Biological Opinion

Interstate 24 Choice Lane Improvements in Davidson and Rutherford County, Tennessee

FWS ECOSphere Project Code: 2024-0109689

Prepared by:

U.S. Fish and Wildlife Service
Tennessee Ecological Services Field Office
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Daniel Elbert 4/24/2026

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ACKNOWLEDGEMENTS

With sincere gratitude, we would like to acknowledge the following for their significant contributions to this Opinion:

Christopher DeVore, Fish and Wildlife Biologist, Tennessee Ecological Services Field Office

Madison Mueller, Fish and Wildlife Biologist, Tennessee Field Office

D. Wesley Giddens, Fish and Wildlife Biologist, Tennessee Ecological Services Field Office

Nicole Sikula, Deputy Field Supervisor, Raleigh Ecological Services Field Office

Dale McGinnity, Ectotherm Curator, Nashville Zoo

David Withers, Zoologist, Tennessee Department of Environment and Conservation

The Service appreciates the cooperation of the FHWA during this consultation. We would like to continue working with you and your staff regarding this project. For further coordination, please contact Christopher DeVore or my staff 207-949-0596.

Notice of Use of Pre-2019 Section 7 Implementing Regulations (50 CFR 402, effective on and prior to 10/27/2019)

On March 30, 2026, the U.S. District Court for the Northern District of California issued a summary judgment ruling in Center for Biological Diversity v. U.S. Department of the Interior, No. 4:24-cv-04651 (N.D. Cal.), finding several provisions of the 2019 and 2024 ESA Section 7 regulations unlawful. The Court vacated four key regulatory changes: 1) including the revised "effects of the action" definition, 2) the revised adverse modification of critical habitat standard,3) the likelihood standard for implementation of conservation measures, and 4) the revisions to reinitiation responsibility. The Court also ordered the pre-2019 versions of those provisions (50 CFR 402, effective on/prior to 10/27/2019) be reinstated and applied in place of the 2019 and 2024 versions. In accordance with the Court's opinion, this Biological Opinion (BO) used the pre-2019 versions of those applicable provisions in order to align with the Court's instructions in issuing this BO.

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CONSULTATION HISTORY

This section lists key events and correspondence during the course of this consultation. A complete administrative record of this consultation is on file in the U.S. Fish and Wildlife Service's (Service) Tennessee Ecological Services Field Office (TNFO).

Date	Event	Participants	Discussion Topic
December 18, 2024	Draft Biological Assessment	Pierce, Nordone, and Erin McGehee prepared BA for TDOT Reviewers Austin Meadows, Alex Terry, Joy Riley	Draft Biological Assessment
January 25, 2025	Final BA submittal	Pierce Nordone to TDOT staff	Final Biological Assessment
March 24, 2025	BA submitted for TNFO preliminary review	Bailey Vernon (FHWA) submitted BA to TNFO	BA submitted for TNFO Review
April 25, 2025	TNFO response to BA Review	Nicole Sikula, Bailey Vernon	BA submitted approved
August 28, 2025	TDOT informs TNFO new BA to be submitted	Rita Thompson, Christopher DeVore	TNFO acknowledges pause on current BA. Awaits updated BA.
December 22, 2025	TDOT submits revised BA with updated information	Rita Thompson, Christopher DeVore	Proposed Action, Environmental Baseline, Action Area updated
April 22, 2026	TNFO delivers Biological Opinion to FHWA and TDOT	Dan Elbert, Christopher DeVore, Brandon Chance, FHWA Contact	TNFO issues opinion on LAA determination for Nashville crayfish and acknowledges conclusion of formal consultation

BIOLOGICAL OPINION

1. INTRODUCTION

Notice of Use of Pre-2019 Section 7 Implementing Regulations (50 CFR 402, effective on and prior to 10/27/2019)

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A biological opinion (BO) is the document that states the opinion of the U.S. Fish and Wildlife Service (Service) under the Endangered Species Act (ESA or Act) of 1973, as amended, as to whether a Federal action is likely to:

a) jeopardize the continued existence of species listed as endangered or threatened; or
b) result in the destruction or adverse modification of designated critical habitat.

The federal action agency for this consultation is the Federal Highway Administration (FHWA). This BO addresses effects to the Nashville crayfish (*Faxonius shoupi*) resulting from the proposed widening of Interstate 24 over 26 miles in the Mill Creek watershed in Davidson County and Rutherford County, Tennessee.

Critical habitat: Because the entire known range of the species at the time of listing was contained within the boundaries of Metropolitan Nashville, where significant residential and commercial development had occurred prior to its listing, it was determined that designation of critical habitat would substantially increase the potential for take of the species (USFWS 1989). Therefore, critical habitat was not designated for the Nashville crayfish and will not be considered as part of this consultation or discussed further in this BO.

Section 9 of the Act and regulations issued under section 4(d) of the Act prohibit the taking of endangered and threatened species, respectively, without special exemption. Incidental take is take that results from a federal action but is not the purpose of the action. Federal agencies may obtain an exemption from section 9's prohibitions through an "Incidental Take Statement" (ITS) that can be attached to a BO when the BO concludes the effects of the action (including incidental take) are not likely to jeopardize the continued existence of listed species. The ITS

includes the amount or extent of anticipated take due to the federal action, and may contain reasonable and prudent measures (RPMs) and terms and conditions (T&Cs) to minimize incidental take, which must be implemented for the exemption from the section 9 prohibitions to apply.

In this BO, the Service has determined that the proposed Action is not likely to jeopardize the continued existence of the Nashville crayfish, but incidental take of Nashville crayfish is reasonably certain to occur. **For this BO, the anticipated amount of incidental take would be exceeded when more than 650 Nashville crayfish present in approximately 24,624 m² of suitable habitat are actually incidentally taken.** All incidental take up to, but not exceeding 650 Nashville crayfish, is exempted from being considered as prohibited take under section 9 of the ESA.

2. PROPOSED ACTION

The proposed action for the project that would impact Nashville crayfish habitat includes the removal or widening of existing bridges, construction of new bridges, and the extension of culverts. Per the Biological Assessment submitted by the Tennessee Department of Transportation (TDOT), a total of 46 structures on Interstate 24 (I-24) are slated for improvements. The entire I-24 study area was surveyed for delineated perennial and intermittent streams. Many streams in the study area did not fall in the direct action area, and only a portion that did display elements of suitable Nashville crayfish habitat. A total of eighteen streams in the action area displayed suitable Nashville crayfish habitat. Therefore, not all structures encompassed within the action area are expected to have impacts on Nashville crayfish. Structures on those 18 streams will be the focus of the remainder of the biological opinion. General construction activities for bridges include dewatering of work areas, temporary stream diversions, foundation preparation (excavation, sheet piling, form work), retaining wall construction, haul roads, bank armoring, the installation of proposed bents, and construction of footings in and near streams. General construction activities for the extension of culverts include dewatering of work areas, temporary stream diversions, reconstruction of headwalls and wingwalls, armoring, permanent stream relocations, and stream encapsulation by the new structures.

General Itemized Bridge Construction Activities would include:

1) Haul roads/work pads will be required to provide access for demolition and construction activities. The haul roads/work pads will extend into the ordinary highwater mark (OHWM) of streams; therefore, in-stream diversions and crayfish sweeps will be required before each haul road/work pad is installed.

2) De-watering of work areas or temporary stream diversions to either demolish or construct piers within the streams. Instream diversions will use sandbags, jersey barriers (with plastic, as needed), or equivalent diversion measures per TDOT standards. Appropriate Erosion Prevention and Sediment Control (EPSC) measures will be used to prevent any

sediment or material within the stream diversion work area from entering streams when the enclosure is de-watered.

3) Foundation preparation for spread footings and/or driving of piles following dewatering of work areas.

4) Demolition of existing spread footings or piers down to bottom of stream elevation.

5) Once in-stream construction is complete, haul roads and stream dewatering/diversions will be removed, and the areas stabilized.

6) All material (concrete, dirt, and rock, etc.) resulting from demolition and construction will be hauled to an approved waste site per the TDOT waste and borrow procedures.

7) Portions of the stream banks under and around the new bridges will be stabilized using Class "B" riprap.

8) No blasting within streams is proposed to construct this project. Some piers will be set on bedrock and some chipping of the rock or leveling concrete may be needed for the footers.

9) Following the completion of each required crayfish sweep, the contractor shall ensure all employees and subcontractors initiate installation of instream diversions within one hour of removal of crayfish.

10) Prior to onset of construction or dewatering within the perimeter of any in-stream diversion, an additional sweep must be completed to remove any remaining crayfish.

11) If an in-stream diversion fails or is overtopped, additional crayfish sweeps will be required. All in-stream work must cease immediately and cannot resume until additional sweeps have been completed.

12) Upon locating dead or injured crayfish, the TDOT Environmental Division Ecology Section must be immediately notified.

13) Proposed bridge drains will not discharge directly over any streams included in this report.

In addition, for streams where culvert extensions are proposed, the following general itemized construction activities will also occur, following dewatering/diversions (all required crayfish sweeps, methodology for restoration of dewatered/diverted stream areas, and notification requirements for dead or injured crayfish, as listed above, would also apply to culvert activities):

General Itemized Culvert Extension Activities would include:

1) Stream bed excavation and preparation for the installation of pre-cast concrete or cast in place culvert extensions.

2) Installation of pre-cast concrete or cast in place culvert extensions, also referred to as stream encapsulation.

3) Reconstruction of headwalls and wingwalls at the ends of the proposed culvert extensions, including stream bed preparation and pouring of concrete for aprons.

4) Installation of armoring along stream banks and within the channels for culvert outfall locations.

5) At areas where culvert extensions must be skewed to match the existing culvert alignment under the roadway, some areas of permanent stream relocation are required to connect stream flow from the new culvert inlet/outlet to the existing natural/undisturbed stream reach. While the stream is diverted around the work area, the permanent relocation channel would be excavated and stabilized. The temporary stream diversion would then be removed to restore stream flow through the culvert and the newly constructed stream channel.

The construction activities described above represent the anticipated methods and typical sequence of work. Minor variations in construction means and methods may occur, provided they do not increase the type or extent of effects analyzed in this Biological Opinion.

