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Dreissena veligers in western Lake Superior - inference from new low-density detection

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Abstract

The notion that Lake Superior proper is inhospitable to dreissenid mussel survival has been challenged by recent finds on shipwrecks and rocky reefs in the Apostle Islands region. Motivated by concerns surrounding these finds, we conducted an intensive sampling campaign of Apostle Islands waters in 2017 to understand *Dreissena* prevalence and distribution. The 100-site effort combined random and targeted sites and collected zooplankton, benthos, video, environmental DNA, and supporting water quality data. We did not find settled *Dreissena* in any video footage or benthos samples, and quantitative PCR applied to eDNA samples was negative for *Dreissena*. *Dreissena* veligers were found in almost half the zooplankton samples but at orders of magnitude lower densities than reported from other Laurentian Great Lakes. Veligers were most prevalent around the western islands and associated with shallower depths and slightly higher phosphorus and chlorophyll, but did not spatially match known (still very localized) settled *Dreissena* colonies. This is the first study to conduct veliger-targeted sampling in western Lake Superior and the first to report consistent detection of veligers there. We speculate that these Apostle Islands veligers are not a new locally-spawned component of the zooplankton community, but instead are transported from an established population in the St. Louis River estuary (~100 km away) by longshore currents; i.e., low-density propagule pressure that may have been present for years. Small-mesh zooplankton data collected along a gradient from the Apostle Islands to the St. Louis River estuary and enumerated with thorough veliger searching would help elucidate these alternatives.

Keywords

Dreissenid mussels; early detection; aquatic invertebrate survey; Apostle Islands National Lakeshore

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Introduction:

Invasive dreissenid mussels have established extensive populations on the lower Laurentian Great Lakes and in smaller water bodies across North America (NAS 2018). *Dreissena polymorpha* (zebra mussel) is thought to have become established in Lakes Erie and St. Clair as early as 1986 and by 1990 had spread across all four lower lakes, while *Dreissena bugensis* (quagga mussel) was first found in Lake Erie in 1989, and had spread across much of the lower lakes by 1992 (Benson 2013) where it has largely replaced the zebra mussel in deeper waters. Bottom-settled *Dreissena* currently play a dominant role in the benthic community of the lower Great Lakes (Burlakova et al. 2018), and Bowen et al. (2018) suggest that planktonic *Dreissena* veligers also seasonally dominate the small-bodied zooplankton community. The well-documented ecological and economic impacts of *Dreissena* in the Great Lakes include substantial changes to water clarity, food-web structure, and nutrient and energy cycling, and greatly increased infrastructure maintenance costs (e.g., Hecky et al. 2004; Higgins and Vander Zanden 2010; Karatayev et al. 2015; Nalepa and Schloesser 2014; Pimental et al. 2005).

Lake Superior stands as an exception to the other Laurentian Great Lakes when it comes to *Dreissena*. Dreissenid mussels have been established in the St. Louis River estuary (also known as the Duluth-Superior harbor) at the far western end of Lake Superior since 1989 (Grigorovich et al 2008; Trebitz et al. 2010) but barring isolated reports representing a few individual mussels (GLANSIS 2018), had not colonized Lake Superior proper. An introduction vector is clearly present -- *Dreissena* are readily transported by boats (adults attached to hulls, veligers in live wells and ballast water) and Lake Superior is regularly transited by commercial and recreational boats from the lower Great Lakes and the St. Louis River estuary (De Ventura et al. 2016; Holeck et al. 2004) -- so researchers had thought the failure of *Dreissena* to establish was because Lake Superior proper was inhospitable to their survival and growth (too cold, too unproductive, insufficient calcium for shell formation; Grigorovich et al. 2003; Whittier et al. 2008). That notion, however, has been repudiated by recent (beginning 2015) finds of small numbers of *Dreissena* on shipwrecks and rocky substrates around the Apostle Islands National Lakeshore (GLANSIS 2018; Lafrancois et al. 2019), which suggests at least some Lake Superior habitats are vulnerable to *Dreissena* invasion.

