



Procedures for Applying the Mussels-Absent Ammonia Criteria to Maryland Surface Waters

Incorporated, by reference, in Code of Maryland Regulations 26.08.02.03-2.



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Water Quality Standards Section
Environmental Assessment & Standards Program (EASP)
Water and Science Administration (WSA)
Maryland Department of the Environment
1800 Washington Boulevard
Baltimore, MD 21230
Phone: 410-537-3958

Principal Author: Timothy Fox
Water Quality Standards Section
Environmental Assessment & Standards Program

Acknowledgements

Reviewers

Matthew Ashton, Maryland Department Natural Resources, 580 Taylor Ave, Annapolis, MD

Yen-Der Chang, Maryland Department of the Environment, 1800 Washington Blvd, Baltimore, MD

Lisa Ochsenhirt, Maryland Association of Municipal Wastewater Agencies

Fred Pinkney, United States Fish and Wildlife Service, Chesapeake Bay Field Office, 177 Admiral
Cochrane Dr., Annapolis, MD

Matthew Stover, Maryland Department of the Environment, 1800 Washington Blvd, Baltimore, MD

Melinda Cutler, Maryland Department of the Environment, 1800 Washington Blvd, Baltimore, MD

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Introduction

The United States Environmental Protection Agency published updated aquatic life water quality criteria for ammonia in 2013 (USEPA 2013a). The derivation of the 2013 criteria incorporated toxicity data for several sensitive freshwater mussel species in the Family Unionidae (and other gastropods) that had not previously been available. The updated criteria are more stringent than the previous national criteria because these species are comparatively sensitive to ammonia (USEPA 1999).

The diversity of freshwater mussels (Bivalvia: Unionidae) in North America is unmatched globally, yet they are among the most imperiled aquatic fauna on the continent. The high rate of imperilment of mussels has been linked to habitat and flow alteration, invasive species, loss of host fish, increased siltation, and degradation of water quality. This decline has likely had major implications on the function of aquatic ecosystems along with the conservation and restoration of aquatic species. Freshwater mussels have long been considered indicators of good water quality. More recently, their sensitivity to pollutants, such as ammonia, has been recognized as greater than that of other freshwater fauna previously used to derive water quality standards.

The recommended national ammonia criteria are protective of freshwater mussel species and will apply to all of Maryland's fresh surface waters. The updated criteria magnitudes are a function of pH and temperature and are bifurcated to take into account the presence or absence of salmonids. Specifically, the salmonids-absent criteria are applied to Class I(P) streams while the salmonids-present criteria are applied to Class III(P) and Class IV(P) streams. The criteria are codified in 26.08.02.03-2 and the criterion magnitudes are expressed as the following equations:

Equation 1: National Acute Criterion Magnitude for Ammonia (salmonids-present)

$$CMC = MIN \left[\left(\frac{0.275}{1 + 10^{7.204 - pH}} + \frac{39}{1 + 10^{pH - 7.204}} \right), \right. \\ \left. \left(0.7249 * \left(\frac{0.0114}{1 + 10^{7.204 - pH}} + \frac{1.6181}{1 + 10^{pH - 7.204}} \right) * (23.12 * 10^{0.036 * (20 - T)}) \right) \right]$$

Equation 2: National Acute Criterion Magnitude for Ammonia (salmonids-absent)

$$CMC = \left[0.7249 * \left(\frac{0.0114}{1 + 10^{7.204 - pH}} + \frac{1.6181}{1 + 10^{pH - 7.204}} \right) * MIN \left(51.93, \right. \right. \\ \left. \left. 23.12 * 10^{0.036 * (20 - T)} \right) \right]$$

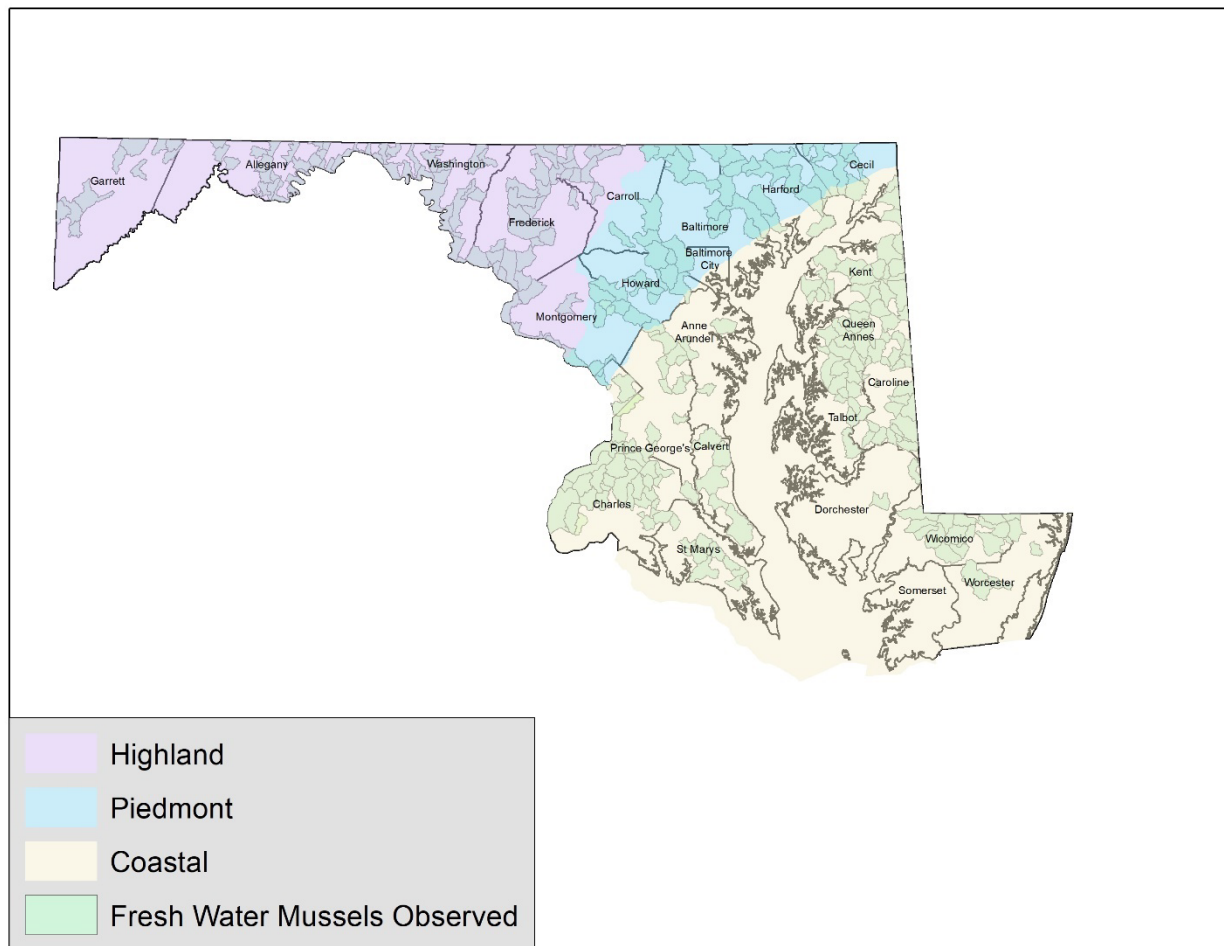
Equation 3: National Chronic Criterion Magnitude for Ammonia

$$CCC = \left[0.8876 * \left(\frac{0.0278}{1 + 10^{7.688 - pH}} + \frac{1.1994}{1 + 10^{pH - 7.688}} \right) * (2.126 * 10^{0.028 * (20 - MAX(T, 7))}) \right]$$

USEPA (2013a, appendix N) also provides recalculated site-specific water quality criteria that are intended to be applied to water bodies where freshwater mussels are absent. Data have shown that mussels have been observed throughout the state of Maryland (Figure 1), and have likely been historically present in most 12-digit watersheds. Furthermore, the distribution of mussels is not limited

by the Use Class of the surface waters. However, other abiotic factors are likely to limit freshwater mussel populations in Maryland and as a result, the mussels absent ammonia criteria, calculated without freshwater mussel toxicity data, are also codified under 26.08.02.03-2.

Figure 1: Confirmed Freshwater Mussel Observations within each Ecoregion.