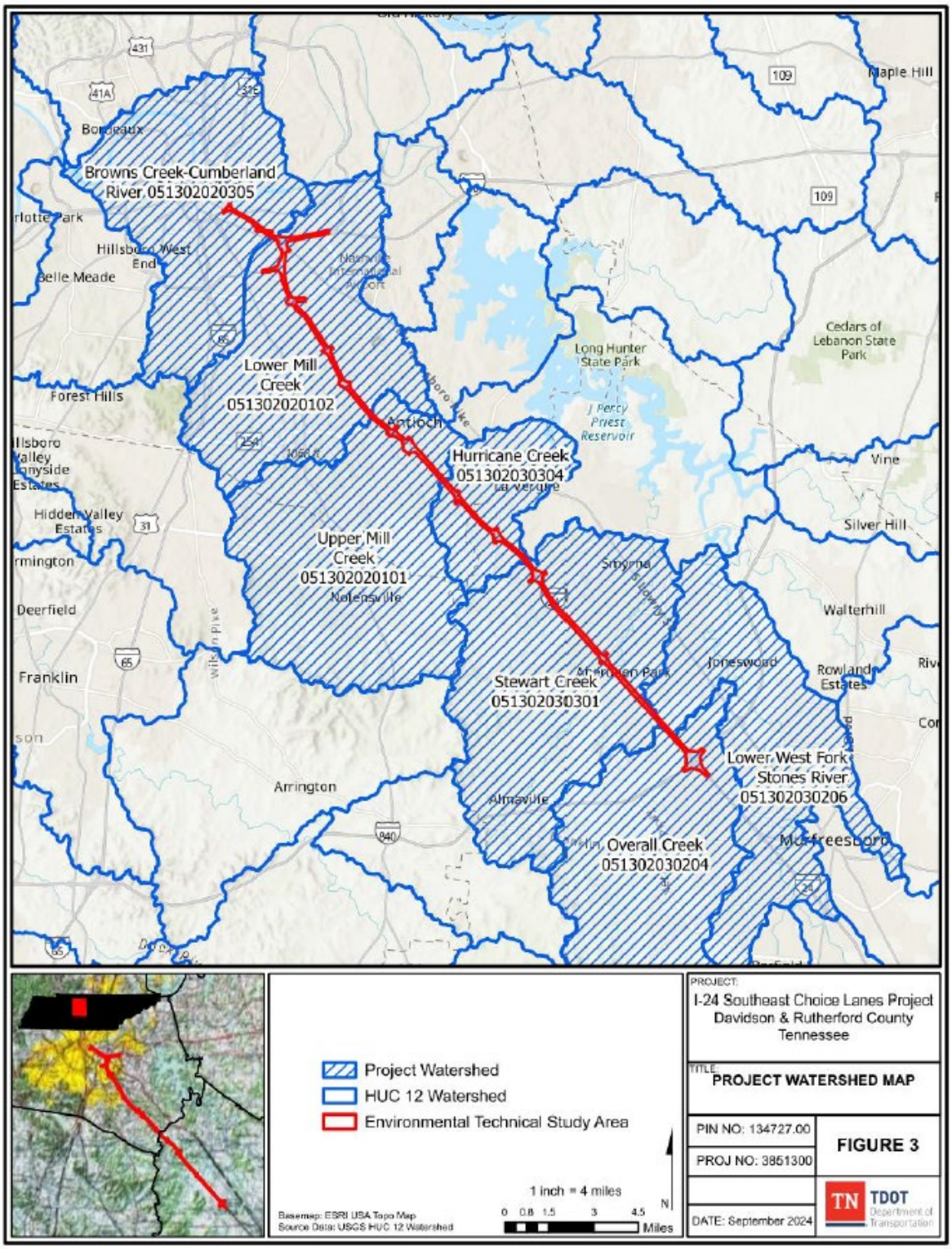

Figure 1. Approximate location of I-24 Roadway Improvements in the Mill Creek Watershed in Davidson and Rutherford Counties, Tennessee

2.1 Action Area

The action area is all areas to be affected directly or indirectly by the Federal action and not merely the immediate area involved in the action [50 CFR 402.02] that modify land, water, or air.

Activities associated with the project may occur during three phases:

Pre-Implementation activities
These activities occur prior to instream construction and include:

- Collection and relocation of Nashville crayfish from the construction footprint,
- Installation of temporary stream diversions,
- Dewatering of isolated work areas,
- Placement of block nets or exclusion devices as needed.

These activities are likely to temporarily disturb substrate and aquatic habitat as personnel access the stream channel and relocate individuals.

Implementation activities
Implementation includes all construction actions with potential to directly or indirectly influence aquatic habitat, such as:

- Equipment staging and maintenance near stream channels,
- Installation and removal of rock pads, work pads, or temporary crossings,
- Operation of heavy equipment in or near the channel,
- Demolition of existing bridge or culvert structures,
- Excavation, formwork, foundation preparation, pile driving, and construction of new structures,
- Placement of armoring or riprap for stabilization.

Post-implementation conditions
Following construction, short-term effects may occur as stream flow is restored to previously diverted or dewatered areas, including:

- Temporary increases in suspended sediment,
- Localized changes in substrate stability,
- Adjustments in channel morphology near construction locations,
- Potential project-related failures such as bank sloughing or erosion where soils were disturbed and not yet fully stabilized

These effects may extend briefly into downstream habitat as the system equilibrates.

Spatial Extent of the Action Area
Because construction-related activities are likely to modify land, water, and air beyond the immediate footprint, the action area includes upstream and downstream buffers around each instream crossing. For bridge structures, the action area extends 150 feet upstream and 1,320 feet

(0.25 mile) downstream of the construction footprint. For culvert extensions, the upstream extent is 100 feet, with the same downstream distance applied. These distances are based on the likely reach of short-term turbidity and disturbance associated with instream construction.

Across all applicable crossings, approximately 6.08 acres of streambed (24,624 square meters) are contained within the aquatic portion of the action area. This area includes suitable habitat for Nashville crayfish as well as transitional areas that may experience indirect effects from project activities.

The action area defined here is used throughout this Biological Opinion to evaluate potential effects and to estimate incidental take. The action area will be further discussed in the **"EFFECTS OF THE ACTION"** section of this Opinion.

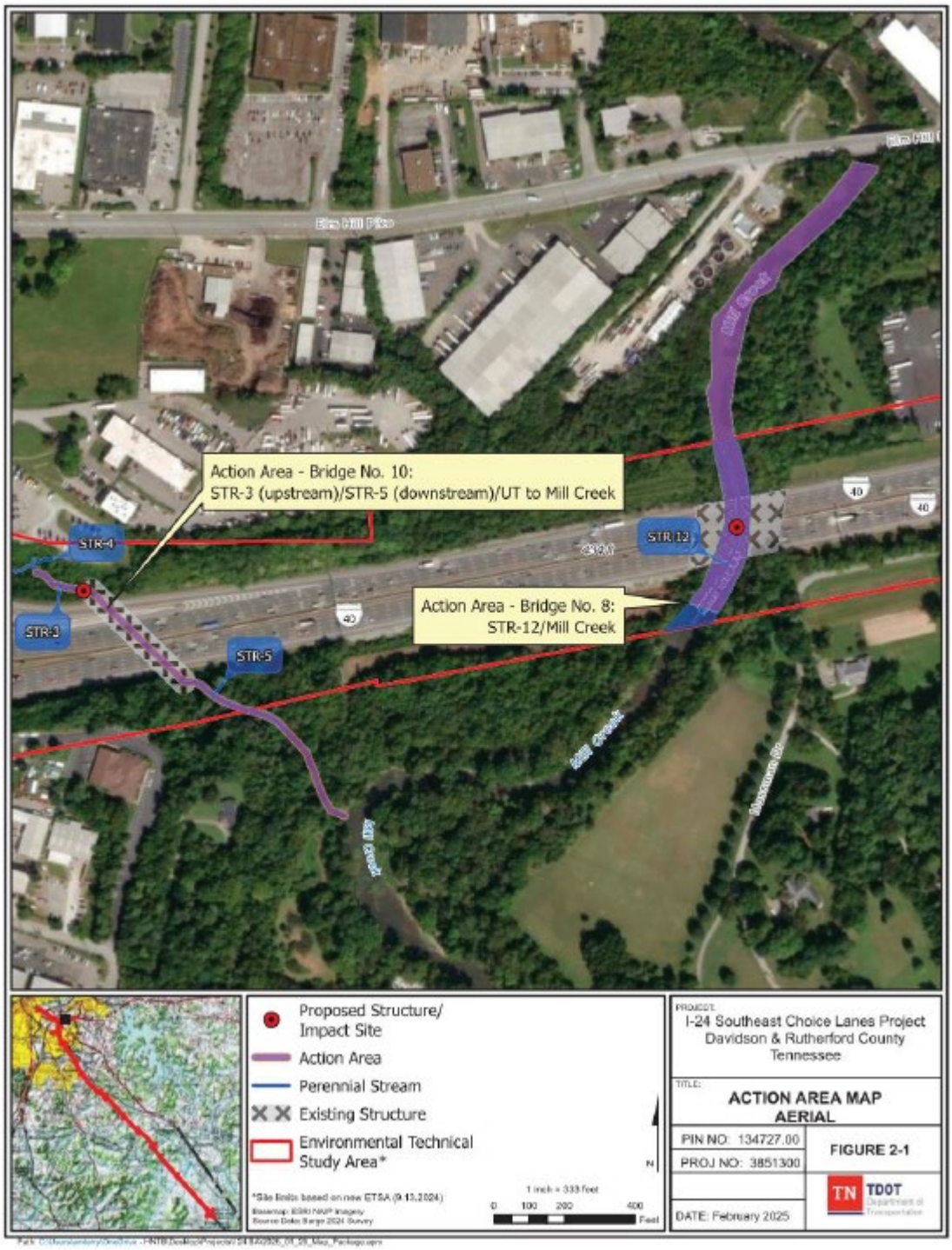

Figure 2. Map shown is representative of two proposed construction sites within the action area. The biological assessment provides multiple maps showing proposed construction activities along the I-24 corridor in Rutherford and Davidson County, Tennessee.

2.2 Conservation Measures

TDOT will implement the following Conservation Measures as part of the proposed action. These measures are integral components of the project and are designed to reduce impacts to aquatic habitat and minimize disturbance to Nashville crayfish where practicable.

