

Calcium and pH Dynamics: Potential Influence on Invasive Mussel Establishment Risk in Lentic Waterbodies

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14. ABSTRACT Under an Interagency Agreement, the USGS created a risk mapping web application for dreissenid mussels in the Columbia River Basin. Additional analysis was conducted investigating the usefulness of using mean calcium and pH measurements from the Water Quality Portal for estimating establishment risk. Lentic waterbodies are the most likely initial establishment sites. Local lotic data is not a good predictor of lentic risk. Calcium is a more stable and reliable variable that should be used when available. Lentic pH data can be highly variable with daily mean values in all three risk categories for many individual sites. A single mean pH below 7.3 of a full lentic profile can be used as an indicator of calcium levels in the moderate or low risk category when calcium data is not available. If the application is expanded to other geographic areas, an analysis of local pH dynamics should be conducted prior to using it as a risk factor. Overall, this application should be used as a high-level screening tool. A deeper dive into the data should be done before making management decisions based on the risk categories displayed in the application.					
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Cover photograph: Jackson Lake, Wyoming during 2021 National Park Service/Reclamation cooperative dreissenid mussel sampling. Photo credit Anthony Prisciandaro.

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**Research and Development Office
Science and Technology Program
Final Report No. ST-2022-19007-01**

prepared by

**Anthony Prisciandaro
Fish Biologist/Aquatic Invasive Species Coordinator
Snake River Area Office
Boise, Idaho**

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Peer Review

Research and Development Office Science and Technology Program

Final Report ST-2022-19007-01

**Calcium and pH Dynamics: Potential Influence on Invasive Mussel Establishment
Risk in Lentic Waterbodies**

**Prepared by Anthony Prisciandaro
Fish Biologist
Snake River Area Office**

**Peer Review by Cavan Gerrish
Water Quality Coordinator
Columbia-Pacific Northwest Region**

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Acronyms and Abbreviations

Acronym or Abbreviation	Definition
CO ₂	Carbon dioxide
CRB	Columbia River Basin
O ₂	Oxygen
PDO	Pend Oreille
Reclamation	Bureau of Reclamation
S&T	Science and Technology
USGS	U.S. Geological Survey
WQP	Water Quality Portal

Measurements

Unit	Measurement
mg/L	milligrams per liter

Executive Summary

Introduction risk and establishment risk are the two main types of risk that are used to prioritize invasive species monitoring locations. If conditions aren't adequate to support a self-sustaining population (establishment risk) then, even if introduction risk is high, the overall risk of mussel infestation could be low. Both pH and calcium influence mussel establishment risk through the ability of mussels to build shells. Literature shows strong relationships between both variables and mussel survival in laboratory environments with near-constant pH and calcium levels. Less data is available to describe the relationships between mussel establishment risk and the more dynamic natural environment. This is especially true for pH, which varies greatly both spatially and temporally within lentic waterbodies. Sample collection methods, timing, and analysis can also influence how well the data represents the real-world conditions.

Most initial infestations occur in lentic waterbodies with lotic systems only becoming infested once an upstream source population exists. The risk mapping application provides data for both lotic and lentic environments. Lotic data in the risk mapping application should be used to investigate potential expansion of established lentic populations rather than locations at risk of an initial infestation. This paper focuses on establishment in lentic environments.

Although the overall range in calcium levels among Bureau of Reclamation (Reclamation) reservoirs is high, the variability seen over time and at a range in depths at individual sites is relatively low. However, pH levels in an individual profile on an individual day can range from being too low to support mussels at depth to being too high to support mussels at the surface, with optimal conditions somewhere in the middle. Within-reservoir variability in calcium levels only includes a handful of sites where spatial or temporal variability extends from high risk to low risk or vice versa. An understanding of the variability of calcium and pH relative to the values used as break points in risk categorization should improve the usefulness of risk categorization in prioritizing early detection sampling efforts.

The field studies in the literature only use lentic waterbodies, but these studies do not detail their methods for obtaining a mean pH value for each waterbody. Sites where surface readings or lab analysis are the only available pH data do not represent a true mean pH. Confidence in the use of pH as a risk factor for mussel establishment in lentic waterbodies can be increased by limiting the pH data used to full profiles collected in the field. For Columbia River Basin data in the Water Quality Portal, only using data with a minimum of five pH readings per sampling date removes much of the data that is not from full profiles. This does remove some profiles at shallow waterbodies and leaves some data that is not from full profiles, but it allows for analysis of a pH value that more closely represents a true mean of the waterbody.

When pH data is analyzed in this manner, a relationship between mean pH and mean calcium becomes apparent. Only one site with calcium data and pH data with more than five pH readings per day has calcium in the high risk category where mean pH is in the low or moderate category. This suggests the lack of mussels in field studies where mean pH is less than 7.3 may be due in part to low calcium and not simply a direct consequence of the mean pH. This addresses the concern

that some areas with optimal pH can exist in waterbodies with mean pH in the low or moderate risk categories.

Overall, when any amount of lentic calcium data is available it can reliably be used as an indicator of mussel establishment risk. If calcium data is not available but full profiles of pH readings are available, mean pH values in the low or moderate risk category can also be interpreted as meaning calcium risk is likely in the low or moderate risk category. This could either be used to more confidently categorize risk indirectly with pH, or to prioritize additional calcium sampling if an even higher degree of confidence is needed.

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Introduction

This document is intended as a supplement to the user guide, literature review, and Columbia River Basin Dreissenid Mussel Risk Mapping Application developed by the U.S. Geological Survey (USGS) under Reclamation's Science and Technology (S&T) Grant funding. These documents are included as Appendix 1 and Appendix 1-A. The documents and the application itself can be found at <https://aiswaterquality.net/aiswq/index.html#crb>. The user guide and literature review provide more details for how and why the risk mapping application was developed.

Zebra and quagga mussels pose a significant threat to the environment as well as to recreation and infrastructure. These invasive mussels became established in the Great Lakes in the 1980s after introduction through ballast water from Eastern Europe. Both species of mussel have since spread to many locations across the United States. The Columbia River Basin (CRB) is the only remaining major river basin in the Continental United States that does not have a documented infestation. Monitoring for these invasive mussels is important to protect the environment, economy, and infrastructure of the Pacific Northwest.

The USGS web-based risk mapping application created under this S&T project uses three risk categories (high, moderate and low) for both pH- and calcium-based establishment risk (Table 1). The risk categories for pH have both upper and lower bounds with optimal, high risk pH levels in the middle. Calcium levels, on the other hand, have no upper bounds. At the site level in the risk mapping application, both pH and calcium data are summarized to a mean of daily means before being assigned a risk category. Mean pH and calcium levels are used for risk categorization in the literature. An understanding of the range and variability of pH and calcium will allow for an informed decision-making process as this establishment risk information is combined with other risk factors to improve sampling prioritization.

Table 1. Risk category break points for calcium and pH used as default levels in the risk mapping application.

Risk Factor	Low Risk	Moderate Risk	High Risk
Calcium Concentration	<8 mg/l	8-20 mg/L	>20 mg/L
pH	<7.0 or >9.6	7.0-7.3 or 9.4-9.6	>7.3 - <9.4

Prioritizing high risk lentic waterbodies for monitoring is critical in the effort to detect any new infestation of zebra or quagga mussels. The spatial and temporal extent of monitoring needed to detect invasive species early on in an infestation often exceeds the financial or practical capabilities of those conducting the monitoring (Counihan and Bollens 2017). Current sampling effort in the CRB is spread among all risk categories (Counihan and Bollens 2017). Most entities conducting sampling are taking the conservative approach to consider waterbodies without calcium data as high risk. A large amount of sampling still occurs annually at low risk waterbodies. Understanding and developing confidence in which waterbodies are truly at low risk of establishment, even if introduction occurs, should help better prioritize sampling effort. This supplemental report aims

to increase confidence in the use of calcium and pH as direct or indirect indicators of low establishment risk.

The variability in pH over space and time in lentic waterbodies is mainly driven by biological processes that convert CO₂ to O₂ (photosynthesis) and O₂ back into CO₂ (respiration). The volume of CO₂ dissolved in the water (creating carbonic acid) changes on both daily and seasonal time steps and can vary greatly from the surface to the bottom of a waterbody. The spatial and temporal sampling of pH is therefore important to understand when interpreting pH data related to the risk of mussel establishment. The extent of this variability in individual waterbodies is mainly regulated by alkalinity. Alkalinity does vary among Reclamation reservoirs but is relatively constant throughout space and time within individual reservoirs.

Although pH has been shown to influence survival in lab studies and is correlated to mussel presence in some field studies, the dynamic nature of pH in lentic waterbodies leads to limited confidence in its use as an establishment risk factor. Static pH levels in lab studies have shown increased mortality at the same levels where mean pH values show a lack of mussels in field studies. This agreement is concerning since the spatial variability in field measured pH often leads to some depths with pH found to be optimal in the lab when the mean is at levels that cause mortality in the lab.

Measurements of pH occur both in the field and with water samples brought back to the lab. Lab pH measurements are often collected as inputs for other analysis. Many factors, including temperature changes, exposure to the atmosphere, and biological activity in the sample bottle, can cause a change in pH between the field and lab. The ability, or inability, to identify pH values as field or lab measurements is important to understand while developing confidence in the use of pH data in risk analysis.

This document details the dynamics and interactions between pH and calcium levels in lentic waterbodies. This could provide managers with higher confidence and justification for prioritizing monitoring activities at high risk waterbodies. Combining this work with other risk factors such as water level fluctuation and boater movement could provide even more confidence in determining a true risk of mussel establishment.

Water Quality Portal

The Water Quality Portal (WQP) is a cooperative service sponsored by the USGS, the Environmental Protection Agency, and the National Water Quality Monitoring Council that integrates publicly available water quality data into an online searchable database. The data in the WQP is collected for a variety of reasons including general surveillance and modeling. A small subset of the data however is collected in response to uncommon events. At certain sites the data may have been collected in response to expected contamination, toxic algal blooms, fire, or other events. Some of the data is collected as part of lab processes and is not representative of field conditions. For example, pH is measured in the lab during alkalinity analysis and sometimes recorded in the dataset. Therefore, not all data in the WQP is representative of normal conditions in the field.

With multiple agencies contributing data, sampling methods are not always consistent. Some agencies collect data at different depth intervals and may be limited by equipment where the maximum sample depth is not the maximum water depth. Some sites and/or dates only have field measurements or lab measurements while others contain both field and lab data. The risk mapping application uses this imperfect data and is a great resource to look at the risk levels in general. The cleaning process described in the user guide does remove some of this data, but some data used in the risk map application likely still represents abnormal conditions. Users should consider investigating the data in more detail during any decision-making process.

Some cleaning and interpretation of the dataset beyond that done for the risk mapping application may allow for higher confidence in using pH data for risk analysis. Limiting pH analysis to sites with five or more readings per day can remove many of the potential issues with pH data in the WQP. This allows for a better representation of the mean of a full profile. Lab analysis, for example, is often conducted on only one or two samples. Some sites have tens or even hundreds of readings, but only one or two per date. Many sites have two lab readings and two field readings per sampling date. There are some sites with five or more readings per day that do not have full profiles. There are also shallow sites where five readings is a full profile. Some analysis later in this document uses this minimum of five readings per date as a cutoff for representing data that mostly consists of full profiles. Individuals may want to look at the raw pH data for their sites of interest before using means for any decision making.

Lentic vs. Lotic Environments

The free-floating veliger stage of the mussel life cycle can aid in the spread of an established population. This free-floating stage can also hinder the initial establishment of a mussel population. Mussels are dioecious and eggs and sperm may only be viable for two hours after spawning (Sprung 1987). Successful establishment requires the spread of veligers from an initial seeding population to be contained to an area small enough for successful external fertilization between males and females of the next generation. Even if a slow-moving lotic (flowing) environment allows for successful external fertilization, veligers have 3-4 weeks of floating around before they settle on substrate. By definition, lotic waters are more likely to spread veligers too far away from each other by the time they settle for successful external fertilization of the next generation and population establishment.

Although mussels can establish in lotic environments, this has mostly occurred after an upstream population has established and provides frequent seeding (Horvath et al. 1996). In any environment, the seeding population has to be large enough, or their distribution contained enough within the receiving water, for the offspring of an introduced population to settle close enough to each other for subsequent successful external fertilization. Manmade marinas or natural coves on larger rivers that increase residence time may provide the conditions for a small population to establish within a larger lotic system. However, most introductions that have established a self-sustaining population have been in lentic waters.

Even with the understanding that an initial infestation is not likely to occur in a lotic environment, it may be tempting to use local lotic data to predict lentic risk where no lentic data is available. Extreme caution is urged before using local lotic pH or calcium data to estimate establishment risk in a connected lentic waterbody. Where WQP data from the CRB has been reviewed at a local scale, both pH and calcium have been shown to differ between nearby lotic and lentic environments. Lotic waters are often not stratified and gases are at or close to equilibrium with the atmosphere. CO₂ and the associated carbonic acid dynamics in stratified lentic waters often lead to pH differences between lentic waters and their lotic inflows and outflows. In general, calcium has shown more consistency between nearby lotic and lentic environments while pH has shown more differences. Lotic outflow from a lentic waterbody is often more similar to the lentic waterbody than the inflowing water. In areas where only lotic data is available, it may be best to assume high risk until lentic data can be obtained rather than assigning risk based on the available lotic data.

Examples of the differences between local lentic and lotic pH and calcium can be seen in data available in the WQP. Lake Pend Oreille for example, shows that both pH and calcium from different inflowing tributaries may not be representative of conditions in the local lentic waterbody (Figure 1 and Figure 2). The mean pH and nearly all of the individual readings in Lake Pend Oreille are in the high risk category. Only two of the five nearby lotic sites also have pH means in the high risk category. Calcium within the lake gives a high risk rating, while two of the five local lotic sites with calcium data are in the low risk category. This suggests that local lotic data is not a good substitute for lentic data. Lentic waterbodies without calcium or pH data should be considered unknowns or high risk for establishment until lentic data becomes available. There may be areas where lotic data is representative of nearby lentic environments; however, this is not always the case. The rest of this report will focus on data and establishment risk in lentic environments.