In streams where salmonids are present (Use Class III or Use Class IV) but mussels are absent, the following equation can be used to replace the acute ammonia criterion when calculating aquatic life-based discharge limits:

Equation 4: National Acute Criterion Magnitude for Ammonia (salmonids-present, mussels-absent)

$$MIN \left[\left(\frac{0.275}{1 + 10^{7.204 - pH}} + \frac{39}{1 + 10^{pH - 7.204}} \right), \left(0.7249 * \left(\frac{0.0114}{1 + 10^{7.204 - pH}} + \frac{1.6181}{1 + 10^{pH - 7.204}} \right) * (62.15 * 10^{0.036 * (20 - T)}) \right) \right]$$

In streams where salmonids are absent and mussels are absent, the following equation can be used to replace the acute ammonia criterion when calculating aquatic life-based discharge limits:

Equation 5: National Acute Criterion Magnitude for Ammonia (salmonids-absent, mussels-absent)

$$\left[0.7249 * \left(\frac{0.0114}{1 + 10^{7.204 - pH}} + \frac{1.6181}{1 + 10^{pH - 7.204}} \right) * \text{MIN} \left(51.93, 62.15 * 10^{0.036 * (20 - T)} \right) \right]$$

In streams where early life stage (ELS) protection is necessary but mussels are absent, the following equation can be used to replace the chronic ammonia criterion when calculating aquatic life-based discharge limits:

Equation 6: National Chronic Criterion Magnitude for Ammonia (ELS-present, mussels-absent)

$$\left[0.9405 * \left(\frac{0.0278}{1 + 10^{7.688 - pH}} + \frac{1.1994}{1 + 10^{pH - 7.688}} \right) * \text{MIN} \left(6.920, 7.547 * 10^{0.028 * (20 - T)} \right) \right]$$

In streams where early life stage (ELS) protection is not necessary and mussels are absent, the following equation can be used to replace the chronic ammonia criterion when calculating aquatic life-based discharge limits:

Equation 7: National Chronic Criterion Magnitude for Ammonia (ELS-absent, mussels-absent)

$$\left(0.9405 * \left(\frac{0.0278}{1 + 10^{7.688 - pH}} + \frac{1.1994}{1 + 10^{pH - 7.688}} \right) * \left(7.547 * 10^{0.028 * (20 - \text{MAX}(T, 7))} \right) \right)$$

Although both the “mussels present” and the “mussels absent” versions of the criteria are adopted into Maryland regulations, the Department will use the default assumption that freshwater mussels are present in all surface waters because of the ecosystem services these sensitive organisms provide and the fact that freshwater mussels have the ability to inhabit a variety of aquatic habitats. However, as this document outlines, the presence of freshwater mussel habitat and freshwater mussels themselves can be limited by natural stream characteristics. Therefore, in certain instances, it may be appropriate to use site-specific mussels absent criteria to derive Water Quality Based Effluent Limits (WQBEL) in surface waters where freshwater mussels are highly unlikely to be found under natural conditions.

A “performance-based” approach will be used to determine the application of the Maryland ammonia criteria in order to streamline criteria implementation. According to USEPA, a performance-based approach has the following characteristics:

- A performance-based approach relies on adoption of a process (i.e., a criterion derivation methodology) rather than a specific outcome (i.e., concentration limit for a pollutant).
- A performance-based approach must be sufficiently detailed and have suitable safeguards to ensure a predictable and repeatable outcome.
- A performance-based approach relies on the State specifying an implementation procedure (methodologies, minimum data requirements, and decision thresholds) in its water quality standards regulations. This ensures that the structure or decision-making framework is binding,

clear, predictable, and transparent. The implementation procedure should consider any special needs of federally listed, threatened, or endangered species and/or their critical habitat.

- Performance-based implementation procedures should also include a public participation step to provide all stakeholders and the public an opportunity to review the data and calculations supporting the site-specific application of the implementation procedure.
- If a performance-based approach is used to implement a criterion, MDE would be required to maintain a publicly available, comprehensive list of all site-by-site decisions made using the procedure. However, such a list would not need to be codified into regulations.

The purpose of this document is to outline a detailed process for determining when and to what scale the mussels absent criteria could be applied. This document provides the process, minimum data requirements, and decision-making framework. It also specifies how federally endangered species are to be protected and how the public will be engaged in this process.

Typically, the absence of freshwater mussel communities is demonstrated through on-site surveys conducted by certified biologists. Maryland has decided to not rely exclusively on such surveys to demonstrate the absence of freshwater mussels for several reasons. Reasons include:

- Mussel sampling designs and surveys are not appropriate for all types of streams. For instance, to have a high confidence that mussels are not present in a small stream, a significant area of coverage is required. In small streams with limited habitat this could require that a survey extend for hundreds of meters beyond the area influenced by a particular discharge.
- A false negative is possible when conducting a freshwater mussel survey. For example, a survey can fail to observe a freshwater mussel when one is present. This could be due to very low abundance and cryptic behavior (i.e. concealment by burying themselves). Furthermore, freshwater mussels may be extirpated from suitable habitat due to other anthropogenic factors. Such streams may be naturally or artificially repopulated if environmental conditions improve.
- Not all permit holders may be able to afford the cost of comprehensive mussel surveys.

In order to ensure that the mussels absent criteria are only applied to surface waters that cannot sustain freshwater mussel populations under natural conditions, abiotic stream characteristics will be quantified to estimate the likelihood of the presence of freshwater mussel habitat. In cases where it is unlikely but possible that freshwater mussel habitat is present, an onsite mussel survey will be required to confirm the absence of freshwater mussels.

The procedure described below must be implemented to demonstrate (to the satisfaction of the Department) that a receiving waterbody associated with a point-source discharge does not contain freshwater mussel habitat. If it can be shown that a receiving waterbody does not contain freshwater mussel habitat, the Department may allow the use of the mussels absent criteria to calculate water quality-based effluent limits for ammonia. The determination whether to allow the use of the mussels absent criteria will be made by MDE's Wastewater Permit Program after consultation with the applicant.

Determining Where the Mussels Absent Criteria May be Appropriately Applied

Appendix N of USEPA (2013) states that:

“In the case of ammonia, where a state demonstrates that mussels are not present on a site-specific basis, the recalculation procedure may be used to remove the mussel species from the national criteria dataset to better represent the species present at the site.”

In certain cases, MDE feels that allowing the use of the mussels absent criteria to calculate ammonia discharge limits is scientifically justified based on analysis of Maryland’s freshwater mussel distribution. MDE derived a logistic regression model using data from approximately 1300 sites that was collected by the Maryland Department of Natural Resources’ Maryland Biological Stream Survey (MBSS). The MBSS collected these data from randomly selected stream locations throughout Maryland with each station consisting of a 75-meter stream section. The MBSS database has recorded mussel observations along with several other habitat and abiotic factors at each station. The model derived from the MBSS data used the probability of mussel observations as a response variable and the following abiotic factors as predictors:

- Log Stream Gradient (Slope)
- Log Discharge Rate (Flow)
- Log Average Stream Width (Width)
- Log Upstream Catchment Area (Area)

MDE evaluated several variations of the logistic regression models based on the Akaike Information Criterion (AIC), Type I and Type II error rate, and significance of error reduction. In addition, MDE also evaluated a dummy variable (also known as a categorical variable) representing the ecoregion of each site (Coastal Plain, Piedmont, and Highlands).

