Running head: Decline of particulates along mussel bed

Suspended particulates decline along a dense, small-stream mussel bed

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Abstract:

Water filtration by freshwater mussels is a valued ecosystem service; however, it has not been well studied in natural settings. To examine the potential influence of mussel filtration on suspended particulates, we measured the concentration of *Escherichia coli*, chlorophyll-a, and total suspended solids along a stream reach with a dense mussel assemblage (Mussel Site) and a stream reach with no mussels (Reference Site). We predicted that these particulates would show greater declines along the Mussel Site than the Reference Site because of mussel filtration. We collected three replicate water samples at upstream, midpoint, and downstream stations at both sites in August, September, and October 2022 to measure concentration values. In accordance with our predictions, concentrations of particulates declined from upstream to downstream at the Mussel Site but not at the Reference Site. We used linear mixed-effect modeling to determine that the interaction between mussel presence (Mussel Site, Reference Site) and sample location (upstream, midstream, downstream) best explained these patterns. There was lower support for the total suspended solids interactive model (AIC weight = 0.45) compared to the other two particulates (AIC weight > 0.95). Selective feeding by mussels may help to explain the lower support of the total suspended solids model. Our results suggest that mussels can appreciably reduce suspended particulate concentrations including harmful bacteria. This study provides a useful example of the ecosystem services mussels provide and why their conservation is needed.

Keywords: water filtration, freshwater mussels, *E. coli*, total suspended solids, chlorophyll-a

Introduction:

The water quality of freshwater ecosystems is highly valued by the public and may be naturally maintained by freshwater mussels (Castro et al. 2016). Freshwater mussels (Order Unionida) often dominate benthic biomass in streams and rivers (Negus 1966; Vaughn et al. 2008; Haag 2012) and are omnivorous suspension feeders, consuming varieties of phytoplankton, detritus, and bacteria from the water column (Haag 2012). Suspended materials that are captured but not digested (e.g., contaminants, heavy metals, harmful bacteria) may be retained in soft tissues and shells or egested as pseudofeces (de Solla et al. 2016; Binkowski et al. 2019; Demircan et al. 2022). Retention of contaminants by mussels removes them from an ecosystem for a mussel's lifetime (Vaughn 2018) and bacteria may be inactivated by the process of mussel filtering (Ismail et al. 2016). By conserving and restoring healthy freshwater mussel assemblages, along with other threat management strategies, we may improve water quality and protect the ecosystem services provided by freshwater mussels. To inform the efficacy of mussel conservation for water quality mitigation, we need to continue to study the ability of freshwater mussels to remove particulates of concern.

While mussel filtration is generally well studied, many studies have focused on the ability of individual mussel species to clear specific particulates in lab settings (Goldsmith et al. 2021; Luck and Ackerman 2022; Brower et al. 2023). Controlled settings are often unrepresentative of natural settings because mussel filtration is naturally modulated by the flow rate and volume of a system as well as the presence of other mussel species (Spooner and Vaughn 2006, 2008; DuBose et al. 2024). It is possible that these forcing factors explain why filtration rates for mussels can vary by orders of magnitude between laboratory and field studies (Riisgård 2001; Vanden Byllaardt & Ackerman 2014). Building a convincing body of evidence that emphasizes

43 the link between water quality and mussel filtration in natural settings (e.g., Welker and Walz
44 1998; Ismail et al. 2016) may provide more evidence of mussels' importance to the greater
45 public and drive support for their conservation.

46 We examined the ability of mussels to filter *Escherichia coli* (*E. coli*), chlorophyll-a (chl-
47 a), and total suspended solids (TSS) in a stream reach in Minnesota. We chose these particulates
48 because bacteria (*E. coli*), phytoplankton (chl-a), and suspended solids (TSS) are frequently used
49 measures of water quality (e.g., Ondokor and Ampofo 2013; Alford and Caporuscio 2020; He et
50 al. 2022) and are all filtered by mussels. The removal of *E. coli* by freshwater mussel species has
51 been demonstrated in multiple laboratory studies (e.g., Silverman et al. 1997; Campos et al.
52 2022; Shah et al. 2022), yet field studies are rare (but see Ismail et al. 2016; Saavedra et al.
53 2022). Chl-a is a proxy for measuring algae biomass and algae cell densities (He et al. 2022), or
54 food for mussels, and has been used to study juvenile mussel clearance rates, phytoplankton
55 reduction by mussels in streams and lakes, and changes in sediment due to mussel bioturbation
56 (Cahoon and Owen 1996; Welker and Walz 1998; Spooner and Vaughn 2006; Fung and
57 Ackerman 2020). Finally, TSS are non-dissolved particles greater than four microns in size and
58 is a proxy of sediment pollution (Tuttle-Raycraft and Ackerman 2018). High concentrations of
59 TSS (> 8mg/L) are considered harmful because they inhibit photosynthesis, impede visibility for
60 predators, and prevent mussels from feeding efficiently (Madsen et al. 2001; Cranford et al.
61 2011; Gascho Landis et al. 2013; Barkalow & Bonar, 2015; Tuttle-Raycraft et al. 2017).
62 Freshwater mussels filter TSS relative to its quality and size, potentially ameliorating these
63 effects (Tuttle-Raycraft and Ackerman 2018).

64 In this study, we examined if water flowing over a dense mussel bed would show greater
65 reductions in *E. coli*, chl-a, and TSS along its length compared to a site with no mussels. We

hypothesized these three particulates, *E. coli*, chl-a, and TSS would show greater decreases at the site with a mussel bed and would not change at a reference site with more than could be expected with natural settling. Furthermore, we hypothesized that the interaction between site (mussels/no mussels) and stream station (upstream-downstream) would be the best predictor of these patterns in particle concentration.

Methods:

Study Sites

This study occurred at the Sunrise River in east-central Minnesota (Figure 1). The Sunrise River spans 6,475 km^2 and drains into the St. Croix River, a National Wild and Scenic River that is of great recreational value to Minnesota's constituents (MPCA 2022). While the St. Croix watershed is generally considered to have high quality waterways, there have been concerns regarding sedimentation and phosphorous loading particularly from the Sunrise River, a 6th order tributary of the Saint Croix River (Chisago County 2013). Additionally, the North Branch of the Sunrise River is listed as impaired for *Escherichia coli (E. coli)* (Donatell et al. 2014) while the mainstem of the Sunrise River downstream of Kost Dam nears the threshold for bacterial impairment (MPCA 2022). Mussel density in the Sunrise River immediately downstream of Kost Dam is exceptionally high (86.4/m^2), whereas surveys in the North Branch of the Sunrise River have detected few mussels (Hornbach et al. 2014). These reaches provided a unique opportunity for us to examine the influence of a mussel bed to improve water quality.

