



1 April 2025

Draft procedure for applying mussels absent ammonia criteria in Missouri surface waters

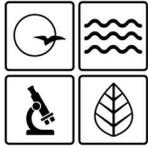
Part I. Introduction

Section 304(a) of the Clean Water Act requires the U.S. Environmental Protection Agency (EPA) to develop and publish water quality criteria recommendations that reflect the latest scientific knowledge. In 2013, EPA updated their 304(a) recommended water quality criteria for the protection of aquatic life from ammonia toxicity (USEPA 2013a). The 2013 toxicity dataset included data for freshwater mussel species (family Unionidae) and snails—these data were not available in 1999 for inclusion in the previous criteria derivation (USEPA 1999). Because these taxa are relatively more sensitive to ammonia than those included in the 1999 criteria derivation, the 2013 criteria are more stringent and protective.

With approximately 300 native species, North America has the highest diversity of freshwater mussels (order Unionida) in the world. However, freshwater mussels are also the most imperiled taxa in the United States—approximately 70 percent of species are either endangered, threatened, species of conservation concern, or presumed extinct. Humans derive numerous ecosystem services from freshwater mussels (Vaughn 2018). Mussels function as biofilters, cleaning water and improving water quality as they filter feed on algae, bacteria, and other particulate matter in the water column. Mussels also play key roles in biodiversity maintenance by cycling nutrients, modifying substrate, creating habitat for other organisms, and impacting numerous links in aquatic and terrestrial food webs. Declines in mussel abundance mean a loss of these ecosystem services.

Mussels are the second-most endangered fauna in Missouri (following crayfishes), primarily due to physical habitat alteration and pollution. Missouri has nearly 70 species of mussels; nearly 42 percent (29 species) are species of conservation concern (McMurray et al. 2012). Mussels occur statewide and can live in any permanent body of water, including ponds, lakes, and reservoirs, as well as in permanent pools in intermittent waters. While some species have narrow ranges, others are widespread and found statewide.

The purpose of this document is to describe the performance-based approach the Missouri Department of Natural Resources (MoDNR) will use to determine the application of mussels absent criteria (defined below) in Missouri surface waters (excluding the Mississippi and Missouri Rivers). This approach is designed to protect aquatic life use in receiving and downstream waters. This document details the process, minimum data requirements, and decision-making framework.



Part II. Criteria

Based on survey data and historical information, and following consultation with the Missouri Department of Conservation (MDC) and the U.S. Fish and Wildlife Service (USFWS), MoDNR has determined that the majority of Missouri water bodies are likely to contain, or have contained in the past, mussel species (order Unionida). For these reasons, MoDNR presumes mussels are present in all Missouri surface waters designated for aquatic life use and shall apply the 2013 ammonia criteria to these waters. The criteria magnitudes are a function of pH and temperature as expressed in the following equations. The acute criteria are bifurcated based on the presence or absence of trout (*Oncorhynchus* sp.); this corresponds to Missouri's cold versus cool and warm water habitat designations.

2013 acute criterion magnitude for cold water [Eq. 1]

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

2013 acute criterion magnitude for cool and warm water [Eq. 2]

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

2013 chronic criterion magnitude [Eq. 3]

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

For instances where natural stream conditions limit mussel habitat and presence, MoDNR has also adopted ammonia criteria that were recalculated with mussel toxicity data removed (mussels absent criteria). MoDNR may apply mussels absent criteria in instances where a permit applicant demonstrates "absence" following the methods described in this document. Mussels absent criteria magnitudes are calculated with the following equations:

Acute criterion magnitude for cold water recalculated with mussels absent [Eq. 4]

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

Acute criterion magnitude for cool and warm water recalculated with mussels absent [Eq. 5]

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

Chronic criterion recalculated with mussels absent, early life stages of fish present [Eq. 6]

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

Chronic criterion recalculated with mussels absent, early life stages of fish absent [Eq. 7]

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

Part III. Mussel presence and absence

The recalculation of aquatic life criteria is based on the composition of species that occur at a site. In accordance with the *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria* (EPA 2013b), MoDNR considers species to "occur at the site" if any life stage meets one of the following elements:

- are usually present at the site;
- are seasonally present at the site during migration;
- are intermittently present at the site because they periodically return to or extend their ranges into the site;
- were present at the site in the past, are not currently present at the site due to degraded conditions, but are expected to return to the site when conditions improve, or;
- are present in nearby bodies of water, are not currently present at the site due to degraded conditions, but are expected to be present at the site when conditions improve.