Results showed that the best approach was to include dummy variables for each of the three ecoregions, which resulted in three separate equations. The models that had the lowest probability of falsely classifying a site as mussels absent are expressed in the following equations:

Equation 8: Mussel-absent likelihood equation (Coastal Plain)

$$\frac{e^{\left[\frac{LN(area)*0.98 - LN(slope)*1.3 + LN(flow)*0.9 - LN(widt\ h)*0.26 - LN(flow)*LN(area)*0.12 + LN(slope)*LN(area)*0.14 + LN(flow)*LN(widt\ h)*0.2 - 7.7}{LN(slope)*LN(area)*0.14 + LN(flow)*LN(widt\ h)*0.2 - 7.7} \right]}}{1 + e^{\left[\frac{LN(area)*0.98 - LN(slope)*1.3 + LN(flow)*0.9 - LN(widt\ h)*0.26 - LN(flow)*LN(area)*0.12 + LN(slope)*LN(area)*0.14 + LN(flow)*LN(widt\ h)*0.2 - 7.7}{LN(slope)*LN(area)*0.14 + LN(flow)*LN(widt\ h)*0.2 - 7.7} \right]}}$$

Equation 9: Mussel-absent likelihood equation (Piedmont)

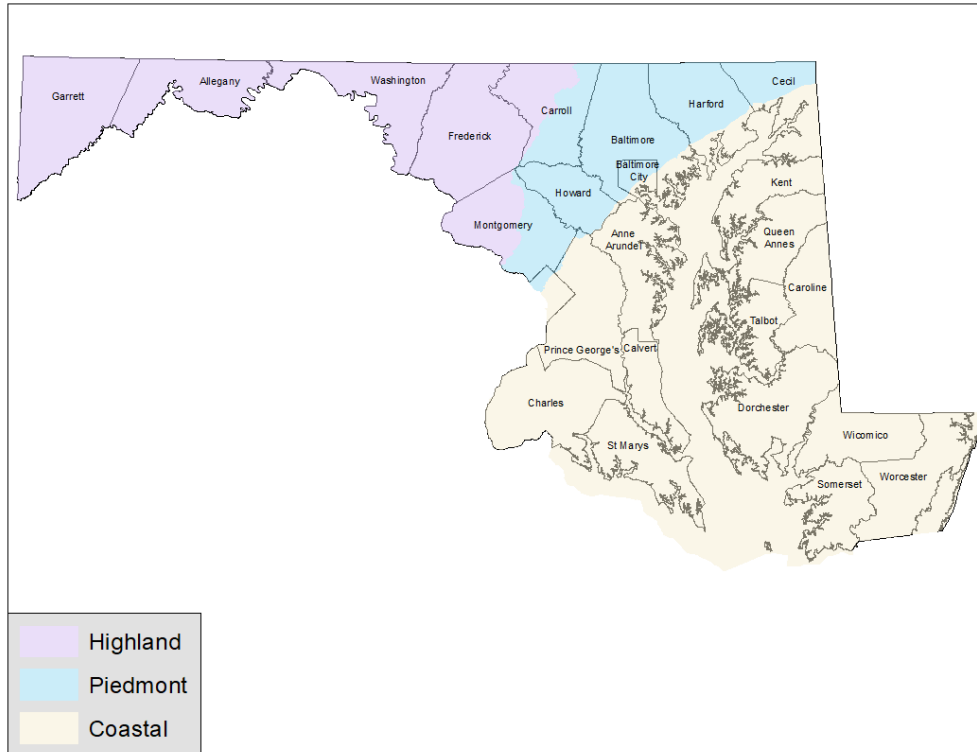
$$\frac{e^{\left[\frac{LN(area)*0.3 - LN(slope)*1.7 + LN(flow)*0.5 + LN(width)*1.5 - LN(flow)*LN(area)*0.12 +}{LN(slope)*LN(area)*0.14 + LN(flow)*LN(width)*0.2 - 8} \right]}}{1 + e^{\left[\frac{LN(area)*0.3 - LN(slope)*1.7 + LN(flow)*0.5 + LN(width)*1.5 - LN(flow)*LN(area)*0.12 +}{LN(slope)*LN(area)*0.14 + LN(flow)*LN(width)*0.2 - 8} \right]}}$$

Equation 10: Mussel-absent likelihood equation (Highlands)

$$\frac{e^{\left[\frac{LN(area)*0.98 - LN(slope)*1.3 + LN(flow)*0.5 + LN(width)*1.5 - LN(flow)*LN(area)*0.12 +}{LN(slope)*LN(area)*0.14 + LN(flow)*LN(width)*0.2 - 13.8} \right]}}{1 + e^{\left[\frac{LN(area)*0.98 - LN(slope)*1.3 + LN(flow)*0.5 + LN(width)*1.5 - LN(flow)*LN(area)*0.12 +}{LN(slope)*LN(area)*0.14 + LN(flow)*LN(width)*0.2 - 13.8} \right]}}$$

In the equation above, “LN” is the natural log function. Upstream catchment area (area) is measured in acres. Stream gradient (slope), which is the change in height over the 75-meter sampling station, is measured in meters to two significant digits. Stream width (width) is measured in meters. Discharge (flow) is measured in cubic feet per second. A map delineating the boundaries of the three ecoregions is shown in Figure 2. A complete description of the derivation of Equations 8, 9 and 10 is provided in MDE (2021).

Figure 2: Maryland Ecoregions



When the measured values of the abiotic predictor variables are entered into these equations, a value between 0 and 1 is calculated.¹ Data have shown that the streams with predictor variables that result in a value less than 0.03 are streams that are very unlikely to contain freshwater mussel habitat under natural conditions (MDE 2021). For example, out of 1309 MBSS sites in Maryland, 438 had a predicted value less than 0.03, and only 4 of those sites had freshwater mussel observations. A further review of these four sampling events revealed that these observations were outliers and did not represent suitable mussel habitat under natural conditions.

Streams with abiotic predictor variables that result in a value less than 0.03 are usually small first order streams with very low flow and/or high gradient. This observation is expected because the life history of freshwater mussels prevents them from inhabiting small streams. Thus, in order to use the mussels absent criteria to calculate discharge limits, MDE requires that the receiving water have associated abiotic factors that result in a value less than 0.03 based on Equations 8, 9, or 10.

¹ The number e is an irrational number that can be approximated by using 2.718.

Historic Ranges and Threatened and Endangered Mussels

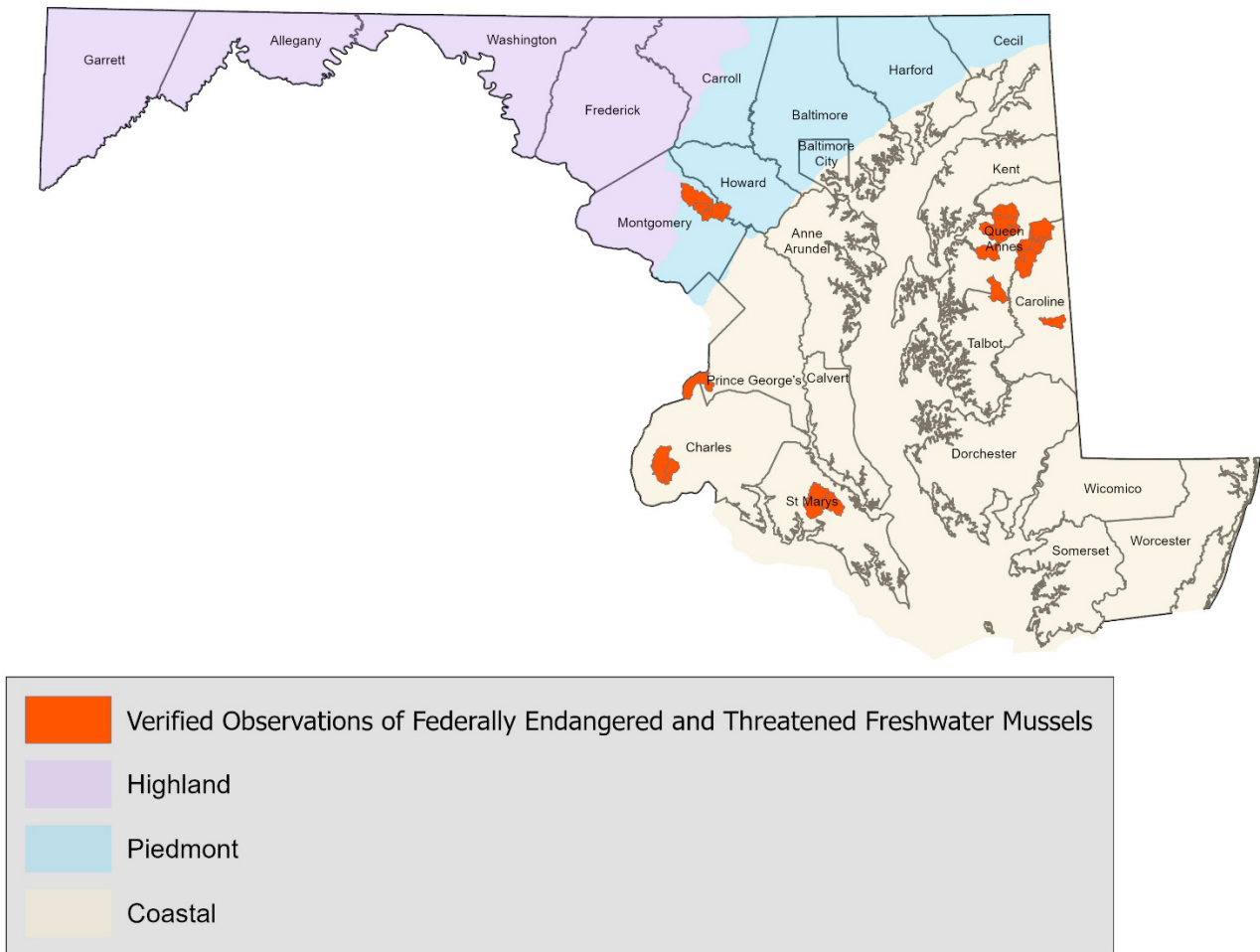
Historic ranges of federally threatened or endangered species were not taken into consideration when developing the statistical model. However, even when rare or threatened freshwater mussel species are lost, other species typically remain, and the presence of mussel habitat is supported by both the statistical model and the actual presence of mussels. There are only a few instances in Maryland where the entire freshwater mussel community has been lost from a particular stream or watershed due to anthropogenic factors (e.g., Gwynns Falls). Here, suitable stream habitat characteristics still exist and are predicted by the model. Furthermore, the numerical values of the abiotic factors are independent from the anthropogenic disturbance.

