All remaining compartment types had zero veligers as a median value. Within any of the eight compartment types, veliger counts were poorly correlated with residual water volumes (Figure 5), but because compartments retaining larger volumes of residual water tended to contain larger numbers of veligers, there was a significant relationship between residual water volume and veliger counts ($F_{[1,377]} = 174.92$, $P < 0.001$; Figure 6) across the entire data set.

During each recreational boating season dating back to 2012 DNR watercraft inspectors have collected survey data on watercraft entering and exiting the study lakes. We compared these data to residual water samples, and created an estimate for the likelihood of transporting veligers in the compartments sampled. During the timeframe of the present study, watercraft inspectors inspected a total of 11,123 exiting watercraft across the state. They classify watercraft into eight general categories (fishing boat, runabout or ski-boat without ballast, pontoon, personal watercraft, wakeboard boat with ballast, Jon boat, canoe/kayak/similar, and sailboat). Fishing boats were the most common watercraft type inspected (60.5%). Inspections at Minnetonka and Gull Lakes closely match inspections across the entire state of Minnesota (Figure 9). During 2016 and 2017 watercraft inspectors inspected over 865,000 watercraft; 66% of those were fishing boats (MN DNR Watercraft Inspection Data).

Of the eight compartments sampled, ballast tanks and sterndrive engines ranked 1st and 2nd for the greatest likelihood of transporting at least 1 veliger, with a P(1) = .97 and a mean of 347 ± 398 veligers per sample, and P(1) = .89 and a mean of 232 ± 789, respectively, for the two compartment types. Small volume compartments (live well, foot well, splash well, bilge, and jet) had low risk of transporting at least one veliger per trip (Table 4). After pooling compartment types by watercraft type, wakeboard boats had the highest estimated likelihood of transporting veligers in a single event based on the compartments they contain (Table 5).

Discussion

Current watercraft designs do not allow 100% of the water present within a watercraft to be drained prior to leaving a water body. We used samples taken *in situ* from watercraft leaving zebra mussel infested waters to quantify the likelihood that zebra mussel veligers were leaving infested waters in small amounts of residual water. Natural resource managers looking to prevent the movement of AIS, including zebra mussel veligers, will need to ensure prevention programs are in place to aid boaters in additional decontamination steps. Watercraft manufacturers can help watercraft owners and resource managers by continuing to create watercraft systems that will minimize the movement of water between locations.

Because we collected samples from watercraft using public water accesses as they were leaving, and on a voluntary basis, we did not have control over which compartments were sampled. DNR inspection staff only sampled one compartment per watercraft, so none of the results measured the total contribution of one watercraft because each one will have multiple compartments that potentially contained veligers. Given the fact that there are also countless configurations of watercraft across numerous manufacturers, actual volumes found in any given watercraft may differ from the values estimated from samples taken from a different watercraft of the same type.

It would be expected that watercraft using zebra mussel infested waters during peak spawning times would present the greatest risk for veliger movement. Residual water sampling was conducted in June, July, and August, which coincides with the times of highest in-lake reproduction for Minnesota waterbodies. Veliger densities were recorded on Lake Minnetonka by the Minnehaha Creek Watershed District (MCWD) from 2011 through 2014. Densities varied in each of the different bays within the lake, with a maximum density of 36.97 veligers/liter recorded at “Lower Lake South” bay in 2014 (MCWD data, unpublished). But in each bay in which densities were high enough to evaluate, veliger counts varied seasonally and peaked in summer, from late June through late July, depending on the bay (MCWD data, unpublished).

Zebra mussel veligers were found in all compartments that were sampled, although 51% of samples (194) contained no veligers. Compartments that contained more residual water on average had larger numbers of veligers compared to the compartments with smaller volumes of water and had a greater likelihood of finding one or more veligers. Differences in veliger counts found in each compartment could be related to the way each compartment receives water. Some compartments sampled (bilge areas, PWC footwells, transom splash wells) do not actively pump water from the water column into enclosed compartments, while others actively pump large volumes of water into/through the system (ballast, sterndrive, outboard). Water is deposited in these smaller compartments through a variety of ways.

Bilge areas could collect water from recreational activities (fishing, swimming, etc.), adverse weather conditions (large waves, rain, etc.), or structural failures on the watercraft (leaking hull, seals, etc.). Bilge water rarely moves from the lake to the bilge immediately. Sampled watercraft typically have raised flooring, and the water would need to move past this initial boundary in order to be deposited in the bilge area. Splash wells at the stern of watercraft can hold water from waves created by backtrolling, and PWC footwells can collect surface water via the actions of the operator or wave action.

None of these compartments pulls water from deeper in the water column where veliger densities can be greater, as found in one study of another MN lake (Montz et al., 2016).

Live wells also contained small amounts of residual water and small numbers of veligers. While these systems do have pumps, they are only used if the operator turns a pump on to fill the compartment with water. If the pump is not activated, water can still partially fill the live well with water. Boaters were not asked if the pump was used or not. Fishing boats sampled at the project lakes typically have a shallow draft, and if activated, the live well pump would be pulling water from the top half meter of the water column. Similar to fishing boats, PWC's have a shallow draft that is typically less than 0.5 meters and the intake for the jet propulsion system is internal.

The large volume compartments contained larger numbers of veligers on average when compared with small volume compartments, though there was no clear relationship between water volume collected and veliger counts within a given type of compartment. Large volume compartments (sterndrive engines, ballast tanks, outboard motor lower units) are situated on watercraft in such a way to pull subsurface water, where veligers densities may be greater than at the surface (Montz et al., 2016). Engine intakes are typically the lowest part of recreational watercraft, and ballast tank intakes are located on the underside of wakeboard boat hulls. One sterndrive engine had the greatest maximum veligers per sample (4,567). During engine sampling, sand and other unidentified debris were found in the water samples. This debris was not analyzed to determine origin, but some sand can be left inside the engines from the manufacturing and casting process (personal communication, Volvo engineer). Because debris can be contained inside the water holding compartments inside engines, it is also possible that engine systems are capable of concentrating veligers, which will sink given sufficient time, and localized areas within engines with little water movement. This may explain how some engine samples were found to have many more veligers per liter that what naturally occurs within Lake Minnetonka, which had a greatest reported density of 36.97 veligers per liter in 2014 (MCWD, unpublished). Ballast tank pumps are capable of pumping large volumes of water in short periods of time, with the pump used for this study pumping at 74 kg/minute. It appears as though the systems that pull large volumes of water from deeper in the water column have a greater likelihood of collecting larger numbers of veligers.

Conclusion

Recreational watercraft compartments can hold zebra mussel veligers after boaters have taken all necessary steps as required by Minnesota statues including cleaning and physical removal of all visible plants and invasive animals and draining water by removing all drain plugs and associated equipment. Not all compartments sampled had the same likelihood of containing veligers, and the mean numbers of veligers is significantly different across compartments sampled.

Based on the results of this study, recreational equipment that contains one or more ballast tanks poses the greatest likelihood of moving high numbers of veligers given our findings that ballast tanks were the compartment with the greatest likelihood of containing veligers and had the greatest mean number of veligers per sample. Wakeboard boats are the type most likely to contain ballast tanks, and some boat models can have multiple ballast tanks. Given the amount of veligers ballast tanks may contain, extra steps (such as hot water decontamination,) should be taken by boat owners to minimize the likelihood of introducing live veligers to new water bodies via overland transport.

Chapter 2: Zebra Mussel Veliger Mortality in Live Well and Ballast Tank Residual Water

Introduction

Zebra mussels (*Dreissena polymorpha)* were initially discovered in North America in Lake Erie in 1986 (Carlton, 2008). By the end of 2010, they invaded a total of 744 natural lakes, impoundments and reservoirs and 131 rivers in the US and Canada (Benson, 2014). Zebra mussels have two primary means of spread; transport of larvae (veligers) and adults via the flow of water through downstream connections (Horvath et al. 1996; Bobeldyk et al., 2005; McCartney and Mallez, 2018) or via human activities that carry them overland between unconnected water bodies (Johnson and Carlton, 1996; Buchan and Padilla, 1999; Johnson et al., 2001). Recreational watercraft are often cited as the primary vector for overland dispersal of adults or veligers.

Zebra mussels were first discovered in Minnesota in 1989 in the Duluth/Superior harbor and have continued to spread throughout the state. In Minnesota there are 218 water bodies (as of October 26, 2018) with confirmed populations of zebra mussels and an additional 156 precautionary designations due to connectivity to upstream zebra mussel infested water bodies (MN DNR 2018, October 26: Retrieved from: https://www.dnr.state.mn.us/invasives/ais/infested.html). The State of Minnesota has legislation in place to minimize the movement of aquatic invasive species between water bodies. Prior to transporting the equipment on public roadways, watercraft users are required to fully inspect all equipment, remove all plants and invasive animals, and open or remove all drain plugs to allow the equipment to drain (Minn. Stat § 84D.10). Draining of watercraft in this manner is not 100% effective, however, and “residual” water often remains trapped inside the watercraft after all drain plugs are removed (Montz et al. 2016; Campbell 2016).