MoDNR considers mussels "present" if a mussel species meets any one of the elements to "occur at the site". Specifically, mussel presence can be determined by the existence of live mussels, mussel shells, mussel tracks, or historical presence data. Additionally, if mussels are present within one mile of the site and both suitable habitat and fish hosts are present, MoDNR considers mussels present.

As there is no way to determine the absence of mussels with 100 percent certainty, it is more accurate to understand "absence" as "not detected". Mussel surveys may result in false negatives (i.e., failure to observe a mussel when one is present). This could be due to very low abundance or cryptic behavior. Specific survey designs linked to statistical models can increase the probability that mussels, if present, are detected and limit the likelihood of a false negative (Smith 2006). The permit applicant must demonstrate mussel absence through field surveys as described in this document.

Part IV. Overview of performance-based approach to apply mussels absent criteria

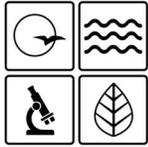
In instances where natural stream conditions limit mussel habitat and presence, it may be appropriate to apply mussels absent criteria. MoDNR will determine the appropriate ammonia criteria based on information provided from the performance-based approach detailed in this document.

Characteristics of a performance-based approach

A performance-based approach relies on the adoption of an implementation procedure into water quality standards regulation, rather than a specific outcome. This allows MoDNR to make site-specific determinations that do not require adoption into regulation. The implementation procedure must be transparent and sufficiently detailed to ensure a predictable and repeatable result. It must also address the presence of federally endangered or threatened species and their critical habitat. Additionally, it must include a public participation step to provide all stakeholders and the public with an opportunity to review the data and calculations supporting the site-specific application of the implementation procedure.

Steps in the performance-based approach

1. Applicant delineates the area of impact and study area.
2. Applicant checks literature, reports, and databases for mussel records.
 - This includes checking for the presence of any federally listed species or critical habitat.
3. Applicant submits results from steps 1 and 2 to MoDNR for review.
4. If step 2 does not indicate mussel presence, applicant retains surveyor and obtain permit(s).
5. Surveyor conducts a preliminary study area assessment.
6. If step 5 does not indicate mussel presence, surveyor submits full survey plan to MoDNR for approval.
7. Following MoDNR plan approval, surveyor conducts full mussel surveys.
8. If mussels are still not detected after step 7, MoDNR reviews final survey report and makes a determination.
9. Following a mussels absent determination, MoDNR calculates ammonia effluent limits for NPDES permit based on mussels absent criteria.
 - The draft permit and final survey report are available to the public during the permit public notice.
10. MoDNR reevaluates mussels absent determination upon every permit renewal.



Part V. Methods for performance-based approach to applying mussels absent criteria

Step 1. Delineate the area of impact and the study area.

Area of impact:

This includes the entire area that could be impacted by the ammonia discharge(s) during critical conditions for ammonia toxicity (high pH and temperature, low flow). The area of impact may extend to downstream water bodies. To delineate the area of impact, establish an end-of-pipe water quality-based effluent limit using the mussels absent criteria and identify the downstream location at which mussels present criteria will eventually be met. This will be the mussels absent criteria boundary.

Applicants may employ one of two methods to identify the mussels absent criteria boundary:

1. Conduct instream sampling to characterize ammonia concentrations downstream of the discharge. Each sampling event must include at least one sample upstream of the outfall, one effluent sample, and samples downstream of the outfall, collected at least every 0.5 miles for a minimum of 3 miles downstream of the outfall. Sampling must be conducted during the months of July and August at base flow (or low flow) conditions and during normal plant operation. Sampling must be repeated a minimum of two more times at least seven days apart.
2. Model ammonia concentrations using an EPA supported water quality model. Model and guidance are available on USEPA's *Surface Water Models to Assess Exposures* webpage (<https://www.epa.gov/hydrowq/surface-water-models-assess-exposures>). Field data will be required to calibrate the water quality model.