In order to ensure the protection of federally endangered and threatened species, the State has identified a list of 12-digit watersheds that have verified observations of federally endangered and threatened freshwater mussels. These watersheds are listed below and will be updated as new information becomes available. List last updated November 25, 2025.

- 021304050522
- 021304050534
- 021304050535
- 021304050536
- 021304050537
- 021304050542
- 021304050541
- 021305080399
- 021305080401
- 021305080402
- 021402010792
- 021402010793
- 021305070397
- 021305080403
- 021401100777
- 021401100776
- 021401040721
- 021401040724
- 021401040723
- 021304040490
- 021311070942
- 021311070945
- 021311070944
- 021311070943

A map displaying the locations of these 12-digit watersheds is shown below in Figure 3. The mussels absent criteria will not be applied to these watersheds.

Figure 3: Map of areas where mussels absent criteria will not apply. Last updated November 25, 2025.



Process for Justifying and Using the Mussels Absent Criteria to Calculate Water Quality Based Effluent Limits

The use of the Mussels Absent criteria to calculate water quality-based effluent limits will require the completion of several steps that are enumerated below. These steps are to be completed prior to the Discharge Permit Application Processing requirements codified in COMAR 26.08.04.01-2.

1. Initial Review by Permit Writer
2. Determine location of sampling station(s)
3. Collection of Site-Specific Abiotic Predictor Variables at All Applicable Sampling Locations
4. Conduct Freshwater Mussel Survey (if applicable)
5. Submit data and results to MDE
6. Review by Maryland Department of Natural Resources and United States Fish and Wildlife Service
7. Mussels absent criteria is applied to stream segment
8. Include Ammonia limits in the draft NPDES permit and in the public participation process for permit review

It will be the responsibility of the discharger to provide MDE with all of the necessary information, obtained in accordance with this document, for the justification of the use of the mussels absent criteria to calculate water quality-based effluent limits (WQBELs).

These steps are further described below.

Step 1: Initial Review by Permit Writer

If an applicant would like to pursue the possibility of using the mussels absent criteria to calculate WQBELs, they will need to petition the MDE Wastewater Permits Program. The MDE permit writer will reference the MBSS database to obtain any relevant information such as the presence of mussel observations or sampling events showing that the probability of mussel observation is low in the receiving stream. Furthermore, the permit writer may notify the Maryland Department of Natural Resources Environmental Review Program to determine if there are any issues with implementing the mussels absent criteria in this receiving water. The permit writer will also reference the list of 12-digit watersheds that are not eligible for the mussels absent criteria. The decision regarding which criteria to use to develop ammonia limits will be made on a case-by-case basis. The MDE permit writer will consider a number of factors including but not limited to the past performance of a facility, and options for managing various characteristics of the effluent. In most cases, the mussels absent criteria will only be made available when the MDE Wastewater Permits Program determines that the more stringent mussels present criteria cannot be met, even when considering the localized pH and the temperature post-mixing with the discharge. If the MDE Wastewater Permits Program determines that the use of the mussels absent criteria may be justified, the applicant will be able to quantify the likelihood that the receiving stream provides freshwater mussel habitat using Equation 8, 9 or 10.

Step 2: Determine Location of Sampling Station(s)

If the MDE Wastewater Permits Program determines that the use of the mussels absent criteria to calculate permit limits may be justified, the geographic extent to which the less stringent criteria applies must be delineated. To determine the geographic extent, the permittee must collect data from one or more sampling locations downstream from the point-of-discharge. Ammonia as a water quality parameter is non-conservative in surface water and will decrease downstream as the ammonia is converted to other nitrogen species. When the end-of-pipe water quality-based effluent limit is established, the MDE Wastewater Permits Program will determine the downstream location (mussels absent criteria boundary) where the mussels present criteria will eventually be met using a water quality model based on the Streeter-Phelps equation. Field data may also be required to calibrate the water quality model or demonstrate that the mussels present criteria will be met closer to the point of discharge.

In keeping with the 75-meter stream sampling protocols used to develop the logistic regression model (MDE 2021), 75-meter segments will be sampled for gathering the abiotic data (e.g. stream gradient, average stream width, etc.) used in the model equations. The number of 75-meter sampling stations required to justify the use of the mussels absent criteria is determined based on the distance between the point-of-discharge and the mussels absent criteria boundary. The following set of rules describe and illustrate the sampling requirements as a function of distance between the point-of-discharge and the mussels absent criteria boundary. Please note that if access to any desired sampling location is not available, the Department will work with the applicant to find an alternative sampling location on a case-by-case basis.

Less than 150 meters from the point-of-discharge: If the distance between the point-of-discharge and mussels absent criteria boundary is less than 150 meters, then the applicant is only required to sample one 75-meter sampling station located at the edge of the boundary (Figure 4).

Figure 4: Sampling requirement when the distance between the point-of-discharge and the mussels absent criteria boundary is less than 150 meters



Between 150 meters and 300 meters from the point-of-discharge: If the distance between the point-of-discharge and the mussels absent criteria boundary is greater than 150 meters but less than 300 meters, there must be a 75-meter sampling station located at the point of discharge and another at the edge of the boundary (Figure 5).

Figure 5: Sampling requirement when the distance between the point-of-discharge and the mussels absent criteria boundary is between 150 and 300 meters.



Between 300 meters and 600 meters from the point-of-discharge: If the distance between the point-of-discharge and the mussels absent criteria boundary is greater than 300 meters but less than 600 meters, there must be a 75-meter sampling station located at the point of discharge, the edge of the boundary, and at the midpoint between the point of discharge and the boundary (Figure 6).

Figure 6: Sampling requirement when the distance between the point-of-discharge and the mussels absent criteria boundary is between 300 and 600 meters.



Between 600 meters and 900 meters from the point of discharge: If the distance between the point-of-discharge and the mussels absent criteria boundary is greater than 600 meters but less than 900 meters, there must be four sampling locations in total. These four 75-meter sampling stations must be located at the point of discharge, the edge of the boundary, and at the first and third quartiles between the point-of-discharge and the boundary (Figure 7).

Figure 7: Sampling requirements when the distance between the point-of-discharge and the mussels absent criteria boundary is between 600 and 900 meters.



Prior to sampling any 75-meter sampling station, applicants can estimate the likelihood of the mussels absent criteria being applicable to that area using the tables found in Appendix A. These tables are provided as a guide to help applicants determine whether it may be worth their time and effort to sample each of the 75-meter segments prior to incurring that expense.

If applicants decide to sample all of the 75-meter sampling stations for the four abiotic factors and the statistical model output is below 0.03, the mussels absent criteria can apply from the most downstream 75-meter sampling station (at the mussels absent criteria boundary) upstream to the point-of-discharge (See the example in Appendix B). On the other hand, if only a portion of the 75-meter sampling stations result in a model output of 0.03 or less, and other stations result in a model output of 0.10 or more, the mussels absent criteria boundary must necessarily be set closer to the point-of-discharge so as to avoid impacting potential mussel habitat. In this case, the WQBEL will be written so that the mussels present criteria will be met where the model output is above 0.03. In a third scenario where a station results in a model output between 0.03 and 0.10, the Department may allow the applicant to conduct a mussel survey to determine if mussels exist in the receiving stream. The results of this survey will then be used to determine if and where the mussels absent criteria applies (See Step 4).