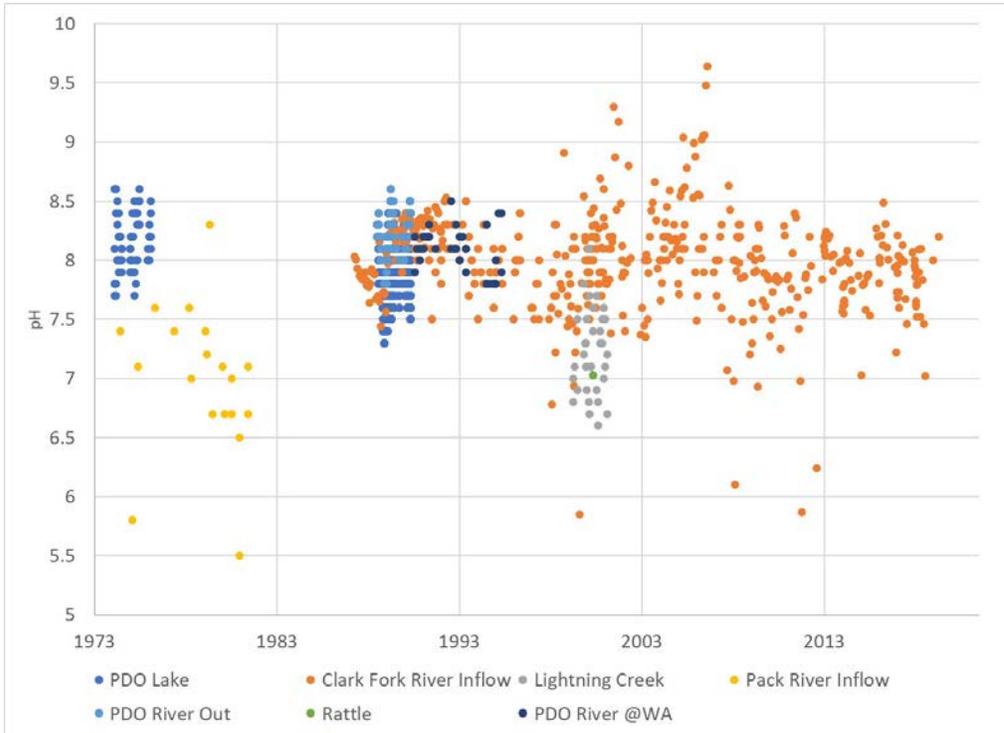


Figure 1. Available pH data for lake Pend Oreille (PDO), tributaries, and outflow

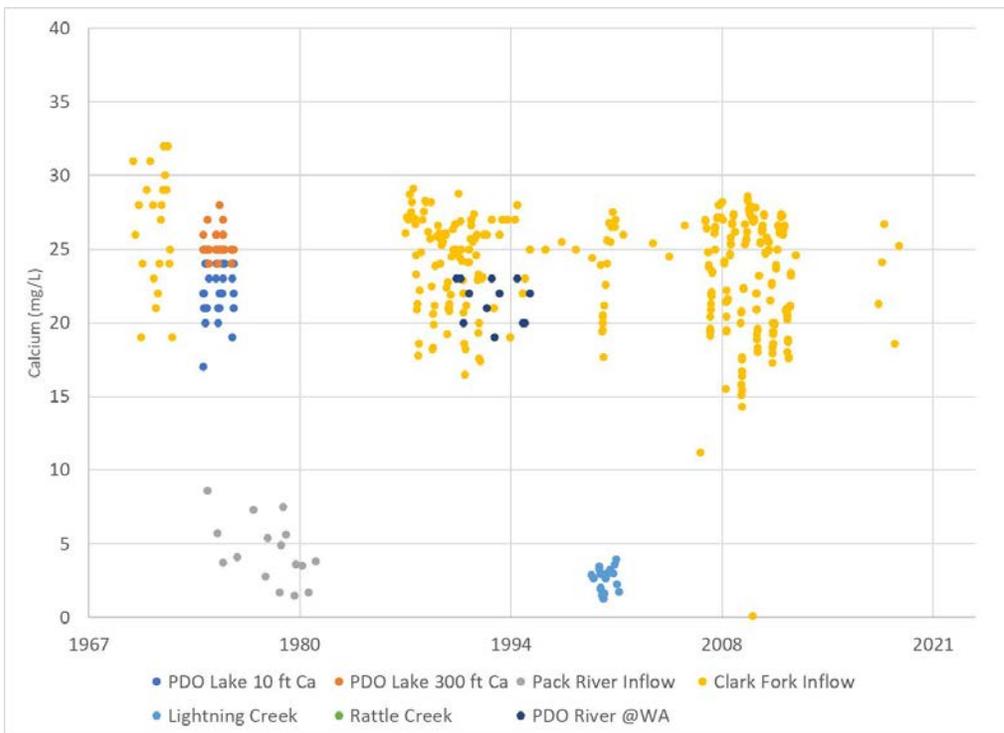


Figure 2. Available calcium data for lake Pend Oreille (PDO), tributaries, and outflow. Note that Rattle Creek calcium levels were measured at 101 mg/L in 2000 but are out of the range of this chart.

Variability of Establishment Risk Parameters

pH Variability

An understanding of the spatial and temporal variability in pH is critical when interpreting risk categorizations depicted in the risk mapping application. The risk mapping application uses a mean of daily means of pH values to categorize each site into an establishment risk category. Since mussels are not present in the CRB, establishment risk category break points were developed from field studies in Canada and Europe where risk values are derived from relationships between mussel presence and mean pH (Claudi and Prescott 2011; Ramcharan et al. 1992). The high spatial and temporal variability documented in the WQP for pH within individual lentic waters invites the question of a direct relationship between mean pH and mussel establishment risk or an indirect relationship between pH and some other factor influencing mussel establishment. Understanding the potential causal relationships between mean pH and mussel establishment could improve the confidence in using pH as a risk factor in environments that experience different climate or geological conditions than the limited examples in the literature. Since the default level of sampling effort for a waterbody with unknown risk is often the same as those known to be high risk, understanding how a site can have a mean pH in the low risk category is especially important.

Variability of pH within a lentic waterbody is caused in large part by biological activities of photosynthesis (decreasing dissolved CO₂ and increasing pH) and respiration (increasing dissolved CO₂ and decreasing pH) (Minor et al. 2019; Stumm and Morgan 1996; Tucker and D'Abramo 2008). These biological activities result in higher pH levels near the surface or thermocline where photosynthesis is highest, with lower pH values at depth where respiration is highest (Figure 3). Changes in other variables can cause differences in specific pH values within and among years, but the general trend of higher pH above the thermocline and lower pH below the thermocline is consistent. This illustrates the need for using only data from full profiles in any analysis using mean values. The overall mean for the data in Figure 3 is 7.1 while the mean of only surface measurements is 8.1.

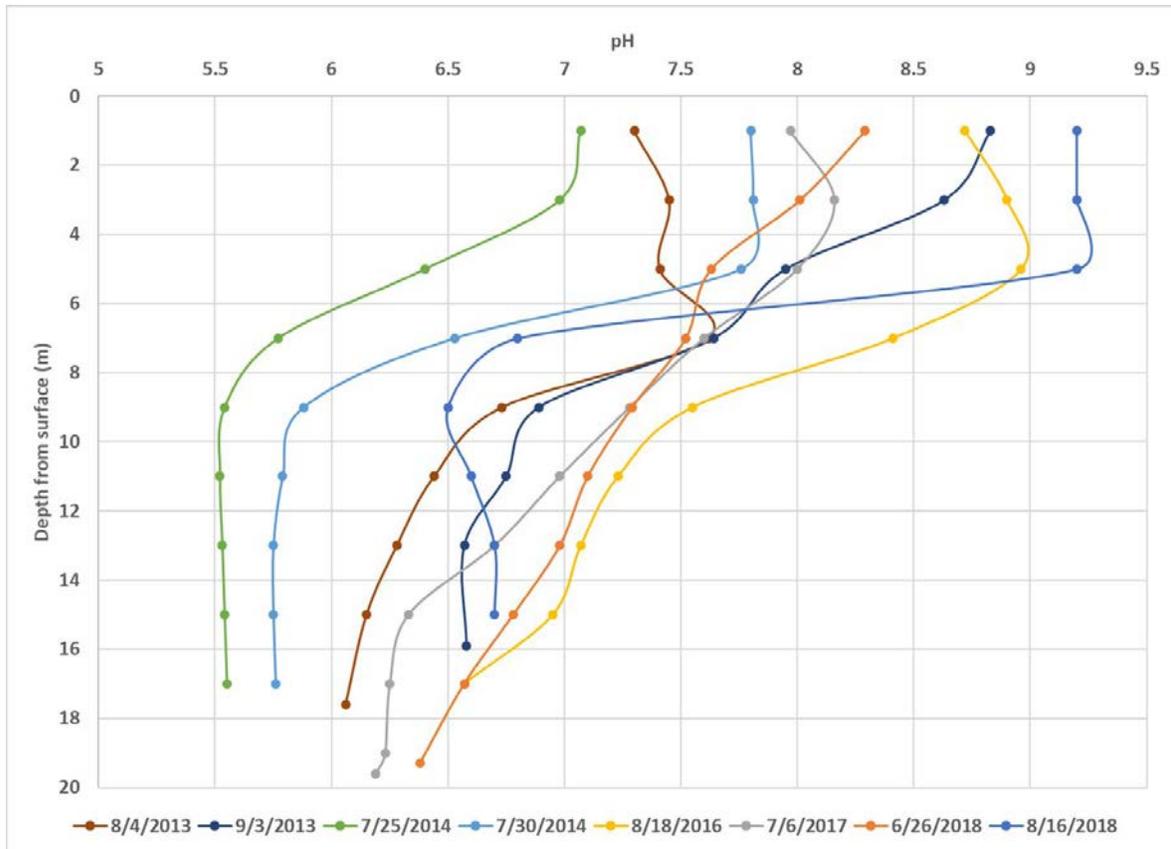


Figure 3. Depth profiles of pH at site 1119USBR_WQX-GAR053 on Cascade Reservoir, 2013-2018

The literature also shows that pH can change on shorter time scales than Reclamation’s data set can demonstrate. Diel variation in pH occurs in large part due to patterns in photosynthesis (photosynthesis decreases dissolved CO₂) (Tucker and D’Abramo 2008). Maberly (1996) documented diel variation in pH up to 1.8 units. Diel variation is often not this extreme but is frequently larger than the 0.2 and 0.3 pH unit differences between low risk and high risk used in the mapping application. This means that it is possible for a mean pH of a morning profile to be in the low risk category and the mean pH of a profile that afternoon to be in the high risk category.

Many sites in the risk mapping application are represented by very few sampling events. Understanding the variation in pH over space and time at sites with more data will help interpret data from sites with fewer sampling events. Variation in pH over space and time can lead to different sampling methods or different sample timing to result in different risk values at the same location. Looking at the average pH of each of the 34 individual profiles taken at Cascade Reservoir between 1999 and 2018 (Site: 1119USBR_WQX-GAR053) shows that 9 have a mean pH in the low risk category, 10 have a mean pH in the moderate risk category, and 15 have a mean pH in the high risk category. The mean of daily means at this site is 7.24, suggesting Cascade Reservoir would be in the moderate risk category. Most sites in the CRB have only a few profiles worth of data available in the WQP. If only 1 of the profiles from Cascade were available instead of 34, there would be a 26 percent chance of results in the low risk category, a 29 percent chance

of results in the moderate risk category, and a 44 percent chance of results in the high risk category. This calls into question the reliability of a mean of daily means pH based risk category determination at other waterbodies where fewer profiles are available. Without knowing the spatial and temporal range of data used in the field studies to compare waterbodies with and without mussels, it is difficult to determine what data is needed to feel confident in risk categorization in the CRB. However, if pH (at the risk category break points used here) is an indirect indicator of risk pH, even a single profile with a mean pH in the low risk category could be indicative of a true low risk waterbody.

The WQP contains pH values measured both in the field and laboratory. Measurement of pH in the lab is needed in order to measure some of the other water quality parameters. However, these laboratory measurements are not always representative of pH in the field. Temperature changes, exposure to the atmosphere, and a reduction in pressure between the sampling location/depth and the lab can lead to lab pH measurements not representing field conditions. The accuracy of field and lab equipment also varies. The DS5 Hydrolab sondes that Reclamation used for field measurements have an accuracy of +/- 0.2 units as defined by the manufacturer (Hach 2006). This draws attention to the small range of moderate risk. On the low end of the pH range, low risk goes up to 7.0 and high risk starts just 0.3 units away at 7.3. On the high end of the pH range, the difference is only 0.2 units with 9.6 being low risk and 9.4 being high risk.

Information on whether a pH value is from a field measurement or laboratory analysis is not always available in the WQP. When both field and laboratory pH values are available from sampling on the same date/depth, data from the WQP shows that laboratory pH can be both higher and/or lower than field measurements. USGS data was summarized where paired lab and field data was available. Lab pH data ranged from 2.6 units lower than field data to 3 units higher than field data with a mean absolute difference of 0.37 units. This mean difference is greater than the 0.3 and 0.2 unit difference between the high and low pH risk categories. Nearly half (43.7 percent) of the paired lab/field pH values had a difference greater than 0.3 units (Table 2). When samples are brought to the lab for the purpose of pH measurements, handling requirements and holding times under 2 hours allow for lab measured pH to be representative of field conditions (NEMI 2022a). The purpose of some of the pH values in the WQP are likely for use in analysis of other constituents in the lab. For example, monitoring of pH levels is needed for alkalinity analysis (NEMI 2022b). It is not always easy to identify pH data from the WQP as a field or lab measurement. Some of Reclamation's data, for example, is only identifiable as lab measurements because the Analysis Start Date is after the Activity Start Date. With the analysis date for some pH measurements being days after the sample collection date, at least some lab analyzed pH measurements did not make the 2-hour hold time requirement. Other sites with pH data labeled as lab analysis are more than a 2-hour drive from the closest lab that could do the analysis. This added complication with pH data shows the importance of looking at the raw pH data when making important decisions. If full profiles are available for the sites of interest, the data can still be useful in risk analysis.

Table 2. Differences between paired lab and field pH values. Out of 634 total sites, the table shows the number of individual sites where field and lab pH match, as well as how many have a higher lab or a higher field pH. The count and percent of these sites with absolute differences greater than the listed values are also included.

Difference	Count	Total Sites
No difference	65	10.3
Lab > field	268	42.3
Field > lab	301	47.5
> 0.3 difference	277	43.5
> 0.5 difference	147	23.2
> 1.0 difference	28	4.4
> 1.5 difference	9	1.4

The veliger life stage is the most sensitive to pH levels with optimal pH for veligers between 7.9 and 8.5 (Sprung 1987; Ramcharan et al 1992; Hinks and Mackie 1997; Churchill 2013). Data from the WQP shows that 27 percent of low and moderate risk sites with at least five pH readings per date (>5 readings suggest field profiles) have some depths and/or seasons within this optimal pH range (Figure 4). At low or moderate risk sites with at least five readings per date, 74 percent have some readings in the high risk category. When interpreting a low or moderate risk site, it is important to understand that there are often some depths or time frames with pH in the high risk or even optimal range for veliger development.

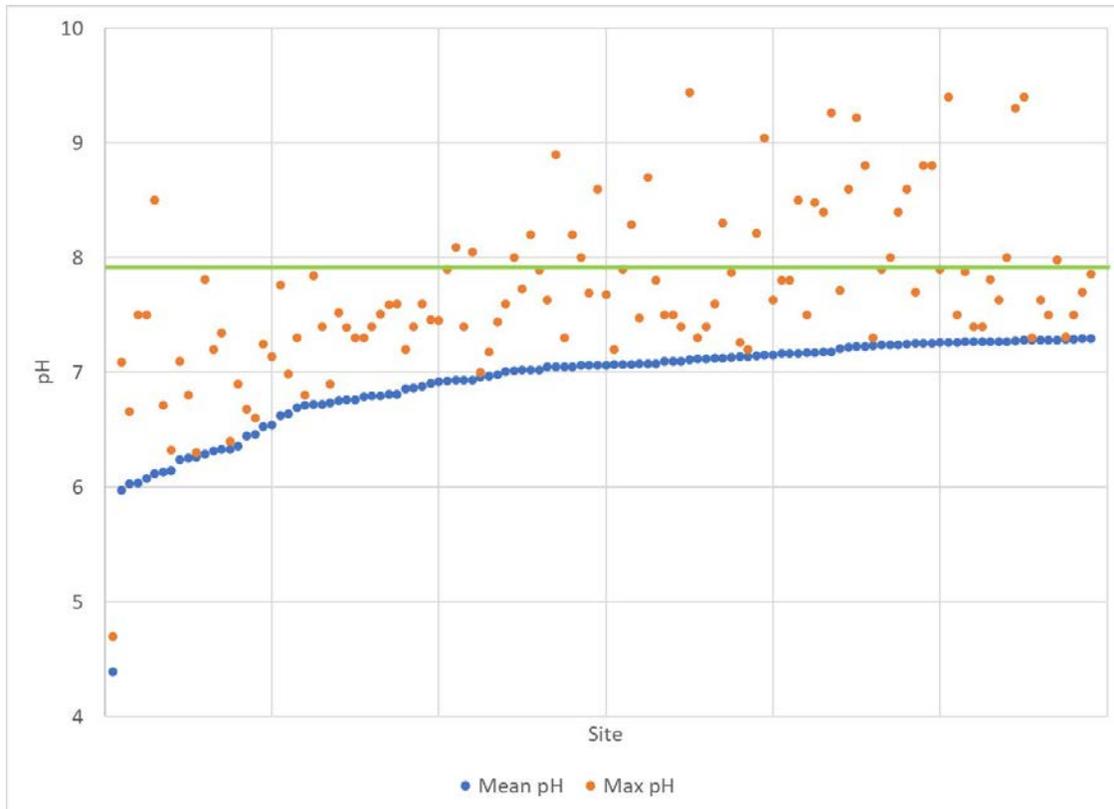


Figure 4. Mean and maximum pH levels for all low and moderate risk sites with at least five pH measurements per sampling date. All sites with a maximum above the green line (27% of all sites) have some times and/or depths within the optimal pH range.