Although there are many studies that document the ability for adult mussels to survive out of water for extended periods of time (Callas et al., 2018; De Ventura, 2016; Johnson et al., 2001; Riccardi, 1995), there is limited information available to evaluate the potential survival rates of veligers in residual water within watercraft. A few earlier studies provide some data on survival times of *Dreissena* larvae in laboratory trials, but only a narrow range of non-stressful temperatures have been tested, because the studies focused either on chemical toxicity (Fisher et al., 1994) or on conditions that affected growth, development and survival of larvae in culture (Stoekel et al., 2004).

Survival of veliger larvae of the quagga mussel (*Dreissena rostriformis* “bugensis”), a related species that now dominates the Great Lakes and is the only species reported from the Colorado River system in the western US, has also been studied experimentally to evaluate whether quagga veligers could survive transport in watercraft. By exposing quagga veligers collected from Lake Mead to controlled temperatures (in sealed tubes containing 30 μL water), Snider et al. (2014) showed that > 60% of larvae were able to survive 20 hour exposures to 30°C. Choi et al. (2013) found that 5 days were required to reach 100% mortality of Lake Mead quagga veligers in 20 L buckets that were placed in ambient “summer” conditions, where air and water temperatures averaged > 30°C, day and night. Together, these studies indicate that quagga mussel veligers are at least capable of surviving “short trips” in watercraft (Snider et al., 2014).

No comparable studies of zebra mussel veligers have yet been published to refine these conclusions. Upon exposure to hot water spray, adults of the two species show differences in heat tolerance, with zebra mussels being more heat tolerant (Morse, 2009; Comeau et al., 2015). These results underscore the need for experimental work to determine survival of zebra mussel larvae, especially under conditions most often experienced during transport in trailered boats. We studied zebra mussel veliger survival within three watercraft compartments commonly found on many recreational watercraft: live wells, ballast tanks, and the sterndrive engines (“inboard/outboard” motors) at exposure temperatures typically encountered during Minnesota summers. By using actual watercraft compartments with small volumes of water, we aimed to better understand the risk posed by transient recreational watercraft moving from one water body to another.

Methods

Trailered watercraft temperature monitoring

Data loggers (iButton Thermachron Temperature loggers, Maxim Integrated Products, San Jose CA), each enclosed within aquarium silicon sealant, were placed inside a live well and a bilge to record the air temperature of these compartments within a watercraft (Lund model 1775) that was dry and stored on a trailer outdoors on a gravel surface. Data loggers recorded the temperature every ten minutes over a 24-hour period on August 20, 2017 in Saint Paul, Minnesota.

Veliger collection

Veligers were field-collected from Lake Minnetonka, Minnesota for use in the live well and ballast tank mortality trials. Veligers were collected off the public docks in the City of Wayzata (44.9686°N, -93.5121°W) at a site where water depth is 10 m. Veligers were collected using a plankton net (30 cm mouth, 80-micron mesh, 90 cm length); lowering the mouth of the net to a depth of 3 m with a retrieval rate of less than 1 meter per second (Wong, 2013). This process was repeated three times, moving the net to a new undisturbed location each time, to ensure that enough veligers were collected for mortality trials. The 3 tows were pooled, added to 18 liters of 80 micron filtered lake water in a bucket, and maintained at a constant temperature of 20C. This "veliger stock" was collected and used within 48 hours for each live well mortality trial. Veliger stock buckets were aerated, using air stones, between trials. Veliger stock for ballast tank mortality trials was collected at the start of each mortality trial.

Scoring live and dead larvae

Veligers were identified using cross polarized light microscopy (Johnson, 1995) and visually inspected to determine if they were alive or dead. Each veliger was examined for a maximum of 10 seconds to look for motility, ciliary motion on the velum (the ciliated tissue fold used for swimming and feeding), or movement of the stomach, gut, or other internal organs. If no movement was observed within 10 seconds veligers were scored as dead.

Live well mortality trials

The live wells and housings used for these trials were constructed and supplied by the Lund Boat Company in New York Mills, MN. All materials used to create the housings were supplied by the factory and are used in production model watercraft. Two separate housings were constructed with marine grade wood; one was wrapped with marine vinyl and the other with carpet. Each housing included three live wells with drains and lids, for a total of 6 live well chambers. Each live well was identical and measured 55 cm long x 28 cm wide x 26 cm tall and is typical of live wells used in the construction of new Lund boats.

One liter of veliger stock was added to each live well and the initial temperature was recorded. The live well test chambers were kept indoors in a climate-controlled room held at a constant 20°C. Determining the amount of residual water to use during experiments was a challenge. The median volume of water remaining in live wells collected during our at-access residual water study (Chapter 1) was 62 mL, but this volume is too small to effectively conduct our mortality trials. One liter of water allowed us to completely cover the entire bottom of the live well with water and allowed us to have a sufficient number of veligers to permit their sampling over an extended time period. One liter of water was the maximum volume collected during our residual water study of live wells (Chapter 1), and could be viewed as a possible “worst-case” scenario for water left in a live well after removing the drain plug. Once the veliger stock was placed in the live well, heat was applied to the internal air space of the live well using a ceramic heater (Flukers Ceramic Heater, 100W) screwed into a heating lamp socket. Air temperature was monitored with a digital thermometer and the ceramic heater was controlled by a digital thermostat. The thermostat would trigger the heater to turn back on if the temperature dipped below the desired test temperature. Live wells were exposed to a maximum temperature of 20°, 27°, 32°, and 38°C. These temperatures were chosen to reflect similar real-world temperatures that a live well would be exposed to if a watercraft was removed from the water and placed on a trailer.

Each live well was completely drained every hour by opening the valve. The entire water volume was filtered through an 80 micron Nitex mesh cylindrical filter, which was gently rinsed into a petri dish to harvest the larvae. The water collected from the live well was set aside after the initial draining, and filtered water was used to gently rinse the inside of the live well to collect as many remaining veligers as possible. Once this step was completed the filtered live well water was returned to the live well, removing any excess rinse water to maintain a volume of 1,000 ml. Veligers were counted and identified with cross polarized light microscopy (Johnson, 1995) and all veligers were returned to the live well as quickly as possible after counting to resume the trial. Trials were run, and samples taken at hourly intervals up to a maximum of five hours.

Ballast tank mortality trials

For the mortality trials, we used two identical soft ballast tanks (Fly High Pro X Series, Fatsac, Milwaukee WI). Each bag held a maximum of 167.8 kg (167.8 L) of water and measured 157.5 cm L x 42.6 cm W x 25.4 H. Veliger stock was collected daily using the methods described above and four liters of veliger stock was used for each trial. Each day, we simultaneously conducted a control trial at 20°C and a heated trial at 32°C, and we conducted live-dead veliger counts at 0, 2, 4, 6, 8, 10, 12, 24, and 48 hours.

Four liters of 20°C veliger stock was slowly poured into each ballast tank and each tank was sealed shut. The control trial was conducted in a temperature-controlled laboratory with a constant temperature of 20°C. Heated trials were placed into an environmental chamber set to a constant 32°C. This temperature was chosen as the maximum exposure temperature because ballast tanks are internally located within factory-built wakeboard boats and do not receive exposure to direct sunlight, so we expect temperatures within the tanks to not rise as much as residual water within live wells. Ballast tanks used for control and heated trials were both laid flat throughout the entire duration of the trial. Each ballast tank was agitated gently to mix the water and suspend any settling veligers before sampling for live and dead larvae. This agitation was the only disturbance to the ballast tanks throughout the trial period. Sub-samples (100mL) were collected at each time point without replacement. Veligers counted in each sample were scored as either alive or dead as above, and as either D-stage or umbonal, based on the easily-scored presence of the umbo (shell peak above the hinge: Ackerman et al. 1994). The appearance of the umbo marks the deposition of an additional larval shell layer (i.e. the prodissoconch II), atop the single shell layer in D-stage larvae. We reasoned that this event might be associated with a change in temperature tolerance.

Sterndrive Engine Sampling

Sterndrive engines (i.e. the engines in inboard/outboard systems) were drained and sampled with the assistance of professional watercraft mechanics. Mechanics at Tonka Bay Marina drained water directly from the engine block to collect as much water as possible from inside the engine. Water was drained from sterndrive watercraft stored on-site in enclosed “dry-stack” storage sheds. Dry stack storage is a common way for boaters to keep their watercraft at a body of water without having to own dock space. Watercraft are kept in enclosed buildings on racks when not in use. A specialized fork-lift removes the boat from storage and places it in the water at the owner’s request. This service keeps watercraft protected from environmental exposure when not in use and is an efficient way to store multiple watercraft in a smaller location. The watercraft chosen had been operated in the lake within 24 hours prior to the time of sampling. Samples were transported back to the lab inside a cooler and analyzed on the same date of sampling.

Statistical Analysis

We fit a generalized linear mixed effect model to the veliger survival data from the live well water experiment. We modeled veliger mortality as a function of time, and temperature, and an interaction between the two (EQ 1).