Lake Superior is often used as a reference state for marking environmental change in the Great Lakes, because it remains much less impacted by aquatic invasive species and other stressors (Allan et al. 2013). The establishment of dreissenid mussels in Lake Superior presents a potential threat to that important status. Motivated by concerns surrounding these finds, we in 2017 conducted a spatially intensive survey of Apostle Islands coastal waters to describe *Dreissena* distribution, test whether presence was related to site-specific environmental conditions, and gather baseline invertebrate community data against which to compare potential future impacts from dreissenid mussels. The survey included sampling pelagic and benthic macroinvertebrates with a suite of gear types, collecting environmental DNA (eDNA) samples for genetic testing of *Dreissena* signal, and collecting supporting data on factors such as temperature, productivity, and calcium concentration that might limit *Dreissena* survival and growth. This paper reports the results of this survey and discusses

distribution patterns relative to environmental gradients, previously known *Dreissena* occurrences, and potential veliger distribution mechanisms.

Methods:

Survey description:

Our survey encompassed coastal waters within the Apostle Islands National Lakeshore unit of the U.S. National Park Service (NPS) system, along the north and west shore of Madeline Island (part of the island chain but outside the park boundary), and along the north and east shore of the Chequamegon Peninsula mainland. Within this area, referred to hereafter as APIS (Apostle Islands), we selected 50 sampling stations according to a depth-stratified random design and another 50 stations targeting likely *Dreissena* introduction points (boat docks and moorings) and potentially desirable settlement habitat (where river-plumes might elevate nutrient or calcium levels; Delvaux 2017, Lafrancois et al. 2018). The random-site design was generated with the SpSurvey package in R (Kincaid and Olsen 2017) and placed 25 points each in the 5-30 m and 30-100 m depth strata within a 1 km coastal buffer around NPS islands (Fig. 1). The targeted sites included all NPS docks, areas identified as popular mooring sites by NPS staff, known settled *Dreissena* colonies (except on shipwrecks, which we avoided), a spatially distributed subset of private docks and marinas along the mainland and Madeline Island (from O'Malia et al. 2018), and at the 5m and 30m depth contours along lines extending from the pour-points of the four largest rivers in the study area (Fig. 1).

The primary sampling effort took place from 10-15 September 2017, with benthic colonization samplers deployed and additional water chemistry measurements taken approximately 8 weeks earlier (26-28 July). We completed sampling at 99 sites, with one targeted site (a NPS dock) dropped because of proximity to an adjacent mooring site. Although some of the colonization samplers went missing and some PONAR and video samples could not be collected, every station received a water chemistry sample and an eDNA sample and at least one kind of benthos sample, and all but one station received a zooplankton sample. The types and numbers of samples obtained are summarized in Table 1.

Zooplankton:

Planktonic *Dreissena* veligers and other zooplankton were collected with a flow-meter equipped 64-micron 0.5-m diameter zooplankton net towed either vertically to the surface from 2 m above the bottom (i.e., full water-column tows), or obliquely for 1 minute if the station depth was <5 m (applies to only 4 stations). Organisms from the zooplankton tows were preserved in 95% ethanol and enumerated for crustaceans according to the Great Lakes National Program Office method LG403 (GLNPO 2016), which consists of full counts from two 200-400 organism sample fractions and searches of two additional larger sample fractions for crustaceans from less-abundant species. Importantly, we also had *Dreissena* veligers counted in all the sample splits examined for crustaceans rather than per the rotifer enumeration part of method LG403 which calls for only 1 mL aliquots from the two smallest splits. We made this protocol change because we suspected that any veligers present would be at low abundances, easily missed if only small aliquots were examined for them.

Typically, the veliger search began at 40x magnification, increasing up to 100x as needed to confirm identification. Zooplankton densities were computed based on tow length and net mouth dimension (i.e., volume swept).

Benthic invertebrates:

A suite of benthic macroinvertebrate collecting gear was used at each site as depth and proximity to boat traffic lanes allowed. Benthic invertebrates were collected with PONAR grabs (0.052 m² area) at soft-bottom sites, with Hester-Dendy multi-plate colonization samplers deployed 1m off the bottom at sites <30m deep, and with rock-bag colonization samplers (mesh bags filled with ~10 fist-sized smooth rocks) bottom-deployed at APIS docks. Upon retrieval, Hester-Dendy samplers and rock bags were gently pulled into a 5-gallon submerged bucket (to avoid organisms washing off upon breaking the surface) where they were disassembled and scraped clean, and the lines and cinder blocks used to anchor them were visually inspected for *Dreissena*. PONAR samples were elutriated through a 500-micron mesh sock. All invertebrate samples were rinsed through 64 micron sieves and preserved in 95% ethanol in the field, with ethanol later refreshed in the lab to ensure adequate preservation. Organisms collected in benthic invertebrate samples were enumerated according to protocols described in Trebitz et al. (2010), which consist of complete counts and best-possible taxonomic resolution for all taxa except oligochaetes and chironomids where a subsample is slide-mounted for best-possible resolution identification.