We established two sampling sites within the Sunrise watershed (Figure 1). Prior to beginning the study, we determined that the dense mussel bed in the Sunrise River stretched about 715 m downstream of the dam (+ 10 m immediately downstream of the dam that was unsurveyable) and this was established as the mussel site (MS). For a reference site (RS) with

89 low mussel abundance, we chose a 715 m reach of the North Branch Sunrise River because of its
90 proximity in the same watershed and anecdotal evidence that mussels were absent which was
91 confirmed by reconnaissance.
92 Both study sites had intact forested corridor and no obvious evidence of streambank
93 erosion. Furthermore, both watersheds for the MS and the RS had similar proportions of forested
94 (21-23%) and developed urban area (~ 0.1%) (https://streamstats.usgs.gov; accessed November
95 18, 2023). Biological monitoring stations near our study sites had fish and invertebrate IBI scores
96 higher than the impairment threshold (MS, Minnesota Pollution Control Agency (MPCA) Station
97 ID 96SC065; RS, MPCA Station ID 09SC004). Finally, both study sites shared similar substrate
98 composition although water characteristics were somewhat different (Appendix 1). It is
99 important to note that watershed area upstream of the MS was larger (698 km^2) than the RS (202
100 km^2), cultivated crop was greater in the RS (56%) than the MS (36%) which may be an
101 additional source of contaminants like *E. coli* to the RS, and the MS had a small, low head dam,
102 the Kost Dam while the RS did not (https://streamstats.usgs.gov; accessed November 18, 2023,
103 Figure 1).
104 *Mussel Sampling*
105 Mussel density, species richness, and size regimes were estimated for both study sites
106 using a systematic grid design. Since mussels are patchily distributed, systematic grids are
107 advantageous over random sampling so that mussel beds are not randomly excluded from
108 sampling efforts (Smith 2006; Newton et al. 2011). We mapped both 715 m reaches in ArcGIS
109 and spaced target sampling points 9 m apart at the RS and 11 m apart at the MS to achieve
110 around 100 samples per site as a baseline. A power analysis of preliminary samples from the MS
111 indicated that 165 samples were needed to achieve a 1.5 mussels/m^2 standard error in the mean

mussel density; therefore, we overlaid an additional grid of 65 target sampling points at the MS. Sample efforts were not increased in the RS as there was no variation in the abundance of mussels found per quadrat.

We sampled for mussels on 27 August 2022 at the RS and from 15-22 September 2022 at the MS (n=107, n=165). We sampled mussels at least 10 days before collecting water samples to allow time for disturbed sediment to settle and for mussels to reburrow and resume normal filtering activity. A 0.25 m^2 quadrat was dropped and excavated to 15 cm of depth at each sample point. All substrate and mussels were placed in an attached bag with 6.35 mm mesh. The bag was vigorously shaken to remove fine sediment and the remaining substrate and mussel mixture was carefully searched. All live mussels were identified, measured (length mm), and returned to the sample point. Nomenclature followed Williams et al. (2017).

Suspended Particulate Sampling

We collected water samples at the Mussel Site and Reference Site on 3 August, 8 September, and 3 October 2022 during low flow conditions (< 2.3 m^3/sec) following Minnesota Pollution Control Agency standard operating procedures and chain of custody guidelines (MPCA 2018). We collected samples at three permanent stations at both sites: upstream (0 m), midpoint (357 m), and downstream (715 m), starting at the downstream station and wading upstream. At each station, we took three replicate samples for *E. coli*, chl-a, and TSS from the left, middle, and right of the stream, for a total of 9 samples/site/month. Samples were collected in the middle of the water column while facing upstream. Samples for *E. coli* were collected by opening a submerged Whirl-Pak bag midway in the water column. Samples for chl-a and TSS were collected by submerging an inverted 2L Nalgene bottle midway in the water column and tilting

134 the bottle upright to fill it. Samples were placed in a cooler with ice and transported on the same
135 day for analysis to the Metropolitan Council Environmental Services Lab in St. Paul, Minnesota.
136 *E. coli* concentration was measured using the Most Probable Number (MPN) method
137 (Bari and Yeasmin, 2022). Chl-a concentration was measured using the ASTM D3731-87
138 method (ASTM International, 2020). TSS concentration was measured by pouring the sample
139 over a rinsed glass-fiber filter with a pore size of 1.5 μm and drying the filter overnight at 105°C
140 (Guy, 1969). TSS samples were not ashed, therefore, measurements include suspended organic
141 and sediment material.
142 To monitor potential confounding variables between the sites, we measured pH,
143 temperature, dissolved oxygen, and conductivity with a YSI probe (YSI ProQuatro) at each
144 sampling point (n=3 per station per visit/month). Water depth was measured at each point with a
145 depth stick. The flow of both rivers was monitored at a stream gauge downstream of the Sunrise
146 River-North Branch Sunrise River confluence (Minnesota Pollution Control Agency, gauge ID
147 37030001) to inform sampling at low discharge conditions of less than 2.3 m^3/s (Figure 1). We
148 also estimated discharge at each site river using the orange method, which is considered
149 acceptable for small streams with relatively uniform depth (Dobriyal et al. 2017). These data are
150 summarized in the Appendix 1. Additionally, we sampled for substrate during mussel
151 excavation. At each 0.25 m^2 quadrat, we visually estimated the composition of materials
152 excavated at each sample point including substrate (Wentworth Scale), detritus, submerged
153 vegetation, and empty shells (Appendix 1).
154
155 *Statistical Analysis*

Mussels.—Mussel density and species data were analyzed by calculating the mean density (±SE) and percent of each species present for the MS and RS in RStudio version 4.0.4. Population estimates were calculated by multiplying the average density by the estimated area of each reach. The density of mussels along the MS reach was mapped in ArcGIS Pro 3.2.0 using the Natural Jenk's method to create density classes and the Inverse Distance Weighted tool to interpolate density along the reach. We calculated and mapped the average size of mussels collected along the MS reach using the same methods.