EQ 1: Live well mortality

$$Y_{i,j} \sim Binomial(n_{i,j}, p_{i,j})$$

$$logit(ProportionSurvived_i) = \left(\beta_0 + b_{0i,j}\right) + \beta_1 Treatment_i + \beta_2 Time_i + \beta_3 Treatment{:}Time_i$$

$$b_{0i,j} \sim Normal(0, AmongLivewellVariance)$$

$$Y_{i,j} = number\ of\ alive\ veligers\ at\ the\ ith\ time\ point\ in\ the\ jth\ trial$$

$$n_{i,j} = number\ of\ total\ veligers\ in\ the\ ith\ sample\ of\ the\ jth\ trial$$

$$p_{i,j} = proportion\ of\ veligers\ in\ the\ ith\ sample\ of\ the\ jth\ trial\ that\ were\ alive$$

We also fit a generalized linear mixed model to the survival data from the ballast tank experiment (EQ 2). We analyzed veliger mortality as a function of time, temperature treatment (20C and 32C), and life stage size, allowing an interaction between treatment and life stage. The interaction term (treatment:lifestage) was incorporated to allow for the possibility that the effect of treatment differed between the two life stages. We found that the time trends in the generalized linear mixed effect model were non-linear on the logit scale, and therefore difficult to fit and interpret. Therefore, we also fit a logistic regression model, including just the data collected at either 6 hours (*t*=6) or at 24 hours (*t=24*) (Table 6, Table 7). Results from both models were then back-transformed for interpretation and visualization.

EQ 2: Ballast tank mortality

$$Y_i \sim Binomial(n_i, p_i)$$

$$logit(ProportionSurvived_i) = \beta_0 + \beta_1 I(Treatment)_i + \beta_2 I(LifeStage)_i + \beta_3 I(Treatment): I(LifeStage)_i$$

$$n_i = total\ veligers\ in\ sub\ sample\ at\ the\ ith\ time\ point$$

$$p_i = proportion\ of\ veligers\ alive\ at\ the\ ith\ time\ point$$

Results

Trailered watercraft temperature monitoring

Temperatures within the study areas during this project ranged from 18°C to 37°C (National Weather Service, Retrieved from: https://www.weather.gov/mpx/). On August 20, 2017, we recorded maximum air temperatures of 41.5°C and 35.5°C in our sample live well and enclosed bilge, respectively. The sample live well had an internal air temperature of 37°C or greater for a total of five hours, while the bilge area maintained an air temperature of 32°C for a total of five hours. The high temperature for the day was 29°C, and it is likely higher temperatures can be observed in both compartments during periods of warmer weather. Therefore, this study closely mimicked real-world conditions that transient watercraft are exposed to in typical Minnesota summertime conditions (maxima of 38°C for the live wells and 32°C for the ballast tanks).

Survival of veligers in sterndrive engines

We sampled sterndrive engines at Tonka Bay Marina on Lake Minnetonka between 2016 and 2017. Their owners operated all watercraft within 24 hours of sampling, and we analyzed all samples on the same day. In all cases, no zebra mussel veligers were observed alive when sampled. A total of 38 sterndrive engine samples were collected. The median number of veligers found was 247 and the maximum was 2,053. Median volume of water collected in sterndrive engines was 4,945 milliliters with a maximum of 13,400 milliliters. One engine was drained completely, and then allowed to run for two minutes after the boat was launched into the lake. This was to simulate a watercraft moving from dock to trailer for transport, without reaching operating temperature—a worst-case scenario of risk of moving live veligers. In this trial 25 dead veligers were found in a total of 13.4 liters of engine water recovered, which was the full volume of water present within the engine. The water temperature in this engine had reached 32°C after two minutes of engine run time. This engine had a density of 1.87 veligers per liter; an average of 41.5 veligers per liter were found across all sampled sterndrive engines.

Survival of veligers in live wells

The number of living zebra mussel veligers in all live well trials declined rapidly over time, with the greatest rate of decline observed in trials exposed to 38°C, in which 100% mortality was reached at three hours of exposure (Figure 11). Mortality of veligers exposed to either the 32°C or 38°C temperature treatments was higher than mortality in the control treatment at 20°C (*P* < .001), but the treatment at 27°C was not significantly different from the controls. We observed 100% mortality at 3 hours in the 38°C treatment, and high, but not complete, mortality after only 3 hours of exposure at the other treatment temperatures (85% mortality at 32°C, 70% mortality at 27°C, and 67% mortality at 20°C).

Survival of veligers in ballast tanks.

The number of living zebra mussel veligers declined over time in all experiments at both temperatures (Figure 12). Time was a significant factor (*P* < .001) for all exposure treatments and both life stages, and both life stages died significantly faster when exposed to 32°C as compared to 20°C. Umbonal veligers showed a lower rate of mortality when compared to D-stage veligers. At 6 hours of exposure to 20°C 40% of D-stage and 32% of umbonal veligers were observed dead. Exposure to 32°C for six hours increased observed mortality to 62% for D-stage and 53% for umbonal (Table 6). After 24 hours exposure to 20°C, 100% of D-stage and 85% of umbonal-stage veligers were noted to be dead (Table 7). At 48 hours 90% mortality was achieved for umbonal stages. Forty-eight hours of exposure to 32°C surrounding air temperature resulted in 100% mortality for both umbonal and D-stage veligers. One sub-sample was collected from each of 2 of the 20°C trials after 5 days (120 hours). In one of these sub-samples, 2 umbonal veligers (out of *n* = 105) were observed alive. In this instance, no motility or velum movement was seen, only internal gut movement was observed.

Discussion

At least some zebra mussel adults can survive exposure to 30°C water temperatures, with some surviving at 35°C (McMahon et al. 1994; Spidle et al. 1995). Exposure to higher temperature water has been demonstrated to quickly kill adult mussels (e.g. 60°C for 10 seconds) (Morse 2009). As a result, hot water has been recommended as the primary method for decontaminating watercraft for zebra mussel prevention (Comeau et al. 2011). Transient trailered watercraft with small amounts of residual water may not receive decontamination when moving between water bodies, and there is a possibility of transporting residual water that contains zebra mussel veligers to the next-visited water body (Johnson 2001; Campbell 2016; Montz et al. 2016).

During our live well experiments, veligers exposed to an air temperature of 38°C died much faster than at any other temperature (32°, 27°, 20°), although mortality was 95% or greater for all temperatures after 5 hours. Veligers in ballast tank residual water survived longer than their live well counterparts. In all trials exposed to 32°C air temperature, 100% mortality was achieved in D-stage and umbonal veligers when sampled at 48 hours. The rate of mortality of zebra mussel veligers was significantly faster at 32°C compared to 20°C in our controlled trials ($P < .001$). Veliger life stage was also a significant factor for survival, with umbonal veligers surviving longer than the D-stage larvae. Based on our results, watercraft equipped with ballast tanks are at a greater risk of transporting live veligers to new water bodies over short trips (e.g. 6 hours).

During both the live well and ballast tank experiments, residual water temperature increased rapidly from the starting temperature of 20°C at T = 0 and reaching the trial temperature within 2 hours. In previous studies adult zebra mussels have shown 100% mortality when tested at 35°C, which included slow increase in temperatures of 1°C per 5-60 minutes (Spidle, 1995). In other work, McMahon and Ussery (1995) showed that the temperature at which 100% mortality was achieved (LT_{100}) was actually higher when temperatures were increased more rapidly (over a range from 1°C increase per 5 minutes to 1°C per 60 minutes). In our case, temperature increases were at the fast end of this range, or faster. It is reasonable to conclude that stress from the rapid change in temperature could increase the rate of mortality of the veligers within residual water and could help account for the high levels of mortality at lower temperatures.

The findings of this study differ with previous research on the thermal tolerances of quagga mussel (*Dreissena rostriformis "*bugensis*"*) veligers in simulated overland transport via recreational watercraft (Choi et al. 2013; Snider et al. 2014). In Choi et al. (2013), veligers were collected from Lake Mead using similar collection methods, and by transferring the contents of the plankton tows to 15-liter plastic water containers. Sample containers were placed under a table outdoors in summer and autumn to mimic the conditions residual water would be exposed to in watercraft compartments. Under these conditions quagga mussel veligers survived approximately 5 days during summer temperatures (air temperatures of 25° to 40°C) and 27 days under autumn temperatures (air temperatures of 6° to 18°C).

Snider et al. (2014) simulated overland transport conditions in volumes much smaller than did Choi et al. (as small as 31 μl) and immersed these samples in various air temperatures. At air temperatures of 35°C or above they found no veliger survival, regardless of the size/age of the larvae; however, they observed survival of 0-3% for larger-sized larvae (≥ 150 μm shell length) and 63-91% of smaller-sized larvae (between 63 and 150 μm) at 20 hours when exposed to 30°C air temperature. At 25°C quagga mussel veligers showed moderate survival for at least 7 days; when the experiment was terminated: ≥ 7% of large size veligers, and ≥ 45% of small-size veligers were still alive.

Our results show faster rates of mortality for zebra mussel veligers in residual water of live wells and ballast tanks when compared to these studies. The volumes used for the present study were based on measurements of residual water in live wells and ballast tanks collected in watercraft leaving Lake Minnetonka and Gull Lake, Minnesota, USA during a three-year collection period (Chapter 1). The volumes of water used in our study are small when compared to the overall storage capacity of the compartment, and as a result, small volumes of residual water are spread thinly across the entire compartment bottom. Under more extreme temperatures it is possible that the smaller amounts of residual water we used caused increased mortality in zebra mussel veligers when compared to 15 liters of water used in Choi's study due to the rapid increase in temperature over a short time period. It is also possible that sampling the entire volume of water hourly during our live well experiments caused increased mortality due to handling of the veligers. This would not be an issue, however, in our ballast study because the larger volume of water allowed us to sub-sample and avoid repeatedly handling veligers. It is unclear why veliger mortality was faster in our residual water experiments when compared to Snider's study, which used volumes as small as 31 µl of water.