Sampling methods can also influence mean values depicted in the mapping application. In the data set available on the WQP, some lentic sites only have surface pH. These sites with only surface pH are not representative of the mean pH of the waterbody and are not comparable to the means identified in the literature. The distribution of samples within an individual depth profile can also influence the mean. Some methods require the same depth interval between all measurements (i.e., 1-meter intervals from surface to bottom). Since water quality parameters below the thermocline are often less variable than above the thermocline, others require more frequent measurements above the thermocline (1-meter intervals until thermocline and then 5-meter intervals below). Some sampling may also be limited by the maximum length of the cable used. All of these complications bias the data towards higher pH. These are additional reasons to look at the data in more depth if making significant decisions. Even with these complications, relationships between pH and calcium described later in this document provide additional confidence in using the mean pH of a profile as an indicator of low establishment risk.

There are also variables present in the CRB that may not be represented in the literature used to develop the risk category break points. Although carbonic acid from dissolved CO₂ is a major influence over pH in most lentic waters, geothermal inputs and acid mine drainage can alter the pH of surface waters through other means (Arnorsson et al. 1995). Seasonal changes in the

relative volume of surface lotic inflows compared to the flow of water (or gases) influenced by mines or geothermal can lead to higher variability in pH than is seen at other lentic waters in the CRB. It is important to determine if the sites of interest are near geothermal or mining influence. These sites should be investigated in more detail rather than relying on the mean value used in the risk mapping application. In particular, sites near mines are often only sampled when there is a potential problem. This could lead to mean values that are only representative of the abnormal conditions.

Mines can act as either a long-term constant input of low pH or an event can cause a short-term drop in pH. By either creating paths for oxygen to access underground mineral deposits or by bringing those deposits to the surface, mining allows for minerals to be oxidized at a higher rate than would occur due to natural weathering (Banks et al. 1997). This oxidation of minerals such as pyrite lowers the pH and can influence local surface water and cause changes to downstream lentic waterbodies directly through pH or indirectly through the lower pH dissolving minerals that are then transported downstream. Flooding and/or a breach in containment at mines can cause short-term changes in pH.

Gases associated with geothermal influence are often the underlying factor influencing the pH of geothermal water. Geothermal waters are often heated by magma sources. Magma releases gases including CO₂, hydrogen sulfide, sulfur dioxide, and hydrogen chloride which all can lower the pH of water (Arnorsson et al. 1995). The rate of degassing as well as the seasonal changes in the relative volume of geothermal water compared to the local lotic inputs can cause fluctuations in lentic pH.

Even outside of the influence of mines and geothermal waters, pH still exhibits high variability. The relationships identified in the field studies however are significant. This could be explained by an indirect relationship between pH and mussel survival. The relationship between pH and calcium may support the indirect relationship between pH and mussels. This relationship is discussed further in subsequent sections of this report and should provide confidence in the ability to use full pH profiles as an indicator of low establishment risk.

Calcium Variability

Mean calcium levels are also used to determine risk categories in the mapping application. Relative to their respective risk category break points in the mapping application, calcium is less variable over space and time than pH. Dissolved calcium is a result of the interaction of water with the local geology as well as some internal cycling in lentic waters. Rain water is typically acidic with a range from around pH 5.0 up to 5.6 across the CRB (USGS 2002). As this precipitation interacts with the surficial geology, the low pH causes calcium to dissolve into solution (Stumm and Morgan 1996). The surficial geology influences how much calcium is available to dissolve into the water. Sedimentary rock typically has more calcium than igneous rock (Stumm and Morgan 1996). Although calcium levels are relatively stable in most lentic waters, some situations can lead to higher variability.

Conditions to be aware of when using calcium as an establishment risk indicator include lentic waters influenced by mines, geothermal inputs, and extreme evaporation. Under these conditions, fluctuations in pH can influence calcium levels by altering the carrying capacity or dissolving more calcium into solution. The fluctuation in carrying capacity can cause the precipitation of calcite (Larson and Buswell 1942; Stumm and Morgan 1996). Calcium levels in lentic waters influenced by mines or geothermal activity can fluctuate seasonally as spring runoff masks the influence compared to summer base flows. Events like eruptions, magma movement, or disturbance of mining sites can also cause short term alterations to calcium levels as discussed below. Although rare in the WQP data for the CRB, lentic waters with extremely high evaporation rates can experience higher levels of calcium fluctuation due to precipitation of calcite at high pH levels. The influence of evaporation may be important if this type of risk analysis is expanded outside of the CRB to areas like the southwestern U.S. This process is explained in the section titled “Interactions between pH, Calcium and Alkalinity.”

Looking at summary statistics for calcium variability by site may initially bring up concerns about the average range of calcium variability being greater than the difference between the high and low risk categories. With the difference between high and low risk being 12 mg/L, any sites with variability greater than 12 mg/L may be concerning. However, this variability increases with increasing calcium levels and few sites contain data in both the high and low risk categories. At sites in the CRB that have at least 25 calcium readings available in the WQP, the range in calcium concentrations increases significantly as the minimum reading increases ($p < 0.01$; Figure 5 and Figure 6). Variability in this data set shows some sites with data in two risk categories; however, only one (Wildhorse Reservoir) contains data in both low and high risk categories (Figure 6). Sites in Figure 6 that display a larger range in calcium compared to sites with similar minimum calcium levels are labeled for discussion here. The low variability of calcium data among low risk sites can assist in developing confidence in identification of low risk sites. Taking into account the exceptions described below, a site with only one available data point (in the low risk category) is not likely to return any samples in the high risk category if additional sampling is conducted.

Most of these sites with a larger range in calcium readings than other sites with similar minimum calcium readings can be explained by local conditions. Heart Lake is located in Yellowstone National Park and has a geyser basin along the inflowing stream. Variations in the volume of geothermal water entering Heart Lake, as well as mixing rates, are expected to be causes of this variability. More details on geothermal influence of calcium are provided below. Wildhorse Reservoir is in an arid climate with high evaporation and can have pH levels over 10 that lead to precipitation of calcite. The two Hilltop Lake sites are less than a mile from an open pit silica and diatomaceous earth mine. Some white outcroppings are visible from aerial imagery on the shoreline of the lake. Another site is a tailings pond for other mining activities.

The calcium variability at these sites makes sense when local conditions are taken into account. However, the sites at Dworshak Reservoir and Flathead Lake are not easy to explain. The outliers in these data sets are a small portion of the overall data but consist of multiple samples taken over multiple different days. At Dworshak Reservoir, only 4 of the 74 measurements (5.4 percent) have calcium levels over the 8 mg/L break point. At the 4 Flathead Lake sites that showed variability in 1979, only 10 out of 262 measurements (3.8 percent) had calcium levels below the 20 mg/L break

point and only 1 of the 262 measurements (0.4 percent) was below the 8 mg/L break point. Despite these outliers, the overall mean of means at these sites still represent the overall establishment risk levels. The low number of calcium readings in multiple risk categories for sites with more than 25 readings should provide confidence in using calcium data from sites with less data.

Looking at all sites within the WQP, no matter the sample size, there are a few sites with calcium values in all three risk categories. Other than the sites on Flathead Lake that have already been discussed, all of the sites with calcium levels in all three risk categories are associated with mines or have volcanic or other geothermal influence. When using the risk mapping application or other WQP data for establishment risk assessments, it is important to be able to identify sites that may have mining or geothermal influence.

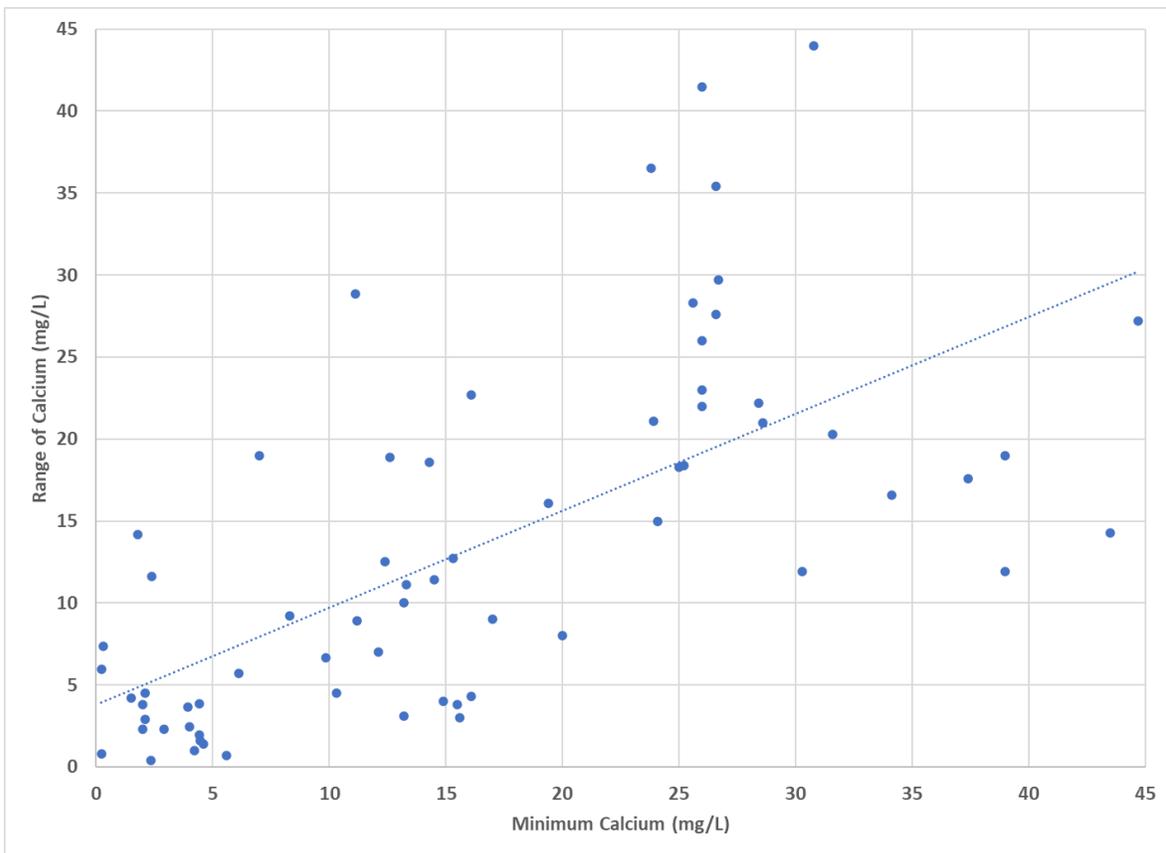


Figure 5. Range in calcium readings (maximum - minimum) and minimum calcium readings for sites with greater than 25 calcium readings

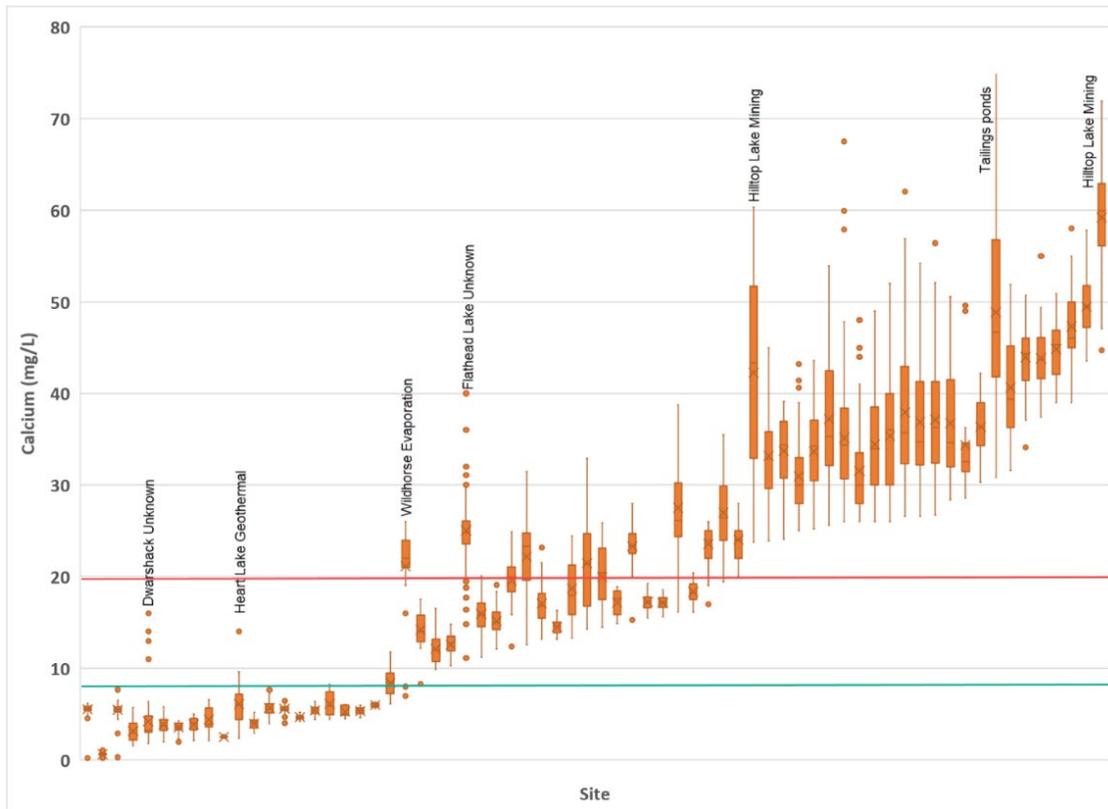


Figure 6. Calcium mean and range for all sites labeled as lake, reservoir, or impoundment within the Columbia River Basin with 25 or more sample results in the water quality portal. Sites are displayed in order by their minimum calcium reading. Anomalies discussed in the report are labeled with text.

Similar to pH, mining activity can lead to temporary or periodic fluctuations in calcium levels. The lower pH often found associated with mines, as described above, can influence calcium concentrations. The lower pH will dissolve calcium at a higher rate than would naturally occur and increase concentrations in lotic and downstream lentic environments (Jeziorski et al. 2008). Over time, the low pH can deplete the calcium source and cause a decline in calcium below pre-mining levels (Jeziorski et al. 2008). As the distance from the mine influence increases, calcium is diluted by other waters and can be precipitated out of solution by increases in pH.

Some sampling events in the WQP dataset are in response to specific events like the 1998 incident at the Bunker Hill Superfund site upstream from Lake Coeur D'Alene. In 1998, metal-laden sediments were disturbed and suspended into the water at the Superfund site (ATSDR 2007). This caused a spike in lotic calcium near the incident upstream from lake Coeur D'Alene that was detected in the lake as well as in the Spokane River below the lake (Figure 7). Some of these sites in the Coeur D'Alene system have been monitored in many years besides 1998 when this incident took place. Some, however, were only monitored for a short time after the event and show higher calcium levels as the only data available to assess risk. This reiterates the need to look at the data at the specific sites of interest in more detail after obtaining the big-picture information from the risk mapping application.