Underwater video:

Underwater video footage was collected with SeaViewer or GoPro cameras (depending on vessel) mounted in pairs (one pointing vertically downward, another angled obliquely downward) along with a light source in a steel frame that was lowered and left sitting on the bottom for at least 1 minute. Video footage was inspected for evidence of mollusks and other fauna, and classified for dominant substrate type and presence of other structure (e.g., aquatic vegetation, woody debris). Any videos that included objects that might be mussels or shells were flagged and reviewed by two additional analysts to confirm their identities.

Environmental DNA:

A 500 mL eDNA water sample was taken from 2 m above the lake bottom with a Niskin sampler at every site, and sites >30m deep received an additional eDNA sample collected from 1 m below the surface with a sterile bottle attached to a clean pole. To prevent contamination, eDNA samples were collected prior to any other samples and handled only with gloved hands. We obtained one eDNA negative field control sample per day, by taking along a 1-L bottle pre-filled with sterilized deionized water and opening and re-capping it at a randomly selected APIS site. We also (later) collected eDNA water samples from the St. Louis River estuary where *Dreissena* mussels are abundant to serve as positive controls. All eDNA samples (including positive and negative controls) were preserved in the field by adding 150 mL of Longmire's buffer solution (Longmire et al. 1997; Williams et al. 2016), then transported and stored at ambient temperature and away from UV light.

Water samples and negative and positive controls were centrifuged within 28 days of collection to prevent DNA degradation (Williams et al. 2016). Before processing, the outside

of each sample bottle was rinsed with sterilized deionized water and wiped dry. Then, each sample was centrifuged to concentrate the DNA (Caldwell et al. 2007) via a three-step process: 1) Dividing into twelve 50 mL sterile tubes (1 full centrifuge tray), centrifuging at 9000 RFC (Relative Centrifugal Force) for 15 min, and partially decanting the supernatant; 2) Combining the pellet and remaining liquid from the twelve tubes into two to three 15 mL sterile tubes that were centrifuged at 9000 RFC for 15 min; and 3) swabbing all three tubes with a single sterile swab that was preserved in 700 μ L Longmire's buffer and stored in a 2 mL sterile microcentrifuge tube until DNA could be extracted. Between samples, centrifuge inserts were soaked in a 50% bleach solution and thoroughly rinsed with sterilized deionized water. Laboratory blanks were not included on the 50 mL tube runs (where each tray only contained tubes from a single sample), but one lab blank per tray of 15 mL tubes was included as a check on cross-contamination (because these trays spanned tubes from multiple samples).

Centrifuged samples and controls were sent from the EPA Duluth laboratory to the EPA Cincinnati laboratory for DNA extraction and quantitative polymerase chain reaction (qPCR) analysis. This analysis used the probe developed by Gingera et al. (2017), which is specific for the *Dreissena* genus but does not differentiate between the species *D. polymorpha* and *D. bugensis*. DNA was extracted from samples and controls using a chloroform - isoamyl alcohol protocol (Renshaw et al. 2015). Eight qPCR replicates were then run for each eDNA extract in 20 μ L reactions consisting of 3 μ L of extracted DNA, 10 μ L of TaqmanTM Environmental Master Mix 2.0 (from Thermo Fisher Scientific), 1 μ L of each PCR primer (forward primer Dre16SrRNAF TGGGGCAGTAAGAAGAAAAAATAA, reverse primer Dre16SrRNAR CATCGAGGTGCGAAACCG, 10 μ M concentration each), 2 μ L of probe (Dre16SrRNAP VIC-CCGTAGGGATAACAGC -MGB, 2.5 μ M concentration), and 3 μ L of sterile water. Replicates were thermal cycled on a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) using an initial cycle of 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds alternating with 60°C for 1 minute, during which the reaction was monitored for fluorescence indicating the probe finding and binding to *Dreissena* DNA. Additionally, inhibition tests were performed following the manufacturer's protocol (Applied Biosystems®TaqMan® Exogenous IPC) on all controls and samples. Water samples can contain various materials that might inhibit qPCR and thereby prevent DNA detection, but all our tests were negative for inhibition. We also tested qPCR sensitivity using serial dilutions of DNA extracted from *Dreissena* tissue collected at the St. Louis River estuary, starting with 10 ng DNA/ μ L and diluting by factors of 10 down to a 1:1,000,000 dilution.