Suspended Particulates.— Mean (± SE) of particulate concentrations were calculated for the upstream, midpoint, and downstream stations for both sites to show overall trends in particulates. Particulate data was analyzed by linear mixed-effect modeling using R 4.0.4 packages "bblme" and "lme4" (Bates et al. 2022, Bolker 2022). Linear mixed-effects models are extensions of regular linear models that can account for repeated measures by including "fixed" and "random" effects (Bolker et al. 2009). Because water samples were taken at the same points within stations every month, our study lacks spatial independence, making linear mixed-effect modeling an effective method to statistically analyze our data. We ran five models for the three particulates, ranging from simple to complex. The simplest model, or null model, compared the dependent variable (i.e., TSS) to the "random effect" of date. From here, two fixed effects were added (stream station, site) to determine which dependent variable, or combination there-of (additive/interactive) best predicted observed patterns of particulate concentrations among the sites. The best model for each dependent variable was selected using Akaike's Information Criterion (AIC). The difference between AIC values were calculated for each set of models, and

AIC values were weighed to choose models with the lowest Δ AIC (dAIC) value. Particulate concentrations were log transformed prior to analysis to increase linearity.

Results:

Sampling sites

There were some differences in the water quality measures at the two sites. Water temperature was lower at the RS (15.7 + 0.5°C) than the MS (20.5 + 0.8°C) during our sampling period (Appendix 1). The dissolved oxygen level was higher at the RS due to the lower water temperature. Water depth at the RS was shallower than the MS. Substrate at both sites was composed mainly of sand and gravel with the RS having a greater percentage of sand.

Mussel Assemblage

Mean mussel density at the MS was 43.9 /m^2 $\pm$ 3.5 SE with an estimated 829,146 mussels $\pm$ 66,552 SE within the assemblage. The distribution of mussels was patchy at the MS, and density was higher in the upstream half (56.2 mussels/m^2 $\pm$ 5.4 SE) than the downstream half (30.1 mussels/m^2 $\pm$ 3.8 SE, Figure 2A). Seventeen species were collected, with *Actinonaias ligamentina* making up 77.3% of the assemblage (Table 1). In contrast, we did not observe unionid mussels or shells at the RS during density sampling or other site visits.

Mussels were quite large on average, with the mean length of mussels being 82.15 mm $\pm$ 0.64 SE. Mean mussel size was greater in the upstream half (84.33 mm $\pm$ 2.38 SE) than the downstream half (73.81 mm $\pm$ 2.10 SE) of the reach (Figure 2B).

Water Particulate Sampling

We determined the interaction between site and stream station was the most parsimonious explanation of observed particulate concentrations from our linear mixed models. Mean values for *E. coli* and chl-a decreased from upstream to downstream in the MS (Figure 3), whereas TSS

decreased from the upstream to the midpoint with no change from the midpoint to the downstream point (Figure 3). Neither *E. coli,* chl-a nor TSS changed along the RS (Figure 3). The interaction between site and stream station for *E. coli* and chl-a was the most parsimonious explanation of these trends (dAIC weight > 0.95, Table 2). For TSS, the interactive model was similarly the most parsimonious explanation (dAIC weight = 0.45, Table 2), yet this model had only half the weight of the other two particulates.

Discussion:

Consistent with our predictions, we observed greater declines in *E. coli*, chl-a, and TSS concentrations at the MS where mussel density was high compared to the RS where mussels were absent. Declines at the RS were minimal (Figure 3) and were likely due to natural settling. Because discharge was similar between both sites during our sampling period (Appendix 1), the greater declines at the MS were likely due to mussel filtration. Our results are consistent with the concept that as water flows over a mussel bed, mussels filter and reduce particulate concentrations from upstream to downstream. Our results add to and corroborate with previous studies on mussel filtration that have demonstrated exponential declines of chl-a (Welker and Walz 1998) and *E. coli* (Ismail et al. 2016) in natural settings. Additionally, this study is a valuable example of the impact of mussels on TSS concentrations in natural conditions.

TSS declined from upstream to the midpoint in the MS but not from the midpoint to downstream unlike other particulates along the reach (Figure 3). This could be due in part to the inability of the laboratory to measure TSS below 3 mg/L or some modulation of mussel filtration. All TSS samples in October were below the 3 mg/L threshold of the MPCA lab, skewing the data collected. Alternatively, mussels in the lower half of the reach may have been less efficient at removing remaining TSS particles due to the type of TSS left unfiltered by the

upper half, lower density, and lower average size of the lower assemblage (Figure 2). Our measures of TSS included organic particles, which may have been selectively filtered by the upper reach while non-preferential particulates continued to float down the river. Finally, there may have been some allochthonous input of solids in the MS between the midpoint and downstream stations of the MS lending to the lack of change of TSS while filtration continued as normal. However, we did not observe obvious erosion and TSS values were low throughout the reach.

It is possible that other organisms at the MS contributed to the observed decline of particulates along the reach. For example, caddisfly larvae nets can capture suspended particulates from the water column whereas organisms like crayfish and bottom feeding fish can resuspend particulates from the benthos via burrowing and feeding (reviewed by Mason and Sanders 2021). While the MS and RS were both reported to have "thriving" aquatic communities (MPCA 2021), our general observations during site visits suggested the MS had greater abundance of macroinvertebrates and fish compared to the RS. However, given the dominance of mussels (biomass and density) at the MS and their high filtering capacity, it seems unlikely other organisms could appreciably influence results by comparison. We believe the presence of a dense mussel bed is the biggest difference between the MS and the RS sites, lending to the decrease in particulates in the MS and lack thereof in the RS.

While our predictions were largely supported, we recognize limitations in our study that demonstrate the need for more field studies on mussel filtration. Furthermore, due to the spatial constraints of this study, our results are technically only applicable to the Sunrise River. The unusually dense mussel assemblage of large individuals just below the dam (Hornbach et al.2014) may have influenced the large decrease in particulates in the upper reach of the MS.

Increasing the scale of this study to include more assemblages could examine latitudinal, longitudinal, and density patterns regarding particulate filtration by mussels, making this study more applicable. Additionally, using a means to track particulates in a water column (e.g., flow cytometry, radioactive labeling) could directly attribute reductions in particulates to mussel filtration, rather than to the presence of a mussel bed.