- **Seasonal Instream Work Restrictions**: No instream construction activities will occur between October 1 and May 31. Work completed behind effective stream diversions or in dewatered areas may occur during this period if isolation from flowing water is maintained.
- **Steam Isolation and Dewatering**: All work below the ordinary high water mark will be isolated from flowing water using appropriate stream diversions and erosion prevention and sediment control measures. Dewatering structures will be installed, monitored, and maintained to prevent releases of sediment or materials into the channel.
- **Crayfish Sweeps and Relocation**: Prior to each phase of instream work, TDOT will conduct crayfish sweeps within the construction footprint to salvage and remove Nashville crayfish and relocate them to suitable habitat upstream or downstream. Additional sweeps will occur following diversion failures or before reestablishing flow in previously dewatered areas.
- **Containment of Construction Materials**: TDOT will implement measures (e.g., nets, tarps, platforms) to capture debris, dust, and materials associated with bridge demolition or construction. All material will be collected and disposed of at appropriate off-site locations.
- **Equipment Staging and Spill Prevention**: Construction equipment will be staged away from stream channels where practicable, and spill containment devices will be used to minimize the potential for contaminants to reach the water. Spill or releases will be addressed immediately in accordance with TDOT protocols.
- **Soil Stabilization and Waste Areas**: Disturbed soils and waste/borrow areas will be stabilized as soon as practicable to prevent erosion, and will be located and managed to prevent sediment from entering streams.
- **Compensatory Mitigation**: TDOT will partner with the U.S. Fish and Wildlife Service, Tennessee Wildlife Resources Agency, Tennessee Department of Environment and Conservation, and the Nashville Zoo to implement compensatory mitigation that support Nashville crayfish conservation. These voluntary measures are part of the proposed action but are not relied upon to minimize incidental take.

These Conservation Measures are foundational components of the proposed action. Compliance with them is required for the action to remain consistent with what is analyzed in this BO and this BO's conclusions regarding the likelihood of the action jeopardizing the continued existence of the species are conditioned upon the implementation of the conservation measures as described above. If the conservation measures are not implemented, then the conclusions in this BO may no longer be valid because the information it was based on would not be an accurate reflection of how the action is being implemented.

3. STATUS OF SPECIES

3.1 Nashville crayfish

This species was listed as an endangered species on September 26, 1986 (51 FR 34410). A recovery plan was approved on August 12, 1987, and a revised plan was approved on February 8, 1989 (USFWS 1989). Critical habitat has not been designated for the Nashville crayfish.

The Nashville crayfish was listed as endangered due to siltation, stream alterations, and water quality deterioration resulting from urban development pressures (USFWS 2019). At the time of its listing, the Nashville crayfish was known only from Mill Creek and five of its tributary streams in Williamson and Davidson counties, Tennessee. Historical collection records also reported occurrences in Big Creek (Elk River drainage), and in Richland and South Harpeth Creeks (Cumberland River drainage), although those records were likely either erroneous or the result of an isolated introduction which did not persist. Extensive sampling efforts in subsequent decades documented this species in the following tributaries to Mill Creek: Collins Creek, Owl Creek, Edmonson Branch, Sims Branch, Seven Mile Creek, Sorghum Branch, Whittemore Branch, Turkey Creek, Indian Creek, Holt Creek, four unnamed tributaries to Mill Creek, and one unnamed tributary to Owl Creek (Miller et al. 1990, TDEC DNA 2009, Walton 2008).

Recent survey efforts outside of the Mill Creek basin discovered a disjunct population of Nashville crayfish in the Lower Tennessee River, in the tailwaters of Pickwick Dam in Hardin County, TN. The discovery of additional viable populations outside of the Mill Creek basin would suggest that this species may not be as vulnerable as previously thought; however, it remains unclear whether this disjunct population is the result of an anthropogenic release or a native, recently discovered population that was recently discovered (Hurt et al., 2022).

This species continues to face risks from degraded water quality and catastrophic spills associated with increasing human populations and urbanization. The Metropolitan Nashville area is experiencing significant growth, with numerous residential, commercial, utility, and other infrastructure developments occurring in the watershed. Despite steady pressure from urban growth, Nashville crayfish have been in found in large numbers at locations that are already heavily developed. Additionally, there have been consistent stormwater and sediment inputs to the Mill Creek watershed, as well as frequent spills/releases of raw sewage and hazardous substances, yet the Nashville crayfish persists in high numbers. Overall, Nashville crayfish populations have been documented to be stable or increasing in size.

Due to its high degree of resistance to disturbance, the Nashville crayfish has exhibited a low susceptibility to threats and a high degree of stability (USFWS 2018). As a result of high resilience across the majority of population segments, high representation, and high redundancy (numbers have increased in all three representative units in the Mill Creek Watershed), and the species' demonstrated ability to adapt to changing environmental conditions over time from both anthropogenic and natural disturbances, the most recent status review determined that the Nashville crayfish no longer meets the definition of an endangered or threatened species in

accordance with sections 3(6) and 3(20) of the ESA. The Nashville crayfish was proposed for delisting on November 26, 2019.

In the spring of 2026, the Tennessee Department of Transportation in conjunction with the U.S. Fish and Wildlife Service, Tennessee Department of Environmental Conservation, Tennessee Wildlife Resources Agency, and the Nashville Zoo at Grassmere agreed to undertake additional conservation measures to offset impacts on the species caused by Interstate 24 roadway improvements. The offsets included three separate but related efforts to support the recovery and delisting of the crayfish. The first element of the collaborative includes expanded surveying for the crayfish into additional Mill Creek tributaries and backwaters where the species is expected to exist, which enhances surveying efforts have not been conducted before. The second element includes restoring a silt-inundated pond on Nashville Zoo property to create crayfish habitat. Nashville crayfish will be placed in this pond to create an exhibit for the public which will be viewed by approximately 2 million visitors annually and can help inform the public about the Mill Creek watershed and its unique species. The pond further serves as an assurance population and habitat source. The final element of the collaborative will be to take lessons learned from the backwater surveying and pond restoration to inform best management practices for local stormwater agencies to enact to conserve crayfish populations found near their structures, and to potentially create more habitat in the watershed. All three elements of the collaborative efforts serve to further advance the recovery of the species in the Mill Creek watershed.

More information about the species life history, distribution, population dynamics, threats, and recovery can be found in the listing rule (51 FR 34410), recovery plan (USFWS 1989), and the proposed delisting rule (84 FR 65098).

4. ENVIRONMENTAL BASELINE

Mill Creek originates in Williamson County, Tennessee, and flows in a northerly direction for 29.8 mi (Cook and Walton 2008). It crosses into Davidson County at approximately Mill Creek river mile (RM) 7.5 and continues to flow north for approximately 15.5 miles before joining the Cumberland River at Cumberland RM 194.5. The drainage area is 108 mi^2 and is located within the Central Basin Physiographic Region, an area approximately 6,000 to 7,200 mi^2 in size, which is comprised predominately of Ordovician limestones and shales. Five major tributaries contribute to the system, including Owl Creek, Indian Creek, Turkey Creek, Sorghum Branch, and Seven Mile Creek. Instream habitat consists of bedrock, gravel, small cobble, sand, and silt (Cook and Walton 2008).

The headwaters of Mill Creek flow primarily through rural, sparsely populated, agricultural lands. The lower reach is located within the heavily developed metropolitan area of Nashville. As the city has grown, development has spread toward the headwaters of the drainage, and agricultural lands have been increasingly converted to residential and commercial developments (USFWS 1986, USFWS 1989, USFWS 2018). These developments have affected stream quality and encroached on the floodplain. Potable water, sewer lines, and transmission lines have been

constructed along and across Mill Creek and its tributaries to provide those utilities to homes, businesses, and industrial facilities.

Of the 20 total miles of Mill Creek, over 16 miles are listed as impaired by the Tennessee Department of Environmental and Conservation (TDEC). Causes of impairment include Escherichia coli (*E. coli*), sediment/siltation, other anthropogenic substrate alterations, flow regime alteration, nitrate/nitrite, total phosphorous, propylene glycol, low dissolved oxygen, alteration in stream-side or littoral vegetative cover, and nutrient enrichment (TDEC 2022).

Throughout the entire Interstate 24 study area there are multiple reaches of delineated perennial and intermittent streams, as well as adjacent floodplain wetlands and wetland karst features. Per the October 2025 Draft Environmental Boundary Report, there are 96 streams, 25 wet weather conveyances/ephemeral streams, 45 wet weather conveyances/upland drainage features, 78 wetlands, 6 man-made ponds, and 32 constructed stormwater ponds that were delineated within and immediately adjacent to the study for the proposed project. Upon review of potential aquatic resources within the Mill Creek HUC-8 Watershed, there are 60 previously delineated streams within the watershed. These 60 streams were assessed for suitable habitat for Nashville crayfish.

The habitat assessment surveys for the crayfish were conducted on all 60 previously delineated streams within the Mill Creek HUC-8 watershed, throughout the entirety of the inspected reach within and immediately adjacent to the study area boundary. Inspections were performed starting from the downstream end of the reach and working towards the upstream point source or study area limit to avoid siltation while working within the channel. Observed habitat parameters were recorded, such as in-stream habitat (riffle, pool, run and glide), available substrate (bedrock, cobble, gravel, silt), approximate water depth within the channel, embeddedness, and presence of fish and crayfish species identified to family or genus classification by visual observations.

Confirmation, or lack thereof, of suitable Nashville crayfish habitat was determined based on the observed in-stream habitat parameters per each of the 60 previously delineated streams within the Mill Creek HUC-8 Watershed. Streams that were observed with the presence of preferred substrate, anticipated annual surface water flow, and potential observation of Nashville crayfish or other crayfish species, were determined to be suitable habitat for the federally listed species. Whereas, streams observed with anticipated seasonal very shallow surface flow, very high embeddedness, or entirely silty clay lined channel, were determined to be non-suitable habitat for the species.