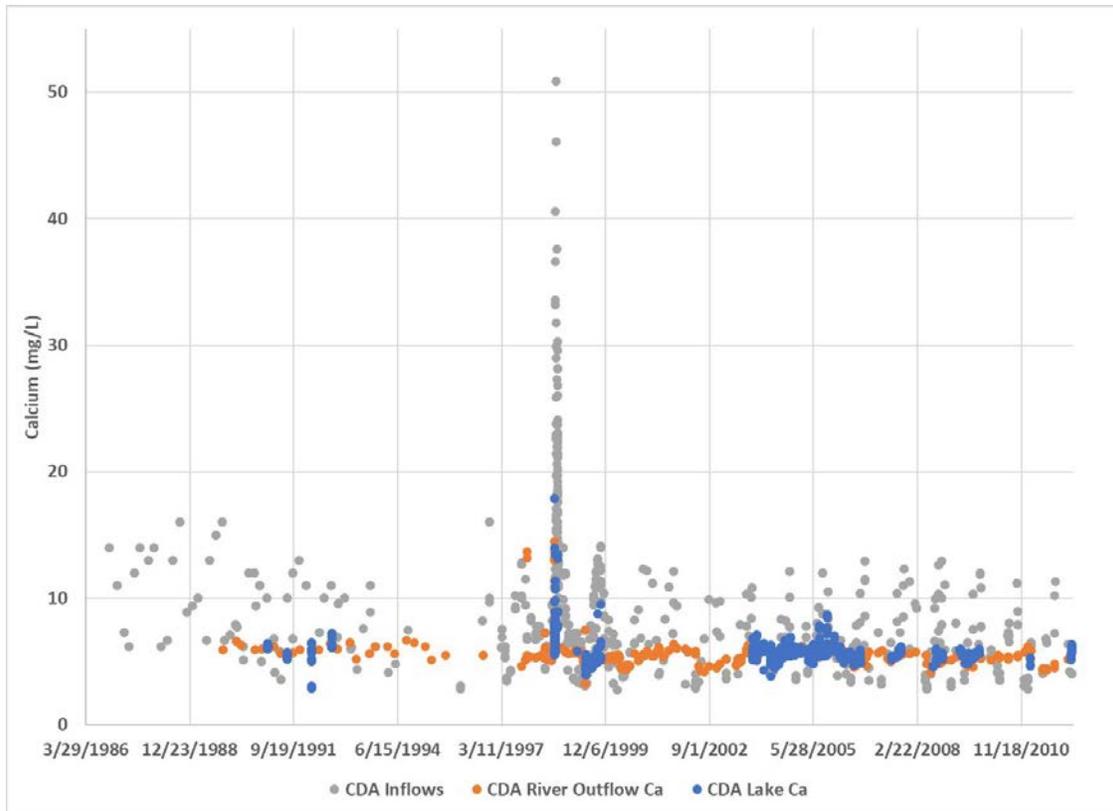


Figure 7. Calcium levels showing background calcium levels and calcium levels after disturbance at an upstream Superfund site in 1998

Geothermal influences can also impact calcium concentrations in lentic waters. The concentration of dissolved solids, such as calcium, in geothermal water can be ten times that of surface water (Arnorsson et al. 1995). As the geothermal flows mix with surface water, the carrying capacity can be decreased from a drop in pressure and/or temperature, and/or a change in pH. Similar to mines, the geothermal inflow may be a small portion of the total inflows to a lentic waterbody in the spring but a much higher portion at summer base flows. This can cause a single sampling event to not be representative of normal conditions at sites influenced by mines or geothermal.

Alkalinity

It is important to understand the variability in alkalinity because variabilities of pH and calcium levels are related to alkalinity. Alkalinity is the buffering capacity of water. In most areas, a major component of alkalinity is calcium carbonate, which is also a source of calcium (Stumm and Morgan 1996). Lentic sites in the CRB, for example, show a significant correlation between calcium and alkalinity ($p < 0.05$; Figure 8). Although calcium carbonate is often a major component of alkalinity, other compounds also contribute to overall alkalinity. The main processes that regulate alkalinity are interactions in the watershed prior to entering the lake/reservoir, in-lake processes, and evaporation (Psenner 1988; Schindler 1986). Catchment geology and pH of precipitation influence the ratio of alkalinity from the catchment vs in-lake production (Schindler

1986). Local climate, surface area, and lentic residence time will determine the relative influence of evaporation.

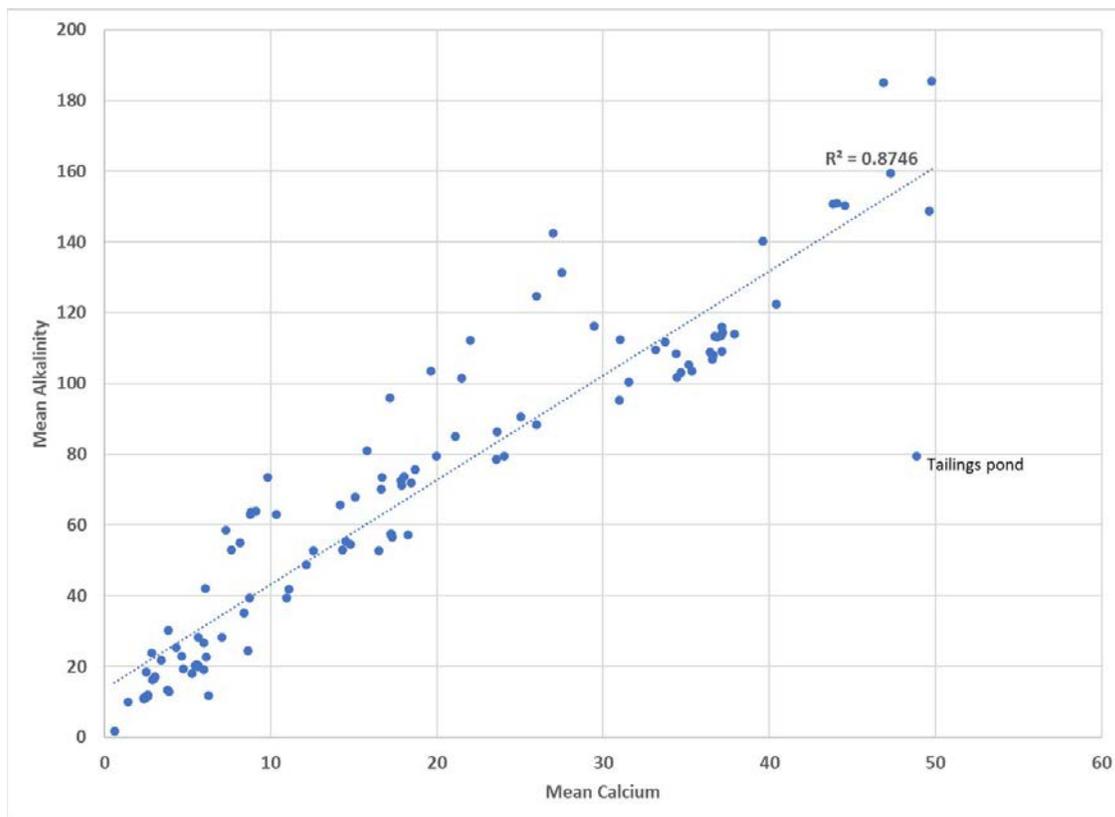


Figure 8. Calcium and alkalinity relationship at sites in the Columbia River Basin with at least 25 samples

Interactions between pH, Calcium and Alkalinity

The variability of pH within a waterbody is regulated by spatial and temporal differences in CO₂. The range of that variability is regulated by alkalinity through its buffering capacity. The significant relationship between calcium and alkalinity shown in Figure 8 suggests that calcium carbonate is a major component of both dissolved calcium and alkalinity in most CRB lentic waterbodies. In lentic environments, sites with mean pH (from profile measurements) on the low end of the pH establishment risk scale (<7.3 for moderate risk or <7.0 for low risk) often have low alkalinity (USGS 1961). The data available in the WQP shows a significant relationship between calcium and alkalinity (Figure 8). Low risk pH levels (mean <7.0) require low alkalinity and low alkalinity is correlated to low calcium risk. This relationship between pH and calcium, through alkalinity, means that when pH is in the low or moderate risk category, calcium is usually also in the low or moderate risk level. This suggests the relationships between mean pH and mussel presence in field studies may be an indirect relationship through calcium.

The WQP dataset can be used to show relationships between pH and calcium at sites in the CRB with at least five pH readings per day (Table 3). Of the 59 sites with low or moderate risk for pH, only three (5 percent) have calcium levels in the high risk category. Two of these sites are mines,

leaving just one site with an unexpected relationship between pH and calcium. An additional seven sites with low or moderate pH risk have moderate calcium risk. The remaining 49 sites (83 percent) with low or moderate pH risk also have low calcium risk. This relationship can be used to predict calcium risk for sites with at least five pH readings per day but no calcium data. This suggests the additional 59 sites with mean pH in the low or moderate pH risk, and that do not have calcium data, are not likely to be in the high risk calcium range if data were to be obtained.

Table 3. Data from sites with low or moderate pH risk and moderate or high calcium risk

Monitoring Location Identifier	Mean Calcium	Mean pH	Sample Days	pH Sample Size	pH readings per Day	Site Name	Lat	Long
NARS_WQX-NLA12_WA-108	8.1	6.6	1	21	21.0	Sacheen Lake	48.15395251	-117.31853
NARS_WQX-NLA12_ID-165	8.5	7.1	1	17	17.0	Kelso Lake	48.01025724	-116.707884
NARS_WQX-NLA12_ID-112	8.8	6.9	2	16	8.0	Perkins Lake	48.7562708	-116.090386
NARS_WQX-NLA12_WA-107	9.2	7.0	1	21	21.0	Lacamas Lake	45.61684794	-122.4258
NARS_WQX-NLA12_WA-112	10.0	7.0	1	10	10.0	Bayley Lake	48.41822976	-117.66407
NARS_WQX-NLA12_WA-118	11.0	6.8	1	7	7.0	Ravensdale Lake	47.35148384	-121.991254
21NEV1_WQX-NV03-105-R-001	14.0	6.1	1	8	8.0	Dry Creek Reservoir	41.8025017	-116.240303
USGS-471848113345301	22.0	7.2	1	20	20.0	Lake Alva	47.31326908	-113.582305
USGS-475705118053000	202.7	7.2	4	22	5.5	MIDNITE MINE PIT 4	47.9512731	-118.092754
USGS-475639118052000	305.0	4.4	3	16	5.3	MIDNITE MINE PIT 3	47.9440508	-118.089976

There are some common traits between six of the seven waterbodies with low or moderate pH risk and moderate calcium risk. The one outlier of the group is a small reservoir in the upper Owyhee River drainage. The rest all have marshy shorelines and/or visible plants/algae on the surface. Figure 9 and Figure 10 show surface vegetation on two of the lakes that are representative of conditions at the other waterbodies. Although the six sites have this surface vegetation in common, the connection between that surface vegetation and their relationship between pH and calcium is unknown. It does, however, provide another tool to improve the confidence in using mean profile pH as a predictor of calcium levels in areas without surface vegetation.



Figure 9. Surface vegetation on Perkins Lake in northern Idaho

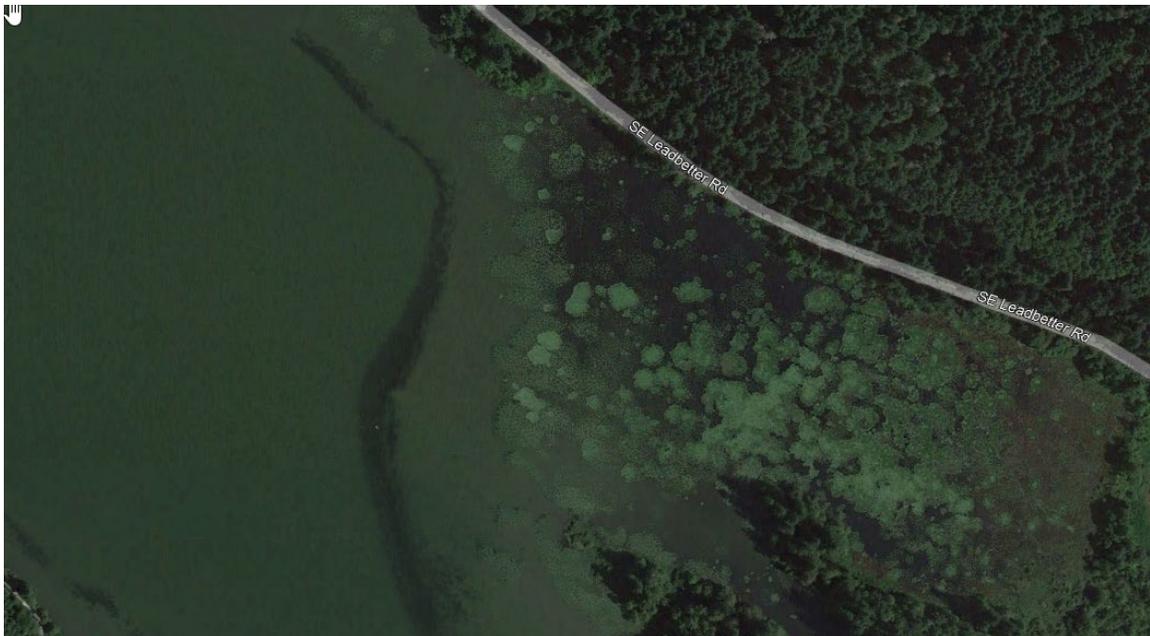


Figure 10. Surface vegetation on Lacamas Lake in Southern Washington

At the higher end of the pH risk levels (above 9.4 for moderate and above 9.6 for low risk), the carrying capacity of calcium is relatively low (Larson and Buswell 1942; Stumm and Morgan 1996). With the strong correlation between calcium and alkalinity seen in the CRB (Figure 8), relationships described by the USGS (1961) would suggest a carrying capacity for calcium of 10 mg/L at a pH of 9.4. Any pH level above 9.4 would therefore lead to supersaturation and eventual precipitation of calcium at all of the high risk and almost all of the moderate risk calcium

concentrations. The higher the pH, the more likely fluctuations in pH are to result in fluctuations in calcium.

Fluctuations in maximum pH caused by photosynthesis or precipitation events can result in higher calcium variability at these high pH (>9.4) sites than other lentic waterbodies with mean pH in the high risk category (7.3-9.4). This is a result of the high evaporation rates mentioned in the section discussing calcium variability and, although rare in the CRB, it may become more applicable as this type of risk assessment is expanded to other geographic areas. In certain circumstances (lentic areas in terminal basins; shallow waterbodies in arid climates with long residence times or extreme winds) where extremely high rates of evaporation lead to high levels of salts, calcium levels can show higher fluctuation than in other environments.

Higher salt concentrations increase pH levels. In the summer, evaporation can increase salt concentrations which causes an increase in pH and leads to precipitation of calcite and a reduction in dissolved calcium. When it rains or when snow melts, calcium is dissolved from the deposits and the process starts over. With the data available in the WQP for the CRB, this only seems to be a major influence in reservoirs in the upper Owyhee River drainage. For example, Mountainview Reservoir has calcium levels ranging from 7.0 to 32.9 mg/L among all the sites and data collection dates at the reservoir. This is one of the few areas in the CRB where individual calcium samples from the same site can produce concentrations in all three risk categories. Relative to other data sets in the CRB, this is a large range in calcium levels (Figure 6). High pH levels up to 10.1 have been documented here.

There is not enough associated data on salts or residence time available for Mountainview Reservoir to prove this is the process causing fluctuations in calcium. A search of the WQP for sites in Utah showed the highest within site range in calcium levels at lentic sites in drainage basins that terminate in the Great Salt Lake or salt flats. Other Reclamation regions that are more arid than the Columbia-Pacific Northwest may have a higher percentage of waterbodies that experience relatively high fluctuation in calcium due to these relationships.

pH as a Direct vs Indirect Indicator of Establishment Risk

Although the literature shows a correlation between mean pH and a lack of mussel establishment in the field (Ramcharan et al. 1992; Claudi and Prescott 2011), this may not simply be a direct cause of the pH levels. Lab studies have shown that pH can limit growth and survival of mussels at similar stable values to the mean values in the above field studies (Claudi et al. 2012). As discussed for pH variability, pH levels above the thermocline are higher than the overall profile mean due to photosynthesis reducing dissolved carbon dioxide. At sites with a full profile mean pH in the moderate or low risk category, pH levels above the thermocline are often suitable (74 percent) if not optimal (27 percent) for mussel survival based on lab experiments. Identifying an indirect relationship between pH and establishment of a self-sustaining mussel population could actually improve confidence in the use of pH as an establishment risk factor.