In a future study, selecting a more similar reference site that had more water quality and physical similarities (i.e., a low head dam) could be helpful. Temperature was lower at the RS than the MS; this may explain the lack of mussels at this site since mussels cannot undergo gametogenesis and exist in waterbodies that exhibit temperatures below 15°C (Clarke 1973; Heinricher and Layzer 1999). The largest difference between our two sites was the presence of the Kost dam directly upstream of the MS. It is possible that accumulation of algae in the reservoir above this dam provided for enhanced sources of chl-a, compared to the RS. By the time the water reached the downstream site at the MS, the chl-a levels were close to that of the RS. While there was greater initial chl-a at the MS compared to the RS, the other two particulates showed no differences in initial values. This was surprising since there could have been settling of TSS in the reservoir above the dam which might have led to an expectation of lower TSS and the upstream MS and despite the North Branch being listed as impaired for *E. coli* (Chisago Soil and Water Conservation District 2013). Including a reference site with a small reservoir could help explore the influence of these differences.

Since freshwater mussels are incredibly imperiled (Loped-Lima et al. 2018) and their filtering behavior is a crucial ecosystem service (Vaughn 2018) more field-based studies that examine their impact on the removal of harmful (*E. coli*) and disruptive (TSS) particulates are needed. These results demonstrate the ability of a mussel bed to result in a measurable decline of

E. coli and TSS along a stream reach. We believe these results are transferable and easily understood by a large stakeholder community due to the public knowledge of these contaminants and common use of them as measures of water quality. Conveying the benefits of freshwater mussels to stakeholders is essential to influence policies to conserve these species. Thus, more studies investigating the filtration capacity of mussels are imperative to amplify the importance of Unionidae to freshwater ecosystems and human health.

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Literature Cited

Alford, J. B., and E. Caporuscio. 2020. Effectiveness of stormwater best management practices in headwater streams to mitigate harmful algal blooms: a case study of the San Bernardino National Forest, California. Case Studies in the Environment 4:1233521. https://doi.org/10.1525/cse.2020.1233521.

Anderson, E. P., S. Jackson, R. E. Tharme, M. Douglas, J. E. Flotemersch, M. Zwarteveen, C. Lokgariwar, M. Montoya, A. Wali, G. T. Tipa, T. D. Jardine, J. D. Olden, L. Cheng, J. Conallin, B. Cosens, C. Dickens, D. Garrick, D. Groenfeldt, J. Kabogo, D. J. Roux. A. Ruhi, and A. H. Arthington. 2019. Understanding rivers and their social relations: A critical step to advance environmental water management. Wiley Interdisciplinary Reviews: Water 6:e1381. https://doi.org/10.1002/wat2.1381

Atkinson, Carla L., G. W. Hopper, D. A. Kreeger, J. W. Lopez, A. N. Maine, B. J. Sansom, A. Schwalb, and C. C. Vaughn. 2023. Gains and gaps in knowledge surrounding freshwater mollusk ecosystem services. Freshwater Mollusk Biology and Conservation 26:20-31.

Atkinson, C.L. and C.C. Vaughn. 2015. Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. Freshwater Biology 60: 563-574. https://doi.org/10.1111/fwb.12498

ASTM International. 2020. Standard practices for measurement of chlorophyll content of algae in surface waters, D3731-20. Annual Book of Standards, Vol. 11.02, ASTM International, West Conshohocken, Pennsylvania. Available at: https://www.astm.org/d3731-20.html (accessed December 9, 2022). Bari, L., and S. Yeasmin. 2022. Microbes culture methods. Pages 77-98 *in* N. Rezaei, editor. Encyclopedia of Infection and Immunity, Vol 4. Elsevier Inc.

Barkalow, S. L. C. and S. A. Bonar. 2015. Effects of Suspended Sediment on Early-Life Stage Survival of Yaqui Chub, an Endangered USA–Mexico Borderlands Cyprinid. Transactions of the American Fisheries Society, 144:2 345–351.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–135.

Brower, S., P. Humphries, A. Holland, and N. McCasker. 2023. Effect of suspended sediment concentration on the clearance and biodeposition rates of and Australian freshwater mussel (Hyriidae: *Alathyria jacksoni*). Freshwater Biology 68: 1413-1427. https://doi.org/10.1111/fwb.14137.

Cahoon, L. B., and D. A. Owen. 1996. Can suspension feeding by bivalves regulate phytoplankton biomass in Lake Waccamaw, North Carolina? Hydrobiologia 325:193-200.

Campos, M., L. Lobato-Bailon, R. Merciai, O. Cabezon, I. Torres-Blas, R. Araujo, and L. Migura-Garcia. 2022. Clearance and persistence of Escherichia coli in the freshwater mussel *Unio mancus*. Scientific Reports 12:12382. https://doi.org/10.1038/s41598-022-16491-x.

Castro, A. J., C. C. Vaughn, M. Garcia-Llorente, J. P. Julian, and C. L. Atkinson. 2016. Willingness to pay for ecosystem services among stakeholder groups in a South-Central US watershed with regional conflict. Journal of Water Resources Planning and Management 142:9. https://doi.org/10.1061/(ASCE)WR.1943-5452.0000671.

Chisago Soil and Water Conservation District. 2013. Sunrise River watershed: total maximum daily load study. Report wq-iw6-06e. https://www.pca.state.mn.us/sites/default/files/wq-iw6-06e.pdf [accessed January 10, 2025]

Clarke, A. H. 1973. The freshwater molluscs of the Canadian Interior Basin. Malacologia 13:1-509.

Cranford, P.J., Ward, J.E. and Shumway, S.E. 2011. Bivalve Filter Feeding: Variability and Limits of the Aquaculture Biofilter. Shellfish Aquaculture and the Environment, S.E. Shumway (Ed.). https://doi-org.wv-o-ursus-proxy02.ursus.maine.edu/10.1002/9780470960967.ch4

Cummings, K. S., and D. L. Graf. 2015. Class Bivalvia. Pages 423-506 *in* J. H Thorp and D. C. Rogers (editors). Ecology and General Biology, Vol I: Thorp and Covich's Freshwater Invertebrates, 4th Edition. Academic Press-Elsevier, New York.