Study area:

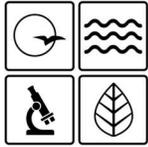
The study area includes the area of impact, as well as adjacent reaches upstream and downstream of the area of impact. The length of each reach will approximate 20 times the mean stream width (MoDNR 2019).

Mussels may be extirpated from the area of impact due to ammonia pollution. Although sedentary as adults, larval mussels may colonize new areas as they disperse within stream networks while attached to fish hosts. Identifying the presence or absence of mussels above and below the discharge can help determine if the discharge is a cause of mussel absence and if there is potential for mussel colonization within the area of impact. Additional guidance for step 1 is provided in Appendix A.

Step 2. Check literature, reports, and databases for mussel records.

Conduct a historical record search to evaluate if mussels have been documented near the study area. To protect existing uses, search for any mussel occurrence records at least as far back as November 28, 1975. At a minimum, searches must include each of the four components listed below.

1. Mussel occurrence records:
 - Invert E Base accessed at <https://www.invertebase.org/portal/index.php>



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2. Missouri distribution maps:
 - McMurray SE, Faiman JS, Roberts A, Simmons B, and Barnhart MC. (2012). *A guide to Missouri's freshwater mussels*. Missouri Department of Conservation, Jefferson City, MO.
3. Scientific literature search for keywords:
 - "mussel" AND "Missouri" AND river basin name
 - "mussel" AND "Missouri" AND receiving water body name
4. Species of conservation concern:
 - NatureServe Explorer at <https://explorer.natureserve.org/>
 - USFWS Information for Planning and Consultation (IPaC) webpage at <https://ipac.ecosphere.fws.gov/>
 - Missouri Natural Heritage Review at <https://naturalheritagereview.mdc.mo.gov/>

Occurrence data provides sufficient justification to determine mussel presence. Conversely, the lack of available occurrence data does not provide sufficient justification to determine mussel absence. If there is no record of mussel presence, the next step is to submit all results from steps 1 and 2 to MoDNR for review (see Appendix A).

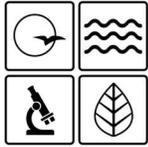
Step 3. Submit results from steps 1 and 2 to MoDNR for review.

Compile all results from steps 1 and 2 (see checklist in Appendix A) and submit to MoDNR for review. Following receipt, MoDNR will review submission and consult MDC and USFWS. MoDNR will provide applicant with a recommendation to proceed or discontinue process. If the recommendation is to proceed, the applicant will need to retain a qualified surveyor to conduct a preliminary study area assessment (steps 4 and 5 below). MoDNR may opt not to apply mussels absent criteria to impaired waters or those with sensitive species.

Step 4. Retain surveyor and obtain permit(s).

While MoDNR does not require biologist certifications, surveyors must have expert knowledge of Missouri mussel species (order Unionida), including their habitats and life histories, and be experienced with mussel sampling. Experience is critical because surveyors must be able to separate small, juvenile, or cryptic mussels from a variety of substrate types. An experienced sampler will also be able to identify sections of the study area most likely to support mussel populations. Targeting likely mussel habitat in a preliminary study area assessment may eliminate the need to conduct subsequent comprehensive surveys. Any inexperienced crew members must be supervised at all times by an experienced investigator.

In addition to the expert knowledge surveyors should possess, surveyors must also have: adequate field experience; the ability to execute mussel survey methods independently; the ability to locate and identify federally-listed species; experience in the safe care and handling of threatened and endangered species and Missouri species of conservation concern. Prior to any sampling, the permit applicant must



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provide MoDNR with documentation of the surveyor's qualifications, accompanied by the appropriate permits (described below) from state and federal permitting authorities.

For the collection or handling of freshwater mussel specimens, the State of Missouri requires a current Wildlife Collector's Permit for Special Collections of Wildlife from MDC. The permit application is available at: <https://mdc.mo.gov/permits/wildlife-collector-permits>. Note that a research proposal must accompany requests for a Missouri Wildlife Collector's Permit for biomonitoring or environmental activities.