Conclusion

Mortality of zebra mussel veligers increased over time in our live well and ballast tank residual water temperature experiments, with no veligers observed alive at temperatures of 32°C or higher in either compartment at time = 48 hours. Two umbonal veligers were observed alive after 5 days ($n = 105$) in one 20 C control ballast tank trial. It is unknown if these veligers would be viable if introduced to a new water body.

In Minnesota, watercraft users are required by law to remove all aquatic plants and invasive animals before leaving the access and travelling on the road and are also required to drain all water holding devices by opening or removing the drain plug. Watercraft are complex machines, and small amounts of water can be trapped in bilge areas, engines/motors, live wells, ballast tanks, trailers, etc. that are nearly impossible to drain completely. It can also be difficult to ensure that all compartments are dry before entering other bodies of water, especially engine systems that hold large volumes of water that would not evaporate as in other compartments.

The results of this study can help resource managers evaluate the potential risks that watercraft in compliance with state laws may pose. Providing on-site or on-call decontamination services to watercraft users can help reduce the risk of transporting aquatic invasive species. These systems are often expensive, however, and not all agencies or organizations have the funding necessary to offer these services. Based on the results found in this study, the nationally recognized suggestion to dry your watercraft for five days or more (Stop Aquatic Hitchhikers!^{TM} 2017 Retreived from: http://stopaquatichitchhikers.org/prevention/) drastically reduces the risk of transporting living zebra mussel veligers to new water bodies. For boaters planning on moving between water bodies within shorter periods of time, extra precautions such as hot water decontamination could be done to minimize the risk of moving living veligers.

Tables

Table 1*:* Residual water sampling locations and details. Each site was sampled in June through August of 2016 and 2017.

Lake Name	Access Name	County	UTM coordinates	Year infestation found
Gull	East	Crow Wing	400557, 5147107	2010
Gull	Government Point	Cass	395411, 5140712	2010
Gull	Gull Narrows	Cass	396851, 5151622	2010
Minnetonka	Gray's Bay	Hennepin	460751, 4977546	2010
Minnetonka	Maxwell	Hennepin	451936, 4977912	2010
Minnetonka	North Arm	Hennepin	451303, 4977602	2010
Minnetonka	Spring Park	Hennepin	450483, 4975887	2010
Minnetonka	Tonka Bay Marina	Hennepin	454178, 4973901	2010

Table 2: Residual water volumes and number of veligers found in residual water recovered from each of 8 compartment types. “Sterndrive” refers to an inboard/outboard engine. The minimum number of veligers for all compartments was zero.

		Veligers			Residual Water Volume (mL)		
Compartment	Samples	25% Quartile	Median	75% Quartile	25% Quartile	Median	75% Quartile
Ballast	34	87	247	522	1294	2650	3450
Bilge	57	0	0	0	48	120	237
Foot wells	20	0	0	1	59	145	202
Jet	8	0	0	0	56	79	110
Live well	127	0	0	2	40	62	177
Lower unit	67	0	1	5	133	292	517
Splash well	28	0	0	1	14	40	52.8
Stern Drive	38	3	13	79	2349	4945	7099

Table 3: Frequency of veliger presence in each of 8 compartment types.

Compartment type	Total samples	Number with veligers present	Number with veligers absent	Percent containing veligers
Ballast	34	33	1	97
Sterndrive	38	34	4	89
Outboard	67	37	30	55
Live well	127	59	68	45
Foot well	20	8	12	40
Splash well	28	9	19	32
Bilge	57	13	44	23
Jet	8	1	7	13

G-test of independence with Williams correction: $G_{adj} = 92.113$ $df = 7$, $P < 0.001$

Table 4: Estimated likelihood of transporting at least 1, 10, or 100 veligers in residual water for each of the 8 compartment types sampled. The likelihood for each compartment is assigned based on the probability of transporting *x* or more veligers in a single transport event based on the samples collected. Compartments receive a score of "Low" if P(x) is less than .33 "Moderate" if P(x) is between .33 and .66 and "High" if P(x) is greater than .66.

Location (*n*)	P(1)	Likelihood of transporting 1 veliger	P(10)	Likelihood of transporting 10 veligers	P(100)	Likelihood of transporting 100 veligers
Ballast (34)	0.97	High	0.85	High	0.71	High
Bilge (57)	0.23	Low	0.04	Low	0	Low
Foot well (20)	0.4	Moderate	0	Low	0	Low
Jet (8)	0.13	Low	0	Low	0	Low
Live well (127)	0.46	Moderate	0.09	Low	0	Low
Outboard (67)	0.55	Moderate	0.09	Low	0.03	Low
Splash well (28)	0.32	Low	0.04	Low	0	Low
Sterndrive (38)	0.89	High	0.61	Moderate	0.21	Low

Table 5: Estimated likelihood of veliger transport for each watercraft type as defined by MN DNR watercraft inspection protocols. Watercraft components vary by manufacturer make and model, and it is possible for watercraft to have multiple compartments of the same type (e.g. ballast tank and live well).

	Boat Type				
	Fishing Boat	Wakeboard Boat	Pontoon Boat	Personal Watercraft	Runabout
Compartments	Bilge Live well Splash well Outboard	Bilge Sterndrive Ballast	Outboard	Foot well Jet	Bilge Outboard *or* Sterndrive
Likelihood	Moderate	High	Low	Low	Moderate

Table 6: Predicted zebra mussel veliger mortality at 6 hours in residual ballast water.

Treatment	Live Stage	Predicted Mortality (%)	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)
32	D-stage	61.5	54.5	68.1
20	D-stage	39.6	33.6	45.8
32	Umbonal	53.0	47.4	58.5
20	Umbonal	31.6	27.1	36.4

Table 7: Predicted zebra mussel veliger mortality at 24 hours in residual ballast water.

Treatment	Live Stage	Predicted Mortality (%)	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)
32	D-stage	99.9	99.2	99.9
20	D-stage	99.4	96.4	99.9
32	Umbonal	96.7	94.4	98.1
20	Umbonal	85	81.5	88.1

Figures

Figure 1: Boxplot of residual water volumes collected from recreational watercraft compartments. Small volume compartments are shown in the boxplot below. The median value is shown by the line that divides the box. The box represents the middle 50% of samples collected, and the whiskers extend to 1.8x the interquartile range. Note the difference in Y-axis scaling.

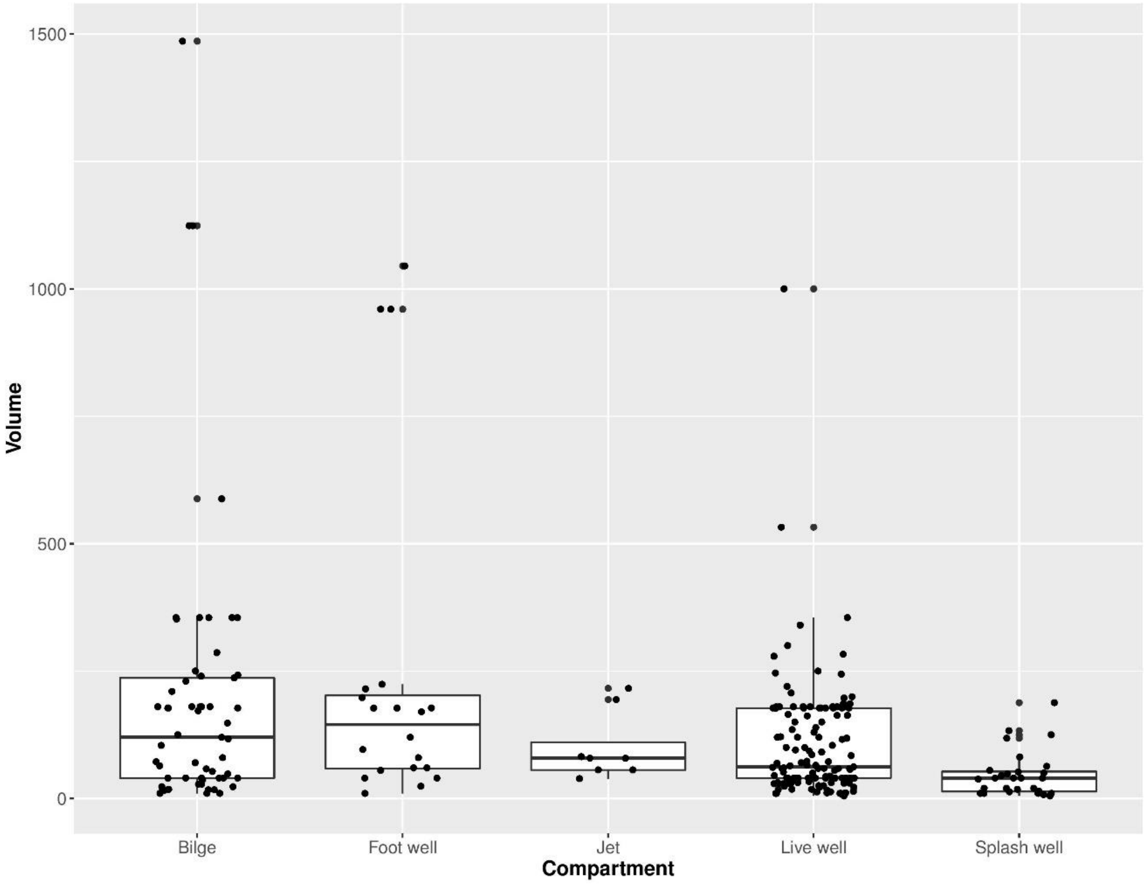


Figure 2: Boxplot of residual water volumes collected from recreational watercraft compartments. Large volume compartments are shown in the boxplot below. The median value is shown by the line that divides the box. The box represents the middle 50% of samples collected, and the whiskers extend to 1.8x the interquartile range. Note the difference in Y-axis scaling.