After inspection of each reach and review of observations made during the onsite assessment of each stream within the Mill Creek (05130202) HUC-8 Watershed for the project, 18 streams were concluded to provide potentially suitable habitat for Nashville crayfish. These streams were observed with suitable in-stream habitat in the form of riffles, glides, runs or pools; a channel bottom of either a bedrock or clay with a suitable presence of boulder, slab rock, cobble, or gravel substrate; little to no substrate embeddedness; and anticipated year-round flow of surface water. Whereas the remaining 42 stream channels within the Mill Creek (05130202) HUC-8 Watershed were observed with a lack of suitable habitat for Nashville crayfish. These 42 streams were either observed as intermittent or headwater (first order) perennial streams with little to no

surface water flow and depth, a lack of suitable substrate to shelter Nashville crayfish within the channel, and/or a significant presence of silt or clay embeddedness. Therefore, the analysis for impacts to Nashville crayfish reflect the proposed actions found on the 18 streams identified with having suitable crayfish habitat.

4.1. Action Area Numbers, Reproduction, and Distribution

Nashville crayfish

Nashville crayfish are known to be present throughout the Mill Creek watershed, including in the tributaries which fall within the larger action area. The primary tributaries encompassed by the action area include mainstem Mill Creek, Seven Mile Creek, Sorghum Creek, Collins Creek, and Whittemore Branch. The most reliable Nashville crayfish population surveys for this Opinion in the action area have been conducted on an annual basis by the Nashville Zoo (McGinnity 2024). Two monitoring sites in particular fall adjacent to the action area, Wimpole Mill and Antioch, and are adjacent to and most representative of the action area. Incidental take calculations in Section 8.1 are based on Nashville crayfish densities found at these sites.

Table 1. Nashville Crayfish densities at three monitoring sites from 2024 study in Mill Creek watershed.

Sample site (Mill Creek River Mile)	**Average Stream width (m)**	**Average number of Nashville crayfish under 25 rocks**	**Number of 0.10 m^2 or larger rocks per 100-meter sampling site**	**Estimated population estimate per 100-meter sampling site**	**Crayfish density per square meter**
Wimpole (5.5)	24.4	38.8	**431 rocks** 38.8/25x431=	669	**0.27**
Antioch (13.6)	17	54.6	**344 rocks** 54.6/25x344=	751	**0.44**
Concord (20.8)	13	143.4	**300 rocks** 143.4/ 25x300=	1721	**1.3**

As noted in the table, the headwaters of Mill Creek show the highest density of Nashville crayfish in the watershed. However, the action area is most represented of the first two sites and the densities at these two sites will be later used in incidental take calculations in Section 8.1

4.2. Action Area Conservation Need and Threats

Within the action area, the Nashville crayfish is being affected by runoff from highways, pesticides and fertilizers from crop fields, organic matter from livestock pastures, and residential

and industrial development sites (USFWS 2018). The primary threat to Nashville crayfish populations comes from siltation associated with poor land-use practices.

As the Mill Creek Basin continues to develop, streams without evident impacts from development are becoming scarce. Farmlands in the headwater areas to the southeast of downtown Nolensville are experiencing rapid conversion from agricultural land to residential neighborhoods, requiring new and upgraded utilities to service this growth (Withers 2009). Development is associated with pollutant and sediment discharges into stream systems that contribute to stream impairment, destruction of riparian vegetation, bank collapse, excessive instream sediment deposition, and increased water turbidity and temperatures. The streams in the Mill Creek headwaters continue to receive runoff of pesticides and fertilizers from home sites and the remaining agricultural lands. Significant mortality of aquatic organisms, including Nashville crayfish, has been documented from introduction of chemicals into the stream from yard applications. These threats are likely to increase in the future as residential development increases (USFWS 2018).

Due to the presence of major roadway arteries in the action area, and the established network of roads within the vicinity of the action area, the potential also exists for accidental toxic chemical spills. Sources of such spills include potential accidents involving vehicles transporting chemicals over road crossings of streams inhabited by the Nashville crayfish and runoff of contaminants (de-icing chemicals, etc.) from roads during winter months.

5. EFFECTS OF THE ACTION

This section evaluates all direct and indirect effects of the proposed construction activities on the Nashville crayfish within the action area. Effects considered include those occurring within the immediate construction footprint and those occurring upstream and downstream from each crossing where land, air, or water was modified by the action's activities.

The assessment of the effects of the action on the species incorporates species behavior, habitat use, hydrology, turbidity transport, sediment dynamics, and anticipated construction practices. The analysis below describes how individual stressors, such as instream construction, sediment disturbance, equipment operation, vegetation removal, and crayfish handling, may affect Nashville crayfish and their habitat.

5.1. Stressor: Crayfish Sweeps and Relocations

Relocating crayfish has long been considered a viable option to protect individuals occurring within the footprint of a project.

Applicable Science – Crayfish Sweeps and Relocations: Little is known about the long-term relocation effects to Nashville crayfish, but relocation of collected individuals into an area already occupied by crayfish likely results in some take, as larger crayfish vigorously defend

their home territories and drive smaller crayfish from their selected cover (USFWS 2018). Relocated crayfish can be displaced through competition, and, lacking suitable cover, may be exposed to an increased risk of predation or starvation (USFWS 2023).

Following crayfish relocations efforts for a Tennessee Valley Authority (TVA) emergency bank stabilization on Owl Creek, the project area was resampled to assess post-construction recovery for Nashville crayfish. Biologists from the TVA, TWRA, and the Service recovered nearly three times as many crayfish (adults and juveniles) as were relocated the year prior (Giddens, pers. comm., 2023).

Surveys between the years of 2014 and 2016 by the Nashville Zoo at the Concord Road Bridge crossing to Mill Creek also showed positive recovery post-construction and suggested that the bridge replacement project in 2014 had minimal effects on Nashville crayfish populations. Rather than the expected reduction in numbers, crayfish observations downstream of the bridge were higher in 2015, from 83 in 2014 to 120 individuals in 2015. The author concluded that this may have been a result of crayfish migrating from the project footprint to avoid construction impacts or possibly relocated individuals, although the latter is unlikely because the recipient site was located upstream of the bridge. In 2016, individuals observed below Concord Road Bridge dropped slightly below preconstruction numbers, down from 83 individuals to 63 (McGinnity 2016).

The results of these monitoring efforts suggest that Nashville crayfish are resilient and can quickly recolonize an area following construction. The species exhibits a high degree of resistance to disturbance, indicating the species has a low susceptibility to threats and a high degree of stability (USFWS 2018).

Effects Pathway 1

Action Categories – Preconstruction crayfish sweeps

Stressor – Collection, handling, and establishment at the recipient site

Exposure (time) –Likely consisting of a cumulative period of less than 24 hours, involving multiple sweep and relocation efforts prior to commencement of each phase of instream construction or demolition activities.

Exposure (space) - The construction footprint for each rock pad, stream diversion/cofferdam, and the existing bridge or culvert construction.

Resource affected – Mill Creek watershed, Nashville crayfish

Individual response –Short-term physiological stress and displacement from cover, and disruption of feeding during collection, handling, and relocation efforts. Attempt to establish at the recipient site.

Effect –Mortality or harm to individuals that are overlooked during the salvage effort. Efforts to establish at the recipient site may result in adverse effects including mortality, reduced fitness, and diminished reproductive success.

Conservation and Minimization Measures –
All crayfish collected will be relocated to suitable habitat, either upstream or downstream of the project area. Multiple sweeps may be required. The crayfish sweeps will be conducted by personnel approved by the USFWS and Tennessee Wildlife Resources Agency (TWRA).
To the extent practicable, relocated crayfish will be released in small numbers and spread out across available habitats, to reduce competition with resident crayfish in relocation areas.

Interpretation – Multiple crayfish sweeps in the construction footprint would likely overlook some individuals and result in effects to those recovered. Due to the extent of bedrock present within this section of Mill Creek, the action area is expected to recover quickly and be recolonized by Nashville crayfish following construction. Most relocated individuals are expected to recover and establish at recipient sites. However, relocated Nashville crayfish could experience a short-term reduction in fitness, growth, and reproductive success.

5.2. Stressor: Instream Construction

Installation/removal and operation on rock pads in Mill Creek watershed may result in inadvertent crushing, burial, or injury of Nashville crayfish that are overlooked during crayfish sweeps. Also, disturbance of the streambed could create sedimentation and cause settled contaminants to re-suspend and obstruct crayfish gills, reducing their ability to respire or feed. However, crayfish, including the Nashville crayfish, have the ability to swim to other areas to avoid water quality disturbances and associated potential effects under most circumstances.

Applicable Science – Instream Construction: The size of a population and its natural variance over time are important characteristics affecting a species' response to construction disturbance. Large populations of Nashville crayfish have higher resilience, as they are better able to return to pre-disturbance numbers after stochastic events (USFWS 2018). Information is lacking regarding population characteristics such as critical size (i.e., a population threshold that is too low to recover from environmental stochasticity), variability (sex ratios, density, genetic viability, recolonization rates) and stability (the species' ability to resist change or dramatic fluctuations over time).

Sensitive periods for Nashville crayfish include the reproductive period (mating, spawning, egg release, and egg incubation and hatching), which occurs during the period from October 1 to May 31 (Barrociere 1986).

Suspended sediment (turbidity), originating from disturbed upstream areas, has the potential to cause dispersal of Nashville crayfish from the project area into areas with less cover, which would increase vulnerability of crayfish to predation (Cook and Walton 2008).

Effects Pathway 2

Action Categories – Rock Pads Installation/Removal

Stressor – Crushing, Sedimentation, and Burying

Exposure (time) – Year-round construction. However, installation/removal of cofferdam structures and rock pads is limited to June 1 to September 30 in order to avoid the Nashville crayfish reproductive period (October 1 - May 31).

Exposure (space) – The entire aquatic portion of the action area, including up to 150 feet upstream of the construction area for bridges, 100 upstream for culvert extensions and 1,320 ft (0.25 mile) below proposed construction areas.

Resource affected – Mill Creek, Nashville crayfish

Individual response –Reduced feeding activities and dispersal from the action area during sediment releases.

Effect – Mortality or harm to Nashville crayfish that are crushed. Temporary reduction in habitat availability from suspended sediment.

Conservation and Minimization Measures – All work below the Ordinary Highwater Mark (OHWM) of streams will be separated from flowing water using stream diversions or other standard best management practices. Appropriate EPSC measures will be implemented during any dewatering and stream diversion activities. Rock pads and stream diversions/cofferdams would be used to separate the work area from flowing waters. No in-stream work will occur from October 1 to May 31. Work from behind installed stream diversions or dewatering structures may continue during the restricted period, if separation from flowing waters of streams is maintained.