Some populations of quagga mussel have the ability to survive at depths up to 130 meters, but most populations of quagga and zebra mussels are concentrated above the thermocline (Mills et al. 1993; Yu and Culver 1999; Wacker and Elert 2003; Churchill 2013). Twenty seven percent of

sites with full profile mean pH values in the moderate or low risk category have some pH values in the optimal range (7.9-8.5) above the thermocline, where an initial infestation would be expected (Figure 11). Besides two mine tailings ponds, only one site with a full pH profile in the moderate or low risk category had calcium levels in the high risk category. Claudi and Prescott (2011) show a similar relationship in data from Ontario lakes (Figure 12). The remainder of the sites in the risk mapping application with low risk pH levels and high risk calcium levels do not have full pH profiles. Confidence in sites risk categorization at sites with no calcium data and low or moderate risk based on pH should be high as long as they are not associated with a mine or volcano.

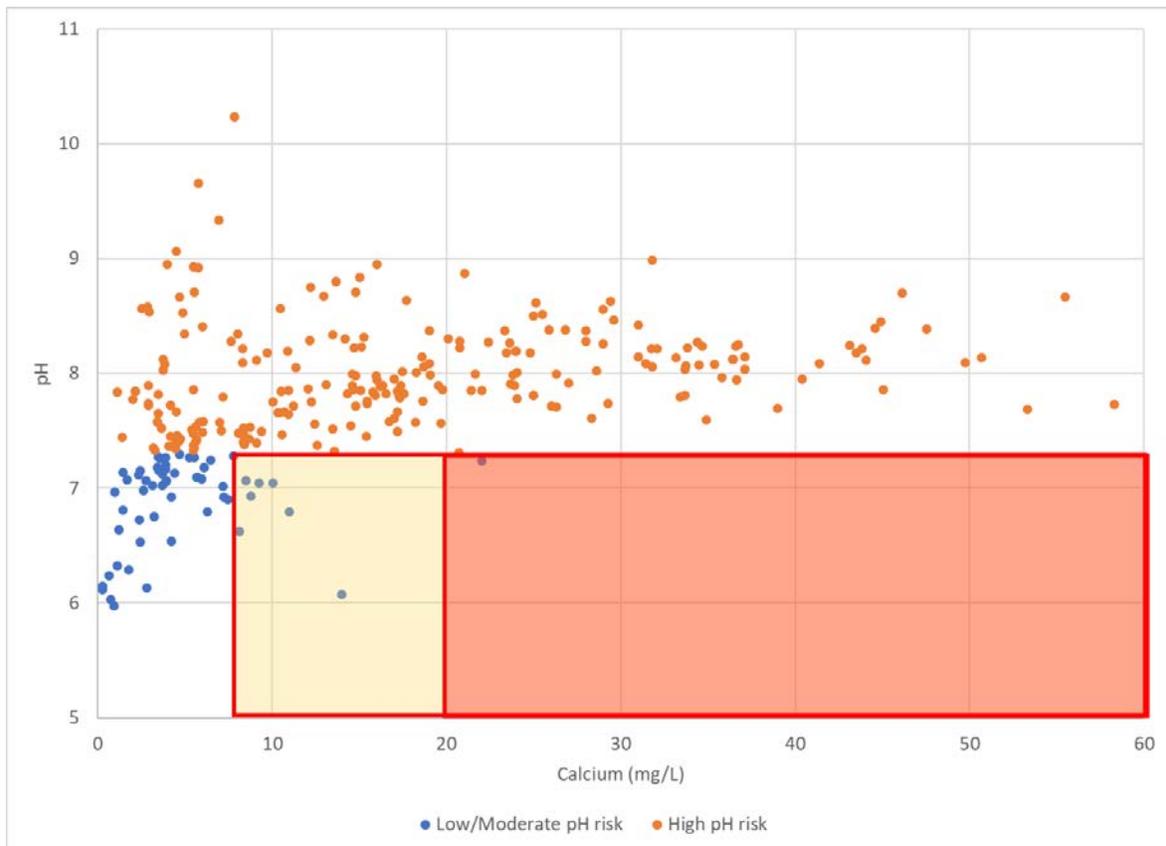


Figure 11. All sites in the Columbia Basin with at least five pH readings per sample date and at least one total calcium reading. The box indicates sites where pH is in the low or moderate risk category while calcium is in the moderate (yellow) or high (red) risk category. Data for two mine sites that would be in the red box are of the chart for calcium.

Establishment of adult zebra mussels in Ontario lakes in relation to mean pH and [Ca]

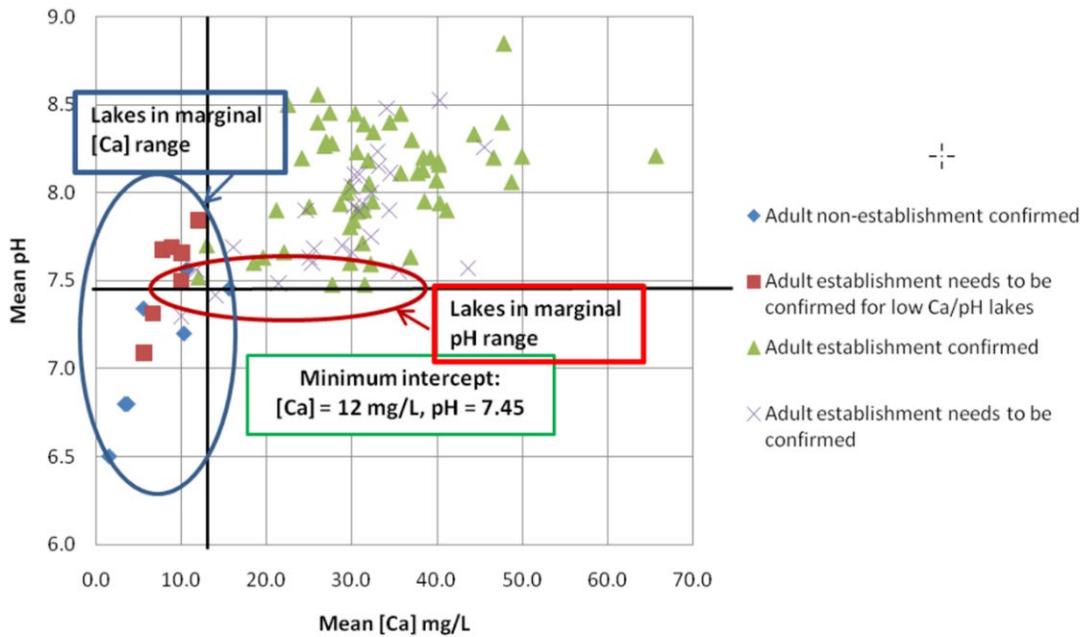


Figure 12. Relationship between calcium and pH in Ontario lakes where mussel presence is known. From Claudi and Prescott (2011).

At the higher end of the pH scale where moderate risk pH levels are defined as $pH > 9.4$ and low risk is $pH > 9.6$, pH again may not be a direct cause of poor mussel survival. Calcium levels in the moderate or high risk categories become supersaturated at mean pH levels in the moderate or low risk categories. Since calcium becomes supersaturated at these levels, it is hard, even in a lab environment, to determine if pH or calcium is playing more of a role in the lack of mussels. Either way, this should provide confidence that sites identified as low risk due to $pH > 9.6$ are truly at low risk of establishment.

The number of profiles collected at a site does not seem to play a role in the relationship between sites with moderate or low pH having either moderate or low calcium levels. At sites where large numbers of pH profiles have been collected, the range in individual daily means can span all three risk categories. This suggests a site with at least one full profile in the moderate or low risk category (even if the overall mean of means is high risk) is likely to be in the moderate or low risk category for calcium.

The mapping application's use of mean values provides a great starting point for assessing risk on a large scale. When using the mapping application to investigate establishment risk at particular waterbodies, it is important to think about the known causes of high variability. Special care should be taken if working on a waterbody in an arid basin with high evaporation rates that may cause large fluctuations in calcium. Calcium may be a more reliable and discriminating variable to use when data is available, but in the absence of calcium data, pH data can provide some information.

Future Work

If funding and staff time become available, there is potential to further evaluate establishment risk using pH. With more pH data being available than calcium, developing additional ways to predict calcium based on pH could increase the number of low risk sites where sampling effort can be reduced in favor of sampling at higher risk sites. The relationship between pH values below 7.3 and low risk calcium levels could be investigated further. There are many sites in the CRB with daily or even annual means in the low risk category for pH, but the overall mean of means displayed in the risk mapping application has a mean in the high risk category. Do sites with any individual profile mean pH below 7.3 (instead of the mean of daily means) correlate to calcium levels in the low (<8mg/L) or moderate (8-20 mg/L) risk range? Are there any exceptions to this trend besides volcanoes, mines, and bogs? Optimally, this analysis could be done by obtaining the raw data used in Ramcharan et al. (1992) or Claudi and Prescott (2011). Data from the WQP could also be used for this type of analysis in areas of the United States that are currently infested with mussels.

Python and Java code for the web application could easily be modified to expand to other geographic areas. If this risk mapping application is updated and/or expanded to other geographic areas, it should include a count of pH samples per day. This would allow for some estimation of sites with full pH profiles rather than just surface readings or a few lab samples. For the CRB, removing sites with less than five samples per day would reduce the number of sites by about half. This would however greatly improve the confidence in pH-based risk assessments. Prior to expansion or updates to the risk mapping application, it may also be worth investing the time and funding to determine if sites with at least one individual profile in the low or moderate risk category have low or moderate risk for calcium. This could increase the number of sites with moderate or low risk where sampling could be reduced in favor of high risk sites.

Other work could be done to integrate additional water quality aspects such as dissolved oxygen or physical characteristics of lentic waters such as the presence of marinas or mooring buoys for multi day use. USGS is currently working on integrating boat check station data to better inform recreational boat movement and infer introduction risk.

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Appendix 1. User Guide

1 **Title.** Columbia River Basin Dreissenid Mussel Risk Assessment Web Interface

2 **Authors.** Adam Sepulveda^{1*}, Tim Counihan², Josh Gage³, Anthony Prisciandaro⁴

3 **Affiliations.**

- 4 1. U.S. Geological Survey, Northern Rocky Mountain Science Center, Bozeman MT
5 59715. (406) 404-9155. asepulveda@usgs.gov
- 6 2. U.S. Geological Survey, Western Fisheries Research Center Columbia River
7 Research Laboratory, Cook WA 98605. (541) 645-0923. tcounihan@usgs.gov
- 8 3. Gage Cartographics LLC, Bozeman MT 59715. (406) 570-1060.
9 info@gagcarto.com
- 10 4. U.S. Bureau of Reclamation, Snake River Area Office, Boise ID. (208) 871-3529.
11 aprisciandaro@usbr.gov

12 *Correspondence to asepulveda@usgs.gov

13 **Disclaimer.** These data are preliminary or provisional and are subject to revision. They
14 are being provided to meet the need for timely best science. The data have not received
15 final approval by the U.S. Geological Survey (USGS) and are provided on the condition
16 that neither the USGS nor the U.S. Government shall be held liable for any damages
17 resulting from the authorized or unauthorized use of the data.

18 **Overview.** Zebra and quagga (*Dreissena polymorpha* and *D. rostriformis*; dreissenids)
19 mussels are prolific aquatic invaders that now occur in most major water basins in
20 eastern North America but are not yet established in the northwest. Early detection of
21 dreissenid mussels is a major pillar of managing these invaders because early detection
22 helps minimize detrimental impacts and contain their spread to other waters.
23 Determining how to best allocate dreissenid mussel early detection monitoring effort has
24 been identified as a need by DOI's "Safeguarding the West from Invasive Species"
25 Initiative, the Dreissenid Mussel Research Priorities Workshop and the 100th Meridian
26 Columbia Basin Team Dreissenid Mussel Monitoring Forum workshop. We developed a
27 web interface for viewing waterbodies with elevated dreissenid mussel establishment
28 risk in the Columbia River Basin. We estimated elevated establishment risk (minimal,
29 moderate and high) as functions of calcium concentrations or pH, which are analytes
30 critical for mussel growth and physiology and widely viewed as limiting factors. Site-
31 specific water analyte data are obtained daily from the National Water Quality Portal,
32 cleaned, summarized and then mapped on to the USGS National Hydrography
33 Dataset's stream reach segments. Our web interface allows users to easily (1) view
34 elevated establishment risk at local (e.g., stream reach) to regional (e.g., Columbia
35 River Basin) spatial scales; (2) modify analyte values that delimit minimal, moderate and
36 high establishment risk categories; (3) apply criteria to filter the quality and relevance of
37 the analyte data; (4) extrapolate risk over space; and (5) query and download the raw
38 and summarized analyte data. Principle limitations of our web interface are incomplete
39 data, as not all organizations provide water quality to the National Water Quality Portal,
40 and the unknown reliability of water analytes as predictors of dreissenid mussel risk in

41 this region, given that dreissenid mussels are not yet present. Thus, our web interface
42 should be used with caution when referencing it to make resource allocation decisions
43 about dreissenid mussel early detection efforts. The Python scripts used to execute this
44 project are publicly available so that others can advance our work or customize invasive
45 species risk assessments.

46

47 **Introduction.**

48 Zebra and quagga (*Dreissena polymorpha* and *D. rostriformis bugensis*; dreissenids)
49 mussels are prolific aquatic invaders that now occur in most major water basins in
50 eastern North America, but are not yet established in the northwest and are still patchily
51 distributed in the southwest. Once established, dreissenids can cause significant
52 economic and ecological impacts that are predicted to result in annual expenditures of
53 100s of millions of dollars for control and mitigation efforts in the Columbia River Basin
54 (Prescott et al. 2013; Nelson et al. 2019).

55 The costs of dreissenid mussel invasions are especially high for the US Bureau of
56 Reclamation (hereafter Reclamation), since mussels settle on or within water facility
57 infrastructure required for hydroelectric energy production and water storage, delivery
58 and diversion. To mitigate these costs and to prevent spread to other waters and
59 facilities, Reclamation has established an early detection program where they collect
60 over 1500 samples at 223 water bodies in 16 western states. For Reclamation and for
61 many other federal, state and tribal agencies in the West, conducting effective early
62 detection monitoring is a challenge since the resources available for monitoring are
63 disproportional to the number and size of waterbodies in the West. Indeed, determining
64 how to best allocate dreissenid mussel early detection monitoring efforts has been
65 identified as a need by DOI's "Safeguarding the West from Invasive Species" Initiative,
66 the Dreissenid Mussel Research Priorities Workshop and the 100th Meridian Columbia
67 Basin Team Dreissenid Mussel Monitoring Forum workshop. For these reasons,
68 Reclamation requested assistance to develop a regional risk assessment of dreissenid
69 mussel establishment in the Columbia River Basin (CRB), especially at or near waters
70 with Reclamation facilities.

71 Risk assessments provide a valuable framework for guiding early detection efforts
72 across broad landscapes because they summarize the landscape suitability of novel
73 areas for invading species. Greater early detection effort is usually allocated to more
74 suitable areas, where invaders are more likely to reach high abundances and cause
75 larger, more costly impacts. Suitability is characterized as the subset of environmental
76 conditions needed for the species in question to maintain self-sustaining populations
77 (i.e., the niche). These environmental conditions can be derived from a combination of
78 physiological experiments (i.e., the fundamental niche) and observational studies (i.e.,
79 the realized niche) of the species in its native and invaded areas.