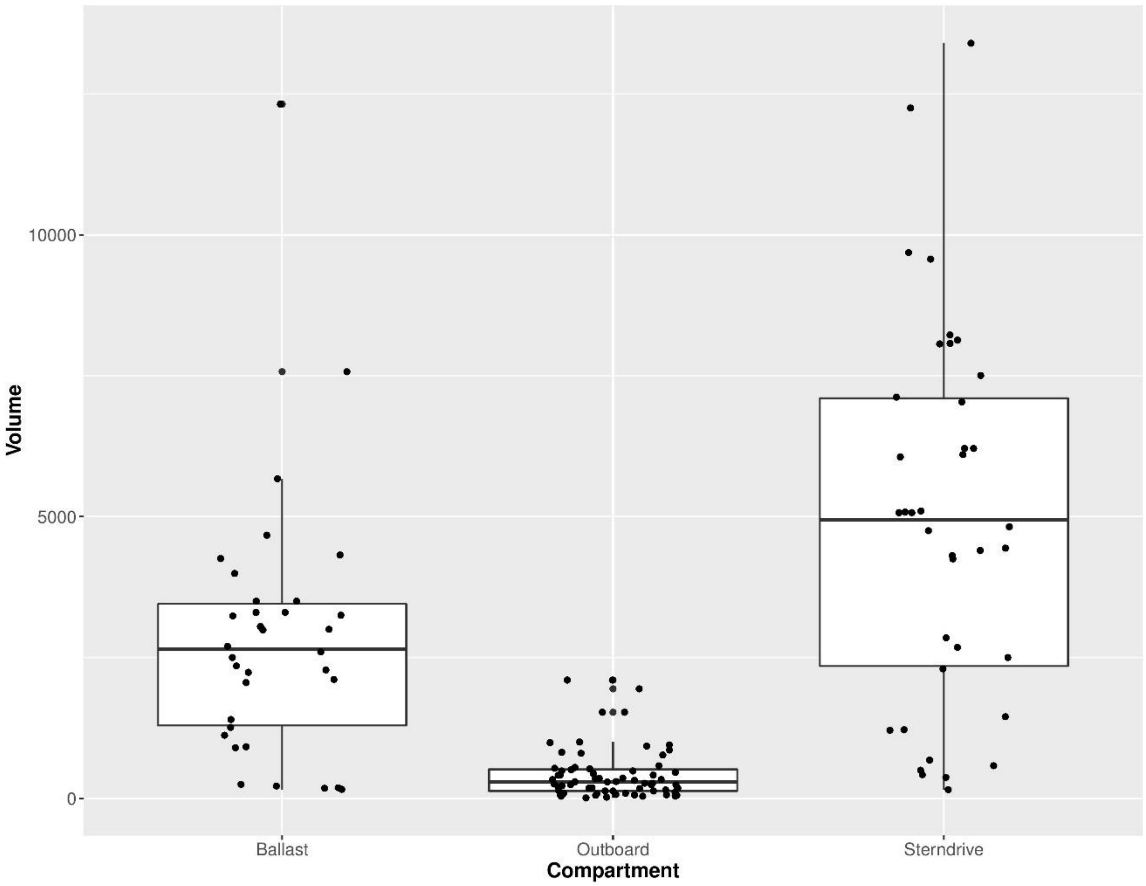


Figure 3: Boxplot of veligers found in residual water by recreational watercraft compartment. Small volume compartments are shown in the boxplot below. The median value is shown by the line that divides the box. The box represents the middle 50% of samples collected, and the whiskers extend to 1.8x the interquartile range. Note the difference in Y-axis scaling.

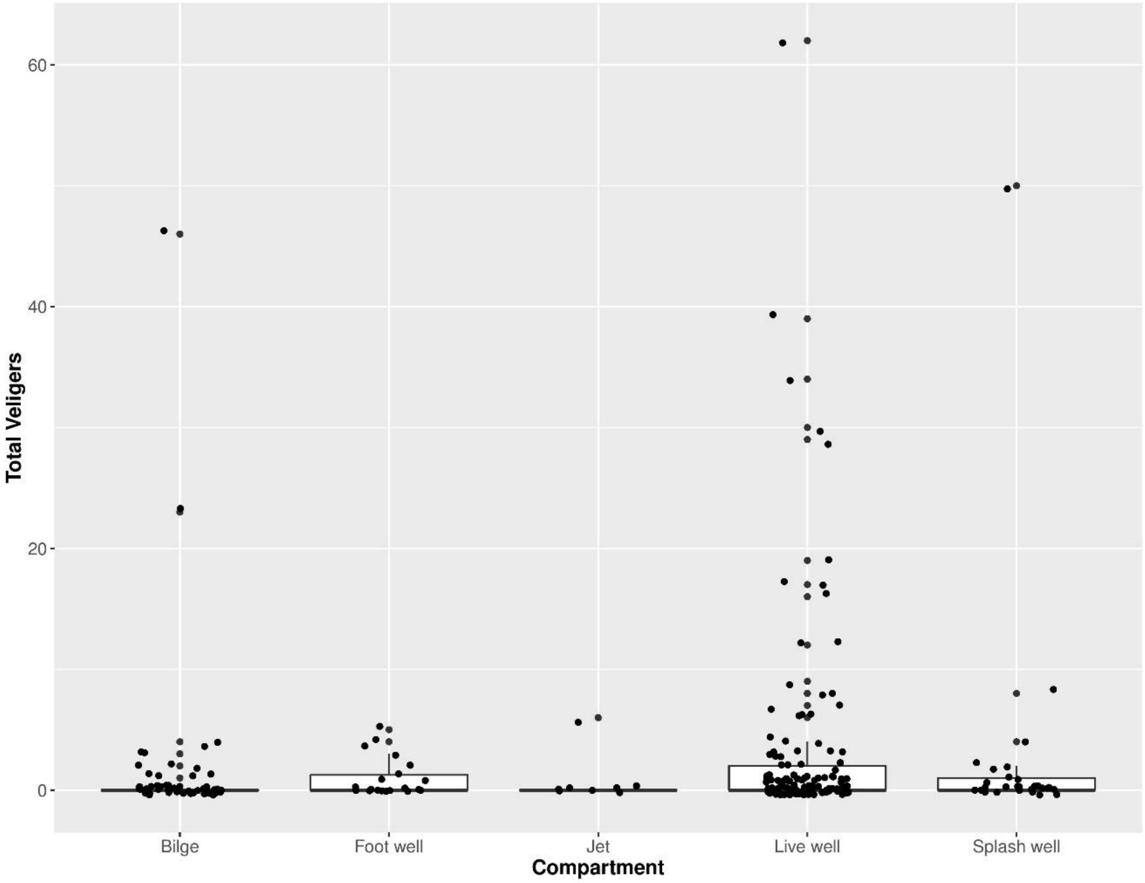


Figure 4: Boxplot of veligers found in residual water by recreational watercraft compartment. Large volume compartments are shown in the boxplot below. The median value is shown by the line that divides the box. The box represents the middle 50% of samples collected, and the whiskers extend to 1.8x the interquartile range. Note the difference in Y-axis scaling.