Interpretation – Nashville crayfish that are overlooked during the project reimplementation sweeps could be harmed or killed by installation/removal of stream diversions/cofferdams, the placement of riprap instream for the rock pads, and/or heavy equipment operation on the rock pads. Construction may temporarily mobilize fine sediment during installation and removal of temporary structures. This could result in crayfish dispersal from the area during construction, or a short-term reduction in fitness, growth, and reproductive success for Nashville crayfish within the action area. Any crayfish that disperse from the project area due to construction pressures may be forced to select sub-optimal habitat, thereby increasing susceptibility to predation and/or starvation.

5.3. Stressor: Heavy Equipment Operation

Use of heavy equipment along and adjacent to waterways increases the risk for introduction of fuels, lubricants, coolant, and hydraulic fluids into the riparian zone or water where they can

injure or kill aquatic organisms. Additionally, noise and vibrations can have deleterious effects on Nashville crayfish by causing dispersal from the action area into sub-optimal habitat.

Applicable Science – Heavy Equipment Operation: Petroleum-based contaminants, such as fuel, oil, and some hydraulic fluids, contain polycyclic aromatic hydrocarbons, which can cause lethal or acute and chronic sublethal effects on aquatic organisms (Neff 1985; Eisler 1987). The National Research Council (1992) indicated that sediment may act as a vector for delivering contaminants to streams. These substances may result in mortality to aquatic organisms, or they may accumulate in their body tissues and result in subsequent adverse chronic effects.

Effects Pathway 3

Action Categories – Operation of Construction Equipment

Stressor – Petroleum Leaks and Noise

Exposure (time) – Year-round, including sensitive periods for the Nashville crayfish.

Exposure (space) – The entire aquatic portion of the action area, including both instream rock pads, the area underneath bridges, and up to 150 feet upstream of the rock pads and 1,320 feet (0.25 mile) below the construction sites.

Resource affected – Mill Creek, Nashville crayfish

Individual response –temporary behavioral disruption, including reduced foraging or displacement.

Effect – Mortality or harm to Nashville crayfish that are unable to avoid exposure to petroleum pollutants if a leak or spill occurs. Construction noise and vibration could affect crayfish normal behavior patterns (including feeding and/or sheltering), increasing stress, and therefore, vulnerability to disease or predation. Habitat could be affected by reduced water quality and flow alterations resulting from construction.

Conservation and Minimization Measures – If spillage or leakage of concrete into a stream is observed, concrete pouring shall cease immediately and the TDOT Environmental Division Ecology Section must be immediately notified. In turn, the TDOT Environmental Division will also notify the Service.

Interpretation - Leaking equipment or spills of petroleum-based contaminants (including grease, oil, petroleum, and detergents) from hydraulic, fuel, or power systems could also result in mortality to Nashville crayfish in the vicinity of the spill and downstream due to resultant rapid water quality changes and inability of individuals to rapidly adjust their physiology. These toxic materials have the potential to affect water quality, food sources, and in turn, respiration and feeding capabilities. If individuals are unable to avoid exposure by burying or dispersing from

the area, they would likely be harmed or killed from exposure to water containing toxins. Noise and vibration may cause displacement into suboptimal habitat, with associated increased risk.

5.4. Stressor: Riparian Removal/Soil Disturbance

Site preparation associated with construction activities would involve removal of vegetation, including riparian habitat, and soil disturbance in the footprint of the bridge, approaches, rock pads, and equipment staging/maintenance areas. These exposed areas, if not properly stabilized, could experience significant erosion during rainfall events, and result in sediment entering Mill Creek watershed.

Applicable Science – Riparian Removal/Soil Disturbance: Riparian vegetation removal can elevate temperatures, destabilize banks, and increase sediment inputs (Johnson and Jones 2000, Sutherland et al. 2002). Nashville crayfish are highly photosensitive and are usually found under cover during the day (Bouchard 1976). All the sites surveyed by O'Bara et al. (1985) for crayfish had significant canopy cover, varying between 60% and 90%. Where canopy removal and ground disturbance has occurred for construction, runoff can additionally result in heavy input of sediment into the stream, resulting in bank collapse, excessive instream sediment deposition, and increased water turbidity. These conditions could make crayfish habitat unsuitable within the action area.

Potyondy et al. (1991) indicated that most of the surface erosion associated with construction in general (whether temporary or permanent) occurs in the first year and continues at a reduced but relatively high rate for several years, depending upon local weather conditions. In general, the highest erosion rates are associated with construction activities, which can contribute sediment at a 300 times greater rate than from forested lands (USDA 1977).

Silt deposition in streams contributes to several of the impairments in the Mill Creek watershed, and can be a risk factor for crayfish. Sediment has been shown to abrade and suffocate benthic organisms by clogging gills and reducing aquatic insect diversity and abundance (Waters 1995). Stream channelization and silt deposition has been reported to be directly responsible for the permanent loss of some crayfish populations (Reynolds et al. 2013). Sediment settling and the filling in of crevices and interstitial spaces under slab rocks may result in increased biological oxygen demand and longer term or permanent loss of habitat for crayfish (Cook and Walton 2008). As crayfish are primarily active at night, the chief requirement of all size classes is for hiding spaces during the daytime. Where loss of hiding spaces occurs through bank reconstruction or siltation from natural or human causes, the habitat's carrying capacity for crayfish diminishes (Reynolds et al. 2013).

Dissolved oxygen (DO) levels are also an important water quality parameter for crayfish. Oxygen is dissolved into the water in streams through diffusion, aeration, and as a byproduct of photosynthesis. The amount of DO present in water can vary due to several factors including water temperature, level of pollutants, and water velocity. Elevated water temperatures from removal of the riparian area could result in a reduction in DO. If DO levels are very low, it is harder for individual crayfish to take in oxygen and, in extreme cases, the lack of DO results in

death. Although the tolerance level of Nashville crayfish for DO is not known, levels below 2.0 mg/L typically result in invertebrates abandoning the area (Fondriest 2013).

Effects Pathway 4

Action Categories –Removal of Vegetation and Site Preparation

Stressor – Elevated Temperatures, Soil Destabilization and Erosion

Exposure (time) – Site preparation, including canopy removal, would likely be a one-time event occurring at the beginning of the project implementation phase. Surface and streambank erosion could occur year-round if eddying occurs upstream of the rock pads, disturbed areas are not properly stabilized, or BMPs are inadequate or not functioning properly.

Exposure (space) - The entire action area, totaling approximately 469,163 ft^2 (27,590 m^2) of terrestrial and aquatic habitat. This includes the project footprint and up to 150 feet upstream of the construction site and 1,320 feet (0.25 miles) below the project site.

Resource affected – Mill Creek watershed, Nashville crayfish

Individual response –Reduced feeding activities or dispersal from the action area during sediment releases and periods of elevated turbidity.

Effect –Loss of daytime hiding spaces required by Nashville crayfish from siltation, lowering the carrying capacity within the action area. Reduction in DO from removal of riparian vegetation.

Conservation and Minimization Measures – Measures including, but not limited to, nets, tarps, etc. will be implemented to prevent materials from the proposed construction activities from entering streams. These materials include, but are not limited to asphalt, metal and concrete debris, dust, paints, sealers, materials from sandblasting activities, etc. These materials will be contained properly and disposed of in an appropriate manner at an approved waste site. Waste and/or borrow areas, if needed, will be established in non-wetland areas and are to be of sufficient distance from area streams such that no soil material is allowed to enter these streams. Waste or borrow areas will be stabilized as soon as practicable. Appropriate EPSC measures will be utilized in these areas to minimize soil loss. To minimize impairment to streams, the contractor shall adopt as many (at least one) of the following measures as practicable: 1) Equipment shall be staged a minimum of 150 feet from all streams, 2) Equipment shall be staged in upland areas with no direct drainage to streams, 3) Catch pans and/or spill containment "diaper" shall be installed on all construction equipment.

Interpretation – Site preparation for construction and removal of riparian areas can cause erosion to occur and sediment to enter Mill Creek and its tributaries during periods of rainfall and higher flows if the disturbed areas are not properly stabilized. Excessive sedimentation can eliminate daytime refugia, reduce habitat quality, and impair essential behaviors. These impacts range

from a short-term reduction in fitness, growth, and reproductive success to chronic effects or even mortality.

5.5. Stressor: Bridge and Culvert Maintenance

Seasonal and routine maintenance activities are required on bridges to ensure safety, preserve the integrity of structural components, and extend their useful life. Routine maintenance often requires application of chemical products such as paint, sealants, lubricants, epoxy, or concrete. Seasonal maintenance includes use of roadway salts and other chemicals to improve safety for the motoring public. Accidental entry of these materials can impair water quality and harm aquatic organisms. Removal of vegetative regrowth within a 10-foot perimeter of bridges is also necessary to maintain structural integrity and ensure bridge access.

Applicable Science – Maintenance Activities: Fresh concrete in contact with water can result in significant increases in alkalinity in streams. Levels of as high as 11 on the pH scale have been recorded in samples where fresh concrete makes contact with water. High levels of alkalinity following construction of concrete culverts resulted in observations of dead fish and eels (Setunge et al. 2009).

Due to concerns about the negative effects of salt on aquatic systems during wintertime de-icing, some consideration is being given to the use of alternatives such calcium chloride and magnesium chloride. These have shown promise in their ability to better lower the freezing point while also providing important ions for plant cells (Coldsnow and Relyea 2018).

Treatment of stormwater runoff that cannot be properly diverted is a significant challenge. There are a number of pollutants associated with stormwater runoff that pose a concern for the environment. Examples of these are polycyclic aromatic hydrocarbons, heavy metals, oil, grease, nutrients, suspended solids, fecal coliform, oxygen-demanding organics, perfluorinated compounds, and polychlorinated biphenyls (Malina et al. 2005; Bakr et al. 2020). Due to the ephemeral nature of runoff, it is difficult to fully comprehend the toxic effects of stormwater discharge on the environment. Chronic exposure may affect organisms that can ordinarily handle higher levels of toxins for short periods of time. Factors that are subject to hydrologic changes, weather patterns, and other conditions include toxicant dose, duration of the exposure, and timing. Bioassay studies on highway runoff have not generally demonstrated acute nor chronic toxicity from heavy metal bioaccumulation, sediments, or other pollutants associated with stormwater runoff (Malina et al. 2005).