80 Calcium and pH are considered to be important environmental variables that delimit
81 dreissenid mussel habitat suitability in its native and invaded areas (Whittier et al. 2008;

82 Jones et al. 2005). Calcium is required for basic metabolic function and shell building
83 and dreissenid mussels have higher calcium requirements than most other freshwater
84 mussels. The physiological importance of pH is less clear, as changes in acidification
85 can manifest in myriad and interacting ways, including shifts in ionic balance,
86 weakening of byssal threads, reduced calcification rates and shell dissolution
87 (Vinogradov et al. 1993, Claudi et al. 2012). The exact calcium and pH values that have
88 been used to delimit unsuitable from suitable habitat have been largely derived from
89 observational studies describing the dreissenid mussel realized niche. These values
90 vary across the geographic distribution of dreissenid mussels for multiple reasons,
91 including: autoecological nuisances, potential for local adaptation to a broader range of
92 environmental conditions, and unaccounted for spatiotemporal variability in
93 environmental conditions. Consequently, water quality-based risk assessments should
94 be used as a starting place for assessing elevated risk of establishment rather than as a
95 final solution. Please see **Appendix 1-A** for a thorough review on water chemistry-based
96 risk analyses for dreissenid mussels.

97 In general, water quality-based risk assessments have found that dreissenid mussels do
98 not spread to calcium-poor waters, very cold water or very warm waters and that fine-
99 scale water quality data are required for accurate assessments (Whittier et al. 2008,
100 Strayer 2009, Therriault et al. 2013, Davis et al. 2015). For example, Whittier et al. 2008
101 related broad-scale depictions (i.e., ecoregions) of alkalinity and calcium values to zebra
102 mussel occurrences in the USA. They found that most zebra mussels occurred in
103 ecoregions with higher alkalinity and calcium values, but that exceptions occurred when
104 lower calcium areas were downstream of higher calcium waters. Importantly, they also
105 found that there was too much local variability (and data gaps) in calcium data in the
106 Northwest to broadly depict this region's dreissenid mussel habitat suitability. Similarly,
107 Therriault et al. conducted broad-scale risk assessments by summarizing calcium data
108 at the sub-drainage level in Canada. They also found that local variability in calcium can
109 be high and broad scale depictions can be misleading, as sub-drainage variation in
110 calcium values was often greater than among sub-drainage variation. Finally, Davis et
111 al. 2015 found that quagga mussels have a higher risk of establishment in low calcium
112 lakes if there are adjacent habitats with slightly elevated calcium. Taken together, these
113 studies underscore that risk assessments are strongest when fine-scale water quality
114 data are used.

115 The National Water Quality Portal (WQP) provides an elegant solution to the challenge
116 of aggregating, standardizing and serving fine-scale water chemistry data collected by
117 many of the federal, state, tribal and NGO organizations in the CRB. The WQP provides
118 a single point of access to the largest standardized water quality database in the United
119 States, with over 300 million water quality records (Read et al. 2017). The WQP
120 aggregates data from multiple federal portals and numerous state, tribal and monitoring
121 organizations portals and then reconciles the various water quality data formats into a
122 common format. These data can be queried using web services, which allows for the
123 rapid exchange of data between applications and the ability to rapidly integrate new
124 data into applications. However, these water quality data were not explicitly collected for
125 inference about dreissenid mussel habitat suitability so they require scrutiny prior to
126 being used for decision-making.

127 Here, we used calcium and pH data queried from the WQP to build a dynamically-
128 updated regional risk assessment of dreissenid mussel establishment in the Columbia
129 River Basin (CRB), especially at or near waters with Reclamation facilities. We display
130 these data on a web interface mapping application with dashboard features that allow
131 users to customize the risk assessments. The intended uses of the web interface are to
132 (1) allow users to view dreissenid mussel establishment risk at different spatiotemporal
133 scales and (2) allow users to identify potential water chemistry data gaps that limit
134 confidence in risk assessments.

135

136 **Approach**

137 We used Python scripted web-serviced tools to query the WQP for applicable water
138 quality data using the following terms:

139 *HUC* = 17;
140 *Site type* = Lake, Reservoir, Impoundment, Stream;
141 *Characteristic name* = Calcium, pH.

142 We limited calcium data to just those values that described dissolved calcium and total
143 calcium concentrations. Most dreissenid mussel risk assessments use dissolved
144 calcium, however, we included total calcium in order to maximize the amount of data
145 available for this risk assessment. Dissolved and total calcium are strongly correlated
146 since dissolved calcium is a constituent part of total calcium. We then cleaned these
147 data by:

- 148 • filtering to 'ActivityMediaName' = Water;
- 149 • dropping rows with 'NA' values;
- 150 • removing white spaces;
- 151 • removing duplicated entries;
- 152 • removing negative controls (e.g., field and lab blanks);
- 153 • removing sites associating with mining activity or mining reclamation,
- 154 • standardizing units (mg/L for calcium);
- 155 • removing values outside of a sensical range (e.g., 0-14 for pH);

156 These cleaned water quality data were uploaded into a cloud-hosted PostgreSQL
157 database, where they were associated with WQP geo-referenced site metadata and
158 USGS NHD reach and HUC codes. To ensure that risk assessments are based on the
159 most up-to-date data, our scripts query the WQP every 24 hr for new data. Any new
160 data are then cleaned and passed to the cloud-hosted database. All Python scripts are
161 provided electronically and in an appendix.

162 We used calcium and pH values described in Table 1 to characterize site-specific risk of
163 dreissenid mussel establishment as minimal, moderate or high. This table was
164 developed by Wong and McMahon in consultation with the Columbia River Basin
165 Monitoring Forum. Calcium breakpoints between risk categories differed for zebra

166 mussel and quagga mussel values, so we used the zebra mussel values as the default
 167 since they provide a more conservative estimate of risk. When more than one
 168 observation occurred at a site, we calculated the mean of the analyte daily means and
 169 associated a risk category with the mean of the daily mean values. as discussed below,
 170 mean of the daily mean summary statistics resulted in comparable risk categorization as
 171 use of daily mean or medians and raw observations.

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Analyte	Species	Minimal	Moderate	High
Calcium concentration (mg/l)	ZM	< 8	8 - 20	>20
	QM	< 12	12 - 20	>20
pH	ZM	<7 or >9.6	7.0 - 7.3 or 9.4-9.6	>7.3 - <9.4
	QM	<7 or >9.6	7.0 - 7.3 or 9.4-9.6	>7.3 - <9.4

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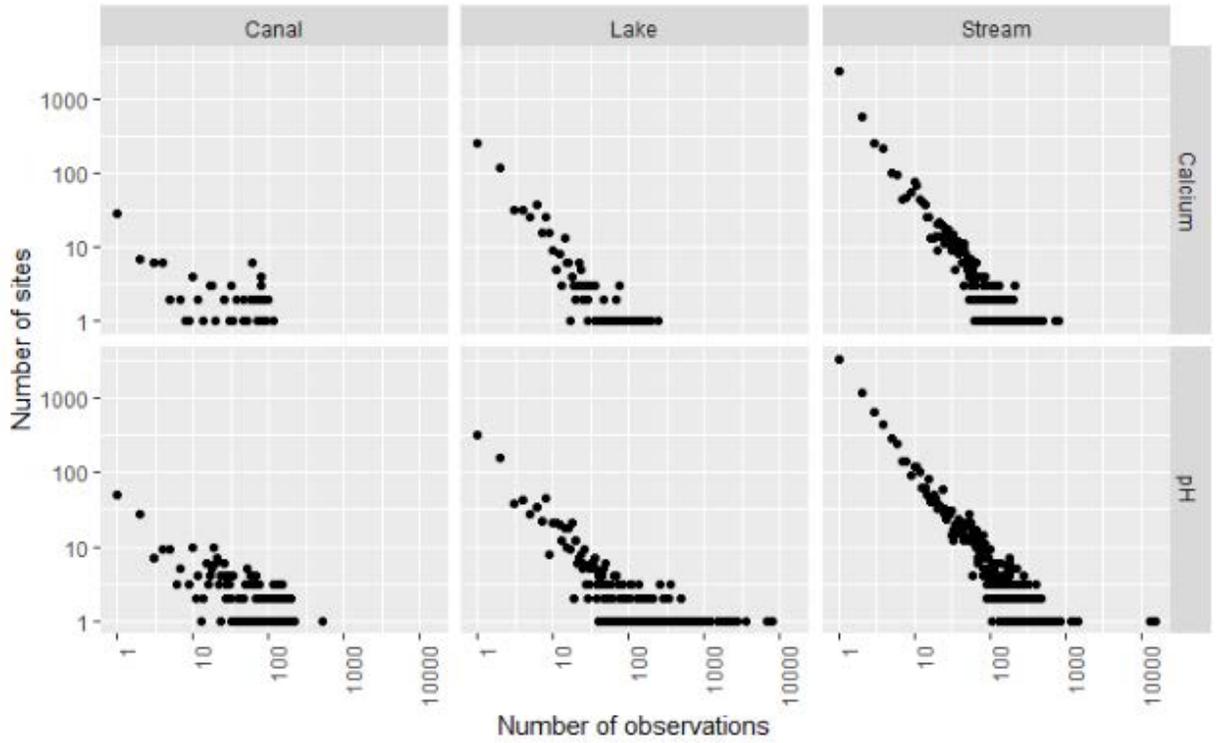
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182 *Data summary*

183 Here, we briefly summarize attributes of data that were retrieved from the WQP on 01
 184 Feb 2021 and cleaned according to our scripts. Calcium and pH data were available for
 185 5,590 and 10,577 sites, respectively. These sites were associated with the following
 186 habitat types, based on the 'MonitoringLocationTypeName' field in the WQP database:
 187 Streams and Rivers (hereafter Streams, Calcium: n = 4,756; pH: n= 8,981), Lakes and
 188 Reservoirs and Impoundments (hereafter Lakes, Calcium: n = 699, pH: n =1225),
 189 Canals (Calcium: n = 134, pH: n =369), and other (Calcium: n =1; pH : n = 2). The
 190 number of observations at a site ranged from 1 – 15,231, though most sites had
 191 between 1 – 10 observations (Figure 1). The number of daily observations at a site also
 192 ranged from 1- 15,231, though most sites only collected data for fewer than five days
 193 (Figure 2). The year that samples were collected ranged from 1900 – present, though
 194 the majority of samples have been collected since 1980 (Figure 3). Approximately 10-
 195 20% of sites per habitat type had both calcium and pH data (Table 2).

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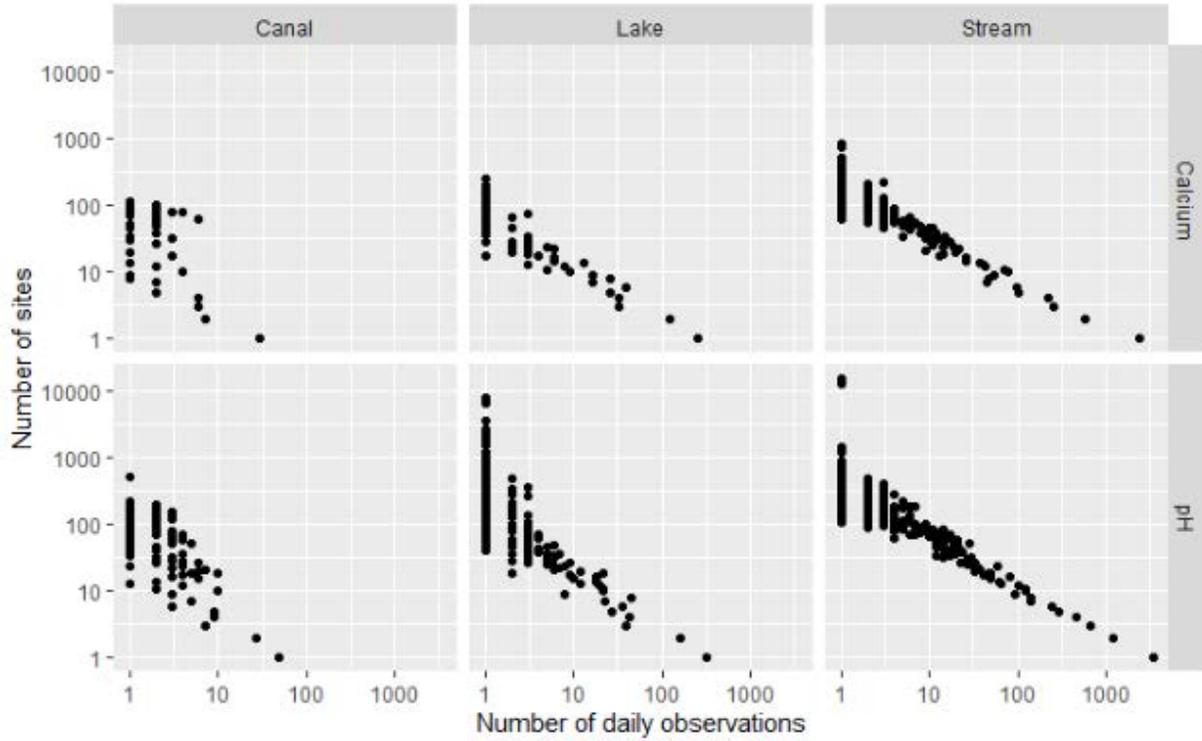


Figure 2. The number of daily calcium or pH water observations at a site in canal, lake and stream habitat types. Note the log scales on the x- and y-axes.

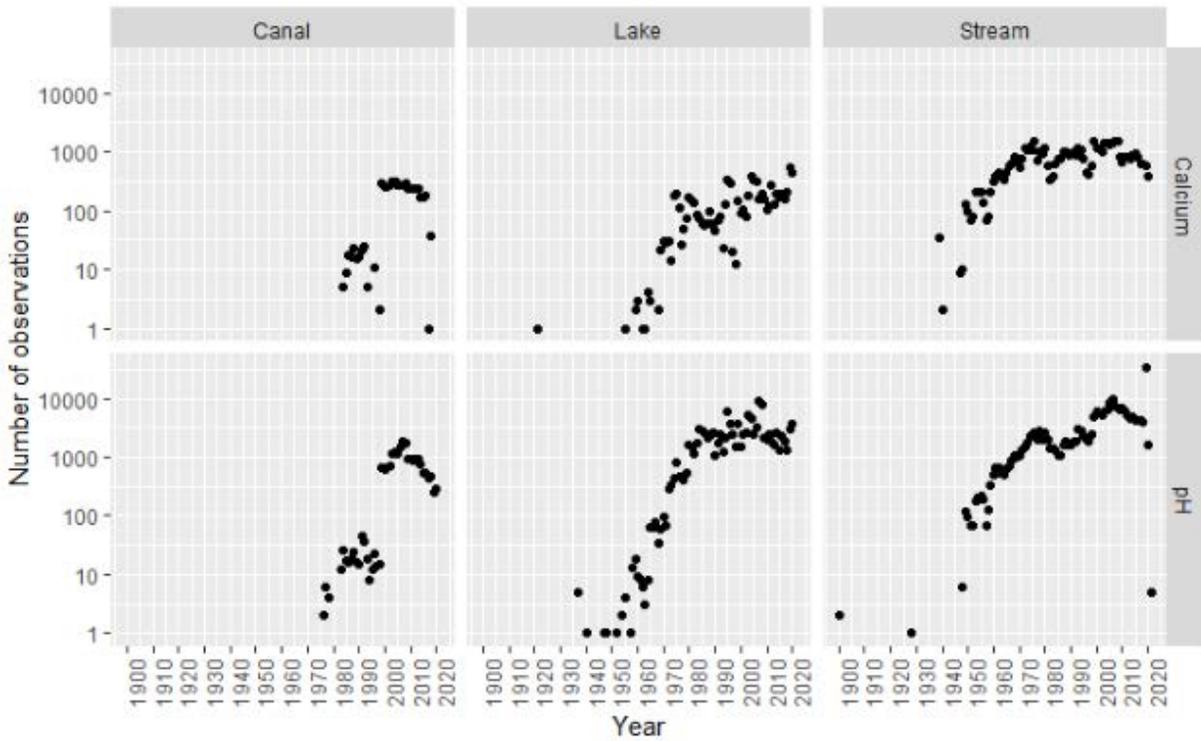


Figure 3. The number of individual calcium or pH water observations collected each year at a canal, lake and stream habitat types. Note the log scales on the y-axis.