Step 3: Collection of Site-Specific Abiotic Predictor Variables at All Applicable Sampling Locations

When the locations of the sampling stations are identified, four abiotic stream variables must be quantified at each station in order to demonstrate that the site does not provide suitable freshwater mussel habitat:

- Stream Gradient
- Average Stream Width
- Upstream Catchment Area
- Discharge

These parameters must be quantified in the 75-meter stream segment. Protocols for measuring stream gradient, average stream width, acreage of upstream watershed, and discharge are provided below. These protocols and their descriptions were copied or adapted from the Maryland Biological Stream Survey Sampling manual (MDDNR 2019). Individuals collecting these data must have attended Spring and Summer MBSS sampling training. Data should be reported to 2 significant digits.

Stream Gradient

The stream gradient parameter is a measure of the change in elevation between the start and end of the 75-meter segment. This measurement is obtained by recording the difference in water surface height from the 0-meter to the 75-meter location in the stream segment. Laser levels and other equipment may be used to obtain this value, but any technique used to measure the stream gradient must provide data accurate to at least the nearest 5 centimeters.

The calibration and proper functioning of the instrument that is used for determining stream gradient must be verified no less than one week prior to use. Height measurements must be taken from the water's surface and not from the stream bottom or any bank locations. Measurements can be taken at any number of locations if the 0-meter and 75-meter locations cannot be seen at the same time, from the same location. However, if the level must be relocated, height measurements must be taken again from the next closest location where a measurement was already taken.

If a culvert is present within the 75-meter stream segment and the stream level drops below the culvert due to the presence of the culvert, then the stream gradient should be measured without considering the unnatural drop caused by the culvert. This requires two separate sets of height measurements, one downstream from the culvert and one upstream of the culvert. The height difference over the span of the culvert should not be measured in this case.

Average Stream Width

The average stream width must be taken during the summer from June 1st to September 30th. To estimate the average width of the stream, MBSS protocol dictates that the "wetted width" be taken at four transects along the 75-meter stream segments. The four transects must be taken at the 0-meter,

25-meter, 50 meter and 75-meter locations (MDDNR 2019). The average width is calculated using the arithmetic mean of these measurements. Wetted width is measured from bank to bank (perpendicular to the direction of the stream flow) to the nearest 0.1 meter and includes only the wetted portion of the stream. Islands or other large features in the stream that would not be covered by water during base-flow should not be included in the measurement of wetted width. Features that would be covered by water, during higher base-flow should be included in the wetted width measurement.

To account for flow variability over the summer, four average stream widths should be calculated. Specifically, averaged stream widths should be calculated during the first two weeks of June, July, August and September. All stream width measurements should be taken during base flow conditions. The arithmetic mean of the four samples will be used as the final average stream width.

Upstream Catchment Acreage

The upstream acreage parameter is the total area of the upstream catchment starting at the downstream end of the 75-meter stream segment. This value can be calculated using GIS or other scientifically defensible method. For example, upstream acreage can be calculated using Stream Stats, an online mapping service hosted by the United States Geological Survey (<https://streamstats.usgs.gov/ss/>). The recommended method is to utilize United States Geological Survey 7.5-minute quarter quad topographic maps using ArcMap and calculate drainage area (in acres) from the perimeter of each polygon. Digital Elevation Models (DEMs) are not recommended for delineating catchments for streams in the Coastal Plain physiographic region. The Maryland Department of the Environment can be consulted to confirm the value of the upstream acreage.

Rate of Discharge (flow)

The average rate of discharge must be taken during the summer from June 1st to September 30th. The calibration and proper functioning of the instrument that is used for determining discharge must be verified no less than one week prior to use. In order to measure the flow rate of the stream, a suitable transect must be located. A suitable transect approximates a “U” shaped channel to the extent possible. The most accurate measurements are acquired by avoiding transects with boulders or other irregularities that create backflows and cross flows. The stream channel can be modified to more closely approximate a “U” shaped channel (by temporally moving rocks and other objects) and provide a laminar flow with adequate depth for taking velocity measurements.² Unless the stream is very small (less than 0.5 meters wide), a minimum of 10 measurements should be taken. As many as 25 measurements can be recorded. In general, more measurements are required in larger streams. The measurements consist of depth (to the nearest 0.5 cm) and velocity (to the nearest 0.001 m/sec) and should be recorded at regular intervals. Velocity measurements should be taken at 0.6 of the distance from the water surface to the bottom (measured from the surface), making sure to orient the sensor to

² If any modifications were made to the stream channel while taking measurements, the stream channel should be restored to its original state.

face upstream and taking care to stand well downstream to avoid deflection of flows. Depth and velocity measurements should be taken at the exact same locations.

To account for flow variability over the summer, four average stream flows should be calculated. Specifically, stream flow should be calculated during the first two weeks of June, July, August and September. All flow measurements should be taken during base flow conditions. The arithmetic mean of the four samples will be used as the final average stream discharge.

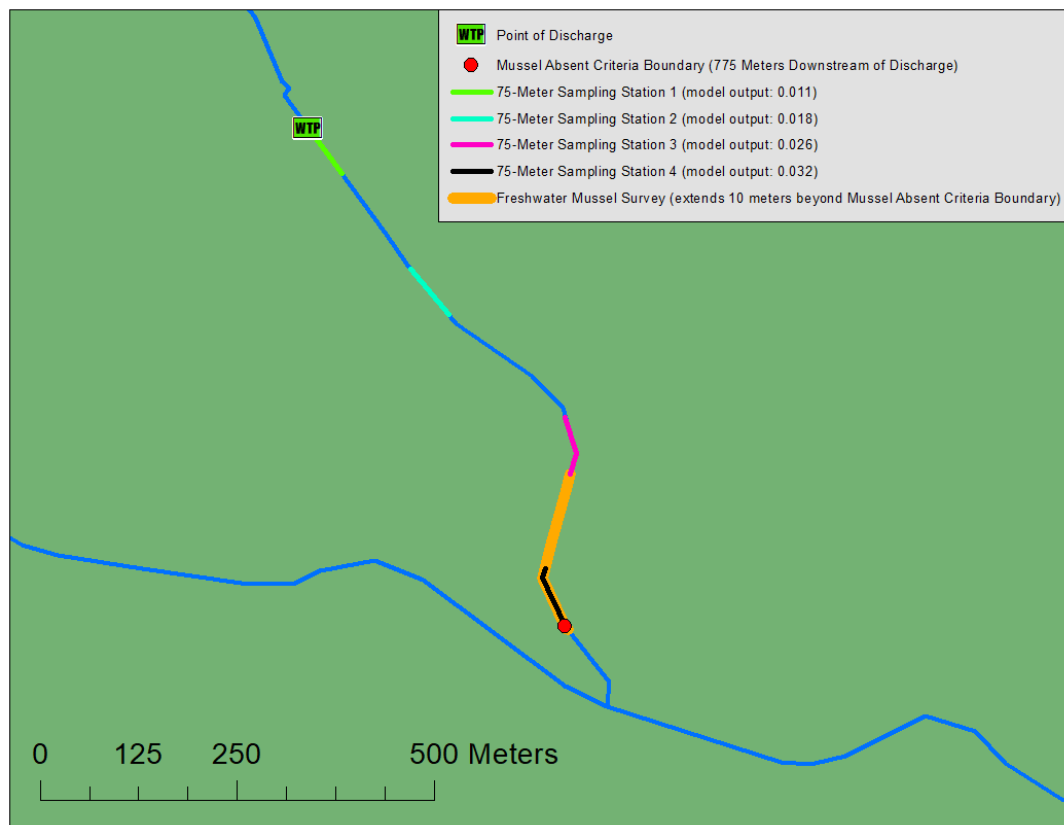
The final reported abiotic characteristic values will be input into Equation 8, 9 or 10, (depending on ecoregion). If the result of the calculation is less than 0.03, the receiving water may not provide mussel habitat. If the result of the calculation is between 0.03 and 0.1, a mussel survey can be used to justify the use of the mussels absent criteria (See Step 4 below). If the results of the calculation are greater than 0.1, the use of the mussels absent criteria to calculate WQBELs will not be permitted at that location.