Dobriyal, P., R. Badola, C. Tuboi, and S. A. Hussain. 2017. A review of methods for monitoring streamflow for sustainable water resource management. Applied Water Science 7:2617-2628. Donatell, J., C. Klucas, P. Anderson, D. Duffey, B. Monson, G. Flom, J. Chirhart, K. Parson, and D. Christopherson. 2014. Lower St. Croix watershed monitoring and assessment report. Minnesota Pollution Control Agency document number: wq-ws3-07030005b. https://www.pca.state.mn.us/sites/default/files/wq-ws3-07030005b.pdf. (Accessed November 26, 2023).

Downing, J. A, P. Van Meter, and D. A. Woolnough. 2010. Suspects and evidence: a review of the causes of extirpation and decline in freshwater mussels. Animal Biodiversity and Conservation 33:151-185.

DuBose, T.P., C.C. Vaughn, G.W. Hopper, K.B. Gido, and T.B. Parr. 2024 Habitat engineering effects of freshwater mussel in rivers across spatial scales. Hydbrobiologia 851: 3897-3910. http://dx.doi.org/10.1007/s10750-024-05545-y.

Fago, D., and J. Hatch. 1993. Aquatic resources of the St. Croix River Basin. Pages 23–56 *in* L. W. Hesse, C. B. Stalnaker, N. G. Benson, and J. R. Zuboy, editors. Proceedings of the Symposium on Restoration Planning for the Rivers of the Mississippi River Ecosystem. Biological Report 19. US Department of Interior, National Biological Survey, Washington, DC.

Fung, V., and J. D. Ackerman. 2020. The Effects of River Algae and Pore Water Flow on the Feeding of Juvenile Mussels. Journal of Geophysical Research-Biogeosciences 125:1. https://doi.org/10.1029/2019JG005302.

Gascho Landis, A. M., W. R. Haag, and J. A. Stoeckel. 2013. High suspended solids as a factor in reproductive failure of a freshwater mussel. Freshwater Science 32:70-81.

Goldsmith, A.M., F.H. Jaber, H. Ahmari and C.R. Randklev. 2021. Clearing up cloudy waters: a review of sediment impacts to unionid freshwater mussels. Environmental Reviews 29: 100-108. https://doi.org/10.1139/er-2020-0080.

Guy, H. P. 1969. Laboratory theory and methods for sediment analysis: Techniques of Water-Resources. Investigations of the U.S. Geological Survey, 5:Cl:58.

Haag, W. R. 2012. North American freshwater mussels: natural history, ecology and conservation. Cambridge University Press, New York.

Hansen, A.T., J.A. Czuba, J. Schwenk, A. Longjas, M. Danesh-Yadi, D.J. Hornbach and E. Foufoula-Georgiou. 2016. Coupling freshwater mussel ecology and river dynamics using a simplified internation model. Freshwater Science 35:200-215 https://doi.org/10.1086/684223.

He, Y., X. Wang, and F. Xu. 2022. How reliable is chlorophyll-a as algae proxy in lake environments? New insights from the perspective of n-alkanes. Science of the Total Environment 836:155700. https://doi.org/10.1016/j.scitotenv.2022.155700.

Heinricher, J. R., and J. B. Layzer. 1999. Reproduction by individuals of a nonreproducing population of *Megalonaias nervosa* (Mollusca: Unionidae) following translocation. American Midland Naturalist 141:140-148.

Hornbach, D. J., M. C. Hove, H. T. Liu, F. R. Schenck, D. Rubin, and B. J. Sansom. 2014. The influence of two differently sized dams on mussel assemblages and growth. Hydrobiologia 724:279-291.

Hornbach, D. J., D. C. Allen, M. C. Hove, and K. R. MacGregor. 2018. Long-term decline of native freshwater mussel assemblages in a federally protected river. Freshwater Biology. 63:243-63.

Howard, J. K., and K. M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460-474.

Ismail, N. S., J. P. Tommerdahl, A. B. Boehm, and R. G. Luthy. 2016. *Escherichia coli* reduction by bivalves in an impaired river impacted by agricultural land use. Environmental Science and Technology 50:11025-11033.

Lopes-Lima, M., L. E. Burlakova, A. Y. Karatayev, K. Mehler, M. Seddon, and R. Sousa. 2018. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. Hydrobiologia 810:1-14.

Luck, K., and J. D. Ackerman. 2022. Threats to freshwater mussels: the interactions of water temperature, velocity and total suspended solids on ecophysiology and growth. Science of the Total Environment 821:153101. https://doi.org/10.1016/j.scitotenv.2022.153101.

399 Madsen, J.D., Chambers, P.A., James, W.F., Koch, E.W., and D. F. Westlake. 2001. The
400 interaction between water movement, sediment dynamics and submersed macrophytes.
401 Hydrobiologia 444:71–84. https://doi.org/10.1023/A:1017520800568
402 Martin, M. S. 2018. The role of freshwater drum as a host of freshwater mussels, Unionidae.
403 Master's thesis. Missouri State University, Springfield.
404 Mason, R. J., and H. Sanders. 2021. Invertebrate zoogeomorphology: a review and conceptual
405 framework for rivers. Wiley Interdisciplinary Reviews: Water 8:e1540.
406 https://doi.org/10.1002/wat2.1540.
407 MNDNR (Minnesota Department of Natural Resources). 2016. Minnesota's Wildlife Action
408 Plan 2015-2025. Division of Ecological and Water Resources, Minnesota Department of
409 Natural Resources.
410 https://files.dnr.state.mn.us/assistance/nrplanning/bigpicture/mnwap/wildlife-action-plan-
411 2015-2025.pdf. (Accessed November 18, 2023).
412 MPCA (Minnesota Pollution Control Agency). 2021. Surface water data. Available at
413 https://webapp.pca.state.mn.us/surface-water/search. (Accessed December 9, 2022).
414 MPCA (Minnesota Pollution Control Agency). 2018. Standard operating procedures: intensive
415 watershed monitoring – stream water quality sampling. Available at
416 https://www.pca.state.mn.us/sites/default/files/wq-s1-18.pdf, revised April 2023 (accessed
417 June 22, 2024)
418 Negus, C. L. 1966. A quantitative study of growth and production of unionid mussels in the
419 River Thames at Reading. Journal of Animal Ecology 35:513-532.
420 Neves, R. J., and M. C. Odom. 1989. Muskrat predation on endangered freshwater mussels in
421 Virginia. Journal of Wildlife Management 53:934-941.