Other environmental data:

Supporting environmental data collected included station coordinates and depth, substrate type, and water chemistry. Substrate categories recorded (based on PONAR grabs and video imagery) were sand, silt, clay, cobble, and rock, which were simplified for this analysis as soft, hard, or mixed. Water quality data collected included temperature, pH, total phosphorus (TP), planktonic chlorophyll-a (CHL), and dissolved Calcium (Ca⁺⁺). Water samples for laboratory analyses were taken only in September but temperature and pH were also measured in July when the colonization samplers were deployed. Temperature and pH were

measured at 1 m below the surface and 2 m above the bottom with a Hydrolab sonde (discrete depths) or a SeaBird profiler (from which depth-specific data were later extracted). Water samples for laboratory analysis were obtained from 2 m above the bottom and later analyzed according to methods summarized in Bellinger et al. (2016). We chose to obtain near-bottom rather than surface-water samples to represent the layer in which *Dreissena* would settle, and collected a single “shared” water sample for some clustered sites to reduce field processing time (Table 1 and Fig. 1). Samples falling below the TP detection limit (1.22 µg/L) were set equal to this limit (CHL and Ca⁺⁺ measurements were all above detection limits), and results for shared water samples were applied to all relevant stations prior to statistical analysis.

Results:

No settled *Dreissena* found:

We did not find any settled dreissenid mussels in this survey. None of the 152 benthic invertebrate samples (PONAR dredges, Hester-Dendy colonization plates, or rock bag samplers) yielded any *Dreissena* during taxonomic enumeration. None of the cinder-block anchors or ropes and floats used to deploy the Hester-Dendy plates and rock bags had visible *Dreissena* upon retrieval. And none of the 92 sites where we obtained benthic videos had visible *Dreissena* in either the down-looking or oblique video footage. Silt and algae on the bottom might have obscured small individual *Dreissena* in the videos, but lighting and water clarity were good, and colonies would have been evident if present. Non-dreissenid mussels found in our benthos samples were from families *Unionidae* (river mussels) and *Sphaeriidae* (pea and fingernail clams). The oblique video at one soft-bottom site (south side of Oak Island; Fig. 1) showed bivalves of undeterminable identity, but their large size and deeply-buried position was not consistent with *Dreissena* and later diver inspection at this site found them to be *Unionidae* (T. Lafrancois, pers. comm.).

No *Dreissena* eDNA found:

We also did not find any *Dreissena* DNA in the APIS eDNA samples; i.e., all tests with the *Dreissena* probe were negative. Our positive eDNA control samples (from St. Louis River estuary water) did test positive for *Dreissena*, confirming the efficacy of the Gingera et al. (2017) probe. DNA extracted from actual *Dreissena* tissue for qPCR sensitivity testing was detected as low as 1:100,000 but no longer detected in the 1:1,000,000 dilution. The qPCR cycle threshold values were the same for the 1:100,000 tissue dilution and the eDNA positive control samples, which suggests the concentration of *Dreissena* DNA was near 1:100,000 in the St. Louis River eDNA positive controls.

Veligers found in low density:

We did, however, find *Dreissena* mussel veligers in the zooplankton samples albeit in very low numbers. Almost half (44%) of the zooplankton tows had veligers present. The number of veligers counted per sample ranged from 1 to 40, with 357 veligers counted in total. The maximum computed veliger density was 39.1 per m³, but only 6 of the 43 samples with veligers had densities >5 per m³ and half the samples with veligers had densities <1 per m³. Veligers observed in the plankton samples were all quite small (< 100 microns). It is not

possible to tell whether the veligers are *D. polymorpha* or *D. bugensis* with either morphology (Nichols and Black 1994) or the *Dreissena* DNA marker (Gingera et al. 2017).

Veliger finds exhibited distinct spatial patterns across the APIS area (Fig. 2). Veligers were found in almost all zooplankton tows taken west of the Raspberry river mouth (see Fig. 1 for location references) whereas veliger occurred much less frequently further east in APIS. Veliger densities in zooplankton tows were highest around Sand Island, and somewhat elevated at the northern edge of the study area (e.g., north of Bear Island) compared to locations in the center of the archipelago (Fig. 2). Veligers that we did find in eastern APIS zooplankton tows occurred along the northwest rather than the southeast shorelines of the diagonally oriented Madeline, Michigan, Stockton, and Outer Islands (Fig. 2).