Effects Pathway 6

Action Categories – Bridge and Culvert Maintenance Activities

Stressor – Chemical Applications and Stormwater Runoff

Exposure (time) – Seasonal maintenance activities would occur during winter months. Routine maintenance activities could occur year-round.

Exposure (space) – If chemical treatments from seasonal or routine maintenance activities enter the water, they could affect the area underneath the bridge and an indeterminable distance downstream from the bridge before being diluted by water.

Resource affected – Mill Creek watershed and Nashville crayfish

Individual response – Reduced feeding activities or dispersal from the action area until chemicals have flushed from the system or have been diluted to a tolerable level.

Effect – Mortality or harm to Nashville crayfish that are unable to avoid exposure to chemical treatments if a leak or spill occurs.

Conservation and Minimization Measures – Containment measures would be required for all routine maintenance that occurs on the bridge to prevent chemicals and other construction materials from entering the river. All construction wastes would be contained and properly disposed of at an approved waste disposal site.

Interpretation - Maintenance activities are periodically performed on bridges to ensure proper function and extend their useful life. Water quality measures and containment of chemical treatments are required at all times during construction by permits and TDOT's standard specifications for bridge construction. If the decks drains are installed and functioning properly and construction materials are contained during routine maintenance activities, we would not anticipate chemical exposure at a level that would result in measurable effects to Nashville crayfish.

5.7. Summary of Effects

Construction activities within Mill Creek and its tributaries are expected to result in a range of temporary effects to Nashville crayfish. Within the construction footprints, individuals may be harmed or lethally take despite relocation efforts. Outside the footprints, individuals may be disturbed by short-term increases in turbidity, sediment deposition, noise, or displacement from cover. These indirect effects decrease with distance from the work area and are expected to be temporary due to the species' documented resilience and the presence of stable habitat conditions throughout much of the watershed. These upstream and downstream behavioral or short-term physiological responses do not constitute take under the ESA because they are not reasonably certain to result in injury or death to individual Nashville crayfish.

Taken together, the effects include short-term behavioral disruption, temporary reduction in habitat quality, and localized potential for injury or mortality. The magnitude and duration of these effects will be limited by the Conservation Measures incorporated into the project design and the additional protective measures included in the Incidental Take Statement.

As described throughout the Effects of the Action (Section 5), the number of Nashville crayfish subject to capture, injury, or mortality during construction is directly related to the spatial extent

of the instream construction footprint. Each stressor, crayfish sweeps (Section 5.1), instream construction (Section 5.2), and heavy equipment operation (Section 5.3), affects only those individuals located within areas where stream substrate is disturbed, excavated, dewatered, or directly overlain by temporary or permanent structures. Because the project area consists of extensive slab-bedrock and complex interstitial spaces, a portion of individuals within this footprint cannot feasibly be detected during pre-construction sweeps. Consequently, although numerical estimates of capture and harm/lethal take can be calculated based on observed densities and sweep efficiency, the best predictor of the magnitude of incidental take is the areal extent of streambed disturbed, which can be accurately measured, flagged, and monitored.

This causal relationship forms the basis for the Service's use of the instream construction footprint as a surrogate measure in the Incidental Take Statement.

6. CUMULATIVE EFFECTS

Cumulative effects include the effects of future state, tribal, local, or private actions that are reasonably certain to occur within the action area of this consultation. Future Federal actions are not considered here because they require separate consultation under section 7 of the ESA. The action area, as defined in Section 2, is limited to stream segments directly upstream and downstream of each instream construction location. This area consists solely of the aquatic footprint affected by turbidity, sediment transport, and other short-term project-related stressors. At this time, we are not aware of any non-Federal actions that are reasonably certain to occur within this aquatic action area. While residential and commercial development is occurring elsewhere within the broader Mill Creek watershed, these activities fall outside the boundaries of the action area for this consultation and therefore do not meet the definition of cumulative effects under 50 CFR 402.02.

Accordingly, no cumulative effects, as defined by the ESA, are anticipated to occur within the action area.

7. CONCLUSION

In accordance with section 7 of the ESA and its implementing regulations [50 CFR 402], this BO considers the current status of the Nashville crayfish across its range, the environmental baseline within the action areas, the effects of the proposed I-24 construction activities, and the absence of cumulative effects, to determine whether the proposed action is likely to jeopardize the continued existence of the species or result in the destruction or adverse modification of critical habitat.

As described in the *Status of the Species* section, TDOT, the Service, TDEC, TWRA, and the Nashville Zoo have developed a draft multi-agency conservation initiative intended to support Nashville crayfish recovery within the Mill Creek watershed. Although the agreement establishing these actions has not yet been finalized, the draft includes defined roles, detailed deliverables, and a proposed funding structure, which collectively indicate a strong likelihood that these conservation actions will be implemented once the MOU is executed. Because the

agreement is still in draft form and has not been incorporated into the proposed action, these conservation efforts are not relied upon to offset effects or to reach the jeopardy conclusion for this project. Instead, they are described in this BO solely because they contribute to the species' overall conservation context and reflect ongoing interagency planning that is reasonably certain to occur upon finalization of the agreement.

Based solely on the proposed action, the applicant's Conservation Measures, and the nondiscretionary Terms and Conditions contained in this BO, the Service concludes that the project, as proposed, is not likely to jeopardize the continued existence of the Nashville crayfish.

This determination is based on:

1. relocation and salvage efforts that minimize the number of individuals exposed to direct construction impacts;
2. project-specific Conservation Measures and enforceable Terms and Conditions that limit the magnitude, duration, and spatial extent of disturbances;
3. the species' documented resilience and demonstrated ability to recolonize disturbed areas; and
4. the limited geographic and temporal scope of effects relative to the species' current range-wide distribution and stable or increasing population trends.

Accordingly, when the effects of the proposed action are added to the environmental baseline and considered within the broader context of the species' conservation status, the Service determines that the proposed action is **not likely to jeopardize** the continued existence of the Nashville crayfish

8. INCIDENTAL TAKE STATEMENT

Section 9 of the ESA and regulations issued under section 4(d) prohibit take of listed wildlife without special exemption. "Take" is defined to include capture and harm (among other forms). Incidental take is take that is incidental to, and not the purpose of, carrying out an otherwise lawful activity. This Incidental Take Statement provides the extent of incidental take exempted under section 7(o)(2) for the proposed I-24 project and the conditions attached to that exemption.

The Service anticipates incidental take of Nashville crayfish in two forms:

1. capture during systematic pre-construction relocation sweeps, and
2. harm or lethal take of individuals that remain undetected within the instream construction footprint.

Based on our careful evaluation of the best available information as described in the Effects of the Action section of this BO, incidental take is reasonably certain to occur within the instream footprint only. In terms of habitat that is upstream and downstream of the construction areas, the short-term turbidity, short-term minor noise, and minor substrate disturbance related to short-term changes in flow areas are not reasonably certain to injury or kill individual crayfish because

their feeding, breeding, and sheltering within the stream is not likely to be impaired. Therefore, no incidental take is reasonably certain to occur outside the footprint.

8.1 Amount or Extent of Take Anticipated

The Service anticipates incidental take of Nashville crayfish to occur within the instream construction footprint only, where individuals may be captured during relocation sweeps or may be overlooked and subsequently exposed to crushing, burial, or impaired respiration. Calculation of take includes both (1) estimated captures and (2) estimated injury or mortality of undetected individuals, as described in the Effects of the Action section.

However, due to the complex slab-bedrock substrate and the inability to directly observe mortality beneath riprap or within interstitial spaces, the actual number of individuals harmed or killed cannot be feasibly monitored during construction. Therefore, while numerical estimates are provided below based on best scientific information, the Service expresses anticipated take using the spatial extent of the instream footprint as the enforceable surrogate measure.

Use of a Surrogate (50 CFR 402.14(i)(1)(i))

A surrogate is necessary because it is not practical to detect or monitor take of all individual Nashville crayfish during construction. Many individuals occupy deep interstitial spaces that cannot be accessed during sweeps, and mortality from crushing or burial beneath riprap cannot be directly observed.

The instream construction footprint provides a direct causal link to incidental take because it defines the exact area where crayfish are reasonably certain to be exposed to injury or death. It also establishes a clear, objective, and enforceable standard: if construction activities exceed the spatial footprint analyzed in this BO—approximately 24,624 m^2 of suitable habitat—then the level of anticipated take is exceeded and reinitiation under 50 CFR §402.16 is required.

Because the footprint can be delineated in the field with stakes, GPS, or physical markers, this surrogate provides a reliable and enforceable measure of take extent.

Numerical Estimates (Retained for Analytical Transparency)

Although the footprint surrogate serves as the enforceable trigger, the Service retains numerical estimates to document the analytical basis for evaluating take:

- Estimated capture: 350–475 individuals
- Estimated undetected individuals likely harmed/killed: up to 175 individuals
- Total estimated incidental take: no more than 650 individuals

Basis for the Capture Estimate

The Owl Creek salvage effort conducted in 2023 (USFWS 2023) provides the best available quantified information on expected sweep yields for Nashville crayfish. Applying the Owl Creek density (0.021 captured individuals/ft^2) to the I-24 instream footprint (~22,097 ft^2) yields a point estimate of approximately 464 individuals captured.

Because I-24 structures include more complex slab/bedrock interstices than Owl Creek, field conditions may reduce sweep efficiency and thereby reduce capture yields. To reflect this uncertainty while remaining consistent with the best scientific and commercial data available, the Service anticipates a capture range of 350 to 475 individuals across the instream construction footprint. This range brackets the Owl Creek-based estimate and reasonably represents sweep performance expected across multiple structures.

Basis for the Harm/Lethal Take Estimate
Not all individuals within the footprint will be detected during sweeps. Those that remain undetected are likely to be injured or killed during construction due to crushing, burial, or impaired respiration in confined interstitial spaces. The number of undetected individuals depends on the total number of crayfish present and the efficiency of the sweep effort.