Newton, T. J., S. J. Zigler, J. T. Rogala, B. R. Gray, and M. Davis. 2011. Population assessment and potential functional roles of native mussels in the Upper Mississippi River. Aquatic Conservation: Marine and Freshwater Ecosystems 21:122-131.

Omernik, J. M. and G. E. Griffith. 2014. Ecoregions of the conterminous United States: evolution of a hierarchical spatial framework. Environmental Management 54:1249-1266.

Ondokor, S. T. and J. K. Ampofo. 2013. *Escherichia coli* as an indicator of bacteriological quality of water: an overview. Microbiology Research 4:e2. https://doi.org/10.4081/mr.2013.e2.

Riisgard, H. U. 2001. On measurement of filtration rates in bivalves – the stony road to reliable data: review and interpretation. Marine Ecology Progress Series 211:275-291.

Saavedra, M. J., C. Fernandes, A. Teixeira, X. Alvarez, and S. Varandas. 2022. Multiresistant bacteria: Invisible enemies of freshwater mussels. Environmental Pollution 295:118671. https://doi.org/10.1016/j.envpol.2021.118671.

Shah, F., A. A.Mamun, M. T. Hossain, M. Moniruzzaman, S. Yeasmine, H. Uddin, and M. J. Uddin. 2022. Clearance of *Escherichia coli* by the freshwater mussel *Lamellidens marginalis* in laboratory conditions. Molluscan Research 42:128-134.

Silverman, H., J. S. Cherry, J. W. Lynn, and T. H. Dietz, S. J. Nichols, and E. Achberger,. 1997. Clearance of laboratory-cultured bacteria by freshwater bivalves: differences between lentic and lotic unionids. Canadian Journal of Zoology 75:1857-1866.

Smith, D. R. 2006. Survey design for detecting rare freshwater mussels. Journal of the North American Benthological Society 25:701-711.

Spooner, D. E., and C. C. Vaughn. 2006. Context-dependent effects of freshwater mussels on stream benthic communities. Freshwater Biology 51:1016-1024.

445 Spooner, D.E. and C.C. Vaughn. 2008. A trait-based approach to species' roles in stream
446 ecosystems: climate change, community structure and material cycling. Oecologia 158: 307-
447 317. http://www.jstor.org/stable/40309748
448 Taskinen, J., P. Berg, M. Saarinen-Valta, S. Valila, E. Maenpaa, K. Myllynen, and J. Pakkala.
449 2011. Effect of pH, iron and aluminum on survival of early life history stages of the
450 endangered freshwater pearl mussel, *Margaritifera margaritifera*. Toxicological and
451 Environmental Chemistry 93:1764-1777.
452 Tuttle-Raycraft, S., and J. D. Ackerman. 2018. Does size matter? Particle size vs. quality in
453 bivalve suspension feeding. Freshwater Biology 63:1560-1568.
454 Tuttle-Raycraft, S., T. J. Morris, and J. D. Ackerman. 2017. Suspended solid concentration
455 reduces feeding in freshwater mussels. Science of the Total Environment 598:1160-1168.
456 Vanden Byllaardt, J., and J. D. Ackerman. 2014. Hydrodynamic habitat influences suspension
457 feeding by unionid mussels in freshwater ecosystems. Freshwater Biology 59:1187-1196.
458 Vaughn, C. C. 2010. Biodiversity losses and ecosystem function in freshwaters: emerging
459 conclusions and research directions. Bioscience 60:25-35.
460 Vaughn, C. C. 2018. Ecosystem services provided by freshwater mussels. Hydrobiologia 810:15-
461 27.
462 Vaughn, C. C., K. B. Gido, and D. E. Spooner. 2004. Ecosystem processes performed by unionid
463 mussels in stream mesocosms: species roles and effects of abundance.
464 Hydrobiologia 527:35-47.
465 Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of
466 freshwater mussels. Journal of the North American Benthological Society 27:409-423.

Welker, M., and N. Walz. 1998. Can mussels control the plankton in rivers? – a planktological approach applying a Lagrangian sampling strategy. Limnology and Oceanography 43:753-762.

WHAF (Watershed Health Assessment Framework). 2016. National Land Cover Dataset Land Cover Tool. Available at https://arcgis.dnr.state.mn.us/ewr/whaflanduse/ (Accessed December 9, 2022).

Williams, J.D., A.E. Bogan, R.S. Butler, K.S. Cummings, J.T. Garner, J.L. Harris, N.A. Johnson and G.T. Watters. 2017. A revised checklist of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. Freshwater Mollusk Biology and Conservation 20: 33-58. https://doi.org/10.31931/fmbc.v20i2.2017.33-58.

478 TABLES

479 **Table 1.** Density estimates of mussel species at the Sunrise River study site. No live unionid
480 mussels or shells were detected at the North Branch Sunrise River study site.

Species	Number of mussels/m^2 ± SE
Actinonaias ligamentina	33.89 ± 2.81
Eurynia dilatata	2.04 ± 0.36
Amblema plicata	1.89 ± 0.29
Lampsilis cardium	1.89 ± 0.32
Lasmigona costata	1.14 ± 0.20
Lampsilis siliquoidea	0.82 ± 0.17
Potamilus alatus	0.56 ± 0.13
Ligumia recta	0.46 ± 0.10
Cyclonaias pustulosa	0.24 ± 0.10
Cyclonaias tuberculata	0.24 ± 0.10
Alasmidonta marginata	0.15 ± 0.06
Strophitus undulatus	0.15 ± 0.07
Pyganodon grandis	0.12 ± 0.06
Toxolasma parvum	0.12 ± 0.05
Fusconaia flava	0.05 ± 0.03
Pleurobema sintoxia	0.05 ± 0.03
Truncilla truncata	0.05 ± 0.03
Total	43.90 ± 3.50

481

Table 2. Summary of linear mixed-effects modeling results testing the effect of site and stream station on *E. coli,* chl-a, and TSS concentrations. Data were log transformed prior to analysis. Bolded rows indicate the most parsimonious models for each dependent variable.