We found several environmental covariates for veliger presence vs. absence in the zooplankton tows. Bottom temperatures were substantially warmer at stations with veligers present than absent ($p < 0.05$ in ANOVA, Fig. 3). Stations with veligers also had slightly higher bottom-water TP and CHL and lower Ca^{++} concentration than stations without veligers ($p < 0.05$ in ANOVA, Fig. 3), although the ranges in these analytes were small. These patterns are related to and may be driven by bathymetry, as stations with veligers in the zooplankton tows were substantially shallower than stations without veligers (inset to Fig. 2), and bottom temperature and CHL declined while Ca^{++} increased with station depth (plots not shown). Note the western APIS region, where veliger occurrence rates and densities were higher, lacks the deep between-island channels occurring on the eastern side (Fig. 2). Surface temperature did not differ between stations with veligers present versus absent in the tows, lending support to the bottom-water chemistry difference having to do with depth rather than simply with location.

Discussion:

To our knowledge, our 2017 survey is the first to have consistently found veligers in small-mesh zooplankton tows of Lake Superior proper. Veligers have occasionally been reported from Lake Superior since 2002 (GLANSIS 2018) but previous finds are very sporadic. The twice-annual GLNPO open-lake survey has had only 16 veliger occurrences - widely-scattered in time and space - across the almost 600 samples taken over the years 2001 to 2016 (U.S. EPA unpublished data per James Watkins pers. comm., station map in Barbiero et al. 2018), which constitute $< 2\%$ of samples. The 2016 Lake Superior Coordinated Science and Monitoring Initiative (CSMI) covering 52 stations found veligers at only one site near the St. Louis River estuary and one in Nipigon Bay but none around the APIS, and no veligers were found at all in the 2006 and 2011 CSMI surveys covering ~ 75 stations (U.S. EPA unpublished data per Matt Pawlowski pers. comm.; station map in Pratt et al. 2016). None of these prior surveys come close to the 44% occurrence frequency for veligers we documented in our APIS survey.

Nevertheless, the veliger densities we found in the 64-micron zooplankton nets (< 1 to 39 ind/m^3) are multiple orders of magnitude lower than what is typically reported from the lower Great Lakes. For example, Fraleigh et al. (1992) report veliger density peaks in nearshore Lake Erie of 100,000 to 300,000 ind/m^3 , and Bowen et al. (2018) report densities

averaging ~14,000 ind/m³ in Lake Erie and 16,500 ind/m³ nearshore and 13,600 ind/m³ offshore in Lake Ontario across the months June through October. For Lake Michigan, Nalepa et al. (2010) report *Dreissena* veliger peaks of 15,000 to 30,000 ind/m³ in open waters and Thomas et al. (2017) report average densities of almost 100,000 ind/m³ across river-mouth, harbor, and near-coastal stations. Our densities are also orders of magnitude below the one published veliger dataset for Lake Superior's St. Louis River estuary, where average densities reached 30,000 to 40,000 ind/m³ in mid-July in a 100-micron net survey (TenEyck 2014). The very low veliger density at APIS also explains why we got only negative results for eDNA samples tested with the Gingera et al. (2017) *Dreissena* marker. Comparisons between our tissue extract-based sensitivity tests and St. Louis River estuary positive controls suggest we were at the edge of our detection capabilities for the eDNA sample volume (500 mL) even in a system with much higher veliger densities than APIS.

The low veliger densities we found are not for lack of searching, as our collection methods were appropriate, and samples were deeply searched for veligers. We used 64-micron mesh to retain small veligers (Bowen et al. 2018; Thomas et al. 2017), conducted full-water column zooplankton tows rather than restricting the tows to the upper 20 m as GLNPO's routine small-mesh zooplankton surveys do (Barbiero and Warren 2011), and enumerated veligers across substantial sample fractions rather than only in small aliquots. All samples containing veligers had counts based on at least 1/8th of the sample volume inspected and all four splits were always examined before concluding that veligers were absent. We could easily have missed these low-density veligers entirely had we looked for them only in 1-mL aliquots of the sample splits, as the GLNPO method for small-mesh zooplankton enumeration calls for. Because previous Lake Superior plankton surveys did use aliquot-based enumeration, we do not know whether our much higher veliger occurrence rate is only due to deeper sample searching or also due to recently increased veliger abundance.