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Table 2. Proportion of sites with only Ca data, only pH data or both Ca and pH data

Site type	Ca	pH	Both	Total
Canal	0.19	0.71	0.09	493
Lake	0.25	0.59	0.16	1953
Stream	0.21	0.62	0.17	14120

211 *Summary metrics*

212 We evaluated the tradeoffs of assigning risk categories (minimal, moderate, high) to
213 sites based on the raw data, daily means, daily medians, the mean of daily means or
214 the mean of daily medians. Averaging observation values within or across days should
215 reduce the influence of anomalous values or errors on site risk scores but could also
216 lesson important spatiotemporal variation that is meaningful to mussel habitat suitability.
217 Averaging water quality observations across space and time has been the dominant
218 approach in previous dreissenid mussel risk assessments (e.g., Ramcharan 1992,
219 Whittier et al. 2008, Therriault et al. 2013).

220 We assigned calcium and pH risk categories to a site based on the values associated
221 with each metric (data retrieved from the WQP on 01 Feb 2021). For raw data, we
222 assigned a site the highest risk category associated with any single observation. We
223 then calculated daily mean and medians of calcium and pH observations for each site
224 and assigned a site the highest risk category associated with these daily metrics.
225 Finally, we calculated the mean of daily mean and medians for each site and used
226 these estimates to determine the site risk category.

227 Approximately 48% (n = 3024) and 36% (n = 3728) of sites with calcium and pH data,
228 respectively, had only one observation; by default, metrics resulted in the same risk
229 category for these sites. There was 40-100% agreement in risk category assignment
230 whether using mean of the daily means, daily means or raw data (Tables 3-4).
231 Agreement was notably higher for calcium risk assignments than for pH. When
232 disagreement occurred, summarizing at a raw or daily time step usually resulted in a
233 one-step (e.g., High to Moderate or Moderate to Low) downgrading of the site's risk
234 category and seldom resulted in a two-step risk category change (e.g., High to Low).

235 Given the minimal differences among most metrics used to assign site-risk and the
236 potential for errors in the WQP database (Read et al. 2017), we chose to summarize
237 observations using the mean of daily mean metric.

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Table 3. Proportion of sites that changed Ca-based risk categories when comparing risks bases on the Mean of the daily mean to risks based on raw observations or daily means

Site type	Summary metric	Risk category	Number sites	Metric comparisons	To high	To moderate	To low	No change
Canal	Mean of the daily mean	Low	2	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00
		Moderate	32	Raw	0.31	0.00	0	0.69
				Daily mean	0.28	0.00	0	0.72
		High	107	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00
Lake	Mean of the daily mean	Low	346	Raw	0.00	0.05	0	0.95
				Daily mean	0.00	0.05	0	0.95
		Moderate	184	Raw	0.25	0.00	0	0.75
				Daily mean	0.20	0.00	0	0.80
		High	262	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00
Stream	Mean of the daily mean	Low	1414	Raw	0.00	0.12	0	0.88
				Daily mean	0.00	0.12	0	0.88
		Moderate	1382	Raw	0.19	0.00	0	0.81
				Daily mean	0.19	0.00	0	0.81
		High	2543	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00

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Table 4. Proportion of sites that changed pH-based risk categories when comparing risks bases on the Mean of the daily mean to risks based on raw observations or daily means

Site type	Summary metric	Risk category	Number sites	Metric comparison	To high	To moderate	To low	No change
Canal	Mean of the daily mean	Low	8	Raw	0.25	0.25	0	0.50
				Daily mean	0.25	0.25	0	0.50
		Moderate	10	Raw	0.60	0.00	0	0.40
				Daily mean	0.60	0.00	0	0.40
		High	351	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00
Lake	Mean of the daily mean	Low	180	Raw	0.16	0.14	0	0.70
				Daily mean	0.06	0.13	0	0.81
		Moderate	179	Raw	0.47	0.00	0	0.53
				Daily mean	0.33	0.00	0	0.67
		High	866	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00
Stream	Mean of the daily mean	Low	1255	Raw	0.09	0.09	0	0.82
				Daily mean	0.08	0.08	0	0.84
		Moderate	807	Raw	0.50	0.00	0	0.50
				Daily mean	0.47	0.00	0	0.53
		High	6919	Raw	0.00	0.00	0	1.00
				Daily mean	0.00	0.00	0	1.00

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257 *Limitations*

258 Our risk assessment is limited to only those CRB sites with WQP data that met our
259 inclusion and cleaning criteria. Not all agencies and organizations (especially in the
260 Canadian portion of the CRB) submit water quality data to the databases that feed the
261 WQP. In addition, the WQP does not collate and serve continuously-collected water
262 quality data (e.g., multimeter probes). Consequently, the data gaps that appear in our
263 web interface may not be indicative of reality. The WQP data are also imperfect; we
264 removed entries that contained missing or ambiguous metadata and duplicate sites and
265 results. However, our cleaned and filtered data may still have unknown errors. Thus,
266 the recommended use of our web interface is as a starting place for assessing broad-
267 scale patterns (HUC 2-10) in dreissenid mussel habitat suitability. Fine-scale
268 assessments (i.e. HUC12 - site) require additional scrutiny of these data and exploration
269 of other data not available in the WQP.

270

271 *Default values in web tool*

272 Calcium data and its associated breakpoints are used as the default analyte for
273 dreissenid mussel risk assessments. Selection of this default was identified by Bureau
274 of Reclamation partners as preferred because its importance as a dreissenid mussel
275 limiting factor is strongly supported by peer-reviewed studies and it is ubiquitously used
276 to inform risk including by most agencies in the Columbia River Basin. We still include
277 the option of evaluating risk using pH because it can provide insight about dreissenid
278 mussel habitat suitability, especially when pH values are low (< 7) and calcium data are
279 not available. In the WQP, approximately two times as many sites have pH data than
280 have calcium data. However, we urge additional caution when using pH to inform risk
281 because pH values can be influenced by biological activity so can have high
282 spatiotemporal variability; consequently, sites with only a few pH observations may miss
283 relevant pH values. For example, data collection for pH often includes a profile with
284 multiple measurements at different depths; pH is often higher near the surface where
285 CO₂ (which dissolves into carbonic acid) concentrations are reduced by photosynthesis.

286

287 *pH inference cautions*

288 Ramcharan et al. (1992a) displays the relationships between mussel presence and
289 calcium separately from pH. Data from Ontario lakes (Claudi and Prescott 2011)
290 describe a relationship between pH<7.45 and a lack of mussels. However, they have no
291 data from locations with pH<7.45 and calcium >10mg/L. The biological processes that
292 mediate carbon dioxide dynamics, mainly photosynthesis and respiration, are a major
293 source of pH fluctuation in lakes and reservoirs (USGS 1961). The extent of pH
294 fluctuation is mediated by the buffering capacity (alkalinity) of the water. Calcium
295 Carbonate is a major source of both alkalinity and dissolved calcium in most surface
296 water systems. At low calcium levels, low alkalinity allows reductions in carbon dioxide,

297 through photosynthesis, to significantly reduce pH (Strum and Morgan 1995). At high
298 calcium levels, high alkalinity buffers this reduction in pH and results in more stable and
299 higher mean pH levels (Strum and Morgan 1995). Although rare in the CRB, some other
300 acid sources can lower pH even at high calcium levels. These sources include
301 volcanoes, pyrite oxidation and acid rain (Talling 2010).
302

303 Additional support for the ability of a mean pH to predict mussel establishment risk
304 comes from a relationship between pH and mussel density as described in Ramcharan
305 et al. (1992a). Ramcharan et al. (1992b) on the other hand did not find any relationship
306 between mussel populations with variable densities and pH even though pH is one of
307 the more dynamic water quality constituents. The literature is unclear on whether the
308 relationship between pH and mussel presence is mediated by pH itself or some yet to
309 be understood interaction between pH and other water quality constituents like calcium.
310 Since low calcium levels are often needed for a site to have a low mean pH there is a
311 lack of information to support mussel establishment risk in the rare occasions when
312 volcanoes, pyrite oxidation or acid rain cause low pH at sites with high calcium. Hinks
313 and Mackie 1997 note that there is also a lack of data informing the models when both
314 calcium and pH are high, greater than 25 mg/L and 9 respectively. The datasets used in
315 this mapping tool show high spatial and temporal variability in pH. This dynamic nature
316 of pH in the field allows one day or one year of data to support one risk categorization
317 with the next day or year falling in a different risk category. Special care should be taken
318 when interpreting pH risk, especially in areas with low pH and high calcium as there is
319 little evidence to suggest mussels could or could not survive under these conditions.

320

321 *Source code for data collation, synthesis, and web display*

322 The source code for downloading, filtering and cleaning data from the WQP portal can be
323 accessed at: <https://github.com/gagecarto/aiswaterquality/tree/main/pyScripts>

324 Source code for the entire project, including the web application can be accessed at:
325 <https://github.com/gagecarto/aiswaterquality>

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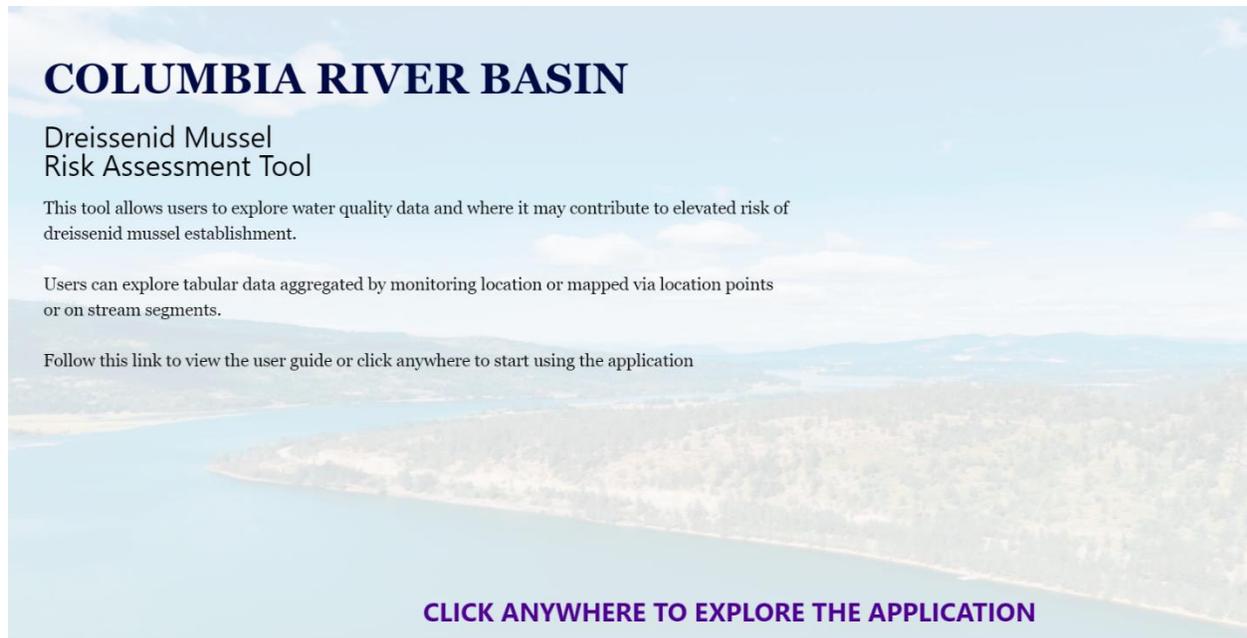
327 **User Guide**

328 Accessing the web interface

329 Use a web browser to navigate to <https://aiswaterquality.net/aiswq/index.html#crb>.

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331 Welcome page

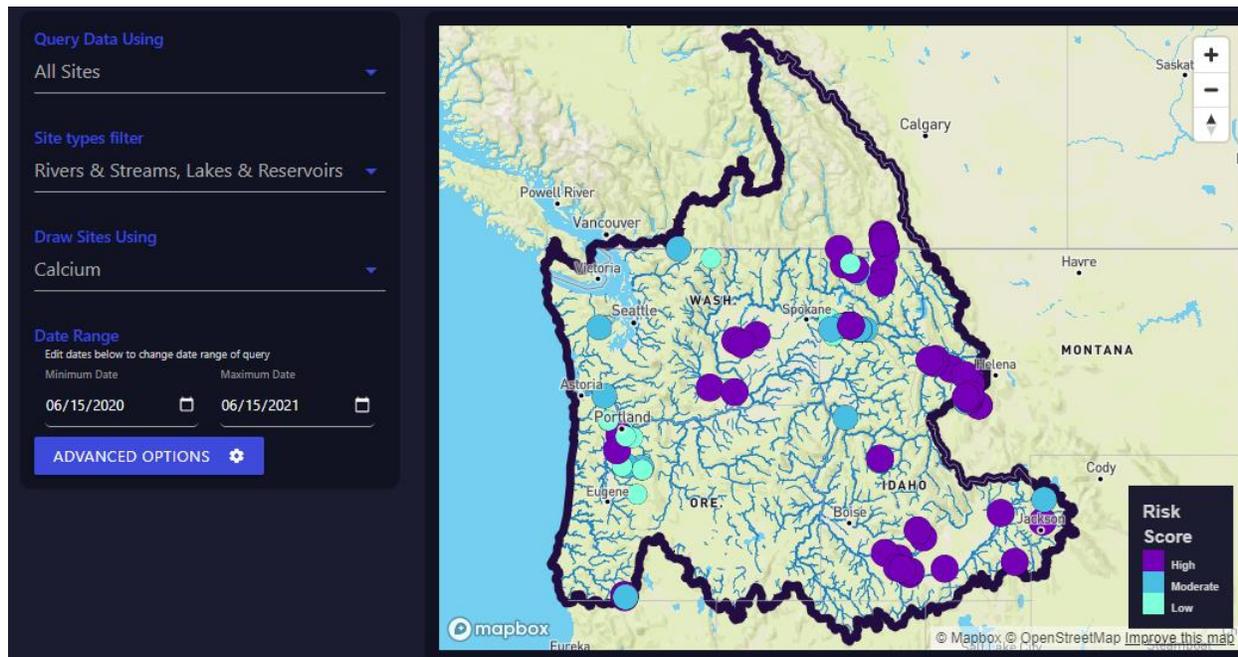


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- The user will be greeted with a welcome page that provides a brief overview of the web tool. Click anywhere on the page to proceed to the actual application.

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336 Navigating the web interface



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- Default entries are used for the initial display to expedite loading. Defaults entries include a map view of the entire CRB and a display of the lake and riverine sites with calcium data from the previous year. Users can change these defaults by selecting from dropdown menus and advanced options as described below.
 - Colored circles on the map indicate the site risk based on the water quality analyte selected in the **Draw Sites Using** dropdown menu.
 - The legend on the right of the map associates these colors with a risk category, which correspond with the default values shown in Table 1. These default values can also be viewed by clicking on **ADVANCED OPTIONS**.
 - Circles with colder colors (light green) indicate lower risk sites and warmer colors (dark purple) indicate higher risk sites.
 - Grey circles appear when zoomed in and indicate sites that have either calcium (Ca) or pH water quality data, but do not fit the queried criteria (e.g., Date Range).
 - Summarized data from the query are provided in a table at the bottom of the web interface. These data can be downloaded as a .csv file.

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- Default entries can be changed by selected the pertinent drop down menus for each major query attribute or by clicking on **ADVANCED OPTIONS**. Major query attributes include:

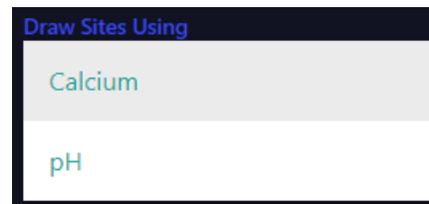
- Query Data Using

- All Sites – displays all sites with applicable WQP data in the CRB
- HUC 8 Boundaries – limits the display to only those sites within a selected HUC 8. To select a HUC 8, zoom in to the area of interest, pan over HUC 8 boundaries and double click within the HUC 8 of interest.