Dependent Variable	Model	df	AIC	dAIC	dAIC Weight
E. coli	1: Null model	3	161.8	124.1	0
	2: Site model	4	62.2	24.9	0
	3: Stream station model	5	162.9	126	0
	4: Additive model (site and stream station)	6	43.2	6.8	0.03
	5: Interaction model (site * stream station)	**8**	**34.97**	**0**	**0.97**
Chl-a	1: Null model	3	115.44	56.8	0
	2: Site model	4	89.39	31.1	0
	3: Stream station model	5	114.74	56.9	0
	4: Additive model (site and stream station)	6	84.95	27.6	0
	5: Interaction model (site * stream station)	**8**	**55.93**	**0**	**1**
TSS	1: Null model	3	57.70	11.6	0
	2: Site model	**4**	**47.58**	**11.4**	**0.18**
	3: Stream station model	5	56.72	1.8	0
	4: Additive model (site and stream station)	**6**	**45.18**	**0.4**	**0.37**
	5: Interaction model (site * stream station)	**8**	**43.38**	**0**	**0.45**

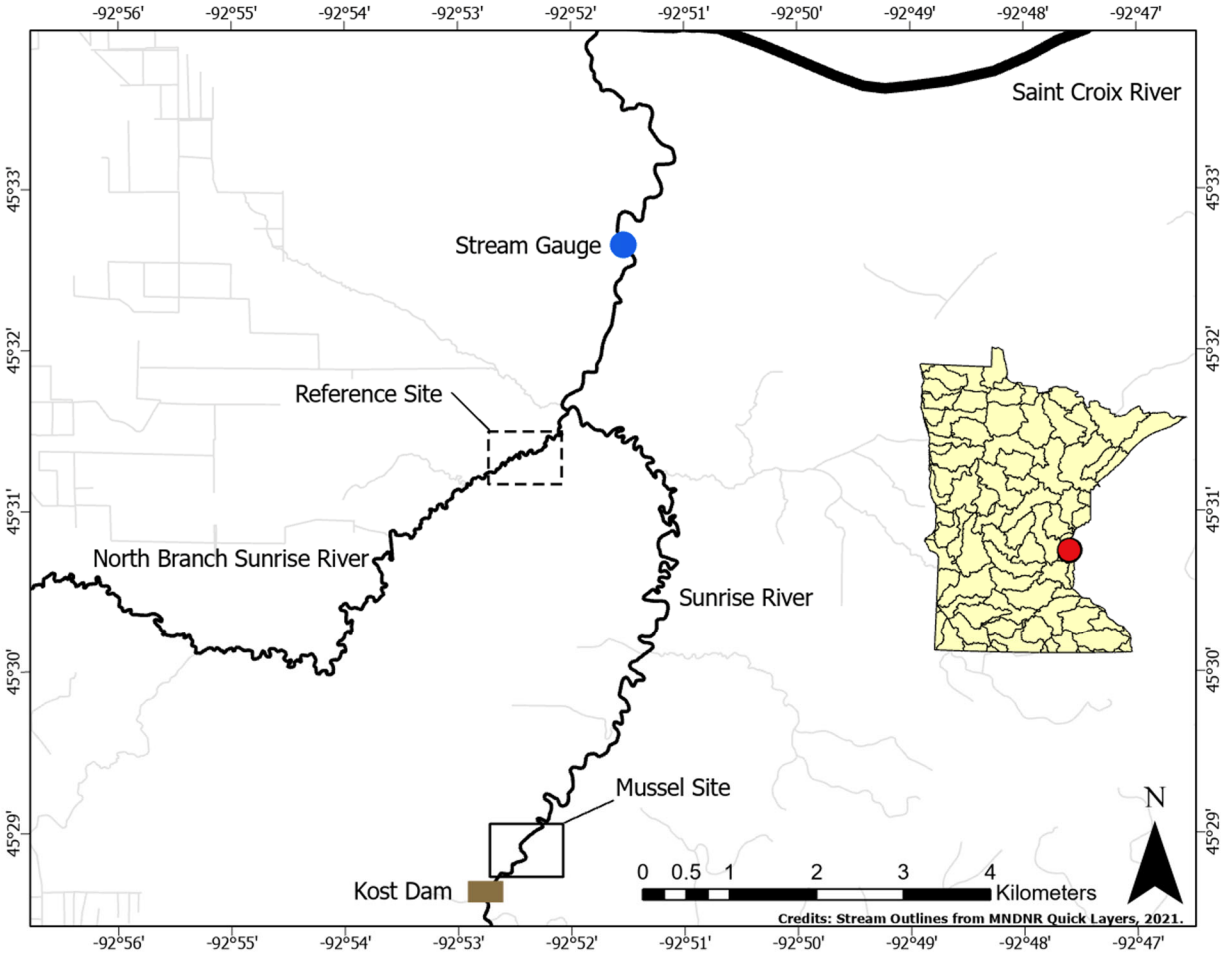
FIGURE LEGENDS

Figure 1. Map showing study sites on the Sunrise River and North Branch Sunrise River. Rivers flow to the north.

Figure 2. Distribution of mussel density (panel A) and mussel length (panel B) at the Sunrise River study site. Colors correspond to increasing density classes produced by Natural Jenk's assortment. Interpolation of density between points was done using ArcGIS Pro's Inverse Distance Weighted tool.