Early September is an appropriate sampling window for veligers based on Lake Superior temperatures. Spawning is initiated in Great Lakes waters by *D. bugensis* at temperatures as low as 4 to 5°C (Glyshaw et al. 2015, Roe and MacIsaac 1997) and by *D. polymorpha* at temperatures of 12 to 15°C (Fraleigh et al. 1992; Sprung 1992) and for both species continues across summer and into autumn (Bowen et al. 2018; Nalepa et al. 2010). Surface temperatures across our APIS sites averaged 17.9°C in July and 17.3°C in September, and bottom temperatures exceeded 15°C at all but the deepest stations by September, so temperatures were suitable for finding veligers of either *Dreissena* species.

Beyond temperature, calcium is the factor most likely to be limiting *Dreissena* survival, growth, and settlement in APIS waters. Ca⁺⁺ concentrations in APIS bottom-water samples ranged from 11 to 14 mg/L, on average exceeding the 12.0 mg/L threshold for very low *Dreissena* invasion risk proposed by Whittier et al. (2008) but well below their 20 mg/L threshold separating low from moderate risk. Laboratory trials by Baldwin et al. (2012) suggest that *Dreissena* limitation in waters exceeding about 13 mg/L Ca⁺⁺ is due to inhibited spawning and veliger survival rather than viability of already-settled mussels of either species. We did not find any Ca⁺⁺ above 15 mg/L, a number used by Lafrancois et al. (2018) as a lower threshold for moderate potential to support *Dreissena*. The pattern of slightly lower (rather than higher) Ca⁺⁺ where veligers are present (Fig. 3) is contrary to the

expected role of Ca^{++} for *Dreissena*, but the median difference is very small and likely not biologically significant. All our APIS-water pH measurements exceeded the 7.5 lower threshold for *D. polymorpha* infestation suggested by Mackie and Schloesser (1996), ruling out pH as limiting. Low productivity has also been suggested as limiting *Dreissena* - APIS waters are oligotrophic based on TP and CHL using NALMS (1990) thresholds - but the continued dominance of *Dreissena* in Lakes Huron and Michigan despite their recent “oligotrophication” (Barbiero et al. 2012; Dove and Chapra 2015) belies this explanation, and the median TP and CHL difference between sites with and without veligers in zooplankton tows (Fig. 3) seems biologically trivial.

Low numbers of adults capable of reproducing might also be limiting veliger numbers. Settled zebra and quagga mussels now occur and persist within APIS waters but remain very localized and low-density (Lafrancois et al. 2019), and our own benthos sampling effort yielded no new finds. It could be argued that PONAR samplers, whose effectiveness is limited to soft substrates, would miss *Dreissena* that preferentially occupy hard substrates (where diver surveys found them; Lafrancois et al. 2019), but our Hester-Dendy and rock-bag samplers also yielded no *Dreissena* at APIS (despite proving attractive to them in other locations; Trebitz et al. 2010), and none of the videos over hard or mixed hardness substrates (N=36) had *Dreissena* either. Furthermore, we found no *Dreissena* settling on the ropes, buoys, and cinderblock anchors used to deploy our samplers, even though multiple previous studies show *Dreissena* settlement on such materials (e.g., Kobak 2004; Muetig et al. 2010). And we found no *Dreissena* eDNA despite application of a qPCR probe that has proven effective elsewhere (Gingera et al. 2017). Settled *Dreissena* densities (and their released DNA) may simply still be too low in APIS for reliable detection with gear that covers only small areas, and veligers may not be surviving long enough to settle. Supporting this notion, Lafrancois et al. (2019) reported APIS *Dreissena* catch-per-effort in dive surveys of only 23 per diver hour at the Sevona wreck and <10 per diver hour at other sites -- extremely low compared to *Dreissena* densities in the other Great Lakes - and noted a lack of evidence of mussel growth or veliger production. Also supporting this notion is the very small size of all the veligers we found (< 100 microns), suggesting that they may not be exhibiting growth.

We did not necessarily expect close correspondence between sites with planktonic veligers and the locations of known settled *Dreissena*. Still, we are struck by the marked differences in spatial patterns between the two. Both veligers and settled *Dreissena* occurred around Sand Island, but settled *Dreissena* have not been found in the northern fringe of APIS where we had multiple veliger finds, and conversely are concentrated in the area between Stockton, Madeline, and Michigan Island where veligers were mostly absent from zooplankton tows (compare Fig. 2 to Fig. 4). The predominant winds over Lake Superior blow from west to east, and summer surface currents typically curl from the northwest to the southeast of the Apostle Islands (Fig. 4 arrows; Beletsky et al. 1999). Wind and currents should thus transport locally-spawned veligers to the southeast side of APIS, yet this is not where most of our veliger finds occurred.