In areas where threatened or endangered species may be present, additional federal permitting requirements apply. Section 10 of the Endangered Species Act regulates activities that could affect endangered or threatened plants and animals and their habitats. The law generally prohibits activities affecting listed species and their habitats unless authorized by a permit from the USFWS. Permits in Missouri are issued by:

U.S. Fish and Wildlife Service, Endangered Species Permit Office
5600 American Blvd. West, Suite 990
Bloomington, MN 55437-1458
Phone: (612) 713-5343
Email: permitsR3ES@fws.gov

More information regarding permitting requirements, including an online permit application form, is available at: <https://www.fws.gov/service/3-200-59-scientific-purposes-enhancement-propagation-or-survival-permits-recovery-permits>.

Step 5. Conduct a preliminary study area assessment.

Following the engagement of an experienced surveyor and receipt of appropriate state and federal permits, conduct a preliminary assessment to characterize the study area. The preliminary assessment may identify mussels without the expense of a full survey. However, the permit applicant may choose to forego the preliminary assessment in preference for a full mussel survey. The preliminary assessment should include a combination of desktop and field reconnaissance to identify likely mussel habitats in the study area followed by implementation of rapid, low-cost sampling to detect mussels in these habitats. Methods may include: shoreline searches performed by walking along the water body margins or islands looking for live mussels in the water and for shells on the shore; and cursory visual searches while wading, with eyes alone or aquascopes, or snorkeling (EPA 2013c). The preliminary assessment should include a minimum search time of one person-hour and cover the entire study area. If the surveyor finds any mussels, mussel tracks, or mussel shells at this stage, the survey shall end; the 2013 ammonia criteria shall apply. If no evidence of mussels is found during the preliminary assessment, the next step is to submit a full survey plan to MoDNR for approval.



Step 6. Submit a full survey plan to MoDNR for review.

Submit results from the preliminary study area assessment along with a full survey plan in accordance with Appendix A.

Step 7. Conduct full mussel surveys.

Sampling design

This design comprises a timed search in ideal habitat (minimum search time of one person-hour), followed by a complete coverage survey. For the complete coverage survey, the surveyor shall divide the entire study area into equally sized cells (maximum cell size is 100 m²) based on stream channel morphology. The surveyor shall spend an equal amount of time searching each cell with a minimum search time of 20 minutes per 100 m². The minimum total search time (for the entire study area) shall be four person-hours.

Mussel surveys cannot verify mussel absence with 100 percent certainty—mussels may be difficult to detect in areas of low abundance and due to cryptic behavior. However, a survey designed to ensure a high probability of detection may help limit false negatives (failure to detect mussels where they are in fact present). Smith (2006) modeled the detection probability of a survey as a function of mussel abundance (or density), search area, and search efficiency:

$$\text{Probability (of detecting at least one individual)} = 1 - e^{-\beta a \mu} \quad [\text{Eq. 8}]$$

where β is search efficiency, a is search area, and μ is mussel density. Using this model, MoDNR has established a minimum search area of 1,151 m² regardless of the size of study area. The minimum search area is based on the following assumptions:

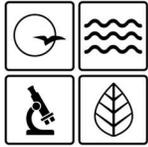
- Minimum probability of detection = 0.90
- Search efficiency (β) estimated as proportion of mussels on substrate surface = 0.20
- Mussel density (μ) = 0.01/m²

$$a = \frac{\ln(1-0.90)}{-0.2*0.01} = 1,151 \text{ m}^2$$

The minimum search area is increased to 2,300 m² for any tributaries to downstream waters identified by USFWS as having federally listed mussel species or critical mussel habitat. This is based on a higher detection probability (0.99).

Sampling methods and frequency

As in the preliminary assessment, if at any point during the survey, surveyors find mussels, mussel tracks, or mussel shells in or along the water body, the survey shall end immediately; the 2013 ammonia criteria shall apply. Surveyors shall examine the sample area visually and tactilely. Tactile searches include moving cobble and woody debris, hand sweeping away silt, sand, or detritus, and probing at least the upper 5 cm of loose substrate. No additional substrate excavation is required. Search methods may include any of those described in EPA (2013c) except excavation. These include wading, snorkeling,



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or diving using SCUBA equipment or Surface Supplied Air (SSA). Wading and snorkeling should be limited to water depths less than 1 m. Diving with the use of SCUBA or SSA should be used in water > 1 m in depth. Water column visibility must be at least 0.5 m (~ 20 inches) (Ohio Department of Natural Resources and USFWS 2020). If suitable visibility is not present at the intended time of survey, the survey must be rescheduled. If the normal flow conditions of a water body are low visibility, the visibility requirement may be waived following consultation with the appropriate state or federal agency, and tactile searches with additional search time may be employed as the primary means of mussel search. Additional search time is also required for areas with off channel habitats, such as backwaters, forewaters, or oxbow ponds that can reasonably become inundated by higher flows.