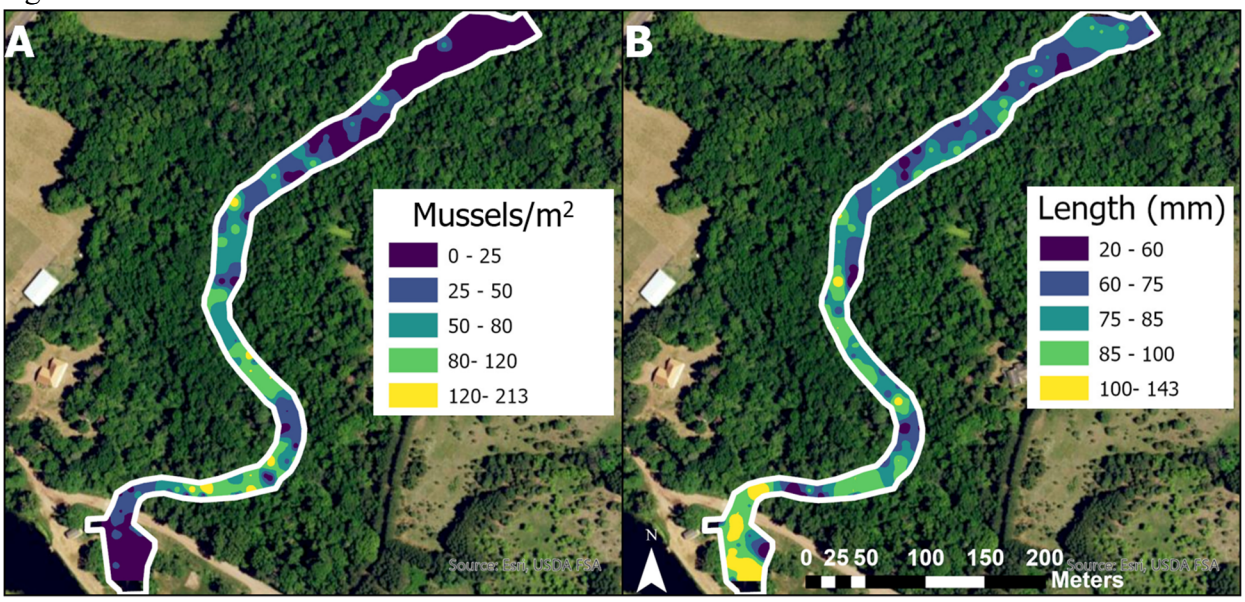
Figure 3. Mean and standard error values for *E. coli*, TSS, and chl-a in the Sunrise River (mussel site) and North Branch Sunrise River (reference site) using combined data from August, September, and October samples. Sampling stations are U=upstream, M=midpoint, and D=downstream.

498 Figure 1.



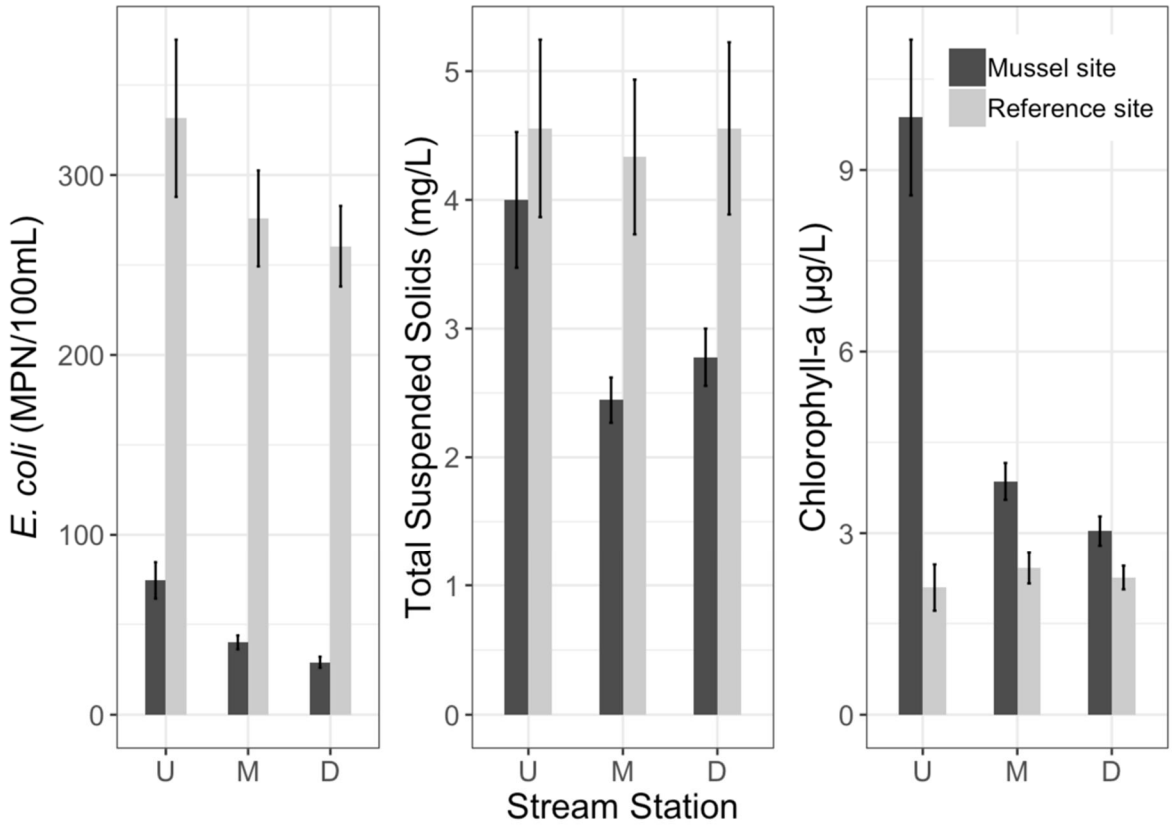
499

500 Figure 2.



501

502 Figure 3.



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APPENDIX 1

Substrate and water characteristics of study sites in mean and standard error. Substrate composition was visually estimated from excavated quadrat samples using the Wentworth scale. Water chemistry, depth, and discharge were measured during suspended particulate sampling.

		Mussel site	Reference site
Substrate	Bedrock (%)	0 ± 0	0 ± 0
	Boulder (%)	4 ± 1	1 ± 1
	Cobble (%)	13 ± 1	4 ± 2
	Gravel (%)	31 ± 2	24 ± 3
	Sand (%)	39 ± 2	51 ± 3
	Silt (%)	7 ± 1	9 ± 2
	Clay (%)	2 ± 1	3 ± 1
	Shells (%)	1 ± 1	0 ± 0
	Detritus/wood (%)	3 ± 1	6 ± 1
	Vegetation (%)	1 ± 0	1 ± 1
Water	Depth (cm)	60.62 ± 5.05	38.07 ± 2.19
	Discharge (m^3/s)	0.31 ± 0.04	0.3 ± 0.01
	Water Temperature (˚C)	20.46 ± 0.82	15.74 ± 0.53
	pH	8.63 ± 0.03	8.39 ± 0.02
	Dissolved Oxygen (mg/L)	8.64 ± 0.26	10.38 ± 0.13
	Conductivity (ms/cm)	379.8 ± 5.15	399.09 ± 4.49