Step 4: Conduct Freshwater Mussel Survey (if applicable)

The MDE Wastewater Permits Program may allow the use of mussel field surveys to demonstrate the absence of mussel habitat in cases where Equation 8, 9 or 10 results in a value between 0.03 and 0.1. Since Maryland does not have a mussel survey protocol developed for this purpose, applicants must follow the West Virginia Mussel Survey Protocol (Clayton, Douglas and Morrison 2018) when conducting mussel surveys in Maryland. In addition, staff performing the mussel survey must be certified in the survey method by the West Virginia Department of Natural Resources and a scientific collection permit must be obtained from the Maryland Department of Natural Resources prior to any field collection efforts. The Maryland Department of the Environment recommends that applicants contact the Department early-on if they are interested in conducting a mussel field survey to ensure that applicants use the most up-to-date survey protocols.

The area of the mussel survey must include the continuous stream segment starting 10 meters downstream of the 75-meter station that had a model output between 0.03 and 0.1 and extending upstream to the next 75-meter station with a model output below 0.03. In other words, if the applicant submits data from two or more sampling stations and an upstream station shows model output below 0.03 while the downstream station shows an output between 0.03 and 0.1, the mussel survey should extend from 10-meters below the downstream station to the upstream station. Figure 8 shows an example that demonstrates how the mussel surveys should be delineated. In this example, the upstream sampling stations (1, 2, and 3) have a model output below 0.03, while the most downstream sampling station (station 4) has a model output above 0.03 but below 0.1. The mussel survey should extend from the end of station 3 to 10 meters beyond the proposed mussels absent criteria boundary.

Figure 8: Example of Freshwater Mussel Survey Requirement



The results of this mussel survey will then help inform the decision of whether the mussels absent criteria should be applied and, if so, to what geographic extent.

Step 5: Submit Data and Results to MDE

All raw data, applicable summary reports, results, and proof of certifications must be submitted to the MDE Wastewater Permits Program for review. The MDE Wastewater Permits Program will respond to the applicant within two months of receiving the material. Based on the information provided, MDE will either recommend that the material be forwarded to the Maryland Department of Natural Resources and United States Fish and Wildlife service for further review or determine that the mussels absent criteria should not be used.

Step 6: Review by the Maryland Department of Natural Resources and the United States Fish and Wildlife Service

The Maryland Department of Natural Resources (DNR) and United States Fish and Wildlife Service (USFWS) will review the material submitted by the applicant. If both DNR and USFWS affirm that the

use of the mussels absent criteria to calculate WQBELs is justified, the MDE Wastewater Permits Program can move forward with using them in the permit.

Step 7: Mussels absent criteria is applied to stream segment

If the Maryland Department of Natural Resources and USFWS agree that the use of the mussels absent criteria is justified, the location of the stream segment to which the less stringent criteria will apply will be published on the MDE website and displayed within an interactive online map. Other information such as stream name, coordinates, criteria magnitudes and supplementary permitting information will also be available on the MDE website. Within the stream reach, the mussels absent ammonia criteria will be used to determine if the segment is attaining the aquatic life designated use.

Step 8: Include Ammonia limits in the draft NPDES permit and in the public participation process for permit review

The draft permit will specify that the ammonia limits were derived from the mussels absent ammonia criteria. When the above steps are completed to the satisfaction of the Department, the Discharge Permit Application Processing can proceed as codified in COMAR 26.08.04.01-2. The Maryland Department of the Environment will maintain an online list of all locations (including coordinates) and permits where the mussels absent criteria apply. This list will be available to the public and will be updated immediately when applicable permits are approved.

Literature Cited

- Ashton, M. J. (2010). *Freshwater mussel records collected by the Maryland Department of Natural Resources' Monitoring and Non-tidal Assessment Division (1995-2009): Investigating environmental conditions and potential host fish of select species*. Maryland Department of Natural Resources, Resource Assessment Service, Monitoring and Non-Tidal Assessment Division.
- Ashton, M. J. (2012). How a Statewide Stream Survey Can Aid in Understanding Freshwater Mussel (Bivalvia: Unionidae) Ecology: Examples of Utility and Limitations from Maryland. *Freshwater Mollusk Biology and Conservation*, 15(1), 1-11.
- Baldigo, B. P., Riva-Murray, K., & Schuler, G. E. (2004). Effects of environmental and spatial features on mussel populations and communities in a North American river. *Walkerana*, 14(31), 1-32.
- Beaver, J. R., Crisman, T. L., & Brock, R. J. (1991). Grazing effects of an exotic bivalve (*Corbicula fluminea*) on hypereutrophic lake water. *Lake and Reservoir Management*, 7(1), 45-51.
- Campbell, C. A., & Hilderbrand, R. H. (2017). Using maximum entropy to predict suitable habitat for the endangered dwarf wedgemussel in the Maryland Coastal Plain. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27(2), 462-475.
- Cohen, R. R., Dresler, P. V., Phillips, E. J., & Cory, R. L. (1984). The effect of the Asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography*, 29(1), 170-180.
- Grabarkewicz, J. & W. Davis. 2008. An introduction to freshwater mussels as biological indicators. EPA-260-R-08-15. U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC. 140 pp.
- Hakenkamp, C. C., Ribblett, S. G., Palmer, M. A., Swan, C. M., Reid, J. W., & Goodison, M. R. (2001). The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. *Freshwater Biology*, 46(4), 491-501.
- Hakenkamp, C. C., & Palmer, M. A. (1999). Introduced bivalves in freshwater ecosystems: the impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia*, 119(3), 445-451.
- Lauritsen, D. D. (1986). Assimilation of radiolabeled algae by *Corbicula*. *American Malacological Bulletin*. 1986.
- Maryland Department of Natural Resources. 2019. Maryland biological stream survey round four sampling manual.
- Maryland Department of the Environment. 2021. Development of a Model to Estimate the Likelihood of Observing Freshwater Mussels in Maryland Streams. Water Quality Standards Section.
- McMahon, R. F. (1983). Ecology of an invasion pest bivalve, *Corbicula*. *The mollusca*, 6, 505-561.

Mynsberge, A. R., Strager, M. P., Strager, J. M., & Mazik, P. M. (2009). Developing predictive models for freshwater mussels (Mollusca: Unionidae) in the Appalachians: Limitations and directions for future research. *Ecoscience*, 16(3), 387-398.

Phelps, H. L. (1994). The Asiatic clam (*Corbicula fluminea*) invasion and system-level ecological change in the Potomac River estuary near Washington, DC. *Estuaries*, 17(3), 614-621.

Sepkoski Jr, J. J., & Rex, M. A. (1974). Distribution of freshwater mussels: coastal rivers as biogeographic islands. *Systematic Biology*, 23(2), 165-188.

Southerland, M. T., G. M. Rogers, M. J. Kline, R. P. Morgan, D. M. Boward, P. F. Kazyak, R. J. Klauda, and S. A. Stranko. 2005. New biological indicators to better assess the condition of Maryland streams. DNR-12-03-05-0100. Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division, Annapolis, Maryland, USA

Strayer, D. L. (1993). Macrohabitats of freshwater mussels (Bivalvia: Unionacea) in streams of the northern Atlantic Slope. *Journal of the North American Benthological Society*, 12(3), 236-246.

Strayer, D. L. (2008). *Freshwater mussel ecology: a multifactor approach to distribution and abundance* (Vol. 1). Univ of California Press.

United States Environmental Protection Agency. 1999. 1999 Update of ambient water quality criteria for ammonia. EPA-822-R-99-014.

United States Environmental Protection Agency. 2013a. Aquatic life ambient water quality criteria for ammonia-freshwater. EPA-822-R-18-002.

United States Environmental Protection Agency. 2013b. Flexibilities for states applying EPA's ammonia criteria recommendations. EPA-820-F-13-001.

United States Environmental Protection Agency. 2013c. Revised deletion process for the site-specific recalculation procedure for aquatic life criteria. EPA-823-R-13-001.