Based on the Owl Creek salvage experience and the more complex substrate conditions expected at I-24, the Service anticipates that sweep efficiency will generally fall between 70% and 85%. When this range of sweep efficiency is applied to the capture range described above, the total number of crayfish expected to be present within the footprint at the time of construction is estimated to fall within a moderate range that exceeds the number actually captured. The difference between the total number present and the number captured represents the group of individuals that are reasonably certain to remain undetected.

Using this method, the Service estimates that the number of undetected individuals across all instream construction sites will fall within a range of roughly 60 to 200 individuals. Based the size of the area, the length of time construction will be taking place, and the intensity of the construction activities, the mechanisms of injury associated with instream construction would involve crushing beneath equipment and burial beneath riprap or sediment. The Service anticipates that 60% to 85% for undetected individuals would be injured or killed.

Applying the proportion of crayfish that are reasonably certain to be killed or injured to the total estimated number of undetected crayfish results in the take of an estimated 40 to 170 individuals under realistic field conditions. To provide a clear estimate on the amount or extent of incidental take for the purposes of determining if take has been exceeded and thereby setting a trigger for reinitiation of consultation, the Service estimated that up to 175 individuals are reasonably certain to be injured or killed (taken) during construction.

Total Amount of Incidental Take that is Reasonably Certain to Occcur
The Service estimates no more than 650 Nashville crayfish will be incidentally taken during the action. This total reflects the combined estimates for both capture and harm/lethal take described above. Depending on actual sweep performance, the total amount of take may be lower, but it is not expected to be exceeded.

If the amount or extent of incidental take approaches or exceeds any of the estimated amounts or extent, including the total take estimate, the capture range, or the harm/lethal allowance, FHWA and TDOT must immediately reinitiate consultation with the Service pursuant to 50 CFR 402.16.

Any incidental take that exceeds the amount of take described above is not exempt from being considered as prohibited take, pursuant to section 9 of the ESA.

8.1.1 Methodology for Estimating Take

The Owl Creek salvage effort conducted in 2023 provides quantifiable information on expected sweep yields (0.021 captured individuals/ft²). Applying this to the I-24 footprint (~22,097 ft²) yields ~464 individuals captured. Sweep efficiency is expected to fall between 70–85%, reflecting more complex substrate conditions at I-24. The difference between estimated total individuals and those captured represents the estimated undetected individuals, of which 60–85% are reasonably certain to be injured or killed. From these calculations, up to 175 individuals are expected to incur harm or lethal take.

These numerical values inform but do not limit take; the enforceable limit is the spatial footprint surrogate.

8.1.2 Effect of the Take

The impacts of the anticipated take on the listed entity and the local population is localized and temporary, with high resilience documented for Nashville crayfish in Mill Creek.

8.2. Reasonable and Prudent Measures

The following Reasonable and Prudent Measures (RPMs) are necessary and appropriate to minimize the amount or extent of incidental take of Nashville crayfish. These RPMs are non-discretionary and apply in addition to the Conservation Measures that are integral components of the action (Section 2.2). Failure to implement the Conservation Measures constitutes a change to the action and may require reinitiation pursuant to 50 C.F.R. § 402.16(a). To avoid redundancy, the RPMs below focus on implementation, verification, and monitoring, rather than re-stating conservation measures already described as part of the proposed action.

RPM 1. **Ensure complete and effective implementation of pre-construction crayfish sweep and relocation efforts.** FHWA and TDOT must ensure that pre-construction sweeps are performed systematically across the full instream construction footprint, that sweep methods are applied consistently, and that relocation and handling methods minimize stress and injury. Sweep teams must also document substrate types encountered, inaccessible interstitial areas, and efforts attempted to access those areas, in order to verify sweep completeness and support assumptions used in take estimates.

RPM 2. **Minimize water-quality impacts during instream construction.** FHWA and TDOT must ensure that isolation measures, EPSC controls, and pollutant-prevention practices committed to in the Proposed Action are functioning as intended, and that any water-quality exceedances or pollutant releases are detected and corrected promptly.

RPM 3. **Detect, contain, and respond rapidly to failures of stream diversions, BMPs, or equipment.** FHWA and TDOT must ensure that structural failures, overtopping, pollutant releases, or any deviations that could result in additional take are identified and addressed immediately.

RPM 4. **Restore disturbed substrate and banks to pre-project physical condition.** FHWA and TDOT must ensure that disturbed streambed areas are restored to approximate pre-project elevations and physical configurations to support rapid recolonization by Nashville crayfish. Restoration must ensure that the spatial extent and depth of substrate disturbance do not exceed the instream footprint analyzed in Appendix B and used as the take surrogate.

RPM 5. **Maintain monitoring, documentation, and reporting sufficient to detect whether estimated incidental take is exceeded.** FHWA and TDOT must ensure complete, accurate, and timely documentation of sweeps, relocations, BMP performance, water-quality conditions, and any potential injury or mortality. Monitoring and documentation must include verification that instream disturbance remains within the 24,624 m^2 instream footprint used as the surrogate measure for incidental take.

8.3 Terms and Conditions

The following Terms and Conditions (T&Cs) implement the RPMs in Section 8.2. They are non-discretionary and FHWA must ensure that TDOT and all contractors implement them through enforceable mechanisms such as permits, contracts, and construction specifications. Compliance with these T&Cs is required for FHWA and TDOT to remain exempt from the prohibitions of section 9 of the ESA under section 7(o)(2).

T&C 1. **Crayfish Sweep Verification and Documentation (implements RPM 1)**

- FHWA and TDOT must ensure that Sweep Logs are maintained for every pre-construction sweep, including:
    - The date and time of each sweep;
    - Personnel conducting the sweep;
    - Area covered;
    - Sweep Logs must also document substrate features that were inaccessible or unsafe to access, including deep slab-rock interstices, and describe efforts taken to attempt access using approved methods;
    - The number of Nashville crayfish captured; and
    - Release locations.
- FHWA and TDOT must ensure that the Sweep Logs are retained on site and available to the Service upon request throughout the duration of the project.

T&C 2. **Water-Quality Monitoring and Corrective Action (implements RPM 2)**

- FHWA and TDOT must ensure that turbidity, clarity, and other water-quality parameters identified in the project-specific SWPPP are monitored at intervals sufficient to detect water-quality exceedances associated with instream construction.
- If monitoring detects water-quality conditions that could impair crayfish respiration (for example, persistent turbidity above background conditions or visible suspended sediment escaping containment), FHWA and TDOT must:
    - Immediately halt the instream activity contributing to the exceedance
    - Identify and correct the cause of the exceedance (e.g., tighten diversion seals, repair cofferdams, adjust pumping rates, remove accumulated fines), and
    - Confirm that water-quality conditions have returned to background or pre-construction levels before resuming work.
- All water-quality monitoring results, exceedances, and corrective actions must be recorded in a Water-Quality Monitoring Log maintained on site and made available to the Service upon request.
- Verification must include documented turbidity readings or photographic evidence of water-quality recovery.

T&C 3. Immediate Response to Sweep Failure or Exclusion Failure (implements RPM 3)

- If a sweep cannot be completed due to safety or access limitations, FHWA and TDOT must document the reason, notify the Service within 24 hours, and coordinate on additional measures if needed.
- If any exclusion device (such as block nets) fails, is overtopped, or becomes ineffective, FHWA and TDOT must:
    - Immediately halt instream work,
    - Restore the device to proper function, and
    - Conduct a supplemental sweep before work resumes.
- All failures and corrective actions must be recorded in an Exclusion Failure Log maintained on site.

T&C 4. Injury and Mortality Reporting (implements RPM 3)

- FHWA and TDOT must notify the Service within 24 hours of discovering any injured or dead Nashville crayfish.
- Injured or dead individuals must be photographed in place prior to movement, to support later assessment of cause.
- When feasible, specimens must be preserved to allow identification and evaluation of potential causes.
- All injury and mortality events must be recorded in a Take Tracking Log.

T&C 5. Substrate Restoration Verification (implements RPM 4)

- FHWA and TDOT must document the condition of the instream substrate following removal of temporary construction features.
- Documentation must include photographs comparing post-construction substrate to pre-construction photographs taken during initial site preparation, and a brief description of how substrate was restored to approximate pre-construction conditions.
- This documentation must be provided to the Service upon request.

T&C 6. Take Accounting and Monitoring (implements RPM 5)

- FHWA and TDOT must maintain a Take Accounting Sheet that tracks:
  - Cumulative captured individuals;
  - Cumulative observed injured or dead individuals; and
  - Dates of sweeps and construction phases.
  - Verification that instream disturbance remains entirely within the 24,624 m² instream footprint serving as the surrogate take limit.

T&C 7. Post-Construction Reporting (implements RPM 5)

- Within 60 days of completing instream construction at each structure, FHWA and TDOT must submit a brief Post-Construction Report that includes:
  - Sweep Logs;
  - The Take Accounting Sheet;
  - Injury/mortality logs; and
  - Documentation of substrate restoration.
- The final report must affirm that instream disturbance remained within the analyzed instream footprint and must include site maps or GPS-referenced photographs showing disturbance boundaries.

8.4 Amount/Extent of Take Covered by This ITS

Pursuant to ESA §7(o)(2), the Service estimates incidental take of no more than 650 Nashville crayfish including both captured and undetected individuals likely harmed or killed. These numerical estimates are retained to document the analytical basis for evaluating take.

Surrogate as the Enforceable Trigger

Because individual mortality beneath riprap or within complex slab substrates cannot be feasibly detected, the enforceable limit of incidental take for this ITS is the total instream construction footprint analyzed in this BO.

Incidental take is exceeded, and reinitiation under 50 CFR §402.16 is required, if:

1. Construction activities disturb streambed outside the 24,624 m² footprint analyzed;
2. Disturbance within the footprint is more extensive, deeper, or longer in duration than evaluated;
3. New instream work occurs at locations not included in the action evaluated.

This surrogate approach satisfies 50 CFR 402.14(i)(1)(i) because:

- There is a direct causal link between substrate disturbance and take;
- It is not practical to monitor take of individuals directly;
- It provides a clear, objective, enforceable standard.