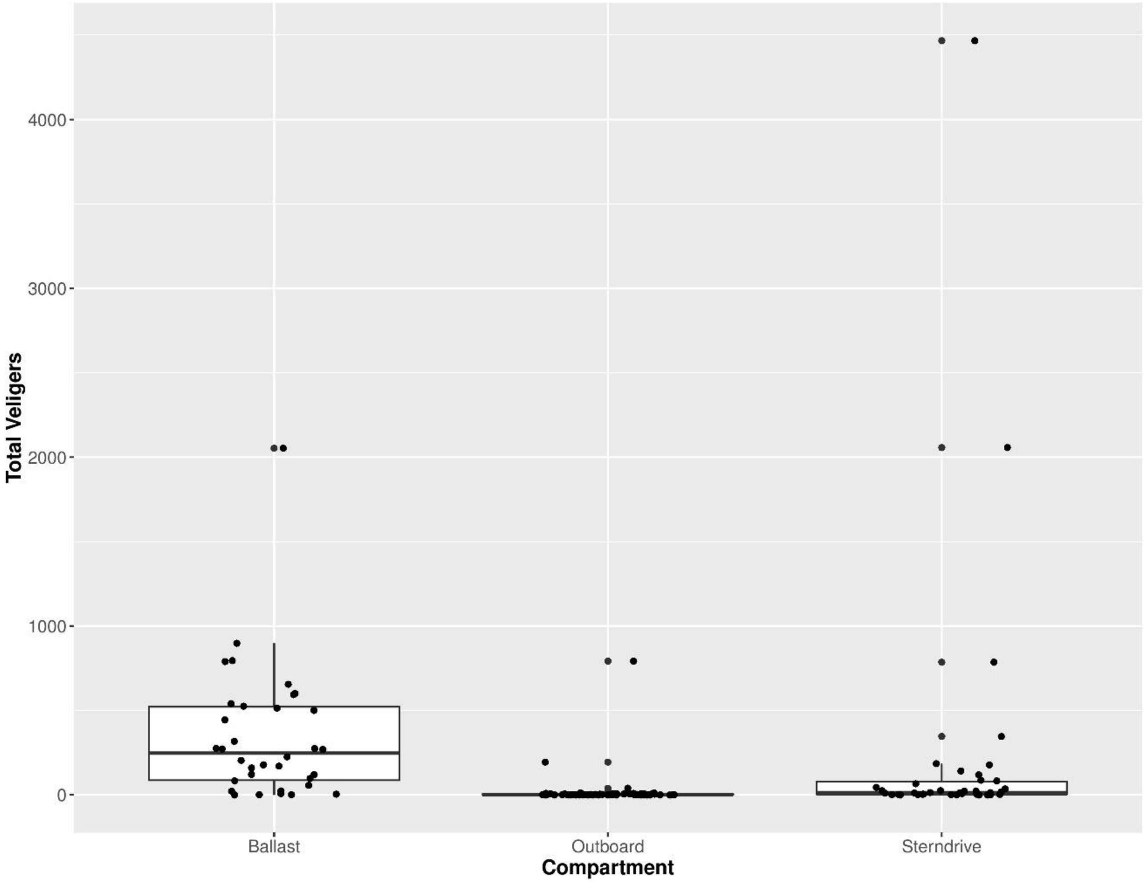


Figure 5: Plots of the number of veligers found per sample (Y-axis) as a function of the volumes of residual water collected within each of 8 different compartment types. Though larger volume compartments tended to have higher counts of veligers, veliger counts were poorly correlated with residual water volume collected within each of the compartment types (blue line is the line of linear fit; grey shaded area shows pointwise 95% confidence interval).

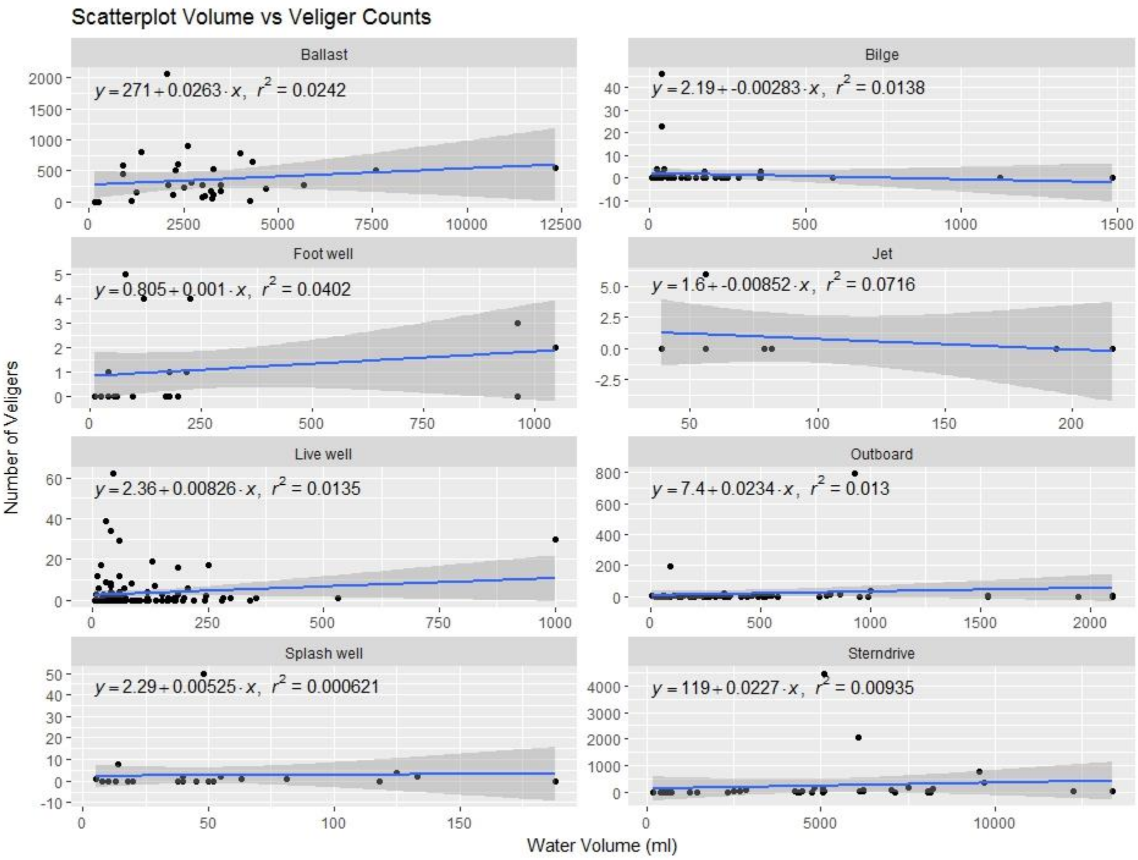


Figure 6: Relationship between residual water volumes and veliger counts collected across all compartment types, pooled. Data with zero veligers has been removed, and the x and y-axis are displayed on a log scale.

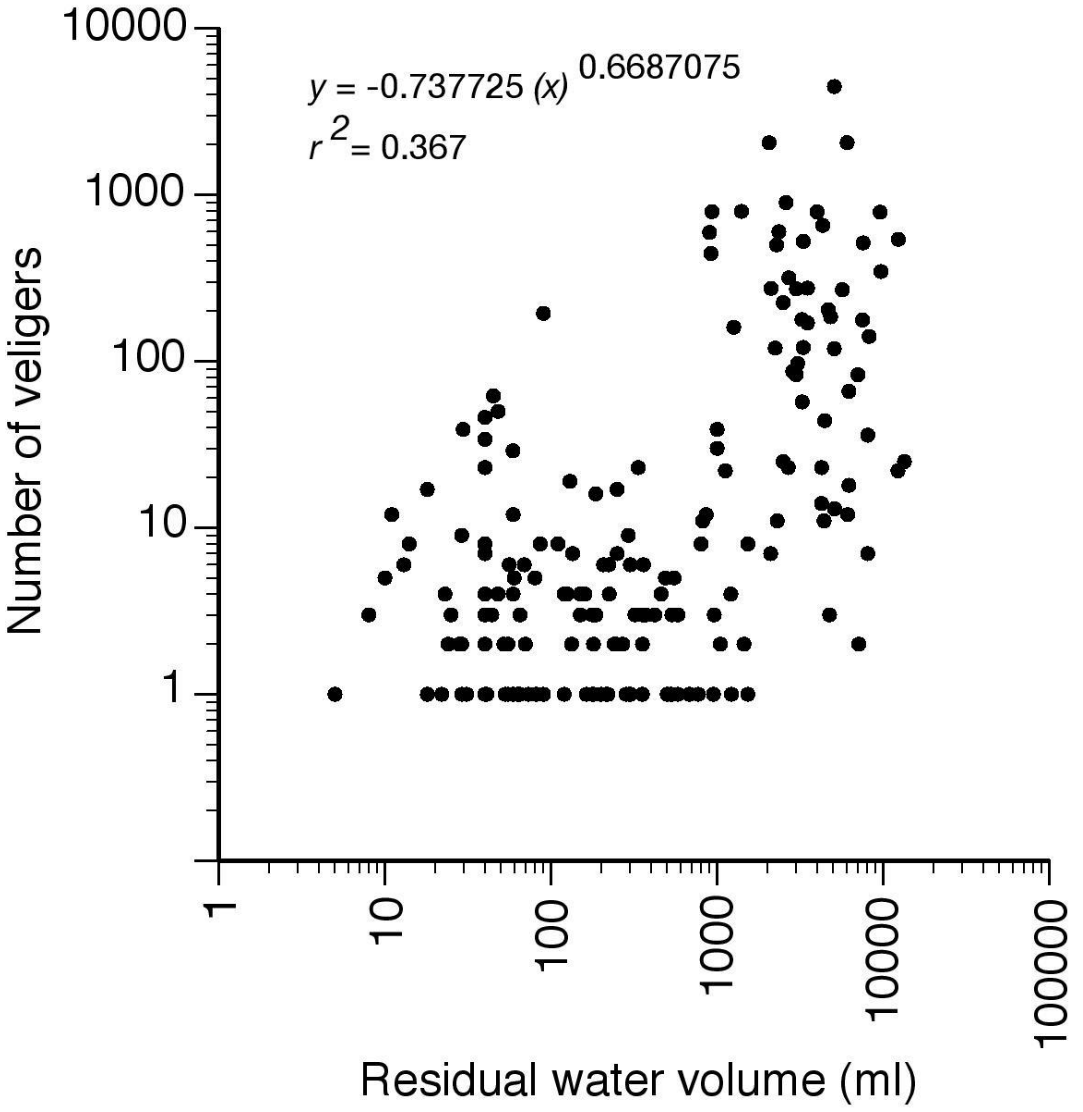


Figure 7: Mean veligers found per sample, per small-volume compartment. Error bars represent one standard error.