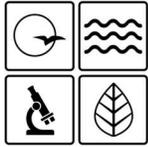
All mussels collected during the survey shall be brought to the surface for identification. Mussels shall be kept inundated in water except for short periods of close examination or photography (for later identification) that should last no longer than one minute. Stress caused by handling and exposure to high air temperatures can cause rapid mortality in freshwater mussels. Mussels removed from the streambed shall be placed by hand back in the substrate in their original filtering position (i.e. with the umbos up).

The ability to detect mussel presence varies across seasons and from year to year. All surveys should be conducted during base flow (or low flow) conditions between May 1st and October 15th, or when water temperatures are greater than 50°F. At this temperature, mussels are more likely to be at the substrate surface versus burrowed into the streambed. To account for seasonal variability, surveyors must conduct two sampling events per year, at least 3 months apart between May 1st and October 15th, as temperatures allow.

Mussel “absence” at a single point in time does not guarantee mussels will not be present even a few months later. There are numerous reasons to reevaluate study areas where surveys do not detect mussels. Juvenile mussels spend at least the first year of life buried deeply in the substrate; thus, juvenile mussels may be missed by certain sampling methods, as might other species that tolerate more silted conditions. Additionally, the proportion of mussels at the surface of the substrate varies with mussel species, size, gender and time of year (e.g. vertical migration for reproduction), as well as water temperature and water level. All of these factors contribute to a high degree of year-to-year variability with regard to sampling efficiency. To mitigate interannual variability in sampling efficiency, MoDNR has selected a relatively low sampling efficiency ($\beta = 0.20$) for use in the probability of detection equation presented above. However, this lower sampling efficiency will not always account for years with very low or no occurrence of mussels at the surface. Therefore, MoDNR will only make a mussels absent determination if a no mussels survey result is confirmed the following year with a subsequent survey.

Safety considerations

If surveyors deem any portion of the study area unsafe for sampling, and the unsafe condition is not temporary, the application of this performance-based approach may be infeasible. In such a case, the permit applicant may wish to consult MoDNR to explore alternate methods of mussel presence or absence determinations and the development of site-specific ammonia criteria. Alternate methods would require both MoDNR and EPA review and any site-specific criteria would require EPA approval.



Step 8. MoDNR reviews final report and makes determination

MoDNR may make a mussels absent determination provided the following criteria are met:

1. If after year two of sampling, surveyors do not detect mussels in the study area; and
2. the permit applicant submits adequate documentation to MoDNR for review and approval at each approval step (see Appendix A); and
3. all surveys were conducted in accordance with the approved survey plan.

Step 9. Implementation and public participation

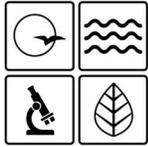
Mussels absent criteria shall apply only to the area of impact as originally delineated (from the discharge to the mussels absent criteria boundary). MoDNR will use mussels absent criteria to calculate effluent limits for that site. Prior to issuing a final NPDES permit decision, MoDNR will make the survey plan, results, and final report available for public comment as part of the draft permit public notice.

Step 10. Reevaluation

MoDNR will reevaluate this determination upon each permit renewal. At a minimum, the permit applicant will be required to repeat steps 1 – 3 upon each permit renewal (every five years) and steps 4 – 9 upon every other permit renewal (every ten years). MoDNR may require additional mussel surveys as indicated by any new information.

Part VI. References

- McMurray, S. E., Faiman, J. S., Roberts, A., Simmons, B., Barnhart, M. C. (2012). *A guide to Missouri's freshwater mussels*. Missouri Department of Conservation.
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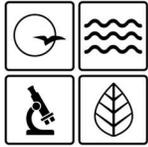


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Vaughn, C. C. (2018). Ecosystem services provided by freshwater mussels. *Hydrobiologia*, 810, 15–27.

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Appendix A. Additional guidance and minimum data requirements

IN DEVELOPMENT!!! This Appendix will provide additional guidance and resources for each step, as well as a checklist of required documentation.

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