Appendix A: Upstream Acreage Associated with Having a Low Likelihood of Mussels Being Observed

Prior to conducting onsite sampling of abiotic characteristics or mussel surveys, an applicant may want to assess the likelihood that a 75-meter stream segment would show a low probability of having freshwater mussel habitat. The upstream acreage parameter is required for the model and can be estimated using free online resources or by consulting with the Maryland Department of the Environment. The following tables and figures can be used by applicants to estimate the likelihood of being able to use the mussels absent criteria by calculating the upstream acreage of a location in a stream. These tables enumerate the percentage of MBSS sampling events (from 1995 to 2016) that had a model output below 0.03 given a range of upstream acreage values. For example, in the Piedmont ecoregion, 93% of MBSS samples with upstream acreage between 430 and 925 had a model output below 0.03 and would potentially qualify for the less stringent criteria. So, if an applicant is considering requesting that the Department have the mussels absent criteria apply to a particular stream segment in the Piedmont ecoregion that has a watershed area of 700 acres (which is between 430 and 925), it is likely that this stream would qualify based on the MBSS data. This information could therefore help applicants decide to conduct a survey of abiotic factors to formally justify the use of the mussels absent criteria for the calculation of the WQBEL. In all cases, a full survey of the abiotic factors must be conducted in order to use the less stringent criteria.

Table 1: Distribution of sites with low model output given upstream acreage (Coastal Plain)

	Coastal Plain Upstream Acreage			
Range	8-112	113-159	160-232	232-252
Percentage < 0.03	92%	55%	45%	21%

Figure 9: Scatterplot of upstream acreage vs. model output (Coastal Plain)

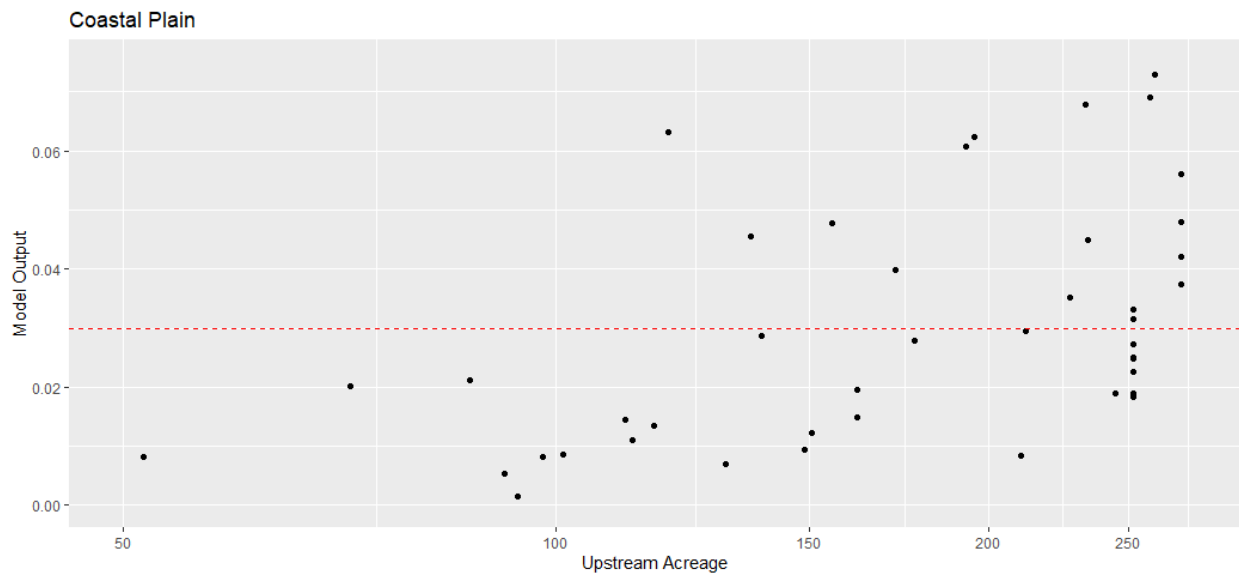


Table 2: Distribution of sites with low model output given upstream acreage (Piedmont)

	Piedmont Upstream Acreage			
Range	50-162	163-429	430-925	926-2047
Percentage < 0.03	98%	98%	93%	19%

Figure 10: Scatterplot of upstream acreage vs. model output (Piedmont)

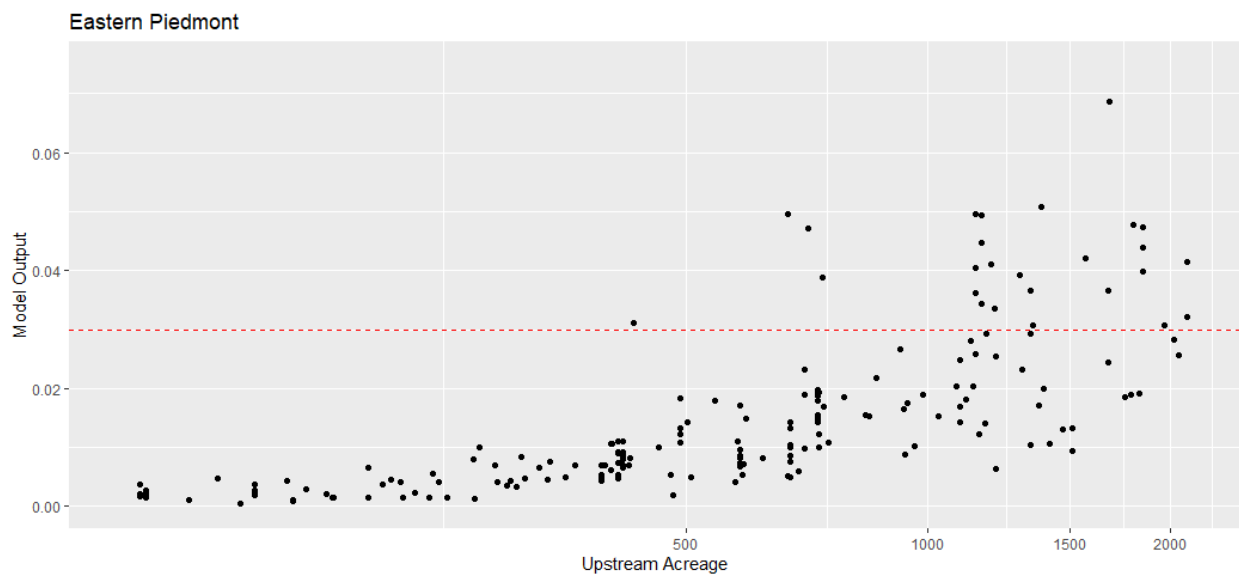
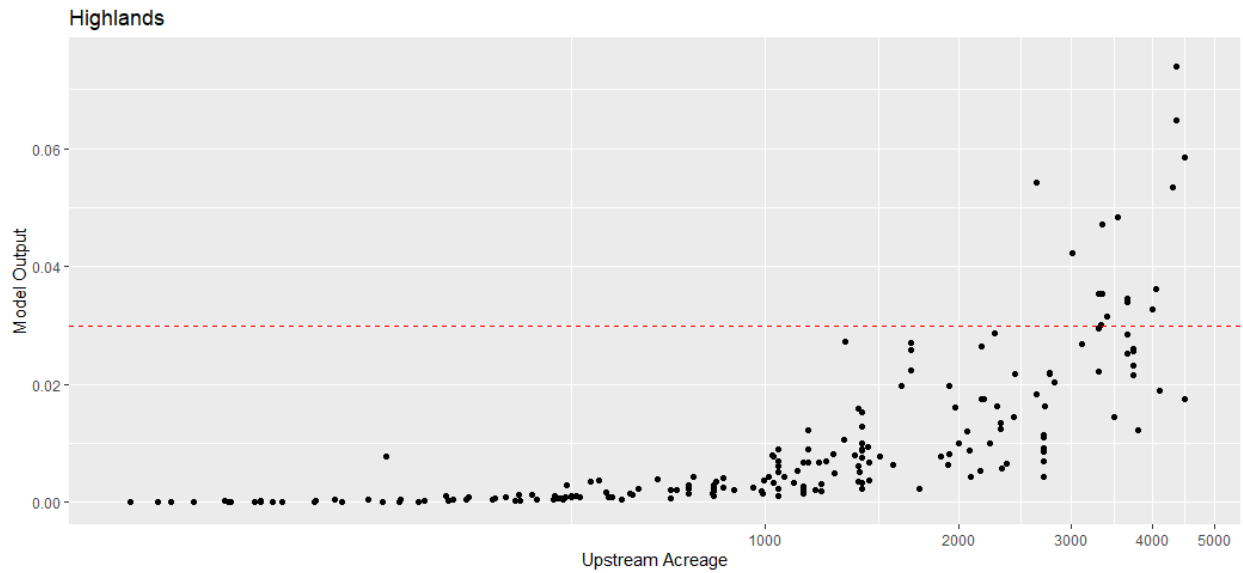