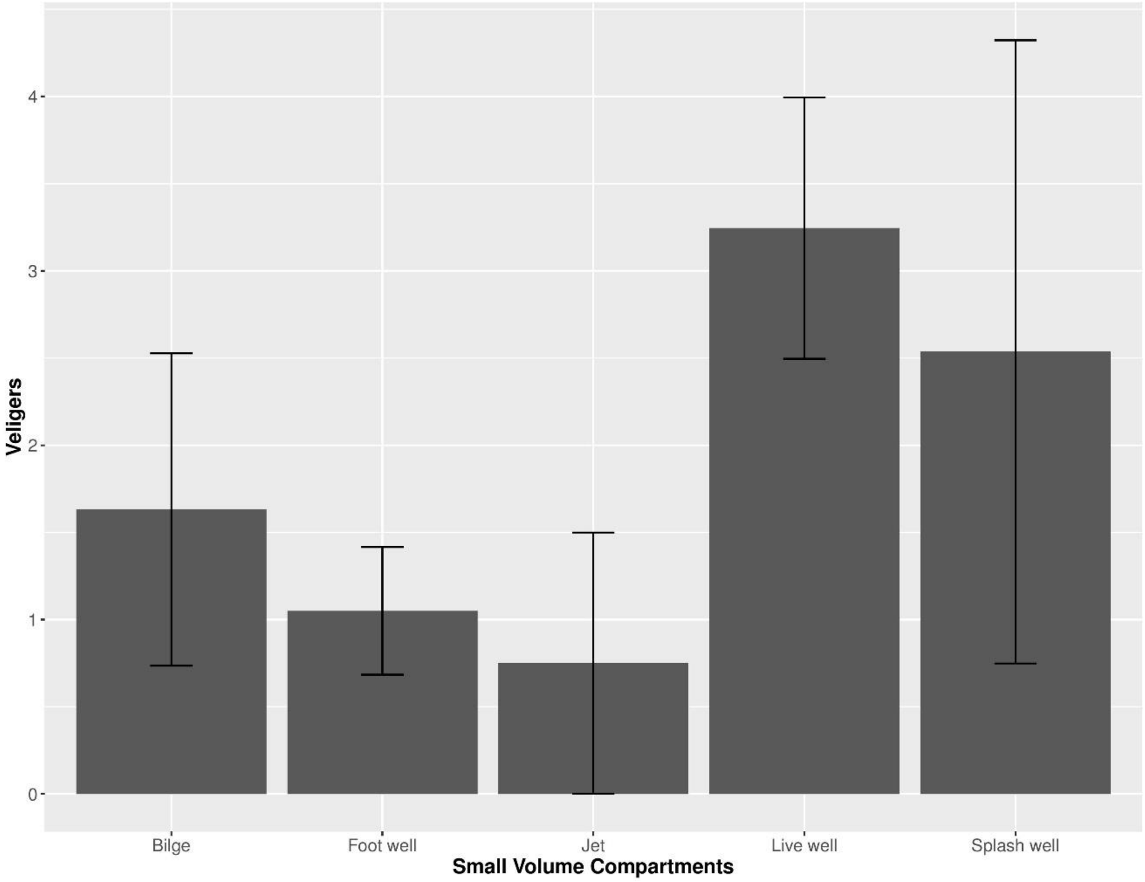


Figure 8: Mean veligers found per sample, per large-volume compartment. Error bars represent one standard error.

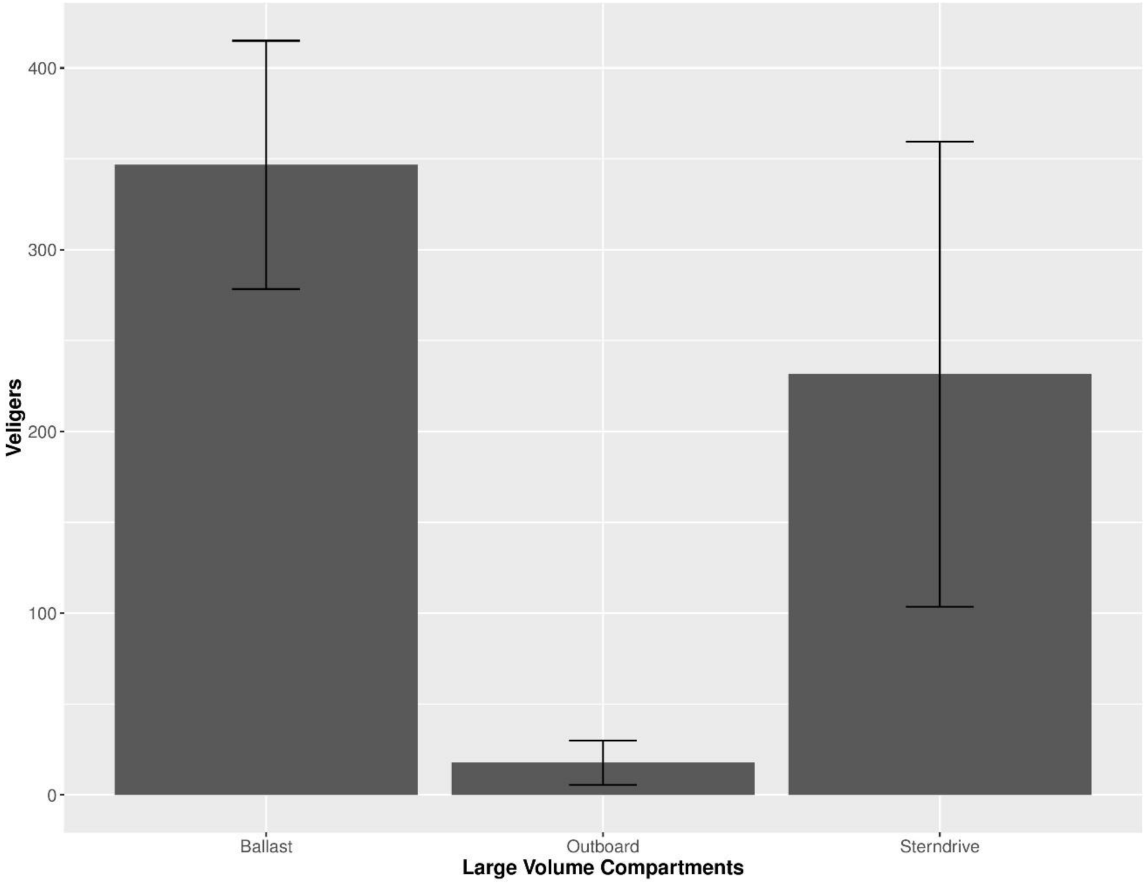


Figure 9: Watercraft inspected by DNR watercraft inspectors leaving Gull and Minnetonka during the project time-period (June – August 2016 and 2017).

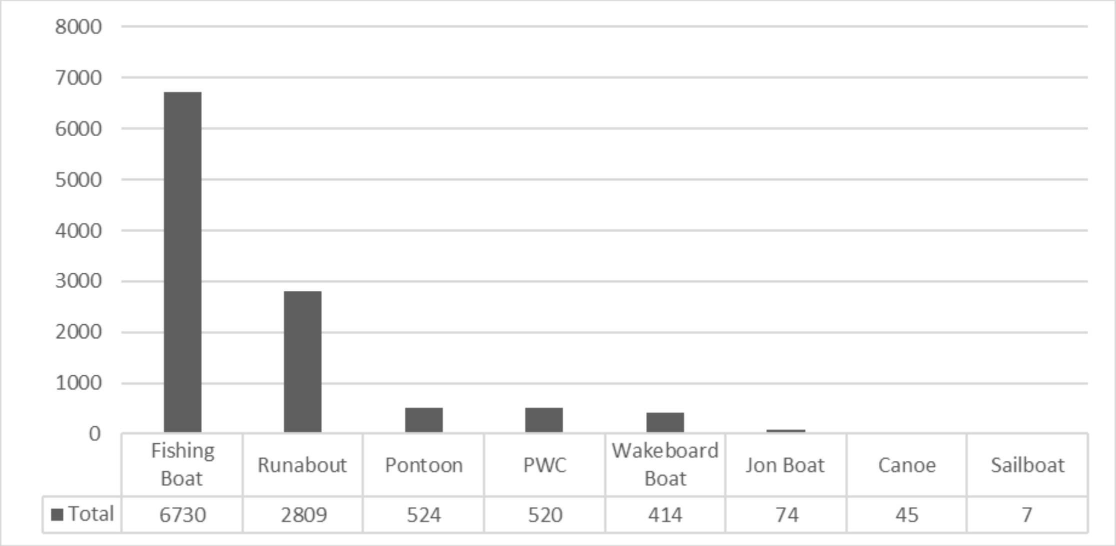


Figure 10: Air temperatures recorded inside a live well and a bilge compartment on a trailered watercraft over a 24-hour period on August 20, 2017. The red line shows the ambient air temperature.