These summer current directions, together with the pattern of veliger presence and abundance (Fig. 2) and what we know of other populations in Lake Superior lead us to wonder whether the veligers come from *Dreissena* spawning in APIS waters, or instead are

transported there from the St. Louis River estuary about 100 km to the southwest. Hydrodynamic models for Lakes Erie and Michigan have shown currents to be capable of spreading planktonic life stages of other non-native aquatic species (Beletsky et al. 2017), and plume studies in Lake Superior have documented substantial eastward long-shore transport of suspended materials from the St. Louis River estuary towards APIS (Delvaux 2017; McKinney et al. 2018). Summer currents along this shore of up to 5 cm/s (Bennington et al. 2010) would suffice to transport materials over the ~100 km distance in about 3 weeks - within the typical time-span of the veliger stage yet long enough that initial spawn densities would have been greatly depleted by predation and natural mortality (Ackerman et al. 1994). These long-shore currents would reach Sand Island first and then travel along the northern APIS fringe, offering a viable alternative explanation for the pattern of veligers being most prevalent in western and northern APIS. The St. Louis River estuary holds by far the largest and most-established *Dreissena* population in Lake Superior, with documented June through September veliger production (TenEyck 2014), and water quality conditions adequate to support them. Compared to APIS, the St. Louis River estuary has approximately 5-fold higher growing-season TP and CHL-a (Bellinger et al. 2016) and Ca⁺⁺ mostly in the 15 to 25 mg/L range (Bellinger et al. unpublished). If the St. Louis River estuary is indeed a veliger source to APIS waters, this low-density (by the time they reach the islands) propagule pressure may have been present for two decades but not been noticed due to lack of targeted sampling. Knowing whether the veligers we found are of the species *D. bugensis* or *D. polymorpha* would help inform the likelihood of them being produced within APIS (where both species occur; Table 2) versus at the St. Louis River estuary (where *D. polymorpha* is heavily dominant; Trebitz et al. 2010) but neither morphological identification of veligers nor the DNA marker we used offers this information (Gingera et al. 2017; Nichols and Black 1994).

Summary and Conclusions:

Our study is the first to report consistent (rather than isolated) *Dreissena* veliger finds in Lake Superior proper. Nevertheless, the veliger densities we found are extremely low compared to densities reported from the other Great Lakes. We could easily have missed finding these veligers had we searched only a small zooplankton sample aliquot (as per typical veliger enumeration protocols) rather than a substantial fraction of the entire sample. Lake Superior appears not to be entirely inhospitable to *Dreissena*, but calcium and other water quality characteristics probably remain marginal for settled-mussel growth and veliger production and survival. Given the very low veliger densities, the still very small population of settled *Dreissena* in the APIS region (we found no additional *Dreissena* in benthic samples or videos or eDNA despite a large sample size), and the lack of spatial match between our veliger finds and known *Dreissena* colonies, we lack strong inference that veligers were spawned locally. Current-driven transport of veligers produced by the established *Dreissena* population in the St. Louis River estuary is a plausible alternate explanation for the observed APIS veliger distribution, and would constitute a low-density propagule pressure that may have been present for up to two decades. Small-mesh zooplankton data collected along a gradient from APIS to the St. Louis River estuary and enumerated with thorough veliger searching protocols would help elucidate the likelihood of

this explanation, as would development of genetic markers capable of discerning among *Dreissena* species and source populations.

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Figure 1:

Map showing our 2017 sampling sites (with upper left inset giving general location). Blue-filled circles indicate the 50 random points (light vs dark blue for shallow vs deep strata) while other symbols indicate the 50 targeted points (legend indicates target type). Known *Dreissena* colonies (stars) are only those known prior to 2017 and not on shipwrecks or navigation buoys (i.e., those we could aim to sample). Islands in green are part of the Apostle Islands National Lakeshore and undeveloped except for docks, campsites, and trails, whereas Madeline Island (in pink) and the mainland have roads, houses, and private docks along the shore. Pourpoints for the four major tributaries are labeled. Ovals around point groups show locations that share a laboratory water quality sample.

Figure 2.

Map showing the spatial distribution of *Dreissena veligers* in zooplankton tows, and inset box plot showing depth distribution of stations where veligers were absent vs. present. Blue crosses indicate tows with veligers absent, whereas red circles indicate tows with veligers present with circle size proportional to veliger density (smallest density $<1 \text{ ind/m}^3$, largest density $\sim 40 \text{ ind/m}^3$). Water shading corresponds to lake bathymetry (darkest colors = greatest depths).