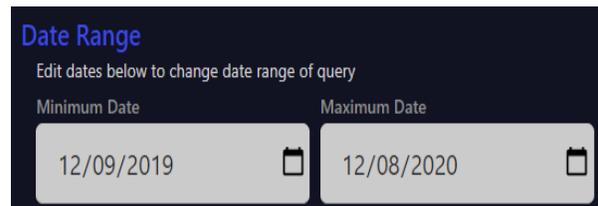
- Draw Sites Using

- Calcium – displays risk color based on calcium breakpoints for all applicable sites with calcium data.
- pH – displays risk color based on pH breakpoints for all applicable sites with pH data



- Date Ranged

- Select the month and type the desired minimum or maximum dates using the MM/DD/YYYY format. Or select the calendar symbol and then select a specific month-day-year.



402 Map Navigation

403 1. Zooming

- 404 • Left click map plus symbol to zoom in
- 405 • Left click map minus symbol to zoom out

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407 2. Panning

- 408 • Select anywhere on the map, hold down mouse
- 409 and move in the desired direction (up, down, left,
- 410 right).

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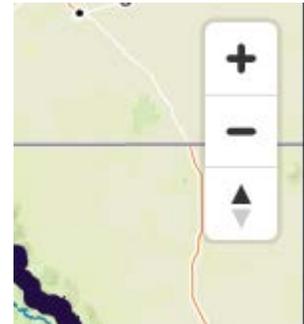
412 3. Rotating display

- 413 • Select the up/down arrow (below the zoom buttons), hold down the mouse
- 414 and rotate in the desired direction (left, right).
- 415 • To reset bearing to the North, click on the up arrow.

416

417 4. Site identification

- 418 • Slowly mouse over any hydrologic or
- 419 site-level feature to get more information.
- 420 Hydrologic information includes the
- 421 stream reach name and length. Site-
- 422 level information includes the site or
- 423 stream reach name, length and pH and
- 424 Calcium risk scores.



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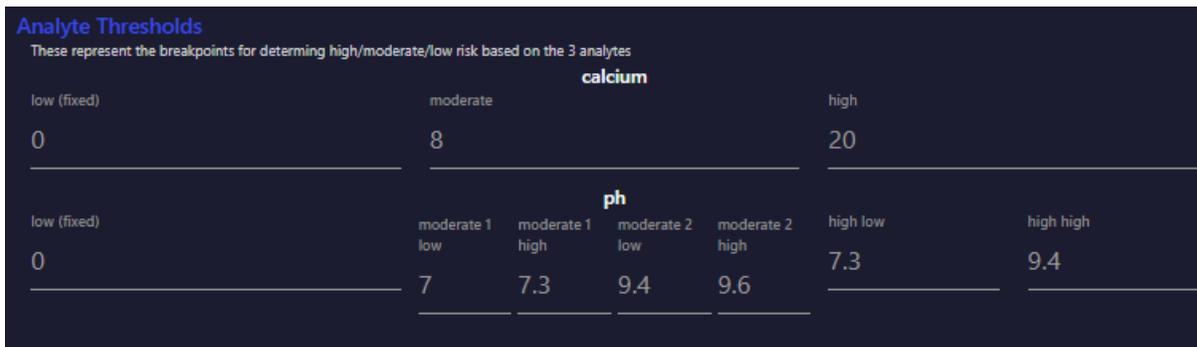
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441 Advanced Options

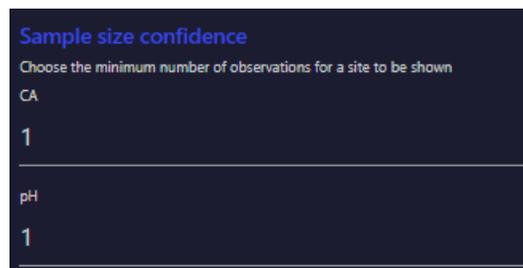
442 Users can click on the **ADVANCED OPTIONS** button to further customize the data
443 display as follows:

- 444 • Analyte Thresholds
 - 445 ○ Alter the breakpoints used for characterizing calcium and pH as high,
446 moderate, and low risk.
 - 447 ○ Select the value and type over it; or mouse over the value and then click
448 the up/down arrows to increase/decrease the value. Please notice that pH
449 breakpoints are not continuously ordered.



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- 454 • Sample size confidence
 - 455 ○ Filter by sample size confidence for
456 each analyte, so that only sites with
457 a minimum number of observations
458 are displayed.
 - 459 ○ Default is set at 1, which includes
460 all sites.
 - 461 ○ Select the value and type over it or
462 mouse over the value and then



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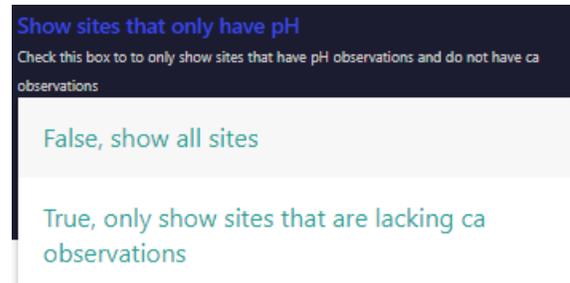
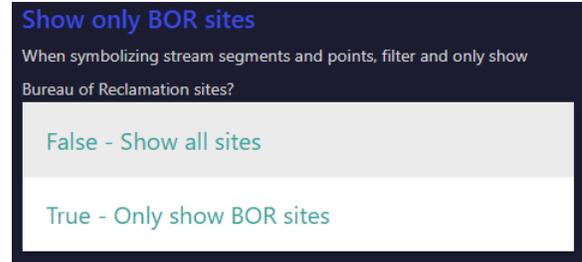
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- Show only BOR sites
 - False - Show all sites: This is the default; all sites with applicable data regardless of agency jurisdiction are displayed.
 - True - Only show BOR sites: Only BOR sites with applicable data are displayed.
- Show sites that only have pH, but lack calcium data
 - False – Show all sites: This is the default; all sites with pH data are show.
 - True – Only show sites that are lacking Calcium observations; Only pH sites without Calcium data are displayed.



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558 **Appendix 1-A.**

559

560 **Dreissenid Mussel Establishment Risk Categories**

561

562 Many environmental parameters, and combinations of parameters, have been used to
563 predict dreissenid mussel distribution potential including calcium, pH, salinity, dissolved
564 oxygen, phosphate and nitrate, air temperature, water hardness, river geomorphology,
565 and substrate type (Cohen and Weinstein 1998; Drake and Bossenbroek 2004; Jones
566 and Ricciardi 2005; Karatayev 1995; Strayer 1991; Karatayev et al. 2007; Whittier et al.
567 2008). Ramcharan et al. (1992a) distinguished lakes with established dreissenid
568 populations from those without using dissolved calcium concentration and mean pH at
569 an accuracy of 92.7%. Other environmental parameters such as maximum summer
570 bottom temperature, maximum summer surface temperature, minimum summer bottom
571 dissolved oxygen concentration, Secchi depth, and concentrations of magnesium,
572 chlorine, bicarbonate, phosphate, total phosphorus, and nitrate were evaluated by
573 Ramcharan et al. (1992a) but ultimately excluded from the model based on model
574 selection criteria. Neary and Leach (1992) used calcium, pH, and road access data to
575 evaluate the potential for introduction and establishment in Ontario lakes. Cohen and
576 Weinstein (1998) used calcium, pH, water temperature, dissolved oxygen, and salinity
577 to predict dreissenid occurrence in California water bodies, but weighted calcium and
578 pH over the other environmental parameters.

579

580 Entities participating in the Columbia River Basin Dreissenid Mussel Monitoring Forum
581 were queried about what environmental parameters they were using to assess
582 establishment risk (DeBruyckere et al. 2018). The results of the query suggest that
583 Columbia River Basin states and provinces used a range of factors that were combined
584 in different ways to form their risk assessments (Table 1). For example, Portland State
585 University conducts monitoring for various entities in the Pacific Northwest and used
586 only dissolved calcium concentrations provided in Wells et al. (2011). Montana Fish
587 Wildlife & Parks used data primarily from internal sources, considered eight risk factors
588 (Table 1), ranked them, summed scores for each factor, and then divided by the number
589 of factors to determine the composite score.

590

591 For the Dreissenid Mussel Risk Assessment Web Interface, we used dissolved calcium
592 concentrations (mg/l) and pH to characterize dreissenid mussel establishment risk at
593 water quality monitoring stations predominately in the U.S. portion of the Columbia
594 River Basin (Table 2). Dissolved calcium was selected given its support in the literature
595 and its current use by most entities in the Columbia River Basin (Table 1). We also
596 selected pH given its support in the literature and its ubiquity across water quality
597 monitoring stations, even though it is only used by a few entities in the Columbia River
598 Basin for risk assessments. Below we summarize what is known about dissolved
599 calcium and pH as dreissenid mussel limiting factors.

600

601 Dissolved calcium and pH affect metabolic functions and shell-building in dreissenid
602 mussels (Hincks and Mackie 1997, McMahon 1996) and therefore have been frequently
603 used to predict the distribution of dreissenid mussels (Cohen 2005). Calcium has

604 ubiquitously been used in forecasting potential dreissenid mussel establishment based
605 on water quality (Cohen 2005). Strayer (1991) found zebra mussels present in lakes
606 with calcium levels above 20-40 mg/l, and absent from lakes with < 20 mg/l. Sprung
607 (1987) suggested that zebra mussel larvae needed hard water with a minimum of about
608 20 mg/l of calcium. Ramcharan et al. (1992a) evaluated 76 lakes and found that zebra
609 mussels are present only where calcium concentrations are at least 28.3 mg/l. In a
610 study of 500 lakes in the former Soviet Union, Padilla (1997) found similar results. In
611 North America, however, zebra mussels have established at calcium levels ranging
612 from 10 to 25 mg/l (Mellina & Rasmussen 1994, Cohen and Weinstein 2001, Frischer et
613 al. 2005, Jones and Ricardi 2005). Ruhmann (2014) found that survival, growth, and
614 settlement of quagga mussels decreased with decreasing dissolved Calcium but that
615 survival of veligers was 24% in water with relatively low calcium (i.e., 13.4 mg/l).
616 Similarly, Davis et al. (2015) demonstrated that adult quagga mussels survived, grew
617 and showed reproductive potential in low calcium water (i.e., 12 ppm) and that veligers
618 were also able to survive, grow and settle in low calcium water.

619
620 With respect to pH, Ramcharan et al. (1992a) found that zebra mussels were absent
621 from waterbodies with pH below 7.3. Vinogradov *et al.* (1993) found that loss of sodium
622 and calcium exceeded uptake at pH levels below 6.8-6.9, and that zebra mussels were
623 generally more vulnerable than other freshwater bivalves to disruption of ion metabolism
624 from reductions in pH level. Sprung (1993) reported that in laboratory experiments a pH
625 of 7.4 to 9.4 is needed for veliger development, with peak success at around pH 8.4 in
626 18-20° C. Baker and Baker (1993) suggest that pH levels below about 7.0 preclude
627 large zebra mussel populations. Claudi et al. 2012 found that approximately 40% of
628 adults died at a pH of 6.9 after 10 weeks of exposure, that the visual loss of calcium
629 was the greatest at a pH of 6.9, and new settlement was essentially prevented at a pH
630 of 7.1. Different authors reviewing the literature have selected minimum pH
631 requirements ranging from 6.5 to 7.5 and maximum pH requirements ranging from 9.0
632 to 9.5 (Cohen 2005). We chose to use a tiered approach that characterized risk across
633 the continuum of possible pH values (Table 2). This approach has been used by others
634 (e.g., Cohen and Weinstein 1998)

635
636 To formulate risk categories of high, moderate, and minimal risk we used existing
637 literature summarized above and consultation with two prominent dreissenid mussel
638 biology subject matter experts; Dr. David Wong, Massachusetts Department of
639 Environmental Protection and Dr. Robert McMahon, University of Texas at Arlington.
640 Risk categories for quagga (*Dreissena rostriformis bugensis*) and zebra (*Dreissena*
641 *polymorpha*) mussels are listed separately in Table 2 because literature suggests that
642 these two species may have different requirements for dissolved Calcium (e.g.,
643 Karatayev 2007; Davis et al. 2015).

644
645 While we use dissolved calcium and pH as indicators of dreissenid mussel
646 establishment risk, these data need to be interpreted with the understanding that 1) the
647 data used in these assessments were not collected with intent of characterizing the
648 suitability of a waterbody for dreissenid mussel establishment and 2) there are
649 interactions of calcium and pH with other factors that could affect dreissenid mussel

650 biological requirements that remain poorly understood. Calcium and pH levels can vary
651 substantially in some water bodies, changing with location (e.g., near a river inlet to a
652 lake), depth (e.g., epilimnion vs. hypolimnion), and/or time (e.g., winter versus summer
653 or low vs. high river discharge in lentic systems)(Wetzel 1995, Cohen 2005, Hamid and
654 Jehangir 2020).

655
656 Dreissenid mussels' calcium requirements may vary with changes in other
657 environmental factors (Cohen and Weinstein 1998). Several studies conclude that zebra
658 mussels' calcium threshold varies with pH, mainly declining with increasing pH (e.g.,
659 Ramcharan et al. 1992a; Hincks & Mackie 1997). Zebra mussels' higher survival in
660 waters with naturally high calcium concentrations may possibly be due to higher
661 magnesium levels, rather than higher calcium levels (Cohen 2005). Zebra mussels may
662 also obtain some calcium from their diet. Vinogradov et al. (1993) observation that 20–
663 30% of calcium demands that can be supplied by food to mollusks may allow dreissenid
664 adults to survive at lower dissolved Calcium levels. Davis et al. (2015) found that that
665 higher levels of natural seston biomass appeared to improve adult quagga mussel life
666 history performance in low calcium water. Pynnonen (1991) suggests that more than a
667 third of dreissenid calcium demands may be met by food consumption or a combination
668 of mechanisms. Davis et al. (2015) suggest that research to determine the role of food
669 quantity, food quality, and filtration activity using across a calcium gradient would help
670 understand what factors, or combination of factors, affect mussel survival, growth and
671 reproductive potential. Davis et al. (2015) further suggest that using a single parameter
672 to assign establishment risk, given the complexity of variables in specific waterbodies
673 that may influence life history performance of introduced species, should be viewed with
674 caution.

675 Table 1. Environmental parameters used to assign dreissenid mussel establishment risk by agencies in the Columbia River Basin
 676 during 2018.

677

	BC Ministry of Environment & Climate Change Strategy	Idaho State Department of Agriculture	Montana Fish, Wildlife & Parks	Portland State University	Washington Department of Fish and Wildlife
Calcium	X	X	X	X	X
Conductivity			X		
Dissolved oxygen			X		
Hardness			X		
pH	X		X		
Secchi depth	X				
Substrate composition			X		
Water temperature	X	X	X		X
Water velocity			X		

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Table 2. Ranges of environmental parameters used to assign risk levels of high, moderate, and minimal dreissenid mussel establishment risk.

Physical Risk Factor	Species	Minimal Risk	Moderate Risk	High Risk
Dissolved Calcium Concentration	<i>Dreissena polymorpha</i>	<8 mg/l	8-20 mg/l	>20 mg/l
	<i>Dreissena rostriformis bugensis</i>	<12 mg/l	12-20 mg/l	>20 mg/l
pH	<i>Dreissena polymorpha</i>	<7.0 or >9.6	7.0-7.3 or 9.4-9.6	>7.3 - <9.4
	<i>Dreissena rostriformis bugensis</i>	<7.0 or >9.6	7.0-7.3 or 9.4-9.6	>7.3- <9.4

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