8.5 Triggers for Reinitiation

Reinitiation of formal consultation is required and shall be requested by the Federal agency or by the Service, where discretionary Federal involvement or control over the action has been retained or is authorized by law and:

a) If the amount or extent of taking specified in the incidental take statement is exceeded;
b) If new information reveals effects of the action that may affect listed species or critical habitat in a manner or to an extent not previously considered;
c) If the identified action is subsequently modified in a manner that causes an effect to the listed species or critical habitat that was not considered in the biological opinion; or
d) If a new species is listed or critical habitat designated that may be affected by the identified action.

9. CONSERVATION RECOMMENDATIONS

The following conservation recommendations are discretionary measures that the FHWA and TDOT may undertake to further minimize impacts on the Nashville crayfish or to promote conservation of the species and its habitat. These measures are not required for compliance with this Biological Opinion or for the incidental take exemption under section 7(o)(2) of the ESA. Their implementation would support ecosystem resilience within the Mill Creek watershed and further the purposes of ESA section 7(a)(1) by encouraging actions that promote conservation of the Nashville crayfish, contribute to recovery planning, and support the development of information beneficial to listed species and their habitats.

We offer the following conservation recommendations for consideration. Together, these conservation recommendations support long-term, post-construction watershed resilience by promoting habitat quality, water-quality protection, and coordinated stewardship across the Mill Creek drainage.

1. Avoid discretionary instream work during the Nashville crayfish reproductive period (October 1 through May 31). Although required instream construction work must adhere to the terms of this BO and the Proposed Action, the Service recommends that any optional or non-critical activities be scheduled outside this reproductive window to further reduce potential impacts on sensitive life stages, including mating, egg-bearing, and early juvenile development.

2. The FHWA and TDOT should actively pursue habitat restoration opportunities upstream or downstream of the structures. These efforts should be guided based on the Tennessee Wildlife Action Plan, specifically the actions identified in the Mill Creek Conservation Opportunity Area (COA). Examples of habitat

restoration include riparian restoration; livestock exclusion fencing; trash removal; eradication of non-native, noxious vegetation, etc.

3. The FHWA and TDOT should integrate information about the presence and recovery success of Nashville crayfish in the Mill Creek drainage and the benefits of maintaining good water quality and habitat into their existing public outreach and community awareness efforts. Enhancing the public's understanding of various water quality parameters, such as water temperature, dissolved oxygen, alkalinity, hardness, turbidity, pH, bacterial concentrations, and macroinvertebrates, should be beneficial for Nashville crayfish and the habitats upon which they depend, but also to the overall environmental quality for local communities.

4. The FHWA and TDOT should use their authorities, to the extent practicable, to support the Tennessee State Wildlife Action Plan (TWRA 2025), the established Nashville crayfish recovery plan, and local initiatives in the Mill Creek watershed that promote good water quality and fish and wildlife habitat.

5. The FHWA and TDOT should integrate project outcomes into statewide watershed planning by sharing data and lessons learned from crayfish relocation, water quality monitoring, and habitat restoration associated with this project with the Duck River Watershed Planning Partnership and other statewide forums established under EO 108. This will support Tennessee's goal of holistic watershed management by using this project as a model for aquatic species conservation in developed areas.

6. The FHWA and TDOT should advance holistic watershed strategies beyond the Duck River by collaborating with TDEC, TWRA, and USFWS to apply EO 108 principles within the Mill Creek watershed. Actions may include riparian buffer enhancement, stormwater best management practices, and aquatic organism passage improvements to maintain connectivity and water quality. These efforts should be coordinated with statewide watershed planning initiatives to ensure consistency with EO 108 objectives.

7. The FHWA and TDOT should promote public engagement consistent with EO 108 by incorporating outreach on crayfish conservation and water quality into existing community engagement programs, and emphasizing how this project supports EO 108's vision for resilient watersheds across Tennessee. Public education should highlight the ecological importance of crayfish and the benefits of maintaining water quality for both wildlife and local communities.

8. The FHWA and TDOT should leverage EO 108 funding and technical support by exploring opportunities for state-level funding or technical assistance under EO 108 initiatives to expand conservation measures beyond the immediate project footprint. Potential activities include upstream habitat restoration, invasive species

control, and additional water quality improvements that benefit listed and proposed aquatic species.

9. The FHWA and TDOT should actively pursue opportunities to collaborate with Nashville Metro Stormwater Services and the Nashville Zoo to evaluate stormwater impoundment areas in the Mill Creek watershed supporting the recovery of the Nashville crayfish. This could include the development of new impoundment areas at existing or proposed structures, or the protection of existing areas Nashville crayfish utilize as habitat.

Nearly all these conservation recommendations will be addressed to some extent by a three-part compensatory mitigation project the Tennessee Department of Transportation approved in March 2026 in partnership with the Service, the Nashville Zoo, Tennessee Department of Environment and Conservation, and the Tennessee Wildlife Resources Agency.

In order for us to be kept informed of actions to minimize or avoid adverse effects or benefit listed species or their habitats, we request notification if any of these conservation recommendations are carried out, or if any other conservation programs benefiting the species considered under this BO are implemented in fulfillment of FHWA's obligations under section 7(a)(1).

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APPENDIX A. Incidental Take of Nashville Crayfish Provided from Previous Consultations

Biological Opinions Issued	Incidental Take Number	Habitat
1994	No more than 25% of the total number of individuals present in the project area	None specified
2002	No more than 2% of the total number of individuals present in the project area	None specified
2002	No more than 3 individuals at each crossing	None specified
2002	None specified	No more than 0.5-ac of suitable habitat
2003	None specified	None specified
2004	No more than 5 individuals	None specified
2008	None specified	No more than 0.5-ac of suitable habitat
2008	None specified	No more than 620 linear ft of suitable habitat
2008	None specified	No more than 320 ft of suitable habitat
2009	None specified	No more than 3,145 linear ft of Mill Creek affected, resulting in the loss of 315 linear feet of suitable habitat
2009	None specified	No more than 4,320 ft^2 of habitat at Mill Creek crossings or 1,120 ft^2 of habitat at the tributary crossings
2009	None specified	No more than the Nashville crayfish occupying 215 ft^2 of the bottom of the Sims Branch pond
2010	None specified	No more than 1,260 ft^2 of Nashville crayfish habitat
2011	No more than 20 individuals per year as a result of collection, holding, and relocation	Loss of 0.5% of suitable habitat from which crayfish are removed at all project sites per year
2011	No more than 2 individuals	None specified
2012	None specified	No more than 45,654 ft^2 (6,906 linear ft) Nashville crayfish habitat
2012	None specified	All Nashville crayfish in 33,023 ft^2 of stream bottom
2012	None specified	No more than 603 ft^2 of Nashville crayfish habitat
2012	None specified	No more than 519 ft^2 of Nashville crayfish habitat
2012	None specified	No more than 532 ft^2 of Nashville crayfish habitat
2012	None specified	No more than 1,077 ft^2 of Nashville crayfish habitat
2013	None specified	No more than 4,466 ft^2 (1,833 linear ft) Nashville crayfish habitat
2013	None specified	No more than 2,082 ft^2 of Nashville crayfish habitat
2013	None specified	No more than 260 ft^2 of Nashville crayfish habitat
2013	None specified	No more than 1,275 ft^2 of Nashville crayfish habitat
2015	None specified	All Nashville crayfish within 2,175 ft^2 of suitable Nashville crayfish habitat.
2015	None specified	All Nashville crayfish in 34,989 ft^2 of stream bottom
2015	None specified	All Nashville crayfish within 1,300 linear ft
2022	146 individuals	Within 7,280 ft^2 of instream habitat
2024	3,328 individuals	Within 48,577 ft^2 of instream habitat

APPENDIX B. Instream Footprint Areas and Habitat Characteristics for Structures in the Action Area

This appendix provides a summary of instream footprint areas for each structure included in the proposed action. These areas represent the portions of streambed directly affected by instream construction activities and therefore constitute the zones where incidental take may occur. This appendix is non-regulatory and serves only as a reference for footprint documentation. The enforceable incidental take estimates are found exclusively in Section 8.4 of this Biological Opinion.

Structure / Location	Stream or Tributary	Type of Work	Instream Footprint Area (ft²)	Notes on Habitat Characteristics
I-40 over Mill Creek	Mill Creek	Bridge replacement (demolition, dewatering, foundation work)	1,179	Mainstem Mill Creek; slab and cobble substrate; high-quality habitat.
East of Massman Drive	UT to Mill Creek	Culvert extension with armoring and diversion	25	Narrow tributary; moderate embeddedness; intermittent habitat patches.
I-40 WB over Spence Lane	UT to Mill Creek	New bridge construction	8	Small tributary with low embeddedness; limited cover.
I-24 EB Connector Ramps	UT to Mill Creek	New bridge construction	38	Moderate-quality riffle/run habitat; scattered cover objects.
Sorghum Branch Crossing	Sorghum Branch	Culvert installation and permanent stream relocation	152	Boulder/cobble substrate; shallow riffles; high-quality tributary habitat.
East of Harding Place	UT to Sorghum Branch	Culvert extension	28	Small tributary; gravel and slab rock present; moderate habitat quality.
East of Blue Hole Road (Bridge 1)	Mill Creek	New bridge construction	80	Mainstem Mill Creek; stable bedrock slab habitat.
East of Blue Hole Road (Bridge 2)	Mill Creek	New bridge construction	98	Mainstem Mill Creek; footprint overlaps with adjacent structure.
Blue Hole Road Mill Creek Replacement	Mill Creek	Bridge replacement	653	Large instream footprint; complex slab-rock interstices.
Collins Creek Crossing	Collins Creek	New bridge construction	214	High-quality tributary habitat; slab and cobble substrate.
Collins Creek Temporary Access	Collins Creek	Temporary stream crossing	37	Overlaps main bridge footprint; no additional area counted.
East of Bell Road Off-Ramp	Collins Creek	New bridge construction	54	Overlaps main Collins Creek footprint.
Bell Road Culvert	Collins Creek	Culvert extension	47	Overlaps bridge footprint; small localized impact.
East of Bell Road (Culvert)	Collins Creek	Culvert extension with minor realignment	144	Mixed cobble and gravel habitat; moderate quality.
Old Franklin Road UT	UT to Collins Creek	Culvert extension and minor relocation	8	Low-quality tributary habitat; shallow intermittent flow.

Note: Some structures are listed with "overlap" notes; these represent locations where action areas or footprints intersect with other project components. In such cases, impacts are not double-counted.