Figure 3.

Notched box plots comparing the distribution of bottom temperature, total phosphorus (TP; truncated at lower detection limit of 1.22 ug/L), calcium ion (Ca++), and planktonic chlorophyll-a (CHL) between stations where veligers were absent (N=55) vs. present (N=43) in the zooplankton tows. Differences between the medians (printed on graph) of the two groups are statistically significant for all four water chemistry measures ($p < 0.05$ in one-way ANOVA), but biologically small for all but temperature.

Figure 4.

Map showing reported locations of settled *Dreissena* in western Lake Superior (symbol indicates species and abundance), the established *Dreissena* population in the St. Louis River estuary (polygon in lower left), and arrows summarizing the dominant summer current circulation pattern (redrawn from Beletsky et al. 1999). Specific locations plotted here are compiled into more generalized entries in Table 2. The St. Louis River estuary has both *Dreissena* species but is colored red because the vast majority are *D. polymorpha*.

Table 1:

Summary of sample types and numbers collected in September 2017 (we also took meter chemistry at 68 sites in July). The final column also reports (in parentheses) the samples we failed to collect: lost = gear deployed in July not found in September, rocky or too wavy = factors preventing sample collection, clusters = one laboratory chemistry sample standing in for several close-together stations.

Type of sample	Target stations	Analysis intent	Sample size (missing sites)
<i>Macroinvertebrates</i>			
PONAR grab	All	Benthos community	89 (11 rocky)
Hester-Dendy (HD), 1m above bottom	<30m deep	Benthos community	52 (16 lost)
Rock bag, on bottom	APIS docks	Benthos community	11 (4 lost)
Zooplankton tow, 63µ mesh eDNA water, bottom and surface	All Bottom all, surface if >30m	Zooplankton community <i>Dreissena</i> PCR marker	98 (1 missed) 99 bottom, 33 surface
<i>Environmental data</i>			
Bottom video, down-looking and oblique	All	Substrate, visible mussels	92 (7 too wavy)
Meter chemistry, surface and bottom	Surface all, bottom if >5m	Temperature, pH	99 surface, 85 bottom
Lab chemistry, bottom water	All	Nutrients, chlorophyll, calcium	85 (13 clusters, 1 missed)

Table 2:

Occurrences of settled *Dreissena* (not veligers) within Lake Superior waters through 2017, as reported by studies besides ours. Entries are sorted by species, then year, and locations are not repeated after the first year where the number reported exceeds “single” (blank = number not specified). Finds within APIS waters are no earlier than 2011 and mostly since 2015.

General Location	Year	# reported	How found	Information source
<i>D. polymorpha</i> (zebra mussel)				
St. Louis River estuary, MN	1989	multiple		GLANSIS 2018
North of Keweenaw peninsula, MI	1989			GLANSIS 2018
West of Brule Point, WI	1989			GLANSIS 2018
Thunder Bay, ON	1990	single		GLANSIS 2018
Marquette, MI	1992	single		GLANSIS 2018
Two Harbors, MN	1993	12		GLANSIS 2018
Thunder Bay, ON	1998	100s		GLANSIS 2018
Ashland, WI	1998	single		GLANSIS 2018
Off Whitefish Point, MI	1999	<5		GLANSIS 2018
Mouth of Ontonagon River, MI	2002	single	on stump	GLANSIS 2018
Upper Marquette Harbor, MI	2004	single		GLANSIS 2018
Isle Royale, MI: Mott & Washington harbor	2009	one each		GLANSIS 2018
APIS: Sevona shipwreck	2015	multiple	diver	B. Lafrancois pers. comm.
APIS: Around Sand island	2016	multiple	diver	B. Lafrancois pers. comm.
APIS: Bays south side of Stockton island	2017	multiple	dive survey	Lafrancois et al. 2019
APIS: Reef east side of Madeline island	2017	<5	dive survey	Lafrancois et al. 2019
APIS: Red Cliff Bay & Ottawa wreck	2017	<20	dive survey	Lafrancois et al. 2019
<i>D. bugensis</i> (quagga mussel)				
St. Louis River estuary, MN	2005	5	PONAR	Grigorovich et al. 2008
APIS: Reef east side of Madeline island	2011	12	fish nets	S. Sapper, pers. comm.
APIS: West of Michigan island	2016	single	benthic sled	E. Hinchey pers. comm.
APIS: West of Michigan island	2016	single	PONAR	J. Hoffman, unpublished

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