Table 3: Distribution of sites with low model output given upstream acreage (Highland)

	Highland Upstream Acreage			
Range	103-477	478-1049	1050-2170	2170-4504
Percentage < 0.03	100%	100%	100%	23%

Figure 11: Scatterplot of upstream acreage vs. model output (Highlands)



Appendix B: Example Sampling Plan and Calculations

A wastewater treatment facility discharges into a Piedmont Use Class III stream with a 7Q10 of 2.93 cfs and 30Q5 of 1.63 cfs. The receiving stream has a pH of 8 and temperature of 25 degrees Celsius. The effluent has a flow of 0.49 mgd, a 90th percentile pH of 8 pH of 7.8 and a temperature of 25 degrees Celsius. Based on the pH, temperature, and instream mixing, the resulting WQBEL are:

- acute: 7.1 mg/L and
- chronic: 1.07 mg/L

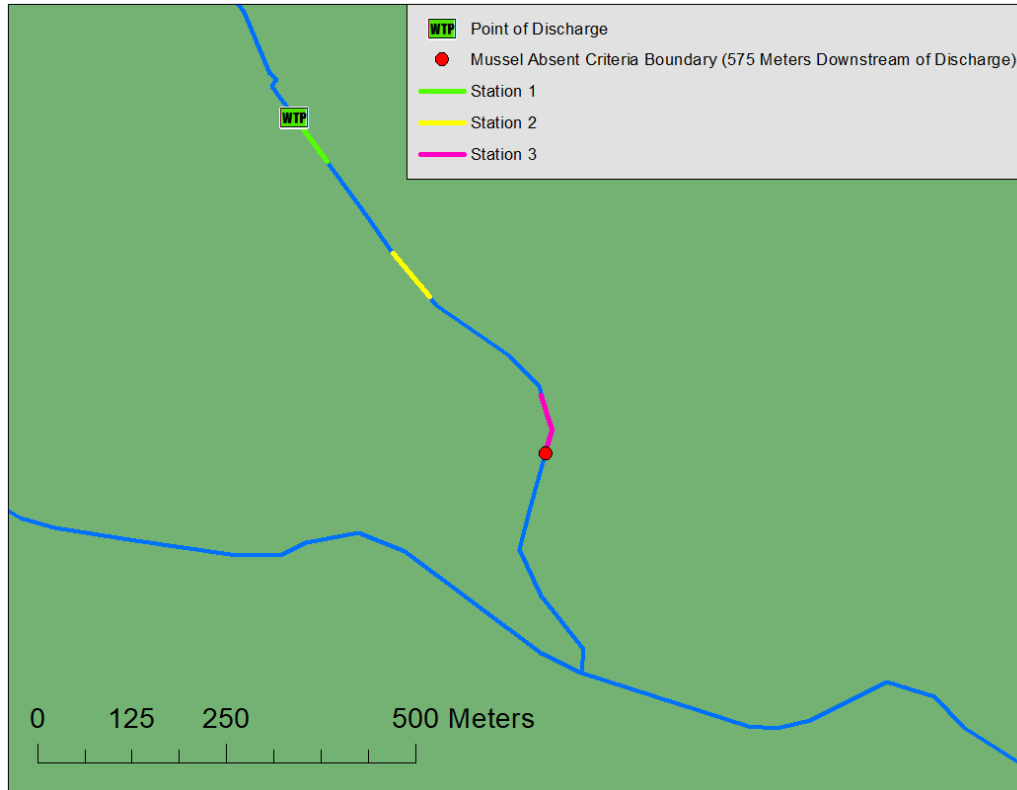
If, however the WQBEL were calculated using the mussels absent criteria, the resulting WQBEL would be:

- acute: 15.45 mg/L and
- chronic: 4.02 mg/L

The facility may not be able to meet the WQBEL calculated using the mussels present ammonia criteria at the point-of-discharge. The operators believe that the mussels present criteria will be met at a downstream location. The Maryland Department of the Environment Wastewater Permits Program determines that if the facility discharges at the mussels absent water quality criteria (15.45 mg/L for acute and 4.02 mg/L for chronic) at the point-of-discharge, the mussels present criteria (7.1 mg/L for acute and 1.07 mg/L for chronic) would be met 575 meters downstream (shown as “mussels absent criteria boundary” in Figure 11).

Because the distance from the mussels absent criteria boundary to the point-of-discharge is between 300 and 600 meters, the permit applicant must collect the abiotic factors in three 75-meter sampling stations downstream of the discharge as shown in Figure 11. Specifically, the facility collects abiotic factors in a 75-meter sampling station starting at 500 meters and ending 575 meters downstream of the point-of-discharge, a 75-meter sampling station located at the point-of-discharge, and a third sampling station located near the midpoint. Figure 11 also shows the locations of the 75-meter sampling locations.

Figure 12: Example Sampling Plan



The facility provides the abiotic factors (collected in the specified 75-meter stream sections) to MDE to determine the likelihood of freshwater mussel habitat being present. Table 4 lists the abiotic factors for each station and the resulting model output using Equation 11 (which is the mussels absent likelihood equation for the piedmont ecoregion).

Table 4: Example Model Output Calculations

	STREAM GRADIENT	DISCHARGE/FLOW (CFS)	AVERAGE STREAM WIDTH	UPSTREAM ACREAGE	MODEL OUTPUT
STATION 1	1.23	3.28	2.1	3500	0.009988
STATION 2	1.21	3.28	2.3	3550	0.011825
STATION 3	1.2	3.28	2.8	3610	0.016681

Equation 11: Mussels absent likelihood equation for the Piedmont ecoregion.

$$e^{\left[\frac{LN(area) * 0.3 - LN(slope) * 1.7 + LN(flow) * 0.5 + LN(wid h) * 1.5 - LN(flow) * LN(area) * 0.12 + LN(slope) * LN(area) * 0.14 + LN(flow) * LN(wid h) * 0.2 - 8}{LN(area) * 0.3 - LN(slope) * 1.7 + LN(flow) * 0.5 + LN(wid h) * 1.5 - LN(flow) * LN(area) * 0.12 + LN(slope) * LN(area) * 0.14 + LN(flow) * LN(wid h) * 0.2 - 8} \right]}$$

$$1 + e^{\left[\frac{LN(area) * 0.3 - LN(slope) * 1.7 + LN(flow) * 0.5 + LN(wid h) * 1.5 - LN(flow) * LN(area) * 0.12 + LN(slope) * LN(area) * 0.14 + LN(flow) * LN(wid h) * 0.2 - 8}{LN(area) * 0.3 - LN(slope) * 1.7 + LN(flow) * 0.5 + LN(wid h) * 1.5 - LN(flow) * LN(area) * 0.12 + LN(slope) * LN(area) * 0.14 + LN(flow) * LN(wid h) * 0.2 - 8} \right]}$$

Equation 12: Example calculations for Station 1.

$$\frac{e^{\left[\frac{LN(3500)*0.3 - LN(1.23)*1.7 + LN(3.28)*0.5 + LN(2.1)*1.5 - LN(3.28)*LN(3500)*0.12 +}{LN(1.23)*LN(3500)*0.14 + LN(3.28)*LN(2.1)*0.2 - 8} \right]}}{1 + e^{\left[\frac{LN(3500)*0.3 - LN(1.23)*1.7 + LN(3.28)*0.5 + LN(2.1)*1.5 - LN(3.28)*LN(3500)*0.12 +}{LN(1.23)*LN(3500)*0.14 + LN(3.28)*LN(2.1)*0.2 - 8} \right]}} = 0.009988$$

Based on the abiotic factors, the model output for all three stations is less than 0.03. After interagency review (MDDNR and USFWS), MDE determines that the mussels absent criteria will apply from the point-of-discharge to 575 meters downstream. Figure 12 displays the first order stream and segment to which the mussels absent criteria apply. The mussels present criteria will apply to all other surface waters.

Figure 13: Example of Mussels absent Criteria Extent