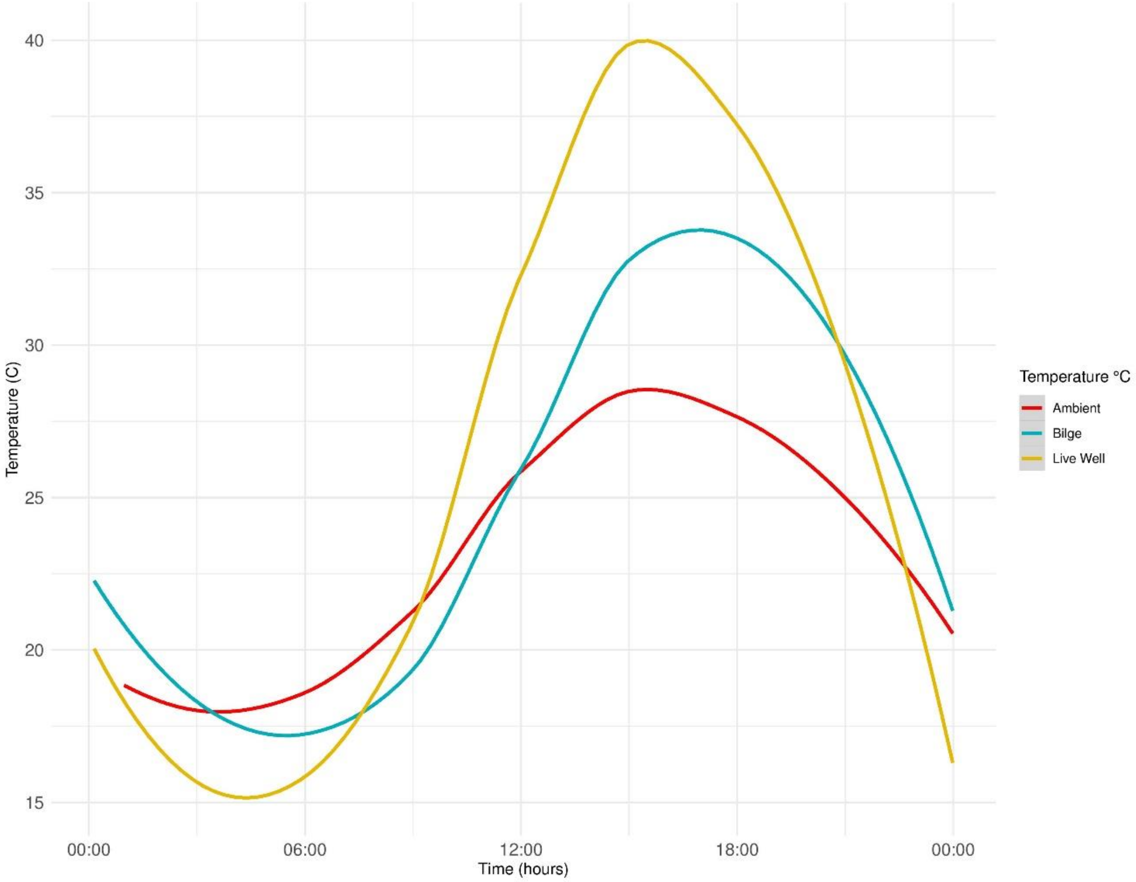


Figure 11: Proportion of surviving zebra mussel veligers in residual live well water over time at various summer temperatures. Solid lines indicate modeled mean survival over time. Shaded regions represent 95% confidence interval of the means.

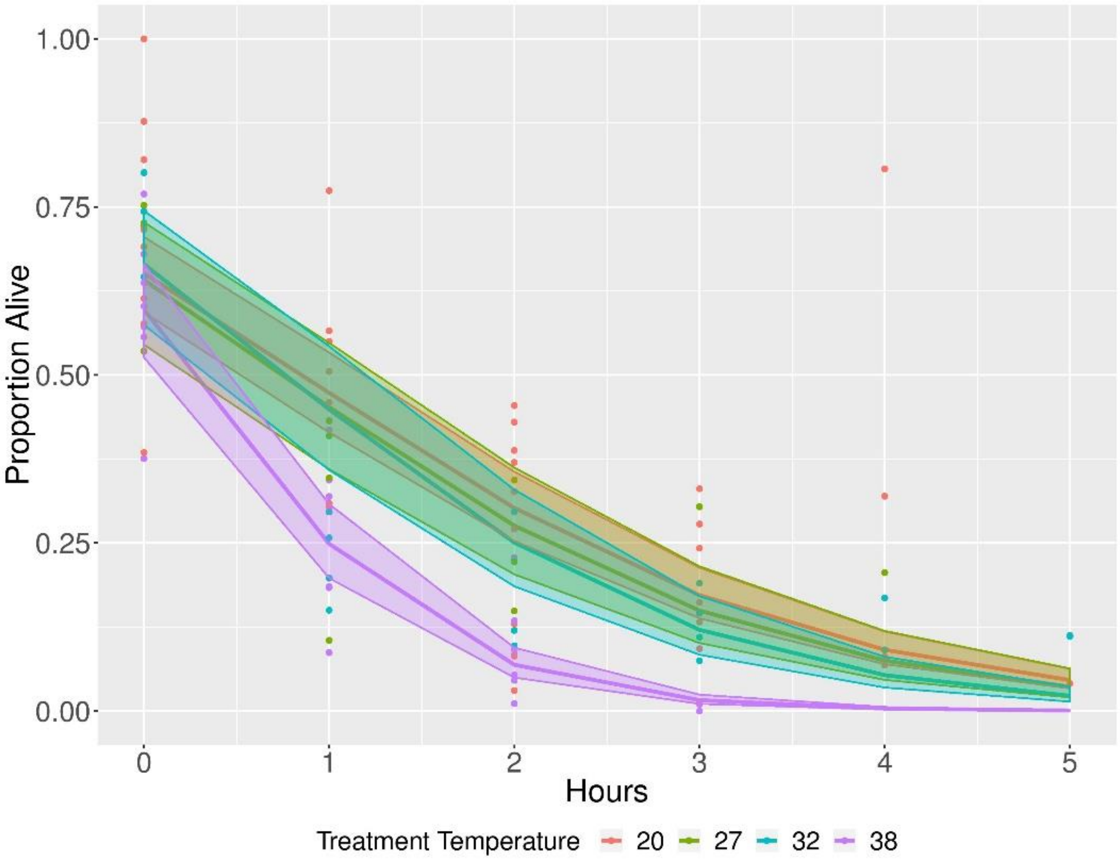
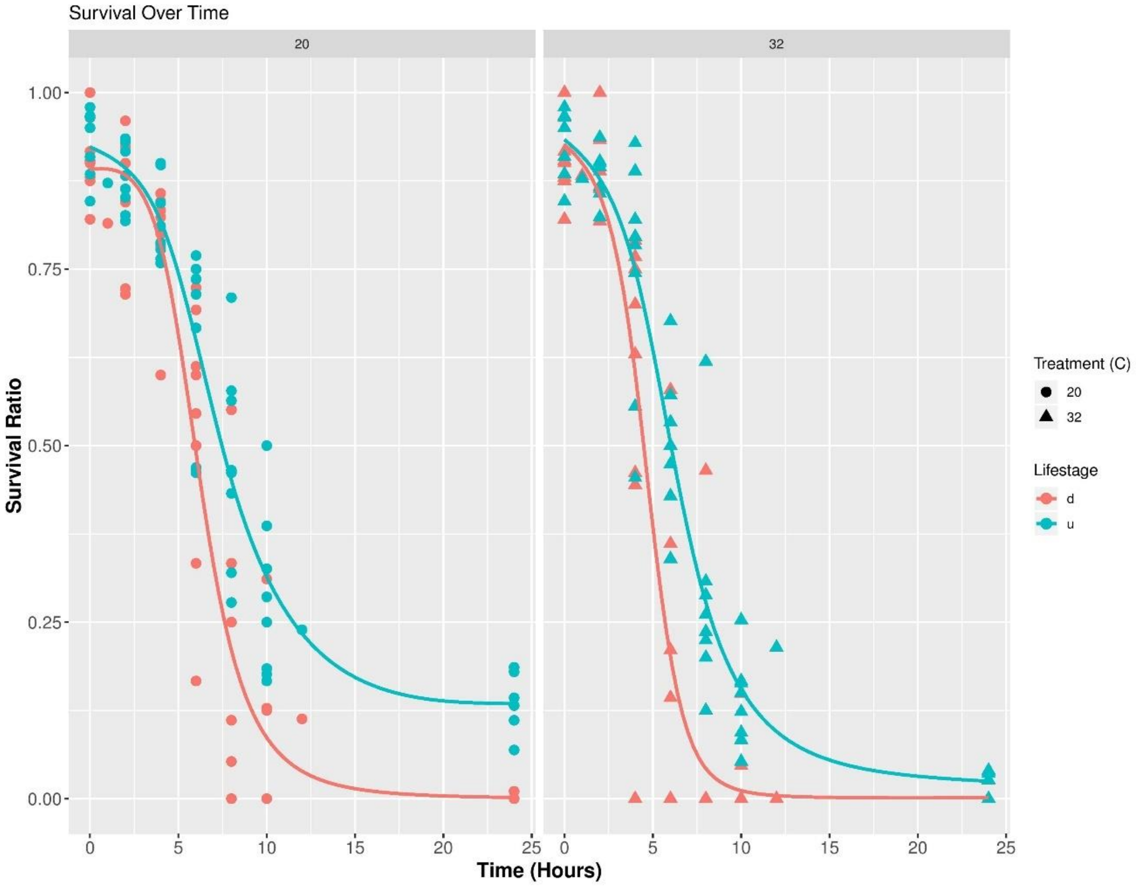


Figure 12: Proportion of surviving veligers alive in ballast tank residual water samples exposed to 20C and 32C air temperature for 24 hours. Umbonal veligers are represented in blue and D-stage veligers represented in red. Curved lines fitted by a generalized linear model with binomial distributed error.



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